6th Norwegian National Proteomics Meeting

9th -10th December, Trondheim

Abstract Book

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Introduction to Trondheim

Founded by the Viking king Olaf I Tryggvason in the 10th century, Trondheim is Norway's third-largest city and was the country's capital until the early 1200s. Scenic and pleasant, it's a bustling university center, with expansive avenues created after a fire razed most of the town in 1681. The city lies on the south bay of the Trondheim Fjord, at the mouth of the Nidelven River.Noted for its timbered architecture, Trondheim retains much of its medieval past, notably the Gothic-style Nidaros Cathedral. Pilgrims came from all over Europe to worship at the shrine of Olaf, who was buried in the cathedral and canonized in 1031.

The city's fortunes declined during the Reformation. Under the Nazi occupation Trondheim became the base of German naval forces in northern Norway, with U-boats lurking deep in its fjord.

Today Trondheim is a progressive city with a rich cultural life, as well as a high-technology center for research and education. Its town center is compact and best explored on foot; most of the historic core of Trondheim lies on a small triangular island surrounded by water but linked via bridges.

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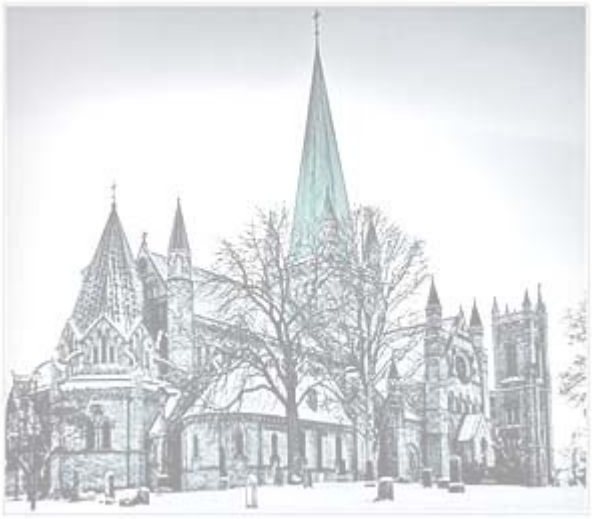

6th Norwegian National Proteomics Meeting

Invited speakers:
Ole Nørregaard Jensen, University of Southern Denmark
Ulf Hellman, Uppsala University

Local organizing comittee for Norwegain Proteomics Society:
Lars Hagen, NTNU
Mirta Sousa, NTNU
Kamila A. Zub, NTNU
Morten Beck Rye, NTNU
Geir Slupphaug, NTNU

Sponsors:

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Program

Thursday, December 09th

08:45 – 9:40 Registration, poster, exhibition mounting and coffee
09:40 – 09:45 Welcome by **Lars Hagen**
09:45 – 10:00 Keynote speech by **Geir Slupphaug**

Session 1: Chaired by Cathrine B. Vågbø

10:00 – 10:45 **NordForsk supported lecture**
Ulf Hellman, Uppsala University: "Use of MALDI-TOF/TOF mass spectrometry in a signal transduction research environment"
10:45 – 11:10 **Mirta Sousa**, NTNU: "Antibody crosslinking and target elution protocols used for immunoprecipitation significantly modulate signal-to noise ratio in downstream 2D-PAGE analysis"

11:10 – 11:30 Coffee break

11:30 – 12:05 **Peter Lindqvist**, Sigma Aldrich**:** "Revealing Low Abundant Targets in the Proteome"
12:05 – 12:30 **Karin Gilljam**, NTNU: "Identification of a new and widespread and functionally important PCNA-binding motif"
12:30 – 13:15 **Kerstin Eriksson**, Instrument & Applications Specialist,Bio-Rad: "Faster, Easier, Better: Innovations in the area of protein electrophoresis and documention"

13:00 – 14:30 Lunch with poster session

Session 2: Chaired by Anastasia Galashevskaya

14:30 – 15:05 **Thorleif Lavold**, Biomotif AB: "Find the needle in the haystack with on-line pI selection of proteins in complex samples with a new HX-IA instrumentTM coupled to HR-API-mass spectrometry"
15:05 – 15:30 **Trygve Kjellsen**, VISTA: Metaproteomics as a tool for increased oil recovery

15:30 – 15:45 Coffee break

15:45 – 16:10 **Madalina Oppermann**, Thermo Fisher Scientific: "Triple quadrupole mass-spectrometry-based peptide assays using inteligent SRM"
16:10 – 16:35 **Ida Ericsson**, NTNU: Mechanism for nuclear import of activation-induced deaminase
16:35 – 17:00 **NPS annual meeting**

19:00 Dinner at Rica Hotel

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Friday, December 10th

Session 3: Chaired by Mirta M. L. Sousa

09:00 – 09:45 **NordForsk supported lecture**
Ole Nørregaard Jensen, University of Southern Denmark: “Post-translational modification of proteins: The eternal frontier of proteomics?”
09:45 – 10:10 **Vibeke Bull**, Biotechnology Center of Oslo: “Quantitative proteomics revealed that sorafenib induce mitochondria mediated apoptosis in human neuroblastoma cells”
10:10 – 10:35 **Magnus Arntzen**, University of Oslo: “Isobaric peptide termini labelling (IPTL) and IsobariQ as a quantitative proteomics workflow”

10:35 – 11:15 Coffee break

11:15 – 11:40 **Kamila A. Zub**, NTNU: “A proteomics and genomic approach to identify factors associated with melphalan resistance in multiple myeloma cells.”
11:40 – 12:05 **Cathrine B. Vågbø**, NTNU: “Biological applications of mass spectrometry in the analysis of DNA and RNA modifications”
12:05 – 12:30 **Linda Helander**, NTNU: “Use of proteomic techniques within photodynamic therapy research”
12:30 – 13:00 **Gustavo Souza**, Rikshospitalet of Oslo: “A proteomic insight into *Mycobacterium tuberculosis* evolution and fitness”

