ABSTRACTS

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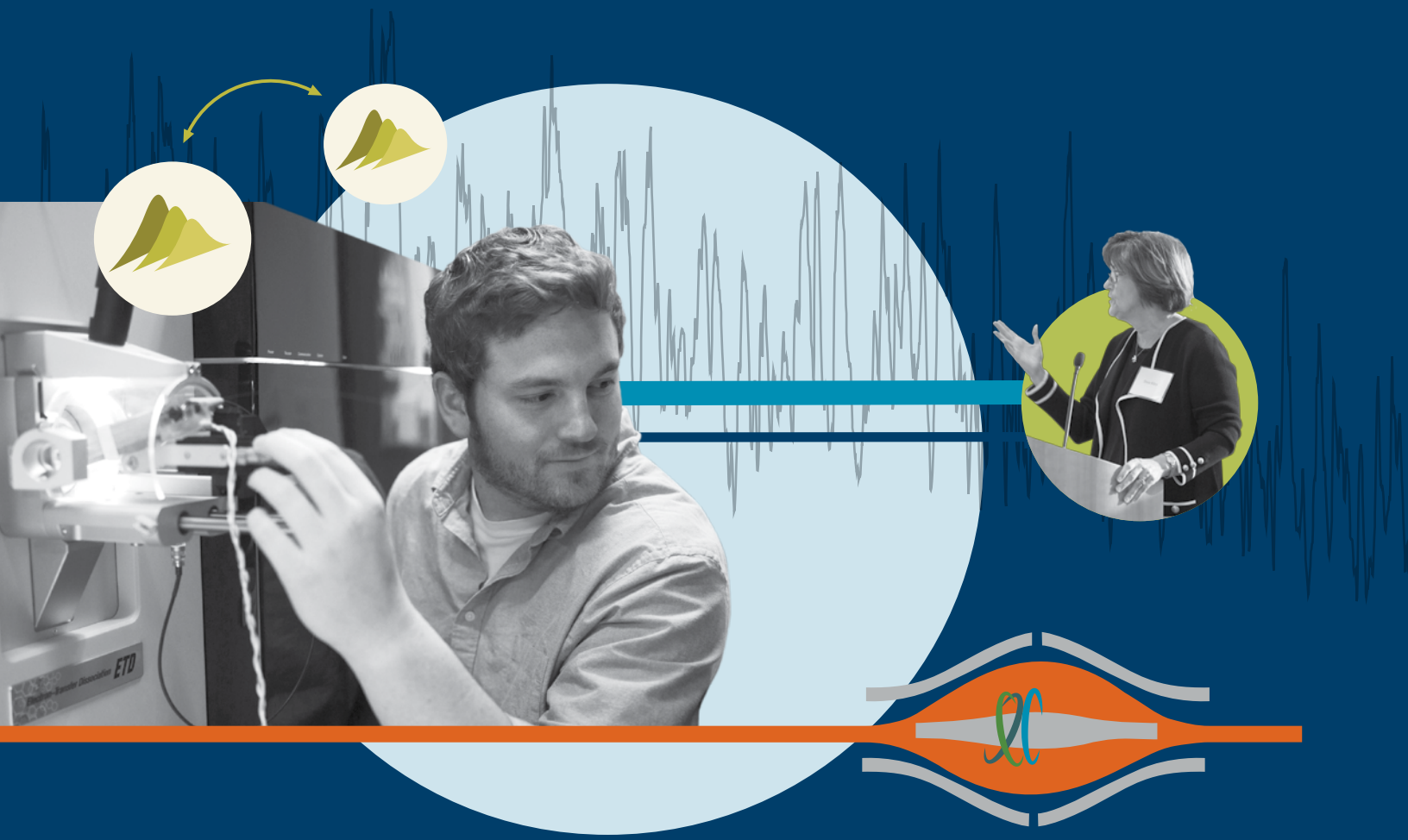
Organized by: Joshua Coon, Michael Sussman, Lingjun Li, David Pagliarini



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SPEAKER'S BIOGRAPHIES

STEVEN BRIGGS
Michigan State University

Briggs earned his PhD at Michigan State University in 1982 and then worked at Pioneer Hi-Bred Int'l; Novartis; Syngenta; and Diversa until 2004 when he accepted an appointment at the University of California San Diego. From 1987-1990 he was Senior Staff Investigator at Cold Spring Harbor Laboratory. Briggs studies plant immunity and genome biology with an emphasis on proteome dynamics. He discovered the first plant disease resistance gene; sequenced the first crop genome; developed the first reverse genetics system for plants; and discovered the lack of concordance between transcriptome and proteome networks. He is a member of the National Academy of Sciences, a Fellow of the American Association for the Advancement of Science, and a Fellow of the National Academy of Inventors.

SIXUE CHEN
Thermo Fisher Scientific

Professor Sixue Chen completed his Ph.D. in China and postdoctoral studies in Germany, Denmark, and University of Pennsylvania, USA. He is the Colonel Allen R. and Margret G. Crow Term Professor in Department of Biology, and Director of Proteomics and Mass Spectrometry at Interdisciplinary Center for Biotechnology Research of University of Florida, USA. Dr. Chen's areas of expertise fall in Biochemistry, Plant Metabolism, Functional Genomics, Proteomics, Metabolomics, and Mass Spectrometry. He learned mass spectrometry when he was collaborating with a senior chemist at the Danish Royal Veterinary and Agricultural University 20 years ago. Dr. Chen carried out a lot of small molecule work at that time. Since joining University of Pennsylvania in 2001, he has worked on many different projects using proteomics and mass spectrometry. Dr. Chen has accumulated experience with different separation and fractionation technologies (e.g., 2-DE), different HPLC instruments including the nanoflow ultra performance LC, as well as mass spectrometers. During his tenure as the Proteomics Facility Director at the Danforth Center in Missouri, USA, Dr. Chen has developed a high throughput protein identification technology. Dr. Chen has successfully administered projects, trained students and scientists, collaborated with other researchers, and produced more than 200 peer-reviewed publications. As a result of the valuable experience, Dr. Chen is aware of the importance of collaboration and implementing powerful proteomics and metabolomics technologies in solving challenging biological questions. At University of Florida, Dr. Chen has established three major research projects: plant guard cell hormone and CO2 signaling, stomatal innate immunity and glucosinolate metabolism. These projects have been funded by the US National Science Foundation, Department of Agriculture and National Institute of Health. Based on findings from large-scale "omics" studies, many novel, testable hypotheses have been derived. Another major component of Dr. Chen's research program has been hypothesis driven and testing, i.e., characterizing molecular, biochemical and physiological functions of specific genes and proteins in plant interaction with the environment. The integration of hypothesis generation and hypothesis driven research will ultimately lead to a holistic view of cellular molecular networks that connect environmental factors to physiological and phenotypic output. Dr. Chen serves as Associate Editor of Metabolomics (specialty section of Frontiers in Molecular Biosciences), Associate Editor of Frontiers in Plant Proteomics, Editorial Advisory Board Member of Journal of Proteome Research, Editorial Board Member of Journal of Proteomics, and Review Editor of Frontiers in Plant Metabolism and Chemodiversity.

JOSH COON
University of Wisconsin–Madison

I grew up in rural Michigan and during these formative years greatly enjoyed flyfishing and woodworking. Putting the latter interest to practical use, I constructed several riverboats (for fishing) while in high school and college. Chemistry interested me, especially Analytical Chemistry, as it offered an avenue to continue "building". Not boats, but chemical instrumentation. To escape the cold I joined the Chemistry graduate program at the University of Florida and worked with Willard Harrison. Professor Harrison didn't just guide my research, he taught me how to write, present, and think like a scientist. He was a gentleman in every sense of the word. Upon graduation in 2002, I moved to Charlottesville, Virginia to join the laboratory of Professor Don Hunt. At Virginia I met John Syka. Don and John both shared a passion for science that was as infectious as it was inspiring. Together we worked to develop electron transfer dissociation (ETD). ETD worked just as we had hoped and the dissociation technique is now commonly used for proteomics and has been commercially introduced by no fewer than four major instrument vendors. In 2005 I moved to Wisconsin to start my own program. And though we have been productive and impactful with ~ 200 published manuscripts, I am most proud to have produced nearly 20 Ph.D. scientists, and our academic family continues to grow.

The sequencing of the human genome marked the beginning of a collective scientific expedition to understand complex organisms. Genes, of course, merely contain the instructions for which proteins will populate the cell. Untangling the multi-faceted networks that regulate complex organisms and their diseases will require innovative technologies to globally monitor many classes of biomolecules, including nucleic acids, proteins, and metabolites. High-throughput technologies for gene and transcript measurement are well-developed and broadly accessible, and, as such, have had a fantastic and transformative impact on modern biology and medicine. For numerous reasons, methods for global analysis of proteins and metabolites – crucial biological effector molecules – are less evolved and markedly less accessible. The overarching mission of my program is to (1) facilitate expedient, comprehensive analysis of proteins and metabolites by innovating new mass spectrometric technologies and (2) apply these techniques to advance biomedical research.

OLIVER FIEHN
University of California, Davis

Prof. Oliver Fiehn has pioneered developments and applications in metabolomics with over 260 publications to date, starting in 2000 as group leader at the Max-Planck Institute for Molecular Plant Physiology in Potsdam, Germany.

Since 2004 he is Professor at the University of California, Davis, Genome Center. He is overseeing his research laboratory and the satellite core service laboratory in metabolomics research. Since 2012, he serves as Director of the NIH West Coast Metabolomics Center, supervising 35 staff operating 17 mass spectrometers and coordinating activities with three UC Davis satellite labs.

Professor Fiehn's laboratory members develop and implement new approaches and technologies in analytical chemistry for covering the metabolome. He applies metabolomics to metabolic questions in a range of human, animal and plant models. He also studies fundamental biochemical questions from metabolite damage repair to the new concept of epimetabolites, the chemical transformation of primary metabolites that gain regulatory functions in cells.

For his work, Professor Fiehn has received a range of awards including the 2014 Molecular & Cellular Proteomics Lecture Award and the 2014 Metabolomics Society Lifetime Achievement Award. He served on the Board of Directors of the Metabolomics Society 2005–10 and 2012–15.

STEVEN GOTTLIEB
Gottlieb Group Communications

Over the course of his career, Steven has worked with a broad and diverse set of organizations from Fortune 100 companies to founders of emerging technologies companies; scientists working in the fields of materials science, energy storage, and vaccines; financiers of innovation and real estate; top administrators of health care systems and university presidents; artists and athletes; elected officials and government institutions; and global aid and health professionals.

Earlier in his career, he held various positions at the Anti-Defamation League, in Denver and San Diego, where he oversaw regional civil rights initiatives and managed public opinion on a variety of First Amendment issues.

Gottlieb received Highest Honors from the University of California, Santa Cruz, earning a Bachelor of Arts in Modern Society and Social Thought. His paper about the American Press during the Holocaust resides at the United States Holocaust Museum and Archives in Washington DC.

Competition has always played an important part in his life:Steven is a two-time NCAA All-American and 1989 NCAA tennis champion. And he provides pro bono counsel to the International Tennis Hall of Fame in Newport, Rhode Island.

Two central components of Gottlieb's life are business and sports — he works with an aggressive and enthusiastic spirit. And listening to jazz is something he enjoys doing in his free time. Jazz is also an important connective tissue for how he marries business focus and athletic roots. In business and sports there is an improvisational necessity that occurs within set and agreed upon boundaries. He uses "Giant Steps" in his company's tagline ("Take Giant Steps In Impact and Influence") for a reason. Giant Steps was recorded by John Coltrane in 1959 and is one of the most complex and difficult jazz songs to solo over, but Coltrane makes it sound easy by finding the patterns and connections between chords and structures that remain hidden to most.

JO HANDELSMAN
University of Wisconsin–Madison

Over the course of his career, Steven has worked with a broad and diverse set of organizations from Fortune 100 companies to founders of emerging technologies companies; scientists working in the fields of materials science, energy storage, and vaccines; financiers of innovation and real estate; top administrators of health care systems and university presidents; artists and athletes; elected officials and government institutions; and global aid and health professionals.

ALEX HEBERT
University of Wisconsin–Madison

I started studying enzyme kinetics as an undergraduate researcher at Albany. Following graduation I moved to Philly to work for Merck in the vaccine development department. There I developed methods for all sorts of vaccines and got experience with separations and other analytical techniques. After four years I decided to go to UW for a Ph.D. in biochemistry. I joined the Coon lab as a graduate student and worked mostly on creating new proteomic methods. I graduated in 2013 and was fortunate enough to stay on as a scientist.

LINGJUN LI
University of Wisconsin–Madison

Dr. Lingjun Li is a Vilas Distinguished Achievement Professor and the Janis Apinis Professor of Pharmaceutical Sciences and Chemistry at the University of Wisconsin–Madison (UW–Madison). Dr. Li received her B.E. degree in Environmental Analytical Chemistry from Beijing University of Technology, China and her Ph.D. degree in Analytical Chemistry/Biomolecular Chemistry from the University of Illinois at Urbana-Champaign under Jonathan Sweedler in 2000. She then did joint

postdoctoral research at the Pacific Northwest National Laboratory (w/ Richard Smith) and Brandeis University (w/ Eve Marder) before joining the faculty at UW–Madison in December 2002. Dr. Li's research interests include the development of novel mass spectrometry (MS)-based tools such as new isotopic and isobaric labeling strategies that enable hyperplexing for quantitative proteomics, glycomics, microscale separations, in vivo microdialysis and imaging MS for functional discovery of neuropeptides and protein biomarkers in neurodegenerative diseases. Her lab also explores novel use of ion mobility MS to address technical challenges in peptidomic research, including site-specific peptide epimer analysis and improvement of isobaric tandem MS quantitation. Professor Li has established a highly productive research program and published more than 250 peer-reviewed research journal papers. She has given more than 200 invited talks. She has been recognized with numerous awards, including ASMS Research Award, NSF CAREER Award, Sloan Fellowship, PittCon Achievement Award, and ASMS Biemann Medal, and was named one of the Top 50 most influential women in the analytical sciences and was included in the 2016 Analytical Scientist Power List. Dr. Li served as an Associate Editor for Analytical Methods during 2013–16. She is currently serving as an Associate Editor for the Journal of the American Society for Mass Spectrometry (JASMS). Dr. Li has served on the ASMS Education Committee, Asilomar Conference Committee, the US HUPO Board of Directors, and the President for the Chinese American Society for Mass Spectrometry (CASMS) (2015–17).

GRAEME MCALISTER
Thermo Fisher Scientific

Graeme McAlister is a senior R&D scientist working for Thermo Fisher Scientific. He is part of the Tribrid team who support the Fusion, Lumos and ID-X mass spectrometers, as-well-as develop next generation Tribrid instruments. Prior to joining Thermo, Graeme earned his PhD in Joshua Coon's lab, where he was involved in the development of ETD. Between Wisconsin and Thermo he held a postdoctoral researcher position in the lab of Prof. Steven P. Gygi, where he developed new methods to improve the accuracy, precision, sensitivity, and capacity of TMT-based multiplexed quantitation.

ALEXEY NESVIZHSKII
University of Michigan

Alexey Nesvizhskii received his Ph.D. from the University of Washington in 2001 for his studies on theoretical solid state physics. After joining the laboratory of Ruedi Aebersold at the Institute for Systems Biology in Seattle, his research interests focused on the emerging fields of mass spectrometry-based proteomics and proteome bioinformatics. He established his own laboratory (www.nesvilab.org) in 2005 at the University of Michigan, Ann Arbor, where he is Professor in the Departments of Pathology and Computational Medicine & Bioinformatics. Dr. Nesvizhskii's research contributions include the development of concepts and computational methods implemented in such widely used bioinformatics tools as PeptideProphet and ProteinProtein, PeptideAtlas, CRAPome, SAINT, DIA-Umpire, and MSFragger. His lab actively collaborates with technology developers, biologists, and clinical scientists. He currently serves on the Board of Directors for the American Society of Mass Spectrometry (ASMS), US HUPO, and on the Scientific Advisory Board for Swiss Institute of Bioinformatics. At the University of Michigan (UM), Dr. Nesvizhskii directs the NIH funded T32 Proteome Informatics of Cancer Training Program. He also directs the Proteomics Resource Facility which provides cutting-edge proteomics capabilities to UM investigators.

DAVE PAGLIARINI
University of Wisconsin–Madison

Dave Pagliarini is the Director and Arthur C. Nielsen Jr. Chair of Metabolism at the Morgridge Institute for Research at the University of Wisconsin–Madison and is also an Associate Professor of Biochemistry. He is a graduate of the University of Notre Dame where he performed research in organic synthesis as an HHMI fellow in the lab of Paul Helquist. Dave began his graduate studies in chemical biology with Michael Marletta at the University of Michigan, and received

a Ph.D. in biomedical sciences under the direction of Jack Dixon at UC San Diego. For his postdoctoral fellowship, Dave joined Vamsi Mootha's laboratory at Harvard Medical School/The Broad Institute where he studied systems approaches to mitochondrial biology. At the Morgridge Institute, Dave leads an interdisciplinary team of scientists focusing on a range of leading-edge challenges in mitochondrial protein biochemistry and metabolism. Dave has received a number of honors for his work, including a Searle Scholar Award, the Protein Science Young Investigator Award from the Protein Society, and the Presidential Early Career Award for Scientists and Engineers (PECASE). He lives in Madison, WI with his wife, Carrie, and their two sons..

EVGENIA SHISHKOVA
University of Wisconsin–Madison

For my PhD, I worked on post-translational modifications (PTMs), specifically arginine methylation. Our lab had not studied this PTM before, so my project involved a lot of independence and innovation. I also worked on mammalian global proteome analysis, trying to develop techniques and optimize methods for analyzing more complex proteomes. We focused on separations as a key technological development that will enable this analysis.

In my current position, I'm continuing the project in mammalian global proteome analysis, which is a collaboration with the Pagliarini lab. We're aiming to characterize over 100 human knockout cell lines—all deletions of mitochondrial proteins, many of unknown functions. The goal is to gather insight into cellular functions of the knockout proteins by characterizing and interpreting the proteomics phenotypes. At this stage, our collaborators are starting some biochemical follow up trying to confirm our suggestions and we're determining what to look at in the future based on our analysis.

I'm also starting new projects with collaborators from all over the country through the NCQBCS. I take more direct positions of communication and sharing my expertise with them in experiment design compared to when I was a graduate student, but the highly collaborative aspect of the Coon Group research does prepare its students to be effective communicators. Being able to explain your knowledge and listen to what collaborators know and need is a necessary and transferable skill, in my experience.

DANIELLE SWANEY
University of California, San Francisco

Danielle Swaney got her PhD at the University in the laboratory Joshua Coon where she focused on applications of ETD fragmentation. She then went on to the University of Washington to do a postdoc in the laboratory of Judit Villen where she focused on developing MS methods to characterize PTM crosstalk and ubiquitin signaling. In her current position at UCSF, she now focuses using using proteomic approaches to understand cancer network biology.

W. ANDY TAO
Purdue University

Prof. W. Andy Tao leads an active research group focusing on new developments in proteome analyses using systems biology approaches. For the past several years, a number of novel proteomic strategies and techniques have been developed in his research group to analyze proteins and their modifications involved in specific biological functions. Current research projects include the identification of protein biomarkers for early onset of cancer from biofluids and dissecting kinase-substrate pathways in multiple cellular systems.

Tao received his mass spectrometry training through his dissertation work on gas-phase chiral analysis in the Aston Lab at Purdue University, headed by Dr. R. Graham Cooks. After receiving his Ph.D. in December 2001, he became a Damon Runyon Postdoctoral Fellow in the Institute for Systems Biology at Seattle, under the supervision of Drs. Leroy Hood and Ruedi Aebersold. He started his own research group in the Department of Biochemistry at Purdue University in 2005, and currently is Professor in ranking. He also has courtesy appointments in the Department of Chemistry, Department of Medicinal Chemistry & Molecular Pharmacology, and Purdue Center for Cancer Research.

CHARLIE TREVOR
University of Wisconsin–Madison

Charlie Trevor is the Pyle Bascom Professor in Business Leadership in the Department of Management and Human Resources at the Wisconsin School of Business. He currently serves as Department Chair. Earlier, he was a faculty member at Pennsylvania State University. He earned his Ph.D. in Industrial and Labor Relations from Cornell University in 1998 and has a couple of master's degrees from Michigan State. He teaches courses on negotiations, employee compensation, research methods, statistics, and Human Resource systems. His research focuses on employee turnover (particularly of the employees that companies can least afford to lose), the determinants and consequences of employee compensation, and employee downsizing.

OLGA VITEK
Northeastern Univerisity

Olga Vitek holds a BS degree from the University of Geneva, Switzerland, and a MS in Mathematical Statistics and a PhD in Statistics from Purdue University. She interned at Eli Lilly & Company in Indianapolis and held a position of post-doctoral associate in the Aebersold Lab at the Institute for Systems Biology in Seattle. Between 2006–14 she was an assistant professor, and then an associate professor with tenure at Purdue University, with a joint appointment in the Department of Statistics and Department of Computer Science. In the summer of 2014 she joined Northeastern University, with a joint appointment in the College of Science and the College of Computer and Information Science.

Professor Vitek was named the Sy and Laurie Sternberg Interdisciplinary Associate Professor at Northeastern University, and University Faculty Scholar at Purdue University. While at Purdue, she was recognized with an Outstanding Assistant Professor Teaching Award, a Graduate Student Mentoring Award, and a Teaching for Tomorrow Award. She is a recipient of the National Science Foundation CAREER Award. She serves on the Board of Directors of the US Human Proteome Organization.

MICHAEL WESTPHALL
University of Wisconsin–Madison

Measurement forms the foundation of the scientific method. Scientific theory may be validated through accurate measurement of physical and biological phenomenon; however, it is not possible to measure everything which theory predicts. This limitation has always motivated my research and has led to a lifelong career pursuing new techniques and instruments to perform measurements which currently cannot be made. This interest guided the selection of my first job after obtaining my Bachelor's degree in Physics, where I developed instruments to measure color. It further influenced my choice of discipline in graduate school. I received a Ph.D. in astrophysics, not because of my interest in astrophysics, but because of the difficulty in making the desired measurements and lack of instrumentation to do it. My career at the University of Wisconsin has been guided by the same principle; however, my research now focuses on the development of techniques and instrumentation for measurements in biotechnology.

ALICIA WILLIAMS
Rutgers University

Alicia Williams holds a PhD in English literature from Rutgers University, where she is a Henry Rutgers Postdoctoral Lecturer in the Writing Program. She studies nineteenth-century British fiction and the history of reading, and her work has appeared in *Victorian Poetry* and is forthcoming in *NOVEL: A Forum on Fiction*—literary equivalents of *JASMS* and *Nature Methods*. She was once in-house editor and writing mentor at the Coon lab, for which she now works on a freelance basis.

JOHN R. YATES
The Scripps Research Institute

John R. Yates is the Ernest W. Hahn Professor in the Departments of Molecular Medicine and Neurobiology at The Scripps Research Institute. His research interests include development of integrated methods for tandem mass spectrometry analysis of protein mixtures, bioinformatics using mass spectrometry data, and biological studies involving proteomics. He is the lead inventor of the SEQUEST software for correlating tandem mass spectrometry data to sequences in the database and developer of the shotgun proteomics technique for the analysis of protein mixtures. His laboratory has developed the use of proteomic techniques to analyze protein complexes, posttranslational modifications, organelles and quantitative analysis of protein expression for the discovery of new biology. Many proteomic approaches developed by Yates have become a national and international resource to many investigators in the scientific community. He has received the American Society for Mass Spectrometry research award, the Pehr Edman Award in Protein Chemistry, the American Society for Mass Spectrometry Biemann Medal, the HUPO Distinguished Achievement Award in Proteomics, Herbert Sober Award from the ASBMB, and the Christian Anfinsen Award from The Protein Society, the 2015 ACS's Analytical Chemistry award, 2015 The Ralph N. Adams Award in Bioanalytical Chemistry and the 2018 Thomson Medal from the International Mass Spectrometry Society. He was ranked by Citation Impact, Science Watch as one of the Top 100 Chemists for the decade, 2000–10. He was #1 on a List of Most Influential in Analytical Chemistry compiled by *The Analytical Scientist* 10/30/2013 and is on the List Of Most Highly Influential Biomedical Researchers, 1996-2011, *European J. Clinical Investigation* 2013, 43, 1339-1365 and the Thomson Reuters 2015 List of Highly Cited Scientists. He has published over **900** scientific articles with **~118,000** citations, and an H index greater than **172 (Google Scholar)**. Dr. Yates is the Editor in Chief at the *Journal of Proteome Research*.

HUI ZHANG
Johns Hopkins University

Prof. Hui Zhang joined Johns Hopkins University in 2006 and established Mass Spectrometry Core Facility, the Center for Biomarker Discovery and Translation. Her research interests are centered on the structural and functional analyses of proteins and protein modifications by glycosylation, phosphorylation, and acetylation. Recently, her laboratory has focused on glycoprotein analysis and developed several novel glycoproteomic and glycomic technologies to study structures and functions of cell surface glycoproteins and secreted glycoproteins. These technologies enable the characterization of glycoproteins, glycosites, glycans associated with each glycosite and their occupancies. Researchers in the group participate in several key national programs in characterization of glycoproteins including the Early Detection Research Network (EDRN), Clinical Proteomic Tumor Analysis Consortium (CPTAC), and Programs of Excellence in Glycosciences (PEG).

ABSTRACTS

PRESENTERS – names alphebatized by last name; numbers refer to poster number

1. JAZMIN ABRAHAM

Protein-protein interaction profiles of MADS-box dimer evolutionary variants

Biological processes are accomplished by proteins that depend on interactions with other proteins to carry out their function. These specific interactions are often maintained during evolution. The regulatory network that controls floral development is complex, and still is not completely understood, especially in monocots. Between the homeotic genes specifying floral organ identity, MADS-box transcription factors are the most important. MADS-box interactions between themselves and with other proteins form quartets, which are critical to DNA-binding. In most species, it is still unclear whether floral quartets assemble in the same way, and what is the identity of proteins in these complexes. Grass B-class MADS-box proteins differ in their protein–protein interaction profiles, and such differences might have profound effects in floral morphology. B-class proteins APETALA3 (AP3) and PISTILLATA (PI) bind DNA as obligate heterodimers with one another, but interestingly, both homo and heterodimerization occurs particularly in monocots. In some cases, as maize, only ancestral PI can form both. We are using the maize PI ortholog STERILE TASSEL SILKY EAR1 (STS1) and evolutionary variants of STS1 to determine the mechanistic basis of the altered protein complexes of differential dimerization, both in planta and in vitro, by immunoprecipitation, BiFC and gel shifts. IPs using an Anti-GFP antibody were analyzed in a Quad-Orbitrap hybrid MS. Identified protein complexes show differences between ancient and modern STS1. Quantitative proteomics is being used for further analysis. Our experiments will reveal the consequences of shifting protein-protein interactions to gene regulatory evolution, a fundamental, but poorly understood evolutionary process.

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2. MEASHO ABREHA

Comprehensive mapping of Alzheimer's disease brain ubiquitylome

Measho H. Abreha[4], Eric B. Dammer[1,4], Lingyan Ping[1,4], Duc M. Duong[1,4], Marla Gearing[3,4], James J. Lah[2,4], Allan I. Levey[2,4], and Nicholas T. Seyfried[1,2,4]

[1]Department of Biochemistry, [2]Department of Neurology, [3]Department of Pathology and Laboratory Medicine, [4]Center for Neurodegenerative Diseases, Emory University School of Medicine, Atlanta, GA, 30322

Introduction: Alzheimer's Disease (AD) is characterized by the deposition of protein aggregates such as neurofibrillary tangles (NFTs) and amyloid (Aβ) plaques in the brain. Post-translational modification (PTM) through ubiquitylation plays critical roles in maintaining protein homeostasis and clearing misfolded protein aggregates. Investigating global ubiquitylation changes in AD brain may provide insights regarding the underlying mechanisms of proteostasis and disease pathogenesis.

Methods: Here, we utilized an immunoaffinity approach to specifically enrich ubiquitinated (di-glycine) peptides from postmortem human control and AD brain tissues coupled with mass spectrometry (MS) analysis.

Result: MS based proteomic analysis identified global ubiquitylation changes associated with AD and hierarchical clustering of the human brain ubiquitylome clearly classified AD cases from healthy controls based on ubiquitylation patterns. Polyubiquitin linkage analysis identified increased levels of all seven polyubiquitin linkage types as well as total ubiquitin in the AD brain tissues. We also report novel ubiquitylation sites in the microtubule binding repeat of Tau with potential implications for Tau aggregation. Furthermore, KXGS motifs within the microtubule binding region (MTBR) of Tau protein, which represents the core of NFTs, are modified through both ubiquitylation and phosphorylation highlighting potential cross-talks between PTMS. Collectively, these studies highlight the utility of an immunoaffinity enrichment approach coupled with MS analysis for mapping AD associated global ubiquitylome changes as well as to gain insights into underlying proteostasis pathways altered in AD brain

3. KEVIN ADAM

Chasing the elusive phosphoramidate bonds by mass spectrometry using a non-acidic enrichment method.

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[4,] Department of Molecular Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037, USA.

Introduction: Phosphorylation is one of the most important post-translational modifications for the cell function. In living organisms, four types of phosphate-protein linkage (named the SONAtes) exist on nine different phosphoresidues – phospho-Ser/Thr/Tyr/His/Lys/Arg/Asp/Glu/Cys. Histidine phosphorylation in bacteria and plants has been established, and the existence of histidine kinases in mammalian cells is known since 50 years. Their functions are poorly understood and this phosphoproteome is largely unexplored. Indeed, the acid-lability of phosphohistidine (pHis) means that techniques applied to study O-phosphate or phosphate esters (phospho-Ser/Thr/Tyr) cannot be used or must be modified for the N-phosphate or phosphoramidate (phospho-His/Arg/Lys). Advances, including anti-pHis monoclonal antibodies, improvement of mass spectrometry methods and development of stable mimics of pHis, facilitate the study of phosphoramidate bonds.

Methods: Synthetic peptides chemically phosphorylated on His, confirmed by peptide dot blot with anti-pHis mAbs, thin-layer chromatography and MS/MS analysis, allowed us to develop and validate a method for purifying pHis-containing peptides from tryptic digests of a human cancer cell line (HeLa). This purification consists of a strictly non-acidic method compatible with hydroxyapatite (HAP) for global phosphopeptides enrichment, which conserves pHis, pArg and pLys. Subsequently, immunoaffinity purification (IAP) is done using 1- and 3-pHis mAbs crosslinked to protein A resin. pHis-enriched peptides are analyzed by LC-MS/MS using a nanospray ionization (NSI) source with CID fragmentation on an LTQ Velos Orbitrap and identified using a specific configuration of MaxQuant software, simultaneously considering Ser, Thr, Tyr, His, Lys, and Arg phosphoresidues.

Preliminary data: To test our enrichment method, a panel of known 1/3-pHis-containing sequence was synthesized: the 1-pHis isomer specific NME1 H118 site (RNIIHGSDS-amidated), the 3-pHis isomer specific PGAM1 H11 site (VLIRHGESA-amidated), and the non-defined 1- or/and 3-pHis HistH4 H18 site (GAKRHRKVL-amidated). Each peptide was analyzed by direct-injection into the Orbitrap, with subsequent manual validation of the peaks for the correct m/z ratio of the non-phosphorylated and the chemically phosphorylated forms. Specific parameters were applied to MaxQuant software, allowing successful automatization of the detection of histidine phosphorylation within these synthetic peptide sequences (>95% probability of localization).

The strictly non-acidic condition used to enrich pHis containing peptides from human cancer cell lines (HeLa and Alva31) conserved the histidine phosphorylation. pHis were detectable by immunoblot prior to the trypsin digest and then by dot-blot using pHis mAbs. Subsequently, global phosphopeptide enrichment using HAP in non-acidic conditions and the MaxQuant interrogation, revealed conventional and non-conventional phosphorylation sites allowing the coverage of all potential SONAtes phosphorylation, with about 5% pHis but only 57% conventional Ser, Thr and Tyr residues phosphorylated. More specifically, the known pPGAM H11 peptide was enriched by 3-pHis IAP from human cells and detected by mass spectrometry with a b/y ions series corresponding to a phosphorylation on H11. Whereas the combination of HAP and IAP increased the proportion of phosphoramidate bonds purified/detected, it can still lead to hydrolysis of His-phosphate bonds and suggests that we still should consider the use of IAP alone.

So far, a list of 20,865 peptides for 2,974 proteins were extracted from RAW data of HAP or/and pHis enrichment. 594 phosphorylation sites were detected including NME1 and PGAM1 peptides, two well-known pHis-containing proteins. 59 of these phosphorylation sites were localized to a His, 44 to an Arg and 50 to a Lys, corresponding altogether to 153 new phosphoramidate bonds.

Novel aspect: A non-acidic enrichment method allows the detection of N-phosphate residues by mass spectrometry.

4. PAUL ADAMCZYK

Combined ^{2}H and ^{13}C metabolic flux analysis enables novel discoveries in *Zymomonas mobilis* metabolism

Paul A. Adamczyk[a,b], Tyler B. Jacobson[b,c], Jennifer L. Reed[a,b], and Daniel Amador-Noguez[b,c]

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[c]Department of Bacteriology, University of Wisconsin—Madison

Zymomonas mobilis is an alpha-proteo bacterium ideal for metabolic engineering of biofuels such as isobutanol from renewable feedstocks owing to its unique properties; namely, strong resilience to lignotoxins present in lignocellulose-derived feedstocks, a simple metabolic network, fast and efficient glucose utilization, and low biomass yield. In this study, metabolic flux analysis (MFA) of wild-type *Z. mobilis* was performed using ^{2}H- and ^{13}C-labeled glucose tracers and subsequent measurement of downstream intracellular metabolites by high-resolution LC-MS. With successful, simultaneous fitting of data from seven

different isotopic tracers (1-^{13}C, 3-^{13}C, 6-^{13}C, 1,2-^{13}C, 2-^{2}H, 4-^{2}H, and 5-^{2}H glucose) into a single, statistically acceptable flux map, we were able to *i*) identify succinate dehydrogenase (SDH)-like activity and inactivity of a tricarboxylic acid (TCA) cycle shunt, *ii*) elucidate pathway thermodynamics, *iii*) determine select enzyme co-factor specificity and their substrate stereo-specificity and, *iv*) confirm a metabolic imbalance preventing efficient xylose catabolism in *Z. mobilis*.

Firstly, ^{2}H and ^{13}C MFA has identified SDH-like activity in *Z. mobilis*. Specifically, before this study, it was not clear whether *Z. mobilis* has SDH activity because only one out of four SDH subunits was annotated in the *Z. mobilis* genome. Upon addition of an SDH reaction to the model, non-zero flux and correct succinate labeling patterns were observed. Unexpectedly, although two Z. *mobilis* enzymes have strong sequence similarity to known cyanobacterial enzymes responsible for a TCA cycle shunt—driven by α-ketoglutarate decarboxylase and succinic semialdehyde dehydrogenase—MFA confirms that this shunt is inactive in *Z. mobilis* and succinate is not derived from it under the conditions tested.

We then inferred the thermodynamic favorability of many *Z. mobilis* reactions after calculating Gibbs free energies (ΔG) from forward and reverse fluxes as determined accurately by MFA. Specifically, we demonstrated the large *in vivo* thermodynamic favorability of the Entner-Doudoroff (ED) pathway that is supported by measured, high intracellular concentrations of ED pathway intermediates—before now, this was only a computational hypothesis.

Cofactor specificity of select *Z. mobilis* enzymes and their unknown substrate stereo-specificity were identified—possible only from ^{2}H labeling experiments. MFA reveals that *i*) glucose-6-phosphate dehydrogenase is only NADP^{+}-dependent, ii) glyceraldehyde-3-phosphate (GAP) dehydrogenase is only NAD^{+}-dependent with, *iii*) the C1 hydrogen from GAP being transferred exclusively to the *si* face of NAD^{+}, and *iv*) transhydrogenase-like activity is present in *Z. mobilis* and operates by transferring a hydrogen from NADPH exclusively to the *si* face of NAD^{+}.

Lastly, MFA confirms there is a metabolic imbalance in *Z. mobilis* pentose phosphate pathway due to an absent transaldolase preventing it from metabolizing xylose as a sole carbon source and causing an overflow of erythrose-4-phosphate, excretion of shikimate, and an intracellular shikimate concentration 1000-fold higher than in *E. coli*. Future MFA experiments to investigate this imbalance would enable rational engineering of *Z. mobilis* strains to obtain higher product yields from glucose- and xylose-rich renewable feedstocks.

5. MD SUHAIL ALAM

Molecular Proteomic Analyses of Chronic HDACi Treatment of Neurodegeneration

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Histone deacetylase (HDAC) inhibitors are of significant interest as drugs. However, their use to treat neurological disorders has raised concern because HDACs are required for brain function. We have previously shown that a triple combination formulation (TCF) of the pan HDACi vorinostat (Vo), 2- hydroxypropyl-beta-cyclodextrin (HPBCD) and polyethylene glycol (PEG) 400 improves pharmacokinetic exposure and entry of Vo into the brain. TCF treatment significantly delayed both neurodegeneration and death in a mouse model of Niemann-Pick Type C (NPC) disease (*Alam et al, 2016, Science Translational Medicine*). The TCF causes no metabolic toxicity. In addition, TCF administered in healthy mice for 8–10 months was not detrimental to the brain or neuromuscular functions based on quantitative analyses of Purkinje neurons, neuroinflammation, neurocognitive/muscular disease symptom progression, cerebellar/hippocampal nerve fiber-staining, and *Hdac* gene-expression. The TCF also improved delivery of Vo to lungs and reduced accumulation of foamy macrophages in *Npc* mice, with no injury. Together, these data support the feasibility of tolerable, chronic administration of an HDACi formulation that treats murine NPC neurological disease and lung pathology (*Alam et al, 2018, Scientific Reports*).

We have shown that TCF stimulates an increase in *Npc1* transcript and protein levels by 3 and 8 fold respectively (*Alam et al. 2016 Science Translational Medicine*). However, since vorinostat is a broad acting drug, we hypothesized that the TCF stimulates expression of a large number of additional targets, in particular chaperones that stimulate better folding and therefore increased NPC1 function. Our preliminary findings with RNAseq suggest that within 4 hours of administration, the TCF stimulates high levels of a unique chaperone and anti-inflammatory signatures associated with hundreds of genes. However, cellular and organellar functions are defined at the level of the proteome and metabolome. Therefore, we plan mass spectrometry based proteomic and metabolomics studies at the level of well-defined neurons (such as Purkinje) as well as microglia (whose levels are known to decrease in the hippocampus in response to TCF).

The novelty of these studies lies in the fact that TCF is a new formulation that delivers an efficacious dose of an FDA approved HDACi to prevent neurodegeneration in the brain. Moreover, it appears to detoxify the HDACi, suggesting for the first time that through TCF, chronic HDACi may be deployed to create proteomic and metabolic contextual networks that promote proteostasis and prevent neurodegeneration

6. W. TEMPLE ANDREWS

Metabolomic and proteomic analysis of GluN2D-/- and wild-type mouse brains after ischemic stroke using MALDI MSI.

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Introduction: During pathological conditions such as ischemic stroke, excess of neurotransmitters causes hyperactivation of N-methyl D-aspartate (NMDA) receptors in the neuronal cleft triggering unfavorable signaling events and eventually neuronal death. The NMDAR is a heterotetramer composed of two GluN1 subunits and two GluN2 subunits (GluN2A, 2 B, 2C, 2D). Our collaborators in the Castellino laboratory (University of Notre Dame) are studying the effects of a GluN2D deficiency, that contributes to protection against ischemic strokes. The present study will use MALDI MSI to detect proteomic changes in localization in GluN2D$^{-/-}$ and wild-type mouse brains. In addition, we will also investigate changes in metabolite abundance and localization within the GluN2D$^{-/-}$ mouse brains, as ischemic strokes typically cause depletion of oxygen and nutrients.

Methods: Stroke was induced using the middle carotid artery occlusion/reperfusion model using isofluorane as the anesthetic. The main and internal carotid arteries are ligated to prevent blood flow, and the middle carotid artery occluded using a coated-tip suture. Ninety minutes post-occlusion, the suture is removed to allow for reperfusion. *Ex vivo,* post-ischemic brains of GluN2D$^{-/-}$ and wild-type mice were sectioned into 15 micron thick coronal sections using a cryostat, and coated with a matrix solution using an HTX-Imaging TM-Sprayer. For metabolomics studies, brain sections are coated with CHCA, while SA will be used for proteomic studies. Matrix-coated brain sections are then imaged at 50-micron spatial resolution using MALDI MSI and analyzed to look at metabolite and protein distributions.

Preliminary Data: Ischemic strokes typically cause depletion of oxygen and glucose to the infarcted region. We therefore, set out to study glycolytic metabolite distributions, as well as, changes in protein distributions, in mouse brains at 24 hours post-ischemia. In this study, post-ischemic brains of GluN2D$^{-/-}$ and wild-type mice have been removed, sectioned, and analyzed using MALDI MSI. Changes in protein and metabolite distributions will be compared between the stroked ipsilateral hemisphere and the non-stroked contralateral hemisphere that serves as a robust internal control. By analyzing protein and metabolite distributions in GluN2D$^{-/-}$ and wild-type post-ischemic mouse brains, we will gain a better understanding of how the GluN2D subunit of the NMDA receptor contributes towards ischemic stroke pathology.

Brain sections of GluN2D$^{-/-}$ and wild-type mice have been coated with CHCA, and imaged using MALDI MSI to analyze changes in glycolytic metabolite distributions. Preliminary data has shown increased levels of pyruvate, 3PG, and F1, 6P (full forms) in the ischemic part of the brain compared to the contralateral region. Although the identities of these compounds need to be confirmed using MS/MS, these results are promising nonetheless. The same tissue section will then be re-coated with SA to analyze protein distributions as well. Because of the low shot count required to acquire metabolic data, sample loss is minimal, making this technique very feasible. Serial tissue sections will also be analyzed to determine medial changes throughout the mouse brains as well.

Novel Aspect: This study allows us to gain a better understanding of how the GluN2D subunit contributes to protection against ischemic strokes.

7. FOLAGBAYI AROWOLO

Effects of Prepubertal Oxidized Dietary Fat Consumption on Body Weight, Adiposity and Adipose Distribution in a Swine Model

High-fat diets, which are increasingly common in developed and developing countries, have been associated with an increased chronic disease risk. The effects of these diets take stage early in life with childhood consumption of fats increasing, and associated incidence of obesity, increasing in these countries. However, what is often neglected when studying the impact of fats in diets is that modern processing and western cooking (deep-frying) practices generate lipid oxidation products (LOPs), and thus fats are not always consumed in their native forms. **In this study, we examined whether consumption of high-fat diets containing lipid oxidation products have a different effect on body weight, adiposity and adipose distribution using a**

swine model. The overwhelming genetic, anatomical, physiological, and pathophysiological similarities to humans make swine an ideal model for translational studies.

