Joint Conference
3rd Annual Canadian National Proteomics Network
and
5th International Symposium on Enabling Technologies for Proteomics

May 8-11, 2011
Fairmont Banff Springs Hotel, Banff AB

NOTES:

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Event organization and coordination by ETP Symposium Inc. and Dr. Christoph Borchers, UVic Genome BC Proteomics Centre

Welcome from the Chairs

Liang Li, University of Alberta (CNPN 2011)
David Schriemer, University of Calgary (CNPN 2011)
Joel Weiner, University of Alberta (CNPN 2011)
Randal Johnston, University of Calgary (ETP 2011)

On behalf of the Organizing Committee, we wish to welcome everyone to the joint symposium of the Third Canadian National Proteomics Network on “Proteomics in Human Health” and the 5th International Symposium on Enabling Technologies for Proteomics. These two symposia bring together world-renowned scientists in the field of proteomics and related disciplines.

We are delighted to host this venue, and to share with you the outstanding contributions of our speakers. Together, these symposia showcase the latest developments in proteomics research from both the technical and applied perspectives. While international in breadth and impact, the symposiums are intended to favor collegial spirit where researchers may engage in active discussion in a stimulating environment. We wanted to keep a regional flavor by focusing on the work of scientists in the western provinces and engaging trainees in both oral and poster contributions.

The scientific events will begin on May 8 with pre-conference activities, involving a workshop in quantitative proteomics followed by a lunchtime job fair, to promote networking among researchers, trainees and companies. A special symposium on structural proteomics will take place in the afternoon followed by an opening reception in the Garden Terrace for all attendees. The CNPN symposium will begin on May 9th in the Alberta Room and will be followed by the ETP symposium starting in the afternoon of May 10th. The award dinner commemorating the contributions of outstanding researchers will take place on the evening of May 10th. The joint symposia will end on Wednesday May 11th at noon. If you have any question on logistics, please contact Janette Champ, Conference Organizer, at janette@etpsymposium.org or 905-252-2856 during the symposia.

We have assembled an exciting program taking place in the majestic environment of the Banff National Park.

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.

ETP Symposium is a non-profit corporation committed to organizing and operating educational seminars and forums and to develop interdisciplinary opportunities for international researchers and scientists to develop technology in the life sciences area.

Au nom du Comité d'organisation, nous vous souhaitons la bienvenue aux colloques conjoints du Réseau Canadien de la Protéomique «Protéomique et sante humaine» et du 5e symposium International des technologies innovatrices en protéomique. Ces deux colloques réunissent des scientifiques de renommée mondiale dans le domaine de la protéomique et des disciplines connexes.

Nous sommes ravis de vous accueillir et de partager avec vous les contributions exceptionnelles de nos conférenciers. Ces colloques présenteront les derniers développements technologiques et leurs applications en protéomique. Bien que ces colloques regroupent des chercheurs de renommée internationale, ils visent également à favoriser un environnement collégial où les chercheurs peuvent discuter librement de leurs travaux dans une ambiance stimulante et enrichissante. Nous avons voulu garder une saveur locale en sélectionnant des contributions de scientifiques des provinces de l’ouest et en permettant aux étudiants et postdoctorants de présenter leurs travaux sous forme de contributions orales ou par affiches.

Les présentations scientifiques commenceront le 8 mai avec un atelier portant sur la protéomique quantitative qui sera suivie d'une foire de l'emploi à midi qui favorisera les entretiens entre les chercheurs, les étudiants et postdoctorants. Un symposium spécial sur la protéomique structurale aura lieu dans l'après-midi et celui-ci sera suivi d'une réception pour tous les participants. Le colloque du CNPN débutera le lundi 9 mai dans la salle Alberta et sera suivi du colloque ETP le mardi 10 mai en après-midi. Une réception honorant les contributions de chercheurs exceptionnels aura lieu durant la soirée du 10 mai.

Welcome from the Chairs

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Les colloques conjoints se termineront le 11 mai à midi. Si vous avez des questions concernant ces événements, n'hésitez pas à contacter Janette Champ, coordonnatrice des conférences, au 905-252-2856 ou par courriel à janette@etpsymposium.org

Nous avons assemblé un programme passionnant qui se déroulera dans le décor majestueux du parc national Banff.

CNPN est un organisme sans but lucratif à charte fédérale favorisant la coopération entre différents laboratoires pour développer une infrastructure de recherche en protéomique au Canada et une meilleure intégration de la protéomique dans les sciences de la vie. Le CNPN parraine des conférences scientifiques, des séminaires et des colloques pour créer un regroupement national facilitant la collaboration scientifique et l'éducation.

ETP Symposium est une corporation sans but lucratif, dédié à l'organisation et à la coordination des séminaires de formation, de forums et au développement d'opportunités interdisciplinaires pour les chercheurs internationaux impliqués dans le développement de technologies dans le secteur des sciences de la vie.

AGENDA - DAY 1

Sunday, May 8, 2011 **Fairmont Banff Springs Hotel, Banff, AB**	
"Hand-on" Workshop on Quantitative Proteomics and Data Mining Organized by Dr. Christoph Borchers, UVic Genome BC Proteomics Centre **Sponsored by Agilent Technologies** **Ivor Petrak Room**	
7:00 am	**Registration; Coffee**
7:30 am	Welcome & Opening Remarks
	Lecture: Introduction and Theory of MRM-based Protein Assays
	Lecture: Software tool for labeling and label-free quantifications Presented by Bin Ma, University of Waterloo
	Lecture and "hands-on": ○ LC-MS/MS in MRM mode ○ Internal isotopic standard peptides ○ Assay development ▪ Selection of target peptides ▪ Synthesis of standard peptides ▪ Optimization of MS parameters ▪ Sample preparation ▪ Validation of specificity ▪ Balancing of endogenous to standard peptide ▪ Determining limit of detection/quantitation ▪ Determining standard curves & dynamic range ▪ Determining %CV ▪ Multiplexing ○ Data handling and interpretation ○ Analysis of Plasma samples using a MRM-assay for 70 proteins using nano and normal flow LC
12:00	Workshop Ends

Sunday, May 8, 2011 Fairmont Banff Springs Hotel, Banff, AB	
JOB FAIR **Norquay Room**	
12:00 pm	**Job Fair Opens**
12:30 pm	Welcome & Opening Remarks
	A 2-hour Job Fair will be held from noon-2PM on Sunday, May 8, in the Norquay Room at the Banff Springs Hotel. Lunch will be served for registered job-fair participants only. There will be "positions available" and "positions wanted" bulletin boards where resumes and job descriptions can be posted. There will also be confidential sign-up sheets with contact information so that prospective employers can select times for interviews with candidates during the Job Fair, or during the rest of the meeting. Students and other candidates should bring ~10 print copies of their resumes for distribution to prospective employers. In addition, please bring an electronic copy with you, or email a pdf version to carol@proteincentre.com. There will also be facilities set up so that both employers and candidates can give short informal presentations during the job fair. We have allotted 15 min time slots for the employer presentations, and 5 min time slots for each student presentation. If you would like to give a presentation at the job fair, please send an email to carol@proteincentre.com.
2:00 pm	Job Fair Ends

AGENDA - DAY 1

Sunday, May 8, 2011
Fairmont Banff Springs Hotel, Banff, AB

Structural Proteomics Symposium
Sponsored by BC Proteomics Network
Ivor Petrak Room

12:00 pm	**Registration**	
1:00 pm	Welcome & Opening Remarks	
1:05 pm	*Introduction to Structural Proteomics*	**Juergen Kast** University of British Columbia
1:15 pm	*Challenges in top-down analysis of protein complexes*	**Catherine E. Costello**, Boston University School of Medicine
1:45 pm	*Towards a High Throughput Structural Genomics Platform Based on Ion Mobility-Mass Spectrometry*	**Brandon Ruotolo**, University of Michigan
2:15 pm	*Folding of Globular and Membrane Proteins Studied by Oxidative Labeling and Mass Spectrometry*	**Lars Konermann**, University of Western Ontario
TRAINEE SESSION		
2:45 pm	*Structural Interrogation of the Transferrin Receptor Complex from Actinobacillus*	**Leslie Silva** University of Calgary
3:00 pm	*Using Proteinase K for Non-Specific Digestion and Comprehensive Identification of Interpeptide Crosslinks: Application to Prion Proteins*	**Jason Serpa,** University of Victoria
3:15 pm	*Coffee Break*	*Coffee/Tea served in the foyer*
3:30 pm	*Informatic Approahces for Detecting Chemically Crosslinked Peptides*	**David Goodlett**, University of Washington
4:00 pm	*Enabling high-throughput identification of tandem mass spectra from cross-linked peptides*	**Nuno Bandeira** University of California in San Diego
4:30 pm	*TBA*	**David Wishart,** University of Alberta
5:00 pm	*General Discussion, Closing Remarks*	

AGENDA - DAY 1

Sunday, May 8, 2011
Fairmont Banff Springs Hotel, Banff, AB

CANADIAN NATIONAL PROTEOMICS NETWORK
Annual General Meeting - Members
Ivor Petrak Room

6:00 pm	**Annual General Meeting Starts**	
6:00 pm	Welcome & Opening Remarks	Christoph Borchers, President
6:05 pm	Report by Board of Directors	K.W. Michael Siu, Chairman
6:10 pm	Report from Treasurer	John Marshall, Treasurer
6:15 pm	Business Items requiring voting	Membership
6:30 pm	Annual General Meeting Ends	

OPENING RECEPTION
Garden Terrace / Mt. Stephen Hall

6:30 pm	**Opening Reception**
8:30 pm	Reception Ends

AGENDA - DAY 2

Monday, May 9, 2011 **Fairmont Banff Springs Hotel, Banff, AB**		
Canadian National Proteomics Network Symposium		
7:00 am	Registration, Coffee with Exhibits Poster Set Up	Riverview Lounge & New Brunswick Room
8:00 am Conference Begins **ALBERTA ROOM**		
8:00 am	Welcome, Opening Remarks	**Dr. Liang Li**,
Plenary Lecture		
8:10 am	*Animal models of human disease: what can metabolomics in worms and yeast tell us about mitochondrial disease?*	**Dr. Brian Sykes,** University of Alberta
9:00 am Proteomics in Biology, Chair: Professor John Wilkins		
Keynote		
9:00 am	*Systems-level biological analysis using data-integrated computational models*	**Dr. Stephen Fong**, Virginia Commonwealth University
9:30 am	*An integrated genomic, proteomic, and biochemical analysis of (+)-3-carene biosynthesis in Sitka spruce genotypes which are resistant or susceptible to white pine weevil*	**Dr. Joerg Bohlmann,** University of British Columbia
9:50 am	*Chemical and Electrical Guidance of Immune Cell Migration*	**Dr. Francis Lin** University of Manitoba
10:10 am	*Functional Genomic and Proteomic approaches to the study of lymphocyte migration*	**Dr. John Wilkins** University of Manitoba
10:30 am Coffee Break - Exhibits, Riverview Lounge **Poster Session: Proteomics in Biology, New Brunswick Room**		
Keynote		
10:50 am	*Signalling networks dynamics: a targeted proteomics view of GRB2-mediated events*	**Dr. Nicolas Bisson** Samuel Lunenfeld Research Institute
11:20 am	*Metabolomics as a platform for high-throughput biomarker discovery in cancer*	**Dr. Aalim Weljie,** University of Calgary
11:40 am	*A systems biology approach to understanding organelle biogenesis: a voyage from gene transcription to organelle inheritance*	**Dr. Richard Rachubinski,** University of Alberta

AGENDA - DAY 2

Monday, May 9, 2011 Fairmont Banff Springs Hotel, Banff, AB		
Canadian National Proteomics Network Symposium		
12:00 pm	*Identification and characterization of intact proteins in complex mixtures using online fragmentation on an Orbitrap mass spectrometer*	**Dr. Shannon Eliuk** Thermo Fisher Scientific
12:10 pm	*Metabolomic Investigation into the Biofilm and Planktonic Response to Copper Stress*	**Dr. Sean Booth** University of Calgary
12:20 to 2:00 pm - Tech Talks - Alberta Room Lunch with Exhibits - Riverview Lounge		
1:30 pm	*Applying High Sensitivity LC/MS and Automated Liquid Handling Technology to Peptide Quantitation*	**Shane Tichy, Ph.D.**, Agilent Technologies
1:45 pm	*Ultra-High Performance Nano LC for Analysis of Complex Proteomic Samples*	**Gurmil Gendeh** Dionex
2:00 pm Proteomics at the Interface of Biology and Medicine, Chair: Professor David Schriemer		
Keynote		
2:00 pm	*Analysis of nuclear receptor and GPCR signaling pathways with differential HDX MS*	**Dr. Patrick Griffin** The Scripps Research Institute
2:30 pm	*Building a Structural Understanding of DNA Damage Repair – a Mass Shift Analysis of DNA Ligase IV Regulation*	**Dr. Martial Rey** University of Calgary
2:50 pm	*Identification of an RNA helicase as a global regulator of gene expression through iTRAQ analysis of the proteome of the Lyme disease spirochete*	**Dr. George Chaconas** University of Calgary
Award Winner - Southern Alberta Cancer Research Institute sponsored trainee		
3:10 pm	*How does increased tRNA synthesis drive growth? An unbiased proteomic and metabolomic approach*	**Ms. Elizabeth Rideout** University of Calgary
3:30 pm Coffee Break - Exhibits, Riverview Lounge Poster Session: Proteomics at the Interface of Biology and Medicine - New Brunswick Room		

AGENDA - DAY 2

Monday, May 9, 2011 **Fairmont Banff Springs Hotel, Banff, AB**		
Canadian National Proteomics Network Symposium		
	Keynote	
3:50 pm	*Recent advances in the use of activity based probes for biomarker discovery, target validation, drug screening and molecular imaging*	**Dr. Matthew Bogyo** Stanford University
4:10 pm	*Industrial scale biomarker verification/validation through process automation*	**Dr. Konstantinos Petritis** TGen
4:30 pm	*Methylglyoxal-modified proteins and their role in metabolic syndrome*	**Dr. Andrew Ross** Fisheries and Oceans
4:50 pm	*Phosphatase editing defines functionally important phosphorylation sites*	**Evgeny Kanshin** IRIC
5:10 pm - Closing Remarks - Day 1		
Free Time		
8:30 pm - Poster Session - All; Wine & Beer **New Brunswick Room**		

AGENDA - DAY 3

Tuesday, May 10, 2011 Fairmont Banff Springs Hotel, Banff, AB		
Canadian National Proteomics Network Symposium		
7:00 am	Registration, Coffee with Exhibits	
8:00 am Conference Begins - Alberta Room		
8:00 am	Welcome, Opening Remarks	**Dr. Liang Li** University of Alberta
Plenary Lecture		
8:05 am	*Clinical Proteomics: Translation of biomarker discovery into clinical practice*	**Dr. Daniel W. Chan** Johns Hopkins Medical Institutions
9:00 am Proteomics for Clinical Applications, Chair: Professor K.W. Michael Siu		
Keynote		
9:00 am	*Plasma protein biomarkers for assessment of risk for common diseases*	**Dr. Samir M. Hanash** Fred Hutchinson Cancer Research Centre
9:30 am	*Clinical Applications of Selected Reaction Monitoring-Based Mass Spectrometry Assays*	**Dr. Vathany Kulasingam** University Health Network
9:50 am	*Quantitative statistical analysis of blood proteins by liquid chromatography, electrospray ionization, and tandem mass spectrometry*	**Dr. John Marshall** Ryerson University
10:10 am	*Digging Deep into the Proteome to Enable Discovery of Potential Disease Biomarkers*	**Dr. Liang Li** University of Alberta

AGENDA - DAY 3

Tuesday, May 10, 2011 **Fairmont Banff Springs Hotel, Banff, AB**		
Canadian National Proteomics Network Symposium		
10:15 am Coffee Break - Exhibits, Riverview Lounge **Poster Session: Proteomics for Clinical Application - New Brunswick Room**		
10:50 am	*Diagnostic, Prognostic and Therapeutic Significance of Head and Neck Cancer Biomarkers Discovered by Mass-Spectrometry Based Proteomics*	**Dr. K.W. Michael Siu** York University
11:20 am	*Can we distill value for clinical management from the human proteome?*	**Gabriela Cohen Freue** PROOF Centre of Excellence
11:40 am	*Measurement of protein C inhibitor (PCI) in human plasma by a SISCAPA-MALDI (sMALDI) technique: a prospective test for monitoring prostate cancer*	**Morteza Razavi** University of Victoria
12:00 pm	*De Novo Sequencing of Minor Histocompatibility Antigens*	**Waffa Yahyaoui,** IRIC
12:20 pm	*Closing Remarks*	
12:30 to 2:00 pm - Tech Talks - Alberta Room **Lunch with Exhibits - Riverview Lounge**		
1:30 pm	*Tech Talk #1*	Bruker Daltonics
1:45 pm	*Complex Mixture? Check. Low Sample Concentration? Check. PTMs? Check. PPH (proteins per hour)? Smokin'*	**Brenda Kesler** Thermo Scientific

JOINT CNPN-ETP 2011 SYMPOSIUM
Banff, AB, Canada

AGENDA - DAY 3

Tuesday, May 10, 2011 **Fairmont Banff Springs Hotel, Banff, AB**		
5TH INTERNATIONAL SYMPOSIUM ON ENABLING TECHNOLOGIES FOR PROTEOMICS		
2:00 PM Conference Begins - Alberta Room		
2:00 pm	*Welcome, Opening Remarks*	**Dr. Randy Johnston**, University of Calgary
Technology Development		
2:15 pm	*Molecular Mass—A Universal Chromophore for Proteomics*	**Dr. Alan Marshall** Florida State University
2:45 pm	*High Performance Separations and their Importance for the Mass Spectrometric Characterization of Proteomes*	**Dr. Richard D. Smith** Pacific Northwest National Laboratory Sponsored by Agilent Technologies
3:15 pm	*Exploration of plasma protein profiles with next generation affinity arrays*	**Dr. Jochen M. Schwenk** School of Biotechnology, KTH
3:45 pm Coffee Break - Exhibits, Riverview Lounge **Poster Session: Proteomics Technology and Other Omics - New Brunswick Room**		
Novel 'Omics Technology		
4:15 pm	*New approaches to glycomic analysis*	**Dr. Catherine Costello** Boston University School of Medicine
Ken Standing Award Winner Presentation		
4:45 pm	*Proteomics and lipidomics technologies to study human diseases*	**Dr. Daniel Figeys** University of Ottawa
5:15 pm - Closing Remarks - Day 3		
6:30 pm Reception - Riverview Lounge & **7:00 pm - Award Dinner & Presentations - Cascade Ballroom**		
6:30 pm	*Reception*	**Riverview Lounge with Exhibitors**
7:00 pm	*Dinner*	**Cascade Ballroom**
8:00 pm	*Award Presentations; Featuring Dr. Jack Greenblatt Winner of CNPN Distinguished Contributions in Proteomics*	**Cascade Ballroom**
End of Day 3		

AGENDA - DAY 4

Wednesday, May 11, 2011 Fairmont Banff Springs Hotel, Banff, AB		
5TH INTERNATIONAL SYMPOSIUM ON ENABLING TECHNOLOGIES FOR PROTEOMICS		
8:00 am	Registration, Coffee with Exhibits	Riverview Lounge
8:45 AM Conference Begins - Alberta Room		
8:45 am	Welcome, Opening Remarks	**Dr. Randy Johnston**, University of Calgary
Proteomics and Informatics		
9:00 am	*The Diagnostic Proteome: Challenges and Opportunities in the Discovery and Clinical Implementation of Protein Biomarkers*	**Dr. N. Leigh Anderson** Plasma Proteome Institute
9:30 am	*Using Mass Spectrometry to Understand Protein Misfolding Diseases*	**Dr. John Yates** The Scripps Research Institute
10:00 am	*Peptide De Novo Sequencing when There Is a Sequence Database*	**Dr. Bin Ma**, University of Waterloo
10:30 am Coffee Break - Exhibits, Riverview Lounge		
Higher Order Structural Analysis		
11:00 am	*Protein Structure and Function Studied by Hydrogen Exchange Mass Spectrometry*	**Dr. Lars Konermann** University of Western Ontario
11:30 am	*Unravelling biomolecular assembly pathways by mass spectrometry*	**Dr. Alison Ashcroft** University of Leeds, UK
12:00 - Closing Remarks - ETP Conference Ends		

AGENDA - DAY 4

Wednesday, May 11, 2011 **Fairmont Banff Springs Hotel, Banff, AB**		
Symposium on the Canadian Human Proteome Project		
2:00 pm - 5:00 pm - Alberta Room		
2:00 pm	Welcome, Opening Remarks	
	A separate program is being prepared for this session.	
5:00 pm - Closing Remarks - Day 4		

Please note that the Agenda is subject to change.

Plenary Lecture

Brian Sykes,
University of Alberta

Biography:

Dr. Brian Sykes obtained his B.Sc. in Chemistry at the University of Alberta and Ph.D. in Physical Chemistry from Stanford University, California. Before coming to Alberta, he was Associate Professor of Chemistry at Harvard University. At Alberta, he has been Chair of the Department of Biochemistry, holder of a Canada Research Chair in Structural Biology, Director of the Canadian Institutes of Health Research Group on Protein Structure and Function, Director of the Alberta Regional Centre of the Protein Engineering Network of Centres of Excellence (PENCE), and Director of the National High Field Nuclear Magnetic Resonance Facility (NANUC).

His research is in the area of biophysical studies of protein structure and function, especially the use of high resolution nuclear magnetic resonance spectroscopy to study protein structure, dynamics and biomolecular interactions. He has applied NMR spectroscopic techniques in conjunction with other biophysical approaches to a wide variety of biological systems. The overall focus of the research can be summarized as biomacromolecular recognition; that is, unraveling the structure, dynamics, and kinetics that contribute to the mechanisms, affinity and specificity of recognition and interaction in biological systems. These include enzymatic mechanism, protein structure and interactions, protein dynamics, NMR chemical shift methods, muscle and calcium regulatory proteins, chemokine structure and function, and antifreeze proteins. Present research is focused on cardiovascular function and disease through elucidation of the molecular mechanism of the thin filament based regulation of contraction in cardiac muscle.

Over the years Dr. Sykes has published over 480 scientific papers, and has trained numerous Undergraduate, Graduate Students and Postdoctoral Fellows.

Presentation:

Animal models of human disease: what can metabolomics in worms and yeast tell us about mitochondrial disease?

S.N. Reinke, S.W. Szeto, B.D. Lemire, and B.D. Sykes

Metabolomics has proven to be a powerful tool in identifying and understanding the connection between disease origin and pathology. Metabolic studies can examine both intracellular and extracellular molecules, known as metabolic fingerprinting and footprinting, respectively. Metabolomics can be applied to many biofluids or tissues. These applications will be reviewed.

The metabolome is extremely sensitive to both internal factors, such as genome and microbiome, and external factors, such as diet, exercise, and chemical exposure. It is therefore difficult develop a mechanistic understanding of a disease, using metabolomics, in the human population. Utilizing model systems provides the advantage of maintaining greater experimental control. The yeast and nematode model systems provide the added advantage of being isogenic.

Mitochondrial disease provides a unique challenge in its diagnoses, understanding, and treatment due to the multi-factorial nature of its pathology. Included in these factors is the central role mitochondria play in metabolic processes. Shifts in the delicate balance of energy metabolism can have catastrophic effects. Using the yeast and nematode model systems, we have established that energy metabolism and nitrogen metabolism are intricately linked. Upon examining the metabolic footprint of yeast with mitochondrial respiratory chain (MRC) mutations, we discovered that metabotypes could be used to discriminate between the different mutants, including those phenotypically indistinguishable. We also found a high degree of correlation between metabotype and growth yield of the various mutants. Using these two model systems, we have gained some insight into how mitochondrial disease affects metabolism.

Plenary Lecture

Daniel Chan,
Johns Hopkins Medical Institutions

Biography:

The focus of Dr. Chan's research is the development and application of proteomic and immunologic techniques in the diagnosis, management, and understanding of cancer. His work has demonstrated the importance of analytical methodologies for tumor markers, endocrine or clinical chemistry assays to determine the clinical outcomes of a patient's disease process. He is an internationally recognized expert in immunoassay, clinical proteomics, and biochemical tumor markers, particularly prostate, breast and ovarian cancer biomarkers. He has edited and written five books on immunoassay, immunoassay automation, diagnostic endocrinology, and tumor markers. Dr. Chan has published over 200 articles including the tumor markers chapters in the Tietz Textbook of Clinical Chemistry and in Tumor Markers: Physiology, Pathobiology, Technology, and Clinical Applications, which he also co-edited. Dr. Chan is actively involved in the Human Proteome Organization (HUPO) and its Plasma Proteome Initiative. He serves as chairman of a HUPO committee responsible for the development of reference specimens for a global comprehensive study of the human proteome.

Presentation:

Clinical Proteomics: Translation of biomarker discovery into clinical practice

During the last decade of proteomics research, significant progress has been made in our understanding of the human proteome. Many potential biomarkers have been discovered. However, little progress has been achieved in the translation of these biomarkers into clinical practice. Most human diseases, such as cancer, are often diagnosed in the late stages when the chance of cure is relatively low. What we need is to detect aggressive cancer in early stages. Proteomic biomarkers offer the best opportunity for making significant impacts in the war against cancer.

In my presentation, I will discuss our strategies from cancer biomarker discovery to translation. Since glycosylation is one of the most important modifications to extracellular proteins, we believe that glycan modifications of glycoproteins could serve as an example for biomarker discovery. There are four key steps for biomarker translation. First, we need to define clearly a specific clinical “intended use” (for unmet clinical needs). Second, we need to generate sufficient evidence in preliminary studies to support the investment for a large-scale validation study. Third, we need to select and develop assays with analytical performance suitable for clinical laboratory. Finally, we need to conduct clinical trial to demonstrate clinical utilities in order to obtain regulatory approval and gain acceptance by the clinical community. Specific examples will be shown to demonstrate the opportunities and challenges for the development of clinical proteomic diagnostics. The successful translation of these cancer biomarkers into clinical practice will require close collaboration between researcher and clinician.

Proteomics in Biology Keynote Speaker

Nicolas Bisson,
Samuel Lunenfeld Research Institute

Biography:

Dr Nicolas Bisson received his Ph.D. in Molecular and Cellular Biology from the Université Laval in Quebec City in 2007. He is the recipient of Canadian Institutes for Health Research (CIHR), National Cancer Institute of Canada (NCIC) and National Sciences and Engineering Research Council (NSERC) research fellowships, as well as the Max M. Burger Endowed Scholarship and the Thomas and Elizabeth Grave Scholarship. He was awarded the Hans-Selye Award of the Quebec Clinical Research Association and the Bernard-Belleau Award of the French Association for Knowledge (ACFAS). Dr Bisson is an alumnus of the Marine Biological Laboratory in Woods Hole, MA. He currently is a post-doctoral fellow in the laboratory of Dr Tony Pawson at the Samuel Lunenfeld Research Institute in Toronto where he is investigating the coordination of signalling networks by adaptor proteins using a combination of mouse genetics, molecular biology and targeted proteomics.

