CANADIAN NATIONAL PROTEOMICS NETWORK

Symposium 2010

GenomeQuébec

Joint Conference of the Canadian National Proteomics Network & Genome Quebec

New Frontiers in Proteomic Research

Nouvelles frontières en recherche protéomique

CNPN

MAY 9-10, 2010

CONTENTS

Welcome from the Chairs

Pierre Thibault
Guy Poirier

On behalf of the Organizing Committee of the joint conference of the Canadian National Proteomics Network (CNPN) and Genome Quebec, we wish to welcome everyone to the Second Symposium of the Canadian National Proteomics Network. This symposium entitled "New Frontiers in Proteomic Research" will bring together world-renown scientists from different disciplines to highlight important contributions of proteomics to health sciences. These presentations will be made as part of six thematic sessions on:

Technology development
Neurobiology
Personalized Medicine
Stem cells
Blood & Blood Cells
Protein interactions

We are delighted to host this venue, and to share with you the outstanding contributions of our speakers.

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

Génome Québec's vision is to put genomics at the heart of the scientific and socioeconomic development of Quebec. Its mission, through the partnerships with key players in life sciences on national and international levels, Génome Québec contributes to strengthening the competitiveness of the system for innovation in genomics. The objective is to maximize the socioeconomic impact in Québec, by funding major genomics research initiatives and putting in place the tools necessary for scientific and strategic development in the field.

De la part du comité organisateur de la conférence conjointe du Réseau Canadien de la Protéomique (CNPN) et de Génome Québec, nous vous souhaitons la bienvenue au Deuxième Symposium du Réseau Canadien de la Protéomique. Ce Symposium intitulé Nouvelles frontières en recherche protéomique, regroupe des scientifiques de renommée internationale de différentes disciplines afin de souligner les importantes contributions de la protéomique aux sciences de la santé. Les conférences seront présentées dans 6 sessions dont les thèmes sont :

Développement technologique, neurobiologie, médecine personnalisée, cellules souches, sang et cellules sanguines, interactions protéiques.

Nous sommes ravis de vous accueillir à ce Symposium et de partager avec vous les contributions exceptionnelles de nos conférenciers.

Le Réseau National Canadien de Protéomique (CNPN) est un organisme sans but lucratif et ayant personnalité morale au Canada. Cet organisme a pour mission de fournir un mécanisme coopératif permettant l'élaboration d'une infrastructure de recherche en protéomique tout en favorisant la dissémination des connaissances de cette discipline au sein de notre communauté scientifique canadienne des sciences de la vie. Le CNPN parraine également des conférences scientifiques, des séminaires et des ateliers de discussion pour favoriser les collaborations scientifiques et l'éducation.

Génome Québec's Vision,
Inscrire la génomique au cœur du développement scientifique et socioéconomique du Québec.

En partenariat avec les acteurs des sciences de la vie à l'échelle nationale et internationale, Génome Québec contribue à renforcer la compétitivité du système d'innovation en génomique afin d'en maximiser les retombées socioéconomiques au Québec, en finançant des initiatives majeures de recherche en génomique et en mettant en place les outils nécessaires au développement scientifique et stratégique du domaine.

AGENDA - DAY 1

Sunday, May 9, 2010 Fairmont Queen Elizabeth Hotel - St. Francois Room, Lobby Level, 900 Rene Levesque Blvd. W.		
7:00 am	Exhibitor & Poster Set Up	Hochelaga 5/6, Saguenay, St. Maurice
7:30 am	Registration, Coffee with Exhibitors & Sponsors	Hochelaga 5/6, Saguenay, St. Maurice
8:15 am Conference Begins St. Francois Room		
8:15 am	Welcome, Opening Remarks and Introduction of Keynote Speaker and Session Chair	Pierre Thibault/Guy Poirier
8:30 am	*The FertiChip® project: Identification and validation of markers for male reproductive disorders in the human seminal plasma* ***Sponsored by Genome Quebec***	**Charles Pineau**, Proteomics Core Facility Biogenouest
9:15 am Technology Development Session – Liang Li		
9:15 am	*Top Down 2020*	**Neil Kelleher**, University of Illinois
9:45 am	*Development of Mass Spectrometric Methods for Whole Proteome Analysis*	**Liang Li,** University of Alberta
10:15 am Coffee Break - Poster Session & Exhibits, Hochelaga 5/6, Saguenay, St. Maurice		
10:45 am	*Discovering Differentially Expressed Proteins among Subtypes of Endometrial Cancer Tissues*	**K.W. Michael Siu**, York University
11:05 am	*In silico interactomics based on MSMS data acquired from high resolution native electrophoresis gels*	**Kurt Dejgaard**, McGill University
11:25 am	*Proteome-wide Analysis of Protein Carboxy Termini: C-Terminomics*	**Oliver Barre**, University of British Columbia
11:45 am	*An Isotopically-coded CID-cleavable biotinylated crosslinker CBDPS*	**Jason Serpa**, UVic Genome BC Proteomics Centre
12:05 to 1:00 pm - Lunch & Tech Talks - Poster Session & Exhibits - Hochelaga 5/6, Saguenay, St. Maurice		
12:30 pm	British Columbia Proteomics Network Structure, Activities and Availability of Resources	**Juergen Kast,** UBC **Christoph Borchers,** UVIC GBC Proteomic Centre
12:45 pm		Thermo Fisher
1:00 pm Neurobiology Session, John Kelly, Chair		
1:00 pm	*Discovery to Diagnostics: From Proteomics to Molecular Imaging of Neurovascular Biomarkers*	**Danica Stanimirovic,** NRC, Institute for Biological Sciences
1:30 pm	*Genomics of Brain Diseases*	**Guy A. Rouleau,** Université de Montréal
2:00 pm	*Label-free quantitative proteomics of surgically isolated brain vessels from Alzheimer's mice*	**AmanPreet Badhwar**, McGill University

AGENDA - DAY 1

Sunday, May 9, 2010 Fairmont Queen Elizabeth Hotel - St. Francois Room, Lobby Level, 900 Rene Levesque Blvd. W.		
2:20 pm	*The Proteome of the Master Circadian Clock using a Novel Automated Online Proteomics System*	**Daniel Figeys**, University of Ottawa
2:40 pm	*The new ZIP on the block analyses of the ZIP-PrP complex*	**Gerold Schmitt-Ulms**, University of Toronto
3:00	*Composition of protein-aggregates induced upon inhibition of the proteasome revealed by quantitative mass spectrometry*	**Inga Wilde**, University of British Columbia
3:30 pm Coffee Break - Poster Session & Exhibits, Hochelaga 5/6, Saguenay, St. Maurice		
3:45 pm Personalized Medicine Session – Tommy Nilsson, Chair		
3:45 pm	*CALIPHO: computer and laboratory approaches targeted toward human proteins*	**Amos Bairoch,** Swiss Institute of Bioinformatics
4:15 pm	*Proteomics analyses played a key role in our understanding of the contribution of phagocytosis and autophagy to antigen presentation*	**Michel Desjardins,** Université de Montréal
4:45 pm	*Plasma Protein and Glycoprotein Profiling in Triple-Negative Breast Cancer*	**Devanand Pinto**, National Research Council
5:05 pm	*Multiple reaction monitoring of amino acid polymorphisms in ovarian carcinoma tissue*	**Declan Williams,** York University
5:25 pm	*Design of a Selected Reaction Monitoring Mass Spectrometric Immunoassay*	**Bryan Krastins**, Thermo Fisher Scientific
5:45 pm	*Quantitative Analysis of IGF-1R signaling pathway activation in FFPE Tissue*	**Julian Saba**, Thermo Fisher Scientific
6:05 pm - Closing Remarks - Day 1		
6:30 pm Reception - Poster Session & Exhibits, Hochelaga 5/6, Saguenay, St. Maurice		
7:30 pm - Award Presentation, St. Francois Room		
8:00 Award Dinner		

AGENDA - DAY 2

Monday, May 10, 2010 Fairmont Queen Elizabeth Hotel - St. Francois Room, Lobby Level, 900 Rene Levesque Blvd. W.		
8:30 am	Coffee with Exhibitors & Sponsors	Hochelaga 5/6, Saguenay, St. Maurice
9:00 am Conference Begins St. Francois Room		
9:00 am	Welcome, Opening Remarks, Day 2	Pierre Thibault/Guy Poirier
9:10 am Stem Cells Session, Pierre Thibault, Chair		
9:10 am	*Phosphoproteomic analyses of leukemia stem cells identify a potential role for ROS detoxification in stem cell self-renewal.*	**Guy Sauvageau,** Université de Montréal
9:40 am	New mass spectrometry tools for the study of ubiquitin and ubiquitin-like proteins	**Brian Raught**, Ontario Cancer Institute
10:15 am Coffee Break - Poster Session & Exhibits, Hochelaga 5/6, Saguenay, St. Maurice		
10:45 am	*Growth factor independence 1b (Gfi1b) restricts dormancy and peripheral blood mobilization of hematopoietic stem cells*	**Cyrus Khandanpour,** IRCM
11:05 am	*Enhancing progenitor cell therapy for vascular repair*	**Annie Bourdeau**, Sunnybrook Research Institute
11:25 am	*BAF Switching: a Novel Epigenetic Mechanism Essential for Normal and Leukemic Hemopoiesis ?*	**Buscarlet**, Manuel; IRIC, Universite de Montreal
11:45 am	*HOXA4 induces self-rewal expansion of Hematopoietic Stem Cells*	**Janetta Bijl**, HMR Research Center
12:05 to 1:00 pm - Lunch & Tech Talks - Poster Session & Exhibits - Hochelaga 5/6, Saguenay, St. Maurice		
12:30 pm	*A New Era in Ultra-High NanoLC Performance*	**Gurmil Gendeh**, Dionex
12:45 pm		**David Craft**, BD
1:00 pm Blood & Blood Cells Session, Juergen Kast, Chair		
1:00 pm	*Quantitative proteomics analyses: Large-scale and targeted analyses*	**Jerome Garin,** Laboratoire d'Etude de la Dynamique des Protéomes (EDyP)
1:30 pm	*Molecular Definition Of The MHC I Peptidome Using A High-Throughput Quantitative Mass Spectrometry approach*	**Claude Perreault,** Université de Montréal
2:00 pm	*Positional N-terminal and C-terminal proteomics deciphers protein terminal and proteolytic post-translational modifications in complex proteomes in vivo.*	**Christopher Overall**, University of British Columbia
2:30 pm	*Control of erythropoiesis through the dynamics of transcription factor interactions: a quantitative proteomics study*	**Margorie Brand**, Sprott Center for Stem Cell Research-OHR
2:50 pm	*Proteomic Biomarkers of Acute Kidney Allograft Rejection*	**Gabriela Cohen Freue**, PROOF Centre of Excellence

AGENDA - DAY 2

Monday, May 10, 2010 **Fairmont Queen Elizabeth Hotel - St. Francois Room, Lobby Level, 900 Rene Levesque Blvd. W.**		
3:10 pm Coffee Break - Poster Session & Exhibits, Hochelaga 5/6, Saguenay, St. Maurice		
3:45 pm Protein Interactions Network Session – Benoit Coulombe, Chair		
3:45 pm	*Improving interaction proteomics – Application to the kinase and phosphatase interactomes*	**Anne-Claude Gingras,** Mount Sinai Hospital, SLRI
4:15 pm	*A lentiviral-based functional proteomics approach identifies chromatin remodelling complexes important for the induction of pluripotency*	**Jason Moffat,** University of Toronto
4:45 pm	*Systematic Identification by Affinity Purification and Mass Spectrometry of Protein-Protein Interactions and Protein Complexes for the Membrane Proteins of Saccharomyces cerevisiae*	**Jack Greenblatt**, University of Toronto
5:05 pm	*In Silico Protein Interaction Analysis Using Archived Proteomics Experiments in the Global Proteome Machine Database*	**Juergen Kast**, University of British Columbia
5:25 pm	*Quantitative Analysis of the GRB2 Adaptor Protein Network Dynamics Using a Scheduled Multiple Reaction Monitoring (sMRM) Assay*	**Nicolas Bisson**, Samuel Lunenfeld Research Institute
5:45 pm	*Identification of poly(ADP-ribose) binding proteins by mass spectrometry.*	**Guy Poirier**, Laval University
5:55 pm	*Modeling Contaminants in Tandem Affinity Purification Experiments*	**Mathieu Lavallee-Adam**, McGill University
6:05 pm - Guy Poirier, Closing Remarks - Day 2		

Please note that the Agenda is subject to change.

Keynote Speaker

Charles Pineau, Proteomics Core Facility Biogenouest

Biography:

Dr. Pineau received his Ph.D. in Biology from the University of Rennes in 1990. Dr. Pineau has held several positions. Since 2006 has held the position of Director at the Proteomics Core Facility Biogenouest. In 2002 he co-founded Innova Proteomics and holds the position of Chief Scientific Officer. Dr. Pineau's research interest is The proteome of Mammalian spermatogenesis. In 1992 Dr. Pineau received the Merit Award from The American Society of Andrology and in 1993 the Medal of the City of Rennes.

Presentation:

The FertiChip® project: Identification and validation of markers for male reproductive disorders in the human seminal plasma

The chance for proposing intracytoplasmic sperm injection (ICSI) to infertile couple in case of non obstructive azoospermia (NOA) depends on the possibility to retrieve live sperm from testis biopsy fragments. Unfortunately, there is currently no powerful predictor of the probability to obtain a positive sample with most of biopsies resulting in sperm recovery failure. In its first phase, this project aimed at investigating whether specific protein biomarkers of post-meiotic germ cells can be detected in seminal plasma in order to select for a testis biopsy only patients for which there are high probabilities to find spermatozoa.

An approach was used that combined protein pre-fractionation, iterative mass spectrometry analysis, and “combinatorial Omics” to identify germline biomarkers in the seminal plasma proteome. The set of proteins identified was compared, merged and completed with yet published human seminal plasma protein datasets, leading to a list of non-redundant unique proteins. A tissue-profiling analysis using Affymetrix microarray expression data was then used to mine the protein dataset and allowed the identification of potentially relevant functional biomarkers for each one of the organs participating in the seminal plasma protein composition (testis, epididymis, seminal vesicles and prostate). In the first part of the project, a focus was given to proteins specific to the presence within the testis of meiotic and postmeiotic germ cells. Specificity of these germ cell biomarkers was then validated on normal and pathological seminal plasmas (NOA, bilateral agenesis of deferent ducts, Sertoli cell-only syndrome, post-vasectomy).

The human seminal plasma can be seen as an important source of potential biomarkers for male reproductive disorders. The continuation of the project has already lead to the identification of relevant markers of epididymis and prostate function in the seminal plasma of healthy fertile men and patients with distinct reproductive pathologies. With this study, we demonstrated that “combinatorial Omics” is a powerful strategy to identify relevant biological markers in complex biofluids without the need for heavy differential proteomics approaches.

An antibody array, named FertiChip®, is currently under development. The relevance of the array will be tested through a yet established network of Fertility & IVF Centers in France on large cohorts of normal or pathological seminal plasmas. The FertiChip® array should become a powerful diagnostic decision tool for clinicians in the field of male infertility and reproductive disorders.

Technology Development Session Invited Speakers

Neil L. Kelleher,
University of Illinois at Urbana-Champaign

Biography:

Professor Neil Kelleher received a B.S. and B.A. from Pacific Lutheran University in 1992, a Fulbright Fellowship the following year, and a Ph.D. with Tadhg Begley and Fred McLafferty (Cornell University) in 1997. After a NIH Postdoctoral Fellowship with Chris Walsh (Harvard Medical School), Kelleher joined the faculty at the University of Illinois in 1999. He has received several awards including a Packard Fellowship, the NSF CAREER Award, the Lilly Analytical Chemistry Award, the Presidential Early Career Award in Science and Engineering (PECASE), the Biemann Medal (ASMS), and the Camille Dreyfus Teacher Scholar Award. He is a Sloan Fellow and has received support from the Burroughs-Wellcome, the Searle, and the Dreyfus Foundations. Kelleher has interest in Mass Spectrometry-based enzymology and "Top Down" proteomics using intact proteins for efficient detection of their post-translational modifications (such as those found in chromatin). Professor Kelleher begins his tenure at Northwestern University in January 2010 where he will serve as Professor of Chemistry and Biochemistry, Molecular Biology and Cell Biology (BMBCB). Within the Chemistry of Life Processes Institute, Neil will serve as the Faculty Director of the Proteomics Center of Excellence.

Presentation:

Top Down 2020

The year is 2020 and with the capability to identify and characterize protein isoforms up to 200 kDa routinely, Top Down Mass Spectrometry has become an equitable partner with digestion-based methods for most researchers undertaking targeted protein studies or full bandwidth proteomics. Certainly, bench top systems with 10e6 resolving power at 100 kDa have enabled a widespread adoption of the Top Down strategy, but breakthroughs in automated intact protein separations allow robust and predictive access to the endogenous proteome of <10e6 cells. Recent developments of protein supercharging coupled to real-time database searching and reliable label-free quantitation now allow deep dives into the proteome for systems biology and personalized biomedicine in the globalized biotechnology sector.

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.

Presentation:

Development of Mass Spectrometric Methods for Whole Proteome Analysis

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 proteome sample preparation and mass spectrometric detection for characterizing the whole proteome will be described.

Technology Development Session Oral Abstract Presentations

Discovering Differentially Expressed Proteins among Subtypes of Endometrial Cancer Tissues

Siu, K.W. Michael
York University

Contributing Authors:

S.N. Voisin, O. Krakovska, L.V. DeSouza, B.A. Clarke and K.W.M. Siu

Endometrial cancer (EmCa) is the most common gynecologic malignancy in North America and the fourth most frequently diagnosed cancer in North American women. It is a heterogeneous disease with different phenotypes and aggressiveness. Classification of EmCa is currently based solely on the histology of tumor cell type, and in the setting of endometroid cell type, grading. This classification is then used to determine the extent of surgery, and the need for as well as the type of adjuvant therapy. Unfortunately, histopathologic classification is subjective, and is made more difficult when mixed EmCa cell types are present. Variants of some cell types are sometimes difficult to differentiate; a wrong diagnosis would mean that non-optimal adjuvant therapy could be prescribed. Herein we report an investigation aiming to identify potential biomarkers that will permit differentiation of high-grade EmCa at a molecular level as an aid to histopathology. Six FIGO (International Federation of Gynecology and Obstetrics) grade III endometrioid, five clear cell, and eight serous carcinomas were compared using iTRAQ labeling and LC-MS/MS analysis. Sample sets were analyzed following an iterative approach to improve and increase the numbers of proteins identified. Using this approach, we identified eighteen proteins that showed differential expression in different histological types of high-grade EmCa. In addition, fourteen proteins showed a similar trend of differential expression for all samples, and may be considered potential candidates as EmCa biomarkers. Verification of these differential expressed proteins in a larger cohort of patient samples will transform these proteins into biomarkers that permit objective and molecular subtyping of endometrial carcinomas. Three proteins, chaperonin 10, alpha-1-antitrypsin, and pyruvate kinase M2, had already been verified by means of immunohistochemistry in a cohort of 146 patients. Verification on a comparable scale will need to be implemented for the rest of the differentially expressed proteins in the future. This will be accomplished on formalin-fixed paraffin-embedded tissues for which supply is readily available and more plentiful.

In silico interactomics based on MSMS data acquired from high resolution native electrophoresis gels

Dejgaard, Kurt
McGill University

Contributing Authors:

Kurt Dejgaard, Robert Kincaid, Amy Wong and David Y. Thomas

Current native electrophoresis techniques may resolve proteins and protein complexes, ranging in size from 10 kDa to 10 MDa at a resolution approaching that of SDS gels. We have previously used MS-based information from select regions of such gels to decipher the complex organization of various assembly states of megadalton sized ribosome:ER-translocon complexes (Dejgaard et al., J. Proteome Res., 2010). Expanding on this to analyze a variety of complexes across entire native gels, a software tool, MassVis, has been developed that can reconstruct synthetic 2D gel images based on quantitative LC-MSMS data. Correlating patterns and integrating normalized relative quantities, computationally, we demonstrate how native gel data may be clustered to predict protein interaction partners. The resolution of the approach is defined by the sensitivity and dynamic range of the mass spectrometer and by sample complexity versus electrophoretic resolution. The accuracy is defined by the reliability of integrated, summed and normalized precursor ion intensities as estimates of relative abundance, across protein species.

Technology Development Session Oral Abstract Presentations

Proteome-wide Analysis of Protein Carboxy Termini: C-Terminomics

Barre, Olivier
UBC

Contributing Authors:

Oliver Schilling, Pitter Huesgen and Christopher Overall

The sequence and nature of the amino (N) and carboxy (C)-termini of proteins provides important functional annotation of proteomes since their modification or truncation affects all proteins, often influencing protein fate and function. Although characterization of the N-terminus is relatively well studied, relatively little is known of such modifications of the C-terminus. Proteome-wide analysis of protein N-termini has recently become the focus of intense research effort providing information on protein isoforms, acetylation, the functional state of the protein, and can be used to identify protease cleavage sites. However, this development has not been complemented by similar progress towards the proteome-wide analysis of protein C-termini by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Present approaches employ anhydrotrypsin, a combination of LysC protease and amine-capture, carboxypeptidase ladder sequencing or diagonal electrophoresis. Generally, these techniques are not used for complex proteome samples and their application is restricted to samples consisting of only few proteins. We present a polymer-based approach for the enrichment of carboxy-terminal peptides from complex proteomes and their identification by liquid chromatography-tandem mass spectrometry. The workflow optionally incorporates isotopic labeling to distinguish and quantitatively compare carboxy-termini from different samples, thus enabling applications in protease substrate profiling.