Forty-eight domestic swine (at twenty-one days of age, immediately post weaning) were divided into three equal study groups and placed on the following diet regimens for five months: (1) **Low-Fat/Low-Calorie Diet** (LF/LC Diet, N=16, 9% of calories from fat). (2) **High-Fat/High-Calorie Diet containing LOPs** (HF/HC+LOPs Diet, N=16, 44% of calories from fat). (3) **High-Fat/High-Calorie Diet without LOPs** (HF/HC–LOPs Diet, N=16, 44% of calories from fat). Body weight (BW) measurements were made weekly. Total adiposity and distribution were assessed at four different time points on eight of the animals from each diet group using dual energy x-ray absorptiometry (DXA). The results from the study suggests that fats containing LOPs do not have the same physiological effects as fats in the native form and the presence of LOPs should be considered in dietary studies. We are currently analysing differences in gene expression in tissues from the study animals to develop mechanistic hypothesis for the observed effects of LOPs.

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8. KASSIE BALESTRIERI

Multiple Mass Spectrometry Approaches Identify Galectin-1 Overexpression in Murine Primary and Metastatic Triple Negative Breast Tumors

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Introduction: Triple negative breast cancer (TNBC) lacks expression of estrogen and progesterone receptors and has reduced expression of tyrosine kinase receptor HER2. Metastases and mortality among TNBC patients remain high due to less effective, highly toxic therapies compared to other breast cancer subtypes, indicating an urgent need for targeted therapies. Our objective is to identify proteins associated with the development of TNBC metastases to gain insight into pathways and mechanisms that could help identify biomarkers or targets for intervention. The present study uses the murine T11 tumor model, which displays gene expression profiles mirroring human claudin-low TNBC. Multiple mass spectrometry (MS) analyses identified Galectin-1 (Gal-1), a β-galactoside binding protein, as an overexpressed protein in TNBC that merits further study.

Methods: MS analysis was conducted on cultured murine T11 tumor cells, primary orthotopic tumors and lungs containing metastases (n≥2) developed in syngeneic mice, as well as mammary fat pad and lung tissue (n≥2) from naïve mice (healthy contemporary controls). Nano-LC/MS, using a 90-minute gradient, was performed on trypsin-digested lysates and analyzed with Protein Pilot software. To spatially map peptides, MALDI/MSI was conducted on 10 μm sections of flash-frozen tissues which were trypsin-digested using a sprayer, incubated for 2 and 4 hours at 37°C, and coated with SA matrix. MALDI/MSI data was analyzed using FlexImaging and Scils software. Gal-1 identification and expression patterns were investigated by Western immunoblot, immunohistochemistry and by analysis of published murine and human genomic data sets.

Preliminary Data: Nano-LC/MS analysis of T11 cultured cells, primary tumors and lung metastases detected high levels of the N-acetyllactosamine-specific binding protein Galectin-1 (Gal-1) with >95% confidence. In cultured cells, ten distinct Gal-1 peptides were identified with protein coverage of 88%. A consistent Gal-1 peptide was identified in lysates of all primary tumors tested, but absent from normal mammary fat pad. Two consistent Gal-1 peptides with 28% protein coverage were detected in metastatic lungs, but not in lungs from healthy control mice. MALDI/MSI detected Gal-1 peptides with Mascot scores >126 after MS-Bridge in all tumor and lung sections. Gal-1 expression (intensity) levels were higher in primary tumors compared to tissue from healthy naïve mice and higher in metastatic areas of lung tissue, compared to adjacent areas. Eleven Gal-1 peptides identified in MALDI/MSI were present in >67% of tumor samples (n=3) and >75% of metastatic lung samples (n=4) with four consistent peptides in 100% of samples. Western blot and immunohistochemistry analysis demonstrated higher Gal-1 expression in T11 primary tumors compared to control mammary fat pad, and higher Gal-1 expression in T11 lung metastases compared to normal lung. Immunohistochemistry also confirmed spatial distribution of Gal-1 in primary tumors and lungs with increased expression within metastatic tumor foci of lung tissue. These findings that Gal-1 is highly expressed in TNBC are further supported by our analysis of both murine and human genomic data sets, which show Gal-1 overexpression in TNBC compared to other breast cancer subtypes and normal breast tissue. To date, there are few studies of Gal-1 expression or function in TNBC, although several reports in mouse models have suggested a role for Gal-1 in tumor cell migration, metastasis and/or immune

evasion. Our findings support the need for further investigations that focus on determining the role and significance of Gal-1 overexpression in TNBC progression.

Novel Aspect: Nano-LC/MS and MALDI/MSI identified Gal-1 overexpression in primary and metastatic TNBC, confirmed by Western blot, immunohistochemistry, and genomic analysis.

9. STEPHANIE BERG

Fourier-transform ion cyclotron resonance mass spectrometry to characterize dissolved organic matter and describe observed photoreactivity at the molecular level

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Dissolved organic matter (DOM) is a heterogeneous mixture of organic molecules found in all natural waters. Its composition dictates its reactivity in many environmental processes including photochemical reactions which can control the fate of persistent organic contaminants in sunlit surface waters. We collected diverse water samples along the St. Louis River in order to relate the differences in DOM composition to observed photochemical reactivity. Bulk properties such as total organic carbon and UV-visible absorbance spectra were measured. Additionally, DOM was isolated from the whole water samples using solid phase extraction and directly injected into a Bruker SolariX XR Fourier-transform ion cyclotron resonance mass spectrometer. Chemical formulas were assigned to exported *m/z* values using a script written in the *R* programming language. Photochemical reactivity was assessed using kinetic probe experiments to quantify steady-state concentrations and quantum yields for the formation of photochemically produced reactive intermediates (PPRI) including triplet DOM (^{3}DOM), singlet oxygen ($^1O_2$) and hydroxyl radical (•OH). Steady-state concentrations of all of the PPRI decreased downstream in the river as organic carbon concentrations decreased. Quantum yields for ^{3}DOM and $^1O_2$ production increased while the quantum yield for •OH production decreased. Spearman rank correlations between formula intensities and quantum yields showed that less oxygenated and more saturated formulas, which were enriched downstream in the river, were generally most efficient at ^{3}DOM and $^1O_2$ and least efficient at producing •OH. This signifies a link between DOM composition and photochemical reactivity at the molecular level.

10. PEDRO BESCHOREN DA COSTA

Network analysis highlights taxa in microbial communities that are often ignored: a microcosm study in plant growth promoting bacteria efficiency

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Application of bacterial inoculants in crops has been proven to improve crop yields with greatly favorable cost-benefit ratios, but wide variations in plant growth promoting bacteria (PGPB) efficacy continues to be a problem difficult to solve. As plant-microorganism interactions are very complex, the use of new analysis tools may be important in revealing natural patterns and associations henceforth undetectable. In this work, we tested a PGPB model that anticipates inoculation efficiency according to different bacterial taxa, strain phenotypic traits, and soil types, over a diversity gradient microcosm. We use network analysis, a relatively new tool in microbial community studies, to suggest ecological and functional roles from different taxa and complement other big data analysis strategies applied in NGS and -omics. A dilution to extinction approach was employed to generate a diversity gradient microcosm, by inoculating sterile soils with progressive dilutions of natural soils (dilutions of 10^{-1}, 10^{-3} and 10^{-6}). After stabilization of the microbial communities in both low-nutrient sandy soil (poor soil) and high-nutrient clay soil (rich soil), rice seeds were planted and inoculated with *Burkholderia vietnamiensis* strain 45 and *Enterobacter asburiae* strain 68. Bulk pot soil samples were obtained immediately before planting, 16s rDNA amplicons were sequenced under the MiSeq platform, and plants developed over 30 days. Sequencing data was analyzed in the MENA pipeline (http://ieg4.rccc.ou.edu/mena/login.cgi) under its standard configuration to generate co-ocurrence networks for poor and rich soils that were evaluated in CytoScape 3.6.0. As according to the expectations of the model we were testing, *B. vietnamiensis* 45 was a better

PGPB in the poor soil, while *E. asburiae* 68 was a better PGPB in the rich soil, when compared to non-inoculated controls. The diversity gradient strongly affected inoculation effects, most notably for the *E. asburiae* 68 in the rich soils. At the 10^{-1} dilution, *E. asburiae* 68 decreased plant shoot length and dry weight compared to control; at the 10^{-3} dilutions it was similar to control, and at the 10^{-6} dilution it increased plant shoot and dry weight compared to control. On-going network analysis of the microbial communities before planting indicate that three Actinobacteria and one Acidobacteria OTUs were highly connected to different network modules (sets of taxa that co-occur between themselves more highly than between other members of the community, representing shared niches or ecological function); these four OTUs frequency progressively reduced as diversity in the microcosm reduced. This suggests that their intense competition with the inoculant on the 10^{-1} microcosm was harmful for plant development, but the inoculant recovered the function of these highly connected OTUs when they were absent from the 10^{-6} microcosm. In addition, one of the network modules dominated by Firmicutes had a single negative co-occurrence with a module hub (a taxa that is strongly associated with a single module structure and stability). This module hub was central to the largest module, and also decreased in frequency as diversity diminished. Mantel tests show that Verrucomicrobia was positively correlated with plant shoot length and dry weight ($r=0.759$; $p= 0.05$). We are currently attempting to correlate specific OTUs and modules to plant biomass and soil traits. None of these results were noticeable under NMDS or PERMANOVA tests, suggesting that network analysis could complement big data evaluation. Network analysis reveals surprising correlations, complementing other statistical methods; it could also be applied in mass spectrometry to great effect

11. FEI BIAN

Investigating the dynamics of multi-protein enzyme complexes during metabolic adaptation in *Escherichia coli* K-12.

Introduction Our previous work indicated that a vast majority of *Escherichia coli* proteins with enzymatic activities are found in multi-protein complexes or have unexpected protein-protein interactions (PPIs). Many of the macromolecular assemblies and as sociations we uncovered in *E. coli* grown in rich media are linked to specific biochemical pathways known to subject to stringent regulatory controls(Nature Biotechnology. 2014, **32**(3):285-90.;Nature Biotechnology 2018, **36**(1):103-12.). In this current project, as a new member of the Emili group, a world leader in proteomics and global protein interactome mapping, I plan to examine the dynamics of enzyme-protein complexes and PPI networks more generally in response to limiting culture conditions (carbon, nitrogen, phosphorus, sulfur or metal sources) that are known to perturb core metabolic processes. By doing this, I expect to define a previously over looked molecular basis underlying metabolic control.

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Methods After cultivating *E. coli* K-12 strain under different culture conditions, the cells will be lysed, then the membrane and cytosolic protein lysates will be fractionated using our multi-dimensional separation system. The stably co-eluting protein fractions will be isotopically labeled, sequenced and quantified using a quadrupole-Orbitrap Q-Exactive HF-X mass spectrometer. Spectra will be mapped against reference *E. coli* protein sequences, and combined with other more precise measurements will allow us to accurately quantify changes in complex memberships, as well as post-translational modifications and subunit stoichiometries across conditions.

Preliminary Data I just started in the Emili lab and my new project in Feb. 2018 and have not got my own data yet. That said, the lab has ample co-fractionation data for *E. coli* under standard (rich media) growth conditions.

Novel Aspect To illustrate that *E. coli* has evolved adaptive mechanisms to regulate multi-protein enzyme assemblies and hence metabolism in response to environmental perturbations.

12. JACOB BIBIK

Discovery of diterpene synthases with novel activity facilitated by transcriptomics

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Diterpenes are a structurally diverse group of natural compounds that have broad applications in the pharmaceutical, cosmetics, and bioenergy industries. Engineering specific diterpene synthesis pathways in organisms for bioproduction is of great interest in synthetic biology. The enzymes responsible for the diterpene skeleton formation are diterpene synthases (diTPSes). DiTPSes are divided into two classes and catalyze sequential steps in the synthesis of diterpenes. While class II diTPSes synthesize diphosphate intermediates from geranylgeranyl diphosphate (GGDP), class I diTPSes use the class II products as substrates for conversion into diterpene skeletons. Our study was conducted to expand our diTPS toolbox to broaden the types of diterpenes that can be produced by engineered organisms. The *Lamiaceae* plant family is rich with a variety of terpenoids, but many of the diTPSes are unknown. We mined *Lamiaceae* transcriptome data from 48 divergent species and selected candidate genes distant from previously characterized diTPSes. Candidate genes were cloned and transiently expressed in *Nicotiana benthamiana.* Class II enzymes were expressed with and without a class I partner, while class I enzymes were always co-expressed with class II enzymes. Crude leaf extracts were analyzed by GC-MS and novel diterpenes elucidated by NMR. Of the genes studied, we found diTPSes with activities previously unknown in the *Lamiaceae* family and with activities unknown by any enzyme. These added tools allow us to now begin engineering biological systems for production of novel diterpene compounds.

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13. ISAAC BISHOF

Identification of methylation and phosphorylation in arginine-rich domains by electron transfer dissociation mass spectrometry

A number of RNA binding proteins with important roles in splicing have highly basic arginine rich (R- domains). Evidence suggests that post-translational modifications (PTMs) within R-domains, such as phosphorylation and methylation, play important roles in regulating protein–protein and protein–RNA interactions. However, identification of PTMs within R-domains after trypsin digestion is extremely challenging due to the high density of arginine residues and because collision induced dissociation (CID) or higher energy collisional dissociation (HCD) peptide fragmentation do not efficiently fragment multiply protonated peptides. To overcome this limitation a middle-down proteomic approach coupled with electron transfer dissociation (ETD) mass spectrometry was utilized to map previously unknown sites of phosphorylation and methylation within R-domains. It was found that R-domains are densely modified suggesting a possible role in RNA binding protein regulation

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14. ANNE-SOPHIE BOHRER

Control of sulfur metabolic pathway in *Arabidopsis thaliana* through enzyme complex formation in the cytosol.

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Sulfate is an essential macronutrient for plant growth and development. Sulfate is taken up from the soil *via* plasma membrane-localized sulfate transporters, and is subsequently activated by ATP sulfurylase (ATPS) in plastids and cytosol to form adenosine 5'-phosphosulfate (APS). APS can then be either reduced in plastids by APS reductase to form primary metabolites such as cysteine and methionine, or phosphorylated in both cytosol and plastids by APS kinase (APK) to produce 3'-phosphoadenosine 5'-phosphosulfate (PAPS). In mammals, PAPS biosynthesis is catalyzed by PAPS synthase, a bifunctional enzyme containing ATPS and APK domains. Since orthologs for PAPS synthase are not found in plants, interaction between ATPS and APK may be postulated as a metabolic pathway control mechanism modulating PAPS biosynthesis.

Cytosol-specific cytATPS2/APK3 interaction was assessed by BiFC in *Arabidopsis* protoplasts. The cytATPS2/APK3 complex

formation is further analyzed through an epitope tagging approach in *Arabidopsis* protoplasts and yeast expression systems. Point mutations affecting the catalytic efficiencies of cytATPS2 (ΔF209L) and APK3 (ΔG42A) were introduced to determine their effect on the enzyme complex formation. Moreover, the functionality of cytATPS2 and APK3 fusion proteins (wild type or mutants; with or without split-YFP and epitope tags) was assessed by complementation in yeast (*met3* (Δatps) and *met14* (Δapk) single mutants or *met3met14* (ΔΔatps-apk) double mutant).

When cytATPS2-nYFP and cYFP-APK3 fusion proteins are co-expressed in *Arabidopsis* protoplasts, a strong fluorescent signal was detected, indicating that cytATPS2 and APK3 interact. The point mutation, which corresponds to F209L in cytATPS2, reduces catalytic efficiency to produce APS (forward reaction); however, it enhances its capability to perform the reverse reaction (ATP synthesis) (*Hermann et al, JBC, 2012*). In this study, we examined if this F209L mutation in cytATPS2 complements the lack of ATPS in yeast *met3met14* mutant. Co-expression of cytATPS2 and APK3 restored the growth of *met3met14* on minimal medium lacking methionine, while co-expression of cytATPS2^{F209L} with APK3 only had a marginal effect on growth recovery. However, co-expression of cytATPS2^{F209L}-nYFP and cYFP-APK3 for BiFC in *Arabidopsis* protoplasts did not seem to affect their interaction

A previous study on *Arabidopsis* APK1 isoform indicates that the point mutation ΔG113A causes the protein to be catalytically inactive without affecting its affinity for APS (*Ravillous and Jez, JBC, 2014*). When APK3^{G42A}, the amino acid substitution identical to APK1^{G113A}, was expressed in yeast *met14* mutant, it showed no ability to restore the yeast growth on minimal medium lacking methionine, verifying APK3^{G42A} is inactive. When cytATPS2-nYFP and cYFP-APK3^{G42A} were co-expressed in *Arabidopsis* protoplasts, no signal for BiFC was detected. The loss of APK3 function due to G42A mutation therefore prevents cytATP2/APK3 complex formation.

The cytATPS2/APK3 interaction is further tested in yeast *met3met14* double mutant and *Arabidopsis* co-expressing cytATPS2-mycHis and APK3-HA. Both epitope-tagged proteins are functional as their co-expression restores the growth of *met3met14*. Their interaction will be examined by pull-down assay and western blot analysis.

We hypothesize that cytATPS2 and APK3 are assembled to an enzyme complex when the APS concentration in the vicinity of the enzyme is low. As production of APS increases, cytATPS2 and APK3 dissociate, allowing APK3 to produce PAPS in the cytosol.

A proposal of a sulfur metabolic pathway control mechanism implicating ATPS/APK enzyme complex formation and dissociation

15. ELI BORREGO

Volatile and non-volatile oxylipins regulate systemic defense against insects in maize

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Unlike dicotyledonous plant species (e.g., Arabidopsis), where leaves are connected to each other via the stem, monocotyledonous seedlings (e.g., rice, maize, and wheat) possess a distinct anatomy that necessitates the movement of any leaf-to-leaf mobile signal to travel through the roots before becoming systemic. In response to pathogen attack, two fatty acid oxygenation products, known as oxylipins, serve as priming signals (i.e., jasmonic acid and azelaic acid) that travel from the site of infection to systemic tissue to prepare uninfected tissue for defense. In terms of systemic defense against insect herbivores, much remains to be elucidated for any plant species, but oxylipins are excellent candidates to explore this phenomenon.

In plants, the lipoxygenase (LOX) pathway is the principle producer of oxylipins. LOX10 is the sole maize isoform responsible for production of green leaf volatiles (GLVS) which directly and indirectly provide defense against insects. Alongside a defective herbivory defense response in local tissue, knockout mutants of *lox10* are also unable to accumulate 16 defense related oxylipins, termed systemic oxylipins signals (SOS), in systemic tissue determined by liquid chromatography- mass spectrometry. In response to herbivory, *lox10* mutants were unable to accumulate normal concentrations of oxylipins in phloem-enriched sap compared to WT. Remarkably, RNAseq analysis revealed that roots but not leaves of *lox10* mutants are perturbed in normal expression of oxylipin biosynthesis compared with WT. In accordance, concentrations of specific oxylipins in xylem-enriched sap from roots are induced by herbivory. In addition to the proposed *LOX10*-dependent SOS, another potential mode of action for LOX10 during long-distance systemic signaling is through direct LOX10 protein movement via the vasculature. Taken together, these results provide evidence for an oxylipin-mediated interaction between leaves and roots that regulates systemic defense against insects.

16. EMILY BRITT

Quantitative characterization of metabolic reprogramming during macrophage activation by LC-MS based metabolomics.

Introduction: Stimulated by classical activation signals, macrophages rapidly undergo a series of functional changes, including the production of different cytokines and changes in phagocytosis ability. This enables macrophages to properly function in immune defense against pathogens. Macrophage activation is accompanied by dramatic and coordinated rewiring of transcription and signaling pathways. Emerging evidences suggest this process also requires reprogramming in metabolism to support functional changes. However, the metabolic activities being regulated and how this is related to functional transitions remains largely unknown.

Methods: Using metabolomics and isotopic labeling approaches, we characterized metabolic changes as macrophages respond to classical activation stimuli (LPS and interferon-gamma) throughout a time course of stimulation. First, we used LC-MS based metabolomics to quantify the changes in total levels of over 100 intracellular metabolites. To investigate the metabolic flux alterations which drive the dynamic changes observed in central carbon metabolism, we applied isotope tracers including U-C13-glucose and 5-C13-glutamine. To validate the important regulation points identified in the tracer experiments and further study the mechanisms for such regulation, we performed targeted biochemical assays.

Preliminary data: We discovered that upon stimulation with classic stimuli, macrophages undergo systematic and highly dynamic metabolic shift. Levels of intermediates in nucleotides degradation, arginine metabolism, pentose pathway and central carbon metabolism show profound changes. Particularly, several metabolites in in TCA cycle show transient increase during early activation phase, which correlate with the dynamic changes in HIF signaling and proinflammatory cytokines such as IL1β, IL10, and TNFα. Isotopic labeling experiments with glucose and glutamine tracers specifically revealed two novel regulation points in the TCA cycle, PDC and OGDC, are important in driving this dynamic shift. The dynamic regulation of these enzymes was further validated by enzyme activity assay.

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17. LEANN BUHROW

Plasmodium phospholipid biosynthesis as a novel metabolic target for antimalarial discovery

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Introduction: Malaria remains one of the most serious infectious diseases, threatening half the world population and causing >400,000 deaths annually. Despite management efforts imploring insecticides and antimalarial drugs, parasite resistance to the most effective drug régimes continues to be reported. Therefore, it is widely accepted that a pipeline of drug candidates with distinct molecular targets lacking cross resistance with clinically used antimalarials is required. Phospholipid metabolism is reported to be critical for all stages of *Plasmodium falciparum* lifecycle yet no current drugs target these processes. Based on likely essentiality and druggability of choline kinase (CK), which catalyzes the first step of phosphatidylcholine biosynthesis, we propose this protein may be an excellent novel drug target.

Methods: A robust expression and purification scheme to isolate milligram quantities of homogeneous *P. falciparum* CK protein is outlined. Kinetic properties of CK proteins were characterized using an enzyme-coupled assay. High-resolution x-ray crystallographic structures of apo-, choline-bound, and ADP-bound CK were solved. Promega's Kinase-Glo HTS assay was adapted to screen for inhibitors within a chemically-diverse UTSW 8K library, a triazolopyrimidine antimalarial library, and the GSK Published Kinase Inhibitor Set (PKIS). *P. falciparum* 3D7 cells were cultured in RPMI media supplemented with human erythrocytes. Experimental parameters for parasite synchronization, lifecycle isolation, metabolite extraction, and metabolite detection using the Shimadzu HPLC / QTRAP 6500 MS are outlined.

Results: Recombinant expression constructs for full-length CK and for an N-terminal truncated kinase domain (Δ78CK) generated >10 mgs of >95% purified protein/liter of culture. The K_m and k_{cat} parameters for purified CK were K_m choline = 84±6.7 μM, K_m ATP = 310±20 μM and k_{cat} = 6.1±0.1 s^{-1} and were similar to Δ78CK. Δ78CK x-ray crystallographic structures of the apo-, choline-bound, ADP-bound were solved to 2.1 (R_{work}/R_{free} 0.18/0.22), 1.9 (R_{work}/R_{free} 0.16/0.21), and 2.3 (R_{work}/R_{free} 0.185/0.219) Å resolution, respectively. Structural alignment of Δ78CK with human CK demonstrates variable residues in the ATP binding sites including differences in the kinase hinge (*Pf*CK 184-186), the phosphate coordinating loop (*Pf*112-113), and at the base of the

pocket (*Pf*295, 292 and 190), potentially allowing for species-selective inhibition. Due to these variations and druggability of kinase ATP-binding pockets, the Kinase-Glo Assay Platform was adapted to screen for inhibitors competitive with ATP ([ATP] = K_m/10), rather than choline ([choline] = K_m). HTS method was validated for enzymatic linearity (5 - 50 nM), reaction linearity (40 – 90 min), and signal window and noise ratio (Z' = 0.73 ± 0.028). The validated hit rate upon re-purchase within the screened UTSW 8K, triazolopyrimidine, and PKIS libraries were 0.76%, 2.3%, and 2.3%, respectively, where hits were defined as able to inhibit activity by 30% compared to vehicle. Two inhibitors with low micromolar activity on both CK and *P. falciparum* 3D7 cells were identified. The on-target antimalarial activity will be validated by monitoring accumulation of PC biosynthetic metabolites upon inhibitor treatment. As lifecycle stage and deconvolution of host vs parasite metabolites is critical for mode of action, methods for parasite synchronization and parasite-erythrocyte or parasite-only isolation compatible with metabolite extraction are presented. Seven water-soluble precursors and metabolites were detected and quantifiable to the low micromolar range.

18. CODY BUTLER

The role of the tRNA degradation pathway in plants, metabolome analysis of Arabidopsis AtIPT2 ans AtIPT9 null mutants

Cytokinins (CKs) are a group of phytohormones that promote growth of plant roots and shoots. Zeatin is a noteworthy form of cytokinin, in that it is the most active type, essential to the growth of a plant. Zeatin exists as two isomers: *trans*-zeatin (tZ) and *cis*-zeatin (cZ). tZ activity in *Arabidopsis thaliana* is relatively well understood, however, the cZ isomer is less well defined. Morphological differences between cZ supressed *Arabidopsis* mutants and WT are not always visible. One way to characterize these mutants is to analyze the changes in metabolism between WT and mutant plants. *Arabidopsis thaliana* WT and two tRNA-IPT mutants were grown in an environmental chamber. Plant tissue was harvested at 4 growth stages: 1.04 (entire plant), 6.00 (basal leaf tissue), 8.00 (immature siliques), 9.70 (mature seeds) to monitor metabolite changes over the life cycle of Arabidopsis. The CK profiles of the mutant plants were analyzed to confirm *cis*-CK suppression. Metabolite extraction was performed with 80% MeOH. Obtained extracts were then dried and re-dissolved in a 50/50 methanol-water solution. Samples were analyzed by HPLC-MS, and data was analyzed with XCMS Online software to determine significant metabolite features that were further characterized by PRM mode. Different metabolite profiles were observed between tRNA mutants and WT, as well as between plant developmental stages.

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19. HANNAH CARLSON

Dietary Polyphenols and Their Metabolites in Human Urine by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS): Variation and Next Steps

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Introduction: Polyphenols are antioxidant compounds, abundant in plant based foods and beverages. As antioxidants they are of particular interest for human disease research because of their potential abilities to systemically reduce free radicals, thus reducing oxidative damage, and in turn possibly preventing diseases such as diabetes and cancers. Polyphenols encompass a large group of compounds, over 500 known in foods, and thus their measurement in humans has previously been limited, both in number of analytes, and reliability of data. Working from two papers both with a subset of our analytes of interest, we created a hybrid method for an expanded LC-MS/MS polyphenol panel. Unfortunately our method has also shown great variability, primarily due to issues of too few internal standards used.

Methods: A mix of two enzymes, β-glucuronidase and sulfatase, were washed with ethyl acetate to remove endogenous phenols. Urine samples are incubated at 37°C for 45 minutes, and then undergo a liquid-liquid extraction with ethyl acetate. The resulting supernatant is dried down under nitrogen and reconstituted with 25% aqueous methanol supplemented with metaphosphoric acid and dithiothreitol to prevent oxidation of phenolic compounds. Samples are injected on a LC system and separated on an Agilent Eclipse XDB-C18 column, and then detected on a triple quad tandem MS.

Preliminary data: Our polyphenols panel includes a total of 31 compounds: 3,4-dihydroxyphenylacetic acid , 4-hydroxybenzoic acid, hippuric acid , 4-hydroxyphenylacetic , vanillic , 3-hydroxybenzoic , homovanillic , p-coumaric , 3(3-hydroxyphenyl)propionic acid , 3-(4-hydroxyphenyl)propionic acid , ferulic , enterolactone , gallic, catechin , chlorogenic , epicatechin , quercetin , 2,5-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid , caffeic, 3-hydroxyphenylacetic , 3-hydroxycinnamic acid , 3,4-dihydrohydroxycinnamic acid , phenylacetic acid , daidzein , enterodiol , naringenin , phloretin, genistein , hesperetin , and isorhametin. For quantification of those 31 analytes, our method used 3 internal standards. We

believe this to be the main cause of the high level of variability for some phenols with our method. Analytes with structures most similar to those of our internal standard were the least variable. Analytes with structures not as closely related to any of the 3 internal standards used demonstrated high variability. A high and a low control material was run across 150 batches of urine samples. Across those runs, the high control coefficient of variations (CVs) ranged from 19% to 74%, with a median value of 39%; the low control CVs ranged from 23% to 361%, with a median value of 68%. These results indicate that the method developed to date is too variable to use in human studies, as all results for studies of that nature must be far more precise in order to distinguish subtle differences in polyphenols across a population. Moving forward, we intend to pursue a different method for urinary polyphenol measurement that would involve differential isotope labeling of all of our compounds of interest, to theoretically improve our ability to quantify each compound with greater reproducibility.

Novel aspect: Exploration of an enhanced urinary polyphenol panel, with the ultimate goal of confirming dietary intake profiles in large studies.

20. LINDSAY CAESAR

Hierarchical Cluster Analysis of Technical Replicates to Identify Interferents in Untargeted Mass Spectrometry Metabolomics

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Introduction: Data filtering prior to statistical analysis is a critical step to extract reliable information from mass spectral datasets. This is particularly important when conducting untargeted metabolomics studies, in which analytes of interest are not known. It is often assumed that compounds that do not originate from the analytical sample are consistent across samples and can be removed by subtracting ions detected in blank injections. With this study, we illustrate that certain chemical contaminants vary in abundance across injections, potentially resulting in their misidentification as important sample constituents and skewing metabolomics analysis. With this study, we provide a novel methodology, utilizing hierarchical cluster analysis (HCA) of replicate injections (technical replicates), that allows for the removal of this type of chemical interference.

Methods: The botanical *Angelica keiskei* was spiked with known compounds and pooled into chromatographic fractions with varying complexity. Resulting fractions were analyzed at two concentrations in triplicate using ultraperformance liquid chromatography coupled to mass spectrometry (UPLC-MS). Mass spectra were subjected to baseline subtraction and peak alignment, after which HCA was used to evaluate chemical similarity. A dataset was considered sufficiently filtered when technical replicates clustered together before clustering with other samples in the dendrogram. If replicate injections did not cluster, spectral variables were inspected. Ions demonstrating peak area variability within triplicate sets higher than a selected threshold were removed from analysis, as were their associated isotopes, fragments, and in-source clusters. This process was repeated until expected clustering of triplicates was observed.

Preliminary Data: We predicted that triplicate injections of the same sample would show high chemical similarity and cluster together using HCA. Before data filtering, however, HCA was unsuccessful in clustering replicates. Upon inspection of spectral variables, a filtering process identified 128 ions with high peak area variability across technical replicates. We predicted that these ions represented chemical interferences originating from the UPLC-MS instrumentation itself, since all other interferences would be consistent across triplicate injections. Indeed, of the 128 ions removed from our metabolomics datasets, 22 were tentatively identified by accurate mass as polysiloxanes. Polysiloxanes are commonly incorporated into column packing materials and silica capillary tubes, supporting our prediction that these contaminants were introduced post-injection. It is of critical importance to identify and remove this type of contamination, given that metabolomics experiments rely on the assumption that compounds showing variability among samples are likely to have chemical or biological relevance. Datasets collected using a greater number of samples with relatively simple chemical composition were more highly influenced by these types of chemical contaminants, as were datasets analyzed at a low concentration. Following the removal of chemical interferents from these datasets, the expected clustering of technical replicates was observed. These results challenge the assumption that chemical interference in mass spectral analysis is consistent across samples. Many metabolomics studies do not include technical replicates in their data analysis, and the results of this project illustrate the limitations of such an approach and demonstrate a simple alternative strategy.

Novel Aspects: Hierarchical cluster analysis of triplicate injections removes chemical interferents that vary across samples, allowing for improvement of untargeted metabolomics studies.

21. CALVIN CHAN

Interfacing Laser Microbeam Lysis with Digital Microfluidics for Single Cell Analysis

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Cellular heterogeneity is crucial for biological processes, including cancer and stem cell differentiation. Conventional cell-based studies measure the average response from a population of cells, however important information from low abundance cells are masked by high abundance features. Recently microfluidic methods have enabled cell isolation and single cell genomic analysis. Yet, cellular heterogeneity at the protein level remains unknown because protein quantification of individual cells by mass spectrometry is challenged by losses during sample preparation. Here, a new platform combining laser microbeam lysis with digital microfluidics for targeted single cell lysis is presented to study cellular proteome heterogeneity *in situ*.

Digital microfluidics is a technology that manipulates droplets across electrode arrays on a device by applying electrostatic forces controlled with a DropBot system. Dropbot was interfaced to a microscope and coupled with a Nd:YVO_4 laser to enable automated droplet control with simultaneous imaging. Laser microbeam lysis was performed through an iterative process, releasing cell contents into droplets for further analysis. Primers corresponding to expressed fluorescent proteins were identified with DNA agarose gel electrophoresis to demonstrate the precision, selectivity, and sensitivity of the platform. The use of this platform will be expanded towards parallel genome sequencing and mass spectrometry-based proteome profiling to investigate cellular heterogeneity at the single cell level.

22. SHAURYA CHANANA

Bacterial Strain Prioritization and Natural Product Discovery Using Automated Hierarchical Clustering – Principal Component Analysis (HC-PCA) in R

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Introduction: Rediscovery of known natural products often hinders discovery efforts aimed at identifying new and interesting structural scaffolds for drug discovery and synthetic chemistry purposes. Liquid chromatography / mass spectrometry in combination with principal component analysis (LC/MS-PCA) based metabolomics has been successfully used to address this challenge. However, the inherent limitations of PCA necessitate the development of a precursor step in determining appropriate group sizes for analysis. Hierarchical clustering analysis (HCA) is able to cluster similar bacterial strains based on their LC/MS chromatograms after which PCA can be successfully employed. This new workflow allows for a more robust analysis of large collections of bacterial strains.

Methods: Bacteria were isolated from sponge and ascidian specimens collected in the Florida Keys. MeOH extracts from these isolates were then grown on solid phase ISP2 media, subjected to solid phase extraction to remove media components, and finally transferred to clean LC/MS vials. LC/MS data was acquired using a Bruker maXis 4G ESI-Q-TOF mass spectrometer coupled with a Waters Acquity UPLC system. MeOH/acidified H_2O was employed at 0.3mL/min on a C-18 column over a 12min gradient followed by a 2min wash. Full scan mass spectra (m/z 150–1550) were measured in positive ESI mode. The spectral intensity table was constructed using Bruker ProfileAnalysis software. PCA and HCA were performed using an automated script written in R.

Preliminary Data: A spectral intensity table is generated using Bruker ProfileAnalysis from LC-HRMS chromatograms of bacterial samples. samples are placed along rows and retention time-mass/charge pairs (*rt-m/z*) along the columns. Each cell of the matrix contains an intensity value for that particular sample at that *rt-m/z* value. This matrix is then Pareto scaled, $x\tilde{}_{ij}=(x_{ij}-\bar{x}_i)/\sqrt{(s_i)}$, where x_{ij} is the i^{th} rt-m/z pair for the j^{th} sample, $\bar{x}_{ij}$ is the average of a column and s_i is the standard deviation. Using the equation for correlation between X and Y, $\rho_{XY}=(E[(X-\mu\_X)(Y-\mu\_Y)])/(\sigma_X \sigma_Y)$, where X and Y are two rows of the matrix previously described, an M x N distance matrix is made. Then a new dissimilarity matrix $(1 - \rho_{XY})$ is calculated. For clustering, most similar samples (least dissimilar) are successively linked together (agglomerative clustering) and a new average sample is made. This is repeated until a tree is formed. From this tree, sub-clusters of 30-40 strains are then further investigated using PCA. The entire process after forming a spectral intensity table is automated using an R script. We used 1046 bacterial samples and 300,000+ *rt-*

m/z pairs in our analysis. Due to the nature of PCA, metabolites that stand out, even if they are fragments, are likely to be novel metabolites. An interesting m/z value was found such as *m/z* 1185.7445, at a retention time of 9.65 min (a potential putative novel peptide) and more are being investigated at this time.

Novel Aspect: Application of HCA prior to PCA for clustering bacterial LC/MS chromatograms to prioritize strains for natural products drug discovery

23. DEVIKA CHANNAVEERAPPA

Mass Spectrometry based Proteomics to Investigate the Molecular Changes in Rat Atria during Obstructive Sleep Apnea

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Introduction: Obstructive sleep apnea (OSA) affects up to 24% of the adult population and is associated with several atrial diseases. OSA is characterized by transient cessations in respiration lasting >10 seconds as a result of narrowing of the upper airway during sleep. An estimated 20% of adults have mild OSA and 7% have moderate to severe OSA, with 85% of patients remaining undiagnosed. OSA has been associated with electrical and structural abnormalities of the atria. Although clinical evidence linking OSA to proarrhythmaic atrial changes is well known, the specific molecular mechanisms by which OSA causes atrial disease remain elusive. To study the OSA-induced cardiac changes, we have implemented a recently developed rat model which closely recapitulates the characteristics of OSA.

Methods: Rats, aged 50-70 days, received surgically implanted tracheal balloons which were inflated to cause transient airway obstructions. Apnea groups experienced 60 apneas per hour of either 13 seconds (moderate) or 23 seconds (severe) for 2 weeks. Control rats received surgeries but no inflations. Proteomics analysis was done on the rat atria homogenates to identify dysregulated proteins in moderate and severe apnea when compared to control. Both 1-dimentional (1-D) and 2-dimentional (2-D) electrophoresis was performed to separate the proteins and the peptide mixtures. The separated gel spots were trypsin digested and analyzed by a Nano-Acquity UPLC coupled with Xevo G2 Mass Spectrometer. Data analysis was done using ProteinLynx Global Server (PLGS 2.4), Mascot server and Scaffold 4.1 software.

Preliminary Data: Rats were given transient apneas for two durations: 13 and 23 s, we termed 'moderate' and 'severe,' respectively. These two apnea durations are within the range of those experienced by people with OSA. ECG recordings showed that the P wave durations (associated with left and right atrial depolarization) and T wave amplitudes (associated with ventricular repolarization) were increased by 2 weeks of chronic OSA. This was important since OSA patients show atrial electrical remodeling. The apnea model recapitulated the pathophysiological characteristics of OSA. The proteomics analysis using 1-D and 2-D electrophoresis revealed that 3 of the 9 enzymes in glycolysis and 2 proteins related to oxidative phosphorylation were down-regulated in the severe apnea group. In contrast, several structural and pro-hypertrophic proteins were up-regulated with chronic OSA. The data suggests the chronic OSA causes proteins changes which lead to cessation of glycolysis, a diminished capacity to generate reducing equivalents (i.e. NADH) as well as promotion of cardiac hypertrophy.

Novel Aspect: We have developed a novel OSA model to study the molecular changes occurring in the atria during apnea.

24. ELIZABETH CHATT

Sex-dependent variation of pumpkin (*Cucurbita maxima* cv. Big Max) nectar and nectaries as determined by proteomics and metabolomics

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Nectar is a floral reward that sustains mutualisms with pollinators, which in turn, improves fruit set. While it is known that nectar is a chemically complex solution, extensive identification and quantification of this complexity has been lacking. *Cucurbita maxima* cv.

Big Max, like many cucurbits, is monoecious with separate male and female flowers. Attraction of bees to the flowers through the reward of nectar is essential for reproductive success in this economically valuable crop. In this study, the sex-dependent variation in composition of male and female nectar and the nectaries were defined using a combination of GC-MS based metabolomics and LC-MS/MS based proteomics. Secondarily, potential metabolic links between the nectar proteomes and nectar metabolites are proposed. This rich dataset significantly expands the known complexity of nectar composition and supports the hypothesis of H+-driven nectar solute export.

25. TSUNG-CHI CHEN

PTPL, a novel protein tyrosine phosphatase-like enzyme functions as a positive regulator in *XA21*-mediated immunity

XA21 encodes a pattern recognition receptor (PRR) that confers robust and broad-spectrum resistance to most strains of the Gram-negative bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). *XA21*-mediated immunity is triggered by recognition of a small sulfated microbial protein called RaxX-sY (required for activation of *XA21*-mediated immunity X) [1]. Here, we report the characterization of a fast-neutron mutagenized rice mutant, *sxi2* (*suppressor of XA21-mediated immunity-2*), which is susceptible to *Xoo*. *sxi2* carries a deletion of the *PTPL* gene, which encodes a novel protein containing three protein tyrosine phosphatase-like domains (PTP-A, PTP-B, and PTP-C). Expression of PTPL proteins in *sxi2* is sufficient to complement the susceptible phenotypes. PTPL proteins are localized in cytosol and interact with full-length XA21 *in vivo* in the absence of RaxX-sY. A conserved cysteine residue in the PTP-A domain of PTPL is critical for XA21 binding in yeast. RaxX-sY-induced Mitogen-Activated Protein Kinase (MAPK) activation is compromised in *sxi2* plants compared to parental XA21 plants, while RaxX-sY-induced reactive oxygen species (ROS) production and expression of pathogenesis-related (PR) genes are not affected in *sxi2*. Together these results suggest that PTPL is a positive regulator required in XA21-mediated immunity.

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26. JENNY CHENG

Myocardial Mechanoefficiency and Mitochondrial Structure in Right Ventricular Failure Secondary to Chronic Pressure Overload

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Introduction: Right ventricular failure (RVF) secondary to pressure overload is the main cause of death in pulmonary arterial hypertension (PAH). Reduced cardiac output and ejection fraction are the clinical hallmarks. Animal studies have shown RVF is associated with downregulation of energy metabolism. Mitochondria produce ATP to sustain cardiac contraction and relaxation. The phenotype of dysfunctional mitochondria includes altered structure and diminished capacity for ATP production. Changes in mitochondrial structure are associated with mitochondrial biogenesis, a process regulated by peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α). Downregulation of PGC-1α has been demonstrated in RVF. However, the link between mitochondrial structure and RV function has not been fully investigated. We hypothesize that RV hemodynamic changes are linked to abnormalities in mitochondrial structure in RVF.

Methods: Pulmonary artery banding (PAB) was used to induce RVF in rats. Rats with sham surgery were used for comparison. Hemodynamic function of the RV was determined using right heart catheterization. RV fibrosis, mitochondrial ultrastructure, and gene expression were determined using histology, transmission electron microscopy, and quantitative real-time PCR respectively.

