FIRST SYMPOSIUM OF THE CANADIAN NATIONAL PROTEOMICS NETWORK

Advancing Canadian Proteome Research into Human Health

The Westin Harbour Castle
Toronto, Ontario
September 25, 2009

First Symposium of the Canadian National Proteomics Network

Advancing Canadian Proteome Research into Human Health

MEETING AGENDA
September 25, 2009

7:30 AM	Registration /Coffee		
8:00 AM	Pier 7/8 - Plenary Session: Michel Desjardins, University of Montreal		
8:45 AM	Break		
	Session 1 – Pier 4		Session 2 – Pier 5
	Neurobiology: John Kelly Chair		Blood & Blood Cells: Juergen Kast
9:00 AM	Dr. Arsalan Haqqani, NRC Institute for Biological Sciences	9:00 AM	BruceMc Manus, University of British Columbia – St. Paul's Hospital
9:35 AM	Dr. Alexandre Prat, CHUM Research Centre, Notre-Dame Hospital	9:45 AM	Leonard Foster, University of British Columbia
10:10 AM	Dr. Daniel Figeys, University of Ottawa	10:15 AM	John Wilkins, University of Manitoba
10:45 AM	Break – Pier 9	10:45 AM	Break – Pier 9
11:00 AM	Dr. Peter McPhersen, Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University	11:00 AM	Juergen Kast, University of British Columbia
11:35 AM	Milijana Vojvodic, Dept. of Molecular Genetics, University of Toronto	11:30 AM	Thomas Kislinger, University of Toronto
12:05 AM	Dr. Elise Stanley, Toronto Western Research Institute, Toronto Western Hospital	12:00 AM	John Marshall, Ryerson University
12:35 PM	Discussion and wrap-up	12:30 PM	Discussion and Launch of National Interest Group in Blood Proteomics
12:45 PM	Lunch – Harbour Ballroom		
	Session 1 – Pier 4 Stem Cells: Pierre Thibault		Session 2 – Pier 5 Personalized Medicine: Tommy Nilsson
1:30 PM	Julie Lessard, Université de Montréal	1:30 PM	Rick Rachubinski, University of Alberta
2:00 PM	Lynne-MariePostovit, Schulich, University of Western Ontario	2:00 PM	Jeff Wrana, Samuel Lunenfeld Research Institute
2:30 PM	Julie Audet, University of Toronto	2:30 PM	Jim Woodgett, Samuel Lunenfeld Research Institute
3:00 PM	Pierre Thibault, Université de Montréal	3:00 PM	Claus Jorgensen, Samuel Lunenfeld Research Institute
3:30 PM	Break Pier 9		
3:45 PM	John Dick, University of Toronto	3:45 PM	Tommy Nillsson, RI MUHC
4:15 PM		4:15 PM	Christoph Borchers, University of Victoria
4:45 PM		4:45 PM	Tom Hudson, OICR
5:15 PM	Break		
5:30 PM	Pier 7/8 - Plenary Session: John Bergeron, RI-MUHC		
6:15 PM	Reception		
7:00 PM	CNPN – Annual General Meeting		

WELCOME

On behalf of the CNPN Symposium Organizing Committee, we wish to welcome everyone to the First Symposium of the Canadian National Proteomics Network. This inaugural symposium is entitled ***Advancing Canadian Proteome Research into Human Health***. We have an excellent line up of speakers and are fortunate that to have them with us today.

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.

Professor K.W. Michael Siu
Chair, Board of Directors, Canadian National Proteomics Network

ORGANIZING COMMITTEE:

Juergen Kast, University of British Columbia
John Kelly, National Research Council
John Marshall, Ryerson University
Tommy Nilsson, McGill University
Pierre Thibault, Université de Montréal

SPONSORS:

We wish to thank our Sponsors for their Support:

SPEAKERS:

Keynote Speakers:

John Bergeron, McGill University

Michel Desjardins, University of Montreal

Session 1

Neurobiology (Chair John Kelly) am

Dr. Arsalan Haqqani, NRC Institute for Biological Sciences	
Talk	**Discovering Novel Molecular Targets for Neurological Diseases by Label-free Proteomics** Neurological diseases, including stroke, Alzheimer's disease, multiple sclerosis and brain tumors, have a significant impact on Canadian economy, costing ten-billion dollars a year. Currently, there is no accurate diagnosis for early detection and onset of these diseases. In stroke, diagnosis is mostly done using 'classical' imaging techniques (e.g., CT scans), which are limited to examining anatomical changes. With recent advancements in molecular imaging technology, there is a push towards development of more 'molecular' diagnostics of these diseases. However, there is a need to identify novel diagnostic targets. Compounding this problem is the fact that unlike many other diseases of similar impact, including myocardial infarction and cancer, there is no safe and effective treatment for neurological diseases. Thus, there is a need to identify novel therapeutic targets. Changes in the brain vasculature (including the blood brain barrier) and inflammation of the central-nervous system (CNS) are some of the early signs in most neurological diseases. The inflammation process involves 'activation' of brain vasculature, including changes in specific molecules on the luminal surface of the vessels. This leads to recruitment and entry of inflammatory cells into the brain leading to neuronal damage. Inhibition of leukocyte recruitment and entry into the brain via the vasculature are emerging as attractive strategies for the treatment of neurological disorders. Recent advancements in nanoLC-MS technologies and bioinformatics have made label-free proteomic methods more quantitatively reproducible and high throughput. Coupling of these advanced proteomics technologies with enrichment methods such as subcellular fractionations and isolation of specific protein subsets (e.g., glycoproteins) have given rise to opportunities in identifying novel targets. Examples will be provided on how we have utilized these technologies to identify adhesion molecules, cytokines and chemokines as potential inhibitory targets for inhibition of inflammation in neurological disorders.

