Proteomics: from protein structures to clinical applications

4th Annual Symposium
Canadian National Proteomics Network
April 23-25, 2012
MTCC 255 Front St. W., Toronto, Canada

Sponsored by:
On behalf of the Organizing Committee, it is a pleasure to welcome all participants of the Forth Annual Canadian National Proteomics Network Symposium on “Proteomics: From Protein Structure to Clinical Application”. This symposium will present cutting-edge research contributions in proteomics and its application to cancer research. This international event will also feature two specialized workshops on mass spectrometry imaging and targeted proteomics using multiple reaction monitoring. In this symposium, renowned scientists will share recent findings on the use and applications of proteomics to unveil important molecular mechanisms and protein biomarkers of importance in cancer and disease development. Four thematic sessions (e.g. chemical and structural proteomics, protein modifications and cell signaling, protein interactions network, and translational proteomics) are directly related to cancer research and one session is dedicated to the emerging area of cardiovascular proteomics. Through major breakthrough in mass spectrometry and affinity purification strategies, proteomics has provided novel insights in cell biology, and different oral and poster presentations will feature emerging technological developments in these fields. In addition to the different scientific presentations, this symposium will also provide the opportunity to present the recipient of the 2012 CNPN distinguish research award and to acknowledge the contributions of future investigators during an award ceremony that will take place on the evening of April 24. While international in breadth and impact, this symposium is intended to favor collegial spirit where researchers can engage in active discussions in a stimulating environment and foster collaborations in proteomics research. We are delighted to host this venue, and to share with you the outstanding contributions of our presenters.

Have a great meeting,

Pierre Thibault
Program chair
2012 CNPN Symposium

CNPN is a not-for-profit federally incorporated organization created to provide a cooperative mechanism for building a proteomics research infrastructure in Canada to further a better understanding of proteomics in the Canadian life sciences community. The CNPN sponsors scientific conferences, seminars and forums to create a national focus for scientific collaboration and education.
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### Oral presentations

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### Posters presentations

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Monday April 23

WORKSHOPS

8:00 Registration

Targeted proteomics analyses; Hands-on MRM – room 205 B

8:30 Welcome by co-chairs
Christine Miller, Agilent Technologies
Derek Smith, University of Victoria

8:35 Development of MRM-based Assays
Derek Smith, University of Victoria

10:10 Break

10:30 Improved Sensitivity of Plasma Protein MRM Quantitation with Reduced sample Loading
Christine Miller, Agilent Technologies

12:30 Lunch

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thinkforward
Monday April 23

WORKSHOPS

8:00  Registration

Mass spectrometry-based tissue-imaging: improving diagnostic and health care – room 205 D

8:30  Welcome by co-chairs
Pierre Chaurand, Université de Montréal
Christoph Borchers, University of Victoria
Vassilios Papadopoulos, McGill University Health Centre

8:35  MALDI imaging: Adding a Molecular Dimension to Histology
Sören-Oliver Deininger, Bruker Daltonics

9:05  Molecular Imaging of Tissue Sections by Mass Spectrometry: Looking Beyond the Microscope
Richard Caprioli, Vanderbilt University School of Medicine

9:35  Application of MALDI imaging in clinical proteomics research of gastric cancer
Benjamin Balluff, University of Munich

10:05 Coffee break

10:20  Peptide Tissue Imaging Mass Spectrometry for Easy Identification of Regulated Proteins via Complementary LC-MS/MS Data
Peter Hoffmann, University of Adelaide

10:50  Exploring the Potential of Phospholipids for Histological Classification by MALDI Imaging MS
Pierre Chaurand, Université de Montréal

11:20  MALDI Imaging MS: Bridging Biology and Chemistry in Drug Development
Stephen Castellino, GlaxoSmithKline

11:50  Mass Spectrometry Imaging under Ambient Conditions
Demian Ifa, York University

12:20  The Canadian Imaging Mass Spectrometry Consortium initiative
Christoph Borchers, University of Victoria

12:30 Lunch
### Monday April 23

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<th>Time</th>
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<tr>
<td>13:30</td>
<td>Welcome address — room 205 BD – Pierre Thibault, Program Chair</td>
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<tr>
<td>13:40</td>
<td><strong>Recent advances in cardiovascular proteomics</strong></td>
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<td></td>
<td><strong>Parveen Sharma, University of Toronto</strong></td>
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<tr>
<td>14:20</td>
<td><strong>MRM-based Quantitative Analyses of Mitochondrial Protein</strong></td>
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<td><strong>Phosphorylation in Murine and Human Heart</strong></td>
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<td>Maggie Pui Lam, University California Los Angeles</td>
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<tr>
<td>14:40</td>
<td><strong>Discovery of Cardiovascular Disease Biomarkers in Human Plasma</strong></td>
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<td><strong>using MRM-based Multiplexed Quantitation</strong></td>
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<td>Gabriela Cohen-Freue, PROOF Centre of Excellence</td>
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<td>15:00</td>
<td><strong>Targeted Protein Quantification using High-resolution MS</strong></td>
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<td><strong>--&gt; MS/MS Transitions to Validate MicroRNA Targets Related to Heart Failure</strong></td>
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<td></td>
<td>Johannes Hewel, University of Toronto</td>
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<td>15:20</td>
<td>Break</td>
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<tr>
<td>15:40</td>
<td><strong>Caspase / Metacaspase Control of Protein Aggregation</strong></td>
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<td><strong>During Cell Adaptation</strong></td>
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<td><strong>Lynn Megeney, Ottawa Hospital Research Institute</strong></td>
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<tr>
<td>16:20</td>
<td><strong>Detection of Novel Myosin- or Cardiac-associated Proteins in the</strong></td>
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<td><strong>Arteries and Veins of Human Subjects During Myocardial Infarction</strong></td>
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<td></td>
<td>Amir Ravandi, Ryerson University</td>
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<tr>
<td>16:40</td>
<td><strong>Plasma Protein Analyses for Assessment of Atherosclerotic Process</strong></td>
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<td><strong>in ApoE Deficient Mice Exposed to Ozone</strong></td>
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<td>Dharani Das, Health Canada, Mechanistic Studies Division, EHC, HECSB</td>
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<td>17:00</td>
<td><strong>New Matrix Candidates for High Spatial Resolution Imaging</strong></td>
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<td><strong>Mass Spectrometry of Lipids</strong></td>
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<td></td>
<td>Aurélien Thomas, Université de Montréal</td>
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<tr>
<td>17:20</td>
<td>CNPN annual assembly – room 205 BD</td>
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<td>18:00</td>
<td>Poster viewing – room 206</td>
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<td>19:00</td>
<td>Reception and mixer – room 206</td>
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Confidence means superior performance and reproducibility across your entire LC/MS-based protein quantitation workflow — from sample preparation to LC/MS analysis.

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<tr>
<td>8:00</td>
<td>Registration</td>
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<tr>
<td>8:30</td>
<td><strong>CHEMICAL &amp; STRUCTURAL PROTEOMICS</strong> — room 205 BD</td>
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<td></td>
<td>Session Chair: Christoph Borchers, UVic-GBC Proteomics Centre</td>
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<tr>
<td>8:30</td>
<td><strong>MS23D: From Mass Spectra to 3D Protein Structures</strong></td>
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<td>David Wishart, University of Alberta</td>
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<td>9:10</td>
<td><strong>Multiple Structural Proteomics Approaches for the Characterization of Protein Complexes</strong></td>
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<td>Evgeniy Petrotchenko, UVic-GBC Proteomics Centre</td>
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<tr>
<td>9:30</td>
<td><strong>Steric and Allosteric Factors Contribute to Instability of the Transferrin Binding Protein Ternary Complex</strong></td>
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<td>Leslie Silva, MD Anderson Cancer Center</td>
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<td>9:50</td>
<td><strong>Kinetic Folding Mechanism of A1-Antitrypsin Probed by Hydroxyl Radical Labeling</strong></td>
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<td>Bradley Stocks, University of Western Ontario</td>
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<td>10:10</td>
<td>Break</td>
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<tr>
<td>10:30</td>
<td><strong>Structural Biology by Mass Spectrometry: 3D Proteomics of Supramolecular Assemblies</strong></td>
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<td>Juri Rappsilber, University of Edinburgh</td>
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<td>11:10</td>
<td><strong>Approach for Large-scale Identification of Linked Peptides From Tandem Mass Spectra</strong></td>
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<td></td>
<td>Jian Wang, University of California, San Diego</td>
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<tr>
<td>11:30</td>
<td><strong>Structural Characterization of the SH3 Domain of AHI-1 in Regulation of Cellular Resistance to Tyrosine Kinase Inhibitors in BCR-ABL-transducedLeukemia Cells</strong></td>
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<tr>
<td></td>
<td>Xiahu Liu, British Columbia Cancer Agency</td>
</tr>
<tr>
<td>11:50</td>
<td><strong>Fetal Liver Tyrosine Kinase 3 Receptor (CD135) is a Co-receptor of the Fc Receptor Complex and its Ligand Regulates IgG-mediated Phagocytosis</strong></td>
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<td></td>
<td>Lauren Ago, Ryerson University, Dept. of Chemistry and Biology</td>
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<td>12:10</td>
<td>Lunch and Tech talk (Thermo Fisher) – Summit Room</td>
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<td><strong>Mass Spectrometric Immunoassays</strong></td>
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<td>Dobrin Nedelkov, Thermo Fisher Scientific</td>
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<td><strong>Practical Discovery to Quantitation Workflows in the Proteomics Laboratory</strong></td>
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<td>Paul Taylor, Hospital for Sick Children</td>
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You are ready

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## Tuesday April 24

### PROTEIN MODIFICATIONS & CELL SIGNALING

— room 205 BD  
Session Chairs: Gilles, Lajoie, University of Western Ontario  
& Pierre Thibault, IRIC, Université de Montréal

**13:30**  
**Invited Speaker**  
*Interrogating a Single Cell Type: From the Genome to the Proteome and Everything Else in Between…*  
Akhilesh Pandey, Johns Hopkins University

**14:10**  
*Comprehensive Analysis of Cancer Somatic Mutations in Phosphorylation Signaling Predicts Novel Cancer Driver Genes and Pathways*  
Juri Reimand, University of Toronto, Donnelly Centre

**14:30**  
*The SH2 Domain and the Phosphotyrosine Signaling Network*  
Shawn Li, University of Western Ontario

**14:50**  
*Systematic Chemical Proteomics and Functional Proteomics Strategies for Validation of Protein Kinase Inhibitors: Application to Unbiased Evaluation of Inhibitors of Protein Kinase CK2*  
David Litchfield, University of Western Ontario

**15:10** Break

**15:30**  
**Invited Speaker**  
*Weak Interactions, Dynamics and Allostery in the Ubiquitin System*  
Mike Tyers, IRIC, Université de Montréal

**16:10**  
*A Targeted Proteomic Approach for the Study of the Human Acetylome*  
Jean-Philippe Lambert, Samuel Lunenfeld Research Institute

**16:30**  
*Protein Termini and Their Modifications Identified by Positional Proteomics and Linked to Protein Function in Cancer*  
Philipp Lange, University of British Columbia

**16:50**  
**Invited Speaker**  
*Mapping the Universal Animal Protein Interactome*  
Andrew Emili, University of Toronto

**17:30** Poster viewing – room 206

**18:30** CNPN distinguish award presentation & invited lecture – room 205 BD

**19:15** Dinner and student/PDF awards presentation – Summit Room
Dr. Michel Desjardins, recipient of CNPN’s Award for Outstanding Contribution and Leadership to the Canadian Proteomics Community

We are pleased to announce the 2012 recipient the CNPN Distinguished Researcher Award Dr. Michel Desjardins of the Université de Montréal. This prize recognizes the remarkable achievement on the fundamental understanding and/or practice of proteomics in biological sciences. Dr. Desjardins’ pioneering work on subcellular proteomics led to the first molecular definition of the phagosome, an organelle that evolved from a phagotrophic compartment into a cellular structure fully competent for antigen presentation. His seminal contributions provided further understanding of the molecular mechanisms conferring specialized functions to mammalian phagosomes linking innate and adaptive immunity.

Award lecture:
How proteomics shaped our understanding of phagosome biogenesis and function

Phagocytosis, the process by which cells eat other cells, plays key roles in a variety of biological processes in mammals including embryogenesis, tissue remodeling, and immunity. Remarkably, the molecular mechanisms allowing phagocytosis to exert these various functions have largely been inherited from unicellular organisms where phagocytosis is used as a feeding mechanism. In my presentation I will highlight how proteomics has had, and continues to have, a significant impact on our understanding of phagosomes. From the handful of proteins identified on this organelle by Edman sequencing in the early 1990’s, to the quantitative data following several hundred proteins in our current analyses, proteomics is unveiling novel aspects of the molecular mechanisms regulating the role of phagosomes in immunity.

Travel award recipients

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<th>Young investigators</th>
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<td>Jordan Klaiman, University of Guelph</td>
<td>Danielle Caron, Université de Montréal</td>
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<td>Jessie Lavoie, University of Ottawa</td>
<td>Cheng-Kang Chiang, University of Ottawa</td>
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<td>Xiaohu Liu, BC Cancer Research Center</td>
<td>Philipp Lange, University of British Columbia</td>
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<tr>
<td>Peter Podobed, University of Guelph</td>
<td>Huadong Liu, University of Guelph</td>
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<tr>
<td>Abul Sardar, McGill University</td>
<td>Leslie Silva, University of Calgary</td>
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<td>Krishnaraj Tiwari, University of Guelph</td>
<td>Ming Zhang, Université Laval</td>
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<td>Aurélien Thomas, Université de Montréal</td>
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<td>Andrew Chambers, University Victoria</td>
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<td>Andrew Percy, University Victoria</td>
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Sponsored by:

[Genome British Columbia]
[Genome Canada]
### Wednesday April 25

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<tr>
<td>8:00</td>
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<tr>
<td>8:30</td>
<td><strong>Navigating the Landscape of Integral Membrane Proteins Interaction:</strong></td>
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<td>Applications for Human Health and Disease</td>
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<td></td>
<td>Igor Stagliar, Terrence Donnelly Centre for Cellular and Biomolecular</td>
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<td></td>
<td>Research, University of Toronto</td>
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<td>9:10</td>
<td><strong>Towards the $1,000 Interactome</strong></td>
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<td>Nozomu Yachie, University of Toronto</td>
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<td>9:30</td>
<td><strong>Systematic Genetic Perturbation of Protein Complexes and Modules</strong></td>
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<td>in Living Cells</td>
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<td>Christian Landry, Université Laval</td>
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<td>9:50</td>
<td><strong>Exploring the Relationship Between Genetic and Protein-protein</strong></td>
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<td>Interaction Networks</td>
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<td>Michael Costanzo, Banting and Best Department of Medical Research</td>
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<tr>
<td>10:00</td>
<td><strong>Break</strong></td>
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<tr>
<td>10:30</td>
<td><strong>Mass Spectrometric Methods to Measure Dynamic Protein</strong></td>
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<td>Interaction Networks</td>
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<td>Ruedi Aebersold, ETH Zurich</td>
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<td>11:00</td>
<td><strong>Mapping the PP1 Phosphatome</strong></td>
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<td>Laura Trinkle-Mulcahy, University of Ottawa</td>
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<tr>
<td>11:30</td>
<td><strong>A New High-throughput Approach to Detect Protein Complexes</strong></td>
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<td>Reveals Temporal Changes in the Interactome in Response to Epidermal</td>
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<td>Growth factor</td>
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<td>Anders Kristensen, University of British Columbia</td>
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<tr>
<td>11:50</td>
<td><strong>Cancer Protein Interactomes: Towards a Fast Mapping of Regulated</strong></td>
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<td>Interactions</td>
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<td>Steve Tate, AB/Sciex</td>
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<tr>
<td>12:10</td>
<td><strong>Lunch</strong></td>
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### Wednesday April 25

**PROTEIN INTERACTIONS NETWORK** — room 205 B  
Session Chair: Anne-Claude Gingras, Samuel Lunenfeld Research Institute

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<tr>
<th>Time</th>
<th>Event</th>
<th>Speaker/Institution</th>
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</table>
| 13:30  | **Invited Speaker**  
Predicting Protein Interactions from the Genome  
Gary Bader, Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto |                                                                                      |
| 14:10  | **iRefWeb: Analysis of Consolidated Protein Interactions and their Confidence Scores**  
Shoshana Wodak, Hospital for Sick Children |                                                                                      |
| 14:30  | **Understanding Spatio-temporal Control of Rho Protein Signaling by RhoGAP/GEF Proteomics**  
Rick Bagshaw, Mount Sinai Hospital, Samuel Lunenfeld Research Institute |                                                                                      |
| 14:50  | **SUMO System Interactome and the Role of SUMO Chains in S. cerevisiae**  
Tharan Srikumar, University of Toronto |                                                                                      |
| 15:10  | Break |                                                                                      |
| 15:30  | **Invited Speaker**  
Physical and Functional Mapping of the Higher Order Structure of Cell Signalling Networks  
Jeff Wrana, Samuel Lunenfeld Research Institute |                                                                                      |
| 16:10  | **PDZ Domain Containing Protein RADIL Interacts with the Kinesin Family Protein KIF14 and Modulates Breast Cancer Cell Motility**  
Syed Mukhtar Ahmed, University of Toronto |                                                                                      |
| 16:30  | **Functional Redundancy of the Drosophila p38 MAP Kinases Probed by Mass Spectrometry-based Interaction Proteomics**  
Vladimir Belozerov, York University |                                                                                      |
| 16:50  | **Proteomic Analysis of Tetrahymena Thermophila Protein Complexes Involved in Chromatin Dynamics**  
Jeff Fillingham, Ryerson University Department Chem/BIO |                                                                                      |
<p>| 17:10  | Closing remarks End of symposium |                                                                                      |</p>
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<tr>
<td>8:00</td>
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<tr>
<td>8:30</td>
<td><strong>TRANSLATIONAL PROTEOMICS</strong> — room 205 D</td>
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<td>Session Chairs: Andrew Emili, University of Toronto</td>
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<td>Eleftherios Diamandis, Mount Sinai Hospital</td>
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<tr>
<td>8:30</td>
<td>Invited Speaker</td>
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<tr>
<td>8:30</td>
<td><em>Discovery of Novel Pancreatic Cancer Biomarker Using in Proteomics</em></td>
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<td>and Mass Spectrometry</td>
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<td></td>
<td><em>Eleftherios Diamandis, Mount Sinai Hospital</em></td>
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<tr>
<td>8:30</td>
<td><strong>Identification of Differentially Expressed Proteins in Direct</strong></td>
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<td><em>Expressed Prostatic Secretions of Men with Organ-confined Versus</em>*</td>
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<td><em>Extracapsular Prostate Cancer</em></td>
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<td><em>Yunee Kim, University of Toronto</em></td>
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<tr>
<td>9:10</td>
<td><strong>Identification of Novel Disease-Specific and Membrane-Associated</strong></td>
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<td><em>Protein Markers in a Mouse Model of Multiple Sclerosis</em></td>
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<td><em>Laura Gianni, CCBR University of Toronto</em></td>
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<td>9:50</td>
<td><strong>A Systems Level View of the Central Dogma in Lung Cancer</strong></td>
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<td><em>Yuhong Wei, Hospital for Sick Children</em></td>
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<td><strong>Integration of Cardiac Proteome Biology and Medicine By A Specialized</strong></td>
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<td><em>Peipei Ping, University of California, Los Angeles</em></td>
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<td><strong>Proteomic Analysis of Patient-specific Blood Outgrowth Endothelial</strong></td>
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<td><em>Cells in Heritable Pulmonary Arterial Hypertension: Insight into Novel</em></td>
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<td><em>Jessie Lavoie, Ottawa Hospital Research Institute, Sprott Stem Cell</em></td>
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<td><em>Centre and Regenerative Medicine Program</em></td>
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<td>11:30</td>
<td><strong>A Census of Human Soluble Protein Complexes</strong></td>
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<td><em>Pierre Havugimana, University of Toronto</em></td>
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<td><strong>Target Identification by Chromatographic Co-elution: New Chemical</strong></td>
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<td><em>Proteomics Method for Protein Target Deconvolution of Drugs and Bioactive Molecules</em></td>
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<td><em>Dajana Vuckovic, University of Toronto</em></td>
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# Wednesday April 25

**TRANSLATIONAL PROTEOMICS** — room 205 D  
Session Chairs: Andrew Emili, University of Toronto,  
Eleftherios Diamandis, Mount Sinai Hospital

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<td><em>New Developments in Quantitative Proteomics Leveraging the Orbitrap</em></td>
<td>Bruno Domon, Luxembourg Clinical Proteomics Center, CRP-Santé, Strassen, Luxembourg</td>
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<td><strong>Invited Speaker</strong></td>
<td><strong>Verification of Male Infertility Biomarkers in Seminal Plasma by Multiplex SRM Assay</strong></td>
<td>Andrei Drabovich, Samuel Lunenfeld Research Institute, Mount Sinai Hospital</td>
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<td>14:10</td>
<td><strong>Verification of Male Infertility Biomarkers in Seminal Plasma by Multiplex SRM Assay</strong></td>
<td>Andrei Drabovich, Samuel Lunenfeld Research Institute, Mount Sinai Hospital</td>
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<td>14:30</td>
<td><em>Intra-protein Variability Revealed by Absolute Quantification of Plasma Proteins using Dimethyl Labelling</em></td>
<td>Devanand Pinto, National Research Council, Halifax</td>
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<td>14:50</td>
<td><em>Nrf2 Target Proteins are Regulated by Angiotensin II in Kidney Cells</em></td>
<td>Ana Konvalinka, University of Toronto</td>
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<td>15:30</td>
<td><em>The New ZIP on the Block: Significance of Evolutionary Descent of Prion Genes From a Zinc Metal ion Transporter</em></td>
<td>Gerold Schmitt-Ulms, University of Toronto</td>
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<td>16:10</td>
<td><em>Novel MS-based Proteomics Platform to Uncover Minor Histocompatibility Antigens</em></td>
<td>Dev Sriranganadane, IRIC, Université de Montréal</td>
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<td>16:30</td>
<td><em>Clinical Application of SWATH: Discovering Novel Biomarkers in Threatened Preterm Labour</em></td>
<td>Jan Heng, Samuel Lunenfeld Research Institute</td>
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<td>16:50</td>
<td><em>Translational Analysis of Mouse and Human Placental Protein and mRNA Reveals Distinct Molecular Pathologies in Human Preeclampsia</em></td>
<td>Prof. Brian Cox, University of Toronto</td>
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<td>17:10</td>
<td>Closing remarks End of symposium</td>
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13:30 Recent advances in cardiovascular proteomics

Invited Speaker

Parveen Sharma, University of Toronto

Authors: Parveen Sharma, Jake Cosme, Anthony O. Gramolini

Dr. Gramolini’s group is interested in the cellular consequences of dysregulation of Ca2+ uptake and in the cellular mechanisms that result in the proper formation of a specialized membrane domain within muscle, termed the sarcoplasmic reticulum. They employ a range of current molecular and cellular approaches, as well as advanced proteomic and genomic methods, to investigate the physiology and biochemistry of several key Ca2+ regulatory proteins in cardiac and skeletal muscle.

Abstract: Cardiovascular diseases (CVDs) are the major source of global morbidity and death and more people die annually from CVDs than from any other cause. These diseases can occur quickly, as seen in acute myocardial infarction (AMI), or progress slowly over years as with chronic heart failure. Advances in mass spectrometry detection and analysis, together with improved isolation and enrichment techniques allowing for the separation of organelles and membrane proteins, now allow for in depth analysis of the cardiac proteome. Here we outline current insights that have been provided through cardiovascular proteomics, and discuss studies that have developed innovative technologies which permit the examination of the protein complement in specific organelles including exosomes and secreted proteins. We highlight these foundational studies and illustrate how they are providing the technologies and tools which are now being applied to further study cardiovascular disease; provide new diagnostic markers and potentially new methods of cardiac patient management with identification of novel drug targets.

14:20 MRM-based Quantitative Analyses of Mitochondrial Protein Phosphorylation in Murine and Human Heart Principal

Maggie Pui Lam, University California Los Angeles

Authors: Maggie P.Y. Lam, Sarah B. Scruggs, Tae-Young Kim, Chenggong Zong, Edward Lau, Ding Wang, David A. Liem, Christopher M. Ryan, Kym F. Faull, Peipei Ping

Abstract: The regulation of mitochondrial function is vital for cardiomyocyte adaptation to cellular stress in health and disease. While mounting evidence supports the concept of reversible phosphorylation as a regulator of mitochondrial protein functions, molecular details on how phosphorylation modulates mitochondrial protein functions remain scarce. Protein phosphorylation is a transient event, and typically only presents in sub-stoichiometric amounts, therefore determining site-specific occupancy is technically challenging. An adaptable, sensitive, specific, and robust method for quantifying site-specific phosphorylation in mitochondria would aid in overcoming these challenges. Here we report a multiple reaction monitoring-based mass spectrometric method for quantifying site-specific phosphorylation of mitochondrial proteins. Chromatographic and mass spectrometric conditions were optimized for transitions derived from murine and human phosphopeptides, and their corresponding unmodified peptides. Our analyses enabled the quantification of endogenous peptides and phosphopeptides from the outer mitochondrial membrane protein VDAC, the inner membrane protein ANT, as well as proteins in the TCA cycle and respiratory chain. The development of this quantitative methodology is a crucial step for advancing our knowledge and understanding of the regulatory effects of mitochondrial protein phosphorylation in cardiac physiology and pathophysiology.
14:40 **Discovery of Cardiovascular Disease Biomarkers in Human Plasma using MRM-based Multiplexed Quantitation**

**Gabriela Cohen-Freue, PROOF Centre of Excellence**

**Authors:** Gabriela V. Cohen-Freue, John S. Hill, Dominik Domanski, Derek Smith, David Lin, Janet Wilson-McManus, Gordon Francis, Bruce McManus, and Christoph H. Borchers

**Abstract:** Cardiovascular disease, ranging from atherosclerosis to myocardial, is the leading cause of morbidity and mortality in adults worldwide. The development of a minimally-invasive test based on plasma protein biomarkers can improve patient care options, and reduce a significant burden on the healthcare system.

**Methods:** A total of 223 peptides from 99 protein candidates were measured by MRM-MS on 73 plasma samples from patients with angiographic coronary artery disease and subsequent cardiovascular mortality (case), and 75 from patients with no coronary artery disease and no subsequent mortality (control). The maximum peptide relative ratio was used for proteins with more than 1 peptide available. Moderated t-tests (robustLIMMA) were used to rank proteins according to their differential concentrations. The top proteins (p value<0.5) were used to generate a protein classifier score using Elastic Nets. The performance of the classifier score was evaluated by the average performance of 50 runs of a 5-fold cross-validation.

**Results:** A total of 14 proteins showed a significant differential concentration between patients with and without coronary artery disease. The top-33 proteins were used to generate a protein classifier score that aggregates the protein levels of these proteins into a single number, which can be used to classify new samples. The estimated sensitivity and specificity to classify test samples from the cross-validation was 70% and 73%, respectively, and the area under the receiving operating curve (AUC) was 0.76.

**Conclusions:** This study provides evidence that protein concentrations in plasma may provide a relevant measure for monitoring and diagnosing cardiovascular diseases.