Results: At 8 weeks post-surgery, pressure-volume analysis demonstrated RVF (35% decrease in ejection fraction and 47% reduction in cardiac output, $p<0.05$). RVF in PAB was associted with RV hypertrophy (1.5-fold increase in RV mass, $p<0.05$), ventricular-vascular uncoupling, diastolic dysfunction (75% decrease in ventricular compliance, $p<0.05$), and fibrosis. There was a significant, 2-fold increase in energy consumption by the RV as measured by pressure-volume area which was not matched by the increase in external work, which resulted in mechanical inefficiency in the PAB group. Transmission electron microscopy demonstrated decreased RV mitochondrial cross-sectional area in the PAB group (0.585 vs. 0.444 μm^2, PAB vs. Sham, $p<0.05$), which modestly correlated with RV ejection fraction. Associated with these changes in mitochondrial structure, PAB rats exhibited a 37% decrease in PGC-1α gene expression ($p<0.05$). PGC-1α levels were modestly correlated with RV contractility.

Novel Aspect: This work quantifies mitochondrial ultrastructural remodeling in RVF and demonstrates decreased energy consumption and mechanical inefficiency as features of RVF.

27. KEVIN CLARK

Ion-tagged Oligonucleotides as Hybridization Probes for Sequence-specific Nucleic Acid Analysis

The analysis of nucleic acids (NAs) has provided key insights into specific sequences that are associated with disease, establishing the basis for NA biomarkers in clinical diagnosis. The innate ability of NAs to recognize complementary sequences *via* hybridization is often leveraged using synthetic oligonucleotide probes to enrich specific NAs and improve method sensitivity. Magnetic beads can be functionalized with oligonucleotide probes for the selective isolation of NAs, but suffer from drawbacks including unwanted particle aggregation, clogging of devices, and complicated/expensive probe immobilization chemistries. In this study, synthetic oligonucleotide probes known as ion-tagged oligonucleotides (ITOs) were coupled with a magnetic liquid support and applied for the capture of specific DNA sequences from biological samples thereby minimizing interferences caused by untargeted NAs.

A series of imidazolium-based ITO probes with varying alkyl chain lengths were synthesized using photoinitiated radical thiol-ene "click" chemistry. ITOs were characterized using denaturing PAGE, LC-TOFMS, and melt curve analysis. The ITO with the longest alkyl chain length (C_8) in the ion tag structure facilitated loading of the ITO onto a hydrophobic magnetic ionic liquid (MIL) support to generate a magnetic liquid extraction phase capable of capturing specific DNA sequences. When compared to a commercially available magnetic bead-based method, the MIL-supported ITO approach extracted similar amounts of target nucleic acid as determined by real-time quantitative polymerase chain reaction (qPCR, n=6).

One major limitation of qPCR analysis is that physiologically relevant covalent modifications to NA sequences cannot be detected using this technique. Mass spectrometry (MS) is an ideal tool for rapidly quantifying biomolecules and their covalent modifications, but the notoriously poor response of NAs in MS has rendered this powerful approach applicable only for highly abundant NAs. It is expected that the tunable structures of ITOs can be leveraged in future work to enhance the response of NAs in MS and enable methods capable of detecting modifications to low abundance NAs.

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28. YUSI CUI

Isobaric Labeling-based Quantitative Studies of Protein Expression and N-glycosylation of AT-1 sTg Mouse Model Reveal Molecular Basis of Aging

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Introduction: Quantitative proteomics is a powerful tool to study the molecular basis of physiological states by quantifying the protein expression of the model system in the control versus experimental state. Isobaric tagging is a frequently-used high throughput, mass spectrometry (MS)-based proteome quantification technique. The Puglielli group found the membrane transporter *AT-1*/*SLC33A1* can import acetyl-CoA into the endoplasmic reticulum (ER) lumen, and its expression status is crucial to the progress of aging. The recent development in higher plexing of N,N-dimethyl leucine (DiLeu) isobaric tags, along with the oxonium ion-triggered targeted MS approach enable the study of proteome changes and N-glycosylation alterations in AT-1 sTg mouse model, which systemically overexpresses *AT-1*/*SLC33A1* and has a progeria-like phenotype that mimics an accelerated form of aging.

Methods: General Proteomics: The cortex of both male and female AT-1 sTg mice and their wild type (WT) controls were lysed in the presence of acetylation inhibitor, digested with trypsin and then labeled with 12-plex DiLeu tags, which were assigned in a way to minimize deuterium shift effect in LC-MS. Following labeling, the samples were combined followed by strong cation exchange (SCX) cleanup and high pH fractionation.

N-glycosylation: An aliquot of labeled tryptic peptides was enriched for N-glycosylation with custom made strong anion exchange-hydrophilic interaction liquid chromatography (SAX-HILIC) stage tips. Oxonium ion triggered targeted LC-MS/MS were acquired with EThcD fragmentation on Orbitrap Fusion Lumos. Collision energy of EThcD fragmentation was optimized for both N-glycopeptide identification and reporter ion intensity for quantitation.

Preliminary Data: We first compared the proteome in the cortex of AT-1 sTg mice with the wild type control. The proteome showed marked gender difference. Within the biological triplicates investigated, systemically overexpressing *AT-1*/*SLC33A1* showed greater impact on male mice. 129 proteins were significantly changed in expression level of male, while 69

were in female. Functional annotation and pathway analysis showed that a large number of these significantly proteins were phosphoproteins and glycoproteins. Gene ontology cellular component analysis showed that they contain membrane proteins of ER, cytoplasm and mitochondria. They were also closely related to acetylation, transporting, secretory, protein binding and alternative splicing. The analysis indicated the *AT-1*/*SLC33A1* gene impacts aging by a synergistic action of ER, mitochondria and nucleus, and offered more insights into the crucial regulation factors at the molecular level.

Although glycosylation defects have been observed in patients that carry geriatric diseases such as Alzheimer Disease (AD), the role of protein glycosylation in the progress of aging has not been thoroughly investigated. To further quantitatively evaluate glycosylation alteration, 12-plex DiLeu labeled peptides were enriched for N-glycosylation with the custom-made SAX-HILIC stage tips. A total of four fractions were collected with an increased gradient of aqueous phase, which largely decreased the complexity of the sample. The enrichment and the oxonium ion triggered targeted LC-MS/MS method enabled a deep coverage of glycosylation. For unlabeled samples, 2000 N-glycopeptides were identified from 200µg peptides. Compared to the enrichment without fractionation, the N-glycopeptide coverage almost doubled. For DiLeu labeled glycopeptides, the collision energy of EThcD fragmentation was optimized to ensure a wealth of backbone fragments for confident N-glycopeptide identification and a decent reporter ion yield for accurate quantitation.

Future work will include other tissues and more mouse models centered on *AT-1*/*SLC33A1* gene to provide a more complete description of molecular players involved in various phenotypes.

Novel Aspect: Applying 12-plex DiLeu labeling, SAX-HILIC and targeted MS to quantitatively examine proteome and glycosylation pattern changes in animal model.

29. EDUARDO DE LA TOBA

Label-free quantitation of peptides involved in itch

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Chronic itch, also known as pruritus, is a condition that affects a large percentage of the population and is associated with various disorders such as eczema and skin damage caused by excessive scratching. Various neuropeptides have been implicated in their role in pruritus, and they include substance P, endothelin-1, neuromedin B, calcitonin-gene related peptide (CGRP), natriuretic peptide B, and kappa-opioid dynorphin. These neuropeptides are secreted by the dorsal root ganglia, therefore peptidomic studies of the dorsal root ganglia of mice through the use of mass spectrometric methods, particularly with liquid chromatography-electrospray ionization- mass spectrometry, will be employed in order to gain a more comprehensive understanding of the levels of these peptides in control samples of skin versus itch-induced skin models of the mice. These neuropeptide quantification measurements will be performed in a label-free fashion using the SKYLINE platform in which we will be able to monitor the changes in neuropeptide amounts between the itch model samples (DRG, dorsal horn and skin) and the untreated control samples.

30. KATE DECK

Understanding how renal erythropoietin (Epo) producing cells sense iron and oxygen and control erythropoiesis

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Introduction: Red cell production requires coordinated oxygen- and iron-dependent regulation of Epo. We study the

interplay between iron, oxygen and aspects of metabolism in controlling the transcription factor hypoxia inducible factor 2α (HIF-2α), the central transcriptional regulator of Epo, in rare cells that are 1% of kidney cells. HIF-2α synthesis is controlled by a translational repressor, iron regulatory protein (IRP) 1, binding to an iron responsive element (IRE) in HIF-2α mRNA. HIF-2α protein stability is controlled by prolyl hydroxylases (PHDs) that hydroxylate HIF-2α in an iron, oxygen and α-ketoglutarate dependent manner. Our studies may provide unique insights for new therapies for treating erythroid disorders associated with premature birth, renal anemia of the aged and other disorders traced to defective Epo expression.

Methods: We use genetically altered mice to elucidate the genetic, biochemical and physiological control of Epo production by iron and oxygen. To date we have used *Irp1*$^{-/-}$ mice and mice lacking the IRE in HIF-2α mRNA. Our genetic, biochemical, molecular and nutritional studies have largely been at the whole animal/tissue level including flow cytometric, targeted transcriptomics, metabolomic (NMR) and *in vivo* imaging for PHD activity. To understand the function of the rare renal Epo-producing cells we are making a new mouse line (*Epo*$^{tdTomato/ER\text{-}Cre}$) that expresses a reporter (tdTomato) and also Cre in these cells. This mouse line will allow us to genetically probe Epo producing cells and isolate them by flow cytometry for studies of their biochemical and metabolic architecture.

Preliminary data: We seek to elucidate the mechanisms through which iron, oxygen and specific metabolites (e.g., α-ketoglutarate) modulate HIF-2α action in renal Epo producing cells. We hypothesize that through its dual actions as a sequence-specific RNA binding protein and the cytosolic aconitase IRP1 influences pathways that dictate HIF-2α action, Epo production and erythropoiesis.

1. Epo mRNA and serum protein level was strongly de-repressed in *Irp1*$^{-/-}$ mice causing overproduction of red cells and a clinically very strong polycythemia (*Cell Metab*. (2013) 17:282-90). HIF-2α mRNA is translationally activated in *Irp1*$^{-/-}$ kidney as early as 1 week of age along with Epo mRNA overexpression during the neonatal period. Dysregulation of erythropoiesis in bone marrow and spleen and associated disturbances in body iron stores and systemic iron regulation was observed. By 10 weeks of age the elevated Epo expression and polycythemia resolves yet HIF-2α mRNA remains translationally de-repressed indicating compensatory mechanisms to suppress HIF-2α hyperactivity and Epo gene transcription.

2. Interestingly, in IRE$^{-/-}$ mice (where IRP1 is intact) renal HIF-2α mRNA translation is similarly de-repressed and polycythemia ensues but with a notably blunted phenotype, suggesting other actions of IRP1 within Epo producing cells modulate Epo transcriptional control. This may involve other mRNA targets of IRP1 that modulate cellular iron status or the metabolic function of the inactive RNA binding form of IRP1 which is the [4Fe-4S] enzyme cytosolic aconitase (c-acon). c-Acon interconverts citrate and isocitrate; deficiency of this enzyme could increase cytosolic citrate and acetyl CoA or reduce α-ketoglutarate. Work from others demonstrated a major impact of the so-called "acetate-switch" in controlling HIF-2α acetylation state and transcriptional potency (Nature Med. 20:1018-1026). Furthermore, α-ketoglutarate is a required substrate not only of PHDs but also other members of this oxygenase gene family involved in chromatin modification (J. Biol. Chem. 290:20712).

Novel Aspect: Combining MS proteomic and metabolomic techniques with novel genetic models to elucidate the unique sensory/regulatory wiring of erythropoietin-producing cells.

31. LINDSAY DEMERS

A molecular exploration of seed vigor in low and normal phytate near-isogenic soybean seeds

Phytic acid is the primary storage form of seed phosphorus. As phytic acid is not digestible by humans and non-ruminants, it is considered an anti-nutrient that can result in mineral deficiencies as well as phosphorus pollution of water systems. While low phytic acid (LPA) soybean lines have been developed to ameliorate these problems, LPA lines exhibit poor seedling emergence. In an effort to better understand the processes that lead to poor emergence, we performed untargeted LC-MS analyses of the lipid component of four near-isogenic soybean lines that confer either low or normal phytic acid contents. By comparative analysis of these lines, we identified metabolic variations in the lipid profiles that could be affecting seedling emergence in LPA soybeans.

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32. JARED DEYARMIN

Stress-omics – A non-targeted multi-omics approach to discriminate stress states in a marine mammal

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Repeated or chronic stress, such as that caused by anthropogenic activity and environmental disturbance, may affect animal health and fitness and contribute to population declines by consequential changes in food webs. However, the physiological impacts of repeated stress have not been extensively studied in wild animals, hindering development of biomarkers that conservation practitioners can use to identify chronically stressed individuals. Baseline endocrine measurements are commonly used for stress diagnosis, but they may be less robust indicators of stress than their downstream molecular mediators. We used a non-targeted, multi-omics approach to profile global changes in target gene and protein abundance in response to acute and repeated stress in a marine mammal study system, the northern elephant seal (*Mirounga angustirostris*). We simulated chronic stress by administering adrenocorticotropic hormone (ACTH) to juvenile seals once daily for four days and collected blubber before and after the first ("acute") and last ("repeated") ACTH administrations. We then sequenced the blubber transcriptomes and proteomes using Illumina RNA sequencing and liquid chromatography tandem mass spectrometry, respectively. We developed computational pipelines to compare transcript and protein expression between stress states and identify cellular consequences and unique molecular markers of repeated stress in a marine mammal. We identified 61 annotated, unique (all transcript isoforms collapsed into a single gene) differentially expressed genes (DEG) in response to the first ACTH administration, 12 DEG in response to the fourth ACTH administration, 99 DEG in the overall ACTH response, 24 DEG in the baseline comparison, and 12 DEG in the ACTH response comparison. Protein-protein interactions (PPI) analyses were applied to each DEG set and one significant network was generated from the overall ACTH response, containing many genes associated with lipid metabolism. Using a proteogenomics approach with a reference translated transcriptome coupled with tandem mass spectrometry, we identified differentially expressed proteins in different stress conditions, as well as built a reference proteome for future studies. Both approaches captured transcripts/proteins that affect adipocyte development, lipid metabolism and immune function and other cell maintenance pathways in response to repeated stress. The stress markers identified in this study may be used to assess stress states in vulnerable marine mammal populations using targeted assays.

33. SHACHINTHAKA DISSANAYAKA

A pathogenic circular noncoding RNA interacts with ribosomal protein L5 to modulate the expression of Transcription Factor IIIA for RNA replication

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Emerging evidence began to illustrate regulatory roles of some noncoding RNAs (ncRNAs), but the functional mechanisms of most ncRNAs are overlooked, in contrast to the explosive discoveries of such noncoding sequences in many organisms. Potato spindle tuber viroid (PSTVd) is a pathogenic circular non-coding RNA that resembles endogenous ncRNAs for function via interacting with cellular proteins. Recent research illustrated that PSTVd utilizes a special splicing form of plant Transcription Factor IIIA (TFIIIA-7ZF) to enhance its replication. However, it is unclear how PSTVd manipulates the alternative splicing of TFIIIA gene to favor its replication. Interestingly, a recent report discovered ribosomal protein L5 (RPL5) as a specific regulator of TFIIIA gene. Here, we used Electrophoretic mobility shift assays (EMSA) to confirm the direct affinity of RPL5 with TFIIIA intron, PSTVd, and the well-established substrate (5S rRNA). We further showed that RPL5 interacts with PSTVd *in vivo*. Interestingly, RPL5 displayed a reduced affinity to PSTVd mutant with a disruptive RNA motif residing in the central regions but showed similar affinities to mutants with disruptive RNA motifs at either one of the terminal regions. Because RPL5 represses TFIIIA-7ZF, we over-expressed RPL5 and observed significant reduction in PSTVd accumulation, which is attributable to the affected expression of TFIIIA-7ZF. Together, our results provide novel insights into the functional mechanisms for circular ncRNAs regulating gene expression at the post transcription level and point to a novel means to combat viroid.

34. MATTHEW DORRIS

Cranberry and grape juice anthocyanins and proanthocyanins decrease with accelerated aging to varying degrees

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Introduction: The regular consumption of polyphenols including anthocyanins and proanthocyanidins (PACs) is associated with reduced rise of all-cause mortality. The reduced risk of urinary tract infection from regular consumption of cranberry products is dependent on the quality of PACs. Further, the color quality of anthocyanin-rich juices depends on the stability of anthocyanins and related polyphenol pigments. However, the storage stability of these compounds is not well understood. We characterized the anthocyanin and proanthocyanidin content of cranberry and grape juices. We looked at the stability of the polyphenols in juices subjected to accelerated aging and the implications on juice color quality.

Methods: Cranberry juice was purchased from a local grocery and used as purchased. A grape juice was prepared in-house from a commercially available frozen concentrate following manufacturer's instructions. Aliquots of each juice were subjected to accelerated aging by incubating at 50° C for 10 days. Reverse phase (RP) high performance liquid chromatography (HPLC) analysis was used to measure the anthocyanin content of each juice. Quantitation was performed based on 520 nm absorbance peak area. Proanthocyanidin fractions of each juice were purified by column chromatography using Sephadex LH-20 gel. The PAC fractions were analyzed by hydrophilic interaction liquid chromatography (HILIC). Quantitation was performed using 230 nm excitation and 321 nm fluorescence on an FLD detector. Absorbance at 520 nm was also collected.

Preliminary data: RP-HPLC showed cranberry juice had a total of 99 ± 7 µg/mL cyanidin-3-glucoside equivalents of anthocyanins including cyanidin, delphinidin, peonidin, and petunidin galactosides, glucosides, and arabinosides. After incubation, the total anthocyanin content decreased to 10. ± 7 µg/mL cyanidin -3-glucoside equivalents. Grape juice had a total of 152 ± 3 µg/mL cyanidin-3-glucoside equivalents. The grape juice had a complex mixture of cyanidin, delphinidin, malvidin, peonidin, and petunidin glucosides, and diglucosides with coumaroyl and acetoyl substitutions. With accelerated aging, the total anthocyanin content of grape juice fell to 9 ± 2 µg/mL cyanidin-3-glucoside equivalents. Changes in concentrations showed some grape and cranberry juice anthocyanins were more stable to accelerated aging than others.

According to HILIC-HPLC fluorescence detection, total PACs in cranberry juice decreased from 21.32 ± 0.03 µg/mL to 2.80 ± 0.02 µg/mL catechin equivalents after accelerated aging, an 87 % loss. Grape juice PACs decreased from 44 ± 2 µg/mL to 7 ± 1 µg/mL catechin equivalents with accelerated aging, an 84 % loss. Exact rates of loss depended on degree of polymerization. Analysis of 520 nm HILIC chromatograms showed the Sephadex PACs fraction of each juice had different impact on each juice's color. The monomer PAC peak of unaged cranberry juice had absorbance, but no other peaks were apparent in aged or unaged cranberry juice fractions. Grape juice had absorbance associated with the peak for PACs of degree of polymerization greater than 11 in the chromatograms of aged and unaged juice. The 42 % loss of the 520 nm absorbance was less than the 94 % loss of 520 nm peak area observed in the RP analysis of anthocyanins. This shows that, in grape juice, there were pigments associated with polymerized PACs that were more stable than monomeric anthocyanins.

Novel Aspect: Our study looks specifically at relative stability of polyphenols in fruit juice. We explore the implications on fruit juice color.

35. JAMES DRAPER

New software for automated downstream analysis of Proteome Discoverer output comparing PTM and protein relative abundances.

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Introduction: We have developed a unique post-search software module for reduction of quantitative proteomics data. This software, termed *Omin*, rapidly normalizes quantitation for PTM-enriched fraction(s) to the linked input fraction(s) assessing protein abundance. *Omin* also leverages the Intermine API to link UniProt Accessions to other databases, such as MitoCarta2.0, to maximize the associations between experimental data and metadata. *Omin* output can be presented as a visual framework linked back to UniProt to facilitate user-friendly data-mining.

Methods: *Omin* is a Python software library developed to streamline the analysis of Proteome Discoverer (PD) 2.2 output for expediting biological discovery, with a focus on high throughput analysis of post-translation modifications (PTMs) of mitochondrial proteins using Tandem Mass Tags (TMT). *Omin* can be called either by the command line, python script, or in a Jupyter Notebook environment. The software leverages data structure and metadata to rapidly normalize PTM-containing peptide measurements to protein abundance changes, perform quantitative comparisons across contrasting experimental conditions, and to provide a visual framework for those comparisons to aid in understanding biological implications. We have automated the annotation of mitochondrial proteins in our datasets, based on their presence in the *MitoCarta* database—here*Omin* uses the Intermine API to convert UniProt Accessions to MitoCarta2.0 (Nucleic Acids Res. 2016 Jan 4;44(D1):D1251-7.) status, retrieving missing identifiers that are needed for comprehensive associations. This approach is amenable for retrieval of other types of Metadata.

Preliminary Data: As an example of its utility, *Omin* was used to analyze protein malonylation levels in normal control mice as compared to those harboring a genetic deletion of malonyl coA decarboxylase (MCD KO), a model of extreme hyper-malonylation of mitochondrial proteins in both skeletal muscle and heart. Of the 3,641 high confidence proteins identified with PD 2.2, 3,481 had Entrez Gene IDs— a result that was increased to 3,595 proteins upon subsequent analysis with *Omin*. When the original PD output was linked to MitoCarta2.0 (without use of *Omin*), 807 proteins were annotated as mitochondrial. Application of *Omin* increased that result to 821 proteins, which in turn yielded a greater number of mitochondrial-associated malonylated peptides. To improve quantitative accuracy, *Omin* can also normalize multiple PTM-containing fractions to the corresponding input material. In addition to whole proteome load normalization, *Omin* normalizes PTM-containing peptides to the relative abundance of the corresponding proteins. These analyses identified 243 and 257 hyper-malonylated peptides in skeletal muscle and heart tissues, respectively, with 216 and 164 meeting a significance threshold of 10% FDR in the MCD KO tissues. Omin output can be exported directly to CSV or Excel formats for further analysis, or piped to other software workflows with open access source-code, such as Protein Set Enrichment Analysis (PSEA) (*Curr Protoc Bioinformatics*. 2016 Mar 24;53:13.28.). The program has robust command line and scripting support, as well as a growing library of cutting edge visualization tools.

Novel Aspect: *Omin* uses Proteome Discoverer output and provides rapid quantitative normalization of TMT-labeled PTM enriched fractions to the respective input material, maps the data to associated metadata, and provides a visual framework for user-friendly data-mining.

36. KYLE DUBIAK

Proteomic and Transcriptomic Analysis of Neural Cell Fate in Developing *Xenopus laevis* Embryos and Explants

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Xenopus laevis has long been one of the most useful and important model organisms for developmental biologists. Detailed fate maps exist for each of the blastomeres in 16- and 32-cell embryos. While no organ or tissue arises exclusively from one particular bastomere, the totipotent D1 blastomere in the 8-cell embryo is the main contributor to the brain, retina, and spinal cord. By the 32-cell stage, the D1 descendants are committed to a neural fate. We have tracked the earliest events in the emergence of the *Xenopus* nervous system at the protein level using isobaric labeling combined with mass spectrometry and at the transcript level using RNA-seq technology. The D1 blastomere of 8-cell embryos were injected with fluorescent (Alexa Fluor 568) dextran and descendant cells isolated from 16- and 32-cell embryos and at four subsequent developmental stages: midblastula (initiation of zygotic transcription), gastrula (formation of the three primary germ layers), early neurula (transcriptional reprograming), and mid-neurula. In order to understand how extrinsic factors released from surrounding cells influence cell fate, D1 blastomeres were explanted and allowed to develop in parallel with sibling embryos to the same developmental stages. Preliminary analysis of the data shows that we have detected the expression of neural-specific genes, including markers of neural stem cells at the expected

developmental time points. Decreasing correlation between explant and progeny cells as a function of time has allowed the identification of candidate genes that are under the control of extracellular signaling. A correlation of mRNA and corresponding protein levels is planned in order to evaluate translational regulation during the earliest stages of neural development.

37. CRISTIANA DUMBRAVEANU

Mass Spectrometry based Proteomics Approach to Identify Alterations of Rat Brain and Liver during induced Obstructive Sleep Apnea

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Introduction: Obstructive sleep apnea (OSA), a sleep related breathing disorder that involves the repetitive obstruction of the upper airway during sleep, is found to affect adult population, mostly middle-aged men. There are many symptoms of OSA and the disorder is strongly associated with several cardiovascular diseases, but the majority of the patients remain undiagnosed. Beside cardiovascular diseases, OSA presence was also reflected in liver and brain damages, like non-alcohol fatty liver disease, severe fibrosis or metabolite changes in hippocampus that lead to inflammatory response. In this study, we aim to determine the molecular changes occurring in brain and liver after inducing OSA, by using a tracheal balloon as the obstruction device to produce apneas in conscious and free roaming rats.

Methods: Male rats (50-70 days), where induced apnea by inserting a tube in their trachea which inflated caused blocking of the airway for 13 seconds (moderate apnea) and 23 seconds (severe apnea). The control rats underwent surgery but did not have the balloon inflation. The moderate and severe apneas were induced in rats every hour for two weeks and the brain and liver samples were obtained. These samples were lysed, separated on SDS-PAGE, digested with trypsin and investigated by Nano Acquity UPLC coupled with Xevo G2 Mass Spectrometer. Data analysis was done using ProteinLynx Global Server (PLGS 2.4), Mascot server and Scaffold 4.1 software. Proteomics approach allowed identification of dysregulated proteins in moderate and severe apnea when compared to control.

Results: The proteomic analysis showed downregulation of proteins involved in aerobic metabolism as well as upregulation for those involved in the functioning of liver and brain such as Myelin basic protein, which promotes nerves insulation.

Novel Aspect: The OSA model used provides new insights regarding the molecular changes occurring in liver and brain following obstructive apnea.

38. EMMALYN DUPREE

Proteomic analysis of the lake trout (*Salvelinus namaycush*)

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Introduction: The Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) has placed great importance on the species *Salvelinus namaycush* (lake trout) in monitoring of toxic compounds. Legacy chemicals such as polychlorinated biphenyls and organochloride pesticides are shown to reside in the Great Lakes at alarming concentrations and the chemical structure of these compounds allows them to bioaccumulate in organsims such as fish. Bioaccumulation of these compounds can lead to changes in transcribed genes, translated mRNAs, and thus, proteins produced and post-translational modifications of these proteins. Although lake trout is used widely in this area of study, there is currently little information available about the proteome of this species.

Methods: In this study, samples from the liver and blood of *Salvelinus namaycush* were separated by SDS-PAGE, followed by in-gel trypsin digestion and finally analyzed by nanoLC-MS/MS. The data was searched against different NCBI and Swissprot databases in Mascot Daemon. This output was then analyzed by Scaffold 4.3 software where many novel proteins were found.

Preliminary Data: Analysis with a nanoAcuity UPLC coupled to a QTOF Xevo G2 (both from Waters) revealed many novel proteins for the *Salvelinus* genus. These proteins were found in more developed databases from NCBI and Swissprot, like Actinopterygii, Salmonidae, and the highly studied *Danio rerio*. This preliminary study using a 150 minute gradient gave rise to 8330 proteins in the liver, and 782 proteins in the blood alone. Further analysis with longer gradients and longer analytical columns will potentially give rise to even more novel proteins.

Novel Aspect: Develop a thorough, comprehensive proteome database for lake trout for further studies on legacy chemical influence in the Great Lakes.

39. GAELEN DWYER

Recipient conditioning contributes to IL-33-driven Th1 alloimmune responses following rapid ST2 upregulation on donor CD4+ T cells during lymphopenia-induced proliferation

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Allogeneic hematopoietic stem cell transplantation (alloHSCT) is used as a therapeutic cure for hematological malignancies. Unfortunately, graft-vs. host disease (GVHD) remains an often-fatal side effect that arises when donor T cells recognize and destroy recipient tissue. Clinical protocols for alloHSCT requires recipient conditioning, which includes removal of their immune and bone marrow (BM) cells through irradiation and/or chemotherapy. This conditioning not only targets immune and BM cells, but also damages barrier tissues like the gastrointestinal (GI) tract or lung epithelium and causes lymphopenia-induced proliferation (LIP) and releases microbial products. Damaged cells release small molecules called "alarmins" that alert the immune system to local tissue damage (3). Interleukin (IL)-33, an alarmin and stromal cell-derived cytokine, is augmented by alloHSCT conditioning in host tissues and is a requirement in donor T cell stimulation through its receptor, ST2, leading to GVHD. We have found that B6 *Il33*$^{-/-}$ recipients or B6 recipients of BALB/c *St2*$^{-/-}$ donor T cells have profoundly reduced GVHD We hypothesize that donor CD4$^+$ T cells undergo IL-33-independent upregulation of ST2, the IL-33 receptor, in the secondary lymphoid organ and it is IL-33 in the GVHD target tissue that represents the critical stimulus for effector T cell functions leading to GVHD. To test the impact of lymphopenia and IL-33 stimulation on T cells, ST2$^-$Foxp3$^-$CD4$^+$ T cells alone or with ST2$^+$ Treg were transferred into B6 *Rag2*$^{-/-}\gamma c^{-/-}$ mice. Mice received PBS or IL-33 on days 1-8. ST2 is quickly induced on CD4$^+$ T cells in response to conditioning induced lymphopenia, but independently of IL-33. Additionally, CD4$^+$ T cells preferentially expand in response to IL-33 present promoting a detrimental Th1 immune response and GVHD pathology. Not only is IL-33 expressed in target tissues, such as the GI tract, it is also expressed by fibroblastic reticular cells (FRC) of the secondary lymphoid organs (SLO). It is unknown whether recipient target tissues, SLO, or both are the source of pathogenic IL-33 initiating or sustaining GVHD. To determine the critical source of IL-33 in GVHD pathology, I will use high-throughput proteomic and transcriptional analysis to characterize the response of syngeneic or allogeneic donor *St2*$^{+/+}$ and *St2*$^{-/-}$ CD4$^+$ T cells isolated from *Il33*$^{-/-}$ or *Il33*$^{+/+}$ recipient small intestine (SI) lamina propria or SLO.

40. PO HIEN EAR

Maternal NR supplementation during lactation promotes NAD metabolites for milk production and neonatal development

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Introduction: NAD$^+$ is an essential metabolite that serves both as a cofactor and a substrate for many cellular enzymes, including the sirtuins. Homeostasis of the NAD$^+$ metabolome is challenged during DNA damage, certain disease models, and during normal tissue aging but can be reversed by the administration of nicotinamide riboside (NR), an NAD$^+$ precursor.

Interestingly, previous rodent studies were done using male mice. Little is known about how NR affects the female systems. Lactation is a highly energy demanding process that requires coordination between multiple organ systems to generate protein, fatty acids, lactose, and vitamins for milk production. NAD precursors such as NR and nicotinamide (Nam) have previously been found in milk but little is known about how NAD metabolism is regulated during lactation.

Method: Here we investigate the effect of boosting NAD levels in female mice during lactation. We used targeted-NAD metabolomics approach to identify where NAD metabolites are synthesized for milk production. In addition, we combined our NAD metabolomics data with gene expression, functional enzymatic assays, histological and physiological analysis in order to generate an integrative map of the lactation process.

Preliminary Data: We found that lactation is an NAD^+ demanding process. The NAD^+ precursor, NR stimulates milk production and mammary gland development. NR increases the fatty acid, protein, lactose, and NAD biosynthetic pathways in the mammary glands. Interestingly, the liver also plays a key role in providing NAD precursors for milk biosynthesis. Pups from NR-supplemented dams exhibit improved neonatal development in terms of increased body weight, mobility, and liver maturation.

Novel Aspect: Since NR improves milk production, it can potentially benefit obese or older females with lactation difficulties.

41. DANI FAIVRE

Analysis of peptide acetylation by new acquisition methods combining DIA and DDA

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Introduction: The purpose of this project was to combine the strengths of DIA and DDA to create a more sensitive method for analyzing peptide ions containing specific PTM's. We tested two methods, Data Independent Acquisition Dependent Acquisition (DIADA) and Data Independent Acquisition Data Dependent Acquisition (DIADDA). The default mode of these methods is DIA, but the observation of a diagnostic fragment ion triggers other scans. After observing the diagnostic ion, DIADA acquires narrow isolation MS2 scans covering the wide isolation region of the DIA scan that contained the diagnostic ion. In contrast, DIADDA acquires a MS1 SIM scan covering the precursor range of the DIA scan containing the diagnostic ion and then allows standard DDA over this narrow mass range.

Methods: We chose eta-Lys acetylation as the PTM of interest and examined the presence of acetylated peptides with a dilution series of chemically acetylated yeast in a yeast background. The samples were analyzed with a Thermo Fusion Lumos mass spectrometer. The DIA parts of the hybrid methods were performed with the Orbitrap. When the diagnostic fragment ion for acetyl lysine (m/z 126.0919) was observed, the dependent acquisition portions of the hybrid methods were triggered. The dependent parts used the linear ion trap to perform the narrow isolation MS2 scans. The dependent acquisition data was analyzed with Comet, and the independent acquisition data was analyzed with EncyclopeDIA. We compared the results of the new hybrid methods to DDA, DIA, and each other.

Preliminary Data: Because the data is analyzed differently depending on the method of acquisition, the data from the hybrid methods had to be split in order to analyze it. The dependent acquisition parts were analyzed by Comet and compared to standard DDA while the independent acquisition parts were analyzed with EncyclopeDIA and compared to DIA. Based on the preliminary results, standard DDA produced a higher number of identifications than the dependent parts of DIADA and DIADDA at all concentrations. On the other hand, the DIA parts of the hybrid methods were similar to normal DIA at high concentrations (~300 unique acetylated peptides) and slightly better at lower concentrations (215 vs. 202 for the third dilution). When compared to each other, the DIA parts of DIADA and DIADDA are equivalent at the higher concentrations (~300 unique peptides each), but DIADA outperforms DIADDA at the lower concentrations (195 vs. 161 for the lowest concentration). At the highest sample concentration, DIA and DDA had 280 and 47 unique acetylated peptides specific to each method, respectively, and we found 26 acetylated peptides by both methods. The relatively small overlap gave us hope that the hybrid methods would be able to identify more peptides than either standard method alone. Our results support that the dependent acquisition parts of the hybrid methods identified some acetylated peptides not seen by the independent acquisition parts. However, it only added between 2 and 10 unique acetylated peptides to the total at any given concentration. As a next step, we would like to check the quantification quality of the DIA data and analyze the LOD and variance of the methods. In the future, we can apply the methods to neutral loses or test them with other PTMs, amino acids, or N-terminal amino acid pairs as diagnostic ions.

Novel Aspect: Combining DIA with DDA is novel

42. PAULO FALCO COBRA

Metabolomic characterization of *Leishmania major* and *Leishmania donovani* by ^{1}H and ^{1}H-^{13}C HSQC NMR

Cobra, P.F; Fathi, F; Markley, J.L; Thiemann, O.H; Colnago, L.A.

Neglected tropical diseases (NTDs) are a group of 17 diseases that thrive mainly on developing countries. According to the World Health Organization (WHO), they are endemic in 149 countries and affect more than 1.4 billion people. Leishmaniasis is among these diseases, and its agent of infection, *Leishmania sp* protozoan, is transmitted through the bites of infected *Phlebotomus* sandflies. The commonest form of leishmaniasis is the cutaneous, which usually causes ulcers on the face, arms and legs and has 1 million cases reported in the last 5 years. The most disfiguring form is the mucocutaneous as it destroys the soft tissues of the nose, mouth and throat. Another form of the disease, visceral leishmaniasis, attacks the internal organs, usually is fatal within 2 years and causes 20 thousand deaths annually.

We aim, in the current poster, to ascertain the metabolic profile of *Leishmania major* Friedling and *Leishmania donovani* DD8 and the metabolic differences caused by the addition of miltefosine and paromomycin drugs to the cell cultures, by nuclear magnetic resonance (NMR) spectroscopy. A Bruker 600 Avance III (600 MHz for ^{1}H) equipped with a 5 mm cryoprobe was used to obtain 1D 1H spectra and a Avance III (600 MHz for ^{1}H) equipped with a 1.7 mm cryoprobe was used to acquire the 2D 1H-1H TOCSY and 1H-13C HSQC spectra. Excitation sculpting was used to suppress the water signal on the 1D experiments. ChenoMX® and Topspin software were used to assign the 1D and 2D peaks.

The ^{1}H spectra obtained presented around 400 peaks, and the 1H-13C HSQC presented around 150 peaks. Assigning these peaks to correspondent metabolite is a major step in the study of the organism and allowed for a better characterization of the two different species studied. About 50 metabolites were identified, such as adenine, arginine, glutamine, glutamate, mannose, isoleucine, leucine and tyrosine. Chemometric techniques (PCA, PLS-DA and heatmaps) were used to investigate the metabolites responsible for the differentiation of the two species and the metabolic response of the protozoa in the presence of the drugs. PLS-DA identified putrescine, leucine and isoleucine as the major metabolites diverging between L. major and L. donovani with a Q2 of 0.75. However, during the test with drugs, both drugs had similar effects on the metabolism of the Leishmania species, contrary to what was expected. Although both drugs affect mitochondrial functions, miltefosine is only used to treat visceral leishmaniasis while paromomycin can treat both forms. The PCA and heatmaps used in these experiments highlighted a decrease in glutamate and an increase in glutamine indicating that the viability of 2-oxoglutarate, needed in the TCA cycle, was affected by the drugs. It was also observed a decrease of mannose levels. Mannose is converted to β-D-frutose-1,6P2 that is used in the glycolysis cycle, where it will turn into pyruvate, also needed in the TCA cycle. The increased usage of mannose by the Leishmania treated with drugs groups indicates a mitochondrial disfunction and an attempt to compensate said disfunction by tapping into other sources of energy. This data corroborates with the information existent in literature, where it is known that paromomycin and miltefosine interfere in the TCA cycle direct or indirectly, but it also adds information on where these drugs are working, and which metabolic pathways are being affected.

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43. FARIBA FATHI

Identification of metabolites in polar and non-polar part: protocol for liver tissue preparation

Fariba Fathi , Antonio Brun, Katherine H. Rott, Paulo Falco Cobra, Marco Tonelli, Hamid R. Eghbalnia, Enrique Caviedes-Vidal, William H. Karasov and John L. Markley

Introduction: Due to the important role of liver in digestive system, researchers have been working at developing methods to inspect the different liver fractions. Metabolites present in liver provide important clues regarding the physiological state of an organism. The methanol/chloroform/water protocol was applied for extraction of metabolites for high-throughput NMR-based analysis of both polar and non-polar metabolites from avian liver tissue. It has been found that the carbohydrate/higher protein diet for three days could increase their maltase and sucrase activity within 24 hours of a switch to a diet higher in carbohydrate. This study characterize and compare the hydrophobic metabolites of the liver when 6- to 7-day-old nestlings are fed two carbohydrate/ protein diets.

Methods: We analyzed the metabolic profile of livers from birds raised on two different diets: 100 mg of intact frozen liver tissues from 10 birds grown on a high carbohydrate diet and livers from 10 birds grown on a high protein diet. This protocol separates

polar and non-polar metabolites. After separating these phases, the solvents were removed from the samples. The dried polar and non-polar fractions were dissolved in 70 µL deuterated solvents and then transferred to 1.7 mm NMR tubes. All spectra were recorded on a Bruker Advance III 600 MHz spectrometer and ZGESGP and ZG30 were the pulse program for the polar and non-polar fraction, respectively. The relative concentration levels of non-polar metabolites were measured using Topspin software.

Preliminary Data: We have presented a simple protocol for simultaneously characterizing the lipidic and hydrophilic metabolic profiles in liver tissue of nestling birds. We used NMR spectroscopy to identify and quantify 61 polar and non-polar metabolites through an array of 1D and 2D NMR experiments. Using the NMR software, we were able to identify 52 metabolites from the polar fraction. Because most of the peaks from the 52 polar metabolites are overlapped in ^{1}H NMR spectra, we used 2D ^{1}H,^{13}C HSQC spectra to assist in resolving them. To identify the compounds, the chemical shifts from 1D ^{1}H NMR and 2D ^{1}H,^{13}C HSQC spectra were compared with those from reference spectra deposited in the Biological Magnetic Resonance data Bank and the Human Metabolome Database. We were able to identify and confirm a larger number of metabolites from liver tissue than previously reported in the literatures. Also, the spectra contain valuable information on nucleotides and cofactors, including ATP, GTP, AMP, IMP, UMP, NAD, and NADP+. In the lipid fraction, 23 lipid-related resonances from nine forms of non-polar molecules were identified by analyzing our NMR spectra in light of literature data. These include: glycerophospholipid backbone, esterified cholesterol, glycerol backbone, phosphatidylcholine, sphingomyelin, fatty acyl chain, free cholesterol, total cholesterol and multiple cholesterol protons.

We also analyzed differences in the metabolic profile of liver tissue from birds fed two different diets. In addition to being able to analyze the hydrophilic fraction, our method has the added potential of identifying and quantifying differences in liver lipids between groups of nestling birds raised on different diets. We found significant differences in the relative concentration levels of non-polar metabolites in the two groups. The results of our studies confirm that the protocol described here can be exploited for high-throughput screening in clinical diagnostic.

Novel Aspect: Our metabolomics approach enables us to detect the altering of hydrophobic metabolites in liver which respond differently to different diets.

44. ELIZABETH FELDEVERD

Characterizing the role of protein disulfide isomerase-9 in pollen development in Arabidopsis exposed to endoplasmic reticulum stress

Healthy plant cells maintain a balance of protein folding and export within the endoplasmic reticulum (ER). Protein disulfide isomerases (PDI) are protein-folding enzymes that catalyze the isomerization, creation and breakage of disulfide bonds. However, environmental factors, such as heat stress, can overwhelm folding capacity, causing unfolded and misfolded proteins to accumulate in the ER. When proteostasis is disrupted, the unfolded protein response (UPR) is activated by special transcription factors such as bZIP60. bZIP60 is spliced to its active transcription factor form by IRE1, a transmembrane protein with a sensing domain in the ER lumen and splicing domain in the cytosol. We have shown that the ER resident protein disulfide isomerase-9, PDI9, is activated during the UPR and is highly expressed in developing and mature pollen of *Arabidopsis thaliana*. We aim to characterize the role of PDI9 in *Arabidopsis* pollen development under ER stress. Double mutant seedlings with both PDI9 and its homolog PDI10 genes knocked out (*pdi9-pdi10*) were evaluated alongside wild type seedlings after induced ER stress with DTT using qPCR. The treated mutant showed an increase in the expression of spliced bZIP60 relative to treated wild type. Interestingly, the untreated mutant showed an increase in the expression of UPR genes, including two other PDIs, relative to untreated wild type. Separately, *pdi9-pdi10* mutant, a new PDI9 over-expressor and wild type plants were subjected to prolonged heat stress and studied using a variety of methods. Preliminary results suggest an important role for PDI9 in mediating ER stress as a consequence of heat. Future experiments, including a proteomics analysis of wild type and mutant pollen, will define the molecular role and physiological phenotype of PDI9.