An Isotopically-coded CID-cleavable biotinylated crosslinker CBDPS

Serpa, Jason
UVic-GBC Proteomics Centre

Contributing Authors:

Evgeniy V. Petrotchenko; Christoph H. Borchers

Successful application of crosslinking combined with mass spectrometry for structural proteomics demands specifically-designed crosslinking reagents. A combination of affinity enrichment, isotopic coding, and cleavage of the crosslinker is beneficial for detection and identification of the peptide crosslinks. Here we describe a novel crosslinker, CBDPS (CyanurBiotinDiPropionylSuccinimide), which allows affinity enrichment of the crosslinker-containing peptides with avidin. Affinity enrichment eliminates interfering uncrosslinked peptides, and allows the researcher to focus on the analysis of the crosslinked peptides. The crosslinker is also isotopically-coded and CID-cleavable. The cleaved fragments still contain a portion of the isotopic label and can therefore be distinguished from unlabeled fragments by their distinct isotopic signatures in the MS/MS spectra. This allows rapid determination of the crosslink type, in addition to facilitating identification of the individual peptides constituting the inter-peptide crosslinks. We use in house developed ICCLMSMS software for analysis of tandem mass spectra of CID-cleavable isotopically coded crosslinks to flag immonium ions and hydrolyzed crosslinker fragments signals as dead-end crosslinks, and to identify the CID-cleaved portions of inter-peptide crosslinks. We satisfactorily tested CBDPS with HIV-RT, a protein heterodimer consisting of two subunits, 55 kDa and 61 kDa. Seventy-eight crosslink candidates were detected. Software analysis of the MSMS spectra of these candidates resulted in the elimination of dead-end crosslinks from further analysis, and assignment and identification of five inter-peptide crosslinks. Thus, affinity enrichment combined with isotopic coding and CID cleavage allows facile in-depth mass spectrometric analysis of the peptide crosslinks.

Neurobiology Session Invited Speakers

Danica Stanimirovic,
NRC Institute for Biological Sciences

Biography:

Dr. Danica Stanimirovic obtained M.D. degree in 1986, and Ph.D. degree in Neurochemistry in 1990, from the Faculty of Medicine, University of Belgrade. After clinical internship in neurology, she joined the Stroke Branch at the National Institute for Neurological Disorders and Stroke (NINDS), NIH in Bethesda, and subsequently NRC's Institute for Biological Sciences in Ottawa in 1993. She established and led the NRC's Cerebrovascular Research Group and was appointed Director of the NRC's Neurobiology Program in 2001. The Neurobiology Program (>100 scientific staff) caries out fundamental and applied research to develop new diagnostics and therapeutics for neurodegenerative diseases. Dr. Stanimirovic is also Adjunct Professor at the Department of Cellular and Molecular Medicine at the University of Ottawa.

Dr. Stanimirovic published over 100 peer-reviewed manuscripts, 25 book chapters and 10 patent applications. She leads large-scale national and international collaborative initiatives and has been involved in organizing several international meetings. She serves on Scientific Advisory Boards of biotechnology companies, and on NIH and Canadian Heart and Stroke Foundation review panels. Her areas of expertise include brain drug delivery, neuroproteomics and molecular imaging.

Presentation:

Discovery to Diagnostics: From Proteomics to Molecular Imaging of Neurovascular Biomarkers

Brain vasculature is affected by and functionally implicated in a majority of brain diseases including among others stroke, Alzheimer's disease, multiple sclerosis and brain tumors. Changes in molecular 'make-up' (or functional properties) of brain vasculature are often an early 'sign' of disease that could be exploited as a biomarker for diagnosis or monitoring the evolution of disease. This opportunity is particularly attractive, because many brain vascular biomarkers could be accessed from the circulatory compartment. However, validated brain endothelial biomarkers are few, principally because brain vascular compartment has been less studied using advanced discovery technologies. In this lecture cerebrovascular biomarker discovery and validation paradigm which links genomics and proteomics discovery workflows with the development of molecular imaging agents against selected targets and their evaluation in vivo by non-invasive imaging will be presented. Discovery workflows include proteomic and glycoproteomic interrogation of cultured brain endothelial cells and their membrane compartments (luminal, abluminal, lipid rafts) as well as laser-capture microdissected vascular tissues from the brains of animals or humans affected by disease. Single domain antibody screening platform was then applied to select and develop biomarker binding moieties. Targeting sdAbs are engineered to carry imaging contrast agent(s) (for optical and MRI imaging modalities) to the target site (s). The in vivo validation of diagnostic/predictive value of cerebrovascular biomarker is achieved by a non-invasive, prospective in vivo imaging during the course of the disease or disease treatment. Validated cerebrovascular biomarkers could become surrogates for patient stratification and efficacy monitoring in clinical trials, reducing the length and cost of drug development cycle.

Neurobiology Session Invited Speakers

Guy Rouleau,
Centre of Excellence in Neuromics of Université de Montréal, and Department of Medicine, Université of Montréal,

Biography:

Dr. Guy Rouleau is the Director of the Research Centre of the CHU Sainte-Justine. He is a tenured professor in the Department of Medicine at Université de Montréal. He is the creator and Director of the Centre for excellence in neuromics and director of the Réseau de Médecine Génétique Appliquée – FRSQ. He is the Chairholder of the Canada Research Chair in Genetics of the Nervous System. At McGill University he is an Adjunct Professor in the Department of Human Genetics and holds cross appointments in the Departments of Psychiatry, Biology, and Human Genetics. He has published more than 400 articles in peer-reviewed journals, as well as 35 review articles and book chapters.
Dr. Rouleau received his MD (Magna Cum Laude) in 1980 from the University of Ottawa. He went on to pursue a PhD in Genetics at Harvard University which he completed in 1989. His post-graduate research was done in Neuroscience at the Montreal Neurological Institute as well as at Massachusetts General Hospital. His clinical work was done in Internal Medicine at the Montreal General Hospital, in Neurology (residency) at the Montreal Neurological Institute, and in Neurology (Research Fellow) at the Massachusetts General Hospital. He has received numerous awards, the most recent of which are the Prix d'excellence, from the Département de médecine, Université de Montréal, the Prix Henry-Friesen from the Royal College of Physicians and Surgeons of Canada / The Canadian Society for Clinical Investigation. Most recently he was made an Officer of the National Order of Québec. He sits on the editorial boards of Neurology of Disease, Canadian Journal of Neurological Sciences, Annal of Human Genetics, and performs ad hoc reviews for more than 12 journals. He is or was a member of the Scientific Advisory Board of Généthon, Evry France; of the Medical and Research Advisory Board of the National Ataxia Foundation; of Human Genome Organization; of the Scientific Advisory Board, Fondation Jean Dausset; of the Board of directors, Fondation des Jumelles Coudé; of the Board of directors, Fondation de l'ataxie de Charlevoix; of the Board of directors, Cole Foundation; of Scientific Advisory Board of the Centre for Applied Genomics, Hospital for Sick Children; of the Science Advisory Council (SAC), Neuroscience Canada; of the Canadian Academy of Health Sciences and of the Science, Technology and Innovation Council, Government of Canada. He has also served on numerous grant panel committees (ex. CIHR, NIH, ALSA, NNFF, FRSQ) and Company Boards (Xenon Genetics Inc, Emerillon Therapeutics Inc, Biocapital Inc.). He has supervised many students (9 Master's, 32 PhD, and 30 post-doctoral fellows). He is a founder of RGS Genome Inc. (1998-2000), director of Xenon Genetics Research Inc. (2000-2003) and founder/director/president of Emerillon Therapeutics Inc (2003-2009).
Over the last 20 years his work has focused on understanding the genetic basis for diseases of the brain. Specifically he has mapped over 20 disease loci, significantly contributed to the identification of over 10 genes causing diseases as well as to a better understanding of the pathogenesis of numerous diseases. He works on numerous neurological and psychiatric diseases, including amyotrophic lateral sclerosis, stroke, familial aneurysms, cavernous angiomas, epilepsy, spinocerebellar ataxia, spastic paraplegia, autism, Tourette syndrome, restless legs syndrome, schizophrenia and bipolar disorder. His laboratory endeavours to understand the pathogenesis of amyotrophic lateral sclerosis, oculopharyngeal muscular dystrophy and CAG repeat disorders using cell and animal models.

Presentation:

Genomics of Brain Diseases

The brain is a complex organ for which we know very little. This complexity has hampered the application of genomic approaches to the study of this organ, and the diseases that affect it. In particular the complex anatomy, the limited access to high quality tissue, the plasticity, and the fact that humans show rather unique abilities (ex. speech) make classical approaches difficult. In addition, diagnoses are based on

Neurobiology Session Invited Speakers

diagnostic criteria that involve subjective judgments, defining syndromes more than specific diseases. Nonetheless, numerous epidemiological studies have clearly implicated genetic factors in the etiology most brain diseases, suggesting that genomic strategies may prove fruitful, if they can overcome the difficulties inherent to the field.

With improving technology, it has become possible to explore a deep resequencing approach to the identification of the genetic factors underlying brain disease. We first chose to study ion channels in episodic brain diseases. Specifically, we sequenced 150 brain expressed ion channel genes in 368 individuals with either epilepsy, migraine, bipolar disorder, Tourette syndrome or essential tremor. Results of this study will be presented, highlighting the salient results and particularly the lessons learned.

We next chose to perform a deep resequencing study schizophrenia (SCZ), autism (AUT) and mental retardation (MR), all common, devastating and poorly treated brain disorders. Converging evidence suggests that genetically disrupted synaptogenesis and plasticity during development may underlie their pathogenesis. We hypothesized that a significant fraction of SCZ, AUT and MR cases are a result of novel mutations in many different genes involved in synapse formation and function. To identify genetic factors predisposing to MR, AUT and SCZ we have adopted a two-step strategy: direct re-sequencing of 1000 genes encoding proteins acting at the synapse, followed by functional validation of variants in zebrafish, drosophila or mouse hippocampal cell models.

We established a list of 5,079 synaptic and potentially synaptic genes, and prioritized them using a ranking system. We next sequenced on one strand DNA samples from 96 MR, 143 SCZ and 142 AUT unrelated probands. To date we have screened 488 genes and have identified over 8000 variants, of which 5990 are novel and not found in public SNP databases. All unique variants are confirmed in the appropriate trio (proband + both parents) to determine whether it is de novo. In this way we have identified a total of 15 de novo variants in 13 different genes. The direct neutral mutation rate is similar to previous indirect estimates, but we find a significant excess of de novo point mutations that are protein-disruptive and highly deleterious in ASD and SCZ individuals with no family history. In addition, we have identified a further 12 protein-truncating variants in 10 genes that are transmitted from either the mother or father. To further genetically validate each of these variants or candidate genes, we sequence additional case and control samples to identify additional variants. Finally, we test whether the variant has a functional effect on the protein using several animal models. Using these approaches, we have identified strong candidate genes for AUT, SCZ and MR. A combination of high-throughput sequencing, automated SNP discovery, genetic and biological validation strategies can be used to identify SCZ and AUT genes. This approach can also be used to identify genes involved in other common diseases, especially as next-generation sequencing technologies become more available to screen a larger number of candidate genes in more patient samples. However the challenge remains in robustly linking a DNA variant to a clinical phenotype.

Neurobiology Session
Oral Abstract Presentations

Label-free quantitative proteomics of surgically isolated brain vessels from Alzheimer's mice

Badhwar, AmanPreet
McGill University

Contributing Authors:

A. Badhwar, R. Brown, C.E. Delaney, D.B. Stanimirovic, E. Hamel and A.S. Haqqani

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BACKGROUND: Cerebrovascular dysfunction appears prior to A-plaque deposition and clear memory deficits in Alzheimer disease patients and in transgenic mice expressing amyloid precursor protein (APP). We have demonstrated that this dysfunction is manifest in young APP-transgenic mice (Badhwar et al, 2010) and persists with age (Nicolakakis et al, 2008). Here we examine the molecular effect of the APP transgene in pial arteries of wildtype (WT) and APP-transgenic mice using label-free MS-based quantitative proteomics. Our aim is to identify proteins and networks that orchestrate the cerebrovascular deficit in Alzheimer's disease mouse models.
METHODS: Pial arteries were isolated from WT and APP-transgenic mice. Proteins were extracted from the vessels, trypsin-digested, fractionated by SCX and analyzed by nanoLC-MS/MS using nanoAcquity UPLC (Waters) and ESI-LTQ Orbitrap (Thermo). MatchRx software (Haqqani et al, 2008) version QnD-2.0 was used to define peptide peaks, quantify and normalize intensities, and align LC-MS runs among fractions. Differentially-expressed proteins were identified as >1.5-fold change at p<0.05. Three biological replicates and two technical replicates were carried out.
RESULTS: More than 6,200 peptides corresponding to 3,283 proteins were identified. Compared to WT, 164 proteins showed differential expression in APP-transgenic mice. Many of the differentially-expressed proteins were associated with pathways modulating oxidative stress, amyloidosis, transport and cytoskeletal processes.
CONCLUSIONS: We have characterized the effect of the APP transgene on the cerebrovascular proteome. Altered protein levels in the APP mice suggest that the altered cerebrovascular function occurs via modulation of the amyloid cascade and oxidative stress pathways.

The Proteome of the Master Circadian Clock using a Novel Automated Online Proteomics System

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Contributing Authors:

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Nearly all organisms have evolved an internal timekeeping mechanism know as the "circadian"™ clock, which allows them to adapt their physiological needs to the constraints of a cyclic 24-hr world. The suprachiasmatic nucleus (SCN), the center of the master circadian clock in mammals, has endogenous pacemaker activity that runs at near-24-hour cycles, and can be reset by changes in environmental light cycle. Abnormal circadian rhythm has been associated with different pathophysiological disorders, such as cancer, cardiovascular disease, metabolic syndrome and various neurological diseases. The SCN is composed of only ~20,000 neurons which control the circadian timing mechanism. Here, we reported for the first time an automated online proteomics sample processing and mass spectrometry identification system, termed automated online proteomics system (AutoProteomic system), for proteomic samples from the SCN ranging from 2 Î¼g to 200 Î¼g. All of the necessary steps for proteomics sample processing, including protein preconcentration, buffer exchanging, reduction\alkylation, digestion were fully integrated. Furthermore, the system includes online two-dimensional chromatography separation and mass spectrometry identification (2D LC-MS/MS) based on a nanoflow HPLC-ESI-MS/MS. We used this automated system to study the light induction of the protein expressed in the SCN region of mouse brain. Over 4,000 unique proteins were identified in the SCN and the level of 218 proteins was statistically changed following light induction. Systematic function analysis revealed their tight connection and distribution in multiple pathways related to circadian rhythm.

Neurobiology Session
Oral Abstract Presentations

The new ZIP on the block analyses of the ZIP-PrP complex

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Prion diseases are fatal neurodegenerative diseases of humans and animals which, in addition to sporadic and familial modes of manifestation, can be acquired via an infectious route of propagation. Quantitative mass spectrometry based on isotopic tagging of peptides was used to characterize the interactome of the prion protein in a murine neuroblastoma cell line (N2a), an established cell model for prion replication. Extensive bioinformatic analyses subsequently established the evolutionary link between the prion gene family and the family of ZIP (Zrt-, Irt-like Protein) metal ion transporters. More specifically, sequence alignments, structural threading data and multiple additional pieces of evidence placed a ZIP5/ZIP6/ZIP10-like ancestor gene at the root of the PrP gene family. The objectives of our current work are to complement this finding with critically needed insights into the biology of ZIP transporters and to evaluate the significance of the PrP-ZIP relationship in health and disease. Our recent data constitute the first biochemical characterization of members of the LIV-1 subfamily of ZIP proteins in N2a cells. Efforts to solve the structure of the prion-like domain within a ZIP transporter are ongoing.

Composition of protein-aggregates induced upon inhibition of the proteasome revealed by quantitative mass spectrometry

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Contributing Authors:

Inga Wilde, Maria Brack, Esther Gies, Jason Winget, Carolina Arias Novoa, Thibault Mayor

Protein aggregates are hallmarks of some of the most common age-related neurodegenerative diseases, like Alzheimer's and Parkinson's diseases. Impairment of the ubiquitin proteasome system (UPS) has been associated to aggregate formation and ubiquitin is among the major components of these aggregates. Here we aim at identifying the composition of protein aggregates induced upon proteasome inhibition in neuroblastoma cells. Inhibition of the proteasome resulted in the rapid formation of ubiquitin-enriched inclusions in the cells. Concurrently, we observed that most ubiquitinated proteins displayed a lower solubility after proteasome inhibition. We presumed that a large fraction of proteasome substrates were misfolded ubiquitinated proteins that formed aggregates when the proteasome was inhibited. Combining a sucrose-gradient enrichment of these ubiquitin-positive aggregates with a SILAC analysis, we identified over 350 proteins specifically enriched after proteasome inhibition. By mixing the SILAC labeled cells prior to the biochemical enrichment, we ensured that the identified proteins were associated to the ubiquitinated proteins during the aggregation process and not after cell lysis. Ubiquitin and several other UPS related proteins were specifically enriched from proteasome-inhibited cells including sequestosome 1/p62, UCHL1 and UBA1. In addition, we identified a large number of cytoskeletal and chaperone proteins, like hsp70. We therefore hypothesized that a large fraction of identified proteins were co-factors that were likely sequestering ubiquitinated aggregating proteins instead of being ubiquitinated themselves. By using triple SILAC-labeling, we then performed a time-course experiment to differentiate between proteins present in early (i.e. 6h) or in late (i.e.12 h) aggregates. We found out that some chaperone proteins, like hsp90, are already present in early aggregates, while others, like hsp70, are only enriched in later structures. The identification of aggregating proteins, as addressed in this study, will contribute to enlighten the role of UPS impairment for the cell in neurodegenerative disorders.

Personalized Medicine Session Invited Speakers

Amos Bairoch,

Swiss Institute of Bioinformatics

Biography:

Professor of Bioinformatics and chairman of the Department of Structural Biology and Bioinformatics at the Faculty of Medicine of the University of Geneva, Amos Bairoch is group leader of the CALIPHO group which he co-directs with Dr Lydie Lane. In 1986 he founded the Swiss- Prot protein knowledgebase and was director of the Swiss-Prot group of the SIB from April 1998 to July 2009. He was also responsible for the development of the PC/Gene sequence analysis software package, as well as the PROSITE and ENZYME databases. Prof. Bairoch is also co-responsible for the development of ExPASy (www.expasy.org), the world's first web site dedicated to protein sciences. He is one of the co- founders of the SIB as well as scientific co-founder of two biotechnology companies in Geneva: GeneBio and GeneProt. In the framework of the SIB and University of Geneva CALIPHO group, he directs his efforts toward the advance in our knowledge of human proteins through the combined use of bioinformatics and experimental methodologies.

Presentation:

CALIPHO: computer and laboratory approaches targeted toward human proteins

In September 2008, the Swiss-Prot group achieved a major milestone: the first complete manual annotation of what is believed to be the full set of human proteins (derived from about 20'300 genes). This corpus of data is already quite rich in information pertinent to modern biomolecular medical research, but there is a huge gap in our knowledge of human proteins in terms of functional information as well as protein characterization (PTMs, protein/protein interactions, subcellular locations, etc).

This observation led the University of Geneva and the Swiss Institute of Bioinformatics (SIB) to jointly create a group called CALIPHO (Computer Analysis and Laboratory Investigation of Proteins of Human Origin). CALIPHO aims to use a combination of bioinformatics and experimental methodologies to increase our knowledge of human proteins. The three missions of CALIPHO are the development of a neXtProt, a human protein knowledge database, discovering the function of selected sets of uncharacterized proteins of human origin through laboratory experiments, and organizing a collective effort to pool available resources around the world in order to functionally characterize all these proteins.

Capturing and representing high-quality proteomics-derived data is quite crucial to our understanding of human proteins. We therefore want to work in close collaboration the emerging worldwide efforts to characterize the human proteome.

Personalized Medicine Session Invited Speakers

Michel Desjardins,
Université de Montréal

Biography:

Michel Desjardins is a Professor at the Département de pathologie et biologie cellulaire and Département de microbiologie et immunologie from Université de Montréal. He is the recipient of the senior Canadian Research Chair in Cellular Microbiology. The research focus of the Desjardins' laboratory in the last few years has been centered around the understanding of the role played by phagocytosis in antigen cross-presentation, a process enabling the elicitation of a sustained CD8+ T cells response against infectious microorganisms. More recently, the group of Desjardins has also highlighted the contribution of autophagy, the process by which cells degrade some of their own constituents, in the processing and presentation of viral antigens on MCH class I antigens. An important part of their research involves the complementary use of a cell biology approach with large-scale proteomics analyses.

Presentation:

Proteomics analyses played a key role in our understanding of the contribution of phagocytosis and autophagy to antigen presentation

Luc English, Magali Chemali, Kerstin Radtke, Pierre Thibault and Michel Desjardins

Antigen presentation is a key process in our ability to fight infectious diseases and cancer. Microorganisms like bacteria and parasites, internalized by macrophages through phagocytosis, are killed and then degraded in phagosomes to generate peptides that can be presented on MHC class II molecules. This process, triggering a CD4+ T cell response, can be complemented by the elicitation of a MHC class I-mediated CD8+ T cell response by a process called cross-presentation. Proteomics and biochemical analyses have shown that the molecular machines required for antigen processing and loading on MHC class I molecules are present in phagosomes. Autophagy, the process by which cells degrade part of their own constituents in response to stress, is also playing a key role in the presentation of endogenous antigens (self antigens and viral antigens expressed in infected cells) on both MHC class I and class II molecules. During Herpes simplex virus 1 (HSV-1) infection in macrophages, a novel type of autophagy involving the nuclear envelope is triggered, enabling the delivery of viral antigens to lysosomes and autophagosomes. This process was characterized, in part, by proteomics approaches showing that the interaction of intracellular compartments derived from the endoplasmic reticulum with lysosomes and phagosomes plays a key role in the ability of autophagy to contribute to the presentation of endogenous peptides on MHC class I molecules.