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15:00 **Targeted Protein Quantification using High-Resolution MS-->MS/MS Transitions to Validate microRNA Targets Related to Heart Failure**

**Johannes Hewel, University of Toronto**

**Authors:** Johannes A Hewel, Daniele Merico, Ruth Isserlin, Jian Liu, Anthony Gramolini, Gary Bader, Andrew Emili

**Abstract:** Targeted protein quantification by selected reaction monitoring (SRM) using peptide surrogates has increasingly become standard for the validation of biomarker candidates and development of protein therapeutics. In a proteomic experiment many more similar features can be detected in an LC-SRM run and hence such assays need to be developed mostly with using known peptide standards. Drawbacks with LC-SRM assays are that 1) they need to be validated and 2) that signature ion identification need to be interpreted reliably with more or 5 SRM-transitions. To address this issue, we show a new approach with using high-resolution Orbitrap-VELOS for the detection of product ions. We also present the feasibility and general strategy for such approach. As a help for identification is the chromatographic profile of a precursor and at least one production necessary, both with high accuracy mass scans in the Orbitrap. First, we show the evaluation of Orbitrap-VELOS scanning parameters. Second we created tools to measure the uniqueness of each product ion compared to the biological background across an LC-MS/MS run. Third, we show the implementation of labeled and unlabeled peptide standards for the absolute quantification of target peptides. Last we apply our approach to a small set of putative biomarkers related to heart failure in transfected cells overexpressing microRNA-1. Finally, we validated several predicted microRNA-1 target proteins by this accurate quantitative platform. The clear benefit of this method is the possibility to extract selected ion chromatograms of target analytes by using extraction windows as small as 0.01 amu.
15:40 Caspase/Metacaspase Control of Protein Aggregation During Cell Adaptation
Invited Speaker

Lynn Megeney, Ottawa Hospital Research Institute

Authors: Shrestha, Amit., Lee, Robin E., Brunette, Steve, and Megeney, Lynn A.

Dr Megeney’s group is interested in defining the organizing principles and biologic impact of signal transduction pathways in stem cell differentiation and cell fate. He has used proteomic analyses to characterize the regulatory pathways in myogenesis and embryonic stem cell differentiation.

Abstract: Caspase enzymes have garnered considerable attention for the respective role in both basic biology (as arbiters of developmentally controlled or immune-induced cell death) as well as disease pathology. In the latter case inappropriate caspase activation has been linked to the excessive cell death exemplified by ischemia induced tissue injury and various proteo-toxic pathologies. As such, devising strategies to disrupt or limit caspase function has become a priority area of investigation for translational medicine. Despite the longstanding evidence that these enzymes act as primary conveners of cell death signaling, recent observations strongly suggest that these proteins retain equally conserved physiologic non-death roles. Here, using multiple proteomic screening strategies (iTRAQ and TAP-tag protein interactions) we demonstrate that the yeast caspase/metacaspase Yca1 interacts with proteins involved in the control of protein folding, protein misfolding and aggregate formation. Loss of Yca1 leads to increased expression of proteins that control vacuole formation and protein degradation, concurrent to the accumulation of insoluble protein aggregates. Based on these observations we propose the general hypothesis that caspase/metacaspase proteases evolved (in part) to effect protein quality control, and that the activation of these enzymes, as exemplified in numerous disease conditions is initially a beneficial response to tame protein aggregates. We discuss ongoing efforts to address this hypothesis using multiple disease models including cardiac hypertrophy and proteotoxic neuro-degeneration.

16:20 Detection of Novel Myosin- or Cardiac-associated Proteins in the arteries and Veins of Human Subjects During Myocardial Infarction

Amir Ravandi, Ryerson University, Dept. of Chemistry and Biology, Toronto, Canada

Authors: Amir Ravandi, Eva Lonn, Angeline Florentinus, Thanusi Thavarajah, Eric Stanton and John G. Marshall

Affiliations: Ryerson University, Dept. of Chemistry and Biology, Toronto, Canada
McMaster University, Cardiology Division, Department of Medicine, Hamilton, Canada
University of Manitoba, Institute of Cardiovascular Sciences, St. Boniface Hospital, Winnipeg

Abstract: Heart attack, or Acute Myocardial infarction (AMI), is a leading source of morbidity and mortality in the Western World. Human blood plasma was collected from AMI patients immediately upon presentation at the emergency ward compared to control patients. The diagnosis of AMI was confirmed by ECG, CK-MB, Roche cardiac-specific -Troponin T tests and coronary angiography. The sampling scheme was 10 arterial AMI, 10 venous AMI, and 30 venous normal. The samples were fractioned into 17 parts prior to identification of tryptic peptides using disposable apparatus with no cross contamination between patients (50 x 17 = 850 LC-ESI-MS/MS runs). Structured query language (SQL) was used to summarize the randomly and independently sampled peptides from control and AMI plasma prior to normality testing and ANOVA with the statistical analysis system (SAS). Some 72,000 proteins, isoforms and splice variants from human plasma were identified with an expectation value of type I error of 1/100 or less by proteome discoverer. A total of 55 proteins were observed in both the arteries and veins of AMI patients with four or more independent detections that were never observed in the normal controls. Many of the proteins detected specifically from the AMI samples were known to be cardiac ESTs, myosin binding or regulatory factors or proteins linked to the muscle contractile pathways that may be relevant to the differential diagnosis of AMI. It remains to be confirmed if the myosin or cardiac proteins were potential diagnostic molecules for AMI by independent biochemical or spectrometric methods.
16:40 *Plasma Protein Analyses for Assessment of Atherosclerotic Process in ApoE Deficient Mice Exposed to Ozone*

Dharani Das, Health Canada, Mechanistic Studies Division, EHC, HECSB

**Authors:** P. Kumarathasan, D. Das, E. Blais, A. Bieleck, S. Mohottalage and R. Vincent

**Abstract:** It is well known that air pollution exposure can cause cardiovascular health outcomes, especially in susceptible individuals. Ozone is one of the main gaseous air pollutants and is a known pulmonary irritant. The aim of this work was to investigate by proteomic analyses whether ozone exposure modulates the progression of atherosclerosis in ApoE^{-/-} mice. Animals were in placed on a western diet for 10 weeks and then exposed to ozone (0, 0.4, 0.8 ppm) on 6 hours/day, 5 days/week for 4, 8, and 13 weeks. Animals were terminated immediately after exposures or after 1 week recovery in clean air. Whole blood samples were stabilized for any post-mortem changes. Aliquots of plasma and bronchoalveolar lavage fluid were analyzed for target biomarkers (e.g. inflammatory cytokines, CRP, ICAM) by multiplex protein analysis. Plasma treated with anti-proteases was fractionated on 30 kDa and 100 kDa cut-off filters. The < 30 kDa fraction and the tryptic digests of the 30-100 kDa and >100 kDa fractions were analyzed by MALDI-TOF-TOF-MS. Levels of CRP and ICAM were elevated in lung lavage fluid of animals exposed to ozone. Furthermore, exposure to ozone increased plasma fibrinogen and adiponectin. Increased plasmatic fibrinogen has been implicated in atherosclerosis. Data mining of mass spectral data by K-nearest neighbor analysis identified several time-related and ozone dose-related plasmatic markers. These findings are consistent with an interaction of ozone in the progression of atherosclerosis.

17:00 *New Matrix Candidates for High Spatial Resolution Imaging Mass Spectrometry of Lipids*

Aurélien Thomas, Université de Montréal

**Authors:** Aurélien Thomas, Jade Laveaux Charbonneau, Erik Fournaise, Pierre Chaurand

**Abstract:** Recently introduced as a novel approach for matrix application, sublimation has shown to be very powerful for the analysis of low-molecular-weight compounds such as phospholipids by imaging mass spectrometry (IMS). Within few minutes, this process forms a very thin and exceptionally homogeneous film of matrix on thinly cut tissue sections. This work presents a comprehensive study aiming to evaluate current and novel matrix candidates for high spatial resolution MALDI IMS of lipids from tissue section after deposition by sublimation. Amongst investigated candidates, DAN has demonstrated to be of high efficiency providing rich lipid signatures in both positive and negative polarities with high vacuum stability and sub-20µM resolution capacity. Negative MALDI IMS offers the possibility to visualize several important lipid classes including phospholipids, sulfatides, ceramides, cardiolipins and gangliosides. After DAN sublimation, the sequential acquisition of ion images in both polarities at 100µM spatial resolution was achieved yielding a significant amount of information from various tissues including adult mouse brain, whole-body fish and prostate cancer tissue sections. High spatial resolution ion images were also acquired with high scanning resolution. For example, at 10µM scanning resolution, we demonstrated that some lipids were expressed in certain brain cerebellum histologies such as white matter for PE-p 34:1 and ST 22:0(OH), whereas CerP 18:0 was only expressed in the molecular layer. In another exciting project, high resolution IMS after DAN sublimation is used to evaluate the differential expression of lipids after injuries to rat brains by microbeam radiation therapy.
CHEMICAL & STRUCTURAL PROTEOMICS

Session chair: Christoph Borchers, UVic-GBC Proteomics Centre

8:30 **MS23D: From Mass Spectra to 3D Protein Structures**

*Invited Speaker*

David Wishart, University of Alberta

Dr Wishart's research interests are focused on structural biology and on the development of computational tools to facilitate the determination of protein structures using NMR and protein cross-linking with MS. He has active research programs in bioinformatics, prion biology, synthetic biology, nanotechnology and metabolomics.

**Authors:** David Wishart, University of Alberta

**Abstract:** There are 2 conventional methods for determining the 3D structure of proteins: NMR and X-ray crystallography. However, not every protein is amenable to characterization by NMR nor is every protein crystallizable. Is there another way? We postulate that mass spectrometry may allow the 3D structures of proteins to be determined at atomic resolution. With continuing improvements to mass spectrometry, H-D exchange measurements, chemical modification and cross-linking as well as improvements to sequence-based protein structure prediction, we believe it is now possible to generate protein structures using MS-derived data. In this presentation I will describe our ongoing efforts to develop and test a computer program called MS23D for generating atomic resolution protein structures using sequence and easily acquired MS-derived data. In particular I will give a brief description of the program, the typical inputs and discuss the type or quality of structures that it has generated. I will also talk about our ongoing efforts to adapt MS23D to the determination of oligomeric structures of the prion protein. This is a particularly challenging problem that has required both computational and manual interpretation as well as the development of some novel protein modification and cross-linking strategies.

9:10 **Multiple Structural Proteomics Approaches for the Characterization of Protein Complexes**

Evgeniy Petrotchenko, UVic-GBC Proteomics Centre

**Authors:** Evgeniy Petrotchenko, Jason Serpa, Jingxi Pan, Jun Han, David Wishart, Christoph Borchers

**Abstract:** We are applying a combination of limited proteolysis, surface modification, chemical cross-linking and hydrogen/deuterium exchange for the structural characterization of the proteins and protein complexes. We illustrate this approach for the characterization of the prion protein conformational changes and the 3D structure of prion aggregates.

A different pattern of limited proteolysis with pepsin was observed between native (PrPC) and β-oligomeric (PrPβ) forms of the prion protein, indicating to an increased exposure of the H1-β2 region in the β-oligomeric state.

Analysis of the PrPC and PrPβ by HDX using top-down ECD-FTMS, which provides single-residue resolution data, showed differences in the exchange rates in the H1 and H1-β2 regions.

Using water-soluble PCASS reagent, we detected multiple residues, that were differentially-modified between the native and β-oligomeric forms in the β1, H1-β2, β2, β2-H2 regions.

We detected multiple crosslinks, several of which were unique to each form of the prion protein. Some crosslinks in the C-terminal portion of the protein, which would have had to transverse the β1 and β2 region in the native structure, were specific to the β-oligomeric form, indicating a rearrangement of the β1-H1-β2 region in the β-oligomeric conformation of the protein.

Thus, data from these multiple structural proteomics approaches points to the same conformational change and indicates a rearrangement of the β1-H1-β2-H2 region as the major difference between
the native and β-oligomeric prion protein conformations. These changes are all consistent with the β1-H1-β2 loop moving away from the H2-H3 core during the conversion from PrPC to PrPβ.

9:30 **Steric and Allosteric Factors Contribute to Instability of the Transferrin Binding Protein Ternary Complex**

Leslie Silva, MD Anderson Cancer Center

**Authors:** Leslie P. Silva, Rong-hua Yu, Charles Calmettes, Xue Yang, Trevor F. Moraes, Anthony B. Schryvers, David C. Schriemer

**Abstract:** The bacterial transferrin binding protein (Tbp) receptor complex, comprised of TbpA and TbpB, plays an important role in facilitating iron acquisition from host transferrin (Tf) during meningococcal infection. While the interaction of surface-exposed TbpB is well studied, how integral-membrane protein TbpA binds Tf and facilitates iron release is less understood. Insight into this binding interaction and iron release process is crucial for vaccine development, as TbpA is required for the transfer of iron across the outer membrane. Hydrogen/deuterium exchange mass spectrometry allows the ability to obtain structural information on the interaction and mechanism of iron release from this large (~170kDa) membrane protein complex.

A bottom-up H/DX-MS method was optimized to allow for the efficient online removal of C8E4 surfactant. The interaction of both apo and holo porcine Tf with TbpA and TbpB were mapped, and results indicate that both interactions take place on the C-lobe of pTf, in adjacent and partially overlapping regions. The predicted interactions have been verified by site directed mutagenesis of pTf and surface Plasmon resonance. Rosetta-docking and computational modeling were used to develop models and outline mechanisms of iron acquisition and release by the Tbps. H/DX-MS results also suggest that peptides involving key binding determinants for the TbpB interaction become destabilized in the presence of TbpA. Together, these findings allow us to propose a mechanism in which TbpB first binds holo-transferrin, and uses a ‘baton hand-off’ type mechanism allowing TbpA to abstract it. TbpA then transports iron across the outer membrane, into the bacterial periplasm.

9:50 **Kinetic Folding Mechanism of A1-Antitrypsin Probed by Hydroxyl Radical Labeling**

Bradley Stocks, University of Western Ontario

**Authors:** Bradley Stocks, Patrick Wintrode, Lars Konermann

**Abstract:** The folding pathway of the metastable serpin A1-antitrypsin (A1AT) remains poorly understood. This study utilizes hydroxyl radical labeling to monitor the temporal changes in side chain solvent accessibility during folding. Previous optical and HDX studies indicate that A1AT folding goes to completion on the order of 30 min with a substantial lag phase of ~5 s. To mirror those studies, oxidative labeling was carried out at between 0.5 s and 24 hr post GdnHCl dilution from 6 M to 0.3 M. Peptide mapping covered 93% of the protein sequence, and MS/MS revealed 69 oxidation sites. Our results have been complemented by kinetic HDX measurements. Our findings indicate a rapid collapse of the structure at the earliest labeling point with high protection in helices B and C. These helices form a scaffold onto which additional elements pack on a timescale of minutes. These tertiary interactions disrupt the reactive center loop (RCL) insertion site, allowing specific hydrogen bonds in strand C1 to form and lock the RCL in an open conformation. After 30 min much of the protein has attained native solvent accessibility levels. Regions that retain elevated accessibility at this time point include the RCL, the loop insertion site in A²-sheet A, and helices F and I. This kinetic species bears striking resemblance to a previously characterized equilibrium unfolding intermediate [Krishnan et al. Nat. Struct. Mol. Biol. 2011, 18, 222]. The use of complementary labeling strategies has provided a more thorough understanding of how A1-AT can fold to a metastable state.
10:30 **Structural Biology by Mass Spectrometry: 3D Proteomics of Supramolecular Assemblies**  
Juri Rappsilber, University of Edinburgh

*Dr. Rappsilber’s research activities aim at developing and applying novel methods to study the conformation and interaction dynamics of proteins in complex mixtures (3D proteomics), and chromatin-associated processes (e.g. RNA polymerase II in complex with transcription factor IIIF) in health and disease.*

**Authors:** Rappsilber, Juri; Tahir, Salman; Fischer, Lutz; Combe, Colin; Bukowski-Wills, Jimi-Carlo; Chen, Zhuo Angel

**Abstract:** Current structural biology methods leave an information gap in the mid-resolution range at which protein interactions or conformation changes are defined at domain or sub-domain level. Mass spectrometry in conjunction with cross-linking is providing exactly this information. We have applied our tools to complexes up to 670 kDa in size, tagged complexes and even whole cell lysates. We have furthermore analysed conformation changes in solution using stable isotope labelling for quantitative analyses.

We have transformed cross-linking/mass spectrometry from an expert approach to routine application by establishing an integrated workflow through having: (1) developed an enrichment strategy for cross-linked peptides based on charge; (2) characterised in detail the fragmentation behaviour of cross-linked peptides in a high resolution mass spectrometer; (3) derived lessons from this for a search algorithm that does not require isotope-labelled cross-linkers and overcomes the n^2 problem of database searching for cross-links; and (4) written user friendly web-based search software that includes a revolutionary spectrum viewer for match evaluation with implications reaching beyond this field and a cross-link map viewer for fast hypothesis generation that expands the current visualisation concepts of protein network viewers such as used in STRING.

We believe that cross-linking/mass spectrometry is now ready for deployment into structural and molecular biology laboratories for routine application.

11:10 **Approach for Large-scale Identification of Linked Peptides From Tandem Mass Spectra**  
Jian Wang, University of California, San Diego

**Authors:** Jian Wang, Veronica Anania, Jeff Knott, John Rush, Jennie R Lill, Philip E Bourne, Nuno Bandeira

**Abstract:** Chemical cross-linking and mass spectrometry have been shown to constitute a powerful tool to study protein-protein interactions and to help elucidate the structure of large protein complexes. However computational methods to interpret the convoluted MS/MS spectra from linked peptides are still in their infancy, thus making the high-throughput application of this approach largely impractical. Here we use disulfide-linked peptides as an example to describe a generic procedure to (a) efficiently generate large mass spectral reference data for linked peptides, and (b) use this data to automatically train an algorithm that can efficiently and accurately identify linked peptides from MS/MS spectra. In order to obtain a large MS/MS dataset of linked peptides, we designed and synthesized three combinatorial peptide libraries, each with a cysteine at different positions along the peptides. Disulfide bond formations are promoted to allow the libraries peptides to form dimers which were then analyzed with an LTQ-Orbitrap-Velos mass spectrometer. Analysis of the identified MS/MS spectra from disulfide-bridged peptides demonstrated fragmentation patterns for the cross-linked peptides are different from those of their corresponding unlinked peptides. This led us to build a scoring function specific to linked peptides and derive a efficient filtration strategy that allow us to reduce the search space by ~10,000 fold. Using this new database search tool we identified a total of ~3500 MS/MS spectra from disulfide-linked peptides (corresponding to ~2500 unique peptide pairs). Our informatics approach is general and can be used to build tools for the identification of other types of cross-linked peptides.
11:30 Structural Characterization of the SH3 Domain of AHI-1 in Regulation of Cellular Resistance to Tyrosine Kinase Inhibitors in BCR-ABL-transduced Leukemia Cells

Xiaohu Liu, British Columbia Cancer Agency

Authors: Xiaohu Liu, Min Chen, Paolo Lobo, Jianghong An, SW. Grace Cheng, Annie Moradian, Gregg B. Morin, Filip Van Petegem and Xiaoyan Jiang

Abstract: Chronic myeloid leukemia (CML) is a clonal multilineage myeloproliferative disorder characterized by the presence of the fusion gene BCR-ABL with increased tyrosine kinase activity. Tyrosine kinase inhibitor (TKI) therapy induces clinical remission in CML patients but early relapses and later emergence of TKI-resistant disease remain problematic. We have recently demonstrated that AHI-1 oncogene physically interacts with BCR-ABL and JAK2 and mediates cellular resistance to TKI in CML stem/progenitor cells. We now show that deletion of the SH3 domain of AHI-1 significantly enhances apoptotic response of BCR-ABL+ cells to TKIs compared to cells expressing full-length AHI-1. Using the AHI-1 SH3 domain as protein "bait" in immunoprecipitation/mass spectrometry, Dynamin-2, a GTPase mainly involved in trafficking process, was identified as a novel interacting partner of AHI-1. The crystal structure of the AHI-1 SH3 domain at 1.53-Å resolution reveals that the SH3 domain adopts a canonical SH3 folding, with the exception of an unusual C-terminal α-helix. PD1R peptide, known to interact with the PI3K SH3 domain, was used to model the binding pattern between the AHI-1 SH3 domain and its ligands. These studies showed that an “Arg-Arg-Trp” stack may form within the binding interface, providing a potential target site for designing specific drugs. The crystal structure of the AHI-1 SH3 domain thus provides a valuable tool for identification of key interaction sites in regulation of drug resistance and for the development of small molecule inhibitors for CML.

11:50 Fetal Liver Tyrosine Kinase 3 Receptor (CD135) is a Co-receptor of the Fc Receptor Complex and its Ligand Regulates IgG-mediated Phagocytosis

Lauren Agro, Ryerson University, Dept. of Chemistry and Biology

Authors: Lauren Agro, Jeff Howard, Albana Kume, John G. Marshall

Abstract: Cell surface receptors are a key a focus of therapy. We have previously shown that it is possible to capture the activated Fc receptor complex from the surface of live RAW 264.7 cells using microbeads coated with the receptors’ cognate ligand, followed by digestion and liquid chromatography-tandem mass spectrometry. The fetal liver tyrosine kinase 3 (FLT3R) was specifically detected on IgG coated beads incubated with live cells, but not on those incubated with crude extract or control beads for non-specific binding. However, it remained possible that the FLT3R was passively collected from the plasmalemma micro-domain that surrounds the activated Fc receptor complex. Alternatively, proximal co-receptors may form unique supramolecular complexes in response to combinations of ligands in order to integrate different signals and thus mount the correct physiological response. We tested the hypothesis that FLT3R plays a role in Fc mediated phagocytosis by cloning and expressing the activation domain of its ligand (FLT3L) in Pichia pastoris with affinity purification as confirmed by Western blot and LC-ESI-MS/MS. FLT3L induced a dendritic cell morphology and clearly enhanced the phagocytic function of mouse macrophages at ~50 to 100 nM concentrations added to media with fetal calf serum for 12 hours. We report for the first time the capture of a receptor supramolecular complex from the surface of live cells with the identification of a physiologically relevant co-receptor using controlled affinity chromatography and mass spectrometry. The controlled affinity capture of a co-receptor from live cells and demonstration of its physiological relevance is of profound medicinal and economic importance.
TUESDAY April 24

PROTEIN MODIFICATIONS & CELL SIGNALING
Session chairs: Gilles, Lajoie, University of Western Ontario & Pierre Thibault, IRIC, Université de Montréal

13:30 \textit{Interrogating a Single Cell Type: From the Genome to the Proteome and Everything Else in Between}
Akhilesh Pandey, Johns Hopkins University

\textit{Dr. Pandey’s lab uses Systems Biology approaches that combines molecular biology, analytical chemistry and computational biology with various “Omics” technologies including genomics and proteomics to understand signaling pathways and to identify therapeutic targets and biomarkers in a number of cancers.}

Author: Akhilesh Pandey

\textbf{Abstract:} High resolution mass spectrometry-based proteomics has the potential to revolutionize genome annotation. We have initiated a systematic proteogenomics analysis to carry out genome annotation of various sequenced and unsequenced genomes of biomedical importance using mass spectrometry. The promise of personalized medicine in humans generally revolves around using genomic and transcriptomic information with less emphasis on many other ‘Omes’ such as the methylome, proteome and the metabolome. Each of these Omics datasets is quite complex in itself. For example, transcriptomic analysis includes analysis of mRNA, miRNA and various other non-coding RNAs while proteomic analysis includes analysis of proteins but also a host of other post-translational modifications such as phosphorylation, acetylation and glycosylation. An integrated view of various Omics type of datasets is likely to be more meaningful in the context of personalized medicine. However, given the diversity of data types and platforms and the lack of a standard computational pipeline, it is not trivial to integrate multi-Omics datasets. I will discuss our recent efforts in combining data from multiple ‘Omes’ – from genomic sequence and methylome to transcriptome and proteome – of a single human cell type and the insights we have gained.

14:10 \textit{Comprehensive Analysis of Cancer Somatic Mutations in Phosphorylation Signaling Predicts Novel Cancer Driver Genes and Pathways}
Juri Reimand, University of Toronto, Donnelly Centre

\textbf{Authors:} Juri Reimand, Gary Bader

\textbf{Abstract:} The complex landscape of somatic mutations in cancer genomes is dominated by few frequently mutated genes and numerous rare mutations. Identification of cancer driver mutations is an important challenge. We hypothesize that frequent cancer-associated alterations in a biological system are unlikely unless the system is important in cancer. We analyzed single nucleotide alterations (SNAs) that specifically alter protein phosphorylation, an important signaling mechanism in cancer, from ~800 samples across 8 cancer types. Using novel computational procedures, we predicted cancer driver genes and pathways, and kinase-focused sub-networks with significantly mutated phosphorylation sites and clinical correlations. We highlight a mutation desert in TP53 phosphorylation sites, a mutually exclusive mutation pattern in EGFR signaling in two cancers, an immune-related network associated with prolonged survival in ovarian cancer, and numerous candidate cancer genes and systems. Our study demonstrates new ways of analyzing cancer genomes and identifies biochemical mechanisms likely important in tumor biology.
14:30 **The SH2 Domain and the Phosphotyrosine Signaling Network**

Shawn Li, University of Western Ontario

**Authors:** Shawn Li, Tomonari Kaneko, Haiming Huang, Huadong Liu

**Abstract:** The Src homology 2 or SH2 domain is a prevalent protein module in the human genome with 120 copies and plays an essential role in tyrosine kinase signaling. Many SH2 domain-containing proteins are involved in human diseases, suggesting that they could be targeted for therapy. In order to realize this potential, we have determined the specificity profiles and interaction network mediated by the human SH2 domains. Using peptide arrays, we defined the specificity for the majority of the human SH2 complement and by comprehensive structural bioinformatic analysis, we uncovered a new paradigm for encoding SH2 domain specificity by surface loops. In addition, we examined systematically how the tyrosine kinase, the SH2 domain and the accompanying phosphotyrosine signaling network had evolved from our ancestors to the present state found in humans. Our analysis not only uncovered the key features of phosphotyrosine network evolution but also identified hot-spots within the network through which cancer cells signal. Our work provides a framework and valuable resource on which to systematically characterize the SH2 domain, the tyrosine kinase and the underlying the phosphotyrosine signaling network for disease intervention.

14:50 **Systematic Chemical Proteomics and Functional Proteomics Strategies for Validation of Protein Kinase Inhibitors: Application to Unbiased Evaluation of Inhibitors of Protein Kinase CK2**

David Litchfield, University of Western Ontario, Department of Biochemistry

**Authors:** Laszlo Gyenis, Jacob P. Turowec, Maria Bretner, David W. Litchfield *(Presenting)*

**Abstract:** The universal role of protein kinases in cellular regulation and as potential targets for molecular-targeted therapy in a number of human diseases has spurred efforts to design novel protein kinase inhibitors. While the potential utility of novel inhibitors can certainly be appreciated, a major limitation in this field has been a lack of systematic strategies to validate both inhibitor specificity and the ability of inhibitors to neutralize intended target(s) within living cells. To address these limitations, our efforts have focused on development of chemical proteomics and functional proteomics strategies to evaluate inhibitor specificity and to identify cellular substrates that can be used as biomarkers for protein kinase inhibition. These strategies have been applied to an investigation of inhibitors for protein kinase CK2, a constitutively-active enzyme that has recently emerged as a potential anti-cancer target in clinical trials. Chemical proteomics strategies verify interactions between CK2 and its inhibitors in complex mixtures, and also reveal potential off-targets. Complementary functional proteomics studies have led to the identification of proteins that exhibit inhibitor-dependent decreases in phosphorylation. Using this strategy, CK2 substrates are identified as those proteins that are directly phosphorylated by CK2 in vitro and where phosphorylation is rescued with inhibitor-resistant CK2. Collectively, our studies provide novel platforms for evaluating CK2 inhibitors that will facilitate efforts to elucidate its biological functions and to evaluate its potential as a candidate for molecular-targeted therapy.

