EuPA 2012 SCIENTIFIC CONGRESS - ABSTRACTS

Oral Presentations

Plenary lecture IO01

CURRENT STATE AND PERSPECTIVES OF PROTEOMICS– ARE WE HEADING IN THE RIGHT DIRECTION?

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Proteomics has now been around for almost two decades. During this period it has undergone a dramatic development. The number of genomes sequenced, which is the basis for proteomics, has expanded exponentially. New generations of mass spectrometers and LC-equipments dedicated to the use in proteomics have been developed. New bioinformatics tools to handle the huge amount of data produced are constantly emerging. Presently several thousands of proteins are routinely identified and quantified in a high throughput proteomics experiment and the information stored in databases. There has been a strong focus on the search for mechanisms behind diseases and for reliable diagnostic biomarkers. However, there are still limitations. The dynamic range of the proteins in the cells is 107-108 and in serum around 1011 and no analytical technique can handle such a dynamic range. In spite of the huge investments in search for reliable biomarkers only a few if any have been identified using proteomics. Better understanding of the basic biology and new concepts for identification of the relevant markers or combinations of markers are needed. Improved methods for enrichment of specific low abundant proteins must be developed. The present state of art of proteomics will be described and the need for rethinking the perspectives of the present trends in proteomics discussed.

IO02

CHEMICAL PROTEOMICS FOR BASIC BIOLOGY AND PRECLINICAL DRUG DISCOVERY

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Mass spectrometry based proteomics has undoubtedly revolutionized the way biological systems are investigated at the protein level. Thousands of proteins can nowadays be charted in parallel both qualitatively and quantitatively and under multiple physiological conditions. Chemical approaches have proven particularly useful when addressing questions around protein activity and, in basic biology research, such studies have provided valuable insights into cellular signalling pathways, the protein folding and degradation machinery, protein homeostasis and many other areas.

Preclinical stages in the drug discovery process require a multitude of biochemical and genetic assays in order to characterize the effects of drug candidates on cellular systems and model organisms. Early attempts to apply unbiased proteomic techniques to the identification of protein targets and off-targets as well as to elucidate the mode of action of candidate drug molecules suffered from a striking discrepancy between scientific expectations and what the technology was able to deliver at the time. Dramatic technological improvements in mass spectrometry-based proteomic and chemoproteomic strategies have radically changed this situation.

This presentation will highlight a number of recent examples that illustrate how chemical proteomics is used in our laboratory to identify novel cancer drug targets and biomarkers, decipher individual tumor biologies of cancer patients, investigate the mechanism of action as well as the selectivity of small molecule drugs.

1. Wu, Z., Moghaddas Gholami, A. & Kuster, B. (2012) Systematic identification of the HSP90 regulated proteome. Mol Cell Proteomics. 2012 Feb 14. [Epub ahead of print] 2. Schirle, M., Bantscheff, M. & Kuster, B. (2012) Mass spectrometry-based proteomics in preclinical drug discovery. Chem. Biol. 19, 72-84.
3. Wu, Z., Doondeea, J.B., Gholami, A.M., Janning, M.C., Lemeer, S., Kramer, K., Eccles, S.A., Gollin, S.M., Grenman, R. Walch, A., Feller, S.M. and Kuster, B. (2011) Quantitative chemical proteomics reveals new potential drug targets in head and neck cancer. Mol Cell Proteomics, Sep 28. [Epub ahead of print] 4. Mallick, P., and Kuster, B. (2010) Proteomics: a pragmatic perspective. Nat Biotechnol 28, 695-709. 5.Bantscheff, M., Eberhard, D., Abraham, Y., Bastuck, S., Boesche, M., Hobson, S., Mathieson, T.,Perrin, J., Raida, M., Rau, C., Reader, V., Sweetman, G., Bauer, A., Bouwmeester, T., Hopf, C., Kruse, U., Neubauer, G., Ramsden, N.,

Rick, J., Kuster, B., Drewes, G. (2007) A quantitative chemical proteomics approach reveals novel modes of action of clinical ABL kinase inhibitors. Nat. Biotechnol. 25 1035 – 1044.

IO03

STRUCTURAL BIOLOGY BY MASS SPECTROMETRY: QUANTITATIVE 3D PROTEOMICS REVEALS PROTEIN DYNAMICS

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We deployed a powerful combination of mass spectrometry, isotopic labelling and chemical cross-linking, termed quantitative 3D proteomics, to interrogate conformational rearrangements such as those accompanying formation of C3(H2O), the spontaneously arising hydrolytic product of C3, an abundant plasma complement protein. Purified protein in different stable conformations is cross-linked, digested and linkage sites are identified through mass spectrometry and database searching. Using isotope-labelled cross-linkers allows for a direct and quantitative comparison of data obtained for different conformations. This approach builds on a high/high data acquisition strategy for confident identification of cross-linked peptides, which frees isotope labelling from its current use in cross-linking as an identification support. We experienced that rather than adapting existing software for the specific needs of our project, general computational tools are such advanced that it was easier to write own code, XiQ.

Formation of C3(H2O) initiates antibody-independent activation of the complement system, a key first line of antibacterial defence. Our data support a model whereby, rarely, the wedge-like anaphylatoxin domain – excised when C3 is enzymatically activated to C3b – undergoes conformational change but remains in a C3-like position. Associated rearrangements of nearby macroglobulin domains disinter a thioester and release the thioester-containing domain that swings to the other end of the molecule creating a stable C3b-like platform for binding the zymogen, factor B, or the regulator, factor H. Domain rearrangements driving complement initiation exemplify binary switching in protein-protein interaction networks; quantitative 3D proteomics accurately monitors such conformational dynamics in solution.

IOO4

INTERWINING (CHEMICAL) PROTEOMICS AND CLASSICAL BIOCHEMISTRY TO CHARACTERIZE CARDIAC SIGNALING

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Introduction: Over the past decade, proteomics has provided a plethora of novel biological insights regarding the involvement of specific proteins in biological processes. However, these need to be combined with classical biochemical assays to further characterize the functionality of specific proteins. Here we show an example based on cAMP signaling components of how such efforts can go hand-in-hand.
Methods: We have recently established several cAMP based-chemical proteomics approaches to screen tissues for presence of protein kinase A regulatory subunit (PKA-R) isoforms and their interacting A kinase anchoring proteins (AKAPs). In this way, the cardiac sphingosine kinase type 1-interacting protein or SPHKAP was defined as a novel AKAP. Further screening in ventricular tissue with a different chemical proteomics tool revealed a unique feature of SPHKAPs; it is specific towards PKA-RI isoform, which was unprecedented. Subsequent biochemical characterization confirmed SPHKAP's specificity *in vitro*.

Results: The molecular environment of SPHKAP was investigated. For this, cAMP-based affinity chromatography was combined with blue native-PAGE and MS analysis to show that SPHKAP forms a single megadalton complex in mammalian heart and PKA RI is one of the complex member.
To further characterize the constituents of this complex, the HA-tagged SPHKAP was immunoprecipitated to reveal Apoptosis-inducing factor (AIF) as a SPHKAP complex contituent. Immunolocalization studies in heart-like H9C2 cells revealed that GFP-SPHKAP colocalizes with AIF in mitochondria. Submitochondrial fractionation showed that SPHKAP colocalizes with AIF and PKA RI in inner membrane. We are now in the process of studying the contribution of SPHKAP in apoptosis to validate SPHKAP's functionality.
Conclusion: Together, the multi-angular (chemical) proteomics approaches intertwined with biochemical and cell biological is a powerful combination to provide for the discovery and functional interpretation of low abundant signaling proteins, such as SPHKAP.

IO05

DISSECTING HIGHLY SIMILAR SIGNALING PATHWAYS BY CHEMICAL PROTEOMICS
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Introduction: The chemically quite similar cyclic nucleotides cAMP and cGMP are two second messengers that activate the homologue cAMP- (PKA) and cGMP- (PKG) dependent protein kinase respectively. *In vitro* studies demonstrated that PKA and PKG are also activated by the 'wrong' cyclic nucleotide, albeit at around a ~100- fold higher concentration. To achieve specificity, PKA is tightly co-localized with its substrates through interaction with the large and diverse class of A-kinase anchoring proteins (AKAPs). Thus far very little G-kinase anchoring proteins (GKAPs) have been identified, likely because they are lower in abundance. Here we set out to build a method to specifically dissect PKA and its associated AKAPs, from PKG and its GKAPs, ideally to study both pathways in a single experiment.
Methods: For the enrichment and discovery of low abundant interactors of PKA and PKG, different chemical proteomics approaches based on immobilized cAMP/cGMP were developed. However, due to the promiscuity of PKA and PKG towards each other's ligands in such an experiment, it was thus far not possible to define which proteins were specific interactors of PKA or PKG, or actually background proteins. For the isolation of PKG/GKAP complexes, we use a single cAMP-based affinity resin on a protein lysate supplemented either with a low concentration of cAMP or cGMP. Combined with a quantitative LC-MS/MS read-out, we could specifically dissect PKG/GKAPs (competed by free cGMP) from PKA/AKAPs (competed by cAMP) and background proteins. In this way we took advantage of the mentioned 100-fold difference in binding affinity.
Results: The novel method allowed us to isolate PKG and known GKAPs, from a set of 15 much more abundant AKAPs, as well as from a large set of background proteins. Application of the method to a set of complementary rat tissues led to the identification of several new putative GKAPs, but also a novel AKAP.
Conclusion: With this versatile method we can start to screen for specific PKG anchoring proteins, but also explore in much more detail the cAMP/PKA/AKAP interactome.

IO06

MODELING OF CANCER KINOME NETWORKS
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Abstract: Biological systems are composed of highly dynamic and interconnected molecular networks that drive biological decision processes. The goal of network biology is to describe, quantify and predict the information flow and functional behaviour of living systems in a formal language and with an accuracy that parallels our characterisation of other physical systems such as Jumbo-jets. Decades of targeted molecular and biological studies have led to numerous pathway models of developmental and disease related processes. However, so far no global models have been derived from pathways, capable of predicting cellular trajectories in time, space or disease. The development of high-throughput methodologies has further enhanced our ability to obtain quantitative genomic, proteomic and phenotypic readouts for many genes/proteins simultaneously. Here, I will discuss how it is now possible to derive network models through computational integration of systematic, large-scale, high-dimensional quantitative data sets. I will review our

latest advances in methods for exploring phosphorylation networks. In particular I will discuss how the combination of quantitative mass-spectrometry, systems-genetics and computational algorithms (NetworKIN [1] and NetPhorest [4]) made it possible for us to derive systems-level models of JNK and EphR signalling networks [2,3]. I shall discuss work we have done in comparative phospho-proteomics and network evolution[5-7]. Finally, I will discuss our most recent work in analysing genomic sequencing data from NGS studies and how we have developed new powerful algorithms to predict the impact of disease mutations on cellular signaling networks.

References: http://www.lindinglab.org
1. Linding et al., Cell 2007. 2. Bakal et al., Science 2008. 3. Jørgensen et al., Science 2009. 4. Miller et al., Science Signaling 2008. 5. Tan et al., Science Signaling 2009. 6. Tan et al., Science 2009. 7. Tan et al., Science 2011.

IO07

PROTEOMEXCHANGE – COORDINATED DISSEMINATION OF PROTEOMICS DATA
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Over the last few years the field of proteomics has evolved into a prolific data producer, and the value of public access to proteomics data for validation, re-interpretation, and tool development, for example spectral libraries or proteotypic peptide determination, is widely accepted. However, the complexity of proteomics workflows, the lack of community-wide agreements like the famous "Bermuda principles" in genomics, as well as funding issues, have so far hampered the development of co-ordinated, large scale proteomics repositories which capture the majority of published proteomics data. Proteomics repositories for different data types and work flows are supported to different degrees by different journals and funding agencies, leading to potentially difficult situations for prospective data providers, lowering overall data deposition rates.
The international ProteomeXchange consortium aims to address this situation by unifying existing efforts into a co-ordinated data deposition and exchange strategy for proteomics data, ensuring a user-friendly single site deposition as well as maximum potential for re-use and citation of the data. By capturing raw data, identifications/quantitations, and metadata, with subsequent automated notification to all interested resources and users, we aim to support different views of the same data, fulfilling requirements of a large user community, from results-oriented to technology-oriented.
From initial concepts in 2008, the ProteomeXchange concept gained traction with dedicated EU funding from 2011, and the first ProteomeXchange depositions were processed in spring 2012. Specific "Stakeholder" meetings for community participation resulted, among others, in the assignment of Digital Object Identifiers (DOIs) to submitted datasets, as well as stronger inclusion of use cases for raw data only depositions. Here, we will outline the typical ProteomeXchange workflow, available tools and limitations, as well as the current status, data access, and dissemination. Initial results of large-scale, cross-experiment data integration for error correction and quality control will provide a practical use case.

IO08

TRAWLING THROUGH PROTEOMICS DATA: ANALYZING AND LEARNING FROM THE CATCH
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Abstract:
With growing amounts of mass spectrometry (MS) based proteomics data becoming available for analysis and (re-) interpretation, it becomes increasingly interesting to mine these data for patterns or information. These investigations can be used both for introspection, allowing us to learn about the strengths and weaknesses of our approaches, as well as for novel discoveries. The latter can consist of better algorithms or methodologies to process MS based proteomics data, but can also deliver actual biological knowledge derived from the accumulation of large amounts of results.
We will here illustrate these points by showing a few examples that together cover the abovementioned gamut of the potential of the mountains of proteomics data. We can investigate the strengths and weaknesses of MS2-based label-free quantification algorithms [1], along with the influence that search engines have on their performance [2]. It is also possible to test the limits of identification algorithms, showing us where they break down [3]. We can devise better algorithms for protein quantification [4; 5], and for retention time

prediction [6]. We can even start thinking about the construction of a human proteomics expression atlas, and the issues we'll face along the way [7].
With these examples, we'll thus show that the data mountain need not be a threatening prospect. In fact, climbing such a data mountain can teach you a lot about yourself, while it will also open up new and unexplored vistas full of promise and discoveries.

References

[1] Colaert N, Vandekerckhove J, Gevaert K & Martens L. A comparison of MS2-based label-free quantitative proteomic techniques with regards to accuracy and precision Proteomics (2011) 11: pp. 1110-1113. [2] Degroeve S, Staes A, De Bock PJ & Martens L . The effect of peptide identification search algorithms on MS2-based label-free protein quantification OMICS (2012), in press. [3] Colaert N, Degroeve S, Helsens K & Martens L. Analysis of the resolution limitations of peptide identification algorithms J. Proteome Res. (2011) 10: pp. 5555-5561. [4] Colaert N, Van Huele C, Degroeve S, Staes A, Vandekerckhove J, Gevaert K & Martens L. Combining quantitative proteomics data processing workflows for greater sensitivity Nat. Methods (2011) 8: pp. 481-483. [5] Colaert N, Gevaert K & Martens L. RIBAR and xRIBAR: Methods for reproducible relative MS/MS-based label-free protein quantification J. Proteome Res. (2011) 10: pp. 3183-3189. [6] Moruz L, Staes A, Foster J, Hatzou M, Timmerman E, Martens L & Käll L. Chromatographic Retention Time Prediction for Post-translationally Modified Peptides Proteomics (2012), in press. [7] Gonnelli G, Hulstaert N, Degroeve S & Martens L. Towards a human proteomics atlas Analytical and Bioanalytical Chemistry (2012), in press.

IO09

UNIPROT - REFERENCE PROTEOMES DATABASE FOR MASS SPECTROMETRY ANALYSIS

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Introduction:
Theanalysisandinterpretationofmassspectrometry(MS)datareliesontheidentificationofproteinsequencesavailabl einpublicdatabases.The success of this identification is highly dependent on the completeness and quality of the database used. UniProt has recently released comprehensive good quality protein data sets for a number of reference proteomes that will hugely benefit proteomics research
Methods: With the increasing number of complete genomes sequenced, it is important for databases to organise this data in a way that allows users to effectively search the growing number of protein sequences encoded in these genomes. To meet this challenge, UniProt has recently released a set of Reference proteomes.
In the development of the reference proteomes data sets, a special emphasis has been put on providing complete, non-redundant and quality sequences. The generation of these data sets is outlined here, using Homo sapiens as an example. Approximately 20,000 protein-coding genes have been manually annotated in the UniProt Knowledgebase (UniProtKB) and are represented by one canonical protein sequence, with some entries describing multiple isoform sequences. To increase the coverage of the human complete proteome and include all known annotated isoforms, gene product predictions in Ensembl and NCBI RefSeq have been identified and incorporated in this data set. The identification of protein isoforms not present in UniProtKB was possible through a process of mapping all human protein sequences in the database to the protein translations in Ensembl, based on 100% amino acid identitity over the full length of the sequence.
Results: Currently, UniProt has defined 455 reference proteomes in collaboration with Ensembl and NCBI Reference Sequence collection.
Conclusion: Comprehensive, non-redundant and high quality annotated databases are important for protein research. UniProt strives to provide reference proteome data sets for a wide range of species ensuring completeness by harvesting all available protein sequences from a diverse number of resources.

IO10

MEMBRANE PROTEIN "SOCIAL" NETWORKS PROVIDE INSIGHTS INTO THE PUZZLING BIOLOGY OF CANCER METASTASIS

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Introduction:Proteomics promises delivery of comprehensive membrane proteomes. Our recent studies on engineered colorectal cancer (CRC) cell lines illuminate changes associated with the metastatic phenotype. IP proteomics identified the uPAR-interacting metastasome1 and we are now examining how these may be involved in the metastatic phenotype and/or the Jekyll/Hyde TGFβ1 suppressor->promoter switch.
Methods:Analyses of sites of interaction between uPAR and αvβ6 (peptide arrays & ELISA competition assays) suggest interesting biologies associated with these interactions. Although little is known about patient outcome when co-expressed, these lynchpin proteins (uPAR and αvβ6) are independent poor CRC prognostic indicators. In addition, using peptide IPG-IEF shotgun MS/MS, label-free quant and glycan analyses of cachectic and non-cachectic mice we have examined liver proteome changes in tumour bearing mice2,3.
Results:Collectively, our data demonstrate that combination(s) of methods including "shotgun" peptide IPG-IEF, glycoprotein enrichment, glycan analyses and membrane protein complex IPs can result in deep, comprehensive mammalian membrane proteome coverage.
Conclusion:Studies like these and previous ones we have conducted using human CRC tissues4 are providing vital new information regarding complications involved in CRC metastasis and treatment (e.g., cachexia), whether (glyco)protein biosignatures are associated with CRC and which proteins drive the process of malignancy as well as which protein interactions may potentially be new therapeutic targets.
References: 1. Saldanha RG, et al., Proteomic Identification of Lynchpin Urokinase Plasminogen Activator Receptor Protein Interactions Associated with Epithelial Cancer Malignancy. J Proteome Res, 2007 6, 1016-28. 2. Lee, A, et al., Rat liver membrane glycoproteome: enrichment by phase partitioning and glycoprotein capture. J. Proteome Res, 2009; 8:770-81. 3. Lee, A, et al., Liver Membrane Proteome Glycosylation Changes in Mice Bearing an Extra-hepatic Tumor. Mol. Cell Proteomics, Sep;10(9): M900538MCP200, 2011. 4. Jankova L, et al., Proteomic comparison of colorectal tumours and non-neoplastic mucosa from paired patient samples using iTRAQ mass spectrometry, Mol. BioSyst., 2011, 7, 2997–3005

IO11

NOVEL BREAST CANCER SUBTYPE MARKERS REVEALED USING DEEP PROTEOMIC ANALYSIS AND SUPER-SILAC

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Classification of breast tumors has been a long lasting research aim, which attempts to identify novel diagnostic and prognostic markers and novel therapeutic targets. Classical breast cancer diagnosis is based on a three-marker classification: Estrogen receptor or progesterone receptor positive (ER+/PR+) tumors, Her2/ErbB2 positive (Her2+) tumors or triple negative (TN) tumors when none of these receptors is identified. Multiple analyses of the mRNA level have divided breast tumors into five main subtypes: basal-like tumors, luminal A, luminal B, Her2-over-expressing and normal breast-like tumors. These subtypes complement and expand the classical ones, but despite the extensive transcriptomics work, they are still ambiguous. We propose that protein-based analysis may alter the classification and highlight novel markers of breast cancer subtypes. To this end we performed in-depth proteomic analysis using high-resolution MS and MS/MS with the Q-Exactive mass spectrometer and data analysis with MaxQuant. We used the recently developed super-SILAC mix, which is a mixture of lysates of five SILAC-labeled cell lines that serves as an internal standard for accurate tissue quantification. We analyzed 40 formalin-fixed paraffin-embedded human breast cancer tissue samples, which were ER+, Her2+ or triple negative.
Deep proteomic analysis identified and quantified in total more than 12,000 proteins, and more than 8,000 proteins in each tumor sample. Classification according to the known breast cancer subtypes using support vector machine created a proteomic signature that distinguishes between the breast cancer subtypes, and

highlights differences in cell proliferation, cell adhesion and metabolism. This signature highlights novel aspects of each of the breast cancer subtypes with the potential to alter breast cancer diagnosis and classification in the future.

IO12

SUBTYPING BREAST CANCER: CORRELATING MRNA AND PROTEIN CLASSIFICATIONS

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Breast Cancer Subtyping: We have completed a study, starting with over 600 breast tumours, to define markers that allow the type of tumour to be established using a protein signature that has been adapted for use in SRM assays (Waldermarson et al. submitted). We can rapidly define the Sørlie status (Luminal A/B, Basel, Normal, ErbB2) as well as the nature of the tumour (normal, benign or malignant). The separation into groups is the same as is found with mRNA analysis however the classifiers are not the same and forcing the classification with those found with mRNA onto the protein data does not work and visa-versa.

We are presently finishing off an in depth analysis of the tumours used in this study to define proteotypic peptides for each of the Sørlie classes and for tumour staging. We have also finished two detailed analyses of protein expression in cell lines that are representative of each of the Sørlie classes and compared this to mRNA expression (Cifani et al., submitted) and a detailed analysis of the sub-cellular location of these proteins (Antberg et al., submitted). The Breast Cancer Index and the Breast Cancer Atlas form a unique reference containing proteotypic peptides for most proteins that are found in breast tissue.

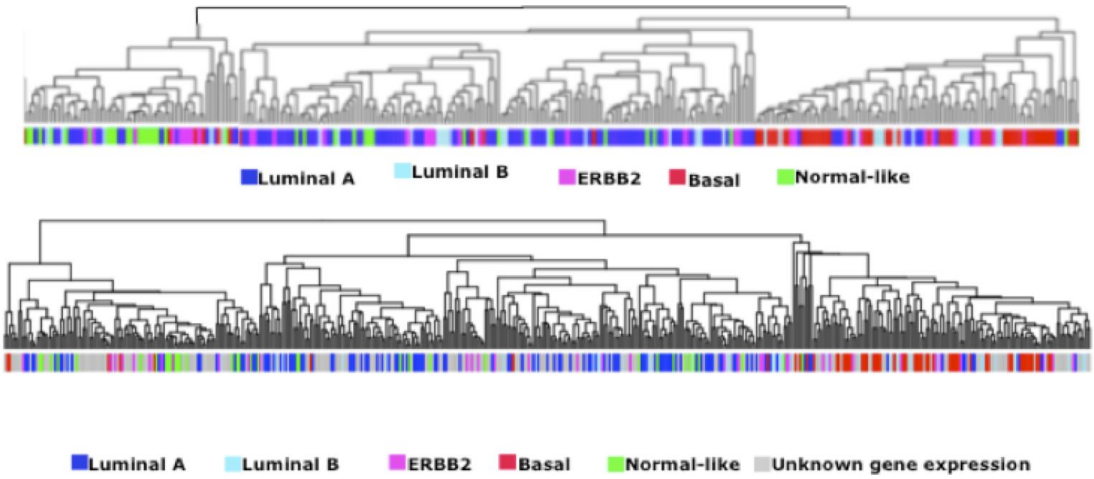

Figure 1. The top panel shows the non-supervised Pearson clustering of the mRNA analysis. The bottom panel shows the same clustering of the protein analysis. The colour codes represent Sørlie groups and grey indicates tumours not analysed due to mRNA degradation.

IO13

HUMAN CORNEA PROTEOME: IDENTIFICATION AND QUANTITATION OF THE PROTEINS OF THE THREE MAIN LAYERS INCLUDING EPITHELIUM, STROMA AND ENDOTHELIUM

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Introduction: Diseases of the cornea are common and refer to conditions like infections, injuries and genetic defects. Morphologically, many corneal diseases affect only certain layers of the cornea and separate analysis of the individual layers is therefore of interest to explore the basic molecular mechanisms involved in corneal health and disease.

Methods: The three main layers including the epithelium, stroma and endothelium of healthy human corneas were isolated and the proteins were (i) separated by SDS-PAGE followed by in-gel trypsinization, (ii) in-

solution digested without prior protein separation or, (iii) in-solution digested followed by cation exchange chromatography. The resulting peptides were separated by LC-MS/MS and analysed on a TripleTOF 5600 mass spectrometer. Proteins were identified in the Swiss-Prot database using the Mascot algorithm and quantified using Mascot Distiller. Data extraction and processing was done using MS Data Miner.
Results: A total of 3221 unique Swiss-Prot annotated proteins were identified in human corneas, 2713 in the epithelium, 1672 in the stroma and 866 in the endothelial layer. Of these, 1759 proteins have not previously been identified in the human cornea by mass spectrometry. In total, 771 proteins were quantified, 157 based on in-solution digestion and 770 based on SDS-PAGE separation followed by in-gel digestion of excised gel pieces. Protein analysis revealed that many of the identified proteins were human plasma proteins involved in the complement system, coagulation and defence against pathogen infections.
Conclusion: The separation of human corneas into the three main layers combined with modern mass spectrometry provides new insight into the proteins present in the individual layers and the relative abundance in each layer. This provides a useful reference dataset when exploring basic molecular mechanisms involved in corneal diseases, many of which are restricted to a specific corneal layer.

IO14

IDENTIFICATION OF NEW MATRIX METALLOPROTEINASE SUBSTRATES IN THE VASCULATURE
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Introduction: Matrix metalloproteinases (MMPs) play a key role in vascular remodeling and cardiovascular disease. MMPs degrade and reorganize the vascular extracellular matrix (ECM). In atherosclerosis MMP activity is associated with plaque rupture leading to heart attacks and stroke. Surprisingly little is known about the substrates of MMPs in the vasculature. In the present study, we used a proteomics approach to identify vascular substrates for three members of the major classes of MMPs: MMP-3 (a member of stromelysins), MMP-9 (a gelatinase) and MMP-14 (a member of the membrane–bound MMPs).
Methods: We incubated human arteries with MMP-3, -9 and -14 and analyzed the released proteins by gel-LC-MS/MS. New substrates were confirmed by digestion of recombinant proteins with the same MMPs. Further analysis focused on the identification of new MMP cleavage sites of ECM proteins by searching for non-tryptic peptides. Newly identified degradation products were validated in human aortic tissue with high MMP-9 activity to relate protein degradation *in vitro* with exogenous MMPs to endogenous proteolytic activity *in vivo*.
Results: Using this innovative approach, we identified 17 novel substrates, including ECM proteins associated with the basement membrane (collagens, nidogen-1, agrin), elastic fibers (emilin-1, transglutaminase-2, TGF b-induced protein ig-h 3) and other extracellular proteins (galectin-1, tenascin-X, tenascin-C). In total, we detected 74 cleavage sites, many of which were shared among different MMPs.
Conclusion: Our current classification of MMPs based on few substrates (collagenases, gelatinases, etc) is an oversimplification of a complex area of biology, and our proteomics study is an attempt to provide a more comprehensive assessment of MMP substrates in the vasculature.

IO15

PROTEIN NETWORK CHANGES IN DIFFERENT TIME POINTS OF HUMAN LACTATION
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Introduction: Human milk is a rich source of bioactive proteins that supports the growth and immunological defences(1) of the newborn. There are several proteomic studies about human milk whey proteins(2) and fat globule proteins(3) during the lactation period , but at the moment there are no protein interaction data from all human milk proteins expressed in lactation period. In the present study we analyzed protein expression

and the associated protein network at several time point in lactation period; 7, 15, 30 and 60 days from partum. Eighteen mothers for each time point with fixed criteria of recruitment were included in study.
Methods: Samples were subjected to 1-DE and 2-DE and all proteins were separated from each experimental condition were identified by UPLC MS/MS. Biological networks were generated with STRING.
Results: STRING analysis highlighted several GO-biological processes and GO-molecular functions mainly involved in defense response to bacteria and enzymatic activities. Classification of proteins and generation of biological networks were done .
Conclusion: Present study provides the first evidence for protein network modulation in different stages of human lactation. Results indicated several possible biological processes and molecular functions modulated in each stage. Proteomic and bioinformatic investigations are necessary to well describe network role in modulation of nutraceutical and nutritional characteristics of human milk.
References: 1. Hettinga K, van Valenberg H, de Vries S, Boeren S, van Hooijdonk T, et al. 2011. *PLoS One* 6: e19433 2. Liao Y, Alvarado R, Phinney B, Lonnerdal B. 2011. *J Proteome Res* 10: 1746-54 3. Liao Y, Alvarado R, Phinney B, Lonnerdal B. 2011. *J Proteome Res* 10: 3530-41
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IO16

SUPER EXPERIMENTS: STUDYING CELL BIOLOGY USING QUANTITATIVE PROTEOMICS
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Abstract:
We are studying the functional organization of mammalian cell nuclei using a dual strategy that combines mass spectrometry (MS) based proteomics with live cell fluorescence imaging (see www.LamondLab.com). We use these complementary, quantitative techniques to analyse protein dynamics and chromatin organisation, providing a rigorous approach combining data from multiple experimental approaches, including MS-based proteomics, FLIM-FRET, time-lapse microscopy, FISH and deep sequencing.
We are using SILAC based methodology to create a flexible suite of assay formats to characterize, system-wide, 'Protein Properties', including abundance, subcellular protein localization, turnover rates, post-translational modifications and specific protein interaction partners. A major aim of our work is to determine how such protein properties vary between cell types and how they change within cells as they progress through the cell cycle and respond to different growth conditions, external stimuli and stress. These assays are performed either on cultured human cell lines, or on model organisms amenable to convenient genetic manipulation, such as yeast and nematodes. We have also used human cells with defined genetic backgrounds to investigate the effects of specific genes, including p53 and p14Arf, on proteome responses to oncogene activation, DNA damage and cellular stress.
The systematic analysis of protein properties using quantitative proteomics inevitably generates very large data sets. An integral part of our work therefore has been the parallel development of a dedicated software environment, called PepTracker (see: http://www.peptracker.com/), to manage the collection, mining and visualization of these large scale, 'second generation' proteomics datasets. We have used computational approaches developed in the business intelligence field to model the proteomics data in n-dimensional OLAP cubes to facilitate rapid and complex data mining. The combination of quantitative proteomics and integrated data management facilitates the development of the "Super Experiment" strategy for studying mechanisms and responses in cell biology at the system-wide level.

IO17

USING QUANTITATIVE PHOSPHOPROTEOMICS TO CONNECT GENETICS TO PHENOTYPE
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To effectively monitor protein phosphorylation events governing signaling cascades, we have developed a mass spectrometry-based methodology enabling the simultaneous quantification of tyrosine phosphorylation of specific residues on dozens of proteins at multiple time points under a variety of perturbations. We have

recently applied this technique to identify key signaling nodes regulating EGFR, Insulin Receptor, and T Cell Receptor signaling network response to stimulation. Using this technology, we have performed an in-depth characterization of signaling networks in glioblastoma cell lines, human patient tumor xenografts, and flash-frozen human glioblastoma tumors. In cell lines, we have quantified the adaptive response to single point mutations at the signaling and phenotypic level through analysis of phosphorylation and cell growth rates. Computational analysis of these data sets has identified the ERK 1/2 MAP kinases as critical for cell growth control in a unique way, as activation of the ERK MAP kinases leads to increased apoptosis, presenting a potentially novel method for treating glioblastoma tumors. To understand the mechanisms by which the ERK 1/2 MAP kinases regulate multiple distinct phenotypes we have performed an in-depth analysis of ERK2 substrates using mass spectrometry combined with analog sensitive kinases. This analysis led to the identification of 80 substrate proteins, including 67 novel substrates. Phosphorylation of one of the novel substrates regulates transcription by preventing binding to DNA at promoters for several thousand genes, including targets involved in negative feedback regulation of itself and of upstream signals.

IO18

A QUANTITATIVE INTERACTION SCREEN FOR NEURODEGENERATIVE DISEASE PROTEINS

Matthias Selbach,

Neurodegenerative diseases are devastating multifactorial disorders that affect millions of people worldwide. As disease progression is mainly driven at the protein level, knowledge about protein-protein interactions may provide important insights into disease mechanisms. We used affinity purification and quantitative mass spectrometry to identify interaction partners of 16 proteins involved in Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD) and spinocerebellar ataxia type 1 (SCA1). SILAC-based quantification allowed us to directly compare interaction partners of wild-type proteins and disease-associated variants. We identified more than 400 mostly novel interactions with little overlap between diseases. 20% of the interactions showed differential binding between the wild-type form of the protein and disease-associated variants, providing potential links to disease mechanisms. To test the functional relevance of our data we employed a Drosophila model of SCA1. We found that a highly significant fraction of identified Ataxin1 interaction partners affected neurodegeneration in vivo. We also compared our data with genome-wide association studies (GWAS) for AD. This analysis revealed a significant enrichment of single nucleotide polymorphisms in interaction partners of amyloid precursor protein (APP). Functional follow-up for a novel APP interactor revealed a link to mitochondrial dysfunction in early-onset Alzheimer's disease. Collectively, our data shows that quantitative interaction proteomics can provide insights into molecular pathogenesis of neurodegenerative diseases.

IO19

AN EXPERIMENTAL PLATFORM WITH INCREASED THROUGHPUT AND DATA ROBUSTNESS FOR ANALYSIS OF DYNAMIC MAMMALIAN PROTEIN INTERACTION NETWORKS

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Introduction: To understand the organization of protein complexes and how biological information is propagated via protein-protein interaction is becoming increasingly important to get new functional insights on cellular processes.
Methods: Affinity purification coupled to mass spectrometry (AP-MS) has been successfully used in the past to characterize protein complexes. However, mainly due to significant experimental limitations the success and power of the AP-MS strategy has been underrated to date. Here, we describe an integrated workflow for characterization of mammalian protein complexes. The experimental pipeline comprises rapid generation of isogenic mammalian cell lines, protein complex analysis using a rapid and efficient single step affinity purification strategy followed by state-of-the-art nano-LC-Orbitrap-MS analysis, label free quantitation and protein frequency filtering.
Results: We investigated p53 complex composition and p53 complex composition in treatments effecting p53 directly with nutlin-3A and indirectly with cisplatin. Nutlins are inhibitors of the p53 specific ubiquitin ligase MDM2. Nutlin-3A was shown to interfere with the interaction of p53 with MDM2 in in vitro experiments and results in apoptosis. Our approach identifies specific effects that nutlin-3A executes on p53 complexes in vivo.

Cisplatin is one of the most effective chemotherapeutic agents, widely used for the treatment of malignant cancer. Cisplatin induces cross-linking of DNA, which results in DNA damage, cell cycle arrest and finally cell death. Cisplatin treatment of human cells, leads to an increase of phosphorylated p53 and elevated p53 levels.
Conclusion: Using AP-MS, we can follow nutlion and cisplatin induced changes in p53 complex composition.

IO20

QUANCAT: A METHOD FOR INVESTIGATING IMMEDIATE/EARLY PROTEOME DYNAMICS
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Introduction: Several techniques are available to researchers studying the dynamics of mRNA expression which allow the sensitive detection of rapid changes in mRNA levels in response to stimuli. However, the dynamics of a particular mRNA is not necessarily an accurate predictor for the changes in the corresponding protein product. To form a complete picture of cellular responses, it would therefore be useful to have a proteomics technique which would enable the accurate and sensitive quantitation of immediate/early changes in the proteome in response to stimuli. To date this has not been possible, since changes in protein levels <2hrs after application of a stimulus are lost against the large background of pre-existing proteins.
Methods: We have used Bio-Orthogonal Non Canonical Amino acid Tagging (BONCAT) to isolate newly synthesised proteins, combined with pulse SILAC to provide accurate quantitation. We have named this method Quantitative Non Canonical Amino acid Tagging (QuaNCAT) and applied it to primary human T-lymphocytes, successfully measuring the response of the proteome after 2 hrs of stimulation.
Results: We discovered a number of proteins reproducibly up and down regulated after 2 hrs of stimulation of primary T-lymphocytes, including some proteins known to be of importance to T-lymphocyte activation during an immune response.
Conclusion: This method is likely to be of interest to cell biologists in numerous fields, since it brings the power of SILAC to enable measurement of immediate/early changes in proteome dynamics in both primary cells and cell lines.
References: Dieterich DC *et al*. Proc Natl Acad Sci U S A. 2006 Jun 20;103(25):9482-7

IO21

PROTEOME ANALYSIS OF SACCHAROMYCES CEREVISIAE AT NEAR-ZERO GROWTH CONDITIONS
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Introduction: Near-zero growth cultivation of yeast is not only interesting for industrial purpose but also it might shed light into chronological aging. Non-growing microorganisms build up to around 60% of the biomass on earth (Gray, 2004). Cells cultured in a retentostat stay viable and metabolically active without cell divisions (Boender, 2009). This “quiescent” state reflects also the conditions for aging processes in higher eukaryotes.
Methods: The effects of this “quiescent” state on the physiology of *S. cerevisiae* and on its transcript levels have been studied recently (Boender, 2011). However, the responses at the level of the proteome are as yet not known. We therefore explored the changes in the proteome of *S. cerevisiae* to cultivation at extremely low growth rates (below 0.01 h^{-1}), i.e. during transition from growing to non-growing state using retentostat cultivation and sampled the cellular proteomes over a period of 21 days. We performed quantitative proteome analysis using the 6-plex tandem mass tag (TMT) labeling technique and a UPLC-coupled Quadrupole-Orbitrap mass analyzer.
Results: The changes in protein levels revealed some clearly distinguishable patterns, which will be discussed. Surprisingly, an induction of protein expression of respiratory chain complexes and enzymes of the

TCA cycles were detected. This may be explained by a release of glucose repression during retentostat cultivation.
Conclusion: Results from this study will shed light into the dynamic changes in the proteome during the transition into quiescence.
References: Gray, J.V. et. al., *Microbiol. Mol . Biol. Rev.*, 68, 187-206. 2004
Boender, L.G.M. et al., *Appl. Environ. Microbiol.,* 75, 5607-5614. 2009
Boender, L.G.M. et al., *FEMS Yeast Res*. 11(8), 603-20. 2011

IO22

PROTEOMICS AND FARM ANIMALS: A TALE OF MUSCLE AND MEAT
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Introduction:Proteomics has been extensively applied to the study of muscular tissues in several species covering a wide variety of topics, from physiology to diseases. Muscles are also the key component of meat and meat-based products. Accordingly, proteomics has been used to characterize the process by which muscles are transformed into meat as well as from meat to meat product. Particular relevance has been given to two species: cattle (Bos taurus) and swine (Sus scrofa) in the context of meat maturation and aging and the processes for the production of hams (dry-cured and cooked). Additionally, proteomics has been applied to study meat quality in other farm animal species such as small ruminants, poultry and rabbits.
Methods:In this presentation authors will present an overview of research activities carried out coupling proteomics and meat science. Particular emphasis will be given to the research efforts of the three teams involved in this presentation.
Results:The three teams involved in this presentation have studied muscle and meat proteomics during recent years. Three major areas of research arise:
1) Meat maturation and breed differentiation in cattle and swine (Norway);
2) Muscle characteristics and breed differentiation in rabbits and sheep as a consequence of weight loss (Portugal);
3) Dry-cured and cooked ham processing techniques (Italy).
Results presented will reflect not only the protein expression profiles but also the intricate metabolic pathways involved in these processes.
Conclusion:Proteomics is of extreme importance to the study of the processes by which muscle is transformed in to meat and by which meat is processed into meat products. The application of proteomics to meat science surpasses the mere study of these processes, being of key importance to issues such as product certification, characterization and the detection of adulterations.

IO23

RESOLVING PROTEIN PHOSPHATASE COMPLEXES FROM THE HIGHER PLANT ARABIDOPSIS THALIANA
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Abstract
Phosphoproteomic analysis of the model higher plant Arabidopsis thaliana has revealed abundant phosphorylation of serine, threonine and tyrosine residues, consistent with over 1000 protein kinases and 150 protein phosphatases encoded in its genome. Affinity chromatography and TAP-tag approaches were employed to identify complexes that contain serine/threonine phosphatases of the PPP family, which includes PP1, PP2A and several novel plant specific enzymes. In line with genomic studies we have identified many phosphatase regulatory subunits that were first characterized in humans and yeast. We have uncovered a PP2A complex localized to microtubules that provides a link between protein phosphorylation and acetylation. Microtubule localized histone deacetylase 14 (HDA14) specifically associates with the scaffolding A-subunit of PP2A (PR65) and we demonstrate that HDA14 can deacetylate K40 of microtubules. This complex also

contains the histone acetyltransferase ELP3, which in humans functions as the □-tubulin acetylating K40 enzyme. Bioinformatics indicates that HDA14 is most like human HDAC6, the enzyme that deacetylates □-tubulin. Taken together these findings suggest an ancient origin for □-tubulin acetylation and phosphorylation that is conserved in humans and plants.

IO24

FOOD PEPTIDOMICS: THE QUEST FOR BIOACTIVE SMALL NON-TRYPTIC PEPTIDES

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Today, proteomics deals with thousands of peptide identifications per hour. One key aspect is the generation of adequate peptides through specific hydrolysis (e.g. trypsin). However, in the field of food research, processing or protein digestion generates very heterogeneous peptide mixtures. While peptides of practical size even if not tryptic can be well characterized with slight modifications to classical proteomics methods, peptides of smaller size (<6 amino acids) are very challenging. However, such peptides often account for >30% in hydrolyzed ingredients (e.g. hypo allergenic infant formula) as well as represent the largest source of bioactive peptides. Food-derived protein and peptides are not only macronutrients and building blocks for protein synthesis, but also bioactive molecules that modulate physiological functions. Biological activities, such as antihypertensive, antimicrobial, immunomodulating, and others have been described. Nevertheless, many such peptides are small non-tryptic peptides (<6 residues long) and represent a particular challenge for classical proteomic approaches, in terms of qualitative and quantitative analysis.
Such a challenge will be exemplified by the development of a mass spectrometric assay to measure 117 di-, tri- and tetra-peptides made out of branched chain amino acids. Because of leucine and isoleucine, the 117 peptides are clustered into 11 isobaric clusters with the largest containing 32 molecules. Moreover, because all three amino acids (Leu, Ile and Val) share similar physico-chemical properties, the mean hydrophobicity of these peptides is 4.26 +- 0.13 as measured by the GRAVY method. Therefore, this peptide pool was selected as an adequate complex mixture to test the feasibility of such an assay for small peptide.
Finally, bioinformatics is an essential element to study food components and functional properties. In order to help identify potential new bioactive molecules within food ingredients or products, better use and further improvement of existing databases for food bioactives is crucial; as well as the application and development of functional prediction tools (in-silico approach).

IO25

DETERMINING PROTEOME TURNOVER IN FISH SPECIES

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Introduction: Fish is a vital source of food for people and farmed fish represents a growing portion of the world protein source as marine caught fish supplies dwindle. The ability to define protein dynamics in fish has particular relevance to the aquaculture industry. We have developed a proteomics-based strategy to measure the rates of synthesis of individual proteins in the tissues of fish.
Methods: Common carp (*Cyprinus carpio*) were fed with an experimental 'heavy' diet, in which 50% of the L-leucine in the diet was replaced with crystalline [$^{2}H_{7}$] L-leucine. The fish were sacrificed and muscle samples collected over seven weeks. Soluble extracts of muscle homogenates were separated by 1-D SDS-PAGE. Protein bands were excised from the gel, subjected to in-gel digestion with trypsin and the resultant peptides were analysed by LC-MS/MS. The relative isotope abundance (RIA) of the precursor pool was determined and used to calculate the rates of synthesis of individual skeletal muscle proteins.
Results: The precursor RIA was 0.51 ± 0.01. The turnover rates of nearly 200 individual proteins from the muscle of common carp were measured. The data were fitted to a single order of exponential revealing the absolute rate of synthesis of skeletal muscle proteins. The rates differed by several orders of magnitude under steady state conditions. The rate of synthesis of the identified proteins ranged from $2.5x10^{-3}d^{-1}$ (muscle cofilin 2) to $0.428d^{-1}$ (proteosome subunit alpha type).

Conclusion: We have demonstrated the feasibility of this experimental approach by determining the turnover of proteins on a proteome-wide scale in the skeletal muscle of common carp, a key species of farmed fish. This work has addressed a number of analytical and technical challenges. The methodology can be readily adapted to monitor the rate of turnover of muscle proteins in other commercially important species, which could be of benefit to the fish farming industry.

IO26

PROFILING THE HUMORAL IMMUNE RESPONSE – APPLICATIONS IN UNDERSTANDING DISEASE AND BIOMARKER DISCOVERY

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Abstract. Immunoproteomics applies proteomics technologies to study immune responses and systems. One application is to profile the antibody repertoire to identify disease-associated immune profiles and their applications to understand disease and in biomarker discovery. Results will be presented of our recent improvements in methods of interaction screening on high content arrays including profiling the autoantibody repertoire of disease subjects, healthy controls, disease & inflammatory controls subjects, with the aim of developing candidate biomarkers & biomarker panels (see references 1-11). We have identified potential biomarkers & biomarker panels for disease diagnosis. Sample sizes are relatively low in our current data set. The sensitivities & specificities produced for these panels are descriptive rather than prescriptive. Our aim is to further assess the significance, specificities & sensitivities using a larger testing cohort. Results of our initial results will be presented and further analysis of the biomarkers identified needs to be performed.

Recent Publications:

1. Murphy, M.A., D.J. O'Connell, S.L. O'Kane, J. K. O'Brien, S. O'Toole, C. Martin, O. Sheils, J. J. O'Leary, D. J. Cahill. Journal of Proteomics. April 2012. PMID:22415278 doi:10.1016/j.jprot.2012.02.031 2. Murphy, M.A., JJ. O'Leary, DJ. Cahill. Journal of Proteomics. March 2012. PMID:22300580 3. Staquicini, F.I., Cardó-Vila, M., Kolonin, M.G et al., O'Connell, D.J., Cahill, D.J et al., Renata Pasqualini1 & Wadih Arap. PNAS Proceeding of the National Academy of Sciences (2012) MS Number 2011-14503. 4. Olsson N, Wingren C, Mattsson M, James P, O'Connell D, Nilsson F, Cahill DJ & Borrebaeck C. Molecular and Cellular Proteomics. (2012) Epub 2011. 10.1074/mcp.M110.003962 PMID: 21673276 5. O'Kane, S.L., O'Brien, J.K and Cahill, D.J. (2011) Vol. 785: Pps 331 – 344. Editor: Ulrike Korf. Humana Press/Springer NY. ISBN 978-1-61779-285-4. 6. Bauer MC, O'Connell DJ, Maj M, Wagner L, Cahill DJ, Linse S. Molecular Biosystems (2011) Molecular Biosystems 7: 521 – 532. 7. O'Connell DJ, Bauer M, Linse S & Cahill DJ. Methods in Molecular Biology. Protein Microarray (2011) Vol. 785: Pps 289 - 344 Editor: Ulrike Korf. Humana Press/Springer NY. ISBN 978-1-61779-285-4 8. Merino A, Bronowska, AK, Jackson, DB & DJ Cahill. Drug Discovery Today (2010) 15(17-18):749-56. 9. O'Connell DJ, Bauer M, O'Brien J, Berggard T, Merin A, Akerfeldt K, Linse S & Cahill DJ. (2010) Molecular and Cellular Proteomics Jun; 9(6):1118-32.10. Gibson, DS, Banha, J., Penque D, Costa L, Conrads TP, Cahill DJ, O'Brien JK, Rooney ME. Journal of Proteomics (2010) Volume 18 Issue 73(6) Pages:1045-60.11. Bauer, Mikael ,Magdalena Maj, Ludwig Wagner, Dolores J. Cahill, Sara Linse & David J.O'Connell. Methods in Molecular Biology (2011), edited by Andrew Emili &Gerard Cagney.

IO27

INCORPORATING SIRNA APPROACHES INTO PROTEOMIC RESEARCH

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Expression of individual genes in higher eukaryotic cells can be effectively ablated using small interfering RNAs (siRNA). In practise this can be achieved using stable integration of expression vectors encoding small hairpin RNAs (shRNA) or transfection mediated delivery into cells of chemically synthesised siRNAs. These approaches are now very widely used in biological research and I will discuss how they can be incorporated into proteomic workflows. Large scale siRNA screening using either full genome coverage or employing focused libraries that cover an area of interest (such as ubiquitin modification or phosphorylation) can be used with an appropriate cell based assay to identify new components of biological pathways. Such screens are complementary to "interactome" based proteomics and there is considerable value in using both approaches

to increase confidence in determining which “leads” to follow up. siRNA mediated approaches can also be used directly in large scale quantitative proteomic analysis to ablate expression of a single gene and determine the consequences on the proteome under study. In our own studies we have used large-scale siRNA mediated ablation of genes involved in the SUMO modification pathway coupled with SILAC based proteomics to identify substrates for SUMO proteases and E3 ligases. The utility and potential pitfalls of these approaches will be discussed.

IO28

SPATIAL PROTEOMICS: METHODS TO LOOK FOR GLOBAL TRAFFICKING ROUTES IN CELLS.

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Proteins increase their functional diversity through interactions with other molecules. Many exist as part of multi-protein complexes, with role-dependent, variable compositions. Proteins also increase their diversity by existing in unique sub-cellular niches which are vital for creating controlled microenvironments where diverse biological functions can take place. Knowledge of both sub-cellular localization and membership of multi-protein complexes is thus of fundamental importance in the analysis of protein function. Many approaches to study the components of multi-protein complexes start with cell lysis, which destroys sub-cellular compartmentalisation, and also make use of reagents, which do not distinguish between different protein isoforms that may relate to different sub-cellular compartments and interaction partners. Furthermore, many biochemical approaches to study protein localization are dependent upon total purification of the sub-cellular niche of interest, concentrate on determining the protein content of few sub-cellular structures, or require the use of large panels of immuno-reactive reagents. The similar physical properties of many organelles, however, result in significant contamination of purified fractions. Moreover, purification of a single organelle provides no information about the steady-state location of a protein, which may be present in a preparation simply as cargo, with the majority of the protein being located elsewhere.

We have developed sets of tools which allow reliable determination of protein localization across multiple organelles in a single experiment without the need for pure organelle preparations. Using these tools, we are also able to glean information about the sub-locations of protein complexes. The methods employed rely heavily on accurate and precise quantitative proteomics methods and also on the application of state-of-the-art machine learning approaches to pattern recognition in complex multi dimensional data sets.

In this presentation, I will describe the tools that we have developed: proteomics pipelines and their reliance upon accurate quantitation and also novel semi-supervised machine learning approaches to improve organelle and sub-organelle complex resolution. I will discuss how we have now applied these to a variety of sample types including adherent and suspension cell culture, tissues, and whole organisms.

This research was supported by BBSRC grants BB/HO24247/1and BB/D526088/1 and the 7th Framework Programme of the European Union (Contract no. 262067- PRIME-XS)

IO29

HIGH THROUGHPUT IDENTIFICATION OF NOVEL LOW-AFFINITY EXTRACELLULAR PROTEIN INTERACTIONS USING A PROTEIN MICROARRAY RECEPTOR SCREENING PLATFORM

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Introduction: Extracellular interactions involving cell surface receptor proteins are essential to initiate signaling pathways that orchestrate cellular behaviors. Identifying extracellular protein interactions, however, remains experimentally difficult due to the biochemical nature of the proteins involved: membrane-tethered

proteins are difficult to solubilise and their interactions are extremely transient, often having half-lives ($t_{1/2}$) that are less than a second.
Methods: To address this, we have developed an assay called AVEXIS (for AVidity-based EXtracellular Interaction Screen), which is designed to detect direct protein interactions between recombinant soluble ectodomain fragments expressed as either biotin-tagged baits or highly avid pentameric ß-lactamase-tagged preys in mammalian cells. Using this approach, we can detect very transient interactions ($t_{1/2} \leq 0.1$ sec) with a low false-positive rate.
Results: The current implementation of the assay is based on a 96-well microtitre plate and is limited by the requirement for relatively large amounts of protein and labour-intensive sample preparation. We will show how we have miniaturized the assay onto a convenient protein microarray format which reduces the amount of protein required by several orders of magnitude whilst maintaining the sensitivity of the assay. We are now using this technology together with large protein libraries that represent the cell surface receptor repertoire of interacting cell types to determine the molecular basis of cellular recognition processes. We will show how we have used this approach to systematically identify an erythrocyte receptor that is essential for erythrocyte invasion by the major malarial parasite, *Plasmodium falciparum*, and a more recent screen to identify novel receptor-ligand interactions involved in platelet aggregation.
Conclusion: The miniaturization of our assay and development of new sample preparation procedures now permit systematic screening for novel extracellular receptor-ligand interaction on a genome-wide scale.

IO30

A DEFINABLE "STRUCTURE" FOR THE IMMUNE SYSTEM AND CANCERS AT THE SINGLE CELL LEVEL IN THE POST-FLUORESCENCE ERA
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It is insufficient to state that cancer is "heterogeneous" in nature. This is akin to stating the problem without suggesting a solution. We focus on the development of intracellular assays of signaling that correlate subsets of cells in complex populations with functional signaling and clinical states. Such correlations allow for documentation and ordering of the inherent heterogeneity in leukemias and other cancers into understandable progressions. Using a next-generation single-cell "mass cytometry" platform we quantify surface and cytokine or drug responsive indices of kinase target with 45 or more parameter analysis (e.g. 45 antibodies, viability, nucleic acid content and relative cell size). We have recently extended this parameterization to mRNA with the capability to measure down to 5 molecules per cell in combination with any other set of previously created markers.
I will present evidence of deep internal order in immune functionality which demonstrates that differentiation and immune activities have evolved with a definable "shape". A hierarchy of functional trans-cellular modules is observable that can be used for mechanistic and clinical insights, including manners by which these modules become transposed during cancer progression. I will focus upon AML and ovarian cancer in the presentation and demonstrate the apparent existence of reproducible ordering of cellular substates that define a limited boundary condition of "what is" a given cancer.

IO31

PROTEIN ARRAYS AND PERSONALISED PROTEOMICS
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The recent advances in next generation high-throughput DNA sequencing are bringing complete genome and transcriptome sequencing within reach for routine diagnosis. What is missing is a means of converting individual genomes and their expressed transcriptomes quickly into corresponding proteomes. Since cellular function is protein-driven, a technology for arraying the altered proteins resulting from coding SNPs, splicing variations and somatic mutations, and further up to the level of entire cellular proteomes, would represent a significant advance, allowing the interrogation of a large number of possible disease-related protein markers simultaneously. We aim to display sequence-specified proteomes from individuals, tissues and cells by linking genomic DNA sequences and expressed RNA with our 'DNA array to protein array' (DAPA) system (1,2). Cell

free protein expression makes it possible to copy DNA into protein quickly in an array format (3), so that perturbations of biological networks can be followed through proteome-wide protein function determinations. This approach could have wide applicability, regardless of species or cell type. The combination of high throughput sequencing and cell free protein expression opens up the possibility of 'personalised proteomics'. This and other methods of cell free protein arraying will be presented.

1. He M, Stoevesandt O, Palmer EA, Khan F, Ericsson O, Taussig MJ. (2008) Printing protein arrays from DNA arrays. Nature Methods. 5:175-7. 2. Stoevesandt O, Vetter M, Kastelic D, Palmer EA, He M, Taussig MJ. (2011) Cell free expression put on the spot: advances in repeatable protein arraying from DNA (DAPA). New Biotechnology 28:282-90. 3. He M, Stoevesandt O, Taussig MJ. (2008) In situ synthesis of protein arrays. Current Opinion in Biotechnology 19:4-9.

IO32

SOMASCAN™: A QUANTITATIVE MULTIPLEX PROTEOMIC PLATFORM THAT MEASURES >1000 ANALYTES IN COMPLEX MATRICES

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Introduction: SomaLogic presents a transformative proteomic biomarker discovery and diagnostic application technology that measures 1 to ~1000 human proteins in low sample volumes (~15-75 µL of serum/plasma, tissue homogenate) with a high-performance, high- throughput, and cost-effective assay. This technology is enabled by a new class of DNA aptamers – "SOMAmers™" – that contain novel chemically-modified nucleotides, which greatly expand the physicochemical diversity of the large combinatorial SELEX libraries from which they are selected.
Methods: Proteins were measured with a process that transforms a signature of protein concentrations into a representative DNA concentration signature, which can be quantified by DNA microarray, bead- based hybridization, or qPCR.
Results: The assay achieves low limits of detection (1 pM average), ~7 logs of overall dynamic range, and ~5% average coefficient of variation. We have demonstrated the utility of this technology in a large clinical biomarker discovery study for a non-small cell lung cancer diagnostic (Ostroff et al., 2010).
Conclusion: A panel of biomarkers discovered in this effort has been converted into a streamlined prototype lung cancer diagnostic assay.
References: Ostroff et al., Nature Preceedings: hdl10101/npre.2010.4537.1

IO33

DIRECT CHARACTERIZATION OF THE PHYSICOCHEMICAL PROPERTIES OF SINGLE PROTEIN COPY USING AFM

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Introduction: Atomic force microscopy (AFM) is a nanotechnological multifunctional molecularplatform for measuring of functional properties of proteins at single copy level. AFM was used for visualization of oligomeric state, activity, elasticity and electron transfer properties of protein from cytochrome P450 family on example of CYP102A1 (BM3) enzyme.
Methods: scanning tunneling microscopy (STM), atomic force microscopy
Results: It was shown that BM3 oligomeric states are monomer, dimer, trimer, tetramer and oligomers of higher order by use sharp and supersharp AFM probes. Functional activity of single oligomers of BM3 was measured by AFM. The height BM3 fluctuations amplitude during catalytic cycle is much larger than the height fluctuations amplitude of the same enzyme molecules in the inactive state. Activity of cytochrome P450 BM3 was calculated in terms of enzyme globule fluctuations in unit time, which was 5±2 Å/s. It was obtained that the height fluctuation amplitude of single globule of cytochrome P450 BM3 depends on temperature, and 22^{o}C is a peak of this temperature profile.
Elasticity of single protein was measured based on deformation of this protein under AFM probes with various radii of curvature. Young's modulus of BM3 molecules was estimated.

Electrical properties of electron transfer in flavocytochrome BM3 was measured using conductive AFM probe. The increasing of electron transfer through protein globule on AFM support at voltages |U| > |REDOX POTENTIAL of BM3| (for negative voltage) was demonstrated. It was supposed that an additional electron transfer channel through heme (AFM probe-heme-AFM support) was realized.
Conclusion: Based on the obtained data, the following conclusions may be made: an enzyme catalytic activity can be measured as an amplitude of enzyme globule fluctuations; elasticity of single protein can be measured based on deformation of this protein under AFM probes and
electron transfer properties of BM3 can be detected using STM/AFM.

IO34

QUANTITATIVE MS-BASED (PHOSPHO)PROTEOMICS

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Abstract
Massspectrometry (MS)-based phosphoproteomics has achieved extraordinary successes in qualitative and quantitative analysis of cellular protein phosphorylation. Considering that estimates place the level of phosphorylation present in a cell at above 100,000 sites, there is, however, still much room for improvement. In our laboratory we attempt to extend the depth of phosphoproteome coverage while maintaining realistic aspirations in terms of available material, robustness and instrument running time. Wedeveloped several strategies, which will be discussed here and further highlighted by key-applications. The use of a novel material for the enrichment, namelypolymerized Ti4+-IMAC, will be described and its use in analyzing phosphoproteomes of human cancer cells. Moreover, the use of peptide pull-downs and stable isotope dimethyl labeling targeting tyrosine phosphorylation will be described and how we used it to explore new targets of Gleevec in a CML model cell. Finally, we describe the use of FACS sorting of adut stem cells directly from mouse intestine, combined with dimethyl labeling to define the intestinal stem cell signature.

IO35

ACCOUNTANCY 101 IN GLOBAL PROTEOMICS: QUANTIFICATION AND TURNOVER

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Simply stated, the specification of the absolute abundance of every protein in a cell or subcellular system seems straightforward. Yet, most proteome studies emphasise relative quantification studies that are very informative, but which do not permit detailed modelling and ultimately, the emergence of a predictive biology of the system. We therefore need robust and scalable approaches to absolute quantification. Whilst there is growing interest in label-free proteome analyses, the strategy of choice remains stable isotope mediated internal standardisation in high-resolution instruments, or for greater sensitivity, triple quadrupole instruments. I will discuss our approach to global protein quantification, covering the generation of multiplexed standards, their deployment in MS based assays and the value of enhanced signal to noise in deeper proteome coverage.
A second parameter that will be required, especially in dynamic systems, is the rate of turnover of each protein in the proteome under study. Turnover can occur in the absence of detectable change in protein abundance, and thus, in the absence of tagged constructs, can only be accessed by monitoring flux of labelled amino acids through a protein pool and in proteome studies, the isotopes are usually stable, although this brings some complications. Whilst it is relatively straightforward to gain turnover parameters from cells grown in culture (dynamic SILAC) it is significantly more complex to acquire these values in complex systems such as intact animals. I will address approaches and pitfalls in the measurement of proteome turnover.

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IO36

PROTEOMICS TOOLS FOR STUDYING THE POTENTIAL INTERPLAY BETWEEN VARIOUS POST-TRANSLATIONAL MODIFICATIONS

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Virtually all cellular processes are tightly regulated by proteins with distinct functions. One of the fastest ways to alter the function of a protein is by post-translational modifications (PTMs). These can modulate many properties: conformational state, stability, activity, interaction partners and subcellular distribution. Many of the control mechanisms involved in the dynamic regulation of cellular expression patterns in health and disease are dependent on specific PTMs within key proteins. Therefore, the determination and quantitative assessment of PTMs on proteins is fundamentally important for elucidation of the complex processes that govern cellular events such as cell growth, division, differentiation and migration. As PTMs are usually mediated by tightly regulated, low abundance proteins, a small change in their expression or activity will result in a significant change in PTMs. This will likely be associated with a change in activity, partners, or cell location of the affected proteins and consequently a change in the dynamics and stability of the cell.

In the recent years an exponential amount of methods have been developed, which allow researchers to specifically isolate and characterize proteins and peptides carrying several kind of PTMs, such as phosphorylation, glycosylation and acetylation. With these methodological development researches now have the tools to elucidate complex cellular signalling events and the effect of various PTMs on the dynamic of cells.

There is increasing evidence that phosphorylation, glycosylation and acetylation play important roles in cellular signaling networks during development and transformation of cells. For example, the nervous system contains an abundant array of sialylated glycoproteins and it is therefore not surprising that changes in the sialiome (the content of sialylated glycoproteins) of a neuron can regulate activity and change important signalling pathways through altered phosphorylation. Interestingly, removal of sialic acids from membrane proteins by the enzyme NEU3 in primary neurons leads to actin depolymerization and axonal growth through TrkA-mediated signaling.

