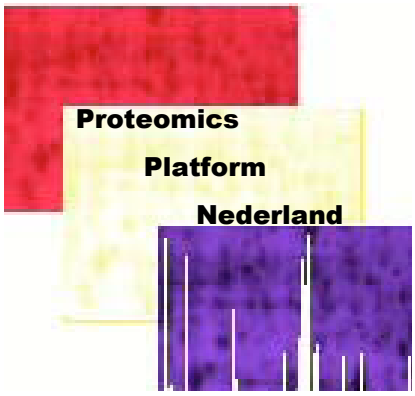


## FALL MEETING OF

## THE NETHERLANDS PROTEOMICS PLATFORM

# Biomarker Discovery

## in the post-genome era

**Monday October 4, 2004**
**Auditorium**
**Vrije Universiteit Amsterdam**

The Netherlands Proteomic Platform is part of the Nederlandse Vereniging voor Biochemie en Moleculaire Biologie (NVBMB)

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**Fall meeting of**

**The Netherlands Proteomics Platform**

# Biomarker Discovery in the post-genome era

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**Auditorium**
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**Twan America**
**Rob van der Heijden**
**Connie Jiménez**
**Jeroen Krijgsveld**
**Monique Slijper**

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## Symposium Program

## Biomarker Discovery in the post-genome era

9.00-9.30 Registration

9.30- 9.35 **Welcome**
Connie Jimenez, Vrije Universiteit Amsterdam

9.35-10.05 **Methods for biomarker discovery applied to chronic obstructive pulmonary disease and cervical cancer**
Rainer Bischoff, Groningen University

10.05-10.35 **Analytical technologies for the characterization of protein interactions: fundamentals and applications in drug discovery**
Hubertus Irth, Vrije Universiteit Amsterdam

10.35-11.00 PAUZE

11.00-11.30 **The use and discovery of biomarkers to support pharmaceutical drug development at Organon**
Alain J. van Gool, N.V. Organon, Oss

11.30-12.00 **Imaging mass spectrometry of cells and tissues**
Sander Piersma, Amolf, Amsterdam

12.00-13.30 PAUZE LUNCH/ POSTERS/ STANDS

PLENARY LECTURES

13.30-14.30 **Focused Serological Proteomics for Clinical Diagnostics and Biomarker Discovery**
Niels Heegaard, Serum Statens Institute, Copenhagen, Denmark

14.30-15.00 PAUZE

15.00-16.00 **Peptidomics Analysis of the Metabolic Syndrome**
Peter Schulz-Knappe, Biovision, Hannover, Germany

16.00-17.00 **Advanced strategies for profiling protein abundances using FTICR mass spectrometry**
Ljiljana Pasa-Tolic, Pacific Northwest National Laboratory, USA

17.00-18.00 BORREL

# Methods for Biomarker Discovery: Chronic Obstructive Pulmonary Disease and Cervical Cancer

Barroso, B., Govorukhina, N.I., Reijmers T.H.2, Nyangoma S.O.2, Jansen, R.C.2, Verheij, E.R.3, van der Greef, J.3, van der Zee, A.G.J.1, Postma, D.S.4 and Bischoff, R
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*4Department of Pulmonary Diseases, University Hospital Groningen, Hanzeplein 1, 9700 RB, Groningen, The Netherlands*

