4th International Symposium on
Enabling Technologies for Proteomics
May 14, 2009, Four Seasons Hotel Vancouver

Poster Abstracts

ETP 2009 Symposium
POSTER ABSTRACTS

Poster	1 - (Confirmation No. 2660)
Submitted by	Zarrin Eshaghi, Payame Noor university
Contributing Authors	
Abstract Title	Extraction And Determination Of Fatty Acid Ethyl Esters In Hair As Biomarkers For Quantitative Assay Of Alcohol
Abstract	Fatty acid ethyl esters (FAEEs) are products in blood of non-oxidative ethanol metabolism. After incorporation in hair, they should be suitable long-term markers of alcohol abuse. This research focuses on basic aspects and recent studies of hair analysis for alcohols. Firstly, biology of hair and sampling of hair specimens have been commented for the sake of correct interpretation of the results from hair analysis. Then the washing method of hair samples and the extraction method for FAEEs in hair have been shown and commented on. Analytical method for analysis have been discussed by the method, namely hollow fiber liquid phase microextraction and gas chromatography-flame ionization detection, HF-LPME-GC-FIDâ€. The outcomes of hair analysis studies have been reviewed into; ethyl palmitate, ethyl oleate and ethyl stearate in hair. Applications of hair analysis to the estimation of alcohols have also been reviewed. Finally, the promising prospects of hair analysis have been described. Keywords: Hair, Alcohol, Fatty acid ethyl esters (FAEEs), hollow fiber liquid phase microextraction (HF-LPME), GC-FID.

Poster	2 - (Confirmation No. 2709)
Submitted by	Mike Moran, University of Toronto
Contributing Authors	M Moran, J St-Germain, P Taylor, J Tong, L Jin, I Stewart, R Ewing, M Dharsee, S Trudel
Abstract Title	Phosphorylation profiles associated with FGFR3 kinase expression, ligand activation, and drug inhibition
Abstract	Signaling by activated growth factor receptor tyrosine kinases is manifest through networks of proteins that are phosphorylated by, and/or bind the autophosphorylated receptors. FGF Receptor-3 (FGFR3) is a drug target in a subset of human multiple myeloma (MM) tumors, and is mutationally activated in some cervical and colon and many bladder cancers, and certain skeletal dysplasias. In order to determine the FGFR3 network in MM, mass spectrometry was used to identify and quantify protein pY sites modulated by FGFR3 activation and inhibition. Label-free quantification of MS ion currents and by multiple reaction monitoring revealed an FGFR3 network including 45 proteins phosphorylated in response to FGF ligand and sensitive to FGFR3 inhibition. These results demonstrate pharmacodynamic monitoring by label-free quantitative phospho-proteomics. This research was supported in part by funding from the Canada Research Chairs Program; Canadian Institutes of Health Research; and the Canadian Cancer Society and National Cancer Institute of Canada.

Poster	3 - (Confirmation No. 2710)
Submitted by	Lalit Agrawal, NIPGR
Contributing Authors	Subhra Chakraborty, Dinesh Kumar Jaiswal, Sonika Gupta, Asis Datta, and Niranjan Chakraborty
Abstract Title	Comparative Proteomics of Tuber Induction, Development and Maturation Reveal the Complexity of Tuberization Process in Potato (Solanum tuberosum L.)

Abstract	Tuberization in potato (Solanum tuberosum L.) is a developmental process that serves a double function, as a storage organ and as a vegetative propagation system. It is a multistep, complex process and the underlying mechanisms governing these overlapping steps are not fully understood. To understand the molecular basis of tuberization in potato, a comparative proteomic approach has been applied to monitor differentially expressed proteins at different development stages using two-dimensional gel electrophoresis (2-DE). The differentially displayed proteomes revealed 219 protein spots that change their intensities more than 2.5-fold. The LC-ES-MS/MS analyses led to the identification of 97 differentially regulated proteins that include predicted and novel tuber-specific proteins. Nonhierarchical clustering revealed coexpression patterns of functionally similar proteins. The expression of reactive oxygen species catabolizing enzymes, viz., superoxide dismutase, ascorbate peroxidase and catalase, were induced by more than 2-fold indicating their possible role during the developmental transition from stolons into tubers and suggest that the generation of ROS may be one of the early and determinant events during tuber initiation in potato, hitherto undiscovered. We demonstrate that nearly 100 proteins, some presumably associated with tuber cell differentiation, regulate diverse functions like protein biogenesis and storage, bioenergy and metabolism, and cell defense and rescue impinge on the complexity of tuber development in potato.

Poster	4 - (Confirmation No. 2711)
Submitted by	John M. Lindsay, Denator AB
Contributing Authors	Marcus Svensson, Karl Sköld, Maria Fälth, Per E Andrén Per Svenningsson, Mats Borén
Abstract Title	Heat Stabilization of the Tissue Proteome: a new technology for improved proteomics
Abstract	Immediately after sampling, proteases and other protein-modifying enzymes change proteome composition. The results from subsequent analyses reflect a mix of in vivo proteome and degradation products. Important information about the pre-sampling state of the tissue may be distorted or destroyed, leading to reduced reproducibility between samples and even faulty conclusions. This problem is addressed by rapid sample inactivation in a novel tissue stabilization system (Stabilizor T1, Denator AB) which halts post-sampling modifications irreversibly by heat induced protein denaturation. Tissue samples were collected from freshly sacrifice mouse and either snap frozen or immediately stabilized using the Stabilizor T1 system, special focus was placed in keeping the post-mortem times as low as possible. Samples were homogenized frozen using a Retsch ball with 5 mm steel balls. After treatment tissue samples were analyzed with downstream techniques such as western blotting, MALDI-MS or Nano-LC-MS according to established and published protocols. When looking at the low mass content (< 10 kDa), the results show a large number of detected peptides in the untreated samples identified as protein degradation fragments from highly expressed proteins such as hemoglobin, dynamin, NADH dehydrogenase. In contrast, the peptides detected in the stabilized samples were identified as known neuropeptides, endogenous peptides and small proteins. The inhibition of phosphorylase is shown to be improved compared to a common chemical inhibitor. After stabilization treatment the levels of phosphorylated CREB, GSK and MAPK were maintained up to 2 hours in room temperature treatment whilst the levels in untreated tissue decreased.

Poster	5 - (Confirmation No. 2713)
Submitted by	Miriam Lynn, University of British Columbia
Contributing Authors	Miriam A. McAndrew-Lynn, W. Robert McMaster
Abstract Title	Quantitative differential proteomic analysis of Leishmania membrane proteins to identify potential drug targets
Abstract	Like many protozoan parasites, Leishmania, the causative agent of leishmaniasis, poses an enormous public health predicament in many developing countries throughout the world, impacting a possible 350 million people worldwide. Presently front line drugs are outdated, have serious side-effects and some associated drug resistance, emphasizing the need for safer, more effective drugs to combat leishmaniasis. In the search for more effective drug targets against Leishmania, studies are identifying genes that are upregulated in the infective lifecycle stage, the amastigote. However, recent studies have shown that the Leishmania genome is essentially constitutively expressed, such that most protein regulation occurs post-translationally. Therefore future studies need to focus on Leishmania proteome profiling to identify potential upregulated pathogenesis targets and consequently drug targets. By focusing on Leishmania membrane proteome profiling, novel accessible targets that are differentially regulated at the protein level throughout the Leishmania lifecycle may be identified. Specifically, the stable isotope labeling iTRAQ technology combined with quantitative mass spectrometry is being applied to identify differential membrane proteomic profiles between the promastigote and the amastigote lifecycles in Leishmania infantum. Comparative studies in L. mexicana will identify common membrane targets in both species upregulated in the infective amastigote lifecycle stage as possible drug targets.

Poster	6 - (Confirmation No. 2715)
Submitted by	Dominik Domanski, University of Victoria Proteomics
Contributing Authors	Dominik Domanski, Michael Kuzyk, Leanne Ohlund, Tyra Cross, Christoph Borchers
Abstract Title	Absolute Quantitation of Phosphorylation Dynamics in the Analysis of Human Breast Cancer Signaling Pathways Using Multiple Reaction Monitoring MS
Abstract	Breast cancer is often related to genetic defects resulting in aberrations in the function of the epidermal growth factor receptor (EGFR) and estrogen receptor (ER) signaling pathways. Despite the fact that signal transducing phosphoproteins are centrally involved in tumorigenesis, cancer progression, and drug susceptibility, indirect methods from genomic technologies that only assess genetic aberrations are still primarily used in cancer research. Individualized treatment selection and drug development, however, will require a thorough and quantitative understanding of actual protein expression and phosphorylation dynamics of signaling pathways. We describe a novel mass spectrometry (MS)-based proteomic assay to directly investigate phosphorylation levels of select signal transducing cancer-related phosphoproteins involved in the EGFR and ER signaling pathways with absolute quantitation for clinical relevance. Our method, termed phosphatase-directed phosphopeptide quantitation (PPQ) with multiple reaction monitoring (MRM) MS detection, can obtain absolute quantitation of select proteins and their site-specific phosphorylation levels.

	We tested a number of phosphopeptides of interest from ER and EGFR pathway proteins. These were synthesized as natural and heavy-isotope-labeled standards in both, phosphorylated and non-phosphorylated form, and then combined at different ratios and different amounts in different sample matrix backgrounds. The PPQ-MRM method was able to absolutely quantify levels of phosphorylation with CVs similar to the direct MRM approach, with sensitivity in the fmol range. This method could potentially allow the profiling of EGFR and ER signaling networks at a high throughput, quantitation accuracy, and sensitivity, allowing the creation of detailed profiles or maps of signaling networks in breast cancer tumor samples.