Presentation:

Signalling networks dynamics: a targeted proteomics view of GRB2-mediated events

Signalling pathways are commonly organized through inducible protein-protein interactions, mediated by adaptor proteins that link activated receptors to cytoplasmic effectors. However, we have little quantitative data regarding the kinetics with which such networks assemble and dissolve to generate a specific cellular response. We have identified 90 proteins and 36 phosphorylation sites associated with the GRB2 adaptor protein in human cells. We have found that GRB2 nucleates a remarkably diverse set of protein complexes, involved in multiple aspects of cellular function. To comprehensively and quantitatively investigate changes in GRB2-based protein interactions in growth factor stimulated cells, we have designed a targeted mass spectrometry method, AP-SRM (affinity purification-selected reaction monitoring). The data define context-specific and time-dependent networks that form around GRB2 following stimulation, and reveal core and growth factor-selective interaction subsets. These results illustrate the reliability of AP-SRM in the quantitative analysis of dynamic signalling networks. They also suggest that capturing a key hub protein and dissecting its interactions by SRM is an approach that can be broadly applied to quantify signalling dynamics.

Stephen Fong,
Virginia Commonwealth University

Biography:

Stephen Fong received his Ph.D. in Bioengineering from the University of California, San Diego in 2004. Dr. Fong's undergraduate degree is in Chemical Engineering with a minor in Biomedical Engineering from Worcester Polytechnic Institute (WPI). Dr. Fong joined the Department of Chemical and Life Science Engineering at Virginia Commonwealth University in the Fall of 2005 and is currently an Assistant Professor and Associate Department Chair.

Presentation:

Systems-level biological analysis using data-integrated computational models

The desire to gain a better understanding of systems-level function in biology has concurrently spurred the development of experimental and computational systems biology tools. A potentially effective approach to studying biological systems is through integration of genome-scale experimental data sets and constraint-based computational models. In a computational-experimental analysis paradigm, the computational model can provide detailed predictions for a breadth of cellular functions and experimental data can inform model predictions to align with in vivo reality. In this talk, the framework for a computational-experimental systems biology approach will be discussed starting with the formulation of constraint-based models and associated algorithmic approaches to data integration. Biofuel and health-related examples will be demonstrated where model integration of transcriptomic and/or proteomic data were used to elucidate system function.

Proteomics in Biology Invited Speaker

Joerg Bohlmann,
University of British Columbia

Biography:

Dr. Jörg Bohlmann is a Professor and Distinguished University Scholar in the Michael Smith Laboratories at the University of British Columbia, Vancouver, Canada (www.michaelsmith.ubc.ca/faculty/bohlmann/). He received his Ph.D. in 1995 from the Technical University of Braunschweig, Germany, did postdoctoral studies as a Feodor Lynen Fellow of the Alexander von Humboldt Foundation at Washington State University, USA (1995-1998) and was a research associate at the Max Planck Institute for Chemical Ecology, Germany (1998-2000). In 2000 he joined the UBC Michael Smith Laboratories (formerly Biotechnology Laboratories) as Assistant Professor, was promoted to Associate Professor in 2004, and to Full Professor in 2008. He also has appointments in the Department of Botany, Department of Forest Sciences, and is an associate of the UBC Wine Research Centre. Dr. Bohlmann's research deals with the biochemistry of terpenoid natural products, plant defense against insets, and functional genomics of conifers, poplars, grapevines and medicinal plants.

His research is funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) and other sources. He has been the project leader of four large-scale genomics projects (Treenomix I and II, www.treenomix.ca/; Tria I and II, http://thetriaproject.ca/) funded by Genome Canada, Genome British Columbia and Genome Alberta. He is co-PI on two other Genome Canada projects on Grape and Wine Genomics and Synthetic Biology of Natural Products. Dr. Bohlmann has published more than 100 peer reviewed papers and has given more than 130 invited and conference talks. He has received several national and international awards and distinctions including the C.D. Nelson Award of the Canadian Society of Plant Physiologists, the Charles A. McDowell Award for Excellence in Research awarded by UBC, the E.W.R. Steacie Memorial Fellowship of the Natural Sciences and Engineering Research Council of Canada; he is an elected Fellow of the American Association for the Advancement of Science (AAAS).

Presentation:

An integrated genomic, proteomic, and biochemical analysis of (+)-3-carene biosynthesis in Sitka spruce genotypes which are resistant or susceptible to white pine weevil

Dawn E. Hall1, Jeanne A. Robert1, Christopher I. Keeling1, Dominik Domanski2, Alfonso Lara Quesada1, Sharon Jancsik1, Michael A. Kuzyk2, Britta Hamberger1, Christoph H. Borchers2,3 and Jörg Bohlmann1

1 Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada
2 University of Victoria – Genome BC Proteomics Centre, Victoria, BC, Canada
3 Department of Biochemistry, University of Victoria, Victoria, BC, Canada

Conifers are extremely long-lived plants which have evolved complex chemical defenses in the form of oleoresin terpenoids to resist attack from pathogens and herbivores. Terpenoid diversity is determined by the size and composition of the terpene synthase (TPS) gene family, and the single- and multi-product profiles of these enzymes. The monoterpene (+)-3-carene is associated with resistance of Sitka spruce (Picea sitchensis) to white pine weevil (Pissodes strobi). We used a combined genomic, proteomic and biochemical approach to analyze the (+)-3-carene phenotype in two contrasting Sitka spruce genotypes. Resistant trees produced significantly higher levels of (+)-3-carene than susceptible trees, in which only trace amounts were detected. Biosynthesis of (+)-3-carene is controlled, at the genome level, by a small family of closely related (82-95% amino acid sequence identity) (+)-3-carene synthase (PsTPS-3car) genes. Transcript profiling identified one PsTPS-3car gene (PsTPS-3car1) which is expressed in both genotypes, one gene (PsTPS-3car2) expressed only in resistant trees, and one gene (PsTPS-3car3) expressed only in susceptible trees. The PsTPS-3car2 gene was not detected in genomic DNA of susceptible trees. Target-specific selected reaction monitoring substantiated this pattern of differential expression of members of the PsTPS-3car family on the proteome level. Kinetic characterization of the recombinant PsTPS-3car enzymes identified differences in the activities of PsTPS-3car2 and PsTPS-3car3 as a factor for the different (+)-3-carene profiles of resistant and susceptible trees. In conclusion, variation of the (+)-3-carene phenotype is controlled by PsTPS-3car gene copy number variation, variation of gene and protein expression, and variation of catalytic efficiencies.

Hall DE, Robert JA, Keeling CI, Domanski D, Qesada AL, Jancsik S, Kuzyk M, Hamberger Br, Borchers CH, and J Bohlmann (2011) An integrated genomic, proteomic, and biochemical analysis of (+)-3-carene biosynthesis in Sitka spruce (Picea sitchensis) genotypes which are resistant or susceptible to white pine weevil. The Plant Journal, in press.

Proteomics in Biology Invited Speaker

Francis Lin,
University of Manitoba

Biography:

Francis Lin obtained his Ph.D. in Physics in 2004 from the University of California, Irvine. He then received his postdoc training at Stanford University School of Medicine from 2005 to 2008. He joined the University of Manitoba as an Assistant Professor in the Department of Physics and Astronomy in December 2008. Prior to his Ph.D. study, Francis obtained his B.S. in Applied Physics from Beijing University of Aeronautics and Astronautics, China, and his M.S. in Physics from California State University, Fullerton. Francis' research interest is in applying biophysical and bioengineering approaches together with biological and immunological methods to understanding immune cell trafficking in complex tissue environments.

Presentation:

Chemical and Electrical Guidance of Immune Cell Migration

Cell migration is important for many fundamental biological and physiological processes such as host defense, wound healing and cancer metastasis. Particularly, immune cell migration critically mediates tissue-specific cell trafficking and immune responses. Such migratory behaviors of immune cells are guided by various environmental cues such as chemical gradients and electric fields and these guiding mechanisms are complex. Combinations of microfluidics-based experimentation and mathematical modeling enable quantitative studies of immune cell migration in complex cellular guiding environments. In this talk, I will highlight a few our recent research on investigating the guiding mechanisms for immune cell migration using such combined approaches. In the first study, we explored the receptor desensitization mechanism for the multi-step chemotactic navigation model of cells in complex chemoattractant gradient fields. In the second study, we showed a novel combinatorial guiding mechanism by CCR7-ligands for leukocyte migration and trafficking in co-existing chemokine fields. Lastly, we investigated T cell migration directed by direct current (DC) electric fields and its potential underlying cellular mechanisms.

Richard Rachubinski,
University of Calgary

Biography:

Dr. Richard Rachubinski is Distinguished University Professor and Chair of the Department of Cell Biology at the University of Alberta. After receiving a Ph.D. in cell biology in the laboratory of Dr. John Bergeron at McGill University in 1980, he pursued postdoctoral research at McGill with Dr. Gordon Shore and then at The Rockefeller University in New York with Dr. Paul Lazarow, where he began his continuing interest in peroxisome biogenesis and function. He was Assistant, Associate, and Full Professor in the Department of Biochemistry at McMaster University before assuming his current position at the University of Alberta in 1993. In 2001, he was named Canada Research Chair in Cell Biology and Senior Investigator of the Canadian Institutes of Health Research. In 2002, he was appointed a fellow of the Royal Society of Canada. He has been an International Research Scholar of the Howard Hughes Medical Institute since 1997. His work centers on defining the molecular pathways controlling the biogenesis of the peroxisome, a cellular organelle that performs a variety of important biochemical functions, notably in lipid metabolism and reactive oxygen species detoxification.

Presentation:

A systems biology approach to understanding organelle biogenesis: a voyage from gene transcription to organelle inheritance

Richard A. Rachubinski, Department of Cell Biology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

John D. Aitchison, Institute for Systems Biology, Seattle, Washington 98103, USA

The peroxisome is an ubiquitous organelle that compartmentalizes a variety of important biochemical functions, notably the oxidation of fats and the inactivation of reactive oxygen species. The requirement for functional peroxisomes is underscored by the lethality of a group of inherited peroxisomal disorders, collectively called the peroxisome biogenesis disorders, in which peroxisomes fail to assemble. One of the exceptional properties of peroxisomes is their highly dynamic nature with respect to changing environmental conditions.

Proteomics in Biology Invited Speaker

We have undertaken a systems biology approach using baker's yeast, Saccharomyces cerevisiae, to achieve a global and predictive understanding of peroxisome assembly, function and dynamics. In our practice of systems biology, we aim to enumerate and quantify all relevant molecular constituents and their interactions involved in the global response governing peroxisome formation; computationally integrate different data types; mathematically model our biological system; and test predictions arising from our systems biology analysis of peroxisomes. Our systems biology approach has centered on 1) transcriptional networks, with a focus on parallel combinatorial control to control timing of the transcriptional response with respect to changes in the environment; 2) quantitative proteomics to evaluate the enrichment of proteins in peroxisomes and define bona fide time- and condition-specific constituents of peroxisomes (proteins move); and 3) a comprehensive screen of the yeast gene deletion library expressing a fluorescently labeled peroxisomal protein to reveal the complexity of the peroxisome biogenic response through quantitative imaging and to identify novel aspects of, and players in, peroxisome biology. Our ultimate goal is a reliable and predictive model of the peroxisome with regard to its biogenesis, function and response in an ever changing environment.

This work was supported by grants from the Canadian Institutes of Health Research and the Howard Hughes Medical Institute to R.A.R. and the National Institutes of Health to J.D.A.

Aalim Weljie,
University of Calgary

Biography:

Dr. Weljie is currently the co-Director of the Metabolomics Research Centre at the University of Calgary, where his primary focus is developing a program in cancer metabolism using a combination of highly sensitive mass spectrometry methods and quantitative nuclear magnetic resonance spectroscopy. In addition to methods development, projects in Dr. Weljie's group are focused on i) elucidating the connection between energy and lipid metabolism and cancer, and ii) the impact of environmental toxicants on carcinogenesis and cellular proliferation. Dr. Weljie's group is also involved in several multidisciplinary clinical collaborations focusing on serum metabolomics in cancer. This program builds on his previous expertise in nuclear magnetic resonance metabolomics, which has been productive in both methods development and elucidating biological phenomena both at the University of Calgary, with Chenomx Inc, and at the University of Cambridge in the lab of Dr. Jules Griffin.

Presentation:

Metabolomics as a platform for high-throughput biomarker discovery in cancer

Metabolomics methods hold promise as a platform which is highly complementary to other systems biology tools such as proteomics, transcriptomics and genomics. The two main analytical platforms employed are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). These two platforms have unique advantages which suggest that acquiring data from both platforms would be advantageous. In this presentation, several examples of biomarkers derived from a combination of NMR and GC-MS in clinical cancer studies will be presented, highlighting the relative advantages of each analytical platform. In this pattern-driven approach, techniques which are both quantitative and high-throughput are favoured, providing information on a 'biopattern' of disease as opposed to comprehensively attempting to characterize the entire metabolome. The resulting multivariate patterns are characterized by concerted changes in multiple markers, in contrast with traditional biomarker-driven approaches that rely on single markers. Finally, several challenges in the evolution of the field will be discussed, including interpreting coherent biological meaning from a combination of both NMR and MS data, and reliable assessment of candidate markers using multivariate statistics.

Proteomics in Biology Invited Speaker

John Wilkins
University of Manitoba

Biography:

John A Wilkins is a Professor in the Departments of Internal Medicine and Biochemistry and Medical Genetics at the University of Manitoba. He is Director of the Manitoba Centre for Proteomics and Systems Biology and the Director of Research at the Health Sciences Centre. His research interests include the application of high content proteomic and functionality approaches to understanding the molecular basis of lymphocyte migration and effector functions and to the study of human physiology and disease.

Presentation:

Functional Genomic and Proteomic approaches to the study of lymphocyte migration

The movement of cells is a critical aspect of the biology of multicellular organisms, playing central roles in development and tissue patterning, wound repair and host surveillance. Lymphocytes exhibit a particularly nomadic life style as they circulate through the tissues and lymphoid organs. This requires transitioning between migratory and sessile states in response to positional queues such as chemotactic stimuli. Our studies examine use proteomics to determine the composition of isolated leading edges of migrating lymphocytes. We have subsequently employed microscopic (live and fixed cell) gene silencing and interaction analysis to define the roles of different molecules in cell migration. The results (successes and failures) of these studies will be presented.

Proteomics in Biology
Oral Abstract Presentations

Shannon Eliuk
Thermo Fisher Scientific

Identification and characterization of intact proteins in complex mixtures using online fragmentation on an Orbitrap mass spectrometer

Contributing Authors:

Shannon Eliuk, John F. Kellie, Neil L. Kelleher, Vlad Zabrouskov

Characterization of intact proteins by mass spectrometry often uncovers biological variations necessary to understanding biological functions which are otherwise not easily discernable using standard proteomics practices involving protein digestion. This includes the ability to discern protein isoforms, localize combinations of post-translational modifications, and determine in vivo protease cleavage sites. By combining speed, sensitivity, high resolution and accurate mass capabilities of the Orbitrap system with ETD, CID and HCD fragmentation, we have improved the analysis of intact proteins in relatively complex mixtures on an LC timescale. In this study we evaluated a complete top down proteomics workflow from sample preparation to data analysis providing a robust and sensitive method for identification and characterization of proteins in a mixture.
Fractionation of yeast lysate by molecular weight was reproducibly performed on the Gelfree 8100 Fractionation System (Protein Discovery) allowing the collection of fractions up to 70 kDa. All samples were analyzed using an ion trap-Orbitrap hybrid mass spectrometer. A variety of sample preparation and instrument conditions were tested to optimize analysis parameters. ETD fragmentation regularly generates a greater frequency of protein backbone cleavages than conventional CID, particularly on proteins with higher charge density, enabling identification with higher confidence using ProsightPC 2.0. As a result, we were able to identify and more fully characterize nearly twice as many proteins in each fraction when fragmented by ETD compared with CID. Combining data acquired using ETD, CID and HCD improved fragment ion coverage and overall protein characterization.

Sean Booth
University of Calgary

Metabolomic Investigation into the Biofilm and Planktonic Response to Copper Stress

Heavy metals are highly toxic, and understanding this toxicity has important environmental implications as they are not only a pollutant themselves, but they also interfere with the biodegradation of organic xenobiotics. While the general effects of metal toxicity have been studied, the underlying mechanisms are not clear. Surface-bound assemblages of cells, called biofilms, are much more tolerant to toxic concentrations of heavy metals than free-swimming planktonic populations. In order to understand the mechanisms of metal toxicity and the increased tolerance of biofilms, we undertook a metabolomic investigation of these phenomena. Metabolomics is the identification and quantification of a cells' metabolites. By examining the changes of the metabolome of planktonic and biofilm cultures in response to copper, we hoped to garner an understanding of how metal toxicity affects the cell and whether biofilms respond differently. After multi-variate statistical analysis our data showed that the metabolic response to copper depends on the growth mode. The altered planktonic metabolites were in disparate metabolic pathways whereas those in the biofilms represented a concerted response to copper stress. No clear pathways were affected in the planktonic cells but several pathways related to oxidative stress were altered in the biofilms. This suggests that metabolism is a factor in the increased metal resistance seen in biofilms and that heavy metals exert their toxicity through different mechanisms in each culture type. Further work will elaborate on these findings using proteomic and genomic techniques to investigate how this toxicity affects xenobiotic metabolism.

Proteomics at the Interface of Biology and Medicine Keynote Speaker

Matthew Bogyo
Stanford University School of Medicine

Biography:

Dr. Bogyo received his Ph.D. in Chemistry from the Massachusetts Institute of Technology in 1997. After completion of his degree he was appointed as a Faculty Fellow in the Department of Biochemistry and Biophysics at the University of California, San Francisco. Dr. Bogyo served as the Head of Chemical Proteomics at Celera Genomics from 2001 to 2003 while maintaining an Adjunct Faculty appointment at UCSF. In the Summer of 2003 Dr. Bogyo joined the Department of Pathology at Stanford Medical School and was appointed as a faculty member in the Department of Microbiology and Immunology in 2004. His interests are focused on the use of chemistry to study the role of proteases in human disease. In particular his laboratory is currently working on understanding the role of cysteine proteases in tumorgenesis and also in the life cycle of the human parasites, Plasmodium falciparum and Toxoplasma gondii. Dr. Bogyo currently serves on the Editorial Board of Biochemical Journal, Chemistry and Biology. Molecular and Cellular Proteomics and Drug Discovery Today: Technologies. Dr. Bogyo is a consultant for several biotechnology and pharmaceutical companies in the Bay Area.

Presentation:

Recent advances in the use of activity based probes for biomarker discovery, target validation, drug screening and molecular imaging

Activity-based probes (ABPs) are small molecules that covalently modify target enzymes in an activity-dependent manor. In their most direct application to proteomic methods, probes can be added to complex proteomes in order to isolate proteins with specific enzymatic function. However, the past decade has seen a dramatic increase in both the total number of validated ABPs available for use as well as in the range of possible applications for these reagents. In this presentation, I will discuss recent advances in probe development as well as specific applications where ABPs have been used to validate enzymes in disease pathology, to screen for lead compounds using HTS methods and to image enzyme activities as a means to monitor disease progress or improve diagnosis.

Patrick R. Griffin,
The Scripps Research Institute**,**

Biography:

Patrick R. Griffin, Ph.D., is Professor and Chair of Molecular Therapeutics, and as Director of the Translational Research Institute, plays a critical role in Scripps Florida's Discovery Pipeline. As a graduate student at the University of Virginia, Dr. Griffin was involved in ground-breaking work in biological mass spectrometry. After a postdoctoral fellowship at Caltech with Lee Hood and an appointment at Genentech, he joined Merck Research Laboratories as a biochemist working on inflammation, infectious diseases, and metabolic disorders. Later, as Merck's Senior Director of Chemistry, he led a team of over 40 scientists focused on drug discovery, resulting in the development of an investigational drug for treatment of pulmonary disease, and sitagliptin (MK-0431), an FDA-approved oral medication for the management of Type 2 diabetes. In 2002, Dr. Griffin left Merck to head a biotech startup, ExSAR Corp. before his recruitment to The Scripps Research Institute in 2004 as Professor of Biochemistry. In June 2006, he was named Director of the Translational Research Institute which focuses on drug discovery in the areas of Parkinson's disease, glaucoma, metabolic disorders, cancer, autoimmune disorders, and drug addiction. In March 2007, Dr. Griffin was named Professor and Chair of the Department of Molecular Therapeutics. Dr. Griffin's lab is focused on structure function analysis of nuclear receptors and G protein coupled receptors. Dr. Griffin also serves as Co-PI of The Scripps Research Institute Molecular Screening Center (SRIMSC), a NIH Roadmap funded program.

Presentation:

Hydrogen/deuterium exchange coupled with mass spectrometry (HDX-MS) has emerged as a powerful technology for analysis of protein conformational dynamics and ligand interactions

Proteomics at the Interface of Biology and Medicine Keynote Speaker

The regulation of transcriptional output by nuclear receptors (NRs) is driven by alterations in the conformational ensemble of the receptor upon ligand binding and previously we have shown that HDX can be used to determine a novel mechanism of ligand activation of PPARg, detailed analysis of binding modes of ligands within the ligand binding pocket of two ER isoforms, and how ERa ligands can be classified and correlated to their pharmacology based on receptor HDX signatures. More recently, we have applied HDX to probe the conformational dynamics of intact full length nuclear receptor complexes upon interaction with DNA and coactivator proteins. These studies have demonstrated that DNA binding alters conformational dynamics of the nuclear receptor heterodimer in regions remote of the DBD. These alterations in conformational selection appear to be important for coactivator binding to the heterodimer suggesting that DNA acts as an allosteric ligand. In addition to work on NRs, the lab recently demonstrated the use of HDX for probing ligand interaction with G protein coupled receptor (GPCRs) and kinases. We have extended these studies to probe differential receptor perturbation upon interacting with functionally selective ligands, and with kinases probed both ligand and co-regulatory protein interactions. Results from these studies will be presented.

Proteomics at the Interface of Biology and Medicine Invited Speakers

Martial Rey
University of Calgary

Biography:

Martial Rey is a post-doctoral fellow in the laboratories of Drs. Susan Lees-Miller and David Schriemer of the Southern Alberta Cancer Research Institute at the University of Calgary. He graduated in 2004 in Chemistry and Biology at the University of Grenoble in France. After a Masters in structural biology obtained in 2006 also at the University of Grenoble, he began a Ph.D. in the area of membrane protein analysis and mass spectrometry. During his Ph.D. he developed a new method to quickly remove detergent from a membrane protein digest in an H/D exchange-compatible manner (Rey et al., Anal Chem. 2010). This technique allowed the structural analysis by H/D exchange of an integral membrane α-helix protein, the ADP/ATP mitochondrial carrier in complex with inhibitors (Rey et al., J Biol Chem. 2010). In September 2010, he joined David Schriemer's laboratory and began a collaborative study with the Lees-Miller laboratory on the nonhomologous end-joining pathway using advanced methods in H/D exchange.

Presentation:

Building a Structural Understanding of DNA Damage Repair – a Mass Shift Analysis of DNA Ligase IV Regulation

DNA double-strand breaks are a form of DNA damage that, if unrepaired, could lead to tumorigenesis or cell death. They are induced by ionizing radiation (IR) or topoisomerase poisons but also occur naturally in the V(D)J recombination process. In human cells, the major mechanism for repairing IR-induced breaks involves the nonhomologous end-joining pathway. We are seeking to define the structural and functional properties of protein complexes involved in this pathway. Such an understanding may support a new approach to cancer treatment, by increasing the sensitivity of cancer cells to radiation treatment. To map the topography of damage-repair complexes, we combine conventional techniques in structural biology with newer techniques in data-directed structure building, involving SAXS and mass-shift mapping with H/D exchange methods. This presentation will review our progress in defining the structural features of DNA Ligase IV interactions, a protein which plays a key role in the final joining step of the pathway. Its activity is regulated by numerous additional proteins but in a poorly understood fashion. We present a mass-shift analysis of scaffolding proteins (XRCC4 and XLF) and the BRCT domain of DNA Ligase IV, and describe our findings in the context of available structural data and modeling efforts. Our data highlights a filamentous superstructure created by XRCC4-XLF interactions and strongly suggests a mechanism for phosphoregulation of DNA Ligase IV function.

George Chaconas
University of Calgary

Biography:

George Chaconas obtained his Ph.D. in the Division of Medical Biochemistry at the University of Calgary in 1978. This was followed by postdoctoral work at Cold Spring Harbor Laboratory in New York with the late Dr. Ahmad Bukhari, one of the international pioneers in the DNA transposition field. In 1981 George returned to Canada to take up a position as an Assistant Professor in the Department of Biochemistry at the University of Western Ontario. His laboratory focused on the molecular mechanism of Mu DNA transposition. In 2002 he took a position in the Department of Biochemistry & Molecular Biology and the Department of Microbiology & Infectious Diseases at the University of Calgary in Alberta, Canada.

In the 1999-2000 year George spent a sabbatical year in the laboratory of Dr. Patricia Rosa at the National Institute of Allergy and Infectious Diseases Rocky Mountain Labs in Hamilton, Montana, USA. This sabbatical, funded by a Guggenheim Fellowship, was the start of a new research interest on the Lyme disease spirochete.

Dr. Chaconas is currently holds the Canada Research Chair in the Molecular Biology of Lyme Borreliosis and is a Scientist of the Alberta Heritage Trust Fund for Medical Research.

Proteomics at the Interface of Biology and Medicine Invited Speakers

Presentation:

Identification of an RNA helicase as a global regulator of gene expression through iTRAQ analysis of the proteome of the Lyme disease spirochete

Aydan Salman-Dilgimen, Pierre-Olivier Hardy, Ashley Dresser and George Chaconas1
Departments of Biochemistry & Molecular Biology and Microbiology & Infectious Diseases, University of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta, T2N 4N1,

Spirochetes causing Lyme borreliosis are obligate parasites that can only be found in a tick vector or a vertebrate host. The ability to survive in these two disparate environments requires up and downregulation of specific genes by regulatory circuits which remain largely obscure. In this work on the Lyme spirochete, B. burgdorferi, we show that a disruption of the hrpA gene, which encodes a putative RNA helicase, results in a complete loss in the ability of the spirochetes to infect mice by needle inoculation. Studies of protein expression in culture by 2D gels revealed a change in the expression of 33 proteins in hrpA clones relative to the wild-type parent. Quantitative characterization of protein expression by iTRAQ analysis revealed a total of 187 differentially regulated proteins in an hrpA background: 90 downregulated and 97 upregulated. Sixty-eight of the 90 downregulated and 86 of the 97 upregulated proteins showed a different pattern of regulation in an hrpA mutant than they did in previously reported studies with rrp2, rpoN, rpoS or rrp1 mutants. Downregulated and upregulated proteins also fell into distinct functional categories. We conclude that HrpA-mediated regulation is a new and distinct global regulatory pathway in B. burgdorferi gene expression. Because an HrpA orthologue is present in most bacteria, its function as a global regulator in B. burgdorferi may have relevance in other bacterial species where its function remains obscure. A role for an RNA helicase as a global gene regulator is particularly timely with the recent growth of the field of RNA regulation of gene expression in bacteria.

