Singapore Society for Mass Spectrometry

"For the Advancement and Sharing of Knowledge"

SSMS Day 2019

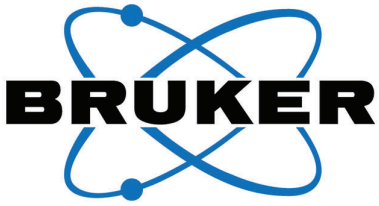
August 15
Exploration Theatrette
Matrix, Biopolis

in conjunction with

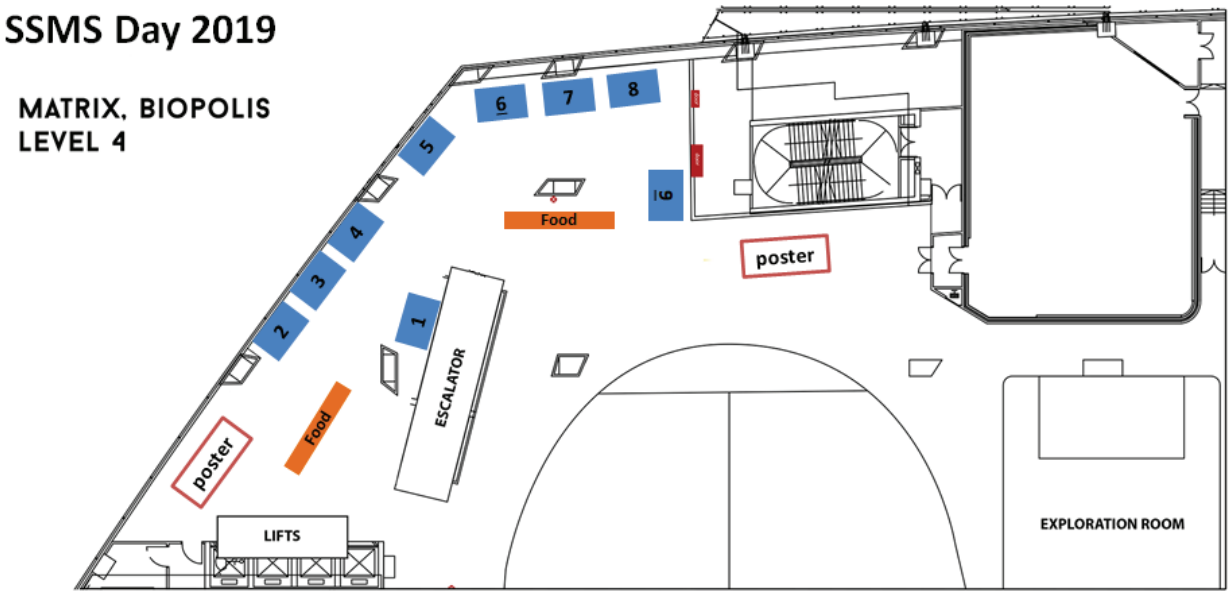
SSMS Day Young Scientist Forum

August 14
Exploration Theatrette

Corporate Exhibitors



Floorplan



Booth number	Corporate exhibitor
1	Thermo Fisher Scientific
2	ChemoPower Technology
3	Shimadzu
4	Agilent
5	Waters
6	SCIEX
7	Scientific Resources
8	Promega
9	Lab Science Solution / Bruker

SSMS Day Young Scientist Forum - 14th August 2019	
Exploration Theatrette, Matrix	
1.30 - 2.00 PM	**Registration**
2.00 - 2.40 PM	**Assistant Professor Dahai Luo** Lee Kong Chian School of Medicine, Nanyang Technological University
2.40 - 3.20 PM	**Aaron Zefrin Fernandis, Director and Singapore Site Lead** MSD
3.20 - 3.50 PM	**Tea Break**
3.50 - 4.30 PM	**Zhanxiong Fang, Research Scientist/Section Leader** National Environment Agency
4.30 - 5.10 PM	**William Chong, Assistant Sales Manager** Thermo Fisher Scientific
5.10 - 5.30 PM	**Q&A Open Panel Discussion**

