

Moving MS-based proteomics closer to the clinic

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Mass spectrometry based proteomics has advanced tremendously in the last few years. We describe a shotgun proteomics workflow that allows us to detect and quantify the large majority of the proteins expressed in a biological system such as cancer cell lines and even formalin-fixed, paraffin embedded material. This included streamlined and highly efficient sample preparation, analysis with very high sequencing speed using modern mass spectrometers and bioinformatic analysis using the MaxQuant and Perseus platforms. Efforts in our group have focused on 'single shot' analysis and we demonstrate very high coverage in this mode (Mann et al., *Mol. Cell*, 2013). We have also extended this concept to the analysis of interactomes (Hein et al. *Cell* 2015) and phosphorylation, where the 'EasyPhos' method now allows acquiring large numbers of phosphoproteomes, for instance for the analysis of in vivo signaling (Humphrey et al. *Nat. Biotech*, 2015). Finally, we are currently re-visiting body fluid proteomics with a view to obtain protein profiles of large numbers of individuals in health and disease. Together such developments make proteomics increasingly relevant to translational research as I will discuss in this talk.

SIRT4 is a host mitochondrial lipamidase that restricts human cytomegalovirus

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Publish consent withheld

1. Koyuncu E, Budayeva HG, Miteva YV, Ricci DP, Silhavy TJ, Shenk T et al. Sirtuins are evolutionarily conserved viral restriction factors. *MBio* 2014; 5.
2. Mathias RA, Greco TM, Oberstein A, Budayeva HG, Chakrabarti R, Rowland EA et al. Sirtuin 4 is a lipamidase regulating pyruvate dehydrogenase complex activity. *Cell* 2014; 159: 1615-1625.

Asn347-Glycosylation of Corticosteroid Binding Globulin Fine-tunes the Host Immune Response by Modulating Proteolysis by *P. aeruginosa* and Neutrophil Elastase

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Corticosteroid binding globulin (CBG) delivers anti-inflammatory cortisol to inflamed tissues upon elastase-based proteolysis of an exposed reactive center loop (RCL). However, the molecular basis of the RCL proteolysis by co-existing host and bacterial elastases and mechanisms regulating these events in inflamed and infected tissues remain unknown. We document that the RCL-localized Asn347-glycosylation is ideally positioned to regulate the RCL cleavage by human neutrophil elastase (NE) and *P. aeruginosa* elastase (PAE) and that it does so by different mechanisms. NE- and PAE-generated fragments of native and exoglycosidase-treated blood-derived CBG of healthy individuals were monitored by gel electrophoresis and LC-MS/MS to determine the cleavage site(s) and N-glycosylation status as a function of NE/PAE incubation time. The site-specific (Val344-Thr345) and rapid (seconds-minutes) NE-based RCL proteolysis was significantly antagonized by several volume-enhancing Asn347-glycan features i.e. site occupancy, triantennary β 1,4/6-GlcNAc branching, β -galactosylation and α 1,6-fucosylation and strongly augmented by Asn347 α 2,3-sialylation (all $P < 0.05$). In contrast, the inefficient (minutes-hours) PAE-based RCL cleavage occurring equally well at Thr345-Leu346 and Asn347-Leu348 was completely antagonized by the Asn347-glycan, but received beneficial electrostatic interactions from sialoglycans of other CBG glycosylation sites. In addition, virulent *P. aeruginosa* growth was augmented by the free cortisol, which may explain the weak PAE efficiency in cleaving glycosylated RCL during host infection. Finally, molecular dynamics simulations of relevant CBG Asn347-glycoforms, carried out using a homology model of uncleaved CBG, largely confirmed the experimental observations and suggested additional interesting relationships between Asn347-glycosylation and RCL cleavage. In conclusion, we are the first to show that site-specific CBG N-glycosylation regulates the bioavailability of cortisol in inflamed environments by fine-tuning the RCL proteolysis by endogenous and exogenous elastases via different mechanisms. This study offers new molecular insight into host- and pathogen-based manipulation of the human immune system.

The N-terminome of *Mycoplasma hyopneumoniae* reveals functionally-diverse proteins as targets of endoproteolysis.

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Mycoplasma hyopneumoniae is a primary colonising agent and economically-devastating porcine respiratory pathogen that is controlled by partially-effective bacterin vaccine formulations and widespread antibiotic therapy. Adherence and colonisation is in part mediated by the P97 and P102 adhesin families that directly interact with the host extracellular matrix, via glycosaminoglycans, fibronectin and the fibrinolytic protein, plasminogen. Post-translational proteolytic cleavage is essential to the production of functional, mature proteoforms on the surface of this ubiquitous pathogen. We aim to determine whether proteolytic cleavage is restricted to the adhesin families using a high-throughput, proteome-wide methodology.

Using protein dimethyl labelling, reversed-charge enrichment of N-termini and 'shotgun' mass spectrometry, the N-terminal sequences of mature *M. hyopneumoniae* proteins were characterised. Our data interrogated protein start sites and identified the precise location of protein cleavage in functionally diverse proteins. These data were combined with surfaceome studies, affinity chromatography using diverse host molecules as bait and mass mapping by SDS-PAGE to confirm the existence of proteoforms and to investigate their putative functions.

Our approach confirmed the existence of multiple cleavage events, previously characterised in the adhesin families, validating our approach. Additionally, we identified internal cleavage events in lipoprotein families and proteins with well-studied canonical functions in the cytosol. The majority of the proteins targeted by endoproteolysis were identified in our surfaceome studies. Furthermore, our chromatography studies suggest that these proteins are multifunctional, with implications for the function of moonlighting proteins on the surface of *M. hyopneumoniae*. Consistent with this hypothesis, homologues of these 'moonlighting' proteins have been identified as virulence factors in other pathogenic bacteria but the data reported here is the first description of them being targets of endoproteolysis. The sequence motifs associated with the characterised cleavage events in this study are similar to those already described in studies of the adhesin families suggesting that the same protease(s) process functionally-diverse proteins on the cell surface. These observations have widespread ramifications for microbial pathogenesis and vaccine design.

005

Large-scale Phosphorylation Study of the Type 2 Diabetic Heart subjected to Control and Infarct Conditions

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Type 2 Diabetes (T2D) significantly increases the risk of a cardiovascular event, with acute myocardial infarction (AMI) the leading cause of mortality in these patients. The impact of T2D on the myocardial molecular mechanisms is however, poorly understood. Previous studies have demonstrated the essential role of protein phosphorylation to the outcomes following AMI. The current study uses large-scale phosphoproteomics to offer insight into the global impact of T2D on the heart and aims to elucidate the altered signalling cascades mediated by phosphorylation in response to AMI in the T2D setting.

Rats were fed a CHOW (12% fat) or high fat (HF) (42% fat) diet for 8 weeks, with T2D induced in 50% of the animals after 4 weeks utilising a low dose of Streptozocin (STZ; 35mg/kg) a pancreatic β -cell toxin. At the cessation of the feeding protocol, hearts were excised and subject to Langendorff perfusion to produce either non-ischemic time control (NITC) or ischemia / reperfusion (I/R) injury (30I/30R). Peptides were labelled with iTRAQ prior to enrichment of phosphopeptides utilising the TiSH method for analysis by tandem MS.

HF T2D hearts showed a significant decrease in functional recovery post-I/R ($6.91 \pm 2.42\%$ versus $32.68 \pm 4.98\%$ in CH controls). This study identified 16,845 and 11,803 unique phosphosites, originating from 3,586 and 2,786 unique proteins from hearts subjected to NITC and I/R respectively. Using z-score cutoff $>+1$ or <-1 , we observed 9,086 phosphosites significantly regulated following NITC and 6,432 phosphosites regulated by I/R. These regulated phosphosites were mapped to functional pathways utilising Kyoto Encyclopedia of Genes and Genomes (KEGG), identifying components of vital regulatory pathways such as the PI3K/Akt signalling cascade. By elucidating potential mechanisms behind the severely reduced capacity of the T2D heart to recover from AMI, improved outcomes and novel therapeutic targets are possible for these patients.

006

The proteome of blister fluid from paediatric burn injuries

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Burn injury is highly traumatic for paediatric patients. Scar formation is a nature outcome in severe burn and dependent on the severity of the burn injury. The early and accurate diagnosis of medium to severe burn is different according to current clinical assessment which is mainly based on the clinician's experience. These wounds represent a grey-area in scar formation and skin grafting. Thus, a more objective and quantitative measure to distinguish between burn severities is urgently required. Burn blister fluid is a viable study matrix that reflects systemic responses as well as the local microenvironment. This project seeks to characterise the blister fluid from children with different degrees of burn severity and determine severity specific protein markers to potentially aid in future clinical practice.

Blister fluid (BF) was collected from superficial thickness (n=12), deep-partial thickness (n=14), and full-thickness burn wounds (n=4). In order to characterise and identify BF proteins, the samples within each depth classification were pooled and subjected a variety of fractionation methods, including: filter aided sample preparation (FASP), lithium dodecyl sulphate polyacrylamide gel

electrophoresis (LDS-PAGE), and Isoelectric OFFGel. Another 11 individual samples were processed by Multiple Affinity Removal Spin (MARS) cartridge. Then the enzymolytic products were analysed using LC-MS/MS to profile protein composition. The underlying biological processes were discovered using a gene ontology analysis.

We have demonstrated the strength of fractionation approaches to facilitate a deeper characterisation of burn blister fluid proteome. We identified, 228 proteins using the FASP, 365 by LDS-PAGE, 460 by MARS and 551 by OFFGel. Importantly, there were 812 different proteins identified in total including 107, 84, and 146 unique proteins in different burn severities, respectively, and 317 common to all three severities. The differences in proteins and biological processes demonstrated that there are subtle, yet significant, differences in the biochemistry of burn wounds according to severity. Moreover, this study represents the most comprehensive study of paediatric burn wound microenvironment to date.

007

A combination of bottom-up and top-down mass spectrometry provides insights on the post-translational regulation of yeast protein methyltransferases

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In recent years, protein methylation has been established as a major intracellular post-translational modification (PTM). Over twenty protein methyltransferases have been identified in *Saccharomyces cerevisiae* that target histone and non-histone substrates such as RNA-binding proteins, elongation factors and ribosomal subunits. The question remains as to how these enzymes are regulated. We overexpressed and purified six *Saccharomyces cerevisiae* protein methyltransferases (Hmt1, Rkm1, Rkm3, Rkm4, Set5, Efm7) in their native host and subjected them to digestion by either trypsin or LysargiNase, followed by analysis with either LC-HCD-MS/MS or LC-ETD-MS/MS. Additionally, undigested samples were analysed via top-down mass spectrometry with FT-ICR MS to investigate the co-occurrence of PTMs and the modform distribution of protein methyltransferases. This multi-protease, multi-fragmentation approach revealed a range of PTMs on several protein methyltransferases including phosphorylation, acetylation and methylation. Of particular interest is a range of PTMs on Rkm1, Set5, and the N-terminal tail of Hmt1. The N-terminal tail of Hmt1 has been shown to be implicated in modulating its oligomerisation state, which in turn regulates its activity. The close proximity of certain PTMs on Hmt1 and Set5 also raises the possibility of crosstalk between PTM pairs. In summary, the identification of several PTMs on protein methyltransferases suggests that at least some methyltransferases are regulated post-translationally and are end points of signalling pathways.

008

Mitochondrial Changes Contribute to the Longevity of the daf-2 Mutant

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We analyzed the mitochondrion organelle in the long-lived daf-2 mutant to find out whether reduced insulin/IGF-1 signaling affects mitochondria and if so, whether these changes contributed to the longevity phenotype. Compared to wild-type *C. elegans*, the long-lived daf-2 mutant showed a better ability in maintaining a youthful mitochondrial morphology and respiratory function at an old age. We therefore asked whether a young daf-2 mutant worm is equipped with more young mitochondria (therefore, more healthy mitochondria) to withstand aging. However, on adult day 1 the total amount of mitochondrial proteins in a daf-2 mutant worm was found to be about the same as that in a wild-type worm. From quantitative proteomic analyses of purified mitochondria of wild type, daf-2 and daf-2; daf-16 mutant worms, we identified 81 up-regulated and 44 down-regulated mitochondrial proteins in the daf-2 mutant, and most of these changes were dependent on daf-16. These differentially expressed proteins revealed a shift of metabolic activity, especially in the metabolism of reactive oxygen species, branched-chain amino acids, β -alanine, propionate, and fatty acids. Further experiments suggested that altered metabolism contributed to the longevity of the daf-2 mutant. No abundance changes were detected in the electron transport chain proteins. There was a slight (10-20%) decrease of mitochondrial ribosomal proteins in the daf-2 mutant; although these changes were not significant for individual proteins, they were as a group. Consistently, by ribosome profiling, we found that the amount of assembled 28S mitochondrial ribosomes decreased by about 20% in the daf-2 mutant, suggesting a decrease in protein synthesis inside mitochondria and echoing with previously reported decrease of protein synthesis in the cytoplasm. We did not find evidence of mitonuclear protein imbalance in our data. Collectively, our results suggest that insulin/IGF-1 signaling regulates mitochondria and mitochondrial changes contribute to the longevity of the daf-2 mutant.

009

Proteomic-scale approaches for identifying reversible and irreversible cysteine redox post-translational modifications in myocardial ischemia / reperfusion

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Redox post-translational modifications (PTM) are emerging as important regulatory mechanisms in signaling and pathogenesis. Cysteine (Cys) is the most redox active amino acid and is a target for these PTM, some of which are biologically reversible (e.g. disulfides, sulfenic acid) while others (sulfenic [Cys-SO₂H] and sulfonic [Cys-SO₃H] acids) are considered irreversible. We have

developed enrichment methods to examine these PTM on a proteome-wide scale. Rapid and specific alkylation of free Cys, followed by thiol-based reduction and resin capture by thiol-disulfide exchange chemistry was applied to isolate reversibly modified Cys-containing peptides. The method was applied to a complex protein lysate generated from rat myocardial tissue and 6559 unique Cys-containing peptides from 2694 proteins were identified by tandem mass spectrometry (MS/MS). We next developed an enrichment method to isolate Cys-SO₂H/SO₃H-containing peptides from complex tissue lysates. The method is based on electrostatic repulsion of Cys-SO₂H/SO₃H-containing peptides from cationic resins (i.e. 'negative' selection) followed by 'positive' selection using hydrophilic interaction liquid chromatography (HILIC). We identified 181 Cys-SO₂H/SO₃H sites from rat myocardial tissue subjected to physiologically relevant concentrations of H₂O₂ (<100 μM) or to ischemia / reperfusion (I/R) injury via Langendorff perfusion. I/R significantly increased Cys-SO₂H/SO₃H-modified peptides from proteins involved in energy utilization and contractility, as well as those involved in oxidative damage and repair. Finally, we have combined these methods to enable multiplexed quantitative analysis of reversible/irreversible Cys redox PTM in response to I/R and in the presence of a broad-spectrum antioxidant (*N*-2-mercapto-propionyl glycine, MPG). We quantified >1350 Cys sites that are reversibly and/or irreversibly oxidized by I/R, including many sites that are protected by MPG. This technique allows for the quantitative profiling of reversible/irreversible Cys PTMs in response to oxidant / antioxidant stimulus, and their delineation within the context of protein abundance, during I/R injury and cardioprotection.

010

Starvation Regulates Ubiquitin-like Modifiers Critical for Stress Responses

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We have shown previously that nutrient deprivation in the model animal *C. elegans* will alter signalling pathways that control longevity¹. These responses are known to be regulated by protein-protein interactions, but such changes are largely uncharacterised. To identify the pathways regulated during mammalian starvation responses, we have used proteome-wide analysis of protein abundance and interactions in mouse tissues. We analysed liver, white adipose tissue, spleen and skeletal muscle after starvation (16 hours), compared to animals fed *ad libitum*. This label-free analysis allowed quantitation of >6000 proteins, the most comprehensive thus far.

One of the most surprising findings was the decreased abundance of the ubiquitin-like protein UFM1. The total abundance of UFM1 (including conjugated and unconjugated forms) was significantly ($P < 0.000015$) decreased more than 2-fold in starved mouse liver. Other proteins in the UFM1-conjugation pathway were not significantly changed in abundance. Quantitative immunoblotting pinpointed the UFM1-UFC1 (E2) conjugate as significantly decreased in abundance in starved mouse liver. In addition, we were able to identify novel direct substrates of UFM1 conjugation involved in liver metabolism, using high resolution tandem MS analysis. We hypothesise that UFM1-conjugation is detrimental to their activity.

Previously, we developed a workflow for the analysis of native protein-protein interactions using protein correlation profiling². We have now applied this method to analyse the starvation response in liver. These data show, for the first time, protein complex regulation is significant among the ~4000 proteins analysed, even in the absence of protein abundance changes. We observed a significant decrease in the interaction of UFM1 and its corresponding E1 (UBA5) and E2 (UFC1) enzymes after starvation. Like the UFM1 immunoblotting, this suggests a significant reduction in UFM1 pathway activation in response to starvation stress. Interestingly, UFM1 has previously been shown to have a role in stress responses such as ER stress and oxidative stress. Our finding that starvation regulates this pathway and UFM1-target proteins has provided new mechanistic insight into the nutrient deprivation response.

1. Larance M, Pourkarimi E, Wang B, Brenes Murillo A, Kent R, Lamond AI, Gartner A. (2015). Global Proteomics Analysis of the Response to Starvation in *C. elegans*. *Mol. Cell. Proteomics*. 14(7):1989-2001.
2. Kirkwood KJ, Ahmad Y, Larance M, Lamond AI. (2013). Characterization of native protein complexes and protein isoform variation using size-fractionation-based quantitative proteomics. *Mol. Cell. Proteomics*. 12(12):3851-3873.

011

Higher-Order Structural Characterization of Therapeutic Antibodies at Residue Level by Middle-Down HDX-MS

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(HDX-MS) strategies currently being used for protein structure and dynamics measurements include bottom-up and top-down approaches. The peptide-based bottom-up approach has no limit in protein size, but the small size of peptic peptides can lead to significant back exchange and incomplete sequence coverage, and the spatial resolution is often limited to peptide level. The top-down approach tackles these issues by analyzing intact proteins, but its success decreases rapidly as the protein size increases. In this presentation, we will discuss our development of a new 'middle-down' approach and its application in antibody characterization with ca. 100% sequence coverage.

Disulfide linkages in the antibody limit the ETD cleavage in top-down experiments and thus need to be. By performing ECD on the light chain and heavy chain separately after reduction and HPLC separation, we achieved complete sequence coverage for the light chain, but only 50% for the heavy chain (45 kDa). To expand the applicability of top-down HDX to larger proteins, we attempted to digest antibodies under HDX quench conditions into a limited number of specific fragments. We found three very large protein fragments after a brief proteolysis (1 min) of HER with a molecular weight of 12-25 kDa, that were well separated by HPLC within a 12 min run. The specific fragments obtained this way covered 100% of the light chain, and 95.3% of the heavy chain, representing a total coverage of 96.8% for the whole antibody. ETD fragmentation on these reached a spatial resolution

of less than two residues and complete coverage for every fragment. The effect of deglycosylation on the HER antibody structure as revealed by HDX with top-down ETD and the structural characterization of intact antibodies will be presented, and the advantages and disadvantages of different HDX-MS strategies will be discussed.

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Quantitative Proteomics Reveals that Plant Plasma Membrane Microdomains are Involved in Molecular Transport, Stress Responses and Callose Biosynthesis

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The plasma membrane (PM) is one of the most interactive and dynamic membrane structures of the cell. It represents an interface between the cell and the extracellular environment and, as such, it is involved in many biological processes, e.g., metabolite and ion transport, endocytosis, defense against pathogens, cell differentiation and proliferation. The PM contains microdomains enriched in sphingolipids and sterols that are resistant to certain concentrations of detergents. The aim of this work was to determine the main functions of such microdomains in poplar cells through quantitative proteomics, using gel-based and solution (iTRAQ) approaches. Compared to PM, 80 proteins related to cell wall biosynthesis, transport, stress responses and signaling processes were found to be significantly enriched in the microdomains. The majority of these proteins were predicted to contain up to 16 transmembrane domains and/or membrane-anchoring acylation sites. The occurrence of S-acylation sites in PM and microdomain proteins was experimentally determined using an acyl-biotin exchange method and mass spectrometry. Out of the 450 S-acylated proteins in PM, 24 were enriched in microdomains, suggesting S-acylation plays a key role in the localization of these proteins to specialised PM subdomains. In addition, the number of transmembrane domains and their length were higher in the proteins isolated from the microdomains than in the total PM proteins. An important proportion of the most enriched microdomain proteins corresponded to cell wall biosynthetic enzymes, namely (1 \rightarrow 3)- β -glucan (callose) synthases and related proteins, indicating that the isolated microdomains are the site for callose biosynthesis and regulation. The proteins identified most likely reflect the biological specialisation of the isolated microdomains in cell surface specific responses that trigger callose formation, e.g. stress responses, as well as a potential role in plasmodesmata formation and structure. The data will be discussed in relation with these key biological processes in plant cells.

012

Harnessing Targeted Proteomics to Enhance Yield, Salinity Tolerance and Thermal Tolerance of Wheat

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Publish consent withheld

013

Proteome profiling of persistent honey bee (*Apis mellifera*) primary cells

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Honey bee (*Apis mellifera*) research has been limited by a scarcity of resources for molecular biology. Cell lines in particular are important tools for studying, e.g., signalling cascades or host-pathogen interactions; however, only one stable honey bee cell line has been reported in the literature¹ and it is not widely used. Here we provide proteomic characterization of honey bee primary cells which can be maintained in cell culture for up to one year. The cells are a mixed population derived from worker pupae, so we were first interested in determining what proteins are regularly expressed and what organ or tissue type has the most overlap in expression profile. In this analysis, we identified 2,498 protein groups and the expression profile unequivocally overlaps with the thoracic salivary gland. We are now developing a lipid nanoparticle transfection method and are exploring the utility of the T2A self-cleaving peptide system in these cells. The T2A sequence can be used to liberate protein concatamers, allowing for expression of multiple proteins from one promoter. This system has been shown to be effective in Sf21 cell extracts for *in vitro* translation,² but has never before been investigated in the honey bee. The work described here represents important developments in the biochemical tools available to honey bee researchers, and will facilitate further research on this important insect.

1. Goblirsch MJ, Spivak MS, Kurtti TJ (2013) A Cell Line Resource Derived from Honey Bee (*Apis mellifera*) Embryonic Tissues. PLoS ONE 8(7): e69831. doi:10.1371/journal.pone.0069831
2. Wang QS, Jan E. Switch from Cap- to Factorless IRES-Dependent 0 and +1 Frame Translation during Cellular Stress and Dicitrovirus Infection. Preiss T, ed. PLoS ONE. 2014;9(8):e103601. doi:10.1371/journal.pone.0103601.

015

Limitations on a biochemical arsenal — do centipede venoms evolve under morphological constraint?

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Venoms are complex biochemical arsenals, often containing hundreds to thousands of unique proteins and peptides. Despite their utility for prey capture, venoms are energetically expensive commodities, and consequently it is hypothesized that venom complexity is inversely related to the capacity of a venomous animal to physically subdue prey¹. Centipedes, one of the oldest yet least studied venomous lineages, appear to defy this rule². We examined a taxonomically diverse range of centipedes using a multidisciplinary approach that included proteomics, transcriptomics, MALDI imaging, histology, immunohistochemistry, MRI, mCT, and electron microscopy to provide the first comprehensive insight into the composition and evolution of these ancient and unique venoms. Our results reveal a rich set of both novel and convergently recruited toxin types, but also substantial variation in toxin diversity between higher-order taxa. Surprisingly, despite lacking the formidable physical weaponry of scolopendrid centipedes, scutigermorph venoms appear relatively non-complex. We show that the observed disparity in venom complexity between scolopendrid and scutigermorph centipedes likely stem from morphological limitations of the venom gland, and that scolopendrids may be unique among centipedes in having evolved venom glands that can accommodate the peptide diversity characteristic of many arthropod venoms^{3,4}. Moreover, the same morphological adaptations that allow great venom complexity in scolopendrid centipedes also appears to enable exquisite control over the composition of secreted venom. Our results provide further support for the concept that toxins are not universally expressed throughout the glands of venomous animals, and that this heterogeneity of toxin production can enable modulation of the composition of secreted venom. Our finding of morphological constraint on toxin diversity has substantial implications for centipede venoms as a biodiscovery resource, and our results are a clear example of an interdisciplinary approach to understanding the evolution of venom systems may guide such efforts.

1. Morgenstern, D., King, G.F. (2013) The venom optimization hypothesis revisited. *Toxicon* 63:120-128.
2. Undheim E.A.B., Jones A., Holland J.W., Clauser K., Pineda S.S., King G.F., Fry B.G. (2014) Clawing through evolution: toxin diversification and convergence in the ancient lineage Chilopoda (centipedes). *Molecular Biology & Evolution* 31, 2124–2148
3. Undheim E.A.B., Sunagar K., Hamilton B.R., Jones A., Venter D.J., Fry B.G., King G.F. (2014) Multifunctional warheads: diversification of the toxin arsenal of centipede venoms via novel multidomain transcripts. *Journal of Proteomics* 102: 1–10
4. Undheim E.A.B., Hamilton B.R., Kurniawan N., Bowlay G., Cribb B., Merritt D., Fry B.G., King G.F., and Venter D.J. (2015) Production and packaging of a biological arsenal: centipede venom evolution under morphological constraint. *Proceedings of the National Academy of Sciences USA*, 112: 4026–4031

016

Tissue specific metabolite and lipid analysis using MALDI-FT-MS – linking spatial distribution to metabolic function

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Imaging Mass Spectrometry (IMS) is a novel technique allowing the measurement of the spatiotemporal distribution of many biomolecules, including lipids and metabolites, in thin sections of biological tissues. The growing field of spatial lipidomics and metabolomics is developing and adopting IMS as a complementary analytical tool to conventional 'omics analyses of crude tissue extracts. Here we present the advantages and disadvantages of Ultrahigh Mass Resolution Matrix Assisted Laser Desorption Ionisation Fourier Transform Ion Cyclotron Resonance IMS and describe potential applications of spatial lipid and metabolite analysis in different biological tissues such as from mammals or plants. We will discuss how IMS will contribute to our increasing knowledge base of biological systems under investigation by providing information on the spatial distribution of lipids and metabolites.