Poster	7 - (Confirmation No. 2716)
Submitted by	Geraldine Walsh, Biomedical Research Centre
Contributing Authors	Geraldine M. Walsh, Arash Khosrovi-Eghbal, Jason C. Rogalski and Juergen Kast
Abstract Title	Extensive Temporal Analysis of Platelet Storage Lesion using Quantitative Proteomics
Abstract	Platelets are key components of the haemostatic system and an essential transfusion product. Their storage time prior to transfusion is limited to 5 days due to the storage temperature (22°C), which leads to increased risk of bacterial contamination, and to the Platelet Storage Lesion (PSL), a progressive loss of platelet function observed during storage, whose underlying mechanisms are not well elucidated. Here, we expand on our previous work by conducting more extensive temporal analyses of stored platelet concentrates, provided by Canadian Blood Services. Platelets were sampled on storage days 1, 5, 7 and 10 (medium time course) and days 1, 7, 12 and 15 (long time course), isolated, washed in physiological buffer and lysed, followed by in-solution digestion with trypsin. Peptides were labeled with 4-plex iTRAQ reagents, separated by SCX, analyzed on an LC QSTAR-XL and quantified using Protein PilotTM software (all Applied Biosystems). For the medium time course, an average of 287 proteins were quantifiable (n=4 experiments), with 145 quantifiable proteins found to be common to all 4 medium time course datasets. For the long time course, on average 406 proteins were quantifiable (n=2 experiments). In both time courses, between 25-30% of the quantifiable proteins were found to be significantly up- or down-regulated at one or more of the time-points. Key hallmarks of PSL include alterations in glucose metabolism, release of granules and increase in activation. Supporting this, the results show an increase in the metabolic enzyme GAPDH, particularly by day 10. Also, a progressive decrease in secreted proteins including thrombospondin is observed, and is particularly dramatic at the longer time points. Many of these proteins have not been previously been reported as playing a role in PSL and are currently undergoing further investigation as potential biomarkers.

Poster	8 - (Confirmation No. 2717)
Submitted by	Rachel Kozlowski, UVic GBC Proteomics Centre
Contributing Authors	Jun Han, Christoph Borchers
Abstract Title	Comprehensive Profiling of Human Plasma Phospholipids by Combining Direct Infusion and LC FTMS.
Abstract	**Introduction** Irregularity of lipid abundances, dyslipidemia, is associated with many

Abstract	Integrins are membrane spanning heterodimers, which play a major role in cell signalling and physiology. They relay signals across the membrane by interaction with intracellular adaptor proteins, which bind to their small intracellular tails. Several interaction partners of integrins have been described, their binding being dependent on the activation status of the cell. However, the detailed mechanism of regulation of these signalling processes is not fully understood and requires further investigation. Formaldehyde cross linking of cells paired with affinity enrichment and mass spectrometry is a powerful method to study protein complexes and was established successfully in our lab to study myc-tagged proteins. However, to analyze integrin complexes we wanted to use antibodies against the endogenous proteins. This requires that the corresponding epitopes are not destroyed by formaldehyde modification. We tested eight different monoclonal antibodies for immunoprecipitation of integrin beta 1 from formaldehyde treated Jurkat cells. Two of them did not precipitate cross linked complexes, whereas the remaining six were successfully applied. Investigation of one cross linked complex was performed by mass spectrometry and revealed that it was composed of integrin beta 1 and integrin alpha 4, which is a known heterodimer present in Jurkat cells. This proves that formaldehyde cross linking together with immunoprecipitation by antibodies against endogenous proteins can be used for investigation of complexes. We are currently investigating further integrin beta 1 complexes and analyzing changes in composition of these complexes following stimulation of the integrins, which will help to understand the signalling processes taking place through these heterodimers.

Poster	10 - (Confirmation No. 2722)
Submitted by	Lindsay Rogers, University of British Columbia
Contributing Authors	L.D. Rogers, Y. Fang, E.C. Boyle, L.J. Foster
Abstract Title	Phosphoproteomic analysis of host signaling events induced by Salmonella.
Abstract	Salmonella enterica is an intracellular pathogen which causes enteritis or typhoid fever by invading epithelial and macrophage cells and persisting in a vacuole which avoids degradation by the host. Virulence is governed largely by effector proteins which the bacteria deliver into the host cytosol using two secretion systems. While several of these effectors have been identified, their mechanisms of action within the host remain largely unknown. Protein phosphorylation is a reversible modification involving the addition of a phosphate to amino acid side chains, and is known to regulate a vast array of biological functions. Interestingly, host tyrosine phosphorylation has been shown to accompany invasion by Salmonella, and a few effectors are known to influence phosphorylation events in host cells. However the exact targets of these effectors, as well as a myriad of potential other downstream signaling events remain unknown. Here we describe a method for the global analysis of phosphorylation signaling from whole cell tryptic digests. After mechanical lysis, cell lysates are separated into membrane, nuclear and cytosolic fractions in the presence of a cocktail of phosphatase inhibitors, followed by immediate heat denaturation. Each fraction is digested with trypsin, and the resulting peptides are fractionated further by isoelectric focusing (IEF). IEF fractions are enriched for phosphorylated peptides using metal oxide chromatography and the eluates are analyzed by LC-MS/MS on an LTQ-Orbitrap. Experiments comparing MS2 versus MultiStage Activation suggest that the additional cycle time required for MultiStage Activation does not result in significant improvements in either protein identification scores or

	prevalent diseases in North America such as diabetes, metabolic syndrome and cardiovascular disease. The identification and study of various lipids and their irregularities in key human tissues as well as blood plasma is important to a complete understanding of the role lipids play in these diseases. The development of efficient, accurate diagnostic techniques is therefore necessary for efficient, accurate treatments. To this end, the development of a comprehensive profile of lipids, such as phospholipids, in human blood plasma will pave the way towards accurate evaluation of diseases associated with dyslipidemia. **Method** Phospholipids were isolated from 3 different sets of human plasma samples by liquid-liquid extraction with CHCl3/H2O/CH3OH. These extracted lipids were analyzed by direct infusion- and LC-FTMS on a 12-Tesla FTICR mass spectrometer in (+) and (-) ESI mode. After internal mass calibration, custom software was used for monoisotopic peak pick up and subsequent peak alignment. Rational molecular formulae were generated using accurate masses and searched against lipid and metabolome databases (Lipid MAPS, HMDB and METLIN). For peaks that returned hits in multiple phospholipid classes or subclasses, offline LC fractionation and FTMS/MS was conducted to further confirm phospholipid identities. **Preliminary Results** Our preliminary results have shown that >200 and >500 phospholipids have been assigned using direct infusion and LC-FTICRMS, respectively. The phospholipid class distribution remained well correlated across LCMS and direct infusion data. After combining all unique masses obtained from each set of spectra, over 500 phospholipids were identified with mass errors of < 2ppm. However, approximately 80% of these hits had associated errors of < 1ppm. The most abundant lipid classes in human plasma were found to be glycerophosphocholines, glycerophosphoethanolamines and glycerophosphoserines. In positive ion mode, these classes represented approximately 50%, 30% and 10% of peaks identified, respectively. In negative ion mode, these classes made up approximately 25%, 15% and 50% of total peak composition respectively. The less abundant classes, in order of abundance, included glycerophosphoinositols, glycerophosphates and glycerophosphoglycerols. In addition to these identified lipids, there were also a significant number of peaks (> 900) for which no hits were returned. A preliminary analysis of a number of these peaks revealed highly probable candidates for unidentified phospholipids. Currently, MS/MS experiments are being undertaken to elucidate the structure of these potential phospholipids. The preliminary results of this study have shown that combining direct infusion and LCMS with ultrahigh resolution of FTICR MS, has provided one of the most comprehensive profiles of human plasma phospholipids in a single experiment. These phospholipid identities will ultimately be used as a model list for targeted screening of potential phospholipid biomarkers for dyslipidemia against 20 human metabolic syndrome patient samples and control patient samples. Currently we are in the process of identifying and analyzing the potential difference in lipid profiles of these 20 human plasma samples.

Poster	9 - (Confirmation No. 2719)
Submitted by	Cordula Klockenbusch, Biomedical Research Centre
Contributing Authors	Juergen Kast
Abstract Title	Investigation of integrin beta 1 complexes by formaldehyde cross linking and mass spectrometry

	phosphorylation site localization. Combining the described assay with stable isotopic labeling of amino acids in cell culture (SILAC), we are currently tracking thousands of phosphoryation events which are induced in HeLa cells during Salmonella infection. Cells are harvested between 0 and 20 min post infection to generate a dynamic profile for each phosphorylation event detected as Salmonella invades its host. Several interesting pathways not previously linked to Salmonella infection have been identified. Future experiments will compare results from wild type Salmonella infections to heat killed Salmonella and mutant strains for specific Salmonella effectors. The described work is expected to represent one of the largest phosphoproteomic studies to date, and also to yield a vast amount of information regarding phosphorylation events induced by specific players in Salmonella virulence.

Poster	11 - (Confirmation No. 2725)
Submitted by	Chris Hao Pu, UBC ChiBi
Contributing Authors	
Abstract Title	Fishing for parkin ubiquitin ligase substrates
Abstract	Ubiquitylation is a major post-translational modification which relies on a vast network of about five hundred E3 ubiquitin ligases in human cells. It is involved in several processes such as proteolysis (via the ubiquitin proteasome system; UPS), vesicle trafficking and DNA damage. Mutations in PARK2, which encode parkin E3 ligase, account for half of autosomal dominant juvenile parkinsonism cases, an early form of Parkinson√¢,Ç¨,Ñ¢s disease. Multiple PARK2 mutations underlie the RING domain, which promotes E3 ligase activity suggesting an inability for substrate degradation may trigger neurodegeneration. We first employed parkin immunoprecipitation to identify dynamic interactors of which a proportion will potentially be substrates. Preliminary MS data suggested enrichment for proteasomal components as well as stress proteins in parkin transfected cells, in accordance with parkin√¢,Ç¨,Ñ¢s UPS connection and the possibility of parkin targeting misfolded proteins. We have also undertaken a second approach relying on purification of the ubiquitin proteome using His-biotin-tagged ubiquitin. The rationale is that overexpression of wild-type parkin should lead to an enrichments of putative substrates due to increased parkin mediated ubiquitination. The challenge is to establish a purification scheme enabling identification of low abundant ubiquitinated proteins to increase the chance of detecting putative parkin substrates.

