

PL 1**Global analysis of genes and proteins that convey the action of drugs**

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Introduction: Biology relies on the concerted action of hundreds of gene products fitting together functional modules operationally organized in molecular machines and larger networks. To understand how human cells react to perturbation of external agents, such as drugs, it is important to use global, unbiased assessments followed by rationalization by computational analysis and functional validation.

Methodology: We use a number of approaches: 1) interaction proteomics, chemical proteomics and thermal-shift proteomics, 2) chemical genetics (mutagenesis of genome of near-haploid human cells and genome editing), 3) ex vivo high content imaging analysis on cells, 4) computational network analysis and modelling, and 5) validation by focused gene inactivation (RNAi and genome editing).

Results and Discussions: We present results on the mechanism of action of known and experimental anticancer drugs, including tyrosine kinase inhibitors. We identify genes that affect the differential activity of these drugs and identify potential new medical use. We also classify drugs based on their ability to affect the interactions among cells of the hematopoietic system. Finally, we genetically identify transporters required for drug entry and resistance.

Conclusions: Our integrated approach allows for a new type of pharmacology, where the individual target is as important as the global effects on dozens and hundreds of gene products that in turn have a strong impact on the action of the pharmacological agent. The profile of genes that are functionally required for drug action allow for advanced pharmacogenomics considerations in therapeutic evaluation. Temperature shift proteomics allows for the systematic mapping not only of drugs, but also of cellular metabolites to the cognate cellular proteins, allowing a whole new unbiased re-evaluation of basic biochemical networks in human physiology.

Biography**Superti-Furga G**

Scientific Director, CeMM Center for Molecular Medicine of the Austrian Academy of Sciences

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**Systems biology and Systems medicine, molecular and network pharmacology**

Interested bringing the genomic and systems-views close to the clinical world to pioneer precision medicine and improve medical practice and. Among his major achievements to date are the elucidation of basic regulatory mechanisms of tyrosine kinases in human cancers, the discovery of fundamental organization principles of the proteome and lipidome of higher organisms, the characterization of the molecular machinery involved in innate immunity and the development of integrated approach to understand the mechanism of action of drugs at the molecular level. His work on the organization of the eukaryotic proteome is among most highly cited in the field.

Knight Officer Order of Merit of the Republic of Italy
Advanced grant holder of the European Research Council
Member of the European Molecular Biology Organization
Member of the Austrian Academy of Sciences
Member of the German Academy of Sciences Leopoldina
Member of the European Academy of Cancer Sciences
Member of the Academia Europaea
Austrian Scientist of the year 2011
Prize of the City of Vienna for Natural Sciences

PL 2**Human Protein Atlas enabled neuroproteomic profiling of body fluids****Peter Nilsson**

Affinity Proteomics, SciLifeLab, School of Biotechnology, KTH-Royal Institute of Technology, Stockholm, Sweden

Abstract

The Human Protein Atlas (www.proteinatlas.org) currently contains more than 24.000 validated antibodies targeting 17.000 proteins corresponding to approximately 83% of the encoded human proteins. The publicly available portal contains several millions of high-resolution images generated by immunohistochemistry on tissue microarrays and confocal microscopy for subcellular localization. The antibodies are antigen-purified and the long-term objective is to generate paired antibodies towards all human protein targets. A systematic biomarker discovery approach has been implemented, utilizing various array-based platforms in

combination with the massive antigen and antibody resource. Proteomic profiling of serum, plasma and CSF in multi-disease cohorts are performed with large number of peptides and antigens on planar microarrays for the analysis of autoimmunity repertoires with subsequent verifications with suspension bead arrays.

Large set of samples are also profiled with massive numbers of antibodies on highly multi-parallel suspension bead arrays which utilizes magnetic color-coded beads functionalized with antibodies to generate protein profiles from labeled samples for biomarker discovery.

Furthermore, the initial protein profiling and exploration of a large serum RPPA will also be described. We have generated what is according to our current knowledge the largest serum microarray ever produced. It contains more than 12 000 serum samples collected within the TwinGene cohort (2004-2008, Sweden) and it comprises both monozygotic and dizygotic twin pairs.

The results from both autoimmunity and antibody-based neuroproteomic profiling of body fluids with analysis both within and between several neurological disorders utilizing the different platforms will be presented.

Biography

Peter Nilsson



P N i s
Professor in
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He has since
2 0 0 2 b e e n



heading the Protein Microarray group within the Human Protein Atlas project. He is the executive director of the Affinity Proteomics national platform at SciLifeLab and the site director of the Human Protein Atlas at SciLifeLab Stockholm. He is also the vice dean of the School of Biotechnology at KTH.

The main research focus is within development and utilization of various protein microarray formats for biomarker discovery through peptide, antigen and antibody based proteomic profiling of body fluids. See <http://publicationslist.org/nipef> for a complete list of publications.

IL 1

Proteomic approaches to study the dynamics of antiviral innate immune signaling

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Introduction: The complex and evolutionarily adapted cellular response to antimicrobial threats such as viral infection, is a characteristic model for studying perturbation of biological systems. Our previous study sampled various viral strategies to perturb the host molecular network using an interaction proteomics approach (Pichlmair et al., Nature 2012). We were further interested in understanding the dynamics of the host response to a single viral infection by which we could assess the extent and the molecular logic of the cellular response.

Methodology: We performed a temporal genome-wide transcriptomics, proteomics and phosphoproteomics analysis of the cellular response of mouse macrophages to Vesicular Stomatitis Virus (VSV) infection, followed by integrative bioinformatics analyses to get a global overview of the cellular response. A novel phosphorylation site as well as four other genes were functionally validated for their role in type-I interferon activation, NFkB activation and VSV life cycle.

Results and Conclusions: The vast and complex molecular changes measured could be decomposed in a limited number of clusters within each category (transcripts, proteins, protein phosphorylation), each with its own kinetic parameters and characteristic pathways and processes, suggesting multiple regulatory options and a specific process logic within the overall sensing and homeostatic program. Overall, the data highlighted a predominant executive function to phosphorylation, likely evolved due to the requirement of a fast response to pathogens. Functional validation of a novel phosphorylation site on the innate immunity adaptor MAVS, identified its essential role in activation of type-I interferon and NFkB response. Additionally, we found that the RAF1 was required for NFkB activation, while ARAF and AKT2 kinases were, together with the SLC7A11 transporter, among the genes involved in controlling VSV life cycle. The dataset represents a large and unique starting platform for further systems-level as well as targeted mechanistic investigations on the functional organization of the response of macrophages to viral infection.

Biography

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Systems immunology, antiviral networks and inflammation

Richard completed his Masters in Life Sciences and Advanced Post-Graduate Diploma in Bioinformatics from Pondicherry University, Pondicherry, India. He did his Ph.D. in Bioinformatics & Biotechnology under the mentorship of Prof. Akhilesh Pandey at Institute of Bioinformatics, Bangalore, India and Johns Hopkins University, Baltimore, USA. His major contribution during his Ph.D. include

development of web-based resources such as Human Protein Reference Database, Human Proteinpedia, NetPath and PathBuilder; systematic analysis of mass spectrometry fragmentation methods for detection of PTMs such as phosphorylation; and computational framework for proteogenomic analysis. He joined the laboratory of Prof. Superti-Furga, Center for Molecular Medicine, Vienna, Austria in 2011, where he applies cutting-edge OMICS as well as genome-editing technologies to understand the systems-levels aspects of antiviral signaling and inflammation. He is now starting his own research group on systems inflammation at the Center for Molecular Inflammation Research (CEMIR), Norwegian University of Science and Technology (NTNU), Trondheim, Norway.

Awards

2012: European Molecular Biology Organization (EMBO) Long-Term Fellowship

PI3

Quantitative proteomics to study oncogenic signalling pathways

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Introduction: Understanding signal transduction pathways is important for understanding normal biologic processes as well as for studying diseases such as cancer. Phosphorylation is a key post-translational modification that regulates the activities of a number of molecules in cells. Aberrant activation of kinases has been linked to a number of cancers and a substantial number of newer drugs for cancer therapy are directed against kinases. Our goal is to study signalling pathways that are aberrantly activated by oncogenic drivers using global quantitative proteomics approaches.

Methodology: By employing quantitative labelling approaches (SILAC or TMT), we have examined global changes in phosphorylation in a number of cellular systems including triple negative breast cancer cell lines, tamoxifen-resistant estrogen receptor positive cell line model, cancer epithelium/fibroblast co-cultures and isogenic lines with a variety of oncogenic drivers. More recently, we have also initiated studies on proteomic analysis of formalin fixed paraffin-embedded tissues.

Results and Discussion: From our tyrosine phosphoproteome profiling studies of a panel of triple negative breast cancer cell lines, we identified a number of tyrosine kinases as potential targets. Some of these kinases were further tested in cell culture and animal studies which confirmed that their inhibition could diminish tumor growth. From the cell line model of tamoxifen resistance of an ER positive cell line, MCF-7, we identified focal adhesion kinase, FAK2, as a novel molecule that could induce tamoxifen-resistance. Our experiments on co-culture systems and several

isogenic cell line sets have generated exciting data that reveal activation of novel signalling pathways and crosstalk.

Conclusions: High resolution mass spectrometry coupled to quantitative approaches are a powerful tool to dissect signalling pathways and can be used for addressing number of biomedical questions in health and disease.

Biography

Akhilesh Pandey, M.D., Ph.D.

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Areas of interest: Signal transduction, Mass spectrometry, Systems biology, Molecular medicine

Dr. Pandey has a broad background in medicine, pathology, molecular biology, proteomics, mass spectrometry and bioinformatics. He developed the SILAC method for quantitative proteomics, which is now a gold standard for accurate quantitation of proteins and post-translational modifications. He is the Founder and Director of the Institute of Bioinformatics, a non-profit research institute that he founded in Bangalore in 2002. His laboratory at Johns Hopkins, along with scientists at the Institute of Bioinformatics, recently developed a draft map of the human proteome.

DOD Era of Hope Scholar Award, U.S. Department of Defense

Human Proteome Organization (HUPO) Discovery in Proteomic Sciences Award

PL4

Proteomics and Precision Health: A Phenotypic Approach to Precision Cancer Detection

Sudhir Srivastava, Ph.D., MPH

Division of Cancer Prevention, NCI, USA

There is an increasing trend toward developing target-specific approach to early detection, prevention and treatment. This effort is being facilitated by the development of a vast array of enabling technologies and cancer-specific biomarkers that are helping in identifying patient-specific prevention and treatment strategies. The Speaker will discuss some of the proteomic approaches that are being developed by the NCI Early Detection Research Network (EDRN). The EDRN ensures that there is a synergy among various technologies and the potential to integrate them for biomarker development and results clearly demonstrate the value of the EDRN in delivering a product that is greater than the sum of the individual projects. Integrated genomic and proteomic technologies are yielding a highly innovative strategy for

identifying candidate biomarkers for early detection that draws upon the multiple disciplines represented within EDRN (i.e., clinical and basic science, technology development, biostatistics and bioinformatics). An efficient and cost effective way to rapidly verify potential candidate biomarkers developed by EDRN researchers and further refine a biomarker panel in pre-clinical validation studies is provided by employing highly sensitive targeted mass spectrometry-based technologies, such as SRM and PRISM-SRM, before further investment in the development of expensive, clinical-grade immunoassays. The Nucleic Acid-Programmable Protein Array (NAPPA) platform opens the possibility of exploiting the natural tumor-antigen signal amplification provided by autoantibodies to identify novel targets that could be used to develop more sensitive early detection biomarker assays. Some examples will be highlighted to illustrate EDRN's integrated approach that simply could not have been without concerted efforts.

Biography

Sudhir Srivastava,
Ph.D., MPH, MS

Dr. Srivastava received his PhD. Degree in biological science from Banaras Hindu University in 1977. Subsequently, he received his M.S. degree in Computer Science from the Virginia Commonwealth University in 1987 and a MPH degree from the Johns Hopkins University in 1997. He did postdoctoral work at the University of Osaka, Japan; the University of California at San Francisco; and the University of Arizona, Tucson.

Dr. Srivastava is Chief of the Cancer Biomarkers Research Group in the Division of Cancer Prevention, National Cancer Institute. He joined the National Cancer Institute in 1988. Since 1990, he has served as program director in the Division of Cancer Prevention and focused his responsibility in developing molecular signatures of cancer cells for cancer detection research programs with primary emphasis on cancer screening, early detection, risk assessment and informatics.

Dr. Srivastava is an internationally recognized leader in cancer biomarker research. He is best known for his seminal contributions to improving systems approach to biomarker discovery, development and validation. Under his leadership the network has begun translating biomarkers into clinical tests for early detection and diagnosis, risk assessment, and prognosis. He has played a key role in conceptualizing and implementing informatics infrastructure for the EDRN in collaboration with NASA (Jet Propulsion Laboratory), a model collaboration being followed elsewhere in NIH.

Dr. Srivastava is best known for his work on developing medical guidelines on the diagnosis of Hereditary Non-polyposis Colorectal Cancer (HNPCC). He played a pivotal role in the development of the Bethesda Guidelines for diagnosing HNPCC, which is in clinical practice worldwide. He has received several honors and awards and is a member of a number of scientific committees world-wide. In 1995, he was elected to the American Joint Committee on Cancer (AJCC) which is responsible for developing staging criteria for



cancers for worldwide use and currently serves on the AJCC Executive Committee. He was featured in Wired magazine in August 2003 for his leadership in cancer diagnostics. He has been a visiting Professor at several medical and academic institutions, and has delivered several inaugural and keynote addresses.

He is Editor-in Chief of the journal *Cancer Biomarkers*, published by the IOS press and serves as Associate Editors and reviewers for several internally known journals. He has received many prestigious awards in science and beyond by receiving a Team Science Award for Informatics by the Jet Propulsion Laboratory, NASA for his visionary and innovative use of NASA Data System Technologies in biomedical science.

L1

Hyperplexing enables study of strain-specific temporal dynamics of host response to mycobacterial infection

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Introduction: Quantitative proteomics is limited by throughput in its multiplexing capability. Metabolic labeling by SILAC allows a 3-plex experiment while isobaric chemical labeling by iTRAQ/TMT allows up to 8 to 10-plex respectively. This makes studying temporal proteome dynamics an intangible and elusive proposition.

Methodology: We have developed a new variant of hyperplexing method, combining triple SILAC with 6-plex iTRAQ to achieve 18-plex quantitation in a single MS run. THP1 macrophages were infected with different strains of Mtb (H37Rv, H37Ra, BND433, and JAL2287) and the newly translated proteins in response to different strains were temporally studied for twelve time points. For quantitation, the strains were encoded with two sets of triple SILAC-H37Ra & H37Rv in one and BND433 & JAL2287 in another with a control in each. These sets were then iTRAQ-labeled to encode for temporal profiles across twelve time points in two sets of 6-plex iTRAQ. Effectively a 72-plex design with 4 replicates of each set, these experiments could have taken months on mass spectrometer, which was completed within few days.

Results and Discussion: Using MaxQuant and in house developed tools and pipelines, we have analyzed the data to map the temporal and strain specific dynamics of newly translated proteins in host. The commonalities and differences in these proteins and their expression will act as direct indicator of how virulence is manifested in response to Mtb

infection. This will help in targeting specific perturbed proteins as prospective targets in devising an effective drug.

Conclusion: Hyperplexing enables large scale spatio-temporal systems biology studies where large number of samples can be processed simultaneously and in quantitative manner. With faster mass spectrometers, expanded coding capabilities of labeling methods and improved algorithms, proteomics is swiftly moving towards large scale quantitative biology to realize the systems biology dream.

YSL1

Emergent properties of post-translational modifications in the human disease proteome

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Introduction: Post translational modifications (PTMs) regulate many crucial functions of proteins including structural stability, signalling, localization, structure, enzymatic activation & inhibition and protein-protein interactions. In disease conditions, the changes in PTM status may result in aberrant protein function and disruption of protein-protein interactions.

Methodology: The highly curated neXtProt database on human proteins was downloaded from <ftp://ftp.nextprot.org>. The protein information from XML and text files was parsed and information integrated from different files into tabular format using Perl programs. The data was analyzed to identify global patterns with respect to PTMs. Also distribution of PTMs in disordered regions was analysed to explore if disorder has a role in harbouring PTM hotspots and their role in promoting protein-protein interactions.

Results and Discussions: All chromosomes harbour a high proportion of PTM containing proteins (~60-70 %) demonstrating their global importance. Multi-PTM proteins have a high propensity to be present in diseased conditions ($p<<0.001$). Some non-disease proteins containing multiple PTMs are housekeeping in nature suggesting the importance of PTMs in growth, metabolism and energy generation. We defined - Functional diversity index (FDI) of a protein (based on biological processes, protein-protein interactions (PPIs) and PTM frequency), which is an indicator for disease susceptibility of a human protein. We are currently exploring if domains and disorder also have a disease prediction propensity.

Conclusion: PTMs in non-housekeeping proteins may be source of variation at the phenotypic level which although may result in metabolic diseases, is also a driver for dynamic

adaptation in changing environment. This helps in rewiring of metabolic networks, providing a mechanism for adaptive evolution. Understanding how PTMs regulate the adaptive evolution of human proteome may help find the appropriate hub proteins as drug targets or biomarkers of disease progression through network inference.

PL5

Proteomics analysis of recombinant antibodies purified by affinity chromatography or precipitation, are they different?

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Detailed methods must be employed to discriminate recombinant proteins purified by different methods. Still a therapeutic protein is defined by the process. Protein A affinity chromatography is the standard method for separation of recombinant antibodies in laboratory and industrial scale. An alternative method for capture of antibodies is precipitation with ethanol or PEG. Precipitation methods are only feasible for culture supernatants with a titer above 1-2 g antibody/Liter. It has been argued that precipitation may harm the integrity of the antibody molecule and thus antibody precipitation was not considered in the past. We have developed a batch and continuous method for capture of antibodies from clarified culture supernatant using PEG or cold ethanol precipitation. In case of cold ethanol precipitation the culture supernatant is concurrently cooled and ethanol is added. The precipitate is harvested in depth filtration and dissolved by back flushing. In case of PEG, a concentrated PEG solution is added in a tubular reactor and the precipitate harvested by microfiltration and the precipitate dissolved by diafiltration. The antibodies have been also purified by conventional protein A affinity chromatography using SuRe as stationary phase. The integrity of the antibody molecules was checked by isoelectric focusing, analytical ion-exchange chromatography, SEC-HPLC, FTIR spectroscopy and CD spectroscopy and 2D-DIGE. Furthermore we have checked the affinity to Fc gamma III receptor by Biacore. A significant difference between the antibodies purified by protein A affinity chromatography, PEG precipitation, and cold ethanol precipitation could not be detected. This was the basis for a development of a capture process for antibodies by precipitation in pilot scale level.

Biography

AloisJungbauer

Professor

Department of Biotechnology
University of Natural Resources and Applied Life Sciences Vienna, Austria
Austrian Centre of Industrial Biotechnology



Professor Alois Jungbauer received his PhD in Food Technology and Biotechnology from the University of Natural Resources and Life Sciences Vienna, Austria 1986. He serves since then as a professor at the Department of Biotechnology. He teaches Protein Technology and Downstream Processing and Bioprocess Engineering. He also acts as area head and Dep. Director of Research in the Austrian Centre of Industrial Biotechnology. He is currently working in the field of bioengineering of proteins, plasmids and viruses with special focus on expression, downstream processing and characterization of large biomolecules.

As a prolific researcher he has more than 270 publications on recombinant protein production and bioseparation, 15 patents and 12 book contributions and recently a monograph entitled "Protein Chromatography, Process Development and Scale Up". He is executive editor and co-founder of Biotechnology Journal, and member of editorial boards from numerous journals in the area of biochemical engineering.

IL 2

Protein Quantitation- Which LCMS method is right for my experiment?

Andreas Huhmer

Director Marketing Proteomics, Life Science Mass Spectrometry, Thermo Fisher Scientific.

Abstract

Proteomics has become the preferred tool for the precise quantitation of many proteins in a sample. In addition, MS-based protein quantitation tools are complementary to genetic tests and are rapidly become the preferred method for targeted protein quantitation over traditional methods, such as Western blotting, ELISA and others, because it allows biologists to simultaneously quantitate targeted proteins and their relevant isoforms, post-translational modifications (PTMs), and interaction partners in a single experiment. Proteomics has recently enabled Biologists to conduct protein quantitation by a number of different LCMS-based methods, such as targeted methods based on SRM (single reaction monitoring) or PRM (parallel reaction monitoring) as well as higher-throughput, multiplexed methods such as TMT (tandem mass tag) and DIA (data-independent methods). The presentation will discuss the individual strengths of the various protein quantitation methods with respect to their specific application to biologically oriented questions.

Biography

Andreas FR Hühmer

He is currently the Marketing Director for Proteomics at Thermo Fisher Scientific in San Jose, CA. In his current role, he directs the day-to-day marketing business as well as the long-term strategy for the business. He



collaborates closely with colleagues in product marketing within the business unit and across the division to deliver innovative and enabling solutions to customers. In a previous role, Dr. Hühmer was instrumental in commercializing the ProteomeX ion trap product, the first highly integrated turnkey solution for Mudpit experiments. He also was responsible for the development of the next generation biosoftware products, such as Proteome Discoverer and SIEVE. Dr. Hühmer holds a Ph.D. (1997) and M.S. (1996) in Pharmaceutical Chemistry from the University of Kansas. He also received a B.S. (1991) and a M.S. (1993) in Analytical Chemistry from the Free University of Berlin, Germany.

