ABSTRACT BOOK

16th ANNUAL
US HUPO Conference

Novel Proteomic Perspectives on Aging, Cancer and Disease

March 8–11, 2020

The Westin Seattle, Seattle Washington, USA

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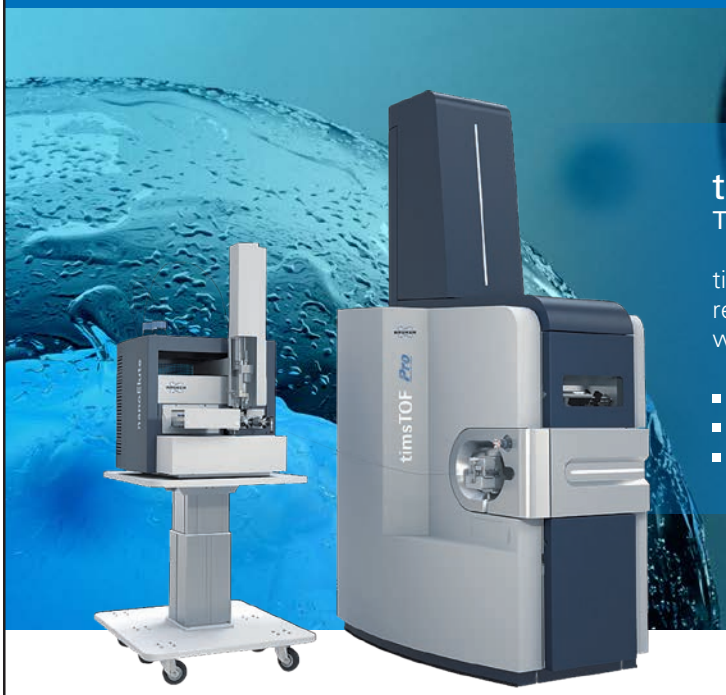

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Meet the Experts Booth #204

Bruker Lunch Seminar
Monday, March 9th, 12:15pm – 1:30pm; Cascade Room #2

diaPASEF: combining improved ion usage efficiency with data independent acquisition to quantify proteomes
Dr. Ben Collins, Reader in the School of Biological Sciences at Queen's University Belfast

Building protein interaction networks to understand KRAS lung cancer
Dr. Peter Jackson, Professor, Stanford University School of Medicine

Register here or at our Booth #204

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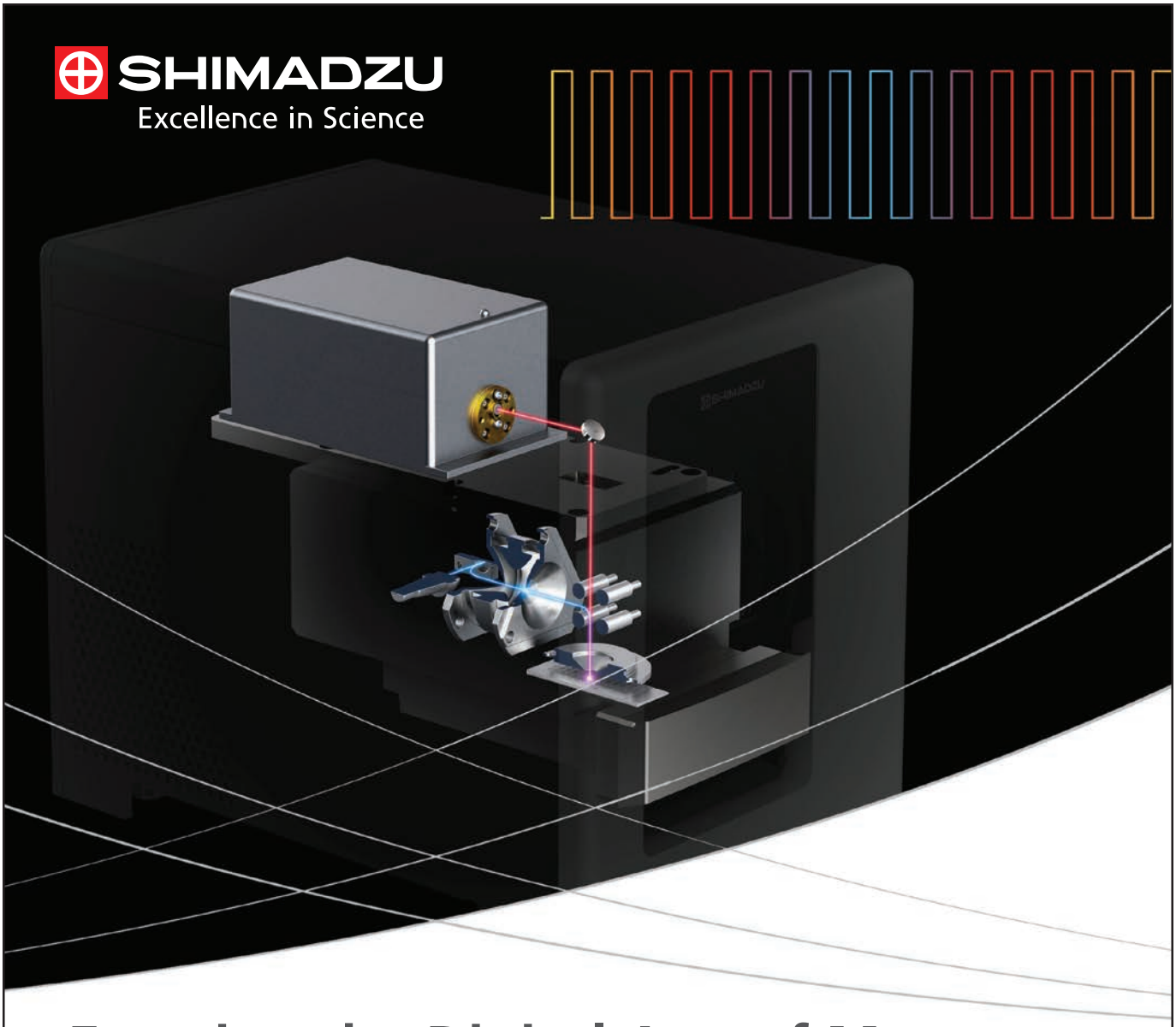

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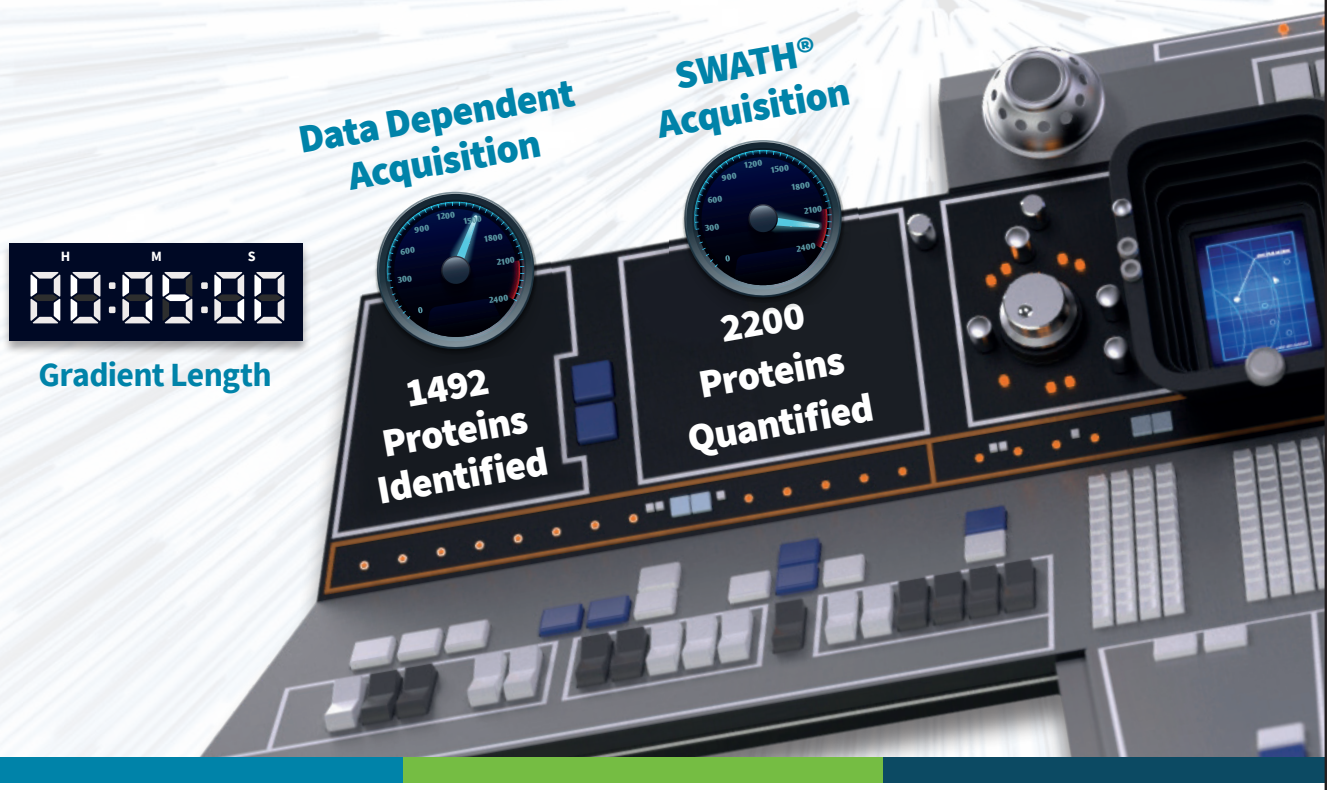

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AGENDA AT A GLANCE

SAT, MARCH 7 - SUN, MARCH 8	MON, MARCH 9	TUES, MARCH 10	WED, MARCH 11
SATURDAY	**8:00 – 8:30 am** Early Morning Coffee *Exhibits* *Grand 2&3*	**8:00 – 8:30 am** Early Morning Coffee *Exhibits* *Grand 2&3*	**8:00 – 8:30 am** Early Morning Coffee *Grand Foyer*
9:00 am - 4:00 pm Full-Day Course, Day 1 **Design and Analysis of Quantitative Proteomics Experiments** *Vashon*	**8:30 – 9:20 am** Plenary Session **Catherine E. Costello Lifetime Achievement in Proteomics Award Lecture** Ruedi Aebersold *Grand 1*	**8:30 – 9:20 am** Plenary Session **New Investigator Award** Si Wu and Nicolas Young **Computational Proteomics** Jimmy Eng *Grand 1*	**8:30 – 9:20 am** Plenary Session **Tips & Tricks Lightning Session** *Grand 1*
SUNDAY	**9:20 – 9:50 am** Coffee Break with Exhibits *Grand 2&3*	**9:20 – 9:50 am** Coffee Break with Exhibits *Grand 2&3*	**9:20 – 9:50 am** Coffee Break *Grand Foyer*
9:00 am - 4:00 pm Full-Day Short Course, Day 2 **Design and Analysis of Quantitative Proteomics Experiments** *Vashon*	**9:50 – 11:10 am** Parallel Sessions **Cascadia Proteomics Symposium Session** *Grand 1* **Cell Biology: Proteomic Analysis of PTMS and Proteoforms** *Fifth Avenue*	**9:50 – 11:10 am** Parallel Sessions **Computation & Analysis: Computational and Statistical Methods** *Grand 1* **Cell Biology: Systems Approaches for Cellular Signaling** *Fifth Avenue*	**9:50 – 11:10 am** Parallel Sessions **Computation & Analysis: Novel 'omics and Multiomics: Data Integration and Applications** *Fifth Avenue* **Cell Biology: Regulation and Function of Protein Phosphorylation** *Grand 1*
9:00 am - 12:00 pm Morning Short Courses **Cross-Linking Mass Spectrometry** *Cascade 1* **Precision Medicine: From Biomarker Discovery to FDA** *Cascade 2*	**11:10 am – 12:00 pm** Plenary Session **Lightning Talks – Round I** *Grand 1*	**11:10 am – 12:00 pm** Plenary Session **Lightning Talks – Round II** *Grand 1*	**11:10 am – 12:00 pm** Plenary Session **Donald F. Hunt Distinguished Contribution in Proteomics Award Lecture** Steven Gygi *Grand 1*
1:00 – 4:00 pm Afternoon Short Course **Stable and Transient Protein-Protein Interactions** *Cascade 2*	**12:15 – 1:30 pm** **Bruker Lunch Seminar** *Cascade Room 2* **Thermo Lunch Seminar** *Cascade Room 1*	**12:15 – 1:30 pm** **SCIEX Lunch Seminar** *Cascade Room 2* **Thermo Lunch Seminar** *Cascade Room 1*	
4:30 – 5:45 pm Workshop **Career Development Event/Workshop**	**1:30 - 3:00 pm** **Poster Session & Break** *Grand 2 & 3*	**1:30 - 3:00 pm** **Poster Session & Break** *Grand 2 & 3*	
6:00 – 7:00 pm Opening Plenary Session **Carol Robinson** *Grand 1* *Vashon*	**3:00 – 4:20 pm** Parallel Sessions **Advances in Technology** *Grand 1* **Proteomics of Disease: Aging and Age-Related Diseases** *Fifth Avenue*	**3:00 – 4:20 pm** Parallel Sessions **Technology: New Developments in Structural Proteomics** *Grand 1* **Proteomics of Disease: Cancer and Precision Medicine** *Fifth Avenue*	
7:00 – 8:30 pm **Welcome Reception** *Mixer with Exhibits* *Grand 2 & 3*	**4:30 – 5:50 pm** Parallel Sessions **Technology: Innovations in Biological Sample Processing** *Grand 1* **Proteomics of Disease: Alzheimer's and Neurodegenerative Diseases** *Fifth Avenue*	**4:30 – 5:50 pm** Parallel Sessions **Technology: Chemical Proteomics and Drug Discovery** *Grand 1* **Proteomics of Disease: Immunity and Infectious Disease** *Fifth Avenue*	
	5:50 – 6:30 pm **Mixer** with Exhibitors *Grand 2 & 3*	**6:00 – 7:30 pm** Workshops **Biomarkers for Cancer, Early Detection** *Cascade 1* **Putting Humpty Dumpty Back Together Again: What Does Protein Quant Mean in Bottom-Up Proteomics?** *Cascade 2*	
	Workshops **Proteomics of Aging** *Cascade 2* **Data-Independent Acquisition (DIA) Workflows** *Cascade 1*	**7:30 – 9:00 pm** **Evening Social & Music with Exhibits** *Grand 2 & 3*	

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VENDOR LUNCH SEMINARS

The following companies offer lunch seminars to conference participants on Monday and Tuesday. Seating is limited. Please reserve a seat at company exhibit booths.

Monday, March 9
12:15 PM – 1:30 PM

Bruker, Cascade 2

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Tuesday, March 10
12:15 PM – 1:30 PM

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AWARDS

Catherine E. Costello Lifetime Achievement in Proteomics Award Lecture

Award presentation and talk, Monday 8:30 AM – 9:20 AM, Grand 1

The Lifetime Achievement in Proteomics Award recognizes a career of discovery that has made a lasting impact on the field of proteomics.

2020 Recipient: **Ruedi Aebersold**
ETH Zurich

Ruedi Aebersold is a Swiss and Canadian scientist trained at the Biocenter, University of Basel. He completed his education at Caltech. He is a Professor at ETH Zurich and the University of Zurich. Ruedi was on the faculties of the Universities of British Columbia and Washington and co-founded, with Lee Hood and Alan Aderem, the Institute for Systems Biology in Seattle. He has co-founded several companies and holds several public service appointments. Ruedi was awarded an honorary doctorate of the University of Lund, Sweden and the work of the group was recognized with numerous prizes and awards.

The research focus of his group is the proteome. The group has pioneered several widely used techniques and generated a range of open access/open source software and statistical tools that have contributed to making proteomic research results more transparent, reproducible and accurate. In his group, these techniques have been applied to a wide range of projects in basic and translational research.

Donald F. Hunt Distinguished Contribution in Proteomics Award

Award presentation and talk, Wednesday 11:10 AM – 12:00 PM, Grand 1

The Donald F. Hunt Distinguished Contribution in Proteomics award recognizes a focused or singular achievement in the field of proteomics. Eligibility is restricted to members of US HUPO.

2020 Recipient: **Steven Gygi**
Harvard Medical School

Steven Gygi received his Ph.D. from the University of Utah in Pharmacology and Toxicology performing small molecule mass spectrometry. He went on to pursue postdoctoral work with Ruedi Aebersold at the University of Washington in 1996. A revolution in biological mass spectrometry was occurring which allowed for the measurement of protein expression levels and a new field, Proteomics, was born. In 2000, Dr. Gygi moved to Harvard Medical School and joined the Department of Cell Biology. Currently, he is the faculty director of two MS core facilities (Taplin Biological MS Facility, and the Thermo Fisher Center for Multiplexed Proteomics—TCMP@HMS).

Research in the Gygi lab centers around developing and applying new technologies in the field of mass spectrometry-based proteomics. These include the systematic and proteome-wide measurements of many protein properties including their expression levels, modification states, structure, localization, function, and interactions. For example, the Gygi lab, together with the Harper lab, is creating a genome-scale map of the protein-protein interaction landscape in cells (termed BioPlex). In addition, sample multiplexing techniques like Tandem Mass Tags (TMT) are being improved to allow up to 16 proteomics samples to be analyzed simultaneously using high resolution mass spectrometry.

Gilbert S. Omenn Computational Proteomics Award

Award presentation and talk, Tuesday 8:30 AM – 9:35 AM, Grand 1

This award recognizes the essential nature of computational methodology and software in proteomics. Specifically, this award acknowledges the specific achievements of scientists that have developed bioinformatics, computational, statistical methods and/or software used by the proteomics community, broadly defined.

2020 Recipient: **Jimmy Eng**
University of Washington

Jimmy Eng earned his Master's degree in Electrical Engineering from the University of Washington. He then joined the Yates lab in 1993 at the University of Washington, where he wrote a number of tools including SEQUEST. In 2000, he joined the Institute for Systems Biology where, as a member of the Aebersold lab, he initiated the development of the mzXML data format and spearheaded the open source Trans-Proteomics Pipeline suite of tools. In 2004, he joined the McIntosh lab at the Fred Hutchinson Cancer Research Center, working on computational proteomics approaches applied to the early detection of cancer. And in 2007, Jimmy returned to the University of Washington as the computational lead for the university's Proteomics Resource, continuing to develop open source tools for the proteomics community. He has co-authored over 150 peer-reviewed journal articles and book chapters.

Robert J. Cotter New Investigator Award

Award presentation and talk, Tuesday 8:30 AM – 9:35 AM, Grand 1

This award was established to honor the memory of Bob Cotter, a founding member of US HUPO, for his many contributions to scientific research and for his legacy as a mentor to young scientists.

2020 Co-Recipient: **Si Wu**
University of Oklahoma

Dr. Wu received her B.S. in Chemistry from Anhui University, and Ph.D. in Analytical Chemistry at the Washington State University with Professor James E. Bruce. She conducted her postdoctoral research on top-down proteomics at the Pacific Northwest National Laboratory (PNNL) with Drs. Ljiljiana Paša-Tolić; and Richard D. Smith. Later, she worked as a research scientist at Battelle Toxicology Northwest and a senior scientist at PNNL. In 2015, she joined the Department of Chemistry and Biochemistry at the University of Oklahoma as an assistant professor. Her research interests focus on developing and applying high-throughput quantitative top-down proteomics techniques for addressing important clinical and biological questions.

2020 Co-Recipient: **Nicolas L. Young**
Baylor College of Medicine

Nicolas Young is an Assistant Professor at Baylor College of Medicine since 2016. Dr. Young received his Ph.D. in Chemistry from the University of California, Davis with Professor Carlito Lebrilla. He received postdoctoral training at Princeton University in the Department of Molecular Biology with Professor Benjamin Garcia. He has also worked as the Director of Biological Applications at the National High Magnetic Field Laboratory with Professor Alan Marshall, as a Staff Scientist at Lawrence Livermore National Laboratory, and in the biotechnology industry. Dr. Young uses proteomics to elucidate the mechanisms by which protein post-translational modifications modulate gene transcription and gene expression. In this endeavor he has developed methods for the identification and quantitation of extensively modified proteins by top and middle down proteomics. This work has involved the development of chromatographies that separate structural isomers of large peptides and intact proteins, mass spectrometry methods, data analysis algorithms for mixed tandem mass spectra, and methods for data interpretation to reveal mechanisms and biological function. His results have unveiled fundamental biological mechanisms and informed novel therapeutic routes for the treatment of disease.

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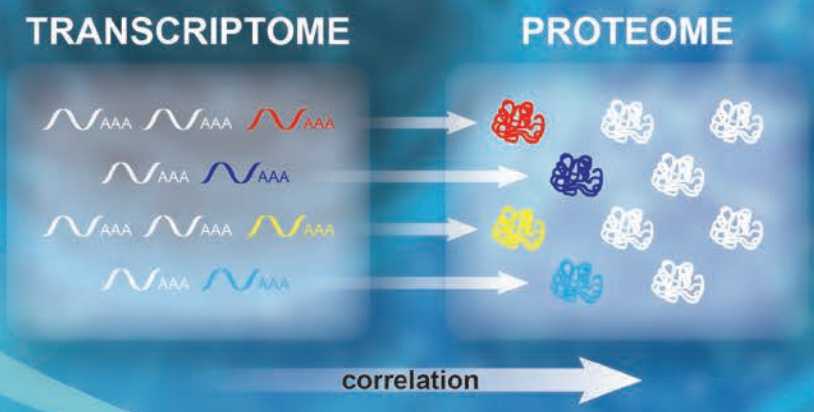

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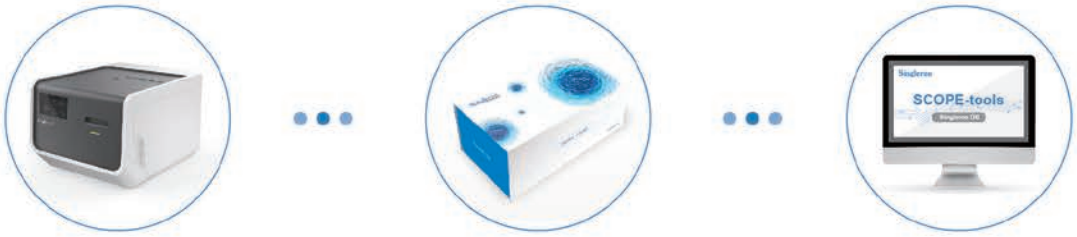

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DAY 1

8:00 AM – 6:30 PM **Registration** *Grand Foyer*

8:00 – 8:30 AM **Morning Coffee with Exhibits** *Grand 2 & 3*

4:30 – 5:45 PM **Career Development Event/Workshop** *Vashon*
Laurie Parker, University of Minnesota

6:00 – 7:00 PM Conference Opening Keynote *Grand 1*

Session Chair: Jennifer VanEyck, Cedars-Sinai Medical Center

Mass Spectrometry: From Plasma Proteins to Mitochondrial Membranes
Carol Robinson, *University of Oxford, Oxford, UK*

7:00 – 8:30 PM **Welcome Reception with Exhibits** *Grand 2 & 3*
Sponsored by: Shimadzu

DAY 2

7:00 – 8:00 AM **Fun Run** *Meet in the Lobby*
Sponsored by: The Buck Institute

8:00 AM – 6:30 PM **Registration** *Grand Foyer*

8:00 – 8:30 AM **Morning Coffee with Exhibits** *Grand 2 & 3*

8:30 – 9:20 AM Catherine E. Costello Lifetime Achievement in Proteomics Award Lecture *Grand 1*

Session Chair: Ileana Cristea, Princeton University

8:30 – 9:20 AM **Proteomic in 2020: Much achieved—much left to learn**
Ruedi Aebersold, *ETH Zurich, Zürich, CH*

9:20 – 9:50 AM **Break with Exhibits** *Grand 2 & 3*

9:50 – 11:10 AM PARALLEL SESSION Cascadia Proteomics Symposium Session *Grand 1*

Session Chair: Robert Moritz and Eric Deutsch, Institute for Systems Biology

9:50 – 10:05 AM **Sample Preparation of OCT-embedded Brain Tissue for Quantitative Proteomics**
Julia Robbins[1]; Gennifer Merrihew[1]; Tom E. Montine[2]; Michael MacCoss[1], *[1]University of Washington, Seattle, WA; [2]Stanford University, Palo Alto, CA*

10:05 – 10:20 AM **The proteome landscape of childhood cancers Implications on xenograft models, drug sensitivity and optimal diagnostic timepoint**
Amanda Lorentzian[1, 2]; Anuli Uzozie[1, 2]; James Lim[1, 2]; Christopher Maxwell[1]; Gregor Reid[1]; Philipp Lange[1, 2], *[1]University of British Columbia, Vancouver, Canada; [2]BC Children's Hospital, Vancouver, Canada*

10:20 – 10:35 AM **Miro: a method for the proteome-wide identification of amino acid substitutions that are deleterious for protein function**
Ricard Rodriguez-Mias; Kyle Hess; Bianca Ruiz; Stephanie Zimmerman; Ian Smith; Anthony Valente; Yang Lu; William S. Noble; Stanley Fileds; Judit Villen, *University of Washinngton, Seattle, WA*

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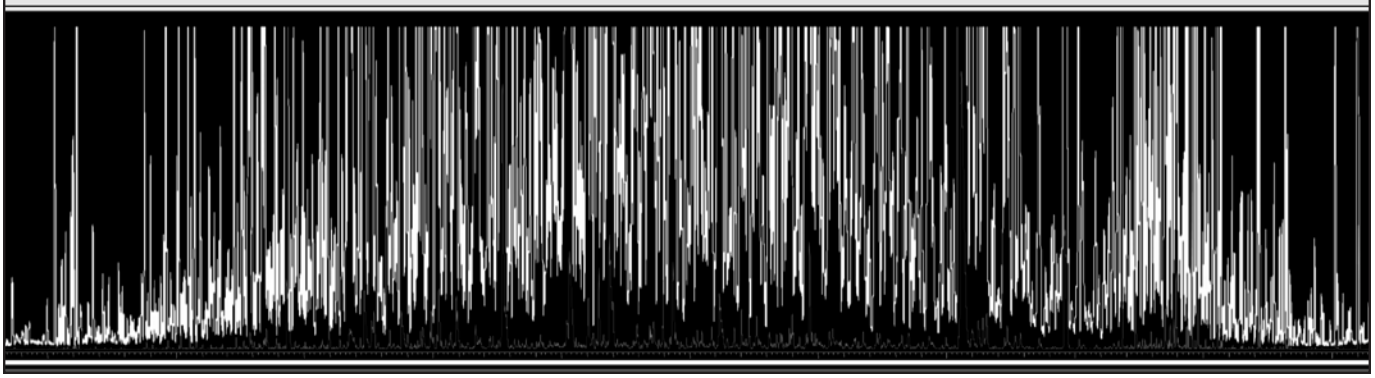

10:35 – 10:50 AM **Absolute quantification of transcription factors reveals principles of gene regulation during human erythropoiesis**
Mark A. Gillespie*[1]; Carmen G. Palii*[2]; Daniel Sanchez-Taltavull*[3]; Theodore J. Perkins[2]; Marjorie Brand[2]; Jeffrey A. Ranish[1], *[1]Institute for Systems Biology, Seattle, WA; [2]Ottawa Hospital Research Institute, Ottawa, ON; [3]Visceral Surgery, University of Bern, Bern, Switzerland*

10:50 – 11:00 AM **Comparative proteomics of serum from 31 mammalian species**
Benjamin Neely[1]; Magnus Palmblad[2]; Phillip Wilmarth[3]; Alison Bland[4, 5]; Michael Janech[4, 5], *[1]National Institute of Standards and Technology, Charleston, SC; [2]Leiden University Medical Center, Leiden, The Netherlands; [3]Oregon Health & Science University, Portland, OR; [4]Hollings Marine Laboratory, Charleston, SC; [5]College of Charleston, Charleston, SC*

11:00 – 11:10 AM **"Borrelia PeptideAtlas" a resource for Lyme disease research**
Jaipalreddy Panga[1]; Zhi Sun[1]; Michael R. Hoopmann[1]; David S Campbell[1]; David Baxter[1]; Strle Klemen[2]; Leroy Hood[1]; Kai Wang[1]; Piesman J[4]; Allen Steere[3]; Robert L. Moritz[1], *[1]Institute for Systems Biology, Seattle, WA; [2]Massachusetts General Hospital, MA & Wadsworth Cen, New York, MA; [3]Massachusetts General Hospital, New York, MA; [4]CDC, Salt Lake City, UT*

9:50 – 11:10 AM

PARALLEL SESSION
Cell Biology: Proteomic Analysis of PTMs and Proteoforms

Fifth Avenue

Session Chair: Hui Zhang, John Hopkins University

9:50 – 10:15 AM **Novel Top-Down Proteomics Strategies for Comprehensive Analysis of Proteoforms**
Ying Ge, *University of Wisconsin-Madison, Madison, WI*

10:15 – 10:40 AM **Profiling System Dynamics and Crosstalk Between the Ubiquitin and Nedd8 Systems**
Donald Kirkpatrick, *Genentech*

10:40 – 10:55 AM **Site-specific analysis of the Asp- and Glu-ADP-ribosylated proteome by quantitative mass spectrometry**
Yonghao Yu, *UT Southwestern Medical Center, Dallas, TX*

10:55 – 11:10 AM **EXoO-Tn: Tag-n-Map the Tn Antigen in the Human Proteome**
Weiming Yang, *Johns Hopkins University, Baltimore, MD*

11:10 AM – 12:00 PM

LIGHTNING SESSION
Round 1

Grand 1

Lightning Sessions will be 60 seconds.

Session Chairs: Karin Rodland, Pacific Northwest National Laboratory and Birgit Schilling, Buck Institute on Aging

Poster 01.01 **Cartridge and bead-based sample preparation for proteome analysis: Assessing robustness of protein recovery and digestion efficiency**
Jessica Nickerson; Alan Doucette, *Dalhousie University, Halifax, Canada*

Poster 01.14 **Spray-capillary Based Capillary Electrophoresis Mass Spectrometry Analysis of Metabolites in Live Cells**
Lushuang Huang; Zhe Wang; Kellye Cupp-Sutton; Si Wu, *University of Oklahoma, Norman, OK*

Poster 02.02 **Region-Specific N-Glycome Mapping of the Human Brain in Alzheimer's Patients by nanoLC chip-Q-TOF MS Analysis**
Jennyfer Tena; Mariana Barboza; Maurice Wong; Carlito B. Lebrilla, *University of California, Davis, Davis, CA*

Poster 02.03 **Quantifying Proteostasis: An *In Vivo* Protein Quality Assay Based On Fold Stability**
Hsien-Jung Lavender Lin; Stella Park; Nathan Zuniga; John Price, *Brigham Young University, Provo, UT*

Poster 02.04 **Improved Isolation Strategies to Increase the Yield and Purity of Human Sera Exosomes for Protein Biomarker Discovery**
Sandip Kumar Patel[1]; Roland Bruderer[2]; Francesco Neri[1]; Nathan Basisty[1]; Lukas Reiter[2]; Judith

Campisi[1]; Birgit Schilling[1], *[1]Buck Institute Research on Aging, Novato, California; [2]Biognosys AG, 8952 Schlieren, Switzerland*

Poster 02.05 **Tissue specific changes in protein metabolism associated with Alzheimer's risk**
Nathan Zuniga; Joseph Creery; John Price, *Brigham Young University, Provo, UT*

Poster 02.07 **Understanding brain aging in non-human primates using high resolution proteomics**
Zeeshan Hamid[1]; Cun Li[2, 3]; Peter Nathanielsz[2, 3]; Laura Cox[1, 2]; Michael Olivier[1], *[1]Wake Forest University School of Medicine, Winston-Salem, NC; [2]Southwest National Primate Research Center, San Antonio, TX; [3]Department of Animal Science, Univ. of Wyoming, Laramie, WY*

Poster 02.08 **Global Analysis of Protein Folding Stability Changes During Parkinson's Disease in a Mouse Model**
Renze Ma[1]; Julia Johnson[1, 2]; Michael FItzgerald[1], *[1]Duke University, Durham, NC; [2]Washington University in St. Louis, St. Louis, MO*

Poster 02.09 **Comparative N-Glycoproteomics Reveals New Molecular Players in Alzheimer's Disease**
Xiaofang Zhong; Zhengwei Chen; Qinying Yu; Lei Lu; Lingjun Li, *University of Wisconsin-Madison, Madison, WI*

Poster 02.12 **Identifying molecular signatures of neurodegeneration – integrative proteomics and transcriptomics quantifies differences between induced cranial and spinal motor neurons**
Shuvadeep Maity[1]; Disi An[2]; Justin Randleman[2]; Dylan Iannitelli[2]; Esteban O Mazzoni[1, 2]; Christine Vogel[1, 2], *[1]Center For Genomics and Systems Biology(CGSB), NYU, New York, USA; [2]Department Of Biology, NYU, New York, USA*

Poster 02.13 **Proteomic Profiling of Extracellular Vesicles Isolated from Human Alzheimer's Disease Brain Tissue.**
Manveen K Sethi; Satoshi Muraoka; Annina M. DeLeo; John D Hogan; Tsuneya Ikezu; Joseph Zaia, *Boston University School of Medicine, Boston, MA*

Poster 02.18 **A multiomic study uncovers the interplay between mitochondrial sirtuin 4 antiviral function and virus inhibition of host defense**
Cora Betsinger; Elizabeth Rowland; Ileana Cristea, *Princeton University, Princeton, NJ*

Poster 02.19 **Protein structural accessibility differences associated with Alzheimer's disease and aging in cerebrospinal fluid and brain tissue by limited proteolysis-mass spectrometry**
Danielle A. Faivre; Eric L. Huang; Michael J. Maccoss, *Genome Sciences, University of Washington, Seattle, WA*

Poster 02.21 **Proteome-wide differences in turnover rates among mammals are correlated to their lifespans and energetic demands**
Kyle Swovick[1]; Denis Firsanov[1]; Kevin Welle[2]; Jennifer Hryhorenko[2]; Andrei Seluanov[1]; Vera Gorbunova[1]; Sina Ghaemmaghami[1], *[1]University of Rochester, Rochester, NY; [2]University of Rochester Mass Spectrometry Center, Rochester, NY*

Poster 02.22 **A quantitative proteomics strategy that accommodates multiple quantitative values for each protein coding gene: Applications in Alzheimer's disease**
Deanna Plubell[1]; Lukas Käll[2]; Gennifer Merrihew[1]; Thomas Montine[3]; Michael MacCoss[1], *[1]University of Washington, Seattle, ; [2]KTH Royal Institute of Technology, Stockholm , Sweden; [3]Stanford University, Palo Alto, CA*

Poster 02.23 **Mass spectrometry analysis reveals altered proteins across cerebellar lobules in the Niemann-Pick, type C1 mouse model**
Melissa Pergande; Thu Nguyen; Fernando Tobias; Rathnayake A. C. Rathnayake; Stephanie Cologna, *Univ of Illinois at Chicago, Chicago, IL*

Poster 03.03 **Correlation of the plasma ΔS-Cys-Albumin integrity marker with changes in clinical analyte measurements founded on molecular interactions**
Erandi Kapuruge; Chad Borges, *Arizona State University, Tempe, AZ*

Poster 03.04 **A sensitive targeted proteomics approach to quantify low abundant proteins of the unfolded protein response pathway in glioblastoma cells**
Chi DL Nguyen[1]; Sebastian Malchow[1]; Stefan Reich[2]; Sascha Steltgens[3]; Konstantin V Shuvaev[1]; Stefan Loroch[1]; Christin Lorenz[1]; Albert Sickmann[1, 7]; Christiane B Knobbe-Thomsen[3]; Björn Tews[4, 5]; Jan Medenbach[2]; Robert Ahrends[1, 6], *[1]Leibniz Institute for Analytical Science ISAS-e.V., Dortmund, Germany; [2]Translational Control Group UniversityofRegensburg, Regensburg, Germany; [3]Institute of Neuropathology University Düsseldorf, Düsseldorf, Germany; [4]University of Heidelberg and DKFZ, Heidelberg, Germany; [5]Molecular Mechanisms of Tumor Invasion, DKFZ, Heidelberg, Germany; [6]Department Analytical Chemistry UniversityofVienna, Vienna, Austria; [7]Medizinische Fakultät, Ruhr-Universität Bochum, Bochum, Germany*

Poster 04.04 CANCELLED

Poster 04.12 **Proteomic analysis reveals TNF-α derived different regulation of mitochondrial complexes in ER/PR positive and negative breast cancer cells**
Hye Ryeon Jung[1]; Anjali Shinde[2]; Ha Yun Lee[1]; Kritarth Singh[2]; Jin Woo Jung[1]; Seo Jin Yang[1]; Han Byeol Kim[1]; Rajesh Singh[2]; Eugene C Yi[1], *[1]Seoul National University, Seoul, South Korea; [2]The MS University of Baroda, Vadodara, India*

Poster 04.15 **Characterizations of acute leukemia, myeloid and lymphoid, proteomes using proteogenomics and OpenProt database to identify new proteins and possible biomarkers.**
Noé Guilloy; Marie Brunet; Xavier Roucou, *Univerité de sherbrooke, Sherbrooke, Canada*

Poster 05.02 **Boosting statistical power in small-scale experiments with Percolator**
William Fondrie; William Noble, *University of Washington, Seattle, WA*

Poster 08.01 **Proteome-wide detection of function misannotation by taxon-specific rate ratio comparison**
Chengxin Zhang[1]; Xiaoqiong Wei[1, 2]; Gilbert S. Omenn[1]; Peter L. Freddolino[1]; Yang Zhang[1], *[1]University of Michigan, Ann Arbor, MI; [2]West China Hospital, Sichuan University, Chengdu, Sichuan, China*

Poster 08.06 **Clustering and identifying hundreds of millions of tandem mass spectra using deep learning**
Wout Bittremieux[1]; Damon H. May[2]; Jeffrey Bilmes[2]; William Stafford Noble[2], *[1]University of California San Diego, La Jolla, CA; [2]University of Washington, Seattle, WA*

Poster 08.08 **MSstatsSampleSize: Simulation tool for optimal design of high-dimensional MS-based proteomics experiments**
Ting Huang[1]; Meena Choi[1]; Tiannan Guo[2, 4]; Yansheng Liu[3]; Ruedi Aebersold[4, 5]; Olga Vitek[1], *[1]Northeastern University, Boston, MA; [2]Westlake Institute for Advanced Study, Hangzhou, China; [3]Yale University School of Medicine, West Haven, CT; [4]ETH Zürich, Zürich, Switzerland; [5]University of Zürich, Zürich, Switzerland*

Poster 08.09 **Phosphopedia 2.0, a modern targeted phosphoproteomics resource**
Anthony Valente; William S Noble; Judit Villen, *Genome Sciences, UW, Seattle, WA*

Poster 08.11 **Fast and quantitative analysis of timsTOF PASEF data with MSFragger and IMQuant**
Fengchao Yu; Sarah Haynes; Guo Ci Teo; Andy Kong; Dmitry Avtonomov; Felipe Leprevost; Hui-Yin Chang; Daniel Geiszler; Dan Polasky; Alexey Nesvizhskii, *University of Michigan, Ann Arbor, MI*

Poster 10.01 **Matching peptides to data independent acquisition mass spectrometry data**
Yang Lu; Wenruo Bai; Jeff Bilmes; William Noble, *University of Washington, Seattle, WA*

Poster 10.02 **Longitudinal DIA-MS reveals key phosphorylation nodes dedicating cell proliferation and differentiation**
Wenxue Li[1]; Anatoly Kiyatkin[1]; Barbora Salovska[2]; Chongde Wu[1]; Erli Gao[1]; Archer Hamidzadeh[3]; Qian Ba[1]; Yansheng Liu[1], *[1]Yale Cancer Biology Institute, West Haven, CT; [2]Department of Genome Integrity, Institute of Molec, Prague, Czech Republic; [3]Yale Systems Biology Institute, West Haven, Conneticut*

Poster 12.01 **Ion Mobility Separation of Glycoforms with Isomerism on the Peptide and Glycan Levels.**
Pratima Pathak; Matthew A. Baird; Alexandre A. Shvartsburg, *Wichita State University, Wichita, KS*

Poster 12.03 **Identification of O-glycopeptides with MetaMorpheus**
Lei Lu[1]; Nicholas Riley[2]; Michael Shortreed[1]; Carolyn Bertozzi[2]; Lloyd Smith[1], *[1]University of Wisconsin-Madison, Madison, WI; [2]Stanford University, Stanford, CA*

Poster 12.04 **Optimized Liquid Chromatography Separation of INLIGHT® Derivatized N-linked Glycans for Isomer Identification and Structure Elucidation in Alzheimer's Disease Brain Samples**
Jaclyn Kalmar[1]; Karen Butler[1]; Erin Baker[1]; Brendan MacLean[2]; Michael MacCoss[2]; Edward Fox[3]; Thomas Montine[3]; David Muddiman[1], *[1]N. Carolina State University, Raleigh, NC; [2]University of Washington, Seattle, WA; [3]Stanford University, Stanford, CA*

Poster 15.01 **Identification of HLA Class I Bound Peptide for Behcet's Disease by mass spectrometric analysis**
Kyung-Cho Cho, *Seoul National University, Seoul, South Korea*

Poster 15.02 **caATLAS: an immunopeptidome atlas of human cancer**
Xinpei Yi; Yuxing Liao; Kai Li; Bo Wen; Bing Zhang, *Baylor College of Medicine, Houston, TX*

Poster 16.02 **Using Metabolomics, CE-MS and Isotope Incorporation to inform Alzheimer's Research**
Kendra Adams[2]; W. Kirby Gottschalk[3]; Joan Wilson[3]; David Millington[1]; Arthur Moseley[2]; J. Will Thompson[2]; Carol Colton[3], *[1]Duke University, Durham, North Carolina; [2]Duke University School of Medicine, Durham, NC; [3]Duke University Department of Neurology, Durham, NC*

12:15 – 1:30 PM Monday Lunch Seminars

Open to all attendees, but RSVP strongly encouraged (at booth or online) in advance as space is limited.

12:15 – 1:30 PM **BRUKER** *Cascade Room 2*
If you have not registered in advance, please stop by the booth.

12:15 – 1:30 PM **THERMO FISHER SCIENTIFIC** *Cascade Room 1*
If you have not registered in advance, please stop by the booth.

1:30 – 3:00 PM Poster Session & Refreshment Break *Grand 2, 3 & Foyer*

Odd-numbered poster boards present from 1:30-2:15 PM and even-numbered poster boards present from 2:15-3:00 PM.

3:00 – 4:20 PM PARALLEL SESSION
Technology: Advances In Technology *Grand 1*

Session Chair: Si Wu, University of Oklahoma

3:00 – 3:25 PM **Data-independent Acquisition Combined with Parallel Accumulation – Serial Fragmentation Combined (diaPASEF): Bottom-up Proteomics With Increased Ion Usage**
Ben Collins, *Queens University Belfast, Belfast, UK*

3:25 – 3:50 PM **A View of Multiscale Protein Organization and Function in Cancer**
Joe Gray, *Oregon Health & Science University, Portland, OR*

3:50 – 4:05 PM **New Directions for IR-MALDESI Mass Spectrometry Imaging: From Cells, Bones and Neurotransmitters to Drift Tube Ion Mobility**
David Muddiman; Crystal Pace; Sitora Khodjaniyazova; Caleb Bagley; Anqi Tu; Hongxia Bai; James Dodds; Erin Baker, *NC State University, Raleigh, NC*

4:05 – 4:20 PM **Isobaric Quantitative Protein Interaction Reporter Technology for Comparative Interactome Studies**
Juan Chavez; Andrew Keller; Jared Mohr; Jimmy Eng; James Bruce, *University of Washington, Seattle, WA*

3:00 – 4:20 PM PARALLEL SESSION
Proteomics of Disease: Aging and Age-Related Diseases *Fifth Avenue*

Session Chair: Matt Kaeberlein, University of Washington

3:00 – 3:25 PM **Proteomics and Metabolomics in Studies of the Biology of Aging: Connecting Mitochondrial Energetics to Physiological Function**
Peter Rabinovitch, *University of Washington, Seattle, WA*

Sponsored By:

3:25 – 3:50 PM **A High Throughput Proteomics Platform to Investigate Aging**
Fiona McAllister, *Calico Labs*

3:50 – 4:05 PM **Senescence-Based Biomarker Signatures of Aging and Senescence Burden**
Nathan Basisty[1]; Abhijit Kale[1]; Okhee H Jeon[1]; Chisaka Kuehnemann[1]; Therese Payne[1]; Chirag Rao[1]; Anja Holtz[1]; Samah Shah[1]; Vagisha Sharma[3]; Luigi Ferrucci[4]; Judith Campisi[1, 2]; Birgit Schilling[1], *[1]The Buck Institute, Novato, CA; [2]Lawrence Berkeley Laboratory, Berkley, CA; [3]University of Washington, Seattle, WA; [4]National Institute on Aging, Baltimore, MD*

4:05 – 4:20 PM **Identification of Proteins with Reduced Solubility in Aging Mouse Brains**
Cristen Molzahn; Mang Zhu; Lorenz Nierves; Aly Karsan; Philipp Lange; Thibault Mayor
University of British Columbia, Vancouver, Canada

4:30 – 5:50 PM

PARALLEL SESSION
Technology: Innovations in Biological Sample Processing

Grand 1

Session Chair: Stephanie Cologna, University of Illinois at Chicago

4:30 – 4:55 PM **Microscaled Methods for Proteogenomic Analysis of Patient-derived Tumors**
Steve Carr, *Broad University, Cambridge, MA*

4:55 – 5:20 PM **Sample Processing and Analysis Platform for In-Depth Single-Cell Proteomics**
Ryan Kelly, *Brigham Young University, Provo, UT*

5:20 – 5:35 PM **Single Cell Proteomics and the Carrier Proteome Effect**
Atticus McCoy; Christopher M. Rose, *Genentech, South San Francisco, CA*

5:35 – 5:50 PM **Multidimensional Cross-linking With a Tetra-reactive Cross-linker**
Jared Mohr; Juan Chavez; James E. Bruce, *University of Washington, Seattle, Washington*

4:30 – 5:50 PM

PARALLEL SESSION
Proteomics of Disease: Alzheimer's and Neurodegenerative Diseases

Fifth Avenue

Session Chair: John Price, Brigham Young University

4:30 – 4:55 PM **The Human Brainome: Genome, Transcriptome and Proteome Interaction in Human Cortex**
Amanda Myers, *University of Miami, Coral Gables, FL*

4:55 – 5:20 PM **An Integrated Proteomics Approach to Resolve Brain-based Biomarkers in Alzheimer's Disease**
Nicolas Seyfried, *Emory University, Atlanta, GA*

5:20 – 5:35 PM **Proteogenomic analysis reveals *FUS* gene as dual-coding with both proteins united in molecular hallmarks of amyotrophic lateral sclerosis**
Marie A Brunet; Jean-Francois Jacques; Amina Lekehal; Xavier Roucou, *Université de Sherbrooke, Sherbrooke, Canada*

5:35 – 5:50 PM **A Consensus Proteomic Analysis of Alzheimer's Disease Brain and Cerebrospinal Fluid Reveals Early Changes Associated with Microglia and Astrocyte Activation**
Erik Johnson[1]; Eric Dammer[1]; Duc Duong[1]; Lingyan Ping[1]; Maotian Zhou[1]; Luming Yin[1]; Lenora Higginbotham[1]; Andrew Guajardo[2]; Bartholomew White[2]; Juan Troncoso[2]; Madhav Thambisetty[3]; Thomas Montine[4]; Edward Lee[5]; John Q. Trojanowski[5]; Thomas Beach[6]; Eric Reiman[7]; Vahram Haroutunian[8]; Minghui Wang[8]; Eric Schadt[8]; Bin Zhang[8]; Dennis Dickson[9]; Nilufer Taner[9]; Todd Golde[10]; Vladislav Petyuk[11]; Philip De Jager[12]; David Bennett[13]; Thomas Wingo[1]; Srikant Rangaraju[1]; Ihab Hajjar[1]; Joshua Shulman[14]; James Lah[1]; Allan Levey[1]; Nicholas Seyfried[1], *[1]Emory University, Atlanta, GA; [2]Johns Hopkins School of Medicine, Baltimore, MD; [3]National Institutes of Health, Bethesda, MD; [4]Stanford University, Palo Alto, CA; [5]University of Pennsylvania, Philadelphia, PA; [6]Banner Sun Health Research Institute, Sun City, AZ; [7]Banner Alzheimer's Institute, Phoenix, AZ; [8]Mount Sinai School of Medicine, New York, NY; [9]Mayo Clinic, Jacksonville, FL; [10]University of Florida, Gainesville, FL; [11]Pacific Northwest National Laboratory, Richland, WA; [12]Columbia University, New York, NY; [13]Rush University, Chicago, IL; [14]Baylor College of Medicine, Houston, TX*

DAY 2

5:50 – 6:30 PM **Mixer with Exhibits** *Grand 2 & 3*

6:30 – 7:30 PM

EVENING WORKSHOP
Proteomics of Aging

Cascade 2

Sponsored by: University of Washington Nathan Shock Center of Excellence in the Basic Biology of Aging

Organized by: Peter Rabinovitch, *University of Washington,* Michael MacCoss, *University of Washington*, Judit Villen, *University of Washington* and Birgit Schilling, *The Buck Institute*

Discussion on latest developments and applications in the aging field.

6:30 – 7:30 PM

EVENING WORKSHOP
Data-Independent Acquisition (DIA) Workflows

Cascade 1

Organized by: Brian Searle, Institute for Systems Biology
How to navigate your way through DIA workflows and data processing.

7:00 AM – 8:00 AM	**Sponsor Breakfast (Private Meeting)**	*Grand Crescent*
8:00 AM – 7:30 PM	**Registration**	*Grand Foyer*
8:00 – 8:30 AM	**Morning Coffee with Exhibits**	*Grand 2 & 3*

8:30 – 9:20 AM — Gilbert S. Omenn Computational Proteomics Award and Robert J. Cotter New Investigator Award Talks

Grand 1

Session Chair: Gil Omenn, University of Michigan

8:30 – 9:35 AM

High-throughput Quantitative Top-down Proteomics
Si Wu, *University of Oklahoma, Norman, OK*

Temporarily and Spatially Resolved Quantitative Top Down Proteomics for Neuroepigenetics
Nicolas Young, *Baylor University, Waco, TX*

My Pathway in Computational Proteomics
Jimmy Eng, *University of Washington, Seattle, WA*

9:35 – 9:50 AM — **Coffee Break with Exhibits** — *Grand 2 & 3*

9:50 – 11:10 AM — PARALLEL SESSION Computation & Analysis: Computational and Statistical Methods

Grand 1

Session Chair: Olga Vitek, Northeastern University

9:50 – 10:15 AM

Intelligent Data Acquisition for Multiplexed Proteomics
David Schweppe, *Harvard Medical School, Boston, MA*

10:15 – 10:40 AM

Measuring Phosphorylation State in the Human Proteome
Brian Searle, *Institute for Systems Biology, Seattle, WA*

10:40 – 10:55 AM

Use of Gas Phase Fractionation Data Independent Acquisition and Spectrum Centric Searching to Build Matrix Specific Libraries for Quantitative Analysis
Lilian Heil; Michael Maccoss, *University of Washington, Seattle, WA*

10:55 – 11:10 AM

MassIVE.quant: a community resource of curated quantitative mass spectrometry-based proteomics datasets; Meena Choi[1]; Jeremy Carver[2]; Cristina Chiva[3]; Manuel Tzouros[4]; Ting Huang[1]; Tsung-Heng Tsai[1]; Benjamin Pullman[2]; Oliver M. Bernhardt[5]; Ruth Hüttenhain[6]; Guo Ci Teo[7]; Maria Pavlou[8]; Erik Verschueren[6]; Bernd Wollscheid[8]; Alexey Nesvizhskii[7]; Lukas Reiter[5]; Tom Dunkley[4]; Eduard Sabidó[3]; Nuno Bandeira[2]; Olga Vitek[1], *[1]Northeastern University, Boston, MA; [2]University of California, San Diego, San Diego, CA; [3]Center of Genomics Regulation, Barcelona, Spain; [4]Hoffmann-La Roche Ltd, Basel, Switzerland; [5]Biognosys, Zürich, Switzerland; [6]University of California, San Francisco, San Francisco, CA; [7]University of Michigan, Ann Arbor, MI; [8]Institute of Molecular Systems Biology, ETH, Zürich, Switzerland*

DAY 3

9:50 – 11:10 AM **PARALLEL SESSION**
Cell Biology: Systems Approaches for Cellular Signaling *Fifth Avenue*

Session Chair: Weiguo Andy Tao, Purdue University

9:50 – 10:15 AM **Proximity Dependent Sensors Define a Role for HOPS in Macropinocytosis-dependent Control of Cell Growth**
Anne Claude-Gingras; The Lunenfeld-Tanenbaum Research Institute, *University of Toronto, Toronto, Canada*

10:15 – 10:40 AM **Identification of ligand-dependent GPCR protein interaction networks with temporal and spatial resolution**
Ruth Huttenhain, *University of California, San Francisco, CA*

10:40 – 10:55 AM **R2-P2 rapid-robotic phosphoproteomics enables multidimensional cell signaling studies**
Mario Leutert; Ricard Rodriguez-Mias; Noelle Fukuda; Judit Villén, *University of Washington, Seattle, WA*

10:55 – 11:10 AM **Identification and characterization of lactate-mediated histone lactylation pathway by proteomics approaches**
Di Zhang[1]; Zhanyuan Tang[2]; He Huang[1]; Guolin Zhou[1]; Chang Cui[1]; Wenchao Liu[1]; Mathew Perez Neut[1]; Robert G.Roeder[2]; Becker Lev[1]; Yingming Zhao[1], *[1]The University of Chicago, Chicago, IL; [2]The Rockefeller University, New York, NY*

11:10 AM – 12:00 PM **LIGHTNING SESSION**
Round 2 *Grand 1*
Lightning Sessions will be 60 seconds.

Session Chair: Judit Villen, University of Washington and Lan Huang, University of California, Irvine

Poster 17.01 **Exploration of stress biomarkers in gilthead seabream (*Sparus aurata*) liver by integrative multi-omics analysis**
Pedro Rodrigues[1]; Cláudia Raposo de Magalhães[1]; Denise Schrama[1]; Marco Cerqueira[1]; Ana Paula Farinha Resende[1]; Annette Kuehn[2]; Dominique Revets[2]; Surintorn Boonanuntanasarn[3]; Chatsirin Nakharuthai[3], *[1]CCMAR, Universidade do Algarve, Faro, Portugal; [2]Luxembourg Institute of Health, L-4354 Esch-sur-Alzette, Luxembourg; [3]Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand*

Poster 18.01 **Implementation of every-other-day fasting in aged mice effectively transforms protein sulfhydromes in multiple tissues**
Nazmin Bithi; Yoko Henderson; Christopher Link; Belinda Willard; Christopher Hine, *Cleveland Clinic, Cleveland, OH*

Poster 18.02 **Acetylation of the nuclear lamina regulates nuclear periphery stability and function**
Laura Murray-Nerger; Xinlei Sheng; Pranav Rekapalli; Ileana Cristea, *Princeton University, Princeton, NJ*

Poster 18.03 **Uncovering Dehydroamino Acids within the HIV-1 Viral Proteome**
Rachel M. Miller[1]; Rachel A. Knoener[1, 3]; Nathan M. Sherer[2, 3]; Lloyd M. Smith[1], *[1]Chemsitry Department, UW-Madison, Madison, WI; [2]McArdle Laboratory for Cancer Research, UW-Madison, Madison, WI; [3]Institute for Molecular Virology, UW-Madison, Madison, WI*

Poster 18.10 **Derivatization of lysine with a commercial reagent to survey its post-translational landscape**
Martin Mathay; James Bruce, *University of Washington, Seattle, WA*

Poster 18.13 **Large-scale open modification searching reveals the role of post-translational modifications in protein-protein interactions**
Charlotte Adams[1]; Kris Laukens[1]; Wout Bittremieux[2], *[1]University of Antwerp, Antwerp, Belgium; [2]University of California San Diego, La Jolla, CA*

Poster 18.14 **Coenzyme A Binding Sites Induce Semi-Enzymatic Acylation**
Andrew Cruz; Chris Carrico; Xueshu Xie; Samah Shah; Cameron Wehrfritz; Birgit Schilling; Eric Verdin, *Buck Institute, Novato, CA*

Poster 19.03 **Multiple Biomarker Panels to Predict Resposne to Tocilizumab(anti-IL6R) in Rheumatoid Arthritis Patients Using High-precision Proteomics Approach**
Jinwoo Jung; Byoung-Kyu Cho; Kyung-Cho Cho; Ara Cho; Yeong Wook Song; Eugene C Yi, *Seoul National University, Seoul, South Korea*

Poster 20.03 **Connect the dots: proteogenomics and protein interaction networks.**
Sebastien Leblanc; Marie Brunet; Michelle Scott; Xavier Roucou, *Universite de Sherbrooke, Sherbrooke, Canada*

Poster 20.05 **Quantitative ProteoCellomic Analysis of a Serial Captured Affinity Purified Complex**
Xingyu Liu[1]; Ying Zhang[1]; Jeffrey Lange[1]; Brian Slaughter[1]; Jay Unruh[1]; Tim Wen[1]; Yan Hao[1]; Charles Banks[1]; Laurence Florens[1]; Jerry Workman[1]; Michael Washburn[1, 2], *[1]Stowers Institute, Kansas City, MO; [2]Department of Pathology and Laboratory Medicine, U, Kansas City, KS*

Poster 20.06 **Novel protein interaction analysis platform reveals a virus-driven mechanism for regulating peroxisome morphology**
Michelle Kennedy; Joel Federspiel; Samvida Venkatesh; Katelyn Cook; Clayton Otter; Pierre Jean Beltran; Ileana Cristea, *Princeton University, Princeton, NJ*

Poster 20.07 **Investigating Protein Quality Control Mechanisms of an Endocrine Protein Misfolding Disorder**
Madison Wright; Logan Kouba; Lars Plate, *Vanderbilt University, Nashville, TN*

Poster 20.08 **Degronomics: Mapping the Interacting Peptidome of a Ubiquitin Ligase Using an Integrative Mass Spectrometry Strategy**
Daniele Canzani[1]; Domnița-Valeria Rusnac[2, 3]; Ning Zheng[2, 3]; Matthew Bush[1], *[1]Department of Chemistry, University of Washington, Seattle, WA; [2]HHMI, University of Washington, Seattle, WA; [3]Dept of Pharmacology, University of Washington, Seattle, WA*

Poster 20.11 **Native Protein-Protein Interaction Profiling by SEC-DIA-MS**
Yuefan Wang; Yingwei Hu; Naseruddin Hoti; Hui Zhang, *Johns Hopkins University, Baltimore, MD*

Poster 21.01 **Defining kinase-substrate relationships using targeted protein degradation and phosphoproteomics**
Rufus Hards[1]; Juan C Mercado Del Valle[1]; Lincoln Howarth[1]; Ian LaCroix[2]; Mark Adamo[2]; Andrew Holland[3]; Scott Gerber[1], *[1]Geisel School of Medicine at Dartmouth, Lebanon, NH; [2]Norris Cotton Cancer Center, Lebanon, NH; [3]Johns Hopkins School of Medicine, Baltimore, MD*

Poster 21.02 **Quantitative proteomics approach to study the dynamic changes in phosphorylation signaling networks of phosphoprotein phosphatases**
Isha Nasa[1, 2]; Lauren E. Cressey[1]; Thomas Kruse[3]; Emil P. T. Hertz[3]; Lee M. Graves[4]; Jakob Nilsson[3]; Arminja N. Kettenbach[1, 2], *[1]Geisel School of Medicine at Dartmouth College, Hanover, NH; [2]Norris Cotton Cancer Center, Lebanon, NH; [3]Novo Nordisk Foundation for Protein Research, Copenhagen, Denmark; [4]University of North Carolina School of Medicine, Chapel Hill, NC*

Poster 21.04 **Dali: a method to identify phosphosites that alter thermal stability proteome-wide**
Ian Smith; Kyle Hess; Anna Bakhtina; Anthony Valente; Ricard Rodriguez-Mias; Judit Villen, *University of Washington, Seattle, WA*

Poster 21.05 **Proteomic and phosphoproteomic changes in rat skeletal muscle induced by acute endurance exercise**
James Sanford[1]; Paul Piehowski[1]; Wei-Jun Qian[1]; Joshua Adkins[1]; MoTrPAC Research Group[2], *[1]Pacific Northwest National Lab, Richland, WA; [2]NIH Common Fund, Bethesda, MD*

Poster 22.03 **Measuring the effect of missense variants on protein stability proteome-wide**
Stephanie Zimmerman; Ricard Rodriguez-Mias; Kyle Hess; Bianca Ruiz; Judit Villen; Stanley Fields, *Genome Sciences, University of Washington, Seattle, WA*

Poster 23.01 **Characterizing bacterial translational reprogramming under stress using a synthetic tmRNA platform**
Randi Turner[1]; Yan Wang[1, 2]; Daniel Dwyer[1], *[1]University of Maryland, College Park, MD; [2]NIH/NIDCR, Bethesda, MD*

Poster 23.03 **Functional Changes in the Murine Intestine Elicited by Fungal Colonization**
Veronika K. Pettersen[1, 2]; Erik van Tilburg Bernardes[1]; Antoine Dufour[1]; Marie-Claire Arrieta[1], *[1]University of Calgary, Calgary, Canada; [2]UiT - The Arctic University of Norway, Tromsø, NO*

Poster 24.01 **Identification of early candidate urine biomarkers for measure Escitalopram treatment response from major depressive disorder**
Yuhang Huan[1]; Jing Wei[1]; Jingjing Zhou[2, 3]; Min Liu[2, 3]; Jian Yang*[2, 3]; Youhe Gao*[1], *[1]Beijing Normal University, Beijing, China; [2]Beijing Anding Hospital, Beijing, China; [3]Capital Medical University, Beijing, China*

Poster 24.05 **Differential Regulation of Proteoforms in Human Hypertrophic Cardiomyopathy Revealed by Top-down Proteomics**
Trisha Tucholski[1]; Wenxuan Cai[1]; Stanford D. Mitchell[1]; Zachery R. Gregorich[1]; Elizabeth Bayne[1]; Sean McIlwain[1]; Max Wrobbel[1]; Hannah Karp[1]; Zachary Hite[1]; Petr G. Vikhorev[2]; Steve B. Marston[2]; Sean Lal[3]; Amy Li[3, 4]; Cristobal dos Remedios[3]; Takushi Kohmoto[1]; Joshua Hermsen[1]; J. Carter Ralphe[1]; Timothy J. Kamp[1]; Richard L. Moss[1]; Ying Ge[1], *[1]UW - Madison, Madison, WI; [2]Imperial College London, London, UK; [3]University of Sydney, Sydney, Austrailia; [4]La Trobe University, Melbourne, Austrailia*

Poster 24.06 **Proteomic-level *Helicobacter pylori* protein array and immuno-profiling of gastric cancer patients**
Lusheng Song[1]; Minkyo Song[2]; Charles S. Rabkin[2]; Jennifer Van Duine[1]; Linda M Liao[2]; Kailash Karthikeyan[1]; Weimin Gao[1]; Jolanta Lissowska[3]; M. Constanza Camargo[2]; Ji Qiu[1]; Joshua LaBaer[1], *[1]Biodesign institute, ASU, Tempe, Arizona; [2]Division of Cancer Epidemiology and Genetics, NCI, Rockville, Maryland; [3]MCMCC, Warsaw, Poland*

Poster 24.08 **Identification of novel cancer stem cell specific transcription factors for B7H3 based on DNA affinity purification coupled to mass spectrometry**
Han Byeol Kim[1]; Yuri Seo[1]; Eunhee G. Kim[2]; Jieun Jeong[2]; Junghyeon Lee[2]; Eugene. C. Yi[1]; Kristine M. Kim[2], *[1]Seoul National University, Seoul, South Korea; [2]Kangwon National University, Chuncheon, South Korea*

Poster 24.09 **Treating enamel defects: A porcine model for leveraging the role of saliva in enamel hardening based on protein remova**
Ana Gil-Bona[1, 2]; Maren Teichmann[1, 2]; Baptiste Depalle[1, 2]; Fabian Schulte[1, 2]; Markus Hardt[1, 2]; Megan K Pugach[1, 2]; Felicitas B Bidlack[1, 2], *[1]The Forsyth Institute, Cambridge, MA; [2]Harvard School of Dental Medicine, Boston, MA*

Poster 24.10 **Transcriptomic and proteomic analysis of HD medium spiny neurons derived from human-iPSCs to identify keys molecular determinants of HD neuropathogenesis**
Kizito-Tshitoko Tshilenge[1]; Carlos Galica Aguirre[1]; Nathan Basisty[1]; Sicheng Song[2]; Swati Naphade[1]; Kevin Perez[1]; Ashley Loureiro[1]; Cameron Wehrfritz[1]; Anja Holtz[1]; Alejandro Lopez-Ramirez[1]; Sean Mooney[2]; Simon Melov[1]; Birgit Schilling[1]; Lisa M Ellerby[1], *[1]The Buck Institute for Research on Aging, Novato, CA; [2]University of Washington, Seattle, WA*

Poster 24.12 **An integrated quantitative proteogenomics pipeline reveals an increase in non-canonical protein variants in inflamed mouse colon tissue**
Andrew Rajczewski; Qiyuan Han; Subina Mehta; Praveen Kumar; Pratik Jagtap; Natalia Tretyakova; Tim Griffin, *University of Minnesota, Minneapolis, MN*

Poster 24.14 **Developing Proteins and phosphoproteins in urine extracellular vesicles as biosignatures for Parkinson's disease diagnostics**
Marco Hadisurya[1]; Li Li[2]; Shalini Padmanabhan[3]; Anton Illiuk[2]; W. Andy Tao[1, 2], *[1]Purdue University, West Lafayette, Indiana; [2]Tymora Analytical, West Lafayette, IN; [3]The Michael J. Fox Foundation, New York City, NY*

Poster 24.15 **HIV-Tat exposure impairs neural development via Ras-ERK pathway dysregulation**
Brandon Murugan; Tariq Ganief; Jonathan Blackburn, *University of Cape Town, Cape Town, South Africa*

Poster 24.17 **Multi-omic characterization of bone marrow interstitial fluid (BMIF) and peripheral blood plasma (PB) of pediatric Acute Lymphoblastic Leukemia patients**
Lorenz A. Nierves[1, 2]; Jian Guo[1]; Tao Huan[1]; Philipp F. Lange[1, 2], *[1]University of British Columbia, Vancouver, Canada; [2]BC Children's Hospital Research Institute, Vancouver, Canada*

Poster 24.18 **Discovering aberrant splicing events in cancer proteomics using isobaric mass tags**
Daniel Roeth; Meiling Jin; Wu Yiming; Lili Wang; Markus Kalkum, *City of Hope, Duarte, CA*

Poster 24.20 **Characterizing the Effects of Fish Oil on High-Density Lipoprotein Proteome and Cholesterol Efflux Capacity**
Paul Mueller; Sara Rosario; Elisabeth Yerkes; Melissa Heard; Nathalie Pamir, *Oregon Health and Science University, Portland, OR*

Poster 26.02 **A Quantitative Single-Cell Proteomics Approach to Characterize an Acute Myeloid Leukemia Hierarchy**
Erwin M. Schoof[1, 2]; Nicolas Rapin[1, 2]; Benjamin Furtwängler[1, 2]; Nil Uresin[1, 2]; Simonas Savickas[3]; Coline Gentil[1, 2]; Khatereh Motamedchaboki[6]; Aaron Gajadhar[6]; Romain Huguet[6]; Daniel Lopez-Ferrer[6]; Eric Lechman[4, 5]; Ulrich auf dem Keller[3]; John E. Dick[4, 5]; Bo T. Porse[1, 2], *[1]The Finsen Laboratory, Copenhagen, Denmark; [2]Biotech Research and Innovation Centre (BRIC), Copenhagen, Denmark; [3]Technical University of Denmark, Lyngby, Denmark; [4]Princess Margaret Cancer Centre, Toronto, Canada; [5]University of Toronto, Toronto, Canada; [6]Thermo Fisher Scientific, San Jose, United States*

Poster 27.02 **High-density chemical cross-linking for modeling protein interactions**
Julian Mintseris; Steven Gygi, *Harvard Medical School, Boston, MA*

Poster 28.02 **Application of FAIMS and top-down mass spectrometry to brain cortex samples from individuals diagnosed with Alzheimer's disease**
James Fulcher[1]; Ronald Moore[1]; David Bennett[2]; Philip De Jager[3]; Vladislav Petyuk[1], *[1]Pacific Northwest National Lab, Richland, WA; [2]Rush University Medical Center, Chicago, IL; [3]Columbia University Medical Center, New York, NY*

Poster 28.04 **Cell-Free Synthesis of Full-Length Internal Standard Proteins for Top-Down Proteomics**
Ayako Takemori[1]; David Butcher[2]; Lissa C. Anderson[2]; Nobuaki Takemori[1], *[1]Ehime University, Toon, Japan; [2]National High Magnetic Field Laboratory, Tallahassee, FL*

Poster 28.07 **Optimization of Quantitative Top-Down Proteomics in Complex Samples using Protein-Level Tandem Mass Tag (TMT) Labeling**
Dahang Yu[1]; Zhe Wang[1]; Kellye Cupp-Sutton[1]; Yanting Guo[1]; Mulin Fang[1]; Kenneth Smith[2]; Xiaowen Liu[3]; Si Wu[1], *[1]University of Oklahoma, Norman, OK; [2]Oklahoma Medical Research Foundation, Oklahoma City, OK; [3]Indiana University–Purdue University Indianapolis, Indianapolis, IN*

12:15 – 1:30 PM Tuesday Lunch Seminars

Open to all attendees, but RSVP strongly encouraged (at booth or online) in advance as space is limited.

12:15 – 1:30 PM **SCIEX** *Cascade Room 2*
If you have not registered in advance, please stop by the booth.

SCIEX
The Power of Precision

12:15 – 1:30 PM **THERMO FISHER SCIENTIFIC** *Cascade Room 1*
If you have not registered in advance, please stop by the booth.

ThermoFisher
SCIENTIFIC

1:30 – 3:00 PM Poster Session & Refreshment Break *Grand 2, 3 & Foyer*

Odd-numbered poster boards present from 1:30-2:15 PM and even-numbered poster boards present from 2:15-3:00 PM.

3:00 – 4:20 PM PARALLEL SESSION
Technology: New Developments in Structural Proteomics *Grand 1*

Session Chair: Joseph Loo, University of California, Los Angeles

3:00 – 3:25 PM **Visualizing the Interactome in Living Systems**
Jim Bruce, *University of Washington, Seattle, WA*

3:25 – 3:50 PM **Next-generation Antibody Proteomics: Technologies, Curiosity-driven Science and Drug Therapeutics**
Yi Shi, *University of Pittsburgh, Pittsburgh, PA*

3:50 – 4:05 PM **Optimizing a proteome scale measurement of protein fold stability**
Ji Sun Stella Park; Hsien-Jung Lavender Lin; Nathan Zuniga; Kim Wagstaff; John Price, *Brigham Young University, Provo, UT*

4:05 – 4:10 PM **Visualizing the Cancer Conformational Landscape with Covalent Protein Painting**
Casimir T. Bamberger; Jolene Dietrich; Salvadore Martinèz-Bartholomé; John Yates, *Scripps Research Institutes, La Jolla, CA*

3:00 – 4:20 PM **PARALLEL SESSION**
Proteomics of Disease: Cancer and Precision Medicine
Fifth Avenue

Session Chair: Henry Rodriguez, National Institute of Health, National Cancer Institute

3:00 – 3:25 PM **Clinical Potential of Targeted Mass Spectrometry-based Proteomics in Personalized Oncology**
Amanda Paulovich, *Fred Hutchinson Cancer Research Center, Seattle, WA*

3:25 – 3:50 PM **Expression and Turnover of Proteoform in Cancer Aneuploidy**
Yangsheng Liu, *Yale University, New Haven, CT*

3:50 – 4:05 PM **Multiplex targeted MRM mass spectrometry for quantification of immunomodulatory proteins in tissue and plasma**
Jeffrey Whiteaker; Lei Zhao; Richard Ivey; Regine Schoenherr; Jacob Kennedy; Amanda Paulovich, *Fred Hutchinson Cancer Research Center, Seattle, WA*

4:05 – 4:20 PM **Excellent sensitivity through excellent recovery – ERLIC for absolute quantification of low abundant protein phosphorylation events in cancer patient tissue**
Stefan Loroch; Albert Sickmann, *ISAS, Dortmund, Germany*

4:30 – 5:50 PM **PARALLEL SESSION**
Technology: Chemical Proteomics and Drug Discovery
Grand 1

Session Chair: Keriann Backus, University of California, Los Angeles

4:30 – 4:55 PM **Expanding the Bioorthogonal Toolbox for Next Generation Chemoproteomics**
Keriann Backus, *University of California, Los Angeles*

4:55 – 5:20 PM **Lysine-targeted Covalent Inhibitors and Chemoproteomic Probes**
Jack Tauton, *University of California, San Francisco, San Francisco, CA*

5:20 – 5:35 PM **Mapping Cell Surface Lectin-Glycoprotein Interactions *in situ* using Oxidation Proteomics**
Yixuan (Axe) Xie; Ying Sheng; Qiongyu Li; Seunghye Ju; Carlito B. Lebrilla, *University of California, Davis, Davis, California*

5:35 – 5:50 PM **Chemical modulation of ER proteostasis to inhibit Dengue and Zika virus propagation**
Lars Plate; Katherine Almasy; Jonathan Davies, *Vanderbilt University, Nashville, TN*

4:30 – 5:50 PM **PARALLEL SESSION**
Proteomics Of Disease: Immunity And Infectious Disease
Fifth Avenue

Session Chair: Katrina Waters, Pacific Northwest National Laboratory

4:30 – 4:55 PM **Probes for Functional Proteomic Analysis of the Microbiome and Infectious Diseases**
Matthew Bogyo, *Stanford University, Palo Alto, CA*

4:55 – 5:20 PM **Discovering Tyrosine Phosphorylation in Mycobacterium Tuberculosis**
Ulrike Kusebauch, *Institute for Systems Biology, Seattle, WA*

5:20 – 5:35 PM **Recombinant MHC class I protein with isotope coded peptides enables relative and absolute quantification of the immunopeptidome**
Lauren Stopfer[1, 2]; Josh Mesfin[1]; Forest White[1], *[1]Massachusetts Institute of Technology, Cambridge, MA; [2]Koch Institute for Integrative Cancer Research, Cambridge, MA*

5:35 – 5:50 PM **Temporal dynamics of protein complex formation and dissociation during viral infection**
Xinlei Sheng; Yutaka Hashimoto; Laura Murray-Nerger; Ileana Cristea, *Princeton University, Princeton, NJ*

6:00 – 7:30 PM **EVENING WORKSHOP**
Biomarkers for Cancer, Early Detection *Cascade 1*

Organized by: Sudhir Srivastava NCI, NIH and Karin Rodland PNNL
Discussion on opportunities and challenges of different approaches for biomarker discovery.

6:00 – 7:30 PM **EVENING WORKSHOP**
Putting Humpty Dumpty Back Together Again: What Does Protein Quant Mean in Bottom-Up Proteomics? *Cascade 2*

Organized by: Michael MacCoss, University of Washington
In person discussions and exchange on legitimacy or value of combining peptide quantities into a protein quantity.

7:00 – 9:00 PM **Evening Social & Music with Exhibits** *Grand 2 & 3*

DAY 4

7:00 AM – 8:00 AM	**Meet with NIH Program Managers (Pre-registration required)**	*Grand Crescent*
8:00 AM – 12:00 PM	**Registration**	*Grand Foyer*
8:00 – 8:30 AM	**Morning Coffee**	*Grand Foyer*

8:30 – 9:20 AM

LIGHTNING SESSION
Tips and Tricks (Technology Focus)

Grand 1

Five-minute presentations selected from poster presentations.
Focus is on work-arounds, hacks, and new technology. Posters will be on display in foyer during the coffee break.

Session Chair: Robert Moritz, Institute of System Biology

Poster 01.15 **Quantifying thermal stability for thousands of protein variants in parallel using multiplexed proteomics**
Kyle Hess; Ricard Rodriguez-Mias; Stephanie Zimmerman; Bianca Ruiz; Ian Smith; Anthony Valente; Ariadna Llovet; Yang Lu; William Noble; Stanley Fields; Judit Villen, *University of Washington, Seattle, WA*

Poster 05.01 **Strategies for correctly controlling the false discovery rate when a subset of peptides in a sample is relevant**
Andy Lin[1]; Uri Keich[2]; William Noble[1], *[1]University of Washington, Seattle, WA; [2]University of Sydney, Sydney, Australia*

Poster 18.06 **Leveraging diagnostic ions for targeting acyl-lysine modifications in proteomic datasets**
Janine Fu; John Muroski; Rachel Loo; Joseph A Loo, *University of California, Los Angeles, Los Angeles, CA*

Poster 20.09 **Exploring the Genomic and Proteomic Basis for Cell-specific Remodeling within two Proteome-scale Protein Interaction Networks**
Ed Huttlin[1]; Laura Pontano Vaites[1]; Jose Navarrete-Perea[1]; Fana Gebreab[1]; Melanie Gygi[1]; Alexandra Thornock[1]; Sipei Fu[1]; Arvene Golbazi[1]; Eila Maenpaa[1]; Keegan Stricker[1]; Sanjukta Guha Thakurta[1]; Ramin Rad[1]; Joshua Pan[2]; David Nusinow[1]; Joao Paulo[1]; Devin Schweppe[1]; J. Wade Harper[1]; Steven Gygi[1], *[1]Harvard Medical School, Boston, MA; [2]Broad Institute, Cambridge, MA*

Poster 25.07 **High Quantitative Accuracy and Sensitivity in PRM with Multiplexing and Asynchronous Fill-Time Correction**
Sebastian Müller; Tejas Gandhi; Lukas Reiter, *Biognosys AG, Schlieren, Switzerland*

Poster 26.03 **Capillary electrophoresis ion mobility mass spectrometry for single-neuron proteomics**
Sam Choi; Peter Nemes, *University of Maryland, College Park, MD*

Poster 28.01 **Improving Proteoform Identifications and Post-Translational Modification Localizations Through Large-Scale Integration of Bottom-Up and Top-Down Data**
Leah Schaffer; Robert Millikin; Rachel Miller; Michael R. Shortreed; Lloyd Smith, *UW-Madison, Madison, WI*

Poster 28.03 **Characterization of cyclic peptide aggregation behavior using biophysical, intact mass, and top-down disulfide mapping approaches.**
Elizabeth S. Hecht[1]; Tao Chen[1]; Shijia Tang[1]; Yury V. Vasilev[2]; Maelia Uy-Gomez[1]; Joseph Meeuwsen[2]; Peter Liu[1]; Valery G. Voinov[2]; Jason Gruenhagen[1]; Joseph S. Beckman[2]; Wendy Sandoval[1]; David Arnott[1], *[1]Genentech, Inc., South San Francisco, CA; [2]eMSion, Inc., Corvallis, OR*

9:20 – 9:50 AM	**Coffee Break**	*Grand Foyer*

9:50 – 11:10 AM

PARALLEL SESSION
Computation & Analysis: Novel 'Omics and Multiomics: Data Integration and Applications

Grand 1

Session Chair: David Fenyo, Fenyo Labs (NYU lang one Health)

9:50 – 10:15 AM

Integrated Machine Learning of Complex Multi-Omic Data and Clinical Risk Factors to Build Interpretable Predictive Models for Type 1 Diabetes
Bobbie-Jo Webb-Robertson, *Pacific Northwest National Laboratory Richland, WA*

10:15 – 10:40 AM

FDR Control In Very Large Proteomic Data Sets and Proteome-wide Prediction of Peptide Tandem Mass Spectra by Deep Learning
Bernhard Kuster, *Technical University of Munich, Germany*

10:40 – 10:55 AM

Alternative Splice Isoforms in Cardiac Aging Defined by Multi-Omics
Erin Yu Han[1, 2]; Julianna Wright[1, 2]; Sara A. Wennersten[1, 2]; Rushita Bagchi[1, 2]; Edward Lau[3, 4]; Maggie PY Lam[1, 2], *[1]School of Medicine - University of Colorado AMC, Aurora, CO; [2]Consortium for Fibrosis Research & Translation, Aurora, CO; [3]Stanford Cardiovascular Institue, Palo Alto, CA; [4]Stanford University, Palo Alto, CA*

10:55 – 11:10 AM

Integrative profiling of human plasma proteomes enables precision phenotyping
Cecilia E Thomas; Tea Dodig-Crnkovic; Matilda Dale; Annika Bendes; Claudia Fredolini; Mun-Gwan Hong; Jochen M Schwenk, *Science for Life Laboratory, Solna, Sweden*

9:50 – 11:10 AM

PARALLEL SESSION
Cell Biology: Regulation and Function of Protein Phosphorylation

Fifth Avenue

Session Chair: Natalie Ahn, University of Colorado

9:50 – 10:15 AM

Substrate Specificity and Kinase Opposition of PP2A Holoenzymes
Arminja Kettenbach, *Dartmouth College, Hanover, NH*

10:15 – 10:40 AM

Methods to illuminate the function of the phosphoproteome
Judit Villen, *University of Washington, Seattle, WA*

10:40 – 10:55 AM

Dynamic Lysosome Interactomes In vitro and In vivo in Aging and Neurodegeneration
Saadia Hasan[2]; Ashley Frankenfield[1]; Michael Ward[2]; Ling Hao[1], *[1]George Washington University, Washington, DC; [2]NIH/NINDS, Bethesda, MD*

10:55 – 11:10 AM

Phosphoproteome analysis of metastatic breast cancer cells following treatment with a tumor-selective NQO1 Bioactivatable Drug
Naveen Singh[1]; Gitanjali Roy[1]; Emma Doud[1]; Edward Motea[1]; Xiumei Huang[1]; Paul Hergenrother[2]; David Boothman[1]; Amber Mosley[1], *[1]Indiana University School of Medicine, Indianapolis, IN; [2]University of Illinois at Urbana-Champaign, Urbana, IL*

11:10 AM– 12:00 PM

Donald F. Hunt Distinguished Contribution in Proteomics Award Lecture

Grand 1

Session Chair: John Yates, Scripps Research

How sample multiplexing magically converts proteomics into a biological assay
Steven Gygi, *Harvard Medical School, Boston, MA*

12:00 PM

Conference Concludes

MONDAY 9:50 AM – 11:10 AM
CASCADIA PROTEOMICS SYMPOSIUM SESSION

Room: Grand 1

Time: 9:50 AM – 10:05 AM

Sample preparation of OCT-embedded brain tissue for quantitative proteomics

Julia Robbins[1]; Gennifer Merrihew[1]; Tom E. Montine[2]; Michael MacCoss[1]

[1]University of Washington, Seattle, WA; [2]Stanford University, Palo Alto, CA

Alzheimer's Disease is a growing public health burden with limited interventions. Further therapeutic development rests on a more detailed understanding of molecular pathogenesis. The vast majority of data for AD pathogenesis in humans is from standard neuropathologic assessments or biochemical measurement of a limited number of analytes directly related to neuropathologic features (amyloid β, hyperphosphorylated tau). Although the current standard neuropathologic evaluation is highly valuable because it remains the only tool that provides comprehensive assessment of diseases that afflict an individual brain, it also is severely limited as a way to investigate molecular pathogenesis.