Poster	12 - (Confirmation No. 2727)
Submitted by	Nancy Fang, CHIBI, UBC
Contributing Authors	Nancy Fang, Mayumi Iwashita and Thibault Mayor
Abstract Title	Heat-Shock Induced Misfolded Cytoplasmic Proteins Are Ubiquitinated by a Network of E3s in Saccharomyces cerevisiae.
Abstract	A significant portion if not the majority of UPS substrates corresponds to misfolded and damaged proteins that are targeted by protein quality control (PQC) machinery. Dysfunction of the UPS can result in the accumulation of misfolded proteins and formation of protein aggregates that can cause extensive cellular damage. This has been associated to various human diseases, including neurodegenerative diseases, cancer, heart disease and diabetes. To gain a better understanding of the pathogenesis of these diseases, it is necessary to investigate how the UPS targets PQC substrates

	for degradation. To obtain a better insight of the PQC system in cytosol, we employed heat-shock stress to globally increase misfolded protein levels in yeast. A vast increase of total poly-ubiquitination levels is the landmark of this dramatic change. Using quantitative mass spectrometry, we first showed that heat-shock induced poly-ubiquitination is a good model to study PQC machinery targeting cytosolic misfolded species. For quantitative comparison, untreated and heat-shock treated cells that are expressing his8-tagged ubiquitin were differentially labeled with 14N and 15N and were subsequently subjected to enrichment of ubiquitinated proteins by nickel chromatography. We showed that the majority of proteins displaying an increase ubiquitination signal were localized in the cytoplasm. We then screened for genes defective in heat-shock induced ubiquitination using a selected deletion library of UPS genes. We identified several putative E3 ligases involved in heat-shock response, including VPS8 and GRR1 that displayed a reduced viability after heat-shock and HUL5. After comparing wild type and HUL5 cells by quantitative mass spectrometry, we determined that about a fifth of the proteins ubiquitylated after heat-shock required HUL5. The current data suggests that there is likely a network of several E3s that is involved in targeting wide range of PQC substrates in cytosol in yeast.

Poster	13 - (Confirmation No. 2731)
Submitted by	Yu Zi (emma) Zheng, CHiBi, BMB, CPS
Contributing Authors	Cecile Boscher, Michelle M. Hill, Robert G. Parton, Ivan R. Nabi, Leonard J. Foster
Abstract Title	Quantitative Proteomics Analysis of Cell Surface Caveolae in Mammary Epithelial tumor Cells
Abstract	Caveolae are a class of membrane microdomains that are microscopically distinguishable stable invaginations of the cell plasma membrane. The formation of caveolae depends on the presence of specific structural proteins caveolin-1 in non-muscle cells and others like the recently reported caveolae associated protein Cavin/Ptrf. Caveolae have well-characterized roles in signal transduction and endocytosis, however non-caveolar roles for caveolin have also been proposed. Caveolae and caveolin-1 are closely associated with detergent-resistant membranes (DRMs) whose detergent-resistance and low density allow them to be enriched biochemically. We performed quantitative proteomic analysis on DRMs from cell lines expressing caveolae, caveolin-1 but no caveolae and limiting amounts of caveolin-1 in order to characterize the different protein composition of DRMs in cells expressing caveolae and/or caveolin-1. We found that the loss of caveolae on the plasma membrane has a dramatic effect on the DRM content of a cell. Multiple proteins were identified to be selectively associated with DRMs in caveolae-expressing cells, such proteins included known raft/caveolae associated proteins caveolin-1, Ptrf and flotillins, signaling proteins such as MAP kinase, tyrosine protein kinase Fyn, R-Ras and multiple G protein subunits, as well as actin and filamin indicating the connection between rafts/caveolae and microfilaments networks.

Poster	14 - (Confirmation No. 2733)
Submitted by	Chengcheng Zhang, University of British Columbia
Contributing Authors	Daniel Evans, Ronald Beavis, Juergen Kast
Abstract Title	Protein Interaction Environments Comparison Using The Global Proteome Machine database

Abstract	By definition, protein interaction environments are represented by all of the proteins interacting with a protein of interest, or sharing its cellular localization and having the potential to form such interactions or to block them. Their identification for a given protein is the focus of functional proteomics studies. With the number of such studies rapidly increasing, proteomics databases are increasingly likely to already contain this type of information. The Global Proteome Machine database (GPMDB) is the largest curated and publicly available data repository for proteomics information derived from tandem mass spectrometry. The GPMDB could potentially allow a novel way to observe protein interaction environment, i.e. by parsing the database for proteins that are commonly coincident and have highly correlated occurrences and intensities. Using the stringent criteria we developed, we have compared the protein interaction environments of certain GTPases, i.e. Rap1, RhoA and CDC42, particularly the proteins that are shared by these GTPases and the ones that unique to them, and also the significance of these proteins, which reveals the different and overlapping context where these enzymes function. Our results demonstrate that GPMDB can be used to gain insight into the protein interaction environment individually, and for the comparison among environments.

Poster	15 - (Confirmation No. 2736)
Submitted by	Yuan Fang, Center for High-Throughput Biology, UBC
Contributing Authors	Lindsay D. Rogers, Leonard J. Foster
Abstract Title	Global Ser/Thr/Tyr phosphorylation in Salmonella
Abstract	Currently, few studies are done on the phosphorylation events in prokaryotes. It was traditionally believed that phosphorylation in bacteria is at least 10-fold less than in eukaryotes and mainly on His/Asp residues. With the recent advances in mass-spectrometry based proteomics, global phosphorylation studies in E. coli and B. subtilis have revealed evolutionarily conserved phosphorylation sites on Ser/Thr/Tyr residues in bacteria (1,2). Salmonella enteric is an intracellular bacteria pathogen that causes enteritis and typhoid fever, and is an important model organism for studying host-pathogen interactions. Salmonella uses two type III secretion systems to inject effector proteins into the host cell to facilitate invasion and intracellular survival, however, little is known about whether phosphorylation plays a role in this process. We have used metal-oxide chromatography to enrich phosphopeptides from Salmonella lysates for subsequent analysis on a LTQ-Orbitrap. With optimized conditions in peptide fractionation by isoelectric focusing and phosphopeptide enrichment, we are able to detect 150 non-redundant Salmonella phosphopeptides. By comparing the phosphorylation profiles between the stationary and log phase cultures (the latter yields invasive Salmonella), we have identified several phosphorylation sites on invasion and motility related proteins. To our knowledge, this represents the first evidence in the association between phosphorylation and Salmonella virulence. Reference: 1. B,M. et al. Mol Cell Proteomics. 2008 Feb;7(2):299-307. 2. B,M., et al. Mol Cell Proteomics. 2007 Apr;6(4):697-707.

Poster	16 - (Confirmation No. 2742)
Submitted by	Farzin Khosrow-Khavar, Department of Biochemistry, UBC
Contributing Authors	Alex Ng, Dr. Thibault Mayor (principal investigator)

Abstract Title	Characterizing the Saccharomyces cerevisiae Cytoplasmic Protein Quality Control System Using Model Substrates
Abstract	A significant pool of aberrant proteins is targeted by the protein quality control (PQC) machinery of the ubiquitin system in the cytoplasm of eukaryotic cells. The array of substrates targeted by the PQC system remains elusive. The ubiquitin system relies on a vast network of proteins. For instance, in the human genome there are over 500 putative E3 ligases. We will use temperature-sensitive model substrates to characterize the PQC system targeting aberrant proteins within the cell. We are currently building a library of temperature-sensitive mutants corresponding to cytoplasmic proteins that are specifically degraded at the non-permissive temperature. In a preliminary screen, we identified two potential candidate substrates that are temperature-sensitive alleles of glutamyl-tRNA synthetase (GUS1) and pyrroline-5-carboxylate reductase (PRO3) with half-lives of 45 and 40 minutes respectively, as determined by cycloheximide chase experiments. The PQC system targeting these substrates will be identified by screening for all known and putative E3 ligase deletion strains and chaperone proteins (CPs) that prevent degradation of these essential proteins and thus restore viability at the non-permissive temperature. Upon identification of E3 ligases and chaperone proteins targeting the model substrates for turnover, the physiological targets of ligases and CPs will be determined using quantitative mass spectrometry.

Poster	17 - (Confirmation No. 2743)
Submitted by	Vincent Chen, University of British Columbia
Contributing Authors	Christian C. Naus, Leonard J. Foster
Abstract Title	Specific Sites of Ubiquitylation and Components of the Ubiquitin Proteasome System (UPS) Associated with Gap Junction Protein Connexin43
Abstract	The aberrant expression of the gap junction protein connexin43 (Cx43) is associated with a range of debilitating pathologies including the propagation of stroke damage and the progression of malignant tumors. Using quantitative peptide-level dimethylation and LC-MS/MS, we have identified components of the ubiquitin-proteasome system (UPS), a regulatory network implicated in the trafficking and degradation of ion channels and membrane receptors. Amongst the proteins found to associate with Cx43 at gap junction in C6 cells and primary mouse astrocytes, we have identified ring finger-protein TRIM a component of the cullin-ring ubiquitin ligases, ubiquitin, and unexpectedly, lid components of the (26s) proteasome. Serving as further evidence, we additionally identified the specific sites of Cx43 ubiquitylation and associated K48-linked polyubiquitin chains, which we speculate serves to regulate the intracellular localization and retrograde turnover of the gap junction protein. Findings presented within this study provide mechanistic insights into the post-translational regulation and trafficking of gap junction proteins, and more generally will serve as a foundation for future investigations related to aberrant levels of Cx43 in a variety of diseases. Acknowledgements: Funded by a grant from the Canadian Institutes of Health Research; V.C.C. holds a Heart & Stroke Foundation of Canada Post-doctoral Fellowship; L.J.F. holds a Canada Research Chair in Organellar Proteomics; C.C.N. holds a Canada Research Chair in Gap Junctions and Disease.