Dr. Alexandre Prat, CHUM Research Centre, Notre-Dame Hospital	
Bio	Dr Prat obtained his B.Sc. degree in biochemistry from Université de Montréal in 1990, an M.Sc. degree in physiology in 1994 and an MD degree from the same university in 1995. Dr Prat completed his Neurology residency (clinical) training at McGill University in 2003 after having completed a Ph.D. degree in the laboratory of Dr Jack P. Antel in 2000. He was interested in developing an in vitro model of the human BBB, to study the immunology of the human BBB in MS. Dr

	Prat is a member of the Royal College of Physician and Surgeons of Canada (Neurology).
Talk	**Novel Therapeutic Targets in Multiple Sclerosis Revealed by Proteomics**

Dr. Daniel Figeys, University of Ottawa	
Bio	Daniel Figeys is a professor in the Department of Biochemistry, Microbiology and Immunology, the Director of the Ottawa Institute of Systems Biology, and a Tier-1 Canada Research Chair in proteomics and systems biology. Daniel obtained a B.Sc. and a M.Sc. in chemistry from the Université de Montréal. He obtained a Ph.D. in chemistry from the University of Alberta and did his postdoctoral studies at the University of Washington. Prior to his current position, Daniel was Senior VP of Systems Biology with MDS-Proteomics. From 1998 to 2000, he was a Research Officer at the NRC-Canada. Daniel's research involves developing proteomics technology and their applications in systems biology.
Talk	**Proteomics and lipidomics of the brain.** One of our research interests is the study of the molecular mechanisms linked with neurodegeneration in Alzheimer's disease (AD), Parkinson disease (PD), and stroke. In particular, we are studying the proteomic and lipidomic changes that occur during neurodegeneration and studying the roles and functions of these biomolecules by mapping their interaction networks. At the tissue level, we are interested in pinpointing the molecular changes that occur in specific regions of the brain. For the proteome, we have focused our efforts on the development of the microfluidic proteomic reactor that greatly simplifies the processing of complex proteomic samples by combining multiple proteomic steps. We will present the development of a fully automated proteomic reactor for analyzing of specific regions of mouse brain. At the lipidomic level, we have developed a series of technologies that allows the quantitative study of specific subsets of the lipidomes. We will present results from our study of the lipidomics changes that occur in the hippocampus and cortex regions of the brain during AD. Finally, at the mechanistic level we are interested in understanding the roles that these proteins and lipids play in the cell. To do this, we have developed analytical approaches for the mapping of protein-protein interactions and protein-lipid interactions. Here we will present results from the study of protein-protein and protein-lipid interactions of proteins implicated in AD and PD.

Milijana Vojvodic (Dept. of Molecular Genetics, University of Toronto)	
Bio	Milijana is a graduate student in the Department of Molecular Genetics, University of Toronto, and a member of the Comprehensive Cancer Centre, Hospital For Sick Children. Milijana is co-supervised by Professors David Kaplan and Michael Moran.
Talk	**Using phosphoproteomics to identify novel druggable neuroblastoma stem cell survival pathways**

Dr. Peter S. McPhersen (Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University)	
Bio	Peter McPherson received his Ph.D. in Neuroscience from the University of Iowa in 1993. He subsequently moved to Yale University for post-doctoral training in the laboratory of Pietro De Camilli. He is currently a James McGill Professor at the Montreal Neurological Institute at McGill University.
Talk	**Proteomic analysis of clathrin-coated vesicles**

	Clathrin-coated vesicles (CCVs) form at the plasma membrane and the trans-Golgi network (TGN) to ferry cargo to the endosomal system. We have determined the protein complement of highly enriched rat brain CCVs using 1D-SDS-PAGE coupled to LC-MS-MS. By counting the total number of MS-MS spectra assigned to peptides within each protein we pioneered a method (peptide counting) that reveals the relative levels of proteins within complex mixtures (Blondeau et al., PNAS, 2004). Applying peptide counting to CCVs purified from rat liver revealed that the 1:1 stoichiometry of clathrin-heavy chain to clathrin-light chains, long held as universal was instead restricted to the nervous system. Subsequent studies revealed that in non-neuronal cells, clathrin-light chains are in fact not required for clathrin-mediated endocytosis but instead have a more restricted and non-stoichiometric function in clathrin trafficking at the TGN (Poupon et al., 2008). Eight of the proteins in the original brain CCV analysis were uncharacterized open-reading frames, seven of which have since been linked to clathrin-mediated trafficking. One such protein, which we have named clavesin (clathrin vesicle associated Sec14 protein) is neuron specific and localizes to the TGN and the endosomal system. Clavesin interacts specifically with PtdIns(3,5)P_2 and lentiviral-mediated clavesin knock down in hippocampal neurons causes lysosomal defects. Decreased levels of PtdIns(3,5)P_2 in the infantile gliosis (*ingls*) mouse cause lysosomal dysfunction and neurodegeneration. Thus, clavesin appears to be a neuron-specific PtdIns(3,5)P_2 effector required for neuronal survival. Our studies reveal the utility of proteomics to the analysis of membrane trafficking.