017

Discovery of mechanisms of action for antimalarial drugs by medium-throughput metabolomics screening

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New drugs are urgently required for the treatment of malaria, due to the emergence of multi-drug resistant forms of the causative parasite, *Plasmodium falciparum*. Recent drug discovery programs utilising high-throughput phenotypic screens have identified thousands of small molecules that have antiparasitic activity against *P. falciparum* in vitro, and a representative subset of these, known as the 'Malaria Box', have been released openly to the research community. However, little is known about the mechanism of action of these compounds, restricting further development of these hit compounds.

Systems biology techniques offer an ideal platform to investigate mechanisms of drug action in a hypothesis-free manner. This approach is ideal for the *de novo* identification of drug mechanisms where no prior knowledge about the mode of action is available. Specifically, metabolomics provides a snapshot of the metabolic state of the parasite after drug treatment, and can reveal the direct impact of a test compound on metabolism.

LCMS-based untargeted metabolomics methods for *P. falciparum* cultures were optimised for 96-well plate format, allowing analysis of the metabolic phenotype induced by 100 known and novel antimalarials. Many of the novel compounds induced metabolic phenotypes that clustered with known antimalarials. In particular, inhibition of pyrimidine biosynthesis was a common mechanism of antimalarial action. Furthermore, depletion of unique parasite peptides and lipids indicated inhibition of parasite-specific pathways that may provide attractive new targets for drug discovery. This work demonstrates the suitability of metabolomics approaches for medium-throughput screening of the impact of drug candidates on cellular biochemistry, and revealed mechanisms of action associated with many of these novel antimalarial compounds.

018

MALDI imaging analysis of the aging human lens metabolome

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The transparent ocular lens focusses light onto the retina in order to form a sharp image. The lens is remarkable in that it is able to remain transparent over many decades of life despite the lack of a blood supply or *de novo* protein synthesis and turnover in the lens centre. Cells in the lens centre are formed *in utero* and maintained throughout life, and contain long-lived proteins whose function must also be maintained. To protect against oxidative-stress induced protein damage, the lens contains high levels of antioxidants, such as glutathione and vitamin C. The development of age-related cataract is thought to be related to changes in lens antioxidants and other metabolites in specific lens regions, and leads to the protein damage, insolubilisation and opacification that characterises lens cataract. In this study, lens small molecules and metabolites have been mapped in the aging human lens to define lens metabolome changes associated with normal lens aging.

Frozen donor human lenses were sectioned at 20mm and collected on gold MALDI targets using methanol soft-landing. NEDC matrix (7mg/ml in 90% MeOH) was applied using a TM-Sprayer (HTX Technologies). MALDI imaging data sets of all lens ages were analysed simultaneously at 50-150um spatial resolution using a MALDI-TOF/TOF mass spectrometer (Bruker UltrafleXtreme) in negative ion mode. Data sets were normalised to total ion current, and MALDI images plotted using flexImaging v4.1 with pixel interpolation on. Where possible, on-tissue tandem mass spectrometry was used to confirm metabolite identities. Over one hundred signals for small molecules were detected in the m/z range 80-1000, including major lens antioxidants, amino acids and nucleotides. Generally, ion signal for lens antioxidants decreased with increasing lens age, while other unidentified ion signals increased in specific lens regions. This study highlights the metabolic changes that take place in the aging human lens, providing a baseline measure for age-related changes to be compared with metabolic changes associated with cataract formation.

019

Glycomics in Health and Disease

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Every cell has multiple types of glycan on its surface where they play vital roles in cell-cell and cell-matrix interactions. Structural determination is essential for understanding the roles that glycans play in biological systems. Mass spectrometry (MS), with its ultra-high sensitivity and ability to analyse complex mixtures of glycans, is the most powerful tool currently available for glycan structure analysis. Our laboratory is engaged in numerous world-wide collaborations in which we exploit high sensitivity glycomic and glycoproteomic methodologies for the structural characterization of glycans found in a diverse range of biological material. This presentation will describe MS strategies for defining the glycomes of cells, tissues and purified glycoconjugates, as well as establishing glycoprotein site-specific glycosylation. The broad range of our research activities will be illustrated by data from ongoing collaborative projects embracing host pathogen interactions and human health and disease.

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020

Alterations in Glycosylation in Ovarian Cancer

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Altered glycans have long been associated with cancer and are displayed on such cancer protein markers as CA-125, CA19-9, a fetoprotein, MUC 1, PSA, etc but have never been fully exploited as indicators of cancer in their own right or as an adjunct to other biomarkers.

We now have the mass spectrometric technology to detect the glycosylation changes on proteins and lipids and to accurately assign glycan structures including sequence and linkage in both cell lines and tissue samples¹. We can also now correlate these

changes with the genes and epigenetic changes responsible for the aberrant glycans observed. Furthermore, we have shown that specific glycan masses can be used to precisely image the localisation of different tissue types in formalin fixed paraffin embedded tissue sections ².

Ovarian cancer is a cancer which is not only difficult to detect at early stage but is also controversial in regard to its tissue origin and metastatic progression. We show that there are significant changes in glycosylation in proteins and lipids between ovarian cancer cell lines that are reflected by changes in expression of the glycosylation pathway genes. We also show that glycan structures can differentiate between patient tissues of ovarian, peritoneal and tubal origin subtypes. Furthermore we demonstrate that specific single glycan structures can localise tissue types in formalin fixed paraffin embedded tissue sections of ovarian cancer samples.

1. Everest-Dass AV, Kolarich D, Campbell MP, Packer NH (2013) Tandem mass spectra of glycan substructures enables the multistage mass spectrometric identification of determinants on oligosaccharides. *Rapid Commun Mass Spectrom*. 27:931-9
2. Gustafsson OJ, Briggs MT, Condina MR, Winderbaum LJ, Pelzing M, McColl SR, Everest-Dass AV, Packer NH, Hoffmann P. (2015) MALDI imaging mass spectrometry of N-linked glycans on formalin-fixed paraffin-embedded murine kidney. *Anal Bioanal Chem*. 407:2127-39.

021

MALDI mass spectrometry imaging on formalin-fixed paraffin-embedded tissue: A spoonful of sugar of the *N*-glycome

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In humans, it is estimated that 50-60% of proteins are glycosylated. *N*-linked glycans are of particular interest in diseases such as ovarian cancer and osteoarthritis because structural alterations have been observed. Further investigation of these alterations may unearth novel biomarkers for early stage diagnosis. A novel method for investigating tissue-specific *N*-linked glycans was recently developed by our group on formalin-fixed paraffin-embedded (FFPE) murine kidney. Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) spatially profiles *N*-glycans in tissue-specific regions, while through liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) the corresponding glycol compositions are structurally characterized.

Regions of interest such as tumour, stroma, adipose and necrotic tissue were isolated from FFPE serous ovarian cancer tissue sections while cartilage and bone marrow tissue were isolated from FFPE osteoarthritic bone tissue sections. *N*- and *O*-linked glycans were structurally characterized through enzymatic peptide-*N*-glycosidase F (PNGase F) release of *N*-glycans, followed by β -elimination of *O*-glycans. The released glycans were analyzed through porous graphitized carbon liquid chromatography (PGC-LC) and collision induced negative mode fragmentation analysis. Following structural characterization, high resolution MALDI-IMS revealed the spatial distribution of these identified *N*-glycans and their tissue-specificity on consecutive FFPE tissue sections. For example, tumour and non-tumour tissue regions established clear demarcation based on their *N*-glycan distribution.

MALDI-IMS and LC-ESI-MS/MS were used as complementary techniques to generate high resolution images and structural information of tissue-specific *N*-glycans. Furthermore, *O*-glycoforms were characterized for the first time in FFPE ovarian tumour tissue sections. Application of this method enabled the first steps in the glycomic quest for the Holy Grail: an early stage diagnosis biomarker.

022

Probing human white blood cells for a novel type of protein *N*-glycosylation

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Mammalian asparagine (*N*)-glycosylation covers three well-established classes of high mannose, hybrid and complex type glycans expressed on membrane or secreted glycoproteins. We have recently proposed paucimannosylation (Fuc₀₋₁Man₁₋₃GlcNAc₂) as an unconventional *N*-glycan class in human neutrophils [1,2]. Despite their suggested immuno-functions, the presence of these truncated *N*-glycoproteins in other white blood cells has received little attention. We here explore the expression of paucimannosylation across multiple white blood cell types involved in the innate and adaptive immune response. Utilising PGC-LC-MS/MS, the *N*-glycomes of neutrophils, lymphocytes, monocytes and platelets isolated from whole blood of a healthy individual were profiled. Existing glycome data of white blood cells from multiple donors deposited in the Consortium for Functional Glycomics repository were also reinterpreted for the existence of these novel structures. In both approaches, paucimannosidic *N*-glycans were found to be highly enriched in the granulocytes i.e. eosinophils and neutrophils relative to the other immune cells in the myeloid and lymphoid lineages. Paucimannosidic glycoproteins were confirmed in isolated neutrophils with immunofluorescence using paucimannose-recognising antibodies. Co-localisation of paucimannosidic epitopes with β -hexosaminidase A and myeloperoxidase yielded clues to their subcellular localisation in the azurophilic granules of the neutrophil. Collectively, this suggests that protein paucimannosylation is a granulocyte-enriched glycosylation feature arising from cell-

specific mechanisms during granulopoiesis. This map of protein paucimannosylation in white blood cells is important to understand the immunological role(s) of this novel class of human glycoproteins.

1. Thaysen-Andersen, M., Venkatakrishnan, V., Loke, I., Laurini, C., Diestel, S., Parker, B.L., Packer, N.H., 2015. Human Neutrophils Secrete Bioactive Paucimannosidic Proteins from Azurophilic Granules into Pathogen-Infected Sputum. *Journal of Biological Chemistry* 290 (14), 8789-8802.
2. Loke, I., Packer, N.H., Thaysen-Andersen, M., 2015. Complementary LC-MS/MS-Based N-Glycan, N-Glycopeptide, and Intact N-Glycoprotein Profiling Reveals Unconventional Asn71-Glycosylation of Human Neutrophil Cathepsin G. *Biomolecules* 5 (3), 1832-1854.

023

MSCypher: A High-Throughput Peptide Identification Strategy for Complex Mixtures

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Shotgun proteomics based on high-resolution mass spectrometry (MS) is transforming biological research in the post-genomic era and offers an unbiased analytical profiling method that complements existing targeted MS and antibody-based approaches. However, using current technology only about 15-30% of the >100,000 observable individual peptide isotopic patterns¹ are ever identified. During traditional data-dependant MS acquisition (DDA) of complex samples, nearly all MS/MS spectra generated contain contaminating fragment ions that arise from the isolation and fragmentation of multiple co-eluting peptides. Identification of these chimeric spectra is limited by the 'one MS/MS spectrum—one peptide' strategy used in nearly all available MS/MS search algorithms.

Here we report MSCypher, a new proteomics workflow that combines an improved tandem MS acquisition strategy with a novel search algorithm. Our initial benchmarking of MSCypher shows significant improvements in speed and sensitivity of peptide detection and identification. This high-resolution MS approach takes advantage of co-eluting peptides and uses a dynamic isolation window to evenly distribute the number of co-fragmented species during acquisition. Processing these multiplexed spectra datasets has required a redesign of the current analysis workflow to include retention time prediction, dynamic probabilistic fragment ion matching and a supervised ensemble learning method for peptide feature identification.

Our initial results indicate that this multiplexed MS/MS approach provides both enhanced sensitivity and a gain in coverage. Each of these improvements was proportional to the number of peptides simultaneously analysed. We also maintain high confidence levels for peptides identified and our latest experiments indicate that identification of modified peptides will also be improved using this strategy.

1. Michalski, A., Cox, J., & Mann, M. (2011). More than 100,000 detectable peptide species elute in single shotgun proteomics runs but the majority is inaccessible to data-dependent LC- MS/MS. *Journal of proteome research*, 10(4), 1785-1793

024

Characterization of the protein complex landscape of murine tissues

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The recent improvements in proteomics technologies have enabled the distribution and relative abundances of proteins to be characterized across tissues in unprecedented detail. These studies have shown that even tissues from unrelated developmental lineages are composed of extremely similar protein repertoires, suggesting that additional layers of co-ordination are responsible for tissue functionality. As the ability of proteins to form higher order associations, also known as protein complexes, allows for dramatic changes in functionality by the exchange of subunits global differences within tissues interactomes may account for tissue functionality. As traditional high-throughput protein-protein interaction (PPI) approaches are untenable for tissues, we have applied an alternative means to explore this hypothesis by use of size exclusion chromatography protein correlation profiling SILAC (SEC-PCP-SILAC). Using a super-SILAC based approach, we profiled the interactome landscape of seven tissues (Brain, Lung, Liver, Heart, Skeletal Muscle, Thymus and Kidney), and we generated quantitative profiles of protein distributions across both SEC fractions and between tissues. Protein chromatograms across the SEC gradients were assessed based on correlation and co-enrichment, to determine PPIs using the principle of guilt by association. Using our PCP-SILAC approach, 7054 protein groups were identified across the seven tissues, with 5231 enabling the mapping of protein profiles. From these profiles, 36554 protein interactions could be determined with a precision of ~68% based on comparison to the CORUM database., Over 80% of proteins were observed in all tissues, indicating that at the protein level these tissues were extremely similar, however of note only ~30% of interactions were shared between tissues. Between functionally related tissues, this overlap increased to >60% consistent with the interactome shaping function. In total, 3304 protein complexes were mapped across tissues with the majority showing high similarity in membership (>0.75), however tissue specific exchanges of subunits were common. Taken together, we observed that complex heterogeneity between tissues is extremely common, and may shapes the functionality of tissues.

025

Extending the Reach of Data Independent Acquisition – Microflow SWATH® Acquisition

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Data independent acquisition (DIA) strategies have been used to increase the comprehensiveness of data collection while maintaining high quantitative reproducibility. In DIA, larger fixed-width Q1 windows are stepped across the mass range in an LC timescale, transmitting populations of peptides for fragmentation, and high resolution MS and MS/MS spectra are acquired. Previous work has shown that using more narrow variable width Q1 windows can improve peptide detection and increase sample coverage. Many labs are now using DIA to perform larger scale quantitative proteomic experiments with solid reproducibility on 1000s of proteins in complex matrices. As this technique increasingly proves to be a solid tool for biomarker research, larger sample sets are being analyzed, driving the need for further investigation of workflow improvements for throughput and robustness.

Here microflow LC was investigated in combination with SWATH® acquisition on a number of complex matrices, to assess depth of coverage and robustness relative to current nanoflow strategies. The MS analysis was performed on a TripleTOF® 6600 System (SCIEX) equipped with an nanoLC™ 425 system (SCIEX) with microflow modules. A number of acquisition parameters were explored to understand the workflow options in this flow regime. Key workflow recommendations have been established to provide researchers additional strategies for performing large scale, higher throughput SWATH acquisition studies.

026

IS-PRM: A Smarter MS Method for Reproducible and Sensitive Pathway Characterization

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Publish consent withheld

027

MALDI mass spectrometric imaging in an Alzheimer disease mouse model

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Mass Spectrometry Imaging (MSI) is an emerging technology for biomarker discovery and for enabling comprehensive molecular imaging in complex biological matrices. It is a suitable technique for the identification of compounds without an *a priori* knowledge of the composition of the sample. Since the location of compounds on tissue sections is preserved, MSI allows the identification of local abundant species in cases where spatial distribution would be lost if the whole tissue is homogenised.

In our study, we aimed to visualize the molecular distribution of molecular entities in various brain regions during the progression of Alzheimer's disease using a well-known mouse model. This neurodegenerative disease was investigated by comparing the CRND8 mouse model to the wild type. A pilot study of nine mice was carried out. We performed in-situ digests on tissue sections which were subsequently analysed by MALDI TOF-TOF. High resolution images were acquired on various regions of interest including the hippocampus, cortex and cerebellum.

We examined molecular changes and mapped targeted entities according to their location. These data were investigated in parallel with relative quantification experiments performed using complementary techniques i.e. LC-MS.

Statistical analyses were performed on the mapped species and correlated to the brain regions. Spectra from the regions of interest, such as cerebellum, were investigated.

Our aim is to highlight specific candidates that may be linked to Alzheimer's disease.

029

A Trans-omics Approach to Identify Novel Regulators of Hepatic Metabolism

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Background: The liver controls numerous pathways central to the maintenance of whole body lipid and glucose metabolism. Accordingly, disruption of these pathways promotes diseases including hepatosteatosis, insulin resistance and cardiovascular disease. However, even though these ailments are amongst the leading causes of death in developed countries, their mechanistic underpinnings are still not well defined.

Aims & Approach: In this study we sought to use a trans-omics approach utilising genetics, metabolomics, phenomics, lipidomics and proteomics to identify novel pathways involved in regulating hepatic metabolism. To do this we took advantage of our exclusive access to a panel of >100 genetically diverse inbred mouse strains, which to our knowledge is the largest of its kind in the world, known as the hybrid mouse diversity panel (HMDDP) at UCLA.

Methods: We collected livers (n=3) from male mice of 107 HMDDP strains that were all housed, fed and sacrificed under the same conditions. We performed deep proteomic analysis on livers (~320) by performing 34 separate TMT-10plex multidimensional LC-MS/MS experiments with SPS-MS3 acquisition on an Orbitrap Fusion. On the same samples we performed quantitative lipidomics analysis using LC-MS/MS on an AB Sciex API4000 Q/TRAP and Analyst 1.5 data system.

Results: Proteomic analysis resulted in quantification of >5,000 proteins with excellent reproducibility within strains and significant variance in ~2500 proteins between the 107 strains. Lipidomics analysis resulted in quantification of 312 lipid species across 23 lipid classes in which significant variation was observed in >100 lipid species. Bioinformatics analysis has identified numerous protein-lipid associations. Once combined with pre-existing phenomics and metabolomics data from the HMDP, we will begin dissecting the interacting networks and their influence on disease phenotypes.

Conclusions: We have established a high-resolution trans-omics network for the identification of novel regulators of hepatic metabolism. When considering both the depth in quantitative analyses and the unique sample sets utilized, we believe this to be the most comprehensive of its kind in Australia, if not internationally.

030

Proteomic characterisation of Clostridium fermentation

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Clastridia offers numerous advantages for the production of sustainable fuels and chemicals without compromising food security or being dependent the availability of arable land. Advantages over the use of conventional (sugar, oil or algae) feedstocks also include minimal water and nutrient demand and the capability to capture greenhouse gases that would otherwise be emitted into the atmosphere. Practically all carbon-containing feedstocks, including industrial off-gases and gasified agricultural and municipal waste, can be readily utilised or converted to syngas for subsequent transformation into fuels and chemicals.

Acetogens such as *Clostridium ljungdahlii* or *Clostridium autoethanogenum* use the reductive acetyl-CoA (Wood-Ljungdahl) pathway as a terminal electron-accepting, energy-conserving, CO₂-fixing process. This pathway is speculated to be the first biochemical pathway in existence on Earth and continues to play a key role in the global acetate cycle with annual acetogenesis in sediments and termite hindguts estimated to amount to several trillion kg of acetate. Energy metabolism in acetogens is complex and many aspects are only partly understood.

While all acetogens use the Wood-Ljungdahl pathway to fix CO₂, they vary in terms of redox coupling (cytochromes, sodium translocating Rnf or proton translocating Rnf) and co-factor use in the bifurcating hydrogenase (NADPH or NADH). Understanding the complex energy metabolism is critical, since ATP availability is a fundamental limitation in engineering acetogen metabolism. *C. autoethanogenum* offers a robust engineering system and a flexible platform for syngas fermentation. Fermentation of *C. autoethanogenum* has high product selectivity, tolerates a broad range of gas compositions and accepts contaminants well, making it the preferred microorganism for industrial gas fermentation.

Using *C. autoethanogenum* as the system for this study, we used a multi-omics approach in order to gain a measure of enzyme capacity (transcriptomics and proteomics) and thermodynamic driving force (metabolomics). By comparing gene expression and transcription when cells are grown autotrophically and heterotrophically (CO, CO₂ and H₂ vs fructose fermentation) we found that, compared to *C. ljungdahlii* in *C. autoethanogenum* the Rnf complex is extremely efficient and is able to maintain high ATPase activity at the transcriptional (RNA-seq) and translational (iTRAQ) levels even when cells are fermented exclusively using gas. Furthermore, our RNA-sequencing data show that under autotrophic conditions, genes in the Rnf complex are highly transcribed, resulting in equi-molar quantities of ATP (metabolomics data). This high efficiency of the electron chain transfer, coupled to the recently discovered electron bifurcating hydrogenases is potentially what makes *C. autoethanogenum* so unique and a great industrial platform for the conversion of syngas into valuable fuels and chemicals.

031

Membrane Proteomics: Surface Markers & Horizontal Signaling

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Solid tumors are complex tissues composed of different cell types (i.e. cancer cells, fibroblasts, immune cells, endothelial cells, etc.) and noncellular components such as the extracellular matrix, soluble factors, pH, oxygen tension and interstitial pressure. While decades of research have identified key oncogenic signaling pathways and more recently through next-gen sequencing (epi)genetic alterations, current therapeutic strategies are still inefficient in curing most cancers. It has become evident that the highly complex crosstalk within the tumor microenvironment actively modulates tumor growth, metastasis and response to therapy. This intracellular crosstalk is mediated by cell-surface receptors, soluble ligands and extracellular vesicles. In-depth studies of membrane proteins have proven to be difficult because of their low abundance and hydrophobicity. However, recent advances in proteomic technologies make it possible to investigate proteins in this cellular compartment, to an impressive depth. Here, I will present our recent work using in-depth proteomics to obtain novel insights into head and neck cancer. Cell-surface labeling strategies, such as silica-bead coating, have enabled us to interrogate the head and neck cancer surfaceome to an unprecedented depth. Integration of these data with publicly available genomics data enabled us to discover novel, previously unstudied surface markers that could serve as novel drugable targets. Functional data on some surface markers will be presented. Cancer-associated fibroblasts (CAFs) represent the most abundant cell type of the stroma and are key components involved in regulating carcinogenesis. Recently, CAF-secreted exosomes were shown to be important mediators of paracrine signals that promote motility, and metastasis in breast cancer. To investigate stromal heterogeneity in OSCCs we isolated matched pairs of human primary fibroblasts from resected tumors (CAFs) and adjacent tissue (AFs) and characterized them according to established CAF markers. Comprehensive proteomics identified a signature of CAF-enriched exosomal proteins potentially involved in pathways mediating tumor-stromal crosstalk. Functional experiments in the context of migration, metabolic coupling and radiation response are currently in progress.

032

Quantitative proteomics of lipoproteins: insights into individual differences of lipoprotein associated proteins in the context of cardiovascular disease.

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A disordered balance between plasma lipoproteins is a major risk factor for developing cardiovascular disease (CVD). High plasma levels of Lipoprotein(a) (Lp(a)) is another, independent risk factor for developing premature CVD. Various underlying disease mechanisms are not fully understood and details of Lp(a) physiology are still unclear.

We utilised different quantitative proteomics approaches to gain further insights into the biochemistry and pathophysiology of different lipoproteins with a main focus on Lp(a). Using absolute quantification (AQUA) of proteins by mass spectrometry we elucidated the stoichiometry of proteins per particle for the different lipoprotein classes. We then quantified the protein cargo of Lp(a) isolated from a cohort of 40 subjects using SWATH-MS in conjunction with AQUA.

The AQUA approach provided accurate numbers of lipoprotein-associated proteins per particle and showed significant individual differences in the apolipoprotein profiles of various particle classes such as strong variations in the numbers of apoC1, apoC2 and apoC3, which may indicate alterations in triglyceride metabolism. Proteins involved in immune response and complement activation which have been discussed in the context of lipoprotein-related CVD risk were only detected in very low numbers. Quantification of Lp(a)-associated proteins using AQUA and SWATH-MS identified a group of subjects with high levels of Lp(a)-associated histidine-rich glycoprotein (HRG). Those subjects also showed significantly higher levels of known HRG-interactors that are involved in coagulation and immune response. We hypothesise that Lp(a)-HRG interaction competes with HRG-plasminogen promoted regulation of fibrinolysis at sites of atherosclerotic plaques. However, in the low number of investigated subjects we could not detect any correlation of HRG-rich Lp(a) with disease associated factors such as high Lp(a) levels, high triglyceride levels or CVD events. More detailed information about the biochemistry of Lp(a)-HRG interactions is required and first insights into the influence of the apolipoprotein(a) isoforms on the HRG-interaction will be presented.

033

A Distinct Proteomic Signature of Latently Infected Cells Reveals New Drug Targets and Immunotherapeutic Targets for HIV

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Combination antiretroviral therapy (cART) has vastly improved the quality of life of those suffering from HIV. However, interruption of therapy leads to re-emergence of the virus from the latent cellular reservoirs that include macrophages, dendritic cells and particularly CD4+ T cells. J-Lat cells are a well-established CD4+ T cell line (derived from Jurkat cells) that harbour one integrated copy of the HIV provirus. These cells have been used to study both the factors that help maintain latency and to identify signals that can break latency and lead to viral reactivation. Previous studies have examined this at the transcript-level or attempted limited protein pull-down experiments. As a first step towards understanding the persistence of HIV and activation of latent provirus, analysis of the changes in host cell protein expression at the early stages of activation is crucial and may provide vital clues to develop new ways to control or eliminate viral reservoirs as well as defining the mechanisms that maintain HIV persistence. This study has provided a comprehensive quantitative protein expression map of latently infected J-Lat cells and follows changes in protein expression after viral reactivation of J-Lat clones by TNF-alpha. More than 9000 proteins and their modifications were identified and quantitated in latent and reactivated cells, making this study the largest of its kind. Unexpectedly, statistical analyses of the data revealed perturbation of several pathways in latently infected cells including proteins involved in cell signalling, energy generation and key transcription factors. This data highlights that latently infected cells are not "invisible" or indistinguishable from uninfected cells and that they have a discrete proteomic signature which has unveiled new drug targets and immunotherapeutic targets that could be used to eliminate HIV.

