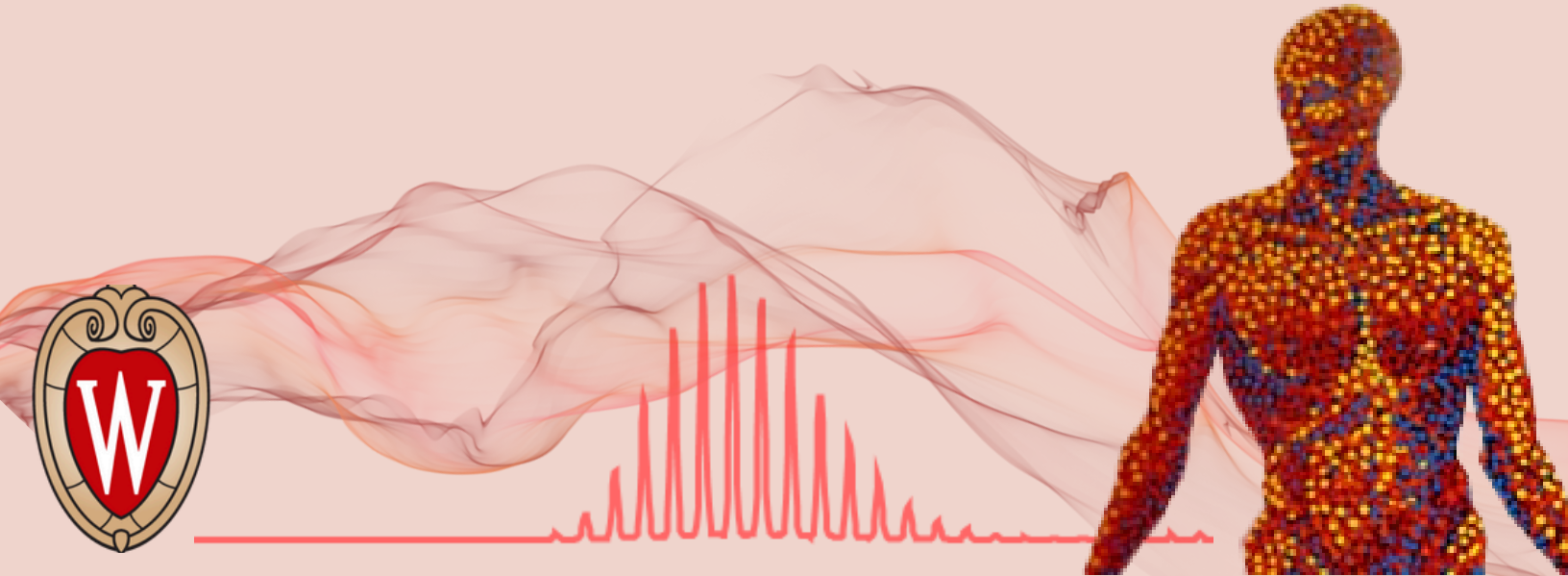
2024 Wisconsin Human Proteomics Symposium

New Frontiers in Clinical Proteomics

Madison, WI | July 31 - August 1, 2024



Welcome Letter

It is our great pleasure to welcome you to the 2024 Wisconsin Human Proteomics Symposium! We are delighted to host this event at the University of Wisconsin-Madison Health Science Learning Center and we are thrilled to bring together a diverse group of researchers, clinicians, and industry professionals dedicated to advancing the field of human proteomics.

This year's symposium, themed New Frontiers in Clinical Proteomics, promises to be an inspiring forum for the exchange of ideas and discoveries. Clinical Proteomics is a burgeoning interdisciplinary research domain that brings together researchers from diverse biomedical fields, offering a promising avenue for translating fundamental scientific insights into practical clinical applications to improve patient outcomes.

Our program includes two keynote presentations from internationally recognized leaders, fourteen invited oral presentations, and numerous poster and exhibitor sessions. We are particularly excited about the rising star sessions designed to promote young scientists. Moreover, Women's initiative and DEI session with subsequent panel discussion is dedicated to supporting women scientists and promoting diversity, equity, and inclusion.

We extend our heartfelt gratitude to our generous sponsors whose support has been instrumental in making this symposium possible. We also thank our speakers for their invaluable contributions and our attendees for their enthusiasm and engagement. Special thanks to our staff and committee members for their hard work in helping organize this symposium. Your participation is vital to the success of this event and the advancement of our shared scientific goals.

While the conference has brought you here, we hope you will also take the time to enjoy the vibrant and welcoming atmosphere of Madison. Take the opportunity to walk around our beautiful campus, visit local attractions, have a taste of our signature dairy products, and experience the unique charm of our city.

Thank you for being a part of the 2024 Wisconsin Human Proteomics Symposium. We look forward to a productive and inspiring event, filled with rich discussions and meaningful connections.

Sincerely,

Symposium organizing committee

Ying Ge, Ph.D.

Allan Brasier, M.D.
Honorary Symposium Chair

Judith Simcox, Ph.D.

Lingjun Li, Ph.D.

Zhicheng Jin, Ph.D.

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Helpful Information

Important Information for the Poster Session:

- **Poster Boards:** Each poster board measures 4x8 feet.
- **Materials Provided:** Push pins and poster numbers will be provided.
- **Setup:** Please set up your poster on Wednesday, July 31, starting as early as 8:00 AM. Poster presentation and competition will be on July 31 4-6pm.
- **Takedown:** Please take down your poster on Thursday, August 1, after the conference has concluded. Any remaining posters will be discarded after Thursday evening.

The symposium program, along with further details about your presentation, will be available on our website: https://labs.wisc.edu/hpp_web/symposium/2024/index.html

Things to do in Madison, WI!

With five lakes, multiple parks, museums, trails, and a world-renowned university, Madison has something for everyone. For more, see *Destination Madison* (https://www.visitmadison.com)

Biking, Walking, Hiking

Take a spin around our lakes or on the many bike paths using an ebike https://madison.bcycle.com. Walk or run from the Memorial Union along the Temin Lakeshore Path. Or take an Uber to the UW-Madison Arboretum (https://arboretum.wisc.edu), where you can walk around and enjoy a brilliant curation of trees and wildlife.

Paddling

Get out on the water and discover our beautiful lakes. Kayak, canoe, and SUP with Bringham

Boast on Monona Bay. (https://www.visitmadison.com/lisngs/paddle-on-lakehttps://www.visitmadison.com/listings/paddle-on-lake-monona/183530/monona/183530/)

Wet your Whistle

Sample craft brews, cocktails, and mocktails at nearby restaurants like Merchant, Tornado Room, Nattspil, and the Robin Room. Or make a reservation at Fairchild, home of the 2023 James Beard's Best Chef Midwest Award!

Art

Check out the Chazen Museum of Art on the UW-Madison campus or on State Street, tour MMOCA, Madison's modern art museum.

DAY 1 | July 31

8:00am – 5:00pm	Conference Registration	HSLC Atrium

Women's Initiative and DEI Session
(Sponsored by UW Carbone Cancer Center)
Chairs: Lauren Fields and Anna Grace
9:00 am – 12:30 pm (HSLC 1306, Virtual option available in Zoom)

9:00 am – 9:05 am	**Welcome** ***Ying Ge, PhD*** University of Wisconsin – Madison
9:05 am – 9:30 am (20 min presentation, 5 min Q&A)	***Judith Simcox, PhD*** University of Wisconsin-Madison *"Harnessing diverse human populations to explore the function and regulation of plasma lipids"*
9:30 am – 9:55 am (20 min presentation, 5 min Q&A)	***Amber Mosley, PhD*** Indiana University *"The Stress of Cellular Origami: Defining the Unfolded Protein Response with Thermal Proteome Profiling"*
9:55 am – 10:20 am (20 min presentation, 5 min Q&A)	***Michelle Kimple, PhD*** University of Wisconsin-Madison *"Elucidating the Role of Circulating Lipids and Their Metabolites in Diabetes Pathophysiology Using Targeted and Untargeted Mass Spectrometry Approaches"*
10:20 am– 10:45 am (20 min presentation, 5 min Q&A)	***Stefani Thomas, PhD, DABCC*** University of Minnesota *"Overcoming Barriers to Develop Diagnostic Serum Protein Biomarkers for High-Grade Serous Ovarian Cancer"*
11:00 am– 12:30 pm	**Panel Discussion and Lunch** **Panelists** • Dr. Si Wu- Professor, University of Alabama • Dr. Emma Doud- Assistant Research Professor, Indiana University School of Medicine • Dr. Savannah Snyder- Field Sales Representative, Bruker Daltonics • Dr. Judith Simcox, Assistant Professor, University of Wisconsin-Madison • Dr. Michelle Kimple, Professor, University of Wisconsin-Madison

	• Dr. Andrea Galmozzi, Assistant Professor, University of Wisconsin-Madison • Dr. Héctor H. Valdivia, Professor, University of Wisconsin-Madison	
Special Session on Spatial Proteomics via Imaging Mass Spectrometry ***Chair: Lingjun Li, PhD*** 1:00 pm – 2:25 pm (HSLC 1306, Virtual option available in Zoom)		
1:00 pm – 1:45 pm (40 min presentation, 5 min Q&A)	**Keynote Speaker** ***Ron Heeren, PhD*** Distinguished Professor and Scientific Director of M4I Maastricht University *"'Omics' and imaging in single cell spatial biology"*	
1:45 pm– 2:10 pm (20 min presentation, 5 min Q&A)	***Peggi Angel, PhD*** Medical University of South Carolina *"Extracellular Glycoproteins as Pathological Differentiators in Tissue and Fluids"*	
2:10 pm– 2:25 pm (12 min presentation, 3 min Q&A)	***Peter Verhaert, PhD*** ProteoFormiX *"HRMS Secretomics Approach to Map True Biological Activity of Human Cells in Culture"*	
2:25 pm– 2:45 pm	**Break & Exhibits**	HSLC Atrium
Rising Star Session, Senior Group **(Sponsored by UW Carbone Cancer Center)** ***Chair: Daniel Matson, MD, PhD*** 2:45 pm – 3:50 pm (HSLC 1306, Virtual option available in Zoom)		
2:45 pm – 3:00 pm (12 min presentation, 3 min Q&A)	***Irene Ong, PhD*** University of Wisconsin-Madison *"Breastfeeding and Traditional Farming Lifestyle Promotes Predominant Bifidobacterium in Infants"*	
3:00 pm– 3:15 pm (12 min presentation, 3 min Q&A)	***Jia Fan, PhD*** Tulane University *"Advances in Mass Spectrometry for Precision Diagnostics and Pathogen Identification"*	
3:15 pm – 3:30 pm (12 min presentation, 3 min Q&A)	***Qing Yu, PhD*** UMass Chan Medical School *"High-throughput intelligent chemoproteomics for massive protein-ligand insight and targeted drug discovery"*	
3:30 pm– 3:45 pm (12 min presentation, 3 min Q&A)	***Tao Chen, PhD*** Absea Biotechnology Ltd. *"Platforms and Resources for Proteomics and Beyond"*	
4:00 pm – 6:00pm	**Reception, Poster Competition & Exhibits**	HSLC Atrium

DAY 2 | August 1

8:00am – 12:00pm	Conference Registration	HSLC Atrium
Session I ***Chair: Ying Ge, PhD*** 9:00 am – 10:50 am (HSLC 1306, Virtual option available in Zoom)		
9:00 am – 9:10 am	**Welcome** ***Allan Brasier, MD*** Senior Associate Dean Clinical and Translational Research, School of Medicine and Public Health University of Wisconsin - Madison	
9:10 am – 10:00 am (45 min presentation, 5 min Q&A)	**Keynote Speaker** ***Matthias Mann, PhD*** Director, Max-Planck Institute of Biochemistry *"Advances in MS-based clinical proteomics: from large cohorts analysis to deep visual proteomics"*	
10:00 am–10:25 am (20 min presentation, 5 min Q&A)	***Akhilesh Pandey, MD, PhD*** Mayo Clinic *"From Exploring Biochemical Pathways in Glycosylation to Translation"*	
10:25 am– 10:40 am (12 min presentation, 3 min Q&A)	***Michael Greig, PhD*** Bruker *"Expanding Capabilities for a Top-Down World"*	
10:40 am – 11:00 am	**Break & Exhibits**	HSLC Atrium

Session II

Chair: Jonathan Sweedler, PhD

11:00 am – 12:05 pm (HSLC 1306, Virtual option available in Zoom)

11:00 am – 11:25 am (20 min presentation, 5 min Q&A)	***Hanno Steen, PhD*** Harvard Medical School *Advances in Body Fluid Proteomics: More from Less and that Much Faster (and Cheaper)*
11:25 am – 11:50 am (20 min presentation, 5 min Q&A)	***Lee Eckhardt, MD*** University of Wisconsin – Madison *"Proteomics in Inherited Arrhythmic Conditions; a clinical perspective"*
11:50 am – 12:05 pm (12 min presentation, 3 min Q&A)	***Mike Rosenblatt, PhD*** Promega *"Toward Optimal Workflow Solutions for Bottom-Up Proteomics"*

Lunch Seminars

12:05 pm – 1:20 pm (HSLC 1335 and 1345, Virtual option available in Zoom)

12:20 pm – 1:20 pm	**ThermoFisher Scientific (HSLC 1335)** *"From Compounds to Clinics: New Innovations in Proteomics"* ***Amanda Lee, PhD*** Thermo Fisher Scientific Tribrid Product Marketing Manager ***Matthias Mann, PhD*** Professor of Proteomics and Signal Transduction Director, Max Planck Institute of Biochemistry ***Akhilesh Pandey, M.D., Ph.D.*** Professor, Mayo Clinic
12:20 pm –1:20 pm	**Agilent Technologies (HSLC 1345)** ***Jonathan Sweedler, PhD*** University of Illinois Urbana Champaign *"Single-Cell Metabolomics via CE-NanoESI-MS"* ***Anna Anders*** University of Michigan *"Development of Native Ion Mobility-Mass Spectrometry (IM-MS) and Collision-Induced Unfolding (CIU) for the Structural Characterization of Non-coding Ribonucleic Acids (ncRNAs)"* ***Gary Patti, PhD*** Washington University at St. Louis *"Spatial Analysis of Metabolic Fluxes by Mass Spectrometry Imaging"*

Rising Star Session, Junior (Predoc- and Postdoc Trainees)

(Sponsored by UW Carbone Cancer Center)

Chairs: Judith Simcox, PhD and Tom Gao, PhD

1:30 pm – 3:10 pm (HSLC 1306, Virtual option Available in Zoom)

Each finalist will give a 10 min presentation plus 2 min Q&A.