Personalized Medicine Session Oral Abstract Presentations

Plasma Protein and Glycoprotein Profiling in Triple-Negative Breast Cancer

Pinto, Devanand
National Research Council

Contributing Authors:

Devanand M. Pinto, Alejandro Cohen, Kenneth Chisholm, and Maureen O'Connor

Breast cancer can be subdivided into several sub-types, the most aggressive of which is referred to as basal like or triple-negative phenotype. The debate continues as to which term more accurately describes the disease and also whether these terms describe distinct sub-types of breast cancer. This debate points to the need for additional molecular characterization of the triple-negative phenotype (TNP). TNP is characterized by a lack of ER, PR and HER2 receptors, which also limits the treatment options. The research presented here is part of a broader strategy to develop therapeutic antibodies against TNP. Targets are selected using detailed proteomic and glycoproteomic analysis of cell lines and clinical samples. As with all targeted therapies, quantitative assessment of targets in each patient is required prior to commencement of therapy. This presentation will focus on target profiling in the serum of breast cancer patients using immunodepletion, glycoprotein enrichment and LC-MRM mass spectrometry. The advantage of this approach lies in the ability to measure dozens of protein targets, in additional to other markers that can be used for optimizing or monitoring therapeutic benefit. Seventy-six patients with confirmed diagnosis of breast cancer were profiled for a total of 34 proteins. Several tryptic peptides or glycopeptides were monitored for each protein and two or three MRM transitions per peptide were monitored in order to maintain specificity. In total, several hundred MRM transitions were monitored for each patient sample. Preliminary analysis revealed that unmodified peptides were relatively consistent among the various breast-cancer sub-types. However, greater variation was observed among the glycopeptides isolated from patient plasma. In addition to plasma profiling, some preliminary data on the analysis of circulating tumor cells in triple negative patients will also be presented.

Multiple reaction monitoring of amino acid polymorphisms in ovarian carcinoma tissue

Williams, Declan
York University

Contributing Authors:

Declan Williams, Leroi DeSouza, Olga Krakovska, Sohrab Shah, David Huntsman and K.W. Michael Siu

Cancer pathology is characterized by hundreds or thousands of mutations throughout the genome, which are heterogeneous in patient populations. Missense mutations, those causing amino acid substitutions at the protein level, may alter protein function and promote carcinogenesis. Because changes in mRNA levels do not necessarily translate to changes in protein expressions, methods to detect and quantify specific mutant proteins in the tumor are needed. We present herein a proof-of-principle study in which mutations initially identified using genetic methods were targeted at the protein level, using two-dimensional liquid chromatography and a hybrid triple quadrupole-linear ion trap mass spectrometer. Using multiple reaction monitoring (MRM) with a minimum of two MRM transitions per peptide, both wild-type and mutant proteins that had previously been identified by nucleic acid sequencing of tumor tissue from two ovarian cancer patients were observed. The peptides targeted in the MRM experiments were selected based on their primary sequences and gene annotation in order to address analytical constraints and maximize throughput. In the absence of peptide standards, chromatographic conditions and data acquisition parameters were optimized using existing MS/MS fragmentation prediction software and data from replicate MRM experiments. The approach applied here may permit the simultaneous interrogation of hundreds of candidate diagnostic or therapeutic targets in patient samples. Furthermore, this workflow illustrates an approach in which genomic data directed biomarker discovery at the protein level, thereby providing complementary evidence and independent verification.

Personalized Medicine Session Oral Abstract Presentations

Design of a Selected Reaction Monitoring Mass Spectrometric Immunoassay

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Contributing Authors:

M. Lopez, A. Prakash, T. Rezai, M. Athanas, D. Sarracino, R. Singh, D. Barnidge, P. Oran, R. Nelson

Parathyroid hormone (PTH) assays able to distinguish between full length PTH (PTH1-84) and N-terminally truncated PTH (PTH7-84) are of increasing significance in the accurate diagnosis of endocrine and osteological diseases. However, there are indications that greater microheterogeneity exists within PTH, which has yet to be fully characterized to determine the potential clinical utility and/or confounding effects it may have on present-day assays. The accurate examination of known PTH variants, while simultaneously evaluating other possible variants, requires a degree of analytical freedom that universally escapes conventional assays. Here we describe mass spectrometric immunoassays that while specifically tooled for the detection of PTH1-84 and PTH7-84, are able to simultaneously discover and evaluate unforeseen consequences of microheterogeneity in PTH. Mass spectrometry-based selective reaction monitoring assays (SRM) are rapidly becoming a preferred technology for the development of quantitative protein or peptide assays for clinical research. SRM assays deliver high sensitivity, selectivity and throughput, and when taken together, these parameters provide a breakthrough quantification methodology. During LC MS/MS, multiple charge states and fragmentation ions are generated from each fragment, resulting in upward of 1,000 different parent/daughter transitions possible for the digested PTH. Due to time and cost restrictions, the empirical investigation of each transition is not efficient. Therefore, a workflow was developed that incorporated predictive algorithms, utilization of previously acquired high-resolution MS trap-based discovery data and iterative optimization. This workflow was applied to predict and refine the optimal transitions for monitoring of target peptides, including peptides for routine monitoring of full length PTH (aa1-84) and peptides specific for clinically observed variants (aa7-84, 34-84). The strategy facilitates the translation of empirically obtained peptide intensity and fragmentation behavior from high resolution LC-MS/MS to triple quadrupole MS SRM assays. Inherent to the success of the workflow is the similarity of peptide ion fragmentation behavior in trap and triple quadrupole instrument. Using this approach, an initial list of transitions was generated and queried empirically to produce an LC-MS/MS profile based on four target peptides that collectively spanned > 50 % of the full-length PTH sequence (45 of 84 amino acids). In addition, transitions for two peptides, were added to the profile to monitor for truncated variants PTH7-84 and PTH34-84, respectively. The optimized SRM assay was then used to interrogate a small cohort of clinical patient samples.

Quantitative analysis of IGF-1R signaling pathway activation in FFPE Tissue

Saba, Julian
Thermo Fisher Scientific

Contributing Authors:

Amol Prakash, Bryan Krastins,David Sarracino, Michael Athanas, Taha Rezai, Mary Lopez

Multiple Ewing's sarcoma clinical trials targeting insulin-like growth factor receptor (IGF-1R) with IGF-1R small molecules and antibody inhibitors are currently underway. The IGF-1R signal pathway is commonly activated in many cancer types so this targeted therapeutic approach holds great promise for not only treating Ewing's sarcoma but other types of cancers as well. There is currently no good method to quantitatively measure the IGF-1R protein, its phosphorylation status, or the immediate downstream signal pathway target IRS-1. Being able to quantify these proteins and determine their phosphorylation status directly in patient tissue biopsies could help identify and select patients most likely to benefit from anti-IGF-1R therapies. We have developed an approach to determine both absolute IGF-1R and IRS-1 levels and the phosphorylation status of both proteins' directly in formalin-fixed paraffin-embedded (FFPE) patient tissue. This approach is based on the Liquid Tissue Æ-SRM technology platform, a combination of tissue microdissection, Liquid Tissue Æ processing which turns dissected tissue to a complete solubilized tryptic digest, and mass spectrometry-based selected reaction monitoring (SRM). This approach enables relative and absolute quantification of proteins and their phosphorylation status directly in formalin fixed tissue. This approach was used to measure the IGF-1R protein and specific phosphorylation sites as well as the IRS-1 protein and its phosphorylation sites in formalin fixed tissue culture cells and xenograft tumors.

CNPN Award Winner

John J.M. Bergeron,
McGill University

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

Biography:

Dr. J.J.M. Bergeron is a leader in the development and dissemination of proteomic techniques and is considered one of Canada's leading cell biologists, particularly in the areas of protein maturation, trafficking and function where he has applied proteomic techniques to study the biochemistry of specific cellular compartments including elements of the secretory apparatus.

Dr. Bergeron is a Co-Founder, Scientific Advisor and past CSO to Caprion Pharmaceuticals. He is a Fellow of the Royal Society of Canada, recipient of the McLaughlin Gold Medal from this Society for important research of sustained excellence in any branch of medical sciences. He has given the David L. Thomson Lecture at McGill University, the Theo Hoffmann Biochemistry Lecture at the University of Toronto, the Nan Qiang Lecture at Xiamen University in China and Raymond Chiu Memorial Lecture at Albert Einstein College of Medicine.

He was the Chair of the Scientific and Organizing Committees of past HUPO World Congresses, a member of its council and past Co-Chair of its International Liver Proteome Project, Chair of the Mouse Models of Human Disease efforts in proteomics and President of Human Proteome Organisation from 2003-2006. He was the chair of the HUPO Initiatives (2007-2008) whose mission has been to accelerate the successful application of proteomics to the solution of important biomedical problems.

Presentation:

Cell Biology Through proteomics

All human cells are compartmentalized into structures we learn in introductory biology as the nucleus, endoplasmic reticulum, Golgi apparatus, cell membrane , endosomes , lysosomes , peroxisomes, mitochondria and a myriad of coated and uncoated vesicular structures . The human genome is itself fully enclosed within the nucleus of each and every cell with only about 20,000 genes which encode proteins. These are modified and come together to form the nervous, muscular and skeletal systems as well as all our body fluids including blood. Currently , we understand the proteins from only a small fraction of these genes , the disease significance of even fewer and target drugs to only a few hundred proteins from these 20,000 genes. It is the job of proteomics , our discipline, to fill in the missing knowledge gap and characterize all proteins in the context of biological significance and human disease. How do we accomplish this goal? One approach is to match the strength of one of our technologies, tandem mass spectrometry, with highly homogenous samples representing the different compartments of the cell (endoplasmic reticulum, Golgi apparatus etc). In this way the high protein concentrations in these compartments overcome the single greatest bottleneck in proteomics namely the dynamic range of proteins which can be characterized in a sample (currently about 3 orders of magnitude). Furthermore , immediate biological significance is realized by this strategy . Combined with simple bioinformatic tools to visualize protein distributions in isolated compartments of the cell enables a further extension to what we have called that of a protein microscope. Already considerable biological insight as well as disease relevance has been realized by the application of proteomics to cell biology.

Stem Cells Session Invited Speakers

Guy Sauvageau,
Université de Montréal

Biography:

Guy Sauvageau is the founding scientific director and CEO of the systems biology institute in Montreal called IRIC. He is also a clinician-scientist specialized in bone marrow transplantation and scientific director of the leukemia cell bank of Québec. He holds the Canada Research Chair in the Molecular Genetics of Stem Cells and is Full Professor at Université de Montréal and adjunct in Experimental Medicine at McGill. He is a world leader in hematopoietic stem cell (HSC) biology. His work revealed the importance of developmental genes of the Hox and Pbx families in the regulation of hematopoiesis and their important contribution to myeloid leukemia. He also pioneered studies which demonstrated the critical function of several Polycomb group (PcG) genes such as Bmi1 and Eed in self-renewal divisions of normal and leukemia stem cells.

Several of his findings are now in the arena of hit identification or optimization for anti-cancer treatment or stem cell expansion, respectively. Of these, the TAT-HOXB4 fusion protein can efficiently penetrate the cellular and nuclear membrane of target HSC and be used as a ‘growth factor’ for these cells. G.S. is the leader of the CIHR team grant on HSC expansion, which includes an integrated group of international investigators who are developing new reagents, tools and protocols to initiate phase I clinical trials using recombinant proteins to expand hematopoietic stem cells ex vivo.

Guy Sauvageau. has received several national and international awards. Among these, the Till and McCulloch award from the international society of hematology (2006), the Stolmann award from the LLSA (2007) and Till and McCulloch award from the Canadian stem cell network (2009). He was recently nominated as a fellow of the Academy of Science of Canada.

Presentation:

Phosphoproteomic analyses of leukemia stems cells identify a potential role for ROS detoxification in stem cell self-renewal.

Stem Cells Session Invited Speakers

Brian Raught,
Ontario Cancer Institute

Biography:

Brian Raught received his Ph.D. in Cell Biology from Baylor College of Medicine, and conducted his post-doctoral training with Nahum Sonenberg at McGill University. He then joined the Institute for Systems Biology (Seattle) as a Senior Scientist, working with Dr. Ruedi Aebersold on developing mass spectrometry-based approaches for the study of ubiquitin and ubiquitin-like proteins (Ubls). Brian opened his lab at the Ontario Cancer Institute in 2006. His most recent work has focused on the function of the small ubiquitin-related modifier (SUMO) proteins, and their important roles in cellular stress responses and protein degradation.

Presentation:

New mass spectrometry tools for the study of ubiquitin and ubiquitin-like proteins

Tharan Srikumar[1], Stanley M. Jeram[1], Xiang-Dong Zhang[2], Patrick G.A. Pedrioli[3], Michael Matunis[2], Henry Lam[4] and Brian Raught[1]

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Ubiquitin (Ub) and the ubiquitin-like proteins (Ubls) comprise a remarkable assortment of polypeptides that are covalently conjugated to target proteins (or other biomolecules) to modulate their intracellular localization, half-life and/or activity. Identification of Ub/Ubl conjugation sites on a protein of interest can thus be extremely important for understanding how it is regulated. While mass spectrometry (MS) has become a powerful tool for the study of many classes of post-translational modifications, the identification of Ub/Ubl conjugation sites presents a number of unique challenges. Here, we present an improved Ub/Ubl conjugation site identification strategy, utilizing our software tool SUMmOn as a complement to standard approaches. As compared to standard database search protocols alone, the addition of SUMmOn analysis can; (a) identify Ubl conjugation sites that are not detected by standard database searching methods, (b) better preserve Ub/Ubl conjugate identity, and (c) increase the number of identifications of Ub/Ubl modifications in lysine-rich protein regions. Using this methodology, we identified a number of novel Ubl linkages and conjugation sites. We have also developed and validated a Ub/Ubl spectral library. The use of this library with a spectral matching tool yields improved performance over database search engines, and can successfully identify many types of Ub/Ubl chain-derived peptides that cannot be identified by standard database search algorithms. Together, these tools have significantly improved our ability to use mass spectrometry in the study of Ub/Ubls.

Stem Cells Session
Oral Abstract Presentations

HOXA4 induces self-rewal expansion of Hematopoietic Stem Cells

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HMR Research Center

Contributing Authors:

Marilaine Fournier, Charles-Etienne Lebert-Ghali

Although homeobox (Hox) gene HOXB4 can induce hematopoietic stem cell (HSC) expansion in vivo and in vitro, mutant mouse models showed that this gene is not required for the generation and function of HSCs. These contradictory observations might be explained by functional redundancy between HOX genes, and has been shown for these genes in embryonic development. Actually paralog 4 group member Hoxa4 is 10-fold higher expressed in HSC enriched fetal liver (E14.5) fractions than Hoxb4. At this time point of ontogeny, HSC are undergoing their principal expansion and thus suggests a potential role for Hoxa4 in HSC self-renewal. To test whether HOXA4 can expand HSCs in vitro we stably over-expressed HOXA4 in primary bone marrow (BM) cells using MSCV-IRES-GFP based retroviral vectors. HOXA4 BM cells grew significantly faster than control BM cells attaining an 87-fold higher cell number after three weeks. This explosive growth was not only sustained by a higher expansion of myeloid progenitors in HOXA4 BM cultures than in control (316x vs 11x in eleven days, respectively), but these were also more primitive maintaining multi-lineage potential. CRU assays performed at day 0 and 6 showed a 6-fold expansion of HOXA4 HSCs in vitro. Moreover, the size and ratios between mature lymphoid and myeloid populations derived from HOXA4 HSCs were comparable to wild type mice, contrarily to HOXB4 HSCs, which show slight skewing towards the myeloid lineage. Thus HOXA4 can induce self-renewal of HSCs in vitro and is a very promising candidate for ex vivo expansion of HSCs.

Enhancing progenitor cell therapy for vascular repair

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Contributing Authors:

Sebastien Trop, Karen M. Doody, Maribelle Cruz, Daniel J. Dumont, Michel L. Tremblay

We identified T cell protein tyrosine phosphatase as a key molecule for the regulation of bone marrow progenitor activity. In tc-ptp-/- mice, the number of bone marrow and circulating progenitors was increased 5 to 9-fold. Treatment of murine or human bone marrow ex vivo for 48 h with TC-PTP inhibitors augmented the number of progenitors 3-fold. In a murine model of hind limb ischemia, augmenting the availability of progenitors by inhibition of TC-PTP resulted in increased incorporation of these cells into nascent vessels and accelerated collateral vessel development. The mechanism whereby inhibition of TC-PTP exerts its effect on progenitor cells involves autocrine stimulation with IL-18, initiating an inflammatory cascade leading to expression of IL-18 binding protein, activation of Stat1 and c-kit. The ability to rapidly expand progenitors through pharmacological inhibition of TC-PTP may have potential clinical use to augment the number of progenitors for regenerative therapies.

Stem Cells Session
Oral Abstract Presentations

BAF Switching: a Novel Epigenetic Mechanism Essential for Normal and Leukemic Hemopoiesis ?

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Contributing Authors:

Krasteva, V., Diaz Tellez, A., Simon, C., Thibault, P. and Lessard, J.

Multicellular organisms depend on epigenetic mechanisms to develop specialized tissues and cell types, processes that constitute a heritable code, excluding the genomic sequence, implicated in the control of gene expression by modifying how DNA is packaged into chromatin. ATP-dependent SWI/SNF-like BAF chromatin remodeling complexes are composed of a dozen of subunit families that are combinatorially assembled to form a variety of tissue- and cell type-specific complexes with specialized functions. In embryonic stem cells, a complex referred to as esBAF is essential for ES cell self-renewal and pluripotency. In the nervous system, subunit exchange within a neural-specific complex, nBAF, is deterministic for the transition from proliferating progenitor cells to post-mitotic neurons and, later on, for neuronal maturation. Despite their essential functions in embryonic and neural development, very little is known about the roles and mechanisms of regulation of these complexes in hemopoietic cells. Using a combination of quantitative proteomics and genomics approaches, we show that a novel family of combinatorially assembled hemopoietic BAF (hBAF) complexes also orchestrates hemopoietic development and leukemic transformation. Conditional deletion of the hemopoietic stem cell (HSC)-specific subunits BAF45a and BAF53a causes important defects in HSC self-renewal, proliferation and myeloid differentiation leading to severe anemia and death. Strikingly, the actin-related protein (ARP) BAF53a subunit seems to be essential for proper chromosome segregation during mitosis. We now aim to define the molecular basis of the transcriptional and biologic specificity produced by combinatorial assembly of these complexes in hemopoietic cells.

Growth factor independence 1b (Gfi1b) restricts dormancy and peripheral blood mobilization of hematopoietic stem cells

Khandanpour, Cyrus
IRCM

Contributing Authors:

Lothar Vassen, Marie-Claude Gaudreau, Christian Kosan, Tarik Moroy

The donor matched transplantation of bone marrow or hematopoietic stem cells (HSCs) is a widely used strategy to treat haematological malignancies, but is a procedure associated with high mortality. The expansion of HSC numbers and their mobilisation into the bloodstream of a donor could significantly improve this therapeutic approach. We report here that the transcription factor Gfi1b is highly expressed in HSCs and is required to both restrict HSC numbers and their egress from the bone marrow to the blood. We have generated a Gfi1b deficient mouse by conditionally inactivation of the Gf1b gene in HSCs. We observe a 30 fold enrichment of HSCs in bone marrow and an almost 100 fold enrichment of HSCs in peripheral blood, when Gfi1b is absent. Gfi1bko/ko HSCs retain their ability to self renew and to initiate multilineage differentiation, but are no longer quiescent and display high levels of reactive oxygen species not detectable in wild-type HSCs indicating their activation. Gfi1b deficiency also affects the expression of integrins and adhesion molecules, for instance CXCR4, VCAM-1, which are required to retain HSCs in a dormant state in the endosteal niche. Moreover, expression of the Gfi1b paralogue Gfi1 is up regulated after Gfi1b deletion in HSCs and the deletion of both Gfi1 and Gfi1b is deleterious for HSCs, suggesting that a compensatory mechanism exists between Gfi1 and Gfi1b and that at least one Gfi1 protein is required to generate HSCs.
Our results suggest that Gfi1b is new regulator of HSC dormancy, pool size and mobilization.

Blood & Blood Cells Session Invited Speakers

Jerome Garin,
Laboratoire d'Etude de la Dynamique des Protéomes (EDyP)

Biography:

Jérôme GARIN, PhD in Molecular and Cellular Biology. Heads the "Commissariat à l'Energie Atomique" Research Institute for life Science and Technology (Grenoble, France), and the EDyP Laboratory. EDyP has for vocation to contribute to the understanding of biological processes through the development of large-scale protein analysis methodologies. EDyP research programs are strongly oriented towards technological developments for the use of proteomics for functional genomics approaches and biomarker discovery. Jérôme Garin is member of the editorial board of Molecular and Cellular Proteomics. Since 2008, he is coordinating the DECanBio program, a European FP7 project dedicated to the set up of a biomarker discovery pipeline for cancer bladder using up to date mass spectrometry technologies.

Presentation:

Quantitative proteomics analyses: Large-scale and targeted analyses

A wide variety of strategies, using a range of methods, exist to carry out quantitative proteomic analyses. The nature of the biological material, and the type of question asked, determine the choice of one method over another. Two types of complementary strategies can be distinguished: large-scale proteomics studies which may be with or without a priori, and quantitative studies targeted towards proteins of interest.
Large-scale studies are, by their essence naïve; they can, for example, be implemented for the analysis of the dynamics of a biological system as part of a kinetic study, or to determine the consequences of a mutation. This type of approach allows the discovery, with no preconceptions, of molecular actors involved in the biological mechanisms studied. In the clinical context, the same naïve approaches can be used to compare biological samples from healthy control patients to samples from patients suffering from a particular pathology; they will lead to the discovery of candidate biomarkers for the pathology in question.
As a second step, it is necessary to refine the knowledge derived from large-scale approaches by carrying out new analyses targeting the proteins of interest revealed by the naïve approach using methods which allow better quality data to be acquired. This can be done by using SRM ("Selected Reaction Monitoring") mass spectrometry combined to adequate internal isotopically labeled standards.
My talk will present different quantitative analytical strategies that are currently being used in the EDyP Lab, especially in the context of the discovery and evaluation of bladder cancer biomarkers.