A major challenge of performing proteomics on human brain is the complexity of the mixture and the abundance of lipids in the tissue. Furthermore, Optimal Cutting Temperature (OCT) compound is often used to embed sample tissue prior to frozen sectioning on a microtome-cryostat. Unfortunately, the analysis of OCT-embedded tissues has been difficult because of the interference of OCT in the mass spectrometry analysis. OCT binds to the C18 reversed phase chromatography column, elutes during the peptide separation, and creates ion suppression during electrospray ionization. Consequently we systematically evaluated different homogenization, solubilization, digestion, and sample cleanup methods with the goal of performing quantitative proteomics measurements. We wanted a methodology that not only improved the number of total peptides that could be detected from digested brain tissue but also improved the quantitative precision. We will present the different strategies that were evaluated using data-independent acquisition mass spectrometry. The result is a protocol that reproducibly minimizes OCT and lipid interference and maximizes peptide digestion and recovery.

Time: 10:05 AM – 10:20 AM

The Proteome Landscape of Childhood Cancers Implications on Xenograft Models, Drug Sensitivity and Optimal Diagnostic Timepoint

Amanda Lorentzian[1, 2]; Anuli Uzozie[1, 2]; James Lim[1, 2]; Christopher Maxwell[1]; Gregor Reid[1]; Philipp Lange[1, 2]

[1]University of British Columbia, Vancouver, Canada; [2]BC Children's Hospital, Vancouver, Canada

Personalized treatments have significant potential to improve therapy options for high-risk cancers and reduce late effects in general. The approach is still in its infancy and much work is needed to improve its utility and efficacy. This is particularly true for childhood cancers for which positive outcome rates remain in the low percent range. To better understand the molecular mechanisms contributing to this we investigate the validity of three key concepts and tools upon which precision oncology is built.

We employ a proteogenomic workflow combined with in vitro drug sensitivity assays and murine patient derived xenografts. Deep sequence coverage of relevant genome variants is enabled by a childhood cancer specific targeted sequencing panel, which we first validated against whole genome sequencing. Data independent acquisition, a newly developed N termini enrichment approach (HUNTER) and sensitive phosphopeptide enrichment allows us to derive functional information beyond protein abundance from only 20µg of limited patient specimen per analysis. We present unpublished data from >100 biopsies from patients diagnosed with diverse pediatric malignancies spanning sarcomas, neuroblastomas and acute lymphoblastic leukemias.

Based on integration of the genomic, proteomic and PTM data we, for the first time, show that the proteome landscape of primary pediatric acute leukemias is largely recapitulated in murine patient derived xenografts. We also present data showing that select pathways differ between primary and xenograft cells and that the host environment has greater impact on post translational modifications than protein abundance. We then explore if protein and PTM level variation can explain observed disconnects between genomic biomarkers and in vitro or in vivotreatment response. Lastly, we explore the stability of genome and proteome variants between initial diagnosis and later relapses. Together these findings support xenografts as excellent model system for most studies and point out areas where addition of proteome analysis would likely improve treatment stratification.

Time: 10:20 AM – 10:35 AM

Miro: a Method for the Proteome-wide Identification of Amino Acid Substitutions That are Deleterious for Protein Function

Ricard Rodriguez-Mias; Kyle Hess; Bianca Ruiz; Stephanie Zimmerman; Ian Smith; Anthony Valente; Yang Lu; William S. Noble; Stanley Fileds; Judit Villen

University of Washington, Seattle, WA

DNA sequencing methods have led to the discovery of millions of mutations that change the encoded protein sequences, but the impact of nearly all of these mutations on protein function is unknown. We addressed this scarcity of functional data by developing Miro, a technology that leverages mistranslation of non-canonical amino acids to produce protein variants en masse followed by their biochemical characterization using high throughput proteomics assays.

I will present early in vitro and in vivo applications of the Miro technology, in conjunction with a diverse suit of biochemical assays, to reveal deleterious proline to azetidine-2-carboxylic acid substitutions that impact protein structure, ligand-binding, protein-protein interactions, post-translational modifications and protein thermal stability.

Additionally, we have screened a panel of non-canonical amino acids and demonstrated that Miro is cost effective, scalable to most types of amino acids, and flexible with respect to the model system. Miro is also versatile in terms of protein functions that can be probed; beyond the assays presented here, Miro can readily be extended to study the effects of amino acid substitutions on protein aggregation, enzymatic activity, subcellular localization, as well as other activities.

Further development of Miro will enable the generation of proteome-wide mutational sensitivity maps for human proteins as an essential companion to the human genome These maps will allow us to understand human variation and to interpret the clinical significance of millions of mutations in the human genome.

Time: 10:35 AM – 10:50 AM

Absolute Quantification Of Transcription Factors Reveals Principles Of Gene Regulation During Human Erythropoiesis

Mark A. Gillespie*[1]; Carmen G. Palii*[2]; Daniel Sanchez-Taltavull*[3]; Theodore J. Perkins[2]; Marjorie Brand[2]; Jeffrey A. Ranish[1]

[1]Institute for Systems Biology, Seattle, WA; [2]Ottawa Hospital Research Institute, Ottawa, ON; [3]Visceral Surgery, University of Bern, Bern, Switzerland

Dynamic changes in transcription factor (TF) abundance and stoichiometry drive cell state changes. However, our understanding of these complex relationships is limited by the paucity of nuclear protein concentration information. To address this, we developed and employed SRM-based targeted mass spectrometry assays, together with stable isotope labeled standard peptides, to quantify the absolute abundances of 103 TF and cofactors across 13 sequential time points during human erythropoiesis. We observe a range of correlations between protein and mRNA abundances, suggesting a role for post-transcriptional regulatory mechanisms. In addition, we define the protein concentration (copies/nucleus) for master regulators of hematopoiesis/erythropoiesis, as well as coregulators of transcription, thereby providing a quantitative scale for TFs in the nucleus. Notably, we observe that corepressors are significantly more abundant than coactivators at the protein level, but not at the RNA level. Finally, we integrate these absolute protein abundances with mRNA measurements to generate a dynamic gene regulatory network of erythroid commitment. These data provide unique and essential information for understanding the transcriptional regulatory programs controlling erythropoiesis, as well as general mechanisms that may regulate other cell fate decisions.

Time: 10:50 AM – 11:00 AM

Comparative Proteomics Of Serum From 31 Mammalian Species

Benjamin Neely[1]; Magnus Palmblad[2]; Phillip Wilmarth[3]; Alison Bland[4, 5]; Michael Janech[4, 5]

[1]National Institute of Standards and Technology, Charleston, SC; [2]Leiden University Medical Center, Leiden, The Netherlands; [3]Oregon Health & Science University, Portland, OR; [4]Hollings Marine Laboratory, Charleston, SC; [5]College of Charleston, Charleston, SC

Systematically characterizing the diversity of mammalian proteomes will improve our understanding of biologically interesting phenotypes and recent adaptation. Our first step to build the molecular cartography of mammalian blood proteomes and provide a foundational understanding for future biomimicry studies has focused on 31 species with annotated genomes spanning three placental superordinal clades: Afrotheria, Laurasiatheria, and Euarchontoglires. Undepleted sera from up to four individuals from each species were digested with trypsin and analyzed by data-dependent acquisition. The NIST SRM 909c human serum was used throughout the experiment to confirm digestion consistency and provide a human reference. Data were analyzed in both an ID-free and ID-based manner. For the former, the compareMS2 molecular phylogenetic approach was employed that used the fraction of shared spectra to calculate pairwise distances between all samples. The calculated relationships are based not only on the sequence-dependent fragmentation spectra, which includes some of the genetic variation, but also the relative abundance and post-translational modifications of the proteins. The resulting phylogenetic tree correctly recapitulated mammalia, with the major exception that cetacean blood proteomes were more similar to carnivora than even-toed ungulates. The data was also searched using standard database approaches and the resulting identified proteins were matched to human ortholog sequences using BLAST. On average, 342 proteins were identified (240 to 502) with 57 being shared across all species, though within each clade there were more shared proteins (*i.e.*, pinnipeds shared 205 proteins). Combining an ID-free method with ID-based methods is particularly useful when covering a large number of species whose genomes are not necessarily assembled and annotated to the same standard, as it provides an independent overview of the quality of and similarity between all datasets in a study. Ongoing analysis will identify differences in the blood proteome that may be linked to phenotypes within or between clades.

Time: 11:00 AM – 11:10 AM

"Borrelia PeptideAtlas" a Resource for Lyme Disease Research

Jaipalreddy Panga[1]; Zhi Sun[1]; Michael R. Hoopmann[1]; David S Campbell[1]; David Baxter[1]; Strle Klemen[2]; Leroy Hood[1]; Kai Wang[1]; Piesman J[4]; Allen Steere[3]; Robert L. Moritz[1]

[1]Institute for Systems Biology, Seattle, WA; [2]Massachusetts General Hospital, MA & Wadsworth Cen, New York, MA; [3]Massachusetts General Hospital, New York, MA; [4]CDC, Salt Lake City, UT

Despite discovering the causative agent for Lyme disease and the availability of the *B. burgdorferi* B31 reference genome sequence, there is no truly sensitive and specific test for detecting Lyme disease at early stages of infection. Currently, the approved two-step diagnostic test only detects post-infection immune response to the Lyme disease pathogen, with limited sensitivity and specificity. A comprehensive genomic and proteomic characterization of infective *B. burgdorferi* isolate is essential to correlate Lyme disease severity with these isolates. Comprehensive plasmid analysis of over thirty infective isolates of *B. burgdorferi* obtained from around the US provides evidence that the genome structure is quite different in plasmid number. Further, analysis of the *B. burgdorferi* B31 reference isolate (ATCC) has identified new plasmids previously unseen. To enable the development of new proteomic based signatures of *B. burgdorferi* infection, we have created the "Borrelia PeptideAtlas", which is derived from multiple clinical isolates and consolidates all these data into a comprehensive and publicly accessible web based, searchable resource for the Lyme research community. Furthermore, we are using this information to develop pathogen specific biomarker panels to detect *B. burgdorferi*.

MONDAY 9:50 AM – 11:10 AM
CELL BIOLOGY: PROTEOMIC ANALYSIS OF PTMS AND PROTEOFORMS

Room: Fifth Avenue

Time: 9:50 AM – 10:15 AM

Novel Top-Down Proteomics Strategies for Comprehensive Analysis of Proteoforms

Ying Ge

University of Wisconsin-Madison, Madison, WI

Top-down mass spectrometry (MS)-based proteomics is arguably the most powerful method to comprehensively characterize proteoforms that arise from genetic variations, alternative splicing, and post-translational modifications (PTMs), but myriad challenges remain. We have been developing novel strategies to address the challenges in top-down proteomics in a multi-pronged approach. To address the protein solubility challenge, we have recently identified a photo-cleavable anionic surfactant ("Azo") that can be rapidly degraded upon UV irradiation, for top-down proteomics. Azo is MS-compatible and can effectively solubilize proteins with performance comparable to SDS. Importantly, Azo-aided top-down proteomics enables the solubilization of membrane proteins for comprehensive characterization of PTMs. To address the proteome complexity challenge, we have been developing novel strategies for multi-dimensional liquid chromatography (MDLC) to separate

intact proteins. We developed novel hydrophobic interaction chromatography (HIC) materials for high-resolution separation of intact proteins under non-denaturing mode and demonstrated the potential of online HIC/MS for top-down proteomics. Given the difficulty in detecting large proteins in top-down MS, importantly, we developed a serial size exclusion chromatography strategy for size-based protein separation that can be coupled with online reverse phase chromatography and high-resolution MS which enabled the top–down MS analysis of large proteins (>200 kDa). Furthermore, we established a robust top-down LC/MS-based targeted proteomics platform for quantification of protein expression and PTMs concurrently in complex mixtures with high throughput and high reproducibility. To address the proteome dynamic range, we have been developing novel nanomaterials that can bind low abundance proteins and PTMs with high specificity. We developed an intergrated top-down phosphoproteomics work flow that coupled phosphoprotein enrichment by functionalized nanoparticles with online top-down LC/MS/MS to enrich, identify, quantify, and charaterize intact phosphoproteins directly from cell lysates and tissue homogenates. We have also recently successfully enriched low abundance proteins from blood for comprehensive analysis of proteoforms by top-down proteomics.

Time: 10:15 AM – 10:40 AM

Profiling system dynamics and crosstalk between the ubiquitin and Nedd8 systems

Donald Kirkpatrick

Genentech

Ubiquitin (Ub) and ubiquitin-like (UBL) proteins play a critical role in regulating dynamic cellular processes. Most notably, the Ub system functions to sculpt the cellular proteome through proteasomal degradation and autophagy. In addition, post-translational modification of proteins by Ub and UBLs can alter their activity, localization and interaction partners. A family of enzymes operating at this critical intersection are the Cullin-RING E3 ligase (CRL) complexes that promote ubiquitination and degradation of numerous substrates. Activation of a CRL complex requires post-translational modification of the cullin subunit by the UBL protein Nedd8, which in turn stabilizes association of the substrate recruitment module to permit efficient substrate recruitment. Despite recent advances, key unanswered questions remain. For example, what factors trigger incorporation of different substrate recruitment modules into functional CRL complexes? Also, do functionally relevant Nedd8 substrates exist besides the cullins? Recently developed mass spectrometry proteomics technologies shed light on these and other important questions. This talk will focus on two technologies that together elucidate the interplay between ubiquitination, neddylation, and the machineries controlling these interrelated processes. Protein Interaction Kinetics and Estimation of Stoichiometries (PIKES) analysis is a systematic proteomic profiling platform that integrates cellular engineering, affinity purification, chemical stabilization and quantitative mass spectrometry to investigate the dynamics of interchangeable multiprotein complexes. Serial NEDD8-Ubiquitin Substrate Profiling (sNUSP) employs Nedd8-R74K knock-in cells and K-εGG-peptide enrichment following non-classical proteolysis to discriminate between endogenous NEDD8- and Ub-modification sites. Using these two approaches, we show that Cand1 is both a novel neddylation substrate as well as critical exchange factor required for efficient substrate degradation by CRL4 E3 ligases.

Time: 10:40 AM – 10:55 AM

Site-specific Analysis Of The Asp- And Glu-adp-ribosylated Proteome By Quantitative Mass Spectrometry

Yonghao Yu

UT Southwestern Medical Center, Dallas, TX

ADP-ribosylation is a protein post-translational modification that is critically involved in a wide array of biological processes connected to cell stress responses. Enzymes known as poly-ADP-ribose polymerases (PARPs) catalyze the addition of the ADP-ribose units to amino acids with various side chain chemistries. In particular, the PARP family member PARP1 is responsible for the modification of a large number of proteins and is involved in initiation of the DNA damage response. Despite the progresses of the PARP1 inhibitors in the clinic (4 PARP1 inhibitors have been approved for the treatment of human cancer), the mechanisms through which PARP1 functions are still incompletely understood. The analysis of protein ADP-ribosylation is challenging because PARylation is a low-abundance, labile and heterogeneous protein modification. Recently, we developed an integrative proteomic platform for the site-specific analysis of protein ADP-ribosylation on Asp and Glu residues. Herein, we describe the method, and demonstrate its utility in quantitative characterization of the human Asp- and Glu-ADP-ribosylated proteome.

Time: 10:55 AM – 11:10 AM

EXoO-Tn: Tag-n-Map the Tn Antigen in the Human Proteome

Weiming Yang

Johns Hopkins University, Baltimore, MD

Tn antigen (Tn), a single N-acetylgalactosamine (GalNAc) monosaccharide attached to protein Ser/Thr residues, is found on most solid tumors yet rarely detected in adult tissues, featuring it one of the most distinctive signatures of cancer. Although it is important in cancer, Tn-glycosylation sites are not entirely clear owing to the lack of suitable technology. Knowing the Tn-glycosylation sites may empower the future development of prevention, diagnostics, and therapeutics of cancer and other diseases associated with the expression of Tn. Here, we introduce a technology named EXoO-Tn for large-scale mapping of Tn-glycosylation sites. EXoO-Tn utilizes glycosyltransferase C1GalT1 and isotopically-labeled UDP-Gal($^{13}C_6$) to tag and convert Tn to Gal($^{13}C_6$)-Tn, which gives rise to a unique glycan mass. This exquisite Gal($^{13}C_6$)-Tn structure is then recognized by a human-gut-bacterial enzyme, called OpeRATOR, that cleaves N-termini of the Gal($^{13}C_6$)-Tn-occupied Ser/Thr residues to yield site-containing glycopeptides. The two enzymes C1GalT1 and OpeRATOR could be used concurrently in a one-pot reaction. The effectiveness of EXoO-Tn was benchmarked by analyzing Jurkat cells, where 947 Tn-glycosylation sites from 480 glycoproteins were mapped. Bioinformatic analysis of the identified site-specific Tn-glycoproteins revealed conserved motif, cellular localization, the relative location of Tn in proteins. Given the importance of Tn in diseases, EXoO-Tn is anticipated to have broad utilities in the translational and clinical studies.

MONDAY 3:00 PM – 4:20 PM
TECHNOLOGY: ADVANCES IN TECHNOLOGY

Room: Grand 1

Time: 3:00 PM – 3:25 PM

Data-independent acquisition combined with parallel accumulation – serial fragmentation combined (diaPASEF): Bottom-up proteomics with increased ion usage

Ben Collins

Queens University Belfast, Belfast, UK

Bottom-up proteomics produces complex peptide populations that are identified and quantified at the precursor or fragment ion level. Data independent acquisition (DIA) modes isolate and concurrently fragment populations of different precursors by cycling deterministically through segments of a predefined

precursor m/z range. Although the selection windows of DIA collectively cover the entire mass range of interest, only a few percent of the ion current are sampled due to the consecutive selection of acquisition windows. Making use of the correlation of molecular weight and ion mobility in a trapped ion mobility device (timsTOF Pro), we here devise a novel scan mode that increases the global ion utilization. We analyze the acquired data by extending established targeted data extraction workflow for the analysis of DIA data by the additional ion mobility dimension, providing additional specificity in the precursor identification. Data acquired from simple protein mixtures verify the expected data completeness and data in single runs of a whole proteome digest demonstrate deep proteome coverage with sample loads ranging from 200ng down to 10ng. Quantitative benchmarking data using samples such as mixed species proteomes in defined ratios, as well as preliminary data in applied projects, will be presented.

Time: 3:25 PM – 3:50 PM

A View of Multiscale Protein Organization and Function in Cancer

Joe Gray

Oregon Health & Science University, Portland, OR

The talk will focus on recent work using multiplex immunofluorescence microscopy, super resolution fluorescence microscopy and 3D scanning electron microscopy to explore the multiscale proteomic organization of human cancers with emphasis on elucidation of functional inter- and intercellular interactions. The interactions will be presented in the context of treatment with drugs that modulate the interactions.

Time: 3:50 PM – 4:05 PM

New Directions for IR-MALDESI Mass Spectrometry Imaging: From Cells, Bones and Neurotransmitters to Drift Tube Ion Mobility

David Muddiman; Crystal Pace; Sitora Khodjaniyazova; Caleb Bagley; Anqi Tu; Hongxia Bai; James Dodds; Erin Baker

NC State University, Raleigh, NC

The mass spectrometry imaging field is moving into the realm of clinical analysis and this is largely based on MALDI and DESI ionization methods. We have developed a novel ionization method called Infrared Matrix Assisted Laser Desorption Electrospray Ionization (IR-MALDESI) which can be readily adaptable to many different sample types. Here, we demonstrate the effectiveness of IR-MALDESI for a wide-variety of applications and new directions using different mass spectrometry platform technologies. Specifically, a home-built IR-MALDESI sources has been coupled to high resolution accurate mass (Orbitrap) and drift tube ion mobility time-of-flight mass spectrometers. A diverse array of sample types will be presented from HTS/HCS, bones and diverse array of tissues. For HTS/HCS screening applications, zero sample preparation is required from very complex mixtures with the throughput of 2 samples per second. For bones, we developed a method that does not require decalcification prior to imaging which is critical because HCl will degrade/alter molecular information present in the sample. Finally, a wide range of ongoing studies will be presented including anti-malaria medications, HIV drugs and their co-localization with viral RNA and immune cells, how a fetus responds to exposure to fire retardants. IR-MALDESI has demonstrated to be extraordinarily adaptable to different sample types and requires no sample preparation. This has allowed us to carry out mass spectrometry imaging for a wide-range of applications that relate to human health and exposure.

Time: 4:05 PM – 4:20 PM

Isobaric Quantitative Protein Interaction Reporter Technology For Comparative Interactome Studies

Juan Chavez; Andrew Keller; Jared Mohr; Jimmy Eng; James Bruce

University of Washington, Seattle, WA

Chemical cross-linking with mass spectrometry (XL-MS) has emerged as a useful tool for the large scale *in situ* study of protein structures and interactions from complex biological samples including intact cells and tissues. Quantifying cross-linked peptides provides unique information on protein conformational and interaction changes resulting from perturbations such as drug treatment and disease state. Previous quantitative XL-MS studies have relied on incorporation of stable isotopes into the cross-linker (primarily deuterium), metabolic labeling of amino acid residues or incorporation of additional labeling steps. Here we report on the development of isobaric quantitative Protein Interaction Reporter (iqPIR) technology which utilizes stable isotopes selectively incorporated into the cross-linker design, allowing for cross-linked peptide pairs originating from different samples to have exactly the same mass in MS1 measurements, yet display distinct quantitative isotope signatures in tandem MS. This affords multiple benefits over other existing quantitative XL-MS strategies including increased signal-to-noise due to additive contributions of MS1 signals, generation of multiple fragment ions carrying quantitative information in a single tandem MS spectrum, avoidance of chromatographic alignment and peak assignment problems, elimination of retention time shifts between isotope partners as commonly observed with deuterium, applicability to systems where metabolic labeling is impractical, and potential for multiplexed quantitation.

We will present details on the molecular design and synthesis of iqPIR cross-linkers, development of custom informatics capabilities, and their application to model systems as well as pharmacological-induced interactome changes in human cultured cells. Data will be compared and contrasted with a previous quantitative cross-linking study using SILAC to evaluate the effects of the Hsp90 inhibitor 17-AAG on the interactome. Data from these initial applications of iqPIR indicate a high level of quantitative information with over 90% of cross-linked peptides identified producing quantitative values with excellent accuracy and precision.

MONDAY 3:00 PM – 4:20 PM
PROTEOMICS OF DISEASE: AGING AND AGE-RELATED DISEASES

Room: Fifth Avenue

Time: 3:00 PM – 3:25 PM

Proteomics and metabolomics in studies of the biology of aging: connecting mitochondrial energetics to physiological function

Peter Rabinovitch

University of Washington, Seattle, WA

For many decades a prominent theory of aging has centered on the damaging effects of reactive oxygen species (ROS). Mitochondria generate the bulk of cellular ROS as a byproduct of oxidative phosphorylation and the generation of ATP. This was the rational

for our studies that began with creation of transgenic mice that express catalase in mitochondria to effectively scavenge ROS. These mice were found to be longer lived and protected against multiple health challenges, including cardiac aging, sarcopenia and some cancers. In search of a parallel pharmacologic approach, similar benefits were found to be conferred by treating mice with the mitochondria-targeted tetrapeptide SS-31 (elamipretide), a drug now in phase II and III clinical studies in man. In recent years it has become evident that the free radical theory of aging is over-simplistic, as the role of ROS and mitochondrial energetics is much more multi-faceted. SS-31 was found to not act as an antioxidant, but instead integrates into the mitochondrial inner membrane, improving the efficiency of oxidative phosphorylation in aged and damaged mitochondria. However, how improvements in mitochondrial energetics in old animals results in improvements in a spectrum of physiologic functions, including heart, muscle and vision, remains to be established. We hypothesize that the acute enhancement of mitochondrial function is followed by persistent structural, proteomic and metabolic remodeling to effect these changes. The application of metabolomics and proteomics (including study of protein abundance, turnover, and post-transactional modifications) has provided essential tools to discover and characterize these mechanisms.

Time: 3:25 PM – 3:50 PM

A High Throughput Proteomics Platform to Investigate Aging

Fiona McAllister

Calico Labs

Sample preparation is often the rate limiting part of proteomics experiments and hinders the application of proteomics to large cohorts of samples. To address this and allow the processing of thousands of samples we have set up an automated workflow on a Hamilton liquid handler. We evaluated a number of different sample preparation techniques to identify one that does not rely on precipitation and is compatible with automation. Our workflow is based on 16plex TMT. We have investigated various post translational modifications and evaluated how the modifications change with age. We are comparing the magnitude of the post translational modification changes in young and old mice with the aim to determine which modification changes the most with age.

Time: 3:50 PM – 4:05 PM

Senescence-Based Biomarker Signatures of Aging and Senescence Burden

Nathan Basisty[1]; Abhijit Kale[1]; Okhee H Jeon[1]; Chisaka Kuehnemann[1]; Therese Payne[1]; Chirag Rao[1]; Anja Holtz[1]; Samah Shah[1]; Vagisha Sharma[3]; Luigi Ferrucci[4]; Judith Campisi[1, 2]; Birgit Schilling[1]

[1]The Buck Institute, Novato, CA; [2]Lawrence Berkeley Laboratory, Berkley, CA; [3]University of Washington, Seattle, WA; [4]National Institute on Aging, Baltimore, MD

Introduction: The senescence-associated secretory phenotype (SASP) has emerged as both a driver of, and promising therapeutic target for, a multitude of chronic age-related conditions, ranging from neurodegeneration to cancer. The complexity of the SASP has been greatly underappreciated and a small set of factors cannot explain the diverse phenotypes it produces in vivo. Here we present a comprehensive proteomic analysis of SASPs driven by multiple inducers of senescence in different human cell types. We propose that SASP proteins are promising biomarkers to assess senescent cell burden in aging and disease.

Methods: The 'core' and inducer-specific secretomes of senescent cells were profiled by comparing the secreted proteomes of senescent fibroblasts to quiescent fibroblasts following three senescence-inducing stimuli: irradiation-, oncogenic RAS-, or HIV drug atazanavir. Secreted proteins were obtained from the medium of cells cultured for 24 hours in serum-free conditions. Data-independent acquisitions (DIA) were performed on a TripleTOF 6600.

Results: We identified over 1000 unique SASP proteins, including 'inducer-specific' and 'core' SASP signatures. The 'core' SASP was composed of 151 proteins, with GDF15, CXCL1, MMP1, and STC1 consistently among the top increased proteins in response to all inducers. About 25% of the core SASP was enriched among plasma biomarkers of aging in humans and are proposed as senescence-based biomarkers candidates. To develop oncogene-induced senescence (OIS) signature, we performed multi-omic analysis to identify proteins exclusively expressed by RAS-induced senescent cells at both the protein level (based on our secretomes) and RNA-level (based on published RNA-seq data). We developed an OIS-specific signature composed of 26 proteins, a subset of which is proposed as an *in vivo* signature of OIS cells.

Discussion: This work will aid in identifying the proteins that drive senescence-associated phenotypes and provide comprehensive catalogs of potential biomarkers for assessing the burden and origin of senescent cells in vivo.

Time: 4:05 PM – 4:20 PM

Identification of Proteins with Reduced Solubility in Aging Mouse Brains

Cristen Molzahn; Mang Zhu; Lorenz Nierves; Aly Karsan; Philipp Lange; Thibault Mayor

University of British Columbia, Vancouver, Canada

Normal cellular function relies on maintaining the collection of proteins, known as the proteome. The cell depends on the balance between protein production, folding and degradation to maintain the homeostasis of the proteome (proteostasis). As an organism ages, cellular response to unfolded proteins declines which increases unfolded protein stress; this results in a decline of proteostasis. The diminished folding capacity can cause an increase in aggregated proteins, which have been associated with neurodegenerative diseases. Whereas, the age-related decline in proteome maintenance has been relatively well documented in the model organism *C. elegans*, a lack of information remains around the link between aging and aggregation in mammalian cells. Further investigation into the changes at the protein level is necessary to better characterize the link between aging and loss of proteostasis in mammals.

This study focuses on the insoluble portion of the proteome defined as those proteins which pellet after high-speed centrifugation. It is expected that inclusion in higher molecular weight aggregates is will result in certain proteins becoming more enriched in the pellet fraction upon aging. Using mass spectrometry-based proteomics approaches we analyzed cortex and hippocampus tissue from mice ages 4, 15 and 23 months revealing age-induced changes in protein solubility. Proteins enriched in the insoluble fraction upon aging show common functions such as chaperone proteins, oxidoreductases and neurofilament proteins (previously associated with amyotrophic lateral sclerosis). Additionally, such proteins generally have fewer disordered regions as compared to the proteome and have longer half-lives. These result suggest common features that may predispose proteins to become less soluble upon aging. By identifying proteins that are affected by aging in the absence of pathology, we can observe how proteostatic decline begins and creates conditions favorable to the formation of neurodegenerative diseases.

MONDAY 4:30 PM – 5:50 PM
TECHNOLOGY: INNOVATIONS IN BIOLOGICAL SAMPLE PROCESSING
Room: Grand 1

Time: 4:30 PM – 4:55 PM

Microscaled methods for proteogenomic analysis of patient-derived tumors

Steve Carr

Broad University, Cambridge, MA

Current gene-expression-based approaches for cancers are largely for prognostication, not prediction of individual drug responses. A major obstacle to progress in NGS-based diagnostics is a fundamental one: we poorly understand how complex cancer somatic genomes drive clinical phenotypes and drug vulnerabilities. Key issues such as therapeutic resistance, the contribution of the tumor microenvironment and the metastatic process belie single gene/mutation explanations. The new field of proteogenomics provides an opportunity to generate new insights by melding the complexity of cancer genomics with cancer proteomics to more completely understand how somatic genomes activate aberrant signal transduction events that drive cancer pathogenesis. Our group tackles this with both broad discovery efforts and targeted assays in the context of preclinical and clinical core biopsies, well-annotated cohorts and clinical trial samples in an iterative design to identify predictive tests that can be feasibly developed into clinical tests. We postulate that this microscaled and integrated approach will produce sounder therapeutic hypotheses and a new generation of accurate predictive biomarkers. Here we will present on novel protocols for simultaneous extraction of DNA, RNA and protein from single core biopsies and microscaled proteomics approaches that enable multiplexed, quantitative and deep-scale profiling of the extracted protein from the core biopsy tissues at proteome and phosphoproteome levels. We will also describe our recent advances in multiplexed, deep-scale analysis of the acetylome and ubiquitylome of tissue samples and the utility of FAIMS for improving quantitative accuracy.

Time: 4:55 PM – 5:20 PM

Sample Processing and Analysis Platform for In-Depth Single-Cell Proteomics

Ryan Kelly

Brigham Young University, Provo, UT

Biological tissues are highly heterogeneous, consisting of a variety of cell types, states and subpopulations, and understanding heterogeneity at the single cell level is of great interest for biomedical research. We have developed a microfluidic platform to minimize sample losses normally incurred during sample processing which, in combination ultra-low-flow nanoLC separations and latest-generation MS, achieves in-depth proteome coverage for samples comprising few or single cells. The sample processing platform, termed nanoPOTS (Nanodroplet Processing in One pot for Trace Samples), utilizes a robotic platform to dispense nanoliter volumes of reagents into photolithographically patterned nanowell reaction vessels, greatly reducing adsorptive losses. NanoPOTS-prepared samples are then analyzed using low-flow nanoLC-MS. To maximize sensitivity, in-house-packed nanoLC columns having internal diameters of 20–30 µm are employed. To date, we have identified >1000 proteins from single mammalian cells. Single cells and other small samples are readily isolated into nanowells using widely available fluorescence-activated cell sorting (FACS) and laser capture dissection (LCM). We have applied the platform to profiling proteins in tissue subsections with high spatial resolution (≤100 µm) and high depth of coverage (up to ~2600 protein groups) including profiling pancreatic islets from type 1 diabetic and nondiabetic donors, brain, liver, uterine and plant tissues. The ability to profile single cells and map the proteome with high spatial resolution across tissue regions provides a fundamental way to understand the tissue microenvironment, substructure, and cellular organization from a global proteome perspective. We will discuss recent advances in sample processing, separations and mass spectrometry, as well as prospects for further improving the platform for single-cell and other low-input proteomics studies.

Time: 5:20 PM – 5:35 PM

Single Cell Proteomics and the Carrier Proteome Effect

Atticus McCoy; Christopher M. Rose

Genentech, South San Francisco, CA

Single cell proteomics (SCP) by mass spectrometry (SCoPE-MS) is a recently introduced method that utilizes isobaric labels to quantify multiplexed single cell proteomes. While this technique has generated great excitement, the enabling technologies underlying SCoPE-MS - isobaric labels and mass spectrometry - comprise technical limitations with the potential to unfavorably impact data quality and biological interpretation if not considered properly. Here, we provide a detailed characterization of the relationship between the level of carrier proteome and quantitative accuracy of SCoPE-MS.

Through the analysis of a sample containing four aliquots each of HeLa and K562 cells and various levels of carrier proteome we find that the relationship between the number of ions sampled and the quantitative accuracy of the measurement is dependent on the level of the carrier proteome. Low levels of the carrier proteome (e.g., 5x) display accurate quantification (≤20% CV) even when a small number of ions are sampled, while high carrier proteome levels (e.g., 400x) require many more ions to be sampled for accurate quantification. By diluting our samples to levels at or below the protein amount found in single cells, we also demonstrate that common mass spectrometer settings tend to under sample ions for SCP and lead to inaccurate data when utilizing high levels of carrier proteome.

Taken together, these data demonstrate that increasing the level of carrier proteome requires a concomitant increase in the number of ions sampled in order to maintain quantitative accuracy within SCoPE-MS experiments – we term this the “carrier proteome effect”. This observation has implications for SCP experiments where high levels of carrier proteome could lead to insufficient sampling of ions and inaccurate quantification. This is particularly troublesome for single cell proteomics analyses where variability in single cell proteomes may falsely be attributed to cell heterogeneity, rather than poor measurements.

Time: 5:35 PM – 5:50 PM

Multidimensional Cross-linking With A Tetra-reactive Cross-linker

Jared Mohr; Juan Chavez; James E. Bruce

University of Washington, Seattle, WA

Cross-linking mass spectrometry (XL-MS) is a powerful and increasingly popular tool for the discovery and characterization of protein-protein interactions. Cross-linker molecular design is not inherently limited to two reactive groups and additional groups can yield multidimensional distance constraints and enable the unambiguous identification of protein complexes with more than two proteins.

We will present “Bisby”, a new CID-labile cross-linker with four amine-reactive n-hydroxyphthalamide (NHP) functionalities, as well as a method for enriching and identifying tetralinked species. Bisby can link up to four proximal lysine residues, providing enhanced distance constraints and greater insight into protein complexes compared to a bifunctional linker. A novel instrument control method was written

in ion trap control language to efficiently identify a set of four peaks in MS^2 spectra that fulfill an expected mass relationship. Each released peptide was then targeted with MS3 analysis to produce fragment data. Tetralinking experiments with purified histones yield numerous fully identified 4 peptide cross-links. These four peptide cross-links offer enhanced structural information compared to their six constitutive binary cross-links as they are generated from a single molecule at one point in time, guaranteeing six distance constraints compatible with at least a single conformer or complex. Tetralinks between H1 and up to 3 core nucleosome proteins allow for tightly constrained docking from only a single tetralink. Beyond these fully identified multidimensional links, there are more than 100 additional cross-links with 2 or 3 peptides identified, showing that a tetralinker provides structural data despite hydrolysis or unidentified spectra. Preliminary tests of whole proteome tetralinking of bacterial samples yields identified multi-dimensional links in abundant proteins, indicating application in live cells is feasible for at least some set of proteins. Live cell applications are particularly appealing due to the ability to unambiguously characterize novel complex interfaces of three or more proteins without additional information.

MONDAY 4:30 PM – 5:50 PM
PROTEOMICS OF DISEASE: ALZHEIMER'S AND NEURODEGENERATIVE DISEASES

Room: Fifth Avenue

Time: 4:30 PM – 4:55 PM

The Human Brainome: Genome, Transcriptome and Proteome Interaction in Human Cortex

Amanda Myers

University of Miami, Coral Gables, FL

Our hypothesis is that changes in gene and protein expression are crucial to the development of late onset Alzheimer's disease. Previously we examined how DNA alleles control downstream expression of RNA transcripts and how those relationships are changed in late onset Alzheimer's disease. We have now examined how proteins are incorporated into networks in two separate series and evaluated our outputs in two different cell lines. Our pipeline included the following steps: 1. Predicting expression quantitative trait loci, 2. Determining differential expression, 3. Analyzing networks of transcript and peptide relationships and 4. Validating effects in two separate cell lines. We performed all our analysis in two separate brain series to validate effects. Our two series included 345 samples in the first set (177 controls, 168 cases; age range 65-105; 58% female; KRONOSII cohort) and 409 samples in the replicate set (153 controls, 141 cases, 115 mild cognitive impairment; age range 66-107; 63% female; RUSH cohort). Our top target is Heat Shock Protein Family A Member 2 (HSPA2), which was identified as a key driver in our two datasets. HSPA2 was validated in two cell lines, with overexpression driving further elevation of Abeta40 and Abeta42 levels in amyloid precursor protein mutant cells as well as significant elevation of microtubule associated protein Tau (MAPT) and phospho-Tau in a modified neuroglioma line. This work further demonstrates that studying changes in gene and protein expression is crucial to understanding late onset disease and further nominates HSPA2 as a specific key regulator of late onset Alzheimer's disease processes.

Time: 4:55 PM – 5:20 PM

An Integrated Proteomics Approach to Resolve Brain-based Biomarkers in Alzheimer's Disease

Nicolas Seyfried

Emory University, Atlanta, GA

There is a need for novel biomarkers of Alzheimer's disease (AD) and other neurodegenerative disorders that are minimally invasive and that more broadly serve as accurate indicators of the underlying pathophysiological processes in brain. The Accelerating Medicine Partnership AMP-AD target discovery consortium is performing large scale multi-omics profiling and systems level integration of more than 2,000 postmortem human brains, establishing an unprecedented understanding of the pathophysiological processes driving cognitive decline, pathological burden, and other disease traits. The Emory AMP-AD team has focused on large scale proteomic analyses using unbiased label-free and isobaric tandem mass tag (TMT) based mass spectrometry methods to quantify thousands of proteins in brains from several different cohorts. Systems based network approaches reveal highly conserved modules of co-expressed proteins, many of which correlate strongly with clinical and pathological phenotypes, including those reflecting key mechanisms strongly correlated with impaired neuronal and synaptic function, neuroinflammation, and neurodegeneration. Preliminary studies were also performed to determine whether hub proteins representing these brain-based modules are found in cerebrospinal fluid (CSF). Following albumin depletion, we analyzed CSF samples from well-characterized AD and control patients and reliably quantified ~3,000 proteins by TMT based mass spectrometry across all samples. Of these, ~70% of the proteins were also identified in brain tissue, including members of phenotype-associated modules. Hence, large-scale proteomics with systems analyses provides a comprehensive dataset of brain-based protein changes linked to AD. This establishes a pipeline for targeting brain-based proteins in CSF as biomarkers for diagnosis, staging and therapeutic

Time: 5:20 PM – 5:35 PM

Proteogenomic Analysis Reveals Fus Gene As Dual-coding With Both Proteins United In Molecular Hallmarks Of Amyotrophic Lateral Sclerosis

Marie A Brunet; Jean-Francois Jacques; Amina Lekehal; Xavier Roucou

Université de Sherbrooke, Sherbrooke, Canada

The emergence of proteogenomics highlighted that current genome annotations do not capture the full coding potential of eukaryotic genomes. Novel coding sequences (alternative ORFs, altORFs) are camouflaged in "non-coding" RNAs, UTRs of mRNA or within annotated sequences in an alternative reading frame. We built OpenProt, a proteogenomic resource, which retrieves experimental evidence of altORFs from large-scale mass spectrometry (MS) and ribosome profiling data to better define proteomes.

Using OpenProt, we discovered a conserved altORF, named altFUS, nested in the FUS CDS; thus demonstrating the dual-coding nature of the Amyotrophic Lateral Sclerosis (ALS)-associated *FUS* gene. AltFUS is endogenously expressed in human tissues, notably in the motor cortex and motor neurons of healthy controls and ALS patients. AltFUS inhibits autophagy, a pathological hallmark presently and incorrectly attributed to the FUS protein. AltFUS is also pivotal in the loss of mitochondrial membrane potential and accumulation of FUS/TDP-43 cytoplasmic aggregates. Suppression of altFUS expression in a FUS-ALS Drosophila model protects against neurodegeneration. Some mutations found in ALS patients, overlooked because of their synonymous effect on the FUS protein, exert a deleterious effect via their missense consequence on the overlapping altFUS protein. Hence, both proteins, FUS and altFUS, are involved in the aetiology and pathological hallmarks of ALS.

Furthermore, we used size exclusion chromatography and affinity purification MS (AP-MS) to explore altFUS interactome, demonstrating its interaction with prohibitins, known regulators of autophagy. AP-MS in differentially SILAC-labelled cells

highlighted protein-protein interaction differences between ALS-linked mutants and wild-type altFUS. Functional annotation revealed in altFUS mutants a loss of proteins involved in the stress response.

FUS dual-coding nature is not an exception. 56% of ALS-associated genes possess an altORF with experimental evidence in OpenProt. Re-analysis of ALS proteomic studies reveal altORFs as hidden players and potential biomarkers, emphasizing the importance of proteogenomic pipelines to better understand human diseases.

Time: 5:35 PM – 5:50 PM

A Consensus Proteomic Analysis of Alzheimer's Disease Brain and Cerebrospinal Fluid Reveals Early Changes Associated with Microglia and Astrocyte Activation

Erik Johnson[1]; Eric Dammer[1]; Duc Duong[1]; Lingyan Ping[1]; Maotian Zhou[1]; Luming Yin[1]; Lenora Higginbotham[1]; Andrew Guajardo[2]; Bartholomew White[2]; Juan Troncoso[2]; Madhav Thambisetty[3]; Thomas Montine[4]; Edward Lee[5]; John Q. Trojanowski[5]; Thomas Beach[6]; Eric Reiman[7]; Vahram Haroutunian[8]; Minghui Wang[8]; Eric Schadt[8]; Bin Zhang[8]; Dennis Dickson[9]; Nilufer Taner[9]; Todd Golde[10]; Vladislav Petyuk[11]; Philip De Jager[12]; David Bennett[13]; Thomas Wingo[1]; Srikant Rangaraju[1]; Ihab Hajjar[1]; Joshua Shulman[14]; James Lah[1]; Allan Levey[1]; Nicholas Seyfried[1]

[1]Emory University, Atlanta, GA; [2]Johns Hopkins School of Medicine, Baltimore, MD; [3]National Institutes of Health, Bethesda, MD; [4]Stanford University, Palo Alto, CA; [5]University of Pennsylvania, Philadelphia, PA; [6]Banner Sun Health Research Institute, Sun City, AZ; [7]Banner Alzheimer's Institute, Phoenix, AZ; [8]Mount Sinai School of Medicine, New York, NY; [9]Mayo Clinic, Jacksonville, FL; [10]University of Florida, Gainesville, FL; [11]Pacific Northwest National Laboratory, Richland, WA; [12]Columbia University, New York, NY; [13]Rush University, Chicago, IL; [14]Baylor College of Medicine, Houston, TX

Our understanding of the biological changes in the brain associated with Alzheimer's disease (AD) pathology and cognitive impairment remains incomplete. To increase our understanding of these changes, we analyzed dorsolateral prefrontal cortex of control, asymptomatic AD, and AD brains from four different centers by label-free quantitative mass spectrometry and weighted protein co-expression analysis to obtain a consensus protein co-expression network of AD brain. This network consisted of 13 protein co-expression modules. Six of these modules correlated with amyloid-β plaque burden, tau neurofibrillary tangle burden, cognitive function, and clinical functional status, and were altered in asymptomatic AD, AD, or in both disease states. These six modules reflected synaptic, mitochondrial, sugar metabolism, extracellular matrix, cytoskeletal, and RNA binding/splicing biological functions. The identified protein network modules were preserved in a community-based cohort analyzed by a different quantitative mass spectrometry approach. They were also preserved in temporal lobe and precuneus brain regions. Some of the modules were influenced by aging, and showed changes in other neurodegenerative diseases such as frontotemporal dementia and corticobasal degeneration. The module most strongly associated with AD pathology and cognitive impairment was the sugar metabolism module. This module was enriched in AD genetic risk factors, and was also highly enriched in microglia and astrocyte protein markers associated with an anti-inflammatory state, suggesting that the biological functions it represents serve a protective role in AD. Proteins from the sugar metabolism module were increased in cerebrospinal fluid from asymptomatic AD and AD cases, highlighting their potential as biomarkers of the altered brain network. In this study of >2000 brains and nearly 400 cerebrospinal fluid samples by quantitative proteomics, we identify proteins and biological processes in AD brain that may serve as therapeutic targets and fluid biomarkers for the disease.

TUESDAY 9:50 AM – 11:10 AM
COMPUTATION & ANALYSIS: COMPUTATIONAL AND STATISTICAL METHODS

Room: Grand 1

Time: 9:50 AM – 10:15 AM

Intelligent Data Acquisition for Multiplexed Proteomics

Devin Schweppe

Harvard Medical School, Boston, MA

High-throughput quantitative proteomics requires efficient sample handling, fast mass spectrometric acquisition, and precise quantitation. Modern methods for multiplexed proteomics enable the utilization of isobaric chemical labeling to compare up to 16 samples simultaneously. However, acquisition of multiplexed proteomic quantitative data generally suffers from either slow acquisition speeds (SPS-MS3) or limited dynamic range (HRMS2). To bridge this divide we developed intelligent data acquisition strategies to acquire high accuracy quantifying scans based on real-time spectral matching. Real-time database searching of spectra during data acquisition enabled a 100% increase in data acquisition speed and improved quantitative accuracy. When applied to diverse human cell lines models, the real-time search strategy reproducily quantified 8000-9000 proteins enabling robust comparison of complex samples. Intelligent data acquisition in combination with quantitative proteomics offers new avenues to explore and understand the proteome across conditions, tissues of origin or species of interest.

Time: 10:15 AM – 10:40 AM

Measuring phosphorylation state in the human proteome

Brian Searle

Institute of Systems Biology, Seattle, WA

While mass spectrometry has made a far-reaching impact towards understanding cellular signaling, there is still a huge limitation in analyzing phosphosites occurring in close proximity. Indeed, accumulating phosphoproteomic data shows that phosphorylation sites cluster together in multi-phosphorylated proteins, where over half of sites are within four amino acids of each other. These neighboring sites result in phosphopeptide positional isomers that can sometimes be chromatographically resolved, but because they have the same precursor mass, dynamic exclusion settings often cause these peptides to be overlooked in data-dependent acquisition (DDA) experiments. This, coupled with the stochastic nature of DDA, often results in replicate quantitative experiments that exhibit very poor overlap. Here we propose Thesaurus, a new search engine that detects clusters of phosphopeptide positional isomers from Parallel Reaction Monitoring (PRM) and Data-Independent Acquisition (DIA) experiments. Using the insulin signaling pathway as a model, we demonstrate we can computationally extract distinct quantitative signaling effects of different positional isomers, even if those isomers do not separate chromatographically.

Time: 10:40 AM – 10:55 AM

Use of Gas Phase Fractionation Data Independent Acquisition and Spectrum Centric Searching to Build Matrix Specific Libraries for Quantitative Analysis

Lilian Heil; Michael Maccoss

University of Washington, Seattle, WA

Due to significant advances in mass-spectrometry instrumentation, data-independent acquisition (DIA) has emerged as a powerful acquisition scheme to sample all peptides in a specified mass range. Because of limitations in scan speed, there is a trade-off between the mass range covered and the width of the isolation window. Wider isolation windows offer more complete mass-range coverage but highly chimeric spectra may complicate data analysis. To address the challenges of complex spectra, these data are normally analyzed using a peptide-centric strategy, making use of prior information about 1) which peptides are likely to be found in the sample, 2) the expected retention time, and 3) fragment ion intensity. Bruderer et al. showed that it was valuable to produce matrix specific libraries to perform this analysis to limit searches to peptides likely present in the sample and to improve the statistical power. A limitation of this strategy is that library information is collected by biochemically fractionating the sample and collecting spectra using data dependent acquisition (DDA). We and others have shown that DDA is a poor predictor of the best peptides to use for quantitative analysis. Additionally, the use of a fractionated sample means that indexed retention time prediction will be performed in a different matrix than the unfractionated samples used for quantitation. Searle et al. expanded upon this concept by using gas-phase fractionation to acquire a small number of narrow window DIA runs of a pooled sample to facilitate sample-specific library preparation. Library assembly from these narrow window data typically implements peptide-centric approaches or builds off existing spectral libraries, but these searches have limited sensitivity, especially in detecting post-translational modifications. Here, we implement a sensitive spectrum-centric search approach to generate sample-specific spectral libraries and demonstrate the ability of these libraries to increase peptide detections and improve quantitative analyses in biological samples.

Time: 10:55 AM – 11:10 AM

MassIVE.quant: A Community Resource Of Curated Quantitative Mass Spectrometry-based Proteomics Datasets

Meena Choi[1]; Jeremy Carver[2]; Cristina Chiva[3]; Manuel Tzouros[4]; Ting Huang[1]; Tsung-Heng Tsai[1]; Benjamin Pullman[2]; Oliver M. Bernhardt[5]; Ruth Hüttenhain[6]; Guo Ci Teo[7]; Maria Pavlou[8]; Erik Verschueren[6]; Bernd Wollscheid[8]; Alexey Nesvizhskii[7]; Lukas Reiter[5]; Tom Dunkley[4]; Eduard Sabidó[3]; Nuno Bandeira[2]; Olga Vitek[1]

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We present MassIVE.quant (http://massive.ucsd.edu/ProteoSAFe/static/massive-quant.jsp), a tool-independent repository infrastructure and data resource for reproducible quantitative mass spectrometry-based biomedical research. MassIVE.quant is an extension of MassIVE (the Mass Spectrometry Interactive Virtual Environment) to provide the opportunity of large-scale deposition of heterogeneous experimental datasets and facilitate a community-wide conversation about the necessary extent of experiment documentation and the benefits of its use. It supports various reproducibility scopes, such as the infrastructure to fully automated the workflow, to store, and to browse the intermediate results. First, MassIVE.quant supplements the raw experimental data with detailed annotations of the experimental design, analysis scripts, and results, which enable the quantitative interpretation of mass spectrometry-based experiments and the online interactive exploration of the results. A branch structure enables to view and even compare reanalyses of each experiment with various combinations of methods and tools. Second, the curated alternative workflows can be used off-line and online reanalyses of the data starting from an intermediate output in MassIVE.quant. MassIVE.quant is independent of data acquisition types and of computational tools used to complete the analyses. To exemplify the utility of storing, sharing, reanalyzing, and curating data from quantitative experiments, we present the first compilation of proteomic datasets from benchmark controlled mixtures and biological investigations, interpreted with various data processing tools and analysis options. The extensive documentation for workflow and data submission, including video tutorials, is available.

TUESDAY 9:50 AM – 11:10 AM
CELL BIOLOGY: SYSTEMS APPROACHES FOR CELLULAR SIGNALING

Room: Fifth Avenue

Time: 9:50 AM – 10:15 AM

Proximity dependent sensors define a role for HOPS in macropinocytosis-dependent control of cell growth

Anne-Claude Gingras

The Lunenfeld-Tenenbaum Research Institute, University of Toronto, Toronto, Canada

The mechanistic Target of Rapamycin Complex 1 (mTORC1) couples nutrient sufficiency to cell growth, and is an important therapeutic target, notably in cancers. Amino acids can be up-taken in free form from transporters, and detected by different protein complexes that control the nucleotide-binding status of small GTPases of the Rag families. Nucleotide-bound Rag proteins recruit mTORC1 to the surface of the lysosome to mediate its full activation. A less well-characterized pathway of amino acid uptake is through macropinocytosis, in which extracellular proteins are endocytosed and delivered to the lysosome where they are degraded to amino acids, which also results in mTORC1 activation. Macropinocytosis is importantly enhanced by oncogenic KRAS mutations, and is thought to contribute to cancer survival in conditions of low free nutrient supply. Leveraging a proximity-dependent map of a human cell (Go, Knight et al., submitted), we generated proximity-dependent biotinylation "sensors" to study the recruitment of mTORC1 components to the surface of the endolysosomes (Hesketh et al., submitted). This revealed an unsuspected role for the HOPS trafficking complex in the activation of mTORC1 specifically in macropinocytic contexts. We also define a mechanism by which free nutrient availability represses macropinocytic mTORC1 activation. Together, our results help understanding the mechanisms of growth regulation by macropinocytosis, and may offer new therapeutic avenues for cancers in which this pathway is upregulated.

Time: 10:15 AM – 10:40 AM

Identification of ligand-dependent GPCR protein interaction networks with temporal and spatial resolution

Ruth Huttenhain

University of California, San Francisco, CA

G protein-coupled receptors (GPCRs) represent the largest family of signaling receptors and drug targets. Following ligand-induced activation of GPCRs signal transduction is mediated by protein interaction networks operating on short timescales and across multiple cellular locations. While temporal dynamics of protein interactions have been previously characterized, a major challenge remains largely unmet: how to interrogate the protein interaction networks engaged by GPCRs while capturing both their spatial and temporal context. Previously, we have developed an analytical approach combining APEX-based proximity labeling with quantitative proteomics and a system of spatial references, which delivers, with sub-minute temporal resolution, protein interaction networks and subcellular location of the receptor. We not only validated capture of proteins known to interact with the receptors, including those with transient or low affinity interactions, but demonstrated that our approach can be used to discover new network components regulating receptor function. We recently extended the approach to understand how chemically distinct agonists targeting the same GPCR produce different receptor based effects. Specifically, we determined cellular location and protein interaction networks engaged by the mu-type opioid (MOR) after stimulation with full, partial, and G protein biased agonists. The data revealed distinct intracellular trafficking comparing the agonists, while we observed rapid endocytosis for the full, unbiased agonist, the G protein biased agonist did not provoke any endocytosis. Interestingly, as interactors of MOR we discovered EYA4 and KCTD12, which were common for all three agonists, and have not been linked previously with MOR. These two novel interactors might play a role in regulating signaling downstream of receptor activation.

Time: 10:40 AM – 10:55 AM

R2-p2 Rapid-robotic Phosphoproteomics Enables Multidimensional Cell Signaling Studies

Mario Leutert; Ricard Rodriguez-Mias; Noelle Fukuda; Judit Villén

University of Washington, Seattle, WA

Recent developments in proteomics have enabled signaling studies where >10,000 phosphosites can be routinely identified and quantified. Yet, current analyses are limited in throughput, reproducibility, and robustness, hampering experiments that involve multiple perturbations, such as those needed to map kinase-substrate relationships, capture pathway crosstalks, and network inference analysis. To address these challenges, we introduce rapid-robotic-phosphoproteomics (R2-P2), an end-to-end automated method that uses magnetic particles to process protein extracts to deliver mass spectrometry-ready peptides for proteome and phosphoproteome analyses. R2-P2 is robust, versatile, high-throughput, and achieves higher sensitivity than classical protocols. To showcase the method, we applied it, in combination with data-independent acquisition mass spectrometry, to study signaling dynamics in the mitogen-activated protein kinase (MAPK) pathway in yeast. Our results reveal broad and specific signaling events along the mating, the high-osmolarity glycerol, and the invasive growth branches of the MAPK pathway, with robust phosphorylation of downstream regulatory proteins and transcription factors. Our method facilitates large-scale signaling studies involving hundreds of perturbations opening the door to systems-level studies aiming to capture signaling complexity.

Time: 10:55 AM – 11:10 AM

Identification And Characterization Of Lactate-mediated Histone Lactylation Pathway By Proteomics Approaches

Di Zhang[1]; Zhanyuan Tang[2]; He Huang[1]; Guolin Zhou[1]; Chang Cui[1]; Wenchao Liu[1]; Mathew Perez Neut[1]; Robert G.Roeder[2]; Becker Lev[1]; Yingming Zhao[1]

[1]The University of Chicago, Chicago, IL; [2]The Rockefeller University, New York, NY

The Warburg effect, originally describing augmented lactogenesis in cancer, is associated with diverse cellular processes such as angiogenesis, hypoxia, macrophage polarization, and T-cell activation. This phenomenon is intimately linked with multiple diseases including neoplasia, sepsis, and autoimmune diseases. Lactate, a compound generated during Warburg effect, is widely known as an energy source and metabolic byproduct. However, its non-metabolic functions in physiology and disease remain unknown. In this presentation, we will report identification of histone lysine lactylation by mass spectrometric analysis as a new epigenetic modification (Nature, 2019, Oct;574(7779):575-580). The histone mark was extensively verified by mass spectrometry, HPLC coelution, and biochemical methods. We identified and quantified histone lysine lactylation sites among diverse cells and under variant cancer-associated environments. In total, we identify 28 lactylation sites on core histones in human and mouse cells. Hypoxia and bacterial challenges induce production of lactate through glycolysis that in turn serves as precursor for stimulating histone lactylation. Using bacterially-exposed M1 macrophages as a model system, we demonstrate that histone lactylation has different temporal dynamics from acetylation. We demonstrate that histone lactylation directly stimulates gene transcription from chromatin. Histone lactylation is a new type of epigenetic mechanism that links metabolism with gene activity, representing a new avenue for understanding the functions of lactate and its role in diverse pathophysiological conditions, including infection and cancer.

TUESDAY 3:00 PM – 4:20 PM
TECHNOLOGY: NEW DEVELOPMENTS IN STRUCTURAL PROTEOMICS

Room: Grand 1

Time: 3:00 PM – 3:25 PM

Visualizing the Interactome in Living Systems

Jim Bruce

University of Washington, Seattle, WA

Proteins carry out nearly all function inside cells critical to support life. Many proteins can be isolated and studied independently in vitro or in complex mixtures to shed light on function. However, natural selection has resulted in evolution of all proteins to confer their critically beneficial functional properties within a specific densely packed subcellular environment. It is under these conditions that intra- and inter-molecular interactions are primary determinants of function. Therefore, improved comprehension of functional pathways important for cell survival could be achieved if greater information on how molecular interactions exist within complex living systems were attainable. Our lab is developing in vivo chemical cross-linking technologies and informatics with

the goal of improved visualization of the set of intra- and inter-molecular interactions that exist inside cells, collectively referred to as the interactome. This presentation will highlight our recent developments and applications to visualize interactomes and dynamics in living systems.

Time: 3:25 PM – 3:50 PM

Next-generation antibody proteomics: technologies, curiosity-driven science and drug therapeutics

Yi Shi

University of Pittsburgh, Pittsburgh, PA

Antibodies are among the most widely used biologics for basic research and have been the major driving force for disease diagnosis and therapeutics. Despite the importance, high-quality antibodies remain very sparse. Camelid VHH single-chain antibodies (nanobodies (Nbs)) are compelling new class of antibodies that are characterized by their small size, ease of bioengineering and production, superior solubility and thermo-stability, as well as low toxicity. Although Nbs are considered promising next generation agents for biomedical research and drug therapeutics, reliable analysis of Nb proteomes remain challenging. Here we report the development of hybrid proteomics and informatics tools that enable in-depth, high-throughput identification, characterization and structural mapping of antigen-specific Nb repertoires. With our approach, thousands to tens of thousands of high-quality, antigen-specific Nb families can be confidently identified and characterized based on their physicochemical properties. A significant fraction has been demonstrated to be outstanding binders that obtain ultrahigh affinity and molecular specificity. In addition, by integrative structural proteomics approaches, we have systematically investigated the structural basis and molecular underpinnings of antigen-antibody interactions, and have revealed the exceptional diversity and surprising structural versatility of humoral immunity. Finally, we will briefly discuss our efforts towards developing novel Nb therapy. We envision that these methods and stories of our exploratory journey, from discovery of novel biomolecules to drug therapeutics- all guided by mass spectrometry - will benefit proteomics research.

Time: 3:50 PM – 4:05 PM

Optimizing a Proteome Scale Measurement of Protein Fold Stability.

Ji Sun Stella Park; Hsien-Jung Lavender Lin; Nathan Zuniga; Kim Wagstaff; John Price

Brigham Young University, Provo, UT

Age-related diseases, such as neurodegenerative disorders, heart disease, and cancer, are increasing with the average lifespan of people. Understanding the mechanisms of aging that contribute to the development of the diseases will eventually lead to treatment of diseases. Aging is directly related to the homeostasis of proteins (proteostasis), where loss of proteostasis becomes more profound as we age. Interventions that reduce the aging rate preserve proteostasis. We want to measure the fold stability as a metric of quality of each protein found in the body. The assay will be used to compare the abnormally functioning protein's stability and quality to the one found in the assay to see how we can improve its functions back to normal. Our method consists of creating a denature curve. Data collected by denaturing proteins at different concentration and incubation time of denaturant Guanidine chloride (GdmCl) will give a denature midpoint of each protein. The midpoint is where the ratio between folded and denatured protein is at 1:1 and the stability of each protein will be determined by the location of the midpoint on the denature curve. To make the denature curve we are using different modifications to target amino acids to measure how much of the protein is denatured at each concentration. We tested a variety of amino acid modifiers for efficiency in labeling amino acids during denaturation. The results of these tests are compared to identify an optimal labeling method that efficiently and reproducibly measured amino acid accessibility and accurately reported protein fold stability.

Time: 4:05 PM – 4:20 PM

Visualizing the Cancer Conformational Landscape with Covalent Protein Painting

Casimir T. Bamberger; Jolene Dietrich; Salvadore Martinèz-Bartholomé; John Yates

Scripps Research Institutes, La Jolla, CA

Because protein-protein interactions and protein conformations change during tumorigenesis, there may be cancer-specific structural changes that hold promise for the development of a novel class of anti-cancer drugs based on structural alterations rather than protein abundance. To scan a proteome for aberrant protein folds we developed "covalent protein painting" (CPP). CPP labels solvent exposed lysine residues in proteins in vivo with two isotope-defined methyl groups and subsequently labels solvent excluded lysine residues with a second, isotopically distinct set of methyl groups. Subsequent bottom-up proteomics with mass spectrometry is used to identify lysine residues and quantify the relative proportion of protein molecules in which a lysine residue was inaccessible. We compared the breast cancer cell line MCF10A to MCF10A-H1045R, an isogenic cell line in which a single tumorigenic mutation in the catalytic subunit of the phosphatidyl inositol kinase p110a, or PI3KCA(H1045R), was inserted. 2,821 lysine sites in 1,566 distinct protein groups were detected and compared between the two cell lines. We identified 24 significantly altered lysine sites in different proteins, several of which suggested altered dynamics of the cytoskeleton. Next, we extended the number of different tumor cell lines measured with CPP to all 60 NCI cancer cell lines and quantified > 8,000 lysine sites in > 3,000 proteins. Data analysis revealed a complex and mostly redundant relationship between 461 protein sequence-altering somatic mutations in all NCI60 cells and the conformational changes in the proteome they induced, which was limited to < 100 lysine sites in the current dataset. Finally, we identified 49 lysine sites that are potentially predictive of the cytotoxicity of 300 different small molecules. These lysine sites are further evaluated as conformational biomarkers to predict drug efficacy or as novel targets to develop cancer conformational landscape specific anti-cancer drugs.

TUESDAY 3:00 PM – 4:20 PM
PROTEOMICS OF DISEASE: CANCER AND PRECISION MEDICINE

Room: Fifth Avenue

Time: 3:00 PM – 3:25 PM

Clinical Potential of Targeted Mass Spectrometry-based Proteomics in Personalized Oncology

Amanda Paulovich

Fred Hutchinson Cancer Research Center, Seattle, WA

Personalized oncology aims to match each patient to a specific therapy based on the molecular characteristics of their tumor. Currently, tumor DNA is sequenced, and genomics reports are given to physicians on tumor boards to help select targeted therapies for patients. While this approach has found success in extending the lives of subsets of patients, many patients do not respond to the

selected therapy, and even those who do initially respond have a high chance of recurring as resistant disease. Therefore, a deeper, more comprehensive readout of tumor biology is required in order to predict tumor phenotype with respect to drug response.

The majority of molecularly targeted therapies (e.g., kinase inhibitors, poly(ADP- ribose) polymerase (PARP) inhibitors, and therapies targeting immunomodulatory proteins) do not directly target the cancer genome but rather target proteins in cancer cells or the microenvironment. Thus, understanding and quantifying the expression of target proteins and their network throughout all phases of personalized oncology, from drug development to patient selection, are critically important. This presentation will discuss the added value of proteogenomics over the current genome-driven approach to the clinical characterization of cancers and summarize current efforts to incorporate targeted proteomic measurements based on selected/multiple reaction monitoring (SRM/MRM) mass spectrometry into the clinical laboratory to facilitate clinical proteogenomics.

See also: Nat Rev Clin Oncol. 2019 16(4):256-268. PMID: 30487530

Time: 3:25 PM – 3:50 PM

Expression and Turnover of Proteoform in Cancer Aneuploidy

Yansheng Liu

Yale University, New Haven, CT

Chromosome- or chromosome arm- scale DNA copy number alternations (CNAs) are called aneuploidy, which is a near-universal characteristic of human cancers. Gene expression analyses such as proteomic measurement will play a key role in understanding the effect of aneuploidy in cancer. The term 'proteoform' is now used to designate different molecular forms in which the protein product of a single gene can be found, including changes due to genetic variations, alternatively spliced RNA transcripts, and post-translational modifications. We will present our work using data independent acquisition mass spectrometry (DIA-MS), pulse-chase stable isotope-labeled amino acids in cells (pSILAC) approach, and genome-wide correlation analysis for quantifying both abundance and turnover rate of proteins in aneuploidy models. We will further discuss the results of two major proteoforms, namely alternatively spliced protein isoform groups and protein phosphorylation, and reveal their genetic and non-genetic quantitative determinants in aneuploidy.

Time: 3:50 PM – 4:05 PM

Multiplex Targeted Mrm Mass Spectrometry For Quantification Of Immunomodulatory Proteins In Tissue And Plasma

Jeffrey Whiteaker; Lei Zhao; Richard Ivey; Regine Schoenherr; Jacob Kennedy; Amanda Paulovich

Fred Hutchinson Cancer Research Center, Seattle, WA

Quantifying the many immuno-modulatory proteins in the "cancer-immunity cycle" is critical for both patient selection and development of novel immunotherapies. Conventional technologies to measure proteins (IHC, ELISA, Western blot, Flow Cytometry) are often semi-quantitative, not multiplexable, feature poor specificity, and are difficult to develop. Due to these limitations, investigators have turned to mRNA-based measurements to *infer protein expression* of immuno-modulatory factors. However, as has been unequivocally demon-strated for human breast, colorectal, and ovarian cancers (Pubmed IDs: 27251275, 25043054,27372738), RNA levels can be poor predictors of protein levels or activity. Thus, direct quantification of proteins is desirable.

As part of the National Cancer Institute's Cancer Moonshot, we are developing >300 multiplex assays for quantifying immunomodulatory proteins in tissue and plasma using targeted multiple reaction monitoring mass spectrometry (MRM-MS). MRM-MS complements traditional immunoassays by enabling highly specific, precise, harmonizable quantification of proteins in biospecimens, even at high multiplex levels.

As proof-of-concept, we will present a novel multiplex assay for quantification of 47 immuno-modulatory proteins in plasma and/or tumor tissue. The technology uses immunoaffinity enrichment coupled with MRM-MS (immuno-MRM assay). Assay bioanalytical validation was conducted in accordance with industry standards for use in FFPE tissue and plasma matrices and included determination of limits of detection, linear range of response, intra-day and inter-day repeatability, stability of analytes in sample processing, and characterization of reproducibility of endogenous analyte measurement. The mean linear range was over three orders of magnitude with median intra- and inter-day CVs less than 10%. The characterized assays are being applied to a panel of tumor specimens from a variety of cancer types to document detection and determine minimum sample requirements. Additional assay panels are under development to provide measurements of up to 350 immunomodulatory proteins, including signaling in the T-cell receptor network.

Time: 4:05 PM – 4:20 PM

Excellent Sensitivity Through Excellent Recovery – Erlic For Absolute Quantification Of Low Abundant Protein Phosphorylation Events In Cancer Patient Tissue

Stefan Loroch; Albert Sickmann

ISAS, Dortmund, Germany

Quantification of low abundant phosphorylation events from minute amounts of sample is a prerequisite for establishing phosphoprotoemics workflows in the clinics circumventing costly generation of antibodies. However, workflows need excellent recovery to overcome problems with low phosphoprotein stoichiometry and the limitations of sample amount (e.g. biopsies). Here we demonstrate that Electrostatic repulsion-hydrophilic interaction chromatography (ERLIC) allows improved analyte recovery and grants access to nearly all phosphopeptides in a digest. Consequently, we employed ERLIC enrichment of low abundant protein phosphorylation events in minute amounts of tissue from colon cancer patients.

To assess quantitative losses during enrichment, we spiked a "heavy"-labeled phosphopeptide-enriched fraction into an unlabeled-digest followed by another round of enrichment. Phosphopeptide ratios reflected the recovery of each phosphopeptide.

In case of TiO2-affinity purification, quantification of 1,800 different phosphopeptides revealed an average recovery of 38 ± 2% with poor recovery rates (< 10%) for a large portion of short and basic phosphopeptides. In contrast, ERLIC demonstrated a 1.7-fold higher recovery with 65 ± 15%, as determined by quantification of 1,100 different phosphopeptides. Most notably, recovery was independent of physicochemical peptide properties rendering ERLIC an excellent method for efficient purification of nearly any phosphopeptide from a digest. Since antibody-based detection repeatedly failed to work, we applied an ERLIC-based targeted assay to quantify S125 phosphorylation levels of PHD2 (mediating Hif1α degradation) in healthy and cancerous tissue of 10 colon cancer patients. PRM analysis revealed fairly stable PHD2 expression levels (358 ± 154 amol/µg) with phosphorylation levels of 2.5 ± 0.9% in all healthy tissue samples. Surprisingly, we detected diminished

S125 phosphorylation levels in cancerous tissue of all 10 patients down to 0.9 ± 0.4% ($p \leq 0.001$), even though total PHD2 expression remained unchanged. Subsequent, biochemical validation revealed a concomitant elevation of Hif1α expression levels rendering S125 an "on/off-switch" for PHD2 activity.

TUESDAY 4:30 PM – 5:50 PM
TECHNOLOGY: CHEMICAL PROTEOMICS AND DRUG DISCOVERY
Room: Grand 1

Time: 4:30 PM – 4:55 PM

Expanding the Bioorthogonal Toolbox for Next Generation Chemoproteomics

Keriann Backus

University of California, Los Angeles, Los Angeles, CA

The exploration of thiol-based redox regulation and signaling offers a grand challenge for achieving a molecular-level understanding of its unique role in physiology and pathology. Redox biology also represents a frontier for developing new therapeutics for cancer, neurodegenerative, and metabolic diseases. We are developing novel small-molecule probes as a way to identify and study the underlying chemistry that governs thiol-based redox signaling. This talk will present our latest results in the discovery and understanding of reactive oxygen species as emerging new chemical signals and their influence on protein function vis-à-vis the oxidative post-translational modification of pivotal cysteine residues.

Time: 4:55 PM – 5:20 PM

Lysine-targeted Covalent Inhibitors and Chemoproteomic Probes

Jack Tauton

University of California, San Francisco, CA

Most targeted covalent drugs have been designed to react with cysteine. However, many ligand binding sites in protein targets of interest lack an accessible cysteine. An alternative strategy is to target lysine, which is more prevalent yet less reactive than cysteine. Here, I will describe our efforts to design chemoproteomic probes that selectively modify catalytic and noncatalytic lysines in living cells. We have developed a chemoproteomic workflow that enables direct identification of probe targets and modification sites by mass spectrometry. Our lysine-targeted probes have shown utility in cell biological and target engagement experiments.

Time: 5:20 PM – 5:35 PM

Mapping Cell Surface Lectin-Glycoprotein Interactions *in situ* Using Oxidation Proteomics

Yixuan (Axe) Xie; Ying Sheng; Qiongyu Li; Seunghye Ju; Carlito B. Lebrilla

University of California, Davis, Davis, CA

The cell membrane is composed of a network of glycoconjugates, including glycoproteins and glycolipids, that presents a dense matrix of carbohydrate playing critical roles in many biological processes. Microarray and confocal imaging with fluorescent lectins have been widely used to investigate these glycoconjugates. However, the detailed information regarding the protein targets of lectins in their native environment has generally not been studied due to the weak and transient nature of these interactions.

Here, we describe an oxidative mapping strategy for identifying cell surface lectin-targeted glycoproteins in situ. Nine commonly-used lectins from plants and humans have been modified with the oxidation probe containing Fe(III) and treated to the living cell culture. By introducing hydrogen peroxide, the proteins which were in close proximity to the lectins were oxidized and detected using mass spectrometry. As a result, more than 150 proteins were oxidized, and more than 70% of the proteins were confirmed with the respective glycosylation using glycoproteomic analysis. The specificity of each lectin was compared. Interestingly, the targets of sialic acid-binding lectin, including Sambucus Nigra (SNA) and Maackia Amurensis II (MAL-II), indicated a great deal of specificity of compared to other lectins such as Hippeastrum Hybrid (HHL). The non-glycosylated proteins were investigated and recognized as the glycoprotein-associated proteins using STRING software, and five central proteins, G3P, ANXA2, EF2, EF1A1, MYH9, and ACTB, were commonly oxidized across the experiments. This result reveals their pivotal roles in the cellular processes through association with glycoproteins such as integrins. Additionally, most of the oxidized proteins were found enriched in the lipid raft microdomain, which is consistent with the results from visualization studies using confocal imaging. By elucidating the details of lectin-glycoprotein interactions in situ, more effective therapeutics may be developed.

Time: 5:35 PM – 5:50 PM

Chemical Modulation of ER Proteostasis to Inhibit Dengue and Zika Virus Propagation

Lars Plate; Katherine Almasy; Jonathan Davies

Vanderbilt University, Nashville, TN

The flavivirus family is responsible for some of the most common tropical and subtropical transmitted diseases. Development of effective antiviral therapies and safe vaccines against Dengue (DENV) and Zika (ZIKV) virus has been challenged by high mutation rates and antibody-dependent enhancement of secondary infection with related serotypes, which frequently leads to more severe disease progression. This motivates efforts to find alternative therapeutic approaches. Here, we focus on common host cell processes that are required for Dengue, Zika, Yellow Fever, West Nile or Hepatitis C virus infection and propagation. Flavivirus replication and assembly occurs at the endoplasmic reticulum (ER) membrane. The virus extensively remodels the organelle through activation of unfolded protein response (UPR)-associated transcription factors. The UPR induces expression of distinct ER chaperones and protein folding factors that maintain protein homeostasis and aid in the assembly and secretion of mature virions. To probe the individual roles of the UPR branches in the context of DENV infection, we take advantage of recently developed pharmacologic agents. We discovered that treatment with the small-molecule ER proteostasis regulator **147**, a preferential activator of the ATF6 branch, results in a significant reduction of DENV propagation. Using chemoproteomics for target identification, we define that the molecule activates ATF6 through perturbation of ER redox signaling processes. Furthermore, we will discuss results from global proteomics profiling to determine other cellular pathways in DENV infected host cells that are impacted by the small molecule. Our ongoing efforts are directed at characterizing how modulation of these cellular redox processes is involved in regulating DENV propagation and the ability to assembly and secrete new virions. Our results suggest that treatment with **147** could be an effective strategy to impair proliferation of other flaviviruses, including Zika virus, offering a broadly-applicable therapeutic strategy to target essential host cell processes to impair viral infections.

TUESDAY 4:30 PM – 5:50 PM
PROTEOMICS OF DISEASE: IMMUNITY AND INFECTIOUS DISEASE
Room: Fifth Avenue

Time: 4:30 PM – 4:55 PM

Probes for Functional Proteomic Analysis of the Microbiome and Infectious Diseases

Matt Boygo

Stanford University, Palo Alto, CA

Hydrolases are enzymes (i.e. proteases, esterases, lipases) that often play important regulatory roles in many diverse types of infections by pathogens as well as in the regulation of commensal bacteria communities with the host. Therefore, tools that allow dynamic monitoring of their activity can be used as diagnostic agents, as imaging contrast agents and as proteomic tools for the identification of novel enzymes as drug leads. In this presentation, I will describe our efforts using small molecule activity-based probes (ABPs) to identify, inhibit and image various hydrolase targets in species of both commensal and pathogenic bacteria. We believe many of these enzymes are ideal targets to visualize and disrupt aspects of bacterial colonization and community formation inside a host.

Time: 4:55 PM – 5:20 PM

Discovering Tyrosine Phosphorylation in Mycobacterium Tuberculosis

Ulrike Kusebauch

Institute for Systems Biology, Seattle, WA

Mycobacterium tuberculosis is the causative agent of tuberculosis, an infectious disease that remains a global health concern. While estimated one-quarter of the world's population has latent tuberculosis and is at risk of developing the disease, the human pathogen continues to claim ~1.5 million lives every year. Reversible protein phosphorylation is a well-known major regulatory mechanism across many life forms, including bacteria, which use this mechanism to sense and respond to changes in their environment. In M. tuberculosis, protein phosphorylation is the main signaling mechanism underlying the dynamic adaptive responses necessary for survival in its host. It is known that at least eleven two-component systems and eleven serine/threonine protein kinases mediate phosphorylation on aspartate, histidine, serine, and threonine. However, there was no conclusive evidence for protein phosphorylation on tyrosine, and tyrosine phosphorylation was thought to be absent in M. tuberculosis. I will discuss the discovery of a previously unrecognized phosphorylation system in M. tuberculosis using a combination of bacterial lysis, phospho-enrichment and highly sensitive mass spectrometry. We conclusively show extensive protein tyrosine phosphorylation of diverse M. tuberculosis proteins including serine/threonine protein kinases. Several serine/threonine protein kinases function as dual specificity kinases that phosphorylate tyrosine in cis and in trans, suggesting a major role of dual specificity kinases in bacterial phosphosignaling. Protein sequence point mutation of an activation segment phosphotyrosine site of the essential serine/threonine protein kinase PknB reduces PknB activity in vitro and in live Mtb, indicating a functional role for tyrosine phosphorylation in serine/threonine protein kinase regulation of bacterial growth. Together, our study provides the basis to understand how this new M. tuberculosis posttranslational modification affects physiology and pathogenesis.