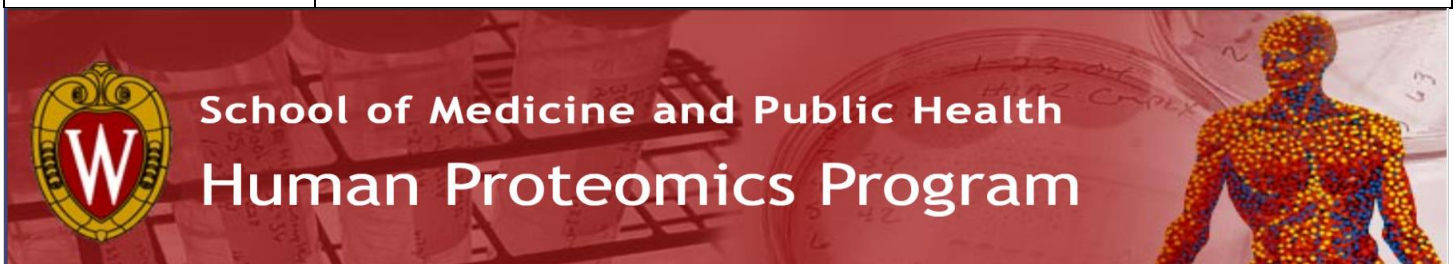
1:30 pm – 1:42 pm	***Isabela James (Predoc)*** University of Wisconsin - Madison) *"Ceramides increase energy expenditure in brown adipose tissue"*	
1:42 pm – 1:54 pm	***Cory Matsumoto (Predoc)*** University of Illinois-Chicago *"Automated Container-less Sample Processing System for Single Cell Multi-omics"*	
1:54 pm – 2:06 pm	***Yanqi Tan (Predoc)*** University of Illinois Urbana – Champaign *"Proteomics Assessment of Plasma-Derived Extracellular Vesicles in Exercised Models by nanoLC-DDA-MS"*	
2:06 pm – 2:18 pm	***Andrew Zhu (Predoc)*** University of Wisconsin – Madison *"Deep Structural Characterization of Metabolites and Metabolic Regulation of Protein Post-Translational Modifications in Diseases"*	
2:18 pm – 2:30 pm	***Zhan Gao, PhD (Postdoc)*** University of Wisconsin – Madison *"Global Proteoform Analysis in Human Hypertrophic Cardiomyopathy through Top-Down Proteomics"*	
2:30 – 2:42 pm	***Morgan Mann, PhD (Postdoc)*** Stanford University *"High Resolution Liquid Chromatography – Mass Spectrometry (LC-MS) For Rapid, High-Throughput Clinical Testing of β2-Transferrin in Human Nasal Secretions"*	
2:42 – 2:54 pm	***Pei Su, PhD (Postdoc)*** Northwestern University *"AutoPiMS: spatially-resolved top-down mass spectrometry enables proteoform-level spatial biology of ovarian cancer"*	
2:54 – 3:06 pm	***Lyndsay Young, PhD (Postdoc)*** Medical University of South Carolina *"Multi-Omic Application of MALDI-MSI and MALDI-IHC for Biomolecular Profiling of Immune Cells in Tissues and Single Cells"*	
3:10 pm – 3:30 pm	**Break & Exhibits**	HSLC Atrium

Session III

Chair: Zhicheng Jin, PhD and Xin Yi, PhD

3:30 pm – 5:00 pm (HSLC 1306, Virtual option Available in Zoom)

3:30 pm – 3:55 pm (20 min presentation, 5 min Q&A)	***David Murray, MD, PhD*** Mayo Clinic *"Bringing Mass Spectometry to the Care of Patients with Monoclonal Gammopathies"*
3:55 pm – 4:20 pm (20 min presentation, 5 min Q&A)	***Ruben Luo, PhD, DABCC*** Stanford University *"Clinical Diagnosis on Hemoglobin Variants and Monoclonal Immunoglobulins: Application of Top-Down Mass Spectrometry and Exploration of Single-Molecule Protein Sequencing"*
4:20 pm – 4:45 pm (20 min presentation, 5 min Q&A)	***Si Wu, PhD*** University of Alabama *"Ultrasensitive top-down using spray-capillary CE-FAIMS-MS"*
4:45 pm – 5:00 pm	**Awards & Closing Remarks**



Keynote Speaker

Matthias Mann, Ph.D.

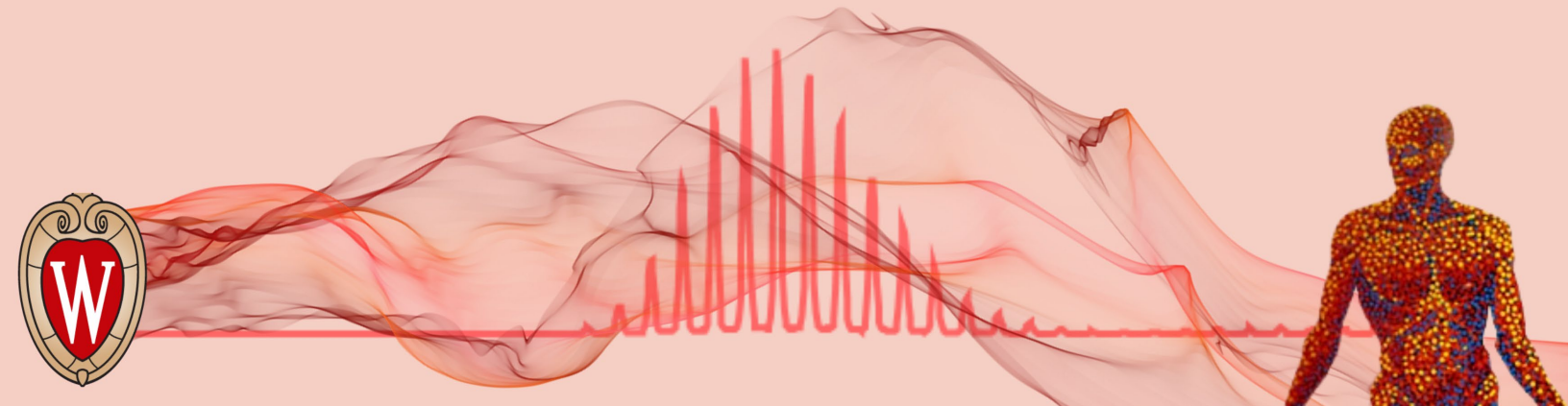
Professor of Proteomics and Signal Transduction
Director at the Max-Planck Institute of Biochemistry
Munich, Germany

Dr. Mann obtained his Ph.D. in chemical engineering at Yale, contributing to the Nobel Prize for his supervisor John Fenn for the development of electrospray ionization. Currently, he heads the Proteomics and Signal Transduction department at the Max-Planck Institute of Biochemistry in Munich, while also directing the Proteomics Program at the Center for Protein Research at Copenhagen University.

As a pioneer in his field, Dr. Mann has made numerous groundbreaking technological advances and has developed powerful computational and proteomics workflows for a wide range of biomedical applications, increasingly emphasizing translational research. His team increasingly focusses on clinically relevant questions, such as body fluid proteomics, single-cell level tissue heterogeneity, signaling, and post-translational modifications.

With a remarkable record of over 850 publications, Dr. Mann is Germany's most cited researcher, with an h-index of 265 and more than 300,000 citations according to Google Scholar. His mentorship has fostered the success of many researchers, and his group at the MPIB has spurred the formation of three start-up companies.

Dr. Mann's outstanding contributions have garnered him numerous prestigious accolades, including the Dr. H.P. Heineken award for Biochemistry and Biophysics, Otto-Warburg Medal, Germany's Leibniz Prize, the Körber European Science Prize, the Louis-Jeantet Foundation Prize for Medicine, the HUPO Distinguished Achievement Award in Proteomic Science, and 'The Order of Dannebrog Knights Cross' conferred by the Queen of Denmark.



ABSTRACT OF KEYNOTE PRESENTATION

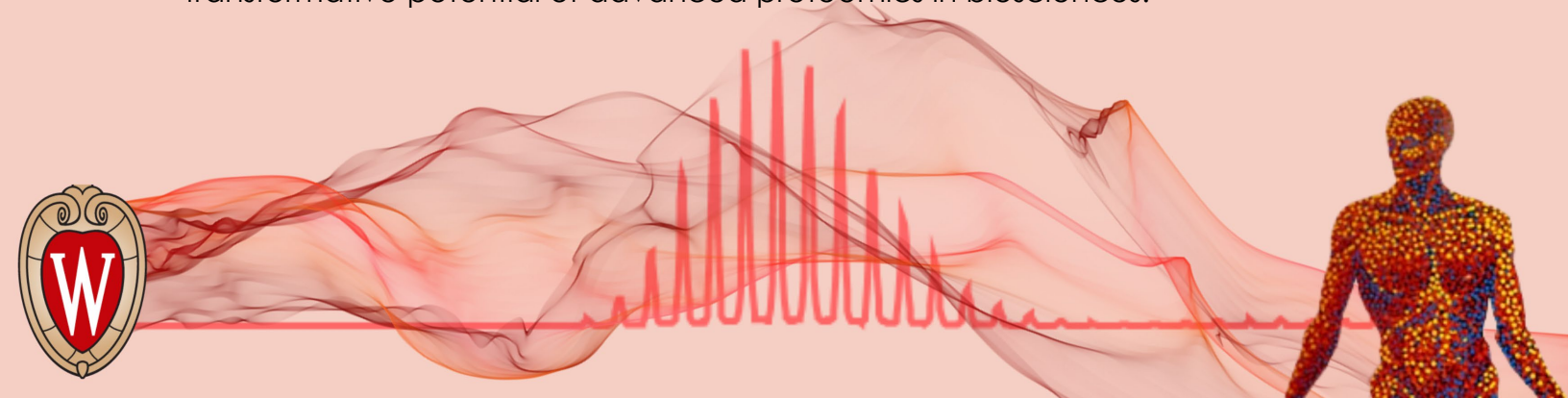
ADVANCES IN MS-BASED CLINICAL PROTEOMICS: FROM LARGE COHORTS' ANALYSIS TO DEEP VISUAL PROTEOMICS

Matthias Mann

Mass spectrometry (MS)-based proteomics has made tremendous progress in recent years and is enabling deep, comprehensive analysis of proteins in any biological context. This talk will highlight the technological advances and computational strategies developed by our group. I will introduce our Python-based open-source AlphaX software suite, designed for rapid and in-depth analysis of large MS datasets. This now includes alphaDIA, our innovative platform that leverages machine learning to handle high dimensional raw data from the mass spectrometers directly, thereby significantly improving analysis efficiency and accuracy of DIA data processing.

On the technology front, the development and integration of low flow chromatography on the Evosep platform with advanced MS technologies on the Bruker and Thermo platforms enable large-scale interactomics studies and plasma proteomics more high-throughput and economical. We are currently applying our robust workflow to study adverse pregnancy outcomes (APOs) in low- and middle-income countries in a very large cohort in order to identify biomarkers for improved prediction aiding clinical decision-making.

Finally, our Deep Visual Proteomics (DVP) and single-cell DVP (scDVP) approaches enable spatial proteomics offering novel and sensitive methods to study cellular heterogeneity in tissue. DVP combines high-content microscopy, AI-driven image recognition, segmentation, laser microdissection, and ultrahigh sensitivity MS to link visual, spatial, and molecular proteomics data. ScDVP extends sensitivity through non-isobaric multiplexing with data-independent acquisition, enabling single-cell resolution. Applied to rare drug rashes and diseases such as melanoma and liver diseases, DVP and scDVP reveal cellular functions at a spatially and cell-type resolved level. Taken together, these efforts highlight the transformative potential of advanced proteomics in biosciences.