Here we illustrate a novel comprehensive multidimensional proteomics and PTMomics strategy which allow quantitative assessment of non-modified peptides, phosphopeptides, sialylated glycopeptides and acetylated peptides from the same sample. This allows the study of potential crosstalk between PTMs. We show the application of this multidimensional strategy to various samples ranging from the developing mouse brain to isolated nerve-endings (synaptosomes) and ischemic hearts.

IO37

INTEGRATING ORBITRAP SHOTGUN PROTEOMICS WITH ILLUMINA GENOME SEQUENCING FOR INVESTIGATING MICROBIAL COMMUNITY PROTEOGENOMES: INSIGHTS FROM A SYNTHETIC MICROBIOME

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Introduction: Community proteogenomics, or the “omic” approach to the study of microbial communities associated with single environments, has the ability to provide information on population diversity and metabolic activity. As such, it holds a huge potential for discovering microbial combinations and protein functions useful for health, environmental, and industrial applications. However, the information depth that can be reached with integrated “omic” approaches on such complex systems remains largely unexplored. Here, shotgun proteomics, genome sequencing, and bioinformatics were combined for the proteogenomic characterization of a defined synthetic microbiome, with the aim of optimizing experimental pipelines and of assessing extent and quality of the gathered proteomic information.

Methods: Five bacteria with different cell walls, biochemical peculiarities, and genomic coverages were selected. First, the proteomic workflow was optimized on E. coli by applying an array of protocols combining different detergents, separation procedures, chromatography conditions and mass spectrometry methods on an LTQ Orbitrap Velos. The three best performing workflows were applied to an isobaric mixture of the 5 bacteria, by using the same amount of total proteins and the same overall duration of LC-MS/MS runs. In parallel, DNA extraction was optimized, two different procedures were implemented, and the 5 bacteria were

subjected to Illumina HiSeq sequencing, assembly and annotation. Then, proteomic data were analyzed with Proteome Discoverer against Uniprot, NCBI, and the HiSeq de-novo sequencing datasets.
Results: The best proteomics workflow, the best genome analysis procedures, and the extent of proteomic coverage increase after de-novo sequencing were defined for the model microorganism; then, the results that can be obtained upon application of the whole analytic pipeline to the five organism synthetic microbiome were assessed.
Conclusion: This study defined the information depth that can be reached when applying an optimized, integrated proteogenomic pipeline to a simple isobaric microbiome. Further studies will be needed to assess its performance with complex and heterogeneous microbial communities.

IO38

ION MOBILITY ASSISTED DATA INDEPENDENT LABEL-FREE LC-MS ANALYSIS OF CITROBACTER RODENTIUM INFECTED MOUSE COLON

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Introduction: *Citrobacter rodentium* (CR) is a murine-specific pathogen used to study human infection with *E. coli*. In mice, CR colonises the gut via formation of attaching and effacing lesions, causing transmissible colonic hyperplasia and a self-limiting disease. Here we used quantitative proteomics to examine the mouse colonic proteome at two key time points of infection, day 8 (bacterial colonisation peak) and day 14 (colonic hyperplasia peak).
Methods: At day 8 or day 14, mice (4 infected, 4 PBS-treated control) were euthanized, 6 cm of colon removed and pooled (2 colons per sample) for protein extraction and trypsin digestion. Peptide samples were randomized and analysed in triplicate using nanoLC (100ng/90 min). Data were acquired in HDMSE mode where raising the collision energy following ion mobility separation produced fragments that exhibit the same drift time as their precursor. Precursors and fragments were correlated and searched using post processing software.
Results: Initial experiments were conducted to determine the optimum extraction procedure of proteins from mouse colon. For the comparative study, a total of 2852 proteins comprising at least 1 proteotypic peptide were identified from the entire sample set (8 samples), with approximately 1000 proteins from 10,000 non-redundant peptides being identified in each injection and >1250 proteins in two out of the three injections for each sample. Reproducibility was demonstrated with over 800 proteins consistently identified in each of the QC injections. Further interrogation of the dataset allowed for the calculation of the relative in-sample abundance for each identified protein. Thereafter, unsupervised hierarchical clustering of the relative abundance calculated values demonstrated a clear classification of CR infected and uninfected mice and afforded identification of both host and pathogenic infection related proteins.
Conclusion: This study describes the comparative analysis and classification of CR infected mice by mobility assisted data independent label-free LC-MS.

IO39

IDENTIFICATION OF POTENTIAL MARKERS OF ABSENCE EPILEPSY BY MALDI IMAGING USING A "MIRROR" MOUSE MODEL

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Introduction: MALDI MS imaging was used for discovering potential markers of childhood absence epilepsy (CAE), a prototypic form of generalized non-convulsive epilepsy, using an original mouse model derived from a genetic selection. Here, an innovative classification method was developed and adapted for processing large MALDI imaging brain datasets and validating potential markers of CAE.
Methods: MALDI images were acquired from frozen brain sections obtained from the BS (sensitive) and BR (resistant) absence epilepsy mouse lines. After washing and drying of tissue sections, matrix was applied by vibrational vaporization. Protein mass spectra were acquired on whole brain sections using MALDI-TOF mass

spectrometry at 100µm lateral resolution. Each MALDI imaging whole brain dataset was reduced taking only each 16th spectrum. Null spectra were excluded and mass spectra recalibration was performed. A new type of cross-classification based on a combined discrete wavelet transformation (DWT) - support vector machine (SVM) classification was developed to classify all data sets of one type vs. each data set of the other type. Identification of candidate biomarkers was performed on extracts from specific mice brain regions collected by microsurgery (thalamus, cortex and somatosensory cortex). Total proteins were fractionated by reversed phase HPLC. An aliquot of each collected fraction was analyzed by MALDI-TOF mass spectrometry and selected fractions containing intact proteins with m/z values of interest were then loaded onto a nanoLC column coupled to an LTQ-Orbitrap XL mass spectrometer for top-down analysis.
Results: Very high recognition rates were obtained to classify the BS and BR mice brains with 19 m/z ratios highlighted as potential biomarkers. Among the identified markers, Synapsin-I was chosen to evaluate our classification method. Immunohistochemistry and Western blotting further validated the localization and differential expression of Synapsin-I observed by MALDI imaging.
Conclusion: The involvement of Synapsin-I in the molecular mechanisms underlying absence epilepsy is currently being performed using functional assays.

IO40

MASS SPECTROMETRY BASED PROTEOMICS AND NETWORK BIOLOGY
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In the life sciences a new paradigm is emerging that places networks of interacting
molecules between genotype and phenotype. These networks are dynamically modulated by a multitude of factors and the properties emerging from the network as a whole determine observable phenotypes. Often such networks are characterized by proteins (network nodes) that are connected to each other in different ways (indicated as network edges). This paradigm is usually referred to as systems biology, network biology or integrative biology.

Mass spectrometry based proteomics has made significant progress towards the identification, characterization and quantification of proteins in complex samples, i.e. towards the characterization of network nodes. The quantitative analysis of network edges has been considerably more difficult.

In this presentation we will discuss how quantitative mass spectrometry can be used to directly measure network edges as in the case of physical protein-protein interactions. We will also discuss emerging methods to generate proteomic datasets from which network edges can be inferred in cases where they are not directly measurable, e.g. for functional interactions. The technical advances will be illustrated with selected examples.

References:
1.Gillet LC, Navarro P, Tate S, Roest H, Selevsek N, Reiter L, Bonner R, Aebersold R. (2012) Targeted data extraction of the MS/MS spectra generated by data independent acquisition: a new concept for consistent and accurate proteome analysis. Mol Cell Proteomics. 2012 Jan 18. Mol Cell Proteomics. 2012 Jan 18.
2. Bensimon A, Heck AJ, Aebersold R. (2012) Mass Spectrometry-Based Proteomics and Network Biology. Annu Rev Biochem. 2012 Mar 22. [Epub ahead of print]

Young investigator presentations

YIP01

SALIVARY ORAL SQUAMOUS CELL CARCINOMA BIOMARKERS – EXPLORING POTENTIAL INDICATORS IN TYPE 2 DIABETES

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Introduction: The early detection of oral squamous cell carcinoma plays a crucial role in the prevention but also in the treatment strategies. Recent conducted epidemiological studies and animal experiments showed that there is a relationship between diabetes and oral squamous cell carcinoma. Our goal was to develop an easy, non-invasive and non-expensive method, which is able to screen the risk for possible oral malignancies in diabetic patients.
Methods: Under standardised circumstances we collected saliva samples of diabetic patients (n=17). As a control group we used saliva from healthy subjects (n=15). The samples were analysed using SDS PAGE and MALDI-TOF/TOF mass spectrometry after tryptic digestion. The resulted peptides were identified by peptide mass finger printing. The peptide masses were searched by utilising the MASCOT Server 2.2 search engine ($p<0.05$).
Results: The analysis demonstrated a different pattern of protein biomarker expression between diabetic and control subjects. A significant difference was measured in the expression of annexin-A11, tyrosine-protein phosphatase and peroxiredoxin-2, which were previously found also in oral cancer patients.
Conclusion: Our study requires further validation with a larger population, but the detected overexpressed peptides and proteins are possible predisponating biomarkers for an early - stage cancer.
References: (1).Greenlee RT: Cancer statistics, 2000. CA Cancer J Clin 50(1): 7-33, (2). M Ujpál, O Matos, Gy Bíbok, A Somogyi, Gy Szabó, Zs Suba: Diabetes mellitus and oral tumors in Hungary. Epidemiological correlations.Diabetes Care 2004. 27. 770-774, (3). Vairaktaris E, Diabetes and Oral Oncogenesis, Anticancer Research 2004. 27: 4185-4194**, (**4). Mandel ID. Salivary Diagnosis: more than a lick and a promise. JADA 1993;124(1):85-7**,** (5). R Duncan, B Carpenter, LC Main, C Telfer, GI Murray: Characterisation and protein expression profiling of annexins in colorectal cancer. British Journal of Cancer (2008) 98, 426-433**,** (6). Ostman A: Protein-tyrosine phosphatases and cancer. Nat.Rev.Cancer.2006 apr;6(4):302-20**,** (7). Z.A Wood, L.B Poole, P.A Karplus: Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. Science 2003 (300) 650–653

YIP02

A QUANTITATIVE PROTEOMICS AND METABOLOMICS PLATFORM FOR BIOMARKER DISCOVERY IN PSYCHIATRIC DISORDERS: FOCUS ON MITOCHONDRIAL DYSFUNCTION

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Introduction: Biomarker discovery for psychiatric disorders is of utmost importance since no molecular markers are currently available. Hypothesis-free approaches such as -omics technologies constitute a robust methodology for biomarker identification and the elucidation of disease pathophysiology.
Methods: We have developed a quantitative proteomics and metabolomics platform based on *in vivo* ^{15}N metabolic labeling and quantitative mass spectrometry (1). We labeled mice with ^{15}N (2) and used the ^{15}N-labeled specimens as internal standards to analyze mouse models of (i) behavioral extremes in trait anxiety (3, 4) and (ii) schizophrenia-like symptoms. We established an experimental and bioinformatics workflow (5) for relative quantification and identification of candidate biomarkers and affected pathways.
Results: We applied our platform to compare:
(i) the cingulate cortex synaptosomal proteomes and metabolomes of high and low anxiety-related behavior mice. We found differences in ~300 proteins and metabolites. *In silico* pathway analysis, immunochemical

and enzymatic validation confirmed alterations in mitochondrial pathways, including oxidative phosphorylation, oxidative stress and mitochondrial import/transport.
(ii) the cerebellar and hippocampal cytoplasmic proteomes of transgenic mice carrying the G72/G30 locus that is implicated in schizophrenia, and wild type controls. We identified alterations in mitochondrial pathways, including oxidative stress and energy metabolism that were validated with immunochemical and enzymatic assays.

Conclusion: We found mitochondria as a common denominator for alterations observed in anxiety-related and schizophrenia-like behavior. Our multi -omics approach is an accurate platform that can shed light on psychiatric disorder pathobiology and provides an example how unbiased -omics methods can pinpoint molecular correlates of disease at the systemic/organellar level.

References: (1) Filiou & Turck, 2012 Methods Mol Biol 829:531-9, (2) Frank et al, 2009 PLoS One 4:e7821
(3) Filiou et al, 2011 Biol Psychiatry 70:1074-82, (4) Zhang et al, 2011 Mol Cell Proteomics 10:M111.008110
(5) Haegler et al, 2009 J Proteomics 71:601-8

YIP03

CIRCULATING PROTEIN BIOMARKER SIGNATURES FOR DIAGNOSIS, THERAPY SELECTION, AND PROGNOSIS OF COLORECTAL CANCER

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Introduction: Early detection and monitoring of colorectal cancer (CRC) would greatly benefit from reliable and non-invasive biomarkers. A biomarker development pipeline was established to discover specific CRC proteins, to initially verify them and to validate biomarker candidates in clinical cohorts of plasma samples.
Methods: Tissue epithelia were dissected from surgically excised tumours (n=16) and adjacent normal mucosa (n=16). Patient plasma samples (n=50) were selected for screening. The clinical cohort (n=355) was comprised of CRC, benign, healthy, lung and pancreatic cancer subjects. *N*-linked glycopeptides were isolated from samples and analysed with high resolution LC-MS or with selected reaction monitoring. Statistical and machine learning methods were employed for data analysis.
Results: More than 300 glycoprotein candidates were discovered from tumour epithelia of CRC patients, which were verified in 50 patient plasma samples, and validated in a unique clinical cohort of aforementioned cases. The robust SRM platform enabled the generation of one of the largest clinical SRM datasets to date, with accurate quantification of 80 candidates in more than 350 samples. Cancer-specific abundance changes, consistent across disease stages, were characterised. A 4-protein biomarker signature with a high diagnostic accuracy to discriminate between CRC and controls was obtained. To characterise prognostic biomarkers, multivariate regression analysis identified multi-protein signatures able to discriminate between patients with a better and worse prognosis by association with 5-year disease free and overall survival. Furthermore, multiple candidate biomarkers were also significantly associated with genetic factors such as the KRAS gene status, to directly predict the response to EGFR antibody therapy and to identify patients with a better prognosis.
Conclusion: In this study we discovered, verified, and validated a concise list of biomarker candidates for CRC with the potential of non-invasive diagnosis, prognosis, as well as therapeutic relevance.

Posters Abstracts

P001

HIGHER MULTIPLEXING WITH ISOBARIC MASS TAGS ENABLED BY 15N AND 13C CONTAINING REPORTER IONS AND HIGH RESOLUTION MASS SPECTROMETRY

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Introduction: Isobaric mass tag-based quantitative proteomics strategies such as iTRAQ and TMT utilize reporter ions in the low mass range of tandem-MS spectra for relative quantification. The number of samples that can be compared in a single experiment (multiplexing) is limited by the number of different reporter ions that can be generated by differential stable isotope incorporation (15N, 13C) across the reporter and the mass balancing parts of the reagents. Here we demonstrate that a higher multiplexing rate can be achieved by utilizing the 6 mDa mass difference between 15N and 13C containing reporter fragments in combination with high resolution mass spectrometry.
Methods: Tryptic protein digests were labeled with TMT (Thermo Fisher). For labels generating reporter ions with the nominal masses 127 and 129 2 different variants were used, which differed in the distribution of 13C and 15N, respectively. Peptides were separated on a nano-HPLC system coupled to an Orbitrap Elite mass spectrometer. Affinity enrichment experiments were performed as described (1).
Results: A tryptic digest of BSA was labeled with either standard TMT127, 129 or variants where a 13C atom in the reporter part was substituted with a 15N atom (TMT127*, TMT129*). HCD tandem mass spectra were acquired using different nominal resolution settings. At 30,000 nominal resolution corresponding to a resolution of 55,000 in the reporter ion region of the spectra, baseline separation of TMT127, TMT127* and TMT129, TMT129* was observed. The high mass accuracy of the instrument enabled unambiguous identification of all reporter ions, thus enabling accurate quantification in standard samples.
Next we applied TMT127* and TMT129* in conjunction with the standard 6-plex reagents to enable 8-plexing of complex samples, thus establishing precision and accuracy of quantification. Finally, 8-plexing was used to measure potency and selectivity of kinase inhibitors using a kinobeads-based chemoproteomic assay(1).
Conclusion: High resolution MS enables higher multiplexing with isobaric mass tags without changing the over-all structure of the molecule.
References: (1) M. Bantscheff et al, Nat Biotechnol. 2007 Sep;25(9):1035-44

P002

A HIGH-PERFORMANCE BENCHTOP QUADRUPOLE-ORBITRAP LC-MS/MS WORKFLOW SOLUTION FOR INTACT MONOCLONAL ANTIBODY CHARACTERIZATION

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Introduction: Monoclonal antibodies (mAbs) are increasingly developed and utilized for the diagnostic and therapeutic of diseases including cancer. Due to the heterogeneity of mAb products, thorough characterization is necessary for their reproducible as well as safe production. Among the analytical tools used for the analysis of therapeutic mAb, mass spectrometry has become more and more important in providing valuable information on various protein properties, such as intact mass, amino acid sequence, post-translational modification including glycosylation form distribution, minor impurities due to sample processing and handling and high order structure, etc.
Methods: In this study, a high resolution LC-MS based workflow solution was developed and evaluated for robust, accurate and comprehensive intact mAb characterization using the Q Exactive, a routine bench-top orbitrap mass spectrometer. Full MS spectra were analyzed using Protein Deconvolution software for intact mass determination.ProSightPC software was used for top-down sequencing.
Results: The results indicated that the high mass accuracy, stability and high resolution of the Q Exactive produced accurate and reproducible intact mAb mass measurement with a mass error of less than 10 ppm. To obtain sequence information of the intact mAb, a unique, high throughput HCD capability of the Q Exactive was used to generate high resolution, top-down spectra on intact light or heavy chain on the LC time scale.

Analysis of the top-down MS/MS spectra using ProSightPC software presented extensive sequence coverage with a mass error of less than 5 ppm for fragment ions.
Conclusion: Results from this study indicated that both precise mass measurement and extensive, high confident sequence information can be obtained for mAb using the same workflow solution. The fast chromatography, the superior resolution, mass accuracy and the high throughput MS/MS combined with the easy, accurate data analysis of this workflow solution provide a high-confident screening tool to accelerate biopharmaceutical product development cycles.

P003

IODOACETYL TANDEM MASS TAGS FOR CYSTEINE PEPTIDE MODIFICATION, ENRICHMENT AND
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Introduction: Tandem Mass Tag® (TMT®) Reagents enable concurrent identification and multiplexed quantitation of proteins using tandem mass spectrometry. We report the development of an irreversible, cysteine-reactive TMT reagent containing an iodoacetyl reactive group (IodoTMT™). IodoTMT reagent can be used for quantifying cysteine modifications such as S-nitrosylation, oxidation and di-sulfide bridges.
Methods: Cell lysates and purified proteins were denatured and reduced for total cysteine alkylation experiments. Samples were then labeled with excess IodoTMT, mixed and desalted prior to enzymatic digestion. For cysteine S-nitrosylation quantitation, samples were treated with nitrosylation donor agents (e.g. GSH-NO) and alkylated with MMTS to block unmodified sulfhydryls. After desalting, S-nitrosyl groups were then selectively reduced using ascorbate to generate free sulfhydryls for Iodo TMT reagent labeling. IodoTMT-labeled peptides were enriched using an immobilized anti-TMT antibody resin and analyzed using Orbitrap hybrid mass spectrometers. Data analysis was performed with Proteome Discoverer™ 1.3 software.
Results: An IodoTMT reagent was developed to irreversibly label sulfhydryls of cysteine-containing peptides for multiplex quantitation by LC-MS. Reagents showed efficient and specific labeling, with reactivity similar to iodoacetamide. The reagent was used as a probe for labeling S-nitrosylated cysteines in a modified S-nitro switch assay. IodoTMT reagents successfully labeled S-nitrosylated cysteines after selective reduction using ascorbate. Addition of 1mM copper sulfate to the switch reaction buffer inhibited IodoTMT reagent labeling. We characterized an anti-TMT antibody developed against the reporter region of the TMT reagent for Western blot detection of IodoTMT-labeled proteins and immuno-enrichment of IodoTMT-labeled proteins and peptides.
Conclusion: IodoTMT labeling with anti-TMT enrichment has several advantages: 1) more specific labeling of sulfhydryl groups; 2) choice of six-plex isobaric multiplexing or two-plex isotopic quantitation; 3) isobaric tagging for more efficient MS data analysis; 4) a simpler workflow using antibody-based capture for labeled peptide enrichment.

P004

DECONSTRUCTING THE ROLE OF ANTERIOR GRADIENT-2 PROTEIN EXPRESSION UTILISING SYNTHETICALLY ENGINEERED CELL LINES AND QUANTITATIVE MASS SPECTROMETRY
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Introduction: Proteomic techniques investigating normal squamous and metastatic epithelium first uncovered anterior gradient-2 (AGR2) as a p53 inhibitor and as being overexpressed in the premalignant oesophageal disease, Barretts epithelium. Subsequent studies have implicated AGR2 in tumour growth, cell migration and metastasis. The mechanisms whereby AGR2 signals to suppress p53 has not been discovered and in this study we present approaches involving isogenic cell model construction and SILAC screening to begin to define how AGR2 protein can function as a proto-oncogene and suppress p53 function.

Methods: Isogenic cell lines were engineered to constitutively express the gene of interest in A375 skin melanoma cells. These cells were chosen since they express no endogenous AGR2 and maintain a wild-type p53 pathway.

Relative protein expression could be quantified using stable isotope labelling by amino-acid in cell culture (SILAC). Cells were cultured in media containing stable isotope labelled lysine and arginine, before cell lysis, alkylation and reduction, followed by 1D SDS-PAGE. The protein lane was dissected to 10 slices, and each slice was subject to in-gel trypsin digestion. The resulting peptides were then separated by nano-LC before ESI-MS/MS. Quantification was performed by MaxQuant, before normalisation and the derived peak list was search using the Mascot search engine. Normalised SILAC ratios were then analysed using Ingenuity pathway analysis. Results were followed up by immunoblot.

Results: AGR2 protein expression appears to reprogram cells to become proliferative. Key modulated proteins demonstrate influential transcriptional activity promoting the capacity of AGR2-expressing cells to increase growth rate compared to the parental cell.

Conclusion: This study demonstrates how we can use quantitative MS techniques in well-constructed synthetic cell models in order to define the specific function of an identified oncoprotein on cell signalling. Ongoing studies investigate the level of regulation AGR2 expression exhibits on the cellular transcriptome and this is coupled to and distinct from the proteome to paint a systems biology snapshot of how AGR2 reprograms a cell to become proliferative.

P005

QUANTITATIVE 3D PROTEOMICS USING ISOTOPE-LABELLED CROSS-LINKERS AND XIQ SOFTWARE

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Introduction: Dynamic aspects of proteins play a pivotal role in many if not all biological processes. Unfortunately, the analysis of protein dynamics remains a challenge. 3D proteomics is emerging as a new and already highly successful tool in the structural analysis of proteins and complexes. Adding quantitative measurements to 3D proteomics will allow expanding this success to the analysis of protein dynamics, such as conformational changes and protein-protein interaction dynamics.
Methods: Human serum albumin (HSA) was cross-linked in triplicates using BS3-d0 and deuterium labelled BS3-d4 in 1:1, 1:2 and 1:4 ratios. LC-MS/MS analysis was conducted using an LTQ Orbitrap Velos with a high-high strategy and 1+ and 2+ precursors excluded. Cross-linked peptides were identified using Xi software. Quantitation was done manually in Xcalibur, using MaxQuant or own software XiQ. XiQ was implemented in C++ using the MSFileReader library.
Results: 3D proteomics is increasingly successful in providing medium resolution data on static proteinaceous structures. Here we add the possibility of recording dynamic processes with this novel tool, using isotope-labelled cross-linkers for quantitation, BS3-d0 and BS3-d4. Using isotope-labelled cross-linkers focuses quantitation on cross-linker containing species and places minimal restriction on the protein source. Consequently we can use HSA as a model system, exposed to different ratios of BS3-d0/4 to test data analysis approaches: manual quantitation, MaxQuant and XiQ, a new software for the quantitation of cross-linked peptides introduced here. Manual quantitation returned the known mixing ratios of our test samples with large accuracy. While MaxQuant does not identifiy cross-links it could be exploited to get quantitation for many albeit not all identified cross-links. Where it did, we noticed very inconsistent performance of MaxQuant, as it could not compensate the isotopic effect of deuterium on the chromatogram. XiQ overcomes this limitation and provides data on all identified cross-links with an accuracy that compares to that of manual quantification. This lays the foundation for quantitative 3D proteomics.
Conclusion: XiQ enables quantitation of crosslinked peptides, thus extending the capabiliteis of 3D proteomics to provide insights into dynamic processes.

P006

ATP-CAPTURE COMPOUND MASS SPECTROMETRY: A NOVEL FISHING POLE IN ATP-BINDING PROTEIN SUBPROTEOMICS

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Introduction: Chemical probes suitable to specifically isolate and identify ATP-binding proteins at high sensitivity from biological sample would cover a wide range of important regulatory proteins in a functional proteomics approach. We here explored the capability of the ATP Capture Compound Mass Spectrometry (CCMS) approach to display the subproteomics of ATP-binding proteins.
Methods: In the CCMS workflow, the selectivity group, i.e. gamma-modified ATPs, provide equilibrium binding of Capture Compounds (CCs) to the target proteins. Second, upon irradiation, the reactivity function freezes this interaction through covalent cross-link to the target proteins. Third, using streptavidin magnetic beads, the CC-protein conjugates can be easily isolated from the complex protein mixture *via* the sorting function of the CC. The captured proteins are then directly subjected to proteolytic on-bead cleavage and automated LC-MS/MS based protein identification. We applied this workflow to protein mixtures from human derived HepG2 cells, and rat brain synaptosomes.
Results: From HepG2 cell lysate, many different classes of ATP and other nucleotide binding proteins were specifically captured, such as kinases and heat shock proteins. Most interesting is that of the total ca 300 identified proteins in the capturing from the solubilized rat synaptosomes, 20-40% of the identified proteins are specific binders of the ATP-CC or at least protein-protein interaction partners of specific binders as deduced from competition experiments using ATP as competitor. Moreover, more than 50% of those specific binders are annotated ATP-binding proteins, such as ATPases, ATP synthase, or the ATP-binding cassette sub-family. The analytical power of CCMS is demonstrated by the robust identification of KATP channels (Kir 6.2 and SUR1) directly from solubilized synaptosomes.
Conclusion: We here demonstrate that CCs with derivatives of gamma-modified ATPs as selectivity group are unique tools to specifically target proteins in the ATP-binding proteins. Notably, ATP-CCMS can successfully address even low-abundant transmembrane ion channels and the interacting partners at an unprecedented sensitivity from minute amounts of complex protein mixtures.

P007

IDENTIFICATION OF SKIN PROTEINS HAPTENATED BY A PANEL OF CHEMICAL SENSITISERS USING STABLE ISOTOPE LABELLING AND RPLC-MS/MS

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Introduction: Contact allergy is a delayed-type hypersensitivity response elicited following the covalent modification of specific amino acid residues of skin proteins by a chemical sensitizer. Modified peptides derived from these proteins are antigenic and provide maturation signals for dendritic cells, triggering a T-cell response when the chemical is subsequently encountered, however the identities of the peptides are currently unknown. The aim of this study was to identify the sites of modification by chemical sensitizers; dinitrochlorobenzene, cinnamaldehyde, p-phenylenediamine, 5-chloro-2-methylisothiazol-3-one and 6-methylcoumarin using a model protein, human serum albumin (HSA) and lysates of a keratinocyte cell line (HaCaT).
Methods: Either HSA or HaCaT cell lysates were modified at 37°C with a mixture of test chemical and stable isotope labelled chemical to a final concentration of 1.5mM (100:1 molar ratio of test chemical:protein). The mixtures were sampled at different time points, excess chemical removed and the proteins digested with trypsin for analysis by RPLC-MS/MS. Multi-dimensional separations were employed for the fractionation of HaCaT lysates prior to analysis.
Results: Modification of proteins by sensitizers is not stoichiometric and the high background ions from unmodified peptides often mask the low ion intensities of modified peptides. This makes the analysis of the MS fragmentation spectra for identification and assignment of modification sites challenging. By reacting proteins with both unlabelled and stable-isotopically labelled sensitiser, the LC-MS spectra can be analysed

for a label dependent isotopic signature, thus identifying modified peptides. Upon identification, peptide inclusion lists are used to acquire fragmentation data for extended periods of time, improving spectral quality and confidence in assigning the site of modification. Using a dual-labelling approach proteins can be analysed in a relatively high-throughput manner, enabling complex protein lysates to be investigated for the presence of covalently modified (haptenated) peptides.
Conclusion: The data confirmed that specific nucleophilic residues were modified in a highly selective and differential manner by each chemical tested.

P008

HIGHLY ORTHOGONAL OFF-LINE STRONG ANION EXCHANGE FRACTIONATION FOR PROTEOMIC ANALYSES

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Introduction: The proteomes of eukaryotic cells exhibit such a high complexity that current large-scale proteome analyses require orthogonal pre-fractionation prior to mass spectrometry analysis. Recently, reversed-phase (RP), Off-Gel and strong cation exchange chromatography (SCX) have been extensively applied for separation of peptide mixtures while strong anion exchange (SAX) has seen relatively little use.
In this work, we tested the performance of 3 SAX columns, differing in their hydrophobicity/capacity, and compared them with a high-resolution high-pH/low-pH RP approach. The off-line SAX fractionation of tryptic peptides resulted in high orthogonality identifying more peptides and proteins compared to a high-pH RP/ low-pH RP system.
Methods: Tryptic digests of murine RAW264.7 cell lysates (60ug) were fractionated (Δ 60 seconds) on a modular-UltiMate™3000 titanium-HPLC System (Thermo Fisher) by either SAX (IonPac® AS24,AS11HC,or AS15, 2x250mm,pH 8.0, Thermo) or RP (Acclaim®RSLC PAII, 2x150mm, pH 10.0, Thermo) and automatically re-injected for second dimension separation on a C18-column (Gemini3x250mm, Phenomenex).
Each fraction was separated online (300 minutes) on Acclaim®-PepMap100 column (75umx500mm, Thermo) and peptides measured by a Thermo Orbitrap Velos-Pro. Data were analysed by the Trans-Proteome Pipeline (TPP), searching against a forward and reverse IPI mouse v3.87 using X!Tandem and filtering the results at 1% Protein FDR as estimated by ProteinProphet.
Results: Using 2D retention maps (Chromeleon®, Thermo) we could show that IonPac® AS24 exhibited a higher orthogonality than the other two SAX columns tested. Furthermore, the SAX approach also outperformed high-pH/low-pH RP, showed better run-to-run reproducibility and exhibited higher robustness.
Mass spectrometric analysis of 34 fractions of each of the approaches identified >8500 unique proteins (1% FDR); however, SAX/RP resulted in >5% unique proteins and >15% unique peptides compared to RP/RP. In combination, the approaches demonstrated an identification of >9000 proteins using 2 weeks of instrument time.
Conclusion: SAX showed superior orthogonality, higher robustness and greater reproducibility than the well-established high-pH RP approach on separation of tryptic peptides.

P009

QUANTITATIVE 3D PROTEOMICS REVEALS PROTEIN CONFORMATION CHANGES USING ISOTOPE-LABELLED CROSS-LINKERS

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Introduction: 3D proteomics, a combination of chemical cross-linking, mass spectrometry and database searching, has been increasingly successful in providing medium resolution data on static protein structures. Here we demonstrate quantitative analyses of protein conformational changes using isotope-labelled cross-linkers.

Methods: C3, C3b and C3(H2O) samples were each divided into two aliquots which were cross-linked with BS3-d0 or BS3-d4. After digestion, cross-linked proteins were mixed pair-wise with equal molar ratio. Cross-linked peptides were enriched using SCX-Stage-Tips. LC-MS/MS analysis was conducted on an LTQ-Orbitrap Velos instrument with a high-high acquisition strategy and 1+ &2+ ions exclude. Cross-linked peptides were identified using Xi and quantitation using XiQ at cross-link level**.**
Results: In each sample, BS3-d0 cross-linked peptides and their BS3-d4 cross-linked counterparts were derived from different isoforms of the same protein; the d0/d4 ratio reflects different yields of a cross-link in two conformations. In the C3-C3b combination, cross-link data revealed both conformational similarities (doublet signals) and differences (singlet signals) between C3 and C3b and showed consistency with crystallographic data. This dataset provided a benchmark for further investigation on C3(H2O), a hydrolyzed analogue of C3. C3(H2O) is essential for initiation of alternative complement activation, however its structure remains unsolved. Pairwise comparison of C3(H2O) against C3 and C3b revealed that C3(H2O) exhibited significant domain rearrangement in alpha-chain in respect to C3, but its beta-chain remained similar to both C3 and C3b. In combination with crystal structures of C3 and C3b, a model of C3(H2O) domain architecture was proposed, where the C3b like TED position exposes a surface that enables hypothetic binding of interaction partners in complement processing.
Conclusion: Quantitative 3D proteomics using isotope-labelled cross-linkers reveals protein conformation changes as demonstrated by solving the previously unknown topology of C3(H2O).

P011

STAGETIP-BASED ENRICHMENT OF CROSS-LINKED PEPTIDES FOR 3D PROTEOMICS

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Introduction: 3D proteomics (protein cross-linking coupled with mass spectrometry) is a powerful technique to further structural understandings of large macromolecular complexes. Unfortunately, the current repertoire of commercially available cross-linkers is not designed for affinity purification of cross-linked peptides and at present, mainly size-exclusion chromatography is used, relying on the approximately doubled size of cross-linked peptides compared to linear peptides. Here we present an alternative approach, using a quick single-step tip-based ion exchange chromatography in which the majority of cross-linked peptides are enriched into one or two fractions with good recovery and reproducibility.
Methods: We used StageTip format SCX salt-based and SAX salt- and pH-based fractionation (3M Empore ion exchange material) to enrich for cross-linked peptides in complex samples. For cross-linking we used C3b protein and BS3 cross-linker (Pierce). Following trypsin digestion, peptides were enriched and analysed by LC-MS using an LTQ Orbitrap Velos. Cross-linked peptides were identified with data analysis platform Xi.
Results: With salt-based SCX- and SAX-StageTip fractionation linear peptides were identified in all four fractions whereas cross-linked peptides were only found in the two high salt fractions, with substantial overlap of identifications between SCX and SAX fractionation.
Interestingly, with standard pH-based SAX fractionation no cross-linked peptides were identified in the default elution fractions, meaning they were still be bound to the SAX matrix. We discovered that organic modifier was needed to release cross-linked peptides from the SAX matrix and this additional elution step resulted in cross-linked peptides being eluted with an extensive identification overlap between salt-based and pH-based fractionation.
Finally, since the peak intensities of the identified cross-linked peptides between different fractionation strategies were mostly at a similar level, pH-based SAX fractionation is preferred as this results in only one fraction of cross-linked peptides.
Conclusion: In conclusion, we have shown that cross-linked peptides can be enriched from a complex mixture by a simple and quick tip-based ion exchange chromatography with good recovery and reproducibility.

P012

ZERO-LENGTH CROSS-LINKING TO EXPAND THE REPERTOIRE OF 3D PROTEOMICS

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Introduction: The frequently used cross-linker BS3/DSS targets lysine pairs, which do not reflect actual contacts, and has a spacer length of 11.4 Å, which is not ideal for modelling. Alternative chemistry would furthermore be desirable to increase the sometimes sparse local density of cross-links. Consequently, we explored the use of zero-length cross-linker 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). As EDC connects salt bridges formed between E/D and K, it captures actual interaction points between and within proteins. The cross-linker is not incorporated into the final product. This feature improves the spatial resolution of cross-linking and facilitates structure modelling.

Methods: Human C3 was cross-linked by EDC with *N*-hydroxysulfosuccinimide stabilization of an unstable *O*-acylisourea intermediate in 0.1 M MES buffer (pH5.0) overnight at 4°C. The reaction mixtures were separated by SDS-PAGE. The protein bands corresponding to cross-linked monomer and cross-linked alpha and beta chains were reduced, alkylated and trypsin digested. Cross-linked peptides were enriched on StageTips and analyzed by LC-MS/MS (LTQ Orbitrap Velos). The mass spectrometric raw files were processed into peak lists using MaxQuant, and searched against the sequence of human C3 using in-house Xi software.

Results: For EDC cross-linked C3, 9698 spectra matched with the database and 330 of them have been auto-validated with scores between 5.5-13.6. The results were 55, 14 and 16 unique cross-links for the alpha-chain, beta-chain and between the two chains, with score cut-off 7.0. In contrast, cross-linking C3 using BS3, 5729 spectra matched the database and 139 of them were auto-validated with scores between 7.3-13.5. Using a global score cut-off 7.0, we found 31, 6 and 6 unique cross-links in alpha chain, beta chain and between the two chains respectively. The higher density of cross-links returned by EDC facilitates the modelling process and gives a more detailed picture of the topology of C3.

Conclusion: This work indicates that EDC has the potential to substantially improve on the already very informative and useful results obtained by BS3/DSS.

P013

AN INVESTIGATION INTO THE USE OF POROUS GRAPHITIC CARBON AS A STATIONARY PHASE FOR THE OFF-LINE FRACTIONATION OF AN ENRICHED POPULATION OF PHOSPHOPEPTIDES IN A 2D-LC-MS/MS WORKFLOW

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Introduction: We present the first investigation into the applicability of Porous Graphitic Carbon (PGC) as a stationary phase for the off-line fractionation of phosphopeptides. The robust nature of PGC renders it an ideal choice for fractionation of phosphopeptides eluted from [Fe(III)] or TiO_2 since the aggressive eluant (1% ammonia) does not degrade this stationary phase unlike others such as silica based C18 phases or PolySULFOETHYL A. Consequently, no further treatment of the sample, such as evaporation and solvent exchange, is required prior to fractionation.
Methods: Approximately 1 mg of SD1 (human ALL cell line) was lysed, reduced, alkylated and digested using trypsin. The sample was subjected to a SIMAC phospho enrichment strategy [1] using IMAC with Phos-Select™ [Fe(III)] beads in conjunction with TiO_2. Peptides were eluted from both enrichment media in 1 % ammonia and applied directly, without evaporation and solvent exchange, to a PGC (Hypercarb™) column for fractionation [2]. Fractions were collected at 30s intervals and analysed by LC-MS/MS using an LTQ Orbitrap XL mass spectrometer (50% of fractionated material used). Data were analysed using the Mascot™ search engine with an ion score cut-off of 22.

Results: Preliminary data revealed approximately 650 unique phosphopeptides from the IMAC fractions and around 1000 unique phosphopeptides from those enriched with TiO2. The combined total number of unique phosphopeptides was 1500 from 500 ug of material loaded onto the second dimension.
Conclusion: Porous graphitic carbon was found to be an excellent substrate material for the off-line fractionation of phosphopeptides. The robust nature of PGC enables enriched phosphopetides in 1 % ammonia to be applied directly to the column with no adverse affect on stationary phase. In turn, any losses associated with drying down samples prior to fractionation were eliminated.
References: [1] T.E. Thingholm, O.N. Jensen, P.J. Robinson, M.R. Larsen, Mol. Cell Proteomics (2007) 661.
[2] J.R. Griffiths, S. Perkins, Y. Connolly, L. Zhang, M. Holland, V. Barattini, L. Pereira, A. Edge, H. Ritchie, D.L. Smith, J. Chrom. A (2012) 276.

P014

EFFECT OF INCUBATION AND ELUTION CONDITIONS ON PHOSPHORYLATION ANALYSIS.
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Introduction: Protein phosphorylation is an important regulator of protein function, including cell proliferation and signal transduction. Phosphopeptides are typically detected at lower levels than the corresponding unphosphorylated peptides by mass spectrometry. Consequently an efficient phosphopeptide enrichment protocol is essential to determine the global profiling of phosphopeptides present in complex mixtures. Different elution conditions for immobilized metal affinity chelate (IMAC) and titanium dioxide (TiO_2) were evaluated using a total cell lysate sample.
Methods: A total cell lysate sample was reduced and alkylated before digestion with trypsin. The resulting peptides were desalted before phosphopeptide enrichment using IMAC and/or TiO_2. Different incubation, washing and elution conditions were evaluated as well as the effect of sequential elutions on the total number of phosphopeptides detected.
Results: A higher acid content and sequential elutions increased the overall number of phosphopeptides identified. The incubation time reached a plateau where no further improvement to both the selectivity and total number of phosphopeptides was achieved.
Conclusion: Improved phosphopeptide analysis is achieved with a higher acid content and sequential elutions using IMAC and/or TiO_2.

P015

IS THERE A PROTEASE BIAS IN ABSOLUTE PROTEIN QUANTITATION USING SPECTRAL COUNTS
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Introduction: There is a strong demand for reliable ways to determine absolute protein abundances ideally at a proteome wide scale. Label free mass spectrometric approaches may ideally fulfill this need, they are cost-efficient, require little sample pre-treatment and can be used on almost any sample. In such approaches the spectral counts and/or intensity of (tryptic) peptides detected in the LC MS/MS analyses are correlated to the overall amount of the protein from which these peptides originated. Trypsin is the most popular enzyme used in proteomics and huge efforts are currently undertaken to create spectral atlases of so-called proteotypic tryptic peptides for quantitative assays. A potential drawback is trypsin's reported irreproducibility in peptide formation, and its limited functionality under harsher digestion conditions. This may introduce a bias in the determination of protein abundance when based solely on tryptic data.
Methods: To evaluate the extent of such a bias we present label-free spectral count based quantitative proteomics data on *Saccharomyces cerevisiae*. In-depth quantitative data originating from 14 2D-LC-MS/MS datasets was obtained using four proteases (trypsin, chymotrypsin, Lys-C and Lys-N).
Results:
We detected 4895 proteins (4475 protein groups, 76 % of the verified and predicted open reading frames, including an unprecedented 44% sequence coverage for the 1000 most abundant proteins). Our data indicate that quantitation is indeed biased when solely relying on trypsin, affecting even the top 100 most abundant proteins, sometimes by more than a factor of 1000.

Conclusion: Our data stresses that the best proteotypic peptides are not necessarily tryptic, which also potentially impacts other quantitative assays such as selected reaction monitoring or emerging pseudo-SRM strategies.

P016

INCREASING PROTEIN IDENTIFICATIONS ON A LC-QTOF MASS SPECTROMETER

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Introduction: Efficient protein identification by LC-MS/MS depends on both the chromatographic separation of the peptides and optimized MS/MS analysis. Improved separation decreases the number of peptides presented to the mass spectrometer at any given instant and also increases the instantaneous signal for the same amount injected, yielding better detection of lower abundance species. Improved data dependent acquisition efficiently selects and fragments precursors to produce 'information rich' spectra. This work describes the combined benefit of improved LC and data-dependent MS/MS algorithms.
Methods: Experiments were performed on a nanoflow LC QTOF mass spectrometer with a multilayer polyimide microfluidic chip. Proteins from E. coli lysate were used for analysis by one-dimensional nanoflow LC-MS/MS. A 1% global peptide FDR was used for database searching of results. A new peptidic isotope cluster algorithm was implemented which improves precursor identification of the monoisotopic *m/z* and centering of the quad isolation window for optimal transmission. Precursor candidates are ranked based on their abundance and precursor "purity". Precursors that would co-isolate with other peptides, yielding chimeric peptide spectra, were filtered based on low purity score. MS/MS accumulation time was modulated to achieve a user-specified target for MS/MS TIC.
Results: Results showed a 44% increase in peptides identified and 42% increase in proteins identified (at a <1% FDR). The improved chromatography resulted in a 28% increase in peptides identified and 18% increase in proteins identified.
Conclusion: Improved precursor selection combined with improved chromatographic separation results in greatly enhanced protein identification results.

P017

PHOTO CROSS-LINKING TO EXPAND THE REPERTOIRE OF 3D PROTEOMICS.

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Introduction: Thus far, chemical cross-linking approaches have been focussed on the utilisation of homobifunctional cross-linkers, such as BS3, which are limited to reaction with lysine (as well as known side-reactions with tyrosine, serine and threonine). We have employed a diazirine photo-activatable cross-linker, which overcomes this limitation and introduce this cross-linker as a complementary tool for the chemical cross-linking approach.
Methods: Reaction conditions for cross-linking the two chains of human complement protein C3b using the photo-activatable cross-linker SDA were optimised. Purified C3b was mixed with SDA in cross-linking buffer to initiate lysine reaction with the NHS ester component of the cross-linker. The diazirine group was then activated using UV irradiation. The resulting cross-linked mixture was separated using an SDS-PAGE gel, bands were excised and the proteins reduced, alkylated and digested using trypsin following standard protocols. Cross-linked peptides were fractionated using SCX-StageTips and desalted using StageTips prior to MS analysis by ES-LCMS using an LTQ-Orbitrap and identification of cross-links using Xi software.
Results: Chemical cross-linking with BS3 is limited by the availability of two lysine side chains <11.4 Å apart. The disadvantage with using such a homobifunctional NHS-ester lysine reactive cross-linker is that both lysine residues need to be in the right conformation and near the interface for cross-linking to occur. A further key limitation is the non-homogenous distribution of cross-links in the network map of protein interactions, with subsequent areas of lacking data within that map. The advantage of using the heterobifunctional cross-linker SDA, containing only one NHS-active ester, is that only one lysine is required. The major gain of having

a diazirine derivative as the secondary photoreactive functionality is the promiscuity of the resulting carbene following UV irradiation, which inserts indiscriminately into any amino acid. The result is the creation of a much richer network map of protein interactions, in comparison with the result obtained with utilisation of the homobifunctional cross-linker BS3.
Conclusion: The diazirine photo-activatable cross-linker SDA provides complementary structural data on proteins to the highly successful NHS-activated cross-linker BS3.

P018

AUTOMATED N-TERMINAL PEPTIDE DERIVATIZATION FOR HIGH-THROUGHPUT DE NOVO SEQUENCE ANALYSIS OF THE ECTOCARPUS SILICULOSUS PROTEOME
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Introduction: PMF and peptide fragmentation have become powerful tools for the identification of proteins isolated from well known organisms. However, they often don't provide sufficient information for the analysis of proteins isolated from unsequenced organisms or the characterization of PTM's. In latter cases, de novo sequence analysis of peptides, by interpretation of fragmentation data, will increase the identification rate significantly. It has been demonstrated that interpretation of MS/MS data can be facilitated by N-terminal sulfonation in combination with guanidination of Lys side chains [1].
Methods: Proteins were extracted from brown algae tissue using phenol extraction and separated using 2D PAGE. Spots were picked randomly, derivatized and digested with trypsin on a Tecan Freedom EVO, prior to MS/MS analysis. In-gel guanidination prior to tryptic digestion was performed with O-methylisourea and sulfonation of extracted peptides was done with 4-sulphophenyl isothiocyanate (SPITC). Derivatized peptide mixtures were spotted and washed on the probe by the robot and analyzed by MALDI-TOF/TOF. *De novo* sequence interpretation was done with PEAKS and proteins were identified by homology using the FASTS and MS-Homology algorithms.
Results: The chemical derivatizations, tryptic digestion and MALDI spotting, were implemented on a robotic liquid handling system to increase sample throughput. Since MALDI MS/MS spectra contained only singly charged y-ions, automated *de novo* sequencing by PEAKS was executed fast and with a low error rate. This automated approach has been applied successfully to study the *Ectocarpus siliculosus* proteome with protein identification rates of over 50%.
Conclusion: We have demonstrated that *de novo* sequence analysis can be performed in a completely automated fashion. As demonstrated before, using a manual approach, identification rates up to 50% can be achieved [2]. Although 2D spots were picked randomly, we observed that identification was biased towards the more abundant proteins. Finally, we will prove that the *de novo* derived information can be used in a proteogenomic study of the *Ectocarpus* genome [3].
References: [1] Keough, T., *et al*. *Anal. Chem.* 75, 156A-165A (2003)
[2] Samyn, B., *et al. J. Prot. Research* 6, 70-80 (2007)
[3] Cock, J. M., *et al. Nature* 465**,** 617-621 (2010)

P019

THIRD GENERATION PROTEOMICS AND THE CHALLENGES OF PTM CHARACTERISATION
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Introduction: Proteomics has rapidly evolved from large-scale cataloguing of the protein complement of a cell (First Generation Proteomics) through quantitative characterisation of proteins and protein/protein interactions (Second Generation Proteomics) to the comprehensive quantitative study of protein dynamics and their complex post-translational modifications in space and time (Third Generation Proteomics). The latter will undoubtedly revolutionise our understanding of cellular function.
One of the most common post-translational modifications used to regulate protein function in multicellular organisms is phosphorylation. While phosphorylation sites with 100% stoichiometry are likely to be extremely important, the importance of low stoichiometry phosphorylation is not well understood and could potentially be a rapid and dynamic mechanism of fine-tuning protein activity and function. Determination of phosphorylation

stoichiometry may therefore be as significant as phosphosite mapping in our understanding of cellular function.

Methods: The SILAC approach was used to characterise changes in the proteome of MCF10A cells; control cells were grown in media containing natural abundance arginine and lysine, while treated cells were grown in media containing $^{13}C_6{}^{15}N_2$-lysine and $^{13}C_6{}^{15}N_4$-arginine. Cell lysates were mixed in a 1:1 ratio prior to sub-cellular fractionation and then subjected to tryptic digestion.
The phosphopeptides were enriched using affinity-based techniques and analysed by nano-LC-MS/MS on the LTQ-Velos Orbitrap and Q-Exactive instruments. Protein/peptide identification and quantification was carried out using the MaxQuant software package. Visualisation was enabled through PepTracker.
Results:
We have carried out the large-scale quantitative study of protein phosphorylation in human breast epithelial cell line MCF10A across the different cellular compartments (space) as cells progress from normal to malignant cells upon transformation (time).
Conclusion:
By using Third Generation Proteomics techniques, we greatly increased our coverage of the proteome and characterised changes in protein phosphorylation accompanying disease progression in two dimensions.

P021

SPATIAL PROTEOMICS: EXPLORING THE PROTEOME AT THE SUBCELLULAR LEVEL
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Introduction: The ultimate goal of proteomics is to accurately describe the protein composition of cells. Many proteomics experiments concentrate on finding as many of the proteins present in a cell as possible with little thought for their subcellular distribution. This approach, while sufficient for most purposes, fails to address the proteome's spatial complexity, i.e. the anisotropic distribution of proteins, isoforms and post-translational modifications in the different cell compartments. Alternatively, a single subcomponent (complex/compartment) is purified and analysed. However, while this approach can yield highly detailed information about a specific element of the cell, it misses the greater part of the proteome. This could be solved by using several compartment-specific purification protocols in parallel to cover the whole cell, but the resulting fractions may partially overlap due to protocol-specific purity issues. Thus, an ideal spatial proteomics fractionation protocol should yield from a single experiment reasonably pure and enriched fractions covering the main subcellular compartments. In several previously published studies, our group used a single protocol to isolate, describe and compare the proteome of the cytoplasm, the nucleus (minus the nucleolus) and the nucleolus.
Methods: We expand the original fractionation method into an integrated subcellular fractionation protocol that can yield eight different fractions corresponding to distinct cellular compartments. The composition of the fractions obtained from U2OS, HeLa, MCF10A and NB-4 cells is analyzed by western blot and SILAC proteomics (U2OS only).
Results: Western Blot analysis shows that fraction purity ranges from average to excellent (nucleolus). While not all fractions correspond to a single compartment, all are distinct and most strongly enriched for the corresponding target compartments. Importantly results vary very little between cell lines. Mass spectrometry analysis yields more comprehensive and quantitative data about fraction composition. Whenever fractions correspond to several compartments, correlation analysis with specific markers may be carried out to determine the compartment distribution of a protein.
Conclusion: This method can be combined with other techniques as part of the third generation of proteomics.

P022

PHOSPHPROTEOMIC ANALYSIS BY ION TRAP MASS SPECTROMETRY WITH COMBINED CID-ETD FRAGMENTATION
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Introduction: Mass spectrometry approaches currently used in phosphoproteomic suffer from problems represented by the sub-stoichiometric amount of phosphopeptides in complex mixtures of peptides, the low ionization efficiency of phosphorylated peptides in ESI mode, and the peptide sequencing and localization of the phosphorylation site. In this study, the performances of tandem mass spectrometry approaches, based on CID and ETD as alternative or complementary fragmentation processes, on peptides formed by a range of enzymatic digestions have been carefully evaluated.
Methods: Standard caseins were subjected to in-solution reduction, alkylation and, finally, enzymatic digestion by using four different proteases (LysC, trypsin, chymotrypsin and a combination of both). Chromatographic analyses were performed by a nano-HPLC apparatus (Ultimate 3000, Dionex), connected to a high resolution ion trap mass spectrometer, coupled with an ESI source and a chemical ionization source (Bruker Daltonics). MS/MS analysis (CID, ETD or alternating CID/ETD) was performed using unattended data-dependent acquisition mode. MS and MS/MS datasets were submitted to database searches by using MASCOT.
Results: For each enzymatic protocol the percentage of sequence coverage, including the number of identified peptides, their size and charge state, and the percentage of identified probabile phosphorylation events were determined. A systematic investigation of tandem mass spectrometry methods based on CID-only, alternating CID/ETD and ETD-only was reported for the phosphoproteomics analysis. The best results were achieved with a mix of trypsin and chymotryspin that allowed the obtainment of the highest information on the peptide sequences and phopshorylation sites, by the combined use of CID and ETD in an alternatine mode. The potential of this novel strategy has been tested by the analysis of both αS- caseins from bovine milk and non-phoshorylated peptides from bovine serum albumine as the control system.
Conclusion: The powerful capabilities of the combined use of CID and ETD as orthogonal fragmentation approaches along with the application of a more appropriate enzyme digestion protocol were demonstrated. This strategy provides an excellent platform to carry out phosphoproteomics analysis.

P024

THE DEEP PROTEOME OF YOUR MACÉDOINE AS DETECTED VIA COMBINATIONAL PEPTIDE LIGAND LIBRARY CAPTURE

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Introduction: It's hard to deny the health benefits of a diet rich in fruits. A huge amount of studies has been focused in the determination of fruit metabolite composition. However, in comparison with small-size components, proteins have scarcely been investigated even though they affect both biological as well as functional properties of food products. In fact, it has been demonstrated that proteins play an important role in food allergy and stability, they are a source of biologically active peptides, and they can provide information about product authenticity.
Methods: Fruit proteomics is limited by the two main handicaps of plant proteomics studies: the scarce database due to the lack of a sequenced genome and the difficulty in obtaining high-quality protein extracts. To the usual problems regarding biological sample proteomics, such as the high dynamic range in protein concentration, one has to add additional problems specific to plant tissues such as the presence of different plant-specific cellular components (polysaccharides, lipids, polyphenols) and secondary metabolites that can interfere with protein separation and analysis. As a consequence, new tools for the isolation of proteins from complex vegetable matrices are required. In this field, the use of combinational peptide ligand libraries (CPLLs), able to dramatically amplify the signal of low-abundance species, has been demonstrated to be an excellent tool.
Results: The capture with different CPLLs with diverse chemical properties has been employed in the search of very-low abundance protein in olive fruit, avocado and banana, three species that contain high levels of polyphenols, lipids and carbohydrates, respectively. Protein extracts were separated by SDS-PAGE, stained with Coomassie, digested, and analyzed by nanoLC-MS/MS.
Conclusion: Thanks to the use of CPLLs, 231, 1284 and 752 species were detected in olive fruit, avocado and banana respectively, an increase up to 400 % of what could be identified in the controls.

P025

EFFICIENT AND RAPID MULTIENZYMATIC LIMITED DIGESTION METHOD FOR COMPLETE PROTEIN CHARACTERIZATION.

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Introduction: This method relies on a combination of limited and multienzymatic reproducible proteolytic digestions on a purified protein, for complete sequence and posttranslational modification (PTM) characterization. It generates numerous different peptides with miss-cleavages, greatly increasing the probability of analyzing the entire protein sequence. Strong identification confidence was obtained due to multiple overlapping peptide matches with the given sequence tag and to a high number of overlapping peptides assignments.
Methods: HSA, Myoglobin, Lysosyme, Invertase and α-Amylase were digested by proteases mixtures in standard buffers and 2 enzymes mixtures were tested, consisting of Trypsin, GluC and Chymotrypsin. Each digest was analyzed by nanoLC-MS/MS. β-Lactoglobulin modifications (NAPQI addition) were induced by activated acetaminophen using an electrochemical flow-through cell.
Results: By controlling and limiting digestion reaction time, miss-cleavages were efficiently generated and the number of unique peptides was highly increased. HSA, Myoglobin and Lysozyme were identified with 100% sequence coverage (S.C.) with respectively 421, 108, and 49 unique peptides; Invertase: 75.9% S.C. - 158 unique peptides, α-Amylase: 78.5 % S.C. - 60 unique peptides. The missing sequence coverages in the last two proteins mainly correspond to sequences known to contain post-translational modifications (PTM) sites (N-glycosylation in the case of Invertase). A comparison analysis of NAPQI modified and unmodified β-Lactoglobulin clearly indicatedwhich cysteine residues were modified by NAPQI. Moreover, this simple and rapid method could also find helpful applications in protein sequence assembly of novel protein. For this purpose, a software allowing performing de novo sequencing based on these overlapping peptides data sets was created and will be presented.
Conclusion: Multienzymatic limited digestion method provides better sequence coverage and highly accurate protein and PTM characterization. The protocol improves the general "bottom-up" strategy applied for highly confident protein identification and would allow better protein characterization, even for those having PTMs, such as *N*-glycosylations.

P026

THE ARABIDOPSIS THALIANA CYCLIC NUCLEOTIDE-DEPENDENT RESPONSE – A QUANTITATIVE PROTEOMIC AND PHOSPHOPROTEOMIC ANALYSIS

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Introduction: Protein phosphorylation governs many regulatory pathways and in particular is involved in cyclic nucleotide signaling. One of the cyclic nucleotides, cyclic adenosine monophosphate (cAMP), has been shown be a second messenger in abiotic and biotic stress responses. However, not much is known about the exact role of cAMP and its role in the down-stream activation of kinases and hence cAMP-dependent phosphorylation.
Methods: We analyzed the proteomic and phosphoproteomic profiles of *Arabidopsis thaliana* callus cells treated with two different concentrations of 8-Bromo-cAMP (1 µM and 100 nM) at five different time-points (0, 5, 10, 30 and 60 minutes). We employed a comparative quantitative approach using two-dimensional gel electrophoresis (2-DE) analysis coupled with comparative analysis using Delta 2D software (DECODON). In addition, a non-gel based approach utilizing peptide separation by strong cation exchange and phosphoproteome enrichment with titanium dioxide is performed to further confirm the phosphorylation status of the cAMP-dependent phosphoproteome. Proteins were identified using tandem mass spectrometry and protein prophet and will be functionalized using protein bioinformatics tools.

Results: Preliminary results showed that treating Arabidopsis cells with 1 µM and 100 nM 8-Bromo-cAMP was sufficient to induce differential protein and phosphoprotein expressions in response to this external stimulus. However, a different pattern in the phosphoproteome profile was observed overtime between 1 µM and 100 nM cAMP treatments. To deduce differential protein expression variations, quantitative comparative analysis will be employed.
Conclusion: This study is providing further insights into biological effects of cAMP-dependent responses and link them to phosphorylation events at the systems level. It also facilitates the discovery of novel proteins and phosphoproteins directly or indirectly responsive to cAMP.

P028

WATER-SOLUBLE AND WATER-INSOLUBLE PROTEINS IN THE RAT LENS DURING AGING

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Introduction: Cataract is the leading cause of blindness in the world. The disease may be induced by PTMs following insolubilization of proteins in the mammalian lens – α-, β- and γ-crystallins. The turnover of crystallins in the lens is negligible, thereby PTMs accumulate throughout life. Such protein transformations lead to the increase of the water-insoluble protein fraction and to the lens opacification.

Methods: This report provides the data on the lens proteomic analysis of age-dependency of two rat strains – Wistar and senescence-accelerated OXYS rats. The WS and WIS lens proteins were separated; the WIS/WS ratio was measured and the relative abundances of the water-soluble and water-insoluble lens proteins were calculated. The identity of each spot was determined by MS-analysis; modified peptides were determined by MS/MS technique. This work contains the technique of the protein elution from the 2-D gels and the protein mass determination using ESI-MS.

Results: Rodents such as rats are often used to study cataractogenesis, since human lenses are difficult to obtain. OXYS rats appear to be a suitable model of cataractogenesis. It was shown that the WIS protein content increases significantly at the aged animal lenses. 2-DE maps of the young rat lenses (20 days) showed single spots for each lens protein while in 3-months-old lenses a number of modified crystallins has been observed. The analysis showed that one of the most pronounced age-dependent effects is the insolubilization of γ-crystallins, and this process proceeds faster in OXYS lenses. The PTMs identified for the proteins of interest in the 3-months-old OXYS rat, such as methionine oxidation, N-term acetylation and asparagines deamidation, can be attributed to the cataract-specific modifications.

Conclusion: The presented data on crystallins insolubilization may correspond to the lens opacification. The obtained PTMs for OXYS rat lens proteins may lead to the protein insolubilization and cataract formation.We appreciate: RFBR projects 11-04-00143, 11-0300296, FASI state contract 14.740.11.0758, grant № 11.G34.31.0045, grant NSh-2429.2012.3, RAS № 21.13, CCU.

P030

PROTEOMIC IDENTIFICATION OF OOCYTE QUALITY PROTEINS IN THE PACIFIC OYSTER CRASSOSTREA GIGAS.

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Introduction: To improve Pacific oyster seed productionobtained in controlled conditions in hatcheries, we conducted a proteomic approach to identify proteins linked to oocyte quality.
Methods: Oocyte quality was evaluated by its fertilization and developmental success until larval-D stage.Differentially expressed proteins between good and bad quality oocytes were analysed using two-dimensional electrophoresis and nano-liquid chromatography tandem mass spectrometry.

Results: Identification of 12 up-accumulated spots in bad quality oocytes revealed 9 distinct proteins. Among them, 2 proteins were previously over-expressed at the mRNA level in the *Crassostrea gigas* selected line sensitive to summer mortality. Identification of 8 up-accumulated spots in good quality oocytes revealed 6 distinct proteins. Among them was the protein PCNA, proliferating cell nuclear antigen, over-expressed in good quality oocytes and validated by immunodetection on western-blot.
Conclusion: Results obtained from this work increase the current understanding of the molecular mechanisms involved in oocyte quality in this economically important bivalve, and shed further light on the proteomic processes involved in oocyte developmental competence in this model.

P031

MASS SPECTROMETRICAL ANALYSIS OF CUTICULAR PROTEINS FROM THE WING OF HEBOMOIA GLAUCIPPE (LINNAEUS, 1758) (LEPIDOPTERA: PIERIDAE)

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Introduction: In Lepidoptera a series of cuticular protein families are known and consist of CPR with the R&R consensus sequence, CPF/CPFL, Tweedle (TWDL), CPLCP, CPG, CPAP3, BcNCP1 orthologs, 18 aa motif, CP with less than 3 AAP and dumpy family members. Although several insect cuticular genes and proteins are annotated and an arthropod cuticular database is available, mass spectrometrical data on cuticular proteins and their post-translational modifications are limited.

