Welcome Message

On behalf of your Canadian National Proteomics Network (CNPN), we are pleased to welcome you to the 16th annual CNPN symposium: CNPN 2025! The theme of the annual symposium: Proteomics Innovations Inspiring Health and Discovery. All conference events will take place at the Westin Harbour Castle Hotel and Conference Centre in Toronto, Ontario, Canada in Piers 4 and 5. This year, as CNPN is hosting the Human Proteome Organization (HUPO) World Congress 2025, the largest international proteomics meeting in the world, we are thrilled for CNPN 2025 to serve as the kick-off event!

CNPN 2025 will feature national and international proteomics experts. Dr. Thomas Kislinger (Princess Margaret Cancer Center, University of Toronto) will deliver the keynote address on ‘Clinical Proteomics: Biomarker Discovery and Therapeutic Targets’. We also welcome Drs. Isabelle Sirois (Université de Montréal), Philipp Lange (BC Children’s Hospital, University of British Columbia), Stuart Cordwell (University of Sydney), and Ling Hao (University of Maryland). Additionally, abstract selected talks will align with conference sessions on: Proteomics Driving Innovation in Health Research and Proteomics Technology and Innovation. Lastly, we are very excited to bring you a high-energy, fun, and dynamic lightning talk session for trainees and sponsors to share

their cutting-edge research in quick two-minute bursts. This year, we are pleased to offer industry-sponsored awards for best abstract selected talk and best lightning talks! During the symposium, you will also meet and hear from our 2025 Tony Pawson and 2025 New Investigator Awardees, along with plenty of opportunities to discuss new innovations with our sponsors and strategies to advance your research.

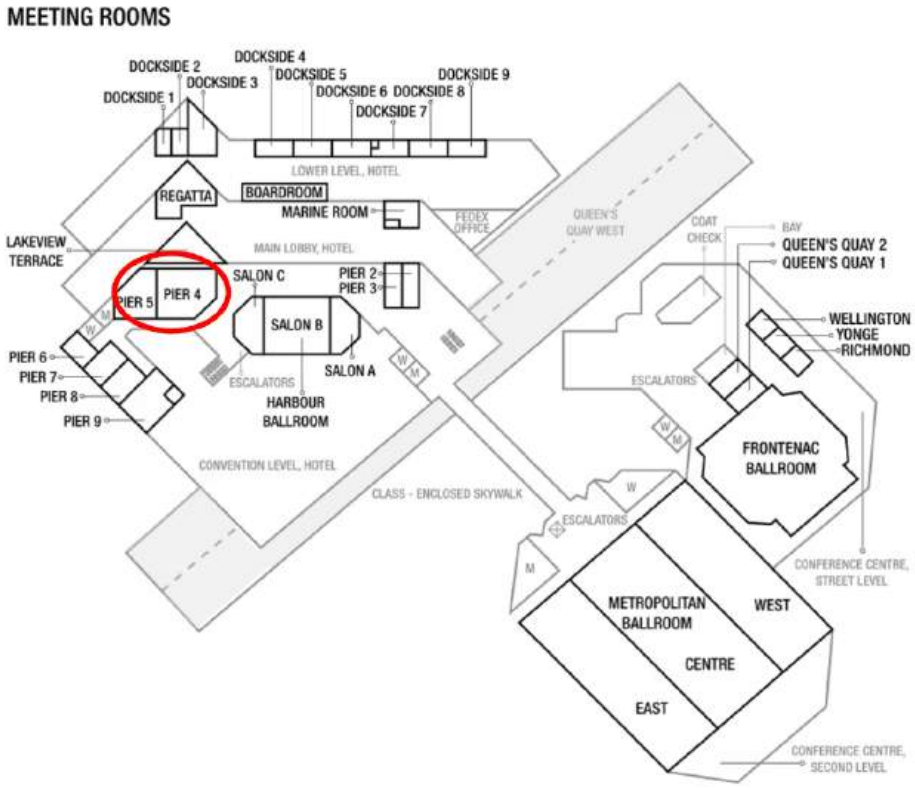
We hope you enjoy the meeting, connecting with your colleagues, and making new connections!

Jennifer Geddes-McAlister, PhD
President, Canadian National Proteomics Network

Housekeeping

Conference venue: Westin Harbour Castle Hotel and Conference Centre in Toronto, Ontario, Canada. 11 Bay Street, Toronto, Ontario, Canada, M5J 1A6.

Room: Pier 4 & 5. On the Conventional level of the hotel.



Presenters & Chairs: Thank you for contributing to CNPN 2025. The keynote talk is scheduled for 40 min talk + 5 min for questions. Invited talks are scheduled for 17 min talk + 3 min questions. Abstract selected talks are scheduled for 12 min talk + 3 min questions. Lightning talks are scheduled for 2 min (maximum 3 slides); you may include a QR code to a virtual poster, if you like. Talks must be uploaded prior to each session to the conference laptop (you may send your talk ahead of time to jgeddesm@uoguelph.ca). Please keep to your allotted time for talks and questions so we keep the day on schedule.

Meals: A light breakfast will be served from 9:00 – 9:45 am in Pier 4 & 5. A buffet lunch will be served from approximately 12:30 – 1:20 pm during which the CNPN Annual General Meeting will be held. Coffee breaks with light snacks will be provided twice during the day. A networking reception with hors d'oeuvres and a complimentary drink will be provided (followed by a cash bar).

Wifi: Provided by the hotel.

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Program

9:00 – 9:45 am	**Registration (light breakfast and refreshments provided)**
9:45 – 10:00 am	**Welcome Remarks**
10:00 – 10:45 am	**Chair: Dr. Nicole Hansmeier**
	Keynote speaker: Dr. Thomas Kislinger (Princess Margaret Cancer Center, University of Toronto)
	Clinical Proteomics: Biomarker Discovery & Therapeutic Targets
10:45 am – 12:20 pm	**Session 1: Proteomics Driving Innovation in Health Research**
	Chair: Dr. Jean-Francois Trempe
	ECR co-chair: Bianca Dupont
10:45 – 11:05 am	**Invited Speaker: Dr. Isabelle Sirois (Université de Montréal)**
	Uncovering Gene Fusion Neoantigens (GF-NEOs): How the ProteoFusion NEO Platform and Mass Spectrometry-Based Immunopeptidomics Reveal Novel Targets for Pediatric Cancer Immunotherapy
11:05 – 11:25 am	**Invited Speaker: Dr. Philipp Lange (University of British Columbia)**
	Single-cell and spatial proteomics to improve personalized precision medicine
11:25 – 11:40 am	**Abstract-selected talk: Chongyang Li**
	SUMO isopeptidase SENP6 reshapes ERE-derived antigen presentation via SUMOylation-mediated epigenetic modulation
11:40 – 11:55 am	**Abstract-selected talk: Laleh Ebrahimi Ghahnavieh**
	NexProQ: Multiplexed Quantitation of 500+ Proteins in Whole Blood and Dried Blood Spots Using PRM-PASEF and TMTpro

11:55 am – 12:10 pm	**Abstract-selected talk: Shawn Li** *Ultra-deep phosphoproteomics using sh2 superbinders: Applications in cancer research*
12:20 – 1:20 pm	**Lunch and CNPN Annual General Meeting (provided)**
1:30 – 3:15 pm	**Session 2: Proteomics Technology and Innovation** **Chair: Dr. Ji-Young Youn** **ECR co-chair: Davier Gutierrez-Gongora**
1:30 – 1:50 pm	**Invited Speaker: Dr. Stuart Cordwell (University of Sydney)** *Integrated Multi-omics for Linking Mass Spectrometry to Bacteria Pathogen Phenotypes*
1:50 – 2:10 pm	**Invited Speaker: Dr. Ling Hao (University of Maryland)** *Advancing Proteomics Strategies to Capture Lysosome Dynamics in Human Neurons*
2:10 – 2:25 pm	**Abstract-selected talk: Florence Roux-Dalvai** *MICROB-AI-R+: combining advanced proteomic data and machine learning for the ultrafast identification of pathogens and their resistance profile*
2:25 – 2:40 pm	**Abstract-selected talk: Andrei Drabovich** Redefining Antibody Diagnostics for Viral Infections through Proteome-Wide Serology
2:40 – 2:55 pm	**Abstract-selected talk: Valeriia Vasylieva** *Advancing proteomic discoveries with open modification search*
2:55 – 3:10 pm	**Abstract-selected talk: Thibault Mayor** *Mapping Protein Quality Control Networks: A Proteomics Approach to Chaperone and Degron-Driven Degradation*
3:15 – 3:45 pm	**Coffee Break (submit votes for best abstract-selected talk)**
3:45 – 5:00 pm	**Award Presentations** **2025 Tony Pawson Awardee: Dr. Thomas Kislinger**