Prof. Elise F. Stanley (Toronto Western Research Institute, Toronto Western Hospital, University Health Network, Toronto)	
Bio	Dr. Stanley obtained her undergraduate and graduate education at the University of East Anglia, Norwich, England in 1976. She did her postdoctoral fellowship and Assistant Professorship in the Department of Neurology, Johns Hopkins Hospital (JHH), Baltimore, making summer excursions to Woods Hole, MA at the Marine Biology Laboratories. She moved to the Neurology Institute, NIH in Bethesda MD in 1984 as a Visiting Fellow and advanced to GS15 (equivalent to full professor) and Head of the Synaptic Mechanisms Section. At the end of 1999 Dr. Stanley moved to Toronto to participate in the reorganization of the Toronto Western Research Institute as the Head of the Cellular and Molecular Division and member of the Research Council in addition to setting up a laboratory in synaptic research. In 2005 she stepped down as Division Head and currently runs a multi-functional laboratory with 5-10 members. Prof. Stanley holds a Canada Research Chair in Molecular Brain Science and the Tanebaum Chair in Molecular Neuroscience.
Talk	**Proteomics and the Analysis of the Presynaptic Transmitter Release Site Organelle** N type calcium channels (CaV2.2) play a key role in the gating of transmitter release at presynaptic nerve terminals. These channels are generally regarded as parts of a multimolecular complex that can modulate their open probability and ensure their location near the vesicle docking and fusion sites. However, the proteins that comprise this component remain poorly characterized. We have carried out the first open screen of presynaptic CaV2.2 complex members by an antibody-mediated capture of the channel from purified rat brain synaptosome lysate followed by mass spectroscopy. 589 unique peptides resulted in a high confidence match of 104 total proteins and 40 synaptosome proteome proteins. This screen identified several known CaV2.2 interacting proteins including

	syntaxin 1, VAMP, protein phosphatase 2A, GOα, Gβ and spectrin and also a number of novel proteins, including clathrin, adaptin, dynamin, dynein, NSF and actin. The unexpected proteins were classified within a number of functional classes that include exocytosis, endocytosis, cytoplasmic matrix, modulators, chaperones, and cell-signaling molecules and this list was contrasted to previous reports that catalogue the synaptosome proteome. The failure to detect any postsynaptic density proteins suggests that the channel itself does not exhibit stable trans-synaptic attachments. Our results suggest that the channel is anchored to a cytoplasmic matrix related to the previously described particle web.

Stem cells (Chair Pierre Thibault) pm

Julie Lessard, Université de Montréal	
Bio	Julie Lessard is an Associate Professor with the Department of Pathology and Cellular Biology at the Université de Montréal and Principal Investigator IRIC. Julie obtained her PhD in Molecular Biology in 2003 at the Université de Montréal and her Fellow in Developmental Biology at Stanford University in 2007.
Talk	**BAF Chromatin Remodeling Complexes: Tackling The Epigenome In Pluripotent Stem Cells** Embryonic and adult stem cells are unique in their abilities to self-renew and to differentiate into many, if not all, cellular lineages. New high-throughput techniques, as well as increasingly accurate loss-of-function approaches, are allowing us to dissect the interplay among genetic, epigenetic and signaling mechanisms involved in the maintenance of self-renewal, pluripotency, and differentiation of stem cells with ever-increasing fidelity. For example, recent progress in proteomics techniques now enables us to take a global, unbiased and comprehensive view of the covalent modifications of histone tails that contribute to gene expression regulation and cell identity during diverse developmental stages. Another epigenetic mechanism underlying the modification of chromatin structure and the regulation of stem cell fate relies on the activity of the SWI/SNF-related ATP-dependent chromatin remodeling complexes. We recently demonstrated that this remodeling complex has dedicated functions at different stages of neural and hemopoietic development that appear to arise by combinatorial assembly of its subunits. This talk will summarize recent findings on the importance of this family of chromatin remodeling complexes in the epigenetic regulation of stem cell identity and function.