Methods: electron microscopy analysis ,Sample preparation for two-dimensional gel electrophoresis
Two-dimensional gel electrophoresis (2-DE),In-gel digestion, Phophatase treatment
Nano-LC-ESI-CID/ETD-MS/MS
Results: A complex wing skeleton and the cuticle of *Hebemoia glaucippe* were demonstrated. Cuticle protein 18.6, isoform A, pupal cuticle protein, cuticular protein CPR59A and two putative proteins, putative cuticular protein B2DBJ and putative cuticle protein CPG31 with two expression forms were identified. Two phosphorylation sites, T213, S214, were identified on putative cuticle protein CPG31, quinone formation was observed at Y76 on cuticular protein CPR59A probably indicating the presence of posttranslational modifications
Conclusion: cuticular proteins could be important for insecticide resistance, drought resistance or resistance against heavy metals and for butterfly aerodynamics to name a few. Studying *Hebemoia glaucippe* wing proteins systematically, we demonstrated soluble CPs primary structure, cross-linking aminoacids and post-translational modifications by a gel-based mass spectrometrical technique.
References: 1. Awolola, T. S.; Oduola, O. A.; Strode, C.; Koekemoer, L. L.; Brooke, B.; Ranson, H., Evidence of multiple pyrethroid resistance mechanisms in the malaria vector Anopheles gambiae sensu stricto from Nigeria. *Trans R Soc Trop Med Hyg* **2009,** 103, (11), 1139-45.
2. Vontas, J.; David, J. P.; Nikou, D.; Hemingway, J.; Christophides, G. K.; Louis, C.; Ranson, H., Transcriptional analysis of insecticide resistance in Anopheles stephensi using cross-species microarray hybridization. *Insect Mol Biol* **2007,** 16, (3), 315-24.

P032

MASS SPECTROMETRICAL ANALYSIS OF BILIN-BINDING PROTEIN FROM THE WING OF HEBOMOIA GLAUCIPPE (LINNAEUS, 1758) (LEPIDOPTERA:PIERIDAE)

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Introduction: The bilin-binding protein (BBP), a member of the lipocalin protein superfamily, is a blue pigment protein which was well-characterized from its crystal structure and amino acid sequence. The BBP is predominantly present in haemolymph, fat body and epidermis in the last instar larval and in the wings of the adult insect of *Pieris brassicae*. Recently it was observed that the combination of BBP and the yellow-related gene corresponds to larval color patterns in three swallowtail butterflies, but additional physiological functions remain elusive.

Methods:
Sample preparation for two-dimensional gel electrophoresis,

Two-dimensional gel electrophoresis (2-DE), In-gel digestion,

nano-LC-ESI-CID/ETD-MS/MS, Western blotting
Results: Two spots were identified from the butterfly wing as BBP (P09464) with high sequence coverages. Nitrotyrosination (Y163; as aminotyrosine) was observed and nitration was verified using immunoblotting. Additional post-translational modifications as hypusine, carboxylation, kynurenine, aminoadipic acid, were proposed.The presence of BBP-immunoreactive protein was also observed in mouse brain.The characterisation of BBP showed high sequence similarity with mouse apolipoprotein D and the findings suggest a tentative function of BBP comparable to apolipoproteins. The role of the post-translational modifications remain elusive but nitration, in analogy to nitration effects reported in literature, proposes a role for mechanoelastic proteins and protein-protein interactions.

Conclusion: The study extends knowledge on Lepidoptera bilin binding protein on wings but not on body of hebomoia glaucippe and suggests a link to mammalian BBPs based upon protein sequence and immunoreactivity. Tyrosine nitration was shown and represented by aminotyrosine, the reduction product of nitrotyrosine and verified by immunoblotting. A series of additional modifications are proposed including the presence of hypusine, an indicator of highly conserved proteins.
References: [1] Huber, R., Schneider, M., Epp, O., Mayr, I., Messerschmidt A., Pflugrath, J., Kayser, H., *J. Mol. Biol.* 1987, *195,* 423-434
[2] Suter, F., Kayser, H., Zuber, H., *Biol. Chem. Hoppe. Seyler* 1988, *369,* 497-505.
[3] Kayser, H., Krull-Savage, U., Rilk-van Gessel, R., *Insec. Biochem. Mol. Biol.* 2005, *35,* 165-174.

P033

MITIGATING FALSE BIOMARKER DISCOVERY: SAMPLE MAPPING VECTORS IN NSCLC PROTEIN BIOMARKER DISCOVERY AND VALIDATION USING SOMAMER™ TECHNOLOGY
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Introduction: Hidden preanalytical variability in biological samples used for biomarker discovery can contaminate the apparent disease-specific information in the biomarkers, and worse, prevent the translation from biomarker discovery to clinical utility. This problem can arise from differences in blood sample processing between study sites or in samples collected differently at the same study site.
Methods: To better understand the effect of different blood sample processing procedures, we evaluated protein measurement bias in a large multi-center lung cancer study using our SOMAscan™ technology, which measures over 1000 proteins across 7 logs of concentration in a single assay. These analyses revealed that perturbations in serum collection and processing result in changes to families of proteins from known biological pathways. We subsequently developed protein biomarker signatures of cell lysis, platelet activation and complement activation and assembled these preanalytical signatures into quantitative multi-dimensional Sample Mapping Vector (SMV) scores. The SMV score provides critical evaluation of the quality of every blood-based sample used in discovery and also enables the evaluation of candidate protein biomarkers for resistance to preanalytical variability.
Results: Despite uniform processing protocols for each clinic, the SMV analysis revealed unexpected case/control bias arising from collecting case and control serum from different clinics at the same academic centers, an effect that created false or bias-contaminated disease markers. We therefore used the SMV score to remove bias-susceptible analytes and to define a well-collected, unbiased training set.
Conclusion: An improved classifier was developed, resistant to common artifacts in serum processing. The performance of this classifier to detect lung cancer in a high-risk population is more likely to represent real-world diagnostic results. This work has been repeated for several other diagnostic development programs (including multiple cancers), validating the general applicability of this method, and the data will be presented. We believe this approach is generally applicable to clinical investigations in all fields of biomarker discovery and translational medicine.

P034

PROTEOMICS ANALYSIS OF CARDIAC EXTRACELLULAR MATRIX REMODELING IN A PORCINE MODEL OF ISCHEMIA-REPERFUSION INJURY

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Introduction: Following myocardial ischemia, extracellular matrix (ECM) deposition occurs at the site of the focal injury and at the border region.

Methods: We have applied a conceptually novel proteomic method for the analysis of ECM in cardiovascular tissues to a porcine model of ischemia-reperfusion injury. ECM proteins were sequentially extracted and identified by liquid chromatography tandem mass spectrometry.
Results: For the first time, ECM proteins, such as cartilage intermediate layer protein 1 (CILP-1), matrilin-4, extracellular adipocyte enhancer binding protein 1 (AEBP-1), collagen alpha-1 (XIV) and several members of the small leucine-rich proteoglycan family, including asporin and prolargin, were shown to contribute to cardiac remodeling. A comparison in two distinct cardiac regions at day 15 (the focal injury in the left ventricle and the border region close to the occluded coronary artery) revealed a discordant regulation of protein and messenger RNA levels: while gene expression for selected ECM proteins was similar in both regions, the corresponding protein levels were much higher in the focal lesion. Certain cartilage-related proteins, such as aggrecan, were only found at day 60 post injury. Principal component analysis and network inference based on more than hundred ECM proteins delineated a signature of early and late stage cardiac remodelling with TGFβ-1 signalling being at the centre of the interaction network. Notably, interactions for CILP-1, asporin or aggrecan had to be inferred from experiments on other tissues, i.e. cartilage, since these proteins are currently not in public cardiac matrix interaction databases. Finally, novel cardiac ECM proteins identified by proteomics were validated in human left ventricular tissue acquired from ischemic cardiomyopathy patients at cardiac transplantation.
Conclusion: Our proteomic analysis of cardiac ECM remodeling revealed previously unknown cardiac ECM components. This bio-signature of early and late stage ECM remodeling after myocardial ischemia-reperfusion injury may have clinical utility as prognostic markers and modifiable targets for drug discovery.

P035

PREDICTION OF THE CLINICAL OUTCOME IN INVASIVE CANDIDIASIS PATIENTS BASED ON MOLECULAR FINGERPRINTS OF FIVE ANTI-CANDIDA ANTIBODIES IN SERUM

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Introduction: Better prognostic predictors for invasive candidiasis (IC) are needed to minimize its high morbidity and mortality. We investigated whether molecular profiling of IgG-antibody response to the whole soluble *Candida* proteome could reveal a prognostic signature that may serve to devise a clinical-outcome prediction model for IC and contribute to known IC prognostic factors.
Methods: By serological proteome analysis and data-mining procedures, serum 31-IgG antibody-reactivity patterns were examined in 45 IC patients randomly split into training and test sets.
Results: Within the training cohort, unsupervised two-way hierarchical clustering and principal-component analyses segregated IC patients into two antibody-reactivity subgroups with distinct prognoses that were unbiased by traditional IC prognostic factors and other patients-related variables. Supervised discriminant analysis with leave-one-out cross-validation identified a 5-IgG antibody-reactivity signature as the most simplified and accurate IC clinical-outcome predictor, from which an IC prognosis score (ICPS) was derived. Its robustness was confirmed in the test set. Multivariate logistic-regression and receiver-operating-characteristic curve analyses demonstrated that the ICPS was able to accurately discriminate IC patients at high risk for death from those at low risk and outperformed conventional IC prognostic factors. Further validation of the 5-IgG antibody-reactivity signature on a multiplexed immunoassay supported the serological proteome analysis results. The five IgG antibodies incorporated in the ICPS made biologic sense and were

associated either with good-prognosis and protective patterns (those to Met6p, Hsp90p, and Pgk1p, putative *Candida* virulence factors and antiapoptotic mediators) or with poor-prognosis and risk patterns (those to Ssb1p and Gap1p/Tdh3p, potential *Candida* proapoptotic mediators).
Conclusion: We conclude that the ICPS, with additional refinement in future larger prospective cohorts, could be applicable to reliably predict patient clinical-outcome for individualized therapy of IC. Our data further provide insights into molecular mechanisms that may influence clinical outcome in IC and uncover potential targets for vaccine design and immunotherapy against IC.

P036

THE CANDIDA CELL SURFACE IMMUNOME DURING YEAST-TO-HYPHA TRANSITION AS A MINE OF BIOMARKERS FOR EARLY AND ACCURATE DETECTION OF INVASIVE CANDIDIASIS
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Introduction: Invasive candidiasis (IC) remains a major cause of disease and death in hospitalized patients because its early diagnosis is extremely difficult, resulting in delayed therapy and ensuing fatal outcomes. We postulated that a better knowledge of the *Candida* cell surface immunome during yeast-to-hypha transition (an important virulence attribute) in invasive infection might provide a useful biomarker panel for early and accurate detection of IC.
Methods: To address this hypothesis, we performed 2-DE followed by Western blotting with human sera, mass spectrometry and bioinformatic analyses to assess seroreactivity patterns to *Candida* yeast and hyphal cell surface proteins (Y-CSPs and H-CSPs, respectively) in IC and non-IC patients, as well as capture ELISAs to validate immunoproteomics-based results.
Results: A total of 28 immunogenic CSPs were differentially detected in yeast and hyphal forms by serum IgG antibodies from IC patients. Unsupervised clustering methods revealed two antibody-reactivity signatures that separated IC samples hybridized with Y-CSPs and H-CSPs into two discrete groups. Principal component and gene ontology analyses uncovered common and specific biologic processes in both morphological forms that were differentially triggered and IgG-targeted during the infectious process. Pairwise correlation mapping across serum specimens further highlighted that distinct biologic changes underlay commensal-to-pathogen and yeast-to-hypha transitions. Interestingly, these 28-IgG antibody-reactivity patterns could also accurately distinguish IC from non-IC patients. Supervised classification analyses identified a 3-IgG antibody-reactivity signature as the best diagnostic predictor of IC. Its discriminatory power was then validated by capture ELISAs in an independent group of IC and non-IC patients. These assays confirmed that this diagnostic signature could reliably predict the IC risk in the tested patients.
Conclusion: Our results provide a molecular signature of IC that may early and accurately discriminate IC from non-IC patients. These further enhance our understanding on pathogenic mechanisms underlying the infectious process during dimorphic transition.

P037

SPATIAL PROTEOMICS: A NEW LC-MS/MS TISSUE IMAGING WORKFLOW PROVIDING PROTEIN DISTRIBUTIONS AND THEIR IDENTITIES IN TISSUE
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Introduction: MALDI-Imaging of proteins in tissue sections represents a powerful new approach to biomarker discovery and histopathological research. However, the lack of direct identification strategies continues to be an obstacle preventing its broader use in Proteomics studies. Initial studies that utilized *in situ* digestion followed by MALDI-MS/MS analysis typically provided 5-50 peptide IDs of only 1-5 high abundant proteins.
Methods: Here we introduce a novel proteomics technology that combines the spatial information with the routine identification of proteins from tissue sections. Highly resolved protein digests are generated by applying trypsin onto two subsequent tissue sections by supersonic nebulization. One of the sections is then analyzed by MALDI imaging mass spectrometry. Peptides are extracted from the other section and submitted to routine LC-MS/MS analysis using MALDI-TOF/TOF. The identified peptide list is then matched to the image and the co-localization of 2 or more tryptic peptides confirm their protein association.

Results: We analyzed rat organs using the new Spatial Proteomics approach yielding peptide distributions at the 50-100 µm level. In brain, more than 100 peptides were identified and more than 20 proteins localized without the need for MS/MS analysis directly from the tissue. The intensity, co-localization of 2 or more peptides and the degeneracy of molecular weight of peptide-to-protein mapping were used as primary validation tools beyond significant mascot scores from peptide identification.
Conclusion: As an extension of the established top-down imaging strategy, this bottom-up Spatial Proteomics approach may facilitate the identification and simultaneous localization of a much greater number proteins than it was previously possible.

P038

SCIATIC NERVE INJURY EVOKED PROTEIN S-NITROSYLATION AND DENITROSYLATION IN THE MOUSE SPINAL CORD

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Introduction: Nitric Oxide has emerged as an important modulator of pain signaling and processing and is known to exert its influence through two primary pathways: by stimulation of soluble guanylyl cyclase and by direct S-nitrosylation (SNO) of target proteins.
Methods: To investigate potential SNO target proteins in neuropathic pain we analyzed the SNO-proteome with two methods, two-dimensional S-nitrosothiol difference gel electrophoresis (2D SNO-DIGE) and SNO site identification (SNOSID). The SNO proteome of the ipsilateral L4-L6 mouse spinal cord was analyzed at baseline in naïve mice and at 24 h after sciatic nerve injury (SNI) with/without pretreatment with the nitric oxide synthase inhibitor L-NAME.
Results: At 24h after SNI, SNO-DIGE revealed 30 proteins with increased S-nitrosylation and 23 proteins with decreased S-nitrosylation. SNO-sites were identified for 17 out of these 53 proteins with SNOSID. L-NAME pretreatment substantially reduced both constitutive and induced S-nitrosylation. For the top candidates S-nitrosylation was confirmed with the biotin switch technique which however failed to detect quantitative changes of S-nitrosylation following nerve injury.
Conclusion: The identified SNO modified proteins are primarily involved in mitochondrial function, protein folding and transport, synaptic signaling and redox control.

P039

COMPARISON OF ERYTHROCYTE MEMBRANE PROTEINS EXPRESSION LEVELS IN POLYCYTHEMIA VERA PATIENTS AND HEALTHY CONTROLS

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Introduction: Polycythemia Vera (PV) is a myeloproliferative disorder arising in a pluripotent stem cell and characterized by increased production of red blood cells independently of the normal regulatory processes of erythropoiesis, and a few cases may progress to acute leukemia. As erythrocytosis is a cardinal feature of the disease, protein expression changes in erythrocyte membrane fraction would be an ideal target to study mechanisms of the disorder. Up to our knowledge this is the first time proteomics application has been used to study erythrocyte membranes in PV patients.
Methods: Erythrocyte membrane fractions from PRV patients and healthy controls were isolated by osmotic lysis of washed erythrocytes, followed by several high speed centrifugations, and washing steps. Membrane proteins were solubilized by 4% Triton X-100. Protease inhibitors were added in each solution. Equal amount of proteins were separated on 1-D SDS-PAGE, visualized by silver staining, and intensities of proteins were compared between patient(s) and control samples. Proteins having different expression levels were cut from the gel, in-gel digested, and analyzed by LC-MS/MS (Qstar Elite). Database searches were performed by Mascot (version 2.2) against Swiss-Prot database (2011_08) to identify proteins. In parallel the whole gel lane

was cut into 10 pieces and all proteins were identified to check the efficiency of membrane protein isolation method.
Results: Proteins, separated on SDS-PAGE, were identified well as erythrocyte membrane proteins using LC-MS/MS. In total about 40 proteins could be identified. Some differences in protein expressions between PRV and healthy controls were observed. However, preliminary results show only minor quantitative differences. Results of the initial study will be presented.
Conclusion: Some minor quantitative differences only were observed between PV patients and healthy controls. As the number of samples was limited in this study, our aim is to include a larger number of PV patients and use quantitative techniques as well during the next stage of the study.

P040

IDENTIFICATION OF BIOMARKERS FOR CERVIX CANCER IN THE CERVICOVAGINAL FLUID
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Introduction: Cervicovaginal fluid (CVF) is composed of secretions originating from organs that are part of the female genital tract, including vagina, cervix, endometrium and ovaries. As a result, this fluid probably contains a wealth of information (eg. biomarkers) concerning the status of these organs. Cervix cancer is caused by an infection of the cervix with an oncogenic form of human papillomavirus (mostly HPV 16 and 18). Today several screening methods exist (Pap-smear, HPV genotyping, colposcopy) but each has its own disadvantages. The need for a specific and sensitive biomarker that can easily be harvested is therefore very high.
Methods: Six CVF samples from healthy postmenopausal women and six samples from precancerous women were run separately. The samples were concentrated and run over a 2D-LC-MS/MS proteomics platform (micro SCX, capillary RP, automated spotting on MALDI targets and identification by MALDI-TOF-TOF and Mascot). Quantification was performed by spectral counting. We used Scaffold to quantify and compare the different results. For validation by ELISA, samples from healthy and precancerous pre-menopausal women were used.
Results: After comparison of the 2x6 samples, we identified one protein that was present and absent in all CVF samples coming from precancerous and healthy women, respectively. Moreover, we also found four proteins that showed a markedly up- or downregulation in one of the two conditions (at least 3-fold). ELISA experiments on 2x7 samples from healthy and precancerous pre-menopausal women confirmed the clear concentration difference of the above mentioned 'qualitative' biomarker. Additional ELISA experiments on the other biomarkers are ongoing.
Conclusion: Although further research on higher patient numbers is needed to validate our results, the 'discovery phase' of biomarkers for identification of the precancerous state in CVF looks promising. If such a CVF biomarker(s) could indeed be put forward, applications such as combination with current screening techniques and/or development of a self-diagnosis test could be considered.

P041

THE EFFECT OF INSULIN ON PHOSPHORYLATION OF MITOCHONDRIAL PROTEINS IN HUMAN SKELETAL MUSCLE.
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Introduction: Mitochondrial dysfunction is linked to insulin resistance in skeletal muscle in obesity and type 2 diabetes (T2D). This includes impaired insulin stimulation of ATP synthesis. We have recently demonstrated that multiple key proteins involved in β-oxidation, tricarboxylic acid cycle and oxidative phosphorylation are phosphorylated in human skeletal muscle in vivo, suggesting a regulatory role for phosphorylation in these molecular processes. Here, we report the first targeted mass-spectrometry based quantification of the effect

of insulin on the phosphorylation of 185 peptides from 81 proteins in isolated mitochondria from human skeletal muscle.
Methods: Skeletal muscle biopsies were obtained from 12 healthy volunteers both before and after a 4-hour euglycemic hyperinsulinemic clamp, which raised serum insulin levels to ~900 pmol/l. Mitochondria from these biopsies were immediately isolated, lysed and trypsin digested. An equal amount of heavy labelled standard phosphopeptides was added to the digest for normalization. The peptide mix was enriched for phosphopeptides using an optimized TiO_2 protocol. Each sample was analyzed by Selected Reaction Monitoring (SRM) on a QqQ giving relative quantification, and each peptide was normalized to its standard peptide. Peptide ratios between samples obtained before and after insulin treatment were calculated for each subject.
Results: A total of 140 phosphopeptides originating from 74 proteins were each quantified in more than 3 subjects. Nine phosphopeptides increased in abundance in response to insulin, whereas five decreased (confidence level = 95%). These phosphorylation sites were present in proteins involved in import processes, beta oxidation and oxidative phosphorylation.
Conclusion: These results provide evidence that insulin in physiological concentrations causes regulation of phosphorylation of mitochondrial proteins in human skeletal muscle, and suggest the possibility that mitochondrial dysfunction in obesity and T2D is a cause rather than a consequence of insulin resistance.

P042

NEW CLINICAL PROTEOMICS APPROACHES FOR DISCOVERING BIOMARKERS: SEARCHING FOR LIVER FIBROSIS MARKERS IN HEPATITIS C PATIENTS

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Introduction: Two-dimensional gel electrophoresis (2-DE) is often used to separate plasma or serum proteins in an attempt to identify novel biomarkers. A major difficulty with this approach is due to high abundant plasma/serum proteins which limits the detection of low abundance features. To overcome this problem a novel proteomics approach was developed and used to identify new fibrosis biomarkers in patients with different stages of liver fibrosis.
Methods: Plasma samples from healthy individuals and patients with hepatitis C virus (HCV) induced cirrhosis were analysed using 2-DE over a narrow pH 3-5.6 range, a range outside the pH of highly abundant albumin, transferrin and immunoglobulins. Novel markers identified by this approach were validated across all fibrosis stages by Western blotting.
Results: 44 candidate biomarkers were revealed of which 20 were novel. Western blot analysis with newly identified biomarkers showed a consistent change with increasing fibrosis stage and were promising when compared to the markers used in established fibrosis tests.
Conclusion: This is the first time the pH 3−5.6 range has been used to separate plasma by 2-DE. This pH range is useful for discovering novel biomarkers in all diseases. The novel fibrosis markers identified by this new clinical proteomics approach may help to assess hepatic fibrosis and eliminate the need for invasive liver biopsies.
References:
Gangadharan et al., 2011, Two dimensional gel electrophoresis using narrow pH 3-5.6 immobilised pH gradient strips identifies potential novel disease biomarkers in plasma or serum. Nature Protocol Exchange, doi:10.1038/protex.2011.261
Gangadharan et al., 2011, New approaches for biomarker discovery: The search for liver fibrosis markers in hepatitis C patients, J Proteome Research, 10, 2643-50
Gangadharan et al., 2011, Discovery of novel biomarker candidates for liver fibrosis in hepatitis C patients, Manuscript in preparation
Gangadharan et al., 2010, Clinical diagnosis of hepatic fibrosis using a novel panel of low abundant human plasma protein biomarkers. Patent US 2010/0291602 A1
Gangadharan et al., 2008, Clinical diagnosis of hepatic fibrosis using a novel panel of human serum protein biomarkers. Patent WO/2008/031051
Gangadharan et al., 2007, Novel serum biomarker candidates for liver fibrosis in Hepatitis C patients. Clin Chem, 53, 1792-9

P043

A QUANTITATIVE EXPLORATION OF HEALTH AND GINGIVITIS: GINGIVAL CREVICULAR FLUID COMPOSITION

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Introduction: Gingivitis, a mild form of gum disease, affects approximately 50% of the population. Understanding the protein composition of locally derived gingival crevicular fluid (GCF), a tissue exudate and serum transudate, will help in the elucidation of the mechanisms of this disease. Our objective was to quantify changes in the GCF proteome collected from periodontally healthy volunteers (n=10) and volunteers with gingivitis (n=10).
Methods: Samples were taken at six sites per volunteer and were immediately snap frozen. Prior to assay, GCF samples were defrosted, vortexed and the supernatant was retained. All samples in each group were pooled and digested with Lys-C and trypsin. The pooled samples were labelled with iTRAQ (ABSciex) labels. The labelled samples were then combined and pre-fractionated using strong cation exchange liquid chromatography prior to analysis by LC-CID/HCD-MS/MS (Thermo Scientific LTQ-Orbitrap-Velos). HCD data was used solely for quantitation, CID data was searched against the IPI human database (supplemented with oral bacterial families) using the SEQUEST algorithm.
Results: 216 human were identified by $\geq$ 2 peptides, of which 178 were more highly abundant in gingivitis. A number of neutrophil associated proteins were greatly increased, including plastin-2, which has previously been found in both proteomic interrogation of experimental gingivitis (Grant et al) and periodontitis (Bostanci et al al) GCF samples. Increases in neuronal associated proteins were also confirmed, as has been shown in previous analysis of an experimental gingivitis derived GCF proteome (Grant et al).
Conclusion: The proteins detected may have diagnostic utility and may shed light on disease mechanisms.
References: Grant et al J Proteome Res 2010, 9, 4732, Bostanci et al al J Proteome Res 2010, 9, 2191

P044

NOVEL CANDIDATE PROTEIN BIOMARKERS FOR CISPLATIN RESPONSE PREDICTION IN NON-SMALL CELL LUNG CANCER

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Introduction: The five-year survival rate for non-small cell lung cancer (NSCLC) is still less than twenty percent in part due to treatment failure and lack of biomarkers for personalized therapy. Most NSCLC patients are treated by chemotherapy and cisplatin is still the major component of most chemotherapy regimens. However, resistance to cisplatin is a common phenomenon. Aim of this study was to identify protein biomarkers that can be used for cisplatin response prediction.
Methods: The proteomes and secretomes[1] of a series of human NSCLC cell lines with a range of IC50-values for cisplatin (1.5 – 15 µM) were analyzed by label-free proteomics based on GeLC-MS/MS and quantified by spectral counting. Significant differential expression of proteins was determined by the in-house developed beta-binomial statistical test[2]. Network and pathway analysis was performed to reveal cellular and molecular functions associated with the differential proteins. A cohort of patients with known response to cisplatin will be used for validation.
Results: In total, 2885 and 2342 proteins were detected in the cell lysates and secretomes, respectively. The proteins with significantly higher expression in the resistant cell lines were associated with response to therapeutics, while the proteins with higher expression in the sensitive cell lines were known to be involved in DNA damage repair. The most promising candidates are being validated in patient tissues and sputum by targeted mass spectrometry and antibody-based methods.
Conclusion: To our knowledge, this is the first proteomics study to identify numerous known and novel potential protein biomarkers for cisplatin response prediction in NSCLC patients.

References: This research was supported by the Center for Translational Molecular Medicine (AIRFORCE project).
[1] Piersma SR *et al.*. *J Proteome Res. 2010 Apr 5;9(4):1913-22.*
[2] Pham TV *et al.*. *Bioinformatics. 2010 Feb 1;26(3):363-9.*

P045

OPPORTUNITIES AND CHALLENGES FOR BIOMARKER DISCOVERY IN CIRCULATING CELLS

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Introduction: Currently, the contribution of protein biomarkers to define the course of treatment for cardiovascular diseases is minimal. The reasons as to why may be based in the methodology. For instance if the observed change is too general to attribute to a single disease state, or due to the induction of significance by unaccounted inhomogeneity in plasma/cell purity combined with a low sample size. Here we set out to investigate how minor, unavoidable changes in sample homogeneity can influence biomarker discovery in circulating cells and lead to false biomarker discovery.
Methods: The carefully isolated platelets for two cohorts of carefully matched cases and controls with clinical symptoms of coronary artery disease were analyzed using high-end quantitative proteomics technology (2D-chromatography, stable isotope labeling, ETD and HCD-fragmentation). For verification in individual patients we use a stable Chip-LC-MS system.
Results: Out of 2440 quantified platelet proteins, 41 were found differentially expressed using a stringent confidence interval. Despite our attempts to ensure sample equality, contamination with plasma, erythrocyte and leukocyte components became apparent and these were found to differ in single patient samples in the verification phase. To correct for this, the 41 differential proteins were correlated to the high abundant quantitative proteomes of plasma, erythrocytes and leukocytes, reducing the number of biomarker candidates for verification to a manageable number of 19. In fact more than half of the differential proteins could be marked as potential false biomarkers.
Conclusion: To deduce protein expression changes related to disease, we present how correlating our in-depth patient/control study with quantitative plasma, erythrocyte and leukocyte reference proteomes is essential to avoid taking false positive 'novel biomarkers' into the costly and time-consuming verification and validation phases.

P046

SILAC-MIX QUANTITATIVE PROTEOME ANALYSIS OF HUMAN SKELETAL MUSCLE CELLS RELATED TO GLUCOSE DISTURBED METABOLISM

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Introduction: Insulin resistance in skeletal muscle is an early event in the pathogenesis of type 2 diabetes. Approaches that give a more global picture of abnormalities in insulin resistance are useful in pointing out new directions for research and responses to several therapies.
Methods: We compared the global protein level of patients with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) using a mixture of cell lines labeled by stable-isotope labeling by amino acids in cell culture (SILAC) as internal standard for accurate and comprehensive human tissue proteome quantification [Geiger et al., Nat Methods. 2010]. Human primary skeletal muscle cells (hpSMC) derived from biopsies from M. vastus lateralis were cultivated in SILAC-medium to acquire the internal standard. Furthermore, we investigated the protein abundance during cell differentiation from

myoblast [$^{13}C/^{15}N$] to myotubes [^{13}C] *in vitro* by time-dependent sampling (control, 2, 4, and 7 days). Additionally, our approach allows the identification of the hpSMC secretome during cell proliferation and direct comparison of myokines serum samples of NGT, IGT and T2DM.
Results: Analysis of muscle protein patterns revealed decreased abundance of mitochondrial proteins and altered expression of proteins involved in glucose and lipid metabolism. Furthermore, the proteins concerned with muscle cell proliferation showed distinct expression profile in T2DM compared to healthy controls. The comparison of myokines in serum revealed several disease related expression patterns.
Conclusion: Our approach enables to compare muscle specific protein pattern, proteins involved in cell proliferation and comparison of myokines in serum.

P047

METAPROTEOME AND MOLECULAR GENETICS OF RAT MICROBIOTA REVEALS GUT SECTION RESOLVED SPECIES DISTRIBUTION AND ENZYMATIC FUNCTIONALITIES

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Introduction: The digestion of many food ingredients depends on the action of the gut microbiota that has a significant influence on the health of the host organism. The metabolic activity depends on the structure and functionalities in the microbiota. The spatial resolution of microbial consortia and the functionalities in the different gut sections of rat are mostly unknown.
Methods: Samples along the intestinal track of rats were investigated using metaproteomics, T-RFLP and 16S rRNA gene clone library. The procedures for harvesting bacteria from the gut were optimized yielding 1803 non-redundant bacterial proteins in total identified by LC-MS/MS. The samples from stomach to distal colon were analysed resulting in a spatial resolution that allowed pinpointing changes in the community of gut microbiota to specific metabolic capacities.
Results: In the mucus the Firmicutes, Bacteroidetes, Proteobacteria und Actinobacteria were dominating in the gut sections caecum, proximal colon and distal colon respectively. This is in accordance with our genomic data. The most prevalent proteins were those involved in posttranslational modification, energy conversion, amino acid metabolism and carbohydrate metabolism. In respect to the functionalities the most striking difference reveals that samples from the gut content had a higher percentage of proteins involved in amino acid metabolism then mucus samples and mucus samples were enriched in energy conversion proteins.
Conclusion: This study demonstrates that the spatial resolution both in respect to the organs but also in terms of gut content and mucus has a strong impact on the microbial community and thereby on the metabolomic processes within the microbiota.

P048

MULTISITE AAV-IGF1 GENE TRANSFER INDUCES MASSIVE MODIFICATION OF THE MUSCLE PROTEOME

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Introduction: The development of gene therapy has provided the opportunity for a potential misuse of the gene transfer in sports to increase the athletic performance. Several genes, when overexpressed, have the capacity to induce muscle hypertrophy and improve muscle function; among others the insulin-like growth factor-I (IGF-I) plays a major role [1]. In this work we describe the functional and structural effects of gene doping, based on adeno-associated virus (AAV)-mediated delivery of the IGF-I cDNA to *gastrocnemius* muscle of adult mice, by proteomic approach.

Methods: Proteomic study was performed on *gastrocnemius* muscle biopsies and from three sets of mice (control, AAV-IGF1 15 days, AAV-IGF1 30 days); the investigation was carried out by fluorescence SDS, 2D-DIGE and mass spectrometry [2].
Results: The proteomic analysis of the IGF-I overexpressing muscles revealed an increment of several structural proteins involved in muscle hypertrophy, force and velocity control; resulting in remarkable fast-to slow transition in muscle fiber types and myosin isoforms. This change is similar to the adaptation that occurs in response to increased neuromuscular activity by exercise training or mechanical loading [3]. Associated with the increase in the number of oxidative-slow fibers, IGF-I delivery influences the levels of key proteins involved in energy metabolism. At the earlier time point, enzymes controlling glycogen mobilization and anaerobic glycolysis were induced, replaced later by proteins involved in aerobic metabolism, including enzymes related to the Krebs cycle and oxidative phosphorylation.
Conclusion: By inducing hypertrophy, as profiled by proteomic analysis, IGF-I gene delivery can be considered as a doping agent in order to obtain greater athletic performance.
Acknowledgments This work was supported by grant 06B6MG from WADA to M.G. and from the Italian Ministry of University and Scientific Research (grant FIRB RBRN07BMCT to C.G.).
References: [1] Barton-Davis, E.R., Shoturma, D.I., Musaro, A., et al. Proc. Natl. Acad. Sci. U.S.A. 95:15603–15607; 1998.
[2] Alban, A., David, S.O., Bjorkesten, L., et al. Proteomics 3, 36–44; 2003.
[3] Pette, D., and Staron, R.S. Cell Biol. 115, 359–372; 2001

P049

EFFECT OF CHEMOTHERAPEUTIC AGENTS ON DNA REPAIR PROTEINS IN TWO BREAST CANCER CELL LINES

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Introduction: Recently many new drugs have appeared in clinical trials that target DNA repair enzymes to enable the selective killing of cancer cells. The overall aim of this project is to study the expression of DNA repair proteins in response to chemotherapeutic agents. Profiling of all the DNA repair proteins in a patient is central to understanding how a patient will respond to (neo) adjuvant therapy and if the patient should be treated with a targeted chemotherapy
Methods: In order to study the impact of chemotherapeutic agents on the DNA repair proteins both a benign breast cancer cell line (MCF10A) and an aggressive basal-like breast cancer cell line (MDA-MB-231) were treated with Methyl methanesulfonate and Doxorubicin. The nuclei from the cells were purified, ran on a 1D gel and analyzed on an Orbitrap XL. Protein identification and quantification was done in a label-free set-up.
Results: Over 100 DNA Repair proteins were identified and for 50 of them there are quantification data as well. The protein expression changes, found in this experiment, will be confirmed by MRM assays.
Conclusion: We have used two breast cancer cell lines, one aggressive and one benign, to catalogue protein expression changes in response to chemotherapeutic treatment and have created MRM assays covering all DNA Repair pathways for validation of the results.

P052

QUANTITATIVE LIVER PROTEOMICS FOR THE DISCOVERY OF NOVEL BIOMARKERS FOR NON-ALCOHOLIC FATTY LIVER DISEASE

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is now the most common liver disease worldwide. Given that NAFLD can progress from steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and potentially hepatocellular carcinoma, early diagnosis and accurate disease staging are primary clinical concerns.[1] The objective of these experiments was to identify novel biomarkers of NASH using apolipoprotein E knockout mice ($ApoE^{-/-}$) as an animal model of NAFLD.

Methods: ApoE$^{-/-}$ and wild type animals were fed normal chow or high fat diet (HFD) for 4, 8, 12 or 16 weeks. Membrane and cytosolic liver fractions were trypsin-digested and peptides were iTRAQ labelled and fractionated by OFFGEL before ESI-MS/MS. Peak lists were generated by MassHunter and spectral extraction performed by Spectrum Mill prior to protein identification and quantitation using Scaffold 3.0 Q+. Pathway analysis utilized MetaCore. Differential expression was confirmed independently by western blotting and immunohistochemistry.
Results: After 12 weeks of HFD, ApoE$^{-/-}$ animals had clearly developed the histological features of NASH and this time point was used for liver proteome analysis. Across biological replicates, an average of 249 cytosolic and 343 membrane proteins were identified with high stringency (0.05 FDR; ≥2 peptides), and quantified by Scaffold. Of these, 229 and 331 were identified in all animals (12; n=3/group); out of which, 43 cytosolic and 39 membrane proteins were found differentially expressed (P<0.05, two-way ANOVA). Liver fatty acid binding protein, previously found down-regulated in NASH patients[2], was amongst those found significantly down-regulated (-1.5; P=1.2E-06) in the HFD-fed ApoE$^{-/-}$ animals. Pathway analysis demonstrated enrichment in the RXR-dependent regulation of lipid metabolism (P=6.55E-05) and several lipid metabolic networks (4/10).
Conclusion: ApoE$^{-/-}$ mice fed a HFD for 12 weeks are a valid animal model of NASH. Novel candidate biomarkers for NAFLD, identified by proteomics and independently confirmed in these experiments, are currently being further tested in clinical biopsy samples.
References: [1]Moore JB (2010), Proc Nutr Soc 69:211-20. [2]Charlton M et al. (2009) Hepatology 49:1375-84.

P056

USING EXCLUSION LISTS TO DRILL DOWN INTO THE NASAL EXOSOME PROTEOME.
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Introduction: Nasal lavage fluid (NLF) is a wash of the nasal cavity, commonly used for analysing the presence of mediators related to nasal diseases. We have recently established the presence of exosomes in the NLF. Exosomes are nano-sized vesicles released by cells, which can participate in cell-to-cell communication by transferring proteins and RNA between cells. The aim of this study was to drill deep into the proteome of nasal exosomes using exclusion lists, which may provide clues as to the cellular origin and role of these exosomes in the nose.
Methods: Exosomes were isolated from two pools of NLF, each consisting of two healthy individuals. The exosomal proteins were extracted and divided into 8 chromatographic fractions before each was run on an Orbitrap Velos. Following this, two exclusion lists were applied to each pool to drill deeper into the proteome. Ingenuity Pathways Analysis (IPA), ExoCarta (an exosome protein database) and PANTHER were used to analyse the functions of the exosomal proteins.
Results: Mass spectrometry analysis of the exosomal protein identified 382 proteins in pool A (87 µg) and 451 proteins in pool B (67 µg) in the first run (identified at FDR 1%, 1 peptide). The second run yielded 30 and 50 additional new proteins respectively in pool A and B, while the third run resulted in 11 and 39 new proteins. Between the two pools, 315 proteins were common. A new database search of all generated spectra, resulted in a nasal exosome proteome of 604 proteins. PANTHER revealed that 32% of the proteins had catalytic activity. Compared to the ExoCarta database, 167 of the 604 proteins had not been identified in exosomes previously and were uniquely identified in NLF exosomes. IPA revealed that the proteins were associated with several biological functions and pathways, including cell movement, immune cell trafficking and the clathrin-mediated endocytosis signalling pathway.
Conclusion: This study shows that exclusion lists can be used on exosomal samples to drill deeper into the proteome, and lead to the identification of low abundance proteins. The baseline proteome of nasal exosomes was analysed under healthy conditions. As it was associated with normal host defence activities, this proteome may be altered under the influence of nasal diseases.

P057

ANTI-INFLAMMATORY AND CELL STRESS RESPONSE IN HUMAN OBESE SUBJECTED TO AN EXERCISE PROTOCOL

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Introduction: Physical exercise is highly prescribed for the management of obesity and insulin resistance. The aim of our study is to investigate the effect of a defined exercise protocol on the pro/anti-inflammatory and stress responses, with emphasis on the cross-talk between immune regulation and heat shock response.
Methods: A total of 600 obese and normal-weight individuals are recruited and divided into six groups: diabetic obese, non-diabetic obese, diabetic overweight, non-diabetic overweight, diabetic normal-weight, non-diabetic normal-weight. After various clinical and physical tests, the volunteers are enrolled into a defined physical exercise for 6 months. Blood samples and abdominal subcutaneous adipose biopsies are collected at 0, 3 and 6 months of the exercise program. For comparing clinical and cellular biomarkers at the baseline between the six groups as well as the effect of the physical exercise on those biomarkers, these samples are analyzed using various cellular and proteomic platforms including ELISA, Luminex, Flow cytometry, RT-PCR, Immunohistochemistry, Western blot, 2D-PAGE gel, MALDI-TOF and ESI-Orbitrap.
Results: Baseline information and time-serial data were collected and the expression levels of some systemic anti-inflammatory & pro-inflammatory responses indicators including IL-10 and TNF-α were found to be correlated with obesity and diabetes. Serum, plasma, PBMCs, and fat tissue biopsies of a first set of volunteers were investigated and have shown changes in many key inflammatory, metabolic and stress mediators after the defined exercise protocol in obese and overweight groups as compared to the normal-weight control group. These include Hsp-60, Hsp-72, GRP-78, ATF-6, TBARS and ROS. Mass Spectrometry-based plasma and PBMC profiling resulted in a list of differentially-expressed proteins which are still under validation.
Conclusion: The benefits of physical exercise on obese and/or diabetic persons are involving changes in various inflammatory and cell stress mediators. The modulation of the latter could be beneficial in the management of obesity and diabetes.

P059

FUNCTION OF BRAIN PDZ PROTEIN GIP ASSESSED BY PROTEOMIC APPROACHES

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Introduction: Glutaminase-interacting protein (GIP) is a PDZ domain-containing protein which seems to play pivotal roles in many aspects of cellular signalling, protein scaffolding and modulation of tumour growth. We have devised a proteomic methodology to identify GIP interacting proteins to get insights into its physiological role in brain nuclei.
Methods: Heterologous expression and affinity purification of human GIP. Expression and purification of recombinant human GIP tagged with 6xHis was done by IMAC as previously reported.Isolation of pure nuclear extracts from rat brain. Nuclei from rat brain tissue were prepared following a standard procedure. Soluble nuclear extracts were obtained by using a lysis buffer containing 1% TX-100 and protease inhibitor cocktail.Capture of GIP-interacting partners by GIP-Sepharose affinity chromatography. A GIP-Sepharose affinity chromatography column was built by coupling CNBr-activated Sepharose 6MB with purified human recombinant GIP, according to standard protocols. A bovine serum albumin (BSA)-Sepharose column was constructed in a similar manner and used to clear the brain nuclear extracts.Identification of GIP-interacting partners by SDS-PAGE and nHPLC-ESI MS/MS. Fractions eluted from the GIP-affinity column were analysed by SDS-PAGE. The gel was silver-stained and selected bands excised for MS analysis. Gel slices were subjected to in-gel tryptic digestion. Extracted peptides from each slice were analyzed by reversed-phase nHPLC (Agilent) coupled to a HCT Ultra ESI-ion trap mass spectrometer (Bruker). Mass spectra were submitted to MASCOT search program.

Results: Relevant protein bands were analysed by LC coupled with electrospray ionization MS/MS. Most protein hits were assigned to four different functional groups: Transcriptional Regulation, Signalling Cascades, Neurofilaments & Nuclear Membrane and Scaffolding/Membrane Transport Vesicles.
Conclusion: Tandem GIP affinity chromatography and LC-MS analysis has proven to be a proteomic methodology very efficient for searching protein interacting partners of GIP in brain nuclei. Our data support roles of GIP in transcriptional regulation and cellular signalling, in agreement with previously reported binding partners, but also suggest novel functions for this PDZ protein in brain.

P060

DIAGNOSTICALLY USEFUL ANTIBODY-REACTIVITY PROFILES IN NON-NEUTROPENIC PATIENTS WITH INVASIVE CANDIDIASIS: FROM BIOMARKER DISCOVERY TO ASSAY VALIDATION

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Introduction: To be able to diagnose invasive candidiasis (IC) in non-neutropenic patients is still a challenging task for physicians. The goal of this study was to identify and validate useful serological biomarkers for IC diagnosis in non-neutropenic patients.
Methods: Serum specimens from non-neutropenic patients with and without IC were profiled for IgG-antibody reactivity to *Candida* cytoplasmic proteins by serological proteome analysis and computational biology tools. Selected biomarker candidates were then expressed in a heterologous system and validated by prototype immunoassays.
Results: Eighteen immunogenic *Candida* cytoplasmic proteins were differentially imunorecognized by serum IgG antibodies from non-neutropenic IC patients as compared to controls. Two-way hierarchical clustering and principal-component analyses of these 18-IgG antibody-reactivity patterns discriminated IC patients from controls reliably and independently of baseline characteristics of the study population. Selected serological biomarker candidates were next validated in an independent non-neutropenic patient cohort using established prototype immunoassays. An IC diagnostic score (ICDS) was generated from this reduced antibody-reactivity signature. Receiver-operating-characteristic curve analyses revealed that this ICDS showed a good ability to discriminate IC from non-IC patients. Multivariate logistic-regression models highlighted a positive association between the ICDS and IC risk that was independent from known predisposing factors for IC and other baseline variables.
Conclusion: We conclude that if confirmed in prospective cohort studies, this serum 18-IgG antibody-reactivity signature could be valuable for early detection of IC in non-neutropenic patients. In particular, our ICDS, as measured by our prototype immunoassays, might be useful and complement blood cultures for early IC detection in these high-risk patients, since these selected serological biomarkers were detected in the absence of candidemia.

P061

ELECTRON TRANSFER DISSOCIATION DIFFERENTIATES THE ISOBARIC DEAMIDATION PRODUCTS ASPARTATE AND ISOASPARTATE

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Introduction: Biopharmaceuticals, in particular antibodies, are set to revolutionize future healthcare. By 2014 very likely 5 of the top 10 pharmaceuticals will be proteins or peptides. Degradation of protein drugs results in reduced potency and increased immunogenicity. E.g., deamidation of asparagine and isomerization into isoaspartic acid is a major cause of protein degradation and leads to protein folding disorders such as Alzheimer's disease. CID fragmentation cannot discriminate aspartate from isoaspartate residues while ETD produces a different signature backbone cleavage.
Methods: Monoclonal antibodies were incubated in 4 M guanidium Cl, ph 8, and reduced with 10 mM DTT for 15 min, followed by carbamidomethylation for 30 min. The resultant heavy and light chains were separated and purified via SEC (TSK-GEL G400SW column, Tosoh Bioscience). Proteins were trypsin-digested and the

peptides were separated by a RP-C18 (4.6 mm x 250 mm, Grace-Vydac) column and injected online to an ion trap (HCTultra ETD, Bruker). CID and ETD were performed and results compared.
Results: The known peptides containing Asp were specifically investigated for a change in mass and retention time. An increase in mass of 1Da was detected in two of the Asp-peptides in the heavy chain. The RP-LC was able to separate three related species from both peptides; these were the original Asn containing peptides and the suspected Asp and IsoAsp containing ones. Examination of the b and y type ions from CID of the suspected Asp and Iso Asp peptides conclusively confirmed the inclusion of an increased mass of 1 Da which enabled identification of the site of deamidation.
Conclusion: However, due to the isobaric nature of the peptides, CID products from the Asp and IsoAsp peptides were found to be identical; therefore CID was unable to differentiate between the aspartate isomers. Formation of isoaspartate results in an extra methylene unit in the peptide backbone. Under ETD fragmentation, the isoaspartyl peptides displayed a unique fragmentation signature (C_n + 58 Da and z_{l-1} - 57 Da) which was not observed in the Asp peptides and could be used for clear differentiation between both modifications and unambiguous identification of the iosasp peptides.

P062

IDENTIFICATION OF NOVEL CANDIDATE NEURITE OUTGROWTH FACTORS SECRETED BY UNRESTRICTED SOMATIC STEM CELLS FROM UMBILICAL CORD BLOOD

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Introduction:
Unrestricted Somatic Stem Cells (USSC) generated from cord blood are a source for the generation of induced pluripotent stem cells and for stem cell therapy. Cell culture as well as animal models revealed that these cells promote neurite outgrowth and neuronal regeneration after spinal cord injury (Schira *et al.* 2012). It has been shown that the one process contributing to increased neurite outgrowth is caused by secretion of stimulating factors. Here, we will present our approach to identify protein factors by an exhaustive secretome analysis of USSCs applying high resolution mass spectrometry in combination with nano HPLC analysis.

Methods:
Supernatant of serum-free cultured USSC were taken, cell debris were removed and proteins were concentrated using TCA-precipitation with sodium lauroyl sarcosinate. The resulting proteins were separated on a 1D-PAGE, silver stained and further analysed with RP-HPLC-MS/MS. Therefore a RSLCnano system online coupled to a LTQ Velos Orbitrap were used. For processing of RAW-Data and result files we used Proteome Discoverer and the Mascot algorithm, biofunctional clustering was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID).
Results:
In cell culture as well as in animal model neural cells showed an upregulated neurite outgrowth when incubated with secretome from USSC but not from fibroblasts and other cell types. We identified 1848 unique proteins in seven independently analysed USSC secretome. A stringent filtering based on the biological function of the proteins (Gene Ontology) extracted 726 proteins already discussed in this context. In addition to the identified growth factors and low abundance molecules like cytokines, these proteins are relevant candidates for the further functional elucidation of the process of neuronal regeneration.
Conclusion:
Our study shows the first view on the USSC secretome. We identified potential neurite outgrowth factors relevant for the therapy of SCI.
References: Schira J, Müller HW *et al.*; Significant clinical, neuropathological and behavioural recovery from acute spinal cord trauma by transplantation of a well-defined somatic stem cell from human umbilical cord blood. Brain 2012, 135 (Pt 2), 431-46

P064

CONSEQUENCES OF ENVENOMATION BY VIPERA BERUS BERUS IN URINARY PROTEIN PROFILE IN DOGS.

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Introduction: Between April and September every year many dogs in Scandinavia are bitten by *Vipera berus berus*, the only venomous snake in the area. Exposure to snake bite venom causes local and systemic symptoms and in severe cases can lead to death.
Methods: Urine samples were collected from four dogs bitten by *Vipera berus berus* and treated in the intensive care unit of the Veterinary Teaching Hospital at the University of Helsinki. The inclusion criteria were a strong suspicion of viper bite no more than two days before admission and clinical signs of a viper bite. Exclusion criteria were defined as: ongoing treatment with glucocorticoids or known history of liver or kidney diseases. Six privately owned, healthy dogs were obtained for controls. Samples were subjected to 2D DIGE analysis. Image analysis was performed with DeCyder 7.0 2D software and protein spots demonstrating minimum 1.5-fold difference in average spot volume ratios between envenomed and control dogs using Student's *t* test *p*-value of less than 0.05 were picked and identified with LC-MS/MS.
Results: In 2D DIGE analysis seven proteins were significantly ($p < 0.05$) over expressed in urine of dogs bitten by *Vipera berus berus* compared to control group. From those, five proteins were identified: beta-2-microglobulin (b2MG), alpha-1-antitrypsin (AAT), albumin, fetuin-B and superoxide dismutase (SOD1).
Conclusion: Results indicate that snake bite by *Vipera berus berus* alters the urinary protein profile in dogs. Identified proteins participate amongst other things in immune responses, regulation of proteolysis, cysteine-type endopeptidase inhibitor activity and oxidation-reduction processes.

P065

PROTEOMICS OF CORNEAL DYSTROPHIES

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Introduction: Transforming growth factor beta induced protein (TGFBIp) is a major component of the human corneal proteome. More than 40 different mutations, in the TBFBIp gene lead to various types of protein aggregates in the cornea including both amyloid deposits as well as non-amyloid deposits. Investigation of the deposit proteome of the various phenotypes may shed light on the molecular mechanism causing these corneal dystrophies.
Methods: Diseased tissue from various genotypes of TGFBIp related corneal dystrophies were isolated from paraffin embedded corneal tissue sections using a Veritas Laser Capture Microdissection system. Trypsinized samples were analyzed on either a nanoLC-LTQ-Orbitrap XL or a nanoLC-TripleTOF 5600 instrument. Data were searched against the Swissprot database using the Mascot algorithm and processed using MS Data Miner software. Spectral counting of TGFBIp peptides was performed to expose any viabilities in *in vivo* TGFBIp processing.
Results: Using Exponentially Modified Protein Abundance Index the most abundant proteins for each TGFBIp phenotype were compared across as well as to healthy corneal tissue. Mutations leading to amyloid deposits revealed accumulation of serum amyloid p component, clusterin, apolipoproteins A-IV and E and HtrA1, when compared to the non-amyloid deposits. Further, spectral counting of TGFBIp peptides suggested high *in vivo* processing of TGFBIp associated with amyloid deposits compared to non-amyloid deposits.
Conclusion: The newly obtained insight into the protein composition of the deposits associated with corneal dystrophies has provided new knowledge that may be used to explain the mechanism leading to these diseases.

P067

QUANTITATIVE PROTEOMIC STRATEGIES FOR THE DISCOVERY AND EVALUATION OF EARLY DIAGNOSTIC BIOMARKERS FOR PANCREATIC CANCER IN SERUM

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Introduction: Pancreatic cancer (PC) is the fourth most prevalent cause of cancer-related mortality globally, primarily due to its late-stage diagnosis. It is anticipated that early detection and screening of high risk patient populations may improve overall survival rates from the disease. Consequently, accurate biomarkers for PC detection are urgently required. The blood serum proteome may be an ideal source of such biomarkers, although its huge complexity necessitates novel strategies to enrich and quantitate the low-abundance proteins most likely to have utility.

Methods: For biomarker discovery, 100 pre-diagnosis PC serum samples (UKCTOCS) were pooled into 5 groups (n = 20 per group) based on time-to-diagnosis (0-6 months to 3+ yrs) and compared to a matched healthy control pool (n = 100). Initially, serum samples were immunodepleted of high abundance proteins, followed by enrichment of glycosylated proteins by multiple lectin-affinity chromatography. In addition to the immunodepleted sample, both bound and unbound lectin-affinity fractions were subjected to reduction, alkylation, tryptic digestion and sixplex TMT-labeling. TMT-labeled peptide samples were then subjected to a multi-dimensional LC strategy of high-pH RP separation (20 fractions), followed by nanoLC-MS/MS. PC and control samples were compared using LC-MS/MS based on TMT reporter ion quantitation.

Results: Proteins were ranked according to the pattern and magnitude of fold-change between groups, quality of MS data and association with disease. Statistically significant changes between PC and healthy control samples were observed for several proteins in the lead-up to PC diagnosis. For biomarker verification, selected candidates were measured by ELISA across the entire cohort. Protein expression differences due to PC were confirmed for several candidate biomarkers of the disease.

Conclusion: A proteomic workflow of multiplexed serum protein profiling was developed for the discovery of novel candidate biomarkers of PC. Further validation of candidate biomarkers will be performed using immuno- and MS-based assays.

P068

PROTEOMIC PROFILING OF RECURRENT GLIOBLASTOMA USING DATA-INDEPENDENT LABEL-FREE QUANTIFICATION

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Introduction: Glioblastoma multiforme is the most common and malignant primary brain tumor and recurrence is often inevitable. Hence, the elucidation of underlying molecular mechanisms of tumor recurrence, disease progression and resistance to therapy is important. Together with the German Glioma Network, we are characterizing molecular aberrations in pairs of primary and recurrent glioblastomas using a data-independent label-free proteomic approach.

Methods: Frozen tissue samples were homogenized and proteins precipitated using TCA, separated by short-SDS-PAGE and in-gel digested using trypsin. Peptide concentration after extraction was determined using amino acid analysis. Dried samples were redissolved and randomized before undergoing data-independent label-free ion mobility LC-MS/MS ($HDMS^E$) by an extension of previously described methods [1-3]. Essentially, analysis was performed using a nanoAcquity LC and a quadrupole-IMS-ToF mass spectrometer (Synapt G2 HDMS, Waters). ProteinLynx Global Server (v2.5; Waters) was used for protein identification and label-free quantification using yeast ADH as an internal standard. Protein quantification was also performed using Progenesis LC-MS.

Results: Twenty paired-brain samples were analysed; 10 primary tumors, and 10 matched recurrent tumors from the same patient. We identified approximately 1000 proteins per run resulting in a total number of 2962 non-redundant proteins being identified in more than one run. 392 proteins were determined in all runs

exhibiting average sequence coverage of 34.5% spanning from 6.8% to 91.3% sequence coverage. Quantitative analysis revealed 59 differentially regulated proteins with p-value <0.05, mean ratio of >2 or <0.5 and represent in at least two of three technical replicates. Applying the AMRT approach [1], we were able to absolutely quantify protein amount over a dynamic range of four orders of magnitude.
Conclusion: These investigations will help to better understand and treat glioblastoma.
References: 1. Silva, JC. Anal Chem (2005);77:2187
2. Silva, JC. Mol Cell Proteomics (2006);5:144
3. Li, G-Z. Proteomics (2009);17:1696

P069

AN ION MOBILITY-ENABLED DATA INDEPENDENT MULTI-OMICS APPROACH TO QUANTITATIVELY CHARACTERIZE URINE FROM CHILDREN DIAGNOSED WITH IDIOPATHIC NEPHROTIC SYNDROME
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Introduction: Idiopathic nephrotic syndrome (INS) is the most prevalent glomerular disease in children. Despite ongoing progress, its pathogenesis is still unknown and the therapy options are confined to gross immune modulation. A variety of methods for diagnostic and treatment purposes are available for the patients; however, the lack of understanding regarding the pathogenic mechanisms underlying INS can lead to poor therapeutic response and adverse side-effects. Here, we describe quantitative proteomic approach to reveal new molecular factors involved in pathogenesis of INS with potential diagnostic and therapeutic significance.
Methods: Urine samples were collected from 10 children diagnosed with INS receiving no therapy, and 10 healthy children. Samples were affinity depleted of albumin before digestion with trypsin. Label-free protein expression data were acquired with Synapt G2 using an ion mobility data independent approach, whereby the collision energy was switched between low and elevated energy state during alternate scans and associate precursor and product ions by means of retention and drift time alignment. Data were processed using ProteinLynx Global Server and searched against a human database. Normalized label-free quantitation results were generated using Progenesis LC-MS. In a similar fashion, diluted neat urine samples were analysed using a small molecule profiling approach. The resulting data was analysed using MarkerLynx XS.
Results: The results of this study showed a significant number of proteins to be over-expressed in the urine from INS patients. More interestingly, the majority of proteins identified as being regulated were secreted glycosylated proteins (e.g. zinc-alpha-2-glycoprotein and cerruloplasmin) in relatively low abundance. Metabolomics analysis showed some statistically significant changes in small molecule content, with homocysteine and uridine elevated, and glutamate, indoline, phenylalanine and glucose reduced in the urine of INS patients.
Conclusion: These proteins and small molecules are biomarker candidates that may prove to be useful in the monitoring and treatment of INS.

P070

SRM QUANTITATION OF LOW NG/ML LEVELS OF CALCYCLIN IN SERUM – A CANDIDATE PRE-ECLAMPSIA MARKER
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Introduction: Pre-eclampsia (PE) is a pregnancy-specific disease that complicates 2-8% of all pregnancies. It is associated with serious perinatal and maternal morbidity and mortality. We previously demonstrated that in PE placenta, calcyclin expression is significantly higher compared to controls. The sensitivity of this analysis was improved by SRM assay of trophoblast cells. Next, a method was developed to quantify calcyclin in digested sera from PE patients to evaluate calcyclin as a potential serum biomarker.
Methods: An SRM assay was developed for quantitative measurements of calcyclin in serum using two stable isotopic labeled peptides LMEDLDR and LQDAEIA (samples from 5 PE patients and 5 controls). Off-

line (SCX) fractionation of digested sera with a Luna 5µm, 150 x 2.00mm SCX column was performed. Subsequently, the samples were measured using a nanoACQUITY LC system equipped with a BEH130 C18, 1.7µm, 75µm x 250mm column connected to a Xevo TQ-S triple quadrupole mass spectrometer. The linearity, limit of detection (LOD) and limit of quantification (LOQ) of the assay were determined
Results: We observed an LOD and LOQ for LMEDLDR corresponding to a serum calcyclin concentration of 0.5 and 1 ng/ml, respectively. For LQDAEIAR the LOD and LOQ was 0.25 and 0.5 ng/ml serum, respectively. The endogenous levels of calcyclin were for both peptides in all measured samples above the LOD. The concentration of calcyclin calculated based on LMEDLDR for controls and PE patients is 23.5 +/- 8.5 and 70.3 +/- 33.7 ng/ml (p-value 0.0004), respectively and for LQDAEIAR 29.66 +/- 5.6 and 33.0 +/- 15.6, respectively. All measurements were performed in triplicate for which an average CV of 5% (range 0-13%) was obtained. The reason for the strong deviation between both peptides needs to be further investigated. In addition also the observed concentration difference will be further validated in a larger patient cohort.
Conclusion: In conclusion, we have developed and validated a method that is sufficiently sensitive and robust to quantify endogenous levels of calcyclin in serum of PE patients.

P071

IDENTIFICATION AND VALIDATION OF TYROSINE PHOSPHORYLATED PROTEINS THAT MAY BE USED TO PREDICT RESPONSE TO NEOADJUVANT CHEMOTHERAPY TREATMENT.
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Introduction: A significant proportion of patients with locally advanced breast tumours show a poor or partial response to neoadjuvant chemotherapy consisting of Taxol/AC (Adriamycin and Cyclophosphamide).There are no reliable markers that can accurately predict patients' response to this treatment. The aim of this study is to identify and validate a panel of novel phospho-proteins and their pathways which may be used to predict responsiveness to NAC treatment.
Methods: Frozen tissues collected before (core biopsies) neoadjuvant chemotherapy (LREC approved study: 04/Q0303/27) were categorised by pathological response (complete, no response and progressive disease). Lysates were enriched for tyrosine-phospho proteins and separated by 2D electrophoresis. Proteins showing consistent differences between response groups were identified by mass spectrometry (MALDI-TOF) and using the NCBInr sequence database (ProFound). Functional pathway analysis was performed using Ingenuity Pathway Analysis. Proteins specific to each response group were identified and validated further in a new cohort of NAC clinical tissues by western blot, immunohistochemistry and immunoprecipitaion techniques.
Results: Phospho-protein expression profiles were successfully established from core biopsies. Proteins involved in cell division, microtubule formation and cellular transport were identified from the different response groups. Pathway analysis suggested the TP53 and tumour necrosis factor pathways may be involved in NAC non-responsiveness. Validation in tumours showed expression and subcellular localisation of proteins varied between response groups.
Conclusion: Proteins and pathways identified by the proteomic screen showed scientific and clinical relevance to NAC responsiveness. Subsequent validation suggests protein subcellular localisation may be important in imparting response or resistance to NAC.