SSMS Day - 15th August 2019	
Exploration Theatrette, Matrix	
8.30 - 9.00 AM	**Registration & Poster Setup**
9.00 - 9.05 AM	**Welcome address and Opening Remarks**
9.05 - 9.15 AM	News and Views on SSMS events
9.15 - 9.35 AM	**Development of New Metabolomics Methods for MS-Driven Research Applications** Andrew Percy (Cambridge Isotopes Laboratories), Scientific Resources
9.35 - 9.55 AM	**Emerging technology for virus detection and typing** Jayantha Gunaratne, Institute of Molecular and Cell Biology
9.55 - 10.15 AM	**Next generation Mass Spectrometry Imaging; a paradigm shift in cancer imaging** Glenn Bonney, National University Health System
10.15 - 10.35 AM	**Redefining the boundaries of Mass Spectrometry: From mass spec for masses to mass spec with unlimited experimental possibilities** Dhaval Patel, Waters
10.35 - 11:10 AM	**Tea Break**
11.10 - 11.30 AM	**Shimadzu's recent launch of DPiMS-8060 and MALDImini-1 for MS without LC** Shimadzu
11.30 - 11.50 AM	**Interlaboratory Performance Metrics from MAM Consortium New Peak Detection Round Robin Study** Trina Mouchahoir, National Institute of Standards and Technology
11.50 - 12.10 PM	**Lipid protein dynamics by MS** Palur Venkata Raghuvamsi, National University of Singapore
12:10 - 12:30 PM	**SCIEX: It's all about speed!** Jason Neo, SCIEX
12.30 - 1.30 PM	**Lunch**
1.30 - 1.50 PM	**Improved Ruggedness for Omics Applications with Trapped Ion-Mobility Spectrometer (TIMS-TOF) for a MALDI-guided Omics Workflow** Jaran Jainhuknan (Bruker), Lab Science Solution
1.50 - 2.10 PM	**A Unique Glycan Motif and Why Structural Elucidation Needed Three Mass Spectrometers** Edward Pallister, University of Manchester / Bioprocessing Technology Institute
2.10 - 2.30 PM	**Processing and QC of large-scale mass-spectrometry lipidomics data** Bo Burla, National University of Singapore
2.30 - 2:50 PM	**Metabolomics-based discovery of a metabolite that enhances oligodendrocyte maturation** Thomas Mingliang Fang, Nanyang Technological University
2.50 - 3:10 PM	**Accelerating capabilities with the new Agilent 6546 LC/Q-TOF** Erhan Simsek, Agilent Technologies
3.10 - 4.20 PM	**Poster session + Tea Break**
4.20 - 4.40 PM	**Advancement in the Orbitrap Technology** Xin Li, Thermo Fisher Scientific
4.40 - 5.00 PM	**GlycopeptideGraphMS: augmenting the conventional database approach for identification of glycopeptides** Matthew Choo, Bioprocessing Technology Institute
5.00 -5.20 PM	**Metabolic Profiling in Diabetic Kidney Disease (DKD) and Diabetic Retinopathy (DR)** Lei Zhou, Singapore Eye Research Institute
5.20 - 5.40 PM	**MS Data Analysis Solution** Chun Kiang Chua, ChemoPower
5.40 - 6.00 PM	**Clinical lipidomics: are we ready yet?** Anne K Bendt, National University of Singapore
6.00 - 6.10 PM	**Closing Remarks**
6.30 PM onwards	**Evening Reception**

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Scientific Posters

S.No	Theme	Poster No.	Author	Abstract Title	Email	Institute
1	Glycomics	G-1	Edward Pallister	In Vitro Sialylation of Recombinant Alpha-1-Antitrypsin using α2,6 Sialyltransferase from Photobacterium Damselae Produces Di-sialyl Galactose Motifs in N-Glycans	edward.pallister@gmail.com	Bioprocessing Technology Institute, A*STAR
2		G-2	Matthew Choo	GlycopeptideGraphMS: augmenting the conventional database approach for identification of glycopeptides	matthew_choo@bti.a-star.edu.sg	Bioprocessing Technology Institute, A*STAR
3	Lipidomics	L-1	Erhan Simsek	A New Lipidomics Software Workflow Demonstrates Disrupted Lipogenesis Induced with Drug Treatment in Leukemia Cells	erhan.simsek@agilent.com	Agilent Technologies
4	Metabolomics	M-1	Erhan Simsek	Food classification and authenticity testing using a new high-resolution LC/QTOF and novel classification software	erhan.simsek@agilent.com	Agilent Technologies
5		M-2	Jeremy Jiang	Multi-feature based data processing of Data Independent acquisition metabolomics data without retention time information	Jeremy.Jiang@sciex.com	SCIEX
6		M-3	Anna Karen Laserna	Taking the metabolomics approach to look at multiple organ dysfunction syndrome (MODS) in trauma patients	chmakcl@nus.edu.sg	National University of Singapore
7		M-4	Dongxiao Yang	Metabolomics insights into key factors influencing CHO glycosylation	yang_dong_xiao@bti.a-star.edu.sg	Bioprocessing Technology Institute, A*STAR
8	Proteomics	P-1	Prem Prakash Das	In planta proximity-dependent biotin identification (BioID) identifies a TMV replication co-chaperone NbSGT1 in the vicinity of 126 kDa replicase	premprakashdas@u.nus.edu	National University of Singapore
9		P-2	Nayana Prabhu	Cellular Thermal Shift Assay (CETSA): From drug targets to horizontal cell biology	nain.prabhu@gmail.com	Nanyang Technological University
10		P-3	Purushottam Kathiresan	Systematic identification of yolk-associated proteins in early embryonic development of zebrafish	kathiresan.purushothaman@nord.no	Nord University
11		P-4	Jaran Jai	Identification and Quantitation of Phosphopeptide Positional Isomers using Trapped Ion Mobility Spectrometry and PASEF	jaran.j@bruker.com	Bruker
12		P-5	Tianhua Wang	Serotyping of Salmonella via proteotyping by MALDI-TOF	tianhua@shimadzu.com.sg	Shimadzu Asia Pacific
13	MS Technology Advances	T-1	Jeremy Jiang	SCIEX: It's all about speed!	Jeremy.Jiang@sciex.com	SCIEX
14		T-2	Jeremy Jiang	A High Throughput Mass Spectrometry Plate Reader: Acoustic Droplet Ejection to an Open-Port Probe Sampling Interface	Jeremy.Jiang@sciex.com	SCIEX
15		T-3	Lv Yunbo	The Detection of Unknown Compounds in GC-MS Analysis with Entropy Minimization	mabel.lv@chemopower.com	ChemoPower Technology Pte Ltd
16		T-4	Jade Aw	A Modified Orbitrap Tribrid Mass Spectrometer with Real Time Search and Advanced Spectral Processing Enhances Multiplexed Proteome Coverage and Quantification Accuracy	jade.aw@thermofisher.com	Thermo Fisher Scientific
17		T-5	Jade Aw	Performance Evaluation of a Modified Quadrupole Orbitrap Mass Spectrometer	jade.aw@thermofisher.com	Thermo Fisher Scientific
18	Biotherapeutics	O-1	Sharon Goh	An Improved Immunoaffinity LC-MS/MS Workflow for the Quantitation of IgG's during Preclinical PK Studies	Sharon.goh@promega.com	Promega Pte Ltd