Time: 5:20 PM – 5:35 PM

Recombinant MHC Class I Protein With Isotope Coded Peptides Enables Relative And Absolute Quantification Of The Immunopeptidome

Lauren Stopfer[1, 2]; Josh Mesfin[1]; Forest White[1]

[1]Massachusetts Institute of Technology, Cambridge, MA; [2]Koch Institute for Integrative Cancer Research, Cambridge, MA

Major histocompatibility (MHC) class I peptide antigens play a critical role in the recognition of malignant cells by the immune system. Recent work has demonstrated that cancer cells modulate surface MHC levels in response to therapy, thereby affecting antitumor immunity. However, understanding the peptide repertoire response to treatment remains challenging, and is limited by quantitative strategies that lack the standard normalization controls used in other mass spectrometry-based methods.

Here, we describe a novel quantitative immunopeptiomic approach that leverages recombinant heavy isotope coded peptide MHC complexes (hipMHCs) for relative and absolute quantitation of peptide repertoires using ultra low sample input (1x10^7cells). HipMHCs improve quantitative accuracy between analyses for both label free and multiplexed (TMT labeled) analyses by normalizing for variation and enabling regression against internal calibrants. Furthermore, for the first time we demonstrate absolute quantification using an internal standard curve for profiling copies per cell of any antigen of interest.

We applied this platform to a biologically relevant context to interrogate how the immunopeptidome shifts with CDK4/6 inhibition, a known modulator of antigen presentation. We discovered that the intracellular response to CDK4/6i is directly reflected in the immunopeptidome, with peptides derived from proteins implicated in CDK4/6i response selectively positively and negatively enriched following stimulation. CDK4/6i mediated upregulation of MHC-I is thought to be modulated by the IFN-g response. Intriguingly, IFN-g stimulation did not recapitulate the quantitative changes seen with CDK4/6i, but instead showed selective upregulation of IFN-g response peptides.

Improved quanitative accuracy provided by hipMHCs reveal alterations in peptide repertoires that connect the intracellular response to extracellular immune presentation. Data generated by this method defines repertoire changes that in turn highlight targetable antigens of interest. We propose this platform can be leveraged to identify treatment modulated antigens for targeted immunotherapy, and inform combination therapy trial design.

Time: 5:35 PM – 5:50 PM

Temporal Dynamics Of Protein Complex Formation And Dissociation During Viral Infection

Xinlei Sheng; Yutaka Hashimoto; Laura Murray-Nerger; Ileana Cristea

Princeton University, Princeton, NJ

The co-evolution and co-existence of viral pathogens with their hosts for millions of years is reflected in dynamic virus-host protein-protein interactions (PPIs) that are intrinsic to the spread of infections. The spatial and temporal regulation of protein complexes and PPIs is fundamental to every step of an infection process, underlying both host defense and virus replication mechanisms. As a master manipulator of host cells, the ancient and large herpesvirus human cytomegalovirus (HCMV) induces a wide range of alterations to protein functions, accomplished primarily via finely-tuned protein

interactions. Therefore, identifying these functional interactions and capturing their dynamics is critical for understanding viral infection and for developing antiviral therapies for this widely spread human pathogen. Here, we tackle the question of system-wide protein complex dynamics during the complete replication cycle of HCMV. To accomplish this, we use thermal proteome profiling (TPP) and thermal proximity coaggregation (TPCA) paired with multiplexed quantitative mass spectrometry, in conjunction with functional molecular virology and microscopy analyses. We monitor the temporal formation and dissociation of hundreds of functional protein complexes, as well as the dynamics of host-host, virus-host, and virus-virus PPIs during infection. We further overlay this dataset with our knowledge of protein subcellular localization and translocation during infection. Using this multi-disciplinary approach, we discover pro-viral roles for cellular protein complexes and translocating proteins. We show the HCMV receptor integrin beta 1 dissociates from extracellular matrix proteins, becoming internalized with CD63, which is necessary for virus production. Moreover, this approach facilitated characterization of essential viral proteins. For example, we uncover and validate the enhanced interactions of the viral packaging protein pUL52 with the interferon-inducible proteins IFIT1 and IFIT2 at the late stage of infection. Altogether, this global analysis of temporal protein complex dynamics provides insights into mechanisms of HCMV infection and a resource for biological and therapeutic studies.

WEDNESDAY 9:50 AM – 11:10 AM
COMPUTATION & ANALYSIS: NOVEL 'OMICS AND MULTIOMICS: DATA INTEGRATION AND APPLICATIONS

Room: Grand 1

Time: 9:50 AM – 10:15 AM

Integrated Machine Learning of Complex Multi-Omic Data and Clinical Risk Factors to Build Interpretable Predictive Models for Type 1 Diabetes

Bobbie-Jo Webb-Robertson

Pacific Northwest National Laboratory, Richland, WA

Type 1 diabetes (T1D) is a chronic autoimmune disease that results from autoimmune destruction of insulin-producing pancreatic beta-cells. T1D progresses through stages and clinical diabetes is generally preceded by the presentation of diabetes-related autoantibodies (IA), but no symptoms. As the cause of the disease remain elusive, multiple diabetes cohorts, such as the Diabetes Autoimmunity Study in the Young (DAISY; http://www.daisycolorado.org/), have been established to collect information longitudinally to gain insights into the biological mechanisms driving changes in the progression of the disease from a pre-symptomatic IA to symptomatic T1D state. These prospective cohort studies have reported potential demographic, immune, genetic, metabolomic, and proteomic markers associated with IA or the progression from IA to T1D. Each of these studies offer insight into environmental or mechanistic drivers of the disease, but from a single source perspective. We present an approach to machine learning-based data integration and ensemble-based feature extraction to enable the development of precision diabetes models that can simultaneously utilize and evaluate the large collection of disparate data available. At the global level, due to ensemble-based learning, we can evaluate the features most important to the model associated with the diabetes endpoints of interest. These can be evaluated to identify clinically relevant biomarkers or to understand the biological pathways leading to IA and/or diabetes. At the individual subject level, we can acquire the probability of a specific diabetes endpoint of interest, as well as interrogate the specific features driving the prediction to acquire distinct profiles and better understand where the model works well and needs improvements.

Time: 10:15 AM – 10:40 AM

FDR Control In Very Large Proteomic Data Sets and Proteome-wide Prediction of Peptide Tandem Mass Spectra by Deep Learning

Bernhard Kuster

Technical University of Munich, Munich, Germany

Estimating false discovery rate (FDR) is a challenge when analyzing very large proteomic data sets because there are limitations inherent to the classic target-decoy strategy that lead to an over-representation of decoy identifications. We compared the classic to a novel target-decoy-based protein FDR estimation approach using ∼19,000 LC-MS/MS runs available in ProteomicsDB (https://www.proteomicsdb.org). The "picked" protein FDR approach treats target and decoy sequences of the same protein as a pair rather than as individual entities and 'picks' either the target or the decoy sequence depending on which receives the highest score. This simple and unbiased strategy eliminates a conceptual issue in the "classic" protein FDR approach that causes over prediction of false-positive protein identification in large data sets. The approach scales from small to very large data sets without losing performance, consistently increases the number of true-positive protein identifications and is readily implemented in proteomics analysis software. The identification of peptides relies on sequence database searching or spectral library matching. The lack of accurate predictive models for fragment ion intensities render this process imperfect. We extended the ProteomeTools synthetic peptide library to 550,000 tryptic peptides and 21 million tandem mass spectra to train a deep neural network, termed Prosit, resulting in chromatographic retention time and fragment ion intensity predictions that exceed the quality of the experimental data. Integrating Prosit into database search pipelines led to more identifications at >10× lower FDR. We show the general applicability of Prosit by predicting spectra for other proteases, generating spectral libraries for DIA and improving the analysis of metaproteomes. Prosit is integrated into ProteomicsDB, allowing search result re-scoring and custom spectral library generation for any organism on the basis of peptide sequence alone.

Time: 10:40 AM – 10:55 AM

Alternative Splice Isoforms in Cardiac Aging Defined by Multi-Omics

Erin Yu Han[1, 2]; Julianna Wright[1, 2]; Sara A. Wennersten[1, 2]; Rushita Bagchi[1, 2]; Edward Lau[3, 4]; Maggie PY Lam[1, 2]

[1]School of Medicine - University of Colorado AMC, Aurora, CO; [2]Consortium for Fibrosis Research & Translation, Aurora, CO; [3]Stanford Cardiovascular Institue, Palo Alto, CA; [4]Stanford University, Palo Alto, CA

Introduction Aging-associated changes in alternative splicing (AS) have been implicated in the functional deterioration of multiple tissues including the aged heart. However, how AS changes lead to the expression of downstream alternative protein isoforms in cardiac aging remains incompletely examined to-date. In this study, we utilized an in-house developed multi-omics workflow to investigate the alterations in cardiac protein isoforms in a mouse model of cardiac aging.

Method We extracted RNAs and proteins from heart ventricles from young (12 weeks) and old (78 weeks) C57BL/6J mice. Transcript expression was quantified using short-read RNA-seq. Reads were mapped to mouse genome mm10 using STAR and splice junctions nominated with rMATS were translated into peptide sequences using an in-house algorithm to generate age-specific protein isoform databases. We then digested the cardiac proteins and performed tandem mass tag (TMT) 2D LC-MS/MS analysis on a Q-Exactive HF mass spectrometer coupled with the

Nano-UHPLC. The MS spectra were searched against the UniProt mouse proteome and the custom age-specific cardiac isoform proteome to quantify changes between proteins expressed in the young and old hearts.

Result (1) A bioinformatics analysis using deep-coverage RNA-seq datasets identified new age-associated changes in the expression of mRNA splice junctions; (2) We present the first age-specific cardiac protein isoform databases, which include novel protein isoforms that are not currently documented in public proteome databases; (3) We discovered 65 protein isoforms differentially expressed (FDR < 0.1) in young vs. aged mouse ventricles. Among these age-associated isoforms, we found a number of promising cardiac proteins that have been known to play vital structural or functional roles in the heart and have been implicated in cardiovascular diseases.

Conclusion Our work uses a proteotranscriptomics approach to reveal widespread proteoform changes between young and aged hearts, and further identifies candidate aging-regulated targets with potential implications in cardiac dysfunction.

Time: 10:55 AM – 11:10 AM

Integrative Profiling of Human Plasma Proteomes Enables Precision Phenotyping

Cecilia E Thomas; Tea Dodig-Crnkovic; Matilda Dale; Annika Bendes; Claudia Fredolini; Mun-Gwan Hong; Jochen M Schwenk

Science for Life Laboratory, Solna, Sweden

Advances by different plasma proteomics approaches provide ever growing opportunities to study human biology. These efforts are being fuelled by growing depth and breadth of complementary multi-omics data as well as extensive clinical information. However, other types of bias have to be tasken Ingo account including technical, pre-analytical as well as meditation data.

We apply a variety of sensitive, multiplexed affinity proteomics systems to profile > 1000 proteins circulating in plasma: Olink's proximity extension assays, Luminex-based assays, automated microfluidic SimplePlex ELISAs, or ultra-sensitive Quanterix Simoa assays. For exploratory screenings, we previously developed antibody bead arrays and built a comprehensive antibody validation pipeline including (i) paired antibodies, (ii) dual capture assays, (iii) sandwich immunoassays, (iv) immunocapture MS and (v) antibody-free targeted MS assays. We also use statistical and computational tools for data analysis such as to associate plasma protein levels with genetic variants (pQTLs).

From large-scaled and systematic explorations of plasma proteomes, we found that sample, technical, personal and health related parameters affect the protein levels. We observed a substantial impact from pre-analytical variables (sampling, needle-to-freezer, storage time, collection date, study centre) and the need to control for batch-effects when profiling 100s of samples. Longitudinal collections are particularly attractive as resampling patients enables to determine personal baselines and from there monitor the individuals' progression. Traits like sex, BMI, age, medication, genetics and clinical disease definitions should also be assessed prior to integrating proteomics with other omics data types. Considering these aspects, and the growing number of proteomics assays as well as population biobanks, profiling plasma will contribute to our understanding of human biology, and if applied in a considerable manner also deliver sustainable features for patient stratification, health management and prediction of treatment outcomes.

WEDNESDAY 9:50 AM – 11:10 AM
CELL BIOLOGY: REGULATION AND FUNCTION OF PROTEIN PHOSPHORYLATION

Room: Fifth Avenue

Time: 09:50 AM – 10:15 AM

Substrate specificity and kinase opposition of PP2A holoenzymes

Arminja Kettenbach

Dartmoth College, Hanover, NH

Protein Phosphatase 2A (PP2A) is an essential protein phosphatase that regulates most cellular processes. PP2A forms heterotrimers composed of one catalytic, one scaffolding, and one of several regulatory subunits. In mitosis, specific B regulatory subunit-containing PP2A holoenzymes are inhibited to allow entry into mitosis, while others are active to ensure faithful chromosome segregation and progression through mitosis. While the role of PP2A in many aspects of cellular physiology is well established, only a small number of PP2A dephosphorylation sites are known. This is in part due to the lack of specific and selective inhibitors. Recently it was shown that substrate identification by PP2A occurs through short linear motifs (SLiMs). The PP2A regulatory subunits recognize SLiMs in the substrates resulting in PP2A and substrate interaction. However, it remains unclear how, upon substrate binding, specific phosphorylation sites are selected for dephosphorylation. To address this gap in knowledge, we have developed phosphoproteomic strategies to identify PP2A regulatory subunit-specific dephosphorylation sites. Using our approach, we have identified thousands of dephosphorylation sites of PP2A holoenzymes containing the B55 or B56 regulatory subunit in different cell cycle phases. We discovered that the B-subunits regulate the phosphorylation site preference and thereby kinase opposition of the PP2A catalytic subunit. Furthermore, we discovered that in addition to direct SLiM-mediated interactions, SLiM-mediated scaffolding is a major mechanism of PP2A-B56 substrate recruitment. These differences in site-specific dephosphorylation contribute to our understanding of the differential regulation of PP2A holoenzymes in mitosis, which is essential for successful chromosome segregation.

Time: 10:15 AM – 10:40 AM

Methods to Illuminate the Function of the Phosphoproteome

Judit Villen

University of Washington, Seattle, WA

Protein phosphorylation functions as a highly versatile signaling unit integral to almost every cellular process. Protein phosphorylation and cellular signaling have been studied for decades, with mass spectrometry developments enabling signaling studies at a proteome-wide scale. These proteomic studies have been crucial for cataloguing thousands of protein phosphorylation events and their regulation. However, as phosphoproteomics experiments have increased in scale, new challenges have emerged, including data sparsity, inaccurate phosphosite localizations, challenges in elaborating network models, and the limited knowledge on which phosphosites may be functionally relevant. I will present some of our recent technological developments in phosphoproteomics that aim to bridge some of these gaps with the final goal of achieving a systematic and accurate characterization of the phosphoproteome.

Time: 10:40 AM – 10:55 AM

Dynamic Lysosome Interactomes In Vitro and In Vivo in Aging and Neurodegeneration

Saadia Hasan[2]; Ashley Frankenfield[1]; Michael Ward[2]; Ling Hao[1]

[1]George Washington University, Washington, DC; [2]NIH/NINDS, Bethesda, MD

Autophagy-lysosomal pathways are critical to cell survival by recycling misfolded proteins and damaged organelles. This is especially true for neurons, which cannot dilute theses insults by cell division. Lysosome dysfunctions have been associated with aging and neurodegeneration, but the molecular mechanisms remain unclear. **Here, we developed lysosomal proximity-labeling proteomics strategies both in vitro (human neuron culture) and in vivo (fixed mouse brain tissues) targeting on the bait protein, lysosomal-associated membrane protein1 (LAMP1), to evaluate lysosome interacting proteins in neurodegeneration.**

In human iPSC-neuron culture, we stably expressed an ascorbate peroxidase (APEX2) enzyme on the LAMP1 protein, which biotinylates proteins within ~20 nm of lysosomes in living neurons. In fixed mouse brain tissues, we stained the tissue slices with LAMP1 antibody and then developed a LAMP1 antibody-guided proximity labeling method which biotinylates lysosomal adjacent proteins. Biotinylated proteins were enriched by streptavidin magnetic beads for subsequent untargeted proteomic analysis. Comprehensive method optimization and validation were conducted for the optimal sensitivity, specificity and confidence of lysosome interacting proteomics. The proximity-labeling results demonstrated specific and complementary identification of lysosome interacting proteins between neurons in culture and mouse brain tissues, in comparison to a cytosolic bait as a spatial control to exclude nonspecific bindings. Using LAMP1-APEX proximity labeling combined with live cell imaging, we have discovered that Annexin A11 (an ALS-associated protein) is used as a molecular tether that links RNA granules to lysosomes during the long-distance transport of RNA binding proteins in neuronal axons (***Cell, 2019***). Annexin A11 also showed reduced recruitment to the lysosome when we knocked out GRN gene, whose loss-of-function mutations lead to frontotemporal dementia. **In summary, the established lysosome proximity labeling methods provide unique insights of dynamic lysosome interactome both in vitro and in vivo with great specificity and sensitivity for the study of lysosome dysfunctions in neurodegeneration.**

Time: 10:55 AM – 11:10 AM

Phosphoproteome Analysis Of Metastatic Breast Cancer Cells Following Treatment With A Tumor-selective Nqo1 Bioactivatable Drug

Naveen Singh[1]; Gitanjali Roy[1]; Emma Doud[1]; Edward Motea[1]; Xiumei Huang[1]; Paul Hergenrother[2]; David Boothman[1]; Amber Mosley[1]

[1]Indiana University School of Medicine, Indianapolis, IN; [2]University of Illinois at Urbana-Champaign,, Urbana, IL

Proteins that are constitutively overexpressed as a consequence of gene amplification or other cellular oncogenic transformation mechanisms are potential targets for precision cancer therapy. One such protein, NAD(P)H:quinone oxidoreductase-1 (NQO1), has been found to be over-expressed in multiple solid cancers including triple negative breast cancer. The NQO1 enzyme overproduction can be targeted using specific quinones which are bioactivated by NQO1 including β-lapachone, deoxynyboquinone, and isobutyl-DNQ (IB-DNQ). In this work, we have explored the phosphoproteome of triple negative breast cancer cells treated with IB-DNQ, the poly ADP ribose polymerase (PARP) inhibitor Rucaparib, or a combination of IB-DNQ and Rucaparib. Numerous significant changes were observed in the phosphoproteome following combination therapy, including large decreases in phosphorylation associated with active RNA Polymerase II transcription.

P01: ADVANCES IN TECHNOLOGY

Poster 01.01

Cartridge and bead-based sample preparation for proteome analysis: Assessing robustness of protein recovery and digestion efficiency

Jessica Nickerson; Alan Doucette;
Dalhousie University, Halifax, Canada

Poster 01.02

Validation of a disposable biospecimen collection system with integral refrigeration for preserving the phosphoproteome

Jacob Kennedy[1]; Amanda Paulovich[1]; Scott Thielman[2]; Amanda Woodcock[2]; Richard Ivey[1]; Guy Corral[2]; Eli Hooper[2]; Gregory Martin[2]; Gina Longman[2]; Blake Stancik[2]; Jeff Whiteaker[1]; Lei Zhao[1]; Travis Lorentzen[1]
[1]Fred Hutchinson CRC, Seattle, ; [2]Product Creation Studio, Seattle, WA

Poster 01.03

Development of an Online 2D Ultra-High-Pressure Nano-LC System for High-pH and Low-pH Reversed Phase Separation in Top-Down Proteomics

Zhe Wang[1]; Dahang Yu[1]; Xiaowen Liu[2]; Kenneth Smith[3]; Si Wu[1]
[1]University of Oklahoma, Norman, OK; [2]Indiana University-Purdue University Indianapolis, Indianapolis, IN; [3]Oklahoma Medical Research Foundation, Oklahoma City, OK

Poster 01.04

Proteograph: Efficient and Automated Multi-Nanoparticle Platform for Deep, Unbiased Plasma Protein Profiling and Protein-Protein Interaction Biological Insight

Patrick A. Everley[1]; Shadi Ferdosi[1]; Daniel Hornburg[1]; William C. Manning[1]; Asim Siddiqui[1]; Greg Troiano[1]; Omid C. Farokhzad[1]; Matthew E. K. Chang[2]; Mark R. Flory[2]; John E. Blume[1]
[1]Seer, Inc., Redwood City, CA; [2]Cancer Early Detection Advanced Research Center, Portland, OR

Poster 01.05

Achieving maximal protein extraction, solubilization and digestion via combination of high temperature and novel mass spec compatible surfactant.

Valerie Ressler; Sergei Saveliev; Wenhui Zhou; Joel Walker; Jean Osterman; Mike Rosenblatt; Poncho Meisenheimer; Marjeta Urh
Promega, Madison

Poster 01.06

Scanning SWATH Acquisition – The Next Step in the Data Independent Acquisition (DIA) Evolution

Christie Hunter[1]; Stephen Tate[2]; Nic Bloomfield[2]
[1]SCIEX, Redwood City, CA; [2]SCIEX, Toronto, Canada

Poster 01.07

diaPASEF in Mixed Proteomes is Deep, Quantitative and Reproducible

Stephanie Kaspar-Schoenefeld[2]; Kristina Marx[2]; Christopher Adams[1]; Tejas Gandhi[3]; Lukas Reiter[3]; Florian Meier[4]; Andreas Brunner[4]; Markus Lubeck[2]; Oliver Raether[2]; Stefan Tenzer[5]; Reudi Aebersold[6]; Ben Collins[7]; Hannes Roest[8]; Matthias Mann[4]
[1]Bruker, San Jose, CA; [2]Bruker Daltonik GmbH, Bremen, Germany; [3]Biognosys, Schlieren, Switzerland; [4]Max Planck Institute of Biochemistry, Martinsried, Germany; [5]University of Mainz, Mainz, Germany; [6]ETH Zurich, Zurich, Switzerland; [7]Queen's University, Belfast, UK; [8]University Toronto, Toronto, Canada

POSTER LIST

Poster 01.08 **BAC-DROP: A High-Throughput Sample Preparation Workflow for Bottom-Up Proteomics Using Dissolvable Polyacrylamide Gel Electrophoresis**
Nobuaki Takemori; Ayako Takemori; Jun Ishizaki; Hitoshi Hasegawa
Ehime University, Toon, Japan

Poster 01.09 **High throughput proteome and phosphorpoteome sample processing coupled to fast gradient DIA**
Stoyan Stoychev; Isak Gerber; Justin Jordaan
ReSyn Biosciences, Pretoria, South Africa

Poster 01.10 **Precision Medicine Recommendation For Breast Cancer Using Machine Learning Techniques**
Reena Lokare; Dr. Sunita Patil
University of Mumbai, Mumbai, India

Poster 01.11 **Increasing the Ease of Use of Nanoflow with Plug and Play Low Flow Source**
Christie Hunter[1]; Leroi De Souza[2]; Carmai Seto[2]; Yang Kang[2]; Leigh Bedford[2]; Bradley Schneirder[2]; Arianna Jones[1]
[1]SCIEX, Framingham, MA; [2]SCIEX, Toronto, CANADA

Poster 01.12 **Reversible chemistry for universal protein extraction and cleanup of whole proteins and peptides for fast, high yield proteomics sample preparation**
Stephanie Biedka[1]; Jonathan Minden[1, 2]; Nolan Frey[2]; Amber Lucas[1]
[1]Impact Proteomics, Pittsburgh, PA; [2]Carnegie Mellon University, Pittsburgh, PA

Poster 01.13 **Deeper proteome coverage of musculoskeletal samples**
Emma Doud[1]; Xiaoling Zhong[1]; Joseph Rupert[1]; Matthew Willetts[3]; Shourjo Ghose[3]; Teresa Zimmers[1, 2]; Amber Mosley[1]
[1]IU School of Medicine, Indianapolis, IN; [2]Richard L. Roudebrush VA Medical Center, Indianapolis, IN; [3]Bruker Scientific, Billerica, MA

Poster 01.14 **Spray-capillary Based Capillary Electrophoresis Mass Spectrometry Analysis of Metabolites in Live Cells**
Lushuang Huang; Zhe Wang; Kellye Cupp-Sutton; Si Wu
University of Oklahoma, Norman, OK

Poster 01.15 - WTT **Quantifying thermal stability for thousands of protein variants in parallel using multiplexed proteomics**
Kyle Hess; Ricard Rodriguez-Mias; Stephanie Zimmerman; Bianca Ruiz; Ian Smith; Anthony Valente; Ariadna Llovet; Yang Lu; William Noble; Stanley Fields; Judit Villen
University of Washington, Seattle, WA

Poster 01.16 **Affordable automated proteomics and multiomics sample preparation**
Richard Lam[1]; John Wilson[2]; John Laycock[1]
[1]Tecan SP, Inc., Baldwin Park, CA; [2]ProtiFi, LLC, Farmingdale, NY

P02: AGING AND NEURODEGENERATIVE DISEASES

Poster 02.01 **Cellular Senescence A Driver of The Pro-aging Side Effects of Antiretroviral Therapies and Chemotherapy in Humans**
Chisaka Kuehnemann[1]; Samah Shah[1]; Abhijit Kale[1]; Nathan Basisty[1]; Christopher Wiley[1]; Birgit Schilling[1]; Judith Campisi[1, 2]
[1]The Buck Institute, Novato, ; [2]Lawrence Berkeley Laboratory, Berkeley, CA

Poster 02.02 **Region-Specific N-Glycome Mapping of the Human Brain in Alzheimer's Patients by nanoLC chip-Q-TOF MS Analysis**
Jennyfer Tena; Mariana Barboza; Maurice Wong; Carlito B. Lebrilla
University of California, Davis, Davis, CA

Poster 02.03 **Quantifying Proteostasis: An *In Vivo* Protein Quality Assay Based On Fold Stability**
Hsien-Jung Lavender Lin; Stella Park; Nathan Zuniga; John Price
Brigham Young University, Provo, UT

Poster 02.04 **Improved Isolation Strategies to Increase the Yield and Purity of Human Sera Exosomes for Protein Biomarker Discovery**
Sandip Kumar Patel[1]; Roland Bruderer[2]; Francesco Neri[1]; Nathan Basisty[1]; Lukas Reiter[2]; Judith Campisi[1]; Birgit Schilling[1]
[1]Buck Institute Research on Ageing, Novato, California; [2]Biognosys AG, 8952 Schlieren, Switzerland

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM. WTT = Wed. tips & tricks talk.

Poster 02.05 **Tissue specific changes in protein metabolism associated with Alzheimer's risk**
Nathan Zuniga; Joseph Creery; John Price
Brigham Young University, Provo, UT

Poster 02.06 **ATF4 Promotes Skeletal Muscle Atrophy by Forming a Heterodimer with C/EBP-beta**
Jacob Rose[1]; Scott Ebert[2, 3]; Steven Bullard[2, 3]; Nathan Basisty[1]; George Marcotte[2, 3]; Zachary Skopec[2, 3]; Jason Dierdorff[2, 3]; Asma Al-Zougbi[2, 3]; Kirstin Tomcheck[2]; Austin Delau[2, 3]; Jacob Rathmacher[2, 3]; Sue Bodine[2, 4]; Christopher Adams[2, 4]; Birgit Schilling[1]
[1]Buck Institute, Novato, ; [2]University of Iowa, Iowa City, IA; [3]Iowa City Veterans Affairs Medical Center, Iowa City, IA; [4]Emmyon, Inc., Coralville, IA

Poster 02.07 **Understanding brain aging in non-human primates using high resolution proteomics**
Zeeshan Hamid[1]; Cun Li[2, 3]; Peter Nathanielsz[2, 3]; Laura Cox[1, 2]; Michael Olivier[1]
[1]Wake Forest University School of Medicine, Winston-Salem, NC; [2]Southwest National Primate Research Center, San Antonio, TX; [3]Department of Animal Science, Univ. of Wyoming, Laramie, WY

Poster 02.08 **Global Analysis of Protein Folding Stability Changes During Parkinson's Disease in a Mouse Model**
Renze Ma[1]; Julia Johnson[1, 2]; Michael FItzgerald[1]
[1]Duke University, Durham, NC; [2]Washington University in St. Louis, St. Louis, MO

Poster 02.09 **Comparative N-Glycoproteomics Reveals New Molecular Players in Alzheimer's Disease**
Xiaofang Zhong; Zhengwei Chen; Qinying Yu; Lei Lu; Lingjun Li
University of Wisconsin-Madison, Madison, WI

Poster 02.10 **Deamidation at N14 and N143 in S-crystallin in age-related cataractous lenses results predominately in isomerization without racemization**
John Klimek; Keith Zeintek; Ethan Makinster; Erin Marble; Kate Halverson; Kirsten Lampi; Larry David
OHSU, Portland, OR

Poster 02.11 **Characterization of changes in the Insolublome with Aging and Alzheimer's Disease models with DIA-MS**
Xueshu Xie; Manish Chamoli; Dipa Bhaumik; Anwen Lin; Kathleen Dumas; Renuka Sivapatham; Suzanne Angeli; Julie Andersen; Gordon Lithgow; Birgit Schilling
Buck Institute, Novato, CA

Poster 02.12 **Identifying molecular signatures of neurodegeneration – integrative proteomics and transcriptomics quantifies differences between induced cranial and spinal motor neurons**
Shuvadeep Maity[1]; Disi An[2]; Justin Randleman[2]; Dylan Iannitelli[2]; Esteban O Mazzoni[1, 2]; Christine Vogel[1, 2]
[1]Center For Genomics and Systems Biology(CGSB), NYU, New York, USA; [2]Department Of Biology, NYU, New York, USA

Poster 02.13 **Proteomic Profiling of Extracellular Vesicles Isolated from Human Alzheimer's Disease Brain Tissue.**
Manveen K Sethi; Satoshi Muraoka; Annina M. DeLeo; John D Hogan; Tsuneya Ikezu; Joseph Zaia
Boston University School of Medicine, Boston, MA

Poster 02.14 **Assessing data and system quality in DIA analyses of Alzheimer's disease post-mortem brain tissue**
Gennifer Merrihew[1]; Julia Robbins[1]; Jea Park[1]; Deanna Plubell[1]; Vagisha Sharma[1]; Thomas Montine[2]; Michael MacCoss[1]
[1]University of Washington, Seattle, WA; [2]Stanford University, Stanford, CA

Poster 02.15 **Proteomic Profile of Metformin Treated Senescent Mesenchymal Stem Cell Secretome**
Mustafa Burak Acar[2]; Serife Ayaz-Guner[1]; Huseyin Guner[1]; Nicola Alessio[3]; Musa Karakukcu[2]; Umberto Galderisi[3]; Servet Özcan[2]
[1]Abdullah Gul University, Kayseri, Turkey; [2]Erciyes University, Kayseri, Turkey; [3]University of Campania "Luigi Vanvitelli", Naples, Italy

Poster 02.16 **Deep Multilayer Brain Proteomics Identifies Molecular Networks in Alzheimer's Disease Progression**
Junmin Peng
St Jude Children's Res. Hosp, Memphis, TN

Poster 02.17 **Altered post-translational modifications in aged muscle is associated with reversal of mitochondrial dysfunction**
Matthew Campbell; Miguel Martin-Perez; Jarrett Egertson; Gennifer Merrihew; Lu Wang; Theo Bammler; Judit Villen; Michael MacCoss; David Marcinek
University of Washington, Seattle,

Poster 02.18 **A multiomic study uncovers the interplay between mitochondrial sirtuin 4 antiviral function and virus inhibition of host defense**
Cora Betsinger; Elizabeth Rowland; Ileana Cristea
Princeton University, Princeton, NJ

Poster 02.19 **Protein structural accessibility differences associated with Alzheimer's disease and aging in cerebrospinal fluid and brain tissue by limited proteolysis-mass spectrometry**
Danielle A. Faivre; Eric L. Huang; Michael J. Maccoss; *Genome Sciences*
University of Washington, Seattle, WA

Poster 02.20 **Panning for substrates of the inflammatory response kinase TBK1.**
Junqiang Ye[2]; Adnan Ahmed[1]; Jared Johnson[1]; Tomer Yaron[1]; Lew Cantley[1]; Tom Maniatis[2]; Noah Dephoure[1]
[1]Weill Cornell Medical College, New York, NY; [2]Columbia University College of Phys. & Surgeons, New York, NY

Poster 02.21 **Proteome-wide differences in turnover rates among mammals are correlated to their lifespans and energetic demands**
Kyle Swovick[1]; Denis Firsanov[1]; Kevin Welle[2]; Jennifer Hryhorenko[2]; Andrei Seluanov[1]; Vera Gorbunova[1]; Sina Ghaemmaghami[1]
[1]University of Rochester, Rochester, NY; [2]University of Rochester Mass Spectrometry Center, Rochester, NY

Poster 02.22 **A quantitative proteomics strategy that accommodates multiple quantitative values for each protein coding gene: Applications in Alzheimer's disease**
Deanna Plubell[1]; Lukas Käll[2]; Gennifer Merrihew[1]; Thomas Montine[3]; Michael MacCoss[1]
[1]University of Washington, Seattle, ; [2]KTH Royal Institute of Technology, Stockholm , Sweden; [3]Stanford University, Palo Alto, CA

Poster 02.23 **Mass spectrometry analysis reveals altered proteins across cerebellar lobules in the Niemann-Pick, type C1 mouse model**
Melissa Pergande; Thu Nguyen; Fernando Tobias; Rathnayake A. C. Rathnayake; Stephanie Cologna
Univ of Illinois at Chicago, Chicago, IL

Poster 02.24 **Identification of 4R-Tau splicing factors in progressive supranuclear palsy**
Sansi Xing[1]; Kevin Strang[2]; John Crary[2]; Yu Lu[1]
[1]McMaster University, Hamilton, Canada; [2]Icahn School of Medicine at Mount Sinai, New York, NY

P03: BIOMARKERS AND TARGETED MS ASSAYS

Poster 03.02 **MitoPlex: A Targeted Multiple Reaction Monitoring Assay for Quantification of a Curated Set of Mitochondrial Proteins**
Aleksandr Stotland[1]; Weston Spivia[1]; Amanda Orosco[1]; Allen Andres[1]; Roberta Gottlieb[1]; Jennifer Van Eyk[2]; Sarah Parker[3]
[1]Smidt Heart Institute, Los Angeles, CA; [2]Cedar Sinai Medical Center, Los Angeles, CA; [3]Cedars Sinai Medical Center, Los Angeles, CA

Poster 03.03 **Correlation of the plasma ΔS-Cys-Albumin integrity marker with changes in clinical analyte measurements founded on molecular interactions**
Erandi Kapuruge; Chad Borges
Arizona State University, Tempe, AZ

Poster 03.04 **A sensitive targeted proteomics approach to quantify low abundant proteins of the unfolded protein response pathway in glioblastoma cells**
Chi DL Nguyen[1]; Sebastian Malchow[1]; Stefan Reich[2]; Sascha Steltgens[3]; Konstantin V Shuvaev[1]; Stefan Loroch[1]; Christin Lorenz[1]; Albert Sickmann[1, 7]; Christiane B Knobbe-Thomsen[3]; Björn Tews[4, 5]; Jan Medenbach[2]; Robert Ahrends[1, 6]
[1]Leibniz Institute for Analytical Science ISAS-e.V., Dortmund, Germany; [2]Translational Control Group UniversityofRegensburg, Regensburg, Germany; [3]Institute of Neuropathology University Düsseldorf, Düsseldorf, Germany; [4]University of Heidelberg and DKFZ, Heidelberg, Germany; [5]Molecular Mechanisms of Tumor Invasion, DKFZ, Heidelberg, Germany; [6]Department Analytical Chemistry UniversityofVienna, Vienna, Austria; [7]Medizinische Fakultät, Ruhr-Universität Bochum, Bochum, Germany

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Poster 03.05 **Delta-S-Cys-Albumin: A Lab Test that Quantifies Cumulative Exposure of Archived Human Blood Plasma and Serum Samples to Thawed Conditions**
Joshua Jeffs[1]; Nilojan Jehanathan[1]; Stephanie Thibert[1]; Shadi Ferdosi[1]; Linda Pham[2]; Zachary Wilson[2, 3]; Christian Breburda[3]; Chad R. Borges[1]
[1]Arizona State University, Tempe, AZ; [2]Valleywise Health, Phoenix, AZ; [3]University of Arizona College of Medicine, Phoenix, AZ

Poster 03.06 **Will you still love the data tomorrow? – Futureproofing Experimental Designs for Translatable Research in Proteomics and Metabolomics**
Will Thompson; Matthew Foster; Lisa St. John-Williams; Erik Soderblom; Sarah Hiles; Greg Waitt; Tricia Ho; Laura Dubois; Kendra Adams; Arthur Moseley
Duke University School of Medicine, Durham, NC

Poster 03.07 **DEVELOPMENT OF A TARGETED LC-MS/MS METHOD FOR THE ABSOLUTE QUANTIFICATION OF LIPOPROTEIN(a) IN PLASMA**
Tomas Vaisar[1]; Noemie Clouet-Foraison[1]; Marlys L. Koschinsky[2]; Michael Boffa[3]; Mark S. Lowenthal[4]; Santica M. Marcovina[1]
[1]University of Washington, Seattle, WA; [2]Robarts Research Institute, London, ON, Canada; [3]University of Western Ontario, London, ON, Canada; [4]National Institute of Standard and Technology, Gaithersburg, MD

Poster 03.08 **Targeted proteomic assays for characterization of human plasma lipoproteins**
Claudia Seidl; Zachary Flaten; Danni Li
University of Minnesota, Minneapolis, Minnesota

Poster 03.09 **Developing Serum Multi-marker Panels for Diagnosing Hepatocellular Carcinoma using Multiple Reaction Monitoring-Mass Spectrometry**
Injoon Yeo[1]; Jae Nyeon Kim[1]; Areum Sohn[1]; Gi-Ae Kim[2]; Young-Suk Lim[3]; Youngsoo Kim[1]
[1]Seoul National University, Seoul, South Korea; [2]Kyung Hee University, Seoul, South Korea; [3]Asan Medical Center, Seoul, South Korea

Poster 03. 10 **Revealing Proteomic Subgroups with Clinical Classification and Prognostic Prediction in Pancreatic Ductal Adenocarcinoma using MRM-MS**
Minsoo Son[1]; Yoseop Kim[1]; Jae-Nyeon Kim[1]; Hongbeom Kim[2]; Jin-Young Jang[2]; Youngsoo Kim[1]
[1]Seoul National University College of Medicine, Seoul, South Korea; [2]Seoul National University Hospital, Seoul, South Korea

Poster 03.11 **Quantitative, comprehensive multi-pathway signaling analysis using an optimized phosphopeptide enrichment combined with an internal standard triggered targeted MS assay**
Bhavin Patel[1]; Penny Jensen[1]; Aaron Gajadhar[2]; Sebastien Gallien[3]; Jae Choi[1]; Romain Huguet[2]; Graeme McAlister[2]; Shannon Eliuk[2]; Markus Kellmann[4]; Tabiwang Arrey[4]; Alexander Harder[4]; Andreas Huhmer[2]; Kay Opperman[1]; John Rogers[1]
[1]Thermo Fisher Scientific, Rockford, IL; [2]Thermo Fisher Scientific, San Jose, CA; [3]Thermo Fisher Scientific, Paris, France; [4]Thermo Fisher Scientific, Bremen, Germany

Poster 03.12 **High throughput plasma proteomics with PASEF and 4D feature alignment**
Thomas Kosinski[1]; Lucy Woods[1]; Tharan Srikumar[2]; Scarlet Koch[1]; Christian Meier-Credo[1]; Christoph Gebhardt[1]; Heiner Koch[1]
[1]Bruker Daltonik GmbH, Bremen, Germany; [2]Bruker Ltd., Milton, Canada

P04: CANCER PROTEOMICS

Poster 04.01 **Title : Inducible knock-down coupled to quantitative LC-MS/MS to identify novel substrates of the oncogenic deubiquitinating protein USP7/HAUSP**
Ahood Aleidan[1, 2]; Paul Skipp[1]; Rob Ewing[1]
[1]University of Southampton, Southampton, United Kingdom; [2]Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Poster 04.02 **Endophytic fungi in cancer therapy.**
Thulasi G Pillai
KJSIEIT Institute, Sion, Mumbai, Maharashtra

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Poster 04.03 **Real-time, High Density Monitoring of pTyr Signaling Targets in Human Tumors Using SureQuant Internal Standard Targeted Protein Quantitation Solution**

Sally Webb[1]; Lauren Stopfer[2, 3]; Cameron Flower[2]; Aaron Gajadhar[1]; Bhavin Patel[4]; Sebastien Gallien[5]; Daniel Lopez-Ferrer[1]; Andreas Huhmer[1]; Forest White[2]

[1]Thermo Fisher Scientific, San Jose, California; [2]1.Massachusetts Institute of Technology, Cambridge, MA; [3]Koch Institute for Integrative Cancer Research, Cambridge, MA; [4]Thermo Fisher Scientific, Rockford, IL; [5]Thermo Fisher Precision Medicine Science Center, Cambridge, MA

Poster 04.04 **CANCELLED**

Poster 04.05 **Characterization of the substrate profile of Tyro3 using phosphoproteomics**

Naomi Widstrom; Laurie L. Parker

University of Minnesota, Twin Cities, Minneapolis, MN

Poster 04.06 **Proteomic analysis of paired primary and recurrent ovarian cancer tumors identifies biomarkers of ovarian cancer and disease recurrence.**

Kruttika Dabke[1]; Simon Coetzee[1]; Nicole Gull[1]; Alberto Reyes[1]; Jasmine Plummer[1]; Brian Davis[1]; Stephanie Chen[1]; John Govindavari[1]; Jenny Lester[1]; Benjamin Berman[2]; Beth Karlan[3]; Simon Gayther[1]; Sarah Parker[1]; Michelle Jones[1]

[1]Cedars Sinai Medical Center, Los Angeles, CA; [2]The Hebrew University of Jerusalem, Rehovot, Israel; [3]David Geffen School of Medicine at UCLA, LOS ANGELES, CA

Poster 04.07 **A data independent acquisition workflow enables the profiling of thousands of human cancer tissues for precision oncology**

Jakob Vowinckel[1]; Karel Novy[1]; Thomas Corwin[2]; Tobias Treiber[1]; Roland Bruderer[1]; Lukas Reiter[1]; Eike-Christin von Leitner[2]; Oliver Rinner[1]; Claudia Escher[1]

[1]Biognosys AG, Schlieren, Switzerland; [2]Indivumed GmbH, Hamburg, Germany

Poster 04.08 **Profiling of non-small cell lung cancer patient-derived xenografts reveals proteome and phosphoproteome remodeling associated with patient outcome**

Shideh Mirhadi[1, 2]; Jiefei Tong[2]; Jessica Weiss[3]; Quan Li[3]; Nhu-An Pham[3]; Nadeem Moghal[3]; Ming-Sound Tsao[3]; Michael Moran[1]

[1]University of Toronto, Toronto, Canada; [2]Hospital for Sick Children, Toronto, Canada; [3]Princess Margaret Hospital, Toronto, Canada

Poster 04.09 **Discovery and Multi-center Verification of Prostate Cancer Protein Biomarkers using Single-shot Short Gradient Microflow SWATH Acquisition and MRM^{HR} Workflow**

Christie Hunter[1]; Rui Sun[2]; Chen Chen[3]; Ruedi Aebersold[4]; Tiannan Guo[2]

[1]SCIEX, Redwood City, CA; [2]Westlake University, Hangzhou, China; [3]SCIEX, Shanghai, China; [4]ETH Zurich, Zurich, Switzerland

Poster 04.10 **Proteomic and Genetic Interaction Mapping Reveals New Ras Pathway Effectors and Regulators**

Marcus Kelly[1]; Kaja Kostyrko[2]; Kyuho Han[1]; Alejandro Sweet-Cordero[2]; Michael Bassik[1]; Peter Jackson[1]

[1]Stanford University School of Medicine, Stanford, CA; [2]University of California, San Francisco, San Francisco, CA

Poster 04.11 **Extending nano-surface and molecular-orientation limited proteolysis (nSMOL)-based antibody detection to high throughput for rapid patient screening**

John Cha[1]; Yoshinobu Koguchi[1]; Noriko Iwamoto[2]; Hong-Ming Hu[1]; Bernard Fox[1]; Eric Tran[1]; William Redmond[1]; Takashi Shimada[2]; Brian Piening[1]

[1]Providence Cancer Institute, Portland, OR; [2]Shimadzu Bioscience Research Partnership, Bothell, WA

Poster 04.12 **Proteomic analysis reveals TNF-α derived different regulation of mitochondrial complexes in ER/PR positive and negative breast cancer cells**

Hye Ryeon Jung[1]; Anjali Shinde[2]; Ha Yun Lee[1]; Kritarth Singh[2]; Jin Woo Jung[1]; Seo Jin Yang[1]; Han Byeol Kim[1]; Rajesh Singh[2]; Eugene C Yi[1]

[1]Seoul National University, Seoul, South Korea; [2]The MS University of Baroda, Vadodara, India

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Poster 04.13 **PROTEOMIC CHARACTERISATION OF SMALL CELL LUNG CANCER MOLECULAR SUBTYPES**
Beata Szeitz[1]; Nicole Woldmar[1, 2]; Zsuzsanna Valko[3, 4]; Zsolt Megyesfalvi[3]; Nandor Barany[3]; Sandor Paku[5]; Viktoria Laszlo[3, 4]; Luciana Pizzatti[2]; Gyorgy Marko-Varga[1]; Balazs Dome[3, 4]; Melinda Rezeli[1]
[1]Dept. of Biomedical Engineering, Lund University, Lund, Sweden; [2]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [3]National Korányi Institute of Pulmonology, Budapest, Hungary; [4]Medical University of Vienna, Vienna, Austria; [5]Semmelweis University, Budapest, Hungary

Poster 04.14 **Kinome-centric pharmacoproteomics identifies signaling pathways underlying cellular responses to targeted cancer drugs**
Martin Golkowski[1]; Ho-Tak Lau[1]; Marina Chan[2]; Heidi Kenerson[1]; Venkata Narayana Vidadala[1]; Anna Shoemaker[1]; Dustin J. Maly[1]; Raymond S. Yeung[1]; Taranjit S. Gujral[2]; Shao-En Ong[1]
[1]University of Washington, Seattle, WA; [2]Fred Hutchinson Cancer Research Center, Seattle, WA

Poster 04.15 **Characterizations of acute leukemia, myeloid and lymphoid, proteomes using proteogenomics and OpenProt database to identify new proteins and possible biomarkers.**
Noé Guilloy; Marie Brunet; Xavier Roucou
Univerité de sherbrooke, Sherbrooke, Canada

Poster 04.16 **Proteomic profiling of triple-negative breast cancer tumors and cell lines**
Natasha C. Mariano[1]; Evelien Schaafsma[1, 2]; Johnathan D. Marotti[2, 3]; Youdinghuan Chen[1, 2]; Brock C. Christensen[1, 2]; Chao Cheng[1, 2]; Todd W. Miller[1, 2]; Arminja N. Kettenbach[1, 2]
[1]Geisel School of Medicine at Dartmouth College, Hanover, NH; [2]Norris Cotton Cancer Center, Lebanon, NH; [3]Department of Pathology and Laboratory Medicine, Lebanon, NH

Poster 04.17 **Investigation of targetable Biomarkers for Non-small-cell lung carcinoma (NSCLC) in human blood plasma**
Barbara Helm[1]; Alexander Gorol[1]; Simon Heming[1]; Marc Schneider[2, 3]; Thomas Muley[2, 3]; Ursula Klingmüller[1, 3]
[1]German Cancer Research Center, Heidelberg, Germany; [2]Translational Research Unit, Thoraxklinik, University Hospital Heidelberg, Germany; [3]Translational Lung Research Center (TLRC), Member of the German Center for Lung Research(DZL), Germany

P05: CASCADIA PROTEOMICS SYMPOSIUM

Poster 05.01 - WTT **Strategies for correctly controlling the false discovery rate when a subset of peptides in a sample is relevant**
Andy Lin[1]; Uri Keich[2]; William Noble[1]
[1]University of Washington, Seattle, WA; [2]University of Sydney, Sydney, Australia

Poster 05.02 **Boosting statistical power in small-scale experiments with Percolator**
William Fondrie; William Noble
University of Washington, Seattle, WA

Poster 05.03 **N-terminomics of lymph nodes during active immune responses identifies multiple cleavage events**
Yoan Machado; Nestor Solis; Isabel Pablos; Georgina Butler; Christopher M Overall
University of British Columbia, Vancouver, Canada

Poster 05.04 **New functionality for the Trans-Proteomic Pipeline: tools for the analysis of proteomics data**
Luis Mendoza[1]; David D. Shteynberg[1]; Michael R. Hoopmann[1]; Henry Lam[2]; Jimmy Eng[3]; Eric Deutsch[1]; Robert L. Moritz[1]
[1]Institute for Systems Biology, Seattle, WA; [2]Hong Kong University of Science and Technology, Hong Kong, China; [3]University of Washington, Seattle, WA

Poster 05.05 **CANCELLED**

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P06: CELLULAR SIGNALING

Poster 06.01 **LPS-Tolerance Induces Global Changes in the Macrophage Secretome**
Joseph Gillen[1]; Thunnicha Ondee[2]; Jiraphorn Issara-Amphorn[2]; Asada Leelahavanichkul[2]; Aleksandra Nita-Lazar[1]
[1]NIAID, NIH, Bethesda, MD; [2]Chulalongkorn University, Bangkok, Thailand

Poster 06.02 **Tbx18 Orchestrates Cytostructural Transdifferentiation of Cardiomyocytes to Pacemaker Cells by Recruiting the Epithelial-Mesenchymal Transition Program**
D. Brian Foster[1]; Jinmo Gu[2]; Elizabeth Kim[2]; Robert O'Meallly[3]; Robert Cole[3]; Hee Cheol Cho[2]
[1]Johns Hopkins School of Medicine, Baltimore, MD; [2]Department of Pediatrics, Emory University, Atlanta, GA; [3]Mass Spectrometry Facility, Johns Hopkins School o, Baltimore, MD

P07: CHEMICAL PROTEOMICS

Poster 07.01 **A novel way for the data analysis of limited proteolysis coupled to mass spectrometry-based on machine learning**
Roland Bruderer[1]; Nigel Beaton[1]; Ilaria Piazza[1]; Paula Picotti[2]; Lukas Reiter[1]
[1]Biognosys AG, Schlieren, Switzerland; [2]ETH, Zurich, Switzerland

P08: COMPUTATION AND ANALYSIS

Poster 08.01 **Proteome-wide detection of function misannotation by taxon-specific rate ratio comparison**
Chengxin Zhang[1]; Xiaoqiong Wei[1, 2]; Gilbert S. Omenn[1]; Peter L. Freddolino[1]; Yang Zhang[1]
[1]University of Michigan, Ann Arbor, MI; [2]West China Hospital, Sichuan University, Chengdu, Sichuan, China

Poster 08.02 **Misbehavin' Trypsin: Selectivity of Atypical Cleavages by Trypsin**
Meghan Burke; Yuxue Liang; Stephen E Stein
NIST, Gaithersburg, MD

Poster 08.03 **Fast, Flexible and Feature-Rich Computation of Peptide and Proteoform Posterior Error Probabilities Using Binary Decision Trees**
Michael R. Shortreed; Lei Lu; Robert J. Millikin; Rachel M. Miller; Leah V. Schaffer; Zach Rolfs; Lloyd M. Smith
University of Wisconsin, Madison, WI

Poster 08.04 **Progress on Identifying and Characterizing the Human Proteome: 2020 Metrics from the HUPO Human Proteome Project**
Gilbert Omenn[1]; Lydie Lane[2]; Christopher Overall[3]; Fernando J. Corrales[4]; Jochen Schwenk[5]; Young-Ki Paik[6]; Jennifer Van Eyk[7]; Siqi Liu[8]; Stephen Pennington[9]; Michael Snyder[10]; Mark Baker[11]; Eric Deutsch[12]
[1]University of Michigan, Ann Arbor, MI; [2]SIB Swiss Institute of Bioinformatics, Geneva, Switzerland; [3]University of British Columbia, Vancouver, BC; [4]Centro Nacional de Biotecnologia, Madrid, Spain; [5]Science for Life Laboratory, Solna, N/A; [6]Yonsei University, Seoul, South Korea; [7]Cedar Sinai Medical Center, Los Angeles, CA; [8]BGI Group-Shenzhen, Shenzhen, China; [9]University College Dublin, Dublin, Ireland; [10]Stanford University, Stanford, CA; [11]Macquarie University, Sydney, Australia; [12]Institute for Systems Biology, Seattle, WA

Poster 08.05 **PASER: Parallel Database Search Engine in Real-Time**
Robin Park[1]; Patrick Garrett[1]; Michael Krause[2]; Sven Brehmer[2]; Titus Jung[1]; Peter Hufnagel[2]; Christopher Adams[2]; Casimir Bamberger[1]; Jolene Diedrich[1]; John Yates[1]
[1]The Scripps Research Institute, San Diego, CA; [2]Bruker Corporation, Bremen, Germany

Poster 08.06 **Clustering and identifying hundreds of millions of tandem mass spectra using deep learning**
Wout Bittremieux[1]; Damon H. May[2]; Jeffrey Bilmes[2]; William Stafford Noble[2]
[1]University of California San Diego, La Jolla, CA; [2]University of Washington, Seattle, WA

Poster 08.07 **Ultra-Sensitive Differential Quantification with MS-EmpiRe**
Constantin Ammar; Markus Gruber; Gergely Csaba; Ralf Zimmer
Ludwig-Maximilians-University, Munich, Germany

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Poster 08.08 **MSstatsSampleSize: Simulation tool for optimal design of high-dimensional MS-based proteomics experiments**
Ting Huang[1]; Meena Choi[1]; Tiannan Guo[2, 4]; Yansheng Liu[3]; Ruedi Aebersold[4, 5]; Olga Vitek[1]
[1]Northeastern University, Boston, MA; [2]Westlake Institute for Advanced Study, Hangzhou, China; [3]Yale University School of Medicine, West Haven, CT; [4]ETH Zürich, Zürich, Switzerland; [5]University of Zürich, Zürich, Switzerland

Poster 08.09 **Phosphopedia 2.0, a modern targeted phosphoproteomics resource**
Anthony Valente; William S Noble; Judit Villen
Genome Sciences, UW, Seattle,

Poster 08.10 - WTT **Using Protein Expressible Range to Define Biological Significance in Quantitative Proteomics**
Joe Creery; Nathan Zuniga; John Price
Brigham Young University, Provo, UT

Poster 08.11 **Fast and quantitative analysis of timsTOF PASEF data with MSFragger and IMQuant**
Fengchao Yu; Sarah Haynes; Guo Ci Teo; Andy Kong; Dmitry Avtonomov; Felipe Leprevost; Hui-Yin Chang; Daniel Geiszler; Dan Polasky; Alexey Nesvizhskii
University of Michigan, Ann Arbor, MI

Poster 08.12 **Bibliometrics Analysis of Proteomics Applications and Global Collaboration Networks Across Biomedical Fields from 2008 to 2018**
Sara A. Wennersten[1, 2]; Maggie P.Y. Lam[1, 2]
[1]University of Colorado AMC, Aurora, CO; [2]Consortium for Fibrosis Research & Translation, Aurora, CO

Poster 08.13 **A Comprehensive and Straightforward Web Application To establish a clinical utility integrating clinical factors and proteomic features**
Jae Nyeon Kim[1]; In Joon Yeo[1]; Young Soo Kim[1, 2]
[1]Interdisciplinary Program of Bioengineering, SNU, Seoul, South Korea; [2]Seoul National University College of Medicine, Seoul, South Korea

Poster 08.14 **SimpliFi: a data-to-meaning analytics engine to bring omics understanding to all**
Jim Palmeri[1]; Darryl Pappin[2]; John Wilson[1]
[1]ProtiFi, LLC, Farmingdale, NY; [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Poster 08.15 **FragPipe: from LCMS data to protein identifications, quantitation and PTM localization and in a few clicks**
Dmitry Avtonomov; Andy Kong; Guo Ci Teo; Fengchao Yu; Felipe Leprevost; Hui-Yin Chang; Daniel Geiszler; Sarah Haynes; Alexey Nesvizhskii
University of Michigan, Ann Arbor, MI

Poster 08.16 **PASEF and Bolt: enabling comprehensive sample analysis via high MS/MS acquisition and MS/MS sequencing through vast protein databases in minutes**
Amol Prakash[1]; Shadab Ahmad[1]; Swetaketu Majumder[1]; Jeifie Tong[2]; Shenheng Guan[2]; Matt Willets[3]; Shourjo Ghose[3]; Tharan Srikumar[5]; Sven Brehmer[4]; Micheal Moran[2]
[1]Optys Tech Corporation, Shrewsbury, MA, USA; [2]Sick Kids Research Center, Toronto, ON, Canada; [3]Bruker Scientific, Billerica, MA, USA; [4]Bruker Daltonik GmbH, 28359 Bremen, Germany; [5]Bruker Ltd., Milton, Canada

P09: CROSS-LINKING

Poster 09.01 **Leveraging the Entirety of Protein Data Bank to Enable Improved Structure Prediction based on Cross-Link Data**
Andrew Keller; Juan D. Chavez; Xiaoting Tang; James E. Bruce
University of Washington, Seattle,

Poster 09. 02 **Crosslinking mass spectrometry mapping mitochondrial protein interaction landscape of SS-31**
Juan Chavez; Xiaoting Tang; Matthew Campbell; Gustavo Reyes; Philip Kramer; Rudy Stuppard; Andrew Keller; David Marcinek; James Bruce
University of Washington, Seattle, WA

Poster 09.03 **Kojak 2.0: New Features For the Analysis of Cross-linked Proteins**
Michael R. Hoopmann[1]; Alex Zelter[2]; Michael Riffle[2]; Jimmy K. Eng[2]; Trisha N. Davis[2]; Robert L. Moritz[1]
[1]Institute for Systems Biology, Seattle, WA; [2]University of Washington, Seattle, WA

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P10: DATA-INDEPENDENT ACQUISITION PROTEOMICS (DIA)

Poster 10.01 **Matching peptides to data independent acquisition mass spectrometry data**
Yang Lu; Wenruo Bai; Jeff Bilmes; William Noble
University of Washington, Seattle, WA

Poster 10.02 **Longitudinal DIA-MS reveals key phosphorylation nodes dedicating cell proliferation and differentiation**
Wenxue Li[1]; Anatoly Kiyatkin[1]; Barbora Salovska[2]; Chongde Wu[1]; Erli Gao[1]; Archer Hamidzadeh[3]; Qian Ba[1]; Yansheng Liu[1]
[1]Yale Cancer Biology Institute, West Haven, CT; [2]Department of Genome Integrity, Institute of Molec, Prague, Czech Republic; [3]Yale Systems Biology Institute, West Haven, CONNECTICUT

Poster 10.03 **CANCELLED**

Poster 10.04 **Systematic parallel reaction monitoring of the human and cynomolgus macaque proteome**
Robert Lawrence; Rachael Hu; Esther Trueblood; Travis Biechele; Bill Arthur
Seattle Genetics Inc., Bothell, WA

Poster 10.05 **Nanoscale HILIC peptide fractionation for library building and diaPASEF data acquisition**
Brett Phinney[1]; Michael Krawitzky[2]; Chris Adams[3]
[1]UC Davis, Davis, California; [2]Bruker Daltonik, San Jose, CA; [3]Bruker, San Jose, CA

Poster 10.06 **Introducing Deepsearch: A supercomputing informatics system that can maximally deconvolve, within minutes, a *modi ied* DIA protocol, to achieve selectivity up to ~0.1Th**
Gautam Saxena[1]; Aleksandra Binek[2]; Simion Kreimer[2]; Aaron Robinson[2]; Jennifer Van Eyk[2]
[1]Deepsearch Services, Bethesda, MD; [2]Cedar Sinai Medical Center, Los Angeles, CA

Poster 10.07 **Human Identification Using Genetically Variant Peptides From Touch Samples using Data Independent Acquisition**
Paul Rudnick[1]; Brian Searle[2]; Richard Johnson[3]; Daniel Chelsky[1]; Sanne Aalbers[4]; Bruce Weir[4]; Michael Maccoss[3]
[1]Spectragen Informatics LLC, Bainbridge Island, WA; [2]Institute for Systems Biology, Seattle, WA; [3]Univ of Washington Genome Sci, Seattle, WA; [4]Biostatistics, University of Washington, Seattle, WA

P11: DRUG DEVELOPMENT

Poster 11.01 **Discovery of a Novel Plasmodium Protein Target of the Antihisatamine Clemastine using Parralel Chemoproteimic Strategies**
Michael C. Fitzgerald; Kuan-Yi Lu; Baiyi Quan; Kayla R. Sylvester; Tamanna Srivastava; Emily R. Derbyshire
Duke University, Durham, NC

P12: GLYCOPROTEOMICS AND GLYCOMICS

Poster 12.01 **Ion Mobility Separation of Glycoforms with Isomerism on the Peptide and Glycan Levels.**
Pratima Pathak; Matthew A. Baird; Alexandre A. Shvartsburg
Wichita State University, Wichita, KS

Poster 12.02 **CellSurfer Platform for semi-automated cell surface N-glycoprotein profiling of human primary cells reveals chamber-specific cardiomyocyte surface maps**
Linda Berg Luecke[1]; Matthew Waas[2]; Rebekah Gundry[2]
[1]Medical College of Wisconsin, Milwaukee, WI; [2]University of Nebraska Medical Center, Omaha, NE

Poster 12.03 **Identification of O-glycopeptides with MetaMorpheus**
Lei Lu[1]; Nicholas Riley[2]; Michael Shortreed[1]; Carolyn Bertozzi[2]; Lloyd Smith[1]
[1]University of Wisconsin-Madison, Madison, WI; [2]Stanford University, Stanford, CA

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Poster 12.04 **Optimized Liquid Chromatography Separation of INLIGHT® Derivatized N-linked Glycans for Isomer Identification and Structure Elucidation in Alzheimer's Disease Brain Samples**
Jaclyn Kalmar[1]; Karen Butler[1]; Erin Baker[1]; Brendan MacLean[2]; Michael MacCoss[2]; Edward Fox[3]; Thomas Montine[3]; David Muddiman[1]
[1]N. Carolina State University, Raleigh, NC; [2]University of Washington, Seattle, WA; [3]Stanford University, Stanford, CA

Poster 12.05 **Creating an Analytical Workflow to Assess N-linked Glycan Species and Their Alterations in Alzheimer's Disease Brain Tissue**
Karen Butler[1]; Jaclyn Kalmar[1]; Brenden MacLean[2]; Michael MacCoss[2]; Edward Fox[3]; Thomas Montine[3]; David Muddiman[1]; Erin Baker[1]
[1]North Carolina State, Raleigh, NC; [2]University of Washington, Seattle, WA; [3]Stanford University, Stanford, CA

P13: HIGH RESOLUTION MASS SPECTROMETRY

Poster 13.01 **Enhanced Label-Free and Targeted Proteomics Performance with Orbitrap Exploris 480 Mass Spectrometer**
Khatereh Motamedchaboki[1]; , Aaron Gajadhar[1]; Aman Makaju[1]; Aaron M. Robitaille[1]; Tabiwang Arrey[2]; Sebastien Gallien[2]; Sega Ndiaye[2]; Min Huang[3]; Yue Zhou[3]; David M. Horn[1]; Alexander Harder[2]; Daniel Lopez-Ferrer[1]
[1]Thermo Fisher Scientific, San Jose, CA; [2]Thermo Fisher Scientific, Bremen, Germany; [3]Thermo Fisher Scientific, Shanghai, China

P14: IMAGING

Poster 14.01 **Advancing MALDI Imaging Mass Spectrometry of Proteins**
Danielle Gutierrez[1]; Audra Judd[1]; Tina Tsui[1]; Carrie Romer[1]; Jamie Allen[1]; Zachary Jenkins[1]; Elizabeth Neumann[1]; Melissa Farrow[2]; Jeremy Norris[1]; Richard Caprioli[1]
[1]Vanderbilt University, Nashville, TN; [2]Vanderbilt University Medical Center, Nashville, TN

Poster 14.02 **SpatialOMx**
combining MALDI imaging with 4D-proteomics
Lucy Woods; Janina Oetjen Oetjen; Stephanie Kaspar-Schoenefeld; Alice Ly; Arne Fuetterer; *Bruker, Bremen, Germany*

P15: IMMUNOPEPTIDOMICS

Poster 15.01 **Identification of HLA Class I Bound Peptide for Behcet's Disease by mass spectrometric analysis**
Kyung-Cho Cho
Seoul National University, Seoul, South Korea

Poster 15.02 **caATLAS: an immunopeptidome atlas of human cancer**
Xinpei Yi; Yuxing Liao; Kai Li; Bo Wen; Bing Zhang
Baylor College of Medicine, Houston, TX

Poster 15.03 **Immunopeptidomic Studies of Hepatitis B Infected Human Liver Tissues Detect Strain Specific HBV-HLA Associated Peptides**
Mayank Srivastava; Darshit Shah; Augustine Choy; Richard Copin; Lisa Chen; Olav Olsen; William Olson; Robert Salzler
Regeneron Pharmaceuticals Inc., Tarrytown, NY

P16: METABOLOMICS

Poster 16.01 **Targeted Transcriptomic and Metabolomic Analyses Identifies Roles of Altered NAD Metabolism and NMRK in Diabetic Cardiomyopathy**
Ying Ann Chiao[1]; Xiaojian Shi[2]; Haiwei Gu[2]; Chi Fung Lee[1]
[1]OMRF, Oklahoma City, ; [2]Arizona State University, Phoenix, AZ

Poster 16.02 **Using Metabolomics, CE-MS and Isotope Incorporation to inform Alzheimer's Research**
Kendra Adams[2]; W. Kirby Gottschalk[3]; Joan Wilson[3]; David Millington[1]; Arthur Moseley[2]; J. Will Thompson[2]; Carol Colton[3]
[1]Duke University, Durham, North Carolina; [2]Duke University School of Medicine, Durham, NC; [3]Duke University Department of Neurology, Durham, NC

P17: MULTIOMICS AND NOVEL ''OMICS

Poster 17.01 **Exploration of stress biomarkers in gilthead seabream (*Sparus aurata*) liver by integrative multi-omics analysis**
Pedro Rodrigues[1]; Cláudia Raposo de Magalhães[1]; Denise Schrama[1]; Marco Cerqueira[1]; Ana Paula Farinha Resende[1]; Annette Kuehn[2]; Dominique Revets[2]; Surintorn Boonanuntanasarn[3]; Chatsirin Nakharuthai[3]
[1]CCMAR, Universidade do Algarve, Faro, Portugal; [2]Luxembourg Institute of Health, L-4354 Esch-sur-Alzette, Luxembourg; [3]Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

Poster 17.02 **iModMix, a network-based tool for multi-omics analysis**
Paul Stewart; Christopher Wilson; Elsa Flores; Brooke Fridley
Moffitt Cancer Center, Tampa, FL

Poster 17.03 **Skyline – Vendor-Neutral Software for Quantitative Proteomics and Metabolomics**
Cameron Wehrfritz[1]; Kendra Adams[2]; Brian Pratt[3]; Arthur Moseley[2]; Michael MacCoss[3]; Brendan MacLean[3]; Will Thompson[2]; Birgit Schilling[1]
[1]Buck Institute for Research on Aging, Novato, CA; [2]Duke University School of Medicine, Durham, NC; [3]University of Washington, Seattle, WA

Poster 17.04 **Multi-omic profiling of a large CRISPR knockout collection elucidates functions of uncharacterized mitochondrial proteins and their role in human disease**
Evgenia Shishkova[1]; Jarred Rensvold[2]; Ian Miller[1]; Yurij Sverchkov[1]; Dain Brademan[1]; Paul Hutchins[1]; Sean Peters[1]; Katherine Overmyer[2]; Adam Jochem[2]; Mark Craven[1]; David Pagliarini[2]; Joshua Coon[1, 2]
[1]University of Wisconsin - Madison, Madison, WI; [2]Morgridge Institute for Research, Madison, WI

Poster 17.05 **PALLID: Functional Integration of Lipidomics and Proteomics**
Hugh Mitchell; Jennifer Kyle
Pacific Northwest National Lab, Richland, Washington

Poster 17.06 **Application of omics mass spectrometry approaches to understand *Salmonella* pathogenesis**
Angela Di Capua[1]; Jikang Wu[1]; Mikayla A. Borton[2, 4]; Anice Sabag-Daigle[3]; Brian M. Ahmer[3]; Kelly C. Wrighton[2]; Vicki H. Wysocki[1]
[1]Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH; [2]Department of Microbiology, The Ohio State University, Columbus, OH; [3]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH; [4]Department of Soil and Crop Science, Colorado State University, Fort Collins, CO

Poster 17.07 **Multi-omic analysis reveals cannabidiol (CBD) disruption of cholesterol homeostasis and mitochondrial respiration**
Steven Guard[1]; Douglas Chapnick[1]; Christopher Ebmeier[1]; Jeremy Jacobsen[1]; Travis Nemkov[2]; Kerri Ball[1]; Kristofor Webb[1]; Helen Simpson[1]; Stephen Coleman[1]; Eric Bunker[1]; Zachary Poss[1]; Michael Stowell[1]; Angelo D'Alessandro[2]; Xuedong Liu[1]; William Old[1]
[1]University of Colorado Boulder, Boulder, CO; [2]University of Colorado Denver, Denver, CO

P18: POST-TRANSLATIONAL MODIFICATIONS

Poster 18.01 **Implementation of every-other-day fasting in aged mice effectively transforms protein sulfhydromes in multiple tissues**
Nazmin Bithi; Yoko Henderson; Christopher Link; Belinda Willard; Christopher Hine
Cleveland Clinic, Cleveland, OH

Poster 18.02 **Acetylation of the nuclear lamina regulates nuclear periphery stability and function**
Laura Murray-Nerger; Xinlei Sheng; Pranav Rekapalli; Ileana Cristea
Princeton University, Princeton, NJ

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Poster 18.03 **Uncovering Dehydroamino Acids within the HIV-1 Viral Proteome**
Rachel M. Miller[1]; Rachel A. Knoener[1, 3]; Nathan M. Sherer[2, 3]; Lloyd M. Smith[1]
[1]Chemsitry Department, UW-Madison, Madison, WI; [2]McArdle Laboratory for Cancer Research, UW-Madison, Madison, WI; [3]Institute for Molecular Virology, UW-Madison, Madison, WI

Poster 18.04 **Global protein phosphorylation identification and quantification by liquid chromatography mass spectrometry**
Ling Li; Dongmei Zhang; Belinda Willard
Cleveland Clinic, Cleveland, OH

Poster 18.05 **Deciphering the Landscape and Compartmentalization of the Thiol Redox Proteome via Stoichiometric Quantification of PTM Site Occupancies**
Tong Zhang[1]; jicheng Duan[1]; Matthew Gaffrey[1]; Karl Weitz[1]; Ronald Moore[1]; David Marcinek[2]; Wei-Jun Qian[1]
[1]Pacific Northwest National Lab, Richland, WA; [2]University of Washington, Seattle, wa

Poster 18.06 - WTT **Leveraging diagnostic ions for targeting acyl-lysine modifications in proteomic datasets**
Janine Fu; John Muroski; Rachel Loo; Joseph A Loo
University of California, Los Angeles, Los Angeles, CA

Poster 18.07 **Proteomic Strategies to Study Oxidative Post-Translational Modifications**
Aaron Maurais; Eranthie Weerapana
Boston College, Chestnut Hill, MA

Poster 18.08 **Regulation of Phosphoprotein Phosphatases by Carboxymethylation**
Scott Lyons; Lauren Cressey; Arminja Kettenbach
Dartmouth College, Hanover, NH

Poster 18.09 **Warburg effect and histone lactylation in human melanoma cells**
Kevin Huang; Ziyuan Li; Lu Yang; Di Zhang; Yingming Zhao
the university of chicago, Chicago, IL

Poster 18.10 **Derivatization of lysine with a commercial reagent to survey its post-translational landscape**
Martin Mathay; James Bruce
University of Washington, Seattle, WA

Poster 18.11 **Detection of Cysteine-to-Thiazole Modifications on Engineered Peptides**
Kimberly Wagstaff; Joshua McPhie; Diana Gabriela Calvopina Chavez; John Price; Joel Griffitts
Brigham Young University, Provo,

Poster 18.12 **Citrullination-specific fragmentation features facilitate confident identification of citrullinated peptides**
Min-Sik Kim
DGIST, Daegu, South Korea

Poster 18.13 **Large-scale open modification searching reveals the role of post-translational modifications in protein-protein interactions**
Charlotte Adams[1]; Kris Laukens[1]; Wout Bittremieux[2]
[1]University of Antwerp, Antwerp, Belgium; [2]University of California San Diego, La Jolla, CA

Poster 18.14 **Coenzyme A Binding Sites Induce Semi-Enzymatic Acylation**
Andrew Cruz; Chris Carrico; Xueshu Xie; Samah Shah; Cameron Wehrfritz; Birgit Schilling; Eric Verdin
Buck Institute, Novato, CA

Poster 18.15 **The 2020 Human Phosphoproteome PeptideAtlas**
Eric W Deutsch[1]; Zhi Sun[1]; David D Shteynberg[1]; Lydie Lane[2]; Paula Duek[2]; Luis Mendoza[1]; Tami Leppert[1]; Robert L Moritz[1]
[1]Institute for Systems Biology, Seattle, WA; [2]SIB Swiss Institute of Bioinformatics, Geneva, Switzerland

Poster 18.16 **Fast and sensitive ubiquitination determination through highly efficient immunoaffinity purification and trapped ion mobility mass spectrometry.**
Matthew Willetts[1]; Shourjo Ghose[1]; Charles Farnsworth[2]; Matthew Stokes[2]; Kimberley Lee[2]
[1]Bruker Scientific, Billerica, MA; [2]Cell Signalling Technology, Danvers, MA

Poster 18.17 **A novel automated and highly selective phosphopeptide enrichment strategy for successful phosphopeptide identification and phosphosite localization**
Shuai Wu[1]; Kenneth Newton[1]; Linfeng Wu[1]; Jordy Hsiao[1]; Valery Voinov[2]; Joseph Beckman[2, 3]
[1]Agilent Technologies, Santa Clara, CA; [2]e-Msion Inc., Corvallis, OR; [3]Oregon State University, Corvallis, OR

P19: PRECISION MEDICINE

Poster 19.01 **Machine Learning and Next Generation Proteomics Driving Precision Medicine**
Simon Dillon[1]; Xuesong Gu[1]; Hasan Otu[2]; Nezam Afdhal[1]; Michelle Lai[1]; Detlef Schuppan[3]; Joern Schattenberg[3]; Harland Winter[4]; Towia Libermann[1]
[1]Beth Israel Deaconess Medical Center, Boston, MA; [2]University of Nebraska-Lincoln, Lincoln, NE; [3]Johannes Gutenberg University Mainz, Mainz, Germany; [4]Massachusetts General Hospital, Boston, MA

Poster 19.02 **CANCELLED**

Poster 19.03 **Multiple Biomarker Panels to Predict Resposne to Tocilizumab(anti-IL6R) in Rheumatoid Arthritis Patients Using High-precision Proteomics Approach**
Jinwoo Jung; Byoung-Kyu Cho; Kyung-Cho Cho; Ara Cho; Yeong Wook Song; Eugene C Yi
Seoul National University, Seoul, South Korea

Poster 19.04 **Deep, reproducible and high-throughput FFPE analyses: moving toward large-scale automated clinical omics applications**
John Wilson[1]; Shourjo Ghose[2]; Matthew Willetts[2]
[1]ProtiFi, LLC, Farmingdale, NY; [2]Bruker Crop., Billerica, MA

P20: PROTEIN COMPLEXES AND INTERACTOMICS

Poster 20.01 **Characterization of DNA Damage, Phosphorylation, and Domain-Specific Interactors of the p53 Transactivation Domains**
Lisa Jenkins[1]; Andres Thorkelsson[1]; Supdipto Das[2]; Harichandra Tagad[1]; Thorkell Andresson[2]; Ettore Appella[1]
[1]National Cancer Institute, Bethesda, MD; [2]Frederick National Laboratory, Frederick, MD

Poster 20.02 **Lateral organization of CD147 with a B7 protein Confers Resistance Phenotype to Docetaxel in Breast Cancer**
Sohyun Kim[1]; Jieun Jeong[2]; Yuri Seo[1]; Eunhee G. Kim[2]; Kristine M Kim[2]; Eugene C. Yi[1]
[1]Seoul National University, Seoul, Korea; [2]Kangwon National University, Chuncheon, Korea

Poster 20.03 **Connect the dots: proteogenomics and protein interaction networks.**
Sebastien Leblanc; Marie Brunet; Michelle Scott; Xavier Roucou
Universite de Sherbrooke, Sherbrooke, Canada

Poster 20.04 **Proteomic Analysis of Immune-Challenged Macrophage Myddosome**
Caleb Bridgwater[1]; Joseph Gillen[1]; Sara Jones[1, 2]; Aleksandra Nita-Lazar[1]
[1]FCNS-LISB, NIAID, NIH, Bethesda, Maryland; [2]ODSET-OSMO, NIAID, NIH, Rockville, Maryland

Poster 20.05 **Quantitative ProteoCellomic Analysis of a Serial Captured Affinity Purified Complex**
Xingyu Liu[1]; Ying Zhang[1]; Jeffrey Lange[1]; Brian Slaughter[1]; Jay Unruh[1]; Tim Wen[1]; Yan Hao[1]; Charles Banks[1]; Laurence Florens[1]; Jerry Workman[1]; Michael Washburn[1, 2]
[1]Stowers Institute, Kansas City, MO; [2]Department of Pathology and Laboratory Medicine, U, Kansas City, KS

Poster 20.06 **Novel protein interaction analysis platform reveals a virus-driven mechanism for regulating peroxisome morphology**
Michelle Kennedy; Joel Federspiel; Samvida Venkatesh; Katelyn Cook; Clayton Otter; Pierre Jean Beltran; Ileana Cristea
Princeton University, Princeton, NJ

Poster 20.07 **Investigating Protein Quality Control Mechanisms of an Endocrine Protein Misfolding Disorder**
Madison Wright; Logan Kouba; Lars Plate
Vanderbilt University, Nashville, TN

Poster 20.08 **Degronomics: Mapping the Interacting Peptidome of a Ubiquitin Ligase Using an Integrative Mass Spectrometry Strategy**
Daniele Canzani[1]; Domnița-Valeria Rusnac[2, 3]; Ning Zheng[2, 3]; Matthew Bush[1]
[1]Department of Chemistry, University of Washington, Seattle, WA; [2]HHMI, University of Washington, Seattle, WA; [3]Dept of Pharmacology, University of Washington, Seattle, WA

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Poster 20.09 - WTT **Exploring the Genomic and Proteomic Basis for Cell-specific Remodeling within two Proteome-scale Protein Interaction Networks**
Ed Huttlin[1]; Laura Pontano Vaites[1]; Jose Navarrete-Perea[1]; Fana Gebreab[1]; Melanie Gygi[1]; Alexandra Thornock[1]; Sipei Fu[1]; Arvene Golbazi[1]; Eila Maenpaa[1]; Keegan Stricker[1]; Sanjukta Guha Thakurta[1]; Ramin Rad[1]; Joshua Pan[2]; David Nusinow[1]; Joao Paulo[1]; Devin Schweppe[1]; J. Wade Harper[1]; Steven Gygi[1]
[1]Harvard Medical School, Boston, MA; [2]Broad Institute, Cambridge, MA

Poster 20.10 **Mapping B7H3 cytosolic interactome using proximity labeling approach**
Seung Ju Moon[1]; Junghyeon Lee[2]; Han Byeol Kim[1]; Yuri Seo[1]; Jieun Jeoung[2]; Eunhee G. Kim[2]; Sumin Huh[1]; Kristine M. Kim[2]; Eugene C. Yi[1]
[1]Seoul National University, Seoul, South Korea; [2]Kangwon University, Chuncheon, South Korea

Poster 20.11 **Native Protein-Protein Interaction Profiling by SEC-DIA-MS**
Yuefan Wang; Yingwei Hu; Naseruddin Hoti; Hui Zhang
Johns Hopkins University, Baltimore, 0

Poster 20.12 **Visualizing HDAC1/2 Protein Interaction Networks in Live Cells**
Cassandra Kempf[1]; Charles Banks[1]; Janet Thornton[1]; Laurence Florens[1]; Michael Washburn[1, 2]
[1]Stowers Institute, Kansas City, MO; [2]University of Kansas Medical Center, Kansas City, MO