Poster	18 - (Confirmation No. 2750)
Submitted by	Inga Wilde, Dept. of Biochemistry and Molecular Biology, UBC
Contributing Authors	Maria Brack, Thibault Mayor: Dept. of Biochemistry and Molecular Biology, UBC, Ca
Abstract Title	Mass Spectrometric Analysis of the Composition of Protein Aggregates Induced upon Proteasome Inhibition
Abstract	Protein aggregation in the cell is connected to certain neurodegenerative disorders, e.g. Parkinson's Disease (PD). Protein aggregates can also be induced by proteasome inhibition in cell culture. Most proteins targeted for proteasomal degradation are labelled with ubiquitin, which is also found enriched in protein aggregates in PD. Using human neuroblastoma cells, we determined that upon chemical proteasome inhibition ubiquitin-enriched aggregates are induced. We established a procedure implementing a sucrose gradient to enrich for these induced aggregates. Preliminary MS analysis showed that a large number of proteins could be identified in sucrose fractions derived from cells treated with MG132 but not from control DMSO-treated cells, e.g. several heat shock proteins, the ubiquitin and ribosomal protein S27a precursor, dynein, vimentin and internexin. These data suggest that proteasome inhibition has a broad impact on the proteome and that it induces the aggregation of a large variety of proteins. We are currently repeating the experiment using SILAC.

Poster	19 - (Confirmation No. 2752)
Submitted by	Julian Saba, Thermo Fisher Scientific
Contributing Authors	Zhiqi Hao, Jae C Schwartz, Andreas Huhmer
Abstract Title	Protein N- and C-terminal Sequencing Using Electron Transfer Dissociation Mass Spectrometry
Abstract	Electron transfer dissociation (ETD), compared to collisional activation, is relatively insensible to the size, the amino acid composition and post-translational modifications of peptides or proteins. The fact that ETD randomly cleaves backbone bonds makes it an advantageous tool for large peptide and intact protein analysis. ETD of intact proteins performs with high efficiency, generating very informative, yet extremely complex spectra which contain highly charged product ions that are difficult, or even impossible to resolve at unit resolution. ETD was recently implemented in a hybrid linear ion trap - Orbitrap mass spectrometer. The high resolution and accurate mass of the Orbitrap would greatly facilitate the analysis of intact proteins using ETD. For unit resolution instruments, proton transfer reaction (PTR) following ETD was developed to reduce spectral complexity. PTR removes protons from the multiply charged product ions, generating a simplified spectrum that contains product ions of resolvable charge states at unit resolution. PTR has recently implemented in LTQ XL under instrument control software. In this study, ETD was applied to proteins top-down analysis both in hybrid linear ions trap and in unit-resolution linear trap. ETD combined with accurate mass and high resolution was employed to study optimized conditions for intact protein sequencing. The performance of PTR for ETD spectra simplification was evaluated. The utility of ETD-PTR approach for intact protein analysis in unit-resolution linear trap was also investigated. Using ETD with accurate mass and high resolving power, standard intact proteins ranging in size from 8 kDa to 46 kDa were analyzed. The resulting spectra are information rich, containing multiply charged c/z. type of product ions which are well resolved. The optimized ratio of analyte cation and ETD reagent anion was investigated for maximum sequence coverage. The

	optimized ETD reaction time for maximum sequence coverage was found to be significantly shorter than ETD reaction time for peptide fragmentation. Longer ETD reactions lead to decreased overall sequence coverage while N- and C-terminal sequence coverage was increased. When ETD of intact proteins was performed in unit-resolution instrument, multiply charged product ions in the resulting spectra could not be resolved adequately. Thus, reducing product ion charge state to produce simplified spectra is necessary for data interpretation. Charge reduction can be achieved either by extended ETD reaction, or by PTR. Our results indicated that c/z. type ions generated by extended ETD reaction contain one or more extra hydrogen than expected, due to charge reduction through multiple electron transfers. These ions are excluded from identification by database search software due to the unexpected mass shift. Furthermore, extended ETD reaction generated secondary fragmentation products which interfere with data analysis. PTR, which reduces product ion charge state by subtracting protons, generates c/z. type ions of expected mass for data analysis software. Our data from intact proteins up to 30 kDa indicated that PTR following ETD in linear trap significantly improves sequence coverage when compared to ETD alone. The utility of ETD-PTR in unit resolution linear trap for intact protein analysis, as well as its limitations, will be discussed.

Poster	20 - (Confirmation No. 2755)
Submitted by	Alain Doucet, UBC
Contributing Authors	Alain Doucet and Christopher Overall
Abstract Title	ATOMS: A Protease Substrate Validation Method Using Quantitative Proteomics
Abstract	Recently, several methods to discover in vivo protease substrates based on high throughput screening of biological samples were developed. These techniques reveal candidate protease substrates that need to be validated. The direct action of the protease on the target protein and the cleavage sites are confirmed by incubation of the candidate substrate and the protease of interest in vitro. The protease cleavage sites are usually identified by N-terminal sequencing of the protein fragments resolved by electrophoresis. To be successful, pure preparation of the targeted protein is required and N-terminal sequencing reactions should be performed separately for each proteolytic fragment. This is particularly challenging when analyzing high molecular weight, complex proteins generating multiple fragments upon limited proteolysis. Moreover, the low resolution of SDS-PAGE does not allow the separation of fragments differing only by a few residues. Here, we developed a quantitative proteomics method named ATOMS (Amino Terminal Oriented Mass Spectrometry of substrates) as an alternative/complement technique to identify protease cleavage sites generated by complex, high molecular weight proteins in vitro. ATOMS is based on the fact that upon proteolysis, protein fragments present new N-termini that are not found in the undigested protein. The goal of ATOMS is to identify these neo N-termini using isotopic labeling of protein amino groups (N-terminal and lysines) and tandem mass spectrometry analysis. Peptides generated by proteolysis and bearing a neo N-termini are represented by a heavy-labeled singleton in our quantitative tandem mass spectrometry analyses while protein natural N-termini are found as doublets. All other peptides are ignored. Sequencing the heavy-labeled singletons by tandem mass spectrometry reveal the proteolytic sites. To test our method we used laminin and fibronectin, two high molecular weight and complex

	proteins found in extracellular matrices as target proteins. A control experiment shown a heavy to light (H/L) ratio centered to 1 for peptides not affected by protease activity. A small number of outliers were identified and eliminated from future analyses. Digestion of denatured fibronectin and laminin with GluC, a serine protease specifically cleaving after glutamate residues, resulted in the identification of 21 neo N-termini and all of them conformed to the GluC cleavage specificity. Proteolysis of native fibronectin and laminin resulted in the identification of 2 and 3 cleavage products, respectively. Digestion of the target protein with human neutrophil elastase, a serine protease with broad cleavage specificity, resulted in the identification of 68 peptides and were in good agreement to the known elastase specificity. Interestingly, no peptide presented a basic residue in P1, which is in accordance with published results. To compare the performance of N-terminal sequencing and ATOMS, fibronectin was digested separately with two enzymes of very broad cleavage specificity, namely matrix metalloprotease (MMP)-2 and -8 and the samples were analyzed using both methods. ATOMS identified 17 cleavage sites while 15 N-terminal sequencing reactions revealed 9 sites. Three cleavage sites were common to both methods. A total of 23 cleavage sites were identified. We can conclude that ATOMS can be used successfully to identify protease cleavage site using difficult target proteins and proteases of broad cleavage specificity. ATOMS performed better than the N-terminal sequencing when proteolysis of complex, high molecular protein generated multiple cleavage products, but the best results are achieved by a combination of both methods.

Poster	21 - (Confirmation No. 2757)
Submitted by	John Kelly, NRC-Institute for Biological Sciences
Contributing Authors	Ally Pen, Tammy-Lynn Tremblay, Phuong Le, Maureen O'Connor-McCourt, Anne Lenferink
Abstract Title	Characterization of protein expression in LCM-captured vessels from matched tumour and non-malignant breast tissue.
Abstract	While several proteomic studies have used whole tumor tissue for protein expression analyses, relatively few have analyzed protein expression in a defined population of cells within the tissue (i.e. endothelial cells). These cells likely express markers/proteins that may be important for tumor pathogenesis. A few years ago we began to study the proteome of microvessels isolated by laser-capture microdissection (LCM) from matched non-malignant and tumor breast tissues (highly vascularized and invasive ductal carcinoma, grades 1-3). The analytical challenges were significant not the least of which has been adapting our analytical protocols to accommodate the tiny quantities of proteins typically provided by LCM. Using a combination of direct on-cap tryptic digestion of the LCM-captured microvessels coupled with label-free nanoLC-MS-based proteomic analysis, we have identified several proteins that are differentially expressed in non-malignant and breast tumor microvessels. A number of these proteins have been validated by immunofluorescence. Interestingly, differential protein expression was similar across the majority of the clinical samples analyzed. This study demonstrates the utility of using LCM-extracted microvessels coupled with protein profiling methods to identify potential vascular breast tumor markers.

Poster	22 - (Confirmation No. 2758)
Submitted by	Maryvonne Rosamont-Ursulet, UBC
Contributing Authors	Angus Murray, Miriam Lynn, W. Robert McMaster
Abstract Title	Elucidation of the role of the Leishmania mexicana A600.4 gene in the regulation of amastigote growth
Abstract	Leishmania, a protozoan parasite from the family Trypanosomatidae, is responsible for the disease leishmaniasis. This disease represents a major public health risk in many tropical and subtropical regions of the world and is endemic in 88 countries on 4 continents. There is currently an urgent need for new therapies against leishmaniasis; most anti-leishmanial drugs are costly and are becoming less effective and no effective vaccine is currently available. The parasite Leishmania has a two-stage life cycle. In the insect vector, Leishmania resides as uniflagellated promastigotes. After transmission to the mammalian host, promastigotes infect macrophages where they differentiate and replicate as amastigotes. There, the parasite can evade the immune system and persist for a long time, resulting in the chronic symptoms of the disease. Because amastigotes are responsible for chronic infection of host cells in humans, research has been focused on the identification and functional analysis of amastigote-expressed genes. Elucidating their role in Leishmania persistence and pathogenesis may allow the identification of new strategies to control leishmaniasis. Comparison of global gene expression profiles between promastigotes and amastigotes indicates that very few genes are differentially expressed between the two stages. The A600.4 gene was identified as one of the rare genes upregulated at the amastigote stage in Leishmania mexicana. This led to the hypothesis that the A600.4 gene is important in amastigote development. Previous studies revealed that A600.4 is part of a gene cluster, A600, and focused on the characterization of this gene family. Current work is investigating the role of the A600.4 gene by identifying A600.4 interacting proteins, using the reductive dimethylation method. This may allow the identification of a new pathway in Leishmania that regulate amastigote proliferation and help in the development of new therapies.