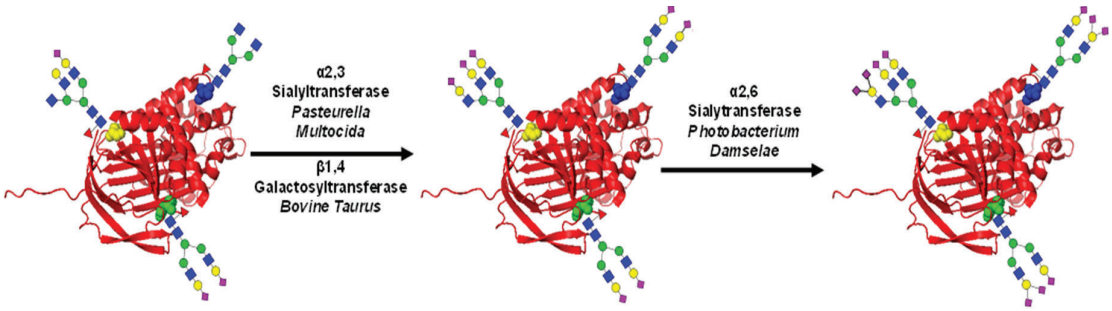
In Vitro Sialylation of Recombinant Alpha-1-Antitrypsin using α2,6 Sialyltransferase from *Photobacterium Damselae* Produces Di-sialyl Galactose Motifs in N-Glycans

Edward G. Pallister†‡ Matthew S. F. Choo†, Jien-Nee Tai†, Dawn S. Z. Leong†, Wen-Qin Tang†, Say-Kong Ng†, Kun Huang‡, Andrea Marchesi‡, Peter Both‡,Christopher Gray‡, Pauline M. Rudd†, Sabine L. Flitsch‡ and Terry Nguyen-Khuong†

†Bioprocessing Technology Institute, Agency for Science Technology and Research, Singapore

‡School of Chemistry & Manchester Institute of Biotechnology (MIB), The University of Manchester, United Kingdom

ABSTRACT: Sialic acids are cell surface sugars present in many animal glycoproteins and are of particular interest in biopharmaceuticals, where lack of sialylation can reduce bioactivity. Here we describe how α-2,6-sialyltransferase from *Photobacterium Damselae* can be used to markedly increase sialylation of CHO produced alpha-1-antitrypsin. Detailed analysis of the sialylation products showed that in addition to the expectedα-2,6-sialylation of galactose, a second di-sialyl galactose motif was produced, which had never been recorded on a mammalian glycoprotein. The influence of this unique di-sialylation on the in vitro activity of alpha-1-antitrypsin was studied and a toolkit of mass spectrometry methods to identify this new di-sialyl galactose motif in complex mixtures was developed.



GlycopeptideGraphMS: augmenting the conventional database approach for identification of glycopeptides

Matthew S.F. Choo[1], Corrine Wan[1], Pauline M. Rudd[1], Terry Nguyen-Khuong[1]
(matthew_choo@bti.a-star.edu.sg)
[1] Analytics Group, Bioprocessing Technology Institute, Agency for Science, Technology and Research, Singapore

GlycopeptideGraphMS is a useful algorithm to comprehensively identify glycopeptides from LCMS data of uncharacterised biologics or biomarkers carrying heterogeneous and with multiple-site glycosylation.