15:30 **Weak Interactions, Dynamics and Allostery in the Ubiquitin System**

Mike Tyers, IRIC, Université de Montréal

**Authors:** X. Tang, S. Orlicky, D. Ceccarelli, V. Csizmok, T. Mittag, T. Pawson, J. Forman-Kay, F. Sicheri and M. Tyers
Abstract: The ubiquitin-proteasome system relies on exquisitely regulated protein interactions to achieve the specific and dynamic regulation of its many targets. The SCF ubiquitin ligases recruit a host of substrates through specific receptors called F-box proteins, often in a phosphorylation-dependent manner. In many signaling networks, phosphorylation governs protein interactions via modular phospho-epitope binding domains that engage short phosphopeptide epitopes with high affinity. However, multisite phosphorylation can lead to more complex effects such as cooperative binding, as for example in the interaction between the CDK inhibitor Sic1 and its cognate ubiquitin ligase SCFCdc4. Our biophysical and genetic evidence suggests that dispersed weak Sic1 phospho-epitopes engage Cdc4 in dynamic equilibrium. This multisite phosphorylation requirement engenders a switch-like dependence on kinase concentration for Sic1 recognition and ubiquitination by SCFCdc4 in a fully reconstituted equilibrium reaction system. Composite high affinity interactions mediated by the equilibrium binding of low affinity sites may be a general means to adjust phosphorylation thresholds and optimize specific biological responses. Low affinity interactions can also underpin allosteric control, a regulatory mode that is likely to be prevalent in the ubiquitin system. In two unexpected examples of allosterity, we identified small molecule inhibitors that either block substrate recognition by Cdc4 or inactivate the E2 enzyme Cdc34 by a non-catalytic site mechanism. The further interrogation of these systems by proteomic methods will be discussed.

16:10 A Targeted Proteomic Approach for the Study of the Human Acetylome
Jean-Philippe Lambert, Samuel Lumenfeld Research Institute

Authors: Jean-Philippe Lambert, Brett Larsen, Beatriz Gonzalez Badillo, Tony Pawson and Anne-Claude Gingras.

Abstract: Lysine acetylation was recently recognized as an abundant protein post-translational modification with critical roles in chromatin structure and function. Recognition of acetylated lysines is mediated by the bromodomain. Consistent with key roles in cellular signalling within chromatin, bromodomain-containing proteins are amplified in many cancers and directly involved in numerous diseases. Because of their deep pocket to accommodate a variety of mono and polyacetylated peptides, bromodomains constitute attractive therapeutic targets, yet their specificity remains to be fully elucidated. We recently developed a standardized proteomic platform based on the previously reported mChIP method enabling the study of chromatin associated proteins in mammalian cells. This novel approach is being utilized to define the specificity of the 44 human proteins containing bromodomain. Following the affinity purification of bromodomain-containing proteins along with their acetylated interaction partners, an additional peptide enrichment step using anti-acetyl lysine antibodies is then used prior to analysis by quantitative mass spectrometry. Our data shows a wide diversity in bromodomain targets. Here we will present data on the optimization of the method using both TripleTOF and Orbitrap-Velos mass spectrometers using affinity purified BRPF3, a bridging subunit of the KAT7 acetyltransferase complex. By employing a targeted approach, we were able to reproducibly detect polyacetylated peptides from our samples at a much greater frequency than in reported proteome-wide studies. Furthermore, we will discuss our ongoing characterization of the BET bromodomain family and of their acetylated substrates. This project is helping us define the landscape of the acetyl lysine system in human cells.

16:30 Protein Termini and Their Modifications Identified by Positional Proteomics and Linked to Protein Function in Cancer
Philipp Lange University of British Columbia

Authors: Yuanmei Lou, Ulrich auf dem Keller, Reinhild Kappelhoff, Pitter F. Huesgen, Paul C. McDonald, Shoukat Dedhar, Christopher M. Overall

Abstract: The functional state of a proteome is defined by the structures, interactions and post-translational modifications of the proteins present at any given time. Among the most fundamental characteristics of a protein are its termini. Protein termini are highly variable as proteins are trimmed at their ends or undergo precise internal proteolytic processing generating stable fragments often
showing altered or new functions. Additionally, protein termini are frequently altered by chemical modification, such as acetylation, with profound effects on protein stability and function. We developed a unique set of techniques to identify protein termini including their modifications and to derive the functional competence of the proteins from these identifications. Protein termini are di-methylated or isotope labeled, enriched and analyzed by our mass spectrometry based N- and C-TAILS approaches. We now present the quantitative profiling of termini and their modifications in a mouse model for breast cancer metastasis. We then correlate the terminome data with gene expression and proteolytic cleavage site specificities and integrate it with curated protein annotation using our newly developed public knowledge base termed Terminome-oriented-protein-Function-Infenred-Database as recently published in Nature Methods. TopFIND positional cross-correlation analysis links cancer promoting somatic mutations in p53 to reduced cellular apoptosis upon loss of cleavage by caspases. Comparative analysis reveals distinct proteolytic post-translational modification processes to be prevalent in tumors of increasing metastatic potential. Extending our approach to human tissues we complement the human proteome project and provide access to termini as a rich, largely untapped resource for analytical, diagnostic and biomarker discovery purposes.

16:50 Mapping the Universal Animal Protein Interactome
Andrew Emili, University of Toronto

Dr. Emili’s lab is interested in comprehensive analysis of proteins expressed by the genome of eukaryotic cells using comprehensive, and complementary proteomics methods. His research aims are to understand the function and integration of the complex biochemical circuitry that underlies cell growth and proliferation.

Author: Emili, Andrew

Abstract: Evolutionarily conserved cellular processes often depend on stable physical associations between proteins. Despite progress in certain model systems, knowledge of the global composition of multiprotein complexes present among all extant animals remains limited and no large-scale comparative experimental studies have been reported. To close this gap, we are applying a systematic integrative proteomic approach to biochemically enrich and systematically identify putative soluble multi-protein complexes from representative cell extracts isolated for a diverse set of animal species. The comprehensiveness, centrality and modularity of this probabilistic physical interaction map, which encompasses core biological systems, should facilitate interpretation of fundamental animal cell biology.
**Wednesday April 25**

**Proteins Interactions Network**

Session Chair: Anne-Claude Gingras, Samuel Lunenfeld Research Institute

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**Navigating the Landscape of Integral Membrane Proteins Interaction: Applications for Human Health and Disease**

Igor Stagljar, Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto

*Dr. Stagljar’s research activities are focused on interaction proteomics and the maintenance of genome stability. He is internationally known for the development of the split-ubiquitin membrane yeast two hybrid technology.*

**Authors**: Igor Stagljar

**Abstract**: A focus of my lab is to understand the function of the majority of “druggable” human integral membrane proteins involved in cell signaling and membrane transport at a systems level. Despite extensive research in the past decade, there is a lack of in-depth understanding of protein networks associated with these integral membrane proteins because of their complex biochemical features, enormous complexity and multiplicity. This is a major obstacle for designing improved and more targeted therapies, and importantly, understanding the biology of deregulation of these integral membrane proteins which leads to numerous human diseases.

To that end, we are applying an in vivo genetic system previously developed in my lab, called the membrane yeast two-hybrid (MYTH) assay, to identify and characterize protein interactors of all yeast ABC transporters, human receptor tyrosine kinases (RTKs) as well as the selected cancer stem cell receptors (CSCRs) and G-protein coupled receptors (GPCRs). During my talk, I will discuss exciting new findings indicating that the newly identified RTK-, CSCR- and GPCR-interacting proteins play novel roles in regulating the activity of these integral membrane proteins in vivo and in vitro. Our systematic approach offers an unbiased systems level view that may identify novel drug targets and contribute to therapeutic research.

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**Towards the $1,000 Interactome**

Nozomu Yachie, University of Toronto

**Authors**: Nozomu Yachie, Sedide B. Ozturk, Joseph C. Mellor, Anna Karkhanina, Haiyuan Yu, Pascal Braun, David Hill, Marc Vidal, Frederick P. Roth

**Abstract**: Cancer and many other human diseases are not the products of defects in a few genes, proteins or single pathways. Rather, they involve a complex web of molecular interactions that are dynamically regulated by processes such as post-transcriptional modification. Thus, a technology that captures the regulated dynamics of a global-scale protein interaction network would be important to accelerate our understanding of complex cellular circuits such as those involved in diseases. Although recent technologies have enabled us to take a snapshot of interaction maps, no current method has the ability to economically produce many “conditional interactome” maps of a given organism with the presence of different co-factors (e.g. oncogenic activated kinases or other post-translational modifiers). Based on our previous successes using high-quality yeast two-hybrid (Y2H) assays, high-throughput DNA barcoding of cells, Cre-loxP based DNA barcode fusion and deep sequencing, we here propose a new technology BFG-Y2H (Barcode Fusion Genetics-Y2H) to allow the mapping of dozens of conditional interactomes by one technician within one year with the cost of $1,000 per interactome.
9:30  **Systematic Genetic Perturbation of Protein Complexes and Modules in Living Cells**  
Christian Landry, Université Laval

**Authors:** Christian R Landry, Guillaume Diss, Luca Freschi, Jose Torres, Alexandre Dube

**Abstract:** A major goal of fundamental and medical research is to describe and model how complex networks of protein interactions are organized and operate in the cell. This will lead to a better understanding of how cells make decisions, how they respond to stimuli, and why they may be robust to many but not all genetic perturbations. Protein interaction networks have been extensively described for some model species but because the experiments have been performed in a single set of genetic backgrounds or growth conditions, we still don’t know how plastic they are or how robust they are to genetic perturbations. We developed a quantitative approach to study the robustness of protein-protein interactions in living cells using the budding yeast *Saccharomyces cerevisiae* as a model. We applied our approach to the retromer, a protein complex involved in the recycling of transmembrane receptors from endosomes to the trans-Golgi network that is conserved across eukaryotes and that has been shown to be deregulated in neurodegenerative diseases such as Alzheimer’s. We measured pairwise protein-protein interactions among the 5 protein subunits of the complex in 28 distinct genetic backgrounds. Our results allowed us to reconstitute the known structure of the retromer, supporting previous observations made in vitro and in vivo, as well as to add new information on how the complex may assemble in living cells. Our approach, when applied to a large scale, will allow to map uncharted dimensions of protein interaction networks.

9:50  **Exploring the Relationship Between Genetic and Protein-protein Interaction Networks**  
Michael Costanzo, Banting and Best Department of Medical Research, Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto

**Authors:** Michael Costanzo, Anastasia Baryshnikova, Chad L. Myers2, Brenda Andrews, Charles Boone

**Affiliations:** 1. Banting and Best Department of Medical Research, Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, 160 College St., Toronto ON, Canada M5S 3E1; 2. Department of Computer Science & Engineering, University of Minnesota-Twin Cities, 200 Union St., Minneapolis MN, U.S.A. 55455

**Abstract:** Most genes are nonessential for eukaryotic life suggesting that organisms are highly buffered from genetic and environmental perturbations. Understanding how genes interact with one another to manifest complex phenotypes remains a daunting task. To address this challenge systematically, we used Synthetic Genetic Array (SGA) to measure genetic interactions among ~5.5 million double mutant combinations in *Saccharomyces cerevisiae*. In doing so, we generated quantitative genetic interaction profiles for ~75% of all yeast genes revealing a functional map of the cell in which genes of similar biological processes cluster together in coherent subsets and functionally interconnected bioprocesses map next to each other. Highly correlated genetic interaction profiles define protein complex and pathway membership enabling prediction of novel gene functions and physical interactions between gene products. Systematic analysis revealed a complex relationship between genome-wide genetic and physical interaction networks and highlighted an important role for genetic suppression interactions in connecting functionally diverse protein complexes.

10:30  **Mass Spectrometric Methods to Measure Dynamic Protein Interaction Networks**  
Invited Speaker  
Ruedi Aebersold, ETH Zurich, Switzerland

*Ruedi Aebersold is one of the world’s leaders in MS-based proteomics, and the recipient of numerous international awards for his innovative contributions in this field. Well known for his development of isotope coded affinity tag (ICAT) reagents, Dr. Aebersold continues to implement new approaches for quantification, both for global profiling of proteomes and for in-depth functional proteomics to characterize cell signaling regulation in health and disease.*
Author: Ruedi Aebersold

Abstract: It is widely appreciated that most biological functions are not carried out by single polypeptides, but rather by macromolecular complexes, some of which dynamically assemble or disassemble as a function of cellular state. Results from systems biology studies have indicated that such macromolecular complexes are further ordered in extensive networks of interacting molecules and that important properties of such networks are the direct consequence of network structure. In every cell, at any given time, numerous types of molecular networks are concurrently present. Among these, protein:protein interaction networks are of particular interest because they contain the modules that directly control and catalyze most cellular processes. In this presentation we will discuss wet lab and computational modules of a comprehensive technology for the analysis of the composition and topology of protein complexes. Specifically, we will describe methods for the quantitative analysis of protein complexes that allows us to determine complex component stoichiometry and to detect changes in protein complex composition and we will describe the use of isotope tagged cross linking reagents and mass spectrometry to generate distance constraints for the computational modeling the topology and structure of complexes. Collectively, these methods constitute important steps towards the analysis of the cellular interaction proteome in space and time.

11:10 Mapping the PP1 Phosphatome
Laura Trinkle-Mulcahy, University of Ottawa

Authors: Delphine Chamousset, Michèle Prévost

Abstract: As our appreciation of the importance of regulated protein phosphatase activity in cellular pathways grows, so does the need to identify and functionally characterize these essential enzymes so that we can take advantage of their therapeutic potential. Unlike kinases, most protein phosphatases are ubiquitous enzymes that gain their substrate specificity not from their sequence or structure but from regulatory proteins with which they associate in holoenzyme complexes. Thus, in contrast to the catalytic site-targeted small molecule inhibitors that work effectively for many protein kinases, inhibition of a specific phosphatase complex relies on targeting the regulatory proteins that confer substrate specificity/localization on the catalytic subunit. To date, using a combination of biochemical, bioinformatic and proteomic approaches, our lab and others have identified >200 regulatory proteins for the serine/threonine protein phosphatase 1 (PP1). Based on biochemical data linking PP1 to a diverse range of cellular pathways, it is clear that many more remain to be discovered. Our laboratory applies a combined fluorescence imaging/quantitative proteomics approach to map the functional interactomes of the 3 human PP1 isoforms, PP1a, b and g, both under steady-state conditions and in response to cellular perturbations. As we continue to improve our ability to fractionate cells and extract proteins while preserving protein-protein interactions for downstream interactome analyses, we have mapped a series of isoform-, cell compartment- and cell cycle-specific PP1 complexes that can be added to our list of therapeutic targets for diseases in which their respective cellular roles are disrupted.

11:30 A New High-throughput Approach to Detect Protein Complexes Reveals Temporal Changes in the Interactome in Response to Epidermal Growth Factor
Anders Kristensen, University of British Columbia

Authors: Anders R. Kristensen, Joerg Gsponer, Leonard J. Foster

Abstract: The interactome is highly ordered and can respond to external stimuli, however current techniques for monitoring the interactome provide very limited stoichiometric and temporal information. We used a novel approach to study temporal interactome changes by combining Protein correlation profiling- stable isotope labeling by amino acids in cell culture PCP-SILAC with Size Exclusion Chromatography (SEC). Three populations of cells are SILAC incorporated, and the heavy (H) population is stimulated with epidermal growth factor (EGF) for 20 min before being combined with
the medium (M) population. Protein complexes are separated by SEC and aliquots from the light (L) population are spiked into each H/M fraction functioning as an internal standard before analysis by LC-MS/MS. By quantifying the M/L ratio in the fractions, chromatographs can be generated for the individual proteins, which can be deconvoluted into component Gaussian curves. Together with the Euclidian distances among the chromatograms these component Gaussian curves can be used to assign binary protein interactions and protein complexes. This method detects similar numbers of protein interactions without overexpression or creating fusion proteins as other high throughput techniques but also quantifies protein stoichiometry. Using the H/M ratio in the fractions we identified 351 proteins displaying temporal changes following EGF stimulation. These proteins correlate strongly with proteins found to be phosphorylated in response to EGF stimulation and proteins known to bind to the EGF-receptor, highlighting that PCP-SILAC combined with SEC is an accurate tool to reveal temporal changes in the interactome.

11:50 Cancer Protein Interactomes: Towards a Fast Mapping of Regulated Interactions
Stephen Tate, AB/Sciex

Authors: Jean-Philippe Lambert, Zhen Yuan Lin, Brett Larsen, Lorne Taylor, Quan Zhong, Marc Vidal, Tony Pawson, Steve Tate, Anne-Claude Gingras

Abstract: Deregulation of the phosphorylation balance controlled by the coordinated action of kinases and phosphatases is implicated in multiple diseases, including cancer. As cancer sequencing projects are progress, there is a disconnect between the speed of genomic data acquisition and functional understanding of the mutations. Kinases and phosphatases frequently associate with regulators, scaffolding molecules and substrates; a possible outcome of mutation is a change in these physical interactions. A strategy to rapidly identify regulated interactions in an unbiased fashion within the context of a human cell would have multiple benefits, including the development of pharmacologically active compounds. We demonstrate data-independent quantification with SWATHTM coupled to affinity purification for rapid, accurate and unbiased quantification of the interactome changes. Two dominant mutations in the kinase CDK4 associated with melanoma, a previously reported loss of interaction with CDK4 inhibitors p15INK and p16INK is easily detected| loss of these interactions would be expected to maintain the kinase in a non-repressed state, accelerating the cell cycle progression. In contrast to p15INK and p16INK, multiple interactions remain constant in the CDK4 mutants, and interactions with Cyclin D and with a member of another family of CDK inhibitors are modestly increased, perhaps as a compensatory mechanism for loss of INK binding. Interactions with the HSP90 machinery, and in particular with the kinase specific co-chaperone CDC37, are drastically increased in the mutants, suggesting potential therapeutic avenues. With its high sensitivity, reproducibility and rapidity, AP-SWATH is an ideal companion to genomic-wide sequencing efforts.

13:30 Predicting Protein Interactions from the Genome
Gary Bader, Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto

Dr Bader is a world leader in the development of open-source biological pathway, network databases, visualization and analysis software for protein-protein interactions. He studies the organization and evolution of biological systems using computational biology and bioinformatics techniques to predict specific molecular interactions including mutations that cause disease.

Authors: Gary Bader

Abstract: We aim to accurately predict biologically relevant protein-protein interactions mediated by peptide recognition domains, such as PDZ, WW and SH3 domains, directly from the genome. First, we need to predict the binding motif preference of a domain given its amino acid sequence. We do this using machine learning techniques. We can then scan a proteome to find potential protein partners of the domain that contain a recognized binding motif. Second, we need to predict if a potential protein interaction actually occurs in the cell. We use available biological context informa-
tion (e.g. gene expression, gene function annotation, conserved binding sites across species) to infer a likelihood that two given proteins interact. Our computational methods require a set of binding preferences for each domain family to learn from. This information is now available for multiple domain families across a few species, determined using phage-display and peptide-chip experimental methods. Protein-protein interactions involving these domains are also available from the literature and from large-scale protein-protein interaction mapping experiments (e.g. yeast two-hybrid). Interestingly, the networks generated using this approach have binding sites for many predicted protein interactions and are available across species, enabling the study of protein interaction network rewiring via binding site evolution as function evolves.

14:10 iRefWeb: Analysis of Consolidated Protein Interactions and Their Confidence Scores
Shoshana Wodak, Hospital for Sick Children

Authors: Andrei L. Turinsky, Brian Turner, Shuye Pu, Thomas Switzer, Paul Boddie, Sabry Razick, Ian M. Donaldson, Shoshana J. Wodak

Abstract: We present iRefWeb, a web interface to over 500,000 non-redundant protein interactions curated from literature, which have been consolidated from 14 major public databases. iRefWeb enables users to extract a full interaction network for an organism of interest, identify interactions for specific genes and proteins, and examine various supporting evidence for an interaction using a range of data filters. A new feature of iRefWeb is the provision of a numerical interaction-confidence score, which conveniently aggregates several types of supporting evidence for each interaction: the number of experimental studies in which the interaction was detected, the different detection methods used, the degree of conservation of the interaction in other organisms or among paralogs in the same organism, and the scale of each study (high- or low-throughput). The computation of each score value is fully detailed and linked to the original pieces of supporting evidence, which reveals the relative contribution from different data sources and experiments to the overall score. The availability of the confidence scores allows researchers to quickly build a high-confidence interaction network in human, mouse, yeast or other model organisms. Such network may then be used either directly, or as a literature-curated benchmark for the statistical validation of new interaction sets (e.g. those derived from affinity-purification mass spectrometry experiments). We provide an overview of the consolidated protein-protein interaction landscape, and examine the effect of various types of supporting evidence and confidence scores on the generation of meaningful organism-specific interactomes. iRefWeb can be accessed at http://wodaklab.org/iRefWeb.

14:30 Understanding Spatio-temporal Control of Rho Protein Signaling by RhoGAP/GEF Proteomics
Rick Bagshaw, Mount Sinai Hospital/ Samuel Lunenfeld Research Institute

Authors: Rick Bagshaw, Evangelia Petsalakis, Oliver Rocks, Tony Pawson

Abstract: The Rho family of GTPases is well known to have regulatory effects on a variety of fundamental cellular processes. This functional breadth indicates the activity of an individual Rho protein is likely determined by its ‘spatio-temporal’ properties. RhoGEF and RhoGAP proteins can directly control the activation state of Rho proteins and are key nodes in determining functional specificity. We are interested in how RhoGEF/GAPs accept and integrate cellular inputs to achieve a decision on the activation state of Rho proteins. We have developed a cDNA library of 143 ‘full-length’ RhoG/ GAP proteins for expression studies to characterize their sub-cellular localizations, protein binding-partners by co- immunoprecipitation/ Mass spectrometry, and RhoGTPase specificity. From these data, ectopic expression of RhoGEF/GAPs can give rise to ‘cellular-phenotypes’ that can be used to categorize Rho protein specificity. From our interactome study, novel binding partners identified in this project appear to direct the localization of RhoGEF/GAPs which may underlie the foundation of spatio-temporal specificity. Clustering of RhoGEF/GAP baits according to properties of their interacting partners correctly groups RhoGEF/GAPs known to be involved in similar cellular pathways and
suggests functions for uncharacterized RhoGEF/GAPs. Our data also suggest that RhoGEF/GAPs have different interaction partner types, which correlates with the fact that certain ‘cellular phenotypes’ segregate with either RhoGAPs or RhoGEFs. Upon completion, a combined RhoGTPase-GEF/GAP network should implicate functional roles for otherwise uncharacterized RhoGEF and RhoGAP proteins and will help identify novel nodes of control and regulation for Rho-dependent processes.

14:50 **SUMO System Interactome and the Role of SUMO Chains in S. cerevisiae**
Tharan Srikumar, University of Toronto

**Authors:** Tharan Srikumar, Michael Costanzo, Kyle Tsui, Harm van Bakel, Erica Johnson, Brenda Andrews, Charlie Boone, Corey Nislow and Brian Raught

**Abstract:** The ubiquitin-like proteins (Ubls) are small polypeptides that function as post-translational modifications. Like ubiquitin, most Ubls are covalently attached to a lysine residue on target proteins. The small Ubiquitin-related modifier (SUMO) plays important roles in a number of critical cellular processes, such as proliferation and regulation of the cell cycle, yet their specific cellular functions remain poorly understood. Like ubiquitin, SUMO proteins can form oligomeric “chains”, but the functions of these structures are even less well understood. We have utilized a robust affinity purification-mass spectrometry (AP-MS) technique to create high-density protein-protein interactomes. Here we describe the first global analysis of SUMO chain function, and the first comprehensive SUMO system protein-protein interactome. In total, 347 high confidence interactions were detected, encompassing a total of 180 interacting partners. Yeast SUMO system components were found to interact with proteins involved in a number of processes and our mapping effort has increased the number of known SUMO system interacting partners >50-fold. The SUMO E3 ligases (Siz1 and Nfi1) as well as the SUMO proteases (Ulp1 and Ulp2) were found to have non-overlapping sets of interactions, highlighting the specificity of our screen. We found a number of transcriptional co-repressors and chromatin remodelling proteins interact physically and genetically with SUMO system components. Indeed, inhibition of SUMO chain synthesis leads to severe chromatin condensation defects, which in turn leads to chromosome missegregation, unscheduled transcription of stress-and nutrient-regulated genes, and aberrant intragenic transcription.

15:30 **Physical and Functional Mapping of the Higher Order Structure of Cell Signalling Networks**
Jeff Wrana and Liliana Attisano, Samuel Lunenfeld Research Institute, Department of Molecular Genetics, University of Toronto

**Invited Speaker**

**Jeffrey Wrana is the recipient of multiple National awards for his transformative research on cancer. He uses high-throughput proteomics and genetic tools, to understand how morphogen cell signalling pathways and the higher organizational structure controls cell behavior in development and cancer.**

**Abstract:** Early work in understanding the extrinsic cues that govern a broad array of developmental and disease processes has revealed a relatively small toolkit of cell signalling pathways that are conserved across all animals. Therefore, to manifest the diversity of form and function required for the development and homeostasis of multicellular organisms, the biological response is highly contextualized. For example, the TGFbeta family of secreted morphogens are employed repeatedly in all animals from the earliest stage of development to the regulation of homeostasis in the adult. Moreover, these contextualized signals are abused in complex diseases such as cancer. Using mass spectrometry and high throughput proteomics tools coupled with systematic functional genomics screens, we are systematically mapping how individual signalling pathways interact with each other to provide the contextual cues that control the biological output to morphogen signalling. This has uncovered a higher order network in which pathways that mediate morphogenesis (TGFbeta and WNT pathways) interact with tissue size control signalling (Hippo) and polarity complexes. In this presentation I will describe recent work from my lab that has uncovered a mechanism whereby stromal, fibroblast-derived factors interact with breast cancer cells to mobilize autocrine WNTR planar cell polarity during breast cancer metastasis.
16:10 **PDZ Domain Containing Protein RADIL Interacts With the Kinesin Family Protein KIF14 and Modulates Breast Cancer Cell Motility**

Syed Mukhtar Ahmed, University of Toronto

**Authors:** Syed M. Ahmed¹, Brigitte L. Theriault², Maruti Uppalatti³, Raffi Tonikian⁴, Brenda Gallie², ⁵, Sachdev Sidhu⁴, Stephane Angers¹, ²

**Affiliations:** ¹Graduate Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy; ²Department of Biochemistry; ³Department of Medical Genetics; ⁴Banting and Best Department of Medical Research, Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto; and ⁵Ontario Cancer Institute, Princess Margaret Hospital, Toronto, ON, Canada.

**Abstract:** Recently we demonstrated that the Radil protein forms a complex with the heterotrimeric G-protein subunits Gβγ and Rap1a-GTP via its RA domain to promote cell-matrix adhesion via inside-out activation of integrins. Using two different proteomic approaches we found KIF14 as a novel interaction partner of Radil. KIF14 is a kinesin family protein that is overexpressed in several cancers including breast cancers. Our data indicate that an atypical PDZ ligand located at the c-terminus of KIF14 interacts with the Radil PDZ domain. KIF14 translocates Radil onto the microtubule network thereby regulating its spatial availability as a Rap1 effector to promote adhesion and spreading. Activated Rap1a leads to the recruitment of Radil from microtubules to the plasma membrane where it can activate integrins. Both knockdown of KIF14 or Radil result in decreased breast cancer cell motility and invasion in vitro through opposite mechanisms. The depletion of KIF14 leads to hyperactive Rap1-Radil signalling, to increased integrin activation and cell-matrix adhesion whereas Radil knockdown inhibits integrin activation and cell-matrix interaction. Furthermore, the depletion of Radil robustly blocks the metastasis of breast cancer cells to the lung in mouse xenografts experiments. Our findings provide a molecular basis underlying the frequent upregulation of Rap1a activity and KIF14 levels found in several cancers by suggesting that it is required to reach the optimal balance of Rap1a-Radil signalling and cell-matrix interaction needed to promote breast cancer cell migration and invasion during metastasis.