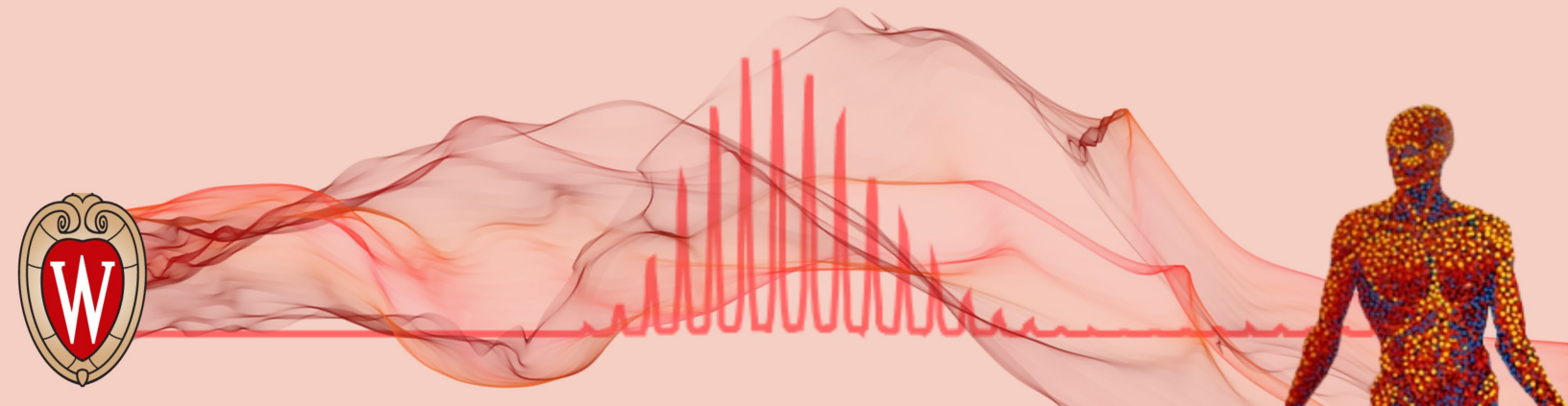


Keynote Speaker

Ron M.A. Heeren, Ph.D.

Distinguished Professor and Scientific Director
Multimodal Molecular Imagine Institute
Maastricht University, The Netherlands

Dr. Ron M.A. Heeren obtained a PhD degree in technical physics in 1992 at the University of Amsterdam on plasma-surface interactions. He started to work on molecular imaging instrumentation and its application as a research group leader at FOM-AMOLF, Amsterdam. In 2001, he became professor at the chemistry faculty of Utrecht University lecturing on the physical aspects of biomolecular mass spectrometry. In 2014 he started as distinguished professor and Limburg Chair at Maastricht University. He is the founder and scientific director of M4I, the Maastricht MultiModal Molecular Imaging institute on the Brightlands Maastricht Health campus. He was awarded the prestigious 2019 Physics Valorization prize by the Dutch organization for scientific Research, NWO and the 2020 Thomson medal of the international mass spectrometry foundation. In 2021 he was elected as a member of the Royal Dutch Academy of Sciences, KNAW. His academic research interests are mass spectrometry based personalized medicine, translational molecular imaging and "omics" research, high-throughput bioinformatics and the development and validation of innovative molecular analytical imaging techniques across the scientific disciplines.

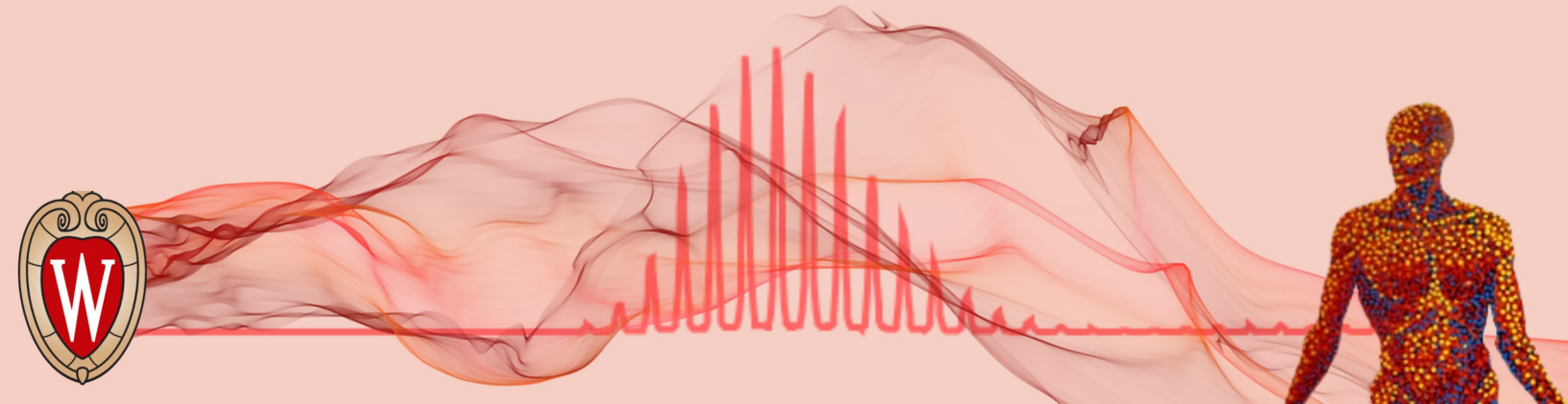


ABSTRACT OF KEYNOTE PRESENTATION

‘OMICS’ AND IMAGING IN SINGLE CELL SPATIAL BIOLOGY

Ron M.A. Heeren

Proteomics technologies are continuously improving and have found their way into clinical diagnostics. Most of these applications are based on protein, lipid or metabolite extracts of homogenized clinical samples. Technological advances have increased methodological sensitivity allowing researchers to acquire multiple levels of ‘omics’ information of smaller and smaller samples. The biggest challenge is to put that concerted information in the spatial context of the problem the biological samples originate from. This lecture describes how innovative “omics” & imaging technologies, based on mass spectrometric innovations have impacted translational clinical research. Or: how a mass spectrometer can be used as a sensitive and selective microscope in molecular pathology. Innovative imaging technologies now offer new insights in life’s complexity. These can be employed for precision medicine, the understanding of new (bio)materials and cellular processes in health and disease. Innovations in mass spectrometry based chemical microscopes have now firmly established themselves in translational molecular research. One key aspect of translational success is the ability to obtain this spatial molecular information on thousands of molecules at the single cell level on a process relevant timescale. Modern mass microscopes can now rapidly acquire images of metabolites, lipids, peptides and proteins, depending on the spatial resolution chosen. Single cells can be analyzed in great molecular detail and in the context of their native tissue with MS based spatial biology. Combined targeted and untargeted proteome imaging offers a truly precision multi-omics approach that reveals contextual molecular complexity in biological and material environments. The lecture will also provide an insight in what lies ahead in the future. The possibilities of high throughput targeted and untargeted MSI are seemingly endless, and will revolutionize the way we deal with spatial biology in the future.



Invited Speakers

Day 1. Women's Initiative and DEI Session

Judith Simcox, Ph.D.

Judith Simcox PhD is an HHMI Freeman Hrabowski Scholar and an Assistant Professor in Biochemistry at the University of Wisconsin-Madison. She grew up in rural Montana, obtaining her BA at Carroll College in Helena Montana. She then pursued her PhD under the mentorship of Dr. Donald McClain at the University of Utah studying the regulation of circadian glucose metabolism by dietary iron. She then completed a postdoctoral fellowship in the lab of Dr. Claudio Villanueva where she discovered that plasma acylcarnitines regulate energy homeostasis. In 2019, Dr. Simcox started her lab at the University of Wisconsin-Madison where her lab works to identify functions for plasma lipids, determine how they are regulated, and discovery the function of plasma lipids in metabolic disease. This work has led to the receipt of the ASBMB Walter A Shaw Young Investigator Award in Lipid Research and the JLR Rising Star in Lipid Research Award. Beyond research Dr. Simcox has worked to increase access to science through building programs such as the Native American Research Internship (NARI) at the University of Utah and the Center for Indigenous Research Collaboration and Learning Excellence (CIRCLE at the University of Wisconsin-Madison. She is currently on the Society for the Advancement of Chicanos and Native Americans in Science (SACNAS) Native American/Indigenous Affairs Committee (NAIAC), the co-mentor for the University of Wisconsin-Madison American Indian Science and Engineering Society (AISES) Chapter, and a mentor for the Native American Research Internship.

Harnessing diverse human populations to explore the function and regulation of plasma lipids.

Cardiovascular disease (CVD) is the leading cause of mortality in the USA. Current clinical biomarkers of LDL, triglycerides, and HDL are inadequate to determine CVD risk in people from diverse groups including African American and Native American populations who disproportionately suffer from CVD. We performed lipidomics on samples from diverse populations to identify biomarkers that can inform on the distinct etiology of CVD in these populations. One of the major lipid classes that was increased with CVD is the lysosomal lipid bis(monoacylglycero)phosphate (BMPs). Combining targeted lipidomics, molecular biology, and animal physiology we determined that the liver is a major producer of plasma BMP lipids. BMP lipid changes occurred independently of lysosomal abundance but were dependent on the lysosomal transcriptional factor EB (TFEB). Knockdown of TFEB decreased BMP lipid levels and altered energy homeostasis. To identify how TFEB regulates BMP lipids, we used a combinatorial approach to identify that *Pla2g15,* a lysosomal phospholipase, regulates BMP lipid levels through degrading BMP lipids. Knockout of *Pla2g15* in mice led to increased BMP lipids in the liver and plasma, and a liver specific rescue of Pla2g15 expression restored liver and plasma BMP lipid levels. Together, our studies uncover a required role of TFEB in mediating plasma BMP levels and identified *Pla2g15* as an enzyme that regulates BMP lipid catabolism. Our future studies are focused on understanding the functional role of plasma BMPs in CVD.

Amber Mosley, Ph.D.

Dr. Amber Mosley grew up in the foothills of the Appalachian Mountains in East Tennessee where she attended East Tennessee State University earning a B.S. degree in Biology and Philosophy. She then pursued her PhD under the mentorship of Dr. Sabire Özcan at the University of Kentucky College of Medicine in the Department of Molecular and Cellular Biochemistry. Her dissertation work focused on the regulation of transcription and histone modification by glucose signaling pathways in both yeast and mammals. She then moved to the Stowers Institute in Kansas City, MO to continue her training under the guidance of Drs. Michael Washburn and Jerry Workman. At the Stowers Institute, Dr. Mosley began her work in mass spectrometry-based proteomics, computational biology, and bioinformatics. Dr. Mosley moved to Indiana University in 2010 where she is currently a Professor of Biochemistry and Molecular Biology and Director of the IU School of Medicine Center for Proteome Analysis. Dr. Mosley also serves as the Director of the IU Simon Comprehensive Cancer Center Proteomics Core. The Mosley lab studies fundamental pathways related to RNA Polymerase II transcription, RNA processing, PTM-dependent protein-protein interactions, and proteostasis from a systems-level perspective while developing novel approaches to interrogate biological pathways using quantitative proteomics.

The Stress of Cellular Origami: Defining the Unfolded Protein Response with Thermal Shift Assays

The Unfolded Protein Response (UPR) is used by eukaryotes to address excess of mis-properly folded protein in the endoplasmic reticulum (ER). The response is essential for normal physiology and maladaptive UPR has been implicated in human diseases including cancer, diabetes, and neurodegeneration. The UPR is induced by unfolded protein being sensed by ER membrane proteins which leads to global attenuation of translation coupled with increased expression of select targets such that are necessary for addressing the protein load. Despite the fact that the response has been delineated over the last several decades, many of the phenomena considered to be integral to the response have yet to be directly observed. We show that proteome-wide measurement of biophysical changes using a thermal shift assay approach such as PISA (Proteome Integral Solubility Alteration), can be used to characterize changes in the UPR that have yet to be shown by other methods. In our work, we treated HEK293A or human hepatic stellate cells with Tunicamycin, an agent that is widely used to induce the UPR through its inhibition of N-linked glycosylation. We show system-wide changes in protein biophysical state that led to highly selective changes in proteostasis revealed through a combination of PISA and global proteomics.

Michelle Kimple, Ph.D.

Michelle Kimple, PhD is a faculty member in the Department of Medicine at the University of Wisconsin-Madison. She is also a Research Health Scientist at the William S. Middleton Memorial Veterans Hospital. Dr. Kimple's research program is focused on understanding how the beta-cells of the pancreas respond to nutrient and hormonal stimulation to affect biological changes. Her group is especially interested in elucidating how dysfunctional G protein-coupled receptor signaling pathways contribute to the pathophysiology of diabetes and in translating these insights into new and improved diabetes therapeutics. Dr. Kimple is heavily involved in the training of future researchers and physician-scientists. While a post-doctoral fellow, she received a Preparing Future Faculty Fellowship, where she learned the skills necessary to be a successful mentor and teacher while maintaining excellence in research. She is also a passionate advocate for the destigmatization of mental health disorders and has written articles and given talks on how to survive and thrive as a woman faculty member with mental health challenges. Dr. Kimple is a member of numerous professional organizations and currently serves as Chair of the American Society for Pharmacology and Experimental Therapeutics (ASPET) Division for Molecular Pharmacology Executive Committee. Among the many awards Dr. Kimple has received are the UW-Madison Department of Medicine's Puestow Research Award and the Vilas Life Cycle Professorship from the UW-Madison Women in Science & Engineering Leadership Institute and Office of the Provost.