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45. ERLU FENG

From Vinyl Cation to Distonic Carbene – An Ion Molecule Reaction Study

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Introduction: Vinyl cations are highly reactive species that are of great scientific interest, but were believed too unstable to exist in any significant quantity. On the other hand, gas-phase ion/molecule reactions are used to observe the reactions

of highly reactive species. This study explored the reactivity of a series of vinyl cations in gas phase using linear quadrupole ion trap mass spectrometer (LQIT). It was discovered that some of the vinyl cations exhibited carbene-like reactivity. Together with novel reactions observed for the first time, these results shined light on the important but poorly-understood reactive intermediates.

Methods: Synthesized vinyl cation precursors were ionized by protonation in positive ion mode via atmospheric pressure chemical ionization (APCI) in a Thermo Scientific LQIT mass spectrometer. After isolating the precursor ions, collision-activated dissociation (CAD) was used to generate vinyl cations in the ion trap. The gas-phase reactivity of these ions was investigated towards various reagents introduced by using an external reagent mixing manifold. Reaction kinetics were obtained by plotting the abundances of the reactant and product ions against reaction time. Quantum chemical calculations were used to identify feasible pathways for the generation of the vinyl cations and determine their ground state multiplicities, thus gaining insights into their reaction mechanisms.

Preliminary data: Upon sequential CAD, ionized vinyl cation precursors can be fragmented to generate corresponding vinyl cations. Plain vinyl cation showed proton transfer with high efficiencies when reacted with all reagents studied here. For the β-mono- and di-phenylvinyl cations, literature suggests that the initially formed primary vinyl cations will spontaneously rearrange to α-substituted phenylvinyl cations. To prevent this facile rearrangement and thus to examine the initially formed primary vinyl cation, we studied methylenecyclopropenyl cations having both β-substituents incorporated into a cyclopropenyl ring. Notably, phenylvinyl and methylenecyclopropenyl cations reacted with cyclohexene, benzene, acetonitrile and 2-butyne, producing covalent adducts, indicating the reactivity of vinyl cations towards unsaturated bonds; such reactivity can be explained by the mechanisms involving the distonic carbene resonance structures. Furthermore, substituted vinyl cations formed a covalent adduct with dimethyl disulfide, instead of SCH_3 abstraction – characteristic for radical-type reactions, indicating a singlet reactivity. Another supportive evidence for the singlet reactivity were the reactions of methylenecyclopropenyl cations with cyclohexene oxide/thiirane, yielding an oxygen/sulfur atom abstracted product. More interestingly, upon reaction with alkylbenzene, methylenecyclopropenyl cations formed benzylic abstraction products besides simple addition, demonstrating a unique reactivity of substituted vinyl cations. Kinetic studies on the distonic carbenes with different substituents showed that the reaction efficiency is affected by the electron withdrawing/donating nature of the substituents. Generally, electron donating substituents decrease the reaction efficiency of the substituted carbene. The possible mechanisms for above reactions are being explored by quantum chemical calculations.

Novel aspect: Gas-phase ion/molecule reactions of vinyl cations have demonstrated carbene-like reactivity toward various chemical reagents.

46. KELSEY FISHER-WELLMAN

Mitochondrial Diagnostics: A multiplexed biochemical assay platform for comprehensive assessment of mitochondrial energy flux in a single mouse.

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Introduction: While the advent of "omics" technologies has spawned an extraordinary new era of discovery science, challenges pertaining to data interpretation are increasingly recognized as a potential pitfall, that in turn poses a barrier to meaningful medical progress. Inarguably, unbiased analyses of SNPs, transcripts, proteins and metabolites offer great potential to identify molecular signatures of health and disease, but the biological relevance of those signatures cannot be clearly understood without similarly comprehensive approaches for phenotyping of cellular and/or organelle function. To address this methodological gap, the present study describes the development and application of a mitochondrial diagnostics platform for deep phenotyping of respiratory fluxes and function at a level that is highly complementary to most molecular *omics* tools.

Methods: Multiplexed analysis of numerous aspects of mitochondrial energy transduction (e.g., respiratory control, energetic potentials, $\mathcal{J}H_2O_2$ emission, dehydrogenase flux and ATP synthesis) were carried out under physiological energy constraints

using isolated mitochondria prepared from hearts of multiple murine models of hyper-acylation (malonyl-CoA decarboxylase deficiency, Sirt5 and Sirt3 deficiency). Complementary analysis of mitochondrial lysine acylation *via* label-free quantitative nLC-MS/MS was also performed using the same population of mitochondria employed for functional characterization.

Preliminary Data: The work here aimed to bridge the gap between molecular and functional phenomics by developing and validating a multiplexed assay platform for comprehensive assessment of mitochondrial energy transduction. The diagnostic power of the platform stems from a modified version of the creatine kinase energetic clamp, performed in parallel with high-throughput analyses of specific dehydrogenase activities and ATP synthesis rates. Together, these assays provided diagnostic coverage of the mitochondrial network at a level approaching that gained by molecular *omics* technologies. In an effort to validate the diagnostic potential of the assay platform, the biochemical consequences of mitochondrial lysine hyper-acylation were investigated in the setting of biological stoichiometry (i.e., in isolated mitochondria) across three distinct genetic models of hyper-acylation (Mcd, Sirt5 and Sirt3 deficiency). Without question, the vast majority of hyper-acylation events did not negatively impact biochemical flux. In fact, the activity of several affected enzymes and associated metabolic pathways were up-regulated rather than impaired in the setting of increased lysine acylation. Interestingly, of the greater than 60 experimental readouts generated for each model system, partial loss of ATP synthase (i.e., CV) activity was the only biochemical phenotype observed consistently across all models. With respect to the acyl-lysine residues mediating the observed CV inhibition, label-free quantitative analysis of the CV "acylomes" from each mouse line revealed strikingly little overlap in the specific lysine residues found to be hyper-malonylated, succinylated and acetylated. Taken together, these data provide direct evidence of functional redundancy within the acylome and suggest that metabolic regulation induced by these PTMs likely stems from cumulative effects induced by multiple modified residues within susceptible protein targets or metabolic pathways.

Novel Aspect: This platform opens exciting opportunities to unravel the connection between mitochondrial PTMs and bioenergetic flux in the context of disease.

47. LAUREN FORGRAVE

TDP-43 Proteinopathies

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Introduction: The transactive response DNA-binding protein 43 kDa (TDP-43) is a ubiquitously expressed nuclear protein, involved in RNA processing and metabolism. TDP-43 has been associated with several neurodegenerative disorders, collectively referred to as TDP proteinopathies. In these protein misfolding disorders, TDP-43 aggregates in the cytoplasm or nucleus of cells, and can be biochemically modified (i.e. ubiquitinated, phosphorylated, sumoylated, acetylated, oxidated, and truncated). TDP-43 inclusions can be the defining proteinaceous aggregate or be observed in conjunction with another proteinopathy, defining primary and secondary TDP proteinopathies, respectively. To date, the mechanism of TDP-43 pathogenesis remains elusive. The objective of this review was to synthesize current experimental evidence related to primary and secondary TDP proteinopathies to gain insight into the mechanism of TDP pathology.

Methods: We performed a literature review of primary and secondary TDP proteinopathies with a focus on histopathological characteristics. Additionally, we compared epidemiological data and clinical findings in individuals with secondary proteinopathies, with and without TDP-43 pathology.

Results: Abnormal TDP-43 aggregation has been documented in over 20 conditions including several forms of dementia and motor neuron disease. Features of pathological TDP-43 include cytoplasmic mislocalization and loss of normal nuclear localization, proteolytic cleavage, misfolding, and aggregation. The primary TDP proteinopathies are frontotemporal degeneration, amyotrophic lateral sclerosis, and Perry syndrome. Secondary TDP proteinopathies include Alzheimer's disease, chronic traumatic encephalopathy, hippocampal sclerosis, and corticobasal degeneration where TDP-43 aggregates with tau and Parkinson's disease and dementia with Lewy bodies where TDP-43 aggregates with α-synuclein. We observed TDP-43 pathology to present with three main spatial patterns within the brain: limbic distribution, diffuse distribution, and in association with motor neurons. In secondary TDP proteinopathies, a substantial portion of concomitant TDP-43 aggregates, were not found within the primary pathological aggregates. Additionally, the presence and extent of TDP-43 co-pathology positively correlated with age, disease comorbidity, and disease severity for Alzheimer's disease, argyrophilic grain disease, progressive supranuclear palsy, and Parkinson's disease dementia. Abnormal TDP-43 aggregation has also been found in cognitively normal individuals over 65 years of age.

Similar to aggregating proteins in related neurodegenerative diseases, findings support age-dependent aggregation of TDP-43 that occurs with a threshold-like mechanism, in which symptoms do not present until pathology has progressed past a certain stage. Given the correlation between age, disease severity, and disease comorbidity, TDP-43 likely plays an important role in disease pathology even when present as a secondary co-pathology. Limitations of this work include small sample size of studies, overlap of disease diagnoses, lack of clinical data available in several studies, and the potential lack of reporting of negative findings.

Novelty: TDP-43 pathogenesis has largely been explored within closely related disorders, but not across all disorders, as demonstrated herein

48. LEIGH FOSTER

Quantitative analysis of signaling pathways using 11plex TMT Reagents and comprehensive phosphopeptide enrichment strategies

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Introduction: Post translational modifications (PTM) are crucial in controlling key aspects of protein function. Identification and quantitation of phosphorylation is critical for the elucidation of cellular function. Despite improvements in MS instrumentation, phosphoproteomics still faces challenges that are often neglected, e.g. recovery of phosphopeptide enrichment, assessment of phosphorylation stoichiometry, label-free quantification, etc. Advances in multiplexed quantitation utilizing isobaric tags have the potential to create a new paradigm in quantitative phosphoproteomics. Amine-reactive isobaric TMT tags enable concurrent identification and quantitation of proteins in different samples using LC-MS/MS. In this comprehensive phosphoproteomics study, we have combined TMT11plex quantitation, SMOAC method (Sequential enrichment of Metal Oxide Affinity Chromatography) and high pH RP fractionation to evaluate network dynamics in HeLa cells under different stimulation conditions.

Methods: HeLa cells were treated under 10 different conditions and then lysed, quantitated, reduced, alkylated, and digested with LysC and trypsin. Digested samples were quantitated and equimolar ratios of TMT11plex labeled samples were combined prior to desalting. Sample was phospho-enriched with TiO_2, and TiO_2 flow-through and wash fractions were enriched by Fe-NTA. Phosphopeptides were fractionated with a high pH reverse-phase method. LC-MS analysis was performed on a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer using a 210 min gradient. Thermo Scientific™ Proteome Discoverer™ 2.2 software was used to search MS/MS spectra with the Byonic™ search engine including PhosphoRS node. A custom TMT11plex quantification method was used to calculate the reporter ratios with mass tolerance ±10 ppm and apply isotopic correction factors.

Preliminary Data: Previously, isobaric TMT multiplexing was expanded from 6plex to 10plex using high resolution mass spectrometry (>50K at m/z 200) to separate 15N and 13C stable isotope variants. Using the same principle, we synthesized the full 13C isotope variant of the TMT-131 reporter, called TMT11-131C. This tag increases isobaric tag multiplex quantitation to 11 samples in a single LC-MS analysis without any changes in reagent structure or LC-MS/MS analysis. Our optimized TMT11plex phosphoenrichment workflow resulted in identification of 33,000 phosphopeptides including ~ 24,000 with quantitation and site localization (confidence score >90). Furthermore, this comprehensive phosphoproteomics analysis allowed quantitation of phosphorylation changes for multiple signaling pathways proteins under different conditions.

The complexity of samples was significantly reduced using a custom fractionation gradient prior to LC-MS analysis. The observed dynamic range for TiO2 enriched and fractionated samples was ~5-fold, whereas dynamic range for SMOAC enriched and fractionated samples was ~7-fold, increasing the number, confidence, and accurate quantitation of identified peptides and phosphorylation events. Furthermore, TiO_2 enriched fractions achieved 79.01% phosphospecificity and 39.83% of peptides identified per fraction were non-redundant. SMOAC enriched fractions achieved 88.87% phosphospecificity and 49.59% of peptides identified per fraction were non-redundant. Phosphospecificity was best in hydrophilic fractions; fractions 6-8 of TiO2 samples and fractions 7-8 of SMOAC samples were determined to be less than 94% specific.

Out of 122 phosphorylation sites of interest from proteins in the AKT/mTOR, RAS/RAF/MAPK and p53 signaling pathways, 59 phosphosites were identified in at least one of the ten conditions evaluated, and 57 of these phosphosites were quantitated. For each identified phosphosite of interest, at least one stimulation technique was identified as having significantly higher expression than other evaluated stimulations. The most frequently identified stimulation techniques for these target phosphosites were from PDGF stimulated lysate, unstimulated lysate, and EGF stimulated lysate, respectively.

Novel Aspect: Unique, integrated and novel workflow using TMT 11-plex quantitation with specialized phosphoenrichment and custom fractionation to monitor signaling pathways

49. XINYU FU

Probing the Structure and Dynamics of Acetyl-CoA Metabolic Network in Plants Using Stable Isotope-Assisted Metabolomics

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The acetyl-CoA metabolic network is at the core of the metabolic processes for synthesizing energy-dense phytochemicals (i.e., fatty acids, hydrocarbons, terpenes), and many bioactive compounds (e.g., polyketides and flavonoids). As sessile organisms with highly compartmentalized cellular structures, plants have evolved distinct acetyl-CoA pools due to the impermeability of membranes to this key intermediate. The compartmentalized acetyl-CoA metabolism in plants is far from being understood due to the difficulty of determining the subcellular locations of metabolites. Our research goal is to use genetic and metabolomics tools to understand the dynamic changes and spatial organization of acetyl-CoA metabolism, which is fundamental to precise control and regulation of carbon fluxes to bioengineer plants for biofuels and bio-based products. To fingerprint the carbon flux through the plant acetyl-CoA metabolic network, we use mass spectrometry-based analytical platforms coupled with stable isotope tracer techniques. *Arabidopsis* plants were cultivated in liquid media with ^{13}C-acetate to produce isotopically labelled plant material. To measure the kinetics of the isotope labelling, sampling was performed at different time-points after the start of the incubation. Quantitative analysis of the stable isotope labelling patterns of fatty acids, amino acids, and organic acids were performed using GC-EI-MS using different chemical derivatization strategies. Non-targeted analysis is being tested using ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF-MS). Mass spectrometry imaging (MSI) via electrospray laser desorption ionization (ELDI) is being developed to map the spatial distribution of isotopically labelled metabolites. With multiple reverse genetic mutant strains, we can manipulate the activation of different isotope-labeled precursors at different cellular compartments. A global investigation of the cellular fate of the precursor metabolites using multiple targeted and non-targeted metabolomics platforms will advance our understanding of compartmentalized metabolic networks by providing quantitative flux information.

50. FABIAN GISKA

The use of mass spectrometry to identify interactors of the Pti1b kinase – a positive regulator of plant immunity in tomato

Plant innate immunity is triggered when pattern recognition receptors (PRRs) are activated upon detection of pathogen-associated molecular patterns (PAMPs). Activated PRRs initiate downstream signaling pathways that lead ultimately to the development of an effective immune response. Key components of PRR-dependent pathways are receptor-like cytoplasmic kinases (RLCK). Pti1a and its paralog Pti1b are RLCKs from tomato with autophosphorylation activity. Tomato plants with CRISPR-generated mutations in Pti1a/Pti1b are impaired in pattern-trigged immunity against *Pseudomonas syringae* and produce lower amounts of reactive oxygen species after treatment with two bacterial PAMPs: flg22 and flg28, flagellin-derived peptides. To decipher the molecular mechanisms that Pti1 uses to regulate plant immunity we carried out a mass spectrometry screen to find interactors of Pti1b. Data analysis showed that Pti1b co-purified with three other plant proteins. Interestingly, in common with Pti1b, each of these proteins is predicted to be associated with the plasma membrane. One of the proteins is a phosphatase belonging to the PP2c family and we hypothesized that this phosphatase might modulate the phosphorylation status and function of Pti1b. In subsequent experiments we found that PP2c interacts *in vivo* with Pti1b and is able to reduce that autophosphorylation of Pti1b *in vitro.* Finally, we discovered that PP2c blocks generation of reactive oxygen species (ROS) in *N. benthamia*na after treatment with different PAMPs of bacterial flagellin. These observations indicate that PP2c acts as a negative regulator of Pti1b and therefore of plant immunity.

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51. LINUS GOG

Can a plastidial retrograde signal be harnessed to improve plant bioenergy content?

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Plant chloroplasts generate cellular signals that influence how nuclear encoded genes are expressed. Although many particulars of the signal transduction pathway between plastid and nucleus remain unknown, we hypothesize that such retrograde signals could be directed to meet societal demand for sustainable bioenergy. The methylerythritol phosphate (MEP) pathway, localized in chloroplasts, generates methylerythritol cyclodiphosphate (MEcDP), which has been identified as a metabolite in retrograde signaling. Downstream from MEcDP production, the MEP pathway generates isoprene (C_5H_8), the repeating molecular unit that forms the isoprenoid class of compounds. Considering that MEcDP acts both as regulator of the MEP pathway as well as a retrograde signal, experimental manipulation of MEcDP flux might influence plants to store more isoprenoid hydrocarbons. While a number of experimental approaches can direct how MEcDP is produced and transduced to plant nuclei, the critical measurement common to each manipulation concerns how much MEcDP is localized in chloroplasts relative to how much MEcDP is present in cytoplasm. Therefore, we intend to grow plants under a variety of experimental conditions, separate plastidial MEcDP from cytosolic MEcDP through non-aqueous fractionation of plant tissue, then quantify MEcDP present in each fraction via mass spectrometry. Our ultimate objective is to generate a variety of poplar, *Populus* sp., that stores an increased amount of isoprenoid hydrocarbons useful for harvest as a biofuel.

52. KRZYSZTOF GORYNSKI

Novel sample preparation techniques applied in prohibited substances analysis from various matrices – which criteria are most important for analysis?

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Introduction: Even though the range of analytical protocols are available for anti-doping screening, there is still a need for improvement of new technologies that can provide better sample preparation from various matrices for simultaneous multi-compound analysis. Ever since there are a number of compounds that can be possibly used as performance enhancers by sportsmen, the selected method should be capable of screen as many drugs of interest as possible very fast and in one single analysis, without sacrificing sensitivity or time length of analysis. Presently dilute-and-shoot, protein precipitation, liquid-liquid extraction or solid phase-extraction are commonly used in analytical laboratories and two decades ago solid-phase microextraction was introduced into analytical community and have recently made significant advances when applied to variety of exo- and endogenous compounds including drugs and metabolites analysis. Recent preliminary studies have shown pros and cons bench of sample preparation techniques applied on the analysis of doping substances in urine, blood and plasma, as regular matrices, as well as saliva, as alternative fluids.

Methods: Different sample preparation techniques (SPME, dilute-and-shoot, protein precipitation) were applied before LC-MS analysis. We determined limit of detection, lower limit of quantification, upper limit of quantification, linear dynamic range, relative standard deviations through the dynamic range, absolute and relative recoveries and matrix effect. The validation was completed according FDA procedure and WADA requirements. Finally SPME was coupled to: high sensitive triple quadrupole platform and high-resolution instrument.

Preliminary data: The applicability of SPME mainly for alternative matrix, saliva, will be discussed. Up-to-date results show limits of detection at pg/mL levels, thus showing potential of the approach to be used as fast and sensitive tool for screening of prohibited substances in alternative matrices e.g. oral fluid/saliva. The comparison of different sample collection technique (salivette vs. spitting) and two different sample preparation method (SPME vs. PP) in combination with high resolution mass spectrometer provide extend information about quality and quantity of metabolites presence depends which configuration was applied. The results demonstrate strengths and weaknesses different analytical approaches suitable for simultaneous multi-compound bioanalysis of analytes with a wide range of polarities. Results from comparison to commercially available saliva test against narcotics vs. tailored in laboratory protocol will be presented. Also data obtained from matrix effect studies using different sample preparation techniques will be discussed.

Novel aspects: Ongoing studies expend utilization of novel sample preparation techniques for saliva during doping control testing.

53. LAURA GREELEY

Comparing proteomic changes during PAMP responses and MKP1-requiring genetic pathways

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Introduction: Plant disease plays a major role in disruption of food security worldwide. Understanding how plants defend themselves is vital to developing strategies to improve plant defense systems. *Arabidopsis thaliana* plants lacking mitogen-activated protein phosphatase 1 (MKP1), a negative regulator in plant immunity, have increased resistance against the pathogen *Pseudomonas syringae* due to decreased levels extracellular plant chemical signals required by bacteria to induce virulence. Unlike most mutants that elevate plant resistance, *mkp1* mutants do not have a fitness penalty in the Wassilewskija (WS) ecotype. The reduction in chemical signals occurs externally; indicating a possible role of membrane bound transporter proteins. A detailed view of modulations on the membrane associated proteins is key to understanding how *mkp1* regulates plant immunity.

Methods: Wild-type and *mkp1* knockout lines of 7 day-old seedlings in WS background are treated with the pathogen associated molecular pattern (PAMP) elf18 for 1 h to stimulate plant defense responses. Seedlings are flash-frozen, ground with liquid nitrogen, and fractioned to enrich for total microsomes. These total membrane fractions are then enriched for plasma membranes (PMs) using Brij-58 and ultracentrifugation. Enrichment is verified via immunoblots with marker proteins for different compartments. Enriched PM proteins are separated by SDS-PAG and in-gel digested. Data will be acquired on a Q-Exactive MS, and a data analysis pipeline of MASCOT and Scaffold will be used to identify and quantitate proteins.

Preliminary Data: To be fully virulent, bacterial pathogens must recognize a potential host to induce their Type III Secretion System (T3SS) in order to inject effector proteins that suppress host immunity. Pretreatment of plants with PAMPs or genetic mutations in *mkp1* both lead to enhanced resistance, and both processes appear to involve host restriction of specific, extracellular chemical signals (i.e. metabolites) that bacteria require to activate the T3SS. A possible mechanism for decreasing these chemicals is through rapid degradation of plant PM transporters for these metabolites. Thus, performing a quantitative PM proteomic analysis of control and PAMP-treated plants of WT vs *mkp1* mutants should identify similar proteins (e.g. transporters) that are decreased or absent in PAMP-treated WT as in the untreated *mkp1* mutant (which already has restricted metabolite accumulation). An alternative hypothesis is that differential protein phosphorylation may control transporter activity, and this could be tested by performing a phosphoproteomic comparison of PM proteins.

We have selected a 1 hr time treatment time for the PAMP (elf18) because it has previously been reported that endocytic removal of another PAMP receptor, FLS2, occurs within 1 hr to desensitize the plants. However, this possibility has not been reported for the elf18 receptor, EFR; and unfortunately, no antibody for EFR exists (we must use elf18/EFR because our mutants are in Ws, which does not have a functional FLS2 receptor). Therefore, as a proxy, we treated plants with elf18 for 1 hr followed by a 2nd treatment with either elf18 or pep1, an unrelated PAMP. We found that plants would not respond to the 2nd elf18 treatment with MAPK activation but responded normally to pep1. Thus, it appears that EFR is also endocytically removed, which means that even if our hypothesis is incorrect, we will learn if other PM proteins are also removed together with EFR.

Novel Aspect: Transporters controlling levels of extracellular metabolites have not been identified, and experiments can identify these key mediators of plant resistance

54. MYRON GROSS

Mass Spectrometry and Standardization of Blood Vitamin D in Large-Scale Human Studies. Is it Posible?

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Introduction: Liquid chromatography-mass spectrometry mass spectrometry (LC-MS/MS) is a commonly used method for the measurement of vitamin D in blood and is accepted as a state-of-the-art measurement for vitamin D. While LC-MSMS is generally quite accurate, variation does exist between and within instruments. Thus, large-scale studies with multiple sites and instruments,

should utilize the standardization of results to reduce variability of results between instruments. This is particularly true when the results are used for the establishment of guidelines such as the occurrence of hypovitaminosis. A standardized laboratory measurement is one that is accurate and comparable over time, location, and laboratory procedure. Standardization depends on the identification of a reference measurement procedure (RMP) and its effective utilization in each instrument of the study.

Method: We reviewed the current literature on the quality assurance of vitamin D analysis. The goal of the search was the identification of a reference measurement procedure for vitamin D and associated proficiency testing programs. Reference measurement procedures are laboratory methods that have been judged to conform with the guidelines developed by the International Organization for Standardization (ISO) and are listed in the database of the Joint Committee for Traceability in Laboratory Medicine. The proficiency programs are essential for the transfer of standardization information from the RMP to the proficiency programs.

Preliminary Results: We found a program, the Vitamin D Standardization Program (VDSP), has been established recently for the promotion of standardization procedures for vitamin D. These procedures have been developed and are readily available in the literature. A calibration chain was found between the RMP values and the routine methods of measurement. The procedures yield results that are accurate and comparable over time, location, and laboratory procedure to the values obtained using reference measurement procedures (RMPs) developed at the National Institute of Standards and Technology (NIST) and Ghent University . This information is utilized through the establishment of proficiency programs. Proficiency programs with a validated linkage to the RMPs for total vitamin D include: NIST Standard Reference Materials or SRMs, CDC's Vitamin D Standardization-Certification Program, Accuracy-Based Proficiency Testing/Quality Assessment Programs including College of American Pathologists (CAP) and Vitamin D External Quality Assessment Scheme (DEQAS). NIST provided reference materials, the CDC provided high-level proficiency testing, CAP and DEQAS provided routine proficiency testing materials. Each of these proficiency testing systems has been used in my laboratory and has yielded excellent and consistent results, we will provide these for the poster in August 2018. Thus, it appears feasible that standardization of vitamin D values with the materials currently available and consideration of logistics is possible.

Novel Aspect: A method has been proposed for the world-wide standardization of vitamin D and its use in establishment of clinical guidelines.

55. VALERIA GUIDOLIN

A High resolution/accurate mass data dependent-constant neutral loss-MS^3 (DDA-CNL/MS^3) DNA adductomic method for the investigation of alcohol-related DNA damage

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Introduction: Acetaldehyde is the major metabolite of alcohol and has recently been classified by IARC as a group-1 human carcinogen. Despite a clear association, the underlying mechanisms of alcohol-induced carcinogenesis remain unclear. Acetaldehyde is a DNA modification-inducing carcinogenic compound. If not eliminated or repaired, these modifications can result in miscoding events leading to mutations and ultimately to cancer initiation. To comprehensively characterize all acetaldehyde-derived DNA modifications, we developed an untargeted DDA-CNL/MS^3 DNA adductomic methodology based on identifying DNA adducts through monitoring the constant neutral loss of features common to all DNA adducts: the 2'-deoxyribose or one of the four bases. This approach has resulted in the simultaneous characterization of numerous acetaldehyde-derived DNA modifications.

Methods: DNA samples treated with or without $NaBH_3CN$ were enzymatically digested and purified on C18 RP-column. Our DNA adductomics approach used an Orbitrap Fusion analyzer interfaced to a nanoUPLC (Ultimate3000) with NanoFlex source, operating in positive mode. A linear gradient of MeCN in aqueous HCO_2H (0.05%v/v) was used to separate the analytes on a PicoFrit emitter, home-packed with a C18-phase. The Orbitrap operated in a gas phase fractionation mode, performing 5 untargeted partial scans. For each scan, the top 5 ions were isolated and fragmented during MS^2 acquisition, a further fragmentation was triggered by the NL-signal of the deoxyribose or one of the four bases. Isotopically-labeled standards were synthesized to confirm the structure of two of the major DNA adducts identified.

Preliminary Data: Two batches of DNA samples were respectively exposed to native and ^{13}C isotopically labelled acetaldehyde (50 mM). Data analysis was performed through an in-house software, which identified DNA adducts upon the observation of an isotopic distance equal to $^{13}C_2$ (2.0067 Da ±5 ppm) and a retention time difference less than 0.2 minutes. The developed method was able to identify about 20 new DNA adducts, as well as all previously observed DNA adducts associated with acetaldehyde exposure. Schiff bases were found in samples reduced with $NaBH_3CN$, meanwhile cross-links and Mannich reaction products in non-reduced samples. In reduced DNA samples, the profile was dominated by the signals of three adducts (primary DNA adducts): the known biomarker N^2-Ethyl-dG and two uncharacterized DNA adducts, N^6-Ethyl-dA and N^4-Ethyl-dC. In non-reduced samples, other unknown DNA adducts were detected (secondary DNA adducts). Elucidation of the structure of the secondary DNA adducts is ongoing, while the two unknown primary adducts were identified and characterized through the synthesis of isotopically-labeled standards. N^6-Ethyl-dA, N^4-Ethyl-dC, D_5-N^6-Ethyl-dA and D_5-N^4-Ethyl-dC standards were synthesized, purified and structurally characterized by 1-D and 2-D NMR (^1H, ^{13}C, COSY and HMBC spectra) and HRMS analyses. A dose-response relationship between the three primary DNA adducts and acetaldehyde exposure was observed by exposing DNA samples to different acetaldehyde concentrations (0 mM, 5 mM, 25 mM, 50 mM). Finally, the presence of these adducts was investigated and confirmed in oral DNA samples from humans exposed to alcohol. In conclusion, we developed a method able to identify numerous DNA adducts simultaneously and screen for unknown DNA modifications deriving from acetaldehyde exposure. Moreover, we discovered two new major DNA adducts, which may be used as additional biomarkers of alcohol and acetaldehyde exposure.

Novel Aspect: An innovative untargeted DDA-CNL/MS^3 methodology was developed to discover new candidate biomarkers of DNA damage induced by acetaldehyde exposure.

56. JANA HAVLIKOVA

Development of Liquid Extraction Surface Analysis Mass Spectrometry for Identification of ESKAPE Pathogens

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Introduction: The ESKAPE pathogens are a group of microorganisms (the Gram-positive bacteria *Enterococcus faecium* and *Staphylococcus aureus* and the Gram-negatives *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa* and *Enterobacter* species) which together account for most nosocomial (hospital-acquired) infections. Levels of antibiotic resistance within this group are rising and therefore fast and accurate bacterial analysis is of high importance. Currently, the examination of microbes takes hours or days. In contrast, analysis by LESA mass spectrometry offers the potential for rapid (minutes) analysis of bacteria cultured on various substrates directly from the Petri dish at ambient conditions without any specific sample preparation.

Methods: Bacterial strains *S. aureus* MSSA476, *P. aeruginosa* PS1054, *K. pneumoniae* KP257 were grown on Lysogeny broth agar (LBA) and blood agar, *E. faecium* E745 and *E. faecalis* V583 were cultured on brain heart infusion medium and incubated for 24 h at 37 °C prior to MS analysis. LESA extraction was performed using an Advion Triversa Nanomate coupled with a Thermo Orbitrap Elite mass spectrometer. The extraction solvent system comprised acetonitrile, water and formic acid at different ratios depending on the type of bacterial species. Top-down analysis of bacterial proteins was performed using collision induced dissociation in the ion trap using helium gas at a normalized collision energy of 35%. Fragmentation mass spectra were analysed in the ProSight software (Thermo Scientific).

Preliminary data: The LESA MS analysis of bacterial species *K. pneumoniae* KP257, *E. faecium* E745 and *E. faecalis* V583 was performed. The extraction solvent system for each bacterial strain was optimized prior to MS analysis. A number of proteins have been identified including DNAbinding protein HUα, 50S ribosomal protein L29 and two uncharacterized proteins from KP257, general stress response protein, 50S ribosomal protein L29, 30S ribosomal protein S20 and two uncharacterized proteins from E745 and 50S ribosomal protein L29, UPF0337 protein and one uncharacterized protein from V583. The molecular masses of the 50S ribosomal protein L29 differed between the two *Enterococci* strains, suggesting the protein may be a useful biomarker for identification of *Enterococcus* strains.

The LESA mass spectra obtained from three bacterial species *S. aureus* MSSA476, *P. aeruginosa* PS1054 and *K. pneumoniae* KP257 grown on different substrates (LBA, blood agar) were compared. The results revealed that the same bacterial proteins were identified regardless of substrate complexity, suggesting that the approach may be suitable for bacterial identification from a variety of substrates. Moreover, when cultured on blood agar, additional protein peaks were detected in the LESA mass spectra. Top-down protein mass spectrometry by use of CID identified these peaks as horse alpha and beta hemoglobin subunits. A strain of *S. aureus* NCTC13435 was grown on 3D living skin model. Experiments showed that it was possible to identify in the mass spectra bacterial protein peaks as well as the peaks representing skin proteins. These results demonstrate the ability to identify microbial proteins together with the substrate proteins by LESA mass spectrometry.

Novel aspect: LESA mass spectrometry identification of proteins from ESKAPE pathogens on various substrates: *Klebsiella* and *Enterococcus*

57. SPENCER HAWS

Mass Spectrometry Based Approaches to Investigating Chromatin-Metabolism Dynamics

Numerous metabolic pathways are capable of influencing histone methylation patterns as the enzymes needed to "write" and "erase" this post-translational modification (PTM) rely on metabolite cofactors. For example, alpha-ketoglutarate is a required cofactor for JmjC demethylases while S-adenosylmethionine (SAM), the downstream product of methionine (MET), is required by histone-methyltransferases (HMTs). This connection between metabolism and epigenetic regulation is particularly important regarding the MET cycle, as altered pathway metabolism is associated with several physiological phenotypes. Here we use mass spectrometry-based approaches to identify the global epigenetic response to altered MET cycle metabolism, as well as its supporting mechanism, and determine the relevance of this response *in-vivo.*

To investigate the global histone PTM response under altered MET metabolism, we developed a tissue culture model of complete MET restriction (MR). Metabolic perturbations in this model were verified by LC-MS metabolomics, which revealed a complete loss of detectable MET and SAM after 5 and 90 minutes, respectively. Loss of these metabolites correlated with global losses in histone methylation as determined by LC-MS/MS histone proteomics. Follow-up analyses identified SAM to be driving the MR histone PTM signature as deprivation of this metabolite alone, through either decreased synthesis or increased consumption, recapitulated the MR histone PTM response.

Despite the global decrease in histone methylation, H3K9me1 abundance was persistent during SAM depletion in each of these 3 unique *in-vitro* systems, as well as in liver from mice fed a 5- week total MR diet. *In-vitro* analyses revealed H3K9me1 was maintained through preferential utilization of residual SAM for *de-novo* methylation of both cytoplasmic and nuclear histones. This conserved maintenance of H3K9me1 across diverse cellular and *in-vivo* systems suggests that this PTM mediates a significant biological function during severe SAM depletion.

As H3K9 methylation is known to support constitutive heterochromatin stability, we hypothesized H3K9me1 was being actively maintained during SAM depletion to provide a mono- methyl substrate for H3K9 di/tri-methyl HMTs in order to attenuate heterochromatin loss. Prevention of active H3K9me1 methylation during MR resulted in greater MNase DNA accessibility and further de-repression of repetitive DNA elements, both signs of decreased heterochromatin stability. H3K9me1 maintenance during MR was also shown to be required for global histone PTM recovery upon SAM repletion.

These results describe a conserved, whole-cell mechanism which supports epigenetic persistence during severe metabolic perturbations. Ongoing work will determine how this mechanism links to *in-vivo* MR phenotypes or may be dysregulated in biological models characterized by heterochromatin instability.

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58. DANNING HUANG

Serum Metabolic Profiling of a High-Grade Serous Ovarian Cancer (HGSOC) Mouse Model: Insights into Disease Progression

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Introduction: Ovarian cancer (OC) is the most lethal gynecologic malignancy, and the seventh most commonly diagnosed cancer among women in the world. High-grade serous ovarian cancer (HGSOC) accounts for 70–80% of OC deaths and the survival has not changed for several decades. This relatively low survival rate is largely due to the fact that this disease lacks specific symptoms until late stages when most patients are diagnosed. Yet, the cellular origin and tumor pathogenesis of HGSOC are poorly understood. *p53-Dicer-Pten* triple-knockout (TKO) mouse model, develops HGSOC that presents clinical and molecular similarities to human cancer. Serum metabolic profiling of this mouse model allows the discovery of HGSOC related biomarkers, as well as providing a deep insight into the disease progression.

Methods: Serum samples were collected from 22 TKO-Pre mice (precursor lesion), 19 TKO-control mice (developed no cancer), 10 TKO-ET mice (early-stage tumor without metastasis), 16 TKO-LT mice (late-stage full-blown metastases observed) and 17 cp53PtKO mice (control mice with late-stage uterine tumors). Isopropanol or methanol were mixed with the serum samples (3:1 v/v) to precipitate proteins. Following centrifugation, the supernatant was collected for analysis. ThermoFisher Scientific Ultimate 3000 UPLC was equipped with an Acquity UPLC BEH C18 1.7 µm column for reverse phase or an Acquity UPLC BEH HILIC 1.7µm column for HILIC separation and coupled to ThermoFisher Scientific Q Exactive HF MS. Data Dependent Acquisition (DDA) and Parallel Reaction Monitoring (PRM) methods were used to collect MS/MS data for feature identification.

Preliminary results: A total of 10,077 spectral features were extracted from UPLC-MS RP ESI+, RP ESI-, HILIC ESI+, and HILIC ESI- datasets by Compound Discoverer V2.1. Following background and solvent front signals removal, a combined total of 5937 features were analyzed with unsupervised Principal Component Analysis (PCA). The TKO-Pre and TKO-control mice samples were clustered together, suggesting only minor observed differences between the TKO-Pre mice and the healthy control mice, thus the TKO-Pre mice were not considered as part of the HGSOC group for the next steps of analysis. TKO-ET and TKO-LT mice were combined to form the HGSOC group, and then compared with the TKO-control and cp53PtKO mice groups. After a two-tailed Welch's t-test with Benjamini Hochberg correction, 2440 features were considered statistically significant between HGSOC and TKO-control mice groups, and 1675 features were statistically significant between HGSOC and cp53PtKO mice groups. Twenty nine features were selected from pool of features that were different from both control and cp53PtKO mice groups using a Genetic Algorithm (GA). Orthogonal Partial Least Squares Discriminant Analysis (oPLS-DA) model built using 29 features yielded a high accuracy of 96.1% (96.2% sensitivity, 96.1% specificity) for discriminating HGSOC from TKO-control and cp53PtKO mice samples. For binary classification, by using the same 29 features, the oPLS-DA model yielded an even better performance of 98.1% accuracy (96.2% sensitivity, 100% specificity) for classifying HGSOC from TKO-control mice samples, and a high accuracy of 95.4% (96.2% sensitivity, 94.7% specificity) for classifying HGSOC from cp53PtKO mice samples. Identified metabolites include species such as cholesteryl ester (CE), cholesterol, phospholipids (PLs), fatty acids (FAs), bilirubin, uridine, N-acetylneuraminic acid (NeuAc), hydroxysebacic acid, and amino acids.

Novel aspect: This study investigates the metabolic alternations in TKO mice, provides useful HGSOC biomarkers that could potentially be transferred to humans.

59. MADHURI JAYATHIRTHA

Mass Spectrometry based Proteomics to Investigate and Characterize the Jumping Translocation Breakpoint (JTB) Protein using Cancer Cell Lines

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Introduction: Human JTB (hJTB) is a gene located on the human chromosome 1 at q21 which is involved in the unbalanced translocation in various types of cancer. It encodes a conserved transmembrane protein of 16.4 kDa. JTB protein is ubiquitously present in normal cells and is overexpressed in various types of cancer including prostate and breast cancer. Hence this protein could be a biomarker for tumor malignancies and a potential target for their treatment. However, the pathway through which this protein causes increased cell growth and differentiation is not clear. Investigation and comparison of the proteomes of cells with upregulated and downregulated JTB can be a good approach to understand the function of the protein and its contribution to tumorigenesis.

Methods: MCF 7 breast cancer cell lines were transfected with the sense and antisense orientation of the JTB cDNA in HA,

His and FLAG tagged CMV expression vector. Transiently transfected cells were selected and were lysed, centrifuged and the proteins were extracted from the cells. Anti-JTB antibody was used to confirm the expression of JTB by western blotting. Immunoprecipitation was done to study the protein complexes and protein-protein interactions. The extracted proteins were then separated by SDS-PAGE. Individual gel bands were trypsin digested and the peptides were analyzed by a Nano Acquity UPLC coupled with QTOF Ultima Mass Spectrometer. The data analysis was done using ProteinLynx Global Server (PLGS 2.4), Mascot server and Scaffold 4.1 software.

Preliminary data: We produced highly affinity purified two sets of antibodies against the protein JTB. The cells were successfully cultured with both upregulation and downregulation of JTB for proteomics analysis. The proteins in transiently transfected cells were analyzed and the differences were compared. Furthermore, stable transfection will also be done for large scale protein production and to evaluate the JTB gene function and regulation mechanism. Two other isoforms of JTB will be analyzed and characterized to study their function and will be compared with the wild type JTB. Validation and functional studies will also be performed. These studies could help us elucidate the mechanism through which JTB induces cell proliferation and test the JTB protein as a potential drug target for malignancies with overexpression of the protein.

Novel aspect: Our study focuses on testing a new target for diagnosis and treatment of breast cancer and other types of cancer.

60. PENNY JENSEN

Targeted Mass spectrometry Assays for Quantitation of Signaling Pathway Proteins

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It is known that the investigation of signaling proteins is very challenging due to poor reproducibility, unreliable quantitation, a lack of standardized workflow solutions and poorly validated methods/reagents, and a reliance on semiquantitative methods (WB and ELISA). Our group is trying to alleviate these obstacles using Mass Spectrometry in combination with antibodies. Immunoprecipitation to mass spectrometry (IP-MS) targeted assays overcome these challenges and open new avenues for a broad range of applications, from cancer diagnosis and prognosis to drug development and personalized medicine. Our proposed targeted MS assays include multiplexed IP modules (antibodies & positive control lysate), an MS sample prep module, AQUA peptide standards, and a data analysis template. IP-MS methods were utilized to determine how various targets change expression in response to hIGF-1 in two different cancer cell lines. Multiple total and phosphorylated targets from AKT/mTOR, TP53 and RAS signaling pathways were analyzed using these optimized IP-MS workflows. These data were then compared to the current semi-quantitative western blot procedure.