16:30 **Functional Redundancy of the Drosophila p38 MAP Kinases Probed by Mass Spectrometry-based Interaction Proteomics**

Vladimir Belozerov, York University

**Authors:** Vladimir Belozerov, Zhen-Yuan Lin, Anne-Claude Gingras, John McDermott, and KW Michael Siu

**Abstract:** The p38 MAPK pathway is a key evolutionarily conserved mediator of an organism’s response to stressful environmental stimuli. In mammals four p38 kinases form a robust signaling module believed to be supported by considerable functional redundancy. In Drosophila the p38 MAPK family consists of two highly homologous kinases, Mpk2 and p38b, and a third putative kinase, p38c. Recent genetic analyses of various Mpk2 and p38b alleles suggest that the two kinases are at least partially redundant. However, the extent of this redundancy, and possible non-overlapping roles remain to be defined. To reveal common and unique molecular functions of individual p38 kinases we generated a high-resolution protein interaction map of Mpk2, p38b, and p38c in S2 cells. The use of an optimized single-step affinity purification procedure followed by gel-freeLC-MS/MS analysis allowed us to detect both abundant complex components, and low-abundance interacting proteins, likely representing more transient and/or lower-affinity interactions. The results of our study suggest limited functional overlap between Mpk2 and p38b, primarily restricted to the regulation of mRNA processing. Another finding is a previously unacknowledged link between the p38 pathway and the regulation of carbohydrate metabolism. Validation of these new functional connections in the context of larval muscle and gut will be presented. More broadly, our results illustrate the use of mass spectrometry-based interaction mapping for assigning shared and unique molecular functions to individual members of redundant protein families.
16:50 Proteomic Analysis of Tetrahymena Thermophila Protein Complexes Involved in Chromatin Dynamics
Jeffrey Fillingham, Ryerson University Department Chem/BIO

Authors: Jeffrey S. Fillingham, Jyoti Garg1, J.P. Lambert, Abdel Karsou, Ernest Radovani Matthew Cadorin, Syed Shah, Anne-Claude Gingras and Ronald E. Pearlman

Abstract: The identification of the complete set of protein-protein interactions for a given protein (its interactome™) provides novel insight into its function. Although the global set of protein-protein interactions have been well-studied in the budding yeast Saccharomyces cerevisiae model, we believe that the comparative study of targeted interactomes in different unicellular microbial systems offers the potential for mechanistic and functional insight that are not possible with the exclusive use of a single organism. We have initiated a project investigating the efficacy of the ciliated protozoan Tetrahymena thermophila as a model for identifying interactomes of proteins involved in chromatin dynamics, particularly associated with RNAi/heterochromatin dependent irreversible genome silencing. Our specific goal is to identify Tetrahymena interactomes in order to determine novel protein-protein interactions of several well-conserved eukaryotic proteins involved in chromatin dynamics using affinity purification coupled with mass spectrometry (AP-MS). To this end we present data and proteomic characterization of the T. thermophila Proteasome, SWI/SNF complex, and putative Mediator through AP-MS of affinity tagged tDss1, tSnf5, and tSoh1/Med31 respectively. We also used AP-MS to characterize the interactome of the tAsf1 H3-H4 histone chaperone. Reciprocal AP-MS of tAsf1 and one interaction partner (Importin β) identifies a protein complex that is potentially involved in the import of histone H3-H4 to the micronucleus. This work was supported by grants to JSF from NSERC and a Ryerson University Health Sciences Internal Grant, and to REP by grants from NSERC and CIHR.
**WEDNESDAY April 25**

**TRANSLATIONAL PROTEOMICS**

Session chairs: Andrew Emili, University of Toronto
Eleftherios Diamandis, Mount Sinai Hospital

8:30 Invited Speaker

*Discovery of Novel Pancreatic Cancer Biomarker Using in Proteomics and Mass Spectrometry*

Eleftherios Diamandis, Mount Sinai Hospital

Dr Diamandis’s research interests are centered on the discovery and validation of novel cancer biomarkers, proteomics, translational research and the role of kallikrein enzymes in health and disease; basic and translational aspects of tumor metastasis. A focus of his work is on the role of quantitative proteomics in pancreatic cancer biomarker discovery and validation.

Authors: DIAMANDIS, Eleftherios P., BLASUTIG Ivan, CHAN Alison, DIMITROMANOLAKIS Apostolos, GALLINGER Steven, HUAN Randy, MAKAWITA Shalini

Abstract: Pancreatic cancer is the 4th leading cause of cancer-related deaths with a 5-year survival rate of <5%. Early detection improves mortality rates and there is an urgent need for discovery of novel biomarkers for early pancreatic cancer detection as well as patient monitoring. We applied a comprehensive proteomics-based strategy capable of identifying candidate biomarkers. We then quantitatively analyzed many of these candidate biomarkers in serum to determine their efficiency, in comparison to the well established biomarker CA19.9. Our proteomic discovery effort included proteomic analysis of pancreatic cancer cell lines, pancreatic juice, pancreatic ascites and pancreatic tissue. This analysis revealed >3,500 proteins, identified with high confidence. Candidates were selected by multiple criteria, such as quantitative comparisons, tissue specificity and microarray data integration. By using such strategies, we identified 12 candidates which were preliminary validated in serum of normal and pancreatic cancer patients (20 patients per group). A more detailed validation was further conducted on 480 samples from healthy individuals (165), pancreatic ductal adenocarcinoma (183), benign pancreatic diseases (44) and patients with other tumours (88 samples). Biomarkers were analyzed by ELISA both individually and in combinations. We found that the most promising of these biomarkers were REG1B, SYCN and AGR2. Combination of CA19.9 + REG1B + SYNC provided an area under the curve of 0.89, which was superior to the one provided by CA19.9 alone (0.82).

We conclude that an integrated proteomic approach may reveal novel biomarkers for pancreatic adenocarcinoma. Further validation of these biomarkers is warranted.

9:10

*Identification of Differentially Expressed Proteins in Direct Expressed Prostatic secretions of Men with Organ-confined Versus Extracapsular Prostate Cancer*

Yunee Kim, University of Toronto


Abstract: Current protocols for the screening of prostate cancer cannot accurately discriminate clinically indolent tumors from more aggressive ones. One reliable indicator of outcome has been the determination of organ-confined versus non-organ-confined disease but even this determination is often only made following prostatectomy. Fluids that are proximal to the prostate, such as expressed prostatic secretions (EPS), are attractive sources of potential prostate cancer biomarkers as these fluids likely bath the tumor. Direct-EPS samples from 16 individuals with extracapsular or organ-confined prostate cancer were used as a discovery cohort, and were analyzed by a 9-step MudPIT. A total of 624 unique proteins were identified by at least two unique peptides (0.2 % FDR). Using spectral counting, we identified 133 significantly differentially expressed proteins in the discovery cohort. In-
tegrative data mining prioritized 14 candidates, including two known prostate cancer biomarkers: prostate-specific antigen and prostatic acid phosphatase, which were significantly elevated in the direct-EPS from the organ-confined cancer group. These and five other candidates were verified by Western blotting in an independent set of direct-EPS from patients with biochemically recurrent disease (n = 5) versus patients with no evidence of recurrence upon follow-up (n = 10). Lastly, we performed SRM-MS- based relative quantification of four candidates using unpurified heavy isotope-labeled synthetic peptides spiked into pools of EPS-urines from men with extracapsular and organ-confined prostate tumors. This study represents the first efforts to define the direct-EPS proteome from two major sub-classes of prostate cancer using shotgun proteomics and verification in EPS-urine by SRM-MS.

9:30 Identification of Novel Disease-specific and Membrane-associated Protein Markers in a Mouse Model of Multiple Sclerosis
Laura F. Gianni, CCBR University of Toronto

Authors: Laura F. Gianni, Anthony W. Purcell and Andrew Emili

Abstract: Multiple sclerosis (MScl) is a primary example of a complex autoimmune disease that can benefit from the elucidation of biomarkers by the application of novel unbiased discovery tools such as proteomics. A label-free quantitative proteomics approach was used to identify disease-specific markers in a mouse model of MScl known as experimental autoimmune encephalomyelitis (EAE). We compared protein expression profiles in the spinal cord and peripheral blood mononuclear cells (PBMCs) of EAE and sham-injected (‘vehicle’ control) mice to identify changes at the protein level which could be implicated in disease pathogenesis. Several time-course studies on EAE support a caudal-to-rostral progression of disease driven by the vulnerability of the spinal cord thus it is expected that biochemical changes are taking place here. PBMCs are largely comprised of T and B cells, the major cellular components of the adaptive immune response in MScl and EAE. Membrane protein-enriched fractions from PBMCs and soluble protein lysate from spinal cord tissue were prepared for nanoflow liquid chromatography tandem mass spectrometry (nLC-MS/MS) using the filter-aided sample preparation (FASP) method. After statistical evaluation of the data, 431 differentially expressed proteins (p-value<0.05, Kolmogorov-Smirnov test) were identified in the comparative spinal cord analysis (peptide FDR=1.1%) whilst 207 statistically significant changes were discovered in the PBMC comparison (peptide FDR=1.2%). Gene enrichment analyses have revealed the most statistically relevant and overrepresented biological annotations in EAE to be processes involving the proteasome, complement and coagulation cascades. The biological significance of candidate biomarkers is currently being elucidated through literature searches and rigorous validation studies.

9:50 A Systems Level View of the Central Dogma in Lung Cancer
Yuhong Wei, Hospital for Sick Children

Authors: Yuhong Wei, C. To, L. Li, P. Taylor, V. Ignatchenko, D. Strumpf, N.A. Pham, I. Jurisica, L. Muthuswamy, T. Kislinger, M.S. Tsao, and Michael F. Moran

Abstract: Non-small cell lung carcinoma (NSCLC) represents 80% of lung cancers, the deadliest cancer worldwide. NSCLC, like most cancers, continues to be classified largely based on histology, and clinical outcomes remain poor. Using an integrated genomics/proteomics platform spanning the central dogma DNA→RNA→Protein, 36 samples from patient-matched NSCLC primary tumours, xenografts, and normal lung were analyzed. Clustering according to gene copy number, mRNA expression, and comprehensive proteome profiles validated the xenografts, but not kindred cell lines for their reiteration of primary tumour molecular signatures, and precisely resolved the NSCLC major histological subtypes. Protein expression from chromosomes was highly correlated with gene densities, and a set of proteins upregulated in tumors (verified by multiplexed selected reaction monitoring, SRM-MS) was expressed to a significant extent from regions of gene copy number gain, a phenomenon enriched in xenografts. Proteome analysis revealed distinctive indexes of tumour and stroma metabolism, and stratified tumours according to their expression of known and
postulated anti-folate targets in a pattern consistent with clinical observations. Increased gene copy number and/or elevated protein expression and activation of the NSCLC drug target EGFR was observed in a subset of tumors and xenografts. This integrated genomics-proteomics analysis validates a primary xenograft model, represents an initial systems level perspective on the central dogma in cancer, and reinforces the proteome as a distinctive and clinically relevant molecular feature for lung tumor stratification and potentially a basis for personalized treatments.

10:30 Integration of Cardiac Proteome Biology and Medicine By A Specialized Knowledgebase
Peipei Ping, University of California at Los Angeles

Dr Ping’s research program focuses on the dynamic alterations of proteome biology in cardiovascular medicine. She is interested in how altered molecular pathways or organelle proteomes contribute to the pathogenesis of myocardial ischemic injury and how regulatory mechanisms activate cardioprotection to minimize injury.

Authors: Nobel C. Zong, Haomin Li, Rafael C. Jimenez, Ning Deng, Allen Kim, Ivette Zelaya, David Liem, Jacob Odeberg, Caiyun Fang, Anna Zolyan, James Weiss, Tao Xu, Huilong Duan, Mathias Uhlen, John R. Yates III, Rolf Apweiler, Henning Hermjakob, Peipei Ping

Abstract: Proteomic technologies enable an unprecedented prospect of characterizing cardiovascular biology; a successful application of these technologies mandates a specialized bioinformatics platform. The integration of diverse proteomics datasets into a contextualized and accessible form will catalyze the development of consensus knowledge, minimize redundant research efforts and leverage novel insights to scientists of multidisciplinary backgrounds.

The central goal of this project is to develop a consolidated knowledgebase that fosters a synergistic feed-forward proteome paradigm in cardiovascular biology and medicine. Orthogonal datasets of functional proteomics and cardiovascular biology were contextualized in a MySQL relational database. This database is configured around cardiac organelle modules to form the Cardiac Organelar Protein Atlas Knowledgebase (COPaKB). Thus far, four modules with 3,171 proteins have been compiled, which include newly acquired datasets on human mitochondria and proteasomes. Using these library modules as references, a COPaKB Client script communicates between investigators, the COPa knowledgebase server, UniProt, and Human Protein Atlas (HPA), via robust web services to orchestrate highly sensitive and reproducible protein annotation across the globe. In addition, this workflow assembles sub-proteome properties and biological information of interest in automation by submitting targeted queries to the COPa knowledgebase, UniProt, and HPA.

Collectively, COPaKB (www.HeartProteome.org) provides an accessible and user-interactive resource to support hypothesis-driven as well as discovery-targeted investigations. It presents an array of innovative tools, in the format of a single web server, effectively translating proteomics information to advance our understanding of cardiovascular biology and medicine.

11:10 Proteomic Analysis of Patient-specific Blood Outgrowth Endothelial Cells in Heritable Pulmonary Arterial Hypertension: Insight into Novel Mechanisms
Jessie Lavoie, Ottawa Hospital Research Institute

Authors: Jessie R. Lavoie1,2, Mark Ormiston3, Carol Perez-Iratxeta1, Baohua Jiang1, David W. Courtman1,2, Nicholas W. Morrell1, Duncan J. Stewart1,2

Affiliations: 1 Ottawa Hospital Research Institute, Sprott Stem Cell Centre and Regenerative Medicine Program, Ottawa, Ontario, K1Y 8L6, Canada; 2 Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada; 3Department of Medicine, Box 157 Addenbrooke’s Hospital, University of Cambridge School of Clinical Medicine, Hills Road, Cambridge, CB2 0QQ UK.
Abstract: Background: Pulmonary arterial hypertension (PAH) is a lethal disease, characterized by excessive proliferation of pulmonary vascular cells. Hereditary PAH is usually caused by “loss-of-function” mutations in the bone morphogenetic protein type II receptor (Bmpr2). However, the mechanisms by which these mutations cause PAH remain unclear.

Aim: To identify dysregulated proteins in blood-derived endothelial cells of PAH patients with Bmpr2 mutations compared with healthy controls.

Methods: Blood-derived endothelial cells (ECs) were expanded ex vivo from peripheral blood mononuclear cell samples of four PAH patients and four healthy subjects. Protein isolates were subjected to 2D gels and analyzed using PDQuest software. Specific proteins were identified by mass spectrometry (LC-MS/MS).

Results: Out of the 416 proteins detected, 11 were downregulated, (p-value ≤ 0.05) in PAH cells, including some proteins previously implicated in PAH (i.e. PKA and guanine nucleotide-binding protein); and 11 were upregulated, including a number of cell growth regulatory proteins such as DNA replication licensing factor MCM7 and translationally controlled tumor protein (TCTP). Increased expression of TCTP was validated in patient-derived ECs in vitro. As well, lung endothelial TCTP was shown to be markedly upregulated by immunofluorescence staining in a clinically relevant model of PAH (Sugen) compared to control animals, localized exclusively to proliferative and obliterative intimal lesions.

Conclusions: Bmpr2 mutant ECs exhibited dysregulation of key proteins controlling vasodilation and EC growth. TCTP could be playing a central role in the development of the obliterative arteriolar lesions that may drive disease progression in PAH, representing a novel therapeutic target.

11:30 A Census of Human Soluble Protein Complexes
Pierre Havugimana, University of Toronto


Abstract: Cellular processes often depend on stable physical associations between proteins. Despite recent progress, knowledge of the composition of human multi-protein complexes remains limited and no proteome-scale experimental maps have been reported. To close this knowledge gap, we applied a global integrative proteomic approach to biochemically enrich and systematically identify 622 putative native soluble multi-protein complexes from cultured human cell extracts, many of which are linked to core biological processes and disease. Strikingly, whereas larger assemblies tend to be more extensively annotated and evolutionarily conserved, human protein complexes with 5 or fewer subunits are far more likely to be functionally un-annotated and/or restricted to vertebrates, suggesting more recent functional innovations. The comprehensiveness, centrality and modularity of this probabilistic physical interaction map should therefore facilitate interpretation of human cell biology.

11:50 Target Identification by Chromatographic Co-elution: new chemical proteomics method for protein target deconvolution of drugs and bioactive molecules
Dajana Vuckovic, University of Toronto


Abstract: The mechanism-of-action and protein target(s) of bioactive small molecules such as drugs are often not well-characterized, and present an important challenge in both phenotype-based and target-centric discovery pipelines. The classical affinity-based biochemical methods, where the compound of interest is immobilized or labeled in order to isolate the interacting proteins, relies on chemical modification of the small molecule which may adversely affect its biological activity.
and binding. We propose a novel method for the determination of protein targets of bioactive molecules using chromatographic fractionation. The method relies on the observable shift of the elution profile of a small ligand when the ligand is bound to a protein binding under non-denaturing chromatographic conditions. This shift is detected by monitoring all of the collected fractions for the presence of bioactive molecule using targeted liquid chromatography-mass spectrometry assay after offline protein-precipitation. Bound drug fractions are then subjected to shotgun proteomic profiling to identify target protein candidates. Multiple chromatographic fractionations allow the isolation of true protein target because the true target and drug have highly correlated elution profiles under a variety of complementary fractionation conditions. We present the validation of this novel approach using well-studied ligands with known interacting protein partners, including interaction of trichostatin A with HDAC1 (Ki=3.4 nM) and sordarin with ELF2 (Kd = 1.26 µM). We also present the successful identification of ERG6 as a new secondary target for an antifungal compound 4513-0042, and ASC1 and DAK1 as novel targets of A77636 showing the capability of technique for de novo target deconvolution.

13:30 New Developments in Quantitative Proteomics Leveraging the Orbitrap Technology – Application to Clinical Samples
Bruno Domon, Luxembourg Clinical Proteomics Center, CRP-Santé, Strassen, Luxembourg

Dr Domon heads the laboratory for clinical proteomics at the CRP Santé, in Luxembourg. He is interested in the development of quantitative proteomics and has been pioneering targeted methods leveraging novel mass spectrometry techniques. He is developing and applying these techniques for routine clinical applications.

Authors: Bruno Domoni

Abstract: The generation of accurate and comprehensive data sets has become an essential element of systems biology and biomarker studies. While large scale discovery experiments one hand and targeted quantitative analyses on the other are nowadays widely used in proteomics, we are proposing an alternative approach, which is based on a novel quadrupole/orbitrap instrument. The high acquisition speed and the exquisite sensitivity of such a hybrid mass spectrometer allow performing reliable qualitative and quantitative experiments. The additional selectivity intrinsic to the high resolution orbitrap mass analyzer enables the development of novel quantification methods. Quantitative experiments can be performed either in full scan mode (using the high-resolution / accurate mass capability) or in MS/MS mode by analyzing simultaneously specific fragment ions (i.e. SRM-like mode). The different modes of operation, their advantages and limitations will be presented in details.

This technique has been applied to precisely quantify biomarker candidates in bodily fluids, and more specifically in urine samples. The quantitative analyses were performed in conjunction with stable isotope dilution, using second generation synthetic polypeptides, composed of one universal reporter fragment facilitating the systematic, precise quantification of multiple analytes in complex biological samples.

14:10 Verification of Male Infertility Biomarkers in Seminal Plasma by Multiplex SRM Assay
Andrei Drabovich, SLRI, Mount Sinai Hospital

Authors: Andrei Drabovich, Ihor Batruch, Keith Jarvi and Eleftherios P. Diamandis

Abstract: Azoospermia, one of the medical conditions of male infertility, is diagnosed in 20% of non-fertile men and has two forms: obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). Currently, a diagnostic testicular biopsy is used to distinguish between NOA and OA. We initiated a seminal plasma proteome project aimed at identifying proteins differentially expressed in infertile men. More than 2000 proteins were identified in seminal plasma by mass spectrometry. A list of candidates was selected based on the spectral counting analysis, and 30 proteins were verified.
by a stable-isotope dilution SRM assay in 30 seminal plasma samples from healthy, OA and NOA individuals (Mol Cell Proteomics, 2011, 10, M110.004127). Most promising 18 proteins were subjected to further verification by SRM assay in 150 seminal plasma samples: normal, OA, post-vasectomy (simulated OA) and NOA. Our method development workflow and sample preparation protocol for quantitative SRM measurements will be presented. Levels of all 18 proteins were found in the range 1-500 µg/mL in normal seminal plasma and were significantly decreased in OA/PV samples (2-1000 fold). Three proteins (LDHC, TEX101 and ECM1) were able to differentiate normal vs OA/PV vs NOA groups with absolute or near-absolute specificities and sensitivities. The performance of ECM1 protein was later confirmed by a commercially available ELISA. Our findings allowed us to propose a panel of biomarkers for differential diagnosis of azoospermia. Pending the development of a routine clinical assay, proposed markers will eliminate in infertility clinics the need for testicular biopsy to diagnose categories of male infertility.

14:30 Intra-protein Variability Revealed by Absolute Quantification of Plasma Proteins using Dimethyl Labelling
Devanand Pinto, National Research Council, Halifax

Authors: Devanand M. Pinto; Kenneth Chisholm; Andrew Leslie; Alejandro Cohen; Rita Kostyleva

Abstract: Highly multiplexed assays are a major advantage of MS quantification. Efforts underway to develop multiplexed assays often include an antibody step. The cost these reagents often limits the number of peptides per protein to one. Single peptides are insufficient to characterize splice variants and variable PTMs.

Plasma from 115 breast cancer patients and was depleted of 14 high-abundance proteins. 23 proteins were selected for analysis and 129 peptides were selected based on an in-house derived algorithm for ranking of MRM intensities followed by selection based on acceptable analytical performance. Using linear discriminant analysis, we were able to correlate the histology of the tumor with the proteomic profile obtained. Correlation between proteomic and clinical parameters was strong at the peptide level; however, for some proteins, significant variation in fold-change was observed between peptides belonging to the same protein. The variation was greater than the expected due to analytical variation. Clusterin, for example, is cleaved into two chains: clusterin beta and alpha chains. Three of the peptides in our MRM methods belong to the beta chain (TLLSNLLEAK, EIQNAVNGVK and ASSIDDELFRQ) and the remainder to the alpha chain (LFDSPITVTVPVEVSR and EILSVDCSTNPSQAK). The discriminant peptides were derived from both chains. These results suggest that moderate level plasma proteins are differentially processed, perhaps by tumor proteases, in the tumor microenvironment. At the very least, these results indicate significant variability in plasma peptides and that the minimum number of peptides need quantify plasma proteins remains to be established.

14:50 Nrf2 Target Proteins are Regulated by Angiotensin II in Kidney Cellss
Ana Konvalinka

Authors: James W. Scholey, Eleftherios P. Diamandisi

Abstract: PURPOSE: Angiotensin II (ANGII), an effector of the renin angiotensin system leads to kidney disease progression, but there are no measures of renal ANGII activity. Accordingly, we sought to define an ANGII-stimulated proteome in primary human proximal tubular cells (PTEC) in order to identify potential markers of ANGII activity in the kidney.

METHODS: We utilized stable isotope labeling with amino acids (SILAC) in PTECs to compare proteomes of ANGII treated to Control cells. ANGII and Control PTEC lysates were processed together. Desalted peptide fractions were analyzed using LTQ-Orbitrap. Peptide/Protein identification and calculation of ANGII:Control ratios was performed by MaxQuant on the human IPI database. Ingenuity Pathway Analysis (IPA) and Cytoscape identified protein networks and enriched Gene Ontology (GO) terms.

RESULTS: Of 5010 identified proteins, 4968 were quantified in 5 replicates. Eighty six proteins were differentially regulated in more than one replicate with p<0.01. IPA uncovered differential regula-
tion of proteins in antioxidant response (Nrf2) pathway by ANGII. The most consistently upregulated protein in response to ANGII, heme oxygenase-1 (HO-1), is downstream of Nrf2. HO-1 protects from oxidative stress and limits fibrosis. Enriched GO term network of differentially expressed proteins was overlaid with gene expression set from an analogous study in mice. “Response to stress” was an enriched GO term in both sets and contained six proteins from Nrf2 network, including HO-1. CONCLUSIONS: SILAC and bioinformatic analyses have identified Nrf2 related proteins, which may serve as markers of ANGII activity in the kidney, and represent potential therapeutic targets.

15:30 The new ZIP on the Block: Significance of Evolutionary Descent of Prion Genes From a Zinc Metal Ion Transporter
Gerold Schmitt-Ulms, University of Toronto

Dr Schmitt-Ulms work contributes to two strands of research at the interface of proteomics and neurodegenerative disease research: the development of strategies for the study of protein interactions and the application of these strategies to dissect the early etiology of Alzheimer’s disease and prion diseases.

Authors: Gerold Schmitt-Ulms

Abstract: Prion diseases are fatal neurodegenerative diseases of humans and animals which, in addition to sporadic and familial modes of manifestation, can be acquired via an infectious route of propagation. Following the in vivo crosslinking of neuroblastoma cells, quantitative mass spectrometry based on isotopic tagging of peptides identified members of the ZIP (Zrt-, Irt-like Protein) metal ion transporter family in proximity to the prion protein. Surprisingly, extensive bioinformatics analyses subsequently established an evolutionary link between the prion gene and the ancient family of ZIP transporters. Our recent data suggest about 0.5 billion years ago a genomic insertion of a spliced transcript coding for a prion-like ZIP ectodomain may have created the prion founder gene in a vertebrate ancestor. Similar genomic insertions involving ZIP transcripts, and probably relying on retropositional elements, have indeed occurred more than once throughout evolution. The objectives of our current work are to complement these findings with critically needed insights into the structure of ZIP transporters and to evaluate the significance of the PrP-ZIP relationship in health and disease. Starting from an observation of abnormal LZT immunoreactive bands in prion-infected mice, subsequent cell biological analyses uncovered a surprisingly coordinated biology of ZIP10 and prion proteins that involves alterations to N-glycosylation and endoproteolysis in response to manipulations to the extracellular divalent cation milieu. Data from this work provide a first glimpse into a hitherto neglected molecular biology that ties PrP to its LZT cousins and suggest that manganese or zinc starvation may contribute to the etiology of prion disease in mice.

16:10 Novel MS-based Proteomics Platform to Uncover Minor Histocompatibility Antigens
Dev Sriranganadane, IRIC, Université de Montréal

Authors: D. Sriranganadane, Diana Paola Granados, Wafaa Yahyaoui, Céline M. Laumont, Tariq Daouda, Tara Muratore-Schroeder, Caroline Côté, Claude Perreault, Pierre Thibault

Abstract: Cells present at their surface major histocompatibility complex I (MHCI)-bound peptides, commonly called immunopeptidome, that mirror the proteome and modulate the immune response. Cytotoxic CD8 T lymphocytes survey the immunopeptidome for unexpected peptide antigens, which upon recognition induce apoptosis of antigen presenting cells. The immunopeptidome can also display peptides harbouring mutations arising from non-synonymous polymorphic variants. These peptides are collectively referred to as minor histocompatibility antigens (mHAgS) and are often associated to graft rejection. The identification of mHAgS by mass spectrometry (MS) is significantly more challenging than common MHCI-bound peptides since they cannot be matched in human reference databases and are present at low frequency at the cell surface. To address this challenge, we developed a novel high throughput MS approach that combines proteomics and transcriptomic data
to profile the mHAg repertoire of human cells. MHC peptides eluted from B cells of HLA-identical siblings were analyzed by high resolution LC-MS/MS on a LTQ-Orbitrap Velos and searched against human protein databases using traditional search engine (Mascot) and de novo peptide sequencing software (PEAKS). To validate PEAKS results, we used a modified Mascot approach based on “personalized” protein databases for each subject that were constructed from their corresponding transcriptomes. Peptides identified in each sibling were compared and enabled the identification of several peptide pairs differing by 1 amino acid, and representing differentially abundant mHAg. Altogether, these data represent the first comprehensive immunopeptidome repertoire of human B cells enabling the profiling and distribution of mHAg from HLA-identical individuals.

