

Canadian National Proteomics Network



17th
ANNUAL
SYMPOSIUM

QUEBEC CITY
MAY 4-6, 2026



PROGRAM
EVENT GUIDE



Dear Proteomics Colleagues,

Welcome to the 16th Annual Canadian National Proteomics Network Conference (CNPN 2026). We are delighted to welcome you to Québec City and hope that you will find this year's conference inspiring, engaging, and intellectually stimulating.

This year's meeting takes place at the Monastère des Augustines, a remarkable heritage site located in the heart of Old Québec. Founded in the 17th century by the Augustinian sisters who established one of the first hospitals in North America, the Monastère stands as a reminder of Québec's long tradition in health, science, and knowledge. For centuries, this place has been dedicated to understanding and caring for human health making it a particularly meaningful setting for a gathering of researchers working at the frontiers of biomedical discovery.

The scientific program features leading proteomics researchers from across Canada alongside international experts from academia, government, and industry. Over the coming days, the conference will include keynote lectures, scientific sessions, poster presentations, and vendor activities that highlight recent advances and emerging directions in proteomics and related omics disciplines.

CNPN 2026 will also include activities dedicated to trainees, providing students and postdoctoral fellows with opportunities to present their work and interact with members of the broader proteomics community. We encourage participants to attend the poster sessions and engage with presenters to discuss the many exciting projects being showcased.

Beyond the scientific program, we hope that you will enjoy discovering Québec City. With its historic streets, fortified walls, and views over the St. Lawrence River, Québec offers a setting where centuries of history meet a dynamic research community. In many ways, the city reflects the spirit of scientific exploration itself revealing new layers of knowledge built upon the foundations laid by those who came before.

Finally, we would like to thank our sponsors for their generous support, which makes CNPN 2026 possible and helps sustain a strong and collaborative proteomics community in Canada.

We are delighted to welcome you to Québec City and wish you a stimulating and enjoyable conference.

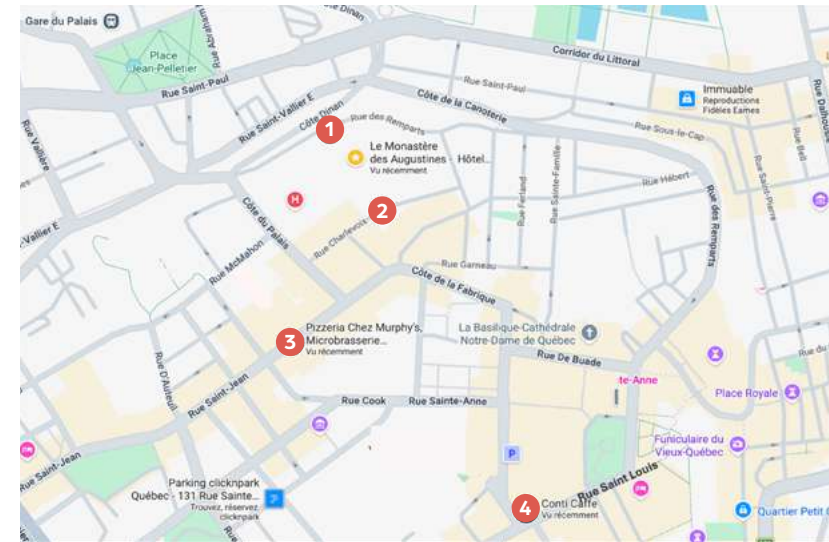
The CNPN 2026 Local Organizing Committee

Arnaud Droit, Florence Roux-Dalvai
and Marie Brunet

The CNPN 2026 Trainee Pre-symposium Organizing Committee

Eloise Coyle, Jordan Ortona, Félix-Antoine Trifiro
and Erik Gomez Cardona

Location Map	3
Venue Map	4
Housekeeping	5
Activities.....	6
Program	7
CNPN Award.....	14
Keynotes Speakers.....	16
Plenary Speakers.....	22
Short talk abstracts	28
Poster abstracts – Session A.....	44
Poster abstracts – Session B.....	54



VENUE

Monastère des Augustines

- 1 Main entrance with parking
77 rue des Remparts, Quebec City, G1R 0C3
- 2 Pedestrian entrance
32, rue Charlevoix, Quebec City, G1R 0C3

TRAINEE SOCIAL EVENT

(Open to the Trainee Pre-symposium participants)

- 3 Chez Murphy's, Microbrasserie
1095 Rue Saint-Jean, Québec City, G1R 4H2

SPEAKER'S DINNER

(On invitation only)

- 4 Conti Caffè
32 Rue Saint-Louis, Québec City, G1R 4L6

▶ **1ST FLOOR**

La Portière dining room

▶ **2ND FLOOR**

Conference floor
(Talks, Poster, Exhibits)



BREAKFASTS

Breakfast will be served continuously from 8:00 a.m. to 8:45 a.m. in the **La Portière dining room** (1st floor).

LUNCHES

Boxed lunches will be provided. You may eat in the **La Portière dining room** (1st floor) or in the conference area (2nd floor).

PRESENTERS & CHAIRS

Slides must be uploaded to the conference laptop at least 30 minutes before the start of your session block. Please note that you will not be able to use your own computer. Therefore, we strongly recommend uploading and reviewing your presentation well in advance of your session.

Presentations can be uploaded during any scheduled break, including the day(s) prior to your talk. Simply place your file in the folder corresponding to your session block so that the session chair can easily locate it.

Please refer to the table below for timing details.

Talk type	Presentation	Q&A
Award	15	5
Keynote	30	10
Plenary	20	5
Short	12	3

POSTERS

Scientific posters must be set up before 8:30 a.m. on the day of your presentation and removed before 6:30 p.m.

- ▶ **Session A** (Tuesday): Posters A01 to A26
- ▶ **Session B** (Wednesday): Posters B01 to B25

Presenters are expected to be at their posters during their assigned presentation time:

- ▶ **Morning session** (Part 1): odd-numbered posters
- ▶ **Afternoon session** (Part 2): even-numbered posters

YOGA (30 MIN)

Available for free

Start your day with a morning yoga session held in a truly unique setting—the Monastery's ancestral vaults.

- ▶ **Tuesday, May 5, 7:15** | Location : Les Voûtes
- ▶ **Wednesday, May 6, 7:15** | Location : Les Voûtes

10 to 25 persons per group.

Information on how to register will be provided during the conference.

GUIDED VISIT OF THE MONASTÈRE (30 MIN)

Available for free

Discover the Monastère des Augustines, founded in the 17th century, where this guided visit will invite you to explore, among other highlights, the historic choir and the ancestral vaults.

- ▶ **Tuesday, May 5, 13:00**
- ▶ **Wednesday, May 6, 13:00**

10 to 25 persons per group.

Information on how to register will be provided during the conference.

SELF-GUIDED MUSEUM VISIT

Available for free

Explore the Monastère Museum at your own pace through the self-guided exhibition Resonances, an immersive journey connecting the Augustines' heritage with themes of care and well-being.

More information : monastere.ca/en/museum

Available at your convenience

Opening hours : 10:00 – 17:00

The museum is located on the 1st floor.

OLD QUÉBEC, A UNESCO WORLD HERITAGE TREASURE

Discover this historic city through its iconic sites such as Petit-Champlain, Place Royale, and Dufferin Terrace, along with its many restaurants.

More information: www.quebec-cite.com/en

MONDAY MAY 4TH**CNPN TRAINEE PRE-SYMPOSIUM**

8:00 – 8:30 Welcoming Breakfast and Registration

8:30 – 10:00 Student Talks

Elmira Shajari, Université de Sherbrooke

A Nested Cross-Validation Framework for Robust Peptide Biomarker Selection from DIA Stool Proteomics in Inflammatory Bowel Disease

Eileen Tudorica, Segal Cancer Proteomics Centre Lady Davis Institute for Medical Research, Jewish General Hospital, McGill University

Discovery and Targeted Validation of Erythrocyte Proteomic Biomarkers of Recombinant Human Erythropoietin (rhEPO) Misuse and Hypoxic Exposure at High Altitude

Michael Woods, University of Guelph

High-throughput drug screening combined with Proteome Integral Solubility Alteration defines mechanisms of action for novel antifungal compounds against Cryptococcus neoformans

Elodie Logerot, Segal Cancer Proteomics Centre, Lady Davis Institute, Jewish General Hospital, McGill University

A Simplified Workflow for Robust, High-Throughput, and Quantitative Plasma Proteomics through Streamlined In-Tip Proteolysis

Jaden Chen, University of Guelph

Proteomic Profiling of Antifungal Resistance in Clinical Isolates of Cryptococcus Neoformans

10:00 – 10:30 Coffee Break

10:30 – 12:00 Student Talks

Mukhayyo Sultonova, Department of Biology, University of Prince Edward Island

Integrating hydrophobic interaction chromatography with TMT-based quantitative proteomics for systematic mapping of metabolite-protein interactions

Carla-Marie Jurkovic, Department of Immunology and Cell Biology, Faculty of Medicine and Health Sciences, Université de Sherbrooke
Time-resolved proximity proteomics reveals a non-canonical role for ANP32A in heterochromatin-dependent DNA repair and replication stress adaptation

Gwendoline Marbach, Department of Immunology and Cellular Biology, Faculty of Medicine and Health Sciences, Université de Sherbrooke
Targeted mass spectrometry for detecting the low-abundance ubiquitin variant UbKEKS

Danielle Simons, McGill University

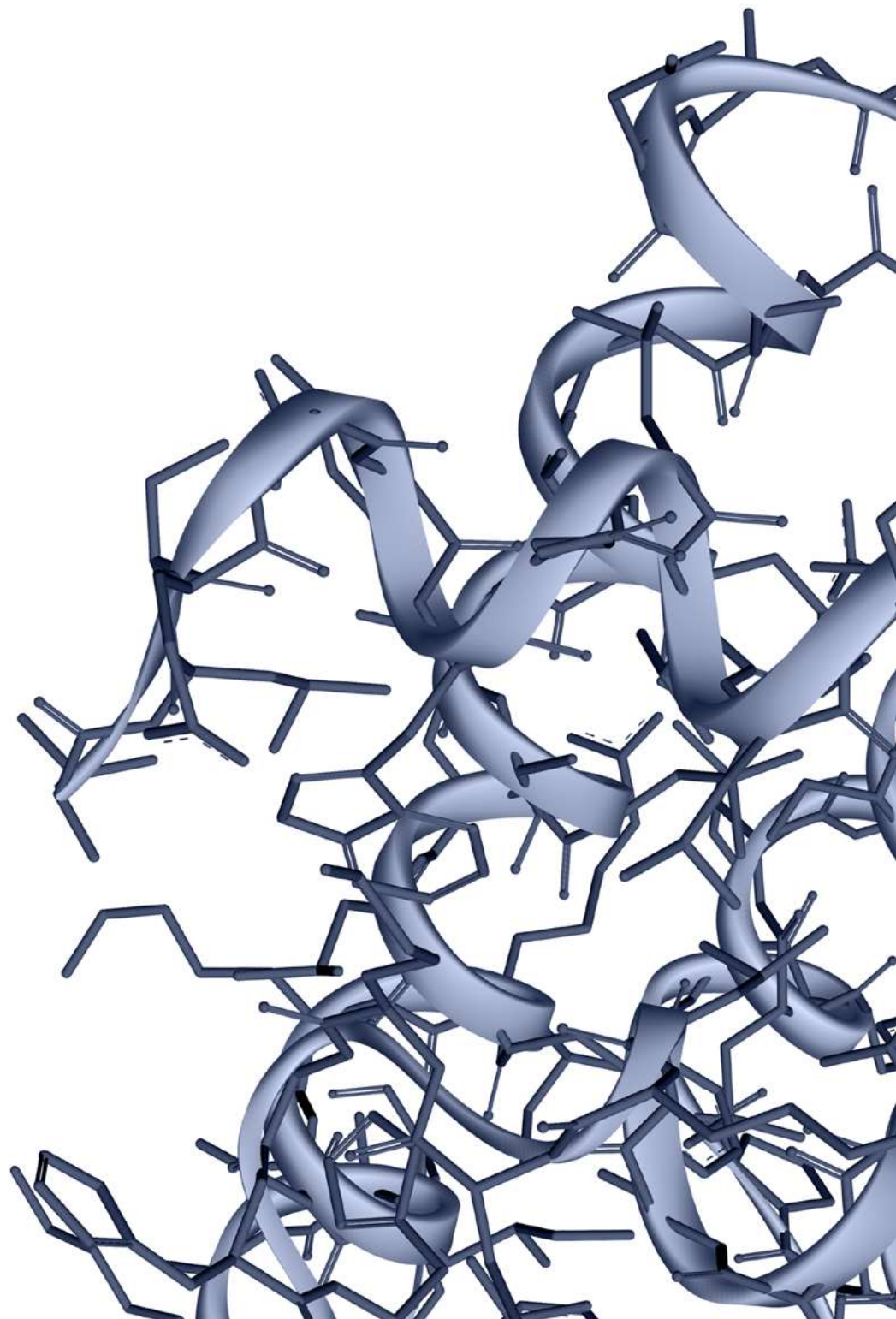
Functional characterization of pathogenic ataxia mutations in the mitochondrial processing peptidase

Nisha Owens, Department of Biology, University of Prince Edward Island
Kynurenine pathway regulation and function in breast cancer

- 12:00 – 13:00 **Lunch Break**
- 13:00 – 13:15 **Flash talks**
- 13:15 – 13:30 **Prize announcement**
- 13:30 – 14:30 **Invited Speaker** – Ji-Young Youn
Hospital for Sick Children, Toronto, ON, Canada
Exploring phase transition as governing principles of cellular organization and life
- 14:30 – 15:30 **Career Talks**
Ji-Young Youn
Hospital for Sick Children, Toronto, ON, Canada
Michael J. MacCoss
University of Washington, Seattle, WA, USA
- 15:30 – 16:00 **Career Panel**

CNPN ANNUAL SYMPOSIUM

- 14:00 – 16:00 **Registration**
- 16:30 – 16:45 **Opening remark**
- CNPN Award Ceremony**
Chair : Jennifer Geddes-McAlister
- 16:45 – 17:15 **Tony Pawson Award 2025** – Thomas Kislinger
Princess Margaret Cancer Centre, Toronto, ON, Canada
- 17:15 – 17:45 **New Investigator Award 2026** – Ji-Young Youn
The Hospital for Sick Children, Toronto, ON, Canada
- 17:45 – 18:15 **Tony Pawson Award 2026** – Judith A.J. Steen
Harvard Medical School, Boston, MA, USA
- 18:30 – 22:00 **Welcome Reception**
Communauté room – Open to all
- 20:00 – 23:00 **Trainee Social event**
Open to the Trainee Pre-symposium participants



TUESDAY MAY 5TH

CNPN ANNUAL SYMPOSIUM

8:00 – 8:45	Breakfast
	Computational Proteomics & Artificial Intelligence session <i>Chair: Mathieu Lavallée-Adam & Valeriia Vasylieva</i>
8:45 – 9:30	Keynote Speaker – Can Cenik The University of Texas at Austin, Austin, TX, USA <i>Invited by the Centre de Recherche Données Massives de l'Université Laval</i> An Integrative Approach to Predictive Modeling of Translation
9:30 – 9:45	Short Talk – Maysa Niazy McMaster Immunology Research Centre and Department of Medicine, McMaster University, Hamilton, ON, Canada Are We Ready for the Next Pandemic? Beyond Severity: Proteomic Immune Signatures Predict Time-to-Event Outcomes in COVID-19
9:45 – 10:00	Short Talk – Elloise Coyle Computational Biology Laboratory, Axe Endo-Nephro, Centre de recherche du CHU de Québec-Université Laval, Quebec City, QC, Canada Rapid Machine Learning-Guided Proteomics for Identification of Infection-Causing Bacteria
10:00 – 10:15	Sponsor Lightning Talks
10:15 – 11:00	Coffee Break & Poster Session A – part 1
	Computational Proteomics & Artificial Intelligence session (continue) <i>Chair: Mathieu Lavallée-Adam & Valeriia Vasylieva</i>
11:00 – 11:30	Plenary Speaker – Amrit Singh The University of British Columbia, Vancouver, BC, Canada Dissecting Disease Mechanisms through Multiomics Data Integration
11:30 – 11:45	Short Talk – Félix-Antoine Trifiro Université de Sherbrooke, Sherbrooke, QC, Canada FOMonet predicts non-canonical proteins validated by MS and Ribo-Seq
11:45 – 12:00	Short Talk – Maram El-Azouni Department of Biochemistry, Microbiology and Immunology and Ottawa Institute of Systems Biology, University of Ottawa, Ottawa, ON, Canada A novel machine learning approach for real-time determination of peptide identifiability in mass spectrometry-based proteomics experiments
12:00 – 12:15	Short Talk – Jordan Ortona Computational Biology Laboratory, Centre de recherche du CHU de Québec, Université Laval, Quebec City, QC, Canada PeptiDIA: A Machine Learning Framework for Enhanced Peptide Identification in Fast-Gradient Data-Independent Acquisition Proteomics

12:15 – 13:30

Lunch

12:30 – 13:30

Moms in Proteomics lunch meeting

Clinical Proteomics : Health & Disease session

Chair: Philipp Lange & Alyzee Minichini

13:30 – 14:15

Keynote Speaker – Michael J MacCoss
University of Washington, Seattle, WA, USA
Next Generation Translational Proteomics of Alzheimer's Disease

14:15 – 14:45

Plenary Speaker – Pierre Thibault
Institut de Recherche en Immunologie et en Cancérologie de l'Université de Montréal, Montréal, QC, Canada
Plasticity of tumor immunopeptidomes: Pharmacological rewiring of antigen presentation

14:45 – 15:00

Short Talk – Charlotte Jacquet
Molecular Medicine, The Hospital for Sick Children (SickKids), Peter Gilgan Centre for Research and Learning, Toronto, ON, Canada
A Proteomic Study Elucidating the Role of Mitochondrial Aconitase in Health and Disease.

15:00 – 15:45

Coffee Break & Poster Session A – part 2

Clinical Proteomics : Health & Disease session (continue)

Chair: Philipp Lange & Alyzee Minichini

15:45 – 16:00

Short Talk – Zoe Turner
University of Alberta, Edmonton, AB, Canada
Proteome-wide Serology Diagnostics for Viral Infections

16:00 – 16:15

Short Talk – Olivier Hinse
The University of British Columbia, Vancouver, BC, Canada
Biochemical Determinants of cathepsin K-mediated cis-transpeptidation in rheumatoid arthritis

16:15 – 16:30

Short Talk – Pascaline Bories
Plateforme Protéomique et laboratoire de Biologie Computationnelle - CHU de Québec - Université Laval, Quebec City, QC, Canada
From Serum to Urine: Proteomic Profiling for Mother and Child Health Monitoring During Pregnancy

16:30 – 16:45

Short Talk – Henrique dos Santos Seckler
Northwestern University, Evanston, IL, USA
PTMs as Covalent Signatures of Metabolism: How the Proteoform Diversity of Apolipoprotein A-I is Associated with Human Cardiometabolic Health

16:45 – 17:15

Plenary Speaker – Neeloffer Mookherjee
University of Manitoba, Winnipeg, MB, Canada
Decoding the Lung Proteome: Host Defence Adaptations to Environmental Exposures

17:15 – 18:00

CNPN General Assembly

19:30 – 22:00

Speaker's Dinner

On invitation only

WEDNESDAY MAY 6TH

CNPN ANNUAL SYMPOSIUM

8:00 – 8:45	Breakfast
	Technological Advances & Single Cell Proteomics session <i>Chair: Thibault Mayor & Mukhayyo Sultonova</i>
8:45 – 9:30	Keynote Speaker – Isabelle Fournier PRISM Inserm, University of Lille, France; Institut Universitaire de France, Paris, France Advancing precision medicine with spatial proteomics
9:30 – 9:45	Short Talk – Jason Rogalski Proteomics & Metabolomics Core Facility, Life Sciences Institute, University of British Columbia, BC, Canada Simplicity and Depth in Single-Cell Proteomics: A Cost-Effective Workflow and Expanded Framework for Data Evaluation
9:45 – 10:00	Short Talk – Kyle Tomaro Department of Biochemistry, Microbiology and Immunology, and Ottawa Institute of Systems Biology, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada A novel antimicrobial peptide drug discovery pipeline to combat the antibiotic resistance crisis.
10:00 – 10:15	Sponsor Lightning Talks
10:15 – 11:00	Coffee Break & Poster Session B – part 1
	Technological Advances & Single Cell Proteomics session (continue) <i>Chair: Thibault Mayor & Mukhayyo Sultonova</i>
11:00 – 11:30	Plenary Speaker – David C Schriemer University of Calgary, Calgary, AB, Canada Transforming hydrogen-deuterium exchange mass spectrometry into a powerful assay technology through gas-phase chemistry
11:30 – 11:45	Short Talk – Lekha Sleno Université du Québec à Montréal, Montréal, QC, Canada Multimomics of Hunter syndrome in a mouse model by LC-HRMS/MS
11:45 – 12:00	Short Talk – Huan Zhong University of British Columbia, Vancouver, BC, Canada Same-Cell Proteomics and Lipidomics Reveal Sex-Linked Baseline States and Infection-Driven Cross-Omic Rewiring in Human Astrocytes
12:00 – 12:15	Short Talk – Bianca Dupont University of Ottawa, Ottawa, ON, Canada Probing CAR-T interaction networks using proximity proteomics for improved immunotherapies
12:15 – 13:30	Lunch

13:30 – 13:45

Student Selected Talk #1

13:45 – 14:00

Student Selected Talk #2

Systems Biology and Functional Proteomics session

Chair: J Patrick Murphy & Naomie Linteau

14:00 – 14:45

Keynote Speaker – Aleksandra Nita-Lazar
NIH/NIAID, Bethesda, MA, USA
Invited by CHU de Québec – Université Laval Research Center
Protein Networks in Innate Immune Signaling

14:45 – 15:00

Short Talk – Romain Brailly
Département d'immunologie et de biologie cellulaire, Institut de Recherche sur le Cancer de l'Université de Sherbrooke, Sherbrooke, QC, Canada
Proteomic analysis of TRIM28-dependent pathways in fluorouracil-resistant colorectal cancer

15:00 – 15:45

Coffee Break & Poster Session B – part 2

Systems Biology and Functional Proteomics session (continue)

Chair: J Patrick Murphy & Naomie Linteau

15:45 – 16:00

Short Talk – Saya Sedighi
University of Toronto, Toronto, ON, Canada
A Next-Generation Human Cell Map Across Multiple Human Cell Lines

16:00 – 16:15

Short Talk – Jean-Francois Trempe
McGill University, Montréal, QC, Canada
Integrated proteomics, metabolomics, and structural biology to elucidate molecular mechanisms of mitochondrial quality control

16:15 – 16:30

Short Talk – Isabelle Perron-Lépine
Pediatrics Department, University of Sherbrooke, QC, Canada
GAPDHP1, an overlooked actor of mitochondrial cristae and bioenergetics

16:30 – 16:45

Short Talk – R. Glen Uhrig
University of Alberta, Edmonton, AB, Canada
Towards understanding Plant Translation: Bio-Orthogonal Non-Canonical Amino acid Tagging (BONCAT) for low-disruption labeling of Arabidopsis proteins in vivo.

16:45 – 17:15

Plenary Speaker – Ugo Dionne
Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada
Divergent Signalosomes and a Shared Assembly Mechanism Define EML4::ALK Variants Oncogenicity

17:15 – 17:45

Trainee Award Ceremony
Sponsored by the Canadian Institutes of Health Research (CIHR), the Institut de l'Intelligence des Données (IID) and Moms in Proteomics

17:45 – 18:00

Closing remarks

CNPND TONY PAWSON AWARD 2025



Thomas Kislinger

Princess Margaret Cancer Centre, Toronto, ON, Canada

Thomas Kislinger received his MSc in Analytical Chemistry from the University of Munich, Germany (1998). He completed his PhD in 2001, investigating the role of Advanced Glycation Endproducts in diabetic vascular complications at the University of Erlangen, Germany and Columbia University, New York. Between 2002 and 2006 he completed a post-doctoral fellowship at the University of Toronto. In 2006 he joined

the Princess Margaret Cancer Centre as an independent investigator. Dr. Kislinger is a Senior Scientist at the Princess Margaret Cancer Centre and a Professor at the University of Toronto in the Department of Medical Biophysics. Dr. Kislinger serves as Associate Editor for the Journal of Proteome Research. The Kislinger lab applies proteomics technologies to translational and basic cancer biology. This includes the development of novel proteomics methodologies, identification of liquid biopsy signatures and the molecular identification of novel cell surface markers. Dr. Kislinger has published 200 manuscripts that have been cited over 32,000 times.

CNPND NEW INVESTIGATOR AWARD 2026



Ji-Young Youn

The Hospital for Sick Children, Toronto, ON, Canada

Dr Ji-Young Youn is a Scientist in the Molecular Medicine Program at the Hospital for Sick Children (SickKids) Research Institute (RI), with a cross-appointment as an Assistant Professor in the Department of Molecular Genetics at the University of Toronto (UofT). She earned her B.Sc. in Bioengineering from Yonsei University, followed by a Ph.D. in Molecular Genetics from the Faculty of Medicine at the University of Toronto in 2013,

under the supervision of Dr. Brenda Andrews. She subsequently joined the proteomics laboratory of Dr. Anne-Claude Gingras at the Lunenfeld-

Tanenbaum Research Institute, where she investigated the proteomes of mammalian RNA-rich biomolecular condensates using cutting-edge quantitative proteomics. Since starting her independent position in 2020, Dr. Youn's lab has applied quantitative proteomics methods to study several key areas of research: 1) Investigating the role of RNA-rich condensates and their dysregulation in human diseases, 2) Elucidating the molecular targets of human pathogen effectors and toxins, and 3) Development and application of proteomics tools. Dr. Youn holds a Tier 2 Canada Research Chair in membraneless organelle proteomics.

She has trained 15 students (7 graduate and 8 undergraduate) and 2 postdoctoral fellows across a wide range of disciplines. Her trainees have gone on to pursue graduate school, study medicine, secure positions in industry and government in Canada.