Poster	23 - (Confirmation No. 2759)
Submitted by	Anna Prudova, Centre for Blood Research, UBC
Contributing Authors	Anna Prudova*, Ulrich auf dem Keller*, Christopher M. Overall
Abstract Title	Multiplex System-wide Discovery of Matrix Metalloproteinase Substrates and their Cleavage Sites
Abstract	Matrix metalloproteinases (MMPs) are zinc-containing endopeptidases that play an important role in development, wound healing, normal tissue turnover and have been implicated in heart disease, cancer, arthritis and neurodegenerative diseases. Defining a complete list of each MMPs substrates, or degradome, is warranted, in order to understand many roles MMPs play in health and disease. Moreover, identification of exact location of cleavages is warranted as this determines functional outcome of proteolytic processing. To address these questions we developed Terminal Amine Isotopic Labeling of Substrates (TAILS), as a novel proteomic screen for substrate discovery using iTRAQ multiplex reagent protein labeling, and MMP-2 as a model protease. In the TAILS approach, the sample is enriched for N-terminal peptides of each protein. Specifically, the proteomes of two samples containing active and inactive protease (control) are first reduced, alkylated and labeled with

	iTRAQ, which modifies lysine residues and protein N-termini. Following mixing the sample in 1:1 ratio and trypsin digestion, the internal unblocked peptides are selectively removed by an amine reactive polymer. The remaining N-terminome fraction that consists of protein original N-termini and proteolytically derived neo-N-termini is then analyzed by MS/MS. The resulting datasets were searched against the IPI database using Mascot and X!Tandem search engines and Trans Proteomic Pipeline (TPP). Proteins secreted by fibroblasts derived from Mmp2 knockout mouse were treated with MMP-2 in vitro and analyzed by TAILS approach. We identified 2200 peptides corresponding to 750 proteins including a number of known MMP-2 substrates, thus validating the technique. The resulting MMP-2 cleavage site specificity was in excellent agreement with the previously reported consensus sequence. We have also employed TAILS for in vivo substrate discovery in murine skin. To induce inflammation and therefore MMP activity, FVB/N wild type and Mmp2 knockout mice were treated with 12-O-tetradecanoyl-phorbol-13-acetate (TPA) or vehicle alone for 48 hours. TAILS analysis of mouse skin yielded 719 peptides corresponding to 450 proteins. Identified MMP-2 substrates are being presently validated. A cluster of ~50 proteins involved in inflammatory response was upregulated by the TPA treatment. In conclusion, iTRAQ-TAILS approach enables a multiplex analysis of protein N-termini, identification of protease substrates with the corresponding cleavage sites, as well as annotation of proteome N-terminal processing. * These authors provided equal contributions to this work.

Poster	24 - (Confirmation No. 2760)
Submitted by	Grace Cheng, Genome Sciences Centre
Contributing Authors	S.-W. Grace Cheng, Michael A. Kuzyk, Annie Moradian, Sarah E. Vollett, Takaaki Ichu & Gregg B. Morin
Abstract Title	The CrkRS/CDK12 Kinase Complexes with a Novel Isoform of Cyclin K and Phosphorylates the C-terminal Domain of RNA Pol II
Abstract	CrkRS (Cdc2-related kinase, Arg/Ser), or CDK12, is a ser/thr kinase believed to coordinate transcription and RNA splicing. While CrkRS complexes were known to phosphorylate the C-Terminal Domain (CTD) of RNA Pol II, the responsible cyclin was not known. However, others showed that co-expression of CrkRS and Cyclin L could affect the alternative splicing of a model substrate. Using immunoprecipitation and mass spectrometry (IP-MS), we identified the protein constituents of endogenous CrkRS complexes. In addition to several spliceosome-related proteins, we identified a novel 65 kDa isoform of Cyclin K, whereas the known isoform is 43 kDa. We did not observe Cyclin L. Previous studies using yeast-two hybrid and co-expression experiments identified CDK9 as a Cyclin K partner and that CDK9/Cyclin K could regulate transcription. Using IP-MS, we show that Cyclin K complexes isolated from mammalian cells contain CrkRS but do not contain CDK9, and that CDK9 complexes contain Cyclin T1 and not Cyclin K, within detection limits. The co-expression of CrkRS with either isoform of Cyclin K showed increased CTD kinase activity compared to controls. Our data suggest the primary cyclin partner for CrkRS is Cyclin K and that the Cyclin K/CrkRS complex phosphorylates the CTD.

Poster	25 - (Confirmation No. 2761)
Submitted by	Annie Moradian, Genome Sciences Centre
Contributing Authors	Annie Moradian, S.-W. Grace Cheng, Takaaki Ichu, Michael A. Kuzyk &

	Gregg B. Morin
Abstract Title	Analyzing CDK/Cyclin Sub-complexes by Multiple Reaction Monitoring
Abstract	CrkRS (Cdc2-related kinase, Arg/Ser), or CDK12 (Cyclin Dependent Kinase 12), is a ser/thr kinase believed to coordinate transcription and RNA splicing. While CrkRS complexes were known to phosphorylate the C-Terminal Domain (CTD) of RNA Pol2, the responsible cyclin was not known. However, previous studies show that co-expression of CrkRS and Cyclin L could affect the alternative splicing of a model substrate. We have isolated CrkRS protein complexes and identified a novel 65 kDa isoform of Cyclin K in endogenous CrkRS protein complexes, whereas the known isoform of Cyclin K is 43 kDa. The MS/MS data contained spectra which were assigned to a tryptic peptide in an alternative Cyclin K open reading frame that could be generated by alternative pre-mRNA processing. We did not observe Cyclin L in CrkRS protein complexes by LC-MS/MS. Using Multiple Reaction Monitoring, we have analyzed CrkRS protein complexes for sub-complexes containing Cyclin K1, Cyclin K2 and Cyclin L1. Our MRM studies show that Cyclin L1 in not present in CrkRS protein complexes and that the predominant complex is CrkRS/Cyclin K1. CDKs are known to have multiple cyclin partners which may modulate its function. Using this method, we can look at the relative abundance of specific CDK/Cyclin complexes within a cell.

Poster	26 - (Confirmation No. 2762)
Submitted by	Robert Parker, UBC
Contributing Authors	Too long for here, so in the main body
Abstract Title	Apis mellifera Proteomics of Innate reSistance (APIS): Quantitative proteomic analysis of honey bee populations within Canada.
Abstract	Robert Parker1, Marta Guarna1, Amy Tam1, Nikolay Stoynov1, Andony Melathopoulos2, Stephen F. Pernal2 and Leonard J. Foster1. 1UBC Centre for Proteomics, University of British Columbia, Vancouver, BC, Canada. 2Agriculture and Agri-Food Canada, Beaverlodge, AB, Canada. The honey bee (Apis mellifera) is the most economically valuable pollinator of agricultural crops in the world today. Bees can suffer from a variety of diseases that can significantly reduce the health of the hive. In Canada the current bee colony losses are consistently 2-fold the normal long term trends. A major factor associated with increased losses has been parasitism by the mite Varroa destructor, while American foulbrood (AFB) continues to be a disease of leading concern. Complex host/pathogen relationships exist within the hive, and mechanisms have evolved allowing bees to tolerate infestation (Annu. Rev. Entomol. 2009. 54:405√¢,Ç¨,Äú23). We have selected diverse populations of bees in Canada and are investigating the presence of heritable traits and their underlying molecular mechanisms. Our aim is to identify and quantitate protein biomarkers that are expressed in bee populations exhibiting desirable physiological and behavioural characteristics. In order to carry out the global analysis of the bee proteome we are developing tools enabling us to efficiently identify and quantitate hundreds of proteins across multiple bee tissues. Analysis of protein expression data will be insightful for understanding the distribution of phenotypes within breeding bee populations, provide markers for selection of disease resistance and suggest novel hypotheses for molecular mechanisms of disease resistance.

Poster	27 - (Confirmation No. 2763)
Submitted by	Fang-Xiang Wu, University of Saskatchewan
Contributing Authors	Jiarui Ding[1], Guy G.Poirier[2] and Fang-Xiang Wu[*1, 3] [1]Department of Mechanical Engineering, University of Saskatchewan, 57 Campus Dr., Saskatoon, SK, S7N 5A9, Canada [2]Health and Environment Unit, Laval University Medical Research Center (CHUL), Faculty of Medicine, 2705 Boul. Laurier, Quebec, QC, GIV 4G2, Canada [3]Division of Biomedical Engineering, University of Saskatchewan, 57 Campus Dr., Saskatoon, SK, S7N 5A9, Canada
Abstract Title	A real-time control workflow for tandem mass spectrum acquisition
Abstract	Tandem mass spectrometers often generate redundant spectra consisting of multiple spectra of the same peptides as well as un-interpretable poor quality spectra. To improve the efficiency and accuracy of mass spectrometry, a real-time control workflow is proposed to select peptide ions for further fragmentation and acquire tandem mass spectra for peptide identification. After a tandem mass spectrum is generated, a trained classifier is used to predict the quality of the spectrum. If the spectrum is a high quality spectrum, it is putted into a 'dynamic exclusion list'. The peptide ions of the spectra in the exclusion list will not be selected for further fragmentation for a while. Therefore, the chance for producing redundant spectra is decreased. As time elapses, the spectra in the dynamic exclusion list will be updated accordingly. In addition, only the high quality spectra are used for peptide identifications and thus much time is saved for not identifying the un-interpretable poor quality spectra. We simulate the proposed workflow and the results are very promising. This workflow will be a key component for the development of real-time control methodologies of spectrometers.