	Presented by: Dr. Michael Moran, 2024 Tony Pawson Award Winner
	2025 New Investigator Awardee: Dr. John Patrick Murphy
	Presented by: Dr. Marie Brunet, 2024 New Investigator Award Winner
5:00 – 6:15 pm	**Lightning Talks**
	ECR co-chairs: Sujani Rathnayake, Félix-Antoine Trifiro, Anthony Glaude, Isabelle Lépine
	Each talk is 2 mins (max 3 slides)!
5:00 – 5:33 pm	**Sandra Spencer**
	Zeshan Ali
	Thermo Fisher Scientific (sponsor)
	Sachini Senarathna
	Iryna Abramchuk
	IonOptiks (sponsor)
	Rachel Nadeau
	Jay Savaliya
	Illumina (sponsor)
	Elmira Shajari
	Mayara da Silva
5:33 – 5:40 pm	**Vote and stretch!**
5:40 – 6:15 pm	**Kiki Huang**
	Shanshan Zhong
	Sciex (sponsor)
	Leon Xu
	Huan Zhong

	Seer (sponsor)
	Karl Schreiber
	George Xie
	Bioinformatics Solutions Inc (sponsor)
	Zoe Turner
6:15 – 7:30 pm	**Networking reception**

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Tony Pawson Award 2025

Dr. Thomas Kislinger

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.

New Investigator Award 2025

Dr. John Patrick Murphy

Dr. J Patrick Murphy specializes in quantitative proteomics and its application to study cancer and immune cell biology. He has been an Assistant Professor at the University of Prince Edward Island (UPEI) since 2019. Dr. Murphy completed his PhD in biology from Dalhousie University with a quantitative proteomics focus in Dr. Devanand Pinto's laboratory at the National Research Council Institute for Marine Biology. He then studied in Dr. Steve Gygi's laboratory at Harvard Medical School where he worked on the development of tandem mass tag (TMT)-based quantitative proteomics approaches and their application to understanding fundamental processes in cell biology. Following this, Dr. Murphy joined Dr. Patrick Lee's group at Dalhousie where he focused on the application of quantitative proteomics to understand cancer metabolism and immunology. His current research group at UPEI uses quantitative proteomics to understand the functions of both endogenous and exogenous metabolites that are produced or encountered by cancer and immune cells. Dr. Murphy has authored or co-authored more than 40 publications and has been named a Canadian Cancer Society Emerging Scholar, and a Banting Research Foundation Discovery Awardee. Dr. Murphy also teaches undergraduate courses at UPEI where he introduces students to new ways that biology may be understood using technology, such as proteomics.

Abstracts

Keynote Speaker

Kislinger, Thomas
University of Toronto

Clinical Proteomics; Biomarker Discovery & Therapeutic Targets

Biography: 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.

Invited Speakers
Siroirs, Isabelle
Université de Montréal

Uncovering Gene Fusion Neoantigens (GF-NEOs): How the ProteoFusion NEO Platform and Mass Spectrometry-Based Immunopeptidomics Reveal Novel Targets for Pediatric Cancer Immunotherapy

Gene fusions (GFs) are critical events in pediatric oncology, serving as oncogenic drivers and valuable biomarkers for diagnosis and risk stratification. However, fusion proteins and their derived neoantigens (GF-NEOs) remain underexplored in the context of targeted immunotherapy. In this study, we developed the ProteoFusioNEO computational tool for systematic in silico translation of large transcriptomic datasets from pediatric cancers, analyzing data from 5,190 patients and 935 cell lines. This yielded 382 unique fusion protein sequences from clinical samples and 446 from cell lines. Our study highlights that GF generate multiple translational outcomes, including in-frame and out-of-frame sequences, 97% of them being in-frame in pediatric cancer patients. We present a multipronged approach that utilizes both in vitro and in vivo systems to prioritize and validate the processing and presentation of GF-NEOs as physical molecules. This approach applies mass spectrometry-based proteomics and immunopeptidomics and specifically highlights the physical detection of GF-NEOs encoded by the ETV6-RUNX1 fusion. Together, our GF-NEO discovery and analysis platform contribute valuable insights into the biology of fusion proteins and their implications for targeted immunotherapy in pediatric oncology.

Biography: Following the completion of a B.Sc. in Biochemistry and the Cooperative Program at the University of Ottawa, Dr. Isabelle Sirois earned a Ph.D. in Biochemistry under the supervision of Dr. Marie-Josée Hébert and Dr. Alexey Pshezhetsky at Université de Montréal. During her doctoral studies sponsored by the CIHR, she characterized the secretome of apoptotic and autophagic endothelial cells using mass spectrometry, revealing unconventional secretory patterns relevant to chronic renal allograft rejection. As a recipient of the Eileen Iwanicki/CIHR Breast Cancer Fellowship, she pursued postdoctoral research in Dr. Mark Basik's lab at McGill University, where she identified novel molecular vulnerabilities in chemoresistant triple-negative breast cancer. Since 2021, Dr. Sirois has been co-investigator and group leader of the CQDM/SynergiQC pediatric oncology project, a multidisciplinary initiative aiming to identify fusion-derived neoantigens for pediatric leukemia treatment, involving around 20 scientists. In 2023, Dr Sirois became Head of the Proteomics and Immunopeptidomics Pediatric Platform at CHU Sainte-Justine, where she leads pioneering research in neoantigen discovery and immunotherapy development for both adult and pediatric cancers.

Lange, Philipp
BC Children's Hospital, University of British Columbia

Single-cell and spatial proteomics to improve personalized precision medicine

Launching from the first case in Canada where proteomics informed the treatment of a cancer patient, I will discuss how we advance spatial and single-cell proteomics to tackle the challenge of tumour heterogeneity and evolution.
We investigated the spatial complexity of tumors by integrating pathology guided laser-capture microdissection into the routine personalized proteomics workflow. We identified a high level of proteome and drug target stability through disease progression and even across phenotypically distinct tumour cells. Next, we investigated the potential role of single-cell proteomics in the personalized assessment of pediatric leukemia patients. To enable this, we developed high-sensitivity diagonal PASEF acquisition methods to support the proteome analysis of single leukemic cells that have only 1/10th the protein content of epithelial cells. In patients we find sub-populations with distinct phenotypes that are not apparent by clinical flow cytometry and show integration of single-cell proteomics into our personalized proteomics workflow to inform treatment selection. Lastly, I will present our progress in the nation-wide integration of pre-clinical proteomics evidence into pediatric precision oncology and patient care.

Biography: Dr. Philipp Lange is Canada Research Chair in Translational Proteogenomics of Pediatric Malignancies and Associate Professor of Pathology at the University of British Columbia (UBC). He is also a Scientist in the Michael Cuccione Childhood Cancer Research Program at the BC Children's Hospital and the BC Cancer Research Institute.
Dr. Lange's research focuses on precision oncology and oncoproteoforms, proteins with cancer-specific post-translational modification, their altered function, role in cell-cell communication and drug resistance, and their potential use as drug targets and biomarkers. His team drives the development and translation of proteomics platforms to advance molecular pathology and guide precision treatment for kids with cancer.
Dr. Lange is Director, Childhood Diseases Research at BC Children's Hospital Research Institute and provides leadership for Cancer Biology in the Canadian Pediatric Cancer Consortium ACCESS and co-leads the multi-centre proteomics team in the Canadian Precision Oncology For Young People (PROFYLE) study. He has won several awards for his advances in cancer research including the CIHR Early Career Investigator in Cancer Award and the Great Canadian Innovation Award by the Canadian Cancer Society.
Dr. Philipp Lange received his PhD in Biochemistry from the Free University Berlin, Germany after earning an MSc in Molecular Biology, Microbiology and Computer Sciences from the University of Hamburg, Germany. During his PhD with Dr. Dr. Thomas Jentsch at the Max Delbruck Centre for Molecular Medicine, Berlin, Germany he studied the molecular causes of hereditary osteopetrosis in children and patented a new drug target for the treatment of osteoporosis in adults. He conducted his postdoctoral work with Dr. Christopher Overall at the Centre for Blood Research, UBC developing computational and proteomics approaches to study post-translational modification in cancer.