16:30 Clinical Application of SWATH: Discovering Novel Biomarkers in Threatened Preterm Labour

Jan Heng, Samuel Lunenfeld Research Institute

Authors: Yujing J Heng, Lorne Taylor, Monika Tucholska, Peter Kupchak, Moyez Dharsee, Kenneth Evans, Stephen Tate, Craig E Pennell, Tony Pawson, Stephen J. Lye

Abstract: Threatened preterm labour (TPTL) accounts for about 30% of pregnancy-related hospital admissions. Only 5% of these symptomatic women will deliver a premature baby within 2-10 days. Increasing evidence demonstrates that peripheral leukocytes can be used to monitor a variety of biochemical and physiological processes occurring in the body. SWATH was employed to quantify leukocyte lysate proteins from peripheral blood in women with TPTL with the aim to develop a unique proteomic signature to predict imminent preterm delivery (PTB). Heparinised blood was collected at point of hospital admission. We analysed samples collected from 16 women who had PTB within <48 hr and 24 women who did not deliver within 48 hour Leukocyte lysates were subjected to solid phase reduction, akylation and tryptic digestion. Peptides were injected into a TripleTOF 5600 mass spectrometer coupled to an Eksigent cHiP-LC system for information dependent acquisition and MS2 quantification using SWATH. Data were processed using Peakview®. Samples were randomized to avoid analytical bias and pooled peptides were interleaved into the sample queue to assess analytical reproducibility. Data were extracted from the top ranked peptides (>95% confidence) for each protein (max 100 peptides/protein), selecting the top 5 abundant ion transitions. The data were then manually triaged to only select the best 3 eXtracted ion currents (XIC)/peptide for further analysis. Quantitative data were analysed using principal component analysis (MarkerView®) and forward stepwise logistic regression. Preliminary analysis indicates putative proteomic signatures are predictive of PTB within 48 hours of hospital admission.

16:50 Translational Analysis of Mouse and Human Placental Protein and mRNA Reveals Distinct Molecular Pathologies in Human Preeclampsia

Brian Cox, University of Toronto

Authors: Brian Cox, Parveen Sharma, Andreas I. Evangelou, Kathie Whiteley, Vladimir Ignatchenko, Alex Ignatchenko, Dora Baczyk, Marie Czikk, John Kingdom, Janet Rossant, Anthony O. Gramolini, S. Lee Adamson

Abstract: Preeclampsia (PE) is the second leading cause of maternal death, adversely impacts 5-10% of all pregnancies and often requires premature delivery as the only treatment option. Despite extensive research, no consistent biomarkers or new treatments have emerged, suggesting that different molecular mechanisms may cause clinically similar disease. To address this, we undertook a proteomics study mouse placenta and translated and integrated these results with human mRNA microarray data to identify subgroups of human preeclampsia. Plasma membrane proteins at the blood tissue interfaces were extracted from placenta using intravascular silica-bead perfusion, and then identified using shotgun proteomics. We identified 1181 plasma membrane proteins, of which 171 were enriched at the maternal blood-trophoblast interface and 192 at the fetal blood-endothelial interface with a 70% conservation of expression in humans. Our translational integrated analysis
identified three distinct molecular subgroups of human preeclampsia by using expression patterns of trophoblast-enriched proteins. Analysis of all misexpressed genes revealed divergent dysfunctions including angiogenesis (subgroup 1), MAPK signaling (subgroup 2), and hormone biosynthesis and metabolism (subgroup 3). Subgroup 2 lacked changes in known preeclampsia markers (sFLT1, sENG) and uniquely over-expressed GNA12. In an independent set of 40 banked placental specimens, GNA12 was over-expressed during preeclampsia when co-incident with chronic hypertension, often undiagnosed prior to presentation with preeclampsia. We conclude that clinically similar preeclampsia patients exhibit divergent placental gene expression profiles thus implicating divergent molecular mechanisms in the origins of this disease. We suggest potential biomarkers to molecularly classify preeclampsia patients, towards personalized medicine treatment options.
CARDIOVASCULAR PROTEOMICS

Cardiac Proteomics Study in LDLr-/-/ApoB100/100xIGF-II Mice, a Model of Diabetic Cardiomyopathy

Presenting author: Ming Zhang, Université Laval
Co-authors: Ming Zhang, Le Quang Khai, Rita Kohen, Andre Marette

Abstract: Metabolic syndrome increases the risk of cardiac dysfunction, however the discovery of underlying mechanisms is hindered by a lack of representative animal models. We assessed the effects of metabolic syndrome on cardiac functions and the underlying pathological changes in proteome level by using low-density lipoprotein receptor-deficient apolipoprotein B100-only mice overexpressing insulin-like growth factor-II in pancreatic beta cells (LDLr-/-/ApoB100/100xIGF-II). Methods and Results: Nine-month-old male LDLr-/-/ApoB100/100xIGF-II mice fed a standard diet showed hyperglycemia, hyper-insulinemia and glucose intolerance versus age-matched C57BL/6 (WT) mice. In LDLr-/-/ApoB100/100xIGF-II mice, the ratio of heart weight/tibia length, an index of cardiac hypertrophy, was significantly increased compared to WT mice (7.3 ± 0.2 vs 6.3 ± 0.3, P<0.05). Moreover, transthoracic echocardiography revealed a reduction of left ventricular systolic performance (decreased fractional shortening and ejection fraction, P<0.01) in LDLr-/-/ApoB100/100xIGF-II mice. We also employed the 2D LC-MS/MS to label free quantify the proteome level in left ventricles in six LDLr-/-/ApoB100/100xIGF-II mice and six WT mice. We found 259 proteins up-regulated and 80 proteins down-regulated at a 2-fold change cutoff in LDLr-/-/ApoB100/100xIGF-II mice. Ingenuity pathway analysis revealed altered proteins are significantly enriched to TCA cycle, glycolysis, mitochondrial dysfunction, PI3K/AKT pathway, fatty acid oxidation and calcium influx.

Conclusions: Diabetic LDLr-/-/ApoB100/100xIGF-II mice represent metabolic syndrome, and develop significant cardiac remodeling with a reduction of cardiac contractility that may be a consequence of impaired TCA cycle, mitochondrial dysfunction, insufficient fatty acid oxidation, decreased AKT and calcium homeostasis.

Analysis of Protein Interactions Involved in Rear Polarization of Rat Artery Smooth Muscle Cells in Atherosclerosis Identify Spectrin as a Possible Interacting Partner of RHAMM

Presenting author: Lorelei Silverman, University of Toronto
Co-authors: Rosalind Silverman-Gavrila[1], Lorelei Silverman-Gavrila[2], Hasan Bilal[1], Milton P. Charlton[2], Michelle Bendeck[1][1] Department of Laboratory Medicine and Pathobiology, University of Toronto

Abstract: Using a phosphoproteomic screen followed by mass-spectrometry we showed that 6 proteins have a higher phosphorylation degree in neointimal smooth muscle cells compared to medial smooth muscle cells. Further analysis showed that RHAMM and ARPC5 play an important role in rear polarization of microtubule organization centres during migration of smooth muscle cells (Silverman-Gavrila et al., 2011). Using bio-informatics programs, the list of proteins obtained from mass spectrometry was analyzed to reveal addition potential interacting partners. One such target protein that was validated is spectrin, an actin binding protein that play a role in the production of actin nets. Relative distribution of spectrin to actin, RHAMM, and a-tubulin in neointimal smooth muscle cells was further assessed using confocal microscopy in order to identify the localization of spectrin during cell cycle as well as its relative distribution and possible colocalization with members of the identified pathway that play a role in rear polarization of the MTOC. SMCs but there is less colocalization of spectrin and a-tubulin. This suggests that spectrin acts as a linker between actin cytoskeleton and RHAMM. The putative interactions will be further validated by biochemical (co-IP), siRNA, and mutagenesis approaches. Our data show that this multifaceted approach can be a useful tool for identifying interacting partners and decoding pathways involved in polarity regulation during migration of smooth muscle cells in atherosclerosis process that can be pursued as targets for preventing migration and thus progression of atherosclerosis plaque formation.
The Effect of Cold Acclimation on the Cardiac Proteome of Rainbow Trout (O. mykiss)  
Presenting author: Jordan Klaiman, University of Guelph  
Co-authors: Jordan M. Klaiman, Sarah L. Alderman and Todd E. Gillis

Abstract: In most endothermic species, a decrease in body temperature can lead to heart failure. The primary cause of this is a decrease in Ca2+ sensitivity of the cardiac myocytes. Interestingly, the heart of the rainbow trout remains functional at 4 µ°C. Previous work has shown that cold acclimation results in cardiac hypertrophy and an increase in the maximal rates of the cardiac actin-myosin ATPase activity in trout. The purpose of this study was to investigate how the cardiac proteome remodels in response to cold acclimation, specifically the role of phosphorylation and/or differential expression of the cardiac contractile proteins. To accomplish this, 2D-DIGE was used to examine for changes in the phosphorylation state and isoform expression of the cardiac contractile proteins, and 1D gel electrophoresis coupled with tandem mass spectrometry was used to identify the troponin I (TnI) isoforms. Furthermore, we used semi-quantitative real-time PCR (qPCR) to characterize the expression of seven different TnI genes. The results indicate that cold acclimation significantly altered the expression pattern of TnI genes in the heart. Despite the expression of seven TnI transcripts, only three protein isoforms were identified in the heart. In addition, cold acclimation caused subtle changes in the phosphorylation state of the slow skeletal isoform of troponin T, as well as of cardiac myosin binding protein C. Together these results demonstrate the complexity of the trout cardiac proteome and suggest a functional value to remodeling the contractile proteins in response to cold acclimation.

Functional Comparison of Phagocytic Immunoglobulin-G versus Oxidized Low-Density Lipoprotein Phagocytic Receptors in Human U937 Macrophages  
Presenting author: David T. Vance, Ryerson University, Dept. Chemistry and Biology  
Co-authors: David T Vance, Jeff Howard, Peter Bowden, Angelique Florentinus, Monika Tucholska and John G Marshall

Abstract: The first step of atherosclerosis occurs when macrophages bind to solid, oxidized low-density-lipoprotein (oxLDL) deposited on the arterial intima. The macrophages become activated, releasing free radicals that further oxidize LDL, leading to foam cell formation and atherosclerosis. The phagocytosis of oxLDL-coated microbeads were compared to Immunoglobulin G (IgG) beads using immunofluorescent staining and laser confocal assays in CD36 (+) human U937 macrophages. The engulfment of IgG-micro beads was initially more rapid, but the accumulation levelled by 2 h whereas engulfment of oxLDL beads started slowly but accelerated till 4 hours. Thus, oxLDL coated beads were engulfed by a qualitatively different internalization pathway than IgG beads. The actin inhibitors Cytochalasin D (10µM) and Latrunculin B (5µM), the Src tyrosine kinase inhibitor MNS (20 µM), the phospholipase C inhibitor U73122 (5 µM), and the Janus kinase inhibitor AG 490 (25 µM) all prevented phagocytosis of both oxLDL-coated and IgG-coated beads. The general protein tyrosine kinase inhibitor genistein (35 µM) showed greater inhibition of oxLDL versus IgG mediated phagocytosis. The spleen tyrosine kinase inhibitor Bay 613606 (1 µM) significantly inhibited oxLDL, but not IgG, mediated phagocytosis. The IgG verses oxLDL receptor complexes were captured from the surface of live cells alongside anti-CD36 beads, or controls for non-specific binding with analysis by LC-ESI-MS/MS. Conmensurate with the pharmacological profile, specific isoforms of tyroosine kinases were only observed in the oxLDL receptor complex but not the IgG or other controls. This experiment indicates that proteomics can accurately identify the drug targets of the oxLDL receptor complex.
Discovery of the Circadian Cardiac Proteome by Two-Dimensional Difference in Gel Electrophoresis and Mass Spectrometry

Presenting author: Petr Podobed, University of Guelph
Affilliation:* Cardiovascular Research Group, Department of Biomedical Sciences, University of Guelph, Guelph, Canada.** Division of Cardiovascular Diseases, Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA.

Abstract: Introduction: Circadian rhythms are crucial for coordinating thousands of biochemical processes in the heart on a daily schedule to facilitate the healthy structure and function of the cardiovascular system. However, little is known about the temporal profiles of cardiac proteins, designated as the cardiac circadian proteome. We hypothesized that cardiac proteins exhibit robust and coordinated rhythms in expression across 24h daily cycles, and investigated this in normal C57Bl/6 murine heart, in a model of heart disease (pressure overload induced cardiac hypertrophy), and mechanistically in two distinct models of circadian rhythm disruption (dyssynchrony, transgenic mice).

Methods/Results: Heart tissues were collected every 4h across 24h daily cycles (n=3 per timepoint). Soluble proteins were isolated with Urea/CHAPS lysis buffer. The circadian proteome was detected using two-dimensional difference in gel electrophoresis and Cy3/Cy5 dyes, scanned with a Typhoon 9410, and analyzed by DeCyder software. Selected spots were subjected to trypsin digestion, hybrid triple quadrupole/linear ion trap mass spectrometer (Q TRAP 4000) and MASCOT searching. Protein identification was validated by Western blot. Functionally, Langendorff analyses revealed increased left ventricular developed pressure in wake vs. sleep hearts, and Actomyosin MgATPase assays demonstrated greater ATP consumption. These time-of-day differences were ablated when the daily rhythms were disrupted by dyssynchrony or in transgenic mice.

Conclusions: This is the first study to detect the cardiac circadian proteome, including a functional component. This approach is generally applicable to tissue proteomics, and provides significant new opportunities for understanding disease physiology.
CHEMICAL & STRUCTURAL PROTEOMICS

Using Ordered Mesoporous Carbon Based Proteomic Reactor to Selective Enrichment of Endogeneous Peptides in Cell Lysates
Presenting author: Cheng-Kang Chiang, BMI, University of Ottawa
Co-authors: Cheng-Kang Chiang, Rui Chen, Hanfa Zhou, Daniel Figeys

Abstract: Analyzing of low-abundance endogeneous peptides in complicated biological samples has been one of the major challenges in proteomics due to their high complexity and huge dynamic range of protein concentrations. Here, we have examined the general applicability of using ordered mesoporous carbon (OMC) for the enrichment of endogeneous peptides in cell lysates. OMC with well-ordered nanoscale pores (i.e. 4.8 nm), larger specific surface area (i.e. 639 m²g⁻¹), and stronger hydrophobicity implies the superior size-exclusion property to only detect peptides with a molecular weight less than or equal to 10 kDa. While using BSA tryptic digests as testing model, around 10 unique peptides with 14% of sequence coverage could be identified by OMC extraction even in the presence 500-fold amount of protein mixtures. Compared with conventional SCX proteomic reactor, OMC also reveal its benefit to capture more (~3-fold) unique peptides either in Neuron 2A or human hepatocyte (HuH7) cell lysates. In addition, the physical characteristics (pI, molecular weight, and grand average hydrophobicity (GRAVY) index, and number of acid and basic amino acid) of the peptides obtained from the OMC proteomic reactor reveal unique properties in comparison with SCX one. Those results indicate this strategy provide a new avenue for the efficient enrichment of endogenous peptides in clinical samples.

Glycosylation Improves Thermotolerance in a Site-Dependent Manner in a Bacterial Xylanase expressed in Pichia pastoris
Presenting author: Raquel Maldonado, Medical School of Ribeirao Preto - USP

Abstract: The relationship between enzyme stability and glycosylation in the GH11 xylanase A from Bacillus subtilis (XylA, a 1,4-β-O xylanohydrolase) has been studied using heterologous protein produced in both Pichia pastoris (XylAPp) and in Escherichia coli (XylAEc). Amino acid sequence analysis of the XylA revealed six potential Asn-type glycosylation sites, however intact mass analyses indicate 4 predominant GlcNAc2Man9 glycosylation events in the XylAPp. LC-MS/MS analyses of the XylAPp chymotrypsin digestion products support glycosylation at N20, N25, N141 and N181. Single and double site-directed mutants that eliminated the N-glycosylation sites revealed that in mutants N20Q, N25Q, N141Q and N181Q, the N29 (which is not modified in wild-type XylAPp) is glycosylated. A substantially higher thermotolerance of the XylAPp and some mutants was observed in the glycosylated enzymes, where at 55°C the half-life of the glycosylated XylAPp was 24 min, as compared to 8 min for the unglycosylated XylAEc. Stability is reduced by 90% in
Proteomic Analysis of the Highly Detergent Resistant Radial Component of Myelin
Presenting author: Vatsal Patel, Wilfrid Laurier University, Chemistry Dept
Co-authors: Patel, V. and DeBruin, L.

Abstract: The wrapping of multiple layers of myelin membrane sheets around an axon plays an important role in normal neuronal function. In central nervous system (CNS), oligodendrocytes are responsible of forming multiple myelin internodes which consist of lipids and proteins. Within these regions, there are various membrane microdomains including the radial component/tight junctions. The radial component is a junctional complex believed to stabilize the myelin membranes in the CNS myelin. In this study, we have attempted to extract radial component from bovine and mouse myelin using differential detergent extraction. A highly detergent resistant fraction containing previously identified proteins of the radial component was examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), 2D electrophoresis (with immobilized pH gradient (IPG) strip or liquid-phase isoelectric focusing (IEF) followed by SDS-PAGE), traditional western blot and multiplex fluorescent western blot, and mass spectrometry. The fraction was found to be significantly and consistently enriched in some high and low abundant proteins. Furthermore, evidence implicates the presence of plasmalemmal voltage dependent anion channel (VDAC) proteins in the myelin membrane. This study indicates that radial component of myelin can be isolated successfully and proteomic study reveals crucial information about high abundant cytoskeletal and transport proteins and low abundant signalling proteins. This information will aid in deciphering the roles of various proteins of the radial component in health and disease.

Cloning, Expression and Functional Characterization of the C2 Domain from Tomato Phospholipase D alpha
Presenting author: Krishnaraj Tiwari, University of Guelph
Co-authors: Krishnaraj Tiwari, Gopinadhan Paliyath

Abstract: C2 domain exist as a 130 amino acid long, highly conserved N-terminal or C-terminal calcium and lipid binding motif, responsible for recruiting proteins to the membrane during signal transduction. In this study, the sequence corresponding to the N-terminal 150 amino acids of a full length cDNA of phospholipase D alpha from tomato fruit was cloned in pET28(b) vector and expressed in E.coli as a His-tagged protein. Recombinant C2 domain showed micromolar affinity towards Ca++ with a maximum of 2 high-affinity binding sites. Interaction of C2 domain with synthetic unilamellar vesicles, evaluated by protein- lipid fluorescence resonance energy transfer showed maximum affinity towards phosphatidic acid, and virtually no binding with phosphatidylcholine. The binding towards phosphoinositides was reduced with increasing degree of phosphorylation. Acid- and chaotropic salt- titrations indicated an electrostatic rather than a hydrophobic mode of interaction between C2 domain and the phospholipid vesicles. Conformational analyses of the recombinant C2 domain showed a much longer calcium-binding loop region, a far less electro-positive phosphoinositide binding region, unique calcium-binding pockets with high electro-negativity, and other features that are distinct from the typical C2 domains of phospholipase A2 and Protein kinase C alpha, signifying the uniqueness of Phospholipase D alpha in fruit developmental events.
Protein Control of Biomineralization: Deciphering the Dental Enamel Proteome

Presenting author: Bernhard Ganss, University of Toronto Faculty of Dentistry
Co-authors: Ganss, Bernhard; Evangelou, Andreas; Starostin, Andrei

Abstract: Dental enamel is the most highly mineralized tissue in mammals. A network of hydroxyapatite crystals gives this bioceramic material its remarkable hardness and flexural strength. If properly formed and cared for, enamel, unlike restorative materials, is designed to last a lifetime under constant challenges by mechanical stress, temperature and pH fluctuations, and cariogenic microorganisms. Enamel is formed in a typical biomineralization process under the guidance of structural proteins and proteolytic factors. Although many of the key proteins in enamel biomineralization have been identified, comprehensive analyses of the enamel proteome have not been conducted. We used Multidimensional Protein Identification Technology (MudPIT) to identify previously described, significantly enriched enamel proteins such as Amelogenin, Ameloblastin, Enamelin, Enamel matrix serine proteinase 1 and Matrix Metalloprotease-20, and used the GOFFA (Gene Ontology for Functional Analysis) approach to determine significant protein enrichment based on biological processes and molecular function gene ontology identification. Significantly enriched GO terms based on the GOFFA tool applied to all proteins identified with a SEQUEST Score of 50 or greater, included extracellular matrix structural constituent conferring compression resistance, structural constituent of tooth enamel, hydroxyapatite binding, extracellular matrix structural constituent, enamel mineralization, and tooth mineralization, based on molecular function and biological processes. While these findings confirm the presence of previously characterized enamel proteins, our MudPIT analyses have also identified several novel components of the enamel proteome. We expect these new components to play important roles in designing strategies for natural enamel regeneration and the rational design of biocompatible, hydroxyapatite-based biomaterials.

Revealing Protein Structural Transitions by Top-Down HDX-MS with Electron Capture Dissociation

Presenting author: Jingxi Pan, UVic-Genome BC Proteomics Centre
Co-authors: Christoph H. Borchers, Jingxi Pan, Jun Han

Abstract: Hydrogen/deuterium exchange (HDX) coupled with mass spectrometry (MS) is a powerful tool for structural proteomics. Traditional HPLC-based HDX-MS usually gives peptide-level resolution and incomplete sequence coverage. Recently, we have developed an electron capture dissociation (ECD)-based “top-down” approach that can reach close to amino acid spatial resolution (JACS, 2009, 131, 12081). Additional advantages include negligible back-exchange and complete sequence coverage. However, application of this approach has thus far been limited to small proteins (< 17 kDa). Here we used top-down HDX to decipher drug-induced structural transitions of the 29-kDa carbonic anhydrase (CAII), as well as the conformational changes during prion protein (PrP) oligomerization. CAII contains 240 amide hydrogens, and global HDX has shown that 163 were protected. After forming a 1:1 complex with furosemide (FSM), CAII displayed 18 additional protected hydrogens. To locate these differentially-exchanged sites, we used our “ECDstitching” method to obtain full sequence coverage at a resolution of 2 residues. Most of these residues are located in domains that are in close contact with FSM. This finding is in sharp contrast to X-ray crystallographic studies, which showed that the structures of drug-free and FSM-bound CAII were identical. The second system is PrP, whose oligomerization plays a crucial role in prion disease. Our Top-down ECD data has shown that the structural differences between PrP monomer and oligomer are located in the middle portion of the protein, a region encompassing helix 1 and sheet 2. This result indicates that PrP oligomerization may require unfolding of helix 1 in the monomer.
The Integrins of the Activated IgG-Fc Receptor Supramolecular Complex Isolated From Live Human U937 Macrophages With Analysis by Liquid Chromatography and Tandem Mass Spectrometry

Presenting author: Jaimie Dufresne, Ryerson University, Dept of Chemistry and Biology
Co-authors: Jaimie Dufresne, Jeffrey C Howard, John G. Marshall

Abstract: Integrins have been shown to be associated with the Fc receptor complex that binds to and recognizes Immunoglobulin G (IgG). The Fc receptor complex was captured using 2um polysterene beads coated in IgG and incubated with live human U937 macrophage cells. Beads coated in oxLDL and anti CD36 served as controls. The cells were then disrupted using a French press and the Fc-IgG-beads isolated with ultracentrifugation over a sucrose gradient. The same ligand beads were incubated with crude extracts for classical ligand affinity chromatography. Uncoated beads incubated with crude extracts or used experimental medium served as controls for non-specific binding. The receptor complexes were fractionated with increasing salt of acetonitrile in step gradients and digested with trypsin. Insoluble proteins were digested directly on the beads with and without organic solvents. The peptides were collected over C18 preparative chromatography prior to analytical separation over C18 HPLC and tandem mass spectrometry (LC-ESI-MS/MS). The resulting peptides were identified using the Proteome Discoverer algorithm (p<0.01). The resulting peptide and protein identifications with the associated MS, and MS/MS spectra, including the ion m/z and intensity data, was subsequently stored in an SQL database. Certain integrins were found to be specific to the Fc receptor complex. The functional role of the specifically detected integrins will be examined by silencing RNA and quantitative phagocytosis assays using laser confocal microscopy.

PAK7 Associates with IgG-activated FcRs in RAW 264.7 Mouse Macrophages and is Functionally Important in Fc Mediated Phagocytosis

Presenting author: Jeffrey C. Howard, Ryerson University, Dept of Chemistry and Biology
Co-authors: Howard, Jeffrey C.; Petrenko, Veronica; Florentinus, Angelique; Tucholska, Monika and Marshall, John G.

Abstract: Phagocytosis of immunoglobulin G (IgG) coated foreign particles and microbes are important innate immune responses initiated by specific ligand-receptor interactions. These IgG-coated particles bind to and activate Fc receptors (FcRs) resulting in dynamic changes in the underlying actin cytoskeleton that regulates particle uptake. P21 activated kinases (PAKs) are known effectors of Cdc42 and Rac, two small GTPases that play a key role in regulating the cytoskeletal changes associated with FcR mediated phagocytosis. However, the exact role of various PAKs in IgG-activated FcR phagocytosis is poorly understood. In this study the FcR complex was captured using IgG bound to 2 micron polystyrene beads by live cell affinity receptor chromatography (LARC). FcR protein complexes isolated using these microbeads were extracted using salt gradients and organic solvents and digested with trypsin. The resulting tryptic peptides were analyzed by LC-ESI-MS/MS using a linear ion trap. CID fragmentation of precursor peptides were correlated by Proteome Discoverer and the resulting data used to construct a SQL protein database. Overall, PAK7 was unique to the IgG-activated FcR complex in mouse macrophages (RAW 264.7). Fluorescent Pak7 (EGFP-PAK7wt) co-localizes with phagocytized IgG-coated microbeads and also displays a distinctive vesicular cytoplasmic distribution that is largely absent when PAK7 is mutated (K478M, S573N). Moreover, a constitutively activated form of PAK7 (S573N) along with mutant forms of PAK2 (K278R, T402E) significantly inhibited phagocytosis of IgG-coated microbeads by 41%, 22.1%, and 27.5% respectively. Together, these results indicate that PAK7 has a unique function in the FcR receptor supramolecular complex.
Monitoring the Chemical Hydroxylation of Complex Phenolic Compounds by LC-ESI-MS/MS

Presenting author: Wagday Samrgandi, Ryerson University, Dept of Chemistry and Biology
Co-authors: Samrgandi, Wagday Mohammed; Safi, Frozan; Florentinus, Angelique; Marshall, John G.

Abstract: It might be industrially important to monitor the hydroxylation or modification of a range of phenolic and other substrates by mass spectrometry. Since phenolic amine compounds may easily enter the gas phase by electrospray, a number of different chemical reactions might be monitored and confirmed by LC-ESI-MS/MS. We have shown the large scale chemical reaction of L-tyrosine with dihydroxyfumaric acid (DHFA) at 0 °C in the presence of bubbling O2 in a 400 ml flask results in the product dopamine (L-DOPA). The production of (DOPA) was confirmed by UV-VIS spectroscopy, thin layer chromatography with staining for amines ninhydrin and LC-ESI-MS/MS. The L-DOPA product was predominantly observed at an m/z value of 198 [M+1H]. The molecular identity of L-DOPA was confirmed by MS/MS analysis with the expected major product ions at 181 m/z and 151 m/z. Monitoring the 181 m/z product ion permitted the quantification of L-DOPA over time to ≤1 pM in the reaction vessel with respect to external standards. The crude reaction product was simply diluted (1/100) in 0.1% acetic acid prior to isocratic C18 LC-ESI-MS/MS at 2 µl per minute with a 300 micron ID x 15 cm column. The effect of varying tyrosine versus DHFA concentration or the presence of O2 on the reaction progress was directly monitored by LC-ESI-MS/MS. Similar hydroxylation reactions were observed with dihydroquinone (DHQ), 3,3',5,5'-Tetramethylbenzidine (TMB) and phenylalanine. We conclude that LC-ESI-MS/MS will be directly applicable to monitoring the industrial modification of a wide class of phenolic amines.