CNPND TONY PAWSON AWARD 2026



Judith A.J. Steen

Harvard Medical School, Boston, MA, USA

Dr. Judith A. Steen is Professor of Neurology at Harvard Medical School and Director of the Neuroproteomics Laboratory at Boston Children's Hospital. A Canadian citizen and graduate of the University of Toronto, where she received the Seeley Junior Fellowship at Trinity College in two consecutive years and an NSERC Graduate Fellowship. Dr. Steen leads the world's most vertically integrated neuroproteomics program, spanning mass spectrometry technology development, molecular disease mechanism discovery, animal models, biomarker translation,

and drug development. Her laboratory's FLEXI Platforms have provided key clinical information for biomarkers and drug discovery in neurodegenerative diseases. Dr. Steen discovered that during the progression of Alzheimer's disease, PTMs on the protein tau change and accumulate in a stage-dependent ordered fashion and that the p-tau217 is an early Alzheimer's disease biomarker (US Patent 11,698,378), now FDA-approved for clinical diagnosis, and has published landmark multi-omics work in Cell, Nature Medicine, and Neuron, among others. Dr. Steen maintains deep ties to the Canadian proteomics community, attending Canadian proteomics meetings and continuing to leverage her NSERC Industrial Research Fellowship connections, including collaborations on instrumentation development with MDS-Sciex and potential pharmaceutical partnerships advancing IND-track therapeutics for neurodegeneration. In 2004, she was personally invited by Tony Pawson to join and build the proteomics at Mount Sinai Hospital — a vision whose spirit she has carried forward at Harvard for over two decades. The 2026 Tony Pawson Proteomics Award is presented to Dr. Steen in recognition of her extraordinary contributions to the field that Tony Pawson sought to champion.



Can Cenik

The University of Texas at Austin, Austin, TX, USA

Can Cenik is an Associate Professor in the Department of Molecular Biosciences at the University of Texas at Austin. He obtained his Ph.D. in Genetics from Harvard Medical School, where he trained with Dr. Frederick Roth, and completed his postdoctoral work with Dr. Michael Snyder at Stanford University. His research focuses on the regulation of mRNA translation and its role in development and disease. His laboratory develops and applies innovative

experimental and computational approaches, including ribosome profiling and single-cell technologies, to study translational control at transcriptome-wide scale. His work has provided key insights into how translation efficiency is coordinated across cell types and how its dysregulation contributes to cancer, neurodevelopmental disorders, and other diseases. Dr. Cenik has received multiple awards, including the RNA Society Moderna Award for Biomedical Innovation (2026), and is supported by major funding from the NIH and the Welch Foundation.

Keynote talk

Tuesday May 5th
8:45 – 9:30

An Integrative Approach to Predictive Modeling of Translation

The overarching vision of our research program is to construct predictive models that explain how cells determine their protein abundance.

Achieving this goal involves two major components: (1) higher-resolution and higher-precision measurements of gene expression modalities, and (2) computational and theoretical advancements capable of integrating these quantitative measurements into cohesive, predictive frameworks. Towards these goals, we have compiled measurements of translation from more than 3,500 experiments and introduced the concept of translation efficiency covariation (TEC), revealing that transcripts associated with shared biological functions and those that are members of the same protein complexes exhibit TEC. We then leveraged our expansive compendium of translation efficiency measurements to develop a deep neural network model, called RiboNN, capable of predicting mRNA translation rates across numerous cell types based solely on the full-length mRNA sequence. R RiboNN can evaluate the impact of genetic variants in the human population, providing insight into diseases driven by abnormal mRNA translation. This approach has implications for bioengineering applications, genetic diagnostics as well as the design and optimization of mRNA therapies.



Michaël MacCoss

University of Washington, Seattle, WA, USA

Michael MacCoss has been working with mass spectrometry instrumentation since 1994. He became interested in biomedical applications during summer internships at Merck Research Laboratories in 1995 and 1996. In 2001, he completed a Ph.D. in Analytical Chemistry with Professor Dwight Matthews developing stable isotope and mass spectrometry methodologies for measuring human amino acid and protein metabolism. As a postdoctoral fellow with

proteomics pioneer John R. Yates III at The Scripps Research Institute, Dr. MacCoss developed methodologies and software for characterizing post-translational modifications and quantitative analysis of complex protein mixtures.

Dr. MacCoss joined the University of Washington in 2004 as an Assistant Professor of Genome Sciences and was promoted to Professor in 2014. Recognizing that software was a major bottleneck limiting quantitative accuracy and reproducibility in proteomics, Dr. MacCoss established a software engineering effort with Brendan MacLean. This effort produced Skyline, a widely-adopted open-source platform for quantitative mass spectrometry that has become essential for workflows across the field, and Panorama, a web-based repository for mass spectrometry data sharing and collaboration. The laboratory's software is noted for its robustness, versatility, extensive support, and user friendliness. The MacCoss lab has also contributed to key computational tools in the field through close collaborations, including but not limited to the development of Percolator with the Noble lab for semi-supervised learning of peptide identification and Comet with Jimmy Eng for sequence database searching. Throughout his career, Dr. MacCoss has been a strong advocate for mass spectrometry-based quantitative proteomics, championing the development and adoption of targeted proteomics and data-independent acquisition (DIA) methods to improve the rigor, reproducibility, and clinical relevance of protein measurements. Beyond software development, the MacCoss lab has made significant methodological advances in data-independent acquisition, targeted proteomics, sample preparation, and instrumentation, applied to aging, cancer, cardiovascular disease, diabetes, and neurodegeneration.

His contributions have been recognized with several awards, including the ASMS Research Award (2004), the Presidential Award for Scientists and Engineers (PECASE, 2007), the Biemann Medal from the American Society

for Mass Spectrometry (2015), the HUPO Award for Discovery in Proteomics Sciences (2016), and the Donald F. Hunt Distinguished Contribution in Proteomics Award from US HUPO (2026). The MacCoss lab's research operates at the intersection of biochemistry, instrumentation, engineering, computer science, and statistics, with sustained focus on advancing quantitative proteomics capabilities.

Keynote talk

Tuesday May 5th
13:30 – 14:15

Next Generation Translational Proteomics of Alzheimer's Disease

Michael MacCoss, Deanna Plubell, Gennifer Merrihew,
Michael Riffle, Dirk Keene, Kathleen Poston, Tom Montine,
and Christine Wu

University of Washington, Seattle, USA; Stanford University, Palo Alto, USA

Alzheimer's disease (AD) is a looming public health disaster with limited interventions. Alzheimer's is a complex disease that can present with or without causative mutations and can be accompanied by a range of age-related comorbidities. This diverse presentation makes it difficult to study molecular changes specific to AD. To better understand the molecular signatures of disease we constructed a unique human brain sample cohort inclusive of autosomal dominant AD dementia, sporadic AD dementia, and those without dementia but with high AD histopathologic burden, and cognitively normal individuals with no/minimal AD histopathologic burden. All samples are clinically well characterized, and brain tissue was preserved postmortem by rapid autopsy. All data were collected using data independent acquisition-mass spectrometry. Furthermore, we also performed similar analyses in cerebral spinal fluid (CSF) and from plasma extracellular vesicles using a well characterized cohorts containing individuals with AD dementia, Parkinson's disease dementia, Parkinson's disease cognitively normal, and healthy cognitively normal. I will also present strategies we have been developing to make these measurements fully targeted and cost effective using the new Stellar mass spectrometry platform.



Isabelle Fournier

PRISM Inserm, University of Lille, France
Institut Universitaire de France, Paris, France

Professor Fournier is a Distinguished Professor at the University of Lille, where she is also co-director of the PRISM Inserm U1192 laboratory. She is a bioanalytical chemist specializing in clinical mass spectrometry and proteomics. She began her research career by working on the fundamentals of the MALDI desorption/ionization process during her PhD at the University Pierre & Marie Curie in Paris, and then as a postdoctoral fellow within the group of Prof. Michael Karas in Frankfurt from 2000 to 2001. In 2002, she established her own research group at the University of Lille, where she began developing

MALDI MS imaging. She has pioneered this field through various developments, including strategies for imaging FFPE tissues and proteins, as well as the creation of tagged probes for targeted MS imaging of mRNA and proteins for MALDI IHC and ISH. In 2009, she was appointed as a full professor and awarded a junior position at the Institut Universitaire de France. Since 2012, she has been interested in developing Spatially Proteomics guided by MALDI MS Imaging for applications in oncology. Over the past five years, she has also worked on developing in vivo MS for guided surgery and intraoperative analysis. She co-founded the Imabiotech company (now Aliri Bioanalysis), which provides services in MALDI MSI, and more recently Celeos, which is set to commercialize the SpiderMass technology. She was recently honored with the distinguished international award from MSACL and was awarded a senior position at the Institut Universitaire de France in 2019, which was renewed in 2024 on an Innovation Chair.

Keynote talk

Wednesday May 6th
8:45 – 9:30

Advancing precision medicine with spatial proteomics

Mass spectrometry (MS)-based spatial proteomics offers a powerful framework to investigate tumor ecosystems by identifying markers that distinguish cancer cells from immune and stromal cell populations, while also resolving phenotypically distinct tumor clones within the same lesion. By preserving spatial context, these non-targeted approaches provide biologically and clinically relevant information that can support diagnosis, prognosis, and ultimately treatment selection in precision oncology. Recent advances in MS instrumentation, including improved sensitivity, throughput, and robustness, are bringing these strategies closer to clinical implementation. In this context, coupling mass spectrometry imaging (MSI) with large-scale, non-targeted spatial proteomics is particularly promising. MALDI-MSI now enables high-resolution molecular mapping, including protein information derived from tryptic peptides in workflows compatible with formalin-fixed paraffin-embedded tissues. Image segmentation can then guide in-depth proteomic analyses of specific cell phenotypes and tissue niches. In parallel, machine-learning approaches make it possible to bridge lipid MSI data with protein networks, signaling pathways, and candidate therapeutic vulnerabilities. These strategies can be translated further using patient-derived organoids, in which rapid MALDI-MSI lipid profiling may support drug repurposing and treatment prioritization. Applied to longitudinal breast cancer cohorts, spatial proteomics reveals that tumor evolution follows a limited number of phenotypic trajectories and suggests that clonal dynamics are driven more strongly by tissue microenvironment than by tumor subtype or treatment alone. These observations can be cross-validated using targeted MS-based proteomics through high-plex MALDI immunohistochemistry, with click-chemistry-enabled antibodies or aptamers allowing multiplexed panels including immune populations and immune checkpoints. Altogether, these developments are extending spatial proteomics from tissue-scale analysis towards subcellular resolution through tissue expansion, and towards intraoperative applications with the ambient ionization SpiderMass technology developed for real-time surgical monitoring.



Aleksandra Nita-Lazar

Functional Cellular Networks Section, Laboratory of Immune System Biology
National Institute of Allergy and Infectious Diseases,
National Institutes of Health (NIAID/NIH), Bethesda, USA

Dr. Aleksandra Nita-Lazar received her Ph.D. in biochemistry in 2003 from the University of Basel for studies performed at the Friedrich Miescher Institute for Biomedical Research, where she analyzed atypical protein glycosylation using mass spectrometry and protein biochemistry methods. After postdoctoral training at Stony Brook University and Massachusetts Institute of Technology (Ludwig Cancer

Foundation Fellow), where she continued to investigate post-translational protein modifications and their influence on cell signaling, she joined the Program in Systems Immunology and Infectious Disease Modeling, now the Laboratory of Immune System Biology, DIR, NIAID, NIH, in April 2009, as an independent investigator and Chief of the Cellular Networks Proteomics Unit. Dr. Nita-Lazar was granted tenure in December 2018 and she now continues her work as Senior Investigator and Chief of the Functional Cellular Networks Section. Her main research interests are protein state changes and networks regulating the host-pathogen interactions and macrophage activation.

Keynote talk

Wednesday May 6th
14:00 – 14:45

Protein Networks in Innate Immune Signaling

Toll-like receptor (TLR) signaling in macrophages is essential for generating effective innate immune responses. Quantitative differences dependent on the dose and timing of the stimulus critically affect cell function and often

involve proteins that are not components of widely shared transduction pathways. Mathematical modeling is an important approach to better understand how these signaling networks function in time and space. We have successfully modeled the S1P signaling pathway in macrophages using selected reaction monitoring (SRM) to measure the absolute abundance of the pathway proteins. The resulting values became parameters in a computational pathway model. To model the TLR signaling networks, we developed assays for the canonical TLR signaling pathway and related proteins and phosphoproteins and used parallel reaction monitoring (PRM) with heavy-labeled internal peptide standards to quantify protein and phosphorylated protein molecule numbers per cell in untreated and LPS-stimulated macrophages. The absolute protein abundance values were entered into a model of the TLR pathway developed using Simmune, the rule-based modeling tool with a visual interface. The TLR signaling network model identified missing negative feedback hub. This hub is being experimentally tested and combined with global proteomic approaches to discover biologically important new proteins, protein complexes and PTMs involved in the innate immune pathways (examples will be given). The protein and PTM levels are quantified in macrophages under diverse, but well-defined conditions (different pathogen-associated molecular patterns, whole pathogens, and cells with mutations in specific signaling molecules). Together, the interconnected projects lead to the better understanding how the immune signaling pathways are regulated and activated during an infection. This research was supported by the Intramural Research Program of NIAID, NIH.

Mass spectrometry

Realize a universe of possibilities

Orbitrap Astral Zoom Mass Spectrometer



Achieve the next generation of breakthroughs in proteomics, metabolomics, and BioPharma research with the Thermo Scientific™ Orbitrap™ Astral™ Zoom Mass Spectrometer featuring:

Increased flexibility for higher performance across more applications with additional capabilities

Faster throughput with higher data quality using pre-accumulation and acquisition rates up to 270 Hz

Deeper coverage to uncover more analytes with enhanced dynamic range mode

Higher sensitivity to improve performance for single-cell analysis using low input mode

Accurate and precise quantitation over an extended quantitative dynamic range

Explore more molecular space
thermofisher.com/orbitrapastralzoom

thermo scientific



Amrit Singh

The University of British Columbia, Vancouver, BC, Canada

Dr. Singh is an Assistant Professor of Heart and Lung Pharmacogenomics at the University of British Columbia and a Principal Investigator at the Centre for Heart Lung Innovation. He leads a computational biology lab focused on biomarker discovery and the development of methods and tools for multiomics data integration and visualization. His research aims to identify clinically relevant biomarkers across a range of

complex diseases using high-throughput biological (“omics”) data. Through close collaborations with clinicians and experimental scientists, his work integrates patient cohorts and model systems with single-cell and spatial technologies to dissect disease mechanisms and advance therapeutic discovery, including drug repurposing.

Plenary talk

Tuesday May 5th
11:00 – 11:30

Dissecting Disease Mechanisms through Multiomics Data Integration

I will begin by motivating the field through the growing ability to profile multiple layers of biology from the same system, and by introducing key concepts in multimodal integration, including horizontal, vertical, and mosaic approaches. I will then highlight software and methods developed in my lab for data integration and interpretation, including DIABLO and caretMultimodal, biologically informed models such as BRUNO, benchmarking pipelines such as MESSI, and interactive web applications for omics data exploration such as OmicsBioAnalytics. Finally, I will discuss how these tools have been applied to benchmark and analyze multiomics data in both health and disease, with examples from heart failure, dysferlinopathy, and vaccine studies.



Pierre Thibault

Institut de Recherche en Immunologie et en Cancérologie de l'Université de Montréal, Montréal, QC, Canada

Pierre Thibault is a Professor at Université de Montréal with appointments in the Departments of Chemistry and Biochemistry. He is also a founding member of the Institute for Research in Immunology and Cancer (IRIC) where he leads the Centre for Advanced Proteomic Analysis (CAPA). Prior to joining the Université de Montréal, he was executive

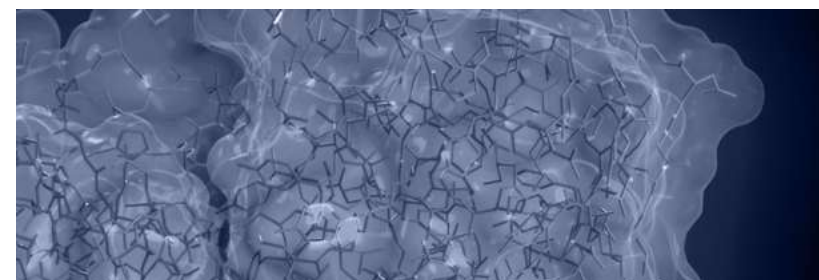
director of protein analysis at Caprion (now CellCarta) and served as a Senior Research Officer at NRC Institute of Biological Sciences in Ottawa and the Institute for Marine Biosciences in Halifax. His laboratory develops new technologies in proteomics and immunopeptidomics, integrating bioanalytical chemistry, protein chemistry, biochemistry, and cell biology to elucidate how post-translational modifications control protein function, dynamics, and localization in immunity and cancer signaling. His team integrates experimental and computational workflows for sensitive immunopeptidome profiling, quantitative comparisons across perturbations, and prioritization of candidate antigens, including mutated and non-coding-derived peptides presented by MHC class I and II. This work supports antigen-directed immunotherapies for personalized medicine.

Plenary talk

Tuesday May 5th
14:15 – 14:45

Plasticity of tumor immunopeptidomes: Pharmacological rewiring of antigen presentation.

Epigenetic regulation shapes the repertoire of tumor antigens by controlling which genomic programs are transcribed, how RNAs are processed, and which proteins ultimately feed the antigen-processing machinery. As a result, the tumor immunopeptidome is highly plastic: pharmacologic perturbations can increase MHC-I display, redirect antigen-source gene usage, and unmask “cryptic” peptides from non-coding or aberrantly processed transcripts thus creating transient windows of targetability. Recent literature converges on two broad trends: (i) drugs that remodel chromatin and transcription can diversify and amplify antigen presentation without changing DNA sequence, and (ii) stress pathways that impinge on RNA metabolism and innate immune signaling can expose non-canonical antigen sources that are otherwise silent. In this presentation, we highlight two complementary examples of drug-driven antigen rewiring. CDK4/6 inhibition in breast cancer remodels Rb-dependent transcription and chromatin to enhance MHC-I presentation, including peptides from non-coding regions. In acute myeloid leukemia, SUMO-pathway inhibition activates interferon-linked programs, boosts antigen-processing machinery, and enriches for non-canonical antigens. Together, these studies motivate rational combinations of epigenetic targeting agents with antigen-directed immunotherapies.





Neeloffer Mookherjee

University of Manitoba, Winnipeg, MB, Canada

Dr. Neeloffer Mookherjee is a Professor within the Departments of Internal Medicine and Immunology, at the University of Manitoba. Her research uses Systems-level approaches to identify molecular hubs within inflammatory networks of chronic diseases such as asthma and arthritis. Dr. Mookherjee is has pioneered research on the immunity-related functions of host defence antimicrobial peptides. She was the inaugural CIHR Sex and Gender Science Chair in Respiratory Health, leading research on the influence of biological sex in respiratory disease and response to therapy. Dr. Mookherjee also Co-leads AirSAFE, a multidisciplinary facility dedicated to research on the health impacts of air pollution. For more information on Dr. Mookherjee's research group see mookherjeelab.com

Plenary talk

Tuesday May 5th
16:45 – 17:15

Decoding the Lung Proteome: Host Defence Adaptations to Environmental Exposures

Environmental exposures such as allergens and air pollution are major risk factors of pulmonary infections and inflammatory respiratory disease. Using both animal models

and controlled human exposure studies, we have characterized changes in the lung proteome following exposure to aeroallergens (e.g. house dust mites) and diesel exhaust (as a paradigm for air pollution). Across these studies, we showed that inhaled allergens and air pollution dysregulate proteins in three functional categories: antimicrobial host defence peptides, inflammation, and oxidative stress. Further examination of proteome changes in human bronchial epithelial cells demonstrated that pro-inflammatory cytokines elevated in the lungs in response to allergen or air pollution selectively alter the abundance of immunodulatory host defence peptides, specifically those linked to neutrophilic airway inflammation. We have also validated the functional relevance of these peptides in neutrophil migration. Collectively, these studies provide functional and mechanistic insights into the adverse health effects associated with inhaled environmental exposures.



David Shriemer

University of Calgary, AB, Canada

Dr. Shriemer is an academic and entrepreneur. He graduated with degrees in organic chemistry and bioanalytical chemistry and received further training in biochemistry during postdoctoral work. He was the founder of INH Technologies Inc. and served as Research Director in MDS Proteomics Inc. before joining the Department of Biochemistry & Molecular Biology at the University of Calgary in 2001. Dr. Shriemer's laboratory investigates structure-function relationships in large multicomponent protein systems of relevance to cancer and builds tools

to probe protein interactions at the cellular level. Dr. Schriemer has been a Canada Research Chair in Chemical Biology and a Senior Scholar of the Alberta Heritage Foundation for Medical Research. He served as the Director of the SAMS Centre for Proteomics until 2017. He is currently the Chief Science Officer of Nepetx LLC (a company that is developing a therapy for celiac disease), and the Director of APACE (Advanced Protein Analytics Centre of Excellence), a new core facility for structural proteomics in Calgary's Cumming School of Medicine.

Plenary talk

Wednesday May 6th
11:00 – 11:30

Transforming hydrogen-deuterium exchange mass spectrometry into a powerful assay technology through gas-phase chemistry

Hydrogen-deuterium exchange mass spectrometry (HX-MS) has long been considered a niche biophysical technique. This is unfortunate, as the technique is enchantingly simple: just add heavy water to protein, and measure deuterium uptake. Measuring the rate of H/D exchange in the backbone amides of a protein unlocks rich structure/function information, but conventional HX-MS technology requires long data acquisitions and large amounts of protein. Furthermore, manual data analysis requires a highly trained specialist and lengthens the experiment considerably. If the technique could be made faster, with lower limits of detection, HDX-MS could become a preferred protein assay technology. In this presentation we will describe how a curious gas-phase reaction (deuterium scrambling) is the key to unlocking high performance, by enabling HX-MS2 modes of analysis that simultaneously simplifies data analysis, improves data quality, and decreases detection limits by orders of magnitude. We propose that HX-MS2 has a role to play in drug discovery pipelines involving complex drug targets.



Ugo Dionne

Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada

Ugo Dionne completed his PhD at Université Laval under the supervision of Drs. Nicolas Bisson and Christian Landry, where he studied the regulation of protein-protein interactions, with a focus on SH3 domains. He is currently a postdoctoral research fellow in the laboratory of Dr. Anne-Claude Gingras at the Lunenfeld-Tanenbaum Research Institute, where his research focuses on the dysregulation of phosphorylation-dependent signaling pathways in cancer.

Plenary talk

Wednesday May 6th
16:45 – 17:15

Divergent Signalosomes and a Shared Assembly Mechanism Define EML4::ALK Variants Oncogenicity

Phosphorylation-dependent signaling pathways are frequently dysregulated in cancers, often due to genetic aberrations such as chromosomal translocations that produce oncogenic fusion proteins. Kinase domains are notably enriched in fusions,

with over 1,400 kinase fusions identified to date. Therapies that directly target overactivated kinases with small inhibitors usually lead to the emergence of resistance mechanisms. In addition, patients can express different variants of a given kinase fusion, which depends on the location of the chromosomal breaks. This is the case for EML4::ALK driven non-small cell lung cancers (NSCLC) where the short variant 3 (V3) is notably more aggressive and resistant to ALK tyrosine kinase inhibitor (TKI) treatments than the canonical longer variant 1 (V1), through mechanisms that remain poorly understood. We integrated quantitative proteomics (BioID, AP-MS, total and phosphoproteomics) with high-resolution imaging and functional genetics to define the variant-specific and shared signalosomes of EML4::ALK and how they are rewired following ALK TKI treatments. We show that while V1 preferentially recruits and activates canonical RTK and PI3K effectors, V3 reconfigures cellular signaling and activates a non-canonical interferon response that is unresponsive to acute ALK targeted treatments. Our results also suggest that treatments with ALK TKIs reconfigures EML4::ALK signalosomes instead of simply disassembling them. In addition, we identify a drastic rewiring of phospho-dependent signaling unique to V3, characterized by a massive expansion of the phosphotyrosine landscape and a simultaneous attenuation of global pSer/pThr networks. Despite these divergent signaling architectures, we show that both variants require SHC1, GAB1 and PTPN11 (SHP2) for the biogenesis of EML4::ALK signalosomes. Crucially, pharmacologic inhibition of PTPN11 disassembles these oncogenic signaling hubs across variants. Our work strongly deepens our understanding of EML4::ALK oncogenicity and of variant specific signaling rewiring. Importantly, we highlight PTPN11 as a critical, variant-agnostic vulnerability for potential therapeutic intervention in ALK-driven malignancies.



Evosep Eno

An **easy-to-use, robust and reliable** chromatographic solution for high performance and **standardized proteomics**, preparing for next generation workflow integration.

evosep.com/evosep-eno



EVOSEP

The short talks for each session were selected by two independent reviewers, specialists in the relevant field and external to the local organizing committee.

COMPUTATIONAL PROTEOMICS & ARTIFICIAL INTELLIGENCE SESSION

Maysa Niazy

McMaster Immunology Research Centre and Department of Medicine, McMaster University, Hamilton, ON, Canada

Short talk

Tuesday May 5th
9:30 – 9:45

Are We Ready for the Next Pandemic? Beyond Severity: Proteomic Immune Signatures Predict Time-to-Event Outcomes in COVID-19

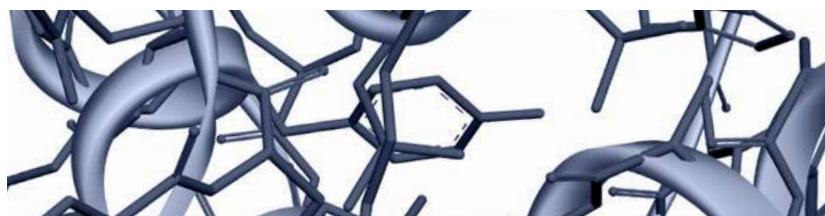
Maysa Niazy, Sierra Vanderkamp, Byram Bridle
McMaster Immunology Research Centre and Department of Medicine, McMaster University, Hamilton, ON, Canada

Coronavirus disease 2019 (COVID-19) exhibits heterogeneous clinical trajectories driven by dysregulated host immune responses. While previous studies, including our own, have characterized cytokine and chemokine profiles associated with disease severity, the temporal dynamics linking these proteomic signatures to clinical progression remain unclear.

In this study, we applied a time-to-event framework to longitudinal plasma proteomic data to identify biomarkers associated with disease progression. Using Survival Analysis, specifically Cox proportional hazards models, we evaluated associations between cytokine concentrations and time to severe or fatal outcomes, adjusting for age and comorbidities.

Elevated levels of interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), pentraxin 3 (PTX3), and a decreasing level of soluble tumour necrosis factor receptor II (sTNF-RII), were significantly associated with increased hazard of progression, indicating their role as early drivers of adverse outcomes. In contrast, higher levels of interferon gamma-induced protein-10 (IP-10), IL-10, and soluble tumor necrosis factor receptor I were associated with delayed progression and milder disease trajectories.