Blood & Blood Cells Session Invited Speakers

Claude Perreault,
Université de Montréal

Biography:

Claude Perreault is Full Professor at Université de Montréal (Department of Medicine) and is one of the founding members of the Institute for Research in Immunology and Cancer (IRIC), the ranks of which he joined as a Principal Investigator in 2005. In addition to his research and training activities at IRIC, Perreault is a clinical practitioner at Maisonneuve-Rosemont Hospital where he set up both the Histocompatibility Laboratory and the Bone Marrow Transplant Unit. Before joining IRIC, Perreault worked at the Maisonneuve-Rosemont Hospital Research Centre, which he also directed from 1992 through to 2001. Perreault holds a Canada Research Chair in Immunobiology and was awarded the Murray Margarit Memorial Award by The Leukemia and Lymphoma Society of Canada in 2009.

Presentation:

Molecular definition of the MHC I Peptidome using a High-Throughput Quantitative Mass Spectrometry Approach

An ongoing collaborative effort involving chemists, biologists and bioinformaticians has led to the development of an MS-based high-throughput quantitative method to study the structure and abundance of MHC I-associated peptides. Our work has yielded four main findings. 1) By comparing peptides eluted from primary dendritic cells and thymocytes, we found that that the MHC I peptide (MIP) repertoire conceals a cell type-specific signature and mirrors signaling events occurring inside the cells. 2) We showed that immunoproteasomes substantially increase the abundance and diversity of MIPs. Our analyses suggest that immunoproteasomes lead to enhanced MHC I presentation of peptides adjoining the unstructured proteome. 3) About 25% of MIPs were differentially expressed on normal versus neoplastic cells. Differentially expressed peptides derived from genes implicated in several biological processes such as cell cycle progression, apoptosis, signal transduction, cytoskeleton assembly, differentiation as well as regulation of transcription and translation. Overexpression of numerous MIPs on neoplastic cells entails posttranscriptional mechanisms. 4) We found that after treatment of neoplastic cells with rapamycin, cell surface levels of 70% of MIPs increased progressively over time. Overexpression of thirty of the latter peptides was particularly dramatic. Eighteen of them derived not from mTOR itself, but from other proteins that are components or targets of the mTOR pathway. We conclude that dysregulation of the mTOR pathway can have a profound impact on the MIP repertoire. We also infer that activation of an oncogenic pathway can increase the expression of MIPs that derive from numerous proteins that interact with or are regulated by the oncogene.

Blood & Blood Cells Session Invited Speakers

Christopher Overall,
Centre for Blood Research, UBC

Biography:

Dr. Overall is a Professor, and Tier 1 Canada Research Chair in Metalloproteinase Proteomics and Systems Biology, at the University of British Columbia Center for Blood Research. He was the 2002 CIHR Scientist of the Year, Chaired the 2003 MMP and the 2010 Proteolytic Enzmes and Inhibitors Gordon Research Conferences, and won the University of British Columbia Killam Senior Researcher Award (Science) 2005. He was a visiting scientist at British Biotech, Oxford (1997-1998) and at the Centre for Proteomic Chemistry, Novartis, Switzerland (2004, 2008). His research interests are in positional proteomics for N- and C-terminome analysis, protease proteomics (degradomics) and breast cancer metastasis. He is the pioneer of degradomics, with 6 Nature Review Papers on this and protease genomics, drug target validation, MMP therapeutics, and substrate discovery. He has pioneered numerous approaches to decipher the protease and substrate degradomes by quantitative proteomics and N-terminome analysis in cell-based systems and in animal models.

Presentation:

Positional N-terminal and C-terminal proteomics deciphers protein terminal and proteolytic post-translational modifications in complex proteomes in vivo.

Protein termini are truncated by proteolysis, but the extent to which this molds the proteome in vivo is unknown. In addition to constitutive proteolysis during protein synthesis and maturation, the processing of a mature protein often irreversibly changes its activity. Specific degradomics techniques are needed to rapidly identify and quantify the N- and C-terminomes in order to reveal the extent of post-translational modifications of protein termini and therefore the functional state of key molecules, the extent of proteolysis in a system, and to identify new protease substrates. Broad coverage N-terminome analysis necessitates a negative selection procedure as the variety of original mature protein N-terminal blocked peptides each present individual chemical hurdles for their enrichment by positive selection strategies. We developed a combined N-terminomics and protease substrate discovery degradomics platform for the simultaneous quantitative analysis of the N-terminome and proteolysis on a proteome-wide scale called Terminal Amino Isotopic Labelling of Substrates (TAILS, Kleifeld et al Nature Biotech 28, 281-288; Prudova et al Mol Cell Proteomics in press; auf dem Keller et al Mol Cell Proteomics in press). By using a novel polymer to deplete the internal tryptic peptides, TAILS suffers little from sample loss and low yields, so requiring only 100 microgram of sample and one MS/MS analysis per sample. By a three-day procedure with flexible labelling options, TAILS can be adapted to a variety of experimental situations including cell culture and complex biological sample analysis. Incorporating iTRAQ labelling iTRAQ-TAILS also provides wide coverage of all forms of naturally blocked N-terminal peptides and allows for their quantification through labelling of lysine side-chains in up to 8 samples. In addition to providing valuable proteome annotation this has several unique advantages. It permits exploitation of the acetylated and other blocked mature protein N-terminal peptides as a statistical classifier that is then used to set isotope ratio cut offs that reveal protease activity. We introduce a novel parameter evaluating ion intensity dependent quantification confidences of single peptide quantifications. Being a quantitative procedure, TAILS can analyse the substrate degradome of a broad specificity protease or one with no known specificity without manual data parsing, in the same experiment, and also do this in vivo. We have applied TAILS to a variety of metalloproteases and compared protease knock out mice, analysed inflamed skin, breast cancer and pancreatic carcinoma in the RIP-Tag model. Typical analyses identify over 3000 N-terminal peptides from which we found that the removal of the N-terminal methionine is dependent upon the amino acid at position 2 with distinct preferences found for valine, glycine, alanine and serine. In one experiment, acetylation occurred on 731 original mature protein N-terminal peptides but at the initiator methionine in only 153 of these instances. In 578 cases, acetylation was at position 2 in the protein after removal of ^{1}Met, with alanine, serine and methionine being the preferred acetylated residues. Finally N-terminal positional proteomics enables MS sample simplification with proteins identified in bronchoalvelar fluid having abundances spanning a range greater than six orders of magnitude. This underscores the potential of TAILS to tackle the dynamic range analysis problem in complex proteomes such as blood.

Blood & Blood Cells Session Oral Abstract Presentations

Control of erythropoiesis through the dynamics of transcription factor interactions: a quantitative proteomics study.

Brand, Marjorie
Sprott Center for Stem Cell Research-OHRI

Contributing Authors:

Chandra-Prakash Chaturvedi, Jeffrey A. Ranish

Erythropoiesis is a tightly regulated process controlled at least in part by complex interactions between transcription factors and cofactors. To decipher the regulation of gene expression driving differentiation towards a particular lineage, it is important to understand the dynamics of the interactions between transcription factors and cofactors that occur during differentiation. The NF-E2 heterodimeric complex is a hematopoietic-specific transcriptional activator that plays an essential role in mediating the activation of e-globin gene transcription. Previously we have shown that this complex forms on the e-globin locus via an exchange of the MafK bZIP heterodimerization partner from the repressor Bach1 to the activator p45. Using quantitative proteomics, we have now identified a number of transcription factors and cofactors that differentially interact with this cell specific DNA-binding activator during erythroid differentiation. Using the e-globin locus as a model system, follow-up studies on some of these factors have revealed key features of the spatio-temporal regulation of gene expression. Here, we will present our latest results on the regulation of e-globin gene expression by the H3K9 histone methyltransferase complex G9a/GLP, showing notably that this complex plays a dual role in adult erythroid cells by maintaining the embryonic e-like gene in a silent state while simultaneously activating the adult e-globin gene counterpart. These studies illustrate the importance of quantitative proteomics to decipher dynamics biological processes occurring during cell differentiation.

Proteomic Biomarkers of Acute Kidney Allograft Rejection

Cohen Freue, Gabriela
PROOF Centre of Excellence

Contributing Authors:

Sasaki M,Meredith A, Günther P,Takhar M, Balshaw R,Ng TR, Borchers C, McManus BM,Keown PA,McMaster R

Background: The aim of the Biomarkers in Transplantation (BiT) initiative is to discover diagnostic plasma proteomic biomarkers of acute kidney allograft rejection to reduce invasive monitoring procedures and allow personalized treatment.
Methods: Plasma samples from 11 patients with biopsy-confirmed acute rejection (BCAR) and 21 with non-rejection (NR) post-transplant were depleted of the 14 most abundant proteins and processed by iTRAQ-MALDI-TOF/TOF methodology. Candidate protein markers were selected by a robust moderated t-test (LIMMA, pval<0.05) and a fold-change threshold of 1.15. A classifier score was developed using Support Vector Machines.
Results: A total of 12 plasma proteins that encompassed processes related to inflammation, complement activation, blood coagulation, and wound repair exhibited significantly different relative concentrations between BCAR and NR samples. Longitudinal monitoring of the identified proteins over the first 3 months post-transplant illustrated the resolution of the rejection episode following rejection treatment. Results were validated using ELISA where possible and initial cross-validation estimated a sensitivity of 80% and specificity of 90% for classification of BCAR. Further refinement of the analytical pipeline expanded the candidate list of markers to 26 proteins, which enhances the biological understanding of acute rejection. Multiple reaction monitoring (MRM) assays for the candidate list are being developed for the validation phase.
Conclusion: This study provides evidence that protein concentrations in plasma may provide a relevant measure for the occurrence of renal rejection. If confirmed in broader studies, results from the current study hold great potential to transition from proteomic discovery to routine clinical use for transplant monitoring.

Protein Interaction Session Invited Speakers

Anne-Claude Gingras,
Mount Sinai Hospital, SLRI

Biography:

Anne-Claude Gingras received her Ph.D. from McGill University in 2001 for her studies on translational control, performed under the guidance of Nahum Sonenberg. She then joined the laboratory of Ruedi Aebersold at the Institute for Systems Biology in Seattle, where she developed proteomics approaches to study protein-protein interactions. She established her own laboratory in 2005 at the Samuel Lunenfeld Research Institute at Mount Sinai Hospital in Toronto, where she currently holds the Canada Research Chair in Functional Proteomics and the Lea Reichmann Chair in Cancer Proteomics. She is an Assistant Professor in the Department of Molecular Genetics of the University of Toronto. Her group focuses on the identification and functional characterization of protein interaction modules surrounding serine/threonine phosphatases. Active areas of research include understanding how the PP4 phosphatase modulates gene expression via regulation of transcription elongation, mRNA capping, and splicing. The Gingras lab is also investigating the function of a novel protein complex that they have identified surrounding the and CCM3, a gene which is mutated in familial cases of angioma. Anne-Claude Gingras is also involved in the development of robust approaches for the analysis of protein interaction networks in yeast and human and for the study of enzyme-substrate relationships within the interaction networks.

Presentation:

Improving interaction proteomics – Application to the kinase and phosphatase interactomes

Protein phosphorylation mediates cellular responses to growth factors, environmental signals, and internal processes, by the regulation of protein interactions, enzyme activity or protein localization. However, the protein interactions of kinases, phosphatases, their regulatory subunits, and substrates remain sparsely mapped. Two interaction mapping projects will be presented: 1) To chart the budding yeast kinase and phosphatase interaction (KPI) network, we systematically characterized protein kinase and phosphatase complexes by sensitive affinity purification coupled to mass spectrometric identification (AP-MS). We identified a KPI network of 1,844 interactions. Notably, we found that the cell cycle phosphatase Cdc14 is associated with multiple kinases, revealing roles for this phosphatase in mitogen-activated protein kinase signaling, the DNA damage response and metabolism. We also uncovered new effector kinases linking the target of rapamycin complex 1 (TORC1) to nitrogen and carbon metabolism. An extensive backbone of kinase-kinase interactions thus cross-connects the proteome, and may serve to coordinate diverse cellular responses. 2) To identify potential substrates, regulators and targeting subunits for the human PP2A phosphatase, we performed an iterative high density AP-MS approach. This allowed us to uncover an interaction network containing 365 proteins, including the largest stable PP2A containing complex identified to date, STRIPAK. This complex is evolutionarily conserved, regulates cytoskeletal dynamics, and is linked to the target of rapamycin complex 2 (TORC2). These two projects will be presented within the context of the development of bioinformatics and statistical analysis tools for protein-protein interactions.

Protein Interaction Session Invited Speakers

Jason Moffat,
University of Toronto

Biography:

Dr. Jason Moffat is an Assistant Professor at the Banting & Best Department of Medical Research at the University of Toronto and an Assistant Professor in the Department of Molecular Genetics. He is a Member of the Donnelly Centre for Cellular & Biomolecular Research at the University of Toronto. Dr. Moffat is interested in developing and using new technologies to annotate mammalian gene function and to understand the molecular mechanisms that relate to cancer. His lab develops lentiviral based technologies for functional genetics and proteomics to identify and characterize genes important for tumor growth.

Presentation:

A lentiviral-based functional proteomics approach identifies chromatin remodelling complexes important for the induction of pluripotency

Protein complexes and protein-protein interactions are essential for almost all cellular processes. Here, we establish a mammalian affinity purification and lentiviral expression (MAPLE) system for characterizing the subunit compositions of protein complexes. The system is flexible (i.e. multiple N- and C-terminal tags, multiple promoters), compatible with Gateway™ cloning, and incorporates the potential for quantitative proteomics. Its major advantage is that it permits efficient and stable delivery of affinity-tagged open reading frames into most mammalian cell types. We have benchmarked MAPLE with a number of human protein complexes involved in transcription, including the PAF, NELF, P-TEFb, SWI/SNF, and MLL complexes. In addition, MAPLE was used to identify an interaction between the reprogramming factor Klf4 and the Swi/Snf chromatin remodelling complex in mouse embryonic stem cells. We show that the Swi/Snf catalytic subunit Smarca2/Brm is upregulated during the process of induced pluripotency and demonstrate a role for the catalytic subunits of the Swi/Snf complex during somatic cell reprogramming. Our data suggest that the transcription factor Klf4 facilitates chromatin remodelling during reprogramming.

Protein Interaction Session Invited Speakers

Jack Greenblatt,
University of Toronto

Biography:

Dr. Greenblatt is Professor, Banting and Best Department of Medical Research, Faculty of Medicine, University of Toronto. He received his Ph.D. in biophysics from Harvard University. He undertook research training in molecular biology at the University of Geneva, Switzerland, and at the Pasteur Institute, France. Following his work in Europe, he became Assistant Professor, Banting and Best Department of Medical Research, University of Toronto, and later received a professorship. Dr. Greenblatt holds an Ayerst Award from the Canadian Biochemical Society and was elected Fellow of the Royal Society of Canada in 1992. He received several awards from the Medical Research Council of Canada, including a Scientist Award (1984-1989) and a Distinguished Scientist Award (1995-2000). Dr. Greenblatt was named as an Howard Hughes Medical Institute International Research Scholar (1991-1996).

Dr. Greenblatt is also cofounder of a leading proteomics company Affinium Pharmaceuticals.

Presentation:

Systematic Identification by Affinity Purification and Mass Spectrometry of Protein-Protein Interactions and Protein Complexes for the Membrane Proteins of Saccharomyces cerevisiae

Mohan Babu[1], James Vlasblom[2], Franco Vizeacoumar[1], Shuye Pu[2], Charles Boone[1], Nevan Krogan[3], Brenda Andrews[1], Andrew Emili[1], Shoshana Wodak[2], Jack Greenblatt*[1].
1) Banting & Best Dept. of Med. Res., Univ. of Toronto, Toronto, ON, Canada; 2) Molecular Structure and Function Program, Hosp. for Sick Children, Toronto, ON, Canada; 3) Dept. of Cellular and Molecular Pharmacology, Univ. of California San Francisco, San Francisco, CA.

Protein tagging *in vivo* followed by tandem affinity purification (TAP) and mass spectrometry (APMS), used previously for soluble yeast proteins, has now been used to define protein-protein interactions (PPI) and protein complexes for ~1600 predicted yeast membrane proteins (YMPs) and ~700 associated proteins. Each YMP was solubilized and purified in the presence of three different detergents (Triton X100, DDM, C12E8). A Bayesian networks approach based on purification enrichment (PE) scores was used to expand the yeast APMS-derived PPI network by many new YMPs and several thousand previously unidentified PPI, and a Markov clustering algorithm was used to define several hundred new YMP-containing complexes. In comparison to the same gold standards, this PPI network for the YMPs is similar in quality to or better than the PPI networks defined previously by APMS or other approaches for the soluble yeast proteins. It was found previously that about half the gene pairs encoding physically interacting proteins involved in chromosome biology have correlated genetic interaction patterns identified using synthetic genetic arrays. Similarly, about half the gene pairs encoding physically interacting YMPs involved in the early secretory pathway, lipid metabolism, endocytosis and other membrane-based processes have correlated genetic interaction patterns, again supporting the quality of our PPI network for the YMPs. As well, a minority of ~50 YMP complexes we tested affect the morphology of the subcellular compartment in which they are located (ER, Golgi, vacuole or mitochondrion). In these cases, typically all the subunits of the complex, either previously known or newly identified in this study, affect compartment morphology in similar ways.

Protein Interaction Session Oral Abstract Presentations

In Silico Protein Interaction Analysis Using Archived Proteomics Experiments in the Global Proteome Machine Database

Kast, Juergen
University of British Columbia

Contributing Authors:

Chengcheng Zhang, Jason C. Rogalski, Daniel M. Evans, Ronald C. Beavis, and Juergen Kast

With protein interaction studies being the focus of functional proteomics, an increasing number of such experiments will be archived in proteome databases. We used the Global Proteome Machine Database (GPMDB), the largest tandem mass spectrometry-based proteomics data repository (over 130,000 experiments stored), to develop a strategy for in silico protein interaction analysis. Using hist1h2bd, a member of histone H2B family, as model for method development, we designed four filters to eliminate experiments that were repetitive, represented large-scale studies, single gel bands or LC fractions, or had low confidence in hist1h2bd identification. Proteins identified in the remaining experiments were merged and ranked by their frequency of occurrence. This identified 79 proteins, including 15 histone proteins, with at least one member of each histone family forming the histone octameric complex. This validated strategy was then applied to integrin alphaIIb, integrin beta3, talin1 and kindlin-3, proteins playing critical roles in platelet activation and aggregation. Upon removal of background proteins, 28 proteins were shared by all four. These include Rap1b, fibrinogens, von Willebrand factor, thrombospondin-1, platelet basic protein, and platelet glycoprotein Ib, which might be part of a larger protein interaction network required for platelet activation and aggregation. In analogous fashion, we are currently generating comprehensive interaction networks for other proteins, including other histones. Although the exact cause of the protein co-occurrence remains to be elucidated, our results demonstrate that in silico protein interaction analysis is a novel tool for identifying candidate protein interactions that could be assessed further in subsequent targeted experiments.

Quantitative Analysis of the GRB2 Adaptor Protein Network Dynamics Using a Scheduled Multiple Reaction Monitoring (sMRM) Assay

Bisson, Nicolas
Samuel Lunenfeld Research Institute

Contributing Authors:

Nicolas Bisson, Andrew James, Steve Tate, Lorne Taylor, Gordana Ivosev, Tony Pawson

Signals from cell surface receptors are often relayed through adaptor proteins. These proteins serve as hubs to recruit appropriate targets in order to stimulate specific cellular pathways. Therefore, adaptor proteins are thought to play sophisticated roles in the coordination of cellular responses to extracellular cues. The growth factor receptor-bound protein 2 (GRB2) is one such adaptor and acts downstream of receptor tyrosine kinases (RTKs).

We have performed standard affinity-purification combined with mass spectrometry to identify 90 proteins and 36 phosphosites associated with GRB2 in human cells. We have used the data to design a sMRM assay comprising 1157 transitions to quantify their abundance and changes in their phosphorylation state under different cellular conditions. We have affinity-purified GRB2 mutants to separate interactors into sub-networks. We have further precised the organization of the network by doing siRNA knockdowns of GRB2 direct interactors. In order to understand how the GRB2 network is modulated following activation of specific RTKs, we have stimulated cells with various growth factors (EGF, HGF, IGF, FGF, PDGF and insulin). Finally, we have performed a time course (0-100 min) of Epidermal Growth Factor (EGF) stimulation of cells to reveal the distinct kinetics of recruitment of proteins to GRB2 upon activation of the EGFR pathway.

This study represents the first MRM analysis of the composition, organization and modulation of a protein-protein interaction network in living cells. The data also show the usefulness of targeted proteomics in cell biology for monitoring the abundance of multiple proteins and their modifications in a single assay.

Protein Interaction Session Oral Abstract Presentations

Identification of poly(ADP-ribose) binding proteins by mass spectrometry.

Poirier, Guy
Laval University

Contributing Authors:

Gagne JP Gagne P, Isabelle M, Pic E, Boutin M, Poirier GG. Laval University, Quebec.

In presence of DNA lesions, PARP-1 is very rapidly activated and signals the presence of damage by attaching ADP-ribose units to chromatin-associated proteins. The central role of poly(ADP-ribose) (pADPr) in the recruitment and assembly of multiprotein repair complexes strenghten the role of DNA-dependent PARPs as regulators of genome maintenance pathways.