The profiling of glycosylation requires high resolution techniques. Although glycosylation profiling using LC-MS/MS is routine for glycoproteins with one glycosylation site and low heterogeneity (i.e. the IgG heavy chain), the profiling of glycoproteins with multiple glycosylation sites and with high heterogeneity remains a difficult and manual task requiring deep expertise and comprehensive MS2 fragmentation.

To address these problems, we augmented the conventional LC-MS/MS database method by exploiting mass and retention time patterns that were specific to glycopeptides. We developed the GlycopeptideGraphMS to use these patterns to infer the identities of hundreds of glycopeptides based on only a handful of confident identifications made using conventional database software. To derive the test dataset, glycopeptides from the heavily glycosylated cancer-associated protein AXL receptor tyrosine kinase were resolved on C18 chromatography and analysed using a Thermo Orbitrap Fusion Tribrid mass spectrometer. GlycopeptideGraphMS identified > 500 unique glycopeptides, triple the number of glycopeptides found by a typical database search. 2.2% FDR was empirically calculated using glycan-digesting enzymes to delete peaks. The Python software and Windows .exe is open source at https://bitbucket.org/glycoaddict/glycopeptidegraphms.

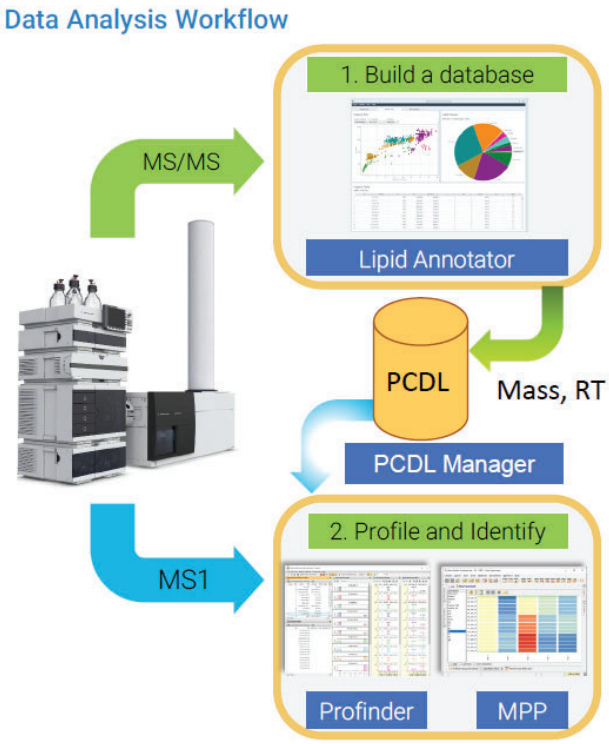
Keywords: Glycomics, LCMS/MS, Bioinformatics

A New Lipidomics Software Workflow Demonstrates Disrupted Lipogenesis Induced with Drug Treatment in Leukemia Cells

Mark Sartain[1], Genevieve Van de Bittner[1], Xiangdong Li[1], Jeremy Koelmel[2], Adithya Murali[1], and Sarah Stow[1]

[1]Agilent Technologies, Inc., Santa Clara, CA, USA
[2]Department of Chemistry, University of Florida, FL, USA

While shotgun lipidomics has advanced the field of lipid analysis, it suffers from limitations that have led to a shift towards chromatographic-based lipid profiling approaches using HPLC coupled to HRMS. To improve these workflows, we demonstrate a novel software tool that enables product-ion spectral matching against an in-silico generated databased to annotate iterative-mode MS/MS spectra. The tool takes special care not to over-annotate lipid entities and quickly generates an accurate-mass retention time (AMRT) database in an automated fashion. The resulting database annotates MS1 lipid profiling data for downstream differential analysis and incorporates new lipid-specific visualization tools. We applied this novel workflow to study lipidome alterations of an acute-myeloid-leukaemia cell line in response to a drug treatment combination.



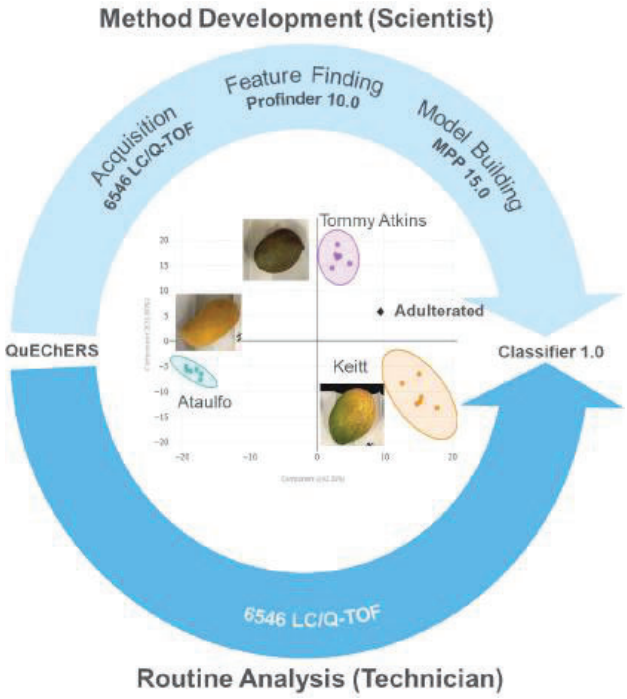
Food classification and authenticity testing using a new high-resolution LC/QTOF and novel classification software