These findings demonstrate that integrating proteomic immune profiling with time-to-event modeling provides critical insight into the timing of disease progression. This approach enables identification of dynamic biomarkers for early risk stratification and supports precision clinical management in COVID-19 and future emerging infectious diseases.



Eloise Coyle

Computational Biology Laboratory, Axe Endo-Nephro, Centre de recherche du CHU de Québec-Université Laval, Quebec City, QC, Canada

Short talk

Tuesday May 5th
9:45 – 10:00

Rapid Machine Learning-Guided Proteomics for Identification of Infection-Causing Bacteria

Eloise Coyle^{1,4}, Pascaline Bories^{1,2}, Antoine Lacombe-Rastoll^{1,2}, Pierre Nival¹, Clarisse Gotti^{1,2}, Ève Bérubé³, Mickaël Leclercq¹, Florence Roux-Dalvai^{1,2}, Sandra Isabel³, Arnaud Droit^{1,2,4,5}

¹Computational Biology Laboratory, Axe Endo-Nephro, Centre de recherche du CHU de Québec-Université Laval, Québec, QC, Canada | ²Proteomics Platform, Axe Endo-Nephro, Centre de recherche du CHU de Québec-Université Laval, Québec, QC, Canada | ³Centre de Recherche en Infectiologie de l'Université Laval, Axe Maladies Infectieuses et Immunitaires, Centre de Recherche du CHU de Québec-Université Laval, Québec City, Québec, Canada | ⁴Département de médecine moléculaire, Faculté de médecine, Université Laval, Québec, QC, Canada | ⁵Inria, Maasai team-Université Côte d'Azur, Nice, France.

Rapid identification of infection-causing pathogens is critical for guiding appropriate antibiotic treatment and limiting the spread of antimicrobial resistance (AMR). Diagnostic delays contribute to unnecessary or ineffective therapy in both human medicine and agriculture. Within a One Health context, improving the timely detection of urinary tract infections in humans and livestock diseases such as bovine mastitis is essential for responsible antimicrobial use.

Using artificial intelligence with LC-MS/MS, our work aims to shorten the time from sample collection to reliable pathogen identification. Data-independent acquisition (DIA) is performed on inoculated and clinical samples to identify peptides that reliably distinguish bacterial species. These peptides are used to train machine learning models that classify samples by causative organism. In urinary tract infections, two complementary approaches have been developed: one using MS1-only spectra from patient urine, and one using targeted PRM to monitor selected peptides. The MS1-only strategy enables rapid, culture-free classification of eight common uropathogens, while the PRM approach tracks informative peptides across a broader panel of 28 pathogens. For bovine mastitis, 13 bacterial species are classified using DIA-defined signatures followed by PRM monitoring.

Targeted proteomics introduces the challenge of manual peak curation, which limits throughput and reproducibility. Previous work showed that machine learning can improve peak detection in SRM data; current efforts extend this principle to PRM through automated models that reduce manual review.

Together, these studies demonstrate how computational proteomics and AI-guided analysis can accelerate diagnostics and support faster, more informed antimicrobial use across human and agricultural settings.



Félix-Antoine Trifiro

Université de Sherbrooke, Sherbrooke, QC, Canada

Short talk

Tuesday May 5th
11:30 – 11:45

FOMOnet predicts non-canonical proteins validated by MS and Ribo-Seq

Francis Bourassa, Xavier Lapointe, Anthony Glaude, Arielle Bedard, Joelle Vincent, Marie A. Brunet

Félix-Antoine Trifiro, Université de Sherbrooke

The advances in proteogenomic and ribosome profiling revealed thousands of unannotated coding sequences, called non-canonical ORFs (ncORFs). Even with translation evidence, peptide identification by mass spectrometry (MS) is the gold-standard to identify novel proteins. Reliable mapping of the non-canonical proteome by MS requires accurate database generation, controlled false discovery rates (FDR), and rigorous spectral validation. Here we present how we combined deep-learning predictions and a robust proteomic pipeline to unveil the non-canonical proteome.

We built FOMOnet, a one-dimensional convolutional network that assigns coding probabilities to each nucleotide in a transcript, followed by kernel density estimation to derive high-confidence CDS predictions. FOMOnet outperforms existing approaches, achieving 0.999 ROC-AUC and 0.998 precision-recall AUC without overfitting. It retrieves 97.8% of annotated CDS by learning features relevant to translation, measured by ribosome-profiling (ribo-seq). Our tool predicts over 70,000 high-confidence ncORF, with an unprecedented validation rate of 75% by ribo-seq.

To obtain proteomic evidence, we analyzed whole-cell and acetonitrile-precipitated lysates from 7 cancer cell lines using a Thermo Orbitrap Astral with a 45-min gradient. Entrapment-based analysis ensured robust group-level 1% FDR filtering. We detected 484 ncORFs with at least 1 unique peptide with a third of them being detected in multiple cell-lines. Our robust methodology gave us confident PSM with >0.9 aNDP to predicted peptide.

FOMOnet predictions combined with stringent MS-based proteomic revealed unknown proteins and set a standard for confident ncORF identification. Our results mend the gap from current annotations and demonstrate that a significant fraction of ncORF is detectable and likely biologically relevant.

Maram El-Azouni

Department of Biochemistry, Microbiology and Immunology and Ottawa Institute of Systems Biology, University of Ottawa, Ottawa, ON, Canada

Short talk

Tuesday May 5th
11:45 – 12:00

A novel machine learning approach for real-time determination of peptide identifiability in mass spectrometry-based proteomics experiments

Maram El-Azouni¹, Giorgio Freije¹, Jonathan Krieger², Tharan Srikumar², Mathieu Lavallée-Adam¹

¹Department of Biochemistry, Microbiology and Immunology and Ottawa Institute of Systems Biology, University of Ottawa, Ottawa, Ontario, Canada | ²Bruker Ltd., Milton, Ontario, Canada

While mass spectrometry-based proteomics is the preferred technique to detect proteins in complex biological samples, it still cannot identify all proteins in these. This is in part due to the mass spectrometer not having the time to fragment all peptides in samples, leaving many unidentified. Moreso, the instrument often fragments peptides that the post-hoc software analysis will be unable to identify for different reasons,

including sub-optimal software parameter selections or poor peptide fragmentation and low abundance. Still, current mass spectrometry data acquisition methods select peptides for fragmentation irrespective of their downstream identifiability.

Peptide precursor ion properties measured prior to fragmentation can inform on a peptide's potential fragmentation quality and identification likelihood. Hence, we present a machine learning algorithm that uses peptide precursor ion properties to predict in real-time, during mass spectrometry experiments, the probability of identifying a given peptide if fragmented. Specifically, we trained an artificial neural network with 16 HeLa cell lysates analyzed on a Bruker timsTOF Pro instrument to predict peptide identifiability.

We show that our artificial neural network can effectively predict peptide identifiability (area-under-the-curve=0.72). We also demonstrate that if the mass spectrometer only acquires data for peptides deemed identifiable by our algorithm, the instrument can identify 95% of proteins from the original experiment using only 87% of the mass spectra. Overall, our machine learning algorithm enhances mass spectrometry efficiency by reducing analysis of unidentifiable peptides, while preserving most protein identifications. These freed instrument resources can be repurposed to detect more proteins and further characterize biological processes.

Jordan Ortona

Computational Biology Laboratory, Centre de recherche du CHU de Québec, Université Laval, Quebec City, QC, Canada

Short talk

Tuesday May 5th
12:00 – 12:15

PeptiDIA: A Machine Learning Framework for Enhanced Peptide Identification in Fast-Gradient Data-Independent Acquisition Proteomics

Jordan Ortona¹, Mickaël Leclercq¹, Florence Roux-Dalvai², Arnaud Droit^{1,2,3}

¹Computational Biology Laboratory, Centre de recherche du CHU de Québec, Université Laval, Québec City, Québec, Canada | ²Proteomics Platform, Centre de recherche du CHU de Québec, Université Laval, Québec City, Québec, Canada | ³Inria, Maasai team-Université Côte d'Azur, Nice, France

Data-independent acquisition (DIA) mass spectrometry has become increasingly prevalent in proteomics as advances in instrumentation, chromatography, and computational analysis have enabled robust proteome identification across complex biological samples. However, analytical depth achieved with fast chromatographic gradients remains lower than that obtained using long-gradients, reflecting a throughput-depth trade-off. Here, we present PeptiDIA, a machine learning framework that enhances peptide identification in fast-gradient DIA data by leveraging paired fast and long-gradient acquisitions from identical samples. PeptiDIA processes DIA-NN outputs generated at relaxed false discovery rate thresholds to obtain expanded candidate peptide pools and trains gradient-boosted decision tree models using long-gradient identifications as reference labels. The model integrates DIA-NN features with engineered peptide descriptors and applies isotonic regression to calibrate probabilities, enabling controlled peptide recovery relative to the long-gradient reference. Applied to human and murine datasets spanning six tissues acquired on an Orbitrap Exploris 480, PeptiDIA increased peptide identifications by 25-34% at 1% target reference-discordance rate (RDR) and increased the number of protein groups containing at least one rescued peptide by 15-17%. Overall, PeptiDIA improves the identification depth of fast-gradient DIA-NN workflows without altering acquisition strategies.

The framework is available as a web application and command-line tool at <https://github.com/Jordano700/PeptiDIA>.

CINICAL PROTEOMICS & ARTIFICIAL INTELLIGENCE SESSION

Charlotte Jacquet

Molecular Medicine, The Hospital for Sick Children (SickKids), Peter Gilgan Centre for Research and Learning, Toronto, ON, Canada

Short talk

Tuesday May 5th
14:45 – 15:00

A Proteomic Study Elucidating the Role of Mitochondrial Aconitase in Health and Disease

Charlotte Jacquet¹, Shokouhian, Mohammad¹, Peter Gilgan^{2,3,4}, Michael Moran¹

¹Molecular Medicine The Hospital for Sick Children (SickKids) | ²Centre for Research and Learning Toronto Canada | ³Molecular Genetics University of Toronto Toronto Canada | ⁴Princess Margaret Cancer Centre University of Health Network Toronto Canada

Iron-sulfur clusters (ISC) and ISC proteins are involved in a broad range of biological processes. Defects in their machinery are observed in diverse human pathologies, ranging from cancer to pediatric neuropathies. Nearly 100 human proteins are involved in ISC biogenesis and delivery or contain an ISC. Among them is mitochondrial aconitase (ACO2), that convert citrate to iso-citrate as part of the TCA cycle. (Lill et al., 2020)

Beyond TCA cycle, ACO2 has key implications for health and disease. In the context of non-small cell lung cancer, high levels of ACO2 inhibit tumor growth and increase iron levels, linking ACO2 to ferroptosis, an iron-dependent type of cell death. (Mirhadi et al., 2023; Jiang et al., 2021)

Proteomic changes in response to ACO2 levels, as well as the role of ACO2 as an essential modulator of cell proliferation, iron homeostasis and ferroptosis in lung cancer and pediatric neuropathies will be discussed.

Iron-sulfur clusters (ISC) and ISC proteins are involved in a broad range of biological processes. Defects in their machinery are observed in diverse human pathologies, ranging from cancer to pediatric neuropathies. Nearly 100 human proteins are involved in ISC biogenesis and delivery or contain an ISC. Among them is mitochondrial aconitase (ACO2), that convert citrate to iso-citrate as part of the TCA cycle. (Lill et al., 2020)

Beyond TCA cycle, ACO2 has key implications for health and diseases. In the context of non-small cell lung cancer, high levels of ACO2 inhibit tumor growth and increase iron levels, linking ACO2 to ferroptosis.

Zoe Turner

University of Alberta, Edmonton, AB, Canada

Short talk

Tuesday May 5th
15:45 – 16:00

Proteome-wide Serology Diagnostics for Viral Infections

Zoe Turner¹, Egor Tchesnokov², Oluwafemi Adu², Dana Kocincova², Ross Edwards², Kalyan Das², Matthias Gotte², Andrei Drabovich¹

¹Department of Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta | ²Department of Medical Microbiology and Immunology, University of Alberta

Introduction: Antibodies play a crucial role in the immune response to infections, enabling the evaluation of immunity. Current clinical immunoassays provide only limited information, restricting their use to diagnostic purposes. Our approach is based on

targeted and untargeted Immunoaffinity–Mass Spectrometry (IA–MS) and enables complete characterization of human antibodies in serum (“Proteome–Wide Serology”). Proteome–Wide Serology gives information on antibody isotypes, subclasses, diversity, absolute concentrations, and variable region sequences.

Methods: We have developed informative in–depth Proteome–Wide Serology assay for human serum antibodies against Influenza A hemagglutinin subtypes (H1, H3, H5) and neuraminidase (N1) and Respiratory syncytial virus (RSV) antigens, by coupling high–throughput antigen–specific immunoaffinity enrichments with targeted and untargeted MS (Bruker timsTOF Ultra 2). 10 purified recombinant antigens and over 200 blood serum samples were selected to: (1) characterize immune response and cross–reactivity of antigens and (2) identify optimal antigens for vaccine development.

Results: Using our Proteome–Wide Serology assays, we have found IgG1, IgA1 and IgM as the predominant isotypes for Influenza A hemagglutinin subtypes (H1 and H5) and regional variants (including recent British Columbia H5N1). Comparatively, fusion glycoprotein of another seasonal virus RSV had detectable levels of all antibody isotype and subclasses (except IgE). Comparison of the full antibody repertoires between similar pathogens revealed distinct immune responses which can have important implications in vaccine development.

Conclusions: Proteome–Wide Serology assays can be included in a pipeline for rapid response to emerging pathogens. Ultimately, our work aims to improve public health preparedness and response to infectious diseases through accurate assessment of population immunity.

Olivier Hinse

The University of British Columbia, Vancouver, BC, Canada

Short talk

Tuesday May 5th
16:00 – 16:15

Biochemical Determinants of cathepsin K-mediated cis-transpeptidation in rheumatoid arthritis

Olivier Hinse¹, T S. Yasin abatabaei Dakhili¹, Lee Freiburger², Eliot Mar¹, Jason Rogalski³, Sriram Subramaniam², Leonard Foster⁴, Dieter Brömme¹

¹Faculty of Dentistry, Department of Oral, Biological and Medical Sciences, University of British Columbia, Vancouver, BC, Canada | ²Faculty of Medicine, Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC, Canada | ³The Proteomics Core Facility, University of British Columbia, Vancouver, BC, Canada | ⁴Faculty of Medicine, Department Biochemistry & Molecular Biology, Michael Smith Laboratories, Life Sciences Institute, University of British Columbia; Vancouver, BC, Canada

Many modified forms of peptides from self- and foreign proteins have been implicated in the induction of autoimmune diseases. Among these, hybrid neo-peptides have been shown to potently activate inflammatory responses in models of type I diabetes. Cathepsins have been implicated as key enzymes catalyzing their formation. Nevertheless, the biochemical determinants governing the cis-transpeptidation reaction underlying the synthesis of these hybrid products remain poorly understood. Here, we demonstrate cathepsin K-mediated cis-transpeptidation (peptide fusion) of fragments derived from self-antigens relevant to rheumatoid arthritis. Using LC-MS/MS and de novo peptide sequencing, we characterized the biochemical features of this reaction, including sequence conservation at fusion sites, pH-dependent modulation of reaction efficiency, and the distribution of fusion events across protein primary sequences such as type II collagen and fibrinogen. Additional experiments

using the SARS-CoV-2 Spike protein further demonstrate the broader substrate permissiveness of this reaction. Cis-transpeptidation preferentially occurs at sites containing small amino acids at the P1 position, increases significantly at mildly acidic pH, and predominantly targets intrinsically disordered regions of proteins. In silico binding simulations demonstrate the capability of hybrid neo-peptides to bind to MHC class II molecules from the RA-associated HLA-DRB1*04:01 allele (shared epitope) with affinities comparable or superior to known antigenic peptides. Ongoing work is focused on demonstrating the enzymatic transpeptidase activity in antigen-presenting cells and on developing proteomics workflows enabling the detection of hybrid neo-peptides in synovial tissues from RA patients. Ultimately, these findings could illuminate novel mechanisms underlying autoimmunity and highlight new targets for the management of rheumatoid arthritis.

Pascaline Bories

Plateforme Protéomique et laboratoire de Biologie Computationnelle – CHU de Québec – Université Laval, Quebec City, QC, Canada

Short talk

Tuesday May 5th
16:15 – 16:30

From Serum to Urine: Proteomic Profiling for Mother and Child Health Monitoring During Pregnancy

Pascaline Bories¹, Florence Roux-Dalvai¹, Marie-Pier Scott-Boyer¹, Geneviève Laforest², Emmanuel Bujold², Arnaud Droit¹

¹Plateforme Protéomique et laboratoire de Biologie Computationnelle – CHU de Québec Université Laval | ²Département d'Obstétrique Gynécologie et Reproduction – CHU de Québec Université Laval

The Great Obstetrical Syndromes (GOS), largely linked to abnormal placental development, affect more than 15% of pregnancies and significantly increase perinatal mortality. Among them, preeclampsia (PE) is a leading cause of maternal and neonatal morbidity and mortality worldwide, responsible for up to 100,000 deaths annually. Although PE can be predicted using ultrasound and blood biomarkers, such screening remains complex and often inaccessible in remote or low-resource settings. As a result, many high-risk cases remain undiagnosed. We propose that urinary proteome profiling could provide a non-invasive, accessible approach for early detection of PE and other GOS by revealing molecular changes that precede clinical symptoms.

To explore urinary proteome variations associated with GOS and assess urine as a source of pregnancy biomarkers, we first established the longitudinal proteome profile of healthy pregnancy. Serum and urine samples were collected from healthy women at different gestational stages and analyzed using an Orbitrap Astral mass spectrometer for protein identification and quantification. Linear mixed-effect models were then applied to construct a reference trajectory of the healthy pregnancy proteome.

We identified shared protein expression patterns in serum and urine, particularly involving immune adaptation pathways. Notably, 53 placental proteins were detected in maternal urine, supporting its relevance as a biomarker source.

These findings demonstrate that urine is a reliable biofluid for proteomic monitoring of pregnancy. This non-invasive strategy could improve prenatal care, especially in low-resource settings and remote areas, by enabling early detection of GOS and timely intervention to reduce risks for both mother and child.

Henrique dos Santos Seckler

Northwestern University, Evanston, IL, USA

Short talk

Tuesday May 5th
16:30 – 16:45

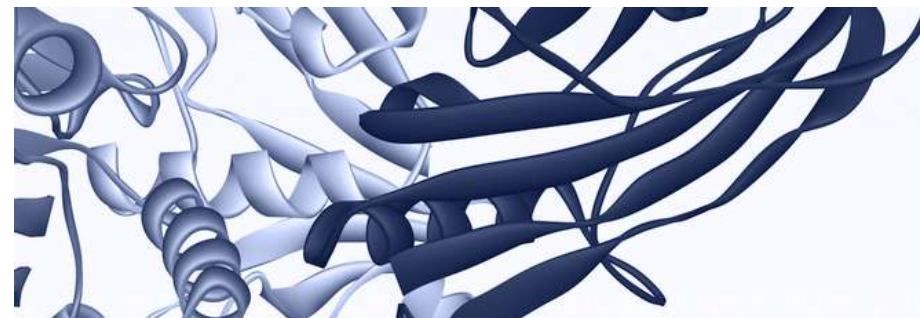
PTMs as Covalent Signatures of Metabolism: How the Proteoform Diversity of Apolipoprotein A-I is Associated with Human Cardiometabolic Health

Henrique dos Santos Seckler¹, John T. Wilkins¹, Cameron Lloyd-Jones¹, Luca Fornelli², Jonathan Rink¹, Philip D. Compton¹, C. Shad

Thaxton¹, Rich LeDuc¹, David Jacobs³, Peter F. Doubleday¹, Allan Sniderman⁴, Donald M. Lloyd-Jones¹, Neil L. Kelleher¹

¹Northwestern University | ²University of Oklahoma | ³University of Minnesota | ⁴McGill University

Apolipoprotein A-I (ApoA1) is the main structural and functional protein of HDL particles, and a marker of HDL metabolism, associated with lower cardiovascular risk. We employed top-down proteomics to characterize HDL apolipoprotein proteoform diversity and quantify proteoform-level associations with human cardiometabolic differences. We optimized collision-assisted electron-transfer dissociation methods to precisely localize PTMs on apolipoprotein backbones. We characterized 15 ApoA1 proteoforms, created by C-terminal truncation, oxidation, glycation and lysine-bound fatty-acid additions (acylations). Using intact-mass label-free quantitation, we investigated proteoform distribution in 150 individuals with varied cardiometabolic phenotype. Among 50 significant associations observed, glycated ApoA1 abundance was associated with fasting blood glucose ($R=0.45$, $p\text{-value}=1E-10$) and diabetes. Fatty-acylated ApoA1 proteoforms were strongly associated with HDL-related metrics, like HDL cholesterol ($R=0.72$, $p\text{-value}=3E-25$), and negatively associated with obesity. These proteoform-level associations to phenotype were significantly stronger than those of total ApoA1. Notably, the distribution of fatty-acid chains acylating ApoA1 closely mirrors the HDL lipidome, suggesting acylating moieties originate inside particles. Consistently, size fractionation showed that non-HDL-bound ApoA1 is acylation-free and that acylations are more abundant in longer-lived, larger HDL particles. Overall, these data paint PTMs as signatures of ApoA1's exposures in metabolism. Glycation, similar to clinical diabetes marker Hemoglobin A1C, presumably arises from ApoA1's extended exposure to elevated blood sugar. Likewise, acylations are likely markers of ApoA1's continued function in physical association with HDL, explaining their strong statistical association with HDL cardiometabolic indices. These results highlight the close relationship between PTMs and metabolism, underscoring the potential of proteoforms as markers of cardiovascular health and disease.



TECHNOLOGICAL ADVANCES & SINGLE CELL PROTEOMICS SESSION

Jason Rogalski

Proteomics & Metabolomics Core Facility, Life Sciences Institute, University of British Columbia, BC, Canada

Short talk

Wednesday May 6th
9:30 – 9:45

Simplicity and Depth in Single-Cell Proteomics: A Cost-Effective Workflow and Expanded Framework for Data Evaluation

Jason Rogalski¹, Shuxin Chi¹, Jason Rogalski¹, Huan Zhong¹, Esperanza Garcia¹, Arpa Ebrahimi², Rachel Wong¹, Melanie

Bailey¹, Marco Marra¹, Claudia Maier², Terrance Snutch¹, Leonard Foster¹

¹University of British Columbia | ²Oregon State University

Single-cell proteomics (SCP) offers direct insight into functional protein states that drive cellular heterogeneity, complementing genomic and transcriptomic analyses. Although recent reports have demonstrated improved proteome coverage, their reliance on specialized instrumentation limits broader.

adoption. Additionally, current evaluation practices remain largely centered on protein and peptide identification counts, which alone do not fully reflect data quality or biological interpretability. Here, we describe an accessible, label-free SCP workflow which implements easily accessible laboratory equipment: a single-cell dispenser, conventional multiwell plates, and an incubator with water-bath-based humidity control. Using trapped ion mobility spectrometry-time-of-flight mass spectrometry (timsTOF), we systematically optimize key sample preparation variables, including trypsin concentration, incubation time, reduction/alkylation, digestion conditions, and plate types, which together maximize data quality and reproducibility. We further introduce a data-quality framework that moves beyond identification counts, emphasizing quantitative consistency and biological interpretability via individual protein coverage completeness across cells, coefficients of variation across technical replicates, peptide-to-protein ratios, and single-cell-to-bulk correlations. Collectively, our approach lowers technical barriers to accessing SCP while enabling more rigorous, interpretable, and scalable SCP analysis across diverse research contexts.

Kyle Tomaro

Department of Biochemistry, Microbiology and Immunology, and Ottawa Institute of Systems Biology, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

Short talk

Wednesday May 6th
9:45 – 10:00

A novel antimicrobial peptide drug discovery pipeline to combat the antibiotic resistance crisis.

Kyle Tomaro¹, François-Xavier Campbell-Valois¹, Mathieu Lavallée-Adam²

¹Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, and Department of Chemistry and Synthetic Biology, Faculty of Science, University of Ottawa, Ottawa, ON | ²Department of Biochemistry, Microbiology and Immunology, and Ottawa Institute of Systems Biology, Faculty of Medicine, University of Ottawa, Ottawa, ON

Drug-resistant infections are predicted to cause 10,000,000 deaths worldwide in 2050, emphasizing the need for conventional antibiotics alternatives. Antimicrobial peptides

are components of the innate immune system across many species that represent promising candidates for combating pan-drug-resistant bacteria. However, these peptides normally exhibit broad-spectrum activity. Therefore, safe therapeutic use would rely, in part, on stricter target specificity.

Herein, we propose a two-phase pipeline for the discovery of bacterial target-specific antimicrobial peptides. The first phase implements a machine learning model trained on the DBAASP antimicrobial peptide database to predict peptide toxicity toward specific bacterial species. We optimized a previously published predictor of peptide toxicity toward *Staphylococcus aureus* (Khabaz et al., 2023), improving its specificity and accuracy by 5% and 1%, respectively. The predictor can therefore predict the toxicity level for *S. aureus* of novel peptide sequences not reported in antimicrobial peptide databases. Using these predictions and known antimicrobial peptides, we will derive sequence motifs associated with *S. aureus* toxicity. Such motifs will guide the generation of novel peptide libraries that will be filtered using our toxicity prediction model to reduce downstream screening resources.

The second phase involves high-throughput in vitro screening using AMPSEC, an AntiMicrobial Peptide SEcRetion system exploiting colicin V's type I secretion system to produce toxic peptides directly into the surrounding environment. We show that AMPSEC can inhibit growth of *Listeria innocua* using a *Listeria*-specific antimicrobial peptide. This pipeline integrates computational and experimental approaches to accelerate the discovery of antimicrobial peptides and improve our understanding of their sequence-function relationship.