Konstantions Petritis
TGen

Biography:

Dr. Petritis received his Ph.D. degree in Analytical Chemistry from the University of Orleans, in France in 2002. In April of 2002, Dr. Petritis joined the Biological Separations and Mass Spectrometry division at the Pacific Northwest National Laboratory as a post-doctoral fellow for two years followed by 5 years as a senior research scientist. In 2009, he joined the Translational Genomic Research Institute (TGen) as an associate professor and laboratory head of the Center for Proteomics. Dr. Petritis' research interests include high throughput, automated, proteomic sample preparation, development of mass spectrometry – based bioanalytical and bioinformatic methods for the characterization and relative/ absolute quantification of peptides and proteins and biomarker discovery, verification/validation.

Presentation:

Industrial scale biomarker verification/validation through process automation

Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA), which uses antibodies to capture proteotypic peptides from complex sample matrices and quantify them by Selected Reaction Monitoring-Mass Spectrometry (SRM-MS), provides the throughput, specificity, and sensitivity required for biomarker verification and validation. One disadvantage of the approach is that biological sample preparation is extensive and involves several critical steps that can affect assay reproducibility. The large number of samples (especially after considering biological replicates) that need to be prepared for biomarker verification/validation studies further undermines process reproducibility. To overcome this bottleneck, we have developed an ultra high-throughput automated proteomic sample preparation system capable of robust clinical sample preparation for mass spectrometry analysis. The platform can prepare ~1,000 samples in less than 24 hours in the SISCAPA workflow. The automated platform can receive samples in cryovials, aliquot them into 96 well-plates, denature, reduce, alkylate, and digest the proteins to peptides capture the peptide interest with anti-peptide antibodies, and elute those peptides into 96-well plates a format ready for mass spsectrometric analysis. The platform performs protein quantitiative (e.g. BCA assays) and calculates the amount of trypsin to be added to each sample based on the protein concentration. The system performs several QA/QC functions (e.g., assessment of trypsin activity, assessment of chemical stability, etc.).

Proteomics at the Interface of Biology and Medicine Oral Abstract Presentations

Evgeny Kanshin
IRIC

Phosphatase editing defines functionally important phosphorylation sites

Contributing Authors

Kanshin, Evgeny; Louis-Philippe Bergeron Sandoval; Michnick, Stephen;Thibault, Pierre

In eukaryotes, the reversible protein phosphorylation is a central regulatory mechanism. Recent evidences indicate that phosphorylation sites evolved slowly when associated with specific functions. However, the large repertoire of phosphorylation sites reported from yeast, mouse and human phosphoproteome studies suggests a varying degree of plasticity of protein phosphorylation where a large proportion of sites are neither conserved nor associated with known functions. We hypothesized that functional phosphorylation sites should be direct substrates of at least one kinase and phosphatase and not arise from fortuitous events. To gain further insights on the conservation of functionally relevant phosphorylation sites, we used quantitative phosphoproteomics to profile the changes in abundance of phosphorylation sites of control yeast cells and cells treated with FK506, a highly specific inhibitor of the protein serine/thereonine phosphatase calcineurin. Phosphopeptides were enriched using TiO2 prior to their 2DLC-MS/MS analysis on a LTQ-Orbitrap-Velos. Label-free quantitative proteomics enabled the identification and abundance profiling of 4091 phosphorylation sites (localization confidence >95%) on 1594 proteins with a false discovery rate less than 1%. A total of 315 sites showed statistically significant change in abundance in response to FK506 treatment, of which 225 sites were upregulated including sites on known substrates of FK506 - CRZ1 and FRT1. Analysis of evolutionary rates of phosphosites regulated by calcineurin showed increased conservation compared to proteins with random distribution of serine/threonine residues. Correlation between evolutionary rates and fold change increase in phosphorylation was also observed. Altogether, these analyses provide important insights into the mechanism of action of FK506.

Andrew Ross
Fisheries and Oceans

Methylglyoxal-modified proteins and their role in metabolic syndrome

Contributing Authoris

Tuanjie Chang, Xuming Jia, Rui Wang, Darrell Mousseau, Douglas Olson, Lingyun Wu

Like certain other sugar metabolites, methylglyoxal (MG) can react non-enzymatically with particular residues (lysine, arginine, cysteine) to form advanced glycation end-products, altering the structures and functions of susceptible proteins. Such modifications have been implicated in the development of insulin resistance and hypertension, both of which are associated with metabolic syndrome. The identification and structural characterization of MG-modified proteins can lead to a better understanding of the mechanisms involved in such diseases and, potentially, to the development of more effective treatments. We present two examples: insulin, for which MG modification reduces activity, leading to insulin resistance/type-2 diabetes; and Akt1, for which MG modification increases activity and hence, the proliferation of vascular smooth muscle cells. MALDI mass spectrometry and high-resolution tandem mass spectrometry were used to identify MG-modified proteins and component peptides, and to locate sites of MG attachment. By combining this information with the results of experiments designed to measure the expression of modified, unmodified and associated proteins in model systems (rats, cell cultures) we were able to establish the role of these modifications in altering the structure and function of insulin and Akt1 in vivo. Our findings suggest that proteome-wide analysis of MG-modified proteins (e.g. using immunoaffinity-based methods, and database searches incorporating appropriate fixed-mass modifications) may help to elucidate mechanisms, and identify therapeutic targets, for related diseases. They also illustrate the importance of post-translational proteomics in applying knowledge of the human proteome to the diagnosis and treatment of these and other medical conditions.

Proteomics at the Interface of Biology and Medicine
Oral Abstract Presentations

SOUTHERN ALBERTA CANCER RESEARCH INSTITUTE SPONSORED TRAINEE - AWARD WINNER

Elizabeth Rideout
University of Calgary

How does increased tRNA synthesis drive growth? An unbiased proteomic and metabolomic approach

Presentation:

How does increased tRNA synthesis drive growth? An unbiased proteomic and metabolomic approach.

Elizabeth J. Rideout and Savraj S. Grewal

Deregulation of the conserved insulin/Target-of-Rapamycin (TOR) pathway is a common feature of cancerous tumours and may drive the uncontrolled growth characterizing this disease. However, it remains unclear how TOR alters cellular metabolism to support this overgrowth. We use Drosophila as a model to identify downstream effectors of the TOR pathway. Ultimately, we hope to identify proteins and metabolic processes which can be exploited to manipulate growth at the cellular, tissue and organismal levels. I have identified tRNA synthesis as a limiting factor for TOR-dependent growth. Larvae with elevated levels of a single tRNA (initiator methionine) grow more rapidly and achieve a larger size. tRNA synthesis drives growth by promoting high levels of protein synthesis, and by stimulating the production and release of insulins. However, it remains unclear whether this enhanced growth is due to a global increase in protein synthesis, or whether specific growth-promoting proteins such as Myc are preferentially upregulated. Furthermore, the metabolic changes occurring as a consequence of increased tRNA synthesis are unknown. Therefore, I want to use an unbiased approach to determine how protein expression and metabolism are altered by elevated levels of tRNA synthesis. Using the flies with an extra copy of a single tRNA(iMet) as a tool, I can analyze protein expression and metabolite production. These questions are pertinent to the field of cancer biology as tRNA synthesis is upregulated in nearly all transformed cells, suggesting that a better understanding of how deregulated tRNA synthesis promotes uncontrolled growth may offer new therapeutic avenues.

Proteomics for Clinical Applications Keynote Speakers

Sam Hanash,
Fred Hutchison Cancer Research Centre

Biography:

Dr. Hanash's interests and expertise focus on the development and application of integrated approaches to the molecular profiling of cancer, with particular emphasis on proteomics. Dr. Hanash's Ph.D. training is in Human Genetics and clinical training in Pediatric Oncology. He has been a program principal investigator (PI) for multi-investigator projects funded by the National Cancer Institute (NCI) while at the University of Michigan, including program projects and most recently, PI for an NCI-funded Director's Challenge program, which focuses on molecular profiling of lung, colon and ovarian cancer and PI of an NCI-funded Cancer Biomarker Development program, which focuses on the application of proteomics to the discovery of protein markers for the early diagnosis of lung and GI cancers. Dr. Hanash has organized and participated in several workshops sponsored by the NCI related to cancer diagnostics and molecular profiling. Dr. Hanash relocated from the University of Michigan to the Fred Hutchinson Cancer Research Center in August 2004 to lead a newly developed program in Molecular Diagnostics.

Presentation:

Plasma protein biomarkers for assessment of risk for common diseases

Implementation of in-depth quantitative plasma protein profiling has provided an opportunity for unbiased discovery of protein marker panels indicative of risk for specific common diseases. We have implemented strategies for the discovery of risk markers for coronary heart disease (CHD), stroke, breast and lung cancer among women using plasma samples collected several years prior to diagnosis as part of cohort studies. The availability of plasmas from several hundred cases and matched controls for each disease necessitated implementation of a pooling strategy followed by expensive fractionation prior to mass spectrometry to achieve sufficient depth of analysis to identify proteins related to disease incidence. Coverage of the plasma proteome spanned no less than seven logs of protein abundance. Some of the candidate markers identified have been successfully validated using immunoassays applied to independent sets of samples that were not part of the discovery sets. The experience gained, the findings from these studies, the resulting extensive database of the plasma proteome and the challenges in developing high-throughput assays with the pre-requisite sensitivity for marker validation will be presented.

K.W. Michael Siu
York University

Biography:

Professor K.W. Michael Siu is Distinguished Research Professor and NSERC / AB SCIEX Senior Industrial Research Chair, York University. Siu's research interests range from fundamentals of mass spectrometry (MS), MS instrumentation, to applications of MS in proteomics, in particular the discovery, identification and verification of cancer biomarkers; some of these biomarkers have been shown to have prognostic values. Siu is a Fellow of the Royal Society of Canada.

Presentation:

Diagnostic, Prognostic and Therapeutic Significance of Head and Neck Cancer Biomarkers Discovered by Mass-Spectrometry Based Proteomics

Shotgun proteomics with iTRAQ labeling on trypsinized lysates of head and neck cancer tissues, oral premalignant lesions, and normal tissues has led to the discovery of a panel of five biomarker candidates that has subsequently been verified on a cohort of 366 patients using immunohistochemistry. The diagnostic and prognostic significance of the biomarkers were evaluated by means of statistical analyses that also examined clinicopathological parameters, including tumor stage, spread, and differentiation. Survival data of 77 head and neck cancer patients in a follow up study that spanned seven years were also available. Short interfering RNA knockdown of a key biomarker in cultured head and neck cancer cells was found to sensitize the cells to common chemotherapeutic agents. A natural product was found to impact upon key proteins and pathways in head and neck carcinogenesis, and offer the potential of chemoprevention.

Proteomics for Clinical Applications Invited Speakers

Dr. Vathany Kulasingam
University Health Network

Biography:

Dr. Vathany Kulasingam completed her PhD at the Department of Laboratory Medicine and Pathobiology, University of Toronto, under the leadership of Dr. Eleftherios P. Diamandis. Her doctoral thesis focused on discovery and validation of novel breast cancer biomarkers by use of tissue culture model systems, in association with quantitative mass spectrometry. Following her PhD, she completed a post-doctoral training diploma program in Clinical Chemistry at the University of Toronto. Dr. Kulasingam is currently an academic clinical biochemist at the University Health Network and an Assistant Professor at the Faculty of Medicine, University of Toronto. Her current interests include novel tumor biomarker discovery and application of proteomics to clinical practice.

Presentation:

Clinical Applications of Selected Reaction Monitoring-Based Mass Spectrometry Assays

Contributing Authors:
Vathany Kulasingam, Andrei P. Drabovich and Eleftherios P. Diamandis

While numerous strategies exist for biomarker discovery, the bottleneck to routine use at the clinic is in the verification and validation phases of candidate biomarkers. In particular, a major limiting factor in getting markers to the clinic is the lack of assays and other methods for quantitative validation of discovered candidates. Using selected reaction monitoring (SRM)-based mass spectrometry (MS), multiple proteins in the low ug/mL concentration can be monitored and quantitated simultaneously in unfractionated digests of complex mixtures. However, to quantify very low abundance proteins (low ng/mL), immuno-mass spectrometry based methods are necessary and have been developed for some analytes. In addition, reliable quantification requires that the MS-based assays are robust, selective, and reproducible. Thus, the development of standardized protocols is also essential in order to introduce protein quantification by MS into clinical laboratories. In this presentation, multiplex SRM assay to verify biomarkers for differential diagnosis of azoospermia will be discussed to highlight the ability to simultaneously quantify 30 proteins using MS, along with an assay utilizing immuno-enrichment to extract 4 proteins from complex mixtures to allow quantification in the low ng/mL range. Finally, the results of a study assessing the transferability and reproducibility of SRM assays between clinical research laboratories will be highlighted.

Dr. Liang Li
University of Alberta

Biography:

Dr. Li obtained his B.Sc. degree in Chemistry from Zhejiang (Hangzhou) University, China, in 1983, and his Ph.D. degree in Chemistry from the University of Michigan, Ann Arbor, Michigan, in 1989, under the supervision of Professor David M. Lubman. After graduation, he joined the Department of Chemistry at the University of Alberta in July 1989, where he is now a Professor of Chemistry, Adjunct professor of Biochemistry and Tier 1 Canada Research Chair in Analytical Chemistry. Dr. Li's research interest is in the area of analytical mass spectrometry for biomolecule and polymer analysis. He has won several awards including the Rutherford Memorial Medal in Chemistry from the Royal Society of Canada (2003), the F.P. Lossing Award from the Canadian Society for Mass Spectrometry (2006) and the Maxxam Award from the Canadian Society of Chemistry (2009). Dr. Li has served on editorial boards of several journals and is an editor of Analytica Chimica Acta since 2005.

Presentation:

Digging Deep into the Proteome to Enable Discovery of Potential Disease Biomarkers

Proteomics has played an important role for discovery of potential biomarkers for diagnosis and prognosis of diseases such as cancer. Ideally, in proteome analysis, all proteins present in a biological system such as cells or tissues are detected, identified and quantified. However, because of limitations of current analytical techniques, only a fraction of the whole proteome is profiled in a typical proteomics application. In this presentation, some recent progress in my laboratory on the development of new analytical techniques related to sample preparation and mass spectrometric detection for comprehensive proteome profiling of cells and tissues will be described. Applications of these techniques for cancer biomarker discovery will be presented.

Proteomics for Clinical Applications Invited Speakers

John Marshall,
Ryerson University

Biography:

Dr. Marshall is an analytical biochemist, whose main interest is the study of proteins, peptides, and enzymes located at or near the cell surface, and their involvement in extra-cellular processes. The research program in the protein biochemistry laboratory at Ryerson University includes isolation, primary sequence identification and/or quantification of polypeptides and their activities by biochemical, biophysical, immunological or cytological methods. Our goal is to discover the proteins from blood, white blood cells, and their receptors on the cell surface that are of central importance in the disease diagnosis and therapy. Our analytical approach is to combine biochemical, enzymological, mass spectrometric (MS), cytological, laser confocal, immunological and statistical methods into a comprehensive analytical system.

Presentation:

Quantitative statistical analysis of blood proteins by liquid chromatography, electrospray ionization, and tandem mass spectrometry

The parent and fragment ion m/z and intensity data from liquid chromatography, electrospray ionization and tandem mass spectrometry of human blood 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 replicate parent and fragment ions from 429 peptides of 196 proteins in the SQL database were subject to transformation, normality testing, and statistical analysis using the generic Statistical Analysis System (SAS). Transformation of both parent and fragment intensity values by logarithmic functions yielded intensity distributions in SAS that closely approximate the log normal distribution. It was possible to compute whole ANOVA models of the transformed parent and fragment intensity values across all treatments, proteins, peptides and fragment ion types with large degrees from many ions producing powerful statistical tests that showed a low probability of false positive results. One-way ANOVA of the transformed peptide and fragment intensity values over sample treatments, proteins or peptides from multiple LC-ESI-MS/MS experiments were corrected by the Tukey-Kramer Honestly Significant Difference test. The ANOVA analysis at the level of treatments or proteins yielded much greater statistical power than measurements of individual peptides. The approach provided a complete and quantitative statistical analysis of LC-ESI-MS/MS from human blood.

Proteomics for Clinical Applications Oral Abstract Presentations

Gabriela Cohen Freue
PROOF Centre of Excellence

Can we distill value for clinical management from the human proteome?

Contributing Authors
Anna Meredith, Derek Smith, Zsuzsanna Hollander, Mandeep Takhar, Axel Bergman, Mayu Sasaki, David Lin, Janet Wilson-McManus, Robert Balshaw, Raymond T. Ng, Scott Tebbutt, Paul A. Keown, Christoph H. Borchers, Bruce McManus, W. Robert McMaster

Background: Despite increased efforts to demonstrate the potential of proteomic biomarkers to predict, and/or diagnose various diseases, only a few protein markers have completed the path from discovery to clinical utility. The PROOF Centre of Excellence has developed a proteomic biomarker pipeline which starts with an unbiased search using iTRAQ-LC-MALDI-TOF/TOF methodology followed by validation with ELISA and LC-MRM assays. Initial results are based on a study in cardiac transplantation.

Methods: Depleted plasma samples (n=20) were analyzed using iTRAQ to identify markers of acute allograft rejection. Results were validated using commercially available ELISA assays on an extended cohort (n=43) run following manufacturerâ€™s protocols. A multiplex LC-MRM assay was developed to measure the identified proteins on tryptic digests of non-depleted plasma. SIS peptides were added immediately following tryptic digestion.

Results: Five potential proteins were identified that discriminated rejection (n=6) from non-rejection samples (n=14) (p-value<0.01). Results were corroborated for three out of four proteins measured by ELISA, and for four out of five proteins measured by LC-MRM with similar discrimination power. The correlations between iTRAQ and ELISA measurements range from 0.45 to 0.78, while those between LC-MRM and ELISA range from 0.69 to 0.99. Initial estimates of sensitivity and specificity of 100% were demonstrated using a classifier score based on LC-MRM measurements of the 5 markers.

Conclusion: This study provides a proof-of-principle for proteomic biomarker studies by identifying a proteomic panel using an untargeted methodology and developing a multiplex targeted assay appropriate for biomarkers validation to allow translation of results into clinical practice.

Morteza Razavi,
University of Victoria

Measurement of protein C inhibitor (PCI) in human plasma by a SISCAPA-MALDI (sMALDI) technique: a prospective test for monitoring prostate cancer

Contributing Authors:

Morteza Razavi (1), N. Leigh Anderson (2), Terry W. Pearson(1)

(1) Department of Biochemistry and Microbiology,
(2) Plasma Proteome Institute

Advances in mass spectrometry have revolutionized the world of biomarker discovery. Although hundreds of putative biomarkers are being discovered every year, only a small fraction of them are validated, which introduces a serious bottleneck to their application for disease diagnosis and monitoring. To address this problem we have designed a quantitative MALDI-TOF-based assay called SISCAPA-MALDI (sMALDI) that combines SISCAPA (Stable Isotope Standards and Capture by Anti-Peptide Antibodies) with high throughput MALDI-TOF MS analysis. SISCAPA employs high affinity anti-peptide antibodies to enrich peptide surrogates of protein biomarkers from complex mixtures and identifies and quantifies them by tandem mass spectrometry. By combining SISCAPA with MALDI-TOF MS we were able to make the procedure amenable to automation and thus suitable for biomarker validation.
As a proof of principle model we have chosen to examine human protein C inhibitor (PCI), a serpin protease inhibitor found in plasma. PCI has been reported to fluctuate in prostate cancer patients and is thus a putative biomarker. Using the sMALDI technique employing a high affinity monoclonal antibody to enrich a surrogate peptide of PCI, we measured PCI in the plasma of 20 normal humans with a balanced distribution of age, race and gender. The average concentration of PCI was determined to be 3.2 ± 0.1 µg/mL, which correlates well with a reported sandwich ELISA immunoassay value of 5.0 µg/mL. No clear differences were found based on age, race or gender. The overall coefficient of variation, including sMALDI assays run on different days, was about 10 percent.
Initial sandwich ELISA immunoassays on 20 controls and 5 prostate cancer patients revealed that PCI levels were elevated in the latter. Two of the patients had stage 2 prostate cancer and had not received any medication whereas 3 others had either stage 3 or 4 cancer and were treated with several different chemotherapeutic agents.

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Based on the above results, we are using sMALDI to measure the levels of PCI in plasma from a larger number and variety of patients with prostate cancer. In this way we will determine whether or not PCI can serve as a biomarker for monitoring of prostate cancer. Since the sMALDI method is amenable to multiplexing, PCI, PSA and several other putative biomarkers for prostate cancer are also being tested on the same plasmas.

Wafaa Yahyaoui
IRIC

DE NOVO SEQUENCING OF MINOR HISTOCOMPATIBILITY ANTIGENS

Contributing Authors:
D. Granados, C. Cote, O. Caron-Lizotte, A.Bramoulle, C. Perreault, P. Tibault

Allogenic haematopoietic stem-cell transplantation is commonly used for eukemic patients refractive to chemotherapy. The immune reaction of the donor T lymphocytes provides a beneficial graft versus leukaemia (GVL) effect to the diseased recipients, though a large number of patients develop a graft versus host disease (GVHD), a severe autoimmune reaction. Appropriate GVL requires the identification of minor histocompatibility antigens (MiHA) specific to the leukemic cells. MiHA are polymorphic peptides which correspond to genetic variations (e.g. SNPs). In the present study, we describe a novel high-throughput peptidomic method to identify and characterize MiHAs that can be used for immunotherapy. Peptides eluted from B cells of HLA-identical individuals were fractionated by liquid chromatography and subjected to mass spectrometry analyses on a LTQ Orbitrap Velos instrument. de novo sequencing on a high resolution mass spectrometer was performed to identify peptide sequences with high accuracy. We analysed the data by comparing the peptide lists of the two members of the pair. Peptides differentially expressed in one member but not the other are putative MiHAs. We identified 28 potential MiHA candidates. We also correlated the occurrence of potential MiHA sequences using database of human SNPs. In conclusion, by using high-throughput mass spectrometry, we profiled and sequenced more than a thousand native MHCI peptides, generating the first comprehensive peptidome repertoire of human B cells. More importantly, the use of high resolution mass spectrometry opens new avenues for identification of MiHAs that can be used for immunotherapy.

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Alan Marshall
Florida State University

Biography:

Alan G. Marshall completed his B.A. degree with Honors in Chemistry at Northwestern U.in 1965, and his Ph.D. in Physical Chemistry from Stanford U. in 1970. He joined the Chemistry faculty at the University of British Columbia (Vancouver, Canada) in 1969. He moved to Ohio State University in 1980 as Professor of Chemistry and Biochemistry and Director of the Campus Chemical Instrument Center. In 1993, he moved to Florida State University, where he is Robert O. Lawton Professor of Chemistry and Biochemistry and Director of the Ion Cyclotron Resonance (ICR) Program, an NSF national user facility for mass spectrometry.

He co-invented and leads the continuing development of Fourier transform ICR mass spectrometry. His current reseach spans FT-ICR instrumentation development, fossil fuels and environmental analysis, and mapping the primary and higher-order structures of biological macromolecules and their complexes.

His major recognitions include: Fellow, American Physical Society; Fellow, American Association for the Advancement of Science; Fellow, Society for Applied Spectroscopy; three American Chemical Society national awards (Chemical Instrumentation, Field-Franklin Award, and Analytical Chemistry Award); two Spectroscopy Society of Pittsburgh Awards (Hasler Award and Spectroscopy Award); American Society for Mass Spectrometry Distinguished Contribution Award; International Society for Mass Spectrometry Thomson Medal; Chemical Pioneer Award from American Institute of Chemists, Eni Frontiers in Hydrocarbon Research Award, and Fellow of the American Chemical Society. He is a former President of the American Society for Mass Spectrometry, and serves on several editorial boards. He has published 4 books, 5 patents, and 500 refereed journal articles, and has presented more than 1,600 talks/posters at conferences, universities, government labs, and industry. His papers have been cited more than 19,000 times. Of his 113 former Ph.D.'s and postdocs, 32 have gone on to academic positions.

Presentation:

Molecular Mass—A Universal Chromophore for Proteomics

Until recently most mass analysis relied on "nominal" mass accuracy (i.e., to within 1 Da). However, more and more applications are based on much more accurate mass measurement. With high mass resolving power (m/Dm50% > 500,000 over a wide mass range), it becomes possible to separate complex mixtures without prior chromatographic or gel separation, as well as to identify amino acid composition and phosphorylation or glycosylation based on accurate (to ~100 ppb) mass measurement. Examples include post-translational modifications, bio-markers, mapping contact surfaces in protein assemblies for drug targets, and metabolomics. Examples uniquely addressable by Fourier transform ion cyclotron resonance mass spectrometry derive from its 10-100x higher mass resolving power relative to other mass analyzers. [Supported by NSF DMR-06-54118, NIH (GM 78359), and the State of Florida.]

Richard D. Smith
Pacific Northwest National Laboratory

Biography:

Dr. Richard D. Smith is a Battelle Fellow and Chief Scientist in the Biological Sciences Division and Director of Proteomics Research at Pacific Northwest National Laboratory (PNNL). His research interests have involved the development and application of advanced methods and instrumentation for applications in biological research. Dr. Smith was responsible for the development of the first combinations of capillary supercritical fluid chromatography and capillary electrophoresis with mass spectrometry, and capillary LC with high performance accurate mass spectrometry. His recent work has emphasized the development and application of capabilities for making ultra-sensitive and high performance proteomics, metabolomics, and related ‘pan-omics’ measurements. Dr. Smith is

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Director of the NIH Biomedical Technology Resource Center for Integrative Biology and the U.S. Department of Energy High Throughput Proteomics Facility at PNNL. He is an adjunct faculty member of the Departments of Chemistry at Washington State University, the University of Utah, and the University of Idaho. Dr. Smith has presented more than 350 invited or plenary lectures at national and international scientific meetings, and is the author or co-author of more than 750 publications. Dr. Smith holds thirty-nine patents and has been the recipient of nine R&D 100 Awards, the 2003 American Chemical Society Award for Analytical Chemistry, and the 2009 HUPO Discovery Award in Proteomics Sciences.

Presentation:

High Performance Separations and their Importance for the Mass Spectrometric Characterization of Proteomes

Advances in the quality, resolution, and the speed of separations for proteomics samples have arguably been as important as mass spectrometric developments in improving the sensitivity and coverage of proteomics measurements. However, challenges related to: the coverage of very low abundance proteins, sample size limitations, the need for sufficient throughput to address large numbers of samples, and the desire to broadly cover protein modifications still pose significant challenges. This presentation will discuss the role of advanced separations, including multidimensional nanoscale LC and/or ion mobility separations, in addressing these challenges in the context of plasma biomarker discovery.

Dr. Jochen M. Schwenk
School of Biotechnology, KTH

Biography:

Dr. Jochen M. Schwenk is facility manager for biobank profiling at the Science for Life Laboratory in Stockholm, Sweden (www.scilifelab.se) and principal investigator for the plasma profiling group within the Human Protein Atlas project (www.proteinatlas.org). He studied Biochemistry at the University of Tuebingen, Germany, and began his scientific career with the development of protein microarray applications with Dr. Thomas Joos at the NMI.

After receiving his PhD in 2005 he joined the Human Protein Atlas project headed by Prof. Mathias Uhlén with funding from the Wallenberg Foundation and F.Hoffmann-La Roche. Dr. Schwenk was recently nominated as “KTH star of 2009” for the WCN symposium on Frontiers in The New Biology. He was also appointed as project leader in the ProNova Centre for “Plasma Analysis for Biomarker Discovery”, where his groups is working on high-throughput analysis of body fluids using suspension bead arrays.