IL 3

On-chip analysis and proteomics studies of blood microparticles with label free detection techniques

Dr Wilfrid Boireau

Micro Nano Sciences and Systems Dept., FEMTO-ST, France
 Blood microparticles (MPs) are small membrane vesicles, coming from degradation processes from various cell types. They are recognized to play important role in various biological processes and are also recognized as potential biomarkers in the diagnosis and prognosis of various health disorders. At the present time, the technic of choice used for the detection of MPs is flow cytometry (FC). But the main drawback of FC is the lower detection limit (about 300 nm at best); consequently, only a small fraction of microvesicles can be detected ($\geq 300\text{nm}$). Our work aims to use the potential of surface plasmon resonance (SPR) method to quantify and qualify nanoparticles in complex media. This label free technology is capable to detect a large panel of biological targets, from the nano- to the micro-scale, and allows to determine the presence of different subpopulations of microparticles via their immunocapture onto the surface of biochip using different specific ligands. The capture level of MPs (2D quantification) is related to their concentration in the sample (3D quantification), and can be determined from the SPR response. To reach the quantification of MPs in solution (3D), we propose to use internal standards. Additionally, the specific capture of our calibration particles has been validated, allowing to consider them as internal standards; these results paved the way of the quantification of physiological MPs in biological and complex samples. Moreover, a combination between SPR, AFM and MS approaches appears to be suitable for a deep analysis of captured MPs on the chip. Expected from this investigation at the nanoscale, the discrimination of all the subpopulations of MPs was achieved and proteomics analysis were engaged. Especially, we have adjusted these analytical platform to analyze, qualify and compare, non-activated and collagen-activated platelet MPs. Promising results suggest that this bioanalytical platform follow a suitable analytical pathway for a sensitive, reproducible and label-free characterization and quantification of whole MPs subpopulations, even the smallest MPs.

Biography

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Wilfrid Boireau obtained his Ph.D degree in enzymatic engineering from the University of Technology of Compiègne (UTC, France) in 1999. He joined the CNRS as researcher in 2001 after a post-doctoral position in the Center of Molecular Genetics (CGM, Gif/Yvette, France). Head director of the Micro Nano Sciences & Systems department (MN2S) of FEMTO-ST Institute, Besançon, France (www.femto-st.fr), his current researches are based at the interface of bio-engineering, microtechnologies and nanostructured materials for the development of sensors and analytical platforms in the fields of proteomics and diagnosis. Moreover, he co-founds the "Clinical - Innovation Proteomic Platform" (CLIPP) in 2008, a laboratory devoted to the investigation of deep proteomes with clinical prospects.

Co-author of 49 publications and of 4 patents since 1998, Dr Wilfrid Boireau has also presented, during the last decade, around 120 communications in national and international conferences and has been awarded twice for Outstanding Poster Presentation (2010 & 2011).

and unknown drug. Our group is working to develop novel anticancer drug against different specific cancers. We have investigated successfully the efficiency of various novel natural and synthetic metabolites on different human cancer like retinoblastoma, leukaemia, melanoma, and lung using cell culture and mouse model with the help of quantitative proteomics. Mode of action and signalling mechanism of these novel drugs has been revealed by using quantitative proteomics.

Biography

Dr. Suman S.Thakur

Affiliation: Centre for Cellular and Molecular Biology, Hyderabad, India

Designation: Senior Scientist

Started New Group: Proteomics and Cell Signaling

Education: University of Delhi, Ph.D. (2002)

Professional Appointments:



Walter Reed Army Institute of Research, Washington \ D.C., USA (Postdoc, 2002- 2004)

Indian Institute of Science, Bangalore, India (Postdoc, 2005 2009)

Max Planck Institute of Biochemistry, Munich Germany (Postdoc, 2009- 2011)

Centre for Cellular and Molecular Biology, Hyderabad, India, (Senior Scientist, from 2011)

Research Interests: Mass Spectrometry based Quantitative Proteomics, Cancer Biology, Stem Cell, Diabetic and body fluid proteomics.

Academic Honors & Awards: 2002- 2004, National Research Council Fellow of National Academies, USA

IL4

Importance of chromatography in quantitative proteomics and drug discovery

Dr. Suman S. Thakur CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India

Abstract

Chromatography is one of the important aspects of modern proteomics. High throughput quantitative proteomics using modern mass spectrometry with high resolution chromatography has found its important applications in the field of life sciences and medical biology. One of the powerful techniques that we had developed in the field of high throughput quantitative proteomics is single-run analysis without any pre-fractionation to quantify accurately the changes at the level of proteome. In depth quantitative proteomics able to find the cause of disease especially narrowing down to particular gene and it is coming closer to transcriptomics and genomics. The quantitation of proteins using stable isotope labeling with amino acids in cell culture (SILAC) would be discussed in details especially to find the target in disease. Isobaric tags for relative and absolute quantitation (iTRAQ) has been applied to find the biomarker of especially infectious disease and metabolic disorder-diabetes and its complications.

Quantitative proteomics including label free approach is very helpful to understand the mode of action of different known

II5

A proteomic analysis of endurance augmenting activities of Cerium oxide nanoparticles

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Introduction: Cerium oxide nanoparticles (Nanoceria) are being extensively used in industrial applications like glass polishing, fuel additives, catalytic materials, solid-oxide fuel cells and solar cells. Interestingly, nanoceria also possesses multi-enzyme mimetic activities such as superoxide dismutase, catalase and peroxidase. In the present study, we investigated redox modulating properties of nanoceria in exercised muscle with an emphasis on mitochondrial function.

Methodology: Cerium oxide nanoparticles were synthesized using a micro-emulsion method. The size and shape were

determined using electron microscopy, XRD and IR-spectroscopy. The hydrodynamic properties were determined using particle size analyzer. Male SD rats (6 week old) were injected with 5 µg/kg BW weekly dose of nanoceria for 5 weeks along with exercise training. At the end of 5 weeks, running endurance was measured by measuring the time of exhaustion on a trade mill. We also measured muscle weight, fiber composition, myosin content, ATP, glycogen and lactate levels along with in vivo particle localization. We also used iTRAQ –based proteome profiling to understand the underlying molecular pathways.

Results and Discussion: Spherical nanoparticles with 4 nm particle diameter were synthesized with fluorite structure. The nanoparticles were suspended in PBS without any aggregation with hydrodynamic diameter of 80 nm and zeta potential of +50 mV. The IM injection of nanoceria resulted in 4.1 fold increases in running endurance over sedentary rats and 1.7 fold increases over exercised rats. We also observed higher muscle mass, increase in slow myosin fibers, myosin, glycogen and ATP content. In contrast we observed low l-lactate content in nanoceria supplemented muscles post exercise. The Muscle proteome analysis resulted in the identification of more than 700 proteins. We observed higher abundance of NDUS1, NDUA2, NDUA9, NDUF12 in complex I, SDHA in complex II, UCCRFS, UCCRC2 in complex III and ATP5B, ATP5D in complex V in Fr-CNP treated exercised muscles over exercised muscles. IPA-based pathway analysis also identified oxidative phosphorylation, tricarboxylic cycle, and ubiquinone metabolism as significant canonical pathways for Fr-CNP treated exercised muscles.

Conclusion: Cerium oxide nanoparticles augment muscle endurance by promoting oxidative phosphorylation. These nanoparticles have the potentials for preserving muscle functions in several clinical conditions where strenuous exercise is not advised.

Biography

Dr. Kalpana Bhargava

Scientist 'F' & Division Head

Peptide and Proteomics

Defence Institute of Physiology and

Allied Sciences (DIPAS)

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Dr. Kalpana Bhargava completed her Ph.D in Peptide Chemistry from Banaras Hindu University in a joint collaboration with Indian Institute of Science, Bangalore in 1999. After her Ph.D she pursued 10 years of multidisciplinary research in academics and R & D environment from USA.

Dr. Bhargava joined DIPAS, DRDO, Delhi in April 2008. As scientist in Defence Institute of Physiology & Allied Sciences (DIPAS), she is heading the Division of "Peptide and Proteomics". She has twenty years of research experience in a wide range of areas which includes: anti-oxidant peptides, anti-microbial peptides, free radical biology, mitochondrial biology, nano-conjugate chemistry, protein biochemistry, proteomics, ribosomal biology and synthetic medicinal chemistry. She has almost 50 international, peer reviewed

journal publications, one book, two book chapters, three patents and several scientific awards/honours to her credit. Her current research work includes, proteome profiling of plasma/serum exposed to hypoxia at high altitude and Nano-material application for maladies caused at extreme environment and drug delivery.

II6

A Comparison of Peptide Quantification Using a Novel Integrated Microfluidics Device and a Nanoscale UPLC with MRM Detection.

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Introduction: Biomarker validation is technically challenging requiring high throughput, high sensitivity, high resolution, wide dynamic range and high selectivity. Miniaturized LC systems offer improved mass-sensitivity but often lack throughput, robustness and reproducibility. A comparison of a novel integrated microfluidic UPLC device with a conventional nanoscale UPLC system, using both high& low resolution MS/MS (MRM) detection (for the quantification of biomarker peptides) is summarized in this presentation.

Methods: Stable Isotope Labeled (SIL) peptides, that are analogues of putative biomarkers for cardiovascular disease, were spiked at various levels into digested human serum. A reversed phase gradient was employed on both a novel integrated microfluidic UPLC device and a conventional nanoscale UPLC system. MS/MS (MRM) detection using both low resolution tandem quadrupole (QxQ) and high resolution Q-ToF instruments was employed.

Preliminary Results: MRM chromatograms, using both high & low resolution, showed peptides to be resolved from matrix background over the entire dynamic range for the candidate biomarkers. For example, <100 amol TAENFR on-column was readily detected with no background matrix interference using the microfluidic UPLC device and low resolution MS/MS (QxQ) detection under high flow and throughput conditions. In comparison, 30 amol of ESDTSYVSLK on-column was detected using nanoscale UPLC and high resolution MS/MS (Q-ToF) detection. Ultimate sensitivity was <10 amol on-column using conventional nanoscale UPLC with either low or high resolution MRM detection (with a minimum of two transitions per peptide). Typical retention time reproducibility with either the microfluidic UPLC device or the nanoscale UPLC equaled 0.02 min standard deviation.

Conclusions:

1. The novel integrated microfluidic UPLC device enabled twice the throughput (at reduced sensitivity) compared to the conventional nanoscale UPLC.

2. Detection limits were in the 10's of amol on-column for the novel integrated microfluidic UPLC device and <10 amol on-column for conventional nanoscale UPLC.

3. Overall, the optimum combination of throughput, sensitivity, linearity and reproducibility was provided by the novel integrated microfluidic UPLC device (Waters IonKey/MS) coupled to the highest sensitivity QxQ mass spectrometer (Waters Xevo TQ-S).

Biography

Mark A. McDowall

Dr. Mark McDowall obtained his BSc in Chemistry (1980) and his PhD in Biological Mass Spectrometry (1984) from the University of Wales working on the development and application of 'primitive' LC/MS technologies. He obtained a post-doctoral fellowship from the Royal Society (of Great Britain) in 1984 to research the mechanism of thermospray ionisation at the University of Bonn (Germany).



Mark joined the LC/MS development team of VG Instruments in 1985 and was involved in the mass spectrometry operations and management of the company for 30 years - throughout its evolution to Micromass, where he was Director of Marketing, and more recently Waters Corporation.

Dr. McDowall served two terms of office on Waters Corporation's Scientific Advisory Board before he 'semi-retired' in March 2014. Today Mark continues his career-long association with Waters as a Consultant Mass Spectrometrist. Dr. McDowall is a member of the executive management committee of the British Mass Spectrometry Society (BMSS) and a fellow of the Royal Institution (Ri) of Great Britain.

YSL2

Proteomic characterization of two elapid snake venoms: Indian cobra (Najanaja) and common krait (Bungaruscaeruleus)

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Introduction: Snake venom is a complete mixture of proteins and peptides and a number of studies have been carried out that describe the biological properties of several venomous proteins. Despite these studies, a complete proteomic profile of many snake species is not available. Indian cobra (Najanaja) and Common krait (Bungaruscaeruleus) are highly venomous elapids common in India.

Methodology: To assess the venom proteomic profiles of the

two snakes, three different approaches were included- i)one-dimensional SDS/PAGE and in-gel digestion coupled with ESI-LCMS/MS (electrospray ionization-liquid chromatography tandem mass spectrometry), ii)In-solution digestion coupled with ESI-LCMS/MS, iii)Gel-filtration coupled with digestion and ESI-LCMS/MS.

Results and Discussion- In the current study 117 and 65 different proteins were identified from Najanaja and Bungaruscaeruleus venoms, respectively. These proteins were classified into 19 different venom protein families. Both venoms were found to contain a large number of phospholipase A2 enzymes and three finger toxins. Whereas snake venom metalloproteases were in higher quantity in Najanaja compared to Bungaruscaeruleus, the analysis identified smaller number of other snake venom proteins such as L-amino acid oxidases, Cysteine-rich secretory proteins, 5'-nucleotidases, Venom nerve growth factors, etc. We also detected in the cobra venom, the presence of Kunitz-type inhibitors, Cobra venom factor, Phosphodiesterases and Aminopeptidases unlike the Krait venom where enzymes such as Acetylcholinesterases and Hyaluronidases were identified.

Conclusion: Understanding the proteome composition of Najanaja and Bungaruscaeruleus can provide useful information on the unique and important toxins and non-toxin proteins in the venoms. This knowledge will also find use in the management of cobra and krait envenomation as these two members of the Big Four snakes cause the highest number of snakebite deaths in India every year.

IL17

Conjoint IMAC device (disk to microfluidics): a pre-fractionation approach for plasma proteomics

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The primary goal of biomarker proteomics is to enable the development of simple non-invasive tests that could lead to rapid and accurate diagnosis of disease progression in patients. Despite advances in quantitative proteomics, the present methods are unable to differentiate structural differences associated with certain pathologies, such as: (i) Mutations and structural variations, (ii) Simple 3D structure variations induced by post-translational modifications, (iii) Isoforms, (iv) Minor truncation, (v) modifications in protein-protein interactions very important to interactomics,etc. The Human Protein Atlas covering most of the proteins by antibody recognition also has limitations in identifying such subtle structural variations.

In order to overcome limitations caused by the broad dynamic range of proteins in biological samples and structural

variations, multi-dimensional separation techniques have been proposed prior to MS analysis. These multi-dimensional separation techniques include ion exchange, reverse phase, affinity chromatography, which utilize different binding and elution conditions limiting their continuous 'on-line' utilization. Hence, simple technologies, which can be tailored for on-line pre-fractionation and identification of these variants based on specific recognition sites like reporter amino acids, are the need of the hour.

In this context, the immobilized metal-ion affinity (IMA) concept, based on the recognition of accessible Histidine residues on a protein by divalent transition metal ions can serve as an excellent tool for simple detection of variants. This recognition is dependent on both qualitative and quantitative aspects of Histidine. Different metal ions (Co^{++} , Zn^{++} , Ni^{++} , Cu^{++}) exhibit different demands in terms of Histidine surface topography of the protein under identical adsorption/desorption parameters. This enables the possibility of passing the same sample through different metals in tandem in decreasing order of Histidine-requirements ($\text{Co}^{++} \rightarrow \text{Zn}^{++} \rightarrow \text{Ni}^{++} \rightarrow \text{Cu}^{++}$).

In this work, we present a few preliminary data in terms of discriminating the individual proteins and protein-protein interaction complexes, as well as oxidative stress-induced structural modifications of proteins involved in cardiovascular disorders.

Furthermore, the ability to integrate this tool into microfluidic systems makes them more suitable for a rapid on-line selection of protein variants prior to mass spectrometric analysis for protein identification, owing to advantages such small sample volumes, multiplexing of microchannels in a single chip, efficient separation, rapid & reliable on-chip analysis, etc. We are prototyping the above discussed conjoint-IMA system into a microfluidic device that can serve as a rapid and efficient on-line analytical pre-fractionation tool to identify proteins from plasma using less plasma sample volumes.

Biography

Dr. Kali Kishore Reddy Tetala

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After successful completion of M.Sc (Organic Chemistry) from Andhra University, India (2001), Dr. K.K.R. Tetala moved to Southampton

University, Southampton, U.K to pursue M.Phil in Organic Chemistry (2004). He obtained his PhD degree in Bio-Analytical Chemistry from Laboratory of Organic Chemistry, Wageningen University, Wageningen, The Netherlands (2009). His PhD thesis was on "Microfluidic devices for sample clean-up and screening of Biological samples". He was employed by Institute of Sustainable Process technology, Amersfoort, The Netherlands to work as post doctoral researcher at Food and Bioprocess Engineering department, Wageningen University, Wageningen, The Netherlands (Oct'08-Mar'09) and Biomaterials Science and Technology group, University of Twente, Enschede, The Netherlands

(Apr'09-Jun'12). Afterwards, he worked for 1 year as post doctoral researcher at Inorganic membrane group, University of Twente, Enschede, The Netherlands. Since Jan'14, he is working as Assistant Professor at Centre for Bioseparation Technology, VIT University, Vellore. His research expertise are: Synthesis of organic molecules, Development of Microarrays, Monolithic columns in both capillary and microfluidic chip formats, Polymer Membranes, Surface Chemistry (Chemical modification) and Biomolecule interaction studies.

PL 6

So many sphingolipids -what do they all do?

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Sphingolipids (SLs) are ubiquitous components of eukaryotic cell membranes and are found in species as diverse as fungi to mammals, and in some bacteria and viruses. Interest in SLs has blossomed over the past couple of decades due to two discoveries, namely that (i) in addition to their well-established roles as structural components of cell membranes, SLs also turnover in a number of cellular signaling pathways, and (ii) SLs are essential component of membrane-rafts/microdomains. Together, these findings have revolutionized SL biology and stimulated research directions that could not have been foreseen twenty years ago. In addition, great interest has been shown in the past few years on the role of SLs in human diseases, since SLs have been implicated to play vital roles, either as the direct cause of human disease, such as in the lysosomal storage diseases, or down-stream to the causative disease mechanism; nevertheless, even in the latter case, intervention in the SL pathway has beneficial effects on disease pathology, in diseases as wide-ranging as cancer, diabetes and psychiatric and neurological diseases.

The acyl chain composition of sphingolipids (SLs) is determined by the specificity of the ceramide synthases (CerS), integral membrane proteins of the ER. Six mammalian CerS exist, with each synthesizing ceramides with defined acyl chain lengths. CerS2 synthesizes ceramides using C22-C24-acyl CoAs as substrate. Knock-out mice to all of the CerS have been generated over the past few years, including CerS2. The major phenotypes of the CerS2 null mouse involve the brain, liver, lung and the adrenal gland. In the liver, specific rather than general pathways of liver function are disrupted. Thus, CerS2 null mice exhibit glucose intolerance despite normal insulin secretion from the pancreas, the mice are resistant to LPS/galactosamine-mediated fulminant hepatic failure, and generation of reactive oxygen species is increased due to hepatic mitochondrial complex IV dysfunction. Chronic hepatocarcinoma, which progresses to hepatocellular carcinoma in older animals, is observed in most mice. CerS2 is also expressed at high levels in the lung, particularly in the epithelial layers of the bronchi, and CerS2 null mice show high susceptibility to bacterial infection in the lung. I will discuss the mechanisms by which changes in the SL acyl chain length influence the phenotypes described above, and may impact on human diseases related to CerS dysfunction.

Biography

Tony Futerman

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Professor Tony Futerman received his BSc in biochemistry at the University of Bath, England (1981), and obtained his PhD from the Weizmann Institute in 1986, where he discovered that acetylcholinesterase, a key enzyme in terminating neuronal signaling in the brain, is attached to the cell membrane via a novel mechanism. From 1987-1990, he was a postdoctoral fellow at the Carnegie Institution (Baltimore, Maryland, USA), where he analyzed the sites of synthesis of lipid molecules in the cell. In 1990, he joined the staff of the Weizmann Institute and is currently Full Professor. He runs a laboratory of ~15 scientists, postdoctoral fellows and students, and has multiple international collaborations. Tony Futerman is the Joseph Meyerhoff Professor of Biochemistry in the Weizmann Institute of Science in Rehovot, Israel. In 2006 he chaired the Gordon conference on Glycolipid and Sphingolipid Biology, chaired the Lysosomal Diseases Gordon Conference in 2011, and was a member of Journal of Biological Chemistry editorial board for two terms, from 2000-2012.

modelling and analysis methodologies to deliver the relevant biochemical information. These chemometric platforms employed vary from simple multivariate analysis to highly complex model based analysis and is presented in a format ready for interpretation by medics.

This facility comprises of high field NMR instruments, accurate mass LC/MS instruments, tandem quadrupole LC/MS systems as well as dedicated training facility. In this presentation we will discuss the development of analytical platforms both LC/MS and NMR as well as a detailed discussion on the workflow, validation, reporting and decision making process. The presentation will cover the development and validation of the "discovery" screening methods for polar, non polar metabolites and lipid profiling using LC/MS methodology, as well as describe the use of proton NMR as an initial screen to eliminate contaminated samples. The quantitative targeted LC/MS assays will also be discussed the various compounds classes such as bile acids, amino acids, eicosanoids, and acyl carnitines.

Biography

Prof. Robert Plumb

*Dept Surgery and Cancer,
Imperial College, London*



Prof Robert Plumb is the Director of Metabolic Phenotyping and Stratified Medicine in the Waters Health Sciences Business Operations Division, based in Milford, Massachusetts.

Prof Plumb has published over 100 papers on the subject of HPLC/MS and NMR for bioanalysis, metabolomics and metabolite identification. He is a recognized expert in the use of liquid chromatography with mass spectrometry, capillary scale LC, purifications scale LC and metabolomics, giving many invited papers at international meetings around the world.

After obtaining an honors degree in Chemistry from the University of Hertfordshire in 1992, he started work in at Glaxo Research and Development Drug Metabolism Department. During his time at Glaxo and later GlaxoWellcome he continued his research in liquid chromatography combined with NMR and mass spectrometry for metabolite identification and bioanalysis obtaining his PhD in 1999. Dr Plumb continued his work for GlaxoWellcome with the responsibility of metabolite identification using HPLC/MS/NMR and new analytical technology development. In 2001 he moved to Waters Corporation in Milford, MA, USA where he was responsible for the Life Science Chromatography group and latterly LC/MS applications in the Pharmaceutical Market Development Group before becoming the Director of Metabolic Phenotyping. He is currently a visiting Professor in Analytical Chemistry at Kings College London, visiting Professor at Imperial College in the Dept Surgery and Cancer and a Fellow of the Royal Society of Chemistry. In 2014 he was awarded Highly Cited Researcher by Thompson Reuters.