Lekha Sleno

Université du Québec à Montréal, Montréal, QC, Canada

Short talk

Wednesday May 6th
11:30 – 11:45

Multimiomics of Hunter syndrome in a mouse model by LC-HRMS/MS

Lekha Sleno², Nathan Ghafari¹, Maggy Lépine¹, Christiane Auray-Blais¹

¹CERMO-FC/Chemistry department, UQAM, Montreal, Quebec, Canada

²Department of Pediatrics, Division of Medical Genetics, University of

Sherbrooke, Sherbrooke, Quebec, Canada

Hunter syndrome, or mucopolysaccharidosis type 2 (MPS II), is a rare disease occurring mainly in boys, with a prevalence of 1/125 000. It is caused by a deficiency of the iduronate sulfatase (IDS) enzyme, resulting in the accumulation of glycosaminoglycans (GAGs), dermatan and heparan sulfate, in lysosomes of cells. This accumulation leads to multiple symptoms, such as hydrocephalus, enlargement of the liver and hearing loss as well as neurological symptoms. In this study, an approach combining proteomics and metabolomics by untargeted LC-MS/MS workflows was used to observe metabolic variations in liver caused by MPS II in an IDS knock-out mouse model. Some known



perturbations observed in the mouse model showed similarities to previous reports in humans with Hunter syndrome, including altered levels of dimethylarginine, as well as disruptions in purine metabolism. Perturbations in amino acid metabolism, previously reported in other types of MPS, were observed, likely linked to reduced hepatic function. Correlation analyses allowed specific biological pathways to be highlighted in this complex dataset, including those involved in acyl-carnitine metabolism and oxidative stress. Additionally, 29 changing lysosomal proteins were implicated in either lysosome organization, transport and degradation. Among them, five are directly implicated in GAG degradation. This work indicated that ERT treatment, in this MPS II model, did not reverse most of the observed metabolic perturbations in the liver. This result further suggests that MPS may cause severe metabolic damage to the liver, likely necessitating a complementary therapeutic approach.

Huan Zhong

University of British Columbia, Vancouver, BC, Canada

Short talk

Wednesday May 6th
11:45 – 12:00

Same-Cell Proteomics and Lipidomics Reveal Sex-Linked Baseline States and Infection-Driven Cross-Omic Rewiring in Human Astrocytes

Huan Zhong, Shuxin Chi, Armando Alcazar, Jason Rogalski, Leonard J Foster

Sex dimorphism shapes antiviral immunity in the central nervous system, yet sex-dependent responses at the single-cell proteome-lipidome level remain poorly resolved. Here, we generated same-cell paired measurements of proteomics and lipidomics from individual primary human cortical astrocytes under control versus HCoV-229E exposure, enabling direct cross-omic integration without separate-cell matching. We then applied integrative multi-omics modeling to separate baseline sex programs from infection-associated responses.

A joint latent-factor analysis (MOFA2) resolved two dominant signal classes: sex-linked baseline programs (Factors 2–3) and condition-driven infection responses (Factors 6–7), with weaker but detectable Sex×Condition modulation. Variance partitioning indicated modality-specific structure, including a proteome-dominant background axis (Factor 1) and complementary infection axes with lipid/proteome-dominant contributions. Factor 6 captured coordinated lipid remodeling during viral exposure, whereas Factor 7 reflected a nuclear stress and reprogramming program in the proteome. Factor-space embeddings revealed strong donor-to-donor variability and a continuum of infection-associated states rather than a simple binary split, underscoring the value of factor-level decomposition.

To extract compact cross-omic signatures with maximal discriminatory power, we applied DIABLO and identified two components linking subsets of proteins and lipids with $|p| \geq 0.3$. Lipid loadings emphasized membrane and sphingolipid/glycolipid-like features, and protein-lipid correlation networks and heatmaps revealed reproducible cross-omic blocks and mixed positive/negative modules that shift with infection. Cross-omic alignment was significantly higher under true cell pairing than under 1,000 random permutations, establishing the importance of same-cell linkage. These findings establish same-cell multi-omics as a powerful strategy for dissecting sex-specific and infection-driven reprogramming in human astrocytes.

Bianca Dupont

University of Ottawa, Ottawa, ON, Canada

Short talk

Wednesday May 6th
12:00 – 12:15

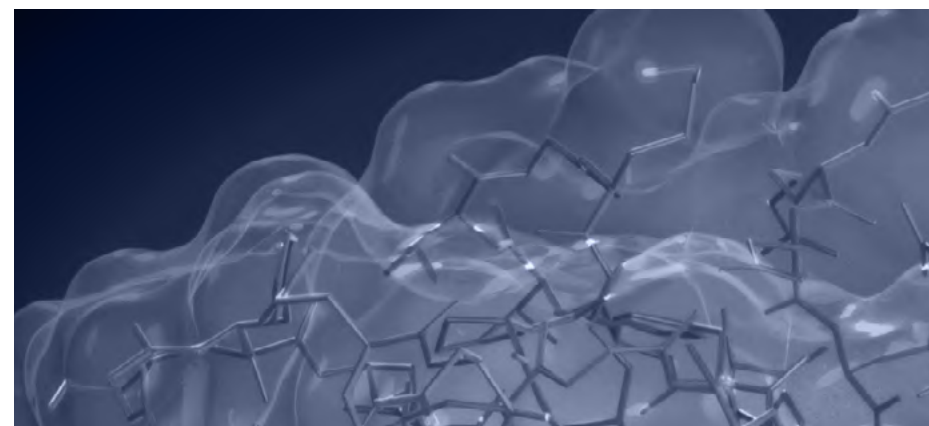
Probing CAR-T interaction networks using proximity proteomics for improved immunotherapies

Bianca Dupont^{1,2}, Tammy-Lynn Tremblay², Scott McComb^{1,2}, Jennifer Hill^{1,2}, Mathieu Lavallee-Adam¹, Joey Sheff^{1,2}

¹University of Ottawa | ²National Research Council of Canada

B-cell cancers see resistance rates of up to 40–60% when treated with standard chemotherapies. Chimeric Antigen Receptor T-Cell Therapy (CAR-T) is a promising alternative. It functions by modifying patient immune T-cells to express a Chimeric Antigen Receptor (CAR), allowing for specific tumour cell killing. Recent findings show that CAR-T efficacy varies according to CAR structural design, including the nanobody-binding and transmembrane domains. However, the precise cellular mechanisms that drive CAR-T function (i.e. activation, persistence, and cytotoxicity) remain uncharacterized. Identifying CAR-proximal proteins associated with enhanced CAR-T function can shed light on such mechanisms. Here, we used cross-linking coupled with mass spectrometry to identify the CAR-proximal proteome of four CAR constructs with variable efficiencies, to further describe the relationship between CAR design, CAR-proximal proteins, and their associated functional properties.

We identified 63 CAR-proximal proteins common to all four constructs. These had roles in activation, metabolism, and regulation, including CD28, CD45, integrins, and solute carriers. We found the CAR-proximal protein environment to largely depend on CAR transmembrane domain identity rather than nanobody-binding domain. Constructs with transmembrane domains associated to lower CAR-T functions showed minimal differences between their proximal profiles, contrary to those associated to higher-efficiencies. The construct with the greatest in vivo activity showed the most unique proximal interactome. Overall, we applied a novel cross linking-based proximity proteomics method to characterize the CAR-interactome of four unique CAR-environments. The enriched proteins that participate in efficient CAR-T function will inform strategies for engineering CAR-T cellular signaling, and enable the development of future, highly effective CAR-Ts.



SYSTEMS BIOLOGY & FUNTIONAL PROTEOMICS SESSION

Romain Brailly

Département d'immunologie et de biologie cellulaire, Institut de Recherche sur le Cancer de l'Université de Sherbrooke, Sherbrooke, QC, Canada

Short talk

Wednesday May 6th
14:45 – 15:00

Proteomic analysis of TRIM28-dependent pathways in fluorouracil-resistant colorectal cancer

Romain Brailly, Jennifer Raisch, François-Michel Boisvert
Département d'immunologie et de biologie cellulaire, IRCUS (Institut de Recherche sur le Cancer de l'Université de Sherbrooke)

Colorectal cancer (CRC) is the third most diagnosed cancer and the second leading cause of cancer-related deaths worldwide. Standard treatments include surgery, radiotherapy, and fluorouracil (5-FU) with adjuvants. However, many patients develop resistance to 5-FU, resulting in disease progression.

We have previously demonstrated that CRC cell lines with acquired 5-FU resistance exhibit significant downregulation of KRAB domain zinc finger proteins, the largest family of transcriptional repressors that canonically interact with co-repressor TRIM28 to silence transposable elements of DNA. Moreover, new transcriptomic analyses we performed revealed that TRIM28 is upregulated in hepatic metastases compared with corresponding healthy margins in relapsed CRC patients not yet treated with 5-FU.

To understand the potential role of TRIM28 in the acquisition and maintenance of 5-FU resistance in CRC, we characterized TRIM28's integration into CRC signaling pathways by comparative proteomics. Using CRISPR-Cas9, we generated TRIM28-knockout variants of three CRC cell lines: DLD1, HCT116, and HT29. We performed a comprehensive differential proteomic analysis of TRIM28-knockout CRC cell lines relative to their wild-type counterparts. We identified key factors of p53 signaling and apoptosis: BAX, BCL2, and cyclin D1, differentially expressed in HT29-TRIM28-KO toward cell death evasion and cellular proliferation. We also identified several differentially overexpressed oncogenic factors including SOX9, which promotes a stem-like phenotype associated with chemotherapy resistance, and several metastasis drivers including FN1, a component of the TGF- pathway overexpressed in 5-FU-treated CRC cells, in DLD1-TRIM28-KO and HCT116-TRIM28-KO. These results show that changes in TRIM28 expression may play a key role in acquired 5-FU resistance in CRC.

Saya Sedighi

University of Toronto, Toronto, ON, Canada

Short talk

Wednesday May 6th
15:45 – 16:00

A Next-Generation Human Cell Map Across Multiple Human Cell Lines

Saya Sedighi, Vesal Kasmaeifar, Brendon Seale, Anne-Claude Gingras
Lunenfeld-Tanenbaum Research Institute; Department of Molecular Genetics, University of Toronto.

The spatial organization of proteins within cells is essential for cellular function, yet how proteome diversity across cell types shapes subcellular architecture remains

poorly understood. Proximity-dependent biotinylation (BioID) enables systematic mapping of protein neighbourhoods in living cells and formed the basis of the original Human Cell Map, which assigned over 4,400 proteins to subcellular compartments in HEK293 cells.

Here, we expand this framework to generate a next-generation Human Cell Map across seven widely used human cell lines (HEK293, U2-OS, A549, HCT116, HeLa, RPE-1, and K562) representing diverse tissue origins. Using 60 baits selected with the GENBAIT algorithm and the ultraID biotin ligase, we performed BioID, followed by data-independent acquisition (DIA) mass spectrometry on a timsTOF Pro 2. To date, comprehensive maps have been generated for four cell lines, each containing over 20,000 high-confidence bait-prey interactions, corresponding to approximately 5,800–6,200 unique proteins assigned across more than 20 subcellular compartments. Matched total proteome measurements were performed in parallel to quantify protein abundance.

Protein localization was assigned using unsupervised clustering of prey profiles, enabling proteins to be placed into primary, secondary, or tertiary compartment associations. Comparison across cell lines reveals conserved localization patterns alongside cell-type-specific differences, particularly within nuclear subcompartments and cytoskeletal structures. Additionally, over 50 proteins lacking prior subcellular annotation in UniProt were assigned to specific compartments.

This work generates the first multi-cell-line next-generation Human Cell Map, integrating spatial maps with expressed proteome data to interpret localization differences. The resource will be publicly available through humancellmap.org.

Jean-Francois Trempe

McGill University, Montréal, QC, Canada

Short talk

Wednesday May 6th
16:00 – 16:15

Integrated proteomics, metabolomics, and structural biology to elucidate molecular mechanisms of mitochondrial quality control

Jean-François Trempe
McGill University

Mitochondrial quality control (MQC) is critical for the maintenance of cellular functions, and dysfunction in MQC can lead to severe diseases including neurodegenerative disorders. For instance, mutations in PINK1 and PARKIN cause early-onset Parkinson's disease (Bayne & Trempe, TIBS, 2026), and mutations in the mitochondrial processing peptidase (MPP), which cleaves nuclear encoded mitochondrial precursors (Bayne et al, JBC, 2025), cause non-progressive cerebellar ataxia. Using affinity purification mass spectrometry (AP-MS) combined with structure prediction by AlphaFold, we discovered that PINK1 binds to the translocase of the outer membrane subunit TOM20 (Eldeeb et al, PNAS, 2024). PINK1 induces the phosphorylation of ubiquitin, which triggers mitochondrial protein turnover via PARKIN, an important mechanism of MQC. Using unnatural and isotopic amino acid labeling in cell culture combined with LC-MS, we find that PINK1 impact mitochondrial protein synthesis and turnover and undergoes non-canonical translation initiation, thereby evading inhibition by the integrated stress response (Romanelli & Trempe, JBC, 2025). Extending the AP-MS/AlphaFold approach to MPP, we discover that it binds NUDT8, a coenzyme

A hydrolase of unknown function. Applying deep quantitative proteomics and metabolomics to NUDT8 knockout cells, we discover major perturbations pointing towards a dysregulation of purine synthesis, glycolysis, and maturation of respiratory chain complexes. Overall, our findings highlight that combining MS-based omics and structural biology is a powerful and fast approach to elucidating the function of proteins in cells.

Isabelle Perron-Lépine

Pediatrics Department, University of Sherbrooke, QC, Canada

Short talk

Wednesday May 6th
16:15 – 16:30

GAPDHP1, an overlooked actor of mitochondrial cristae and bioenergetics

Isabelle Perron-Lépine¹, Joelle Vincent¹, Antoine Lévènes¹, Félix-Antoine Trifiro¹, Guillaume Provost⁴, Benoit Laurent⁴, Marie A Brunet^{1,2,3}

¹Pediatrics Department, University of Sherbrooke, Canada | ²Cancer Research Institute (IRCUS), University of Sherbrooke, Canada | ³Centre de Recherche du Centre hospitalier universitaire de Sherbrooke (CRCHUS), Canada | ⁴Biochemistry and Functional Genomics Department, University of Sherbrooke, Canada

Thousands of non-annotated coding sequences are translated in human. These novel ORFs are excluded from reference repositories, shadowing an entire layer of the proteome. Using the OpenProt resource, we discovered a novel protein, P1, encoded within a pseudogene of GAPDH on the X chromosome, with robust mass spectrometry (MS) detection: >2 unique peptides, in >18 independent studies on human cell lines and tissues.

P1 is an inner mitochondrial membrane (IMM) protein detected by MS, high-resolution confocal microscopy and biochemical assays. It interacts with Mic60, a key component of the MICOS complex, which is essential for the formation and maintenance of cristae, IMM invaginations crucial for efficient oxidative phosphorylation. We induced specific knockdowns of P1 (shP1) in undifferentiated neuronal cells. Our shP1 cells show a collapsed mitochondrial network, with perinuclear aggregation. shP1 cells also display disorganized and swollen cristae in transmission electron microscopy. This structural phenotype correlates with a significant decrease in basal (68%) and maximal (67%) respiration, and ATP production (57%). Our results suggest a partial destabilization of the MICOS complex in shP1 cells, defining P1 as an overlooked regulator of MICOS.

Mitochondrial architecture and bioenergetic changes are observed upon stress or cell differentiation. We leveraged the neuronal differentiation model to track P1 expression. P1's expression (RT-qPCR) and transcription (CAGE-seq) is significantly downregulated in progenitors, alongside other MICOS subunits. Furthermore, P1 is significantly downregulated in Alzheimer's patients, highlighting its potential as a biomarker.

Through functional proteogenomic, we discovered a novel protein, important for mitochondrial health.

R. Glen Uhrig

University of Alberta, Edmonton, AB, Canada

Short talk

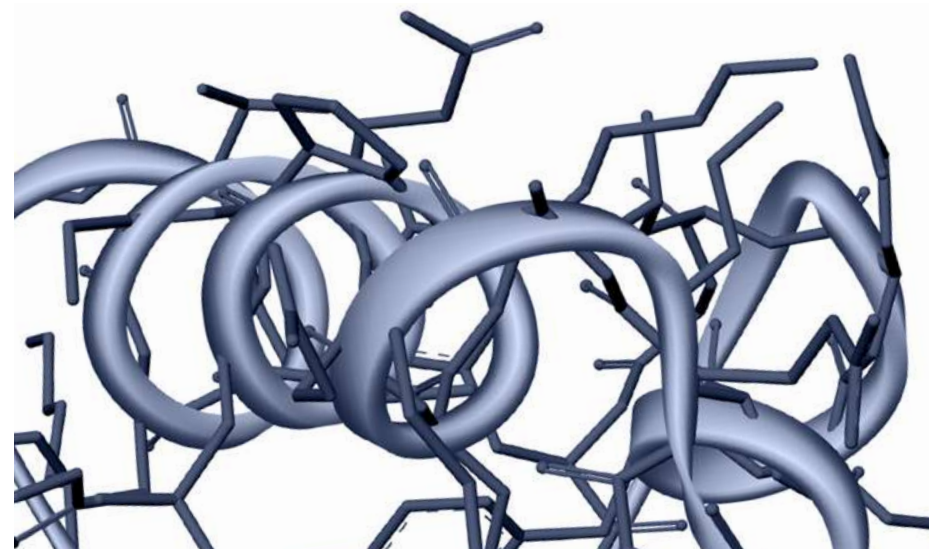
Wednesday May 6th
16:30 – 16:45

Towards understanding Plant Translation: Bio-Orthogonal Non-Canonical Amino acid Tagging (BONCAT) for low-disruption labeling of Arabidopsis proteins in vivo

R. Glen Uhrig¹, Nicholas Hassan¹, Shelly Braun², Mohana Talasila¹, Curtis Kennedy^{1,3}, Luke Yaremkó³, Richard Fahlman², R. Glen Uhrig^{1,2}

¹Department of Biological Sciences, University of Alberta | ²Department of Biochemistry, University of Alberta | ³University of Alberta; Department of Computer Science, University of Alberta

As sessile organisms, plants require protein synthesis responses to adapt to a variety of environmental conditions. Measurement of newly synthesized proteins across several organisms has been successfully facilitated with Bio-Orthogonal Non-Canonical Amino acid Tagging (BONCAT). Here, we use non-canonical amino acids (NCAAs) L-azidohomoalanine (AHA) or L-homopropargylglycine (HPG) incorporation in place of methionine residues into the actively translating Arabidopsis (*Arabidopsis thaliana*) proteome, allowing for that subset of proteins to be enriched for quantification by mass spectrometry. We provide evidence for successful BONCAT implementation through the liquid immersion of seedlings in AHA- or HPG-containing media that functions with significantly lower concentrations than the literature standard. Our approach splits acute exposure and the incorporation phase of labeling to mitigate potential negative impacts of prolonged NCAA exposure without compromising effective enrichment capacity. This method results in an unperturbed growth phenotype for AHA-treated seedlings. Finally, we demonstrate the capacity of this modified approach to enrich newly synthesized proteins from the whole proteome under standard stress conditions. These improvements allow for a broader use of BONCAT technologies in molecular plant research, affording a deeper understanding of the newly synthesized proteome without negatively impacting plant health.



▶ **P.A01 – Leanne Wybenga-Groot**

The Hospital for Sick Children

LIGAND-AL; an opportunity to identify chemical modulators for your protein of interest

Mike Tyers, SickKids

Target 2035 is a global open science initiative led by the Structural Genomics Consortium (SGC). The mission of Target 2035 is to develop pharmacological modulators for every human protein by the year 2035. Artificial intelligence will enable this mission, but it needs large-scale, open datasets that do not exist yet. In the next five years, LIGAND-AL and its 18 global partners will generate high-quality protein-ligand interaction datasets that will be shared openly and used to train next-generation AI models capable of predicting drug-like molecules for thousands of proteins, thereby expediting the mission of Target 2035.

Many of us have done the proteomics experiments and determined proteins or biomarkers of interest, but lack a chemical modifier to expand our investigations. Or, perhaps you have purified your protein for structural proteomics or determination, and could learn more about its biology if only there was an inhibitor. This is your opportunity to identify chemical modulators for your protein of interest! Consider joining the Protein Contribution Network and submit purified, high-quality proteins to be screened for small molecule binders. Allow the screening data to be shared and in turn receive validated hits for your protein of interest. Community contributions will help expand the diversity of targets screened in industry-standard platforms, as well as provide collaborative opportunities with the SGC consortium, including industry partners. Find out more at my poster or <https://ligand-ai.org/about>.

▶ **P.A02 – Valeriia Vasylieva**

University of Sherbrooke

Enhancing OpenProt with mass offset search

Vasylieva, Valeriia, Pediatrics Department, University of Sherbrooke; Trifiro, Felix-Antoine, Pediatrics Department, University of Sherbrooke; Bourassa, Francis, Pediatrics Department, University of Sherbrooke; Roucou, Xavier, Biochemistry and Functional Genomics Department, University of Sherbrooke; Brunet, Marie, Pediatrics Department, University of Sherbrooke;

Non-canonical proteins, defined as novel proteins absent from reference repositories, are recorded in OpenProt through closed search proteogenomic analysis of mass spectrometry (MS) data. Up to a third of the MS-detectable proteome carries modifications, which can lead to mis- or lack of identifications. However, searching for the 1,574 known modifications drastically raises the chance of random high-scoring matches, inflating the false discovery rate (FDR).

Here, we implemented detailed mass offset search with entrapment-based FDR filtering in OpenProt. Three human datasets were analyzed with MSFragger using modifications from Unimod and PhosphoRS for modifications' localization rescoring. DMO was run with 3 entrapment databases to calibrate the posterior error probability (PEP) score for an empirical 1% FDR for each peptide class (canonical/non-canonical).

Our entrapment analysis showcased the importance of class-based FDR calibration as non-canonical proteins displayed an empirical FDR 37-fold greater (22.2%) than canonical proteins (0.6%) under global 1% FDR filtering. Entrapment-based calibration controlled both canonical and non-canonical proteins at 1% FDR, yielding 73% fewer non-canonical and 2% greater canonical identifications. 45.6% of 6,120 non-canonical proteins detected with at least two unique peptides contained 633 distinct mass offsets. The most frequent modifications include iodoacetamide derivative (26.6%), oxidation/hydroxylation (17.2%), acetylation (9.5%) as well as residue substitutions. 9.6% of modified non-canonical peptides were supported by ≥ 2 spectra in ≥ 2 samples. Non-canonical proteins were more frequently modified (1.6-fold), highlighting how modification-aware search strategies can accelerate functional discoveries.

Our approach, implemented on 183 datasets in OpenProt, will foster the discovery of non-canonical proteins and provide functional insights.

▶ **P.A03 – Elmira Shajari**

Université de Sherbrooke

A Nested Cross-Validation Framework for Robust Peptide Biomarker Selection from DIA Stool Proteomics in Inflammatory Bowel Disease

Shajari, Elmira, Université de Sherbrooke, Sherbrooke, Canada; Gagné, David, Université de Sherbrooke, Sherbrooke, Canada; Malick, Mandy, Université de Sherbrooke, Sherbrooke, Canada; Roy, Patricia, Université de Sherbrooke, Sherbrooke, Canada; Noël, Jean-François, Sherbrooke, Canada; Gagnon, Hugo, Allumias, Sherbrooke, Canada; Delisle, Maxime, Université de Sherbrooke, Sherbrooke, Canada; Boisvert, François-Michel, Université de Sherbrooke, Sherbrooke, Canada; Brunet, Marie A., Université de Sherbrooke, Sherbrooke, Canada; Beaulieu, Jean-François, Université de Sherbrooke, Sherbrooke, Canada.

Monitoring disease activity in inflammatory bowel disease (IBD) is essential for guiding therapy and preventing irreversible tissue damage. While colonoscopy remains the clinical gold standard, its invasive nature limits frequent use, and fecal calprotectin shows reduced accuracy within its diagnostic gray zone (100–250 $\mu\text{g/g}$). Mass-spectrometry-based stool proteomics offers a non-invasive strategy to capture molecular signatures of

intestinal inflammation. Here, we evaluated whether DIA-derived stool peptides can classify IBD activity using a rigorously nested cross-validation framework designed to identify stable peptide biomarkers.

A total of 174 stool samples from IBD patients were analyzed using SWATH-DIA mass spectrometry. Feature selection was restricted to training partitions within a nested cross-validation workflow to prevent information leakage. Boruta, LASSO, and recursive feature elimination were applied across repeated subsampling iterations, retaining peptides selected in $\geq 70\%$ of runs. Stable peptides were used to train four classifiers (GLMNet, SVM-Radial, SVM-Linear, Naïve Bayes) with hyperparameter tuning performed in inner 5-fold loops. Model performance was evaluated on unseen outer test folds, and predictive performance was further assessed in samples within the fecal calprotectin gray zone.

Results: Nested cross-validation identified a consensus panel of nine peptides from five proteins. GLMNet achieved the best overall performance (outer-fold AUC 0.93, balanced accuracy 0.88, F1-score 0.85) with minimal evidence of overfitting. Within the gray-zone subgroup ($n=34$), the model retained discriminative power (balanced accuracy 0.78, AUC 0.80).

DIA stool proteomics combined with nested cross-validation enables robust identification of stable peptide biomarkers for non-invasive assessment of IBD activity, supporting future targeted assay development and clinical validation.

▶ **P.A04 – Mathieu Lavallée-Adam**

University of Ottawa

MealTime-MS 2.0: A novel supervised learning approach for the real-time optimization of mass spectrometry data acquisition increases proteome coverage.

Abramchuk, Iryna, University of Ottawa; Chung, Yun-En, University of Ottawa; Petrova, Alona, University of Ottawa; St-Germain, Jonathan, Princess Margaret Cancer Centre; Decker, Jens, Bruker Daltonics GmbH & Co. KG; Raught, Brian, Princess Margaret Cancer Centre; Krieger, Jonathan, Bruker Ltd.; Sri Kumar, Tharan, Bruker Ltd.; Lavallée-Adam, Mathieu, University of Ottawa

While data-independent acquisition has improved protein identification, data-dependent acquisition (DDA) remains valuable for proteomics applications such as multiplexed samples and crosslinked peptide analyses. However, DDA often collects redundant mass spectra from abundant proteins, leaving many low-abundance proteins uncharacterized. To address this limitation, we previously presented a software tool (MealTime-MS) that prevents, in real-time, redundant data acquisition from proteins confidently identified during an experiment. Nevertheless, MealTime-MS was only evaluated in simulations.

Here, we present MealTime-MS 2.0, which controls data acquisition on Bruker timsTOF mass spectrometers. The tool uses a logistic regression classifier to assess, on-the-fly, the confidence of a protein identification based on the mass spectra collected for it. Peptides from confidently identified proteins are then excluded from further redundant data collection, allowing the instrument to acquire data from less abundant proteins.