P072

COMPARATIVE HELICOBACTER PROTEOMICS: PROTEIN MARKERS FOR BACTERIAL COLONISATION
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Introduction: *Helicobacter pylori,* an important human pathogen, primarily infects the stomach causing gastric infections including ulcers and gastric cancer. Other members of the Helicobacter genus infect sites

throughout the GI tract. We are looking at those features that promote bacterial infection at the different sites in the GI tract.
Methods: Eight species, including *H. pylori,* from the *Helicobacter* genus were grown under standard conditions *in vitro* and total cellular proteins extracted by direct cell lysis. The soluble cellular proteins were analysed by 2D gel electrophoresis under conditions optimised for *H. pylori*. A minimum of 3 biological replicate analyses were processed. The protein profiles were compared using Progenesis SameSpots through the use of intermediate co-electrophoresis gels. Selected protein spots were identified by peptide fragment fingerprinting.
Results: Characteristic protein profiles were obtained for each Helicobacter species. Principal Component Analysis (PCA) of the cellular proteins clearly clustered replicate gels and demonstrated clustering of the bacterial species, broadly correlating with the site of colonisation (gastric and enterohepatic). The 3 gastric isolates showed more diversity than the 5 enterohepatic isolates analysed. Further mining of the data demonstrated the discrimination of the bacterial isolates according to the colonisation site at the proteome level. Selected proteins differentially expressed between the gastric and enterohepatic groups were identified to look for putative markers defining bacterial colonisation.
Conclusion: Traditional 2DGE technology remains a robust method for bacterial proteomics. Only limited genomic sequence data are available for most of the *Helicobacter* species we analysed. Nevertheless, 2DGE coupled to LC-MS identified protein species that differentiated the bacteria and can be targeted further to investigate bacterial pathogenesis. The majority (66%) of the identified proteins differentially expressed between the gastric and enterohepatic groups fell within 3 COG groups; energy production, amino acid metabolism and post-translation modification. The identification of the proteins will promote our understanding of this diverse group of bacterial pathogens.

P074

THE APPLICATION OF ATMOSPHERIC PRESSURE MATRIX-ASSISTED LASER DESORPTION/IONIZATION TO THE ANALYSIS OF LONG-TERM CRYOPRESERVED SERUM PEPTIDOME

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Introduction: The atmospheric pressure matrix-assisted laser desorption ionization/time of flight mass spectrometry (API-MALDI/TOF MS) is an attractive alternative solid phase ionization technique because of its ability to be coupled to a wide range of analyzers. Here, we describes the effects of medium and long-term storage on peptidomic profiles of cryopreserved serum samples from healthy women.
Methods: Low molecular weight peptides were purified using magnetic Dynabeads® RPC 18 either from fresh sera, or after serum storage at -80 °C for 18 months or at -20 °C for 8 years. MS data were pre-processed using newly developed bioinformatic tools and then subjected to statistical analysis and class prediction.
Results: Fresh samples showed a significant presence of peptides fragments, mostly deriving from the α and β chains of fibrinogen, which drastically decreased in samples stored at -80 °C for 18 months, and completely disappeared in the samples stored at -20 °C for 8 years. In the samples conserved for short term at -80 °C, it was observed the appearance of several new signals, such as C3f and C4a complement fragments, α1 antitrypsin, kininogen, inter-α-trypsin inhibitor heavy chain 4 and bradykinin, absent in fresh sample. Some fragments such as clusterin were observed only in long-term stored samples.
Conclusion: Many authors suggest that peptide fragments are generated from specific precursor proteins by activity of serum exopeptidases and these enzymes have been found to be active even at low temperatures. Some precautions should be applied in the use of serum samples, cryopreserved in biobanks, for retrospective studies to avoid storage-induced bias. For instance, our studies put in evidence that the long-term conservation of serum samples has a crucial effect on the serum degradome by generating peptides that could be incorrectly considered as biomarkers for pathological status.
References: Steiner WE, *et al*. Rapid Commun Mass Spectrom 2004;18:882-888.

Mangerini R, *et al*. Anal Biochem 2011; 417:174-181.
Gislefoss RE, *et al*. Scand J Clin Lab Invest 2008;68:402-409.
Villanueva J, *et al*. J Clin Invest 2006;116:271-284.

P075

PLASMA PROTEOME CHANGES IN WORKERS OCCUPATIONALLY EXPOSED TO TOBACCO SMOKE: A 2D-DIGE APPROACH

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Introduction: Environmental Tobacco Smoke (ETS) exposure is a major threat to public health. After partial smoking restriction in Portuguese hospitality venues, ETS exposure of their workers remains high. DNA damage analysis on blood cells after challenge with EMS suggested an adaptive response elicited by the previous exposure to low levels of ETS. The aim of this work was to disclose by proteomics the biological effects of ETS on the plasma proteome collected from occupationally exposed workers.
Methods: Workers of Lisbon restaurants were evaluated for ETS acute exposure, genotoxic lesions, and respiratory function. Global proteome changes were evaluated on pooled plasma samples according to average age, smoking habits, ETS exposure and level of genotoxic lesions representing four groups' conditions, each represented by three biological replicates: smokers and non-smokers exposed, or not, to ETS. Plasma samples were depleted of the 14 most abundant proteins using a multiple affinity removal system and resulting protein extract were analysed based on a 2D-DIGE strategy. Protein profiles were compared using SameSpots software. Protein identification was based on MALDI-TOF/TOF MS for tryptic peptide analysis.
Results: Among the 967 spots detected, 6.3% spots exhibited expression variation. Identified proteins are being annotated according to molecular function and associated pathways to disclose altered mechanisms induced by ETS on exposed workers. Post-translational modifications, namely oxidative ones, will be searched on several differently expressed protein isoforms.
Conclusion: This interdisciplinary study on occupational health already deliver information regarding ETS exposure at Lisbon restaurants and may contribute to a better understanding of the biological effects of ETS exposure leading to pathogenesis mechanisms preceding pulmonary dysfunction.

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P076

ENHANCE ERCR CLASSIFICATION SCORES OF FERTILITY IN CRYOCONSERVED BULL SPERM USING PROTEOMIC DATA

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Introduction: The major problem of herd is represented by reproductive management and breeding of dairy cattle to obtain high production. This factors account for large parts on costs of food production. Otherwise is important to assess the fertility of a bull breeder. Estimated relative conception rate (ERCR) is a method of evaluating relative bull fertility (1). Several proteins have been described in stallion sperm but at the moment not in sperm bull with ERCR scores(2). Aim of this study is to evaluate, through differential proteome analysis, changes in protein expression profiles of spermatozoa from bulls with high fertility (high ERCR score) and low fertility (low ERCR score) in order to identify possible protein markers to be used as indices of fertility
Methods: Bulls were classified with ERCR score and four groups were created (from very low to very high fertility) for proteomic analysis. Sperm proteins were separated by 2-DE and digitized maps from each class

analyzed with image analysis (Progenesis SameSpot). Statistically different spots (Mann-Whitney $p<0.05$) were analyzed by MALDI-TOF/TOF.
Results: Image analysis highlighted three significantly up and down regulated proteins in ERCR groups Alpha-enolase was found to be strongly up-regulated in very high fertility (ERCR++) group. Isocitrate dehydrogenase subunit alpha (IDH-a) and triosephosphate isomerase (TPI) showed highest expression in ERCR-/- group which is associated with a very low score of fertility.
Conclusion: Present study provides the first evidence for protein variations linked to ERCR values in bull sperm proteome and results will be useful for the identification of biomarkers and evaluation the level of fertility. The present data have indicated several possible candidate protein biomarkers for high and low ERCR. Further investigations will be necessary to evaluate possible use of these markers in fast screening of bull semen (by flow cytometry).
References: 1.Clay JS, McDaniel BT. 2001. *J Dairy Sci* 84: 1238-45
2.Gaviraghi A, Deriu F, Soggiu A, Galli A, Bonacina C, et al. 2010. *Vet Res Commun* 34 Suppl 1: S33-6

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P077

TRANSCRIPTOMIC AND PROTEOMIC DATA FROM BRTL MOUSE, A MODEL OF OSTEOGENESIS IMPERFECTA, POINTED TO CELLULAR MALFUNCTION AS CONTRIBUTING FACTOR FOR DISEASE OUTCOME.
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Introduction: Osteogenesis imperfecta (OI), a prototype for skeletal dysplasias, is mainly caused by mutations in the genes coding for type I collagen. OI is characterized by phenotypic variability, properly resembled in the Brtl murine model. Even if the skeletal system is primarily affected, several evidence suggested OI being a more systemic disease. We recently applied microarray and functional proteomic approaches to address the study of phenotypic variability in Brtl bone and skin. Our data suggested that the OI molecular basis was not only based on abnormal bone extracellular matrix but also on a general cellular machinery malfunction. Thus, we investigated the functional correlation existing between the differentially expressed proteins detected in bone and in skin.
Methods: Data from differential analysis of bone and skin samples were processed by the MetaCore program and hypothetical networks were generated. According to their functional occurrence in the nets as well as to literature, interesting proteins, identified by MS or added by the program to cross-link the differentially expressed proteins, were further investigated by Western blot.
Results: Even if not many proteins/mRNAs have been detected to significantly differ in expression among mutants and the wild type, a number of the program added interesting factors were present in bone and skin paths. Moreover, some factors found as differentially expressed only in skin or in bone when reciprocally added to the built paths become central hubs. The biological reliability of such crossed results was then assessed by Western blot and interesting expression patterns were delineated in the investigated tissues.
Conclusion: We demonstrated that tissue specific response is activated in OI as confirmed by the differential expression of some proteins, as GADD153 or Galectin-7, in skin versus bone, although common pathways can be modulated as well, *e.g.* Oct3/4 and Stathmin data. This study represents a further step toward the understanding of the molecular bases of the disease, and of their tissue specificity.

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S-GLUTATHIONYLATION PROFILE OF MICROGLIA STIMULATED BY AMYLOID OLIGOMERS.
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Introduction: Microglia are the resident macrophage-like cells of the central nervous system with a broad role in the brain's innate immunity and in inflammatory neuropathologies. A central role for these cells in the

onset and progression of neurodegenerative pathologies, such as Alzheimer's disease, has been assessed. Since oxidative stress is a condition associated with this disease, we investigated if treatment of microglia by amyloid beta (Aβ) oligomers could affect cellular redox balance and alter the profile of redox-dependent post-translational modification.
Methods: Murine microglial cell line BV2 were treated with 50 μM of $A\beta_{25-35}$ peptide. Glutathione was measured by HPLC equipped with an electrochemical detector. Carbonyls content was assessed by colorimetric assay with 2,4-dinitrophenylhydrazine. S-glutathionylated proteins were identified isolating glutathionylated peptides by an affinity column. Proteins were then identified by a "bottom up" approach using an LTQ-Orbitrap mass spectrometer.
Results: BV2 stimulated by Aβ oligomers showed an increase in reactive carbonyls of proteins and in oxidized glutathione while the total content of glutathione was significantly decreased indicating a steady redox unbalance. Thus we further investigated whether this scenario might change the redox-dependent S-glutathionylation profile in these cells. For this purpose, we use a combined approach of affinity labelling and mass spectrometry. We were able to indentify more than 20 modified proteins specific for the proteome of activated BV2 with respect to control cells. These differentially modified proteins belong to different functional classes, such as cytoskeletal and chaperone known to be involved in microglia activation.
Conclusion: These findings indicate that the redox unbalance induced by Aβ oligomers in microglia may cause a novel pattern of S-glutathionylation of cellular proteins, that can modulate their function and possibly switch towards new signalling pathways.

P079

ANALYSIS OF CARDIAC MITOCONDRIAL SUBPROTEOME IN A PORCINE MODEL OF ISCHEMIA-REPERFUSION INJURY
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Introduction: As mitochondrion is the principal source of reactive oxygen species, it play a key role in post ischemic processes in cardiac tissue. The aim of this study was to investigate, in the region surrounding ischemic tissue, changes in mitochondrial protein expression 3 and 15 days after ischemia-reperfusion injury (I/R).
Methods: Eleven three-month-old pigs were randomly assigned into the 3 days post-I/R (n=5) group or the 15 days post I/R group (n=6). Non-operated healthy pigs constituted the control group (n=6). Mitochondrial proteins were extracted from non-ischemic cardiac tissue surrounding the focal lesion. Protein samples were run in 1D gels, cut and digested. Tryptic peptides were separated using a nanoflow LC system on-line with an ion trap mass analyzer (Orbitrap Velos). Peptides were searched against a porcine/human database using the Mascot and X! Tandem algorithms. Quantification was based on the adjusted spectral count, and ANOVA tests were used to search for differences in protein expression between experimental groups.
Results: In total, 296 mitochondrial proteins were identified with a minimum of 2 high confidence peptides. Among the identified proteins, 72 were significantly regulated at least in one of the groups ($p>0.05$). As a probable early response to compensate the energetic charge in areas next to the ischemic lesion, an increment was observed after 3 days for proteins from the mitochondrial electron transport chain (EMTC). Proteins involved in the response against oxidative stress were also found to be upregulated. Interestingly, the protein expression pattern had dramatically changed after 15 days. A decreased expression for EMTC proteins was observed in parallel with that observed for proteins related to oxidative stress and apoptosis, suggesting a bias towards cell stabilization processes. Remarkably, the novel mitochondrial brain protein 44 (BR44) was significantly overexpressed after 3 days, and this was validated by alternative techniques.
Conclusion: Mitochondrial proteomics can be used to discriminate stage-specific protein changes after myocardial ischemia, which are important to control cell survival and injury expansion.

P080

ANALYSIS OF THE SEROLOGIC RESPONSE TO SYSTEMIC SACCHAROMYCES INFECTION IN MURINE MODEL
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Introduction: The beneficial effects derived from the consumption of *Saccharomyces cerevisiae* are well known. Nevertheless, the possible undesirable effects have not been studied due to the consideration of this yeast as a safe micro-organism. In the last 20 years, an increase in the number of the cases of infections by *S. cerevisiae* in humans has been reported [1], mainly in immunodepressed patients.
In previous studies, we have characterized a *S. cerevisiae* strain isolated from dietary supplements (D14) that showed to be virulent in a murine model of systemic infection. In the present work, we analysed the mice humoral response along *S. cerevisiae* D14 infection by immunoproteomics in order to study the interaction of this yeast strain with the host.
Methods: ICR/Swiss mice were inoculated with 10^7 cells of two *S. cerevisiae* strains, the potentially virulent strain D14 and the avirulent laboratory strain W303. Sera from inoculated mice 15 and 30 days after infectionand from non-infected mice were obtained.
Total protein extracts of *S. cerevisiae* were separated by 2D-PAGE, transferred to nitrocellulose membranes and western blot were performed using the different sera. Immunogenic proteins were identified by MALDI TOF/ TOF (Proteomic Facility at UCM-PC, Madrid).
Results: In this study, we analysed the pattern of *S. cerevisiae* immunogenic proteins. Large differences were observed depending on the *S. cerevisiae* strain inoculated. 76 immunogenic protein spots were identified from the 15 days sera of D14 infection versus 20 protein spots from the15 days sera of W303 infection.
Conclusion: The pattern of *S. cerevisiae* immunogenic proteins were compared with those obtained in previous studies of *Candida albicans* infections, showing that the humoral response of mice inoculated with D14 strain (potentiallyvirulent) shows greater similarities to *C. albicans* mice humoral response than to the response to the avirulent W303 strain.
References: [1] Enache-Angoulvant, A. y Hennequin, C. Clin Infect Dis 2005; 41, 1559-1568.
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P081

ANALYSIS OF SURFACE PROTEINS DURING HUMAN SERUM-INDUCED DIMORPHIC TRANSITION IN CANDIDA ALBICANS

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Introduction: There are a variety of diseases, from local mucous membrane infections to invasive systemic infections that are caused by *Candida albicans*. Serum induces *C. albicans* to make a rapid morphological change from the yeast cell form to hyphae. This morphological transition thought to be essential for virulence. The study of exposed surface proteins in hyphal *C. albicans* cells is of the great interest in the search for new therapeutic targets, diagnostic and prognostic markers or vaccines.
The main objective was to characterize the exposed surface proteins during human serum-induced dimorphic transition in *C. albicans* by a rapid proteomic approach based on trypsin digestion in whole cells, avoiding sample cell wall fractionation.
Methods: Cells were grown either in Lee media pH 6.7 with 10% human serum at 37°C for 6 hours. Whole hyphae cells were subjected to trypsin treatment in mild conditions in order to avoid cellular lysis. Extracted peptides were properly clean-up and analysed by a LTQ Orbitrap XL. The cell integrity after trypsin digestion was evaluated with propidium iodide stain. The analyses were performed in three biological replicates.
Results: Analysis of surface proteins in *C. albicans* allowed the identification of 61 proteins present in three biological replicates.Among these, ten i proteins related with cell wall organization (Phr1p, Ecm33p, Rbt5p, Rbt1p, Bgl2p, Ssr1p, Als3p, Pir1p, Mp65p and Ywp1p) and several proteins described as immunogenic cell surface proteins (Eno1p, Pgk1p, Met6p, etc..) were identified. Besides, more than 100 human proteins including different proteins of the complement system and several apolipoproteins were detected
Conclusion: This study allowed the identification of *C. albicans* surface proteins and human serum proteins attached to fungi cell surface showing the usefulness of this approach to study host-pathogen interaction.
References: Hernáez, M. L., et al., 2010. J Proteomics. 73, 1404-9.
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P082

MODULATION OF RAT BRAIN PROTEIN EXPRESSION DURING ACUTE AND CHRONIC FINASTERIDE ADMINISTRATION

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Introduction: Preliminary data suggest that S5AR inhibitors like finasteride (FIN) may elicit therapeutic effects in psychotic disorders(1) and Tourette syndrome(2) producing very limited side effects. Behavioral and neurochemical effects of FIN have been described in rat (3), but at the moment there are no proteomic data about effects of FIN administration on rat brain protein expression. In the present study we evaluated, by gel based and gel free methods, the impact of acute and chronic FIN treatment on global protein expression in specific rat brain areas.
Methods: Samples were subjected to 2DE. Gels were analyzed using Progenesis SameSpot. Differentially expressed spots (Mann-Whitney $p<0.05$) were identified by MALDI-TOF/TOF. Samples were also analyzed using a shotgun approach (LC–MS^E). Proteins were classified using PANTHER and STRING for generation of biological networks
Results: Image analysis highlighted several significantly up- and down regulated proteins in acute and chronic group with respect to controls. Shotgun data from nucleus accumbens also showed nine proteins unique to chronic group and 22 proteins unique to acute group. Classification of proteins and generation of biological networks are in progress
Conclusion: Present study provides the first evidence for protein variations in rat brain proteome following FIN treatment. Results indicated several possible candidate proteins modulated by FIN treatment. Further proteomic and bioinformatic investigations will be necessary to evaluate their role as possible molecular targets of drug.
References: 1.Koethe D, Bortolato M, Piomelli D, Leweke FM. 2008. *Pharmacopsychiatry* 41: 115-6
2.Muroni A, Paba S, Puligheddu M, Marrosu F, Bortolato M. 2011. *Mov Disord* 26: 2146-7
3.Bortolato M, Frau R, Orru M, Bourov Y, Marrosu F, et al. 2008. *Neuropsychopharmacology* 33: 3146-56

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P085

EFFECTS OF THYROID FUNCTION ON POSTTRANSLATIONAL MODIFICATIONS OF TRANSTHYRETIN

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Introduction: Transthyretin (TTR) is visceral protein, which is involved in the transport of thyroid hormones. The protein reveals free cysteine residues, which are subjected to multiple posttranslational modifications such as sulfonation, cysteinylation and glutathionylation. Since modifications of TTR have been associated with changes in health status, modifications might also be modulated by thyroid function.
Methods: In serum of patients with hypothyroidism, hyperthyroidism and euthyroidism (10 per group) concentration of thyroid stimulating hormone (TSH) was determined as parameter of thyroid function. Additionally serum concentration and posttranslational modifications of TTR were evaluated by ELISA and immunoprecipitation with subsequent MALDI-TOF-MS analysis, respectively. The amount of posttranslationally modified TTR was assessed as relative intensity in percent of intensity of native TTR.
Results: Study groups did not differ in age (median 39 years) but were discriminated by TSH serum concentration with 0.03 IU/mL in hypothyroidism, 2.4 IU/mL in euthyroidism and 14.2 IU/mL in hyperthyroidism. The median TTR serum concentration was 3.4 µmol/ L and did not differ among groups. However, concerning posttranslational modifications of TTR hyperthyroitic patients revealed a significant increase of relative intensity of Gly-TTR (15.5%), sulfonated TTR (21.4%) and glutathionylated TTR (7.6%) in

comparison to the hypothyroitic group (6.8%, 11.1% and 0.0%, respectively), but no difference could be detected to the euthyroitic group either (10.4%, 11.5% and 4.1%, respectively).
Conclusion: Changes in thyroid function result in significant changes of posttranslational modification of TTR, which might be associated with modulation of TTR function and should be matter of future research.

P087

THE NASAL EXOSOME PROTEOME OF HEALTH, ASTHMA AND CHRONIC RHINOSINUSITIS: A QUANTITATIVE PROTEOMICS APPROACH.

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Introduction: Nasal lavage fluid (NLF) is a non-invasive sample commonly used for assessing mediators related to nasal diseases. Exosomes are small vesicles (less than 100 nm), released by cells and present in many body fluids, participating in cell-to-cell communication. We have recently established the presence of exosomes in the NLF[1]. The aim of this study was to conduct the first quantitative proteomic study on exosomes and determine differences in the proteome of nasal exosomes from asthma, chronic rhinosinusitis (CRS) and asthma/CRS subjects.
Methods: Exosomes were isolated from the NLF of four subsets of subjects; control (n=14), CRS (n=6), asthma (n=13) and asthma/CRS (n=15). All but the CRS group were divided into 2 pools. The tryptic peptides of the extracted exosomal proteins underwent a quantitative proteomics workflow. Ingenuity Pathways Analysis (IPA) was used to analyse the composition and functions of the proteome.
Results: 45 proteins were quantified in all seven samples. IPA analysis (1.2 fold change threshold) of these proteins indicated that mucins were increased in asthma/CRS and asthma compared to healthy controls. Asthma/CRS and asthma had similar top networks, containing the functions of free radical scavenging, lipid metabolism and small molecule biochemistry. CRS displayed a distinctly different network, containing cellular movement, haematological system development and function, and immune cell trafficking. The top biological function of asthma/CRS was connective tissue disorder, while asthma was associated with organismal injury and CRS was associated with inflammatory response.
Conclusion: Proteins associated with asthma susceptibility and the airways, such as mucins, were observed in the asthma groups. The nasal exosome proteome of CRS was shown to be distinctly different to that of the asthma related diseases. This study shows that it is possible to conduct quantitative proteomics on nasal exosomes from respiratory diseases. Further investigations are needed to determine the role of exosomes in the pathogenesis of airway diseases.
References: Lasser C, O'Neil SE, Ekerljung L, Ekstrom K, Sjostrand M, Lotvall J: **RNA-containing exosomes in human nasal secretions.** *Am J Rhinol Allergy* 2011, **25:**89-93.

P088

MEASURING THE GROUP A STREPTOCOCCI SURFACE PROTEOME DYNAMICS USING DATA DEPENDENT MS/MS AND SRM-MS

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Introduction: Pathogenic bacteria can rapidly alter the composition of their surface proteome to accomplish host environment adaption and immune response evasion, a process that is critical for bacterial virulence. The goal of this project is to use a combination of mass spectrometry based proteomic techniques to quantify changes on the surface of different strains of Group A Streptococci (GAS).
Methods: To account for differences in the surface composition during different growth phases, all 10 strains used were grown to both exponential and stationary phase. In addition we included a mix of invasive, non-invasive, mutant and isogenic GAS strains. The surface proteome was enriched by a short incubation with trypsin that cleaves the surface proteins while leaving the bacterial cell wall intact. For statistical accuracy and to account for any biological or technical differences, each sample was done in quadruplicates resulting in 80 individual proteins pools. All 80 protein pools were analyzed using both shotgun MS and SRM resulting in a total of 240 MS runs.

Results: Roughly 500 proteins were identified with MS/MS and of these a subset with 20 of the highest abundant proteins accounts for 60% of the total surface proteome concentration. Looking at the distribution within these 20 high abundant proteins one protein in particular stands out, the M protein. This protein is a major virulence factor that accounts for over 50% of the total concentration of the 20 highest abundant proteins.
The dynamic composition of the surface proteome is evident when comparing bacteria in different growth phases. When growing exponentially the bacteria are actively dividing and the 20 proteins accounts for 50-60% of the total surface proteome. Comparing this to the stationary phase where the bacterial turnover is balanced, these same proteins only accounts for 30% showing how radical the bacteria can alter its own surface to fit it´s needs.
Conclusion: Our data shows that the surface proteome consists of a large collection of proteins where a small subset of proteins constitutes a major part. Further investigations will look at how these high abundant protein interacts with human proteins which would give an insight into how the GAS interact with the human host, a mechanism that is crucial for virulence.

P089

URINARY PROTEOMICS REVEALED PROSTAGLANDIN H2D-ISOMERASE, NOT ZN-ALPHA2-GLYCOPROTEIN, AS A BIOMARKER FOR ACTIVE LUPUS NEPHRITIS

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Introduction: Introduction: Although renal histopathology is the gold standard for the diagnosis and prognosis of lupus nephritis (LN), the invasiveness of renal biopsy warrants the discovery of novel non-invasive diagnostic and prognostic biomarkers.

Methods: Method: Urine samples from 10 LN patients were precipitated and analyzed by two-dimensional gel electrophoresis (2-DE). Differentially expressed protein spots were identified by electrospray ionization quadrupole time-of-flight tandem mass spectrometry (ESI-Q-TOF). PGDS and ZAG were selected to validate by enzyme-linked immunosorbent assay (ELISA) for potential biomarkers of active LN.
Results: Result: Quantitative analysis and statistics revealed 16 protein spots whose levels significantly differed between groups. These proteins were successfully identified by ESI-Q-TOF MS/MS. Among these potential candidates, differential levels of urinary Zn-α2-glycoprotein (ZA2G) and prostaglandin H_2D-isomerase (PGDS) were further validated by ELISA in another independent group of 70 subjects, including 30 active LN, 26 inactive LN, 14 non-LN glomerular diseases, and 8 healthy normal individuals. Whereas ZA2G levels were elevated in urine of patients with active LN and non-LN glomerular diseases, PGDS was elevated only in the urine of the active LN group.
Conclusion: Conclusion: Our data indicate that urinary PGDS, not ZA2G, may serve as a biomarker for active LN and may become one of the non-invasive tests to evaluate the disease activity in future management of LN.

P090

THE SERUM PROTEOME OF ATLANTIC SALMON DURING PANCREAS DISEASE

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Introduction: Salmonid Alphaviruses (SAVs) are the aetiological agent of Pancreas disease (PD) which infects farmed Atlantic salmon, *Salmo salar* L. in the marine environment. Whilst the pathology of PD is well

understood, , little is known of the humoral responses to the disease or how its pathology affects the serum proteome.
Methods: A cohabitation trial was carried out using SAV3 Trojan shredders with tissues (pancreas, heart, white and red muscle) and blood sampled 0, 2, 3, 4, 5, 6, 8, 10 and 12 weeks post challenge from fish. Lesion severity of tissues was assessed using a semi-quantitative histopathological scoring system. Protein concentrations of these were determined and pools diluted to an equal protein loading of 208µg in rehydration buffer for 2-dimension electrophoresis analysis in duplicate (equipment & reagents from Biorad Ltd, Hemel Hempstead UK) using 11cm immobilized pH Gradient strips with a pH range of 3-10. Strips were then run on SDS-PAGE gels with XT MOPS running buffer, stained in Coomassie and scanned analysis using Progenesis SameSpots 2D gel image analysis software (Nonlinear Dynamics Ltd, Newcastle, UK), to identify protein spots which were differentially expressed through time (Power >0.8 and ANNOVA significance score of <0.05 between replicate gels). Spots were excised manually and subjected to trypsin digest prior to identification via ion trap mass-spectrometry. The relationship between protein spot expression profiles and tissue pathologies was investigated via regression analysis of both data sets with a significance of <0.05 used to indicate a link.
Results: A total of 72 proteins were differentially expressed and identified via mass spectrometry. Analysis of these spots' expression profile and lesion scores of tissues were used to differentiate between proteins which either leaked from tissues due to pathological damage such as creatine kinase or secreted as components of the innate immune system (eg complement).
Conclusion: This study has identified potential biomarkers of PD which associate with differing stages of disease progression.

P091

PROTEOMIC AND BIOCHEMICAL INVESTIGATION OF BOVINE NASAL SECRETION
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Introduction: Bovine nasal secretion has not been well characterized but is likely to provide useful indicators of immune function and status. The aim of this study was to establish a proteomic and biochemical analysis of normal bovine nasal secretion, with a view to identification of biomarkers for immune function of the nasal mucosa.

Methods: Nasal secretions were collected from thirty eight clinically healthy Holstein-Friesian cows aged 2-5 years on the University of Glasgow Cochno Farm. The nasal secretion proteome was separated with 2-dimension electrophoresis followed by SDS-PAGE on 4-12% polyacrylamide gels and stained with Coomassie blue. Protein spots were excised and subjected to tryptic in-gel digestion. Samples were then analysed using MALDI-TOF/TOF mass spectrometer. Analysis of the MS and MS/MS spectral data was performed using *SwissProt* database version *57.15*. The nasal secretions were also examined for biochemical composition using an automatic biochemical analyzer, protein concentration using a Bradford assay and IgA and IgG concentrations using specific ELISAs.

Results: Protein concentrations in bovine nasal secretion ranged from 8.21 to 33.7 g/L. Seven major proteins were identified. Serum proteins: albumin, fibrinogen, apolipoprotein A1 and serotransferrin were identified in the nasal secretion. In addition, lactotransferrin, an anti-bacterial protein known to occur in secretions such as milk and saliva, odorant binding protein, known to be involved in scent recognition and glutathione-S-transferase, an enzyme capable of detoxifying noxious compounds were putatively identified. Alkaline phosphatase (ALP) activity was significantly higher in all samples, mean of 1193 ± 500 U/L compared to the reference range for bovine serum which is 20-280 U/L (laboratory reference range). Other biochemical analytes were within or near the reference range for serum. The concentrations of IgA and IgG in nasal secretion were between 0.45 to 1.82 g/L and 0.17 to 1.88 g/L respectively.

Conclusion: The proteomic, biochemical and immunological results from nasal secretion samples were analyzed to establish a baseline parameter of normal bovine nasal secretion. This will enable the development and validation of biomarkers in this fluid to study the pathophysiological responses of the host against respiratory diseases.

P092

PROTEOMIC SCREEN FOR GHB EXPOSURE IN HUMAN THP-1 CELLS

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Introduction: Gamma hydroxybutyric acid (GHB) is a natural neurotransmitter found in the brain at low concentrations. GHB has been used in general anaesthesia and is currently used to treat narcolepsy and alcoholism. The abuse of GHB, especially in date rape sexual assaults, has increased in recent years. GHB has a rapid rate of metabolism, causing it to disappear within 12 hours, and criminal cases are often difficult to prosecute. This study is aimed at extending the window of detection of GHB beyond 12 hours by measuring the GHB-dependent proteomic changes in a monocytic blood cell line.
Methods: Two-dimensional gel electrophoresis was carried out on whole cell extracts to determine the effect of 10 µM and 900 µM GHB concentrations on the proteomic profile of human THP-1 monocytic cell line after 24 hr exposure to GHB drug. After digestion of the spots with trypsin, electrospray ionization ion trap mass spectrometry (ESI-TRAP) was used for the identification of altered spots.The induction or repression of these proteins was validated by Western blotting using specific antibodies.
Results: A total of 21 spots out of 903 spots analyzed appear altered by at least 2-fold. 9 of these were over-expressed and 12 were under-expressed after treatment with GHB.The output revealed that several of the altered proteins are glycolytic enzymes.
Conclusion: The results reveal that GHB induced changes in protein expression in blood THP-1 cells and these results may be useful in identifying markers for GHB exposure that can be used in forensic toxicology.
References: 1. Larson et al, 2007. *J. Anal. Toxicol*. 31: 15-22. 2. Kemmel et al, 2010. Physiol Genomics *41:146-160.* 3. Schnackenberg et al, 2010. Neuroscience. 170(2):523-41.

P093

QUANTITATIVE PROTEOMICS OF GINGIVAL CREVICULAR FLUID: HEALTH VS MILD AND SEVERE PERIODONTITIS

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Introduction: Periodontitis is an inflammatory disease which affects approximately 50% of the population and is linked to several other inflammatory diseases including coronary heart disease.
Gingival crevicular fluid (GCF) is a tissue transudate and has been used for disease biomarker analysis for several years. This study aims to compare protein abundance of GCF from healthy volunteers and volunteers with mild and severe periodontitis pre- and post-treatment.
Methods: GCF was collected from three groups: periodontally healthy volunteers (n=10), and those with mild (n=10) and severe (n=10) periodontitis. GCF was collected from six sites per volunteer and immediately frozen. Samples were taken pre- and post-periodontal therapy, providing a total of five groups. Samples in each group were pooled and digested with Lys-C and trypsin before labelling with an iTRAQ 8-plex. The sample was pre-fractionated using strong cation exchange (SCX) liquid chromatography prior to analysis by LC-CID/HCD-MS/MS (Thermo Scientific LTQ-Orbitrap-Velos). HCD data was used solely for quantitation, CID data was searched against the IPI human database (supplemented with oral bacterial families) using the SEQUEST algorithm.
Results: 216 human and 4 bacterial proteins were identified by $\geq$2 peptides. Bacterial proteins identified were either *P.gingivalis*- or *T.denticola*-derived. Of the proteins identified 189 and 192 were more abundant in mild and severe periodontitis respectively. Of these 148 and 169 were observed in lower abundance post-treatment.
Conclusion: The data shows that multiple proteins are observed in greater abundance for patients with both mild and severe periodontitis compared to health. Many of these proteins decrease in abundance post-treatment and could be used as potential biomarkers for disease progression or therapeutic outcome measurement.

P094

RESPONSES OF THE 14-3-3-BINDING PHOSPHOPROTEOME TO INSULIN/IGF1 AND SMALL MOLECULE INHIBITORS OF PI 3-KINASE, MTORC1, AND MTORC2

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Introduction: The PI 3-Kinase/mTOR signalling pathway plays central roles in development, cellular responses to stimuli such as insulin and growth factors, and cancer progression. Small molecule inhibitors of pathway components have been developed that are showing clinical promise as cancer therapeutics.Rapamycin, which inhibits the mTORC1 complex, is already being used clinically, while other compounds used in this study also have a potential in cancer therapies. Here we deploy quantitative proteomics, bioinformatics analyses and biochemical experiments for the dissection of the PI 3-kinase/mTOR pathway to reveal that this signalling pathway induces the phosphorylation of large and overlapping sets of proteins, which are then captured by phosphoprotein-binding proteins named 14-3-3s.
Methods: Starved HEK293 cells were exposed to insulin and various inhibitors. 14-3-3 binding proteins were captured using 14-3-3 affinity chromatography, tryptic in-gel digested followed by dimethyl labelling with heavy and light formaldehyde respective to their stimuli. The peptide mixture was analyzed using a nano-LC coupled to an Orbitrap mass spectrometer. Data was quantified using MSQuant.
Results: Our screens identified hundreds of novel and well known proteins binding to 14-3-3 proteins under insulin stimulation. A bioinformatics analysis clustered these data and compared it with previously published datasets on high-throughput 14-3-3 binding phosphoproteomics experiments and well-defined 14-3-3 binding phosphorylated sites. Several proteins with the highest +/- insulin scores in our experiment have already been demonstrated in literature to be phosphorylated in response to insulin/IGF1 and then binding directly to 14-3-3, with 14-3-3 binding sites identified in most cases. The analysis of samples treated with different inhibitor/insulin combinations revealed very different responses of the 14-3-3 interactome. Whereas the addition of rapamycin showed only small and PI-103 moderate changes in the 14-3-3 binding proteome, we could observe dramatic changes with Ku-0063794.
Conclusion: This study opens up the way for global high throughput identification and quantitation of novel biomarkers indicative for diseases such as cancer, diabetes and neurodegenerative disorders in mammalian cells.

P095

USING MULTIPLE REACTION MONITORING TECHNIQUES AS AN ALTERNATIVE TO ANTIBODY BASED ASSAYS FOR THE VALIDATION OF BIOMARKERS OF PANCREATIC CANCER.

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Introduction: There remains an urgent need for the earlier detection of pancreatic cancer to improve patient prognosis. We have used proteomic techniques to identify potential blood-borne biomarkers and using immune-based assays have validated several potential biomarkers. However validation of some promising candidates has proved impossible due to the lack of suitable antibodies, hence we have sought to use Multiple Reaction Monitoring (MRM) - mass spectrometry as an alternative validation strategy.
Our aim is to establish MRM for the detection and quantification of candidate serum biomarkers of pancreatic cancer.
Methods: iTRAQ discovery data were used to select fully characterised peptides that generate optimal MS responses for the candidate biomarkers, with unique MRM transitions chosen for each peptide. Standard curves were generated using synthetic peptides spiked in dilute human serum with corresponding stable-isotope labelled peptides utilised as internal standards. The use of micro, rather than nano flow rates was also investigated, with the objective of shorter run times whilst retaining the sensitivity to detect the candidate peptides in un-depleted serum. Our initial validation set contained 20 serum samples from each of our

pancreatic ductal adenocarcinoma groups (patients with/without biliary obstruction), chronic pancreatitis patients and healthy controls samples.
Results: Preliminary data showed each synthetic peptide was detected in un-depleted serum using a 12min gradient (100µl/min) over a linear range of 0.25-100 fmole (on column). Subsequent analysis of digested serum from our patient cohorts demonstrated this sensitivity was sufficient to detect the candidate biomarker peptides.

Conclusion: MRM method development is proving useful as an alternative validation technology when a suitable antibody is not available.

P096

COMPARISON OF NANO AND NARROW BORE LIQUID CHROMATOGRAPHY SYSTEMS FOR THE DETECTION OF LOW-LEVEL BIOMARKERS IN BIOLOGICAL FLUIDS BY MRM
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Introduction: Detection of low-abundance proteins in biological samples is usually hampered by limited sensitivity and dynamic range of mass spectrometers. In the Selected Reaction Monitoring (SRM) mode, the dynamic range problem is alleviated, as target parent and fragment ions are both selected in a narrow mass range window, filtering out other highly abundant peptide ions. Nonetheless, SRM analysis of weakly concentrated molecule results in low ion currents and intrinsic sensitivity of the instrument may then become the limiting factor. Injecting higher amounts of analyte could thus in this case help to improve the detection of species present at very low level in a complex matrix.
Here, we present the comparison of nano and narrow bore LC-MS platforms for the analysis of biomarker candidates in serum samples in terms of sample capacity, robustness, limits of detection and quantification.
Methods: Serum samples were loaded either on a 75µm I.D. capillary column coupled to a nanospray source, or on a 2mm I.D. column coupled to an ESI source. A 300µm I.D. C18 precolumn was used in front of the nanocolumn. A set of proteins was monitored by SRM on a 5500QTrap™ mass spectrometer.
Results: Increasing amounts of serum samples were injected to determine the optimal loading capacity of each chromatographic system. The results show that ~100 times more material has to be injected on the narrow-bore column than on the nanocolumn to measure identical peak intensities. Interestingly, the loading capacity of the nanoLC system is peptide dependent and higher amounts of material can be injected for the detection of peptides with higher retention times. Both systems show equivalent robustness. The limit of detection by SRM of candidate biomarkers spiked at different concentrations in serum was determined. The results indicate that the loss of sensitivity of conventional HPLC compared to nanoLC is balanced by the higher amount of sample that can be injected.
Conclusion: Narrow-bore HPLC is an interesting alternative for the detection of low-abundance species, as long as the absolute quantity of starting material does not represent a limiting factor.

P097

CHARACTERISATION OF MRFAP1 INTERACTIONS AND LOCALISATION
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Introduction: NEDD8 is a small ubiquitin-like peptide which can be conjugated to proteins and act as a post-translational modification to mediate altered protein function. Recently a small molecule inhibitor of NEDD8 conjugation (MLN4924) was generated and it is currently in Phase 1 clinical trials for the treatment of acute myeloid leukaemia (AML), melanoma and other non-haematological malignancies. However, detailed analysis of the biochemical effects of this inhibitor remain to be elucidated.

Methods: We have performed a quantitative proteomics study using stable isotope labelling of amino acids in cell culture (SILAC) to identify proteins regulated downstream of the NEDD8 pathway, utilising MLN4924 as a specific inhibitor of NEDD8 conjugation.
Results: Our SILAC-based analysis highlighted that the abundance and stability of the protein MRFAP1 is increased after NEDD8 inhibition. Characterisation of the interaction partners of each of these proteins showed binding of MRFAP1 to a number of E3 ubiquitin ligases, including EDD1. In addition, we observed that MRFAP1 may regulate the ability for MORF4L1 to interact with chromatin modifying enzymes, such as those in the NuA4 complex, by binding in a mutually exclusive manner with MRGBP. Analysis of MRFAP1 human tissue expression by immunostaining with a validated MRFAP1-specific antibody revealed it was detectable in only a small number of tissues. Strikingly, analysis of the seminiferous tubules of the testis showed highest nuclear staining in the spermatogonia and much weaker staining in the spermatocytes and spermatids. MRGBP was inversely correlated with MRFAP1 expression in these cell types, indicating a gradient of MORF4L1 activity may exist as cells progress through meiosis in the testis.
Conclusion: These data suggest that MRFAP1 may have a potential role in spermatogenesis, particularly the process of radical histone modification and chromatin remodelling. A greater understanding of proteins involved in spermatogenic development may provide valuable insight into the aetiologies of testicular tumours and infertility.

P098

PROTEOMIC ANALYSIS OF CIRCULATING PLASMA MICROPARTICLES IN B-CELL LYMPHOMAS
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Introduction: One of the major difficulties in large scale proteomic analysis (crude extracts, blood samples…) concerns the complexity of the sample and the large dynamic range of protein abundances. To overcome this problem, one solution is to add prefractionation steps or to work on subproteomes in order to enrich the sample in proteins of interest. Membrane proteins are of particular interest for routine diagnostic in hospitals, as they can easily be used for immunological validation.
Methods: In the present work, we investigate circulating plasma microparticles (MPs) as potential biological material enriched in membrane proteins. MPs are small vesicles that are naturally generated by cells. Our proteomic approach consists of 1D SDS PAGE gel separation, followed by optimized nanoLC-MS/MS and protein identifications in databases. To ensure maximal confidence, the identifications were validated by two search engines (Mascot and Omssa).
Results: In a previous work, we focused on artificially induced MPs (not circulating ones) originating from either lymphocytic cell cultures or from patients suffering from different B-cell lymphomas. This former study i) showed that induced MPs preparations are enriched in plasma membrane proteins compared to the classical preparation(1) and ii) allowed us to identify new specific B-Cell lymphoma biomarkers, among which the CD148 for mantle cell lymphoma(2). As circulating MPs are naturally present in blood, we hypothesized that they could originate from specific tumor cells and contain relevant biomarkers. The present work aims at evaluating circulating plasma MPs as a source of biomarkers. Our results enlighten for the first time that the amount of circulating plasma MPs is compatible with proteomic analyses, as we have already identified a few hundred proteins.
Conclusion: Among all those proteins, discussions will focus on plasma membrane proteins and especially on clusters of differentiation, which could be of major interest for diagnosis and prognosis purposes.
References: 1. Miguet L, et al. 2006. *Proteomics* 6: 153-71
2. Miguet L, et al. 2009. *J Proteome Res* 8: 3346-54

P099

PROTEIN PROFILING IN COLORECTAL CANCER (CRC) USING LABEL-FREE ANALYSIS
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Introduction: Colorectal cancer is prevalent in asymptomatic people of 50-75 years old and is associated with poor prognosis. Polyps in the colon or rectum are considered as a precancerous situation in CRC carcinogenesis schema. While early diagnosis of CRC is associated with enhanced survival rate, there is an urgent need for specific biomarkers for CRC detection. The aim of this work is to build up a library of potential protein markers of CRC for evaluation using AIMS and future validation by targeted proteomics
Methods: A valuable biological collection in the field of CRC was constituted and located at the Henri Mondor Hospital. Sera from 3 conditions were tested: normal colonoscopy (N), patients with 1 polyp of 1 cm or more (A) and patients with invasive carcinoma (K). Samples were submitted to immuno-depletion and enzymatic fragmentation. Sample from each condition were injected 8 times in the chromatography and detected by the LTQ-Velos Orbitrap.
Raw data files were submitted to label-free comparison using the Progenesis LC-MS program. After alignment of LC-MS runs, features detection, and statistical analysis, LC-MS/MS spectra were searched using Mascot against the Human SwissProt database with a reversed decoy database (for FDR calculation). Final analysis was performed using Cluster 3.0 and Ingenuity Pathway Analysis (IPA) to identify enriched functional-related protein groups which are involved in the colorectal carcinogenesis.
Results: LC-MS/MS analysis allowed identification and quantification of 852 proteins. Based on N as control reference, 80 discriminative proteins (5% FDR, Fold 2.0) were quantified (A/N=40 K/N=77 K/A=27). The main biological pathway of these proteins was “Cell Death, Cellular development, Growth and Proliferation”. The majority of these proteins interact with P53.
Conclusion: Using Label-free Quantification, we are now able to explore the serum proteome of CRC patients in greater depth. Cellular growth and proliferation are stimulated early in carcinogenesis. Common cancer associated proteins and CRC biomarkers are detected in our analysis. Potential protein biomarkers are now under evaluation in different clinical situations including patients with normal and abnormal colonoscopy.

P100

BREAST CANCER TUMOUR TRANSFORMATION FROM PRIMARY TUMOUR TO SECONDARY SITE

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Introduction: Breast cancer is a very heterogeneous disease and some patients are cured simply by surgical removal of the primary tumour while other patients suffer from recurrence and spreading of the disease. A number of treatment predictive factors have been identified such as tumour size, estrogen (ER) and progesterone (PgR) receptor status and human epidermal growth factor receptor 2 (HER2) status. Lymph node involvement is also assessed during surgery to determine if the tumour has started spreading and thus determine if lymph node stripping is required. The predictive factors assessing the nature of the tumour are all based on the status of the primary tumour. However, it could be anticipated that the cancer cells undergo a molecular transformation allowing the spreading to a secondary site. If the lymph nodes are positive for cancer cells or if distant metastases are identified, this disease would likely be more successfully treated by assessing predictive markers characterizing the cells having undergone spreading.
Methods: We are analysing a unique tumour material comparing a set of 18 primary breast cancer tumours with matched axillaries positive for breast cancer cells and a set of 20 primary tumours with matched distant metastases spread to different sites in the body to further understand the molecular changes during the spreading and identify novel predictive markers. Protein glycosylation is predominant in both membrane proteins and secreted proteins. Importantly, changes in glycosylation of these proteins have been shown to correlate with cancer states. Glycoprotein capture was used in this study to selectively isolate and quantifies N-linked glycopeptides from mixtures of glycoproteins. The captured glycoproteins were digested into peptides, and subjected to mass spectrometry analysis.
Results: Protocols for glycoprotein and glycopeptide capture were tested and optimized and it was concluded that the glycoprotein capture gave the most satisfactory results with 50 proteins identified in one single analysis. We are now processing all above samples with optimized workflow for glycoprotein analysis.
Conclusion: Glycopeptide capture is a highly sensitive and specific method that will allow IHC analysis of the tissue but also allow affinity capture to be used to analyze for biomarkers in serum.

P101

QUANTITATIVE PROTEOMICS ON NASAL LAVAGE FLUID FROM ASTHMA PHENOTYPES

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Introduction: Asthma is a complex disease composed of many phenotypes with different underlying mechanisms. In line with the united airways concept, identification of proteins in nasal lavage fluid (NLF) could reflect the mechanisms in the lung. This study aimed to explore differences in mechanisms between asthma phenotypes using a quantitative mass spectrometry approach to study the NLF proteome.
Methods: NLF was collected from 3 groups of asthmatics; aspirin intolerant asthma (AIA, n=9), multi-symptom asthma (MSA, n=9), and MSA with chronic rhinosinusitis (CRS, n=9), and a group of healthy subjects (n=9). Samples were randomised into 9x6-plex experiments with 1 sample from each group and a common reference pool, for normalisation purposes, in each set. Tryptic peptides of the extracted proteins were labelled with 6-plex tandem mass tag reagents and after SCX fractionation were subjected to nano LC-MS/MS. The resulting spectra were searched against the human Swiss-Prot database using Mascot algorithms in the Proteome Discoverer software. Peptides were included if they had a ≤ 1% false discovery rate. Ingenuity Pathways Analysis was used to identify global proteome differences.
Results: In all, 474 proteins could be identified and quantified in at least 3 samples per group. In total, 93 proteins in AIA, 113 proteins in CRS and 75 proteins in MSA met a fold change cut off of 1.3 compared to healthy. Of these, 21 proteins were found in all asthma phenotypes, which could reflect common inflammatory processes in asthma, while 33 proteins were unique to AIA, 56 to CRS and 26 to MSA. Analysis of the global proteomes revealed mechanistic differences between the phenotypes. CRS was more associated with epithelial associated conditions than AIA and MSA. AIA was more associated with carbohydrate metabolism than CRS and MSA. MSA had more proteins associated with cellular movement and signalling.
Conclusion: Evidence of inflammatory processes can be found in different asthma phenotypes compared to healthy individuals and a comparison between asthma phenotypes revealed mechanistic differences. These results indicate that relevant mechanisms can be detected in NLF from different asthma phenotypes using a quantitative proteomics approach.

P105

A NOVEL CLINICAL PROTEOMIC TECHNIQUE FOR PLASMA BIOMARKER DISCOVERIES BASED ON PROTEOMIC REACTOR USED TO STUDY PLASMA PROTEOME OF KUWAITI DIABETIC PATIENTS.

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Introduction: The high incidence of diabetes and obesity in Kuwait has prompted a national campaign to combat them by establishing a high calibre education and research institute. A clinical research program has been established to investigate the effect of exercise on obesity and diabetes in Kuwait.
Methods: In this study we are attempting to investigate the effect of exercise on diabetes and obesity and to discover novel biomarkers that can generate future drug targets. To do so, we have developed a method that combines the centrifugal proteomic reactor published by Zhou et al. and the use of the exclusion list generated by Proteome Discoverer software to analyze plasma proteome. Briefly, we used strong cationic exchange beads to digest and fractionate 5ul of human plasma into 10 different fractions eluted at different pHs. The Different fractions were further separated by reverse phase and analyzed using the LTQ-Orbitrap velos. Database searches were done by Proteome Discoverer, which uses Sequest and Mascot engines to identify peptides at 1% false discovery rate.
Results: 230 Proteins were identified from the first run, which was dominated by the major plasma proteins. Peptides identified for the major proteins were used to generate an exclusion list containing about twenty thousand ions. This list was then integrated in the xcalibur method before reanalysis of the same fractions by MS leading to identification of 979 proteins. Combining both runs resulted in the identification of 1159 proteins. Proteins identified by this technique spanned more than 5 orders of magnitudes identifying low

abundance proteins such as IL16 as well as those with higher abundance such as HMGB1, MIF and APO proteins.
Conclusion: Proteins from a wide dynamic range were identified from a small amount of plasma with minimal processing steps at a reduced coast. This new approach is now being used in our laboratory to profile plasma samples from diabetic volunteers as compared to non-diabetic controls undergoing exercise.
References: Zhou H et al., Mol Cell Proteomics. 2011 Oct;10(10).

P106

PROTEOMIC APPROACH TO HETEROGENEITY OF PROTEOME CONTENTS IN TUMOR TISSUES USING LASER MICRODISSECTION AND 2D-DIGE IDENTIFIED METASTASIS-ASSOCIATED PROTEINS IN COLORECTAL CANCER
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Introduction: Tumor tissues generally include various different cell populations. Previous studies demonstrated that the molecular profiles were different among the tumor cells, depending on their localization in the tumor tissues and reflecting malignant potentials such as invasion and metastasis. Therefore, by focusing on the cells at the specific site in tumor tissues, we may be able to identify the proteins with unique characters for malignant characters of tumor cells. We aimed to identify the proteins for malignant potentials in colorectal cancer (CRC).
Methods: This study included tumor tissues from 19 CRC patients. Using laser microdissection, we recovered CRC cells from frozen tissue sections according to the localization of tumor cells such as the superficial, center and invasive front area of tumor tissues. The protein expression profiles were created by two-dimensional difference gel electrophoresis (2D-DIGE) with CyDye DIGE Fluor saturation dye (GE Healthcare) and our original large format gel apparatus. We selected the protein spots with significant differences ($p < 0.01$, fold difference > 2.0) between normal epithelial and tumor cells for mass spectrometric protein identification. The results were validated using specific antibodies.
Results: Among 3578 protein spots observed, we identified 189 protein spots with different intensity between normal and tumor cells. The identified 88 protein spots depended on the tissue localization of tumor cells. Mass spectrometric protein identification demonstrated the site specific protein profiles. We found that metastasis-associated protein was up-regulated in the invasive front.
Conclusion: By recovering tumor cells according to their localization in tumor tissues before protein extraction, we could give unique interpretations of the identified proteins. The similar approach may be applied for the other types of malignancies which have complex histology.

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PROTEOMIC APPROACH TOWARDS BIOMARKER DISCOVERY FOR EARLY INTRA-HEPATIC RECURRENCE IN HEPATOCELLULAR CARCINOMA
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Introduction: Prediction and prevention of early intra-hepatic recurrence have been required for the risk stratification therapy in hepatocellular carcinoma (HCC). Early intra-hepatic recurrence in HCC is attributable to the dissemination of tumor cells through venous structures, and venous invasion of HCC cells is one of the independent prognostic factors. Although many lines of evidence suggested the presence of molecular signatures for venous invasion in HCC (1, 2), conclusive discovery was not achieved yet. We aimed to identify the biomarker candidates for early intra-hepatic recurrence in HCC by proteomic approaches.
Methods: Proteomic profiles of primary tumor tissues were created by two-dimensional difference gel electrophoresis (2D-DIGE) and CyDye DIGE Fluor saturation dye using our original large format electrophoresis apparatus (3). The profiles were compared between the 12 HCC patients with venous invasion and the 14 ones without it. Non-tumor tissues of the HCC patients were also included in the comparative proteomic studies.

Results: We observed 3549 protein spots. Among them, we found the protein spots, the intensity of which was quantitatively unique to the venous invasion. Mass spectrometric protein identification and literature mining determined the proteins which were not correlated with early recurrence nor venous invasion in HCC. Validation study using antibody demonstrated the significant correlation between the identified protein and venous invasion.
Conclusion: Proteins for the presence of venous invasion apparently included the metastasis-associated proteins. Proteomic studies based on clinical and pathological observations will lead novel discovery of proteins with clinical utilities.

References: 1. Orimo et al, Proteomic profiling reveals the prognostic value of adenomatous polyposis coli-end-binding protein 1 in hepatocellular carcinoma. Hepatology. 2008;48(6):1851-63.
2. Yokoo et al, Protein expression associated with early intrahepatic recurrence of hepatocellular carcinoma after curative surgery. Cancer Sci. 2007;98(5):665-73.
3. Kondo et al, Application of highly sensitive fluorescent dyes (CyDye DIGE Fluor saturation dyes) to laser microdissection and two-dimensional difference gel electrophoresis (2D-DIGE) for cancer proteomics. Nat Protoc. 2007;1(6):2940-56.

P108

PRIDE INSPECTOR: IMPROVING DATA QUALITY IN THE PROTEOMICS IDENTIFICATIONS (PRIDE) DATABASE

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Introduction: The PRoteomics IDEntifications database (PRIDE, http://www.ebi.ac.uk/pride) is one of the most prominent public repositories of mass spectrometry (MS)-derived proteomics data. Here, we introduce the PRIDE Inspector tool as an open source rich client application for inspecting MS proteomics data (http://pride-toolsuite.googlecode.com).
Methods: PRIDE Inspector is developed using Java Swing and Java 2D API. The XML handling makes extensive use of the Java Architecture for XML Binding (JAXB) and uses the jmzML library to access mzML files. In addition, the visualisation module for all spectra, chromatograms and charts utilises the jFreeChart library with custom additions for enhanced graphics. Moreover, it provides APIs/libraries which can be reused independently by the scientific community: the PRIDE JAXB library (for quick parsing of PRIDE XML files), and the PRIDE mzGraph Browser library (for the visualisation and annotation of spectra and chromatograms).
Results: Using PRIDE Inspector, MS proteomics experiments can be examined based on different views emphasising either metadata, identified proteins or peptides, mass spectra, or quantification results. Apart from its powerful visualization features, the major strength of PRIDE Inspector is the possibility to perform a first assessment on data quality using the 'Summary charts', which are generated based on different aspects of the data. Currently, PRIDE Inspector supports fast data retrieval on standard file formats (mzML and PRIDE XML, mzIdentML support is close to completion) and it also gives the user direct access to a PRIDE public database instance. As a key point, it provides journal reviewers/editors access to (privately available) experiments during the review process.
Conclusion: With PRIDE Inspector, users can now review their own data before the submission, or access data already in PRIDE for data mining purposes. Data can be examined based on different views: metadata, spectra centric, protein/peptide identification centric and quantification centric views. PRIDE Inspector has already become an essential tool for both PRIDE staff and external researchers, able to highlight and identify errors in complex datasets.

P109

PRIDE AND THE PROTEOMEXCHANGE CONSORTIUM: FACILITATING THE DEPOSITION AND SHARING OF MS PROTEOMICS DATA

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Introduction: The sharing of mass spectrometry (MS) proteomics data is not yet a generalized fact in the field despite its multiple potential applications, such as the validation of experimental results, the development of spectral libraries and fragmentation models, or the improvement in the design of SRM assays, among others.
Methods: The PRIDE (PRoteomics IDEntifications) database (http://www.ebi.ac.uk/pride) at the European Bioinformatics Institute has become one of the main public repositories of this kind of information. PRIDE stores peptide/protein identifications, mass spectra, protein expression information, and technical/biological metadata.
However, PRIDE is not working in isolation. PRIDE is a founding partner of the ProteomeXchange (PX) consortium, together with other key repositories such as PeptideAtlas (ISB, Seattle, USA) and UniProt.
Results: The PX members are currently beta-testing the implementation of a system that allows proteomics data sharing between all the members, with PRIDE as the initial submission point for MS/MS data. Draft guidelines for PX submissions are available at http://www.proteomexchange.org and some pilot submissions have already been performed.

The new challenges related to the growing and heterogeneity of the incoming data, have triggered the development of the new PRIDE system (called PRIDE 3), where major architectural improvements are being carried out. They include supporting the Proteomics Standards Initiative (PSI) data standards mzML and mzIdentML, increasing its extensibility and scalability (moving experiment data to a Lucene-indexed file system), together with automating the submission pipeline. Major improvements in user experience are also expected thanks to indexation and database schema changes. The redesign of the front-end architecture has already increased the productivity of the development team and reduced the time to accommodate changes and modifications.
Conclusion: The international ProteomeXchange initiative, with participation from key resources like PRIDE, PeptideAtlas, and UniProt, aims to maximise the availability, impact, and re-use of public proteomics datasets.

P111

APOPTOPROTEOMICS: AN INTEGRATED DATABASE FOR ANALYSIS OF PROTEOMICS DATA OBTAINED FROM APOPTOTIC CELLS

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Introduction: Apoptosis is the most commonly described form of programmed cell death and dysfunction is implicated in a large number of human diseases. Many quantitative proteome analyses of apoptosis have been performed to gain insight in proteins involved in the process. These proteomic studies resulted in large and complex datasets which are difficult to evaluate. Therefore, we developed the ApoptoProteomics database for storage, browsing, and analysis of the outcome of large scale proteome analyses of apoptosis derived from human, mouse and rat.
Methods: The proteomics data of 52 publications were integrated and unified with protein annotations from UniProt KB, the Caspase substrate database homepage (CASBAH) and gene ontology (GO).
Results: Currently, more than 2.300 records of more than 1.500 unique proteins were included, covering a large proportion of the core signalling pathways of apoptosis. The database is available at http://apoptoproteomics.uio.no.
Conclusion: Analysis of the dataset revealed a high level of agreement between the reported changes in directionality reported in proteomics studies and expected apoptosis related function and may disclose proteins without a current recognised involvement in apoptosis based on GO. Comparison between induction of apoptosis by the intrinsic and the extrinsic apoptotic signalling pathway revealed slight differences. Furthermore, proteomics has significantly contributed to the field of apoptosis in identifying hundreds of caspase substrates.
References: Arntzen, M.Ø. and Thiede, B., *Mol. Cell. Proteomics*, 2012, 11(2): M111.010447

P112

AN ADAPTIVE ALIGNMENT ALGORITHM FOR IMPROVED LABEL-FREE DATA ANALYSIS

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Introduction: As the label-free mass-spectrometry workflow is rapidly gaining popularity, improving robustness of data analysis is essential. Quantification using precursor ion intensities in LC-MS runs contains two focus points; feature detection and alignment. The process of alignment compensates for retention time differences from chromatographic separation of samples for differential expression studies. Misalignment introduces missing values and systematic bias into the results.
Methods: We have implemented an alignment algorithm in the Proteios Software Environment [1] that estimates parameters from the sample cohort, thereby avoiding addition of technical variation due to varying software settings or reference runs. Quality control [2] is incorporated into the analysis as a parameter regulator.
Results: Label-free analysis has been performed on high-resolution LC-MS datasets and alignment and quantification accuracy were extensively evaluated and confirmed the benefits of the new algorithm. Independence of feature detection module and incorporation into a multi-user platform extends the flexibility of the alignment algorithm compared to stand-alone program suites.
Conclusion: We show that a combination of an adaptive alignment algorithm and continuous quality control evaluation leads to improved label-free data analysis.
References: [1] *Häkkinen et al.*, The Proteios Software Environment: An Extensible Multiuser Platform for Management and Analysis of Proteomics Data, **J. Proteome Res.**, 2009, 8 (6), pp 3037–3043
[2] *Sandin et al.*, Generic workflow for quality assessment of quantitative label-free LC-MS analysis, **Proteomics**, 2011, 11 (6), pp 1114-1124

P113

MYPROMS, A BIOINFORMATICS SOLUTION FOR COLLABORATIVE PROCESSING AND ANALYSIS OF MASS SPECTROMETRY-BASED PROTEOMICS DATA

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Introduction: Proteomic Mass Spectrometry (MS) generates complex data that require multiple steps of computational and manual processing to convert raw MS-data into meaningful biological information. Typical tasks to be performed include protein identification, data curation, protein quantification, gene ontology analysis as well as the cumbersome data management. To successfully achieve this challenging goal, different partners with complementary skills (MS specialists, bioinformaticiens, biologists …) need to share their expertise.
We have developed *my*ProMS, a comprehensive bioinformatics environment, to rationalize this data processing workflow while allowing multiple users to interact with the data according to their expertise level.
Methods: *my*ProMS relies on a web server coupled with a MySQL database for efficient data access and management. It is developed in Perl-CGI, HTML, JavaScript, JAVA and relies on R statistical language for quantitative data analysis.
Results: Multiple output files from MASCOT or Proteome Discoverer search engines can be easily imported into *my*ProMS within a defined experimental context. Protein identifications and post-translational modification sites are then available for automated or manual validation by MS specialists. Only curated results become accessible to other collaborators for further analysis. *my*ProMS supports diverse labelled and label free-quantification methods and ease quantitative data visualization with helpful interactive graphical displays.
Further biological interpretation of the results is possible using customized Gene Ontology analysis tools and extensive linking to external resources.
Conclusion: *my*ProMS is a mature solution for proteomic MS collaborative projects. It is used by multiple laboratories and platforms and benefits from users' feedbacks for continuous improvement. The software can be evaluated and downloaded freely for academic users at http://myproms-demo.curie.fr.