PL7

Understanding Human Health and Disease With LC/MS Based Metabolic Phenotyping

Prof. Robert Plumb

Dept Surgery and Cancer, Imperial College, London

Abstract

The MRC-NIHR National PhenomeCentre, Imperial College London, is the first of its kind facility. Born out of the UK Olympic Legacy its mandate is to provide "high throughput, forensic quality, metabolic phenotyping to support large scale epidemiological studies as well as basic medical research into disease understanding and patient stratification". As global life-styles change we are seeing increasing cases of obesity, diabetes, and mental health issues. This not only affects a person's quality of life but also places increased strain on the health-care systems to provide the right treatment whilst managing costs closely.

Metabolic Phenotyping offers a valuable and unique insight into the underlying biochemistry of diseases as well as the patients individual biochemistry "phenotype", diet, health status, age and stress. To deliver this information the analytical data generated in processed via a variety of chemometric

IL8**CCPM v3.4: Towards Collaborative Metabolomics**

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Assisted by advances in high-throughput instrumentation, metabolomics has emerged as a popular research area, which is witnessing rapid generation of large volumes of high quality data. This demands ready availability of advanced analytical and computational methods for generating analyzed datasets, as well as advanced tools for data analysis, visualization and integration; within a collaborative platform which addresses the unique interdisciplinary character of metabolomics.

The Computational Core for Plant Metabolomics, CCPM v3.4, is a fully functional, open, state of art web application, addressing the issues of storage, processing, analysis and sharing of data and results of metabolomics experiments. With a python web framework (web2py) backend, CCPM allows rapid prototyping, easy maintenance and role-based access control without compromising on security, scalability and efficiency. Accessible through a user friendly bootstrap-based GUI, CCPM functions as a secure repository with a pipeline for end-to-end analysis of LC/GC-MS data involving raw data capture, data pre-processing, putative metabolite characterization (linking to METLIN and GOLM), data pretreatment, and a wide range of statistical analysis, with option for customization of parameters from the web interface itself. Bypassing the pipeline, the modular architecture of CCPM also allows inputting of externally preprocessed/pretreated data, directly, to available analysis tools. In contrast with task size restrictions, associated with usual stateless or session-oriented web applications, CCPM supports long-running tasks featuring task completion alerts. Other features include support for handling data in all popular file formats, user friendly tools for on the fly data analysis and visualization, and provision for publication quality image download.

Biography**Abhijit Mitra**

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Professor Abhijit Mitra (PhD 1978; Indian Institute of Technology, Kanpur), Alexander von Humboldt fellow (1984-1986), is a physical organic chemist by training who has primarily been involved in chemistry education in his early career. He joined IIIT Hyderabad in July 2002 to develop its Bioinformatics program and has been involved in the development of the Center for Computational Natural Sciences and Bioinformatics (CCNSB) [erstwhile Bioinformatics Research Center (BiRC)], which spearheads research and educational activities in the interface of computational sciences and natural sciences. His research interests are centered on understanding molecular mechanisms of functional biomolecules using quantum chemical computations and molecular dynamics simulations. His major contributions have focused on geometries and interaction energies of noncanonical base pairs and structure and dynamics of riboswitches. Currently he is also interested in the broad area of computational metabolomics.

IL9**Proteomics and Metabolomics Analysis by CESI-MS: Application to Biologics Characterisation, Bio similarity Assessment and Biomarker Discovery**

Jim Thorn, PhD, EMEA Marketing & Applications Leader, SCIEX Separations

Abstract

The analytical challenges for Mass Spectrometry in the field of Proteomics, Metabolomics and Biopharmaceutical Biosimilarity assessment are well known. These include complex workflows to study protein PTMs and charge variants, the need for high resolution separation for intact protein characterization, solubility issues with charged and polar metabolites in HILIC compatible buffers and the inability to perform multiple analysis from sample volumes of only a few microliters. CESI-MS is the integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into a single process in a single device. CESI-MS operates at low nL/min flow rates with sample injection volumes below 50nL, offering several advantages including increased ionization efficiency and a reduction in ion suppression from small sample volumes.

This presentation will review the latest data using CESI-MS for assessment of Biosimilarity with examples of candidate biosimilars for Trastuzumab, Rituximab and Bevacizumab. Examples will be discussed comparing nanoLC-MS and CESI-MS for the study of phosphoproteomics and other PTMS (glycosylation, acetylation, methylation, Citrullination, etc), showing how the orthogonal nature of these two techniques expands the information which may be obtained from a sample. Finally, results of recent experiments in the field of metabolomics discovery will be presented, showing the use of CESI-MS in negative ion mode for the analysis of metabolites difficult to analyze by other means (isobaric phosphorylated sugars and low molecular weight

organic acids).

In conclusion, CESI-MS provides the capabilities to improve the separation resolution, sensitivity, speed, and depth of characterization of both intact proteins and digests as well as metabolites involved in the cellular energy cycle. These analysis can now be performed with injection of 50nL of sample from volumes as low as 1uL, allowing multiple analysis from one precious sample.

Biography

Jim Thorn,
PhD, EMEA Marketing &
Applications Leader, SCIEX
Separations



Jim Thorn completed his PhD in the field of Medicine at St Bartholomew's Hospital, University of London in 1991, investigating genetic predisposition to human atherosclerosis and cardiovascular disease. Following postdoctoral fellowships in the field of membrane transport, Jim joined Beckman Coulter in 1998. Over the course of 15 years Jim held a range of roles including European and then Global Capillary Electrophoresis Product Manager. In January 2014, the separations business from Beckman Coulter was integrated into AB SCIEX to create a new SCIEX Separations unit which combines the microscale separations from both companies. In this organization, Jim has taken the role of EMEA Marketing and Applications Support Leader, responsible for the scientific support and promotion of current and new technologies

IL10

Proteomic analysis of circulating immune complexes in diabetes: Targeted analysis of glycated albumin using fragment ion library

Mahesh J Kulkarni

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Introduction: Diabetes is a chronic condition characterised by persistent hyperglycemia. The unavoidable consequence of hyperglycemia is glycation, a non-enzymatic reaction between glucose and proteins. This process is accelerated during poorly controlled diabetes. Glycation of proteins results in altered protein structure and function. Sometimes glycated proteins cause aberrant immune response leading production of autoantibodies. Autoantibodies bind to the modified proteins and form immune complexes (IC). When system fails to remove them, lead to inflammation and immune mediated pathologies.

Methodology: Immune complexes from control and diabetic plasma of mice and clinical samples were isolated using

Protein-G sepharose columns, followed by in solution digestion and identification, quantification and characterization by nano LC MS/MS. Fragment ion library was generated from synthetically glycated albumin using high resolution accurate mass spectrometer.

Results and Discussions: The associated proteins of Immune complexes from different stages of diabetes in animal and clinical plasma were identified, characterised and quantified by nano LC MS/MS. Serum albumin was the protein found to be predominantly increased in immune complexes of diabetic plasma , which was confirmed by western blot analysis with anti serum albumin antibody. Serum albumin in IC was also found to be AGE modified in diabetic plasma. This was corroborated with presence of diagnostic fragment ions of AGE modified albumin.

Conclusions: Advanced glycation end products modify native proteins and elicit autoimmune response in the system. This may lead to abnormal clearance of the native proteins as well as leading to the development of severity and complications associated with diabetes. Further experimental work is in progress to explore these AGE modified proteins associated with immune complex, in depth.

Biography

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Research Interests: Mass spectrometry and Proteomics, Advanced Glycation End Products and Diabetes

Our group's research activity involves developing mass spectrometric application for the proteomic research, as well as to understand the molecular mechanisms of biological process using proteomic approaches. The main focus of my lab is to elucidate the role of advanced glycation end products (AGEs) in diabetes and its complication. The long term goal is to identify a diagnostic marker for diabetic complications, identify drug targets and develop intervention strategies.

Awards/ Recognition/Membership

NCL Research Foundation - Scientist of the year, 2010
Elected as Young Associate of Maharashtra Academy of Sciences, 2007
Life member of Indian Society for Mass spectrometry.
Joint Secretary, Proteomics Society, India from, 2014

Relevant Publications: 49

II11

Glycoproteins Analysis Challenges and Solutions

Dr. Jaran Jainhuknan

Bruker Daltonics

Frontier of analysis of biological molecules is now at analysis of polysaccharides. They are responsible for functions of proteins and are important for immune response as they are binding sites of pathogens. Analysis of glycoprotein and glycan is challenging because of the diversity of connectivity, chirality of sugar and other modifications on the molecules. MS analysis and fragmentation has provided tools for accelerated pace of progress in the field. We discuss the update of technology and methodology of glycoprotein and glycan analysis.

Methodology: Released Glycans are analyzed directly by MALDI-TOF and TOF/TOF. Fragment ions are subjected for spectral library search. Library can be update from various public domain sources.

Glycopeptides are analyzed preferably by MALDI-TOF and TOF/TOF because sample can be revisited for additional measurement. However, if sample is a mixture and require LC separation, LCMS measurement can be performed. For LCMS/MS runs, fragmentation information of glycans can be obtain from standard CID, but peptide sequence information require electron-transfer dissociation (ETD). The method and experimental setup as well as results will be discussed.

Biography

Dr. Jaran Jainhuknan

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Research interests: Application of Mass Spectrometry Achievements: Visiting Lecture and Trainer for Mass Spectrometry Memberships: American Society for Mass Spectrometry

Jaran Jainhuknan obtained his Ph.D. in Analytical Chemistry studying structures of peptides in gas phase and fragmentation of peptides under MALDI-PSD conditions. He joined Bruker as an application scientist in 1998. Over the past 10 years, he has enjoyed helping scientists develop LCMS methods for various applications including proteomics and protein identification, metabolite profiling and identification, metabolite quantitation and screening. Seeing well-planned experiments work out satisfies him.

IL12

Nuclear envelope resident, CaSUN1, participates in dehydration signaling by modulating unfolded protein response

Niranjan Chakraborty

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The complexity in the perception of dehydration driven stimuli and induced adaptive responses, especially in the context of membrane proteins, is poorly understood. Dehydration affects almost all the physiological processes including those that result in the accumulation of misfolded proteins in the endoplasmic reticulum (ER), which in turn elicits a highly conserved signaling, the UPR (unfolded protein response). We investigated the dehydration-responsive membrane proteome of a food legume, chickpea, which led to the identification of a non-canonical SUN domain protein, henceforth designated CaSUN1 (CicerarietinumSad1/UNC-84). SUN proteins reside in the inner nuclear envelope, and are involved in the formation of LINC (linker of nucleoskeleton and cytoskeleton) complex. The SUN proteins belong to a multigene family and have been extensively studied in animal systems, but little is known about their function in plants. Here we describe the genomic organization, expression, and stress-responsive function of CaSUN1. CaSUN1 predominantly localized to the nuclear membrane and ER, besides small vacuolar vesicles. The transcripts were downregulated by both abiotic and biotic stresses, but not by abscisic acid treatment. Overexpression of CaSUN1 conferred stress tolerance in transgenic Arabidopsis. Furthermore, functional complementation of the yeast mutant, slp1, could rescue its growth defects. We propose that the function of CaSUN1 in stress response might be regulated via UPR signaling.

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Biography

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Area of expertise

Plants, being sessile, have evolved a wide spectrum of adaptations to counteract various environmental stresses. Our aim is to determine the molecular circuitry that operates in cellular response to such stresses. The alteration of protein synthesis and/or degradation is one of the fundamental metabolic processes that may influence stress tolerance. We are developing high-throughput proteomics approaches to study stress perception, signal transduction and metabolic responses of plants to multivariate stresses. We are focusing on subcellular proteomics using a number of protein mining tools in order to understand the role of protein modifications and/or their differential expression under stress conditions.

Research interests

My laboratory is developing high-throughput proteomics approaches to study stress perception, signal transduction and metabolic responses of plants to adverse environmental conditions. We are focusing on subcellular proteomics and using a number of protein mining tools to understand the role of differentially regulated proteins and/or their posttranslational modifications. The major focus is on identification of the dynamics of stress-responsive proteins (SRPs), which would not only aid in elucidation of the mechanism underlying stress tolerance, but also serve as a valuable inventory for crop improvement program.

L2**Proteomic analysis of *Podophyllum hexandrum***

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Podophyllum hexandrum Royle, containing Podophyllotoxin (PTOX), is an endangered medicinal herb of enormous economic importance. PTOX has been used as the starting compound for the production of the semi-synthetic anticancer drugs viz. etoposide (VP-16-213), teniposide (VM-26) and ethopos, as well as the newer versions, tafluposide, TOP53, NK611, GL-331 and the hybrid drug azatoxin. To meet the commercial demand, up till now PTOX has been extracted from the rhizomes of *P. hexandrum* and *P. peltatum* collected in the wild; chemical synthesis of PTOX is possible, but not economically feasible. Till date, little advances have been made in the characterization of the PTOX biosynthetic pathway, especially the later steps. The major focus of our research is to identify the proteins involved/modulate the PTOX biosynthesis. A MeJA elicited cell suspension culture of *P. hexandrum* has been established in a day-dependent mode and the PTOX content was determined by HPLC. Significant enhanced amount of PTOX was noted in 12-days old cell suspension culture which was used. Further, a differential proteomic analysis through 2-DE and MALDI TOF-TOF MSMS identification followed by functional annotation from a biological point of view through KEGG was

performed to focus on the altered abundance of protein in control and elicited cultures. Results showed that in addition to several stress-responsive proteins viz. NBS/LRR resistance protein, LRR kinase protein, glutathione transferase, PR-10, etc., a number of early as well as late phenylpropanoid pathway proteins viz. polyphenol oxidase, caffeoyl CoA 3-O-methyltransferase, S-adenosyl-L-methionine-dependent methyltransferases, caffeic acid-O-methyl transferase etc. were also identified successfully which has been explored further to unravel the PTOX biosynthetic pathway in *P. hexandrum* will be discussed.

L3**Understanding the Microbial Responses to Quorum Sensing and Biofilm Inhibitors –A Proteomic Approach**

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Abstract

Biofilm mediated infectious diseases have been found to be the second leading cause of mortality worldwide. Quorum sensing (QS) has been shown to regulate the virulome, biofilm formation and antibiotic resistance in most of the Gram-negative and Gram- positive bacteria. Ability to form the highly protective biofilm and inappropriate use of antibiotics in health care settings led to the emergence of Multidrug Resistance (MDR), Pandrug Resistance (PDR) and Extreme Drug Resistance (XDR) in an alarming number of human pathogens. Henceforth, interference with bacterial QS has emerged as an alternative therapeutic/ anti-pathogenic strategy to combat the infections caused by these pathogens. QS inhibition selectively targets the virulence factors production, biofilm formation and exerts relatively low evolutionary pressure on bacteria to develop resistance. For more than a decade, research in the field of anti- pathogenic strategy has culminated in identification of numerous anti-QS and anti-biofilm agents. In-depth knowledge of the mechanism of action and molecular targets of QS inhibitors and antbiofilm agents remain unclear. Traditional microbiological research coupled with the Two-Dimensional Gel Electrophoresis (2-DGE) and Mass Spectrometry (MS) based microbial pathogenicity research is expected to throw more light on understanding the molecular mechanism and identification of novel key proteins targeted by the QS and biofilm inhibitors. Intracellular proteome profiling and identification of differentially expressed proteins of *Pseudomonas aeruginosa* revealed that modulation of iron homeostasis, pyoveridine and pyochelin biosynthesis pathways by curcumin is responsible for reduction of virulence factor production and biofilm formation. In addition, proteomic analysis of *Streptococcus pyogenes* grown in the presence and absence of antbiofilm agents (3-furancarboxaldehyde and limonene) showed that the proteins involved in cell wall biogenesis, amino acid metabolism,

regulation of cell shape were down regulated. Further studies involving the identification of interacting partners of differentially expressed proteins would help to unearth the mechanisms underlying the antibiofilm activity and would pave the way for identifying the ideal targets for anti-QS and antibiofilm agents.

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L4

Proteomic analysis in *Caenorhabditis elegans* to understand bacterial infections

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Abstract

Purpose: Understanding host-pathogen interaction at protein level is a complex process, the outcome can be defined using information from both host and pathogen perspective in different dimensions. Infections caused by specific bacteria with increased resistance to available antibiotics raise alarm for better understanding of microbial infections and host susceptibility at proteome level. The recent molecular tools shed more light on the bacterial virulence factors and host immune effector molecules providing in depth knowledge about infection and immune response. *Caenorhabditis elegans* is a well established model for studying the bacterial infections and recently enormous data has been generated using transcriptomics and proteomics analysis using this model against different pathogens. Using *C. elegans* as a host, we have analyzed the host proteome changes against set of Gram negative pathogens including *Salmonella Typhi*, *Vibrio alginolyticus*, *Proteus Spp.*, *Shigella Spp.* and *Pseudomonas aeruginosa*. The role and contribution of identified regulatory proteins were confirmed by specific *C. elegans* mutants based studies.

Experimental description: Regulation of *C. elegans* total proteins was analyzed against Gram negative bacterial pathogens using quantitative proteomic approach. Total proteins were separated using two-dimensional differential gel electrophoresis (2D-DIGE) and differentially regulated proteins were identified using PMF and MALDI TOF-TOF analyses. Validation experiments for the expression of candidate proteins were done by Western blot analysis and their mRNA counterpart were analyzed by qPCR. The interacting partners of the short listed regulatory proteins were identified by bioinformatics tools and subsequently validated.

Results: The results suggested that *C. elegans* displayed pathogen specific response by differentially regulating proteins and specific innate immune pathways against different pathogens. Identified proteins are found to be key

regulators of essential pathways which are highly conserved in higher organisms.

Conclusions: For the first time our studies report the role of *C. elegans* proteins PDI-2, DAF-21 and EEF-2 in regulating host immune system against bacterial infections.

Key Words: *Caenorhabditis elegans*, *Salmonella*, *Vibrio*, *Shigella*, *Pseudomonas*, *Proteus*, Proteomics

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PI8

Translational Tissue and Plasma Biosignatures of Colorectal Cancer

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Current methods used for colorectal cancer (CRC) screening (e.g., FOBT, FIT and/or colonoscopy) are grossly inadequate on both sensitivity and specificity grounds. Here, we first report a program evaluating numerous (SWATH-MS and Proseek® Oncology) proteomics biomarker discovery methodologies. In addition, we discuss how proteomics allows identification of interacting membrane proteins (i.e., the metastasome) and demonstrate how proteins involved in the metastasome might regulate the cancer invasive phenotype and can be used as clinical biomarkers. Expression of 92 potential plasma cancer biomarkers were measured in pooled CRC Dukes' staged (i.e., A-D and controls) EDTA plasmas utilizing Olink's PEA based Proseek® Multiplex Oncology I kit, where duplicate samples were analysed using Bio-Plex Pro™ human cytokine 27-plex immunoassays. Expression of CEA (a diagnostic biomarker for CRC) was found to be significantly high in malignant stages C and D, whilst IL 8 and prolactin expression changed significantly between control, benign and malignant stages. We also, employed SWATH-MS a data independent acquisition (DIA) method that allows a complete and permanent record of all fragment ions of detectable peptide precursors from pooled plasmas (n=20 per stage) that were previously immuno- (i.e., MARS-12) from the same Dukes' stage A-D CRC patients with age-, sex- and other criteria matched control EDTA-plasmas. The results of these plasma biomarker studies aim for early detection of CRC and the differences between Dukes' stages will be discussed.

In addition, shotgun proteomic studies suggest that integrin (avb6) and protease receptor (uPAR) expression are changed in CRC, allowing us to understand changes associated with the metastatic phenotype. Analyses of the interaction between uPAR and avb6 has allowed us to develop lead iPEPs that antagonise this metasome. Both proteins (uPAR and epithelial-restricted avb6) were examined as intra-stage tissue biomarkers of patient survival in a large retrospective 20 year rectal cancer tissue biomarker study.

Biography

Mark S. Baker

Born 1956 Maroubra, Sydney. Completed PhD 1985 at Macquarie University and subsequent research focused on biochemistry of proteins in human health and disease (e.g., arthritis, breast, ovarian, prostate and colorectal cancer). Mark has made contributions to the growth of the science of proteomics and to the organisation of scientific societies at national, regional and global levels, as well as having a successful stint in the US biotech sector. Mark returned to serve as APAF CEO, supporting Australia's national proteomics effort and securing NCRIS for national "omics" infrastructure. Mark has served the HUPO Board and Executive and is the current HUPO President. Mark co-chaired the 9th HUPO World Congress in Sydney with Ed Nice, Ian Smith and Marc Wilkins where the global Human Proteome Project was launched and he currently drives many HUPO scientific and management activities alike, sits on various journal Editorial Boards and industry Advisory Boards. Mark was recognised in 2012 for the legacy he leaves the proteomics community with the HUPO Distinguished Service Award. He has published >140 peer-reviewed papers, supervised more than 40 students and is the inventor of IgYultradepletion patents. He remains an advocate for industry:academic collaborations, media engagement, and the promotion of career paths for young researchers.



II13

High-throughput Spatial Proteomics of Tissue using rapifleX MALDI Tissue types

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Introduction: MALDI Imaging massspectrometry (IMS) is a unique analytical tool that allows simultaneous label-free visualization of hundreds of endogenous compounds expressed in tissue. In combination with histology MALDI-TOF IMS can reveal correlation of many lipids/ peptides/ proteins to pathological features, creating a multi-dimensional scale for molecular histology that promises to aid in our understanding of disease diagnosis and treatment. Further,

MALDI imaging following enzymatic reactions on tissue provide additional depth to the molecular information that can be extracted. TOF mass analyzers provide the widest analyte versatility for MALDI imaging but existing system designs are incapable of meeting the demands for acquiring imaging datasets at a time consistent with clinical relevance. Here we describe a newly designed MALDI-TOF imaging system which offers a significant speed advantage over existing systems and makes possible the goal of tissue typing.

Methodology: The new rapifleX MALDI-TOF platform is capable of acquiring upto 50 spectra per second in imaging mode. It has a 5µm focused laser coupled to a novel mirror system which scans the laser beam while the target is in constant motion. Called smartbeam 3D, this optical system is capable of analyzing square regions of the sample as small as 10µm, the size of which the user chooses via software, which corresponds to the image resolution.