61. FUNDA M KAR

Post-translational Modifications during Meiosis

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Meiosis produces four haploid gametes from a single diploid cell and is the fundamental basis for sexual reproduction which is conserved from yeast to human. During meiosis, events leading to correct segregation of chromosomes into gametes require strict regulation. Inability to coordinate these events or the checkpoint activities may result in formation of defective gametes. Post-translational modifications (PTMs) control protein activity, stability, localization, and interaction partners in most cellular processes, including meiosis. Several examples for modified key meiosis regulators are known. To better understand the global impact of PTMs on coordination of meiosis, we will conduct a comprehensive mass spectrometry study mapping several PTMs and time points. We will use budding yeast for these studies as it can be synchronized and several relevant mutant strains exist. We will discuss first insights we gained from analysis of this PTM crosstalk and their possible temporal and causal relationships.

62. WILLIAM KASBERG

Activation of oncogenic signaling by TFG fusion proteins in thyroid cancer

The metazoan secretory pathway involves packaging of cargoes into COPII-coated transport carriers, which emerge from sites on the endoplasmic reticulum (ER) and eventually fuse with ER-Golgi intermediate compartments (ERGIC). Trk-fused gene (TFG) has been identified as a key regulator of COPII carrier trafficking at the ER/ERGIC interface, regulating integrity of the COPII coat and preventing isotropic diffusion of transport carriers. TFG has also been implicated in cell transformation, as a fusion partner with several receptor tyrosine kinases following chromosomal translocation events. However, the mechanisms by which these fusion proteins lead to cancer remain unclear. Global phosphoproteomics will be performed using 8505c thyroid cells that have been engineered to inducibly express either the active or kinase dead variant of the TFG-NTRK1 fusion, which has previously been implicated in thyroid cancer. These studies will define substrates of the fusion protein and identify the key signaling pathways activated when an oncogenic kinase is directed into the early secretory pathway to modulate protein secretion and potentially enhance tumor microenvironment. We previously showed that expression of TFG-NTRK1 was sufficient to promote cell transformation in vitro. This ability requires the fusion protein to accumulate at the ER/ERGIC interface, suggesting that targets in the early secretory pathway are phosphorylated to facilitate oncogenic growth. These could include components of the machinery responsible for producing COPII transport carriers and/or cargoes that are prematurely activated in the early secretory pathway and promote oncogenic signaling. We hypothesize that modulation of the early secretory pathway plays a key role in promoting establishment of a tumor microenvironment. We will leverage our ability to inducibly express TFG-NTRK1 for various lengths of time to elucidate the direct and indirect targets of the oncogenic fusion protein that enable cell transformation. Ultimately, defining these targets should highlight key factors that promote oncogenesis, which may have therapeutic value for many types of cancer. The mechanism by which a secretory pathway component contributes to oncogenesis has never been described.

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63. ERIKA KELMER

Comprehensive characterization of protein abundance, stability and aggregation during vertebrate brain aging

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A large number of studies have investigated the effects of aging on transcript regulation, but how these changes translate into protein levels is unclear. We combined high-resolution mass spectrometry-based proteomics with RNA-seq and a novel approach for high-throughput measurement of protein stability to characterize proteome dynamics during brain aging of *N. furzeri.*

To study total proteome changes, we compared the profiles of young (5 weeks), adult (12 weeks) and geriatric (39 weeks) fish of a longer-lived strain, in two TMT10 experiments. RNA-seq data from the same samples were also obtained. To evaluate age-dependent changes in protein stability, we employed the thermal proteome profiling (TPP) protocol both in *N. furzeri* and mice brain. To evaluate protein biophysical properties we used *in silico* methods. In addition, we investigated aggregation propensity *in vivo*, by analyzing SDS insoluble protein aggregates of young and geriatric brains. The main results of this study are:

1. Proteins coded by long transcripts are preferentially down-regulated;

2. Correlation between protein abundance and transcript level is reduced during aging (decoupling) and depends on transcript length;

3. There are many cases of discordant regulation between transcript and protein (i.e. RNA up-regulated and protein down-regulated or vice versa). These proteins are highly enriched for functions related to biosynthesis of macromolecules, such as RNA processing and translation;

4. Several protein complexes, such as the ribosome, lose their stoichiometry during aging, with some subunits being up-regulated and others down-regulated;

5. Increased protein aggregation in aging could be shown by biochemical methods;

6. We could correlate enrichment in protein aggregates with experimentally-measured thermal stability, and computationally inferred solubility, disorder and chaperone requirement;

7. Ribosomal proteins have very low thermal stability and their presence in aggregates could be confirmed by immunostainings.

These networks are likely to have a central role in the decline of cognitive function and neurodegenerative processes during aging.

64. KYUNGMIN (ALYSSA) KIM

The effect of soil pore size distribution on plant root decomposition and N_2O emission from decomposing plant roots

Kyungmin Kim and Alexandra Kravchenko

Soil pore size distribution is known to influence diverse soil processes (e.g., decomposition) associated with presence of incorporated plant residues. It was reported that decomposition of plant leaves is faster when the leaves are surrounded by large (>30 μm) soil pores rather than small (<10 μm) soil pores (Negassa et al., 2015), and the amounts of N_2O emitted from soil with incorporated leaves is higher in presence of such pores (Kravchenko et al., 2017). One of the mechanisms by which decomposing leaf residues interact with soil pore characteristics, subsequently enhancing decomposition and N_2O emissions, is the '*sponge effect*' - greater water absorption by the residue from the surrounding soil, especially when it is dominated by large pores. However, while the sponge effect by incorporated aboveground plant residues has been well investigated, that by belowground plant residues (i.e., plant roots) has not been explored yet. Thus, in this study, whether the presence of large soil pores plays a similar role in affecting decomposition of dead plant roots and N_2O emissions from them as that observed for plant leaves, was investigated.

For the study, two contrasting soils were prepared, with dominance of >30 μm pores and <10 μm pores. Young soybean plants were grown for one week in microcosms constructed from these materials. The plants were cut, air-dried and subjected to X-ray computed micro-tomography (μCT) scanning at 5 μm resolution. Then the microcosms were brought to two contrasting soil moisture conditions, namely 50% and 75% of water filled pore space (WFPS), incubated for 21 days, and subjected to the second μCT scanning. The images obtained from μCT was used to measure the root decomposition and attached minerals. CO_2 and N_2O measurements were collected from the microcosms on a regular basis during the incubations.

Preliminary results indicate that at 50% WFPS, the condition similar to which used in our past experiments (45% WFPS) with leaf microcosms, greater N_2O emissions were observed in microcosms with large than with small pores. This finding suggests that effect of large pores on N_2O emission related processes is ubiquitous, similarly affecting decomposing roots as well as leaves. Root μCT images from before and after the incubation scans also supported the result, showing greater decomposition in large-pore soil compared to small-pore soil at 50% WFPS. However, at 75% WFPS, the result of N_2O emission showed opposite tendency; small-pore soil released a lot more N_2O, especially in the initial stage. On the contrary, root μCT images showed no significant difference of decomposition between soil pore sizes. Discrepancy of N_2O emission and decomposition can be explained by considering microbial process. It is possible that large-pore soil has greater sponge effect as same in the microcosm with lower WFPS, but greater synthesis of microbial enzymes reducing N_2O into N_2 could have led the lower release of N_2O rather than small-pore soil. Supplementary experiment for microbial activity is being planned to test this hypothesis.

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65. ANNA KISIALA

Targeted PRM approach and High Resolution Accurate Mass Metabolite Profiling for Detection of Wide Spectrum of Plant Hormones Cytokinins (max 20 words)

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Introduction: Phytohormone profiling requires highly sensitive detection tools as these compounds are often present in trace levels and sample matrix complicates their precise quantification in biological materials. Classic methods for cytokinin (CK) detection use MRM-based, targeted quantitative analysis performed on triple-quadrupole mass spectrometers. We developed a new method for plant CK analysis using parallel reaction monitoring (PRM) combined with sample scanning for a range of molecular masses on a high resolution accurate mass (HRAM) quadrupole-Orbitrap mass spectrometer. The PRM technique has several potential advantages over the traditional MRM approach. Our method makes use of the PRM to monitor all product ions of a mass-selected cytokinin coupled with full mass range Orbitrap analysis to increase specificity and facilitate identification of other metabolites.

Methods: CKs were purified from *Arabidopsis thaliana* leaf tissue. The HPLC-MS/MS assay was conducted using an Ultimate 3000 LC equipped with Kinetex reversed-phase C18 column. The separated CKs were monitored using a Q Exactive mass spectrometer equipped with a HESI source optimized for small molecules. A full MS scan was acquired in the mass range of 50-550 m/z at a resolution of 35,000, with an AGC target of 1×10^6 and injection time of 128 ms. For PRM measurements, the Q Exactive was operated with a resolution of 17,000, AGC of 1×10^6 and max injection time of 64 ms. Data analysis was performed using Xcalibur 3.0 software. Labelled CK standards were used to verify transitions, determine retention times and quantify hormone levels.

Results: The results obtained from phytohormone analysis in the leaves of *A. thaliana* by the developed PRM method indicate, that Q Exactive mass spectrometer has high sensitivity, and specificity for accurate quantification of over 30 different types of naturally occurring CKs. Furthermore, we applied a new method to effectively profile CKs in various tissues of different plant species, as well as in microorganisms and nematode cultures. Up to date results suggest that the precision of concurrent PRM measurement of multiple compounds is comparable with the results provided by MRM experiments.

Novel aspect: The presented work is the first method that uses Orbitrap in PRM mode combined with a full MS scan for precise profiling of phytohormones cytokinins.

66. SAMANTHA KNOTT

Top-Down Proteomics of the Breast Cancer Proteome Enabled by Multi-dimensional Liquid Chromatography

Samantha Knott[1], Trisha Tucholski[1], David Inman[2], Suzanne Ponik[2], Ying Ge[1, 2, 3]

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Top-down mass spectrometry (MS)-based proteomics is a powerful tool for the comprehensive analysis of combinatorial post-translational modifications (PTMs) together with amino acid sequence variants in complex mixtures. Operated in discovery mode, top-down proteomics provides insight into unknown proteoforms or PTM changes that can be implicated in disease mechanisms and serve as biomarker candidates. Development of robust and reproducible top-down proteomic analysis of tumor tissue is urgently needed for mapping of the cancer proteome and understanding molecular mechanisms in cancer. However, methods for protein extraction from tumor tissue and the subsequent sub-fractionation of intact proteins are underdeveloped. Herein, we first established an extraction and online 1D RPC/MS top-down proteomics and MS/MS method to yield important identification of proteins relevant to studying cancer progression, including histone proteoforms. However, only the small (<30 kDa) and most abundant proteins were detected and identified- calling for further fractionation of the tissue lysates. To alleviate this detection challenge, we developed and utilized a multi-dimensional liquid chromatography (MDLC) top-down proteomics strategy utilizing size-exclusion chromatography (SEC) fractionation prior to reverse-phase chromatography (RPC) to increase the coverage, especially for those > 50 kDa. Overall utilizing the separation provided by 2D SEC-RPC/MS, we saw an increase in the detection of proteoforms including high molecular weight (MW) species due to reduced co-elution with small and high-abundance proteins. Overall performance coverage showed the number of unique proteoforms detected between 5-50 kDa using 1D RP-LC/MS (791 proteoforms) and 2D SEC-RP-LC/MS (4,137 proteoforms). This MDLC approach provided a much more comprehensive view of the cancer proteome and is a highly desirable top-down proteomics platform with the potential to provide access to potential biomarkers and species of interest for analysis that may not be otherwise accessible.

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67. SLAVA KUZNETSOV

Analysis of substrate specificity and cellular function of Sirt7 protein de-acetylase.

Introduction: Sirtuins constitute a family of 7 protein de-acetylases with remarkably diverse cellular localization, substrate specificity, and functions. Sirt6 and Sirt7 are two nuclear de-acetylases associated with both normal and pathological epigenetic events. While Sirt6 has been extensively characterized both *in vivo* and *in vitro* the true function of Sirt7 remains largely unknown. *E.coli* recombinant Sirt7 is inactive against recombinant acetylated histones, and synthetic peptides suggesting its functional activity involves more physiologically relevant substrates (acetylated chromatin) or additional interaction partners.

Preliminary data: Our preliminary data suggest that both enzymes strongly associate with DNA, RNA, and nucleosomes. However, our data disproves previous claims that association with nucleic acids can stimulate Sirt7 activity. We developed and validated a new Sirt7 expression system based on Sf9 baculovirus infection. Our kinetic data with purified recombinant Sirt7 WT and its C- and N- terminal truncations revealed it's active against synthetic histone peptides with a strong for long- chain de-acylation over de-acetylation.

Methods: Sirt7 functional role will be probed in vitro and in vivo using mass spectrometry as a primary method. Native chromatin will be purified from SAHA-treated MCF 7 cell using our newly developed chromatographic approach. Purified chromatin fragments will be used as a substrate for recombinant Sirt7 *in vitro*. LC-MS/MS analysis of Sirt7-treated chromatin will reveal its natural histone substrate specificity.

CRISPR-Cas9 system will be used to create several Sirt7 knockout mammalian cell lines. HA- tagged Sirt7-WT and its catalytically inactive mutants will be re-introduced under the tight control of TET promoter using lantiviral vector system. Using LC-MS/MS a global and nuclear acetylome will be analyzed under physiological level of Sirt7 expression using catalytically inactive enzyme mutant for control.

Using HA-specific antibody selective pull-down will be used to identify Sirt7 interactome and potential activating partners. Whole cell lysate will be used after DNAse and RNAse treatment, and MS/MS analysis of purified proteins will be conducted.

Novel aspect: Newly identified physiological Sirt7 substrates and interaction partners will provide an invaluable information regarding it's natural functions. The subsequent *in vitro* kinetic analysis will provide important mechanistic details underlying its activation that will allow for better understanding of sirtuins as a family.

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68. CHARIS LAM

Digital microfluidics coupled to liquid chromatography/tandem mass spectrometry as a tool for metabolite quantitation in human tissue samples

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Core needle biopsy (CNB) improves upon conventional surgical biopsy by reducing invasiveness. However, the clinical advantages come with increased analytical challenges. The small sample size—only a few milligrams—raises technical difficulties during sample handling and preparation.

We have previously shown that digital microfluidics (DMF) provides a semi-automated platform for metabolite extraction from CNB samples and for subsequent extract clean-up. DMF moves microlitre-sized droplets of liquid across an array of hydrophically-coated electrodes using electrodynamic forces. It reduces direct operator handling during sample preparation, while providing comparable analyte recovery to macroscale methods.

Here, we apply DMF tissue handling to a new biological problem—investigating markers of fertility in endometrial tissue—further demonstrating its utility for the measurement of scarce biological samples. We measured nine hormones in endometrial biopsy samples from women about to undergo in vitro fertilization, and found low variability in hormone concentrations across single biopsy samples.

69. CHRIS LAWSON

Carbon metabolism of versatile anaerobic ammonium-oxidizing bacteria resolved by isotope labeling and systems-level metabolic flux analysis

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Anaerobic ammonium-oxidizing (anammox) bacteria play a central role in global nitrogen cycling and mediate energy-efficient wastewater treatment processes for autotrophic nitrogen removal. While significant progress has been made on understanding the energy metabolism of anammox bacteria, limited physiological studies have been done to confirm their central metabolism beyond genomic predictions. Here, we employ a systems biology approach to resolve the metabolic network of the anammox bacterium *Candidatus* Kuenenia stuttgartiensis using stable isotopes combined with quantitative metabolomics and metabolic network modeling. ^{13}C-labeled bicarbonate was fed to planktonic cultures of *Candidatus* Kuenenia stuttgartiensis growing in membrane bioreactors, followed by rapid metabolite quenching and extraction over a time course of three hours. Samples were analyzed via high-resolution liquid chromatography mass spectrometry (LC-MS) to follow ^{13}C incorporation into the metabolome. Metabolite labeling patterns were used to resolve the metabolic network structure of *Ca.* K. stuttgartiensis and compute intracellular fluxes via isotopic non-stationary metabolic flux analysis. In-vivo fluxes and absolute metabolite levels were used to calculate pathway thermodynamics, and were combined with transcriptomic data to refine a genome-scale metabolic reconstruction of *Ca.* K. stuttgartiensis. Our findings reveal novel deviations in the central metabolism of anammox bacteria from what was predicted based on genomics alone and represents a platform for incorporating quantitative systems biology into environmental biotechnology and biogeochemistry.

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70. ALEXIS LAWTON

Defining the Mechanisms of Rapid Changes in Protein Acetylation in Response to Cell Stimulation

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Introduction: Acetylation of the ε-amino of lysine residues is a widespread, reversible post-translational modification that regulates many cellular functions, including protein-protein interactions, protein-DNA interactions, cellular localization, protein stability, and enzymatic activity. While the first identification of acetylation was found on histone tails, previous proteomics analyses have identified thousands of acetylation sites on non-histone proteins. In order to answer the question of which acetylation sites are functionally relevant, our lab has developed a complementary proteomics method to calculate global and site-specific acetylation stoichiometry. Using the acetylation stoichiometry analyses, the goal of this study is to understand acetylation dynamics in serum stimulated MCF7 cells and to define the cellular mechanisms involved in regulating the unique responses and rapid changes in protein acetylation.

Methods: Cell lysates were denatured, alkylated, chemically acetylated using isotopic D_6-acetic anhydride, and digested with Trypsin and GluC. Peptides are pre-fractionated using high pH reverse phase (HPRP) chromatography, analyzed in data independent acquisition (DIA) mode using a Q-Exactive, and processed using Spectronaut™ v11. A spectral library is generated from a ^{12}C-acetic anhydride labeled sample and analyzed in data-dependent acquisition (DDA) mode. The spectral library is generated in MaxQuant, and inflated *in silico* to contain both light and heavy peptides. Acetylation stoichiometry are calculated for unique acetylation sites by matching light and heavy fragment ion pairs and dividing the light fragment ion peak area by the total (light plus heavy) fragment ion peak area, with a correction for isotopic purity.

Preliminary Data: Using this method, we analyzed acetylation stoichiometry dynamics over time in response to serum stimulation. MCF7 cells were synchronized by serum depletion for 24 hours before adding back serum and harvesting cells after 0 (serum-free), 1, 2, and 4 hours. We calculated acetylation stoichiometry on nearly 3000 unique lysine sites and over 1200 proteins. Additionally, we had robust coverage across all four time points, including nearly 2000 unique lysine sites on over 900 proteins. Pattern recognition analysis using fuzzy c-means clustering revealed several clusters representing distinct,

dynamic acetylation responses. Functional annotation of each cluster revealed overlapping and unique pathways and biological processes. For example, the largest cluster (160 unique lysine sites), which included sites that rapidly increased and maintained after the first hour, was enriched for proteins involved in translation, splicing, and protein folding.

Novel Aspect: Cell stimulation induces rapid changes in protein acetylation stoichiometry, revealing distinct responses in major cellular pathways.

71. JOSH LENSMIRE

Examination of sulfur catabolism by the antibiotic resistant pathogen, *Staphylococcus aureus*

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Antibiotic resistant pathogens cause considerable mortality and represent a significant threat to human health. Treating these infections is exceedingly challenging, necessitating the development of new therapeutic strategies that impair bacterial growth within host tissues. One potential avenue for therapeutic intervention is targeting how pathogens obtain nutrients during infection. *Staphylococcus aureus* is an important human pathogen due to its ability to cause infective endocarditis, and its evolving antibiotic resistance strategies. During infection *S. aureus* must acquire nutrients from host reservoirs. This work focuses on *S. aureus* catabolism of oxidized and reduced glutathione (GSH/GSSG), important organosulfur molecules for eukaryotic cell homeostasis, as a sulfur source. We hypothesize *S. aureus* imports and catabolizes organosulfur compounds from host reservoirs during infection to proliferate in the host.

Numerous methods were devised to start to test this hypothesis. For determination of GSSG and GSH levels, metabolites were extracted from mid-exponential phase cultures of *S. aureus* grown in a chemically defined medium with either cystine, GSSG, or GSH added. Metabolites were extracted with methanol:water mix (80:20) and analyzed by LC/MS/MS. Growth curves were performed in a chemically defined medium with the base medium containing methionine and sulfate, compounds *S. aureus* cannot utilize as sulfur sources. Growth on potential sulfur sources was tested by adding potential sulfur sources to the base chemically defined medium and examining growth.

A metabolomics examination of *S. aureus* grown in the chemically defined medium with GSSG, GSH, and cystine as sulfur sources, found intracellular levels of GSSG and GSH in *S. aureus*, compounds *S. aureus* lacks the ability to synthesize, when grown in a medium containing GSH and GSSG. *Since S. aureus* lacks the ability to utilized sulfate, we hypothesized *S. aureus* is importing and catabolizing GSSG and GSH to use as a sulfur source. Previously, GSSG was not known to serve as a sulfur source for *S. aureus*, and as a consequence no transporters of GSSG in *S. aureus* have been elucidated. To examine import of GSSG by *S. aureus* a screen utilizing a transposon mutant library was with GSSG as the sole utilizable sulfur source present was devised. This screen yielded transposon mutants in an ABC transporter operon *gisABCD* and a γ-glutamyl transpeptidase. This operon consists of an ATPase, two permease proteins, a lipoprotein, and a γ-glutamyl transpeptidase, an enzyme known to cleave the γ-peptide bond present in GSSG and GSH. Upon further screening the *gisABCD* operon displayed reduced growth on both GSSG and GSH as sulfur sources. Notably, these mutants can proliferate with cysteine. Future directions will further enhance our knowledge of the import mechanisms GSSG, and GSH specifically examining localization of Ggt and determination of the mechanism in which *S. aureus* catabolizes GSSG and GSH Additionally, future directions will explore the *S. aureus* metabolome grown on different sulfur sources to determine the effects of sulfur source on downstream metabolism and physiology. This research is novel because it represents the unexplored area of sulfur metabolite transfer and utilization at the host *S. aureus* interface.

72. FURONG LIU

Molecular characterization of rice immune signaling mediated by pattern recognition receptors that confer resistance to Xanthomonas oryzae pv. Oryzae

In plant cells, receptors localized on the cell membrane activate the immune responses upon recognition of extracellular signals derived from pathogens. The rice XA21 and XA3 receptor kinases confer robust resistance to the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the causal agent of rice bacterial blight disease. I characterized both plant and bacterial genetic factors that are important for the rice response to *Xoo.* On the plant side, there must be genes encoding factors responsible recognition of the microbial molecules, transmission of the signal, and proper regulation of the immune response. On the bacteria side, genes encoding the machinery for synthesis and regulation of a microbial molecule required for activation of immunity must be present. Here, I report the identification of the tyrosine-sulfated protein RaxX, required for activation

of XA21-mediated immunity x, and we show that RaxX is sulfated by the tyrosine sulfotransferase RaxST. I also describe the characterization of *Xoo* field isolates that overcome XA21-mediated immunity, and I identify the genetic basis for their virulence on XA21 rice. I also carried out experiments to identify components involved in XA21- and XA3-mediated immunity. Using the model rice cultivar Kitaake, I assessed whether genes known to act in XA21-mediated immunity are also involved in XA3-mediated immunity. The results demonstrated that XA21 and XA3 have both shared and distinct components in their signaling pathways. In order to identify novel signaling components, I characterized a rice mutant line which shows suppression of XA21-mediated immunity (*sxi4*). Genetic analysis and a whole genome re-sequencing approach were used to identify the causative mutation(s) of *sxi4* as a translocation which results in overexpression of the Dicer-like genes *OsDCL2a*.

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73. PAUL LIZHNYAK

Traumatic brain injury proteomics guides novel KCC2-targeted therapy

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There remains a need for more effective therapeutics in the treatment of traumatic brain injury (TBI). This study tested whether post-translational effects of TBI revealed by temporal proteomics would serve as an effective guide for TBI interventions. Of interest were delayed-onset processes that could be managed after patient stabilization on the intensive care unit. Self-organizing map analysis was applied to the post-TBI temporal proteome, from which we focused on a cohort of proteins involved in ionic dysregulation that exhibited a decrease beginning one day following injury. From this map, we identified neuron-specific K+-Cl cotransporter 2 (KCC2) as an amiable target, an essential component for maintaining chloride homeostasis that is critical to inhibitory neurotransmission. The proteomic results further defined a potential therapeutic window of opportunity around phosphorylation events preceding the escalated functional loss of KCC2. Testing this window, we administered the KCC2-targeting compound CLP290 (50 mg/kg, p.o.) before, at, and after the identified KCC2 post-translational processing. The intervention was most effective at the predicted 1-day time point in preserving plasmalemmal KCC2 within perilesional somatosensory neocortex. Results from a pentylenetetrazole assay validated restored chloride homeostasis and inhibitory function with CLP290 therapy. Furthermore, treatment significantly improved functional recovery of the injured somatosensory cortex on rotarod and whisker adhesive removal task assessments. Study findings demonstrate how temporal proteomic profiling may guide therapeutic development through identifying novel targets and defining windows of intervention. Furthermore KCC2 represents a promising target in TBI and potentially other neurological insults known to involve chloride dysregulation such as epilepsy and stroke.

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74. YOU LU

Identification of candidate components in protein complexes with Arabidopsis transcription factors CBP60g and SARD1

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Plant immune responses activated through perception of microbe-associated molecular patterns (MAMPs), leading to pattern-triggered immunity (PTI), are highly effective for resistance against non-adapted pathogens. Responses during PTI are often tightly regulated, enabling both disease resistance and minimal impact on plant fitness. Two members of the Arabidopsis *CALMODULIN-BINDING PROTEIN 60* family transcription factors, *CBP60g* and *SARD1*, are crucial positive regulators of plant immunity through targeting the promoter regions of a suite of important immune-related genes. The transcription of *CBP60g* and *SARD1* are transiently induced by pathogens or MAMPs. However, how the activities of CBP60g and SARD1 are regulated at the post-translational stage is yet to be elucidated. We hypothesized that 1) the DNA-binding specificity CBP60g and SARD1 may be modulated through interacting with other transcription factors and co-activators; 2) the activity of CBP60g and SARD1 may be regulated by post-translational modifications (PTMs). To test these hypotheses, we have established Arabidopsis stable transgenic lines expressing HA-tagged *CBP60g* or *SARD1*. The transgenic plants are treated with water or flg22, a MAMP derived from bacterial flagellum, and samples are collected following a time course after treatment. The DNA-

binding specificity will be determined using Chromatin immunoprecipitation sequencing (ChIP-Seq) and transcription activity will be measured by RNA-Seq. Proteins co-immunoprecipitated with CBP60g and/or SARD1 will be identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS)

SARD1-HA are subject to ubiquitination-mediated degradation. Correlations between presence of certain candidate interacting proteins and changes in DNA-binding specificity and transcription activity will be used as foundation for further investigating molecular basis on modulation of CBP60g and SARD1 activity during plant immune signaling.

75. JESSICA LUKOWSKI

Multifaceted Imaging Approach for Liposomal Drug Delivery in Tumor Spheroids

Jessica K. Lukowski, William T. Andrews, Amanda B. Hummon

Introduction: This study aims to develop an approach to image liposomal drug delivery systems in a multifaceted way that would enable evaluation of the efficacy of chemotherapeutics, in tumor spheroids. Imaging liposomal bilayer distribution in our tumor spheroid model becomes problematic, as these lipids are also endogenous to our model system. To resolve this, we are modifying our liposomes by chemically cross-linking fluorescent tags to their outer lipid bilayer of the liposome using click chemistry. In doing so, we hope to be able to observe differences in localization between our lipid bilayer and free drug over time, providing information on where the drug is delivered in our model system and how the drug distributes throughout treatment using fluorescence microscopy and MALDI-MSI.

Methods: Liposomes were created through extrusion and composed of phosphatidylcholine (PC), hexynoyl phosphatidylethanolamine (HPE), phosphatidylethanolamine (PE), and cholesterol at a 80:10:5:5 ratio respectively to a final lipid concentration of 1mM in solution. Doxorubicin, 400 μM, was added to solution and becomes encapsulated in the hydrophilic core of the liposome during extrusion. Click chemistry reaction was then performed and liposomes were dialyzed overnight. HCT 116 tumor spheroids were then dosed with either liposomes containing doxorubicin, liposomes containing doxorubicin and underwent the click chemistry reaction, empty liposome that underwent the click chemistry reaction, free drug, or no treatment (control) for 24, 48, and 72 hours before being embedded in gelatin and cryosectioned for imaging experiments.

Preliminary Results: In this study a multifaceted imaging approach using click chemistry allowed for the successful detection of entrapped chemotherapeutic and liposome bilayer using both fluorescence microscopy and MALDI-MSI methodologies. The click chemistry liposome system was first tested in traditional two-dimensional cell culture to verify the success of the click reaction between the fluorophore and HPE and to ensure limited signal in the doxorubicin channel using fluorescence microscopy.

This study aims to develop an approach to image liposomal drug delivery systems in a multifaceted way that would enable evaluation of the efficacy of chemotherapeutics, in tumor spheroids. Imaging liposomal bilayer distribution in our tumor spheroid model becomes problematic, as these lipids are also endogenous to our model system. To resolve this, we are modifying our liposomes by chemically cross-linking fluorescent tags to their outer lipid bilayer of the liposome using click chemistry. In doing so, we hope to be able to observe differences in localization between our lipid bilayer and free drug over time, providing information on where the drug is delivered in our model system and how the drug distributes throughout treatment using fluorescence microscopy and MALDI-MSI. Alternating 16 μm slices of the dosed spheroids were collected during cryosectioning so a comprehensive picture of drug penetration and liposome bilayer fate could be generated.

Tumor spheroids were dosed with liposomes containing doxorubicin, liposomes containing doxorubicin and underwent the click chemistry reaction, empty liposome that underwent the click chemistry reaction, free drug, or no treatment so the appearance or absence of specific peaks in MALDI-MSI allows for the determination of the liposome bilayer fate because of the mass shift that the fluorophore creates. Further efforts will focus on the localization of doxorubicin metabolites and the elucidation of release mechanism of the liposome.

Novel aspect: This new methodology allows for multifaceted imaging of not only the entrapped chemotherapeutic but also the liposome bilayer

76. PETER LUONG

Identification of key factors modulated in Hereditary Spastic Paraplegia rat model expressing TFG (p.R106C) mutation

Hereditary spastic paraplegias (HSPs) are a group of gait disorders characterized by progressive loss of voluntary movement in the lower extremities. HSPs can be defined as either complicated or pure. Complicated HSPs are associated with additional symptoms such as ataxia, neuropathy, and optical atrophy. In this project, we focus on one type of complicated HSP that is caused by a single point mutation (p.R106C) in Trk-Fused Gene (TFG). Although previous work has demonstrated that TFG plays a key role in secretory protein trafficking from the endoplasmic reticulum (ER), the mechanisms by which the TFG (p.R106C) mutation causes HSP in patients remains unclear. Through CRISPR-mediated genome editing, we developed a rodent model of HSP harboring the TFG (p.R106C) mutation. Mutant animals are born normally but exhibit progressive hind limb spasticity and ataxia starting at less than 5 weeks of age. Using brain tissue harvested from control and mutant animals at various timepoints during disease progression, we will conduct a series of mass spectrometry studies to identify lipids, peptides, and metabolites that differ significantly when the TFG (p.R106C) mutation is present. These studies will determine the impact of the TFG (p.R106C) mutation to brain physiology, which may highlight potential therapeutic targets for disease intervention. Our previous findings have demonstrated a key role for TFG in regulating cargo transport in the early secretory pathway. In particular, TFG functions at the interface between the endoplasmic reticulum (ER) and the ER-Golgi intermediate compartment (ERGIC), regulating the anterograde movement of COPII-coated transport carriers. Since our discovery, several studies have been published that implicate TFG in neurological diseases. In particular, the TFG (p.R106C) mutation has been identified in several patients that suffer from hereditary spastic paraplegia (HSP). In preliminary studies, we have developed human induced pluripotent stem cells and rats through CRISPR-mediated genome editing to introduce the TFG (p.R106C) mutation. Rats expressing only TFG (p.R106C) are born in normal Mendelian ratios and exhibit no motor/gait defects during early development. However, between 4 and 5 weeks of age, we observe the initial signs of gait dysfunction, as measured using a computer-aided kinematic analysis system. The impairment worsens with age, and by 4 months, animals exhibit hind limb paralysis. In parallel, we have found that neurons differentiated from iPSCs harboring the TFG (p.R106C) mutation exhibit pathfinding defects and a loss of axon bundling. We hypothesize that these deficits arise from reduced secretory efflux from the ER in the long motor neurons that emanate from the cortex. To explore this idea, we have initiated a collaboration with the Coon laboratory at UW–Madison. Using brain tissue from control and mutant animals at 15 weeks of age, we have conducted a pilot study and identified 979 proteins that are differentially expressed in our mutant rats. A combination of bioinformatics tools are now being used to determine whether specific classes of proteins are preferentially affected. For the first time, we expect to identify key factors (lipids, proteins, and/or metabolites) that are modulated in HSP

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77. LUIS MACIAS

Analysis of chemically crosslinked proteins by ultraviolet photodissociation

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Introduction: By covalently linking interacting residues, chemical crosslinking of proteins allows for the identification of transient interactions and conformations that are otherwise difficult to characterize. After crosslinking and proteolysis, MS/MS strategies are employed for characterization of the crosslinked peptides. While collisional activated dissociation (CAD) is the standard activation method in crosslink analysis, identification can be impeded by insufficient fragmentation of often large crosslinked products. Ultraviolet photodissociation (UVPD) is an alternative activation method useful for the characterization of modified peptides and often provides more extensive sequence coverage than CAD for large peptides. This study focuses on evaluating UVPD as a complementary dissociation technique to enhance identification of chemically crosslinked residues within proteins.

Methods: A series of proteins (myoglobin, serum albumin, aprotinin) were chemically crosslinked by four crosslinkers (BS3, DSSO, DC4, DSP) at experimentally determined optimized conditions. The cysteine residues on the crosslinked proteins were carbamidomethylated before purifying the proteins via molecular weight cutoff filters (3 or 10 kDa) and digesting with trypsin. 50 ng of digested protein was separated on a nano-LC system using 30 cm column packed with C18 resin coupled to a Thermo Scientific Instruments Fusion Lumos Orbitrap mass spectrometer equipped with an excimer laser for UVPD. MS1 spectra were collected at 60,000 resolving power and MS2 spectra at 30,000 resolving power in data dependent mode. Activation energies were optimized for each system. Data were analyzed using Kojak, StavroX, or MeroX.

Preliminary Results: BS3 is a conventional bis-succinimidyl amine-to-amine crosslinker with an 8-carbon spacer arm. In contrast, DSSO, DC4 and DSP are cleavable crosslinkers designed to incorporate one or more bonds that are labile to CAD or a chemical agent, thus facilitating MS3 strategies in which the linked peptides are separated in the first step and the released peptides are characterized in a second CAD step. Since absorption of 193 nm photons in UVPD is a higher energy activation method that accesses excited electronic states, the ability to cleave the crosslinked peptides and obtain extensive sequence information was evaluated in comparison to HCD. Upon crosslinking and trypsin proteolysis of aprotinin (6.5 kDa), peptides ranging from 1.9 kDa to 3.8 kDa were generated. Analysis of the resulting mixture by CID, HCD, and UVPD resulted in the identification of 4, 5, and 3 crosslinks, respectively, with 1 being uniquely identified by HCD. Crosslinking aprotinin with the cleavable crosslinker DSSO resulted in the identification of 6, 7, and 5 via CID, HCD, and UVPD, respectively with 1 being uniquely identified by HCD. The data also showed that UVPD is capable of cleaving DSSO (as CAD does) to produce the diagnostic doublets that aid in data analysis and in MS3 strategies. Analysis of the larger protein BSA, crosslinked by BS3, identified 36 and 32 crosslinks by HCD and UVPD, respectively. In this system, 9 crosslinks were unique to HCD and 5 to UVPD, offering evidence of complementary information. In all cases, both UVPD and HCD produced high sequence coverage across both peptides involved in a crosslink.

Novel Aspect: UVPD used for enhanced identification of chemical crosslinks to map protein interactions

78. NEHA MALHAN

Rapid Isolation of Native Ribosomes by Strong Anion Exchange Chromatography

Because of its essential role in the cell and principal structural differences between human and bacterial ribosomes, structural characterization and understanding of key regulatory mechanisms of the ribosome is critical to deciphering a wide variety of biological processes ranging from microbial dormancy to antibiotic resistance. Traditional methods for isolation of ribosomes, such as density gradient ultracentrifugation, are very long processes and hinder large scale, sensitive characterization of ribosomal proteins and the associated posttranslational modifications. In this poster I will describe how we have addressed the need for a rapid method for the isolation and characterization of ribosomes using strong anion exchange chromatography coupled with top-down proteomics.

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79. JOSHUA E. MAYFIELD

Identification and characterization of secretory pathway kinase substrates

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Introduction: Phosphorylation is a conserved post-translational modification that regulates nearly all-cellular processes. Current knowledge of phosphorylation centers on phosphorylated proteins within cells. Secreted proteins are also phosphorylated, however the lack of identified kinases hampers our investigation of its importance. Recently, our lab and others identified a suite of kinases that phosphorylate substrates in the secretory pathway. These include the protein kinase Fam20C, which phosphorylates the majority of the phospho-secretome in several cell types; Fam20B, a sugar kinase; and Fam20A, a pseudo-kinase. Other kinases include Fam69A-B, DIA1, and Fam198A-B. Mass spectrometric identification of substrates and functional characterization of these will increase our understanding of phosphorylation in the extracellular space and shed light on this relatively unexplored facet of biology.

Methods: Knock-out strains of cell lines and mice will be generated utilizing CRISPR genome editing or Cre-Lox recombination. These strains will provide material for mass spectrometry analysis. Mass spectrometry will be utilized to identify differentially phosphorylated proteins between treatment groups. These initial hits will be verified using *in vitro* kinase assays, cell biology and *in vivo* experiments. The functional consequences of these modifications will be analyzed at the level of protein structure, molecular interactions, signaling networks, and cellular/organismal phenotypes.

Preliminary Data: We have published phospho-proteomic analysis of proteins secreted into media for a variety of human cell-lines with or without Fam20C including HepG2 (liver), MDA- MB-231 (breast epithelial), and U2OS (osteoblast-like)

cells. Currently, we are characterizing identified substrates in *vitro* and *in vivo*. However, Fam20C and several other secretory kinases are expressed across cell types and their substrate repertoire is highly cell-type dependent. Our identified substrates likely only represent a fraction of physiological substrates. Furthermore, the majority of our knowledge only pertains to proteins that are secreted. Secretory kinases interact with proteins retained in the Golgi and Endoplasmic Reticulum, and embedded into the cell membrane. To better understand the role of secretory kinases, with an initial focus on Fam20C, across tissues and in different subcellular locations we have generated mouse models lacking these kinases and used subcellular fractionation strategies to enrich for proteins sent through the secretory pathway to different destinations. Whole body and tissue specific knock-outs of some of these kinases have been generated. Those of specific interest include nervous system, macrophage, and cardiac specific knock-outs of Fam20C and whole body knockouts of Fam69C. These tools will allow us to identify substrates and discover novel proteins in different *in vivo* contexts.

An example of initial progress made using these tools is the use of Fam20C knock-out mice to generate mouse embryonic fibroblasts (MEFs). Characterization of their secreted phospho-proteome revealed 124 differently phosphorylated proteins compared to wild-type MEFs. Of these, 36 represented novel phospho-sites not previously identified on proteins involved in processes ranging from induction of cellular senescence to collagen biosynthesis. These proteins are currently under investigation and these large portions of novel substrates exemplify the importance of investigating secretory kinase function across cell types.

Novel Aspect: Secretory pathway kinases are poorly understood. Characterizing their substrates will provide a molecular framework to understand the extracellular environment.

80. ELI MCCOOL

Deep top-down proteomics using capillary zone electrophoresis-tandem mass spectrometry: identification of 5 700 proteoforms from the *Escherichia coli* proteome

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Introduction: Capillary zone electrophoresis (CZE)-MS/MS has been recognized as a useful tool for top-down proteomics due to the high resolution of CZE for separation of intact proteins and the high sensitivity of CZE-MS/MS for detection of intact proteins. Furthermore, loading and peak capacities for CZE have recently been improved to ~1 µL and 280 respectively utilizing dynamic pH junction based sample stacking methods. CZE-MS/MS has been shown to efficiently separate and identify intact proteins in complex mixtures such as *Escherichia coli* lysates (~600 proteoform identifications in a single run). Additional separation dimensions in front of CZE-MS/MS including size exclusion chromatography (SEC) and reversed-phase liquid chromatography (RPLC) is proposed herein to improve peak capacity and thus the number of proteoform identifications.

Methods: SEC was performed on a column (4.6 x 300 mm) and HPLC system from Agilent. Five fractions were collected from 1 mg starting material (*E. coli*) and then separated by RPLC (C4, 2.1 x 250 mm, Sepax Technologies). All RPLC fractions (100 total, 5×20) were redissolved (5 µL 50 mM NH4HCO3, pH 8.0) for CZE-ESI-MS/MS. An automated CZE-ESI-MS system and a commercialized electro-kinetically pumped sheath flow interface (CMP Scientific) was coupled to a Q-Exactive HF mass spectrometer (Thermo Fisher Scientific). A 1-meter-long fused silica capillary (50 µm i.d., 360 µm o.d.) coated with linear polyacrylamide (LPA) was used with BGE (10% AA) and sheath buffer (0.2% FA, 10% MeOH). Separation and ESI voltage was 20 kV and 2-2.3 kV respectively.

Preliminary Data: The peak capacity of the SEC-RPLC-CZE-MS/MS platform was around 4 000 based on the full width at half maximum (FWHM) of protein peaks. We identified over 58 000 proteoform-spectrum matches (PrSMs), 5 705 proteoforms and 850 proteins from the *E. coli* proteome using spectrum-level FDR less than 1%. The dataset represents an order of magnitude improvement in the number of proteoform IDs compared with previous CZE-MS/MS studies (5 700 *vs.* 300-600 proteoforms). The dataset also represents the largest bacterial top-down proteomics dataset reported to date. More importantly, the performance of our CZE-MS/MS based platform is comparable to the state-of-the-art RPLC-MS/MS based system in terms of proteoform IDs. Cumulative proteoform IDs increased steadily with the increase in the number of RPLC fraction or SEC fraction, indicating the efficient pre-fractionation performance of SEC and RPLC. The majority of proteoforms had mass in a range of 10–20 kDa and 52 proteoforms with mass bigger than 30 kDa were identified, indicating the potential of the platform for top-down characterization of large proteins. Proteoforms per gene ranged from 1 to 345 and the detected mass errors from the identified proteoforms ranged from -600 Da to 600 Da, corresponding to various modifications. We observed good linear correlation between PrSMs and the abundance (ppm) of 20 randomly

selected proteins in a mass range of 6–20 kDa. We used the number of PrSMs to estimate the relative abundance of various proteoforms derived from a gene and the results highlight the capability of the CZE-MS/MS based top-down proteomics for accurate characterization of proteins in cells. We further compared the identified proteins (850) with the proteins in UniPort *E. coli* database (~4 000 proteins) in terms of the gene ontology (GO) information and SEC-RPLC-CZE-MS/MS platform had no obvious bias in protein ID with respect to the biological process and molecular function distributions.