Most diseases manifest themselves by measurable changes in human physiology. This forms the basis for clinical chemistry and its value in helping to diagnose disease correctly and in following therapeutic interventions. Presently, many biochemical and cellular parameters (biomarkers) are routinely measured in blood, plasma, serum or urine in any major hospital and the results of these measurements support decision making by clinicians. One of the major challenges in using body fluid analyses for diagnostic or therapeutic purposes is that there is a large natural variation in the concentration of proteins, peptides or metabolites between individuals over time.
Discovery of biomarkers or biomarker patterns depends on quantitative analytical methods that can provide reproducible data from samples of healthy subjects and patients at different stages of disease. Well-controlled collections of samples need to be available that allow correlation of the clinical history with the biomarker patterns. Here we will report about biomarker studies into cervical cancer and chronic obstructive pulmonary disease (COPD).
Cervical cancer is the second most prominent cause of death for women from any cancer. It is most prevalent in developing countries and is generally associated with infection by human papilloma virus (HPV). In order to analyse an extensive serum bank at the University Hospital Groningen, major serum proteins are depleted and the protein mixture digested with trypsin. Peptide mixtures are analyzed by reversed-phase HPLC-MS and the pre-processed data compared by multivariate statistics.
COPD is a major cause of mortality and morbidity in industrialised countries. Most cases of COPD are caused by cigarette smoking but only 15-20% of heavy smokers develop the disease within their lifetime. It is thus of interest to find biochemical markers that can identify the high-risk population and allow to followtherapeutic interventions. Degradation products from extracellular matrix proteins as well as phospholipids may constitute relevant biomarkers of inflammation and tissue destruction during COPD. Work is ongoing to analyse bronchoalveolar lavage fluid (BAL) from patients with COPD and Bronchiolitis Obliterans.
* the cervical cancer project is being supported by the Dutch Cancer Fund (KWF)

# Analytical technologies for the characterization of protein interactions: fundamentals and applications in drug discovery

H. Irth

*Vrije Universiteit Amsterdam, Department of Analytical Chemistry and Applied Spectroscopy, De Boelelaan 1083, 1081 HV Amsterdam, (The Netherlands) and Kiadis BV, Zernikepark 6-8, 9747 AN Groningen (The Netherlands)*

The present lecture gives an overview on our attempts to develop analytical screening technologies, where capillary LC-MS is used to measure simultaneously the chemical and biochemical characteristics of bioactive compounds. MS-based biochemical assays are equivalent to fluorescence HTS assays by using appropriate reporter molecules and enzyme substrates for the development of receptor-ligand binding and enzyme inhibition assays. Electrospray MS is used to detect concentration changes of these reporter molecules upon interaction with receptors or enzymes and consequently allows the detection of (unknown) active ligands or biomarker with biological activity. Next to the biochemical readout, molecular mass information on the active compound is generated simultaneously and allows the rapid identification of the chemical species involved.

The applicability of the integrated screening technology will be presented for key areas in drug discovery. Special attention will be paid to case studies from lead optimization and early ADME projects with a focus active metabolite screening. Both ligand binding and substrate conversion assays will be shown using proteases from the cathepsin family as well as monoaminoxidases as biomolecular targets. Teh applicability of the current technologies for the discovery of biomarkers will be discussed.

## The use and discovery of biomarkers to support pharmaceutical drug development at Organon.

Alain J. van Gool, N.V. Organon, Oss

The development of pharmaceutical drugs is a costly process, lasting at least 12 years. Biomarkers can aid several aspects of this process, ranging from the selection of compounds to the support of clinical development. Different types of biomarkers exist and examples will be given. Although many biomarkers are known and are being used, the need for novel biomarkers remains. Strategies to identify and validate such biomarkers will be discussed.

## Imaging mass spectrometry of cells and tissues

Sander Piersma, Amolf, Amsterdam

Mass spectrometry is a key technology in the structural characterization of peptides and proteins. This development is mainly due to the advent of the soft ionization techniques MALDI and ESI. For many mature spectroscopic techniques (e.g. fluorescence, IR and Raman spectroscopy) technological innovations have produced imaging instruments allowing determination of spectral features in a spatially resolved manner. In mass spectrometry SIMS has long been the only imaging MS application allowing imaging of elements and small molecules at submicron resolution. In recent years also MALDI-ToF has emerged as an MS imaging technique using the scanning probe approach. In this contribution a novel MALDI-ToF mass microscope is used to study the spatial distribution of biomolecules on biological surfaces, such as cells and tissue sections. After matrix deposition a surface area of 190 x 240 micron is homogeneously illuminated by a UV laser pulse and the ions produced are mass separated and imaged with retention of their initial spatial distribution on the detector. In its present geometry the instrument delivers mass selective peptide and phospholipid images at the single cell level. Molecular imaging mass spectrometry is a powerful technique that combines the chemical information of mass spectrometry with the spatial distribution information of a microscope.