Daniel Cuthbertson[1], Karen Yannel[2], and Frank Kuhlmann[2]

[1]Agilent Technologies Inc, Seattle, WA, USA
[2]Agilent Technologies Inc, Santa Clara, CA, USA

Adulteration of food products is a growing issue and manufacturers are increasingly interested in checking the quality and authenticity of ingredients used in products. Chemometric profiling of the ingredient's chemical components with mass spectrometry and using multivariate statistical models to classify an unknown sample is a desirable way to determine if an ingredient is pure or adulterated.

In order to simplify the analysis for a technician to routinely perform the multivariate modelling, new software has been introduced to complete the food authenticity workflow. A method development scientist builds the model using traditional feature extraction and statistical modelling software but then hands the analysis method and model file off to a technician to use with a simplified software for routine analysis, MassHunter Classifier. This workflow was demonstrated with a proof of concept study using three mango varieties: Keitt, Ataulfo and Tommy Atkins. The model was developed using method development workflow and then deployed for routine analysis in Classifier 1.0.



Abstract Title: Multi-feature based data processing of Data Independent acquisition metabolomics data without retention time information

Pradeep Narayanaswamy[1], Adam Lau[2], Lyle Burton[2], Stephen Tate[2]
(pradeep.naraya@sciex.com)
[1]Singapore, SCIEX, [2]Concord, SCIEX

Mass spectrometry coupled with liquid chromatography is increasingly the most popular method for untargeted metabolomics that detects thousands of features from complex samples, but only a fraction of them are annotated. Applying a Data Independent Acquisition (DIA) workflow like SWATH® acquisition will allows unbiased MS/MS data to be generated for the metabolite standards [Mass Spectrometry Metabolite Library (MSMLS) kit] with different concentrations. Here, we developed a novel scoring model using multiple features and data processing algorithms for confident identification of these metabolites acquired using SWATH-MS on our TripleTOF® system even without prior retention time information. We further developed a prediction model to generate in-silico MS/MS spectra for other features (ISF) based on the MS/MS of known features of M+H/M-H and then matched the observed fragments of in-silico spectra for sequence coverage against MS/MS spectra of molecular ion (M+H/M-H) to enhance the scoring of metabolites. In complex mixtures, two or more peak groups representing product ions can located at different RTs along with the expected peak group. To address this challenge, peak groups correlates across SWATH windows using multiple features to identify the correct metabolite with reduced false positive.

Keywords: SWATH-MS, Metabolomic

Taking the metabolomics approach to look at multiple organ dysfunction syndrome (MODS) in trauma patients

Anna Karen Carrasco Laserna[1], Jorming Goh[4], Lynette Loo[3], Raj Kumar Menon[3], Mikael Hartman[3], Jialing Neo[4], Pavandip Singh Wasan[4], Mahesh Uttamchandani[2], Philip Tsau Choong Lau[3], Sam Fong Yau Li[1] and Shabbir M Moochhala[2,3]
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[1]Department of Chemistry, National University of Singapore (NUS),
[2]Defence Medical & Environmental Research Institute (DMERI), DSO National Laboratories,
[3]Department of Surgery, National University Hospital (NUH),
[4]Work done under prior affiliation with DMERI, DSO National Laboratories

The developments in trauma care have led to a decrease in immediate and early mortality among trauma patients. However, a new cause of death in the intensive or post-operative care units was found, wherein patients succumb to complications in organs unrelated to the initial injury. This condition was called multiple organ dysfunction syndrome (MODS). In this pilot study, we take a metabolomics approach to assess the development of MODS through time and try to identify the association of metabolites with organ-specific or multiple organ dysfunction. The study was done with 23 trauma patients admitted to the surgical intensive care and high dependency units having different degrees of injury severity at admission. Plasma samples were collected within the first 12 hours of inclusion in the study and every 12 hours thereafter until the 48th hour. Patient condition was evaluated using the sequential organ failure assessment (SOFA) scoring system. Untargeted metabolomic analyses were performed using reverse phase and hydrophilic interaction LC-MS. Multivariate analysis revealed that a metabolomics approach can be used to establish a total SOFA score threshold at which MODS and non-MODS patients can be distinguished. Certain metabolites were also found to have significant correlation with MODS and organ-specific dysfunction.