Poster 20.13 **HSP70 family molecular chaperone clients identified via UBAIT proteomics show differential specificities and stress response**
Seung Woo Ryu[1]; Rose Stewart[1]; Chase Pectol[2]; Nicolette Ender[1]; Oshadi Wimalarathne[1]; Ji-Hoon Lee[1]; Carlos P. Zanini[1]; Antony Harvey[3]; Maria D. Person[1]; Jon Huibregtse[1]; Peter Meuller[1]; Tanya T. Paull[1]
[1]The University of Texas at Austin, Austin, TX; [2]Texas A&M University, College Station, TX; [3]Thermo Fisher Scientific, Austin, TX

P21: PROTEIN PHOSPHORYLATION

Poster 21.01 **Defining kinase-substrate relationships using targeted protein degradation and phosphoproteomics**
Rufus Hards[1]; Juan C Mercado Del Valle[1]; Lincoln Howarth[1]; Ian LaCroix[2]; Mark Adamo[2]; Andrew Holland[3]; Scott Gerber[1]
[1]Geisel School of Medicine at Dartmouth, Lebanon, NH; [2]Norris Cotton Cancer Center, Lebanon, NH; [3]Johns Hopkins School of Medicine, Baltimore, MD

Poster 21.02 **Quantitative proteomics approach to study the dynamic changes in phosphorylation signaling networks of phosphoprotein phosphatases**
Isha Nasa[1, 2]; Lauren E. Cressey[1]; Thomas Kruse[3]; Emil P. T. Hertz[3]; Lee M. Graves[4]; Jakob Nilsson[3]; Arminja N. Kettenbach[1, 2]
[1]Geisel School of Medicine at Dartmouth College, Hanover, NH; [2]Norris Cotton Cancer Center, Lebanon, NH; [3]Novo Nordisk Foundation for Protein Research, Copenhagen, Denmark; [4]University of North Carolina School of Medicine, Chapel Hill, NC

Poster 21.03 **Phosphoproteomic profiling and causal analysis reveal signaling relations in GPVI-mediated platelet activation programs in health, aging and disease**
Özgün Babur; Jennifer Cunliffe; Jessica Minnier; Jiaqing Pang; John Klimek; Ashok Reddy; Phillip Wilmarth; Larry David; Joseph Aslan
Oregon Health & Science University, Portland, OR

Poster 21.04 **Dali: a method to identify phosphosites that alter thermal stability proteome-wide**
Ian Smith; Kyle Hess; Anna Bakhtina; Anthony Valente; Ricard Rodriguez-Mias; Judit Villen
University of Washington, Seattle, WA

Poster 21.05 **Proteomic and phosphoproteomic changes in rat skeletal muscle induced by acute endurance exercise**
James Sanford[1]; Paul Piehowski[1]; Wei-Jun Qian[1]; Joshua Adkins[1]; MoTrPAC Research Group[2]
[1]Pacific Northwest National Lab, Richland, WA; [2]NIH Common Fund, Bethesda, MD

POSTER LIST

P22: PROTEOFORM BIOLOGY

Poster 22.01 **Discovering Alternative Protein Isoform Switches in iPSC Cardiomyocytes by Integrated Omics and JCast.py**
Edward Lau[2]; Erin Yu Han[1]; Damon R. Williams[2]; Cody T Thomas[1]; Rajani Shrestha[2]; Maggie Pui Yu Lam[1]
[1]University of Colorado Anschutz Medical Campus,, Aurora, CO; [2]Stanford University, Palo Alto, CA

Poster 22.02 **Proteoform Enzymology: Quantitation of the Differential Proteoform Substrate Specificity of p300 and CBP**
Tao Wang[1]; Nikit Venishetty[2]; Matthew V. Holt[1]; Nicolas Young[1]
[1]Baylor College of Medicine, Houston, TX; [2]Rice University, Houston, TX

Poster 22.03 **Measuring the effect of missense variants on protein stability proteome-wide**
Stephanie Zimmerman; Ricard Rodriguez-Mias; Kyle Hess; Bianca Ruiz; Judit Villen; Stanley Fields
Genome Sciences, University of Washington, Seattle, WA

P23: PROTEOMICS IN MICROBIOLOGY

Poster 23.01 **Characterizing bacterial translational reprogramming under stress using a synthetic tmRNA platform**
Randi Turner[1]; Yan Wang[1, 2]; Daniel Dwyer[1]
[1]University of Maryland, College Park, MD; [2]NIH/NIDCR, Bethesda, MD

Poster 23.02 **The metabolic state of a syntrophic bacterium affects the proteomic acylation profile**
John Muroski[1]; Hong Hanh Nguyen[1]; Michael McInerney[2]; Robert Gunsalus[1]; Joseph A Loo[1]; Rachel Loo[1]
[1]University of California, Los Angeles, Los Angeles, California; [2]The University of Oklahoma, Norman, OK

Poster 23.03 **Functional Changes in the Murine Intestine Elicited by Fungal Colonization**
Veronika K. Pettersen[1, 2]; Erik van Tilburg Bernardes[1]; Antoine Dufour[1]; Marie-Claire Arrieta[1]
[1]University of Calgary, Calgary, Canada; [2]UiT - The Arctic University of Norway, Tromsø, NO

P24: PROTEOMICS OF DISEASE

Poster 24.01 **Identification of early candidate urine biomarkers for measure Escitalopram treatment response from major depressive disorder**
Yuhang Huan[1]; Jing Wei[1]; Jingjing Zhou[2, 3]; Min Liu[2, 3]; Jian Yang*[2, 3]; Youhe Gao*[1]
[1]Beijing Normal University, Beijing, China; [2]Beijing Anding Hospital, Beijing, China; [3]Capital Medical University, Beijing, China

Poster 24.02 **Lipid and apolipoprotein changes in response to inflammation with Type 2 diabetes**
Bryan A. Parks; Michael Gardner; Lisa McWilliams; Zsuzsanna Kuklenyik; John Barr
CENTERS FOR DISEASE CONTROL AND PREVENTION, Atlanta,

Poster 24.03 **Proteomic Analysis of 200 Barrett's Esophagus Cells Identifies Novel Biomarkers Induced by Lithocholic Acid**
Naomi Uwugiaren[2]; Ashita Singh[3]; Sarai Williams[4]; Mowei Zhou[4]; Ronald Moore[4]; Javier Alfaro[2, 3]; Ljiljana Pasa-Tolic[4]; Ryan Kelly[4, 5]; Ted Hupp[2, 3]; Ying Zhu[4]; Irena Dapic[2]; David Goodlett[1, 2]
[1]University of Maryland, Baltimore, MD; [2]University of Gdansk, Gdansk, Poland; [3]University of Edinburgh, Edinburgh, UK; [4]Pacific Northwest National Laboratory, Richland, WA; [5]Brigham Young University, Provo, UT

Poster 24.04 **A streamlined protocol for protein extraction from bone.**
Maggie Stainback; Ellen Quillen
Wake Forest School of Medicine, Winston Salem, NC

Poster 24.05 **Differential Regulation of Proteoforms in Human Hypertrophic Cardiomyopathy Revealed by Top-down Proteomics**
Trisha Tucholski[1]; Wenxuan Cai[1]; Stanford D. Mitchell[1]; Zachery R. Gregorich[1]; Elizabeth Bayne[1]; Sean McIlwain[1]; Max Wrobbel[1]; Hannah Karp[1]; Zachary Hite[1]; Petr G. Vikhorev[2]; Steve B. Marston[2]; Sean Lal[3]; Amy Li[3, 4]; Cristobal dos Remedios[3]; Takushi Kohmoto[1]; Joshua Hermsen[1]; J. Carter Ralphe[1]; Timothy J. Kamp[1]; Richard L. Moss[1]; Ying Ge[1]
[1]UW - Madison, Madison, WI; [2]Imperial College London, London, UK; [3]University of Sydney, Sydney, Austrailia; [4]La Trobe University, Melbourne, Austrailia

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Poster 24.06 **Proteomic-level *Helicobacter pylori* protein array and immuno-profiling of gastric cancer patients**
Lusheng Song[1]; Minkyo Song[2]; Charles S. Rabkin[2]; Jennifer Van Duine[1]; Linda M Liao[2]; Kailash Karthikeyan[1]; Weimin Gao[1]; Jolanta Lissowska[3]; M. Constanza Camargo[2]; Ji Qiu[1]; Joshua LaBaer[1]
[1]Biodesign institute, ASU, Tempe, Arizona; [2]Division of Cancer Epidemiology and Genetics, NCI, Rockville, Maryland; [3]MCMCC, Warsaw, Poland

Poster 24.07 **Effects of Protein Structure on Rheumatoid Arthritis**
William Thompson; John Holman; Hsien-Jung Lavender Lin; John Price
Brigham Young University, Provo,

Poster 24.08 **Identification of novel cancer stem cell specific transcription factors for B7H3 based on DNA affinity purification coupled to mass spectrometry**
Han Byeol Kim[1]; Yuri Seo[1]; Eunhee G. Kim[2]; Jieun Jeong[2]; Junghyeon Lee[2]; Eugene. C. Yi[1]; Kristine M. Kim[2]
[1]Seoul National University, Seoul, South Korea; [2]Kangwon National University, Chuncheon, South Korea

Poster 24.09 **Treating enamel defects: A porcine model for leveraging the role of saliva in enamel hardening based on protein removal**
Ana Gil-Bona[1, 2]; Maren Teichmann[1, 2]; Baptiste Depalle[1, 2]; Fabian Schulte[1, 2]; Markus Hardt[1, 2]; Megan K Pugach[1, 2]; Felicitas B Bidlack[1, 2]
[1]The Forsyth Institute, Cambridge, MA; [2]Harvard School of Dental Medicine, Boston, MA

Poster 24.10 **Transcriptomic and proteomic analysis of HD medium spiny neurons derived from human-iPSCs to identify keys molecular determinants of HD neuropathogenesis**
Kizito-Tshitoko Tshilenge[1]; Carlos Galica Aguirre[1]; Nathan Basisty[1]; Sicheng Song[2]; Swati Naphade[1]; Kevin Perez[1]; Ashley Loureiro[1]; Cameron Wehrfritz[1]; Anja Holtz[1]; Alejandro Lopez-Ramirez[1]; Sean Mooney[2]; Simon Melov[1]; Birgit Schilling[1]; Lisa M Ellerby[1]
[1]The Buck Institute for Research on Aging, Novato, CA; [2]University of Washington, Seattle, WA

Poster 24.11 **Plasma Proteomics in Non-Human Primates**
Sumaiya Nazli; Zeeshan Hamid; Michael Olivier
Wake Forest University School of Medicine, Winston Salem, NC

Poster 24.12 **An integrated quantitative proteogenomics pipeline reveals an increase in non-canonical protein variants in inflamed mouse colon tissue**
Andrew Rajczewski; Qiyuan Han; Subina Mehta; Praveen Kumar; Pratik Jagtap; Natalia Tretyakova; Tim Griffin
University of Minnesota, Minneapolis, MN

Poster 24.13 **Metabolomic and Proteomic Analysis of Type II Diabetes Mellitus Progression Using Human Aqueous Humor**
Jessica Skeie[1, 2]; Christopher Fortenbach[1]; Gregory Schmidt[1, 2]; Darryl Nishimura[1, 2]; Mark Greiner[1, 2]
[1]University of Iowa, Iowa City, Iowa; [2]Iowa Lions Eye Bank, Coralville, IA0

Poster 24.14 **Developing Proteins and phosphoproteins in urine extracellular vesicles as biosignatures for Parkinson's disease diagnostics**
Marco Hadisurya[1]; Li Li[2]; Shalini Padmanabhan[3]; Anton Illiuk[2]; W. Andy Tao[1, 2]
[1]Purdue University, West Lafayette, Indiana; [2]Tymora Analytical, West Lafayette, IN; [3]The Michael J. Fox Foundation, New York City, NY

Poster 24.15 **HIV-Tat exposure impairs neural development via Ras-ERK pathway dysregulation**
Brandon Murugan; Tariq Ganief; Jonathan Blackburn
University of Cape Town, Cape Town, South Africa

Poster 24.16 **The HDL Proteome Predicts Incident Cardiovascular Disease in Patients with Chronic Kidney Disease**
Baohai Shao[1]; Anna V. Mathew[2]; Carissa Thornock[1]; Subramaniam Pennathur[2]; Jay W. Heinecke[1]
[1]University of Washington, Seattle, WA; [2]University of Michigan, Ann Arbor, MI

Poster 24.17 **Multi-omic characterization of bone marrow interstitial fluid (BMIF) and peripheral blood plasma (PB) of pediatric Acute Lymphoblastic Leukemia patients**
Lorenz A. Nierves[1, 2]; Jian Guo[1]; Tao Huan[1]; Philipp F. Lange[1, 2]
[1]University of British Columbia, Vancouver, Canada; [2]BC Children's Hospital Research Institute, Vancouver , Canada

Poster 24.18 **Discovering aberrant splicing events in cancer proteomics using isobaric mass tags**
Daniel Roeth; Meiling Jin; Wu Yiming; Lili Wang; Markus Kalkum
City of Hope, Duarte, CA

Poster 24.19 **Differential proteomics in a mouse model of Niemann-Pick Type C following 2-hydroxypropyl-beta-cyclodextrin treatment**
Melissa R. Pergande[1]; Antony Cougnoux[2]; Forbes D. Porter[2]; Stephanie M. Cologna[1]
[1]Univ of Illinois at Chicago, Chicago, IL; [2]National Institutes of Health, Bethesda, MD

Poster 24.20 **Characterizing the Effects of Fish Oil on High-Density Lipoprotein Proteome and Cholesterol Efflux Capacity**
Paul Mueller; Sara Rosario; Elisabeth Yerkes; Melissa Heard; Nathalie Pamir
Oregon Health and Science University, Portland,

Poster 24.21 **Label free detection strategies of ovarian cancer in the vaginal microenvironment**
Gordon Luu; Thu 'Mi' Nguyen; Melissa Galey; Stephanie Cologna; Joanna Burdette; Laura Sanchez
University of Illinois at Chicago, Chicago,

Poster 24.22 **Smyd5 is a novel regulator in cardiac hypertrophy**
Ryan Bia; Mickey Miller; Caiyi C. Li; Aman Makaju; Anna Bakhtina; Samuel Hickenlooper; Emilee Horiuchi; Steven Valdez; Li Wang; Stephen T. Smale; Sarah Franklin
University of Utah, Salt Lake City,

P25: QUANTITATIVE PROTEOMICS

Poster 25.01 **Double barrel ESI source and novel tandem nanoLC-MS setup enables 24/7 deep proteome profiling**
Runsheng Zheng[1]; Thomas Lanzinner[2]; Georg Voelkle[3]; Christopher Pynn[1]; Jan Linnemann[2]; John Modrow[2]; Wim Decrop[1]; Andreas Tebbe[2]; Peter Jehle[1]; Oleksandr Boychenko[1]
[1]Thermo Fisher Scientific, Germering, Germany; [2]Evotec (München) GmbH, Martinsried, Germany; [3]Sonation GmbH, Biberach, Germany

Poster 25.02 **Towards Turnkey Targeted Proteomics Solutions Using SureQuant Internal Standard Targeted Protein Quantitation**
Sally Webb[1]; Sebastien Gallien[2]; Aaron Gajadhar[1]; Bhavin Patel[3]; Markus Kellman[4]; Tabiwang Arrey[4]; Alexander Harder[4]; Andreas Huhmer[1]
[1]Thermo Fisher Scientific, San Jose, California; [2]Thermo Fisher Scientific, Paris, France; [3]Thermo Fisher Scientific, Rockford, IL; [4]Thermo Fisher Scientific, Bremen, Germany

Poster 25.03 **An Orbitrap Eclipse Tribrid mass spectrometer with real time search enhances multiplexed proteome while improving quantitation accuracy and precision.**
Aaron Robitaille[1]; Romain Huguet[1]; Graeme McAlister[1]; Arne Kreutzmann[2]; Daniel Mourad[2]; Daniel Lopez-Ferrer[1]; Vlad Zabrouskov[1]
[1]Thermo Fisher Scientific, San Jose, CA; [2]Thermo Fisher Scientific, Bremen, DE

Poster 25.04 **Reliable and Deep Proteome Coverage by Gas-Phase Fractionation of Peptides with a FAIMS Pro Interface on a Novel Quadrupole Orbitrap**
Julia Kraegenbring[1]; Tabiwang Arrey[1]; Michael Belford[2]; Satendra Prasad[2]; Aaron Robitaille[2]; Markus Kellmann[1]; Martin Zeller[1]; Alexander Harder[1]
[1]Thermo Fisher Scientific, Bremen, DE; [2]Thermo Fisher Scientific, San Jose, CA

Poster 25.05 **Development of an accurate quantitative method for post-translational modifications on histone by parallel reaction monitoring**
Byoung-Kyu Cho; Young Ah Goo
Northwestern University, Chicago, IL

Poster 25.06 **Maximizing shotgun proteomics workflow to enable unmatched proteome profiling**
Joshua Nicklay[1]; Aaron Robitaille[2]; Scott Peterman[2]; David Horn[2]; Romain Huguet[2]
[1]Thermo Fisher Scientific, Somerset, NJ; [2]Thermo Fisher Scientific, San Jose, CA

Poster 25.07 - WTT **High Quantitative Accuracy and Sensitivity in PRM with Multiplexing and Asynchronous Fill-Time Correction**
Sebastian Müller; Tejas Gandhi; Lukas Reiter
Biognosys AG, Schlieren, Switzerland

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Poster 25.08 **Quantifying more than 500 human plasma proteins with SureQuant and PQ500 in a single run**
Tejas Gandhi[1]; Sebastian Müller[1]; Jan Muntel[1]; Sebastien Gallien[2]; Shannon Eliuk[3]; Oliver M. Bernhardt[1]; Lukas Reiter[1]
[1]Biognosys, Schlieren, Switzerland; [2]Thermo Fisher Scientific, Paris, France; [3]Thermo Fisher Scientific, San Jose, CA

Poster 25.09 **Evaluating label-free protein quantification strategies for integral membrane proteins**
Jeffrey Enders; Weijue Chen; Michael Hyman
NC State University, Raleigh, NC

Poster 25.10 **An Optimized Sample Preparation Method of Formalin-Fixed Paraffin-Embedded Tissues for Mass Spec Applications**
Kara Zehr[1]; Bhavin Patel[2]; Amarjeet Flora[2]; Penny Jensen[2]; Sergei Snovida[2]; Ryan Bomgarden[2]; Kay Opperman[2]; John Rogers[2]
[1]University of Illinois at Urbana-Champaign, Champaign, IL; [2]Thermo Fisher Scientific, Rockford, IL

Poster 25.11 **Development of a prmPASEF Approach on a tims-Q-TOF Instrument for Targeted Proteomics**
Antoine Lesur[1]; Joseph Longworth[1]; Pierre-Olivier Schmit[2]; Michael Krawitzky[3]; Sven Brehmer[4]; Jens Decker[4]; Gunnar Dittmar[1]
[1]Luxembourg Institute of Health, Strassen, Luxembourg; [2]Bruker Daltonique S.A., Wissembourg, France; [3]Bruker Daltonik, San Jose, CA; [4]Bruker Daltonik GmbH, Bremen, Germany

Poster 25.12 **Optimized Sample Preparation for TMTpro-labeled Proteomics Samples.**
Sergei Snovida; Amarjeet Flora; Leigh Foster; Ryan Bomgarden; Kay Opperman; John Rogers
Thermo Fisher Scientific, Rockford, IL

Poster 25.13 **Mechanism of adrenergic CaV1.2 stimulation revealed by proximity proteomics**
Marian Kalocsay[1]; Steven Marx[2]; Guoxia Liu[2]
[1]Harvard Medical School, Boston, MA; [2]Columbia University, New York, NY

Poster 25.14 **NanoTPOT: Enhanced Sample Preparation for Quantitative Nanoproteomic Analysis**
Ruilin Wu; Akshat Pai; Lina Liu; Sansi Xing; Yu Lu
McMaster University, Hamilton, Canada

P26: SINGLE-CELL PROTEOMICS

Poster 26.01 **Ultra-sensitive LC/MS workflow for high-throughput single-cell proteomics**
Khatereh Motamedchaboki[1]; Maowei Dou[1]; Yongzheng Cong[2]; Romain Huguet[1]; Aaron M Robitaille[1]; David M Horn[1]; Yufeng Shen[3]; Daniel Lopez-Ferrer[1]; Ryan T Kelly[2, 4]; Ying Zhu[4]
[1]Thermo Fisher Scientific, San Jose, California; [2]Brigham Young University, Provo, UT; [3]CoAnn Technologies LLC, Richland, WA; [4]Pacific Northwest National Laboratory, Richland, WA

Poster 26.02 **A Quantitative Single-Cell Proteomics Approach to Characterize an Acute Myeloid Leukemia Hierarchy**
Erwin M. Schoof[1, 2]; Nicolas Rapin[1, 2]; Benjamin Furtwängler[1, 2]; Nil Uresin[1, 2]; Simonas Savickas[3]; Coline Gentil[1, 2]; Khatereh Motamedchaboki[6]; Aaron Gajadhar[6]; Romain Huguet[6]; Daniel Lopez-Ferrer[6]; Eric Lechman[4, 5]; Ulrich auf dem Keller[3]; John E. Dick[4, 5]; Bo T. Porse[1, 2]
[1]The Finsen Laboratory, Copenhagen, Denmark; [2]Biotech Research and Innovation Centre (BRIC), Copenhagen, Denmark; [3]Technical University of Denmark, Lyngby, Denmark; [4]Princess Margaret Cancer Centre, Toronto, Canada; [5]University of Toronto, Toronto, Canada; [6]Thermo Fisher Scientific, San Jose, United States

Poster 26.03 - WTT **Capillary electrophoresis ion mobility mass spectrometry for single-neuron proteomics**
Sam Choi; Peter Nemes
University of Maryland, College Park, MD

Poster 26.04 **Performance of the VICI Valco TrueNano U/HPLC system and sample preparation workflow for single cell level shotgun proteomics**
Guoting Qin[1]; Rufeng Li[1]; Hong Shao[1]; Qiulin Li[1]; Jennifer Copeland[2]; Martin Brisbin[2]; Hal Barnet[2]; Huamin Cai[2]; Stan Stearns[2]; Chengzhi Cai[1]
[1]University of Houston, Houston, ; [2]VICI Valco Instruments, Houston, TX

Poster 26.05 **Single-cell proteomics reveals TMSB4X downregulation upon hair-cell differentiation to release actin for building stereocilia**
Ying Zhu[1]; Mirko Scheibinger[2]; Daniel Ellwanger[2]; Jocelyn Krey[3]; Dongseok Choi[3]; Ryan Kelly[4]; Stefan Heller[2]; Peter Barr-Gillespie[3]
[1]PNNL, Richland, Washington; [2]Stanford University, Stanford, CA; [3]Oregon Health & Science University, Portland, OR; [4]BYU, Provo, UT

P27: STRUCTURAL PROTEOMICS

Poster 27.01 **Phosphorylation patterns on the Neurotensin Type I Receptor required for arrestin recruitment and receptor trafficking as demonstrated in 4D.**
John Janetzko[1]; Asuka Inoue[3]; Michael Krawitzky[2]; Guillaume Tremintin[2]; Christopher Adams[2]; Brian Kobilka[1]
[1]Stanford University, Mol. and Cell. Phys., Stanford, CA; [2]Bruker, San Jose, CA; [3]Tohoku University, Aobaku, Sendai, Miyagi, Japan

Poster 27.02 **High-density chemical cross-linking for modeling protein interactions**
Julian Mintseris; Steven Gygi
Harvard Medical School, Boston, MA

P28: TOP-DOWN PROTEOMICS

Poster 28.01 - WTT **Improving Proteoform Identifications and Post-Translational Modification Localizations Through Large-Scale Integration of Bottom-Up and Top-Down Data**
Leah Schaffer; Robert Millikin; Rachel Miller; Michael R. Shortreed; Lloyd Smith
UW-Madison, Madison, WI

Poster 28.02 **Application of FAIMS and top-down mass spectrometry to brain cortex samples from individuals diagnosed with Alzheimer's disease**
James Fulcher[1]; Ronald Moore[1]; David Bennett[2]; Philip De Jager[3]; Vladislav Petyuk[1]
[1]Pacific Northwest National Lab, Richland, WA; [2]Rush University Medical Center, Chicago, IL; [3]Columbia University Medical Center, New York, NY

Poster 28.03 - WTT **Characterization of cyclic peptide aggregation behavior using biophysical, intact mass, and top-down disulfide mapping approaches.**
Elizabeth S. Hecht[1]; Tao Chen[1]; Shijia Tang[1]; Yury V. Vasilev[2]; Maelia Uy-Gomez[1]; Joseph Meeuwsen[2]; Peter Liu[1]; Valery G. Voinov[2]; Jason Gruenhagen[1]; Joseph S. Beckman[2]; Wendy Sandoval[1]; David Arnott[1]
[1]Genentech, Inc., South San Francisco, CA; [2]eMSion, Inc., Corvallis, OR

Poster 28.04 **Cell-Free Synthesis of Full-Length Internal Standard Proteins for Top-Down Proteomics**
Ayako Takemori[1]; David Butcher[2]; Lissa C. Anderson[2]; Nobuaki Takemori[1]
[1]Ehime University, Toon, Japan; [2]National High Magnetic Field Laboratory, Tallahassee, FL

Poster 28.05 **MASH Explorer, a Universal, Comprehensive, and User-friendly Software Environment for Top-down Proteomics**
Sean J. McIlwain[1]; Zhijie Wu[1]; R. Kent Wenger[1]; Molly M. Wetzel[1]; Jake A. Melby[1]; Yiran Yan[1]; Xiaowen Liu[2]; Ruixiang Sun[3]; Irene M. Ong[1]; Ying Ge[1]
[1]UW-Madison, Madison, WI; [2]Indiana University - Indianapolis, Indianapolis, IN; [3]Chinese Academy of Sciences, Beijing, China

Poster 28.06 **Exploring proteomics data from multi-sample experiments**
Iobani Godinez
PNNL, Richland

Poster 28.07 **Optimization of Quantitative Top-Down Proteomics in Complex Samples using Protein-Level Tandem Mass Tag (TMT) Labeling**
Dahang Yu[1]; Zhe Wang[1]; Kellye Cupp-Sutton[1]; Yanting Guo[1]; Mulin Fang[1]; Kenneth Smith[2]; Xiaowen Liu[3]; Si Wu[1]
[1]University of Oklahoma, Norman, OK; [2]Oklahoma Medical Research Foundation, Oklahoma City, OK; [3]Indiana University–Purdue University Indianapolis, Indianapolis, IN

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ADVANCES IN TECHNOLOGY
P01.01 – P01.16

Poster Number: P01.01

Cartridge And Bead-based Sample Preparation For Proteome Analysis: Assessing Robustness Of Protein Recovery And Digestion Efficiency

Jessica Nickerson; Alan Doucette

Dalhousie University, Halifax, Canada

Introduction: Detergents including sodium dodecyl sulfate (SDS) are becoming increasingly accepted in proteomics workflows, owing to a growing list of semi-automated approaches, designed to purify and digest protein mixtures ahead of bottom-up LC-MS/MS analysis. Among the cartridge and bead-based technologies are Suspension Trapping S-Trap, in-Stage Tip (iST), Single-Pot, Solid-Phase-Enhanced Sample Preparation (SP3), and the ProTrap XG, all reporting successful acquisition of peptide lists from SDS-containing samples. [1, 2, 3, 4, 5] Here, we evaluate these sample preparation strategies, comparing the recovery from varying sample concentration and type (membrane fraction vs. whole), as well as purity and relative digestion efficiency.

Methods: Samples will be prepared within FASP, S-Trap, iST, SP3, and ProTrap XG. Two test proteome mixtures will be prepared: an aqueous extract of S. cerevisiae, and a membrane fraction extracted with 1% SDS. Protein concentration will be varied to assess the effectiveness of the preparation at different loadings. Protein recovery will be quantified by the bicinchoninic acid (BCA) assay, as well as SDS PAGE, and LC-UV analysis. Digestion will be performed with a standard enzyme:protein ratio (*wt/wt*) to assess relative digestion efficiency of each product. The resulting peptide mixtures will be subject to bottom-up LC-MS/MS analysis, assessing the number of peptides, degree of missed cleavage and coverage of the proteome.

Results: Methods are in place to evaluate performance (recovery, purity, digestion efficiency) of each sample preparation strategy.

Conclusions: Multiple strategies exist for bottom-up MS analysis of detergent-containing samples. This study provides unbiased assessment of the performance characteristics of several popular strategies.

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Poster Number: P01.02

Validation Of A Disposable Biospecimen Collection System With Integral Refrigeration For Preserving The Phosphoproteome

Jacob Kennedy[1]; Amanda Paulovich[1]; Scott Thielman[2]; Amanda Woodcock[2]; Richard Ivey[1]; Guy Corral[2]; Eli Hooper[2]; Gregory Martin[2]; Gina Longman[2]; Blake Stancik[2]; Jeff Whiteaker[1]; Lei Zhao[1]; Travis Lorentzen[1]

[1]Fred Hutchinson CRC, Seattle, ; [2]Product Creation Studio, Seattle, WA

The cancer phosphoproteome is highly susceptible to preanalytical variables, such as ischemic time prior to chemical fixation or flash freezing. The current gold standard for collecting and processing cancer biopsies is to flash freeze tumor samples in liquid nitrogen. However, in many clinical settings liquid nitrogen is not readily available, and neither the personnel nor the infrastructure is generally available to rapidly process the tumor samples. As a result, tumors are often subjected to prolonged ischemia and/or chemical fixatives, altering the phosphoproteome such that it may no longer reflect the true *in vivo* state of the tumor. There is need for an economical, single-use device that can be stored at room temperature then activated at point of care to rapidly freeze the specimen.

Our proof-of-concept quick-freeze prototype device focused on the key requirements including cooling performance, device safety, minimize use error, and the ability to ship via existing cold chain logistics pathways. We have demonstrated that our device can cool a core sample below 0 degrees C in less than 70 seconds, below -8 degrees C in less than 150 seconds, and maintain that sample below 0 degrees C for greater than 70 minutes.

The performance of our prototype was benchmarked against liquid nitrogen using melanoma-bearing PDX mice that were subjected to total body irradiation. Tumors were harvested and quadrisected. Two parts of the tumor were snap frozen in liquid nitrogen, and the remaining two parts were rapidly cooled in the prototype quick-freeze biospecimen containers for 1 hour. Protein lysates will be subjected to untargeted LC-MS/MS phosphoprofiling as well as targeted multiple reaction monitoring (MRM) MS-based quantification of a panel of phosphopeptides that respond to DNA damage. Preliminary MRM results demonstrate that measured phosphosite levels are comparable in samples frozen in the device to those snap frozen in liquid nitrogen.

Poster Number: P01.03

Development of an Online 2D Ultra-High-Pressure Nano-LC System for High-pH and Low-pH Reversed Phase Separation in Top-Down Proteomics

Zhe Wang[1]; Dahang Yu[1]; Xiaowen Liu[2]; Kenneth Smith[3]; Si Wu[1]

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The development of novel high-resolution separation techniques is crucial for advancing the complex sample analysis necessary for high-throughput top-down proteomics. Recently, we developed an offline 2D high-pH RPLC/low-pH RPLC separation method and demonstrated good orthogonality between these two RPLC formats. To further improve the throughput and sensitivity of the offline approach, we developed an online 2D ultra-high-pressure nano-LC system for high-pH and low-pH RPLC separations in top-down proteomics.

System Setup: The automatic online 2D system was built through customizing a high-pressure normal flow LC system with two Thermo Accela LC pumps (operational pressure 12,000+ psi, operation flow rate 10-1000 µL/min), two high-pressure 6-port switching valves and splitting columns, and one Thermo Accela autosampler. A micro-trap column with an online dilution setup was used to online collect eluted proteins from the high-pH separation and to inject fractions for 2nd dimension column. Specifically, ultra-high-pressure long capillary column RPLC separation has been applied to the second dimensional low-pH RPLC separation for the improvement of separation resolution (peak capacity of 200).

Results: In total, 1,507 non-redundant proteoforms in 308 unique proteins were identified in 5 µg intact *E. coli* cell lysate with our online 2D separation, which is comparable with our previous reported results in 500 µg intact E. coli cell lysate using the offline 2D separation. Good orthogonality between 1st dimension high-pH RPLC and 2nd dimension low-pH RPLC were demonstrated using the online 2D system, which is consistent with our previously reports using the offline 2D system. Overall, we have demonstrated that our online 2D pH RP/RPLC system coupled with top-down proteomics holds the potential for deep proteome characterization of mass-limited samples because it allows the identification of hundreds of intact proteoforms from complex biological samples at low microgram sample amounts.

Poster Number: P01.04

Proteograph: Efficient and Automated Multi-Nanoparticle Platform for Deep, Unbiased Plasma Protein Profiling and Protein-Protein Interaction Biological Insight

Patrick A. Everley[1]; Shadi Ferdosi[1]; Daniel Hornburg[1]; William C. Manning[1]; Asim Siddiqui[1]; Greg Troiano[1]; Omid C. Farokhzad[1]; Matthew E. K. Chang[2]; Mark R. Flory[2]; John E. Blume[1]

[1]Seer, Inc., Redwood City, CA; [2]Cancer Early Detection Advanced Research Center, Portland, OR

The proteomic complexity of biological samples limits the scale and speed of robust biomarker discovery studies. Complex workflows for deep profiling of the plasma proteome have limited study size and compromised validation and replication potential, while necessitating trade-offs among depth and breadth of coverage, efficiency, precision, and accuracy.

Herein we describe Proteograph, an efficient, automated multi-nanoparticle (NP) platform for deep and unbiased proteomic profiling. NPs reproducibly form protein coronas driven by their engineered biophysicochemical properties, and those protein signatures provide insights into protein-protein interaction (PPI) in a biological sample. Specific and reproducible protein-NP binding is the product of binding affinities and a sample's protein concentrations. The result is compression of concentration range that improves detection of low-abundance proteins without loss of linearity (demonstrated by spike-recovery experiments) or measurement precision. As proof-of-concept, we used our panel of 10 specifically engineered superparamagnetic NPs to identify >2,000 protein groups (1% protein and peptide FDR, 84% with 2 or more peptides) from 16 individual plasma samples. Proteograph allows parallel processing of 80 NP-sample combinations, from sample to purified peptides, in less than 8 hours. We also compared Proteograph to a typical workflow including high-pH reverse-phase fractionation, and found higher protein group detection (1,949 vs 658) with better precision (27% vs 41% CVs). In mapping the 1949 and 658 protein groups to a PPI map derived from the STRING database, we identified, respectively, 15/21 and 1/21 interaction clusters with >10 protein members where >10% of the members were covered.

Compared to typical fractionation, the Proteograph platform has superior coverage, depth, precision, and accuracy in unbiased proteomic profiling. Furthermore, this NP-based system has the potential to discover PPIs at a level not possible using conventional fractionation. Proteograph may enable the large-scale studies necessary for protein biomarker discovery and yield new biological insights into the human proteome.

Poster Number: P01.05

Achieving Maximal Protein Extraction, Solubilization And Digestion Via Combination Of High Temperature And Novel Mass Spec Compatible Surfactant

Valerie Ressler; Sergei Saveliev; Wenhui Zhou; Joel Walker; Jean Osterman; Mike Rosenblatt; Poncho Meisenheimer; Marjeta Urh

Promega, Madison, WI

Mass spectrometry (MS) compatible surfactants are the popular means for preparation of biological samples for MS analysis. Despite significant advantages, MS compatible surfactants often fall short of expectations and fail to match SDS in terms of protein extraction and solubilization. In addition, these surfactants cause peptide precipitation upon degradation. In this study, we addressed these shortcomings using a novel surfactant. This surfactant is designed to assist sample preparation in which all steps – protein extraction, solubilization and digestion – are performed at high temperature. To address peptide precipitation problem, this surfactant was optimized to produce soluble degradation products.

In our study, we created a library of over a hundred MS compatible surfactants. The surfactants were screened for compatibility with trypsin, protein extraction, solubilization and denaturation, long-term stability at high temperature, degradation to MS compatible products and additional criteria. The most promising candidates were structurally optimized. Surfactant 2-hydroxy-3-(((2-methyltridecan-2-yl)oxy)carbonyl)amino)propane-1-sulfonate was selected as the best candidate. In addition to overall advanced performance it showed a unique property: it maintained trypsin activity at high temperature (50°C). SDS inhibited trypsin at these conditions.

We tested this surfactant in proteomics studies involving *E. coli* and human protein extracts and porcine lung tissue. In the study, all steps of sample preparation – protein extraction, solubilization, digestion and surfactant degradation – were performed at high temperature. The surfactant assured maximal protein extraction and solubilization, matching or exceeding the corresponding SDS efficiencies. In contrast, RapiGest®, a commonly used MS compatible surfactant, afforded for only ~25% protein recovery. Our surfactant provided significant increase in peptide and protein IDs and protein sequence coverage. Special investigation was performed to assure that the surfactant degradation products did not cause deterioration of critical MS parameters. Results of the study and advantages of our procedure compared to common sample preparation methods will be discussed.

Poster Number: P01.06

Scanning SWATH Acquisition – The Next Step in the Data Independent Acquisition (DIA) Evolution

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As the field of quantitative proteomics moves towards large sample cohorts, the use of microflow LC in combination with SWATH® Acquisition has been growing steadily as it provides better robustness and higher throughput. While variable window SWATH provides very good quantitative results on faster gradients, more narrow peak widths require faster cycle times and therefore present an opportunity for further acquisition improvements. With Scanning SWATH acquisition, a narrow Q1 window can be very rapidly scanned across a Q1 mass range and MS/MS is acquired at every small step. This adds an extra dimension to the DIA data that can be used to improve data quality.

Microflow LC was performed on the SCIEX TripleTOF® 6600 System with the OptiFlow® Source using the nanoLC® 425 system. A trap-elute workflow was used and a range of gradient lengths from 5 – 45 mins was explored, with key acquisition parameters for Scanning SWATH Acquisition varied to optimize and compare to variable window SWATH acquisition using a research prototype Analyst® Software. Data was processed with research prototype SWATH software and results were analyzed using the SWATH Replicates Template.

Using a variety of complex matrices, both single species and mixtures of digests, acquisition parameters for Scanning SWATH Acquisition was explored. Not surprisingly, using a more narrow Q1 window provided improvements in # of peptides quantified over variable window SWATH for the microflow gradients tested. Optimization results will be presented.

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

Poster Number: P01.07

diaPASEF in Mixed Proteomes is Deep, Quantitative and Reproducible

Stephanie Kaspar-Schoenefeld[2]; Kristina Marx[2]; Christopher Adams[1]; Tejas Gandhi[3]; Lukas Reiter[3]; Florian Meier[4]; Andreas Brunner[4]; Markus Lubeck[2]; Oliver Raether[2]; Stefan Tenzer[5]; Reudi Aebersold[6]; Ben Collins[7]; Hannes Roest[8]; Matthias Mann[4]

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Introduction: Trapped ion mobility spectrometry (TIMS) focuses ions by mobility before they are introduced into a Q-TOF mass spectrometer. Mobility has a strong correlation with *m*/*z*, this additional dimension aligns ions and affords selectivity such that the ion cloud is efficiently sampled. DIA approaches have an inherent advantage of being nonrandom, reducing the missingness in datasets. Here we apply DIA in concordance with PASEF (termed diaPASEF) on mixed proteomes to achieve deep and quantitative sampling.

Methods: Whole-cell proteomes from human, yeast and E. coli were digested and analyzed via nanoLC coupled to a timsTOF Pro. A library was built by HpH reverse-phase fractionation and run in a PASEF dda fashion. From the projected mobility and mass aligned ion cloud a windowing schema covering the bulk of 2+ and 3+ ions was selected. This diaPASEF schema was run against mixed proteomes of known admixtures. Data was processed by Spectronaut (Biognosys) and filtered to a 1% FDR.

Results: The experiment specific spectral library consisted of 13,013 protein groups (6,790 HeLa; 2,423 *E. coli*; and 3,800 Yeast proteins) and 136,682 unique peptide. From total protein loads of 200ng with a 100 min. gradient in a diaPASEF schema more than 8000 protein groups were identified and quantified. Background human proteins were spiked in equal amounts resulting in a theoretical ratio of 1:1. Human proteins were centered at a log2 ratio of sample HYE-A versus HYE-B of 0. For yeast and *E. coli* we achieved a very good global accuracy on the timsTOF Pro with regulation ratios measured close to the theoretical ones (Median ratio sample HYE-A/HYE-B for yeast: 1.9 (expected value: 2.0) and for *E. coli*: 0.3 (expected value: 0.25).

Conclusion: diaPASEF for mixed proteomes is highly sensitive, quantitative and reproducible

Poster Number: P01.08

BAC-DROP: A High-Throughput Sample Preparation Workflow for Bottom-Up Proteomics Using Dissolvable Polyacrylamide Gel Electrophoresis

Nobuaki Takemori; Ayako Takemori; Jun Ishizaki; Hitoshi Hasegawa

Ehime University, Toon, Japan

Background: SDS polyacrylamide gel electrophoresis (SDS-PAGE) is a high-resolution separation technique for complex protein samples and is commonly used for sample pretreatment in mass spectrometry-based proteomics. Proteins separated by SDS-PAGE are trapped in the gel matrix and are difficult to recover. In conventional proteomics, proteins are digested with trypsin in the gel, and the digested peptides recovered from the gel are subjected to mass spectrometry. This "in-gel" digestion involves a laborious process, and complete peptide recovery is often difficult.

Objective: To recover proteins after SDS-PAGE without loss and continuously perform proteolytic digestion in solution, we developed a novel proteomic workflow using a dissolvable, bis-acrylylcystamine (BAC)-cross-linked gel, which we named BAC-DROP (BAC-gel dissolution to digest PAGE-resolved objective proteins).

Methods: SDS-PAGE using a BAC gel (BAC-PAGE) was performed as previously reported (Takemori *et al*., Analytical Chemistry 2017). After electrophoresis, the gel was stained with CBB and gel bands containing proteins of interest were excised. An excised gel piece in 50 µL of 50 mM dithiothreitol solution was completely dissolved by shaking for 5 minutes and dissolved proteins were digested with trypsin in the solution. After protein digestion, 50 µL of acetonitrile was added to the sample and the precipitated acrylamide filaments were removed by centrifugation. The obtained peptides were purified using a Stage tip and subjected to selective reaction monitoring (SRM) with SCIEX QTRAP5500 mass spectrometer.

Results: We conducted SRM quantification of CRP in human serum using the BAC-DROP workflow. Sensitive detection of CRP was possible by separating CRP from major serum proteins using BAC-PAGE. We have demonstrated the detection of trace amounts of CRP (~0.5 mg/L) from a 0.5 microliter serum. BAC-DROP can be applied to the simultaneous processing of multiple samples, and in combination with quantitative mass spectrometry, it is expected to be a useful approach in clinical biomarker analysis.

Poster Number: P01.09

High Throughput Proteome And Phosphorpoteome Sample Processing Coupled To Fast Gradient Dia

Stoyan Stoychev; Isak Gerber; Justin Jordaan

ReSyn Biosciences, Pretoria, South Africa

This work focusses on the application of high throughput workflows for proteome and phosphoproteome profiling coupled to fast gradient liquid chromatography (LC) and data-independent mass spectrometry analysis. Automation of sample preparation increased throughput and reproducibility covering all steps from protein extract to mass spectrometry analysis allowing for parallel processing of up-to 96 samples in less than 6 hours (excluding digest time).

We show the adaptation of the sample preparation methods including protein capture, clean-up and on-bead digestion as well as phosphopeptide enrichment for use in a magnetic handling stations (Thermo Scientific King Fisher Flex) allowing for semi-automated sample processing. The methods are readily transferable to a range of liquid handling robots with magnetic handling stations (capabilities).

Efficient protein isolation from 12 mouse tissues was achieved using 5% SDS followed by aggregation-based protein capture method (PAC) on MagReSyn® Amine magnetic microparticles allowing for efficient contaminant removal followed by on-bead digestion. For phosphoproteome profiling the PAC-generated peptides were desalted and further processed using MagReSyn® Ti-IMAC HP magnetic microparticles. 500 ng peptides or enriched phosphopeptides were loaded directly on Evotips and further processed using 21 minute gradients on Evosep LC coupled to Orbitrap Exploris equipped with FAIMS source allowing for up-to 60 samples per day to be analysed. Over 8,000 proteins and 22,000 unique phospho-peptides were quantified across the 12 tissues in a total mass spectrometry time of 28 hours.

Poster Number: P01.10

Precision Medicine Recommendation For Breast Cancer Using Machine Learning Techniques

Reena Lokare; Dr. Sunita Patil

University of Mumbai, Mumbai, India

A lot of research on cancer especially breast cancer has significantly reduced the number of deaths worldwide. Next-generation sequencing (NGS) has made it possible to sequence individual genome sequences. Due to rigorous research in breast cancer, molecular classification into subtypes such as luminal A, luminal B, Triple Negative\Basal-like and HER2-enriched is possible. Gene mutation in all these subtypes of breast cancer is also discovered. The proposed work aims at personalized therapy for breast cancer. A patient's breast cancer subtype can be found from NGS sequencing. Machine learning techniques such as Deep Neural Network is proposed here. The proposed model is trained using gene-drug correlation data available in repositories. This model will predict suitable and accurate drugs for a patient. Model accuracy can be enhanced by using a Genetic algorithm. If a patient can not afford NGS, gene-specific tests are also available. The doctor can predict a subclass of breast cancer and propose gene-specific test which will reduce the cost of treatment. The proposed work can be used in cancer hospitals, research institutions.

Poster Number: P01.11

Increasing the Ease of Use of Nanoflow with Plug and Play Low Flow Source

Christie Hunter[1]; Leroi De Souza[2]; Carmai Seto[2]; Yang Kang[2]; Leigh Bedford[2]; Bradley Schneirder[2]; Arianna Jones[1]

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When performing electrospray ionization mass spectrometry, reducing chromatographic flow rates can improve sampling efficiency and therefore increase sensitivity. When operating in the nanoflow regime (50-500 nL/min) very high sampling efficiencies are possible. However, nanoflow ionization can require significant user expertise and advanced tuning to get best performance. Significant research was done to determine whether most source parameters could be optimized and locked in, to take out most of the user interactions. Using Design of Experiments, x,y,z positioning, tip protrusion, ionization voltage, nebulization gas and other parameters were examined. Spray electrodes were developed for maximal robustness. This led to the development of the OptiFlow® Source, a single source that would cover the full spectrum of low flow rates for high sensitivity LC-MS analysis.

Performance was evaluated relative to a highly tuned NanoSpray® Source III on both a TripleTOF® 6600+ system and a QTRAP® 6500+ system. Equivalent chromatographic performance was observed comparing peak width and area. 30 day testing was performed to check spray electrode robustness and minimal change in backpressure was observed. The SWATH® Acquisition Performance kit was run with both sources and very equivalent performance was again observed, within 5% in IDA and SWATH acquisition modes. Application to large sample studies will be performed next.

Poster Number: P01.12

Reversible Chemistry For Universal Protein Extraction And Cleanup Of Whole Proteins And Peptides For Fast, High Yield Proteomics Sample Preparation

Stephanie Biedka[1]; Jonathan Minden[1, 2]; Nolan Frey[2]; Amber Lucas[1]

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High quality protein extraction and cleanup during sample preparation is critical for achieving the coverage, yields, and reproducibility required for a successful proteomics experiment. Current technologies for proteomics sample preparation generally rely on precipitation or filtration-based technologies, both of which can suffer from sample loss, long processing times, incompatibility with certain buffers that aid in extraction, and limitations for automation. Recently, new sample preparation technologies have made improvements to filter- and precipitation-based sample preparation and have come closer to creating efficient protein sample preparation workflows. However, these methods still suffer from many of the aforementioned issues, which can only truly be addressed by taking a novel approach to protein sample preparation

Biochemical tags have been used for decades for preparation of single protein samples, with affinity chromatography being a fast and simple way to separate a target protein from other cellular material. Biochemical tags have not previously been used for proteomic sample preparation in a similar manner due to numerous technological challenges, such as the tags altering protein size and charge, making mass spectrometry analysis complicated or impossible. We have created a covalent, reversible chemical tag, which we refer to as ProMTag, that is used for tagging, capture, and cleanup of whole proteome samples using a complimentary resin that allows for extensive washing without sample loss. This reversible tagging method allows the convenience of affinity purification without permanent modification of the protein sample, ensuring fast, high yield, and easy to perform sample preparation that yields highly reproducible results and requires less than 30 minutes of hands on time. With this novel approach to sample preparation, this technology can be used to cleanup samples for single protein projects, whole protein gel-based proteomics workflows, or peptide mass spectrometry-based proteomics workflows without compromising yields or coverage.

Poster Number: P01.13

Deeper Proteome Coverage Of Musculoskeletal Samples

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Bottom-up proteomic analysis of skeletal muscle samples is hindered by the presence of abundant and large proteins such as Titan and Myosin which create hundreds to thousands of unique tryptic peptides. As a result, despite offline fractionation, significant MS time in data dependent acquisition (DDA)-based analysis is spent cataloging a small number highly abundant peptides rather than allow for increased depth of proteome coverage. Expanding the number of high pH basic fractions by 50% resulted in only slight changes in the total number of proteins identified. In this work, we have compared our standard global proteomics workflow to the PASEF acquisition method on the timsTOF Pro (Bruker). PASEF acquisition allows a significant increase in sequencing speed by prefractionating peptides based on their collision cross section (CCS). These studies found that peptide-spectral matches were doubled and protein identifications increased by one third using PASEF acquisition. Although these results were striking, we will continue to explore additional prefractionation methods to improve the overall depth of coverage of the musculoskeletal proteome for discovery proteomics of mouse and human skeletal muscle disease states.

Poster Number: P01.14

Spray-capillary Based Capillary Electrophoresis Mass Spectrometry Analysis of Metabolites in Live Cells

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Single-cell capillary electrophoresis mass spectrometry (CE-MS) serves as a promising platform for the analysis of cellular contents and probing cell heterogeneity. Advanced technologies such as automated microchip tools, high-quality coated separation capillary, and highly sensitive interface have boosted the performance of the single-cell CE-MS analysis. However, current single-cell CE-MS methods often rely

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on offline micro-sampling processes which can decrease the sampling precision and accuracy of the sample introduction process.

We have recently developed an electrospray-assisted device for quantitative low-volume sample extraction, referred to here as "Spray-Capillary". The electrospray formed at the MS end of the spray-capillary provides the driving force to quantitatively draw low-volume samples from the sample inlet end of the spray-capillary (*e.g.,* ~ 15 pL/s injection flow rate). In addition, the spray-capillary can directly serve as a CE capillary for online separation and MS detection with no additional devices.

In this study, we further redesigned and applied the spray-capillary to the analysis of the cellular contents of living cells. Specifically, the sample inlet end was laser-pulled into a 15 µm-diameter tapered-tip so that it can be directly inserted into living single cells (*e.g.,* single onion cells) for micro-sampling. We also implemented a micro-manipulator to control the position of the sample inlet end of the spray-capillary and used an inverted microscope to monitor the sample injection process. The single cell injection volume was estimated using a pressure-based sample elution process followed by MS detection. Furthermore, after micro-sampling, we evaluated and optimized our spray-capillary based CE-MS platform for quantitative metabolite analysis of onion cells.

Poster Number: P01.15

Quantifying Thermal Stability For Thousands Of Protein Variants In Parallel Using Multiplexed Proteomics

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Characterizing the impact of amino acid substitutions on protein structure and function remains a monumental challenge in genome analysis. In order to accelerate variant interpretation, we recently developed Miro, a novel mass spectrometry-based workflow that assesses the impact of amino acid substitutions across hundreds of proteins simultaneously by relying on mistranslation with non-canonical amino acids (ncAAs). Here, we showcase an application of Miro that couples proteome-wide mistranslation with multiplexed proteomics to quantify the impact of ncAA incorporation on protein thermal stability. Specifically, we induced proteome-wide mistranslation in Saccharomyces cerevisiae by supplementing growth media with the proline analog azetidine-2-carboxylic acid (azetidine). We then measured the impact of these proline-to-azetidine substitutions on protein thermal stability using both Thermal Proteome Profiling and Proteome Integral Solubility Alteration. In total, we quantified the impact of 1,662 proline-to-azetidine substitutions on the stability of 460 yeast proteins. Globally, azetidine substitutions tended to decrease protein thermal stability. However, these differences were subtle, with only 79 of the 1,662 substitutions significantly altering stability, most of which were destabilizing. We applied alanine scanning to validate several of these substitutions in the essential glycolytic enzyme Pgk1, and found that positions that were destabilized by azetidine were also sensitive to alanine. Taken together, these findings suggest that ncAA sensitivity is indicative of sites within proteins that may also be sensitive to natural amino acid substitutions. Ultimately, we envision Miro being a highly-generalizable method that can be coupled with a variety of amino acid substitutions, biochemical assays, and model organisms to accelerate genome interpretation across species.

Poster Number: P01.16

Affordable Automated Proteomics And Multiomics Sample Preparation

Richard Lam[1]; John Wilson[2]; John Laycock[1]

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Sample processing in omics analyses is obligatory and has traditionally been the largest source of variability. Resultantly, many biomarker "discoveries" are subsequently traced to batch or run order effects. Such errors unfortunately continue to the present day, thus compelling serious improvements in reproducibility especially in sample processing. Similarly, the growing use of omics analyses makes efficiency of sample processing ever more important. Automation can efficiently address issues of both throughput and variability, however access to automation has traditionally been limited by expense, expertise and sometimes the size requirements of the automation platform.

Here we present an affordable, accessible option for automated proteomics and multiomics sample preparation by implementing ProtiFi S-Trap and Si-Trap workflows on the Tecan Resolvex A200. Many researchers have found S-Trap sample processing to have great utility and speed[1-3], benefits extended by the Si-Trap workflow to multiomics sample processing for analysis, at a minimum, of small molecules and proteins. The Tecan A200 is an affordable, fully programmable positive pressure workstation which can process partly filled or full plates from 4 – 96 samples. It dispenses solvents such as wash, digestion and elution buffers, using proven reliable technologies to give reproducibility and processing uniformity following touchscreen activation and walkaway.

We present optimal pressure settings, flow rates, and processing times to reduce overall hands-on time and enhance analyte recovery for S- and Si-Trap workflows. Subsequently, using quantitative biomarker assays from plasma, we show excellent well-to-well, column-to-column, plate-to-plate and day-to-day reproducibility. We anticipate that the affordable Tecan A200 platform will make accessible automation and its commensurate high reproducibility available to the majority of research and clinical labs thereby helping to deploy omics tool more widely in both laboratory and clinical settings.

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[3]DOI: 10.1021/acs.jproteome.8b00891

AGING AND NEURODEGENERATIVE DISEASES P02.01 – P02.24

Poster Number: P02.01

Cellular Senescence; A Driver of The Pro-aging Side Effects of Antiretroviral Therapies and Chemotherapy in Humans

Chisaka Kuehnemann[1]; Samah Shah[1]; Abhijit Kale[1]; Nathan Basisty[1]; Christopher Wiley[1]; Birgit Schilling[1]; Judith Campisi[1, 2]

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Antiretroviral Therapy (ART) and chemotherapy has dramatically improved the prognosis of HIV-infected patients and certain cancer patients. However, these treatment regimens are associated with multiple serious side effects and signs of premature aging in humans, including lipodystrophy, osteoporosis, type 2 diabetes, cardiovascular disease, clotting and neurocognitive decline.

In HIV treatment, two drug classes are implicated in progeroid effects: nucleotide reverse transcriptase inhibitors (NRTIs), which inhibit the mammalian mitochondrial DNA polymerase gamma (PolG), resulting in mitochondrial dysfunction, and protease inhibitors (PIs), which can inhibit the mammalian protease ZMPSTE24. ZMPSTE24 processes the nuclear protein lamin A (LMNA), defects in which cause Hutchinson-Gilford progeria syndrome (HGPS). Both mitochondrial dysfunction and ZMPSTE24 inhibition induce cellular senescence, a state of permanent growth arrest and significantly altered gene expression. In cancer treatment, genotoxic chemotherapies such as doxorubicin are known to drive cells into senescence. Senescent cells accumulate with age and are thought to contribute to a multitude of aging phenotypes, primarily through the senescence-associated secretory phenotype (SASP), which includes numerous pro-inflammatory molecules.

To determine whether ART or chemotherapy promotes age-related phenotypes by inducing cellular senescence, we cultured human fibroblasts with NRTI's (tenofovir/emtricitabine), PI's (atazanavir and ritonavir), or genotoxic chemotherapy (doxorubicin). Treated cells arrested growth and expressed several markers of cellular senescence and the SASP. We tested the hypothesis that ART and chemotherapy causes the accumulation of senescent cells in vivo, which contribute to aging phenotypes through the SASP. Using unbiased mass spectrometry and data-independent acquisitions, we characterized the complement of known and unique SASP factors associated with ART and chemotherapy. These studies will help identify biomarkers for aging phenotypes linked to senescent cells and determine whether patients receiving ART or chemotherapy might benefit from senolytic drugs, which reduce senescence burden, and uncover disease processes associated with the presence of senescent cells in aging and age-related diseases.

Poster Number: P02.02

Region-Specific N-Glycome Mapping of the Human Brain in Alzheimer's Patients by nanoLC chip-Q-TOF MS Analysis

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that currently affects over five million Americans. However, the role of N-glycosylation in AD has not been thoroughly investigated. To understand the mechanism of neurodegeneration in AD, this research is focused on identifying and quantifying alterations in human brain N-glycans and their corresponding glycoproteins. In this study, we report the region-specific N-glycome map of post-mortem human brain from controls (non-AD) and AD patients using a nanoLC chip-Quadrupole Time of Flight Mass Spectrometry (nanoLC chip-Q-ToF MS) method.

Eleven brain samples were obtained from the UC Davis Alzheimer's Disease tissue bank. The N-glycans were released enzymatically from the cell membrane and analysis on a nanoLC/Chip-Q-ToF-MS. Glycan compositions were identified using MS and MS/MS data utilizing the MassHunter software with an in-house library.

The region-specific N-glycome was obtained for all brain areas analyzed. In total, more than 200 glycan compositions were detected in each brain region and variations between control (non-AD) and AD were observed. Overall, sialylated and fucosylated complex/hybrid glycans were found to be the most abundant N-glycans. The relative abundance of the sialylated and fucosylated complex/hybrid glycans were between 73% and 90% in the frontal cortex, temporal cortex, parietal cortex, and occipital cortex in both control (non-AD) and AD samples. Strikingly, in the AD brain samples, a decrease in total sialylation was observed throughout four important brain regions with relative abundances between 73% and 79%. The N-glycans accounting for this decrease include sialylated complex/hybrid glycans and fucosialylated complex/hybrid glycans with relative abundance of < 3% and <76%, respectively.

In this work, we have performed a comprehensive region-specific N-glycome and glycoproteomic profiling across different brain regions in control and AD brains. The region-specific N-glycome analysis results provide promising correlations between N-glycosylation and AD thus, shedding light into a new perspective in understanding AD.

Poster Number: P02.03

Quantifying Proteostasis: An *In Vivo* Protein Quality Assay Based On Fold Stability

Hsien-Jung Lavender Lin; Stella Park; Nathan Zuniga; John Price

Brigham Young University, Provo, UT

Aging is one of the primary risk factors of many diseases. While the symptoms of aging and its related functional decline are obvious, the biochemical mechanism is not well-defined. A well accepted explanation of aging is decline in proteostasis, a functional balance between protein synthesis, folding, degradation, localization, and modification. A kinetic model of proteostasis can describe the proteostasis change systematically. While there are assays to study many individual components of the proteostasis maintenance network, there is not yet a robust method to access the quality of protein itself at the proteome level. We are developing a protein fold assay that measures in vivo protein stability (DGfolding) via covalently modifying interior amino acid residues as the protein is denatured. By monitoring changes between states of high quality and lower quality proteostasis control we can understand how fold stability changes with quality. Here we present the application of the protein quality assay on human cardiac amyloidosis. It is a disease related to amyloid deposit in heart tissue, a result of decline in proteostasis. Transthyretin (TTR) amyloid is responsible for the disease. Its DGfolding shifts between its monomer, tetramer (functional protein), and amyloid (disease state). Different concentration of TTR stabilizer, Diflunisal, is used on control and diseased human serum to learn how protein stability of TTR and other protein react to the treatment and the protein quality control change with the disease. The result of this study validates the protein quality assay, which can be used in the Kinetic Proteostasis model for aging study. Furthermore, it establishes a baseline for future study in treatment effectiveness and early biomarker identification for cardiac amyloidosis.

Poster Number: P02.04

Improved Isolation Strategies to Increase the Yield and Purity of Human Sera Exosomes for Protein Biomarker Discovery

Sandip Kumar Patel[1]; Roland Bruderer[2]; Francesco Neri[1]; Nathan Basisty[1]; Lukas Reiter[2]; Judith Campisi[1]; Birgit Schilling[1]

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Exosomes (EVs) are small, lipid-bilayer enclosed, cell-derived nanoparticles (30 to 150 nm) that mediate various cell-cell communication. Protein, metabolite, and nucleic acid cargo (rRNA, tRNA, miRNA, lncRNA and in some cases DNA) within EVs can be stably shuttled toward target cells in a directed manner. EV production and release are tightly regulated processes, which vary between physiological and pathological conditions. A bio-pool of EVs present in plasma thus represents the physiological state of the individual and could serve as potential diagnostic and prognostic markers. However, the major challenge in the clinical application of exosome lies in the successful isolation of the exosome from the biological fluids.

In the present study, we (i) develop a robust workflow for highly pure isolation of clinical-grade exosomes and compare it with existing methods (ultracentrifugation, precipitation, filtration, chromatography, immunoaffinity-based, etc), and (ii) prepare a comprehensive 'exosome protein atlas' using combined DDA/DIA based approach. Exosomes isolated by various methods were acquired on the TripleTOF 5600 and Orbitrap Exploris 480 and analyzed with Spectronaut (Biognosys). Thus far, we successfully isolated exosome proteins from total protein (90% enrichment) using a combination of size exclusion chromatography and ultrafiltration. The proteins thus isolated were subjected to high-resolution mass-spectrometry and a total of 588 protein was reproducibly identified with 1% FDR. We are currently using different age sera (young 20-30 and old >60yr) to enhance the exosome protein coverage for our exosome protein atlas and develop preliminary EV-based biomarkers of aging in humans.

In the future, we will apply our rigorously optimized protocols to develop precision biomarkers in disease models and human cohort studies of aging and Alzheimer's disease. In the future, this work could be extended to clinical set-up and has a wide implication in diagnostics.

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Poster Number: P02.05

Tissue Specific Changes In Protein Metabolism Associated With Alzheimer's Risk

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Brigham Young University, Provo, UT

The strongest genetic risk factor for development of late onset Alzheimer's disease (AD) are polymorphisms in the lipid transport protein ApoE. Lipid transport is critical to health because in addition to energy storage, lipids comprise a wide array structural and signaling functions. Synthesis of APOE occurs in hepatocytes where it is used to export lipids throughout the body. In the brain, APOE is synthesized by astrocytes and microglia to support neuronal cells. It is not clear how modified lipid transport leads to the most recognized pathogenic marker of AD, namely protein aggregation.

Research suggests that different isoforms of APOE are linked to neurodegenerative and cardiovascular disease. For example, APOE4 has been associated with increased risk for atherosclerosis, Alzheimer's disease, and cognitive decline. However, further research is required to validate the effects of different APOE genotypes in lipid homeostasis and the careful interplay between different organs (i.e. liver and brain). We have used mass spectrometry (MS) to compare metabolic differences in APOE mouse cohorts. Our analysis of both membrane and cytosolic components have allowed us to observe how isoforms of APOE alter different components of the lipid metabolism. Using D2O tissue-labeling and state of the art LC/MS technology we compare changes in protein metabolism between APOE variants.

Poster Number: P02.06

ATF4 Promotes Skeletal Muscle Atrophy by Forming a Heterodimer with C/EBP-beta

Jacob Rose[1]; Scott Ebert[2, 3]; Steven Bullard[2, 3]; Nathan Basisty[1]; George Marcotte[2, 3]; Zachary Skopec[2, 3]; Jason Dierdorff[2, 3]; Asma Al-Zougbi[2, 3]; Kirstin Tomcheck[2]; Austin Delau[2, 3]; Jacob Rathmacher[2, 3]; Sue Bodine[2, 4]; Christopher Adams[2, 4]; Birgit Schilling[1]

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Skeletal muscle atrophy is a highly prevalent and debilitating condition that remains poorly understood at the molecular level. Previous work found that aging, fasting and immobilization promote skeletal muscle atrophy via expression of Activating Transcription Factor 4 (ATF4) in skeletal muscle fibers. However, the direct biochemical mechanism by which ATF4 promotes muscle atrophy was unknown. ATF4 is a member of the basic leucine zipper transcription factor (bZIP) superfamily. Because bZIP transcription factors are obligate dimers, and because ATF4 is unable to form highly stable homodimers, we hypothesized that ATF4 may promote muscle atrophy by heterodimerizing with a different bZIP family member.

We designed an in vivo tandem affinity purification (TAP: FLAG and S-tag) method for proteomic identification of protein-protein interactions in mouse skeletal muscle fibers, generating a stable and suitable TAP construct by eliminating the regions outside the ATF4 bZIP domain and using only the ATF4 bZIP domain as bait. We used in vivo electroporation to transfect tibialis anterior (TA) muscle fibers of 16 mice with plasmid DNA encoding the ATF4 bZIP TAP construct. In each mouse, the contralateral TA was transfected with plasmid DNA encoding the empty TAP construct.

After immuno-affinity enrichment and data-independent acquisition (DIA) workflow was performed to identify ATF4 bZIP domain interacting proteins. The MS analysis revealed, that ATF4 forms at least 5 distinct heterodimeric bZIP transcription factors in skeletal muscle fibers. However, only one of these heterodimers, composed of ATF4 and C/EBPbeta, mediates muscle atrophy. Within skeletal muscle fibers, the ATF4-C/EBPbeta heterodimer interacts with a previously unrecognized and evolutionarily conserved ATF-C/EBP composite site in exon 4 of the Gadd45a gene, encoding a critical mediator of muscle atrophy. We present a biochemical mechanism by which ATF4 induces skeletal muscle atrophy and provide new insight into the way that skeletal muscle atrophy occurs at the molecular level.

Poster Number: P02.07

Understanding Brain Aging In Non-human Primates Using High Resolution Proteomics

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Aging commonly results in a decline in normal biological functions, often leading to diseases and health complications. The specific age of an individual when biological functions start declining is variable, and dependent on various factors including genetic predisposition, diet, lifestyle, and other environmental factors. Since one of the key age-related health complications is cognitive decline, often progressing to neurodegenerative disorders like Alzheimer's and Parkinson's disease, the aim of our study was to characterize protein changes in the brain of animals at different ages.

Our study included 45 brain frontal cortex samples, consisting of 35 females in the age range of 6-23 years (22-80 human-equivalent years) and 10 males in the age range of 8-23 years (28-80 human equivalent years). Samples were homogenized in Tris buffer followed by reduction, alkylation and tryptic digestion of proteins. Resulting peptides were analyzed using a Fusion Lumos Orbitrap mass spectrometer, acquiring high resolution Orbitrap data both in MS and MS/MS with high-energy collision dissociation for fragmentation. Data analysis was performed using MetaMorpheus which has been shown to process proteomics data much faster compared to other search engines. Also, its added features like internal data calibration and G-PTM analysis improve the quality of data search resulting in improved and robust peptide identification for a large number of posttranslational modifications.

Across all samples, we obtained quantitative data for 8084 peptides corresponding to 1577 proteins. Our initial results suggest that proteins specific to energy metabolism like creatine kinase or fructose bisphosphate aldolase, are upregulated in middle-aged adult male baboons, while in younger or older baboons the levels tend to decrease. A wide range of additional proteins show age-correlated changes, highlighting the complex cellular changes in the frontal cortex as animals age. To our knowledge, this is the first detailed high-resolution proteomics study characterizing aging-related changes in healthy baboons.

Poster Number: P02.08

Global Analysis of Protein Folding Stability Changes During Parkinson's Disease in a Mouse Model

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The lack of appropriate protein biomarkers makes the early detection of Parkinson's disease (PD) challenging, and it thwarts efforts to develop drug therapies for this incurable disease. Thus, new approaches are needed for biomarker discovery and for better understanding the molecular basis of PD progression. Here, we utilize proteome-wide protein folding stability measurements to characterize the progression of PD in a mouse model of the disease in which the human α-synuclein protein with an A53T mutation was overexpressed. Using the Stability of Proteins

from Rates of Oxidation (SPROX) technique, the thermodynamic stabilities of proteins in brain tissue cell lysates from PD mice were profiled at three time points including at 1 Month (n=9), at 6 Months (n=7), and at the time (between 9 and 16 Months) a mouse became Symptomatic (n=8). The thermodynamic stability profiles generated here on over 1000 proteins were compared to the thermodynamic stability profiles generated on the same proteins from similarly aged wild-type mice. Reported here are the results of a series of comparative analyses designed to identify proteins with thermodynamic stability changes during the course of PD progression, as well as proteins with thermodynamic stability differences in age-matched PD and wild-type mice. The differentially stabilized proteins hits identified here, which represented 10-40% of the assayed proteins, have the potential to not only aid in the early diagnosis of PD, but also to contribute to a better molecular level understanding of the disease that in turn could lead to novel therapeutic strategies.

Poster Number: P02.09

Comparative N-Glycoproteomics Reveals New Molecular Players in Alzheimer's Disease

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Research advances have elucidated the neuropathological hallmarks of AD, amyloid plaques and neurofibrillary tangles, but no profound disease-modifying treatment nor reliable protein biomarkers for early detection are available in mild cognitive impairment (MCI) or AD so far. Given the controversial discovery of protein biomarkers in AD, it is pivotal to define new molecular players to aid in accurate diagnosis of this devastating disease. Protein glycosylation is one of the most common and complex post-translational modifications, playing a fundamental role in many key biological processes. Here, we developed large-scale comparative N-glycoproteomics of cerebrospinal fluid in AD to understand the site-specific microheterogeneity across the glycoproteome and how such heterogeneity and dysregulation patterns contribute to this neurodegenerative disease.

In this study, cerebrospinal fluid collected from healthy controls, patients at MCI and AD were compared individually via 12-plex N,N-dimethyl leucine (DiLeu) tagging strategy (n=48). 1519 intact N-glycopeptides mapping to 178 glycosites on 107 glycoproteins were quantified, representing the most in-depth N-glycoproteome characterization in AD to date. The intact N-glycopeptide analysis enables investigation of system-wide glycosylation patterns. More than half of the glycoproteins were characterized with only one glycosylation site, but about 70% of glycosylation sites have more than one glycan modifying them, demonstrating the high-degree of N-glycosylation microheterogeneity. Compared to age-matched control, 61 and 118 N-glycopeptides were significantly regulated in patients at the stage of MCI and AD ($p < 0.05$), respectively. Among them, 19 N-glycopeptides were dysregulated in both MCI and AD with consistent alteration trend. The majority of these aberrant N-glycopeptides were sialylated, indicating the correlation of sialylation with AD. Notably, our data revealed 25 aberrant N-glycopeptides in MCI were modified into 54 different glycoforms at the same site of a given protein in AD, termed as disease stage-specific N-glycopeptides. These aberrant glycosylation forms may participate in the transition of protein structural changes.

Poster Number: P02.10

Deamidation At N14 And N143 In γs-crystallin In Age-related Cataractous Lenses Results Predominately In Isomerization Without Racemization

John Klimek; Keith Zeintek; Ethan Makinster; Erin Marble; Kate Halverson; Kirsten Lampi; Larry David

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Deamination of crystallin proteins in human lens has been previously associated with cataract formation and protein insolubilization, and γS-crystallin is especially prone to this modification. Asparagine and preexisting aspartyl residues can undergo succinimide ring formation and subsequent hydrolysis that creates isoaspartates (β-linkages) and L to D racemizations. The purpose of this experiment was to assign the isomerization and stereochemical configurations of aspartyl residues of γS-crystallin peptides 7-18 and 131-145 in cataractous lenses. Insoluble proteins from the center (nucleus) of human cataractous lenses were trypsinized, separated using a 4 hour UPLC gradient, and analyzed using an Orbitrap Fusion Mass Spectrometer with 500,000 FWHM SIM scans to resolve deamidated and non-deamidated peptides using a 19 mDa mass difference between isotopic peaks, data-independent acquisition of fragment ions to confirm peptide identities, and electron-transfer dissociation to assign sites of isomerization. Stereochemical configurations were determined by spiking in heavy-labelled versions of synthetic peptides containing different combinations of isoaspartyl residues in L and D configurations. The major modified form of peptide 7-18 in cataractous lenses contained isomerization at both D12 and deamidated D14, with both residues remaining in L-configuration. Similarly, deamidated N143 in the major form of modified peptide 131-145 was isomerized, but also remained in L-configuration. These data suggest that isomerization alone may play a more important role in γS-crystallin insolubilization than does racemization. These results will be used to inform the semi-synthetic synthesis of γS-crystallins containing isomerized D residues so changes in protein structure and stability can be determined. These experiments aim to better understand the role of deamidation, isomerization, and racemization in age-related cataract.

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Poster Number: P02.11

Characterization Of Changes In The Insolublome With Aging And Alzheimer's Disease Models With DIA-MS

Xueshu Xie; Manish Chamoli; Dipa Bhaumik; Anwen Lin; Kathleen Dumas; Renuka Sivapatham; Suzanne Angeli; Julie Andersen; Gordon Lithgow; Birgit Schilling

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Studies have shown that protein insolubility increases with age. The proteins that become insoluble with age are enriched for lifespan determining functions. Knocking down some of the genes encoding the insoluble proteins has shown extension of lifespan in nematodes. Age-related Diseases, e.g. Alzheimer's disease (AD) are known to be associated with protein aggregation, but it is not clear how normal aging processes and age-related disease processes are related. In this study, we investigated the insolublome (1% SDS insoluble proteins) of C. elegans in aging model (young vs. old for N2 and Ju775 strains) and disease model (human Ab expressing worms vs. control). We aim to decipher the significant changes and the possible correlation of insolublome in aging and age-related disease. Our preliminary results indicate normal aging as an important driver, and relevant observations to reveal the molecular mechanism of AD pathology.

Poster Number: P02.12

Identifying Molecular Signatures Of Neurodegeneration – Integrative Proteomics And Transcriptomics Quantifies Differences Between Induced Cranial And Spinal Motor Neurons

Shuvadeep Maity[1]; Disi An[2]; Justin Randleman[2]; Dylan Iannitelli[2]; Esteban O Mazzoni[1, 2]; Christine Vogel[1, 2]

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Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurodegenerative disease without cure or understanding of the molecular basis. The disease encompasses selective, highly progressive loss of both spinal and upper motor neurons. In contrast, cranial motor neurons– which are very similar to spinal motor neurons -- are much less sensitive and survive until the late stages of the disease. Despite extensive research, the actual cause of the disease remains elusive.

We established a system to produce highly pure populations of induced cranial and spinal motor neurons (iCrMN, iSpMN) which are highly similar to primary motor neurons and show differential sensitivity to protein misfolding stress: iCrMN are more stress-resistant than iSpMN consistent with findings in primary neurons. Using this system, we quantified >8,200 mRNAs and proteins in their expression changes in response to stress in both cell lines. While the expression profiles confirmed the high similarity between the two cell lines, we identified many statistically robust, but often subtle expression differences at either or both the RNA and protein level. The affected pathways spanned the entire functions of a neuronal cell, ranging from gene expression regulation, splicing, metabolism, to cytoskeletal and synaptic differences.

In addition to these global findings, we investigated expression differences in proteolytic pathways in more detail. We identified counterintuitive roles of the core proteasome, consistent with recent findings on its localization to the neuronal plasma membrane to support synaptic function. Further, we discovered links between the core proteasome and other protein processing pathways such as endosome/lysosome formation, autophagy, and vesicular transport.

Combined, the results demonstrate that unbiased, large-scale, integrative analysis of proteomic and transcriptomic differences enables us to step outside the beaten track and identify non-canonical roles of cellular machineries that underlie subtle differences in the stress sensitivity of different cells.

Poster Number: P02.13

Proteomic Profiling of Extracellular Vesicles Isolated from Human Alzheimer's Disease Brain Tissue.

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Alzheimer's disease (AD) is the leading cause of dementia and cognitive decline in aged populations. The two most important proteins involved in AD are amyloid-beta (Aβ) precursor protein and tau protein. Extracellular vesicles (EVs) are small membranous vesicles formed by the invagination of the membrane of endosomal multivesicular bodies around cytoplasmic materials. EVs derived from microglia contribute to tau propagation as previously shown in AD mouse model. We, therefore, examined the protein composition of EVs isolated from the human brain tissue of pathologically diagnosed AD and non-demented control (CTRL) groups. EVs were isolated from the unfixed frozen cortical grey matter of AD (n=20) and CTRL subjects (n=18) using a combination of centrifugation, filtration and discontinuous sucrose gradient ultracentrifugation. Equal concentration of protein from each sample was then subjected to *in-solution* trypsin digestion, followed by nano-LC-MS/MS analysis. The data were further analyzed using the PEAKS software package for label-free quantitative analysis. A total of 949 proteins were quantified. The principal component analysis (PCA) showed a marginal separation of AD and CTRL groups. A total of 289 quantitative proteins were quantified for more than 50% patients (AD: n > 10 and CTRL: n > 9). Among these, 15 proteins were significantly up-regulated, and 3 proteins were significantly down-regulated in AD compared to CTRL. Further, differentially expressed EV proteins were subjected to machine learning tool. A panel of proteins comprising of annexin A5, neurosecretory protein VGF, neuronal membrane glycoprotein M6-A, and alpha-centractin were observed to be differentially expressed in AD EVs compared to CTRLs with 88% accuracy by machine learning. Finally, upregulation of ANXA5 in AD brain-derived EVs compared to CTRL EVs was validated by ELISA using different set of AD and CTRL samples. These data suggest ANXA5, VGF, GPM6A, and ACTZ may serve as potential biomarkers for monitoring the progression of AD.

Poster Number: P02.14

Assessing Data And System Quality In DIA Analyses Of Alzheimer's Disease Post-mortem Brain Tissue

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Quantitative proteomics is being used increasingly for the analysis of tissue samples from valuable clinical cohorts. To ensure that the data is collected of high quality, processes must be put in place to monitor chromatography, quality control the preparation of the samples, and ensure that the quantification of each "batch" meets expectations. We have designed a process to assess the quality of quantitative proteomics data collected using DIA on nearly 500 post-mortem brain tissue samples in a project to study resilience in Alzheimer's disease. Our process assumes a randomized and balanced block design to minimize systematic biases during sample preparation. To minimize and assess batch-to-batch differences, two identical samples are prepared and analyzed in each batch. These samples are: 1) a calibrant, composed of a pool that represents all conditions in the study, to adjust the peak areas to place the signal intensity on the same scale across all batches, and 2) a batch quality control of a similar sample matrix to monitor the quantitative results from the entire acquisition, analysis pipeline and quantitative calibration. Within each sample, prior to sample preparation, we add the undigested proteins and PRTC synthetic peptide mix to assess the sample preparation and provide a retention time calibration. We also use PRTC as a system suitability control to monitor the instrumentation prior to and in-between sample runs to assess the system. The retention time and peak areas of peptides from the pre-digest sample controls, post-digest sample controls and system suitability can be observed in real-time and tracked longitudinally as data is coming off the mass spectrometer using Skyline, AutoQC, and Panorama. The controls and processes we have put in place for this Alzheimer's disease project can be used as a model for the assessment of instrumentation performance, sample preparation quality, and quantitative data quality in real-time.

Poster Number: P02.15

Proteomic Profile of Metformin Treated Senescent Mesenchymal Stem Cell Secretome

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The accumulation of senescent cells and stem cell exhaustion are considered as hallmark of aging. Mesenchymal Stem Cells (MSCs) which are multipotent, adult stem cells found in variety of tissues and function during rescue and restoration of injured organs and tissues. As MSCs enter the senescence, a large amount of Senescence Associated Secretory Phenotype (SASP). molecules will be released into the micro-environment, which in turn spreads senescence. Metformin, is a prescription drug used to treat type 2 diabetes, has been shown to increase lifespan in animal models. However, the

effect of metformin on MSCs and cellular senescence hasn't been elucidated yet.

Here, we treated MSCs with 3mM, 6 mM and 9 mM of metformin for total of 35 days. We performed senescence (β-gal staining), apoptosis, cell cycle analysis, and proliferation assays at 21st, 28th and 35th days. We observed that metformin treatment reduced the percentage of senescence cells and apoptosis at 28th and 35th days compared to control. In order to understand the molecular mechanism of metformin induced interruption in senescence, we collected conditioned media at 21st and 28th days. Using label-free quantitative LC-MS/MS approach, we have compared the composition of the secretome in senescent MSCs in the presence and absence of metformin. Our preliminary results elucidated that metformin interrupt senescence of MSCs and changed the composition of SASP.