034

Data-independent mass spectrometry phenotyping of patient-derived melanoma cancer cell lines correlates with MEK inhibitor resistance independent of genotype and predicts poor patient outcome

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Melanoma accounts for only 5% of all skin cancer variants, but is the most common cause of skin cancer related deaths, and is responsible for killing more young (<40yrs) people than any other cancer. Despite initial success of drugs that target specific enzyme mutations, melanomas rapidly acquire drug resistance. To understand baseline protein processes in melanomas with different genetic mutational status, mitogen activated protein kinase (MAPK) pathway mutant melanomas were compared to MAPK wild type (wt) melanomas using data-independent mass spectrometry (SWATH-MS) profiling.

Ten patient-derived melanomas with known MAPK mutational status (3xBRAF^{mut}, 3xNRAS^{mut}, 3xMAPK^{wt}, 1xMEK^{mut}) were cultured. Tryptic digests were submitted to LC-MS/MS on a TripleTOF 5600 MS (SCIEX) utilizing a multiphasic chip-based LC approach (reverse phase (RP), strong cation exchange, RP), for ion library generation to extract protein information from rapid

1h 1D RP SWATH-MS acquisitions per cell line. Presto Blue Assay was performed to assess ten-day cell viability in presence of the MEK inhibitor (MEKi) AZD6244.

This approach enabled comprehensive protein detection identifying 3200 proteins (10 cell lines; FDR<1%). Data extraction from SWATH-MS datasets revealed 2500 proteins quantifiable among these cell lines. Principal component analysis demonstrated segregation of melanomas based on sensitivity to MAPK inhibition (2 μ M AZD6244), whereas genotype did not. In total, we show 57 proteins whose abundance correlate ($r^2>0.75$) with MEKi cell viability, revealing changes in cell pigmentation, lipid metabolism, and in adherence and inter-cell communication. Kaplan – Meier survival analysis using post-surgery patient survival data demonstrated untreated patients with MEKi sensitive melanoma cells showed significantly ($p=0.01$) lower mortality rates than patients with MEKi resistant tumours (mean survival >7.5 years MEKi sensitive versus 1.7 years MEKi resistant).

Therefore, data-independent MS phenotyping of MAPK pathway mutant and wt melanoma cell lines demonstrated explicitly segregation of MEKi sensitive and resistant cell lines and is associated with patient outcome.

035

Reprogramming biological functionalities for autonomous microbial factories and therapeutics

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Synthetic biology aims to engineer genetically modified biological systems that perform novel functions that do not exist in nature, with reusable, standard interchangeable biological parts. The use of these standard biological parts enables the exploitation of common engineering principles such as standardization, decoupling, and abstraction for synthetic biology. With this engineering framework in place, synthetic biology has the potential to make the construction of novel biological systems a predictable, reliable, systematic process. While the development of most synthetic biological systems remains largely ad hoc, recent efforts to implement an engineering framework in synthetic biology have provided long-awaited evidences that engineering principles can facilitate the construction of novel biological systems. Synthetic biology has so far demonstrated that its framework can be applied to a wide range of areas such as energy, environment, and health care. In this talk, our recent efforts to develop synthetic microbes with programmable behaviors will be presented. In particular, an emphasis will be placed on our recent development of auto-regulatory genetic circuits for microbial chemical production and therapeutic applications. <http://synbiolab.org/>

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Proteotype-based decoding of the viral signaling network orchestrating the assembly of poxvirus

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Poxviruses, the largest mammalian viruses, encode two dual-specificity enzymes, F10 kinase and H1 phosphatase. These enzymes are essential signaling mediators during poxvirus infection. Yet their precise functional role in reversible protein phosphorylation during the virus lifecycle remains enigmatic. Using a phosphoproteomic strategy in combination with F10 and H1 inducible viruses, we established the F10/H1 dependent viral signal network in HeLa infected cells by the prototypic poxvirus, vaccinia. In order to relate defined perturbations of this signaling network to a phenotypic outcome, we performed a detailed characterization of the progeny virions' proteotype. Our phosphoproteomic strategy allowed us to pinpoint relevant phosphorylation sites dependent on F10 and H1 which guided us towards focused functional analysis of two specific factors, the I7 protease required for proteolytic processing during virion maturation, and the viral early transcription factor, A7, showing that virion morphogenesis and the transcriptional competence of newly assembled virions are directly dependent on reversible phosphorylation of S134 in I7 and Y367 in A7. These results establish the molecular mode of action and a key role of F10/H1 in dynamic phospho-regulation during multiple stages of infectious poxvirus assembly.

037

Rapid identification of beer spoilage microorganisms using Biotyper

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Beer spoilage microorganisms present a major risk for the brewing industry and can lead to cost intensive recall of contaminated products, damage to brand reputation or in extreme cases, to the loss of business. Current microorganism detection procedures for bacterial and wild yeast contaminations involve cultivation-based enrichment and optical analyses, coupled with more recent molecular methods such as PCR, riboprinting, rRNA hybridization and antibody-based techniques. However, optical read-out methods are prone to misidentifications, whilst molecular methods like PCR are cost intensive. We investigated the applicability of molecular profiling using mass spectrometry in combination with the Biotyper software for the identification of beer spoilage microorganisms as a rapid and cost effective method for brewery quality control testing. Reference mass profiles for the five most common beer spoilage microorganisms (*Lactobacillus brevis*, *Lactobacillus lindneri*, *Pediococcus damnosus*, *Pectinatus frisingensis* and *Megasphaera cerevisiae*), four commercially-available brewing yeasts (top- and bottom-fermenting)

and *Brettanomyces bruxellensis* wild yeast were established and incorporated into the Biotyper reference library. The method was validated by inoculation of these microorganisms into beer samples and identification using Biotyper. All tested bacteria, bottom- and top-fermenting and the wild yeast strain could be accurately identified and distinguished from one another, as well as from the other approx. 5,600 microorganisms present in the Biotyper database. The applicability of mass spectrometry profiling for the brewing industry was assessed by testing routine quality control samples from a local brewery, where contaminating microorganisms could be reliably identified. This renders the Biotyper platform a promising candidate for routine biological quality control within the brewing industry, especially considering the time-to-result is faster and analysis cost per sample is smaller relative to other molecular methods such as PCR.

S. aureus strain functional diversity as observed through pan-proteomics: an emerging approach for proteome measurement within a background of genetic heterogeneity

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Background

Pan-proteome analysis is an emerging method that seeks to qualitatively and quantitatively compare the proteomes of genetically heterogeneous microorganisms. This approach has been reported in a single paper within the scientific literature;⁽¹⁾ however, there are major technical considerations that need to be addressed in order for this method to progress towards a valuable and accessible analytical technology.

Experimental Approach

Five unique strains of *Staphylococcus aureus*, four of which are unsequenced, were grown under standard conditions and harvested at mid-exponential and stationary points on the growth curve. Cells were processed and comprehensive proteome samples subjected to data-independent acquisition LC-MS/MS as well as data-dependant acquisition LC-MS/MS, following deep proteome fractionation.

Qualitative mass spectrometry data were searched against all *S. aureus* sequences (~125,000) found within the UniProt database (Sept, 2014) using the Paragon algorithm. The resulting data were used to generate a spectral library of empirical *S. aureus* peptide mass spectra; the library was used to extract peptide ion areas from within the data-independent mass spectra. Peak area data were exported and processed in *R* in order to compare the summed protein abundance information under a set of unified pan-protein identifiers, based on protein sequence homology. Pan-protein abundances were normalized then compared across all strains using permutation statistics. Global protein abundance ratios between strains were compared to matched RNAseq quantification data.

Results and discussion

Pan-proteomics offers new opportunities to measure and interpret microorganisms' biochemistry within the context of genetic diversity. In this example, the contribution of protein abundance to unsequenced *S. aureus* strain types were measured and interpreted to reveal potential metabolic and virulence advantages that may underpin pathogenic observation. With robust development, pan-proteomics may prove valuable in the areas of drug development and/or industrial strain selection and, furthermore, could be applied to the proteome measurement of genetically-diverse but related plants and invertebrates.

1. Zhang, L.; Xiao, D.; Pang, B.; Zhang, Q.; Zhou, H.; Zhang, L.; Zhang, J.; Kan, B., The core proteome and pan proteome of *Salmonella Paratyphi A* epidemic strains. *PLoS One* 2014, 9, (2), e89197.

The Advancement of Chemical Cross-Linking/Mass Spectrometry in Structural Proteomics

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During the last 15 years, chemical cross-linking combined with mass spectrometry (MS) and computational modeling has advanced from investigating 3D-structures of isolated proteins to deciphering protein interaction networks [1,2]. Chemical cross-linking relies on the introduction of a covalent bond between functional groups of amino acids within one protein, to gain insight into the conformation of a protein, or between interaction partners to elucidate interfaces in protein complexes. Based on the distance restraints derived from the chemical cross-links, three-dimensional structural models of proteins and protein complexes can be constructed. Most commonly, homobifunctional amine-reactive cross-linkers, such as *N*-hydroxysuccinimide esters, are used for studying protein-protein interactions. One of our goals is to extend the arsenal of existing cross-linkers to obtain complementary 3D-structural information of proteins and protein complexes. To facilitate the identification of cross-linked products, we have designed novel MS/MS cleavable cross-linkers creating characteristic marker ions upon fragmentation [3].

In my talk, I will describe an integrated workflow for the automated identification of cross-linked products based on an MS/MS cleavable cross-linker using the different fragmentation methods available on an Orbitrap Fusion mass spectrometer (CID, HCD, EThcD, and EThcD). For conducting fully automated analyses, we employ our *in-house* developed software tool MeroX [4]. A direct way to probe protein-protein interactions *in vivo* is by site-specific incorporation of genetically encoded photo-reactive amino acids or by non-directed incorporation of photo-reactive amino acids. I will illustrate the different cross-linking strategies based on two protein systems: The tetrameric tumor suppressor protein p53 [5] and the complex between the basement membrane proteins laminin and nidogen-1 [6].

Literature References:

[1] Sinz, A., *Expert Rev. Proteomics* **2014**, 11, 733.[2] Herzog *et al.*, *Science* **2012**,337, 1348. [3] Müller, M.Q. *et al.*, *Anal. Chem.* **2010**, 82, 6958. [4] Götze, M. *et al.*, *J. Am. Soc. Mass Spectrom.* **2015**, 26, 83. [5] Arlt *et al.*, *Proteomics* **2015**,14, 3996. [6] Lössl *et al.*, *PLoSOne* **2014**, 9:e112886.

040

Whole Dengue viral particle breathing dynamics unravels quaternary temperature-specific changes across capsid proteome

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Temperature is one of the most important perturbations for initiating the infectious phase of a virus as it is transmitted from a vector to host. Dengue serotype 2 shows a large expansion in viral capsid size at a temperature of 37 °C corresponding to the environment within the human host. Amide hydrogen/deuterium exchange mass spectrometry (HDXMS) is a powerful method to probe in parallel changes in dynamics across all constituent proteins in the viral capsid proteome at peptide resolution. Our results indicate that DENV2 viral particle shows high intrinsic and non-uniform dynamics across the C, E and M-proteins in the capsid and in alignment to the symmetry related contacts. These dynamic hotspots form the basis for where the largest changes are observed with temperature shift to 37 °C. These temperature-dependent changes in dynamics are largely irreversible and are specific to the viral particle. The breathing dynamics observed solely in whole viral assemblies highlight the importance of protein quaternary contacts, packing of lipid bilayer and RNA genome for the coordinated changes to temperature. The increased dynamics and temperature-dependent expansion measured by HDXMS expose hidden linear epitopes and modulate interactions with neutralizing antibodies. These results have enormous implications for targeted antibody discovery. HDXMS has also been applied to map antibody-whole viral complexes and allows a molecular understanding of antibody-dependent expansion.

041

Structural characterisation of biomolecular assemblies by ion mobility-mass spectrometry

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A precise network of interacting biomolecules, including proteins, DNA and lipids, allow for the function and control of all biological processes. Consequently, to understand these processes and offer potential for intervention, for example in the treatment of human disease, it is useful to have an understanding of the molecular components and their binding interactions. However, structural characterisation of such complex, heterogeneous and dynamic systems remains a challenge, in part due to analytical limitations of current structural biology approaches.

Ion-mobility mass-spectrometry (IM-MS) has emerged as a complementary tool for biomolecule structure determination, and in some cases a structural biology method in its own right. It is capable of defining identity, stoichiometry, size, structural arrangement and subunit interactions in a biomolecular assembly in a single experiment.

Here will be discussed recent advances in IM-MS methodology, and our application of native IM-MS and chemical cross-linking methods, in combination with complementary biophysical methods, to offer new insight into biological assemblies. We have applied these methods to assemblies of interest in human disease, for example; characterising the protein-protein and protein-lipid interactions mediating misfolding and toxicity in amyloid diseases such as Parkinson's and Alzheimer's Disease.

042

Characterizing human organelle proteomes - Towards a complete Cell Atlas

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Compartmentalization of biological processes is a fundamental principle of eukaryotic cells that gathers the relevant molecules and enables multiple processes to occur in parallel. Despite a great deal of research, basic questions about the spatial organization of many proteins and biological processes remain unanswered. The Cell Atlas aims to systematically localize the human proteome using an antibody-based approach as part of the Human Protein Atlas project.

Data generation include automated sample preparation, high-resolution confocal microscopy and computational image analysis. An integrative approach with strict validation criteria is used including gene silencing, paired antibodies and fluorescently tagged proteins. This allows identification of novel protein components of both known and unknown cellular structures.

In total, over 12000 human proteins have been localized to 25 organelles and cellular structures. As much as 50% of all proteins localize to multiple compartments and 20% show cell cycle dependent expression. The high spatial resolution allows pinpointing of protein localization to fine structures such as the cytokinetic bridge, microtubule ends, nuclear bodies, and rods and rings.

Here we discuss the importance of spatial proteomics for cell biology and present the content of the Cell Atlas as well as the path ahead to define the human organelle proteomes.

This research was supported by grants from Knut and Alice Wallenbergs foundation.

043

Measurement and Computational Modelling of ERK-MAPK Signalling *in situ*

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The skin is largely comprised of keratinocytes within the interfollicular epidermis. Over approximately two weeks these cells differentiate and traverse the thickness of the skin. The stage of differentiation is therefore reflected in the positions of cells within the tissue, providing a convenient axis along which to study the signaling events that occur *in situ* during keratinocyte terminal differentiation and over this extended two-week timescale.

We used this system to study the canonical ERK-MAPK signaling cascade (Raf-1, MEK-1/2 and ERK-1/2) which has been implicated in controlling diverse cellular behaviors, including proliferation and differentiation. While the molecular interactions involved in signal transduction through this cascade have been well characterized in cell culture experiments, our understanding of how this sequence of events unfolds to determine cell fate within a homeostatic tissue environment has not been fully characterized.

Thus, we measured the abundance of total and phosphorylated ERK-MAPK signaling proteins within interfollicular keratinocytes in transverse cross-sections of human epidermis using immunofluorescence microscopy. These data show coordinated variation in the abundance of phosphorylated ERK-MAPK components across the epidermis. To investigate these data further, we developed a mathematical model of the signaling cascade using a normalized-Hill differential equation formalism.

The model demonstrates that the spatial profile of activation for ERK-MAPK signaling components across the epidermis may be maintained in a cell-autonomous fashion by an underlying spatial gradient in calcium signaling. The approaches developed for this work may be useful for studying intracellular signaling within other tissue systems.

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Proteome survey of wound fluid from non-healing wounds reveals key biological processes associated with poor healing outcomes

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Non-healing wounds are a significant problem for patients and healthcare systems worldwide. The underlying biochemistry, which drives non-healing outcomes in self-perpetuating leg wounds, is poorly understood. To address this knowledge deficit, a study of the proteins that compose the fluid, which exudates from these wounds, may provide important insight regarding treatment response and healing outcome for patients. In this respect, we have conducted a clinical study that included the collection of biological samples and clinical / psychosocial data over a 24 week period, during which time patients received best-practice care. Biological samples were analysed using mass spectrometry-based proteome measurements to detect and quantify the protein complement of the wound fluid. The resulting data were integrated with clinical measurements and contextualized by gene ontology annotations to enable deeper insight into the dynamic biological processes taking place within non-healing wounds. This identified key biological processes that may indicate specific underlying issues for a sub-set of wounds and their recalcitrant nature towards clinical care. A number of biological markers that are indicative of the wound healing outcome were also derived from these analyses. Unravelling the complex biology of non-healing wounds through proteome and clinical data integration provides some insight into the mechanisms associated with a patient's adverse or positive responses to clinical care. Such information can be developed further to inform clinical practices and enable the meaningful personalisation of wound management.

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Identification of the Fragmentation Features of Dityrosine cross-linked Amyloid beta in ESI-MS/MS

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The use of mass spectrometry coupled with chemical cross-linking of proteins has become one of the most useful tools for proteins structure and interactions studies. One of the challenges in these studies is the identification of the cross-linked peptides. While lysine and cysteine cross-linked peptides have been investigated, identification of fragmentation patterns of dityrosine cross-linked peptides is yet to be understood. Oxidative stress is known to play a key role in the neurodegenerative disease like Alzheimer's disease (AD) and dityrosine in the amyloid plaques in AD brain is a potential biomarker of oxidative stress. Dityrosine cross-linked amyloid beta has been investigated *in vitro* to be toxic but whether its presence in the AD brain has any correlation with the disease progression and neurodegeneration is not well established. A quantitative analysis of covalently cross-linked dityrosine amyloid beta in AD brain using mass spectrometry requires a thorough understanding of the fragmentation pattern of these peptides. In this study we have investigated and characterized the fragmentation pattern of the LysC cleaved synthetically prepared amyloid beta(1-42) using ESI-MS/MS. In this study we report a detailed fragmentation study of the dityrosine containing peptide A β (1-16) generated by various techniques such as CID, HCD, ETD and ECD. The fragmentation features observed here can be helpful in the interpretation and identification of cross-linked dityrosine peptides present in nature and can be further implemented in search engine's algorithms.

A bioinformatic solution for identifying non-genomic peptides in the immunopeptidome

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Human leukocyte antigen (HLA) molecules are cell-surface glycoproteins that present peptides for surveillance by T lymphocytes seeking signs of disease. It has been shown that the sequences of some class I HLA-bound peptides are not found in the genome, such as peptides arising from post-translational peptide splicing in the proteasome (Vigneron and Van den Eynde, 2012). Our group identifies large numbers of HLA-bound peptides by mass spectrometric analysis of purified immunopeptidome (i.e. HLA peptide) samples. However, non-genomic peptides such as spliced peptides are not susceptible to identification by conventional analysis software such as Mascot, since these search methods rely on matching tandem mass spectra to sequences in genome-based databases. This means that peptide sequences which are not present in the databases will be ignored or falsely identified. Here, we introduce software to address this problem by enabling searches to consider non-genomic sequences. Our program generates comprehensive 'artificial databases' which include all of the possible permutations of amino acids for peptides of a given length, then searches spectra using Mascot-based scoring. Our preliminary results, for which we analysed complex immunopeptidome samples by LC-MS/MS and searched for peptides of 8-11 amino acids in length, shows that the program can identify many conventional sequences in agreement with Mascot, ProteinPilot and PEAKS DB searches. Despite statistical challenges necessitating more manual inspection than desired, it has also revealed a number of novel, non-genomic sequences for further investigation by MRM. This technique has potential to aid not only studies of the immunopeptidome but also analyses of other peptide samples for which genomic protein databases are incomplete or inapplicable.

Vigneron, N., Van den Eynde, B.J. (2012). Proteasome subtypes and the processing of tumor antigens: increasing antigenic diversity. *Curr. Opin. Immunol.* 24, 84-91.

Multi-omic analysis of Epithelial-Mesenchymal Transition in pancreatic cancer

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Epithelial to mesenchymal transition (EMT) is an evolutionarily conserved, complex biological process that plays a central role in development, tissue repair, formation of stem cells and cancer progression and metastasis. Accordingly, it has been hailed as an important but yet-to-be-realised opportunity in cancer therapy. EMT has been recently shown to promote formation of pancreatic tumours, for which there is ~5% survival rate (Rim et al, *Cell* 2012). Recognizing the underlying scale and complexity, we hypothesized that EMT can be better characterized using an integrated systems biology approach and undertook a multi-omic study in PANC-1 human pancreatic carcinoma cells. EMT was induced using transforming growth factor beta in a time dependent manner (0-72hrs). Multiple high-throughput technologies were used to profile the epigenome, transcriptome, proteome and phosphoproteome from PANC-1 cells in epithelial and mesenchymal states.

In parallel, we developed an informatics framework called as PGTools for the comprehensive analysis of multi-omic data. The software consists of a suite of programs for efficient analysis and management of high-throughput 'omics' datasets. Using our automated software, proteomic data procured from any mass-spectrometer can be directly compared with genomic and transcriptomic data generated by next-generation sequencing. PGTools provides the informatics framework to study complex biological processes, offers new directions for EMT research and with the potential for therapeutic interventions and improves our understanding of cell state, function, shape and polarity.

DNA methylation arrays (21,000), RNA-sequencing (21,612), SILAC-based mass spectrometry (4,602) and phospho-proteomics (3,818) in epithelial and mesenchymal states were simultaneously measured from the same cell population. Integrative systems analyses recapitulated known EMT signatures as well as identified candidate genes, pathways and regulatory networks underpinning EMT.

We have uncovered previously unknown molecules and molecular events associated with EMT, suggesting that EMT is far more complex than currently perceived.

Proteomics-based approach to elucidate the mechanism of anti-hypercholesterolemia activities of selected herbal medicines

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Introduction:

Hypercholesterolemia is a high incidence disease in developed and under developing societies. Natural products are becoming more popular for treatment of hyperlipidemia as alternatives or complementary medicines [1]. But the mechanisms of action of most of natural products are not clear yet. Recent studies shows that systems biology approaches and Omics techniques could better decipher mechanism of action of natural products. In this study we aimed to find mechanism of action of herbal remedies which used commonly for hyperlipidemia by using quantitative MS-based proteomics.

Materials

Hydroalcoholic extracts from 10 medicinal plant species were prepared and dried. The extracts were dissolved in DMSO and added to HeLa and Huh7 cells (2ug/ml). The cells were harvested 48 hours after treatment and proteins digested into peptides. The samples were measured by selective reaction monitoring (SRM) [2] and 45 proteins involved in control of lipid metabolism were quantified. Analysis was performed with Skyline and MSstats and from three independent biological replicates.

Results:

Our results show 7 significant differential expression proteins by *Glycyrrhiza glabra*, 6 by *Panax ginseng*, 6 by *Achillea wilhelmsii*, 5 by *Apium graveolens*, 5 by *Citrus aurantium*, 4 by *Trigonella foenum-graecum*, 4 by *Allium sativum*, 4 by *Zataria multiflora*, 2 by *Silybum marianum* and one by *Cynara scolymus*.

Conclusion:

Herbal extracts are composed form multiple compounds and it is believed multi-ingredient drugs can interfere by multiple targeting in cells at same time. This study shows that each selected natural compounds can change the concentration of several proteins and control cholesterol metabolism by controlling of different pathways. By using proteomics techniques we will have a better holistic view on different mechanisms that are responsible for herbal remedies pharmacological activities.

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A novel MRM_HR based glycosyltransferase assay

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Protein glycosylation is synthesized through concerted actions of glycosylation enzymes in the secretory pathway. Evidences have shown that functions of these enzymes are not only regulated at gene expression level but also post-transcriptionally at protein level. We have previously developed MRM_HR assays to quantitate differential expressions of endogenous glycosyltransferases. However, robust enzyme activity assays to detect endogenous glycosyltransferase activities is lacking in our proposed systems biology approach to study glycan biosynthesis.

Lectins are glycan binding proteins that could bind and distinguish structural isomers. We previously developed MRM_HR assays to analyze and quantitate degree of sialylation of glycopeptides from glycoproteins. These properties suggest lectin and MRM_HR analysis are useful tools to detect change of glycan structures before and after enzyme reaction.

In this study, we developed a novel glycosyltransferase assay that combines lectin detection and MRM_HR analysis. We demonstrated our method by detecting α 2-6 sialyltransferase activity. We tested binding specificities of three sialic acid binding lectins, SNA, MALI and MALII using glycosidase modified fetuin and confirmed SNA binds specifically to α 2-6 sialic acid. De-sialylated fetuin was immobilized on 96-well plate as substrate for sialyltransferase reaction. Probing by SNA showed only reaction with recombinant ST6Gal1 gave positive signal suggesting addition of sialic acid in α 2-6 linkage on substrate. Trypsin digestion was then conducted in the same wells and peptide mixtures were analyzed by MRM_HR assays. The results confirmed the addition of sialic acid on fetuin and degree of sialylation could be calculated accordingly.

Our results showed this method is superior in that it is robust and compatible with cell lysate so that it could be used for high throughput screening. MRM_HR analysis provided quantitative results and confirmed the signal from SNA detection is truly from immobilized substrate. Thus, the method could be configured to fit different analysis requirements.

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Effect of carbon source on the glycosylation pathway of *Trichoderma reesei* RUT-C30

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Trichoderma reesei has been of industrial interest for several decades for the production of cellulases, resulting in the development of hypercellulolytic strains such as RUT-C30. High protein production and secretion, and eukaryotic glycosylation machinery, make *T. reesei* RUT-C30 a suitable expression host for recombinant proteins. The glycosylation of secreted proteins of RUT-C30 is known to vary depending on culture nutrients and this variability in glycosylation affects stability and activity of CBHI, the main secreted protein. However, the impact of media on the intracellular precursors of glycosylation and on the secreted protein glycosylation has received little attention, and techniques for their analysis in fungi are lacking.