Cordwell, Stuart
University of Sydney

Integrated multi-omics for linking mass spectrometry to bacterial pathogen phenotypes

Mass spectrometry (MS) is an analytical tool that has enabled the proteomics era. In our laboratory, we explore how alterations in the proteome are reflected in corresponding phenotypes across a range of bacterial pathogens when they are subjected to changes in internal (genetic) or external (growth conditions) environments, particularly those that reflect the human host niche. MS allows us to explore phenotypes related to nutrient transport, internal and culture supernatant metabolite levels (metabolomics), changes in protein interactions and protein complex formation (interactomics), and membrane composition (lipidomics, peptidoglycan and lipid A analysis). Here, we discuss the role in human virulence of the *N*-linked protein glycosylation (Pgl) pathway in the helical gastrointestinal pathogen *Campylobacter jejuni*. We show that deletion of *pgl* genes results in the loss of human virulence and define functional pathways that confer this phenotype. We employ MS-based approaches to profile bacterial lipid A and peptidoglycan showing these structures largely reflect environmentally-induced alterations to the proteome. Finally, we explore the interactome of the *C. jejuni* flagellar apparatus and explore quantitative cross-linking mass spectrometry (qXL-MS) to elucidate spatial changes to the chaperone interactome during temperature stress. Collectively, these data show how MS-based approaches can provide an integrated multi-omics viewpoint across many individual parameters that can generate an holistic understanding of pathogen biology in virulence-relevant conditions.

Biography: Stuart Cordwell is a graduate of the University of Sydney and was Senior Research Fellow, and Director, R&D at the Australian Proteome Analysis Facility, Macquarie University from 1999-2004. Since returning to the University of Sydney in 2005, he has been Sesqui Senior Lecturer, Associate Professor and since 2015, Professor of Analytical Biochemistry in the Schools of Life and Environmental Sciences and Medical Sciences. He is a member of the Charles Perkins Centre and is the Director of Sydney Mass Spectrometry. He has been involved in proteomics research throughout his career. He was an author on the manuscript that defined the term ‘proteome’ and established the first laboratory-based undergraduate proteomics course in Australia, in 2025 training >100 final year undergraduates in proteomics and multi-omics science. His research focuses on protein post-translational modifications (PTM) in bacterial and cardiovascular disease. He received the Selby Research Award for work on bacterial membrane proteins and a Danish VELUX Villum Kann Rasmussen Professorship for his work on cardiovascular PTM (undertaken at the University of Southern Denmark). In 2021, he was invited to deliver the Simpson Lecture by the Australasian Proteomics Society. He has published >150 papers with >8000 career citations. He has been invited to speak at many international conferences, including 8 HUPO World Congresses and was Co-Chair of the 2019 HUPO Congress in Adelaide, Australia. He is a Past-President of the Australasian Proteomics Society (serving from 2015-2020). He was Secretary-

General of HUPO from 2023-2024, and is currently a member of HUPO Council (2025-2027), and a Member-At-Large on the HUPO Executive Committee.

Hao, Ling
University of Maryland

Advancing Proteomics Strategies to Capture Lysosome Dynamics in Human Neurons.

Lysosomes function as the cell's waste-disposal and recycling system, degrading diverse intracellular and external cargo. In neurons, lysosomes also play unique roles in signal transduction, nutrient sensing, and axonal trafficking. Lysosomal dysfunction has been linked to numerous neurological disorders. Yet, capturing highly dynamic lysosomal activities remains challenging due to technological limitations. Here, I will present our recent advances in developing and improving multifaceted proteomics methods including proximity labeling, organelle isolation, and dynamic SILAC, to characterize the lysosomal microenvironment and protein turnover in human stem cell-derived neurons. By integrating proteomics, neurobiology, and bioinformatics tools, we aim to elucidate the molecular causes of brain diseases. I will also introduce our newly established contaminant libraries and contaminant-check assays for mass spectrometry workflows, providing practical tools for the broader proteomics community.

Biography: Dr. Ling Hao is an Associate Professor in the Department of Chemistry & Biochemistry at the University of Maryland (UMD), College Park. Before joining UMD, she was an Assistant Professor of Chemistry at the George Washington University. Dr. Hao received her Ph.D. from the University of Wisconsin-Madison in Prof. Lingjun Li's group and completed her postdoctoral training at the National Institutes of Health/National Institute of Neurological Disorders and Stroke (NIH/NINDS) in Dr. Richard Youle's group and Dr. Michael Ward's group. Research in the Hao Lab is at the intersection of analytical chemistry and neurobiology. Since starting her independent career, she has been recognized with several national/international awards, including an NSF CAREER Award, a Cottrell Scholar Award from the Research Corporation for Science Advancement, a Ralph E Powe Junior Faculty Enhancement Award from the Oak Ridge Associated Universities, and an inaugural Rising Star Award from the World Human Proteome Organization. She also serves on the Editorial Board of Molecular Omics journal as well as the Advisory Board of US-HUPO.

Abstract-selected talks

Li, Chongyang
Institute for Research in Immunology and Cancer, Université de Montréal, Montréal, Canada

Pascariu, Cristina Mirela 1, Bonneil, Éric 1, Courcelles, Mathieu 1, Lanoix, Joël 1, Baudouin, Claire 1, Emery, Gregory, Thibault, Pierre 1,2,3
1Institute for Research in Immunology and Cancer, Université de Montréal, Montréal, Canada,
2Department of Chemistry, Université de Montréal, Montréal, Canada,
3Department of Biochemistry and Molecular Medicine, Université de Montréal, Montréal, Canada

SUMO isopeptidase SENP6 reshapes ERE-derived antigen presentation via SUMOylation-mediated epigenetic modulation

Sentrin-specific protease 6 (SENP6) is a SUMO-specific isopeptidase that preferentially cleaves poly-SUMO2/3 chains, thereby maintaining SUMOylation homeostasis and preserving genome stability through the regulation of chromatin architecture. By deconjugating SUMO2/3 from chromatin associated substrates, SENP6 modulates nucleosome mobility and influences the activity of chromatin remodeling complexes, ultimately shaping epigenetic landscape. To map SENP6-dependent SUMOylation events, we performed label-free quantitative SUMO proteomics in HEK293S3m cells following SENP6 knockdown. This analysis identified 374 SENP6- regulated SUMOylation sites on 256 substrates. These include key histone modifying enzymes (EZH2, ATRX, SETDB1) and components of the KRAB-ZFP silencing machinery (ATRX, KAP1), implicating SENP6 as a central modulator of epigenetic repression. To assess the impact of SENP6 loss in a disease-relevant context, we used the human acute myeloid leukemia (AML) cell line THP-1. SENP6 knockdown led to a marked decrease in the repressive histone marks H3K9me3 and H3K27me3, without affecting total protein levels of EZH2, SETDB1, KAP1, or ATRX. Immunofluorescence analyses revealed nuclear relocalization of SETDB1, KAP1, and ATRX in SENP6-deficient cells, providing a mechanistic link to the observed chromatin changes. Transcriptome profiling of SENP6-depleted THP-1 cells showed enriched gene sets involved in epigenetic regulation and KRAB-ZFP-mediated silencing of endogenous retroelements (EREs). Notably, loss of SENP6 resulted in derepression of ERE transcripts and adjacent transcripts, reflecting a breakdown in retroelement silencing. To investigate the immunological consequences of these chromatin changes, we performed immunopeptidomic profiling of SENP6-deficient THP-1 cells. A total of 4,984 MHC class I-associated peptides were identified, including 99 derived from EREs, revealing that loss of SENP6 facilitates the presentation of previously hidden epitopes. Collectively, these findings establish SENP6 as a pivotal regulator of SUMOylation-driven epigenetic control and antigen presentation in AML. By linking chromatin remodeling to immune surveillance, this work highlights SENP6 as a

promising therapeutic target in cancers marked by epigenetic dysregulation and neoantigen generation.