Results & Discussion: RapifleX has acquired images of several hundred thousand pixels in 1-2 hours, a time scale relevant for clinical applications. Areas of the sample that correspond to pixels are square in shape and range in size from 10- 200µm. The unique combination of moving laser and sample ensures that true pixels are created that do not overlap as with over sampling techniques while ensuring maximum analytical sampling of the complete pixel area. 10µm resolution MALDI images of phospholipids in excess of 800 k pixels have been acquired in 3hours. 20µm protein images from large biopsies, >1cm², can be acquired in 2-4 hours. Mega pixel ion images have been acquired in only a few hours.

Conclusions: The new rapifleX MALDI-TOF imaging system is capable of acquiring data ~20x faster than traditional TOF systems. The unique optical system combined with the constantly moving sample provides true square pixels as small as 10µm.

Biography

Shannon Cornett

PhD Applications Development Manager, Bruker Daltonics

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Research interests: MALDI instrumentation, MALDI imaging, Clinical mass spectrometry

Achievements: Special Features Editor, Journal of Mass Spectrometry

Memberships: American Society for Mass Spectrometry, American Society for Investigative Pathology



Shannon Cornett received his Ph.D. in analytical chemistry from the University of Georgia in 1993, working under the mentorship of Jon Amster to develop MALDI applications for FT-ICR and TOF. Following a Post-doctoral Fellowship at City of Hope National Medical Center, Shannon joined Bruker and held positions of Applications Scientist, TOF R&D Manager and Omniplex Product Manager. In 2002, he moved to Vanderbilt University as Research Assistant Professor to work with Professor Richard Caprioli developing new tools

and methodologies for the then-emerging field of imaging mass spectrometry. Shannon rejoined BrukerDaltonics in 2009 and now serves as Applications Development Manager for the Americas and also supports the MALDI TOF and FT-ICR product lines. He also holds an appointment of Adjunct Research Professor in Biochemistry at Vanderbilt University and continues to be active in MALDI research with more than 25 peer-reviewed publications.

II14

Proteome of Nuclear Architecture

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Nuclear Matrix (NuMat) forms the structural basis of nuclear architecture which supports nuclear processes including transcription, replication, etc. We have carried out NuMat proteome analysis of different organisms at different developmental stages. Our findings reveal highly dynamic and developmentally regulated proteome profile of NuMat. Only about 40% of the proteome is constant, referred to as core NuMat proteome, while rest is dependent on the cell type. We also carried out proteome analysis of the mitotic chromosome scaffold (MiCS) and find that much of the MiCS is of NuMat origin. In summary our results suggest that while NuMat is highly dynamic, during mitosis a subset of its components stay with the MiCS, presumably contributing to the cellular memory retention after mitosis. Developmental dynamics and mitotic transition in the context of nuclear architecture will be discussed.

Biography

Dr. Rakesh K Mishra

Senior Principal Scientist and Group Leader
Professor, Academy of Scientific and Innovative Research
Head, Information Technology Group and Coordinator of Bioinformatics Centre
Center for Cellular and Molecular Biology (CCMB), Hyderabad



Academic background: Obtained M.Sc. (Chemistry) in 1981 and Ph.D. (Organic Chemistry) in 1986 from the University of Allahabad

Research experience:

1986-1988: Molecular Biophysics Unit, Indian Institute of Science, Bangalore Studied DNA conformations and DNA topology

1988-1992: Center for Cellular and Molecular Biology (CCMB), Hyderabad Studied transcription initiation in prokaryotes, and Chemical recognition and cleavage of DNA.

1992-1995: University of Bordeaux, France
Antisense oligonucleotide mediated knock down against

protozoan parasites

1995-1996: Saint Louise University, School of Medicine, USA
Role of small nucleolar RNAs in Xenopus oocytes in rRNA maturation

1996 to 2001: University of Geneva, Switzerland
Chromatin organization and regulation of Hox genes in *Drosophila melanogaster*

2001- to date: Center for Cellular and Molecular Biology (CCMB), Hyderabad
Working on:

- 1 Comparative and functional genomics of no-coding DNA
- 2 The evolutionary logic of anterior
- 3 posterior body axis formation in animals
- 4 Epigenetic regulation and development

Elected fellow of:

1. Indian National Science Academy
2. Indian Academy of Sciences
3. National Academy of Sciences, India
4. Andhra Pradesh Academy of Sciences

Publications: >100 articles in international journals

L5

Phosphoproteomic profiling for the identification of novel marker for coronary artery disease

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Abstract

Phosphoproteomics involves the identification and quantification of phosphoproteins among the gel images to allow both qualitative and quantitative analysis. It is a key reversible modification that regulates protein function, subcellular localization, complex formation, degradation of proteins and therefore cell signaling networks. Approximately 30% of proteins are assumed to phosphorylated at threonine, tyrosine and serine residues. The ability to measure the global phosphorylation state of many proteins at various time points makes this approach much more powerful than traditional biochemical methods. Several recent reviews have summarized the development of various phosphoproteomic methodologies. These methods typically combine different separation strategies with mass spectrometry. The successful application of proteomic technologies to biomedical and clinical research of coronary artery disease (CAD) may enable us to discover the disease-specific biomarkers for diagnosis and treatment monitoring, thus offering insight into the underlying pathologies of diseases and identification of new therapeutic targets.

CAD is a leading cause of death in most regions of the world. Over the past 3 decades it has become clear that the initiation and progression of atherosclerosis, the pathological basis of Cardio Vascular Disease results from a combination of abnormalities in lipoprotein metabolism, oxidative stress,

chronic inflammation, and susceptibility to thrombosis. There is now increasing evidence that at the molecular level, the changes that occur during development of heart failure represent a complex series of inter-related events. Rupture of an atherosclerotic plaque is the predominant cause of acute coronary syndromes and peripheral vascular disease. The underlying molecular mechanisms of plaque rupture have been extensively studied, but are still not fully understood. It has been observed that preventive and therapeutic measures have improved the prognosis of these patients to certain extent. But, still some new early diagnostic markers are needed for solving this problem.

In this study, we have explored the phosphopeptide proteome of human blood by enriching the phosphoprotein. The differential protein expression was studied by two-dimensional (2D) gel electrophoresis followed by highly sensitive mass spectrometry analysis, improved software for phosphopeptide identification and subsequent analysis with an elaborate bioinformatics strategy, pathway analysis and protein network analysis. The identified protein has been validated by Western blot analysis, ELISA and Fluorescence activated cell sorting method in 150 patients sample. The rich data from the proteomic analysis will provide the insight into the pathogenesis of CAD.

II15

Proteomics approach to identify host factors involved in Chikungunya virus infection

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Introduction: The recent re-emergence of Chikungunya virus (CHIKV) infection in India after 32 years and its worldwide epidemics with unprecedented magnitude raised a great public health concern globally. During 2005-2008, around 1.3 million people in 13 states of India were affected by CHIKV infection. The clinical manifestations of the patients during these outbreaks were found to be more severe as compared to the classical cases. Thus, the magnitude of this outbreak emphasizes the need to study this virus in detail to understand the reasons of different clinical manifestation and involvement of host factors. In this study, an attempt was made to identify and characterize differentially expressed proteins involved in Chikungunya virus infection in mammalian cells using high throughput differential proteomics approach.

Methodology: In order to address this, Vero cells were mock infected and infected with CHIKV prototype strain, S-27. Further, cells were harvested and 2D gel electrophoresis was performed with 3-11NL, 18 cm IPG strips. The 2D proteome profiles were compared between mock and the CHIKV infected cells using 2D analysis software.

Result and Discussions: In this study, as many as 762 and 883 protein spots were identified in mock and the CHIKV infected cell proteome respectively. By comparing these profiles, many differentially expressed protein spots were identified during CHIKV infection. Out of which, 13 most abundant down regulated protein spots were chosen and these spots were cored from silver stained gel of mock infected proteome. These protein spots were analyzed by LC-MS/MS, many proteins were identified with MS/MS and out of that, 25 proteins were found most significant. GO annotation suggested that the identified proteins were sub-cellularly distributed across different cellular components with majority in mitochondria and cytosol and these proteins were predicted to be involved in molecular functions like formation of cytoskeleton, post translational modification, cell proliferation, stress response, transcription, protein synthesis, protein folding etc. Validations of a few identified proteins were successfully carried out using western blot analysis.

Conclusion: In conclusion, in this study, differential proteomics approach has helped in the identification of 13 most abundant down regulated protein spots after CHIKV infections which were resulted in identification of most significant 25 proteins and few were successfully validated. Similarly, identification of unregulated proteins after CHIKV infection is also in progress. This information will help to identify important host factor for understanding CHIKV biology and also to design control strategies to combat the disease, however further experimental investigation is required.

Biography

Dr. Amol Ratnakar Suryawanshi
Sr. Scientist

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Area of Research Interest
Clinical Proteomics, Infectious diseases, cancer etc

His area of interest is clinical proteomics with main focus on Biomarker discovery in various clinical diseases important this geographic location; decipher the role of important protein/s in disease pathogenesis and identification and characterization of post-translational modifications with respect to their role in particular disease. Presently his laboratory is working on viral diseases mainly on Chikungunya and Dengue virus infection and Nasopharyngeal carcinoma (NPC) as a disease model using proteomics approach. His laboratory uses both gel based and non-gel based proteomics, qualitative and quantitative proteomics for these disease models. He is very much keen to add more disease in future. He also has many collaboration within and outside of the institute wherein he looks after proteomics part involved in project.

1-2 outstanding awards/honours received or prizes won:

He is active member of Human Proteome Organization (HPO) since 2006. He is a Life member and presently Executive council member of Proteomics society of India (PSI) where his main interest is to carry educational activities towards popularization of proteomics. He is also life member of Laboratory Animals Scientist Association, India (LASA), Society of Biological Chemists, India (SBC) and Indian Society for Mass Spectrometry (ISMAS).

II16

Proteomic analysis of human plasma in Rheumatic heart disease

Arun Bandyopadhyay, Somaditya Mukherjee, Tania Banerjee, Sudip Ghosh, Monodeep Biswas, Santanu Duttta, Sanjib Pattari, Prakash Chandra Mandal

Introduction: Rheumatic fever in childhood is the most common cause of Mitral Stenosis in developing countries. The disease is characterized by damaged and deformed mitral valves predisposing them to scarring and narrowing (stenosis) that results in left atrial hypertrophy followed by heart failure. It is mainly diagnosed by imaging techniques. Despite the high prevalence and increased morbidity, no biochemical indicators are available for prediction, diagnosis and management of the disease. Adopting a proteomic approach to study Rheumatic Mitral Stenosis may therefore throw some light in this direction.

Methodology: Six plasma samples, three each from the control and patient groups were pooled and subjected to low abundance protein enrichment. Pooled plasma samples (crude and equalized) were then subjected to in-solution trypsin digestion separately. Digests were analyzed using nano LC-MSE. Label-free protein quantification was performed in crude plasma only.

Results and Discussions: A total of 130 proteins spanning 9-192 kDa were identified. Of these 83 proteins were common to both groups and 34 were differentially regulated. Functional annotation of overlapping and differential proteins revealed that more than 50% proteins are involved in inflammation and immune response. Verification of selected protein candidates by immunotechniques in crude plasma corroborated our findings from label-free protein quantification. Circulating levels of markers of collagen turnover were also monitored by immunoassay. Plasma level of C terminal propeptide of type I collagen (PICP) in rheumatic heart disease subjects ($n=75$) was 400% higher than in controls ($P<0.0001$). Levels significantly decreased one month after valve replacement surgery (240%, $P<0.0001$).

Conclusions: This protein profile of blood plasma, or any of the individual proteins, could serve as a focal point for future mechanistic studies on Mitral Stenosis. In addition, PICP may be candidate biomarkers for disease diagnosis and prognosis.

Biography

Arun Bandyopadhyay

Senior Principal Scientist

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Area of research Interest: Cardiovascular

disease especially in relation to intracellular protein dynamics in cardiac hypertrophy. His group is also actively involved with identification of biomarkers for heart diseases. Coronary artery disease and rheumatic heart disease are some of the interesting areas where Dr. Bandyopadhyay has been employing proteomics for identification of biomarkers and understanding pathophysiology. Important recent publications include Journal of Biological Chemistry, International Journal of Cardiology, PLOS One, Clinical Proteomics etc.

II17

Applications and Advances in Chemical-Proteomics for Drug Target Identification

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Shantani Proteome Analytics Pvt. Ltd., Pune

Identification of a few rightful protein targets of bioactive small-molecule from thousands of cellular proteins is a daunting task. Although small-molecule affinity chromatography followed by mass-spectrometry, termed as 'chemical-proteomics', is paving the path, the identity of the rightful target (the needle) buried in the long list of proteins (the haystack) usually is hard to deconvolute. Utilizing advances in chemical biology and polymer sciences we have developed several cutting-edge technologies to address the need of target-deconvolution. We will be presenting two of our proprietary methodologies and their application in Target Deconvolution and later provide an overview of other technologies that are evolving globally to meet the demand.

Biography

Chaitanya Saxena,

Ph.D.

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Area of Research / Interest: Drug Discovery, Proteome Analysis Technology and Tools



With 10+ years of industrial experience in utilizing

biophysical techniques to solve the relevant problems in life sciences, Chaitanya is a seasoned professional. He currently is working for Shantani Proteome Analytics Pvt. Ltd. as its Chief Executive Officer. Company develops and commercializes drug target deconvolution technologies to drug discovery organizations and proteomics and protein chemistry based path-forward solutions to bio-pharma and other life-sciences based companies. Earlier he worked for Eli Lilly & Company at its Indianapolis based drug discovery unit where he successfully developed numerous novel technologies for drug-target deconvolution and drug-target interaction measurements in intact biological systems. Chaitanya completed a Ph.D. in Biophysics from Dept. of Physics, The Ohio State University, Columbus, Ohio, USA.

II18

Chemical ligation methods for preparation of homogeneous protein biomolecules

Pattabiraman, V. R.

Laboratory of organic chemistry, ETH Zurich, Switzerland.

In an era with a growing number of challenging and unmet medical conditions, the search for new therapeutic agent to address several diseases has become an important pursuit across scientific disciplines. In the past decade, scientists have largely turned to protein-based drugs due to their highly efficacy and low toxicity. For the past several years, more than half the drugs approved by FDA are either protein or antibody therapeutics for various diseases. The increasing success rates and the rich pipeline of promising biopharmaceuticals also demand the development of newer methods to access these rather large complicated biomolecules. So far a number of biopharmaceuticals, including those that are currently in the market, are prepared by recombinant methods. Although largely successful, there are several challenges associated with the development of suitable production and purification methods. Also, recombinant products always pose the risk of biological contaminants and product heterogeneity. On the other hand, chemical synthesis of biotherapeutics, similar to the preparation of small molecule therapeutics, is a promising alternative that can circumvent the challenges associated with recombinant methods. In this lecture, contemporary chemical methods for the preparation of synthetic proteins will be presented. More specifically, the development and application of chemoselective ligation methods for the preparation of both modified and unmodified proteins will be highlighted with few specific examples.

Biography

Dr. Vijay Pattabiraman

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Areas of research: Research interest is at the interface of chemistry and biology with special interest in Protein/peptide chemistry, synthetic organic chemistry, protein biochemistry and the associated technologies.

Awards: Dr. Bert Schram Young Investigator Award (2012) and others.

Vijay Pattabiraman was born in Vellore, India and received much of his early education in Vellore and completed his Bachelor's degree from Voorhees college. After his Master's in Anna University, Chennai, he worked for two years at Dr. Reddy's Laboratories, Hyderabad performing research and development of new drug entities. In 2002, he moved to Canada and completed his PhD under the supervision of Prof. John Vederas at University of Alberta. Following which, he started his postdoctoral studies with Prof. Jeffrey Bode at University of Pennsylvania, USA and moved to ETH Zurich and completed his post-doctoral studies on the chemical synthesis of proteins. Since 2012, he has been working as a Senior Scientist in the laboratory of organic chemistry, ETH Zurich associated with the Bode group. His research interest is at the interface of chemistry and biology with a specific interest in new chemical methods for the preparation of synthetic proteins, unusual peptides and the development of associated technology.

II19

Biosimilars Characterization & Quantitation

Dr. Jaran Jainhuknan

Bruker Daltonics

Patent expiration of therapeutic protein based drugs prompt the growing manufacturing the generic form of these drugs. Manufacturing them requires living organisms, and thus, product quality depends on growing conditions of the living things. Characterizing the end products, compared with the original drugs is extremely crucial to ensure the efficacy. For successful characterization of the product, many techniques are called upon starting from intact protein molecular weight determination, confirmation of modifications at intact and mid-down analysis. In addition, glycan moieties must be verified and confirmed. All results must be traceable and well documented.

Method : Electrospray UHR-Q-TOF is used for confirming intact MW. For antibody (around 140kDa) confirmation, Fabricator is used for cleaving the intact antibody fragments between 20 to 25 kDa where accurate MW measure is achieved. Experimental results are discussed.

Glycan analysis for biosimilar is carried out effectively by MALDI-TOF/TOF. Experimental setup and results are discussed.

Deamidation is detected with Liquid chromatography in

conjunction with ESI-UHR-TOF. Examples of real life samples are presented and discussed.

Biography

Dr. Jaran Jainhuknan

LSC SEA Customer Support
Manager Bruker Daltonics

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Research interests: Application of Mass Spectrometry

Achievements: Visiting Lecture and Trainer for Mass Spectrometry

Memberships: American Society for

Mass Spectrometry Jaran Jainhuknan

obtained his Ph.D. in Analytical Chemistry studying structures of peptides in gas phase and fragmentation of peptides under MALDI-PSD conditions. He joined Bruker as an application scientist in 1998. Over the past 10 years, he has enjoyed helping scientists develop LCMS methods for various applications including proteomics and protein identification, metabolite profiling and identification, metabolite quantitation and screening. Seeing well-planned experiments work out satisfies him.



PL9

Chromatographic monoliths in PAT

Podgornik A.

Faculty of Chemistry and Chemical technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana, Slovenia & The Centre of Excellence for Biosensors, Instrumentation and Process Control - COBIK, Tovarniška 26, 5270 Ajdovščina, Slovenia

Introduction: Process analytical technology (PAT) is a novel paradigm introduced by the United States Food and Drug Administration (FDA) regulatory agency as a mechanism to design, analyze, and control pharmaceutical manufacturing processes through the measurement of Critical Process Parameters (CPP) which affect Critical Quality Attributes (CQA). To fulfill this goal appropriate sensors have to be implemented providing high specificity and short response time what enable besides monitoring also regulation of the process.

Methodology: Among various pharmaceutical processes especially production biopharmaceuticals is very challenging due to complex nature of the product itself which has to be thoroughly characterized. To provide required specificity in many cases chromatographic separation is involved. Being rather slow method, there were in recent decade several advances to overcome this limitation through introduction of high throughput analysis by implementing simultaneous analysis on parallel chromatographic columns as well as by speeding-up analysis through introduction of short

chromatographic columns filled with micron sized particles, however on the expanses applying ultra-high pressures. An alternative approach might be utilization of monolithic stationary phase which enable convective based transport combined with high porosity, resulting in short analysis time at moderate pressure drop. Because of that, one can use conventional HPLC to obtain in-minute or even in-seconds results.

Results and discussion: In this work different concepts will be presented and examples of PAT using monolithic columns will be described. Several examples of IEX monolithic chromatography of proteins, DNA or viruses will be presented but also implementation of monoliths bearing affinity moieties or enzymes will be discussed. They were incorporated for monitoring in up-stream but also in down-stream becoming an integral part of the process.

Conclusion: Due to flow unaffected properties chromatographic monoliths can provide in-second information about the process state without ultrahigh pressure, decreasing equipment cost, therefore increasing accessibility but also safety.

Biography:

Aleš Podgornik

EMPLOYMENT

1992 -2013 BIA d.o.o.; Head of R&D

1998 -2011 BIA Separations d.o.o.;
Head of R&D

2001 - 2012 University of Ljubljana;
Biotechnical faculty:
Assistant professor of
biotechnology

2007 - 2010 BIA Separations d.o.o.;
CSO

2010 - COBIK; head deputy LBA

2011 - 2014 BIA Separations d.o.o.; director consultant

2012 - University of Ljubljana; Biotechnical faculty:
adjunct professor of biotechnology

2014 - University of Ljubljana; Faculty of chemistry
and chemical technology: professor of chemical
engineering



Co-founder of BIA separations d.o.o. in 1998 (producer of monolithic chromatographic columns, currently over 70 employees, headquarter in Villach; Research & Development as well as Production facilities in Ajdovščina, Slovenia, sales offices located in Wilmington, Delaware, US and Shanghai, China; for details see:). Co-founder of LBABiod.o.o. in 2012 (worldwide workshops on down-stream processing, see e.g. <http://btc.lba-bio.com/btc/btc-2013>).

Research

Fields of interest: Chromatography, stationary phases – monoliths, separation processes, biochemical engineering, chemical engineering.

20 years of expertise in biochemical engineering, including bioprocess monitoring and control and down stream processing. Main focus is development of monolithic stationary phases for chromatography called monoliths.

Leader or team member of over 40 national and international research projects (including research programme) and many industrial oriented projects which resulted in several commercial products (mainly related to the trademark CIM – details can be found on www.biaseparations.com). CIM® technology, currently consisting of over hundred difference products (<http://www.biaseparations.com/sp/197/products-and-services>), is widely recognized, and was selected among 100 Technology Offers stemming from EU Biotechnology RTD results (ftp://ftp.cordis.europa.eu/pub/lifescihealth/docs/booklet_100_off.pdf), Boehringer Ingelheim Austria was awarded with »2004 Frost&Sullivan Technology Leadership Award«, for the process of pDNA purification, which was co-developed by Boehringer Ingelheim Austria and BIA Separations d.o.o. and where one of the main inventions was usage of large CIM monolithic columns, while BIA Separations was in 2011 awarded by Kappa Healthcare as one of the most successful research-intensive small companies in Europe (<http://www.kappa-health.org/News/259.aspx>).