This study investigated the effect of carbon source on nucleotide sugars of RUT-C30, important precursors of glycosylation. Methods were developed to extract and analyse these metabolites from mycelia grown on glucose or lactose. In parallel, the glycosylation profile of secreted protein was monitored. Differences were found in the abundance of nucleotide sugars depending on carbon source, not directly reflected in secreted protein glycosylation. In addition, previously unidentified O-glycan structures on the total secreted protein were identified. The work provides insight into the effect of carbon sources on the glycosylation and protein secretion pathways in *Trichoderma reesei* RUT-C30.

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Quantitative proteomics reveals novel diagnostic and prognostic markers of acute rheumatic fever.

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Acute rheumatic fever (ARF), a condition which precedes the development of rheumatic heart disease (RHD), causes 275,000 deaths per year worldwide and has a global prevalence of 3.9 million. In Australia, ARF and RHD incidence remains high in Indigenous communities, where Aboriginal people are up to eight times more likely than other Australians to be hospitalised, and nearly 20 times more likely to die from RHD.

ARF is an autoimmune response that follows infection with group A streptococcus (GAS). RHD develops after repeated episodes of GAS infection and causes progressive heart valve damage. Accurate and timely diagnosis of an initial ARF episode and prophylaxis with antibiotics are the only current approaches for preventing RHD. At present, the diagnosis of ARF is made using the Jones criteria, which relies mainly on clinical features and has changed little in over 50 years. Misdiagnosis of ARF is common and a major contributor to the high rates of RHD seen in Aboriginal communities. Despite a clear need, there is no definitive method for diagnosing ARF.

In this study, we developed a novel peptide-based classification strategy using Random-forest based machine-learning algorithms in the R statistical computing environment. Data was generated using a label-free MS-based proteomics approach using Bruker's new Impact II UHR-QTOF and MaxQuant software. To detect novel diagnostic and prognostic markers of ARF and RHD, we compared affected individuals with healthy individuals and those with alternative diagnoses. A total of 53 plasma samples from patients in the Northern Territory were collected including those with ARF (21), RHD (8), healthy controls (7) and other diagnoses (17). In order to improve the dynamic range of low-abundance proteins, samples were immunodepleted with Agilent's Multi-Affinity Removal System (MARS-14) LC column. Utilising this approach we have identified statistically-relevant peptide signatures that distinguish patients with ARF from all other patient groups.

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Proteogenomic profiling of acute myeloid leukaemia reveals novel insights into leukaemogenesis.

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The Mixed Lineage Leukaemia (MLL) gene located on chromosome 11q23 is a frequent site of translocation that results in a number of oncogenic fusion proteins. MLL-translocations are present in a variety of paediatric and adult leukaemias and are associated with poor survival outcome. Despite intense research efforts the mechanisms underlying the leukaemogenic activity of MLL-translocations are poorly understood. Our aim was to identify proteins involved in leukaemogenesis that are regulated by MLL fusion protein expression through quantitative proteome analysis using a murine leukaemia model. Our model of acute myeloid leukaemia (AML) involves the generation of leukaemic cells *in vivo* and is controlled by the regulated expression of a common MLL-translocation gene, MLL-AF9. Leukaemic cells were collected from mice before analysis of global protein expression using a SILAC quantitative proteomics workflow to generate a profile of proteins that are expressed in the presence or absence of MLL-AF9 expression. We extended this analysis by also profiling changes to the transcriptome using RNA-sequencing during MLL-AF9 regulation. These data underwent bioinformatic analysis to quantify and compare changes in protein and RNA expression before further comparison to human AML patient expression array and survival data to identify proteins and pathways of diagnostic and therapeutic interest. Our analysis has confirmed several known targets in AML and also highlighted novel pathways and targets that are regulated when MLL-AF9 is expressed. These pathways include epigenetic regulators, cell signaling and cell-cell or cell-extracellular matrix interactions. The results from this study not only identify mechanisms by which MLL-AF9 regulates leukaemogenesis, but also demonstrate the potential identification of clinical biomarkers and novel drug targets.

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Differential abundance, but not kinetics, of virus-derived epitopes presented on infected fibroblasts and dendritic cells

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The presentation of peptide epitopes by major histocompatibility complex (MHC) class I molecules is a critical process governing anti-viral CD8⁺ T cell-mediated immunity. Here we have used quantitative mass spectrometry to profile the presentation of 46 vaccinia virus-derived MHC-bound epitopes during infection of dendritic cells and fibroblasts, including a comparison between immortalized cell lines and *ex vivo* primary cells. Our data show that, in general, dendritic cells outperform in terms of absolute presentation capacity, with *ex vivo* fibroblasts the poorest presenters. However, several epitopes remained refractory to this fibroblast deficiency, highlighting the need for such wide-scale analyses. Conversely, despite such differential presentation across

cell lines, the kinetic profile of each epitope remained markedly unchanged – subsequent analysis of vaccinia proteome kinetics strongly supports the notion that nascent protein synthesis drives MHC-peptide display, and that the similarities in infection kinetics across cell types is therefore providing a comparable rate of peptide supply.

In order to try to relate this dataset to the immunogenicity of each peptide, we measured in parallel the CD8⁺ T cell response from infected mice, with the panel of peptides eliciting a robust immunodominance hierarchy. However, despite thorough analysis, the data so far show a complete disconnect between abundance and kinetics and the ensuing T cell response. These results highlight the complexities of epitope presentation during virus infection and the multifaceted processes that drive immunogenicity.

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A new method of validation using the protein quantitation false discovery rate from control/control analysis

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Label-free protein quantitation by spectral counting has proved to be a useful method for accurate protein profiling. However, much work still remains to be done in applying useful and valid statistical methods for validation of shotgun results. Due to the large number of protein identifications involved, any t-test will invariably yield false positives; this is known as the multiple testing problem. My work focuses on the development of a novel form of statistical validation based on control-control experiments, where the statistical parameters for empirically minimising false discovery rates are determined from triplicate analyses of biologically identical material. An appropriate statistical measure (q value) is then carried forward into the analysis of control vs different samples. Through this additional statistical step, the resulting output comprises highly confident protein identifications with a minimised protein quantitation false discovery rate (PQ-FDR), which represents a work-around to solve the multiple testing problem.

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Investigation of SOCS5 signalling complexes by mass spectrometry uncovers a role in breast cancer

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The Suppressor Of Cytokine Signalling (SOCS) family of proteins are critical negative regulators of cytokine and growth factor signalling. Whilst the roles of some SOCS family members have been clearly elucidated, the function of SOCS5 remains poorly characterised. SOCS5 has been postulated to act as a tumour suppressor in both EGFR and JAK/STAT-driven malignancies through expression analyses and functional studies of the *Drosophila* homologue Socs36E. However its precise physiological role and how it acts on these distinct pathways is yet to be elucidated. SOCS5 contains a C-terminal SOCS box motif that forms part of an E3 ubiquitin-ligase complex, a central SH2-domain and large uncharacterised N-terminal region. In this study, we have used mass spectrometry to characterise the SOCS5 interactome and have identified a number of known and novel interacting proteins. These analyses have also revealed that the SOCS5 N-terminal region, which is predicted to be largely unstructured, to be heavily phosphorylated and we hypothesise that SOCS5 acts as a scaffold to support multiple signalling complexes. Additionally, many of the proteins enriched by SOCS5 are known drivers of tumorigenesis, particularly in the context of breast cancer. Expression analysis of SOCS5 in breast cancer revealed that is down regulated in a majority of patients with invasive ductal breast carcinoma (TCGA dataset, top 7% under-expressed genes, $p=2.53E-23$). Excitingly, SOCS5-deficient mice exhibited accelerated tumour onset and growth relative to wild-type mice in the Polyoma Middle T antigen model of breast cancer, thus providing the first *in vivo* evidence that mammalian SOCS5 can act to regulate tumorigenesis. Using our interactome data, we are now seeking to determine which specific protein targets are regulated by SOCS5 in breast cancer cells.

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Life on the Rafts

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Lipid rafts are specialized membrane microdomains enriched in cholesterol and sphingolipids thought to act as dynamic signalling and sorting platforms. Given their fundamental roles in cellular regulation, there is a plethora of information on the size, composition and regulation of these membrane microdomains, including a large number of proteomics studies. To facilitate the mining and analysis of published lipid raft proteomics studies, first we have developed a searchable database RaftProt. Currently it hosts information about 117 proteomes derived from 69 different cell and tissue types across six mammalian species. In addition to variety of different search and browse features, we have captured the lipid raft preparation methods and implemented advanced search option for methodology and sample treatment conditions, such as cholesterol depletion. Furthermore, we have identified a list of high confidence proteins, and enabled searching only from this list of likely bona fide lipid raft proteins. Secondly, with the aim to identify of a common mechanism for lipid raft-mediated tumor progression, we integrated subcellular proteomics with computational systems biology approach. For this, we identified 'core cancer raft proteins' by analysing rafts from ovarian cancer, prostate cancer, breast cancer, renal cell carcinoma and melanoma which may offer novel pan-cancer therapeutic targets/strategies. Our analysis suggest that the proteins from cancer rafts have

enhanced tendency to engage in protein-protein interactions, leading to much larger and more-dense interaction networks. Moreover, we observed strong enrichment of cytoskeletal assemblies in the membrane rafts during cancer progression which was abrogated by tumor suppressors OPCML and PTRF in epithelial ovarian cancer and prostate cancer respectively. Taken together, our results demonstrate the value of integrative computational analysis of subcellular proteomics data in biomedical research.

N-linked glycopeptide analysis of plant glycoproteins

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N-glycosylation of plant proteins has been shown to be important for protein folding, correct subcellular location and secretion, enzyme catalytic activity as well as plant pathogen interactions¹. At the modification site there is (micro) heterogeneity of the glycans attached, making it difficult to detect in routine proteomics studies and the labile nature of the glycan hinders the analysis by mass spectrometry (MS). Plant *N*-glycosylation profiling studies performed have primarily relied on removal of the *N*-glycans to ease both the identification of the peptide and the glycan but at the cost of losing information on site-specific microheterogeneity².

Current MS-based ionisation and fragmentation techniques allow the comprehensive sequencing of intact glycopeptides revealing both the peptide and glycan sequences as well as the *N*-glycosylation site position. We describe a method for the enrichment of *N*-linked glycopeptides from the model plant *Arabidopsis*, followed by LC-ESI-MS/MS analysis using higher-energy collision dissociation product dependent electron transfer dissociation (HCD-pd-ETD) fragmentation for peptide identification, glycan site attachment and glycan sequence.

1. Song, W., Henquet M.G.L., et al. (2011) *N*-glycoproteomics in plants: Perspectives and challenges, *Journal of Proteomics* 74, 1463-1474
2. Ford KL, Zeng W, Heazlewood JL, Bacic A (2015) Characterization of protein *N*-glycosylation by tandem mass spectrometry using complementary fragmentation techniques, *Frontiers in Plant Science* 6: 674

Queensland Unknown (QX) and Winter Mortality (WM) disease of Sydney rock Oysters: A comparative proteomics study.

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Sydney rock oysters (SRO) are indigenous to Australian waters and contribute to the economy greatly. In recent years, however, the SRO industry has witnessed a continuous decline in production, which can be attributed mainly to Queensland unknown (QX) and winter mortality (WM) diseases, in conjunction with environmental factors and anthropogenic pollution. The diseases together are responsible for > 80% of the mortality of oysters in the estuaries in NSW.

In 1990s DPI-NSW initiated a breeding program to tackle the loss by selection of broodstock that survived the disease outbreaks, without any knowledge of the molecular basis of resistance. This project is an attempt to connect the missing links between selection and the biological processes associated with it.

In this baseline study we aim at identifying the proteins differentially regulated in QX and winter mortality selected Sydney rock oysters when compared against non-selected oysters, employing both two dimensional protein gel electrophoresis and shotgun proteomics. We have identified proteins which are differentially regulated in winter mortality and QX. These proteins were found to be associated with different biological processes including stress response, cytoskeleton, cell communication, energy metabolism and protein synthesis. Identification of their biological significance has led to a better understanding of the underlying molecular processes associated with the disease selection. This ongoing study requires further substantiation, but results are already being used to help in breeding better oysters.

A proteomic approach to characterising antigen processing and presentation in bats.

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Bats are a major reservoir of emerging infectious diseases and harbour deadly viruses such as Ebola, SARS, MERS and Hendra virus which pose serious threats to animal and human health. We hypothesise that the bat adaptive immune system contributes to their ability to co-exist asymptotically with these viruses. One of the key adaptive immune responses is the presentation of antigens by major histocompatibility complex (MHC) class I molecules to cytotoxic CD8⁺ T cells. Using a proteomic approach, we

have characterised the proteins and peptides bound to three distinct bat MHC class I molecules. We determined that these bat MHC class I molecules, like their human and mouse homologues, associate with a peptide-loading complex to facilitate optimal ligand selection. Using a peptidomics approach, we also characterised the first repertoires of bat MHC class I bound peptides. These peptides ranged in length from 8-15 amino acid residues and motif analysis of the endogenous peptides revealed strong amino acid biases at various anchor positions. Furthermore, we were able to identify Hendra virus-derived peptides from infected bat cells which also display this binding motif, suggesting these motifs may be exploited to predict epitopes for vaccine development and immunological studies. In conclusion, this study provides fundamental insights into the antigen processing and presentation pathways of bats, which ultimately can be used to understand viral control.

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Antioxidant and anti-inflammatory properties of sugarcane dietary fibre

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Chronic inflammation involves the dysregulation in the synthesis of pro-inflammatory mediators which are associated with several diseases including autoimmune diseases, diabetes, cancer, etc. Some natural plant products are known to possess anti-inflammatory properties based on their high content of phytochemicals. In this study, we demonstrate that sugarcane dietary fibre (SCF) is a potent source of phytochemicals presenting more than two-fold polyphenols, flavonoids and antioxidants compared with raisins and cranberry juice. In vitro studies performed in a cellular model of intestinal inflammation using LPS-stimulated SW480 and HepG2 cells show that extracts from SCF suppresses the phosphorylation of transcription factor NF- κ B, and the protein kinase Akt as has been shown for the well-known polyphenol, resveratrol. *Mass spectrometry based phosphoproteomic analysis is being used to uncover other modes of action to explain the anti-inflammatory events associated with SCF extracts. Preliminary data shows profound changes in the phosphorylation of proteins involved in the inflammatory response.* Our findings suggest that sugarcane fibre is a valuable source of antioxidants with potential to impart health benefits associated with inflammatory-related conditions.

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A Systematic Investigation of CID Q-TOF Collision Energies for Complete Identification of Glycopeptides by Mass Spectrometry

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In most naturally occurring glycoproteins, pools of glycans are attached to one or more glycosylation sites. The analysis of each individual site is challenging and requires the analysis of glycopeptides. For this, quadrupole time of flight (QTOF) mass spectrometers (MS) with high mass accuracy, fast duty cycles, and high m/z range are highly suited due to the usability of multiple collision energies. In this study we systematically investigated the optimum collision energies required for fragmentation of the glycan and the peptide parts of glycopeptides, and present a software-supported approach for data interpretation. For the initial studies of fragmentation energy, synthetic N-glycopeptides were analysed on a QTOF MS instrument (impact II) with CaptiveSpray nanoBooster[™] (Bruker Daltonics). For further testing, tryptically digested standard glycoproteins (fetuin, antibodies) were separated by nano LC before MS analysis using systematically varied collision energies. Glycopeptide spectra were detected and the peptide masses were determined automatically (ProteinScape 4.0, Bruker Daltonics). Glycan structures were identified using the integrated GlycoQuest search engine, and for peptide identification Mascot (Matrix Sciences) was used. The energies required for optimal glycan fragmentation were found to be clearly below the ones necessary for the peptide part. Collision energy stepping allowed identification of both peptide and glycan moieties from a single MSMS spectrum in ProteinScape 4.0. In summary, the optimized parameters were successfully applied on digested monoclonal antibodies and glycoprotein mixtures, which resulted in the identification of complete N- and O-glycopeptides. This improved methodology is particularly useful in the fields of glycoproteomics research as well as biopharmaceutical development and quality control.

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Investigating lipid changes with PI3-K inhibition in colorectal cancer liver metastases by MALDI-MS imaging

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Colorectal cancer (CRC) is one of the leading causes of cancer deaths worldwide, with metastases to the liver being responsible for very poor diagnoses. It is therefore crucial to develop a therapeutic approach to target these metastases. Phosphoinositide-3-kinase (PI3-K) is upregulated in many cancers, including CRC; and its cascades include various downstream lipid effectors.

MALDI-MS imaging has been used to investigate the effect of a PI3-K inhibitor GDC-0941 on lipid profiles in CRC liver metastases.

An in-house mouse model was used to generate CRC tumours. Mice that underwent drug therapy were administered two doses of 50m/kg of GDC-0941. Vehicle and drug treated tumours were flash-frozen and sectioned for MALDI imaging. Adjacent sections were kept for immuno fluorescence. MALDI imaging and MS/MS experiments were carried out on the MALDI-7090 (Shimadzu, UK).

Clear lipid profile changes occur between non-treated and treated tumours, with certain phosphatidylcholines showing a decrease in abundance after drug addition. Comparing MALDI images to H&E stains reveal which lipids are localised to necrotic, tumour, normal tissue; or those which are found heterogeneously in the tumour, and are hence significant for effective therapy. MALDI images have also been compared to immunofluorescence to show which lipids co-localise with immune cell infiltration. Future work will involve elucidating exactly what lipid mechanisms are occurring in these tumours to give the lipid changes seen as a result of PI3-K inhibition.

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The development and implementation of a new microbial pathovariance system.

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There is an ever increasing necessity for the rapid identification of microbiological pathogens in human clinical samples and agricultural samples. Generating information such as strain level identification, in a rapid, robust and automated way, is the way forward for microbiology. Traditional techniques rely on sometimes subjective morphological and biochemical information which requires a high level of technical expertise and expensive reagents. We aim to replace traditional microbiology identification workflows with a single simple mass spectrometry-based protocol that allows walk up users to quickly and accurately identify a disease causing organism from multiple collected samples. We have developed a new pathovariance technique which is an analysis technique that uses Matrix Assisted Laser Desorption Ionisation Mass Spectrometry (MALDI-MS) to identify microorganisms from a range of clinical samples. In short, a small amount of cells are grown (or sampled) and prepared according to a proprietary protocol to extract molecules that can be analysed by the mass spectrometer. Currently this technology is limited, with several major pathogen groups, such as *Staphylococcus* spp., *Streptococcus* spp., and *Escherichia coli*/*Shigella* subgenus indistinguishable beyond the species level. We have significantly improved on this current technology by developing a proprietary sample preparation protocol and computational package that allows the differentiation and identification of a range of organisms at strain and sub strain level with a high level of accuracy. We have termed this new technology the Karora Pathovariance System (patent pending).

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Peptidomic and proteomic comparison of electrically stimulated and manually dissected venom from the South American bullet ant *Paraponera clavata*

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Venom peptides are currently being developed as novel drugs and bioinsecticides. A large number of ant species have evolved a venom rich in peptides and proteins used for predation, defence and communication and therefore represent an untapped source of potential lead compounds, with only 72 currently described peptides. One aspect of ant venoms that has not been investigated is the difference in venom composition obtained using differing venom collection methods and the potential variations in venom composition between ant colonies of the same species. The present study therefore aimed to determine any differences in the peptide and protein components of the bullet ant (*Paraponera clavata*) collected by either manual venom gland dissection or electrical stimulation, as well as difference between ant venoms collected from different colonies. Venom proteins were separated by 2D-PAGE and analysed by nanoESI-MS. Peptidomic analyses were carried out by separating venoms on C18 RP-HPLC then analysing by MALDI-MS. The proteomic experiments revealed numerous proteins that were assigned a biological function (96 in total) of which 70% were common to both collection methods. The remaining 30% of proteins were either structural or metabolic proteins. The peptidomic analysis revealed a large number of peptides (309 in total) of which only 30% were common to both collection methods. This was far lower than what was observed from protein matches. Therefore, we have shown that each method reveals a unique set of peptides and proteins, however, extracting venom via electrical stimulation, which is not destructive to the ants, is the preferred method of collection as it contains all the major components. There were also notable differences in peptide and protein expression between the venoms obtained from the two different *P. clavata* colonies. These findings further demonstrate the richness and diversity of ant venoms as potential sources of bioactive compounds.

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Characterisation of lipoprotein MPN_284, a potential adhesin of *Mycoplasma pneumoniae*

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Mycoplasma pneumoniae is the second most common cause of community acquired pneumonia. Infections are localised to the respiratory tract but can be systemic and affect multiple tissues and organs. *M. pneumoniae* is a strict parasite and must adhere to the respiratory epithelium to survive. Little is known of the mechanism of pathogenesis and attempts to develop efficacious vaccines have been unsuccessful.

M. pneumoniae possesses an unusually large repertoire of lipoproteins and their functions are largely unknown. Our proteome studies identified a number of lipoproteins in *M. pneumoniae*. One of these, MPN_284, was unusual in that it was highly expressed, proteolytically processed and surface accessible. N-terminomics of mature proteoforms and 2D SDS-PAGE followed by LC-MS/MS were used to characterize the cleavage sites of MPN_284. Two recombinant fragments were expressed that span the N-terminal (MPN_284 N-terminus) and C-terminal thirds (MPN_284 C-terminus) of MPN_284. Polyclonal antiserum to each fragment was generated to determine the cellular location of the lipoprotein fragments using 3D-Structured Illumination Microscopy and to confirm the identities of the cleavage fragments of MPN_284 by western blotting. Image analysis suggests that the C-terminal region of MPN_284 is accessible and distributed on the surface of *M. pneumoniae*, while the N-terminal region produced sporadic staining patterns. To investigate the adhesive characteristics of the N- and C-terminal cleavage fragments of MPN_284, inert latex beads were coated separately with MPN_284 fragments and incubated with respiratory epithelial cells (A549). Beads coated with MPN_284 fragments were found to bind to A549 cell monolayers while beads coated with bovine serum albumin did not.

Our data confirms that MPN_284 resides on the extracellular surface of *M. pneumoniae* and is proteolytically processed, generating multiple surface accessible fragments with putative binding functions for host molecules. The bead experiments were consistent with these observations. This work provides new avenues to explore the identities of binding domains in MPN_284 and the development of an efficacious vaccine for *M. pneumoniae*.

Moonlighting proteins in *Mycoplasma hyopneumoniae*: Investigating a pathogenic role for glycolytic enzymes and their cleavage fragments.

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Mycoplasma species are strictly parasitic bacteria from the Mollicutes class that are thought to have undergone extensive genomic reduction from a low G + C Firmicute ancestor. As such, *Mycoplasma* spp. lack genes for the TCA cycle and secretory mechanisms, and the biosynthesis of a cell wall, amino acids, nucleotides, and cholesterol. The pig pathogen *Mycoplasma hyopneumoniae* (*Mhp*) destroys mucociliary function in the respiratory tract and causes significant economic loss to pig production systems globally. Our surfaceome studies show that in addition to the P97 and P102 adhesin families, many lipoproteins and proteins with canonical cytosolic functions are surface-accessible targets of proteolytic processing events.

Proteins that do not contain known secretion motifs are often described on the surface of bacteria including metabolic enzymes, chaperones, and ribosomal proteins. The surface-expression of these proteins has been ignored for many years but has recently garnered widespread attention as their possible role in pathogenesis has developed. Many of these canonically cytosolic moonlighting proteins have since been shown to play important roles in binding to a functionally and structurally diverse array of host molecules and to activate host proteases such as plasminogen¹. In *Mycoplasma pneumoniae*, subunits of the pyruvate dehydrogenase (PDH) complex are known to bind plasminogen and fibronectin^{2,3}.

Here we report processing of the PDH complex subunits that are located on the surface of *M. hyopneumoniae*. We argue that proteolytic processing may be a mechanism to release new, functional domains and expand the adhesive capacity of this pathogen as it colonises the host. To assist with the identification of cleavage fragments, a series of affinity chromatography assays using host molecules (plasminogen, fibronectin, and others) as bait were applied. Protein cleavage fragments were mapped by combining 1D and 2D-SDS-PAGE and LC-MS/MS data and precise cleavage sites within these molecules identified by dimethyl labelling of neo-N-termini. These enrichment strategies are useful for mapping functionally relevant cleavage fragments and assist with the identification of novel binding domains.

1. Kainulainen, V. and Korhonen, T. K., Dancing to another tune—adhesive moonlighting proteins in bacteria. *Biology*, 2014. 3(1): p. 178-204.
2. Dallo, S.F., Kannan, T. R., Blaylock, M. W., Baseman, J. B., Elongation factor Tu and E1 β subunit of pyruvate dehydrogenase complex act as fibronectin binding proteins in *Mycoplasma pneumoniae*. *Molecular Microbiology*, 2002. 46(4): p. 1041-1051.
3. Gründel, A., Friedrich, K., Pfeiffer, M., Jacobs, E., Dumke, R., Subunits of the Pyruvate Dehydrogenase Cluster of *Mycoplasma pneumoniae* Are Surface-Displayed Proteins that Bind and Activate Human Plasminogen. *PLoS ONE*, 2015. 10(5): e0126600

Comparative Proteomic Analysis of Two Rice Genotypes Exposed to Drought Stress and Recovery

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Rice is the major staple food for more than half of world's population. As global climate changes, many places have seen changes in rainfall, resulting in more floods, droughts and severe heat waves. Two rice varieties with contrasting genetic backgrounds, Nipponbare and IAC 1131, were used in this study. Nipponbare, the first fully sequenced agricultural plant, is a drought sensitive rice from Japan while IAC 1131, which is endemic to the hotter climate of upland rice fields in Java, is a drought tolerant upland

tropical rice from the same sub-species as Nipponbare. Four-week-old seedlings of both cultivars were grown in large soil volumes and then exposed to moderate and extreme drought for 7 days by imposing two distinct watering regimes, followed by 3 days of re-watering. Mature leaves were harvested from plants from each treatment for protein extraction and subsequent quantitative proteomic analysis, with validation of selected proteins by Western blotting. Gene Ontology (GO) annotations of differentially expressed proteins provide insights into the metabolic pathways that are involved in drought stress resistance. Higher abundance of stress response proteins was found in IAC1131 than in Nipponbare. Additionally, six proteins involved in the porphyrin and chlorophyll metabolism were found to be significantly decreased in IAC1131 under extreme drought. Our data indicate that IAC 1131 appears to be better able to cope with stressful conditions by up regulating a suite of stress and defence response related proteins. Nipponbare, in contrast, lacks the range of stress response shown by the more stress tolerant variety, and responds to drought stress by initiating a partial shutdown of chlorophyll biosynthesis in an apparent attempt to preserve resources.