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

Richard, Vincent, Segal Cancer Proteomics Centre, Jewish General Hospital, Montreal, QC, Canada; Geib, Timon, Segal Cancer Proteomics Centre, Jewish General Hospital, Montreal, QC, Canada; Kubiniok, Peter, Segal Cancer Proteomics Centre, Jewish General Hospital, Montreal, QC, Canada; Mohammed, Yassene, Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, Netherlands; Hornburg, Daniel, Bruker Daltonics GmbH & Co. KG, San Jose, CA, USA; Willetts, Matt, Bruker Daltonics GmbH & Co. KG, Billerica, MA, USA; Krupa, Gary, Bruker Daltonics GmbH & Co. KG, Bremen, Germany; Borchers, Christoph, Division of Experimental Medicine, McGill University, Montréal, QC, Canada/ Segal Cancer Proteomics Centre, Jewish General Hospital, Montreal, QC, Canada/ Gerald Bronfman Department of Oncology, McGill University, Montréal, QC, Canada.

NexProQ: Multiplexed Quantitation of 500+ Proteins in Whole Blood and Dried Blood Spots Using PRM-PASEF and TMTpro

Introduction: Quantitative proteomics in clinical samples have remained constrained by limited multiplexing capacity, high reagent costs, and low sample throughput. To address these limitations, we developed NexProQ, a novel platform combining TMTpro multiplexing with PRM-PASEF acquisition on the timsTOF HT. This approach enables us simultaneous absolute quantitation of over 500 protein targets in whole blood (WB) and dried blood spot (DBS) samples in a single run.
Methods: Proteotypic peptides were selected from 500+ clinically relevant targets and synthesized as unlabeled NAT standards. Five-point internal calibration curves were prepared using TMTpro 18-plex labels and peptide dilutions (1000× to 1× LLOQ). These were multiplexed with plasma and DBS digests on EvoTips and analyzed at 30SPD using PRM-PASEF. Assay performance was evaluated for linearity, detection limits, reproducibility, and matrix effects.
Results: Assay linearity was strong across targets ($R^2 > 0.98$) with median LLOQs between 0.1–0.5 fmol. Utilizing non-adjacent TMTpro reporter ions boosts quantitation reproducibility and decreases interference due to isotopic overlap. Intra-assay CVs were below 5% for most targets. Comparison with QE+ showed higher mass accuracy for reporter ions, while PRM-PASEF enabled faster throughput. Validation of a 274-peptide panel against MRM confirmed high concordance for abundant proteins.
Conclusion: NexProQ significantly enhances the scalability and accessibility of clinical proteomics, enabling high-throughput quantitation suitable for biomarker discovery, clinical validation, and large-scale cohort studies. Its performance surpasses current antibody- or aptamer-based platforms. A commercial kit is under development for cross-lab standardization. Keywords: Quantitative Proteomics, PRM-PASEF, TMTpro, Dried Blood Spot (DBS), Biomarker Discovery

Li, Shawn
Western University, London, Canada

Kaneko, Tomonori 1, Zhong, Shanshan 1
1Western University, London, Canada

Ultra-deep phosphoproteomics using sh2 superbinders: Applications in cancer research

Aberrant regulation of tyrosine kinases (TKs) plays a crucial role in the pathogenesis of various diseases, including cancer. The human genome encodes approximately 90 TKs, which collectively phosphorylate over 50,000 tyrosine residues. The vast number of pTyr sites that can coexist in a cell poses a significant challenge for their systematic identification and characterization. This challenge is further compounded by the low abundance of tyrosine phosphorylation relative to serine/threonine phosphorylation and the lack of efficient reagents to enrich pTyr for identification using mass spectrometry (MS). To address these challenges, we have developed novel reagents called SH2 Superbinders (SH2S), which can effectively capture and enrich pTyr -containing peptides prior to MS analysis. By combining SH2S with quantitative MS techniques, including TMT labeling and SILAC, we have created an SH2S affinity purification (AP)-MS/MS platform. This platform has been applied to both cellular and patient samples to: (1) map the landscape of the phosphoproteome and kinome; (2) assess dynamic changes in the phosphoproteome and kinome between normal and pathological states, or during disease progression; and (3) identify novel biomarkers or therapeutic targets for cancer metastasis or therapeutic resistance. Using the SH2S AP-MS/MS approach, we have identified kinome and phosphoproteome reprogramming as a central mechanism underlying of a host of conditions, including recurrence of head and neck squamous cell carcinoma, acquired resistance to tyrosine kinase inhibitors in breast cancer and leukemia, and T cell suppression by the programmed death 1 (PD-1). Therefore, our SH2S AP-MS/MS platform provides a powerful tool for the identification of tyrosine phosphorylation and TK activation at a depth and breadth unattained by conventional methods, offering novel insights into the pathogenesis, progression, and therapeutic responses in cancer.

Roux-Dalvai, Florence
AD Lab - Proteomics and Computational Biology laboratory

Bories, Pascaline 1, Lacombe-Rastoll, Antoine 1, Bérubé, Ève 2, Leclercq, Mickaël 1, Sa, Mariana 3, Thibeault, Marie-Ève 1, Bromirski, Maciej 4, Geddes-McAlister, Jennifer 3, Isabel, Sandra 2, Droit, Arnaud 1
1AD Lab - Proteomics and Computational Biology laboratory, CHU De Québec - Université Laval Research Center, Quebec, Canada, 2Infectiology Research Centre, CHU De Québec - Université Laval Research Center, Quebec, Canada, 3Molecular and Cellular Biology, University of Guelph, Guelph, Canada, 4Thermo Fisher Scientific, Bremen, Germany

MICROB-AI-R+ : combining advanced proteomic data and machine learning for the ultrafast identification of pathogens and their resistance profile

According to the World Health Organization, antimicrobial resistance (AMR) could become the
leading global cause of death by 2050. A key contributor to this crisis is the routine over-prescription of antibiotics, driven by slow diagnostic methods. Current gold-standard diagnostics require 24–48 hours due to bacterial culture prior to MALDI-TOF identification. In the meantime, patients receive broad-spectrum antibiotics, which promote resistance. To address this, our team developed MICROB-AI, a novel strategy combining LC-MS/MS with artificial intelligence to identify pathogens directly from clinical samples in under four hours, without culture. We expanded this approach to detect resistance to carbapenems, a last-line antibiotic class used against multidrug-resistant infections. Proteomic profiles of carbapenem-resistant and susceptible Enterobacteriaceae clinical isolates were generated using an Orbitrap Astral mass spectrometer operating in narrow DIA mode. Analyses were performed with both 20-minute (standard) and 5-minute (ultrafast for diagnosis purpose) liquid chromatography gradients, followed by DIA-NN data processing. Various antibiotic concentrations and incubation times were tested to capture proteomic shifts upon drug exposure. Machine learning algorithm (XGBoost) was then used to reveal peptide signatures linked to species identity and resistance genes. Notably, OXA-48-like resistance was associated with rapid changes—within 5 minutes—in proteins involved in transmembrane transport, ion homeostasis, stress responses, and metabolic adaptation, reflecting a rapid and coordinated response to changes in the environment, optimizing bacterial survival and growth. These changes enabled the identification of peptide signatures specific to resistance that could be monitored in very short targeted analyses in Parallel Reaction Monitoring (PRM) mode, suggesting a potential use for clinical diagnosis. Further analyses are underway to extend these signatures to other genes characterizing carbapenem resistance (NDM, KPC) and further optimize the MICROB-AIR+ method for ultrafast clinical diagnosis of pathogens and their resistance profiles without the need for culture.

