Enabling
Technologies

8th International Symposium on Enabling Technologies for Life Sciences

December 1-2, 2015
The Fairmont Chateau Lake Louise
111 Lake Louise Drive, Lake Louise, AB, Canada

Chaired by
Dr. Daniel Figeys
University of Ottawa

Bringing Researchers and Scientists together to develop novel life sciences technologies.

www.etpsymposium.org

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Welcome

On behalf of the Organizing and Scientific Advisory Committees, I would like to welcome you to the 8th International Symposium on Enabling Technologies (ETP 2015). ETP Symposium has been fortunate, once again, to bring together an excellent slate of speakers representing a wide range of novel techniques that have or will have a major influence on research in the life sciences. We wish to thank our sponsors for their continued support as without them, the ETP Symposia would not continue.

The symposium offers several opportunities for discussions between attendees, speakers and sponsors. We urge everyone to take advantage of these chances to get together with others who share your scientific interests.

The ETP Symposium is held every two years in order to permit our participants to focus on their research and develop new technologies. As of 2011, we deviated from our normal course so that we may alter the emphasis from Proteomics to a more broad scope to meet the most recent advances of technology that impact the life sciences. If you have suggestions for topics to be included in future symposia, please let us know. We welcome all comments and suggestions.

Thank you for supporting the ETP Conferences.

Janette Champ
President
ETP Symposium Inc.

Organizing Committee

Dr. Daniel Figeys, University of Ottawa, Symposium Chair
Janette Champ, President, ETP Symposium Inc.

ETP Symposium Scientific Advisory Board

Ruedi Aebersold, Institute of Molecular Systems Biology, ETH Europe
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Chris Dambrowitz, Northern Alberta Institute of Technology (NAIT)
Norman Dovichi, University of Notre Dame
Daniel Figeys, University of Ottawa (Committee Chair)
Randy Johnston, Dept. of Biochemistry & Molecular Biology, University of Calgary
Pierre Thibault, Université de Montréal

History

The ETP Symposium was initiated as part of a Genome Canada project sponsored by MDS Sciex, Genome Alberta and Genome Prairie. Its major theme was to have world-renown scientists present papers on their experiences with novel technologies that have, or will have a major influence on research in the life sciences. The popularity of the original two symposia led to the creation of a not-for-profit entity, ETP Symposium Inc., who now is responsible for organizing the symposium on a biennial basis. The theme remains the same: alerting scientists in the life sciences to advances in new technology that could have a significant impact on their research activities.

The original founders of ETP are Dr. William (Bill) Davidson and Janette Champ.

Historic ETP Symposia, together with full programs, may be found on our website: www.etpsymposium.org

8th International Symposium on Enabling Technologies

Fairmont Chateau Lake Louise
Lake Louise, AB

Tuesday, December 1, 2015	
17:30 -22:00	***Registration, pick up badges*** *Welcome Reception & Dinner with Sommelier & Wine Pairing* **Alpine Room**
Wednesday, December 2, 2015	
7:00 - 8:30	*Breakfast* **Poppy Brasserie**
8:00 - 8:30	***Late Registration, pick up badges***
8:30	*Conference start* **Victoria Room**
8:30 - 8:40	*Introductions*
Conference Sessions	
8:40 - 9:10	***INVITED SPEAKER*** ***Alain Stintzi, University of Ottawa*** *Decoding the host-gut microbiota dialogue in children with inflammatory bowel disease*
	ORAL PRESENTATIONS
9:10 - 9:30	***Eric Dodds, University of Nebraska - Lincoln*** *TGlycan and Glycopeptide Separations by Ion Mobility Spectrometry: Towards Isomer Discrimination and Biomolecule Class Sorting for Glycoproteomics*
9:30 - 9:50	***Irena Peytchev, Queen's University*** *The optimization of cultural and physical conditions in cadmium sulfide quantum dot biosynthesis by the fungus Fusarium oxysporum*
9:50 - 10:20	***INVITED SPEAKER*** ***Joshua Elias, Stanford University School of Medicine*** *Characterizing host-microbe interactions in the gut: host-centric proteomics and beyond*
10:20 - 10:40	*Coffee Break*
10:40 - 11:10	***INVITED SPEAKER*** ***Dr. Leonard Foster, University of British Columbia*** *Inter-tissue differences in the interactome by PCP-SILAC*
	ORAL PRESENTATIONS
11:10 - 11:30	***Sui-Lam Wong, University of Calgary*** *Development of a streptavidin-based system with both reversible binding and immobilization capabilities for recombinant proteins tagged with an engineered streptavidin-binding peptide*
11:30 - 11:50	***Peter Verhaert, Lund University*** *Antibody-free analysis of human secretomes for medical applications. Mass spectrometry based detection of secretory peptides proteins in primary cell culture media.*
11:50 - 12:10	***Lars Konermann, Western University*** *Using MD Simulations to Understand the Mechanism of Protein Electrospray Ionization*

8th International Symposium on Enabling Technologies

Fairmont Chateau Lake Louise
Lake Louise, AB

Wednesday, December 2, 2015	
12:10 - 12:30	*Short Break*
12:30 - 13:30	*Lunch Seminars - lunch served* *12:45 - 13:00 — Thermo Fisher Presentation -* ***Rosa I. Viner*** *Advances in Orbitrap Technology* *13:00 - 13:15 — SCIEX presentation -* ***Brigitte Simons*** *Quantitative and Qualitative Metabolomics for the Investigation of Interacellular Metabolism*
13:30 - 14:00	*Poster Session (sponsored by Waters) Coffee & Desert*
14:00 - 14:30	***INVITED SPEAKER*** ***Olga Schubert, ETH Zurich*** *Genome-wide relative and absolute protein quantification in the human pathogen Mycobacterium tuberculosis by SWATH MS*
	ORAL PRESENTATIONS
14:30 - 14:50	***Jeffrey Smith, Carleton University*** *Enhancing the sensitivity of MS-based proteomics and phosphoproteomics using TrEnDi*
14:50 - 15:10	***Derek Wilson, York University*** *When Proteins Go Rogue: Conformational Disorder in Cancer and Neurological Disease*
15:10 - 15:30	***K.W. Michael Siu, University of Windsor*** *Prognostic Significance of Head and Neck Cancer Biomarkers: Translation into Oral Surgery*
15:30 - 15:50	*Coffee Break*
15:50 - 16:10	***Bill Davidson Student Travel Award Winner*** ***Siavash Vahidi, University of Toronto*** *Uncovering the Dynamic Interactions of the FoF1 ATP Synthase Molecular Machine by H/D Exchange Mass Spectrometry* Sponsored by: SCIEX
16:10 - 16:40	***Ken Standing Award Winner*** ***Dr. Benjamin Garcia, University of Pennsylvania*** *Quantitative Proteomics for Understanding Modified Proteins and Proteomes* Sponsored in part by: University of Manitoba
16:40	***Closing Remarks - Conference Ends***

Symposium Chair

Daniel Figeys

University of Ottawa

Daniel Figeys is a professor in the Department of Biochemistry, Microbiology and Immunology at the University of Ottawa. He is also 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. His laboratory has published over 110 papers and has been cited over 6000 times.