Three protein motifs have been characterized to confer affinity to pADPr: (I) the Macro domain originating from homology to the C-terminus of the macro-H2A histone protein; (II) the PBZ-type zinc fingers found in DNA repair and checkpoint proteins and (III) a pADPr-binding motif composed of interspersed basic and hydrophobic amino acid residues. Evaluation of these pADPr-binding motifs shows a remarkably high noncovalent affinity to pADPr.

Our laboratory is presently engaged in proteome-wide identification of proteins with affinity to pADPr by large-scale mass spectrometry-based proteome analysis and protein microarray analysis of pADPr-binding proteins were used to establish a comprehensive repertoire of pADPr-associated proteins. We are currently using Multiple Reaction Monitoring (MRM) and SILAC to monitor the dynamic behavior of proteins complexes associated with pADPr over a time course of genotoxic stimuli.

Visualization and modeling of these pADPr-associated proteins in networks not only reflect the widespread involvement of poly(ADP-ribosyl)ation in several pathways but also identify protein targets that could shed new light on the regulatory functions of pADPr in normal physiological conditions as well as after exposure to genotoxic stimuli.

Modeling Contaminants in Tandem Affinity Purification Experiments

Lavallee-Adam, Mathieu
McGill University

Contributing Authors:

Mathieu Lavallee-Adam (1), Philippe Cloutier (2), Benoit Coulombe (2) and Mathieu Blanchette (1)

Gel band contamination, faulty purification, and antibody non-specificity are some of the main ways contaminants can be introduced in the Tandem Affinity Purification (TAP) followed by tandem Mass Spectrometry (MS/MS) experimental pipeline. Previously used methods systematically rejecting known contaminants are problematic because a contaminant for one bait might be a true interaction for another [3], suggesting that a finer model of contaminants would be beneficial.

Approaches comparing mass spectrometry confidence scores of the preys of both control and induced experiments of a given bait have been used in the past (Jeronimo et al.) [4]. However, such methods are expensive in terms of time and resources and inaccurate in cases where the bait's expression vector is leaky.

Here, we propose a confidence assessment algorithm using a limited number of high quality controls to build a contaminant presence model in TAP-MS/MS experiments. Our algorithm assigns a p-value and a FDR to the Mascot score [5] of each prey obtained under the induced condition. Choosing a FDR threshold of 5% results in the selection of 3237 high-confidence interactions from a dataset of 89 baits and 11894 interactions [2]. Compared to the Jeronimo method, our approach results in a predicted set of interactions with 30% more overlap with the merged BioGrid [7] and HPRD [6] databases and involves pairs of proteins with at least one GO annotation [1] in common 25% more often. These findings will allow significant reductions in expenses and a greater number of experiments to be conducted with higher accuracy.

POSTER ABSTRACTS
Technology Session

Poster No.23

ProteoConnections: an analysis platform to accelerate proteomes and phosphoproteomes exploration

Courcelles, Mathieu
IRIC

Contributing Authors:

Courcelles M, Lemieux S, Thibault P

Considering the wealth of information generated by large-scale proteomics experiments, improved computational tools are required to manage these data. To this end, we developed ProteoConnections, a bioinformatics platform tailored to address the pressing needs of proteomic analyses. The primary focus of this platform is to organize peptides/proteins identifications, profile abundance changes, evaluate the quality of the acquired dataset and accelerate biological interpretation using bioinformatics applications. All peptides identifications are stored into a database to facilitate data mining. Datasets quality can be controlled by computing peptides FDR (target-decoy database methodology) and is reported by statistics (distributions for number of unique peptides/proteins, charge, mass, delta mass, etc). Many databases and bioinformatics tools were integrated into the platform for the specific purpose of analyzing phosphoproteomics datasets and to assist in the biological interpretation of the data. For convenience, phosphopeptide neutral loss detection and algorithms evaluating the confidence in the localization of phosphorylation sites were integrated in ProteoConnections. Swissprot, Phospho.ELM and PhosphositesPlus databases can be quickly interrogated to reveal previously observed phosphorylation & glycosylation sites and report if the identification of a site is novel. To get more insight on the regulation of phosphorylation sites, potential kinases/phosphatases can be obtained via a motifs scan. Sites can be annotated with structural environment, protein domains or binding motifs. We also implemented a convenient homology comparison tool to determine if phosphorylation sites are evolutionary conserved. The application of these tools will be demonstrated with the phosphoproteome kinetic profiling of IEC6 rat cells upon ERK pathway inhibition experiment.

Poster No.24

PTH Immunocapture Coupled to Robust Low Microflow LC-MS/MS

Rezai, Taha
Thermo Fisher Scientific

Contributing Authors:

Bryan Krastins, Mary F. Lopez, David Sarracino, Amol Prakash, Mihael Athanas

PTH (parathyroid hormone)is a clinically relevant marker for several disease states including renal failure and bone disease. A PTH immunocapture method utilizing serum enriched with IBI tips has been developed. The method can distinguish between several forms of PTH, including the 1-13 and 7-13 fragments. Using high microflow LC rates (180uL/min), a robust and reproducible LC-MS/MS triple quadrupole method has already been developed. In the current work, a LC-MS/MS triple qudrupole method using low microflow rates (2-5uL/min) was developed, capable of enhanced sensitivity. This method is capable of detecting the 7-13 fragment in serum (as well as other fragments) and has equivalent or better sensitivity than currently available immunoassays.

POSTER ABSTRACTS
Technology Session

Poster No.25

Rapid Protein Validation Assay Development Using Intelligent Software and Acquisition Strategies

Hevko, John
ABSciex

Contributing Authors:

Sahana Mollah, Matthew Champion, Sean Seymour, Christie Hunter

The field of biomarker discovery is advancing, but there is a big gap between biomarker identification and translation to clinical applications. Discovery generates too many candidate biomarkers to validate with a high cost of validation assays and long lead time needed to develop them and to generate the necessary reagents. We have addressed a solution to the need for a rapid, more robust and automated MRM assay development providing higher multiplexing. On the QTrap system utilizing the MIDASTM workflow with the aid of MRM PilotTM and MultiquantTM software we can develop an MRM assay at a rate of ~48 peptides/day, thus reducing the time required for developing a large MRM assay from weeks to just a matter of days. Using the mTRAQ reagent triplex labeling strategy we can create a global internal standard (GIS) from a pooled sample. This GIS is used when running many biological samples to enable reproducible and accurate relative quantitation experiments. Labeling of the GIS provides a much more cost effective strategy for internal standard creation when analysing many peptides since the assay development and MRM refinement is all done from biological matrix with no synthetic peptides required for the development strategy, reducing overall project cost.

Poster No.26

Investigation Into the Peptide Selectivity of Turboflow Columns

Rezai, Taha
Thermo Fisher Scientific

Turboflow chromatography is extensively used in small molecule applications. Currently there is an enormous desire to develop high-throughput peptide quantitation assays. Tryptic peptides, however, have a wide range of length and hydrophobicity. This can cause some difficulty in method development. In order to simplify the development of peptide based turboflow methods, a mixture of peptides were run on a set of turboflow columns to investigate what trends exist.A generic quick elute turboflow LC method was developed on a Transcend TLX system coupled to a TSQ Vantage triple quadrupole. Next, a mixture of 63 synthetic tryptic peptides of varying length and hydrophobicity were run on 7 different turboflow columns. A single Hypersil Gold 100x1 1.9um analytical column was utilized. Initially, all transitions were scanned on the instrument with a scan time of 3ms. After retention times for the peptides on each turboflow column were established, a scheduled method (TSRM) with a scan time of 10ms per transition was developed using Pinpoint Software. During each scheduled run, a peptide standard was infused (post column) and used as an internal standard to normalize signal from the sample.

POSTER ABSTRACTS
Technology Session

Poster No.27

Ultra-High-Performance nanoLC-MS/MS Analysis of Complex Proteomic Samples

Gendeh, Gurmil
Dionex Corporation

Contributing Authors:

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Determination of the proteome and identification of biomarkers is 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 by decreasing 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, we report on the performance of nanoLC columns operating at ultra-high 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 nanoLC. 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.

Poster No.28

Monolithic Columns for High-Efficiency LC-MS/MS Peptide Mapping

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Peptide MS analysis applied in proteomics research has made great advances over the past years in terms of sensitivity and scan speeds. However, in order to truly tackle present-day proteomic samples, different strategies have to be followed to increase the separation performance, including the development of novel column technologies.

Polymer monolithic columns have become an attractive alternative for packed columns. Especially for the nanoLC-MS analyses of complex biological samples they offer high-efficiency separations due to the absence of inter-particle mass transfer. The porosity of monolithic materials can be influenced to tune the permeability and reaching maximum separation efficiency. Optimization of the monolithic structure allows preparation of very long columns (1 meter), while maintaining the column pressure at an acceptable level.

The performance of nanoLC columns upto 1 m in length was tested by the gradient-elution of complex tryptic digests. Experiments were conducted at low and high pH mobile phases with different ion-pairing agents. MS detection was conducted by electrospray interfacing with an ion-trap mass spectrometer operated in positive and negative ion mode. The peak capacity was experimentally determined by averaging the peak width at 4-alpha for at least ten peptides.

The maximum peak capacity on long poly(styrene-co-divinyl benzene) monolithic nanoLC columns was obtained at much longer (shallower) gradients than typically applied for conventional (5 cm long) monolithic columns. Peak capacities exceeding 1000 were achieved when using 1 m long monolithic columns with optimized morphology and applying 1 -5 h gradients.

POSTER ABSTRACTS
Technology Session

Poster No.29

Development of a Method for Quantitative MALDI imaging by MRM for Absolute and Spatial Quantitation of Proteins in Breast Cancer Tissues.

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Matrix-assisted laser desorption/ionization (MALDI) imaging is a technique that allows for 2-dimensional spatial resolution of proteins, peptides and small molecules in tissue sections. In order to better understand the biological functions of different cellular regions of tissue, it is highly advantageous to identify the location and abundance of the component proteins. Here we describe a protein quantitation method using MRM MALDI imaging with a triple-quadrupole mass spectrometer fitted with a MALDI source.

We demonstrate 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 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 spotted 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.

The imaging quantitation method is based on spraying a tissue sample with a mixture of SIS peptides followed by in-situ digestion and MALDI-MRM analysis.
This method will be used to quantitate the levels of S100A7 expression in ductal carcinoma in situ (DCIS). The S100A7 protein is highly expressed in DCIS where it promotes many aggressive features leading to invasive breast cancer.

Poster No.30

Mass spectrometry-based relative quantification of potential cancer markers in FFPE samples

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Our previous discovery-phase work using iTRAQ analysis on tissue homogenates from endometrial carcinoma, GBM, and HNCa has resulted in a number of potential biomarkers for each type of cancer. A number of these potential markers were subsequently verified using conventional molecular biology approaches on the same as well as additional cohorts of samples. Current studies using a new variant of the iTRAQ reagent, mTRAQ, in combination with multiple reaction monitoring (MRM) on a hybrid triple quadrupole linear ion trap instrument have been used to determine the expression levels of a number of such potential markers in archived formalin fixed paraffin embedded (FFPE) tissue. Tumor cells from FFPE EmCa tissue were laser microdissected (LMD) and solubilized using a commercial kit. Trypsinized extracts of these samples were then labeled with the mTRAQ reagent and mixed with a similarly processed but oppositely labeled control sample consisting of extracts from a pool of non-malignant endometria. These mTRAQ-labeled pairs were then separated by SCX fractionation into four fractions, each of which was then analyzed using RP LC-MRM analysis. Three MRM transitions specific for each labeled version of the peptides of interest were targeted for monitoring. Using this approach, 13 specifically targeted proteins were detected. The relative expression levels of two of the consistently detected proteins were compared with previous results obtained during the discovery phase and found to correlate favourably. This suggests that such archived FFPE samples can in fact be used as an invaluable resource for mass spectrometry-based verification of potential cancer markers.

POSTER ABSTRACTS
Technology Session

Poster No.31

Using a Complex Proteomics Standard for Comparison of Protein Identification Workflows

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Comprehensive proteome analysis can be very challenging due to complexity and range of protein concentrations. Techniques such as 2D LC, pI-based fractionation and gel electrophoresis are typically employed to increase separation efficiency as a strategy for obtaining more peptide MS/MS spectra and thus increasing the number of proteins identified. Having a standard sample of moderate complexity facilitates comparison of different protein identification workflows. For this work, a complex proteomics standard consisting of a soluble extract from Pyrococcus furiosus is employed to evaluate the effectiveness of different fractionation schemes for increasing protein identification. The fractionation schemes evaluated included protein fractionation by 1D SDS-PAGE and RPLC as well as peptide fractionation by OFFGEL electrophoresis and SCX. A comparison of the proteins identified as well as the total number of proteins and peptides will be done for measuring the effectiveness and relative orthogonality of the different workflows.

Poster No.32

Development of a Multiplexed Immuno MALDI (iMALDI) Mass Spectrometry Assay for Diagnosis of Hypertension Related Diseases

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INTRODUCTION
The renin angiotensin system (RAS) plays an important role in regulating arterial pressure and development of hypertensive diseases. The RAS is regulated by renin, which cleaves angiotensin I from angiotensinogen in response to low arterial pressure. Angiotensin I is then converted into angiotensin II by angiotensin converting enzyme (ACE). Angiotensin II is a powerful vasoconstrictor and regulator of sodium reabsorption, thereby increasing blood pressure. Hypertensive diseases are commonly diagnosed by quantifying angiotensin I levels through plasma renin activity (PRA) radioimmunoassays. However, PRA assays do not provide information pertaining to ACE function or angiotensin II concentrations. Here we demonstrate application of immuno-MALDI (iMALDI) as a highly sensitive and specific method for multiplexed, absolute quantitation of angiotensin I and angiotensin II.
METHODS
Custom synthesized isotopically labeled angiotensin I and angiotensin II peptides were spiked into human plasma as internal standards. Anti-angiotensin I and anti-angiotensin II antibodies were immobilized on magnetic Protein G Dynabeads and used to immunoprecipitate endogenous peptides and isotopically labeled internal standards from non-digested human plasma. Antibody conjugated beads with bound peptides were placed directly on a MALDI plate. An Applied Biosystems 4800 MALDI TOF/TOF mass spectrometer was used to detect and quantify angiotensin I and angiotensin II in MS and MS/MS modes.
PRELIMINARY DATA
Employing the iMALDI approach, we show improved sensitivity in the detection of both angiotensin I and angiotensin II compared to direct analysis (no immuno-affinity enrichment) of these peptides on a MALDI target. By direct MALDI MS analysis, endogenous levels of angiotensin I and angiotensin II in normal human plasma were not detectable. However, using the iMALDI approach we were able to detect endogenous levels of both angiotensin I and angiotensin II in non-digested human plasma. The sequences of the peptides were confirmed by tandem mass spectrometry. Concentration curves for angiotensin I and angiotensin II were created by varying the abundance of natural to isotopically coded forms of the peptides. The sensitivity and linearity of the assays was determined to be within the clinically relevant range for hypertension diagnosis. Radioimmunoassay for plasma renin activity requires lengthy incubation periods and the use of radioactive molecules. In addition, antibody cross reactivity can lead to mis-diagnosis and the assay only provides a limited linear range as a result of a sigmoidal calibration curve. Our iMALDI approach represents an improved method for determining plasma renin activity through increased specificity, a wider range of linearity, ease and robustness. Furthermore, the assay's multiplexing capabilities permit simultaneous detection and quantitation of angiotensin I and angiotensin II for a more comprehensive understanding of the renin angiotensin system.

POSTER ABSTRACTS
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Poster No.33

Comparative HPLC and Ultra High Performance nanoLC-MS/MS analyses of iTRAQ labeled cancer tissue with exclusion list generation

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We have previously identified several biomarkers from endometrial carcinoma tissue using iTRAQ labelling and LC/MS/MS (1). To improve the numbers of identified proteins and investigate lower-abundance peptides, we have begun to incorporate an exclusion list into our existing method, whereby peptides identified in one run are excluded from selection for MS/MS analysis in a subsequent iterative analysis. Herein we compare the protein identification of iTRAQ-labeled endometrial carcinoma tissue sample using a conventional split-flow HPLC operating at 1700 psi and a nanoLC-Ultra HPLC system (UHPLC) operating at 4200 psi to determine whether the inherently improved resolution of UHPLC would afford a larger number of proteins identified.

Four endometrial tissue samples were trypsinized and labeled with iTRAQ tags. The four labeled samples were pooled and separated off-line, by strong-cation exchange chromatography (SCX) into 30 fractions. Two middle fractions were subjected to on-line reverse-phase (RP) chromatography using a 15 cm x 75 µm column packed with either 3 µm C18 beads (HPLC system) or 1.7 µm particles (UHPLC system).

The second run on the HPLC system added 12% more proteins to the first run whereas the third run added an additional 23%. By contrast, the second run on the UHPLC system added 35% more proteins, while the third run added additional 25% more proteins. All in all, we identified 217 non-redundant proteins using UHPLC with two rounds of exclusion list. These data indicate that the use of UHPLC and an exclusion list is an effective strategy to improve the number of identifications from complex proteomic mixtures.

Poster No.34

Parallel Algorithm for Speeding up Peptide-Spectrum Matching

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Peptide-spectrum matching is the most time-consuming portion of the database search method for assignment of tandem mass spectra to peptides. In this study, we develop a parallel algorithm for peptide-spectrum matching by using Single-Instruction Mutliple-Data (SIMD) instructions. Unlike most other parallel algorithms in peptide-spectrum matching, our algorithm parallelizes the computation of matches between a single spectrum and a given peptide sequence from the database. It also significantly reduces the number of comparison operations. Extra improvements are obtained by using SIMD instructions to avoid conditional branches and unnecessary memory access within the inner loop of the algorithm. The implementation of the developed algorithm is based on the Streaming SIMD Extensions (SSE) technology that is embedded in most Intel microprocessors. Similar technology also exists in other modern microprocessors. A simulation shows that the developed algorithm achieves an eighteen-fold speed-up over the previous version of RT-PSM (in Rapid Communication in Mass Spectrometry, 2006, 20: 1199-1208). Therefore, the developed algorithm can be employed to develop real-time control methods for tandem mass spectrometry.

POSTER ABSTRACTS
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Poster No.35

Comparing Subtypes of High-Grade Endometrial Cancer Tissues by Means of Iterative Proteomic Analyses

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The majority of Endometrial carcinoma (EmCa) are classified histologically as being endometrioid, clear cell, serous, or a mixture of these cell types. Grades I and II endometrioid EmCa are considered low grade. By contrast, grade III endometrioid, serous and clear cell EmCa's are considered high grade and treated aggressively. Histological classification is subjective and molecular biomarkers can be of value in aiding cell-type classifications, especially when mixtures of EmCa cells are encountered. Ultimately, biomarkers may determine the treatment regimes.
In this study, the proteomics profiles of six grade III endometrioid, five clear cell and eight serous carcinomas were compared with a reference pool of ten non-malignant proliferative endometrial samples, using iTRAQ labeling and LC-MS/MS analyses. An iterative LC-MS approach was used to increase the numbers of proteins identified. Proteins that displayed iTRAQ ratios 0.67 or 1.5 in a minimum of three samples in at least one of the subtypes, and which have been found in at least three samples in each of the other subtypes with no such differential expression, were considered as biomarker candidates. 1518 proteins were identified; of these, 732 were quantified each with a statistically significant iTRAQ ratio. Eighteen proteins showed differential expressions in different EmCa subtypes. Verification of these differential expressed proteins in a larger cohort of patient samples will transform these proteins into biomarkers that permit subtyping based on the EmCa molecular profiles. Fourteen more proteins showed a similar trend for all subtypes, and may be considered potential candidates as general EmCa biomarkers.

Poster No.36

Protein changes in relation to food quality and safety: from qualitative to quantitative proteomics

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Proteomics is a systematic approach to study changes in proteins, providing an essential linkage between the transcriptome and metabolome. Both two-dimensional electrophoresis (2-DE) and non-gel based quantitative proteomic tools have been available for plant research. Despite the intensive biochemical and physiological studies on fruit with additional genomic and molecular investigations, very few data available at proteomic level. Up to now, fruit ripening and senescence at gene and protein levels have not been fully understood. In this study, protein profiles from apples and strawberries at different developmental stages were investigated using 2-DE. Significant changes in protein population in relation to fruit ripening and senescence have been shown. In addition, a non-gel quantitative proteomic technique based on stable isotope dimethyl labelling at peptide level was developed to investigate the complex protein population during fruit ripening. More than 200 proteins have been identified. Combining with Gene Ontology annotation tools, various metabolic pathways and biological functions of those identified proteins were found to be related to fruit ripening. These proteins played an important role in fruit ripening and senescence. This research developed whole approaches based on gel-based (2-DE) and non-gel based proteomic techniques which were suitable for food/fruit quality research. The technologies developed also demonstrated great potential to study fruit ripening and senescence at proteome level.

POSTER ABSTRACTS
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Poster No.37

Protease Substrates Discovery Using Terminal Amine Isotope Labeling of Substrates (TAILS)

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Determining the individual and relative contributions of proteases in biochemical pathways is elusive for most systems. Identification of protease biological substrates is also critical to understand the biological role of proteases. Lastly, modification of the natural N-terminus of proteins determines the activity, localization and half-life of many proteins. Here we present Terminal Amine Isotope Labeling of Substrates (TAILS), a method for the identification of protease substrates and the simultaneous analysis of the N-termini of proteins in a proteome in a single experiment.

TAILS compares peptides from 2 proteomes that have been subjected to proteolysis or not. Proteolysis generates neo N-termini that are the signature of proteolytic events. Neo and natural N-termini are identified by TAILS using primary amine isotope labeling, N-termini enrichment using a newly developed amine reactive polymer, and quantitative tandem mass spectrometry (MS/MS). Isotopic labeling allows for discrimination of protease neo-N-terminal peptides from background proteolysis and the natural N-terminome.