Elucidating the Role of Circulating Lipids and Their Metabolites in Diabetes Pathophysiology Using Targeted and Untargeted Mass Spectrometry Approaches

Type 2 diabetes (T2D) is a widely prevalent, chronic, and often debilitating disease for which the mechanisms underlying disease pathophysiology are not fully characterized. To address this knowledge gap, in collaboration with Dr. Ying Ge at the University of Wisconsin-Madison, we developed an ultrahigh resolution flow-injection electrospray (FIE) Fourier-transform ion cyclotron resonance (FTICR) mass spectrometry (MS) approach for untargeted plasma metabolomics and applied it to plasma from mice with or without T2D. We observed wholesale changes in the plasma lipidome associated with disease status. One of these lipids, arachidonic acid, is the precursor to a family of bioactive molecules termed eicosanoids. These eicosanoids include the inflammatory mediator, prostaglandin E_2 (PGE_2). Notably, PGE_2 has previously been linked with the pancreatic beta-cell dysfunction of T2D. Applying targeted metabolomics approaches to mouse biosamples, we found PGE_2 levels correlated both with T2D status and the amount of islet insulin secreted from pancreatic beta-cells *ex vivo* in response to PGE_2 receptor agonists and antagonists. Next, we applied the FIE FTICR MS approach to plasma samples from human subjects with and without T2D, replicating the changes in the plasma lipidome observed in our pre-clinical mouse model. Notably, these changes were normalized in subjects with T2D treated with metformin monotherapy as compared to those treated with diet and lifestyle changes only, independent of glucose control. The results of these studies bring us one step closer to our goal of identifying precision medicine approaches to treat individuals with T2D.

Stefani Thomas, Ph.D.

Stefani Thomas, PhD, DABCC, NRCC is an Assistant Professor in the Department of Laboratory Medicine and Pathology at the University of Minnesota. She is also the Associate Medical Director of the M Health Fairview University of Minnesota Medical Center West Bank Laboratory and a board-certified Clinical Chemist. Stefani earned a BA in Biological Sciences from Dartmouth College, a PhD in Pharmaceutical Sciences from the University of Southern California, and she completed a Clinical Chemistry postdoctoral fellowship at Johns Hopkins. While a postdoctoral research fellow and subsequently a Research Associate within the Center for Biomarker Development and Translation at Johns Hopkins, Stefani was engaged in cancer clinical proteomics research, primarily via her participation in the Assay Development Working Group of the NCI's Clinical Proteomic Tumor Analysis Consortium (CPTAC). Through her participation in CPTAC, Stefani led the development and validation of >200 mass spectrometry-based targeted proteomic assays to verify biological insights gained from the global proteomic analysis of ovarian and prostate cancer. Stefani has more than 70 publications, and she is an Associate Editor of the journal *Clinical Proteomics*. Her research program at the University of Minnesota utilizes mass spectrometry-based clinical proteomics for predictive and diagnostic biomarker development.

Overcoming Barriers to Develop Diagnostic Serum Protein Biomarkers for High-Grade Serous Ovarian Cancer

Current clinical high-grade serous ovarian cancer (HGSOC) diagnostic tests rely on measuring serum CA-125 (MUC16) levels to: test women who have vague symptoms of HGSOC, monitor women who have been diagnosed with HGSOC, or monitor women who are at high risk of developing HGSOC. However, this method is not adequately sensitive or specific to screen for HGSOC in the general population. The current lack of a reliable screening test for HGSOC in the general population emphasizes the critical need for more robust diagnostic biomarkers. The frontier of clinical diagnostics is expanding to encompass mass spectrometry-based proteomics assays in clinical laboratories. Encountering challenges during one's training to become an academic research scientist and throughout one's career is not a matter of "if", rather it's a question of "when". How we contextualize and navigate these challenges greatly influences our likelihood of future success. During this Women's Initiatives Session & DEI Workshop, in addition to describing our lab's efforts to develop mass spectrometry-based diagnostic biomarkers for ovarian cancer, I will share some of my experiences while highlighting critical lessons I learned that have enabled me to develop transformative resilience, which is a skill that I argue is particularly essential for scientists from under-represented backgrounds to acquire.

Day 1 Special Session on Spatial Proteomics via Imaging Mass Spectrometry

Peggi M. Angel, Ph.D.

Peggi Angel is tenured Associate Professor at Medical University of South Carolina and Co-Associate Director of Mass Spectrometry Imaging. Dr. Angel's work focuses on the contribution of spatial chemical biology to the external, endogenous environmental and mechanical forces in disparities of disease risk, progression, and therapeutic resistance. She has developed multiple mass spectrometry imaging approaches to spatial biology all of which are designed for use on clinically archived human specimens of tissues, cells and fluids, and are developed working with clinicians. Notably, she is the inventor of a spatial method targeting collagen on formalin-fixed, paraffin-embedded tissues that integrates 2D collagen proteomic maps with spatial transcriptomics and microscopy studies. Dr. Angel is co-founder of Glycopath, Inc., a company leveraging glycosylation patterns as a prognostic or diagnostic tool and serves on the board of N-Zyme Scientifics, a company that produces enzymes for targeted mass spectrometry imaging. She is committed to creating a collaborative mass spectrometry imaging community and serves as President for the Americas Region of the International Mass Spectrometry Imaging Society and Secretary on the USHUPO Board of Directors. Dr. Angel is devoted to coaching and mentoring, particularly for females and minorities, serving on multiple committees to advise and mentor young scientists in entrepreneurship within multidisciplinary teams.

Extracellular Glycoproteins as Pathological Differentiators in Tissue and Fluids

In the era of single cell 'omics, data is showing that the extracellular microenvironment surrounding cells represents novel, unexplored markers of disease. The Angel Lab is supported by multiple NIH and charity grants using mass spectrometry imaging as a platform to measure the extracellular microenvironment in tissue and the release of extracellular protein domains into body fluids. Research in the Angel lab focuses on developing simplified approaches for deeper single cell level sequencing of collagen protein structure, detection of remodeled proteins as markers in tissues and biofluids, and the application of these methods for disease prognosis and diagnosis. Tissue imaging and serum proteomics that includes both the cellular and the extracellular microenvironment are being used to assess cell sources of collagen post-translational modifications as markers of cancer and differentiators of fibrotic disease. Spatially derived techniques allow for sourcing biomarkers to the tumor microenvironment and provide compelling evidence towards future commercialization.

Peter Verhaert, Ph.D.

Peter Verhaert obtained his MSc in Biology (Zoology) and a PhD in Comparative Neurobiology at the University of Leuven [Belgium]. He was Full Professor Analytical Biotechnology and Innovative Peptide Biology at Delft University of Technology [Netherlands, 2005-2016] and is Guest Professor at the Faculties of Medicine and Pharmaceutical Sciences at Leuven University [2024-]. Peter founded ProteoFormiX (www.proteoformix.com) in 2017 at JLABS@BE [J&J Innovation Center, Belgium], where he currently serves as CSO/CEO. He started as Assistant Professor [1983-1987] and, after a year of postdoc research at the Laboratory of Toxicology and Biochemistry in the Biology Department of the University of Waterloo [Canada; 1987-1988], became Associate Professor in Histology and Biological Mass Spectrometry [1988-1999] at Leuven University. After an industrial sabbat year at the Flemish Biotech Innogenetics in Ghent [Belgium; 1998-1999], he was appointed group leader Proteomics at the Organon (now MSD) in Oss [Netherlands; 2000-2004]. He served as co-Director of the Center of Excellence on BioMedical Mass Spectrometry of Lund University (Sweden; 2016] and is Honorary Professor at the Maastricht Multimodal Molecular Imaging Institute (M4i) of Maastricht University [Netherlands; since 2017].

HRMS Secretomics Approach to Map True Biological Activity of Human Cells in Culture

The detection of low-abundant signaling (poly)peptides in a complex background of 'contaminating' high-abundant extracellular matrix and/or serum proteins, represents a major challenge in secretomics, such as in biofluid biomarker discovery and in analyses of conditioned (primary) cell culture media. Conventional workarounds have included a variety of often sophisticated analytical tricks to remove the most abundant proteins downstream from the (*in vivo*) sample collection. For *in vitro* cultures, smart knacks have been developed upstream of sampling, e.g., to transfer the cells under investigation, immediately prior to the experimental stimulus of interest, to serum-free or so-called well-defined media. Although very popular, some evident limitations are unavoidable by both these analytical habits. Abundant protein removal almost inescapably results in the co-depletion of other (protein) analytes from the sample, especially those for which major plasma proteins (like albumin) are known to be carriers. Cultivation of (human) cells in serum-free media for prolonged times (>24 hours) is not possible without introducing significant amounts of cell stress, if not death. With the colossal improvements in analytical performance, especially of high-resolution mass spectrometers, possibilities arise to rethink the above conventional secretomics strategies, e.g., to allow the identification of low abundant secretory proteins in conditioned media of human primary cell cultures kept alive for several days after 'stimulation'. We will present our 'no compromise' proof-of-concept secretome analysis HRMS study showing the specific detection of cytokines and other (signaling) proteins in primary cultures of selected human donor T-cells activated *in vitro* and analyzed 6, 24 and 48h after activation stimulus. Our results demonstrate how highly-specialized isolated *Homo sapiens* immune cells brought into primary culture adapt their secretory behavior when transferred *ex vivo* to cell culturing conditions. The detection of many of the 'usual suspects' in secreted protein identification studies, nicely fit in these somehow less-specialized, i.e. 'dedifferentiated' cellular activities.

Day 2, Aug. 1, 2024

Akhilesh Pandey, MD, Ph.D.

Dr. Akhilesh Pandey is a physician scientist with specialty training in Clinical Pathology. He is currently a Professor in the Department of Laboratory Medicine and Pathology at Mayo Clinic in Rochester, Minnesota where he oversees the Systems Biology and Translational Medicine Laboratory and is also leading efforts to develop mass spectrometry-based novel clinical assays in the Advanced Diagnostics Laboratory. He is the Founder and Director of Institute of Bioinformatics, a non-profit systems biology institute in Bangalore, India. His laboratory at Mayo Clinic employs a broad range of tools and technologies including molecular biology, gene editing (CRISPR/Cas9), animal models, genomics, proteomics, metabolomics, lipidomics and computational biology. In proteomics, he has pioneered methods for quantitative proteomics, analysis of post-translational modifications, protein-protein interactions and proteogenomics. His interests are in using systems biology approaches to investigate a variety of human diseases ranging from cancer and infectious diseases to inherited genetic disorders and in developing novel assays for clinical diagnostics.

From Exploring Biochemical Pathways in Glycosylation to Translation

Although glycosylation is the most abundant post-translational modification, it has only recently become possible to study the glycoproteome on a global scale. Congenital disorders of glycosylation (CDGs) are rare genetic diseases with a wide range of multi-system phenotypes resulting from defects in the enzymatic addition of glycans to proteins and lipids. Over 400 genes are known to play a role in glycosylation and mutations in >150 of these genes have been found to cause different forms of CDG. N-linked glycosylation is the most complex form of protein glycosylation in humans, where oligosaccharide chains or glycans are covalently attached to proteins at asparagine (Asn) residues by an N-glycosidic bond. I will describe our early efforts in the exploration of N-glycoproteome in the context of CDGs using tandem mass spectrometry-based approaches and the discovery of glycopeptides as novel biomarkers that could be developed as clinical assays for accurate diagnosis and therapeutic monitoring of patients. I will also highlight some of the accidental discoveries along this journey that should remind us how little we know and the importance of carefully choosing data analysis approaches.

Hanno Steen, Ph.D.

Hanno Steen, PhD is an Associate Professor of Pathology at Harvard Medical School, Principal Investigator in the Department of Pathology at Boston Children's Hospital (BCH) and its Director of Proteomics. He is a member of the *Neurobiology Program* at BCH, the *Precision Vaccines Program* at BCH, the NIAID-funded *Human Immunology Project Consortium* (HIPC), *Immune Development in Early Life* (IDEaL) and *Immunophenotyping Assessment in a Covid-19 Cohort* (IMPACC) programs, and the NCI/NIDDK-funded *Chronic Pancreatitis, Diabetes, and Pancreatic Cancer* (CPDPC) consortium. After studying chemistry in Freiburg/Germany, Manchester/UK (UMIST) and Zurich/Switzerland (ETH), he received his Ph.D. from the University of Southern Denmark (Odense/Denmark) under the supervision of Dr. Matthias Mann. After postdoctoral training in proteomics and quantitative biology in the laboratories of Drs. Steve Gygi and Marc Kirschner at Harvard Medical School, he joined the faculty at Harvard Medical School and BCH in 2004. At BCH, he established a state-of-the-art proteomics laboratory dedicated to developing and applying quantitative proteomics methods to identify disease-relevant biomarkers in primary human specimens. His laboratory is currently focused on developing high-throughput and sample proteomics methods for detecting biomarkers in small amounts of systemic body fluids such as urine or blood for various inflammatory, infectious and neurodegenerative diseases. He has published more than 190 papers in international peer-reviewed journals.