MealTime-MS 2.0 data acquisition performance was compared with that of a state-of-the-art DDA-PASEF workflow using a K562 whole-cell digest. Experiments guided by MealTime-MS identified, on average, 17.5% more proteins than DDA experiments. MealTime-MS uniquely identified 601 proteins that were never observed with DDA, whereas only 230 proteins were uniquely identified by DDA. Proteins identified with MealTime-MS 2.0 also exhibit lower protein abundance values reported by PaxDb in human cells than those detected with DDA. This shift in the protein abundance distribution suggests that MealTime-MS improves the identification of low-abundance proteins.

Overall, MealTime-MS 2.0 enables a more efficient mass spectrometry resource allocation, detects more low-abundance and under-characterized proteins than state-of-the-art approaches, and improves proteome coverage and biological characterization of samples.

▶ **P.A05 – Rachel Nadeau**

University of Ottawa

Discovery of 3'UTR structural motifs using protein co-localization networks

Nadeau, Rachel (1); Sarrazin-Gendron, Roman (2,3); Waldspühl, Jérôme (2); Lavallée-Adam, Mathieu (1) 1 Department of Biochemistry, Microbiology and Immunology, and Ottawa Institute of Systems Biology, Faculty of Medicine, University of Ottawa; 2 School of Computer Science, McGill University; 3 Département d'informatique, Université du Québec à Montréal

Protein localization is regulated through various molecular mechanisms, including cis-regulatory elements within 3' Untranslated Regions (3'UTRs) of mRNAs. These elements can mediate the transport of transcripts to specific subcellular compartments for translation, often in a structure-dependant manner. However, the functional role of 3'UTR structural motifs remain poorly understood. BioID-based proximity labelling coupled with mass spectrometry has generated comprehensive protein co-localization networks that can help tackle this issue. Proteins that are densely connected in such networks likely localize to similar compartments. If the transcripts of such proteins share a common 3'UTR structural motif, this motif could be directly or indirectly related to the proteins' regulation or localization. We present a novel graph theory-based algorithm to detect 3'UTR structural motifs associated with proteins that are significantly clustered in a co-localization network. We leveraged BoyesPairing2 (Sarrazin-Gendron et al., 2019) and the RNA 3D Motif Atlas to annotate structural motifs within 3'UTRs and mined the Human Cell Map protein co-localization network (Go et al., 2021). Our approach identified four structural motifs that were associated with significantly clustered protein in the network (false discovery rate < 0.19). None of these were detected by a state-of-the-art network clustering tool. Motifs clustered at

similar positions within 3'UTR sequences and many instances matched with known protein binding sites, hinting at their putative functionality. Proteins associated with three motifs showed enrichments for specific cellular compartments and biological processes. Overall, our approach demonstrates the potential of mining protein co-localization networks to functionally characterize structural motifs and shed light on protein localization mechanisms.

► **P.A06 – Peter Kubiniok**
Quantum Inc.

An AI-Assisted End-to-End Workflow for Automated Targeted LC-MS/MS Data Processing and Quality Control

Targeted LC-MS/MS assays such as multiple reaction monitoring (MRM) and parallel reaction monitoring (PRM) are increasingly deployed in clinical and translational laboratories for quantitative proteomics. However, data processing remains fragmented, labor-intensive, and dependent on expert manual review, creating a bottleneck for large-scale studies and routine deployment.

We present MRMPipe, an automated, AI-assisted software framework designed to streamline targeted assay analysis from raw data to report-ready results. MRMPipe integrates chromatogram extraction, smoothing, peak detection, retention-time alignment across runs using dynamic time warping, and transition-level scoring into a unified pipeline. The system supports both discovery-to-targeted assay development and routine clinical-style batch processing.

Machine-learning components are incorporated to assist with peak classification, interference detection, and prioritization of samples requiring expert review, enabling a human-in-the-loop workflow for quality assurance. This approach reduces manual inspection while preserving analytical rigor and regulatory-oriented traceability. MRMPipe also implements reproducibility metrics, automated flagging of low-confidence integrations, and cross-sample consistency checks to support longitudinal studies.

In pilot proteomics datasets comprising repeated measurements across multiple LC-MS batches, MRMPipe achieved >90% quantitative reproducibility and reduced hands-on analysis by 10 fold time compared with conventional manual review workflows. Ongoing work focuses on expanding support for proteomics-scale targeted panels, vendor-agnostic raw data handling, and deployment in regulated laboratory environments.

MRMPipe is designed for both local installation and secure server-based deployment, facilitating integration into existing laboratory information systems.

► **P.A07 – Mickael Leclercq**

Centre de recherche du CHU de Québec – Université Laval

Autonomous AI Agent for De Novo Candidate Discovery and In Silico Multi-Target Validation: From Literature Mining to Replicated Molecular Dynamics Without Human Intervention

Leclercq, Mickael, "Département de médecine moléculaire, Faculté de médecine, Université Laval, Québec, QC, Canada"; "Axe Endo-Nephro, Centre de recherche du CHU de Québec-Université Laval, Québec, QC, Canada"; Léopold, Quitté, "Département de médecine moléculaire, Faculté de médecine, Université Laval, Québec, QC, Canada"; Droit, Arnaud, "Département de médecine moléculaire, Faculté de médecine, Université Laval, Québec, QC, Canada"; "Axe Endo-Nephro, Centre de recherche du CHU de Québec-Université Laval, Québec, QC, Canada"; "Inria, Maasai team-Université Côte d'Azur, Nice, France"

Identifying and validating novel small-molecule candidates against a panel of protein targets is a resource-intensive process that traditionally requires months of iterative human-supervised computational work. Here we present a fully autonomous AI research agent capable of executing the entire discovery-to-validation pipeline end-to-end, from automated literature mining and candidate identification to multi-target docking, replicated molecular dynamics, interaction fingerprinting, and binding free energy estimation, without human intervention between stages.

The agent autonomously retrieves and screens candidates from public chemical and bibliographic databases, applies physicochemical and ADMET filters, prepares protein receptors with co-crystallized ligand redocking as a quality control checkpoint, and launches AutoDock Vina campaigns across panels of protein targets simultaneously. Promising docking hits are forwarded automatically to OpenMM-based molecular dynamics simulations (Amber14/TIP3P, 200 ns, multiple independent replicates per system), followed by ProLIF-based persistent interaction fingerprinting and MM-PBSA binding free energy decomposition.

Built-in error recovery, GPU queue management, cross-session persistent memory, and autonomous logging enable uninterrupted overnight execution. Applied to a repositioning case study spanning 6 candidates and 11 targets (66 ligand-target pairs, 22+ completed MD systems), the full pipeline ran in 40 days of wall-clock time with near-zero manual intervention.

Our results demonstrate that autonomous agent orchestration makes systematic dynamic validation of large docking campaigns practically tractable, and that docking scores alone substantially overestimate binding stability in a significant fraction of cases.

► **P.A08 – Alyzee Minichini**

University of Guelph

Targeted Gene Deletion and Proteomic Characterization to Uncover Roles in Anti-fungal Resistance within *Cryptococcus neoformans*

Geddes-McAlister, Jennifer, University of Guelph; Woods, Michael, University of Guelph; Bermas, Arianne, University of Guelph; Adolfo, Mary, University of Guelph

Cryptococcus neoformans is an opportunistic fungal pathogen that is responsible for approximately 180,000 deaths annually, with the highest mortality rates observed in low-resource countries. This is in part due to the lack of variety in the drugs we have available for treating this infection. As it stands, resistance rates will begin to outpace drug development, as already observed

for the mainstay antifungal, fluconazole (FLC). Previous comparative mass spectrometry-based proteomic analyses by our team revealed six proteins with increased abundance in FLC resistant strains compared to the susceptible wild-type strain. Of these, three proteins have been confirmed to reinstate FLC susceptibility when absent from the genome. The remaining three proteins: xylitol dehydrogenase, succinate semi-aldehyde dehydrogenase, and a hypothetical protein, appear to be promising candidates for drug targeting.

To investigate the contributions of these proteins to anti-fungal resistance, I have generated gene deletion strains in FLC-susceptible and -resistant genetic backgrounds using split-marker PCR and biolistic transformation. Drug resistance of the strains will be assessed in the presence and absence of FLC at 30 and 37 °C. Next, survivability in the presence of macrophages, thermotolerance, polysaccharide capsule and melanin production will be tested. Lastly, I will perform comparative mass-spectrometry-based proteomics to identify downstream targets and pathways that are disrupted by gene loss and observe the global compensation that occurs via changes in protein abundance. Overall, this work showcases our innovative use of proteomics to identify and characterize FLC resistance-associated proteins and explore strategies to make our current arsenal of antifungals effective again.

► **P.A09 – Cameron Ellis**

PreOmics Inc., Billerica, MA, USA

Optimized FFPE Tissue Sample Preparation: Flexible, scalable processing of diverse FFPE tissue formats for in-depth proteome analysis

Johansson, Jasmin, PreOmics GmbH, Planegg/Martinsried, Germany; Wuertenberger, Silvia, PreOmics GmbH, Planegg/Martinsried, Germany; Hartinger, Katrin, PreOmics GmbH, Planegg/Martinsried, Germany; Kulak, Nils A., PreOmics GmbH, Planegg/Martinsried, Germany

Formalin-fixed, paraffin-embedded (FFPE) tissues are invaluable for translational medicine. The FFPE workflow based on BeatBox and iST technology provides a high-throughput solution for FFPE tissue processing in plate format, eliminating separate deparaffinization steps and opening a new avenue for retrospective proteomic studies. However, sample input can be limited, and proteomic laboratories typically receive FFPE tissue in various formats and amounts. We present an adapted tube-based FFPE workflow that overcomes these limitations, enhancing compatibility with various analytical needs.

Non-deparaffinized FFPE curls and 1mm punches from mouse tissues (liver, kidney, cardiac muscle) were homogenized using BeatBox®, with proteins de-crosslinked at 95°C, reduced, and alkylated in LYSE buffer. Followed by iST protocol for trypsin digestion and optimized peptide cleanup for paraffin removal. Samples were analyzed using nanoElite® 2 coupled to timsTOF HT in dia-PASEF® mode. Data was processed with either Bruker ProteoScape™ or Spectronaut® 20.

Protein yields from two curls were comparable across both workflows. Increasing inputs to five full curls increased protein yield and consistent protein identifications for each tissue type, regardless of BeatBox kit format or input amount. Over 5,500 proteins were identified for liver samples, demonstrating that the BeatBox-iST FFPE workflow achieves deep proteome coverage. Coefficients of variation (CVs) within quadruplicates of FFPE curls were <10% for all tissues. LC-MS analysis of FFPE punches yielded similar results to those of FFPE curls, demonstrating workflow flexibility with various FFPE formats.

The tube version of the proven BeatBox-iST workflow for FFPE tissue samples adds flexibility and scalability for deep, reproducible proteomic analysis.

► **P.A10 – Cameron Ellis**

PreOmics Inc., Billerica, MA, USA

Advancing Plasma Proteomics Through a Next-Generation Single-Particle Enrichment Workflow for Deeper and More Quantitative Biomarker Discovery

Limm, Katharina, PreOmics GmbH, Planegg/Martinsried, Germany; Schär, Sandra, Biognosys AG, Zurich, Switzerland; Bruderer, Roland, Biognosys AG, Zurich, Switzerland; Kulak, Nils A., PreOmics GmbH, Planegg/Martinsried, Germany

Human plasma represents an exceptional yet analytically demanding matrix for proteomic biomarker discovery. Its dynamic range and compositional heterogeneity limit detection of low-abundance proteins, which often convey pathophysiological insights. To address these challenges, we present a workflow integrating P2 plasma enrichment technology with iST sample preparation and Spectronaut® 20. The combined platform leverages the selective enrichment power of P2 and the reproducibility of iST-based digestion and cleanup, enabling deep proteome coverage from human EDTA plasma samples. Human EDTA-plasma samples from a colorectal cancer cohort (n=6 per group) were processed using the P2 single-particle enrichment workflow (Biognosys Group), employing proprietary nanoparticles that form transient protein coronas to selectively capture low-abundance proteins while excluding high-abundance proteins. Enriched fractions were digested using the optimized iST technology (Biognosys Group) for standardized peptide generation. Peptides were analyzed on a timsTOF HT (Bruker) in dia-PASEF® mode in a library-free DIA framework for deep, quantitative profiling. Compared to conventional preparations, the P2-iST workflow achieves significantly deeper coverage with substantially higher identification of protein groups and greater quantitative precision (CV <15%). Nearly 6,000 proteins were identified in 12 samples, demonstrating high data completeness of more than 90%. Pathway enrichment shows an emerging biological separation between disease and control groups. In addition, more cytokines, interleukins, and other biomarkers were identified. Integration of P2 enrichment with streamlined iST technology and Spectronaut 20 data analysis defines a next-generation, high-performance platform for deep, precise, and reproducible plasma proteomics. This synergistic approach holds significant promise for advancing biomarker discovery and clinical translational research.

► **P.A11 – Camille Bouchard**

Université Laval

Characterization of Limb–Girdle Muscular Dystrophy Variants Within the Quebec and Canadian LGMD Network and Emerging Therapeutic Correction Strategies

Camille Bouchard, Joël Rousseau, Jing Jiang, Mayra Aldecoa, Pierre Trudel, Mathieu Blais, Dr Nicolas Dupré, Dr Jean-Denis Brisson, Dr Erin O’Ferrall, Dr Jacques P. Tremblay

Limb–Girdle Muscular Dystrophies (LGMDs) are a heterogeneous group of rare neuromuscular disorders characterized by progressive weakness of the shoulder and pelvic girdle muscles. In Quebec, the full genetic landscape of LGMD remains incompletely documented, and many patients carry variants of unknown significance (VUS), complicating diagnosis and therapeutic development.

To address this gap, we conducted a province-wide census of LGMD mutations in collaboration with neurology centers in Quebec. This initiative established a shared clinical–research network and biobank enabling access to patient genetic data, muscle biopsies, and biopsy-derived cell lines. Proteomic analyses are being implemented within this framework to investigate the downstream molecular consequences of deficiency and to support functional interpretation of genetic variants.

Mutation-specific gene correction strategies were also evaluated. Prime editing enabled precise correction of DYSF mutations in vitro, achieving editing efficiencies of up to 31% in HEK293T cells and 23% in patient-derived myoblasts. Ongoing proteomic analyses are being used to determine whether corrected cells recover protein expression signatures consistent with restoration of dysferlin function.

To support preclinical therapeutic testing, we also characterized the DYSF–R1925X mouse model, which reproduces key pathological features of dysferlinopathy and provides a platform for in vivo evaluation of mutation-targeted therapies.

Building on these results, this work will expand into a Canada-wide LGMD mutation census during my postdoctoral research. In collaboration with variant interpretation specialists including Dr. Conrad Wehl and colleagues, functional assays and proteomic signatures will be used to confirm the pathogenicity of VUS and improve molecular diagnosis for LGMD patients.

► **P.A12 – Eileen Tudorica**

Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada; Division of Experimental Medicine, McGill University, Montreal, QC, Canada

Discovery and Targeted Validation of Erythrocyte Proteomic Biomarkers of Recombinant Human Erythropoietin (rhEPO) Misuse and Hypoxic Exposure at High Altitude

Richard, Vincent, Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada; Kubiniok, Peter, Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada, Quantum Inc., Montreal, QC, Canada; Blidjios, Constantinos, Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada, Division of Experimental Medicine, McGill University, Montreal, QC, Canada; Geib, Timon, Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada; Ebrahimi, Laleh, Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada, Division of Experimental Medicine, McGill University, Montreal, QC, Canada; Absar, Foughsadat, Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada; Bonne, Thomas, University of Copenhagen, Copenhagen, Denmark; Bejder, Jacob, University of Copenhagen, Copenhagen, Denmark; Nordsborg, Nikolai, University of Copenhagen, Copenhagen, Denmark; Borchers, Christoph H., Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada, Division of Experimental Medicine, McGill University, Montreal, QC, Canada, Gerald Bronfman Department of Oncology, Jewish General Hospital, McGill University, Montreal, QC, Canada, Department of Pathology, McGill University, Montreal, QC, Canada

Recombinant human erythropoietin (rhEPO) is a prohibited erythropoiesis-stimulating agent used to enhance endurance performance, with microdosing and high-altitude training complicating detection strategies. This study aimed to identify erythrocyte protein biomarkers that selectively reflect rhEPO exposure, independent of altitude-induced hypoxia, and to develop quantitative MRM-assays for their detection. A total of 849 erythrocyte samples were collected longitudinally from 39 athletes assigned to rhEPO (20 IU/kg) or placebo under sea-level or high-altitude training conditions across 4-week baseline, treatment, and follow-up periods. Automated protein extraction and protein aggregation capture (PAC)-based digestion enabled high-throughput sample preparation. Discovery proteomics was performed using dataPASEF acquisition, and data was searched against an erythrocyte specific spectral library with over 17,000 peptides from more than 2,000 proteins. Across the cohort, 1,673 proteins were quantified with 900 consistently detected across all samples. A permutation based linear regression model to establish longitudinal trends in protein expression, followed by fuzzy clustering-based refinement, as well as supported vector machine (SVM) modeling, identified 121 proteins showing rhEPO-specific expression changes independent of altitude effects. These proteins were ranked according to resultant p-values, and a final panel of 40 proteins were selected for targeted MRM assay development and validation in a separate cohort of 191 samples. The resulting assays had a median LLOQ in the mid-attomole range with precision below 15% CV. This integrated DIA-to-MRM workflow establishes a high-throughput and analytically robust method for detecting rhEPO misuse and highlights erythrocyte proteomics as a promising platform for anti-doping biomarker development.

► **P.A13 – Elodie Logerot**

Segal Cancer Proteomics Centre, Lady Davis Institute, Jewish General Hospital, McGill University, Montréal, Québec, Canada

A Simplified Workflow for Robust, High-Throughput, and Quantitative Plasma Proteomics through Streamlined In-Tip Proteolysis

Logerot, Elodie, Segal Cancer Proteomics Centre, Lady Davis Institute, Jewish General Hospital, McGill University, Montréal, Québec, Canada; Geib, Timon, Segal Cancer Proteomics Centre, Lady Davis Institute, Jewish General Hospital, McGill University, Montréal, Québec, Canada; Stoychev, Stoyan, EvoSep Biosystems, Odense, Denmark; Bekker-Jensen, Dorte B., EvoSep Biosystems, Odense, Denmark; Bache, Nicolai, EvoSep Biosystems, Odense,

Denmark; Borchers, Christoph H., Warren Y. Soper Clinical Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montréal, Québec, Canada; Division of Experimental Medicine, McGill University, Montréal, Québec, Canada; Department of Pathology, McGill University, Montréal, Québec, Canada

Blood plasma is an ideal matrix for biomarker discovery and disease monitoring. However, current LC-MS workflows rely on complex, multi-step sample preparation, which limits large-scale and clinical applications by increasing handling time, the risk of sample loss, and variability. Simplified, robust, and standardized workflows are therefore required to ensure reproducible protein measurements suitable for clinical applications. To address this need, we developed a novel sample preparation approach as part of the SysQuan project, combining streamlined handling with the use of stable isotope-labeled (SIL) mouse plasma as a global internal standard. This strategy enables accurate and reproducible absolute quantification of plasma proteins at ~10^x lower cost than synthetic SIL peptides, supporting the routine use of plasma proteomics in research and clinical settings. We developed a simplified “One-Tip” workflow integrating protein extraction, reduction, alkylation, digestion, and peptide desalting within a single C18 disposable trap column (EvoTip). This one-pot strategy eliminated transfer and evaporation steps, reduced handling time by 3 hours, and minimized technical variability while preserving protein identification. Human and SIL mouse plasma were mixed at a 1:1 protein ratio. Digests were analyzed using an Evosep One LC system coupled to a timsTOF HT mass spectrometer operating in DIA-PASEF mode. The optimized One-Tip workflow enabled reproducible protein identification from neat plasma (>325 proteins, median peak intensity CV <20%), with a 4-hour digestion providing the best compromise between proteome coverage, reproducibility, and analytical throughput. Approximately 75% of detected peptides overlapped with the SysQuan panel, demonstrating strong compatibility with SysQuan-based absolute quantification pipelines.

► **P.A14 – Elodie Logerot**

Segal Cancer Proteomics Centre, Lady Davis Institute, Jewish General Hospital, McGill University, Montréal, Québec, Canada

Absolute Quantitation of the Mouse Brain Proteome Using the SysQuan Platform

Baker, Elyssa, Segal Cancer Proteomics Centre, Jewish General Hospital, Montreal, McGill University, QC, Canada ; Logerot, Elodie, Segal Cancer Proteomics Centre, Jewish General Hospital, McGill University, Montreal, QC, Canada ; Richard, Vincent, Segal Cancer Proteomics Centre, Jewish General Hospital, Montreal, QC, Canada; Kubiniok, Peter, Geib, Segal Cancer Proteomics Centre, Jewish General Hospital, Montreal, QC, Canada; Timon, Segal Cancer Proteomics Centre, Jewish General Hospital, Montreal, QC, Canada ; Borchers, Christoph, Segal Cancer Proteomics Centre, Jewish General Hospital, McGill University, Montreal, QC, Canada

Comprehensive characterization of the mouse brain proteome is essential for understanding the molecular mechanisms underlying development, function, aging, and disease. Mass spectrometry-based proteomics enables deep interrogation of complex biological systems, however, most workflows rely on relative quantitation, limiting cross-study comparability and reducing sensitivity to subtle biological differences. Here, we implement SysQuan, a stable-isotope tissue-based strategy for absolute quantitation of the mouse brain proteome.

SysQuan employs metabolically ¹³C-lysine-labelled mouse tissue as a global internal standard. Absolute protein concentrations are determined by calibrating labelled-to-endogenous peptide ratios with synthesized natural (NAT) peptide standards. Brain homogenates were processed using high-pH reverse-phase fractionation to maximize proteome depth, alongside streamlined unfractionated workflows to evaluate scalability and throughput. Samples were analyzed using both DDA and DIA-PASEF acquisition on a TimsTOF HT coupled to an Evosep One LC system. Data were processed using MSFragger, FragPipe, and DIA-NN. Experiments were performed using sex-segregated mouse brain tissue, covering the brainstem, cerebrum, and cerebellum. More than 10,000 proteins were identified in each brain region and approximately 80% of these were predicted to be quantifiable using the SysQuan approach, suggesting broad and robust proteome coverage across distinct anatomical regions.

Beyond the global proteome, we also extended our analysis to the phosphoproteome, given the central role of phosphorylation in regulating neuronal signaling, synaptic plasticity, and disease-associated signaling pathways. To date, several hundred phosphorylation sites have been confidently identified across brain regions, highlighting the feasibility of integrating post-translational modification profiling within the SysQuan framework.

► **P.A15 – Erik Gomez-Cardona**

Department of Pathology and Laboratory Medicine, University of British Columbia

Tracking Cell Surface Remodeling in Leukemia Through Advanced Proteomic Profiling

Nazri, Jehan; Garcia, Maria-Jose; Conrro, Agustina; Stockert, Fabian; Lange, Philipp.

Childhood leukemia remains the most common pediatric cancer, and the limited availability of selective cell surface markers continues to constrain the development of targeted therapies and minimally invasive biomarkers. The cell surface proteome represents an attractive source of disease-relevant targets; however, its comprehensive characterization is technically challenging, particularly for identifying glycosylation sites and proteolytic fragmentation events in cell surface proteins.

Here, we focus on the optimization of complementary proteomic workflows to improve the depth and robustness of cell surface profiling. We systematically evaluated an N-glycocapture strategy for the identification of glycosylation sites, optimizing key steps including surface labeling, enrichment efficiency, and peptide recovery to enhance sensitivity and reproducibility. In parallel, we optimize the HUNTER workflow, a novel N-terminal labeling strategy for improved detection of protease-generated protein N-termini, and the MS acquisition methods that enable broader coverage of cell surface fragmentation events.

These optimized approaches were applied in a pilot study to murine spleen and bone marrow samples to assess their performance in complex biological tissues and to generate an initial map of surface protein fragmentation. We utilize the EuRET mouse model, which recapitulates key features of B-cell leukemogenesis and enables the study of temporal alterations in the pre-leukemic stages preceding malignant transformation.

Overall, this work establishes robust and scalable methodologies for the characterization of cell surface proteins and their processing, providing a foundation for future discovery of biomarkers and therapeutic targets in leukemia.

► P.A16 – Gian Luca Negri

BC Cancer Research Centre Institute

Proteomic profiling identifies muscle-invasive bladder cancers with distinct biology and responses to platinum-based chemotherapy

Contreras-Sanz, A, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; Negri, G L, Canada's Michael Smith Genome Sciences Centre, BC Cancer Research Institute, University of British Columbia, Vancouver, BC, Canada; Reike, M J, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; Oo, H Z, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; Scuri, J M, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; Spencer, S E, Canada's Michael Smith Genome Sciences Centre, BC Cancer Research Institute, University of British Columbia, Vancouver, BC, Canada; Nielsen, K, Canada's Michael Smith Genome Sciences Centre, BC Cancer Research Institute, University of British Columbia, Vancouver, BC, Canada; Ikeda, K, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; Wang, G, Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; Jackson, C L, Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada; Gupta, S, Department of Oncology, The Cleveland Clinic, Cleveland, OH, USA; Roberts, M E, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; Berman, D M, Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada; Seiler, R, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; Department of BioMedical Research, University of Bern, Bern, Switzerland; Department of Urology, Hospital Center Biel, Biel, Switzerland; Morin, G B, Canada's Michael Smith Genome Sciences Centre, BC Cancer Research Institute, University of British Columbia, Vancouver, BC, Canada; Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; Black, P C, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada.

Muscle-invasive bladder cancer (MIBC) is a clinically aggressive malignancy for which platinum-based neoadjuvant chemotherapy (NAC) followed by radical cystectomy has been the standard of care for more than two decades. Even with the advent of new combinatory approaches, cisplatin-based chemotherapy will continue to be used regularly as a subsequent line of therapy. NAC response rates have remained modest and predictive biomarkers are lacking. In this study, we conducted comprehensive proteomic profiling of 107 MIBC tumors using mass spectrometry tandem mass tag on archival formalin-fixed paraffin-embedded tissue, analyzing samples both before and after NAC. Clustering of the pre-NAC proteome revealed four distinct molecular subtypes—Luminal, Nuclear, Basal, and Stroma-rich, each characterized by unique biological features, clinical outcomes, and response to therapy. Post-NAC analysis showed a general increase in extracellular matrix components and a decrease in keratinization, indicating a shift in tumor biology following chemotherapy. It also identified four proteomic clusters, only partially overlapping with the pre-NAC groups, demonstrating significant plasticity. Notably, certain post-NAC clusters were enriched for druggable proteins, such as mTOR and PARP in neuronal-like tumors, suggesting new avenues for targeted therapy. Intra-tumoral heterogeneity (ITH) was assessed using duplicate samples from 36 pre-NAC and 14 post-NAC tumors. ITH scores demonstrated that pre-NAC tumors with above-median heterogeneity correlated with poorer NAC response rates and survival outcomes. Collectively, this work delineates the proteomic landscape of MIBC in the context of NAC, identifies subtype-specific biomarkers and therapeutic targets, and emphasizes the prognostic significance of proteomic heterogeneity.