Keywords: metabolomics, trauma, multiple organ dysfunction syndrome

Metabolomics insights into key factors influencing CHO glycosylation

Dongxiao Yang, Ke Xuan Leow, Shi Ya Mak, Shuwen Chen, Lyn Chiin Sim, Amelia Mak, Gavin Teo, Farouq Bin Mahfut, Ian Walsh, Alison Lee, Andy Tan, Terry Nguyen-Khuong, Yuan Sheng Yang and Ying Swan Ho*

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Glycosylation is a key critical quality attribute in biologics production, with potential impacts on the stability, efficacy and immunogenicity of the product. Consequently, the effect of varying culture conditions on protein glycosylation have been widely studied but the underlying biological mechanisms are still poorly defined currently [1]. As such, there is still limited knowledge on how culture components or factors influence these changes, which in turn has delayed the development of glycosylation-based control strategies during production.

In this study, a liquid chromatography – mass spectrometry based untargeted metabolomics approach was used to obtain an in-depth characterisation of fed-batch CHO cultures producing an anti-TNF alpha antibody under varying operation conditions, including different pH and two different media/feed combinations. Media/feed A resulted in almost 3-fold higher maximum cell density and product titre, while the degree of galactosylation was 10% higher than media/feed B. Additionally, the degree of galactosylation was observed to be highly correlated with the levels of galactosylation precursors and carbohydrate derivatives. More importantly, the correlations were found to persist under different culture conditions regardless of media/feed combination or pH, suggesting their potential utility as indicators of product glycosylation status, leading to future applications in the real-time monitoring of CHO antibody production.

References

[1] Zhang P, Woen S, Wang T, Liau B, Zhao S, Chen C, et al. Challenges of glycosylation analysis and control: an integrated approach to producing optimal and consistent therapeutic drugs. Drug Discov Today. (2016) 21(5):740-65.

In planta proximity-dependent biotin identification (BioID) identifies a TMV replication co-chaperone NbSGT1 in the vicinity of 126 kDa replicase

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[b] Temasek Life Sciences Laboratory, 1 Research Link, 117604, Singapore

[c] National University of Singapore Suzhou Research Institute, Suzhou, Jiangsu, China

Tobacco mosaic virus (TMV) is a positive, single-stranded RNA virus. It encodes two replicases (126 kDa and 183 kDa), a movement protein and a coat protein. These proteins interact with host proteins for successful infection. Some host proteins such as eEF1α, Tm-1, TOM1, 14-3-3 proteins directly interact with *Tobamovirus* replication proteins. There are host proteins in the virus replication complex which do not interact with viral replicases directly, such as pyruvate kinase and glyceraldehyde-3-phosphate dehydrogenase. We have used Proximity-dependent biotin identification (BioID) technique to screen for transient or weak protein interactions of host proteins and viral replicase in vivo. We transiently expressed BirA* tagged TMV replicase 126 kDa in TMV-infected *N. benthamiana* plants. Among 18 host proteins, we identified NbSGT1 as a potential target for further characterization. Silencing of NbSGT1 in *N. benthamiana* plants increased its susceptibility to TMV infection, and overexpression of NbSGT1 increased resistance to TMV infection. There were weak interactions between NbSGT1 and TMV replicases but no interaction between them was found in Y2H assay. It suggests that the interaction might be transient or indirect. Therefore, the BioID technique is a valuable method to identify weak or transient/indirect interaction(s) between pathogen proteins and host proteins in plants.

Keywords: TMV 126 kDa replicase, SGT1, Hsp90

Cellular Thermal Shift Assay (CETSA): From drug targets to horizontal cell biology

Nayana Prabhu[1], Lingyun Dai[1], Tianyun Zhao[1], Yingyu Liang[1], Helen Yu[1], Wendi Sun[1], Pär Nordlund[1,2,3] (par.nordlund@ki.se)

[1]School of Biological Sciences, Nanyang Technological University,
[2]Institute of Molecular and Cell Biology, A*STAR,
[3]Department of Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden

The Cellular Thermal Shift Assay i.e. CETSA is based on the biophysical principle of ligand-induced thermal stabilization of proteins. At inception about 5 years ago, CETSA was considered a tool for drug-target deconvolution and mainly used to study direct target engagement *in situ*. Using quantitative mass spectrometry, proteome-wide CETSA (MS-CETSA) was established, which allows off-target binding as well as downstream effects to be discovered, in addition to direct drug target interactions. MS-CETSA has since then been used widely in the pharmaceutical industry for such studies and is currently a valuable tool for biomedical research and drug development.

Today, MS-CETSA has evolved to encompass several other applications including novel ways to study cell biology. With the advent of a highly sensitive multidimensional implementation of MS-CETSA, comprehensive studies of protein level changes as well as stability changes are plausible. In addition, MS-CETSA can also be used to access binding of physiological ligands to proteins, such as metabolites, nucleic acids and other proteins. Such studies will aid in understanding “Horizontal Cell Biology” where modulations of protein interaction states (PRINTs) are studied (as opposed to the “vertical information” focusing on the protein and RNA levels) to provide new insights on cellular functions and processes.