This work was supported by the The Scientific and Technological Research Council of Turkey (117S216).

Poster Number: P02.16

Deep Multilayer Brain Proteomics Identifies Molecular Networks in Alzheimer's Disease Progression

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Alzheimer's disease (AD) displays a long asymptomatic stage before progressive decline of cognition, but molecular networks underlying AD progression are not well understood. Here we present the characterization of AD stage-associated networks and deregulated signaling via profiling of 14,513 proteins and 34,173 phosphosites in human brain cortical tissue using multiplexed quantitative mass spectrometry, highlighting 173 differentially expressed proteins in 17 pathways. The altered proteins are validated in two independent cohorts (~100 cases from Banner Sun cohort and ~200 cases from Mount Sinai cohort) by additional large-scale proteomics analysis. Interestingly, the majority of these proteins are not well studied in AD context.

Proteome and transcriptome comparison shows that these altered protein levels are partially consistent with RNA abundance. Whereas almost all decreased proteins are associated with decreased RNA levels, only about half of increased proteins link to upregulated transcripts. Further comparison of brain tissue and cerebrospinal fluid proteomes in human reveals a panel of proteins as biomarker candidates.

We have also performed age-dependent omics profiling of the commonly used AD mouse model (5xFAD). In both human and mouse AD, we determine 15 Aβ-correlated proteins (MDK, NTN1, CTHRC1, NTN3, SMOC1, SFRP1, OLFML3, SLIT2, HTRA1, FLT1, SLIT3, CLU, ICAM1, LSP1, and C4B). Interestingly, the AD mouse model shows a molecular signature similar to symptomatic AD, but exhibits strong activation of autophagy and interferon response, and lacks human-specific deleterious events, such as downregulation of neurotrophic factors and synaptic proteins. Final multi-omics integration prioritizes AD-related molecules/pathways, including amyloid cascade, inflammation, complement and coagulation, WNT signaling, TGFβ/BMP signaling, lipid metabolism, iron homeostasis, and membrane transport. Immunostaining shows that some Aβ-correlated proteins are colocalized, at least partially, with amyloid plaques in both AD and the mouse model. Thus, the multilayer proteomics and systems biology approaches identify key proteins and molecular networks during AD progression.

Poster Number: P02.17

Altered Post-translational Modifications In Aged Muscle Is Associated With Reversal Of Mitochondrial Dysfunction

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Muscle redox status and post-translational modifications (PTMs) regulate diverse aspects of skeletal muscle function, including mitochondrial energetics and contractile performance. In aging skeletal muscle mitochondrial oxidative stress contributes to altered redox homeostasis. We have previously demonstrated that reducing protein-S-glutathionylation (PSG) by 8-week treatment with mitochondrial-targeted peptide elamipretide (SS-31) in aged mice was associated with improved skeletal muscle and mitochondrial function. However, the mechanisms by which improving mitochondrial function with SS-31 reverses age-related decline in skeletal muscle function remain unknown. The pronounced decrease in PSG suggests PTMs may play a key role. To investigate the effect of SS-31 on the skeletal muscle proteome we assessed global changes in protein abundance, and phosphorylation in young vs. aged and aged+SS-31 vs. aged mice. In gastrocnemius abundance of only 43 proteins were significantly altered between young and aged animals using and none of these 43 proteins were significantly different between aged and aged+SS-31 suggesting strongly that SS-31 does not alter overall protein expression in aged skeletal muscle. Phosphoproteome analysis revealed 145 sites significantly altered between young and aged animals. Gene ontology revealed the two most affected processes are on proteins that contribute to metabolism (75) and/or muscle contraction (64). Only 90 phosphorylation sites were significantly different between aged and aged+SS-31. However of those 90 sites 42 were significantly altered between young and aged animals. Further analysis revealed 20 of those 42 sites related to metabolism and 19 were related to muscle contraction suggesting that a large portion of phosphorylation sites that are altered with age and contribute to muscle contraction or metabolism are also altered by treatment with SS-31. These results suggest that altered regulation of protein function through redox sensitive PTMs rather than altered protein expression may underlie the improved skeletal muscle performance following a mitochondrial targeted treatment by SS-31.

Poster Number: P02.18

A Multiomic Study Uncovers The Interplay Between Mitochondrial Sirtuin 4 Antiviral Function And Virus Inhibition Of Host Defense

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Alterations of mitochondrial functions and cellular metabolism are hallmarks of nearly all viral infections, and connected to susceptibility to infection and pathologies of aging. As obligate intracellular parasites, viruses rely on mitochondria for the production of metabolites and energy necessary for generating new viral particles. We previously discovered that the mitochondrial enzyme sirtuin 4 (SIRT4) is a broad-spectrum antiviral factor, protecting cells against DNA and RNA viruses. Among these, SIRT4 protects against human cytomegalovirus (HCMV), a beta-herpesvirus known to globally alter cellular metabolism and strongly implicated in age-related alterations in immune function. Although initially thought to be a deacetylase and ADP-ribosyltransferase, we discovered that SIRT4 is a potent lipoamidase. SIRT4 removes the rare but essential posttranslational modification lipoylation from the pyruvate dehydrogenase complex (PDH), thereby inhibiting PDH activity and regulating carbon entry into the TCA cycle. Here, we used a multidisciplinary approach to define the mechanism and regulation of SIRT4 antiviral function during HCMV infection. Specifically, we integrate targeted mass spectrometry, posttranslational modification analyses, affinity purification-mass spectrometry, live-cell metabolic assays, microscopy, enzyme activity assays, and molecular virology. We monitored SIRT4 lipoamidase activity and the regulation of PDH lipoylation and PDH activity throughout HCMV replication.

Our results uncovered that SIRT4 functions in defense by opposing virus-induced changes in cellular metabolism. Furthermore, SIRT4 affinity purification following mitochondrial enrichment identified a previously uncharacterized viral protein, pUL13, as a novel SIRT4 interaction. Functional analyses via generation of virus strain mutants and enzymatic activity assays led us to discover that this interaction represents a mechanism acquired by HCMV to inhibit SIRT4 antiviral functions. By monitoring cellular respiration (oxygen consumption and extracellular acidification rates), we additionally demonstrated that pUL13 upregulates mitochondrial bioenergetics during infection. These findings underscore the pivotal role for mitochondria in human health and age-related disease and highlight mitochondrial processes as potential therapeutic targets.

Poster Number: P02.19

Protein Structural Accessibility Differences Associated With Alzheimer's Disease And Aging In Cerebrospinal Fluid And Brain Tissue By Limited Proteolysis-mass Spectrometry

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Aging and many neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease, are characterized by protein misfolding and aggregation. Protein aggregation occurs when misfolded proteins adopt a conformation that enables polymerization into aggregates and fibrils, but the causes of protein misfolding and structural changes in AD are not understood. We have been working on combining limited proteolysis (LiP) with state-of-the-art proteomics methods to assess quantitative changes in protein conformation in a native complex biological matrix. By using limited proteolysis on cerebrospinal fluid (CSF) and brain tissues, we can assess the protease accessibility of different protein regions and how this accessibility changes under different conditions like age or disease state. We have analyzed CSF and brain samples using a targeted proteomics assay generated towards proteins of AD importance, such as apolipoprotein E (APOE), amyloid precursor protein (APP), and chitinase-3-like protein 1 (CH3L1). For each peptide, a LiP ratio is calculated by dividing the native limited proteolysis peak area by the denatured endoproteinase LysC/trypsin control peak area. Due to this normalization, the LiP ratio is a quantitative measurement of protease accessibility -- the smaller the numerator relative to the denominator, the greater the specific peptide's accessibility in the native structure. Based on the preliminary data, a quarter of our peptides are protease accessible. As expected, different peptides within the same protein have different protease accessibilies. For a few peptides, we can see differences in protease accessibility between AD and healthy patients. Future experiments include performing the experiments on a larger number of samples and using data independent acquisition to discover changes in protease accessibility on a greater scale.

Poster Number: P02.20

Panning For Substrates Of The Inflammatory Response Kinase TBK1.

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Enormous advances in quantitative mass spectrometry and phosphoproteomic methods have enabled the identification of >100,000 unique human phosphorylation sites. Despite the continued maturation of tools, identifying functional protein kinase substrates continues to be extremely challenging. TBK1 is an innate immune response kinase that has been linked to ALS and Fronto-temporal dementia. We set out to explore its substrate landscape by examining quantitative changes in the phosphoproteome after dsDNA stimulation in WT, delTBK1, delIKKe (a related kinase with overlapping target specificity), and double knock-out cells using both label-free and multiplexed TMT methods. Even with effective activating conditions and proper negative controls, the majority of stimulated and TBK1-dependent sites initially identified lacked an expected +1 hydrophobic amino acid, suggesting they were not direct substrates. We subsequently applied strict filtering based on an in vitro determined TBK1 substrate motif and additionally scored each site in our dataset against a panel of >180 human protein kinase motifs all determined from in vitro assays using directed peptide arrays. Thus, sites were scored not only for their similarity to the TBK1 motif, but also for TBK1's selectivity relative to all other assayed kinases. After motif filtering, sites on a number of well-characterized substrates, including SQSTM1 and OPTN, as well as sites on known interactors and down-stream effectors were easily distinguished. Motif filtering enabled us to identify high-quality substrate candidates without extensive manual curation.

Poster Number: P02.21

Proteome-wide Differences In Turnover Rates Among Mammals Are Correlated To Their Lifespans And Energetic Demands

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The constitutive process of protein turnover plays a key role in maintaining cellular homeostasis. By continually degrading old proteins and synthesizing new ones, cells can maintain a healthy pool of undamaged proteins. Recent technological advances in mass spectrometry have enabled the measurement of protein turnover kinetics across the proteome. Using these methodologies, we calculated the global protein turnover rates from mammals with lifespans ranging from 4 (mouse) to 200 (bowhead whale) years. We show that long-lived species overall have slower protein turnover rates in comparison to short-lived species. As a result of reduced energetic demands from continuous rapid protein turnover, these long-lived species have reduced levels of glycolytic enzymes and electron transport chain proteins which leads to a decrease in ATP production. By decreasing ATP production, particularly by decreasing oxidative-phosphorylation, long-lived species have decreased generation of reactive oxygen species. In lieu of relying on constitutive protein turnover to clear damaged proteins, we show through a novel proteomics technique that long-lived species have a more selective method of protein degradation where they are able to more effectively clear damaged proteins than short-lived species. Through heightened selective degradation, long-lived species are able to maintain a healthy proteome while not relying on a more energetically expensive method such as rapid, constitutive turnover thus reducing the generation of reactive oxygen species over the course of their extended lifespans.

Poster Number: P02.22

A Quantitative Proteomics Strategy That Accommodates Multiple Quantitative Values For Each Protein Coding Gene: Applications In Alzheimer's Disease

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As the incidence of Alzheimer's disease continues to rise, it is imperative to have comprehensive molecular profiles to aid in early detection and intervention. Current analysis methods in bottom-up proteomics roll up peptide level measurements to a protein level, potentially obscuring peptide or proteoform differences indicative of disease etiology. Because of this, we propose an alternative to the current standard of reporting a single quantitative measurement for each protein-coding gene. We have developed a method that is not constrained to reporting a single measurement for each protein if the peptide quantities represent different results across conditions. When applied to Alzheimer's disease cohort data, we find novel differences that would have otherwise been missed if peptides were combined into a single value at the protein level.

Proteomics data was collected by data-independent acquisition as described previously by Searle et al. Nature Communications 2018. We use principal component analysis to identify protein-coding genes that have multiple peptide clusters, based on the peptide abundance trends across multiple sample conditions. The resulting eigenvalues are tested for significance, and corrected for multiple hypothesis testing. Using this analysis method we are able to detect protein-coding genes with differential peptide profiles in cohorts of Alzheimer's disease brain tissue and Parkinson's and Alzheimer's cerebrospinal fluid. In brain, we find differences in peptides spanning protein-coding genes with known differential proteoforms in Alzheimer's disease; such as amyloid precursor protein and microtubule associated protein tau. From cerebrospinal fluid samples we detect genes previously proposed as markers; such as secretogranin-2 and apolipoprotein-E variants. In addition to these previously studied genes, we find many genes with differential peptide abundances whose role in neurodegenerative disease is unknown or poorly studied. Although additional follow-up is needed to determine the cause of these differential profiles, we believe this method provides us new targets for further study.

Poster Number: P02.23

Mass Spectrometry Analysis Reveals Altered Proteins Across Cerebellar Lobules In The Niemann-pick, Type C1 Mouse Model

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Introduction: Niemann-Pick, type C1 (NPC1) disease is a fatal, autosomal recessive, lysosomal storage disorder caused by mutations in the *NPC1* gene. The biochemical defect is represented by accumulation of unesterified cholesterol and sphingolipids in the late endosomal/lysosomal. Remarkably, patterned cerebellar Purkinje neuron death is observed in a rostral to caudal manner concomitant to disease progression. While significant progress has been made to better understand NPC1, the molecular mechanisms that cause the patterned neurodegeneration in the cerebella remains unclear. In the current study, we analyzed the altered proteome across cerebellar lobules in the *Npc1* null mouse model during disease progression.

Method: Cerebellar lobules were dissected from $Npc1^{+/+}$ and $Npc1^{-/-}$ mice and analyzed via differential mass spectrometry-based proteomic analysis (i.e., ITRAQ). Protein identification and statistical analysis was performed in the Proteome Discoverer and Scaffold software, respectively. To determine proteins associated with Purkinje neuron death, a positive (0.95 to 1.0) and negative Spearman correlation (-0.95 to -1.0) analysis was performed to identify proteins in each lobule group that show either a similar or opposite trend to calbindin (known Purkinje cell marker).

Results: Analysis of the cerebellar lobules revealed alterations in numerous proteins related to lysosome function, calcium signaling and fatty acid transport, among others. Specifically, we observed significant alterations in lysosomal membrane proteins Limp2 and Lamp1. Additionally, Parv, Pcp2, Pcp4, Fabp7 and Fas were also observed to be altered. Further, many of these proteins were also found to be expressed higher in cerebellar lobule X compared to adjacent lobules (i.e., lobules VIII/IX) at a late stage of disease, suggesting that this lobule may contain a unique neuroprotective component.

Conclusion: In this study, we report alterations of multiple proteins across cerebellar lobules and disease progression in the *Npc1* mouse model. Current efforts are focused on the functional significance of these finding.

Poster Number: P02.24

Identification Of 4R-tau Splicing Factors In Progressive Supranuclear Palsy

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Background: Progressive supranuclear palsy (PSP) is a neurodegenerative disease that occurs in multiple parts of the brain. PSP and a group of other forms of neurodegeneration, including Alzheimer's disease (AD), share the common feature of fibrillar aggregates of the microtubule-associated protein Tau in the brain. These diseases are collectively termed as tauopathies, in which Tau aggregation is central to the progressive brain dysfunction and damage. Aberrant alternative splicing (AS) of Tau exon 10 leads to an imbalance of Tau splicing isoforms and is one of the major causes of Tau aggregates in several tauopathy forms. In PSP patients, post-mortem brain autopsies reveal that Tau aggregates are preferentially enriched with Tau isoforms including the exon 10 region (termed 4R-Tau as these Tau isoforms contain four microtubule-binding repeats). Despite the importance of Tau exon 10 AS in the pathogenesis of PSP, comprehensive understanding of this process in PSP is lacking.

Method: In this study, we utilized a recently published RNA antisense purification (RAP) proteomic method to systematically identify splicing factors mediating Tau exon 10 AS. This is the first time that the RAP proteomic method has been used to study alternative splicing regulatory mechanisms.

Results: Using the RAP proteomic method, we successfully captured Tau primary mRNA and identified 64 proteins (including 12 known Tau exon 10 AS regulators) that bound to Tau primary mRNA around its exon 10 region in SH-SY5Y cells. Among these proteins, hnRNPC and hnRNPA2B1 are the top candidates that support 4R-Tau expression, which we confirmed in our subsequent validation experiments. We mapped the binding region of these two proteins on Tau primary mRNA. Intriguingly, these two proteins are upregulated in post-mortem brain samples of PSP patients. With future animal model and patient studies, hnRNPC and hnRNPA2B1 may represent potential drug targets for treatments of PSP and other tauopathies.

BIOMARKERS AND TARGETED MS ASSAYS P03.02 – P03.12

Poster Number: P03.02

MitoPlex: A Targeted Multiple Reaction Monitoring Assay for Quantification of a Curated Set of Mitochondrial Proteins

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Mitochondria are the major source of cellular energy (ATP), as well as critical mediators of widespread functions such as cellular

redox balance, apoptosis, and metabolic flux. Methods to quantify mitochondrial content are limited to low throughput immunoassays, measurement of mitochondrial DNA, or relative quantification by untargeted mass spectrometry.

Here, we present a high throughput, reproducible and quantitative mass spectrometry multiple reaction monitoring based assay of 37 proteins critical to central carbon chain metabolism and overall mitochondrial function termed 'MitoPlex'. We coupled this protein multiplex with a parallel analysis of the central carbon chain metabolites (218 metabolite assay) extracted in tandem from the same sample, be it cells or tissue. In tests of its biological applicability in cells and tissues, 'MitoPlex plus metabolites' indicated profound effects of HMG-CoA Reductase inhibition (e.g., statin treatment) on mitochondria of i) differentiating C2C12 skeletal myoblasts, as well as a clear opposite trend of statins to promote mitochondrial protein expression and metabolism in heart and liver, while suppressing mitochondrial protein and ii) aspects of metabolism in the skeletal muscle obtained from C57Bl6 mice. Our results not only reveal new insights into the metabolic effect of statins in skeletal muscle, but present a new high throughput, reliable MS-based tool to study mitochondrial dynamics in both cell culture and in vivo models.

Poster Number: P03.03

Correlation Of The Plasma Δs-cys-albumin Integrity Marker With Changes In Clinical Analyte Measurements Founded On Molecular Interactions

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Human plasma/serum (P/S) are commonly used biospecimens in the biomarker discovery field. But a substantial number of P/S proteins are unstable if they are exposed to thawed conditions (> -30°C). Thawed-state exposures can result in the chemical modification of proteins and lead to apparent quantitative changes in clinically important proteins by disrupting the epitope that serves as the basis for quantification. Such modifications can constitute a major problem for biomarker discovery and validation studies utilizing P/S because they can easily go undetected and produce misleading results. Recently our group developed an intact-protein mass spectrometric assay to measure the oxidizability of albumin, termed ΔS-Cys-Albumin which facilitates the detection of P/S samples that have been exposed to thawed conditions. Hypothesizing that the oxidative damage observed in albumin reflects parallel damage to the entire specimen, a set of clinically relevant proteins were analyzed via a molecular-interaction based assay for their apparent concentration changes in P/S samples exposed to different ex vivo storage conditions including 25˚C (1 day (d)), 4˚C (2d, 90d) and -20˚C (10d, 90d, 360d), and then measured in parallel with ΔS-Cys-Albumin. Protein concentrations were measured using the Luminex Bead-based Multiplex Assay. Results indicated that 47% of the analyzed proteins in mistreated samples had statistically significant differences in concentrations compared to their respective paired controls (i.e., matched P/S aliquots kept at -80˚C). Significant protein concentration changes were observed when samples were mistreated to the point where ΔS-Cys-Albumin was below the population reference range for pristine samples. These results indicate that ΔS-Cys-Albumin can serve as an effective surrogate marker for apparent concentration changes in clinically relevant proteins that occur due to poor sample storage/handling.

Poster Number: P03.04

A Sensitive Targeted Proteomics Approach To Quantify Low Abundant Proteins Of The Unfolded Protein Response Pathway In Glioblastoma Cells

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Transcription factors and membrane receptors play important roles in many cellular events, which determine cellular fait and diverse metabolic processes at multiple levels. The detection and quantification of these proteins has been a challenge due to their low protein expression level in the cell and limitation in sensitivity of the detection technique. So far, only the use of exhaustive subcellular fractionation, multidimensional separation or the use of tags for affinity enrichment in combination with mass spectrometry (MS) allowed us the access to reliable information about the absolute quantities of low abundant proteins. To overcome the laborious workflow prior to MS analysis, we optimized a parallel reaction monitoring (PRM) targeted proteomics workflow to maximize the sensitivity and selectivity to the highest level. This workflow enables the detection and quantification of low abundant protein down to attomol range in one simple and straight forward measurement. We applied this workflow to assess the dynamics and copy numbers of the Unfolded Protein Response (UPR) key component upon Endoplasmic Reticulum (ER) stress activation in glioblastoma cells. The UPR has strong impact in progression of many different diseases and play crucial roles in cancer progression, proliferation including angiogenesis, metastasis and drug resistance. Therefore, the quantitative information of its key components including PERK, ATF6, IRE1a, Chop, XBP1, ATF3, GADD34 and ATF4 is of great clinical and prognostic importance. Utilizing our optimized PRM workflow, we could for the first time perform a quantitative analysis for all UPR proteins in a single and straight forward measurement. This allowed us to investigate the UPR signaling pathway before and after triggering the ER stress in glioblastoma cells using different chemical compounds. Our optimized PRM assay presents a potential application and strengthen the employment of MS based analysis in the clinics as a potential alternative to the conventional diagnostic tools.

Poster Number: P03.05

Delta-S-Cys-Albumin: A Lab Test that Quantifies Cumulative Exposure of Archived Human Blood Plasma and Serum Samples to Thawed Conditions

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Exposure of blood plasma/serum (P/S) to thawed conditions (> -30°C) can produce biomolecular changes that skew measurements of biomarkers within archived patient samples, potentially rendering them unfit for molecular analysis. Since freeze-thaw histories are often poorly documented, objective methods for assessing molecular fitness prior to analysis are needed.

We report a 10-µL, dilute-and-shoot, intact-protein mass spectrometric assay of albumin proteoforms called "ΔS-Cys-Albumin" that quantifies cumulative exposure of archived P/S samples to thawed conditions via a multi-reaction rate law established herein. The assay is based on the fact that the relative

abundance of S-cysteinylated (oxidized) albumin in P/S increases inexorably but to a maximum value under 100% when samples are exposed to temperatures > -30°C. The difference in the relative abundance of S-cysteinylated albumin (S-Cys-Alb) before and after an intentional incubation that drives this proteoform to its maximum level is denoted as ΔS-Cys-Albumin. ΔS-Cys-Albumin in fully expired samples is zero.

The range (with mean ± 95% CI) observed for ΔS-Cys-Albumin in fresh cardiac patient P/S (n=97) was, for plasma 12-29% (20.9 ± 0.75%), and for serum 10-24% (15.5 ± 0.64%). The multi-reaction rate law that governs S-Cys-Alb formation in P/S was determined and shown to predict the rate of formation of S-Cys-Alb in plasma and serum samples—a step that enables back-calculation of the time at which unknown P/S specimens have been exposed to the equivalent of room temperature. A blind challenge demonstrated that ΔS-Cys-Albumin can detect exposure of groups (n=6 each) of P/S samples to 23°C for 2 hrs, 4°C for 16 hrs, or -20°C for 24 hrs—and exposure of individual specimens for modestly increased times. An unplanned case study of nominally pristine serum samples collected under NIH-sponsorship demonstrated that empirical evidence—not just a paper trail—is required to ensure accurate knowledge of archived P/S biospecimen storage history.

Poster Number: P03.06

Will you still love the data tomorrow? – Futureproofing Experimental Designs for Translatable Research in Proteomics and Metabolomics

Will Thompson; Matthew Foster; Lisa St. John-Williams; Erik Soderblom; Sarah Hiles; Greg Waitt; Tricia Ho; Laura Dubois; Kendra Adams; Arthur Moseley

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Experimental expectations of the earliest stage of clinical research, so-called T0, are poorly defined. There is a lack of clarity on the experimental designs required in T0 research which will allow the datasets to be utilized in subsequent stages of translational research. While the data from individual experiments may be elegant, without the requisite experimental design and QC samples, these datasets are destined to remain isolated islands.

As part of our lab's goal to advance the use of mass spectrometry in translational research, we have sought to understand those portions of proteomic and metabolomic workflows which contribute the most to pre-/analytical variability. We have implemented design elements in proteomic and metabolomic studies which measure, and where possible control for, these sources of variability, in order to ensure the translatability of datasets across projects, cohorts and laboratories. Some of these elements include the use of study pool QC samples, reference pools, assay-specific system suitability testing, and standardizing workflows using commercial kits where possible. These elements have been incorporated into projects large and small, from our routine proteomic and metabolomics projects to the >10,000 analyses performed as part of the Accelerating Medicines Partnership-Alzheimer's Disease (AMP-AD) and Alzheimer's Disease Metabolomics Consortium (ADMC) initiatives.

This presentation will discuss translatable experimental designs, including multiple types of QC samples, which allow the definition of experimental variability associated with sample processing, LC-MS/MS and biological variability. These QC samples are generated from the cohort samples (SPQC) as well as commercially available sources such as NIST SRM1950 plasma. Use cases, design elements and observed results will be described for nontargeted clinical proteomics measurements of large cohorts of samples, as well as targeted metabolomics assays.

Poster Number: P03.07

Development Of A Targeted Lc-ms/ms Method For The Absolute Quantification Of Lipoprotein(A) In Plasma

Tomas Vaisar[1]; Noemie Clouet-Foraison[1]; Marlys L. Koschinsky[2]; Michael Boffa[3]; Mark S. Lowenthal[4]; Santica M. Marcovina[1]

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Mass spectrometry has become a method of choice for the quantification of proteins as it avoids issues associated with antigen-antibody interactions that affect immunoassay approaches. However, the suitability of LC-MS/MS as a primary reference method for absolute quantification of proteins has not been demonstrated. Here, we describe the development of such a method for the absolute quantification of lipoprotein(a) [Lp(a)], a causal risk factor for cardiovascular disease. Lp(a) is a large LDL-like lipoprotein characterized by the presence of a distinct protein termed apolipoprotein(a) [apo(a)], linked by a disulfide bond to apoB-100. The complex structural characteristics of apo(a) strongly impact measurement of Lp(a) levels resulting in highly discordant results generated by the different immunoassays used in clinical practice.

The method relies on the quantification of apo(a), a protein characterized by a genetically determined high size heterogeneity (MW 200-700 kDa) due to the expression of a variable number of repeats of its kringle IV Type 2 region. The method is based on SRM-LC-MS/MS quantification of two unique apo(a) peptides outside of the KIV-2 region using double isotope dilution with stable isotope-labelled peptides. The method is calibrated with a high purity human recombinant apo(a) expressed in HEK293 cells formed by 14 Kringle IV domains and exhibiting the same structural/functional characteristics as endogenous apo(a). The concentration of the calibrator was determined by amino acid analysis using the NIST AAA reference method ensuring the method accuracy. The LC-MS/MS method was extensively optimized and validated. The equivalence between recombinant and endogenous apo(a) in plasma was established by evaluating the parallelism of responses. Furthermore, digestion completeness, stability of the peptides, and the equivalence in quantification between different apo(a) isoform size were confirmed. The method intermediate precision showed a CV <10% and the linearity was validated from 20 nmol/L to 400 nmol/L (LLOQ 20 nmol/L).

Poster Number: P03.08

Targeted Proteomic Assays For Characterization Of Human Plasma Lipoproteins

Claudia Seidl; Zachary Flaten; Danni Li

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Current proteomic studies in the context for Alzheimer's disease (AD) focus on unfractionated plasma lipoproteins, which fail to consider the uneven distribution of proteins across plasma lipoproteins. The aim of this work was to develop a targeted mass spectrometry (MS) proteomic assay for the analysis of plasma lipoproteins isolated by anion-exchange fast protein liquid chromatography (AEX-FPLC). AEX-FPLC is a less labor-intensive approach when compared to ultracentrifugation-based lipoprotein isolation techniques. Six fractions were obtained from 50 µl of plasma by AEX-FPLC on a diethylaminoethyl column using a 36 min step-gradient elution with sodium perchlorate. Fractions 2 to 5 corresponding to plasma lipoproteins HDL, LDL, IDL and VLDL were digested for LC-MS/MS

analyses. A multiple reaction monitoring (MRM) LC-MS/MS assay was developed for measurement of 5 proteins (ApoA1, ApoB, ApoE, ApoD and ApoJ) present in plasma lipoproteins. The assay was characterized based on Experiments 1, 2 and 5 of the CPTAC guidelines. LLOQ, LOD and linear ranges were obtained using a pooled digest (of fractions 2-5) containing matched stable isotope-labeled peptides added prior to LC-MS/MS analyses. ApoB had a LLOQ (41.68 nM), LOD (33.54 nM), and was linear from 41.68 to 1667 nM; other 4 proteins had lower LLOQs of 8.35 nM, LODs between 0.53-3.11 nM, and was linear from 8.35 to 1667 nM. Intra- and inter-day assay repeatability (CV) ranged between 2.4-11.6% and 3.1-13.1%, meeting the CTPAC recommendation (<20%). Intra-and inter-day reproducibility of the entire workflow including lipoprotein isolation and digestion with respect to protein measurement in fractions 2 to 5 were 28.3%; 20.4%; 17.9%; 59.0% and 22.9%; 24.3%; 23.6% and 77.0%, respectively. In conclusion, we developed a targeted proteomic workflow for measurement of 5 proteins in isolated plasma lipoproteins with a high level of sensitivity, specificity, precision and reproducibility that can be implemented in a large cohort of samples.

Poster Number: P03.09

Developing Serum Multi-marker Panels for Diagnosing Hepatocellular Carcinoma using Multiple Reaction Monitoring-Mass Spectrometry

Injoon Yeo[1]; Jae Nyeon Kim[1]; Areum Sohn[1]; Gi-Ae Kim[2]; Young-Suk Lim[3]; Youngsoo Kim[1]

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Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. It has a poor prognosis as evidenced by being the third most common cause of cancer-related deaths. And Hepatitis B virus (HBV) and liver cirrhosis (LC) are the most relevant risk factor of HCC. Early detection of HCC amongst high-risk groups is paramount in improving prognosis. We aimed to identify and assess a serum biomarker combination that could detect the presence of clinical and preclinical hepatocellular carcinoma in high-risk patients.

A total of 300 serum samples from HBV (n=100), LC (n=100), and HCC patients (n=100) were analyzed multiple reaction monitoring-mass spectrometry (MRM-MS) to verify HCC biomarkers and develop multi-marker panels. The HCC biomarkers and multi-marker panels were validated in 140 independent samples (57 HBV, 43 LC, and 40 HCC).

By comparative analyses of HBV vs. HCC, and LC vs. HCC, and high-risk group (LC+HBV), a multi-marker panels improved the diagnostic power. The serum proteins of patients with HCC were distinct from those with HBV, LC, and high-risk group (LC+HBV) as demonstrated by the area under the receiver operating characteristic (AUROC) curves of 0.970 (accuracy=0.850), 0.940 (accuracy=0.950), and 0.970 (accuracy=0.933) in the training set and 0.851 (accuracy=0.773), 0.923 (accuracy=0.783), and 0.928 (accuracy=0.821) in the validation set, respectively. These panels showed higher accuracy than AFP (cut-off 20 ng/mL) to distinguish individuals with hepatocellular carcinoma from HBV (AUC=0.790), LC (AUC=0.737), and high-risk group (LC+HBV; AUC=0.760) in the validation set.

We have been able to identify a panel of diagnostic biomarkers of HCC by serum biomarkers. These multi-marker panels might predict the risk of HCC development in high-risk populations before clinical diagnosis, which is meaningful for the surveillance of patients with preclinical HCC.

Poster Number: P03.10

Revealing Proteomic Subgroups with Clinical Classification and Prognostic Prediction in Pancreatic Ductal Adenocarcinoma using MRM-MS

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Pancreatic ductal adenocarcinoma (PDAC) is considered as a highly immunosuppressive and heterogeneous neoplasm. Despite improved knowledge about the genetic background and the tumor microenvironment, current clinical classification of PDAC cannot predict risk of relapse or prognosis after surgical curation precisely.

MRM-MS is one of the high-throughput '-omics' technologies that can simultaneously measure the abundance of proteins on the order of hundreds using a triple quadrupole MS. It is highly selective and sensitive approach for quantifying targeted proteins in samples, and very applicable for biomarker study and screening diseases.

The resected tumours of 234-patients in the PDAC cohort [follow-up during 8Y (2010-2018)] were selected to quantify tissue proteins. We listed about two-hundreds of cancer related proteins differently expressed in resected tumours between poor prognostic group (Relapse in 10 months) and good prognostic group (No relapse or relapse after 10 months). And the 122-protein were quantified in all the samples with the level above the LOD (99.9%) and the LLOQ (97.4%) of each protein. We found the prognostic subgroups (low, intermediate and high risk) which were associated with several prognostic factors and the original transcriptomic subtypes (such as basal-like, activated stroma, or classical) using Hierarchical Clustering Analysis. Finally, we developed the Multi-class Classification model for predicting the subtype of tumours.

The proteomic subgroups and the prediction model could be used to improve the prognosis of PDAC patients giving the help to clinicians with decision for cancer therapy. Moreover, some of the proteins which were targets of the targeted therapy could offer a chance to treat cancer more effectively, but that is needed to be studied further.

Poster Number: P03.11

Quantitative, Comprehensive Multi-pathway Signaling Analysis Using An Optimized Phosphopeptide Enrichment Combined With An Internal Standard Triggered Targeted Ms Assay

Bhavin Patel[1]; Penny Jensen[1]; Aaron Gajadhar[2]; Sebastien Gallien[3]; Jae Choi[1]; Romain Huguet[2]; Graeme McAlister[2]; Shannon Eliuk[2]; Markus Kellmann[4]; Tabiwang Arrey[4]; Alexander Harder[4]; Andreas Huhmer[2]; Kay Opperman[1]; John Rogers[1]

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Introduction: There is broad interest in quantifying dynamic protein phosphorylation states in cellular signaling pathways under different conditions. Enrichment is necessary for better detection of the low abundant phosphorylated proteins, and multiplexed quantitation reagents parallelize processing across a multitude of experimental conditions. We have combined SMOAC (Sequential enrichment of Metal Oxide Affinity Chromatography), 146 AQUA™ heavy-labeled phosphopeptide standards, and SureQuant™ targeted MS to evaluate changes in phosphorylated protein abundance under different stimulation conditions. The specific phosphopeptides have been chosen to cover biologically interesting phosphosites from several different signaling pathways.

Method: HeLa/A549 cells were grown with different stimulation conditions (hIGF-1/hEFG) before in-solution digestion. One

milligram of each digest spiked with phosphopeptides standard was subjected to SMOAC analysis using the Thermo Scientific Pierce Hi-Select™ TiO2 and Fe_NTA phosphopeptide enrichment kits. Both eluents were combined before LC-MS analysis using Thermo Scientific Dionex nanoLC® system coupled to Thermo Scientific® Orbitrap Exploris® 480 or Orbitrap Eclipse® Tribrid® Mass Spectrometers. To ensure optimal measurement of each target, a novel SureQuant method was performed where real-time heavy peptide detection triggered high-sensitivity measurement of endogenous targets. Data analysis was performed with Proteome Discoverer and Skyline software.

Results: We have previously described our optimized SMOAC phosphopeptide enrichment method and we have shown with that method significant improvement in the number of phosphopeptides identified. In this study, we developed a targeted assay based upon 146 AQUA heavy-isotope phosphopeptide standards. More than 90% of heavy peptides were quantified with high sensitivity and reproducibility across different MS acquisition methods. The phosphopeptide standards spiked into stimulated HeLa/A549 cell digest, followed by enrichment using the SMOAC method, allowed quantitation of about 60 endogenous phosphopeptides and 134 heavy phosphopeptides by PRM or SureQuant method.

Conclusion This phosphopeptide standard with novel targeted MS analysis allowed quantitation of phosphorylation changes from >80 signaling pathway proteins.

Poster Number: P03.12

High Throughput Plasma Proteomics With PASEF And 4D Feature Alignment

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Body fluids provide an easy possibility to monitor in depth physiological human parameters; they are routinely collected with minimal invasion and are readily available at biobanks from thousands of clinical studies. To maximize throughput, we have optimized MS conditions, column lengths and LC overhead times to obtain runs of 28.8 min, injection to injection (50 samples/day) on the Bruker nanoElute UHPLC system. Additionally, we utilized PEAKS X+ software which aligns features in four dimensions; retention time, intensity, m/z and ion mobility to transfer identifications in a match between run design to enhance data completeness across runs. Similar results can be achieved on the Evosep One together with PASEF, as recently described.

Materials and Method: Plasma proteome (PP) tryptic digests (top 12 depleted) were kindly provided by Roman Fischer (Oxford University). Human colostrum samples (HC) were collected over the first 14 days and digested with trypsin at 1:50 ratio. 50 ng of tryptic digests from PP and HC were delivered directly to a 10cm C18 column (Bruker TEN). Data was recorded on a timsTOF Pro utilizing the PASEF acquisition method with a 0.5 sec cycle time. Data was analyzed using PEAKS X+ (Bioinformatics solution Inc.). Results were filtered to 1% FDR on the PSM level of the ID result. Protein profile plots were generated in Perseus v.1.6.0.6 (Cox group) and the proteomaps on a web-based application (www.proteomaps.net).

Results: We demonstrated that reduced LC overhead times enable efficient use of MS instrument time, particularly with a fast MSMS acquisition due to PASEF. We further demonstrated that transferring IDs between runs by rt, m/z and 1/K0 adds additional confidence and allows boosting ID numbers. Finally, we showed the feasibility of high throughput body fluid sample analysis of 50 samples/day on the Bruker nanoElute & timsTOF Pro combination.

CANCER PROTEOMICS
P04.01 – P04.17

Poster Number: P04.01

Title : Inducible Knock-down Coupled to Quantitative LC-MS/MS to Identify Novel Substrates of the Oncogenic Deubiquitinating Protein USP7/HAUSP

Ahood Aleidan[1, 2]; Paul Skipp[1]; Rob Ewing[1]

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Background The ubiquitin-specific protease USP7/HAUSP is a deubiquitinating peptidase that cleaves ubiquitin chains from its substrates and therefore rescues them from proteasomal degradation. USP7 abundance and stability plays a major role in regulating various cellular processes and USP7 regulates multiple oncogenic pathways by maintaining the stability of key oncogenic proteins. The aim of this study is to comprehensively identify USP7 targets in colorectal cancer through mapping of the USP7-associatied protein network.

Methods This study utilised multiple approaches to characterise the USP7 protein functional network in colorectal cancer cells. Inducible siRNA was used to knock-down USP7 in LS147T colorectal cancer cells and CRISPR/Cas used to knock-out USP7 in HCT116 colorectal cancer cells. Quantitative label-free liquid chromatography-dual mass spectrometry (LC-MS/MS) was performed using these models with EasySpray PepMap coupled to Thermo-Finnigan Orbitrap mass-spectrometry.

Results and Discussion USP7 knockdown significantly decreased cell viability and growth. It also decreased the abundance of known USP7 interaction partners, including DNA methyltransferase I (Dnmt1), while increasing the abundance of p53 and SUMO1. Our LC-MS/MS identified and quantified over 4800 proteins in colorectal cancer cells, and this enabled the identification of many proteins whose abundance was significantly altered in USP7 knockdown cells. Our findings open new avenues to explore USP7 as a potential target for the treatment of colorectal cancer.

Poster Number: P04.02

Endophytic Fungi in Cancer Therapy

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Abstract: An endophytic fungus was isolated from the medicinal plant, *Aerva lanata*, from Western Ghats region of India. The fungus was identified by morphology, scanning electron microscopy and molecular characterisation as *Fusarium equiseti*. The fungus was found to produce terpenoid when cultured in potato dextrose broth for 90 days. The compound was separated by column chromatography and identified by Thin Layer Chromatography. The compound was further characterised by IR, NMR and LC-MS and was finally identified to be beta amyrin, a penta terpenoid. Cytotoxicity assay, cell cycle analysis and Western blotting were taken as end points for the study. The compound was found to induce cell death in colon cancer cell line SW620. In cell cycle analysis the Sub G_0 population was very high compared to the control population indicating cell death. Western blotting results indicated that the drug was having activity against cancer cells. The compound thus ensures the possible development of anticancer drug.

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

Poster Number: P04.03

Real-time, High Density Monitoring of pTyr Signaling Targets in Human Tumors Using SureQuant Internal Standard Targeted Protein Quantitation Solution

Sally Webb[1]; Lauren Stopfer[2, 3]; Cameron Flower[2]; Aaron Gajadhar[1]; Bhavin Patel[4]; Sebastien Gallien[5]; Daniel Lopez-Ferrer[1]; Andreas Huhmer[1]; Forest White[2]

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Tyrosine phosphorylation (pTyr) plays a pivotal role in signal transduction and is commonly dysregulated in cancer. Profiling the tumor pTyr proteome may reveal therapeutic insights critical to combating disease. Existing discovery-based mass spectrometry methods to monitor pTyr networks favor broad coverage while sacrificing reproducibility, while targeted methods attempt to address reproducibility but are focused on a limited subset of sites in the entire pTyr network. To achieve high coverage, high reproducibility analysis of the network, we applied a novel, high-density global targeted approach that leverages isotopically-labeled trigger peptides to reliably quantify several hundred commonly dysregulated pTyr targets in a cohort of 30 human colorectal tumor samples.

Tumor specimens were lysed, and proteins were reduced, alkylated, and digested to peptides. A mixture of stable isotopically-labeled (SIL) peptides, corresponding to several hundred pTyr targets, was spiked into each tryptic tumor digest. The endogenous and SIL forms of pTyr-containing peptides were isolated with a 2-step immunoprecipitation and IMAC enrichment, and LC-MS analysis was performed with IonOpticks Aurora column (250mm x 0.075mm) and an EASY-nLC® 1200 coupled to an Orbitrap Exploris® 480 mass spectrometer. To ensure reproducible measurement of selected pTyr targets, a SureQuant™method adapted from the internal standard triggered parallel reaction monitoring method (IS-PRM) was performed where real-time heavy peptide detection triggered selective and sensitive measurements of endogenous pTyr targets. Data analysis was performed using Proteome Discoverer and Skyline software.

SIL-triggered targeted pTyr analysis quantified over 300 unique pTyr across patient tumor samples, revealing quantitatively distinct proteomic signatures. In some cases, pTyr profiles align with proteomic & transcriptomic molecular subtypes previously reported. However, pTyr profiling also revealed putative patient specific oncogenic driving mechanisms not captured in global proteomics. Heavy peptide triggered and guided acquisition maximizes the efficiency of targeted quantification by enhancing the detectability of targets, significantly improving measurement reproducibility across analysis.

Poster Number: P04.04

CANCELLED

Poster Number: P04.05

Characterization Of The Substrate Profile Of Tyro3 Using Phosphoproteomics

Naomi Widstrom; Laurie L. Parker

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Tyro3 is a poorly studied receptor tyrosine kinase with potential to be a target for cancer therapeutics. Tyro3 belongs to the TAM family of kinases, which are best known for resolving of immune signals and promoting wound healing in innate immune cells. However, the individual role of Tyro3 is not well understood. Tyro3 is overexpressed or ectopically expressed in a number of different cancer types, and is associated with cancer survival, migration, and chemoresistance. This suggests that Tyro3 may be a target for cancer therapeutics to block these key pathways. Additionally, expression of Tyro3 in tumor associated innate immune cells may create an immunosuppressive microenvironment, negatively effecting the efficacy of checkpoint blockade therapies. In order to study the functional role of Tyro3 in cancer as well as evaluate Tyro3 as a potential target for cancer therapeutics, we need to develop tools to study its activity. First, we aim to determine the substrate profile of Tyro3 using phosphoproteomics. In brief, we add active Tyro3 to a naturally derived peptide library, allowing the kinase to preferentially phosphorylate substrates. Using this substrate profile, we will design artificial peptide substrates to assess Tyro3 activity. We can determine the phosphorylation state of the artificial substrate, and thus the activity of the kinase, using both cellular and in vitro assays. These studies will help elucidate Tyro3's biological role, potentially providing insight into Tyro3 signaling in cancer, as well as provide a readout method for drug development and testing.

Poster Number: P04.06

Proteomic Analysis Of Paired Primary And Recurrent Ovarian Cancer Tumors Identifies Biomarkers Of Ovarian Cancer And Disease Recurrence

Kruttika Dabke[1]; Simon Coetzee[1]; Nicole Gull[1]; Alberto Reyes[1]; Jasmine Plummer[1]; Brian Davis[1]; Stephanie Chen[1]; John Govindavari[1]; Jenny Lester[1]; Benjamin Berman[2]; Beth Karlan[3]; Simon Gayther[1]; Sarah Parker[1]; Michelle Jones[1]

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High grade serous ovarian cancer (HGSOC) is the most common form of epithelial ovarian cancer, representing ~70% of cases. While patients initially respond well to platinum-taxane combination chemotherapy, up to 80% relapse and most experience chemotherapy resistance. There are no effective population-level screening methods or biomarkers for ovarian cancer, which significantly contributes to the late stage of diagnosis that occurs in the majority of patients. To answer pertinent questions in this field, we have performed proteomic analysis by using label-free data-independent acquisition mass spectrometry (DIA-MS) in matched chemotherapy-naive and resistant tumors from 12 women with HGSOC and germline mutations in BRCA1/BRCA2 under the CSMC Precision Health Initiative. We hypothesize that unique protein expression signatures can identify biomarkers for early detection and identify pathways that confer chemoresistance in ovarian cancer. Our analysis quantified a total of 2686 proteins in both primary and recurrent tumors. Further, we identified 166 differentially expressed proteins between primary and recurrent tumors; 89 and 77 were overexpressed in the primary and recurrent tumors respectively. The top three differentially expressed proteins; Nuclear factor 1 B-type (NFIB) and Vesicle-associated membrane protein 7 (VAMP7) are upregulated in primary tumors, and Carbamoyl-phosphate synthase mitochondrial (CPSM) is upregulated in recurrent tumors. Dimensional reduction analysis revealed heterogeneity among tumors of individual patients, with no evidence for clustering based on tumor site, patient ID, time to recurrence or BRCA 1/2 germline mutation frequency. Additionally, we identified the protein Thioredoxin, which acts in combination with FOXO1, as a potential driver of chemoresistance in half of the cohort. With our ongoing analysis, we hope to answer multiple biological and clinical questions, as matched serum is available (pre-cancer diagnosis, post-cancer diagnosis, pre-recurrence diagnosis) and are extensively annotated with clinical data from the Women's Cancer Program (WCP) Biorepository.

Poster Number: P04.07

A Data Independent Acquisition Workflow Enables The Profiling Of Thousands Of Human Cancer Tissues For Precision Oncology

Jakob Vowinckel[1]; Karel Novy[1]; Thomas Corwin[2]; Tobias Treiber[1]; Roland Bruderer[1]; Lukas Reiter[1]; Eike-Christin von Leitner[2]; Oliver Rinner[1]; Claudia Escher[1]

[1]Biognosys AG, Schlieren, Switzerland; [2]Indivumed GmbH, Hamburg, Germany

Precision oncology requires deep understanding of molecular mechanisms involved in cancer biology. The combined analysis of several types of omics data will generate knowledge that goes beyond purely genetic approaches on which precision medicine has relied on almost exclusively in the past. With the rise of data-independent acquisition (DIA) mass spectrometry and advances in chromatography, proteomics technology has come into close proximity of the scale and depth of next-generation sequencing required for multi-omics efforts.

Here we present a platform for the acquisition of true large-scale proteomics and phospho-proteomics datasets for a unique collection of biospecimens derived from the IndivuType cohort of Indivumed, Germany. Matching fresh frozen tumor and adjacent normal tissue samples from thousands of patients were obtained from Indivumed's global network of partner hospitals.These samples are of outstanding high quality as they are collected following strictly defined standard operating procedures minimizing variation from pre-analytical variables.

Sample processing from 5 mg of tissue was performed on 96-well plates with the help of a liquid handling platform. Phospho-peptide enrichment was carried out using a Kingfisher Flex device and MagReSyn Ti-IMAC magnetic beads. DIA LC-MS/MS was performed on multiple platforms each consisting of a Thermo Scientific Q Exactive HF-X mass spectrometer coupled to a Waters M-Class LC. Chromatography was operating at 5 µL/min, and separation achieved using 45 min (total proteome) and 60 min (phospho-proteome) gradients. Several thousand samples were analyzed using the platform to date. Quality of raw data is continuously validated using directDIA searching and runs passing QC thresholds are analyzed using Spectronaut software with comprehensive tissue-specific spectral libraries. The resulting high quality proteomics data containing more than 9'000 proteins (6'000 proteins per run) and 20'000 phospho-peptides (14'000 phospho-peptides per run) is integrated into Indivumed's IndivuType multi-omics database, supporting identification and validation of new molecular cancer drug targets and biomarkers.

Poster Number: P04.08

Profiling Of Non-small Cell Lung Cancer Patient-derived Xenografts Reveals Proteome And Phosphoproteome Remodeling Associated With Patient Outcome

Shideh Mirhadi[1, 2]; Jiefei Tong[2]; Jessica Weiss[3]; Quan Li[3]; Nhu-An Pham[3]; Nadeem Moghal[3]; Ming-Sound Tsao[3]; Michael Moran[1]

[1]University of Toronto, Toronto, Canada; [2]Hospital for Sick Children, Toronto, Canada; [3]Princess Margaret Hospital, Toronto, Canada

Lung cancer is the most frequently diagnosed cancer and the leading cause of cancer-related death worldwide. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer and is predominantly composed of two histological types: adenocarcinoma and squamous cell carcinoma. Patient derived xenograft (PDXs) are recognized as the preferred preclinical model because they retain the histology and mutational landscape of their corresponding primary tumors. Additionally, both positive and negative drug response outcomes in patients have been replicated in cognate PDX models. We employed Tandem mass tagged (TMT) based labeling to quantitatively measure the proteome and a two step pY enrichment strategy to measure the phospho(Tyr)-proteome of 131 NSCLC PDX tumors. By leveraging the cross-species nature of tumor and stroma tissue, this study took a novel approach to stratify NSCLC according to both tumor and stromal proteotypes.Tumor proteome profiling resolved the known major histological subtypes and revealed 3 proteome subtypes among adenocarcinoma and 2 in squamous cell carcinoma that were associated with distinct protein-phosphotyrosine signatures and patient survival. Interrogation of these subtypes at proteome and phospho(Tyr) level led to identification of active pathways and promising therapeutic targets. Stromal proteomes were similar between histological subtypes, but two of the identified ADC proteotypes had distinctive stromal proteomes, which suggests the unique molecular signaling of these subtypes specifies tailored stromal microenvironments. Proteotypes comprise tumor and stromal signatures of targetable biological pathways suggesting that patient stratification by proteome profiling may be an actionable approach to precisely diagnose and treat cancer.

Poster Number: P04.09

Discovery and Multi-center Verification of Prostate Cancer Protein Biomarkers using Single-shot Short Gradient Microflow SWATH Acquisition and MRM^{HR} Workflow

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

Christie Hunter[1]; Rui Sun[2]; Chen Chen[3]; Ruedi Aebersold[4]; Tiannan Guo[2]

[1]SCIEX, Redwood City, CA; [2]Westlake University, Hangzhou, China; [3]SCIEX, Shanghai, China; [4]ETH Zurich, Zurich, Switzerland

<p align="left">Discovery and verification of protein biomarkers in biologically derived samples using mass spectrometry is inherently challenging and resource consuming. Here, we developed a 15 min microflow LC approach coupled with SWATH® acquisition and applied it to a quantitative proteomics study of prostate cancer cohorts. Differentially expressed proteins were then further verified using the MRM^{HR} workflow and tissue microarray in additional multi-center cohorts.

<p align="left">Formalin fixed, paraffin embedded (FFPE) tissue-biopsy samples from a prostate cancer cohort PCZA (n=68) were processed in triplicates using pressure cycling technology (PCT), followed by microflow LC SWATH Acquisition analysis with different gradients and window schemes. Potential biomarker candidates were prioritized using random forest analysis and evaluated by Receiver Operating Curve analysis. Selected proteins were verified by MRM^{HR} workflow using the same 15 min microflow LC on a second prostate cancer cohort PCZB (n=54). Potential biomarkers were further verified using TMA on a third cohort PCZD (n=100).

<p align="left">Results from the fast microflow SWATH acquisition were compared to previous SWATH data collected using a more conventional 120 min gradient in PCZA cohort. The two datasets provided quantification on 3800 proteins in common with high quantitative correlation (r = 0.77). MRM^{HR} verification of 140 prioritized proteins showed high quantitative consistency with the 15min SWATH data (r = 0.89). Separation of benign and malignant tissues achieved precision (AUC = 0.99). We demonstrated the practicality and effectiveness of fast microflow SWATH acquisition for biomarker discovery and the use of MRM^{HR} for verification in multi-center clinical studies.

Poster Number: P04.10

Proteomic and Genetic Interaction Mapping Reveals New Ras Pathway Effectors and Regulators

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The failure of targeted therapies for Ras-mutant tumors underscores our incomplete knowledge of Ras effector pathways and demands a better catalogue and characterization of pathways upstream and downstream of Ras. To that end, we used tandem affinity purification of KRas, HRas and NRas and twelve interactors with known regulatory or effector roles to generate a high-confidence protein-protein interaction (PPI) network. Interactions were cross-referenced with public PPI, genetic susceptibility, and patient data to assemble a PPI map (360 proteins with 1000 physical interactions among them) annotated with important functional determinants. This PPI map was used to design a library of 1000 sgRNAs covering 120 genes highly relevant to the Ras pathway. This library was screened for pairwise genetic effects in a dual sgRNA vector system to identify quantitative changes in the growth of the A549 and H23 lung cancer (NSCLC) lines. The combined GI/PPI network points to many compelling hypotheses, and our new discoveries to exemplify the power of the approach. We find that Kras and Nras, but not Hras, interacts strongly with the cell adhesion regulator Radil, presenting a new avenue for Ras to regulate cell motility and adhesion. We identify RIN1 as a major GEF-family effector involved in KRas upregulation of macropinocytosis. We demonstrate Ras interaction with Raf and RalGEF family members is dramatically different for Ras paralogs. We find over 250 synthetic lethal interactions, including a potent Ras-dependent interaction between the GTPase chaperone Rap1GDS1 and the GTPase RHOA, which presents a possible therapeutic target. Components of the Raf-MAP kinase cascade also show some of the strongest synthetic lethal interactions both within the pathway and with other pathways. The surprising density of physical and genetic interactions underscores the value of systematic analysis in defining complex molecular signatures.

Poster Number: P04.11

Extending Nano-surface and Molecular-orientation Limited Proteolysis (nSMOL)-based Antibody Detection to High Throughput for Rapid Patient Screening

John Cha[1]; Yoshinobu Koguchi[1]; Noriko Iwamoto[2]; Hong-Ming Hu[1]; Bernard Fox[1]; Eric Tran[1]; William Redmond[1]; Takashi Shimada[2]; Brian Piening[1]

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Immune checkpoint inhibitor (ICI) monoclonal antibody therapies have recently transformed cancer care and have become standard of care treatments for a variety of tumor contexts. Despite this, there is significant variability in individual responses to these therapies, with one potential factor being variability in pharmacokinetics of the drug. One promising method for the sensitive detection of ICIs in biological samples is nano-surface and molecular-orientation limited proteolysis (nSMOL), which involves the use of resin beads containing IgG-containing nanowells for binding ICI antibodies for the selective cleavage of the Fab region for detection by SRM-MS. Here, we adapted and miniaturized this approach to a high-throughput plate-based format in order to better monitor ICI persistence in blood and tumor tissues in rapid fashion across large cohorts of patient samples. We show that these modifications do not result in the loss of sensitivity of ICI detection in complex biological specimens and exhibits significant increases in detection sensitivity and specificity over prior gold-standard ELISA-based assays.

Poster Number: P04.12

Proteomic Analysis Reveals TNF-α Derived Different Regulation Of Mitochondrial Complexes In ER/PR Positive And Negative Breast Cancer Cells

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Tumor heterogeneity is a major concern about targeted therapy of solid tumors. Especially, breast cancer could be classified as hormone responsive (ER/PR positive) or non-responsive (ER/PR negative), and they have distinctly different characteristics, primary or metastatic. Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine that induces both anti-tumor and pro-metastatic progression depending on the types of breast cancers. TNF-α is strongly found high in the tumor microenvironment, and it undergoes dynamic change for breast cancer heterogeneity, while it alters to cell metabolic reprogamming. However, differential role of TNF-α in regulation of ER/PR positive and negative breast cancer metabolism is still unknown. Here, we investigated TNF-α modulated mitochondrial proteome using mass spectrometry analysis and identified DEPs (differentially expressed proteins) of two different breast cancer cell lines, MCF-7 (ER/PR positive) and MDA-MB231 (ER/PR negative). After isolating mitochondria proteomes and in-gel tryptic digestion, LC-MS/MS results of MCF-7 and MDA-MB231 with or without TNF-α treatment were searched against the UniProt human database. We identified 1,077 and 1,150 mitochondrial proteins (Peptide

probability >95%; Protein probability >99%), respectively, which were categorized in mitcochondria according to the MitoCarta and Gene Ontology cellular component analysis. Among them, 108 and 111 DEPs were identified in each cell lines with TNF-α treatment, and it showed that the alteration of critical proteins of mitochondrial respiratory chain complexes in MDA-MB231 as compared to MCF-7 in presence of TNF-α, which correlated with differential assembly of mitochondrial ETC (Electron transport chain) complexes. Additionally, we performed metobolomic analysis at the same conditions, and obserbved TNF-α induced accumulation of metabolites which is important for anaplerotic pathways supporting growth and migration of MDA-MB231. Collectively, our study suggests that mitochondrial metabolic reprogramming differs in two breast cancer cell lines depending on the presence of ER/PR, the assembly and activity of mitochondria ETC complex altered in TNF-α treated condition.

Poster Number: P04.13

Proteomic Characterisation Of Small Cell Lung Cancer Molecular Subtypes

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Small-cell lung cancer (SCLC), a highly aggressive tumor accounting for approximately 15% of lung cancers, is biologically and clinically very different from other lung cancers. SCLC is characterized by rapid growth, high vascularity, genomic instability, early metastatic dissemination, almost universal inactivation of TP53and RB1 genes and frequent disruption of several characteristic signaling networks (1, 2). SCLC, when diagnosed, is usually widely metastatic and initially responds to cytotoxic therapy but relapses with resistance to further therapies.

Recent studies on primary human tumors, patient-derived xenografts, cancer cell lines and genetically engineered mouse models suggest a new model of SCLC subtypes defined by differential expression of four key transcription regulators: achaete-scute homologue 1 (ASCL1), neurogenic differentiation factor 1 (NeuroD1), yes-associated protein 1 (YAP1) and POU class 2 homeobox 3 (POU2F3)(3). These subtypes exhibit different drug sensitivities.

To further characterize the aforementioned four molecular SCLC subtypes at the proteome level,19 SCLC cell lines, including their secretome, were subjected to in-depth proteomic analysis.In addition, viability assays confirmed the differential drug sensitivity of these cell lines. We investigated the mechanisms and molecular background underlying the differences between subtypes utilizing integrative bioinformatic analyses.

We believe that our study will help to gain a deeper understanding of the oncogenesis of SCLC, the resistance mechanisms and, moreover, to identify markers that may guide treatment decisions to improve treatment efficacy in patients with SCLC.

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Poster Number: P04.14

Kinome-centric Pharmacoproteomics Identifies Signaling Pathways Underlying Cellular Responses To Targeted Cancer Drugs

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Despite the recent successes of kinase-targeted drugs in cancer chemotherapy it remains challenging to determine which kinases control the progression of many cancers – particularly in the absence of dominant driver genes and mutations. Furthermore, many cancers develop resistance to kinase-targeted drugs despite being initially responsive. Therefore, we need novel methods identifying reliable biomarkers that predict drug responses and new targets to overcome drug resistance. Genomics and transcriptomics can identify gene mutations and aberrant gene expression, yet they cannot measure the protein phosphorylation that regulates kinase-dependent signaling network activity. Proteomics measures protein expression and phosphorylation and, therefore, quantifies aberrant signaling network activity directly. We developed a kinome-centric pharmacoproteomics platform to study signaling pathways that determine cancer drug response. Using hepatocellular carcinoma (HCC) as our model, we determined kinome activity with kinobead/LC-MS profiling, and screened 299 kinase inhibitors for growth inhibition. Integrating kinome activity with drug responses, we obtained a comprehensive database of predictive biomarkers, and kinase targets that promote drug sensitivity and resistance. Our dataset specified pathway-based biomarkers for the clinical HCC drugs sorafenib, regorafenib and lenvatinib, and we found these biomarkers enriched in human HCC specimens. Strikingly, our database also revealed signaling pathways that promote HCC cell epithelial-mesenchymal transition (EMT) and drug resistance, and that NUAK1 and NUAK2 regulate these pathways. Inhibition of these kinases reversed the EMT and sensitized HCC cells to kinase inhibition. These results demonstrate that our kinome pharmacoproteomics platform discovers both predictive biomarkers for personalized oncology and novel cancer drug targets.

Poster Number: P04.15

Characterizations Of Acute Leukemia, Myeloid And Lymphoid, Proteomes Using Proteogenomics And Openprot Database To Identify New Proteins And Possible Biomarkers

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The proteome is deeper than expected, and we are still discovering it. Studies have shown evidence of proteins from alternative open reading frames located in untranslated regions or overlapping canonical sequences. We created the OpenProt database to explore this unknown proteome by mass spectrometry (MS). Those new proteins, here referred as alternative proteins (AltProts), are predicted from the 3 frames of all transcripts and have a minimum size of 30 amino acids (AA). We aim to systematize the discovery of AltProts in order to fill the gaps in the currently annotated proteome.

Recently, the rise of proteogenomic studies allowed a new way to investigate the proteome of individuals by generating personalized databases using RNAseq. The whole proteome (WP) of patients can therefore be constructed with the combination of currently annotated proteins (RefProt) with alternative proteins (AltProt) discovered by proteogenomic approaches.

We created personalized protein databases from predicted annotations with OpenProt using transcriptomic data of 38 patients

with acute leukemia, myeloid or lymphoid. The WP of each patient was then characterized by MS.

Altprots are considerably smaller than RefProts (median length of 48 AA) which introduces challenges in their detection. In order to focus on lower molecular weight proteins we used fragmentation by SDS-Page prior to LC-MS/MS. To correct for the increase of false positives due to increased large search space, we used a stringent false discovery rate. We used a peptide centric approach ,pepQuery, to ensure that spectra matching AltProts could not be better assigned to a RefProt with any combination of post translational modifications. Preliminary results show 61 AltProts expressed in AML patients with 21 present in all patients. Four AltProts are encoded in genes known to play a role in Leukemia, including LMO1.

Poster Number: P04.16

Proteomic Profiling Of Triple-negative Breast Cancer Tumors And Cell Lines

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Triple-negative breast cancer (TNBC) is clinically defined by the absence of estrogen receptor (ER) and progesterone receptor (PR) expression and human epidermal growth factor receptor 2 (HER2) overexpression. TNBC accounts for approximately 15% of all breast cancers in the U.S. and is considered to be more aggressive than the other breast cancer types, with lower 5-year survival rates and higher rates of recurrence and metastasis. The poor prognosis and clinical outcomes are attributed to severe heterogeneity and lack of common molecular markers to target within TNBC, which limits patients to chemotherapy as the standard-of-care treatment option.

In the present study, we employ a mass spectrometry-based, quantitative proteomics approach to profile formalin-fixed and paraffin-embedded (FFPE) TNBC patient tumor specimens collected at the Norris Cotton Cancer Center at the Geisel School of Medicine at Dartmouth. To further supplement these profiles, we performed transcriptomic, immunohistochemical and copy number variation analyses to identify novel characteristics of the TNBC tumors. Unsupervised hierarchical clustering of gene transcripts and protein abundances across tumor samples was performed to identify subsets of tumors with common targetable pathway alterations. The correlation between gene expression and protein abundance will be determined to pinpoint the origin of alterations. In combination, we are profiling the proteomes of a broad panel of cell lines that span various TNBC subtypes to evaluate their validity to function as models that recapitulate the aberrant signaling found in the patient tumors. Thus, identifying appropriate representative cell lines to enable drug-sensitivity assays and functional follow-up studies based on our findings. Ultimately, the goal is to utilize the proteomic profiling of the FFPE TNBC patient tumors to gain insight into novel oncogenic pathways and use the appropriate representative cell lines to determine mechanisms that drive specific drug response to current therapies used in the clinic and in trials today.

Poster Number: P04.17

Investigation of Targetable Biomarkers for Non-small-cell Lung Carcinoma (NSCLC) in Human Blood Plasma

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Non-small-cell lung cancer (NSCLC) constitutes the majority of all diagnosed cases of lung cancer. Regardless of recent therapeutically advances, NSCLC still presents higher mortality when compared with other cancer types. From a clinical perspective, early detection is the key factor lowering its mortality rate; therefore, the identification of markers involved in the molecular mechanisms underlying genesis and progression of NSCLC is crucial. For that reason, in the present pilot study, plasma samples were obtained from 18 NSCLC patients (adenocarcinoma stages I and IV) and from 9 controls (e.g. benign lung diseases). The magnetic beads-based SP3 protocol was here adapted and applied to plasma preparation in order to facilitated speed and throughput of our analysis. Therefore, only 20 µg (~2µl) of total protein was necessary for profiling the proteome of NSCLC patients and controls. Subsequently, SP3 recovered peptides were labeled with TMT 9-plex and individual samples were mixed according to groups. Samples were further off-line fractionated (high-pH reverse phase chromatography), analyzed on a Q Exactive HF-X and a data analysis tool was custom-built to process MaxQuant output. Thus, quantitative information was extracted on both MS1 and MS2 levels, making it possible to access (i) major differences among study groups (LFQ-like comparison) and (ii) particular individual variations among individuals patients (TMT ratios). In summary, we here present the application of the SP3 protocol to blood plasma preparation as well as the novel utilization of two levels of quantitative information extracted from a single MS run. By integrating different quantitative approaches, we expect to uncover and establishment proteomic-based biomarkers for NSCLC in human blood plasma.

CASCADIA PROTEOMICS SYMPOSIUM P05.01 – P05.05

Poster Number: P05.01

Strategies For Correctly Controlling The False Discovery Rate When A Subset Of Peptides In A Sample Is Relevant

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In a standard database search, spectra are searched against a database of target and decoy peptides, and target peptide-spectrum matches (PSMs) compete against decoy PSMs to yield a false discovery rate (FDR) estimate. Typically, spectra are searched against a database containing all peptides reasonably expected to be found in the sample, and FDR is estimated on the full set of PSMs. This approach implicitly assumes that all peptides found in the target database are relevant.

We investigate six strategies for analyzing spectra when relevant peptides comprise a subset of peptides in the sample. In the "all-all" strategy, all peptides are searched and all PSMs are assessed. In "all-sub," all peptides are searched but only relevant PSMs are assessed. In "sub-sub," relevant peptides are searched and only relevant PSMs are assessed. In "cascade search," spectra are first searched against irrelevant peptides and then searched against relevant peptides. In "group FDR," spectra are searched against both relevant and irrelevant peptides, but FDR is calculated separately on relevant PSMs. Finally, in "group-neighbor FDR," spectra are searched against both relevant and neighbor peptides, but FDR is calculated separately on relevant PSMs. Here, "neighbor peptides" are irrelevant peptides that match the precursor mass of a relevant peptide and share a similar fragmentation pattern as the relevant peptide.

Our results indicate that out of the six methods, all-all and all-sub do not properly control FDR. Among the remaining methods, a power analysis shows no clear winner and indicates that the best FDR calculation method may be context dependent.

Poster Number: P05.02

Boosting Statistical Power In Small-scale Experiments With Percolator

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The confident assignment of peptide sequences to tandem mass spectra is critical to the success of any proteomics experiment. This assignment task is most often performed using a database search algorithm, yielding a set of peptide-spectrum matches (PSMs). Machine learning methods, such as Percolator and PeptideProphet, have proven invaluable for increasing the sensitivity of peptide detection and providing reliable confidence estimates from database search results. Modern tools often use semi-supervised algorithms to learn models directly from the experiments that they analyze. Although these methods have proven effective for many proteomics experiments, we suspected that they may be suboptimal for experiments of smaller scale; hence, we investigated how the performance of Percolator changed when used to analyze experiments of decreasing size. We found that the statistical power normally gained from Percolator was reduced and the variability of confidence estimates increased when few confident or total PSMs were analyzed. As an alternative, we propose a more traditional approach for using Percolator: learn a model from a large dataset and use the learned model to evaluate the small-scale experiment. We refer to this method as a "static modeling" approach, in contrast to the "dynamic modeling" approach normally used by Percolator, in which a model is learned anew for each experiment. We evaluated the static modeling approach in two example settings where relatively few PSMs are expected: small, gel-based experiments and single-cell proteomics. In both cases, we found that static models increased the yield of PSMs and peptides when compared to the dynamic modeling approach. Additionally, the use of static models eliminated model-induced variability across experiments, resulting in greater consistency in the discovered peptides. Based on these results, we present static models as a powerful tool for bringing the full benefits of Percolator to small-scale experiments.

Poster Number: P05.03

N-terminomics Of Lymph Nodes During Active Immune Responses Identifies Multiple Cleavage Events

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Lymph nodes are immune organs that specialize in collecting antigens at peripheral sites in order to activate specific immune effector cells. During every immune response, the lymph nodes expand its size several times while undergoing major morphological changes to ensure the recruitment, activation, and proliferation of antigen-specific immune cells. In this work, we study the contribution of proteolytic processing to the remodeling of mice lymph nodes. We apply N-terminal amine isotopic labeling of substrates (TAILS) to identify protease substrates on a proteome-wide scale. Skin-draining lymph nodes from mice immunized subcutaneously with Ovalbumin in complete Freund's adjuvant were collected at different time points and N-terminal peptides were enriched from whole lymph node lysates by TAILS. LC-MS was performed in a nanoLC system coupled to a Q-TOF Impact II mass spectrometer. We identify over 3000 proteins and 1000 cleavage sites, many of which are reported for the first time. Identified substrates are involved in regulation of the immune response, platelet activation and hemostasis. We found cleavages on 53 extracellular matrix proteins e.g. collagens, laminin, decorin, which reflects the active lymph node stromal reorganization. Bioinformatics interrogation of the cleavage data using TopFinder (http://clipserve.clip.ubc.ca/topfind/ntermini) suggests a predominant role for matrix metalloproteinases, cathepsins and Granzyme B. These results highlight the role of proteolysis as a regulatory mechanism in the immune system and shed light on protease-substrate interactions that may be critical for eliciting adequate immune responses. Ongoing work is directed to understand the biological mechanisms by which the identified cleaved substrates impact the quality and strength of the immune response.

Poster Number: P05.04

New Functionality For The Trans-proteomic Pipeline: Tools For The Analysis Of Proteomics Data

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High throughput LC-MS/MS is capable of simultaneously identifying and quantifying thousands of proteins in a complex sample; however, consistent and objective analysis of large datasets is challenging and time-consuming. Over the past seventeen years, we have continually developed and provided improvements to the Trans-Proteomic Pipeline (TPP), an open source suite of tools that facilitates and standardizes such analysis. The TPP includes software tools for MS data representation, MS data visualization, peptide identification and validation, protein identification, quantification, and annotation, data storage and mining, all with biological inference. We present an overview of the TPP and describe newly available functionality.

We introduce several new features in version 6.0 of TPP. We have added new tools that extend the utility of the software suite: mzTrace plots extracted ion currents; DidIScanThat returns the set of MS2 spectra in a given run within a precursor mass window of interest. We have also added support for Ion Mobility data, including FAIMS and timsTOF; isobaric quantitation of up to 18 channels, including TMT-16. We also expanded support for variant and unexpected modification searches via the new PEFF format, as well as for search results from de novo algorithms. Updated versions of Comet, Kojak, MSToolkit, Lorikeet, and ProteoWizard are included. Ongoing bug fixes and improvements to the user interfaces have also been made.

The TPP is a collection of more than 30 tools that can be strung together as a pipeline or run individually as needed. The unique fully open source architecture enables other researchers to build and incorporate additional tools to enhance the functionality of the TPP. More information and downloads are available from www.tppms.org.

Poster Number: P05.05

CANCELLED

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

CELLULAR SIGNALING
P06.01 – P06.02

Poster Number: P06.01

LPS-Tolerance Induces Global Changes in the Macrophage Secretome

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While the prolific production of protective cytokines, chemokines, and interferons in response to pathogen exposure is required for host survival, the overproduction of these toxic molecules in response to repeated stimuli can be fatal. In the TLR4 pathway, the process of LPS-tolerance (endotoxin tolerance) inhibits the response to subsequent stimulations following an initial substantial exposure. While LPS-tolerance is characterized by decreased secretion of immunomodulating proteins, sepsis is characterized by increased secretion of these toxic molecules and fatal in approaching 50% of cases. Examination of LPS-tolerance and the factors that regulate it, may produce potential treatments to counter sepsis. To identify the secreted proteins in LPS-stimulated and -tolerant cells, we used mass spectrometry of tryptic-digested proteins collected from the media of RAW 264.7 cells treated with one dose of LPS or two doses of LPS separated by 24 hours. The cells were washed with serum-free media and allowed to incubate for 6 hours prior to collection. While the LPS-stimulated cells secreted proteins strongly associated with lymphocyte chemotaxis and the innate immune response (including TNF, Ccl2, Ccl4, Ccl9, and CXCL10), the LPS-tolerant cells secreted lower amounts of immunomodulating agents with the majority of proteins being related to cell survival and the negative regulation of apoptosis (including Clusterin (Clu), CD44, and Osteopontin (Spp1)). In addition, pathway analysis of the secretome results showed the LPS-tolerant cells had distinct changes in pathways related to proliferation, metabolism, and the production of reactive oxygen species. These changes in the secretomes of LPS-stimulated and -tolerant cells may contribute to the decreased reactivity of LPS-tolerant cells. Identifying and quantifying the secreted proteins related to LPS-tolerance may lead to identification of LPS-tolerance inducing factors that could counteract the rampant secretion associated with sepsis. This research was supported by the Intramural Research Program of NIAID, NIH.

Poster Number: P06.02

Tbx18 Orchestrates Cytostructural Transdifferentiation of Cardiomyocytes to Pacemaker Cells by Recruiting the Epithelial-Mesenchymal Transition Program

D. Brian Foster[1]; Jinmo Gu[2]; Elizabeth Kim[2]; Robert O'Meallly[3]; Robert Cole[3]; Hee Cheol Cho[2]

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Background: Cardiac pacemaker cells are distinct from ordinary cardiomyocytes in their electrogenic profiles and morphologies. Previously, we have demonstrated that re-expression of an embryonic transcription factor, Tbx18, is sufficient to reprogram postnatal ventricular myocytes to induced pacemaker cells (Tbx18-iPMs).

Objective & Methods: In this study, we sought to understand the mechanisms at work in TBX18-iPM reprograming, using quantitative proteomics, pathway, and network analyses to extract salient features and establish a gene-regulatory model. Specifically, Tbx18-iPMs (n=5) were compared with GFP-overexpressing, neonatal rat ventricular myocytes (NRVMs; n=5).

Results: Over 2/3 of proteins changed significantly (4,475 of 6,661; $p<0.05$, FDR 1.6%), consistent with a massive shift in cell identity induced by Tbx18. The emergent pacemaker electrogenic phenotype consisted of upregulation of the Hcn4 ion channel, the small-conductance connexin, Cx45, and mechanosensitive ion channels, including Piezo1, Trp channels Trpp2 (PKD2) and TrpM7. Metabolic pathways, including TCA cycle, oxidative phosphorylation, glycolysis and fatty acid oxidation were downregulated, as were ion channels associated with ventricular excitation-contraction coupling (Na+, Ca2+, and K+ channels). Extensive intracellular cytoskeletal and extracellular matrix remodeling included 96 protein hallmarks ($p<0.05$) of the epithelial-to-mesenchymal transition (EMT), including vimentin and OB-cadherin. EMT-inducing transcription factors Snai1 (snail), Snai2 (slug), Twist1, Twist2 and Zeb2 were all substantially regulated at the protein or RNA level ($p<0.5$). Finally, network diffusion was used to map the proximity of EMT factors to Tbx18, within a STRING network consisting of >200 transcriptional regulators. EMT transcription factors and heart development factors (e.g. Tbx5, Nkx2.5, Gata4 among others) occupy adjacent, yet overlapping, network neighborhoods immediately downstream of Tbx18, but upstream of metabolic control factors (e.g. PGC1a, Esrra).

Conclusion: We submit that heterologous expression of Tbx18 in chamber cardiomyocytes recruits the EMT program to guide morphological transdifferentiation into the pacemaker cells.

CHEMICAL PROTEOMIC
P07.01

Poster Number: P07.01

A Novel Way For The Data Analysis Of Limited Proteolysis Coupled To Mass Spectrometry-based On Machine Learning

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High attrition rates in target-centric drug development approaches, as well as a limited number of targets, have shifted the focus of drug development back towards phenotypic screening. In parallel, novel proteomics-based target deconvolution approaches to drug target identification have gained popularity. Limited proteolysis coupled with mass spectrometry (LiP-MS) is a new target deconvolution technique that exploits protein structural alterations, as well as steric effects driven by drug binding.

Here, we present a data analysis strategy for LiP-MS experiments based on multiple features of the data and machine learning. This algorithm was designed for the analysis of LiP-MS discovery experiments in an unbiased way. We demonstrate this on controlled experiments with drugs and their known targets.

We developed an algorithm based on LDA using R. The features using for machine learning include dose-response correlation testing, t-testing, multiple peptide score and more. This algorithm enables the ranking of the peptides and proteins in the LiP-MS experiment with a combined LiP score. We performed dose-response experiments in HeLa using the drugs Calyculin A, Rapamycin, FK506, Selumetinib, Fostriecin and Staurosporine. Using this data, we trained the machine learning framework using a subset of the experiments. Then we applied it to all controlled data sets. This resulted in candidate lists ranked by the LiP score. In the analyzed experiment, the known drug targets are consistently found in the top ten candidates. We demonstrate that the LiP score ranking outperforms individual scores. 3D visualization of the obtained LiP responding peptides clearly shows their proximity to drug binding sites.

COMPUTATION AND ANALYSIS P08.01 – P08.16

Poster Number: P08.01

Proteome-wide Detection Of Function Misannotation By Taxon-specific Rate Ratio Comparison

Chengxin Zhang[1]; Xiaoqiong We Proteome-wide Detection Of Function Misannotation By Taxon-specific Rate Ratio Comparisoni[1, 2]; Gilbert S. Omenn[1]; Peter L. Freddolino[1]; Yang Zhang[1]

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Many protein function databases, such as UniProt Gene Ontology Annotation (UniProt-GOA) and neXtProt, are built on automated or semi-automated curations and may contain various annotation errors. The correction of misannotations is critical to improving the reliability of these databases and downstream proteomics analyses based on database annotations. We propose a new approach to detect potentially incorrect Gene Ontology (GO) function annotations by comparing the ratio of annotation rates (RAR) for the same GO term across different taxonomic groups, where those with a relatively low RAR usually imply misannotations. As a proof-of-concept, we applied the approach to proteomes of 20 commonly-studied species in two recent UniProt-GOA releases. In release 2018-11-6, 250 potential misannotations were identified, only 25% of which were corrected in release 2019-6-3. Among the misannotations, 56% are annotated in UniProt-GOA with "Inferred from Biological aspect of Ancestor" (IBA) evidence, in contradiction with previous observations attributing misannotations mainly to "Inferred from Sequence or structural Similarity" (ISS) evidence in the database, reflecting an error source shift due to recent developments of annotation protocols. Of the 250 UniProt-GOA misannotations, 13 are included in neXtProt release 2019-08-22, including annotations of "cell wall mannoprotein biosynthesis", "fungal-type vacuole membrane" and "compound eye development" to well-curated human proteins. These 250 misannotations are likely a small fraction of all errors due to our focus on a small set of commonly-studied species representing <0.7% of UniProt-GOA; a simple check of 2 misannotated GO terms across all species reveals another 453 potential misannotations. Our results demonstrated a simple but efficient misannotation detection approach useful for proteome-wide comparative function studies. The software and equations of this study are available at https://zhanglab.ccmb.med.umich.edu/RAR, together with the list of identified misannotations, 13 of which neXtProt has referred to GO consortium for reassessment.

Poster Number: P08.02

Misbehavin' Trypsin: Selectivity of Atypical Cleavages by Trypsin

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The well-known behavior of trypsin cleavage is imperfect and can cleave polypeptide chains C-terminal to amino acids other than lysine and arginine. Although the rate of atypical cleavage is estimated to be approximately 1% of that for the expected specificity, our results show that semi-tryptic peptide formation is a significant source of sample-specific variation. Here, we perform an in-depth characterization of both the variability and selectivity of atypical tryptic cleavage sites across more than 120 2D LC-MS/MS analyses, which include data acquired from multiple laboratories, different sample types and labeling methods (label-free, iTRAQ- and TMT-labeled peptides). Although semi-tryptic peptides are generally lower in abundance than the fully tryptic form, they are found to comprise a significant fraction of the total peptide spectral matches, thereby reducing the dynamic range of a given analysis. Our results also demonstrate how NIST MS Metrics may be used to compare variation across samples to identify significant sources of variation in multiplexed experiments.