12:45 – 14:00 Lunch

Session 4: Chaired by Antonio Sarno

14:00 – 14:25 **Bjarte Bergstrøm**, NTNU: “Identification of a novel in vivo virus-targeted phosphorylation site in interferon regulatory factor-3 (IRF3)”
14:25 - 14:50 **Harald Wiker**, University of Bergen: “A quantitative view on *Mycobacterium leprae* antigens by proteomics”

14:50 – 15:05 Coffee break

15:05 – 15:40 **Volker Kruft**, AB SCIEX: “Qualitative and quantitative workflows on a single MS plataform without compromising speed, sensitivity or resolution”
15:40 – 16:00 **Lars Hagen**, NTNU: “PTMs of the human DNA repair protein Uracil DNA glycosylase, UNG2”
16:00 Concluding remarks by **Ole N. Jensen**

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Abstracts
Thursday, December 09th

Use of MALDI-TOF/TOF mass spectrometry in a signal transduction research environment.

Ulf Hellman

Ludwig Institute for Cancer Research Ltd. ,Uppsala University, Uppsala, Sweden

The Protein Structure Group at our Institute has witnessed a tremendous development in the sensitivity of protein analyses over 25 years. Up until mid nineties, when Edman degradation still was the principal technology, an amount of 1-10 pmoles was required for a successful amino acid sequence analysis. The usage of SDS-PAGE for preparation of samples replaced the tedious classical chromatography and when mass spectrometry was developed for biological samples the combination of the two techniques set a new standard.
Now, when many of the studied species are completely characterized, MALDI-TOF MS is frequently used to determine the identity of a given protein by peptide mass fingerprinting (PMF); a method which requires only low femtomole amounts, *i. e*. corresponding to a silver stained spot from a 2D gel. This has been utilized in several cancer related projects where a comparison between a tumor and a healthy tissue has revealed significant differences and allowed potential cancer markers to be identified.
For the majority of species, still uncharacterized, PMF is not an option and there peptide sequence analysis will be useful for identity suggestions using BLAST for homology searches. Fragmentation analysis of proteolytic peptides by mass spectrometry will produce data that can be utilized to interpret a sequence (or to perform an MS/MS search – this however only applies to known species). True *de novo* sequencing is not an easy task; the outcome is highly sample dependent and many possibilities often appears. A significant breakthrough was the introduction of sulfonation at the N-terminus of tryptic peptides which almost always results in a clean series of exclusively y-ions. The mass difference between peaks represents a unique amino acid (except for the isobaric Leu/Ile!) and peptides up to over 40 residues have been determined.
An important branch off proteomics is the study of post translational modifications (PTM) – principally all proteins are modified, and this must have a functional meaning. We have successfully identified phosphorylation, acetylation, methylation and ubiquitinylation; however, as there are at least 200 PTMs described, a lot more efforts will be spent in this subject. It is obvious that sequence analysis of a modified peptide is superior to PMF as not only the nature but also the position of the modification can be determined.
The talk will cover the topics above and a little more!

Antibody crosslinking and target elution protocols used for immunoprecipitation significantly modulate signal-to noise ratio in downstream 2D-PAGE analysis

Mirta M.L. Sousa, Kristian W. Steen, Lars Hagen, and Geir Slupphaug

Norwegian University of Science and Technology, NTNU, Department of Cancer Research and Molecular Medicine, Trondheim, Norway.

Co-immuneprecipitation and downstream separation by 2D-PAGE combined with mass spectrometry analysis is widely used to identify protein:protein interactions as well as post translational modifications modulating such interactions. This workflow is, however, often hampered by incomplete elution of the target proteins in buffers compatible with the isoelectric focusing step in 2D-PAGE, as well as high levels of unspecifically bound proteins that complicate analysis of the target proteins. Here we have compared coupling of antibodies to Dynabeads® Protein A by using either DMP or BS3 crosslinkers, as well as various conditions for elution of bound proteins prior to 2D PAGE separation. BS3 crosslinking generally resulted in less nonspecific binding than DMP, whereas DMP crosslinking generally gave overall higher yield of target protein. Regardless of the crosslinker used, incomplete elution of the primary target protein was observed when using conventional glycine- or urea-based elution buffers. The most effective elution method compatible with downstream 2D-PAGE was heating of the beads with buffer containing 2 % SDS. Subsequent dilution of the eluted proteins in 2DE buffer containing 4 % CHAPS, to a final SDS concentration of 0.2 % yielded perfectly focused gels suitable for mass spectrometry analysis. In conclusion, careful choice of Ig crosslinker as well as efficient elution of target protein in SDS prior to downstream 2D-PAGE may be key factors when studying low-abundance proteins.

Keywords: elution, immunoprecipitation, nonspecific binding, 2DE.

Revealing Low Abundant Targets in the Proteome

Peter Lindqvist

Sigma Aldrich

Mass spectrometry enables the characterization of proteins in complex mixtures, and is the primary proteomic instrument used in biomarker discovery today. Detection of low abundant proteins is difficult because highly abundant proteins often mask them. To overcome this issue, protein fractionation steps are necessary to deplete abundant proteins.
The Seppro® depletion technology, including the novel Super-mix resin, allows for the removal of highly abundant proteins and most moderately abundant proteins. The technology utilizes avian polyclonal IgY antibodies to allow highly specific partitioning of protein mixtures.
We will present data that demonstrates the effectiveness of this technology and its use in a biomarker study.