Lynne-Marie Postovit, Schulich, University of Western Ontario	
Bio	Lynne-Marie Postovit is an assistant professor in the Department of Anatomy & Cell Biology at the University of Western Ontario. Lynne obtained her PhD from Queen's University in the group of Dr. Charles Graham and received her postdoctoral training with Dr. Mary Hendrix at Northwestern University. Lynne has published over 26 peer-reviewed manuscripts and her work has produced 2 patents; one of which progressed into clinical trials for the treatment of prostate cancer and has led to the development of a start-up company (Nometics Inc.). Dr. Postovit has received doctoral and postdoctoral awards from the Canadian Institutes of Health Research (CIHR) and a scholar in training award from the

	American Association for Cancer Research. In 2009 she was awarded a CIHR New Investigator Award. Lynne studies the microenvironmental regulation of cell phenotype in cancer and stem cells. She is particularly interested in the role of oxygen as a regulator of cell fate and in factors that characterize the human embryonic stem cell niche.
Talk	**Harnessing the Stem Cell Proteome to Predict and Reprogram Aggressive Cancers** Department of Anatomy & Cell Biology, Schulich School of Medicine & Dentistry, University of Western Ontario, London ON, Canada, N6A 5C1 Communication between cells and their microenvironment facilitates cancer progression and embryological development. Cells rely on extracellular cues, such as oxygen (O_2) gradients and growth factors, to direct functions including proliferation, and differentiation. ***Normal stem cells sustain an environment that is conducive to a balance of self-renewal and differentiation. This balance is disrupted in cancer cells, favouring the maintenance of self-renewal in the absence of normal differentiation.*** Based on these commonalities and differences, we propose that 1.Cancer cells use stem cell associated factors to maintain a microenvironment that supports cellular plasticity and; 2.Cancer cells should be able to respond to normal embryonic microenvironments by reprogramming their genomes toward a more differentiated phenotype. We have determined that cancer cells use Nodal, an embryonic stem cell (ESC) associated protein, to support tumourigenesis, and that factors derived from human ESCs (hESCs) can be used to reprogram cancer cells toward less aggressive phenotypes. We are currently examining how biophysical parameters, such as O_2 availability, affect cell fate in cancer cells and in hESCs. We have found that low O_2 supports cellular plasticity and pluripotency in cancer and hESCs, respectively. Proteomic analyses are currently being used to determine a "signature" of O_2-regulated pluripotency so that this phenotype can be predicted in normal stem cells and in cancers. We are also using proteomics to uncover factors that hESCs deposit into the extracellular milieu. By understanding how the microenvironment affects stem cell phenotypes, we hope to uncover therapeutic modalities designed to specifically predict, differentiate and eradicate aggressive cancers.

Julie Audet, University of Toronto	
Bio	Julie Audet first completed an undergraduate (1995) and graduate degree (1996) in chemical engineering at Laval University, in Quebec City. She then moved to UBC for her PhD and joined the Terry Fox Laboratory where she worked on the expansion of hematopoietic stem cells. After the completion of her PhD (2001), she did a short postdoc in the field of bioanalytical chemistry at the University of California-Irvine. She joined the Institute of Biomaterials and Biomedical Engineering (IBBME) at the University of Toronto (2003) and in 2006 she became investigator and occupant of the new Donnelly Center for Cellular and Biomolecular Research (CCBR). Dr. Audet has a strong interest in the development of assays to examine signal transduction at the single cell level and in the mechanisms by which combinations of cytokines jointly control stem and progenitor cell fate decision. As a professor of biomedical engineering, she also teaches Cellular Bioengineering and Statistics courses to undergraduate and graduate engineering students. Dr. Audet was the recipient of a NARSAD Young Investigator Award in both 2006 and 2008.
Talk	**Single-cell capillary electrophoresis and mass spectrometry applied to the measurement and manipulation of kinase activity in live cells.**

	The ability to measure and manipulate kinase activity in live cells is crucial for the identification of molecular mechanisms controlling cell fate decision and for the discovery of new drugs. We have developed a cell-permeable reporter of glycogen-synthase kinase-3 (GSK-3) activity, referred to as TAT-eIF2B. However, while TAT-eIF2B was specifically phosphorylated by GSK-3 in cell extract preparations, single-cell capillary electrophoresis (CE) revealed that it was not consistently phosphorylated in single live cells. The interaction of TAT-eIF2B with GSK-3β in live cells was verified by loading it in supraphysiological concentration (μM range) in cells and by comparing its effect with that of ATP-competitive inhibitors, CHIR99021 and BIO. In cultures of mouse ESCs, the inhibitors increased neurosphere formation; in hematopoietic cultures, they improved the maintenance of erythroid progenitors (BFU-E) during cytokine starvation. Then, further CE analyses indicated that TAT-eIF2B was rapidly cleaved due to the action of intracellular peptidases, which may have precluded the detection of its phosphorylated form in live cells. Subsequent LC-MS analysis revealed four potential cleavage sites in TAT-eIF2B. Interestingly, an all D-isomer version of eIF2B conjugated to TAT was resistant to the action of intracellular peptidases over a 2-hour period. Moreover, it had retained the ability to inhibit of GSK-3, as demonstrated in the neurosphere assay and in the BFU-E assay. Conventional methods to select or optimize kinase substrates using peptide libraries focus on examining the kinetic parameters of phosphorylation (K_m and V_{max}). This study highlights the need for novel approaches to simultaneously screen kinase substrates for their increased phosphorylation and resistance to intracellular peptidases.