Unlike chromatographic beads, the high molecular weight polymers we developed for TAILS have a extremely high binding capacity and no non-specific peptide interactions. Application of TAILS to the secreted proteome of fibroblasts digested with GluC identified 532 GluC-generated peptides; only 70 were identified before the enrichment step. Next, new matrix metalloproteinase-2 (MMP-2) substrates were discovered by TAILS. Following treatment of a secretome of MMP-2-/- embryonic fibroblast with human MMP-2, TAILS identified 35 novel MMP-2 substrates, 96 published MMP-2 candidate substrates that have never been validated and 19 known MMP-2 substrates. Four new MMP-2 substrates were biochemically validated in vitro. Analysis of the proteins N-termini of mouse bronchoalveolar lavages following LPS treatment were also analyzed by TAILS revealing many modifications of the N-termini and multiple cleavage products due to inflammation, so proving the utility of TAILS for both substrate degradomics and N-terminome analysis in in vivo situations.

Poster No.38

Systematic Assessment of the Reproducibility of Relative Quantification Based on LC-MS with Technical Replicates

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The objective of this study was to develop a method to assess and display reproducibility of protein quantification based on LC-MS with replicate analysis.

Technical replicates were used to assess the reproducibility of relative quantification uniquely at three levels:
Data level - Peptide features from each of the replicates were detected and aligned between the replicates. The data reproducibility was analyzed by paired T-test and correlation analysis on the intensities of paired features. Venn diagrams of peptide features between replicates were also used to display the reproducibility.
Identification level - Protein identification was conducted on each replicate. The reproducibility of the identified peptides and proteins between replicates was assessed by paired T-test and correlation analysis. The boxplot diagram was used to represents the distribution of the logarithmic ratios of identified feature pairs.
Quantification level - The relative peptide/protein abundance was computed based on the intensity of each peptide ion. The reproducibility of peptide and protein quantification was assessed by paired T-test and ANOVA. This method was implemented in the PEAKSTM software suite and tested by a standard protein mixture. The standard mixture UPS2 was reduced and alkylated by iodoacetamide, then digested by trypsin overnight. MS data was obtained from a Waters Q-TOF Premier. Two replicate experiments were performed. The results from this experiment demonstrate that this method could efficiently assess and display the reproducibility at three levels, which helps to evaluate both the LC-MS experiment and the quantification algorithm.

POSTER ABSTRACTS
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Poster No.39

The ABRF Proteomics Research Group 2010 Study: Identifying Unforseen Problems in Otherwise Straight-Forward Proteomics Analyses

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An experiment frequently performed in proteomics laboratories today is the analysis of protein complexes isolated by coimmunoprecipitation or affinity enrichment, wherein the primary goal is typically to identify and characterize the individual components of the complex. Such protein complexes are usually isolated from a cell lysate, and often include many unforeseen challenges and confounding factors such as reproducibility and the presence of non-specific binding partners. The Association of Biomolecular Resource Facilities (ABRF) Proteomics Research Group (PRG) 2010 study explores the use of different approaches for determining the identities of several similar affinity-enriched recombinant protein samples. The study was modeled after a real-life scenario, and sufficient information was provided to study participants to mimic an actual submission to an analytical laboratory. Additional challenges were present within these samples, including 15N-labeled proteins, an altered expression construct, and an unanticipated contaminant, making this study amenable to a wide spectrum of expertise levels. The majority of participants reported that the study was of moderate difficulty. Study results will be used to assess the different approaches that are used by the proteomics community to determine the relative identities and composition of the components in the mixture.

Poster No.40

Tandem Affinity Selective Enrichment of Inter-Peptide Crosslinks for Complex Structural Proteomics Crosslinking Applications.

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The successful application of crosslinking combined with mass spectrometry for studying complex protein systems, including proteome-wide crosslinking applications, requires specific methods for the detection, isolation, and identification of inter-peptide crosslinks in complex peptide mixtures. These most-informative inter-peptide crosslinks provide information on the distance between crosslinked sites, as well as information on the identities of the interacting proteins. Introducing an affinity-tag into a crosslinker allows the separation of crosslinks from the excess of non-crosslinked peptides. Unfortunately, even after affinity purification, inter-peptide crosslinks are still overwhelmed by less-informative dead-end and intra-peptide crosslinks. Here, we describe the combination of affinity-tagged crosslinking reagents with selective enrichment of imidazole-modified inter-peptide crosslinks on Ni-NTA supports.
We tested this tandem-affinity purification on model peptides, proteins, and protein complexes of increasing complexity. Initial proof-of-principle experiments using a test mixture of dead-end and inter-peptide CBDPS crosslinks spiked into a BSA tryptic digest resulting in MS detection of only the inter-peptide crosslink which was undetectable prior to this tandem-affinity purification procedure. Applying this approach to the protein heterodimer HIV reverse transcriptase led to detection of inter-peptide crosslinks which also were not otherwise detectable. We are currently evaluating this method for proteome-wide crosslinking applications. Preliminary data obtained so far indicate that this approach of tandem affinity purification of inter-peptide crosslinks could be the breakthrough technique needed to make crosslinking combined with mass spectrometry applicable to large protein complexes and proteome-wide systems.

POSTER ABSTRACTS
Technology Session

Poster No.41

Direct detection and identification of bacteria and micro-organisms from food, water and environmental samples using LC-ESI-MS/MS

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There is a need to directly detect, identify and quantify bacteria and other micro-organisms in human food, water, or environmental samples. Laboratory strains of E. coli, Salmonella, and Pseudomonas were cultured and analyzed directly, or after mixture to model a complex authentic sample. Subsequently a sample from a polluted lake, from urban soil, and from chicken purchased at a retail food store were obtained. Food and soil samples were washed in the sterile water under aseptic conditions. The wash water, lake water or control sterile water were then analyzed directly or after culture for 24 h in Luria broth. Tap water and autoclaved water served as controls. The samples were homogenized in a French press and centrifuged at 12,000 RCF to remove unbroken cells and debris. The lysates were analyzed by Dumbroff or Bradford proteins assays and resolved by 7% tricine SDS-PAGE gels stained with Coomasie blue or diamine silver. The original and cultured liquid-samples were digested with trypsin and analyzed by LC-ESI-MS/MS. Digests of the food, soil and water samples were collected over C18 and separated by 15 cm x 150 micron ID, double-fritted column at 200 nl per minute and ionized through an empty 10 micron ID electrospray emitter into a linear ion trap. The SEQUEST algorithm within the Proteome Discoverer 1.0 application directly calculated the probability of a false positive identification for each peptide sequence. The peptide to protein distributions and protein score distributions were also compared to those of random MS/MS samples from instrument noise or a random MS/MS spectra generator to estimate the probability of false positive results. Some mammalian or eukaryotic proteins were observed in the control water, however, strong spectra for bacteria, fungi and other micro-organisms were observed in the original or cultured food, lake and soil samples. Hence it is apparently possible to directly detect, identify, and quantify levels of bacteria and micro-organism proteins from food, water and soil samples using biochemical and biophysical methods that require no previous culturing, specialized reagents or procedures.

Poster No.42

Combining nanoLC-RePlay-MS/MS with a scheduled precursor list (exclusion/inclusion list) to significantly enhance protein identification from complex

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LC-MS/MS-based bottom-up proteomic approaches try to tackle the common problem of under sampling by using multiple injection strategies, increased gradient run times, multi-dimensional LC separation or faster MS/MS data acquisition at the cost of sample dilution, time consumed per sample, analytical complexity and cost of instrumentation.

Here we investigate the new RePlay technology that allows post-column nanoLC flow splitting for additional mass spectrometric analysis from the same sample injection, without reinjection of the sample, which is a very important aspect when sample amount available for interrogation is limited as is the case for tissue micro dissections or small animal proteomic studies (fly, zebrafish, mouse, etc.). RePlay is a significant advancement for investigating a complex proteomic sample, especially when combined with an exclusion list.

We will show the use of a semi-automatic scheduled precursor list (exclusion/inclusion list) in order to significantly enhance the protein identification from a complex biological sample (e.g. tryptic digest of mouse F9 cell nuclear lysate, Active Motif, CA). Actively excluding MS/MS triggers from previously collected data/information greatly enhances the thoroughness of sample analysis, allowing one to dig deeper into the proteome. We found that a combination of LC-RePlay-MS/MS with a data dependant exclusion list (Bruker HCT IonTrap with SPL macro) reduces the information overlap observed in a multiple injection strategy by 50 %.

Consequently, peptide assignments increased by ca. 90 % and protein assignments increased by 40 % (95 % confidence level for peptide/protein assignments) compared to a standard LC-MS/MS based proteomic analysis.

POSTER ABSTRACTS
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Poster No.43

Combinatorial peptide librariesfacilitate development of multiple reaction monitoring assays for low-abundance proteins

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Low-abundance proteins (present in biological fluids at concentrations lower than 1 ºg/mL) are considered an attractive source of new disease biomarkers. Since such proteins are masked by high-abundance proteins and thus poorly observed in proteome-scale discovery experiments, the development of quantitative multiple reaction monitoring (MRM) assays based on discovery data is a challenging task. Here, we present a strategy that facilitates the development of MRM assays for a large number of unpurified low-abundance proteins (Drabovich, A.P. and Diamandis, E.P., J. Proteome Res., 2010, 9, 1236-1245). Our discovery strategy is based on the reduction of the dynamic range of protein concentrations in biological fluids by means of one-bead one-compound combinatorial peptide libraries (CPL). Our 2D-LC-MS/MS approach allowed us to identify a total of 484 unique proteins in ovarian cancer ascites, and 216 proteins were assigned as low-abundance ones. Interestingly, 74 of those proteins have never been previously described in ascites fluid. Treatment with CPL allowed identification of a significantly higher number of unique peptides for low-abundance proteins and thus provided empirical fragmentation information for development of MRM assays. Finally, we confirmed that MRM assays worked for 30 low-abundance proteins in the unfractionated ascites digest. Using a multiplexed MRM method, relative amounts of five proteins (kallikrein 6, metalloproteinase inhibitor 1, macrophage migration inhibitory factor, follistatin-related protein, and mesothelin) were determined in a set of ovarian cancer ascites. Multiplexed MRM assays targeting large numbers of proteins can be used to develop comprehensive panels of biomarkers with high sensitivity and selectivity.

Poster No.44

Using Ion-Pairing Normal Phase Liquid Chromatography and Mass Spectrometry to Isolate and Characterize Glycopeptides

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Glycoproteomics often requires that glycopeptides be isolated from complex proteolytic digests in order to increase their MS sensitivity and facilitate their analysis. Glycopeptide capture using hydrazide chemistry is effective, at least for N-linked glycopeptides, but does not preserve the glycans. Lectins have restricted specificities and non-specific binding of non-glycopeptides can be problematic. Normal phase LC (NPLC) enriches glycopeptides with various glycan structures but does not recover smaller, less hydrophilic glycopeptides, e.g., high mannose glycopeptides. Here we describe the use of acids as ion-pairing reagents in NPLC (IP-NPLC) to reproducibly isolate high mannose-type and sialylated glycopeptides from complex peptide mixtures with close to 100% recovery. In addition to working with model proteins such as fetuin and ribonuclease b, IP-NPLC was used to isolate N-linked glycopeptides from tryptic digests of proteins extracted from wild-type (WT) and pglD mutant forms of the food-borne pathogen, Campylobacter jejuni. The glycopeptides were isolated from tryptic digests with close to 100% recovery and 90% selectivity. Furthermore, we determined that the glycan PTMs from the pglD mutant differed from the WT by the loss of an acetyl group on the di-N-acetylbacillosamine residue, the sugar that links the N-linked glycan to the asparagine side-chain.

POSTER ABSTRACTS
Technology Session

Poster No.45

Segmented 1D-nanoLC combined with CID/ETD for Increased Sequence Coverage in Biomarker Discovery

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Fractionation is a common methodology for processing complex proteomic samples by mass spectrometry. After gel based methods, 2D-LC is the most common approach, based on online strong cation exchange (SCX) separation in the first dimension and reverse phase (RP) separation in the second. However, this setup is inherently complex. Here, we describe a simple ìsegmenting strategy on a 1D split-free nanoLC using a biphasic pre-column combined with an RP analytical column. This strategy eliminates the requirement for ternary or quaternary gradient systems which simplifies the experimental setup. This split less method does increase the run time of the experiment, which allows for the application of multiple types of tandem mass spectrometry to be performed with out missing peaks.

A standard E. coli cell lysate digest was used to compare the results of a conventional 90 min. nanoLC run with the segmented approach.

When using alternating CID and ETD, most of the time the same peptides are being fragmented twice. Normally in a conventional nanoLC method this means some loss in time, which is lost for the dissociation of additional peptides and may result in a lower number of identified proteins compared to performing CID MS/MS alone. With the extended run time of the segmented experiment, time issues are no longer a major concern, so doing both CID and ETD does not decrease the number of fragmented peptides. CID and ETD often give complementary sequence information, in particular on peptides of different charge states, and increase both sequence coverage and scores of the identified proteins.

This preliminary data shows that this combination of segmented 1D-LC and CID/ETD fragmentation improves the information content on this complex cell lysate.

Poster No.46

Mass Spectrometric Top-Down Protein Sequencing de novo: The Full Determination of a 13.7 kDa Camelid Nanobody Sequence

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Mass spectrometric Top-Down Sequencing (TDS) was used in this work for the first time to establish a complete unknown protein sequence de novo. The sample was made available for participation in a blind study in the course of the Martinsried Conference 2009 on ìMicromethods in Protein Chemistry. A pure ~14 kDa protein was received, which was not present in any database to prevent sequencing by simple annotation to sequences that can be searched. The presented approach provides direct access to N- and C-terminal sequences of proteins such as recombinant antibodies and to fully establish protein sequence if the molecular weight is in the 3-15 kDa range.

POSTER ABSTRACTS
Technology Session

Poster No.47

A combined LC-MS/MS approach for quality control of recombinant proteins

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Bruker Daltonics

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Antibodies represent one of the most important classes of protein drugs. In accordance to common pharma QC requirements, recombinant proteins products need to be characterized in detail. This includes an as complete as possible confirmation by experimental data of the expressed protein sequence as well as the in-depth characterization of modifications.
In this paper we describe the sequence characterization of a recombinant antibody based on a combined strategy involving multiple enzymatic digests in conjunction with LC-MS/MS analyses performed on various MS/MS platforms. Seamless integration and analysis of the resulting data as well as final result compilation is demonstrated employing a unique database software illustrating the crucial importance of sophisiticated bioinformatics tools to the efficiency of in-depth protein characterization tasks.

The application of a combined analytical approach as described here results in large amounts of mass spectrometric data. Approximately 20 LC-MS/MS datasets obtained from the various digests and MS/MS platforms, respectively, were imported and organized in the proteomics-specific database system, and were then matched against the antibody chain sequences considering expected modifications, especially terminal modifications like N-terminal pyroGlu. The sequence information obtained from these individual experiments was then further compiled into a detailed, comprehensive, non-redundant project report integrating all acquired data irrespective of the workflow and MS instruments used. As a result, nearly 100% MS/MS sequence coverage was achieved for both, the antibody's light and heavy chains. When compared to the results of a single dataset, the biggest contribution came from the integration of LC-MS/MS runs of the four different in-solution digestions. Three additional amino acids of the heavy chain could be gained by a combination of data sets from different instruments.

Poster No.48

Ultra-high-performance nanoLC-MS/MS analysis of complex proteomic samples

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Bruker Daltonics

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Identification and quantification of biomarkers is required to monitor dynamic changes in living organisms and predict the onset of an illness. One popular method applying in this research field is 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. The separation of these highly complex peptide samples is one of the major challenges in analytical chemistry. Data quality in label free quantitative proteomics relies on efficient and sensitive LC-MS measurements. In addition as for most chromatographic technique, retention time precision in nano LC is of utmost importance.

Here, we report on the performance of nanoLC columns operating at ultra-high 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 nanoLC. High-resolution LC-MS separations of complex peptide samples are obtained, demonstrating peak capacities over 750 on 50 cm long columns packed with 2 µm particles and applying long gradients of 4-5 h. As an example for a very complex sample 500 -1000 ng of a human cancer cell line were injected per analysis to evaluate the performance of the setup. Very reproducible retention times allowed us to work with very short time windows to exclude or in include peptides as pre-cursors in multiple consecutive LC-MS/MS experiments. Reproducible chromatography will also help data processing and sample comparison in quantitative proteomics by allowing the application of a smaller retention time deviation threshold.

POSTER ABSTRACTS
Technology Session

Poster No.49

Biomarker validation and clinical utility of multiple reaction monitoring (MRM) in cardiac transplantation

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PROOF Centre of Excellence in collaboration with the UVic-Genome BC proteomics Centre has developed a proteomic biomarker pipeline for a discovery and validation approach that is validated with ELISA, the current gold standard. In a recent study, 14 potential plasma proteins associated with cardiac rejection were identified using iTRAQ-MALDI-TOF/TOF mass spectrometry. An initial validation was performed on 23 samples originally analyzed by iTRAQ using ELISA for 4 proteins and MRM for all 14 protein candidates. ELISA tests were run following manufacturer's protocols. Liquid chromatography coupled with tandem mass spectrometry in multiple reaction monitoring (MRM) mode was then performed on tryptic digests of non-depleted human plasma. SIS peptides were added immediately following tryptic digestion. iTRAQ and MRM measurements were strongly correlated ($r>0.70$) for 5 out of 14 validated proteins but weakly correlated ($r<0.50$) for other 3 proteins. Similar results are obtained between iTRAQ and ELISA measurement. MRM and ELISA measurements showed strong correlations ($r>0.83$) for the 4 common proteins. In addition, this study provided a proof of principle by selecting a plasma proteomic panel to diagnose acute cardiac rejection and building a classifier based on MRM measurements of the 14 biomarker candidates. Initial estimates of of sensitivity and specificity by leave-one-out cross-validation were above 82%. MRM is not limited by antibody availability, has the theoretical capability of multiplexing hundreds of markers in a single run, and is cost- and time-effective. For the set of validated proteins, MRM measurements correlated strongly with ELISA showing the potential utility in a biomarker validation pipeline.

Poster No.50

Identification of Lipids in Placentas from Control and Preeclamptic Pregnancies

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Introduction
Preeclampsia is a pregnancy-associated disorder characterized by maternal hypertension and dyslipidemia in the maternal bloodstream. To determine if the placenta is also affected by dyslipidemia, it is necessary to develop a method to profile the major placental lipid species. As a precursor to quantitative analysis of the placental lipid profile in preeclampsia, we have qualitatively analyzed lipids from control and preeclamptic placentas by FTMS.
Methods
Samples of placental chorionic villus were taken immediately after delivery from two control and two preeclamptic pregnancies and lipids were extracted by the Bligh-Dyer method. The organic phase extracts were then ionized by direct infusion nano-electrospray and analyzed in a Varian QFT 12 Tesla FTMS.
Results
In a typical analysis, 78 peaks of m/z between 400 and 900 were detected in negative ion mode and 40 in positive. The LIPID MAPS database was searched using these m/z ratios with a mass tolerance of 0.01 mass units. 57 (48%) of all of the detected peaks could be unambiguously assigned to a unique lipid class and elemental composition, accounting for 69% of the total peak intensity. The identified peaks predominantly represented glycerophospholipids including phosphatidyl cholines, phosphatidyl ethanolamines, phosphatidyl serines, phosphatidyl inositols, and phosphatidic acid. 55 species of lipid were identified as common between all 4 placental samples analyzed.
Conclusion
Qualitative analysis has identified lipid species that can be targeted for further quantitative analysis of placental lipids in preeclampsia. Identifying changes in placental lipid composition associated with preeclampsia will facilitate greater understanding of this disease.

POSTER ABSTRACTS
Technology Session

Poster No.51

Sensitivity and Specificity of a Linear Ion Trap Mass Spectrometer

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The Sensitivity and specificity of a linear ion trap mass spectrometer was determined using complex protein samples. Previously a set of 18 commercial protein standards purified from bacteria, fungi or mammalian species were analyzed to establish cut off scores for confident identification using the decoy library method. However the decoy library method may result in improper inference if the protein standards are not pure. Since complex biological samples may often foul chromatography columns and develop back pressure, a double fritted C18 micro column (150 micron ID) and an empty glass emitter (10 micron ID) were used together to provide sensitivity and protect the nanospray tip from blocking. The robust sensitivity of the nanospray was estimated to be on the order of 1-10 femto Mol or less on column. We observed that many proteins were reliably and reproducibly detected from the commercial standards by LC-ESI-MS/MS that appeared to contain tens to hundreds of protein each. Coomasie brilliant blue gels indicated that the commercial proteins were apparently pure and matched the predicted Mr of the standard. However, staining with diamine silver revealed many apparently contaminating bands in the purchased standards. Using only the listed commercial standard protein as the target led to high rates of estimated false positive results from the decoy library method. Comparison of the distributions MS/MS spectra obtained from the protein standards to instrument noise, or computer-generated random spectra, by Chi square analysis clearly separated the authentic data from random false positive results at expectation values E-1. The expectation values of peptides identified by SEQUEST (Proteome Discoverer 1.0) showed agreement with those from Chi square comparison to random, false positive results. Hence LC-ESI-MS/MS with a linear ion trap shows robust sensitivity to the femto Mol range and two statistical methods showed a well-defined probability of false positive (type I error).