Identification of a new and widespread and functionally important PCNA-binding motif

Karin M. Gilljam, Emadoldin Feyzi, Per A. Aas, Mirta M.L. Sousa, Cathrine B. Vågbø, Rebecca Müller, Tara C. Catterall, Nina B. Liabakk, Geir Slupphaug, Finn Drabløs, Hans E. Krokan and Marit Otterlei

Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, N-7489 Trondheim, Norway

The proliferating cell nuclear antigen (PCNA) is essential for replication as well as orchestration of other cellular processes, including epigenetics, sister chromatid cohesion, cell signalling, cell cycle regulation, translesion synthesis and DNA repair (Moldovan *et al*., 2007). Most proteins interact with PCNA through the PCNA interacting peptide called the PIP-box, first described in 1998 (Warbrick *et al*., 1998). Since then, a second PCNA binding motif called the KA-box has been identified (Xu *et al*., 2001); however this motif remains to be confirmed by experimental data. We have found a novel PCNA interacting motif shared among more than 200 proteins involved in DNA maintenance, transcription, and cell cycle regulation, and we have experimentally verified the functional importance of this motif for five of these proteins. We first identified this motif in the N-terminal of the oxidative demethylase human AlkB homolog 2 (hABH2), and have therefore named it AlkB homolog 2 PCNA Interacting Motif, APIM. Importantly, over-expression of an APIM-containing decapeptide fused to EYFP increased cellular sensitivity against several DNA alkylating agents not accounted for by perturbing only the hABH2-PCNA interaction. This supports a role for this novel PCNA interacting motif in mediating PCNA binding in several proteins involved in DNA repair and / or cell cycle control after genotoxic stress, and may be a potential drug modulator for use in cancer treatment.

" Find the needle in the Haystack" with on-line pI selection of proteins in complex samples with a new *HX-IA Instrument*™ coupled to HR-API-Mass Spectrometry

Thorleif Lavold, CEO, Biomotif AB, Danderyd, Sweden

The new unique ***HX-IA Instrument*™** will enable researchers to study structure, conformation, dynamics and molecular interactions in the liquid phase without tethering.
The Electrocapture Cell consists in a microchannel with two gaps covered with a conductive membrane from where the electric field is applied. Once the injected molecules are captured in the electric field, a new solution can be injected. Using this approach, we can obtain desalting, buffer exchange, removal of contaminants and online multistep microreactions (e.g. cleanup and digestion of membrane proteins). In addition, several microliters of sample can be concentrated and released in a few nanolitres. Furthermore, the system can be used to fractionate complex mixtures of polypeptides by using a voltage-gradient release scheme. This technology can be used for the analysis of protein, peptides, nucleic acids, hormones and charged small molecules. A second technology, called Membrane-based Amide-Hydrogen/Deuterium Exchange (H/D Exchange), utilizes a single-membrane section device to monitor protein/ligand interactions and protein/protein structural changes by mass spectrometry.
Due to the inherent multifunctionality and the broad number of different molecules on which these technologies can be applied, ElectroCapture and Membrane-based H/D Exchange have a great potential to become a useful tool for different bioanalytical and pharmaceutical analysis. The HX-initiative aims to expand the use of the Electrocapture Technology -and potentially merge both technologies- to provide a robust and highly informative analytical tool for molecular interactions.
The ***HX-IA Instrument*™** could potentially become a unique tool for diagnosing patients with Alzheimers, Parkinsons and other neurodenerative diseases in the early stage.
The use of our latest**" Find the needle in the Haystack"** invention coupled to API-MS as a novel analytical tool for on-line pI protein separation, digestion and quantitative analysis of peptides will be discussed.

TRIPLE QUADRUPOLE MASS SPECTROMERY-BASED PEPTIDE ASSAYS USING INTELLIGENT SRM (iSRM)

Reiko Kiyonami[1], Bruno Domon[2], Madalina Oppermann[1]

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[2] *ETH Zurich, Switzerland, name@crp-sante.lu*

Keywords: iSRM, quantitation, triple quadrupole MS

Introduction

The commonly used SRM technique provides sensitive and precise quantitative results by monitoring one or several primary SRM transitions per targeted compound. Recently this technique was extended to simultaneously confirm the identity and quantify multiple compounds in one HPLC/MS run by monitoring eight or more SRM transitions per compound. The bottleneck of this approach is that only a limited number of compounds can be targeted in one run because of the time required to monitor each transition. The newly developed instrument control software can use the specificity of a small subset of SRM transitions to simultaneously quantify and intelligently trigger the full list for confirmation, thereby allowing the analysis of up to 1000 compounds in a single LC-MS run.

Experimental

A triple quadrupole mass spectrometer equipped with a nanoLC pump and a nanospray source was used. The intelligent SRM method utilizes SRM specificity in two ways. The first is compound specific quantification using a time based SRM acquisition, which monitors several primary transitions for each compound. The second is a data dependent SRM acquisition, which monitors both those primary and additional secondary transitions and is triggered only when the intensities of all primary SRM transitions simultaneously exceed the defined intensity threshold. For large scale screening experiments, the dynamic exclusion was used to trigger secondary acquisition only once for each peak for providing sufficient structural information to confirm the compound's identity without perturbing the quantification obtained with the primary SRM list.

Results and discussion

The technique was applied to quantify precisely and confirm identity of peptides in complex biological samples, including yeast cell lysates, and pesticides in orange oil. By monitoring multiple transitions, it is possible to use very low intensity thresholds to accurately trigger the data dependent SRM scan. The trigger is the leading edge of the LC peak and relatively independent of the LC peak intensity. Using known LC peak widths, the strongest intensity point to obtain the data dependant scan is easily anticipated. Instead of a full product ion scan, high quality SRM spectra monitor eight or more transitions at that point. The resulting eight spectra are assembled into a pseudo full scan MS/MS spectra that can be used for confirming the identity of the peptides by matching them to reference spectra stored in a library.