Honors

- 2001 The Jozef Stefan Golden Emblem Prize Award
- 2005 Zois Award for applicative achievements
- 2008 Finalist (among 3) »Emerging leader in Chromatography«
- 2012 Best lecturer on Biotechnology study for year 2010/11 Biotechnical faculty

PL 10

Analytics and PAT in Virus Particle Manufacturing

Peterka M

Center of Excellence for Biosensors, Instrumentation and Process Control, Ajdovščina, Slovenia

Process analytical technology (PAT) initiative promotes the use state-of-the-art science and engineering principles to achieve effective quality control through increased process understanding. The scope of PAT is to design, analyze, and control manufacturing processes through critical process parameters (CPPs). Vaccines manufacturing processes are complex and inherently have bad reproducibility. PAT for vaccine manufacturing is therefore much more challenging, but at the same time more needed.

Number of PAT tools can be applied either on-line or off-line. For most vaccine manufacturing processes, critical quality attributes (CQA) are generally measured using labour and time intensive quality control tests. This is especially true for viral vaccines in the form of infectious, inactivated virus particles and virus-like particles, where complex network of reactions involved in virus multiplication makes the control of the process very challenging. Developments in the field of sensor and biochips indicate that on-line measurements of more CQAs of complex vaccines could be feasible in the near future.

Presentation will focus on virus and virus-like particles (VLPs)

manufacturing processes. Bottlenecks in analytics and PAT for these processes will be described. Available techniques for virus, VLPs, and critical impurities detection, quantification and characterization will be presented and use of these methods for specific manufacturing processes will be demonstrated and discussed. Feasibility of off-line measurement methods to be upgraded and used for in-line measurements will be discussed.

Biography

Dr. Matjaž Peterka

Dr. Matjaž Peterka received a PhD in Microbiology from University of Ljubljana. Afterwards he joined company BIA Separations and has been part of the team for ten years working as a researcher, project manager, department head, and consultant. Matjaž was involved in development and marketing of various products and services and his responsibilities included the development of chromatographic purification methods and processes for recombinant proteins, plasmid DNA, viruses, and vaccines. Matjaž published over 30 papers, reviews and book chapters in international journals in the field of biotechnology and he is a reviewer for several journals from the field. Matjaž is lecturing at different Slovenian and foreign Universities and was a mentor to BSc, MSc and PhD students. Presently, he is CEO at Centre of excellence for Biosensors, Instrumentation and Process Control (COBIK). As a result of efforts at COBIK Matjaž and coworkers established a spin-off company LBaBio focusing on bioengineering solution of biotechnology industry.



IL 20

Novel Comparability Approach and Innovative Quality Control

Anette Persson

GE Healthcare, Uppsala

Establishing Biosimilarity for complex biological products with several critical quality attributes is a challenging task. Accurate, precise and information-rich assays based on surface plasmon resonance have shown to be of great utility in supporting applications in regulated late-stage development, such as comparability assessment and manufacturing quality control.

The objective with this presentation is to give an overview of current SPR trends. Key topics covered include:

Novel label-free binding approach for assessment of comparability

Industry case-study: Improving process development and productivity for Biosimilars

Biography

Anette Persson

Product Manager-Life Science Research, GE Healthcare, Uppsala

Anette Persson has worked with label-free interactions since the earliest years.

With a background in biochemistry and clinical chemistry at Uppsala university, she came to Pharmacia Biosensor in late 1980's, a few years before the commercial launch of the first Biacore instrument. After 10 years in the system department, working with application development with a focus on the integration of sensor surfaces, flow system and application needs in the first biosensor instruments, Since 1997 Anette has held a position of manager in the Knowledge and Training group with responsibility for developing and providing training courses and application support.



L6

Evaluation of expression levels of a signaling protein, p38 β in pancreatic cancer and design of peptide inhibitors against the same

Sharmistha Dey^{1*}, Abhishek Gupta¹, Vishal Sahu¹, Vertica Agnihotri¹, Anoop Saraya²

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Background: Pancreatic cancer is the fourth leading cause of cancer death. The majority of cases are diagnosed in the advanced stages, making curative therapy impossible and leading to poor prognosis and incidence equalling mortality. Molecular marker and imaging have not proven to be accurate modalities for screening of pancreatic cancer. It has been demonstrated that p38 MAPK selectively functions as a sensor of oxidative stress during the initiation of tumorigenesis as its apoptotic and antiproliferative effects are suppressed by uncoupling of the production of ROS (Reactive Oxygen Species) from p38 MAPK activation. This study aims to develop p38 β as a protein marker for pancreatic cancer and to design peptide inhibitor against the same.

Methodology: This study was performed on 35 pancreatic cancer patients and 10 healthy groups and 10 chronic pancreatitis patient as control groups. Blood samples were collected and serum was separated. Serum levels of p38 β expression were evaluated by Surface plasmon resonance (SPR) technology and ELISA, where samples were allowed to flow over anti-p38 β antibody immobilized sensor chip. The peptide inhibitors were designed to inhibit the activity of p38 β and the kinetic assay was done by SPR and ELISA.

Results: The pancreatic cancer patients (n=35) in the study showed two fold higher level of p38 β serum concentration (4.67 ng/ μ l, 95% CI 4.13-5.21) than controls (n=10) group (2.93 ng/ μ l, 95% CI 2.58-3.28) ($P < 0.0009$). Among them, 15 patients continued the treatment and showed significant ($P < 0.0002$) decline in p38 β after treatment (2.92 ng/ μ l, 95% CI 2.58-3.28). The patients with tumor growth (T1+T2) and stage (I+II) after therapy had reduced concentration of p38 β . This study was followed by the design of specific peptide inhibitors based on the structure of active site of p38 β . The kinetic assay had shown the dissociation constant, (KD) to be 3.16×10^{-8} M and IC₅₀, 25 nM by SPR and ELISA, respectively. The peptide inhibitor also significantly reduced viability and induced cytotoxicity in PANC-1 cells.

Conclusions: The expression levels of p38 β increases according to the stage and can evolve as a potential marker in pancreatic cancer. The KD and the IC₅₀ values of the peptide VWCSS showed it to be a highly potent inhibitor and can surely be further analyzed. The SPR technology can set a platform for better manifestation of p38 β levels in pancreatic cancer.

IL21

High Throughput Screening of therapeutic proteins in early stage development

Vivek Halan

Theramoyt Novo biologics Pvt. Ltd., Bangalore

Market for therapeutic proteins such as monoclonal antibodies (mAbs), growth hormones, other recombinant therapeutic proteins has grown tremendously in the last two decades. To compete in the biopharmaceutical market, we should identify, develop the high product producing clone and find the best conditions to purify to highest purity, good recovery with good quality in the early stage of development. Biosimilars that is similar biological product (similar to originator or innovator product) are increasingly being developed by many companies and used as therapeutics for various diseases worldwide. There is a lot of scope to improve in biosimilar story. Biosimilar products are approved through stringent regulatory pathways in highly regulated markets such as the US, EU, Japan, Canada and Australia following loss of exclusivity of their originator reference product.

The development of biosimilar product possesses various challenges such as comparable quality, safety and efficacy to a reference product in addition to other challenges in product development from laboratory to manufacturing scale. Biosimilar from process development, pre-clinical trials and clinical trials up to fill finish meets number of challenges. Quality attributes of monoclonal antibody or bio therapeutic proteins are highly affected by both process and product related impurities. There should be an efficient upstream as well as downstream process to overcome all the bottlenecks and establishing appropriate standards for biosimilarity remains an important area for scientific, legislative and regulatory debate. The barriers to biosimilar entry into the clinic are much more difficult to overcome than challenges

generic manufacturers generally faces.

In addition to monoclonal antibodies, other antibody types such as bispecific antibodies, neutralizing antibodies are also being developed by various biopharmaceutical companies. Antibody fragments such as Fab, ScFv molecules being produced in bacteria. To bring the product to the clinic at the earliest is very essential. High Throughput screening (HTS) platform is one of the key step to achieve the goal within short period. The main goal of early stage development is to rapidly develop bioprocesses to produce materials for Phase 1 or 2 clinical trials and animal toxicology studies. High throughput screening platform can be used efficiently in both upstream as well as downstream process side. HTS helps in rapid identification of target and gives preliminary idea to reach optimum conditions to develop a product. It has been gaining tremendous popularity among new and established biologics manufacturers.

Biography

Vivek Halan

Vivek Halan has a post graduate degree in Biotechnology from Bharathidasan University in Trichy, Tamilnadu. He was awarded Gold medal for securing highest aggregates in M.Sc Biotechnology. He has more than eleven years of research experience in Downstream Process Development of various products in Oncology, Diabetes, Osteoporosis, Metabolic Disorder, Inflammation, Rheumatoid arthritis and Liver associated diseases. He has worked for various companies such as Magene Life sciences Pvt Ltd, Avesthagen Ltd, and Syngene International Pvt Ltd where he was involved in downstream processing of various biologics, biosimilars, innovators and few other proteins such as Kinases, CD molecules as a target. He received few R &D Star awards for his contribution in developing various products during his tenure at Syngene.

Moreover, he was also involved in recombinant protein expression in bacterial cells, monoclonal antibodies expression in mammalian cells lines in laboratory scale. He has been involved in technology transfer for various biosimilars and innovator and few molecules are in different stages of clinical trials. Currently, he is heading downstream process development department at Theramy Novobiologics Pvt Ltd.

P1

Quantitative proteomic and phosphoproteomic analysis of H37Ra and H37Rv strains of *Mycobacterium tuberculosis*

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Introduction: *Mycobacterium tuberculosis*, the causative agent of tuberculosis accounts for 1.5 million annual deaths worldwide. Despite efforts to eradicate tuberculosis, it still remains a deadly disease attributed mainly to the emergence of several multi-drug resistant strains in the recent years. The two well-characterized strains of the parental H37 strain namely, H37Ra and H37Rv show different pathogenic phenotypes. In order to identify factors that may account for virulence, we compared the proteome and the phosphoproteome profiles of virulent (H37Rv) and virulence attenuated (H37Ra) strains of *M. tuberculosis* at logarithmic and stationary phases.

Methodology: The two strains were grown in Middlebrook 7H9 media with OADC supplement and harvested at logarithmic and stationary phase. We used 8-plex TMT (Tandem Mass Tag) *in vitro* labeling for quantitative proteomic analysis. The total proteome fractions as well as the enriched phosphopeptides were analyzed using high resolution Fourier transform LTQ Orbitrap Fusion Tribrid mass spectrometer. The files were searched against combined data developed from the proteome of H37Ra

and H37Rv strains.

Results and Discussions: Our analysis resulted in the identification and quantitation of 2,793 proteins and 505 phosphorylation sites corresponding to 252 proteins. Of which, 423 were unique phosphosites- pS (125), pT (289) and pY (09). Comparative analysis revealed over 5-fold overexpression of several proteins associated with virulence including EsxD protein (Rv3891c), ESX-1 secretion-associated protein EspC (Rv3615c) and pilin (Rv3312A) in H37Rv strain when compared to H37Ra. In addition, we also identified 156 proteins which exhibited changes in phosphorylation levels between the two strains. Bioinformatics analysis of differentially expressed proteins and phosphoproteins revealed enrichment of pathways involved in bacterial secretion system, fatty acid biosynthesis and two-component regulatory system. Furthermore, motif analysis of identified phosphopeptides revealed enrichment of five dominant motifs shared by six *M. tuberculosis* STPKs.

Conclusions: Our data indicates that there are definable molecular differences between H37Rv and H37Ra strains at both the proteome and phosphoproteome levels which may explain the virulence and phenotypic differences.

P2

Protein Quantitation Using Data Independent Acquisition (DIA) on Orbitrap Fusion Platform

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Introduction: Data Independent Acquisition (DIA) is unbiased mass spectrometry acquisition method which fragments all the precursor ions in the form of wide isolation window across the selected mass range. Because of its comprehensive sampling of all the precursor ions compared to DDA, it records fragment ions of all precursors which enables reproducible identification and offers major advantage for label free quantitation. In the last decade, many DIA based methods have evolved across different instrument platforms but gained some momentum with the advent of the SWATH approach which is limited to TripleTOF. Here, we have employed similar acquisition strategy to assess the quantitative performance of DIA using High Resolution Orbitrap Fusion Mass spectrometer.

Methodology: Protein digest obtained from human cell line was analysed in triplicates with different sample amounts from 250ng–2μg. DIA acquisition was obtained using MS scans analysing 400–1000m/z with a resolution of 60k (at 400m/z). 30 MS/MS scans were acquired with 20m/z isolation window and orbitrap resolution of 30k (at 400m/z).

Data Analysis: We used Skyline &DIA-Umpire programs to analyse the raw data. For skyline, the spectral library was created by analysing the same sample in DDA mode

Result and Discussion: For two fold change, we observed relatively better quantitative accuracy for lower protein amount compared to higher. For higher fold changes, we observed good quantitative accuracy irrespective of protein amount.

Conclusion: We have shown preliminary success in using DIA in identification and quantitation of proteins of a complex human cell lysate on an Orbitrap-Fusion platform. Although DIA offers several advantages over DDA, MS instrumentation and informatics tools to deconvolute the data for identification and quantitation need significant improvement.

P3

Unraveling the proteome of human pineal glands

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Introduction: Pineal gland is a neuroendocrine gland located at the center of the brain. It protects the body from the effect of toxic compounds and regulates sleep/wake cycle, body temperature and sexual maturity through the secretion of melatonin. Abnormal functioning of pineal glands is known to be associated with Smith-Magenis syndrome, autism spectrum disorder, delayed sleep phase syndrome and Alzheimer's disease. Characterization of pineal gland proteome will facilitate molecular level investigations on pathophysiological conditions underlying these diseases. We aimed to characterize the proteome of human pineal glands using a high resolution mass spectrometry-based approach.

Methodology: Pineal gland tissues were obtained from road accident subjects at the time of autopsy. Protein extraction and fractionation at the proteins and peptides was carried out separately and analyzed on mass spectrometer.

Results and discussion: A total of 6,062 proteins were identified from human pineal glands for the first time in

this study. Of these, 1,063 proteins contained signal peptide domain. We identified 3 proteins, which have been reported to be missing in neXtProt database. In addition, all the enzymes that are involved in the biosynthesis of melatonin were also identified in this study.

Conclusions: A comprehensive list of proteins identified for the first time from human pineal glands will aid in unraveling the role of pineal gland in sleep disorders and neuropsychiatric and degenerative diseases.

P4

E-pharmacophore mapping, molecular docking and dynamics simulations to propose potential inhibitors of CDK5

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Introduction: Neuropathologically, Alzheimer's disease is characterized by presence of P-amyloid (AP) plaque deposition and neuro fibrillary tangles (NFT). Cyclin-dependent kinase 5 (CDK5) is indispensable for numerous neuronal processes, including higher cognitive functions such as learning and memory function. Dysregulated CDK5 promotes oxidative stress, mitochondrial dysfunction, excessive phosphorylation of Tau correlating with the synaptic loss and neurofibrillary tangles formation in AD. CDK5 also influences AP production by altering APP processing by phosphorylating Presenilin (PS1). Thus inhibiting CDK5 reduces CDK5 induced neuronal cell death in AD.

Methodology: E-pharmacophore model based virtual screening with multiple docking strategies and free energy calculations were employed to propose leads from in-house library containing more than one million small molecules. E-pharmacophore mapping rationalize the structural requirements necessary for the activity of CDK5, thus blocking the active site may reduce the activity. E-pharmacophore models were subjected to shape based similarity screening against in-house library having more than one million compounds. Ligand data set obtained after screening was subjected to multi step rigid receptor docking followed by MM-GBSA calculation. To define the best leads, dock complexes were subjected to QPLD abided by free energy calculations. The best lead was subjected to IFD with MM-GBSA calculation. MD simulations for 50 ns were performed in solvated model system to define the steady nature of lead1 interactions towards CDK5.

Results and Discussion: Generated 5 e-pharmacophores were subjected to shape based similarity screening against in-house library resulted 3853 compounds. RRD and QPLD studies disclosed 30 ligands and were compared to five co-crystallized inhibitors which resulted three the best leads. Potential energy, RMSD and RMSF results showed stable interactions of lead1 throughout 50 ns MD simulations run.

Conclusion: Three leads showed lowest free binding energy, better binding affinity, similar binding orientation with existing inhibitors and favorable ADME/T properties. Thus these three leads can provide a framework to design novel CDK5 antagonist.

Keywords: CDK5, Alzheimer's disease (AD), e-pharmacophore, molecular docking, MD simulations.

P5

Identification of Methylglyoxal Responsive proteins in L6 Rat Muscle Cells

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Introduction: Methylglyoxal, physiologically present in all biological systems, which is highly reactive AGE precursor is formed by the enzymatic and non-enzymatic elimination of a phosphate from triose-phosphate glycolytic intermediates and by oxidation of amino acetone formed in the catabolism of threonine. In type 2 diabetes, altered glucose metabolism leads to increased formation of methylglyoxal. Skeletal muscle and adipose are the major insulin-target tissues responsible for maintenance of whole body glucose homeostasis. Methylglyoxal is highly cytotoxic, causes oxidative stress, generate reactive oxygen species, so it is possible that because of its influence cells may secrete some proteins outside the cell in media. Secreted proteins are important sources for early detection and diagnosis of disease. The proteins secreted by a particular type of cell, secretomes, play important roles in the regulation of many physiological processes and they are of increasing interest as potential biomarkers and therapeutic targets in diseases. Herein, we sought to identify and characterize the Methylglyoxal induced secretome in Rat Muscle cells.

Methodology: This study is carried out in insulin sensitive cells such as muscle cells (L6). Cells are treated with Methylglyoxal and Media is collected. In-Solution Trypsin Digestion is performed for the secreted proteins. LC MS analysis is done to study differential protein expression of methylglyoxal induced secretome.

Results and Discussions: In this study, we have used methylglyoxal, highly reactive dicarbonyl AGE precursor and evaluated its effect on secretome of Rat muscle cells.. Methylglyoxal induces differential protein expression. Upregulated proteins may act as potential Biomarker for diabetes.

Conclusions: This study reveals effect of Methylglyoxal on muscle cells secretome and will give better insights for Biomarkers.

P6

Development of immobilized metal-ion (IMA) monolith multiplexed microfluidic device for plasma proteomics using LC-MS. Ashish Khaparde*, Vijayalakshmi M. A. Kishore.

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Introduction: Current generation of proteomic studies require involvement of miniaturized devices that have the potential of automation, integration, efficient separation and most significantly a parallelized on-line detection system for valuable complex clinical samples. Microfluidic chips incorporated with porous stationary phase materials possessing enhanced surface properties are considered to be an efficient detection tool. These devices can be easily integrated and can generate multi-functional advantages over the conventional methods.

Methodology: An organic monolith is prepared by homogenous polymerization of monomer unit (2-Hydroxyethylmethacrylate), cross-linker units (N,N'-Methylenebisacrylamide & N,N'-Diallyltartaramide) and an initiator (AMPA) in the presence of a suitable porogen. Material modifications to achieve separation of proteins are made to enhance the surface property by multi-step and 2-step approach involving various chemical treatments. The resulting functional groups are utilized to immobilize metal-ion [Cu (II)] on its surface to facilitate affinity based separation. Monoliths (0.12 g) are suspended in individual model protein solution (HSA, IgG & Transferrin) to check non-specific and specific adsorption.

Results and Discussion: Successful development of a methacrylate based monolith with two different modification strategies is achieved. Scanning electron microscope (SEM) images showed the material with highly interconnected porous network. IR-data, confirms the successful immobilization of IDA onto the material. Monolith (without metal ion) is investigated for its non-specific adsorption and Cu (II)-IDA-monolith is tested for specific adsorption of model proteins via static protein adsorption studies. In presence of metal ion (Cu (II)), Protein binding is as follows: IgG > Transferrin > HSA. Formulation of monolith inside the glass capillary was successfully achieved.

Conclusions: A significant amount of model proteins were adsorbed in accordance with their number of surface exposed Histidine residues present on the protein surface. The obtained results show the potential of monolith in microfluidic approaches for pre-fractionation of proteins.

P7

Pre-fractionation and mass spectrometric characterization of HDL-APO A1 from human plasma.

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Introduction: Apolipoprotein A1 (Apo A1) is the major constituent of High Density Lipoprotein (HDL). It plays a vital role in cholesterol metabolism and homeostasis, and also

exhibits anti-inflammatory, anti-oxidative and anti-apoptotic functions. Due to this multifunctional property it is considered as cardio-protective molecule and plays very important role in controlling atherosclerosis, a major pathological condition in cardiovascular diseases (CVD).

Methodology: In this study a two-step purification method was developed and optimized for recovery of ApoA1 from human plasma. In the first step, plasma was subjected to 60% ammonium sulphate precipitation. The supernatant obtained was injected into mixed-mode chromatographic resin HEA (Hexylamine) HyperCel (PALL Life Science). The bound proteins were eluted in decreasing pH gradient (pH 6-3.5). The elution fractions were analyzed by SDS PAGE and western blot for presence of Apo A1. Mass spectrometric analysis was also carried out for determining purity and intact molecular mass.

Result and Conclusion: SDS-PAGE analysis showed enrichment of ApoA1 in 60% ammonium sulphate supernatant. Chromatographic elution at pH-3.5 revealed presence of Apo A1 with good purity in SDS-PAGE and was confirmed by western blot analysis. Mass spectrometric analysis of this fraction gave an intact mass at 28kDa and sequence coverage of 50% confirming the purified protein as Apolipoprotein A1. This method overcomes the need to isolate HDL and facilitates direct purification of Apo A1 from blood plasma and can be used as an alternative method for processing large number of clinical samples.

P8

Green Fluorescent Protein: Eco-friendly dye for leather

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Introduction: The application of dyeing in various industries such as textile and leather plays an important role to impart coloring of materials. However, the dyeing industry is known to be the major contributors of environmental pollution since 10-35% of the dye is lost in the effluent during the dyeing process. Hence there is a growing demand for eco-friendly dyes which is an important alternative to these synthetic dyes. The present study focused on use of Green Fluorescent Protein as an alternative dyeing agent.

Methodology: The Green fluorescent protein (GFPs) is cloned into the plasmid pQE 80L by using specific primers and restriction enzymes and the construct was transformed in to *E.coli* tyrosine auxotroph. The dyeing potential of the purified GFPs has been evaluated for different kinds of leather. The dyeing conditions are optimized process parameters such as percentage of protein absorption on leather and color analysis of leather has been studied.