Research Interest:

Our laboratory focuses on the development and application of proteomics and lipidomics technology. For example, we created a device, called the Proteomic Reactor that allows the concentration and digestion of proteins in 60nl of volume. We are using the technologies developed in our laboratory to study biological processes. For example, we study PCSK9 and its impact on liver cells, the Proteome and lipidome in neuronal diseases, and more recently, we have been working on the proteome of inflammatory bowel diseases.

Invited Speakers

Dr. Joshua E. Elias
Stanford University

ABSTRACT:

Characterizing host-microbe interactions in the gut: host-centric proteomics and beyond

The intestinal microbiome has profound and diverse effects on host physiology, but the precise nature of host-microbe interactions remains difficult to measure. Specific microbial sub-populations, enumerated through 16S sequencing, are often correlated with health and disease states. This approach cannot directly report dynamic host responses to perturbations to the microbiota as mediated by diet, pharmaceuticals, or opportunistic infections, however. We previously developed a mass spectrometry technique, "host-centric proteomics of stool," which directly measures proteins secreted or shed into the GI in response to changing intestinal ecosystems. With this approach, we quantified host responses to antibiotic-associated infections in mice, and demonstrate that the host proteome, as measured from stool, provides a sensitive and specific assay of different microbiotas and inflammation states. In certain cases, proteomic output proved superior to DNA sequencing techniques in distinguishing clinically relevant intestinal states.

As we extend our efforts to humans, we are broadening the reach of this powerful technique with new computational tools. Novel software we developed enables protein discovery at unprecedented depths, from virtually any organism, -- even in the absence of paired metagenomic sequencing. With it, we can characterize dynamic host, microbial and dietary interactions directly from stool. This is a fundamental step towards understanding the forces that shape the gut microbiome, and how it in turn can shift the balance between health and disease.

Leonard Foster
University of British Columbia

ABSTRACT:

Inter-tissue differences in the interactome by PCP-SILAC

Modern mass spectrometers and bioinformatic workflows have enabled researchers to monitor >10000 proteins in a single sample but the interaction network (a.k.a., interactome) connecting these proteins remains challenging to measure. Traditional high-throughput interactome approaches, e.g., affinity-based approaches, are vastly too laborious so we developed an alterative approach based on protein correlation profiling (PCP). Seven tissues (Brain, Lung, Liver, Heart, Skeletal Muscle, Thymus and Kidney) were isolated from SILAC (Lys-6) and normal mice and complexes were resolved by size exclusion chromatography. SILAC labeled samples were then used to generate a global reference mixture that was added to all non-labeled samples and the resulting samples were analyzed on a Q Exactive and processed using MaxQuant. Protein chromatograms were then processed and compared in MatLab to generate the first interactome map of multiple mice tissues. This study was designed

to allow both the identification of protein interactions within specific tissue, demonstrate quality characteristics, e.g., precision, false discovery rate and false negative rate, equal to or better then typical tagged based approaches. Using our PCP-SILAC approach 9063 protein groups were identified across the seven tissues with 8231 protein groups leading to the generation of unique Gaussian fitted profiles. Interestingly a large proportion of proteins were only observed within a limited range of tissues, suggesting interactome of each tissue is highly specialized. From the detected Guassians fitted profiles 31518 protein interactions could be determined with a precision of ~65% as compared to the CORUM database. This dataset represents the largest interactomes to data.

Olga Schubert
ETH Zurich

ABSTRACT:

Genome-wide relative and absolute protein quantification* in the human pathogen *Mycobacterium tuberculosis by SWATH MS

Contributing Authors: Olga T. Schubert[1], Christina Ludwig[1], Maria Kogadeeva[1], Michael Zimmermann[1], George Rosenberger[1], Martin Gengenbacher[2,3], Stefan H. E. Kaufmann[2], Uwe Sauer[1], Ruedi Aebersold[1,4]

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With one third of the world's population latently infected, tuberculosis remains one of the great global health challenges. However, the development of more efficient intervention measures is hampered by the incomplete understanding of the molecular mechanisms underlying basic physiological processes of *Mycobacterium tuberculosis* (Mtb) during infection. Over the past years, targeted mass spectrometric techniques such as Selected Reaction Monitoring (SRM) and SWATH MS have emerged, which use specific mass spectrometric coordinates to achieve highly consistent, sensitive and accurate protein quantification. We established a strategy to build large-scale libraries of high-quality SRM and SWATH MS assays and applied it to develop assays for all 4000 annotated proteins of Mtb. Furthermore, we implemented a label-free method to estimate absolute cellular protein concentrations on a genome-wide scale based on SWATH MS data. Together, these tools enabled us to monitor the global remodeling of the Mtb proteome under clinically relevant stress conditions, providing unprecedented insights into proteome composition and dynamics. Specifically, by relating absolute abundances of metabolic enzymes to corresponding metabolites using a genome-scale metabolic model we could determine the biomass investment of the cell into specific compartments of metabolism and infer the fluxes though metabolic pathways.

The presented methods and data sets may broadly impact Mtb research in the coming years by facilitating system-level approaches addressing complex biological processes, such as host-pathogen interactions. Furthermore, they may open new avenues in key application-related fields of mycobacterial research, such as vaccinology and drug discovery, to improve intervention and prevention measures for tuberculosis.