Using a constant cycle time allows enough points across the peaks from the primary SRM scans for precise quantification. With this approach, the instrument is able to confirm and quantify most targeted peptides in the low attomol range (1).

This method was further employed to large scale screening experiments of peptides. 1000 yeast peptides were targeted in one single LC/MS run. The yeast peptide list and the iSRM method (Two primary transitions and six secondary transitions) were generated automatically by using SRM workflow software based on MS/MS data from the previous proteomics discovery experiments. The targeted peptides were successfully identified and quantified in a single HPLC MS run by using the iSRM software.

References

[1] R. Kiyonami, A. Schoen, A. Prakash, S. Peterman, V. Zabruskov, P. Picotti, R. Aebersold, A. Huhmer, B. Domon, *MCP Papers in Press*, published on September 10 (2010)

Metaproteomics as a tool for increased oil recovery

Trygve D. Kjellsen, Christian Collin-Hansen, Leni L. Grebstad and Hans Kristian Kotlar

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Easily recoverable fossil fuels are declining and the petroleum industry is seeking new ways of recovering oil from reservoirs previously believed to contain oils with too high viscosity for economically sustainable production. These oils are commonly referred to as heavy oils. Given the right conditions some microbial communities are capable of utilizing the hydrocarbons of heavy oils as their main source of energy and thereby decrease the viscosity of these oils. A deeper understanding of this bioconversion at the molecular level could lead to the development of new and more cost efficient and environmentally friendly methods for increased oil recovery. Laboratory experiments have shown that certain microbial communities are capable of bioconverting heavy oils. Extraction of proteins from both oil and water phase from such experiments have given us the opportunity to study changes in the metaproteome of microorganism communities during bioconversion of different heavy oils. Qualitative and quantitative differences in protein accumulation profiles in both water and oil phase at different time points have been analysed by using Difference In Gel Electrophoresis (DIGE). In combination with mass spectrometry this method has successfully been used to find and identify certain proteins that might play a direct or indirect role during bioconversion of heavy oils.

Mechanism for nuclear import of activation-induced deaminase

Ida Ericsson, Yi Hu, Kathrin Torseth, Ottar Sundheim, Nina B Liabakk, Geir Slupphaug, Hans E Krokan, and Bodil Kavli.

Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, 7006 Trondheim, Norway

The mutator enzyme activation-induced deaminase (AID) is the initiating key for somatic hyper mutation (SHM), class switch recombination (CSR) and Ig gene conversion (GC) in B cells. The enzyme does so by deaminating cytosine bases to uracil at the immunoglobulin (Ig) loci. However, AID-catalyzed off-target deamination is also linked to development of cancerous diseases, such as B cell lymphomas. Thus, a tight regulation of expressed AID is crucial to control Ig diversification and to prevent untargeted mutagenesis. Generally, AID displays cytoplasmic localization and must translocate to the nucleus to be able to deaminate DNA. Here we present a mutational analysis screen where we have mapped important residues for nuclear import by a total screen of all positively charged residues studied by live cell confocal microscopy.

Abstracts
Friday, December 10th

"Post-translational modification of proteins: The eternal frontier of proteomics?"
Ole Nørregaard Jensen, professor.

Institut for Biokemi og Molekylær Biologi, Det syddanske Universitet, Odense

More than 300 different types of protein post-translational modifications (PTMs) have been described, many of which are known to have pivotal roles in cellular physiology and disease. Nevertheless, only a handful of PTMs have been extensively investigated at the proteome level. Knowledge of protein substrates and their PTM sites is key to dissection of PTM-mediated cellular processes. The past several years have seen a tremendous progress in developing MS-based proteomics technologies for global PTM analysis. Modification-specific enrichment techniques combined with advanced MS/MS methods and computational data analysis have revealed a surprisingly large extent of PTMs in proteins, including multi-site, cooperative modifications in individual proteins. We review some of the current strategies employed for enrichment and detection of PTMs in modification-specific proteomics.

Quantitative proteomics revealed that sorafenib induce mitochondria mediated apoptosis in human neuroblastoma cells
Vibeke Hervik Bull[1], Krishnaraj Rajalingam[2], Bernd Thiede[1],

[1] The Biotechnology Centre of Oslo, University of Oslo, P.O. Box 1125 Blindern, 0317 Oslo
[2] Institute of Biochemistry II, Medical Faculty of the Goethe University, University Hospital Building 74/75, Theodor-Stern-Kai 7, 60528 Frankfurt am Main, Germany.

Sorafenib (Nexavar, BAY 43-9006) is a multikinase inhibitor that was recently approved for use against renal cell carcinoma and hepatocellular carcinoma. We show that sorafenib induces apoptotic cell death in human neuroblastoma cells through activation of the caspase cascade induced by early activation of caspase 10 and 7. Stable isotope labeling with amino acids in cell culture (SILAC) was combined with high resolution mass spectrometry to detect quantitatively the proteomic changes that occur upon sorafenib-induced apoptosis. Bioinformatic pathway analysis of the identified proteins revealed that mitochondrial proteins were significantly affected. Particularly components of the electron transport chain and mitochondrial ribosome, as well as cell cycle regulators were significantly regulated in response to sorafenib treatment. The electron transport chain is, in addition to producing ATP, responsible for maintaining the mitochondrial transmembrane potential (ψm). We found that the observed down-regulation of the respiratory chain complex I (NADH dehydrogenase) was accompanied with loss of ψm and complete loss of complex I activity. This occurs at an early time point, prior to activation of apoptotic caspases and independently of mitochondrial translation of complex I subunits. Finally, the levels of reactive oxygen species were increased in sorafenib treated SH-SY5Y cells, possibly associated with the impairment of the electron transport chain.