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Combination of metabolomics, peptidomics and proteomics to elucidate mechanisms of drug resistance in the malaria parasite

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Drug resistance in the malaria parasite, *Plasmodium falciparum*, poses a major threat to global plans for the control and elimination of malaria. Resistance has been reported against all the currently approved antimalarial agents, although the underlying mechanisms associated with resistance are poorly understood. Most current drugs act by inhibiting cellular metabolism (e.g. atovaquone and antifolates) or act in the digestive vacuole (e.g. artemisinin and quinolines). Therefore, it is likely that a system-wide approach to monitor metabolism and protein turnover will reveal novel aspects of drug resistance for existing antimalarials. In order to identify key features associated with a delayed parasite clearance phenotype, we have developed a multi-omics platform based on high-resolution mass spectrometry combining proteomics, peptidomics and metabolomics to analyse global differences between drug-resistant and drug-sensitive isolates.

When applied to *P. falciparum* infected red blood cells, our multi-omics platform facilitated the identification of approximately 3000 proteins, 1000 metabolites and 10,915 naturally abundant peptides and integration of these data has provided a detailed view of protein expression and regulation associated with drug resistance. Preliminary findings for artemisinin-resistant isolates revealed that artemisinin resistance is associated with increased expression of proteins associated with the stress response and the unfolded protein response, as well as the accumulation of specific peptides and small molecule metabolites. These findings shed light on the biochemical pathways that are targeted by this critical antimalarial compound, and reveal the molecular adaptations that allow parasites to overcome the antimalarial effects of these front-line therapies.

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Characterization of vulvar cancer progression and metastasis by imaging mass spectrometry from formalin-fixed paraffin-embedded tissues

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To date no tumour specific biomarkers for vulva cancer have been identified and the initiation and progression of the disease is poorly characterised and understood. Here we present an examination of vulva cancer progression in a cohort of 4 patients by the analysis of healthy tissue, vulvar intraepithelial neoplasia stage III (VINIII), vulvar squamous cell carcinoma (VSCC) and inguinal lymph node metastases by MALDI mass spectrometry imaging (MALDI-MSI). 1821 peak groups were detected in the MALDI-MSI experiments, of which 567 were found to have different distributions of signal to noise ratios across the 4 tissue types according to a Kruskal-Wallis test ($P \leq 0.01$). Potential peptide identifications for the 567 peak groups were obtained by matching the MALDI-MSI data back to standard nanoLC-ESI-MS/MS data. Six of the highest intensity differentially expressed MALDI-MSI peak groups were targeted for identification by *in situ* MALDI MS/MS. The 6 *in situ* sequenced peptides matched back to 5 proteins, with one peptide identified from Histone H2A, Haemoglobin subunit β , Histone H4, and Cytoskeletal 10, and 2 unique peptides identified from β -Actin. In order to validate the MALDI-MSI results the spatial expression pattern and differential expression of β -Actin was further verified by immunohistochemistry and data independent nanoLC-ESI-MS/MS.

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Quantitative proteomics of metronidazole drug resistance in *Giardia duodenalis*

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Giardia duodenalis is a protozoan parasite of the gastrointestinal tract and the major global contributor to human diarrheal disease. Treatment of *Giardiasis* is predominately through nitroheterocyclics, specifically metronidazole, which are redox-active compounds believed to induce oxidative damage of DNA and proteins via activation by parasite oxoreductases. However, the effectiveness of metronidazole varies, and resistance is well-documented in both clinical and laboratory settings. Using three

isolates of *G. duodenalis* from the human-infective Assemblage A taxonomic group, we have compared susceptible parent and metronidazole resistant strains of the same isolate using a TMT labelling approach. We identified over 1000 proteins in each isolate, and defined differentially expressed proteins in metronidazole-resistant strains. Though similarities in differential expression between isolates was observed at the level of protein function, there was limited overlap at the protein identification level. This may indicate multiple mechanisms are capable of producing metronidazole resistance in *G. duodenalis*. We observed differential expression in a wide range of oxidoreductases, some which have been previously implicated in metronidazole resistance and others in FAD and NADH dependent pathways, which are not as well-characterised. These oxidoreductases also showed variable combinations of up- and down-regulation between isolates, further implicating alternate patterns of regulation may achieve similar outcomes for drug resistance. Our results also indicate several post-translational modifications may play a role in resistance, with multiple kinases up-regulated in drug-resistant strains. Furthermore, proteins associated with deacetylase activity were up-regulated, including histone deacetylase and a sirtuin homologue, as well as proteins associated with chromatin remodelling and silencing. This is a novel experimental observation which implicates epigenetic regulation in metronidazole resistance in *G. duodenalis*.

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Using proteomics to decipher novel protein function in a genome-reduced pathogen

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Mycoplasma hyopneumoniae is a genome-reduced, cell wall-less, bacterial pathogen with a predicted coding capacity of less than 700 proteins and is one of the smallest self-replicating pathogens. The cell surface is extensively modified by processing events that target the P97 and P102 adhesin families. We analysed the proteome of *M. hyopneumoniae* using protein-centric approaches (1D and 2D GeLC-MS/MS) that also enabled us to examine global processing events in this species. While these approaches only identified 52% of the predicted proteome (347 proteins) we identified 35 surface-associated proteins with widely divergent functions that were targets of unusual endoproteolytic processing events including cell adhesins, lipoproteins and proteins with canonical functions in the cytosol such as metabolic enzymes. Affinity chromatography assays that used key host substrates; heparin, fibronectin, actin, plasminogen and host epithelial cell surface proteins as bait, recovered cleavage products derived from these processed proteins, suggesting these fragments interact directly with the bait proteins and display previously unrecognised adhesive functions. Our combined methods of analysis were able to examine the interplay between structure, function and localisation of proteins. Previously well-characterised glycolytic enzymes were identified to be surface-exposed, bind key host substrates and displayed evidence of cleavage, and together these are strong indicators of protein moonlighting. We hypothesize that protein processing is underestimated as a post-translational modification in genome-reduced bacteria and prokaryotes more broadly and represents an important mechanism for creating cell surface protein diversity.

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A novel protein methyltransferase catalyses conserved N-terminal and lysine methylation on translational elongation factor 1A

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Eukaryotic elongation factor 1A (eEF1A) is an essential protein that facilitates translational elongation by delivering aminoacyl-tRNAs to ribosomes. Protein N-terminal methylation is an under-characterised post-translational modification, with only three, highly homologous, N-terminal methyltransferases known in eukaryotes. Here we report a new eukaryotic protein N-terminal methyltransferase, *Saccharomyces cerevisiae* YLR285W, which methylates eEF1A at a previously undescribed high-stoichiometry N-terminal site and the adjacent lysine. Using parallel reaction monitoring (PRM) and MS/MS/MS (MS3), we unambiguously localised these modifications, showing that N-terminal trimethylation precedes dimethylation at the adjacent lysine. Deletion of YLR285W resulted in the loss of N-terminal and lysine methylation *in vivo*, whereas overexpression of YLR285W resulted in an increase of methylation at these sites. This was confirmed by *in vitro* methylation of eEF1A by recombinant YLR285W. Accordingly, we name YLR285W as elongation factor methyltransferase 7 (Efm7). This enzyme is a new type of eukaryotic N-terminal methyltransferase as, unlike the three other known eukaryotic N-terminal methyltransferases, its substrate does not have an N-terminal [A/P/S]-P-K motif. We show that the N-terminal methylation of eEF1A is also present in human; this conservation over a large evolutionary distance suggests it to be of functional importance. Indeed, proteomic analysis of the knockout of Efm7, with SILAC, reveals the role of this methylation in modulating the function of eEF1A.

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The "SRGG" Protein Interaction Code

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Post-translational modifications (PTMs) can modulate protein-protein interactions. When used in combination, to modulate the specificity of interactions, PTMs can form "interaction codes". To date, interaction codes have been characterised on a small number of proteins; histones, RNA polymerase II, FOXO transcription factors and chaperones. However, PTMs are known to be very widespread on eukaryotic proteins, raising the possibility that they may be a key means by which protein-protein interactions are regulated. We have termed this the "protein interaction code hypothesis".

Finding new protein interaction codes is challenging, due to their complexity. Here we have simplified the study of interaction codes, by analysing paired PTMs, and through this defined a new interaction code based on an identical, recurring amino acid motif in yeast. The "SRGG" motif is found on 32 yeast proteins, many of which have functions in RNA maturation, transport or transcription. Phosphorylation and methylation, of "SRGG" motifs on yeast protein Npl3p has been shown to modulate protein-protein interactions. Kinase Sky1p and methyltransferase Hmt1p are the modifying enzymes. Here we describe a second yeast protein, Nop1p, to also be phosphorylated by kinase Sky1p and methyltransferase Hmt1p on "SRGG" motifs. Via the use of our conditional two-hybrid system we also show these modifications to modulate the protein-protein interactions of Nop1p.

The proximity of phosphorylation and methylation on "SRGG" motifs suggests these modifications may be subject to PTM crosstalk. Using in vitro peptide assays, coupled to LC-ETD MS/MS, we found that phosphorylation of "SRGG" attenuates the methylation by Hmt1p. However, prior methylation has little effect on Sky1p phosphorylation. Together these results characterise an "SRGG" protein interaction code, which recurs across yeast proteins Nop1p and Npl3p. We discuss how this code is likely to be important for nucleo-cytoplasmic shuttling.

Large-scale mass spectrometry-based identifications of enzyme-mediated protein methylation are subject to high false discovery rates

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Post-translational methylation is a protein modification that predominantly occurs on lysine and arginine residues, and a growing number of recent large-scale LC-MS/MS methylation site discovery experiments have indicated that these modifications are widespread and sometimes conserved in eukaryotes. In interpreting these LC-MS/MS data, methylpeptide spectrum matches (methyl-PSMs) must be identified at acceptably low false discovery rates (FDRs) following sequence database searching, and to estimate FDRs and determine methyl-PSM filtering criteria, the target-decoy approach is frequently employed. The efficacy of this methyl-PSM filtering approach has, however, yet to be thoroughly evaluated.

Here we conduct a systematic analysis of methyl-PSM FDRs across a range of sample preparation workflows and mass spectrometric instrument platforms (each employing a different mode of MS/MS dissociation). Through ¹³CD₃-methionine labeling (heavy-methyl SILAC) of *S. cerevisiae* cells and in-depth manual data inspection, accurate lists of true positive methyl-PSMs were determined, allowing methyl-PSM FDRs to be compared to target-decoy approach-derived methyl-PSM FDR estimates.

Our results show that global FDR estimates (i.e. FDR estimates obtained using all PSMs from target and decoy databases) produce extremely unreliable methyl-PSM filtering criteria; we demonstrate that this is an unavoidable consequence of the high number of amino acid combinations capable of producing peptide sequences that are isobaric to methylated peptides of a different sequence. Separate methyl-PSM FDR estimates (i.e. FDR estimates obtained using only methyl-PSMs from target and decoy databases) were also found to be unreliable due to prevalent sources of false positive methyl-PSMs that produce high peptide identity score distributions. Incorrect methylation site localizations, peptides containing cysteinyl-S- β -propionamide, and methylated glutamic or aspartic acid residues can partially, but not wholly, account for these false positive methyl-PSMs.

Together these results indicate that the target-decoy approach is an unreliable means of estimating methyl-PSM FDRs and methyl-PSM filtering criteria. We suggest that orthogonal methylpeptide validation (e.g. heavy-methyl SILAC) should be considered a prerequisite for obtaining high confidence methyl-PSMs in large-scale LC-MS/MS methylation site discovery experiments.

Multiple roles of elongation factor Tu in *Mycoplasma pneumoniae*

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Mycoplasma pneumoniae (*Mpn*) is responsible for 15-20% of cases of community-acquired pneumonia. Up to 25% of infected patients develop extrapulmonary complications that can affect neurological, musculoskeletal, haematological and cardiovascular sites. An extracellular extension called the attachment organelle mediates attachment to the respiratory epithelium via the P1 and P30 adhesins. Vaccines incorporating both adhesins unfortunately exacerbate disease and a successful vaccine is yet to be developed. Our surfaceome studies identify about 160 proteins on the surface of *Mpn* including a number that lack signal secretion sequences. One of these, elongation factor Tu (Ef-Tu), is a protein that moonlights on the surface of Gram positive and Gram negative pathogens. Dallo *et al.*, (2002¹) used immunogold EM microscopy to confirm Ef-Tu on the cell surface of *Mpn* and also described its ability to bind fibronectin (Fn). Subsequent studies identified two Fn binding motifs and six key amino acids in those regions that are needed to bind Fn in Ef-Tu (Balasubramanian *et al.*, 2008², 2009³). Notably, whole cell Labelling studies that target N-terminal amino acids identified nine neo-N-termini within Ef-Tu. LC-MS/MS analysis of protein spots separated by 2D SDS-PAGE and analysis of eluents from affinity chromatography columns loaded with fetuin, actin, plasminogen, heparin and Fn as bait has allowed us to map 15 putative cleavage fragments of Ef-Tu. Preliminary microscale thermophoresis experiments indicate that purified recombinant Ef-Tu binds heparin, plasminogen and actin and further experiments are underway to validate interactions between Ef-Tu and other host molecules implicated in our affinity studies. Our data indicates that Ef-Tu is a multifunctional adhesin that is processed into functional domains on the cell surface of *Mpn*.

1. Dallo SF, Kannan TR, Blaylock MW, Baseman JB: Elongation factor Tu and E1 beta subunit of pyruvate dehydrogenase complex act as fibronectin binding proteins in *Mycoplasma pneumoniae*. *Mol Microbiol* 2002, 46(4):1041-1051.
2. Balasubramanian S, Kannan TR, Blaylock MW, Baseman JB: The surface-exposed carboxyl region of *Mycoplasma pneumoniae* elongation factor Tu interacts with fibronectin. *Infect Immun* 2008, 76:3116-3123.

3. Balasubramanian S, Kannan TR, Hart PJ, Baseman JB: Amino acid changes in elongation factor Tu of *Mycoplasma pneumoniae* and *Mycoplasma genitalium* influence fibronectin binding. *Infect Immun* 2009, 77, 3533–3541.

Proteomics of Cephalopod Venoms

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Coleoid Cephalopods are a diverse molluscan sub-class that includes octopus, cuttlefish and squid. There is strong morphological, behavioural and transcriptomic evidence to suggest that many cephalopod species use a potent cocktail of proteinaceous toxins for predation and/or defense. Despite this, very few cephalopod proteins have been observed at the protein level and even fewer have been well characterised. As a consequence they are a conspicuously under-represented group in the Uniprot toxins database and potentially a rich source of novel toxic proteins.

We present our findings based on bottom-up proteomics on venom glands and toxic secretions from three different cephalopod species (two octopus and one squid). In order to perform this work we relied heavily on integrating information from transcriptomics and proteomics. For all organisms it was necessary to assemble high quality reference transcriptomes and transcriptome maps across multiple tissues. We used these to predict protein sequences and then further refined these predicted protein databases using proteogenomics. New bioinformatic tools were developed to facilitate these analyses. All these tools are open source and are available via the user-friendly Galaxy platform.

Toxins were identified based on well known general features of toxins, including small size, high cysteine richness, existence of a signal and/or pro peptides and sometimes homology to known toxins. Further evidence for toxicity was obtained from approximate quantitative protein expression estimates via intensity based absolute quantitation (iBAQ) in venomous secretions as well as relative expression in different tissues based on transcriptomic data.

Our work highlights the important role that mass spectrometry based proteomics can play in the discovery of novel toxin molecules from poorly studied taxa, and describes the non-standard bioinformatic techniques that enable such work.

Automated *N*-glycopeptide identification in glycoproteomics

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Recent developments in LC-MS/MS-based *N*-glycoproteomics including advances in software-driven glycopeptide identification have facilitated biochemical studies reporting thousands of intact *N*-glycopeptides. In this early phase it is particularly important to scrutinize the automated glycopeptide identification to ensure confidence in the process. Herein, we explore the accuracy of software-assisted site-specific glycoprofiling using the PTM-centric search-engine Byonic (Protein Metrics) relative to manual expert annotation. To allow an appropriately deep comparison, the study utilised glycoproteomics-typic acquisition and data analysis strategies, but of a single glycoprotein, the previously uncharacterised triply (Asn160, Asn268 and Asn302) *N*-glycosylated human basigin. Initially, detailed site-specific reference *N*-glycoprofiles of purified basigin were established using manual annotation and relative quantitation of ion trap (CID) and high-resolution Q-Exactive Orbitrap (HCD) LC-MS/MS data of tryptic *N*-glycopeptides. Conventional *N*-glycome profiling supported the manual annotation. The *N*-glycosylation sites of basigin showed extensive and diverse micro- and macro-heterogeneity. Subsequently, the basigin peptide mixture was glycoprofiled using Byonic with or without a background of complex peptides using the same Q-Exactive Orbitrap LC-HCD-MS/MS data. By monitoring the software-assisted glycoprofiling accuracy and coverage relative to the reference profile, the influence of multiple search parameters and scoring thresholds in Byonic was investigated. In general, the search criteria and confidence thresholds suggested by the vendor provided highly accurate and sensitive automated glycopeptide detection. As expected, several search parameters i.e. search space (proteome and glycome size), mass tolerance and peptide modifications and the confidence thresholds influenced the glycoprofiling accuracy and coverage in particularly for Asn268 basigin peptides displaying extensive peptide heterogeneity formed by incomplete trypsinization and methionine oxidation and, unexpectedly, also carbamidomethylation. The latter produced abundant neutral losses in the HCD-MS/MS spectra that were left unassigned by Byonic reducing the identification scores. These are valuable lessons learned in our ambition to ensure high glycoprofiling accuracy and coverage as we transition rapidly into automated FDR-based *N*-glycopeptide identification in glycoproteomics.

Understanding adaptation dynamics of *Pseudomonas aeruginosa* in cystic fibrosis lungs

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Pseudomonas aeruginosa (PA) is an opportunistic human pathogen and an etiological agent in cystic fibrosis (CF), leading to chronic infection and death. To understand how PA adapts to the harsh micro-environment of the CF host, we recently characterised three novel strains isolated from sputum of CF patients. We conducted genome sequencing, proteome profiling and phenotypic analysis and made comparisons to the laboratory strain PAO1. Genomic and phenotypic analyses revealed significant diversity in virulence, colonization and metabolism related traits in CF strains. 2D-LC-iTRAQ-MS analysis of carbonate-extracted membranes of all four strains of PA, grown in lung nutrient mimicking medium (SCFM) and M9 minimal medium revealed a total of 2,442 (SCFM) and 3,171 (M9) expressed proteins (global protein FDR <0.008%). Gene ontology and bioinformatics predictions assigned 994 (M9) and 765 (SCFM) proteins as membrane proteins. CF strains portrayed heterogeneity in membrane proteome expression which was reflected in phenotypes including biofilms, pigmentation, and virulence. We observed differential expression of key proteins involved in virulence, drug resistance, motility and adhesion. Interestingly, proteins involved in drug resistance (MexY, MexB, MexC, OprM) were up-regulated and chemotaxis, aerotaxis (PA1561, PctA, PctB) motility and adhesion proteins (FliK, FlgE, FliD, PilJ) were downregulated in CF strains. Confirmatory phenotype assays including drug MIC, plate motility assay and sputum binding assay reinforced the observation by portraying increased antibiotic resistance and reduced motility and adhesion. In conclusion, our multi-omic analysis shows that the adaptation strategies of *P. aeruginosa* to CF lungs are both multifactorial and combinatorial and are tailored according to micro-environmental niches.

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Isolation and Characterization of Natural Crosslinks in Animal skins by Liquid Chromatography and Electrospray Ionization-Mass Spectrometry Detection

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Natural crosslinks are important for stabilizing the collagen structure. However, because of their low concentration in collagen compared to amino acids, their isolation, characterization and quantitation are very challenging. To the best of our knowledge, no comparative study of the isolation and characterization of natural crosslinks in different animal skins has been reported. Herein, we describe an efficient and rapid method for the isolation of crosslinks from animal skins by liquid partition chromatography and preparative TLC followed by Reverse Phase High Performance Liquid Chromatography (RP-HPLC) and characterization using Electrospray Ionization-Mass Spectrometry (ESI-MS).

Skin is first treated with sodium borohydride to stabilize the crosslinks to prevent their destruction by acid hydrolysis. Reduced skin is then hydrolyzed using 6 M HCl at 105°C for 24 hours. Crosslinks are separated from the bulk of the amino acid residues by liquid partition chromatography on fibrous cellulose using butanol:water:acetic acid and water as solvents. Water fractions were monitored by thin layer chromatography (TLC) and fractions containing crosslinks were pooled, concentrated, and purified using preparative TLC with ethylacetate:water:acetic acid as solvent. The purity of the crosslinks was analysed by RP-HPLC and the exact mass of each crosslink measured using ESI-MS. Three main crosslinks were identified in sheep, goat, deer and cow skins; hydroxylysinoerleucine (HLNL, 292.1736 m/z), histidinolysinoerleucine (HHL, 445.2207 m/z) and histidinohydroxymerdesmosine (HHMD, 574.2969 m/z). Non-reduced skin lacked HHMD crosslinks and cartilage showed a different crosslinking profile to skin. It contained dihydroxylysinoerleucine (DHLNL, 309.1040 m/z) and pyridinoline (Pyr, 429.1887 m/z) and lacked the ones in skin. This method allows direct analysis of crosslinks HLNL, HHL and HHMD without interfering compounds such as basic amino acids and salts which has been a drawback of previously reported methods. The crosslinks found in sheep, goat, deer and cow skins will be presented and discussed in terms of their contribution to skin strength.

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LC/MS Method Optimization for TMT Quantification using Q-Exactive instruments

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Isobaric mass tagging (e.g., TMT) has become a common technique in mass spectrometry for relative quantification of proteins. However, there are a couple of factors that impede accurate quantification of complex proteomic samples. One of these factors is co-isolation of peptides, which leads to systematic under/overestimation of quantitative ratios of the isobaric tags [2]. Co-isolation interference has been addressed using various kinds of approaches, such as using MS³ experiments [3]. Due to the fast acquisition speed and efficient quadrupole isolation the Q-Exactive Plus/Q-Exactive HF can be used to address some of these challenges. The effect of several parameters (isolation window, normalized collision energy (NCE), source fragmentation, and others) on the protein quantification accuracy and precision is discussed.

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Automated Protein Digestion to Reduce the Sample Preparation Bottleneck

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Today using targeted quantitative proteomics techniques such as MRM analysis or SWATH[®] Acquisition, protein panels can be quantified across a broad dynamic range with very high reproducibility. While these techniques have increased the reproducibility and scale of protein analysis, sample preparation remains a key bottleneck as the number of samples increases. Numerous

processing steps are required, each susceptible to technical variation. Here, we have used automation to improve the throughput and day-to-day reproducibility of the protein digestion portion of the sample preparation workflow.

To increase throughput and reduce technical variation, we have implemented an automated peptide preparation protocol on a liquid handling workstation (Biomek NX^P) coupled with an MRM workflow using a QTRAP[®] 6500 system. Denaturation, reduction/alkylation and digestion were the steps included, total workflow time was ~5-6 hours, depending on # of samples processed. A large number of proteins/peptides were monitored to ensure that good general digestion was occurring. Two different automation workstations were used to confirm the method transferability and multi-day experiments were performed on each to confirm method reproducibility.

First the protocol was validated by performing a very careful manual digestion across 24 wells using MRMs to 150 peptides. Using this benchmark, the automation protocol was optimized to achieve similar or better performance. For off-deck digestion using separate incubators, on average, ~80% of peptides monitored had raw peak area CVs < 10% as monitored by LC/MRM analysis. Next steps will be to optimize the use of on-deck heating (incubators integrated on workstation) and assess variance. Use of internal standards could also be incorporated for specific workflows to further reduce variance and will be assessed.

The automation method provided excellent reproducibility for digestion across multiple days on multiple workstations.

DESI-MS Optimisation for the application of proteomic Imaging

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Desorption electrospray ionisation (DESI) provides a useful alternative to matrix assisted laser desorption ionisation (MALDI) for mass spectrometry imaging (MSI) with the ambient nature of the technique increasing the scope of samples that can be analysed whilst reducing the sample preparation requirements. Furthermore DESI offers the possibility to generate multiply charged ions for biomolecules like peptides and proteins directly from a surface. Here we investigate sample analysis parameters and conditions to optimise the ionisation/desorption of biomolecules directly from glass surface and tissue sections.

Analyses were performed on a SYNAPT G2-Si Q-TOF-IMS-MS, positive MS mode with integrated tri-wave ion guide optics used to separate ions by ion mobility in the gas phase. The DESI (Prosolia) stage was mounted directly using an electrospray inlet.

Initial optimisation and experiments were performed on single peptides/proteins. Extracted proteins were also typically digested spotted onto a Teflon coated glass slide. Various DESI solvent compositions were tested using methanol, Water, acetonitrile, formic acid. The MS spectra produced were in large comparable with those generated via a supporting ESI-MS analyses. However, it was noted that a number of peaks observed in the ESI spectrum were absent for the DESI data. In this case acetonitrile was found to be marginally better as the organic component than methanol. Furthermore the spray solution containing 0.2% formic acid was found to improve ionisation. In the case of the tryptic digest samples, peptide species with charge states of up to 4+ and 5+ were detected and were baseline separated using ion mobility.

Direct analysis of larger m/z molecules directly from tissue has shown to be more difficult because of the other endogenous molecules like lipids present within the tissue that ionise preferentially compare to larger endogenous molecules. Subsequent to these analyses, the focus turned to digested tissues. Trypsin, was reconstituted in 50 mM ammonium bicarbonate, 0.5% Octyl β -D-glucopyranoside with concentration of 20 μ g/mL.