P115

COMPARATIVE BIOINFORMATIC ANALYSIS OF PROTEOME AND TRANSCRIPTOME DERIVED FROM A SINGLE CELL TYPE

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Introduction: With protein identifications of more than thousands of proteins it is essential possible to perform sophisticated bioinformatic analysis of such datasets and their comparison to gene expression data. Here we present a new development in ProteinCenter, which enables such a comparison, using the data derived from publically available sources. We also introduce an option to integrate ProteinCenter with Cytoscape for visualization of the protein-protein interaction (PPI) networks as a part of data analysis tools.
Methods: To test the new capability we used data published as part of a recent article ”Deep proteome and transcriptome mapping of a human cancer cell line” (Nagaraj N, Molecular Systems Biology 7, 548, 2011). Data was logged to ProteinCenter including accession code, ion intensities, iBAQ and FPKM values, absolute quantification in fmol, and number of copies per cell. Genomic information was restricted only for proteins detected in the study. Statistical analysis of datasets was performed based on Benjamini-Hochberg correction of p-values with false discovery rate (FDR) values of 5%. Protein-protein interaction network analysis was performed by Cytoscape (http://www.cytoscape.org/). IntAct, MIPS and STRING ‘network file’ were exported from ProteinCenter. All quantitative values were imported into the Cytoscape nodes as 'node attributes'.
Results: In total 239 pathways were detected out of 245 available from KEGG for human proteins. Among the underrepresented pathways were asthma, autoimmune thyroid disease, allograft rejection, olfactory transduction and some others. All of them are not expected to be functionally relevant in HeLa cells. At the same time some of the over- represented pathways, such as the nucleotide excision repair pathway have more than 92% coverage. All in all, average pathways coverage is around 46% and suggests that the detected proteome covers a very large part of functional pathways. To complete the bioinformatic analysis of the data set we performed PPI analysis using Cytoscape graph algorithms and VizMapper visualization tool, where ProteinCenter data were used to colour-code nodes according to the protein quantitative values.
Conclusion: Software tools for visualization and comparison of proteome and transcriptome on a protein-centric platform

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NOVEL BIOINFORMATICS TOOL: INTERPRETATION OF GLYCAN MASS SPECTRA WITH METAL ADDUCTS AND MULTIPLE ADDUCT COMBINATIONS

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Introduction: Previously we had presented a bioinformatics tool for automated structural interpretation of glycan MS/MS and MS^n data. This was limited to characterizing glycans with single adduct of H and Na ($[M+H]^{+1}$, $[M-H]^{-1}$, $[M+2H]^{+2}$, ($[M+2Na]^{+2}$ etc.). Here we expand our bioinformatics tool, SimGlycan, to support Li and K adducts as well as combination of multiple adducts such as Na + H, Li + H, Na+K etc. In order to demonstrate the utility of the software, a combination of permethylation and MS^n are used to characterize glycans derived from bovine fetuin and human IgG.
Methods: Released glycans from Bovine Fetuin and human IgG were permethylated as described previously. All MS^nexperiments were carried out on a Thermo Scientific Velos Pro linear ion trap mass spectrometer using direct infusion into a nanoelectrospray source. Data analysis was performed using SimGlycan software from PREMIER Biosoft.
Results: Small amounts of alkali metals are added to improve ionization. However, introduction of these adducts can result in spectra with precursors containing multiple adducts which complicate spectra interpretation. We have expanded SimGlycan to support characterization of glycans with Li and K adducts and combinations of multiple adducts. In order to test the performance of the software, glycans from bovine fetuin was chosen. MS profile was acquired for the glycans and specific precursors with different adducts and combination of adducts were targeted for MS/MS and MS^n. Data were imported into SimGlycan software for structural characterization. SimGlycan characterized glycans were verified using manual assignment and previously published data. We were able to correctly identify and assign structures to the MS/MS and MS^n

spectra using this software. We further extended this to glycans released from Human IgG. This is an area of interest as IgG are involved in human circulation as part of the humoral immune responseand the changes in *N*-glycosylation of IgG associate with various diseases and affect the activity of therapeutic antibodies and intravenous immunoglobulins.
Conclusion: Using a combination of permethylation and MS^n more than 30 structures were identified. All of the targeted glycans contain multiple adducts and SimGlyan was used in structural interpretation.

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KINASE - SUBSTRATE ENRICHMENT ANALYSIS: AN ALGORITHM AND COMPUTER PROGRAM TO INFER PROTEIN KINASE ACTIVITY FROM PHOSPHOPROTEOMICS DATA
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Introduction: A common feature of all cancer cells is that they deregulate components of the kinase signaling network. However, it is not known how many of the different >500 kinases in the human genome can contribute to the malignant phenotype. Phosphoproteomics techniques based on mass spectrometry can now quantify thousands of phosphorylation sites per experiment but linking measured phosphorylation to the kinases acting upstream is not straightforward. Here we developed a computational approach to statistically infer kinase pathway activation based on large-scale phosphoproteomics data and applied it to the investigation of protein kinase activities downstream of PI3K and mTOR, a pathway frequently deregulated in cancer cells.
Methods: This approach, termed Kinase Substrate Enrichment Analysis (KSEA), involves (i) grouping phosphopeptides (identified by phosphoproteomics) into substrate groups defined by a common phosphorylation motif or by being phosphorylated by a common kinase and (ii) calculating the extent and significance of enrichment of these substrate groups in the phosphoproteomics dataset. The algorirhms were implemented in a VBA computer program.
Results: To investigate its performance, KSEA was applied to the comparison of two leukemia cell lines showing different sensitivities to kinase inhibitors; the approach revealed that PKB/Akt, PKC and RSK activities were increased in a resistant leukemia cell line whereas ERK and CK were more active in the sensitive one. These results were consistent regardless of the method used to quantify enrichment and of the source of kinase-substrate relationships illustrating the robustness of the approach. Application of KSEA to leukemia cells treated with PI3K and mTOR inhibitors showed activities of PKB/Akt, CDKs, S6K and PAK to be downstream of PI3K/mTOR, thus further validating the approach. In addition, KSEA identified DNA-PK as being activated as a consequence of inhibiting PI3K. Follow up experiments validated this observation and further revealed that activation of DNA-PK was secondary to the activation of apoptosis by the PI3K/mTOR inhibitors.
Conclusion: These results illustrate the robustness and utility of KSEA as a means to statistically and comprehensively infer kinase activation from phosphoproteomics data in cancer cells.

P119

OPTIMIZATION OF ACQUISITION PARAMETERS TO MAXIMISE SPECTRAL COUNTING ACCURACY ON A QTRAP SYSTEM
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Introduction: Spectral counting as label-free abundance measure for relative quantitation have become popular in recent years. Publications on acquisition method optimization exist for Thermo's iontrap and Orbitrap type instruments. We set out to optimize the information dependant acquisition (IDA) parameters on an ABSCIEX 4000QTRAP MS to maximise the SC number for the purpose of more accurate relative quantitation.
Methods: Yeast cell lysate was separated by SDS-PAGE. Each lane was divided into 5 sections and trypsin digested, peptides were RP separated (10-25%B over 60 min) and analysed on the QTRAP. Data was analysed by Mascot and ProteoIQ. The same IDA methods were used with each of 3 biological replicates.

Parameters tested; 1) threshold level {0%, 50%, 100%, 150%, 200%, 250% and 300% of baseline}, 2) number of peptides selected for MS/MS scans {1, 2, 3}, 3) peptide exclusion times {30s, 60s, 90s, 120s} and occurrences {1, 2, 3} before exclusion. Chromatographic gradient and the randomness of peptide selection was also investigated.
Results: The SC across the 3 replicates generated from each method was used for comparison. None of the parameter combinations resulted in a huge increase, only small differences between the methods were recorded. Comparing the average values and favouring small standard deviation resulted in a range of parameters that gives similar maximum SC results. The SC number from the threshold value data remained constant up to 250% of the baseline signal. As expected, more SC were recorded from longer chromatographic gradient runs.
Conclusion: No single combination of parameters yielded a clear combination that can be perceived to be better. Instead, for a 60 min gradient selecting 2-3 peptides, excluding them for 30-90s after 2-3 occurrences resulted in maximum SC amount. The only way to drastically increase spectral count values would be to clean up the sample separation to reduce complexity dramatically and lower background noise to reveal low abundant peptides.

P121

AUTOMATIC CHARACTERIZATION OF LIPIDS FROM MALDI MS/MS DATA USING SIMLIPID™ SOFTWARE

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Introduction: Within lipid research mass spectrometry is one of the most sophisticated technologies for identification and quantification of lipids from biological mixtures. MALDI TOF MS analysis has unique advantages for structural characterization of lipids,however, the major challenge in mass spectrometric analysis of lipids by MS and MS/MS is the huge amount of data generated in the process. In addition, the structural analysis of lipids by mass spectrometry is not routine and often requires tedious, time consuming manual spectral interpretation. SimLipid™ software is a comprehensive informatics tool for characterizing lipids by MS and MS/MS data which streamlines this type of data analysis.
Methods: Lipid samples in different concentrations were prepared using DHB matrix in dichloromethane/isopropanol/acetonitrile (2:1:1, v:v). Lipid standards of known structure were obtained from Avanti Lipids, Alabaster, Alabama. Lipids from commercial fats were analyzed as well. Samples were analyzed using the AB SCIEX TOF/TOF™ 5800 system in MS and MS/MS positive ion mode. Up to 2000 laser shots were averaged for MS/MS mode spectra, 1000 laser shots for MS mode spectra. MS and MS/MS data was analyzed using SimLipid software.
Results: Different lipid standards were analyzed by MALDI TOF acquisition. MALDI MS/MS under high energy-CID conditions supplies highly detailed fragmentation information for the structure elucidation of lipids. The information of the MS/MS data can be used to determine the lipid class or even the lipid species structure using SimLipid software, which utilizes a database of lipids for identification.
Conclusion: SimLipid provides a rapid means of identifying relevant lipid species at high confidence. This will be demonstrated identifying unknown lipids from fats, e.g. m/z 907.74, which was identified as TAG 54:3 within olive oil. Additional lipids could be identified within this workflow as well, which include e.g. m/z 689.75 from butter which was identified as a Diacylglycerophosphogycerol and from Margarine, ID of m/z 901.65, which leads to an unsaturated TAG as well.

P122

AUTOMATED TISSUE STATE ASSIGNMENT FOR HIGH RESOLUTION FTMS MALDI IMAGING DATA

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Introduction: Interpretation of MALDI imaging data can be tedious, especially if many peaks are present. Hierarchical clustering of mass spectra based on their overall similarity is one choice. This is used for the fast

and concise semi-supervised segmentation of MALDI-TOF imaging data (Deininger et. al, J. Proteome Res 2008, 7(12):5230-6). Compared to MALDI-TOF the clustering of FTMS data is more challenging because of many more peaks. In this study several parameters for the hierarchical clustering such as distance and linkage methods and missing value treatment were evaluated and their results compared in respect to computational performance and analytical results.
Methods: MALDI imaging data from several model tissues such as mouse kidney, brain, and pancreas were recorded on an FTMS (Solarix, Bruker). The MW peak lists were binned into one bucket table with a custom C+ program. Clustering was calculated with R. Different parameters for the distance (such as Euclidean, Correlation, Manhattan) and linkage (Ward, Complete, Average) were used for the calculation, for the Euclidean distances data were also pre-treated with Principal Component Analysis (PCA). Segmentation maps were reconstructed from the clustered data and compared to the anatomy of the tissue sections.
Results: The data suggest that different distance and linkage methods lead to overall similar segmentations of the dataset, which were in good agreement with the anatomy of the samples. For kidney, the cortex, medulla, and renal pelvis were clustered as the main tissue structures by all approaches. The same was true for the anatomical regions in the brain. This suggests that the differences in the anatomical regions were significantly larger than random pixel-to-pixel variations, thus leading to a robust clustering result.
Conclusion: Differences were observed in the details: Ward linkage led to dendrograms that were easiest to interpret, since they resulted in larger clusters at the top end of the dendrogram. While ward linkage resulted in the clearest segmentation it was computationally not feasible to use this on larger datasets. Correlation was found to be a very appropriate distance method that could be applied without normalization of the data.

P123

AN INTEGRATIVE GENOMICS APPROACH TO ACCESS THE TESTICULAR GERMLINE SECRETOME AND DECIPHER THE GERM CELL-SERTOLI CELL DIALOGUE

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Introduction: The extremely complex structural organization of the mammalian testis creates particular difficulties for studying its organization, function, and regulation. Spermatogenesis that takes place in the seminiferous tubules is an intricate and highly specialized process whose control incorporates juxtacrine, paracrine and endocrine factor information, including numerous testis-specific isoforms. It has been yet established that germ cells modulate somatic Sertoli cell function via diffusible proteins. However, the impossibility to maintain germ cells in vitro makes it difficult to study their secretome. Interestingly, within the seminiferous tubules, germ cells and Sertoli cells are surrounded by the testicular fluid (TF), which probably contains factors involved in the germ cell-somatic cell crosstalk.
Methods: An innovative approach combining TF collection by microsurgery, fluid pre-fractionation, shotgun mass spectrometry and “combinatorial omics” was used to decipher and mine the rat and ram testicular fluids. Over 1400 non-redundant proteins were identified and their presence in TFs was further correlated with the transcriptome of isolated testicular cells so as to confirm their cellular origin.
Results: Secreted proteins were identified and scored using the Secreted Protein Database (SPD). We demonstrate here that a subset of proteins is actively secreted into the TF by germ cells. Potential known partners of these germ cell secreted proteins were proposed using protein network data available via certified public repositories and those known to be expressed on Sertoli cells plasma membranes were selected for further studies. Co-expression of potential germ cell-Sertoli cell protein partners was validated by immunohistochemistry on rat testis sections.
Conclusion: Our results provide new insights into the germ cell-Sertoli cell crosstalk by identifying novel interacting protein partners. We also demonstrate that “combinatorial omics” is a powerful approach to characterize the testicular germ cell secretome that was so far technically inaccessible.

P124

VISUALISING CROSS-LINK/MS DATA AS A HIERARCHICAL GRAPH

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Introduction: Cross-linking/MS acquires information on the physical structure of proteins and on protein-protein interactions, however the data acquired is at the resolution of individual residues. The work presented here addresses the design of a visualisation tool to explore the results of cross-linking/MS experiments.
Methods: We are engaged in an iterative, user-centred design process to develop the visualisation of output from cross-linking/MS experiments.
Results: Researchers working in this area think of the experimental data at different levels of abstraction: interactions between residues, between proteins or between clusters. An initial idea was that there should be distinct views of the data showing it at different scales or 'levels'. Through the design process, we moved to a single view of the data in which it is individual proteins that can be represented at different levels of abstraction. The visualisation shows the topology of the cross-link network as a hierarchical graph: links between proteins are shown but the nodes can be expanded to show (inter and intra protein) cross-links between individual amino acids.
Conclusion: We have developed an effective, web-based software component for viewing protein interaction networks when those networks contain information at both protein-protein and residue-residue resolution. This has been integrated into Xi, a web-based software suite for cross-linked peptide identification. This JavaScript component will also be refactored to form part of the BioJS library (http://code.google.com/p/biojs/), thereby facilitating its reuse in other areas of proteomic and protein-protein interaction research.

P125

A HIGH-HIGH STRATEGY FOR IDENTIFYING CROSS-LINKED PEPTIDES IN 3D PROTEOMICS

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Introduction: Chemical cross-linking paired with mass spectrometry has evolved into a powerful method to investigate protein structure and protein-protein interactions. High accuracy mass spectrometers have greatly improved the automated assignment of MS/MS spectra when performing peptide identification. Similarly, cross-linked peptides are best identified by high-resolution measurement of peptide and fragment masses. By adopting a high–high strategy, we are able to find and validate cross-linked peptides in an efficient and scalable manner without the need for additional tags, such as isotope-labelled reagents.
Methods: Data was generated using chemical cross-linking by BS3 (Pierce) and a set of proteins in our lab including human serum albumin and complement proteins from ongoing investigations. Proteins were digested with trypsin, followed by enrichment of cross-linked peptides using SCX-StageTips and analyses by LC-MS/MS using an LTQ Orbitrap Velos. We used a high-high strategy and excluded 1+ and 2+ precursors from selection for MS2. Cross-linked peptide identification was achieved using Xi software.
Results: Using isotope-labelled cross-linkers, we observed the number of unique cross-links identified depends on the mixing ratio. A 1:1 mixture of H/L cross-linker gave the smallest number of identified cross-links: 17 on average. This number increased to 19 and 32 with a mixing ratio of 1:2 and 1:4 respectively. Using H/L versions of cross-linkers to increase identification confidence may come at the expense of losing 50% of the data on cross-links that would otherwise have been observable. Xi, our software, does not rely on isotope labeling, but solely on high-resolution data and moreover leaves the use of isotopes for quantitative approaches.
Conclusion: High-resolution data, unlike its low-resolution counterpart, maximizes specificity since **1** Dependence on isotope labelling is removed; **2** Spectra can be “charge-reduced” i.e. charge recognition allows us to focus on fewer targeted peaks during database searching; strengthened by the observation that cross-linked peptides tend to have higher charge states; **3** Neutral loss peaks are spotted, e.g. loss of

water/ammonia, which are frequently observed for cross-linked peptides. Lastly, **4** Isotope clusters can be collapsed onto a mono-isotopic peak.

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GEENA, A TOOL FOR MS SPECTRA FILTERING, AVERAGING AND ALIGNING

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Introduction: Geena is a new tool for MALDI/TOF MS data management that aims at automating some of the fundamental elaboration steps involved in the data analysis. It includes: a) preprocessing of spectra replicates, which includes isotopic peaks joining, normalization, and peak selection, b) computing average spectra for replicated analysis of samples, and c) alignment of average spectra.
Methods: The software was written in PHP and partially, for spectra alignment, in perl. Both input and output are managed in simple text files, usually having tab or comma delimited values. Such formats can easily be consumed by MS Excel or any other data management system. Data may be stored on the server in a mySQL database.
Results: The data file, consisting in MALDI/TOF MS spectra, is usually uploaded to the server and removed as soon as it has been used. The presence of a normalization peak and its corresponding m/z value must be specified to normalize data. It is possible to select peaks above a given threshold and to join isotopic peaks of the same molecule. The output consists both in the averaged spectra from replicates and in the alignment of averaged spectra. The alignment is shown in the results page, while all results are available for downloading from the same page and sent by email, if a valid address is provided.
Conclusion: Geena is a public web server (http://bioinformatics.istge.it/geena/) and was developed by taking into account the following assumptions:
- In each spectrum, molecules are present in the form of different isotopic abundances that can be summed together to give a total abundance value.
- Experimental data must be normalized against an internal standard to obtain (semi) quantitative results.
- The selection of signals above a modulated threshold built on the spectra profile may be useful.
- The analysis of sample replicates yields multiple spectra which are different because of marginal errors/changes in the experimental phase. A spectrum representative of the sample may be defined by aligning these spectra along the m/z axis and computing a mean intensity value from the corresponding abundances.
- To compare single or average spectra obtained from different samples the alignment along the m/z axis is required.
References: Mangerini R et al. Anal Biochem 2011;417:174-181

P127

INTEGRATION OF SPECTRAL LIBRARY IN STANDARD DATA PROCESSING PIPELINE FOR SHOTGUN PROTEOMICS DATA

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Introduction: Over the past years the usage of spectral library search tools was established in shotgun proteomics. Instead of identifying the same peptide over and over again by a sequence database search engine like SEQUEST, spectral library search uses a library of spectra identified with high accuracy. The restriction to previously measured spectra avoids a lot of computational overhead. Search tools like SpectraST, MSPepSearch or BiblioSpec are freely available and a lot of work was spent on building accurate and confident libraries, which is the crucial part of the identification procedure. Here we present the integration of a spectral library search engine (SpectraST) into an automated pipeline for shotgun proteomics data analysis (Proteome Discoverer).
Methods: The usability of spectral library search as fast and sensitive first level search to speed up the peptide identification in a standard shotgun proteomics data analysis workflow will be presented. All samples were measured on a Thermo Scientific LTQ Orbitrap Elite instrument coupled to a Thermo Fisher

Easy nano-LC. The data was analyzed using a pre-release version of Thermo Scientific Proteome Discoverer. All spectral libraries used were from NIST. For data analysis different workflows were used. Search results of spectral library searches were compared to classical database search engines like SEQUEST and Mascot. The benefit of the usage of spectral library search as pre search to a standard database search is investigated in a sequential Proteome discoverer workflow.
Results: A HeLa and a E.Coli sample were processed using the different workflows. Comparing spectral library search to classical database search shows a decrease in search time. The number of spectra identified in the spectral library search is smaller compared to the database search due to the limitations of the used libraries to previously identified high confident peptides, but almost all identifications from spectral library search have a corresponding highly confident identification in the database search.
Conclusion: Using a spectral library search as an identification method for a subset of spectra prior to a database search is considerably faster than a method based on identifying peptide sequences by database search alone.

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XI SPECTRUM VIEWER: REVOLUTIONISING READER-ARTICLE INTERACTION IN THE WEB BROWSER BY EMBEDDING DYNAMIC MASS-SPECTRAL DATA

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Introduction: Any mass spectrum of individual scientific importance should be completely available to any scientist for interrogation. Currently, spectra can be made available in online repositories, such as PRIDE, but these require some time to learn and navigate, offer restricted interactions and do not allow exploration of alternative interpretations for a spectrum. The result is that even experts in the field must spend considerable effort, with an array of specialist tools, in order to interrogate published MS data with respect to their interpretation. Non-specialists often only have access to the printed spectra and limited annotations. We hope to remedy this situation with Xi Spectrum Viewer, an intuitive, browser-based viewer for online display and interrogation of spectra and their interpretations by mass spectrometrists and biologists alike.
Methods: Xi Spectrum Viewer is implemented entirely on the client side using XHTML for menus and dialogs, SVG for spectrum and peptide display, and Javascript to drive functionality. The sources and demo are available at http://sourceforge.net/p/spectrumviewer/ .
Results: To overcome current limitations in published MS data accessibility, we introduce a browser-based spectrum viewer that aims to allow exploration of alternative interpretations for MS spectra and additionally: can be operated by non-specialists, is open source, can be integrated into other software, gives appropriate publication-quality output.
Xi Spectrum Viewer works well in most browsers. It correctly identifies peaks for a single peptide, for a pair of peptides and for a cross-linked pair of peptides, while also allowing easy exploration of the data and alternative explanations, such as different peptide sequence, different linker position (for cross-linked peptide pairs) and different modification positions. It can run completely stand-alone but is also a good candidate for any database front-end.
Conclusion: We hope that publishers will acknowledge the untapped power of the modern browser and adopt Xi Spectrum Viewer in online publications as a first step towards in-line interactive data display. We believe that Xi Spectrum Viewer is only the first of many such tools that will revolutionise the peer review and scientific reading experience.

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PROTEOMICS AND FARM ANIMALS: A TALE OF MUSCLE AND MEAT

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Introduction: Proteomics has been extensively applied to the study of muscular tissues in several species covering a wide variety of topics, from physiology to diseases. Muscles are also the key component of meat and meat-based products. Accordingly, proteomics has been used to characterize the process by which muscles are transformed into meat as well as from meat to meat product. Particular relevance has been given to two species: cattle (*Bos taurus*) and swine (*Sus scrofa*) in the context of meat maturation and aging and the processes for the production of hams (dry-cured and cooked). Additionally, proteomics has been applied to study meat quality in other farm animal species such as small ruminants, poultry and rabbits.
Methods: In this presentation authors will present an overview of research activities carried out coupling proteomics and meat science. Particular emphasis will be given to the research efforts of the three teams involved in this presentation.
Results: The three teams involved in this presentation have studied muscle and meat proteomics during recent years. Three major areas of research arise:
1) Meat maturation and breed differentiation in cattle and swine (Norway);
2) Muscle characteristics and breed differentiation in rabbits and sheep as a consequence of weight loss (Portugal);
3) Dry-cured and cooked ham processing techniques (Italy).
Results presented will reflect not only the protein expression profiles but also the intricate metabolic pathways involved in these processes.
Conclusion: Proteomics is of extreme importance to the study of the processes by which muscle is transformed in to meat and by which meat is processed into meat products. The application of proteomics to meat science surpasses the mere study of these processes, being of key importance to issues such as product certification, characterization and the detection of adulterations.

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PROTEOMIC ANALYSIS OF GEOSMIN PRODUCING STRAINS OF PENICILLIUM EXPANSUM FOR COMPREHENSION OF THE MOLECULAR MECHANISMS OF EARTHY-MUDDY FLAVOUR FORMATION IN WINE

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Introduction: During 2000's vintages, some luxembourgish wines were spoiled by earthy-muddy flavour. Several fungi occurring in grapes cause these off-flavours; their frequencies are highly dependent on the meteorology during the maturation period. *Penicillium* spp. have been associated with earthy-muddy flavour (geosmin, MIB). We selected four *Penicillium expansum* strains, that produce different amounts of geosmin, and we analyzed their proteome through 2D gel electrophoresis to understand the molecular basis and regulation of geosmin production.
Methods: Four *P. expansum* strains (P4, P8, P21 and P23) and 4 replicates for each strain, were grown on malt-peptone medium at 25 °C in the dark for 3 days. The obtained mycelia were filtered on filter paper and proteins were extracted by TCA/Acetone/DTT, quantified by the Bradford method, labeled with CyDyes (30 mg per sample) and separated by 2D-PAGE (1^{st} dimension: pH 3-10 NL strips; 2^{nd} dimension: 12,5% pre-cast polyacrylamide gel). Gels were scanned with a 9400 Typhoon and analyzed by Decyder 7.0 software.
Results: Production of geosmin was quantified on the liquid media, after 3 days of growing, by GC-MS/MS: the lowest amount of geosmin was detected for the strain P4 (249 ng/L), the strains P21 and P23 showed the highest production (2519 and 5621 ng/L of culture medium respectively). The strain P8 produced both low and high concentrations on identical conditions. 250 spots were picked and 162 proteins were identified. Among them, 87 showed differentiated expression patterns between strains. PCA analysis highlighted the differences between the geosmin-production-ability of each strain. This allowed us to cluster the strains for the production of geosmin: the two high producer strains (P21 and P24) form a unique cluster together and they are indistinguishable among each other, while P4 and P8 strains are forming individual clusters which are clearly separated.
Conclusion: The analysis allowed to identify several differentially expressed proteins within the 4 *P. expansum* strains included in the study. Several proteins linked to secondary metabolism were identified and suggest that the production of geosmin is related to oxidative stress, carbon metabolism and protein degradation.

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EXPLORING THE USE OF DIFFERENTIAL PROTEIN EXPRESSION PROFILING FOR THE DETECTION OF BSE IN OVINE PLASMA.

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Introduction: Transmissible spongiform encephalopathy describes a range of diseases affecting both man (vCJD) and animal (e.g. BSE and scrapie). Current tests for diagnosis of these diseases are based on post mortem detection of an abnormal form (PrP^d) of the host prion protein. Recent concern over the sensitivity of this marker, coupled with an urgent need for a pre-clinical live animal test, has led to the search for new markers of disease. We have shown in previous studies that differential protein expression profiling using surface enhanced laser desorption and ionisation time of flight mass spectrometry had potential as a novel testing platform for the detection of TSE disease in brain tissue homogenates. In this present study we sought to extend this methodology to examine ovine blood plasma.
Methods: Ovine plasma samples collected temporally from groups of both BSE infected and non-infected sheep were analysed to determine differential protein expression profiling between groups. Resultant data were statistically tested utilising a two - level cross validation scheme to build predictive models of disease for BSE at each time point taken throughout the course of disease (training set). Each disease model was tested using linear discriminant analysis (LDA) on a "test set'' of samples (*toxoplasma gondi* infected plasma and plasma from animals of unknown BSE status).
Results: The terminal stage model was predictive of disease (90% sensitivity 75% specificity). LDA of the "test set'' revealed all *Toxiplasma gondi samples* and all but two of the BSE infected animals still alive at the time of analysis*,* predicted as negative. Some predictive power was also apparent at earlier time points. Further purification of proteins from the "Best Peaks'' profiles resulted in identification of plasminogen/fibrinogen complex, ApoN and IgG lambda light chain.
Conclusion: Results suggest that a predictive model might be achieved however for a more robust model; a larger sample set would be required, particularly in the control sample set. From our previous study in cattle, sample sets of at least fifty in each of the diseased and non-diseased groups are required to build a robust model.

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PUSHING THE LIMITS OF PROTEOMICS TECHNIQUES TO UNDERSTAND STRESS AND TOLERANCE IN AN ALLOPOLYPLOID CROP

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Introduction: Polyploidy and allopolyploidy have played an important role in the evolution of many crops. Two-dimensional electrophoresis is still the workhorse for proteomics in non-model plants (Carpentier et al., 2008) and is an excellent tool to quantify and analyze the different protein isoforms (Carpentier et al., 2011). However, trans membrane proteins are hardly detected on classical 2-DE gels (Vertommen et al., 2010). Therefore we have developed a peptide based shot gun workflow (Vertommen et al., 2011).
Methods: A peptide based approach in crops demands special precautions to prevent false positive identification of proteins and a false reconstruction of peptides into proteins. Our workflow included (i) optimization of protein extraction and the peptide separation (2DLC), (ii) performing de novo sequencing, (iii) visualization of identified peptide-protein associations using Cytoscape to remove redundancy and wrongly assigned peptides and to visualize the protein inference problem.
Results: The optimization of the 2DLC RPRP was done by adapting the concentration of acetonitrile in the first dimension and the ACN gradient slope in the second dimension. Due to this optimization less than 10 % of the total peptides were identified in more than one fraction. The spectra were initially searched against an inhouse database. Subsequently, automatic *de novo* sequencing was performed on the remaining unassigned spectra.The application of *de novo* sequencing greatly improved the number of identified peptides. On average, 67% of the peptides were identified through *de novo* sequencing.The purpose of most proteomic experiments is not the identification of peptides, but the identification of the proteins present in the

sample before digestion. A problem encountered in a peptide-based approach is the assignment of peptides to proteins to which they may not belong. A visualization program such as Cytoscape facilitated the detection of these peptides and visualized the confidence of the protein identifications.
Conclusion: In this paper a new workflow for the analysis of membrane proteins from poorly sequenced plants using a peptide-based approach is proposed. Using this workflow, integral plasma membrane proteins from banana leaves could be succesfully identified.

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MASS SPECTROMETRY BASED REFERENCE METHODS AND MATERIALS FOR THE ANALYSIS OF FOOD ALLERGENS

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Introduction: Risk management of food allergy, a major public health problem, depends on quantification of allergen proteins in a traceable manner, a task hampered by lack of certified reference materials (CRM). Mass spectrometry (MS) is a powerful technique for quantification of proteins and production of CRM. The feasibility of producing CRM for allergen analysis by MS is discussed. A reference method for quantification of 1 mg kg^{-1} 1of lysozyme in wine is detailed and preliminary results on the analysis of casein and β lactoglobulin in food matrices are discussed.
Methods: 1 mg kg^{-1} of lysozyme in white wine was spiked with isotopically labelled peptides, tryptic digested and analysed by liquid chromatography mass spectrometry (LC-MS). A sample clean up step based on high pH reverse phase chromatography was performed prior to analysis. Standard peptides were quantified by amino acid analysis to ensure results are traceable to the SI.
Results: White wine spiked with 1 mg kg^{-1} lysozyme was quantified by exact matching isotope dilution mass spectrometry (IDMS) with an uncertainty below 10%. Tryptic digestion was optimized to assure complete release of the three monitored peptides. The stability and recovery of the labeled peptides was verified by spiking equimolar amounts of unlabelled peptides after each step of the digestion and sample clean up. The characterization of the aspecific binding of the peptides with wine tannins played a major role in the optimization of peptide recovery after tryptic digestion and in development of the reverse phase clean up step.
Preliminary experiments assessed the feasibility of producing CRM for milk allergens. The effects of the matrix, glycosylation and Maillard reaction on the selection of the peptides to be used as standards were evaluated.
Conclusion: Exact matching IDMS has been successfully applied for the development of a method to be employed for the production of CRM for quantification of lysozyme in wine. Casein and β lactoglobulin have also been studied. This is a major step for the production of biological reference materials in the food area which in turn can improve robustness, confidence and comparability of analytical results.

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NEW INSIGHTS IN DIAGNOSES OF BOVINE SUBCLINICAL AND CLINICAL MASTITIS

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Introduction: Research for biomarkers discovery of bovine mastitis aims to identify reliable biomarkers for early detection and drug efficacy. Most of proteomic studies on mastitis have been performed on milk and somatic cells[1]. Although proteomic profile of bovine milk whey proteins have been well characterized still poor information have been provided on serum and plasma proteomics of bovine mastitis. Aim of this work is to extend the current knowledge on molecular circulating biomarkers of mastitis including both sub-clinical and clinical animal group. Collected evidences showed complementary data between oxidative stress response, lipid metabolism and the differential protein expression.

Methods: The study has been performed using a classical biochemistry approach to evaluate inflammatory process and through a comparative proteomic analysis for the detection of differentially expressed proteins among subclinical and clinical groups.
Results: Inflammatory status was evaluated analyzing PON1 and PAF-AH that were found to be down-regulated during clinical mastitis. A shotgun-MS approach was used to evaluate the differences in high abundant proteins. Moreover using 2D eletrophoresis were found ten differentially expressed proteins. Among them, vitronectin-like protein and serpin A3-1 have been described because of their increased expression in subclinical mastitis group.
Conclusion: Vitronectin production is linked to the process of bacterial opsonization that involves the complement cascade. It indicates the presence of a strong bacterial infection. Serpin A3-1 is a protease inhibitor and because of its overexpression during inflammation it has been well characterized. Evaluation of profiling and concentration of these biomarkers during subclinical mastitis could be helpful for the early diagnoses of this pathology.
References: 1. Alonso-Fauste, I. et al. Proteomic characterization by 2-DE in bovine serum and whey from healthy and mastitis affected farm animals. *Journal of Proteomics* (2011).

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THE CONTRIBUTION OF PROTEOMICS TO LIPIDOMICS AND BIOMETRICS FOR ASSESSING QUALITY AND ZOOTECHNICAL PERFORMANCE OF GILTHEAD SEA BREAM (SPARUS AURATA, L.)
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Introduction: The characterization of differential expression profiles in farmed fish tissues can contribute to the understanding of fish biology, growth dynamics, product quality, food safety and authentication, traceability, and shelf-life, facilitating monitoring, control, and optimization of aquaculture practices. In this study, the impact of different commercial feeds on the zootechnical performance of gilthead sea breams (*Sparus aurata*) was evaluated with a multidisciplinary approach combining lipidomics, biometrics and proteomics.
Methods: Two feeding trials were carried out on three groups of fish in a pilot aquaculture plant. The first trial evaluated three different commercial feed formulations, while the second trial evaluated two different feeding schedules with the best performing feed. Morphometric parameters were assessed, and lipidomic analysis was performed on fish tissues by NMR and GC. Liver and blood serum were subjected to 2D DIGE and DeCyder analysis, LC-MS/MS identification, and Ingenuity Pathway Analysis.
Results: The best performing feed formulation and feeding regimen in terms of fish growth, lipid composition and liver metabolism were identified with this integrated approach. By proteomics, feed-specific and regimen-specific alterations were identified, especially concerning carbohydrate and lipid metabolism, that helped the identification of the best performing feed. Furthermore, information on how the feed formulation can be changed to increase its compatibility with fish metabolism were obtained. In addition, significant variations in serum apolipoprotein levels were identified in relation to the feed and the feeding regimen.
Conclusion: The proteomic analysis of liver and serum can contribute significantly to traditional morphometrics and lipidomics in increasing productivity and quality of aquaculture plants. In addition, the investigation on serum protein expression profiles can enable the identification of serum markers useful to track fish metabolism and its alterations.

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COMPARING SIMPLIFICATION STRATEGIES FOR THE FISH MUSCLE PROTEOME
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Introduction: Proteomic analyses are increasingly being adopted to monitor the quality, safety and authenticity of fish muscle. However, the protein complement of fish muscle is very complex with a few highly

abundant proteins typically dominating the expressed proteome. In this study, we have compared simplification strategies in order to characterise the fish muscle proteome more extensively.
Methods: Skeletal muscle was collected from common carp (*Cyprinus carpio*). Soluble extracts of muscle homogenates were fractionated either using ProteoMiner combinatorial peptide ligand library beads (Bio-Rad) or an OFFGEL fractionator (Agilent) prior to separation by 1-D SDS-PAGE. Protein bands were excised from the gel, subjected to in-gel digestion with trypsin and the resultant peptides were analysed by LC-MS/MS using a LTQ-Orbitrap XL mass spectrometer (Thermo) coupled to a nanoAcquity UPLC system (Waters). The number of unique proteins identified using each method was compared to that obtained by 1-D SDS-PAGE alone. Different search engines and sequence databases were also evaluated.
Results: The 1-D SDS-PAGE analysis revealed a large dynamic range in the distributions and intensities of protein bands. Using the ProteoMiner beads it was possible to abstract a substantial proportion of the high abundance proteins, allowing visualisation of additional protein bands by 1-D SDS-PAGE. OFFGEL analysis resulted in sequential fractionation of different protein populations with a concomitant increase in protein identifications. These simplification strategies enhanced the number of proteins identified from the fish muscle. In addition, greater sequence coverage was attained for many of the proteins, leading to increased confidence in assignment.
Conclusion: This study has compared different experimental approaches for proteome simplification in fish skeletal muscle. These strategies are able to improve the identification of low abundance soluble proteins by reducing the complexity of the protein complement of the tissue.

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IMMUNOREACTIVE PROTEINS OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS
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Introduction: *Mycobacterium avium* subsp*. paratuberculosis* (MAP) cause chronic enteritis of ruminants (bovine paratuberculosis-Johne disease) associated with enormous worldwide economic losses for the zootecnical industries.
Eradication programs for this pathology have been hampered by the lack of simple and specific diagnostic tests. Diagnosis is based on detection of antibodies in milk or serum, or by bacterial culture from feces, these diagnostic methods are usually applicable only when the disease is already in an advanced status.
A good and sensitive diagnostic method is required to avoid the spread and for the eradication of this pathology that afflicts most ruminant species of farm animals.
Methods: Mycobacterial lysis was performed using both freeze-thaw cycles and bead beating method. MAP proteins were immunoblotted respectively against control and MAP infected bovine sera.
The spots of interest were excised from the gel and analyzed through mass spectrometry.
Results: Among detected immunogenic spots two proteins were identified through mass spectrometry as major membrane protein-1 (MAP2121c) and hypothetical protein MAP3061c. MAP2121c has already being described, it is a surface protein in mycobacteria with a high immunogenicity[1]. MAP3061c is a predicted protein with putative electron carrier activity function.
Conclusion: Identified immunoreactive proteins could be used as tools for the detection of bovine paratuberculosis or as targets for ELISA tests or as epitopes for developing antibodies for MAP detection. Sequence of both protein is currently being aligned with similar bacterial proteins in order to find the non-conserved epitopes for MAP specific detection.
References: 1. Bannantine, J.P., Huntley, J.F.J., Miltner, E., Stabel, J.R. & Bermudez, L.E. The Mycobacterium avium subsp. paratuberculosis 35 kDa protein plays a role in invasion of bovine epithelial cells. *Microbiology* **149**, 2061 (2003).

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SPECIES IDENTIFICATION OF GELATINES IN YOGURT AND DAIRY DESSERTS BY UPLC-ESI-QTOF-MS

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Introduction: The use of unspecified gelatines in food products is a problem for people with specific diet wishes, such as people with an allergy, a specific religious background or a vegetarian diet. Accurate and truthful labelling of the source of gelatin is therefore needed and this requires a robust method that can identify and discriminate between the gelatines derived from different animal species.
Methods: In this study a generic ultra performance liquid chromatography - electrospray - time of flight mass spectrometry (UPLC-ESI-QToF-MS) method for protein identification was used to identify gelatines in fruit yogurt and (dairy) desserts. The amino acid sequence of collagen differs between species, which allows identification of the source of the gelatin used in food products. Gelatin was extracted and enriched from the dairy products and subjected to trypsin digestion. After reversed solid phase extraction of the peptide mixture, samples were analysed by UPLC-ESI-QToF-MS/MS. Identification of the gelatines was done by data analysis using Mascot.
Results: Fifteen yogurts, obtained from various retailers in the Netherlands, declared with and without gelatin, were analysed for gelatin content. It was found that six products did contain the gelatin as was declared on the product: two contained bovine gelatin, one contained porcine gelatin and three did not contain gelatin. Out of the nine products with unspecified gelatin, six contained gelatin from bovine source, two from porcine source and in one product no gelatin could be detected.
Conclusion: This study shows the applicability of an LC-MS/MS method for the identification of gelatin from porcine or bovine source in dairy products. Further research on the gelatin extraction from different (processed) food products might allow extension of the method to other gelatin containing (food) products, such as different types of candy, (canned) meat products or pharmaceuticals, allowing accurate control of product authenticity.

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PROTEOMICS AS A TOOL TO EVALUATE THE BIOACTIVITY EFFECTS OF FARMED SENEGALESE SOLE FED WITH A NEW DEVELOPED MICROENCAPSULATED FEED

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Introduction: Mass production of healthy, high quality juveniles is one of the main constraints to aquaculture development. In the early stages of development, weaning marine fish larvae with microdiets is still a bottleneck. This is due to difficulties in meeting larval nutritional requirements and the small size of the particles that leads to important losses of nutrients by leaching. However, recent studies show that meeting the fish larval nutritional requirements through inert microdiets brings very significant improvements in growth and quality of juveniles.
Methods: Our aim is to develop a microencapsulated feed for fish larvae of marine and small freshwater species that satisfies simultaneously a nutritionally balanced, with a high content of easily digestible protein (soluble protein), limited and controllable losses of water-soluble nutrients, attractive and well ingested by fish larvae and easily digestible.
Results: The new product will be based on the compatibility of all these requirements, through a critical evaluation of different microencapsulation technologies. The application of these technologies in the feed for fish larvae is an important element of innovation.
Conclusion: A proteomics evaluation of the potential bioactivity effects of the peptidic fractions included in the diets will be done. This bioactivity potential of peptidic fractions is already described both in fish larvae and mammals. A standard 2D-DIGE experiment will be performed and the proteome of larvae fed with a successful microdiet and a standard live feed, compared. Information on the nutritional metabolism and immune system of the larvae can be taken from the differentially expressed proteins between conditions.

References: This work is part of project 13380-MICALA co-financed by FEDER through PO Algarve 21 in the framework of QREN 2007-2013.The authors of the presented work declare no conflict of interest.

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COMPARATIVE PROTEOMIC ANALYSES BETWEEN VIRULENT AND ATTENUATED E.RUMINANTIUM: TOWARDS HEARTWATER VACCINE DEVELOPMENT

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Introduction: Ehrlichia ruminantium (ER) is an obligateintracellular bacterium that causes Heartwater, a fatal tick-borne disease inruminants, being a major limitation to livestock production in sub-SaharanAfrica and in some Caribbean islands. Currently tick control is performed using acaricidesand several vaccine candidates are under evaluation. Still the development of a fully effective vaccinehas been hindered due to the lack of knowledge on ER biology. We expect tocontribute to an increase of the knowledge of this subject that foster newvaccine strategies.
Methods: In thiswork, we compare the proteome of a virulent and an attenuated ER phenotype (obtainfrom the Gardel strain), both being currently used as inactivated andattenuated vaccines, respectively. The attenuated ERGardel phenotype derives from the virulent ERGardel isolatedin Guadeloupe and was obtained through over 200 laboratory passages in bovineaortic endothelial cells (BAE).
Results: Herein, ERwas harvested from infectedBAE monolayers, purified by a multistep centrifugation process and preparationof total ER protein extracts was optimized. Trials were done using a microdismembratorand sonication. 1DE gel profile with defined bands was obtained only using sonication.Differential protein migration patterns were clearly seen between attenuatedand virulent ER phenotypes. Protein bands will be excised, digested and submittedto microLC-MALDI-TOF/TOF-MS analysisfor protein identification.Complementary,differentiallyexpressed proteins of the two ER phenotypes will be analyzed from a total of 1300 well resolved spots by2DE and detected by cydye staining. Proteins spots then be identify and itbiological function acessed.
Conclusion: Amongstthe differentially expressed proteins in both phenotypes, we aim aim toidentify i) proteins related to attenuation mechanisms in ER Gardel and ii) someantigens with potential to elicit a protective cellular immune response inorder to develop a more effective immunization strategy against heartwater.Globally these results will contribute to improve livestock production insusceptible countries and increase food safety.

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THE PICO ALGAE OSTREOCOCCUS TAURI UNDER OXIDATIVE STRESS: A PROTEOMIC STRATEGY TO EXAMINE THE CELLULAR STRESS RESPONSE

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Introduction: The unicellular algae *Ostreococcus tauri* (*O.tauri*) is one of the most primitive eukaryotes and has the potential to serve as a key model organism for plant biology. However, the *O. tauri* genome lacks genes coding for catalase and other proteins known to participate in cellular oxidative damage repair. Despite this fact, O. tauri is found abundantly in the upper layer of the euphotic zone where reactive oxygen species (ROS) are produced (photochemical reactions with dissolved organic matter). In this work, we examined the ability of *O.tauri* to cope with various forms of oxidative stress using mass spectrometry-based proteomics as well as other complementary strategies.
Methods: *O.tauri* was grown in artificial sea water and subjected to different forms of oxidative stress (H_2O_2 and glufosinate). Cell viability and integrity was verified by FACS analysis and enzymatic assays. Samples were digested and analysed on an LC-coupled LTQ-Orbitrap XL mass spectrometer. Global label-free quantitation as well as enrichment of different redox states of cysteine using streptavidin biotin pull down were performed.
Results: FACS analysis of H_2O_2 –treated cells shows an initial disruption of the chloroplast followed by loss of cell integrity. In parallel, glutathione peroxidase activity decreased under oxidative conditions. A cluster of

enzymes involved in carbon fixation and oxidative phosphorylation pathways have been found with a significantly higher number of oxidised cysteines. Several other enzymes were found to be both up-regulated and containing a higher number of oxidised cysteines such as thioredoxin, thioredoxin/protein disulfide isomerase, and thioredoxin reductase.
Conclusion: The abundance of several different thioredoxin-based complexes identified in this study suggests that they play a key role in the cellular protective mechanisms against ROS.

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PROTEIN ADSORPTION ON ULTRA-HIGH MOLECULAR WEIGHT POLYETHYLENE JOINT - PROTEOMICS CORRELATED TO MALDI IMAGING MASS SPECTROMETRY DATA
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Introduction: Ultra-high molecular weight polyethylene (UHMW-PE) is used for artificial joints. Considerable benefits of biocompatibility, friction coefficient and wear resistance makes this material attractive, but patients suffer from implant failure. This aging is related to material oxidation, maybe enhanced by biomolecule adsorption *in vitro*. Understanding the failure mechanism on the molecular level requires a time-dependent study and identification of adsorbed biomolecules. MALDI imaging mass spectrometry (IMS) enables two-dimensional localization and identification of adsorbed components.
Methods: After incubating UHMW-PE blocks in synovia, excessive biofluid was removed. After slicing UHMW-PE samples into 15 µm sections (cryo-microtomy) they were mounted on indium-tin oxide glass slides. For protein identification (ID) trypsin was deposited on top, followed by incubation (37°C) before MALDI matrix application. IMS was performed on MALDI-TOF/RTOF-MS (Shimadzu Biotech Kratos Analytical) and MALDI-QqRTOF-MS (Waters) instruments. Biomap (Novartis) and MATLAB (Mathworks) were used to visualize analyte distributions. Incubated UHMW-PE was also leached and extracted proteins were separated by 2D gel electrophoresis (2D-GE). Again protein ID is based on in-gel digestion with trypsin. Peptide mass fingerprinting and/or peptide sequencing and Mascot search was used for final protein ID.
Results: IMS successfully visualized synovia related peptide distributions on UHMW-PE. Lubrication related proteins were identified and despite taking biological degradation into consideration statistical evaluation of the results revealed compound class pattern changes with prolonged incubation time. The identified analyte clusters are correlating with hydroperoxide, the most relevant material-inherent degradation marker. An orthogonal experimental setup separated adsorbed proteins by 2D-GE. Again time-dependent changes of the electrophoretic protein pattern were observed correlating nicely with IMS results. Protein IDs from 2D-GE and IMS matched.
Conclusion: We show that synovia related biological compounds adsorb on UHMW-PE surfaces in a time-dependent process. The degree of cross-linking of UHMW-PE influences adsorption patterns, adsorption preferences and diffusion dynamics.

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A HIGH RESOLUTION/ACCURATE MASS TARGETED APPROACH FOR KINASE INHIBITION SCREENING USING A QUADRUPOLE ORBITRAP MASS SPECTROMETER
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Introduction: Development of novel protein kinase inhibitors remainss a strong focus of the pharmaceutical industry. Performing multiplexed protein kinase detection/quantification presents many challenges. Recently, probe-based assays were introduced to increase kinase enrichment and provide a selective screening approach for kinase inhibitor analysis using tandem mass spectrometry. Here, we present a high resolution/accurate mass targeted approach for kinase inhibitor screening in A549 cells within a high-throughput workflow.
Methods: A549 cell lysates were treated with staurosporine(0-10 uM). Each sample was reacted with desthiobiotin ATP or ADP probes and then processed according to the manufacturing instructions to enrich labeled kinase active-site peptides. All mass spectrometry experiments were performed on a Q Exactive

(Thermo Scientific, Bremen). Initial kinase identification and quantification were performed in data dependent acquisition mode, followed by database searching and spectral library creation. Targeted quantification/verification was performed by narrow mass range multiplex SIM. Orthogonal experiments were performed by Western Blots.
Results: Desthiobiotin-ATP and -ADP are two nucleotide derivatives that have been shown to selectively label kinase active sites. Using these probes to enrich kinase active-site peptides, we identified 126 kinases from A549 cell extracts using high resolution, accurate mass (HR/AM) spectrometry. Targeted SIM multiplexing enables shorter acquisition cycle times and is fully compatible with LC constraints of targeted proteomics experiments. We also assessed specificity of kinase inhibitors - staurosporine by determining IC50 values for kinase active-site peptides after drug treatment. The results for the staurosporine treated A549 cell line showed over 70% kinases demonstrated strong inhibition by the drug as expected. In addition, we validated kinase inhibitor targets using a parallel Western blot workflow.
Conclusion: Kinase inhibition measured using targeted HR/AM spectrometry had strong correlation with Western blot data, previously published results, and enabled global profiling of kinase inhibitor target and off-targets.

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COMPREHENSIVE TARGETED QUANTITATIVE PROTEOMICS – TAKING MULTIPLEXED ASSAYS TO A NEW LEVEL
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Introduction: The goal of quantitative proteomics is to both identify and quantify a broad range of proteins and peptides. The extreme complexity and dynamic range of proteins in typical proteomic samples challenges traditional data dependent workflows by requiring very high speed MS/MS acquisition to reproducibly and deeply interrogate the sample. The application of data independent acquisition strategies to increase the reproducibility and comprehensiveness of data collection has been limited by the speed of current mass spectrometers. Recent QqTOF innovations providing high speed acquisition of high resolution MS/MS spectra have enabled a new data independent acquisition strategy.
Methods: In this workflow (called MS/MS^{ALL} with SWATH™ Acquisition), the Q1 quadrupole is stepped at 25 amu increments across the mass range of interest, passing a 25 amu window through into the collision cell. The transmitted ions are fragmented and the resulting fragments are analyzed in the TOF MS Analyzer at high resolution. This is done in a looped fashion with an LC compatible cycle time, such that MS/MS spectra has been acquired on every peptide in the sample. Because the fragment ions are collected at high resolution, high quality XICs can be generated post-acquisition to produce the MRM-like data.
Results: The utility of this workflow for highly multiplexed targeted quantification in complex proteome samples will be explored. Working from peptide MS/MS spectral libraries, XICs for target proteins and peptides can be generated from this comprehensive dataset. Initially, XICs to large #s of peptides and proteins were extracted and analyzed for quantitative reproducibility. Impact of various acquisition conditions on quantitative specificity was explored and optimized. Finally, this technique will be compared to more common quantitative techniques such as MRM quantification.
Conclusion: SWATH™ acquisition has the potential to generate MRM specificity quantitative data for all detected peptides in a complex dataset. This represents a significant advance in quantitative analysis, as high specificity quantitative data can be acquired in a non-targeted fashion, allowing quantitation to be performed retrospectively on proteins of interest.

P150

DEVELOPING PEPTIDE BASED BIOMATERIALS FOR STEM CELL DIFFERENTIATION IN VITRO
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Introduction: The innate physical characteristic of the cell niche is known to play an important role in lineage commitment of mesenchymal stem cells as they undergo differentiation. An understanding of how to mimic physical traits of the extracellular matrix is a valuable tool in being able to achieve targeted stem cell

differentiation in vitro. The study aims to elucidate changes in the cell activity as it undergoes early stage differentiation.
Methods: Biomaterials used for directing stem cell differentiation were made by converting short chain peptide solution of diphenylalanine and serine into hydrogel substrates. The rigidity of these hydrogel substrates were tuned accordingly to mimic those of naturally occurring cell niches using pH alteration. Resultant Young's modulus for hydrogels were 2kPa (soft), 6kPa and 36kPa mimicking that of adipose, muscle and osteoid (demineralised bone) tissue types.
Cells were cultured on these substrates for up to one week after which samples were assessed for differentiation biomarkers using polymerase chain reaction (PCR) analysis and immunofluorescent labelling. Samples were then assessed using liquid chromatography coupled to mass spectrometry (LC-MS) for metabolite identification and subsequent profiling study.
Results: Cells were found to metabolically quiescent when maintained in culture and distinctly active on the hydrogel substrates. Cultured cells on the soft 2kPa hydrogels supported differentiation to towards soft tissue types while cells cultured on the 6 and 36kPa hydrogels showed a strong tendency toward cartilage and bone formation (harder tissue types). These differences in cellular behaviour could also be ascertained from examination of the cellular metabolome.
Conclusion: Being able to track and profile distinct changes in cell activity over time as they respond to a number of external stimuli allows stem cell behaviour to be subject to detailed scrutiny. The plethora of information obtained from a metabolomics approach toward studying stem cell behaviour in vitro opens up the possibility of identifying small molecules that can play a key role in stem cell differentiation. This also possesses value with regards to the manner in which future biomaterials for cell culture is developed.

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TO DIGEST OR NOT TO DIGEST, THAT'S THE QUESTION

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Introduction: Top-down MALDI-Imaging Mass Spectrometry (IMS) of proteins in tissue sections represents a powerful approach to biomarker discovery. However, lack of direct identification strategies is preventing its broader use in Proteomics studies. Shown here is a new workflow that combines the spatial information in IMS with the routine identification of proteins from tissue sections by LC-MS/MS. Tryptic protein digests were generated on two subsequent tissue sections maintaining the spatial distribution of the peptides. One section is analyzed by IMS, peptides from the other section are identified by routine LC-MALDI-MS/MS.
Methods: Fresh frozen rat brain / testis samples and human breast cancer biopsies were cut into 10 µm thick slices. Each section was analyzed in parallel with and without tryptic digestion. For the digests, two subsequent slices were placed onto one glass slide and a trypsin solution was applied by supersonic vibration. Tryptic peptides were MALDI-imaged from one section. The other section was analyzed by LC-MALDI for peptide identification by Mascot database search. A software tool groups the peptide masses by their associated protein and links it to the peptide masses in the bottom-up image for visual validation of their co-localization.
Results: In the rat organs, more than 200 peptides in the image (= 80 %) were identified by LC-MALDI and ca. 120 proteins localized. Biologically relevant proteins were identified such as thymosin-α4 and LCFA-CoA from rat testis. CRIP1, a novel marker for metastatic cancer, was identified in the breast cancer biopsies as one in 150 proteins. Its Arg-68 was present predominantly in the methylated form and to a lower extent in the non-modified form. Arginine methylation heterogeneity present in CRIP1 was in agreement with the unexplained top-down imaging peak pattern [Rauser 2010 JPR 9:1854-63] in breast cancer biopsies.
Conclusion: The new "Spatial Proteomics workflow" allows routine identification of more than 100 proteins from tissue sections as well as their spatial distribution at the 50 µm level. The method may also be applied to FFPE tissue which is largely accessible from tissue banks, but where top-down analysis is made impossible by protein crosslinks.

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LABEL-FREE PHOSPHOPEPTIDE QUANTITATION AND OCCUPANCY DETERMINATION FROM A SINGLE PROTEIN USING LC-MALDI

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Introduction: Protein phosphorylation has gained much attention in proteomics. Most work focuses on establishing catalogues of phosphopeptides in a proteome of interest and determining whether a certain peptide is phosphorylated or not, but this research stops short of checking for the occupancy of individual residues while verifying multiple phosphorylation sites. We describe a new approach for the quantitative assessment of phosphorylation occupancy at various previously known target sites within a single isolated protein resembling the situation after immunoprecipitation of a protein in question.
Methods: The protein KAPCA_HUMAN contains 10 known phosphorylation sites, whose occupancy was quantified. The sample was trypsinized and split into two aliquots. One of the aliquots was enzymatically de-phosphorylated, so that a native (N) and a dephosphorylated (D) peptide aliquot were obtained. Aliquots were analyzed in replicates by nanoLC-MALDI-TOF/TOF for peptide identification and the label-free quantification of the relative abundance of the free, unphosphorylated peptides. The intensity of the D-peptides was assumed to be 100 % free peptide. By subtracting the lower intensity of the N- peptides (1-x), the occupancy x [%] could be determined
Results: KAPCA_HUMAN contains 10 phosphorylation sites, 8 partial and 2 stoichiometric. For the determination of the occupancy, 3 replicate LC runs of 1 pmol of each the native (N) and the dephosphorylated (D) tryptic digest were done. MALDI-MS/MS and Mascot search provided peptide sequence verification. The native sample yielded pairs of phosphorylated and non-phosphorylated peptides with the same sequence. Unique to this method, only the non-phosphorylated peptides were used for the occupancy determination.
Conclusion: As expected, the peptides containing the stoichiometrically phosphorylated residues were detected with 100 % phosphorylation. Three other sites were non-stoichiometrically phosphorylated at Ser10 (93 %), Ser139 (37 %), and Ser259 (23 %). For 5 residues, phosphorylation was not detectable, implicating that the degree of phosphorylation at these 5 sites was less than 2 %. The coefficients of variation (CVs) of the label-free dephosphorylation-LC-MALDI method are within a range of 10 to 30 % in agreement with established and cost intensive isotope labeled reference methods.

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NEW NANO ESI SOURCE DEVELOPMENT FOR INCREASED PERFORMANCE NANOLC-MS WITH PLUG-AND-SPRAY CONFIGURATION

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Introduction: Nano-flow LC-MS is widely used for qualitative and quantitative proteomics studies due to its high sensitivity and specificity;Improper connections often result in leaks and large swept volumes that cause substantial peak broadening and thus poor sensitivity while a poor high voltage connection will yield poor data because of unstable spray. In order to address these common issues, we have developed a new nano-electrospray source and emitter concept in which, a column, column heater, high voltage electrode and an emitter are combined in one ready-made assembly. The performance of this new source concept was evaluated.
Methods: A prototype of new developed nano-electrospray source was installed on the Orbitrap Elite mass spectrometer and connected to an Easy-nLC 1000 nanoLC pump. Three column (50 µm id x 15 cm, 2 µm) assemblies were used for evaluating column-to-column and run-to-run reproducibility. Both complex peptide mixtures and simple digest mixture were used. The retention time reproducibility, peak shape, resolution and peak capacity in different temperature ranges were evaluated. In order to test the device flexibility, several flow rates from 150 nl/min to 1000 nl/min were used. The mobile phases were 0.1% FA/H2O and 0.1% FA/MeCN. A 60 min linear gradient was used for the complex sample separation. A 15 min linear gradient was used for the simple digest sample separation

Results: The column assembly is positioned in the source without the need to adjust the X,Y, or Z positions of the emitter. A high spray stability was achieved over all LC runs with three different columns. The obtained chromatographic resolution, sensitivity, and reproducibility matches other state-of-the-art data without need for adjustments (or expert intervention) beyond plugging in the column/sprayer assembly. More than a thousand proteins were identified with increased component detection from the complex protein digest mixtures. The reproducibility of the retention time from column to column for the targeted peptides from the simple standard mixtures was less than one percent.
Conclusion: Newly developed nano-electrospray source allows non-LC-MS experts to get optimized nanoLC-MS results by using an integrated column-emitter design.

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IMPROVING INTACT PROTEIN AND TOP-DOWN ANALYSIS BY ORBITRAP MASS SPECTROMETRY
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Introduction: The ultra high resolution of the Orbitrap Elite is one of the most important pre-requisites for intact protein characterization and top-down analysis. Resolving power of intact protein mass measurements is limited by the pressure in the Orbitrap mass analyzer due to fast transient decay caused by collisions with residual gas. A standard Orbitrap Elite ETD instrument has been modified to accomplish high molecular weight ion characterization.
Methods: All sample measurements were performed on a modified Thermo Fisher Scientific Orbitrap Elite ETD instrument coupled to an LC. The modifications include a switching valve to control the pressure in the HCD collision cell and changes in the instrument control software. An intact monoclonal antibody in non-reduced and reduced form was used for the instrument performance characterization. ProSight PC 2.0 and Protein Deconvolution 1.0 were used for data evaluation.
Results: Reduction of gas load in the C-trap/HCD collision cell reduces pressure in the Orbitrap mass analyzer and thus reduces of the decay of transients for intact proteins. However, lowered pressure in the C-trap/HCD collision cell results in undesired reduction of trapping efficiency in the C-trap. This effect is compensated by trapping ions in the HCD cell for all modes of operation with subsequent gentle transfer of cooled ions into the C-trap prior to injection into the Orbitrap analyzer. The reduced pressure in the Orbitrap mass analyzer leads to the detection of more discrete beats in the transient of high molecular weight ions thus facilitating the upper mass limit of isotopic resolution up to around 50 kDa. In addition, lower number of collisions during ejection from the C-trap was found to result in an increased signal even for the first beat. This increases signal-to-noise in spectra of larger proteins such as antibodies and improves analysis of their glycosylation status. Top-down analysis using SID, CID, HCD, and ETD is achieved for the reduced antibodies.
Conclusion: Modifications to a standard ion trap-FTMS hybrid instrument for improved intact protein analysis and top-down analysis.