Advances in Body Fluid Proteomics:

More from Less and that Much Faster (and Cheaper)

Meaningful biomarker studies that account for genetic, environmental and lifestyle variation require large cohorts – too large for the current proteomics platforms. Various NIH-funded consortia, the Steen Lab is involved in, aimed at analyzing thousands of plasma samples to understand better the impact of infection, vaccination and development on the immune system. These projects motivated us to develop a high-throughput plasma proteomics pipeline that can handle thousands of samples while concomitantly mapping more than a thousand plasma proteins at throughputs of 60 to 100 samples per day. The pipeline, with reagent costs in range of less than $5 per sample, has been tested and validated in the meantime on more than 10,000 samples from cohorts in various disease and development contexts, showing exquisite robustness. Interestingly, the concepts of this pipeline are widely applicable to a range of many other body fluids even in a sample-sparing environment such as samples from premature newborns.

Lee Eckhardt, MD, MS

Dr. Lee L. Eckhardt is the Gary and Marie Weiner Professor of Cardiovascular Research and Professor of Medicine in the Division of Cardiovascular Medicine within the Department of Medicine. She has had a long-standing fascination with cardiac electrophysiology and trained as a clinical as well as cellular electrophysiologist. Her a clinical practice solely dedicated to preventing and treating arrhythmic sudden death syndromes as director of the University of Wisconsin Inherited Arrhythmias Clinic. Her lab's goals mirror the clinical practice and aim to advance the investigative platform to better understand diverse arrhythmia syndrome phenotypes and genotype relationships. Dr. Eckhardt serves as the immediate past President of the Cardiac Electrophysiology Society, a standing member of MPPB NIH study section, and is Chairperson of the Heart Rhythm Society Research Fellowship Committee.

Proteomics in Inherited Arrhythmic Conditions; a clinical perspective

Membrane proteins represent a large family of proteins that perform vital physiological roles and represent key drug targets. Despite their importance, bioanalytical methods aiming to comprehensively characterize the post-translational modification (PTM) of membrane proteins remain challenging compared to other classes of proteins in part because of their inherent low expression and hydrophobicity. The inward rectifier potassium channel (Kir) 2.1, an integral membrane protein, is critical for the maintenance of the resting membrane potential and phase-3 repolarization of the cardiac action potential in the heart. The importance of this channel to cardiac physiology is highlighted by the recognition of several sudden arrhythmic death syndromes, Andersen−Tawil and short QT syndromes, which are associated with loss or gain of function mutations in Kir2.1, often triggered by changes in the β-adrenergic tone. Therefore, understanding the PTMs of this channel (particularly β-adrenergic tone-driven phosphorylation) is important for arrhythmia prevention. We developed a proteomic method, integrating both top-down (intact protein) and bottom-up (after enzymatic digestion) proteomic analyses, to characterize the PTMs of recombinant wild-type and mutant Kir2.1, successfully mapping five novel sites of phosphorylation and confirming a sixth site. Lamin A/C (LMNA) is an important component of nuclear lamina. Mutations cause arrhythmia, heart failure, and sudden cardiac death. While LMNA-associated cardiomyopathy typically has an aggressive course that responds poorly to conventional heart failure therapies, there is variability in severity and age of penetrance between and even within specific mutations, which is poorly understood at the cellular level. Further, this heterogeneity has not previously been captured to mimic the heterozygous state, nor have the hundreds of clinical LMNA mutations been represented. We compared the proteomic profiles of cardiopathic LMNA variants of (1) aggregating Q353 K alone, (2) Q353 K coexpressed with WT, (3) aggregating N195 K coexpressed with WT, and (4) nonaggregating E317 K coexpressed with WT to help capture some of the heterogeneity between mutations. We analyzed each data set to obtain the differentially expressed proteins (DEPs) and applied gene ontology (GO) and KEGG pathway analyses. We found a range of 162 to 324 DEPs from over 6000 total protein IDs with differences in GO terms, KEGG pathways, and DEPs important in cardiac function, further highlighting the complexity of cardiac laminopathies. Pathways disrupted by LMNA mutations were validated with redox, autophagy, and apoptosis functional assays in both HEK 293 cells and in induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs) for LMNA N195 K. These two studies highlight the value of detailed proteomic profiles to expand our repertoire for mutation-specific downstream cellular effects that may become useful as druggable targets for personalized medicine approach.

David L. Murray, Ph.D.

David L. Murray MD, PhD is a physician scientist who oversees the clinical protein immunology laboratory at The Mayo Clinic, Rochester. Before attending medical school, he spent 10 years performing research related to macromolecules for Dow Corning and Eastman Chemical Company. He is also a board certified anatomical and clinical pathologist. His research goals center on the adoption of proteomics into the clinical lab. His work had resulted in over 100 peer reviewed articles, 60 patents and mass spectroscopic proteomic assays adapted into the clinical laboratory.

Bringing Mass Spectrometry to the Care of Patients with Monoclonal Gammopathies

Serum protein electrophoresis methods are widely employed to detect, quantify and isotype M-proteins for multiple myeloma patients. Increasing clinical demands to detect residual disease and interferences from new therapeutic monoclonal antibody treatments have stretched electrophoretic methods to their analytical limits. Newer techniques to detect M-proteins using mass spectrometry (MS) are emerging with improved clinical and analytical performance. These techniques are beginning to gain traction within the routine clinical lab testing. This presentation will describe these MS methods with attention to the current and future roles such testing could play in the care of patients with monoclonal gammopathies.

Ruben Y. Luo, Ph.D.

Ruben Y. Luo, PhD, DABCC, FAACC is an Assistant Professor of Pathology at Stanford University and the Associate Director of Clinical Chemistry Laboratory at Stanford Health Care. He received PhD in chemistry from Stanford University, worked in the clinical diagnostic industry for several years, and then completed clinical chemistry fellowship at the University of California San Francisco. Dr. Luo is dedicated to innovations in clinical diagnostics. His research focuses on (1) discovering the clinical diagnostic value of molecular characteristics of protein biomarkers, and (2) applying top-down mass spectrometry and label-free optical sensing immunoassays to characterization and accurate measurement of biomarkers. He has been invited to present in international conferences consecutively for years, e.g., American Association for Clinical Chemistry (AACC), American Society for Mass Spectrometry (ASMS), Mass Spectrometry & Advances in Clinical Lab (MSACL). His research awards include 2022 AACC George Grannis Award for Excellence in Research and Scientific Publication, 2021 NACCCA Outstanding Research Award, 2020 ASCP "40 Under Forty" Honoree, etc.

Clinical Diagnosis on Hemoglobin Variants and Monoclonal Immunoglobulins: Application of Top-Down Mass Spectrometry and Exploration of Single-Molecule Protein Sequencing

Protein markers are being increasingly measured in clinical laboratories for disease diagnosis. With the majority of measurements focusing on quantity, the structural characteristics of protein markers are typically not acquired. These additional characteristics could aid in the diagnosis and treatment of patients by providing more specific and accurate information. Structural characterization of hemoglobin (Hb) variants, particularly the mutant forms of α- and β-subunits, is of significant value in the clinical diagnosis of hemoglobinopathy. The conventional methods for identification of Hb variants in clinical laboratories can be inadequate due to the lack of detailed structural characterization. Top-down mass spectrometry (TD-MS) has been demonstrated for superior analytical performance in identification of Hb variants. In addition, a silicon chip-based single-molecule protein sequencing technology (Quantum-Si) was explored to sequence proteotypic peptides to confirm Hb variants. Monoclonal gammopathies are plasma cell dyscrasias with a significant prevalence, for which monoclonal immunoglobulins are diagnostic markers, i.e., M-proteins and monoclonal free light chains (mFLCs). The common laboratory tests for M-proteins are unable to characterize the proteoforms of M-proteins. TD-MS is ideal for characterizing the proteoforms of M-proteins as the deconvolution step in top-down analysis automatically extracts monoclonal immunoglobulin signals and removes polyclonal immunoglobulin background. Therefore, HR-MS was employed to study the glycosylation patterns of M-proteins to broaden the understanding of monoclonal gammopathies.

Si Wu, Ph.D.

Dr. Wu received her Ph.D. in 2006 from Washington State University under the direction of Prof. James Bruce, working on developing novel MS tools for studying proteins in complex samples. She then worked as a postdoctoral fellow at the Pacific Northwest National Laboratory with Drs. Ljiljiana Pasa-Tolic and Richard D. Smith on top-down proteomics and later became a research scientist at PNNL in 2008. In 2015, she joined the Department of Chemistry at the University of Oklahoma as an assistant professor and was later promoted to associate professor. In 2023, she joined the University of Alabama as a full professor. She was named a "2017 ASMS Emerging Investigator" by the Journal and American Society for Mass Spectrometry (JASMS) and received the USHUPO "Robert J. Cotter New Investigator Award" in 2020. She is also the editor of the Journal of Mass Spectrometry (JMS). Her current research focuses on developing and applying high-throughput quantitative top-down and functional proteomics techniques for addressing important clinical and biological questions.

Ultrasensitive top-down using spray-capillary CE-FAIMS-MS

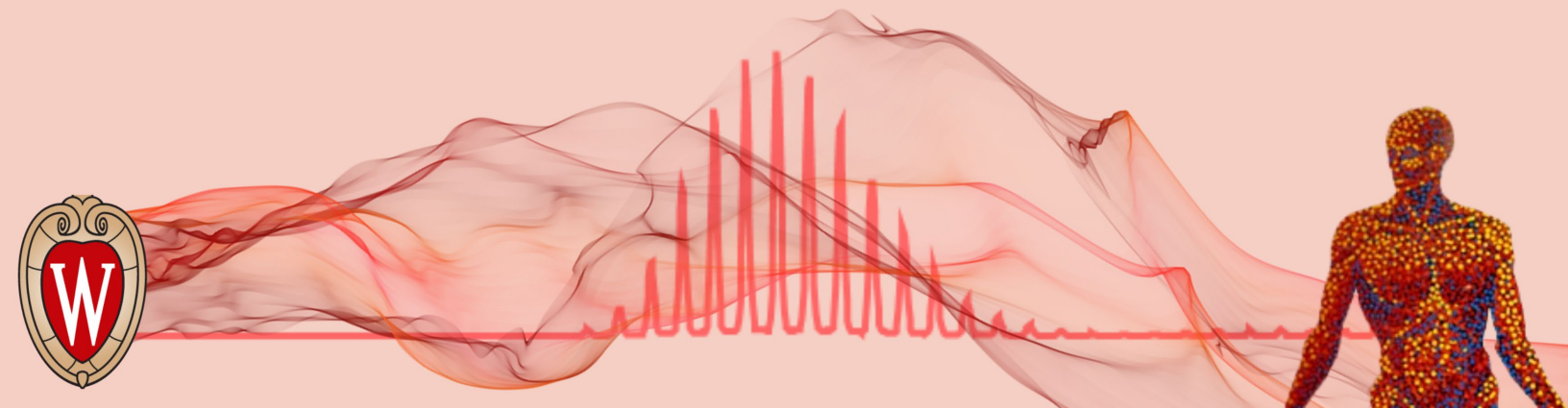
Analyzing intact proteins using top-down proteomics is important for characterizing intact proteoforms with modifications. CE-MS is a promising technique due to its high resolution and sensitivity compared to LC-based separation techniques. We have developed an ESI-assisted device, 'Spray-Capillary', for quantitative ultralow volume sampling and online CE-MS separation. Multidimensional separation can improve top-down proteomics analysis by reducing sample complexity and increasing separation efficiency. High-field asymmetric waveform ion mobility spectrometry (FAIMS) is a gas-phase separation technique that applies compensation voltages (CVs) to separate ions based on their characteristic differences in mobility in high and low electric fields. In this study, we have coupled our automated sensitive top-down spray-capillary CE-MS platform with FAIMS fractionation for improved proteoform characterization in pg-level E. coli lysate. A group of 5-6 CVs were evaluated, and the results were compared with our original spray-capillary CE-MS platform. We demonstrated that the proposed spray-capillary is capable of single-cell metabolomics or proteomics via the direct coupling of CE separation and MS detection, which requires no additional devices. Overall, we envision these techniques as novel analytical tools to apply to the rapidly expanding field of clinical proteomics.

Rising Star Finalists

Isabella James, Predoc, University of Wisconsin - Madison
Cory Matsumoto, Predoc, University of Illinois - Chicago
Yanqi Tan, Predoc, University of Illinois Urbana-Champaign
Zhijun Zhu, Predoc, University of Wisconsin - Madison
Zhan Gao, Postdoc, University of Wisconsin - Madison
Morgan Mann, Postdoc, Standford University
Pei Su, Postdoc, Northwestern University
Lyndsay Young, Postdoc, Medical University of South Carolina

Honorable Mention

Mario Alba, Predoc, University of Southern California
Yutong Bao, Predoc, University of Wisconsin - Madison
William Beimers, Predoc, University of Wisconsin - Madison
Lauren Fields, Predoc, University of Wisconsin - Madison
Hannah Miles, Predoc, University of Wisconsin - Madison
Margaret Robinson, Predoc, University of Wisconsin - Madison
Holden Rogers, Predoc, University of Wisconsin - Madison
Kalina Rossler, Predoc, University of Wisconsin - Madison
Jack Shireman, Predoc, University of Wisconsin - Madison
Feixuan Wu, Predoc, University of Wisconsin - Madison
Dominique Baldwin, Postdoc, University of Wisconsin - Madison
Haiyan Lu, Postdoc, University of Wisconsin - Madison
Min Ma, Postdoc, University of California, San Diego
Jericha Mill, Postdoc, University of Wisconsin - Madison
Dylan Tabang, Postdoc, Boston Children's Hospital – Harvard Medical School



Rising Star Session (Senior Group)

Irene Ong, Ph.D.