Characterization and Collision Cross Section Determination of Lipids from Metabolic Syndrome Disorders

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Introduction

Obesity is a risk-factors associated with metabolic syndrome, causing excess body fat to be accumulated to the extent that it adversely affects health and life expectancy. This work provides additional characterization of the associated lipids using ion-mobility with collision cross section (CCS) databases.

Methods

Lipid were extracted from a variety of sources including plasma and liver tissue of control, obese and diabetic subjects. Extracts were separated over a 20 min reversed-phase LC gradient and data acquired using a data independent acquisition approach utilizing ion mobility. Data were processed and searched using Progenesis QI and dedicated lipid compound databases, providing normalized label-free quantitation results with additional specificity of CCS measurement.

Preliminary Data

Interrogation of the LC-IM-DIA-MS data revealed over 5000 potential features for further investigation as a result of positive and negative ion acquisitions combined. Data were further interrogated using multivariate statistical analyses, showing clear distinction between control and metabolic syndrome groups. OPLS discriminant analysis revealed 795 potential features that were of significant correlation and covariance. Database searching resulted in 163 candidates. Identifications were scored according to mass accuracy, isotopic fit, CCS and MS/MS fragmentation. Additional filtering to curate the data was based on mass errors less than 2 ppm, fold change >2, 5% CCS tolerance and ANOVA p-value.

Improvements in accurate mass GC-MS based Metabolomics: A novel atmospheric pressure GC-APCI Source increases quantitative and qualitative performance for metabolic profiling

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Gas chromatography coupled to mass spectrometry (GC-MS) is one of the most widely used analytical techniques in metabolomics. While electron ionization (EI) is the common analytical standard for GC-MS, atmospheric pressure chemical ionization (APCI) became more prominent in recent years. The soft APCI ionization preserves molecular information and opens the doors to the world of unidentified metabolites which could not yet be annotated due to missing library data.

We report the application of a novel GC-APCI design coupled with high resolution oTOF-MS to analyse derivatized metabolite extracts and reference standards. Compared with earlier results [1], an improved analytical performance resulted in a higher number of compounds which could be identified in human cancer cell extracts.

Upon comparing previous APCI-I [1] and novel APCI-II ion sources, GC-APCI-II-TOFMS analysis resulted in improved peak shapes and much better peak area reproducibility for fatty acid methyl esters (FAMES). Furthermore, overall decreased lower limits of quantification in the sub-micromolar range were found for twenty metabolites due to reduced background in the ion source. Meanwhile, the analytical linear working range was either maintained or slightly increased. The improved analytical performance enabled to approximately double the number of extracted peaks with signal-to-noise ratios >20 in cell culture supernatant samples of pancreatic cancer cells. Injecting PFTBA automatically into the source before each GC/MS run, lead to low ppm mass deviations for the standard compounds analyzed in this study. The higher number of peaks extracted and improved mass accuracy resulted in 36% more compounds which could be identified compared to the previous setup.

In summary, APCI-II has a number of notable improvements over APCI-I and holds great promise for further studies in metabolomics.

1. C. J. Wachsmuth, M. F. Almstetter, M. C. Waldhier, M. A. Gruber, N. Nürnberger, P. J. Oefner, K. Dettmer, *Anal Chem* 83 (2011) 7514-22.

EasyPhos – a high-performance, scalable and universal phosphoproteomics platform

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Tremendous advances in MS-based proteomics has revealed the pervasive nature of protein phosphorylation¹. The widespread utility and application of existing approaches to quantifying signalling in vivo and en-masse however remains hampered by poor scalability of enrichment and labelling workflows, large input-material requirements, and inadequate coverage of key signaling networks, particularly in the context of tissue samples. Here, we set out to develop and refine a phosphoproteomics pipeline that was scalable, without compromising performance. Such a pipeline would enable processing of large sample numbers in parallel, and without using proprietary reagents to ensure its widespread applicability.

Here we describe EasyPhos², a high-performance, scalable and extensible phosphoproteomics workflow that greatly streamlines the study of signalling networks across large sample numbers. We evaluated our platform in cells and tissues, measuring the phosphoproteomes of mouse liver cell lines, where replicate single-shot measurements quantified ~20,000 distinct phosphopeptides in one day. In liver, brain and kidney tissues, half-day measurements together quantified 24,000 phosphopeptides. Deep phosphoproteome coverage in the absence of fractionation was facilitated by high enrichment specificity (>95% of identified peptides phosphorylated). Compared with our previous studies in the same systems we achieved 3x the depth, despite using 10x less material and 1/3rd of the measurement time.

EasyPhos requires minimal input material and measurement time without compromising depth, making large-scale signaling studies much more practical. Just as cheap and rapid sequencing technologies have driven an explosion of data in the genomics fields, we envisage an acceleration of MS-driven signalling studies providing rich and complex insights about cellular network function in physiological and patho-physiological contexts.

1. Humphrey SJ, James DE, Mann M. Protein Phosphorylation: A Major Switch Mechanism for Metabolic Regulation. *Trends in Endocrinology and Metabolism* (2015). 26(12):676-87.
2. Humphrey SJ, Azimifar SB, Mann M. High-throughput phosphoproteomics reveals in vivo insulin signaling dynamics. *Nature Biotechnology* (2015). 33(9):990-5.

New elements of the chondrocyte stress response identified in a model of cartilage degeneration

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The destruction of articular cartilage in osteoarthritis involves chondrocyte dysfunction and imbalanced extracellular matrix (ECM) homeostasis. Pro-inflammatory cytokines such as interleukin-1 α (IL-1 α) contribute to osteoarthritis pathophysiology, but the effects of IL-1 α on chondrocytes within their tissue microenvironment have not been fully evaluated. To redress this we used label-free quantitative proteomics to analyse the chondrocyte response to IL-1 α within a native cartilage ECM. Mouse femoral heads were cultured with and without IL-1 α and both the tissue proteome and proteins released into the media were analysed. Mass spectrometry identified 728 proteins and 368 proteins in the cartilage proteome and secretome, respectively. New elements of the chondrocyte response to IL-1 α related to cellular stress included markers for protein misfolding (Armet, Creld2 and Hyou1), enzymes involved in glutathione biosynthesis and regeneration (Gstp1, Gsto1 and Gsr) and oxidative stress (Prdx2, Txn, Atox1, Hmox1 and Vnn1). Other proteins previously not associated with the IL-1 α response in cartilage included ECM components (Smoc2, Kera and Crisp1) and cysteine proteases (cathepsin Z and legumain), while chondroadherin and cartilage-derived C-type lectin (Clec3a) were identified as novel products of IL-1 α induced cartilage degradation. This comprehensive view of the cartilage IL-1 α response identified candidate biomarkers of cartilage destruction and novel targets for therapeutic intervention in osteoarthritis.

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The N-Glycosylation Profile of Metastatic Melanoma Lymph Node Tumours

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Metastasis accounts for the majority of mortality associated with melanoma, as limited treatment options exist for advanced stages of the disease. Alterations in cell surface glycosylation, in particular, increases in highly branched tetra-antennary N-glycan structures contribute to the invasive and metastatic potential of melanoma cells. Despite a growing understanding of the role of tetra-antennary N-linked oligosaccharides in melanoma biology, there has been little progress in using these glycans as a screening tool for the early diagnosis of metastasis and predictor of patient prognosis.

Here, we demonstrate a targeted method combining PGC-LC-MS with exoglycosidase digestion for the complete structural characterisation and relative quantitation of N-glycans released from metastatic melanoma lymph node tumours. Released glycans were treated with a full array of exoglycosidase enzymes to assign monosaccharide linkage and confirm terminal epitopes on pools of good and poor prognosis patient samples. The global membrane N-glycosylation profile of tumour tissue from individual patient samples have been compared and glycan structures quantitated before and after selected exoglycosidase combinations to confirm differences in structural features including the degree of branching, sialylation and fucosylation. Over 80 glycan structures were identified, including high mannose, pauci mannose, hybrid and complex type glycans.

This study contributes to our understanding of glycosylation alterations in melanoma metastasis towards using specific glycosylation changes as prognostic markers and specific targets for therapeutic intervention.

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Proteomic and Degradomic Analysis of a Virulence-Associated Serine Peptidase from *Campylobacter jejuni*

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Campylobacter jejuni is one of the leading causes of acute gastroenteritis in the developed world, and a major antecedent for a number of debilitating autoimmune disorders. Recently, the serine peptidase Cj0511 has been identified as being required for optimum virulence and is associated with number of virulence mechanisms including biofilm formation, stress tolerance and pancreatic amylase triggered α -dextran secretion. As a component of outer membrane vesicles, which are a key mechanism for delivery of a number of classical mediators of pathogenicity including components into host cells, there is also an implication that Cj0511 may also modify host proteins during infection.

Here, we employed iTRAQ-based labelling to determine the effect of loss of the peptidase on whole protein abundance in a hypermotile population of the original sequenced strain; *C. jejuni* strain 11168H. Of the 1306 *C. jejuni* proteins quantified, only 67 were deemed to have a significant change in abundance in the Δ cj0511 strain relative to the wild-type isolate. Of these, 41 were reverted to WT levels in a complemented strain, Δ cj0511::cj0046 including a number of proteins from the *dccRS* regulon, flagellar components and protein secretory systems all of which have known associations with virulence. N-terminal amine isotopic labelling of substrates (N-TAILS) was also employed for a pair wise comparison of the N-degradome of wild-type 11168H and the cj0511 knock out strain to both identify biological targets of the protease as well as attempt to elucidate the sequence specificity of the protease. These proteomics-based approaches were complemented with various standard phenotypic tests to further establish the role of the protease towards *C. jejuni*'s physiology.

1. Karlyshev, A. V. et al.. 2014. *Campylobacter jejuni* gene cj0511 encodes a serine peptidase essential for colonisation. FEBS Open Bio 4:468-72
2. Jowiya, W. et al.. 2015. Pancreatic amylase as an environmental signal for regulation of biofilm formation and host interaction in *Campylobacter jejuni*. Infect Immun 83(12):4884-95

3. Kleifeld, O. et al.. 2010. Isotopic labelling of terminal amines in complex samples identifies protein N-termini and protease cleavage products. *Nat Biotechnol* 12(3):281-288

Towards a blood test for oesophageal adenocarcinoma: Serum glycoprotein biomarker candidates for oesophageal adenocarcinoma

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While the incidence of most cancers are now steady or declining, oesophageal adenocarcinoma (EAC) continues an upward trend. Rapid increase in EAC is not due to improved diagnosis, but attributed to the increased prevalence of risk factors gastro-oesophageal reflux and obesity. Despite aggressive treatment, the survival rate for EAC is low at 9-24% five years post-diagnosis. The precursor condition, Barrett's oesophagus (BE), affects 0.2-2% of the adult population and increases EAC risk 30-100 fold. However, due to the low conversion rate of BE to EAC, studies indicate that current endoscopic screening programs may not be beneficial. Furthermore, a significant proportion of EAC patients do not have prior BE diagnosis, hence there is an urgent need for better detection of EAC. Our goal is to develop blood biomarker panels that can be used to screen at-risk patients, with positive or suspicious results triggering follow-on endoscopic screening.

We focused on alterations in circulatory protein glycosylation, using a panel of 20 lectins to enrich serum glycoproteins based on their glycan structures. Serum samples from healthy, BE and EAC patients (n=108) were analyzed by lectin magnetic bead array (LeMBA)¹, ²-coupled biomarker discovery (LeMBA-QTOF-GlycoSelector <http://glycoselector.di.uq.edu.au/index.php>) and verification (LeMBA-MRM-MS-Shiny mixOmics <http://mixomics-projects.di.uq.edu.au/Shiny/>) pipeline.³

We have identified a list of putative serum glycoprotein biomarker that distinguish a) EAC from BE and b) EAC from healthy phenotypes. A multivariate panel achieved area under the receiver operating curve (AUROC) over 0.9, indicative of high diagnostic value. Selected biomarker candidates were further validated using an orthogonal technique, immunoblotting. The results indicate alteration of complement pathway in EAC pathogenesis. Currently we are evaluating the biomarker candidates in an independent patient cohort including early stage EAC patients. Continuing work will also evaluate performance of the biomarker panel to predict therapeutic response.

[1] Loo et al., *J Proteome Res* 2010

[2] Choi et al., *Electrophoresis* 2011

[3] Shah et al., *Mol Cell Proteomics* 2015

MALDI Imaging of primary endometrial cancers reveals proteins associated with lymph node metastasis

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Metastasis is a crucial step of malignant progression and remains the primary cause of death for patients with endometrial cancer (EC). However, clinicians presently face the challenge that conventional surgical-pathological variables, such as tumour size, depth of stromal invasion, histological grade, FIGO stage, lymphovascular space invasion or radiological imaging are unable to predict metastatic potential of the primary tumour with accuracy. In the current study, we have compared differential protein expression using primary tumour samples of EC patients diagnosed with (n=24) and without (n=34) lymph node metastasis (LNM). Using matrix assisted laser desorption and ionisation imaging mass spectrometry (MALDI-MSI), we have identified protein signatures from primary tumours to accurately predict LNM. By applying canonical correlation analysis (CCA) based variable ranking approach; we have generated a list of *m/z* values that could predict the status of LNM in EC. Protein identifications were achieved by *in situ* MALDI MS/MS in combination with LC-MS/MS. SCI LS lab software was used to visualize the spatial distribution of potential molecular discriminators and their AUC values. Using this approach, we could reliably identify two proteins, which can discriminate EC with and without LNM with an AUC score of 0.2-0.3. Further, the differential expression of proteins was validated by immunohistochemistry. In summary, MALDI-MSI has the potential to identify discriminators of metastasis using primary tumour samples.

Post translational modifications in the type 2 diabetic liver and their contribution to the diseased state

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Insulin resistance and oxidative stress are hallmarks of type 2 diabetes (T2D), commonly observed in insulin sensitive tissues including the liver. Insulin resistance has been attributed to impaired insulin signalling which normally promotes glucose uptake and utilisation through the IRS/PI3K pathway. Oxidative stress involves the increased generation/reduced clearance of reactive oxygen species (ROS), with high levels of ROS oxidising proteins producing redox modifications which may alter structure, functionality and signalling pathways. To investigate the changes in signalling mediated by phosphorylation and/or redox modifications with T2D, we investigated a rat model using global phosphoproteomics and redox proteomics. Rats were subjected to a high fat diet and streptozotocin to generate the T2D pathology. To quantify alterations in PTM status, samples were isobarically tagged and subjected to various enrichment strategies prior to mass spectrometry (MS). Phosphopeptides were enriched by a combination of TiO₂ and IMAC while redox modified peptides were enriched by thiol disulfide exchange for reversibly oxidised cysteines. Enriched and non-captured fractions were subjected to hydrophilic interaction chromatography prior to MS analysis. The insulin signalling pathway showed significant reduction in phosphorylation confirming impairment in T2D. Pathways regulating energy metabolism also showed significant perturbations in phosphorylation, possibly in response to or contributing to the energy imbalance. Oxidative modifications to the insulin receptor suggests contribution of redox modifications to insulin resistance, while modifications to proteins which maintain redox balance suggests dysregulation, contributing to the oxidative stress in T2D. The current study has identified changes in protein phosphorylation and redox state indicative of dysregulated signalling and energy utilisation while isolating species prone to oxidative damage, all able to contribute to the pathogenesis of T2D.

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The myocardial degradome following ischemia/reperfusion injury

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Myocardial ischemia and the subsequent return of normal blood flow by reperfusion can both lead to significant injury in the heart. Ischemia / reperfusion (I/R) injury is characterized biochemically by an influx of reactive oxygen species (ROS) and calcium overload, which are both thought to induce proteases (e.g. matrix metalloproteinases and calpains) that target contractile proteins and other metabolic processes that are necessary for uninhibited function of the myocytes. Such proteases are therefore assumed to contribute to contractile dysfunction, apoptosis and necrosis during I/R injury. Here we describe a high-throughput approach to identifying and quantifying the targets of proteolysis in I/R. An *ex vivo* rat heart model of brief I/R was employed in the presence and absence of ROS scavengers. N-terminal peptides were enriched and quantified by N-terminal amine isotopic labelling of substrates (N-TAILS) and analysed by LC-MS/MS. Degradation products (neo-N-termini) were identified that corresponded to previously identified markers of proteolytic damage in I/R, including the troponins (TnI and TnT) and myosin light chains 1 and 2, as well as completely novel sarcomeric proteins such as desmin and myomesin. Cleaved proteins were also identified in key metabolic pathways including oxidative phosphorylation, the TCA cycle, and redox regulation. Finally, cysteine and glycine rich protein 3 (CSRFP3) was confirmed as a target for degradation during I/R, which correlates with our previous work showing the release of CSRFP3 peptides into the coronary perfusate post-I/R in both animal models and human STEMI patients. Collectively, our data confirm the increased activity of proteases during I/R injury and identify new molecular proteolytic targets that may contribute to the functional consequences of I/R in the myocardium.

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Neuronal differentiation and analysis of Multiple sclerosis patient adipose derived stem cells

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Multiple sclerosis (MS) is a chronic demyelinating disease that typically results in cognitive and physical disability. The high incidence, early onset and long-term morbidity presents an extensive problem for public health as well as decreasing the quality of life for MS patients, due to substantial psychological stress resulting from the impairment of daily function and unpredictable nature of the disease. Whilst there are treatments available to alleviate symptoms, treat attacks and prevent relapses, there are no treatments that have the capacity to recover neurological function.

This project has further explored the capacity of autologous non-MS and MS patient adipose derived stem cells (ADSCs) potential to differentiate into neuronal cells and identifies mechanisms with links to the disease progression. This proteomics and systems biology approach will assist in neuronal regenerative medicine by providing more targeted research with the hope of restoring neurological function, and overcoming the nervous systems limited capacity to regenerate.

ADSCs are an ideal target for neuronal regenerative therapy due to their ability to transdifferentiate into ectodermal lineages and consequently form neuronal cells, and they can be sourced ethically through liposuction of adipose tissue. Characterisation of the cells was achieved by analysis of cell morphology, secreted cytokines, the proteome and validation of known protein markers.

The proteomic analysis demonstrated that ADSCs from both non-MS and MS patients differ by a large number of proteins prior to differentiation. Non-MS neuronal differentiation was confirmed and the comparison to MS patient stem cells revealed that the latter has a reduced neurogenic potential. The explored function of unique and shared proteins at the variable time-points revealed an extensive difference between the two population's stem cells and their differentiated progenies. The identified proteins reveal mechanisms that play pivotal roles within neurogenesis and roles in the disease progression. Elucidating these proteins

functionality is vital for understanding the disease and developing a targeted therapy which could utilise stem cells in a regenerative capacity.

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Towards improving the genome annotation of the honey bee (*Apis mellifera*)

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Honey bees (*Apis mellifera*) are important pollinators in managed agriculture as well as natural ecosystems. Recently, the sequencing and annotation of the honey bee genome has allowed proteomics to become a powerful technique to probe aspects of honey bee biology; however, one troubling trend that emerged from these studies is that honey bee samples consistently result in lower peptide identification rates compared to other organisms. This suggests that either the genome annotation can be substantially improved, or some atypical biological process is interfering with the mass spectrometry (MS) workflow.

We used a publically available MS dataset (Peptide Atlas; 1,472 raw files) in a proteogenomic approach to search for missing genes, new exons, and to revive discarded annotations. To do this, we searched the data against a six-frame genome translation, a three-frame refSeq RNA translation and a database including sequences that were removed from previous annotations. We also considered unexpected post-translational modifications (PTMs), high genetic diversity and endogenous proteolysis as alternative explanations for low peptide identification rates.

While we found no significant effects of PTMs, sequence diversity or proteolysis in tissues other than the gut, we did discover 1,454 new coding regions matched by two or more peptides (1% FDR), including twelve sequences that were previously annotated as non-coding RNAs. In a separate search, we matched 748 previously annotated proteins that were not retained in the current official gene set (OGS). Importantly, when the sequences were added to the OGS protein database (increasing the database size by 13.5%), this improved MS identification rates across tissues.

Using this proteogenomic strategy, we have improved the completeness of the honey bee genome annotation. The information we present here can facilitate further research on this important insect and this workflow template can be used to aid the genome annotation of other under-studied species.

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The N-linked glycosylation system of *Campylobacter jejuni*: a functional proteomics approach

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Campylobacter jejuni is the most common cause of food-borne illness worldwide, accounting for ~225,000 infections in Australia per year. Infection is zoonotic and is associated with consumption of under-cooked or poorly prepared poultry, in which *C. jejuni* is a commensal. A unique molecular feature of *C. jejuni* is the ability to post-translationally modify membrane-associated proteins by the N-linked addition of a heptasaccharide glycan. Glycan biosynthesis is performed by proteins encoded within the *pgl* (protein glycosylation) locus, and attachment is mediated by the PglB oligosaccharyltransferase. Disruption of *pgl* genes reduces chicken colonization and adhesion to human epithelial cells, however the proteins that mediate these phenotypes, and indeed the overall function of the N-glycan, remain to be determined. We developed mass spectrometric techniques that have allowed the identification of many novel N-linked glycoproteins in *C. jejuni*, and identified a non-canonical glycan that is itself modified by addition of phosphoethanolamine (pEtN) to terminal GalNAc. Quantitative proteomics and glycoproteomics have examined *C. jejuni* model strains NCTC11168 O (O), a clinical isolate, and NCTC11168 GS (GS), a laboratory-adapted avirulent strain derived from NCTC11168 O. Analysis of these strains demonstrated alterations in proteins responsible for the production of the N-linked glycan and its transfer. Increased Pgl proteins leads to elevated N-glycan biosynthesis and attachment, but this targets specific proteins rather than a general increase. Quantitative glycoproteomics also revealed glycopeptides that lack the typical *C. jejuni* glycosylation sequon (D/E-X-N-X-S/T). Recently, we have been working on the function of the N-glycosylation system, and hypothesize a role in protein stability, mediating protein complexes, and survival in diverse environments.

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Applying a Proteoform profiling method for neurological disorder biomarker discovery

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Measuring the intact masses of proteins from biological samples provides important insight into biological events which cannot be analyzed by standard bottom-up strategies. Despite technical limitations, mainly due to the large size of the biomolecules being measured, it has gained significant momentum over the last couple of years from improvements in both the general workflows and the associated technologies. Being able to analyze intact proteins rather than their proteolytic peptides enables direct measurements of the major and very important biological processes like alternative splicing, proteolytic processing and post translational modifications – including the complex patterns and stoichiometry of multi-modified proteins. The first step in

this approach is to establish a set of quantitative proteoform profiles. The differentially expressed features are then targeted and subsequently identified through a separate top-down MS/MS experiment and if needed a more comprehensive characterization. In this study we have used a quantitative proteoform profiling experiment to detect and identify several potential biomarkers for Alzheimer's disease based on cerebrospinal fluid (CSF). We were able to comfortably and reproducibly detect more than 1500 proteoforms in each sample leading to the detection and identification of 77 differentially regulated proteoforms (potential biomarker candidates) which are currently being identified and validated through a scheduled top-down MS/MS approach. Many of those correspond to proteoforms of proteins carrying a specific modification, a mutation or having undergone a proteolytic modification which would have made their characterization much more challenging, if not impossible, with a bottom-up workflow. One clear example of this is the diagnostic 1:2 distribution of the truncated and mutated versions of Secretogranin 2 which also clearly illustrates the big potential of the proteoform profiling approach. We are currently extending this study to larger cohorts of patients to both validate our current findings and potentially expand the list of new potential biomarkers.

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Label-free Proteomic Analysis of Microdissected Formalin-fixed and Paraffin Embedded Cutaneous Squamous Cell Carcinoma Tissue

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BACKGROUND: Cutaneous Squamous Cell Carcinoma (cSCC) is one of the most common types of non-melanoma skin cancer. Histopathologic variants of cSCC show significantly different clinical behavior and their correct classification is critical for diagnosis, prognosis and therapy.

METHOD: In the present study, we employed laser-capture microdissection coupled with liquid chromatography–tandem mass spectrometry (LC-MS) to test the feasibility of using archival formalin-fixed paraffin embedded (FFPE) cSCC material (five patients) for proteomic investigations that could provide reliable biomarkers for the cSCC clinical management. Pair-wise comparisons were performed between proteomes extracted from tumor and adjacent morphologically normal tissue for each patient. Ingenuity Pathway Analysis (IPA) bioinformatics suite was used to interpret the roles of altered proteins in cSCC pathophysiology. Oncomine database was used to assess the gene expression levels of proteins changed in our dataset.

RESULT AND DISCUSSION: In total, 1310 unique protein species were identified across all five patients. Of these, an average of 144 proteins were significantly increased and 88 proteins decreased in the tumor samples compared to their normal counterparts ($p < 0.05$). IPA analysis revealed the disruptions are mainly in proteins and upstream regulators associated with the regulation and control of cell proliferation, apoptosis, and cell movement. Genes corresponding to 18 proteins were also differentially expressed at the transcript level in silico, supporting our initial proteomic measurements.

CONCLUSION: Our findings confirmed that label-free mass spectrometry-based quantitative proteomics is a useful platform for analyzing FFPE cSCC tissues.

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Proteomic Analysis of an Artelinic Acid-Resistant *P. falciparum* strain

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Resistance to artemisinin and its derivatives is becoming a serious threat to current antimalarial drug therapy. Understanding the molecular mechanisms underlying artemisinin resistance will aid drug development and malaria control. Recently, genetic screens have identified the involvement of the Kelch13 gene in screens of clinical samples with a slow parasite clearance time, and PfMDR1 gene amplification in laboratory-derived strains of *P. falciparum* resistant to 80 ng/ml artelinic acid, a semi-synthetic derivative of artemisinin. In these resistant strains, the resistance phenotype has been demonstrated to have (i) decreased sensitivity of mature-stage parasites, (ii) decreased sensitivity of the ring stage to the induction of dormancy, and (iii) a faster recovery from dormancy. In an effort to identify proteins and pathways involved in the resistance phenotype, we have used Orbitrap mass spectrometry-based proteomics to compare global protein expression levels in parallel cultures of resistant and wild-type strains. This has identified 11 proteins whose expression is significantly associated with artelinic acid resistance. These proteins and their potential involvement in artemisinin resistance will be discussed.