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SEPARATION OF PHOSPHOLIPIDS FROM BIOLOGICAL EXTRACTS USING A SOLID CORE REVERSE-PHASE LC COLUMN
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Introduction: The role of lipids on cell surfaces is known to be of key importance to cell function and inter-cellular communication. Lipidomics is rapidly growing in interest and understanding the lipids present within cells is an important aspect of biological studies. The separation, detection and classification of lipids by conventional LC-MS methods is not without its challenges. Lipids belong to many different classes and generally include a polar head group and at least one attached hydrocarbon chain. Their analysis is complicated by the wide variation in composition and structures present in any biological extract and high retention on C18 reverse phase stationary phases. Solid-core stationary phases have shown to be able to yield UHPLC-levels of resolution due to the small depth of the porous shell resulting in faster more limited

diffusion within the particle and more uniform paths through the LC column. This can be attained without the UHPLC side effect of elevated backpressure which is associated with small particle sizes. A separation protocol for the analysis of lipids by LC-MS using a solid-core stationary phase will be presented.
Methods: *E.Coli* and *yeast* lipid extracts were analysed by reverse phase HPLC with a methanol gradient. An Accucore RP-MS column (2.1 x 100 mm dimension, 2.6 µm particle size) was used for the HPLC separation with negative ion MS detection.
Results: Separation of lipids from *E.Coli* and *yeast* lipid extracts were achieved using conventional, MS-compatible conditions. Analysis of the data revealed separation according to lipid-class within the *E.coli* lipids (phosphatidylethanolamine and phosphatidylglycerol lipids formed two distinct clusters within the chromatographic separation). Separation of residual peptides from a lipid sample can also be achieved using this technique, showing that any peptides which may be present in a biological sample can be removed without impacting the lipid separation. Excellent chromatographic resolution of the lipids enables optimal detection and minimises any MS peak suppression due to co-elution.
Conclusion: We propose that solid core stationary phases offer a number of advantages for the analysis of lipids including excellent peak shape and separation of a wide range of lipids present in biological sources without high backpressures.

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TRYPTIC DIGEST IN PROTEOMIC WORKFLOWS: AN EASY TO USE TEST TO MONITOR REPRODUCIBILITY OF TRYPTIC SAMPLE PREPARATION
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Introduction: The complete tryptic digest of proteins in biological samples is an important step in MS-based proteomic workflows. Incomplete digestion will impair the qualitative and quantitative results of such experiments. Different protocols for tryptic digestion have been described and available trypsins vary in quality. Therefore, an easy to use test to prove efficiency of tryptic digestion and reproducibility of sample processing would enable monitoring of this important step.
Methods: A set of model peptides to control tryptic digestion has been prepared. The design criteria were as follows: (i) different resistance to tryptic cleavage, (ii) all peptides and cleavage products to produce clear MS-signals and to be easily separable by MS, (iii) sequences do not occur in native proteins and (iv) the retention times of cleavage products to span a wide range of typical proteotypic peptides and to be associated with an SSRT value estimation.
The model peptides were synthesized and digested as mixtures under varying incubation conditions. Additionally, the digestion performance of trypsin lots from different suppliers was compared.
Results: The generated peptide set was well suited to determine differences in digestion conditions and between trypsin lots from various suppliers. The fact that all peptides and cleavage products do not occur in natural proteins allowed performing trypsin efficiency evaluation in the same vial as the protein digests. Additionally, the peptide set provided reference peptides for retention time calibration.
Conclusion: The developed set of model peptides enables efficiency determination of trypsin cleavage, which is essential for all MS based proteomics approaches. The "*in situ digestion"* performed as part of routine protein digestion workflows provides a measure for reproducibility of sample processing in different experiments. No additional equipment is necessary for the measurement.
References: Schnatbaum, K., Zerweck, J., Nehmer, J., Wenschuh, H., Schutkowski, M., Reimer, U., SpikeTides - proteotypic peptides for large-scale MS-based proteomics, application note in *Nature Methods* 8 (2011).

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THE 15N ISOTOPE EFFECT: A MEANS FOR CORRELATING PHENOTYPE AND PATHWAY ALTERATIONS
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Introduction: Stable isotope metabolic labeling is frequently used for quantitative proteomics comparisons. Could the introduction of a heavy stable isotope *per se* affect biochemical pathways and the phenotype of an organism?
Methods: To investigate the stable isotope effect, we metabolically labeled *in vivo* high anxiety-related behavior (HAB) mice with the heavy nitrogen isotope ^{15}N and compared them with ^{14}N (unlabeled) HAB mice at the behavioral (1) and proteome (2) level. We analyzed several brain regions (hippocampus, cingulate cortex, cerebrum) and subproteomes (cytosol, synaptosomes) followed by *in silico* analysis to identify altered pathways in ^{15}N-labeled HAB mice.
Results: At the behavioral level, ^{15}N-labeled HAB mice display a decreased depression-like behavior compared to ^{14}N HAB mice. At the brain proteome level, ^{15}N-labeled mice exhibit alterations in major metabolic pathways pathways, including oxidative phosphorylation and the citric acid cycle.
Conclusion: The correlation of the proteomic and behavioral alterations induced by the ^{15}N isotope introduction may provide information on the neurobiological underpinnings of a behavioral phenotype. Thus, ^{15}N metabolic labeling apart from being a robust tool for quantitative proteomics has the potential to shed light on molecular mechanisms of behavior.
References: (1) Frank et al, 2009 PLoS One 4:e7821
(2) Filiou et al, 2012 Proteomics (in press)

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NITROGEN STRESS INDUCED LIPID PRODUCTION IN THE MODEL ALGA CHLAMYDOMONAS REINHARDTII - A PROTEOMIC INVESTIGATION
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Introduction: Nutritional manipulation of microalgae has commonly been employed to increase the relative abundance of energy storage molecules, such as lipids. Although desirable for lipid accumulations and hence biofuel production, such crude manipulations may have a deleterious effect on growth and culture health. In this study, we utilised the 8plex variant of iTRAQ (isobaric tags for relative and absolute quantitation) to analyse *Chlamydomonas reinhardtii* proteomic extracts over the course of nitrogen starvation, in order to understand the effects of nitrogen starvation on the biochemical machinery of *C. reinhardtii.*

Methods: *C .reinhardtii* (CCAP 11/32CW15+) was cultured for 24 hours in TAP media containing 375 mg ml^{-1} NH_4Cl. Cultures were grown at 25˚C under 350 µE m^{-2} sec^{-1} light on an orbital shaker. The cells were harvested and resuspended in nitrogen-free TAP media. Samples were then taken at 0,6 and 39 hours post re-suspension, representing periods of normal, high carbohydrate and high lipid production. Protein samples were prepared and labelled with iTRAQ labels following manufacturer's guidelines. Labelled peptides were either fractionated by HILIC or SCX before being run on a QToF. Protein assignment was conducted using ProteinPilot and statistical analysis in R.

Results: 587 proteins were identified (>3 peptides) of which 70 and 311 were found to be significantly changed ($p<0.05$) during nitrogen stress induced carbohydrate and lipid production. Several trends could be observed including: an increase in energetic pathways, a decrease in translation machinery, increase in cell wall production and a change of balance between photosystem I and II.

Conclusion: This study provides an initial overview of the proteomic changes in *C. reinhardtii* under nitrogen starvation and highlights several candidate proteins for further investigations. The study also reveals how crude biochemical manipulation dramatically affects the culture. This further suggests that through continued understanding off the lipid induction mechanisms within *C. reinhardtii,* significant improvements can be made in the manipulation of cultures for optimal biofuel production.

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OPTIMIZING NANOLC OF COMPLEX PROTEOME DIGESTS FOR HIGH-SPEED, HIGH-RESOLUTION MASS SPECTROMETERS TO OBTAIN THE HIGHEST COVERAGE IN A SINGLE ANALYSIS.

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Introduction: 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 hardware with high sensitivity and large dynamic range capabilities. High-resolution peptide separations are a prerequisite for diving deeper into complex proteomes. Coupled with a high speed Qq-TOF system that is capable of high acquisition speeds while maintaining high resolution and spectral quality in MS and MS/MS modes, LCMS can truly capitalize on increases in chromatographic peak capacity, and yield dividends in the push for whole-proteome coverage.
Methods: Yeast and Human cell lysate digests were analyzed using an Eksigent nanoLC-Ultra® 2D system coupled to a TripleTOF™ 5600 system. Varying amounts of lysate were loaded onto various HPLC and UPLC Columns (75 to 200µm x 15-50cm ChromXP C18-CL 3µm 120Å) and washed for 10min at 200nL/min. Elution gradients of 5-35% acetonitrile (0.1% formic acid) in 120-240 min were run. Eluent was analyzed using the TripleTOF™ 5600 system with a nanoflow source. One TOF MS and 20 to 50 MS/MS scans/second were acquired. Datasets were searched against the Swissprot database using ProteinPilot™ software.
Results: Various sample loadings on column were run to explore the optimal sensitivity and speed of the instrument for in-depth sample interrogation. The optimal acquisition rate on the smallest column (75µm x 15cm ChromXP C18-CL 3µm 120Å) was achieved with 2 µg of protein digest on column, where an average of 46 MS/MS in a second were acquired. With 2 µg of human cell lysate digest in a 120 min gradient, >24,616 peptides and > 3368 proteins were identified at a 1% global FDR rate. Doubling the column length (75µm x 30cm ChromXP C18-CL 3µm 120Å) resulted in a 14 % increase in the number of protein identifications and a 20% increase in the number of peptides identified.
Conclusion: Preliminary results indicate that significant improvements in the number of proteins and peptides identified in a single acquisition have been achieved through increases in column length and chromatographic resolution.

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DETERMINATION OF CORE FUCOSYLATION IN GLYCOPEPTIDES OF MONOCLONAL ANTIBODIES

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Introduction: Fucosylation is a glycan modification which affects glycoprotein confirmation and function. α-1,6 fucosylation of the chitobiose core found on N-linked glycans plays an important role in hysiological processes. Involvement of core fucosylation has been shown in pathological processes like cancer and described as biomarker for liver cancer malignity. Here we describe a fast and reliable approach to unambiguously determine core fucosylation directly on the glycopeptides in tryptic digests by MALDI-TOF/TOF and ESI-IT.
Methods: Glycoproteins like asialofetuin, horseradish peroxidase and a panel of therapeutic and standard monoclonal antibodies (e.g., MOPC21 from Sigma) were reduced, alkylated and trypsinized. Tryptic peptides and glycopeptides were separated by RP-LC and fractions were analyzed by ESI-IT-MS and MALDI-TOF/TOF-MS in positive ion mode. A dedicated software algorithm for N-linked glycopeptide analysis automatically classified MS/MS spectra taking the specific fragmentation patterns of MALDI and ESI CID into account, clearly distinguishing glycopeptides with or without core fucose. Database searches provided glycan structures and peptide sequences.
Results: The fragmentation patterns derived from N-linked glycopeptide fragmentation by MALDI and ESI analysis was used to filter glycopeptides rom a large number of MS/MS spectra. Both, peptide and the glycan masses of individual glycopeptides were determined and enabled detection of fucosylation at the chitobiose core. In ESI-IT, the peptide fragment mass including the first GlucNAc of the chitobiose is detected in the

majority of N-glycopeptides. In addition, various typical fragments for core (non-)fucosylated N-glycans, for peptide & GlucNAc, for peptide & GlucNAc & fucose and for the peptide & 2 GlucNAc & fucose are observed.
Conclusion: This information was used to adapt the glycoprotein analysis software by extending lists of fragmentation patterns to distinguish different glycan structures. That capacity enabled us to identify the high glycan micro heterogeneity at HC-Asn294 in murine IgG1 MOPC21. Core fucosylation was found to be a common modification in more than 50 % of the identified N-glycans. The main glycan in MOPC21 is G0F1 (GlucNAc4 Man3 Fuc1), followed by the same structure lacking the core fucose (G0).

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LABEL-FREE QUANTITATIVE PROTEOMIC ASSESSMENT OF STRESS-RESPONSES IN CANDIDA ALBICANS USING MOBILITY ASSISTED DATA INDEPENDENT LC-MS
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Introduction: *Candida albicans* (*CA*) survives in humans most commonly as a commensal organism in the flora of the GI and urogenital tracts. In subjects that are immunocompromised, *CA* can cause life-threatening infections. Stress adaptation is critical for pathogenicity. Here we use LC coupled with ion mobility mass spectrometry in order to gain a greater understanding and measure of the salt-stress response of *CA* at the proteomic level.
Methods: Samples were trypsinized and separated over a 90 minute linear RP-LC gradient. Data were acquired using an ion mobility data independent HDMSE approach, processed using ProteinLynx Global SERVER, and searched against a *CA* database. Normalized label-free quantitation results were generated using Progenesis software.
Results: Six *CA* digest samples (three based on normal conditions and three subjected to salt-stress) had 10 fmol/µl phosphorylase B digestion standard added and were analysed in triplicate, allowing for protein abundance levels to be measured. The search results revealed more than 1500 protein identifications per run. Quantitative results were achieved over a wide dynamic range using the three most intense peptides of the internal standard to determine a response factor for quantification of other proteins in the sample. The data was interrogated further using Progenesis LC-MS software. Data interpretation returned confident expression ratios for over 1000 proteins and indicated that enzymes involved in glycerol metabolism and heat-shock proteins are some of the most highly up-regulated proteins during the salt-stress. Low abundance regulated proteins identified in the salt-stress samples were targeted in a separate acquisition on the new high sensitivity Synapt G2-S platform. A comparison of the correlation variance (CV) and fold change between the two groups will be presented.
Conclusion: Mobility assisted data independent label-free LC-MS has been used to quantify the expressed proteome of *CA* which have been exposed to normal and salt-stress conditions.

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HIGH DEFINITION ION MOBILITY MALDI IMAGING FOR THE VISUALISATION AND IDENTIFICATION OF PROTEINS DIRECTLY FROM ON-TISSUE TRYPTICALLY DIGESTED SECTIONS
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Introduction: A data independent MALDI imaging acquisition method is presented, where MS and MS/MS data are acquired within a single experiment, without any precursor selection requirements. Post acquisition, the precursors and the fragments are correlated on the basis of their common drift time, which is further refined utilizing spatial distribution commonality.
Methods: Data were acquired using a MALDI SYNAPT G2 with tri-wave ion guide optics to separate ions according to their gas phase mobility. Within the same experiment, the MS was set to apply alternate collision energies to the transfer cell between low energy (peptide data) and elevated collision energy ramp (peptide fragment data). As fragmentation took place post-ion mobility separation, the precursors at low energy had

the same drift time as their associated fragments from the elevated energy experiments. The data were processed using novel High Definition Imaging (HDI) MALDI software.
Results: In order to digest the proteins directly from tissue, several coats of a trypsin were applied to rat brain using a SunCollect sprayer. After incubation, CHCA matrix was also applied. The MALDI imaging experiment was designed such that adjacent pixels had low or elevated collision energy applied. Using HDI MALDI software, the low and elevated energy functions were independently processed with Apex 3D detection software and independently visualised. Using the drift time alignment functionality, tryptic peptide precursors were associated with product ions that share similar drift times. For additional and supplementary refinement, another level of correlation was achieved on the basis of spatial distribution commonality. Further work will be conducted and shown with the automatically HDI generated output (i.e. pkl file) for the automatic sequence annotation of multiple multiplex peptide spectra and identification of proteins from a single MALDI imaging experiment.
Conclusion: Here we present a data-independent method allowing multiple proteins localisation and identification in a single experiment using unbiased fragmentation, IMS and spatial correlation.

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ULTRA-HIGH SENSITIVITY IN PROTEOMICS USING NANOUHPLC AT 20 NL/MIN
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Introduction: In nanoLC-ESI-MS, sensitivity can be increased by limiting in-column dilution of the sample, typically achieved by reducing the inner diameter (ID) of the column. This results in higher ion counts detected with MS, allowing more peptides to be sequenced and thereby improve protein identification. At their limit of detection, the number of peptides detected in complex samples can increase by more than a factor of 100 just by decreasing the column diameter from 75 µm to 30 µm ID.
Columns of 30 µm ID (or less) require flow rates well below the performances of most commercially available nanoLC pumps. So far, these flow rates are achieved by home-made flow splitters. This approach is not easy and prone to human errors. Therefore, a splitless pump delivering flows below 100 nL/min is highly appealing. Low flow rates increase the efficiency of the electrospray process, contributing to the overall gain in sensitivity.
Methods: Packing high quality columns with ID of 50 µm or narrower is not easy. One drawback is that they usually need to be fitted to the switching valve by the sleeve-ferule-nut paradigm, which is not ideal because extremely low flow rates dictate extremely low dead volumes. Therefore the column needs to be carefully and precisely tight in order to avoid extra-column volume. Column damage and solvent leaks are additional drawbacks related to this procedure.
Results: Here, we describe an easy and robust solution to nanoLC-ESI-MS at ultra-low flow rates, tackling both the flow delivery system and the column limitations of the current state of the art nanoLC. The results are a splitless pump capable of accurate and precise gradient formation below 75 nL/min, and 25 µm ID UHPLC columns with outstanding performances, stability, and column to column reproducibility. The columns were implemented with UHPLC compatible, finger tight, leaking proof connections.
Conclusion: Separation of peptide mixtures with different complexity is shown using an Orbitrap MS/MS. An extensive series of tests using medium to low quantities of tryptic peptides from a HeLa cell lysate were performed to assess the robustness and improved sensitivity of the ultra-low flow delivery system used with 25 µm ID columns.

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GLOBAL DYNAMICS OF THE E. COLI PROTEOME AND PHOSPHOPROTEOME DURING IN VITRO GROWTH
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Introduction: Recent phosphoproteomic studies have generated large datasets of bacterial phosphoproteins, enabling physiological characterization of processes regulated by Ser/Thr/Tyr phosphorylation; however, most

phosphoproteomics studies in bacteria were so far qualitative. Here we perform a global, quantitative analysis of proteome and phosphoproteome dynamics of *E. coli* during *in vitro* growth.
Methods: We combined two SILAC experiments to analyze five different growth phases: lag/exponential transition (T1), exponential (Exp), exponential/stationary transition (T2), early stationary (ES) and late stationary phase (LS). The SILAC experiments included three cell cultures each, labeled with Lys0 (Exp), Lys4 (ES) and Lys8 (LS); and Lys0 (T1), Lys4 (ES) and Lys8 (T2). Protein extracts were mixed, digested by endoproteinase Lys-C and subjected to global (phospho)proteome analysis. The samples were analyzed on an LTQ-Orbitrap Elite mass spectrometer. MS data were processed by MaxQuant software.
Results: We detected a total of 2244 proteins, which represent about 80% of the *E. coli* proteome considered to be expressed during *in vitro* growth. Of detected proteins, 1982 were quantified in all measured phases of growth. In the phosphoproteomic experiment we quantified the dynamics of 161 S/T/Y phosphorylation sites. Downstream bioinformatic analysis revealed distinct clusters of co-regulated proteins and phosphorylation sites for each analyzed phase of growth. Interestingly, the quantified phosphorylation sites showed a general up-regulation in the late stationary phase. Among them were proteins related to stress (superoxide dismutase, cspC stress protein) and other important cellular processes, such as carbon metabolism and protein biosynthesis. An example is EF-Tu, previously suggested to be a substrate of HipA kinase and implicated in antibiotic resistance, whose phosphorylation increased by 4 fold during the LS phase.
Conclusion: Ser/Thr/Tyr phosphorylation is an emerging regulatory modification in bacteria. Our data reveal protein expression and phosphorylation profiles in the individual growth stages for an unprecedented number of *E. coli* (phospho)proteins, providing a detailed insight into regulatory processes involved in bacterial growth.

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ON LINE TLC-MALDI FOR THE CHARACTERIZATION OF NEUTRAL AND ACIDIC GLYCOSPHINGOLIPIDS: QUANTITATIVE AND QUALITATIVE ANALYSIS.
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Introduction: Glycosphingolipids are a wide class of ubiquitous lipids, characterized by a great structural and functional variety. Importantly, altered levels of these lipids have been correlated with different diseases, suggesting their crucial role in health. Classical methods for the characterization and quantification are mainly based on often unspecific antigen-antibody reactions or by cumbersome radioactive labelling followed by TLC and retention factor (R_f) comparison with known standards. Herein we set up an online analytical methodology which combines the HPTLC chromatography and the high resolving power and mass accuracy of MALDI mass spectrometry, directly performed on the HPTLC plate.
Methods: Total lipids from wild-type and NEU3 sialidase silenced C2C12 murine myoblasts, were extracted with 20:10:1 (v/v/v) chloroform/methanol/water. The aqueous and organic phases were analysed by HPTLC, followed by MALDI-TOF and results compared to [^{3}H]sphingolipids radiolabeled HPTLC. To improve GSLs detection, a number of matrices and methods for matrix delivery were tested. Quantitative analysis were conducted with serial dilutions of GSLs standards.
Results: Analysis of myoblast sphingolipid patterns by the HPTLC-MALDI gave comparable sphingolipid profiles to those obtained by radiolabeling. However, mass spectrometry resolution allowed to identify several species with similar R_f on the HPTLC plate not resolved with the radiolabelling method. Several sphingolipids that differed for their fatty acid chains could be distinguished. In particular, neutral GSLs (SM, Gb3, LacCer, GlcCer) were identified as C16:0, C22:0, C24:1 and C24:0 chains, whereas gangliosides (GM1, GM2, GM3, Gd1a) as C16:0, C18:0 and C20:0 chains. Quantitative analysis showed a linear trend comparable to radioactive measurements.
Conclusion: Compared to traditional measurements, on line TLC-MALDI is an easy and high-throughput analysis for the qualitative and quantitative characterization of GSLs suggesting its use for their profiling with high specificity and sensitivity.
Acknowledgment: Supported by IIT (SEED IPG CHIP 21531) and FIRB RBRN07BMCT

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LIPIDHOME: A DATABASE OF THEORETICAL LIPID STRUCTURES RELEVANT TO HIGH THROUGHPUT LIPIDOMICS.

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Introduction: Protein sequence databases are the pillar upon which modern proteomics is supported, representing a stable reference space of predicted and validated proteins. The main example of such resources is UniProt, enriched with both expertly curated and automatic annotations. While taken largely for granted, mature resources such as UniProt in proteomics are far from a reality in some other 'omics' fields, lipidomics being one of them. While having a seasoned community of wet lab scientists, lipidomics lags significantly behind proteomics in its adoption of data standards and other core bioinformatics concepts. This work aims to fill the gap for an equivalent resource to UniProt, called 'LipidHome', that provides theoretically generated lipid molecules and useful metadata.
Methods: Using the Chemical Development Kit Java Library, a MySQL database was populated with theoretical lipid species, generated from a set of community agreed upon chemical bounds. In parallel to the database, a dynamic web application was developed to present the information and provide flexible computational access via a web service. Underpinning the web application is the SPRING framework with an ExtJS 4 client presenting the data.
Results: Designed specifically to accommodate high throughput mass spectrometry based approaches, lipids are organised into a hierarchy that reflects the variety in the structural resolution of lipid identifications. From 'species' e.g. PC 36:2 via 'sub species' e.g. PC 18:0/18:2 to isomers e.g. PC 18:0/18:2[3,6]. On top of this data cross references to other lipid related resources were integrated and text mining used to annotate lipids with relevant papers that mention them. The web application encompasses a browser for viewing lipid records and a tools section where an MS1 search engine is currently implemented. The code is open source and the live web application currently hosted at www.ebi.ac.uk/apweiler-srv/lipidhome.
Conclusion: The field of lipidomics stands a lot to gain from the example set by proteomics. This work is hopefully a step in the right direction towards closing the gap between these 'omics' fields.

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COILED-COIL PROTEINS – ANALYSIS OF STRUCTURALLY CHALLENGING TARGETS BY 3D PROTEOMICS

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Introduction: Knowing the structural arrangement of protein complexes is vital for understanding their function. Some structural features of proteins and protein complexes are notoriously difficult to analyse by traditional means. The coiled-coil is one such feature. Unfortunately for structural biologists, coiled-coils are common to many proteins, including those involved in essential biological processes like kinetochore and spindle assembly during mitosis. 3D proteomics offers a means to analyse these challenging structures. Importantly, this can be achieved on microgram amounts of affinity-purified endogenous protein complexes. This gives us the opportunity, in a straightforward way, to understand the *in vivo* architecture of highly important protein complexes.

Methods: Endogenous Mad1-Mad2 and Ndc80 complexes were affinity-purified from *S. cerevisiae* and BS3 crosslinked on-beads. Trypsin-digested crosslinked peptides were enriched on SCX-StageTips and analysed by LTQ Orbitrap Velos LC-MSMS. Crosslinked peptide identification was achieved using our algorithm and Xi software.

Results: Analysis of recombinant human Ndc80 and Ndel1 complexes indicated that coiled-coil regions are favourably covered by crosslinking[1,2]. We have now analysed endogenous Ndc80 and Mad1-Mad2 complexes from *S. cerevisiae*. We observe that crosslinks within one helix of a coiled-coil tend to bridge 3, 7,

10 amino acids, linked to the dyad of the alpha helix that brings amino acids onto the same side of the helix in this order. This also links with helices in coiled-coils having a hydrophobic and a hydrophilic side, the latter being rich in lysines among other hydrophilic amino acids. Coiled-coils tend to be solvent exposed with a large surface area, giving easy access to crosslinkers. Crosslinks between helices in a coiled-coil are equally favoured as they tightly interact via the hydrophobic cores. Only lysines within the same or adjacent turns are near enough for crosslinking, meaning that helices can be positioned with single-turn accuracy on each other based on crosslink data.
Conclusion: The properties that make coiled-coils difficult to tackle by traditional means make them favourable and information-rich targets for crosslinking.

References: 1. Maiolica *et al*, MCP, 2007**,** 2. Cifferi *et al*, Cell, 2008

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RECOVERING TEMPERATURE SIGNATURE ON NATURAL POPULATIONS OF AQUATIC INSECTS
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Introduction: Temperature is one of the most important abiotic variables that influence the limits of species distributions, in addition to behaviour, metabolism, growth, and reproduction rates. To understand the processes involved in the response to thermal stress of a narrowly distributed species we set an experiment analysing the differences in protein expression between two natural populations.
Methods: Fifty-four individuals of *Agabus ramblae* MILLÁN & RIBERA (Dytiscidae: Coleoptera) of two populations (Murcia, SE Spain, and High Atlas, central Morocco) were collected in the field and brought back to the laboratory, where they were acclimated for one week at the same temperature and pH, and fed the same prey (frozen *Chyronomidae* larvae). We then applied for a subset of each population three temperature treatments (4ºC, control at room temperature and 27ºC) in the range of what they may experience in the field. We then froze the specimens (three replicas for each temperature treatment, three individuals in each replica). The experimental design allowed us to make comparisons at replica, treatment and population level. We made total protein extraction and a DIGE experiment of each population, and analysed the images with SameSpots and different statistical methods, e.g. heuristic analysis, principal component analysis and Eisen method using the normalized volume of spot proteins.
Results: In a first overview of the data, after analysing both populations, we found similarities in the number of protein spots, and at different levels of significance ($p < 0.05$ and $p < 0.01$). After a heuristic analysis we recovered the signature of temperature treatments, which were perfectly separated in different clusters for both experiments independently. By using principal component analysis and the Eisen method we could make a pre-selection of spots for future sequencing and identification.
Conclusion: With these experiments we show that it is possible to recover the signature of the temperature treatments in two different natural populations of a non-model species. We also show the replicability of our experimental design in recovering this response even when using wild specimens of unknown general condition.

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EXPLORING THE DYNAMIC RANGE OF LABEL-FREE MASS SPECTROMETRIC QUANTIFICATION IN COMPLEX SAMPLES
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Introduction: The proteome is characterized by its high complexity and wide concentration range which are constantly in flux due to developmental and stress-related processes. Thus, discovery approaches are required which are completely unbiased to cover the whole proteome. Accurate quantification of differently expressed proteins is still challenging and relies heavily on a stable analytical platform. In this study we will investigate the quantitative performance of label-free approaches with a focus on the dynamic range.

Methods: Tryptic peptides were separated on a nanoUHPLC system in a 120min gradient. Data for label-free quantification was acquired with an Ultrahigh Resolution Q-TOF system (maXis impact, Bruker) equipped with CaptiveSpray. A standard of 48 proteins spanning over 6 concentration decades (UPS-2 standard, Sigma) was used to determine the dynamic range for label-free quantification. This standard was spiked at two different concentrations A and B at a relative ratio of 2:1 each into *E.coli* background (500ng) mimicking the complexity typically found in biological samples. The label-free quantitation workflow was based on signal intensities from LC-MS runs for quantification. Data from LC-MS/MS runs were used for identification information.
Results: The experimental setup covered a concentration range from 500fmol to 2.5amol. Prerequisites for label-free quantification are a high retention time and signal intensity stability. Quantitative information was obtained from MS signal intensities. Signals originating from the same peptide, like different charge stages and isotopes were combined. Results show almost no variation for the E.coli background, whereas regulation ratios were observed for the standard spiked peptides. In total 27 proteins from the UPS2 mixture were detected as significantly regulated ($p<0.05$).
Conclusion: Very accurate quantification was obtained down to the low fmol range, but the results also show that the label-free approach provides correct quantification of peptides in the amol range. The high dynamic range of this approach allows in-depth studies of quantitative changes in biological systems

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STRAIN-SPECIFIC PEPTIDES IN MOUSE URINE: BASIS FOR OLFACTORY DISCRIMINATION OF INDIVIDUALITY

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Introduction: Besides their core function in immune recognition, genes of the major histocompatibility complex (MHC) are considered to influence mating preference and recognition of individuality in mice. The olfactory MHC signal is present in urine and has been proposed to consist of MHC class I peptides. Synthetic MHC class I peptides can mediate the pregnancy block effect in mice of the respective MHC type and murine nasal sensory neurons detect these peptides with very high sensitivity (up to 10^{-14} M). The sensory neurons can discriminate changes in peptide amino acid side chains including the MHC anchor amino acid residues required for peptide binding to MHC class I molecules. However, a natural source for MHC-dependent peptides accessible for nasal recognition has not been identified so far.
Methods: We developed an MHC-based enzyme-linked immunosorbent assay to measure urinary concentrations of an MHC model peptide in mice with or without functional MHC class I molecules. Peptide sequencing was done on an LTQ Orbitrap XL mass spectrometer employing isotope labelled synthetic peptides for absolute quantifications.
Results: We show that the MHC class I model peptide SIINFEKL occurs in urine of ovalbumin-transgenic mice in an MHC-dependent manner. It is present in a free form at a median concentration of $4 \cdot 10^{-12}$ M. In addition, 10-35% of total urinary SIINFEKL is bound to so far undefined carrier molecules. We also find MHC-independent urinary peptides with MHC class I binding motifs at concentrations of up to 10^{-6} M in all five mouse strains investigated. These MHC-motif peptides are produced by proteolysis of abundant proteins of urine, plasma, kidney and other origins. As shown for two examples, they bind to the respective MHC molecules. Furthermore, we provide evidence for urinary peptides that differ between various mouse strains in single amino acids as a result of genomic single nucleotide variations.
Conclusion: We demonstrate at least two classes of strain-specific urinary peptides in mice delivering essential basic information for future neurobiological and behavioural experiments concerning genotype specific individual recognition in mice. The results might also provide leads for biomarker identification in humans.

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THE APPLICATION OF PROTEOMIC METHODOLOGIES TO LIPID RESEARCH

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Introduction: Lipids have long been implicated as antibody targets in autoimmune diseases such as Guillain-Barré Syndrome (GBS), Multiple Sclerosis and Multifocal Motor Neuropathy. These disorders are characterised by inflammation, weakness and paralysis as a result of an autoimmune response against common membrane glycosphingolipids (GSL) that are enriched in the nervous system. Recent research has demonstrated how the targeting of this immune response is further complicated by *cis*-interactions between lipids generating heteromeric lipid complexes which can strongly influence antibody affinity. The unravelling of these subtle lateral interactions between lipids and their significance in autoimmune disease requires the creative development and application of novel technology.

This project aims to adapt several proteomic-associated methodologies including liquid chromatography-tandem mass spectrometry (LC-MSMS), mass spectrometry imaging (MSI) and microarrays to lipid research.

Methods: MSI has been coupled directly to thin layer chromatography (TLC); a work-horse preparative and analytical method in GSL research enabling direct detection and identification of GSLs without the need for destructive stains, comparative standards or lengthy immunodetection protocols. High mass accuracy LC-MSMS has also been applied to GSL profiling in commercial and biological extracts making profiling possible to a previously unattainable degree of detail.

Results: Workflows for both methods have been developed using lipid standards and commercial ganglioside extracts.

Conclusion: TLC-MSI and LC-MSMS will now be used in a proof-of-principle study to create in-depth GSL profiles of various glycosyltransferase knock-out and rescue mice of relevance to GBS research. Following validation in mouse models, these methods could be used to identify tissue lipid biomarkers of human GBS disease, including those outside of the GSL fraction, which contribute to lipid-epitope presentation. Lipids of interest can then be included on novel combinatorial heteromeric lipid microarrays that are currently being used to screen patient samples for anti-lipid antibody profiles.

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MULTIPLEXED LABEL-FREE BIO-AFFINITY ANALYSIS AND MALDI-MS CHARACTERISATION OF BOUND ANALYTE

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Introduction: The coupling of Surface Plasmon Resonance imaging (SPRi) and Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) is an innovative approach for biomarker discovery in biological fluids.
Methods: Multiplexed SPRi analysis allows the direct visualization and thermodynamic analysis of molecular avidity, and is advantageously used for ligand fishing of captured biomolecules on multiple immobilized receptors on a SPRi-Biochip surface. MALDI-MS is a powerful tool for the identification and characterization of molecules by their molecular weight and peptide sequence. Therefore, the combination of SPRi and MS into one concerted procedure, using a unique dedicated surface, is of great interest for functional and structural analysis of bound molecules.
Results: Results will be shown using the Lymphocyte Activation Gene 3 (LAG3) protein, a potential biomarker of breast cancer and tuberculosis. LAG3 was captured in human plasma by SPRi down to several

femtomoles/mm². Then, after MS pre-processing, LAG3 was successfully identified by MALDI-MS directly on the SPRi biochip.
Conclusion: The coupling of SPRi to MS analysis is possible and is a valuable tool for biomarker identification.

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LAYER BY LAYER: EXPLORATION OF THE MUCIN CORE-1 TYPE O-GLYCOPROTEOME OF BOVINE SERUM

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Introduction: Readily available bodyfluids have been studied in the hope of obtaining biomarkers. Recently more of this research is directed towards post-translational modifications. The huge dynamic range of serum components hinders the characterization of low level proteins. There is added difficulty for O-glycosylation analysis, because of the lack of consensus motif, and the carbohydrate heterogeneity as well as the "hostile" mass spectrometry behavior of glycopeptides.
We will demonstrate how much information can be gained about mucin core-1 type glycopeptides using increasingly complex enrichment protocols, improved instrumentation and data processing.
Methods: Fetal bovine serum was subjected to glycoprotein enrichment applying Jacalin, a plant lectin for affinity chromatography followed by
Experiment 1: tryptic digestion and a second peptide-level affinity chromatography [1]
Experiment 2: mixed bed ion exchange chromatography, tryptic digestion and peptide-level affinity enrichment [2]
Experiment 3: tryptic digestion, ERLIC (electrostatic repulsion hydrophilic interaction chromatography) fractionation and peptide level affinity enrichment [2]
Experiment 4: Proteominer treatment, tryptic digestion and ERLIC fractionation.
All samples were subjected to partial enzymatic deglycosylation prior to mass spectrometry analysis applying electron transfer dissociation and higher energy collisional activation.
Results: A comparison of the sample preparation protocols in terms of the number of identified glycoproteins, glycosylation sites and nonspecific background will be presented.
Conclusion: Focusing on a well defined core-structure we were able to identify a significant number of novel glycoproteins and glycosylation sites in a high throughput manner. We also outline an approach where HCD and ETD data could be combined for more successful glycopeptide characterization.
References: [1] Z. Darula and K.F. Medizhradszky. Affinity-enrichment and characterization of mucin core 1-type glycopeptides from bovine serum. *Mol. Cell. Proteomics* **8,** 2515-2526 (2009)
[2] Z. Darula, J. Sherman, K.F. Medzihradszky. How to dig deeper? Improved enrichment methods for mucin core-1 type glycopeptides. *Accepted for publication in MCP*

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EVALUATION OF TARGETED PROTEOMIC APPROACHES ON A Q-TOF SYSTEM

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Introduction: MS-based quantitative proteomics is a popular tool for biomarker discovery. The improved LC separation speed and the MS data quality also enable the subsequent validation of discovered protein biomarkers in complex biological matrices by targeted approaches. Typically, MRM approaches are applied for validation tasks. However, those methods require detailed target knowledge and do not allow the detection of unknowns. We describe here targeted proteomics by the high resolution and accuracy on an Ultrahigh Resolution QTOF system.
Methods: Target protein quantification is done in a complex *E.coli* background (500ng) spiked with different amounts of a proteomic standard, which spans a concentration range of 6 decades in total (UPS-2, Sigma). Protein identification information, including m/z values and retention times, used as targets for quantification is

obtained out of the equimolar proteomic standard (UPS-1, Sigma) containing the same set of proteins as the dynamic range mixture. Tryptic peptides are separated on a nanoUHPLC system (RSLCnano, Dionex) and data acquisition is done using an Ultrahigh Resolution (UHR)-TOF (maXis impact, Bruker) equipped with a CaptiveSpray ionization MS source
Results: Peptide targets for quantitative proteomics are obtained out of an equimolar proteomic standard by a data-dependent MS/MS approach. Results of different identification runs are combined and reveal successful identification of all 48 proteins. Creation of high resolution extracted ion chromatograms used for quantification is done based on the information of identified peptides with regard to m/z values and retention. Quantification of the target peptides spiked into *E.coli* background based on pure MS data acquisition reveals reliable results of targeted proteomics. In total 28 proteins were significantly regulated ($p<0.05$) demonstrating a high quantification efficiency from 500 fmol down to the amolar range.
Conclusion: Results of targeted proteomics approaches using broad band and middle band CID will not only provide quantitative but also qualitative data in single runs. Quantification efficiency with regard to the coverage of a high dynamic range of these methods will be compared to the pure MS-based acquisition.

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A NEW BIOADHESIVE MATERIAL FROM FISH PARASITES
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Introduction: Monogenoids are fish parasites which are able to quickly and reversibly attach to their host in the presence of strong water currents through the secretion of a proteic glue. The adhesive secretion is produced by glands located in the antero-lateral region of the animal. Unlike most adhesive secretions found in the animal world which stick to abiotic substrates, monogenoidean adhesive secretion works on living tissues. All these features make it a very promising material for exploitation in the surgical field. The aim of this work is the characterization of the bio-adhesive material by means of a proteomic approach. Since no genomic data for monogenoids are available, an MS/MS based de novo sequencing strategy was applied.
Methods: The secreted material was obtained by electrostimulation of the parasites in a 50% PBS solution using 40 volts electric field and 2 Hz frequency. The secretion of 30 parasites was collected in a test tube and the SDS soluble and unsoluble components were analysed separately. Following to 2D-electrophoresis, each spot was trypsin digested and analysed by nano-LC-MS/MS using an LTQ Orbitrap Velos (Thermo Scientific, Germany) mass spectrometer. De novo sequence analysis and protein identification were obtained using Peaks Studio 5.3 (Bionformatics Solutions, Canada).
Results: Separate analysis of SDS soluble and unsoluble components of the glue suggests that more than 90% of the proteinaceous adhesive material is SDS unsoluble. Identification of proteins following de novo sequencing and database similarity searches using Peaks Studio allowed concluding that the glue is composed by a relatively small number of proteins, as expected for this type of material.
Conclusion: De novo sequencing by high resolution MS/MS allowed the characterization of the proteic components of a new bioadhesive material with potential medical applications.
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PARYS, A WEB SERVER FOR MANAGING REVERSE-PHASE PROTEIN ARRAY PLATFORM DATA
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Introduction: The Reverse-Phase Protein Array (RPPA) is an emerging high-throughput technology relying on highly specific antibodies to quantify proteins in nanogram-amounts of spotted cell or tissue lysates. This technology has thus become a promising approach for proteome analysis of cancer patients. A RPPA platform was set up at Curie Institute (Paris, France) as a result of a partnership with Servier pharmaceutical company. Typical research projects utilizing this platform focus on finding new therapeutic targets by examining the expression and activation (eg. phosphorylation state) levels of multiple proteins in hundreds of tumour biopsies.

To fully exploit the potential of RPPA technology, we have developed PARYS (Protein Array Server), a comprehensive bioinformatics environment for the platform.
Methods: PARYS is developed in Perl-CGI, HTML and JavaScript. It relies on an Apache web server coupled with a MySQL database. Data analysis is performed using R statistical language.
Results: PARYS is organised into two main sections:
- A LIMS-like section to track major laboratory reagents (e.g. antibodies, tumour samples, cell lines, proteins extracts, arrays) and key processes such as extracts preparation, spotting, antibody labelling and spot quantification.
- A Project section to coherently organize, mine and analyze the quantitative data generated.
PARYS implements an extended data annotation scheme coupled with differential and exploratory analysis tools to provide the platform collaborators with means to extract relevant biological information from their data.
Conclusion: PARYS is now fully integrated to our RPPA platform activity. To date, over 1400 antibodies targeting critical biological pathways are referenced with detailed information on their suitability for RPPA experiments. Use of the PARYS server has significantly reduced the delay between raw data generation and their biological interpretation.

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SIMPLE WESTERN ANALYSIS OF NF KAPPA B SIGNALING CASCADE PROTEINS
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Introduction: Aberrant expression and signaling of multiple proteins in the NF kappa B pathway are commonly associated with inflammatory and stress-induced diseases, including many cancers. Understanding how NF kappa B signaling impacts disease progression is important to the development of novel therapeutics. Cell signaling events are routinely assessed using traditional Western blot analysis. The Western blot technique is very labor intensive and generally yields results that are semi-quantitative. The Simple Western™ platform described here completely automates the manual steps involved in traditional Western blot protocols and can analyze up to 96 samples in a single experiment. Because Simple Western protocols consume only nanoliter sample volumes, reproducible and quantitative results can be generated from precious or limited quantity samples.
Methods: Whole cell and nuclear extract lysates were prepared from cultured Hela cells. Lysates were prepared in SDS Sample buffer and loaded in duplicate into a 384-well assay plate at a final concentration of 1 mg/mL. Eight replicate analyses were generated from each 5µL sample.
Results: Targets from the NF kappa B pathway, including I kappa B, NF kappa B subunits c-Rel, p65, and p50/p105 from both whole cell and nuclear lysates were screened on the Simple Western platform with normalization to a loading control (alpha-tubulin). In response to TNF-alpha treatment, statistically significant changes in signal and localization were clearly observed for each of the key targets. Moreover, comparisons between Simple Western and traditional Western results are shown.
Conclusion: The Simple Western permits the rapid and quantitative high throughput analysis of signaling pathways at a level of efficiency and reproducibility not obtainable by traditional Western blots. The platform's ability to sample and generate data from extremely small sample volumes enables new approaches to the characterization of biomarker targets involved in the progression of disease states.

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PROTEIN NITRATION IS TRIGGERED BY NANOSTRUCTURED TIO2 DURING PC12 DIFFERENTIATION
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Introduction: Cells in their natural environment interact with extracellular matrix components in the nanometer scale and respond to nanoscale features when grown on synthetic substrates. Therefore, in studying neuronal proliferation and differentiation processes, nanoscale topography of synthetic materials has been receiving increasing attention because of its resemblance to in vivo surroundings. It has been recently proposed that the physical properties of the substrates can be considered as a new kind of stimulus based on

the finding that surface energy distribution, through cell-substrate interactions, triggers neuritogenesis of PC12 cells in the absence of nerve growth factor (NGF) or other inducers. However the molecular mechanisms of the triggered signalling cascade has not been identified, yet.
Methods: In order to detect nitrated proteins, cellular extracts were submitted to SDS-PAGE; gels were sliced and proteins reduced, carbamidated, digested with trypsin and analysed by nano-LC-MS/MS with an LTQ Orbitrap Velos (Thermo Scientific) mass spectrometer.
Results: To investigate the potential role exerted by protein nitration, we studied the behaviour of PC12 cells on nanostructured TiO_2 films of different thickness in the presence and in the absence of NGF while cells grown on PLL (poly-L-Lysine) coated glass were taken as the control. Since it has been demonstrated that NGF induces NO production by nitric oxide synthases (NOS) and that differentiation in PC12 cells grown on PLL-glass in the presence of NGF is associated to an increase in protein nitration, our investigation was aimed at detecting whether increased protein nitration is also observed during PC12 differentiation triggered by nanostructed TiO_2 films in the absence of NGF.
Conclusion: Altogether the data suggest that protein nitration is involved in the differentiation process induced by nanotopography similarly to what is described in PC12 cells differentiated upon NGF.

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LARGE SCALE ANALYSIS OF RNA AND PROTEIN EXPRESSION VARIATION THROUGH THE CELL CYCLE

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Introduction: Proliferating cells progress through temporal stages that are largely defined by cell growth, genome duplication and cell division. The transitions between each stage are tightly regulated to ensure high fidelity replication and deregulation of the cell cycle is a characteristic of all cancers. We have used a combined proteomics and transcriptomics approach to analyze gene expression dynamics through interphase in a human leukemia cell line, NB4. Whereas the majority of cell cycle studies on mammalian cells have relied on drug-induced synchronization, we have used a physical method of obtaining cells enriched at specific cell cycle stages, namely counterflow centrifugal elutriation.
Methods: Enrichment of G1, S, or G2/M cell cycle stages were validated by flow cytometry and immunoblot analysis of known cell cycle markers. Total RNA and protein were extracted from each cell cycle-enriched fraction for sequencing (Wellcome Trust Cancer Genome Project) and MS-based proteomics (Wellcome Trust GRE). A total of 3 x 3 biological and technical proteomics replicates were performed. Proteomics data were analyzed using PepTracker, a data management and visualization tool developed in the Lamond laboratory.

Results: A total of 4,000 proteins and 48,000 transcripts were identified in this dataset so far. We find that approximately 3% of the detected genes vary in intensity through the cell cycle, including well known cell cycle regulatory proteins. We find moderate correlation between RNA and protein expression (Spearman $r \approx 0.5$) in each of the three stages of interphase. The RNA and protein expression trajectories of certain protein clusters, like G2/M-associated cyclins, are highly correlated. A significant proportion of proteins that vary across interphase do not share similar protein and RNA expression profiles, which may suggest that the expression of these genes are post-transcriptionally regulated.

Conclusion: We have compared the variation of the proteome and transcriptome across the mammalian mitotic cell cycle without drug arrests.

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QUANTITATIVE ANALYSIS OF THE PROTEOME AND PHOSPHOPROTEOME OF CELLS EXPRESSING CONSTITUTIVELY ACTIVE H RAS

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Introduction: The Ras pathway regulates proliferation, differentiation and survival of cells. In this study we analyse the changes on the nuclear proteome and phosphoproteome due to the expression of a constitutively active H Ras.
Methods: The Tet-On Gene Expression System is used to regulate the expression of the constituvely active HRasV12 by doxycycline in NIH 3T3 cells. Nuclear protein extracts from cells grown in the presence and absence of doxycycline were digested and analysed by data-independent LC-MS/MS. In paralell, phosphopeptides present in both protein digests were enriched by TiO_2 and analysed by LC-MS/MS. Finally, quatification of the identified phosphopeptide was performed by manual inspection of the spectra.
Results: Out of the 348 proteins confidently identified, 48 were significantly deregulated by the expression of active HRasV12 ($p<0.05$; ratio>1.5) and another 28 proteins were identified only in one condition. In addition, 196 phosphopeptides, corresponding to 130 proteins were identified (Mascot score ≥ identity). A subset of 33 of these proteins was selected for the quantitative analysis of their identified 72 phosphopeptides showing that 19 of these proteins have at least one phosphopeptide whose level is significantly changed in the cells expressing HRasV12 ($p<0.05$; ratio>2).
Conclusion: The proteomic analysis carried out in this work has allowed the identification of 76 differentially expressed proteins and 19 proteins with changes in their phosphorylation state as a consequence of the expression of a constitutively active H Ras.

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CHARACTERISING RAPID PROTEIN TURNOVER USING SILAC-MS
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Introduction: Numerous biological processes, such as oncogenic transformation, are partly controlled at the level of protein degradation. This is exemplified by the oncogene Myc which, if stabilised, can induce cellular proliferation and contribute to cancer development. Stable isotope labelling with amino acids in cell culture (SILAC) has provided a useful approach for the quantitative analysis of protein degradation with high accuracy. In this study, we have used in-depth fractionation and high sensitivity LC-MS/MS to detect rapidly degraded proteins of low abundance in human cells.
Methods: We have utilised the drug cycloheximide, which effectively blocks protein synthesis, to detect those proteins that are rapidly degraded (half-life <6 h) in U2OS human osteosarcoma cells. Cells labelled with either heavy or light SILAC media were treated with either cycloheximide or DMSO (control) respectively, for 6 hours. After mixing of the SILAC labelled lysates, sub-cellular fractionation was performed and each fraction further separated by denaturing (SDS) size exclusion chromatography. This yielded 24 fractions, which were digested with trypsin, de-salted and subjected to LC-MS/MS. 4 hour gradients were used on a 50cm C18 column coupled via ESI to a Q Exactive mass spectrometer (Thermo Scientific). These data were subject to quantitation and protein identification using the Maxquant software package.
Results: We have established a fractionation workflow for dividing the proteome into 24 fractions and using high sensitivity mass spectrometry analysis with the Q Exactive we have identified and quantified over 5,000 proteins with two or more peptides. Proteins that are rapidly degraded will quickly decrease in abundance in the heavy cycloheximide treated cells compared to the light control samples. These data revealed numerous proteins previously characterised to have rapid turnover rates such as Myc, which act as positive controls for this study. In addition, we have identified several novel proteins showing rapid turnover which are uncharacterised.
Conclusion: The data we have collected to date have provided significant insight into protein turnover in human cells. Further work will characterise how the rapid turnover of these proteins is altered in disease states, such as cancer, using high-throughput techniques including reverse-phase protein arrays (RPPA).

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DEEP PROTEOMIC ANALYSIS OF SILAC WORM GIVES FUNCTIONAL INSIGHTS INTO TRANSLATIONAL REGULATION BY RNA-BINDING PROTEIN GLD-1

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Introduction: Animal mRNAs are regulated by hundreds of RNA binding proteins (RBPs) and identification of their targets is crucial for understanding their function. The recently introduced PAR-CLIP method was modified to allow identification of thousands of targets genes of RBPs *in vivo* (iPAR-CLIP) to determine transcriptome-wide target sites of GLD-1, a conserved, germline-specific translational repressor in C. elegans (1).
Methods: In order to proof its functionality as translational repressor, we used GLD-1 knockdown and a reference stable isotope labeled (SILAC) *C. elegans* worms to detect targets, which are specifically up-regulated at protein but not at mRNA level. Therefore an in-solution isoelectric fractionation of peptides before the LC-MS/MS analysis was applied.
Results: Using this technique 6,000 proteins could be identified and a fold change for more than 4,000 could be measured; among these, 2,795 proteins were germline-expressed. We could measure the up-regulation for 217 out of 439 reproducible GLD-1 targets.
Conclusion: GLD-1 targets showed a highly significant shift toward higher expression levels, while their mRNA remained constant. With this refined method for protein quantification together with metabolic labeling of proteins we were able to resolve the translational repressive activity of GLD-1 *in vivo*.
References: (1) Jungkamp, A., Stoeckius, M., Mecenas, D., Grün, D., Mastrobuoni, G., Kempa, S., Rajewsky, N. (2011) In Vivo and Transcriptome-wide Identification of RNA Binding Protein Target Sites. Molecular Cell, Volume 44, Issue 5, 828-840

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A QUANTITATIVE PHOSPHOPROTEOMICS APPROACH REVEALS A NEW MECHANISM IN THE FUNCTIONAL SELECTIVITY OF SEROTONIN RECEPTOR 5-HT2A

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Introduction: The serotonin 5-HT2A receptor is a primary target of psychedelic hallucinogens such as lysergic acid diethylamide (LSD), which reproduce some of the core symptoms of schizophrenia. An incompletely resolved paradox is that only some 5-HT2A receptor agonists exhibit hallucinogenic activity, whereas structurally related agonists with comparable affinity and activity do not.
Methods: Using quantitative phosphoproteomics combining stable isotope labelling by amino acids in cell culture (SILAC), phosphopeptide enrichment by hydrophilic interaction chromatography (HILIC)/immobilized metal affinity chromatography (IMAC) and high resolution mass spectrometry, we compared the phosphoproteome in HEK-293 cells transiently expressing the 5-HT2A receptor under three conditions: no-stimulation; exposure to the phenethylamine hallucinogen 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) and exposure to the non-hallucinogenic 5-HT2A agonist lisuride.
Results: Among the 5,996 identified phosphopeptides (FDR < 1%), 454 sites were differentially phosphorylated upon exposure to DOI vs. lisuride (ANOVA p value < 0.05). These include a serine residue phosphorylated upon exposure to DOI but not to lisuride and located in the i3 loop of the 5-HT2A receptor, a region important for receptor desensitization. Mass spectrometry analysis of immunopurified receptor further confirmed differential phosphorylation of this residue upon exposure to several hallucinogenic (DOI and LSD) vs. several non-hallucinogenic (lisuride and ergotamine) agonists. Interestingly, exposure to hallucinogens induced less pronounced receptor desensitization than exposure to non-hallucinogenic agonists and mutation of the serine residue decreased the difference of desensitization between the two classes of ligands.
Conclusion: this phosphoproteomics analysis reveals that 5-HT2A receptor stimulation by hallucinogenic vs. non hallucinogenic agonists induces contrasting phosphorylation patterns that may be related to their distinct

behavioural responses. It also provides one the first demonstrations of differential phosphorylation of a G protein-coupled receptor upon stimulation by “biased” agonists.

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DYNAMIC COMPLEXOME AND SECRETOME ANALYSIS FOR THE STUDY OF TYPE III PROTEIN TRANSLOCATION AND SECRETION MECHANISM

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Introduction: The type III secretion system (T3SS) is a specialized bacterial protein secretory pathway that plays an essential role in the pathogenesis of Gram-negative bacteria (e.g. Enteropathogenic *E. coli*, EPEC) [1]. It is encoded by the Locus of Enterocyte Effacement (LEE), and injects effector proteins into the host cell, modulating key cellular processes [2]. The precise mechanisms of T3SS remain poorly understood.
Methods: Cytosolic protein complexes were isolated from wt and selected deletion mutant and fractionated by Native polyacrylamide gel electrophoresis (N-PAGE) and size-exclusion chromatography (SEC). In a targeted approach, His-tagged T3SS-related proteins were used for the isolation of protein complexes which were fractionated by N-PAGE and SEC. The secretome was collected and concentrated by acid-mediated precipitation. Complexome and secretome were analyzed by “bottom-up” proteomics. Protein identification, validation and relative quantitation was performed by Proteome Discoverer, Scaffold, and iBAQ [3], respectively.
Results: More than 1500 proteins from a wide range of cellular processes were identified, that corresponds to >80% of the expressed cytosolic proteome. As a proof of principle, ~150 known and novel protein complexes were determined. 38 predicted as non-transmembrane T3SS-related proteins, were identified as components of several T3SS protein complexes and interactions. Immuno-detection, pull downs and deletion mutants were used for the verification and the elucidation of the precise function of these complexes during T3 protein secretion.
Conclusion: The combination/optimization of global and targeted approaches for the characterization of protein complexes shed light on the dynamics of T3SS-related cytosolic complexome. Known and novel complexes and interactions were determined. Further in depth study of the identified complexes will provide us with new insights on the poorly understood T3SS mechanisms.
References: [1] Pallen, M. J., and B. W. Wren, Nature, 449:835–842 (2007)
[2] Garmendia, J., G. Frankel, and V. F. Crepin, Infect. Immun., 73:2573–2585 (2005)
[3] Schwanhaeusser, B., et al. Nature, Nature 473 (7347): 337-342 (2011)

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SEARCHING FOR BIOMARKERS OF PHYSIOLOGICAL STATE IN HUMAN SWEAT

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Introduction: During emergency interventions, workers or servicemen must face extreme situations that can deeply impact their physiological behaviour. Real-time monitoring of biomarkers reflecting subject state would allow to prevent any medical accident by anticipating their pullback from the danger zone. In this context, sweat represents a biological fluid of choice for non-invasive detection of that kind of markers. We therefore undertook a quantitative proteomic analysis of human sweat.
Methods: Sweat was collected from healthy donors submitted to various environmental constraints. Proteins contained in sweat were extracted using ethanol, TCA or acetone precipitations as well as ultrafiltration. After SDS-PAGE separation, digested proteins were submitted to liquid chromatography coupled to tandem mass spectrometry. Biological and technical replicates were conducted. Peptide and protein identifications were validated, filtered, structured and compared using in-house developed softwares (IRMa[1], MSIdb, hEIDI). Statistical analysis of spectral counting data was carried out using a R package based on a beta-binomial model[2].

Results: Our results indicated that ultrafiltration of crude sweat combined with SDS-PAGE and in-gel digestion gave the best results in terms of protein recovery and number of identifications. To date, a set of more than 1000 different proteins have been identified with high confidence in this biological fluid.
After highly stringent statistical analyses, 10 putative biomarkers of constraints were selected for validation by MRM-MS experiments. Preliminary results showed a good correlation between results obtained from discovery and validation phases.
Conclusion: The proteomic analysis of human sweat allowed the establishment of a repertoire of more than 1000 proteins; this represents a unique contribution to the establishment of sweat proteome. Several of these proteins were shown to represent biomarkers of physiological state.
References: [1] Dupierris V *et al.*, Bioinformatics, 2009, 25: 1980-1981
[2] Pham T. V. *et al.,* Bioinformatics, 2010, 26 : 363-369.

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COMPARATIVE PROTEOMIC AND PHOSPHOPROTEOMICS ANALYSIS OF BLOODSTREAM AND PROCYCLIC FORM TRYPANOSOMA BRUCEI, THE CAUSATIVE AGENT OF AFRICAN SLEEPING SICKNESS.
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Introduction: The protozoan parasite *Trypanosoma brucei*, the causative agent of African sleeping sickness, has a complex digenetic lifecycle between a mammalian host and an insect vector. The ability of the parasite to sense and adapt to its host environment is essential for its survival and virulence, but the underlying mechanisms are poorly defined at the molecular level.
Methods: We have optimized a procedure for growing *Trypanosoma brucei* cells in conditions suitable for stable isotope labeling by amino acids in culture (SILAC) and describe a comparative proteomic and phosphoproteomic analysis of cultured procyclic form and bloodstream form *T. brucei* cells. The comparative phosphoproteomic analysis uses strong cation exchange chromatography and TiO_2 to enrich for phosphopeptides prior to analysis.
Results: In total we were able to identify 3959 proteins and quantify SILAC ratios for 3553 proteins with a false discovery rate of 0.01 [1]. A large number of proteins (10.6 %) are differentially regulated by more the 5-fold between lifecycle stages, including those involved in the parasite surface coat, and in mitochondrial and glycosomal energy metabolism. Phosphosite data were normalized for changes in protein abundance, and showed widespread variation between lifecycle stages.
Conclusion: Our proteomic data shows surprisingly good correlation with previous transcriptomic studies, but with significantly larger fold changes observed at the protein level than at the mRNA level. Differential phosphorylation between lifecycle stages is widespread, and may provide an additional mechanism by which the parasite adapts to its changing environments. The present work will underpin further studies aiming to define the signaling pathways required for the differentiation between bloodstream and procyclic form parasites.
References: [1] Urbaniak *et al.* (2012) PLoS One, In press.

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IDENTIFICATION OF PHAGOSOMAL HOST PROTEIN SUBSTRATES OF THE MYCOBACTERIAL KINASE PKNG
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Introduction: Cells use phagocytosis as a host defence mechanism against pathogen infection. During the process cells engulf pathogens to form phagosomes which then mature by fusing with lysosomes, where the pathogens are finally degraded. However, many pathogens, such as pathogenic mycobacteria, are able to escape this host defence.An important virulence factor for mycobacteria is the eukaryotic-like serine/threonine protein kinase G, PknG. After entry into macrophages, PknG is secreted into the phagosome, inhibiting phagosome-lysosome fusion and mediating intracellular survival of mycobacteria. Importantly, the kinase

activity of PknG is essential for its regulation of phagosome maturation. The aim of this research is to identify host substrates of PknG and the proteome/ phosphoproteome changes regulated by PknG.
Methods: We used proteomics, cell biology and biochemistry approaches to characterize the mechanism of phagosome maturation arrest mediated by PknG. Immunoprecipitation coupled with LC-MS/MS and Western blot were used to identify proteins interacting with PknG. Moreover, polystyrene beads coated with PknG, kinase-dead mutant K181M and non-coated beads were presented to RAW264.7 macrophages, and phagosomes at various time points following internalization were isolated. Phagosomal proteins were digested using RapiGest. The phagosomal proteome and phosphoproteome were analysed by Orbitrap Velos Pro, followed with label free quantification by MaxQuant. Proteins differentially regulated by PknG were validated by various techniques, such as Western blot and immunofluorescence.
Results: Using biochemistry and cell biology approaches, we have found several host proteins specifically interacting with PknG. We are currently investigating these proteins for their involvement in phagosome biogenesis pathways that PknG may participate in. Furthermore, with our approach we have identified a number of novel autophosphorylation sites of PknG which might be specific for intracellular protein-protein interactions. Finally, analyses of the phagosomal proteome and phosphoproteome upon uptake of PknG-coated beads will be presented.
Conclusion: Results so far have indicated the potential function PknG may have in regulating host phagosome biogenesis.

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DISCOVERY OF BIOMARKERS OF COMPLICATIONS IN DIABETES
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Introduction: Plasma biomarker discovery for diabetes is mainly based on protein and PTM profiling and quantification. For glyco-biomarkers, not only glycosylation site occupancy but also the glycan structure can change during diabetes and complications in diabetes. We applied comprehensive proteomics and glycomics methods to study normal and diabetic plasma glycoproteomes, including quantitative information of glycosylation site occupancy and glycan structure.
Methods: A proteome quantification experiment was first performed to identify differences in protein abundance between diabetic and healthy samples. Pools of healthy and diabetic plasma samples were digested using the FASP method. Peptides from each sample were labelled by iTRAQ and these were mixed and fractionated by HILIC. All the fractions were analysed by LC-MS/MS for protein identification and quantification.
Quantitative glycoproteomics was performed using a glyco-FASP method. The glycopeptides were enriched by trapping on lectin and N-glycans were released by PNGase F. Deglycosylated-peptides were desalted by C18 column. Free glycans were desalted by Envi-Carb SPE column. Comparative glycoproteome data were obtained using iTRAQ labelling and LC-MS/MS analysis to analyse glycosylation site occupancy differences. The glycans were then permethylated and analysed by LC-MS/MS to look for glycan structure changes.
Results: Using quantitative proteomics and glycomics methods, we found several glycoproteins where expression level and glycosylation site occupancies were changed in diabetic samples. Some of these glycoproteins are known as potential biomarkers association with diabetes and complications in diabetes. Validation experiment will be performed to provide more precise quantification and individual differences. Moreover, we are also investigating quantification of glycan structure changes for in those glycoproteins where overall levels and site occupancies do not change in diabetes.
Conclusion: Preliminary data show that some glycoprotein expression levels and their glycosylation status change in diabetic plasma. Further validation experiment will be performed to provide us more detailed information.

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WHAT IS CHROMATIN? DISENTANGLING COMPOSITION AND FUNCTION.