Dr. Irene Ong is an Associate Professor in the Departments of Obstetrics and Gynecology and Biostatistics and Medical Informatics at the University of Wisconsin-Madison. She is a faculty member of the Center for Human Genomics and Precision Medicine and the UW Carbone Cancer Center and an affiliate of the UW Data Science Institute. Dr. Ong's research interests lie in the development and use of bioinformatics, data science, and machine learning techniques to gain insights into complex biological systems to uncover mechanisms, develop therapeutics or identify biomarkers for precision medicine.

Breastfeeding and Traditional Farming Lifestyle Promotes Predominant Bifidobacterium in Infants

The pioneer microorganisms that colonize newborn gastrointestinal systems play a critical role in establishing the foundation and trajectory of microbial colonization, which in turn influence the metabolites, lipids, and development of the infant, including the immune system. Infants with early life farm exposure have unique microbial signatures that have been associated with immune cell phenotypes and protection from allergic disease development, however, specific mechanisms remain unknown. Metagenomic, metabolomic, and lipidomic analysis of stool from two-month-old infants from families who participate in a traditional agrarian lifestyle (TA), live or work on a modern dairy farming operation, or were not engaged in farming were performed. We applied statistical and machine learning feature analysis to identify microbe-metabolite and microbe-lipid associations with robustly predictive features for farm group and diet. These multi-omic features and associations were integrated into a computational network and visualized to provide insights into TA-associated metabolites and lipids that are microbially modulated. All breastfeeding infants had high relative abundances of genus Bifidobacterium. Non-TA infants and infants with formula in their diet had higher microbial diversity than the TA infants, while the metagenomic profiles of the TA infants were dominated by *Bifidobacterium longum subsp. infantis*. The presence of formula in the diet also correlated with reductions in certain bile metabolites. Compared to non-TA regardless of diet, the stool of the TA infants had differences in metabolites essential for cellular function, energy metabolism, membrane structure and signaling. Confirming prior findings, TA infants and non-TA infants with high levels of *Bifidobacterium longum* had elevated levels of indole-3-lactic acid. The results of this study are consistent with, and expand upon, previous studies that identified *B. infantis* and associated metabolites in traditional agrarian communities.

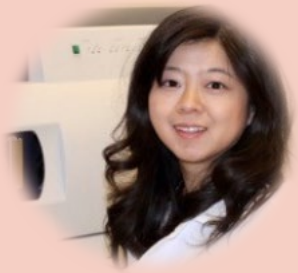
Qing Yu, Ph.D.

Assistant Professor of Biochemistry and Molecular Biotechnology at the University of Massachusetts Chan Medical School. He received his B.S. degree from Tianjin University in China and obtained his Ph.D. under the supervision of Dr. Lingjun Li at the University of Wisconsin-Madison. In 2017, he moved to Boston to pursue postdoctoral training with Dr. Steven Gygi at Harvard Medical School. In July 2024, he concluded his postdoctoral training and joined the faculty at the UMass Chan Medical School. Throughout his training, Dr. Yu developed novel isobaric labeling reagents for sample multiplexing, built intelligent platforms to improve data quality and throughput, and created chemoproteomics strategies to enable large-scale compound screening. He has successfully integrated these technological advancements to systematically understand biological processes and connect underlying genotype to molecular phenotype using various model systems. His research efforts have led to 43 publications. The research in the Yu lab at the UMass Chan Medical School currently focuses on creating innovative proteomics platforms to decipher protein function in health and disease, with the overarching goal of driving therapeutic development and clinical application. Dr. Yu was recently awarded the NIH-NCI K22 Transition Career Development award.

High-throughput intelligent chemoproteomics for massive protein-ligand insight and targeted drug discovery

Chemoproteomics has become a major approach to discover new protein-ligand interactions for biomedical research and drug discovery. The success of multiple covalent drugs against proteins once deemed "undruggable" has fueled growing interest in electrophiles that covalently bind amino acids such as cysteine. However, current technology can routinely access only a small fraction (<25%) of the human cysteinome due to limited sensitivity. This limitation creates bottlenecks in discovering novel ligands for therapeutic application and evaluating the selectivity of molecules. In addition, large-scale compound screening campaigns against therapeutic targets are frequently plagued by missing values, a consequence of the stochastic nature inherent in mass spectrometry-based discovery proteomics. Here, we introduce a platform which integrates tandem mass tag-based sample multiplexing, an intelligent targeted proteomics strategy—GoDig, and a high-depth activity-based cysteine profiling to overcome the challenges and revamp covalent drug discovery. The platform enables high-throughput, targeted interrogation of cysteine engagement and protein abundance within the same experimental run, effectively differentiating engagement from otherwise confounding protein degradation. After identifying confident ligands to proteins of interest, selectivity can be evaluated across ~30K reactive cysteines in a single assay using the platform, which represents a two-fold improvement in depth for proteome-wide cysteine ligandability profiling. We applied the platform to a screening for electrophiles that can ligand cysteines with annotated biological functions (e.g., active-site cysteine), with an eye towards manipulating their enzymatic function for drug discovery. The screening identified compounds that inhibited the ubiquitin-proteasome system, as well as those that caused protein degradation. As an example of the former, we highlight one specific compound, E2913, which engages the active site of the E3 ubiquitin-protein ligase HUWE1, perturbing its function. HUWE1 is a HECT E3 ligase implicated in key biological processes and is associated with cancer and neurodevelopmental disorders.

Jia Fan, Ph.D.



Assistant Professor of Biochemistry and Molecular Biology at Tulane University and a faculty member of the Center for Cellular and Molecular Diagnostics, where she also serves as the Director of the Tulane Proteomics Core. Dr. Fan began her career as an Assistant Research Professor at the Biodesign Institute of Arizona State University after completing her postdoctoral studies at the Houston Methodist Research Institute. She has published over 50 peer-reviewed papers, filed four patents, authored a book chapter, and received a Young Investigator Grant from the Association for Mass Spectrometry: Applications to the Clinical Lab (MSACL). Dr. Fan was a funded Associate Member of the NCI Early Detection Research Network (EDRN) in 2017-2018. Dr. Fan recently received an NIH S10 instrument grant to support purchasing a Tribrid Eclipse mass spectrometry system. Her research focuses on developing and validating integrated mass spectrometry strategies for peptide biomarker discovery and their diagnostic applications. She focuses on developing mass spectrometry-based assays for pathogen peptide detection and computational tools to support the clinical application of mass spectrometry in bacterial identification.

Advances in Mass Spectrometry for Precision Diagnostics and Pathogen Identification

Mass spectrometry (MS) is a powerful tool in precision diagnostics, offering unmatched specificity and sensitivity for detecting biomarkers in clinical samples. Despite its potential, the integration of MS into routine clinical diagnostics is hampered by its complexity and the need for specialized knowledge, which slows adoption and introduces potential biases. To address this, we have developed computational tools to streamline MS data analysis and enhance the precise identification of pathogens. Our first innovation is a nested machine learning (ML) pipeline for targeted MS data analysis, designed to extract and analyze both traditional statistical and novel engineered morphological features. This pipeline includes a nested active learning algorithm (nActL) to refine training set quality dynamically, enhancing model accuracy and robustness. Preliminary results with a Random Forest model demonstrated an area under the curve (AUC) of 0.98±0.01, with external validations confirming robust performance. The nActL proved particularly effective in improving sensitivity across various training data sizes, challenging the assumption that large datasets are essential for robust training. In a subsequent study, we created a Peptide Taxonomy/Organism Checking (PEP-TORCH) pipeline, a decision-tree logic for the identification of mycobacterial pathogens at subspecies resolution by analyzing taxa-specific peptides. This method streamlines the diagnostic process by eliminating the need for multiple laboratory tests and significantly reduces diagnosis turnaround times by efficiently analyzing early culture samples. Validation on a clinical sample cohort achieved a 100% agreement rate with clinical diagnoses, underscoring its potential to improve diagnostic methods significantly. These innovations—the ML pipeline and the PEP-TORCH algorithm—simplify traditional diagnostic processes and enhance diagnostic precision. These novel approaches could accelerate the integration of MS into clinical settings, potentially revolutionizing clinical proteomics.

Diamond Sponsor Oral Presentations

Tao Chen, Ph.D.

Founder & CEO of Absea Group

Tao Chen has extensive experience at the intersection of biology and informatics, with a strong background in developing original methods and translating them into industry. During his graduate studies in the Netherlands, he co-developed transgenic mice producing human nanobodies and the HCAb Erasmus MC, acquired by Harbour BioMed for $85 million. At the Netherlands Cancer Institute, he was a key developer of TIDE, a gene editing evaluation tool licensed to Desktop Genetics, cited about 2,300 times and used by over 2 million people annually. Since 2020, Tao Chen has founded Absea Group (formerly Unogen), developing soluble proteins and monoclonal antibodies to cover the human proteome. Absea also has built an advanced protein interaction characterization platform in Berlin based on cross-linking mass spectrometry (XLMS). In 4 years, Absea has grown into a research team of over 250 members and collaborates globally with leading proteomics companies and consortia. This presentation will cover Absea Group's scientific approach and how its resources and platforms contribute to proteomics and protein sciences.

Platforms and Resources for Proteomics and Beyond

Panoramic detection of proteins with spatial and temporal resolution is crucial for understanding life, driving various proteomics disciplines. Comprehensive availability of proteins, monoclonal antibodies, monoclonal pairs, and oligoclonal antibodies, along with rigorous characterizations, is essential but commercially limited. Collaborating with leading proteomics companies and scientific consortia, we have developed over 13,000 soluble human proteins, covering 65% of the human proteome in the last four years. We are currently developing monoclonal and oligoclonal antibodies targeting over 10,000 human proteins in the next two to three years, at a rate of approximately 200-400 targets per month. These antibodies are characterized for applications including IHC, IF, WB, IP, FACS, and pairing, providing valuable assets for non-MS-based proteomics, MS-based proteomics, cell biology, biochemistry and so on. Additionally, Absea leverages cross-linking mass spectrometry (XL-MS) to study protein-protein interactions (PPI), generating large-scale data assets. Compared to conventional methods, XLMS is 10,000 times more efficient, which is important for driving AI modeling and de novo protein design. In summary, Absea has built unprecedented platforms to generate fundamental resources of molecules and information to facilitate the developments of proteomics and protein sciences.

Michael M. Rosenblatt, Ph.D.

Promega Corporation, Madison WI

Dr. Michael M. Rosenblatt received his B.Sc. (Cum Laude) in chemistry from Towson University and PhD (Chemistry) from the University of Illinois at Urbana-Champaign. For his graduate work, he primarily focused on the biophysical characterization of *de novo* designed heme proteins. He then did his postdoctoral training at the University of Pennsylvania (laboratory of Bill DeGrado) on the *de novo* design of metallo-proteins and was the recipient of an NRSA from the NIH. In 2003, he was the founding director of the Proteomics Core Facility at the Children's Hospital of Philadelphia. In 2007, he joined Thermo Fisher Scientific (Pierce) as a Senior Scientist in the newly formed MS Reagent Research and Development team. In 2011, Dr. Rosenblatt assumed the role of Group Leader (MS Reagents) in R&D at Promega Corporation. In this role, he has overseen the development of multiple new enzymes (Proteases and Glycosidases), Mass Spec Reference materials, as well as workflow solutions using magnetic beads for enrichment and desalting. He is also the primary operator of the Mass Spectrometer within Promega RnD and helps to support the needs of internal Promega RnD projects related to Targeted Protein Degradation, Proximity and IP based proteomics using the HiBiT tagged system, as well as work to support some of the efforts within the Promega Advanced Technology Platform.

Toward Optimal Workflow Solutions for Bottom-Up Proteomics

With increasing interest in quantifying dynamic events within the human proteome, such as between disease states or response to drug treatment, high-quality sample preparation is essential. Low-cost, universal, efficient, and automatable methods for sample cleanup, capture of protein-interactions, and efficient proteolysis are of top priority. Recent improvements in proteolytic enzymes are reflected with the introduction of ProAlanase, a proline- and alanine-specific protease, and Arg-C Ultra, an enzyme with dramatically improved specificity for arginine residues that is so efficient that missed cleavages can virtually be eliminated. Combining Arg-C Ultra with Lys-C may represent a significant improvement on traditional methods that rely on Trypsin or Trypsin and Lys-C. To improve sample cleanup/desalting, we utilized magnetic beads (with varying surface chemistries) combined with precipitating agents for removal surfactants and denaturants in lysis buffers. Our findings indicate that this approach provides multiple advantages over filter-based or precipitation-based methods, including cost reduction, ability to multiplex and automate, improved results with low sample input, and overall time savings. Bead-based sample cleanup can be incorporated into complex workflows, including quantitative proteomic screens following small molecule treatment, PTM enrichment, automation etc. Protein-Protein and Protein-drug interactions are also of increasing interest, particularly under endogenous conditions. We will discuss several approaches focused on improved proteomic methods for identifying endogenous protein-interactions, taking advantage of a small tag called HiBiT. After incorporation of a HiBiT tag fused to a protein of interest via CRISPR, endogenous interactors can be isolated either by traditional IP using anti-HiBiT magnetic beads, or via proximity labeling by way of targeting a proximity-labeling (PL) enzyme to the protein of interest via fusion of the PL enzyme to LgBiT, which then associates with HiBiT to form a functional NanoBiT Luciferase. Finally, we discuss the identification of protein-drug interactions (on and off target) using a novel chemoproteomic-based approach.