Poster Number: P08.03

Fast, Flexible and Feature-Rich Computation of Peptide and Proteoform Posterior Error Probabilities Using Binary Decision Trees

Michael R. Shortreed; Lei Lu; Robert J. Millikin; Rachel M. Miller; Leah V. Schaffer; Zach Rolfs; Lloyd M. Smith

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Search engine results from data dependent acquisition (DDA) proteomics experiments are often provided with a false discovery rate (FDR). The FDR is an estimation of the fraction of PSMs in a set of results that are probable false positives. This can be determined by performing the search with concatenated target and decoy databases and using the count of matches to decoy peptides as proxies for the frequency of false positive matches. A 1% FDR means that for a set of 100 matches, ~1 is likely to be a false discovery. But, which one? FDRs do not provide confidence levels for any specific PSM but refer only to the rate of false positives in the complete ensemble. Computing the probability that a specific PSM is incorrect requires a second level of analysis. Here, we describe the use of binary decision trees (BDTs) to compute posterior error probability of individual peptide spectrum matches. BDTs provide an intuitive approach to computing probability that can employ several different parameters simultaneously (e.g., peptide score, the longest uninterrupted series of matched fragment ions, the score of the next best matching peptide, and so on). We demonstrate how this approach can be applied to data from multiple different search

types (e.g., crosslinking, non-specific, top-down) with equally effective results. This is possible because the process is integrated into the MetaMorpheus search engine, which can perform all those search types. It is interesting to observe how dramatically the composition of results changes upon application of the BDT. The posterior error probability found using the BDT can be used to calculate a new FDR, which yields an increase in the total number of PSMs, peptides and proteoforms. Interestingly, this increase is most substantial (20-50%) for non-specific searches where the size of the decoy database is massive.

Poster Number: P08.04

Progress on Identifying and Characterizing the Human Proteome: 2020 Metrics from the HUPO Human Proteome Project

Gilbert Omenn[1]; Lydie Lane[2]; Christopher Overall[3]; Fernando J. Corrales[4]; Jochen Schwenk[5]; Young-Ki Paik[6]; Jennifer Van Eyk[7]; Siqi Liu[8]; Stephen Pennington[9]; Michael Snyder[10]; Mark Baker[11]; Eric Deutsch[12]

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The Human Proteome Project (HPP) annually reports on progress made throughout the field in credibly identifying and characterizing the complete human protein parts list and making proteomics an integral part of multi-omics studies in medicine and the life sciences. NeXtProt release 2019-01-11 contains 17 694 proteins with strong protein-level evidence (PE1), compliant with HPP Guidelines for Interpretation of MS Data v2.1; these represent 89% of all 19 823 neXtProt predicted coding genes (all PE1, 2, 3, 4 proteins), up from 17 470 one year earlier. Conversely, the number of neXtProt PE2, 3, 4 proteins, termed the "missing proteins" (MPs), has been reduced from 2949 to 2129 since 2016 through efforts throughout the community, including the chromosome-centric HPP. PeptideAtlas is the source of uniformly re-analyzed raw mass spectrometry data for neXtProt; PeptideAtlas added 495 canonical proteins between 2018 and 2019, especially from studies designed to detect hard-to-identify proteins. Meanwhile, the Human Protein Atlas has released version 18.1 with immunohistochemical evidence of expression of 17 000 proteins and survival plots as part of the Pathology Atlas. Many investigators apply multiplexed SRM-targeted proteomics for quantitation of organ-specific popular proteins in studies of various human diseases. The 19 teams of the Biology and Disease-driven B/D-HPP published a total of 160 publications in 2018, bringing proteomics to a broad array of biomedical research. All of these numbers will be updated in early 2020 for USHUPO with the PeptideAtlas 2020-01 and neXtProt 2020-01 releases.

Poster Number: P08.05

PASER: Parallel Database Search Engine in Real-Time

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We developed PASER (Parallel Database Search Engine in Real-Time) to perform a novel database search in parallel with data acquisition by extremely high-speed timsTOF mass spectrometry. We used PASER with a superfast IP2-GPU search engine to demonstrate the simultaneous completion of a database search with mass spectrometry spectra acquisition. The spectra were acquired on timsTOF PRO with digested proteins from cells from the NCI-60 cell lines, and a search was performed on a UniProt human database. The search was completed simultaneously with the acquisition of the final spectrum by the instrument. The speed of the PASER search engine is crucial to keep up with the enormous size of data generated by the timsTOF instrument. PASER collects spectra in real-time and generates threads with small and continuous batches of spectral data, then sends each batch to the GPU computer through a fast network cable, directly initiating database searches that are performed while the instrument continues to acquire spectra. Once the database search is complete, results are stored on the same computer. By eliminating separate data extraction and protein database search steps, PASER saves a significant amount of data analysis time, enabling true high-throughput proteomics data analysis in an automated way. PASER can even perform searches on data from multiple timsTOF instruments without compromising search speed.

Poster Number: P08.06

Clustering And Identifying Hundreds Of Millions Of Tandem Mass Spectra Using Deep Learning

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We have trained a deep neural network, called GLEAMS, using peptide–spectrum assignments to embed spectra in a low-dimensional space such that spectra generated by the same peptide are close to one another. The neural network takes as input three feature types — precursor attributes, binned fragment intensities, and similarities to a set of reference spectra — and consists of a mixture of convolutional and fully connected layers. To train the embedder network, a Siamese network containing two instances of the embedder with tied weights is trained via optimization using gradient descent of the contrastive loss function. The resulting learned embedding captures latent properties of the spectra, such as precursor mass and charge, and protein modifications correspond to translations in the latent space.

We have used GLEAMS to process 31TB of human HCD proteomics data belonging to the MassIVE Knowledge Base dataset, corresponding to 658 million spectra derived from 220 publicly available experiments. Based on this repository-scale public dataset we investigate the "dark matter" of the human proteome. First, we propagate spectrum identifications within high-quality clusters obtained using DBSCAN clustering. Second, we use the ANN-SoLo open modification spectral library search engine to identify modified peptides that are frequently observed but consistently remain unidentified. This combined strategy achieves a 133% increase in identifications relative to the MassIVE-KB standard database search results, providing valuable new insights into previously unlabeled data.

To conclude, the GLEAMS neural network is a powerful, scalable method that enables us to efficiently process hundreds of millions of MS/MS spectra and explore the "dark proteome".

Poster Number: P08.07

Ultra-Sensitive Differential Quantification With MS-EmpiRe

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In MS based proteomics, protein expression changes need to be reliably derived from a large number of measured peptide intensities and their corresponding peptide fold changes. These peptide fold changes vary considerably for a given protein. Numerous instrumental setups aim to reduce this variability, while current computational methods only implicitly account for this problem.

We introduce a new computational method, MS-EmpiRe, which explicitly accounts for the noise underlying peptide fold changes. We derive dataset-specific, intensity-dependent empirical error fold change distributions, which are used for individual weighing of peptide fold changes to detect differentially expressed proteins (DEPs).

In a proteome-wide benchmarking dataset, MS-EmpiRe doubles the number of correctly identified DEPs at correct FDR in comparison to state-of-the-art tools. MS-EmpiRe requires only peptide intensities mapped to proteins and, thus, can be applied to any common quantitative proteomics setup. We apply our method to diverse MS datasets and observe consistent increases in sensitivity with more than 1,000 additional significant proteins in deep datasets, including a clinical study over multiple patients. At the same time, we observe that even the proteins classified as most insignificant by other methods but significant by MS-EmpiRe show very clear regulation on the peptide intensity level.

MS-EmpiRe provides rapid processing (< 2min for 6 LC-MS/MS runs (3h gradients)) and is publicly available under github.com/zimmerlab/MS-EmpiRe with a manual including examples.

Poster Number: P08.08

MSstatsSampleSize: Simulation Tool for Optimal Design of High-dimensional MS-based Proteomics Experiments

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The scientific community is increasingly interested in deepening the insight into the composition of complex proteomes. Additionally, it is widely recognized that the most significant advances in the field will come not from an exhaustive analysis of one or a few samples, but rather from the study of large sample cohorts, e.g., case/control cohorts in translational research. The development of a variety of stable and reproducible mass spectrometry (MS)-based workflows now supports the proteomics analysis at a great proteome coverage and large sample cohorts with high consistency and accuracy. However, the technological limitations of these workflows trade off the depth of proteome coverage and the extent of biological replication, i.e., the size of cohorts that can be processed. We argue that the experimental design of large-scale MS-based proteomics experiments should consider these trade-offs to obtain optimal performance.

To allow researchers to design optimal experiments in terms of statistical power and use of resources, we develop a free and open-source R package **MSstatsSampleSize** (available in Bioconductor). MSstatsSampleSize suggests optimal numbers of replicates and proteins quantified for a specific experimental method. It requires a preliminary dataset as input, which quantifies a list of proteins across a small cohort of samples of interest. MSstatsSampleSize fits a linear model on the preliminary dataset and estimates protein abundance variance from the fitted model. Then it simulates a dataset with a user-defined number of biological replicates and proteins based on the variance estimation. MSstatsSampleSize estimates the classification predictive accuracy and the protein importance of this simulated dataset. While varying the number of biological replicates and proteins to simulate, the number of biological replicates that generates the largest predictive accuracy and the proteins that best separate different phenotypes are recorded. We demonstrate the performance of MSstatsSampleSize through a recently published proteomics study of clear cell renal cell carcinoma.

Poster Number: P08.09

Phosphopedia 2.0, a Modern Targeted Phosphoproteomics Resource

Anthony Valente; William S Noble; Judit Villen

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Protein phosphorylation is a fundamental mechanism of cellular signalling. While discovery mass spectrometry approaches such as data dependent acquisition (DDA) have been the workhorses of phosphopeptide identification and quantification, targeted methods achieve much higher quantification sensitivity and precision. To facilitate the implementation of targeted phosphoproteomic assays, we had built Phosphopedia, a resource that originally contained information for over 200,000 phosphopeptides compiled from nearly 1000 DDA mass spectrometry runs. However in our previous implementation it was not possible to expand Phosphopedia, missing out on a wealth of new phosphoproteomic data that is continuously being generated and made publicly available. Here, we present Phosphopedia 2.0, which allows generating targeted assays for any phosphosite, regardless of previous observation.

This update was achieved through two important developments. First, in order to enable the dynamic expansion of Phosphopedia 2.0, we have automated data injection, enhancing the database with thousands of new DDA mass spectrometry runs. Even with this update, many phosphopeptides may still be missing that are of interest to users, but each identification within the database is a direct link between the sequence of a phosphopeptide and its behaviour in our instruments. Thus, in our second development we employ machine learning to model three fundamental properties of phosphopeptides. We first predict which peptides are likely to exist within a sample, by predicting the cleavage propensity of trypsin at its target sites. We also build models which can aid in assay design once a list of peptides is given, by providing predictions for likely retention time and charge state.

With these developments, we provide the ability to expand phosphorylation assays by effectively targeting novel phosphopeptides or taking into account population genetic variation, and the ability to build assays for any model organism of interest.

Poster Number: P08.10

Using Protein Expressible Range to Define Biological Significance in Quantitative Proteomics

Joe Creery; Nathan Zuniga; John Price

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Protein fold-change calculations do not inherently represent perturbation effect in biological systems. Higher thresholds and statistical analyses have been used to determine whether a protein in a group has been biologically altered with significance. Many of these methods address the problem from analytics without a biological reason for choosing to emphasize the most-changed protein. An overall definition of biological significance has yet to be established. For a system that deals with disturbance and change constantly, this is critical information. Using data-mining tools, we collected and compared protein fold-change values from hundreds of biological perturbations in mouse brain and established the protein expressible range (PER) value. This is defined as the possible range a protein can be expressed due to any perturbation. PER values are intrinsic to each protein within a system and have allowed us

to determine which protein fold changes are biologically significant and which ones are due to random variability within that system. Already we have observed that protein turnover can only be altered in proteins belonging to a specific PER value. This enhances our understanding of both values along with dynamic protein abundance as it is a function of protein turnover rate. To take it one step further we enriched mouse brain samples for membrane bound signaling proteins, which have shown the greatest PER values, and measured turnover. This to determine characterization of signal proteins within the proteome. We hypothesize that normalizing protein fold-change values to PER will give us more biological insight in quantitative proteomic experiments and that even more discoveries can be made to define homeostasis within biological systems.

Poster Number: P08.11

Fast and Quantitative Analysis of TimsTOF PASEF Data With MSFragger and IMQuant

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Ion mobility brings an additional dimension of separation to liquid chromatography-mass spectrometry (LC-MS), improving identification of peptides and proteins in complex mixtures. A recently introduced timsTOF mass spectrometer (Bruker) couples trapped ion mobility separation (TIMS) to time-of-flight (TOF) mass analysis. With the parallel accumulation serial fragmentation (PASEF) method, the timsTOF platform achieves promising results, yet analysis of the data generated on this platform represents a major bottleneck. Currently, MaxQuant and PEAKS are most commonly used to analyze these data. However, due to high complexity of timsTOF PASEF data, both require substantial time (multiple hours per file) to perform even standard tryptic searches. Advanced searches (e.g. with many variable modifications, semi- or non-enzymatic searches, or open searches for PTM discovery) are practically impossible. We have extended our fast peptide identification tool (MSFragger) to support timsTOF PASEF data, and developed a label-free quantification tool, IMQuant, for fast and accurate 4D feature extraction and quantification. These tools are available stand-alone or as part of our easy-to-use software suite FragPipe. With MSFragger and IMQuant, a typical 2h PASEF run can be fully processed in under 50 minutes on a modern desktop (12 cores, 32 GB RAM), significantly faster than other tools. Using four HeLa replicates published by Meier et al. (2018), we demonstrate that MSFragger identifies significantly (~30%) more unique peptides than MaxQuant (1.6.10.43), and performs comparably or better than PEAKS X+ (~5% more peptides). IMQuant outperforms both in terms of number of quantified proteins while maintaining good quantification accuracy. Finally, we demonstrate the application of open database searching, leading to interesting observations and deeper understanding of the data. Overall, our computational tools enable fast, accurate, and scalable analysis of timsTOF data, making it suited for analyzing very large datasets. We believe our tools will accelerate proteomics research based on the timsTOF platform.

Poster Number: P08.12

Bibliometrics Analysis of Proteomics Applications and Global Collaboration Networks Across Biomedical Fields from 2008 to 2018

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Bibliometrics analyses can be applied to summarize the literature output of individual scientific fields and identify research trends and collaborative networks. Here we apply several computational tools to obtain worldwide trends of proteomics studies and highlight key collaborative efforts amongst HUPO Human Proteome Project (HPP) investigators over the past decade.

We wrote custom scripts that utilize the PubMed web API implemented in the R package easyPubMed to import data directly from PubMed records in order to create visualization plots. Twenty-two custom PubMed search queries (e.g., "diabetes OR diabetic AND HUPO OR "human proteome project" OR "missing protein" OR proteome OR proteomic OR proteogenomic OR "mass spectrometry" OR neXtProt OR PeptideAtlas") were designed to retrieve HPP field-specific publications and information on collaborative works programmatically.

In total, we retrieved roughly 160,000 publication records. The results revealed robust upward gains in the application of proteomics research across all HPP Initiative relevant areas. Particularly strong growth was observed in the outputs of cardiovascular-, cancer-, and nephrology-related proteomics publications, which have increased by 156%, 145%, and 75% from the year 2008 to 2018, respectively. In parallel, we identified overlaps in proteomics publications among distinct biomedical fields that reflect key collaborative nodes enabled by cross-disciplinary proteomics investigators. Collaborative network maps are currently being devised to highlight these efforts around the globe.

Poster Number: P08.13

A Comprehensive and Straightforward Web Application To establish a Clinical Utility Integrating Clinical Factors and Proteomic Features

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Discovering biomarkers are a fundamental objective of clinical proteomics. Before biomarkers can be considered qualified, they must be rigorously tested. Typically, factors such as sample size, randomized-controlled trials, and case-control studies are used to evaluate diagnostic efficiency in biomarker validation.

To alleviate the burden on researchers and to reduce errors of manual origin, we developed a web application with a user-friendly interface that can calculate sample size, randomize blocks, combine and validate biomarker classification power using logistic regression, support vector machine, and automatically build a nomogram from the results.

For sample size calculation, users have to submit an anticipated incident rate of each group and value of Type I/II error. For block randomization, researcher have to input specific information, or alternatively, manually index each group. Size or number of blocks can be modified, with the results displayed and available for download as a csv file. To proceed with discriminant analysis, researcher have to upload experimental data following the specified format. Accuracy of diagnostic biomarker tests is reported as ***AUROC***, with sensitivity, specificity, and predictive value calculated at specific cut-offs for the result range. The web application also build up a nomogram based on the best marker group calculated by LR or SVM and with additional factors manual uploaded by the user.

Spring framework was used for web application design with Java. Statistical functions are calculated by using python package. All presented pages for users were developed with html, CSS and JSP. My-SQL was used to manage user data.

Poster Number: P08.14

SimpliFi: A Data-to-meaning Analytics Engine To Bring Omics Understanding To All

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Because of (and despite) our ever-increasing ability to generate large amounts of omics data, limitations in our ability to translate such massive datasets into human-understandable, actionable meaning represent a fundamental and growing bottleneck. Indeed if anything, the burgeoning number of analytics tools – purchased and free, for all platforms, written in every imaginable language, from full pipelines to custom scripts to boutique websites to bespoke software and exorbitantly expensive platforms – hinders rather than helps our attempts to understand. The problem worsens geometrically in multiomics studies which, given their intrinsically interdisciplinary approaches, can be interrogated with factorially growing combinations of analytical tools. Additionally, once successfully navigated, often this digital labyrinth extorts time yet again as the analyses and their meaning must be painstakingly explained to others in attempt to impart their meaning.

Working from exactly this experience, we created SimpliFi, an online, browser-accessible data-to-meaning engine. At its core, SimpliFi allows users of all skill levels to explore, visualize and "touch and feel" their data to understand and form hypotheses of what experimental observations might mean. In SimpliFi, users from new-to-omics biomedical researchers to career bioanalysis data experts can rapidly and intuitively access statistically solid approaches, including nonparametric and resampling techniques. Mono- or integrated multiomics data are simplified into clean interactive displays of pathways, states of tissues, disease, cells and molecular-level classifications. Importantly, results from fold-changes to p-values are always presented with their confidence intervals, informing end-user decisions of the potential risks of the next experimental choices.

SimpliFi is, to our knowledge, the first omics analytics engine designed from the ground up from fundamental first-principles of mathematics, data analysis, visualization and user design. As Simplify enables exploration of data from expert to inexperienced end-user levels, we anticipate it will ease data analysis and help bring meaning to omics data.

Poster Number: P08.15

FragPipe: From LCMS Data To Protein Identifications, Quantitation And PTM Localization And In A Few Clicks

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FragPipe (http://fragpipe.nesvilab.org/) is a graphical wrapper for a comprehensive suite of computational tools for shotgun proteomics data analysis: MSFragger as the proteomic search engine, DIA-Umpire as the pre-processing tool for DIA data independent acquisition data sets, Crystal-C for post-search cleanup of open search results, Philosopher as the encompassing toolkit for further downstream proteomic analysis (including PeptideProphet, ProteinProphet, iProphet, label-free quantification, and modules for PSM/ion/peptide/protein-level FDR filtering and summary report generation), PTM-Shepherd for global PTM characterization, IMQuant for Bruker timsTOF specific quantitation, and SpectraST for spectral library building. Input LCMS data files can be processed separately or in batch mode, combined into experiments/groups and replicates, with options to perform peptide and protein level comparisons between groups.

FragPipe guides the user step by step through configuration stages of the pipeline represented by tabs in the UI. Default pre-configured sets of options can be loaded for common types of searches / experiments, from there the whole pipeline can be run without user intervention - just drag and drop LCMS data files from file explorer to the FragPipe window and click "Run".

The pipeline can adapt to various workflows: high throughput peptide/protein identification, DIA data input, spectral library building, label-free quantitation, common and extended PTM profiling, search for novel mass shifts through open-search, etc. FragPipe enable options for generating REPRINT/CRAPome compatible input files (for AP-MS interactome analysis), files compatible with statistical analysis using MSstats. Furthermore, with recent updates, FragPipe also provides native support for Thermo RAW files and Bruker timsTOF files in addition to common open formats, mzML and mzXML. The overall success of our computational tools is evident from rapidly increasing user base, we are actively looking for user feedback and suggestions for future development of the individual algorithms and the FragPipe graphical wrapper.

Poster Number: P08.16

PASEF and Bolt: Enabling Comprehensive Sample Analysis via High MS/MS Acquisition and MS/MS Sequencing Through Vast Protein Databases in Minutes

Amol Prakash[1]; Shadab Ahmad[1]; Swetaketu Majumder[1]; Jeifie Tong[2]; Shenheng Guan[2]; Matt Willets[3]; Shourjo Ghose[3]; Tharan Srikumar[5]; Sven Brehmer[4]; Micheal Moran[2]

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The timsTOF Pro implements a novel scan mode termed 'parallel accumulation – serial fragmentation (PASEF)', which has been demonstrated to multiply the sequencing speed without a loss in sensitivity (Meier et al., PMID 26538118 & 30385480). In a standard 120 min LC run, more than 800,000 fragmentation spectra are easily achievable, which can be used for near exhaustive precursor selection in complex mixtures or resequencing weak precursors. The increase in acquisition speed and sensitivity are often hindered by sub-optimal data processing pipelines. In almost all MS/MS peptide search engines, users must limit their search space to a canonical database due to time constraints and q value considerations, but this typically does not reflect the individual genetic variations of the organism being studied. In addition, engines will nearly always assume the presence of only fully tryptic peptides and limit PTMs to a handful. Even on high-performance servers, these search engines are computationally expensive, and most users decide to dial back their search parameters. We recently presented Bolt (Prakash et. al, PMID 31452088), a new cloud-based search engine that can search more than 900,000 protein sequences (canonical, isoform, mutations, and contaminants) with 41 post-translation modifications and N-terminal and C-terminal partial tryptic search in minutes on a standard configuration laptop. Here we extend Bolt and Pinnacle to natively support timsTOF Pro datasets fully utilizing the ion-mobility space for label-free quantitation. Furthermore, we compare the performance of Bolt against other software packages (academic and commercial). Finally, we utilize the Pinnacle\Bolt workflow on a single-shot patient derived xenograft sample to illustrate the depth of biological information that can be gained in combination with the timsTOF Pro.

CROSS-LINKING
P09.01 – P09.03

Poster Number: P09.01

Leveraging the Entirety of Protein Data Bank to Enable Improved Structure Prediction based on Cross-Link Data

Andrew Keller; Juan D. Chavez; Xiaoting Tang; James E. Bruce

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

University of Washington, Seattle,

XLinkDB is a fast-expanding public database now storing more than 100,000 distinct identified cross-linked protein residue pairs acquired from samples of 12 species. Mapping identified cross-links to protein structures, when available, provides valuable guidance on protein conformations detected in the cross-linked samples. As more and more structures become available in the Protein Data Bank, we sought to leverage their utility for cross-link studies by automatically mapping identified cross-links to structures based on sequence homology of the cross-linked proteins with those within structures. This enables use of structures derived from organisms different from those of samples, including large multi-protein complexes and complexes in alternative states.

To date, cross-links uploaded to XLinkDB have been mapped to over 43,000 structures, providing one or more structures to 1/3 of inter-protein, and 2/3 of intra-protein, cross-links. Researchers can now easily exploit evolutionary conservation of protein structures by exploring identified sample cross-links in the context of a variety of homologous organism protein structures. An example cross-link between two subunits of mouse mitochondrial Complex I was mapped to 15 structures derived from five mammals, its distances there of 19.6±0.4Å indicating strong conservation of the protein interaction. Structures consistent with distance constraints of most or all identified cross-links can help elucidate the conformations and interactions of sample proteins. Multimeric structures containing multiple instances of proteins furthermore enable re-assessment of presumed intra-protein cross-links as potentially inter-protein.

Large numbers of multi-protein structures to which inter-protein cross-links are mapped present an opportunity to assess docking of protein structures as performed in the past for inter-protein cross-links on XLinkDB using the Integrated Modeling Platform. Cross-linked protein chains are extracted from the structure file, docked using the cross-links as distance constraints, then compared with the original structure. We find that approximately half of docked structure models match very closely to the multi-protein structure.

Poster Number: P09.02

Crosslinking Mass Spectrometry Mapping Mitochondrial Protein Interaction Landscape Of SS-31

Juan Chavez; Xiaoting Tang; Matthew Campbell; Gustavo Reyes; Philip Kramer; Rudy Stuppard; Andrew Keller; David Marcinek; James Bruce

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Mitochondrial dysfunction underlies the etiology of a broad spectrum of human diseases including heart disease, cancer, neurodegenerative diseases, and the general aging process. Therapeutics that restores healthy mitochondrial function holds promise for treatment of these conditions. The synthetic tetrapeptide elamipretide (SS-31), a novel class of therapeutics discovered by serendipity, is currently under clinical trials showing promising results for treatment of multiple mitochondrial diseases, however, its mechanism of action is still unknown. SS-31 is reported to target the inner mitochondrial membrane via selectively binding with phospholipid cardiolipin, but the roles of this specific binding of SS-31 with cardiolipin in restoration of mitochondrial function remain unclear. Most therapeutic interventions target proteins and have pharmacological effects through modulating target activities. Thus, one could expect that SS-31 functions through interactions with mitochondrial proteins. Here we utilize chemical cross-linking with mass spectrometry to identify protein interactors of SS-31 in mitochondria. The SS-31-interacting proteins identified in this study are all known cardiolipin binders, which falls into two groups, i.e., those involved in ATP production through the oxidative phosphorylation pathway and those involved in 2-oxoglutarate metabolic processes. Residues cross-linked with SS-31 reveal binding regions that in many cases, are proximal to cardiolipin-protein interacting regions. These results offer the first glimpse of the protein interaction landscape of SS-31 and provide new mechanistic insight relevant to SS-31 mitochondrial therapy.

Poster Number: P09.03

Kojak 2.0: New Features For the Analysis of Cross-linked Proteins

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Shotgun MS analysis of cross-linked proteins is a versatile tool in proteomics. Data analysis of cross-linked proteins has unique challenges for which specialized algorithms are required. Kojak was initially released in 2015 and designed to perform database searching on MS/MS spectra of cross-linked peptides. Designed to be computationally efficient, Kojak is highly customizable and allows for analysis with many different cross-linkers on both small and large datasets. Its simple interface, combined with adherence to open data standards, enabled Kojak's use with diverse experimental conditions and allowed integration into analytical pipelines. Development of the algorithm continues to build upon these core features. Here we present Kojak version 2.0, a major update to the original Kojak algorithm.

Algorithm improvements include an optimized two stage search strategy that prioritizes identification of the larger peptide in the cross-link in the first pass. In the second pass, only those peptides that can link to the best candidates in the first pass are searched, providing a significant cost savings in computation time as database searches become larger and include increasing numbers of modifications in the parameters. The scoring functions were updated to include calculation of E-values, including individually for each peptide in the cross-link, enabling assessment of the cross-linked PSM using the E-value of its lowest scoring peptide, an invaluable parameter for downstream validation algorithms such as PeptideProphet, xiFDR, and Percolator. Kojak now includes a feature that makes use of 15N-labeled proteins mixed with their natural abundance counterpart, to enable accurate identification of inter-protein and intra-protein cross-links from homomultimers. Pipeline improvements include more open data standards for input (mzML, mzXML, MGF, Thermo RAW) and output (pepXML and mzIdentML), allowing integration into any workflow using these highly ubiquitous formats. Kojak 2.0 remains open-source and multi-platform.

DATA-INDEPENDENT ACQUISITION PROTEOMICS (DIA) P10.01 – P10.07

Poster Number: P10.01

Matching Peptides To Data Independent Acquisition Mass Spectrometry Data

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INTRODUCTION : One core problem in the analysis of tandem mass spectrometry data is to detect patterns within the observed data that correspond to distinct peptide species using database search algorithms. Here, we introduce a method, DIASearch, for addressing the problem of detecting peptide in data independent acquisition (DIA) tandem mass spectrometry data. Specifically, given DIA data and a list of precursors, we aim to induce a ranking on the precursors, where highly ranked precursors are detected in the data set with higher confidence. Compared to existing alternatives, DIASearch copes with highly multiplexed DIA data acquired using wide isolation

windows, without requiring any spectral library.

METHOD : Perhaps the primary challenge in analyzing DIA data is to tackle the interference that occurs when the observed signal associated with one peptide overlaps with signals from another peptide. DIASearch explicitly models this interference by constructing a bipartite graph that jointly matches all DIA spectra and a precursor database. Each edge connects a spectrum and a precursor, and its weight reflects a variety of features, including the XCorr E-value, the observed precursor intensity, the difference between the observed and predicted retention time, charge cooperativity, and elution time cooperativity.

Combining these features is nontrivial because each feature exhibits biases specific to retention time, m/z, charge, peptide length or combinations thereof. DIASearch therefore performs feature-wise calibration before aggregating the scores.

DIASearch then selects confident precursors in a greedy fashion according to this score.

PRELIMINARY DATA : DIASearch is applied to real data sets that cover various settings (different complexity, instruments, gradient lengths, and isolation window sizes). Empirical results show that DIASearch outperforms existing methods such as PECAN, XCorDIA, and DIA-Umpire, when evaluated by the number of distinct target precursors or peptides observed when the number of decoy precursors reaches a specified threshold.

Poster Number: P10.02

Longitudinal DIA-MS Reveals Key Phosphorylation Nodes Dedicating Cell Proliferation And Differentiation

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The differential stimulation of epidermal and nerve growth factors (EGF and NGF) determines cell fate. In a classic cell system, EGF "transiently" stimulates the mitogen-activated protein (MAP) kinases, ERK1 and ERK2, and provokes cellular proliferation. In contrast, NGF stimulation leads to the "sustained" activation of the MAPKs and subsequently to neuronal differentiation. Therefore, such a system presents a strong case for studying how the timing and duration of cellular signaling dedicate phenotypes. Although much previous literature has measured the phosphoproteomic signaling following EGF stimulation, there is lack of a high-resolution, systematic, and quantitatively accurate analysis comparing both EGF and NGF using the state-of-the-art proteomic technologies. Here, we used an optimized data-independent acquisition mass spectrometry (DIA-MS) to measure the both proteomic and phosphoproteomic changes in PC12 cells following respective EGF and NGF stimulations. Particularly, we performed label-free quantification between multiple time points, including 0 sec, 15 sec, 30 sec, 1 min, 2 min, 5 min, 10 min, 30 min, 1 hour, 2-hour, 4-hour, 8-hour, 12-hour, 24-hour, 48-hour, and 72-hours. We utilized a novel PTM-localizing algorithm for DIA-MS and quantified >30,000 phosphopeptides (Class- I) as well as 9,000 proteins (protein FDR 1%) throughout all the samples without any prefractionation. Our data excellently recapitulates the "transient" and "sustained" patterns of EGF and NGF signaling. We uncovered the cascades of 150 kinases at both phosphorylation and bulk protein levels. Furthermore, the data reproducibly reveals certain phosphorylation events as early as 15-30 seconds. For the first time, the difference of EGFR phosphosites activation after NGF treatment was identified, which was further ascribed to the activation of P38 kinase. Finally, the comparison to mRNA profiles dissects the translational and post-translational control of phosphoproteins. In summary, the mechanisms leading to the divergence of cell proliferation and differentiation were illustrated with an unprecedented resolution.

Poster Number: P10.03

CANCELLED

Poster Number: P10.04

Systematic Parallel Reaction Monitoring Of The Human And Cynomolgus Macaque Proteome

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Target expression is a primary driver of activity for next-generation cancer therapeutics such as antibody-drug conjugates that are directed toward antigens on the cell surface. Thus, it is critical to assess target expression in normal healthy tissues with high accuracy, and to understand the differences in expression between human subjects and preclinical toxicology models such as the cynomolgus macaque (*Macaca fascicularis*). Mass spectrometry (MS)-based proteomics complements antibody-based detection methods such as immunohistochemistry (IHC) by offering high precision, dynamic range, and selectivity. Recent advances in instrumentation and software permit near-comprehensive, systematic analysis of the proteome using data-

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independent acquisition (DIA) strategies. In this study, we used gas phase fractionation (GP-DIA) to profile the mass range from 400 – 900 m/z of 48 normal human tissues and 17 cynomolgus tissues following trypsin digestion. Fractions were collected by dividing the mass range into five sequential 100 m/z bins with MS/MS collected at 4 m/z overlapping intervals, providing the selectivity and sensitivity of a parallel reaction monitoring assay across the entire range. EncyclopeDIA was used to query the data using either empirical spectral libraries generated by data-dependent acquisition or using whole-proteome synthetic libraries generated by Prosit. We compare MS-based quantification to public gene expression and IHC datasets to assess multi-assay concordance, and identify potential non-specific antibody binding by examining MS quantification at the peptide/epitope level. We also assess the concordance between cynomolgus and human tissue proteomes, providing a global and detailed view of conservation of protein expression between the two organisms. We show that GP-DIA is a scalable method to systematically interrogate relatively large sets of complex samples with high sensitivity. We anticipate that these data and strategies will lead to better antibody, model, and target characterization for the next wave of precision-guided cancer therapies.

Poster Number: P10.05

Nanoscale HILIC Peptide Fractionation for Library Building and DiaPASEF Data Acquisition

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Fractionation of complex proteomic digests is critical to proteome depth and library building for data independent acquisition (DIA) experiments. When a limited availability of sample is encountered the depth of fractionation is stymied because of the inherent inefficiencies in sample recovery for traditional offline HPLC and column-based SPE. One popular technique is high pH reverse phase fractionation as one does not have to remove large quantities of salt. However, for this and other column-based peptide fractionation methods, sample loss is more prominent for low starting sample amounts and due to this, typically starting amounts between 20-100 ug's are needed. However magnetic bead-based proteomics methods are gaining popularity such as 1pot sample prep methods for single-cell and other low starting protein amount workflows. Here, we evaluate using HILIC ReSyn magnetic microparticles for HILIC based peptide fractionation for sample starting amounts of 1 ugram or less with subsequent DIA analysis using diaPASEF on a Bruker timsTOF Pro.

Poster Number: P10.06

Introducing Deepsearch: A Supercomputing Informatics System That Can Maximally Deconvolve, Within Minutes, a *modified* DIA protocol, to Achieve Selectivity Up to ~0.1Th

Gautam Saxena[1]; Aleksandra Binek[2]; Simion Kreimer[2]; Aaron Robinson[2]; Jennifer Van Eyk[2]

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Introduction: Data Independent Acquisition (DIA) could revolutionize mass spectrometry (MS), if we can solve at least two key challenges: 1) the need to build spectral libraries and 2) the need to rely on any library, spectral-library built or FASTA generated, for accurate quantification of analytes that could lead to differential predictors. We demonstrate here a prototype protocol (termed affectionately eMRM, for "exhaustive MRM"), in which traditional DIA is modified such that *'n'* method files are presented to the MS, as follows:

Methods & Discussion: In traditional DIA, *'n'* samples would have *'n'* identical method files. In eMRM, the *'n'* samples have different method files such that no two method files have identical windows, eg sample 1's DIA windows are {...[640,644], [644,648]...}, while sample 2's are {...[638,642], [642,646]...} and so on. By varying the windows across all *'n'* samples, we can achieve in-silico computed DIA windows that are up to 1/*n*-th the actual window sizes (in practice up to MS's quadrupole sharpness, currently ~0.1Th), without having affected the MS in any way.

This deconvolution is computationally intensive, and so it is run on a supercomputing informatics system we've termed "Deepsearch". The resulting deconvolved, "clean" spectra can not only be searched directly against a FASTA file, but more importantly, immediately quantified and submitted to machine-learning tools to possibly identify a *panel* of analytes (whether initially IDed or not) with maximum predictive power.

Results: We ran 21 mouse liver samples on a Lumos with ~100 heavy peptides spiked in at various dilutions. We show promising identification and quantification results, and compare and contrast these results to alternative protocols, such as traditional DIA (processed via OpenSwath, DIA-NN, and DIA-Umpire) and possibly even triple-quad MRM. (With MRM, we would target only the ~100 spiked-in peptides, whereas Deepsearch/eMRM would attempt to quantify all the analytes in the MS).

Poster Number: P10.07

Human Identification Using Genetically Variant Peptides From Touch Samples Using Data Independent Acquisition

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Proteomics is emerging as a new forensic tool that can be used for human identification. Preliminary studies in hair have set the groundwork for these efforts, which are based on the ability to detect genetically variant peptides (GVPs), corresponding to non-synonomous SNPs and other genetic variations, by mass spectrometry. Minor allele frequencies (MAFs) associated with the detected GVPs can be used to calculate random match probabilities (RMPs) and likelihood ratios (LRs), key metrics in forensic analysis. For this work, we focused on touch samples: residual skin proteins left behind when a person contacts an object. Proteins in touch, as well as hair samples, can be more abundant and more resistant to degradation than DNA, thus establishing their importance and utility in certain cases to forensic investigators within law enforcement and the intelligence community. We initiated our work by establishing the contents of the "touchome" in a pooled sample of 39 individuals by tandem mass spectrometry, using DDA. The resulting database, plus common (>1% MAF) GVPs mapping to the same proteins, was used for DIA analysis of all 39 independent samples on a QE HFX. Data analysis was performed by XCorDIA and EncyclopeDIA, and all variant peptides were filtered to 1% false detection and localization rates. GVP detections in each sample were then matched to exome data from the same subjects to determine positive predictive value and sensitivity. A resulting panel of 65 common GVPs can be used to identify individuals with RMPs less than 1 in 10^10. Our work demonstrates that GVPs can be readily detected in shed skin cells and used to match to a subject's exome with high confidence. Our work supports the use of genetically variant peptides in the forensic analysis of human samples where DNA is limited or degraded.

DRUG DEVELOPMENT
P11.01

Poster Number: P11.01

Discovery of a Novel Plasmodium Protein Target of the Antihisatamine Clemastine using Parralel Chemoproteimic Strategies

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The antihistamine, clemastine, has been shown to inhibit multiple stages of the *Plasmodium* parasite that causes malaria. However, the molecular targets responsible for its mode of action are unknown. Reported here is the use of two complementary mass spectrometry-based chemoproteomics approaches to identify the mode of action of clemastine's anti-malarial activity. The thermal proteome profiling and Stability of Proteins from Rates of Oxidation techniques both identified the *P. falciparum* TCP-1 ring complex (TRiC) as a target of clemastine. TRiC is an essential hetero-oligomeric chaperone complex responsible for *de novo* cytoskeletal protein folding. The TPP technique revealed a clemastine-induced thermal destabilization of all eight TRiC subunits. The SPROX technique revealed a clemastine-induced thermodynamic stabilization of the *Plasmodium* TRiC delta subunit, suggesting a direct interaction between the drug and this subunit. Further biochemical experiments demonstrated that clemastine reduced levels of tubulin, the major TRiC substrate in Plasmodium parasite, and lead to the disorientation of *Plasmodium* mitotic spindles during the asexual reproduction and aberrant tubulin morphology. These findings suggest a clemastine-induced dysfunction of the *Plasmodium* TRiC complex. Additionally, it is noteworthy that such a significant thermal destabilization of the TRiC complex was not observed in the TPP experiment using human liver cells, suggesting that clemastine selectively targets the *Plasmodium* TRiC complex over the human TRiC complex. Our findings indicate that the *Plasmodium* TRiC-complex is a novel therapeutic target to inhibit malaria.

GLYCOPROTEOMICS AND GLYCOMICS
P12.01 – P12.05

Poster Number: P12.01

Ion Mobility Separation of Glycoforms with Isomerism on the Peptide and Glycan Levels.

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Glycosylation is a ubiquitous post-translational modification (PTM) that controls the protein quality and folding and protects proteins from degradation and antibody recognition. Many diseases, including cancer and inflammatory and autoimmune disorders, affect glycosylation patterns that thus can serve as biomarkers. Glycosylation is also the most complex PTM. While all PTMs yield regioisomeric proteoforms, glycans also exhibit isomers within the PTM that differ in the subunit connectivity, link stereochemistry (anomers), and isomeric subunits, e.g., galactosamine (GalNAc) versus glucosamine (GlcNAc). This four-level variability allows numerous isomeric glycoforms with distinct biological activity.

Peptides are normally sequenced by tandem mass spectrometry, commonly via collisional dissociation. Such ergodic methods usually cannot localize glycans, as the cleavage of weak glycosidic bonds before the backbone obliterates the site information. Hence the radical-directed electron transfer dissociation, severing the backbone with intact glycans, is preferred. However, isomers must be fractionated prior to the fragmentation step.

Here we explore the separations of representative glycoforms by field asymmetric waveform ion mobility spectrometry (FAIMS). We investigated the mucin peptide GTT*PSPVPT*TSTT*SAP with O-linked glycans: 3α-GalNAc, 9α-GalNAc, or 13α-GalNAc (localization variants), 9α-GalNAc versus 9β-GalNAc (anomers), or 9β-GalNAc versus 9β-GlcNAc (isomeric subunits). The protonated 2+ ions (m/z = 853) dominating the electrospray ionization spectra were analyzed using a custom planar FAIMS device coupled to the LTQ XL ion trap via a slit-aperture ion funnel interface. We used the 4-kV dispersion voltage and He/N_2 carrier gas with up to 63% He.

The resolution of variants improved upon He addition to baseline by ~60% He, expanding our results for smaller PTMs to glycosylation. The isomerism within glycans proved more challenging, but we fully resolved the anomers by extended filtering at reduced gas flow and distinguished the peptides comprising GalNAc versus GlcNAc. These findings demonstrate the first capability to disentangle glycopeptides with undetached isomeric glycans.

Poster Number: P12.02

Cellsurfer Platform For Semi-automated Cell Surface N-glycoprotein Profiling Of Human Primary Cells Reveals Chamber-specific Cardiomyocyte Surface Maps

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In the heart, cell surface glycoproteins in cardiomyocytes (CM) are essential for sustaining normal cardiac function by facilitating proper conduction of action potentials and subsequent contraction of the myocardium. Improper function and/or expression of cell surface proteins can result in life-threating disorders and sudden cardiac death. Therefore, a variety of cell surface receptors have been targeted in pharmacological therapies for common cardiovascular diseases (e.g. hypertension, angina, arrhythmias, and heart failure). Despite the critical roles that cell surface glycoproteins play in cardiac biology and disease, a detailed cell type- or chamber-resolved view of the cell surface proteome of normal adult human heart does not exist yet. Here, we report the development of a new analytical platform, CellSurfer, which integrates efficient sample handling and streamlined data analysis workflows for rapid, quantitative discovery of cell surface N-glycoproteome. CellSurfer includes a semi-automated sample preparation workflow, Microscale Cell Surface Capture (mCSC) that enables discovery of >300 N-glycosylated surface proteins from less than <1 mg total cellular proteins (1-10 million cells). Integrating CellSurfer with an optimized strategy for isolating intact CM from human heart tissue resulted in the generation of the first chamber-specific map of the cell surface N-glycoproteome of adult human CM. Overall, >600 cell surface N-glycoproteins were identified, including transmembrane, GPI-anchored, and extracellular matrix proteins. Differential analysis of CM from each major myocardial chamber revealed previously unreported differences between left and right sides. These data will enhance our understanding of the distinct phenotypic fingerprint of CM that reside in each of the four chambers of the human heart and represent the first major step towards a comprehensive, cell-type, subtype, and chamber-resolved reference map of cell surface N-glycoproteins in the adult human heart and reveal new potential targets for immunophenotyping, in vivo imaging, and drug delivery.

Poster Number: P12.03

Identification of O-glycopeptides With MetaMorpheus

Lei Lu[1]; Nicholas Riley[2]; Michael Shortreed[1]; Carolyn Bertozzi[2]; Lloyd Smith[1]

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

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O-glycosylation is one type of common and important post-translational modification. Extracellular O-glycosylation plays important roles in biochemical and biophysical interactions that govern cell interactions. It mainly occurs in mucin-type glycoproteins, which are characterized by regions of dense O-glycosylation.

Mass spectrometry has been widely used to identify the corresponding O-glycopeptides, but challenges remain, especially for mucin-type O-glycopeptides. First, mucin-type O-glycans are heterogeneous, meaning that there are many possible modifications for any one glycosite. Additionally, a high degree of O-glycosylation on adjacent sites make assigning site-specific modification to any given residue difficult.

Traditional approaches to identify O-glycopeptides usually employ a restricted search strategy, in which the mass of an identified glycopeptide from a database should agree with the corresponding precursor mass. The construction of such theoretical O-glycopeptide databases is a permutation problem and the vast size of the generated databases limits the search speed and increases the false positive identification rate.

We have implemented an algorithm for O-glycopeptide identification in the open-source search engine MetaMorpheus. MetaMorpheus uses an open search strategy to rapidly identifying O-glycopeptides. To do this, MetaMorpheus first identifies peptide candidates using fragment ion indexing. The mass difference between the peptide and the precursor is used to determine the variety of possible O-glycans present on the peptide. Electron-driven activation (e.g., ETD or EThcD) is often used in conjunction with collisional activation (i.e, HCD), meaning there are paired HCD-ETD/EThcD for the same precursor. MetaMorpheus uses identifications from an open search to perform localization in paired EThcD by enumerating all possible O-glycosylation sites.

MetaMorpheus can analyze O-glycopeptides produced using multiple different fragmentation methods. Our platform is the first to combine an open search on HCD spectra with localization using a paired EThD spectrum. The software program is free, reliable, fast, user-friendly and readily accessible to researchers.

Poster Number: P12.04

Optimized Liquid Chromatography Separation of INLIGHT® Derivatized N-linked Glycans for Isomer Identification and Structure Elucidation in Alzheimer's Disease Brain Samples

Jaclyn Kalmar[1]; Karen Butler[1]; Erin Baker[1]; Brendan MacLean[2]; Michael MacCoss[2]; Edward Fox[3]; Thomas Montine[3]; David Muddiman[1]

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Alzheimer's disease (AD) is the 6th leading cause of death in the United States. There are roughly 5.7 million people, 96% of which are 65 years or older, living with AD in the US. AD is a neurodegenerative disease defined by regional accumulation of amyloid plaques, comprised primarily from protein deposits of beta-amyloid (Aβ), and neurofibrillary degeneration. The primary protein component of largely intraneuronal neurofibrillary degeneration is a pathologic form of tau, called paired helical filament (PHF) tau. Amyloid precursor protein (APP) and tau isoforms, the precursors of Aβ and PHF-tau, have been shown to be glycosylated.

Glycosylation is an essential post translational modification that aids with normal cell function along with the stability and folding of proteins, and can provide insight into pathological processes. Due to the neutral and hydrophilic nature of native glycans, they are poorly retained on standard reversed phase liquid chromatography columns are difficult to ionize, and differentiate between various structural isomers. Chemical derivatization methods, such as with hydrazide reagents like INLIGHT®, draw upon well-established mechanisms for enhancing their separation, sensitivity of analysis, and ionization efficiency during electrospray ionization.

In this work, we identified native and INLIGHT® derivatized N-linked glycans from human plasma and brain tissue samples. To do this, the optimized INLIGHT® derivatization conditions were explored using a high resolution accurate mass (HRAM) mass spectrometer. Multiple chromatographic methods for the INLIGHT® derivatized glycans, including C18 reverse-phase (RP) and hydrophilic interaction chromatography (HILIC) separation techniques, in both positive and negative ionization mode and at both high and nano flow rates, were compared and optimized. These optimized methods were then used for elucidation of structural information of isomeric glycans obtained using deep scan and MSn HRAM data.

Poster Number: P12.05

Creating an Analytical Workflow to Assess N-linked Glycan Species and Their Alterations in Alzheimer's Disease Brain Tissue

Karen Butler[1]; Jaclyn Kalmar[1]; Brenden MacLean[2]; Michael MacCoss[2]; Edward Fox[3]; Thomas Montine[3]; David Muddiman[1]; Erin Baker[1]

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Glycosylation in proteins is a ubiquitous post-translational modification involving the addition of glycans to the amino acids serine, threonine, or asparagine. Specifically, N-linked glycans have known important biological roles including the regulation cellular signaling, protein folding, and inflammation. Because of these functions, their alterations have been linked to various forms of cancer and neurodegenerative diseases such as Alzheimer's disease (AD). However, the analysis of N-linked glycans is not a trivial process. These molecules are very hydrophilic in nature requiring the use of HILIC liquid chromatography (LC) or derivatization procedures in order to use reverse phase LC (RPLC). Furthermore, many N-linked glycans are composed of subunits with the same masses resulting in numerous isomers. Thus, LC combined with mass spectrometry (LC-MS) alone is often not sufficient to fully evaluate each specific glycan alteration.

To increase the sensitivity and enable the assessment of specific N-linked glycan isomers, we incorporated both derivatization and ion mobility spectrometry (IMS) separations into LC-MS evaluations. For the derivatization we utilized the INLIGHT™ strategy or Individuality Normalization when Labeling with Isotopic Glycan Hydrazide Tags. Here, glycans are labeled to increase their hydrophobicity and improve their sensitivity and LC separations. The presence of both a natural and stable-isotope label in this strategy also provides a distinct six Dalton mass difference for confident glycan identifications in complex matrices. IMS was then incorporated into the experimental workflow to enable the separation of isomeric glycans that co-elute or cannot be distinguished with LC-MS. To assess the resulting data quickly, N-linked glycan libraries using the LC, IMS, MS and fragmentation information were created and used in the open source analysis program, Skyline. Finally, the workflow was evaluated by assessing various N-linked glycan species in biological samples including fetuin, human plasma, and human brain tissue samples.

HIGH RESOLUTION MASS SPECTROMETRY P13.01

Poster Number: P13.01

Enhanced Label-Free and Targeted Proteomics Performance with Orbitrap Exploris 480 Mass Spectrometer

Khatereh Motamedchaboki[1]; , Aaron Gajadhar[1]; Aman Makaju[1]; Aaron M. Robitaille[1]; Tabiwang Arrey[2]; Sebastien Gallien[2]; Sega Ndiaye[2]; Min Huang[3]; Yue Zhou[3]; David M. Horn[1]; Alexander Harder[2]; Daniel Lopez-Ferrer[1]

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LC-MS-based proteomics analysis is a powerful analytical tool for identification and quantification of thousands of proteins in complex biological samples. Orbitrap mass spectrometers in past decade has contributed to a track record peer reviewed journal publication providing the sensitivity required for verity of proteomics analysis. In moving from discovery to targeted quantitation in proteomics; there has been a need for a robust mass spectrometry system that provide robust throughput needed to analyze 1000s of samples without compromising on identification and quantitation performance. While at the same time providing ease of use for any level of analytical expertise. Here we present a novel quadrupole-orbitrap hybrid mass spectrometer (Orbitrap Exploris 480 MS) coupled to a FAIMS Pro Interface. The performance of this new benchtop mass spectrometer was evaluated in a data-dependent acquisition (DDA) for sample injection amounts of just a single Hela cell to 5000 HeLa cells (~1ug). The sensitivity of the instrument was evaluated by analyzing 10-1000 ng of a Pierce HeLa digest with different throughputs (30, 60, 80, 120 min gradients. To demonstrate the sensitivity of the instrument we analyzed proteins from single HeLa cell, as well as bulk digest at single cell level. This instrument sensitivity enables identification of ~7000 protein groups from only a 200 ng of bulk HeLa digest and ~800 protein groups from a single Hela cell in 2hr gradient. The method performance was also evaluated across different instruments located in different laboratories around the world for reproducibility in identification and quantitation as well as for the new targeted quantitative workflow SureQuant.

IMAGING P14.01 – P14.02

Poster Number: P14.01

Advancing MALDI Imaging Mass Spectrometry of Proteins

Danielle Gutierrez[1]; Audra Judd[1]; Tina Tsui[1]; Carrie Romer[1]; Jamie Allen[1]; Zachary Jenkins[1]; Elizabeth Neumann[1]; Melissa Farrow[2]; Jeremy Norris[1]; Richard Caprioli[1]

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The ability to visualize molecular pathways directly within tissue will provide important insight into human biology and pathology. Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS), which acquires data in a spatially resolved and untargeted manner, is well-suited for this task since previous biological knowledge about the molecular composition of the tissue specimen is not required. However, an inherent challenge to collecting untargeted data is comprehensive molecular identification. This is a limiting factor for MALDI IMS proteomic imaging. Currently, there is no routine pipeline to translate the *m/z* signals seen in MALDI images to protein identifications that hold meaning for a biologist or clinician and that can lead to molecular network and pathway information. Here, we describe the development of a pipeline to achieve systematic identification of *m/z* signals in MALDI proteomic images. In this workflow, proteins within tissue sections of interest are digested in a manner that maintains spatial localization of the peptides and are subsequently imaged by MALDI IMS. In parallel, sections from the same tissue block undergo bulk homogenization, digestion, and LC-MS/MS analysis. Proteins identified from the bottom-up LC-MS/MS experiments are used to build a tissue-specific peptide database. Finally, to identify the *m/z* signals with high confidence within the MALDI images, peptides in the database are matched to the MALDI data and scored according to the developed algorithm. Three tiers of identification were established: tentative assignments, putative IDs, and confirmed IDs. As a proof-of-concept, this pipeline was applied to colon tissue. Out of 300 monoisotopic peaks detected above threshold within the MALDI image, 228 putative IDs were assigned, representing a 76% identification rate. Future work will investigate automation, driving the identification rate above 90%, construction of databases from publicly available data, and integration with pathway analysis tools and other omic modalities.

Poster Number: P14.02

SpatialOMx; combining MALDI imaging with 4D-proteomics

Lucy Woods; Janina Oetjen Oetjen; Stephanie Kaspar-Schoenefeld; Alice Ly; Arne Fuetterer

Bruker, Bremen, Germany

MALDI Imaging has a broad range of applications in OMICs research. However, a gap exists in instrumentation able to obtain spatial information using high throughput imaging, whilst also being able to deliver in-depth proteomics analyses. Here, we present results from the timsTOF fleX; a timsTOF Pro mounted with a high-resolution MALDI source and stage and a new workflow, SpatialOMx, that is able to maintain spatial information whilst identifying a large number of potential biomarkers.

A timsTOF Pro was mounted with a MALDI source and 10 kHz smartBeam 3D laser featuring electronically controlled spot positioning and beam profile for imaging. Tissue samples for MALDI-MSI were mounted on conductive glass slides, and coated with matrix using standard protocols on a TM Sprayer (HTX Technologies, Chapel Hill, NC, USA). Trapped ion mobility imaging measurements removed isobaric interferences in imaging and was able to identify characteristic signals, signifying several regions of interest across the tissue.

The timsTOF fleX was benchmarked for proteomics performance showing the dual-source design is able to routinely identify over 5000 protein groups from 200ng HeLa extract. This high performance can be maintained during routine MALDI imaging runs over several months. Identifying potential biomarkers from small regions of interest classified using MALDI imaging requires a system with high LC-MS/MS sensitivity to analyse extracts containing low-sample amounts. This can be acheived using the established Parallel Accumulation Serial Fragmentation (PASEF) method, creating the basis for the SpatialOMx workflow.

IMMUNOPEPTIDOMICS P15.01 – P15.03

Poster Number: P15.01

Identification of HLA Class I Bound Peptide for Behcet's Disease by mass spectrometric analysis

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

Kyung-Cho Cho

Seoul National University, Seoul, South Korea

HLA class I (A, B, and C) protein complex plays important roles in inducing CD+8 T-cell by presenting peptide fragments and HLA molecules are circulating in blood vessel. Typically, HLA related immune inflammatory is induced by providing peptide fragments derived from foreign antigens (e.g. virus), but sometimes autoinflammatory is induced by providing peptide fragments derived from endogenous proteins. Behçet's disease (BD) is an autoinflammatory systemic vasculitis of unknown etiology and is characterized by oral aphthae, genital ulcerations, ocular and skin lesions, and sometimes with enterocolitis and arthritis. Even though human leukocyte antigen (HLA)-B*51 is known to be largest risk factor for BD statistically, prevalence of B*51 in BD is only 50 %. .In this study, we conducted HLA peptide analysis of HLA-B*51 Behcet's and HLA-B*51 control patients and identified 3,760 unique peptides (~60% of peptides are 9 amino acids in length) with high confidence (1 % FDR) using DDA approach. To select peptides related to Behcet's disease, we analyzed represented peptides detected at least 4 times in healthy patients and Behcet's patients. The majority of peptides showed signature motifs with hydrophobic amino acids at both ends, and gene ontology analysis revealed that they were derived from proteins related to virus infection. The validity of the peptides is under investigation in association with Behcet's disease.

Poster Number: P15.02

caATLAS: an Immunopeptidome Atlas of Human Cancer

Xinpei Yi; Yuxing Liao; Kai Li; Bo Wen; Bing Zhang

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Identification of tumor antigens and neoantigens is critical for vaccine-based and TCR/CAR T immunotherapies. However, the landscape of antigens presented by major histocompatibility complex (MHC) molecules on human cancer cells remains poorly understood. The vast majority of existing studies to identify tumor antigens and neoantigens in human cancers use whole-exome and/or transcriptome data followed by *in silico* MHC-binding prediction. Mass spectrometry (MS)-based proteomics provides an unbiased method to directly identify MHC-binding peptides. Here we aim to build a human cancer antigen atlas (caATLAS) through extensive collection of publicly available immunopeptidome data and comprehensive data analysis. We identified 39 MS-based immunopeptidome datasets with a total of more than 300 samples from 9 cancer types and more than 200 non-cancer samples. Together, these datasets covered 290 MHC class I alleles and 69 MHC class II alleles. To allow comprehensive identification of peptides including peptides with modifications, we used an open search tool, which we found to outperform four close search tools with regard to both sensitivity and specificity. In total, we identified 350, 000 antigens, which is 50% more than those in SysteMHCAtlas, an existing comprehensive database of MHC immunopeptidomic data. The antigens we identified included a substantial number of cancer testis (C/T) antigens. In addition, comparative analysis between cancer and normal samples as well as across cancer types identified cancer type-specific tumor antigens. To further identify neoantigens, we extended our recently published data analysis tool PepQuery to support immunopeptidomic validation of neoantigens and discovered immunopeptidomic evidence for a number of frequent mutations in the International Cancer Genome Consortium (ICGC) database. We created a web resource named caATLAS to make all these data easily available and accessible to the broad cancer research community.

Poster Number: P15.03

Immunopeptidomic Studies of Hepatitis B Infected Human Liver Tissues Detect Strain Specific HBV-HLA Associated Peptides

Mayank Srivastava; Darshit Shah; Augustine Choy; Richard Copin; Lisa Chen; Olav Olsen; William Olson; Robert Salzler

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The presentation of viral peptides by HLA class I molecules on the cell surface and the recognition of these complexes by $CD8^+$ cytotoxic T cells as well as activation of $CD4^+$ T cells form the basis of antiviral immunity against various infections. Herein, we aimed to identify the viral peptides in Hepatitis B virus (HBV)-infected patient tissues using an optimized immunopeptidomics workflow. We employed a highly selective anti-HLA Class I monoclonal antibody, W6/32 to enrich HLA complexes from ~50 human liver tissues and identified HLA-associated peptides using a Thermo Orbitrap Fusion Lumos on-line with a nanoLC 1200. Our state of the art immunopeptidomics workflow identified, on average, over 7000 HLA class I peptides, with a high percentage of 9-mers and 10-mers. However, differences in the strains of HBV that are present in infected clinical samples pose a major challenge in analyzing these clinical samples. To address this issue, we utilized a novel proteogenomics approach to generate patient-specific databases based on the RNA sequencing of viral transcriptomes from each patient liver sample. Novel strain-specific HBV-HLA associated peptides were identified by mass spectrometry with high confidence and HBV epitopes were further validated using heavy peptide analogues. These newly identified HBV-HLA-associated peptides could assist in the development of a vaccine, bispecific antibody or CAR-T based therapeutics and take us a step closer to the precision medicine.

METABOLOMICS
P16.01 – P16.02

Poster Number: P16.01

Targeted Transcriptomic and Metabolomic Analyses Identifies Roles of Altered NAD Metabolism and NMRK in Diabetic Cardiomyopathy

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Diabetic cardiomyopathy (DC) is linked to altered NAD metabolism. However, the causal roles of NAD metabolism in DC is not established. Chronic diabetes by streptozotocin caused gradual declines in cardiac function and lowered NAD/NADH ratio. To determine whether NAD redox imbalance promotes DC, we employed cardiac-specific Ndufs4-KO mice (cKO), which exhibit lowered NAD/NADH ratio without overt dysfunction. Cardiac dysfunctions were worsened in diabetic cKO mice compared to diabetic control mice, suggesting that NAD redox imbalance promotes DC. We examined how NAD metabolism is altered to accelerate DC. In addition to NAD redox state, NAD metabolism involves metabolites and enzymes in NAD consumption and synthesis pathways, coordinating NAD homeostasis. Analyses of transcripts and metabolites in these pathways were performed by qPCR and quantitative LC-MS/MS analyses. We identified that *Nmrk* mRNA levels were up-regulated in diabetic cKO hearts, compared to diabetic control hearts. Of thirteen cardiac NAD metabolite levels surveyed, product metabolites of NMRK enzymatic reaction showed decreases in levels in diabetic cKO hearts. The transcript and metabolite analyses suggest a role of NMRK in the DC progression. Cardiac fibrosis of diabetic control and cKO hearts were not different, suggesting that the accelerated decline of cardiac function in diabetic cKO hearts is not be due to altered extracellular matrix environment. We analyzed acetylation-dependent pathways to account for the accelerated functional decline. NAD redox imbalance in diabetic cKO hearts promoted protein acetylation including SOD2 acetylation (SOD2-K68Ac), which inhibits its antioxidant function. Therefore, we

observed elevated protein oxidation levels in diabetic cKO hearts. Our results support that altered NAD metabolism is a critical determinant for DC progression and warrants further investigations.

Poster Number: P16.02

Using Metabolomics, CE-MS and Isotope Incorporation to inform Alzheimer's Research

Kendra Adams[2]; W. Kirby Gottschalk[3]; Joan Wilson[3]; David Millington[1]; Arthur Moseley[2]; J. Will Thompson[2]; Carol Colton[3]

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Immune-based metabolic reprogramming in the brain, causing arginine starvation and accumulation of neurotoxic polyamines, progressively leads to neuronal pathology associated with AD. [1] In order to investigate the activity of this pathway in a variety of AD mouse models, we have developed methods using capillary electrophoresis high-resolution mass spectrometry (CE-HRMS) to study arginine metabolism after injecting AD mouse models with uniformly labeled ($^{13}C_6$ $^{15}N_4$) arginine. Tracing the metabolic fate of stable isotope-labeled substrates in metabolomics enables the direct measurement of interconversions between labeled molecules to quantify metabolic flux, allowing us to shed light on how genetic differences or other factors modify those pathways. We developed a pseudo-primed infusion-dosing regimen, using repeated injections, to achieve a steady state labeling of arginine in 135-195 minutes post bolus dose. *In vivo* isotope incorporation into arginine and a host of downstream metabolites was measured using serially sampled tail blood via dried blood spots. In addition to the dried blood spot time course samples, we are able to observe differences in the rate, and in total incorporation of these metabolites by analyzing brain and plasma after 285 minutes. In the CVN mouse, a familial Alzheimer's disease model, heavy isotope enrichment for citrulline produced from the labeled arginine via arginase versus nitric oxide synthase (citrulline+7 vs. citrulline+9) differed between brain and circulation. We also see differences in isotope incorporation in the downstream metabolites between male and female mice of the same genotype and age as well as between sporadic AD mouse models, the human NOS mouse (HN) and the APOE4-HN mouse (E4HN). The technique described herein is suitable for future metabolic flux analyses comparing various mouse genotypes as well as physiological effects of other factors thought to contribute to AD such as stress, exercise and menopause.

1. Kan et. al. *Journal of Neuroscience* **2015**, *35* (15)

MULTIOMICS AND NOVEL ''OMICS P17.01 – P17.07

Poster Number: P17.01

Exploration Of Stress Biomarkers In Gilthead Seabream (Sparus Aurata) Liver By Integrative Multi-omics Analysis

Pedro Rodrigues[1]; Cláudia Raposo de Magalhães[1]; Denise Schrama[1]; Marco Cerqueira[1]; Ana Paula Farinha Resende[1]; Annette Kuehn[2]; Dominique Revets[2]; Surintorn Boonanuntanasarn[3]; Chatsirin Nakharuthai[3]

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Managing the welfare of fish in captivity is crucial to ensure a sustainable aquaculture production. Farmed fish welfare has been studied through the physiological stress response by assessing plasma metabolites such as cortisol, glucose, and lactate. However, their reliability as welfare indicators in cases of long-term chronic stressors has been questioned due to high biological variability, resultant of fish adaptation processes. The aim of this work is to identify and validate more robust fish welfare biomarkers using a multi-omics approach integrating transcriptomics, proteomics, and metabolomics. *Sparus aurata* was reared under different stressful conditions: overcrowding, net handling, and hypoxia, using fish reared under optimal conditions as a control. Fish were sampled after 45 days of trial and protein extracts were prepared from liver samples. Proteins were separated by 2D-DIGE and identified by MALDI-TOF/TOF MS. Candidate welfare biomarkers were then chosen based on their stress-related function, fold-change, and protein score, and used for primer design. Total RNA was extracted from liver samples using Trizol reagent with DNase treatment and used for cDNA synthesis. The mRNA levels of the target genes were assessed by real-time PCR. Gel-based proteomics showed, in the liver, a total of 243 proteins with statistical differential abundance among conditions, whereas protein-protein interaction and gene ontology enrichment analyses revealed interactions between 47 proteins implicated in biological processes such as amino acid and lipid metabolisms and response to a stimulus. From these, 20 were indicated as candidate biomarkers and chosen for their transcription level analysis. Quantitative gene expression analysis reveals that the transcripts' levels of *krt8* and *grp8* were modulated. This work explores the potential of fish protein-based adaptations as reliable signatures of stress allowing for the future development of novel biomarker models to monitor fish welfare and improve aquaculture sustainability.

Poster Number: P17.02

iModMix, a Network-based Tool For Multi-omics Analysis

Paul Stewart; Christopher Wilson; Elsa Flores; Brooke Fridley

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We present iModMix (integrative Modules for Multi-omics data), an integrative framework for analyzing multi-omic datasets. This approach has several advantages over existing frameworks and can handle any combination of omics expression data (e.g. proteomics and metabolomics, transcriptomics and metabolomics). iModMix first uses pairwise partial correlations that measure only direct associations to build biologically meaningful networks from user-provided expression data. Our framework avoids the "hairball problem" by imposing a sparsity constraint to reduce the dimensionality of the network. Fuzzy clustering is then used with the topographical overlap measure (TOM) to identify modules or groups of related molecules (e.g. genes, proteins, metabolites). Fuzzy clustering allows features to belong to more than one module, as for example genes can belong to multiple pathways or gene sets. TOM considers each pair of molecules in relation to all other molecules in the network, while other measures only consider pairs of molecules in isolation. Since modules are empirically derived directly from the data, they are not constrained by pre-defined pathway databases such as KEGG. We then use sparse principal component analysis to condense expression in the module into a single set of values, called "eigen-features" (e.g. "eigen-genes" for gene expression data, "eigen-metabolites" for metabolomics data). Importantly, eigen-features from multi-omics datasets can be related to the phenotype/endpoint and also between omic data types, making our method fully integrative. We apply iModMix to a novel proteometabolomics dataset of KRAS mutant lung tumors from genetically altered mice. Consistent with known biology, we found cysteine metabolism protein modules highly correlated with glutathione metabolite modules. Interestingly, we also found protein modules highly correlated with metabolite modules that consisted entirely of unidentified metabolites. Future work will investigate iModMix's ability to help identify unknown metabolites based on associations with known modules.

Poster Number: P17.03

Skyline – Vendor-Neutral Software for Quantitative Proteomics and Metabolomics

Cameron Wehrfritz[1]; Kendra Adams[2]; Brian Pratt[3]; Arthur Moseley[2]; Michael MacCoss[3]; Brendan MacLean[3]; Will Thompson[2]; Birgit Schilling[1]

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Mass spectrometric experiments are typically associated with the need for quantitative analysis. This is true for both proteomic as well as metabolomic workflows. Skyline has established itself as a vendor-neutral software tool for quantification in proteomics workflows, but more recently also for metabolomics workflows. Easy switching of the user interface from the proteomics quantitative environment to a metabolomics-specific interface enables user-friendly data processing in either mode. This offers unique opportunities for laboratories to integrate their multi-omics workflows, including the direct integration with the Panorama web repository for easy data cataloguing and sharing.

Skyline now includes dedicated support of workflows for small molecule analysis, including selected reaction monitoring (SRM), high-resolution mass spectrometry (HRMS), and calibrated quantification. This fundamental expansion of Skyline from a peptide-sequence centric tool to a molecule-centric tool makes it agnostic to the source of the molecule while retaining Skyline features critical for workflows in both peptide and more general biomolecular research. The data visualization and interrogation features already available in Skyline - such as peak picking, chromatographic alignment, and transition selection - have been adapted to support small molecule data.

We present new features in Skyline to support quantitative metabolomics workflows and demonstrate examples for benchmarking of the new software tools. In addition, we feature new workflows, positive/negative switching, as well as generation of calibration curves using internal standards and surrogate standards. These new data processing capabilities will advance and facilitate data transparency and easy sharing of metabolite assays and results.

This work was supported by the NIH: RF1 AG057358 (MPI: Lithgow, Andersen - BS), R56 AG057895 and R01 AG057895 (MPI: Colton, Thompson), and P41 GM103493, R01 GM103551, P30 AG013280, and R56 AG063885 (to MJM). Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (NIH U01 AG024904) and DOD ADNI (W81XWH-12-2-0012).

Poster Number: P17.04

Multi-omic Profiling of a Large CRISPR Knockout Collection Elucidates Functions Of Uncharacterized Mitochondrial Proteins And Their Role In Human Disease

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Multi-omics is a powerful and versatile approach for probing biological systems. Encompassing many layers of biological information, multi-omic data can holistically describe a living system and its response to perturbations. Mitochondria - central hubs of cellular metabolism - are particularly amenable to multi-omic profiling, as myriad metabolites, lipids, and proteins jointly orchestrate responses to various stimuli. Despite the recent surge in mitochondria- and metabolism-focused research, many mitochondrial processes and their connections to human health and disease remain elusive. To shed new light on this essential organelle, we designed a custom collection of >200 human CRISPR-generated single-gene knockout cell lines, targeting genes encoding functionally characterized and uncharacterized mitochondrial proteins. The targets included members of core mitochondrial pathways, such as oxidative phosphorylation and metabolite transport, and prominently featured proteins linked to human disease. Using shotgun proteomics, metabolomics, and lipidomics, we performed multi-omic profiling of the cell lines in biological triplicate, collecting ~2,200 LC or GC-MS/MS experiments and monitoring abundances of >11,000 diverse biomolecules in each cell line. To facilitate exploration of the collected data, we created a web-based data analysis and visualization portal. The remarkable size, depth, and high quality of the dataset afforded a comprehensive view of knockout-induced molecular perturbations, empowering systems-level bioinformatic analyses, such as t-Distributed Stochastic Neighbor Embedding and Uniform Manifold Approximation and Projection, to reconstruct molecular networks linking characterized and uncharacterized proteins. Capitalizing on multiple data types, we have gained new insights into complex I assembly, CoQ biosynthesis, the MICOS complex, and metal ion transport. These findings showcase the tremendous discovery potential our data and, more broadly, multi-omic profiling hold.

Poster Number: P17.05

PALLID: Functional Integration of Lipidomics and Proteomics

Hugh Mitchell; Jennifer Kyle

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Lipids are at the front line of human health, and, in combination with other biomolecules, are a force multiplier poised to advance infectious disease research and reduce illness. Lipids are chemically diverse with thousands of different lipids being identified within one human cell. They are continuously changing to meet the demands of the host system. Many of the vital functions of lipids and the chemical diversity are tightly linked or regulated by proteins. Lipidomics has revealed that pathogen infection can drastically alter the lipids of the host. The interpretation of these results is often based on general knowledge of lipid function with the underlying enzymes driving these alterations remaining largely undetermined. The function of many lipid-related enzymes is known; however, direct database links between most complex lipids (e.g., phospholipids and glycerolipids) and the enzymes responsible for metabolizing them is severely lacking, creating a critical need for the elucidation of pathway relationships that bridge protein activity and lipid metabolism. Furthermore, this lack of connection stalls the power of systems biology approaches to inform and refine hypotheses of disease progression and severity. We have created a database of directional linkages between lipids and proteins, so that lipidomics and proteomics datasets from the same samples can be fully and functionally integrated according to known directional enzyme/substrate relationships. Our tool, Protein and Lipid Linkage for Integration and Directionality (PALLID) leverages these linkages and matches corresponding specific lipid and protein changes within a dataset. Preliminary results show the utility of PALLID for integrating the two data types. This work provides another much-needed step towards bridging the gap between our ability to collect lipid data and our ability to use it to gain biological insight.

Poster Number: P17.06

Application of Omics Mass Spectrometry Approaches To Understand Salmonella Pathogenesis

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Salmonella is a foodborne pathogen that causes illness and death each year. The Centers for Disease Control and Prevention estimate that in the United States *Salmonella* causes approximately 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths annually. There are no drugs specifically targeted at *Salmonella*, and the use of broad spectrum antibiotics can prolong shedding and cause antibiotic resistance. To better understand how *Salmonella* can influence the metabolic pathway and the microbiota gut environment, we are developing robust protocols for identification of untargeted metabolites and proteins in fecal samples obtained from *Salmonella*-infected mice. In our initial study, 20 mice were infected with *Salmonella* and fecal samples were collected twice per day for 18 days. Small molecules were extracted from selected fecal samples and then the solution was analyzed by liquid chromatography-mass spectrometry (LC-MS). By comparing the MS data pre- and post-*Salmonella* treatment, it is possible to determine whether any new metabolites are identified after *Salmonella* infection. Given the large sample set collected (720 fecal samples for the initial studies), these studies would benefit from sample multiplexing, enabling samples to be combined and run at the same time. Multiplex isobaric tags have been developed for such studies and are a valuable tool in omics studies. For our proteomic studies we will use DiLeu isobaric tag, synthesized in house. The presence of a DiLeu tag in different proteomic samples allows us to increase the number of experiments in a single run, increasing the efficiency and consistency of the final results. Because different classes of compounds are involved in the *Salmonella* infection, we aim to identify and quantify compounds involved in the *Salmonella* infection and their metabolic pathways by labeling the samples with DiLeu tag (for NH_2 group) and other chemical tags specific for each functional group.

Poster Number: P17.07

Multi-omic Analysis Reveals Cannabidiol (CBD) Disruption Of Cholesterol Homeostasis And Mitochondrial Respiration

Steven Guard[1]; Douglas Chapnick[1]; Christopher Ebmeier[1]; Jeremy Jacobsen[1]; Travis Nemkov[2]; Kerri Ball[1]; Kristofor Webb[1]; Helen Simpson[1]; Stephen Coleman[1]; Eric Bunker[1]; Zachary Poss[1]; Michael Stowell[1]; Angelo D'Alessandro[2]; Xuedong Liu[1]; William Old[1]

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Cannabidiol (CBD), a natural product of the *Cannabis* plant, is an FDA approved drug for treatment of several forms of epilepsy in children. Although preclinical data from several other therapeutic applications of CBD exist, little is known about the mechanism of action of CBD in cells, tissues, and organs. Here, we report the use of temporally resolved proteomics, transcriptomics, metabolomics, and phenotypic screening to dissect the molecular mechanism of CBD response in human neuroblastoma cells. Mass spectrometry-based metabolomics revealed relatively slow kinetics of intracellular CBD accumulation, with saturation occurring between 40 and 80 minutes. FRET-based phenotypic screening identified increased cytosolic calcium and activation of AMPK signaling within 1 hour of CBD treatment. Phosphoproteomic analysis revealed signatures of AMPK activation and signaling at 1 and 3 hours post treatment, predicting decreased protein synthesis, decreased fatty acid synthesis and increased flux through Acetyl-CoA. Subcellular proteomics, RNAseq and lipidomics revealed CBD-dependent activation of cholesterol transport and storage, as well as upregulation of cholesterol biosynthesis enzymes on the transcript and protein levels. Although CBD is relatively nontoxic to a variety of normal and transformed human cell lines, CBD sensitizes cells to apoptosis upon additional stress to the cholesterol homeostatic network. We demonstrate the ability of CBD to incorporate into membranes, affect cholesterol orientation, and alter lipid order. Thus, CBD's biochemical consequence was consistent with the diverse therapeutic applications of CBD in diseases that engage cholesterol and membrane processes for progression.

POST-TRANSLATIONAL MODIFICATIONS P18.01 – P18.17

Poster Number: P18.01

Implementation Of Every-other-day Fasting In Aged Mice Effectively Transforms Protein Sulfhydromes In Multiple Tissues

Nazmin Bithi; Yoko Henderson; Christopher Link; Belinda Willard; Christopher Hine

Cleveland Clinic, Cleveland, OH

Introduction: Hydrogen sulfide (H_2S) is a redox-active metabolite that promotes stress resistance and metabolic fitness. A mechanism of action for H_2S is through protein sulfhydration ($R\text{-}SS_nH$). Endogenous H_2S production through catabolism of sulfur amino acids declines with age. However, aging-related changes in sulfhydrome profiles have not been characterized, nor has the impact of anti-aging interventions to preserve the sulfhdyrome in late life. We hypothesized that the dietary intervention of every-other-day (EOD) fasting, which increases lifespan and boosts endogenous H_2S production, would preserve and/or expand functional tissue-specific sulfhydromes in aged mice.

Methods: Ten C57BL/6 mice were kept on *ad libitum* (AL) diets until 20-month of age, then 5 were randomly assigned to 2.5 months of EOD fasting and 5 on AL feeding. Tissues were then harvested and sulfhydrated proteins isolated via the Biotin Thiol Assay followed by in-gel digestion. Untargeted LC-MS/MS proteomic analysis of these proteins on an Orbitrap Fusion Lumos Tribrid analyzer with data processed using multiplatform bioinformatics programs to identify the sulfhydrated proteins and their functional pathway enrichment.

Results: We identified 1158, 1211, 530, 446, 731 and 170 sulfhydrated proteins and their associated biological pathways in the liver, kidney, heart, muscle (quadriceps), brain and plasma, respectively. EOD fasting enriched the number of sulfhydrated proteins up to 98% in liver, kidney, muscle, and brain while it decreased these in heart and had minimal impact in plasma. EOD fasting-induced functional enrichment with sulfhydrated proteins in multiple metabolic and health-related pathways compared to AL feeding.

Conclusion: We defined tissue-dependent sulfhydromes in aged mice and how diet transforms their makeup, underscoring the breadth for EOD fasting and H_2S to impact biological processes and organismal health.

Poster Number: P18.02

Acetylation Of The Nuclear Lamina Regulates Nuclear Periphery Stability And Function

Laura Murray-Nerger; Xinlei Sheng; Pranav Rekapalli; Ileana Cristea

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Regulation of nuclear shape, composition, and function is at the core of cellular life and plays an essential role during viral infection. Many nuclear-replicating viruses need to disrupt the nuclear periphery for virus capsid egress. This includes human

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cytomegalovirus (HCMV), a pathogen that infects 50-90% of the global population and for which there are no effective treatments or vaccine. During its prolonged replication cycle, HCMV viral capsids are assembled within the nucleus. However, as the viral capsid is too large to egress via nuclear pores, the lamina at the nuclear periphery must be disrupted, forming infoldings that allow capsid budding through the nuclear membrane. Here, we report our discovery that acetylation is a critical regulator of viral capsid egress. Using acetyl-peptide enrichment and quantitative mass spectrometry, we find acetylations on nuclear lamina proteins that are temporally regulated during the progression of infection. Among these, K134 acetylation on lamin B1 (LMNB1) displayed a large increase late in infection. To determine the function of acetylation, we generated acetyl-mimic and charge-mimic LMNB1 mutants, and discovered that acetylation induces reductions in both extracellular and intracellular infectious virus. Live cell microscopy showed that LMNB1 acetylation induced the accumulation of viral capsids within the nucleus, and cells expressing the LMNB1 acetyl mimic displayed a reduction in nuclear infoldings and virally-induced nuclear curvature. Additionally, using proximity-labeling by miniTurboID we identified acetylation-dependent temporal alterations in LMNB1 associations during infection. The broad relevance of this finding was demonstrated by our discovery that LMNB1 acetylation also acts in host defense during infection with another virus, herpes simplex virus type 1 (HSV-1), stabilizing the nuclear periphery and reducing infectious virus production. As both HCMV and HSV-1 arrest the cell cycle, we further employed flow cytometry and fluorescence microscopy to demonstrate a regulatory role for LMNB1 acetylation in cell cycle progression.