Alain Stintzi
University of Ottawa

ABSTRACT:

Decoding the host-gut microbiota dialogue in children with inflammatory bowel disease

An intricate and essential partnership is established early in life between the host and the intestinal microbial community. Disturbance of this partnership is often associated with the development of diseases and various pathological conditions including inflammatory bowel diseases (IBD). IBD is characterized by chronic and relapsing inflammation of the intestine, with up to ¼ of IBD patients diagnosed before adulthood. IBD is comprised of two main subtypes, Crohn's disease (CD) and ulcerative colitis (UC). There are no cures for IBD and the ultimate cause also remains unknown. Alterations in the composition of the gut microbiota are thought to play an important role in the pathogenesis of IBD. However, we don't know whether microbial alterations directly cause IBD, are important in the chronicity of CD and/or UC or whether the altered compositions are simply consequences of these diseases. The main objective of our study was to address these keys questions, decode the host-gut microbiota dialogue and assess the causal effect of the microbiota on the host using a combination of meta-genomic and meta-proteomic approaches together with a cohort of clinically well-defined pediatric IBD patients.

Oral Presentations

Eric Dodds
University of Nebraska-Lincoln

ABSTRACT:

TGlycan and Glycopeptide Separations by Ion Mobility Spectrometry: Towards Isomer Discrimination and Biomolecule Class Sorting for Glycoproteomics

Contributing Authors: Abby S. Gelb, Yuting Huang, and Eric D. Dodds

As one of the most taxonomically ubiquitous and functionally pivotal post-translational modifications (PTMs) of proteins, glycosylation has captured a great deal of scientific interest across a number of disciplines. A complete understanding of the structures of glycans and their corresponding glycoconjugates is essential to elucidate their biological functions in molecular detail; however, large-scale, systems-oriented glycoproteomics remains largely unrealized and represents a grand challenge in post-genomic science. Mass spectrometry (MS) has found broad application to glycan and glycoconjugate analysis; however, the distinction of glycan isomers remains a challenging endeavor. Moreover, the analysis of glycopeptides occurring in complex glycoproteomic preparations is complicated by several factors, including the detection of glycopeptides against a background of unmodified peptides which generally have greater ionization efficiency. Ion mobility spectrometry (IMS) has significant potential to contribute novel solutions in the area of isomeric glycan discrimination, as well as in the arena of glycopeptide vs. peptide sorting. This presentation will focus on: (1) the use of IMS in conjunction with metal ion adduction and gas-phase electron transfer reactions to generate conformationally distinct species which allow for improved distinction of glycan isomers by IMS, and (2) IMS studies of the chemical and physical properties which dictate the mobility vs. mass space that is occupied by protonated glycopeptides. Implications for glycoproteomic analysis will be discussed.

Lars Konermann
Western University

ABSTRACT:

Using MD Simulations to Understand the Mechanism of Protein Electrospray Ionization

The mechanism whereby gaseous protein ions are released from charged solvent droplets during electrospray ionization (ESI) remains a matter of debate. Also, it is unclear to what extent electrosprayed proteins retain their solution structure. Molecular dynamics (MD) simulations offer insights into the temporal evolution of protein systems. Surprisingly, there have been no all-atom simulations of the protein ESI process to date. The current work closes this gap by investigating the behavior of protein-containing aqueous nanodroplets that carry excess positive charge. We focus on “native ESI”, where proteins initially adopt their biologically active solution structures. ESI proceeds while the protein remains entrapped within the droplet. Protein release into the gas phase occurs upon solvent evaporation to dryness. Droplet shrinkage is accompanied by ejection of charge carriers (Na^+ for the conditions chosen here), keeping the droplet at ~85% of the Rayleigh limit throughout its life cycle. Any remaining charge carriers bind to the protein as the final solvent molecules evaporate. The outcome of these events is largely independent of the initial protein charge and the mode of charge carrier binding. ESI charge states and collision cross sections of the MD structures agree with experimental data. Our results confirm the Rayleigh/charged residue model (CRM). Field emission of excess Na^+ plays an ancillary role by governing the net charge of the system. Models that envision protein ejection from the droplet are not supported. Most nascent CRM ions retain native-like conformations. For unfolded proteins ESI likely proceeds along routes that are different from the native state mechanism explored here.

Irena Peytchev
Queen’s University

ABSTRACT:

The optimization of cultural and physical conditions in cadmium sulfide quantum dot biosynthesis by the fungus Fusarium oxysporum

Contributing Authors: Irena K. Peytchev and Daniel D. Lefebvre

Nanoparticle biosynthesis by fungi is an emerging branch of nanotechnology as a cost-effective and environmentally benign method of producing commercially viable nanoparticles. Fusarium oxysporum uses extracellular enzymes and precursors to produce cadmium sulfide (CdS) quantum dots (QDs) within the 2-50 nanometer range. In order to produce the greatest yield of CdS QDs, it is necessary to optimize the cultural and physical conditions for biosynthesis. The growth media, fungal biomass, pH, NaCl and CdSO4 concentrations, and temperature each influenced the yield of CdS QDs. Different concentrations of the capping agent, 3-mercaptopropionic acid, and the reducing agent, sodium borohydride, were found to shift the size and colour output from blue to yellow, demonstrating tunability. The aims of the present study were to optimize different conditions of F. oxysporum growth and enhance the secondary metabolism with the purpose of increasing monodisperse QD yield.

K.W. Michael Siu
University of Windsor

ABSTRACT:

Prognostic Significance of Head and Neck Cancer Biomarkers: Translation into Oral Surgery

Contributing Authors:
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There has been much criticism about discovered biomarkers not making it into clinics. A reason for this paucity is that the skill sets for discovery and translation are different, and success requires building collaboration and having the right collaborators at the appropriate time. Here we present our experience in head and neck cancer (H&NC). H&NC biomarkers were identified by MS-based differential proteomics using iTRAQ and verified using immunohistochemistry. Their prognostic values were assessed for oral premalignant lesions' (OPLs') potential for transformation into cancers. A commercial test has been developed for use in oral maxillofacial surgery clinics.

iTRAQ analyses enabled the identification of a suite of biomarkers, including 14-3-3 zeta, 14-3- 3 sigma, S100A7, hnRNPK and prothymosin alpha, for H&NCs and OPLs, and verified on >300 patient samples. We tested their effectiveness on an additional set of 110 patients with biopsy-proven OPLs with known clinical outcomes. The best-performing biomarker in correlating expression with high-risk malignant transformation is S100A7 (p-value = 0.014, odds ratio = 4.706). Patients who overexpressed S100A7 had a mean oral-cancer-free survival period of ~70 months versus ~120 months for those who did not. S100A7's expression impacts upon beta-catenin, and promotes tumor differentiation and secretion of cytokines. The commercial version of the test, Straticyte, has been developed by Proteocyte Diagnostics Inc. Straticyte is an immunohistochemistry-based test that assesses the expression and distribution of biomarkers, compares these to a reference database, and generates a score that expresses the progression risk of a given OPL. The score is intended to aid the surgeon in deciding the best personal therapy: high-risk lesions may require immediate attention, probably surgery plus radio- and/or chemotherapy; low and moderate risk lesions could be monitored closely.