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High-Speed Imaging Mass Spectrometry of N-Linked Glycans Provides a new approach to Molecular Histology and Tissue typing of Tumors

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Introduction: Imaging mass spectrometry (IMS) is a unique analytical tool that allows simultaneous label-free visualization of hundreds of compounds expressed in tissue. When applied to tumor samples, IMS provides a means of correlating many lipids/peptides/proteins to histological features thereby creating a multi-dimensional scale for molecular histology. N-linked

glycosylation of proteins is an important subset of the molecular mechanisms important for cellular homeostasis, and disease state alters certain glycosylation mechanisms. The aim of this study was to use a new high-speed IMS tissue typing platform to map and identify N-glycan markers from prostate and breast tumor samples to determine the suitability using the high-speed platform for N-glycan tissue typing.

Experimental: Sections were cut at 5 micron from a tissue microarray of breast cancer, 48 HER2+/HER2- cores, and FFPE prostate biopsies. These were mounted onto conductive microscope slides. Each slide was processed with standard antigen retrieval methods, followed by aerosol applications of peptide N-glycanase to release N-linked glycans and matrix for matrix-assisted laser desorption (MALDI). Imaging mass spectrometry analysis of the released N-glycans was done on a Bruker rapifleX at image resolutions of 10-50 $\mu\text{m}/\text{spot}$.

Results: Results from the high-speed imaging measurements were found to correlate with data generated from previous studies. A number of histopathology-specific glycan distributions were identified. The high-speed imaging system provided acquisition times similar to that of standard histology, ranging from seconds for each TMA core to a few minutes for the larger biopsies. Images of the most abundant N-glycans (15-20 species) distinguish tumor-related stroma vs normal stroma in the prostate biopsies. In the breast cancer TMA, images show that N-glycan abundances correlate with HER2 status of cancer regions in the cores.

Conclusion: High-speed images of N-glycosylation patterns are characteristic of tissue pathology, making it possible to create large-cohort databases.

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Quantitative peptide assay for optimized and reproducible sample preparations for mass spectrometry applications

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New advances in mass spectrometry (MS) have pushed the limits of sensitivity, enabling comprehensive characterization and accurate quantitation of complete proteomes. Despite rapid advances in analytical instrumentation and software, data quality to a large extent still depends on upstream sample preparation techniques. A significant reduction in variability should be possible by standardizing sample preparation steps and analyzing accurate quantities of peptide material in each LC/MS analysis. In this study, we utilized a recently introduced peptide quantitation assay to monitor the protein and peptide concentration at each step of Thermo Scientific tandem mass tagTM (TMTTM) reagents sample preparation workflow. Standardizing the digestion, labeling and fractionation steps using a peptide quantitation assay significantly improved reproducibility and quality of the MS results.

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Look who's talking: UHPLC-MRM/MS assay for staphylococcal quorum-sensing and virulence peptides

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With the ongoing rise of antibiotic-resistant pathogens such as Methicillin-resistant *Staphylococcus aureus*, and the loss of their effective treatment, novel approaches to tackle this superbug crisis are urgently needed. The exploitation of bacterial quorum sensing (QS), i.e. the regulation of gene expression in response to fluctuations in cell-population density, has previously been discussed as a potential leverage point to combat pathogens. By interfering with the bacterial communication process, also known as quorum quenching, the goal is to shut down virulence production without developing resistance mechanisms. *S. aureus* employs oligopeptides, so-called autoinducer peptides (AIPs), as QS signaling molecules. AIPs consist of about 8 amino acids (AA) with a thiolester linkage between the central cysteine and the C-terminal carboxyl group. AIPs released by different species have divergent AA sequences but conserved thiolactone ring structure. After the detection of a minimal threshold concentration of AIPs, *S. aureus* expresses phenol-soluble modulins (PSMs), a toxin family usually composed of 21-26 AA arranged in an alpha-helix.

To facilitate monitoring of AIPs and PSMs in bacterial communities, the purpose of this project was to establish a high-throughput mass spec method for screening of staphylococcal isolates. To establish multiple reaction monitoring (MRM) methods, selected AIP and PSM peptides were custom-synthesized and used to experimentally compile a Q1/Q3 transition list and optimize collision energies. Finally, we have been able to squeeze the separation of the physico-chemically very different peptides in a 4.5 min gradient on an Agilent Poroshell column. Screening efforts are currently underway to sensitively detect and quantify AIP and PSM peptides in culture media filtrates from bacterial isolates. Furthermore, gathered knowledge about the fragmentation behavior of cyclic peptides will aid to generate transition lists *in silico* in the future.

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Epidermal bladder cells as a single-cell-type system to further our understanding of the molecular mechanisms involved in plant salt tolerance and water use efficiency.

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Crassulacean acid metabolism (CAM) is an alternative photosynthetic mode that achieves high water use efficiency through nocturnal CO₂ uptake and daytime stomatal closure. Crop plants that undergo CAM would be particularly attractive alternatives to C₃ crops for agricultural production in semi-arid, water-limited environments and there is increased interest in engineering CAM into C₃ crops (DePaoli et al., 2014). *Mesembryanthemum crystallinum* is a halophyte and facultative CAM plant, with the ability to switch its pathway of CO₂ assimilation from C₃ metabolism to CAM in response to environmental cues including water deficit, salinity or high light. This ability for photosynthetic plasticity makes it an extremely useful model experimental system for understanding the underlying molecular mechanisms of the CAM pathway and additionally, the mechanisms of environmental induction of complex traits in plants. Our lab has been using the specialized model trichomes called epidermal bladder cells that line the aerial parts of *M. crystallinum* as a single cell type system to gain further understanding of CAM and salt-tolerance mechanisms. Transcriptomics, proteomics, metabolomics and ionomics studies have allowed an integrated understanding of the role of these cells, with results contradicting earlier reports these cells were solely repositories for sodium and water (Oh et al., 2015; Barkla et al., 2012; Barkla et al., submitted; Barkla and Vera-Estrella, 2015). Our findings show that photosynthesis and primary metabolism in the cell supports cell growth, ion accumulation, compatible solute synthesis and CAM.

1. DePaoli HC, Borland AM, Tuskan GA, Cushman JC, Yang X. 2014. Synthetic biology as it relates to CAM photosynthesis: challenges and opportunities. *J. Exp. Bot.* 65: 3381-3393.
2. Oh D-H, Barkla BJ, Vera-Estrella R, Pantoja O, Lee SY, Bohnert HJ, Dassanayake M. 2015. Cell type-specific responses to salinity- the epidermal bladder cell transcriptome of *Mesembryanthemum crystallinum*. *New Phytologist* 15: 207:627-644.
3. Barkla BJ, Vera-Estrella R, Pantoja O. 2012. Protein profiling of epidermal bladder cells from the halophyte *Mesembryanthemum crystallinum*. *Proteomics* 12: 2862-2865.
4. Barkla BJ, Vera-Estrella, Raymond CA. Single-cell-type quantitative proteomic and ionic analysis of epidermal bladder cells from the halophyte model plant *Mesembryanthemum crystallinum*. Submitted manuscript
5. Barkla BJ, Vera-Estrella, Raymond CA. Single-cell-type quantitative proteomic and ionic analysis of epidermal bladder cells from the halophyte model plant *Mesembryanthemum crystallinum*. Submitted manuscript
6. Barkla BJ, Vera-Estrella R. 2015. Single cell-type comparative metabolomics of epidermal bladder cells from the halophyte *Mesembryanthemum crystallinum*. *Frontiers in Plant Science* 6:435.

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Terminal galactosylation and sialylation switching on membrane glycoproteins upon TNF- α -induced insulin resistance in adipocytes

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Insulin resistance (IR) is a complex pathophysiological state that arises from both environmental and genetic perturbations and leads to a variety of diseases including type-2 diabetes (T2D). Obesity is associated with enhanced adipose tissue inflammation, which may play a role in disease progression. Inflammation modulates protein glycosylation in a variety of cell types and this has been associated with biological dysregulation. Here we have examined the effects of an inflammatory insult on protein glycosylation in adipocytes. We performed quantitative *N*-glycome profiling of membrane proteins derived from mouse 3T3-L1 adipocytes that had been incubated with or without the proinflammatory cytokine TNF- α to induce IR. We identified the regulation of specific terminal *N*-glycan epitopes, including an increase in terminal di-galactose- and a decrease in biantennary alpha-2,3-sialoglycans. The altered *N*-glycosylation of TNF- α treated adipocytes correlated with the regulation of specific glycosyltransferases including the up-regulation of B4GalT5 and Ggta1 galactosyltransferases and down-regulation of ST3Gal6 sialyltransferase. Knockdown of B4GalT5 down-regulated the terminal di-galactose *N*-glycans, confirming the involvement of this enzyme in the TNF- α regulated *N*-glycome. SILAC-based quantitative glycoproteomics of enriched *N*-glycopeptides with and without deglycosylation were used to identify the protein and glycosylation sites modified with these regulated *N*-glycans. The combined proteome and glycoproteome workflow provided a relative quantification of changes in protein abundance versus *N*-glycosylation occupancy versus site-specific *N*-glycans on a proteome-wide level. This revealed the modulation of *N*-glycosylation on specific proteins in IR, including those previously associated with insulin-stimulated GLUT4 trafficking to the plasma membrane.

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Modulation of neurotransmitter release by phospho-signalling: Major substrates and master regulators

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To achieve understanding of how neural circuits function, we need to unravel the neuronal integration of incoming signals and modulation of synaptic transmission strength over time. This is a process known as synaptic plasticity and is an essential mechanism behind memory and learning. While focus has largely been on post-synaptic plasticity, pre-synaptic plasticity has been widely neglected. An important part of pre-synaptic plasticity is modulation of the synaptic vesicle cycle, which governs vesicle availability/localization – and thereby neurotransmitter release potential.

Protein phosphorylation modulates several aspects of the synaptic vesicle cycle and is thus a potential widespread mode of presynaptic plasticity. We have isolated presynaptic nerve terminals (synaptosomes) from rats and depolarized them with KCl to investigate the role of activity-dependent phospho-signaling in neurotransmission up to 15 min after depolarization. Performing

enzymatic fluorescence-based assays of glutamate release, we show that this treatment induces synaptic depression. We have quantified >5000 phosphorylation sites in synaptosomes using dimethylation, phospho-peptide enrichment and fractionation via TiO₂, Sequential elution from IMAC and HILIC (TiSH) followed by nanoLC-MS analysis. We observe prolonged reprogramming of the presynaptic phosphoproteome after depolarization. Changes in regulatory phosphosites on phosphatase regulators – key integrators of neuronal signaling networks – suggest a likely link between depolarisation and long-term global presynaptic phosphorylation changes. Importantly, the protein machinery of the active zone controlling the release of neurotransmitter-containing vesicles appears to be regulated by activity-dependent phosphorylation. Amongst these proteins, the key synaptic plasticity protein Rab3 interacting molecule (RIM1), which links calcium influx to neurotransmitter release, contained several regulated phosphorylation sites without known functions. This establishes RIM1 as a prime candidate for being the link between activity-dependent phospho-regulation and presynaptic plasticity.

MS-based protein pulldown experiments using phospho-deficient and -mimetic mutants of RIM1 are currently underway and will reveal the phospho-dependent mediators of presynaptic plasticity. Primary hippocampal neurons will be transfected with these constructs to validate the functional effect of the RIM1 phosphorylation sites using dye uptake/release assays.

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Elongation factor Thermo unstable (Ef-Tu) is proteolytically processed on the extracellular surface of *Staphylococcus aureus*

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Staphylococcus aureus is a leading cause of nosocomial-acquired infections and has rapidly acquired resistance to multiple antibiotics. Pathogenic and commensal bacteria present multifunctional surface adhesins that bind to a wide range of host molecules, particularly those associated with the extracellular matrix. Glycosaminoglycans are components of the extracellular matrix (ECM) that are targeted by bacterial adhesins. Heparin is a glycosaminoglycan that mimics highly sulphated regions of proteoglycans and it has been used as a bait to identify bacterial virulence factors that facilitate adherence, colonisation and invasion of target host cells.

To characterise the surfacome of *S. aureus* we tagged surface proteins with biotin and identified them by streptavidin affinity chromatography and LC-MS/MS. A complimentary set of surface proteins were identified by shaving *S. aureus* with trypsin. Dimethyl labelling of neo-N-termini followed by LC-MS/MS was used to identify cleavage start sites. Unfractionated native WCL were used to identify heparin-binding proteins via heparin-agarose chromatography. We applied this technique to gain insights into the repertoire of *S. aureus* surface proteins that have evolved the ability to bind highly sulphated glycosaminoglycans and correlated the identities of these proteins with those from our surfacome studies.

318 proteins were identified on the surface of SH1000. Notably, Ef-Tu was identified as an intact protein and as cleaved fragments and both the intact molecule and the cleavage fragments bound heparin. Dimethyl-labelling studies of neo-N-termini enabled us to determine precise sites of cleavage within Ef-Tu. For the first time, these data suggest that Ef-Tu and processed fragments of Ef-Tu can perform alternate functions on the surface of *S. aureus*. Our studies indicate that cleavage events target a region of the molecule that separates the major subdomains within Ef-Tu, suggesting that processing may function to increase functional diversity.

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An Integrated Approach to Metabolomics Studies in Pancreatic Cancer Cells: Discovery to “Quantification” on a Single Platform

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Mass spectrometry (MS)-based metabolomics is one approach used to investigate the metabolic profiles of cancer cells. To gain a comprehensive interpretation of the metabolite variation profiles in biological systems, qualitative and quantitative information is required. This typically involves a discovery-driven untargeted profiling analysis using a high resolution accurate mass (HRAM) MS platform followed by a hypothesis-driven targeted analysis using a tandem triple quadrupole MS platform. Here, we propose a metabolomics research paradigm shift which utilizes a hybrid quadrupole-Orbitrap mass spectrometer to achieve an integrative approach of these two methodologies. The discovery and targeted workflows to study the metabolic profiles of *Panc-1* cancer cells will be discussed.

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Insight into protein glycosylation in human capacitated sperm using PGC-LC-MS/MS

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In order to achieve a successful pregnancy, sperm rely on surface glycans, which are constantly remodelled throughout their life cycle. Glycans within spermatozoa are essential for several processes, including passage through cervical mucus within the female reproductive track, protection from female immunity and Capacitation. Capacitation, which begins when a sperm is ejaculated, is the term used to describe the biochemical processes that modifies a sperm and enables it to fertilise an Oocyte. Interestingly, Glycans have shown to be highly modified during the capacitation (1) and as such, may play a major role in male infertility; something that affects 1 in 15 men of reproductive age. However, the role of specific glycans and their structures during sperm capacitation is still unknown.

In this work, *in vitro* capacitated sperm were enriched by Percoll density gradient centrifugation. Here we present glycan analysis of capacitated and non-capacitated sperm using porous graphitised carbon-liquid chromatography-MS/MS. Membrane proteins were extracted by different methods, immobilised on a PVDF membrane then treated with PNGase F to release *N*-linked glycans. The subsequent β -elimination allows the collection of *O*-linked glycans from the same proteins. Preliminary results suggested the presence of high mannose, paucimannose and hybrid-complex structures, and membrane enrichment by ultracentrifugation as the best method for high glycan yields from sperm proteins. The differences in glycosylation profiles between capacitated and non-capacitated samples will be presented. The identification of specific glycans associated with sperm capacitation will allow the selection of healthy sperm for *in vitro* fertilisation applications.

1. Teclé E, Gagneux P. 2015. Sugar coated sperm: unravelling the functions of the mammalian sperm glycocalyx. *Molecular Reproduction and Development*. 82:635–650.

A comparative transcriptomic and proteomic analysis of the infection of mammalian cells with Nelson Bay orthoreovirus

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Nelson Bay orthoreovirus (NBV) is the prototypic member of the Pteropine orthoreovirus family. Previous work in our laboratory has shown that mouse fibroblast cells are capable of restricting the replication of NBV, *in vitro*, compared to other mammalian cells. The mechanism involves suppressing viral induced cell fusion (termed syncytia). Here, we investigate this unique host-pathogen interaction further using an integrated transcriptomic and proteomic approach. Employing RNA sequencing and SILAC, the gene and protein expression profiles of mouse fibroblast and immortalised bat kidney cells following NBV infection was assessed.

The evaluation of the gene and protein expression data demonstrate that mouse fibroblasts respond to NBV through a robust type I interferon response. We show that knockdown of the type I interferon receptor (IFNAR1) results in an increase in NBV replication and in syncytia formation in mouse fibroblasts. In contrast, stimulation of type I interferon signalling in bat cells with recombinant interferon decreases viral replication. Our results suggests type I interferon signalling plays a crucial role in mediating NBV replication and viral induced cell fusion. We are currently investigating which element of the broad type I interferon signalling pathway is involved in mediating this interaction between mouse fibroblast cells and NBV.

By understanding the molecular mechanisms that influence NBV replication, antiviral strategies may be applied to other fusogenic orthoreoviruses.

Multi-omics analysis of primary cytotrophoblasts from second trimester and term placentas

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During human pregnancy, a subset of placental cytotrophoblasts (CTBs) differentiates into cells that aggressively invade the uterus and its vasculature, anchoring the progeny and rerouting maternal blood to the placenta. Defects in this process are the hallmark of the pregnancy complication preeclampsia. While disease-associated genes or transcripts may serve as useful biomarkers, they are not necessarily predictive of disease mechanisms. Thus, we performed global proteomic and transcriptional profiling to measure expression patterns of CTBs from second trimester and term normal placentas to gain further understanding of CTB differentiation in healthy pregnancy.

Primary CTBs were isolated using collagenase and trypsin digestion and Percoll gradient centrifugation. For proteomics analysis, cells were lysed and digested with trypsin. Variable window SWATH[®] Acquisition data were acquired using the TripleTOF 6600 System (SCIEX). A 180 min gradient using a nanoLC[™] 425 with a cHiPLC[®] system (SCIEX) was performed for separation. SWATH data were processed using OneOmics[™] applications in BaseSpace (Illumina). For transcriptomics, a second set of CTBs were analyzed using RNAseq (Kundaje, Nature 2015). iPathwayGuide (Advaita) was used to compare protein and RNA levels for pathway and gene ontology analyses.

Approximately 3000 proteins were quantified and ~400 showed differential expression in second trimester CTBs vs. term. Proteins known to function in CTB differentiation processes, e.g. angiogenesis and hypoxia response, as well as previously uncharacterized processes, e.g., NF- κ B signaling, metal ion transport and muscle contraction, were altered. Integration with RNAseq data showed variations at the molecular level but concordance among biological processes. Multi-omics data sets comprised of SWATH MS protein quantification and RNAseq expression results showed gestation age differences in healthy CTB

populations corresponding to known and novel processes. In future, these methods will be used to study CTBs from patients with preeclampsia is likely to identify aberrations that could contribute to disease and/or serve as diagnostic markers.

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Quantitative proteomic analysis of plasma proteins in healthy neonates, young children and adults using SWATH-MS data independent acquisition

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SWATH-MS data independent acquisition is now commonly used to determine the abundance of proteins in complex biological samples. It is well suited for quantitative studies across many biological samples, but has not yet been fully exploited for applications of human plasma biomarker discovery.

In this paper, we used SWATH-MS to profile changes in plasma proteins associated with healthy human infant development. We profiled 40 individual plasma samples from four sample age groups (neonates, < 1-year olds, 1-5 year olds, adults, n=10 each). A local SWATH peptide library was built by combining 1D nanoLC MS/MS IDA runs from each pooled group, and an extended SWATH peptide library was also evaluated by merging the local library with publicly available MS spectral repository in SWATHAtlas.

Using undepleted plasma, 118 proteins with minimum of two peptides were quantified when the local library was used which contained 146 proteins while the extended library allowed for putative identification and quantification of more than 900 proteins. Overall, the changes in neonatal plasma proteins were the most significant when compared with the other three groups. Using both protein and peptide cut-off criteria to determine proteins changed in expression, the local library yielded 17 and 26 proteins up- and down-regulated respectively when compared with children under one year of age. Correspondingly, 8 and 35 proteins were found to be up- and down-regulated respectively when compared to 1-5 year old children; and 9 and 25 proteins with adults group. Interestingly, the number of differentially expressed proteins was only slightly higher with the extended library. In summary, this is the first description of using SWATH-MS to profile the expression of plasma proteins associated with human infant development.

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SWATH mass spectrometry performance using extended ion assay libraries

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The use of data-independent acquisition methods such as SWATH for mass spectrometry based proteomics is usually performed using peptide MS/MS reference ion assay libraries which enable identification and quantitation of peptide peak areas. Reference assay libraries can be generated locally through information dependent acquisition, or obtained from shared data repositories for commonly studied organisms. However, there have been no studies performed to systematically evaluate how locally-generated or repository-based assay libraries affect SWATH performance for proteomic studies. To undertake this analysis we developed a software workflow, *SwathXtend*, which generates extended peptide assay libraries using a local seed library and delivers statistical analysis of SWATH-based sample comparisons. We designed test samples using peptides from a yeast extract spiked into peptides from human K562 cell lysates at different ratios to simulate common protein abundance change comparisons. SWATH-MS data with 2, 5 and 10% of yeast peptides spiked into the human cell lysate were assessed using several local and repository-based assay libraries of different complexities and proteome compositions. We evaluated detection specificity and accuracy to detect differentially abundant proteins and reporting thresholds for statistical analyses. We demonstrate that extended assay libraries integrated with local seed libraries achieve better performance than local limited assay libraries alone from the aspects of the number of peptides and proteins identified and the specificity to detect differentially abundant proteins; the performance of extended assay libraries heavily depend on the similarity of the seed and add-on libraries; statistical analysis with multiple testing correction can improve the statistical rigor needed when using large, extended assay libraries.

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Diagnosis of Amyloidosis Subtype by Laser-Capture Microdissection (LCM) and Tandem Mass Spectrometry (MS/MS) Proteomic Analysis

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Background & Aims

Correct identification of the protein that is causing amyloidosis is crucial for clinical management. Current standard laboratory methods have limited ability to detect the full range of amyloid forming proteins. We assessed the diagnostic value of LCM-MS/MS, which combines specific sampling of amyloid deposits by LCM with protein identification by MS/MS.

Methods

Biopsy specimens were referred to the Princess Alexandra Hospital Amyloidosis Centre. For all specimens, 10µm sections of formalin-fixed paraffin embedded tissue were stained with Congo Red using a standard technique. LCM was performed using an Arcturus XT instrument with an infrared capture laser. Proteins were extracted with FFPE Protein Extraction Solution, digested with trypsin and peptides were analysed by nano-liquid chromatography-coupled MS/MS using an Agilent Chip CUBE-QTOF.

Results

Biopsies were received for 136 patients from 35 different organ sites. There was insufficient tissue in the block for 7 cases, repeat LCM was required in 15 cases. An amyloid forming protein was identified in 121 out of 129 attempted cases (94%). Of the 121 successfully cases, the Mayo Clinic (at least two of SAP, ApoE and ApoA4) was detected in 92 (76%), and was associated with higher Amyloid protein score and % coverage. We found that vitronectin provided complementary information to the existing 3-protein amyloid signature. In terms of clinical impact, amyloid typing by immunohistochemical stains had been attempted in 87 cases and reported as diagnostic in 39. Five of these were subsequently revealed by proteomic analysis to be incorrect. Overall, the clinical diagnosis of amyloid subtype was altered by proteomic analysis in 24% of cases.

Conclusions

LCM with LC-MS/MS successfully identified an amyloid forming protein in 94% of clinical biopsy samples. Amyloid deposits often contain small amounts of other amyloid forming proteins. Because of this, results need to be interpreted in the context of full clinical information to enable correct diagnosis of amyloid subtype.

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Phosphoproteomics reveals Aurora kinase B (AurkB) as a regulator of neuronal development

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Protein kinases are utilized by cells during cell division and development, cytokinesis, and for the maintenance of metabolic homeostasis. Given the well-established role of Aurora kinase B (AurkB) in cellular division and cancer development, we have made a surprising recent discovery – that AurkB has a critical role in axonal outgrowth of neurons (a non-dividing cell). We have recently identified AurkB in neuronal regeneration [1] but its substrates involved in neurite outgrowth remains to be investigated. Here, we aim to explore the potential phosphorylated substrates of AurkB and their involvement in neurogenesis. Utilizing titanium dioxide enrichment and LC-MS/MS, differentiated RGC-5 cells grown in SILAC neurobasal media were treated with AurkB inhibitor, AZD1152-HQPA, and cell lysates were digested with trypsin and enriched for phosphopeptides. We identified >10,000 phosphopeptides, in which ~650 phosphopeptides were differentially down-regulated upon treatment. Preliminary analysis revealed that some key proteins involved in axonal transport are under the regulation of AurkB. The role of AurkB was further supported by in vivo studies of transgenic zebrafish, demonstrating that overexpression of AurkB regulates axonal elongation and neurite outgrowth. Collectively, this data suggests that apart from cellular division, AurkB could play a critical role in the development of neurons.

- 1) Ng, J. M., Chen, M. J., Leung, J. Y., Peng, Z. F., Manikandan, J., Qi, R. Z., . . . Chung, R. S. (2012). Transcriptional insights on the regenerative mechanics of axotomized neurons in vitro. *J Cell Mol Med*, 16(4), 789-811. doi:10.1111/j.1582-4934.2011.01361.x

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A slimy situation: Using proteomics to highlight mechanisms of slime secretion in the southern bottletail squid, *Sepiadarium austrinum*.

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Cephalopods comprise over 800 species, possess advanced nervous, cardiovascular and visual systems, and are masters of camouflage. They are also thought to use toxic secretions widely for defence and predation but very little is known about the proteomic composition of these secretions. One such species, *Sepiadarium austrinum*, the Southern bottletail squid, secretes viscous slime from its underside. This substance is thought to be toxic to predators such as crabs but the physical structure of slime itself is also of interest because it is remarkably voluminous and rapidly secreted.