Presentation:

Exploration of plasma protein profiles with next generation affinity arrays

There is need for high-throughput methods for screening patient samples in the quest for potential biomarkers for diagnostics and patient care. With more than 15,000 well-characterised affinity reagents accessible through the Human Protein Atlas (www.proteinatlas.org), a systematic protein profiling of body fluids stored in biobanks is now on-going. To facilitate this, antibody suspension bead arrays have been developed that are performed in microtiter plates and employ magnetic beads to profile of up to 384 proteins in parallel. The procedure is multiplexed in the dimensions samples as well as antibodies and it was recently found that retrieving target epitopes by heat allowed promising differences in comparative studies to be identified. Protein profiles are derived from labelled, non-fractionated samples such as serum, plasma or other body fluids. Support from liquid handling systems allows up to 150,000 immunoassays to be performed per day, and these assays consume minimal amounts of sample with limits of detection into sub-ng/ml ranges. These characteristics of this assay suggested that applying the method to a proteome-wide screening would allow exploring and identifying new targets for further validation studies, thus we have started to profile human serum and plasma in a systematic manner. In a hypothesis-free pilot study, more than 4,600 validated antibodies have been applied to determine profiles across 576 samples representing 24 disease categories. The subsequent stages will then technically and biologically verify interesting findings to ultimately develop streamlined sandwich assays. In addition to disease related profiling efforts, our lab also focusses on generating protein profiles across ages and genders towards describing landmarks in an atlas of plasma proteins.

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Dr. Catherine Costello
Center for Biomedical Mass Spectrometry
Boston University School of Medicine

Biography:

Dr. Costello is a Professor in the Departments of Biochemistry and Biophysics at Boston University School of Medicine and the Department of Chemistry at Boston University, and is the founding Director of both the NIH-NCRR Mass Spectrometry Resource and the NIH-NHLBI Cardiovascular Proteomics Centre at BUSM. She has served as Vice-President and President of the American Society for Mass Spectrometry and is currently President-Elect of HUPO and Vice-President (Society) of the International Mass Spectrometry Foundation; she is a Councilor for the American Chemical Society, a member of the US National Committee for IUPAC, the boards of the Human Proteome Organization, the Human Disease Glycomics/Proteome Initiative and the US Human Proteome Organization, and numerous editorial, review and advisory boards.

Dr. Costello is the 2010 winner of the Frank H. Field and Joe L. Franklin Award for Outstanding Achievement in Mass Spectrometry. Dr. Costello's research aims to refine and extend mass spectrometry methods for proteins and glycans and to elucidate structure-activity relationships as they influence or reflect processes related to health, growth and development, and disease.

Presentation:

New approaches to glycomic analyses

Nature deploys a wide diversity of intricately varied and dynamically changing glycans for a myriad of purposes that can determine both the structural properties and interactions of individual molecules, subcellular components, cells, tissues and whole organisms. This fascinating and complex repertoire presents challenges to the analyst. New techniques and emerging methods are now allowing mass spectrometry to penetrate deeper into this previously impenetrable realm. In particular, electron-based dissociation methods show great promise for glycan and glycoconjugate analysis because they generate many linkage-defining products that arise from cross-ring cleavages, in addition to sequence-specific glycosidic fragments that are easily obtained by CID and IRMPD. Multistage dissociation experiments that combine established and newer fragmentation methods are particularly useful for complete structural elucidations of highly branched glycans and glycoconjugates, *e.g.,* glycolipids and glycoproteins. Investigations of the sometimes quite novel fragmentation mechanisms are required before these capabilities can be fully and confidently exploited. This lecture will discuss the application of established and novel dissociation methods for the delineation of biologically and biomedically relevant interactions of glycans.

Acknowledgements: Many members of the BUSM CBMS and its collaborators stimulate and contribute to our progress. Our research is supported primarily by the National Institutes of Health, National Center for Research Resources and National Heart Lung and Blood Institute.

Dr. N. Leigh Anderson
Plasma Proteome Institute

Biography:

Leigh Anderson, Ph.D. is Founder and CEO of the Plasma Proteome Institute, Washington D.C. (www.plasmaproteome.org). The Institute aims to foster a comprehensive exploration of the proteins of human blood plasma (the plasma proteome), improved quantitation of potential disease markers, and the rapid application of novel protein measurements in clinical diagnostics. Dr. Anderson obtained his B.A. in Physics with honors from Yale and a Ph.D. in Molecular Biology from Cambridge University (England) where he worked with M. F. Perutz as a Churchill Fellow at the MRC Laboratory of Molecular Biology. Subsequently he founded (with Dr. Norman Anderson) the Molecular Anatomy Program at the Argonne National Laboratory (Chicago) where his work in the development of 2-D electrophoresis and molecular database technology earned him, among other distinctions, the 1983 Pittsburgh Analytical Chemistry Award.

Prior to founding PPI, Dr. Anderson was Chief Scientific Officer at Large Scale Biology Corporation, whose proteomics division he founded in 1985, and co-led a successful Nasdaq IPO based largely on the proteomics technology platform. More recently Dr. Anderson has developed novel technologies for quantitation of protein biomarkers using mass spectrometry, receiving the 2009 HUPO Distinguished Achievement Award in Proteomic

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Science. Dr. Anderson currently serves as a Principal of Anderson Forschung Group LLC, a member of the Board of Directors of Luna Innovations (a developer of novel sensors and materials), associate editor of the journal Clinical Chemistry and on numerous scientific advisory boards. Dr. Anderson has published more than 150 scientific papers, one book and 32 patents.

Presentation:

The Diagnostic Proteome: Challenges and Opportunities in the Discovery and Clinical Implementation of Protein Biomarkers

The current clinical plasma proteome consists of 109 proteins measured by FDA-cleared or approved assays and 96 proteins measured using widely-available laboratory developed tests, in total about 1% of the baseline human proteome. However the rate at which new protein analytes achieve FDA approval has remained essentially constant over the past 15 years at 1.5 proteins per year, insufficient to meet clear medical needs. The striking shortfall in new protein diagnostics emerging from proteomics research reflects a lack of critical biomarker verification capacity. In order to bridge the gap between biomarker discovery and clinical use, a new approach to verification is proposed: multiplexed panels of specific candidate assays based on hybrid immuno-mass spectrometric (SISCAPA) detection. By combining high sensitivity, high throughput, and precision with use of small plasma samples, a platform for systematic verification of hundreds of candidates in thousands of samples can be implemented. In the clinical laboratory, affinity enrichment with MS quantitation can provide absolute specificity, true internal standardization, and facile multiplexing – thus transcending the limitations of conventional immunoassays. An extension of this approach, the hPDQ project providing a library of specific tests for all 21,300 human proteins, could provide the larger biomedical research community with direct quantitative access to the entire human proteome.

Dr. John Yates
The Scripps Research Institute

Biography:

Dr. Yates is a Professor in the Department of Chemical Physiology at The Scripps Research Institute. His research interests include development of integrated methods for tandem mass spectrometry analysis of protein mixtures, bioinformatics using mass spectrometry data, and biological studies involving proteomics. He is the lead inventor of the SEQUEST software for correlating tandem mass spectrometry data to sequences in the database and principle developer of the shotgun proteomics technique for the analysis of protein mixtures. His laboratory has developed the use of proteomic techniques to analyze protein complexes, posttranslational modifications, organelles and quantitative analysis of protein expression for the discovery of new biology.

Many proteomic approaches developed by Yates have become a national and international resource to many investigators in the scientific community. Dr. Yates led an NIDCR funded Center to characterize the Saliva proteome and has been involved in an NCRR funded Research Resource Center for the last 15 years and was involved in an NSF funded Science and Technology for 10 years. He has received the American Society for Mass Spectrometry research award, the Pehr Edman Award in Protein Chemistry, the American Society for Mass Spectrometry Biemann Medal, the HUPO Distinguished Achievement Award in Proteomics, Herbert Sober Award from the ASBMB, and the Christian Anfinsen Award from The Protein Society. He has published ~500 scientific articles.

Presentation:

Using Mass Spectrometry to Understand Protein Misfolding Diseases

A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. Several major technologies, but especially mass spectrometry, have benefited from large-scale genome sequencing of organisms. The sequence data produced by these efforts can be used to interpret mass spectrometry data of proteins and thus enables rapid and large-scale analysis of protein data from experiments. Advances in multi-dimensional separations as well as mass spectrometry have improved the scale of experiments for protein identification. This has improved the analysis of protein complexes, and more complicated protein mixtures. Quantitative mass spectrometry can be used to study biological processes such as protein-protein interactions, development or the effects of gene mutations on pathways. Recent studies on the loss of function mutant form of the Cystic Fibrosis Transport Regulator (DF508) as it progresses through the folding pathway will be presented. Through the study of protein-protein interactions, we are beginning to understand the critical interactions regulating pathways for export or destruction. Additional studies of gain of cytotoxic function to identify activities that may be important for removal of aggregating proteins will also be presented.

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Dr. Bin Ma
University of Waterloo

Biography:

Bin Ma is an Associate Professor and University Research Chair in the David R. Cheriton School of Computer Science at the University of Waterloo. He received his Ph.D. degree from Beijing University in 1999. During 2000-2008 he worked at University of Western Ontario as Assistant Professor, Associate Professor, and Canada Research Chair. He published over 100 research papers, and is the creator of several popular bioinformatics software packages including PEAKS and PatternHunter. He received Ontario Premier's Catalyst Award for Best Young Innovator in 2009 and the Outstanding Young Computer Science Researcher Prize (Canadian Association of Computer Science) in 2010.

Presentation:

Peptide De Novo Sequencing when There Is a Sequence Database

De novo sequencing is the process of deriving a peptide's sequence from its MS/MS spectrum without the need of a protein sequence database. This is particularly useful for sequencing novel peptides, such as synthesized peptides and peptides from organisms without a sequence database. However, in this talk we explore the unconventional applications of de novo sequencing when there is a protein sequence database, and demonstrate that de novo sequencing can be extremely useful even when a sequence database is provided. The talk will start with a brief introduction of de novo sequencing algorithms. Then the special capabilities of de novo sequencing for validating database search results, finding modifications and mutations, and sequencing/confirming complete proteins are examined.

Dr. Lars Konermann
University of Western Ontario

Biography:

Professor Lars Konermann holds a Canada Research Chair in Biophysical Protein Mass Spectrometry at The University of Western Ontario (UWO). The work in his laboratory is aimed at gaining insights into protein folding mechanisms, and the relationship between protein function and conformational dynamics. These and other areas are being tackled through hydrogen exchange and covalent labeling methods. Another field of interest is the mechanism of electrospray ionization, a process that allows the transfer of intact biological molecules from solution into he gas phase of the mass spectrometer. Konermann is faculty member at the UWO Chemistry Department, and he is cross-appointed to the Department of Biochemistry.

Presentation:

Protein Structure and Function Studied by Hydrogen Exchange Mass Spectrometry

Amide hydrogen/deuterium exchange (HDX) mass spectrometry has become a widely used approach for exploring the structure and dynamics of proteins. H atoms in disordered segments are readily exchangeable, whereas sites that are involved in stable hydrogen bonds are strongly protected. Spatially-resolved HDX measurements usually employ proteolytic digestion with subsequent measurement of peptide mass shifts by HPLC/MS. This traditional approach will be illustrated by discussing phosphorylation-induced structural changes of VraR, a key protein involved in the antibiotic resistance of Staphylococcus aureus. Limitations of traditional proteolysis-based HDX/MS measurements include their relatively low spatial resolution, as well as the occurrence of isotope back exchange during analysis. In collaboration with Jun Han and Christoph H. Borchers (University of Victoria, Genome BC Proteomics Centre) we developed an alternative approach that employs top-down electron capture dissociation (ECD) for HDX measurements, instead of relying on solution phase digestion. For small to medium-sized proteins this ECD strategy appears to be superior in performance compared to traditional proteolysis-based HDX measurements. In addition, top-down ECD allows the conformer-specific HDX interrogation of proteins in structurally heterogeneous samples. The feasibility of this strategy is demonstrated by applying it to Aβ oligomers that co-exist with disordered monomers in solution. These oligomers play a key role in the etiology of Alzheimer's disease. Our data point to similarities between oligomers and mature amyloid fibrils with regard to the Aβ backbone organization.

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Dr. Alison Ashcroft
University of Leeds, UK

Biography:

Alison Ashcroft is Professor of Biomolecular Mass Spectrometry in the Astbury Centre for Structural Molecular Biology at the University of Leeds, UK. Her PhD research at the University of Manchester was followed by a post-doctoral fellowship at the University of Geneva, Switzerland. After this, Dr. Ashcroft worked in industry, spending time with Kratos Analytical, ICI Pharmaceuticals (now AZ), and Micromass/Waters, before joining the University of Leeds in 1997.

Dr. Ashcroft's research is focused on the application and development of mass spectrometry for studying protein folding and protein conformer characterisation, protein-protein and protein-ligand interactions, and biomolecular assembly pathways. Dr. Ashcroft is interested in ion mobility spectrometry - mass spectrometry as a method of separating co-populated protein species in real-time and of characterising tertiary and quaternary protein structure.

Dr. Ashcroft served on the BMSS Committee from 1989-1998 and was Chair from 1996-1998. In 2008 she was appointed President of BMSS. She is also a founding member and past Secretary of the International MS Foundation.

Presentation:

In vivo the majority of proteins function within non-covalently bound macromolecular complexes rather than alone. Unravelling the assembly pathways of these complexes is important for understanding how individual proteins interact with other proteins and ligands and, in cases where such complexes are associated with disease, for devising means of assembly inhibition.

ESI-IMS-MS and ESI-MS/MS are highly suitable methods for probing the assembly pathways of macromolecular complexes including, for example, the self-aggregation of certain proteins into amyloid fibrils. One such protein, beta-2-microglobulin, forms fibrils in vivo which are associated with a condition termed dialysis-related amyloidosis. Monitoring amyloid fibril formation from beta-2-microglobulin in vitro, ESI-MS has been used to compare, in terms of shape and stability, monomeric and oligomeric conformers detected within transient, heterogeneous protein ensembles on two different, fibril-forming pathways. The interactions of this protein with a range of potential inhibitors of self-aggregation have also been evaluated.

Ken Standing Award Winner

Daniel Figeys,
University of Ottawa

Recipient of The Ken Standing Award. This award is presented biennially at ETP to honour a young scientist who has made a significant contribution to technology development in support of research in the life sciences.

Biography:

Dr Figeys is a Systems Biologist and Analytical Chemist. He is the Director of the Ottawa institute of Systems Biology, University of Ottawa and a Canada Research Chair in Proteomics and Systems Biology.
Research efforts are focused on the development and application of proteomics, lipidomics, and technology development. In proteomics, we are interested in developing and applying techniques for 1) the mapping of protein-protein interactions for proteins relevant to human diseases, and 2) the study of quantitative changes in the proteome when limited amount of material is available. In lipidomics, we are interested in developing and applying techniques for the study of changes in the lipidome during diseases. In particular, we are targetting specific subclasses sof lipids such as PC, PE, and PS and are developing techniques for other classes of lipids as well. In collaboration with the laboratory of Dr. S. Bennett we are studying the targetted lipidome in neurodenegrative diseases. Our technology development efforts are focused on developing proteomics technologies to study minute levels of proteins, the development of lipidomic technologies and the development of bioinformatic tools.

Presentation:

Proteomics and lipidomics technologies to study human diseases

My laboratory is interested in the development and application of proteomics and lipidomics technologies to better understand biological processes with a particular focus on neurodegenerative and cardiovascular diseases. These diseases pose some interesting technical challenges for proteomics and lipidomics. For example, the amount of sample is a limiting factor when studying specific regions of the brain. Although, proteomics can routinely identify and quantify 1000's of proteins, this is generally done using a large amount of material. Here, we will present technologies to study minute amounts of specific regions of the brain using proteomics and lipidomics. We are also studying the role of the liver in cardiovascular diseases with an emphasis on the regulation of LDLR. This requires studying biological processes from the ER, Golgi, endosome, and plasma membrane. We will present some of our approach to study the proteome and the lipidome at the sub-cellular level.

CNPN Award Winner

Jack Greenblatt
University of Toronto

Recipient of CNPN's Award for Outstanding Contribution and Leadership to the Canadian Proteomics Community

We are pleased to announce this year's winner of the CNPN Distinguished Researcher Award is Dr. Jack Greenblatt of the University of Toronto. This Award recognizes the remarkable achievement on the fundamental understanding and/or practice of proteomics in biological sciences. Dr. Greenblatt's pioneering work on protein-protein interactions and the fundamental mechanisms regulating gene expression in bacteria, viruses, yeast, and human cells gave rise to remarkable achievements that inspired many others in the field. Recognizing the significance of these contributions, it is thus with great pleasure that we extend our most sincere congratulations for this well deserved award.

Presentation:

Protein-protein Interactions in Molecular Machines and Biological Regulation

I will initially provide historical perspective on our research into the biological importance of protein interactions, beginning with our use of protein affinity chromatography to investigate transcriptional antitermination by the bacteriophage λ N protein and continuing with our identification of general transcription factors interacting with human RNA polymerase II and how viral activators communicate with the transcriptional apparatus.

Over the last decade, we have devoted considerable effort to identify protein-protein interactions and protein complexes for E. coli and S. cerevisiae, in these cases using affinity purification of proteins tagged in vivo followed by mass spectrometry (AP/MS). These experiments began with a focus on transcriptional regulation and chromosome biology in yeast and evolved into an effort to characterize the genome-wide protein interactomes for the soluble proteins of both organisms. I will describe in more detail our more recent use of AP/MS to characterize protein interactions for the membrane proteins of S. cerevisiae.

Most recently, we have focused on identifying protein interactions for human proteins related to chromatin biology. In this case, we use the MAPLE (Mammalian Affinity Purification and Lentivirus Expression) system to deliver tagged ORFs into cultured human cells. Some of our recent findings with this system will be described.

POSTER ABSTRACTS

Poster No. B-1

Proteomics in Biology

Snapshots of protein dynamics and posttranslational modifications in one experiment beta-catenin and its functions

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The proto-oncogene beta-catenin plays a central role in the Wnt signaling pathway and is an integral part of cell-cell adhesion complexes. These different cellular functions - transcription factor or cell adhesion molecule - are mediated by changes in concentration, phosphorylation, and by complex formation with other factors such as cadherins. The Wnt signalling pathway is often found to be hyperactive in liver and other tumours and it has become a main focus of basic and pharmaceutical research.
We have developed a microarray-based assay that uses antibodies and known interaction partners of beta-Catenin to study the phosphorylation status of the protein and its complexation status within the same multiplex assay system. This enables us to investigate the dynamic effects of chemical inhibitors on protein's abundance, on function and on posttranslational modifications. Detailed quantitative and time-resolved analysis of the pathway activity, based on the evaluation of the dynamic changes in the Wnt pathway, is possible and gave insights into the mechanisms of action of the natural ligand Wnt3a and a kinase inhibitor.

Poster No. B-2

Proteomics in Biology

Proteomic Analysis of Platelet Releasate Using Different Stimuli

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Platelet activation plays an important role in hemostasis and is also associated with diseases such as cardiovascular disorders. Different stimuli tend to engage different receptors on the platelet surface, so they are likely to engage different molecular signaling pathways, or to result in different strength of response. The aim of this study is to identify the changes in the platelet releasate proteome during platelet activation using different stimuli. To this end, isolated washed platelets were re-suspended in HEPES buffer containing 1.8mM CaCl2 at physiological concentrations. In this study we used thrombin, collagen and medium ox-LDL as stimuli for platelet activation. The extent of platelet activation and aggregation was determined using flow cytometry and fluorescent antibodies directed against P-selectin and integrin ÃŸ3. The content of platelet releasate was separated into three components: total platelet releasate, platelet microparticles and platelet releasate minus microparticles. The protein content of each component was subject to GeLC separation followed by in-gel trypsin digestion and FT-ICR analysis. We identified over 650 total releasate proteins using thrombin, over 1500 proteins using collagen and over 500 proteins using ox-LDL. Using Ingenuity Pathway Analysis we highlight the pathways that these proteins are involved in. There is ongoing work to use dimethyl labelling to quantitatively study the different components from the different stimuli. By analyzing the proteins released from platelets upon activation a more complete picture of platelet activation will emerge, which will help us understand the pathways and the biological implications of different platelet stimuli.

Poster No. B-3

Proteomics in Biology

Uncovering the Immunoproteasome in Platelets

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The major function of platelets is their role in hemostasis. However, in recent years an involvement of these anucleate cell fragments in the immune system has been discovered. An in-depth global proteome analysis on human platelets in our lab revealed the overrepresentation of the “Proteasome” KEGG pathway. A more detailed investigation discovered that all α and β subunits except β5 were detected in at least one of the four datasets obtained in this study. Instead of β5, the subunit β5i was identified in all datasets and raised the question of the presence of the immunoproteasome in platelets.

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The assembly of this form of the proteasome is induced by interferon-γ, which leads to the replacement of the subunits β5, β2 and β1 with newly-synthesized β5i, β2i and β1i. Western Blot analysis was used to study the presence of the proteasome subunits β5, β5i and β1i. Although we did not detect β5 in our proteomic datasets, we detected the subunit in its cleaved form in platelets. Furthermore we identified the immunoproteasome subunits β5i and β1i in their processed form. Our next step was to prove that the immunoproteasome is assembled in platelets and we performed immunoprecipitation experiments using an antibody against the α2 subunit and mass spectrometry analysis to identify the components of the precipitated complex. Preliminary data suggests that all immunoproteasome subunits are found in this complex, indicating that a fully assembled immunoproteasome is present in platelets and experiments are currently underway to determine if it is also active.

Poster No. B-4

Proteomics in Biology

Extraction of Isoform-specific Protein Information from Public Data Repositories Enables Targeted Proteomic Analyses

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As public available proteomic data repositories continue to grow, we hypothesized that sufficient information on protein isoforms is archived, which allows us to extract information on protein interaction patterns for different isoforms and proteotypic peptides for Multiple Reaction Monitoring (MRM) analysis that enables isoform-specific investigations. Using the in silico protein interaction analysis (http://gpmdb.thegpm.org/thegpm-cgi/pvip.pl), a bioinformatic approach developed in our laboratory that is integrated in the Global Proteome Machine Database (GPMDB), we studied protein interaction environments of the histone family of proteins. For the 15 histone H2B1 isoforms, we observed vastly different numbers of unique identifications. HIST1H2BB and HIST1H2BC showed the highest number of observations (2441 and 1168), while HIST1H2BG and HIST1H2BI had no unique identifications in the GPMDB. Of the 28 and 30 proteins identified for HIST1H2BB and HIST1H2BC using in silico protein interaction analysis, 20 were common to both analyses. This included at least one member of each histone subfamily, indicating comprehensive interaction maps for both isoforms. Different small GTPase isoforms were used to study proteotypic peptide information using the MRM feature of the GPMDB. Three, five and two proteotypic peptides were identified for H-Ras, K-Ras and N-Ras. The most promising candidates for MRM detection were SFEDIHQYR (H-Ras, >5000 observations) and SYGIPFIETSAK (K-Ras, >1000 observations). The limited number of observations of both N-Ras peptides (<250) prevented further analysis. Our preliminary results indicate that isoform-specific information is readily available in public data repositories such as the GPMDB, which allows designing targeted proteomic analyses.

Poster No. B-5

Proteomics in Biology

A PPI-based GO functional enrichment strategy for "iomics" data analysis

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A PPI-based GO functional enrichment analysis for "ìomics" data

With the popularization of high-throughput technology, analyzing tools have been rapidly developed for large-scale "ìomics" data. However, it is still a daunting and challenging task to figure out accurate biological interpretation from data. Funle, few of them take biological concepts into consideration. It is therefore difficult for researchers to assess the quality of GO enrichment results.

In this study, we introduce a new functional enrichment analysis strategy. It integrates (1) comparative genes/proteins quantization from experiments, (2) the GO DAG structures and the evidence code of GO annotation for quality control, (3) the interaction relationship provided by STRING. Our aim is to report multi-concepts biological interpretation more close to the real biological system. In addition, we provide a visualization output of the enriched term's PPI which is considered as functional modules differently regulated within datasets.

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Applying this new functional enrichment analysis to two protein expression dataset of human iPSC/ESC and human iPSC/EB, the result indicates that the biological terms GO: 0060070 (Wnt receptor pathway through beta-catenin). In iPSC are more up-regulated (enriched) than ESC. On the other hand, the GO:0030509 (BMP signaling pathway) in EB biological process are down-regulated (enriched) then ESC. The aforementioned GO terms in the results are supported by most of the previous study. GO term inclusions (gene/protein) have potential to be candidate for biomarker discovery and Interesting target for furthermore basic study.

GO: gene ontology
DAG : Direct acyclic graph
PPI: Protein-protein interaction
iPSC: Induced Pluripotent Stem Cells
ESC : Embryonic Stem Cell
EB: Embryoid Body

Poster No. B-6

Proteomics in Biology

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

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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. This approach was compared to the commonly used ZIC-HILIC based strategies.
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. For method validation, isolated glycopeptides were analyzed in native form or after treatment with PNGase F- H2O18 on a hybrid dual pressure linear ion trap-orbitrap mass spectrometer equipped with ETD. More than 200 N-glycoproteins were identified by using these two approaches.

Poster No. B-7

Proteomics in Biology

Binary Protein Structure Determination through H/DX-MS and Data-driven Molecular Modeling

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Hydrogen/deuterium exchange (H/DX), in combination with mass spectrometry (MS), is commonly employed to probe the structure and dynamics of multicomponent assemblies. Despite its prowess, it may not be clear from the H/DX-MS data alone how to align the proteins in a complex. This criticism becomes particularly valid if conformational changes distal from the point of ligation manifests. To overcome this point of ambiguity and to achieve an accurate structural model of a protein complex, H/DX-MS methods should be partnered with alternatives. We investigated herein the utility of applying computational simulations, via the HADDOCK2.0 program, to the study of the actin-vitamin d binding protein (vDBP) complex using H/DX data during model building to bias the search. Briefly, simulations were driven using the PDB files of the individual proteins and the active/passive Ambiguous Interaction Restraints (AIRs) as structural inputs. Residue-specific AIRs were derived from theoretical controls and bottom-up H/DX-MS data. The latter required filtering of the statistically significant alterations in differential deuteration (based on relative solvent accessible surface area and proximity criteria) then sorting into a multitude of AIR subsets. The cluster and score outputs of the docking runs were subsequently assessed for their accuracy in returning the correct interfacial orientation. Preliminary data suggests that optimizing cluster size using a strategy of iterative AIR generation from theoretical/empirical data can be successful in generating an accurate high resolution structural model of a protein complex. Future work could involve extending this data-driven docking strategy to larger protein systems that lack an accepted structural model.