Drabovich, Andrei
University of Alberta, Edmonton, Canada

Redefining Antibody Diagnostics for Viral Infections through Proteome-Wide Serology

Zoe Turner1, Yasmine Rais1, Anthony Chiu1, Assoc. Prof. Andrei Drabovich1

1Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Canada

Introduction: Serological diagnostics of viral infections relies on detecting specific antibodies
circulating in blood and biological fluids. Human serum antibodies form a highly diverse and dynamic mixture of isotypes, subclasses, and clonotypes that collectively shape the immune response. Conventional antibody diagnostics, typically based on indirect immunoassays, fails to capture the enormous heterogeneity of polyclonal antibodies and is limited by semi-quantitative measurements and insufficient standardization. Here, we introduce a Proteome-Wide Serology approach aimed at the identification and quantification of the full-scale depth and diversity of the human polyclonal antibody response.
Methods: Proteome-Wide Serology was validated with over 20 recombinant antigens representing Respiratory Syncytial Virus (RSV), human Metapneumovirus, SARS-CoV-2 and its variants of concern, and Influenza A H1N1. Serum, plasma, and saliva from over 500 individuals including pre-pandemic, convalescent, and general-population cohorts were analyzed. High-throughput immunoenrichments combined with shotgun proteomics identified the major IGHC isotypes and subclasses and the Fcbinding interactomes. Customized databases of variable regions provided the diversity of IGHV gene locus segments. Rapid targeted proteomic assays (>140 injections/day) and thoroughly designed internal standards enabled absolute quantification (ng/mL) of a complete set of human antibody isotypes (IgG, IgA, IgM, IgE, IgD) and subclasses (IgG1-4, IgA1-2).
Results: Serum levels of antigen-specific IgG1 (1-3 µg/mL) and IgA1 (0.1-0.3 µg/mL) were elevated across all viral infections. RSV elicited a unique response characterized by increased IgG2 and IgG4. Detection of circulating anti-RSV immunoglobulin D was the most significant discovery. Repertoire profiling of antibody variable regions revealed the usage of ~30 IGHV genes in polyclonal response. IgG1 autoantibodies against prostate-specific antigens were detected at markedly lower levels (3-30 ng/mL), demonstrating over a 1,000-fold dynamic range in the IgG1 immune response.
Conclusions: Proteome-wide serology will facilitate rational design of antibody diagnostics and offer novel tools to explore the complexity and diversity of the human polyclonal antibody response.

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Advancing proteomic discoveries with open modification search

Reference proteomes represent curated protein sets but fail to capture the full coding potential of genomes. Proteogenomics revealed thousands of non-canonical proteins, yet reproducible detection of non-canonical proteins by mass spectrometry remains limited. Open modification searches foster identifications through the detection of modified peptides, however the expanded search space combined with large databases hinders their widespread application in proteogenomics. Here, we present the first large-scale open modification search of non-canonical proteins. Ionbot, a machine learning-guided search engine, enables closed and open modification search with high PSM confidence irrespective of the database size. We applied the Trans-Proteomic Pipeline or ionbot for closed search, and FragPipe or ionbot for open search, on publicly available datasets using a common (TrEMBL) or a large proteogenomic database (OpenProt). Ionbot improved identification rates by 6.8% (TrEMBL) and 8% (OpenProt) with no FDR inflation (FPR<1.3%). In open search mode, Ionbot yielded a 2.2-fold increase in non-canonical peptide identification and increased multi-peptide (>=2) detection of non-canonical proteins from 10% to 17.6%. Spectra matched to non-canonical peptides were either unidentified (43.6%), matched to canonical peptides (54.6%), or to decoys (1.8%) when analyzed with the TrEMBL database. Yet, noncanonical PSMs showed significantly higher scores, ion correlations, and explained intensities than their alternative canonical match, highlighting the value of exhaustive databases and the contribution of non-canonical proteins to our proteomes. 85.4% of non-canonical proteins were identified with >=1 modified peptides. 78% of unique non-canonical peptides were identified only in their modified form, whereas only 30% of unique canonical peptides were. 60% of non-canonical peptides had modifications other than hydroxylation, acetylation or carbamidomethylation. They harbored distinct modification patterns, with significant differences in carboxymethylation, deamidation and formylation compared to canonical ones. Our study highlights the importance of exhaustive databases and open modification searches in advancing proteomic discoveries and understanding our biological systems.

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Mapping Protein Quality Control Networks: A Proteomics Approach to Chaperone and Degron-Driven Degradation

Mutations can destabilize proteins, leading to misfolding, degradation, and loss of function. In our recent survey of unstable cytosolic proteins carrying disease-relevant missense mutations, we found that degradation is primarily mediated by the ubiquitin-proteasome system. To identify factors involved in mutant protein turnover, we used BioID proximity labeling and quantitative proteomics to compare the interactomes of wild-type and misfolded variants. This revealed preferential enrichment of core chaperone machinery with the mutant proteins. Among these, the co-chaperone DNAJA2 emerged as a major interactor. Knockdown of DNAJA2 led to reduced levels of several misfolded proteins, suggesting enhanced degradation. While wild-type proteins were also affected, some mutant proteins were disproportionately destabilized, pointing to a buffering role for DNAJA2 that is more critical for specific mutants. Further analyses implicated Bag6 in the degradation of one mutant protein. Bag6 is known to mediate quality control of membrane and aggregation-prone proteins. We identified multiple embedded degron sequences within the misfolded protein, including three elements that drive Bag6-dependent degradation. These findings suggest that mutant proteins can contain redundant degrons, enabling parallel engagement of quality control pathways. Overall, this work highlights how proteomic profiling can uncover chaperone-substrate relationships and degron architectures that govern mutant protein fate.

Lightning talks

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

Introduction: Mass-spectrometry (MS) is commonly implemented for quantitative proteomics of clinical samples. Unfortunately, a lack of proper process controls and poor intra-/inter-laboratory reproducibility has hampered standardization of untargeted proteomic data. We are establishing a highly controlled and standardized untargeted proteomics workflow that will be validated in British Columbia and Nova Scotia and available to proteomics laboratories nation-wide as part of a pan-Canadian personalized medicine program.

Methods: Reproducibility, repeatability, and linearity are assessed at both sites based using best practices established by CPTAC (web portal). Peptide standards for known targets are used to assess linearity, limit of detection, and limit of quantification for known targets in a relevant background. Assay variability is assessed by evaluating the impact of matrix, assessing stability during storage, and reproducibility is assessed by a “5x5”.

Results: Sample preparation is a primary source of variability for proteomics MS experiments. Thus, establishing a workflow that involves minimal sample handling and reduces the potential for variability while maintaining accessibility is necessary for successful implementation of a standardized pan-Canadian workflow. MDA-MB-231 cells were grown and prepared as a FFPE standard for all laboratories. As protein extraction is the most variable procedure for FFPE tissue preparation, we evaluated methods for deparaffinization and extraction of protein from FFPE tissue: xylenes, “safe” xylenes, or hot water for deparaffinization and detergent lysis, acid lysis, or Covaris ™ sonication for protein extraction. Sonication provided the best protein extraction and coverage, resulting in the lowest sample-to-sample variation while eliminating the necessity for deparaffinization.

Conclusion: An effort is underway to establish a highly standardized and controlled workflow for untargeted quantitative proteomics from FFPE for a pan-Canadian personalized medicine program. This protocol is being tested for reproducibility, linearity, stability, sensitivity, and selectivity using established CPTAC guidelines by two separate Canadian laboratories and will be deployed in laboratories throughout Canada.

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Protein biomarkers discovery of lead (Pb) toxicity and adaptive response in House Sparrows from Australian Mining Towns

Lead (Pb) remains a pressing environmental concern in Australian mining and smelting towns such as Broken Hill and Port Pirie. Lead levels in these towns are significantly elevated due to continuous mining activity over the past 150 years. While high levels of lead exposure are toxic, chronic low-dose exposure subtly impairs fitness and reproduction in animals. House Sparrows (Passer domesticus) were introduced to Australia during early European settlement, and a small population established itself in the mining town of Broken Hill. This population has been thriving in the high-lead environment for the past 100 years. Remarkably, it survives with minimal adverse effects on fitness and reproduction. Our prior genomics research has revealed potential genetic resilience mechanisms. We are now investigating the proteomic pathways that may underlie these adaptations and serve as biomarkers of lead toxicity in blood. Mass spectrometry-based blood proteomics is challenging due to the high abundance of haemoglobin. To address this, we have optimised Mitra VAMS micro-sampling tips for avian blood proteomics. Our method works with as little as 2 µL of whole blood or frozen blood pellet, without compromising protein identifications. We identify over 1,500 proteins—six times more than standard plasma protocols—with a method median %CV of less than 20%. Using this protocol, we have analysed 100 samples from two mining towns via LC-MS. Our results indicate a distinct response to lead exposure in the Broken Hill population compared to others. These populations have been geographically separated for over 100 years, leading to independent evolutionary pathways in response to the same environmental stressor. Our analysis identified key proteomic shifts related to lead resistance, including the activation of protein renewal pathways and enhanced glutathione-mediated protection against reactive oxygen species.