Pierre Thibault, Université de Montréal	
Bio	Dr. Pierre Thibault is a renowned bioanalytical chemist specialized in mass spectrometry and proteomics with more than 20 years experience as a principal investigator in academic, government and industry laboratories. Before joining IRIC in 2004, Dr. Thibault was a founding director at Caprion Pharmaceuticals (2001-2004) where he developed an innovative proteomics platform together with bioinformatic tools to identify and quantify proteins differentially expressed in cancer cells as part of immunotherapy programs in partnership with pharmaceutical companies. He was also a Senior Research Officer with the National Research Council of Canada's Institute of Marine Biosciences in Halifax (1990-1996) and Institute of Biological Sciences in Ottawa (1996-2002). He pioneered the use of sensitive high resolution separation methods and microfluidic devices coupled to mass spectrometry and their applications in protein chemistry and cell biology. His scientific achievements in this area have been recognized by numerous awards and distinctions including the National Research Council Outstanding merit award for scientific innovation and the Canada Research Chair in bioanalytical mass spectrometry and proteomics.
Talk	**Quantitative Proteomics of Leukemic Stem Cells Reveal Novel Regulators of Self-Renewal** Most leukemia include a rare population of stem cells that are responsible for tumor development and recurrence after treatment. Leukemic stem cells (LSC) are maintained through self-renewal, a specialized cell division in which one or both of the daughter cells remain undifferentiated and retain the same replication potential of the parent. To date, pathways regulating the maintenance of these LSC remain poorly understood. As part of this study, we create a series of *Hoxa9+ Meis1* LSC derived from purified fetal liver cells. LSC displaying significant changes in their self-renewal capabilities were selected for proteomics

	analyses. Comprehensive proteome analyses of nuclear and cytosol extracts of these LSC revealed more than 2,300 different proteins including 400 differentially abundant proteins. Phosphoproteome analyses of both cell populations enabled the identification of more than 11,000 unique phosphopeptides of which 1,000 were differentially regulated. Differential regulation of chromatin and histone modifiers, including members of the polycomb complexes we clearly identified from network analyses. Further investigation showed that members of the PRC2 polycomb complex are translocated to the cytoplasm in stem cells with lower self-renewal capability. These analyses suggest that post-transcriptional and post-translational modifications, rather than gene expression, regulate cell fate determination in these leukemic stem cells.

Session 2

Blood and Blood cells (Chair Jeurgen Kast) am

John Wilkins, University of Manitoba	
Bio	**B Sc.** Biology and Chemistry (Honours); **M Sc.**, University of Waterloo; Membrane Biology **Ph.D.** University of Manitoba, Cellular Immunology. P**rofessor:** Departments of Internal Medicine, Immunology, Biochemistry and Medical Genetics **Founding and Current Director:** Manitoba Centre for Proteomics and Systems Biology. **Research Areas:** **1)** Lymphocyte cell biology with specific emphasis on the cell surface, cell migration, adhesion and polarization. **2)** Identification of biomarkers using proteomic approaches with emphasis on autoimmune diseases and renal transplantation. **3)** Proteomic and functional genomics approaches to the characterization of cell virus interactions. **4)** Differentiation using epithelial mesenchymal transition as a model system. **5)** Proteomic and activity based profiling for characterization of microbes as potential biofuels sources.
Talk	**Lymphocyte Polarization: Delivering the Kiss that Kills** Cytotoxic T lymphocytes and NK cells protect the host from intracellular infectious agents and the development of abnormal cells. This is achieved in large part through contact dependent cell mediated cytotoxicity. During this process the killer cells form a tight contact region called an immunological synapse with the target cells. The synapse stabilizes cell contact and defines the location where cytotoxic granules are delivered. An equally important aspect of this process is the capacity of immune cells to move to sites where the targets may reside so that contact can be made. This requires directed cell movement in response to adhesive and chemotactic signals. All of these processes require stringent temporal and positional controls to ensure the appropriate polarity and directionality of a cell. These constraints highlight the importance of linking compositional analysis with those examining functionality and topography. I will discuss our experiences of using proteomic, functional genomic and imaging based approaches for the examination of lymphocyte migration and cytotoxicity using several model systems. Examples will include proteomic analysis of isolated pseudopodia and cytoskeletal proteins from NK cells, genome wide screens for proteins involved in T cell migration, and characterization of the immune synapse. Some of the challenges as well as the commonalities and complementarities of the different types of analysis will be discussed.

Bruce McManus, University of British Columbia	
Talk	**Background:** Blood has long considered an optimal source for biomarker sampling, and clinical interrogation of circulating proteins has formed the