POSTER ABSTRACTS

Poster No. B-8

Proteomics in Biology

Cloning, expression and purification of the cytokine FLT3-ligand for protein interaction studies

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FMS-like tyrosine kinase ligand (FLT3L) is a cytokine responsible for the proliferation and differentiation of hematopoietic progenitor and stem cells. Most notably, the FLT3L-FLT3 receptor complex has been shown to be involved in several hematological malignancies including approximately 45% of acute myeloid leukemia cases, 15% myelodysplastic syndrome cases, and 5% of acute lymphoblastic leukemia cases. There is a need to express and purify large amounts of FLT3L to search for protein interactors that might serve as therapeutic targets. A truncated, soluble form of FLT3L that has been shown to be sufficient for ligand binding, was amplified from the full length sequence (ATTC) by PCR and cloned into a genomic integration and expression vector for the yeast Kluyveromyces lactis. Genomic integration of the construct was confirmed by PCR of K. lactis strains grown in antibiotic selective media. Expression and secretion of the 161 amino acid truncated FLT3L was analyzed by SDS-PAGE and Western blot and showed a 28 kDa band consistent with a high level of glycosylation as predicted from the primary sequence and literature. A yield of about 700 micrograms per 50 milliliters of culture was obtained. Purification of the expressed protein in preparation was performed by Ni-affinity chromatography. The purified FLT3 ligand will be utilized for protein-protein interaction studies in human body fluids and cell culture models of myeloid leukemia.

Poster No. B-9

Proteomics in Biology

The Fc receptor-cytoskeleton complex from Human Neutrophils

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The Fc receptor-cytoskeleton complex from Human Neutrophils

The Fc receptor directs the formation of a supramolecular complex within the pseudopods that exert force to reach out and engulf foreign particles. The Fc receptor complex and its associated phagocytic cytoskeleton machinery were captured from the surface of live cells by IgG coated microbeads and identified by mass spectrometry. A subfraction of the cytoskeleton and associated proteins encoded by the human genome was observed to be specific to the Fc receptor complex from human neutrophils. The isolated complex included specific forms of actin, the actin nucleation proteins such as WASP or ARP2/3, the capping protein gelsolin and bundling proteins like ataxin and fascin. Specific isoforms of the nucleotide dissociation proteins such as cofilin, thymosin and LIM were observed with the IgG-Fc receptor complex. Myosin motor proteins, both heavy chain and light chain, with the regulatory kinase were specific to the IgG coated microbeads along with tropomyosin, spectrin, desmin, nebulin, vimentin and desmoyokin. The proteins of the microtubule network, including tubulin, dynein, kinesin with its receptor kinectin and dynactin, were all observed with the activated receptor. Certain isoforms of known or suspected regulatory proteins, such as cortactin, filamin, villin, advillin, supervillin, contactin, as well as FERM domain proteins, were specific to the IgG-receptor complex. The detected cytoskeleton proteins, binding proteins and enzymes were used to predict the network of actin-associated regulatory factors. Signaling factors/proteins PIK3, PLC, GTPases (such CDC42, Rho GAPs/GEFs), annexins and inositol triphosphate receptors were all identified as being specific to the activated receptor complex by mass spectrometry. In addition, the tyrosine kinase Fak was specifically associated with the receptor supramolecular complex. Hence, it is possible to effectively capture an activated receptor cytoskeleton complex and its associated regulatory and effector proteins from the surface of a live human primary cells.

POSTER ABSTRACTS

Poster No. B-10

Proteomics in Biology

Studying Cdc42 Interactions by Formaldehyde Cross-linking and Mass Spectrometry

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The small GTPase Cdc42 plays an important role in regulating cytoskeletal organization and membrane trafficking in physiological processes such as cell proliferation, motility and polarity. Considering the many roles of Cdc42 in various biological processes, it is evident that it would form interactions with numerous proteins within the cellular context. There are yet several of these interactions to be verified and many additional interacting complexes to be characterized. Formaldehyde, a homo-bifunctional reagent, has consistently been used as a cross-linker of proteins in cells, tissue, and even entire organisms. It can readily permeate cell walls and membranes without external manipulation, resulting in efficient cross-linking. The general purpose of this project is to study Cdc42 protein interactions in living cells using formaldehyde cross-linking combined with immunoprecipitation. HEK293 cells transfected with constructs coding for myc-tagged Cdc42 wild type and dominant negative mutant were used in this approach. Large-scale quantitative mass spectrometry analysis was performed to characterize the protein interactions. To verify existing interacting partners and gain more information about novel interacting partners of Cdc42, clones of wild type Cdc42 were compared to empty vector. The results showed around 70 proteins interacting with wild type Cdc42 but not with empty vector, with about 30 of these proteins being known interacting partners of Cdc42. In addition, interactions of Cdc42 were compared between wild type and dominant-negative mutant; finding 51 and 46 interacting proteins respectively in common between two separate data sets.

Poster No. B-11

Proteomics in Biology

Determining the Absolute Stoichiometry of Phosphorylation Sites by Multiplex MRM - Studying Phosphorylation Events of Estrogen Receptor in Breast Canc

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Phosphorylation is a common and significant protein modification for regulating protein function. We have previously developed a method for the accurate and absolute quantitation of phosphorylation stoichiometry, phoshosphatase-based phosphopeptide quantitation (PPQ), based on enzymatic dephosphorylation, combined with specific and accurate peptide identification and quantification by MRM detection with stable-isotope-labeled standard (SIS) peptides. PPQ-MRM needs only one non-phosphorylated SIS peptide and two analyses (one for the untreated and one for the phosphatase-treated sample), from which the expression and % phosphorylation can be determined. This facilitates quantitation since only the abundances of the non-phosphorylated isoforms need to be compared. We have now expanded the estrogen receptor alpha (ER assay to 5 target phosphorylation sites (S154, S167, S282, S294, S559). These include 4 of the 7 target sites that have been found to be relevant to clinical breast cancer prognosis. Additionally, one of these sites, S154, cannot be currently assessed due to lack of an available antibody. We have assessed the assay using breast cancer cell lines and in vitro kinase reactions on recombinant ER protein. PPQ-MRM was compared to direct-MRM which requires the assessment of both phosphorylated and non-phosphorylated peptide isoforms. These assays revealed distinct phosphorylation profiles in breast cancer cell lines and showed the predicted differential kinase preference in in vitro kinase reactions using CK2 and AKT kinases. We plan to extend the assay to include other signaling proteins involved in breast cancer in addition to these and the ones we previously reported (HER2, RAF, and ERK1). We are now analyzing estradiol induced breast cancer cell lines with our new multiplexed assay covering these 9 phosphorylation sites and working on translating the assay to real breast cancer samples.

POSTER ABSTRACTS

Poster No. B-12

Proteomics in Biology

Elucidating Chromatin-Related Protein Networks in Human Cells

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In recent years it has become evident that not all heritable changes in gene activity are attributable to changes in DNA sequence(epigenetics), and that these changes play a critical role in human health and disease. In eukaryotic cells, DNA is wrapped around histone octamer cores to form nucleosomes. The nucleosomes are further compacted into higher order structures to form chromatin. The chromatin structure can either shield or expose the DNA to proteins in the cell and therefore can control any process occurring on the DNA,including replication, transcription and repair. Alterations to the chromatin structure are tightly regulated. Defects in appropriate chromatin regulation have been implicated in a wide range of human disease including cancer, neurological and congenital disorders. In spite of our expanding knowledge of epigenetics, human chromatin modification pathways have not been well characterized. Here we describe a large scale proteomics approach aimed at building a high-quality protein-protein interaction network of chromatin-related proteins in human cells. This approach uses a lentiviral transduction system, developed in our laboratory, to introduce affinity tagged human ORFs into cultured human cell lines, where they are stably expressed. These tagged ORFs are then subjected to protein affinity purification using a two-step protocol, followed by gel-free mass spectrometry(MS) analysis, to identify physically interacting proteins. Our target list consists of approximately 700 genes and includes known and predicted chromatin modifiers as well as other proteins involved in processes which occur in the context of chromatin, such as DNA repair. To date we have made lentiviral constructs for 453 target genes. We have completed purifications for over half of those (267) and have MS results for a total of 205 genes. Purifications done to date have successfully identified known protein complexes and identified new components of known molecular pathways. The recent data as well as validation experiments will be presented.

Poster No. B-13

Proteomics in Biology

Proteomic Assessment of Endogenous Microtubule Regulation in the Mitotic Spindle

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Microtubules are targets of cancer therapy due to their role in the formation of the mitotic spindle. The current understanding of the mechanism of action of microtubule antimitotics involves their ability to disrupt microtubule dynamics. In a recent study, the discovery of a fourth ligand binding site on microtubules was described. This site is located on the exterior of the microtubule and is adjacent to the MAP-binding C-terminal E-hook on √é¬≤-tubulin. It is the first stabilizing binding site on microtubules that is fully removed from the taxoid site. Stabilization of microtubules with the two classes of microtubule stabilizers was found to be indistinguishable from the perspective of conformational dynamics. However, these identical maps should not be taken to indicate an identical mechanism of stabilization as these drugs have been found to have differential effects in vivo. Therefore, the two ligands may induce stability in different ways. The purpose of this study is to identify possible endogenous regulators of microtubules in mitosis in the presence of different microtubule stabilizers. Using a model of mitotic spindle microtubules, proteomics will be used to identify differences in proteins associated with the microtubule in the presence of the two classes of microtubule stabilizers.

Poster No. B-14

Proteomics in Biology

Proteomics based prognostic signature of head and neck cancer

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Clinical cancer proteomics has the potential to discover,

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identify, and quantify novel biomarkers for early detection, diagnosis, prediction of the clinical outcome, and to develop novel therapeutic targets. Despite therapeutic interventions, the five-year survival rate of head and neck cancer patients is less than 50%, resulting in poor prognosis. Using high-throughput proteomics, we identified a panel of biomarkers in head and neck squamous cell carcinoma (HNSCC). This panel of markers was further verified in a large independent cohort of paraffin embedded head and neck cancer tissues (n=100) using immunohistochemistry (IHC). Our results demonstrated overexpression of a panel of proteins including 14-3-3zeta, 14-3-3sigma, heterogeneous nuclear ribonuclear protein K (HNRNPK), S100A7 and prothymosin alpha (PTMA)] in HNSCC patients. Kaplan Meier survival analysis revealed shorter disease-free survival (DFS = 4 mths.) in patients showing overexpression of the panel of proteins in comparison to patient cohort not showing overexpression of this panel (DFS = 41 mths., $p < 0.001$). Positive predictive value (PPV) and Negative predictive value (NPV) further verified the reduced disease free survival of these patients. Logistic regression analysis clearly demonstrated the relevance of this panel as the best prognostic signature for HNSCC patients ($p < 0.001$, Hazards ratio, H.R.=19.6, 95% C.I.= 2.3 - 8.5) in comparison to combinations of 2 - 4 markers and clincopathological parameters. In conclusion we identified, verified and developed a proteomics-based prognostic signature for HNSCCs which may find its utility in follow-up clinics to accurately predict the recurrence of the disease after primary treatment.

Poster No. B-15

Proteomics in Biology

Using Proteinase K for Non-Specific Digestion and Comprehensive Identification of Interpeptide Crosslinks: Application to Prion Proteins

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Using proteinase-K and the isotopically-labeled, affinity-purifiable, CID-cleavable crosslinker CBDPS (Creative Molecules, Inc.), we were able to differentiate crosslinks from the very complex peptide mixture which is produced by this non-specific enzyme. This strategy requires all of the features of this crosslinker, and produced high sequence coverage and a mass distribution of crosslinked peptides which is very suitable for mass spectrometry. Using MALDI-MS and MS/MS, we detected >60 total crosslinks, which include several sets of 'ìconfirmatory' crosslinks corresponding to linkages between the same two amino acids. Three of the 9 crosslinked pairs found in PrPC are exclusive to PrPC;.6 pairs are found in both PrPC and PrP; 6 of the 12 pairs found in PrP are exclusive to PrP. In comparison, previous crosslinking studies using trypsin produced no crosslinks in the 500-4000 Da mass range from prion proteins.

Included among the crosslinked pairs unique to PrP is a K185-K220 crosslink from the C-terminal portion of the protein. In the native structure, the distance between K185 and K220 is over 26, and these residues are situated on opposite surfaces of the protein, separated by the aa127-aa165 loop. A conformational change involving unfolding of the aa127-aa165 loop, however, would expose residues K185 and K220 for crosslinking with CBDPS, thus confirming this conformational change. We are currently in the process of determining whether this is an inter- or intra-molecular crosslink. In either case, this conformer-specific crosslink may be useful for future development of a method for detection of infectious prions in tissue samples.

Poster No. B-16

Proteomics in Biology

Development of a Method for Quantitative MALDI

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Introduction:
Imaging Mass Spectrometry (IMS) is a technique that allows for 2-dimensional spatial resolution of proteins, peptides and small molecules in tissue sections. In IMS a spectrum is acquired at each of a regular series of points across a section of tissue. Each spectrum contains molecular weight and intensity information representative of the analytes present at that position. Specific ion images are generated from a plot of the intensity of any measured ion as a function of individual pixel location. Initially, only MALDI-TOF instruments were used for imaging, however, applications now include MALDI coupled to FTICR, Q-TOF and triple-quadrupole instruments.

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Here we describe a protein quantitation method using MRM MALDI imaging with a triple-quadrupole mass spectrometer fitted with a MALDI source.

Methods:
Four peptides from an in-situ trypsin digestion of a 10-ºm section of rat brain were characterized by MALDI-TOF imaging on the Bruker UltraflexIII. Stable Isotopically-labeled standards (SIS) and non-labeled standards of the peptides were synthesized. The purity and accurate concentration of the standards was determined by amino acid analysis, capillary electrophoresis and MALDI-MS. A standard was sprayed upon a rat brain tissue section with the Bruker Imageprep for a uniform application. An in-situ trypsin digestion was performed on the tissue section followed by CHCA matrix application. The section was then analyzed in MRM mode with the Applied Biosytems QTRAP 4000 equipped with a MALDI source. Data was analyzed using the Bruker fleximaging software as well as custom built software.

Preliminary Data:
Previously we have shown, using MALDI-MRM, that peptides obtained by in-situ trypsin digestion and stable-isotope labeled standard (SIS) peptides can be accurately detected in a multiplexed manner while maintaining the spatial resolution. In a rat brain tissue section an ion signal showing a distinct pattern was observed by MALDI-TOF imaging and identified as myelin basic protein (MBP) by MS/MS analysis. A trypsin digested serial section was sprayed with a SIS peptide version of an MBP peptide. The MALDI MRM image acquired from the section demonstrated the ability to image both the SIS peptide and the peptide from the trypsin-digested MBP. Two-dimensional images were created in heat-map style format. Three-dimensional images were created with x and y coordinates showing ion signal location and z coordinates showing intensity.

The imaging quantitation method is based on spraying the tissue sample with a SIS peptide followed by in-situ digestion and MALDI-MRM analysis. To determine precisely the amount of SIS peptide per area sprayed, a standard curve was made that was based on the ion intensity ratio between the known amounts of unlabeled peptide spotted onto a target to the SIS peptide sprayed onto the target.

In order to define the lower limits of detection and quantification (LOD and LOQ), a second calibration curve was created from conditions that emulate the background relevant to the MBP quantification procedure. The method used to acquire the data for this calibration curve was similar to the quantitation of the SIS peptide, except a larger concentration range of unlabelled peptide was spotted onto thin sections of liver tissue followed by in-situ digestion and MALDI-TOF analysis. The LOD and LOQ will be calculated from the calibration curve using the empirical method described by Armbruster et al. (1994), as well as a statistical approach described by Anderson (1989).

Reference:
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Poster No. B-17

Proteomics in Biology

Mass spectrometric analysis of relative protein expression levels in glioblastoma multiforme tissues and lung cancer brain metastases

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Brain cancer is one of the leading causes of cancer-related death in North America. Canadian cancer statistics reported an incidence rate of 2,600 new cases of brain cancer and 1,750 deaths from it for the year 20101. Approximately 10% of cancer patient develop brain metastasis during the course of illness2. Here we investigate differentially expressed proteins in glioblastoma multiforme (GBM) tissues versus brain tissues of metastasized lung cancer.

Twenty brain tissue homogeneous were divided into five sets each containing two GBM and two brain metastasis samples. Individual samples were trypsinized and labeled with the appropriate iTRAQ tags. The four labeled samples in the same group were pooled and separated off-line by strong-cation exchange chromatography (SCX) into 20 fractions. The fractions were then subjected to on-line reverse-phase (RP) chromatography using a 15 cm x 75 µm column packed with 3 µm C18 beads. For each fraction, the m/z ratios and elution times of resulting peptides from the previous runs were combined and imported into an exclusion list for subsequent analyses.

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Candidate biomarkers should be detected 50% of sample analysed and with 50% showed differential expression. 1,262 unique proteins were identified after three iterative runs, 61 of which were significantly differently expressed; 23 proteins were overexpressed in GBM and 38 in the metastatic samples. A number of these proteins such as GFAP, vimentin and MARCKS are known to be differential expressed3, while others appear to be novel findings including PCM13 and PCM16, both were underexpressed, and PCM59 which was overexpressed.

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Poster No. B-18

Proteomics in Biology

Absolute quantitation and validation of serum C19orf10 levels in Rheumatoid Arthritis patients using Multiple Reaction Monitoring

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Introduction. In rheumatoid arthritis (RA) patients, joint inflammation and destruction has been associated with the deregulation of the fibroblast-like synoviocytes (FLSs). Studies on FLSs have contributed considerably towards an understanding of the mechanisms that underlie synovitis. Our group identified a novel protein product of chromosome 19 open reading frame 10 (C19orf10) through proteomic analysis (2D-PAGE and LC-MS/MS analysis) of FLSs, 1. Immunflouresence and immuno-histopathology confirmed the presence of this protein in cultured FLSs, and in RA and osteoarthritis patient synovial tissue sections 2. In addition, double-staining in situ analysis demonstrated the presence of c19orf10 specifically in FLSs, whereas macrophages, B cells, or T cells produced insignificant amounts of this protein. C19orf10 is secreted into the vascular space and the extracellular matrix surrounding the synovial lining. These observations make C19orf10 an interesting candidate to study for its association in RA.

Method. Due to reagent limitations, we could not establish a quantitative ELISA assay to determine C19orf10 levels in patient serum and synovial fluids. Therefore, we set out to utilize Multiple Reaction Monitoring (MRM) for an absolute quantitation of C19orf10 an alternative approach to ELISA. Using C19orf10 protein sequences of six non-tryptic peptides were selected for an initial MRM screening. Based on the intensity of detection, the three best peptides were finalized for further optimization studies. Isotopically labeled synthetic peptides were custom synthesized, with terminally labeled Lysine (U-13C6, U-15N2), and had a mass difference of 8.014 Da. These isotopic peptides were used for optimization of C19orf10 MRM assay parameters such as collision energy, de-clustering potential, and known amounts of peptides were spiked in reduced, alkylated and trypsin digested serum samples. The detection of both isotopically labeled and endogenous peptides was performed using QTRAP-4000 mass spectrometer.

Preliminary Data. MRM analysis of C19orf10 in 30 healthy control serum samples confirmed the presence of this protein in low femto-mole range (50-100 fmol/√Ç¬µl). However, in RA patients receiving anti-TNF alpha therapy (infliximab treatment) for three months, C19orf10 levels were found to be elevated in the range of 750-1250 fmol/µl. MRM analyses of serum samples from 20 RA patients at base line (i.e. before receiving any treatment) are in progress. Differential regulation of C19orf10 in RA raises a possibility of establishing it as a disease or therapeutic biomarker for RA.

Novel Aspect. We have demonstrated the presence of C19orf10 in serum and its potential association with Rheumatoid Arthritis. We have successfully applied MRM as a method to quantify new candidate molecules such as c19orf10 in low fmol concentration.

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Acknowledgements. The Canada Foundation for Innovation, The Health Sciences Centre Foundation, Winnipeg, Canada.

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Poster No. B-19

Proteomics in Biology

Evaluation of Dithranol as a New MALDI Matrix for Tissue Imaging of Endogenous Metabolites by Fourier-Transform Mass Spectrometry

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MALDI-MS is an important tool for the spatial profiling and imaging of biological macromolecules in situ, but most MALDI matrices generate signals in the low-mass region that interfere with small-molecule analysis.
We found dithranol (1,8-dihydroxy-9,10-dihydroanthracen-9-one) to be the best of the seven matrices studied (3-HPA, SA, CHCA, THAP, DHB, 9-AA, and dithranol) for FTICR-MS MALDI imaging of endogenous metabolites, specifically lipids. Saturated dithranol solutions in CH3Cl:MeOH at high or low pH generated comparable results for detecting metabolites above m/z 300 in positive ionization mode. Several dozen to >100 metabolites were observed in rat heart, liver, and kidney. In contrast, other matrices produced either weak signals or no signals under the same experimental conditions.
In equatorial sections of bovine lens tissue, several compounds were identified as sphingomyelins by accurate mass, including SM (18:1/14:0) [m/z 713.49954; M+K]; SM (18:1/16:0) [m/z 7.41.53078; M+K]; SM (18:1/18:0) [m/z 769.56214; M+K]; SM (18:1/20:0) [m/z 797.59360; M+K]; SM (18:1/22:0) [m/z 825.62486; M+K]; SM (18:1/22:1) [m/z 823.60918; M+K]; and SM (18:1 /24:1) [m/z 851.64037; M +K]. These ions all showed similar spatial distributions in the tissue, with the highest abundances around the inner cortex, and lower abundances elsewhere. In contrast, the detected acylcarnitines (e.g., palmitoylcarnitine and oleoylcarnitine) showed unique localization patterns, with abundant signals around a thin layer in the middle cortex, and were undetectable elsewhere. This region conformed to a previously described hydrophobic barrier in mammalian ocular lenses.
This work has demonstrated the usefulness of dithranol as a matrix for MALDI tissue imaging of endogenous metabolites by FTICR-MS.

Poster No. B-20

Proteomics in Biology

PROTEOMIC PROFILES IN EXCRETED AND CIRCULATING PROTEIN LEVELS ASSOCIATED WITH WEIGHT REDUCTION IN ADULT SAUDI SUBJECTS

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Food is essential for life and provides the nutrients for growth, energy and healing, but regular overeating results in the accumulation of excess fat on the body. Obesity is now a worldwide epidemic that adds billions to health care costs, decreases the quality of life, and lead to a variety of serious conditions including diabetes mellitus, metabolic syndrome, cardio-vascular diseases, and some forms of cancer.1 Although bariatric surgery is a drastic and invasive approach, it is currently considered the most successful therapeutic approach for obesity, especially when other means fail to achieve and maintain a healthy body weight. On a positive note, bariatric surgery successfully reduces not only the body weight, but also the obesity associated co-morbidities. Proteomics tools offer an opportunity to improve our understanding of complex pathophysiologic changes, can aid in the discovery of new diagnostic and prognostic biomarkers of obesity, and also provide an opportunity to identify novel targets for therapeutic intervention.

We have compared changes in proteins' profile following two interventions: i.e., pre- and post- calorie restriction and pre- and post-bariatric surgery, using two approaches; targeted multiplexed ELISA assays and proteomics techniques. The first approach demonstrated that dietary restriction induced improvement of all the proinflammatory markers measured, with the exception of resistin, plasminogen activator inhibitor-1 (PAI-1), and interleukin (IL)-1. On the other hand, post bariatric surgery; resistin, tumor necrosis factor-alpha, IL-1 and adiponectin all increased; C-peptide, leptin and PAI-1 all decreased; and no significant changes were detected in IL-6, insulin and C-reactive protein (CRP) levels. Urinary protein excretion in clinically obese patients shows some distinct changes following surgical intervention. A greater number of proteins change with surgery than by diet, but this could simply reflect the more substantial weight loss in this group rather than a mechanistic difference.To list just few examples; retinol binding protein 4 (RBP4) was significantly increased

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post- surgical intervention, while not in the calorie-restricted group; Zinc-alpha2-glycoprotein levels were differentially affected post surgical intervention, but not in the dietary intervention group; an increase in a series of enzymes which play key roles in carbohydrate and protein metabolic pathways were also observed after bariatric surgery; and multiple variant forms of albumin were detected with diverse abundance in both interventions groups. These changes, and several others, might highlight differences in body responses to weight reduction methods.

In summary, by combining a targeted multiplexed ELISA assays and proteomics approach to analyze several key proteins, we have identified a set of proteins that mark weight loss following dietary and the surgical approaches. These proteins may be thought of as markers that would help in better defining the molecular bases of obesity, and identifying the unique biochemical consequences of bariatric surgery.

Poster No. B-21

Proteomics in Biology

No hiding place: strategies for global absolute proteome quantification

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As proteomics moves from the descriptive, discovery phase to a quantitative phase, there is increasing pressure to provide reliable quantitative data about the abundance of proteins within a cell. Most mass spectrometry-based methodologies are based on the principle of surrogacy, in which a peptide, usually tryptic, is used as a measure of protein abundance. Peptide abundance or number can be used in label-free quantification workflows, but reliable label-free data reach a statistically limited level far higher than that needed for deep proteome quantification.

The gold standard for mass spectrometry based quantification, that which provides maximal sensitivity, is derived from isotope dilution approaches based on stable isotope labelled √¢,Ç¨Àúquantotypic√¢,Ç¨,Ñ¢ peptides as chemically identical, mass resolved, surrogate standards. Whilst simple to state and comprehend, delivery of robust workflows that achieve the extreme sensitivity needed for deep quantitative proteomics is highly challenging, and the pitfalls encountered en route are manyfold and substantive.

We present an overview of the construction of absolute quantification workflows, emphasizing challenges in sample preparation, standard generation and MS based analyses. In 2005 QconCATs were introduced, a new approach to the generation of multiplexed stable isotope labeled standards, creating the peptides as products of artificial, chemically synthesized genes that are expressed and labeled heterologously. Subsequently, co-proteolysis of analyte and QconCAT permits simultaneous quantification of many proteins, for a modest expansion of the protein pool that has to be analysed (typically less than 1%). QconCAT workflows are now being applied to absolute quantification of an entire proteome (using 200 QconCATs for standard generation). This invokes the selection of optimal quantotypic peptides, the critical importance of complete proteolysis, the development of optimal SRM assays for deep proteome quantification and the quantitative relationship between label-mediated and label-free methods.

Poster No. B-22

Proteomics in Biology

Analysis of Protein Profile Changes in Maternal Plasma samples

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Exposure to environmental chemicals is associated with negative health effects including reproductive health. Maternal-Infant Research on Environmental Chemicals (MIREC) project was initiated to define a national profile of in utero and lactational exposure to environmental contaminants and to investigate potential impacts on pregnancy outcomes. The hypothesis of this study is that exposure of mothers to pollutants such as heavy metals will result in oxidative stress and endothelial dysfunction, with potential alteration of utero-placental perfusion, and affect fetal development. Low birth weight is an indicator of the general health of newborns, and a key determinant of infant survival, health and development. We report here on the analysis of the third trimester maternal plasma samples for

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proteomic changes. A subset of plasma samples (N=90) obtained at third trimester from pregnancies in ten medical centres across Canada were stabilized at recovery and analyzed in our laboratory for markers of oxidative stress, endothelial dysfunction and inflammation through analysis of peptide and protein changes (HPLC-fluorescence/EC array, protein array, MALDI-TOF-TOF-MS). Interestingly, our preliminary results revealed that among the maternal plasma proteins analyzed MCP-1, MMP-9, and VCAM exhibited negative associations with infant birth weight. Protein markers of inflammation MCP-1 and MMP-9 were elevated ($p<0.05$) in plasma of mothers with infants in the low 10th percentile of birth weight, <2.7kg. MCP-1 and MMP-9 are implicated in endothelial dysfunction as well as cardiovascular diseases, and are linked to oxidative stress scenarios. Our results support the view that proteomic changes relevant to oxidative stress and inflammation in pregnant mothers is associated with impacts on fetal development, resulting in lower birth weight. The relationship between oxidative stress and inflammation in mothers and their exposure to chemicals remains to be elucidated.