Michael Greig, PhD

Executive Director

Bruker Pharma/Biopharma Business Unit

Michael Greig, Executive Director Pharma/Biopharma at Bruker, is a graduate from the University of Delaware. His first job was at Ionis Pharmaceuticals in Carlsbad, CA synthesizing, purifying, and analyzing oligonucleotides. During this time, he also worked as a visiting scientist at the National High Magnetic Field Lab in Alan Marshall's group studying gas-phase structure of nucleotides and oligos, as well as native interactions of oligos and small molecules. Prior to joining Bruker in 2018, Mike worked for 20 years at Pfizer (San Diego) as an Assoc. Research Fellow in the Protein Dynamics Group where he guided inter-disciplinary global project teams working on native MS and structural proteomics including HDX-MS, drug-target interactions, high-throughput screening, bottom-up proteomics, metabolomics, and interactomics. Mike initiated and coordinated external collaborations with Scripps, Northwestern University, Florida State University, and served on NHMFL ICR Advisory Panel. He was also awarded a Pfizer People Leader Award and a Green Chemistry Award during his tenure at Pfizer. Mike is currently responsible for developing strategy for Bruker's Pharma mass spec business unit. Mike has over 50 scientific publications ranging from PK properties of oligonucleotides in mice, native MS of biological complexes, HDX to identify resistance mechanisms in oncology, to SFC/MS of small molecule libraries.

Expanding Capabilities for a Top-Down World

Top-down protein analysis by mass spec, while being around for decades, is not as routine as bottom-up protein analysis. At Bruker, we are removing two of the major roadblocks to top-down with tools for rapid and precise data analysis as well as advanced instrumentation enabling multiple pathways for intact biologics fragmentation. I will present a brief overview of our new OmniScape top-down sequencing software as well as give a sneak preview of our future instrumentation for molecular analysis and dissection of ions.

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Lunch Seminars, ThermoFisher

From Compounds to Clinics: New Innovations in Proteomics

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Amanda Lee, Ph.D.

Thermo Fisher Scientific
Tribrid Product Marketing Manager

Amanda Lee received her PhD from the University of Maryland under the guidance of Professor Catherine Fenselau in analytical chemistry. There she focused her efforts of the top-down elucidation of poly-ubiquitin chains by mass spectrometery. Her post-doctoral fellowship was spend at the University of Illinois, Chicago in the laboratory of Professor Richard van Breemen working on studying protein biomarkers of natural product by semi-quantitative techniques. Her career at Thermo Fisher Scientific began as a field application scientist for omics and biopharma applications on high-resolution mass accuracy (HRAM) mass spectrometers. Currently, she is the product marketing manager focused on the Thermo Scientific™ Orbitrap™ Tribrid mass spectrometry line and Thermo Scientific™ Direct Mass Technology™ mode products.

Matthias Mann, Ph.D.

Professor of Proteomics and Signal Transduction

Director, Max Planck Institute of Biochemistry

(See bio in the keynote speaker session)

Akhilesh Pandey, M.D., Ph.D.

Professor

Mayo Clinic

(see bio in the invited speaker session)

Lunch Seminars, Agilent Technologies

Jonathan V. Sweedler, PhD

Jonathan Sweedler is the James R. Eiszner Family Endowed Professor of Chemistry, Acting Head of the Department of Chemistry, and affiliated with the Institute of Genomic Biology and the Beckman Institute for Advanced Science and Technology at the University of Illinois Urbana-Champaign. His research interests focus on developing new approaches for assaying small volume samples, including lipidomics, metabolomics and peptidomics using micro- and nanofluidics, miniaturized separations, and mass spectrometry. He has used these tools to characterize small molecules and peptides in a range of animal models across metazoan life and in samples as small as individual cells and cellular domains. Sweedler has published more than 500 manuscripts and presented 600 invited lectures. He has received numerous awards including the Instrumentation Award from the Analytical Division of the ACS, the Pittsburgh Analytical Chemistry Award, and the ACS Award in Analytical Chemistry. He is a fellow of both the American Association for the Advancement of Science and the American Chemical Society. He is currently the Editor-in-Chief for ***Analytical Chemistry***.

Single-Cell Metabolomics via CE-NanoESI-MS

(with Shuangshuang Chen, Stanislav S. Rubakhin, Mike Knierman, Yash Nelavelli3, Noah A. Bender)

Department of Chemistry, University of Illinois Urbana Champaign

Single-cell metabolomics is important for understanding cell-to-cell heterogeneity and link cell neurochemistry to function. Here an Agilent 7100 CE is coupled with the Agilent 6495C triple quadrupole mass spectrometry using 3D-printed parts as the nanoflow sheath liquid interface, and the system used for single cell measurements. Different identified neurons were isolated from the central nervous system of *Aplysia californica*, and the sample preparation steps were performed in vial inserts prepared from commercially available 10 µL pipette tips. Other changes including a nanointerface and a mechanically tapered capillary tip for optimized detectability. The metabolites in individual cells were characterized using the multiple reaction monitoring mode (MRM) with the QqQ-MS. The CE-nanoESI-QqQ MS system demonstrated detection limits ranging from femtomole to attomole levels for a range of amino acids and transmitters. We are extending our metabolomic analysis to isolated rodent neurons, transplant-quality islets, and individual endocrine cells. The combination of nanovial CE-nanoESI-QqQ MS with the 3D-printed interface, an auto sampler, and fast MS2 scanning enables high-throughput, high-sensitivity, and robust metabolite analysis of single cells.

Anna Anders, PhD Candidate

Anna completed her undergraduate studies at Arkansas State University where she obtained a bachelor's degree in biology (BS) and a bachelor's degree in chemistry (BA). She worked in an analytical laboratory leveraging laser-induced breakdown spectroscopy (LIBS) and laser-ablation laser-excited atomic fluorescence (LA-LEAF) for trace arsenic quantitation in rice. Currently, she studies at the University of Michigan where she obtained her master's degree in 2022, and she continues to work on her doctoral degree developing native mass spectrometry-based techniques for the biophysical characterization of ribonucleic acids (RNAs).

Development of Native Ion Mobility-Mass Spectrometry (IM-MS) and Collision-Induced Unfolding (CIU) for the Structural Characterization of Non-coding Ribonucleic Acids (ncRNAs)

Anna G. Anders[1], Ingrid R. Kilde[2], Elizabeth D. Tidwell[3], Varun Gadkari[4], Caela Fedraw,[1] Courteney R. Dufrene,[1] Markos Koutmos[2,3], and Brandon T. Ruotolo[1]

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[3]*Program in Biophysics, University of Michigan, Ann Arbor, MI 48109*
[4]*Department of Chemistry, University of Minnesota, Minneapolis, MN 55455*

Most of the human genome encodes ribonucleic acids (RNAs) that either carry out key biological functions or possess unknown roles in cellular biochemistry. This reality demands the development of new technologies capable of studying the structures of non-coding RNAs (ncRNAs). Current RNA structural probes are time and resource-intensive, often require structural modifications, and critically lack the ability to probe the RNA structural ensemble. Native ion mobility-mass spectrometry (IM-MS) is a gas-phase approach uniquely capable of overcoming the aforementioned challenges. Nanomoles of sample are gently ionized by nano-electrospray ionization (nESI), separated based on size, shape, and charge in a low-pressure drift cell, and analyzed by a time-of-flight mass spectrometer. IM yields collision cross-sections (CCSs), an experimentally derived structural parameter that can be compared to other biophysical techniques, and in combination with MS, is sensitive to subtle differences in solution conformations while readily enabling the observation and quantitation of structural polydispersity. An IM-MS-based method, collision-induced folding (CIU), further enables the measurement of structural unfolding as a function of gas-phase activation (energy input) enabling the measurement of structural stability in addition to structural changes not readily discernible by CCS alone. Here, we present the development of CIU for ncRNA structural characterization. Specifically, we will show the development of this technique in how it is used to probe ncRNA higher order structure (HOS) as it relates to biophysical perturbations due to mutation and/or encapsulation by lipid nanoparticles (LNPs). Next, we address ongoing efforts in the development of high-throughput CIU related to small molecule targeting of ncRNAs (i.e., biologically relevant aptamers), and finally, how these gas-phase results relate to solution-phase studies (via leveraging ultraviolet-visible spectroscopy, or UV-vis, melting curves). Finally, we wrap up this presentation with work surrounding an RNA-binding protein before concluding and addressing what future directions we hope are next for RNA-related CIU.

Gary Patti, PhD

Gary Patti is the Michael and Tana Powell Professor at Washington University in St. Louis, where he holds appointments in the departments of chemistry, genetics, and medicine. Dr. Patti is the Senior Director of the Center of Mass Spectrometry & Metabolic Tracing, Director of the Clinical Research Core, Principal Investigator of the NIH Omics Production Center, Dean's Fellow of Advancement and Entrepreneurship, Director of Faculty Affairs, and the Chief Scientific Officer and Co-Founder of Panome Bio. Professor Patti's research focuses on developing and applying mass spectrometry-based technologies to enhance our understanding of human diseases such as cancer. Professor Patti has been recognized with numerous awards including the Biemann Medal from the American Society for Mass Spectrometry and the inaugural NIH award for revolutionizing, innovative, and visionary environmental health research.

Spatial Analysis of Metabolic Fluxes by Mass Spectrometry Imaging

Gary Patti, Professor of Chemistry and of Genetics and Medicine, Michael and Tana Powell Professor of Chemistry, Washington University School of Medicine

Mass spectrometry-based imaging has emerged as a powerful technology to assess metabolite levels in tissues. Mapping metabolite concentrations, however, only provides a static picture of metabolism. To capture the dynamics of biochemical pathways, isotope tracers must be applied. Although there is an armamentarium of tools to evaluate isotopically labeled samples by gas and liquid chromatography/mass spectrometry, these techniques have not yet been extended to imaging. Here, we present a strategy to assess metabolic fluxes using MALDI and DESI imaging. We describe the major challenge of resolving complete isotopologue patterns to model pathway activities and explore several solutions to overcome interferences including using the MassTech AP-MALDI source on an Agilent 6495C triple quadrupole mass spectrometer. We apply the approach to map fluxes in the brains of mice bearing glioma. The results show that brain tumors increase production of lipid precursors relative to surrounding healthy tissue and other tumors outside the central nervous system.

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SUMMARY OF POSTER COMPETITION ABSTRACTS

(Listed alphabetically by last name)

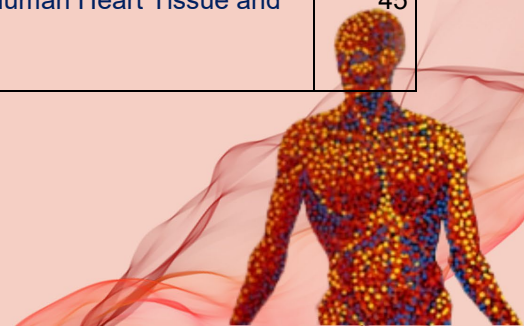
Last Name	First Name	Institution	Year	Title	Poster #
Alba	**Mario**	University of Southern California	Predoc	AKT Isoform Specific Regulation of Hepatic Steatosis Induced by Bioactive Lipids	1
Baldwin	**Dominique**	University of Wisconsin - Madison	Postdoc	Plasma lipids as predictors of metabolic health in people with Type 1 Diabetes	2
Bao	**Yutong**	University of Wisconsin - Madison	Predoc	Profiling of lipid binding protein subcellular translocations in brown adipocytes in response to adrenergic stimulation	3
Beimers	**Wiliam**	University of Wisconsin - Madison	Predoc	Extensive Multi-Omics of Blood Plasma for Molecular Characterization of Acute- and Long-COVID	4
Chan	**Hsin-Ju (Ruby)**	University of Wisconsin - Madison	Predoc	In-Depth Characterization of Protein Kinase Complexes by Hybrid Top-Down Mass Spectrometry	5
Chen	**Shuangshuang**	University of Illinois	Predoc	Exploring D-amino Acids as Biomarkers in Diabetes	6
Chiang	**Hung-Yu**	University of Wisconsin - Madison	Predoc	Extended Characterization and Multiplexed Quantification via a Chemical Proteomics Platform Using High-field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS)	7
Feeney	**Austin**	University of Wisconsin - Madison	Predoc	Multi-omic Characterization of Human Pluripotent Stem Cell-derived Cardiomyocyte Maturation during Long-term 2D Culture	8
Fields	**Lauren**	University of Wisconsin - Madison	Predoc	Unlocking the Crustacean Neuropeptidome through EndoGenius and Data-Independent Acquisition Mass Spectrometry	9
Fischer	**Matthew**	University of Wisconsin - Madison	Predoc	A Multidimensional Native Separation for the Online Analysis of Endogenous Protein Complexes from Human Heart Tissue	10
Gearhart	**Rob**	University of Wisconsin - Madison	Predoc	Discovery of RSV-Inducible Interactome of Interferon Regulatory Factor-1 Using Affinity Enrichment Mass Spectrometry	11
Hoffman	**Julia**	University of Wisconsin - Madison	Predoc	Optimization of isolating synaptic vesicles for comprehensive glycoproteomic and neuropeptidomic analysis in neurodevelopmental disorders.	12
Huang	**Penghsuan**	University of Wisconsin - Madison	Predoc	Deciphering age-dependent global proteome changes in the leaf-cutting ant Acromyrmex echinatior for better understanding of biomineralization process	13
Kizzar	**Thomas**	University of Wisconsin - Madison	Predoc	Modulation of hepatic TFEB activity during cold exposure uncovers direct regulation of bis(monoacylglycero)phosphate lipids by Pla2g15	14