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Quantification of celiac epitopes in oat cultivars by tandem mass spectrometry

Celiac disease is an autoimmune condition driven by specific gluten peptides primarily found in wheat, barley, and rye. While oats are often included in gluten-free diets, their safety for some celiac patients remains debated due to the presence of avenin proteins, which may contain immunoreactive sequences. To address this concern, this study applied a robust proteomics strategy to precisely detect and quantify oat-derived celiac epitopes using advanced tandem mass spectrometry. To achieve this goal, 38 cultivars were sourced from Brandon, Swan River, and Saskatchewan. Avenin proteins were isolated using an optimized Osborne extraction method, then enzymatically cleaved with chymotrypsin to generate peptides. Then the celiac epitopes of the peptides were identified and quantified using an Orbitrap ExplorisTM 480 LC-MS/MS system. Parallel analysis by ELISA measured total gluten levels for comparison. This untargeted LCMS approach detected three celiac epitopes, PYPEQQQPI, PYPEQQPF, and PYPEQQEPF, each expressed in multiple peptide forms. The relative peptide intensities varied significantly among cultivars, indicating genotype-dependent immunogenic potential. Douglas-Brandon exhibited the highest epitope intensity, whereas Hidalgo-Brandon showed the lowest. ELISA indicated total gluten amounts ranging from 5.19 mg/kg (Noranda-Brandon) up to 14.47 mg/kg (Triactor-Brandon). Correlation analysis showed limited alignment between the MS-based epitope quantification and ELISA gluten protein quantification, highlighting the specificity of the proteomic approach for determining immunoreactive sequences. This study demonstrates the suitability of tandem mass spectrometry in characterizing food allergens at the peptide level, supporting efforts to identify low-immunogenic oat varieties. These proteomic findings can guide breeding programs and product development to expand celiac-safe oat options.

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Real-time machine learning detection of protein contaminants in mass spectrometry analysis of affinity purification experiments

Mapping protein-protein interactions is critical to understand the inner workings of cells. A widely used method to detect these interactions is affinity purification, where a target protein is purified with its interacting partners. Mass spectrometry can then detect these interacting proteins by collecting their mass spectra for protein identification. Unfortunately, this approach suffers from poor identification sensitivity for low abundance and transient protein-protein interactions. This is partly due to data-dependent acquisition methods redundantly collecting superfluous mass spectra from abundant proteins at the expense of less abundant ones, which may be more biologically informative. Such abundant proteins also often mask low abundance ones in data-independent acquisition. Additionally, affinity purification is susceptible to contaminants that do not reflect true biological interactions yet further expend mass spectrometry resources for their identification. Herein, we designed a novel artificial intelligence pipeline that can guide mass spectrometry data acquisition to improve protein-protein interaction detection sensitivity in affinity purification. Specifically, we built a Bayesian inference model to identify contaminants in real-time, during mass spectrometry data acquisition, and prevent the instrument from acquiring data from them. Our tool uses 25 GFP-tag negative controls to estimate a baseline contamination abundance null model for each protein. These models are then used in real-time to evaluate whether a protein is significantly more present in an experiment than what is expected as background, thereby assessing experimental protein-protein interaction confidence. We benchmarked our model performances using 24 affinity purification experiments against the state-of-the-art contaminant detection software SAINT (Choi et al., 2011). Our software achieved slightly improved performances over SAINT, with the main advantage of being applicable in real-time to guide mass spectrometry data acquisition and prioritize data collection from bona fide interactions. Ultimately, our method enables a more efficient use of mass spectrometry resources to improve the detection of biologically meaningful protein-protein interactions.

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Discovery of novel 3'UTR regulatory structural motifs using protein colocalization Networks

Protein localization is regulated through various molecular mechanisms, including cis-regulatory elements within the 3' Untranslated Regions (3'UTRs) of mRNAs. These elements can mediate the transport of transcripts to specific subcellular compartments for translation, often acting in a structure-dependent 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 subcellular compartments. If the transcripts of such clustered proteins share a common 3'UTR structural motif, this motif could be directly or indirectly related to the proteins' regulation or localization. We therefore 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. To identify these motifs, we leveraged BayesPairing, a probabilistic RNA structural motif scanning tool (Sarrazin-Gendron et al. 2019), along with the curated RNA 3D Motif Atlas to annotate known structural motifs within 3'UTRs. We mined the Human Cell Map protein co-localization network (Go et al., 2021), identifying four structural motifs (false discovery rate<0.19) that were not detected by a state-of-the-art biological network clustering analysis tool, highlighting the novelty and discovery potential of our approach. Furthermore, proteins associated with three structural motifs showed enrichments for specific cellular compartments and biological processes, suggesting a direct or indirect role for the motifs in protein regulation or localization. All motifs tend to cluster at similar positions within 3'UTR sequences, hinting at their putative binding site role and functionality. Overall, our algorithm demonstrates that mining protein co-localization networks can uncover previously uncharacterized regulatory RNA structural motifs. This work illustrates the potential to leverage such networks to systematically explore post-transcriptional regulation of protein localization.

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The Hidden Cost of Vaping: How E-Cigarette Base Fluid Aerosols Rewire Lung Metabolism and Structure

The rapid increase in e-cigarette use (also known as vaping) has raised urgent questions about the safety of its core ingredients, particularly the carrier base fluids - propylene glycol (PG) and vegetable glycerin (VG). In this study, we examined the biological consequences of repeated inhalation of PG/VG aerosols in a Wistar rat model. Animals were exposed to aerosolized PG/VG or warm air (controls) for 10 minutes, three times per week over six months, followed by a three-month recovery period. Lung tissues were assessed using histopathological staining and quantitative proteomic analysis using a Waters M-Class nano-LC system coupled to a Waters Synapt XS HDMS. Out of 5917 identified proteins, 401 were significantly altered in the exposed group. These changes were strongly associated with dysregulated lipid metabolism, including activation of fatty acid metabolic pathways. Correspondingly, histological analysis revealed lipid-laden macrophages in bronchiolar and alveolar regions, suggesting a link between metabolic reprogramming and immune cell infiltration. Additionally, structural hallmarks of early emphysematous and interstitial lung changes were observed, including localized alveolar destruction, alveolar septal thickening, and fibrotic remodeling. Proteomic signatures further indicated disrupted protease/antiprotease balance and activation of fibrosis-related pathways. Together, these findings contribute to a growing body of evidence suggesting that e-cigarette carrier fluids have harmful physiological effects independent of nicotine or flavoring additives and are sufficient to trigger metabolic, inflammatory, and structural damage in the lungs.

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Stool Proteomic Biomarker Discovery for Resolving the Diagnostic Gray Zone in Inflammatory Bowel Disease

Background: Monitoring inflammation activity in Inflammatory Bowel Disease (IBD) is essential for guiding treatment and preventing long-term complications. While fecal calprotectin is a common noninvasive biomarker, its diagnostic reliability declines significantly within the “gray zone” (50 –300 µg/g), limiting its clinical utility. To address this challenge, we developed a stool -based proteomic biomarker panel for precise classification of inflammation activity in this diagnostically ambiguous range.

Methods: We analyzed 155 stool samples from IBD patients for model training and reserved 53 samples for blind testing. The proteomic profiling was performed using SWATH-MS, a data-independent acquisition (DIA) mass spectrometry technique known for its reproducibility and depth. Protein- and peptide-level datasets were preprocessed separately. Feature selection was conducted using Boruta, LASSO, RF, and RFE. Features identified consistently across both data levels were prioritized. Six machine learning models (SVM, Random Forest, Naïve-Bayes, KNN, GLMnet, and XGBoost) were evaluated with 10-fold cross-validation, focusing on gray zone performance. Model interpretability was assessed using SHAP values and GO enrichment analysis explored the biological relevance of selected features.