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Introduction: Chromatin is the macromolecular architecture in which DNA's functions are largely mediated by proteins. At present, chromatin is not well defined. It is not easy to investigate neither the composition of its constituent proteins or how this arrangement changes. We aim to unravel human chromatin composition to outline the functional and structural changes occurring during the cell cycle.
Methods: Three chromatin snapshots of the cell cycle are analysed by quantitative proteomics. Our procedure couples a SILAC mass spectrometry-based approach with a newly developed biochemical chromatin purification method, which involves fixation of proteins to DNA.
Results: By testing two different fixation times (5 and 10 minutes) and three phases of the cell cycle (G1/S, G2, M), we were able to make a first separation of chromatin proteins known to have a function in DNA-based processes and proteins that are opportunistic binders. In a total of ~2000 proteins quantified in four experiments, human chromatin seems to be occupied by many unexpected proteins (40%). Interestingly, many of those proteins are retained throughout the cell cycle presumably after entering in contact with such a structure during mitosis.
Conclusion: In the present study we offer the first description of chromatin composition across the cell cycle. In addition, we managed to reach a preliminary separation of genuine chromatin proteins from proteins that bind non-functionally to this structure. Ultimately, we hope these results will refine the current view of chromatin protein composition and function.

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INCREASE SUCCESS RATE TO IDENTIFY ENDOGENOUS PEPTIDES IN TISSUE SAMPLES

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Introduction: We describe a general protocol to facilitate reproducible detection and measurement of endogenous peptides. Tissue preparation, extraction and isolation steps have been optimized to achieve high yields of non-degraded extracts ready for analysis.
Methods: A protocol for the isolation of endogenous peptides was developed using rapid heating to stabilize tissue samples from the moment of sampling (Stabilizor system, Denator). Methods were optimized using mouse cortical tissue and the final result verified using mouse thymic tissue. Results were compared with those obtained using conventional snap-freezing.
Three extraction buffers were tested on heat-stabilized tissue at different time points after sampling. Homogenization intensity was varied to investigate the possibility of inducing breakage of peptide bonds. Ultra-filtration filters with a 10 kDa cut-off limit from two suppliers were compared. All samples were analyzed using LC/ LTQ-FT-MS.
Results: In heat-stabilized tissue we found several previously known thymic proteins and hormones such as prothymosin, thymosin beta 4 and - 10. Samples that had been heat-stabilized prior to extraction contained more intact, full-length peptides whereas samples that had only been snap-frozen showed significant degradation. Tissue that was heat-stabilized within 3 min. after removal contained more intact, full-length peptides and the least number of protein degradation products. All snap- frozen tissue samples showed similar degradation patterns irrespective of whether they were snap-frozen immediately or 30 minutes later. Results suggest that most degradation of thymic proteins and hormones occurs at the time of tissue extraction when proteolytic enzymes are released. Inactivation of these enzymes, using heat-stabilization prior to extraction, minimizes proteolytic activity at the time of extraction.
Conclusion: Application of heat stabilization followed by optimal extraction gives less bias in quantitative measurements of peptide levels and more reproducible peptide identifications.
References: Silva A. B. et al, 2006. Immunology, 118, 131-40
Sköld K. et al, 2007. Proteomics, 24, 4445-56

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RELATIVE QUANTITATION OF TMT-LABELED PROTEOMES – FOCUS ON QUANTITATIVE PRECISION AND ACCURACY

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Introduction: Isobaric tagging methods involving 'differential' isotope labeling using chemical tags are universally applicable approaches measuring relative amounts of proteins across two or more different samples. The main focus of the presented work was on assessing the quantitative precision and accuracy for a TMT sixplex-labeled complex proteome sample using modern ion trap-Orbitrap hybrid instrumentation.
Methods: Six aliquotes of a digest of 9 proteins labeled each with one of the TMT sixplex reagents and mixed to obtain the final ratio 10 : 1 : 10 : 2 : 10 : 1.5. Such sample was added to the background of a TMT sixplex-labeled E. coli lysate digest. Peptides were analyzed using nano-LC coupled to the Orbitrap Elite. A data dependent method (Top10 method) as well as an MS3-based method where MS3 multipole collision cell fragmentation was triggered on a strong fragment ion from a particular m/z range of an ion trap fragmentation spectrum (MS3 method) were evaluated. Quantitative precision was expressed as protein ratio variability. Quantitative accuracy was presented as a deviation of a measured ratio value from the expected value.
Results: The percentage of peptides whose fragmentation spectra contained all six reporter ions (quantifiable peptides) exceeded 90% for any sample load tested.
The variability was lower than 10% for approximately 90% of the quantified proteins at a 500 ng sample load, and for about 70% at a 20 ng sample load.
The Orbitrap detection at resolution settings 15,000 corresponds to effective resolution >27,000 for m/z 126-130. Consequently, a very tight mass tolerance (+/- 10 ppm) could be used effectively filtering most reporter ion interferences.
For higher ratios (theoretical ratio 10:1) the median peptide ratio increased from 5.64 (Top10 method) to 7.97 (MS3 method).
Conclusion: An improved mass spectrometer design resulted in a high percentage of quantifiable peptides.
The quantitative precision is signal intensity-dependent.
Very high resolution effectively eliminated isobaric interferences in the reporter ion region.
The impact of peptide precursor co-isolation issue on quantitative accuracy was minimized with MS3 method.

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INVESTIGATION OF PROTEOME CHANGES DURING CAENORHABDITIS ELEGANS LARVAL DEVELOPMENT USING 15N METABOLIC LABELING

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Introduction: The ontogenesis of the nematode *C. elegans* is a highly dynamic process, and although many studies targeted the associated transcriptomic changes, detailed information on protein level is still missing. For this reason, we analyzed changes in the *C. elegans* proteome at several timepoints after hatching using ^{15}N metabolic labeling.
Methods: We employed a cost-effective new labeling method using minimal medium containing ^{15}N ammonia chloride. Two biological replicates of animals 20, 40 and 60 hours after hatching were analyzed by LC-MS/MS, and proteins were identified and quantified using the Mascot Distiller quantitation toolbox against an internal ^{15}N-labeled standard comprising of a mixture of all samples in equal amounts.
Results: Quantitative proteomics analysis revealed 245 proteins that were identified and quantified in all samples and thus could be followed over the analyzed time course. Statistical analysis revealed a significant regulation for 61 proteins. Between 20 and 40 hours after hatching, a strong upregulation of proteins involved in protein biosynthesis and metabolic pathways was observed, while extracellular matrix proteins and muscle proteins were downregulated. Changes from 60 to 40 hours were much less pronounced with only 3 proteins being significantly upregulated. One of them was the egg-yolk precursor protein VIT-6. The observed

progression of VIT-6 abundance is in line with earlier reports of its stage-specific expression with a gradual increase towards adulthood. Of 54 identified ribosomal proteins, 13 were upregulated and one was downregulated between 20 and 40 hours after hatching, while the others showed no significant changes in abundance. These results indicate that the increased abundance of specific ribosomal proteins reflects more than a general increase in ribosomes and hints to an additional regulatory or non-ribosomal function of those proteins.
Conclusion: We report a quantitative proteomics profiling of changes during *C. elegans* larval development. Changes are most pronounced between 20 and 40 hours after hatching, with an increased abundance of proteins involved in protein biosynthesis and metabolism.

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TOWARDS UNRAVELING KEY MOLECULAR COMPONENTS IN ADULT STEM CELL BIOLOGY WITH MASS SPECTROMETRY BASED PROTEOMICS

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Introduction: The proteome is a highly complex dynamic beast that challenges all aspects of every methodology used to interrogate it. In this contribution I will describe our work in sequencing and quantitation and specifically the improvements we have made towards allowing facile comprehensive quantitative proteomics experiments. For a number of years, IT-CID has been the method of choice for peptide identification; However, advances in instrumentation now allow beam-type higher energy collisional activation (HCD) and ETD with comparable performance. In this work we will present a systematic evaluation of all sequencing modes. Furthermore, combined with 'dimethyl labelling', we will show the optimized proteomics strategy for the characterization of FACS-sorted adult stem cells.
Methods: Peptides were analyzed either directly or after SCX-fractionation on an ETD enabled Orbitrap Velos. Data was analysed with Proteome Discoverer 1.3.
Results: Here, we show how HCD and ETD can be used complimentarily to facilitate more efficient peptide sequencing. I will also talk about the isotopic labeling strategy 'Dimethyl labeling' which can now be applied simply and efficiently on any level of material from any origin. Finally, I will describe how these technologies can help identify key molecular components in adult stem cells. The identification of Lgr5 as a definitive intestinal stem cell marker has now made it possible to isolate adult stem cells in order to study their unique biology including *in-vivo* differentiation. Combined with the improvements in sequencing and quantitation we can now compare the proteomes of FACS sorted intestinal stem cells and their immediate daughter cells. This represents the first quantitative proteomic analysis of small intestinal stem cells to an unprecedented depth of ~6,000 proteins, revealing rapid changes in protein abundance in the differentiating daughter cells within very few cell divisions, including several important Wnt target genes.
Conclusion: We demonstrate an optimized sequencing and quantification strategy for the analysis of adult stem cells. Using our strategy we are able to characterize over 6000 proteins from a modest amount of starting material.

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ENHANCING QUANTITATIVE PRECISION IN COMPLEX SYSTEMS UTILIZING A HIGHLY SELECTIVE AND ORTHOGONAL ANALYTICAL WORKFLOW

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Introduction: Quantitative proteomics label-free or labeled is based on the area ratio of matched pairs. The accuracy is limited to how uniquely each ion is measured. Described is a workflow using an ion detection algorithm which assigns a purity value to each ion. Enhanced accuracy is obtained by controlling which ions are utilized in determining the quantitative change.
Methods: Cells were grown in 'heavy' and 'light' media. Each sample was digested, reduced and alkylated both individually and as 1:1 mixtures. Peptides were separated over 120 min and analysed with a MS with

and without ion mobility (IMS). Ion interference rates were determined utilizing the ions' mass and chromatographic peak widths at half-height.
Results: Samples produced ~148K and 178K low-energy ions with and without IMS. Querying the two data sets at +/-10ppm and 15 sec resulted in ~32k common ion detections. Ion interference was determined by querying the light-ion lists with pseudo heavy-m/z values at a mass tolerance reflective of the mass resolving power and Rt tolerances of 15 and 30 sec. With IMS, the interference rate reduced from ~18.6% to 3.4% at 15 sec and 27.9% to 5.3% at 30 sec. To ascertain which ions should be used for quantification a unique identifier was created for each peptide and its replication rate determined. A 0.5% peptide error rate was employed. The mass, Rt and drift times for all injections were compared, outliers removed, and each validated sequence was annotated with its' average mass, Rt and drift time. The resulting list was then matched back to each replicates' ion list to fill in any holes where ions were measured albeit not identified. Here, the algorithm has the area of every isotope of every charge-state to every parent peptide. Ion selection is then determined utilizing the calculated purity scores and the associated area ratios. Limiting the ion selection reduced the average CVs from 20% to 8% with the best reductions at the lower concentration levels.
Conclusion: Use of ion purity measurements for enhancing quantitative accuracy and precision in stable isotope labeled complex "systems"

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THE PROTEOMICS OF FUNCTIONAL COMPLEXES ON REPLICATING CHROMATIN
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Introduction: We have previously established a systems approach based on quantitative timelapse proteomics for analysis of chromatin passing through interphase of the cell cycle. Our data demonstrated the highly dynamic nature of the chromatin proteome and showed that inhibition of DNA replication has unexpectedly large system-wide effects on the chromatin composition (1). We then established an unbiased approach to identify protein complexes assembled on replicating chromatin in *Xenopus laevis* egg extract.
Methods: We isolated chromatin in the middle of S-phase and released protein complexes into solution by complete digestion of the DNA. Solubilised protein complexes were separated based on their molecular size and shape using gel filtration and glycerol gradient techniques. The proteins present in all fractions were identified by quantitative mass spectrometry. Label free quantitative information on peptide abundance was extracted by Maxquant and then used to assemble, analyse and compare protein profiles with in-house written software “Chronoprot”. We calculated the native molecular size of each protein from two hydrodynamic parameters: the Stokes radius from gel filtration and the sedimentation coefficient from glycerol gradient.
Results: Number of identified proteins were analysed by western blotting and all of them confirmed the fractionation profiles obtained from mass spectrometry analysis. The use of correlation analysis with two different fractionation techniques allows independent confirmation of the putative complexes and the estimation of potential complex size. This resulted in determination of many previously described complexes, verifying our approach. Relevant to replication were GINS/Cdc45, RFC, ORC, FACT, RPA and cohesins. We also demonstrated that Mcm2-7 form double hexamers at licensed origins in *Xenopus egg* extract(2). Some of predicted possible interactions are currently under investigation.
Conclusion: We are creating a database of *Xenopus* proteins bound to chromatin during S-phase progression, containing their native molecular size, potential complexes, timing of chromatin binding and behaviour in response to replication inhibitors.
References: 1. Khoudoli et al, (2008) Curr Biol 18, 838-843
2. Gambus et al, (2011) J. Biol. Chem. 286, 11855-64

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PROTEIN SYNTHESIS AND DEGRADATION BY 15N TIME COURSE MASS SPECTROMETRY IN THE SMALLEST KNOWN GREEN ALGA OSTREOCOCCUS TAURI
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Introduction: The continual degradation and synthesis of proteins enables cells to implement rapid adaptation. Plants in particular have evolved sophisticated biochemical tactics to deal with fluctuating living conditions. Tailored turnover rates can eliminate the need for a specific stress response, as a global increase in synthesis will result in "slow" turnover proteins accumulating more rapidly than "fast" ones. Experimentally however, these rates are little known proteome parameters. SILAC-based studies have recently begun to quantify turnover in yeast and animal cells, however these methods are not fully compatible in plants. Here, we present a proteome-scale method to quantify turnover, synthesis and degradation rates of individual proteins in autotrophic organisms such as algae and plants.
Methods: Ostreococcus tauri (OTTH0595) were cultured in artificial sea water with either 14N or 15N nitrogen sources, before exchanging filtered media. 15N incorporation in the 14N culture was monitored over six days. Samples were digested, cleaned and analyzed on an LC-coupled LTQ-Orbitrap XL mass spectrometer. A set of Perl programs were written to automatically extract and fit partial 15N labelled peptide peak data, returning the percentage of 15N incorporation and relative intensities of light and heavy peaks.
Results: We observed an average turnover rate of 5% per day. High relative turnover occurred in chloroplast proteins, in contrast to similar nuclear-encoded proteins. Chloroplast ATPases, core photosystem II proteins, and RbcL were among the fastest proteins (0.34-0.58 %/h). The nuclear-encoded RbcS2, mitochondrial targeted ATPases, photosystem antennae, and histones were comparatively stable (0.07-0.23 %/h). The calculation of degradation and synthesis rate constants confirmed RbcL as the bulk contributor to turnover.
Conclusion: We have demonstrated the automated analysis of partial 15N-labeled MS-level peptide spectra and the quantification of protein turnover. This method lends itself to the integration of dynamic proteome and metabolome studies. Understanding of the control of inorganic nitrogen assimilation and protein dynamics during plant growth could enable crop improvements and result in agronomic applications.
References: SF Martin et al, JPR 2012

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CHROMATIN PROTEOMICS. FROM METHOD DEVELOPMENT TOWARDS AN INVENTORY OF INTERPHASE CHROMATIN

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Introduction: Chromatin contains about twice as much protein as DNA. Chromosomal proteins orchestrate fundamental biological processes such as gene expression, replication and essentially all other biological processes taking place at the level of the eukaryotic genome. Yet surprisingly, no unbiased approach to identifying proteins associated with chromatin exists to date. New chromatin players are often identified biochemically, on the basis of their interaction with known chromatin components, or using functional assays for a particular chromatin-based process. We have developed a radically different approach towards the identification of such chromatin “effector” proteins. We named the resulting chromatin proteomics pipeline “Effector protein identification by chromatin cross-linking” (Epixx). In a second step, we combined Epixx with a machine-learning approach to distinguish between genuine chromatin components and co-purifying contaminants.
Methods: We used a new method for isolating chromatin that is suitable for proteomic analysis.
Results: We identified several hundred previously uncharacterized proteins as novel chromatin components.
Conclusion: Our results provide an inventory of human interphase chromatin.

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QUANTITATIVE PROTEOMIC INVESTIGATION OF DIFFERENCES IN PHAGOCYTOSIS INDUCED BY KEY PHAGOCYTOTIC LIGANDS

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Introduction: Macrophages and other immune cells engulf microbes and cellular debris following recognition of discriminating ligands in phagosomes, where the particles are degraded. Through the production and isolation of phagosomes via differing receptor ligands, including bacterial membrane and fungal cell wall

components, immune opsonins, and apoptotic cell markers, the differential proteome of phagosomes was tracked over a timecourse in order to elucidate the cellular pathways which properly sort diverse phagosomal cargo.
Methods: RAW264.7 murine macrophages were inoculated with macrophage ligand-coated polystyrene beads, and phagosomes were isolated across a 15, 30, 60, and 180 min timecourse. Tryptic peptides were isotopically labelled, and a pooled internal control in all runs provided a universal intensity reference. Samples were analyzed via LC-MS/MS on a Dionex U3000 UHPLC with a 50 cm PepMap C18 column coupled to an Orbitrap Velos Pro. Resulting data were searched and quantified using MaxQuant 1.2.2.5 to produce LFQ intensities, and ligand-induced and temporal changes statistically vetted and visualized through in-house Chronoprot software.
Results: Avidinylated polystyrene beads have been generated to enable consistent attachment of diverse ligands, verified by flow cytometry and the ability to induce an inflammatory response as assayed by induction of NF-κB. Identifications of over 1200 proteins were possible in each triplex run by using a detergent-based preparation method and high resolution chromatographic conditions. Thus far, the preliminary analysis of early phagosomes following uptake of LPS or control coated beads showed high reproducibility and yielded identification of 1649 protein groups and quantification data for 1126 proteins. Moreover, data indicate that LPS-coated beadphagosomes more rapidly mature into phagolysosomes relative to control beads, which are more enriched for endosomal proteins. As LPS has a demonstrated ability to increase the rate of phagosomal maturation, these results validate the present approach to dissect differences in phagosomal processing due to differential ligand-receptor activation.
Conclusion: Time-resolved proteomics analysis of phagosomes shows that receptor-ligand interactions considerably affect phagosome biogenesis.

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PROTEOME-WIDE STUDIES OF PROTEIN UBIQUITINATION USING COFRADIC
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Introduction: Protein ubiquitination regulates various protein functions and steers several pathways including protein degradation, trafficking and signaling. Despite its importance, proteome-wide profiling of ubiquitination sites has remained challenging, amongst others due to the difficulties in enriching for peptides holding ubiquitinated sites. Only a few proteome-wide studies have been published and these typically use over-expressed tagged ubiquitin. Here we present a novel mass spectrometry driven proteomics method to identify ubiquitination sites in proteins using the COFRADIC principle.
Methods: Following lysis of human JURKAT cells, ubiquitin is enzymatically removed from proteins and replaced by a handle. After trypsinisation, this handle allows specific isolation by tandem RP-HPLC (COFRADIC) of those peptides which were ubiquitinated *in vivo*. The isolated peptides are subsequently identified by high-resolution mass spectrometry.
Results: In human JURKAT cells, without over-expressing tagged ubiquitin and without using proteasome inhibitors, we catalogued more than 8000 putative protein ubiquitination sites in 2425 proteins. Besides known sites, our dataset also covers thousands of new sites. To the best of our knowledge, we here identified one of the largest, unbiased and site-specific protein ubiquitination datasets in human cells so far.
Conclusion: Here we show that our Ubiquitin COFRADIC approach is successful in identifying thousands of potential ubiquitination sites, without the need for the overexpression of tagged ubiquitin or the use of proteasome inhibitors. Future experiments will use differentially labeled COFRADIC sorting handles, such that our method allows comparison of two or more biological samples.

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STUDY OF YEAST PLASMA MEMBRANE PROTEINS DYNAMICS BY QUANTITATIVE PROTEOMIC ANALYSIS

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Introduction: Living organisms possess a wide variety of mechanisms to respond to environmental changes. Many studies are interested in signaling pathways that control the regulation of yeast genes expression by the nitrogen source available in the environment. In our lab, we want to understand the mechanisms of regulation of yeast plasma membrane proteins after a sudden change of the nitrogen source available in the medium.
Methods: For this purpose, we developed a gel-free quantitative proteomic approach adapted to plasma membranes. In this procedure, highly purified plasma membrane proteins are digested into peptides; those peptides are then labeled with iTRAQ, separated by reversed-phase chromatography and analyzed by tandem mass spectrometry (LC-MS/MS). We applied this technique to compare the protein contents of purified plasma membrane from yeast cultivated in presence of either ammonium or proline as unique nitrogen source. In order to follow the evolution over time of the plasma membrane proteome after a sudden change in nitrogen source, we designed a kinetic experiment. Cells were first grown on proline, then ammonium was added to the medium and cells were harvested after 15, 45 and 90 minutes.
Results: This gel-free quantitative proteomic procedure allowed the identification of 124 plasma membrane proteins. Among them, 18 were significantly less abundant after growth on ammonium, mostly transporters of nitrogenous compounds, but also the plasma membrane H^+-ATPase, components of microdomains, proteins involved in endocytosis, etc. The quantitative results of the kinetic experiment confirmed that some proteins are down-regulated faster than others, suggesting that different mechanisms of regulation may be involved.
Conclusion: In conclusion, we developed a gel-free quantitative proteomic approach based on iTRAQ labeling of highly purified plasma membrane proteins to monitor changes in the plasma membrane proteome according to the nitrogen source available in the growth medium. This technique circumvents the limitations of traditional gel-based approaches and allowed the identification and quantification of a high number of plasma membrane proteins. Moreover, it offers a global approach to study the dynamics of the plasma membrane proteome in response to a sudden change in the external environment.

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RAPID HEAT INACTIVATION OF PHOSPHATASES AND KINASES IS ESSENTIAL FOR RELIABLE MEASUREMENT OF PHOSPHORYLATION STATES WITHOUT INTERFERENCE FROM POST-MORTEM EVENTS

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Introduction: Recent reports show that a group of protein and peptide biomarkers, regardless of tissue origin or species, is often found expressed differentially in various disease states. We suspect an overlap between proteins commonly identified as changing in 2D proteomic studies and those found to change post-mortem. Proteins with one or more phosphorylation sites are of particular interest since they are over-represented in this group.
Methods: Data was drawn from several proteomic studies in which tissue samples were heat-stabilized[1-4] or snap-frozen[5,6] to create a list of 86 unique proteins reported to be change post-mortem. The protein list was compared with another list of 48 proteins often found in 2DGE experiments when studying disease states[7,8]. Various estimations were made to investigate any apparent overlap of proteins, for example, by examining phosphorylation sites.
Results: Comparing the disease-changing – and the post-mortem changing proteins subset, we found a match of 65%. When focusing on phosphorylations, 81% of the disease-changing proteins subset has known phosphorylation sites. In the list of post-mortem changing proteins, 84% contain at least one known phosphorylation site. This clear over-representation of phosphorylation sites, compared to a total average of 30%, indicates that proteins, and their post-translational modifications, change substantially post-mortem. Taken together with the recent report of the elimination of kinase activity in heat-stabilized samples[9], we

conclude that it is highly important to quickly inactivate both phosphatases and kinases to ensure reliable measurement of phosphorylation states.
Conclusion: We conclude that post-mortem changes, particularly in phosphorylation states, may distort our view of in vivo proteomic profiles. The use of heat stabilization permanently stops enzymatic activity. This approach may help us differentiate true biomarkers from those proteins found in any situation where cells are under stress.
References: 1 Skold, Proteomics. 2007**.** 2 Scholz, MCP. 2011**.** 3 Goodwin, Proteomics. 2010**.** 4 Robinson, Proteomics. 2009**.** 5 Hunsucker, Jn of Neurochemistry. 2008**.** 6 Jackson, Proteomics. 2006**.** 7 Petrak, Proteomics. 2008**.** 8 Wang, Proteomics. 2009**.** 9 Smejkal, Electrophoresis. 2011

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QUANTITATIVE PROTEOMIC ANALYSIS OF POLYPHOSPHATE METABOLISM MUTANTS OF ESCHERICHIA COLI: COMPARISON BETWEEN MRNA AND PROTEIN ABUNDANCE.
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Introduction: Polyphosphates (polyP) is a linear biopolymer found in all living things. In *Escherichia coli* is synthesized by the polyphosphate kinase (PPK1), while its degradation to phosphate is produced by the exopolyphosphatase (PPX). PolyP deficit causes many structural and functional defects in bacterial cells. The link between genotypes and phenotypes observed during polyP deficiency can be the result of complex networks of interaction that can be elucidated by using Omics technologies. We previously studied the global transcriptome changes in *E. coli* polyP mutants deficient in *ppk1*, *ppx* and both genes (*ppk1-ppx*). To complement these studies we performed quantitative proteomics studies in the same polyP mutant strains.
Methods: Proteins extracts prepared from E. coli wild-type and polyP mutant strains were digested and labelled with the ICPL reagent at the peptide level according to the manufacturer's instructions (1).
Results: By using isotope-coded protein labelling (ICPL) in combination with mass spectrometry we identified more than 900 different proteins and about 328 could be quantified as well. Of these proteins, 17% were observed with the same pattern in previous transcriptomic experiments. Moreover, the gene ontology (GO) categories overrepresented among the proteins that change their expression (Krebs cycle, fermentation, homeostatic processes, aerobic and anaerobic respiration) were also observed in previous microarray experiments. Bioinformatic analyses highlight a possible deregulation of ArcA that could explain all metabolic imbalances in both, polyP-synthesis and -degradation mutants.
Conclusion: Despite we only found 17% of concordance in the proteomic and transcriptomics levels, a direct correlation between mRNA changes and protein abundance was observed for several GO categories and metabolic pathways.
References: 1. Paradela A, Marcilla M, Navajas R, Ferreira L, Ramos-Fernandez A, Fernández M, Mariscotti JF, García-del Portillo F, Albar JP. Evaluation of isotope-coded protein labeling (ICPL) in the quantitative analysis of complex proteomes. Talanta. 2010;80:1496-502.

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PROTEOMIC ANALYSIS OF SHEWANELLA-LIKE PPP-FAMILY PROTEIN PHOSPHATASES FROM ARABIDOPSIS THALIANA
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Introduction: *Shewanella*-like (SLP) phosphatases are a recently resolved subgroup of the PPP-family protein phosphatase compliment of photosynthetic eukaryotes. In green plants SLP phosphatases exist as two isoforms, SLP1 (chloroplast targeted) and SLP2 (cytosol targeted), with recent biochemical analysis revealing their unique insensitivity to the classic PPP-family phosphatase inhibitors microcystin-LR and okadaic acid.
Methods: To elucidate the cellular function of SLP phosphatases from *Arabidopsis thaliana*, tandem affinity purification coupled to mass spectrometric analysis was employed to identify SLP phosphatase protein

interactors. *Arabidopsis thaliana* SLP phosphatase tandem affinity purification fusion constructs were stably transformed into both *Arabidopsis thaliana* cell culture and whole plants via *Agrobacterium* mediated transfection techniques. Affinity purification involved: IgG sepharose purification, HRV3c protease mediated protein-A tag removal and Ni-NTA purification followed by on bead trypsinization and whole sample analysis using a ThermoFinnigan LTQ linear ion trap mass spectrometer. All identified tryptic peptides were searched against the *Arabidopsis thaliana* complement of the RefSeq database (http://www.ncbi.nlm.nih.gov/RefSeq/) using the Matrix Science Mascot search engine. Trypsin specificity with one missed cleavage was selected along with methionine oxidation as a variable modification. Search results were parsed into a relational database (ProHits). Filtering of the results was performed by removing hits with a Mascot score <60 and only one unique peptide.
Results: Mass spectrometry resolved a small subset of SLP1 (chloroplastic) and SLP2 (cytosolic) specific protein interactors indicative of SLP phosphatases possessing specialized subcellular functionality. Identified binding partners indicate SLP phosphatases are likely involved in plant cellular energy regulation.
Conclusion: Previous biochemical evidence found purified *Arabidopsis thaliana* SLP1 phosphatase to have a physiologically relevant sensitivity to inhibition by inorganic phosphate, substantiating the identified protein interaction partner dataset and further implicating SLP1 phosphatases as functioning in plant cellular energy regulation.

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PROTEOME PROFILE COMPARISON IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE AND CONGENIC RAT STRAINS

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Introduction: Previous work identified altered sphingosine signalling and blood pressure differences in our stroke-prone spontaneously hypertensive (SHRSP), normotensive Wistar-Kyoto (WKY) and chromosome 2 congenic (SP.WKYGla2a) strains. We aim to extend this observation by comparing their proteome profiles, to examine the potential effect of altered signalling on blood pressure regulation.
Methods: We performed proteome profiling of mesenteric primary vascular smooth muscle cells from 16 week-old male WKY, SP.WKYGla2a and SHRSP, using triple stable isotope labeling by amino acids in cell culture (SILAC). Cells from each strain were grown in differentially labelled media (light:R0K0, medium:R6K6, heavy:R10K8), mixed in 1:1:1 ratio and after SDS-PAGE and in-gel trypsin digestion analysed by liquid chromatography coupled to mass spectrometry (MS) on Orbitrap Velos. MS data was filtered and quantified through MaxQuant software and uploaded to Perseus platform for statistical analysis. To explore relevant biological pathways the data was uploaded to Ingenuity Pathway Analysis database and filtered by fold change (FC: +/- 1.3).
Results: From a total number of 1998 proteins identified in MaxQuant, 254 were found to be differentially expressed between SHRSP versus WKY (comparison A). 172 were in common, with SP.WKYGla2a versus WKY (comparison B). 13 of the common proteins mapped to the chromosome 2 congenic interval, including proteins known to be implicated in biological processes associated with blood pressure regulation such as oxidative stress (glutamate-cysteine ligase (GCLM) FC: +2.0 / +3.3), hypertrophy (natriuretic peptide receptor C (NPR3) FC: +2.2 / +3.8), and fibrosis (collagen type XI alpha 1 (COL11A1) FC: -2.0 / -1.4) (FC: comparisons A / B respectively).
Conclusion: The combination of high throughput proteome comparison with congenic strain construction allows identification of a number of chromosome 2 differentially expressed proteins, that may result in altered sphingosine signaling and blood pressure regulation. Data from this study will facilitate construction of computational models of signaling networks relevant to hypertension in the SHRSP.

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UNRAVELING THE INTERACTIONS BETWEEN PLANTS´ PLASMA MEMBRANE MICRODOMAINS WITH THE CYTOSKELETON ELEMENTS.

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Introduction: In this work we evaluate the interactions between the cytoskeleton and plasma membrane microdomains in plants. Since the cytoskeleton, especially the actin and microtubule filaments, is highly dynamic, it may play an important role in the protein dynamics of the plasma membrane and may have implications for the regulatory processes and signal transduction.
Methods: In our experiments we used *A. thaliana* cell suspension cultures which were treated with the actin and microtubular depolimerizating agents, Cytochalasin D and Oryzalin respectively. Subsequently, we extracted plasma membrane with the use of two-phase system [1]. Then we treated the fraction of plasma membrane with Triton X-100 and separated the fraction of detergent resistant membranes (DRM) by sucrose gradient centrifugation.
Digested and desalted peptides from the different fractions soluble proteins, plasma membrane, DRM and detergent soluble membranes were analyzed by LC/MS/MS using nanoflow HPLC and an LTQ-Orbitrap hybrid mass spectrometer as mass analyzer.
MaxQuant was used as a tool for peptide identification and label free quantification. Obtained peptide lists were statistically evaluated by cRacker [2] which is an in-house application based on R for the statistical analysis of mass spectrometry measurements. Normalization and processing was based on fraction of total normalization and z score scaling.
Results: Among the membrane proteins which responded to actin or microtubule depolymerization we were particularly interested in those proteins known as constituents of the plasma membrane microdomains from sterol-depletion experiments [3]. Among these we identified proteins like ATPases, phosphate or sugar transporters.
Conclusion: Our studies show that in plants the localization of certain proteins in the sterol enriched microdomains is dependent on the interaction with cytoskeleton. We hypothesize that altered localization within the plasma membrane can result in drastic changes of the activity of these proteins. We are in progress of studying these relationships on a functional level by enzymatic activity measurements and GFP-localization studies.
References: [1] Marmagne A, et al; Methods Mol Biol.;323:403-20. 2006.
[2] http://cracker.mpimp-golm.mpg.de/
[3] Kierszniowska S, et al; Mol Cell Proteomics. 2009 Apr;8(4):612-23. Epub 2008 Nov 25.

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STUDYING PROTEOMIC CHANGES IN MICRO DOMAINS OF PLASMA MEMBRANE USING A. THALIANA MUTANTS WITH CHANGED STEROL COMPOSITION

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Introduction: The plasma membrane is the initial point of perception of environmental changes, triggering signal transduction. In this context, analysis of proteins located in microdomains is gaining in importance in mammals and plants as they have shown to be involved in dynamic adaptation to external stress.
In this study we used the plant knockout mutant *ugt80A2;B1,* lacking both sterol glycosyl transferases, as a control and developed a systems approach to validate responding proteins using label free quantitation. We further developed a fully automated R based platform for standardized processing of quantitated data.
Methods: A.thaliana lines include the double knockout *ugt80A2;B1* grown in cell culture. For the analysis of protein locations, protein extracts were fractionated to soluble proteins, intracellular membranes, detergent resistant or soluble membranes (SP, IM, DRM, DSM). Tryptic peptides have been analyzed on a nLC coupled LTQ-Orbitrap. Peptides have been identified and quantified using MaxQuant. Normalization and processing of peptide intensities was done with the self-developed R script suite cRacker, using fraction of total normalization and z score scaling.

Results: From 1861 quantified proteins, 127 were significantly differentially abundant in DRM fractions of *ugt80A2;B1* compared to wt. Co-purifying proteins with significant abundance in IM or SP were filtered out. Particularly kinases and G-proteins showed a significant decreased abundance in DRM fractions. The same was observed for remorin, a marker of micro domains, confirming the role of glycosylated sterols in membrane microdomain formation and protein recruitment.
Conclusion: The use of a mutant providing a membrane microdomain environment with changed physicochemical properties enabled us to define a role of micro domains in signaling events. Thereby systematic comparisons of protein abundances in other fractions like IM and SP is evident. In *ugt80A2;B1* sterol dependent proteins show a decreased affinity to DRM fraction, reflecting the biological relevance of sterol glycosylation for forming functional micro domains.
Following the current analysis strategy we will perform starvation resupply experiments and analyze protein phosphorylation testing for altered signaling properties in *ugt80A2;B1*.

P210

HIGH SELECTIVITY QUANTIFICATION OF PROTEIN ISOFORMS USING MRM3.
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Introduction: Quantitation of proteins by Multiple Reaction Monitoring (MRM) requires selection of peptides which are unique to the sequence of the protein of interest. When a protein is part of a protein family with very high sequence similarity, this can often restrict the choice of unique peptides for quantification to those that are not proteotypic, and are present at lower abundance. MRM^3 is a high selectivity method that provides additional specificity by monitoring secondary product ions of a peptide of interest. In this work, the CYP450 3A5 protein (which shares ~80% amino acid sequence identity with isoform 3A4) is specifically quantified in human liver microsomes by monitoring peptides unique to the 3A5 protein.
Methods: Synthetic tryptic peptides to the CYP450 3A5 protein (both isotopically heavy and light) were used to design MRM and MRM^3 assays. Peptide separation was performed on an Eksigent nanoLC-Ultra® 2D pump equipped with a cHiPLC®-nanoflex system in trap elute mode. Each sample was desalted on a 200 µm x 6 mm trap chip and then eluted onto a 200 µm x 150 mm column chip for MS analysis. Peptides were separated using a linear gradient formed by A (2% ACN, 0.1% FA) and B (98% ACN, 0.1% FA) from 10–30% B over 45 minutes at a flow of 1 µL/min. MS analysis was performed on a QTRAP® 4500 system. Data was processed using MultiQuant™ Software.
Results: Selective quantitation of the CYP450 3A5 protein was achieved by monitoring peptides unique to this specific isoform. The two unique peptides selected: DTINFLSK and SLGPVGFMK showed significant interferences in two or more dominant MRM transitions at concentrations less than 5 fmol on column. To improve selectivity and LLOQ values, MRM^3 analysis was applied to the two peptides by resonance excitation of selected first generation product ions in the linear ion trap to generate second generation fragments, which were scanned out of the trap at 10,000 Da per second. Extracted ion chromatograms were generated for multiple secondary product ions and summed together to produce MRM^3 traces, from which quantitation was performed.
Conclusion: The application of MRM^3 to quantitation of the two unique peptides resulted in LLOQ values lower than the MRM assay, and showed good linearity between 1.2 fmol and 805 fmol on column.

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N-TERMINAL PROTEIN VARIANTS AND THEIR DIFFERENT STABILITIES IN CELLS
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Introduction: Stable isotope labeling of amino acids in cell culture (SILAC) enables mass spectrometry based analyses of proteome dynamics. In contrast to other proteome studies that cannot readily distinguish between protein isoforms encoded by the same gene, our N-terminal COFRADIC positional proteomics provide unique information on the synthesis and degradation rates of protein isoforms deriving from alternative translation initiation events.

Methods: Jurkat cells were grown in the presence of either $^{12}C_6$ (light) or $^{13}C_6$ (medium) variants of the essential amino acid L-Arg. A pulse-chase was performed by changing the $^{13}C_6$ Arg media to $^{13}C_6{}^{15}N_4$ Arg (heavy) containing media, proteomes were harvested at different time points (0.5 to 48 h) after which equal amounts of light and medium/heavy labeled samples were combined. N-terminal peptides were then enriched by COFRADIC.
Results: Triple labeling strategy allowed us to identify three dynamic forms of each N-terminal peptide and to calculate protein half-lifes. The “light” peptides represent the average protein levels that are maintained in a dynamic equilibrium. The decrease in “medium” peptides shows protein degradation while “heavy” peptides reflect the rate of protein synthesis. Proteins with the highest turnover are involved in mitosis, ribosome biosynthesis, apoptosis and cell cycle regulation. Further, numerous protein isoforms deriving from alternative usage of translation initiation sites have distinct stability.
Conclusion: Our results highlight the underestimated incidence and importance of database non-annotated protein N-terminal isoforms. Variation of turnover rates between such protein variants point to their possible differential regulation and yet unrevealed functions.

P212

IDENTIFICATION OF MORE THAN 3000 PROTEINS IN SINGLE LC/MS/MS RUNS FROM A CELL LINE LYSATE

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Introduction: A distinct goal in proteomics is still the survey of an entire cell lysate with a maximum number of unambiguously identified proteins. While high resolution instruments are favored for this application, recent improvements in both MS instrumentation as well as in nanoLC separation lead to a much higher number of identified proteins from such a sample in single LC/MS/MS experiments in general. Important criteria for a successful analysis are a high dynamic range, fast MS/MS and good mass accuracy. Results for various mass spectrometer platforms are demonstrated.
Methods: 1 ug Human colon adenocarcinoma cell line was trypsin digested and the resulting digest was separated by nanoLC (Dionex Ultimate 3000 RSLC nano or Bruker Easy-NanoLC) with a Dionex PepMap C18, 20x0.075 mm trap column and a Dionex PepMap C18, 150 – 500 x 0.075 mm analytical column at a flow rate of 300 nL/min with eluent A / B: 0.1% FA in water, 80 % ACN 0.1% FA. The nanoLC was coupled online either to the maxis impact QTOF or the amazon speed ion trap was done. For MALDI, fraction collection was done on a Proteineer fc II (Bruker) in 1360 fractions, 10s each, on a Bruker MTP AnchorChip 1536 TF. The database search was done in the NCBI database using Mascot (Matrix Science). Data validation was done by ProteinScape (Bruker).
Results: Very restrictive criteria were applied in the database search to largely avoid false positive hits. The peptide identity threshold was set to be at least 35, the peptide false discovery rate to FDR < 1%. The list of hits was further screened for multiple or redundant proteins found for the same peptides which were eliminated. More than 12,000 peptides are identified in single runs, resulting in more than 3000 identified proteins, e.g. for the maxis impact, at a FDR < 1%.
Conclusion: Merging the data for the maxis and the ultraFlextreme, which is partially complementary due to the different ionization techniques, leads to a 20% increase in identified proteins. All different platforms demonstrate the strength of the individual technologies as well as the advantage of merging data from different ionization modes.

P214

THE CGMP-DEPENDENT PROTEOME AND PHOSPHO-PROTEOME OF ARABIDOPSIS THALIANA

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Introduction: Cyclic nucleotides are universal second messengers with key roles in many and diverse physiological responses and processes in prokaryotes, and in both higher and lower eukaryotes. Guanosine 3’,5’-cyclic monophosphate (cGMP), the molecule of interest here, has been implemented in a wide range of

cellular and physiological processes in vertebrates and invertebrates, as well as bacteria, fungi, and algae. In plants, growing evidence points to a central role of cGMP in physiological processes including abiotic and biotic stress-response signaling, the gating of ion channels, light signal transduction and hormone signal transduction. In this study, we investigated the effects of cGMP on the phosphorylation status of proteins.
Methods: To this end, we used a cell suspension culture of *Arabidopsis thaliana* and applied 10 µM of the cell permeable 8-Bromo-cGMP for different length of time. Total protein was extracted by TCA/acetone precipitation and resolubilized in urea-thiourea based buffer. Proteins were then separated by 2D gel electrophoresis and stained with Pro-Q Diamond for visualization of phosphoproteins and subsequently with Sypro Ruby for detection of total proteins. The Delta 2D analysis software was used to compare gels from the untreated and cGMP treated samples.
Results: A total of 127 phosphoproteins and 83 proteins were found responsive to the cGMP treatment and were identified by LC-MS/MS.
Conclusion: Functional categorization of these proteins will provide new insight into plant signaling at the systems level.

P215

SUB-CELLULAR PLASTICITY AND DYNAMICS OF ALL ENDOGENOUS PROTEASOME COMPLEXES UNRAVELED BY A WORKFLOW COMBINING IN VIVO PROTEIN CROSS-LINKING AND QUANTITATIVE PROTEOMICS

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Introduction: The proteasome is a multimeric protease complex which is involved in the regulation of many important cellular processes. Mammalian proteasome is composed by a central catalytic core, the 20S proteasome, which can exist alone or associated with one or two regulatory particles. Various proteasome regulators exist but their precise cellular localization and their distribution within the cell remain to be determined.
The aim of the present work was to determine the distribution of proteasome regulators associated to the catalytic core according to the cellular localization, and their dynamics upon IFNγ stimulation, to allow a better understanding of their functions.
Methods: An in vivo cross-linking procedure using formaldehyde was developed to stabilize labile proteasome networks but also to freeze them in a native and functional state. Three high quality fractions (cytosol, microsol, and nucleus) were then isolated from leukemic cells and proteasome complexes were immunopurified. Robust label-free quantitative proteomics strategies based on high speed, high resolution and high sensitivity Orbitrap MS analyses and dedicated bioinformatics tools were used to determine for the first time the sub-cellular distribution of proteasome complexes in leukemic cells.
Results: In vivo crosslink maintained all regulatory complexes associated to the 20S proteasome and allowed a proper distribution to be determined. We show that free CP constitutes an important fraction of proteasomes. It can be considered as a platform recruiting different activators which relative integration in the whole complex might vary. Whatever the compartment and the cell type, the 19S complex is the main 20S proteasome-associated activator. If we consider all the regulator-associated 20S core complexes as what we call “activated 20S proteasome”, the fraction of activated proteasome represents almost half the total 20S proteasome pool in the nucleus, but a subsequently lower proportion, around one third, in the cytosol.
Conclusion: Important differences in the composition of microsomal proteasomes could be correlated to activity profiles among leukemic cell lines and upon IFNγ stimulation.

P216

ITRAQ−BASED COMPARATIVE PROTEOMICS REVEALS CHANGE IN MACROPHAGE RAFT CONTAINING FERROPORTIN AFTER IRON TREATMENT

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Introduction: Macrophages play a key role in iron homeostasis through the recycling of heme iron from red blood cells during the process of erythrophagocytosis. This process involves the only known mammalian iron exporter ferroportin (Fpn) that is expressed in lipid rafts at the plasma membrane of macrophages. We previously observed that the presence of Fpn into cell surface lipid rafts is strongly enhanced upon iron treatment.
Methods: In order to gain insight into the biological function of Fpn in lipid rafts, we studied the changes of lipid raft proteome after iron treatment using detergent−resistant (Triton X−100) membranes (DRM) containing Fpn, iTRAQ (isobaric tag for relative and absolute quantitation) labeling and LC−MS/MS. J774a1 macrophages were treated or not with 200µM Fe−NTA for 17 hours and DRM were obtained using iodixanol flotation gradients. Lightest DRM containing Fpn (verified by western blot) from untreated and iron−treated cells were precipitated, trypsin digested before i−TRAQ labeling. Labeled peptides were first separated according to their pI and then further separated using Nano−chromatography before tandem mass spectrometry. The resulting spectra were used to identify and to quantify the proteins present in the sample using ProteinPilot analyses.
Results: A total of 823 proteins were indentified in DRM with a high percentage of predicted membrane proteins, or membrane−associated proteins including a number of raft associated proteins (ex; flotilin 1). We identified 66 proteins showing more than a 5−fold increased expression in DRM after Fe−NTA treatment. Among those proteins and accordingly to our biochemical analysis, Fpn was showed to increase importantly with Fe−NTA (ratio of 25).
Conclusion: Expression of signaling proteins including protein kinase and kinase pathway adaptators was altered by iron treatment. Others proteomic changes suggest a cellular response to oxidative stress likely mediated through iron overload. Interestingly, heme oxygenase 1 (the enzyme of heme catabolism) was strongly enriched in DRM after iron−NTA. The physiological relevance of this regulation is under investigation.

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PROTEIN PROFILE OF TWO SUGARCANE GENOTYPES CONTRASTING IN SUCROSE ACCUMULATION

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Introduction: Brazil is the world's largest producer of sugar and ethanol from sugarcane. Growing demands for ethanol production, particularly for the substitution of oil derived fuels in the transport sector, and also the need to reduce carbon dioxide emissions, has driven the growth of the sugarcane industry. Within the context of proteomics and plant breeding, the comparative study between segregating genotypes in sucrose accumulation can indicate major changes in gene expression that occur between these plants.
Methods: Two varieties were selected having contrasting genotypes in sucrose accumulation. The variety SP 80-3280 was selected as a material with high sucrose content and CO 740 variety was selected as having a low content of sucrose. Proteins of leaves from 12 month-old plants were extracted by the method of Kim et al. (2001). After desalinization, proteins were submitted to tryptic digestion. Peptides were sequenced in the Synapt G2 mass spectrometer (Waters) coupled to nanoACQUITY UPLC System with 2D-LC Technology (Waters). The data were processed using the ProteinLynx Global Server® version 2.5, using sugarcane database (SUCEST) and the identified proteins were annotated through the Blast2GO software (http://www.blast2go.com/b2ghome).
Results: Among the proteins/isoforms observed, 173 were exclusive from SP 80-3280 and 62 were exclusive from CO 740. Within SP 80-3280 exclusive results were observed three isoforms of the ADP-glucose pyrophosphorylase, which are involved in the starch and sucrose metabolism, one isoform similar to triose-phosphate isomerase and one phosphoenolpyruvate kinase that is involved in carbon fixation. The UDP-glucose pyrophosphorylase isoform, involved in the starch and sucrose metabolism, was up regulated in SP 80-3280. Three triose-phosphate isomerase isoforms were also up regulated in SP 80-3280.
Conclusion: These isoforms, among others, probably can contribute with the higher sucrose content of this variety.
References: Kim, S.T.; Cho, K.S.; Jang, Y.S.; Kang, K.Y. **Eletrophoresis**, v. 22, p.2103-2109, 2001.
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P219

PROTEOMICS ANALYSIS OF NITRATE/AMMONIUM ASSIMILATION IN THE PICOEUKARYOTIC GREEN ALGAE OSTREOCOCCUS TAURI

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Introduction: A major component of every cell is nitrogen, autotrophic organisms such as plants usually utilize ammonium or nitrate as the nitrogen source. Photosynthetic eukaryotes are responsible for assimilation of nitrogen, and carbon fixation in the oceans. One of such as picoeukaryotic is the unicellular green algae *Ostreococcus tauri* (*O*. *tauri*). Nitrogen assimilation is predominantly performed by the glutamine synthetase/glutamate synthase cycle, ammonium ions are the obvious substrates for the assimilatory reaction. However, nitrate is the nitrogen source most preferred because of its major abundance
Methods: *O. tauri* was grown in artificial sea water supplemented with nitrate or ammonium salts as a sole source of nitrogen. Samples were digested and fractionated in an Off-Gel fractionator prior to analysis on an LC-coupled LTQ-Orbitrap XL mass spectrometer. Quantitative data analysis (label-free) was performed using Progenesis.
Results: A total of 3500 proteins were identified; proteins such as nitrate reductase, nitrate transporters, ammonium transportes and glutamine synthetase were amount the proteins identified. Bioinformatics analysis shows quantitative information regarding functional categories, 330 proteins showed more than 1.5-fold up- or down-regulation. Proteins associated with nitrate assimilation, (nitrate reductase, nitrate high-affinity transporter, nitrite reductase and nitrate high-affinity transporter accessory protein) were found to be more than 2-fold up-regulated compared to ammonium. Other proteins such as glutamine synthetase showed no regulation in both conditions, possibly indicating no influence in its activity by the source of nitrogen. Enzymatic activity assays for glutamine synthetase and nitrate reductase showed similar trend as the proteomic data.
Conclusion: Result of this proteomic study shows a complex mechanism of nitrogen assimilation, with an overall regulation of nitrate assimilation proteins in the nitrate condition, together with stress-associated proteins in the ammonium condition. To our knowledge this is the first time that a qualitative and quantitative proteome analysis is performed to study the mechanism of nitrogen assimilation in *O*. *tauri*.

P220

IDENTIFICATION AND QUANTIFICATION OF >6,000 UNIQUE PHOSPHOPEPTIDE IONS BY LABEL-FREE MASS SPECTROMETRY-BASED PHOSPHOPROTEOMICS WITHOUT SAMPLE PRE-FRACTIONATION

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Introduction: Phosphoproteomics is a powerful platform by which signalling pathways can be profiled and quantified. Label-free quantification is particularly useful for comparing large arrays of biological conditions and replicates, and is therefore emerging as a successful technique with applications to the analysis of the phosphoproteome from a systems biology perspective. Multi-dimensional chromatographic techniques have been applied to phosphoproteomics experiments; however, such multi-dimensional separations complicate quantification by label-free techniques, as peptides may be present in different fractions across the samples being compared. Furthermore, the increase in MS analysis time prohibits comparing multiple samples and replicates needed for robust statistical analysis.
Methods: High coverage of the phosphoproteome from single TiO_2 extractions is a key element of the label-free workflow. Here we investigated TiO_2 and nanoLC-MS/MS conditions that maximised phosphopeptide identifications from 500 µg of protein per sample, without pre-fractionation, from a breast cancer cell-line model. To achieve this, we performed single TiO_2 extractions for each sample; varying the composition of loading, washing and eluting buffers, and additional desalting steps that reduced unwanted peptide modifications.
Results: This method allowed the identification of >6,000 unique phosphopeptide ions from 9 different samples. Furthermore, we established that the combination of both HCD & CID ion activation methods was

beneficial with respect to the number of phosphopeptide identifications confidently assigned from each sample run, as several phosphopeptide identifications were found to be unique to each method.
Conclusion: Overall, we have developed a relatively high-throughput sample-processing and phospho-enrichment method that allows the comparison of multiple experimental conditions and biological replicates in reasonable time-frames, thus expanding the applicability of phosphoproteomics to address questions not previously tractable with this technique.

P221

SUPER EXPERIMENTS IN PROTEOMICS

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Introduction: Looking beyond the sequencing of the human *genome,* the work of the Lamond Laboratory is focusing on the next major frontier, interpreting the human *proteome*. This is of great importance to understanding biological mechanisms and molecular disease, as it is the collection of cell proteins and their *properties* that determine most cellular responses and which are the targets of most drugs and medicines.
Methods: The scale of the data collection in this project is huge, hence we have placed much effort in dealing with the resulting vast and highly complex datasets in a structured, multidimensional database and software environment created called, “PepTracker”. This software provides us with an integrated and customised platform for large-scale proteomics data management and allows us to carry out multi-dimensional analytics on the data we are gathering (http://peptracker.com). This software environment has been central to our development of ‘**Super-Experiments**’.
Results: At present, most experiments are performed in an *ad hoc* fashion and interpreted in isolation. We foresee that the future application of proteomics technology for knowledge discovery lies in the intelligent mining of highly annotated, vast collections of quantitative proteomics data generated from direct experiments on cell systems. We have successfully applied this “Super-Experiment” approach to measure protein turnover and localisation behaviour and apply these inferred protein annotations to the systematic analysis of protein pools, isoforms and modifications (1,2).
Conclusion: Rather than study single datasets, it is imperitive to analyse global proteome changes at the cellular level, including subcellular localisation patterns, post-translational modifications, rates of synthesis and degradation and interactions with partner proteins. PepTracker and the idea of super experiments have thus far highlighted the huge potential for further development of these approaches.
References: 1. Ahmad, Y., Boisvert, F. M., Lundberg, E., Uhlen, M., and Lamond, A. I. (2012) Systematic analysis of protein pools, isoforms, and modifications affecting turnover and subcellular localization. *Mol Cell Proteomics* 11, M111 013680.
2. Boisvert, F. M., Ahmad, Y., Gierlinski, M., Charriere, F., Lamont, D., Scott, M., Barton, G., and Lamond, A. I. (2012) A quantitative spatial proteomics analysis of proteome turnover in human cells. *Mol Cell Proteomics* 11, M111 011429.

P222

HOW SAMPLE LOADABILITY OF THE NANO LC COLUMN INFLUENCES PROTEIN IDENTIFICATION IN LC-MS PROTEOMICS STUDIES

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Introduction: LC column miniaturization has been successfully applied to improve the MS detection sensitivity required for the small sample amounts found in proteomics. Even with these small sample amounts overloading is a risk originating from the wide dynamic concentration range of the samples.
In recent years, research has been directed to improve nano LC separation performance by using smaller particles and longer columns, where the gain in efficiency is often expressed as reduced peak width or increased peak capacity. Sample loadability, directly influenced by stationary phase characteristics like surface area, end-capping, and particle size, is equally important, but rarely investigated in proteomics.
Methods: Here we evaluate the chromatographic performances of nano LC columns (75 µm internal diameter), packed with various reversed phase stationary phases at various sample loads. The evaluation

was performed with synthetic peptides and complex protein digest samples from the fmol to pmol range. Additionally different elution conditions were compared to try to overcome or minimize the negative effects of overloading. Peak shape and peak asymmetry were examined to determine the maximum loading capacity of these columns and were correlated to the number of identified peptides and proteins.
Results: Initial data indicates that the sample mass loadability differs significantly between the different stationary phases. The maximum amount of sample, that could be injected without overloading the column, varied by more than an order of magnitude for the evaluated stationary phases. Significant peak broadening (> 10%) was observed for peptides under overloading conditions.
Conclusion: The use of trifluoroacetic acid (TFA) has resulted in lower MS signals, however the separation improved dramatically with the overload conditions, which resulted in proper identification with the TFA as ion pairing agent, which could not be achieved with formic acid.

P223

PROBING THE DYNAMICS OF THE HOST PROTEOME IN A MURINE MODEL OF SYSTEMIC CANDIDIASIS BY MALDI MS IMAGING

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Introduction: *Candida albicans* is a commensal fungus borne by about 70% of the healthy population, yet in immunocompromised patients it may cause life-threatening systemic infections. We are developing a MALDI MS Imaging approach to investigate proteomic changes associated with systemic candidiasis in the host kidney, in a murine model of the disease.
Methods: BALB/c mice were injected intravenously with *C. albicans* SC5314 cells, and the infection was allowed to proceed for up to four days. Kidneys harvested from healthy and infected animals were cryosectioned and trypsinised *in situ*, after which CHCA matrix was applied by vibrational nebulisation (Bruker ImagePrep). Imaging data were acquired with a MALDI TOF/TOF mass spectrometer (Bruker ultrafleXtreme) and average spectra from healthy and infected tissues were analysed with ClinProTools v2.2 using ROC curve analysis. Peptides of interest were sequenced *in situ* using the LIFT mode of the MALDI mass spectrometer.
Results: A total of ten peptides, including four with contrasting prominence in the average spectra of kidneys from healthy and infected animals, were sequenced in the course of the preliminary analysis and the corresponding proteins identified using MASCOT. The major protein classes represented in the identified set were histones and haemoglobin. Interestingly, peptides from haemoglobin alpha subunit (*m/z* 1529.73, 1819.88) were more pronounced in tissue from infected animals, while the relative prominence in the average spectrum of haemoglobin beta subunit peptides (*m/z* 1274.73, 1302.63, 1312.65) remained constant regardless of the host's infection state.
Conclusion: We demonstrate the potential of MALDI Imaging as a powerful new technology for investigating fungal systemic disease.

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SHOTGUN PROTEOMIC COMPARISON OF THE ACCESSORY GLAND PROTEIN CONTENT OF DROSOPHILA SPECIES RELATED TO THEIR POST-MATING BEHAVIOR.

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Introduction: In male drosophila, accessory glands produce proteins (Acps) transmitted during mating to the female within the seminal fluid. These Acps play an important role in numerous reproductive processes inducing strong changes in female post-mating behavior.The evolution of the Acps genes is thought to be rapid between species because of the strong selection on sexual interactions. It may also favor speciation by creating reproductive isolation.The present work aim to investigate the relation of Acps with mating system in the 9 species of the *Drosophila melanogaster* subgroup. The main challenge is to compare Acps from both sequenced and non-sequenced species to better understand the molecular mechanisms underlying evolution of post-mating interactions between the sexes.

Methods: 4 species have been selected according to their difference in mating systems (poly-or mono-androus system) and their phylogenetic divergence. Males were separated from females early after hatching and left to grow for 7 days in culture. The content of accessory glands was then extracted from individuals and analysed using nano-LC/MS/MS analysis directly after digestion by trypsin.
Results: Among the four species studied, *D. melanogaster, D. yakuba, D. teissieri and D. santomea* only the two first are already sequenced. Acps inventories for each species have been produced using the *D. melanogaster* and *D. yakuba* total sequences databases. Using the gene ontology available for *D. melanogaster*, and selecting the Gene ontology (GO) terms,"Reproduction" and "Response to Stimuli", annotations were also used to characterize potential candidates for further studies on their involvement in post-mating behavior regulation. Finally more than 600 proteins were found in every species. Out of these sets of proteins, around 40 were not shared with any other fruit fly species

Conclusion: These results, associated to experimental tests that allow characterizing the female receptivity to remating with the entire set of Acps or only a fraction (studied in another part of this project), will help to identify candidate genes for new regulators of the female post-mating behavior.

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DROUGHT STRESS INFLUENCE ON THE PROTEOMIC PROFILE OF EUCALYPTUS GRANDIS LEAVES
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Introduction: In Brazil, *Eucalyptus grandis* wood has been widely used as raw material for the pulp and paper industry. The increasing demands for cellulose, particularly short fiber derived from *Eucalyptus spp,* has prompted in recent years the expansion of commercial plantations in marginal land, where drought is the main abiotic limitation for growth. Thus, we set up a preliminary experiment in order to characterize the proteome of *E. grandis* leaves from young plants (6 month-old-trees) submitted to a short period of drought (72h).
Methods: Leaves from ten plants (continuous irrigation and drought) were harvested and proteins extracted by the method of Kim et al. (2001). One gram of material was homogenized in 5 ml extraction buffer containing 1M Tris-HCl pH 8.3, 2% (v/v) NP-40, 20mM $MgCl_2x6H_20$, 2% (v/v) 2-mercaptoethanol, 1 mM PMSF and 1% PVPP. After centrifugation at 12,000x*g* for 15min at 4ºC, the supernatant was collected and precipitated in 10% (w/v) TCA. The precipitate was washed three times with acetone + 0.07% (v/v) 2-mercaptoethanol. The pellet was dried and solubilized in a buffer consisting of 7M urea, 2M thiourea, 0.4% Triton X-100, 10mM DTTand 4% (w/v) CHAPS. Total protein content was obtained through the Agilent Protein 230 Kit. After desalination, proteins were submitted to tryptic digestion. Peptides were separated using MudPIT by LC-MS^E through the nanoACQUITY UPLC® System with 2D Technology. Proteins were identified by the software ProteinLynx Global SERVER®. Then, they were annotated through the software Blast2GO®(http://www.blast2go.com/b2ghome).
Results: Leaves from well-irrigated plants presented 12 unique sequences, with major GO Terms related to photosynthesis (5/23). On the other hand, leaves from plants under drought stress presented 208 unique sequences, from which major GO Terms (19/99) were related to responses to stress.
Conclusion: These results highlight the influence of drought on proteomic profile in *E. grandis* leaves.
References:
1. Kim, S.T.; Cho, K.S.; Jang, Y.S.; Kang, K.Y. *Electrophoresis*, **2001**, *22*, 2103-2109.
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P230

EXAMINATION OF THE ROLE OF PPP1CC2 IN THE FIRST-WAVE OF MOUSE SPERMATOGENESIS VIA PHOSPHOPROTEOMIC ANALYSIS

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Introduction: The first-wave of spermatogenesis begins shortly after birth in mice, and is synchronous, meaning certain germ cell types arise at predetermined developmental time-points. Targeted deletion of the serine/threonine phosphatase gene *Ppp1cc* results in impaired spermatogenesis in mice. Phenotypic abnormalities are first visible in the seminiferous tubules of *Ppp1cc* null mice at 3 weeks of age, and are hypothesized to arise due to the requirement of the testis-specific isoform, PPP1CC2 in late spermatocytes. Expression analysis of PP1 isoforms during the first wave of spermatogenesis shows that *Ppp1cc2* is up-regulated in the testis beginning at 3 weeks of age, while other isoforms exhibit no change in expression level, further supporting a critical role for PPP1CC2 at this point in development. In an effort to identify candidate substrates of PPP1CC2 we are conducting a phosphoproteomic analysis of the 3 week mouse testis.
Methods: Peptide samples were produced from 3 week spermatogenic cell suspensions, designed to minimize somatic cell content. Immobilized metal ion affinity chromatography (IMAC) and/or titanium dioxide affinity chromatography (TiO_2) phosphopeptide enrichment strategies were employed, and isolated peptides were subjected to liquid chromatography-tandem mass spectrometry for identification.
Results: To gain greater knowledge of the testis phosphoproteome we are seeking to identify as many phosphorylated proteins in this tissue as possible. To date we have identified over 350 phosphorylation sites, corresponding to 212 different proteins in mouse spermatogenic cells. Amongst these proteins, we have found that 138 (approximately 200 sites) are phosphorylated in spermatogenic cells at 3 weeks of age. In comparing our dataset to previously published proteomic studies, we have found that 86 (62%) of the identified phosphoproteins are known to be expressed in mouse spermatocytes.
Conclusion: Misregulation of protein phosphorylation due to a lack of the phosphatase PPP1CC2 in mouse spermatocytes leads to a failure of spermatogenesis. Our work of characterizing the phosphoproteome of this cell type will serve as a basis for elucidating the precise role of PPP1CC2. A comparative phosphoproteome analysis between peptides from 3 week old wild-type and *Ppp1cc* testis cell suspensions is currently underway.

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EXPLOITING THE VERSATILITY OF A HYBRID TRIPLE QUADRUPOLE LINEAR ION TRAP MASS SPECTROMETER FOR PROTEIN ANALYSIS.