Poster No. B-23

Proteomics in Biology

Crossing four membranes: Insights from the N-terminome of a diatom producing oxygen on a global scale

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Diatoms are ecologically vital eukaryotic microorganisms that are responsible for 20% of global photosynthesis. Diatoms have acquired their plastids by secondary endosymbiosis. Most of the plastid proteins are nuclear-encoded and must be imported into the chloroplast across four membranes compared to two in higher plants. Diatom plastid proteins therefore have bipartite targeting signals made up of an ER signal peptide followed by a transit peptide sequence which are cleaved off during protein transport. A number of studies showed that the transit peptide generally starts with a Phe and is enriched in positively charged and hydroxylated amino acids. However, there is little information in the literature about the cleavage site because mature N-termini are known for very few diatom proteins.

Our laboratory has recently developed a proteomic method for selective enrichment and identification of protein N-terminal peptides using a novel polymer for proteomics (Kleifeld et al. Nat Biotechnol. 2010). Here we have applied TAILS to characterize the N-terminome of the diatom model organism Thalassiosira pseudonana. Our data provides insight into the N-end rule of diatoms, the extent of protein N-terminal acetylation and the degree of proteolysis as a post-translational modification. N-termini of mature plastid proteins, identified in our datasets based on existing functional annotation information and the presence of a putative bipartite signal sequence, reveal transit peptide sequences and allow us to propose a consensus cleavage site motif for the elusive transit peptide peptidase. This provides a more detailed understanding of how proteins cross all four membranes into the plastid stroma.

Poster No. B-24

Proteomics in Biology

Novel proteomics approach to dissect the interplay between protein SUMOylation and ubiquitinylation

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Small Ubiquitin-related MOdifier (SUMO) is a family of ubiquitin-like proteins that are involved in various cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, and protein stability. Three main SUMO paralogs can modify proteins via the formation of an isopeptide bond between the C-terminal glycine residue of SUMO and an acceptor lysine on the target proteins. Several substrates have been identified including PML, Mdm2, c-Jun, and p53 whose misregulation can lead to tumorigenesis and metastasis. The identification of SUMOylation sites by mass spectrometry (MS) remains challenging due to the low occurrence of this modification and the presence of long SUMO C-termini lacking arginine/lysine residues. In the present work we describe a novel dual affinity approach to enrich SUMOylated peptides in large-scale proteomics experiments. This innovative approach is based on the expression of functional SUMO

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isoforms that comprise a 6xHis-tag at the N-terminus and mutations near the end of the C-terminus of the expressed proteins. These mutations introduce an arginine residue near the C-terminus of SUMO which upon tryptic digestion leave a short five amino acid remnant attached to the lysine of SUMOylated proteins. The modified tryptic peptides can be subsequently enriched using antibodies specific to each SUMO stubs. HEK293-SUMO3 mutant cells exposed to the proteasome inhibitor MG132 enabled the identification of more than 120 SUMOylation sites (83 protein substrates) compared to only 17 sites (12 protein substrates) without MG132. The comparison of cells treated or not with MG132 revealed that 60% of identified SUMOylated proteins showed an increase in abundance upon proteasome inhibition whereas a decrease was observed for less than 5%. These results suggest that a large number of SUMOylated substrates including PML, p53, and parafibromin are degraded via the proteasome pathway. Our analyses also revealed that 35 % of identified sites do not have a consensus KX(D/E) represent an hydrophobic residue) motif recognized by Ubc9 conjugating enzyme. This novel proteomics approach thus provides a unique platform to profile changes in protein SUMOylation from human cells.

Poster No. B-25

Proteomics in Biology

Study of interactive patterns of ZBTB7 gene product with HIV1 viral activator Tat and ARF tumor suppressor

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The transcription repressor protein ZBTB7 (FBI1, BTB-ZF protein LRF) known as a Pok√É¬©mon is critical factor in onco-genesis, which is encoded by ZBTB7 gene. ZBTB7 processes by controlling the pathways that are required to transform normal cell to cancerous one. It produces its effects by repressing the function of other proteins including a tumor suppressor protein ARF leading to lymphomas and other oncogenic effects and present in very high level in certain type of B cell. Also FBI-1, the factor binding to Inducer of Short Transcript (ISt-1) interacts with site on the HIv-1(i.e.HIV1 viral activator Tat). Activation of Tat, represses the synthesis of short RNAs. SMRT domain binds with ZBTB7a and interacts with ARF tumor suppressor gene which leads to the cancer proliferation. In this paper, a method to suppress the activity of ZBTB7a protein (LRF) from accessing ARF protein is devised. The protein-protein interactions between LRF and one of its co-repressor proteins, SMRT (Silencing mediator of retinoic acid and thyroid hormone) as well as between LRF and modified SMRT, which was derived from wild type SMRT, was studied by docking analysis.

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Poster No. I-1

Proteomics at the Interface of Biology and Medicine

Secreteome Database: a cacer-oriented database of secretome proteomes for potential biomarker discovery

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Cancer secretome, proteins secreted by cancer cells or tissues, is a promising source for cancer biomarker discovery. With the efforts of high-throughput technology, large amounts of secretome proteomic data have been accumulated. We have developed SecreteBase, a secretome database, to systematically collect these data for further investigation. Secreted proteins and peptides identified by mass spectrometry are collected. For each protein, annotations and online resources are integrated and proteomic validations such as results of immunohistochemistry and western blot are also included if available. These data can be queried and browsed via a web-based interface. Moreover, lists of proteins can be retrieved according to user preferences and shown visualized comparison. Cancer-specific proteins or common proteins across cancers can thus be selected as potential markers via the comparison. There are now 4,422 proteins identified in 23 cell lines derived from 11 cancer types available in SecreteBase. It is cancer-oriented and web-based that can serve as a reference database for cancer biomarker discovery.

Poster No. I-2

Proteomics at the Interface of Biology and Medicine

Characterization of mitotic centromere-associated kinesin on the microtubule lattice by hydrogen-deuterium exchange mass spectrometry

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The assembly of microtubules is a dynamic process in that microtubules are constantly polymerizing and depolymerizing in a tightly regulated manner. This process is partially regulated by MCAK, a kinesin 13 protein, that localizes at spindle poles, kinetochores, and microtubule ends to facilitate depolymerization. This localization is coupled to the formation and maintenance of the bipolar spindle. MCAK depolymerizes microtubules by individually removing tubulin dimers along the protofilament, which is catalyzed by ATP hydrolysis. The mechanism of microtubule depolymerization by MCAK remains unclear, and it is unknown whether MCAK-MCAK cross-protofilament interactions occur on the microtubule lattice. To investigate the MCAK-tubulin interactions on the microtubule lattice, a co-sedimentation assay was performed to determine the binding stoichiometry, while peptide-based hydrogen-deuterium exchange mass spectrometry was used to map the sites of differential exchange. In regards to the latter, reporters of H/D exchange spanned 80 and 87% of the MCAK and tubulin seuqence, respectively. From the differential analysis, insight into the structure and dynamics of the MCAK-tubulin interaction was achieved, which paves the way toward a better understanding of MCAK depolymerization. The impact of this research could lead toward the development of new drug targets for anti-tubulin chemotherapeutics.

Poster No. I-3

Proteomics at the Interface of Biology and Medicine

Functional comparison of IgG verses oxLDL phagocytic receptors in human U937 macrophages

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Phagocytic macrophages bind to the solid form of oxidized low density lipoprotein (oxLDL) deposited on the arterial intima and become activated releasing free radicals that further oxidize LDL leading to foam cell formation and atherosclerosis. Atherosclerosis is the largest cause of morbidity and mortality in Canada. Upon oxidation LDL is no longer recognized by the native LDL receptor and gains a high affinity for phagocytic scavenger receptors, such as CD36, that are innate (inherited) non-self receptors. Oxidized low-density lipoprotein deposited on the wall of large arteries may be engulfed by scavenger receptors on monocyte-derived macrophages requiring enzymes in the phagocytic pathway including phospholipases, kinases and g proteins associated with the cytoskeleton. The specific isoforms of SRC, SYK, PI3K, PLD, PLC, FAK, PAP-1 and other enzymes that might be drug targets in the oxLDL

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engulfment pathway are unknown. The mechanism of oxLDL internalization was examined with monocyte derived macrophages in vitro using PMA treated U937 cells with latex microbeads coated with oxLDL or Immunoglobulin G (IgG) over the time course of engulfment. The oxLDL and IgG coated beads were quantified using immunofluorescent staining and laser confocal assays. The kinetic of IgG-mediated engulfment was more rapid and complete compared to oxLDL beads indicating a qualitatively different internalization pathway. Both IgG and oxLDL pathways were actin dependent and the filamentous actin inhibitors Cytochalasin D (10μM) and Latrunculin B (5μM) completely inhibited phagocytosis of both oxLDL-coated and IgG-coated beads. The Src and Spleen tyrosine kinase (SYK) inhibitor 3,4-methylenedioxy-beta-nitrostyrene (20 μM), the phospholipase C inhibitor U73122 (5 μM), the Janus kinase inhibitor AG 490 (25 μM) and the general protein tyrosine kinase inhibitor genistein (35 μM) displayed significant inhibitory effects on the phagocytosis of both IgG and oxLDL microbeads. The specific isoforms of the receptor associated SRC, SYK, Janus and PLC enzymes that were associated with the IgG and oxLDL coated microbeads were determined and quantified by nano LC-ESI-MS/MS with an LTQ ion trap.

Poster No. I-4

Proteomics at the Interface of Biology and Medicine

A Secretome Database for Cancer Biomarker Discovery

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Cancer cell secretome, group of proteins released by cancer cells or tissues, is a promising source for discovery of cancer biomarker candidates. With the efforts of high-throughput technology, large amounts of secretome proteomic data have been accumulated. To facilitate the identification of potential cancer markers, we developed a secretome database by systematical collection of cancer-cell secreted proteins detected by mass spectrometry. Each protein is integrated with annotations and online resources; proteomic validations are also included if available. This database can be queried and browsed via a web-based interface. Moreover, lists of proteins can be retrieved according to user preferences and shown visualized comparison. With the comparative tools introduced into the database, specific or common proteins across cancer cells or types can further be selected quickly for evaluation as potential markers. Until now, ~4500 proteins identified in the conditioned media of 23 cancer cell lines derived from 11 cancer types are available in this database. It is a cancer-oriented and web-based database that can serve as a reference repository for cancer biomarker discovery.

Poster No. I-5

Proteomics at the Interface of Biology and Medicine

Development of a CZE-based protein fractionation strategy with off-line peptide mapping for microscale sample preparation of complex mixtures

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Top-down proteomics methods are highly efficient and digestion-free but require high-resolution mass spectrometers that are expensive. Therefore, bottom-up methods are still commonly used. These rely on digestion of all the proteins in a sample and separation by 2D-LC coupled to MS to identify the peptides. The high complexity of the digest requires sophisticated computational methods to infer proteins from the detected peptides. To address complexity, we are developing a small-scale, 4-step protein fractionation/digestion system as follows: 1) a mixture of proteins is fractionated into ‘F’ equal portions under ‘B’ different buffer conditions to obtain an array of B x F fractions that each contain much fewer proteins than the starting mixture; 2) a small portion of each fraction is submitted to MS to identify protein masses (overlap between fractions is inevitable); 3) the remainder of each fraction is digested in an immobilized enzyme microreactor followed by peptide mapping by CE, MALDI-MS or LCMS; 4) the peptide maps and protein masses from each fraction are used to identify proteins using MASCOT and simple chemometrics, e.g., principle components analysis. To test the strategy, a simple mixture of protein standards (BSA, carbonic anhydrase, α-lactalbumin, β-lactoglobulin) and 30 collected fractions was envisioned, i.e., B = 3 buffer conditions and F = 10 fractions for each buffer.

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Multiple injections for a given buffer must be made and the fractions pooled to accumulate sufficient material. This presentation will discuss our efforts to establish a stable electro-osmotic flow using dynamically coated capillaries, assessment of the total protein mass collected in each fraction for native and fluorescently labeled proteins and the efficiency of digestion of fractionated proteins using immobilized enzymes.

Poster No. I-6

Proteomics at the Interface of Biology and Medicine

Structural Proteomics Characterization of Prion Protein Aggregation

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Introduction.
The central element in the development of the prion diseases is the conversion of the cellular prion protein (PrPC) into an aggregated pathological fibril-forming isoform (PrPSc), which leads to the accumulation of amyloid fibrils in the central nervous system and eventually to death. The exact molecular mechanisms which lead to the conformational change, as well as the final structure of the aggregates, are still unknown.

We are characterizing PrPC conformational changes and the 3D structure of the prion aggregates using a structural proteomics approach: a combination of protein chemistry and mass spectrometry (MS). We have applied a combination of limited proteolysis, surface modification, chemical crosslinking, and hydrogen/deuterium exchange with MS methods for the characterization of native prions and prion aggregates.

Methods.
Native and Î²-oligomeric prion protein, PrPC and PrPÎ², respectively, were obtained from PrioNetâ€™s PrP5 facility (University of Alberta). For chemical surface modification, proteins were modified with our isotopically-coded amine-reactive reagent pyridinecarboxylic acid N-hydroxysulfosuccinimide ester (PCASS), then digested with trypsin, chymotrypsin, or pepsin and analyzed by MALDI MS. For limited proteolysis, native and Î²-oligomer forms of prions were digested with trypsin or pepsin. Digestion sites were determined by MALDI-MS and MSMS analysis of in-gel digests of SDS-PAGE separated proteolysis products. HDX using FTMS-ECD top-down analysis was performed on Bruker 12 Tesla FTICR mass spectrometer. Crosslinking analysis was done by LC-MALDI-MS and MS/MS of the crosslinked protein digests on an AB 4800 MALDI TOF/TOF.

Preliminary results.
We detected several residues modified with our water-soluble PCASS reagent that were differentially-modified between the native and Î²-oligomeric forms. In addition to Y149 and Y150, we found differential modification of Y157 and Y162 in the second YYR motif in the Î²-oligomeric form.

We observed a different pattern of limited proteolysis between the native and Î²-oligomeric forms of the prion protein: with trypsin, limited proteolysis of the native soluble form of the protein rapidly removed the flexible N-terminus, while for the Î²-oligomeric sample, this cleavage was markedly slower. With pepsin, virtually no proteolysis was observed for the native form. However, for the Î²-oligomeric form, rapid accumulation of a C-terminal ~6 kDa product was detected.

We also have characterized these PrP preparations using top-down FTMS with ECD. We have been able to achieve 100% sequence coverage, which ensures that we can determine the degree of exchange for every amino acid residue. Preliminary analysis of the native monomeric and Î²-oligomeric samples showed differences in the exchange rates of certain residues which are localized in the C-terminal portion of the protein.

Using BS3-H12/D12, we were able to detect several crosslinks that were different for the native and Î²-oligomeric forms of the PrP 90-232. These crosslinks were between the flexible N-terminal part of the molecule and the C-terminal portion of the molecule. Using our isotopically-coded, biotinylated, and CID cleavable crosslinker CBDPS, we were able to detect several crosslinks that were unique to the each form of the prion protein, including a K185-K220 crosslink in the C-terminal portion of the protein which is specific to the Î²-oligomeric form.

These various structural proteomics approaches have already provided valuable and complementary experimental data on the structure of prions. These approaches have already generated information on the conformational changes involved in the conversion of PrPC to PrPSc.

Novel Aspect.
The complementary data from multiple structural proteomics methods has been generated on prion protein conformational changes.

POSTER ABSTRACTS

Poster No. C-1

Proteomics for Clinical Applications

A quantitative metabolomic study on transgenic mice urine samples of Alzheimer's disease by dansylation labeling and LC-FTICR-MS

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Alzheimer's disease (AD) is one of the common neurodegenerative diseases among older people. Currently there is no definitive biomarker available for clinical use. Metabolomics has been used for biomarker discovery in human samples of AD. However, some confounding factors such as diet, environment, may complicate the human study. Animal models of AD, for example, transgenic mice models, will be useful since it enables a well-controlled study. A differential 13C-/12C-isotope dansylation labeling combined with LC-FTICR-MS and automated data processing method has been developed in our group. Here we report a quantitative metabolomic biomarker discovery study for the transgenic mice urine samples of AD based on dansylation labeling and LC-FTICR-MS. We were able to detect and quantify 950 unique ion pairs in 10 uL samples, which corresponded to 950 metabolites containing primary and secondary amines and phenols. Chemometrics analysis showed PCA model could separate the male and female mice urine samples, which was consistent with previously reported data from other metabolomic studies of mice biofluids. OPLS-DA model showed that there was a distinct separation between the APP mutant group and the wild type group. The metabolomic changes observed between the APP mutant group and the wild type group at different ages were corroborated well with the phenotype changes in the transgenic mice model. Three candidate biomarkers were unambiguously identified as methionine, desaminotyrosine, and taurine, which were confirmed by matching the MS/MS spectra with the authentic standards. The biological significance of these metabolites will be discussed within the context of AD development.

Poster No. C-2

Proteomics for Clinical Applications

Development of a Mass Spectrometry-Based Assay for Measurement of Angiotensin I and Plasma Renin Activity to Diagnose Secondary Hypertension

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The renin-angiotensin-aldosterone system (RAAS) plays an essential role in maintaining plasma volume and arterial blood pressure. Measurement of plasma renin activity (PRA) is a commonly employed method of diagnosing secondary hypertension. PRA is quantified by determining the concentration of angiotensin I generated through the enzymatic cleavage of angiotensinogen by renin. Radioimmunoassay is routinely used to measure PRA; however this method has limitations including antibody cross-reactivity and use of hazardous radioisotopes. An MS-based assay presents the opportunity to improve existing assays by eliminating mis-diagnosis as a result of non-selective antibodies.

An MS-based method was developed to measure angiotensin I concentration and PRA in whole human plasma. Immuno-affinity enrichment combined with MALDI MS (iMALDI) enabled detection of endogenous angiotensin I. Triplicate analysis of eight patient samples demonstrated assay reproducibility (CV <13 % for 7 out of 8 samples) and linearity (R=0.99). Evaluation of the iMALDI assay against the radioimmunoassay demonstrates that it is reproducible and provides a linear response over a diagnostically relevant concentration range.

We have developed an iMALDI assay for measuring PRA that overcomes the limitations of current radioimmunoassay-based methods. The assay requires a total of 5 hours for incubation, while the radioimmunoassay protocol performed in this study can require incubation periods up to 48 hours. Furthermore, it also provides absolute specificity, thereby avoiding complications with antibody interference. In addition, the iMALDI assay can be multiplexed allowing simultaneous measurement of both angiotensin I and II, allowing a more comprehensive analysis of the RAAS pathway.

POSTER ABSTRACTS

Poster No. C-3

Proteomics for Clinical Applications

Development of a Microwave-assisted Shotgun Analysis Protocol for Proteome Profiling of Formalin-fixed, Paraffin-embedded (FFPE) Tissues

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Formalin-fixed, paraffin-embedded (FFPE) tissue samples are widely available as they are traditionally archived with historical diagnostic and patient information for decades. Proteome analysis of these tissue samples is of great biological and medial importance. Comprehensive profiling of the FFPE tissue proteome remains a challenge because formaldehyde cross-linkes proteins, making them difficult to analyze. In this presentation, we report a rapid and efficient protocol using domestic microwave oven to retrieve the proteins and analyze them using off-line 2D-LC MS to generate a relatively comprehensive proteome profile of FFPE tissues. 2% SDS buffer was first used to compare the protein retrieval efficiency by either conventional heat cycles or microwave. The gel image of the extracted proteins indicated that both methods generated identical protein profiles. However, the time for microwave method was shortened to about 1/9 of the heat cycle.
The efficiency of different protein retrieval buffers, including Tris-HCl, RIPA, 2% SDS, GnHCl and thiourea, were then compared. The SDS-PAGE images showed that both Tris-HCl and RIPA buffers were not as efficient as the others. We then analyzed the digests of extracted proteins by 1D LC MS/MS to compare the performance of SDS, GnHCl and thiourea. The MS/MS results showed that over 450 unique proteins and 2500 peptides can be identified in both SDS and thiourea treated samples, whereas only 284 proteins and 1893 peptides were found in the GnHCl sample. The advantage of using thiourea over SDS is obvious as no extra SDS removal step is needed. Finally, we will demonstrate the comprehensive analysis of FFPE samples collected from individuals with heart diseases using 2D LC-MS/MS.

Poster No. C-4

Proteomics for Clinical Applications

Accurate Quantitative Determination of the Reproducibilities and Efficiencies of Cartridge and Column-based Depletion Methods for Plasma Proteins

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Human plasma is an important biofluid for clinical diagnostics. Unfortunately, the analysis of human plasma is challenging because of the ~1010-fold concentration difference between proteins. Fourteen proteins account for ~95% of the total protein concentration, hindering the detection of low-abundance proteins through signal suppression. By removing these abundant proteins the signal from low-abundance plasma proteins can be enhanced. Unfortunately, removal of the abundant proteins may also lead to unintentional removal of additional proteins through non-specific binding or through binding to the targeted proteins. Using isotopically-labeled internal standard peptides (SIS peptides) and MRM-based quantitation of plasma proteins, we are now able to accurately compare depletion methods using the absolute protein concentrations. Human plasma was loaded onto both Sigma Seppro IgY14 depletion column formats (spin and LC) and samples were depleted following the manufacturer's instructions. Additionally, both the Sigma depletion buffer and 25mM Ammonium Bicarbonate pH7.5 (AmBic) were tested. Samples were concentrated, digested with trypsin, and spiked with SIS peptides representing 65 human plasma proteins. Using the spin column format, %CV was measured and averaged at 55% for the Sigma Dilution buffer and 30% when the buffer was changed to 25mM Ambic. For the LC columns an average %CV of 25% was observed using the Sigma Dilution buffer and 26% for Ambic. Upon removal of the high abundance proteins an increase in the PAR for low abundance proteins was observed for all depletion column formats. Using this accurate protein quantification a depletion method can be chosen based on the research project.

POSTER ABSTRACTS

Poster No. C-5

Proteomics for Clinical Applications

Confirmation No. 3156
Separation of proteins secreted from prostate epithelial cells with quantification by peptide fragment ion intensity

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The secreted proteins from a human prostate cancer (CaP) epithelial cell line PC3(AR)6 were separated into fractions by strong anion exchange (SAX) chromatography prior to digestion with trypsin for peptide analysis by liquid chromatography and tandem mass spectrometry (LC-ESI-MS/MS). The protein and peptide information provided by the X!TANDEM algorithm was matched with the MS and MS/MS spectra m/z and intensity data in a Structured Query Language (SQL) database. After log transformation in the Statistical Analysis System (SAS) the parent and fragment intensity data approached a normal distribution. The measured ion intensity values for every peptide and fragment from each chromatography fraction was stored in an SQL database. Statistically significant differences in measured intensity values from 1421 parent and 101,905 fragment ions were observed over 11 column fractions, with 233 proteins and 583 peptides as calculated by SAS. The average intensity of the 14-3-3 proteins in the chromatography fractions were compared and 14-3-3 zeta/delta was most intensely detected in a discrete fraction at about 700 mM NaCl that showed average fragment intensity values at least tenfold greater than the adjacent fractions. A total of five different peptide sequences were detected from 14-3-3- zeta/delta of which YLAEVAAGDDK had the highest intensity values.

Poster No. C-6

Proteomics for Clinical Applications

Confirmation No. 3165
Prognostic significance of DLC-1 expression in Head and Neck Squamous Cell Carcinoma

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In our previous study, used isobaric mass tags for absolute quantitation (iTRAQ), and multidimensional liquid chromatography / tandem mass spectrometry (LC-MS/MS) for comparing the protein expression profile of human HNSCC and non-malignant tissues, deleted in liver cancer (DLC-1), a Rho GTPase-activating protein was discovered, identified, and verified to be under-expressed in HNSCCs. In view of the role of DLC-1 as a tumor suppressor protein, we hypothesized that altered DLC-1 expression may impact disease progression in HNSCCs. To verify this hypothesis, we determined the clinical significance of DLC-1 expression in different stages of development and progression of head-and-neck cancer by immunohistochemistry using a polyclonal antibody against DLC-1, and correlated the findings with clincopathological parameters. Immunohistochemical analysis was carried out in HNSCC (n = 214), dysplasia (n = 51), squamous cell hyperplasia (n = 45), and histologically normal oral tissues (n=80). HNSCC patients were followed for over seven years to determine the association of DLC-1 expression with disease prognosis. Significant loss of DLC-1 expression was observed in squamous cell hyperplasia (22%), dysplasia (31%) and HNSCCs (64%) in comparison with oral normal mucosa (ptrend < 0.001). Kaplan-Meier survival analysis showed significantly reduced disease free survival (median survival 25 months, p = 0.021; Hazards ratio, H.R. = 1.8) in HNSCC patients showing loss of DLC-1 expression as compared to median disease-free survival of 59 months in the patients showing DLC-1 expression. In multivariate Cox-regression analysis, loss of DLC-1 expression emerged as an independent predictor of disease recurrence in HNSCC patients (p = 0.013, H.R.= 2.6) in comparison to other clincopathological parameters including age, gender, histopathological grade, T-stage, nodal status and tumor stage. Thus, loss of expression may serve as a potential marker to identify a subgroup of HNSCC patients with poor prognosis, suggesting its putative utility in clinical management of HNSCC.

POSTER ABSTRACTS

Poster No. C-7

Proteomics for Clinical Applications

Heat shock protein expression is down-regulated in renal proximal tubule cell exosomes upon simulated kidney obstruction.

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Exosomes are small, extracellular vesicles generated by many cell types both in vivo and in vitro and quantitative proteomic analysis using mass spectrometry can elucidate molecular changes experienced within diseased tissues producing these exosomes. In this study, we simulate kidney obstruction in vitro with the rat proximal tubule cell line NRK-52E and analyze protein expression in the exosomal fraction.

Kidney obstruction is simulated through mechanical stretch of cells grown on flexible BioFlex' plates, and measured by cell death ELISA. Exosomes are collected from the cell culture media and isolated by ultracentrifugation and protein expression is then quantitatively measured by spectral counting and isotopic labelling using mass spectrometry. Quantitative analysis shows a complete lack of heat shock proteins (HSPs) in the stretched cell exosomal fraction and a 13 fold increase in expression of the structural protein fibronectin. Of the ~90 other proteins identified, very little quantitative variability was found.

Cellular retention of HSPs during cell stretch may be explained by cell survival mechanisms. HSPs are involved in cellular responses to stress conditions, acting as chaperone proteins and combating the buildup of intracellular reactive oxygen species during stress. Intracellular retention of HSPs could be a survival mechanism in stretched cells, reflected as a decrease in HSPs in the exosomal fraction. Increased fibronectin levels could be a damage repair response by the cells in an effort to retain function during stress. If true, the abundance of these proteins in urinary exosomes could be applied as a diagnostic test for kidney obstruction.

Poster No. C-8

Proteomics for Clinical Applications

GC-MS (gas chromatography - mass spectrometry) accurately distinguishes small physiologic metabolite differences in serum.

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Introduction: Clinically relevant metabolomic differences often consist of very small changes in absolute concentration. GC-MS is sufficiently sensitive to detect such changes in simple solutions and targeted analyses, but its ability to do so in untargeted metabolomic biomarker studies, with complex biofluids such as serum, has been insufficiently explored.