Isobaric peptide termini labelling (IPTL) and IsobariQ as a quantitative proteomics workflow

Magnus Ø. Arntzen1,2,3, Christian J. Koehler1, Harald Barsnes4,5, Frode S. Berven4, Achim Treumann6, Bernd Thiede1*

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[2] Proteomics Core Facility, Oslo University Hospital-Rikshospitalet & University of Oslo, 0027 Oslo, Norway
[3] Proteomics Core Facility, Norwegian University of Life Sciences, 1432 Ås, Norway
[4] Proteomics Unit, Department of Biomedicine, University of Bergen, Bergen, Norway
[5] Computational Biology Unit, Uni BCCS, University of Bergen, Norway
[6] NEPAF, Devonshire Building, Newcastle upon Tyne, NE1 7RU, United Kingdom

Abstract:
Isobaric peptide labelling plays an important role in relative quantitative comparisons of proteomes. Isobaric labelling techniques utilise MS/MS spectra for relative quantification, which can be either based on the relative intensities of reporter ions in the low mass region (iTRAQ and TMT) or on the relative intensities of quantification signatures throughout the spectrum due to isobaric peptide termini labelling (IPTL). Due to the increased quantitative information found in MS/MS fragment spectra generated by the recently developed IPTL approach, new software was required to extract the quantitative information. IsobariQ was specifically developed for this purpose, however, support for the reporter ion techniques iTRAQ and TMT is also included in the software.

A proteomics and genomic approach to identify factors associated with melphalan resistance in multiple myeloma cells.

Kamila Anna Zub[1], Mirta M.L. Sousa[1], Per Arne Åas[1], Clifford Young[3], Erming Tian[1,2], Jostein Johansen[1], Lars Hagen[1] , Ole Nørregaard Jensen[3] and Geir Slupphaug[1]

[1] Norwegian University of Science and Technology, NTNU, Department of Cancer Research and Molecular Medicine, Trondheim, Norway.
[2] Laboratory of Myeloma Genetics, Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, Little Rock, Arkansas
[3] Department of Biochemistry and Molecular Biology, Protein Research Group, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark

One of the most frequently used and effective drugs against multiple myeloma is the alkylating agent melphalan. However, the treatment is often hampered by development of drug resistance. Eventually, nearly all patients develop resistance, and mean survival time is about 3 years. Several factors have been suggested to contribute to drug resistance, including modulated drug transport and metabolism, enhanced DNA repair and decreased apoptotic signalling. Major common determinants associated with melphalan resistance, however, remain elusive. To further identify potential protein biomarkers and/or pathways involved in elevated drug tolerance, microarray and SILAC quantitative profiling was performed using the multiple myeloma cell line RPMI 8226 (sensitive) and its derivative RPMI 8226-LR5 (adapted to growth in melphalan). Comparison as well as grouping into appropriate networks and pathways of identified proteins was performed by GeneGo software. Selected candidate proteins were further validated by quantitative western-blot analysis and viability assays. Results indicate that proteins involved in sensing DNA damage and its repair, in cell cycle regulation, stress and inflammation response as well as drug metabolizing pathway are associated with development of melphalan resistance.

Biological applications of mass spectrometry in the analysis of DNA and RNA modifications

Cathrine Broberg Vågbø
Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, N-7006 Trondheim, Norway

Mass spectrometry analysis of DNA and RNA nucleosides is important in the search for biological functions of proteins, particularly in combination with animal models. We used this approach to study the functions of mammalian AlkB homologs. AlkB is a bacterial enzyme that repairs DNA alkylation damage by a direct, oxidative mechanism. AlkB is highly conserved across the evolution, and as many as nine mammalian AlkB homologs (ALKBH1-9) have been discovered based on sequence similarity. These have distinct subcellular locations, and although *in vitro* enzymatic acitivities have been reported for several of these proteins, their biological roles are only starting to become understood. By mass spectrometry analysis of nucleosides we found that the mouse homolog Alkbh2 is a genuine DNA repair enzyme that functions like AlkB *in vivo*. Moreover, we discovered that ALKBH8 is involved in the biogenesis of natural tRNA modifications, which expand the function of the AlkB homologs beyond nucleic acid repair.

Use of proteomic techniques within photodynamic therapy research

Linda Helander, Yan Baglo, Mirta M. L. Sousa, Lars Hagen, Geir Slupphaug and Odrun Arna Gederaas

Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, N-7006 Trondheim, Norway

Photodynamic therapy (PDT) is a highly selective cancer treatment involving addition of light sensitive compounds, illumination by visible light and presence of oxygen. The light sensitive compound is called a photosensitizer and possesses properties as cancer cell specificity and visible light excitability. Once the sensitizer has absorbed light energy, it has the capacity to induce cell damage, leading to cell death. Nowadays, PDT is used on endoscopically accessible tumours and in dermatology. Since photodynamic therapy is highly selective, it preserves healthy tissue and gives nice cosmetic results.

The molecular mechanisms associated with PDT are studied in details on the purpose to improve clinical treatment. The primary emphasis has been investigation of cell death pathways and results has shown that PDT induces autophagy, apoptosis and necrosis in varying extent. PDT induces reactive oxygen species (ROS) through photochemical reactions. Induced ROS causes oxidative stress and ROS can further react with cellular macromolecules resulting in cellular damages, including oxidation of proteins. PDT effects on protein oxidation and expression have been studied at IKM, NTNU. Some of the used techniques and results will be presented.