Poster	28 - (Confirmation No. 2764)
Submitted by	Devanand Pinto, National Research Council
Contributing Authors	Devanand M. Pinto; Stephen A Tate; Christie L Hunter; Kenneth Chisholm
Abstract Title	Knock down, Drag out Proteomics for Biomarker Validation
Abstract	Proteomic analysis of complex systems, such as breast cancer cell lines and patient tissues, yields detailed information that can be used for diagnostic, prognostic and theranostic purposes. However, biomarkers must first be validated in a prospective trial before they can be used in a clinical setting. Unfortunately, such trials are extremely costly. We present a method that makes use of protein knock down using siRNA, followed by targeted proteomic analysis and in vitro assays as a method to prioritize biomarkers and perform extensive in vitro validation. Proteomic analysis of three breast cancer cell lines (T47D, MCF-7 and MDA-MB-231) and two normal cell lines (HMEC and MCF10A) was performed by label-free analysis. Specifically, 1D-LC-MS/MS was performed on three biological replicated. Six technical replicates were collected for each biological replicate. PCA and N-Dimensional grouping was used to identify biomarkers that were upregulated at both the transcrpit and protein level. These putative biomarkers were then validated in vitro by two functional assays, the wound healing assay and the Matrigel invasion assay. Biomarkers that appeared promising were used to build a quantitative, MRM based method for further in vitro validation using siRNA knock down. This process allowed us to drag out 15 putative proteomic biomarkers for invasive breast cancer. The label-free proteomic analysis allowed for identification and quantification of 700 proteins using a 1D-LC analysis. Data from three biological replicates

	and a six technical replicates was collected. Upregulated proteins were verified by LC-MRM and, in some cases by Western blot analysis. In vitro validation using wound healing and Matrigel invasion assays was also performed. The biomarkers that were verified by in vitro assays were then used to construct a quantitative model of the invasive phenotype. LC-MRM using the mTRAQ√¢,Äû¬¢ reagents was then used to follow the temporal behavior of these biomarkers following knockdown with siRNA. This system allows for the perturbation of the system and monitoring of biomarkers whose expression changes in a coordinated fashion upon siRNA treatment. In this way, biomarkers that are regulated in a similar manner or in the same biochemical pathway could be identified. Overall the combination of knock down using siRNA and targeted LC-MRM analysis is a powerful method for dragging out the valid biomarkers from large proteomic analysis.

Poster	29 - (Confirmation No. 2767)
Submitted by	Jason Serpa, University of Victoria Proteomics Centre
Contributing Authors	Jason Serpa; Evgeniy Petrotchenko; Christoph Borchers
Abstract Title	Use of N-terminal modification with isotopically coded reagents and crosslinkers for selective identification of inter-peptide crosslinks
Abstract	Crosslinking combined with mass spectrometry has great potential for determining three-dimensional structures of protein and protein assemblies. One of the main analytical challenges of the method is the problematic specific detection and identification of the most informative (providing distance information) inter-peptide crosslinks in the peptide mixture obtained from enzymatic digestion of the crosslinked protein complex. We use N-terminal modification (NTM) with isotopically coded reagents, first proposed by Chen et al. (1999) in combination with isotopically coded crosslinkers (ICCL), for specific detection of interpetide crosslinks. Interpeptide crosslinks contain two amino termini, and, upon ICCL combined with NTM, exhibit a distinct isotopic signature (doublets of √¢,Ç¨≈ìtridents√¢,Ç¨¬ù of peaks with 1:2:1 intensity ratios). By using this signature, we can exclude most of the cumbersome free peptides, dead end crosslinks, and intrapeptide crosslinks from further analysis. In-house software was developed to compare ICCL and ICCL + NTM reactions in parallel and to produce a list of potential inter-peptide crosslinks. By combining ICCL with NTM, and using our software, we have developed a method for the rapid generation of a small and manageable list of interpeptide crosslink candidates that can be quickly confirmed by MSMS analysis.

Poster	30 - (Confirmation No. 2769)
Submitted by	Georgina Butler, UBC, Life Science Institute
Contributing Authors	Georgina S. Butler, Richard A, Dean, Eric Tam and Christopher M. Overall
Abstract Title	Pharmacoproteomics to validate MT1 MMP-mediated protein shedding using a hydroxamate inhibitor
Abstract	A systems biology approach to drug target validation is desirable to identify potentially unpredictable effects on the proteome. Here we present a quantitative proteomic evaluation of the targets and effects of the broad spectrum MMP inhibitor (MMPI) AG3340 (Prinomastat) on human MDA-MB-231 breast cancer cells transfected with the membrane-type 1 matrix metalloprotease (MT1-MMP). Proteins in conditioned medium and membrane fractions that were affected by the MMPI were identified by isotope-coded affinity tag (ICAT) labelling and tandem mass spectrometry. A reduction in shedding or release of proteins from pericellular sites in the

	presence of the MMPI led to the identification of a number of novel MMP-14 substrates. We demonstrate that a pharmacoproteomic screen such as this can identify key molecules and pathways which are affected by a drug that may be useful for prediction of drug side-effects, as well as the identification of novel substrates which may be therapeutic targets.

Poster	31 - (Confirmation No. 2770)
Submitted by	Carthene Bazemore-Walker, Brown University
Contributing Authors	Hongbo Gu
Abstract Title	Analysis of the Human Sigma-1 Receptor and Its Interacting Proteins Using Chemical Proteomics
Abstract	The human sigma-1 receptor (Sig-1R) mediates a prosurvival signaling pathway and has been implicated in processes associated with cancer, cardiovascular disease, and neurological disorders. Due to its involvement in a multiplicity of (patho)physiological pathways, this membrane-bound receptor is an important potential target for therapeutic intervention. Comprehensive characterization of the receptor at the protein level, which has not been done, would identify potential sites of posttranslational modification (PTM) or amino acid deviations from the gene sequence. This information is necessary for rational drug development and understanding the functional diversity of Sig-1R. To this end, we have developed a technique that not only allows for the characterization of native Sig-1Rs, but also facilitates identification of its binding partners. Our methodology could easily be adapted for broader use. An affinity resin was prepared by coupling reduced-haloperidol (RHAL), a known Sig-1R ligand, to an epoxide activated silica bead. Sig-1R was affinity-purified from membrane extracts derived from MCF7 breast cancer cells stably-transfected to overexpress the human Sig-1R (referred to as Line 41). The purified proteins bound to the affinity resin were subjected to on-bead digestions using trypsin, chymotrypsin, or Glu-C in the presence of RapiGest or urea. The resulting peptides were analyzed using LC-MS/MS. Low-passage, wild-type MCF7 cells, which do not express Sig-1R mRNA or protein, served as the negative control. Using this RHAL-resin, nonspecific interactions were minimized and purification efficiency was superior when compared to traditional immunoprecipitation (100% vs. ~2%). Based on LC-MS/MS analysis of peptides generated from on-bead digestions, approximately 84% of the Sig-1R primary amino acid sequence was characterized including putative transmembrane domain regions and the cholesterol-binding site. In addition, a novel oxidation site at tryptophan 81 was detected. Furthermore, proteins affinity-purified from the Sig-1R+ cell line (Line 41) and from the Sig-1R cell line (MCF7 cells) were compared and 48 potential binding partners of Sig-1R were identified, including a known interactor (the endoplasmic reticulum chaperone protein, BiP).

Poster	32 - (Confirmation No. 2772)
Submitted by	Hui Qiao, UBC
Contributing Authors	Hui Qiao, Victor Spicer, Werner Ens
Abstract Title	The effect of fluence and spot size on sensitivity in MALDI
Abstract	The influence of incident laser parameters on sensitivity in MALDI has been investigated using an orthogonal-injection TOF instrument. A qualitative comparison was first made between the beam profiles obtained with a N2 laser and a Nd:YAG laser using fibre optics. The N2 laser gives better

	sensitivity, consistent with broader features and therefore better coverage in the N2 laser profile. Most of the difference disappears when the fibres are twisted during irradiation to smooth out the fluence distribution. In more systematic measurements, the total integrated ion yield from a single spot (a measure of sensitivity) was found to increase rapidly with fluence to a maximum, and then saturate or decrease slightly. Thus, the optimum sensitivity is achieved at high fluence. For fluence near threshold, the integrated yield has a steep (cubic) dependence on the spot size, but the saturation fluence is higher for smaller spots. The area dependence is much weaker (close to linear) for fluence values above saturation, with the result that the highest integrated yields per unit area are obtained with the smallest spot sizes.

Poster	33 - (Confirmation No. 2773)
Submitted by	Werner Ens, University of Manitoba
Contributing Authors	Hui Qiao, Gamini Piyadasa, Victor Spicer, Werner Ens
Abstract Title	Analyte distributions in MALDI samples using MALDI imaging mass spectrometry
Abstract	The analyte distribution in MALDI matrices has been studied using MALDI imaging at 10 √Ç¬µm spatial resolution in an orthogonal-injection TOF instrument. The technique is demonstrated by mapping the analyte distribution on typical preparations of MALDI samples using the common matrices 2,5-DHB, sinapinic acid and √é¬±-HCCA, showing evidence of exclusion of impurities, and that smaller matrix crystal size gives better reproducibility from spot to spot. Large single crystals of DHB and sinapinic acid were grown to examine the incorporation of analytes within the crystals. Purified protein analytes were found to be homogenously incorporated in both types of crystal, with no evidence for preferred crystal faces. The distributions of analytes in simple mixtures in single crystals of DHB were also examined. Segregation of some species was observed and appeared to correlate with analyte hydrophobicity, and to a lesser extent analyte mass or mobility. Similar segregation phenomena were observed with confocal laser scanning microscopy of the same analytes labeled with fluorescent dyes in 2,5-DHB single crystals. The above investigations may shed some light on optimizing sample preparation with different matrices.