Tetraspanin Proteins Associated with the Fc Receptor Complex of Human U937 Macrophage

Presenting author: Pardis Pakshir, Ryerson University, Dept. of Chemistry and Biology
Co-authors: Pardis Pakshir, Jeffry C Howard, John G. Marshall*

Abstract: Tetraspanins may act as molecular facilitators, grouping specific cell-surface proteins and thus increasing the formation and stability of functional signaling complexes. The Fc receptors belong to the immunoglobulin superfamily and may cooperate with co-receptors to perform their cellular functions. The ligand of the Fc gamma receptor is immunoglobulin IgG, which triggers the production of reactive oxygen species (ROS) and the phagocytic engulfment of foreign molecules coated by antibodies. Low density lipoproteins (LDL) may be oxidized to oxLDL which is recognized by innate receptors that also trigger the production of free radicals and phagocytic engulfment. The Fc receptor complex was captured from the surface of live cells using IgG, compared to oxLDL coated microbeads prior to disruption with a French press. The same beads were incubated with crude extracts for classic ligand affinity chromatography. Uncoated beads incubated with crude extracts or used experimental medium were used to establish non-specific background binding. The receptor complexes were washed with PBS and isolated using ultra-centrifugation over sucrose-gradients. The soluble proteins were eluted with increasing salt and acetonitrile concentrations prior to tryptic digestions. The insoluble proteins were digested on the beads with and without organic solvents. The resulting peptides were analyzed by liquid chromatography and tandem mass spectrometry. The receptor complex proteins were identified with the Proteome Discoverer algorithm. A number of tetraspanin proteins were found to be associated with the Fc receptor complex. Silencing RNA and quantitative assays of phagocytosis using laser scanning confocal microscopy will be used to establish the function role of tetraspanins in Fc receptor function.
The Presence of Protein Tyrosine Kinases Specifically Associated with the Activated Fc Phagocytic Receptor Complex from Human U937 Macrophages

Presenting author: Marwan Althagafi, Ryerson University, Dept. of Chemistry and Biology
Co-authors: Althagafi, Marwan; Howard, Jeff; Florentinus Angelique; Tucholska, Monika; Marshall, John G.

Abstract: The Fc gamma receptor protein is found on the surface of macrophages and other innate immune cells and specifically binds to Immunoglobulin G (IgG) antibodies. The activated Fc receptor complex was captured from the surface of live human U937 macrophages using IgG bound to 2 micron beads alongside oxLDL or anti CD36 comparator ligands. The same IgG and oxLDL ligands were also incubated with crude extracts for classical ligand affinity chromatography. Uncoated beads were incubated with crude extracts or used experimental media as non-specific binding controls. The receptor complexes were isolated by washing in PBS followed by sucrose-gradient ultracentrifugation. Thereafter soluble proteins were fractionated with increasing salt and then acetonitrile concentrations prior to digestion. The remaining insoluble proteins were directly digested on the beads to yield 17 fractions per experiment. The live cell, ligand affinity and control treatments were randomly and independently sampled in at least three independent experiments in replicate blocks (≥16 treatments x ≥3 replicate blocks x 17 fractions = ≥1088) by LC ESI MS/MS with a linear ion trap (Thermo). The MS and MS/MS data from about 72,000 proteins identified (p≤0.01) with Proteome Discoverer were collected and compared using Structured Query Language (SQL) and the Statistical Analysis System (SAS). Certain Protein Tyrosine Kinases (PYKs) were shown to be specifically associated with IgG versus oxLDL and/or control treatments. Silencing RNA and quantitative assays of phagocytosis using laser scanning confocal microscopy will be used to determine the functional role of the specific proteins.
PROTEIN MODIFICATIONS & CELL SIGNALING

A Comprehensive Proteomics Approach to Determine Rtt109 Chaperone-dependent Enzymatic Specificity
Presenting author: Nebiyu Abshiru, IRIC, Université de Montréal
Co-authors: Abshiru, Nebiyu; Ippersiel, Kevin; Verreault, Alain; Thibault, Pierre.

Abstract: Rtt109 is a yeast histone acetyltransferase (HAT) that play important roles in DNA replication and the maintenance of genome integrity. This HAT associates with either Vps75 or Asf1 histone chaperones to direct the acetylation of specific residues on histone H3. Here we develop a comprehensive proteomics approach to characterize the chaperone dependent specificity of the Rtt109 enzyme. We profiled global changes in acetylation of H3 during in vitro assays with Rtt109 and its chaperones using LC-MS on a Q-TOF instrument. The site and stoichiometry of acetylation were determined upon LC-MS/MS analysis of tryptic peptides generated from acetylated-H3. Our analyses indicated that under high enzyme: substrate ratios Rtt109-Vps75 preferentially acetylate H3 at positions K9, K14, K23, K27, and K56| K9ac being the most abundant. In contrast to previous studies, we observed a stronger specificity of Rtt109-Vps75 for H3-K23 over H3-K27. Importantly, this study revealed that Rtt109-Asf1 displayed a significantly higher substrate specificity for acetylation of H3-K56. For the first time, we also report acetylation of histone H4 by Rtt109-Vps75, at position K12 while Rtt109-Asf1 showed no detectable activity toward H4. In summary, this MS-based approach enabled the profiling of multiple acetylation sites to determine site-specificity of Rtt109 toward its substrate.

Interactome Mapping Reveals a Novel Role for HP1β in DNA Repair
Presenting author: Huadong Liu, University of Western Ontario
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Abstract: Heterochromatin protein 1 beta (HP1β) is a multiple function chromosomal protein involved in a variety of cellular processes, including heterochromatin formation and gene silencing, telomere capping and silencing, and control of gene expression [1]. Recent studies indicate that HP1β also plays a role in DNA damage repair although it is inconclusive due to conflicting results. Herein, we employed mass spectrometry to identify a comprehensive interaction network mediated by the HP1β-chromodomain (HP1β-CD) and developed an approach to systematically identify methyllysine sites in the HP1β interactome by selected reaction monitoring (SRM). The dynamic nature of methylation was studied using deuterium labeled cells under conditions to induce DNA double strand breaks (DSB). Our data indicate HP1β function as an adaptor during DSB repair through a methyllysine-mediated interaction with DNA-PKcs. Knockdown of HP1β or mutation of the methyllysine sites in DNA-PKcs caused dislocation of activated DNA-PKcs from DNA damage foci and increased the sensitivity of cells to ion radiation.

AN INNOVATIVE PROTEOMIC APPROACH TO IDENTIFY AND CHARACTERIZE NEW SUB-STRATE OF SUMOYLATION

Presenting author: Danielle Caron, IRIC, Université de Montréal
Co-authors: Danielle Caron¹, Chantal Durette¹, Frédéric Lamoliatte¹,², Éric Bonneil¹, Mounira Chelbi-Alix³, Pierre Thibault¹,²
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Abstract: Small ubiquitin-related modifier (SUMO) are evolutionary conserved ubiquitin-like proteins that are involved in different regulatory pathways including intracellular trafficking, cell cycle, genomic integrity, cell differentiation and apoptosis. SUMOylation result in the formation of an isopeptide bond between SUMO and the Lys residues on protein substrates and represents a challenge of identification by mass spectrometry. We recently developed HEK293 cells expressing SUMO mutants for mass spectrometry (MS)-based proteomics studies, and implemented a novel immunoaffinity method that selectively enrich SUMOylated tryptic peptides from complex cell extracts. We build upon recent analytical developments in large-scale quantitative proteomics to profile changes in the SUMOylation of nuclear substrates from HEK293 cells (SUMO3 paralog) in response to proteasome inhibitors. This innovative large-scale proteomics method enabled the identification of an unprecedented number of SUMOylation sites (more than 150 SUMO sites on 93 proteins). Among these, we identified a novel SUMO substrate parafibromin/CDC73, a tumor suppressor associated with the Pafl (polymeraseassociated factor 1) complex that regulates transcriptional initiation and elongation, histone modifications and mRNA quality. Moreover, we confirm previously known SUMO sites on HSF2, a DNA-binding protein which activates transcription, but also identify 3 new SUMO site on HSF2. These experiments have been complemented with immunoblots and immunofluorescence analysis on wild type and SUMO-site mutant’s forms of CDC73 and HSF2 to monitor the pattern of modifications in presence or absence of proteasome inhibitors. These data provide a new strategy to identify SUMO site which is essential to conduct functional insights and determined SUMO function and its biological significance.

Quantitative Phosphoproteomics for the Identification of Kinase Substrates

Presenting author: James Knight, Samuel Lunenfeld Research Institute
Co-authors: James Knight, Ruijun Tian, Robin Lee, Derek Ceccarelli, Fangjun Wang, Ariane Beauvais, Hanfa Zou, Lynn Megeney, Frank Sicheri, Daniel Figeys, Rashmi Kothary, Tony Pawson, and Anne-Claude Gingras

Abstract: A major challenge preventing the comprehensive study of phosphorylation-based cell signaling is the difficulty of dirzase-specific inhibitor-treated cells, this in vitro approach can be simultaneously coupled with biologically relevant information to identify direct substrates regulated by the kinase of interest in vivo. Applying this technique to the p38α MAPK and mammalian STE20-like protein kinase 4 (MST4) has resulted in the identification of seven relevant substrate phosphorylation sites for p38, and has defined consensus phosphorylation motifs for both kinases. Additional in vitro comparisons between p38α and p38β has revealed that substrate specificity does not discriminate these two isoforms, while a comparison between full-length MST4 and a C-terminal truncation mutant suggests that the non-cata-lytic C-terminal tail of MST4 is used to target specific substrates. Collectively these data provide information on the biological functions of these kinases, and give insight into the mechanisms kinases can use to achieve substrate specificity. The substrate finding approach presented will serve as a useful tool for studying the hundreds of kinases that exist, and for uncovering the deeper mechanisms of cell signaling.
**Structural Basis for the Diverse Ligand Recognition Specificity of the SH2 Domain**  
Presenting author: Tomonori Kaneko, University of Western Ontario  
Co-authors: Tomonori Kaneko, Haiming Huang, Sachdev Sidhu, Shawn Li

**Abstract:** Cellular functions require specific but dynamic protein-protein interactions that are often mediated by modular interaction domains. Ligand recognition specificity and basal binding affinity are the two complimentary and fundamental factors that maintain proper cell signalling activities. Each domain family shares a conserved domain fold within the family members. It is not fully understood how a pre-defined domain scaffold yields specificity variations within the domain family members, while retaining functional binding affinity. Using the Src Homology 2 (SH2) domain, a prevalent modular domain that only targets phosphotyrosine (pTyr)-containing ligand sequences, we show that surface loops dictate ligand recognition specificity by controlling access to binding pockets. Structural comparison of 63 different SH2 domains revealed that each SH2 domain is equipped with three binding pockets or subsites in addition to the common pTyr-binding pocket. However, not all of these three sites are available for binding in an SH2 domain. We found that two loops on the domain surface are in charge of restraining accessibility of ligand residues to these sites, and simultaneously shaping a defined specificity pocket. Loop residue mutations rationally introduced on these loops successfully altered ligand recognition specificity of an SH2 domain. Therefore, selective blockage of binding pockets or subsites by surface loops provides molecular basis for the diverse modes of ligand recognition specificity by the SH2 domain. Further mutations systematically introduced on the ligand recognition surface may lead to creation of an SH2 domain library that provides a wide range of specificities and affinities towards pTyr-containing targets.

**Targeted Analysis of Bacterial Protein Phosphorylation Sites by Reductive Cleavage and Multiple Reaction Monitoring**  
Presenting author: Declan Williams, York University  
Co-authors: Declan Williams, Michael Fridman, Leroi V. DeSouza, Dasantila Golemi-Kotra and K. W. Michael Siu

**Abstract:** In eukaryotes signal transduction is mediated by protein phosphorylation at serine, threonine and tyrosine residues, while metabolic regulation in prokaryotic systems is largely dependent on aspartate and glutamate phosphorylation. Unlike serine, threonine and tyrosine phosphoesters, acyl phosphates of aspartate and glutamate are highly labile in solution over a wide pH range. Reduction of acyl phosphates by sodium borohydride produces homoserine and 4-hydroxy-1-aminovaleric acid from phosphoaspartate and phosphoglutamate respectively. The characterization of aspartate phosphorylation of a bacterial signalling protein using sodium borohydride reduction followed by trypsin digestion and multiple reaction monitoring-initiated detection and sequencing (MIDAS) on a TempoLC-4000 QTRAP (AB Sciex) is described here. The technique allowed the sequencing of both non-phosphorylated and corresponding derivatized peptides in the same analytical replicate. Derivatized and non-phosphorylated species were chromatographically resolved and could be further differentiated by the relative abundance of their fragment ions both in MRM mode and the associated MS/MS scans. This sensitive method is broadly applicable to the study of sub-stoichiometric bacterial protein phosphorylation.
Investigating the Hippo signalling Pathway Using High-throughput LUMIER Protein-protein Interaction Screens
Presenting author: Ahmed Shibian, University of Toronto
Co-authors: Ahmed Shibian and Liliana Attisano

Abstract: The Hippo pathway, originally discovered in flies but conserved in mammals, plays a key role in controlling organ growth and size. High cell density in cultured cells acts as a stimulus for the activation of the Hippo pathway. In mammals the core components of the pathway consists of the kinases Lats1, Lats2, Mst1 and Mst2. Activation of the Hippo pathway activates Lats and Mst kinases, that eventually leads to the phosphorylation and sequestration of the transcriptional regulators, Taz and Yap, in the cytoplasm. Upstream signaling of the Hippo pathway that leads to activation of the Lats and Mst kinase is still largely unknown. Therefore, in order to understand the regulation of the Hippo pathway we performed high throughput protein-protein interaction LUMIER (LUminescence-based Mammalian IntERac-tome) screens. The LUMIER screens were performed using luciferase tagged Taz, Yap, Lats1, Lats2, Mst1 and Mst2 as baits and their interaction was tested with a cDNA library of 1280 genes. The interacting partners of the core Hippo pathway components obtained from the LUMIER assay enabled us to identify putative novel components of the Hippo pathway. This will eventually help us understand how signals from cell-cell contact activate the Hippo pathway and is transmitted to the nucleus.

A Global Analysis of the Protein Phosphatase/Receptor Tyrosine Kinase Interactome
Presenting author: Zhong Yao, University of Toronto
Co-authors: Zhong Yao, Katelyn Darowski, Marta Wierzbicka, Nicole St. Denis, Anne-Claude Gringas, and Igor Stagljar

Abstract: Receptor tyrosine kinases (RTKs) sense extracellular signals and transmit them into the cell through autophosphorylation and phosphorylation of tyrosines of other proteins. They play crucial roles in many physiological processes and pathological processes such as tumorigenesis. Protein phosphatases (PPs) remove phosphates from RTKs and thereby are heavily involved in the regulation RTK activities. RTKs are transmembrane proteins and there is a lack of efficient interactive proteomic approaches to use in their characterization, so many details of the RTK networks still remains unknown. Our goal is to build the whole interactome for all human protein phosphatases and all human RTKs. For this purpose, we employ membrane yeast two-hybrid (MYTH) system, which was developed in our lab and is an ideal proteomic tool to study membrane interaction. Here, 58 RTKs are expressed as integral membrane bait proteins in yeast, mimicking their native states in mammalian cells and maintaining their structure and functions. They were screened against a complete set of ~150 phosphatases. The preliminary results have already identified several novel and interesting previously reported interactions. Following the preliminary screen, the biological significance of the interactions will be verified in mammalian cell culture system via co-immunoprecipitation, and functional analysis of downstream signalling proteins.
In summary, the MYTH system offers an ideal approach to systematically map protein interaction networks involving RTKs and PPs and may thus lead to the identification of novel drug targets, contribute to therapeutic research, and shed new light on the mechanism of transmembrane signaling.
Proteomic Approaches to Investigating the Phosphorylation Status of the Drug Transporters, ENT1 and ENT2

**Presenting author:** Natalia Grane

Co-authors: Natalia Grane-Boladeras*, Pedram Mehrabi, Declan Williams, Leroi DeSouza, K.W. Michael Si, M. Pastor-Anglada* and Imogen R. Coe.

Affiliations: Institute for Biomedicine, University of Barcelona, Spain; Department of Chemistry, CRMS; and Department of Biology, York University, Toronto, ON, Canada.

**Abstract:** Equilibrative Nucleoside Transporters (SLC29) are a family of transmembrane proteins responsible for bidirectional nucleoside and nucleobase transport across cellular membranes. These transporters are ubiquitously expressed in mammalian tissues and involved in various physiological processes in addition to uptake of nucleoside-derived drugs. Despite their important cellular roles, very little is known about the regulation of ENTs. Recent evidences suggest that phosphorylation by a variety of kinases may regulate ENT activity and the large intracellular loop between transmembrane domains 6 and 7 may be the site of kinase targets. Using a proteomic approach involving mass spectrometry, we have detected the specific phosphoamino acids in His/Ubiquitin tagged ENT-loop peptides. Moreover, in vitro kinase assays with specific kinases have defined which kinases are involved in phosphorylating the ENTs. The results confirm that specific residues in the large loop of m/hENT1 and/or m/hENT2 are phosphorylated by certain kinases and support the concept of a complex regulation of these clinically important drug transporters involving multiple kinases pathways.

A Comparison of TiO2-Graphite vs ZIC-HILIC Glycopeptide Selective Enrichment Strategies

**Presenting author:** Julian Saba, Thermo Fisher Scientific

Co-authors: Julian Saba, Rosa Viner

**Abstract:** Glycosylation is an important post-translational modification (PTM) that plays crucial roles in biochemical processes. However, structural characterization of glycoproteins and glycopeptides remains analytically challenging. Successful application of mass spectrometry (MS) in glycoproteomics greatly depends on the workflows adopted to address specific questions relating to a particular sample type. Targeted enrichment of glycopeptides is one such procedure. In principle, this enrichment step greatly reduces the complexity of the overall sample matrix, thus facilitating more sensitive and accurate analysis of the glycopeptides. Others have explored the use of graphite and TiO2 as means to selectively enrich glycopeptides for MS analysis. Here we report on the selective enrichment and characterization of glycopeptides on the basis of combining TiO2 and graphite. A two-step enrichment strategy was used to isolate glycopeptides from human serum both at the protein and the peptide level. This strategy involves using lectins at the protein level to isolate glycoproteins followed by ZIC-HILIC or TiO2-Graphite based enrichment at the peptide level to isolate the glycopeptides. More than 300 N- and O-linked glycopeptides and 100 N and O-glycoproteins were identified by using these two approaches.
**Mass Spectrometry-Based Proteomics Analysis of the Matrix Microenvironment in Pluripotent Stem Cell Culture Principal**

**Presenting author:** Gilles Lajoie, University of Western Ontario  
**Co-authors:** Hughes, C.S., Radan, L., Stanford, W., Betts, D., Postovit, L.M., Lajoie, G.

**Abstract:** The microenvironment constitutes factors and support cells or matrices that combine to effect cellular behavior. With pluripotent stem cells, the microenvironment is critical for the maintenance self-renewal and the ability to differentiate. In this study we will present the generation and analysis of hESC matrix microenvironments using an MS-based proteomics approach. With the recent observation that a hESC-derived matrix microenvironment could modulate the behavior of metastatic cells, we sought to determine the protein composition and activity of this conditioned matrix (CMTX) [1, 2]. To facilitate proteomic analysis, we utilize quantitative metabolic labeling of the hESCs in defined, feeder-free conditions during generation of the CMTX. We also utilize several methods for efficient fractionation and proteomic analysis of complex extracellular matrices previously developed by our group [3]. With our quantitative proteomic approach we obtain significant depth of coverage for CMTX from multiple pluripotent stem cell lines. Within these matrices, numerous antagonists of core pluripotency pathways are identified, such as soluble frizzled related protein (sFRP) 1 and 2. Utilizing a combination of experimental approaches we investigated the potential role of the sFRP proteins in the regulation of hESC behavior in vitro. [1] Postovit, L. M. et al, Proc Natl Acad Sci U S A 2008, 105, 4329-4334.[2] Postovit, L. M. et al, Stem Cells 2006, 24, 501-505. [3] Hughes, C. S., et al., Proteomics, 11, 3983-3991.

**Identification of a Novel Signaling Pathway that Regulates the Drug Transporter ENT1: Implications for the Effective Delivery of Nucleoside Analog Therapeutic Drugs**

**Presenting author:** Alex Bicket, York University  
**Co-authors:** Alex Bicket, Pedram Mehrabi, Zlatina Naydenova and Imogen R. Coe

**Abstract:** The equilibrative nucleoside transporter 1 (ENT1) is responsible for the bidirectional transport of nucleosides down their concentration gradient and is important for the drug delivery of nucleoside derivative drugs such as gemcitabine, cladribine, cytarabine, fludarabine, zalcitabine, and ribavirin. In vitro analysis of the protein-protein interactions of ENT1 using MYTH screening identified novel interactors of ENT1 and suggested that previously unidentified signaling pathways are involved in the regulation of ENT1 activity. Further evidence has also suggested that this interaction occurs on the unstructured intracellular loop between transmembrane domains 6 and 7. The mechanism(s) underlying regulation of ENT1 have been investigated by modifying the large intracellular loop, for instance, by introducing specific mutations and investigating the functional changes these mutations have on nucleoside uptake. Identifying novel pathways that regulate ENT1 provide promising new strategies to optimize the drug delivery of nucleoside derivative drugs.
Regulation of ENT1, a Member of the SLC29 Family of Transporters by Various Signaling Pathways: a Biochemical-proteomic Approach to Understanding Membrane Protein Regulation.

Presenting author: Alexandre Rodrigues, York University
Co-authors: dos Santos-Rodrigues A; Naydenova Z and Coe IR (Department of Biology, Farquharson Life Sciences Building, York University, Toronto, ON, Canada)

Abstract: Nucleoside Transporters (NTs) are membrane proteins involved in regulation of nucleoside concentrations both intracellularly and extracellularly. They are clinically important because they are responsible for uptake of nucleoside analog drugs which are widely used in anti-cancer, anti-viral and anti-parasite therapies. The SLC29 family comprises the equilibrative nucleoside transporters (ENTs), which are widely distributed in human tissues but poorly understood in terms of their regulation. Previous data (dos Santos-Rodrigues et al., 2011) suggests that various protein kinases can regulate the expression and/or activity of NTs. However, little is known about the underlying mechanisms involved (direct phosphorylation, protein-protein interactions etc.) In this study, we have investigated the role of ERKs on ENT-1 dependent adenosine transport in two different cell lines, HEK293 and SH-SY5Y using a biochemical-proteomic approach to determine if direct phosphorylation is involved in ERK-dependent regulation. Preliminary data suggest that direct phosphorylation may be a mechanism underlying kinase-dependent regulation of full length ENT1. Regulation of ENT1 via signaling cascades provides insights into the fundamental biology of this membrane protein. These observations are additionally important since they may provide new approaches to enhancing the therapeutic efficacy of nucleoside analog drugs transported by ENT1. CAPES fellowship support to A dos Santos-Rodrigues; NSERC support to IRC.

The role of Fc Receptor Isoforms in Phagocytosis in Raw 264.7 macrophages

Presenting author: Jaspreet Jawanda, Ryerson University, Chemistry & Biology
Co-authors: Jawanda, Jaspreet; Florentinus Angelique; Marshall, John G.

Abstract: Fc receptors play a central role in immunity, linking both the adaptive and innate immune systems. Previous studies have shown that IgG coated microparticles are recognized by Fc-gamma receptors (FcRs) that cluster upon activation followed by phagocytosis. However, the role of other Fc receptor isoforms in the engulfment of IgG coated beads remains to be determined. The Fc receptor complex was captured from the surface of live cells using IgG, versus oxLDL, coated microbeads prior to disruption with a French press. The IgG versus oxLDL beads were also incubated with crude extracts for ligand affinity chromatography. As a control, uncoated beads were incubated with crude extracts or used experimental medium to establish non-specific background binding. The receptor complexes were washed with PBS and isolated using sucrose-gradient ultracentrifugation. The soluble proteins were eluted with increasing salt and acetonitrile concentrations followed by tryptic digestion. The insoluble proteins were digested directly on the beads both with and without organic solvents. The resulting peptides were analyzed by liquid chromatography and tandem mass spectrometry. A number of Fc receptor isoforms were shown to be specifically associated with IgG coated microbeads. The phenotypic effects of the FcRs on phagocytosis is being assessed with the use of silencing RNA, followed by quantitative phagocytosis assays in combination with laser scanning confocal microscopy.
Proteomic Analysis of UVB-Induced Protein Expression- and Redox-Dependent Changes in Skin Fibroblasts using Lysine- and Cysteine-Labeling Two-Dimensional Difference Gel

Presenting author: Hong-Lin Chan, National Tsing Hua University
Co-authors: Hsiu-Chuan Chou, Chieh-Lin Wu, Yi-Wen Lo, Jing-Yi Chen, Hong-Lin Chan

Abstract: UVB is the most energetic and DNA-damaging to humans in Ultraviolet radiation. Previous research has suggested that exposure to UVB causes skin pathologies because of direct DNA damage and the generation of reactive oxygen species (ROS). However, the detailed molecular mechanisms by which UVB leads to skin cancer have yet to be clarified. In the current study, normal skin fibroblast cells (CCD-966SK) were exposed to various doses of UVB, and the changes in protein expression and thiol reactivity were monitored with lysine- and cysteine-labeling 2D-DIGE and MALDI-TOF mass spectrometry. Our proteomic analysis revealed that 89 identified proteins showed significant changes in protein expression, and 37 in thiol reactivity. Many proteins that are known to be involved in protein folding, redox regulation and nucleotide biosynthesis were up-regulated under UVB irradiation. In contrast, proteins responsible for biosynthesis and protein degradation were down-regulated. In addition, the thiol-reactivity of proteins involving cytoskeleton, metabolism, and signal transduction were altered by UVB. In summary, these UVB-modulated cellular proteins and redox-regulated proteins might play important roles in the early stages of skin cancer formation and photoaging induced by UVB-irradiation. Such proteins might provide a potential target for the rational design of drugs to prevent UVB-induced diseases.

Analysis of Artemia Franciscana Embryogenesis by Shotgun Proteomics

Presenting author: Shiburaj Sugathan, Department of Biology, Dalhousie University
Co-authors: Shiburaj Sugathan, Jinnie Kim, Svetla Bojikova-Fournier, Thomas H. MacRae

Abstract: Proteomics provides reliable evidence for the identification and function of proteins. With the continued development of proteomics, many techniques, ranging from simple polyacrylamide gel electrophoresis (PAGE) to advanced mass spectrometry such as MALDI-TOF, MS-MS, LC-MS and SELDI-TOF are used to profile proteins. Shotgun proteomics is a recently emerged strategy based on simultaneous digestion of multiple proteins followed by peptide sequencing using tandem mass spectrometry (MS/MS) and automated database searching. Shotgun proteomics has become the method of choice for identifying and quantifying proteins in most large-scale studies (Aebersold and Mann, 2003). Compared with more traditional proteomics involving 2D-gel electrophoresis shotgun proteomics serves as a powerful tool to identify proteins/peptides from complex protein mixtures and possesses the virtues of high efficiency, time, and labour saving. Embryos of the crustacean A. franciscana develop either oviparously, yielding encysted embryos (cysts) that enter a reversible state of dormancy called diapause, or ovoviviparously, resulting in the release of swimming nauplii (MacRae, 2003). To identify specific proteins involved in each developmental pathway embryos were collected from females at different times post-fertilization. Shotgun proteomic analysis was then performed to characterize proteins expressed in both oviparous and ovoviviparous embryos. The authors acknowledge Dr. Alan Doucet, Department of Chemistry for the assistance with mass spectrometry. DST, Govt of India (Shiburaj) and NSERC (MacRae) are acknowledged for generous financial support.