A proteomic insight into *Mycobacterium tuberculosis* evolution and fitness

de Souza G.A.[1],*, Tomazella G.G.[1], Fortuin S.[2], Thiede B.[3], Warren R.W.[2] and Wiker H.G.[1]

[1]The Gade Institute, Section for Microbiology and Immunology, University of Bergen, Norway; [2]Stellenbosch University, Cape Town, South Africa;
[3]The Biotechnology Centre of Oslo, University of Oslo, Norway.
**Current address: Institute of Immunology, University of Oslo, Norway.*

Within the population of the same bacterial species, individual specimens separate into family clusters due to specific genotypic features. In *Mycobacterium tuberculosis*, such features result mostly from transposable elements that differ in number of copies and position in the genome, because horizontal gene transfer is inexistent in this bacterium. Since the pattern of transposable elements is passed on to new daughter cells after cell division, clinical *M. tuberculosis* isolates can be classified in an evolutionary manner, from strains more similar to an ancestral genotype to more modern family clusters. In this work, through the use of label-free proteomic quantitation, we are comparing the proteome from whole cell lysates of nine *M. tuberculosis* strains isolated from clinical cases in South Africa. The clinical strains comprise one ancestral type EAI, three from the Group 1 (including typical and atypical Beijing strains), and four from Group 2 including a Harleem-like strain. Preliminary data shows that we are able to identify over 3000 protein products of the expected 4012 open reading frames, of which 85% of those are quantifiable. We expect to observe expression patterns of key proteins that might help us to understand molecular pathways leading to virulence and increased fitness phenotypes.

Identification of a novel in vivo virus-targeted phosphorylation site ininterferon regulatory factor-3 (IRF3).

Bjarte Bergstrøm, *NTNU*

The transcription factor interferon regulatory factor-3 (IRF3) regulatesexpression of type I interferon-beta and plays an important role inantiviral immunity. Despite the biological importance of IRF3, its invivo phosphorylation pattern has not been reported. In this study, wehave identified residues in IRF3 that are phosphorylated in vivo afterinfection with Sendai virus. We found that Sendai virus inducedphosphorylation of the C-terminal residues Thr(390) and Ser(396), inaddition to either Ser(385) or Ser(386). Moreover, Ser(173) andSer(175) were constitutively phosphorylated. Ser(396) has previouslybeen suggested to be the major target of the IRF3-activating kinaseTBK1 (TANK-binding kinase-1), whereas Thr(390) has not previously beenimplicated in IRF3 regulation. Mutagenesis studies indicated thatphosphorylation of Thr(390) promotes Ser(396) phosphorylation andbinding to the coactivator cAMP-response element-binding protein. Takentogether, our results show that IRF3 is subject to multipleinterdependent phosphorylations, and we identify Thr(390) as a novel invivo phosphorylation site that modulates the phosphorylation status ofTBK1-targeted Ser(396).

A quantitative view on *Mycobacterium leprae* antigens by proteomics

Harald G Wiker [a,b,] *, Gisele G Tomazella [a] and Gustavo A de Souza [a,c]

[a] *The Gade Institute, Section for Microbiology and Immunology, University of Bergen, Norway;*
[b] *Department of Microbiology, Haukeland University Hospital, Bergen, Norway;*
[c] *Proteomic Unit at University of Bergen (PROBE), Norway.*

Leprosy is an ancient disease and the focus of researchers' scrutiny for more than a century. However, many of the molecular aspects related to transmission, virulence, antigens and immune responses are far from known. Initially, the implementation of recombinant DNA library screens raised interesting antigen candidates. Finally, the availability of *Mycobacterium leprae* genomic information showed an intriguing genome reduction which is now largely used in comparative genomics. While predictive *in silico* tools are commonly used to identify possible antigens, proteomic approaches have not yet been explored fully to study *M. leprae* biology. Quantitative information obtained at the protein level, and its analysis as part of a complex system, would be a key feature to be used to help researchers to validate and understand many of such *in silico* predictions. Through a re-analysis of data from a previous publication of our group, we could easily tackle many questions regarding antigen prediction and pseudogene expression as well as mapping of metabolic pathways. Several well known antigens are among the quantitatively dominant proteins, while several major proteins have not been explored as antigens. We argue that combining proteomic approaches with bioinformatic workflows is a required step in the characterization of important pathogens.

Qualitative and Quantitative Workflows on a Single MS Platform without Compromising Speed, Sensitivity or Resolution

Volker Kruft, Christof Lenz

AB SCIEX, Darmstadt, Germany

A major challenge in proteomics research and biomarker discovery by mass spectrometry is the analysis of complex samples. As sample complexity increases, so does the need for powerful hardware with high sensitivity and large dynamic range capabilities. Equally important for a thorough analysis of complex samples is the ability to acquire the maximum number of MS/MS spectra without loss of spectral quality during an LC/MS/MS run.
Most available mass spectrometers will fail to meet the requirements for qualitative and quantitative simultaneous analysis. Triple quadrupoles will deliver extreme speed, sensitivity and linear range, but are limited in resolution and accuracy. TOF or Orbi analyzers will offer ultimate resolution and accuracy, but will - generally speaking - compromise speed and sensitivity at the same time.
The AB SCIEX TripleTOF™ 5600 system was developed to achieve high acquisition speeds while maintaining high-resolution and high mass accuracy in both MS and MS/MS mode. The system can acquire up to 50 MS/MS spectra in a second using IDA (information-dependent acquisition) workflows, and up to 100 MS/MS spectra per second in fragmentation mode only.
We will present data that demonstrate high quality protein identification with simultaneous quantitation in MS or MS/MS mode. Also, the MS/MS(all) workflow for the rapid profiling of components of complex samples will be illustrated for a complex lipid mixture.