Poster	34 - (Confirmation No. 2774)
Submitted by	Charlotte Morrison, UBC
Contributing Authors	Stephanie Mancini, Jane Cipollone, Reinhild Kappelhoff, Calvin Roskelly and Christopher Overall
Abstract Title	Microarray and Proteomic Analysis of Breast Cancer and Osteoblast Co-cultures: Role of Matrix Metalloproteinase (MMP)-13 in Bone Metastasis
Abstract	Proteases and in particular matrix metalloproteinases (MMPs) play a pivotal role in tumor metastasis through modulation of tumor growth, angiogenesis and invasion. The cellular origin of these proteases is not always clear with both tumors and stroma contributing to the protease repertoire. Our goal is to characterize the interaction between metastatic breast cancer tumors and the bone microenvironment and the resulting changes in the protease repertoire. We use an in vitro 2-dimensional culture system in which the highly invasive human breast cancer cell line MDA MB 231 (MDA 231) and a sub-population 1833 (MDA 1833) derived by in vivo passaging with increased propensity for metastasis to bone, were overlaid onto a monolayer of differentiated osteoblast (MC3T3-E1) cells. The changes in the

	complete protease and inhibitor expression profile induced upon co-culturing of these cells were determined using the dedicated murine and human specific microarray chips (CLIP-CHIP√¢,Äû¬¢). An increase in MMP-13 mRNA expression was consistently observed when osteoblast cells were co-cultured with either MDA MB 231 or 1833. The elevation in osteoblast derived MMP-13 was observed when the co-cultured cells were in direct contact, separated by filters or when conditioned medium derived from the MDA MB 231 or 1833 was added, indicating the involvement of soluble factors. Changes in mRNA and protein expression were confirmed by QRT-PCR and Western blot analysis respectively. Proteomic analysis using differential iTRAQ labeling and multidimensional liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) revealed changes in the osteoblast secreteome upon elevation of MMP-13 levels and several novel potential MMP-13 substrates were identified. Our findings demonstrate the influence that metastatic breast cancer cells can have upon the osteoblasts, potentially manipulating the microenvironment to enhance the growth of metastases. Elucidating the dynamic relationship between breast cancer tumors and the microenvironment is essential to understanding this metastatic process.

Poster	35 - (Confirmation No. 2776)
Submitted by	H. Alexander Ebhardt, Dep't of Biochemistry
Contributing Authors	H. Alexander Ebhardt, Angela W. Fung, and Richard P. Fahlman
Abstract Title	Investigating the reaction mechanism and biological function of leucinyl/phenylalanyl tRNA protein transferase by Mass Spectrometry.
Abstract	Leucinyl/phenylalanyl tRNA protein transferase (L/F transferase) catalyzes the transfer of an esterified amino acid from tRNA(Leu) or tRNA(Phe) to polypeptides with an N-terminal lysine or arginine amino acid. Investigating this class of enzyme, which catalyze a reaction analogous to that of the ribosome, may provide insight into the still controversial aspects of ribosome catalyzed peptide bond formation. Currently investigations of these enzymes have been restricted as a result of limitations to current assay methodologies. We have recently developed a method to quantify L/F transferase activity that utilizes stable isotope labelling and quantitative analysis by matrix assisted laser desorption/ionization time-of- flight (MALDI-ToF) mass spectrometry [1]. We present the initial investigations of the wild type L/F transferase in parallel with a series of mutations to aspartic acid 186, which has been proposed to be critical for catalysis. Prokaryotic L/F transerases have been proposed to be involved in N-End rule protein degradation but to date there are no known in vivo substrates of the enzyme. To address this issue we have begun to apply proteomic approaches to understand the biological role of L/F transferases. With affinity purification protocols using L/F transferase as a bait molecule, we have identified several proteins by LC-ESI-MS/MS that associate with L/F transferase, some of which may potentially lead us to the function of L/F transferase in vivo. Reference: [1] Ebhardt,H.A., Xu,Z., Fung,A.W., Fahlman,R.P.: Quantification of the Post-Translational Addition of Amino Acids to Proteins by MALDI-TOF Mass Spectrometry. Anal. Chem. (2009) 81 1937-1943.

Poster	36 - (Confirmation No. 2777)
Submitted by	Jae Kyung Myung, BC Cancer Agency
Contributing Authors	Michael Kuzyk, Dorothy Cheung, Allen Delaney, Christoph Borchers and Marianne
Abstract Title	Quantitative Large-scale Phosphoproteome Dynamics in Rapid Signaling by Androgen
Abstract	Differentiation and function of the prostate, as well as prostate cancer growth and survival are dependent upon androgen. Androgens regulate gene transcription by binding to the androgen receptor (AR), which acts as ligand-dependent transcription factor (genomic signaling). Androgen can also cause rapid activation of protein-kinase cascades through nongenotropic (nongenomic) signaling. These rapid steroid actions are mainly transmitted by phosphorylation events. Nongenotropic signaling has been described for nearly all of the steroid hormone receptors yet to date there are no reports of large-scale analyses of the phosphoproteome induced by steroid until here. We have implemented Stable isotope labeling with amino acids in cell culture (SILAC) for large-scale proteomics coupled to LC-MS/MS to extract unbiased and quantitative data. LNCaP human prostate cancer cells were metabolically labeled prior to stimulating with dihydrotestosterone or vehicle at various time points. Phosphopeptides enriched by TiO2 were analyzed by LC-MS/MS to quantify and identify peptides with differential phosphorylation states in response to dihydrotestosterone. A total of 700 peptides defining 770 phosphorylation sites were determined. Of these, 641 were singly phosphorylated, 53 doubly phosphorylated, and 5 with >2 phosphorylated sites. Bioinformatic analysis revealed that 129 of these sites contain the phosphorylated motif pS/T-X-X-E which may indicate the importance of casein kinase 2 in rapid signaling. Identification of kinases and key substrates may yield new targets for the treatment of prostate cancer and other androgenic diseases.

Poster	37 - (Confirmation No. 2778)
Submitted by	Jun Song, Agriculture and Agri-Food Canada
Contributing Authors	Jun Song, Qifa Zheng, Leslie Campbell, Elden Rowland, Ken Chisholm, Devanand M. Pinto, David M.Byers
Abstract Title	Proteomic analysis of protein changes in fruit ripening using amine-specific isotopic labeling, two-dimensional electrophoresis and LC-MS/MS
Abstract	Proteomics is a systematic approach to study changes in proteins, providing an essential linkage between the transcriptome and metabolome. In the past few years, our research group has established a gel-based proteomic research platform as well as non-gel quantitative proteomic tools to study fruit ripening, quality and nutrition. The purpose of this study is to summarize the major research developments in fruit proteomic research. Total protein extracted from fruit tissues was separated based on protein iso-electric point and size. Due to low protein content and the presence of interfering substances, protein extraction and sample preparation are the most critical step in the two-dimensional electrophoresis (2-DE) proteomic study of fruit. Sample preparation and protein extraction protocols have been compared and optimized for apple, strawberry and banana fruit. With established, reliable and effective protein extraction procedures, protein profiles from apples and strawberry fruit at different developmental stages have been investigated. Significant changes in protein population in relation to fruit ripening and senescence have been shown. Protein that may regulate these processes have also been located and excised from gels and identified using a LC/MS/MS. In addition, a non-gel proteomic tool using amine-

	specific isotopic labeling was also modified to study protein population in apple fruit ripening. Examples of using these proteomic approaches to identify allergens from apples and strawberry fruit during fruit ripening are also shown. The challenges and limitations of using proteomic analysis on fruits and vegetables will be discussed.

Poster	38 - (Confirmation No. 2779)
Submitted by	Juan Chavez, Oregon State University
Contributing Authors	Woon-Gye Chung, Cristobal L. Miranda, Jan F. Stevens and Claudia S. Maier
Abstract Title	Mass Spectrometric Identification of 4-Hydroxynonenal Modified Proteins in THP-1 cells: Protective effects of Ascorbic Acid.
Abstract	**Introduction:** Excessive production of reactive oxygen species can lead to lipid peroxidation resulting in accumulation of lipid peroxides which decomposes to aldehydic end products such as 4-hydroxy-2-nonenal (HNE). It has been shown that ROS is elevated in atherosclerotic plaques suggesting an involvement of ROS-derived lipid aldehydes in the development and progression of atherosclerosis and associated vascular diseases. HNE is an unsaturated alkenal that can react with nucleophilic sites in proteins and DNA yielding Michael adducts and in some cases, a Schiff base with Lys residues. Covalent modifications of proteins by reactive lipid aldehydes may lead to impairment of protein function and disruption of the cellular structure. Our previous studies have demonstrated that exposure of THP-1 monocytic cells to HNE caused the formation of protein carbonyls and that ascorbic acid (Asc) pretreatment lessened the formation of protein carbonyls in the HNE-treated cells detected by ELISA using anti-DNPH antibody (Miranda et al., 2009). However, the nature of the carbonylated proteins produced in HNE-treated THP-1 cells or sites of modification by HNE was not known. In the present study, we addressed these questions by labeling of a Michael-type HNE protein conjugate with an aldehyde reactive probe (ARP) and analyzing the labeled peptides by MALDI-TOF/TOF and LTQ-FT mass spectrometry. **Methods:** THP-1 cells were cultured and incubated with or without 1mM ascorbate and 100 HNE. HNE modified THP-1 cell proteins were labeled with ARP and enriched as described by Chavez et al. (2006). The enriched ARP peptide fraction was concentrated using vacuum centrifugation before being fractionated by reverse phase liquid chromatography and spotted to a MALDI target plate using the LC-Packing Ultimate nano-LC system coupled with a Probot. Mass spectrometry was performed using an Applied Biosystems 4700 MALDI-TOF/TOF instrument and a Thermo Scientific LTQ-FT Ultra. Mascot was used to search the MS/MS data against the Swiss Prot database limited to Human taxonomy. Tandem mass spectra of potential ARP-HNE modified peptides were then manually inspected for verification of the sequence and modification. Additionally 1 and 2D electrophoresis and Western Blot analysis were performed using monoclonal anti-HNE antibodies to visualize HNE modified proteins. **Preliminary data:** Immunoblot analysis using monoclonal anti-HNE antibodies showed eighteen proteins as potential targets for HNE adduction. The levels of these HNE-protein adducts were reduced by ascorbic acid pretreatment of THP-1 cells. Using mass spectrometry the sites of HNE modification were identified at Cys residues in 12 proteins and at His residues in four proteins,

	some of which are considered new sites of HNE modification in proteins, e.g., Cys 295 in tubulin chain, Cys 499 and Cys 351 in actinin-4, Cys 328 in vimentin, Cys 369 in D-3-phosphoglycerate dehydrogenase, and His 246 in fructose-bisphosphate aldolase A. These results show for the first time the protein targets for HNE modification in THP-1 cells and the potential of ascorbic acid pretreatment in preventing HNE-protein adduct formation in these cells. **References:** (1) Miranda, C. L.; Reed, R. L.; Kuiper, H. C.; Alber, S.; Stevens, J. F., Ascorbic Acid Promotes Detoxification and Elimination of 4-Hydroxy-2(E)-nonenal in Human Monocytic THP-1 Cells. Chem Res Toxicol 2009. (2) Chavez, J.; Wu, J.; Han, B.; Chung, W. G.; Maier, C. S., New role for an old probe: affinity labeling of oxylipid protein conjugates by N'-aminooxymethylcarbonylhydrazino d-biotin. Anal Chem 2006, 78 (19), 6847-54.