Jeffrey Smith
Carleton University

ABSTRACT:

Enhancing the sensitivity of MS-based proteomics and phosphoproteomics using TrEnDi

Contributing Authors: Blank, Katrin; Wasslen, Karl, V; Koudrina, Anna; Manthorpe, Jeffrey, M; Smith, Jeffrey, C

Over the past few decades, mass spectrometry (MS) has proven to be a powertool in the analysis of cellular life. Despite numerous advances, many biomolecules remain difficult to analyze by MS either due to their inherent chemical properties or their low concentration. MS-based determination of phosphopeptides remains a challenging field due to dynamic range issues as well as the relatively high hydrophilicity and low proton affinity of phosphopeptides compared to their non-phosphorylated counterparts. Additionally, achieving

high degrees of sequence coverage for proteins remains challenging as increased complexity of co-eluting peptides leads to ion suppression for some analytes. To address this, we have developed a new and simple chemical derivatization strategy termed Trimethylation Enhancement using Diazomethane, or TrEnDi, that dramatically increases the signal strength of peptides and phosphopeptides when analyzed by MS. TrEnDi operates by methylating any functional group with a pKa less than 11, thereby trimethylating primary amines, dimethylating imidazole and phosphate groups, methylating phenol and thiol groups and esterifying carboxylic acids. Both peptides and phosphopeptides are rendered permanently positively charged, eliminating the need for protonation resulting in increased sensitivity when analyzed by MS. Sequence coverage enhancements for proteins have been demonstrated and peptide analytes below the limit of detection prior to modification are observable post-TrEnDi derivatization. TrEnDi also yields a novel highly sensitive neutral loss scan for phosphopeptides via the loss of dimethyl phosphoric acid. TrEnDi has been proven to be fully compatible with chromatographic and microfluidic workflows. TrEnDi-modified peptides also yield predictable fragmentation behavior, permitting the a priori design of multiple reaction monitoring experiments as well as the use of commercial peptide identification software tools.

Peter Verhaert
Lund University
Department of Clinical Sciences, Faculty of Medicine

ABSTRACT:

Antibody-free analysis of human secretomes for medical applications. Mass spectrometry based detection of secretory peptides proteins in primary cell culture media.

Even with the latest bioanalytical technology and the most performant mass spectrometers the unbiased/untargeted analysis of secreted peptides and proteins from living (in particular human) systems (cells, tissues, organs,…) remains quite challenging.
Analytical scientists hunting for (often small and low abundant) native extracellular peptides face apparent difficulties to identify these in the complex biological background of highly abundant large proteins, unless they employ highly specific and selective antibodies. Indeed the enormous difference in concentration between extracellularly secreted signalling peptides and serum proteins like albumin has made the former virtually impossible to detect in conventional mass spectrometry based bottom-up proteome analyses.
In addition, also top-down analyses with smart sample preparation steps including ultrafiltration and/or immunodepletion to get rid of the uninteresting high abundant background proteins failed to result in the successful detection of signalling peptides, let alone the discovery of novel ones.

Over the past years, we have optimized a workflow which now enables us to successfully detect and identify known as well as novel signalling proteins and peptides. We will explain our model system, i.e. the *ex vivo* T-cell, one of the main players in the human immune system, which we analyzed the conditioned cell culture medium of to demonstrate proof of principle.

Naive human T-lymphocytes from two adult male donors were harvested from buffy coats, sorted by FACS (CD4+, CD25-), and put in primary culture. The changes in protein composition of the cell culture medium were studied over time after T-cell activation by anti-CD3 and anti-CD28.

At 3 time points after activation (6, 24 and 48h), conditioned media were analyzed by conventional (bottom-up) proteomics. After centrifugation of the cell culture medium to remove cells, proteins were fractionated by C_4 RP HPLC. The resulting 13 fractions were individually reduced, alkylated, and digested by trypsin. Tryptic peptides were analyzed by C_{18} RP nanoLC MS/MS on a linear ion trap-orbitrap hybrid instrument (LTQ Orbitrap Velos, ThermoFisher). Data were analyzed by 2 different workflows, one including Mascot and spectral counting for label-free protein quantification, the other employing Sequest in combination with 'Quanti', a protein quantitation algorithm developed at Karolinska [Lyutvinskiy Y, Yang H, Rutishauser D, Zubarev RA (2013) Mol Cell Proteomics 12: 2324-31].

From over 512 hours of LC MS/MS analyses, yielding >130 Gb data, more than 95% of the data matched with bovine proteins, evidently originating in the culture medium FCS (fetal calf serum) supplement. Yet the <5% human peptides identified clearly reveal some of the cell biological (re)actions of the *in vitro* cell to its transfer *ex vivo*. In addition, clear activation signals were detected, including various interleukins (IL2, IL9, IL17A, IL17F, IL37…), GDF9, TNFalpha, IFNgamma, CCL4, granulins, and others. For several of these secretory proteins their differential presence in the activated versus non-activated cell media was orthogonally validated by ELISA.

We want to underline that our primary detection of these activation 'signals' was entirely unbiased, using

orbitrap based MS as sole detection device and without employing any antibody.

We conclude that upon rapid adaptation by the *ex vivo* T-cell to an artificial medium, an optimized secretomics approach allows one to subsequently measure many typical extracellular activation proteins, described earlier as T-cell communication signals.

Derek Wilson
York University

ABSTRACT:

When Proteins Go Rogue: Conformational Disorder in Cancer and Neurological Disease

Looking at the beautiful X-ray crystallographic protein structures that are the focus of classical structural biology, one could be forgiven for believing that proteins just sit there and look pretty. But if they were truly as immobile and 'brick-like' as they appear in these images, most would be virtually non-functional. In fact, proteins are constantly undergoing thermally-driven conformational fluctuations - conformational dynamics - in which they briefly adopt higher energy structures that are critical to function. Virtuallly all of the processes that underlie biological activity - ligand binding, allostery, catalysis and functional control through modification - would be impossible without the ability to 'visit' alternate structures through dynamics. Don't believe me? Try getting your favorite hyperthermophile enzyme to work at room temperature (it won't... at least not very well)... But as vital as it may be, all this moving around, morphing from one shape to another is dangerous. What if these conformational fluctuations go too far? What if we get stuck in 'non-native' structures that are pathogenic? The answer is: Bad stuff happens. Usually to your neurons. In this talk, we'll discuss how we characterize high energy, transient structures in protein function and disease, with a view to 'pacifying' the danger they can sometimes represent.