Poster No.52

Modeling false positive identification of tryptic peptides from human blood using tandem mass spectrometry compared to random mass spectra

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To date, the central problem in proteomics has been uncertainty regarding whether tandem mass spectrometry of peptides is a highly reliable method for the identification of primary structure, or whether it is prone to large rates of false positive identification. Identification of peptides by tandem mass spectrometry utilizes estimates of the mass to charge (m/z) ratio of the parent peptide and the many peptide fragments. Uncertainty has been expressed about the sufficiency of the measurement of m/z values in the spectral lines of mass spectra. At the time of the experiment the true m/z values are unknown but are continuous variables. In contrast, there is little doubt about the well-known amino acid sequences in protein libraries that are discrete variables. It is both complex and arbitrary to model the unknown, continuous m/z of ions by varying the known, discrete amino acid sequences in libraries. The expectation of false positive identification of tryptic peptides from human blood was calculated using the goodness of fit test within the X!TANDEM algorithm. In frequency-based statistics, samples from populations are classified with respect to frequency of observations in a category, or along a continuum, and often compared to the distribution of random data. In this study the frequency distributions from authentic blood peptides and proteins were compared to those of random MS/MS spectra obtained from a computer, or instrument noise. Chi square analysis of the frequency distributions clearly distinguished the real data from random false positive correlations. Hence goodness of fit tests of individual peptide fragment spectra, or the resulting populations of peptide and proteins, show unambiguous evidence that even simple ion traps are sufficient to identify thousands of unmodified tryptic peptides with a very low expectation of false positive results ($p<0.0001$).

POSTER ABSTRACTS
Technology Session

Poster No.53

Antibody Colocalization Microarray: validation and clinical application to breast cancer

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Robust, high-sensitivity, high-throughput multiplexed protein profiling technologies are still missing, which constitutes a bottleneck to the widespread adoption of proteomics. We recently reported the development of a sandwich antibody microarray format, which we call antibody colocalization microarray (ACM) as a sensitive and specific tool for profiling multiple proteins simultaneously.
Our platform comprises 36 antibody pairs. We introduced normalization approaches using GFP in a sandwich assay as a calibrant which allows eliminating variation from pin-to-pin and between PBS calibration curve and serum sample analysis. We validated our platform by profiling the concentration of Leptin in 20 serum samples both with the ACM and with a commercial ELISA kit, and obtained an excellent correlation (R2 = 0.93). To demonstrate the potential of our antibody platform, we used it to profile 30 proteins in 15 breast cancer patients and 25 healthy controls. We found IP-10, EGF, IL-6, CCL2, MMP-9, Endoglin and Osteopontin to correlate with the patient status (p 0.1). Heat Map and cluster analyses showed a strong segregation of cancer and normal patients. It strongly suggests that this platform may potentially be used for diagnosis and prognosis purposes.
ACM represent a new reliable tool to quantify low concentration of multiple proteins from small amount of blood without compromise on the sensitivity. It can be easily customized and scaled up because of the independence of each micro-assay. By increasing the number of analyzed biomarkers, it may be possible to further reinforce the statistical correlation with particular patient status and disease outcome.

POSTER ABSTRACTS
Neurobiology Session

Poster No. 11

Quantitative Changes in Proteins and Glycoproteins in Luminal Membranes of Brain Endothelial Cells in Response to TNF-alpha and INF-gamma

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BACKGROUND. Mammalian brain vessels contain a thick-coating of molecules on the luminal side (i.e., facing the blood) called glycocalyx, which are enriched in proteins and glycoproteins. These molecules are attractive therapeutic and diagnostic targets because they are accessible to blood. Here we have isolated luminal membranes from activated human brain vasculature endothelial cells (HBEC) and quantified the changes in proteins and glycoproteins using label-free quantitative proteomics. METHODS. HBEC (HCMEC/D3) were either not activated or activated with proinflammatory cytokines (TNF-alpha, INF-gamma) and two approaches were taken for isolating luminal proteins and glycoproteins. The first involved sucrose-gradient/ultracentrifugation (SG/UC) based separation of luminal/abluminal proteins, and the second involved in situ oxidation of cells followed by hydrazide-capturing of luminal glycoproteins. Both approaches were coupled with label-free nanoLC-MS/MS proteomics using nanoAcquity UPLC (Waters) and LTQ-XL (Thermo), followed by quantification using MatchRx (QnDv2) software. RESULTS. Both approaches identified >250 luminal glycoproteins, although the overlap between them was <100 proteins. Most of the well-known HBEC luminal proteins were identified by both approaches, including P-glycoprotein-1, ICAM1, VCAM1 and ABCC5. The SG/UC approach additionally identified >500 non-glycoproteins in the luminal fractions. In addition, it identified >600 abluminal proteins and glycoproteins, including well-known HBEC abluminal molecules such as ABCC1, SLCs and integrins. Finally, >50 proteins and glycoproteins showed statistically significant quantitative changes when HBEC were activated, several of which were validated by western blotting and immunocytochemistry. CONCLUSIONS. This is the most comprehensive profiling of luminal and abluminal (glyco)proteins from glycocalyx of brain vascular cells. The results demonstrate the power of subcellular fractionation and in situ-hydrazide capturing to selectively identify key molecules that have a potential to be used as targets for therapy and/or molecular imaging agents under cerebrovascular inflammatory conditions.

Poster No. 12

Exploring the potential role of GBM markers discovered by LC-MS/MS analysis of brain tissue homogenates

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Glioblastoma multiforme (GBM) is the most common and devastating brain tumor. The diagnosis of the brain tumors on the basis of tumor type and grade is very important for its management. Neuroimaging is the only non-invasive modality of diagnosis of GBM that may help to differentiate the tumor from other benign mass lesions, abscess, edema etc., but definitive diagnosis is possible only by stereotactic or open brain biopsy. The discovery of GBM-specific biomarkers is of high clinical importance, especially if these biomarkers are secreted into blood. In order to identify a GBM-specific panel of proteins, we compared six non-malignant brain tissue homogenates with six GBM tissue homogenates using LC-MS/MS analysis of iTRAQ-labeled samples. The clarified tissue homogenates were digested with trypsin, labeled with iTRAQ reagents, and subjected to 2D-LC separation followed by MS/MS analysis. The data obtained was analyzed by using the ProteinPilot software (Applied Biosystems Inc) which searched a Celera database of protein sequences comprising NCBI, SWISS-PROT and Trmble databases. The analysis identified more than 1000 non-redundant proteins, amongst which a number of proteins were found to be differentially expressed in the GBM only. Ingenuity Pathway Analysis network mapping algorithm showed the majority of these proteins appeared to map to one network, of which one protein is particularly interesting. This protein, though not detected in the LC-MS/MS analysis, was known to directly interact with the majority of the differentially expressed proteins. Further this protein, while not being directly associated with GBM, has previously been implicated in a number of other neurological diseases. On-going experiments are aimed at elucidating its possible role in GBM.

POSTER ABSTRACTS
Neurobiology Session

Poster No. 13

Mitochondrial localization of the neuronal apoptosis inhibitory protein NAIP.

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The neuronal apoptosis inhibitory protein (NAIP), the founding human inhibitor of apoptosis (IAP) is comprised of three anti-apoptotic BIR domains, a nucleotide oligomerization domain (NOD) and a C-terminal leucine-rich repeat (LRR) domain implicated in intracellular sensing of L. pneumophila and S. thyphimurium derived flagellin. NAIP is unique among IAPs because of its NOD and LRR domains and among NLRs (Nod-like receptors) because of the presence of the BIR domains. These characteristics localize NAIP at the cellular crossroads of apoptosis and innate immunology. Putative neuronal roles for NAIP include a modifying factor for both multiple sclerosis as well as spinal muscular atrophy.
In addition to the full length protein there are four 5'- and 3'-partially deleted versions of the full length gene. Copy number variation in the chromosomal region (5q13.2) encoding NAIP, distinct starting transcription sites scattered along the NAIP gene and the reported expression of not less that three different NAIP isoforms among various organs and cell types, configure a complex NAIP landscape complicating the study of NAIP at transcriptional or protein levels.
The subcellular localization of NAIP has been thought to be cytosolic, Here we present confocal microscopy analysis in HeLa cells and in mouse macrophages demonstrating the colocalization of NAIP with the mitochondrial marker Tom20 (component of the mitochondrial translocase of outer membrane), a finding confirmed at the electron microscopy level by means of colloidal gold immuno-detection. The mitochondrial localization of NAIP is observed with antibodies directed to the first N-terminal third of full length NAIP. We have yet to explore if mitochondrial NAIP is restricted to the IAP signature of NAIP and/or whether the full length version of it or other NAIP isoforms are also present in the mitochondria.
The subcellular localization of NAIP here described constitutes a new and interesting insight in NAIP biology and should therefore be considered in any future experiments addressing the role of NAIP.
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Poster No. 14

Analysis of Proteins in Cerebral Microdialysis Samples of Severe Traumatic Brain Injury Patients

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Severe traumatic brain injury (sTBI) is the leading cause of death in young populations. Diagnostic and prognostic is currently difficult because little is known about the mechanisms of secondary injuries that develop within days after the primary injury. The objective of this study is to qualify several candidate protein biomarkers for sTBI.
This study uses a novel microarray platform called antibody colocalization microarray (ACM), which overcomes cross-reactivity problems normally seen in conventional antibody microarray by segregating detection antibodies on the microarray. This new platform required a custom solution for the analysis of the microarray data as conventional software packages were unable to process our data. We therefore developed the Dot Analysis Tool (DAT), which allows the user to read large spot intensity table files, and quickly generate dilution curves for protein standards and samples. The tool performs outlier removal automatically on replicates, and will be made available to the research community.
Here, we collected samples from a cerebral microdialysis catheter implanted into the brain of patients during the initial decompressive surgery, and compared them to normal human cerebrospinal fluid, using the ACM platform. We have successfully detected 12 proteins in the cerebral microdialysis samples, and in normal cerebrospinal fluid control. We observed the evolution of the inflammatory response in the patient's brain. Samples from additional patients with different outcomes will be analyzed. This study could potentially lead to a better understanding of the secondary injuries in the brain and help with the diagnostic and prognostic of patients.

POSTER ABSTRACTS
Personalized Medicine Session

Poster No.54

SMALL INTERFERING RNA (siRNA) TARGETING 14-3-3 zeta SENSITIZES HEAD AND NECK CANCER CELLS TO CHEMOTHERAPY

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Advancements in treatment modalities [surgery, chemotherapy (CT) and/or radiotherapy (RT)] have not translated into marked improvement in the prognosis of head and neck squamous cell carcinoma (HNSCC) patients. Thus a major thrust is being laid on the development of molecular targeted therapies for HNSCC. Recently, using iTRAQ-labeling and liquid chromatography - tandem mass spectrometry (MS/MS), we identified a panel of biomarkers including 14-3-3 zeta in HNSCC and oral pre-malignant lesions. In this study, we determined the effect of downregulating 14-3-3 zeta expression in head and neck cancer cell lines (SCC4 and HSC2) on proliferation and response to CT agents. Chemosensitivity of transfected cells was determined by measuring cell death using MTT assay. Cell cycle analysis, annexin V dye binding and TUNEL assays were carried out using flow cytometry. Head and neck cancer cells transfected with 14-3-3 zeta siRNA showed G2/M arrest and reduced cell proliferation within 48-72 hours. Importantly, siRNA transfected cells showed a dose-dependent increase in cell death on treatment with chemotherapeutic agents (paclitaxel, doxorubicin, or cisplatin) as compared with non-transfected control cells. Flow cytometry analysis using propidium iodide staining showed an increased fraction of cells in the sub-G0 phase, indicating cell death at lower doses on treatment with chemotherapeutic agents. Annexin V staining and TUNEL assay supported these results. In conclusion, our results suggested 14-3-3 zeta may serve as a novel target to improve the efficacy of chemotherapeutic agents in the management of head and neck cancer.

Poster No.55

Tissue-Based Identification of Renal Cell Carcinoma Markers

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Renal cell carcinoma (RCC) is the seventh most common cancer in men and the ninth in women worldwide. There are no RCC molecular markers currently available to aid clinical diagnosis of the disease. Mass spectrometry-based differential analysis of non-malignant and RCC tissues has the potential to discover such biomarkers. In what is regarded as a √¢,Ç¨≈ìtissue-to-blood√¢,Ç¨¬ù strategy, any such differentially expressed proteins detected in tissue could serve as prospective targets in the eventual development of new blood-based biomarkers for RCC.

For the identification of differentially expressed proteins from tissue, we used tryptic digests of clarified tissue homogenates that were labeled with isobaric tags for relative and absolute quantitation (iTRAQ). Four-plex tags were used to label combinations of non-malignant and tumor samples prior to pooling and separation by offline strong cation exchange chromatography, followed by on-line reverse phase nano-LC-MS/MS analyses in triplicate (QSTAR Pulsar; AB/MDS SCIEX). MS data were analyzed by ProteinPilot to identify differentially expressed proteins.

Our experimental design involves the analyses of six primary RCC, six metastatic, six non-malignant, and one non-malignant pool (comprised of six samples) of tissue homogenates. Thus far, a set comprising two primary RCC, one metastatic RCC, and the normal pool has been analyzed. We identified 11 overexpressed and 10 underexpressed proteins in primary RCC, and 57 overexpressed and 79 underexpressed proteins in the metastatic sample. On completion of MS analyses on all remaining sets, differential expressions of proteins of interest will be verified using independent conventional molecular biology techniques, including immunohistochemical and/or Western analyses.

POSTER ABSTRACTS
Personalized Medicine Session

Poster No.56

Crystal structure of RHCC interacting with the anti-cancer drug (Cis-platin) and its potential as novel chemotherapeutic delivery system in cancer

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Right handed coiled-coil (RHCC) is a 24 kDa tetrameric protein that originates from the archaebacterium Staphylothermus marinus. S. marinus is an extremophile capable of surviving wide ranges of temperature, salt, pressure and pH. The crystal structure of RHCC reveals a new structural motif with four large cavities inside the tetrameric channel. The cavities vary in size (320 Å 360 Å 3) and can be loaded with metallic compounds. Based on our new Cis-platin RHCC crystal structure, we hypothesize that the binding properties of the cavities make RHCC a potential storage and delivery system for one of the most efficient anti-cancer drugs. Here we present the crystal structure of the chemotherapeutic drug Cis-platin bound to RHCC at 3.2 Å resolutions. RHCC was crystallized in space group P3121 with unit cell dimensions of a, b=112.8 Å, c=71.6 Å and √é¬±, √é¬≤=90√Ç¬∞, √é¬≥=120√Ç¬∞. Employing fluorescence microscopy we show that Alexafluor labelled RHCC molecules are internalized by the human hypopharyngeal squamous carcinoma cell line FaDu, human glioblastoma cell line T98G, and primary glioblastoma cells from patients. RHCC may provide a novel mode for the delivery of chemotherapeutic drugs into tumour cells and represent a unique and novel approach in the treatment of cancer patients.

POSTER ABSTRACTS
StemCells Session

Poster No.16

Differential Phosphoproteome Analyses of Leukemia Stem Cells Reveals Novel Post-Translational Regulation of Polycomb Proteins

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We created two acute leukemias by overexpressing Hoxa9+Meis1 in isolated fetal liver stem cells which display leukemic stem cell (LSC) frequencies from ~1/350 (FLB1) to 1/1.4 (FLA2), making them an unique tool to identify pathways regulating cell fate in primary leukemia. As the differentiation, cell death and cell cycle profile and chromosomal, morphological, phenotypical and transcriptomical analyses of the two leukemias revealed no differences, we performed a mass spectrometry-based systems biology approach and quantitatively analyzed proteome and phosphoproteome of nuclear and cytoplasmic extracts. We identified more than 2,300 different proteins including 400 showing differential abundance. The phosphoproteome analyses of both cell populations enabled the identification of more than 11,000 unique phosphopeptides of which 1,000 were differentially regulated. Bioinformatic analyses revealed a strong differential regulation of chromatin and histone modifiers, including members of the polycomb complexes. Further investigation showed that the polycomb protein EZH2 is translocated from the nucleus to the cytoplasm in stem cells with lower self-renewal capability. This translocation seems to be post-translationally modulated by phosphorylation, proteolytic cleavage and sumoylation, some of which appears to be regulated by differential levels of reactive oxygen species (ROS) and p38 MAP kinase activation within the leukemias. Data obtained from this study suggests that post-transcriptional and post-translational modifications, rather than gene expression, regulate cell fate determination in these leukemic stem cells.

Poster No.17

Proteomics studies of the microenvironment of human embryonic stem cells

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Studies characterizing the soluble portion of the human embryonic stem cell (hESC) microenvironment with proteomic methodologies are important to understanding the hESC behavior in vitro. We already reported our study on the dynamic hESC 'niche' wherein paracrine/autocrine signaling between hESCs and human fibroblasts cooperated to maintain the stem cells in an undifferentiated state [1,2]. Based on the findings of Postovit et al. that the hESCs were found to generate a conditioned matrix (CMTX) that caused malignant melanoma cells to exhibit a less metastatic phenotype, we have begun characterization of the 'non-soluble' portion of the hESC microenvironment [3]. Towards this end, we developed a protocol for analysis of a sample as complex as the extracellular matrix (ECM) and demonstrated its utility on Matrigel, a widely used matrix for hESC growth. By employing ammonium sulfate precipitation coupled with iterative exclusion mass spectrometry we identified >1800 proteins present in Matrigel, including many growth and transcription factors that will be discussed here [4].
We have also begun characterization of the hESC pericellular matrix in order to uncover the currently poorly characterized interactions between hESCs and the ECM. This has led to the development and optimization of numerous growth, extraction, and fractionation protocols for the isolation of hESC specific deposited proteins that will be discussed. In this presentation we will describe the current understanding of the HESC microenvironment, as well as the interaction of hESCs with the ECM utilizing a wide range of proteomics methodologies.

[1] Bendall, S. C., Stewart, M. H., Menendez, P., George, D., et al., IGF and FGF cooperatively establish the regulatory stem cell niche of pluripotent human cells in vitro. Nature 2007, 448, 1015-1021.
[2] Bendall, S. C., Hughes, C., Campbell, J. L., Stewart, M. H., et al., An enhanced mass spectrometry approach reveals human embryonic stem cell growth factors in culture. Mol Cell Proteomics 2009, 8, 421-432.
[3] Postovit, L. M., Seftor, E. A., Seftor, R. E., Hendrix, M. J., A three-dimensional model to study the epigenetic effects induced by the microenvironment of human embryonic stem cells. Stem Cells 2006, 24, 501-505.
[4] Hughes, C. S., Postovit, L. M., Lajoie, G. A., Matrigel: A Complex Protein Mixture Required for Optimal Growth of Cell Culture. Proteomics.

POSTER ABSTRACTS
Blood & Blood Cells Session

Poster No. 1

USE OF PROTEOMIC APPROACH TO DETECT BLOOD-BORNE PROTOZOAN INFECTIONS: CASE OF CHAGAS DISEASE

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Millions of people have emigrated to Canada and the United States from regions of the world where protozoan parasitic diseases are endemic. Several of the protozoan parasites can establish latent or persistent infections in their hosts such as Chagas Disease (CD), malaria, and leishmania. The diagnostic tests for these infections have changed little since the discovery of the microscope. Screening tests for blood-borne parasites are not routinely used by transfusion services in any developed country. Current tests cannot be used to follow response to therapy or to identify subjects with progressive disease.
Surface enhanced laser desorption/ionization (SELDI) time of flight-mass spectrometry (ToF) mass spectrometry (MS) was evaluated in an effort to discover biomarkers for diagnostic tests against latent or persistent protozoan infections. Sera from subjects with asymptomatic CD (n=131) were compared to uninfected controls (n=164) and subjects with other parasitic diseases (n=140) using SELDI-TOF MS. Multiple candidate biomarkers were found in CD sera (range of 3 - 75.4 kDa). Many of these biomarkers have been identified as intact or truncated host proteins by MS/MS. Diagnostic algorithms using 2 or more biomarkers achieved up to 100% sensitivity and 98% specificity for CD. These included MIP1 alpha, intact C3a anaphylatoxin and unusually-truncated forms of fibronectin, apolipoprotein A1 and C3. Anti-peptide antiserum against the C-or N-terminus of the fibronectin fragment and apolipoprotein A1 were used in a western blot format to validate the biomarkers. The evaluation of these biomarkers to support feasibility of proteomic approach for detecting seropositive donors from North-America blood bank showed up to 95% sensitivity. These data demonstrate the potential of SELDI-TOF technology for the discovery of unique biomarkers in blood-borne protozoan diseases. Such biomarkers may lead to the development of a new generation of diagnostic tests that will help to ensure the safety of blood supplies throughout the world, to monitor therapies and patients with progressive disease.

Poster No. 2

Neutrophil oxidative burst in response to the bacterial peptide fMLP is regulated by specific forms of PI3K, PLC, PLD, DAGK and p67phox

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Neutrophils are part of the innate defense mechanism against infectious disease. The fMLP receptor triggers the release of free radical oxygen from the phagocytic oxidase (phox) complex in response to the bacterial peptide. Analysis by LC-ESI-MS/MS revealed the presence of specific isoforms of PI3K, PLC, PLD, DAGK and p67phox in primary human neutrophils. Enzymatic inhibition of PI3K, PLD, and PLC attenuated the fMPL-stimulated oxidative burst. In contrast, inhibition of DAGK increased the oxidative burst. Hence it appears that the levels of DAG as regulated by the enzyme DAGK help regulate the oxidative burst. The addition of the PAP-1 and iPLA2 inhibitor halo enol lactone suicide substrate (HELSS) strongly inhibited the oxidative burst but this effect could not be consistently confirmed by the addition of the PAP-1 inhibitor propranolol. Moreveor, the PLA2 inhibitors OBAA, AACOCF3 and MAFP had little effect on oxidative burst ruling out a role for PLA2. The use of a radioactive version of the molecular cross-linker HELSS showed that the alkylating reagent covalently modified many proteins throughout the primary human cells. The inhibitory effect of HELSS was observed to be associated with the apparent covalent modification of p67phox resulting in large shift in its relative migration in Western blots. Hence the inhibitory effect of HELSS on the oxidative burst may be accounted for, at least in part, from its direct effect on the components of the phagocytic oxidase complex. Specific isoform information for the PI3K, PLC, PLD, p67phox and DAGK enzymes that might regulate the oxidative burst were obtained from in primary human neutrophils.