	mainstay of patient management, as evidenced by long-standing laboratory tests such as apolipoproteins, troponins and transaminases for vascular, heart muscle and liver disease, respectively. With evolution of "-omic" technologies, the genomics of blood became an arena of intense investigation, and similarly, recent advances in proteomics and metabolomics have provided novel tools for biomarker discovery and development with an aim of improving patient care. **Biomarkers in Transplantation:** Our own work in the realm of biomarkers in transplantation and immune rejection has taken an initial plasma proteomic discovery approach. Recently there has been a growing understanding of the complexities of blood and plasma proteomes. **Particles and Bodies in the Blood:** In considering plasma proteomic discovery one must remain cognizant of the presence of circulating cell derived vesicles such as exosomes and microparticles which may complicate current plasma proteomic data interpretation. The existence in the plasma of cellular proteins that have been secreted in the form of vesicles, in addition to those released through tissue damage, is important to consider as it represents an opportunity to direct efforts at targeted biomarker discovery based on the cell types involved in disease processes. **Cells in the Blood:** Further, the cellular compartment of blood also adds complexity to proteomic investigation and biomarker development. The involvement of blood cells in the pathogenesis of multiple disease types, particularly through immune and inflammatory processes, indicates that the proteomes of various blood cell types may contribute to molecular signatures considered to be indicative of disease when obtained through analysis of blood. In this context, work establishing reference proteomes for blood cells may be of help in identification of markers specific to disease. **Conclusion:** While blood remains the medium of choice for biomarker discovery efforts due to its ease of sampling and representative nature, there remain multiple issues to consider in the interpretation of plasma proteomic data, particularly the contribution of all blood constituents. These complexities also provide novel avenues of investigation and opportunities to interrogate pathologic processes through the analysis of all components of the blood. **Acknowledgement:** Support from the Networks of Centres of Excellence, Genome Canada, Genome BC, IBM, Novartis, Pfizer, IO Informatics

Leonard Foster, University of British Columbia	
Bio	Dr. Leonard Foster grew up in McBride in the Central Interior of BC and completed his Bachelor's in Biochemistry at Simon Fraser University. From there he went to Toronto to pursue graduate studies in cell biology and biochemistry with Dr. Amira Klip at the Hospital for Sick Children. Following this he moved to Odense, Denmark to learn about mass spectrometry and proteomics in one of the founding labs of this burgeoning field. He returned to BC in January of 2005 to take an assistant professor position in the Department of Biochemistry and Molecular Biology at UBC's Point Grey campus. Dr. Foster's laboratory is in the UBC Centre for High-Throughput Biology in the Networks of Centres of Excellence building and the focus of their work is using proteomics to understand host-pathogen interactions. They are particularly interested in signaling events in macrophages in response to bacteria. Dr. Foster is a Michael Smith Foundation Scholar and he holds the Canada Research Chair in Quantitative Proteomics.
Talk	**Host targets and effects on signaling of Salmonella-secreted effector proteins** Intracellular bacterial pathogens must control specific host cell processes to

	allow their survival within a host. Salmonella enterica serovar Typhimurium manipulates multiple host pathways in order to effect internalization and infection, and then is further able to modify its vacuole. These mechanisms allow avoidance of exposure to host lysosome contents and other anti-microbial factors. Both manipulation and modification are achieved by effector proteins secreted into the host cytosol through type III secretion systems encoded in Salmonella pathogenicity islands 1 and 2 (SPI-1 and SPI-2, respectively). Some aspects of these effectors' impact on host cell functions are well-understood: e.g. SopE/E2 and SptP are known to be guanine nucleotide exchange factors and a GTPase activating protein, respectively, for host Cdc42 and Rac1 GTPases. While several effectors appear to hijack host cell processes, there is limited mechanistic detail linking effector function to observations of downstream effects of Salmonella infection, (e.g., intracellular replication or Salmonella-induced filament formation). Here we have taken a global approach to identify host targets of each secreted effector and then started to systematically dissect how each effector impacts host phosphorylation signaling.

Thomas Kislinger, University of Toronto	
Bio	- Study of Analytical Chemistry(1993-1998): LMU Munich, Germany - PhD (1998-2002): FAU Erlangen, Germany & Columbia University, USA on the biology of advanced glycation endproducts in diabetic vascular disease. - Post-doc (2002-2006): Banting and Best Department of Medical Research, University of Toronto - Scientist at the Ontario Cancer Institute & Ass. Prof. at University of Toronto since 2006
Talk	**Comparative proteomic analysis of human and mouse: Applications to placental and vascular biology** Placental abnormalities are associated with two of the most common and serious complications of human pregnancy, maternal preeclampsia (PE) and fetal intrauterine growth restriction (IUGR), each disorder affecting ~5% of all pregnancies. An important question for the use of the mouse as a model for studying human disease is the degree of functional conservation of genetic control pathways from human to mouse. The human and mouse placenta show structural similarities but there have been no systematic attempts to assess their molecular similarities or differences. Over 7,000 ortholog genes were detected with 70% co-expressed in both species. Several of these phenotype-associated proteins form a tight protein-protein interaction network involving 15 known and 34 novel candidate proteins also likely important in placental structure and/or function. The second part of the talk will cover a proteomics study of the placenta vasculature using the silica-bead coating technology. As normal vascular function is compromised in both preeclampsia and IUGR we applied a shot-gun proteomics approach to gain novel insight in protein expression patterns of the fetal and maternal vasculature of near-term mouse placenta (E17.5), while keeping specific focus on the poorly studied trophoblast-lined maternal vasculature. Comparative analyses identified 98 proteins specifically associated with the maternal vasculature surface, including several known markers. Rigorous data validation using immunohistochemistry (IHC) in human tissue sections provided a panel of 33 novel markers of the maternal vasculature. Western blotting analyses and IHC, in a highly annotated set of clinical samples, demonstrated significant up-regulation of several novel maternal surface markers in both IUGR and preeclampsia.