Results and Discussion: The conditions of dyeing is optimized as 5μM protein concentration, neutral pH, and the time duration 10h. The results showed that maximum uptake of protein is absorbed on post tanned leather with 1.5mm thickness and the percentage was found to be 98.83%. Marked improvement in the color intensity is also observed

in the leather samples. Conclusion: Hence the present study will provide an eco-friendly dye from natural sources for reducing pollution load in dyeing process.

P9

Proteogenomic analysis of H37Ra strain of *Mycobacterium tuberculosis* using high resolution mass spectrometry

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Introduction: *Mycobacterium tuberculosis* (MTB) H37Ra is an attenuated tubercle bacillus closely related to the virulent type strain MTB H37Rv. The genome sequencing of H37Ra strain of *Mycobacterium tuberculosis* was completed in 2008. Although the genome of H37Ra is 99% similar to H37Rv with only little difference in genome size, differences at the level of proteome among the two strains are still lacking. In addition, annotation of its genome remains challenging because of high GC content and dissimilarity to other model prokaryotes.

Methodology: Cell lysates and culture filtrates from H37Ra cultures were digested using trypsin, fractionated and analyzed on high resolution Fourier transform LTQ Orbitrap Fusion Tribrid mass spectrometer. The mass spectrometry-derived data were analyzed using a six-frame translation of genome sequences in addition to searches of a protein database of *M. tuberculosis* H37Ra. We employed gene prediction algorithms and comparative proteomic approach to validate alternative gene models.

Results and Discussions: In all, we identified 2793 proteins from *Mycobacterium tuberculosis* H37Ra representing ~69% of its total predicted gene count. In addition to protein database search, we carried out searches against six frame translated genome database which led to identification of ~400 genome search specific peptides (GSSPs). Based on these novel genome search-specific peptides, we discovered novel protein coding genes, corrected gene models, confirmed existing annotations for translational start sites in the H37Ra genome.

Conclusions: We report creation of a high confidence set of protein coding regions in *Mycobacterium tuberculosis* H37Ra genome obtained by high resolution tandem mass-spectrometry for the first time. This data can further be utilized to identify signatures for virulence in *Mycobacterium tuberculosis*.

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P10

In-silico Analysis of Ubiquitin - Protein Interactions in Human

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Introduction: Ubiquitination is a post-translational modification where ubiquitin- a small regulatoryprotein binds to a target protein destined for degradation. Ubiquitination can affect the target protein in many ways: 1) It can signal for their degradation *via* the proteasome, 2) alter their cellular location, 3) affect their activity, and 4) promote or prevent protein-protein interactions. All intracellular and extracellular proteins are continually being hydrolyzed to their constituent amino acids and replaced by new synthesis which is known as “protein turnover”. Ubiquitination regulates the protein turnover in a cell by regulating the degradation of specific proteins. We know that protein synthesis and abnormal cell division is a key mechanism in cancer cells. Thus, ubiquitination plays an important role in cell cycle regulation, and we are interested in understanding the factors promoting ubiquitin binding to its targets.

Methodology: Using online informatics tools, a set of proteins linked to cancer and undergo ubiquitination was chosen for our analysis by multiple in-silico methods. These methods include Docking, Sequence analysis, Machine learning technique and Clustal W. The results were obtained using each of the above approaches and were compared with previous studies. Immunoprecipitation- based proteomics analysis was performed to confirm ubiquitin-protein interactions.

Results and Discussion: We found some ubiquitin binding signatures as common to the previously predicted ubiquitination sites from yeast as well as human proteins and at the same time we were also able to identify additional sequence signatures that are unique to human proteins. Our approach followed here gave higher ubiquitin binding site predictability when compared to the existing methods. Further work is under way to confirm our findings and to identify a more consensus ubiquitin binding motif in human ubiquitin targets.

Conclusion: The above in-silico approaches are helpful in identifying the consensus sitesfor ubiquitination, especially with reference to human proteome.

P11

Pepdize: web-based comparative in-silico protein digestion tool

Introduction: Protein identification and analysis software performs a central role in investigation of proteins from mass spectrometry. Trypsin is widely used as endopeptidase. However, if a proteome is inaccessible using trypsin, an empirical decision for different endopeptidase such as chymotrypsin, Lys-C, V8 can be inferred using comparative in-silico protein digestion framework. And hence user can acquire information against the target protein database to define the experiment apriori.

Methodology: We have developed a web-based tool using PERL and JavaScript to address aforementioned limitations. User can upload a file containing one or more protein sequences in FASTA format and in-silico digest them with multiple proteolytic enzymes in a single run. Result will consist of graphical representation detailing comparison of peptide number derived using multiple proteolytic enzymes along with list of proteins that can be accessible with one or more unique peptides and table detailing the proteins that cannot be identified using selected enzyme and peptide length range, list of peptides that are shared with two or more proteins and amino acid fraction distribution. User can also acquire and analyze the in-silico digested peptide comprising of target amino acids of various modifications.

Result and Discussion: Although there are numerous programs available for in-silico digestion of proteins and acquiring the list of peptides, existing tools cannot handle entire proteome, cannot provide comparative and combinatorial account on unique peptides for two or more proteolytic enzymes, and also does not attribute to shared peptides.

Conclusion: The user-friendly framework of the tool would allow user to deduce an apriori based on protein families or entire proteome and assist the experiment design.

P12

Human Brain Proteome: molecular insights into regional heterogeneity and neurological disorders

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Introduction: Human brain is considered to be the most complex organ due to its different cell types and its heterogeneous structural and molecular organization. A proteomic map of different sub-regions present in the brain is required for novel insights into the molecular mechanisms regulating neuronal maturation and to understand the pathophysiology of multiple forms of neurological disorders. In this study, we have carried out in-depth proteomic analysis of various anatomically distinct sites from human brain.

Methodology: Various sub-regions from human brain autopsy samples were dissected and processed for LC-MS/MS analysis. SDS-PAGE and basic Reverse phase liquid chromatography technique were employed for protein and peptide level fractionation of the sample. Each fraction was then analyzed on Orbitrap Fusion mass spectrometer followed by searching the data against human protein database. Unassigned MS/MS spectra were then searched against translated non-coding RNA and Pseudogenes.

Results and Discussion: From ten different sub-regions analyzed so far, we confirmed expression of 11,280 proteins in the human brain. Of these, 1,507 proteins were found to be co-expressed in all the tissues. Subsets of the proteins identified were found to be tissue restricted and may be responsible for specific functions of the corresponding tissue. For example – 170 proteins were uniquely identified in the hippocampus region and seven of them are known to be involved in deafness or hearing loss. Similar analysis on the proteins found to be enriched in other regions highlighted their biological functions. In addition, we searched our data against translated non-coding RNAs and Pseudogenes. Interestingly, we found translational evidence for 383 genes, which were annotated as Pseudogenes and their expression varied among different brain regions. These translated products may play an important role in the development of the brain and neurobiological disorders. Similarly, we confirmed protein level evidence for 35 genes, which were termed as ‘missing proteins’ in the neXtProt database.

Conclusions: We present a proteomic map of human brain and confirm translation of protein-coding genes, translated products from several designated non-coding RNAs and Pseudogenes among various brain regions. Our study will provide a platform to understand the molecular mechanism involved in brain development and understand pathophysiology of various neurological disorders.

PL 13

Proteomic analysis of milk from early, mid and late lactation stage of Malnad Gidda breeds

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Introduction: Bovine milk is one of the most consumed mammalian milks worldwide due to its lactase persistency and prevailed cattle domestication among human population. Most common proteins observed in milk are casein and whey protein which constitute around 80% and 16% of total milk protein content respectively. The protein quality and quantity varies in different lactation periods like periods of colostrums secretion, transitional milk secretion, early-lactation, mid-lactation and late-lactation. In this study we have proposed to study altered proteome in three stages of lactation from Malnad Gidda, an indigenous, dwarf breed of cattle in India.

Methodology: Whole milk from early, mid and late lactation stages was collected and centrifuged. Removed the lipid layer, the resulted skim milk was again centrifuged at high speed so as to deplete casein. Protein estimation and normalization using SDS-PAGE was carried out. Equal amounts of protein from each case were subjected to in-solution trypsin digestion and labeled with 10-plex TMT labeling kit. The labeled peptides were pooled, fractionated and analyzed in high resolution Orbitrap Fusion Tribid mass spectrometer.

Results and Discussions: A total of 2,100 proteins were identified from early, mid and late lactation stages, subset of which was found to be differentially expressed in the given three periods of lactation. Gene Ontology analysis from these three stages showed enrichment of proteins involved in immune response, transport, and metabolism. We also observed secretory proteins and growth factors in all the three stages of lactation which suggests developmental role of milk. We observed immune response proteins in early lactation milk which suggests passive immunity promoting factors

Conclusions: Our study has shown relative proteomic changes in different lactation stages which give an insight into varying composition of milk proteins in different lactation stages of bovine.

PL 14

Rapid processing of biological samples for proteomic analysis using Pressure - cycling

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Introduction: Advent of mass spectrometry based proteomics has revolutionized our ability to study proteins

from biological specimen. One of the most crucial aspects of bottom-up proteomics experiments is sample preparation that involves protein isolation and proteolytic cleavage. Here we demonstrate the utility of pressure-cycling technology for rapid processing of biological samples prior to mass spectrometry analysis. We also demonstrate that this method allows efficient protein extraction and proteolytic digestion from formalin fixed paraffin embedded (FFPE) tissue where conventional methods have limited success.

Methodology: 10-20 mg of fresh frozen tissue samples and three 10um FFPE sections from two esophageal squamous cell carcinoma patients were used for extracting proteins and carrying out proteolytic digestion using pressure cyclingtechnology (Barocycler). Peptides were subjected to SCX and SD **Conclusion:** Pressure cycling technology significantly reduces sample preparation time and enables achieving proteome depth that is on par with conventional approaches. It also enables proteomic analysis of FFPE samples providing the opportunity to leverage valuable clinical material archived in several hospitals.

P15

Differential expression and oxidative modification of proteins in the cerebral cortex of physically trained old rats.

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Cerebral cortex is an important brain area associated with the processing of learning and memory. Exercise is a natural form of neurophysiologic stimulation that has known benefits for mental health, maintenance of cerebral function and stress reduction. However molecular mechanisms underlying the effect of exercise are limited. Therefore our study was aimed to evaluate the effect of swimming exercise on the expression pattern and oxidative modification (carbonylation) of proteins in the rat cerebral cortex using a proteomic approach. Male *Wistar* rats of 22 months old were swim trained in a rectangular glass tank (77cm x 38cm x 39cm) filled with water to a height of 26 cm with a load of 3% of their body weight tied to their tails. During the training programme, initially the rats were made to exercise for 5min/day with a gradual increase to 30min/day for a total training period of 4 weeks with 6 days/week. Sedentaries were restricted to cage activity. Swimming training significantly up-regulated ($P<0.05$) proteins associated with various functions like metabolism, cell redox homeostasis, synaptic plasticity, ion transport and chaperone. Protein carbonyl content, a marker of protein oxidation showed reduced levels in response to exercise and 2D-Oxyblot analysis also revealed the reduced carbonylation of the proteins with exercise such as actin cytoplasmic 1(synaptic plasticity) and Guanine nucleotide- binding protein G(I)/G(S)/G(T) subunit beta-1 (signal transduction). The observed changes could imply that swimming exercise may provide an enrichment to activate beneficial compensative mechanisms in the cerebral cortex to cope up with the age-related oxidative stress.

P16

A proteome map of the human eye

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Introduction: Visual impairment and blindness are some of the common ocular problems seen in populations across the world. Some of the causes for visual impairment and blindness include eye diseases such as macular degeneration, diabetic retinopathy, cataract, and glaucoma. The human eye is currently an emerging proteome that may provide key insight into the biological pathways of these diseases.

Methodology: Employing high-resolution mass spectrometry, an in-depth proteomic analysis of the different tissues of the eye including iris, choroid, retina, optic nerve and its associated ocular fluids namely- aqueous and vitreous humor was carried out. The data obtained was searched against human protein database and the resultant unmatched spectra was searched against a series of custom databases including six-frame translated genome, three-frame translated transcript, pseudogene and noncoding RNA databases. Furthermore, the proteome was compared with the list of “missing proteins” obtained from NextProt as well as the human proteome map developed previously by our group. In addition, the identified proteome was also compared with the proteins listed in Plasma proteome database.

Results and Discussion: Deep proteome profiling enabled identification of >12,000 proteins from all the tissues sampled. Proteome expression maprevealed a tissue-restricted pattern of expressionwith retina and iris displaying highest tissue-restricted expression of proteins. Employing proteogenomics analysis pipeline, we identified several novel protein-coding regions in the human genome that have not been reported previously. Furthermore, comparison of our data with the previously published human proteome map as well as the “list of missing proteins” from NextProteable identification of several proteins that currently lack proteomic evidence. The comprehensive dataset generated in this study will serve as the basis for pursuing biomarkers for diseases pertaining to these organs.

Conclusions: This study will provide clues on the variations in protein expression across various ocular tissues and will serve as a baseline to compare against proteome of ocular disorders. From the derived data, a high-confidence human eye proteome reference set will be developed for the identification and quantitation of proteins using MRM mass

spectrometry-based strategies.

P17

Cuticle proteome analysis of *Brassica juncea* leaves show presence of cuticle synthesis and stress signaling proteins.

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Introduction: Cuticle forms the outermost layer of the aerial parts of the plants. Major function of cuticle is to protect from desiccation, UV radiation, pathogens and herbivores. It is the perception site for biotic and abiotic stress signaling. However, cuticle proteome studies are scarce. The leaf cuticle proteome of an economically important oil yielding crop, *Brassica juncea* was analyzed as its yield is decreased by multiple stress.

Methodology: Cuticle proteins were extracted using chloroform-methanol (2:1; v/v) followed by solvent evaporation at 55°C. The residue was re-suspended in chloroform:water (2:1; v/v). The aqueous phase was precipitated and re-suspended in 50mM Tris-Cl pH 7.4 (Pyee et al., 1994). Purity was checked by cytosolic marker enzyme (Glucose-6-phosphate dehydrogenase) and RuBisCO. The proteins were resolved on SDS-PAGE and polypeptides were identified by MALDI-ToF/ToF. Antifreeze activity was detected using nanoliter osmometer.

Results and Discussion: Cuticle protein showed negligible cytoplasmic contamination (1.54%, G6PDH activity) and absence of RuBisCO. In all, 16 polypeptides were observed on a Colloidal CBB stained gel. Proteins such as myrosinase, involved in defense against herbivores; GDSL esterase/Lipase ESM1, required for modification and recycling of cutin; Xyloglucan endotransglucosylase/hydrolase protein 9, involved in cell wall metabolism; Succinate-semialdehyde dehydrogenase, MADS-box transcription Factor 32 and PsbP domain-containing protein 5 were identified. A polypeptide of ~10kDa a probable lipid transfer protein (LTP) was purified using PVPP. Cuticle proteins also showed antifreeze activity.

Conclusion: Protein candidates for cuticle synthesis and stress signaling were identified. A ~10kDa putative LTP is purified and its characterization is underway. Using high throughput techniques more candidates of signaling and stress related proteins would be identified.

P18

Shotgun Proteomics of Saliva in Indigenous Sahiwal Cow Reveals More Than 1400 Proteins

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Introduction: Saliva has emerged as an ideal diagnostic fluid to understand the physio-pathological conditions. It can be collected in non-invasive manner, which harbors numerous molecules in the form of proteins, carbohydrates, mucin and hormones etc. Recently saliva has emerged as an important source for diagnosis of diseases including various physiological conditions that shows strong correlation between local and systemic changes. In animal science specifically in bovine, saliva can be used for diagnosis of pregnancy, estrus, disease diagnosis and other physiological conditions etc. In the present study we have profiled the proteome of cow saliva and analyzed their functional significance.

Methodology: To profile the proteins, saliva was collected from 3 healthy Sahiwal cows (*Bos indicus*) and protein was extracted following standard protocol. Equal amount of protein from each animal was pooled and resolved on 12% SDS-PAGE. The SDS-PAGE lane was divided into 7 major pieces according to the intensity of bands. Four gel pieces showing intense bands were digested with trypsin and subjected to nano-LC attached to ESI-Qtof for identification. The tryptic peptides generated from 3 other gel pieces were pooled and subjected to basic reverse phase liquid chromatography (bRPLC). The bRPLC fractions were pooled into 12 fractions which were further subjected to nano-LC attached to ESI-Qtof for identification. Peptides were fractionated in Nano LC, eluted at 400nl/min over 135 gradient. The compound list of all the nLC MS-MS/MS fractions was converted into MASCOT Generic Files (MGF) in ProteinScape software and identified using IPI Mascot enabled IPI bovin database. Gene ontology of differential expressed proteins was done using PANTHER 4.0 software.

Result and Discussion: A total of 1440 proteins were identified, out of which 338 proteins were unique proteins which we have been reported for the first time. IgG, MUC19, albumin, Ovostatin2, Lactotransferrin and lactoperoxidase are the top representatives of the list. The PANTHER based GO classification reveals that 39% proteins are involved in catalytic activity, 29% in binding, 12% in structural, and 52% in metabolic processes which is true to the biological functions and role of the saliva in cows. Out of these 1440 proteins, 71% proteins were secretory in nature. Thus, these results provide a wide data set of bovine salivary proteome that may serve as important biomarkers in different physiological contexts.

P19

A Proteomic Based Approach to Differentiate Sheep Meat with Buffalo Meat

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Introduction: Honest and accurate labelling of food is an essential component of food safety and choice. In the case of meat products, the most common cases of adulteration deal with substitution of high quality meat with lower value or cheaper meat. Present study was conducted to develop a proteomic based method for detection of buffalo meat in mixes with sheep meat at different proportions. This study is based on the objective of qualitative analysis and to find the limit of detection with the identification of species specific peptide biomarker for sheep and buffalo.

Methodology: Authentic fresh buffalo and sheep skeletal muscle samples were minced and mixed at different proportion (100:0, 95:5, 99:1, 99.5:0.5 and 99.9:0.1) and cooked in a sealed bag at 100 °C for 30 minutes. Myofibrillar proteins were extracted and separated using Agilent 3100 OFFGEL fractionator for qualitative separation of proteins from mixture according to their respective isoelectric pH followed by SDS-PAGE. After this, species-specific peptide biomarkers were identified using mass spectrometry (MS).

Results and Discussions: The OFFGEL electrophoresis of myofibrillar protein extract from meat mixes, upon theoretical pH and molecular mass (MW) calculation, 6th OFFGEL fraction exhibited thick and clear band after SDS-PAGE which was subjected to mass spectrometry (MS) analysis. Selected protein bands were in-gel digested with sequencing grade trypsin and were subjected to MALDI-TOF/TOF mass spectrometry followed by protein identification using PMF. The MS analysis confirmed the species specific peptides between the amino acids 132-138 of myosin regulatory light chain-2, skeletal muscle isoform for sheep with a sequence of "FSQEEIR" with a mass/charge (m/z) 908.4472 and for buffalo the sequence is "FSKEEIK" with m/z of 880.4774. The identification level was found up to 99:1 with high confidence level.

Conclusions: The proteomic based method developed in the present study is robust and simple, comprising the extraction of myofibrillar proteins, enrichment of target proteins using OFFGEL electrophoresis, in-gel trypsin digestion of myosin light chain 2, and analysis of the generated peptides by mass spectrometry.

P20

Proteomic profiling to identify chewing tobacco-induced signalling in esophageal cells

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Introduction: Tobacco consumption is a major risk factor for

cancers of the upper aero digestive tract, including esophagus. In India chewing tobacco is a common practice and is one of the leading causes of esophageal cancer. Epidemiological studies indicate a clear contribution of tobacco chewing and carcinogenesis, explaining, at least in part, the higher incidence of esophageal cancer in India. However, molecular mechanisms that are critical for induction of carcinogenesis by chewing tobacco are not clear.

Methodology: We developed an *in vitro* cell line model using normal esophageal cells, Het-1A to mimic chronic exposure to chewing tobacco. We employed TMT-based quantitative proteomics to identify differentially regulated proteins in esophageal cells chronically exposed to chewing tobacco compared to untreated Het-1A cells.

Results and Discussion: TMT-based quantitative proteomic analysis led to the identification of 201 differentially expressed proteins. Out of 201 proteins, 122 were overexpressed and 79 were downregulated in Het-1A cells chronically exposed to chewing tobacco compared to parental cells. A wide array of proteins known to play an essential role in increased cellular proliferation and migration were found to be overexpressed in the tobacco treated cells. Such proteins include enolase 1(ENO) (2.5-fold), vimentin (1.7-fold) and wingless-type MMTV integration site family member 5B (WNT5B) (2-fold). We observed a decrease in expression of proteins involved in cell adhesion like plakophilin 2 (PKP2) (0.6 fold) and EpCAM (0.5 fold). We also observed a significant reduction in the expression of phosphatases like protein phosphatases (PTPRC) (0.6 fold) which might play an important role in oncogenic transformation. These observations provide an evidence towards altered molecular pathways in tobacco treated cells.

Conclusions: Our study provides comprehensive insights into the proteome level changes and shows widespread molecular perturbations in esophageal cells in response to tobacco.

P21

Understanding the mechanism behind alleviation of cysteine induced toxicity using *Saccharomyces cerevisiae* as a model system

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Introduction: Cysteine, a thiol containing amino acid synthesized via the trans-sulfuration pathway, is involved in a variety of biological functions. It is required for the synthesis of glutathione and maintaining the redox of the cell and is also necessary for the synthesis, structure and function of the proteins. However, besides being biologically important, excess cysteine is known to be toxic in many organisms. Elevated levels of cysteine have been associated with neurological and cardiovascular diseases. So it becomes important to understand how the toxicity induced by cysteine can be alleviated. This study is an attempt to understand how

Saccharomyces cerevisiae responds to high levels of cysteine for survival.