Post translational modifications of the human DNA repair protein Uracil DNA glycosylase, UNG2

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Uracils arise in DNA either as misincorporation of dUMP during replication or as spontaneous deamination of cytosine. Deamination creates mutagenic U:G pairs while incorporated dUMP results in non-mutagenic U:A pairs. Uracils are removed from DNA by error-free base excision repair (BER) in most cells. However, in B-cells uracil is introduced as deamination of cytosine by Activation Induced Deaminase and is essential in increasing immunoglobulin diversity.
The removal of uracils is initiated by a Uracil DNA-glycosylase (UDG) and the UDG encoded by the UNG-gene is the quantitatively dominant of the four known UDGs in humans. The nuclear form UNG2 is dominant in highly proliferating cells while in resting cells the mitochondrial form (UNG1) dominates.
By gel-electrophoresis and mass spectrometry we have been studying the post translation modifications (PTM) of UNG2. The acetylation, phosphorylation and ubiquitinylation found so far are all residing in the unstructured N-terminal part of the protein. The PTMs affects activity, protein turnover and interactions to other proteins.

Keywords: Uracil, uracil DNA glycosylase

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Bacterial proteins with cleaved or uncleaved SPI-signal peptides

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Correct protein compartmentalization is a key step for molecular function and cell viability, and this is especially true for membrane and externalized proteins of bacteria. Recent proteomic reports of *Bacillus subtilis* have shown that many proteins with Sec-like peptide signals and absence of transmembrane domain helix are still observed in membrane-enriched fractions, but further evidence about signal peptide cleavage or soluble protein contamination was still needed. Here we report a proteomic screening of identified peptides in culture filtrate, membrane and intracellular fractions of *Mycobacterium tuberculosis*. We were able to detect peptide sequencing evidence that shows that the predicted signal peptide was kept uncleaved for several types of proteins such as mammalian cell entry (Mce) proteins and PE or PE-PGRS proteins.. Label-free quantitation of all proteins identified in each fraction showed that the majority of these proteins with uncleaved signal peptides are, indeed, enriched in the Triton X-114 lipid phase. Some of these proteins are likely to be located in the inner membrane while others may be outer membrane proteins.

Definition of novel cell envelope associated proteins in Triton-X-114 extracts of Mycobacterium tuberculosis H37Rv

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Background: Membrane- and membrane-associated proteins are important for the pathogenicity of bacteria. We have analysed the content of these proteins in virulent *Mycobacterium tuberculosis* H37Rv using Triton X-114 detergent-phase separation for extraction of lipophilic proteins, followed by their identification with high resolution mass spectrometry. **Results:** In total, 1417 different proteins were identified. *In silico* analysis of the identified proteins revealed that 248 proteins had at least one predicted trans-membrane region. Also, 64 of the identified proteins were predicted lipoproteins, and 54 proteins were predicted as outer membrane proteins. Three-hundred-and-ninety-five of the observed proteins, including 91 integral membrane proteins were described for the first time. Comparison of abundance levels of the identified proteins was performed using the exponentially modified protein abundance index (emPAI) which takes into account the number of the observable peptides to the number of experimentally observed peptide ions for a given protein. The outcome showed that among the membrane-and membrane-associated proteins several proteins are present with high relative abundance. Further, a close examination of the lipoprotein LpqG (Rv3623) which is only detected in the membrane fractions of *M. tuberculosis* but not in *M. bovis*, revealed that the homologous gene in *M. bovis* lack the signal peptide and lipobox motif, suggesting impaired export to the membrane. **Conclusions:** Altogether, we have identified a substantial proportion of membrane- and membrane-associated proteins of *M. tuberculosis* H37Rv, compared the relative abundance of the identified proteins and also revealed subtle difference between the different members of the *M. tuberculosis* complex.

Exploring the influence of protein phosphatase E on *Bacillus subtilis* metabolism: proteomic analysis of small acid soluble spore proteins in the endospores

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Bacillus subtilis (*B. subtilis*) is a model Gram positive sporulating bacterium. Its endospores are highly resistant to environmental stress, such as high temperatures, UV radiation or chemicals. Sporulation has not been entirely defined yet. Protein phosphatase E (PrpE) and small acid soluble spore proteins (SASPs) influence this process, but direct interaction of this enzyme on SASPs occurrence in the spore coat of *B. subtilis* endospores has not been evidenced so far. Therefore, in the aim to explore these relations, a proteomic analysis of changes in SASPs composition of spore coat produced by *B. subtilis* strain, with a deletion of gene encoding PrpE phosphatase (Δ*prpE*), in comparison to standard 168, was performed. The whole spore cell lysates were analyzed by LC-ESI-MS/MS, and then the detected peptides were assigned to corresponding proteins in the SwissProt database using Sequest algorithm incorporated in Bioworks 3.0 (Thermo Fischer Scientific). Additionally to MS/MS ion search parameter, a QSRR-based linear regression model to predict peptides retention times was tested, in terms of its application to enhance confidence of protein identification.

Performed experiment revealed that the strain, lacking PrpE phosphatase, was characterized by higher occurrence of peptides typical for SASPs, in contrast to the standard 168. Moreover their identification reliability was also better. This enabled to prove the α (SspA)- and β (SspB)-SASPs presence in the Δ*prpE* strain with a satisfying level of confidence. In case of γ (SspE), Tlp and I types of SASPs in this strain it was too low to confirm their real occurrence. Furthermore, application of differences between predicted and experimental retention times in peptides analysis revealed to be a useful tool, facilitating their classification to correctly identified, when these values are low, or incorrectly, when high. In 168 strain, only SASPs α and β could be properly defined, however basing only on one characteristic peptide, so their presence is questionable. Assuming above mentioned results, it may be observed that PrpE phosphatase affects SASPs appearance in the endospores lowering their levels.