PROTEINS INTERACTIONS NETWORK

Determining the Biological Role(s) of the Ubiquitin-like Protein Ufm1
Presenting author: Yasmina Tehami, Ontario Cancer Institute
Co-authors: Yasmina Tehami, Teresa Zasowski, Wasan Abada, Janet Wan and Brian Raught

Abstract: Ubiquitin fold modifier 1 (Ufm1) is a member of the ubiquitin-like protein (UBL) family. Like other UBLs, Ufm1 can be conjugated to protein substrates via a cascade of Ufm1-specific E1 (Uba5), E2 (Ufc1) and E3 (Ufl1) proteins, and removed from these substrates via the action of Ufm1-specific proteases (Ufsp1 and Ufsp2). While Ufm1 has been implicated in endoplasmic reticulum (ER) function, its biological roles remain poorly understood. Only a single Ufm1 substrate has been identified to date, DDRGK1 (a.k.a. C20orf116), a protein localized to the cytoplasmic face of the ER. Ufm1 expression is upregulated in type 2 diabetes and ischemic heart disease, along with other ER stress proteins. I have established cell lines stably expressing Flag-tagged versions of Ufm1, all of the known members of the Ufm1 conjugation/deconjugation system, and all previously reported Ufm1 interactors. Affinity purification followed by mass spectrometry (AP-MS) analysis of these proteins has allowed me to establish the first complete Ufm1 system interactome, and has revealed a number of novel interactors previously reported to play a role in ER-to-golgi transport. To better understand the makeup of the numerous protein complexes identified in this screen, I am now conducting in vitro binding assays (using GST- and His-tagged recombinant proteins), and thereby mapping the organization of each complex. Using a similar approach, I am also characterizing a putative Ufm1 interacting motif. Finally, I have identified a putative ufmylation site on Ufc1 (the Ufm1 E2 protein), in addition to several additional putative novel Ufm1 conjugates.

A Novel Mammalian Split-ubiquitin Interaction Proteomics Approach as a Tool for Functional Investigation of Signaling Pathways in Human Cells
Presenting author: Julia Petschnigg, University of Toronto
Co-authors: Julia Petschnigg, Bella Groisman and Igor Stagljar

Abstract: The yeast membrane two-hybrid system (MYTH) is a robust technique for the identification of protein partners of integral membrane proteins. However, its implementation for mammalian proteins is limited, due to differences between mammalian and yeast cells. Here, we present a novel split-ubiquitin-based mammalian membrane two-hybrid (MaMTH) technology that enables investigation of protein-protein interactions (PPIs) in human cells. Briefly, the specific interaction between membrane “bait” and “prey” proteins coupled to ubiquitin halves, allows reconstitution of functional ubiquitin. Subsequently, deubiquitinating enzymes cleave off the transcription factor coupled to the bait, thus activating reporter gene transcription. Using the MaMTH technology, we successfully confirmed known PPIs of various cellular compartments. Here, we focus on known PPIs of the ErbB family members of receptor tyrosine kinases such as the SH2-domain containing proteins Shc1, Crk, Grb2. We demonstrated that wild-type EGFR or ErbB4 bind these adaptor proteins in stimuli-dependent manners, whereas mutated, kinase-active (oncogenic) forms of the receptors resulted in constitutive binding in a stimuli-independent manner. Furthermore, constitutively active EGFR (L858R) interaction with Shc1 was efficiently inhibited by the EGFR small molecule inhibitor Tarceva. We also used MaMTH to detect agonist-specific PPIs between the .2 adrenergic receptor (GPCR) and -arrestin. Our results demonstrate that MaMTH can be used to study PPI in a drug or agonist dependent/independent manner, which is crucial for better understanding of this clinically relevant class of proteins.
Identification of a Novel RAD6-associated E3 Ligase, KCMF1
Presenting author: Jenny Hong, University of Toronto and Ontario Cancer Institute
Co-authors: *Jenny Hong, Etienne Coyaud, Janet Wan1 and Brian Raught

Abstract: Ubiquitin (Ub), a highly conserved polypeptide, is conjugated to proteins by E1, E2, and E3 proteins, which effect a sequential series of activation, conjugation, and ligation reactions. RAD6 is one of 40 human E2 conjugating enzymes. Overexpression of RAD6 is observed in human breast cancer cell lines, and renders cells resistant to chemotherapeutic DNA damaging reagents. RAD6 is also mutated in some cases of X-linked mental retardation (XLMR). RAD6 interacts with a number of different E3 ligases to carry out different cellular functions: N-end rule protein degradation is carried out with UBR1 and UBR2; post-replication repair with RAD18; and histone modification with the RNF20/40 heterodimer. Using affinity purification coupled to mass spectrometry (AP-MS), we identified several additional novel RAD6 interacting partners, including the poorly characterized E3 ligase KCMF1. Interestingly, we also found that a RAD6 point mutation identified in XLMR patients (R11Q) specifically disrupts the KCMF1-RAD6 interaction (but does not affect interactions with any of the other E3 ligases). Using an in vitro binding assay, we demonstrated that KCMF1 binds directly to RAD6 through its C-terminus, and this observation was confirmed in vivo using AP-MS. Other interactors for KCMF1 were also identified using AP-MS. Finally, similar to RAD18, we find that in vitro KCMF1 inhibits the formation of polyubiquitin chains synthesized by RAD6. Together, our data suggest that KCMF1 may play an important role in monoubiquitylation of (unknown) substrates, and that disruption of this process may be involved in XLMR.

Quantitative Proteomics and Dynamic Imaging Reveal that G3BP-mediated Stress Granule Assembly is Poly(ADP-ribose)-dependent
Presenting author: Maxim Isabelle, Université Laval
Co-authors: Maxim Isabelle, Jean-Philippe Gagné, Imed-Eddine Gallouzi, Arnaud Droit, Guy Poirier

Abstract: Poly(ADP-ribose) (pADPr) is heterogenic molecule synthesized from NAD by poly(ADP-ribose) polymerases (PARPs). Multiple cellular functions are affected by pADPr through its network of associated proteins ranging from genome integrity surveillance, cell cycle progression, DNA repair to apoptosis. Using SILAC-based quantitative proteomics, we established a temporal map of pADPr-associated complexes upon genotoxic stress. Results suggested a strong pADPr-association of multiple proteins involved in stress granule formation, notably G3BP, in the late phase of MNNG-stress-induced cells. Further investigation with dynamic imaging clearly demonstrated a pADPr dependent initiation of stress granule assembly originating from the nucleus. The co-transfection of G3BP with poly(ADP-ribose) glycohydrolase (PARG) indicates that pADPr is involved in modulating the nuclear shuttling of G3BP. Moreover, a peptide pADPr blot assay of G3BP revealed that pADPr binds to the glycine-arginine rich domain of G3BP. Thereafter, we established a comprehensive G3BP interactome in presence of pADPr. Our findings establish a novel function for pADPr in the formation of G3BP-induced stress granules upon genotoxic stress.
Systematic Analysis of Kinase Signaling Networks in TRAIL-mediated Apoptosis

Presenting author: Adrian Pasculescu, Samuel Lunenfeld Research Institute
Co-authors: Jonathan So, Pau Creixell, Andrew James, John Sinclair, Anna Yue Dai, Kelly Williton, Erwin Schoof, Vivian Nguyen, Karen Colwill, Claus Jorgensen, Rune Linding, Tony Pawson

Abstract: The TRAIL death-inducing ligand holds great promise as a selective inducer of apoptosis in cancer cells; however, many tumour cells are intrinsically resistant or acquire resistance after treatment. To identify protein kinases that may synergize with TRAIL in overcoming this resistance, and therefore may be putative targets for combination therapy, we systematically investigated the influence that kinases have on TRAIL-induced apoptosis. We perturbed kinase expression levels and monitored apoptotic outcome. Using the human DLD-1 colon adenocarcinoma cell line as our model system, we either reduced the levels of >500 protein kinases by RNA interference or we created cell lines with enhanced expression of >100 kinases (flag-tagged). These cells were then stimulated with TRAIL and the apoptotic response was estimated by measuring CASP3 cleavage. Kinases primarily alter signalling networks within the cell. To map these networks, we immunoprecipitated the flag-tagged kinases from our cell lines and identified interacting partners by mass spectrometry (IP-MS). Additionally we measured overall changes in global phosphorylation after TRAIL signalling using SILAC-labeled cells following 0, 5 and 60 minutes stimulation. A network of protein-protein interactions was then constructed from the IP-MS results, the global phosphorylation network, and from literature-curated interactions. Extensive computer simulation and modeling of the network were conducted to identify clusters of kinases linked to apoptosis and the interdependencies between these kinases. We expect that this network will provide insight into which kinase inhibitors may be used in combination therapy with TRAIL.

A Draft of the Human Phosphatase Interactome Reveals New Biological Functions for Uncharacterized Phosphatases

Presenting author: Nicole St-Denis, Samuel Lunenfeld Research Institute
Co-authors: Nicole St-Denis, Amanda Veri, Zhen Yuan Lin, Beatriz Gonzalez Badillo, Laurence Pelletier, and Anne-Claude Gingras

Abstract: Reversible protein phosphorylation, catalyzed by kinases and phosphatases, is crucial for the proper regulation of cellular processes. Disruption of phosphoregulation results in a variety of disease states, making these enzymes attractive therapeutic targets. There are however crucial gaps concerning the regulatory networks controlling these enzymes, particularly phosphatases. Many phosphatases are regulated through protein-protein interactions, either through combinatorial complex formation with regulatory subunits or through distinct combinations of modular domains. Therefore, the systematic identification of phosphatase interacting proteins and complexes can provide valuable insight on phosphatase function. We are using affinity purification coupled to mass spectrometry (AP-MS) to identify interactors for 151 wild type protein phosphatases in asynchronous cells. To this end, we have generated a near-complete collection of human phosphatases in a Gateway-compatible system. Using our well-established AP-MS pipeline, stable isogenic HEK293 cell lines expressing tetracycline-inducible FLAG-tagged versions of each phosphatase were lysed and subjected to FLAG immunoprecipitation. After digestion to peptides, interacting proteins (prey) for each phosphatase (bait) were identified using LC-MS/MS. Mass spectrometry data was analyzed using MS data management and interaction proteomics analysis software developed in-house (ProHits, SAINT). The current high quality interactome contains 104 phosphatases, and reveals 722 highly significant phosphatase-prey interactions, 76% of which are novel. The results indicate novel roles for phosphatases in a diverse array of cellular processes (specific examples will be presented), placing previously unstudied phosphatases within putative functional contexts.
Dynamics of Protein Phosphatase PP1 Interactions Across the Cell Cycle

Presenting author: Brett Larsen, Samuel Lunenfeld Research Institute
Co-authors: Brett Larsen, Nicole St-Denis, Zhen-Yuan Lin, Stephen A Tate, Ron Bonner, Anne-Claude Gingras

Abstract: The activation of serine/threonine phosphatase PP1 and recruitment to substrates are crucial for progression through the cell cycle. Our group has undertaken an unbiased quantitative survey of the protein interactions for the three genes encoding catalytic isoforms of PP1 to define cell cycle regulated interactions. FLAG-tagged phosphatases were purified with their binding partners from cells arrested in different phases of the cell cycle. Each sample was analyzed on a TripleTOF 5600 mass spectrometer coupled to an Eksigent cHiP-LC system using IDA (20 ions/s) and data independent quantification SWATH (TM). SWATH conditions were 25 amu windows/100 ms over the mass range of 400-1250 amu with a 3.25s cycle. After compiling the IDA data in the ProHits LIMS and filtering using the SAINT statistical tool, we observed between 50-90 high confidence interaction partners for each PP1 catalytic isoform. These included many of the expected partners but also revealed multiple new interaction partners. Using SWATH to quantify detected proteins across the PP1 isoforms and cell cycle phases provided a robust approach, revealing the dynamic formation of PP1 complexes in different phases of the cell cycle. Furthermore, this analysis revealed several interactors with specificity for different PP1 catalytic isoforms, extending previous studies reporting non-redundant roles for these isoforms. From this, we demonstrate that SWATH is complementary to IDA in the ability to globally quantify proteins of interest and provides a sensitive unbiased, unlabeled approach for quantitation of protein complex dynamics.

Comparing Distribution Profiles Reconstructed From SILAC Labeling and Density Gradient Fractionation to Analyze Protein Sub-cellular Translocation Induced by PCSK9

Presenting author: Zhibin Ning, U. Ottawa, Shanghai Institute of Biochemistry
Co-authors: Zhibin Ning, Janice Mayne, Daniel Figeys

Abstract: It is almost impossible to discover and track subcellular translocation of multiple proteins by traditional biochemistry method in a high throughput way. Here we present a strategy using SILAC labeling combined with density gradient fractionation to address this problem. As a testing biological system, we expressed three forms of modified proprotein convertase subtilisin kexin type 9, PCSK9, (WT-v5| GOF: D374Y| LOF: Q154H) to treat Huh7 cell lines to look for downstream changes of protein expression and protein translocation caused by PCSK9. PCSK9 induces LDLR internalization and degradation by route of endosome to lysosome. In this experiment all microtomes are purified and subjected to Nycodenze density gradient fractionation. Distribution profiles of SILAC labeling states among continuous density gradient fractions were reconstructed for quantified proteins. Shift of the profiles indicates protein translocation/trafficking among subcellular locations caused by different treatment. The additional dimension information of distribution profile could enhance the confidence of quantitation in reverse. Many proteins show consistent profile shift according to known biological functions. Several new protein candidates are identified for subsequent analysis.
**Scoring Associations From Affinity Purification-mass Spectrometry Experiments: One Size Does not Fit All**

**Presenting author:** Shuye Pu, The Hospital for Sick Children  
**Co-authors:** Shuye Pu, James Vlasblom, Andrei Turinsky, Mohan Babu, Jack Greenblatt, Andrew Emili, Shoshana J. Wodak

**Abstract:** Affinity purification-mass spectrometry (AP-MS) is the technique of choice for high-throughput analysis of protein-protein associations. AP-MS requires tagging individual proteins (bait), and identifies groups of proteins (prey) that co-purify with each bait. With the improving sensitivity of MS instruments, the task of discriminating spurious binding from genuine associations has become increasingly challenging. In recent studies, competing methods have been proposed for assigning reliability scores to detected associations. The experimental protocols used in these studies differ, and they each probe association landscapes that vary in coverage and binding propensities of the corresponding proteins. This complicates the task of choosing a scoring method that is right for the data at hand. We report a comparative analysis of 6 scoring methods for protein associations identified by AP-MS: Comparative Proteomic Analysis Software Suite (ComPASS), Significant Analysis of Interactome (SAINT), Purification Enrichment (PE), the Hypergeometric Spectral Counts score (HGSCore), Dice Coefficient (DC), and the Hart score. These methods were applied to several datasets collected by different groups, and performance was evaluated against high confidence literature curated protein-protein association datasets retrieved from iRefWeb. We find that the relative performance of the scoring schemes can vary substantially depending on the dataset, and that methods that incorporate bait-prey and prey-prey interaction information display a clear advantage over those that only consider bait-prey interactions. This advantage is further enhanced when relative protein abundance data (spectral counts) is considered. The implications of our findings for extracting meaningful information from AP-MS data will be discussed.

**Optimization of Affinity Purification for Mass Spectrometry**

**Presenting author:** Amber Couzens, Samuel Lunenfeld Research Institute  
**Co-authors:** Zhen-Yuan Lin, Wade H. Dunham, Brett Larsen and Anne-Claude Gingras

**Abstract:** Protein-protein interactions provide key information on the biological function of individual proteins. For example, identifying the interactors of kinases and phosphatases can elucidate the components of signaling pathways and even potential crosstalk between pathways, in addition to activator, repressor and substrate identification. In recent years, affinity purification coupled to mass spectrometry (AP-MS) has become a powerful method for identifying these interactions. Here we present optimization of experimental methods for AP-MS from human cells by using different epitope tags, affinity supports, digestion protocols, fractionation approaches and mass spectrometers. We have found that the use of either anti-FLAG agarose or magnetic bead supports for targeting FLAG-tagged proteins yielded similar results (after background filtering), however, purification using magnetic beads was faster and compatible with direct on-bead trypsin digestion, increasing overall protein recovery. Multidimensional separation of AP-MS samples led to an increase in spectral counts, however, this did not immediately translate into a cost-efficient increase in protein identifications. By contrast, newer generation mass spectrometers may identify more proteins in the same time. A comparison of different AP-MS epitope tags yielded results that were less clear: while the majority of baits tested with FLAG were recovered better (and with more interactors) than HA or eGFP (e.g. coverage of the PP6 phosphatase was 6% with eGFP and 34% with FLAG), there were cases such as the CCM3 protein where eGFP fusion led to protein stabilization and a concomitant increase in interaction partner detection.
Tracking Interactomes: ProHits and SAINT in 2012
Presenting author: Guomin Liu, Lunenfeld Research Institute
Co-authors: Guomin Liu, Jianping Zhang, Hyungwon Choi, Jean-Philippe Lambert, Tharan Srikumar, Brett Larsen, Brian Raught, Mike Tyers, Alexey I. Nesvizhskii, Anne-Claude Gingras

Abstract: Affinity purification coupled to mass spectrometry (AP-MS) is increasingly used to identify interaction partners for proteins of interest. The development of bioinformatics tools to analyze AP-MS data has however lagged behind developments in sample preparation and mass spectrometric analysis. In recent years, our group and others have developed solutions for tracking and scoring AP-MS data. These include the laboratory information management system ProHits and the statistical tool Significance Analysis of INTERactome (SAINT). The complete ProHits solution (available on a Linux platform) performs scheduled backup of mass spectrometry data, can initiate database searches, store search results and link the mass spectrometry data to entries in the relational database ProHits module called “Analyst”. The “Analyst” module functions as an electronic notebook, enabling experimental annotation, and data analysis, visualization and export. The Analyst module is also available as a stand-alone application, called ProHits Lite, in which search results (Mascot, X!Tandem, SEQUEST and the output from the TransProteomics Pipeline) can be uploaded. Here we present major updates to both of these resources: 1) we implemented new options in SAINT that enable adaptation of the scoring to the dataset at hand; 2) we fully integrated SAINT within ProHits for seamless analysis of interaction data; 3) we developed a virtual machine implementation of ProHits “Analyst” which comes preinstalled with protein databases, SAINT and Cytoscape-web. This virtual machine implementation can be easily installed on recent Microsoft Windows or MacOS X computers, without any programming expertise. All tools are freely available at ProHitsMS.

Investigating Novel Interactors of Mammalian Fat and Dachsous
Presenting author: Nicole Liscio, Samuel Lunenfeld Research Institute
Co-authors: Nicole Liscio, Brian Raught and Helen McNeill

Abstract: Fat and dachsous are Drosophila genes encoding large cadherins involved in the regulation of growth via the Hippo kinase pathway and a form of tissue organization called planar cell polarity (PCP), both processes that are conserved to vertebrate systems. However, the mechanisms of action of both mammalian Fat and Dachsous (Dchs) are still unclear. To gain understanding of how Fat and Dachsous are acting in these pathways, I am using a proteomic screening approach to identify novel mammalian Fat and Dachsous interactors via affinity purification coupled with mass spectrometry (in collaboration with Dr. Brian Raught at the Ontario Cancer Institute). I have generated stable, tetracycline-inducible HEK293 T-REx cell lines expressing tagged deletion constructs of various Fat and Dchs orthologs to be used as bait in these studies. Potential interactors will further be investigated for effects on growth and/or PCP signaling, and for any genetic or biochemical interactions with mammalian Fat or Dachsous.

Identifying Novel Components of the Fat Cadherin Pathway
Presenting author: Srdjana Ratkovic, Samuel Lunenfeld Research Institute
Co-authors: Srdjana Ratkovic, Helen McNeill

Abstract: Fat is a Drosophila tumor suppressor gene that encodes a large cadherin involved in regulation of growth and a form of tissue organization called planar cell polarity (PCP). Fat regulates growth through its actions upstream of the Hippo kinase pathway, however the precise mechanism of its action is still unknown and very few proteins that biochemically associate with Fat have been identified. To better understand the biology of Fat signaling, I am using a proteomic screening approach to identify novel Fat interactors using affinity purification coupled with mass spectrometry. I generated flies and KC167 cells that overexpress truncated versions of tagged Fat protein, which are used as bait in these studies. Potential Fat interactors will further be evaluated for effects on growth, and how they genetically and biochemically interact with Fat.
The Role of PPP1CC2 in the Mouse Testis: Transgenic Interactome and Phosphoproteomic Approaches

Presenting author: Graham MacLeod, University of Toronto, Depart. of CSB
Co-authors: Graham MacLeod, Gregory Booth, Susannah Varmuza

Abstract: Deletion of mouse Ppp1cc, a highly conserved Ser/Thr phosphatase leads to male infertility due to impaired spermatogenesis. This phenotype is hypothesized to be due to a loss of the testis specific splice isoform PPP1CC2. To elucidate the function of PPP1CC2 in the mouse testis we have employed interactome and phosphoproteomic approaches. As tissue culture-based modelling of the testis is not currently feasible we have chosen to study the interactome of PPP1CC2 directly in the testis. We have created 3xFLAG-SBP tandem affinity tagged PPP1CC2 knock-in ES cells under the control of the endogenous Ppp1cc promoter. This system is amenable to any gene for which gene-trap ES cell lines exist. Tandem affinity purification (TAP) from knock-in ES cells has identified 8 previously known PP1 interacting proteins, illustrating the functionality of the knock-in construct. From these cells, chimeric mice have been produced, and breeding of transgenic lines for TAP experiments is currently underway. To identify candidate substrates of PPP1CC2 in the testis we have performed a phosphoproteomic analysis of the Ppp1cc mutant testis in an effort to identify hyperphosphorylated proteins. Thus far we have identified over 125 testis phosphoproteins and 350 sites. A comparison between wild-type and Ppp1cc mutant testis phosphoproteomes is currently underway. By using interactome and phosphoproteomic analyses directly in the tissue of interest (testis) we hope to identify biologically relevant protein-protein interaction networks that are important to the study of male infertility, a condition affecting as many as 20% of couples.

Analysis of Shc1 Signaling Network in Time and Space by sMRM

Presenting author: Yong Zheng, Mount Sinai Hospital
Co-authors: Yong Zheng, Cunjie Zhang, Mohamed Soliman, Rick Bagshaw, Adrian Pasculescu, Lorne Taylor, Steve Tate, Yue Dai, Karen Colwill, Jim Dennis, Tony Pawson

Abstract: Cell surface receptors commonly use cytosolic scaffold proteins to selectively recruit downstream effectors required for signaling pathways coordination. Shc1 is a scaffold protein that connects ErbB receptor tyrosine kinases (RTKs) to their targets such as Grb2 and Gab1. Through combined use of its docking sites, Shc1 can extend the range and potency of a receptor’s signaling capacity. Although multifaceted biological effects were established for Shc1, the kinetics with which Shc1 binds different signaling components, and the means by which its functional outputs may change in time and space remain unclear. Using a quantitative targeted proteomic method based on scheduled multiple reaction monitoring (sMRM), we have resolved the local signaling network assembled by the scaffold protein Shc1 and have demonstrated that Shc1 responds to growth factors in an unexpectedly dynamic manner, involving multiple distinct waves of protein interactions and phosphorylation events. Notably, a rapidly binding group of Shc1-interacting proteins is dependent on recruitment of the Grb2 adaptor to Shc1 pTyr sites at the plasma membrane, while a different set of proteins, primarily involved in cytoskeletal organization, is recruited to Shc1 at intracellular membranes with delayed kinetics, and in a Grb2-independent fashion. The results indicate that scaffold proteins can be remarkably versatile in directing information flow during signal transduction.
Hippo and TGF-beta Signalling Converges to Control Pluripotency in Human Embryonic Stem Cells

Presenting author: Alexander Weiss, SLRI, Mt Sinai Hospital
Co-authors: Alexander Weiss, Tobias Beyer, Katherine Huang, Jeffrey Wrana

Abstract: The capacity of embryonic stem cells to self-renew and differentiate into all functional cell types depends on an elaborated transcriptional network. The core members of this pluripotency transcriptional circuitry are NANOG, OCT4, SOX2. Loss of any of these regulators induces differentiation and inevitably loss of pluripotency. Not surprisingly, expression of these core factors is tightly controlled - by NANOG, OCT4 and SOX2 in autoregulatory feedback loops, and by several signalling cascades. It is well established that human embryonic stem cells depend on an active TGF-beta signalling pathway to maintain pluripotency. Recently, members of the newly emerged Hippo tumor suppressor pathway have also been shown to play important roles in controlling stemness. Here we used DNA-protein affinity purification followed by mass spectrometry and chromatin immunoprecipitation followed by high-throughput sequencing (Chip-Seq) to reveal how the Hippo and TGF-beta pathway converge to regulate pluripotency. Using the NANOG promoter as a bait, we identified factors involved in the regulation of NANOG. Besides the already known SMAD proteins, the effectors of the TGF-beta pathway, we detected TEAD proteins, effectors of the Hippo pathway. Subsequent experiments revealed a general theme, in which SMADS and TEADS interact with OCT4 to regulate target genes involved in pluripotency. Importantly, our results imply a novel function of this OCT4/TEAD/SMAD complex as a repressive complex suppressing genes involved in differentiation.

A DNAP-MS Approach to Identify Enhancer-bound Proteins that Poise Interferon Target Genes for BRG1-dependent Induction

Presenting author: Manoja Eswara, Toronto Western Hospital
Co-authors: Manoja Eswara, Alexander Weiss, Brett Larsen, Jeffrey L. Wrana, and Rod Bremner

Abstract: Interferon (IFN) signaling is essential for immune surveillance, which blocks tumorigenesis. However, the detailed mechanisms involved are mostly obscure. Our work showed that the chromatin remodeling ATPase, BRG1, is essential for IFN responsiveness, by facilitating active histone methylation, transcription factor recruitment and chromatin looping. Moreover, we have discovered that BRG1 antagonizes the negative effect of Polycomb Repressive Complex 2 (PRC2) at interferon targets. Interestingly, while BRG1 is defective in ~15% of human cancers, PRC2 components are commonly over-expressed in human tumors, suggesting that we have uncovered a general mechanism to explain resistance to immune surveillance in cancers. Exploring this novel connection has the potential to expose new strategies to manipulate epigenetic regulation of interferon stimulated genes (ISGs) and reactivate IFN responsiveness in cancer cells. Using the CIITA gene as a model ISG, we identified that BRG1 “poises” this locus for IFN induction by binding to remote enhancers. We hypothesize that specific unknown protein(s) may recruit BRG1 to these sites to mediate its effects. To identify these factors, we performed an in vitro DNA-protein affinity purification combined with Mass spectrometry (DNAP-MS) using biotin labeled enhancer DNA elements and HeLa nuclear extracts. Apart from proteins that are known to interact with these enhancers, we identified other novel proteins using this strategy. These proteins will be thoroughly validated using a combination of ChIP and knock down analysis and further studies will aim at understanding their mechanism of action in mediating BRG1 recruitment and IFN responsiveness.
Characterization of Tetrahymena Thermophila Asf1

Presenting author: Jeffrey Fillingham, Ryerson University Department Chem/BIO
Co-authors: Jeffrey S. Fillingham, Jyoti Garg, J.P. Lambert, Abdel Karsou, Ernest Radovani, Anne-Claude Gingras, Ronald E. Pearlman.