Sui-Lam Wong
University of Calgary

ABSTRACT:

Development of a streptavidin-based system with both reversible binding and immobilization capabilities for recombinant proteins tagged with an engineered streptavidin-binding peptide

Contributing Authors: Dawson Fogen, Sau-Ching Wu, Kenneth K. S. Ng and Sui-Lam Wong

Streptavidin-binding peptide (SBP) tag is a short peptide tag that can bind to streptavidin in nano-molar affinity. SBP tagged proteins can be affinity purified using either wild-type streptavidin or an engineered streptavidin (SAVSBPM18) which binds both SBP tag and biotin in a reversible manner. To extend the SBP tag technology from reversible binding for protein purification to reversible immobilization for the development of reusable biosensor chips and other applications, a cysteine residue was introduced to SAVSBPM18 and the SBP tag to generate SAVSBPM32 and the SBP(A18C) tag, respectively. The resulting pair of derivatives can form a disulfide bond in an affinity driven manner. SAVSBPM32 and SAVSBPM18 have comparable binding affinities towards biotin and the wild-type SBP tag. Although the introduction of cysteine in SBP results in lowering the binding affinity between SBP(A18C) and SAVSBPM32, both the cysteine containing tag and the streptavidin mutein can still interact efficiently before they are locked together via disulfide bond formation ? a phenomenon we have named affinity-driven thiol coupling. With SBP(A18C) tags in excess, a tetrameric SAVSBPM32 mutein can bind maximally two SBP(A18C) tags. To illustrate the application of this reversible immobilization technology, optimized conditions were established to use the SAVSBPM32-affinity matrix for the purification of a SBP(A18C)-tagged reporter protein to high purity. Furthermore, we show that the SAVSBPM32-affinity matrix can also be applied to purify biotinylated proteins and a reporter protein tagged with the unmodified SBP-tag. The dual (covalent and non-covalent) binding modes possible in this system offer great flexibility to many different applications.

LUNCHEON TECH TALKS

Brigitte Simons
SCIEX

ABSTRACT:

Quantitative and Qualitative Metabolomics for the Investigation of Intracellular Metabolism

Contributing Authors:
Brigitte Simons; Baljit Ubhi; Douglas McCloskey

Studying intracellular metabolism of model organisms, such as E. coli, is vital to further our biochemical knowledge1, to develop new pharmaceuticals that target harmful pathogens2 and to improve industrial applications that aim to metabolically engineer bacteria in order to produce commodity chemicals from renewable resources3. Paramount to these endeavors is the ability to reliably and accurately measure the intracellular metabolome. By measuring the absolute metabolite levels of such compounds, one is able to calculate reaction and pathway thermodynamics4 and infer in vivo enzyme kinetics5. When microorganisms are grown on a specifically chosen labeled substrate (e.g. 1-13C glucose) during a metabolic labeling experiment, the isotopomer distribution of intracellular compounds can be used to calculate absolute flux through specific reactions of interest6.

Samples of E. coli were grown in 4 g/L glucose or complex M9 minimal media8 with trace elements9 and sampled from an anaerobic chamber4. Samples were extracted using a fast filtration approach and serially extracted using a mix of acetonitrile, formic acid, methanol and water. An LC-QTRAP 5500 System (SCIEX) was used for targeted profiling. Samples were acquired using the Scheduled MRM? Pro Algorithm in Analyst? Software 1.6.2. The information dependent acquisition (IDA) method consisted of a multiple reaction monitoring (MRM) survey scan coupled with an enhanced product ion (EPI) scan for compound identity confirmation. Samples were quantified using IDMS7, 10 with metabolically labeled internal standards from E. coli and processed using MultiQuant? Software 2.1.1.

The quantitative workflow uses the Scheduled MRM? Pro Algorithm to maximize method efficiency. When a signal is detected in the MRM scan, acquisition of an enhanced product ion (EPI) spectrum is triggered via IDA. The acquired MS/MS spectra can then be searched against a library of compound spectra from pure standards to provide greater confidence for compound detection. The compounds targeted in this workflow are involved in pathways that include central carbohydrate metabolism (i.e., glycolysis, the pentose phosphate pathway, and the citric acid cycle), amino acid metabolism and nucleotide and cofactor metabolism. An efficient and cost-effective means to generate internal standards for quantitation is through metabolic labeling10. This involves growing a micro-organism (e.g. E. coli) on a uniformly labeled carbon source resulting in a fully labeled biomass. This can be harvested, extracted and subsequently used as internal standards. In addition to coupling MRM and EPI scans, the qualitative workflow can also include an enhanced resolution (ER) scan which provides a higher resolution MS spectrum of each analyte with accurate isotope ratios. From the ER scan, the isotope distribution of metabolically labeled compounds can be determined in the labeling experiments. The EPI scan triggered provides additional structural information on the location of the heavy labels. These methods were applied to measure the differences in absolute metabolite concentrations in E. coli grown in two different media conditions. The first media condition was a glucose minimal media and the second was a complex media that included glucose and supplemented amino acids and precursors. We found that supplementation with amino acids and their precursors correspondingly increased levels of intracellular amino acids. This finding is consistent with previous studies that found an increase in growth rate for bacteria supplemented with amino acids due to the import of amino acids and their precursors into the cell13.

Rosa I. Viner
Thermo Fisher Scientific

ABSTRACT:

Advances in Orbitrap Technology

Rosa Viner, Thermo Fischer Scientific,

The field of biology has shifted from merely discovery work focused on identification as the primary driver of technology to quantification. Researchers have moved beyond “what” is in the sample to how does a system work, from mapping to functional questions. With the introduction of the Orbitrap technology a decade ago, the high resolution, accurate mass detection capabilities have provided scientist with complete and reproducible proteome coverage that has opened the door for many, new exciting insights into cellular dynamics. Orbitrap Fusion Lumos is the newest Tribrid mass spectrometer

providing highest sensitivity, highest selectivity and lowest detection limit. It was uniquely developed to expand tribrid performance in advanced proteomics, biopharma and metabolomics workflows, including low level quantitation, PTM and top down analyses. In this presentation we will highlight and show some of these advanced workflows that are accessible with Orbitrap Fusion Lumos mass spectrometer.