Novel Aspect: Our SEC-RPLC-CZE-MS/MS platform has 2-5 times higher proteoform identifications than any other top-down LC-CZE-MS/MS system.

81. JUSTIN MCKETNEY

Proteomic Atlas of the Human Brain in Alzheimer's Disease

The brain represents one of the most divergent and critical organs in the human body, making up the chemical basis for our identity and personalities. Yet, it continues to be afflicted by a variety of neurodegenerative diseases specifically linked to aging, about which we lack a full biomolecular understanding of onset and progression, such as Alzheimer's Disease (AD). Here we provide a proteomic resource composed of 9 functionally distinct sections from 3 individuals clinically diagnosed with Alzheimer's, across a spectrum of disease progression. Using state-of-the-art mass spectrometry, we were able to identify a "core" brain proteome that exhibited only small variance in expression, accompanied by a group of proteins that were highly differentially expressed in individual sections and broader regions. AD affected tissue exhibited slightly elevated levels of tau protein with similar relative expression to factors known to be associated with the AD pathology. Significant differences were also identified between previous proteomic studies of mature adult brains and our aged cohort. This resource can serve not only as a snapshot of the brain proteome in AD but also as a point of reference for how specific regions of the brain are affected by aging, helping to expand our understanding of protein expression in the human brain.

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82. ANAIS MEZIANI

Exploring modulation of translation initiation factor complexes in Spinal Muscular Atrophy in mouse and human spinal cords by proteomic analysis

Introduction: Spinal Muscular Atrophy (SMA) is a neurodegenerative disease and the leading genetic cause of infant mortality. Degeneration targets motor neurons, leading to muscle atrophy. SMA is caused by mutation of the SMN1 gene, encoding SMN protein. SMN is implicated in several aspects of RNA metabolism including transport of RNA-protein complexes, snRNP assembly, and splicing. Our study focuses on potential involvement of SMN in another crucial process: translation initiation. Our previous proteo-transcriptomic analyses identified global dysregulation of translation in SMA spinal cords and significantly decreased abundance of translation-associated proteins, including the elongation factor EIF4G3.

Methods: In this study, we investigate EIF4G3 protein complexes during development in SMA mice and expand our study to include proteomic analysis of human spinal cords. Based on wild-type C57/Bl6 mice spinal cord (stages P6 and P10), we optimized a strategy to capture EIF4G3 in a whole mouse spinal cord with 6 mg conjugated beads (with 3 µl antibodies per mg of beads). Beads were conjugated separately, in batches of 2 mg of beads, and for each batch a different antibody was used. This protocol allows an efficient isolation of EIF4G3 and associated proteins, followed by enzymatic digestion with Trypsin and LC-MS/MS analysis on a QExactive mass spectrometry for identification. Interaction specificity was determined using the SAINT algorithm. In parallel, we performed a global proteomic analysis of human post-mortem spinal cord tissue from SMA patients and non-SMA controls using isobaric tagging methods (TMT).

Preliminary Data: After FASP preparation, we have detected EIF4G3 and EIF4G1 on the elution fractions (and not and the flow through or beads fractions), based on our searches on Mascot, we found those two proteins on the top- ranking with a mascot score over 2000. This shows that we are enriching our bait protein successfully. Moreover, we identified groups of proteins that belong to the ribosomal and spliceosome complex.

We performed a preliminary analysis of human spinal cord tissue from SMA patients (n=3) and control (non- SMA cause of death) patients (n=3). These human tissue samples correspond to a late stage of SMA (postmortem tissue represents the end-stage of SMA disease). We compared the results of the human tissue analysis with the results of the mouse tissue time-course analysis and identified conserved changes in regulation of translation initiation complex proteins. We found an up-regulation of the protein EIF1AD across the 3 SMA samples compared to control samples. EIF1AD is a subunit of the translation initiation

complex that is important for the early stage of the translation complex formation. Additional evidence for disrupted translation was found in the group of down-regulated proteins, where EIF2C3 was found to be decreased in abundance in SMA samples. EIF2C plays a role in RNA-mediated gene silencing (RNAi) and repression of mRNA translation. Furthermore, we identified up-regulate the protein Amyloid beta (A4) precursor-binding, family A, member 1 (APBA1). It may be possible that APBA1 is expressed in response to a cytotoxic synaptic environment in SMA, as it is in Alzheimer's disease. This protein prevents Aβ aggregation, by inhibiting of cleavage of its precursor (APP protein). Together, these analyses of both human SMA tissue and SMA mouse tissue shows evidence of altered expression of specific translation initiation factors.

Novel Aspect: This is the first comprehensive proteomic study of translation complex initiation dynamics in mouse and human SMA tissue.

83. GARRET MILLER

Purification and Functional Determination of Diterpene Synthases

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Diterpenes are an incredibly diverse class of molecules with a range of applications including use as pigments, fragrances, biofuels, and pharmaceuticals. Due to their structural complexity, diterpenes are often difficult to synthesize through traditional synthetic methods, which makes identification and optimization of biosynthetic methods an appealing area of research. Diterpene backbones are synthesized from geranylgeranyl diphosphate (GGPP) in a two-step conversion by diterpene synthases (diTPSs), first by class II diTPSs which form a cyclic intermediate, followed by further modification and removal of the diphosphate group by class I diTPSs. In the present study, class II and class I diTPSs from five different plant families were cloned, expressed in *E. coli*, and purified. Enzymes were incubated with GGPP in both class II assays and class II/I combination assays, and following hexane extraction, products were analyzed via GC-MS, resulting in the identification of a wide range of diterpenes. *In vitro* diTPS assays have the advantage of resulting in relatively pure products. It also doubles as a useful platform for unambiguous determination of diTPS function, without the possibility of non-diTPS related modifications to the diterpene skeleton due to endogenous machinery present in *in vivo* platforms. Furthermore, current work on diterpene production scale-up is ongoing in both *in vitro* and *E. coli* systems, which will provide useful methods for production of these compounds in sufficient quantities for further chemical characterization, as well as testing of potential anti-bacterial and anti-cancer activities.

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84. GUSTAVO MONNERAT

Modeling premature cardiac aging by induced pluripotent stem cell from a patient with Hutchinson-Gilford Progeria Syndrome

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Introduction: Hutchinson-Gilford Progeria Syndrome (HGPS) is an extremely rare genetic disorder characterized by accelerated aging and high incidence of cardiovascular complications. However, the underlying mechanisms of this syndrome are not fully understood.

Methods and Results: This study modelled HGPS using cardiomyocytes (CM) generated from induced pluripotent stem cell (iPS) derived from a patient affected by HGPS, characterizing the biophysical, morphological and molecular alterations found in these CM comparing with CM derived from a health donor. Electrophysiology recordings were performed with intracellular recording and the generated CM showed to be functional and the results demonstrates normal cardiac electrophysiology proprieties. Electron tomography and 3D reconstruction suggests structural abnormalities in the mitochondria of Progeria CM. High resolution respirometry performed with isolated CM derived from three distinct cellular differentiations suggests decreased oxygen consumption capacity in Progeria CM, however, none difference was observed in mitochondrial content quantified by Mitotraker. The telomere length was quantified by PCR and no difference was observed neither in the iPS and in the CM. The miR-15a was

found to be upregulated and miR-133 and miR-200a were downregulated in CM from Progeria. Proteomics analysis, performed with Liquid Chromatography tandem mass spectrometry (LC-MS/MS) in a high-resolution system, demonstrate different protein expression between Progeria and Control CM highlights mitochondrial dysfunctions, apoptosis among other alterations.

Conclusions: Our work demonstrate that iPS derived cardiomyocytes from a Progeria patient presents significant molecular and proteomics alterations suggesting novel mechanisms underlying premature cardiac aging.

85. CHRISTIAN MONTES

Identification of differentially expressed and differentially phosphorylated proteins in Arabidopsis in response to brassinosteroid pathway activation using different BIN2 mutants.

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Every organism deals, on a daily basis, with different biotic and abiotic stresses. Plants respond to these different stimuli by modifying and balancing their growth and stress responses in a coordinate manner. The brassinosteroids (BRs) phytohormone family is involved in growth regulation, plant development and stress response. In the presence of BRs and through a series of intermediate signaling, the GSK-like kinase BRASSINOSTEROID INSENSITIVE 2 (BIN2), a BR signaling negative regulator, is phosphorylated to inhibit its function. To reveal the effect of brassinosteroid-induced signaling, *Arabidopsis thaliana* plants carrying either a BIN2 null mutant (BIN2-T), a gain-of-function hypermorphic BIN2 mutant (BIN2-1D) or the wild-type BIN2 gene (Col-0 WT plants) were grown in either H_2O (as mock) or 1μM Brassinolide (BL). Three biological replicates per genotype and condition were used. Each sample consisted on whole rosette leaves, ground in presence of liquid nitrogen. A phenol-based protein extraction was performed on 500 mg of ground tissue, followed by urea-based filter aided sample preparation (FASP). Proteins were reduced, alkylated and, finally, digested with trypsin. Peptides were de-salted using C18 columns and isobarically labeled with Tandem Mass Tags. Purified samples were enriched for phosphorylated peptides using both Fe^{+3} and TiO_2 methods in tandem. Spectra were acquired on a Thermo Scientific Q-Exactive plus hybrid quadrupole Orbitrap mass spectrometer. We are in the initial stages of analyzing this data but we have already uncovered some exciting aspects of brassinosteroid signaling.

86. LAURA K. MUEHLBAUER

Investigating the role of IQGAP1 in head and neck carcinogenesis *in vivo* through mass spectrometry-based phosphoproteomics

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Head and neck squamous cell carcinoma (HNSCC) is a prevalent type of cancer that frequently carries activating mutations in the PI3K/AKT/mTOR signaling pathway, which is downstream of growth factor signaling. Isoleucine-glutamine motif-containing GTPase-activating protein 1 (IQGAP1) is an important scaffolding protein that promotes PI3K/AKT/mTOR signaling. The disruption of IQGAP1-mediated PI3K signaling using a peptide that interferes with the ability of IQGAP1 to bind to PI3K reduces HNSCC cell survival. Furthermore, upon treatment with a synthetic oral carcinogen, 4-nitroquinoline 1-oxide (4NQO), *Iqgap1*-/- mice show significant reductions in the multiplicity and severity of head and neck cancer foci compared to *Iqgap1*+/+ mice. *Iqgap1*-/- mice also show significantly reduced PI3K signaling upon epidermal growth factor stimulation, and PI3K signaling was significantly decreased in the tumors from *Iqgap1*-/- mice compared to those from *Iqgap1*+/+ mice. Clearly, IQGAP1 is important to HNSCC development and progression. However, further insight is needed to learn how IQGAP1 contributes to HNSCC, as IQGAP1 is implicated in multiple other signaling pathways important in carcinogenesis besides the PI3K/AKT/mTOR pathway.

Mass spectrometry-based phosphoproteomics is especially useful for such pathway analysis in cancer systems. Here, we performed comparative phosphoproteomic analysis of *Iqgap1*-/- vs. *Iqgap1*+/+ mice esophagus tissue through multiplexed isobaric labeling combined with high-resolution mass spectrometry. Peptides were TMT-labeled, enriched for phosphopeptides with Ti-IMAC, and fractionated by high-pH reverse phase chromatography prior to LC-MS/MS. Non-enriched peptides were similarly fractionated and analyzed to allow normalization of phospho-sites to protein expression changes. Preliminary data showed identification of 5853 proteins and 9644 phosphorylated sites, of which 414 phospho-sites significantly changed between conditions. Significant changes were mostly related to proteins involved in mRNA splicing, translation, apoptosis, and other pathways in which IQGAP1 is involved, such as Rho-GTPase and MAPK signaling. Interestingly, females had almost no significant changes between conditions, indicating that IQGAP1's role can differ between genders. Deeper analysis of these phosphoproteomic changes will help to provide a better view of the pathways in which IQGAP1 is involved to contribute to carcinogenesis. Future direction includes using mass spectrometry-based phosphoproteomics to compare normal and tumors tissues in *Iqgap1*-/- mice.

87. VINAY NAGARAJAN

RNA degradomes reveal substrates and importance of Arabidopsis XRN4 in the crosstalk between auxin signaling and nitrogen homeostasis

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Introduction: In plants, XRN4 is the 5' to 3' exoribonuclease that functions in cytoplasmic mRNA decay, homologous to XRN1 of yeast and animals. XRN4 preferentially catalyzes 5' monophosphorylated RNA such as those found on miRNA-targets following miRNA-guided cleavage and decapped mRNA. XRN4, also known as Ethylene Insensitive 5 (EIN5), impacts ethylene and ABA signaling, seed dormancy, flowering time, and plant responses to heat stress in addition to being a major player in post-transcriptional gene silencing (PTGS). However, at the transcription level only a modest number of genes (ranging from 20 to 100) are affected in XRN4 deficient lines. There is mounting evidence to indicate XRN4 impacts gene expression at the post-transcriptional level by fine-tuning mRNA stability. Our study investigated XRN4 substrates on a global scale to gain insight about the biological impacts of the enzyme in Arabidopsis.

Methods: Global deep-sequencing approaches to capture (1) RNA degradome (Parallel Analysis of RNA Ends, PARE) that detects populations of RNA decay intermediates and (2) transcriptome (RNA-seq) were utilized to study XRN4 substrates in wild-type (WT) and two T-DNA knockout *XRN4* mutant (*xrn4-5* and *xrn4-6*) seedlings. In our study both the polyadenylated and nonpolyadenylated RNA degradomes were examined separately because in current decay models trimming and removal of the polyA tail (deadenylation) initiates turnover for many mRNAs degraded by XRN1 or XRN4.

Results: RNA-seq and degradome analysis demonstrated that *xrn4* mutants overaccumulate many more decapped, deadenylated intermediates compared to those that are polyadenylated. Among XRN4 substrates, some of the over-represented gene ontology (GO) categories include gene products from primary C and N metabolism, hormone signaling (auxin and ethylene) and abiotic stress responses. Of these XRN4 substrates, auxin-responsive *Aux/IAA* genes were of greatest interest since some code for unstable proteins and are repressors of auxin-responsive gene expression impacting root growth. In *xrn4*, *Aux/IAA* transcripts *IAA2* and *IAA3* showed elevated mRNA levels than the WT. Following transcription inhibition, RNA decay kinetics in seedlings showed that these *Aux/IAAs* are more stable in *xrn4* ($t_{1/2}$>36 min) than the WT ($t_{1/2}$ <20 min), indicating that XRN4 is required for their decay. Intriguingly, validation of PARE using 5'RACE analysis indicated that these *Aux/IAA* transcripts not only overaccumulated decapped mRNAs, but also 5'capped mRNAs in *xrn4*. This suggests that in *xrn4* either the decapping is inefficient or transcription is elevated for

select transcripts. Based on our analyses, we propose that the former is a more likely scenario due to the strong impact of *xrn4* on *Aux/IAA* mRNA stability. Collectively, our results point towards post-transcriptional regulation via XRN4-mediated RNA turnover as one of the mechanisms maintaining cellular levels of Aux/IAA repressors. We further show that *xrn4* mutants are insensitive to the stimulatory effects of optimal N supply and produce significantly fewer (>50%) lateral roots than WT. This root branching defect could be attributed to stable levels of *Aux/IAA* mRNAs, potentially elevating repressor protein levels. Further analysis is underway to examine the global RNA stability levels and protein profiles in the roots of *xrn4* subjected to different N regimes.

Novel Aspects: Our systematic approach has allowed us to capture many more XRN4 substrates and identify novel impacts of this enzyme, e.g. N-specific root branching and additional roles in senescence and osmotic stress responses (not discussed here). Our work opens avenues to identify the molecular basis for known and novel phenotypes and uncover mechanisms (e.g. decapping efficiency) controlling RNA stability and its impact on protein levels in model and crop plants.

88. KATHLEEN NICHOLSON

Deletion of two PE genes delays virulence of *Mycobacterium marinum*

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Introduction: *Mycobacterium marinum*, an infectious agent of poikilothermic fish and an opportunistic human pathogen, can serve as a laboratory model for *Mycobacterium tuberculosis*. These pathogens rely upon the ESX-1 (ESAT-6 System 1) secretion system for virulence in their respective hosts. We have extensively characterized the secreted proteome using targeted and non-targeted mass spectrometry techniques to identify pathways and genes responsible for virulence. Recently, we identified several PE family proteins as candidates for virulence factors related to the ESX-1 system. PE proteins are denoted by their characteristic N-terminal domains containing Pro-Glu motifs in addition to variable C-terminal regions. Although this class of proteins has been previously implicated in mycobacterial pathogenesis, the mechanism by which these specific proteins contributes to virulence remains unknown.

Methods: Genetic deletions of two PE genes were generated in *M. marinum*. To determine if these genes were required for ESX-1 function, a hemolysis assay was performed. Wild-type (WT) strains of *M. marinum* lyse Red Blood Cells in an ESX-1-dependent manner. The hemolytic activity of *M. marinum* strains reflects the activity of the ESX-1 secretion system in different genetic backgrounds. Western blot analysis directly measures virulence factor secretion of *M. marinum* strains. WT strains secrete virulence factors into culture medium, while strains with defects in ESX-1 secretion no longer secrete a subset of proteins during growth. Infection assays using macrophage-like models were used to test immune response against and cytotoxicity of WT and mutant *M. marinum* strains.

Preliminary Data: Strains containing deletions of individual PE genes were subjected to hemolysis assays. Like the $\Delta eccCb_1$ strain, which lacks a functional ESX-1 system, deletions of PE genes ablated hemolysis. Because the mutant strains were non-hemolytic, a secretion assay was performed to determine if ESX-1 virulence factors were being secreted. In strains lacking the PE genes, the secretion of the two major ESX-1 substrates was greatly reduced. These findings indicate that the PE genes may be required for ESX-1 secretion. To determine if these PE genes affect virulence within the host, RAW264.7 cells were infected with WT, $\Delta eccCb_1$, and PE mutant strains at an MOI of 5. After four hours of infection, qRT-PCR data revealed intermediate IFN-β transcript abundance in strains lacking PE proteins, while WT showed elevated IFN-β transcript and $\Delta eccCb_1$ showed almost negligible amounts of IFN-β transcript. However, in a separate RAW264.7 infection assay, PE mutant strains resulted in cytotoxicity on par with infections by the WT strain. The disparity between the secretory and virulence phenotypes are novel, and require further dissection using LFQ MS. Together our preliminary data suggests roles for the PE genes we have analyzed thus far as key undescribed elements of mycobacterial virulence through ESX-1 secretion.

Novel Aspect: This work will employ hypothesis and phenotypic driven mass spectrometry proteomics.

89. AARON OGDEN

Metabolic development of symbiotic nitrogen fixation in the *Medicago-Sinorhizobium* symbiosis and the role of HslUV and ClpXP protease machinery.

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Introduction: Symbiotic nitrogen fixation (SNF) describes a process in which inert atmospheric dinitrogen is reduced to ammonium through the coordinated interaction of bacteria with a host plant. The majority of SNF occurs when soil dwelling bacteria, commonly called rhizobia, infect legumes and form specialized root organs called nodules. The model symbionts *Medicago truncatula* and *Sinorhizobium medicae* are ideal for studying SNF development because as they develop, the nodules contain distinct zones that spatially separate the metabolic progression from free-living individuals into nitrogen fixing partners. The progression of metabolic remodeling within the nodule is in part regulated post translationally via the rhizobial orthologs of the eukaryotic 26S proteasome, specifically HslUV and ClpXP.

Methods: To better understand the coordination of plant and bacterial metabolism within the nodule we used LC and GC MS/MS to create protein and metabolite profiles representative of these developmental zones, as well as profiles of whole-nodules and uninfected roots. The different developmental zones were collected by slicing the nodule before and after the region of conspicuous leghemoglobin accumulation. Protein and metabolite profiles for each zone were analyzed using a Waters Synapt G2S and Leco GC TOF-MS, respectively. To better understand the role of proteases HslUV and ClpXP in remodeling the proteome, we tagged each protein with a C-terminal eGFP. This allowed us to use native coimmunoprecipitation followed by LC-MSMS on a Thermo Orbitrap Fusion to identify many proteins associated with the proteases.

Preliminary data: Our approach confidently identified 361 bacterial proteins and 888 plant proteins, as well as 160 metabolites. Our data confirms many nodule and sub-nodule protein and metabolite localization patterns characterized in previous publications. We used our comprehensive dataset to demonstrate how branches of primary metabolism are coordinated between the symbionts and how zones differ in central carbon, fatty acid, and amino acid metabolism. Our experiments were recently published and provide the scientific community with the largest intra-nodule protein and metabolite dataset to date. We have demonstrated that deleting the HslUV and ClpP protease genes results in a less effective symbiosis, as well as significantly reduced exopolysaccharide production and altered free-living growth rates. eGFP tagging each endogenous gene showed that each protein had no immediate nodule zone specificity, and occasionally formed complexes in symbiosis. Co-immunoprecipitation of eGFP tagged HslUV, and ClpXP from free-living rhizobia, as well as nodules formed from S. meliloti 1021 – M. truncatula symbiosis successfully enriched for many of the already known HslUV and ClpXP interacting proteins, and identified many novel interactors. Of the proteins enriched in the co-IPs of HslU and ClpX, 48% and 76% of those proteins were also enriched in the co-IPs of their partners HslV and ClpP, respectively. Co-IPs of nodules, however, showed only 21% and 36% overlap in the number of enriched proteins for HslUV and ClpXP, respectively. Notable targets include master regulators of exopolysaccharide synthesis, nodule specific cysteine rich peptides, and other molecular chaperones.

Novel aspect: Protein profiling and protein-protein interaction studies facilitated by mass spectrometry are revealing new and exciting aspects of symbiotic nitrogen fixation.

90. KIWON OK

Detecting how gold drugs target zinc finger proteins via native nano-electrospray ion mobility mass spectrometry

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Introduction: Gold complexes exhibit anti-tumor and anti-inflammatory properties, and there are currently several FDA approved gold(I) drugs for the treatment of rheumatoid arthritis. The mechanism of action for gold drugs is unclear making the development of novel gold drugs challenging. There is evidence that gold complexes target thiol ligands in present in zinc finger proteins. One attractive target is the zinc finger protein, tristetraprolin (TTP). TTP contains two Cys_3His (CCCH) zinc finger domains and regulates inflammation by binding to and down regulating cytokine mRNAs. To determine whether gold-complexes can target TTP and identify gold/TTP interactions, optical, fluorescence, X-ray absorption, and mass spectrometric approaches were taken. For the latter, native ion mobility mass spectrometry provided key information regarding stoichiometry and ligand targets.

Methods: A construct of TTP (TTP-2D), was overexpressed, purified in the apo-form, and folded with zinc(II) as previously described (diTargiani, 2006). The model gold(III) complex, Au(terpy), was synthesized using published procedures (Hollis, 1983). UV-visible spectroscopy (PerkinElmer, Lambda 25) and fluorescence anisotropy (K2, ISS) were performed to determine whether gold displaces zinc and analyze the effect on RNA binding. Native mass spectrometry studies were performed on a quadrupole time-of-flight mass spectrometer with a traveling wave ion mobility-mass spectrometry (Synapt G2S, Waters). 10 μM TTP-2D protein sample mixed with Au(terpy) in 10 mM ammonium acetate buffer was directly infused via syringe pump at a flow rate of 50 to 100 nL/min. Nano-electrospray ionization source parameters were adjusted to conserve the metal-complexed protein.

Preliminary Data: Using optical and fluorescence spectroscopies, we determined that Au(terpy) exchanges with the zinc-bound and folded TTP-2D (Zn_2-TTP-2D) and that target RNA (5'-UUUAUUUAUUU-3') binding inhibits this exchange. To understand the stoichiometry and identify ligands involved in gold coordination, inductively coupled plasma-mass spectrometry (ICP-MS) and native protein mass spectrometry were employed. ICP-MS showed that approximately 2.0 equivalent of gold atoms bind to TTP-2D. Using native protein mass spectrometry, the metal-protein complex were preserved in the gas phase and we observed a charge state distribution centered at 5^+ to 8^+ for TTP-2D. Zn_2-TTP-2D was more preserved (>99%) than the gold-bound proteins (~70%) in the gas phase, based upon the relative ion intensity ratio of apo- and metal-complexed proteins. Au(terpy) was titrated into Zn_2-TTP-2D at a molar ratio of 1:1 to 4:1. Under these conditions, two zinc ions were ejected from the CCCH zinc binding domain, and a series of gold-bound proteins were found to be predominant species. At a 2:1 molar ratio of Au(terpy) and Zn_2-TTP-2D, zinc ions were completely displaced with 1~4 gold atoms forming a 'gold finger' (Au_x-TTP-2D). The formation of Au_x-TTP-2D from Zn_2-TTP-2D indicated that Au(terpy) can eject zinc from the metal site of TTP. We examined the effect of RNA by performing a direct titration of Au(terpy) with the Zn_2-TTP-2D/RNA complex. Intense ion peaks of Zn_2-TTP-2D/RNA complex were not inhibited by addition of Au(terpy) until salt-induced signal suppression occurs. These data are consistent with our FA data. For further characterization and comparison of the conformational difference among ion species, dominant charge state ions were analyzed by ion mobility-mass spectrometry. Au_x-TTP-2D showed a longer peak drift time than apo- and Zn_2-TTP-2D, indicating the structure of 'gold finger' is in different conformation. The tandem mass spectrometry (MS/MS) of Au_x-TTP-2D ions is in progress to further identify potential gold ligand protein residues.

Novel Aspect: Native ion mobility-MS utilized to characterize the exchange of gold complexes with zinc in a zinc finger protein.

91. IAN PALMER

Screening for Novel Salicylic Acid Analogs that Resist Degradation by the Citrus Greening Pathogen Candidatus Liberibacter asiaticus

Citrus greening is a disease that has recently appeared in the citrus fields of several US states. The disease is spread by the Asian citrus psyllid, an invasive species. This disease is estimated to have caused a $1B loss to the citrus industry annually, since 2005. It has recently been demonstrated that Candidatus Liberibacter asiaticus (CLas), the pathogen responsible for causing citrus greening, possesses a functional salicylic acid (SA) hydroxylase. By degrading SA, the pathogen can subvert SA-mediated plant defense. In this work, I demonstrate that several SA analogs can enhance the interactions between key SA-mediated defense proteins, that these analogs can induce the master regulator of SA-mediated defense, NPR1, in planta, and that these analogs can induce an obvious defense activation phenotype in Arabidopsis thaliana. I propose cloning CLas SA hydroxlase, expressing SA hydroxylase in E. coli, purifying this enzyme, incubating SA hydroxylase with active SA analogs, then using LC-MS to analyze the degradation products. If successful, this research could provide valuable knowledge of SA-mediated plant defense, and provide the means for citrus farmers to protect their valuable citrus trees.

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92. OPHELIA PAPOULAS

A Map of Conserved Plant Protein Complexes Generated from Co-fractionation Shotgun Proteomics Data.

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Introduction: Plants represent 80% of our planet's biomass and are critical for our survival. They are essential as our food supply, air supply, and major sources of materials and medicines, however our understanding of the protein assemblies in plants lags significantly behind that of animals. This is in part due to the challenges of extracting native proteins from cells with rigid cell walls, the overwhelming levels of photosynthetic proteins in green tissues, and from the focus on a few research species that serve as model genetic organisms or crop species. We are undertaking a systematic approach to generate a comprehensive map of protein complexes conserved in existing plant species spanning the evolutionary tree.

Methods: The strategy we use has proved abundantly successful in identifying protein complexes (both known and previously unknown) in animal species (Wan et al. 2015 Nature, Phanse et al. 2015, Data Brief, Drew et al, 2017, Mol Syst Biol). Briefly, soluble proteins are separated by native chromatographic methods (ion exchange, size exclusion, isoelectric focusing) and the proteins present in each fraction are identified and quantified by mass spectrometry. These data are then analyzed in a computational pipeline that quantifies the extent to which any two proteins co-fractionate across multiple fractionation methods and experiments. The pair-wise co-fractionation data can be integrated with existing public data on known protein interactions to create a high confidence map of the conserved macromolecular entities.

Preliminary Data: We have analyzed nearly 2000 biochemical fractions derived from eudicots (*Arabidopsis*, broccoli, soy, hemp, quinoa), monocots (wheat germ, rice leaves, coconut nuclei), more primitive green land plants (*Selaginella moellendorffii*, *Ceratopteris richardii* fern), and the green algae *Chlamydomonas*.

Plant proteomics is fraught with several unique challenges. The vast majority of previous plant proteomic studies have used denaturing methods to deal with cell walls. We have had to rely on gentler, often low-yield, methods to extract protein complexes in their native states. Secondly the extreme dominance of the proteome by photosynthetic proteins (RuBisCO alone comprises 25-60% of total leaf protein) made it difficult to identify less abundant proteins. To address this limitation we included embryonic tissue (germ and seeds), plants grown in the dark, and isolated nuclei where possible. Finally, the genomes of many plants are polyploid leaving relatively few unique peptides for a given gene. Furthermore, a gene can have widely varying numbers of co-orthologs in other species. To address polypoloidy we devised a method of mapping peptides to orthogroups to best observe conserved protein interactions and annotate protein identifications similarly across species. Our detailed methods and current map and characterization of the plant proteome will be presented.

Novel Aspect: The present study analyzes native endogenous protein interactions to generate the first map of conserved protein complexes in plants.

93. KEVIN PARKER

Gas Phase Study of C-C Coupling Catalyzed by Nickel(II) Complexes

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Introduction: In organic chemistry carbon-carbon coupling reactions play a significant role in synthesis through mechanisms such as the Suzuki coupling and Negishi reactions. Typically metals such as Ru, Rd and Pd are used as the metal centers in the catalysts however Fe and Cu have been shown to be less expensive and more abundant options. Many studies have been done on the effect of various group 10 and 11 transition metals however the effect of varying carboxylic acids and alkanes has not been studied.

Methods: Experiments were done on a ThermoFisher LTQ (San Jose, CA) that had been modified to run ion molecule reactions (IMRs) in the gas phase. A solution containing $Ni(NO_3)_2$, phenanthroline and a carboxylic acid counter ion (-OOCR) were transferred into the gas phase through electrospray ionization (ESI). The ion $[(phen)Ni(-OOCR)]^+$ was trapped in the ion trap and then decarboxylated through collision induced dissociation (CID) giving [(phen)Ni(R)]+ and CO_2 gas. The intermediate ion was then reacted with different carboxylic acids ($R'OCOCH_3$) that were introduced through a bypass on the helium line. The products are monitored through time to estimate the relative rate of reaction.

Differences in reactivity and reaction mechanisms are studied trough DFT calculation performed at the M11L/cc-pVTZ level of theory.

Preliminary data: Ions of the form $[(phen)Ni(R)]^+$ can have different R groups through variation of the carboxylic acid counter ion and subsequent decarboxylation through CID. A reaction scheme for the formation of these ions and subsequent carbon-carbon coupling reaction is as follows:

1) $[(phen)Ni(OOCR)]^+ \longrightarrow [(phen)Ni(R)]^+ + CO_2$
2) $[(phen)Ni(OOCR)]^+ + CH_3COOR' \longrightarrow [(phen)Ni(OOCCH_3)]^+ + R\text{-}R'$

The range of reactivity due to varying the R group (R= CH_3-, C_6H_5-, H_2C=CH-) on the carboxylic acids and the different neutrals was studied in the gas phase using ESI-MS.

Carbon-carbon coupling products were formed for all combinations of three different counter ions and four different neutrals; vinyl acetate, allyl acetate, 2-hydroxyethyl acetate, 4-methoxybenzyl acetate (R'= -HC=CH_2, -H_2CHC=CH_2, -CH_2CH_2OH, -$CH_2C_6H_4OCH_3$).

By varying the reaction time in the ion trap a reactivity study is possible to try and determine kinetics data from experiment. Using the time it takes for the reaction to reach completion, rate constants can be evaluated for each combination of components.

The relative rate of the reactions can then be compared with the information obtained from theoretical calculations for the energy levels of different intermediates and products.

Preliminary DFT results at the M11L/cc-pVTZ level of theory agree with the reactivity trend observed experimentally. The determination of the mechanisms of C-C coupling is still under study.

Novel aspect: The use of nickel as a metal in the catalyst for C-C coupling reactions in place of more expensive and less abundant metals.

94. MICHAEL VAN PARYS

Failures of Hemolysis Testing, Assessment and Remediation Case Studies.

Michael Van Parys, James Farnham, Ted Brus, David Good, Aaron Ledvina, Stephanie Cape

Introduction: Hemolysis is a process, during which lysis of red blood cells releases the contents of the cells into the plasma. These contents, (primarily hemoglobin, bilirubin, and salts) can present unique challenges for validation of bioanalytical methods. Hemolysis can be problematic for quantitative LC/MS/MS methods for a variety of reasons. For example, cellular components that are present in hemolyzed plasma can impart ion suppression on the analytes of interest. This effect can be substantial enough to prevent detection at low analyte concentrations. Also, these components may adversely impact the stability profile of the analytes. Herein, we describe case studies illustrating these challenges and describe mitigating steps taken.

Methods: Often hemolysis based ion suppression manifests itself in a region of the chromatographic spectra that coincides with the elution of the analyte. Examples of identification of this region and methods for shifting analyte elution away from these zones of suppression will be illustrated. Cellular components may be removed from the analyzed samples by improving the pre-analysis sample cleanup. Additionally, mitigation of hemolysis interference using high resolution, accurate mass MS will be explored. When the failure is a result of instability of the compound of interest in the presence of cellular blood components temperature control or stabilizing additives are necessary to prevent further degradation.

Preliminary Data: When hemolysis testing failures are observed and ion suppression is suspected a number of approaches can be taken. It is common to dilute the hemolyzed samples with control plasma but this technique may impart restrictive limitations on the viable LLOQ of the assay. Additionally, there may be significant variability associated with the plasma used from one batch to the next, introducing a potential source of assay variability.

- Case Study #1 (Utilized post-column infusion to identify the suppression zone resulting from cellular components in the matrix and modified LC conditions to shift retention time away from this)
- Case Study #2 (Optimized sample precipitation solvent in order to remove the components causing the matrix effects prior to analysis)
- Case Study #3 (Altered sample extraction chemistry to provide consistent recovery across multiple matrix sources)
- Case Study #4 (Improved the stability of analyte of interest in extracted hemolyzed samples by modifying the matrix treatment protocol)

Novel Aspect: This work demonstrates novel approaches to overcome unique matrix suppression and stability challenges that can be associated with hemolyzed samples.

95. VANESSA PAURUS

Fused silica tubing applications in nano-flow UPLC

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Fused silica tubing is widely used in nano-flow UPLC due to its relative inertness, availability in multiple internal diameters and ease of use. Detailed procedures that are used by the high throughput LC-MS capability at Pacific Northwest National Laboratory are described for the preparation of analytical and on-line trapping columns, along with chemically etched electrospray emitters. Both before and after the installation of the prepared columns and emitters on the instrument, many different methods and metrics are used for evaluating each of their quality. While all these items are available commercially, the ability to prepare them in-house provides considerable flexibility and significant savings.

96. YIMING QIN

Investigating acetylation as a regulatory mechanism for LON protease

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The regulation of mitochondrial homeostasis is crucial for cell function and viability. An important regulator of such process is mitochondrial LON protease. The functions of LON include maintaining mitochondrial proteostasis, binding to G-quadruplex of mtDNA and facilitating OXPHOS complexes formation. So far, several substrates of LON have been identified in human, such as aconitase, TFAM, Twinkle, Cox4-1, many of which are mtDNA binding proteins. LON is thus poised to regulate mtDNA replication and expression, through both proteolytic and mtDNA binding activities. Despite LON regulates essential steps of mitochondrial metabolism, how LON itself being regulated has not been fully addressed yet. Previous studies of mitochondrial acetylome of Sirt3 KO mice and Sirt3-LON co-immunoprecipitation have suggested that LON is a substrate of Sirt3 and acetylation may have functional impacts on LON activity. Coupling chemical acetylation using D6-acetic anhydride with high resolution mass spectrometry and bioinformatics data processing, we have captured the dynamic change of LON acetylation stoichiometry of MCF7 cells during metabolic switching. We observed that after 24 hours serum starvation, more than 50% LON were acetylated at active site K898. Adding serum led to transient decrease in acetylation followed by recovered acetylation of LON K898. While acetylation would abolish K898 nucleophile property and destroy LON proteolytic activity, studies have demonstrated that LON mutant that lacks protease activity can still bind to nucleic acid, and the binding affinity can be increased by the presence of LON substrates. Thus, we propose that acetylation of K898 may alter the functions of LON by inhibiting proteolysis and promoting mtDNA binding.

97. TIAN (AUTUMN) QIU

DNA damage as a toxicity mechanism of NMC ($Li_xNi_{1/3}Mn_{1/3}Co_{1/3}O_2$) nanomaterial exposure to bacterial models

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Introduction: The potential wide application of nanoscale lithium nickel manganese cobalt oxide ($Li_xNi_yMn_zCo_{1-y-z}O_2$, NMC) as a cathode material in lithium-ion batteries calls for risk evaluation of NMC being released into the environment. Using an ecologically beneficial bacterium, our previous studies showed that NMC induced inhibition on bacterial growth via metal ion dissolution. Considering the genotoxicity effect of nickel and cobalt ions, we evaluated bacterial DNA damage upon NMC exposure as a potential toxicity mechanism, along with other toxicity and biochemical assays. Using two microbial models, the Gram-negative bacterium *Shewanella oneidensis* MR-1 and the Gram-positive bacterium *Bacillus subtilis*, we measured DNA strand breakage with single cell gel electrophoresis, and successfully applied a high-resolution mass spectrometry-based approach to identify bacterial DNA adducts upon nanomaterial exposure.

Methods: Toxicity of NMC to both bacteria was determined by monitoring bacterial growth and viability upon nanomaterial exposure. Intracellular reactive oxygen species (ROS) generation and metal ion uptake, namely Ni(II) and Co(II) ions, were measured using two fluorescent dyes, $DCFH_2$-DA and Newport Green DCF diacetate, respectively. Single cell gel electrophoresis was carried out by lysing bacterial cells in situ in low-melting agarose, followed by electrophoresis, DNA staining and image analysis. Bacterial DNA samples were enzymatically digested, purified by fraction collection and analyzed via nano-LC/MS^3. A list of endogenous DNA adducts was used. During nano-LC/MS, precursor ions of masses in the inclusion list (full scan) with a subsequent neutral loss of deoxyribose (MS2) were further fragmented (MS3) to confirm the putative DNA adducts.

Preliminary Data: Results showed that NMC induced dose-dependent toxicity to both bacteria as well as elevated levels of intracellular ROS, although not statistically significant, and metal ion in NMC-treated cells compared to control groups. DNA strand breakage was observed at sub-lethal dosage of NMC (5 mg/L) to both bacteria. Bacterial DNA adducts were successfully observed using the high-resolution LC/MS^3 method, while no significant changes were observed on levels of various DNA adducts in NMC-treated bacterial cells compared to control groups. This indicated that endogenous DNA adduct formation was likely not the mechanism of DNA double strand breakage observed in NMC-treated bacterial cells. Taking current results together, we concluded DNA damage as a toxicity mechanism of NMC exposure to bacterial cells possibly via ROS and transition metal ion uptake.

Novel Aspect: For the first time, we showed the capability of this data-dependent mass spectrometry approach in detecting bacterial DNA adducts upon nanomaterial exposure.

98. ANDREW RICHING

Activation of TGF-β Signaling prevents removal of H3K27me3, thereby impairing cardiomyogenesis.

Introduction: Heart disease is the leading cause of death in the United States. Current therapies demonstrate improved ventricular function and long-term survival in patients, yet are unable to stop or reverse the progression of heart failure due to the inability to regenerate cardiomyocytes (CMs) following coronary events. Direct reprogramming of fibroblasts to CMs offers the potential to regenerate the heart following MI. However, translation to humans faces numerous challenges including increasing the efficiency of generating CMs, generating a homogeneous, mature population of CMs, and overcoming safety concerns for delivery to human hearts. Our lab has previously shown that inhibition of TGF-β receptor 1 (TGFR1) significantly enhances reprogramming efficiency. However, the mechanisms by which TGFR1 inhibition improves cardiomyogenesis efficiency remain poorly understood.

Methods: Our lab has demonstrated that TGFR1 inhibition combined with overexpression of the cardiac transcription factors (cTFs) GATA4, Hand2, MEF2C, and Tbx5 (GHMT), as well as microRNAs miR-1 and miR-133 (GHMT2m) reprograms ~60% of mouse embryonic fibroblasts (MEFs) into functional induced CMs (iCMs). Using biochemical assays including Co-IP and ChIP, I aim to establish the mechanism by which TGF-β signaling impairs cardiomyogenesis. We hypothesize that Smad2/3 compete with cTFs to bind and/or recruit chromatin modifiers to cardiac gene loci, impairing with cardiomyogenesis.

Preliminary Data: Our studies indicate that cTFs physically interact with the H3K27me3-specific demethylase JMJD3. In addition, mass spectrometry analysis identified core components of the SWI/SNF chromatin remodeling complex including Brg1, Baf170, Baf155, Baf180, and Brm also interact with cTFs. Furthermore, TGF-β signaling impairs these interactions and perturbs demethylation of H3K27me3 as well as binding of GATA4 to target genes. Recent publications demonstrate that JMJD3 and Brg1 both also physically interact with Smad2/3. Therefore, physical interactions between cTFs and chromatin remodeling complexes may be disrupted by TGF-β signaling, impairing cardiomyogenesis. Finally, JMJD3 protein expression and demethylase activity are required for cardiomyogenesis, suggesting removal of H3K27me3 at cardiac genes is essential in cardiac reprogramming.

Novel Aspect: Removal of H3K27me3 and GATA4 binding to target genes are prevented by TGF-β signaling, leading to decreased cardiac gene expression.

99. GABRIELLE RIZZO

Proteomic Characterization of Microneedle-Extracted Human Dermal Interstitial Fluid

Abstract Submission for 2018 North American Mass Spectrometry Summer School

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Introduction: With the advent of wearable fitness devices, commercial interest in real-time monitoring of an individual's physiological status using non-invasive techniques has grown. Microneedles have recently been proposed as a minimally-invasive method of sample collection for clinical diagnosis, as they do not reach nerve endings within the dermis and are therefore painless. Using a microneedle array with no suction apparatus, interstitial fluid (ISF) was collected from three healthy human donors along with matching serum and plasma samples. Proteomic analysis confirmed previously published work which indicates that ISF is highly similar to both plasma and serum. ISF may therefore serve as a viable alternative to blood-derived samples for clinical applications including the novel application of wearable real-time sensing devices.