# Focused Serological Proteomics for Clinical Diagnostics and Biomarker Discovery

Niels H. H. Heegaard, 81/536, Statens Serum Institut, Copenhagen, Denmark

Mass spectrometry (MS) is an important tool for the discovery of diagnostic leads in human disease because of its precision and resolution. This is evident from the growing number of publications linking proteome analyses of e.g. serum samples with specific diagnoses. In the initial phase of introducing the approach, however, a number of issues have been overlooked or ignored. This has transformed the initial euphorism into a probably more realistic cautious optimism with regards to using MS-based proteomics for clinical diagnostics. One very important issue is that clinicians and statistical epidemiologists often do not consider that all MS proteomics applications require some kind of focusing (reduction of analyte population) because the complexity, mass span, and dynamic range of biofluid analyte mixtures overwhelm the capabilities of present MS instruments. Conversely, MS operators and data handlers often overlook the clinical reality where diagnostic specificity is crucial, i.e., the importance of being as critical regarding the choice of control samples as regarding the choice of the disease samples. Thus, the task of integrating statisticians, MS analysts, and clinical biochemists into method development is formidable. We use an integrated, focused proteomics approach based on extracting serum proteome subsets for MALDI-MS with derivatized (C8) magnetic beads (MB) or based on even more focused subsets using on-line immunoaffinity (IA) LC-MS. The important interplay between sampling, sample preparation, data reduction, algorithm performance, and model building in the MB approach was tested simply by adding a number of synthetic peptides to one part of a split sample set of normal sera that were then processed with small differences to obtain solid data regarding the most optimal procedures of sampling and processing for serum-proteomics. This, as well as the potential of IA-LC-MS in discovering new modified protein markers will be illustrated in the talk. Focused and ultra-focused proteomics promise to be invaluable for improving laboratory diagnostics and thus also for improving biological understanding and for improving patient care as long as carefully validated approaches are adhered to in every procedural step.

## Peptidomics Analysis of the Metabolic Syndrome

P. Schulz-Knappe and Hans-Dieter Zucht
BioVisioN AG, Feodor-Lynen-Str 5, 30625 Hannover, Germany

Sedentary lifestyle and overly rich nutrition have resulted in a dramatic increase in the incidence of type 2 diabetes, obesity and associated comorbidities. The metabolism and communication of the key tissues involved is widely regulated by peptides and unter intense investigation.
The comprehensive analysis of peptides and proteins from tissues and body fluids in proteomics and peptidomics research bears potential to guide novel discovery to better understand, prevent, diagnose and treat the disorders of the metabolic syndrome.
This presentation will focus on peptidomics technologies and their application to the analysis of model systems and human plasma samples with special emphasis on peptide hormones involved in glucose homeostasis. Differential expression of secreted peptides from adipocytes following thiazolidinedione treatment, the comparative, quantitative analysis of pancreatic peptides from wild type versus ob/ob mice and carboxypeptidase E deficient mice, and results from an oral glucose challenge in healthy volunteers will be presented. The benefit of peptidomics analysis for drug profiling, peptide prohormone processing and biomarker discovery will be discussed. Special emphasis will be put on the sensitive analysis of plasma samples for population based proteomics type of work.