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Introduction: Hybrid triple quadrupole linear ion trap (QqLIT) mass spectrometers are versatile instruments, with their ability to perform both QQQ quantitation experiments and ion trap qualitative experiments. In this study, we carry out a comprehensive investigation of a range of applications to explore the full capabilities of QqLIT technology.
Methods: For protein identification exploration, 1 ug of tryptically digested E.coli sample (Waters) was analyzed on a QTRAP® 4500 system using the nanoflow source interfaced to nanoLC Chip system. MRM triggered MS/MS experiments were used to expand sequence coverage on the low abundant proteins. For quantification exploration, multiple reaction monitoring (MRM) transitions and MRM^3 experiments of human P450 tryptic peptides (AB SCIEX) spiked into digested human microsomes were developed and used to measure the calibration curves in both high and low LC flow regimes. Finally, a series of protein standards were used for intact analysis.
Results: Using the Ecoli digests as a standard matrix, the various experimental settings for a data dependant analysis were optimized for highest protein and peptide identification rates. The 1-2 peptide hit proteins were analyzed using MRM triggered MS/MS to detect additional tryptic peptides for the proteins to confirm the identification and increase the sequence coverage. In many cases, the additional peptide IDs for the single hit proteins turned into higher confidence protein identifications. To explore the utility of targeted MRM and MRM^3 workflows, calibration curves were generated for human P450 peptides spiked into digested human

microsomes and the LLOQs for each technique were determined. Two of these peptides showed significant specific interferences which could be mostly removed using the MRM^3 workflow. For intact analysis, protein standards were analyzed using both quadrupole and trap modes to determine the technique that provides the best protein m/z envelopes and the highest molecule weight deconvolution accuracy
Conclusion: The facility of hybrid QqLIT instruments to perform targeted MRM, untargeted MRM-triggered MSMS experiments and MRM3 allows a range of qualitative and quantitative workflows to be performed on a single instrument.

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PROBING ADVANTAGES OF DIFFERENT SELECTIVITY STRATEGIES FOR TARGETED QUANTITATIVE PROTEOMICS

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Introduction: There has been an exponential increase in the number of 'potential' peptide biomarkers discovered; thus requiring the need for better quantification strategies to confirm or refute their ultimate utility. Also desired is increased throughput with reduced sample preparation and/or accelerated chromatography, which increases the chance of matrix interferences confounding robust quantification. The purpose of this study is to explore a range of new MS analysis methodologies that enable higher selectivity quantification, including MRM, MRM^3 and Differential Mobility Separation (DMS).
Methods: A hybrid triple quadrupole linear ion trap system equipped with Turbo V™ source was used to analyze both a large endogenous peptide, human BNP, and a set of human P450 tryptic peptides spiked in a complex digested proteome. Heavy isotope labeled standards were spiked into the complex matrix and Multiple reaction monitoring (MRM) and MRM^3 experiments were developed to measure calibration curves. Differential mobility separations (DMS) were performed on a hybrid triple quadrupole linear ion trap system equipped with SelexION™ Technology using both BNP and human P450 tryptic peptides in the complex human background matrix.
Results: Three quantitative methodologies were explored using the QTRAP® 5500 System: MRM, MRM^3, and DMS. Initial work with a large peptide, intact human BNP, provided good fragmentation for MS/MS and MS3, thus best sensitivity was obtained using MRM and MRM^3 (LLOQ of 128 pg/ml). For large peptides which do not fragment well, SIM (single ion monitoring) using DMS is an alternative methodology for quantification. Due to the removal of co-eluting interferences, the LLOQ for the SIM using DMS was ~25x lower over the SIM only experiment, a substantial improvement. Further investigation into these strategies were done using tryptic peptides from human P450 spiked into digested liver microsomes.
Conclusion: LOQs were compared for twelve different peptides within the complex matrix using MRM, MRM^3 and DMS and LLOQs were compared. More than 20% of tryptic peptides analyzed in this study displayed matrix interferences in MRM mode which raised the detected LLOQ, requiring the use of a secondary separation strategy such as MRM^3 and/or DMS.

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GLYCOPROTEOMIC ANALYSIS OF THE SECRETOME OF ENDOTHELIAL CELLS

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Introduction: Endothelial cells form the inner lining of blood vessels. Previous studies have partially unravelled the complexity of endothelial protein secretion, but did not investigate post-translational modifications. Glycosylation, in particular, plays a key role in endothelial cell function.
Methods: Human umbilical vein endothelial cells were kept in serum-free medium before activation by phorbol 12-myristate 13-acetate (PMA), a commonly used secretagogue that induces exocytosis of endothelial vesicles. Proteins in the conditioned media were analysed with and without enrichment for glycopeptides by ZIC-HILIC resins. Peptides were either treated with PNGase F and $H_2{}^{18}O$ before identification by an Orbitrap XL or Elite hybrid mass spectrometer (both Thermo Fisher) using an HCD-product dependent ETD method.

Results: In the conditioned medium of PMA-stimulated endothelial cells, 962 proteins were identified, significantly more than in previous proteomic studies investigating endothelial protein secretion either at baseline [1], or in response to mechanical stimulation [2]. After deglycosylation with PNGase F in the presence of $H_2{}^{18}O$, 169 unique peptides displayed O-18 deamidation of asparagines, corresponding to 103 proteins with a total of 161 glycosylation sites, less than half of which were predicted. Direct glycopeptides analysis on an Orbitrap Elite plus Byonic software identified 130 unique glycopeptides from 65 proteins and 123 glycosylation site, of which 55 were predicted and 32 were novel. The mass information of glycans and adducts obtained from the HCD-ETD workflow allowed a calculation of the sugar chain composition.
Conclusion: This study represents the most comprehensive characterisation of the endothelial secretome to date and demonstrates that the HCD-ETD workflow on an Orbitrap Elite offers an important complementary strategy for the identification of post-translational modifications.
References: [1] Tunica DG, Yin X, Sidibe A, Stegemann C, Nissum M, Zeng L, Brunet M, Mayr M. Proteomic analysis of the secretome of human umbilical vein endothelial cells using a combination of free-flow electrophoresis and nanoflow LC-MS/MS. *Proteomics*, 2009; 9(21): 4991-4996.
[2] Burghoff S and Schrader J. Secretome of human endothelial cells under shear stress. *J. Proteome Res.*, 2011; 10(3): 1160-1169.

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QUANTITATIVE CHANGES IN POSTSYNAPTIC DENSITY-ENRICHED PROTEIN FRACTIONS AFTER AVERSIVE AUDITORY CONDITIONING IN MICE

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Introduction: Changes of synaptic efficacy, crucial for learning and memory formation, are thought to be caused by plastic alterations of protein components and their interactions in the synapse. In this study, a quantitative ICPL-quadruplex based proteomic screen was used to investigate molecular modifications during auditory learning.
Methods: Mice were trained in a go/no-go shuttle box paradigm to discriminate between a rising and falling frequency-modulated tone to avoid a mild foot shock. Control-treated mice were subjected to the shuttle box for a corresponding number of sessions but received either the tone stimuli only or the foot shocks only. At 6 and 24 h after the training session the protein composition of a postsynaptic density (PSD) enriched fraction prepared from four brain areas (auditory cortex, striatum, prefrontal cortex, hippocampus) was analysed by quantitative mass spectrometry and compared to data from naive, untreated mice.
Results: In both groups of control-treated animals, the proportion of proteins with reduced appearance in the PSD-enriched fraction was similar to the proportion of proteins with increased appearance. In contrast, in the PSD-enriched fraction of trained mice, the number of down-regulated proteins considerably exceeded that of up-regulated proteins in all brain regions studied. The training-induced changes concerned primarily cytoskeletal and synaptic scaffolding proteins.
Conclusion: These data suggest that learning processes initially induce removal and/or degradation of proteins from presynaptic and postsynaptic cytoskeletal matrices before adapting to new post-learning situations.
References: This work was supported by the Deutsche Forschungsgemeinschaft (SFB 779), the Initiative for Research and Innovation of the Leibniz Association, the Research Center Dynamic Systems (CDS) at the OvGU and the European Structural Funds 2007-2013 (CBBS/ZVOH).

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ABSOLUTE QUANTITATION OF CENTRAL CARBON METABOLISM IN GROWING AND STRESSED BACILLUS SUBTILIS

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Introduction: In its natural habitat, the soil, *Bacillus subtilis* has to adapt to a wide range of environmental insults to ensure survival. The regulatory architecture of transcription of *B. subtilis* has recently been described in a comprehensive transcriptome study analyzing 104 different conditions [1]. This study revealed that sigma factor utilization accounted for ~66% of the variance of transcriptional activity and the major contribution of antisense RNAs. However, beyond transcription regulation of mRNA, protein levels and activity also contribute to adaptation. In this study we wanted to explore the adaptation of central carbon metabolism during nutrient starvation and osmoadaptation, two environmental insults frequently encountered by *B. subtilis* in soil.
Methods: Therefore, we cultivated *B. subtilis* in glucose limited chemostats at different dilution rates with or without imposition of osmotic stress. Samples were collected for the analysis of the transcriptome, proteome, metabolome and fluxome.
Results: For absolute quantitation of proteins involved in central carbon and proline metabolism, a single scheduled SRM method containing three QconCATs that cover 45 proteins (638 transitions corresponding to 206 peptides) was developed.
The data of the different OMICs measurements are being integrated to decipher adaptation of central carbon metabolism to both insults (alone or combined) at the molecular level. The data indicate that glucose starving cells differ from those also simultaneously experiencing osmostress, very likely because osmoadaptation requires additional resources, e.g. for synthesis of high levels of compatible solutes that are necessary for sustaining growth.
Conclusion: The data of these integrated analysis covering transcriptional, posttranscriptional and metabolic regulation will be discussed.
References: 1. Nicolas, P., et al., *Condition-dependent transcriptome reveals high-level regulatory architecture in Bacillus subtilis.* Science (New York, N.Y.), 2012. **335**(b91176ad-0035-51b8-8aaa-034efb070e48): p. 1103-1109.

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THE EFFECT OF COLOSTRUM INTAKE IN NEWBORN LAMBS: PROTEOMIC PROFILES OF BLOOD PLASMA IN THE FIRST 14 HOURS OF LIFE

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Introduction: The colostrum intake by newborn ruminants (calves, lambs and kids) plays a fundamental role in the acquisition of passive immunity. Due to characteristics of ruminant placenta, the transfer of immunoglobulins from the dam to the foetus is not enough to ensure the survival of the newborn ruminant. Knowledge about what other proteins from colostrum, despite immunoglobulins, have an immune function on newborn lambs is therefore necessary in order to try to increase lamb survival rate. For this reason the objective of this study was to evaluate differences in blood plasma proteomic profiles due to the colostrum intake.
Methods: In this study two groups (6 lambs each) were fed with colostrum. One group received three colostrum meals, at 2, 14 and 26 hours after birth. The other group was fed with colostrum at 14 and 26 hours after birth. At the end of the colostral period each animal of both groups took the same amount of

immunoglobulins per live body weight at birth (4mg of Immunoglobulin G/Kg) from colostrum. Blood samples were collected and centrifuge before feeding at 2 and 14 hours after birth. 2-D Differential in Gel Electrophoresis (DIGE) was performed using Immobiline DryStrips with pH 3-10 and 24 cm length (GE Healthcare). Differentially expressed protein spots were detected with the Progenesis SameSpots software (Nonlinear). After digestion, peptides were analyzed using a MALDI-TOF/TOF MS.
Results: A total 11 spots were found to have differential expression as a consequence of colostral intake. They were identified as serum amyloid A, apolipoprotein A-IV, fibrinogen and plasminogen that increased their expression as a consequence of colostrums uptake.
Conclusion: Results show that colostrum intake increased the expression of protein with immune function like serum amyloid A, apolipoprotein A-IV, fibrinogen and plasminogen, reinforcing the knowledge about colostrum importance in the immune passive transfer.

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HUMAN CELL-LINE PHOSPHOPROTEOME MAPPING USING A DUAL-FUNNEL ETD ION TRAP
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Introduction: One of the essential regulatory mechanisms in eukaryotic cell is driven by the action of kinases. They affect the information flow through the signaling processes and consequently exert an influence on cells, tissues and organisms phenotype and functions. Altered regulations of these processes are observed in a variety of diseases, among which are many cancers. Therefore, detecting, characterizing and ultimately quantifying the phosphproteins altered in these pathologies is an important step towards a better understanding of the implicated processes.Presented here are the results obtained after human cell line IMAC phosphopeptide enrichment followed by an ESI CID/ETD Auto MSn experiment.
Methods: Cell lysate from Human Raji B cell has been subjected to gel-assisted digestion. Subsequent phosphopeptide purification was performed as previously reported [J. Proteome Res. 2008, 7, 4058-4069], using an IMAC column capped at one end with a 0.5-µm frit disk enclosed in a stainless steel column-end fitting. Tryptic peptides were reconstituted in loading buffer and loaded onto the IMAC column. Automated purification of phosphopeptides has been performed using Acquity UPLC (Waters). Eluted peptide samples were vacuum dried, reconstituted in 0.1% (v/v) TFA (40 µL) and desalted and concentrated using ZipTips™ (Millipore, Bedford, CA). LC-MS/MS alternated CID/ETD acquisition has been performed on a dual-funnel amZon ETD ion trap (Bruker) coupled to a RSLCnano (Dionex). Three technical replicates have been performed in order to assay the method's robustness and the system capability to deliver reliable and reproducible results.
Results: The analyses led to the identification of 95 proteins, 88 of them being identified in each of the three runs. Among these, 93 are phosphoproteins and 87 of them representing 220 phosphorylated peptides have been found in the three replicates.
Conclusion: The acquisition speed as well as the high quality of the obtained CID and ETD spectra combined with the use of dedicated bioinfomatics has enabled to map efficiently the phosphorylation sites for most of these peptides**.** We are now ready to use this technique on a larger scale to proceed to a more systematic mapping of the phosphoproteins present in our sample.

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IDENTIFICATION OF HETERODIMERS BETWEEN THE PLANAR CELL POLARITY RECEPTORS VANGL1 AND VANGL2
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Introduction: Neural tube defects (NTDs) are very common diseases in humans. At the molecular level, recent advances have highlighted that loss of function mutations in genes regulating planar cell polarity (PCP) play a key role in the emergence of NTDs during early embryonic development. Recent genetic studies have revealed that mutations in the cell polarity receptor *Vangl2* cause PCP defects and craniorachischisis. Interestingly, *Vangl2* genetically interacts with *Vangl1*, a close homolog, as double heterozygous

Vangl1/*Vangl2* mice develop identical PCP defects and craniorachischisis to *Vangl2*$^{-/-}$ deficient mice. It remains unknown whether these proteins belong to a common protein complex or act in parallel pathways
Methods: Experimental biochemical evidence for Vangl2/Vangl1 complexes has been sparse, depicting only ectopic protein expression. In addition, all studies use antibodies incapable of discriminating between Vangl1 and Vangl2, so leading to flawed interpretations. In this study, we have generated a highly specific monoclonal anti-Vangl2 antibody able to efficiently purify Vangl2 from protein extracts. We screened breast cancer cell lines and identified SKBR7 as a cell line co-expressing Vangl1 and Vangl2. We then used an immunoaffinity-based experimental approach using the anti-Vangl2 antibody to isolate endogenous Vangl2 and its associated binding partners from SKBR7 protein extracts. Protein analysis of the immunoprecipitated proteins was undertaken using SDS-PAGE, in-gel trypsin digestion and Orbitrap mass spectrometry analysis
Results: We efficiently identified endogenous Vangl2 with very good peptide sequence coverage and confirmed by analysis of post-translational modifications that the methionine encoded by the predicted start codon is acetylated. Analysis of proteins present in Vangl2 immunoprecipitates showed that Vangl2 copurifies with endogenous Vangl1. Using GFP or myc-tagged Vangl1 and Vangl2 constructs, we further confirmed that these cell polarity receptors are able to form homo- and hetero-complexes in living cells
Conclusion: The data presented here demonstrate that Vangl1 and Vangl2 form protein complexes, and therefore establish a paradigm for the understanding of the morphogenetic defects in which these proteins are implicated

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NUCLEAR PROTEIN PROFILE FROM YOUNG SUGARCANE LEAVES
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Introduction: Sugarcane is a cash crop, cultivated for its stalks which accumulate sucrose, the raw material for products like sugar and bioethanol. Nuclear proteome comprehension is essential for deciphering the mechanisms that governs genome regulation and function. In the present study, we report the isolation and identification by 1D SDS-PAGE, of nuclear proteins from young sugarcane leaves.
Methods: The nuclei were isolated from fresh tissue of one and four-month-old sugarcane leaves, using the modified protocol of Folta and Kaufman (2000). The experiment consisted on a completely randomized design, three biological repetitions each with 18 plants. After purification using a *percoll* gradient, nucleus integrity was evaluated by staining with 1% acetolactic orcein and with DAPI. The nuclear proteins were isolated using TRI Reagent (Sigma) and quantified by Bradford. Western blot analysis was used to prove enrichment for nuclear proteins. Membranes were incubated with RUBISCO, PEPCase, OEE1, Histone and PCNA. For the characterization of nuclear proteome 60 µg of proteins were separated by PAGE and each lane divided into 20 sections, the proteins from each section were digested and purified. Identification was carried out by mass spectrometry (Synaptic G2 HDMS) and analyzed using ProteinLynx and Sucest database. Softwares, such as BaCCello, will also be used to predict cellular localization.
Results: The results obtained reveal nuclei as uniform spheres with an average diameter of 5 µm. They also showed the presence of PCNA and Histone only in the nuclear fraction. RUBISCO, PEPCase and OEE1 were very abundant in the total protein fraction and reduced in the nuclear fraction. Proteins such as histones H2A.2, H2A.8, H2A.6, H2B.5, H2B.1,H2B.2, H3.3 and H4, transcription factors APFI and BHLH, putative topoisomerase VIA were identified.
Conclusion: These results show the applicability of this method to create an accurate sugarcane nuclear proteome profile.
References: FOLTA, K. M.; KAUFMAN, L. S. Preparation of transcriptionally active nuclei from etiolated *Arabidopsis thaliana*. **Plant Cell Reports.** v. 19, p. 504-510, 2000.
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MAPPING POSTTRANSLATIONAL MODIFICATIONS IN SCHIZOSACHAROMYCES POMBE RNA POLYMERASE II

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Introduction: RNA polymerase II (RNA pol II) is a well-studied and highly conserved transcription regulator consists of multiple subunits, yet little is known regarding the sites of posttranslational modifications (PTMs) besides those in the carboxyl-terminal domain of the largest subunit. This study aims to globally identify PTMs in RNA pol II using S. pombe as a model organism and a shotgun proteomic approach.
Methods: S. pombe RNA pol II was immunoaffinity purified. Peptide mixtures were prepared by triple digestion using endoproteinases. Each differentially-digested sample was independently analyzed three times by a 10-step Multidimensional Protein Identification Technology (MudPIT) using a Velos pros-Orbitrap elite hybrid mass spectrometer. Spectra were searched using SEQUEST for PTMs against a S. pombe database along with shuffled sequences and contaminants. Spectra/peptide matches were only retained if they met conservative filtering criteria.
Results: A total of 37 modified proteins including 11 RNA pol II subunits were identified in the S. pombe sample. The sequence coverage amongst the RNA pol II subunits ranged from 67% to 92% and the false discovery rates were less than 1% at the spectral level. For Rpb1, phosphorylation (S366) and acetylation (S760, K1209 and K1252) were confidently identified with more than one spectrum from at least two datasets. Twelve additional acetylation, formylation and phosphorylation sites were also observed from a single dataset, hence less confident. In addition, acetylation was detected definitively in Rpb2 to Rpb6 and Rpb11. Less confident PTMs were observed in Rpb7 and Rpb8. Only oxidation was detected in Rpb9 and Rpb10.
Conclusion: Using S. pombe as a model organism on a MudPIT platform, this study provides evidence for the first time that there are sites for PTMs in 9 out of 11 RNA pol II subunits. Two lysine acetylation sites observed on Rpb1 are near a conserved “trigger loop” and “bridge helix”. Serine 760 (in the “funnel” region of Rpb1) is conserved in human and S. cerevisiae. Additional analyses using S. pombe as well as other organisms would likely increase the number and level of confidence of detection and might provide insights into this “epigenetic” coding scheme in eukaryotic RNA pol II.

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SCREENING OF EXTRACTION BUFFERS FOR 2-D DIGE ANALYSIS OF PLANT PROTEINS

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Introduction: Sample preparation is a crucial part of the 2-D electrophoresis workflow to achieve a large number of well-resolved protein spots. Plant tissue is a challenging material to work with due to the high content of interfering components and low protein content. A novel workflow for common bean leaves (*Phaseolus Vulgaris*) was developed using extraction buffers based on urea / thiourea containing different sets of detergents in combination with a precipitation procedure to remove interfering substances such as polysaccharides, lipids and phenolic compounds.
Methods: Five buffers were screened in this study to find the optimal buffer giving highest number of detected spots. The purified protein fractions were analyzed by 2-D Differential Gel Electrophoresis (2-D DIGE) and DeCyder™ image analysis software.
Results: All tested buffers resulted in overall well-resolved gel images over a wide pH range (pH 3-11). In average 2634 spots were automatically detected by the software (n=10) and 1955 spots were matched to the master gel with small differences between the buffers (RSD 1.3%). The relative intensity of certain spots depended on the extraction buffer used which may be beneficial for preparative purpose.
Conclusion: It can be concluded that the presented workflow is suitable for proteomic studies of common bean.

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TOP-DOWN MASS SPECTROMETRIC ANALYSIS OF GLYCOSYLATED AND PHOSPHORYLATED PROTEINS

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Introduction: Top-down approaches allow for direct analysis of intact proteins using their mass and their fragments for identification and characterization. In contrast to bottom-up techniques, labile PTM's like phosphorylation remain stable in top-down analysis and enable direct modification site determination. MALDI-In-Source-Decay (MALDI-ISD) is a powerful Top-down-Sequencing (TDS) tool and typically provides long N- and C-terminal sequence tags of up to 80 amino acids and more, allowing for the detection of large PTM's like N-linked glycans even at central sites of proteins up to 15 kDa. TDS were applied to standard glycoproteins such as RNAse B, horseradish peroxidase, α- and β-caseins for positive as well as negative ionization mode MALDI-ISD was explored

Methods: Proteins (Sigma) were dissolved in water/ 0.1 % TFA at 10-100 pmol/µl, applied to a MALDI sample plate carrying 2 mm hydrophilic anchor sample spots, allowed to dry and rinsed. This led to higher S/N values and extended mass range for TDS analyses. Matrices were 1,5-diaminonaphtalene (DAN) and super-DHB, dissolved in 50 % ACN/water/0.1 % TFA.1 µl was applied to the rinsed sample spots. Spectra were acquired in positive and negative ion mode. Proteins were identified using ISD signals as virtual precursors and as fragments for MASCOT (Matrix Science, Inc.) searches. Protein sequences were matched to the spectra including proposed modifications.

Results: Glycosylation: With DAN matrix the SS-bridges were reduced, resulting in ISD fragmentation of RNaseB up to residue 40 including the glycosylated Asn34. For Horseradish peroxidase, the N-linked glycan structure typical for plants was identified at Asn13. The pyro-glutamate at the N-terminus of horseradish peroxidase was found. Phosphorylation: Negative ion mode enabled the detection of all 5 N-terminally phosphorylated serine residues. In α-S1-casein, Ser41 was identified as non-phosphorylated in contrast to uniprot description, S46 and S48 were confirmed as being phosphorylated. β-S2-Casein carries 5 phosphorylated serine residues near the N-terminus, all of them were confirmed using negative mode ISD.

Conclusion: As a result, we propose to use negative ion mode ISD in cases where phosphorylation or other negative charged residues effect the formation of fragment ions in TDS analyses.

Trade and educational workshop abstracts

TEW 01

ANALYSIS OF SRC ONCOGENIC SIGNALING IN COLORECTAL CANCER BY STABLE ISOTOPE LABELING OF AMINO ACIDS IN MOUSE XENOGRAFTS UTILIZING MOUSEEXPRESS® LYSINE 13C6 MOUSE FEED.

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The non-receptor tyrosine kinase SRC is frequently deregulated in human colorectal cancer (CRC) and SRC increased activity has been associated with poor clinical outcome. SRC has been implicated in tumor growth by still not well known mechanisms. In nude mice xenografted with human CRC cells, SRC over-expression favors tumor growth and is accompanied by a robust increase of tyrosine phosphorylation in tumor cells. In order to analyze SRC oncogenic signaling in these tumors, we developed a novel quantitative proteomic analysis we have named Stable Isotope Labeling with Amino acids in mouse Xenografts, or SILAX. This method is based on heavy isotope labeling of the tumor proteome by addition of [13C6]-Lysine into mouse food utilizing MouseExpress® Lysine 13C6 Mouse Feed. An incorporation level >88% was obtained in xenograft tumors after 30 days of heavy lysine diet. Quantitative phosphoproteomic analysis of these tumors allowed the identification of 63 proteins which exhibited a significant increase in tyrosine phosphorylation and/or association with tyrosine phosphorylated proteins upon SRC expression. These mainly included molecules implicated in vesicular trafficking, signaling and RNA binding proteins. Most of these proteins were specific targets of SRC signaling in vivo as they were not identified by stable isotope labeling by amino acids in cell culture (SILAC) analysis of the same CRC cells in culture. This observation was experimentally confirmed in the case of the vesicular trafficking protein and SRC substrate TOM1L1. While TOM1L1 depletion affected only slightly SRC-induced proliferation of CRC cells in vitro, it drastically decreased tumor growth in xenografted nude mice. This data suggests that vesicular trafficking plays an important role in SRC-induced tumor growth and validates SILAX as a valuable approach to decipher tyrosine kinase oncogenic signaling in vivo.

TEW 02

STRUCTURAL PROBING OF PROTEIN COMPLEXES BY CHEMICAL CROSS-LINKING AND MASS SPECTROMETRY

Franz Herzog, Abdullah Kahraman, Thomas Walzthoeni, Alexander Leitner and Ruedi Aebersold.
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Established structural biology methods successfully solve structures of individual proteins and subcomplexes, however, fail to reveal the architecture of marcromolecular protein complexes. Our strategy aims at integrating distance restraints identified by mass spectrometry and structural information obtained by X-ray crystallography and electron microscopy in order to gain insights into the topology of protein assemblies. We use chemical agents to cross-link amino or carboxyl groups within a protein or a protein complex and identify the connected amino acids by mass spectrometry. The length of the cross-linker represents a measure for the distance spanned between the two covalently linked residues.
Protein samples are cross-linked with a mixture of isotopically labeled reagents that facilitate identification of all modified peptides by a characteristic isotope pattern. Pairs of isotope-coded peptides are analyzed by LC-MS/MS and the corresponding tandem mass spectra are searched by the dedicated search engine, xQuest.
The analysis of protein complexes purified for crystallographic purposes is routine and detected about 150 inter-protein and 320 intra-protein cross-links on RNA polymerase I. The distance restraints reveal its subunit architecture, support the localization of the transcription initiation factor Rrn3 and provide insights into the structural basis for proof-reading and transcription termination.
Affinity-purification of proteins from human tissue culture cells yields heterogeneous protein complexes at low concentration. A modified cross-linking protocol facilitates identification of inter-protein and intra-protein distance restraints on protein phosphatase 2A (PP2A) complexes elucidating the topology of complexes formed by regulatory subunits of the modular PP2A signaling network.

The detection of spatial restraints by chemical cross-linking and mass spectrometry allows the measurement of structural information on heterogeneous protein complexes and has been established as integral part of hybrid structural biology approaches.

TEW 03

PROTEIN INTERACTION REPORTER: "NEWS" ON PROTEIN TOPOLOGIES IN CELLS

James E. Bruce, Juan D. Chavez, Chunxiang Zheng, Li Yang, Chad Weisbrod, Department of Genome Sciences, University of Washington Seattle, Washington

Life on earth has evolved to rely on proteins as functional molecules due to the diversity and specificity of topological features that are possible with sequences of amino acids. In cells, proteins interact with other molecules either by direct binding or reaction. In all cases, interactions are resultant from protein topological features that are themselves, the products of selective adaptation. These molecular interactions, protein abundance levels and other factors such as posttranslational modifications serve in complex ways to regulate global functional levels in cells. However, topological features of proteins and their interactions are ultimately at the origin of all fundamental biological processes. Therefore, improved technologies that can help visualize topological features of protein interactions in cells will increase understanding of functional networks and their relationships to disease and normal function. This presentation will highlight our efforts to develop and apply Protein Interaction Reporter (PIR) technology (1) to enable topological measurements of proteins and protein complexes in cells. Because of its unique features, PIR technology enables unambiguous cross-linked peptide identification that has resulted in the largest set of identified in vivo cross-linked peptides produced to date (2). Several examples of in vivo PIR data will be discussed to illustrate the excellent agreement with available protein and complex crystal structures. Finally, examples from our PIR data will also be presented to show how this technology can provide novel in vivo topological information that can impact understanding of biological function, even for cases where high resolution structures are not known.

1. Tang X, Munske GR, Siems WF, Bruce JE. Mass spectrometry identifiable cross-linking strategy for studying protein-protein interactions. Anal Chem. 2005 Jan 1;77(1):311-8.
2. Zheng C, Yang L, Hoopmann MR, Eng JK, Tang X, Weisbrod CR, Bruce JE. Cross-linking measurements of in vivo protein complex topologies. Mol Cell Proteomics. 2011, 10(10): 1-14.

TEW 04

HIGH-RESOLUTION MASS SPECTROMETRY AS STEPPING STONE TO LARGE SCALE AND DYNAMIC CROSS-LINK ANALYSES.

Juri Rappsilber,

Cross-linking in conjunction with mass spectrometry has, over recent years and in several labs, reached a maturity that allows the structural analysis of multi-protein complexes such as: RNA polymerase II with transcription factors and the proteasome. Our lab has consistently been at the forefront in terms of the number of identified unique cross-links and we propose that a key contributing factor is the choice of mass spectrometer and its resolution in MS and MS2. I will present data showing the benefits of electrospray ionization over MALDI in this context and on the value of high- over low-resolution fragment data for cross-link analyses. This is in direct competition with an alternative, and also successful, approach that uses isotope-labelled cross-linkers. A key advantage of isotope-free identification of cross-links is a higher identification success rate and the potential combination of cross-linking with quantitation with relative ease.
Expression of opposing experiences and views are encouraged and welcomed as contribution to this talk.

TEW05

AMINO ACIDS: CHEMISTRY, FUNCTIONALITY AND SELECTED NON-ENZYMATIC POST-TRANSLATIONAL MODIFICATIONS

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The ultimate goal of proteomics is determination of the exact chemical composition of protein species, including their complete amino acid sequence and the identification of each modified side chain, in every protein in a biological sample and their quantification. We are still far from achieving this goal due to limitations in analytical methodology and data analysis but also due to the fact that we cannot anticipate all amino acid modifications that occur in nature.
To detect modified side chains and to discover new, still unknown amino acid derivatives, an understanding of the chemistry of amino acids is mandatory. This workshop focuses on introducing the chemistry of amino acid side chains. By highlighting some exemplary reactions a glimpse of the huge diversity of modified amino acids will raise the awareness of the attendees for the complexity of proteomes.
Analytical procedures that address a wider part of the 'invisible proteome' need to be based on dedicated sample preparation and enrichment, high-resolution separation, optimized and often targeted mass spectrometry as well as advanced and unbiased data analysis algorithms. Addressing these points will give ample room for discussion at the end of the workshop.

TEW06

Waters Corporation
TOWARDS A COMMON WORKFLOW FOR METABOLITE AND PROTEIN PROFILING IN UNBIASED DISCOVERY.
Robert Tonge, Jim Langridge, Waters Corporation, Manchester, UK.

Complementary metabolomic and proteomic studies can provide a stereoscopic view of biological processes at the molecular level. Combining optimised 'omics workflows, within a single analytical platform, enable an efficient gateway to 'integrative biology'.

Endogenous metabolite profiling and bottom-up protein profiling are currently performed with highly specific analytical systems. Whilst the core of either system may be the same HDMS instrument, the chromatography and software tools employed will be unique to each workflow. Metabolite profiling conventionally utilises analytical scale UPLC while protein profiling mandates capillary scale UPLC. Moreover, discreet 'bioinformatics ecosystems' have evolved to support either metabolomics or proteomics workflows. Switching between these workflows therefore necessitates exchanging chromatography systems and that the analyst is conversant with a plethora of bioinformatics. Consequently proteomics and metabolomics studies are rarely performed on the same analytical platform.

We propose a novel system solution to enable a common workflow for both endogenous metabolite profiling and bottom-up protein profiling in unbiased discovery research. This unique analytical platform is based on the permanent combination of a nanoACQUITY UPLC, a SYNAPT HDMS and coherent bioinformatics. Metabolomics and proteomics experiments are performed sequentially, on separate batches of samples, requiring only an in-situ change of chromatographic column. The nanoACQUITY UPLC readily accommodates UPLC columns in the 75-300µm i.d range (for optimised proteomics) and 1.0mm i.d. columns (for high sensitivity metabolomics). The SYNAPT HDMS operated in the UPLC/MSE mode provides unprecedented peak capacity and duty cycle efficiency to profile either metabolites or proteins in complex biological extracts. The coherent bioinformatics environment, with intuitive data visualization, mitigates 'software opacity syndrome' (SOS) that has hitherto afflicted analysts seeking a practical solution for combined 'omics analysis.

TEW07

Thermo Fisher Scientific
High Resolution Accurate Mass Orbitrap based mass spectrometry platforms have proved to be useful tools to the research community. This technology has enabled scientists to mine proteomes to new depths, to detect, characterise and quantify more than ever.

With increased confidence, the benefit of HR/AM platforms has been employed to describe changes in the protein complement of human cells. Novel biological modifications have been uncovered due to relentless

technological development encompassing sample preparation methodology, chromatography, mass spectrometry and software platforms, all organised in workflows which deliver robust, cutting-edge functionality.

A brief technology update will be given by Dr. Martin Hornshaw and Dr. Madalina Oppermann, (Thermo Fisher Scientific) you will then enjoy two special guest presentations from recognised specialists in their fields:

• In depth proteomics and phospho-proteomics of a human cancer cell line using the Thermo Scientific Q Exactive
o Dr Kirti Sharma, (Mann Laboratory, Max-Planck Institute of Biochemistry, Martinsried)
• Mass Spectrometric Immunoassays (MSIA)
o Dr Dobrin Nedelkov (Thermo Fisher Scientific) will present data on the development and validation of several mass spectrometric immunoassays toward specific proteins such as Insulin-like Growth Factor 1 (IGF1) and parathyroid hormone (PTH)

TEW08

Ab Sciex
THE COMPLETE PROTEOMIC WORKFLOW: FROM DISCOVERY TO VERIFICATION.
Dr Tom Knapman and Dr Cathy Lane, AB Siex

Advances in instrumentation and experimental approaches have enabled us to dig deeper into the proteome than ever before from a qualitative standpoint. This workshop will focus on how cutting edge technologies and experimental strategies can accelerate the complete proteomic workflow from conventional discovery experiments through to novel targeted and untargeted quantitative analyses, such as MS/MSALL with SWATH™ acquisition.

TEW09

SomaLogic
SomaLogic Announces the European-wide Launch of the SomaSciences Technology at the BSPR/EuPA 2012 Meeting.
SomaLogic, Inc., announces the European launch of SomaSciences making SomaLogic's breakthrough proteomic technology available to researchers and drug developers who seek to improve and accelerate their protein biomarker discovery efforts.
The SomaSciences European launch will take place at the EuPA 2012 Scientific Congress: New Horizons and Applications for Proteomics on Wednesday July 18, 2012 at Glasgow's Royal Concert Hall. Steven Williams, M.D., Ph.D., Chief Medical Officer of SomaLogic, will present the company's technology in a lunchtime workshop talk entitled:
SOMAmers Enable High Throughput Screening for Protein Biomarkers and Diagnostics
"We are delighted to formally launch the European-wide availability of our technology," said Dr. Williams. "We look forward to building on our current strong European collaborations in establishing new ones that will create additional opportunities for building transformative health care products."
A light lunch will be provided for attendees. To register for this event, please e-mail Fintan Steele, at fsteele@somalogic.com

Workshop Talk Agenda:
Introduction to SomaLogic Inc. and SomaSciences
Tony Bartlett, Director European Commercial Operations, SomaLogic, Inc.

Launch Presentation: SOMAmers Enable High Throughput Screening for Protein Biomarkers and Diagnostics
Stephen A. Williams, M.D,. Ph.D., Chief Medical Officer, SomaLogic

A synergistic combination of attributes: >1000 proteins measured simultaneously with ELISA-like performance, sample volumes of a few microliters, increasingly high throughput (currently >30,000

samples/yr.), and the same reagents useable for discovery and routine diagnostic applications. This has enabled an unprecedented productivity breakthrough in mechanistic, diagnostic and prognostic biomarkers.

SomaSciences Business Alliance Offerings
Evan King, Director SomaSciences, Somalogic Inc.

Late Breaking Abstracts

P249

An Investigation into the Optimum Conditions for Separating Low Abundance and Abundant Proteins in Rat Plasma Using a Seppro Column

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The plasma proteome has a large dynamic range of individual protein concentrations (10 orders of magnitude). Identification of low copy number proteins of interest using 2-DE is therefore difficult due to the confounding presence of higher abundance proteins and adversely affects biomarker discovery. Several columns have been developed to deplete the higher abundance proteins. For example, the Seppro Column has frequently been used in the published literature. It can remove the top 7 highly abundant proteins in mouse plasma, and be repeatedly used up to 80 times, according to the manufacturer's documentation. However, there are no independent data on the efficiency of protein extraction after repeated use of the column. The aim of the study is to 1) optimize the concentration methods of depleted protein sample; 2) to test the efficiency of the column after 50 depletion cycles for abundant protein removal. The proteins of the depleted fraction, the non-specifically bound protein, and the specifically-bound protein were measured after Seppro spin column treatment. Protein recovery at each step was: (1) depleted protein 27.7%; (2) non-specifically-bound protein 2.4%; (3) bound protein 25.2%. Protein was further concentrated by using Vivaspin4 5kDa MWCO membranes, trichroroacetic acid (TCA) precipitation, and a RedayPrep 2-D Cleanup Kit. The protein recovery rate was 60%, 67%, and 76%, respectively. 200μg of depleted or bound protein from Vivaspin4 5kDa MWCO, TCA precipitation was loaded onto pH 3-10 IPG strips (18cm) and 8-16% SDS gels in the second dimension. The gels were stained with Coomassie blue and analysed by Progenesis SameSpots software. The number of protein spots was 600 for crude plasma precipitated with TCA, 604 for depleted protein precipitated with TCA, 565 for depleted protein concentrated by vivaspin4 5kDa MWCO, 495 for the bound protein with TCA and 659 spots for the bound protein with vivaspin4 5kDa MWCO concentration. Results also showed that the ratios of depleted protein to total recovered protein decreased from 70% to 60% after 25 depletions and then gradually decreased to 45% after 50 sample depletions.

Acknowledgement:

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P250

Resolving interactions involved in binding of the dynein cofactor Bicaudal-D (BicD) to Rab6

Andrew N Holding; Yang Liu; Hannah Salter; Simon L Bullock; J. Mark Skehel and Elaine Stephens *MRC Laboratory of Molecular Biology, Cambridge, U.K.*

The cytoplasmic dynein-motor is responsible for the minus-end-directed transport of many cargos within the cell, including vesicles, mRNAs and pathogens. The method by which motors like dynein prioritise between different cargos within the cell is, however, poorly understood. To study this process, we investigated the structure and interactions of Bicaudal-D **(**BicD) an evolutionary conserved protein that functions in combination with specific cargo adaptors. To model the interaction of BicD with cargo adaptors, a complex between the Rab6 GTPase and BicD has been reconstituted *in vitro*. Crystallisation trials of this BicD/Rab6 complex have so far proven unsuccessful; therefore alternative methods have been employed.

Chemical cross-linking coupled with mass spectrometry provides a convenient alternative to conventional techniques for the structural analysis of complexes. The method provides a challenging bioinformatics problem, due to the large amount of data generated during a standard experiment and the small quantity of cross-linked peptides produced.

We have developed software that can analyse these data. While other software already exists, many have specific limitations (for example: closed source, mass spectrometer specific or limited to single spectral pairs at a time). We wanted an open and easily configurable application, which can automatically analyse the large data sets generated by this technique.

Data will be presented demonstrating the successful identification of a single cross-linked BicD-Rab6 peptide following the lc-ms/ms analysis of the enzymatically digested sample, combined with our in-house software to process the raw data. Mutagenesis of the residue in BicD that was cross-linked revealed that it was critical for Rab6 binding in *in vitro* pull-down studies. Furthermore, neighbouring residues in BicD were also shown to be essential for binding to Rab6. Where tested, the introduced mutations did not disrupt dimerization of BicD, suggesting that they participate directly in binding Rab6. We conclude that the cross-linking method was able to locate the binding site on BicD for Rab6.

P251

Reference 2D maps of Clostridium *tyrobutyricum* endospores and germinative forms isolated from cow milk used for Grana Padano production

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Introduction. The change which more frequently affects production of semi-hard and hard cheeses such as Grana Padano is known as "late swelling" or "blowing". This phenomenon, if marked, may affect the structure of the cheese and can be accompanied by unpleasant taste and odour due to the production of butyric acid and acetic aldehyde. The late appearance of the swelling is related to the number of spores, in particular from Clostridium *tyrobutyricum*, initially present in milk[1]. Little is known about proteins that enhance germination and metabolic changes in C. *tyrobutyricum* [2]. Thus, proteomic understanding of the initial stages of germination may help to prevent the late blowing phenomenon. Aim of this study is the definition of a 2D reference map for endospores and germinative forms of C. *tyrobutyricum*. **Methods.** Bacterial samples isolated from milk were subjected to 1-DE and 2-DE and all proteins separated from each experimental condition were identified by mass spectrometry. **Results**. The effects of germination on protein expression were studied with two-dimensional protein electrophoresis (2DE). There were about 400 protein spots on the 2-DE maps obtained for both the endospore and germinative form. These 2-DE protein maps were analyzed with the Progenesis SameSpot 3.3 software (Nonlinear Dynamics), which compared and identified proteins with changed expression levels between experimental conditions **Conclusion.** Proteomic data obtained from this study, may provide a reference 2D map of C. *tyrobutyricum* endospores and germinative forms and additional information for elucidating proteins involved in early germination of C. *tyrobutyricum* endospores.

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References

1. Klijn N, Nieuwenhof FF, Hoolwerf JD, van der Waals CB, Weerkamp AH (1995) Identification of Clostridium tyrobutyricum as the causative agent of late blowing in cheese by species-specific PCR amplification. Applied and environmental microbiology 61 (8):2919-2924
2. Mato Rodriguez L, Alatossava T (2010) Effects of copper on germination, growth and sporulation of Clostridium tyrobutyricum. Food microbiology 27 (3):434-437. doi:10.1016/j.fm.2010.01.003

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Scientific session: food security

P252

Development and optimization of a procedure for extracting proteins from fixed archival tissues and assessment of its potential for biomarker discovery in human and veterinary medicine

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Fixed tissue repositories are an ideal information mine for biomarker discovery, with their vast collections of diseased tissues and associated clinical records describing diagnosis, prognosis, therapy, and outcome. Unfortunately, fixation hampers protein extraction, posing considerable limitations to proteomic investigations. In this scenario, and building on the existing literature, several methods were combined and refined to enable extraction of high-quality, full-length proteins from the fixed specimens, leading to the definition of an optimized protocol with unprecedented performances. In order to test its ability to provide meaningful results in terms of differential disease traits and enable biomarker discovery, the optimized method was applied to sample repositories stored in pathology departments. Specifically, diseased human, canine and sheep tissues were retrieved and subjected to the optimized extraction procedure, followed by established proteomic pipelines consisting of electrophoresis, image analysis, mass spectrometry, and data processing by ontology and pathway analysis. The method enabled gel-based proteomic investigations of archival tissues, performing better than commercially available systems. Differential proteins were successfully identified in all cases, and these were clearly related to the disease biology. In conclusion, biomarker discovery strategies based on proteomic investigations can be successfully performed on fixed archival tissues by means of this optimized extraction protocol.

P253

Identification of a new food allergen from hazelnut (Corylus avellana): proteomic and immunoassay based approach

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Hazelnuts (*Corylus avellana*) are one of the most common lifetime lasting IgE-mediated cause of food allergy. Immune reactions to hazelnut range from mild oral allergy syndromes, which are caused by cross-reactivity with tree pollen, to severe life-threatening anaphylaxis. Hazelnut allergy is more frequent in infancy than in adulthood and its prevalence varies among countries (0.4-1%). Over the years different studies aimed to identify the hazelnut allergenic determinant(s) have been carried out. To date several hazelnut allergen proteins have been identified and characterized. The predominance of specific allergens appears strictly related to the geographical origin of the allergic patients [1]. Cor a 1.04, related to the birch aeroallergen Bet v 1 (from *Betula verrucosa*), is considered the major allergen in Northern Europe [3]; 11S globulin (Cor a 9), consisting of a 30–40 kDa acidic and a 20–25 kDa basic subunits linked *via* inter-chain disulfide bridge, is the principal non-pollen related allergen in United States [2]; *non specific*-lipid transfer protein (*ns*-LTP), referred to as Cor a 8, is supposed to be the predominant allergen in Southern Europe [1]. Additional storage proteins, such as 48 kDa-glycoprotein (Cor a 11) and 2S albumin (Cor a 14) are also reported as potential food allergens.

In this study we investigated the IgE immune-affinity of hazelnut proteins and addressed proteomic strategies for investigating the pattern of the immunological response of hazelnut allergic children. Children's sera (N=4) were collected from the South of Italy. Preliminarily a "classical" two-dimensional electrophoresis (2DE)-mass spectrometry (MS) approach was used for characterizing of the protein fraction of three commercial autochthon cultivars from Southern Italy and one from Oregon (USA). Western blot IgE-immunostaining showed that all sera recognized a not yet described protein of about 55 kDa consisting of at least two subunits bound *via* disulfides. Under reductive conditions, a 21 kDa subunit was the unique immunoreactive band. The IgE-binding protein was purified by a sequential two-step chromatography procedure, including size-exclusion-chromatography (SEC) and RP-HPLC. The tryptic peptide mass fingerprinting was unsuccessful in identifying the allergenic subunit due to the incompleteness of the deposited hazelnut genome. Several tryptic peptides were "*de novo*" sequenced by HPLC-electrospray (ESI)-Q-TOF MS/MS, thus evidencing a high homology degree with the 11S globulin-like storage proteins from other seeds. These indications, combined with the quaternary structural protein traits, suggest that the new allergen is a divergent isoform of the canonical Cor a 9. In order to infer information about the epitopes, the purified protein was subjected to simulated *in vitro* gastrointestinal digestion and the peptide pool was tested for the IgE affinity by dot-blot assay. The positive IgE-binding reaction confirmed that antigenic determinants are linear digestion-stable epitope(s). These results open a new scenario for developing innovative diagnostic and therapeutic intervention plans.

References

[1] Pastorello EA et al. Identification of hazelnut major allergens in sensitive patients with positive double-blind, placebo-controlled food challenge results. J Allergy Clin Immunol. 2002; 109(3):563-70.

[2] Beyer K, Grishina G, Bardina L, Grishin A, Sampson HA. Identification of an 11S globulin as a major hazelnut food allergen in hazelnut induced systemic reactions. J Allergy and Clin Immunol. 2002; 110(3):517–523

[3] Lüttkopf D et al. Comparison of four variants of a major allergen in hazelnut (Corylus avellana) Cor a 1.04 with the major hazel pollen allergen Cor a 1.01. Mol Immunol. 2002; 38(7):515-25, Jan.

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P254

In depth proteomic profile of mammary normal and cancer-associated fibroblasts.

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Angiogenesis is the mechanism of blood vessel formation from pre-existing ones and has been linked to tumour progression and metastasis in breast cancer. This fact is known since 1971 when Folkman proposed that tumours cannot grow without blood vessel beyond a critical size or metastasise to other organs. This was the starting point for an intensive research looking for factors which could modulate angiogenesis in tumours. In parallel, the cancer stroma and extracellular matrix have gained importance in cancer progression and tumour angiogenesis, as well. Cancer associated fibroblasts (CAFs) play a pivotal role in tumour angiogenesis by initiating and promoting the progression of mammary epithelial tumours. Indeed, CAFs secrete extracellular matrix (ECM) proteins, various growth factors and cytokines that modify the tumour microenvironment and activate the endothelial cells of the surrounding vessels.

Using SILAC-based quantitative proteomics we have identified the proteome and secretome differentially expressed in immortalized human mammary CAFs (CAF2) compared to normal fibroblasts (Orimo A. et al. 2005. Cell 6; 121(3):335-48). We have determined a proteomic fingerprint for the CAF2 which interestingly includes many proteins involved in angiogenesis. In parallel, we have tested the CAF2 influence on endothelial cells (ECs) using three dimensional co-culture as *in vitro* angiogenesis model. We found that CAF2 altered the ECs sprout size and proliferation. Additionally, we have analysed how the ECM produced by CAF2 affects ECs motility. Finally, to start investigating how CAFs can alter endothelial cells behaviour, and potentially angiogenesis in breast cancer, we performed a mass spectrometry-based analysis of the endothelial surfaceome when plated on ECM produced by CAF2 or normal fibroblasts. The CAFs fingerprint in combination with the altered ECs phenotype and surfacesome described here will contribute to better understanding tumour angiogenesis.

P255

GELFREE: Soluble fractionation of complex protein mixtures. A direct comparison with traditional 1-D electrophoresis sample preparation

Sample fractionation is critically important to generating quality, comprehensive proteomics data. One-dimensional gels (GeLC-MS/MS) have traditionally been the tool of choice for sample preparation as they provide rich and consistent protein identification data. However, this method has significant disadvantages:

- Protein recovery is difficult; the protein must be digested for recovery.
- Conventional 1D gels have limited loading capacity, making detection of low abundance proteins challenging.
- PAGE gels have poor reproducibility, making it difficult to reproducibly isolate proteins of interest.
- Cumbersome and time consuming workflow.

This study compares GELFREE 8100, a new and improved method of sample preparation for LC-MS/MS that utilizes a precast, high capacity PAGE separation to isolate distinct molecular weight fractions in solution phase to traditional GeLC-MS/MS using a 1 mm precast 1D gel and in-gel digestion. Since GELFREE 8100 enables in-solution protein collection, recovery is much higher (>90%). It is shown that this feature, combined with the higher loading capacity (>500 µg), leads to an overall increase in proteome coverage and data quality.

P256

Glycoproteomic characterization of *Trichomonas vaginalis*

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Trichomonas vaginalis, the only medically important protozoan parasite that is sexually transmitted, causes 180 million infections each year worldwide. Primarily a disease of women, Trichomonas causes vaginitis, pre-term delivery, low birth weight, and increased risk of HIV transmission.

Trichomonas causes disease when it adheres to and damages the vaginal or ectocervical epithelium. An important *Trichomonas* virulence factor is the lipophosphoglycan (LPG) that contains chains of Galß1-4GlcNAc (also known as N-acetyllactosamine or LacNAc) on a back-bone of rhamnose (1). LacNAc is bound by the plant toxin ricin and the host lectin Galectin-1, an important component of the innate immune response. Ricin-resistant mutants of *Trichomonas* show decreased quantities of LPG, decreased binding of Galectin-1, and decreased adherence to ectocervical cells in vitro.

We recently showed (using mass spectrometry and 13C-mannose metabolic labelling) hybrid Asn-linked glycans (*N*-glycans) extracted from the parasite, also contain LacNAc, and this LacNAc is reduced in ricin-resistant mutants. Unmodified *Trichomonas N*-glycans are recognized by anti-retroviral lectins that target similar unmodified *N*-glycans on the surface of gp120 of HIV. This finding is important because anti-retroviral lectins, which are included in creams designed to stop the heterosexual transmission of HIV, may also have effects on the transmission of *Trichomonas*.

Using a gel-free proteomic approach we identified numerous unique *Trichomonas* proteins having occupied *N*-glycan sites that are potential vaccine candidates. In order to complete the characterization of this organism, we are focusing on O-linked glycoproteome of *Trichomonas*. O-glycans are interesting because *Trichomonas* is the only parasite that contains homologs of the peptide:O-mannosyltransferases, which are called POMTs in humans and PMTs in yeast.

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References:

[1] Bastida-Corcuera FD, Okumura CY, Colocoussi A, Johnson PJ. Trichomonas vaginalis lipophosphoglycan mutants have reduced adherence and cytotoxicity to human ectocervical cells. Eukaryot Cell. 2005 Nov;4(11):1951-8.

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Quality control for a large-scale study using protein-arrays and protein-beads to measure immune response in serum and plasma

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We report on quality control performed in the context of a large-scale, multi-institutional study of the immune response in blood samples from prostate cancer patients. The measurements were performed by two commercially available techniques/services: protein arrays and an automated bead-based ELISA-like technique on 871 patient samples. The project started with a wide screen using standard arrays with 8302 protein sequences for 113 patients, followed by three studies using custom arrays with 215 selected protein sequences. These studies were followed up by three studies using the bead-based approach on 57 protein sequences chosen from the 215 selected before. We find similar responses in plasma and serum samples. Samples from the two European projects from which the samples originated also appeared comparable. In the data from the high density standard arrays, we see for ~12% of the protein sequences high cross-correlation (R^2 >0.8) with signals from unrelated protein sequences that are physically nearby on the array, suggesting production issues. The custom array and bead-based techniques both have good reproducibility, but the techniques do not agree with each other for ~50% of the protein sequences measured. We discuss the consequences of the observed data quality for the design and interpretation of the study.

This work can be part of Clinical Proteomics session.

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Potential Method for the Quantification of Phosphopeptides

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Abstract

The absolute quantification of phosphopeptides has been investigated by both elemental and molecular mass spectrometry; however, both approaches have difficulties associated with them. In this work, a novel approach to phosphopeptide quantification is described based on the incorporation of a metal tag gallium – N,N-biscarboxymethyl lysine (Ga-LysNTA) in solution by liquid chromatography inductively coupled plasma mass spectrometry (LC-ICPMS). Successful coupling of the phosphopeptides to Ga-LysNTA is shown by electrospray ionisation mass spectrometry (ESI-MS). Excess labelling reagent was removed from the reaction mixture by automated solid phase extraction (SPE) prior to analysis by LC-ICPMS. A recovery of 89% of LysNTAGa-Phosphopeptide was achieved, demonstrating a high tagging efficiency. The detection limit for LysNTAGa tagged phosphopeptides was 250 pg/g, which is over an order of magnitude more sensitive than detection by phosphorus alone.

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Title: Peripheral biomarkers of Alzheimer's disease using Intact protein labeling

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Background:

Cerebrospinal fluid and imaging techniques have been shown to reliably diagnose patients with Alzheimer's disease (AD). Despite this, there is still a need to find a cost-effective and non-invasive test that can be implemented for routine clinical practice.

Therefore the discovery of a blood-based biomarker in recent years has become a prominent area of research. Technology used in previous biomarker discovery projects has been limited to investigate only highly abundant proteins as candidate markers. A more thorough exploration of the plasma proteome will surely yield potentially novel peripheral biomarkers with a greater sensitivity and specificity.

Here we have employed a quantitative gel mass spectrometry method with the aim to increase the quantitative coverage of the plasma proteome.

Materials & Methods:

100ug of raw plasma was labeled with a combination of lysine (Lys-TMT) and cystine (Cys-TMT) isobaric tags (TMT; Proteome Sciences), denatured and resolved on a 1D gel, divided into 10 equal fractions (Gel10) and trypsin digested. Anti-TMT resin was used to selectively enrich peptides that have are labeled with TMT tags thereby increasing the quantitative coverage. LC-MS/Mass analysis of trypic peptides was performed on the Orbitrap Velos Pro and protein assignment were determined by Mascot v2.2 with validation from Proteome Discoverer and Peptide Validator software's.

Results:

Initial data analysis shows that we are able to accurately indentify 290 plasma proteins from 44,000 MS/MS events from a Gel10 experiment, of which half are reliably quantified using Lys-TMT before anti-TMT enrichment.

Results will show if we have employed a novel approach for increased detection and quantitative coverage of the plasma proteome by combining two intact protein labeling technologies. By simplifying our complex sample by enrichment of TMT labeled peptides we hope to significantly increase the number of unique proteins, making it easier to detect and measure low-abundance proteins.

Conclusion:

We have developed methods using intact protein labeling and multiplex TMT labeling of human plasma samples that enables a higher throughput with robust relative protein quantification. Those methods are suitable for large AD plasma biomarker discovery projects.

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Using high resolution mass spectrometry to unravel the effects of matrix stiffness on endothelial cell behaviour.

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Tumours are reliant on their blood supply and consequently exploit the process of new blood vessel growth, angiogenesis, in order to thrive. Termed tumourigenesis, this process results in increased tissue stiffness, which promotes cancer progression. Stiffness is known to have a significant impact on endothelial cell phenotype, however it is unknown how stiffness affects endothelial cell signalling. Our aim is to elucidate the molecular mechanisms behind stiffness mediated effects and its contribution to angiogenesis.

We combine nano-liquid chromatography coupled to an LTQ-Orbitrap Elite with stable isotope labelling by amino acids in cell culture (SILAC) to analyse the quantitative proteome changes of primary human endothelial cells cultured on physiological and pathological stiffnesses.

Using biological assays, we show that increasing stiffness causes an elevated rate of endothelial spreading and adhesion to the substrate. After acclimatisation to their environment, endothelial cells are shown to maintain phenotypical changes for a limited time following removal of this stimulus. For SILAC experiments, we demonstrate that primary endothelial cells can be fully labelled in just three passages without affecting endothelial marker expression. Preliminary triple SILAC results show the differential regulation of proteins with increasing stiffness, some of which are known to be involved in angiogenesis, extracellular matrix maintenance and in regulating endothelial cell function.

Localization is critical for the function of transmembrane and secreted proteins, however protein presence within whole cell lysates does not indicate localisation or activity. Therefore we isolate specific sub-proteomes, the secretome and surfaceome. These contain proteins that are involved in how cells sense and respond to extracellular signals, and therefore are key to the cellular response to matrix stiffness and potentially angiogenesis. Candidates identified from proteomic analysis will be functionally validated by studies including siRNA knock down to determine if the phenotype at increased stiffness is suppressed, allowing us to identify proteins regulated by stiffness that may impact angiogenesis. This is particularly relevant to the stiffening tumour microenvironment and may provide targets to interfere with tumour angiogenesis for anti-cancer therapies.

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Molecular partners of *E. coli* transcriptional modulator AidB

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Upon exposure to alkylating agents *Escherichia coli* increases the expression of four genes, *ada*, *alkA, alkB,* and *aidB*. Three genes (*ada, alkA, alkB*) encode DNA repair proteins whereas the role of AidB remains unclear [1]. AidB belongs to the acyl-CoA dehydrogenases family, shows a weak isovaleryl-CoA dehydrogenase activity and it has been reported to exhibit non-specific DNA binding activity [2]. Even though the precise biochemical mechanism of action remains unclear, our previous data suggests that AidB is not a repair protein. Instead it might prevent alkyl damage by shielding the DNA molecule or by inactivating alkylating molecules thus reducing their local concentration as an alkylation resistance protein [3].

Our studies were focused on the elucidation of AidB functions at the molecular level by functional proteomic approaches. Isolation of the entire multiprotein complexes was performed by pull down strategies using AidB as bait, in the presence and in the absence of methylating agent. This technique exploited the interaction between the histidine tag of the recombinant protein and chelating Ni^{2+} ions coupled to highly cross-linked agarose beads. AidB partners were identified by LC-MS/MS analyses of their tryptic digests. 48 proteins were identified between the 2 different conditions.

According to their reported biological activities, the identified interactors were grouped into different functional categories: FAD and NAD^{+} dependent proteins, resistance and protection families members and components of other known pathways. To validate some of the identified interactions, co-immunoprecipitation experiments of selected partners with AidB were carried out.

Validated interaction between the bait and UvrA protein, a member of the SOS response system, suggests that AidB might be involved in different response complexes in a new cellular strategy. The precise mechanism by which AidB inactivates alkylating agents is still obscure and its determination requires further work.

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References:

[1] Landini P., Hajec L.I., and Volkert M.R. Structure and transcriptional regulation of the E. coli adaptive response gene aidB. J. Bacteriol. 1994.

[2] Bowles T., Metz A.H., Quin J.O., Wawrzak Z. and Eichman B. F. Structure and DNA binding of alkylation response protein AidB. PNAS, 2008.

[3] Rippa V., Duilio A., di Pasquale P., Amoresano A., Landini A., Volkert M.R., Preferential DNA damage prevention by the E. coli AidB gene: a new mechanism for protection of specific genes. DNA repair, 2011.

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Elucidating roles of ANXA1 in murine mammary gland cells by SILAC-based phosphoproteome profiling

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Annexin-1 (ANXA1) is a member of the family of Ca^{2+}-dependent phospholipid binding proteins. Besides mediating inflammation, ANXA1 has also been reported to be involved in important physio-pathological implications including cell proliferation, apoptosis and cancer metastasis. However, with controversies in ANXA1 expression in breast carcinomas, its role in breast cancer initiation and progression remains to be elucidated. Our previous study based on Stable Isotope Labeling of Amino acids in Cell culture (SILAC) analysis on the proteomes of normal mammary gland epithelial cells from mice revealed that ANXA1 is potentially implicated in DNA-damage response, cell adhesion and migration pathways. In our quantitative proteome profiling data, we observed differential regulation of several kinases and phosphatases in the absence of ANXA1. Thus, investigating the impact of ANXA1 on the phosphoproteomes of murine mammary gland cells would reveal possible mechanisms on how ANXA1 regulates above cellular pathways. Here we used SILAC to quantify the phosphoproteomes of normal mammary gland epithelial cells from ANXA1-heterozygous (ANXA1+/-) mice in comparison to those from ANXA1-null (ANXA1-/-) mice followed by bioinformatics analysis to find functional relevance of the differentially regulated phosphorylation sites. We identified 631 up- and 394 down-regulated phosphorylation sites from ~ 5,000 quantified phosphorylation sites (~10,000 identified sites in total). Pathway analysis of these phosphoproteomic data revealed that 'tissue remodeling and wound repair pathway' proteins were enriched in the protein cluster with up-regulated phosphorylation sites. Further analysis disclosed that the phosphorylation sites of PKC substrates, such as marcks, marcks-related protein and calpastatin, which are also involved in cell migration and focal adhesion, are differentially regulated. These results verified our finding in the global proteomic studies that ANXA1 is involved in cell migration/adhesion pathway. Kinase motif analysis indicated that the phosphorylation by CHK1/2, NEK6 and PLK1, kinases which are involved in the cell cycle pathways, are enriched among the up-regulated phosphorylation sites. Phosphorylation by the key kinases in cell cycle and cell proliferation such as Aurora, CDK1/2 and AKT are enriched among the down-regulated phosphorylation sites. All these analyses demonstrated that ANXA1 plays crucial, yet complex roles in the initiation and progression of breast cancer. Functional analyses of these phosphoproteomic outcomes are in progress.