The Ken Standing Award

The Ken Standing Award is generously provided by the sponsors of the ETP Symposium and the University of Manitoba.

Professor Kenneth G. Standing is Professor Emeritus, Dept. of Physics & Astronomy, University of Manitoba. He graduated B.Sc. from the University of Manitoba in 1948, and obtained his Ph.D. in Physics from Princeton University in 1955. Dr. Standing was appointed to the faculty of the University of Manitoba in 1953, where he rose through the ranks and eventually became Professor Emeritus in 1995. Along the way he was Director of the Manitoba Cyclotron Laboratory for the period 1959-1974. Starting in the early 1980s he turned his attention to time-of-flight mass spectrometry and its applications to study of biological macromolecules, particularly proteins and peptides, and is widely regarded as the pioneer in this area. He has received many honours during his distinguished career, including the Canadian Society for Mass Spectrometry Award for Distinguished Contributions to Mass Spectrometry in 1998, the Canadian Association of Physicists Medal for Outstanding Achievement in Industrial & Applied Physics in 2003 and the American Chemical Society's Field and Franklin Award for Outstanding Achievement in Mass Spectrometry in 2004. He was elected as a Fellow of the American Physical Society in 2004, and in the same year as a Fellow of the Royal Society of Canada. He has served as Member or Chair of various US National Institutes of Health Special Study Sections. Dr. Standing is regarded by his many colleagues and friends, including several generations of scientists from around the world, as one of the founders of the still-young discipline of proteomics. Never content to only invent and perfect experimental tools he has sought collaborations with biologists, focused on solution of a wide range of difficult biological problems using his own technological innovations as well as those of others. This led to a continuing collaboration with MDS-Sciex in development of mass spectrometers used by proteomics researchers worldwide, recognized in 2000 by the award of an NSERC/Conference Board Synergy Award for University–Industry collaboration to the Manitoba-MDS-Sciex collaboration. In 2007 the Manitoba-MDS-Sciex team was awarded NSERC's Brockhouse Canada Prize for Interdisciplinary Research in Science and Engineering, a prize established to honour the memory of Bertram Brockhouse, Canada's Nobel Laureate in Physics.

Ken Standing's exemplary career continues to provide an inspiration to new generations of scientists in Canada and elsewhere. Despite the recent curtailment of his alpine skiing activities, his energy level in his research laboratory appears to be undiminished. For these reasons the Sponsors of the Enabling Technologies Symposium decided in 2006 to establish the Ken Standing Award to honour the lifetime achievement of this distinguished Canadian scientist. The award consists of a $5000 (CDN) cash grant and a commemorative sculpture, and is presented at the annual ETP Symposium to a young scientist who has made a significant contribution to the development of technology related to the life sciences.

The winner of this year's Award is presented to

Benjamin A. Garcia
Presidential Associate Professor
Director of Quantitative Proteomics, Epigenetics Program
Department of Biochemistry & Biophysics
Smilow Center for Translational Research
Perelman School of Medicine
University of Pennsylvania

Benjamin A. Garcia obtained his BS in Chemistry at UC Davis in 2000, where he worked as an undergraduate researcher in Prof. Carlito Lebrilla's laboratory. He then received his PhD in Chemistry in 2005 at the University of Virginia under Prof. Donald Hunt and then was an NIH NRSA Postdoctoral Fellow at the University of Illinois under Prof. Neil Kelleher from 2005-2008. From there Ben was appointed as an Assistant Professor in the Molecular Biology Department at Princeton University from 2008-2012, until his recruitment as the Presidential Associate Professor of Biochemistry and Biophysics at the University of Pennsylvania Perelman School of Medicine in 2012. The Garcia lab has been developing and applying novel proteomic approaches for interrogating protein modifications, especially those involved in epigenetic mechanisms, publishing over 155 publications. Dr. Garcia is on the editorial boards for the BMC Genomics and Molecular and Cellular Proteomics journals, and serves on the Board of Directors for the U.S. Human Proteome Organization. He has also been recognized with many honors and awards for his research including the ASMS Research Award, the PITTCON Achievement Award, and the American Chemical Society Arthur F. Findeis Award for Achievements by a Young Analytical Scientist.

Presentation:

Quantitative Proteomics for Understanding Modified Proteins and Proteomes

Histones are small proteins that package DNA into chromosomes, and a large number of studies have showed that several single post-translational modification sites on the histones are associated with both gene activation and silencing. Nevertheless, what type of effect distinct combinations of simultaneously occurring histone modifications (Histone Codes or patterns) have upon cellular events is poorly understood. We have been specifically addressing this deficiency by developing novel mass spectrometry based proteomic methods and accompanying bioinformatics to quantitatively characterize molecular level descriptions of combinatorial Histone Codes. These include both methodology advances and novel technology (i.e. data-independent approaches). Examples of these methods to study how these dynamic Histone Codes influence gene expression from multiple cellular states, especially stem cell pluripotency and cancer will be presented. Additionally, our early work to resolve how signaling pathways crosstalk into chromatin to influence epigenetic mechanisms will also be described. These studies in combination with biological experiments will help provide a systems biology outlook on gene expression that will lay down the basic scientific foundation to advance several applications, such as stem cell reprogramming and cancer progression.

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The Bill Davidson Graduate Student Travel Award

Bill Davidson was a PhD graduate from the laboratory of Paul Kebarle at the University of Alberta, where he acquired the expertise in the physical chemistry of ion-molecule reactions that stood him in good stead in his long career. He joined Sciex in 1978 and very quickly became a key part of the early success of that company, contributing to the hardware development and writing some of the first application software (on a PDP8 computer!) for the TAGA (Trace Atmospheric Gas Analyzer). This instrument incorporated an APCI ion source and the first commercially available triple-quadrupole analyzer. Mobile versions mounted on a truck are still in use to monitor air quality, especially in the event of environmental accidents.
Bill's vision and leadership contributed greatly to development of the Aromic System (used as a non-invasive inspection system for air cargo) and in 1989 the API III LC/MS/MS system. The latter pioneered the commercialization of reliable LC/MS/MS instruments for biomedical and other applications, and was the basis of the subsequent commercial success of Sciex.
Bill developed an extensive network of collaborations and interactions with Canadian and inter- national Universities and research institutes that provided the company with exposure to many new scientific advances and technologies. His later role in business and technology development grew from his extensive contacts in the larger scientific community, where his scientific knowledge and judgment were greatly respected.