John Marshall, Ryerson University	
Bio	Dr Marshall obtained his bachelor degree from the University of Toronto. He won both the NSERC and OGS scholarships for his M.Sc and Ph.D. with Dr Erwin Bernard Dumbroff at Waterloo University. He performed post doctoral fellowships with Dr. Eduardo Blumwald at the University of Toronto; Dr. Paul Walker of Toronto General Hospital; and Dr Sergio Grinstein under the auspices of a fellowship from the Hospital for Sick Children. He was the vice president of R&D at SYNX Pharma under Dr George Jackowski. Dr Marshall joined Ryerson University as an assistant professor in 2003 and was promoted to associate in 2008. Dr Marshall has been a founding board member/executive of Artemis Proteomics, YYZ Pharmatech, the Ontario Cancer Biomarker Network and the Canadian National Proteomic Network. Dr Marshall's research is focused on blood proteins and their receptor complexes on white blood cells. Dr Marshall's published work has been supported by the Natural Science and Engineering Research Council, Federal/Ontario Research and Development Agreements, Imperial Chemical Industries, Astra Zeneca, The Heart and Stroke Foundation, and The Canadian Foundation for Innovation. More information regarding Dr Marshall's lab and the Ryerson University Analytical Center may be found at Marshalllab.org.
Talk	**XTANDEM to SQL Parser permits analysis by SAS** The X!TANDEM algorithm fits tandem mass spectra of peptides to amino acid sequences and directly calculates the expected probability of mis-identification. The product of the peptide expectation values directly yields the probability that the parent protein has been mis identified. A relational database could capture both the mass spectral data and the results of the goodness of fit test, while permitting subsequent calculations by a general statistical analysis system. Thus the many files of the HuPO blood protein data were fit by X!TANDEM to the human proteins of the Ensembl database. The resulting peptides, and associated protein sequences, were collected into a relational database. A redundant set of 247,077 proteins and peptides were fit by X!TANDEM, and collapsed to a set of 34,956 peptides from 13,379 distinct proteins using structured query language. About 6875 distinct proteins were only represented by a single distinct peptide, 2866 proteins showed 2 distinct peptides, and 3454 proteins showed at least three distinct peptides by X!TANDEM. The distribution of peptides per protein from X!TANDEM were significantly different than those expected from the previously established random assignment of peptides, as calculated by the Chi Square test. More than 99% of the peptides were associated with proteins that had cumulative expectation values, i.e. probability of false positive identification, of one in one hundred or less: Only about 500 of the 247,077 data points failed to meet the cut off scores for type I & type II error at the 1 in 100 level. The analysis indicates that there may be a high degree of confidence in the thousands of novel proteins discovered from human blood by mass spectrometry in this study.

Juergen Kast, University of British Columbia	
Bio	Dr. Juergen Kast obtained his PhD in Analytical Chemistry from the University of Konstanz. Germany in 1998, for the development of novel approaches to study protein interactions by mass spectrometry. He continued this line of research as a post-doctoral fellow at the European Molecular Biology Laboratory, Heidelberg, Germany, where he focused on the analysis of protein complexes in cells. In 2001, he joined the University of British Columbia in Vancouver, where he currently holds an appointment as Associate Professor at The Biomedical Research Centre and in the Department of Chemistry. He has been appointed

	Scientific Director of the British Columbia Proteomics Research earlier this year. His research focuses on the characterization of signal transduction mechanisms in mammalian cells and their regulation. He has pioneered novel proteomic methods such as formaldehyde cross-linking in live cells to study protein interactions, and the multiplex detection of post-translational modifications by mass spectrometry.
Talk	**Platelets - dead-end or nexus?** Platelets, one of the cellular blood components, have a well-established physiological role. Upon vascular injury, they halt bleeding by a defined sequence of events involving their adhesion, activation, and aggregation, which result in the formation of blood clots. Aberrant clot formation can cause arterial occlusion and lead to heart attack and stroke. Platelet research has mostly focused on the mechanisms of activation and aggregation, and more recently also on platelet storage. Transfusion of stored platelets is a life-saving measure when the number or activity of platelets in patients is reduced, *e.g.* after chemotherapy or during surgery. Collected platelets can only be stored for five days at room temperature. Extended storage increasingly compromises platelet functionality upon transfusion, an outcome thought to be due to changes in key proteins. As platelets do not possess nuclei and show low mRNA levels, post-translational protein activity is indeed the most likely mediator of platelet functionality. Proteomics is thus well suited to investigate the changes in platelet proteins associated with these events. In my talk, I will present recent results from our proteome studies of stored platelets, and will illustrate two possible strategies for drug intervention in platelet function. Finally, I will discuss our efforts in mapping the platelet proteome, which has allowed us to explore the protein machinery of platelets. Our maps not only confirm known platelet features, but also suggest that platelets may have physiological functions beyond hemostasis.