Keywords: CETSA, drug-target deconvolution, protein interaction states

Systematic identification of yolk-associated proteins in early embryonic development of zebrafish

Purushothaman Kathiresan[1], Prem Prakash Das[2], Teck Kwang Lim[2], Christopher Presslauer[1], Steinar D. Johansen[1], Qingsong Lin[2], Igor Babiak[1]

[1]Faculty of Biosciences and Aquaculture, Nord University, 8049 Bodø, Norway
[2]Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543 Singapore

Zebrafish is a well-established model organism for the investigation of development of vertebrates. Multiple genomics and transcriptomics studies have been performed to analyse zebrafish embryonic development in recent years. However, quantitative proteomics studies of early embryo remained unexplored. Previous proteomics analysis of early embryo was limited due to technological constraints. Besides, high abundance of yolk during early embryonic development also restricts the identification of cellular proteins.

Previously, we have developed an improved protocol to remove yolk from zebrafish embryos of the cleavage stages. As a result, we were able to identify a large number of cytosolic proteins. However, the deyolking process depleted a significant number of yolk-associated proteins. With the help of iTRAQ-based quantitative method coupled with LC-MS/MS, we were able to determine a catalogue of yolk proteins during zebrafish embryonic development. Our study identified 203, 154 and 115 yolk-associated proteins from 1-cell, 16-cell and 32-cell stage embryos, respectively.

Yolk localization of the candidate proteins was verified in another experiment, where portions of yolk were manually dissected, and analysed with shotgun LC- MS/MS. We found 133 (out of 169), 67 (out of 103) and 76 (out of 111) proteins were verified. The significantly overrepresented proteins common in both experiments among all the three developmental stages were associated with protein folding, translation, and peptide biosynthetic processes. GO results clearly co-align with KOG and KEGG functional analyses, which suggest the presence of translational machinery in the yolk. In summary, we systematically identified yolk-associated proteins in their functional context in the early embryonic development of zebrafish, considerably expanding the catalogue of known yolk proteins.

The study was financed by the InnControl project (Research Council of Norway, grant #275786), Nord University, and the National University of Singapore.

Identification and Quantitation of Phosphopeptide Positional Isomers using Trapped Ion Mobility Spectrometry and PASEF

Christopher M. Adams[1], Michael Krawitzky[1], Katherine Tran[2], Baozhen Shan[2], Zac Anderson[2], Charles Farsnsworth[3], Matthew P. Stokes[3], Kimberly Lee[3], Shourjo Ghose[1], Matthew Willetts[1], Gary Kruppa[1], Jaran Jai[1] (Gary.kruppa@bruker.com)

[1]Bruker Daltonics
[2]Bioinformatics Solutions Inc., ON, CA
[3]Cell Signaling Technology, Danvers, MA

Enriched phosphopeptides from human cell lysates were analyzed on a nanoLC connected to timsTOF Pro using PASEF acquisition method. The data analysis was done with PEAKS X. Results showed 18,5000 and 1,100 unique PTM modified peptides, respectively. It is estimated that around 25-30% of PTM peptides are positional isomers most of which are eluted with 15sec time window. This hinder the use of extracted ion chromatogram (EIC) for quantitation. The data also showed there are several positional isomer pairs of phosphopeptide existed as separated by ion mobility collision cross section (CCS). More PTM peptides, thus, are detected using timsTOFPRO PASEF methods.

Serotyping of *Salmonella* via proteotyping by MALDI-TOF

Sheena Wee[1], Tianhua Wang[2], Yin Ling Chew[2], Jayantha Gunaratne[1]
(Jayanthaag@imcb.a-star.edu.sg)
[1]Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, [2]Shimadzu Asia Pacific

Salmonella is the leading bacterial cause of foodborne illness. In Singapore, there are around 2,000 reported cases of Salmonellosis annually (MOH Weekly Infectious Diseases Bulletin). More than 2,600 serotypes have been described for *Salmonella*, with around 100 of them being pathogenic to humans and causing illnesses. *Salmonella* serotyping hence forms the core of public health monitoring.

We are developing a MALDI-TOF based workflow that offers cost-effective serotyping with fast analysis speed and high throughput expandability utilizing a dedicated strain typing software for biomarker library building and searching.

Keywords: *Salmonella*, MALDI-TOF

SCIEX: It's all about speed!

Jason Neo
AB SCIEX (Distribution), Singapore

Label-free quantitation is routinely used in pharmaceutical industries for drug screening applications and in biology research/ precision medicine laboratories for proteomics and metabolomics applications. In this presentation, we would introduce two latest SCIEX's innovations that will speed up your work.

1. Scanning SWATH® Acquisition - the Ultimate Digital Record

   The next chapter in the SWATH story takes this concept even further by allowing every single MSMS iteration to be acquired and directly correlated to the correct precursor. The data produced by Scanning SWATH® Acquisition therefore contains all the information that would be contained in a multitude of different experimental approaches combined in a single all-encompassing dataset

2. Acoustic Ejection Mass Spectrometry (AEMS)

   Acoustic droplet ejection (ADE) is a droplet transfer technology capable of high speed, reproducibility and absolute accuracy. This allows screening speeds to be as fast as 0.4 seconds-per-sample, with superb sensitivity, great reproducibility even without internal standard, good quantitation capability, no matrix effect, and broad compound coverage.