Poster Number: P18.03

Uncovering Dehydroamino Acids within the HIV-1 Viral Proteome

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Human immunodeficiency virus type 1 (HIV-1) remains a deadly infectious disease despite existing antiretroviral therapies. A comprehensive understanding of the specific mechanisms of viral infectivity is necessary for the development of new, effective therapies against HIV. HIV virions consist of two HIV RNA genomes surrounded by the structural viral proteins, Gag and Gag-Pol. The efficient infectivity of these particles is dependent on the cleavage of Gag and Gag-Pol into their protein products and the formation of the genome-encapsulating capsid core. Proper assembly and disassembly of this capsid core is vital for effective viral replication and infectivity, making the capsid protein a potentially important therapeutic target. We have performed a comprehensive proteomic analysis of HIV-1 virions produced from transfected 293T cells, which revealed an unexpected non-coded amino acid, dehydroalanine, at multiple sites in the capsid protein. Dehydroamino acids are post-translationally installed via a) the dehydration of serine or threonine residues, or b) the removal of hydrogen sulfide from cysteine residues. They are rare and have previously been detected mostly in select bacterial and fungal species. Analysis of the HIV-1 virion proteome using MetaMorpheus/GPTMD revealed the conversion of highly conserved capsid cysteine residues, Cys 198 and 218, to dehydroalanine. These dehydroalanine residues were identified at both the peptide and the proteoform level. Chemical labeling of the reactive alkene present in the dehydroamino acids followed by mass spectrometric analysis substantiated the identity and localization of this non-canonical amino acid within the HIV capsid protein. We hypothesize that these residues may be important in viral core assembly and disassembly. These previously undiscovered dehydroamino acids may provide valuable insight into HIV infectivity.

Poster Number: P18.04

Global Protein Phosphorylation Identification And Quantification By Liquid Chromatography Mass Spectrometry

Ling Li; Dongmei Zhang; Belinda Willard

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Phosphorylation is the most commonly studied post translational modifications (PTM) and is of great importance in biological function and mechanisms of disease. In traditional bottom-up proteomic experiments, identification of phosphopeptides is challenging due to the highly complex nature of tissue or cell lysate samples along with the lower abundance of phosphopeptides in these samples. Due to this, the global analysis of phosphopeptides requires enrichment prior to LCMS analysis.

To develop a reproducible protocol for quantitative phosphorylation analysis, we tested several phospho-enrichment strategies on Hela cell lysates. Two different methods for phosphopeptide enrichment were tested including TiO2 (Thermo A32993) and Fe-NTA (Thermo A32992). Consecutive enrichments on the same sample using both enrichment methods were tested in an effort to recover more phosphopeptides. In addition, we have compared two quantitative methods for the quantitation of phosphopeptides including label-free quantitation and an isobaric tagging approach.

Our experiments have shown that a single step phosphoenrichment on 1 mg protein using either TiO2 or Fe-NTA resulted in the identification of 9000 phosphopeptides with 86% specificity and 12000 phosphopeptides with 98% specificity respectively. A consecutive phosphoenrichment using TiO2 followed by Fe-NTA resulted in the identification of over 15000 phosphopeptides. An initial test of phosphoenrichment coupled with isobaric tagging has shown that complete labeling of phosphopeptides was achieved, however, the number of identified phosphopeptides was dramatically reduced to ~50% of non-labeled samples. The ongoing work on this project includes optimizing the acquisition method to increase the number of identified phosphopeptides in the isobaric tagging experiment and a comparison of quantitative accuracy of the label free and isobaric tagging approach using a yeast/Hela cell phosphoproteomics mix.

Poster Number: P18.05

Deciphering the Landscape and Compartmentalization of the Thiol Redox Proteome via Stoichiometric Quantification of PTM Site Occupancies

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Reversible redox modifications of protein thiols represent a fundamental mechanism of redox signaling and regulation. Absolute stoichiometries of fractional site occupancies of redox PTMs provide potentially more accurate functional predictive values for understanding cellular redox state and redox regulatory networks. Herein we present an approach for thiol redox proteome profiling and stoichiometric quantification of site occupancies of both S-glutathionylation (SSG) and total thiol oxidation in cells and tissues with a typical coverage of 7000-10,000 Cys sites. In RAW 264.7 macrophage cells under basal conditions, a mean site occupancy of 4.0% for SSG and 11.9% for total oxidation was observed. Further analyses revealed compartmentalization of the redox state such that the mean site occupancies of redox PTMs in cellular compartments correlates well with their respective redox potentials. For instance, the lowest occupancies were observed in more reducing compartments such as mitochondria and nucleus

and highest occupancies in more oxidizing organelles such as endoplasmic reticulum and lysosome. Vastly different occupancies were also observed across different enzyme classes. For example, site occupancies of the thioredoxin and glutaredoxin systems are typically as low (<15% in total oxidation) while protein disulfide isomerases and caspases being highly oxidized (>50%). Furthermore, mitochondria and ER are less susceptible to low-stress perturbation than nucleus and cytoplasm, presumably due to the differences in basal redox states and antioxidant capacity. Similar redox landscape and compartmentalization were also observed in several tissues such as mouse skeletal muscle and lung. Finally, the quantitative site occupancy data from multiple Cys residues of a given protein also provides a good prediction of active or functional sites. Together, these results reveal the unique values of quantification in site occupancies and novel insights into protein thiol redox regulation across different enzyme classes and compartments.

Poster Number: P18.06

Leveraging Diagnostic Ions For Targeting Acyl-lysine Modifications In Proteomic Datasets

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N-ε-lysine acylation is a common post-translational modification (PTM) that regulates a variety of biological processes ranging from gene expression to cellular metabolism. Proteomic workflows have been developed to investigate the biological roles of these PTMs and remain a powerful tool for identifying them. Current methods, however, have limitations as low abundance peptides may be missed and the presence of additional PTMs complicate spectra assignments. Several PTMs, like lysine acylation, are known to generate unique immonium product ions that indicate the PTM's presence, but these diagnostic ions are not fully utilized for confidently assigning acyl-lysine peptides. Using Orbitrap mass spectrometry, we investigated LC-MS/MS datasets of N-ε-acetyl-lysine and non-acetylated peptides, and found that for acetylated peptides, the intensity of the m/z 126 immonium ion for acetyl-lysine is consistently higher than that of the m/z 129 ion for lysine. In approximately 80% of acetyl-lysine PSMs containing both ions, the m/z 126 ion is more intense. We observed that the diagnostic ion's intensity within a spectrum dramatically increases with larger normalized collisional energy (NCE), from being in the top 20 most intense ions (27 NCE) to the top 3 most intense ions (40 NCE). Increasing the NCE, however, also results in less sequencing information, as we see a five-fold reduction in assigned PSMs. We developed a stepped NCE acquisition workflow that rescues the drop in assignable PSMs while highlighting the diagnostic ion, providing more confident acetyl-lysine assignments. Using the stepped NCE workflow, we see a similar number of acetylated PSMs to those at 27 NCE and the m/z 126 ion remains among the top 5 most intense ions. Our stepped workflow has also been applied to diagnostic ions of other acyl-lysine modifications, with similar increased confidence in the identification of these of acyl-modifications.

Poster Number: P18.07

Proteomic Strategies to Study Oxidative Post-Translational Modifications

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The two sulfur containing proteogenic amino acids, cysteine and methionine, are susceptible to a wide array of oxidative post translational modifications (PTMs). In addition to modifications resulting from oxidative stress, targeted oxidative PTMs have been shown to play important roles in regulating protein function. This poster will describe two projects which seek to elucidate the role of regulatory oxidative PTMs of methionine and cysteine. In the first project we describe the development of a proteomics platform to identify cysteine oxidation sites on interactors of the NADPH oxidase complex in response to EGF activation. The NADPH oxidases (Nox) are the source of H2O2 which acts as a secondary messenger during EGFR activation. Known targets of Nox H2O2 include protein tyrosine phosphatases PTP1B and PTEN. Oxidation of the active site of PTP1B and PTEN temporarily inactivates their phosphatase activity which allows for EGF signal propagation. The envisioned workflow to identify targets of NOX ROS will be to first capture EGF dependent interactors of NOX2, using a temporally controlled, proximity dependent biotinylation platform (BioID), then profile changes in cysteine reactivity (+/-) EGF on the enriched subset of the proteome. In the second project, our goal is to identify proteins which recognize two Met oxidation sites in actin known to play a role in regulating the transition between F and G actin. We utilized a peptide based photo-crosslinking approach to identify PFKL and HSP70s HSPA8 and HSPA1B as punitive "readers" of oxidized or unoxidized methionine in actin respectively.

Poster Number: P18.08

Regulation of Phosphoprotein Phosphatases by Carboxymethylation

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The Phosphoprotein Phosphatases (PPP) are responsible for the majority of serine and threonine dephosphorylation. The PP2A family of PPPs consists of PP2A, PP4, and PP6, which form heterotrimeric holoenzymes consisting of a catalytic, scaffolding, and one of many regulatory subunits. The regulatory subunits are responsible for substrate binding, catalytic activity, and cellular localization. The PP2A-family plays critical roles in DNA damage response, cell cycle control, cell metabolism and survival.

Holoenzyme assembly and activity is in part regulated by carboxymethylation of the C-terminal leucine of the catalytic subunit. This reversible modification is catalyzed by the enzyme LCMT1 and removed by PME-1. While this is well established for PP2A, less is known about the role of carboxymethylation for PP4 and PP6.

Using a chemical proteomics strategy to enrich PPPs, we identify carboxymethylation of PP2A, PP4, and PP6 in several cancer cell lines. We systematically investigate the role carboxymethylation of PP2A, PP4, and PP6 have on holoenzyme composition and activity. Previous studies have employed deletion of the C-terminal leucine as a model for loss of carboxymethylation. We evaluate the validity of this model by comparing it with the effects of loss of function of LCMT1 on PP2A, PP4, and PP6 holoenzyme composition and activity.

We show that the majority of the PP2A regulatory and scaffolding subunits preferentially bind to methylated PP2AC. In contrast, carboxymethylation had only minor effects of PP4 and PP6 holoenzyme composition.

Poster Number: P18.09

Warburg Effect And Histone Lactylation In Human Melanoma Cells

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<p style="margin-left:28.35pt;">The Warburg effect, originally describing augmented lactogenesis in cancer, is associated with diverse cellular processes such as angiogenesis, hypoxia, macrophage polarization, and T-cell activation. This phenomenon is intimately linked with multiple diseases including neoplasia, sepsis, and autoimmune diseases. Lactate, a compound generated during Warburg effect, is widely known as an energy source and metabolic by-product. However, its non-metabolic functions in physiology and disease remain unknown. Recently we reported that lactate-derived lactylation of histone lysine residues serves as an epigenetic modification that

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

directly stimulates gene transcription from chromatin. We had found that hypoxia and bacterial challenges induce the production of lactate by glycolysis, and this acts as a precursor that stimulates histone lactylation. We identified 23 histone marks bearing lysine lactylation in human melanoma A375 cells. Histone lactylation but not acetylation was markedly decreased by a clinical drug PLX4032 (Vemurafenib) in $BRAF^{V600E}$ mutated cells (A375) but not in BRAF wildtype cells (A431). The dynamic changes of histone Kla and Kac were determined by both Western blotting analysis and quantitative proteomics. Collectively, these data may indicate an opportunity to improve our understanding of the functions of lactate and its role in diverse pathophysiological conditions, including immune cells, stem cells and cancer.

Poster Number: P18.10

Derivatization Of Lysine With A Commercial Reagent To Survey Its Post-translational Landscape

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Since the discovery of lysine acetylation in histones, other lysine PTMs have been identified on histones and other cellular proteins. The growing list of unique lysine PTMs demonstrate the wide range of modifications on a single amino acid. The identification and characterization the full extent of lysine modifications remains an elaborate challenge as sensitive quantification methods for each individual modification requires specific and selective purification methods.

We report on the utilization of commercially available biotin n-hydroxysuccinimide ester (Biotin-NHS) for quantification of site-specific lysine occupancy. The reactivity of activated NHS-esters with unmodified lysine side chains at physiological pH has conventionally been used for biotinylation and fluorescent conjugation of proteins. Here, in our current strategy, biotinylation of unmodified lysines assays sites unoccupied by any modification. MS1 signal quantification of exogenously biotinylated precursor ions provides site-specific occupancy changes between a perturbed and normal control.

We will present validations of this quantitative approach with a chemically acetylated protein standard and its application to a complex proteome. This approach is attractive in its capacity to assess global site-specific lysine occupancy and identify novel changes resulting from perturbations such as drug treatment.

Poster Number: P18.11

Detection of Cysteine-to-Thiazole Modifications on Engineered Peptides

Kimberly Wagstaff; Joshua McPhie; Diana Gabriela Calvopina Chavez; John Price; Joel Griffitts

Brigham Young University, Provo,

Post-translational modification (PTM) of polypeptide chains is essential for controlling protein activity in all life forms. In a large family of antimicrobial peptides, PTMs are essential for their biological functions. We are studying the antimicrobial peptide TclE found in the organism Macrococcus caseolyticus. Six Cys residues near the C-terminal end of TclE are converted to thiazoles. These PTMs are installed by the tripartite TclIJN enzyme, which docks with TclE and modifies the six Cys residues, all of which are in distinct sequence contexts. We are researching the mechanism of these post-translational modifications and our current hypothesis is that substrate specificity is conferred on many residues from the modified cysteines, at a docking site on the N-terminal half of the peptide, termed the leader region. Our current goal is to better interpret this docking site. We first co-expressed a GST-TclE fusion and the TclIJN enzyme in E. coli. After purification of TclE and removal of the GST purification tag, we used a targeted MS2 method on an LC-MS Orbitrap Mass Spectrometer. We then used PEAKSX+ proteomics software to identify the peptide TclE along with all installed PTMs. Our data confirms that the TclIJN enzyme installs all six thiazoles with almost 100% efficiency. We are now investigating how the PTM machinery is affected when a series of truncations are introduced to the leader region of TclE. Our preliminary data suggest that within the 35-amino acid leader, the 15 N-terminal residues are dispensable for enzyme-substrate interactions. Refinement of the roles of the remaining 20 amino acids is underway. The work will provide greater understanding of how PTMs are controlled and installed, and provide a foundation for exploring the structure of the TclEIJN complex. This research could lead to the generation of engineered thiazolyl peptides with novel biological activities.

Poster Number: P18.12

Citrullination-specific Fragmentation Features Facilitate Confident Identification Of Citrullinated Peptidese

Min-Sik Kim

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Confident identification of modified peptides in tandem mass (MS/MS) spectra is an abiding challenge in PTM proteomics. For relatively less studied PTMs such as citrullination, database searching followed by manual spectrum validation is often a necessary step for identification of bona fide modified peptides. In order to confidently identify citrullinated peptides, a number of criteria for spectrum validation has been suggested. One example is the use of citrullination-specific diagnostic mass spectral features including immonium ion and neutral loss of isocyanic acid, which have been suggested for confident identification of citrullinated peptides. As a follow up and an extension of this idea, we systematically investigated potential citrullination-specific diagnostic ions and neutral losses using synthetic peptide dataset. We then demonstrated the utility of citrullination-specific diagnostic features in spectrum validation by developing a predictive model to assign confidence level to identified citrullinated peptides. Together, we show that citrullination-specific diagnostic features can facilitate confident identification of citrullinated peptides.

Poster Number: P18.13

Large-scale Open Modification Searching Reveals The Role Of Post-translational Modifications In Protein-protein Interactions

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The analysis of protein-protein interactions is an essential task to understand cellular functions. To this end, recently several ambitious efforts that provide a first large-scale view of the human interactome have been published, such as the BioPlex project. Because the BioPlex project consists of several thousands AP-MS runs, only a single variable modification (oxidation of methionine) was considered during spectral identification to minimize the computational requirements. However, as no further modifications of biological interest were considered, the role of post-translational modifications in protein-protein interactions remains unexplored.

In this work we have used the ANN-SoLo spectral library search engine to reanalyze the BioPlex dataset. ANN-SoLo is optimized for highly efficient open modification searching, which enables us to implicitly consider any type of modification. Compared to the previously published BioPlex results, ANN-SoLo achieves a 53% increase in spectrum identifications corresponding to newly identified modified peptides and proteins. The derived modifications cover both artificial modifications introduced during sample processing, as well as biologically relevant modifications.

Based on the protein identifications resulting from the reanalysis, we have used the SFINX tool to compile an interaction network which was compared to the original BioPlex interaction network. Whereas the new interaction network overlaps to a large extent with the original BioPlex network, we also observe additional interactions and slight changes in network topology driven by the newly identified modified peptides.

To conclude, by utilizing state-of-the-art computational tools for open modification searching we are able to characterize the human interactome at an unprecedented depth to investigate the role of modifications in protein-protein interactions. This leads to highly valuable new insights into how post-translational modifications influence cellular functions.

Poster Number: P18.14

Coenzyme A Binding Sites Induce Semi-Enzymatic Acylation

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N⍰-lysine acylation affects protein in all domains of life. In the mitochondria, N⍰-acylation of proteins is predominately a non-enzymatic process driven by the high pH in the mitochondrial matrix and the high concentration of acyl-CoA species, such as acetyl-CoA and succinyl-CoA. Using previously published datasets generated from mass spectrometry studies mapping acylation in mouse mitochondrial samples (Newman et al. 2013), succinylation (Rardin et al. 2013), and glutarylation (Tan et al. 2015), were downloaded and the fraction of total proteins and total sites found on CoA-binding enzymes were calculated. We found that acetylation, succinylation, and gluterylation are enriched in proteins with a CoA-binding pocket. Using a computational structural model, we showed that acylation is enriched in lysine residues near the CoA binding pocket, suggesting acylation is mediated by Acyl-CoA binding. To confirm that acylation is mediated by Acyl-CoA binding, we co-incubated succinyl-CoA and CoA with Enoyl-CoA Hydratase Short Chain 1 (ECHS1), a mitochondrial protein with a CoA-Binding pocket involved in fatty acid beta-oxidation. We then subjected ECHS1 to mass spectrometric analysis using a TripleTOF 6600 system and data-independent acquisition for quantification of the acylation sites. Overall we monitored and quantified nine ECHS1 acylation sites. We found that succinylation was increased in ECHS1 incubated with succinyl-CoA in a time-dependent manner and found that CoA inhibited succinylation in a concentration-dependent fashion indicating that CoA acts as a competitive inhibitor to succinyl-CoA. Here, we demonstrated a novel 'semi-enzymatic' mechanism of acylation of mitochondrial protein mediated by Acyl-CoA binding. Several metabolic enzymes contain lysine residues that mediate catalytic function near CoA- binding pocket. This may serve as an essential means of regulation of the enzymatic function of CoA-binding protein in the mitochondria.

Poster Number: P18.15

The 2020 Human Phosphoproteome PeptideAtlas

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Tandem mass spectrometry (MS/MS) has become an effective tool for the study of post-translational modifications (PTMs) in proteomics experiments. However, the localization of PTMs where more than one potential site exists is a complex mass spectrum interpretation challenge. Here we present the Human Phosphoproteome PeptideAtlas 2020 build, a compendium of 215 experiments from human phospho-enriched datasets, all uniformly processed to high stringency with the Trans-Proteomic Pipeline (TPP). The processing culminates with PTMProphet, an advanced software tool for the post-processing analysis of MS/MS datasets that contain ambiguous mass modifications. The resulting Human Phosphoproteome PeptideAtlas contains 15.8 million peptide-spectrum matches, double the number of the 2017 build, yet only a 13% increase in the number of distinct peptides at 157,000, and a mere 1.2% increase in the number of canonical proteins. We analyzed the relative proportions of STY residues in various subsets and the localization patterns of nearby phosphosites in order to estimate the total number of human phosphosites accessible with current technologies.

Poster Number: P18.16

Fast And Sensitive Ubiquitination Determination Through Highly Efficient Immunoaffinity Purification And Trapped Ion Mobility Mass Spectrometry

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Protein ubiquitination is a very common posttranslational modification (PTM) within cells. Ubiquitin can be covalently linked to target lysine residues and targets substrates for proteasome-dependent degradation. Ubiquitination can also alter protein function by modulation of protein complexes, localization, or activity, without impacting protein turnover. Ubiquitination imparts critical regulatory control over nearly every cellular, physiological, and pathophysiological process. Affinity purification techniques coupled with mass spectrometry represents an efficient method of identifying PTMs on endogenous proteins. These antibodies recognize the Lys-⍰-Gly-Gly (diGLY) remnant that is generated following trypsin digestion of ubiquitinated proteins, and these peptides can then be identified by standard mass spectrometry approaches. Here we investigate the use of trapped ion mobility (TIMS) for the identification and characterization of lysine ubiquitination (K-ε-GG) peptides immunoenriched from mouse tissue.

Post-translationally modified peptides were prepared from mouse liver protein tryptic digests using PTMScan® kits (Cell Signaling Technology) enriching the ubiquitin remnant K-ε-GG. The resulting extracts were separated by nano HPLC (nanoElute, Bruker) on 250 mm x 75 µm, 1.6 µm (IonOpticks, Australia). 60 min gradients at 400nL/min were analyzed on a trapped ion mobility Q-TOF (timsTOF Pro, Bruker Daltonics) operating in PASEF mode using a modified method optimized for enhanced sensitivity of low level samples. Data were processed in PEAKS X+ software (Bioinformatics Solutions Inc).

Initial analysis of mouse samples enriched for K-ε-GG on the timsTOF Pro resulted in more than 4000 unique modified peptides from 1 mg of starting material using a 60 min gradient. We will apply this method to smaller amounts of starting material.

Poster Number: P18.17

A Novel Automated And Highly Selective Phosphopeptide Enrichment Strategy For Successful Phosphopeptide Identification And Phosphosite Localization

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Phosphopeptide enrichment has remained as a challenge due to the variation of the reproducibility and selectivity caused by manual sample preparation. The labile phosphosite on peptides also makes it difficult to localize the phosphoryl group in a CID experiment. In the Phosphopeptide Challenge of the Human Proteome Project, Agilent AssayMAP Bravo platform provided a fully automated and highly selective phosphopeptide enrichment workflow using high capacity Fe(III)-NTA cartridges. Auto CID of both "Phosphopeptide" sample and enriched sample was performed with triplicate injections. The datasets were searched against Human Swiss-Prot database and the results were combined in Spectrum Mill. HUPO provided 89 peptide sequences for this study. For the "Phosphopeptide" sample, we identified all 89 non-phosphorylated peptides and more than 100 possible phosphopeptides with the number of phosphosites also determined using CID. After enrichment of the "Phosphopeptide-Yeast" sample, all the phosphopeptides were detected from the enriched sample showing >91% selectivity using Fe(III)-NTA cartridges. To preserve and localize the labile phosphosites, targeted ECD experiments were performed with multiple injections of "Phosphopeptide" sample. The targeted MS/MS list was created based on the *m/z*, charge state and retention time of the phosphopeptide. ECD spectra were analyzed with *Byonic* software using the same Human Swiss-Prot database and 96 phosphopeptides including mono-, di-, tri- phosphopeptides with different degrees of phosphorylation and specific locations of phosphosites were successfully assigned. The relative abundance of phosphorylation at each site was analyzed with full MS scan using Skyline software. The ratio of phosphopeptide/non-phosphopeptide were calculated based on ion intensity. The relative quantity of phosphorylation at each site (phosphopeptide/non-phosphopeptide) ranged from zero to about 120 within the "Phosphopeptide" sample.

PRECISION MEDICINE
P19.01 – P19.04

Poster Number: P19.01

Machine Learning and Next Generation Proteomics Driving Precision Medicine

Simon Dillon[1]; Xuesong Gu[1]; Hasan Otu[2]; Nezam Afdhal[1]; Michelle Lai[1]; Detlef Schuppan[3]; Joern Schattenberg[3]; Harland Winter[4]; Towia Libermann[1]

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Proteomics has emerged as the next frontier in precision medicine and several innovative proteomics platforms (SOMAscan, Olink, ScioDiscover) have emerged that are driving biomarker discovery to the next level. We are using the aptamer-based high multiplex SOMAscan and other platforms to discover and independently validate diagnostic, predictive, and prognostic biomarkers for various human diseases. Our efforts are especially focused on non-invasive biomarkers in serum, plasma, urine, and exosomes for a broad range of clinically highly relevant and challenging diagnostic, predictive, and prognostic indications.

The key challenges for biomarker discovery are not the generation of a large dataset, but how to analyze the proteomics data and to generate biomarker models with the highest predictive accuracy. While the majority of diagnostics are still based on single analytes, it is becoming evident that multiplex biomarker panels are more accurate than single analyte diagnostics. However, the challenge is how to select the optimal combination of analytes from a high complexity dataset. We are applying the latest in machine learning approaches for feature selection and for generating predictor models using not only the proteomics data, but also integrating clinical and demographic variables with proteomics data into our modeling strategies.

We will present how machine learning improves development of high accuracy predictor models from proteomics data for applications in disease diagnosis, prediction of therapeutic response and outcome, and monitoring of disease progression. We have developed biomarker models for discriminating NASH from fatty liver disease, different stages of liver fibrosis in NASH, discriminating in IBD Crohn's disease from ulcerative colitis and IBD from non-IBD, as well as identifying biomarkers for early detection of pancreatic cancer.

Poster Number: P19.02

CANCELLED

Poster Number: P19.03

Multiple Biomarker Panels to Predict Resposne to Tocilizumab(anti-IL6R) in Rheumatoid Arthritis Patients Using High-precision Proteomics Approach

Jinwoo Jung; Byoung-Kyu Cho; Kyung-Cho Cho; Ara Cho; Yeong Wook Song; Eugene C Yi

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Rheumatoid arthritis (RA) is a chronical systemic autoimmune disease that cause inflammation in synovial lining layer of the joints. Interleukin-6 (IL-6), along with TNF-alpha and several inflammatory cytokines, acts a vital role in activation of local synovial leukocyte and induction of chronic inflammation. A humanized anti-IL-6 receptor(IL-6R) monoclonal antibody, Tocilizumab (TCZ), has been demonstrated a significant clinical efficacy for RA patients. However, like other inflammatory cytokine blockers such as TNF-alpha, Interleukin-1 (IL-1), or CD20 inhibitors, some patients are still partially responsive or resistant to the treatment. This study therefore aimed at identifying protein biomarkers that could predict clinical response against TCZ in RA patients by implementing high-precision proteomics approach. We first identified 54 serum protein biomarker candidates from a large-scale serum proteome profiling of TCZ responder and non-responder groups. Selected protein biomarker candidates combined with known RA biomarkers from the literature data mining were verified by two different targeted quantification methods; multiple reaction monitoring (MRM) and parallel reaction monitoring (PRM) with Triple-quadrupole (QqQ) and Q-Exactive (QE), respectively. Moreover, we validated the results with 47 individual serum samples using MRM and developed as a multi-biomarker panel. The constructed 4-biomarker panel showed 83% discriminate power in average between response and non-response groups with high AUC value of 0.859. The panel also shows 82% sensitivity and 84% specificity. Collectively, our multi-biomarker panel implies that 4 selected proteins were able to serve as diagnostic assessments to predict the TCZ non-responders in RA patients and possible to supplement serum biomarker discovery-validation process in the clinical field based on integrative proteomic approach.

Poster Number: P19.04

Deep, Reproducible and High-throughput FFPE Analyses: Moving Toward Large-scale Automated Clinical Omics Applications

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Formalin fixation and paraffin embedding (FFPE) tissue preparation is standard in pathology departments worldwide for diagnosis by staining and immunohistochemistry. Analysis of the FFPE samples already collected in current practice is thus a natural entry point for omics methods to inform clinical decisions. Additionally, FFPE samples are uniquely stable at room temperature, resulting in massive retrospective archives often with corresponding diagnoses and disease courses. These collections represent an invaluable resource for retrospective and translational studies.

However, despite this huge potential for research and medicine, proteomic analysis of FFPE samples has lagged. Crosslinked, wax-saturated samples are not in their native state suitable for MS analysis. Additionally, myriad sample processing protocols have not in general been developed to yield flash-frozen results from paired FFPE samples, leaving question as to the validity and utility of their outputs. FFPE sample processing must be automatable and analysis must take place a time scale compatible with future large-scale deployment without making tradeoffs between depth and speed of analysis.

Herein, we combine ProtiFi HYPERsol sample processing and Bruker timsTOF Pro data acquisition to prepare and analyze in one day 50 FFPE samples with great overall reproducibility and proteome depth in an automatable format.

HYPERsol (**H**igh-**Y**ield **P**rotein **E**xtraction and **R**ecovery by direct **SOL**ubilization) sample processing[1] is an FFPE processing system developed specifically to obtain identification rates (97% – 101%) and quantifications (R_{avg} = 0.94) from FFPE that are nearly identical to results from paired portions of the same samples which were simultaneously flash-frozen. The Bruker timsTOF Pro with the PASEF acquisition mode defines new performance standards for shotgun proteomics by affording dramatic improvements in speed, sensitivity and depth of coverage.

We show how the combination of the Bruker timsTOF Pro and ProtiFi HYPERsol FFPE sample processing will potentially facilitate implementation of omics analyses in clinical settings.

[1]https://doi.org/10.1101/632315

PROTEIN COMPLEXES AND INTERACTOMICS P20.01 – P20.13

Poster Number: P20.01

Characterization of DNA Damage, Phosphorylation, and Domain-Specific Interactors of the p53 Transactivation Domains

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The p53 tumor suppressor protein is a multi-domain transcription factor that responds to cellular stresses to induce cell cycle arrest, senescence, and apoptosis. In unstressed cells, p53 is maintained at low levels, but it is stabilized following stress by phosphorylation which abrogates binding of the negative regulator and E3 ligase, MDM2. p53 is phosphorylated on multiple residues in its N-terminal transactivation subdomains (TADs), with different patterns of modification observed in stress-specific responses. Some of the protein interactors of the TADs bind to both subdomains with different effects of phosphorylation between the two domains. Moreover, although both subdomains share some interactors, they are not functionally equivalent. Thus, there should be distinguishing transcriptional cofactors for TAD1 and TAD2 whose interaction is regulated differently by p53 phosphorylation. We have used mass spectrometry combined with affinity purification to identify interactors of the two transactivation subdomains from unstressed and etoposide-treated cells. Additionally, each bait domain peptide was specifically phosphorylated to examine modification-specific interactions. In all, we identified over 100 interactors of p53 TAD1 in both the untreated and etoposide-treated cells present in at least 2 of 3 biological replicates; for the TAD2 experiments, over 400 proteins were identified to be interactors in at least 2 of 3 biological replicates. Similar numbers of interacting proteins were identified for the phosphorylated forms. Comparison of the sets of interactors classified them into those that are domain specific, modification specific, or stress specific. These studies have identified novel interaction partners of p53 that play important roles in regulation of its stability and function. They further open new areas of investigation into the independent functions of the two transactivation domains of p53.

Poster Number: P20.02

Lateral Organization of CD147 with a B7 Protein Confers Resistance Phenotype to Docetaxel in Breast Cancer

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Assembly of integral plasma membrane proteins not only mediates responses to the endogenous and environmental cues that regulate various cellular events, but also maintains intrinsic property of cellular states. Here we aimed to show that the lateral assembly oncogenic proteins with CD147 expressed in CSCs is integral features of breast cancer stem cells. Utilizing the proximal proteomics method with an anti-CD147 antibody probe, we discovered a B7 protein including known stem cell markers, such as CD44, CD133, EGFR that considered to be the nearest neighbor proteins of CD147. This cell surface assembly of CD147 and a B7 protein including other oncogenic proteins was concealed within an assembly of lipid-raft like microdomain, which was further confirmed by confocal microscopy. Sequestration of the unique surface protein assembly confers resistance to docetaxel via Akt-dependent p53 mediated apoptotic signaling pathway. In sum, the lateral interaction between CD147 and proximal partners promotes docetaxel resistance and suggests the key determinant of cancer cell stemness phenotype.

Poster Number: P20.03

Connect The Dots: Proteogenomics And Protein Interaction Networks

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While the Human Proteome Project proposes to identify and characterize a complete picture of the human proteome, its foundation relies on predictions from genomic and transcriptomic information. Indeed, neXtProt and UniProt present useful tools for proteome characterization including extensive curation of evidence. However, a growing literature unveils the existence and functional role of many proteins never before predicted. Here we present a method for the systematic annotation and discovery

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

of yet unannotated novel proteins, OpenProt. Transcriptome wide predictions of alternative open reading frames (altORFs) are combined with existing annotations to augment the tools aimed at a more complete characterization of the human proteome.

OpenProt was used to mine a large affinity purification-mass spectrometry data set with a stringent analytical pipeline and identified over 5,000 novel proteins. These could at once be functionally contextualized within a network of physical interaction. Gene ontology enrichment analysis in the close neighborhood of many revealed possible involvement in a wide array of cellular function.

The implications of this new annotation in proteogenomics studies opens new avenues in the exploration of the genotype-phenotype relationship. SnpEff is a tool for predicting the effects of mutations and single nucleotide polymorphisms on protein and RNA. We adapted it to include putative effects of genetic variations on altORFs and used it to identify over 100 SNVs significantly associated with a variety of pathological phenotypes (including cancers and neurodegenerative diseases). Of these, many align in regions of overlap between altORFs and canonical coding sequences (CDS). Furthermore, 6 were synonymous in the annotated protein but missense in the alternative protein, suggesting that expression of the phenotype may be through the altered altORF rather than the CDS. We show that the integration of sequencing data with MS derived interaction networks reveals potential new players in physiological and pathological modules.

Poster Number: P20.04

Proteomic Analysis of Immune-Challenged Macrophage Myddosome

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Toll-Like Receptors (TLRs), a family of Pattern Recognition Receptors (PRRs), are the first line of defense for the innate immune system. TLRs on macrophages and dendritic cells recognize conserved Pathogen Associated Molecular Patterns (PAMPs) to initiate the expression of pro-inflammatory cytokines and interferons through activating transcription factors. There are ten human TLRs and all are MyD88 dependent, excluding the TLR3 and intracellular TLR4 pathways. Upon stimulation, MyD88 binds to additional proteins to form a large signaling complex known as the myddosome. While nine of the TLRs use myddosomes, each pathway elicits distinct secretomes. Investigation of the formation, composition, and regulation of the myddosome may uncover the cause for the distinct responses. Immortalized Mouse Macrophages (IMM) were metabolically labeled via Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC) for Mass Spectrometry (MS)-based quantitative proteomic measurement of the myddosome during a two-hour time course following stimulation with either LPS, Pam3CK4, R848, or Poly(I:C) that stimulate the TLR4, TLR1/2, TLR7/8, or TLR3 pathways, respectively. Immunoprecipitation of MyD88 and the associated protein complex(es) was performed, followed by tryptic cleavage and analysis with LC-MS on an Orbitrap Velos Pro. Protein identification and quantification was achieved through MaxQuant. Analysis confirmed the IP success and presence of expected associated-proteins in the three MyD88-dependent pathways, such as the IRAK proteins (downstream kinases), as well as proteins used in RNA binding and silencing (such as DICER, Eif2ak2, and Tarbp2). Differing kinetics of the myddosome formation and dissociation have been observed. Proteomic study of the changing temporal profile and post translational modifications of the myddosome will elucidate protein kinetics and expression patterns explaining the previously discovered differences in interferon and cytokine expression after diverse immune challenges. This research was supported by the Intramural Research Program of NIAID, NIH.

Poster Number: P20.05

Quantitative ProteoCellomic Analysis of a Serial Captured Affinity Purified Complex

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Affinity purification followed by mass spectrometry analysis has been a powerful approach to study protein complexes, despite of the problem of non-specific bindings. Here, we established a new workflow to perform pair-wise study of interacting proteins. To validate protein-protein interactions, we combined quantitative proteomics with live cell imaging approach. To improve the quality of affinity purified samples for mass spectrometry analysis, we developed a Serial Capture Affinity Purification method to prepare purified protein complexes containing two interacting proteins of interest for proteomics analysis. Our novel purification approach has successfully enriched the complex containing bait proteins. In addition, the highly purified sample has benefited the application of SCAP purified samples in crosslinking mass spectrometry.

Poster Number: P20.06

Novel Protein Interaction Analysis Platform Reveals A Virus-driven Mechanism For Regulating Peroxisome Morphology

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Protein-protein interactions (PPIs) underlie most protein functions and are fundamental to nearly all biological processes. As PPIs have become a persistent experimental target in biological research, the generation of high-quality protein interaction datasets has become increasingly more accessible and common. However, the integration and visualization of such datasets remains computationally challenging, especially when the goal is to extract information about the temporal and spatial dynamics of PPIs. Here, we developed a web-based platform for the analysis of interaction data and then leveraged this tool to uncover virus-host PPIs that drive virus replication. Our platform enables users to quickly build dynamic interaction networks by synthesizing information across proteomic experiments, automatically integrating protein subcellular localization, abundance, functional annotations, and multiprotein complex information. We first validated our platform by analyzing publicly available interaction datasets and demonstrated its utility for different experimental workflows, including bait affinity purification and proximity labeling (APEX) mass spectrometry approaches. We then leveraged this tool to investigate PPIs at the virus-host interface during infection with the worldwide spread pathogen, human cytomegalovirus (HCMV). We focused on characterizing one of the most important viral proteins, the essential anti-apoptotic protein pUL37x1 (vMIA). By integrating our platform with molecular virology and quantitative mass spectrometry, we showed that spatially- and temporally-controlled PPIs underlie pUL37x1 functions during infection, occurring at the mitochondria and, as infection progresses, at the peroxisome. Using reciprocal isolations, microscopy, and CRISPR-based genetic perturbations, we validated and further characterized the spatiotemporal character and function of these interactions. Our findings expand on known pUL37x1 mechanisms for inducing mitochondrial fragmentation, uncovering its association with the MICOS complex critical for mitochondrial integrity. We further characterize how pUL37x1 regulates peroxisome morphology. Specifically, we discover that pUL37x1 activates the peroxisomal protein PEX11ß to regulate peroxisome shape during infection, a key metabolic aspect of virus assembly and spread.

Poster Number: P20.07

Investigating Protein Quality Control Mechanisms of an Endocrine Protein Misfolding Disorder

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Cells are under pressure to properly fold and assemble thousands of proteins and maintain their proteome integrity throughout changes in cellular conditions and stress. The proteostasis network (PN) helps facilitate the proper folding, trafficking, localization, and secretion of proteins in a process referred to as protein quality control. Perturbations to the PN caused by environmental, age-related, or genetic alterations are linked to a variety of protein misfolding disease states[1]. One such disease is congenital hypothyroidism (CH), resulting from mutations in components of the thyroid hormone biosynthetic pathway. Here, we focus on CH stemming from mutations in the thyroglobulin (Tg) gene encoding a thyroid-specific, large 330 kDa secreted prohormone[2]. We have implemented a multiplexed quantitative affinity purification – mass spectrometry (AP-MS) method to distinguish the wild-type Tg interactome from pathological variants to identify PN dynamics responsible for the aberrant processing of Tg mutant variants[3]. Our results uncover distinct imbalances in PN-client engagement among CH-associated Tg mutations. PN components involved in these imbalances are linked to diverse protein quality control processes including Hsp70/90 assisted folding, disulfide/redox processing, endoplasmic reticulum associated degradation, and N-glycan processing. Using biochemical assays, we have probed the involvement of quality control factors on the dysregulated secretion and degradation of mutant Tg variants. These findings have provided novel insight into the loss of function associated with these mutations and may find broader applicability across other protein folding diseases.

<p style="margin-left:32.0pt;">1. Plate et al. *Trends in Cell Biology* (2017).
<p style="margin-left:32.0pt;">2. Citterio et al. *Nature Reviews Endocrinology* (2019).
<p style="margin-left:32.0pt;">3. Plate et al. *Cell Chem. Biol.* (2019).

Poster Number: P20.08

Degronomics: Mapping the Interacting Peptidome of a Ubiquitin Ligase Using an Integrative Mass Spectrometry Strategy

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Human cells make use of hundreds of unique ubiquitin E3 ligases to ensure proteome fidelity and control cellular functions by promoting protein degradation. These processes require exquisite selectivity, but the individual roles of most E3s remain poorly characterized in part due to the challenges associated with identifying, quantifying, and validating substrates for each E3. We report an integrative mass spectrometry (MS) strategy for characterizing protein fragments that interact with KLHDC2, a human E3 that recognizes the extreme C-terminus of substrates. Using a combination of native MS, native top-down MS, MS of destabilized samples, and liquid chromatography MS, we identified and quantified a near complete fraction of the KLHDC2-binding peptidome in E. coli cells. This degronome includes peptides that originate from a variety of proteins. Although all identified protein fragments are terminated by diglycine or glycylalanine, the preceding amino acids are diverse. These results significantly expand our understanding of the sequences that can be recognized by KLHDC2, which provides insight into the potential substrates of this E3 in humans. We anticipate that this integrative MS strategy could be leveraged more broadly to characterize the degronomes of other E3 ligase substrate receptors, including those that adhere to the more common N-end rule for substrate recognition. Therefore, this work advances "degronomics," i.e., identifying, quantifying, and validating functional E3:peptide interactions in order to determine the individual roles of each E3.

Poster Number: P20.09

Exploring the Genomic and Proteomic Basis for Cell-specific Remodeling within two Proteome-scale Protein Interaction Networks

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Thousands of interactions among proteins organize the human proteome into functional modules whose structures vary dynamically according to cellular context. Defining the full set of interactions and the conditions in which they occur will thus be essential to understanding proteome diversity. To address this challenge, we have employed our high-capacity pipeline for affinity-purification mass spectrometry to create two proteome-scale, cell-specific models of the human interactome. The first, created in 293T cells, results from affinity purification of 10,128 human proteins and encompasses 118,162 interactions among 14,586 proteins; the second results from 5,522 affinity purifications in HCT116 cells and includes 70,966 interactions among 10,531 proteins. Together, these networks reveal extensive customization within each cell line. In general, central core protein complexes are detected equally in both cell lines, while cell-specific interactions link these components together in cell-specific ways, effectively 'rewiring' the interactome to suit each cell's specific needs.

We have sought to understand the biological basis for these cell-specific interactions by integrating complementary genomics and proteomics resources. For example, comparison with gene and protein expression patterns across 29 human tissues (Wang et al. MSB 2019) reveals that proteins with variable interaction profiles also tend to express variably across unrelated tissues; in contrast, components of protein complexes retained across cell lines also express consistently across tissues, confirming their central biological roles. In addition, incorporating genome-scale protein evolution data has revealed that interactions among ancient proteins dating to the dawn of cellular life are conserved at rates 6-8-fold higher than their youngest counterparts. Moreover, integrating genome-wide CRISPR co-essentiality profiles measured across hundreds of cancer cell lines as part of Project Achilles has revealed protein subnetworks that associate both physically and functionally. Finally, we leverage both 293T and HCT116 networks in tandem to confidently link thousands of previously undescribed accessory proteins with core protein complexes.

Poster Number: P20.10

Mapping B7H3 Cytosolic Interactome Using Proximity Labeling Approach

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B7H3 (CD276), an immune checkpoint molecule, is highly expressed on a wide range of human solid tumors and known to inhibit T-cell activation. Despite its crucial role in regulating T-cell function, canonical B7H3 signaling pathways are still elusive. Here we utilize the APEX2 approach to identify interacting cytosolic proteins of B7H3 and putative cell signaling proteins involved in the B7H3 canonical signaling pathways that are associated with B7H3 signaling pathways. We performed proximal proteomics analysis of B7H3 in HEK293 cells by transfecting a construct harboring APEX2 fused at the C-terminus of B7H3 gene. Mass spectrometry analysis of biotinylated proteins in the vicinity of B7H3 cytosolic domains identified 2019 proteins (FDR 1%). KEGG pathway analysis identified a functional interaction network with a significant enrichment of proteins involved in metabolic pathways. The network contains proteins involved in glycolysis and gluconeogenesis pathways such as ALDH1B1, ALDOC, DLAT, DLD, HK2, PDHA1, PFKL, PFKM, PFKP, PGMA, PGK1, and PGM1. Although further investigations are needed to validate the interacting protein complexies of B7H3, we provide a comprehensive repository of potential interactome associated with cytosolic domain of B7H3 in HEK293 cells.

Poster Number: P20.11

Native Protein-Protein Interaction Profiling by SEC-DIA-MS

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Johns Hopkins University, Baltimore, 0

Protein complexes play central roles in all living cells with all aspects. The protein complexes dynamically assemble and dissociate based on cellular functions which provide mechanistic insights into organization of whole biological system and also facilitate to understand disease related pathways. As the technology developed especially mass spectrometry, experimentally large-scale protein-protein interaction research came to scientific research since millennium. However, it is challenging to trap the dynamic weak interactions and no effective method have been reported to globally detect native protein complex in tissue samples.

Typically, comprehensive characterization of protein-protein interactions and protein complexes using mass spectrometry have three different approaches, one is affinity purification of epitope-tagged exogenous proteins with mass spectrometry (AP-MS) which could identify the components of stably human protein-protein interactions and multiprotein complexes. Another approach to characterize protein complexes is using cellular thermal shift assay (CETSA) coupled with multiplexed quantification mass spectrometry that could profile the dynamic protein complexes in cells without exogenous ligands. The third approach is size exclusion chromatography (SEC) to fractionate fixed native protein complexes or native cellular protein extracts with data-dependent analysis (DDA) or (data-independent analysis) DIA mass spectrometry respectively. Either way has its own limitation.

Here, we develop a general integrated experimental and computational SEC-DIA-MS framework to exploit, precisely quantified proteins in each SEC fraction of control and cross-linking fixed protein complexes samples and to systematically compare the retention time change of each protein in SEC for revealing the protein complexes. Through thorough integration of PPI databases, computational analysis strategy and quality control, we created an integrated native PPI and protein complexes profiling approach. Specifically, we use this newly developed approach to mouse liver tissue for the first-time global identification of protein complexes in tissue sample, which would be a great tool for analyzing the native protein complex in clinical samples.

Poster Number: P20.12

Visualizing HDAC1/2 Protein Interaction Networks in Live Cells

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The study of protein interaction networks typically focuses on affinity purifications of protein complexes after cellular lysis. This has been a highly valuable approach for the study of networks but does not necessarily reflect the interactions of complexes in living cells. Here we present an integrated proteomic and microscopy approach to study the assembly of histone deacetylases HDAC1 and HDAC2 into different protein complexes. HDAC1 and HDAC2 are enzymes that tightly regulate the acetylation status of histones and are important targets of anticancer therapeutics. We built HDAC1-HaloTag and HDAC2-HalotTag stable cell lines for the analysis of protein complexes and to determine the proper localization of these enzymes into the nucleus. Next, we developed a quantitative approach to determine the extent of nuclear localization of HDAC1/2 with the HaloTag on different termini. From this analysis, we selected interacting proteins in these networks for additional localization analysis using multifunctional tags in order to develop multicolor approaches for studying HDAC1 and HDAC2 protein complexes and protein interaction networks in live cells.

Poster Number: P20.13

HSP70 Family Molecular Chaperone Clients Identified via UBAIT Proteomics Show Differential Specificities and Stress Response

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HSP70 and HSC70 are members of the heat shock protein 70 (HSP70) family that is expressed in human cells. Both HSP70 and HSC70 are involved in diverse cellular processes including proteostasis, growth, and autophagy, similar to other chaperones. In this study, we use recently developed Ubiquitin-Activated Interaction Traps to identify binding partners and clients of human molecular chaperone: HSP70 and HSC70. Proteins are identified by LC-MS/MS on an Orbitrap Fusion instrument and processed with Proteome Discoverer. Using an ubiquitin-mediated proximity tagging strategy, we show that, despite their high degree of similarity, these enzymes have largely non-overlapping specificities while both proteins show a preference for association with newly synthesized polypeptides but each responds differently to changes in the stoichiometry of proteins in obligate multi-subunit complexes. In addition, expression of an ALS-associated SOD1 mutant protein induces changes in HSP70 and HSC70 client association and aggregation toward polypeptides with predicted disorder. Together these findings show that the ubiquitin-mediated UBAIT fusion system can efficiently isolate the complex interactome of HSP chaperone family proteins under normal and stress conditions.

PROTEIN PHOSPHORYLATION P21.01 – P21.05

Poster Number: P21.01

Defining Kinase-substrate Relationships Using Targeted Protein Degradation and Phosphoproteomics

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Protein phosphorylation is an essential post-translational modification that controls most biological processes. More than three-quarters of all proteins are phosphorylated at one or more sites in human cells. Systematic genome sequencing, gene expression and RNAi studies have implicated deregulation of kinase function in many human diseases, including cancer, diabetes, and neurodegeneration; however, such approaches do not reveal specific signaling pathways and molecular targets. While specific and selective kinase inhibitors exist for a small subset of disease-relevant kinases, most of the kinome remains understudied, and off-target effects of those inhibitors still remain a concern. Thus, there is an unmet need for new technologies to enable the systematic interrogation of human kinase-substrate relationships. Traditional genetic approaches to knock-down specific proteins apply on timescales of days, which precludes these strategies from being used to observe direct kinase substrates. Chemical genetic approaches, such as analog sensitive mutants, provide engineered specificity and rapid inhibition; however, the necessary mutations tend to greatly reduce solubility and activity of the kinase. To address these limitations, we have established a general quantitative chemical proteomics strategy that enables the identification of specific kinase substrates through the use of inducible protein degradation technology. The auxin inducible degradation (AID) system is an emerging approach for the rapid degradation of degron-tagged proteins upon the addition of a plant hormone to mammalian cells. Here, we employ a combination of inducible, rapid protein degradation and mass spectrometry-based proteomics to identify specific kinase substrates of the essential mitotic kinase, Plk1. To benchmark the efficacy of AID degradation to identify kinase substrates, we compare candidate Plk1 substrates generated with inducible degradation to those generated with the Plk1 chemical inhibitor BI2536 and report our findings.

Poster Number: P21.02

Quantitative Proteomics Approach to Study the Dynamic Changes in Phosphorylation Signaling Networks of Phosphoprotein Phosphatases

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Mitosis is characterized by the activation of mitotic kinases and inhibition of phosphoprotein phosphatases (PPPs) resulting in a stark increase in substrate phosphorylation that initiates mitotic progression. This balance of activities is reversed at the metaphase-to-anaphase transition. Furthermore, phosphorylation and dephosphorylation of mitotic kinases and PPPs is crucial for the generation of switch-like transitions that separate cell cycle phases and their distinct phosphorylation thresholds. While kinases have been extensively studied in this regard, we are just starting to elucidate PPP regulation. Here, we used a chemical proteomics approach to profile cell-cycle specific protein phosphatase and kinase interactomes using phosphatase-inhibitor conjugated beads (PIBs) and multiplexed kinase-inhibitor conjugated beads (MIBs) respectively. We further applied quantitative mass spectrometry to globally investigate phosphorylation-dependent regulation of PPPs in mitosis. We demonstrate that combining PPP and kinome profiling can provide a more comprehensive view of the dynamic changes in phosphorylation signaling networks and identify specific actions and counteractions between PPP and kinase family of enzymes. Using this strategy, we were able to identify 392 phosphorylation sites on PPP interacting proteins that significantly (fold-change mitotic/asynchronous > 1.5-fold, p-value < 0.05) increase and 79 sites that significantly (fold-change mitotic/asynchronous < 1.5-fold, p-value < 0.05) decrease in mitosis. Furthermore, we identify that Cyclin-dependent kinase 1 (Cdk1) phosphorylates the catalytic subunit of PP2A (PP2Ac) on threonine 304. This phosphorylation disrupts PP2A-B55 holoenzyme formation and thereby regulates mitotic entry and exit through alteration of substrate phosphorylation. Thus, direct phosphorylation of PP2Ac by Cdk1 contributes to the generation of phosphorylation thresholds that separate cell cycle phases.

Poster Number: P21.03

Phosphoproteomic Profiling and Causal Analysis Reveal Signaling Relations in GPVI-Mediated Platelet Activation Programs in Health, Aging and Disease

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Platelets are essential to vascular repair, responding to endothelial damage through adhesion, secretion and aggregation processes. These rapid platelet responses are initiated by glycoprotein and immunoreceptor tyrosine-based activation motif (ITAM) receptors (i.e., GPVI-FcgR) and progress in specific vascular injury contexts. Despite knowledge of key intracellular mediators critical to platelet function, it remains unclear how multiple signals coordinate platelet responses. We recently developed a high-throughput workflow using multiplex tandem mass tag (TMT) labeling, quantitative proteomics tools and causality analysis algorithms to measure and map signaling events underlying platelet function. Our analyses reproducibly measure over 2,000 significant tyrosine, serine and threonine phosphorylation events specifically in response to the platelet ITAM/GPVI agonist collagen-related peptide (CRP-XL). With a newly developed computational tool, CausalPath, we identify and map over 200 site-specific signaling relations between these events that center on Syk, PLCg2, and PKCd. We also identify activating phosphorylation events on more novel effectors, including KSR1, SOS1 and Rab7, which we characterize as regulators of platelet function. In addition to comprehensively mapping signaling relations and illuminating unrecognized mechanisms of platelet regulation, our work provides a platform to generate novel, mechanistic and testable hypotheses from omics data sets. Subsequent discovery-driven efforts will further identify essential hemostatic effectors as well as biomarker and disease targets specific to aging, inflammation, thrombosis and other pathologies associated with aberrant platelet functional phenotypes.

Poster Number: P21.04

Dali: a Method to Identify Phosphosites That Alter Thermal Stability Proteome-wide

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Proteomics has enabled the cataloguing of 100,000s of phosphosites, however we lack methods to systematically annotate their function. Here we developed Dali, a proteomic method to assess the effects of phosphorylation on protein thermal stability.

Dali applies the Proteome Integral Stability Alteration method where protein extracts are treated with a range of temperatures and the resulting soluble protein is combined prior to MS analysis to measure the area under the protein melting curve. To reliably compare phosphopeptides to proteins, we normalize measurements with a 30°C-treated proteome internal reference, obtaining relative thermal stability measurements for phosphopeptides and proteins.

We applied Dali to the *S. cerevisiae* proteome and obtained reproducible thermal stability measurements for proteins and phosphopeptides. We find that the stability of phosphopeptides correlates well with the stability of their respective proteins, suggesting that most phosphosites do not alter protein stability, challenging previously reported findings. Our analysis yielded 71 phosphoisoforms out of 2,345 (3%) with significantly different thermal stability than their unmodified protein, many of which can be associated with modulating protein structure and interactions.

Among the phosphosites showing significant stability effects, phosphoSer56 on the 20S proteasome subunit PUP2 was destabilizing relative to PUP2 and other proteasome subunits, suggesting Ser56 phosphorylation may dissociate PUP2 from the 20S proteasome. Also, we measured the stability for six phosphosites on PGK1, of which only phosphoThr331 showed significantly decreased stability, agreeing with predicted stability effects of phosphomimetic substitutions on PGK1. Additionally, known functional RPL12 phosphoSer38 was stabilizing, supporting increased binding affinity to known interactor EF-2 at the ribosome. Collectively, Dali enables the identification of phosphosites with effects to protein structure or interactions proteome-wide. Our method can be extended to other model organisms and cell culture systems, as well as to other post-translational modifications, expanding the proteomic toolkit to functionally annotate dynamic protein modifications at scale.

Poster Number: P21.05

Proteomic and Phosphoproteomic Changes in Rat Skeletal Muscle Induced by Acute Endurance Exercise

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Despite the broad health benefits of physical activity, an in-depth understanding of the molecular mechanisms underlying the exercise response remains incomplete. Thus, the Molecular Transducers of Physical Activity Consortium (MoTrPAC) aims to provide a rich, multi-omic framework for investigating the effects of exercise across the body. Elucidation of changes to the proteome and phosphoproteome of skeletal muscle—a key tissue involved in the locomotive and bioenergetic processes of exercise—has been hampered by experimental challenges including small cohort size and low proteome coverage stemming from the issue of high dynamic range/high abundance of giant structural proteins. We present initial findings from an animal model of acute exercise using data available in the MoTrPAC External Data Release. Utilizing tandem mass tag (TMT) labeling and multiplexing in combination with offline sample fractionation and IMAC-mediated phosphopeptide enrichment prior to LC-MS/MS analysis, the global proteome and phosphoproteome of rat gastrocnemius muscle was evaluated at multiple timepoints following an acute endurance exercise bout, ranging from immediate to 24 hours post-exercise. Overall, global protein expression levels showed major differences based on sex, while the effects of acute exercise on protein abundance in this timeframe were minimal. In contrast, we observed major perturbations to the phosphoproteome of skeletal muscle, with the most significant changes detected immediately post exercise. Phosphorylation changes were observed on proteins involved in pathways known to participate in the exercise response—i.e. MAPK signaling and insulin signaling—as well as novel effects, such as changes to the phosphorylation state of sarcomere structural proteins. This work, in combination with multi-omic analyses of multiple additional tissues types, represents an opportunity to significantly expand our knowledge of the effects of physical activity on tissue-specific proteomes while contributing to the efforts of MoTrPAC to build an integrative molecular map of the response to exercise.

PROTEOFORM BIOLOGY
P22.01 – P22.03

Poster Number: P22.01

Discovering Alternative Protein Isoform Switches in iPSC Cardiomyocytes by Integrated Omics and JCast.py

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Introduction. Alternative splicing is a post-transcriptional mechanism that fine-tunes the structures and functions of proteins encoded by the same genes, and is increasingly recognized to be a key regulator in eukaryotic cell development as well as in disease progression. Despite advances in transcriptomics to identify alternative isoforms, the protein-level translational status of alternative splicing events and the functional significance of protein isoform switches remain poorly understood.

Method. In this study, we developed an RNA-seq guided method (JCast.py) to generate tissue-specific protein sequence databases from 12 human tissues. Using these databases, we reanalyzed ~80 million mass spectra in public proteomics datasets retrieved from PRIDE. In parallel, we applied this proteotranscriptomics method to obtain original quantitative mass spectrometry data on a Q-Exactive HF to determine isoform regulation during human induced pluripotent stem cell cardiomyocyte differentiation, and performed PRM validations with synthetic isotope-labeled peptide standards on a number of isoform peptides previously undocumented on TrEMBL and RefSeq.

Result. We recovered >1,500 noncanonical alternative isoforms across the 12 human tissues from public datasets and observed widespread isoform shifts during human iPSC-cardiomyocyte (CM) differentiation (e.g., downregulation of a TPM1 isoform in day 14 vs 7). PRM validated alternative isoform sequences from MYOM1, TENX, SVIL, RYR2, NDUA5, and MYBPC3. We further discovered that splice isoforms preferentially overlap with disordered protein regions and are enriched in known PTM sites, suggesting alternative splicing events may modulate protein functions through direct elimination or addition of PTM sequences.

Conclusion. We describe an integrated omics and computational approach to identify, quantify, and validate protein alternative isoforms. Our work revealed alternative isoforms previously unidentified in public proteomics datasets, and proteome-wide isoform switches during iPSC-CM differentiation from MS experiments.

Poster Number: P22.02

Proteoform Enzymology: Quantitation of the Differential Proteoform Substrate Specificity of p300 and CBP

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Proteoforms arise from a single gene due in part to enzymatically mediated post-translational modifications. Here we present a novel in vitro proteoform enzymology method and use it to reveal the fundamental mechanism by which gives rise to only some proteoforms in vivo: proteoform substrate specificity. Histone acetylation is involved in gene regulation, most notably, aberrant regulation of histone acetylation is a hallmark of several major human diseases. Although lysine acetyltransferases (KATs) have been previously characterized as acetylating multiple lysine residues on histones, how factors such as enzyme complexes or external stimuli such as KAT activators and inhibitors alter KAT specificity remains elusive. We use top-down proteomics in an in vitro enzymology assay with recombinant protein and a complex mixture of substrate proteoforms to quantitate the inherent proteoform specificity of the KATs p300 and CBP and derive kinetic parameters.

We recapitulate much of the apparent in vivo proteoform specificity of acetylation with p300/CBP in a purely in vitro enzyme kinetics method. p300/CBP are ubiquitous KATs that are responsible for the majority of lysine acetylation observed on histone tails. p300 and CBP modify the same sites, H4K5, K8, K12, and K16, and are thus often considered redundant. Here we show significant differences in their substrate recognition and KAT activity. CBP follows a strict hierarchical order of sites and acetylates processively. p300 is also proteoform specific but exhibits lower fidelity to only part of this hierarchy. Surprisingly we note that CBP, and not p300, is selective for K20 methylation states in vitro. K16ac is only added to those substrates containing K20me2/3. Functionally these suggest distinct roles between p300 and CBP: CBP may play a more important role in acetylating heterochromatin while p300 may have more functionality in euchromatin maintenance and facile regulation of active genes.

Poster Number: P22.03

Measuring the Effect of Missense Variants on Protein Stability Proteome-wide

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Experimentally measuring the effect of mutations in diverse proteins would improve how accurately we can predict the consequences of genetic variation. To this end, we are developing a method that measures the functional effects of amino acid substitutions in hundreds of proteins in a single experiment. In this method, we induce mistranslation to create proteome-wide libraries of variants during protein synthesis. We then impose biochemical selections on the pool of variants and use mass spectrometry to quantify each variant and its wild type counterpart before and after selection. To generate variants, we have engineered mutant tRNAs that mistranslate serine at levels of 5-15% at specific non-serine codons in yeast. Next, we used the thermal proteome profiling method to measure the thermal stability of variant proteins that contain one of six different amino acids in place of serine. In total, we measured the effects of 1631 serine variants in 432 proteins. Although most substitutions had no effect on thermal stability, we found that 15% of variants significantly altered stability, most of which were destabilizing. Furthermore, we identified and validated examples of destabilizing variants in homologs of clinically-relevant proteins. We are currently developing selections that interrogate other aspects of protein function, as well as engineering additional tRNAs to substitute diverse amino acids at many different codons. Ultimately, our goal is to measure the functional effects of many different substitutions at all sites in the proteome to predict clinically and biologically relevant variation.

PROTEOMICS IN MICROBIOLOGY
P23.01 – P23.03

Poster Number: P23.01

Characterizing haracterizing Bacterial Translational Reprogramming Under Stress Using a Synthetic tmRNA Platform

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Translational reprogramming is recognized as a key component of the bacterial stress response, affecting the stability of mRNAs and polypeptides. Total proteome measurements provide insight on the stable proteome but can fail to capture dynamic changes. This is particularly true under stress, where incomplete polypeptides will be produced from cleaved mRNAs or stalled translation events. To deal with this stress, bacteria possess transfer-messenger RNA (tmRNA), naturally evolved tRNA-mRNA hybrid that rapidly resolves stalled translational complexes and tags nascent incomplete polypeptides for degradation. A short tag reading frame (TRF) encoded on tmRNA facilitates the addition of a peptidyl degradation tag to the polypeptides. Identification of these incomplete polypeptides has the potential to reveal previously unknown aspects of the bacterial stress response and close the information gap between the proteome and transcriptome. To address this gap, we have developed an engineered tmRNA platform that reprograms the native system to allow for isolation of incomplete polypeptides in live bacteria. To study translational remodeling under stress, we modified the tmRNA sequence by replacing native TRF with a 6x-Histidine isolation epitope to stabilize and isolate incomplete peptide products. We have successfully demonstrated isolation of 6xHis-tagged polypeptides, verified inducible control over tagging, and assessed broad-spectrum tag introduction with mass spectrometry. Our results reveal that tagged protein profiles are markedly different under distinct stress conditions, including peptides unique to tagged proteomes and protein-specific changes in the abundance relationships between tagged and total proteomes. In our most recent study investigating translational stress by serine hydroxymate, we have excitingly found significantly fewer unique peptides identified in challenged versus untreated cells despite identifying the same number of proteins. We envision our synthetic tmRNA platform will become a powerful new tool that dramatically expands our understanding of translational reprogramming in bacteria and explains discrepancies between transcriptome and proteome profiles under stress.

Poster Number: P23.02

The Metabolic State of a Syntrophic Bacterium Affects the Proteomic Acylation Profile

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Post-transitional modifications (PTMs) play a critical role in tailoring protein function. One of the most common PTM classes is lysine acylation, a class that includes acetylation, a ubiquitous modification distributed throughout the domains of life. These can occur either enzymatically or spontaneously in response to the presence of acyl-coenzyme A. Syntrophomonas wolfei are

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Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

a bacterial species of environmental importance. They tend to acquire high concentrations of CoA intermediates. This study examines the extent of acyl modifications present in this bacterium, the conditions that affect their levels, and the putative roles these modifications may play. The acyl modifications investigated were acetyl, butyryl, crotonyl, and others. Cells were grown on crotonate or butyrate, and as either pure cultures or with a methanogen to vary pathway kinetics. Label-free quantitation compared relative levels of the modifications. Data was collected on a QExactive orbitrap mass spectrometer. The proteome was also analyzed using an Exploris480 orbitrap system, revealing more modified proteins in the syntrophic microbe. Several hundred acyl modifications were identified in our system, even without enrichment for modification. The modifications changed with different cultivation conditions, with butyrylation most abundant from butyrate cultures and acetylation more pronounced from crotonate-grown cells. The modifications themselves were most prominent in pathways that utilized CoA intermediates as substrates or in associated pathways. Future efforts will be focused on determining the physiological impact of acylation on metabolically-important proteins and on understanding how cells regulate the extensive acylation.

Poster Number: P23.03

Functional Changes in the Murine Intestine Elicited by Fungal Colonization

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Gut microbiota-derived molecules modulate immune development, yet the contribution of gut fungi remains elusive. Our previous work showed that mice colonized only with fungi displayed features related to <pwa class="pwa-mark pwa-mark-done" data-pwa-category="grammar" data-pwa-dictionary-word="atopy" data-pwa-heavy="false" data-pwa-hint="Possible confused word" data-pwa-id="pwa-4DB3D09C9CE72129C853F6975A78F7C9" data-pwa-rule-id="CTX_CONFUSION" data-pwa-suggestions="atop">atopy</pwa> in early life, increased airway inflammation following an allergen challenge, and metabolite profiles similar to germ-free mice. The latter finding suggested that fungi do not modulate the host immune system via metabolites. To functionally analyze the observed changes, we profiled the mice fecal <pwa class="pwa-mark pwa-mark-done" data-pwa-category="spelling" data-pwa-dictionary-word="metaproteomes" data-pwa-heavy="false" data-pwa-hint="Unknown word: metaproteomes" data-pwa-id="pwa-902CCDD6A5DED31BC6A850A36E83A012" data-pwa-rule-id="SIMPLE_SPELLING" data-pwa-suggestions="metaproteins">metaproteomes</pwa> by LC-MS/MS. Label-free quantification showed broad changes in the amounts of bacterial proteins (60% from 2,834) among the different mice groups, reflecting <pwa class="pwa-mark pwa-mark-done" data-pwa-category="grammar" data-pwa-dictionary-word="ecological" data-pwa-heavy="true" data-pwa-hint="Possible missing determiner" data-pwa-id="pwa-2A42D3B50B0C5B45A28F825904E58352" data-pwa-rule-id="MISSING_DETERMINER_CE" data-pwa-suggestions="the ecological">ecological</pwa> responses of individual bacterial species. Many of the detected fungal proteins (totally 1,454) have <pwa class="pwa-mark pwa-mark-done" data-pwa-category="style" data-pwa-dictionary-word="been shown" data-pwa-heavy="false" data-pwa-hint="Passive verbs make your writing less direct. Try to use an active verb instead." data-pwa-id="pwa-518656CCD08093F1367291CAADD55DDF" data-pwa-rule-id="PASSIVE_VOICE" data-pwa-suggestions="">been shown</pwa> to be excreted via extracellular vesicles and to possess immunomodulating properties. Different microbial colonization elicited substantial changes in the host fecal proteome, as 66% of the quantified mouse proteins (297) showed changes in abundance. We also looked at the mouse proteome in the small intestine by using <pwa class="pwa-mark pwa-mark-done" data-pwa-category="grammar" data-pwa-dictionary-word="quantitative" data-pwa-heavy="true" data-pwa-hint="Possible missing determiner" data-pwa-id="pwa-9DF622F0981D88D0D661057DEEE327DD" data-pwa-rule-id="MISSING_DETERMINER_CE" data-pwa-suggestions="a quantitative">quantitative</pwa> tandem mass tag labeling approach. Changes in the protein levels (4% of 1,514 proteins) <pwa class="pwa-mark pwa-mark-done" data-pwa-category="grammar" data-pwa-dictionary-word="associated" data-pwa-heavy="true" data-pwa-hint="Incorrect verb form" data-pwa-id="pwa-BFE62F9FE280D1B3AEA23B21FF20CCF3" data-pwa-rule-id="WRONG_VERBFORM_CE" data-pwa-suggestions="are associated">associated</pwa> with essential cellular pathways, including lipid metabolism and apoptosis. Overall, the results suggest that fungi can alter host immune homeostasis that is developing during early life, possibly through direct cell-to-cell contact.

PROTEOMICS OF DISEASE
P24.01 – P24.22

Poster Number: P24.01

Identification of Early Candidate Urine Biomarkers for Measure Escitalopram Treatment Response From Major Depressive Disorder

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Major depressive disorder (MDD) is a common mental disorder that can cause substantial impairments in quality of life. Clinical treatment is usually built on trial and error method, which lasts about 12 weeks to evaluate whether the treatment is efficient or not, leading some inefficient treatment measures. We therefore identify early candidate urine biomarkers to predict efficient treatment response in MDD patients. In this study, urine samples were collected from 10 respondent and 10 non-respondent to escitalopram MDD patients with 0-, 2-, and 12-week treatment. Differential urinary proteins were then analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). On week 2, there were 14 differential proteins identified in response group when compared with week 0 and these proteins can't be enriched by random allocation analysis, 10 among which have been associated with the mechanisms of MDD. While in non-response group, there were 35 differential proteins identified on week 2, and 18 among which have been associated with the mechanisms of MDD. In addition, differential urinary proteins on week 2 between respondent and non-response group can be distinguished clearly by using Orthogonal Partial Least Squares Discrimination Analysis (OPLS-DA). Our small pilot results indicated that the urine proteome can reflect early changes between respondent and non-respondent group to escitalopram therapy since on week 2, which will provide potential early candidate urine biomarkers to predict efficient treatment measures in MDD patients.

Poster Number: P24.02

Lipid and Apolipoprotein Changes in Response to Inflammation With Type 2 Diabetes

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Type II Diabetes (T2DM) patients often exhibit elevated levels of pro-inflammatory cytokines including TNF, IL-1, and IL-6. These markers were also associated with altered lipid metabolism, most commonly observed in the form of high triglyceride (TG) levels due to increased supply of fatty acids to the liver. Altered liver functions

also lead to changes in the levels and composition of plasma lipid carrying lipoproteins (HDL, LDL and VLDL). We attempt to correlate lipoprotein composition characteristics with typically observed pro-inflammatory cytokine levels in controls and T2DM patient samples.

We performed quantitative MS measurements of over 20 proteins, including 8 apolipoproteins (ApoA-I, ApoA-II, ApoA-IV, B-100, ApoC-I, ApoC-II, ApoC-III and E), and lipids in HDL and LDL fractions, isolated from serum using size exclusion chromatography. Quantitative cytokine measurements were performed using a commercial multiplex ELISA assay (Luminex).

In comparisons of T2DM and normal donors, initial data shows statistically significant negative (P≤0.05) correlation between ApoA-I/ApoA-II ratio and Leptin levels, the primary hormone responsible for signaling that energy needs are met for the body (i.e. feeling full). ApoC-III per HDL particles were also higher, corresponding with high TG levels, indicating enhanced TG-CE exchange with elevated concentration TG-rich LDL/VLDL particles and reduced lipase activity. SAA1 and SAA4, inflammatory proteins associated with both HDL and LDL, were also elevated in T2DM samples. These conditions are consistent with increased circulation time of LDL particles which likely trigger inflammatory response.

Poster Number: P24.03

Proteomic Analysis of 200 Barrett's Esophagus Cells Identifies Novel Biomarkers Induced by Lithocholic Acid

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Esophageal cancer is the eighth most prevalent cancer in the world with an estimated 456,000 new cases (3% of all cancers) and 400,000 cancer deaths (5% in all cancer deaths) in 2012. Survival rate is less than 20% for 5 years. Barrett's esophagus is a disorder in which the normal squamous mucosa in the esophagus is replaced by metaplastic columnar epithelium and is the highest risk for esophageal adenocarcinoma. Barrett's esophagus is linked to acid reflux disease that is stimulated by bile-acids of which a potent component is lithocholic acid (LCA). Patients with Barrett's esophagus have at least ten times greater risk of developing esophageal adenocarcinoma. In a pilot project designed to work with as few cells as possible we chose the microPOTS proteomic platform (Xu et al. Anal Bioanal Chem. 2019) to analyze samples containing ~ 200 Barrett's esophagus cells (CPA) of different isogenetic status (CPA) to define proteins that are induced by LCA. The microPOTS method enabled the detection of differentially expressed proteins between wild type CPA, p53 knockout (KO) and p53 Smad4 KO CPA treated with lithocholic acid or X-ray, including NDRG1. Specifically, more than 1500 protein groups were identified from CPA samples containing ~ 200 cells increasing to more than 2000 identified protein groups when results from three wells from the same sample type were pooled together. An overlap of 67 – 77% was observed between different sample types. We will present the trade offs of being able to work with so few cells using the microPOTS method and discuss the biological relevance of select key proteins.

Poster Number: P24.04

A Streamlined Protocol for Protein Extraction From Bone

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Identifying shifts in protein composition of bone is fundamentally important to understanding musculoskeletal aging and disease. Existing methods for extracting proteins from bone are either extremely time consuming or inappropriate for downstream mass spectrometry. To shorten the time required to isolate proteins for untargeted proteomic studies, we compared three methods of extraction, with efficacy determined by amount and variation of proteins obtained.

The protocols varied in preparation of the bone (grinding vs. not grinding) and length of incubation with each solvent. All protocols included a PBS wash and four sequential solvents following Jiang et al. 2007 (Journal of Proteome Research): 1.2M HCl, 100mM Tris 6M guanidine-HCl pH 7.4, 100mM Tris 6M guanidine-HCl 0.5M Tetrasodium EDTA pH 7.4, and 6M HCl. This method has been shown to produce large and diverse protein yields suitable for LC/MS analysis.

We collected replicate samples of previously frozen (-20C) baboon lumbar vertebrae. For protocol 1 and 2, we left the 10 x 2 mm sections intact and for protocol 3, we pulverized the sample after freezing in liquid nitrogen. All samples were washed overnight in 1X PBS, while rotating at 4C. Subsequently, we incubated protocol 1 samples sequentially in each solvent for 72 hours at 4C while rotating with two washes of milli-Q water in between. Protocol 2 and 3 samples were incubated for 24 hours. Extracts were stored at -80C until protein was precipitated in acetone. Precipitation was repeated to remove impurities from bone extracellular matrix.

Protein abundance was quantified using a DeNovix Spectrophotometer. Despite a shorter incubation time, the grinding protocol resulted in 56.5% more total protein than the longer incubation without grinding. The shorter incubation without grinding performed worst. Based on SDS-PAGE, similar heterogeneity of proteins was present for all methods. Mass spectrometry results will further evaluate these methods.

Poster Number: P24.05

Differential Regulation of Proteoforms in Human Hypertrophic Cardiomyopathy Revealed by Top-down Proteomics

Trisha Tucholski[1]; Wenxuan Cai[1]; Stanford D. Mitchell[1]; Zachery R. Gregorich[1]; Elizabeth Bayne[1]; Sean McIlwain[1]; Max Wrobbel[1]; Hannah Karp[1]; Zachary Hite[1]; Petr G. Vikhorev[2]; Steve B. Marston[2]; Sean Lal[3]; Amy Li[3, 4]; Cristobal dos Remedios[3]; Takushi Kohmoto[1]; Joshua Hermsen[1]; J. Carter Ralphe[1]; Timothy J. Kamp[1]; Richard L. Moss[1]; Ying Ge[1]

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Hypertrophic cardiomyopathy (HCM) is the most common heritable heart disease. While the genetic causes of HCM are secondary to mutations in genes that encode sarcomeric proteins, the molecular mechanisms by which these mutations precipitate contractile dysfunction and hypertrophy remain exceedingly complex. It is well-established that post-translational modification (PTM) of sarcomeric proteins regulate cardiac contractile function. To better understand the molecular aspects of HCM, we sought to identify changes in sarcomeric protein proteoforms, or all of the protein forms arising from events such as PTMs and alternative splicing, in tissues collected from HCM patients using quantitative top-down proteomics. We characterized a panel of sarcomeric proteoforms in septal myectomy tissues from HCM patients (n=16) and non-diseased tissues from healthy donors (n=16). Altered sarcomeric protein proteoform levels were consistently found in the myocardium of HCM patients compared to those of healthy donors, regardless of HCM-causing mutations. Particularly, we observed a significant decrease in cardiac troponin I and enigma homolog isoform 2 phosphorylation, mediated by protein kinase

A. In addition, phosphorylation levels of the myofilament proteins α-tropomyosin (α-Tpm), myosin light chain 2, and cardiac troponin T were significantly altered in myectomy tissues compared to donor tissues. We identified two phosphorylated isoforms of the key LIM domain-containing Z-disc protein, cypher (isoforms 5 and 6), with cypher 5 phosphorylation significantly up-regulated in HCM tissues relative to donor tissues. Furthermore, we identified cysteine rich protein 2 to be thiol-sulfenylated, a PTM not previously reported for sarcomeric proteins, with significant decrease in HCM tissue. We also observed isoform switching between α-Tpm/β-Tpm, and expected changes in the expression of ventricular/atrial myosin light chain 1 and cardiac/skeletal α-actin isoforms. Taken together, these data suggest HCM-causing mutations activate convergent hypertrophic signaling pathways to yield comparable alterations to sarcomeric protein PTMs, and may underlie the abnormal contractile function and pathological hypertrophy phenotypes.

Poster Number: P24.06

Proteomic-level Helicobacter Pylori Protein Array and Immuno-profiling of Gastric Cancer Patients

Lusheng Song[1]; Minkyo Song[2]; Charles S. Rabkin[2]; Jennifer Van Duine[1]; Linda M Liao[2]; Kailash Karthikeyan[1]; Weimin Gao[1]; Jolanta Lissowska[3]; M. Constanza Camargo[2]; Ji Qiu[1]; Joshua LaBaer[1]

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Background: Although *Helicobacter pylori* (*Hp*) is a known risk factor for gastric cancer (GC), half of the world's population is infected and yet only 1-3% infected individuals develop GC in their lifetimes. Differences in the immune response to *Hp* may modulate GC risk. A full proteomic-level immune-profiling of *Hp* would provide a systematic understanding of humoral response contributing to GC risk.

Methods: We developed a *Hp* nucleic acid programmable protein array (*Hp* NAPPA) with a total of 1527 *Hp* genes (1453/1590 [91%] from strain 26695, and 74 from strain J99). GC patients (n=150) and population-based controls (n=150) were selected from a population-based GC case-control study in Poland. Anti-*Hp* serologic responses for 50 GC cases and 50 controls were measured on *Hp* NAPPA and the seropositivity was defined as MNI (median normalized intensity using the median of the raw intensity of all antigens) ≥2.0. Anti-*Hp* antibodies elevated in either GC cases or controls were selected for further validation with the remaining set of 100 GC cases and 100 controls using ELISA. Discriminatory power for statistically significant antigens was assessed using odds ratios (OR) and a panel was built with lasso logistic regression.

Results: A total of 53 *Hp* proteins with more than 10% seropositivity were discovered in either GC cases (0%-70%) or controls (4%-84%). Interestingly, GC cases had an overall *lower* serological response to immunodominant proteins (median, 12%) compared with population-based controls (median, 25%) (p <0.01). Six anti-*Hp* antibodies (to HP1118, HP0516, HP0243, HP1293, HP0371, HP0875) were significantly decreased in GC with OR<0.5 (p<0.05). A panel based on anti-HP1118, anti-HP0516, anti-HP0243, and anti-CagA had AUC=0.73.

Conclusion: We presented the first comprehensive immunoproteomic assessment of anti-*Hp* humoral profiles in GC. Depressed immune response to multiple *Hp* proteins in GC may reflect the decreased burden of *Hp* proteins and a higher risk of GC.