Methods: Pooled standardized human serum was mixed with two solutions, each with 6 spiked metabolites at target physiologic concentrations (30-300uM), over a series ranging from zero to two-fold of the target concentrations. Triplicate samples of control serum and spiked serum solutions were analyzed by GC-time-of-flight-MS. Resultant data underwent deconvolution and identification against the GOLM database using MetaboliteDetector software. Principle components analysis (PCA) and orthogonal partial least squares (OPLS) analyses were conducted with Umetrics software. All processing and analyses were conducted using protocols developed in our laboratory for the analysis of clinical serum samples.

Results: The 6 spiked metabolites from each solution were identified, along with 126 non-spiked background serum metabolite features. The two spiked serum solutions were clearly distinguished from each other, and from the noise of background metabolites on unsupervised PCA (R2 0.61, Q2 0.31) and supervised OPLS-discriminant analysis (R2 0.41, Q2 0.41, CV-ANOVA p=0.02). OPLS modeling clearly demonstrated each step in the 2-fold dilutional series (R2 0.51, Q2 0.83, CV-ANOVA p<0.001).

Conclusion: Current advanced techniques for multivariate analysis of metabolomic GC-MS data can distinguish very small, physiologic, changes in metabolite mixtures against the noisy background of complex biofluids such as serum. This data validates the use of these methods for clinical serum samples.

POSTER ABSTRACTS

Poster No. C-9

Proteomics for Clinical Applications

Proteomic Signatures of Disease Progression in Chronic Kidney Disease

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Background: Chronic kidney disease (CKD) is costly for patients, and health-care systems. Despite the fact that the consequences of CKD can vary drastically between patients, physicians currently lack tools to assess the likelihood of progression of CKD and treat patients accordingly. We used iTRAQ MALDI-TOF/TOF mass spectrometry to identify biomarkers of CKD progression which, with further validation, could help guide trajectory-specific patient treatment.

Methods: We collected plasma from a cohort of CKD patients (eGFR below 25 mL/min) who were either rapid progressors (eGFR decline of more than 5 mL/min over the last 12 months, n=24) or slow progressors (eGFR decline of less than 2 mL/min over the last 12 months, n=25). Depleted samples were analyzed using iTRAQ-LC-MALDI-TOF/TOF methodology. Proteins were quantified relative to a pooled CKD patient plasma control. Panels of proteomic markers with differential relative concentrations (p below 0.10) were identified using a robust moderated t-test (LiMMa). Classifiers were built using Elastic Net.

Results: We identified a panel of 15 proteins with differential relative concentrations between slow and rapid progressors. Using leave-one-out cross-validation, the panel had a discriminatory performance of 0.77 as measured by the area under the receiver operating characteristic curve, and could detect rapid progressors with sensitivity of 0.75 and specificity of 0.69.

Conclusions: Our results suggest that differential proteomic signatures of disease progression exist in the plasma of CKD patients. If validated in an external cohort, the biomarker panel would allow physicians to identify patients with CKD that will progress rapidly and could significantly impact the diagnosis and treatment of CKD patients.

Poster No. C-10

Proteomics for Clinical Applications

Proteomic and Genomic Changes In Peripheral Blood Of Asthmatics Undergoing Allergen Inhalation Challenge

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Background: Asthmatic individuals respond differently to allergen inhalation challenge (induced allergic asthma attacks). Some individuals develop an isolated, early response while others develop isolated late or dual (early plus late) responses. Human experimental models of allergen challenge provide a unique opportunity to evaluate the mechanisms and pathways leading to each type of response.

Methods: We undertook an initial Luminex-based multiplex analysis of human cytokines and chemokines, using plasma from 16 samples: pre- and post-challenge from four early and four dual response subjects. Using the RNA isolated from PAXgene blood tubes taken from the same eight asthmatic subjects, we have generated preliminary transcriptomics data using Affymetrix GeneChip Human Gene 1.0 ST Arrays. To complement current data, we are now processing depleted plasma samples from the same subjects using iTRAQ-MALDI-TOF/TOF methodology.

Results: The most statistically significant finding (pre- versus post-challenge) was for monocyte chemoattractant protein-1 (p-value below 0.05). Although plasma protein levels of this chemokine were significantly higher pre-challenge versus post-challenge, and also higher than in non-asthmatic healthy controls, there was no difference between the isolated early and dual responder groups. From the gene expression data, 1,965 probe-sets were differentially expressed (FDR of 10%) in an ANOVA model including the pre-/post-challenge and response type. Proteomics data will be available soon for further analysis.

Conclusions: Understanding the molecular changes that underpin the physiological and cellular characteristics of allergic responses to allergen challenge is important for evaluating and treating allergic diseases such as asthma. Results from the plasma proteome will complement current findings.

POSTER ABSTRACTS

Poster No. C-11

Proteomics for Clinical Applications

PROTEOMIC PROFILING OF EXOSOMES DERIVED FROM DIFFERENT PROSTATE CANCER CELL LINES

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Eukaryotic cells secret proteins through either regulated ER/Golgi dependent mechanism or by vesicle secretion. The release of microvesicles provides a novel mechanism for intercellular communication. Exosomes are extracellular nano-vesicles which are secreted upon fusion of multivesicular bodies with the plasma membrane. The function of these 30-100 nm vesicles depends on the cell types that they are derived from. Recent studies have linked cancer initiation and progression with exosome formation, their relevance in prostate tumour growth and progression has yet to be determined. Factors such as stage of cancer, cell type and cell cycle could affect the amount and composition of exosomes formed and secreted. Exosomes contain a spectrum of different lipids and proteins which could play a key role in their function. While the mechanisms underlying exosome formation and secretion are not fully understood, proteomic classifications of exosomes derived from different cell lines indicate many common membrane and cytosolic protein markers. The presence of differential protein markers in and on these entities may provide potential as biomarkers during cancer diagnosis.
The specific aims of this study are:
i) To characterize exosomes derived from different prostate cancer cells and
ii) To delineate the role of specific proteins/lipids present within exosomes which are involved in prostate cancer progression.
This study highlights a potential of differential protein composition of exosomes as a source of diagnostic biomarkers for prostate cancer via non-invasive testing as well as a potential therapeutic target.

Poster No. C-12

Proteomics for Clinical Applications

Comparison of ELISA and mass spectrometry based assays for the quantification of chaperonin 10 in serum and cell cultures

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The complex composition of blood is a barrier to the measurement of plasma proteins of low abundance. Matrix interference may be reduced by removal of extraneous species, direct isolation of the analyte or both. Here the development of assays for chaperonin 10 (cpn10), an intracellular and circulating protein with diagnostic and therapeutic utility, based on ELISA and immunoaffinity capture followed by multiple reaction monitoring (MRM) are described. The methods were applied to normal human serum spiked with recombinant cpn10 as well as lysates of the cell line Hec1a, a source of endogenous cpn10. Glass, polystyrene and polyvinylidene fluoride (PVDF) were compared as immunoprecipitation substrates. The highest signal-to-noise ratio was obtained using PVDF while non-specific binding was lowest for polystyrene. The sensitivity of the immunoprecipitation was dependent on antibody concentration and washing conditions. The sensitivity of the immunoprecipitation based assay was determined using two liquid chromatography-mass spectrometry platforms, a cHiPLC-QTRAP 5500 and a TempoLC-QTRAP 4000 (MDS Sciex). The lower limit of detection (LLOD) of the QTRAP 4000 and 5500 for tryptic cpn10 peptides were 50 and 10 attomoles respectively. The LLOD of the immunodepletion and immunoprecipitation on the QTRAP 4000 were 109 and 1 ng/ml of plasma respectively while that of the ELISA was 0.15 ng/ml plasma. Immunoprecipitation-MRM was the most reproducible method. The MRM signals from selected clusterin and albumin peptides remaining in the immunoprecipitated samples were sufficiently reproducible to be used as internal standards. The techniques reported herein permit the quantification of cpn10 at physiologically significant plasma concentrations.

POSTER ABSTRACTS

Poster No. T-1

Proteomics Technologies and Other Omics

Integrated Strong-Cation Exchange Liquid Chromatographic Procedure for SDS Removal and Peptide Separation for MS-Based Shotgun Proteome Analysis

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SDS can be used to dissolve a wide range of proteins including membrane proteins. However, the resultant peptides containing SDS cannot be analyzed directly using reversed-phase (RP) LC-MS, as SDS can cause interference with RPLC separation and MS analysis. Therefore, efficient removal of SDS is required. In this work, we report a new method based on the use of strong-cation exchange (SCX) liquid chromatography (LC) for removal of SDS as well as peptide separation in an automated setup. We demonstrate that, by purposely increasing the SDS concentration, we could remove SDS effectively while achieving peptide recovery of about 90%.

By using a standard protein, BSA, as the model, several SCX parameters were examined to optimize the SDS removal protocol. The pH of the sample was adjusted to be 2. The composition of the mobile phase A was changed from 10 mM KH2PO4 (pH 2.7), which was normally used, to 10 mM LiH2PO4 (pH 2.7). By using the new mobile phase A, the separation between the salt peak (containing SDS) and the peptide peak in the SCX chromatogram was much better than using the conventional one. In addition, the concentration of DTT and IAA used for the protein reduction and alkylation was optimized to 5 mM and 12 mM, respectively. NH4HCO3 was removed by drying down the sample after the tryptic digestion. By using this optimized method, sample recovery of 90.6% ±1.0% (n=3) could be achieved as long as the SDS concentration in a peptide sample was kept at 0.5%.

Poster No. T-2

Proteomics Technologies and Other Omics

A LC-MS-Based Triplex Stable-Isotope Labeling Method for Quantitative Metabolome Analysis

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Stable isotope labeling (SIL) combined with LC-MS has been widely used in target metabolic profiling. Recently, 12C2-/13C2-DnsCl has been reported to selectively derivatize primary and secondary amines and phenolic hydroxyl groups in MS-based quantification. This labeling method, due to the hydrophobicity of the attached labeling group, apparently improves the chromatography behavior of polar and ionic metabolites in RPLC and enhances their ionization efficiency by 1-3 orders in ESI as well. Herein, we present a new set of DnsCl-like labeling reagents, 12C4-, 12C213C2-, and 13C4-5-diethylamino-naphthalene-1-sulfonyl chloride. Although only two methyl groups of DnsCl in the duplex reagents are replaced by the ethyl groups in the triplex reagents, the increased hydrophobicity greatly influences the derivatization reaction. To this end, we have developed a new protocol with much better labeling efficiency and simpler sample preparation for labeling amines and phenols. After derivatization and combination, the samples were analyzed by RPLC-FTMS, in which the labeled metabolites form the triplex isotopic patterns with each isotope pair differed by two-Dalton. Compared to the duplex reagents (12C2-/13C2-DnsCl), the new method can perform twice quantification for the same analysis time. After data acquisition, an in-house program has been developed to find all potential triplex peak pairs, and list the m/z values of the metabolites and their corresponding signal intensity ratios among the pairs. In this presentation, we will introduce the application of this strategy for quantitative profiling of human urine samples. This will form a baseline for future work on discovery of metabolite biomarkers for various diseases.

POSTER ABSTRACTS

Poster No. T-3

Proteomics Technologies and Other Omics

Development of Microwave-Assisted Acid Hydrolysis Combined with MS and MS/MS for Determining Terminal Amino Acid Sequences of Proteins

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Antibodies have been widely developed as potential therapeutic for treatment and management of diseases. Characterizing the amino acid sequence and modifications is important, they determine an antibody√¢,Ç¨,Ñ¢s biological functions. Microwave-assisted acid hydrolysis (MAAH) has been demonstrated to be useful for hydrolyzing proteins into polypeptides ladders, and generate sequence information by mass spectrometric analysis. However, because of hydrolysate√¢,Ç¨,Ñ¢s complicity, gaps between polypeptides ladders using MS analysis have usually been observed, and the sequence information of terminal peptides is often missing. In this work, we combine MAAH with MS/MS analysis to produce the sequence information of an intact protein, especially its terminal amino acid sequence.
In our experiment, Cytochrome C (12.3 kDa), Myoglobin (16.7 KDa) and Lysozyme (14.3 kDa) were used as protein standards for method development. Both Cytochrome C and Myoglobin do not have disulfide bond, while the structure of Lysozyme is stabilized by four disulfide bonds linking all its eight cystein residues. Cytochrome C and Myoglobin were hydrolyzed by MAAH, dried to remove acid, and reconstructed with 0.1% TFA. Lysozyme was reduced with DTT for 1 hour at 37√¢,Äû∆í after MAAH, and the hydrolysate was desalted on HPLC, which was also reconstructed with 0.1% TFA. About 5 pmol of the hydrolysate were mixed with a matrix and spotted on a MALDI plate using the two-layer method. Samples were analyzed on MALDI TOF/TOF with the linear mode to produce the mass spectrum of peptide ladders containing either the N- or C-terminal amino acid of the protein. MS conditions were optimized to increase resolution and sensitivity, especially for the peptides with m/z between 5000 and 10000. Possible amino acid sequences were constructed by directly reading the ladders. However, sequence gaps where bond breakage products were not detected from the N- or C- terminal peptides ladders were observed. The terminal peptides with uncertain sequences were selected for MS/MS analysis. In order to improve ion fragmentation for the large peptides with m/z from 2000 to 5000, different fragmentation conditions were tested. Note that these peptides are different from the conventional tryptic peptides where the terminal amino acid is usually a basic residue; the amino acid of a hydrolysate peptide can be any one of the 20 amino acids. From our experiment, we found that, for peptides with m/z<2000, it√¢,Ç¨,Ñ¢s good to use the post-source decay with the 1-kV mode setting on the ABI 4800 MALDI TOF/TOF. For peptides with m/z between 2000 and 4000, better results were obtained from fragmentation with CID. For peptides with m/z>4000, 2-kV mode setting with CID fragmentation could be applied. By analyzing MS and MS/MS spectra generated from the standard proteins, 100% sequence coverage could be gained, with information on the terminal amino acid sequences. We are applying this method to sequence antibodies and the results of this work will be presented.

Poster No. T-4

Proteomics Technologies and Other Omics

Core-structure study by MS3 analysis for unknown metabolite identification

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LC-MS is an important tool for studying metabolomics as it's readily adapted to highly sensitive and specific analysis of biological samples. However, in LC-MS applications spectral libraries are less developed than GC-MS and the characterization of unknown metabolites still relies on NMR. Our goal is to develop tandem MS for structure analysis of unknown metabolites.

Metabolites can be sorted into different classes based on their carbon backbones. In some cases, fragmentation generates common core-structures from the common backbone and this can be used to identify the chemical family of unknown metabolites. In this work, the core-structures of benzene-containing compounds are identified and their fragmentation patterns are documented.

We have analyzed a series of benzene-containing standards found in the human metabolome database (HMDB). Based on their MS3 spectra, we were able to identify a number of core structures (or diagnostic ions) and their characteristic fragmentation patterns. These diagnostic ions are indicative of a certain class of compounds.

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We identified diagnostic ions for benzene-containing compounds which upon further fragmentation reveal characteristic fragment ions common to this compound family. For example, for phenylalanine, tyrosine, and L-dopa derivatives (N-substitution or ester/amide), we have identified diagnostic ions at m/z 131 (103, 77), 147 (119, 91, 65), and 165 (145, 135, 117, 107, 89, 77) for each class, respectively (with the fragmentation pattern shown in parentheses). Searching for these diagnostic ions may facilitate identification of unknowns.

Future work will be focused on continuing to study benzene-containing compounds. We can further expand the ring structure to include some other biologically important compounds such as flavonoids. This approach will then be applied to MCF-7 cell extracts to identify endogenous benzene-containing compounds.

Poster No. T-5

Proteomics Technologies and Other Omics

A Method Based on Microwave-Assisted Acid Hydrolysis, LC Fractionation and MALDI and ESI MS for Protein Terminal Sequence Analysis

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Mass analysis of polypeptide ladders of a protein after hydrolysis in HCl with microwave irradiation is fast and sensitive for direct protein sequencing and post-translational modification identifications. However, this method is only applicable to the small proteins whose molecular weight is smaller than 20kDa, not suitable for larger proteins like antibodies due to the sample complexity after hydrolysis.

In this work, we developed a LC fractionation method, combined with microwave-assisted acid hydrolysis to separate the complex hydrolysate mixture into less complicated peptide fractions. The fractionated peptides are subjected to MALDI MS and ESI MS and MS/MS analysis to generate the peptide sequence information with a particular focus on the amino acid sequences of the two terminus of large proteins.

Bovine serum albumen (BSA) was used as a model protein for the method development. For LC fractionation of the BSA hydrolysates, at first the Varian Polaris C8 column (1 mm i.d. 50 mm, with 180 pores) was used. It was found the BSA proteins as well as large peptides were lost in this column due to the small pore size and long carbon chain of the stationary phase. Then the Agilent Zorbax 300SB-C3 column (3 mm i.d. 150 mm, with 300 pores) was tested. The recovery of BSA hydrolysates in this column was found to be about 100%. At least seven fractions were collected from the LC experiment using this column. SDS-PAGE analysis of the fractions indicated in the low organic solvent fractions, low molecular weight peptides were found to be predominant. In the other fractions, large peptides as well as small peptides were present. This presented a challenge in MALDI-TOF analysis, as there is an upper mass limit for detecting polypeptide peaks when they are present in a mixture. The useful mass region is generally limited to below 14000 Da. The peak intensity decreases as the polypeptide mass increases. Thus, for the high organic solvent fractions, only a few small peptides (below 8kDa) were detected and it is believed these low mass peptide ions suppressed the ionization of the large peptides. Nevertheless, compared to the results obtained from direct analysis of the BSA hydrolysate by MALDI-TOF, LC fractionation followed by MS analysis increased the number of peptides detected by about 2-fold, resulting in significant increase in sequence coverage and the confidence of the sequence reading due to overlap sequence information generated by the increased number of peptides. The analysis of the LC fractions of the BSA hydrolysate by ESI MS is currently underway.

Poster No. T-6

Proteomics Technologies and Other Omics

Effective Extraction Method and Stable-Isotope Dansylation Labeling Combined with RPLC-FTMS for the Analysis of the Ginseng Root Metabolome

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Plant metabolomics has become an important area of research for understanding plant biology and developing new or improved plant-related products. One potential application is to study the traditional Chinese medicines (TCMs) due to their low toxicity and good therapeutic performance. In this work, we report our studies of using a newly developed quantitative LC-MS method for study the metabolome of ginseng roots.

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12C-/13C-dansylation reaction has been successfully developed as a stable isotope derivatization method for analyzing amine- and phenol-containing metabolites. In the present work, we have developed a robust metabolite extraction method from ginseng roots tailored to the analysis of amine- and phenol-containing metabolites. For plant metabolome analysis, metabolite extraction is an important step. We focused on the development and application of the microwave-assisted extraction and ultrasonic-assisted extraction methods. The preliminary results showed that microwave-assisted extraction was better than ultrasonic-assisted extraction when methanol was used as the extraction solvent. In order to optimize the extraction conditions, several parameters have been investigated, including solvent-to-sample ratio, solvent composition, extraction time, and the microwave power. A reproducible separation method has been developed for analyzing the isotope-tagged metabolites from ginseng roots. After labeling by the stable isotope dansylation reagents, the reversed-phase chromatographic separation can be easily done in 30 min. In general, we can readily detect over 200 ion pairs or metabolites from a ginseng root extract. We are currently in the process of applying this method to generate the quantitative information on the metabolome profiles of different ginseng roots.

Poster No. T-7

Proteomics Technologies and Other Omics

Diagnostic neutral losses and common fragment ions for putative identification of amine-containing metabolites

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“Clinical metabolomics” is now focusing on assessment and prediction of disease risks by identifying metabolic signatures in biofluids. Analysis of amine-containing metabolites in biological samples is important for disease biomarker discovery. However, identification of unknown metabolites remains a challenge. The goal of this research is to study MS/MS fragmentation patterns of amine-containing metabolites and their corresponding dansylation derivatives and apply this knowledge to determine the structures of unknown amine-containing metabolites found in biofluids.

A total of 35 amine compounds with structures related to amino acids were chosen from Human Metabolome Database. These compounds were subjected to tandem MS analysis, and several fragmentation patterns were proposed.

In molecules containing primary amines, protonation occurs at nitrogen and cleavage occurs at C-N bonding, resulting in a neutral loss of 17, the corresponding amine.

A neutral loss of 17 in MS/MS followed by neutral loss of 28 in MS3 can only be observed when the amines contain “alpha-amino acid” structure (–NH2 and –COOH be connected onto the same carbon atom).

Fragment ion of m/z 72 is observed in MS/MS spectra of long chain primary amines which have amino group at the end of the chain connected with four methylene groups (H2N-CH2-CH2-CH2-CH2-).

For the future work, dansylation labeling will be applied to these amines, MS/MS and MSn spectra will be generated. Fragmentation patterns before and after labeling will be compared to help understand how labeling influences fragmentation patterns. Obtained fragmentation patterns will finally be applied to identify interesting amine metabolites extracted from body fluids.

Poster No. T-8

Proteomics Technologies and Other Omics

Fusing Atmospheric Pressure ECD (AP-ECD) with nanospray to track Post-Translational Modifications with a "standard" mass spectrometer

Carter, Davin
UBC Biomedical Research Centre

Contributing Authors:

Davin Carter; Jason Rogalski; Damon Robb; Michael Blades; Juergen Kast

One concern of current mass spectrometry is the difficulty of analyzing labile Post- Translational Modifications (PTMs) with traditional collision induced dissociation (CID). In contrast to traditional CID, electron capture dissociation (ECD) and its related technique, electron transfer dissociation, offer direct identification and localization of labile PTMs but generally require specialized mass spectrometers. Using a modified PhotoSpray™ photoionization lamp we have recently added the capability of performing ECD to our nanospray CID Q-ToF.

POSTER ABSTRACTS

The modified AB Sciex PhotoSpray™ source was interfaced with a QStar XL Q-ToF so that ions from a nanospray emitter could be exposed to photoelectrons generated from acetone to induce electron capture dissociation. With the photoionization lamp off, and no photoelectrons present, ionized peptides were admitted into the MS as in traditional nanospray - allowing conventional high sensitivity LC-MS. When the photoionization lamp was on, the resulting photoelectrons caused ECD in source. C- and z-type fragment ions were admitted into the mass spectrometer, allowing high sensitivity LC-AP-ECD- MS. AP-ECD is also able to produce fragment ions with labile PTMs retained, allowing for both sequencing and localization of the PTMs, equivalent to modern ECD or ETD available on specialized instruments. The new AP-ECD source can be incorporated in-line on any atmospheric pressure ionization instrument; in fact, switching to this source has enabled our Q-ToF MS to identify labile modifications on peptides at the fmol level with no need for ion trapping.

Poster No. T-9

Proteomics Technologies and Other Omics

Identification of HNO-reactive Cysteines in Platelet Proteins by Isotope-coded Affinity Tag Enrichment and Differential Alkylation

Li, Ru
UBC

Contributing Authors:

Liwen Lin, Juergen Kast

HNO has received intense attention from the research and medical communities because of its effects on many biological functions, including its ability to inhibit platelet aggregation. Cysteines in proteins are the major targets of HNO, which can be modified into either sulfinamide or disulfide groups. In order to elucidate the mechanism of HNO's effects on platelet aggregation, an important step is the identification of HNO-reactive cysteines in platelet proteins. In the present study, an isotope-coded affinity tag (ICAT) approach was combinned with a differential alkylation strategy developed previously in our lab to identify HNO-reactive cysteines and quantify the extent of modification. Compared to the original approach, it was hypothesized that the enrichment of cysteine-containing peptides and the co-elution of Light- and Heavy-ICAT labeled peptides would provide a higher dynamic range and increase the number of HNO-reactive cysteines identified, especially those in the low abundant proteins, which may play important roles in the inhibition of platelet aggregation by HNO. Preliminary results show that the number of detectable cysteine-containing proteins significantly increases, and that this modified approach is capable of identifying cysteines that are HNO-reactive and proteins that show different responses to HNO.

Poster No. T-10

Proteomics Technologies and Other Omics

APFO...The good, the bad and the bubbly

Vieira, Douglas
Dalhousie

Contributing Authors:

Andrew M.J. Crowell and Alan Doucette

Introduction: During last decades an anionic detergent known as Sodium Dodecyl Sulphate (SDS) has been commonly used to improve the extraction and solubilisation of proteins. However, this surfactant can suppress or even eliminate the mass spectrometry signals for both proteins and peptides. Also, the removal of SDS in same circumstances stills a goal to achieve. Then, strategies to replace SDS from proteomics workflow have thus been a subject of ongoing research. An ideal surfactant for proteomics experiments is one that can assist the extraction and/or solubilisation while avoiding signal suppression during mass spectrum analysis. An alternative surfactant for proteome samples might be the ammonium perfluorooctanoate (APFO), a potent solubilising agent that may be removed by evaporation.

Methodology: Mixture of standard peptides or proteins with increasing amount of detergent was analysed using common methods in proteomics analysis such as MS infusion, LC/UV, LC/MS and LC-MS/MS.

Aim: The goal of this current work is establish the interference of APFO detergent in proteomics analysis. Results: We have found that mass spectrometry as well as HPLC or even HPLC-MS seems to be slightly more compatible for APFO than SDS. Furthermore, the great advantage of choosing APFO is based on its evaporation efficiency, which it is related to pH and protein concentration. In other words, the APFO detergent is easily evaporated at low pH and protein (or peptide) concentration.

Conclusion: Despite superficial better performance of APFO in proteomics analysis, the removal of APFO vs all complex step for SDS removal make this a favourable alternative.

POSTER ABSTRACTS

Poster No. T-11

Proteomics Technologies and Other Omics

Understanding Formaldehyde Cross-linking of Proteins by Mass Spectrometry

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Contributing Authors:

Juergen Kast

Formaldehyde cross-linking can be used to study native protein complexes. Complexes are cross-linked in their cellular environment, purified, digested and finally analyzed with Mass Spectrometry (MS). Cross-linked dipeptides in the digest indicate the geometry of the complex but have proven difficult to identify, partially due to a limited understanding of formaldehyde induced cross-linking reactions. We will present a method that identifies cross-linked dipeptides from model proteins and localizes cross-link sites, thereby revealing reactive residues, the reaction mechanism and the stability of cross-links. In the digest of formaldehyde cross-linked model proteins, the masses of known peptides with formaldehyde induced modifications were correlated with a large pool of unknown MS signals. The pool was thereby narrowed down to several cross-linked dipeptide candidates, among which eleven were verified by MS/MS to be cross-linked. Fragmentations occur both at the cross-links and peptide backbones, allowing verification of the precursors, and localization of the cross-link sites to the N-terminus, Lysine (K), Tyrosine (Y) and Asparagine (N). These residues are therefore reactive under in vivo-like reaction conditions applied in this study. The reaction of an amino group (the N-terminus or K) cross-linked to Y was suggested to follow the mechanism of the Mannich reaction, by correlating the appearance of cross-linked dipeptides and modifications on identified sites. We are currently studying the effect of heat treatment in different buffers to determine the stability of cross-linked dipeptides.