► P.A17 – Jaden Chen

University of Guelph

Proteomic Profiling of Antifungal Resistance in Clinical Isolates of *Cryptococcus Neoformans*

Chen, Jaden, University of Guelph; McAlister, Jason, University of Guelph; Woods, Michael, University of Guelph; Deyarmin, Jared, ThermoFisher Scientific; Samra, Stephanie, ThermoFisher Scientific; Geddes-McAlister, Jennifer, University of Guelph.

Introduction: Antimicrobial resistance, specifically antifungal resistance, is a global health burden. The human fungal pathogen *Cryptococcus neoformans* is an emerging health threat due to rapidly-developing resistance against fluconazole (FLC), the only antifungal widely-available in high-incidence areas of cryptococcal meningitis. Therefore, research on FLC resistance mechanisms is necessary and timely.

Methods: FLC-susceptible wild-type *C. neoformans* and six FLC-resistant strains were cultured in presence and absence of FLC. Minimum inhibitory concentrations and growth curves were performed. Cells were collected and proteins extracted at late-log phase, followed by peptide separation on the Vanquish Neo UHPLC system at 60 samples per day and analyzed on the Orbitrap Astral Zoom mass spectrometer using narrow window data-independent acquisition mode. Mass spectra were processed by library-free search using Spectronaut v20 with data analysis and visualization performed using Perseus.

Results: Stable baseline resistance markers were identified by comparing clinical isolates to wild-type in the absence of FLC. Within-strain comparison of FLC-treated/untreated samples revealed activated markers of resistance. In total, 5659 proteins were quantified, where known treatment- and resistance-associated proteins were detected, including drug efflux pumps and ergosterol biosynthesis pathway components. Gene Ontology by Biological Processes revealed higher abundance of proteins involved in carbohydrate metabolic processes in both baseline and activated resistance markers.

Conclusions: Divergent proteomic profiles of FLC resistance vs. susceptibility map to known and novel drug targets and propose a strategy to restore FLC susceptibility in *C. neoformans*. Future work includes validating roles of specific genes in driving resistance and exploring compound inhibitors for tailored therapeutic options.

► P.A18 – Maxim Berezovski

University of Ottawa

Surface Proteome of Extracellular Vesicles and Correlation Analysis Reveal Breast Cancer Biomarkers

Hüttmann, Nico, University of Ottawa; Li, Yingxi, University of Ottawa; Poolsup, Sutinee, University of Ottawa; Zaripov, Emil, University of Ottawa; D'Mello, Rochelle, University of Ottawa; Susevski, Vanessa, University of Ottawa; Minic, Zoran, University of Ottawa; Berezovski, Maxim V., University of Ottawa;

In this study, extracellular vesicles (EV) proteins from small EVs (sEVs) and medium EVs (mEVs) were isolated from breast cancer (BC) MDA-MB-231 and MCF7 and non-cancerous breast epithelial MCF10A cell lines and then analyzed by two approaches: global proteomic analysis and enrichment of EV surface proteins by Sulfo-NHS-SS-Biotin labeling. From the first approach,

proteomic profiling identified 2459 proteins, which were subjected to comparative analysis and correlation network analysis. Twelve potential biomarker proteins were identified based on cell line-specific expression and filtered by their predicted co-localization with known EV marker proteins, CD63, CD9, and CD81. This approach resulted in the identification of 11 proteins, four of which were further investigated by Western blot analysis. The presence of transmembrane serine protease matrilysin (ST14), claudin-3 (CLDN3), and integrin alpha-7 (ITGA7) in each cell line was validated by Western blot, revealing that ST14 and CLDN3 may be further explored as potential EV biomarkers for BC. The surface labeling approach enriched proteins that were not identified using the first approach. Ten potential BC biomarkers (Glutathione S-transferase P1 (GSTP1), Elongation factor 2 (EEF2), DEAD/H box RNA helicase (DDX10), progesterone receptor (PGR), Ras-related C3 botulinum toxin substrate 2 (RAC2), Disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), Aconitase 2 (ACO2), UTP20 small subunit processome component (UTP20), NEDD4 binding protein 2 (N4BP2), Programmed cell death 6 (PDCD6)) were selected from surface proteins commonly identified from MDA-MB-231 and MCF7, but not identified in MCF10A EVs. In total, 846 surface proteins were identified from the second approach.

► P.A19 – Michael Woods

University of Guelph

High-throughput drug screening combined with Proteome Integral Solubility Alteration defines mechanisms of action for novel antifungal compounds against *Cryptococcus neoformans*

Michael J P Woods; Davier Gutierrez-gongora; Mary Adofo; Chelsea Reitzel; Jason A McAlister; Jonathan Sayewich; Jared Deyarmin; Stephanie N. Samra; J Patrick Murphy; Jennifer Geddes-McAlister

The global impact of fungal disease is a growing threat to human health. Specifically, invasive fungal species cause serious systemic infections that are difficult to diagnose and treat, resulting in high mortality rates. Among these is *Cryptococcus neoformans*, which causes life-threatening cryptococcal meningitis in immunocompromised individuals. Treatment options for cryptococcal disease are limited, especially in low resource regions, where the most effective therapy is replaced by suboptimal fluconazole monotherapy. The extensive clinical use of fluconazole, together with cross-resistance arising from environmental azole agrochemicals, has contributed to a growing prevalence of antifungal-resistant isolates, undermining treatment efficacy. New antifungals are needed against diverse fungal cellular targets with mechanisms of action to increase the diversity of treatment options, as well as discovering strategies to target azole-resistant strains. Here, the Proteome Integral Solubility Alteration (PISA) assay was optimized to identify antifungal targets and mechanisms of action of compounds that inhibit fluconazole-resistant *C. neoformans* growth. The proof-of-principle PISA experiment with the antifungal agent, fluconazole, confirmed the known target enzyme, Erg11, and downstream impacts on the ergosterol biosynthesis pathway. A chemical library screen of 2500 compounds against fluconazole-resistant *C. neoformans* identified the anthelmintic drug mebendazole to have potent inhibitory effects on growth. Through PISA, cell cycle regulating proteins Spo12 and Cep57 domain-containing proteins were identified as potential targets of mebendazole, in addition to downstream effects in proteins involved in G2/M phase regulation. Phenotypic assays revealed impaired daughter cell budding, indicating the mechanism of action for mebendazole involves targeting the late stages of the cell cycle.

► P.A20 – Sparsh Makhaik

University of Alberta

Discovery and clinical validation of plasma biomarkers in primary progressive multiple sclerosis using mass spectrometry.

Plemel, Jason, University of Alberta; Power, Christopher, University of Alberta; Camara-Lemarray, Carlos, University of Calgary; Bernardo Alvarez, University of Alberta; Fahlan, Richard, University of Alberta; Olivier, Julien, University of Alberta

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system that causes demyelination resulting in progressive disability, affecting almost 90,000 people in Canada. Primary Progressive Multiple Sclerosis (PPMS) is a distinct clinical subtype of MS characterized by steady progression of neurological decline from disease onset, without clearly defined relapses or remissions. This absence of relapses makes early-stage diagnosis of PPMS particularly challenging. To address this, we developed mass spectrometry-based proteomic approaches to identify plasma protein biomarkers for PPMS diagnosis in clinical settings. Given the high complexity of plasma and the abundance of background proteins such as albumin, nanobead-based protein enrichment was employed to enhance proteomic coverage. This enabled the detection of 4278 proteins in plasma samples from a patient cohort including those with Primary Progressive Multiple Sclerosis (PPMS). Using this approach, we identified a panel of eight proteins that were significantly upregulated in PPMS patients. Interestingly, these markers also effectively distinguished PPMS from relapsing-remitting multiple sclerosis (RRMS). We now aim to validate these candidate biomarkers using targeted proteomic strategies like parallel reaction monitoring (PRM) to assess their potential for clinical application with high specificity and reproducibility.

► P.A21 – Zoran Minic

University of Ottawa

Proteomics Approaches in Discovery of Potential Enzymatic Biomarkers for Early Diagnosis of Breast Cancer

Zoran MINIC, John L. Holmes Mass Spectrometry Facility, Faculty of Science, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5, E-mail: zminic@uottawa.ca

Breast cancer (BC) is one of the leading causes of death in Canadian women, with an average survival rate of 5 years after diagnosis. Early detection of BC can greatly improve patient outcomes and survival. However, a non-invasive BC detection method is not contemporarily available in clinics. Recent studies suggest that proteins in small extracellular vesicles (sEVs) could be promising biomarkers for non-invasive early-stage BC diagnosis. sEVs are membrane-enclosed vesicles secreted by cells,

which drive different stages of carcinogenesis in BC. For proteomics analyses, sEVs were derived from different metastatic BC cell lines and a non-cancerous epithelial breast cell line. The results were generated from three proteomics approaches: quantitative proteomics, phosphoproteomics, and protein acetylation analysis. Enzymes with high abundances in cancerous cell lines were extracted from the quantitative proteomic data. Similarly, phosphorylated and acetylated enzymes present only in the cancer cell lines were extracted. Among these approaches, we proposed a list of enzymes, including their metabolic pathways, that can be explored as potential BC biomarkers. Some of these phosphorylated and acetylated enzymes were validated, showing higher specific enzymatic activity in sEVs isolated from MCF7 (estrogen and progesterone receptor-positive, metastatic) and MDA-MB-231 (triple-negative, highly metastatic) when compared to MCF10A (non-metastatic) cell lines. Future validation of enzymes using both cancer cell lines and blood from BC patients remains to be determined.

▶ P.A22 – Zoran Minic

John L Holmes Mass Spectrometry Facility, University of Ottawa

Application of proteomics to unveil molecular-level disturbances in nanomaterial exposed Zebrafish embryo

Kumarathasan Premkumari^{1,2}, Thomas Jith³, Blais Erica¹, Syama Krishna Priya¹, Nazemof Nazila², Minic Zoran⁴, Li Yingxi⁴, Khraibah Abdullah ⁴, Subedi Sanjeena⁵, Mennigen Jan⁶, Nipu Niepukolie⁴, Kessen Patten⁷

¹Environmental Health Science and Research Bureau, HECSB, Health Canada, Ottawa, ON; ²Interdisciplinary School of Health Sciences, Faculty of Health Sciences, University of Ottawa, Ottawa, ON; ³Regulatory Toxicology Research Division, Health Product and Food Branch, Health Canada, Ottawa, ON; ⁴John L Holmes Mass Spectrometry Facility, University of Ottawa, Ottawa, ON; ⁵School of Mathematics and Statistics, Carleton University, Ottawa, ON; ⁶Department of Biology, University of Ottawa, Ottawa, ON; ⁷INRS, Centre Armand-Frappier Santé Biotechnologie, Laval, QC

Utility of proteomic methodologies in chemical toxicology is evolving. Notably, expression proteomics and affinity proteomics (e.g., interaction) are approaches used in toxicity testing. Here, we used expression proteomics to examine molecular-level perturbations in Zebrafish embryos exposed to nanomaterials (NM) to refine the utility of this experimental model as alternative in vivo NM toxicity testing platform. Zebrafish exhibits similarities with human genome notably, developmental. Application of NMs in electronics, consumer products (e.g., cosmetics, food-additive/packaging) and biomedicine is expanding because of attractive tunable properties. Surge in production/use of NMs in recent years cause health concerns due to exposure potential requiring toxicity testing for risk analysis. Thus, Zebrafish embryos (chorionated) were exposed to well-characterized NMs (nanoSiO₂, TiO₂, ZnO) in E3 medium (dose: 0-100 µg/mL; 24 h-4 dpf). Chorionated, de-chorionated embryos were exposed to tris(2-butoxyethyl)phosphate to examine impact of chorion on exposure-related effects. Phenotypic changes were assessed and Zebrafish embryo proteomic changes were analyzed using LC-Orbitrap MS/MS method, post-lysis, clarification, reduction/alkylation and enzymatic-digestion. Chorionated and de-chorionated embryos exhibited similar survival rates, yet protein-level differences were observed. NM-specific effects were seen in morphology, survival, hatching rates for nanoZnO exposure. Decreased locomotion was noticed for nanoZnO, with trends for nanoSiO₂. NM type-, dose-, developmental stage-specific protein expressions demonstrated sensitivity/specificity of analysis. Mitochondrial effects (e.g., SOD2) pointed to oxidative stress. Enriched pathways included protein processing, mitophagy, metabolism, cellular senescence providing insight into toxicity mechanisms. Our findings suggest that the Zebrafish embryo model in combination with proteomics is promising for NM toxicity testing and warrants further exploration.

▶ P.A23 – Ji-Young Youn

Hospital for Sick Children

PRRC2A, PRRC2B and PRRC2C are Stress Granule Proteins that Promote Translation Initiation Through Association with the eIF3 complex Stress Granule Functions in Translation Control and Post-Transcriptional Regulation

Jie Qi Huang, Eileigh Kadijk, Karl J. Schreiber, Zi Hao Liu, Rachel W. Chiang, Zhixin Zhang, Kevin Guttman, Tian Hao Huang, Sylvia M.T. Almeida, Olena Zhulyin, Alan Moses, Julie D. Forman-Kay, John L. Rubinstein, Ji-Young Youn

Regulation of mRNA translation is essential for cellular homeostasis, and its dysregulation contributes to cancer, neurodegeneration, and developmental disorders. Stress granules are cytosolic condensates that form during stress-induced translation arrest and are enriched in mRNAs, translation factors, and RNA-binding proteins. However, how stress granule proteins modulate translation remains poorly understood. Here, we identify the stress granule components Proline-Rich Coiled-Coil A, B, and C (PRRC2 proteins) as translation regulators. PRRC2 proteins are large, intrinsically disordered paralogs conserved across jawed vertebrates. Functional proteomics revealed that all PRRC2 proteins associate with the 48S translation initiation complex (PIC), whereas PRRC2B additionally interacts with nuclear proteins. Under stress, the proximal interaction network of PRRC2 proteins undergoes dynamic remodeling, including increased interactions with the stress granule scaffold G3BP1. Genetic perturbation shows that the PRRC2 proteins influence stress granule assembly in a context-specific manner and are collectively required for cell growth in basal conditions. Cells with reduced PRRC2 proteins exhibit a significant reduction in the abundance of more than half of the proteome, with a bias toward translational targets of eIF3d and eIF4G2. Interaction domain mapping and AlphaFold3 modeling revealed that an alpha helix within the putative coiled-coil domain of PRRC2C mediates interactions with the eIF3 core complex. This modeling places the PRRC2C alpha helix in a previously unassigned region of a published cryo-EM density map, validating the protein interaction and the mechanistic role of PRRC2C in translation control. Together, these findings establish PRRC2 proteins as components of the translation initiation machinery that regulate translation through their

▶ P.A24 – Sandra Spencer

BC Cancer

Standardization and Validation of Pan-Canadian High Throughput Untargeted Proteomics from Formalin Fixed Paraffin Embedded Tissues

Spencer, Sandra E., BC Cancer Research Institute, Department of Basic and Translational Research, Vancouver, BC, Canada; Hughes, Christopher, Dalhousie University, Faculty of Medicine, Halifax, NS, Canada; Negri, Gian Luca, BC Cancer Research Institute, Department of Basic and Translational Research, Vancouver, BC, Canada; Asleh, Karama, Dalhousie University, Department of Pathology, and Immunology, Halifax, NS, Canada; Cheng, S.-W. Grace, BC Cancer Research Institute, Department of Basic and Translational Research, Vancouver, BC, Canada; Marcato, Paola, Dalhousie University, Department of Pathology, and Immunology, Halifax, NS, Canada; Morin, Gregg B., BC Cancer Research Institute, Department of Basic and Translational Research, Vancouver, BC, Canada, University of British Columbia, Department of Medical Genetics, Vancouver BC, Canada

Untargeted mass-spectrometry is an attractive technique for analysis of archival clinical samples due to the specificity and hypothesis-generating nature of the approach. A lack of proper process controls and poor intra-/inter-laboratory reproducibility has hampered standardization of untargeted proteomics in clinical settings. To address this issue, we are part of an effort to establish an accessible, highly standardized workflow for the analysis of protein from formalin fixed, paraffin embedded (FFPE) breast cancer nationwide for personalized medicine. Consideration was taken to minimize sample handling and reduce the potential for variability between sites and this workflow is being independently validated in British Columbia and Nova Scotia to evaluate the intra- and inter-laboratory reproducibility.

Protein extraction is one of the most variable steps of FFPE tissue preparation for proteomics due to tissue transfer and paraffin removal. We evaluated common methods for deparaffinization and extraction of protein from FFPE tissue and have designed a workflow that combines these steps, eliminating the need for tissue transfer or wax removal. MDA-MB-231 cells were prepared as a FFPE standard to validate the workflow. Peptide standards for known breast cancer targets were used to determine the reproducibility, repeatability, linearity, and LOD/LOQ of the technique as per CPATC guidelines for targeted assay validation. Assay variability is measured by evaluating the impact of matrix, assessing stability during storage, and reproducibility is assessed by a "5x5". Ultimately, we are developing a workflow for quantitative proteomics of archival tissue that is accessible and reproducible nationwide.

▶ P.A25 – Tyler Cooper

Université de Montréal

A Microenvironment to Biofluid Pipeline for Ovarian Cancer Biomarker Discovery

Blanchet, Sophie-Anne, CR-CHUM; Valence, Alexane, Université de Montréal; Cooper, Tyler T, CR-CHUM, Université de Montréal

Early detection and disease stratification remain major challenges in ovarian cancer, particularly for aggressive and understudied subtypes such as high-grade serous and mucinous carcinomas. My research program aims to address this gap by developing a proteomics-driven pipeline that systematically links tumor biology within the microenvironment to clinically actionable biomarkers detectable in biofluids. This framework integrates mechanistic studies in physiologically relevant models with deep proteomic profiling of extracellular vesicles (EVs), the secretome, and tumor tissue. Using cell line models that recapitulate key microenvironmental stressors, including hypoxia and nutrient deprivation, we define how cancer cells remodel their proteome and secretory output. These discoveries are translated into biofluid analyses through optimized EV isolation workflows compatible with low-volume clinical samples, enabling high-resolution proteomic interrogation of plasma and ascites.

To enhance biological and clinical relevance, candidate biomarkers identified in vitro are cross-validated in patient-derived samples and tumor tissues using orthogonal approaches, including targeted proteomics, immunohistochemistry, and machine learning classification models. This microenvironment-to-biofluid strategy enables the identification of protein signatures associated with disease stage, progression, and therapeutic response. By bridging discovery biology with translational proteomics, this program establishes a scalable and clinically adaptable framework for biomarker development. Ultimately, this work aims to improve early detection, refine disease classification, and support precision medicine approaches in ovarian cancer.

▶ P.A26 – Ronan Houssin-Guitière

Centre de recherche du CHU de Québec – Université Laval, Québec, Canada

Identification of the interactome of human herpesvirus 6 (HHV-6) IE1 protein using a proximity-dependent biotinylation approach

Andréanne Blondeau, Centre de recherche du CHU de Québec – Université Laval, Québec, Canada ; Annie Gravel, Centre de recherche du CHU de Québec – Université Laval, Québec, Canada ; Louis Flamand, Centre de recherche du CHU de Québec – Université Laval, Québec, Canada, Department of Microbiology and Infectiology, Faculty of Medicine, Université Laval, Québec, Canada ; Jean-Philippe Lambert, Centre de recherche du CHU de Québec – Université Laval, Québec, Canada, Department of Molecular Medicine, Faculty of Medicine, Université Laval, Québec City, Canada ; Amélie Fradet-Turcotte, Centre de recherche du CHU de Québec – Université Laval, Québec, Canada, Department of Molecular Biology, Medical Biochemistry and Pathology, Faculty of Medicine, Université Laval, Québec City, Canada

Human herpesvirus 6B (HHV-6B) infects 90% of the population and integrates into the telomeres of human chromosomes, where it becomes latent. When the virus integrates into germ cells, it can be inherited vertically, a condition known as chromosomally-integrated HHV-6B (iciHHV-6B). Recent data have shown that iciHHV-6B is the second most important risk factor associated with the development of basal cell carcinoma and that the expression of the viral protein IE1 induces genomic instability in infected cells. Biochemical and cellular approaches have demonstrated that the C-terminal domain of IE1 (called the ATM inhibitor domain, ATMiD) inhibits the activation of the effector kinase ATM and the subsequent activation of DNA repair pathways, suggesting that IE1 contributes to the emergence of genomic mutations that contribute to the development of cancers.

This project aims to determine the molecular mechanism by which IE1 inhibits ATM activation. Since IE1 does not interact directly with the kinase, we hypothesize that a cellular factor enables it to perform this function. In order to identify the IE1 interactome necessary for ATM inhibition, stable cell lines expressing different IE1 domains fused to TurboID biotin ligase were established in UW-BCC1, a cell line isolated from basal cell carcinoma. Under these conditions, TurboID-IE1 inhibits DNA damage signalling, confirming that the protein is functional. Identifying the IE1 interactome dependent on its ATMiD domain will allow us to better understand the mechanisms used by the virus to divert the cellular response to double-strand DNA breaks.

P.B01 – Eileen Tudorica

Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada; Division of Experimental Medicine, McGill University, Montreal, QC, Canada

Comparison of Analytical Methods for Absolute Quantitation of Synthetic Peptides

Kubiniok, Peter, Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada, Quantivum Inc, Montreal, QC, Canada; Trawka, Maria, MRM Proteomics Inc, Montreal, QC, Canada; Petrochenko, Evgeniy V., Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada; Shanker, Adeline, MRM Proteomics Inc, Montreal, QC, Canada; Borchers, Christoph H., Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada, Division of Experimental Medicine, McGill University, Montreal, QC, Canada, Gerald Bronfman Department of Oncology, Jewish General Hospital, McGill University, Montreal, QC, Canada, Department of Pathology, McGill University, Montreal, QC, Canada

Accurate peptide quantitation is essential for bottom-up quantitative proteomics. Amino acid analysis (AAA) is the gold standard for absolute quantitation of synthetic peptides; but it is laborious and subject to errors from poor sample purity, long peptide sequences, and variability in amino acid sequence-specific cleavage efficiencies. Three additional approaches for absolute peptide quantitation were compared: 214 nm UV-HPLC, fluorescamine fluorescence assay (Ex/Em 355/460 nm), and dansyl chloride (DNSCI) 330 nm UV-HPLC-MS. A panel of 600 HPLC-purified and MS-confirmed synthetic peptides were analyzed. AAA was performed by LC-MRM-MS following 18-hour acid hydrolysis. The 214 nm UV-HPLC method quantified peptides using absorbance peak areas (mAU*min) and sequence-derived molar extinction coefficients. The fluorescamine assay enabled 30-minute quantitation in 96-well microplate format using calibration curves ($R^2 > 0.99$). DNSCI-UV-HPLC-MS provided simultaneous UV and MS detection for quantitation of derivatized peptides, but chromatographic analysis of each sample resulted in 12-hour processing time. Due to its comparably labor-intensive workflow and limited high-throughput capacity, this method was not included in the full sample correlation analysis and was instead reserved for problematic samples. Pairwise correlation analyses using an 85% purity cutoff demonstrated strong agreement among methods. The highest correlation was seen between AAA and UV-HPLC ($R=0.95$), then AAA and fluorescence ($R=0.87$), and fluorescence and UV-HPLC ($R=0.84$). These results demonstrate that a combination of 214 nm UV-HPLC and fluorescamine fluorescence assays can be used as an efficient alternative to AAA in high-purity samples, and DNSCI-UV-HPLC-MS as a confirmatory assay for complicated and impure samples.

P.B02 – Gwendoline Marbach

Department of Immunology and Cellular Biology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, QC, Canada.

Targeted mass spectrometry for detecting the low-abundance ubiquitin variant UbKEKS

Marbach, Gwendoline, Department of Immunology and Cellular Biology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, QC, Canada; Raisch, Jennifer, Department of Immunology and Cellular Biology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, QC, Canada; Lévesque, Dominique, Department of Immunology and Cellular Biology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, QC, Canada; Haroune, Louÿs, Department of Chemistry, Faculty of Medicine and Health Sciences, Université de Sherbrooke, QC, Canada; Boisvert François-Michel, Department of Immunology and Cellular Biology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, QC, Canada;

Pseudogenes have long been associated with non-canonical proteins. However, studies in transcriptomics and proteomics have demonstrated that a subset of pseudogenes is transcribed and translated into functional proteins. Among them, ubiquitin pseudogenes have biological functions are largely unknown. Here, we investigate UBB pseudogene 4, which encodes an atypical ubiquitin variant named UbKEKS. Unlike canonical ubiquitin, UbKEKS does not appear to target substrates for proteasomal degradation, highlighting the interest to better characterize its cellular function.

The detection of low-abundance pseudogenes, like UbKEKS, remains technically challenging and requires highly sensitive and specific strategies. This research developed and compared proteomics approaches based on parallel reaction monitoring and multiple reaction monitoring to detect and quantify UbKEKS in cells. To optimize UbKEKS detection and recovery by mass spectrometry, protein extraction strategies were tested in U2OSWT and UbKEKS Knock-Out (KO) cell lines. Proteotypic peptides were selected by in silico prediction and experimental validation. The impact of lysis conditions on protein extraction efficiency and peptide recovery was systematically assessed.

Optimization of extraction strategies significantly enhanced peptide recovery and signal intensity, enabling robust and reproducible detection of UbKEKS specific peptides in WT cell extracts, while no signal was detected in KO cells, confirming assay specificity. Absolute quantification using calibration curves generated from serial dilutions of synthetic peptides enabled accurate measurement of UbKEKS abundance. Both PRM and MRM approaches demonstrated high sensitivity and reproducibility.

This work establishes a highly optimized proteomics strategies for detecting and quantifying low-abundance protein variants. These approaches will facilitate investigations into cellular functions of pseudogenes.

P.B03 – Mukhayyo Sultonova

Department of Biology, University of Prince Edward Island

Integrating hydrophobic interaction chromatography with TMT-based quantitative proteomics for systematic mapping of metabolite-protein interactions.