# Advanced strategies for profiling protein abundances using LC-FTICR/MS

Ljiljana Pasa Tolic, Gordon A. Anderson, Mary S. Lipton, David G. Camp II, Yufeng Shen, Christophe Masselon, and Richard D. Smith

We will describe comprehensive quantitative proteome measurements obtained using accurate mass and time (AMT) tag approach[1] that builds upon the combination of high resolution nano-scale liquid chromatography (LC) with the high performance Fourier transform ion cyclotron resonance mass spectrometry (FTICR/MS). Strategies for LC-based quantitative proteomics are based on the use of stable-isotope labeling methods (e.g., $^{14}N/^{15}N$, $^{16}O/^{18}O$) to obtain precise measures of the relative protein abundances. For very complex samples (e.g., whole cell lysates), a single LC-FTICR analysis (i.e., AMT tag proteomics) can definitely yield more information than a conventional LC-MS/MS experiment (shotgun proteomics) since a large fraction of detected peptides are amenable to AMT tag based identification and quantitation in a single acquisition. The use of FTICR, with its unsurpassed resolving power and mass measurement accuracy, allows one to determine abundance ratios (ARs) for highly complex peptide mixtures either using $^{14}N/^{15}N$ metabolic labeling (where the mass difference between pair members is peptide dependent) or $^{16}O/^{18}O$ labeling (where the isotopic distributions of two versions of the peptide are likely to overlap). Since all of the species detected can be paired with high confidence, an AR-directed MS/MS strategy will likely allow characterization of potential biomarkers with high enough sensitivity and throughput to enable application to clinical samples.
Herein, we describe tools for quantitative AMT tag proteomics and present application of these methods for the analysis of microbial systems of interest because of their possible applications in e.g. bioremediation, and representative mammalian proteomics studies. Advanced sample processing techniques, LC and FTICR instrumentation to enable rapid and precise measurement of protein abundances from limited samples (e.g., clinical specimens) will be presented.

1. L. Pasa Tolic et al., J. Mass Spectrom. 2002, 37, 1185-98.

# POSTER PRESENTATIONS

# Machine learning methods for tumor diagnostic and biomarker detection.

Abstract: This investigation considers proteomic pattern datasets from the NCI/CCR and FDA/CBER Clinical Proteomics Program Databank. Novel machine learning methods for classification and variable subset selection are applied to these datasets. In particular, methods based on support vector machines (SVMs) are considered. SVMs are a popular machine learning approach for robust classification of high input dimension data. Results of computational experiments indicate the effectiveness of these methods for handling proteomic pattern data.

Joint work by Kees Jong, Elena Marchiori, Aad van der Vaart, Vrije Universiteit Amsterdam

# Detailed difference analysis of complex protein mixtures by multidimensional LC-MS using new software for alignment and statistical difference analysis.
# A proteomics study of tomato fruit ripening.

A.H.P. America[1], J.G. Cordewener[1], H.A. van Geffen[1], A. Lommen[1], H. Vissers[2], R.D. Hall[1]
1) Plant Research International, PO Box 16, 6700 AA Wageningen, The Netherlands
2) Waters Corporation - EU Mass Spectrometry Technologies Centre, Almere, Netherlands

Multidimensional HPLC coupled to mass spectrometry is a recently developed method in the field of proteomics research in order to analyse complex protein mixtures in much detail. By trypsin digestion of a complete protein mixture, a highly complex peptide mixture is produced. In this paper a procedure is presented for fast, detailed and quantitative protein profiling based on the multidimensional LC-MS separation of tryptic-derived peptides. We apply the method to analyse protein expression during the ripening of tomato fruit. Digested tomato proteins were analysed by two-dimensional LC-MS/MS. The complex peptide mixture was first separated by strong cation exchange chromatography (SCX). Collected SCX fractions were subsequently separated by reversed phase nano-LC on line coupled to a hybrid Quadrupole-Time-of-Flight (QTOF) mass-spectrometer.