Personalized Medicine (Chair) Tommy Nilsson pm

Rick Rachubinski, University of Alberta	
Bio	Dr. Richard Rachubinski is Distinguished University Professor and Chair in the Department of Cell Biology at the University of Alberta. After receiving a Ph.D. in cell biology with Dr. John Bergeron from McGill University in 1980, he pursued postdoctoral research at McGill with Dr. Gordon Shore and at the Rockefeller University in New York with Dr. Paul Lazarow. He was Assistant, Associate, and Full Professor in the Department of Biochemistry at McMaster University before assuming his current position at the University of Alberta in 1993. In 2001, he was named Canada Research Chair in Cell Biology and Senior Investigator of the Canadian Institutes of Health Research. In 2002, he was appointed a fellow of the Royal Society of Canada. He has been an International Research Scholar of the Howard Hughes Medical Institute since 1997. His work centers on defining the molecular pathways controlling the biogenesis of the peroxisome, a cellular organelle that performs a variety of important biochemical functions, notably in lipid metabolism and reactive oxygen species detoxification.
Talk	**Peroxisomal diseases. What we can learn from yeast.** Peroxisomes are ubiquitous intracellular organelles that perform a myriad of important biochemical functions, most notably in lipid metabolism and the detoxification of reactive oxygen species. The requirement of peroxisomes for normal human development and physiology is underscored by the lethality of the

	peroxisome biogenesis disorders, genetic diseases in which peroxisomes fail to assemble. As the peroxisome biogenic pathway has been highly conserved during evolution, we exploit yeast as a model system to investigate the molecular mechanisms underlying the peroxisome biogenesis disorders. Our research aims for a better understanding of the peroxisome biogenesis disorders to provide for future rational genetic and/or therapeutic intervention.

Jeff Wrana, Samuel Lunenfeld Research Institute	
Bio	Recent efforts to systematically map the network of mammalian protein-protein interactions have led to the discovery of both local and global topological features that confer important functions to the network. We have identified structural features of proteins that define their function within this modular network. Furthermore, by examining how gene expression changes are associated with outcome in breast cancer, we have defined alterations in network modularity that are associated with poor outcome. Global disorganization in network organization may thus be an important aspect to complex human diseases.
Talk	**Disruption of the topological structure of the human interactome is associated with breast cancer outcomes.**

Jim Woodgett, Samuel Lunenfeld Research Institute	
Bio	PhD, Dundee, Scotland 1981-4; post doc Salk Institute, San Diego 1984-7; Scientist, Ludwig Institute for Cancer Research 1987-92; moved to Ontario Cancer Institute in 1992 and was head of the division of experimental therapeutics. Joined MSH in Nov 2005. He is a CIHR Senior Investigator, Howard Hughes Medical Institute International Research Scholar and Fellow of the Royal Society of Canada. Research interests are mechanisms of breast and colon cancer, diabetes and neurodegenerative disorders.
Talk	**Setting traps: amplifying tissue-specific progenitor cells**

Claus Jorgensen, Samuel Lunenfeld Research Institute	
Bio	PhD from University of Southern Denmark, Odense, Denmark. During his studies he was co-supervised by Drs Karsten Kristiansen and Peter Roepstorff. Postdoctoral studies are currently ongoing with Dr Tony Pawson, since 2005.
Talk	Integrated analysis of reciprocal cell signaling

Tommy Nilsson, Professor and Director of Proteomics and Systems Medicine, McGill University	
Bio	PhD work at Uppsala University and Scripps Clinic & Research Foundation, La Jolla California. Post-doc at Cancer Research UK, London, UK. Group Leader at the European Molecular Biology Laboratory, Cell Biology & Biophysics, Heidelberg, Germany and Professor at University of Gothenburg, Medical Faculty, Sweden. Now, Investigator at the Research Institute of the McGill University Health Centre and Professor, Faculty of Medicine, McGill University
Talk	**Opportunities and Challenges of "Omics" in the clinic**

Christoph Borchers, University of Victoria	
Bio	Christoph is the director of the UVic-Genome BC Proteomics Centre and Associate Professor in the Biochemistry and Microbiology Department at UVic. Through his position as director Christoph is interested in several aspects in proteomics with special focus on technology development. He has become particularly interested in developing approaches and methods for structural and targeted quantitative proteomics including MRM techniques and the iMALDI approach. Today he will talk about these techniques and how it can be used for molecular diagnostics as a central part of personalized medicine"
Talk	**Novel MS-based Proteomics Approaches for Molecular Diagnostics**

Tom Hudson, OICR	
Bio	Dr. Thomas J. Hudson is President and Scientific Director of the Ontario Institute for Cancer Research, a new Institute created to support multidisciplinary teams needed to effectively translate research discoveries into interventions for better prevention, detection, diagnosis and treatment of cancer. Dr. Hudson is internationally renowned for his work in genomics and human genome variation. He was a founding member of the International Haplotype Map Consortium, the Public Population Project in Genomics and the International Cancer Genome Consortium. Dr. Hudson is a fellow of the Royal Society of Canada and editor-in-chief of the journal *Human Genetics*. Dr. Hudson has co-authored over 200 peer-reviewed scientific publications.
Talk	Personalized Medicine for Cancer