Poster	39 - (Confirmation No. 2780)
Submitted by	Ulrich auf dem Keller, University of British Columbia
Contributing Authors	Anna Prudova, and Christopher M. Overall
Abstract Title	Skin inflammation degradomics: on the search for MMP-2 substrates
Abstract	Proteolysis is a major component of inflammatory processes that contribute to onset and progression of diseases such as arthritis and cancer. To better understand underlying mechanisms it is crucial to analyze proteolytic processing in complex biological systems. Here we applied Terminal Amine Istotopic Labeling of Substrates (TAILS), a novel proteomic platform for quantitative N-terminome analysis, to the global analysis of proteolysis in TPA (12-O-tetradecanoyl-phorbol-13-acetate) induced skin inflammation. First, we developed and successfully tested a mass spectrometry-compatible protein isolation and purification method for total skin lysates. Applying this method we identified 1569 proteins with high confidence from murine skin samples. In the same experiment we used TAILS to identify MMP-2 substrates in normal and inflamed skin. Wild-type and Mmp2-/- animals were treated with TPA, skin lysates were prepared and subjected to TAILS, comparing their N-terminomes to untreated controls of each genotype. We identified 1783 N-termini from 1087 proteins with a statistically significant enrichment of inflammation-related categories by Gene Ontology (GO) analysis of proteins upregulated upon TPA treatment. Among those proteins were low abundance chemokines like small inducible cytokine B5 (LIX) and macrophage inflammatory protein 2 (MIP2). Notably, the analyses were neither skewed by proteins that are highly abundant in skin, such as keratin and filaggrin, nor by serum proteins (only 23 identified). Serum amyloid A1 protein was identified by a neo-N-terminus four amino acids downstream of the mature N-terminus, which was only present in wild-type skin treated with TPA but not in skin from animals lacking MMP-2. This defines serum amyloid A1 protein as a unique MMP-2 substrate, the cleavage of which is not compensated for by other proteases in vivo. On the global level we identified a higher percentage of proteolytically processed proteins in inflamed compared to normal tissue, hence indicating that inflammation is associated with elevated proteolytic activity. This was expected, since many proteases are secreted by inflammatory cells.

Poster	40 - (Confirmation No. 2781)
Submitted by	Caroline Bellac, Centre for Blood Research, UBC
Contributing Authors	Caroline L. Bellac, Christopher M. Overall
Abstract Title	Proteomic identification of macrophage-related substrates of matrix metalloproteinase Changing the substrate repertoire by triggering inflammation
Abstract	Matrix metalloproteinase 12 (MMP-12) is a macrophage specific elastase. Through the precise cleavage of ELR+-CXC chemokines at E-LR, the critical receptor-binding motif and the specific processing and inactivation of the monocyte chemotactic proteins CCL2, 7, 8, and 13 at position 4-5, MMP-12 has been shown to be involved in regulating the recruitment of immune cells, such as neutrophils and macrophages to the site of tissue damage (or infection). To further study the role of MMP-12 in regulating inflammation, particularly the macrophage activity, we used a multiplex proteomic approach to identify new macrophage-related MMP-12 substrates by tandem-mass-spectrometry. Raw 264.7 cells were stimulated with TNF for 24 h in order to mimic an inflammatory stimulus, or treated with medium only as the control condition. 500 of secretome of each condition were incubated with exogenous recombinant MMP-12 at a ratio 1:100, or with buffer only, so generating four different conditions to be compared by labeling the proteins of each condition separately at the N-termini with four different isotopic iTRAQ-labels. Tandem-mass-spectrometry analysis in combination with database searches enabled the identification and quantification of MMP-12 generated cleavage fragments that occurred in the protease-treated samples with a higher intensity compared to the buffer-treated controls. We identified 2200 peptides in the combined sample, half of which with a ratio >10 in the protease treated samples compared to the controls. In the samples without MMP-12 addition, 50 peptides showed an increased abundance upon TNF stimulation, pointing at the fact that TNF upregulates proteins or triggers proteolysis so that the spectrum of candidate substrates may vary under inflammatory conditions. We now repeated the experiment in vivo by injecting thioglycolate intraperitoneally into MMP-12 deficient and wildtype mice to recruit macrophages into the peritoneal cavity. By isotopic labeling of the proteins present in the different peritoneal washes, we will identify the macrophage-related MMP-12 specific cleavage products occurring only in the wildtype mice and thus confirm candidate substrates found in vitro in the in vivo model.

Poster	41 - (Confirmation No. 2782)
Submitted by	David Golub,
Contributing Authors	Swapan Roy, Ph.D. (Chief Scientific Officer) Matthew Kuruc
Abstract Title	Molecular Profiling With SeraFILE™: Sub-Proteome Pools with both Differential Constituents and Activity States
Abstract	The SeraFILE™ platform – a proprietary, surface-based separations reagent set and associated exploratory protocols - addresses problems of functional proteomic prospecting. Clients and collaborators exploit its usefulness for low-abundance enrichment and prospecting as an adjunct to conventional methods, i.e., Immunoassay and Mass Spec. Preliminary data has established that SeraFILE™ can differentiate conformational variants, suggesting even the characterization of sub-unit equilibrium. Collaborations of interest center around conformational differentiation within specific protein groups of pharmacological importance (e.g., p-53, Proteasome, Kinases,

	Unfolded Protein Response). SeraFILE™ encompasses innovations in surface chemistry and associated process methods that obviate the need for any bio-engineering, greatly reducing cost and increasing throughput. SeraFILE™ separations and protocols are seamless with existing proteomic assay and detection infrastructure. Unique sub-proteomes are generated efficiently and in parallel, while maintaining the functional characteristics that define the conformational variability associated with a crude soluble protein sample.

Poster	42
Submitted by	Brinda Shah, University of Victoria Proteomics
Contributing Authors	Brinda Shah1, 2, Jennifer D. Reid1, 2, Dan Holmes3, Christoph H. Borchers1, 2 1University of Victoria Proteomics Centre, Victoria BC, 2Department of Biochemistry & Microbiology, University of Victoria, Victoria BC, 3St. Paul's Hospital, Vancouver BC
Abstract Title	Development of an Immuno Tandem Mass Spectrometry (iMALDI) Assay for the Analysis of Biomarkers from Clinical Samples
Abstract	iMALDI (immuno-MALDI) is a mass spectrometry-based method combining antibody affinity enrichment, stable isotope labeled standards, and MALDI mass spectrometry for highly sensitive, specific and quantitative analysis of target molecules. Custom synthesized isotopically labeled peptides are spiked into clinical samples as internal standards. Anti-peptide antibodies are immobilized on magnetic Protein G Dynabeads and used to immunoprecipitate endogenous target peptides and the isotopically labeled internal standards from human plasma. Antibody conjugated beads with bound peptides are analyzed directly on a target plate using a MALDI mass spectrometer to detect and absolute quantify endogenous peptides with high specificity in MS and MS/MS modes. Application of the iMALDI approach to the detection and quantitation of epidermal growth factor receptor (EGFR) isoforms in cancer cell lines and determination of plasma renin activity in human plasma for the diagnosis of hypertension indicates several advantages afforded by the approach including: improved limit of detection of MALDI mass spectrometry; provision of the specificity required to distinguish between isoforms; and multiplexed analysis of clinically relevant markers in complex clinical samples. High sensitivity in tissue (<100 cells) and plasma (pg/mL) is achievable with only small sample volumes (20 uL) and good linearity (correlation coefficient of 0.995) demonstrate clinical utility.

Poster	43
Submitted by	Edward Lau, Simon Fraser University
Contributing Authors	Edward G.M. Lau, Xin Zhou Hu, George R. Agnes
Abstract Title	Identification of Secreted Proteins from Lung Cells (A549) Dosed with Designed Particulate Matter in vitro
Abstract	Epidemiological studies have indicated that the inhalation of ambient particulate matter (PM) induce inflammation in the lung, which may lead to many other diseases. When lung cells are injured, signalling proteins are secreted to initiate the inflammation process. What remains unclear is the role of the chemical composition on the over all toxicity of PM. To better study the effects of chemical composition, solutions of known composition are dispensed into a levitation trap to form PM of known composition. Particles are then deposited onto cultures of lung cells, and have the resulting supernatant collected for analysis. Analysis by Matrix Assisted

	Laser Desorption Mass Spectrometry (MALDI-MS) show differential expression of bio-molecules between negative controls and cultures dosed with as few as 20 particles. Tandem mass spectrometry of supernatant digested with trypsin has identified the chemokine protein CXCL-5 in cultures when injury is induced. Once proteins have been identified, MALDI-MS has the potential to screen samples for specific protein expressions quickly.