Poster No. T-12

Proteomics Technologies and Other Omics

Comprehensive Evaluation of Statistical Tools Used for Peptide and Protein Identification in Shotgun Proteome Analysis

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Contributing Authors:
Liang Li

In mass spectrometry-based proteomic studies, a protein is considered identified when at least one of its peptides is matched by sequence searching algorithm (e.g., Mascot). Because of the error-prone property of these algorithms, many statistical means (e.g., ProteinProphet) and rules (e.g., two-peptide rules) have been implemented to gauge search results' quality. With the development of statistical tools, peptide and protein identifications can now be assigned with statistical meanings indicating their confidence levels. But how well do these rules and statistical tools evaluate the peptide and protein identifications? In this work, we experimentally validate the peptide and protein identifications by applying the 18O-labeling strategy and then use these validated results to assess the accuracy of these statistical tools.

Human cells were cultured, harvested, disrupted and subjected to acetone precipitation, reduction, alkylation, protein-level RPLC fractionation and trypsin digestion. Then peptides in each fraction were divided into two equal portions, one of which underwent 18O-labeling. Next, all the unlabeled fractions were analyzed by RPLC-QTOF. After database searching, precursor masses of 18O-labeled peptides were calculated based on the results of the unlabeled peptides. All the 18O-labeled fractions were analyzed with the predicted inclusion lists. By overlapping the unlabeled and 18O-labeled search results, we can distinguish the true and false identifications. Finally, we applied statistical tools (e.g., PeptideProphet) to the unlabeled search results. Since the false identifications were isolated, we were able to assess the accuracy of these statistical tools.

Firstly, the effectiveness of the peptide-inclusion strategy was assessed by analyzing complex digests and their technical replicates. Our results showed that after 3 inclusion-runs the recovery of peptides identified in the original run was as high as 98% at the significance threshold of 0.05. This indicates that highly reproducible LC-MS/MS analyses can be achieved using the inclusion strategy.

Next, with QTOF MS/MS analysis, we identified more than 20000 peptides from the unlabeled fractions at the significance threshold of 0.05, which leads to more than 2000 protein identifications. By applying the inclusion strategy approximately 25000 peptides and 2000 proteins were identified from the labeled fractions. After comparing each unlabeled-peptide with its 18O-labeled counterpart, over 85% of the unlabeled-peptide identifications can be experimentally corroborated. Further analysis showed that the peptides identified by multiple tools are far more likely to be validated than the ones that are only matched by one of them.

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We have compared the performance of different statistical tools. The results showed that 93% of unlabeled-peptide identifications from only Mascot or X!Tandem original results can be verified, while the verification percentages for PeptideProphet or Percolator results are around 88%. This indicates that more false positive identifications have been included after being processed by these statistical tools. Interestingly, if we only recognize the common peptide matches from any two search-engines or statistical tools, the verification percentage increases to above 93%.

At the protein level, our preliminary results showed that the single-hit protein identification is less likely to be verified than multi-hit protein identification. However, over 70% of the single-hit protein identifications can be verified. It clearly indicates that the two-peptide rule was overly conservative. We are currently developing a means of dealing with the single-hit protein identification more intelligently to reduce false negatives and false positives.

Poster No. T-13

Proteomics Technologies and Other Omics

Automated Glycan Structural Isomer Differentiation Using Bioinformatics Tool

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Thermo Fisher Scientific

Contributing Authors:

Amy Zumwalt; Ningombam Sanjib Meitei, Arun Apte and Rosa Viner

Mass spectrometry (MS) has emerged as a powerful tool for the structural elucidation of glycans. The use of permethylaytion in combination with multistage fragmentation (MSn) is a critical aspect for glycan structural characterization. Only MSn truly characterizes a glycan structure as it allows identification of branching patterns, linkages and resolution of isobaric structures which are otherwise indistinguishable in MS/MS spectra. However, MSn analysis is complicated by large number of spectra generated for a single structure. It is very common that one must acquire MS6 or MS7 level of fragmentation to differentiate potential glycan structural isomers. Here we present the use of a bioinformatics tool (SimGlycan) for glycan structural isomer differentiation from MSn data. SimGlycan software was used in combination with MSn and permethylation to differentiate structural isomers present in ovalbumin glycans. The ion trap MS profile was acquired for permethylated ovalbumin glycans and specific precursors were targeted for MSn to differentiate structural isomers. Sequential MSn data were imported into SimGlycan software and various structural isomers of ovalbumin glycans were differentiated. SimGlycan differentiated structural isomers were verified using manual assignment and previously published data.

Poster No. T-14

Proteomics Technologies and Other Omics

Optimized protein recovery and SDS reduction using the Pierce Detergent Spin Column technology

Fitzsimmons, Shayla
Dalhousie University

Contributing Authors:

Mass spectrometry is a powerful tool used in proteomics. However, due to the inherent complexity of the sample, fractionation is necessary before MS analysis. A widely used detergent which facilitates proteome prefractionation (eg GELFrEE/GeLC), and also assists in protein solubilization is sodium dodecyl sulfate (SDS). Unfortunately, the presence of SDS at concentrations exceeding 0.01% prohibits LC-ESI-MS. Thus, methods for SDS removal are paramount to couple front-end manipulations with downstream LC-MS. Multiple techniques are available for SDS reduction, and include electrophoretic or chromatographic approaches, precipitation or filtration. Many of these strategies are available commercially. These products succeed to varying degrees, as consideration is given not only to the degree of SDS reduction, but also towards optimizing protein recovery. A recent product available from Pierce, the Detergent Spin Removal Columns, has been shown to efficiently remove SDS and other detergents from protein solutions. However, column cost is quite high, and protein recovery is variable and often quite poor. Presented here is an alternate, cost-effective protocol, which makes use of the Pierce Detergent Column technology. The method is developed for optimal protein recovery while maintaining efficient SDS removal. The beads are removed from the column and equilibrated in distilled water, and a small volume of the resulting slurry is added to the sample. The slurry/protein mixture is manually agitated, followed by removal of the SDS-reduced supernatant from the beads. This optimized protocol removes SDS to levels below 0.001% while obtaining protein recovery greater than that of the original protocol.

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Poster No. T-15

Proteomics Technologies and Other Omics

A feedback framework for protein inference from peptides identified from tandem mass spectra

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Contributing Authors:

Jinhong Shi and Fang-Xiang Wu

Protein inference is a critical step in proteomics. Although peptides and proteins are naturally nested, protein inference is usually based on but separated from peptide identification. Different from traditional two-stage methods, this study intends to integrate protein inference and peptide identification in a unified framework with a feedback mechanism. By returning beliefs of identified proteins to peptide identification, we developed a statistical model to simultaneously identify proteins and peptides. First, proteins are inferred by grouping identified peptides. Second, the belief of proteins is grouped and passed back to peptides, and the confidence of peptides is recomputed. As such, the confidence of a peptide can be increased (or decreased) with a positive (or negative) feedback from its parent proteins. Besides, signal peak intensity in a tandem mass spectrum is proposed to approach the assignment of shared peptides. The idea is that, for a given peptide shared by protein A and protein B, if the peptide is from protein A, then its intensity will be closer to the intensity of its siblings in protein A than that in protein B. The intensity of a peptide is represented with the signal peak intensity in its matched tandem mass spectra. By testing on a dataset with 30 true proteins, the preliminary results show that the proposed model can identify more true positives (TP) and less false positives (FP) (30TP/9FP; 30TP/12FP) than ProteinProphet (25TP/11FP; 27TP/19FP) when the threshold is 1 and 0.9, respectively.

Poster No. T-16

Proteomics Technologies and Other Omics

WITHDRAWN

Poster No. T-17

Proteomics Technologies and Other Omics

Peptide termini-specific immunoaffinty enrichment for mass spectrometric protein quantification

Poetz, Oliver
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Contributing Authors:

Oliver Poetz, Anke Schnabel, Katrin Marcus, Thomas Joos

Immunoaffinity enrichment of proteotypic peptides coupled with MS has been proven to be a highly specific and sensitive approach to determine the absolute concentration of proteins. However, the “one antibody - one analyte” approach requires a high number of specifically binding reagents and thereby limiting the use of this concept to a proteome-wide scale.

We tackle this issue by using antibodies that are binding a short C-terminal peptide epitope. These antibodies are capable of enriching groups of peptides that share a common terminus. This strategy reduces the number of the antibodies required substantially, while a high sensitivity can still be obtained. Here the ”one antibody - multiple analytes” approach is used in a pharmacokinetic setting. The dynamics of the induction of drug metabolizing enzymes is monitored in a quantitative assay we developed for a set of different toxicologically relevant proteins. Three homologous members of the Cytochrome P450 enzyme system - CYP 3A4, 3A5, 3A7 - and the drug transporter MDR-1 can be quantified by using one antibody that is targeting the same C-terminal epitope present in signature peptides of these proteins. The assay allowed the monitoring of statin induced drug-transforming enzymes in primary human hepatocytes. By combining the immunoaffinity enrichment step with MRM read-out a more than 50-fold increase in sensitivity compared to LC-MS only was achieved.

Thus the developed assay allows the absolute quantification of drug metabolizing enzymes directly from proteolytical digests of human hepatocytes and is well suited to test CYP450 induction by drug candidates.

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Poster No. T-18

Proteomics Technologies and Other Omics

Metabolomics-Guided Phytochemistry for Drug Discovery

Murch, Susan
University of British Columbia

Contributing Authors:

Paula N. Brown and Susan J. Murch

The use of plants as sources of medicines and other valuable phytochemicals is widespread but the vast majority of the estimated 30,000 compounds in a plant tissue have never been isolated, identified or described. Traditional bioassay-guided fractionation approaches to finding bioactive constituents involve beginning with an ethanol extract and following a series of isolation and purification steps with re-assay of activity at each stage. For a large number of traditional plant medicines, this approach has failed to identify a single active constituent and activity is reduced with each purification step until eventually the initial bioactivity is lost. Metabolomics coupled with proteomics technologies offer a new approach to identify bioactive constituents in plant extracts. In our research group, we are using metabolomics approaches to traditional medicines to identify unique “lead” compounds that are present in active bioassay fractions but missing from non-active fractions. The key to making this approach successful is the production of standardized, controlled plant material in controlled environments coupled with preparation of a range of different crude extracts to determine the bioactivity and chemical complexity and detailed proteomic knowledge. This research represents a new approach to drug discovery of bioactive compounds that are less chemically stable and therefore not efficiently recovered from bioassay-guided fractionation approaches. In addition, the problems of polymolecular drugs and chemical synergies in natural products where a single isolated compound does not correlate with bioactivity can be addressed in meaningful, quantitative experiments.

Poster No. T-19

Proteomics Technologies and Other Omics

Mapping and Distinguishing HNO-induced Modifications on Cysteines in Platelet Proteins: Sulfinamides and Disulfides

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Biomedical Research Centre

Contributing Authors:

Geraldine Walsh, Juergen Kast

HNO, a small compound which can be produced upon hydrolysis of Angeli's Salt (AS), is known to be able to inhibit platelet aggregation. Sulfhydryl is the major target of HNO, which can be modified into either sulfinamide or disulfide. The sulfinamide modification is directly detectable by mass spectrometry; however, cysteines converted into disulfide groups by HNO cannot be monitored this way, because the origin of the other cysteine it reacts with is unknown. In the present study we developed a differential alkylation strategy to identify HNO-reactive cysteines regardless of the eventual modification state and evaluated it on a protein model: proteins were treated with N-ethylmaleimide (NEM) to block free thiols, dithiothreitiol (DTT) to reduce sulfinamides and disulfides, and subsequently iodoacetamide (IAA) to label reduced free thiols. To identify HNO-responsive cysteines upon AS treatment, this differential alkylation method was applied to platelet proteins. By quantitating the final products of cysteines in the AS-treated and untreated platelet proteins, we identified 32 HNO-reactive cysteine residues from 18 platelet proteins, each with different reactivities toward HNO. Moreover, we used the mass shift-based method developed previously in our lab that excludes DTT treatment to detect the sulfinamide modification. Comparison of the results showed that among the 32 HNO-reactive cysteine residues discovered, 3 were modified into sulfinamides, and the other 29 cysteine residues were likely converted into disulfide groups.

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Proteomics Technologies and Other Omics

WITHDRAWN

Poster No. T-21

Proteomics Technologies and Other Omics

Evaluation of Gel vs Solution Prefractionation for Universal Proteome Profiling

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Contributing Authors:

Fang Liu, Alan Doucette

To enable comprehensive proteome profiling through LC/MS, additional separation is crucial. Multidimensional separation brings together two or more separation platforms, and can be applied post digestion (i.e. on peptides), or through prefractionation of intact proteins. When the latter is applied, both top-down as well as bottom-up MS is possible. Intact level separation also has greater potential to separate masking abundant proteins, and apply the intrinsic information available from the separation to target specific proteins. However, proteome prefractionation is also considered more challenging than peptide level separation, owing to the greater diversity of proteins over peptides, in terms of size, charge and hydrophobicity. Using yeast as a model system, we evaluate gel- vs solution-based proteome prefractionation strategies, with a goal of enabling universal (comprehensive, top-down or bottom-up) proteome profiling. Thus, unbiased separation and recovery of 'all' samples components is a critical aspect of the separation. Emphasis is placed on the profiling of membrane proteins, and includes the development of a novel bioinformatics approach to discern the yeast membrane proteome. With gel-based separation platforms (GeLC MS and GELFrEE MS), the use of SDS before and during separation translates into exceptional recovery of hydrophobic proteins. Chromatographic separation, employing reversed phase LC, can no longer make use of SDS. Thus, formic acid is used as a compatible solvent system to enhance proteome solubility. However, loss of proteins due to column adsorption results in a greater loss of membrane proteins. Thus, gel-based separation is a preferred approach to separate proteins at the intact level.

Poster No. T-22

Proteomics Technologies and Other Omics

Linking protein termini and their modifications with underlying proteolytic processing and implications for protein function

Lange, Philipp
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Contributing Authors:

Philipp F. Lange and Christopher M. Overall

The functional state of a proteome is defined by the structures, interactions and posttranslational modifications of the proteins present at any given time and place. Among the most fundamental characteristics of a protein are the termini defining the start and end of the polypeptide chain. With recent advances in method development and the advent of the human proteome project, information about the actual N- and C-termini of proteins in vivo and their generation by proteolytic processing is rapidly increasing. A suitable resource to make the vast amount of information originating from the human proteome project termini analyses, large scale in vivo substrate screens and other projects accessible to the scientific community is by now missing but indispensible. Thus we developed TOPCAT, a knowledgebase for protein termini and underlying proteolytic processing. It consists of ~55000 C- and ~60000 N-termini from extracted from published studies and databases and more are continuously added by the community. We compare it to existing resources and perform cross species characterization of the terminome and its modifications. In two scenarios we demonstrate its application to general biological questions.
Data mining reveals a so far unreported cytoplasmic chain of B cell receptor associated protein 31. We suggest a new role of Bap31 in regulation of transcription during apoptosis upon caspase-mediated release of this leucine-like zipper containing cytoplasmic C-terminal domain. TOPCAT cross correlation analysis of cellular tumor antigen p53 suggests loss of caspase cleavage as the functional mechanism in some somatic cancer causing mutations of p53 highlighting it's broad scope.

POSTER ABSTRACTS

Poster No. T-23

Proteomics Technologies and Other Omics

A Pilot Study Characterizing Pre- and Post-treatment Metabolomic Profiles of Lung Cancer Patients

Sarfaraz, M. Omair
Department of Metabolomics-University of Calgary

Contributing Authors:

M. Omair Safaraz, D. Gwyn Bebb, Camelia Lee, Cynthia M. Card, Marilyn David, Anthony M. Magliocco, A

Background: Systems level analysis of serum metabolites represents a potentially non-invasive approach to determine biomarkers of cancer growth and death, tumour type and stage. The serum metabolome may reflect disease-mediated changes, and also register host response to treatment. In this pilot study, we evaluated the pre- and post-treatment metabolomic profiles of 25 non-metastatic lung cancer patients. Methods: Serum specimens were prospectively collected prior to treatment, mid-treatment and six months post-treatment. Metabolites were characterized using nuclear magnetic resonance (NMR) spectroscopy and gas chromatography mass spectrometry (GC-MS). Multivariate statistical analysis was conducted unsupervised principal component analysis (PCA) or orthogonal partial least squares discriminant analysis (OPLS-DA). Model significance was assessed using a cross-validated ANOVA based on a seven-fold cross validation. Results The metabolite profiles were reflective of the binary survival ($p<0.001$ by NMR & GCMS), disease progression (NMR, $p<0.001$; GCMS, $p<0.01$) and stage (NMR, $p<0.001$). The NMR data was also reflective of disease pathophysiology (NSCLC vs SCLC) across all time points ($p<0.005$), and survival from the baseline samples alone ($p<0.005$). A significant model could be built distinguishing baseline from post-treatment samples ($p<0.001$), suggesting that the samples during treatment represent an intermediate state. Smoking status may also be predicted across time points.. Conclusions: This study suggests that there may be differences in the metabolomic profile between NSCLC vs. SCLC, smokers vs. non-smokers and pre- and post-treatment. Intriguingly, it appears as though there remains a metabolic imprint in the serum from baseline, through treatment and post-treatment which can predict survival, disease progression, and pathophysiology.

Poster No. T-24

Proteomics Technologies and Other Omics

Ultrahigh-Performance Nano LC-MS/MS Analysis of Complex Proteomic Samples

Gendeh, Gurmil
Dionex Corporation

Contributing Authors:

Gurmil Gendeh, Evert-Jan Sneekes, Bjorn de Haan, and Remco Swart

Determination of the proteome and identification of biomarkers are required to monitor dynamic changes in living organisms and predict the onset of an illness. One popular method to tackle contemporary proteomic samples is called shotgun proteomics, in which proteins are digested, the resulting peptides are separated by high-performance liquid chromatography (HPLC), and identification is performed with tandem mass spectrometry. Digestion of proteins typically leads to a very large number of peptides. For example, digestion of a cell lysate easily generates 500,000 peptides. The separation of these highly complex peptide samples is one of the major challenges in analytical chemistry.

The main strategy to improve the efficiency of packed columns is either to increase column length or to decrease the size of the stationary phase particles. However, to operate these columns effectively, the LC conditions need to be adjusted accordingly. Naturally, the on-line coupling to MS systems has to be taken into account in the optimization process.

Here, the authors report on the performance of nano LC columns operating at ultrahigh pressure. The effects of column parameters (particle size and column length) and LC conditions (gradient time, flow rate, column temperature) were investigated with reversed-phase (RP) gradient nano LC. High-resolution LC-MS separations of complex proteomic peptide samples are demonstrated by combining long columns with 2 µm particles and long gradients. The effects of LC parameters on performance and the influence on peptide identification are discussed.

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Poster No. T-25

Proteomics Technologies and Other Omics

Reduction of Sample Carryover in Proteomics LC-MS Experiments

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Contributing Authors:

Gurmil Gendeh,

Relevant biomarkers are often present at concentrations near or below the detection limit of current analytical methods. Despite this challenge, several biomarker candidates have been identified and moved into the validation phase. The increase in sensitivity of analytical methods and mass spectrometry, in particular, over the past years is the reason for this accomplishment. However, a sensitivity increase alone is insufficient to accurately identify potential biomarkers; carryover reduction is also important to ensure a marker is actually present in the sample being analyzed. Therefore, reduction of carryover has received increased attention from the proteomics community.

TECH TALKS Abstracts

Monday, May 9 - 1:30

Shane Tichy, Ph.D.,
Agilent Technologies

Presentation:

Applying High Sensitivity LC/MS and Automated Liquid Handling Technology to Peptide Quantitation

Sensitivity is a key requirement for assays that are both specific and quantitative for target proteins. These assays are critical for preclinical validation of putative biomarkers, which may have low concentrations in commonly used biofluids such as serum and plasma. Using a triple quadrupole mass spectrometer with innovative iFunnel technology, we have achieved new levels of sensitivity and dynamic range for detection of target peptides in complex matrices such as human plasma. Peptide quantitation results achieved and details of the instrument design will be discussed. Recent work will be discussed where the Bravo Automated Liquid Handling Platform was used to automate the SISCAPA procedure, where specific anti-peptide antibodies are used to enrich target peptides from a plasma digest (SISCAPA).

Monday, May 9 - 1:45

Gurmil Gendeh
Dionex Corporation

Ultra-High Performance Nano LC for Analysis of Complex Proteomic Samples

Increasingly, biomarkers are being used to monitor dynamic changes in living organisms and predict the onset of illnesses. But, the determination of proteomes and the identification of biomarkers is a challenging and complicated process. Shotgun proteomics is a popular method used to tackle proteomic samples in which proteins are digested, the resulting peptides are separated by high-performance liquid chromatography (HPLC), and then biomarkers are identified with tandem mass spectrometry. But, the digestion of proteins typically leads to a very large number of peptides. For example, digestion of a cell lysate can easily generate 500,000 peptides. The separation of these highly complex peptide samples is one of the major challenges in analytical chemistry. The main strategy to improve the efficiency of packed columns is either to increase column length or to decrease the size of the stationary phase particles. Here, the speaker will describe the use of an ultra-high performance nano LC for separations of complex proteomic peptide samples by combining long columns packed with 2 µm particles and long gradients.

Tuesday, May 10 - 1:30

Bruker Daltonics

TBA

Tuesday, May 10 - 1:45

Brenda Kesler
Thermo Scientific

Complex Mixture? Check. Low Sample Concentration? Check. PTMs? Check. PPH (proteins per hour)? Smokin

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Department of Chemistry, University of Alberta

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Development of Microwave-Assisted Acid Hydrolysis Combined with MS and MS/MS for Determining Terminal Amino Acid Sequences of Proteins
tang, yanan
University of Alberta

Poster No. T-4, Proteomics Technologies and Other Omics Session
Core-structure study by MS3 analysis for unknown metabolite identification
Wu, Yiman
University of Alberta

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Chen, Lu
University of Alberta

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University of Alberta

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University of Alberta

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Dalhousie University

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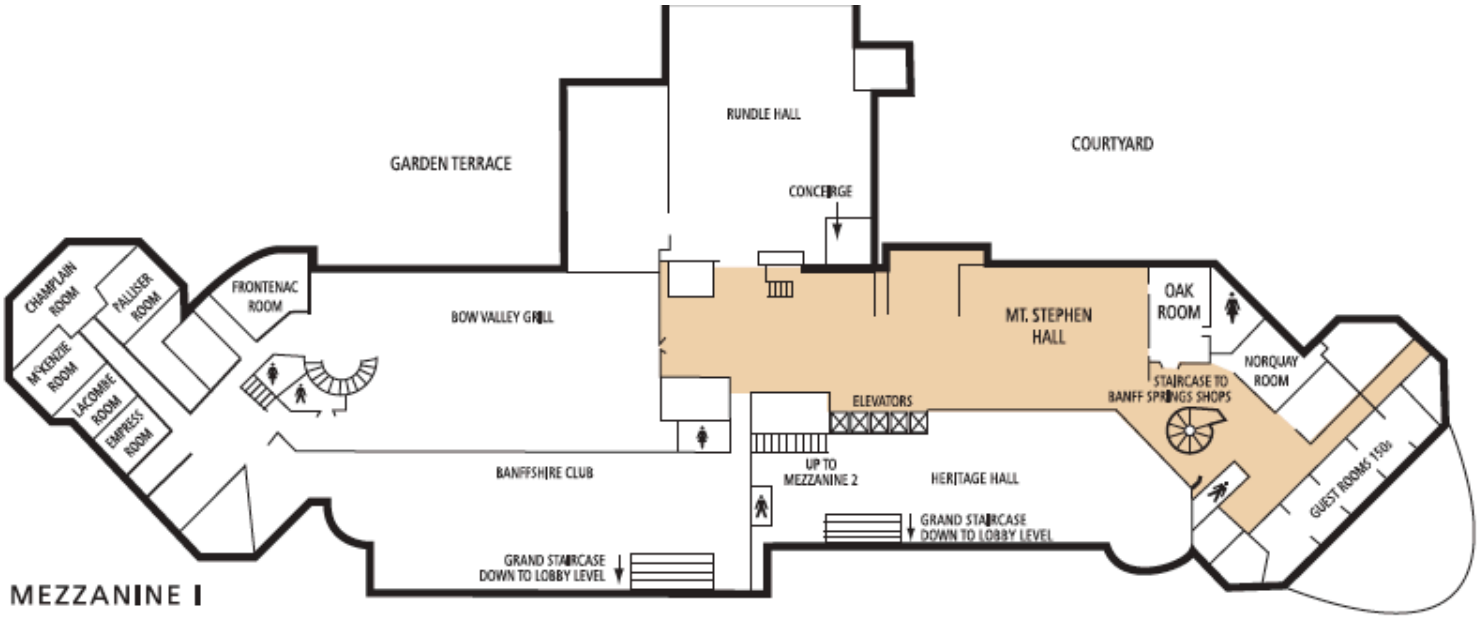
CONFERENCE FLOOR PLANS



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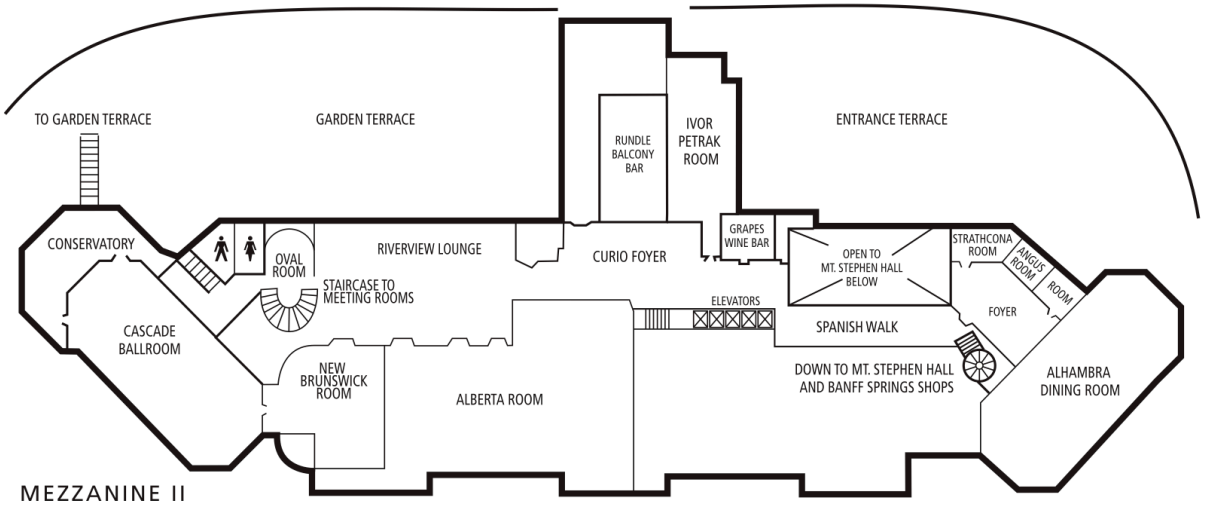
Student Job Fair: Norquay Room
Opening Reception: Mt. Stephen Hall
CNPN Board Meeting (closed door): Oak Room

CONFERENCE FLOOR PLANS



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Student Workshop: Ivor Petrak Room
Structural Proteomics Symposium: Ivor Petrak Room
CNPN Annual Members Meeting: Ivor Petrak Room
Plenary Sessions: Alberta Room
Poster Sessions: New Brunswick Room
Exhibits: Riverview Lounge
Award Banquet: Cascade Ballroom

Notes:

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