Paulo, J.A., Department of Cell Biology, Harvard Medical School; Murphy, J.P., Department of Biology, University of Prince Edward Island
Metabolite-protein interactions underpin fundamental biochemical processes, yet the metabolite-protein

interactome remains insufficiently mapped, in part due to limitations in current profiling methods. Several proteome-wide approaches detect ligand-protein interactions by perturbing proteome stability using heat, chemical denaturation, or proteolysis gradients. While powerful, these strategies may miss weak, transient, or non-stability altering interactions, leaving gaps in our understanding of metabolite function. Complementary approaches are therefore needed to expand the detectable landscape of metabolite-protein interactions.

Here, we establish a proof-of-principal quantitative proteomics workflow integrating hydrophobic interaction chromatography (HIC) with TMT-based mass spectrometry to detect ligand-induced changes in protein surface hydrophobicity. HIC separates proteins under native conditions based on their hydrophobicity, providing an orthogonal readout of ligand binding that has not been systematically applied to global interaction mapping. We first validated the workflow using the well-characterized methotrexate-dihydrofolate reductase (MTX-DHFR) interaction in HEK293T lysate. Both quantitative proteomics and western blotting validations confirmed a reproducible HIC retention shift for DHFR upon MTX treatment, while non-target proteins including ACTB and GAPDH showed no change. We next applied this workflow to map interactions with metabolite NAD⁺. HIC fractions were analyzed using two sets of 18-plex TMTpro experiments, enabling quantifications of 3720 proteins across both bridged MS runs. Analysis of elution profiles identified 184 proteins with ≥ 1 min retention shifts including known NAD-binding proteins such as UDP-glucose 4-epimerase (GALE) and 15 previously annotated NAD-binding proteins.

Together, these results demonstrate that HIC-based quantitative proteomics provides a complementary strategy for systematic mapping of metabolite-protein interactions while preserving proteins in their native state.

P.B04 – Andrei Drabovich

Department of Laboratory Medicine & Pathology, University of Alberta

Proteome-Wide Serology for Viral Diagnostics and the Discovery of High-Affinity Human Antibodies

Zoe Turner, Yasmine Rais, Weize Tang, and Andrei Drabovich

Introduction: Human serum antibodies comprise a highly diverse mixture of isotypes, subclasses, and clonotypes that collectively shape the immune response. Informative sequencing of polyclonal antibodies remains a major challenge due to the lack of universal sequence-matching databases. Novel assays for measuring antigen-specific antibodies have important practical implications for serological diagnostics of infectious diseases. Here, we developed a conceptually new approach of Proteome-Wide Serology aimed at characterization of the full-scale depth and diversity of polyclonal antibody response and rational design of serology diagnostics.

Methods: High-throughput antibody enrichments using recombinant viral antigens were followed by untargeted and targeted proteomic assays. Data-independent acquisition (timsTOF Ultra 2) enabled discovery and label-free quantification of antibody isotypes, variable chain regions, and interactomes. A rapid targeted assay with carefully designed internal standards (OTRAP 6500+) provided absolute quantification of all 11 antibody isotypes and subclasses with a throughput suitable for clinical applications (120 samples/day).

Results: Proteome-Wide Serology was validated with over 20 recombinant antigens from Influenza A, RSV, SARS-CoV-2, and their variants. Blood serum samples from over 500 individuals were analyzed. Antigen-specific IgG1 ($\sim 1-3 \mu\text{g/mL}$) were elevated across all viral infections, while RSV elicited a distinct immune response with elevated IgG4 and IgD. Repertoire profiling of heavy-chain variable regions revealed patient-specific patterns and frequent use of only a small subset of IGHV genes that defined the major 3D scaffolds of antibody clones.

Conclusions: Proteome-Wide Serology facilitates rational design of viral diagnostics and characterization of polyclonal antibody mixtures for selection and sequencing of high-affinity clones directly from patient samples.

P.B05 – Cameron Ellis

PreOmics Inc., Billerica, MA, USA

A versatile enrichment workflow for in-depth biomarker discovery across anticoagulants, species, and biofluids

Limm, Katharina, PreOmics GmbH, Martinsried, Germany; Wurzenberger, Xaver, PreOmics GmbH, Martinsried, Germany; Hartinger, Katrin, PreOmics GmbH, Martinsried, Germany; Kulak, Nils A, PreOmics GmbH, Martinsried, Germany

Biofluid proteomics is a powerful route to biomarker discovery, but its broad use is limited by the high dynamic range of protein concentrations and variability across matrices, species, and collection conditions. A workflow that performs robustly under these diverse conditions is therefore highly valuable for translational and preclinical studies. Here, we demonstrate the versatility of ENRICH-IST across anticoagulants, species, and biofluids, and highlight its value for biomarker discovery.

ENRICH technology increases proteome depth by enriching low-abundance proteins from biofluids on paramagnetic beads. This step is followed by the IST-BCT workflow for standard sample preparation prior to nLC-MS/MS analysis in diPASEF®. The ENRICH-IST was evaluated in human EDTA plasma, citrated plasma, and serum, as well as mouse and rat plasma and mouse CSF. A pilot study in human lung cancer plasma was included as a showcase application. Data was processed either in ProteoScapeTM or Spectronaut® 17.

Across human EDTA plasma, citrated plasma, and serum, ENRICH-IST increased proteome depth relative to neat samples, yielding at least 1.5-fold more protein identifications while maintaining strong repeatability (CVs <10%). The workflow also proved species-independent, enhancing proteome coverage in both mouse and rat plasma. In CSF, increasing input volume further improved depth, achieving ~ 1.7 -fold more protein identifications

at 200 μ L versus neat samples. In the lung cancer pilot study, ENRICH-iST improved group stratification and revealed additional significantly regulated proteins compared with neat plasma. Together, these results demonstrate that ENRICH-iST is a robust and versatile workflow for in-depth biomarker discovery across anticoagulants, species, and challenging biofluids.

► **P.B06 – Adeline Shanker**
MRM Proteomics Inc.

SysQuan Enables Affordable and Comprehensive Tissue-Specific Absolute Quantification of the Human Proteome

Borchers, Christoph H., Warren Y. Soper Clinical Proteomics Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec ; Geib, Timon, Segal Cancer Proteomics Centre, Lady Davis Institute for Medical Research ; Logerot, Elodie, Lady Davis Institute of Medical Research, Jewish General Hospital, Montreal, Quebec ; Kubiniok, Peter, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec ; Spicer, Victor, Evosep Biosystems, Odense, Denmark ; Stoychev, Stoyan, Evosep Biosystems, Odense, Denmark ; Bekker-Jensen, Dorte, Evosep Biosystems, Odense, Denmark, Bache, Nicolai, Evosep Biosystems, Odense, Denmark ; Popp, Robert, MRM Proteomics Inc., Montreal, Canada ; Zahedi, René P., University of Manitoba, Manitoba, Canada

Despite major technological advances, quantitative proteomics remains hindered by matrix and batch effects, complex workflows, and lengthy chromatographic separations, limiting detection of biologically important changes. Cross-laboratory reproducibility is poor, and the high-cost and limited availability of stable isotope-labeled (SIL) standards has prevented widespread adoption of absolute quantification. SysQuan addresses these barriers by leveraging SIL-mice as universal internal standards for absolute quantification of the human proteome. C57BL/6 mice were fed a 13C-lysine-enriched diet to generate SIL reference material. Human plasma and tissues, as well as SIL mouse plasma and tissues, were lysed in SDS buffer and mixed 1:1 based on protein concentration. Following S-Trap digestion, targeted analyses were performed using dynamic-MRM on an Agilent 6495D triple quadrupole coupled to an Evosep One LC-system. Untargeted analyses employed a timsTOF-HT and an Orbitrap Exploris 480, both interfaced with an Evosep One. Mixed human/SIL mouse plasma and tissue digests analyzed by 2D-LC-MS/MS yielded >1,470 human plasma proteins quantifiable from >5,000 co-detected SIL peptide counterparts. In kidney, liver, and lung, 9,830, 7,680, and 9,620 proteins were identified with >66,800, >51,400, and >1,900 paired light/SIL peptides, respectively. Using synthetic light peptides, we developed >2,650 MRM assays for kidney and liver, and >2,580 and 1,786 assays for lung and plasma. Leveraging light peptide standards, we are performing reverse quantification of thousands of SIL mouse proteins with validation following CPTAC guidelines. Targeted MRM analysis of neat plasma enables simultaneous detection of ~700 light/SIL peptide pairs, supporting quantification of >400 plasma proteins within a one-hour LC-MS analysis.

► **P.B07 – Brenna Ing**
Department of Biology, University of Prince Edward Island

Profiling interactions between blueberry phytochemicals and the human proteome

Ing, Brenna, Department of Biology, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada, C1A 4P3; Paulo, Joao, Department of Cell Biology, Harvard Medical School, Boston, MA, 02115; McCallum, Jason, Agriculture and Agri-Food Canada, Charlottetown Research Center, C1A 4N6; Murphy, J. Patrick, Department of Biology, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada, C1A 4P3, Department of Pathology, Dalhousie University, Nova Scotia, BH3 4R2.

Phytochemicals, naturally occurring secondary compounds in fruits and leafy vegetables, are known for their beneficial effects on human health, including anti-inflammatory, antioxidant, and anticancer properties. However, their targets and inherent mechanisms of action are poorly understood, limiting their pharmacological potential. The Proteome Integral Solubility Alteration (PISA) assay uses quantitative proteomics to identify global changes in protein thermal stability imparted by the presence of the ligand of interest, potentially providing an opportunity to identify targets of phytochemicals. We assessed the PISA assay's ability to identify protein thermal shifts from blueberry extracts (abundant in phytochemicals) and observed significant thermal shifts in 455 human proteins (p -value <0.05, log₂ fold change 2), including peptidyl-prolyl cis-trans isomerase (PIN1). PIN1 is a cell signaling protein that changes the conformation of phosphorylated proteins and is involved in various cellular processes, including cell cycle progression, and is being explored as a potential therapeutic target for several diseases. These data suggest PIN1 may be a mechanistic link between phytochemicals and cell phenotypes. To interrogate the specific phytochemicals interacting with PIN1, anthocyanins from the extract were separated from other blueberry phytochemicals using liquid phase extraction, followed by cellular thermal shift assay (CETSA) analysis. We found that anthocyanins commonly interact with PIN1, which was further supported by molecular docking. These results show that PISA may be used to detect phytochemical protein interactions from whole extracts, which may then be validated by fractionation, highlighting a new strategy for identifying protein targets from components of complex natural extracts.

► **P.B08 – Carla-Marie Jurkovic**
Department of Immunology and Cell Biology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, QC, Canada

Time-resolved proximity proteomics reveals a non-canonical role for ANP32A in heterochromatin-dependent DNA repair and replication stress adaptation

Carla-Marie, Jurkovic¹, Department of Immunology and Cell Biology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, QC, Canada; Jennifer, Raisch, Department of Immunology and Cell Biology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, QC, Canada; Dominique, Lévesque, Department of Immunology and Cell Biology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, QC, Canada; François-Michel, Boisvert, Department of Immunology and Cell Biology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, QC, Canada

DNA replication is vulnerable to stress that can stall replication forks, activate DNA damage responses, and ultimately drive cancer progression. Using a large-scale interactome mapping approach, we profiled 17 replication fork-associated protein interactomes under basal and hydroxyurea (HU)-induced stress conditions. ANP32A emerged as a differentially modulated factor at stalled forks, suggesting a previously unrecognized role for ANP32A in replication stress regulation beyond its known function in viral replication.

To investigate the role of ANP32A in replication fork protection and DNA repair, stable U2OS cell lines expressing ANP32A fused

to the AirID proximity labeling system were generated to characterize its proximity interactome under basal conditions and following 24h HU treatment. Quantitative mass spectrometry revealed that ANP32A associates with proteins involved in DNA replication, chromatin remodeling, histone modification, DNA repair, and cell cycle regulation.

To further define the timing of ANP32A function during the DNA damage response, we performed kinetic proximity proteomics at multiple time points following HU treatment. This temporal analysis uncovered a dynamic remodeling of the ANP32A proteome, highlighting stage-specific recruitment of replication stress sensors, checkpoint regulators, and chromatin-associated repair factors demonstrating how ANP32A coordinates replication fork adaptation and stress responses over time.

These findings demonstrate that ANP32A acts as a temporally regulated modulator of replication fork stability. Kinetics proximity proteomics provides novel insight into the temporal organization of the DNA damage response and identifies ANP32A as a key factor in replication stress management. Our results suggest that ANP32A may represent a potential therapeutic target to enhance cancer cell sensitivity

► **P.B09 – Danielle Simons**
McGill University

Functional characterization of pathogenic ataxia mutations in the mitochondrial processing peptidase

Qin, Sibeil, McGill University; Thibodeaux, Christopher, McGill University; Jean-François, Trempe, McGill University

Mitochondria are multifaceted organelles that regulate energy metabolism, apoptosis, immunity, and other cellular processes. Although mitochondria possess their own genome, most mitochondrial proteins are nuclear encoded, synthesized in the cytosol, and imported via N-terminal mitochondrial targeting sequences (MTSS). In the matrix the mitochondrial processing peptidase (MPP) cleaves these MTSS to enable proper protein folding and localization. MPP is a heterodimer, composed of MPP (encoded by PMPCA), responsible for substrate recognition, and MPP β (encoded by PMPCB), a Zn²⁺ metalloprotease responsible for MTS cleavage. In humans, mutations in PMPCA cause autosomal recessive ataxias, while PMPCB mutations result in childhood neurodegeneration associated with multiple mitochondrial dysfunction syndrome. Current knowledge of MPP is primarily derived from yeast studies (Mas1/2), which have clarified the cleavage mechanism but are limited by low homology with human MPP (37% for MPP α and 44% for MPP β). This project aims to define the structural and functional consequences of disease-causing mutations in human MPP. We have generated PMPCA mutations associated with spinocerebellar ataxia, autosomal recessive 2 (SCAR2) for recombinant expression in *E. coli* and assessed protein yield relative to wild type. Functional effects on MTS processing were evaluated using an in vitro FRET assay, while mass photometry will be used to assess dimerization. Preliminary results indicate most mutants exhibit low yield due to aggregation, impaired MTS processing, or both. Ongoing studies using hydrogen-deuterium exchange mass spectrometry (HDX-MS) aim to define allosteric changes upon MTS binding. This work will advance understanding of MPP-related disease mechanisms and inform future therapeutic development.

► **P.B10 – Jeremy Loehr**
Cancer Research Center and PROTEO-Laval Research Center, Université Laval

The characterization of BRD2-dependent mechanisms governing melanoma development and proliferation.

Loehr, Jeremy, Department of Molecular Medicine, Cancer Research Center and PROTEO-Laval Research Center, Université Laval, Quebec, Canada; CHU de Québec Research Center, Quebec, QC, Canada. PROTEO-Quebec Network for Research on Protein Function, Engineering, and Applications; Estavoyer, Benjamin, Institute for Research in Immunology and Cancer (IRIC), Université de Montréal, Montreal, QC, Canada; Department of Pathology and Cell Biology, Faculty of Medicine, Université de Montréal, Montreal, QC, Canada; Saidi, Ismael, Department of Molecular Medicine, Cancer Research Center and PROTEO-Laval Research Center, Université Laval, Quebec, Canada; CHU de Québec Research Center, Quebec, QC, Canada; Lashgari, Anahita, Department of Molecular Medicine, Cancer Research Center and PROTEO-Laval Research Center, Université Laval, Quebec, Canada; CHU de Québec Research Center, Quebec, QC, Canada; Roux, Philippe, Institute for Research in Immunology and Cancer (IRIC), Université de Montréal, Montreal, QC, Canada; Department of Pathology and Cell Biology, Faculty of Medicine, Université de Montréal, Montreal, QC, Canada; Lambert, Jean-Philippe, Department of Molecular Medicine, Cancer Research Center and PROTEO-Laval Research Center, Université Laval, Quebec, Canada; CHU de Québec Research Center, Quebec, QC, Canada. PROTEO-Quebec Network for Research on Protein Function, Engineering, and Applications.

Melanoma is the most aggressive and lethal form of skin cancer. Despite notable advances in treatment strategies, including immunotherapies, the prognosis for patients with advanced melanoma remains poor, with a 5-year survival rate of only 15–20%. A key driver of melanoma is the dysregulation of the RAS/MAPK signaling pathway, which promotes tumor growth by altering downstream effectors. Emerging evidence points to a critical role for epigenetic alterations in driving melanoma progression and treatment resistance, although these processes are not well characterized. We recently found novel mechanistic links between the RAS/MAPK pathway and epigenetic regulation in melanoma. Specifically, we found that the RAS/MAPK pathway promotes the phosphorylation of BRD2, a chromatin reader that binds acetylated histone tails to regulate gene expression, which is also upregulated in melanoma cells and confers a proliferative advantage. Here we will present our efforts to determine: 1) the phospho-regulation of BRD2 in melanoma cells; 2) the role of BRD2 phosphorylation in transcriptional regulation; and 3) whether modulating RAS/MAPK signaling toward BRD2 is a promising therapeutic intervention in melanoma. Our results position the RAS/MAPK-BRD2 axis as a critical driver of melanoma progression, and we believe that its targeting could offer a promising therapeutic strategy for melanoma patients.

► **P.B11 – Albie Gong**
Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, V6T 1Z3

Proteomic Analysis of Glucose Effects on Astrocytic Response to HuCV-229E Virus Infection

Gong, Albie^{1,2}; Chi, Shuxin^{2,3}; Zhong, Huan^{2,3}; Foster, Leonard J.^{2,3,4}

¹Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, V6T 1Z3; ²Michael Smith Laboratories, University of British Columbia, Vancouver, BC, V6T 1Z4; ³Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, V6T 1Z3; ⁴Life Sciences Institute, University of British Columbia, Vancouver, BC, V6T 1Z3

Astrocytes are glial cells affected by hyperglycemia, contributing to altered metabolism and neuroinflammation. Previous

work has shown that under coronavirus infections, such as human coronavirus 229E (HuCV-229E), astrocytes exhibit increased reactivity and stress. Moreover, hyperglycemia exacerbates astrocyte-associated neuropathology in diabetic patients infected with coronaviruses. However, glucose impacts on glucometabolic and inflammatory proteomic responses during HuCV-229E infection remain unclear. Since astrocytes exhibit sex differences such as dimorphic regulation of glucose uptake via glucose transporter 2, we aim to examine glucometabolic and inflammatory protein changes in male and female astrocytes infected with HuCV-229E under normal- and high-glucose conditions. We hypothesize that both glucose and sex would significantly influence astrocytes' proteomic responses to infection.

Primary human astrocytes (ScienCell) from three male and three female donors were cultured under normal (5.5 mM) or high (25 mM) glucose and infected with HuCV-229E at 33°C (MOI = 0.2, 72 h.p.i.), alongside uninfected controls. Samples were processed for liquid chromatography-mass spectrometry (timsTOF SCP), and outputs were processed in DIA-NN. Data analysis was conducted in R.

Differential expression and Gene Ontology analyses revealed that female astrocytes demonstrated greater glucometabolic pathway upregulation such as oxidative phosphorylation under uninfected and high glucose conditions. Males showed increased coagulation-related proteins and reduced response to ER stress. Viral infection led to a greater increase in immune-related protein expression in females, whereas males showed downregulation in apoptotic pathways.

High glucose and viral infection induce sex-specific proteomic alterations in astrocytes, highlighting metabolic and inflammatory differences in male and female responses.

► **P.B12 – Marie-Gabrielle Ayika**

University of Guelph

Determination of hypothetical protein's role in Cryptococcal virulence

Ayika, Marie-Gabrielle, University of Guelph; Geddes-McAlister, Jennifer, University of Guelph

Cryptococcus neoformans is an opportunistic fungal pathogen causing severe disease in immune compromised hosts. The increasing rates of disease prevalence and rising threat of antifungal resistance highlights the need to identify determinants of fungal infection and factors modulating host-pathogen interactions. Macrophages as the first line of defense against C. neoformans were used to uncover proteins important for their critical interaction. Herein, mass spectrometry-based proteomic profiling of co-cultured C. neoformans and bone marrow-derived macrophages identified multiple infection-associated candidates, including the uncharacterized protein, CNAG_03492. Based on reduced capsule size and thermotolerance, CNAG_03492Δ is hypothesized to influence cryptococcal virulence. To define this role, CNAG_03492Δ is being assessed for unknown protein interactors using a co-immunoprecipitation pulldown assay. In addition, quantitative proteomic profiling of CNAG_03492Δ will be used to identify remodeling and compensatory shifts that can occur in the absence of the protein. Further, this remodeling can also detect other cryptococcal proteins and provide biological insight into the functional role of the candidate gene within the host infection. Also, in vivo studies using CNAG_03492Δ and complemented strains in BALB/c mice will be used to assess how CNAG_03492Δ affects fungal dissemination, organ-specific burden, and host survival. These findings will be complemented with in silico prediction modeling of target structure and interactions, as well as functional assays exploring global proteome disruption upon deletion of CNAG_03492. Together, these findings will explore the positioning of CNAG_03492 as a target for therapeutic development against cryptococcal disease.

► **P.B13 – Matthew Rennie**

University of Guelph, Department of Molecular and Cellular Biology

Proteomics-integrated characterization of the mitochondrial electron transport chain as a putative antifungal target in Cryptococcus neoformans

Rennie, Matthew, University of Guelph; Ball, Brianna, University of Guelph; Chan, Norris, University of Guelph; Woods, Michael, University of Guelph; Murphy, J., Patrick, University of PEI; Sayewich, Jonathan, SickKids SPARC BioCentre; Fladd, Chris, SickKids SPARC BioCentre; Deyarmin, Jared, Thermo Fisher Scientific; Samra, Stephanie, N., Thermo Fisher Scientific; Geddes-McAlister, Jennifer, University of Guelph.

The opportunistic fungal pathogen Cryptococcus neoformans primarily infects immunocompromised individuals causing fatal meningitis. Rising antifungal resistance is confounding treatment, thus new antifungal targets are needed. To address this, we previously identified the uncharacterized protein CNAG_05997 (a putative mitochondrial electron transport chain complex 1 subunit) as an antifungal target by mass spectrometry-based infectome profiling.

Various assays were performed to support that CNAG_05997 is a complex 1 subunit. Proteomic analysis of the deletion versus wild type (WT) strains showed large shifts in the global proteome, with increased abundances of electron carrier and oxidoreductase proteins to compensate for the deletion of CNAG_05997. ATP quantification supported this with a decrease in ATP concentration in the deletion strain compared to WT, highlighting a dysfunctional electron transport chain. Fluorescent microscopy confirmed mitochondrial localization of CNAG_05997.

Compound library screening identified several putative electron transport chain inhibitors, the most promising was analyzed through a proteome integral solubility alteration (PISA) assay. PISA, coupled with mass spectrometry-based proteomics, identified electron transport chain related targets identifying our compound as a putative electron transport chain inhibitor. Compound treatment led to a reduction in thermotolerance at 30 and 37 degrees Celsius in WT C. neoformans. Further proteomic analysis identified global proteome shifts upon compound treatment, including an increased abundance of an ABC multidrug transporter, demonstrating drug evasion pathways. This work shows a putative treatment option for C. neoformans, providing new insight into antifungal targeting of the electron transport chain, and highlights the role of mass spectrometry-based proteomics in antifungal target identification.

► **P.B14 – Matthew Stone**

SCIECX

Quantitative proteomics using high-sensitivity data-independent acquisition

Ihor Batruch; Patrick Pribil.

The central goal of proteomics is the robust identification and quantification of proteins to understand biological processes and disease associated changes in protein abundance. Data independent acquisition (DIA) has become a cornerstone of quantitative proteomics due to its reproducibility, analytical depth, and streamlined workflows. However, confident detection and quantification of low abundance peptides and proteins remain persistent challenges, particularly in high throughput and low sample load applications. Addressing these challenges requires advances in MS/MS sensitivity to improve selectivity and quantitative precision.

Here, we demonstrate a high sensitivity DIA workflow using the ZenoTOF 8600 system to enable enhanced qualitative and quantitative proteomic analysis, with particular emphasis on low abundance proteins. Commercial K562 samples were analyzed at low (5 ng) and moderate (50 ng) loadings using high throughput Evosep One separations (200–500 samples per day). DIA data were acquired using either Zeno SWATH DIA or ZT Scan 2.0 DIA and processed using DIA NN or PEAKS Studio with a K562/HeLa spectral library.

Compared to the most sensitive current SCIECX accurate mass platform, the ZenoTOF 8600 system delivered >10 fold LC MS peak area gains, enabling substantially increased protein group and precursor identifications at low sample loadings. Notably, ZT Scan 2.0 DIA provided 1.7–3.8x more identifications than comparable ZT Scan DIA methods on the ZenoTOF 7600+ system at equivalent loadings. These results demonstrate that enhanced MS/MS sensitivity combined with ZT Scan 2.0 DIA enables high throughput, low input quantitative proteomics with improved depth and reproducibility.

► **P.B15 – Naomie Linteau**

Université Laval Cancer Research Center, CHU de Québec-Université Laval Research Center Endocrinology and Nephrology Axis, Department of Molecular Medicine, Faculty of Medicine, Université Laval, Québec, Canada and PROTEO-Quebec Network for Research on Protein Function, Engineering, and Applications

Characterizing the Impact of Modulating Acetyl-CoA Metabolism in Melanoma

Linteau, Naomie, Université Laval Cancer Research Center, CHU de Québec-Université Laval Research Center Endocrinology and Nephrology Axis, Department of Molecular Medicine, Faculty of Medicine, Université Laval, Québec, Canada and PROTEO-Quebec Network for Research on Protein Function, Engineering, and Applications; Loehr, Jérémy, Université Laval Cancer Research Center, CHU de Québec-Université Laval Research Center Endocrinology and Nephrology Axis, Department of Molecular Medicine, Faculty of Medicine, Université Laval, Québec, Canada and PROTEO-Quebec Network for Research on Protein Function, Engineering, and Applications; Fradet-Turcotte, Amélie, Université Laval Cancer Research Center, CHU de Québec-Université Laval Research Center Oncology Axis, Department of Molecular Biology, Medical Biochemistry and Pathology, Faculty of Medicine, Université Laval, Québec, Canada and PROTEO-Quebec Network for Research on Protein Function, Engineering, and Applications; Lambert, Jean-Philippe, Université Laval Cancer Research Center, CHU de Québec-Université Laval Research Center Endocrinology and Nephrology Axis, Department of Molecular Medicine, Faculty of Medicine, Université Laval, Québec, Canada and PROTEO-Quebec Network for Research on Protein Function, Engineering, and Applications

Dietary choices, food additives, medications, and pathologies can drastically alter metabolites that cells need to function properly. Consequently, essential metabolites are often generated from redundant pathways to ensure a sturdy supply. Acetyl-CoA is a central metabolic intermediate and a necessary substrate for lysine acetylation. This epigenetic mark is recognized by bromodomain-containing proteins, which act notably as scaffold proteins for transcription complexes involved in tumoral progression.