In order to perform a difference analysis between the samples of different ripening stages we performed a replicate set of RP-LC-MS analyses of separate fractions from the two SCX separations, of the red and the green tomato sample. Alignment of the obtained high density LC-MS chromatograms appeared to be absolutely essential for a detailed comparison of these data. We used in house developed software for multiple alignment, noise~ and data reduction and statistical variance analysis of these replicate datasets. The reduced dataset of peak retention and intensity data was compared by principal component analysis. This revealed a clear separation of the green vs. the red samples, and clear separation of the different SCX fractions. From a selected set of differential detected peptide masses we collected the MS/MS spectra using data dependent acquisition mode. These spectra were analysed matched to TICGR TC sequence database using ProteinLynx software.This enabled the identification of several hundred proteins from different stages of ripening tomato fruit and a comparison of protein expression patterns.

# Proteomics Analysis of Potato Tuber in Relation to Tuber Quality Characteristics

A.H.P. America, F. van der Wal, H.H. Jonker J.G. Cordewener , R.D. Visser, R.D. Hall
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In this project we aim to establish a link between expression profiles of specific proteins in relation to established differences in potato tuber quality. We report here on a detailed proteomics analysis of the protein composition of ripe potato tuber material from multiple sibblings from the F1 population of two diploid parent lines (CxE population, Dept. Plant Breeding WUR). From the F1 population those clones were selected that display the most extremes in the quality phenotype regarding their protein content value. Samples were prepared from two different harvests from field material. We analysed individual samples from the selected clones as well as bulked samples from the selected clones, separating the low and the high quality group. We performed detailed two-dimensional gel electrophoresis in order to separate up to 2000 protein spots per gel. We employed a multiplex analysis procedure making use of fluorescent protein labeling (DIGE technology). A collection of 16 2D gel samples resulted in 48 high quality gel images , including internal reference samples. By the use of dedicated 2D gel analysis software we were able to detect, match and quantifiy on average 1670 spots per gel.
Statistical analysis of the protein expression data selected 25 spots from the bulked samples that were significantly differential for the high versus the low quality groups. Of these 25 spots some 7 spots were seletive in at least three out of five clones within the bulked sample.
Mass spectrometric analysis using LC-MS/MS will provide peptide sequence information from which the identity of the selected proteins can be derived. The strength of this approach is that complex data sets involving measurements of up to several thousand different components will be used to find linked clusters of elements potentially associated with traits of interest.

# Direct peptide profiling of microdissected breast cancer cells

Arzu Umar[1,2], Mieke Timmermans[2], John Foekens[2], and Theo M. Luider[1]

[1] Department of Neurology and Center for Biomics, Erasmus Medical Center
[2] Department of Medical Oncology, Erasmus Medical Center, Rotterdam, The Netherlands

Breast cancer is the most common cancer in women worldwide and a major cause of death. Since 70% of breast tumors are positive for estrogen receptors (ERs), the anti-estrogen tamoxifen is a widely used treatment for patients with advanced disease. Despite the presence of ERs, not all breast carcinomas respond favorably to tamoxifen treatment. In addition, tumors that initially show a favorable response eventually become resistant. In order to unravel the mechanisms of tamoxifen-resistance, we are aiming to identify responsible key proteins and modifications using direct mass spectrometry. For this study we used two groups of breast carcinomas, the ones that responded favorably to tamoxifen treatment and the ones that did not show any response. Cryosections were prepared from the tissues and both tumor and stromal cells were separately captured by laser microdissection. Tryptic digests were prepared from total cell lysates and directly analyzed by MALDI-TOF-MS and LC-ESI-MS/MS. We were able to obtain peptide profiles from as little as one hundred cells. The observed peptides peaks in both cell types varied between 70 and 100 and distinct differences could be observed, although 40-60% of the profiles were overlapping. Furthermore, significant protein identification by MS/MS was possible using approximately 1000 cells. We are currently in the process of determining peptide profiles of tamoxifen-responders and non-responders, in order to identify proteins that are involved in this process.

Arzu Umar and Theo Luider are supported by the Netherlands Proteomics Center.

# PROTEIN EXPRESSION AFTER IRRADIATION OF HUMAN BRAIN TUMOUR SPHEROIDS

P. Sminia[1], C. R. Jiménez[2], M.V.M. Lafleur[1], B.J. Slotman[1] Department of [1]Radiation Oncology and [2]Proteomics Center, VU University medical center, Amsterdam, The Netherlands.