After retiring from being the Vice-President of Science and Technology at AB SCIEX, Bill co-founded (along with Janette Champ) ETP Symposium Inc. (ETP) to continue to bring researchers together to develop new scientific advances and technologies. ETP is world-renowned as a key conference where international and Canadian researchers get together to collaborate to develop novel technologies to further life sciences research. Bill was an active participant in ETP up until his death in August 2009.

To honour this lifetime achievement, ETP and AB SCIEX have put together an award geared to helping graduate students travel to and participate at the biennial ETP Symposium.

This inaugural award is being presented by
Dr. Brigitte Simons, SCIEX to

Siavash Vahidi
University of Toronto

Siavash Vahidi attended the National University of Iran (Shahid Beheshti University), obtaining a BSc in chemistry (2006-2010). He then moved to The University of Western Ontario (2010-2015) to earn a PhD degree in biophysical mass spectrometry with Prof. Lars Konermann. His interest in protein structure, folding, and dynamics has brought him to the Departments of Molecular Genetics and Biochemistry at the University of Toronto where he is a postdoctoral fellow in the Kay lab.

Presentation:

Uncovering the Dynamic Interactions of the FoF1 ATP Synthase Molecular Machine by H/D Exchange Mass Spectrometry

LS. Vahidi, Y. Bi, S. D. Dunn, and L. Konermann, Western University

The role of conformational dynamics for the function of large protein systems, such as FoF1 ATP synthase, is a question of both fundamental and practical importance. FoF1 is a membrane-bound multi-subunit molecular motor that uses proton-motive force (PMF) to drive the synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (Pi). Reverse operation of the enzyme generates PMF via ATP hydrolysis. Operation in either direction involves rotation of the g and e subunits that connect the a3b3 head and the membrane-anchored cn ring. X-ray crystallography and other techniques have provided detailed insights into the structure and function of FoF1 sub-complexes. However, interrogating the overall conformational dynamics of intact membrane-bound FoF1 during rotational catalysis has proven to be difficult. In this talk, I will outline how hydrogen-deuterium exchange (HDX) mass spectrometry (MS) can be used to probe the inner workings of FoF1 in its natural membrane-bound state. The H-bonding network of key power transmission elements is insensitive to PMF-induced mechanical stress. Unexpectedly, a pronounced destabilization of the g C-terminal helix during rotational catalysis was observed. This destabilization occurs when FoF1 operates against a PMF-induced torque; the effect disappears when PMF is eliminated by an ... uncoupler. The ... behavior of g is ... attributed to ... kinetic friction ... within the apical ... rotor bearing. ... These

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results provide direct evidence that friction-mediated torsional stress is an intrinsic factor associated with FoF1 operation under physiologically relevant conditions. PMF effects on g are analogous to those encountered in combustion engines, where the crankshaft bearings exert much greater forces when operated under load than when the engine is idling.

This award is generously sponsored by

PosterPresentations

Liu Yang
University of Regina

ABSTRACT:

Pesticides in the Atmosphere in the Okanagan Valley Agricultural Region in British Columbia

Contributing Authors: Liu Yang*, Renata Raina-Fulton

A modified gas chromatography-negative chemical ionization mass spectrometry method was developed to analyze atmospheric samples for pesticides including chlorpyrifos, endosulfan I/II, and endosulfan sulfate. Negative chemical ionization in selected ion monitoring mode provides higher sensitivity and reduced issues with interferences or matrix effects as compared to electron impact ionization. Atmospheric samples were collected with a high-volume particle/vapour sampler at Osoyoos in the southern Okanagan Valley of British Columbia. The pesticides were extracted from the solid materials for atmospheric collection using pressurized solvent extraction and then after a drying stage clean-up was accomplished with solid-phase extraction. The Okanagan Valley is an intensive agricultural area with vineyards and orchards for tree fruit crops. During April ?October 2011 maximum atmospheric concentrations were 2880 pg/m3, 7940 pg/m3, 1940 pg/m3 and 334 pg/m3 for chlorpyrifos, endosulfan I and II, and endosulfan sulfate, respectively. The seasonal trends in atmospheric concentrations of these pesticides will be presented. Chlorpyrifos is not recommended for use on tree fruit crops or in vineyards in BC, but has been used in Washington State. A global ban on the manufacture and use of endosulfan was negotiated under the Stockholm Convention in April 2011 and became in effect in the mid-2012. Canada developed a plan to phase-out endosulfan usage by December 31, 2016 and BC is one of the highest usage regions of endosulfan in Canada with 2100 kg of endosulfan sold in 2010. Preliminary data for 2012 will also be presented to determine changes of endosulfan I/II versus endosulfan sulfate concentrations.

Sau-Ching Wu
University of Calgary

ABSTRACT:

A simple and efficient method to prepare a streptavidin mutein based affinity matrix via an orientation specific immobilization approach

Contributing Authors: Sau-Ching Wu, Jonathan Chin and Sui-Lam Wong

A streptavidin mutein, SAVSBPM18, has been engineered for affinity purification of recombinant proteins tagged with a streptavidin binding peptide (SBP). This mutein has the binding affinities (~10-8M) towards both SBP tag and biotin. The bound SBP-tagged proteins can be eluted from the affinity matrix using biotin as the competitor. The greatest strength of this system is that the affinity matrix can be reused many times. To prepare the affinity matrix, SAVSBPM18 was traditionally coupled to a matrix via amine coupling. Since SAVSBPM18 has many lysine residues, it is impossible to couple this protein to the matrix in an orientation specific manner. Furthermore, Lysine-121 plays a critical role in binding both the SBP tag and biotin. Coupling via this residue will essentially inactivate SAVSBPM18. To develop a simple and efficient method for preparing affinity matrix, a 14-kDa dextran binding domain (DBD) derived from the Leuconostoc mesenteroides dextransucrase was used as a molecular handle for matrix coupling. This domain binds to dextran in high affinity (kd = 2.8 x10-9 M). Two SAVSBPM18 fusions (SAVSBPM18-DBD and DBD-SAVSBPM18) were constructed. Both versions can be produced intracellularly from E. coli with the majority of the fusion proteins in the soluble form. Functional characterizations using biotin agarose and Sephadex G100 indicate that both the streptavidin domain and dextran binding domain are functional in SAVSBPM18-DBD. An SAVSBPM18 affinity column can be prepared

easily using crude extract from the SAVSBP-DBD producing strain. SBP tagged lactamase can be affinity purified in one step with high purity using this matrix.