Results: We identified 19 protein-level and 14 peptide-level discriminatory features, with five robust overlapping markers selected for final modeling. The Support Vector Machine (SVM) model achieved the highest performance: 0.96 precision and 0.88 recall during training, and 1.00 precision and 0.86 recall in blind testing. SHAP analysis confirmed biomarker contribution, and enriched GO terms were linked to immune and inflammatory pathways.

Conclusion: This proteomic signature offers a promising non-invasive tool for resolving diagnostic uncertainty in IBD monitoring within the gray zone

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Unbiased protein correlation host-pathogen interactome mapping for Cryptococcus neoformans

Protein-protein interactions (PPIs) mediate disease development, including those between the World Health Organization's priority fungal pathogen, Cryptococcus neoformans, and macrophages. Current methods used to characterize such interactions present technical limitations that restrict analysis to a single protein of interest. Therefore, new techniques that can globally assess these PPIs are needed to obtain a comprehensive knowledge of mechanisms driving cryptococcosis. As a result, I am developing an unbiased approach based on co-fractionation and mass spectrometry to detect and quantify a higher number of PPIs between the pathogen and the host, as well as within each biological system. To date, I have established an in vitro co-culture model for C. neoformans with immortalized macrophages and optimized protein extraction to identify proteins while preserving PPIs. I have also applied size exclusion chromatography to separate proteins within a sample and propose a 'ruler' of protein fingerprinting for each biological system in the presence and absence of infection. A total of 4207 unique proteins were identified among C. neoformans secretome (216) and proteome (1699), uninfected (1838) and infected (2240) macrophages. The infected samples contained 29 unique cryptococcal proteins, of which 20 were also found in the C. neoformans-only samples. Five of these proteins, including the virulence-associated Cipc, showed shifts in the fractionation profiles and a significant increase in the infected proteome. Another 10 non-shifting proteins were upregulated in the infectome, including stress response-associated proteins and PPIases. These results demonstrate proof-of-concept of this novel method, and the identified PPIs will be further validated using co-immunoprecipitation coupled with mass spectrometry and Förster resonance energy transfer. Ultimately, this project contributes to the detection of interacting proteins driving cryptococcal infection. Such PPIs could then be used as targets for drug development and repurposing to combat infection.

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Redundant and Divergent Functions of Proline-rich Coiled-Coil Intrinsically Disordered Proteins

Introduction: The structure-function paradigm proposes that a protein's stable, three-dimensional structure dictates its function. Yet, about 1000 proteins in the human proteome do not possess domains, and their roles remain poorly understood. The Proline-Rich Coiled-Coil A, B, and C paralogs (PRRC2s) are highly conserved intrinsically disordered proteins found across jawed vertebrates. Few studies have implicated PRRC2s in regulating translation. PRRC2s are predicted to localize to stress granules (SGs), a biomolecular condensate that forms when cells are stressed and translation is arrested. However, we lack an understanding of how PRR2Cs function in translation and the extent of their shared functions as paralogs.

Methods: We integrated proteomics, microscopy and genetics to characterize the function of PRRC2 paralogs at steady state and during stress, when translation is arrested. We defined their protein interactors via proximity labelling and affinity purification coupled to mass spectrometry using data-independent acquisition. Using microscopy, we characterized their localization at steady state and upon stress. Finally, we utilized CRISPR/Cas9-mediated knockout cells to examine alterations to the proteome and the functional redundancy of the paralogs.

Results: Functional proteomics revealed that all PRRC2s strongly associate with translational machineries, suggesting they have redundant functions in translation. PRRC2B uniquely shows stress-specific interactions with proteins involved in transcription regulation and DNA damage. We find all PRRC2s partition to SGs, and loss of each PRRC2 protein compromises efficient SG assembly. PRRC2 single knockouts show a moderate decrease in fitness, while triple knockouts display a severe defect, suggesting functional redundancy in cell fitness.

Conclusions: Despite their lack of stable domains, PRRC2 proteins share functions required for cell fitness. PRRC2B uniquely associates with proteins involved in transcription and DNA damage, suggesting functional divergence. We propose the PRRC2s' physical association with translation machineries is important for cell growth and upon cellular stress, partitions into SGs to facilitate SG assembly.

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A Yin-Yang Dichotomy: Temporal and Spatial Rewiring of TCR Signaling by PD-1 in T Cells

Immune checkpoint blockade targeting Programmed Cell Death Protein 1 (PD-1) has revolutionized cancer immunotherapy, yet the molecular mechanisms of PD-1–mediated T cell suppression remain poorly resolved. Here, we employ stable isotope labeling by amino acids in cell culture (SILAC) combined with tandem mass tag (TMT) multiplexed phosphoproteomics to systematically dissect the spatiotemporal dynamics of TCR and PD-1 signaling in a co-culture model of Jurkat T cells and Raji B cells. This high-throughput approach quantified over 15,000 phosphorylation sites in T cells, revealing that T cell receptor (TCR) activation induces widespread and time-dependent remodeling of the phosphoproteome, including oscillatory patterns in key signaling nodes. Strikingly, PD-1 engagement actively reprograms the kinome and phosphoproteome—modulating both proximal TCR signaling and intracellular pathways involved in mRNA splicing and RNA processing. This extensive rewiring uncovers a broader immunoregulatory role for PD-1 in T cells, extending well beyond simple suppression of TCR signal transduction. In parallel, we identify a distinct spatial regulation of signaling: PD-1 selectively modulates proximal signaling events driven by tyrosine phosphorylation—shaping early membrane-associated networks—while exerting minimal effects on distal signaling cascades governed by serine/threonine phosphorylation. This reveals a “Yin-Yang” temporal dichotomy, where PD-1 precisely modulates activation thresholds without globally arresting effector functions. Altogether, our work offers a mechanistic basis for improving immunotherapeutic strategies by targeting discrete phases of T cell activation and may inform combination treatments that optimize T cell functionality in the tumor microenvironment.

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Controllable Peak Detection In Data Independent Acquisition Mass Spectrometry Extracted Ion Chromatograms Using Disentangled Tokens and Cross Attention Transformers

Background : Data Independent Acquisition Mass Spectrometry (DIA-MS) balances the high throughput of Data Dependent Acquisition (DDA) and the reproducibility of targeted methods. The complexity and volume of data from DIA requires preprocessing steps like peak detection, peak scoring, and retention time alignment, yet challenges persist in ensuring consistency across multiple runs/experiments.

Methods: Current peak identification algorithms are limited to single runs and may inconsistently call identified peaks of the same precursor across runs without alignment. We developed a neural network architecture featuring a novel cross-attention based token mechanism and hierarchical layers to decouple related but potentially conflicting tasks (e.g. peak detection vs. ranking). This enables explicit information transfer across runs/experiments, and displays emergent capabilities such as the abilities to: modulate the output score distribution post-training, support arbitrary data from sources like the target library/input sample/additional signal traces as input/conditioning information, and perform internal “soft”-alignment/consistently identify peaks not only across runs/experiments but also through changes in elution order.

Results: We benchmarked on a subset of the LFQBench mixed proteome dataset (Human, Yeast, and E. coli at varying proportions), where our model achieved competitive peptide identification rates as DIA-NN, but better quantification accuracy (tighter log change ratios/lower absolute bias). We also evaluated performance on a synthetic phosphopeptide dataset with manual annotations, where we achieved significant performance gains over OpenSWATH, which struggled due to the existence of confusing signals from peptide isoforms.

Conclusion: Our end-to-end neural network streamlines peak identification and cross-run analysis, reducing errors inherent in multi-step processes by explicitly modelling them. It also demonstrates high levels of adaptability, by being able to easily incorporate arbitrary data modalities to enhance performance and knowledge transfer across runs and experiments. Coupled with drastically improved inference times post-training for large scale datasets, it paves the way for more scalable, controllable, and interpretable DIA data analysis.