We are investigating the hypothesis that altered levels of nucleocytoplasmic acetyl-CoA regulate the use of the human genome through bromodomain-dependent mechanisms. Accordingly, the specific aims are 1) to characterize cellular models in which acetyl CoA and lysine acetylation levels can be modulated, and 2) to define their impact on bromodomain-containing proteins recruitment.

To do so, we knocked out key enzymes respectively converting citrate (ACL1) and acetate (ACSS2) to acetyl-CoA in metastatic melanoma cells. Independent and combined ACL1 and ACSS2 losses were characterized by proliferation tests, liquid chromatography-mass spectrometry and additional molecular biology experiments.

Globally, the loss of ACL1, independently of ACSS2 loss, decreases acetyl-CoA levels, histone lysine acetylation levels, and cell proliferation. Furthermore, preliminary data suggest differential recruitment of bromodomain-containing proteins and differential sensitivity to bromodomain inhibitors.

Developing models with altered acetyl-CoA metabolism will enable the investigation of acetylation changes, their effects on bromodomain-containing proteins, and the contribution of these proteins to cancer initiation and progression. In the long run, these models will help exploring interventions based on the manipulation of acetyl-CoA metabolism to potentiate oncology treatments targeting bromodomain-containing proteins.

► **P.B16 – Nisha Owens**

Department of Biology, University of Prince Edward Island

Kynurenine pathway regulation and function in breast cancer

Sommer, Tayah, Department of Biology, University of Prince Edward Island; Enkhbat, Munkhtuul, Department of Biology, University of Prince Edward Island; Sullivan, Reilly, Department of Biology, University of Prince Edward Island; Sultanova, Mukhavyo, Department of Biology, University of Prince Edward Island; Paulo, Joao, Department of Cell Biology, Harvard Medical School; Murphy, J. Patrick, Department of Biology, University of Prince Edward Island, Department of Pathology, Dalhousie University

The kynurenine pathway (KP) degrades tryptophan and generates precursors for de novo NAD biosynthesis, many of which have bioactive roles. In inflammatory conditions such as cancer, many cell types upregulate the first enzyme in the pathway, indoleamine 2,3-dioxygenase 1 (IDO1), resulting in kynurenine production. Since kynurenine is immunosuppressive, IDO1

inhibitors are being developed to enhance cancer immunotherapies but have thus far performed poorly. Recent work from our group shows that GM-CSF-derived or maintained macrophages induce kynureninase (KYNU), which degrades kynurenine and may alleviate immune suppression. However, both the regulation of KYNU and the resulting effects of kynurenine catabolites in cancer cells remains poorly understood.

Using quantitative proteomic analysis of breast cancer tissues, and validation with western blotting, we have identified subsets of breast tumors with high KYNU, leading us to investigate cellular responses to kynurenine catabolism in breast cancers. We performed metabolomics following treatment with kynurenine in breast cancer cell lines with both high and low KYNU expression. Although no increased contribution to NAD⁺ biosynthesis was detected, we observed kynurenine catabolism to anthranilic acid in high KYNU cell lines in quantities consistent with KYNU expression levels and at a rate corresponding to the reported Michaelis constant ($K_m = 400 \mu M$) for this reaction. We hypothesize that this diversion functions to catabolize elevated kynurenine and mitigate its biological effects. Elucidating this pathway may reveal how breast cancer cells adapt to kynurenine and lead to improved KP-based treatments for breast and other cancers.

► P.B17 – Seraphina Lu

University of Victoria

A multi-omic investigation into the host response of human macrophages to *Treponema pallidum*, causative agent of syphilis

Lu, Seraphina D., University of Victoria; Ranasinghe, Akash, University of Victoria; Waugh, Shay, University of Victoria; Goodyear, Mara C., University of Victoria; Lee, Amy H., Simon Fraser University; Reynolds, Lisa A., University of Victoria; Cameron, Caroline E., University of Victoria, University of Washington.

Cases of syphilis, which is caused by *Treponema pallidum* subspecies *pallidum* (Tp), have increased by 77% (infectious) and 220% (congenital) in Canada between 2018 and 2023. Despite rising syphilis rates in Canada and throughout the world, the mechanisms of pathogenesis used by Tp remain incompletely understood. Our prior multi-omic analyses demonstrated that Tp exposure alters endothelial cell signalling pathways, contributing to Tp dissemination, immune evasion and persistence. In the primary and secondary stages of syphilis, macrophages are a key immune cell responsible for killing Tp via opsonophagocytosis, although Tp clearance is incomplete. Our overarching goal uses multi-omics to determine the response of human macrophages to Tp exposure. Following differentiation of human monocytes into macrophages, time-course flow cytometry, RNA-seq and proteomics were used to detect changes in macrophage gene and protein expression profiles during Tp exposure. We employed a label-free quantitative proteomics approach and LC-MS/MS with data-independent acquisition. Flow cytometry was used to identify macrophage polarization markers, allowing determination of whether Tp induces an M1-like pro-inflammatory or M2-like anti-inflammatory phenotype. Bioinformatic analyses were used to identify differentially expressed genes and proteins in macrophages exposed to Tp, as well as host pathways impacted by Tp. This study is the first time-course multi-omic systems-level analysis of the macrophage response to Tp exposure. Understanding the effect of Tp on a primary immune cell responsible for bacterial clearance increases our understanding of the host mechanisms and pathways driving Tp pathogenesis and how to protect against infection.

► P.B18 – Seyedehsanaz Ramezanzpour

Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Chemotype dependent remodeling of maize immunity and fungal virulence uncovered by dual kingdom DIA proteomics

Seyedehsanaz Ramezanzpour, Nasim Alijanimaghani, Jason A. McAlister, Ashleigh Dale, Stuart Cordwell, David Hooker, Jennifer Geddes-McAlister

Trichothecene mycotoxins produced by fungal pathogens, including deoxynivalenol, threaten global food safety and security by contaminating food and feed sources, causing harmful effects on digestive health, neurological function, and fertility. Emerging NX type trichothecene chemotypes of *Fusarium graminearum*, including 3ANX, are increasing in prevalence across Canadian cereals, yet their effects on maize defense networks remain poorly understood. To characterize chemotype specific virulence strategies under bona-fide agronomic conditions, we performed a field based infection experiment using 20 isolates representing 15ADON (15-acetyl DON) and 15ADON/3ANX chemotypes. Dual kingdom data-independent acquisition LC-MS/MS profiling of infected maize ears quantified 7,242 proteins (6,011 maize;1,231 fungal), enabling a systems level comparison of host immunity and fungal pathogenicity. Network based clustering of 1,080 maize defense associated proteins resolved 92 functional modules organized into a three layer maize defense architecture: direct antifungal mechanisms (chitinases), regulatory control systems (MAPK cascades), and metabolic support functions (photosynthesis). Although global proteome variation was modest, direct chemotype comparison revealed that 15ADON/3ANX-producing isolates selectively suppressed photosystem II components and heat response proteins, indicating targeted disruption of metabolic resilience required for sustained immunity. Fungal proteomes exhibited extensive modular organization that similarly resolved into a three layer virulence model: direct virulence drivers (CAZymes), regulatory layers (kinases), and cellular support systems (ribosomal proteins). NX producing isolates showed distinct modulation of carbohydrate degradation, ergosterol biosynthesis, and redox associated enzymes, suggesting chemotype specific strategies for nutrient acquisition and immune suppression. Together, this dual kingdom proteomic framework reveals that emerging 3ANX producing isolates impose deeper constraints on maize metabolic resilience

► P.B19 – Siham Ait benichou

Research Center CHU de Québec – Université Laval, Quebec, Canada

Study of the Molecular Mechanisms Involved in Primary Sclerosing Cholangitis

Loehr, Jérémy, 1 Research Center CHU de Québec – Université Laval, Quebec, Canada, 2 PROTEO-Québec Network for Research on Protein Function, Engineering and Applications; Lambert, Jean-Philippe, 1 Research Center CHU de Québec – Université Laval, Quebec, Canada, 2 PROTEO-Québec Network for Research on Protein Function, Engineering and Applications, 3 Department of molecular medicine, Faculty of medicine, Université Laval, Quebec, Canada

Primary sclerosing cholangitis (PSC) is a rare chronic hepatobiliary disease with a prevalence of approximately 1 in 10,000–100,000, affecting about 4,000 Canadians. PSC is characterized by progressive inflammation, hepatic fibrosis, and damage to both intrahepatic and extrahepatic bile ducts, leading to irreversible liver injury. To date, liver transplantation remains the only available treatment. The molecular mechanisms underlying PSC remain poorly understood, and new insights are essential for the development of effective therapies. To address this gap, we have implemented an unbiased systems biology approach to study PSC. Specifically, we aim to examine how proteins previously associated with PSC respond to chronic inflammation caused by excess hepatic bile acids. To achieve this, we have begun developing ex vivo hepatic organoids derived from wild-type and *Mdr2^{-/-}* mouse livers, the only validated murine model for PSC research. These innovative models will allow us to characterize PSC-related proteins using a range of molecular, cellular, and omic tools. Notably, we will use these organoids to perform proximity-dependent biotinylation (BioID) experiments and identify the molecular mechanisms potentially contributing to PSC. Our project will shed light on the pathways involved in PSC, support the identification of novel therapeutic targets, and, in the long term, contribute to the development of targeted treatments.

► P.B20 – Fatima Ezzahra Elhaou

Department of Biochemistry, University of Regina, Regina, Saskatchewan, Canada / Faculty of Medical Sciences, UM6P Hospitals, Mohammed VI Polytechnic University, Benguerir, 43150, Morocco.

Mapping L444P Mutation-Specific Protein-Protein Interaction Rewiring in Gaucher Disease

Elhaou, Fatima Ezzahra, Faculty of Medical Sciences, UM6P Hospitals, Mohammed VI Polytechnic University, Benguerir 43150, Morocco / Department of Biochemistry, University of Regina, Regina, Saskatchewan, Canada; El Ayadi, Rachid, Centre Hospitalier d'Auch, Allée Marie Clarac, 32000 Auch, France; El Fatimy, Rachid, Faculty of Medical Sciences, UM6P Hospitals, Mohammed VI Polytechnic University, Benguerir 43150, Morocco; Babu, Mohan, Department of Biochemistry, University of Regina, Regina, Saskatchewan, Canada; Moutaoufik, Mohamed Taha, Faculty of Medical Sciences, UM6P Hospitals, Mohammed VI Polytechnic University, Benguerir 43150, Morocco (corresponding author)

Gaucher disease (GD), the most prevalent lysosomal storage disorder, is caused by biallelic mutations in GBA1, encoding the lysosomal enzyme β -glucocerebrosidase (GCCase). Among pathogenic variants, the L444P mutation, located in the non-catalytic Ig-like domain, destabilizes GCCase folding and is strongly associated with severe neuronopathic forms of the disease. However, the L444P variant can cause different clinical phenotypes depending on the allelic context, suggesting that mechanisms beyond residual enzyme activity contribute to disease heterogeneity.

We hypothesized that L444P drives mutation-specific changes in the GCCase protein-protein interaction (PPI) network that contribute to subtype-specific cellular dysfunction. To test this, we constructed expression-weighted PPI networks integrating TMT-based quantitative interactome data for wild-type and mutant GCCase (PRIDE: PXD032155) with single-cell transcriptomics from GBA1 mutant midbrain organoids (GEO: GSE198033).

Differential interactome analysis identified 52 L444P-associated interactors, whose significance was prioritized by weighting interactions with cell-type-specific transcriptional changes. Functional and spatial annotation of the network revealed four major disrupted modules. ER quality control components (CANX, CALR, PDIA3, HSP90B1) were prominently enriched, consistent with a proteostatic response to GCCase misfolding. Lysosomal trafficking interactors like SCARB2 which is the primary GCCase transporter was also altered, along with IGF2R, MAN2B1, and ACP1. Interestingly, the network also implicated mitochondrial inner membrane organization (TIMMDC1, SLC25A3, SLC25A4, LONP1) and membrane-vesicle trafficking (MTMR3, MTMR4, GOLGA5, TMEM33, TMEM59), indicating that L444P disrupts organelle communication beyond the lysosomal compartment.

These findings suggest that L444P-associated disease mechanisms extend beyond lysosomal dysfunction, involving disruptions in proteostasis, organelle communication, and mitochondrial function.

► P.B21 – Laleh Ebrahimi Ghahnavieh

Division of Experimental Medicine, McGill University, Montréal, QC, Canada/ Segal Cancer Proteomics Centre, Jewish General Hospital, Montreal, QC, Canada

A Multi-Omics Proteogenomic Integrative Analysis Reveals Metabolic Reprogramming and CMS-Related Proteomic Heterogeneity in Colorectal Cancer

Ebrahimi Ghahnavieh, Laleh, Division of Experimental Medicine, McGill University, Montréal, QC, Canada/ Segal Cancer Proteomics Centre, Jewish General Hospital, Montreal, QC, Canada; Gelb, Timon, Segal Cancer Proteomics Centre, Jewish General Hospital, Montreal, QC, Canada; Batist, Gerald, Faculty of Medicine, McGill University, Montréal, QC, Canada/ Gerald Bronfman Department of Oncology, McGill University, Montréal, QC, Canada/ Lady Davis Institute for Medical Research & Segal Cancer Centre, Jewish General Hospital, Montréal, QC, Canada; Borchers, Christoph, Division of Experimental Medicine, McGill University, Montréal, QC, Canada/ Gerald Bronfman Department of Oncology, McGill University, Montréal, QC, Canada/ Segal Cancer Proteomics Centre, Jewish General Hospital, Montreal, QC, Canada/ Lady Davis Institute for Medical Research, Jewish General Hospital, Montréal, QC, Canada.

Colorectal adenocarcinoma (COAD), the third leading cause of cancer-related mortality in Canada, carries a five-year survival of only 11–13% in the metastatic setting. Resistance to anti-EGFR and anti-VEGF therapies, driven by oncogenic KRAS mutations and receptor tyrosine kinase upregulation, constitutes an enduring clinical barrier. The poor correlation between mRNA and protein levels underscores the need for integrated quantitative proteomics to decode functional signaling dysregulation in COAD.

We integrated six omics layers, including TMT proteomics, phosphoproteomics, RNA-seq, somatic copy-number variation, mutational profiles, and miRNA across 94 tumor and 99 adjacent normal samples from the CPTAC COAD cohort. We assessed differential protein expression (DEP) using paired limma-eBayes after median-centring, SVS batch correction, and missing-value filtering. Downstream analyses comprised over-representation analysis (ORA) and gene set enrichment analysis (GSEA) with unsupervised stratification via a multi-omics annotated heatmap.

Proteome-wide comparison of tumor versus normal (93 paired samples) identified 32 up-regulated and 405 down-regulated proteins (BH-adjusted $p < 0.05$, $|\log_2FC| > 1$) out of 6,415 proteins, highlighting clear separation of proteomic profiles in a heatmap. Up-regulated proteins were enriched for neutrophil degranulation and extracellular matrix organization; down-

regulated proteins for actin cytoskeletal and muscle contraction programs. GSEA revealed significant enrichment of MYC target activation and oxidative phosphorylation, fatty acid metabolism, and heme metabolism, as suppression in tumor tissue confirmed a Warburg-type metabolic shift at the protein level.

Proteogenomic integration of the CPTAC COAD cohort reveals MYC-driven proliferative rewiring and coordinated metabolic suppression, providing a protein-level framework for precision oncology stratification in COAD beyond transcriptomic subtyping.

► P.B22 – Mohammad Shokouhian

Program in Cell & Systems Biology, The Hospital for Sick Children, Toronto; Department of Molecular Genetics, University of Toronto

ACO2 regulates export of Fe/S cluster from mitochondria to cytosol

Shokouhian, Mohammad^{1,2}; Jacquet, Charlotte¹; Mirhadi, Shideh^{1,2}; Thomson Tylor, David^{3,4}; Simpson, Craig^{1,5}; Moffat, Jason^{2,4,5}; Moran, Michael F.^{1,2,3}

¹Program in Cell & Systems Biology, The Hospital for Sick Children, Toronto; ²Department of Molecular Genetics, University of Toronto; ³SPARC BioCentre, The Hospital for Sick Children, Toronto; ⁴Institute of Biomedical Engineering, University of Toronto; ⁵Program in Genetics and Genome Biology, The Hospital for Sick Children

Mass spectrometry (MS) analysis of human tumor cells and murine stromal material from non-small cell lung cancer (NSCLC) patient-derived xenograft tumors identified significantly reduced mitochondrial aconitase (ACO2) protein levels associated with engrafting NSCLC. Orthogonal approaches validated low ACO2 expression as a predictor of worse outcomes in NSCLC. ACO2 is a Fe/S cluster-binding protein classically known to function in the TCA cycle, but with roles beyond it, including regulation of iron homeostasis via an incompletely understood mechanism. Proteomic, biochemical, and cell proliferation analyses revealed that ACO2 modulates iron homeostasis by regulating iron regulatory protein 1 (IRP1) access to Fe/S clusters; unbound IRP1 converts to cytosolic aconitase upon Fe/S cluster binding. These data support a model in which CISD1, an outer mitochondrial membrane Fe/S transfer protein, delivers clusters to apo-IRP1. MS-based proteomics analyses of ACO2 knockout (KO) vs CISD1 KO in adenocarcinoma NSCLC line A549 showed coordinated downregulation of cytosolic Fe/S cluster scaffold and targeting machinery specifically in ACO2 KO cells. Moreover, wild type ACO2 overexpression in stably transfected HEK293 T-REX cells, but not a Fe/S-binding-deficient triple Cys-to-Ala mutant, reduced cytosolic and nuclear Fe/S clusters-derived iron levels. Overall, our data shows that ACO2 regulates extra-mitochondrial Fe/S cluster availability, which is essential for cytosolic Fe/S cluster maturation and, specifically, IRP1 function.

► P.B23 – Nicole Hansmeier

Luther College at the University of Regina

From Exposure to Remodeling: Cannabis Aerosol Induces a Proteome-Level Shift Toward Chronic Lung Injury and Dysregulated Repair

Jay H. Savaliya, Department of Biology, University of Regina, Regina, SK, Canada; Josef Buttigieg, Department of Biology, University of Regina, Regina, SK, Canada; Tzu-Chiao Chao, Department of Biology, University of Regina, Institute of Environmental Change and Society (IECS), Regina, SK, Canada; Nicole Hansmeier, Department of Biology, Luther College at the University of Regina, Regina, SK, Canada

The increasing prevalence of inhalational cannabis use, particularly via vaping, necessitates a mechanistic understanding of its impact on lung biology. Here, we investigated the molecular and structural consequences of repeated cannabis aerosol exposure in a Wistar rat model. Animals were exposed to cannabis aerosols or warm air (controls) for seven minutes, three times weekly over six months, followed by a three-month recovery period. Lung tissues were analyzed by histopathology and quantitative proteomics using a Waters M-Class nano-LC coupled to a Synapt XS HDMS.

Across 3,255 identified proteins, 259 were significantly altered. Proteomic profiling revealed a coordinated remodeling program characterized by upregulation of oxidative stress response, xenobiotic detoxification, and translational machinery, alongside downregulation of mitochondrial respiration, vesicle trafficking, proteostasis, and epithelial junctional components. Functional clustering indicated disruption of redox homeostasis, impaired autophagy-lysosomal pathways, and loss of cytoskeletal and barrier integrity. These molecular alterations closely align with established signatures of smoke-induced lung injury and early COPD pathogenesis.

Histologically, exposed lungs exhibited bronchial epithelial hyperplasia and alveolar septal destruction, consistent with concurrent proliferative repair and emphysema-like tissue remodeling. Integration of proteomic and histological data supports a model in which chronic aerosol exposure induces persistent oxidative stress, driving compensatory epithelial proliferation in the context of impaired cellular quality control and disrupted tissue architecture. The observed imbalance between adaptive stress responses and declining homeostatic functions suggests a trajectory toward chronic injury-associated inflammation and tissue degeneration.

Together, these findings provide mechanistic insight into cannabis aerosol-induced lung injury, demonstrating substantial convergence with cigarette smoke-associated pathways and highlighting

► P.B24 – Khiry Patterson

University of Southern California, Los Angeles, CA.

Deep Metaproteomic Profiling of a Mock Ocean Microbial Community Using the Orbitrap Astral Zoom Mass Spectrometer

Held, Noelle, University of Southern California, Los Angeles, CA; Palmer, Maxfield, University of Southern California, Los Angeles, CA; Hartel, Nicolas, Thermo Fisher Scientific, San Jose, CA.

Ocean microbiomes present significant analytical challenges for LC-MS/MS-based metaproteomics due to low biomass, high taxonomic complexity, and a wide dynamic range, which limits detection of low-abundance organisms. Large-scale ocean studies reveal substantial spatial and temporal variability in microbial communities, underscoring the need for sensitive

instrumentation and robust quality control (QC) strategies. Here, we evaluate the performance of the Orbitrap Astral Zoom mass spectrometer using a pooled multispecies ocean proteome (>100,000 proteins; 16 species) designed for QC applications. Data were acquired in both data-dependent (DDA) and data-independent acquisition (DIA) modes, with and without FAIMS.

Tryptic digests from sixteen marine microorganisms were combined to generate a mock metaproteome. LC-MS/MS analyses were performed on a Thermo Scientific Vanquish Neo UHPLC system (trap-and-elute) coupled to the Orbitrap Astral Zoom, using a 75 µm × 25 cm column and a 60-minute gradient. DDA data were processed in Proteome Discoverer 3.3 (CHIMERYS), and DIA data in Spectronaut 19.7.

The platform enabled deep proteome coverage, identifying 20,351 unique proteins, including 14,059 (DIA ± FAIMS) and 17,274 (DDA ± FAIMS). High quantitative reproducibility was observed (CV = 4.3%, n=2), demonstrating robust performance. These results highlight the Orbitrap Astral Zoom as a powerful tool for scalable ocean metaproteomics and support development of standardized QC workflows for complex environmental samples.

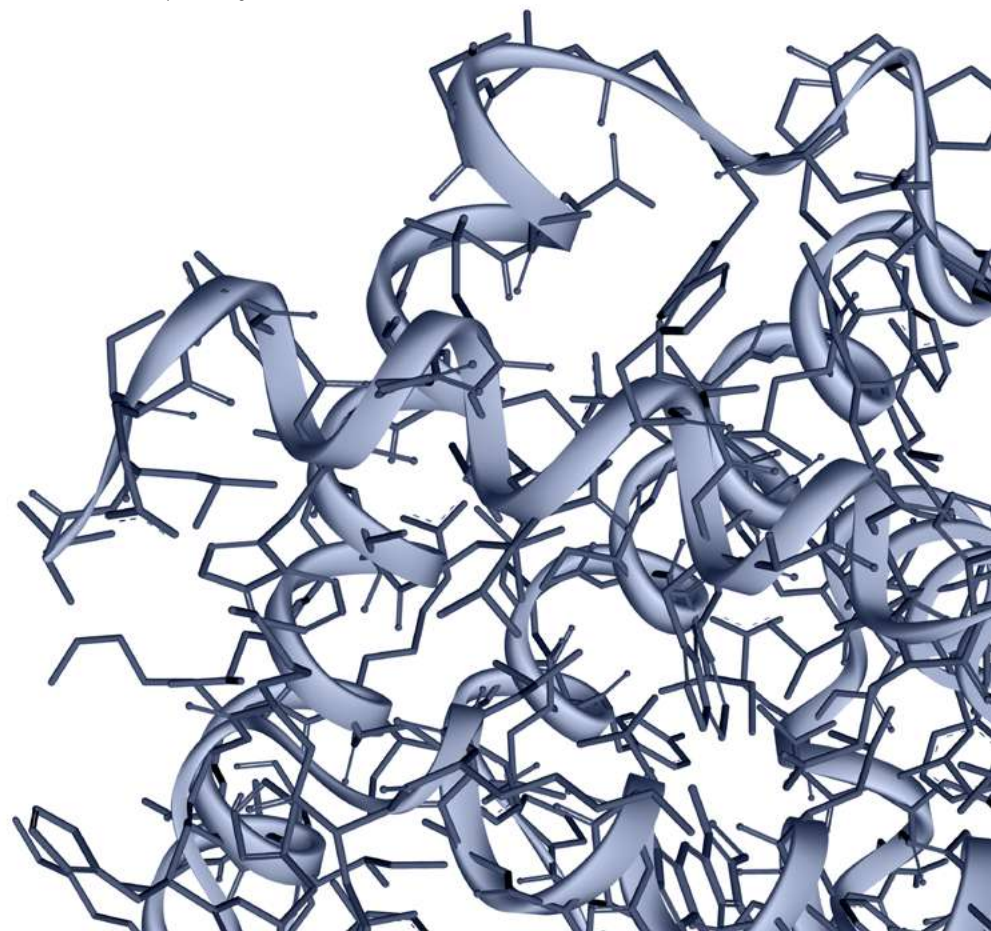
► P.B25 – Maggy Lepine

University of Quebec in Montreal

Integrated LC-MS/MS multiomics profiling of Mucopolysaccharidosis subtypes in *Caenorhabditis elegans* mutants

Lépine Maggy, Ghafari Nathan, Killeen Jeffrey, Bénéard Claire, Sleno Lekha

Mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders caused by deficiencies in specific enzymes responsible for glycosaminoglycan (GAG) degradation. The resulting accumulation of partially degraded substrates leads to progressive multi-systemic pathology, yet the downstream metabolic and proteomic alterations remain incompletely characterized. An integrated multi-omics strategy based on liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS) was used to investigate molecular dysregulation across distinct MPS subtypes using *Caenorhabditis elegans* as a disease model, with mutants involving different genes associated to MPS. Integrating proteomics and metabolomics analyses will provide mechanistic insight into how lysosomal dysfunction reshapes cellular metabolism, mitochondrial function, and stress-response pathways. By identifying molecular signatures across MPS variants, this work may also help uncover candidate biomarkers and novel therapeutic targets.



We *thank* our sponsors

GOLD

ThermoFisher
S C I E N T I F I C

SILVER

EVUSEP

BRONZE



ionopticks

PREOMICS



illumina[®]



FRIENDS