Marcus Kim
Agilent Technologies

ABSTRACT:

Exploring Protein Three Dimensional Structural Conformation Using Native Ion Mobility Mass Spectrometry

Contributing Authors: Ning Tang, Marcus Kim

The coupling of a uniform low field ion mobility drift tube with a quadrupole time of flight mass spectrometer has added the dimensions of shape and size of a molecule to its exact mass and collisional induced fragmentation profile. The separation mechanism of an ion mobility drift tube is orthogonal to chromatography and high resolution accurate mass detection so it offers additional powers of separation. This new dimension of separation increases mass spectral purity for complex mixtures and the low field drift tube minimizes RF induced ion heating to provide native molecule structural information for various protein conformers. This talk will discuss how the Agilent IMS-QTOF is able to detect and identify various protein conformers.

Naomichi Baba
Okayama University, Okayama, Japan

ABSTRACT:

Tracing of Structural Changes in Labeled Phospholipid Molecular Species in Biological Systems by Means of Tandem Electrospray Ionization Mass Spectrometry

Contributing Authors: Naomichi Baba,[1] Hiroko Tominaga,[1] Ai Nagai,1 Tomoe Ishihara,[1] Rumiko Shimizu[2]
[1]Graduate School of Bioscience and Technology, Okayama University, Tsushimanaka, Okayama, Japan
[2] Kobe Gakuin University, Kobe, Japan

Precursor ion scan mode in collision-induced tandem electrospray ionization mass spectrometry is enable to detect specific molecular ion species even in the presence of complex biological systems. Based on the principle of precursor ion scan mode, we synthesized two labeled phosphatidylcholines with deuteriomethyl or ethyl group. They provide unnatural product ions with specific m/z and could be used as molecular probes for typical cell membrane phospholipid. They were applied on human blood, human skin surface and herring skin surface. The recovered samples from the biological systems were analyzed in the precursor ion scan mode. The mass spectra thus obtained were found to afford a lot of information about their structural changes in the biological systems. Simultaneous use of the two molecular probes was also shown to be a useful technique.

Nasir Al Awwad
College of Clinical Pharmacy, Al-Bahah University

ABSTRACT:

PEG nanoparticles decorated with Rabies Virus Glycoprotein cross the BBB

Contributing Authors: Nasir Al Awwad[1], Li Y[2], Lam KS[2].
[1].Department of Clinical Pharmacy, Faculty of Clinical Pharmacy, Albaha University, Albaha 65431 Saudi Arabia.[2].Department of Biochemistry and Molecular Medicine, College of Medicine, University of California at Davis, Sacramento, CA 95817 USA.
A major impediment in the treatment of neurological diseases is the presence of the blood–brain barrier, which precludes the entry of therapeutic molecules from blood to brain. Here we show that a short peptide derived from rabies virus glycoprotein (RVG) enables the transvascular delivery of micellar nanoparticles (NPs) based on linear polyethylene glycol (PEG)-block-dendritic cholic acids (CA) copolymers (telodendrimers), for the targeted delivery of drugs in the treatment of brain infection and inflammation. The micellar NPs have been decorated with (RVG) peptide which facilitated the (NPs) Trojan-horsing the BBB. "Click chemistry" was used to conjugate alkyne group on (RVG) peptide to the azide group at the distal terminus of the PEG chain at a molar ratio of 1:2 (RVG:PEG). The delivery was demonstrated in vivo biodistribution study after intravenous injection into mice.
Therefore, telodendrimers decorated with (RVG) peptide have great potential as a new therapeutic approach for patients with brain infection or inflammation. This 29-amino-acid peptide specifically binds to the acetylcholine receptor expressed by neuronal cells and transduce the (NPs) to neuronal

cells. Controversially, the naked (NPs) could not pass through the BBB. Thus, RVG provides a safe and noninvasive approach for the delivery of (NPs) and potentially other therapeutic molecules across the blood–brain barrier.

Peter Horvatovich
University of Groningen

ABSTRACT:

Critical assessment of LC-MS data pre-processing and feature selection methods for biomarker discovery in clinical proteomics

Contributing Authors: Berend Hoekman[1, 5, 6], Christin Christin[1, 5, 6], Huub Hoefsloot[2], Age Smilde[2], Rainer Breitling[3, 5], Frank Suits[4], Rainer Bischoff[1, 5, 6], Peter Horvatovich[1, 5, 6]

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Performance assessment of LC-MS data pre-processing and selection of discriminating features (peaks) is critical to interpret correctly the outcome of quantitative proteomics profiling studied such as biomarker discovery. The presented performance assessment is performed using two complex proteomics label-free LC-MS datasets spiked with peptides at different concentration levels. Relative performance of different LC-MS data pre-processing workflows is demonstrated using msCompare framework. msCompare framework includes peak detection and peak matching modules from multiple popular open-source LC-MS quantification programs such as MZmine, OpenMS and SuperHirn. msCompare allows to design workflows using all possible combination of integrated peak detection and peak matching modules. Score based on the rank of spiked features amongst the most discriminating features is used to assess the relative performance of the different workflows. The presented method is suitable to select the best workflow with the best peak detection and peak matching modules for a given quantitative LC-MS dataset.
The goal of feature selection statistical methods in biomarker discovery is the accurate selection of feature set, which is able to discriminate between predefined groups of samples. 6 feature selection methods such as *t*-test, Mann-Whitney-Wilcoxon-test, Nearest Shrunken Centroid, Partial Least Squares – Discriminant Analysis, Principal Component – Discriminant Analysis and Support Vector Machine – Recursive Features Elimination are assessed using two spiked complex proteomics LC-MS datasets. The performance for accurate selection of a set of features related to spiked compounds was assessed using the harmonic mean of the recall and the precision (f-score) and the geometric mean of the recall and the true negative rate (g-score). The feature selection methods were applied on data sets with different sample size and extent of sample class separation determined by the concentration level of spiked compounds. The comparison enable the selection of the best performing feature selection method for a quantitative LC-MS dataset with given sample size and class separation.

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