Methodology: iTRAQ-4plex and SWATH-MS based quantitative proteomics approach was used to study the proteome change induced due to cysteine treatment. Further, to understand the genes that could be involved in the alleviation of cysteine toxicity, yeast deletion library was used. Cells were grown with and without cysteine for 12 hrs. and relative cell density was measured using multimode reader.

Results and Discussions: Cysteine induces growth defect in yeast strain BY4741 (MAT_a his3A0 leu2A0 met15A0 ura3A0) in a dose dependent manner. Using both SWATH and iTRAQ based approach we quantified 1368 proteins, among which 146 proteins were up-regulated and 256 were down-regulated. Cysteine treatment leads to upregulation of proteins involved in amino acids synthesis and down-regulation of glycolysis and TCA cycle. Further we found that supplementation of high levels of leucine and pyruvate can rescue cysteine induced toxicity. From the genetic screen of around 4800 non-essential genes we found several genes which are required for survival in high levels of cysteine. Among which pyruvate was not able to rescue in Aleu3 strain (Leu3 is a transcription factor regulates genes involved in branched chain amino acid biosynthesis and ammonia assimilation) and leucine was not able to rescue in Ncl1 deleted strain (Ncl1 is a tRNA m5C-methyltransferase, methylates tRNA_{Leu} (CAA) at the anticodon wobble position).

Conclusions: Leucine and pyruvate synergistically rescue cysteine induced toxicity. Cells try to upregulate the biosynthesis of other amino acids for survival during high levels of cysteine.

P22

PROTEOMICS OF ENDOTHELIAL DYSFUNCTION

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Introduction: Endothelial dysfunction, i.e., imbalance in the regulatory functions of the vascular endothelium, acts as an initial step in the development of cardiovascular diseases. An understanding of the mechanism of its development can be helpful for finding strategies to prevent and manage these diseases. Among the known mediators of endothelial dysfunction are plasma proteins chemically modified *in vivo* by reacting with metabolites and drugs present in blood. One such metabolite capable of modifying plasma proteins is homocysteine which has also been reported as risk factor in cardiovascular diseases. Using homocysteine modified plasma proteins we have tried to dissect the cellular mechanism involved in development of endothelial dysfunction.

Methodology: Serum albumin was chemically modified *in vitro* with homocysteine and added to human umbilical vein endothelial cells (HUVECs) in culture to evaluate the effect

on endothelial viability and function. Differential total cell proteomics of treated cells was performed using SWATH MS.

Results & Discussion: Total cell proteomic analysis gave an overview of proteins and pathways involved in endothelial dysfunction. Differential expression of numerous proteins regulating endothelial function was observed including those affecting barrier function, inflammation and angiogenesis.

Conclusion: This study shows how chemically modified proteins elicit endothelial dysfunction affecting major regulatory functions of endothelial cells and predisposing to increased risk of cardiovascular diseases.

P23

Effect of vitamin B₁₂ restriction on Wistar rats: A Global proteomic profiling

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Introduction: Deficiency of vitamin B₁₂ (which regulates one carbon metabolism including DNAmethylation) is common in Indian population especially in pregnant mothers. In-utero deficiency of vitamin B₁₂ may be an important cause for the fetal programming for complex diseases including cardiovascular disease in the offspring. We are interested in understanding whether these changes are transmitted multi-generationally to set the permanent change in physiology. To assess the effect of maternal deficiency we developed the B₁₂ deficient Wistar rat model. In this study we have elucidated the global proteome change that occurs across different tissues due to vitamin restriction (VR) in 3 months old F1 offspring's of male and female rats.

Methodology: Liver (n = 3 VR males, n = 3 VR females, n = 3 control males and n = 3 control females), kidney (n=3 VR males, n=3 VR females), skeletal muscle (n=3 VR males, n=3 VR females) were collected. Three replicate sets of 8-plex iTRAQ based quantitative tissue proteomic experiments were undertaken in males and females separately to identify the differentially expressed proteins.

Results and Discussions: We have identified a total of 1234, 1654 & 1854 proteins in three different (includes three biological replicates for each group) experiments at 1% FDR in males. In the liver tissue of males 170 (87 up and 83 down regulated) and in females 155 (104 up and 51 down regulated) differentially regulated proteins were found in case of vitamin restriction as compared to control. Among males and females liver tissues 18 proteins are commonly upregulated and 12 are down regulated whereas, 16 proteins showed a reverse

trend. We have found the tissue specific changes in protein in expression in kidney and skeletal muscle as well.

Conclusions: Distinct protein expression among males and females suggest sex specific effects of vitamin B₁₂ restriction. This study will provide a comprehensive understanding of the effect of vitamin B12 deficiency on different tissue types.

P24

AGE modified hemoglobin –A better for marker for monitoring long-term glycemic status in diabetes

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Introduction: Deoxyfructosylated –N1-Val –β-hemoglobin, also referred as HbA1c, is widely used diagnostic test to assess the glycemic status in diabetes. During the lifespan of hemoglobin, it may undergo advanced glycation modifications. Therefore, this study aims to characterize and quantify AGE modified peptides of hemoglobin

Methodology: AGE modified peptides of hemoglobin were identified and characterized by high resolution accurate mass (HR/AM) mass spectrometry, and the modified peptides are quantified by parallel reaction monitoring.

Results and Discussion: We have identified and quantified AGE modification peptides of hemoglobin peptides in healthy control, pre-diabetic, diabetic, and poorly controlled diabetes.

Conclusion: The AGE-Hb peptides are quantified and they show better correlation with the clinical parameters and thus could be a better marker for assessing the long-term glycemic status in diabetes.

P25

Attempts to understand the role of arginine in influencing electrospray ionization collision induced dissociation characteristics: A middle-down proteomic investigation

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Abstract

Introduction: Collision induced dissociation (CID) tandem mass spectrometry (MS/MS) has proven to be successful for bottom-up (BU) proteomics, whereas it has been found to be not well suited for top-down (TD) investigations. Recently, middle-down (MD) proteomic approach has emerged, which seeks to combine the virtues of BU and TD strategies. Therefore, the role of CID MS/MS for MD proteomics requires more probing. Unlike BU method, MD approach involves proteolytic peptides > 20 or 25 amino acids long. Electrospray ionization (ESI) of such polypeptides usually results in protonation states higher than 2 or 3. It has been well noted that ESI CID of highly protonated polypeptide precursor ions yields inadequate sequence coverage. In this context, we chose to focus on arginine, since it can be responsible for producing higher protonation states, owing to high basicity of guanidine moiety. Thus, modifying guanidine might decrease the number of protons on the polypeptide ions. We hypothesize that ESI CID of such arginine-modified polypeptide ions may bring about better sequence coverage.

Methodology: Initial studies were conducted on model peptides, melittin (26 residues) and glucagon (29 residues). 1,2-cyclohexanedione (CHD) and phenylglyoxal (PG) were chosen for arginine modification. ESI MS data were acquired on a triple quadrupole and quadrupole/time-of-flight (Q/ToF), mass spectrometers.

Results and Discussion: Melittin and glucagon possess two arginines, each. ESI mass spectrum of native melittin and glucagon contained prominent signals attributable to protonation states +4/+5/+6 and +3/+4/+5, respectively. Interestingly, no significant changes in the distribution of these charge states were observed, even in the cases of one-arginine and two-arginines modified melittin and glucagon. Almost similar results were noted with both CHD as well as PG.

Conclusions: Despite modifying guanidines of arginines, the number of protons/charges on melittin and glucagon has not decreased. The CID MS/MS experiments of different charge states of native and modified melittin and glucagon are in progress.

P26

A pipeline for large scale mapping of post translational modifications from shotgun proteomics data

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Introduction: Identifying post-translational modifications (PTMs) and their annotation from mass spectrometry data is a daunting computational challenge. Generic database search tools can identify a limited number of anticipated modifications in high throughput manner. Most studies focus on specific modifications by enriching and analyzing one

modification at a time like phosphorylation, ubiquitination and acetylation. More than 300 biological PTM types are already known. Many more are still undiscovered due to lack of reliable methods.

Methodology: Recent developments are beginning to focus on an unrestrictive approach of PTM identification where tandem mass spectra are searched with a generous mass tolerances to account for mass shifts resulting from PTMs. MODa is one of such unrestrictive search tool that searches known and unanticipated PTMs from tandem mass spectra. The results are unwieldy and require manual intervention for complete analysis. We have developed an automated search and analysis pipeline which queues a large number of input files for blind mode search, processes the resultant output, calculates statistical significance by estimating FDR and tabulates the important findings.

Results and Discussion: Using public datasets, we have demonstrated the effectiveness and usefulness of the pipelining in mapping PTMs on large scale from high throughput proteomics data. The pipeline can map modification masses to the corresponding amino acid sites in peptides with high precision, and report the protein wise PTMs discovered in the experiment. We have discovered many modification sites along with the commonly expected chemical artefacts introduced during the experiment.

Conclusion: Our pipeline performs rapid, accurate and automated mapping of PTMs in a turn-key approach, with no limitation on the number of PTMs. The pipeline helps in large scale PTM discovery in automated manner from biological samples of interest, easing the otherwise cumbersome analysis process.

P 27

Cigarette smoke induced molecular alterations in esophageal cells

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Introduction: Esophageal squamous cell carcinoma (ESCC) is the sixth leading cause of cancer. Five year survival rate of ESCC patients remains between 5-20% due to lack of early diagnostic biomarkers and efficient therapeutic targets. Smoking is one of the major risk factors for ESCC. However, there is limited data on the effects of cigarette smoke on the progression of ESCC. Understanding the effects of cigarette smoke on esophageal squamous epithelial cells at a molecular level would lead to a better understanding of the pathobiology of ESCC which has implications for identification of early biomarkers and therapeutic targets.

Methodology: To investigate the effect of chronic exposure to

cigarette smoke on esophageal cells, we developed a cell line model where Het1A cells (non-neoplastic human esophageal epithelial cells) were chronically treated with cigarette smoke condensate (CSC) for 2 months, 4 months, 6 months and 8 months. We carried out TMT based comparative proteomic analysis to identify differentially expressed proteins in CSC-treated cells when compared to non-treated parental cells.

Results and Discussion: We identified 6,383 proteins, of which 35-50 proteins showed differential expression depending on the duration of the treatment. Differentially expressed proteins included cysteine-rich protein 2 (CRIP2), plexin-A3 (PLXNA3), HtrA serine peptidase 1 (HTRA1), GPI ethanolamine phosphate transferase 3 (PIGO), phosphomannomutase 2 (PMM2), myeloid derived growth factor (MYDGF), non-histone chromosomal protein HMG-17 (HMGN2), sialidase-3 (NEU3) and olfactory receptor 2A12 (OR2A12). We are now investigating the role of these differentially expressed proteins in ESCC.

Conclusion: Cigarette smoke induces specific changes in protein expression pattern in esophageal squamous epithelial cells that might play a vital role in development of ESCC.

P28

Proteomic Analysis of Halophilic Bacterium Efficient in Degrading Acid Orange 7 Dye

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Introduction: Microflora associated with sites contaminated with industrial effluents has shown immense capabilities to biotransform/biodegrade xenobiotic compounds. Such species have developed variable enzymatic machineries, to not just resist the stress of chemicals, but also to partake in degradation process. Recent advancement in dye degradation studies has ascertained huge potential in employing enzymes in degrading textile dyes and development of remediation techniques.

Methodology: A highly salt tolerant bacterial strain was isolated from the textile effluent site and employed in degrading Acid Orange 7 (AO7) dye. The degraded products and AO7 dye were then analyzed for their toxicological potential on *Vigna mungo* and *Allium cepa*. Further, proteomic analysis of intracellular bacterial proteins was carried out using SDS-PAGE. Bacterial cells grown in media containing AO7 dye (under static and shaking condition) and media without dye were analyzed for the differential expression of proteins and identified using mass spectrometry analysis (MALDI-TOF/MS-MS). The validation of differentially expressed proteins was done using Western blot analysis.

Results and Discussions: Bacterial strain is capable of degrading AO7 dye (90% degradation up to 500ppm at static condition). However, toxicological assessment (cytotoxicity and mutagenicity) pointed out that metabolites are more toxic than the parent compound. Further corresponding with proteomic analysis, a total of 10 differentially expressed proteins and formation of new bands were recognized in the case of bacterium treated with AO7 supplemented media under static condition. Since, dye was degraded only under

static condition; overexpression can be attributed to either dye decolorization or resistance against toxicity of degraded metabolites as bacterium was able to grow and flourish in such environment.

Conclusions: Proteomic analysis can be very useful in identification of novel enzymes from microbes native to the contaminated sites. Such enzymes from microbes surviving in extreme conditions can play pivotal role in development of efficient dye degradation technology.

P29

Emphasis of Phosphorylated Regulatory protein during *Shigella* spp. infection in model organism *Caenorhabditis elegans*

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Introduction: *Shigella* spp., a Gram negative rod which causes gastroenteritis, diarrhoea and severe inflammation termed as bacillary dysentery or shigellosis in humans particularly in infants. Since virulence proteins of *Shigella* spp. are known to phosphorylate/dephosphorylate host proteins, analyzing the fate of immune regulatory proteins phosphorylation status may reveal further insight into the pathogenesis process. It becomes apparent that the post-translational modifications (PTMs) occurring in the protein molecules provide new dimension to understand the host proteomic changes during bacterial infections. By using *Caenorhabditis elegans*, a soil nematode as a model organism we studied the *Shigella* infection mediated host immune regulatory proteins modification at PTM level. This study confers the proteomic level approach to study the modification in a candidate regulatory protein, c-Jun N-terminal Kinase (JNK) and its involvement during pathogenesis.

Methodology: *C. elegans* adult worms were cultivated in laboratory conditions and infected with *Shigella flexneri* in a time course dependent manner. *E. coli* OP50 was used as the control food source. In the same way cycloheximide treatment (10mg/ml) was given to control and infected worms. By using western blot analysis, total and phosphorylated levels of JNK protein was analyzed. The regulation of JNK protein was further validated by cycloheximide treatment.

Results & Discussions: Immunoblot reveals the regulation in total and phosphorylated levels of JNK protein during early hours of *Shigella* infection. Preliminary data suggest that after 24 hours of infection, no significant correlation in regulation between total and phosphorylated levels of *C. elegans* proteins were observed. Experiments with cycloheximide treatment indicate that JNK protein phosphorylation takes place in the already existing proteins rather than the newly translated protein during infection. These results correlate well with existing reports on phosphorylation/dephosphorylation of regulatory proteins against the bacterial virulence players during their interactions.

Conclusions: Phosphorylation/dephosphorylation of regulatory proteins of a host during pathogenesis appear to

be modulated by the interacting bacterial pathogen.

P30

Deregulated “miRNA and Target” cascades in GBM and their implications in transcriptomic and proteomic data analysis

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Introduction: Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor characterized by high rates of cell proliferation, migration and invasion. New therapeutic strategies are being continuously explored with the hope for better outcome.

Methodology: We used heterogeneous transcriptomic and proteomic data on GBM clinical tissues and generated integrated molecular information at the level of microRNAs and their mRNA and protein targets to lay down regulatory cascades encompassing altered tumor-related functions and processes.

Results and Discussions: Application of this integrated view to differential expression data on GBM revealed 2-dimensional molecular maps of microRNA and their targets building linkages in regulatory and functional dimensions with respect to the molecular networks of cell proliferation and invasion.

Conclusions: The 2-dimensional maps built upon gene expression changes derived from omics-based studies of the tumor tissues would enhance the strength of the data for mapping networks and pathways useful for developing molecular insights and clinical applications for GBM as well as tumors in general.

P 31

In silico analysis of amino acid sequences of bacterial (D/L)hydantoinase and allantoinase for their substrate specificity and physicochemical characterization

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Introduction: Hydantoinase and allantoinase are cyclic amidase, which are involved in the degradation of purines and pyrimidine respectively. The present paper aims to differentiate these two cyclic bacterial amidases on the basis of their amino acid sequences with reference to substrate

specificity and physicochemical properties due to their wide industrial applications. Computational analysis of these cyclic aminohydrolases revealed many detailed important characteristics information.

Methodology: All bacterial amino acid sequences of hydantoinase and allantoinase were extracted from ExPASy and analyzed using ProtParam, pI and molecular calculator. Multiple sequence alignment was performed using Clustal W and examined using Bioedit software. Data were evaluated using statistical tool like SPSS and CDHIT.

Results and discussions: Composition and total number of amino acid clearly indicate the difference among these two subclasses of cyclic amino hydrolases. Hydantoinase was more stable based on an instability index (<40) and aliphatic index. Multiple sequence alignment and statistical analysis proved that Alanine, Glycine and Leucine were found to be higher where Tryptophan was found to be very low in both the amidases. Catalytic triad was conserved in both, which makes them substrate specific. Position specific amino acid variation was found in gram positive as well as gram negative bacteria for both the enzymes and the presence of amino acids like Glutamate, Histidine, Cysteine for stability showed many conserved positions as well as substitutions.

Conclusions: Studied cyclic amidase differ in the total number of amino acids, molecular weights as well as composition. This study has highlighted the differences in the conserved amino acid residues at several positions among these bacterial cyclic amidases. The *in silico* analysis results can be quite useful in prediction and selection of hydantoinase and allantoinase from the reported amidases sequenced from bacterial genome.

P32

Identification and Characterization of Hemoglobins Variants by Novel Methods for Simpler Diagnosis of Hemoglobinopathies

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Introduction: Hemoglobinopathies affect the functioning of red blood cells and are the most common monogenic diseases worldwide. It has been estimated that about 7% of the world population is affected with these disorders. The high frequency and clinical severity of the hemoglobinopathies make them a major public health problem. India is also burdened with hemoglobin disorders that pose several challenges. Probably the best strategy for the prevention and control of the disease is early diagnosis. Analytical and preparative procedures for the characterization of abnormal hemoglobin are complex and time-consuming. The current methods used for screening of hemoglobinopathies have their own strengths and weaknesses, sometimes failing to identify abnormal hemoglobin.

Methodology: Fast, inexpensive, accurate and easier methods that could be successful independently or complement existing methods for screening are highly desirable. Spectroscopy could provide rapid identification of hemoglobin disorders since normal and abnormal hemoglobins and related biomolecules give rise to distinct spectral signatures. We aim

to build on the minimal and preliminary information available in this direction and establish biomolecular spectroscopy (absorbance and FTIR) as the routine method for detection of hemoglobinopathies. In addition, methods/protocols for ligand binding kinetic to study single mutation in hemoglobin disorders was also studied.

Results and Discussions: Absorbance and FTIR spectra showed different pH induced local changes in normal v/s mutant hemoglobin. FTIR spectroscopy seems to provide a concentration independent tool for differentiating between the normal and variant hemoglobins. In addition, methods/protocols for ligand binding kinetic to study single mutation in hemoglobin disorders showed changes but need to be further standardized.

Conclusions: Spectral signatures associated with variant hemoglobin could be used as a diagnostic marker for the detection of these variants for patients having hemoglobin disorders.

P33

MASS SPECTROMETRIC N-TERMINAL SEQUENCING OF PEPTIDES USING A BACTERIAL AMINOPEPTIDASE

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Introduction: Aminopeptidases catalyze the hydrolysis of peptide bonds joining the N-terminal amino acid of any peptide to the next amino acid in the sequence. Processive aminopeptidase can be used to create a population of peptides of different lengths differing by one amino acid mass, so that the entire population can be studied in a single mass spectrum, to determine the peptide's amino acid sequence. Sometimes, structure present in the peptide can also interfere. In this study, we describe the use of a non-specific, processive, deblocking *Bacillus subtilis*-derived aminopeptidase (BsuAP) in a reaction.

Methodology: Glu fibrino peptide (GFP B) from Waters, USA, was used as substrate. BsuAP was expressed in, and purified from, *E. coli* in our own lab. The reaction mixture of enzyme and substrate was prepared by mixing 1 nanomole of the aminopeptidase to 1.6 nanomoles of the substrate (GFP B) in water. The reaction mixture and control reaction (lacking aminopeptidase) were incubated at 70°C for 1 hour. After incubation, both were spotted on the MALDI plate. Samples were analyzed on the Q-TOF Synapt G2S HDMS system (from Waters, USA), with laser desorption achieved by the instrument's 355nm laser.

Result and discussion: The GFP B peptide is 14 amino acid residues-long. Our data suggests, BsuAP is non-specific and quite processive, progressively digesting 11 amino acid residues in the peptide substrate. It may be noted that the differences in masses of successive peaks (moving leftwards from the control GFP B mass in mass spectrum) correspond to masses of the amino acids removed, after addition of the mass of one hydrogen.

Conclusion: Masses are seen to have different relative

intensities which could be indicative of differences in specificity. A sufficiently slow reaction would produce a mass spectrum containing the mass corresponding to the complete peptide, and all smaller masses progressively truncating the peptide from its N-terminus, assuming that the reaction does not proceed to completion on all substrate molecules.

P34

Proteomic analysis of enterococcal biofilms under quercetin stress

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Bacterial biofilms cells adapted well to survive the effects of harsh environmental conditions better than planktonic cells of the same species. The reason for this may be the alteration in the life-style of the biofilms cells in compared to their planktonic counter mates. The changes in life occurs due to changes in expression of genes and hence in proteins expression. This study was designed to dig out the changes in proteome of the *E. faecalis* cells when treated with quercetin. Quercetin was found to be antibiofilm in nature as confirmed by crystal violet assay and confirmed using CLSM microscopy. The 1/8th MIC concentration of the compound was used to treat the bacterial cells to study the changes in cell proteome that may have caused the biofilm suppression. There was a clear cut change were observed in the cellular proteome of the organism after treatment. Identification of differentially expressed proteins spots included proteins necessary for the transcription and translation machinery, indicating that protein production becomes suppressed during biofilm inhibition using quercetin. DnaK protein was also found differentially expressed, as this protein is already known target protein for quercetin. Proteins identified were from glycolysis pathways, serine metabolic pathway. Stress proteins were also seen with an increased expression on treatment with the quercetin. These pathways may be involved in the transition of the planktonic cells to biofilm cells and their differential expression may be responsible for the alteration in the biofilm formation. These conclude that differential proteomic shifts play a role in biofilm-specific formation and tolerance. An altered metabolic response to quercetin toxicity represents a novel addition to a growing list of biofilm-specific mechanisms to resist environmental stress.