Establishing the Proteomic Signature of a Multi-Resistant Clinical *Escherichia coli* Isolate Using Tandem Mass Spectrometry

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The processes by which antibiotic resistance and virulence has evolved remain incompletely understood, even for the common pathogen, *Escherichia coli*. Specific gene clusters are passed on to different strains by horizontal gene transfer, which could lead to evolution of new pathovars, and improve fitness for survival while it coevolves with the host. Using tandem mass spectrometry-based proteomics and a label-free approach, we analyzed a clinical *E. coli* isolate from urine of a patient, which was resistant for antibiotics belonging to □-lactams, aminoglycosides and fluoroquinolones and identified proteins that could be involved in its antibiotic resistance and virulence. To characterize such an isolate with many specific genetic variations we used the recently in-house designed MSMSpdbb software which merges protein databases from several sources of *E. coli* including type strains and other commensal and pathogenic isolates. From the 1,596 identified proteins, we estimated the protein abundance levels of important virulence factors such as iutA, ompA, traT and selected enzymes conferring antibiotic resistance, such as CTX-M-15 (ESBL) and AAC(6')-Ib-cr (Aminoglycoside + fluoroquinolone). We identified several risk factors amongst the highly-abundant proteins. In addition, we could analyze the genome of the clinical isolate and found that 27% of the proteins identified in the present study belong to the pan-genome of *E. coli* species and are only present in a subset of strains. Our approach is a first step towards the identification of risk factor signatures in multi-resistant uropathogenic *E. coli* strain.

Changes in the insoluble protein fraction of bovine *longissimus dorsi* muscle induced by electrical stimulation and post mortem storage

Eva Veiseth Kent, *Nofima Mat*

Changes in protein composition of the insoluble protein fraction of bovine *longissimus dorsi* muscle between electrical stimulated (ES) and non-electrical stimulated (NES) carcass sides were studied by proteomics (2D-PAGE). Changes in the protein composition during the first 24 h post mortem were also analysed for these samples. Most of the variation in the data set was related to protein changes occurring during post mortem storage, however electrical stimulation also contributes to variation in protein abundance. Moreover, the ES treatment seems to induce more protein changes from 1 to 24 h post mortem than the NES treatment.

High Performance at Highest Speeds for Protein Identification in Complex Matrices

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A major challenge in proteomics research and biomarker discovery by mass spectrometry is the analysis of complex samples. As sample complexity increases, so does the need for powerful hardware with high sensitivity and large dynamic range capabilities. Equally important for a thorough analysis of complex samples is the ability to acquire the maximum number of MS/MS spectra without loss of spectral quality during an LC/MS/MS run. However with most high performance mass spectrometer instruments available today, high speed MS/MS acquisition is performed at the expense of high quality data. This is detrimental as higher mass accuracy and higher resolution improves the specificity of peptide identification.
The AB SCIEX TripleTOF™ 5600 system is capable of achieving high acquisition speeds while maintaining high-resolution and high mass accuracy in both MS and MS/MS mode. The system can acquire up to 50 MS/MS spectra in a second using powerful IDA (information-dependent acquisition) workflows, and its performance was investigated to understand the advantages for protein identification from complex samples.
Obtaining the most in-depth interrogation of a proteome also depends on the quality of the LC separation and the power of the database search engine. Here, we use the Eksigent nanoLC Ultra system with the cHiPLC™-nanoflex to obtain the highest quality separations with the highest reproducibility. Digging deeper into a proteomic sampe requires a database search algorithm that is able to broadly identify many different PTMs and other unexpected peptides that will now be observed. ProteinPilot™ Software with its unique Paragon™ Algorithm enables the simultaneous identification of hundreds of modifications, substitutions, and unexpected cleavages.

AB SCIEX TripleTOF™ 5600 System: High Performance for Qualitative and Quantitative LC/MS/MS

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The AB SCIEX TripleTOF™ 5600 System is the first accurate-mass, high-resolution LC/MS/MS system for qualitative analysis, with the speed and sensitivity to deliver high-performance triple quad-like quantitation. The system enables enhanced workflows for comprehensive exploring, profiling, and quantifying of low abundance analytes in very complex matrices. The combination of high mass accuracy and resolution with high sensitivity allows very high acquisition speeds without compromising performance. The system enables advanced workflows that allow conclusive identification and quantification of low abundance compounds in complex samples, all in a single run, making it the ideal platform for transitioning from qualitative workflows such as ID, confirmation or screening to early quantitation experiments.

A Strategy For The Quantitative Analysis of All Lipids With A Single Acquisition Method

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The recent advances in the AcceleratorTOF™ Analyzer in the AB SCIEX TripleTOF™ 5600 System now enables high speed acquisition of MS and MS/MS data while maintaining resolution, mass accuracy and quantitative accuracy. When applied to *shotgun* lipidomics, TOF MS and MS/MS approaches are now capable of characterizing complex lipid extracts by direct infusion without a preliminary need for HPLC or UHPLC fractionation of lipid class and sub-classes1. This is carried out by a simplified acquisition technique called MS/MS(ALL) acquisition, which enables the acquisition of product ion spectra of every precursor in a data independent way, selected at user specified steps across a given mass range.

Because all TOF MS/MS product ions are stored, lipid-class specific profiling can be performed post-acquisition through the extraction of any lipid fragment ion, to determine all precursors in the given mass range that possess the targeted fragment ions. Additionally, a high resolution TOF MS survey scan is also collected during the infusion based acquisition, to supplement the precursor profile with high mass accuracy measurements for optimal lipid identification.

This approach yields lipid identifications as precursor ion selection is carried out at unit resolution and MS/MS is acquired with high resolution and mass accuracy. Also, if proper lipid internal strategies are employed and signals are corrected for isotopic distributions, this workflow can be both quantitative and qualitative through a broad dynamic range. With infusion in both positive and negative polarities, comprehensive lipid profiling can be done in a single acquisition without the requirement for HPLC separations and deconvolution of time aligned precursors and product ions. This *shotgun* MS/MS(ALL) workflow represents a fast and easy acquisition strategy to profile lipids from complex biological extracts.

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