Keywords: AEMS, ADE-OPP-MS, SWATH

A High Throughput Mass Spectrometry Plate Reader: Acoustic Droplet Ejection to an Open-Port Probe Sampling Interface

Chang Liu[1], Hui Zhang[2], Luke Ghislain[3], Jianhua Liu[2], Wenyi Hua[2], Timothy Foley[2], Sammy Datwani[3], Don W. Arnold[4], Thomas R. Covey[1]
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Label-free LC/MS based screening technology is routinely used in pharmaceutical industries for hit discovery and various ADME profiling applications. Although the current analysis speed of <30 seconds per sample is quite promising, it still cannot match the throughput provided by plate-reader based HTS platforms. Acoustic droplet ejection (ADE) is a droplet transfer technology capable of high speed, reproducibility and absolute accuracy. In this work, we successfully couple ADE and the standard ESI ion source of a mass spectrometer with the open-port probe (OPP) sampling interface. Screening speeds as fast as 0.4 seconds-per-sample are demonstrated with superb sensitivity, great reproducibility even without internal standard, good quantitation capability, no matrix effect, and broad compound coverage. Since, the standard ESI source was used for the ionization process, great sensitivity and reproducibility can be achieved for a wide range of compounds, ranging from small drug molecules, peptides and large intact protein, even used directly for the analysis of unprocessed samples.

Keywords: ADE-OPP

The Detection of Unknown Compounds in GC-MS Analysis with Entropy Minimization

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Chemical profiling of complex samples with GC-MS typically leads to the detection of hundreds of components whose identities could not be determined. The difficulty in identifying these unknown components escalate further when they are present as co-eluting and minor components. However, the availability of their pure mass spectra can potentially aid in the process of structural identification. In this study, beet root sample was analysed by GC-MS and entropy minimization was utilized to extract pure spectra of unknown compounds from co-eluted peaks. The possible structure was deduced from the pure structure and computational analysis. The potential of the entropy minimization algorithm in extracting pure mass spectra for minor and co-eluting components could be wide-reaching and beneficial for a wide range of mass spectrometry-based research.

Keywords: Unknown Detection, Entropy Minimization

A Modified Orbitrap Tribrid Mass Spectrometer with Real Time Search and Advanced Spectral Processing Enhances Multiplexed Proteome Coverage and Quantification Accuracy

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Isobaric tagging strategies using Thermo Scientific™ Tandem Mass Tags™ (TMT™) are powerful tools for studying how proteins interact and function in biological systems. Up to 11 samples can be multiplexing in a single high-resolution LC/MS experiment to enable state-of-the-art quantitative analysis of peptide and protein abundance. Here we evaluate the benefits of the Thermo Scientific™ Orbitrap Eclipse ™ Tribrid ™ mass spectrometer including real time search capabilities, advanced spectral processing algorithms, and modified hardware to enhance TMT quantification accuracy and proteome coverage.

Keywords: Orbitrap, Proteome, TMT

Performance Evaluation of a Modified Quadrupole Orbitrap Mass Spectrometer

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The increasing importance of understanding how, when and where proteins are expressed, together with the interaction with other proteins and what functions they perform, is driving advancements in mass spectrometric instrumentation. Since its introduction, Orbitrap-based instruments have and are still playing a pivotal role in many different research areas such as proteomics, metabolomics, biopharma, etc. Each of these applications come with different challenges to mass spectrometric instrumentation. To address some of these challenges, new technological developments as well as improvements for existing mass spectrometers is a necessity. Here we evaluated the Orbitrap Exploris 480 mass spectrometer for proteomics applications with focus on data dependent acquisition and data independent acquisition. Additionally, we assessed the use of the ΦSDM processing algorithm on TMT11plex labeled samples.

Keywords: Orbitrap, Proteomics, Biopharma

An Improved Immunoaffinity LC-MS/MS Workflow for the Quantitation of IgG's during Preclinical PK Studies

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The testing of biotherapeutic proteins (particularly IgG's) in animals is a requirement for approval by regulatory agencies, prior to treatment in humans. PK/PD studies of biotherapeutic protein metabolism have traditionally been performed using ELISA. However, the use of immunoaffinity purification coupled with LC-MS/MS has proven advantageous in part due to its ability to monitor total drug and multiple surrogate peptides. As the number of biotherapeutic proteins in the pipeline increases, so does the need for an optimized workflow. Toward this end, we have developed an optimized workflow which improves the dynamic range of the assay, decreases the digestion time, and increase the overall workflow flexibility. The assay is also compatible with automation.

Keywords: IA LC-MS/MS, Biotherapeutic proteins