Methods: Samples were collected from three healthy human donors. ISF was sampled by pressing an array of five microneedles gently against the forearm; capillaries attached to the microneedles collected ISF. Blood was sampled using a standard clinical venipuncture procedure. Serum and plasma fractions were collected from blood samples via centrifugation with and without coagulant, respectively. Equal amounts of ISF, serum and plasma were submitted to trypsin digestion, 10plex TMT labeling, basic reverse-phase fractionation and LC-MS/MS analysis. Data processing was performed using Proteome Discoverer 2.1. Protein identifications were filtered at a false discovery rate of 1%. Reporter ion intensities were normalized to total ion current and used for calculating relative abundance ratio of proteins. Statistical analysis was performed using Perseus 1.5.5.3.

Preliminary Data: Data analysis revealed that 3270 out of 3506 identified proteins in donor #1 were found to be in common between ISF, serum and plasma—a 93.3% protein overlap. Even higher overlaps were seen in the other donors, with 95% of proteins in common between the fluids of donor #2 and 97.6% in common for donor #3. Additionally, variability between ISF proteomes among the three donors was extremely low; 3101 of 3485 (89%) of proteins identified in ISF were found to be in common across all donors.

Out of a total of 3527 identified proteins, 407 were identified with at least one peptide unique to the protein. These unambiguously-identified proteins were subjected to further statistical analyses to assess the similarities and differences between the three biological fluids. A principal component analysis (PCA) revealed that the proteins identified in ISF were highly distinguishable from serum and plasma proteins. Z-score based hierarchical clustering analysis supported this result, highlighting clusters identified in ISF whose expression levels differed drastically from the clusters identified in serum and plasma.

An analysis of variance (ANOVA) test was performed on the 407 quantified proteins to compare the ratios of ISF proteins to either serum or plasma in all donors. Of these, 26 proteins were differentially expressed consistently across all three donors—22 proteins were found to be more abundant in ISF, while 4 proteins were more abundant in both serum and plasma. Gene annotation enrichment analysis showed that 19 of the 26 proteins were located in the extracellular exosome, and that all but one of these 19 proteins were expressed higher in ISF than in serum or plasma.

Taken together, these results show that the overall protein diversity of ISF is highly similar to that of serum and plasma, with differences appearing mainly at the quantitative level.

Novel Aspect: Microneedle-derived ISF can serve as a minimally-invasive alternative to blood-derived fluids for clinical applications.

100. GINA ROESCH

Matrix Interferences in Solution Cathode Glow Discharge Emission Spectrometry

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With assistance from Andrew Schwartz, Ph.D. and Radislav Potyrailo, Ph.D

The solution cathode glow discharge (SCGD) is a novel plasma source used to determine trace elements in solution by means of atomic emission spectrometry (AES). The SCGD, unlike a traditional glow discharge, operates at atmospheric pressure and utilizes a flowing electrolyte solution as a cathode rather than a conductive metal electrode. This configuration is simple, compact, and inexpensive. It does not require a gas flow and uses low, direct-current power (~70 W). For these reasons and competitive limits of detection, the SCGD is highly attractive for laboratory- and field-based AES measurements.

While there are many practical and operational advantages of the SCGD, the SCGD suffers from numerous interferences caused by the sample matrix, in large part because the sample solution forms part of the electrical circuit. These interferences can result in analytical errors and are difficult to detect without prior knowledge of the sample composition, which is itself usually the purpose of the analysis. To address this problem, we have devised a number of different methods for flagging the existence of an interference. The first to be discussed in this dissertation is a method for flagging the presence of matrix interferences using spatially resolved images of analyte emission. A second method utilizes a statistical approach involving Principal Component Analysis (PCA) and Partial Least Squares (PLS), both of which are chemometric algorithms. In addition to identifying interferences, potential methods to overcome interferences are also proposed for each method.

101. JOANIE RYAN

Fluorescent tracking of *Mycobacterium tuberculosis* extracellular vesicles

Joan M Ryan, Juan Belardinelli, Mary Jackson, and Nicole Kruh-Garcia

Introduction: Extracellular vesicles (EVs) are small vehicles of various biomarkers known to be secreted by nearly every cell type. Recently, EVs have been isolated from Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis infection in humans. The role of EVs in Mtb infection is not yet understood. Here we present a new tool for understanding the interaction between Mtb EVs and their environment by fluorescent visualization. Previous work by the Jackson Laboratory at Colorado State University resulted in a vector system (pBJ+/-) where green fluorescent protein is incorporated into plasmids, with or without an associated protein, and used to determine protein localization in non-pathogenic Mycobacterium smegmatis. This project aims to adapt this system to generate tagged EVs from various mycobacterial species, and follow them through host infection.

Methods: Cloning was performed to modify the pJB+/- vector system so that it contains the far-red reporter mCherry, a longer lasting marker shown to be visible in bacterial tracking studies of *Mycobacterium tuberculosis*. *Mycobacterium smegmatis* with the pBJ plasmid containing mCherry targeted to the plasma membrane was cultured in 1L flasks until log phase growth was achieved. Cellular Filtrate Protein (CFP) containing EVs was collected, then filtered to remove proteins smaller than 100kDa. Size exclusion chromatography was performed to isolate mycobacterial EVs. The EVs were evaluated based on protein through mBCA and western blotting for known EV proteins and size through nanosight particle analysis. Purified EVs were then interrogated for fluorescence using a microplate reader and microscopy. Purified EVs were then applied to human macrophage cell culture and tracked by fluorescence microscopy.

102. KATE SAMMONS

Effects of ploidy level on chemotype in the *Achillea millefolium* complex

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Introduction: The *Achillea millefolium* complex is a species aggregate composed of plants occurring from diploids (2n=18) to octoploids. This genus occurs globally, and has an ancient history of medicinal use as a hemostat, vulnerary, febrifuge, digestive stimulant, anxiolytic, and antimicrobial. It has long been known that the presence of chamazulene in the essential oil is dependent on the ploidy level. However, crude hydro- or hydro-ethanolic extracts of the plant are the most common forms for medicinal use, and little is known about the effects of ploidy level on the presence and abundance of other compounds present in these preparations. We are cultivating 100+ populations of *A. spp.*, determining their ploidy level, and performing analysis by HPLC-MS on leaf and floral tissue.

Methods: Populations of the *A. millefolium* complex from the USDA and Kew MSB are cultivated in a common garden containing 5 blocks. Flow cytometry is used to determine the ploidy level of each population. Leaves and flowers are harvested for each plant at anthesis (5 plants/population), and extracted with 70% ethanol at a rate of 100 mg/mL. Extracts are analyzed with UPLC-HRMS using a reversed-phase column and gradient of 0.1% formic acid (FA) in water and 0.1% FA in acetonitrile. The mass spectrometer is set to positive and negative mode, with a scan range of 150-1000 *m/z*. The data are processed with XCMS and analyzed in Galaxy. Accurate masses and the literature are used to determine putative ID.

Preliminary Data: While flow cytometry work and harvests are underway, data is not yet available for this experiment. A related preliminary experiment surveying the range of chemotypes of *Achillea* available in commerce suggests that European *A. millefoliums* are more chemically similar to each other than to the American sources examined. Crude extracts representative of the two sources (one European and one American) were tested in an antimicrobial activity assay against *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* and visualized with the resazurin viability assay. Based on this, the hexane and water partitions exhibited differential activity against *S. aureus* and *C. albicans*, respectively, suggesting that the chemotypic differences may have meaningful clinical differences. Another preliminary experiment analyzing whether leaf chemotype is predictive of floral chemotype in *Achillea* will also be presented.

103. GRETCHEN SEIM

Analysis of arginine metabolism using LC-MS and isotopic labeling

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Arginine metabolism is linked to several metabolic pathways that are implicated in processes important in immune and vascular function, cancer cell pathology and more. Quantitative analysis of the flux through arginine metabolism pathways in response to different physiological conditions can provide valuable insight into the role of arginine metabolism in affecting fundamental cellular processes. Here we describe a method, using a $^{15}N_4$-arginine tracer, to quantitatively determine the flux through arginine metabolism reactions. Computing values of metabolic flux, begins by using relevant metabolites' labeling patterns to calculate the relative flux of reactions supplying these metabolites. This data is supplemented with the determination of exchange fluxes by measuring changes of metabolites in the media. Lastly, this data is integrated to compute absolute fluxes through relevant arginine metabolism pathways. This isotopic tracer method can reliably quantify the abundance of important intermediates and fluxes of major metabolic reactions in arginine metabolism in a variety of cultured mammalian cell models.

104. MIKAYLA SHANAFELT

Comprehensive Proteomic Analysis of Spider Fibers and Silk-Producing Glands Using Optimized Sample Preparation Methodology

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Introduction: Spider silk is a high-performance material with outstanding strength and toughness, rivaling the mechanical properties of high tensile steel and Kevlar™. In black widow spiders, the tubuliform and aciniform silk-producing glands extrude different fibers that are woven into egg cases. Previous biochemical studies of egg cases have led to the identification of Egg Case Protein 1 (ECP1), Egg Case Protein 2 (ECP2), and Tubuliform Spidroin 1 (TuSp1). For our investigations, we

plan to perform a comprehensive proteomic analysis using diverse solvents to solubilize egg cases. After fiber solubilization, we are attempting to identify new proteins that represent major constituents of egg sacs. Our main objective is to characterize the molecular composition of egg sacs, allowing us to move a step closer towards synthetic silk production. Our long-term goal is to use heterologous expression systems to produce the newly identified factors for artificial silk production.

Methods: Egg cases will be divided and solubilized in a panel of different solvents. Solubilized proteins will be subject to in-solution tryptic digestion and then peptides subject to MS/MS analysis. Several different individuals will be used for egg sac collection. Samples will be analyzed using an Orbitrap Fusion™ Tribrid™ mass spectrometer equipped with nano-HPLC. MS/MS spectra will be subject to *de novo* sequencing with PEAKS 7.5 (Bioinformatics Solutions Inc.), followed by searching against a custom protein database of spider silk proteins and common contaminants. Results will be filtered for two or more unique peptides of a False Discovery Rate of 1%.

Preliminary Data: Analysis of egg cases and glands reveals the presence of peptides corresponding to previously reported tubuliform (cylindrical) silk proteins, which included ECP1, ECP2, and TuSp1. MaSp1 and MaSp2, spidroins that are known components of dragline silk, were also found in egg cases. Additionally, the aciniform silk protein, AcSp1, was identified as a major constituent of egg cases. Furthermore, our results identified the presence of novel peptides that could not be mapped to these structural proteins, suggesting the presence of undiscovered proteins in egg case fibers. In particular, using the newly discovered peptides obtained by *de-novo* sequencing, we used reverse genetics to clone a cDNA coding for a novel protein, which we have dubbed Egg Case Protein 3 (ECP3). The ECP3 cDNA was amplified from a cDNA library prepared from silk-producing glands of black widow spiders by PCR. Translation of the ECP3 cDNA reveals a predicted protein that is approximately 11.8 kDa, consisting of over 31% glycine and serine content. Proteomic studies of the tubuliform glands, as well as quantitative real-time PCR analysis, revealed that ECP3 is exclusively expressed in the cylindrical gland and extruded into tubuliform silk. In addition to the identification of ECP3, the use of different chaotropic solvents yielded the identification of overlapping but distinct proteins in egg cases, implying that proteins in the fiber have different solubility properties.

Novel Aspect: This study is the first comprehensive analysis of spider egg case fibers using proteomics and novel sample preparation methodology.

105. JIANQIANG SHEN

Identification of PIR interactome using a Mass Spectrometry based strategy in Arabidopsis

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More than 10% coding genes in Arabidopsis thaliana genome are annotated with unknown

functions. Previous reports show that many of these genes play important roles in multiple biological processes, including abiotic stress response and hormone signaling pathways. Here we identified a protein of unknown function that acts as a transcriptional repressor. We further showed that this protein interacts with Polycomb Repressive Complex 2 physically and genetically. Polycomb Interacting Repressor (PIR) has no known functional domains. Our studies have implicated a conserved phosphorylated serine as well as phenylalanine residues in the N-terminal domain in PIR function, probably by modulating interaction with protein partners. We therefore next wish to employ Mass Spectrometry to identify the PIR interactome after IP of wild-type and variant PIR and this shed light on functionally important components of the PIR complexes.

To investigate the endogenous transcriptional complexes or chromatin complexes, like PIR complexes, we think immunoprecipitation mass spectrometry and chromatin immunoprecipitation allow us to get information on both the interactome and cistrome. We want to develop a pipeline to employ these two methods in parallel for a given protein in plants. After formaldehyde fixation, we use antibodies to immunoprecipitate the cross-linked complexes and then digest protein into peptide as well as purify the DNA. In addition, we also try to use the mass spectrometry without cross-linking to figure out the components in protein complexes. These methods would provide information to elucidate an unknown function gene, especially chromatin regulatory complexes. Additionally, the combined method will allow us to get insight into the epigenetic control of gene expression.

106. LUDWIG SINN

In Situ Structural Biology in Mycoplasma Pneumoniae Using Cross-linking/Mass Spectrometry

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Introduction: Mycoplasma pneumoniae is a human pathogen that causes atypical bacterial pneumonia and its reduced genome makes it one of the simplest known organisms. Furthermore, it is a prime candidate in the hope to model an entire cell and a large amount of quantitative data has been so far collected on its genome, transcriptome, proteome and metabolism in order to reach this goal. Information on the organization of the proteome has previously been gathered by affinity-pulldowns coupled with mass spectrometry from cell lysates. Here, we use in situ chemical cross-linking/mass spectrometry to investigate the structural organization of the cell.

Methods: Grown cells were incubated with the membrane-permeable cross-linker DSS and subsequently disrupted using an optimized extraction protocol under denaturing conditions employing the proteases LysC and Trypsin. The digests were fractionated by combining several offline-fractionation techniques, such as SCX, SEC and hSAX, to deepen the analysis. Following data acquisition via a Fusion Lumos Tribrid Instrument with optimized acquisition strategies using 100 fractions in total, the data was searched for cross-linked peptide pairs using in-house engine Xi and XiFDR for statistical evaluation. Inspection of the data by XiNET and study of cross-linked protein structures by Pymol and other tools revealed previously unknown functions of individual proteins based on their interactions, as well as their in-cell topology/existence in complexes.

Preliminary data: Here, we present the first in-cell cross-linking mass spectrometry data for this species obtained via multi-dimensional chromatographic fractionation and enrichment of cross-linked peptides from a moderately complex cellular matrix. We found 11,739 cross-links in total covering about 98 % of the quantified proteome of M. pneumoniae with a median of 15 cross-links per protein at applied FDR of 5 %. Of these links, 711 were found between proteins of which 49 % could be validated via matching to previous TAP- tag/MS protein interaction data. Additionally, we used the cross-link data to model proteins and protein complexes on existing pdb-models based on distance restraints gleaned from detected cross-linking sites that emphasized the conformational heterogeneity of proteins in their cellular environment. This is the most comprehensive cross-linking dataset ever reported and demonstrates that in situ structural biology is now possible for much of the proteome.

Novel aspects: This work discloses unprecedented detail on protein structural topology in situ and adds a robust pipeline to structural proteomics research.

107. GWENN SKAR

Identifying Novel CSF Biomarkers in a Rat Model of *Staphylococcus epidermidis* Central Nervous System Catheter Infection

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Introduction: Bacterial infection is a frequent and serious complication of cerebrospinal fluid (CSF) shunt placement for the treatment of hydrocephalus. Diagnosis of these infections relies on the gold standard of CSF culture; however, culture may not always be reliable in the setting of biofilm, slow growing or fastidious organisms, or if patients are treated with antibiotics prior to CSF culture. Novel protein biomarkers from the CSF or serum chemokines may prove useful as alternative strategies for diagnosis of CSF shunt infection, however this has not yet been reported. The objective of the current study was to characterize the CSF proteome following *Staphylococcus epidermidis* CNS catheter infection to explore the utility of identifying potential biomarkers of infection.

Methods: To assess the feasibility of evaluating CSF changes over the course of CSF shunt infection using mass spectrometry proteomics, we adapted our well-established murine CNS catheter infection model to generate infection with *S. epidermidis* in Lewis rats. The rats tolerated the procedure well and catheter placement in the lateral ventricle was verified visually. Qualitative proteomics were performed on pooled CSF samples at days 1 and 5 post-infection.

Preliminary Data: There were no technical difficulties with in gel-digest sample preparation or mass spectrometry reads.

Eighteen distinct proteins were detected in CSF at days 1 and 5 post-infection in animals with *S. epidermidis* infected catheters that were not present in the CSF of non-infected rats at equivalent time points. These 18 proteins were classified into groups that included immune function, signaling, structural proteins, and neurologic disorders and neurodevelopment. Importantly this pilot study proved the feasibility of evaluating the CSF proteome of rats with *S. epidermidis* infected CNS catheters. Qualitative proteomic mass spectrometry studies are currently ongoing in our laboratory.

Novel Aspect: Coupling host response and pathogen-associated biomarkers may be a useful tool for diagnosing shunt infection in early post-operative time periods, which is currently challenging.

108. IAN SMITH

A novel method to identify functional post-translational modification sites across the proteome

Ian R. Smith, Miguel Martin-Perez, Ricard A. Rodriguez-Mias, Kyle Hess, Anthony Valente, Ariadna Llovet, Judit Villén

Introduction: Mass spectrometry is revealing tens of thousands of post-translational modification sites, but we do not know which of these are functionally important. While bioinformatics can predict functional sites to some degree, experimental validation is preferred. Currently, there are no methods to experimentally validate multiple sites simultaneously. To address this major bottleneck, we developed a proteomic method to measure the effects of modifications on protein biochemical properties that associate with function. Among biochemical properties, protein turnover is an ideal candidate for a functional proxy because it's easy to measure on a proteome-scale and it captures all general functions of phosphorylation. Also, protein size is a useful functional proxy to identify phosphorylation events that alter protein complex dynamics.

Methods: We developed our method targeting phosphorylation in *Saccharomyces cerevisiae*. To determine protein turnover, we applied pulsed-SILAC labeling and calculated heavy amino acid incorporation. To determine phosphorylation events that alter protein complex dynamics, we implemented a size exclusion chromatography (SEC) coupled with mass spectrometry to capture protein elution profiles separated on size. We conducted all measurements on phospho-enriched and unenriched peptide samples. The novelty of our approach lies in using peptide-level readouts to assess differences between the modified and unmodified protein. Significant deviations in turnover or size between modified and unmodified peptides encode biological function.

Preliminary Results: We measured protein turnover for ~3,700 proteins and over 6,200 phosphorylation sites. Across three biological replicates, we were able to calculate differences for 2,700 of them. We determined that ~10% of the phosphorylation sites were functional based on a significant deviation in turnover from its unmodified protein counterpart. A significant fraction (75%) of these experimentally identified functional phosphorylation sites demonstrated a slower turnover compared to its unmodified counterpart, suggesting that most functional phosphorylation sites identified are protecting the protein from degradation, likely by mediating protein-protein interactions. The phosphorylation sites that increase turnover showed an enrichment in PEST-like sequence motifs, which have been implicated in promoting protein degradation. Comparing turnover for a phosphorylated peptide to its unmodified peptide counterpart further verified that phosphorylation events that change turnover are of low stoichiometry.

With our size exclusion approach, we identified more than 4,500 proteins and ~11,000 phosphorylation sites across 58 fractions. As preliminary results, we are able to compare ~7,000 phosphorylated proteoforms to their unmodified counterparts. Although we are still developing a robust statistical method to identify phosphorylation events that change protein complex dynamics globally, preliminary results demonstrate that using a size exclusion MS strategy is sensitive for identifying phosphosites that change its unmodified protein's elution profile. By manual validation of the phosphorylation events that significantly changed protein turnover, we observed many of these events had SEC elution profiles that deviated from the unmodified protein. Many slow turnover phosphosites were largely in fractions with larger size, while many faster turnover phosphosites were in fractions with smaller sizes when compared to their respective unmodified protein.

Our study focused on protein phosphorylation and yeast. However, this method and analysis approach can be readily applied to study the functional impact of other post-translational modifications in the proteome of yeast and other organisms.

Novel Aspect: First method to identify functional post-translational modification sites on a proteome-wide scale.

109. DEANDRAE SMITH

Impact of microwave drying on mechanical and functional characteristics of parboiled rough rice

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The volumetric heating phenomenon provided by the use of a microwave offers a means to quickly dry high moisture content (MC) paddy in one-pass with minimal impacts on the kernel quality. The objectives of this research were to investigate the efficacy of an industrial microwave for implementation in parboiling operations. The specific objectives of this research are to investigate the implications of using a 915 MHz industrial microwave process to dry high-MC parboiled rice. Parboiling conditions consisted of soaking temperature 76oC, soaking duration 3 h and steaming durations of 5, 10, 15 and 20 mins. Long-grain rough rice (Cv. Chenier) was dried using a microwave dryer set to transmit energy at power levels that ranged from 1 to 8 kW. The study evaluated the implications of microwave treatments on rice final MC, milled rice yield (MRY), head rice yield (HRY) and pasting properties. Results show that one pass drying of parboiled rough rice was possible (final MC 15% w. b) with minimal impacts on the quality. Additionally, increasing power levels up to 6 kW had minimal effects on the MRY (73%), after which the MRY reduced by 4% point. A similar reduction was seen for HRY. Rice treated at microwave powers <= 6 kW had average HRY of 67%. Beyond 6 kW the HRY reduced by 3, 20, 5 and 11% points for samples soaked for 5, 10, 15 and 20 min duration, respectively.

110. UNNATI SONAWALA

Toward understanding the role of amino acid transporters in nutrient acquisition by biotrophic pathogens

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Hyaloperonospora arabidopsidis (*Hpa*) is a naturally occurring oomycete pathogen on *Arabidopsis thaliana*. It is related to downy mildews of economically important crops such as cabbage, kale and broccoli, belonging to the *Brassicaceae* family. Downy mildew pathogens are obligate biotrophs that extract nutrients exclusively from living plant cells. Similar to other biotrophic pathogens, *Hpa* has lost the ability to assimilate inorganic nitrogen and sulfur. It would thus have to acquire these nutrients from the host in an organic form; possibly amino acids. We have found that loss of function mutants of certain Arabidopsis amino acid transporters display enhanced disease resistance to *Hpa* compared to a wild type plant. We hypothesize that these transporters directly or indirectly play an important role in *Hpa*'s mechanism to acquire nitrogen from the host. It is conceivable that knocking out these host amino acid transporters reduce flux of amino acids towards the pathogen. We have developed transgenic lines using GUS and mCherry reporters for these transporters and are using them to unravel the role of promoter and protein localization in their interaction with *Hpa*. Further, knocking out an amino acid transporter possibly leads to metabolic changes in the plant that adversely affects the pathogen, either through changes in nitrogen pools and/or a change in metabolites involved in defense responses. We are working towards characterizing these changes in our candidate mutants. To successfully colonize a plant, a pathogen must be able to achieve both suppression of plant immunity and acquisition of nutrients from the plant host. While the former has been well studied, research on the latter is sparse. Together, above described studies will pave way to increase our understanding of one set of potential players in nutrient acquisition by pathogens.

111. TORI SPEICHER

A Quantitative Mass Spectrometry Approach to Understanding Cellulose Synthase Complex (CSC) Composition and Protein-Protein Interactions in *Physcomitrella patens*

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Cellulose is the most abundant biopolymer on the planet, and the primary load-bearing polysaccharide component of plant cell walls. Cellulose biosynthesis is catalyzed at the plasma membrane by the cellulose synthase complex (CSC). Seed and non-seed plants both independently evolved from a common ancestor to contain CSCs with a rosette structure and 6-fold symmetry. In the model seed plant *Arabidopsis*, hetero-oligomeric CSCs contain three functionally distinct cellulose synthase A proteins (CESAs). Conversely, *Physcomitrella patens*, a model non-seed plant, contains seven CESA isoforms that span two clades. The CESA composition of non-seed plant CSCs is currently unclear. To address this question, we pursued a quantitative proteomic approach. We generated solubilized extracts from wild-type *Physcomitrella* (Gd11) and HA-tagged transgenic *PpCESA3,* and *PpCESA5* strains. Each of these samples were separately subjected to immunoaffinity chromatography on anti-HA magnetic beads, and the resulting anti-HA eluates of three replicate wild-type control or HA-PpCESA samples were independently labeled with a unique isobaric tag. I performed quantitative mass spectrometry analysis to identify protein-protein interactions between several of the PpCESA isoforms in the resulting mass spectrometry samples. In the PpCESA3 IP experiments, *PpCESA3, 8,* and 6/*7* were identified with an abundance enrichment of 10.9, 7.8, and 6.2 respectively in comparison to wild-type, suggesting that these subunits constitute a stable CSC. Conversely, *PpCESA5* was the only PpCESA identified in PpCESA5 IP samples, with a 40-fold enrichment compared to wild type control samples. These data suggest that non-seed plants, such as *Physcomitrella*, display hetero-oligomeric CSCs and novel homo-oligomeric CSCs depending upon cellular context, which further supports the hypothesis of convergent evolution between seed and non-seed plant CSCs. These data also suggest a specialized role for homo-oligomeric *PpCESA5* CSCs and support a neutral evolution mechanism for the formation of higher plant hetero-oligomeric CSCs.

112. ROBERT SPRUNG

A High-multiplex PRM Assay for Global Kinase Quantification and Pan-cancer Analyses

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Introduction: Kinase drugs have demonstrated remarkable success in the treatment of cancer with 37 approved small inhibitors in the clinic and a pipeline of ~250 under development. Broad specificity of single inhibitors has been demonstrated for many drugs using chemical proteomics with inhibition of multiple kinases and pathways (Science. 2017. (358) 1148-1164). There is a need for absolute quantification of global kinase activities and levels with limited quantities of samples and with throughput to support clinical cohort studies. We have developed a single LC-MS assay for ~200 kinases and have applied this assay to the measurement of kinase levels in breast cancer (primary tumors and patient-derived xenografts), ovarian and uterine cancer, and acute myelogenous leukemia after enrichment with multi-inhibitor beads (MIB-PRM).

Methods: Proteotypic peptides were selected according to CPTAC recommendations (Clin. Chem. 2016 Jan; 62(1): 48–69) and were synthesized as high purity natural abundance and stable isotope-labeled pairs for 440 peptides. Lysates from breast, ovarian, and uterine cancers were prepared from cryopulverized powder as previously described (MCP. 2014. July; 13(7): 1690-704) and tryptic digests were prepared for spiking with labeled peptide standards. LC-MS analysis was performed using Q-Exactive mass spectrometer. Assays were developed as Tier 2 assays (Mol. Cell. Proteomics. 2014; 13:907–917). The 2h PRM method used time windows of 8 min with a minimum of 8 MS2 scans acquired for each peptide.

Preliminary Data: We developed a validated assay panel for 212 kinases using the guidelines of the Clinical Proteomics Tumor Analysis Consortium (http://assays.cancer.gov). The panel includes representatives of 9 kinase families (AGC, atypical, CAMK, CK1, CMGC, lipid kinase, STE, tyrosine kinase, tyrosine kinase-like). Peptide response curves were generated in peptide carrier solution (low complexity matrix), to determine linear response ranges and chromatographic performance of peptides for optimization of precursor ion concurrency, and in patient derived xenografts (PDX; high complexity matrix) to determine the prevalence of interfering signals and determine limits of detection (LOD). Response curves in patient-derived xenograft (PDX) matrix were successfully generated for 415 of the 440 peptides, yielding sub-femtomole limits of detection for 366 peptides. The result is a robust, well-characterized assay panel suitable for sensitive quantitative assessment of kinase expression in patient tumor samples.

Following characterization of the assay panel, kinase quantitation in patient samples was performed by stable isotope dilution. Isotope-labeled peptides corresponding to the 440 peptides of interest were spiked into trypsin digested, patient samples

representing a variety of cancers including 11 ovarian, 11 endometrial, 16 colorectal, 9 leukemia and 9 breast cancer samples. In total, 276 peptides representing 180 kinases yielded quantitative information in at least one cancer sample.

We analyzed 11 breast cancer PDX models and found that the Her2 positive tumors had levels of Her2 that averaged 17.9 fmol/mg protein, much greater than the average of the other non-Her2 tumors where the level of Her2 was not detectable. In contrast, Her2 was detected in 9 of 9 breast cancer patient samples and we observed significant overlap in Her2 quantity between tumors designated +3 and those with no Her2 detectible by IHC. Her2 was also detected in 2 of the 11 endometrioid uterine cancer tumors whereas Her2 was not detected in the ovarian cancer tumors.

113. NILUSHA SUDASINGHE

Characterization of Microalgal Biomass and Co-Products for Optimization of Biochemical Composition in Microalgae to Reduce the Cost of Advanced Biofuels

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Introduction: Microalgae are a promising advanced biofuel feedstock, and their potential for developing valuable co-products increases their promise as an economically viable energy alternative. Major biochemical constituents of algae (i.e. lipids, proteins, carbohydrates) have a direct impact on the biofuel quality, yield and cost, as well as the development of co-products. Within the DOE-funded project, Producing Algae and Co-Products for Energy, we aim to develop genetically engineered microalgae strains with optimized biochemical composition for enhanced coproduction of high-value products and biofuel with the goal of reducing fuel costs to <$5 per gallon of gasoline equivalent. Guar gum, a polysaccharide composed of galactose and mannose is one of the co-products we aim to produce which can reduce the fuel costs by 30-50%.

Methods: Characterization of algal biomass generated in our research efforts of strain development and improvement is performed as follows: Algal lipids are characterized by *in situ* transesterification of lipids to fatty acid methyl esters and analysis by gas chromatography coupled with flame ionization detection. Total carbohydrates are quantified spectrophotometrically by the 3-methyl-2- benzothiazolinone hydrazone (MBTH) method. Protein content is calculated by measuring the elemental nitrogen content and multiplying by a nitrogen-to-protein conversion factor of 4.78. A high performance liquid chromatographic (HPLC) method was developed for the quantification of guar gum in the genetically modified (GM) microalgae strains that will be produced during the project.

Preliminary Data: The proximate analysis methods were established, validated, and utilized to determine the biochemical composition of algae biomass during culture growth and nutrient depletion. The HPLC method developed for guar gum quantification was validated using commercially available guar gum isolated from the endosperm of the guar bean. The experimentally calculated mannose-to-galactose ratio (i.e. 1.6) was within the range of literature values (i.e. 1.3-2.0) reported for guar seed. As the goal of the project is to produce guar gum with a 5% total biomass harvestable yield, the sensitivity of the HPLC method was tested by spiking *C. sorokiniana* biomass with varying levels (0%, 2%, 3.5%, 5%, 10% wt.) of commercial guar gum. The results indicate that the developed HPLC method can be directly applied to quantify guar gum at our goal wt% in the genetically engineered strains. We will also report the data from guar gum quantification in genetically engineered algal strains.

Novel Aspect. Guar gum production in algae is new. This HPLC method can be used to characterize guar gum in GM strains.

114. FANGXU SUN

Global Analysis of Secreted N-Glycoproteins in Human Cells using Click Chemistry-Based Enrichment and Mass Spectrometry

Fangxu Sun, Haopeng Xiao, and Ronghu Wu*

Secreted proteins are of great importance to maintain the intercellular communication and mediate diverse physiological processes including metabolism and immunity. Besides, the status of diseased cells such as cancer metastasis is often reflected by secreted proteins, which can be used to serve as disease biomarkers. It is a daunting task to systematically analyze secreted proteins in cultured cells because secreted proteins are low abundant compared to highly abundant proteins in commonly used Fetal Bovine Serum (FBS). Secreted proteins are frequently glycosylated, which may protect them from degradation

and increase the chance to be detected. Here secreted N-glycoproteins were metabolically labeled with a sugar analog and the analysis of N-glycoproteins was achieved by coupling click-chemistry-based enrichment with MS-based proteomics.

A sugar analog with an azide group, N-azidoacetylgalactosamine (GalNAz), was used to label secreted N-glycoproteins in MCF7 cells. Media was harvested by centrifugation and concentrated with 10 kDa filters. Subsequently the enrichment reaction was performed by using alkyne derivatives including DBCO-sulfo-biotin, DBCO-magnetic beads, DBCO photo-cleavable (PC) linker-magnetic beads and alkyne-magnetic beads. For DBCO-sulfo-Biotin, NeutrAvidin beads were used to capture biotinylated proteins and subsequently were released by PNGase F. After click chemisty or copper-free click chemistry, the beads were wahsed thoughly followed by on-bead digestion. The glycopeptides were eluted by PNGase F and analyzed by LC-MS/MS (LTQ-Orbitrap). For PC modified beads, UV light was used to release the glycopeptides before PNGase F treatment.

The global analysis of secreted N-glycoproteins was achieved by integrating metabolic labeling, click-chemistry-based enrichment and MS-based proteomics. Different modified beads were investigated for enrichment. DBCO PC-magnetic beads outperformed DBCO-sulfo-biotin enrichment and DBCO-magnetic and alkyne-magnetic beads. By using PC DBCO beads, 166 unique glycopeptides from 102 glycoproteins with motif NXS/T (X is any amino acid residue except proline) were identified, while 100 and 32 unique glycopeptides were identified by DBCO and alkyne beads, respectively. An example of the identified glycopeptide is SVHN#VTGAQVGLSCEVR (# represents the glycosylation site). This glycopeptide was confidently identified with a XCorr value of 4.6, and based on the fragments, the glycosylation site was well localized at N166. This glycopeptide comes from secreted IGFBPL1, one kind of IGF-binding proteins, which inhibits or stimulates the growth promoting effects of the IGFs in cell culture and alters the interaction of IGFs with their cell surface receptors. The Gene Ontology clustering of the identified glycoproteins indicates that most of the proteins are located at extracellular region. Protein clustering based on molecular function and biological process shows the proteins possess catalytic activity, and receptor binding, which are closely related with protein extracellular activities. More than 90% of the identified N-glycoproteins contained a signal peptide according to the SignaIP 4.1 prediction, which indicates that the vast majority of the identified proteins might be secreted through the classical secretory pathway.

Without FBS starvation and with the interference from highly abundant FBS proteins, we globally and site-specifically analyze secreted N-glycoproteins in MCF7 cells.

115. DAN TAN

Novel FAHFA-containing Lipids and Their Role in Metabolic and Inflammatory Diseases

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Introduction: FAHFAs (Fatty Acid ester of a Hydroxy Fatty Acids) is a class of endogenous lipids which have demonstrated potent anti-diabetic and anti-inflammatory activity. However, FAHFAs are about 100-fold lower in concertation than free fatty acids, making it unlikely that these lipids are depots for lipid storage. Triacylglycerols are considered to be depots for fatty acid storage during nutritional rich and are hydrolyzed to release fatty acids under fasting conditions. In this work, we report the discovery of a novel class of triacylglycerols which contain a FAHFA in place of one of the three fatty acids (FAHFA-TAGs). This finding reveals a link between TAGs and FAHFAs that could have a role in metabolic health, cardiovascular function, and inflammation.

Methods:

- Lipids were extracted using a modified Bligh-Dyer method. Mouse tissue were Dounce homogenized in a mixture of 1:1:2 PBS: methanol: chloroform. ^{13}C-PAHSA-TAG standards were added to chloroform prior to extraction. The resulting mixture was centrifuged to separate organic and aqueous phases. Organic phase containing lipids was collected and dried.
- 3T3-L1 adipocyte differentiation was induced in DMEM with 10% Fetal Bovine Serum (FBS) using 500 μM IBMX, 5 μg/ml insulin, and 5 μM dexamethasone on day 0. Induction of lipolysis with or without inhibitors was performed on day 10 of induction.
- Lipid extracts were separated on a Kinetex C18 reversed-phase column (2.6 μm, 100 Å, 150 x 2.1 mm, Phenomenex) using a 68 min gradient at flow rate of 0.25 ml/min. FAHFA-TAGs were measured on a TSQ Quantiva mass spectrometry via Multiple Reaction Monitoring (MRM) in positive ionization mode.

Preliminary Data:

- We synthesized a variety of unlabeled and isotopic labeled FAHFA-TAGs, which allows identification and quantification of this new type of lipids.
- We detected various FAHFA-TAG species in the lipid extract from adipose tissue of WT mouse, which differ in the FAHFA moiety and the two other fatty acid moieties attached to the glycerol backbone. To validate this discovery using an orthogonal method, we hydrolyzed a pool of TAGs from adipose tissue under basic conditions that can cleave the ester bond to release the FAHFA from the FAHFA-TAG. FAHFAs were detected after hydrolysis but not before, confirming the existence of FAHFA-TAGs in adipose tissue.
- To determine whether FAHFA-TAGs can be synthesized endogenously, we incubated differentiated 3T3-L1 adipocytes with a ^{13}C isotope labeled FAHFA. We observed that ^{13}C-FAHFA is incorporated into FAHFA-TAG species in lipid extracts from cells treated with ^{13}C-FAHFA but not from cells treated with vehicle, suggesting that adipocytes have the enzymes to synthesize FAHFA-TAGs.
- FAHFAs levels were increased when lipolysis was induced in differentiated 3T3-L1 adipocytes and the increase was blocked when the adipocytes were co-treated with a lipolysis inducer or a lypolysis inhibitor, suggesting that FAHFAs levels can be regulated by FAHFA-TAG metabolism.

Novel Aspect: This work reported a structurally novel class of lipids, which can provide a deeper understanding of metabolic and inflammatory physiology.

116. SYDNEY THOMAS

Mechanisms of Epi-metabolites: discovering how bacteria affect host epigenetic states

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Introduction: Short chain fatty acids (SCFAs), one of the primary fermentation products of gut bacteria, have many beneficial effects on host health. However, the mechanism behind these effects is unclear. We recently discovered that SCFAs can act as epigenetic metabolites, dramatically affecting histone modifications in host tissues. Histones are the fundamental unit of chromatin – the structure that organizes eukaryotic DNA in the nucleus. Modifying histones changes the accessibility of DNA and thus ultimately affects transcription. The fact that bacterial metabolites may change the organization of host DNA is intriguing, but we currently do not understand how these metabolites interact with host cells. To investigate these mechanisms, we use mass spectrometry to track SCFAs through cellular metabolism and onto histones.

Methods: We plan to use ^{13}C-labeled SCFAs to track carbon metabolism using mass spectrometry. To do so, we will treat HCT-116 (colon cancer) cells with fully-labeled ^{13}C-acetate, propionate, or butyrate over a time course from 2-24 hours. Metabolites will be measured using our Orbitrap, while histone deposition will be measured with our in-house histone protocol and newly-developed methods for ^{13}C-labeled acetyl- and acyl-group quantification. Results from these experiments will identify possible pathways, which we will then attempt to disrupt by knocking-out key metabolic proteins. Combining knockout cell lines with ^{13}C-labeling will allow us to pinpoint which metabolic pathways contribute to SCFA metabolism and histone deposition.

Preliminary Data: A dose-curve treatment of HCT-116 cells with unlabeled acetate, propionate, or butyrate resulted in diverse changes in histone modifications on multiple histone peptides. Changes after propionate and butyrate treatment were especially dramatic, producing up to 32-fold increases in histone acetylation. A preliminary analysis of cells treated with 10 mM of ^{13}C-labeled SCFAs revealed ^{13}C deposition on the lysines of histone H4 after propionate and butyrate treatment, suggesting that SCFA carbons may be directly added to histone tails.

Novel Aspect: This project links metabolomics to our in-house histone PTM analysis to track bacterial metabolites through host cellular metabolism.

117. CHRIS THOMAS

Discovery of Natural Product Inhibitors of New Delhi Metallo Beta-Lactamase 1

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Introduction: Carbapenem Resistant *Enterobacteriaceae* (CRE) is a growing health concern in desperate need of new treatment options. New Delhi metallo beta lactamase 1 (NDM-1) is the newest member of the metallo beta lactamase (MBL) family of carbapenemase enzymes that contribute to CRE antibiotic resistance. NDM-1 is the most prevalent MBL CRE infection in the United States. At present, there are no clinically relevant inhibitors for NDM-1. A cell based screen and an LC/MS based screen were developed to identify NDM-1 inhibitors from a library of marine actinomycete derived natural product extracts. Assay guided fractionation of active fractions coupled with HRMS and NMR led to the discovery of new natural product inhibitors of NDM-1.

Methods: BW27749 *E. coli* cells were transformed with pBAD33-NDM1, a vector constructed to homogenously express NDM-1 in a dose-dependent manner with inducer molecule L-arabinose. Natural product libraries generated from marine actinomycetes were screened against these cells in the presence and absence of ceftriaxone, a beta-lactam antibiotic and substrate for NDM-1. Growth inhibition was determined for both conditions to assess the NDM-1 inhibition and antimicrobial potential of fractions. A second screen was employed using LC/MS to confirm inhibition. Inhibitory fractions were incubated with NDM-1 producing cell lysate and substrate ampicillin. The reaction mixture was analyzed using LC/MS to assess the degree of ampicillin hydrolysis and NDM-1 inhibition. Activity guided fractionation from these methods led to the discovery of natural product inhibitors of NDM-1.

Preliminary Data: One 96-well plate of fractionated extracts from 20 marine actinomycetes was screened (n=3) against NDM-1 producing *E coli* cells. Of the 79 fractions screened, 9 showed greater than 50% growth inhibition when screened in conjunction with beta-lactam antibiotic ceftriaxone at 1/4 its MIC. DMSO and L-captopril, an established inhibitor of NDM-1, were used as negative and positive controls respectively. The Z' score for this screen was 0.7. These 9 fractions were screened again in the absence of ceftriaxone to assess antimicrobial activity (n=5). 3 of the 9 fractions were shown to be antimicrobial with a growth inhibition of greater than 50% and were not analyzed further. For the LC/MS based screen, only L-captopril has been assessed so far in the development of this assay. L-captopril was capable of reducing the amount of hydrolyzed ampicillin (nominal *m/z* 368.13 $[M+H]^+$) observed in the chromatogram in a dose dependent manner when incubated with cell lysate containing NDM-1. Our next step is to incubate inhibitory fractions with cell lysate containing NDM-1 and determine inhibition using this method. Activity guided fractionation is currently ongoing for the 6 active fractions with inhibitory potential toward NDM-1 and low antimicrobial activity. Cultures of each of the 6 strains have been grown in 1 L cultures, extracted and fractioned in an identical manner as was done for the libraries to generate more material from which to work from. The active fraction from *Streptomyces* strain WMMB 276 has been fractioned further using HPLC to identify the active component. HRMS analysis led to *m/z* 464.1699. Structure determination using NMR is currently in progress. Results of the two screening methods, as well as all structures found to inhibit NDM-1, will be presented.