**Purpose:** Identification of radiation-induced differential protein expression in human glioblastoma cells.

**Experimental procedure:** Human U87 glioma cells, which are relatively radioresistant, were cultured in vitro as three-dimensional cell aggregates - spheroids - , resembling tumor micrometastases in vivo. Spheroids were _-irradiated with 20 Gy single dose or sham treated. Twenty-four hours after irradiation, cells were lysed and proteins were extracted. Using 2D gelelectrophoresis, protein expression profiles from irradiated and control glioma spheroids were compared. Image analysis software was used to detect significantly regulated proteins (at least 2-fold; T-test). Five highly regulated proteins were selected for identification by Mass Spectrometry.

**Results:** Image analysis showed significant differential expression in 67 of approx. 2200 expressed proteins. Irradiation resulted in an approx. 100-fold upregulation of Macrophage Migration Inhibition Factor (MIF; involved in neovascularization) as well as in upregulation of peptidyl-prolyl cis-trans isomerase A (apoptosis-associated protein). Downregulated proteins included enolase-alpha (cell differentiation), heat shock protein 70 ATPase (stress conditions) and thioredoxin peroxidase (cell proliferation; approx. 5-fold).

**Conclusion:** Protein expression profiling is a novel tool to detect differentially expressed proteins in cancer and evaluate treatment effects. Counteracting the radiation-induced upregulation of MIF, recently described to be a critical protein in neovascularization of glial tumours, might enhance the effectiveness of radiation therapy of glioblastoma multiforme.

# A combination of protein profiling and isotopomer analysis using MALDI-TOF mass spectrometry reveals an active metabolism of the extracellular matrix of 3T3-L1 adipocytes

Freek Bouwman, Johan Renes and Edwin Mariman

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The abstract is:
Differential gel electrophoresis followed by MALDI-TOF mass spectrometry is a commonly used protein profiling method. However, observed changes can be explained in multiple ways, one is by protein turnover rate. In order to easily and rapidly obtain information on both identity and turnover of individual proteins, we applied the combination of protein labeling with L-(ring-2,3,4,5,6 2H5) phenylalanine and MALDI-TOF mass spectrometry. While the spectrum reveals the identity of a protein, mass isotopomer analysis provides information about the rate of protein labeling as a measure for synthesis or turnover.
Using this approach on mature 3T3-L1 adipocytes, we were able to discriminate between rapidly and slowly metabolising proteins. In our isolate, proteins of the cytoskeleton appeared to be slowly metabolising, whereas components of the extracellular matrix, in particular collagen type I alpha 1 (COL1A1) and collagen type I alpha 2 (COL1A2) show a rapid accumulation of newly synthesized proteins. In addition, functionally related proteins were also readily labeled. Taken together, we have shown that a combination of stable isotope labeling and protein profiling by gel electrophoresis and MALDI-TOF analysis can simultaneously provide information on the identity and on the relative metabolic rate of proteins in eukaryotic cells in a simple, non-hazardous and rapid-throughput way.

# Identification of tyrosine-O-sulfation as post-translational modification of melanoma inhibitory activity (MIA) protein.

Marcel de Vries[1], Nicole Rullens-Ligtvoet[1], Wouter Hoeberichts[1], Alain van Gool[1], Peter Verhaert[1,3], and Ebo Bos[2]
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[3] Laboratory of Neuroplasticity and Neuroproteomics, University of Leuven (LEUVEN, Belgium)