POSTER ABSTRACTS
Blood & Blood Cells Session

Poster No. 3

The inhibition of phosphatidic acid phosphohydrolase (PAP-1) prevents Fc mediated phagocytosis in Raw macrophages

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Contributing Authors:

Andy Jankowski, Veronica Barbisan and John G. Marshall

Macrophages are part of the innate defense mechanism against infectious disease. The Fc receptor engulfs IgG coated micro particles as a model of bacteria. LC-ESI-MS/MS revealed the presence of specific isoforms of PI3K, PLC, PLD and potential candidates for the unknown enzyme activity magnesium dependant phosphatidic acid phosphohydrolase (PAP-1) in human nuetrophils. Enzymatic inhibition of PI3K, PLD and PLC inhibited Fc mediated particle engulfment. Inhibition of the enzyme activity PAP-1 with the haloenol lactone suicide substrate (HELSS) or propranolol prevented particle engulfment. Addition of DAG, the product of the enzyme PAP-1, in the form of DiC8 partially recovered the inhibition by HELSS. Transfection of specific protein binding domains for the signal lipids DAG, PI(4,5)P2, and PI(3,4,5)P3 fused to GFP confirmed there was no side effect of HELSS or propranolol on these pathways. HELSS has a known side effect on iPLA2 enzymes but the PLA2 inhibitors OBAA, AACOCF3 and MAFP had little effect on particle uptake or PI(3,4,5)P3 accumulation at the activated receptor In addition to its effects on the enzyme PAP-1, propranolol is also a beta adrenergic receptor antagonist. However, a panel of beta adrenergic antagonists had little effect on particle engulfment. Hence it appears that the levels of phosphatidic acid as regulated in part by the enzyme PAP-1 play a role in Fc mediated phagocytosis. Recently it has been suggested that the proteins of the lipin type comprise the enzymatic activity PAP-1. Multiple isoforms of lipin were observed in Raw macrophages by LC-MS/MS and antibody staining for Lipin-1 shows the protein is associated with the activated Fc receptor complex.

Poster No. 4

Specific isoform(s) of sPLA2 implicated in the release of arachidonic acid by human neutrophils in response to the bacterial peptide fMLP

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Contributing Authors:

PeiHong Zhu and John G. Marshall

Neutrophils are the most abundant leukocytes and an important source of the pro-inflammatory mediator arachidonic acid. The phospholipase A2 (PLA2) enzyme that directly mediates AA releases from human neutrophils in response to fMLP is not clear. LC-ESI-MS/MS of human neutrophils revealed the presence of iPLA2, cPLA2 and sPLA2 enzymes. Stimulating human neutrophils with fMLP produces a release of arachidonic acid as measured by radiometric or mass spectrometric assays. An increase in the release of arachidonic acid in response to fMLP occurs after priming with [2D8] arachidonate. The arachidonic acid analog AACOCF3 is an inhibitor of the enzyme cPLA2 and measured cPLA2 enzymatic activity was significantly inhibited by AACOCF3. Neutrophils showed a strong and rapid release of authentic arachidonic acid in the presence of the arachidonic acid analog and cPLA2 inhibitor AACOCF3. Arachidonic acid release was not effected by the iPLA2 inhibitor HELSS. In contrast, neutrophils permeabilized by streptolysin O toxin showed that arachidonic acid release in response to fMLP was dependant on millimolar levels of calcium and was sensitive to dithiothreotol consistent with a requirment for sPLA2. Hence this study apparently rules out iPLA2 and cPLA2, but implicates specific sPLA2 protein(s), as the enzymes that directly mediate the release of AA from human neutrophils.

POSTER ABSTRACTS
Blood & Blood Cells Session

Poster No. 5

A Complete Evaluation of 3 Peptide Quantitation Strategies in Complex Biological Matrices using LC-MS/MS

Rezai, Taha
Thermo Fisher Scientific

When measuring peptides with triple quadrupole based LC-MS/MS SRM assays in complex biological matrices, there are several issues that need to be dealt with. First, each sample has a unique set of interferences and ionization (enhancement and suppression) effects. These problems are well known in the small molecule quantitation world. With peptide quantitation, however, there is an additional layer of complexity. Unlike small molecules where a BSA solution is typically used as a plasma substitute, there is no equivalent matrix substitute for plasma digest. As a result, peptide calibration curves with a real plasma digest background have endogenous peptides present in them. Methods and post acquisition data analysis have to take the endogenous peptide that is present in background matrix into account.

Poster No. 6

A Heavy Isotope Labeled Protein Combined with AQUA Peptides Provides More Accurate Quantitation when used with an Enzymatic Digest prior to LC-SRM

Rezai, Taha
Thermo Fisher Scientific

Triple quadrupole based peptide quantitation typically requires an enzymatic digest. However, often the digest efficiency for the protein of interest is not 100%. In order to obtain accurate protein quantitation with peptide SRM experiments on a triple quadrupole, the digest efficiency for the protein target must be determined.

Poster No. 7

HDL biology in Chagas Disease: new insights through SEDLI TOF MS

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Research Institute of McGill University Health Ctr

Contributing Authors:

M Ndao, D Nyholt, D Miao, J Genest

Chagas disease (CD) is caused by the protozoan parasite, Trypanosoma cruzi. Endemic in Central and South America, latent infections can persist for decades, causing terminal, cardiomyopathy in ~30% of subjects. However, even those who die from cardiac complications have 'clean' coronary arteries. Levels of HDL and Apo-A1 in CD patients are reported to be normal. Using SELDI TOF MS to screen serum, we have identified intact Apo-A1 as a negative biomarker for CD and several truncated forms of Apo-A1 as positive biomarkers for CD. Apo-A1 is the principle protein found in high density lipoprotein (HDL). We have demonstrated that HDL is collected onto the surface of the blood-stage form (when only glycolytic pathways are active) and that the principal cysteine protease of T. cruzi cleaves Apo-A1 in HDL as predicted by the SELDI data. The HDL is internalized and carried into the target host cell by the parasite (when the parasite switches from glycolytic to lipolytic metaboloism). Mice infected by T. cruzi show similar patterns of Apo-A1 cleavage in vivo (MS confirmation of fragmentation pattern underway). Preliminary data suggest that HDL from CD infected mice is 20% better at cholesterol transport than native HDL. These unanticipated observations give unique insights into the biology of the host-parasite interaction.

POSTER ABSTRACTS
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Poster No. 8

DETECTION AND IDENTIFICATION OF BIOMARKERS FOR DENGUE FEVER AND DENGUE HEMORRHAGIC FEVER USING PLASMA SAMPLES FROM TWO DIFFERENT ENDEMIC AREAS

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Contributing Authors:

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Surface-Enhanced, Laser-Desorption & Ionization, Time-Of-Flight Mass Spectrometry (SELDI-TOF MS) permits the study of the protein/peptide content of diverse biological fluids such as serum, plasma, urine, cell lysates and tissue extracts. This high throughput proteomic platform has been used to identify biomarkers for a wide range of inflammatory, infectious and neoplastic conditions. The promising results obtained in these varied conditions raised the possibility that SELDI could be applied to dengue virus (DV) infection to develop urgently needed diagnostic test capacities.

Plasma from Thai patients with either primary dengue fever (DF) (12 patients) or dengue hemorrhagic fever (DHF) (9 patients), patients with either secondary DF (24) or DHF (27), and 15 samples from Thai patients admitted to hospital with other febrile illnesses (OFI) were analyzed and their proteomic profiles obtained using SELDI-TOF MS technology. Validation was done using 30 confirmed primary DF, 54 secondary DHF and 30 OFI cases from Puerto Rico. Potential biomarkers that discriminated between patients with DV infection and those with OFI were determined (diagnostic biomarkers), as well as biomarkers that discriminate for DHF (prognostic biomarkers) with a p-value 0.01. Using Biomarker Pattern Software (BPS), we were able to select for key biomarkers common for both geographical areas. Guided by these results, candidate biomarkers were identified using SDS-PAGE gels and tandem MS. Moreover, more than 100 candidate biomarkers were found to differentiate between 1 and 2 DENV infection, which suggests, contrary to today's literature, that the plasma proteome of patients with a 1 infection is markedly different than that of a patient with a 2 infection.

This study demonstrates the potential for high-throughput proteomics to develop useful diagnostic and prognostic tests for DF/DHF. Moreover, these biomarkers may give unique insight into the mechanisms underlying the different manifestations of DENV infection.

Poster No. 9

Blood Cell Interactions: Proteomic Analysis of the Interaction Environment of Platelets and Monocytes

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Contributing Authors:

James Xu, Arash Khosrovi-Eghbal, Cordula Klockenbusch and Juergen Kast

Dynamic interactions and signalling between different cell types circulating within the blood stream underlie physiological and pathophysiological events and are of great clinical relevance. For example, circulating platelet-monocyte aggregates are now an established early marker of acute cardiovascular events. We have developed a model system using platelets, isolated from human blood, and a monocyte cell line (THP-1) to explore the global proteome of these cells and several sub-proteomes involved in platelet-monocyte interactions. Initially, reference datasets for the platelet, platelet releasate (PR) and THP-1 cell proteomes were generated. For global proteome analysis, lysed platelets or cells were separated using the GeLC approach followed by high resolution LC-MS/MS, leading to extensive proteome depth, with, on average, 1,420 platelet proteins and 1,492 THP-1 cell proteins identified. PR generated by thrombin-activated platelets was analyzed using the same method, resulting in identification of more than 657 high-confidence proteins. THP-1 cells, cultured in either light (controls) or heavy (treated) SILAC-labelled media, were treated with a vehicle control or platelet releasate for either 4 or 24 hours. After treatment, cells were pelleted and the supernatant retained. Analysis of global protein changes in THP-1 cells after 4 hours of treatment resulted in 1,729 protein identifications, of which 999 were quantified using SILAC, revealing, among others, an increase in CD43 (sialophorin), a major surface protein on THP-1 cells. Analysis of the THP-1 releasate identified 243 heavy-labelled proteins, which are currently being analyzed in more detail. These results aid in the elucidation of changes induced in monocytes upon platelet activation.

POSTER ABSTRACTS
Blood & Blood Cells Session

Poster No. 10

Existence and variation of nucleic acid binding proteins in human blood

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Ryerson University

Contributing Authors:

Christina L. Addison, Peter Bowden, Peihong Zhu, Kenneth R. Evans and John G. Marshall

Transcription factors are intracellular proteins that regulate gene expression resulting in cell growth, proliferation, differentiation, response to physiological signals or cell death. The presence of transcription factors, and other apparently nuclear proteins such as histones and their modification enzymes, was detected in human blood using LC-MS/MS. The presence of transcription factors was confirmed in control and cancer patients using Western blot analysis. Transcription factors were observed in normal human serum samples as well as in serum from patients with prostate, ovarian and breast cancer. In this study, six members of the Ets family (c-Ets 1, c-Ets 2, c-ER81, c-Elk1 and c-ERG1/2/3), two members of the basic region-leucine zipper (bZIP) family (c-Fos and c-Jun), two members of the helix-turn-helix family (c-Myb and c-Myc), the cardiac-enriched transcription factor p53 and the serum response factor (SRF) were compared in normal versus cancer serum. Since the ECL Western blot is only sensitive to the ~10 femto mol range and since only ~1 micro liter of serum was loaded on the blots, these results indicate that large amounts of nucleic acid binding proteins, apparently nano molar amounts or greater, circulate in the blood. The use of plasma from plasmaphoresis of normal human blood confirmed that these transcription factors are not merely released from blood cells during sample collection and processing. While the highly basic histones were well detected by mass spectrometry they were poorly detected from blood by immunological methods. We conclude that transcription factors and other nuclear proteins exist in normal human serum, can be readily detected by mass spectrometry, show variation between physiological states, and are not artifacts of sample collection.

POSTER ABSTRACTS
Protein Interaction Session

Poster No.18

The actin-myosin cytoskeleton of the Fc receptor complex isolated from the surface of live human neutrophils

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Contributing Authors:

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Actin interactions are fundamental for the functions of the cytoskeleton in cell morphology, mobility, signal transduction and phagocytosis. The Fc receptor complex directs the formation of an actin-myosin supramolecular complex within the pseudopods that exert force to engulf foreign particles. The Fc receptor complex and its associated actin-myosin machinery was captured from the surface of live cells by IgG coated microbeads. The cells were homogenized in a French Press and the IgG-Fc receptor complex was isolated by centrifugation through sucrose, elution with salts or organic solvents and digested with trypsin before liquid chromatography with electrospray ionization and tandem mass spectrometry. Microbeads isolated from crude lysates served as a control. Actin and associated proteins such as actinin and dynactin were observed to be specific to the Fc receptor complex from live cells. The Fc receptor complex included Wasp, Arp2/3, myosins, myosin light chain kinase, cofilin, thymosin ≤4, vimentin, nebulin, Band 4.1, FERM, ezrin, moesin and radixin, contactin, cortactin, desmoyokin, ataxin, fascin, Brg1, SWI/SNF, paxillin, filamin, villin, advillin, supervillin and others that attained significant scores with multiple independent peptides and therefore near certain identification. The presence of tubulin, dynein, kinesin and dynactin proteins was also specifically detected. The detected cytoskeleton proteins, binding proteins and enzymes we used to predict the network of actin associated regulatory factors that included PI3K, PLC, GTPases such Rac and CDC42, and Rho GAPs and GEFs and some of these predicted protein types were also found to be specific to the isolated receptor complex. Hence, it is apparently possible to capture an activated receptor-actin supramolecular complex from the surface of a live human primary cell.

Poster No.19

Temporal proteomics of poly(ADP-ribose) binding proteins and poly(ADP-ribose)-associated protein complexes

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CRCHUL

Contributing Authors:

Isabelle M, Gagne J.P, Gagne P et Poirier GG.

Poly(ADP-ribose) (pADPr) is a polymer assembled from the enzymatic polymerization of the ADP-ribosyl moiety of NAD by poly(ADP-ribose) polymerases (PARPs). The dynamic turnover of pADPr within the cell is essential for a number of cellular processes including progression through the cell cycle, DNA repair and the maintenance of genomic integrity, and apoptosis. The present study is aimed at assessing the dynamic interplay of pADPr binding proteins to better comprehend the biological influence of pADPr on cellular pathways.

The combination of affinity purification and tandem mass spectrometry is a powerful method for revealing many potential interacting proteins associated directly or indirectly with proteins of interest. Therefore, it was possible to immunoprecipitate complexes associated with pADPr. ìStable isotope labeling with amino acid in cell culture ì (SILAC) method was used to monitor the dynamic behavior of proteins complexes associated with pADPr over a time course of genotoxic stimuli.

Visualization and modeling of these pADPr-associated proteins in networks not only reflect the widespread involvement of poly(ADP-ribosyl)ation in several pathways but also identify protein targets that could shed new light on the regulatory functions of pADPr in normal physiological conditions as well as after exposure to genotoxic stimuli.

POSTER ABSTRACTS
Protein Interaction Session

Poster No.20

Quantification of Poly(ADP-ribose)-Binding Proteins using Multiple Reaction Monitoring (MRM)

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Contributing Authors:

Jean-Philippe Gagne, Sylvie Bourassa, Maxim Isabelle, Guy G. Poirier

Poly(ADP-ribose) (pADPr) is a polymer assembled from the enzymatic polymerization of the ADP-ribosyl moiety of nicotinamide adenine dinucleotide (NAD) by poly(ADP-ribose) polymerases (PARPs). The dynamic turnover of pADPr is involved in many cellular processes such as DNA repair and apoptosis. During these cellular processes, many proteins bind either in a covalent or noncovalent manner to pADPr. The identification of these proteins and their quantification at different time points after exposure to genotoxic stimuli could shed new light on the regulatory functions of pADPr during DNA repair and thus its implication in the maintenance of genome integrity. Human SK-N-SH cell line was incubated 5 min with the DNA alkylating agent N-Methyl-N'-Nitro-N-Nitrosoguanidine (MNNG) to induce DNA-damage and DNA-dependent PARP activation that result in large elevation of cellular pADPr levels. Specific anti-pADPr antibodies were used to immunoprecipitate pADPr-associated proteins and pADPr-containing protein complexes. More than 300 pADPr-associated proteins were identified by nanoLC-MS/MS on this sample. Proteins of the DNA damage response and repair pathways were collectively over-represented. Among these proteins, 14 were associated to the NHEJ complex (PARP1, DNA-PK, XRCC4, Ku70, Ku80, PNKP, Aprataxin, NBS1, MRE11, RAD50, RAD51, Artemis, NONO and SFPQ). A MRM method was develop to relatively quantify the 14 NHEJ proteins at three time points (Ctrl., MNNG 5 min, MNNG 1hr). An increase of the concentration of the NHEJ proteins was observed after the 5 min MNNG treatment compared to the untreated control.

Poster No.21

Investigating the Interaction Environment of Integrin Beta 1 by Formaldehyde Supported Immunoprecipitation and In Silico Protein Interaction Analysis

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Contributing Authors:

Chengcheng Zhang, Juergen Kast

Protein-protein interactions are the foundation for almost every event taking place in a cell. Analysing these networks is key to understanding physiological and pathophysiological processes. Here we applied two complementary approaches, one biochemical and one bioinformatic, to gain insights into the interaction environment of integrin beta 1. This transmembrane protein forms heterodimers with varying integrin alpha subunits and by interacting with adaptor proteins through its intracellular tail, it triggers signalling cascades. Several of these adaptor proteins are described; however, the knowledge on the spatial and temporal regulation of this interaction network and all its components is still incomplete.
For the biochemical approach, an immunoprecipitation protocol applying formaldehyde cross-linking to preserve weak and transient interactions was used. Human platelets expressing integrin beta 1 were treated with 1% formaldehyde for 10 min and lysed. mmunoprecipitation was performed using the anti-integrin beta 1 antibody JB1B and precipitated Icomplexes were analysed by LC-MS/MS. Known interaction partners such as talin-1 and kindlin-3 and additional putative binding proteins were identified. In parallel an in silico method developed in our lab was used to obtain information about the protein network containing integrin beta 1. This method, termed ìVirtual IP, takes advantage of the large amount of MS/MS data stored in the Global Proteome Machine Database to predict the interaction environment of proteins. We are currently comparing the results of the two complementary approaches and data published in the literature to gain further insights into the interaction network of integrin beta 1.

POSTER ABSTRACTS
Protein Interaction Session

Poster No.22

Nuclear import and biogenesis of RNA polymerase II requires the conserved GPN-loop GTPase RPAP4/GPN1 and microtubule assembly

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IRCM

Contributing Authors:

Andree-Anne Lacombe, Diane Forget, Philippe Cloutier, Annie Bouchard, Benoit Coulombe

Over the past 4 decades, significant efforts have been made to identify and characterize the factors that regulate the activity of RNA polymerase II (RNAPII), the eukaryotic enzyme that synthesizes all the mRNA and several non-coding RNA. Quite surprisingly, very little is known about the molecular machinery that regulates the fate of RNAPII before or after transcription.
In this study, we show that RNAPII-Associated Protein 4 (RPAP4/GPN1) shuttles between the nucleus and the cytoplasm and regulates nuclear import of RPB1, the largest subunit of RNAPII. RPAP4/GPN1 is a member of a newly discovered GTPase family that contains a unique and highly conserved GPN-loop motif that we show is essential, in conjunction with its GTP-binding motifs, for nuclear localization of RPB1 in a process that also requires microtubule assembly.

Poster No.23

High-resolution mapping of the protein interaction network and affinity purification of novel RNA polymerase II-associated chaperone

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Contributing Authors:

Al-Khoury, R. Lavallee-Adam, M. Faubert, D. Poitras, C. Bouchard, A. Forget, D. Coulombe, B.

Numerous reports have described how RNA polymerase II (RNAPII) enacts transcription of protein-coding genes or how this enzyme is regulated by a variety of cues, but paradoxically, little is known about the assembly of this multi-subunit machine or how it travels from the site of its synthesis (the cytoplasm) to the site of its activity (the nucleus). Tandem affinity purification (TAP) of human RNAPII subunits and associated factors have led to the identification of a novel chaperone complex that combines prefoldins and prefoldin-like proteins, the human ortholog of the yeast R2TP complex and some other factors, including RPB5, a common subunit of all three nuclear RNAPs. In this case, the presence of RPB5 does not appear to be a target by which this chaperone interacts with RNAPII, but rather a bona fide component of this complex. Expression of GFP-tagged subunits and immunofluorescence microscopy localized the complex primarily to the cytoplasm. Surprisingly, knockdown of one component of this complex, RPAP3, led to mislocalization of RNAPII to the cytoplasm. Experiments are ongoing to assess the involvement of this chaperone in RNAPII assembly or transport.

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TECH TALKS Abstracts

Sunday, May 9 - 12:30

British Columbia Proteomics Network - Structure, Activities and Availability of Resources

Juergen Kast
British Columbia Proteomics Network
Christoph Borchers
UVic Genome BC Proteomics Centre

Launched in 2006 and funded by the Michael Smith Foudation for Health Research in 2007, the BC Proteomics Network (BCPN) is a growing network of scientists engaged in proteomics research from institutions throughout BC. The BCPN functions both as an educational and training resource for proteomics and as a research platform providing shared instrumentation and resources that enable large-scale collaborative research. Opportunities for interaction with researchers outside of BC will be discussed. The UVic Genome BC Proteomics Centre is one of the core facilities available within the network. The Proteomics Centre offers service, technology development and research in proteomics. One of the focuses of the Centre is the development and application of novel quantitative and structural proteomics techniques with implications for diagnostics and drug development.

Sunday, May 9 - 12:45

Thermo Fisher Scientific

TECH TALKS Abstracts

Monday, May 10- 12:30

A New Era in Ultra-High NanoLC Performance

Gurmil Gendeh
Dionex

Monday, May 10- 12:45

David Craft
BD

Monday, May 10- 12:30

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