Along with identifying the putative toxic proteins within the slime secretions of *S. austrinum*, this study compares its protein composition to the slime of sea stars and planarians, and speculates on potential similarities in slime mechanism to that of hagfish. Proteins involved in the hagfish slime secretion include calcium regulators as well as intermediate filament proteins, both of which are abundant within *S. austrinum* slime.

By combining a de novo assembled transcriptome from various tissues including the slime, with the proteomics of *S. austrinum* slime, our study was able to uncover this remarkable defence mechanism within a relatively unknown species. The method used for identifying proteins in non-model organisms has allowed a greater understanding of the proteins involved in the mechanisms that control the secretion of slime within *S. austrinum*. This method will also be used on other closely related species to create a comparative proteomic study on the secretions of cephalopods and highlight potentially novel proteins.

Identifying the proteins found within *S. austrinum* slime is important both from an ecological and evolutionary perspective as well as having the high potential to produce biomedical products, and biomimetics.

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PromarkerD: Biomarkers for Diabetic Kidney Disease

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Chronic kidney disease is a significant complication of diabetes that affects >35% of patients and is a major cause of renal transplantation globally with 10-20% of diabetics dying of kidney failure. The current diagnostic test for diabetic kidney disease uses a single urine biomarker that measures microalbumin levels (albumin/creatinine ratio, ACR) which has limited utility. Clinicians require better tools that deliver a more effective diagnosis, prognosis and monitor treatment to notably improve health outcomes.

A panel of **12 candidate biomarkers** were identified by mass spectrometry in a pilot study of 90 highly curated clinical samples. A further **diagnostic** study analysing 572 individuals has validated protein biomarkers at high stringency using mass spectrometry, including proteins involved in metabolism, inflammation and oxidative stress. Cross-validation of these findings against antibody-multiplexing approaches has yielded **highly significant correlations between biomarker levels and the ACR and eGFR** measurements of these patients. Specific ROC curves were constructed for combinations of biomarkers that show that the panel can discriminate different risk categories of diabetic kidney disease.

The final **prognostic** dataset was collected from 349 patients at three key time points (Years 0, 2, 4). The statistical analysis of this data revealed the predictive ability of the biomarker panel to **determine which patients were at risk of significant and rapid decline in kidney function** better than any other known measure. The preferred model of 3 biomarkers as a predictor of eGFR decline had an **AUC of 0.83 with 89% sensitivity, 68% specificity**. People who have altered levels of protein from the biomarker panel are up to 7 times more likely to be in the eGFR decliner trajectory group.

PromarkerD as a panel of biomarkers for diabetic kidney disease has the ability to both diagnose existing disease where the current measures are inadequate and predict future significant decline in kidney function. The target outcome is a **point-of-care test** or **companion diagnostic** for diabetic kidney disease.

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Characterisation of a Potential Nutrient Transporter Protein (Cj0025) in *Campylobacter jejuni*

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Campylobacter jejuni is recognized as the most common pathogen responsible for bacterial gastroenteritis in the developed world, with the route of infection involving under-cooked or poorly prepared poultry as the organism resides as a commensal in the intestines of avian species. The ability of *C. jejuni* to survive in a diverse range of environments, colonise chickens and to adhere to and invade human gut epithelial cells has been strongly linked to factors including motility and the ability to uptake and utilise 'unusual' nutrients, especially organic and amino acids, rather than sugars. *C. jejuni* encodes a putative C₄-dicarboxylate transporter (gene designation *cj0025c*) that has been identified as the most up-regulated protein in proteomics analysis of *C. jejuni* grown under conditions of stress that mimic human hosts. The function of Cj0025c however, as well as the substrate it transports, remain unknown. Here, we use quantitative mass spectrometry-based proteomics using both iTRAQ and SWATH methods to compare proteomes of wild-type 11168H and *cj0025c* knockout strains with and without additional treatment conditions.

iTRAQ-based proteomic analysis of the Δ *cj0025c* mutant revealed several functional clusters of proteins changing in abundance, including proteins involved in lipooligosaccharide modification and flagellar motility. SWATH-based proteomics successfully validated the Δ *cj0025c* versus wild-type proteome comparison, and revealed clusters of proteins with altered abundance when cells were subjected to osmotic stress, including ribosomal and tryptophan biosynthesis proteins.

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Investigation of prostasin's role in placental development by proteomic and degradomic approaches

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Prostasin is a membrane-anchored serine protease, expressed in the epithelium of multiple organs and known to regulate the epithelial sodium channel. In several studies it has been linked to pre-eclampsia.

In mice, prostasin deficiency leads to placental malfunction and embryonal lethality from day 13.5 onwards. To understand the consequences of deleting prostasin in the placenta, we quantitatively compared placenta lysates of wildtype and prostasin deficient mice by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in conjunction with stable isotope labeling. Furthermore we were interested in prostasin's substrates and performed "terminal amine isotopic labeling of substrates" (TAILS), an N-terminomic technique for the identification and quantification of native and proteolytically generated protein N-termini.

Prostasin deficiency leads to alterations in the abundance of proteins involved in angiogenesis, placental development and several members of the carcinoembryonic antigen family. TAILS revealed that Prostasin affects a multitude of proteolytic processing sites. To underline these findings validations by immunohistochemistry and western blot analysis will be performed.

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Haptens Happen: Searching for drug modified Human Leukocyte Antigen-Peptide Complexes

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Adverse drug reactions (ADRs) inflict a significant toll on our health care system, preventing the use of key medications. A subset of ADRs involve the inappropriate activation of T cells resulting in immune responses targeted against healthy tissues. The Human Leukocyte antigen (HLA) class I and II molecules are cell surface proteins that present both self and pathogen derived peptides to CD8 and CD4 T cells, respectively. Under immunogenic conditions, peptides that are not part of the normal self-repertoire (for example pathogen derived peptides) stimulate T cell responses to help combat infection. Drugs such as beta-lactam antibiotics (including penicillins) have been shown to modify proteins and T cell responses to antigen presenting cells fed with drug modified human serum albumin have been observed. Here we explore modifications induced by penicillin-G in cell culture media containing either bovine or human serum. We use these data, in combination with the large amount of HLA-peptide ligand data amassed in the laboratory, to explore the potential for both direct haptenation of, and haptenated peptide presentation by, the HLA molecules. These data lay the groundwork for screening for drug-modified HLA-peptide complexes.

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A Simple and Convenient Way to Pack Capillary LC Columns

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Packing your own columns for capillary and nano LC is very common in the research community but has a number of disadvantages over commercially packed columns. In a lab it is harder to get the columns reproducible due the variation in bed height, the manufacture of the frits and the fact that fused silica capillaries are fragile. Packing your own columns also require a pressurised packing bomb and a high pressure gas supply.

We have developed a column packing kit where the fused silica capillaries are replaced by PEEKsil, a PEEK coated fused silica capillary. Columns with the desired ID and length are butt connected to connection capillaries with integrated low-volume screens and slurry packed using the HPLC pump as the packing solvent delivery system. The resulting columns have a precise bed height, are more robust than fused silica columns and can be reversed for column cleaning purposes. Independent test have also shown them to be equal in performance or superior to commercially available columns.

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Systematic Comparison of Integrated Microfluidics/Nanoscale LC Platforms and High/Low Resolution Mass Spectrometers for Quantitative MRM Analysis of Candidate Peptide Biomarkers

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Biomarker discovery and validation are the first steps in understanding disease and drug development. Validation is technologically challenged since it requires analyzing a large number of samples with high-throughput, but also requires high sensitivity, high resolution, large dynamic range and excellent selectivity. Targeted LC-MS based assays afford protein quantification with the reproducibility and throughput required in order to improve biomarker acceptance. MRM, using both low, high and ion mobility enabled high resolution mass spectrometry, are enabling technologies. Miniaturized LC systems offer improved mass-sensitivity but often lack throughput, robustness and reproducibility. Here, we compare the application of an integrated microfluidic device with nanoscale LC, using different MS platforms, for the quantification of marker peptides and proteins, considering speed, sensitivity and selectivity. Important aspects when characterizing LC-MS systems for validation/translation experiments are retention time reproducibility, technical reproducibility of the monitored transitions, and consistent, quantitative measurement accuracy. MRM transitions were inspected ensuring that a minimum of two transitions per peptide were detected. Typical retention time reproducibility for the monitored peptides was shown to be acceptable at microfluidic and nanoscale LC system levels and equaled 0.02 min standard deviation. Ratio measurements experimentally determined for peptides present in the MS Qual/Quant mixture were in good agreement with the manufacturer specified values. For all peptides, the average variation from the expected values was on average 11.5%. The levels of various putative peptide biomarkers in tryptically digested human serum matrix were determined, for example, ITLYGR was present at 650 amol per 200 ng of plasma matrix using the microfluidic LC system in combination with low resolution tandem mass spectrometry, and GYSIFS YATK present at 45 amol per 200 ng of matrix using the nanoscale LC system in combination with high resolution Q-ToF mass spectrometry, operating with and without ion mobility enhancements. Comprehensive comparison of various LC-MS platforms for the quantitative analysis of potential peptide biomarkers, including ion mobility based MRM technology.

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Enhancing the Analytical Capabilities of DESI Imaging Using Ion Mobility Separation - Providing Superior Insights of Biological Samples

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Matrix assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) coupled with ion mobility separation has demonstrated significant utility over the last decade for the separation of matrix ion species from endogenous ion species in the gas phase. Since its introduction ten years ago, desorption electrospray ionization (DESI) has been gathering momentum as a complementary MSI technique to the more traditional SIMS and MALDI-MS approaches, proving especially beneficial for the analysis of metabolites/ lipids localization in tissue. In this study, we compare and contrast DESI imaging with MALDI imaging on the same ion mobility enabled mass spectrometer, with variety of samples. We demonstrate that additional classes of molecules are ionized by DESI which are clearly defined using ion mobility.

Different tissue samples including mouse brain sections and human tumor sections, have been analyzed using the same mass spectrometer by both MALDI and DESI. By keeping the parameters for the ion mobility and mass analyzer constant between the different techniques, the ion distribution overlap could be studied in detail. One advantage of MALDI imaging using an ion mobility enabled MS is ability to differentiate clustered matrix peaks from tissue derived analytes (e.g. lipids) as two distinct nested trendlines are observed in the m/z vs drift time plot. As DESI does not require a matrix compound for the ionization of molecules on the surface of tissue section, it could be expected that DESI MS spectra and 2D-plot m/z vs drift time would be cleaner. However, the DESI spectra show similar strong lipid peaks as observed in MALDI, but also intense fatty acid species were detected at the lower mass range. Closer inspection of the ion mobility dimension revealed further trendlines in the 2D-plot, corresponding to either different classes of molecules or different charge states of ions, present at much lower abundance. Additional information highlighted by the use of the ion mobility separation when DESI imaging was compared to MALDI imaging.

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Compartment specific PTM study of Endogenous Protein Kinase C Using Mass Spectrometry in Human Breast Cancer Cell line

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Protein kinase C's are distributed in different compartment and found at the nexus of the many signalling pathways. PKCs regulate many vital pathways required for cell survival, proliferation and metabolism. Any aberrant alteration in the PKC structure/sequence/chemical modification leads to manifestation of many diseases ranging from inflammation to cancer. Recently, the compartment specific functions of these PKC isoforms are attributed to the post-translational modifications (PTM) at specific sites. PTM plays an important role in regulating their activation and translocation in different compartments of the cell. Most of the study of PKC PTM are done in non-endogenous conditions so necessarily not reflect the natural environment with in the cell. Here, we are trying to isolate endogenous PKC using membrane preparation and cell surface labelling methods.

Mass spectrometry has evolved dramatically, and is now considered a key method for site mapping, quantification of chemical modifications (PTM) and even for detecting protein-protein interactions. The right combination of MS method with maximum enrichment is crucial for mapping of proteins to study their PTMs in specific cell compartment.

Cell surface labelling helps to separate the cell surface protein by labelling the surface protein with biotin non-specifically followed by affinity purification using a neutravidin column. Membrane preparation will enrich the membrane proteins including membrane associated protein like protein kinase C's by using differential ultracentrifugation. By combining these two methods, selective enrichment of low abundant proteins like PKC's can be done. Selectively enriched membrane or membrane associated proteins can then be characterised for its PTM study by new MS method like SWATH.

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Why use Ultra-High Resolution Quadrupole Time of Flight Instruments for Proteomics Applications?

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Introduction

In shotgun proteomics it is desirable to identify and quantify a large number of individual peptides from complex samples in the shortest possible time. Complexity and concentration range, however, pose a great challenge to the MS instrumentation in terms of sensitivity, resolution and dynamic range. Several hardware modifications of a bench-top UHR-TOF instrument were carried out and evaluated addressing these particular performance aspects.

Methods

To test the impact of these modifications on proteomics performance, different complex tryptic digests were analyzed with nano-flow UHPLC and a CaptiveSpray ion source connected to the impact II (Bruker Daltonik). For data processing the MaxQuant software package was used (Nature Biotechnology 26, 1367 - 1372 (2008)).

Results and Discussion

Proteomic capabilities were evaluated on a UHR-TOF instrument and results show that the high MS/MS acquisition speed is very suitable for large sample amounts (e.g. 5µg of tryptic HeLa digest) resulting in more than 4,000 protein identifications during a 90

min gradient. Preliminary quantitative data of 200 ng E.coli lysate spiked with iTRAQ labeled peptides were investigated with regard to the theoretical ratios of these proteins at 10:10:5:5:2:2:1:1. A reference amount of 4 ng was used providing a quantification accuracy of all four proteins at 10:10.2:5.5:5.4:3.3:3.5:2.1:2.8. Additionally, label-free quantitation results as well as identification numbers in very low sample amounts will be shown, further supporting UHR-TOF capabilities for proteomics applications.

Obtained data clearly reveal that hardware changes leading to higher sensitivity at fast acquisition speed and an increased resolution are beneficial for proteomics applications. The improvements result in higher identification rates and a very accurate quantification making the impact II a good choice for proteomics applications.

Conclusion

Improvements to several hardware components allow identification and quantification of complex proteomics samples with very high dynamic range.

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Investigating the role of a new E3 ubiquitin ligase implicated in ALS disease pathology

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Amyotrophic Lateral Sclerosis (ALS) is a progressive and devastating neurodegenerative disease with poor prognosis. Our team has identified novel missense mutations in an *FBX* gene (manuscript submitted), some of which lead to aggressive ALS pathology. At the protein level, FBX is a component of an E3-ubiquitin ligase and is responsible for recognition of specific substrates for ubiquitination [1]. Our hypothesis is that disease-variants of FBX may have altered substrate recognition properties. To date, there are only three known FBX substrates. One of the main challenges of identifying substrates of E3 ligases is the transient and low binding affinity between the ligase and its substrates. Accordingly, one aim of this project is to identify novel substrates of FBX using proximity-dependant biotin identification (BioID) and LC-MS/MS. BioID is a new methodology that involves fusing mutant biotin ligase in frame with the enzyme of interest in order to biotinylate proteins that come into close proximity [2]. This approach has advantages in that it enables the selective isolation and identification of weak and transient protein-protein interactions using standard biotin-affinity capture. Furthermore, it provides a list of candidates for further interaction studies. In addition to identifying novel substrates of FBX, this project will also work toward producing recombinant FBX for structural studies. The structure will help us rationalise the changes in E3-ligase activity caused by mutations, and will provide a basis for structure-based drug design. Overall, this project will investigate the link between, and the degree to which, FBX affects ALS disease progression. It will also work toward identifying specific structural features of FBX that might be therapeutically targetable.

1. [1] Deshaies, R.J. and C.A. Joazeiro, RING domain E3 ubiquitin ligases. *Annu Rev Biochem*, 2009. 78: p. 399-434.
2. [2] Roux, K.J., et al., A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. *J Cell Biol*, 2012. 196(6): p. 801-10.

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Improved capacity trapped ion mobility spectrometry

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In conventional drift tube ion mobility spectrometry, the overall duty cycle is principally limited by the injection of ions into the mobility analyzer. The initial ion pulse must be kept relatively small with respect to the drift time such that the resolving power is not significantly reduced. While multiplexed strategies can improve the sensitivity and duty cycle, peak aliasing still remains problematic.

Over the past few years, trapped ion mobility spectrometry (TIMS) has displayed several attractive features including: (1) rapid gas phase separations on a millisecond timescale, (2) resolving power that can approach ~300, (3) flexibility to tune the separation parameters (resolution vs. separation speed/duty cycle) in accordance with the analytical challenge, and (4) the ability to determine collision cross section values that closely match (within 1-2%) those obtained using drift tube IMS

Here, a new analyzer with an enhanced storage capacity is evaluated. This second generation TIMS analyzer features a tunnel region that is approximately double the length compared to the first generation prototype. An increase in the storage space of the new TIMS analyzer was observed by roughly the geometrical factor (2-fold) resulting in a duty cycle of ~60% at a resolving power of ~100.

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Megapixel tissue imaging at high-speed: Evaluation of a next generation MALDI-TOF instrument

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MALDI Imaging (Mass Spectrometry Imaging, MSI) is a spatially resolved, label-free analytical technique for direct analysis of biological samples. Acquisition speed and sample throughput are a limiting factor in MSI experiments, in particular in clinical research where large cohorts of patients have to be analyzed to encompass the biological variance of human samples. Acquisition speed also limits MSI experiments at high spatial resolution, especially for larger tissue sections.

Here, we present data from a next generation MALDI-TOF instrument that enables acquisition speeds of up to 50 pixel (spectra) per second at pixel sizes of 10 μm and smaller. Data is shown from the most common application areas of MSI, including lipid, peptide and intact protein analysis.

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TMT One Stop Shop: from reliable sample preparation to computational analysis platform

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Publish consent withheld

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The development of barley-specific peptide markers for food testing applications

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Gluten is the collective name for a class of proteins found in wheat, rye, barley and oats. Coeliac disease (CD) is an immune-mediated inflammatory disease of the small intestine in a subset of genetically-susceptible individuals that is triggered by the ingestion of gluten, resulting in intestinal inflammation and damage. The only current treatment for CD- and gluten-intolerants (~70 million people globally) is lifelong avoidance of dietary gluten. Gluten-free (GF) foods are now commonplace, however, it is difficult to accurately determine the gluten content of GF-products using current methodologies as the antibodies are non-specific and show cross-reactivity. In processed products measurement is further confounded by protein modifications and/or hydrolysis. Global proteomic analysis of 14 economically important barley varieties utilising discovery LC-MS/MS was used to characterise the "gluteome" and select peptide markers specific to barley gluten (hordeins) and/or as general markers of barley for targeted quantitative MS assays. Barley-specific peptide markers were detected across 14 barley varieties with nine potential markers showing detection in all cultivars tested. The panel of barley peptide markers were then assessed against 16 commercial grains including those grown in crop rotation or typically processed in the same facility. Of these, seven of the peptide markers were unique to barley and can be used to develop sensitive assays for the presence of barley as an unlabelled food ingredient and show promise for the quantification of gluten to protect consumers suffering from CD.

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A top-down proteomic approach to the identification of early serum biomarkers predictive of spontaneous preterm birth

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Publish consent withheld

1. Wright, E.P., et al. (2014) Top-down proteomics: Enhancing 2D gel-electrophoresis from tissue processing to high sensitivity protein detection. *Proteomics* 14, 872-889.
2. Butt, R.H., Coorsen, J.R. (2005) Postfractionation for Enhanced Proteomic Analyses: Routine Electrophoretic Methods Increase the Resolution of Standard 2D-PAGE. *J. Proteome Res.* 4, 982-991.
3. Gauci, V.J., et al. (2013) Coomassie blue staining for high sensitivity gel-based proteomics. *J. Proteomics* 90, 96-106.
4. Wright, E.P., et al. (2014) Deep Imaging: How much of the Proteome Does Current Top-Down Technology Already Resolve? *PLoS ONE* 9(1): e86058. doi:10.1371/journal.pone.0086058.

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A human stimulome – mapping signalling network intersection downstream of major cell surface receptors

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Reversible protein phosphorylation forms vast signalling networks that rapidly respond to intra- and extra-cellular cues to orchestrate molecular responses, linking the cell's genome with its environment. The misregulation of such signals cause or exacerbate the onset of complex diseases including cancer and ageing, and their rectification is therefore a major area of biomedical research. This is however complicated by the fact that major signaling nodes are functionally pleiotropic, being involved in several biological processes. Indeed, different cellular stimuli often signal through shared network branches. Designing effective therapies against dysregulated signalling therefore requires a more complete picture of the signalling landscapes downstream of cell surface receptors and the extent of network overlap.

Using EasyPhos1 – a new scalable, single-run phosphoproteomics platform – we quantified the response of signalling networks of diverse cell lines to a large panel of ligands, targeting pro-survival (e.g. EGF, IGF, VEGF, PDGF) and pro-inflammatory pathways (e.g. TNF α , TRAIL, IL1 β , IL-6). We quantified over 20,000 phosphorylation sites, from which comparisons of ligand induced cellular signalling networks have been visualized, revealing highly specific network branches as well as large tracts of shared signalling components.

These systems-wide data provide an extensive view of ligand-induced signalling networks in different cell lines, greatly extending the current knowledge of the signalling occurring downstream of major cell surface receptors.

1. Humphrey SJ, Azimifar SB, Mann M. High-throughput phosphoproteomics reveals in vivo insulin signaling dynamics. *Nature Biotechnology* (2015). 33(9):990-5.

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Novel O-glycans containing acidic sugars on fungal secreted protein and their characterisation using ESI-PGC-LC-MS/M

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Trichoderma reesei has been of industrial interest for the production of cellulases, resulting in the development of hypercellulolytic strains such as RUT-C30. High protein production and secretion, and eukaryotic glycosylation machinery, make *T. reesei* RUT-C30 a suitable expression host for recombinant proteins. The N-glycosylation of secreted proteins of RUT-C30 is known to vary depending on culture nutrients but O-glycosylation has been less extensively studied. Under glucose as a sole carbon source, secreted protein glycosylation featured novel O-glycan compositions which made up over a third of the total abundance of O-glycans released from these proteins.

This study characterised these novel compositions, confirming the presence of hexuronic acid in released O-glycans. Negative mode ESI-PGC-LC-MS/MS using a ThermoFisher Velos Pro was used to identify and characterise these O-glycans through annotation of glycosidic bond and cross-ring fragments. Trap-HCD allowed targeted MS3 experiments to be performed on the hexuronic acid substituent of these structures which was not possible with CID, validating the novel O-glycan composition. One site of novel O-glycosylation was identified, revealing attachment to a peptide in the catalytic domain of CBHI. These are the first reported glycans to contain acidic sugars in fungi and could have significant implications for CBHI structure and activity.

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Mass spectrometry-based lipidomics analysis of Barrett's oesophagus and oesophageal adenocarcinoma

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The incidence of oesophageal adenocarcinoma, which has a 5-year survival rate of 15%, has rapidly increased in the last years. The risk factors for oesophageal adenocarcinoma include chronic reflux, male gender, obesity and Barrett's oesophagus, a metaplastic change of the oesophageal squamous epidermal cells which are replaced by columnar cells. There is a need to understand the underlying mechanism of oesophageal adenocarcinoma development and find potential biomarkers. MALDI (Singhal et al. 2013) and spectroscopy (Dave et al. *Eur J Gastroenterol Hepatol*) studies report changes in the lipid profiles of Barrett's oesophagus and/or adenocarcinoma tissue compared to healthy squamous epithelium. Interestingly, cholesterol lowering drugs of the statin family have been reported to attenuate growth and malignant potential of oesophageal adenocarcinoma cells (Sadaria et al. 2011), and a more recent study report statin use as protective against Barrett's oesophagus and adenocarcinoma (Nguyen et al. 2014, 2015). To determine if lipid composition changes during the development of oesophageal adenocarcinoma, we conducted an untargeted mass spectrometry-based lipidomics experiment in five cell lines representing progression from Barrett's oesophagus, dysplasia to oesophageal adenocarcinoma. This poster will report the initial results.

1. Dave et al. 2004 *Eur J Gastroenterol Hepatol* 16:1199-205
2. Singhal et al. 2013 *J Proteomics* 80:207-15
3. Sadaria et al 2011 *J Thorac Cardiovasc Surg* 142:1152-60
4. Nguyen et al. 2014 *Gastroenterology* 147:314-23

Identification of beer spoilage microorganisms using the MALDI Biotyper platform

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Beer spoilage microorganisms present a major risk for the brewing industry and can lead to cost intensive recall of contaminated products and damage to brand reputation. The applicability of molecular profiling using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in combination with Biotyper software was investigated for the identification of beer spoilage microorganisms from routine brewery quality control samples. Reference mass spectrum profiles for top- and bottom-fermenting brewing yeast strains, wild spoilage yeast and common bacterial spoilage organisms were established, incorporated into the Biotyper reference library and validated by successful identification after inoculation into beer. The applicability and integration of mass spectrometry profiling using the Biotyper platform into existing brewery quality assurance practices within industry was assessed by analysing routine microbiology control samples from Coopers Brewery Ltd., where contaminating microorganisms could be reliably identified and distinguished from over 5,600 microorganisms present in the database. This renders the Biotyper platform a promising candidate for biological quality control testing within the brewing industry as a more rapid, high-throughput and cost-effective technology that can be tailored for the detection of brewery-specific spoilage organisms from the local environment.

IS-PRM: A Smarter MS Method for Reproducible and Sensitive Pathway Characterization

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The advent of high resolution Q-Orbitrap MS-instruments allowed for quantification of peptides/proteins with high sensitivity and selectivity using PRM targeted analysis. Time-scheduled PRM acquisition allows reducing the duty cycle time hence increasing the number of peptides that can be targeted. However unavoidable drifts in peptide elution time can lead to missing targets and often chromatographic windows of 2-3 minutes have to be used while actual elution times last around 0.5 min. This results in inefficient use of instrument time (below 25%). Here we present a novel data acquisition scheme that allows for significantly increasing the number of peptides targets in a PRM assay.