Melanoma inhibitory activity (MIA), or cartilage-derived retinoic acid-sensitive protein (CD-RAP), is a 11 kD secretory protein which is normally expressed by cartilage, but is also produced by malignant melanomas, chondrosarcomas and to a lesser extent by adenocarcinomas, including breast and colon cancers.
MIA is translated as a 131 amino acid (AA) precursor and is processed into a mature, non-glycosylated 107 AA protein by cleavage of a secretion signal peptide, and stabilized by disulfide bridges in an SH3-domain resembling tertiary structure.
Biochemical analysis of recombinant human MIA by isoelectric focussing and MALDI mass spectrometry revealed various MIA isoforms, with and without +80 Da modifications. Ion stability studies in MALDI TOF and MALDI quadrupole TOF hybrid instruments of intact and trypsin digested protein, combined with enzymatic treatment (alkaline phosphatase and sulfatase) provided evidence that the modification is Tyr-O-sulfation, and not phosphorylation, on tryptic fragment 59-76 of the mature protein.
Final proof was obtained by producing the recombinant protein in the presence of chlorate, a known inhibitor of protein sulfation in CHO-transfectants, which resulted in a complete absence of the +80 Da modification.

# New Technologies for Expanding the Dynamic Range of Protein Identification in Human Serum

Frans te Welscher*, Kelly Zhang, Gordon Nicol, Nina Zolotarjova, Cory Szafranski, Jerome Bailey, Liang-Sheng Yang, and Barry Boyes
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Proteomics is promising in protein identification for disease markers and/or drug targets. But it is also a challenging field due to the complex nature of proteins and the wide dynamic range of protein mass in biological samples such as human plasma/serum. Dynamic range limitations in protein separation technologies such as gel electrophoresis (2DGE), capillary electrophoresis, and HPLC need to be overcome to enable the detection of lower-abundant proteins of interest in mixtures. Peptide mass mapping by MALDI-MS has the inherent problem for detecting stronger arginine peptide signals than lysine tryptic fragments. In this study, we have used an immunodepletion technology to remove six high-abundant proteins: albumin, IgG, IgA, haptoglobin, transferrin, and antitrypsin to bring low-abundant proteins into detectable levels. Furthermore, we have utilized a novel lysine mass tagging reagent to improve peptide sequence coverage and MS/MS fragmentation of lower-abundant proteins found in human serum. After removal of the six high-abundant proteins (85-90% of total protein mass in human serum), low abundant proteins were efficiently enriched and resolved on 2DGE with ten times increase in mass loading. An increase in level of confidence in MALDI-MS identification of proteins was demonstrated by derivatizing lysine side chains with 2-methoxy-4,5-dihydro 1H-imidazole. This lysine specific mass tagging reaction resulted in a 5 - 20 times increase in lysine peptide signal relative to the underivatized peptides. These technologies, used together, enable improvements in the number of identifiable proteins in human serum/plasma.

# Proteins involved in fat storage and fat depletion in 3T3-L1 cells: a proteomics approach

Renes J., Wang P., Bouwman F. and Mariman E. Maastricht Proteomics Center, Dept. Human Biology, University of Maastricht, Maastricht, The Netherlands

Currently the power of the DNA-arrays is highly appreciated, however the predictive value of mRNA expression is limited with respect to cellular physiology. Consequently, a broader understanding of the adipogenic process requires independent examination of protein expression and protein function complementing the mRNA expression analysis. We aimed to search for proteins that are involved in pre-adipocyte differentiation and adipocyte starvation using a proteomics approach. Mouse 3T3-L1 preadipocytes were differentiated into adipocytes and subsequently adipocytes were subjected to a starvation protocol either in presence or absence of tumor necrosis factor $\alpha$ (TNF$\alpha$). Protein samples were isolated from cell material and from culture media that was used during differentiation/starvation to study excreted proteins. Protein profiles were investigated using 2-dimensional (2D) gel electrophoresis and protein identification was perfomed with matrix assisted laser desorption ionisation Time of Flight-mass spectrometry (MS) or with liquid chromatography-MS. From cellular material 93 differently expressed proteins were observed during differentiation/starvation from which 50 were identified. Culture media from cells showed 227 differently expressed proteins from which 161 were identified. Cellular material revealed up- and downregulation of glycolytic enzymes as well as other metabolic enzymes, proteins with functions in cell growth regulation, protein modification, cytoskeletal re-arrangements and proteins with currently unknown functions in 3T3-L1 differentiation/starvation. Starvation with or without TNF$\alpha$ showed regulation of a different panel of proteins. Profiles of excreted proteins showed extracellular matrix proteins and proteins with growth regulatory properties. In conclusion, we have identified protein markers for and molecular pathways involved in the in vitro differentiation/starvation of 3T3-L1 cells. This may have consequences for future weight gain management.