Poster Number: P24.07

Effects of Protein Structure on Rheumatoid Arthritis

William Thompson; John Holman; Hsien-Jung Lavender Lin; John Price

Brigham Young University, Provo,

Rheumatoid Arthritis (RA) is an autoimmune disease which causes swelling of joints and leads to bone erosion. It can cause premature death, disability and lowers the quality of life. Knowledge of the RA mechanism is minimal, making early diagnosis challenging. Current diagnosis depends on finding a rheumatoid factor which is plagued by low specificity. Research has shown that an early diagnosis for RA can lead to better treatment and a longer life span. Thus, it is important to create diagnostic methods that can detect RA at an early stage of development. We recently compared blood serum heat denaturation curves from 25 RA patients versus 25 healthy controls and saw a significant difference in the curves between the RA and non-RA samples. This difference is a mass average measurement of the most abundant proteins of human serum, where Serum Albumin is the most abundant protein in serum overall. Serum Albumin is a lipid carrier, which is important because lipid cargos might modify the overall structure and the heat of denaturation. It is known that protein modifications can affect the protein structure, and denaturation temperatures. We found evidence that surface accessible amino acids are different between healthy and disease groups. We tested whether an endogenous PTM or a change in lipid cargo carried by Albumin would alter the protein structures first, which would then lead to a difference in the heat denaturation curves. We created samples enriched in Serum Albumin through a Multiple Affinity Removal Column to separate the main proteins, and then examine the lipids associated with Albumin. Post translational modifications of the abundant proteins were also examined for all 50 samples, while lipidomic cargo of Serum Albumin was also examined. Here we will report the PTM and lipid composition differences between the RA and non-RA samples.

Poster Number: P24.08

Identification of Novel Cancer Stem Cell Specific Transcription Factors for B7H3 Based on DNA Affinity Purification Coupled to Mass Spectrometry

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[1]Seoul National University, Seoul, South Korea; [2]Kangwon National University, Chuncheon, South Korea

B7H3, a member of the B7 family, is known as the immune checkpoint moleucule. Recent studies have shown that it has a co-inhibitory role on T-cells, contributing to tumor cell immune evasion. In addition, our previous study showed that the expression of B7H3 was increased in cancer stem cell (CSC) - like cell line derived from MDA-MB453 breast cancer cells compared to non cancer stem cell (NCSC) through a cell surface proteomics study. This suggests that B7H3 protein expression in CSC is linked to cancer stem cell immunity, progression and therapeutic resistance and dissecting the molecular mechanisms the regulate B7H3 expression has great biological and clinical meaning for CSC targeted cancer therapy. However, the mechanism of how B7H3 expression is regulated in CSC is not investigated. In this study, we found that transcription of B7H3 was up-regulated in CSC and tried to identify transcription factors for B7H3. Using the biotinylated DNA of the promoter region of B7H3, we identified the candidates of B7H3 transcription factor by DNA affinity purification coupled to mass spectrometer. Proteomic analysis of the mass spectrometry result showed that many identified candidates was associated with poly ADP-ribosylation. Protein poly ADP-ribosylation (PARylation) is widespread post-translational modification at DNA lesions, which is catalyzed by poly(ADP-ribose) polymerases (PARPs). Here we show that knockdown of the candidates reduced B7H3 expression in CSC. As a result, we found novel transcription factors of B7H3 specifically in CSC and also provide a clear evidence that PARylation is involved with B7H3 expression and function of cancer stem cell.

Poster Number: P24.09

Treating Enamel Defects: a Porcine Model for Leveraging the Role of Saliva in Enamel Hardening Based on Protein Removal

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Molar-incisor hypomineralization (MIH) is a developmental enamel defect affecting in 13-17% children worldwide resulting in decreased mineralization and 3 to 15-fold higher protein content in permanent molar and incisors. While enamel protein levels are unchanged in MIH enamel, serum albumin, collagen and serine proteases inhibitors are different. Since the lack of a disease model has hampered both resolving MIH etiology and improving treatment, we propose the porcine model to develop treatment strategies. Similar to MIH-enamel, porcine enamel erupts soft, with high protein content, including albumin, yet hardens quickly through post-eruptive protein removal and mineralization. This study characterizes the protein composition of porcine whole saliva and enamel at different stages after eruption. Samples were collected from 2-, 4- and 16-week-old pigs, salvia from ropes the pigs chewed on, before sacrifice and collection of dentition. Analyses by LC-MS/MS were performed to identify protein composition and compare protein abundances in saliva and enamel. Preliminary saliva data showed differences in protein profiles and abundances. Serum albumin, antitrypsin and cathepsin D decreased in saliva, but KLK and antithrombin increased with age. The albumin content decreased rapidly within enamel after eruption, observed by ELISA, western blot and visualized by immunohistochemical labelling in teeth and confocal microscopy. Our working hypothesis is that post-eruptive porcine enamel mineralization is mediated by saliva proteases that facilitate removal of enamel matrix proteins and influx of mineral ions. The purpose of this work is to resolve the mechanism of this rapid and marked enamel hardening and to develop new strategies for MIH treatment. Saliva and enamel profiles, including albumin abundance, change markedly with pig age. Removal of albumin and protease inhibitors appear critical for post-eruptive enamel mineralization. Additional analyzes are ongoing to validate these results and test their role in enamel mineralization and for MIH treatment.

Poster Number: P24.10

Transcriptomic and Proteomic Analysis of HD Medium Spiny Neurons Derived from Human-iPSCs to Identify Keys Molecular Determinants of HD Neuropathogenesis

Kizito-Tshitoko Tshilenge[1]; Carlos Galica Aguirre[1]; Nathan Basisty[1]; Sicheng Song[2]; Swati Naphade[1]; Kevin Perez[1]; Ashley Loureiro[1]; Cameron Wehrfritz[1]; Anja Holtz[1]; Alejandro Lopez-Ramirez[1]; Sean Mooney[2]; Simon Melov[1]; Birgit Schilling[1]; Lisa M Ellerby[1]

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Huntington's disease (HD) is a neurodegenerative disease caused by an unstable CAG repeats expansion in the huntington (HTT) gene, which produces a polyglutamine (polyQ) tract that alters the function of the HTT protein. Although the polyQ-expanded HTT is expressed throughout different organs, the medium spiny neurons (MSNs) within the striatum are the cells predominantly affected in HD. Previous studies using induced pluripotent stem cells (iPSCs) to model HD have shown that the disease phenotypes only manifest in the differentiated neural stem cells stage, not in iPSCs. To further study HD neuropathogenesis, we differentiated HD-MSNs to perform omics analysis including transcriptomic and proteomic of HD-MSNs when compared to isogenic corrected MSNs. The transcriptomic analysis utilizing RNAseq identified 6,372 differentially expressed genes between HD and corrected MSNs (FDR <0.01) and illustrated that multiple targets of the polycomb repressive complex 2 (PRC2) were enriched in HD-MSNs. We used single cell RNAseq to characterize heterogeneity within the MSNs-derived iPSCs population and found that DLX1/2 transcription factors involved in maturation of striatum are downregulated in HD-MSNs. We performed quantitative proteomic analysis using two systems: the TripleTOF-6600 data-independent acquisitions (DIA) and Orbitrap Lumos data-dependent acquisitions label free quantification with FAIMS (Lumos-DDA-LFQ-FAIMS). We identified 3,214 proteins with the TripleTOF-6600 system in DIA mode, while the Lumos-DDA-LFQ-FAIMS identified 6,323 proteins with at least two peptides (FDR <0.01). The differential gene expression and pathway analysis using both systems revealed that the dysregulation of proteins involved in the maintenance and regulation of the extracellular matrix are significantly associated with the HD signature. Furthermore, we showed that proteins involved in formation and maintenance of axons, dendrites and synapses such as septin protein members are dysregulated in HD-MSNs. Overall, our omics analysis using HD-MSNs was able to identity relevant pathways that regulate MSNs differentiation and/or maintenance as therapeutic targets for HD.

Poster Number: P24.11

Plasma Proteomics in Non-Human Primates

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Protein biomarker signatures in plasma are often proposed as a less invasive means to assess proteomic changes in organs and tissues, which normally cannot be sampled over time in human patients. However, due to the complex composition of plasma, MS analysis is complicated by the high abundance of a small number of serum proteins (14 most abundant proteins, including albumin and Ig, account for over 90% of total plasma protein), and the correlation between plasma protein changes and target tissue changes often is unclear.

Non-human primates (NHP) resemble humans in their physiology, genetics, and disease pathobiology. They are often used for cardiometabolic interventional studies since tissue samples can be obtained under highly controlled conditions. Here, we proposed to examine whether standard plasma proteomics approaches developed for human samples can be used to assess the plasma proteome of NHP.

Using human NIST plasma, we depleted the top 14 abundant proteins (Thermo A36369), followed by reduction, alkylation and tryptic digestion. Peptides were further fractionated using high pH RP peptide fractionation, and analyzed using a Fusion Lumos Orbitrap mass spectrometer. Based on peptide ion intensities, the depleted proteins still accounted for 17-39% of total proteins with 20mg of protein starting material, or 37-41% for 100mg. Depletion was less efficient when NHP plasma was used, with depleted proteins still accounting for 42-64% of total proteins detected by MS. Overall, MS analysis identified 148 proteins in unfractionated and 182 proteins in pH-fractionated samples with 20mg of protein, and 206 and 289 proteins in unfractionated and fractionated samples with 100mg. Similarly, plasma from NHP resulted in 242 proteins identified with 100mg protein and 162 proteins identified with 20mg starting protein, suggesting that the analysis protocol works effectively in NHP.

Poster Number: P24.12

An Integrated Quantitative Proteogenomics Pipeline Reveals an Increase in Non-canonical Protein Variants in Inflamed Mouse Colon Tissue

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Chronic inflammation is increasingly recognized as a major contributor to the development of oncogenesis. Despite this, the exact mechanisms by which this process occurs are still unknown. To gain insight into the phenotypic changes that occur during chronic inflammation and how they precipitate the development of cancer, proteogenomic analysis was employed on a chronic inflammation model. Samples were collected from proximal colon tissue of Rag2-/- Il-10-/- A/J mice treated with Helictobacter Hepaticus to induce inflammation; protein from these samples (along with complimentary controls) was isolated, digested to peptides, and subjected to differential quantitative proteomic analysis using LC-MS/MS. A bioinformatic workflow in the Galaxy for proteomics (Galaxy-P) platform was used to generate a protein FASTA database using assembled transcriptomes of mice subjected to H. hepaticus-induced inflammation; a second workflow was then implemented to identify non-canonical peptide sequence variants using the FASTA database and the raw MS/MS data. Differential protein abundance analysis showed a significant increase in proteins mediating inflammatory responses. Bioinformatic analysis revealed the presence of peptides in the inflamed protein samples containing sequences that deviate from their canonical isoform with single amino-acid variants and alternative splicing events. Verification of non-canonical peptides identified is being pursued via bioinformatics means using the PepQuery software, as well as analytically using targeted parallel reaction monitoring (PRM) experiments. Collectively, these results will provide new into mechanisms of inflammation-induced colon cancer as well as an integrated bioinformatics and analytical pipeline for the identification and validation of protein-level sequence variants underlying this process.

Poster Number: P24.13

Metabolomic and Proteomic Analysis of Type II Diabetes Mellitus Progression Using Human Aqueous Humor

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Purpose: To map the metabolic and proteomic profiles of human aqueous humor and overlay the results of both datasets to find significant pathways of interest associated with type II diabetes mellitus disease progression.

Methods: Patients were classified as nondiabetic (CTL; N=17), diabetic without advanced features of disease (NAD; N=23), or diabetic with advanced features (AD; N=18). Aqueous humor samples were collected during anterior segment surgeries and frozen immediately for metabolomics (N=42) or collected from donor whole globes within 6 hours post mortem and frozen immediately for proteomics (N=16). Metabolites were identified by GC-MS (Thermo Q Exactive GC) and proteins were identified by UHPLC-MS/MS (Thermo Fisher Scientific Easy-nLC 1000 UHPLC system). Metabolite data was collected in the full mass range (50-700 Da) and identification of metabolites was based on their retention times using Tracefinder 4.1 (Thermo Fisher). Tandem mass spectra from the top 20 ions in the full scan from 400-1200 m/z were acquired and peptides were identified using up-to-date protein sequence libraries available from UniProtKB, X!Tandem, and OMSSA. Pathway and statistical analyses were conducted using MetaboAnalyst 4.0 and Ingenuity Pathway Analysis (IPA; Qiagen).

Results: We detected 71 targeted metabolites and 7,145 unique protein isoforms in our human aqueous samples. Using the statistically significant, differentially expressed metabolites and proteins ($P<0.05$) for pathway analysis, we found the most significant overlapping pathways of interest included FXR/RXR activation, glutathione-mediated detoxification, IL-12 signaling and production in macrophages, arsenate detoxification, sucrose degradation, angiogenesis inhibition, phagosome maturation, growth hormone signaling, and lysine degradation.

Conclusions: This is the first study to cross reference both metabolomics and proteomics datasets to determine significant pathways associated with the progression of type II diabetes mellitus. These pathways provide a detailed map of potential biomarkers and therapeutic targets for the treatment of diabetes related anterior segment eye diseases.

Poster Number: P24.14

Developing Proteins and Phosphoproteins in Urine Extracellular Vesicles as Biosignatures for Parkinson's Disease Diagnostics

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Extracellular vesicles (EVs) have emerged as a rich resource for the discovery of neurodegenerative disease-relevant biomarkers from biofluids. These EV-based disease markers can be identified well before the onset of symptoms or physiological detection of disease, making them promising candidates for early-stage PD. Moreover, EVs are a promising source to develop phosphoproteins as disease markers, considering many phosphorylation events directly reflect cellular physiological status. Here we propose to develop proteins and phosphoproteins in extracellular vesicles as biosignatures for Parkinson's disease diagnostics. Parkinson's disease (PD) is the second most common neurodegenerative disorder among the elderly population. LRRK2 gene mutations have been recognized as genetic risk factors for both sporadic and familial forms of PD. LRRK2 is known to phosphorylate a subgroup of Rab proteins and regulate their ability to bind effector proteins. Rab proteins are the regulators of vesicle formation and exosome secretion. We utilized a total of 164 samples (82 proteomics and 82 phosphoproteomics) available through the LRRK2 Cohort Consortium with and without the common Gly2019 →Ser (G2019S) mutation. We focused on LRRK2 and its substrates because this sample cohort has been uniquely curated for in-depth analysis and comparison of LRRK2 genotype and activity effects on PD. We utilized our unique combination of techniques, several of which we have developed, for effective EV extraction and accurate proteomic biomarker discovery from urine. Our approach to date is the first and only method to successfully demonstrate the feasibility of developing biofluid-derived EV phosphoproteins for disease profiling. More importantly, with our distinctive combination of extensive mass spectrometry expertise and proteomic knowledge, we will also determine biosignatures for Parkinson's disease diagnostics. Our findings will not only enhance the development of novel tools for proteomics-based biomarker discovery but will also create an effective clinical diagnosis strategy for PD.

Poster Number: P24.15

HIV-Tat Exposure Impairs Neural Development via Ras-ERK Pathway Dysregulation

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Background: HIV poses a significant global health burden, with a significant proportion of HIV-positive individuals also affected by the cognitive, motor and behavioral dysfunction that characterizes HIV-associated neurocognitive disorders. We and others have previously shown that HIV transactivator of transcription (HIV-Tat) can regulate cellular signaling in adult neurons and is thought to play a role in the onset of dementia. However, the neurodevelopmental

consequences of fetal exposure to HIV-Tat are largely unknown and may be particularly severe given the sensitivity of the developing CNS. Thus, understanding the early signaling mechanisms involved may inform possible clinical interventions.

Methods: Toward this, we established SILAC-labeled, non-transformed human neuroepithelial stem cell model of HIV-Tat induced neuronal impairment. Using this model, we performed proteomic and phospho-proteomic analysis after 15, 30, 45, and 60 minutes of treatment to understand the dynamics of early signaling events leading to impairment.

Results and Discussion: Regulation in both expression and phosphorylation of proteins central to the Ras-ERK signaling pathway such as Raf1, MEK and ERK were observed as early as 15 minutes post-treatment. Similarly, proteins associated with focal adhesion such as Vinculin and Talin which involved in stem cell development were also dysregulated. As focal adhesion development plays a role in neuronal process outgrowth, its fine regulation is critical to neuronal maturation and synaptic maintenance.

Conclusions: Given the sensitivity of the developing CNS, any overt influence on regulation of the signaling involved can have long-term neurodevelopmental impairment. Our findings indicate that HIV-Tat may disrupt these pathways and dysregulate neuroepithelial stem cell development. Such dysregulation could lead to the developmental deficits seen in children born to HIV-infected mothers. This study identifies potential molecular mechanisms of HIV on fetal neural development and forms the basis for further investigations into the molecular etiology of HIV-Tat induced impairment as well as possible clinical interventions.

Poster Number: P24.16

The HDL Proteome Predicts Incident Cardiovascular Disease in Patients with Chronic Kidney Disease

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Patients with chronic kidney disease (CKD) are at high risk for cardiovascular disease (CVD). However, traditional lipid risk factors do not predict CVD risk in CKD patients. We have previously shown that the high-density lipoprotein (HDL) proteome is markedly remodeled in CKD subjects on dialysis. We therefore determined whether the HDL proteome and two other proposed metrics of HDL's cardioprotective effects, HDL particle number and HDL function, predicted future CVD events in patients with CKD. We prospectively studied 92 subjects (46 incident CVD and 46 one-to-one matched controls) with different stages of CKD, using targeted isotope dilution MS/MS to quantify 31 proteins in HDL. We also measured the cholesterol efflux capacity (CEC) of serum HDL and the concentrations of different sized HDL particles (HDL-P). We tested associations of HDL metrics with incident CVD using matched logistic regression analysis. In unadjusted models, levels of six HDL proteins (APOA1, APOA4, APOC3, LCAT, PON1, PON3) and medium size HDL-P significantly associated with incident CVD, whereas no significant associations were found for HDL-cholesterol, CEC, and total HDL-P. After adjusting the models for clinical confounders, three HDL proteins (APOA4, PON1, PON3) remained significantly associated with CVD risk. Further adjusting the models for the levels of HDL-cholesterol, LDL-cholesterol and triglycerides, the associations between the three HDL proteins and incident CVD remained significant with odds ratios (per 1-SD) of 0.32 (0.13-0.78, 95% CI, P=0.012) for APOA4, 0.38 (0.18-0.97, P=0.042) for PON1, and 0.42 (0.20-0.92, P=0.031) for PON3. Taken together, APOA4, PON1, and PON3 in HDL were significantly and inversely associated with future CVD events in subjects with CKD. Importantly, animal and human studies demonstrated that all three proteins are cardioprotective possibly due to their anti-oxidative and anti-inflammatory activities. Our observations indicate that HDL's protein cargo can serve as a marker-and perhaps mediator-of CVD risk in CKD patients.

Poster Number: P24.17

Multi-omic Characterization of Bone Marrow Interstitial Fluid (BMIF) and Peripheral Blood Plasma (PB) of Pediatric Acute Lymphoblastic Leukemia patients

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Presently, the 5-year survival rate for acute lymphoblastic leukemia (ALL) in children is approaching 90%. But current treatment options are harsh and indiscriminate, leading to secondary cancers, acute side effects, or complications later in life. There is, therefore, a need to seek out more specific therapeutic targets for paediatric ALL. Pediatric ALL, however, has a relatively lower mutational burden; thus making it more challenging to select targets that are specific to cancer cells.

Proteases are enzymes that irreversibly alter proteins and are known to play a role in both normal and pathologic physiology. To overcome the aforementioned challenge, we are interested in exploring the proteolytic proteoforms that result from the presence of a unique microenvironment and deregulated proteases that are exclusive to cancer cells.

In the present study, we obtained matched BMIF and PB samples from 8 pediatric ALL patients from the BC Children's Hospital Biobank. Samples were collected at diagnosis (Dx) and 29 days (D29) after induction treatment. As controls, matched BMIF and PB samples from 2 non-pediatric ALL patients were obtained. Proteomic, terminomic, and metabolomic analyses of the samples were conducted. Protein abundance and proteolytic processing using previously established proteomic workflows called Single-pot, solid phase-enhanced sample preparation (SP3) and High efficiency Undecanal-based N termini enrichment (HUNTER), respectively.

This study will provide a comprehensive examination of the differences between BMIF and PB of pediatric ALL patients. It will further our understanding of how proteases contribute to leukemogenesis as well as offer insight on how proteolytic proteoforms can be used as cancer-exclusive therapeutic targets.

Poster Number: P24.18

Discovering Aberrant Splicing Events in Cancer Proteomics Using Isobaric Mass Tags

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Splicing machinery mutations are a hallmark of Chronic Lymphocytic Leukemia (CLL). The resulting aberrant mRNA splice forms may then give rise to unconventional proteins that can be non-functional or drive cancer development. These proteins might also contain neoantigens that could be targeted by novel cancer therapeutics. Thus, the discovery of such splice-affected proteins may provide insight into cancer development and treatment. However, splicing affected amino acid sequences are not covered by proteomic databases and consequently searches of their corresponding MS/MS signatures will not yield peptide-spectrum matches. Here we present a strategy to identify noncanonically-spliced proteins in proteomic datasets, which relies on the hypothesis that the abundance ratios of all peptides of the same protein should be constant, whereas peptides derived from aberrant splice forms should not correlate. Therein we use Tandem Mass Tags (TMT) to quantify and identify proteins, in which aberrant

splicing has occurred. To detect such unknown splice variants, large LC-MS/MS data sets must be analyzed for all reasons of low correlation. This includes wrong peptide assignment by the search engine, missed PTMs, poor sequence coverage, or low peptide ion counts. We generated a training data set to simulate aberrant splice variants by enriching certain peptide fractions in an otherwise unfractionated mixture of TMT-labeled HEK cell digest peptides. Our approach was then applied to a proteomics dataset from NALM-6 B-cells, that carry mutations in the splicing factor 3B1 (SF3B1) resulting in aberrant splice sites and the production of novel proteoforms. Our model predicted that mutations at K666 led to the highest amount of novel proteoforms, compared to mutations at K700 or H662. In conclusion, we were able to identify dysregulated proteins in cancer relevant pathways such as carbon metabolism and cell signaling.

Poster Number: P24.19

Differential Proteomics in a Mouse Model of Niemann-Pick Type C following 2-Hydroxypropyl-beta-cyclodextrin Treatment

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Niemann-Pick Type C (NPC) disease is a fatal, neurodegenerative lysosomal storage disorder that arises from mutations in the *Npc1* or *Npc2* gene. As a result of the genetic defect, cholesterol trafficking is impaired and accumulates within endo/lysosomal compartments. In vitro and animal studies have revealed that 2-hydroxypropyl-beta-cyclodextrin (HPBCD) reduces cholesterol storage, cerebellar degeneration and extends animal lifespan. Current clinical investigation is underway in patients with NPC. In the current study, we sought to investigate the differential proteome as a result of chronic treatment with HPBCD. Label free quantitative proteomics was carried out using standard flow chromatography and JetStream electrospray ionization coupled to a Q-TOF mass spectrometer. Enriched pathways that are affected by HPBCD in the NPC mouse model include downregulation of the sumoylation, autophagy and neuroinflammation pathways in the cerebellum whereas an up-regulation of toll-like receptor signaling was observed with treatment. Our results show differences induced by HPBCD treatment in wild-type animals including dopamine receptor signaling and synaptic long-term depression. Our current investigations are geared towards understanding how HPBCD affects synapse function in NPC. Results from the proteomic analysis in the NPC mouse model, an in vitro cell culture model, as well as validation and functional studies will be presented.

Poster Number: P24.20

Characterizing the Effects of Fish Oil on High-Density Lipoprotein Proteome and Cholesterol Efflux Capacity

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Fish oil supplements are commonly administered dietary source of two omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Fish oil reduces patient triglycerides and very low-density lipoprotein (VLDL) cholesterol, modestly increases high-density lipoprotein (HDL) cholesterol (HDL-C) and reduces cardiovascular disease (CVD) event risk in high risk patients. While elevated HDL-C is independently associated with decreased risk of CVD events, its mechanism is unknown. Evidence suggests HDL function [i.e. ABCA1-mediated cholesterol efflux capacity (CEC)] rather than its concentration is a stronger indicator of CVD risk reduction; and, further, HDL's protein cargo correlates with its CEC. Thus, we sought to characterize how fish oil supplementation modulates HDL proteome and function. Seven subjects not previously using fish oil and without history of CVD were recruited to take a regiment of fish oil (1125mg EPA/875mg DHA daily) for 30 days followed by a 30-day washout period wherein no fish oil supplements were administered. Fasting whole blood was drawn at baseline, after 30 days on fish oil supplements, and following washout. APOB-containing lipoprotein-depleted serum was used to determine ABCA1-specific CEC using BHK cells overexpressing human ABCA1 under mifepristone control. DDA LC-MS/MS proteomics was performed on ultracentrifugation isolated, [N15]APOA1 supplemented HDL using Thermo Q-Exactive HF. Following fish oil therapy, triglycerides decreased from 109 ± 42.5mg/dL at baseline to 78.2 ± 28.3 mg/dL after therapy ($P<0.05$), VLDL reduced by an average of 25.9 ± 16.1% ($P<0.05$). ABCA1-dependent CEC increased 21.2 ± 15.0% ($P<0.05$) after fish oil therapy. We identified 14 proteins significantly changed ($P<0.05$) following fish oil therapy including HDL-associated proteins apolipoprotein A2 and apolipoprotein F as well as proteins involved in regulated exocytosis such as vinculin, bridging integrator 2, and Fermitin Family Member 3. Herein we present evidence that fish oil supplementation enhances the CEC of HDL potentially via modulation of its proteome.

Poster Number: P24.21

Label Free Detection Strategies of Ovarian Cancer in the Vaginal Microenvironment

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High grade serous ovarian cancer (HGSOC) is typically not discovered until stage 3 or 4; and when diagnosed in these late stages, the five-year survival rate is less than 30%. This low five- year survival rate can be partially attributed to the lack of routine diagnostic tools for early detection of the disease. One strategy to improve early detection is to source diagnostic material in close proximity to the tumor, rather than in the serum, which has typically failed. Given the recent data indicating that HGSOC arise from the fallopian tube it is predicted that sampling from the vagina could improve detection. It has been shown that matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) whole cell fingerprinting could discriminate between cell types in a heterogeneous mixture, and this method has since been tested in complex vaginal lavages sourced from a longitudinal murine model of HGSOC for the detection of cancer cells. In order to closely simulate ovarian cancer in a murine model, xenografts were performed using OVCAR8-RFP, a human-derived cell line of HGSOC. A preliminary *in vivo* experiment with mice xenografted with OVCAR8-RFP was conducted and vaginal lavages were obtained for each mouse throughout the study to collect cells and extracted factors for analysis. This study revealed that it is possible to detect proteins using MALDI-TOF MS from the 4-20 kDa region that accumulate over the course of disease from vaginal lavages across all animals in the study. The identity of these proteins will be elucidated using an LC-MS/MS bottom-up proteomics approach and the method will be translated to study the protein signatures in tampons from women pre- and post tumor debulking surgery.

Poster Number: P24.22

Smyd5 Is a Novel Regulator in Cardiac Hypertrophy

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Epigenetic regulation is the process of altering gene activity without

changing DNA sequence including methylation, acetylation and phosphorylation of histone proteins which modify chromatin structure and allow gene expression or silencing. Heart disease, the leading cause of death in the United States, is accompanied by two hallmark features: specific alterations in gene expression and hypertrophic growth of the myocardium. However, we are only beginning to identify the proteins which regulate these alterations and contribute to heart disease. The Smyd family is a unique class of methyltransferases whose catalytic SET domain is separated by an MYND domain. This family of five has been shown to methylate several unique histone and non-histone proteins and has been implicated in regulating cell growth, cardiac development, sarcomere organization, and muscle differentiation. One specific member, Smyd5, is ubiquitously expressed across various human tissues including both fetal and adult heart. Smyd5 has never been studied in striated muscle, so it is unknown how Smyd5 regulates gene expression in the heart and how this contributes to cardiac physiology and morphology. To characterize the role of Smyd5 we generated inducible, cardiac-specific knockout mice which developed hypertrophy and fibrosis two weeks after Smyd5 knockout and ultimately heart failure at five weeks. These phenotypic changes included a reduction in histone H4 K20 trimethylation, highlighting a mechanism whereby this protein alters gene expression and cardiac physiology. Additionally, proteomic analysis of cardiac tissue from Smyd5 KO mouse at various time points, and subsequent bioinformatics analysis, identified transcriptional regulation and RNA processing as the most perturbed biological processes, and identified specific proteins differentially regulated by Smyd5 in each of these pathways. Overall, these results constitute the first analysis of Smyd5 confirming its role in regulating hypertrophic growth through methylation of histone H4, and begin to identify the downstream pathways modulated by these epigenetic changes.

QUANTITATIVE PROTEOMICS
P25.01 – P25.14

Poster Number: P25.01

Double Barrel ESI Source and Novel Tandem nanoLC-MS Setup Enables 24/7 Deep Proteome Profiling

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NanoLC coupled with high-resolution accurate-mass (HRAM) mass spectrometry (MS) is the gold standard in discovery proteomics that requires deep profiling of complex proteomes. The unmatched sensitivity of nanoLC-MS, however, is often linked with relatively low MS utilization. The limitations can be eliminated by combining tandem nanoLC configuration where samples are separated on 2 columns with the double-barrel source that allows to interface two columns with HRAM MS without post-column flow splitting.

This novel tandem nanoLC separation platform comprises a tandem UltiMate 3000 RSLCnano system (Thermo Fisher Scientific) and a Double Barrel column oven (Sonation GmbH) installed onto the Nanospray Flex Ion Source. The separation platform was configured for direct or pre-concentration injections and coupled with Q Exactive HF-X MS. The peptides were separated at 250 nL/min on analytical columns packed into the emitter (C18, 75 µm I.D. x 40 cm). The optimized nanoLCMS methods incorporated "look ahead" injections and intelligent automated switching between columns. The data were processed with Proteome Discoverer 2.4 for protein and peptide identification at 1% FDR.

We extensively tested the double-barrel ESI source and novel tandem nanoLCMS setups with gradients from 45 to 120 min and MS utilization above 95%. The record performing results were obtained while operating system 24/7. For example, a 90-min method resulted in the identification of more than >73,000 peptide and >6,700 protein groups in each replicate This corresponded to the identification of > 800 unique peptides during each minute of 24/7 operation. More than 97% proteins and 90% peptides overlapped between individual runs that showed excellent result reproducibility.

Overall, the developed novel tandem nanoLC-MS platform with the double-barrel source is a robust solution to increase MS utilization in proteomics to almost 100% while maintaining the advantages of nanoLC-MS sensitivity for deep proteome profiling.

Poster Number: P25.02

Towards Turnkey Targeted Proteomics Solutions Using SureQuant Internal Standard Targeted Protein Quantitation

Sally Webb[1]; Sebastien Gallien[2]; Aaron Gajadhar[1]; Bhavin Patel[3]; Markus Kellman[4]; Tabiwang Arrey[4]; Alexander Harder[4]; Andreas Huhmer[1]

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Targeted quantitative proteomics based on high resolution parallel reaction monitoring (PRM) technique benefits from improved measurement selectivity, allowing more sensitive endogenous peptides quantification in complex samples. Here we introduce an extension of PRM, called "SureQuant" method, which uses spiked-in internal standards (IS) to dynamically control the acquisition process and to maximize its productivity. This novel method has been implemented in the native instrument control software of next-generation Orbitrap instruments, Thermo Scientific® Orbitrap Exploris® 480 and Eclipse® Tribrid® mass spectrometers, to enable a broad access. The SureQuant IS targeted protein quantification method has been adapted from the IS-PRM method in order to improve its usability and robustness (especially against chromatographic variations). Its ability to deliver high-density, ultra-sensitive measurements has benefited to a variety of applications. Applied to the monitoring of signaling pathways in cell lines and tissues specimens supplemented with stable isotopically labeled (SIL) peptides (30-150 IS, including Thermo Scientific® Pierce® SureQuant® kits), the method enabled systematic quantification of endogenous peptides with high precision (<5%-CV for the majority of peptides) and short analysis time (10-40 min LC gradient). In a larger scale application of the method, non-depleted plasma samples supplemented with 804 SIL peptides (Biognosys® PQ500® kit) were analyzed with a 70-min LC gradient for global plasma proteome quantification. More than 550 endogenous peptides, surrogates of around 400 plasma proteins, were reproducibly quantified over a 6 orders of magnitude range. This proteome coverage compared favorably with that of profiling methods, while still benefiting from the enhanced data quality of targeted measurements (including peptide quantification in the low amol range). The multiple analytical benefits of the SureQuant method combined with the ability to embed pre-set (optimized) methods, associated with predefined kits of IS peptides, directly into the instrument control software represents a decisive step towards the provision of turnkey targeted proteomics solutions.

Poster Number: P25.03

An Orbitrap Eclipse Tribrid Mass Spectrometer With Real Time Search Enhances Multiplexed Proteome While Improving Quantitation Accuracy and Precision

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

Aaron Robitaille[1]; Romain Huguet[1]; Graeme McAlister[1]; Arne Kreutzmann[2]; Daniel Mourad[2]; Daniel Lopez-Ferrer[1]; Vlad Zabrouskov[1]

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Quantitative proteomics strategies using Tandem Mass Tags® (TMT®) enable precise measurement of peptides or proteins from samples multiplexed into a single high-resolution LC/MS experiment. Interference can suppress ratio quantitation and thereby mask true differences in abundance. Here we evaluate if an Orbitrap Eclipse Tribrid mass spectrometer including real time search (RTS), advanced spectral processing algorithms, and modified hardware can enhance TMT quantification accuracy and proteome coverage. Synchronous precursor selection (SPS) based methods provided higher accuracy compared to MS2 methods for TMT quantitation. However, depending on which fragments are selected for MS3 quantitation, accuracy can still be distorted. To improve upon this, we implemented RTS between MS2 and MS3 scans. Using this approach, MS3 scans are only triggered if a peptide is identified from the preceding MS2. This increased the number of peptides identified with RTS by 30%. Secondly, RTS selects fragment ions for MS3 quantitation that are generated from the identified peptide on the fly. Thus, quantitation can be improved to be 95% interference free. The Orbitrap Eclipse Tribrid mass spectrometer has an optimized quadrupole that improves ion transmission, enabling narrower isolation widths to improve TMT quantitation accuracy. Overall, the Orbitrap Eclipse® Tribrid mass spectrometer includes features such as TurboTMT and Precursor Fit which facilitate intelligent acquisition methods that improve TMT quantitation accuracy, precision, and proteome coverage.

Poster Number: P25.04

Reliable and Deep Proteome Coverage by Gas-Phase Fractionation of Peptides with a FAIMS Pro Interface on a Novel Quadrupole Orbitrap

Julia Kraegenbring[1]; Tabiwang Arrey[1]; Michael Belford[2]; Satendra Prasad[2]; Aaron Robitaille[2]; Markus Kellmann[1]; Martin Zeller[1]; Alexander Harder[1]

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While great advances have been achieved in the performance of mass spectrometers, deep proteome coverage is still impaired for highly complex samples with high dynamic concentration ranges. To address this challenge a multitude of offline fractionation techniques are employed. However, these are time-consuming and mostly use higher sample amounts. Employing gas-phase fractionation with a FAIMS Pro interface on the new Orbitrap Exploris 480 mass spectrometer mitigates these challenges.

The data demonstrate that the singly charged ion population can be filtered out effectively by applying compensation voltages larger than -40 V. As a consequence, the chemical background can be significantly reduced even in single CV runs. This aspect is especially beneficial for the peptide species eluting at higher organic concentrations because of co-eluting contaminants.

Internal switching between CVs enhances the range of identifiable peptide ion species. With CV switching between -45 V, -55 V, and -65 V, we were able to boost protein identification by as much as 15 % in 90 min LC-gradients and 1 µg HeLa digest accompanied by 30 % more unique peptide IDs. With lower sample loads, we still gain 10% protein IDs even though peptide IDs are slightly decreased which points to the enhanced sampling with FAIMS. In fact, by internal switching of compensation voltages, the number of protein identifications can be increased in a single shot proteomic experiment comparable to a traditional 2D-LC setup.

Poster Number: P25.05

Development of an Accurate Quantitative Method for Post-translational Modifications on Histone by Parallel Reaction Monitoring

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Post-translational modification (PTM) in histones plays a fundamental role in gene regulation by modulating chromatin states. PTM abnormal patterns are involved in various disease pathology. Precise monitoring of histone PTM patterns would provide key information to understand disease specific mechanisms or outcomes of a therapeutic regimen. Selected reaction monitoring (SRM) assay has emerged as a powerful analytical strategy for monitoring the histone PTM dynamics, but it remains challenging to discriminate the coexisting isobaric histone PTMs that are indistinguishable by low resolution mass spectrometers.

We have established a quantitative parallel reaction monitoring (PRM) assay for the lysine site specific acetylation and/or methylation on histone using HeLa cells. By utilizing a high resolution Q Exactive HF mass spectrometer, we have optimized a multiplexed-PRM method with high selectivity/specificity for 65 PTM states corresponding to 18 lysine sites on histone H1.4, H2A, H3.1, H3.3 and H4. To evaluate the analytical performance, we analyzed 25 independent quantitative PRM data sets generated by five biological/technical replicates and confirmed that the target PTM peptides showed less than 10% of coefficient of variation across all PRM assay. More importantly, we can identify the MS/MS fragment ions of target PTM peptides with high mass accuracy (<10 ppm), which allows us to distinguish and quantify each isobaric histone PTM with a much higher confidence compared to SRM-based quantification. In specific, with this PRM approach, the PTM combinations on K27/K36 between histone H3.1 and H3.3 isoforms, which elutes almost simultaneously yet with a major quantitative difference, can now reliably be discriminated.

Although further applications of this technique in diverse biological systems are required to validate our PRM method, it provides a promising tool to track disease specific mechanisms by accurately measuring the histone PTM dynamics caused by altered perturbation.

Poster Number: P25.06

Maximizing Shotgun Proteomics Workflow to Enable Unmatched Proteome Profiling

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Shotgun proteomics promises the capability to perform large-scale global profiling using simple LC-MS based acquisition and data processing methods. The depth and quality of precursor and product ion data is key to enabling accurate and confident peptide and protein assignments over wide dynamic ranges, and governs the resulting peptide/protein search space used for sequencing. The continued goal is to leverage the existing data to expand the proteomics search space for PTMs, and/or enable the identification of point mutations specifically for cancer studies. Uncontrolled increases in the database search space can simply introduce greater number of false positives requiring laborious confirmation steps. We have introduced a workflow to facilitate high quality data acquisition resulting in the greatest proteome coverage reported at low loading amounts. Automated data processing using a novel database search strategy can expand matching considerations to extensive cancer point mutations while maintaining short search times. Additionally, a new tool is used to simulate peptide product ion distribution profiles to help stratify putative peptide candidates. The newly developed shotgun proteomics workflow has been used to analyze 200 ng of HeLa digest on an Orbitrap Eclipse Tribrid

mass spectrometer equipped with a FAIMS Pro interface stepping between two compensation voltage. The resulting triplicate data was searched using the Bolt node in Proteome Discoverer and a database that contains over 800,000 protein entries with cancer splice variants. The search results showed over 8300 proteins and 72000 peptides identified, including over 50 potential splice variants. Overall the workflow we presents here maximizes shotgun proteomics workflows to enable unmatched proteome profiling.

Poster Number: P25.07

High Quantitative Accuracy and Sensitivity in PRM with Multiplexing and Asynchronous Fill-Time Correction

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Parallel reaction monitoring (PRM) is used to monitor peptide quantities robustly across large sample sets. The gold standard for quantification employs stable-isotope standard (SIS) peptides as references to identify endogenous peptides and directly derived absolute quantities. A drawback of this methodology is the doubling of peptides that need to be acquired per analyte, i.e. heavy and light.

One solution to address this is to multiplex the SIS and endogenous peptide acquisition into a single scan. The fill times can be same length for heavy and light peptides, isochronous, or dependent on their abundance, non-isochronous. The latter requires post-acquisition correction of the raw intensities to obtain accurate quantification, as the instrument itself cannot distinguish which fragments are derived of the SIS or the endogenous peptide.

A plasma sample was spiked with PQ500 reference peptide mix, containing 804 SIS peptides representative of 581 proteins and data acquired on a Q Exactive HF-X, with standard or heavy-light-multiplexed PRM, with isochronous or non-isochronous filling. Scheduled method set-up as well as targeted data analysis, including automatic scan-wise non-isochronous fill-time correction, was performed with SpectroDive 9.

We show that non-multiplexed acquisition leads to higher endogenous peptide identifications (619 representative of 419 plasma proteins) than standard multiplexing with isochronous filling (523 IDs; 359 proteins), while the latter shows higher quantitative accuracy with median quantitative CVs of 3.7 % compared to 10.4 % in standard PRM. When employing non-isochronous filling and correcting the intensities, the quantitative accuracy is kept (median CV of 3.2 %) and the sensitivity of the standard method nearly reached (589 IDs; 383 proteins).

We present a plasma proteomics workflow for targeted proteomics on a discovery scale, using PQ500 reference peptides and SpectroDive 9. Ths methodology will be specifically useful to novel targeted proteomics workflows, like MaxQuant.Live, employing optimized fill times for individual analyte groups.

Poster Number: P25.08

Quantifying More Than 500 Human Plasma Proteins With Surequant and Pq500 in a Single Run

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Introduction: Plasma proteome is one of the most challenging biological matrices because of its large dynamic range. A classical untargeted proteomics method suffers from this due to oversampling of abundant proteins. Targeted workflows have a higher dynamic range but are difficult to setup and are not as comprehensive as discovery methods.

Here, we evaluate the use of a plasma protein assay panel containing SIS peptides measured with a new targeted acquisition method. This method uses the spiked-in SIS peptides to trigger the acquisition of the endogenous peptides. This greatly simplifies the classical targeted acquisition workflows by not needing to schedule peptide acquisition by their retention times. However, analyzing data acquired from such a pipeline poses new challenges. Towards this end, we adapted our analysis pipeline in SpectroDive software to optimally analyze SureQuant data.

Methods: A pooled non-depleted plasma sample was spiked with the PQ500 kit (Biognosys) containing 804 SIS peptides for 582 proteins. The sample was acquired in technical triplicates with a 50-minute gradient using a Thermo Scientific Exploris 480 in SureQuant acquisition mode. The data was analyzed with an adapted SpectroDive software with 1% peptide FDR. As a next step, we acquired plasma samples from a cohort of cancer and healthy patients using this newly established pipeline.

Preliminary results: We could obtain absolute quantities for 740 endogenous peptides corresponding to 529 proteins in all 3 replicates with a median CV of 6.5%. Mapping the identified proteins to proteins with known concentration in plasma [Anderson, 2002] shows we measured 8 orders of magnitude in dynamic range of plasma protein concentration.

Summary: This workflow is more comprehensive than a typical discovery workflow while providing the precision of a targeted workflow. The use of AAA quantified peptides allows to estimate absolute protein quantities and comparison of quantities between laboratories and LC-MS setups.

Poster Number: P25.09

Evaluating Label-free Protein Quantification Strategies for Integral Membrane Proteins

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Integral membrane proteins (IMPs) play pivotal roles in various biological processes. However, traditional proteomic sample preparation and analysis of this class of proteins can often lead to reduced coverage or complete loss of this class of proteins. As such, relative quantitation strategies, such as spectral counting, may produce misleading results.

We have taken various cellular fractions of the alkane-oxidizing bacterium, *Mycobacterium vaccae* JOB5, and performed three different filter aided sample preparation (FASP) strategies on these fractions in an attempt to increase coverage of some of the IMPs responsible for its unusual hydrocarbon-oxidizing capabilities. First we have employed a traditional trypsin digestion in aqueous 50mM ammonium bicarbonate. Next, we have performed the digestion in 60% methanol. Despite the fact that trypsin is reduced to about one-fifth of its activity in this level of organic solvent, previous work has demonstrated success with this system. [1] Additionally, we performed a Lys-C/trypsin sequential digestion by modifying a method reported previously. [2]

Using these datasets, we have performed three variants of label free quantitation (predominantly within Thermo Scientific's Proteome Discoverer 2.4) using (1) a spectral counting strategy incorporating normalized spectral abundance factors (NSAF) [3], (2) a summed peptide abundances approach where protein abundances are calculated by summing sample abundances of the connected peptide groups, and (3) a top N average approach where protein abundances are calculated as the average of the N most abundant distinct peptide groups.

Initial conclusions indicate that relative quantities calculated were slightly different, but that using an average of 5 peptide abundances provided the most consistent quantitation for this particular group of IMPs.

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

[1]Blonder et al. 2004 J of Proteome Research
[2]Wisniewski et al. 2009 J of Proteome Research
[3]Zybailov et al. 2006 J of Proteome Research

Poster Number: P25.10

An Optimized Sample Preparation Method of Formalin-Fixed Paraffin-Embedded Tissues for Mass Spec Applications

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Introduction: Formalin-Fixed Paraffin-Embedded Tissues (FFPE) are an underappreciated resource in the field of proteomics. Hospitals and tissue banks worldwide have large archives of biologically relevant information trapped in these little blocks. Currently, there is no standardized or optimized protocol for extracting proteins from FFPE tissues for mass spec (MS) analysis.

Method: We evaluated different paraffin removal protocols and lysis/homogenization techniques using FFPE sections from breast, lung, and liver tissues. We then assessed protein yield to identify the most optimized protocol and verified compatibility with LC-MS/MS analysis. The optimized extraction protocol and the EasyPep® MS Sample Prep Kits (Mini spin-column & 96 well-plate) were used to prepare MS digest from multiple normal and tumor tissues. TMT10plex® isobaric label reagents and Pierce® High pH Reversed-Phase Peptide Fractionation Kit were used to quantify differentially expressed proteins for normal vs tumor samples. MS data acquisition was performed using Thermo Scientific Dionex nanoLC® system coupled to Thermo Scientific® QExactive Orbitrap mass spectrometer. Proteome Discoverer 2.3 software was used for MS data analysis.

Results: First, we optimized paraffin removal method using xylene and sequential ethanol washes to improve decrosslinking and protein extraction. Higher protein yield was observed using the EasyPep lysis buffer and micro pestle-based tissue homogenization. This optimized method coupled to EasyPep MS Sample Prep Kits allowed identification and quantitation of 2,000-3,500 proteins from breast/lung/colon tissue samples. TMT 10plex experiment allowed relative quantification of 5,000 proteins from biological replicate tissue samples (breast, colon, and lung). Differentially expressed proteins were identified in tumor vs normal samples. Using the optimized lysis protocol, fresh frozen tissues were compared to FFPE and similar number of proteins were identified for each sample type.

Conclusion: The optimized protein extraction protocol for FFPE tissue samples and EasyPep MS sample prep kits provided complete workflow solution for MS applications.

Poster Number: P25.11

Development of a prmPASEF Approach on a tims-Q-TOF Instrument for Targeted Proteomics

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Background: Targeted proteomic approaches are commonly used to perform biomarker candidate discovery and validation. We previously evaluated the tims-Q-TOF for targeted proteomics by using the MRM function, and now we have developed a prmPASEF approach, which has the potential to target more ions while maintaining the instrument's exceptional sensitivity and selectivity. In this study, we evaluated the performance of the tims-Q-TOF with a prototype version of the prmPASEF software.

Methods: An equimolar mixture of 259 quantified synthetic peptides labelled with stable isotopes (AQUA) was diluted in a 100 ng/µl human cell line digest. The dilution series covered 6 concentration levels ranging from 31.25 amol/µl to 25 fmol/µl. All samples were separated by nano-HPLC using a 30 min gradient and analysed on a high-resolution timsTOF Pro instrument (Bruker Daltonics) operated in either data dependent acquisition PASEF mode or in the novel prmPASEF acquisition mode. Overall sensitivity, selectivity and detectability of the different acquisition modes were evaluated with the latest version of Skyline Ô software.

Results: PASEF acquisition of cell lysate digest spiked with 25 fmol, 6.25fmol, 1.56 fmol, 500 amol, 125 amol and 31.25 amol of the AQUA mixture identified 253, 235, 205, 104, 48 and 4 of the original 259 AQUA peptides, respectively. Using a novel tims-PRM approach with a 100 ms tims trapping time, and prior to any collision energy optimization, 110, 168, 205, 213 and all 259 of the AQUA peptides could be quantified at the 31, 125, 500, 1562, 6250 amol level, respectively. In comparison to tims-PRM, prmPASEF allowed a 5-fold increase in the number of targets that could be simultaneously monitored, without sensitivity loss. The latest results of the optimization process will be presented.

Conclusions: prmPASEF enables greater sensitive and higher coverage in targeted proteomics experiments.

Poster Number: P25.12

Optimized Sample Preparation for TMTpro-labeled Proteomics Samples.

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Advances in mass spectrometry (MS) instrumentation have enabled routine analysis of complex protein samples. However, sample preparation methods are not standardized with many protocols taking 8-24 hours in addition to suffering from low peptide yields, poor digestion efficiency and low reproducibility. We have recently introduced several easy-to-use sample preparation kits, based on the Thermo Fisher Scientific EasyPepTM sample preparation workflow that enables quick and reproducible proteomic sample preparation form cells, tissue, and fluids, while maintaining excellent sample quality metrics, such as peptide yields, digestion and chemical labeling efficiency, and high protein identification numbers. Together with the use of the new TMTproTM tandem mass tag reagent, which allows multiplexing of up to sixteen samples in a single batch, researchers can quickly process and analyze hundreds of samples in a matter of days. Here, we describe a robust workflow for preparing complex proteomic samples which includes labeling with the tandem mass tag reagents, efficient clean-up, and off-line high pH reversed-phase for a comprehensive comparative analysis. Our new standardized workflow yielded 10-20% higher number of peptides and proteins with lower missed cleavages (<90%) compared to other commercial MS sample prep kits and protein digest standards. We have also evaluated this workflow in a 96-well filter plate format to support higher sample processing throughput amenable to the use with automated liquid handling system for sample preparation and peptide clean-up and have extended it further by incorporating post-translational modifications (phosphopeptide and glycopeptide) enrichment steps for cell and tissue samples, as well as abundant protein depletion steps for plasma samples.

Poster Number: P25.13

Mechanism of Adrenergic CaV1.2 Stimulation Revealed by Proximity Proteomics

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Increased cardiac contractility during fight-or-flight response is caused by b-adrenergic augmentation of CaV1.2 channels. This iconic regulation is assumed to involve phosphorylation of CaV1.2 a1/b-subunits. We here show that in transgenic murine hearts expressing fully PKA phosphorylation-deficient mutant a1C/b, this regulation persists. This suggests involvement of extra-channel factors. Peroxidase-catalyzed labeling in mouse hearts expressing engineered ascorbate peroxidase APEX2 conjugated-a1C or b2B with multiplexed quantitative proteomics allowed tracking of hundreds of proteins in proximity of CaV1.2. We observed that Rad is enriched in the CaV1.2 micro-environment and reduced upon b-adrenergic stimulation. PKA up-regulates cardiac CaV1.2 channels by phosphorylating the small G-protein Rad, relieving constitutive inhibition of CaV1.2. Co-expressing Rad enabled robust heterologous reconstitution of PKA-mediated up-regulation of CaV1.2 that depends on specific Ser-residues in Rad. Single-channel experiments indicate PKA relieves Rad inhibition of CaV1.2 and thus increases channel open probability. These results reveal the mechanism by which b-adrenergic agonists stimulate CaV1.2.

Poster Number: P25.14

NanoTPOT: Enhanced Sample Preparation for Quantitative Nanoproteomic Analysis

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With the ever-growing need for protein-level understanding in biological research, proteomics researchers are starting to examine detailed proteome dynamics using minute amount of samples with crucial biological and clinical relevance. Aside from mass spectrometer instrumentation advancement, a single-tube and high throughput sample processing workflow is imperative to ensure sensitive, quantitative and reproducible protein analysis for these increasingly sophisticated studies. Leveraging the benefits of an acid cleavable detergent, RapiGest SF Surfactant (Waters Corporation), we have optimized a nanoproteomic tandem mass tag (TMT) processing in one-pot (NanoTPOT) quantitative nanoprotoemics workflow. Through the assessment of proteolytic digestion, TMT labeling, online and offline fractionation strategies, our optimized workflow effectively eliminated the need for sample desalting and enabled compatible sample processing for MS analysis. We further applied the NanoTPOT workflow to examine cellular response to stress caused by dithiothreitol, where we identified and quantified more than 7000 proteins in a TMT 10-plex experiment with one microgram of starting material in each channel. Our workflow has been proven to be an effective alternative for current nanoproteomic sample processing to minimize sample loss in biological and clinical applications.

SINGLE-CELL PROTEOMICS
P26.01 – P26.04

Poster Number: P26.01

Ultra-sensitive LC/MS Workflow for High-throughput Single-cell Proteomics

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Traditionally, proteomics experiments are applied to large populations of cells, representing the average protein expression under given biological conditions. However, understanding the cellular heterogeneity provides insights that cannot be gained from bulk studies, such that the analysis of single-cell protein expression is of growing interest. Current LC-MS-based proteomics workflows have not been widely applicable to single cell analysis, mainly due to large sample losses during sample preparation, limited analytical sensitivity and low throughput. To address these challenges, we have combined nanoPOTS (Nanodroplet Processing in One-pot for Trace Samples) technology with tandem mass tag (TMT) isobaric labeling to analyze single mammalian cells containing ~0.2 ng total proteins on the new Orbitrap Eclipse® Tribrid® mass spectrometer with real time search and the FAIMS Pro® Interface to improve single cell proteome coverage and enhance quantitation accuracy. Single cells were isolated from cultured murine and HeLa cells via fluorescence-activated cell sorting and samples were processed on nanoPOTS chip. The UltiMate® 3000 RSLCnano system was used with 20 to 30 µm i.d. columns coupled to the Orbitrap Eclipse. Proteome Discoverer® 2.4 software was used for data analysis. Both label-free and isobaric labelling (TMT10plex)-based protein quantitation were evaluated with a focus on reproducibility and improved throughput. The FAIMS-enhanced label-free workflow resulted in the identification of an average of 925 protein groups from single Hela cells with high-confidence MS^2 spectra. This was 3-fold higher than without FAIMS. The TMT10plex analysis of three cultured murine cell populations were compared with MS^2 and SPS MS^3 method with Real Time Search. We have demonstrated that single cell proteomes can be quantified using label-free or TMT workflows by combing nanoPOTS with the Orbitrap Eclipse Tribrid mass spectrometer, and the FAIMS Pro Interface, enabling researchers to investigate cell heterogeneity as well as rare cells in an ultra-sensitive, higher throughput LC-MS analysis.

Poster Number: P26.02

A Quantitative Single-Cell Proteomics Approach to Characterize an Acute Myeloid Leukemia Hierarchy

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Hematopoietic stem cells (HSC) are the origin of all cell lineages in the human blood hierarchy. Acute myeloid leukemia (AML), a perturbed state of hematopoiesis, is also hierarchically organized, with leukemia stem cells (LSC) at the apex. Successful eradication of AML will likely depend on specific targeting of these tumour-initiating cells, in turn requiring their molecular characterization. Currently, we are able to isolate LSC based on CD34 and CD38 expression levels of primary AML cells. These sorted fractions, however, vary in their levels of LSC, and thus should be considered LSC-enriched, rather than pure LSC. Consequently, this significant level of heterogeneity within the LSC-enriched fractions hampers the molecular characterization of true LSC.

Here, we have taken a well-characterized primary AML patient sample, "8227", and subjected it to fluorescence-activated cell sorting, combined with a newly developed single-cell proteomics strategy to identify the protein landscapes of individual cells within

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the LSC, progenitor and blast populations of this patient. Using the latest state-of-the-art LC-MS instrumentation, combined with the new TMTPro reagents, this has resulted in an unprecedented map of protein expression in individual AML cells. Using our in-house built computational pipeline SCeptre, we found a strong enrichment of stem cell specific proteins in the LSC and progenitor compartments compared to blasts, and the resulting protein signatures clearly distinguish the various differentiation stages that were isolated. Comparing the Real Time Search aided SPS MS3 analysis used in this study with previously generated MS2-level data revealed a clear improvement, both in terms of quantitative accuracy and proteins identified per cell, resolving cell populations at an even higher resolution.

As a fully integrative experimental and computational pipeline, this work paves the way for future single-cell proteomics approaches to dissect e.g. individual tumour hierarchies from AML patients, and potentially find key LSC-specific therapeutic targets using protein-level data.

Poster Number: P26.03

Capillary Electrophoresis Ion Mobility Mass Spectrometry for Single-neuron Proteomics

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Mass spectrometry-based proteomics of single neurons would afford powerful investigative potentials to study normal and impaired development of the brain. Although high-resolution mass spectrometry (HRMS) is the method of choice for discovery proteomics in large populations of cells, this technology is challenged for single cells due to sensitivity limitations. Here, we integrated microanalytical capillary electrophoresis with ion mobility mass spectrometry (IMS) and tested the detectable coverage of the single-cell proteome. Analysis of 1 ng of protein digest from mouse brain sections, which estimates to the total protein content of a single neuron, yielded 700 protein groups. These identifications were double compared to CE coupled to a late-generation orbitrap mass spectrometer. Among the identified proteins were neuron specific or commonly expressed in the brain region that was analyzed in this study. With deeper coverage of the proteome, miroanalytical CE with IMS raises a potential to better characterize proteomic cell heterogeneity in the central nervous system.

Poster Number: P26.04

Performance of the VICI Valco TrueNano U/HPLC System and Sample Preparation Workflow for Single Cell Level Shotgun Proteomics

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Mass spectrometry (MS)-based bottom up proteomics at single cell level have attracted rapidly increasing interest, particularly for revealing the cell heterogeneity related to diseases. A great challenge in this field is to minimize sample loss and degradation during extraction and processing of single cells with sub-nanogram amounts of proteins, and delivery of the resultant peptides to the liquid chromatography (LC) column. Also, to gain a high LCMS sensitivity for maximum proteome coverage, it is vital to use high performance ultralow-flow (<100 nL/min) LC with sub-50 um i.d. columns. To this end, we have developed a high-throughput inline sample preparation workflow to minimize sample loss. Specifically, proteins were loaded onto parallelly prepared capillaries, where the downstream process, including reduction, alkylation, digestion, and cleanup occurred. Furthermore, we utilized a VICI Valco TrueNano U/HPLC system and a 30 um i.d., 15 cm long C18 column for LC separation. The TrueNano U/HPLC system allows a sub 100 nL/min flow rate (down to 10 nL/min) without splitting. It also features fittings for 360 um o.d. capillaries and a 10-port valve with a port-to-port volume of only ~12 nL. The analysis was performed by a Bruker timsTOF Pro MS system with parallel accumulation-serial fragmentation (PASEF) technology. We will present the systematic optimization of the sample preparation workflow, the LC setup and conditions, as well as the PASEF and MS conditions to maximize the proteome coverage.

STRUCTURAL PROTEOMICS
P27.01 – P27.02

Poster Number: P27.01

Phosphorylation Patterns on the Neurotensin Type I Receptor Required for Arrestin Recruitment and Receptor Trafficking as Demonstrated in 4D

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G protein-coupled receptors (GPCRs) are a family of seven transmembrane (7TM) proteins whose functions are to transduce extracellular signals. These intracellular signals are terminated by the recruitment of proteins called arrestins to activated GPCRs. However, arrestin recruitment is subject to an additional layer of regulation and arrestins are only recruited to GPCRs that have been phosphorylated by one or more members of a family of GPCR kinases (GRKs). GRKs phosphorylate agonist-bound (active) GPCRs on their cytoplasmic loops and C-termini. Differences in receptor phosphorylation influence down-stream signaling and the fate of the receptor by imposing distinct conformations on the arrestin transducer. Unequivocally linking distinct phosphorylation sites with downstream signals has remained a challenge. Further, unambiguously assigning the position and stoichiometry for these phosphosites, especially in the context of multiple phosphorylation positional isomers remains a technical challenge.

Recent structural work on the Neurotensin Type I receptor (NTSR1) revealed the first molecular picture for a non-rhodopsin GPCR bound to an arrestin. This study revealed a significant degree of conformational heterogeneity in how arrestin was bound to the receptor, but was unable to directly link different phosphorylated forms of the receptor to discrete conformations of the transducer protein. Using NTSR1 as a model and the advantages of 4D proteomics- including simultaneous identification of m/z, MSMS, RT and CCS we discuss unveiling of distinct phosphorylated forms of the receptor produced in vitro and the use of biophysical reporters of arrestin conformation to link differences in receptor phosphorylation to conformational changes within arrestin. Using these findings we evaluated the phosphorylation state of the NTSR1 in cells and, combined with GRK deletion strains and mutagenesis, propose that there are indeed functionally distinct roles for different GRKs and for different phosphorylation sites within NTSR1.

Poster Number: P27.02

High-density Chemical Cross-linking for Modeling Protein Interactions

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Detailed mechanistic understanding of protein complex function is greatly enhanced by insights from its three-dimensional

structure. Traditional methods of protein structure elucidation remain expensive, labor-intensive and require highly purified starting material. Chemical cross-linking coupled with mass spectrometry offers an alternative that has seen increased use, especially in combination with other experimental approaches like cryo-electron microscopy. Here we report advances in method development, combining several orthogonal cross-linking chemistries as well as improvements in search algorithms, statistical analysis and computational cost to achieve coverage of one unique cross-linked position pair for every seven amino acids at a 1% false discovery rate. This is accomplished without any peptide-level fractionation or enrichment. We apply our methods to model the complex between a carbonic anhydrase (CA) and its protein inhibitor, showing that the cross-links are self-consistent and define the interaction interface at high resolution. The resulting model suggests a scaffold for development of a new class of protein-based inhibitors of the CA family of enzymes. We next cross-link the yeast proteasome, identifying 3893 unique cross-linked peptides in three mass spectrometry runs. The dataset includes 1704 unique cross-linked position pairs for the proteasome subunits, more than half of them inter-subunit. Using multiple recently-solved cryo-EM structures, we show that observed cross-links reflect the conformational dynamics and disorder of some proteasome subunits. We further demonstrate that this level of cross-linking density is sufficient to model the architecture of the 19-subunit regulatory particle *de novo*.

TOP-DOWN PROTEOMICS
P28.01 – P28.07

Poster Number: P28.01

Improving Proteoform Identifications and Post-Translational Modification Localizations Through Large-Scale Integration of Bottom-Up and Top-Down Data

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Cellular functions are performed by a vast and diverse set of proteoforms. Proteoforms are the specific forms of proteins produced as a result of genetic variation, RNA splicing, and post-translational modifications (PTMs). Top-down analysis of intact proteins enables proteoform identification, including those derived from sequence cleavage events and those containing multiple PTMs. In contrast, bottom-up proteomics identifies peptides, which prevents identification of proteoforms and thus necessitates protein inference. We automated the large-scale integration of results from multi-protease bottom-up and top-down analyses and applied it to the analysis of proteoforms from human Jurkat cell lysate. This integrated approach enabled observation of the overlap in identifications and PTM localization between top-down and bottom-up data. Specifically, we assessed the ability of top-down identifications to aid in protein inference in bottom-up analysis, and conversely for the ability of bottom-up peptide identifications to aid in proteoform PTM localization in top-down analysis. Approximately 50% of the bottom-up peptides from proteins that were also identified in the top-down analysis could be derived from more than one identified proteoform, demonstrating the ambiguity inherent in attempts to identify proteoforms from bottom-up data alone. The bottom-up results were also used to generate potential Jurkat proteoforms that had not been identified by top-down analysis; some of these proteoforms were then observed in intact proteoform MS1 data. This large-scale integrated analysis is an important step towards targeted proteoform analysis informed by bottom-up data to improve top-down proteome coverage.

Poster Number: P28.02

Application of Faims and Top-down Mass Spectrometry to Brain Cortex Samples From Individuals Diagnosed With Alzheimer's Disease

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Alzheimer's disease is the most commonly encountered neurodegenerative disease in the US and a significant economic burden, with 2018 costs estimated at $277 billion. The suspected causative agent of Alzheimer's disease has been amyloid beta peptide (Aβ), which is believed to induce formation of toxic fibrils that can cause neuronal cell death. Unfortunately, medications targeting Aβ or other Aβ-associated proteins have failed to reduce cognitive decline, underscoring the need for alternative therapeutic targets. Of the methods available for discovering new therapeutic targets, top-down mass spectrometry (TDMS) is an attractive approach as proteins are observed intact, providing a more direct link to determining their role in disease. Due to instrument limitations, however, current TDMS methodology is constrained to observing proteoforms that are most abundant and less than 30 kDa on average. Overcoming these barriers in TDMS is paramount for describing proteoforms in the context of disease. Although offline fractionation techniques can reduce sample complexity and improve identification of proteoforms, these methods are limited by throughput and protein recovery. An alternative to offline fractionation is gas phase separation through ion mobility, such as with high-Field Asymmetric waveform Ion Mobility spectrometry (FAIMS). FAIMS is capable of separating analytes based on factors such as size, conformation, and shape. Here, we describe application of FAIMS-TDMS to characterization of proteoforms present in the cortex of Alzheimer's disease subjects. Using FAIMS with nano-ESI we were able to identify 420 proteins and 1930 proteoforms, compared to 290 proteins and 1242 proteoforms without FAIMS in a single run. Significant improvements in signal-to-noise for MS1 precursors were found, as well as a correlation between protein molecular weight and FAIMS compensation voltage which allowed for separation of proteins by MW. Based on these results, we believe that FAIMS will find considerable utility in TDMS applications.

Poster Number: P28.03

Characterization of Cyclic Peptide Aggregation Behavior Using Biophysical, Intact Mass, and Top-down Disulfide Mapping Approaches

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Cyclic peptides (CPs) that are rich in disulfides constitute a promising class of biotherapeutics. The unique structure of CPs calls for stress studies to evaluate their stability *in vitro*. A CP was thermally stressed and its products were characterized by native & reducing SDS-PAGE, SEC-MALS, and intact SEC-MS. Aggregates of discrete numbers of peptides, such as a 20-kDa species, were found to be enriched, compared to other multimers, with the highest aggregate detected at ~100 kDa (30-mer). Compared to the control, aggregates did not show a differential change in the frequency of

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 PM; even-numbered posters present 2:15-3:00 PM.

post-translational modifications, as determined by intact mass analysis on the Q Exactive EMR. Interestingly, denaturing RPLC-MS, compared to the native SEC-MS, showed no higher-order aggregates and only the presence of 1-6-mer species. Dissociation of these species was achieved via addition of a reducing agent, suggesting their covalent nature. To decipher the structure of the covalent aggregates, disulfide mapping was desired; CPs, however, are poorly suited to enzymes used in traditional bottom-up workflows. Here, two complementary approaches were developed to determine the linkages. First, the CP was treated with a reducing agent and iodoacetamide labeling was performed as a function of time, with N-isopropyl-iodoacetamide labeling used to capture the remaining cysteines at completion. Top-down analysis was then performed to localize and quantify the modified sites, allowing extrapolation of the bonded cysteines. Second, a Q Exactive modified with an ECD cell (e-MSion Inc.) was used to directly map the amino acid site location and intra/inter chain linkages of disulfide bonds. The totality of this work will provide evidence for the first time of a CP covalent aggregation seeding mechanism and extend existing MS disulfide bond mapping approaches to top-down sequencing.

Poster Number: P28.04

Cell-Free Synthesis of Full-Length Internal Standard Proteins for Top-Down Proteomics

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Background: Standard stable isotope-labeled peptides, *e.g.* AQUA peptides, have been used for absolute quantification of targeted proteins in bottom-up proteomics. In top-down proteomics, however, in which intact proteins are analyzed, full-length proteins labeled with stable isotopes could become the ideal reference standard for protein quantification. However, the high-throughput synthesis of intact protein standards is generally a challenge in cell-based biosynthetic approaches.

Objective: To construct a standard protein library for top-down proteomics, we established a high-throughput workflow for the synthesis of 13C/15N-labeled full-length recombinant proteins using a cell-free synthesis (CFS) system, and evaluated the quality of the synthesized protein standards through top-down mass spectrometry.

Methods: A commercial *E. coli* CFS kit (Taiyo Nippon Sanso, Japan) was used for protein synthesis. Stable isotope-labeled proteins were synthesized using 20 amino acids uniformly labeled with 13C/15N. The CFS reaction was performed in a microdialysis device. After the CFS reaction, the protein components in the reaction mixture were separated by SDS-PAGE and visualized by an aqueous CBB reagent (ATTO, Japan). The bands of the target proteins were selectively excised and recovered from the gels via passive extraction with 0.1% SDS for 10 minutes. Following methanol-chloroform precipitation, the synthesized proteins were analyzed through LC-MS/MS on the 21-tesla FT-ICR mass spectrometer (NHMFL).

Results: To evaluate the established workflow, we conducted CFS synthesis of stable isotope-labeled chloramphenicol acetyltransferase and bacteriorhodopsin. The 100-microliter scale CFS reaction yielded a sufficient amount of protein for top-down mass spectrometry analysis within 6 hours, and its stable-isotope labeling efficiency was approximately 100%. The combination of PAGE and rapid passive extraction allows efficient purification of target proteins without using affinity tags, and the resulting stable isotope-labeled proteins have the potential to be high-quality internal standards in top-down protein quantification.

Poster Number: P28.05

MASH Explorer, a Universal, Comprehensive, and User-friendly Software Environment for Top-down Proteomics

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Top-down mass spectrometry (MS)-based proteomics has emerged as a powerful tool for comprehensive analysis of proteoforms arising from sequence variations, alternative splicing and post-translational modifications. Top-down proteomics has recently gained an important space in the field of proteomics with a rapidly increasing user base. However, the lack of a universal, comprehensive and user-friendly software environment, and the complexity of high-resolution top-down proteomics data are increasingly recognized as major barriers for newcomers. To address these challenges, we have developed MASH Explorer, which is an integrated software environment for top-down proteomics. Our software can process data in various vendor formats and incorporates multiple algorithms for deconvolution and database searching. Furthermore, MASH Explorer incorporates powerful functionality to assist users with tasks such as protein identification, quantification and characterization.

MASH Explorer provides a graphical user interface (GUI) for performing MS data processing workflows. We have designed two workflows to guide users in analyzing top-down proteomics data. In discovery mode (which is used for LC-MS/MS datasets) MASH Explorer integrates several tools to centroid raw data, deconvolute isotopic distributions, and identify proteins. MASH Explorer implements methods to seamlessly convert between the input and output data formats of different algorithms and provides tools for queueing multiple analysis workflows to improve data analysis productivity. In targeted mode (which is used for MS/MS datasets) MASH Explorer allows users to characterize proteoforms by providing visualization tools to investigate matches between theoretical and observed fragment ions and to verify and update annotations. MASH Explorer also includes an ion finding tool to help find missing annotations caused by algorithmic issues or noisy data. Supporting multiple file formats and integrating numerous analysis tools, MASH Explorer is a vital tool for any top-down mass spectrometry experiment.

Poster Number: P28.06

Exploring Proteomics Data from Multi-sample Experiments

Iobani Godinez

PNNL, Richland,

We present a simple, standalone web application for exploring proteomics data from multi-sample experiments. The application takes a set of protein and peptide files as input in the widely-used statistical language R and outputs a javascript-based web application that can be run by itself and shared with collaborators or as supplemental data. The application is based on Trelliscopejs, a versatile R package for data visualization, and is advantageous for quickly filtering and sorting data as well as generating metrics. Using this package, input data is divided into proteins and a visualization panels are built for each protein. Individual proteins are visualized as a heatmap showing the abundance of contributing peptides across conditions and a bar graph summary of abundance. The user can quickly filter and sort which proteins they want to view by name, number of peptides, abundance values, or other qualities, such as comparative metrics such as differential abundance between conditions. Because of the flexible nature of the underlying

Trelliscopejs framework options for network graphs, scatterplots, and comparative boxplots are also available. The simplicity and ease of use of this tool make it useful for sharing proteomics data between experts and with non-proteomics experts.

Poster Number: P28.07

Optimization of Quantitative Top-Down Proteomics in Complex Samples using Protein-Level Tandem Mass Tag (TMT) Labeling

Dahang Yu[1]; Zhe Wang[1]; Kellye Cupp-Sutton[1]; Yanting Guo[1]; Mulin Fang[1]; Kenneth Smith[2]; Xiaowen Liu[3]; Si Wu[1]

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Isobaric chemical tag labeling (e.g., iTRAQ and TMT) has been widely applied for the quantification of peptides and proteins in bottom-up proteomics. However, until recently, the quantification of intact proteins using isobaric chemical tag labeling methods has been limited. Isobaric chemical tag labeling has also yet to be applied to labeling and quantifying complex intact protein samples. Application of isobaric chemical tag labeling methods to top-down proteomics methods has been limited primarily because (1) intact proteins tend to precipitate and "crash" out of solution during the labeling reaction, and (2) under labeling and over labeling issues complicate the analysis of complex samples.

In this study, we describe the optimization of intact protein labeling using TMT reagents. First, to reduce the precipitation of intact proteins under labeling conditions, we evaluated a filter-SEC approach that combines a molecular weight cut-off (MWCO) filter step with high-performance size exclusion chromatography (SEC) to remove large proteins in the complex sample. Our results indicate that this filter-SEC approach decreases the protein precipitation and allows for the identification and quantification of intact proteoforms (< 35 kDa) in complex biological samples. Then, we carefully evaluated and optimized the TMT reaction conditions including the reaction solvent, buffer pH, reduction and alkylation conditions, and protein-to-reagent ratios so that optimal labeling of intact proteins in a complex biological sample was observed. Overall, our results show that our optimized TMT top-down protocol can be applied to quantify intact proteoforms smaller than 35 kDa.

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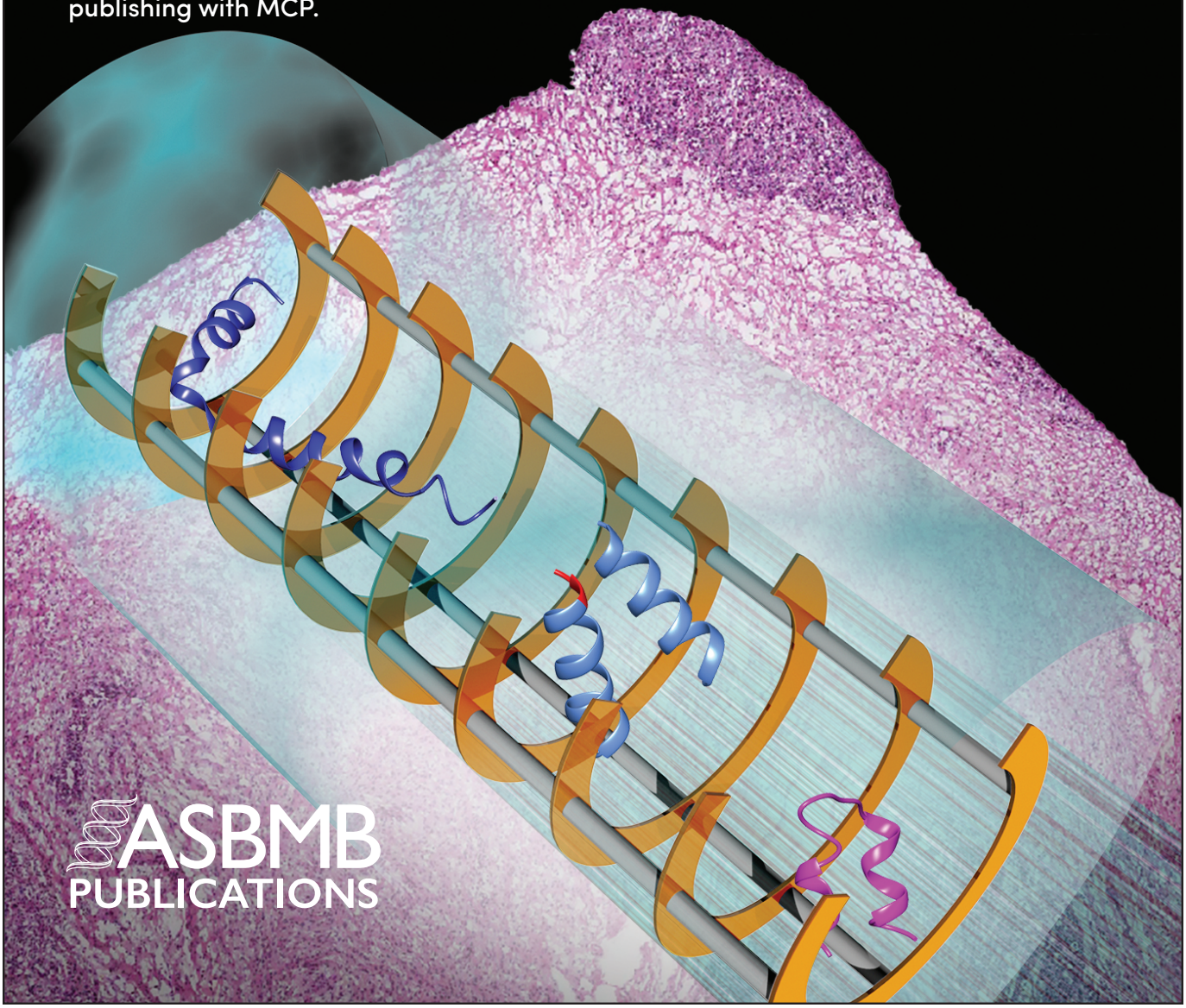

ASBMB PUBLICATIONS

AGENDA AT A GLANCE

SAT, MARCH 7 - SUN, MARCH 8	MON, MARCH 9	TUES, MARCH 10	WED, MARCH 11
SATURDAY	**8:00 – 8:30 am** Early Morning Coffee *Exhibits* *Grand 2&3*	**8:00 – 8:30 am** Early Morning Coffee *Exhibits* *Grand 2&3*	**8:00 – 8:30 am** Early Morning Coffee *Grand Foyer*
9:00 am - 4:00 pm Full-Day Course, Day 1 **Design and Analysis of Quantitative Proteomics Experiments** *Vashon*	**8:30 – 9:20 am** Plenary Session **Catherine E. Costello Lifetime Achievement in Proteomics Award Lecture** Ruedi Aebersold *Grand 1*	**8:30 – 9:20 am** Plenary Session **New Investigator Award** Si Wu and Nicolas Young **Computational Proteomics** Jimmy Eng *Grand 1*	**8:30 – 9:20 am** Plenary Session **Tips & Tricks Lightning Session** *Grand 1*
SUNDAY	**9:20 – 9:50 am** Coffee Break with Exhibits *Grand 2&3*	**9:20 – 9:50 am** Coffee Break with Exhibits *Grand 2&3*	**9:20 – 9:50 am** Coffee Break *Grand Foyer*
9:00 am - 4:00 pm Full-Day Short Course, Day 2 **Design and Analysis of Quantitative Proteomics Experiments** *Vashon*	**9:50 – 11:10 am** Parallel Sessions **Cascadia Proteomics Symposium Session** *Grand 1* **Cell Biology: Proteomic Analysis of PTMS and Proteoforms** *Fifth Avenue*	**9:50 – 11:10 am** Parallel Sessions **Computation & Analysis: Computational and Statistical Methods** *Grand 1* **Cell Biology: Systems Approaches for Cellular Signaling** *Fifth Avenue*	**9:50 – 11:10 am** Parallel Sessions **Computation & Analysis: Novel 'omics and Multiomics: Data Integration and Applications** *Fifth Avenue* **Cell Biology: Regulation and Function of Protein Phosphorylation** *Grand 1*
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4:30 – 5:45 pm Workshop **Career Development Event/Workshop**	**1:30 - 3:00 pm** **Poster Session & Break** *Grand 2 & 3*	**1:30 - 3:00 pm** **Poster Session & Break** *Grand 2 & 3*	
6:00 – 7:00 pm Opening Plenary Session **Carol Robinson** *Grand 1* *Vashon*	**3:00 – 4:20 pm** Parallel Sessions **Advances in Technology** *Grand 1* **Proteomics of Disease: Aging and Age-Related Diseases** *Fifth Avenue*	**3:00 – 4:20 pm** Parallel Sessions **Technology: New Developments in Structural Proteomics** *Grand 1* **Proteomics of Disease: Cancer and Precision Medicine** *Fifth Avenue*	
7:00 – 8:30 pm **Welcome Reception** *Mixer with Exhibits* *Grand 2 & 3*	**4:30 – 5:50 pm** Parallel Sessions **Technology: Innovations in Biological Sample Processing** *Grand 1* **Proteomics of Disease: Alzheimer's and Neurodegenerative Diseases** *Fifth Avenue*	**4:30 – 5:50 pm** Parallel Sessions **Technology: Chemical Proteomics and Drug Discovery** *Grand 1* **Proteomics of Disease: Immunity and Infectious Disease** *Fifth Avenue*	
	5:50 – 6:30 pm **Mixer** with Exhibitors *Grand 2 & 3*	**6:00 – 7:30 pm** Workshops **Biomarkers for Cancer, Early Detection** *Cascade 1* **Putting Humpty Dumpty Back Together Again: What Does Protein Quant Mean in Bottom-Up Proteomics?** *Cascade 2*	
	Workshops **Proteomics of Aging** *Cascade 2* **Data-Independent Acquisition (DIA) Workflows** *Cascade 1*	**7:30 – 9:00 pm** **Evening Social & Music with Exhibits** *Grand 2 & 3*	