Abstract: Asf1 is a histone H3-H4 chaperone that functions in replication-dependent and independent chromatin assembly in eukaryotes from yeast to humans. In the budding yeast Saccharomyces cerevisiae, Asf1 has additionally been shown to function in chromatin disassembly as well as to coordinate histone post-translational modification such as acetylation of histone H3 at lysine 56 (H3K56ac). In order to learn more about the function of Asf1 in a protozoan model we generated transgenic Tetrahymena thermophila strains expressing the FZZ epitope tag at the C-terminus of Asf1. Indirect immunofluorescence shows that tAsf1 localizes to both macro- and micro- nuclei but the majority to the micronucleus. We used affinity purification coupled with mass spectrometry (AP-MS) and determined the set of tAsf1 interacting proteins. tAsf1 physically interacts with an Importin β, as well as tNASP/Hif1, a conserved histone H3-H4 chaperone known to physically interact with Asf1 in yeast and humans, as well as two novel proteins with no similarity to any known proteins. Reciprocal AP-MS of this tagged Importin β co-purified the 2 novel proteins and tAsf1 but not tNASP/Hif1. Functional analysis of tAsf1 suggests that it cannot substitute for budding yeast Asf1 with respect to H3K56ac in vivo or in vitro. This work was supported by grants to JSF from NSERC and a Ryerson University Health Sciences Grant, and to REP by grants from NSERC and CIHR.

Characterization of Tetrahymena Thermophila SOH1/MED31

Presenting author: Jeffrey Fillingham, Ryerson University Department Chem/BIO
Co-authors: Jeffrey S. Fillingham, Jyoti Garg, J.P. Lambert, Matthew Cadorin, Anne-Claude Gingras, Ronald E. Pearlman.

Abstract: SOH1/MED31 is a component of the evolutionarily conserved Mediator transcriptional coactivator protein complex that physically interacts with, and phosphorylates the evolutionarily conserved C-terminal domain (CTD) of the RPB1 subunit of RNAPII in most eukaryotes. Our bioinformatic analyses revealed that T. thermophila Rpb1 has a divergent CTD domain and that SOH1/MED31 is the sole conserved gene encoding a known Mediator subunit encoded in the T. thermophila genome. The significant sequence conservation of Soh1/Med31, but not other Mediator components, argues that in T. thermophila, Soh1/Med31 may have an important Mediator-independent conserved function. To address this, we used affinity purification coupled with mass spectrometry (AP-MS) and determined that Soh1/Med31 physically interacts with a large number of novel proteins none of which have similarity to Mediator components in any system. We also show that tsOh1 is non-essential for vegetative growth in T. thermophila. Data will be presented demonstrating that tsoh1/Med31 and most of the genes encoding its interacting proteins indicates they are expressed at high levels in early conjugation suggesting that tsoh1/Med31 could have an important role in meiosis, a hypothesis we are currently testing. This work was supported by grants to JSF from the NSERC and a Ryerson University Health Sciences Internal Grant, and to REP by grants from NSERC and CIHR.
Proteome Changes Associated Leishmania Donovani Promastigote Adaptation to Oxidative and Nitrosative Stress

Presenting author: Abul Sardar, McGill University
Co-authors: Abul Hasan Sardar, Sudeep Kumar, Ashish Kumar, Bidyut Purkait, Sushita Das, Manish Kumar, Kumar Abhishek, Dharmendra Singh, Armando Jardim, Pradeep Das.

Abstract: Phagocytic cells produce reactive oxygen and nitrogen species (ROS & RNS) as the most common arsenal to kill intracellular pathogens. Leishmania an obligate intracellular pathogen also confronts this antimicrobial assault during the early process of infection but nevertheless is able to survive these attacks and proliferate in macrophage. Adaptation of Leishmania to the toxic effects of ROS and RNS, involves a rapid post-transcriptional change in the parasite proteome in order to combat the host defense response. To understand the events associated with combating ROS and RNS species we performed a proteomic analysis of L. donovani promastigotes treated sub-lethal doses of menadione (ROS), S-nitroso-N-acetylpenicillamine (RNS) or combination of both compounds. Proteomic changes in response to these reagents were evaluated by iTRAQ MS/MS analysis. The analysis identified alterations ~20% of the parasite proteome across 3 stress conditions. Major changes were observed in enzymatic machinery of pathways involved in maintaining redox homeostasis, trypanothione metabolism, oxidative phosphorylation, superoxide metabolism, mitochondrial respiration process and other essential metabolic pathways. These observations shed light on how Leishmania promastigotes counter ROS and RNS affects during the initial stage of infection. Key words: iTRAQ, LC-MALDI-TOF/TOF-MS, redox homeostasis, trypanothione metabolism, superoxide metabolism.
TRANSLATIONAL PROTEOMICS

Discovery and in Silico Verification of a Proteomic Prognostic Panel for Breast Cancer
Presenting author: Maria Pavlou, University of Toronto
Co-authors: Maria P. Pavlou, Apostolos Dimitromanolakis, Eleftherios P. Diamandis

Abstract: More than 50% of patients with early breast cancer are subjected to cytotoxic therapies, without receiving additional benefit, due to a lack of reliable prognostic biomarkers. Proteome profiling may hold promise in elucidating protein signatures for clinically relevant patient sub-classification. We report an extensive mass spectrometry-based secretome analysis of eight breast cancer cell lines representing the three main breast cancer subtypes in search of subtype-specific proteomic signatures. Since clinical outcome differs in the distinct breast cancer subtypes, we hypothesized that subtype-specific proteomic signatures may have prognostic potential. More than 5,200 non-redundant proteins were identified in the conditioned media of eight breast cancer cell lines. Proteins common to all cell lines of one subtype but not present in the other two subtypes were identified. Subtype-specific proteins were filtered to develop a panel of 30 proteins: 26 proposed as unfavourable and four as favourable prognosis markers. A search of the current literature revealed two previously studied prognostic breast cancer biomarkers in our panel. To evaluate the prognostic potential of the identified proteomic panel and further prioritize the candidate biomarkers, an in silico mRNA analysis was performed using publicly available data from four independent experiments including more than 900 patients. The expression profiles of 15 of 30 proteins investigated showed significant power to distinguish subtypes and correlation with estrogen receptor expression, a known prognostic factor. This indicates the potential of the identified proteins to improve the prediction of patient survival in tandem with known clinical variables.

Identification and Quantification of Peptides and Proteins Secreted from Prostate Epithelial Cells by Unbiased Liquid Chromatography Tandem Mass Spectrometry
Presenting author: Angelica Florentinus, chemistry and Biology, Ryerson University
Co-authors: Angelica K. Florentinus, Peter Bowden, Girish Sardana, Eleftherios P. Diamandis, John G. Marshall

Abstract: The proteins secreted by prostate cancer cells (PC3(AR)6) were separated by strong anion exchange chromatography, digested with trypsin and analyzed by unbiased liquid chromatography tandem mass spectrometry with an ion trap. The spectra were matched to peptides within proteins using a goodness of fit algorithm that showed a low false positive rate. The parent ions for MS/MS were randomly and independently sampled from a log-normal population and therefore could be analyzed by ANOVA. Normal distribution analysis confirmed that the parent and fragment ion intensity distributions were sampled over 99.9% of their range that was above the background noise. Arranging the ion intensity data with the identified peptide and protein sequences in structured query language (SQL) permitted the quantification of ion intensity across treatments, proteins and peptides. The intensity of 101,905 fragment ions from 1421 peptide precursors of 583 peptides from 233 proteins separated over 11 sample treatments were computed together in one ANOVA model using the statistical analysis system (SAS) prior to Tukey-Kramer honestly significant difference (HSD) testing. Thus complex mixtures of proteins were identified and quantified with a high degree of confidence using an ion trap without isotopic labels, multivariate analysis or comparing chromatographic retention times. The chromatography resin and salt fractions that best detected the 14-3-3 proteins without antibodies were determined and statistically analyzed. It was possible to statistically analyze proteomic data without the use of any heuristic, pragmatic of practical approaches or proteomic analysis software but rather with the data systems common to all fields of science.
Development of Selected Reaction Monitoring Assays for Quantification of Biochemical Markers of Down Syndrome in Amniotic Fluid Samples

Presenting author: Eduardo Martinez-Morillo, Mount Sinai Hospital
Co-authors: E. Martinez-Morillo, J. L. Shaw, A. Soosaipillai, E. P. Diamandis

Abstract: BACKGROUND: Down syndrome (DS) is the most frequent and recognizable form of mental retardation, appearing in about 1 of every 700 newborns. Current screening strategies have detection rates of 90-95% for a 5% false positive rate. The aim of this study was to discover new biomarkers of DS in amniotic fluid (AF) by using selected reaction monitoring assays.

METHODS: Nine proteins were analyzed: CEL, CPA1, MUC13, CLCA1, MUC5AC, PLUNC, HAPLN1, CGB, as positive control, and transferrin, as negative control. One proteotypic peptide for each protein was selected and isotopically labeled peptides were spiked into the samples. A 35 min. gradient in an EASY-nLC pump (Proxeon A/S) was used to elute the peptides and quantification was carried out in a TSQ Vantage (Thermo Fisher) by monitoring three transitions for all light/heavy peptides. Fifty-four samples from pregnant women carrying normal (n=37) or DS affected (n=17) fetuses were analyzed. Gestational age ranged from 15+0 to 17+5 weeks. Results: The median protein concentrations for DS and normal samples were: 20 and 49 ng/ml (p<0.01) for CEL; 3.7 and 14 ng/ml (p<0.001) for CPA1; 80 and 263 ng/ml (p<0.001) for MUC13; 46 and 135 ng/ml (p<0.001) for CLCA1; 0.65 and 0.93 μg/ml (p<0.05) for MUC5AC; 61 and 73 ng/ml (p>0.05) for PLUNC; 144 and 86 ng/ml (p<0.01) for HAPLN1; 0.89 and 0.54 μg/ml (p=0.05) for CGB; 91 and 87 μg/ml (p>0.05) for transferrin.

CONCLUSION: Statistically significant differences were found in six of the proteins analyzed, reflecting a different regulation in DS.

Quantitative Statistical Analysis of Standard and Human Blood Proteins From Liquid Chromatography, Electrospray Ionization and Tandem Mass Spectrometry

Presenting author: Peter Bowden, Ryerson University, Dept. of Chemistry and Biology
Co-authors: Peter Bowden, Thanusi Thavarajah, Peihong Zhu, Mike McDonnel, Herbert Thiele, and John G Marshall
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Abstract: It will be important to determine if the parent and fragment ion intensity results of liquid chromatography, electrospray ionization and tandem mass spectrometry (LC-ESI-MS/MS) experiments have been randomly and independently sampled from a normal population for the purpose of statistical analysis by general linear models and ANOVA. The tryptic parent peptide and fragment ion m/z and intensity data in the MASCOT generic files from LC-ESI-MS/MS of purified standard proteins, and human blood protein fractionated by partition chromatography, were parsed into a Structured Query Language (SQL) database and were matched with protein and peptide sequences provided by the X!TANDEM algorithm. The many parent and/or fragment ion intensity values were log transformed, tested for normality and analyzed using the generic Statistical Analysis System (SAS). Transformation of both parent and fragment intensity values by logarithmic functions yielded intensity distributions that closely approximate the log normal distribution. ANOVA models of the transformed parent and fragment intensity values showed significant effects of treatments, proteins, and peptides, as well as parent versus fragment ion types, with a low probability of false positive results. Transformed parent and fragment intensity values were compared over all sample treatments, proteins or peptides by the Tukey-Kramer Honestly Significant Difference (HSD) test. The approach provided a complete and quantitative statistical analysis of LC-ESI-MS/MS data from human blood. Here, type I error and relative quantification was achieved using only classical frequency based statistics with the commonly available SQL and SAS data systems and no heuristic, pragmatic or empirical approaches were required.
Multiplexed LC-MS/MS SRM Assay for Parathyroid Hormone (PTH) and Variants: Correlation with Current Clinical Immunoassay Methods

Presenting author: Bryan Krastins, Thermo Fisher Scientific - BRIMS Center
Co-authors: Mary F. Lopez, Amol Prakash, David Sarracino, Dobrin Nedelkov, David R. Barnidge, Randall W Nelson, Paul Oran, Linda Benson, Robert H Bergen, Jolaine Twentyman, Ravinder J. Singh, Andrew Hoofnagle

Abstract: Background: The heterogeneity of PTH has traditionally been an impediment to the development of assays that distinguish full length PTH (PTH1-84) from N-terminally truncated PTH (PTH 7-84 and others). Because intact and truncated forms of PTH vary in their biological activity, assays that can accurately quantify the ratio of intact hormone to its fragments are of increasing significance in the diagnosis of endocrine and osteological diseases. To date, most immunoassays used to monitor PTH levels are based on traditional sandwich ELISA methods and cannot accurately discriminate intact from truncated PTH. In addition, these methods typically employ primary antibodies to the N-terminus of the hormone, thereby preventing quantification of any fragments. Previously, we developed multiplexed SRM assays for PTH that allow quantification of four fully-tryptic monitoring peptides (that span the entire PTH sequence) and two semi-tryptic variant specific peptides (1,2). Using this approach, it is possible to monitor intact PTH and also the degree of N-terminal fragmentation. In this study, the objective was to apply the LC-MS/MS SRM assay in addition to two commercially available immunoassays to a cohort of clinical samples and monitor intact and truncated PTH isoforms. In addition, the correlation between the three assay measurements was determined.

Methods: A single cohort of IRB approved clinical serum samples was distributed between three laboratories. Mass Spectrometry and sample preparation were as previously described (1). Immunoassays (Beckman, Roche) were run according to manufacturer’s instructions.

Results: Peptides exhibited linear responses (R2 = 0.90-0.99) relative to recombinant human PTH concentration. The limits of detection were 8 ng/L and limits of quantification were of 16-32 ng/L depending on the peptide. Comparison of the MSIA-SRM assay with the commercial ELSA assays demonstrated good correlation. However, the data also showed that the commercial immunoassays overestimated the amount of intact PTH as compared with the MSIA-SRM. This is due to the lower specificity of the immunoassays since the capture antibodies bind more than one isoform. In contrast, the MSIA-SRM can distinguish and quantify individual isoforms at the amino acid sequence level.
TECHNOLOGY DEVELOPMENT

**An Introduction of a Novel Atmospheric Pressure Micro/Nano-ESI ion Source**

**Presenting author:** Hui Qiao, Ionics Mass Spectrometry Group Inc.
Co-authors: Hui Qiao, Brett Larsen, Lorne E Taylor, Lisa Cousins, Gholamreza Javahery

**Abstract:** The advent of micro/nano-ESI has considerably extended the applications of ESI in analytical mass spectrometry, offering advantages such as lower sample consumption and higher ionization efficiency. However, conventional micro/nano-ESI systems can suffer from unstable Taylor cone effects, clogging of emitters, and incomplete droplet desolvation, making them more difficult to operate. In the present study, a novel atmospheric pressure source was developed to accommodate nano to micro LC flow ranges (0.5-20 uL/min). Compared to the conventional micro/nano-ESI ion source, this new ion source offers ease of use, 2-fold improvement in signal stability, and higher S/N ratio. The experiments were carried out on an IONICS EP10+ with IONICS HSID interface. Reserpine, testosterone, and BSA digest were used to compare the performance of the new ion source with a conventional microspray ion source. For continuous sample injection of both reserpine and BSA digest, ion signal from Q1 scan shows that the new ion source has 2-fold improvement in stability than the conventional microspray ion source at 1 ul/min flow rate with both syringe and nano-HPLC pumps. For multiple injections of reserpine using a nano-HPLC pump with 75um ID columns, the MRM spectrum of reserpine showed that the new ion source provided two times higher signal reproducibility. Both MRM and Q1 multiple ion (MI) scan spectra from BSA digest clearly showed reductions of chemical noise level of the new ion source due to more complete droplet desolvation. An improved S/N ratio was also found in the MRM spectra of testosterone in serum.

**Mass Spectrometric Immunoassay for Insulin-Like Growth Factor 1**

**Presenting author:** Dobrin Nedelkov, Thermo Fisher Scientific
Co-authors: Dobrin Nedelkov, David Phillips, Bryan Krastins, Eric Niederkofler, Urban Kiernan, Kemmons Tubbs

**Abstract:** Targeted proteomics can facilitate the full assessment of the human proteome. Affinity interactions are utilized to selectively retrieve specific proteins from complex biological fluids, and are followed by mass spectrometric analysis of the targeted proteins. Mass spectrometric immunoassays (MSIA) are similar to traditional enzymatic immunoassays in the use antibodies as reagents for affinity retrieval of specific proteins. However, instead of enzymatic reaction for indirect protein detection, mass spectrometric analysis is used for direct identification of the proteins and their modifications. This rather straightforward concept is realized through affinity pipette devices (MSIA-Tips) that enable high-throughput assaying of thousands of samples. Described here is the development and validation of mass spectrometric immunoassay for insulin-like growth factor 1 (IGF1). MSIA-Tips derivatized with anti-IGF-1 antibody were used for affinity isolation of IGF1 from human samples. Long R3 (LR3) IGF1, spiked into the samples prior to the affinity isolation, served as internal reference standard for signal normalization and IGF1 quantification. Following capture, the proteins were eluted either onto a MALDI plate for intact mass analysis, or into vials and subjected to tryptic digest and LC-MS for surrogate peptides detection. Linear standard curves were built that spanned the range of 5-1,500 ng/mL. The assay exhibited intra- and inter-assay precisions of <10%, and linearity and spiking-recovery in the 90-110% range. Data was compared to that obtained with a commercially available ELISA, yielding good correlation. The data correlation between intact IGF1 and peptide-based IGF1 MS analyses was good, demonstrating the applicability of the IGF1 MSIA across MS platforms.
Re-evaluation of 18 Non-human Protein Standards to Estimate the False-positive Rate of LC-ESI-MS/MS with a Linear Ion Trap

Presenting author: Thanusi Thavarajah, Ryerson University
Co-authors: Thavarajah, Thanusi; Bowden, Peter; Tucholska, Monika; and Marshall, John G.

Abstract: The False Discovery Rate (FDR) was invented by Benjamini and Hochberg as a competition for significance used to correct multiple means comparisons. However, matching MS/MS spectra to peptides is not a multiple means comparison problem but rather a goodness of fit. Eighteen non-human protein standards, similar to those used to create the empirical model for the so called FDR of LC-ESI-MS/MS, were obtained. The standards were resolved by SDS-PAGE and stained with diamine silver to reveal the standards were not pure but visibly contain at least hundreds of proteins. A mixture of three standards; cytochrome c, glycogen phosphorylase b, and alcohol dehydrogenase were digested with trypsin and analyzed by LC-ESI-MS/MS to optimize the search engine parameters for X!TANDEM. The optimal fit parameters were used to identify the peptides from each of the 18 proteins separately after selecting only parent ions that achieved a signal of at least E3 counts. Each standard was observed to contain tens to hundreds of proteins that were confidently identified with multiple peptides by X!TANDEM. However, the empirical FDR model, based on the assumption that the protein standards were pure, indicated that most of the demonstrably correct data should be discarded resulting in a large type II error (false negative) when applied to these standards. The aim of any statistical analysis is to minimize total error (type I + type II). We conclude that the FDR test in proteomics is theoretically inappropriate, based on incorrect assumptions, and in practice leads to a large total error.

Comparison of Standard-flow and Nano-flow LC-MRM/MS Systems for Quantitating Cardiovascular Disease-associated Plasma Proteins

Presenting author: Andrew Percy, UVic - Genome BC Proteomics Centre
Co-authors: Percy, Andrew J.; Chambers, Andrew G.; Yang, Juncong; Domanski, Dominik; Borchers, Christoph H.

Abstract: Accurate plasma protein concentrations of putative cardiovascular disease (CVD)-related biomarkers have been determined through a targeted, multiplexed approach centered on MRM/MS in conjunction with stable isotope-labeled standards (SIS). This has conventionally been performed using a nano-flow and standard-flow LC-MRM/MS platform with a variety of LC systems, ionization sources, and mass spectrometers. While comparable lower limits of quantitation (LLOQ) were achieved in several cases, these methods were developed using different variables (e.g., protein panel, sample type) and different platform conditions (e.g., flow rate, mass spectrometer type), which makes it difficult to determine which system is preferable. This talk will compare the analytical performance of the nano-flow and standard-flow LC-MRM/MS platforms conducted on the same state-of-the-art triple quadrupole mass spectrometer using a panel of candidate CVD-associated plasma proteins. Variables such as the mass spectrometer, the sample origin, the sample preparation, the protein targets, and the SIS peptides were kept constant in this platform comparison study so that only the performance of the standard-flow versus nano-flow systems could be compared. Through inter-/intra-day measurements and per-peptide calibration curves, the reproducibility, sensitivity, LLOQ, and dynamic range obtained from each platform were evaluated. The results demonstrated the standard-flow UHPLC system to deliver superior MRM/MS reproducibility and sensitivity. These findings, along with their implications for future method development in quantitative proteomics of putative CVD-associated biomarkers, will be discussed.
Dried Blood Spot Samples for Multiplexed MRM Analysis of Proteins
Presenting author: Andrew Chambers, UVic Genome BC Proteomics Centre
Co-authors: Andrew G. Chambers, Andrew J. Percy, Alexander G. Camenzind, Juncong Yang, and Christoph H. Borchers

Abstract: Dried blood spot (DBS) sampling was originally developed to address the limited sample volumes available for newborn screening of phenylketonuria. The success of this sampling method has led its implementation in nation-wide screening for several metabolic disorders and its use in viral disease monitoring. Over the last ten years, DBS has increasingly been coupled with quantitative mass spectrometry (MS) for pre-clinical toxicology and pharmacokinetics studies supporting small molecule drug development. More recently, there is interest in developing MS based DBS assays for monitoring therapeutic peptides and proteins. Here we evaluate the potential of integrating DBS methodology into multiplexed clinical assays for the verification of potential protein biomarkers in human blood. Whole blood was collected from multiple donors, pooled, and spotted onto cellulose based collection cards. Proteins were then simultaneous extracted and denatured before enzymatic digestion with trypsin. At least one stable-isotope labeled standard peptide for each of the 60 protein targets was synthesized and added post-digestion to improve the accuracy and precision of the assay. Peptides were separated on an Agilent 1290 Infinity UHPLC and detected with an Agilent 6490 QQQ mass spectrometer using multiple reaction monitoring. Several methods for extracting proteins from DBS cards were investigated to optimize sample preparation reproducibility. Initial results show that both the efficiency of protein extraction from the collection cards and the digestion efficiency are very reproducible. Preliminary data evaluating the stability of proteins in DBS will also be presented.

The Endogenous Peptides of Normal Human Blood
Presenting author: Jaimie Dufresne, Ryerson University, Dept. of Chemistry and Biology
Co-authors: Jaimie Dufresne, Thanusi Thavarajah, Angelique Florentinus, Peter Bowden and John G. Marshall

Abstract: We have previously compared methods to analyze endogenous peptides from human blood including albumin isolation, ion exchange chromatography, solid phase extraction with C18, and organic precipitation with a variety of solvents, acids or bases, separately or in combination. Polypeptides were quantitatively precipitated in 90% acetonitrile followed by the selective extraction of low molecular mass peptides with mixtures of organic solvents and water that dissolved peptides while maintaining proteins in the solid phase. The extracted peptides were decanted, dried and refined over C18 reversed phase. Peptides were analyzed by liquid chromatography with electrospray ionization followed by tandem mass spectrometry (LC-ESI-MS/MS). The MS/MS data was corrected to a protein library of about 137,500 proteins, isoforms, splice variants and predicted expression products by Proteome Discoverer. About 24,316 proteins from normal human blood were correlated with three or more independent peptide observations. Peptides derived from signal transduction molecules including kinases, G-proteins and their adapter and regulatory proteins were identified. Furthermore peptides from interleukins, chemokines cytokines and their soluble or membrane bound receptors were observed. Similarly, growth factors including molecules known to have low nano molar or pico molar concentrations and their corresponding receptors were detected. The peptide ion and fragment ion intensity values were recorded in a structure query language (SQL) database for relative quantification using the statistical analysis system (SAS). Many novel blood peptides from parent proteins that show homology to proteins with medicinally important biological functions were directly discovered with confidence from human blood.
Tissue-Based Identification of Prognostic Marker of Metastatic Clear Cell Renal Cell Carcinoma
Presenting author: Olena Masui, York University
Co-authors: Olena Masui, Nicole M.A. White, Leroi V. DeSouza, Olga Krakovska, Ajay Matta, Shereen Metias, Bishoy Khalil, K.W. Michael Siu, and George M. Yousef

Abstract: We have used high throughput quantitative proteomics for the identification of differentially expressed proteins that can predict the risk of metastasis in clear cell renal cell carcinoma (ccRCC). Primary and metastatic ccRCC samples and non-malignant kidney tissues were analyzed using isobaric tags for relative and absolute quantitation (iTRAQ) analysis. Using ProteinPilot software, a total of the 1256 non-redundant proteins were identified; 12 of which were over-expressed in metastatic, relative to both primary ccRCC and non-malignant samples. Three of these overexpressed proteins, PCM352, PCM353, and PCM70 were targeted for evaluation of prognostic potential. Their overexpression was confirmed by western blot analysis using the same tissue samples. A second pre-validation scale analysis performed to verify their overexpression using immunohistochemical analysis on an independent cohort of paraffin-embedded tissues, confirmed the overexpression of PCM353 in the majority of primary ccRCC samples from patients who subsequently developed metastases, relative those who did not. Our findings suggest that PCM353 may serve as a potential prognostic marker for metastatic ccRCC. Interestingly, to the best of our knowledge, overexpression of PCM353 appears to be unique to ccRCC; it is reportedly underexpressed in human breast cancer, squamous cell carcinoma, pancreatic and hepatic carcinoma cells, and nasopharyngeal cancer cell lines. The altered pattern of differential expression between PCM353 in ccRCC and the other forms of cancer is consistent with its varied role in the different contexts. In metastatic ccRCC PCM353 induces migration by positively regulating the actin-cytoskeleton pathways whereas in metastatic breast cancer cells by negatively regulating phosphatidylinositol (3,4)-bisphosphate.

OpenFreezer: a Reagent Information Management Software System
Presenting author: Marina Olhovsky, Samuel Lunenfeld Research Institute
Co-authors: Kelly Williton, Anna Dai, Adrian Pasculescu, John Paul Lee, Marilyn Goudreault, Clark D. Wells, Jin Gyoon Park, Anne-Claude Gingras, Rune Linding, Tony Pawson, Karen Colwill

Abstract: The rapid growth of large-scale reagent collections that are necessary for systems-level approaches in biology has brought about a concomitant need for extensible and flexible tracking systems to manage such resources. To meet this need, we developed OpenFreezer, an open-source web-based enterprise software application, which maintains detailed and standardized documentation on common laboratory reagents in proteomics and molecular biology. OpenFreezer is designed to track both large-scale laboratory reagent collections and individual reagents via a centralized repository that allows for easy access, sharing and management of data across projects and research groups. This repository is accessed through a graphical interface that provides virtual representations of each reagent and its inherent features such as sequence, database identifiers, and location within the laboratory. Interactive tools such as primer design and automated vector creation facilitate experiment design and planning. Our current development efforts are focused on adapting the software for management of multiple reagents simultaneously (in “batches” ) for faster input and customized output.OpenFreezer has a proven track record at the Samuel Lunenfeld Research Institute where over 150,000 reagents are accessed by users in 17 different laboratories. Since its release in August 2011 as an open-source software package under GNU GPL license V3, OpenFreezer has been adopted internationally by research groups of varying magnitude. OpenFreezer provides a foundation for researchers who wish to build specific workflow applications within an enterprise application framework. For further details or to download OpenFreezer, visit http://openfreezer.org/.