# High Content Proteomics: Fractionation and Identification of Differentially Expressed Alzheimer's Disease Proteins

Mary Lopez, Simon Melov, David Bennett, Scott Kuzdzal, Alvy Mikulskus, Eva Golenko, Suzanne Ackloo

corresponding author: Ewoud Ouwerkerk

Control and Alzheimer's human brain tissue (cortex) samples from a Religious Order study (with complete clinical histories) were fractionated using microscale techniques to enrich for acidic, basic, and calcium-binding proteins. Experiments were carried out on both individual and pooled samples. Over 100 statistically significant differentially expressed protein spots were located in the pooled and individual studies of 2D-gel separations. Thirty-nine protein spots were chosen as the best candidates. Eleven of these protein spots were found in both studies (pooled and individual) and twenty-seven were identified in the individual study only. These spots were excised,digested and analyzed by Matrix Assisted Laser Desorption/Ionization orthogonal-Time of Flight (MALDI o-TOF) mass spectrometry. Previously reported Alzheimer's-associated proteins (such as gamma neuron-specific enolase) were identified, as well as many interesting new proteins. These proteomics results will be compared to genomics array data performed at the Buck Institute.

# A novel qualitative and quantitative and LC-MS based approach to protein profiling and biomarker discovery

Scott Geromanos[1], Keith Richardson[2], Phillip Young[2], Richard Denny[2], Kieran Neeson[2], Therese McKenna[2], Craig Doreschel[1], Marc Gorenstein[1], Guo-Zhong Li[1], Timothy Riley[1], Jeffrey C. Silva[1], Alistair. Wallace[2] and Jim Langridge[2].

[1]Waters Corporation, Milford MA, USA; [2]Micromass MS Technologies Centre, Manchester, UK;

Keywords: proteomics, biomarker, Waters, quantitation, novel, protein profiling

In this presentation we will present a new concept for qualitative and quantitiative protein profiling. This is based upon a simple LC-MS based methodology that will allow for determining the relative change in abundance of proteins in highly complex mixtures. Utilizing a reproducible chromatographic separations system along with the high mass resolution and mass accuracy of an orthogonal time-of-flight mass spectrometer, allows for the identification of tens of thousands of ions emanating from identically prepared control and experimental samples. Using this configuration, we can determine the change in relative abundance of a small number of ions between the two conditions solely by accurate mass and retention time. Our data clearly shows that with respect to digestion, ionization, and chromatographic reproducibility as well as high mass precision we are capable of generating the appropriate levels of reproducibility and mass precision to provide such conclusions and in addition that the associated algorithms are capable of extracting the data and calculating the appropriate responses with careful attention to proper error modelling.

In addition this technology provides a powerful MS based peptide identification alternative to conventional LC-MS/MS strategies. In the course of an LC-MS acquisition the collision energy is continuously switching from low to high energy throughout the entire LCMS acquisition. The resulting high-energy data provides extensive fragmentation information across the entire mass range, including the low mass region, across the entire peak width for every precursor ion detected in the low energy function of the chromatogram. The fragment ions (high energy function) are aligned to their related precursor ions in chromatographic space by retention time and chromatographic peak shape. The results clearly demonstrate the ability to confidently identify significantly more proteins with higher sequence coverage than traditional DDA based approaches as well as minor peptide components within a complex peptide mixture. Results from the study of various biological samples such as E.coli and Human plasma and serum will be presented.