Liu	**Peng - Kai**	University of Wisconsin - Madison	Predoc	13-plex DeAla Isobaric Reagents for High-Throughput Proteome Quantification	15
Lloyd	**Sarah**	AbbVie Inc.	Postdoc	Development and application of highly efficient LC-MS/MS methodology for investigating collagen crosslinking in idiopathic pulmonary fibrosis	16
Lu	**Gaoyuan**	University of Wisconsin - Madison	Predoc	Unveiling Novel L-to-D Amino Acid Residue Isomerization in Neuropeptides via Multi-faceted Single-Cell Mass Spectrometry	17
Lu	**Haiyan**	University of Wisconsin - Madison	Postdoc	FAIMS-DIA-MS-based structural proteomics and N-glycoproteomics to discover Alzheimer’s disease staging biomarkers	18
Miles	**Hannah N.**	University of Wisconsin - Madison	Predoc	Integrating MALDI-MSI and LC-MS/MS for Spatial Localization of Disease Markers in a Model of Benign Prostatic Hyperplasia	19
Mill	**Jericha**	University of Wisconsin - Madison	Postdoc	Investigating rapamycin treatment in older adults with heart failure	20
Perciaccante	**Andrew**	University of Wisconsin - Madison	Predoc	Achieving In-Depth Multi-omic Coverage of Extracellular Vesicles/Exosomes through Systematic Comparison of Multiple Extractions and High-Resolution Mass Spectrometry	21
Robinson	**Margaret**	University of Wisconsin - Madison	Predoc	High-throughput LC-MS/MS enables protein Quantitative Trait Loci mapping in the progression of Metabolic Dysfunction-Associated Steatotic Liver Disease	22
Rogers	**Holden**	University of Wisconsin - Madison	Predoc	Top-down Proteomic Characterization of Dilated Cardiomyopathy in Phospholamban-R14del Patients	23
Rossler	**Kalina**	University of Wisconsin - Madison	Predoc	Integrated Proteomics Analysis of In Vitro Hypertrophic Cardiomyopathy Models	24
Shireman	**Jack**	University of Wisconsin - Madison	Predoc	Transcriptomic and Proteomic Spatial Profiling of Pediatric and Adult Diffuse Midline Glioma H3 K27-Altered	25
Tabang	**Dylan**	Boston Children's Hospital - Harvard	Postdoc	High-throughput platelet proteomics from small amounts of previously frozen samples adds another layer to plasma-based immunophenotyping	26
Towler	**Anna Grace**	University of Wisconsin - Madison	Predoc	Azo-Enabled Extracellular Matrix Proteomics for High-throughput Quantitative Analysis of Lung Tissue	27
Tran	**Vu Ngoc Huong**	University of Wisconsin - Madison	Predoc	Neuropeptide Secretion in Crustacean in Response to Feeding via In vivo Microdialysis	28
Whitworth	**Isabella**	University of Wisconsin - Madison	Predoc	Developing Technologies to Probe RNA-protein interactions	29

Wu	**Feixuan**	University of Wisconsin - Madison	Predoc	High-throughput simultaneous quantification of glycopeptides and phosphopeptides enabled by 12-plex DiLeu isobaric tags and dual-functional Ti(IV)-IMAC material	30
Xu	**Shuling**	University of Wisconsin - Madison	Postdoc	Isobaric Labeling of Multiple Lipid Classes toward Multiplexed LC-MS/MS-based Quantitative Analysis	31
Young	**Lyndsay**	Medical University of South Carolina	Postdoc	Multi-Omic Application of MALDI-MSI and MALDI-IHC for Biomolecular Profiling of Immune Cells in Tissues and Single Cells	32
Zhang	**Jingwei**	University of Wisconsin - Madison	Predoc	Boosting Quantification of N-Glycans by Enhanced Isobaric Multiplex Reagents for Carbonyl-Containing Compound (SUGAR) Tagging Strategy	33
Zhang	**Yanghai**	University of Wisconsin - Madison	Predoc	Sequestration of RNA binding proteins in cytoplasmic granules disrupts posttranscriptional process in RBM20 cardiomyopathy	34

SUMMARY OF POSTER FAIR ABSTRACTS (Not for poster competition)

Angel	**Peggi**	Medical University of South Carolina	Post-translational Regulation of Collagen as a Pathological Differentiator in the Ancestry-defined Breast Microenvironment	35
Arnold	**Randy**	Sciex	Scout triggered MRM (stMRM); a method to acquire large numbers of MRM without predefined retention time	36
Bleiholder	**Christian**	Florida State University	Atomic-level protein structure characterization by tandem-trapped ion mobility spectrometry/tandem-mass spectrometry coupled with UV photodissociation	37
Colquhoun	**David**	Sciex	Improving complex phosphopeptide characterization with hybrid collision-induced dissociation and electron activated dissociation fragmentation	38
Ibarra	**Beatriz**	University of Illinois Chicago	Laser Assisted Spatial Tissue Encoding (LASTE) – a high-resolution method for spatial proteomics.	39
Rosenblatt	**Michael**	Promega	Magnetic Bead based Proteomic Sample Preparation	40
Shafaei	**Mana**	Affinisep	Development and optimization of a method for automated peptide desalting on the DigestPro MSi robot using AttractSPE® C18 tips	41
Tabang	**Dylan**	Boston Children's Hospital - Harvard	Proteoform-Driven Biomarker Discovery for Alzheimer’s Disease Progression in Matched Plasma and CSF	42
Vitko	**Dijana**	Bruker	Increasing throughput while maintaining coverage depths in single cell proteomics using the timsTOF Ultra2	43
Wilson	**Mallory**	University of Wisconsin - Madison	Novel Proteomics Method to Analyze the Maturation of Micropatterned Induced Pluripotent Stem Cell-Derived Cardiomyocyte Co-Cultures	44
Zhu	**Yanlong**	University of Wisconsin - Madison	Comprehensive Metabolomic Analysis of Human Heart Tissue and Application to Diabetic Cardiomyopathy	45



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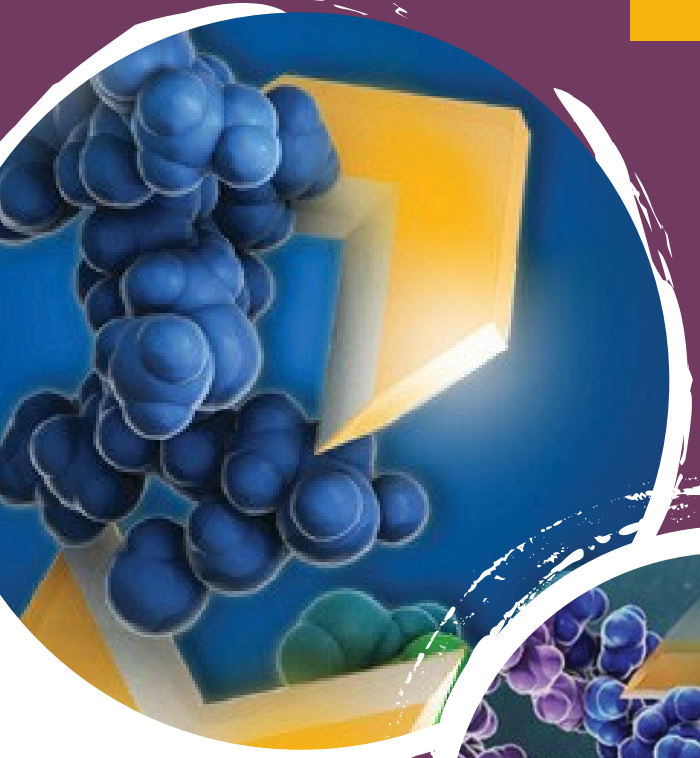

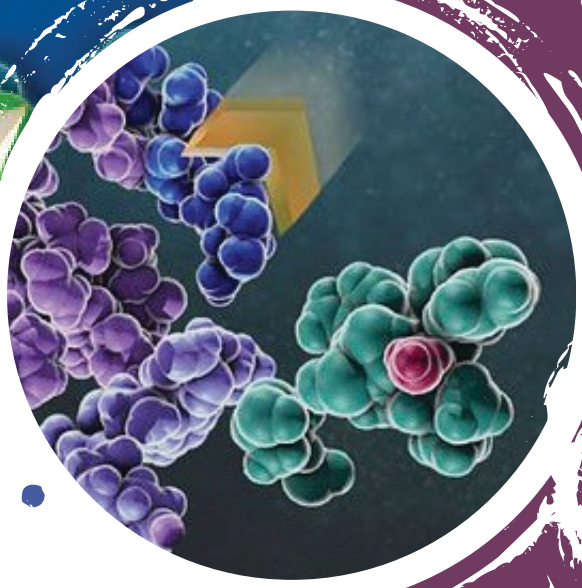

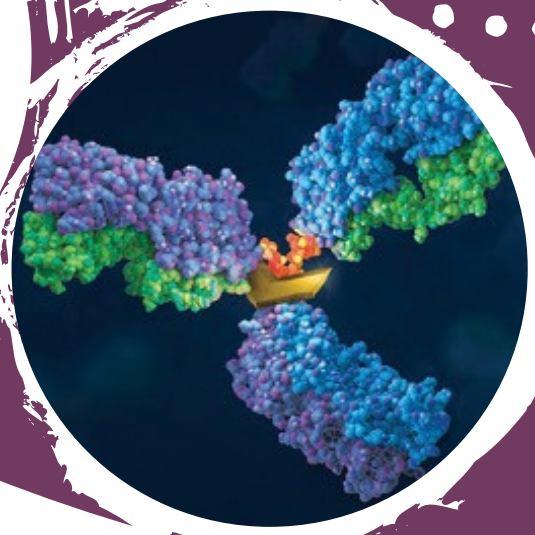

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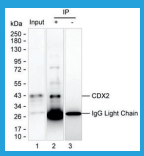

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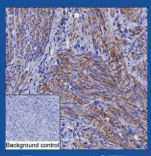
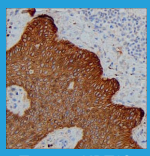
IHC



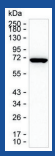
Target: ACTB
Tissue: endometrium

IHC



Target: KRT10
Tissue: skin

WB



Target: HSPA1A

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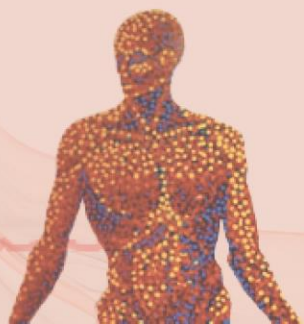
Penghsuan Huang Noah Lancaster
Gaoyuan Lu Daniel Nesbitt



School of Medicine and Public Health

Human Proteomics Program

The UW SMPH Human Proteomics Program (HPP), established in 2006 through support from the SMPH Wisconsin Partnership Program, is dedicated to addressing the needs of researchers and clinicians at UW-Madison in the rapidly growing field of proteomics and metabolomics. Proteomics and metabolomics enable researchers to obtain real-time information on a person's physiological status leading to an in-depth understanding of the causes of diseases, which has great potential for precision medicine.

Mission: The overall mission of the University of Wisconsin-Madison Human Proteomics Program is to develop cutting-edge technologies in proteomics and metabolomics for basic/translational/clinical research to improve the health of the citizens of Wisconsin, the nation, and the world through a program of research collaboration and education.

Goals

- Provide advanced proteomic and metabolomic capabilities featuring state-of-the-art, ultra-high resolution mass spectrometry, top-down proteomics, native mass spectrometry, and multi-omics.
- Provide new opportunities to catalyze basic, translational, and clinical research collaborations for understanding the basis of human health and diseases, discovering novel biomarkers for diagnosis, and identifying new molecular targets for treatment of human diseases.
- Establish educational programs to inform investigators and the public about proteomics and metabolomics, and its potential use for biomedical research and public health. Provide hands-on training to users in proteomics and metabolomics.

Advisory Committee: Dean Allan Brasier, Dean Jon Audhya, Prof. Paul Ahlquist, Prof. Chris Bradfield, Prof. Andreas Friedl, Prof. Lingjun Li, Prof. Timothy Kamp, Prof. Michael Nork, Prof. Michelle Kimple, Prof. Richard Halberg

To learn more, please visit: https://labs.wisc.edu/hpp_web/index.html

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