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scPROT: Context-Specific Embedding of Proteins and Cells through Integration of Single-Cell Proteomics with Prior Knowledge

While transcriptomics reveals the potential for gene expression, proteomics provides a direct readout of the cell's physiological state and dynamic regulatory activities. Understanding cellular function therefore requires direct quantification of proteins—the key drivers of biological processes—rather than relying on mRNA levels, which often fail to capture post-transcriptional regulation, interaction networks, or protein activation states. Unlike bulk proteomics, single-cell proteomics (SCP) further enables the identification of rare subpopulations and transient states, providing critical insights into cellular heterogeneity and dynamic molecular function. Nevertheless, SCP datasets remain highly sparse and noisy, posing challenges for downstream applications such as imputation, functional analysis, and biological interpretation. To address this, we developed scPROT, a biologically informed, context-aware framework that integrates SCP data with diverse prior knowledge—including protein–protein interaction networks, gene ontologies, pathways, and cell-type markers. scPROT uses dual-view encoders and attention mechanisms to learn robust embeddings for proteins and cells, enabling accurate reconstruction of expression patterns and cell clusters. We evaluated its performance on peripheral blood mononuclear cell (PBMC) datasets using a mask-and-recover strategy, comparing against baseline imputation methods such as Random Forest. scPROT significantly reduced prediction error and improved agreement with ground-truth protein expression, highlighting its ability to recover biologically meaningful protein states. It also identified rare cell subpopulations and dynamic transitional states which were undetectable by both bulk proteomics or single-cell RNA sequencing (scRNA-seq). In summary, scPROT provides a biologically grounded, context-aware framework for denoising SCP data and recovering meaningful protein signals, highlighting the unique value of single-cell proteomics in revealing functional cellular heterogeneity.

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Quantitative Proximal Interaction Profiling of TDP-43 Variants Reveals Its Functions in DNA Metabolism

Introduction: TAR DNA-binding protein 43 (TDP-43) is an essential, multifunctional DNA/RNA-binding protein whose nuclear depletion and cytosolic aggregation in motor neurons typifies the fatal neurodegenerative disease, amyotrophic lateral sclerosis (ALS). The contribution of TDP-43 to normal physiological activity and ALS pathogenesis is incompletely understood. To functionally characterize TDP-43, we utilized a functional proteomics approach to compile proximal interactome profiles for wild-type TDP-43 and for variants affecting functionally relevant sequence features.

Methods: We surveyed the proximal interaction networks of ALS-associated TDP-43 point mutants as well as mutations affecting DNA/RNA binding, redox-sensitive cysteines, and predicted phosphorylation sites. Given the role of oxidative stress in ALS pathogenesis, we also examined the impact of this stress on the TDP-43 proximal interactome. We performed proximity-dependent biotinylation using HEK293 stable cell lines inducibly expressing TDP-43 variants fused to the abortive biotin ligase miniTurbo. Biotinylated proteins were purified and analyzed on an Orbitrap Exploris 480 mass spectrometer operated in data-independent acquisition mode.

Results: We observed that wild-type TDP-43 associates with nuclear DNA- and/or RNA-binding proteins with roles in RNA splicing, RNA transport, and transcription. Relative to wild-type TDP-43, ALS-associated mutants exhibit subtle and variable changes in their proximal interactomes. We note a significant reduction in proximal associations with RNA-binding proteins in cells experiencing oxidative stress, as well as with TDP-43 variants that are unable to bind DNA/RNA, have redox-insensitive regulatory cysteines, or harbor phosphomimetic mutations. This coincides with increased associations between TDP-43 and DNA-binding proteins, including those involved in DNA damage responses, chromatin remodeling, and the resolution of DNA:RNA hybrids known as R-loops.

Conclusions: Overall, we demonstrate the utility of proximity-dependent labeling as a tool for monitoring the interactome landscape of a multifunctional protein altered by different genetic contexts. We are currently working to validate specific proximal interactors and examine their functional relevance to cellular stress responses.

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Developing Novel Potent and Specific Pin1 Inhibitors to Probe the Molecular Mechanisms of Tumour Resistance in Pancreatic Ductal Adenocarcinoma

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and lethal cancers, and is notoriously hard to treat, owing to a highly immunosuppressive tumour microenvironment (TME). Remarkably, by targeting the prolyl isomerase Pin1, PDAC can be rendered eradicable by immunochemotherapy. Pin1 is overexpressed in cancers where it drives pathogenesis through regulating the conformation, activity, and stability of over 70 oncogenes and 30 tumour suppressors. In PDAC, Pin1 contributes to maintenance of the immunosuppressive TME; however, the precise mechanism by which Pin1 inhibition sensitizes PDAC to immunochemotherapy is unknown. In this study, we present a novel potent Pin1 inhibitor and degrader, and evaluate its mode of binding, cellular engagement, cellular activity, and specificity. The novel Pin1 inhibitor (PI-1) has nanomolar affinity and was discovered using fluorescence polarization screens. Treatment of MIA-PaCa2 PDAC cells with PI-1 revealed rapid and potent degradation of Pin1 within 12 hours. To verify that PI-1 was binding to intracellular Pin1, parallel reaction monitoring was performed to quantify levels of PI-1 modified and unmodified cysteine containing Pin1 peptides in treated cells. Results indicate PI-1 significantly modifies Pin1 Cys113-containing peptide, while Pin1 Cys57-containing peptide is not modified, suggesting specificity of PI-1 for Cys113. To further assess the cellular activity and specificity of PI-1, cells were treated for 18 hours, then lysed, digested, and injected for DIA LC-MS/MS. Results indicate Pin1 is the most significantly downregulated protein across 6279 identified proteins, suggesting that PI -1 specifically induces degradation of Pin1. To further assess specificity, we aim to perform thermal proteome profiling and reactive cysteine profiling to uncover potential off-target proteins. This study highlights the potency and specificity of PI-1, supporting its use as a molecular tool to probe the molecular mechanisms of tumour resistance in PDAC. Furthermore, the combination of PI-1 and immunochemotherapy presents a novel approach to treat PDAC.

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Discovery and Quantification of the Human Polyclonal Antibody Response Against Viral Antigens

1. Introduction: Indirect immunoassays are the only current method to quantify endogenous polyclonal antibody responses, offering simplicity and high throughput but limited by semi - quantitative measurements, cross-reactivity, and lack of isotype/subclass detail. Advances in mass spectrometry and proteomics now enable 'Precision Serology'—a more precise approach to discovering, quantifying, and characterizing antigen-specific polyclonal antibodies in serum

2. Methods: We present an LC-MS–based workflow combining immunoaffinity enrichment with both targeted (6500+ QTRAP) and shotgun (Orbitrap Elite) mass spectrometry to quantify and characterize antigen-specific polyclonal antibodies from serum. Using heavy-labeled peptide standards targeting antibody heavy chains, we achieved absolute quantification across all human isotypes and subclasses. While the method is broadly applicable, we focused on key respiratory viruses—respiratory syncytial virus (RSV), Influenza A subtype H1N1, SARS-CoV-2, and human metapneumovirus (hMPV). The streamlined experimental design enables robust, sensitive, and high throughput measurements, advancing precision serology.

3. Results: RSV F protein (RSVF) was selected as the model antigen for method development due to the consistent presence of RSVF-specific antibodies in all serum samples tested. In total, 215 samples were analyzed, revealing predominant responses from IgG1 (1709 ng/mL), IgA1 (237 ng/mL), and IgM (110 ng/mL). Interestingly, the rarer isotypes IgG4 (17 ng/mL) and IgD (1.2 ng/mL) were detectable in response to RSVF but not to other RSV or SARS-CoV-2 antigens, suggesting a distinct and specific immune response. Co-precipitation analyses also identified significant enrichment of complement components C1qA, C1qB, C1qC, and CD5L. Interestingly, antigens of influenza A subtype H1N1, hMPV, and SARS-CoV-2 revealed a conventional immune response dominated by IgG1 and IgA1 isotypes.

4. Conclusions: Further developments of IA-MS assays will allow for in-depth characterisation of pathogen-specific polyclonal antibodies and reveal the precise mechanisms of antibody-mediated immune response.