Novel Aspect: Mass spectrometry was employed to assess inhibition of NDM-1 from fractionated natural product extracts.

118. LENA VINCENT

A Chemical Ecosystem Selection Approach for Generating Evolvable Chemical Systems *in Vitro*

Introduction. How does life emerge from non-living components? Some theorists have suggested that adaptive evolution initiated when autocatalytic chemical sets became spatially localized on a mineral surface. These would tend to become better at collective propagation and colonizing new mineral surfaces by neighborhood selection. Under this model, life-like chemical systems (LCSs) that can propagate and evolve adaptively might arise quite easily. Indeed, the main challenge may not be to find conditions that allow LCSs to emerge, but rather to detect them once they have arisen. Motivated by this theory, we have deployed an experimental framework analogous to artificial ecosystem selection used in microbial ecology called *chemical ecosystem selection* (CES) to find life-like systems based on their capacity to respond to selection.

Methods. The CES approach involves incubating complex mixtures of chemicals with mineral grains and performing regular serial transfers to select for surface-associated systems that are better at being transmitted among grains. The composition of

the chemical mix is reminiscent of the results of Miller-Urey-type experiments and includes amino acids, nucleobases, and other organics. The minerals used in our CES experiments are pyrite and montmorillonite, both of which are known to catalyze peptide and nucleic acid polymerization reactions. Sources of chemical energy in the form of inorganic ions out of equilibrium and activating compounds such as ammonium persulfate and ATP are included to fuel the formation and propagation of LCSs.

Preliminary Data. We have identified a number chemical proxies for energy dissipation as first-line indicators that interesting reactions are occurring in a given vessel. To date, we have optimized protocols to track changes in redox state using NADH as a reporter and to quantify the concentrations of inorganic ions, such as ammonium, nitrate, or inorganic phosphate released by the hydrolysis of ATP. These methods allow us to look for directional changes over generations and compare a set of samples after many generations of selection to a control set established just one generation earlier. We have carried out 50 generations of serial transfers in our current CES iteration and have not found LCSs using the chemical proxies described above. However, we are currently improving the throughput of the protocol to facilitate a more efficient search of chemical parameter space and increase the likelihood of finding LCSs, as well as diversifying the methods by which we search for potential LCSs that may have arisen in a given reaction vessel. Because we include amino acids and nucleobases in our chemical mixtures as precursors for the formation of autocatalytic sets composed of small peptides and/or nucleic acids, we are exploring the use of high-performance liquid chromatography (HPLC) combined with mass spectrometry (MS) as a detection method. The formation of polymers in samples exposed to multiple generations of selection of serial transfers would constitute convincing evidence that an autocatalytic set capable of collective propagation and adaptive evolution had emerged. However, the LC-MS results will have to be combined with energy-dissipation profiles to confirm that autocatalysis is occurring, which would manifest as a non-linear consumption of available chemical energy. Even if we are not successful in detecting LCSs in our experiments, the results will advance our understanding as to how evolvable chemical systems could have emerged on prebiotic Earth and how readily they might arise elsewhere in the universe.

Novel Aspect. This approach has the potential to reveal critical new insights into how living systems emerge from abiotic conditions.

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119. BURCU VITRINEL

New protocols to monitor proteome dynamics in the developing chordate heart.

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During chordate development, pluripotent cells are specified to their terminal fates. Differentiation of pluripotent progenitors to tissue-specific cells requires highly dynamic regulation at both the transcriptional and post-transcriptional levels. This regulation occurs in a tissue-specific manner where each lineage giving rise to a single tissue has a unique set of players executing said regulation. While our understanding of transcriptional regulation is rapidly growing, post-transcriptional regulation in development is often overlooked due to the challenging nature of proteomics research when only small samples are available. To investigate the post-transcriptional regulation in heart development, we exploit the simple chordate tunicate Ciona as a model organism. As tissue-specific markers begin to be expressed hours after fertilization, we can distinguish and FACS-purify specific differentiating cells very early on.

We are developing proteomics methods to process these samples with as few as ~1,000 cells which is fewer than has been used in existing small sample protocols. During method development of low-input proteomics using quantitative mass spectrometry, we interrogated many aspects of the benchmarked proteomics workflow from front-end sample preparation, although focused on the mass spectrometry analysis. We have successfully developed a low-input proteomics workflow that allows robust protein identifications using only 1,000 FACS-purified lineage specific Ciona cells with high reproducibility, using isobaric peptide labeling. Using this protocol in ~1,000 FACS-purified cells per condition, I reproducibly identified and quantified ~2,000 proteins. We are now in the process of profiling the cardiopharyngeal lineage with respect to post-transcriptional regulation.

120. KARA R. VOGEL

γ-Hydroxybutyrate (GHB): phase I/II MS-metabolite identification and profiling of novel conjugates in acute and chronic models of GHB exposure

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Introduction: Gamma-hydroxybutyric acid (GHB) use as a recreational drug, adjuvant for drug-facilitated sexual assault, and FDA-approved therapeutic for narcolepsy continues. Misuse of GHB has dictated the need for forensic toxicologists to add GHB to their screening panels, yet the short half-life of GHB (3-5 h) provides challenges in identification. To circumvent this challenge, many groups seek to identify GHB metabolites with longer half-lives. We acquired biospecimens from patients with succinic semialdehyde dehydrogenase deficiency (SSADHD), a defect in GHB metabolism that results in the chronic elevation of GHB in physiological fluids and identified novel metabolites of GHB through combined high-sensitivity multiple reaction monitoring (MRM) on a triple quadrupole mass spectrometer (API 6500) and high-resolution mass spectrometry (Thermo QExactive).

Methods: Human specimens (urine, plasma, and CSF) were obtained (IRB, 12678) from SSADHD patients and healthy volunteers (pooled, n=3-5), serum from SSADHD mice and from mice treated with the GHB prodrug, gamma butyrolactone (GBL), were obtained (IACUC 4232). Prepared samples were injected onto a Schimazdu UPLC system for absolute quantification of GHB-Gluc and $^{2}H_{4}$-GHB-Gluc. Novel metabolites were identified using adapted chromatography and high-resolution MS. For candidate GHB metabolites, MRM methods were optimized by repeat injection of samples, and identification was confirmed either with an authentic calibration standard or by semi-quantification in mouse plasma during a time course following GBL administration.

Preliminary data: First human samples were analyzed, and despite the clear accumulation of GHB in patient urine, we found no correlation between GHB levels and glucuronide or sulfate derivatives. Second, we identified structures consistent with acetylation, trioxidation, and glucose or amino acid conjugation that were elevated in patient urines (>10-fold). High-resolution mass spectrometry could not confirm the identity of these novel conjugates, but did demonstrate that acetylation and trioxidation are likely accurate designations. MS/MS fragmentation was compared to an authentic standard of the trioxidative GHB product, which had not previously been detected in humans. In the absence of additional authentic standards for conjugates and for the acetylated metabolite, SRM methods were successfully developed by repeated injection of samples to optimize MS parameters. Wild-type mice, we then administered a bolus of γ-butyrolactone and metabolites were semi-quantified in the sera at multiple time points (0-6 h). The glucose conjugate and acetylation product steadily increased from baseline levels in mouse sera for six hours following a bolus delivery of GBL. The trioxidation metabolite was at its maximum levels after 20 minutes (the first post-dose timepoint) and decreased in a time-dependent manner over the next 6 h, to approximately 75% the maximum levels, indicating a long half-life in mouse (>12 h). An authentic standard of this metabolite was obtained and MS/MS fragmentation and confirmed similarity. Further optimization is underway to validate this metabolite as a potential biomarker for GHB intoxication.

Novel Aspect: A unique set of patient samples and a metabolite identification framework has identified potential GHB metabolites with longer half-lives.

121. NGOC VU

Ozone-induced dissociation mass spectrometry as a new tool to determine the C=C double bond locations in natural products

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Introduction: Natural products are one of the major source of therapeutic agents in treatment of cancer, viral and bacterial

infection. The location of unsaturation within natural product determines its bioactivity and is a major factor in the observed anticancer, antiviral, and anti-inflammatory activities. Currently NMR is the primary tool in structural elucidation of natural products, but it is time consuming and challenging to identify the location of the double bonds within the complex molecular structure of natural products. Ozone-induced dissociation mass spectrometry (OzID-MS) is well established in identifying the C=C position with the fatty acyls of lipids, herein, we extend this technology to structural analysis of natural products by demonstrating its utilities in C=C position assignment in various environments.

Methods: Purified polyketides containing different chemical motifs: limonene, polyene, aromatic, and conjugated C=C were dissolved in methanol in 10 mM sodium acetate. Each compound was directly infused, electrospray ionized and analyzed using our established OzID-MS approach. OzID-MS was set up in a traveling wave mass spectrometer Synapt G2 (Waters Inc.) An O3MEGA integrated ozone delivery system (MKS Inc.) was used for *in-situ* production of ozone from high-purity oxygen. The generated ozone/oxygen mixture was connected to the mass spectrometer trap/transfer gas supply, with the excess ozone converted back to oxygen before venting to the laboratory exhaust system. To aid in structural assignment of OzID fragment ions, high resolution accurate mass spectrum was acquired for each compound using external calibration.

Preliminary data: The polyketides (Satorypyrone A, AA03390, Sorbicilin and Visnaginone) used in this study were structurally determined by NMR, which contain C=C positioned in linear chain or ring structures, and existed as either single or conjugated C=C. Sodiated adducts generated in electrospray ionization were selected for OzID. To confirm the fragment ions are originated exclusively from OzID and not from collision induced dissociation, we repeated the analysis in argon gas under the identical setting as those of OzID.

Under OzID settings, the C=Cs in the polyene were highly reactive with ozone and resulted in abundant OzID fragment ions (aldehyde and Criegee), which are characteristic ions pinpointing the location of double bond at C2 and C6 for Sartorypyrone A, at C4 and C6 for AA03390, and at C2 and C4 for Sorbicilin. In addition, the conjugated double bonds in polyketides could be easily determined by the presence of ion at $[M+Na+16]^+$, the formation of an epoxide with ozone. Furthermore, we observed that if the polyketides contains an endocyclic C=C, a corresponding ozonide at $[M+Na+ 48]^+$ appears. Alternatively, molecules contains aromatic ring structure can be cleaved to form aldehyde/ketone and Criegee product ions.

Novel aspect: Complementary to NMR, OzID-MS is a promising tool for accurate structural elucidation of C=C unsaturation in natural products.

122. DIANE WALLACE

Detection of contamination by the herbicide triasulfuron in botanical dietary supplements utilizing untargeted UPLC-MS metabolomics

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Introduction: Adulteration and contamination within the botanical dietary supplement industry has become a prevalent issue due to challenges with regulation by the Food and Drug Administration (FDA) of the United States. The FDA itself does not regulate dietary supplements unless adulteration/contamination has been proven to be present. Several targeted approaches have successfully been utilized to detect altered supplements, however, there needs to be a great deal of prior knowledge to determine specific target compounds. The goal of this study is to develop an untargeted technique so that samples that contain adulterants or contaminants may be detected without prior knowledge of contaminant identity.

Methods: Ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS) was used to analyze a sample set of 11 commercial green teas as well as a standard for green tea leaves from NIST. One of the 11 green teas was selected to be intentionally contaminated with triasulfuron, an herbicide used on grass and wheat for cattle. The triasulfuron was added prior to extraction of the green tea leaves, in small volumes varying depending on the desired concentration, and allowed to dry. The samples were then extracted in the same manner, and in triplicate, then reconstituted to a concentration of 1 mg/mL for UPLC-MS analysis. The data were processed using MzMine software and multivariate statistical analysis was performed using the software Sirius.

Preliminary Data: Principal component analysis (PCA) was used to view the metabolomics data. Several of the scores plots (PC1 vs PC2, PC1 vs PC3, PC1 vs PC4) showed a trend in concentration. The more triasulfuron in the sample, the higher the peak area, and the more variance caused by the peak. In contrast, the lower the concentration of triasulfuron, the smaller the variability until the peak was no longer significant within the statistical analysis and the contaminated sample clustered

with the green tea instead of being identified as an outlier. The corresponding loadings plot confirmed that the contaminated samples were distinguished from the others based on the presence of traisulfuron rather than chemical interference or noise. The limit of detection for this compound on a high resolving trap Orbitrap mass spectrometer (Q-Exactive Plus) was a concentration of 10 ppb (expressed as mass triasulfuron per mass of tea leaf). Below this concentration, tea samples spiked with triasulfuron clustered with uncontaminated samples. Additional, higher concentration samples (1.0, 7.5, 15, 25, 50., 60., and 120 ppm) were successfully identified as outliers in the metabolomics analysis.

Novel Aspect: The limit of detection for untargeted metabolomics for detection of adulterants/contaminants is established using green tea spiked with a pesticide as a model system.

123. TAO WEI

IQGAP1, a PI3K Signal Scaffolding Protein, Is Necessary for Efficient Head and Neck Carcinogenesis

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is a common cancer in humans. Phosphoinositide 3-kinase (PI3K)/AKT signaling, along with its downstream effector mTOR, is one of the most frequently altered pathways in HNSCC, and is downstream of growth factor signaling including that mediated by epidermal growth factor receptor

(EGFR), which is also commonly upregulated in HNSCC. Recently, IQ motif-containing GTPase activation protein 1 (IQGAP1) has been reported to function as a scaffold for the enzymes involved in the PI3K/AKT signaling pathway. *Iqgap1* gene expression is increased in human HNSCCs, raising the hypothesis that it acts as an oncogene in this cancer. Whether IQGAP1 is necessary for HNSCC development as well as what is its underlying mechanism are both unknown.

Methods: We tested the role of IQGAP1 in HNSCC by performing a combination of *in vitro* studies using human cancer cell lines using a cell permeable peptide that interferes with the ability of IQGAP1 to bind to PI3K, and *in vivo* studies using a well-validated preclinical mouse model for HNSCC that is known to depend upon EGFR signaling. A synthetic oral carcinogen, 4-nitroquinoline 1-oxide (4NQO), is used to drive head and neck tumorigenesis in mice. Iqgap1$^{+/+}$ (n=19) and Iqgap1$^{-/-}$ (n=16) experimental groups were given 4NQO in their drinking water for 16 weeks, followed by 5 weeks of normal drinking water.

Preliminary Data: Human cancer cells knocked out for IQGAP1 by CRIPSPR-Cas9 lost AKT signaling. Disruption of IQGAP1-scaffolded PI3K/AKT signaling using a peptide that interferes with the ability of IQGAP1 to bind to PI3K reduced HNSCC cell survival. *In vivo* studies utilizing *Iqgap1*-null (*Iqgap1*$^{-/-}$) mice demonstrated that IQGAP1 is necessary for efficient PI3K signaling upon EGF-stimulation. In the 4NQO-induced HNSCC study, *Iqgap1*$^{-/-}$ mice led to significantly lower multiplicities of cancer foci as well as significantly lower numbers of high grade cancers than observed in similarly treated *Iqgap1*$^{+/+}$ mice. IQGAP1 protein was increased in its expression in HNSCCs arising in the *Iqgap1*$^{+/+}$ mice, consistent with that seen in human HNSCCs. We also observed a significant down-regulation of PI3K signaling in 4NQO-induced HNSCCs arising in the *Iqgap1*$^{-/-}$ mice, consistent with IQGAP1 contributing to carcinogenesis by promoting PI3K signaling.

Our studies, therefore, support the hypothesis that IQGAP1 acts as an oncogene in head and neck carcinogenesis, and provide mechanistic insight into its role. Future studies will be focused on determining the mechanism of how IQGAP1 contributes to HNSCC. Considering that IQGAP1 is a multifunctional protein involved in multiple cellular pathways, proteomic studies based on mass-spectrometry will be a useful tool to determine which pathways are affected with the presence and absence of IQGAP1, and how they are contributing to carcinogenesis differently.

Novel aspect: We demonstrate, for the first time, that the signaling scaffolding protein, IQGAP1, contributes HNSCC, which provides a possible therapeutic target.

124. ZHI WEN

Loss of Wild-type Kras promotes early T cell precursor acute lymphoblastic leukemia via Rasgrp1 and Tcof1

Early T cell precursor acute lymphoblastic leukemia (ETP-ALL) is an aggressive subtype of T-ALL with a high risk of treatment failure. Genetic mutations that constitutively activate cytokine receptor and Ras signaling are prevalent in ETP-ALL, but rarely observed in general T-ALL. These mutations, including oncogenic *NRAS* mutations, are characteristic of myeloid leukemias. Not surprisingly, we and others previously showed that hematopoietic expression of $Nras^{G12D}$ and $Nras^{Q61R}$ from the endogenous *Nras* locus drives a highly penetrant myeloid disease in mice. However, these animals rarely develop T-ALL or ETP-ALL. Therefore, it remains unknown how activated Ras signaling contributes to ETP-ALL.

Here, we find that in addition to the frequent oncogenic *RAS* mutations, wild-type (WT) *KRAS* was significantly downregulated via genetic and other mechanisms in ETP-ALL cells. Similarly, loss of WT *Kras* promoted an early onset of ETP-ALL-like disease in $Nras^{Q61R}$ mice. Mechanistic studies revealed profound proteomic and phosphoproteomic changes in $Kras^{-/-}; Nras^{Q61R/+}$ thymocytes and identified key events promoting ETP-ALL. A short-term MEK inhibitor treatment induced a deep remission in a significant fraction of recipients transplanted with $Kras^{-/-}; Nras^{Q61R/+}$ malignant T cells, suggesting that MEK/ERK-targeted therapies may improve the poor outcomes in patients with ETP-ALL.

Our studies reveal a novel mechanism by which WT Kras restricts oncogenic Nras in promoting ETP –ALL. This mechanism is distinct from what we previously published on the tumor suppressor function of WT Kras in oncogenic Kras-driven myeloid disease. Moreover, our data suggest that although loss of WT Kras promotes leukemias via various mechanisms, it also sensitizes these leukemia cells to MEK inhibitors.

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125. CARA WESTMARK

Defining a Proteomic Signature for Soy-Induced Metabolic Changes in Mice.

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Introduction: During the course of studying seizures in $Fmr1^{KO}$ mice, a rodent model for the developmental disability fragile X syndrome, we serendipitously discovered that the type of rodent feed (casein-based purified ingredient diet versus grain/soy-based chow) significantly affects seizure propensity (published) and weight gain (preliminary data). We hypothesize that soy-based diets are contributing to the childhood obesity epidemic. Little is known regarding the molecular signature of soy consumption or the resulting metabolic phenotypes. Herein, we present our findings to date and describe future experiments to identify and validate metabolic phenotypes and proteomic profiles that change in response to dietary consumption of soy.

Methods: The study design for the preliminary data includes: (1) retrospective analysis of prior seizure datasets containing body weight metrics to ascertain differences in weight as a function of age, diet (casein-based purified ingredient versus grain/soy-based chow), and genotype (WT and $Fmr1^{KO}$); (2) comparison of infant growth metrics in response to casein- and soy-based infant formula using the Center for Disease Control and Prevention (CDC) Infant Feeding Practices Study II (IFPSII) dataset; and (3) prospective evaluation of weight gain and plasma biomarker expression in WT and $Fmr1^{KO}$ mice in response to casein- and soy-based infant formula diets.

Preliminary Data: Juvenile mice fed chow throughout gestation and postnatal development exhibit increased weight gain compared to mice fed a casein-based purified ingredient diet. The effects are more pronounced in $Fmr1^{KO}$ mice. Adolescent WT and $Fmr1^{KO}$ mice weaned onto soy-based infant formula diet exhibit increased weight gain compared to mice weaned onto

a casein-based infant formula diet. Adult *Fmr1*^{KO} mice transferred to soy infant formula diet also exhibit excessive weight gain. Thus, at the systems level, our findings indicate that the consumption of grain/soy-based diets increases weight gain in mice irrespective of age with the most pronounced effects in juvenile *Fmr1*^{KO} mice. Retrospective analysis of the CDC IFPSII dataset indicates that consumption of soy-based infant formula may be associated with increased BMI in female infants exclusively fed formula through 12-months of age. At the molecular level, the expression of several mouse plasma proteins including the adipose hormone leptin and the amyloid-beta degrading enzyme neprilysin are altered in response to diet. A pending R01 will: (1) determine the effects of soy protein consumption on metabolic phenotypes (monitoring anthropometrics, energy expenditure, biochemistry, activity, hematology and necropsy phenotypes) in wild type and *Fmr1*^{KO} mice; (2) identify and quantitate proteomic profiles in response to soy protein consumption by proteomic mass spectrometry analysis and *in situ* labeling of MALDI tissue sampling; and (3) validate soy responsive molecular biomarkers by western blot and ELISA.

Novel Aspect: A quarter of infants consume soy-based formula. There is a critical need to identify and validate associated molecular biomarkers.

126. REBECCA WHELAN

Characterizing proteoforms of MUC16 (CA125)

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CA125 is the most widely used biomarker for clinical monitoring of epithelial ovarian cancer. Serum levels of CA125 are monitored during treatment, and increases correlate with cancer recurrence. CA125 is the repeating peptide epitope of the mucin protein MUC16 and is currently detected via double determinant immunoassay. Previous work from the Whelan and Patankar labs indicates that the antibodies used in existing CA125 clinical assays do not respond uniformly to all CA125 proteoforms, suggesting that these assays significantly underestimate levels of this biomarker. We will use capillary-electrophoresis-mass spectrometry (CE-MS) to characterize MUC16 (CA125) from the ascites fluid of patients with known clinical outcome. We will test the hypothesis that heterogeneity in this protein has clinically relevant predictive power.

127. JESSIE WHITAKER

Differentiation and quantification of four metabolites of F_2-Isoprostanes in urine by liquid chromatography/tandem mass spectrometry

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Introduction: Isoprostanes are an important metabolic indicator of oxidative stress. Oxidative stress has been implicated as a risk factor in a wide variety of diseases. Previously, isoprostanes have been measured in plasma, involved a complicated extraction method, and followed by analysis on GC-MS/MS. Urine is a human matrix that involves a less invasive collection, and does not require additional processing before long term storage. These differences in collection and analysis between plasma and urine isoprostane measurement could increase feasibility and efficiency of isoprostanes for larger studies. Our lab was interested in increasing the number of metabolites measured to find a relationship between the types of metabolites and outcomes in study subjects.

Methods: For optimal peak separation of the four metabolites, we worked to optimize the mobile phases, gradients, and column configuration for the LC method. Previous methods focused solely on rapid elution with no consideration for peak separation. For this method, the final run time was increased from 5.5 minutes to 16.5 minutes, allowing for a gradual gradient change during peak separation. The previous method used a single Magic C18AQ column, 25mm in length. We did not alter the column packing material, instead we moved to the Phenomenex Kinetex C18 100mm, and we added a second 100mm C18 column in sequence for increased separation and selectivity. Metabolites were detected with a triple-quad SciEx 5500 MS.

Preliminary Data: With the changes outlined in the methods, we were able to separate four distinct and reproducible peaks. High and low quality control material (n=28) was run for each (8-iso Prostaglandin F2α, 2,3-dinor-8iso Prostaglandin F2α, 8,12-iso iPF2α, iPF2α-VI) analyte, using three separate internal standards (8-iso Prostaglandin F2α-D4, 8,12-iso iPF2α-d11, iPF2α-D4) to facilitate quantification. With this method, we achieved CVs below 10% for all analytes at both high and low levels, with the exception of the low 2,3-dinor-8iso Prostaglandin F2α with a CV of 11.9%.

Novel aspect: With this method, we are able to achieve isoprostane specificity along with a quantitative amount for a more in-depth analysis.

128. MARITES WOON

Functional characterization of Leucine-Rich Repeat Containing Protein 10 (LRRC10) in induced pluripotent stem cell cardiomyocytes

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Introduction: LRRC10 is an understudied protein expressed exclusively in the myocardium. We recently described a novel variant in LRRC10, I195T, associated with early onset dilated cardiomyopathy (DCM) in a human patient. Preliminary electrophysiology recordings in a heterologous expression system provided evidence that LRRC10 may function as an auxiliary subunit of L-type Ca^{2+} channels (LTCCs). However, whether LRRC10 modulates LTCC activity in cardiomyocytes remains unclear. Here we will perform electrophysiology recordings in induced pluripotent stem cell cardiomyocytes (iPSC-CMs) to demonstrate regulation of LTCC current by LRRC10 sequence variants (WT and I195T). Moreover, we propose to use top-down and bottom-up proteomics to identify LRRC10 post-translational modifications (PTMs) and interacting proteins towards a better understanding of the function(s) of this protein in the heart.

Methods: iPSC-CMs were generated from an *LRRC10*$^{I195T/I195T}$ patient. Sanger sequencing analysis was performed using genomic DNA extracted from *LRRC10*$^{I195T/I195T}$ and 19-9-11 iPSCs as our control. Whole-cell patch clamp electrophysiology recordings of L-type Ca^{2+} channel currents were generated from *LRRC10*$^{I195T/I195T}$ and 19-9-11 iPSC-CMs as previously described (Woon MT et al. *J Am Heart Assoc.* 2018;7:e006428). LRRC10 from 19-9-11, patient-derived *LRRC10*$^{I195T/I195T}$ and isogenic control iPSCs as well as interacting proteins, will be isolated via immunoaffinity purification. Intact LRRC10 will be analyzed on an ultra-high-resolution 12T FT-ICR mass spectrometer to characterize PTMs. Eluents will also be in-solution digested and analyzed on an Impact II Q-TOF mass spectrometer to identify interacting proteins.

Preliminary data: iPSC-CMs were generated from a patient harboring the rare, homozygous I195T-LRRC10 variant. Sanger sequencing validated the c.584T>C variant in the iPSC line responsible for p.I195T and *LRRC10*$^{I195T/I195T}$ iPSC-CMs were generated using an established small molecule approach.

Previous whole-cell patch clamp electrophysiology results using a heterologous expression system revealed a decrease in L-type Ca^{2+} channel current ($I_{Ca,L}$) with I195T-LRRC10 coexpression compared to LTCC expression alone. Therefore, to determine whether the I195T-LRRC10 variant impacts $I_{Ca,L}$ in a more physiologically relevant model system, electrophysiology recordings were performed using the *LRRC10*$^{I195T/I195T}$ and 19-9-11 iPSC-CMs. Recent results demonstrated a significant decrease in $I_{Ca,L}$ in the *LRRC10*$^{I195T/I195T}$ iPSC-CMs compared to the control. Moreover, *LRRC10*$^{I195T/I195T}$ iPSC-CMs resulted in a hyperpolarizing shift in the voltage dependence of activation compared to control. While analysis of the voltage dependence of steady-state inactivation of $I_{Ca,L}$ was not significantly different between *LRRC10*$^{I195T/I195T}$ iPSC-CMs and the control 19-9-11 iPSC-CMs, the ratio of $I_{Ca,L}$ remaining at +20 mV to the maximal current is significantly larger in *LRRC10*$^{I195T/I195T}$ iPSC-CMs. Collectively, these results demonstrate a significant decrease in peak Ca^{2+} current and increase in slowly inactivating current in our patient-derived *LRRC10*$^{I195T/I195T}$ iPSC-CMs.

These results support our previous findings in the heterologous expression system and provide additional evidence that LRRC10 functions as an auxiliary subunit of LTCCs. However, many questions remain to be answered. What is the mechanism by which LRRC10 modulates $I_{Ca,L}$? As a scaffolding protein, is LRRC10 involved in the formation of a macromolecular signaling complex that regulates LTCC activity? Is LRRC10 post-translationally modified? To answer these critical questions, we look to mass spectrometry-based proteomics. We propose to employ top-down proteomics to identify PTMs in WT LRRC10 and the disease-associated variant, I195T. Moreover, we will use bottom-up proteomics to identify novel interacting proteins of LRRC10 and determine whether the sequence variant disrupts signaling complex formation.

Novel aspect: This study identifies dysregulation of L-type Ca^{2+} channels in human cardiomyocytes by a DCM-linked LRRC10 variant.

129. SHU-ZON WU

Actin and microtubule crosstalk mediates persistent polarized growth

Coordination between actin and microtubules is important for numerous cellular processes in diverse eukaryotes. In plants, tip-growing cells require actin for cell expansion and microtubules for orientation of cell expansion, but how the two cytoskeletons are linked is an open question. In tip-growing cells of the moss *Physcomitrella patens*, we show that an actin cluster near the cell apex dictates the direction of rapid cell expansion. Formation of this structure depends on the convergence of microtubules near the cell tip. We discovered that microtubule convergence requires class VIII myosin function and actin is necessary for myosin VIII-mediated focusing of microtubules. The loss of myosin VIII function affects both networks, indicating functional connections among the three cytoskeletal components. Our data suggest that microtubules direct localization of formins, actin nucleation factors, that generate actin filaments further focusing microtubules, thereby establishing a positive feedback loop ensuring that actin polymerization and cell expansion occur at a defined site resulting in persistent polarized growth.

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130. XIAOFENG WU

Effective phosphoproteome capture and analysis procedure of urinary extracellular vesicles

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Introduction: In human biofluids, extracellular vesicles (EVs), classified as microvesicles (MVs) and exosomes by size, serve as a wellspring for biomarker discovery and disease diagnosis due to the cell-derived cargo they carry. Vast majority of the current EV studies, however, focus on microRNA and DNA, with virtually nothing reported on their phosphoproteomes. As phosphorylation is a major player in cancer and other disease progression, EV phosphoproteins offer enormous potential as indicators of cellular states and for *in vitro* disease diagnosis. To address this need, we have developed a highly efficient and robust platform to reproducibly capture and detect phosphoproteins from urine EVs. The foundation of the approach will rely on the ability to enrich low-abundant EV phosphoproteins with high yield and low CV.

Methods: Biofluids, such as urine, incubated with in-house functionalized magnetic beads to capture the complete profile of EVs. The bound EVs can then be recovered off the beads or by an on-beads digestion. Following the EV capture, the membranes were lysed using phase-transfer surfactant-based buffer, and the proteins recovered. After digestion, the phosphopeptides were enriched by the PolyMAC method and analyzed by LC-MS. Label-free quantitation was used to compare phosphoproteome recovery to other methods, including the current exosome isolation gold standard of differential ultracentrifugation (DUC). In a parallel experiment, the samples were denatured and analyzed by Western Blot (WB) to quantify the EV recovery yield through marker detection, and by silver staining to compare the purity with other methods.

Preliminary Data: Our research so far has been focused on the human urine EV isolation. Based on the WB results, the new method provided consistent EV recovery of over 95%, a much higher yield than the 10–20% typically obtained from the DUC method. Our improved EV capture and phosphoproteome sample preparation procedure have resulted in identification of ~2,000 unique phosphopeptides and >800 phosphoproteins from only 10-mL urine sample and a single 60-min LC-MS run. Based on these promising results, we believe that a complete profile of EV phosphoproteins can be obtained and used for the discovery of potential disease biomarkers.

Novel aspect: The first effective phosphoproteome analysis of urinary extracellular vesicles

131. KATHARINA YANDROFSKI

Size and Mass Heterogeneity of the NISTmAb: From Reference Values to Reference Cell Line

The NISTmAb IgG1k is a NIST Reference Material (RM 8671) that embodies the quality and characteristics of a biopharmaceutical product, is widely available to the biopharmaceutical community, and is an open innovation tool for development and dissemination of results. It is intended to provide a well characterized, longitudinally available test material

that is expected to greatly facilitate development of originator and follow-on biologics for the foreseeable future. Size exclusion chromatography and intact/middle-down MS were optimized for characterization of the NISTmAb. These methods are now being implemented for characterization and similarity assessment of prototype non-originator NISTmAb materials.

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132. YOU YOU

Discovery and Expression Quantification in Planta by MS of IPD072Aa, a Novel Insecticidal Protein

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Introduction: The western corn rootworm (WCR), *Diabrotica virgifera virgifera*(Coleoptera: Chrysomelidae), is one of the most invasive crop pest in North America and Europe. Currently, BT (*Bacillus thuringiensis*) proteins transgenetically expressed in crops are used to control the insect damage. However, some insect populations have evolved resistance to Bt proteins. Novel insecticidal protein discovery has been an ongoing effort for long-term WCR management. IPD072Aa, discovered from a Gram-negative bacterium, *Pseudomonas chlororaphis*, shows potent and selective insecticidal spectra. During discovery of IPD072Aa, nanoLC-MS/MS data of tryptic peptides is used to search against a database to identify the sequence and quantify transiently expressed protein in plants.

Methods: Soil samples were collected, cultured and isolated. Crude protein extracts from these isolates were screened for activity against WCRs in artificial diet bioassays. One isolate—identified as P. chlororaphis by 16S ribosomal DNA sequence analysis —showed a potent inhibitory effect on WCRs. After multistep chromatography, the major band of ~10 kDa was digested with trypsin and subjected to identification by nanoLC-MS/MS. Database search showed high confidence match to a hypothetical protein encoded by a gene from our specific P. chlororaphis isolate. This gene encodes a small protein of 86 amino acids, which we designated IPD072Aa (GenBank accession number KT795291). This protein was recombinantly expressed in E.Coli and transformed to maize plant. Activity is confirmed.

Novel Aspect: a completely novel non-BT insecticidal protein against WCR

133. TONYA ZECZYCKI

Transglutaminase 2 and α-synuclein macromolecular complex formation in the absence of Ca2+.

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Introduction. Transglutaminase 2 (TG2) activity is crucial for an assortment of biological processes. TG2 catalyzes the Ca2+-dependent, post-translational modification of α-synuclein, tau, huntingtin, and Aβ-amyloid. TG2-mediated modifications of these specific proteins contribute to the formation of the toxic protein oligomers and aggregates in various neurodegenerative diseases. Little, however, is known as to how these specific modifications facilitate the formation of α-synuclein oligomers. Based on preliminary data from our lab, our hypothesis is that, depending on the TG2-mediated modification, TG2 activity can both facilitate and inhibit toxic α-synuclein oligomer formation by stabilizing specific aggregate-competent or resistant conformations of α-synuclein. In addition, we hypothesize that protein-protein interactions and intrinsic protein disorder play significant roles in controlling the allosteric regulation of TG2 activity and α-synuclein aggregation.

Methods. Our goal is two-fold. (1) We wish to map out the protein-protein interactions important to allosteric regulation and (2) determine which protein modifications of α-synuclein result in aggregate competent or incompetent proteins. We will use a variety of proteomic-based, MS techniques (including HDX-MS, LC/MS, and quantitative MS) to determine

(1) how protein-protein interactions control TG2 activity, (2) the effects of complex formation on intrinsic disorder, and (3) the identity of aggregate competent and incompetent TG2-mediated post-translation modifications. Our preliminary MALDI-TOF MS data of the intact TG2:α-synuclein complex shows that the proteins interact in regions of TG2 thought to be intrinsically disordered or contain Ca2+-binding sites, lending support to the idea that protein-protein interactions are important to the allosteric mechanism.

Preliminary data. Using native gels and limited proteolysis (trypsin) we are currently using MALDI-TOF and LC/MS to identify the binding interface between TG2 and α-synuclein in the absence of Ca2+. Preliminary data suggests that the protein-protein interface may stabilize intrinsically disordered regions in TG2 near the putative Ca2+ binding sites, thus facilitating Ca2+ binding and activation of the complex. Similar experiments in the presence of Ca2+ will help reveal both the Ca2+ binding sites and possible conformational changes associated with Ca2+ binding to the complex. Additionally, we have begun characterizing the TG2-mediated, Ca2+-dependent modifications of α-synuclein. Using size-exclusion chromatography, we have separated the protein reaction products and are subjecting them to LC-MS/MS and MADLI-TOF MS/MS analysis to identify where the modifications are occurring. Once identified and characterized, we will use quantitative MS to monitor the relative abundance of each product over time and determine the extent to which each modification promotes or inhibits aggregation.

Novelty. Our research is novel because it aims to determine how intrinsic disorder and protein-protein interactions drive allostery and protein aggregation.

134. BINGQING ZHAO

Novel ETD-Cleavable Cross-linker for Improving Confidence of Cross-linked Peptide Identifications

The previously reported homobifunctional cross-linker diethyl suberthioimidate (DEST), which amidinates primary amines, is shown to be ETD cleavable. DEST has a simple spacer arm of a 6-carbon linear alkyl chain, and it cleaves at the resulting amidine groups created by reaction at primary amines of proteins. DEST cross-links are recognized in ETD MS/MS spectra based on mass pairs of peptide-NH_2 (P-NH_2) and peptide+linker+NH_3 (P+L+NH_3). Backbone cleavages also occur. Dead-ends that can confuse the identifications of cross-links, are diagnosed by strong dead-end reporter ions. ETD mass pairs can be used in MS^3 experiments to confirm cross-link identifications.

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135. YI ZHENG

Effect of pregnancy on raltegravir free concentrations

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Introduction: Raltegravir can be used for the prevention of mother-to-child HIV transmission, especially when a rapid decline of HIV RNA load is necessary. Physiological changes during pregnancy have an impact on raltegravir elimination. Indeed, exposure of total raltegravir was shown to decrease from 29 to 50% during the third trimester of pregnancy compared to postpartum(1). However albumin level is known to be lowered during pregnancy which could increase the active free fraction of the drug and reduce this effect. The objective of this study was to describe the unbound, total and glucuronide raltegravir pharmacokinetics during pregnancy.

Methods: The RalFe ANRS160 study was a non-randomized, open label, multicenter phase II trial in HIV-infected pregnant women receiving raltegravir 400 mg twice daily. Samples were collected during pregnancy (between 30 and 37 weeks of amenorrhea), at delivery and at postpartum (4 to 6 weeks after delivery). None of these women received an antiretroviral drug which could interact with raltegravir. Free raltegravir, total raltegravir and raltegravir glucuronide concentrations were measured using a validated liquid chromatography-tandem mass spectrometry and ultrafiltration methods. Aspartate transaminase, alanine transaminase, creatinine, biribuline and albumin of each sample were systematically measured. Furthermore, raltegravir has been shown to be primarily metabolized by the UDP-Glucuronosyltransferase (UGT1A1) and to be a substrate of the drug efflux transporter P-glycoprotein(PgP)(2). Two polymorphisms, one in UGT1A1 and another in

P-glycoprotein, were also genotyped by sequencing and real time PCR and respectively. A population pharmacokinetic model was developed by using NONMEM software.

Preliminary data: A total of 414 samples were collected from 43 women (aged from 23.3 to 45.9 years old) in which free, total and glucuronide raltegravir concentrations were measured. Free raltegravir was described by a one-compartment model with first order absorption and lag time, evolving either to bound raltegravir (by a linear binding to albumin), or to glucuronide raltegravir (through an additional compartment) or to a first order elimination. The influence of body weight, age, aspartate transaminase, alanine transaminase, creatinnie, biribuline and two polymorphisms were evaluated for the raltegravir pharmacokinetics analysis and no effect was found. Pregnancy increased free raltegravir clearances by 17% . During pregnancy, trough concentrations decreased by 28 for total raltegravir and by 16 for free raltegravir. The decrease was negligible for the glucuronide form.

Novel aspect: This is the first data reporting free and glucuronide raltegravir pharmacokinetics during pregnancy. Pregnancy effect was moderate on the active raltegravir free fraction, especially when compared to its intersubject variability. Our data suggest that this pregnancy effect could be considered not to be of clinical importance, raltegravir does not need to be modified during pregnancy.

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136. YUNYUN ZHU

Proteomic profiling of yeast strains deleted of key components in mitochondrial contact sites

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Introduction: Inter-organelle communication is an emerging field in cell biology. Mitochondria is the hub of the energy metabolism and it can exchange proteins, lipids, small molecule metabolites, calcium and even reactive oxygen species with other organelles. The communication and cargo between organelles is achieved through contact site complexes composed of multiple proteins. Currently, there're three known mitochondria contact sites (MCS) complexes in yeast including vCLAMP with vacuole, ERMES with endoplasmic reticulum and MICOs connecting the inter- and outer-membrane of mitochondria. The components of these MCS complexes and how they mediate exchanges is not fully known and poorly characterized. To address this issue, proteomics of 18 yeast strains including the wildtype with one known component gene-knockout in each strain was performed.

Method: The parental (WT) Saccharomyces cerevisiae strain was haploid W303. Single gene deletion (Δgene) derivatives of W303 were either obtained through gene deletion consortium30 (Thermo #YSC1054) or a KanMX deletion cassette. Deleted genes have been reported as components or regulators in one of the three MCS complexes.

Yeast pellets (1E8 cells) were lysed to extract proteins and proteins were sequentially digested into peptides with LysC (Wako Chemicals, Richmond, VA) and trypsin (Promega, Madison, WI). Peptides were separated in a 120 min liquid chromatography (LC) gradient and analyzed with Orbitrap hybrid Fusion Lumos® mass spectrometer (Thermo Scientific, San Jose, CA). Data searching was carried out with in-house software suite COMPASS. Label free quantification was performed with MaxQuant (v. 1.5.2.8).

Preliminary Data: Depth of proteome reflected in the number of identified peptides and protein groups is one of the criteria that ensures data quality. In this study, we identified ~50,000 in a 120-minute LC gradient. On average, these 50,000 peptides are assigned into ~4,000 proteins or protein groups in each strain. Given the fact that there are about 4500 proteins in the whole proteome of yeast, this study is believed to be comprehensive proteome analysis that detects ~90% of the expressed proteome (≥ 4000 proteins for yeast).

Reproducibility is another critical indicator of data quality, which is measured by the intra-replicate coefficient variance (CV) values. Normally, median CV below 15% is considered decent for proteomics data. In this study, the median CV of the wildtype replicates is 10%, while median CV of some knockout strains are more variant. Gene-knockout can sometimes lead to unpredictable outcomes, making larger biological variances in knockout strains than the wildtype. Those strains include ΔMdm10, ΔMcp1, ΔMcp2, ΔMic12, ΔMic19, ΔMic26 and ΔYlp109c with a higher median CV around 13%. All the other strains have a median CV around 7%. The overall proteomic data is of good quality.

Further data analysis was carried out to identify those differentially expressed proteins in the knockout strains. Among the 17 knockout strains, there're hundreds of proteins that are differentially expressed in ΔGem1, ΔMdm10, ΔMdm12, ΔMdm34 and ΔVps39 compared with wildtype after FDR correction. These strains are considered to be 'Active Strains'. Meanwhile, strain ΔLam6, ΔMcp1, ΔMcp2, ΔMic12, ΔMic19, ΔMic26 and ΔMic27 don't show much changes. For all the other strains, less proteins are altered than active strains, showing a mediate deviation from the wildtype. Interpretation of the protein expression patterns associated with the gene knocked out will be performed later on.

Novelty: This study is the first to explore the yeast proteome with key MCS gene knockouts.