P35

Evolutionary dynamics of N-glycosylation sites in hemorrhagic fever viral envelope proteins

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Introduction: The recent deadly outbreak of Ebola – a hemorrhagic fever virus (HFV) has highlighted the need of viral research to better understand their biology. Many HFVs are “enveloped” and their surface proteins have N-glycosylation sites whose glycosylation is essential for immune evasion and cell invasion. While some envelope glycoproteins such as gp120 of HIV and HA of Influenza are known to have extreme selection and variations in N-glycosylation sites, such insights are lacking in HFV. We analysed thousands of envelope glycoprotein sequences from over 200 HFV species to explore the pattern and possible evolutionary trends of N-glycosylation site variations.

Methodology: HFV envelope glycoprotein nucleotide sequences were retrieved from Los Alamos National Laboratory and NCBI databases. Various computations relating to N-glycosylation sites such as density, shifts, and mutation probability were performed based on amino acid and codon frequencies, and using other sequence analyses methods in Python.

Results and Discussion: HFV envelope glycoproteins show large variation in the N-glycosylation site densities (number of sites per 500 amino acids) – from no preference (for example, in Mojiang virus) to extreme selection in Ebola viruses and Simian HFV. Zaire ebolavirus shows many variations in N-glycosylation sites – both gain and loss, with latter being more common. Both gain and loss of sites based on asparagine, and codon and sequon preferences were also observed. Far more variations in the envelope protein N-glycosylation sites of Marburg ebolavirus hint that this virus could be very adaptive and hence more problematic.

Conclusions: There is large variation in the pattern of N-glycosylation sites in HFV envelope proteins. Zaire and Marburg ebolaviral proteins show numerous gains and losses of N-glycosylation sites which might have relevance in viral biology and vaccine development.

P36

Investigation of phospholipid alterations in breast cancer using mass spectrometry

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Introduction: Breast cancer is the most common malignancy and the second most common cause of cancer related mortality. Phospholipids are major constituents of cell membranes and are also known to have diverse roles in cellular signalling and cell-cell interactions. Hence, the comparative analyses of the phospholipids of normal, benign and malignant samples can provide a better understanding of the molecular events

involved in tumour development.

Methodology: We have analysed alterations in phospholipids by taking 25 tissue and serum samples of each, benign, malignant and respected controls. For phospholipid profiling, we have developed LC-MS/MS based in house MRM phospholipid profiling platform using head group specific loss of Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), Phosphatidylinositol (PI) and Phosphatidylserine (PS) on AB Sciex 4000 QTRAP mass spectrometer. Expression of differential phospholipids in breast cancer samples was analysed by multivariate statistical analysis using MarkerView and SIMCA software. The differentially expressed phospholipids were further confirmed using SWATH analysis on TripleTOF 5600 System.

Results and Discussions: In this work, we applied mass spectrometry based quantitative MRM based MS approach to identify phospholipids involved in breast cancer. LC-MRM-MS/MS experiments revealed that 32 phospholipids out of total 210 were showing statistically significant differential expression. Exploratory PCA of identified lipids was employed to detect intrinsic clustering and possible outliers. PLS-DA, OPLS-DA further maximized the group separation, based on which a statistical model was built. These statistical models showed good discrimination of breast cancer patients with benign and healthy controls. SWATH analysis provided the confirmation of isomeric species using high resolution MS/MS fragmentation.

Conclusions: It is envisaged that understanding the phospholipids changes in breast cancer will not only help to identify new prognostic and predictive biomarkers, but will also be useful in predicting the biochemical pathways involved in breast cancer.

P37

Mass spectrometry-based characterization of the composition of Murrah buffalo milk lipids

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Bovine milk is a significant part of human diet of all age groups. More recently, improved characterization of bovine milk have revealed key bioactives that may be relevant for human health such as unsaturated fatty acids (UFAs) and its relevance to adiposity and anticarcinogenicity. While characterization studies have improved, little is still known analytically about the natural variation of milk fat. Thus, we present a pilot investigation, using high resolution mass spectrometry-based lipidomics on Murrah buffalo milk as a model of study where we seek to profile and statistically pinpoint key lipid components within bovine milk samples. Milk specimens sampled from six healthy Murrah buffalo were collected and processed for lipid isolation as described

by Stefanov et al (2). In brief, whole lipidome were extracted in dichloromethane-ethanol (DM-E) and subsequently lyophilised. Samples were dissolved in absolute methanol prior to LC-MS analysis where UHPLC hyphenation was carried out using Agilent 1200 system with zorbax 2.1*150mm reverse-phase C18 column, at a flow rate of 300pL/min. A 60 minute gradient (5-90% B) was performed using H₂O + 0.1% formic acid (FA) and Solvent B: Methanol + 0.1% FA, and lipid samples were eluted into a Bruker QTOF mass spectrometer, operating in Top5 data-dependent mode from 100-1500 m/z both ESI positive and negative mode. Three methods were tested for extraction efficiency (Bligh-Dyer, methanol-chloroform, DM-E), DM-E was found to achieve the highest recovery and was subsequently used as the extraction method. Milk lipid extractions were performed in analytical triplicates and analyzed using QTOF mass spectrometer in triplicates. Preliminary data using positive ion mode on master pooled samples have revealed a total of 118 high intensity and reproducible lipids signatures. Further identification of the collected MS/MS data was performed using Simlipid® leading to the identification of 65 different lipids species. Identified lipids were grouped into phospholipids (PL), fatty acyls and sterols with majority of the phospholipid being phosphatidylcholine and phosphatidyl inositol. In addition, triacylglycerols derived from fatty acids such as palmitic, oleic acids identified based on their fragment ions. Our preliminary observation from the analysis of polar dairy lipids provides a framework towards understanding the total heterogeneity in dairy derived from Murray buffalo. Future work would include mapping and quantitatively profiling lipid concentrations across breed (versus Sahiwal cow). Findings from the comprehensive quantitative mapping should enable the identification of key lipid features that may be relevant for bioengineering in order to improve both yield and quality of Murrah buffalo based dairy products. Comprehensive lipidome profiling of Murrah buffalo milk to enable identification of lipid markers relevant for bioengineering in dairy products.

P38

Effect of fish oil supplementation on Acyl CoA Cholesterol Acyl Transferase expression in liver of experimental rats

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Introduction: Epidemiological studies led to the hypothesis that marine oils rich in n-3 fatty are hypolipidemic and antiatherogenic. In our study we tried to look at the effect of diet enriched with a high percentage of fish oil in comparison to a diet rich in coconut oil in male albino rats.

Methodology: Wistar rats weighing 150-160 g were separated into three dietary groups of six animals each. The groups were, 4 % groundnut oil Group 1, 20 % coconut oil Group 2 and fish oil Group 3. The experimental diets were

administered for 3 weeks. Rats were sacrificed; blood and liver samples were taken for further analysis.

Total liver tissue fat, fatty acid and triacylglycerol contents and serum plasma cholesterol were assayed by Zak's method. SDS PAGE of serum and liver proteins was carried out. Liver tissue histopathology for lipids was done

Total liver RNA was isolated and reverse transcribed using 1 mg RNA. The mRNA of ACAT was evaluated by real-time PCR using specific primers. Products were amplified and purity confirmed by agarose gel analysis. Changes in mRNA expression were calculated and expressed as fold induction over control group.

Proteomics: Cytosolic protein homogenates were separated by two-dimensional gel electrophoresis. Gels were analysed and spots with densities that significantly differed between treatments were excised and trypsinised and analysed by LC-MS methods.

Results: Body weight gain and food intake expressed as g/week were significantly more in Group 2 and 3 than control group. Total liver fat was significantly less in Group 2 and 3 than control group. There was a slight but significant increase in liver fat content in group 3 over group 2. Group 3 showed a significantly high w-3 fatty acid content than groups 2 and 1. Plasma levels of triglycerides, and NEFA were reduced in the group 3 than in group 2. Further fish oil induced a decrease in the levels of triacylglycerols in the liver. SDS PAGE of liver and serum protein show that intensity of several high molecular weight bands has changed in group 3. In liver, about 2.3 fold reduction in induction of ACAT mRNA was observed in fish oil group over the control group.

Conclusion: Fish oil feeding has resulted in lowering of serum and liver triglycerides and enhanced the level of hepatic n-3 PUFA content. At the same time hepatic expression of ACAT was lowered which is important as this would mean lower rate of conversion of cholesterol into esters.

P39

Extraction and Characterization of Steviol Glycosides from Stevia Plants

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Abstract:

Steviol glycosides are highly sweet triterpene glycosides found in the shrub 'Stevia rebaudiana'. The leaves can be used for their sweetness and are used as non- calorie sweetness in several countries due to their nutritional and pharmacological benefits. The various methods employed in previous research works include HPTLC and Column Chromatography.

Experimental Part:

Extraction and Isolation of Sativoside from Stevia plant involved the leaves of stevia being dried in the shade and then pulverized in grinder. The powder was extracted using water as solvent and was pre-treated with solvent and air dried. Dried crude extract of sample were added to column packed with silica gel. The column was run with a solvent of methanol and chloroform with increasing polarity. Fraction were collected and TLC for each fraction was performed.

Conclusion: The results were awaited and will be discussed.

P40

Profiling of flavonoid-subclasses in *Myristica fragrans* and *Cordyline terminalis* by LC-ESI-MS

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Introduction: Although a lot of research has been conducted on the antioxidant properties of medicinal plants, very few studies have focused on their phenolic composition. Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants. Flavonoids have been shown to have a wide range of biological and pharmacological activities like anti-allergic, anti-inflammatory, antioxidant, antimicrobial, antibacterial, antifungal, and antiviral, anti-cancer, and anti-diarrheal activities. Here, we have carried out investigations on the seeds of *Myristicafragrans* (*M. fragrans*) and leaves of *Cordyline terminalis* (*C.terminalis*). While the seeds of *M. fragrans* are routinely utilized for day-to-day culinary purposes, *C.terminalis* is predominantly an ornamental plant. The antioxidant activity of extracts from seeds of *M. fragrans* and leaves of *C. terminalis* has been determined. The investigation herein is on methanol-water and water extracts of seeds of *Myristicafragrans* and leaves of *Cordyline terminalis*, and the objective is to screen potential subclasses of flavonoids in these extracts.

Methodology: The extracts were investigated by liquid chromatography (reverse phase) coupled to electrospray ionization mass spectrometry (LC-ESI-MS). Interpretation of LC-ESI-MS data was done by using the database of Lipid Metabolites and Pathways Strategy Consortium (LIPID MAPS).

Results and Discussion: In both the plant extracts, the subclass 'Flavones and Flavonols' seems to be predominant. 'Flavones and Flavonols' and 'Anthocyanidins' appear to be more abundant in *C. terminalis* than in *M. fragrans*. Higher content of 'isoflavonoids', 'chalcones and dihydrochalcones' and 'other polyketides' were observed in *M. fragrans* as compared to *C. terminalis*. There may be some isoprenoids too in *M. fragrans*. *C. terminalis* has a higher content of 'Flavones & Flavonols' than *M. fragrans*, which corroborates with the data obtained by AlCl₃ method

Conclusion: To the best of our knowledge, this is the first report that demonstrates the use of LIPID MAPS' database in elucidating the subclasses of flavonoids from the mass spectral data. Such a profiling can help in designing interesting formulations, wherein, different subclasses can be mixed in various proportions that might be useful for preparing dietary supplements and nutraceuticals having antioxidant potential.

P41

Expression dynamics of protein kinases and phosphatases across human tissues

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Introduction: Protein phosphorylation is one of the major post-translational modifications associated with regulation of various cellular processes. There are over 500 serine/threonine and tyrosine kinases and about 200 phosphatases encoded by the human genome that regulate reversible phosphorylation. Dysregulation of protein phosphorylation/dephosphorylation is associated with various diseases including cancers. Several studies have employed high-throughput technologies such as RNA-Seq and mass spectrometry-based proteomics to study gene expression across human tissues. We combined data from transcriptomic and proteomic studies to determine expression pattern of kinases and phosphatases across different human tissues.

Methodology: In this study, we utilized publicly available RNA-Seq and mass spectrometry-based proteomic datasets to explore the expression dynamics of kinases and phosphatases across various tissues.

Results and Discussion: This analysis revealed kinases and phosphatases that showed nearly ubiquitous expression as well as those that showed tissue restricted expression patterns. For example, kinases such as titin (TTN) and obscurin (OBSCN) showed higher expression levels in heart and skeletal muscle tissues as compared to other tissues. Similarly, protein kinase C, gamma (PRKCG) and microtubule associated serine/threonine kinase 1 (MAST1) showed predominant expression in brain tissues. In addition, expression of dual specificity phosphatase 13 (DUSP13) was found to be largely restricted to the skeletal muscle while dual specificity phosphatase 2 (DUSP2) showed higher expression levels in the bone marrow and lymph node.

Conclusions: These results demonstrate the significance of high throughput datasets in understanding gene expression patterns across human tissues. Identification of genes that are expressed in specific tissues are valuable candidates for biomarker investigations and to find putative therapeutic targets.

P42

Chromosomal alterations, differentially expressed gene clusters and potential biomarkers in Oral Cancer: Insights from Proteomics

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Introduction: Oral squamous cell carcinoma (OSCC) is one of the major causes of mortality in India. Differential proteomics data can help identify clusters of genes involved in tumors that can be utilized to identify new biomarkers. Identifying the chromosomal loci of the differentially expressed proteins in OSCC, with the objective of identifying gene clusters and thereby potential biomarkers for clinical applications is the focus of this study.

Methods: Previously published data on OSCC proteomics were compiled to create a database of differentially expressed proteins identified with high confidence. Chromosomal locations of these proteins were mapped using NextProt database as reference. Pathway analysis was done using KEGG analysis tool. The secretory proteins were identified either using prediction databases (Exocarta, SignalP, TMHMM) or experimentally proven to be present in OSCC secretome or saliva.

Results and Discussions: We constructed a database of differentially expressed proteins in OSCC consisting of 850 candidates. Mapping of these proteins to chromosomes revealed Chr 12, Chr 10 and Chr 17 to be the top 3 altered chromosomes. The major clusters localized to Chr1q21.3 which codes for S100 proteins, Chr12q13.13 which code for type II keratins, and Chr17q21.2 which code for type I Keratins. Since chromosome 12 coded for the maximum number of altered proteins, we focused on these 55 proteins; 52 out of these 55 proteins were secretory in nature and proteotypic peptides for these secretory proteins were identified from GPM database and SRM atlas.

Conclusions: Experimental identification of deregulated proteins and their mapping to amplicon regions of the chromosomes suggests consistency in chromosomal changes. Deregulated keratin clusters may represent inter-chromosomal cross talk which would be interesting to investigate, they being associated with progression of OSCC. The proteotypic peptides for the portfolio of the secretory proteins provided would be useful for targeted assays for clinical validation.

P43

Quantitative proteomic analysis of cerebrospinal fluid from paralytic rabies and Guillain–Barré syndrome

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Introduction: Rabies is a viral disease, which affects the central nervous system. Worldwide, more than 55,000 deaths are attributed to rabies, making it a disease requiring medical urgency. Clinically, it is classified into encephalitic rabies and paralytic rabies. It is a fatal disease, where diagnosing it at the earliest is necessary for providing appropriate action in its treatment. GuillainBarré Syndrome (GBS) is an autoimmune disorder resulting in the inflammation of peripheral nervous system. The pathophysiology underlying this disorder is still unclear. GBS and paralytic rabies display similar symptoms at the onset of the disease, making the diagnosis difficult. In case of rabies, incorrect diagnosis will result in fatality. Our proteomics study can be used as a platform to help in the accurate diagnosis of these disorders.

Methodology: In the present study, the cerebrospinal fluid samples of GBS and Paralytic rabies cases were subjected to quantitative proteomic analysis using Tandem mass tags (TMT) labelling and high resolution Orbitrap LC-MS/MS analysis. The proteins identified were analysed using various bioinformatics tools.

Results and discussion: We identified 800 proteins, out of which, 77 proteins were differentially expressed. The differentially expressed proteins include, Haptoglobin, Apolipoprotein A-IV which were up regulated and Transthyretin, Apolipoprotein E were down regulated in the GBS condition.

Conclusion: The identification of differentially expressed cerebrospinal fluid proteins in GBS and paralytic rabies have the potential to reveal the mechanisms involved in their respective disorders. These differentially regulated proteins can be used as candidate biomarkers to differentiate the disorders for accurate diagnosis.

towards breast cancer subtypes. Our main aim of this study is to investigate subtype specific potential protein biomarkers using multipronged proteomic approaches like 2D-DIGE, iTRAQ and SWATH followed by validation using western blotting and MRM assays.

Methodology: In this work, we used mass spectrometry based 4-plex iTRAQ, SWATH and complementary 2D-DIGE to identify the differentially expressed proteins in four subtypes of BC. In 2D-DIGE and iTRAQ the differential expression pattern was analyzed using DeCyder and ProteinPilot software respectively. The statistically significant proteins were subjected to Gene Ontology analysis using multiple databases which revealed important pathways involving in BC. Identified proteins were validated using Western Blot as well as LC-MS-MRM.

Results and Discussions: The differentially expressed proteins identified using iTRAQ, SWATH, and 2D-DIGE was interpreted to identify sub-type specific serum protein biomarkers. Interestingly, we were able to identify a panel of differentially expressed subtype specific protein markers which can not only be used to discriminate subtypes but also can give a deep insight towards disease progression in different subtypes. Our validation data in a different cohort of samples using western blot and LC-MS-MRM are consistent with experimental data.

Conclusions: In this study, we employed complementary proteomic approaches to identify subtype specific serum potential biomarkers in BC. These subtype specific protein signatures are not only helpful as theranostic biomarkers but also enhance our understanding of the different molecular changes at proteomic level.

P45

Repertoire of differentially expressed proteins in cerebrospinal fluid of rabies survivor and non-survivor individuals

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P44

Identification of subtype specific serum potential protein biomarkers in breast cancer using complementary gel-based and gel-free quantitative proteomic approaches

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Introduction: Among all other cancers, Breast cancer (BC) has the highest incidence rate in women around the world. Being a clinically and genetically heterogeneous disease, breast cancer tumor is subdivided into 4 subtypes viz. luminal A, luminal B, HER2-enriched and basal-like subtype. Hence, there is an urgent need to come up with potential biomarkers which can support the clinicians in early diagnosis as well as to develop the targeted therapeutics

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Introduction: Rabies continues to be a severe health issue in most developing countries. The clinical diagnosis for rabies is vaguely defined and complete understanding of neuropathology caused due to rabies infection is lacking.

Rabies infection at times is negligible in terms of cytosolic and inflammatory symptoms and without any considerable blood-brain barrier disruption. Analyzing the cerebrospinal fluid of the affected individual through a proteomics approach therefore, is essential for understanding the underlying mechanism of rabies pathology. The present study compares the protein profile of cerebrospinal fluid from rabies survivors and non-survivors to comprehend the primary mechanisms associated with rabies infection and fatality.

Methodology: We examined lumbar cerebrospinal fluid samples from six individuals with laboratory confirmed rabies. Three of the individuals survived (survivor) after a rabies infection while the other three scurried (non-survivor) after infection with rabies. Cerebrospinal fluid from each individual was depleted for high abundant proteins followed with trypsin digestion and labeling with 6plex Thermo Mass Tag labels. Labeled peptides were analyzed on Thermo LTQ-Orbitrap Fusion mass spectrometer.

Results: A total of 158 proteins were identified from the cerebrospinal fluidsamples considering a false discovery rate (FDR) of <1%. Of these, 94 proteins were found to be differentially regulated. We observed 44 proteins to be expressed in higher amounts (≥ 1.5 fold) in cerebrospinal fluid of rabies survivors. Increased expression (≥ 1.5 fold) of 50 proteins was observed in cerebrospinal fluid of non-survivor individuals. We did not identify any peptide from rabies virus in the present study. The differentially regulated proteins could be mapped to several pathways including energy metabolism and immune response. We observed an increased expression of proteins associated with innate and acquired immune response in case of rabies survivors as compared to non-survivors.

Conclusions: The present study reports the first quantitative proteomics analysis of cerebrospinal fluid from rabies survivors and non-survivors. The findings will significantly enhance the understanding of immune response; metabolism and cellular mechanisms involved in rabies infection and associated neuropathology

in three categories i.e. dilated (DCM), hypertrophic (HCM) and restrictive cardiomyopathy (RCM) depending on their pathophysiological phenotype. Recent studies suggest that gene environmental interactions play a major role in these complex disorders. Here we have used Plasma proteomics approach to look for markers in three different forms of myopathy.

Methodology: Blood samples from angiographically proven DCM (n=3), HCM (n=3), RCM (n=3), and controls (n=3) were taken in this study. Individual plasma samples from each group were immunodepleted and three sets of 4-plex iTRAQ based quantitative LC-MS proteomic experiments was undertaken to identify the differentially expressed proteins.

Results and Discussions: We have identified a total of 208, 191 & 211 proteins in three different (includes three biological replicates for each group) experiments at 1% FDR. A total of 69, 61 and 62 differentially expressed proteins were found, out of which 31, 35 and 31 were up regulated and 38, 27 and 31 were down regulated in DCM, RCM and RCM respectively as compared to controls. Further, a total of 8 up and 8 down-regulated proteins were common among three groups. These commonly up regulated proteins are involved in complement and blood coagulation cascade. The common down regulated proteins are involved lipoprotein metabolism and reverse cholesterol pathway. However, there were several proteins that were differentially expressed specifically in these three groups. DCM patients' show altered acute phase response and blood coagulation. HCM and RCM patients do not show any specific pathway alteration, but show some distinct proteins involved in cardiac dysregulation.

Conclusions: This study indicates that blood coagulation pathway, immune response and reverse cholesterol pathways are being significantly altered in myopathy. However three different form of myopathy show distinct protein alterations in terms of disease progression. Further this study would help us to identify novel markers of cardiomyopathy.

P46

Identification of novel markers of Dilated, Hypertrophic and Restrictive cardiomyopathy using Plasma Proteomics approach

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Introduction: Cardiomyopathy is a complex broad-spectrum disease of the heart myocardium with unknown etiology. It is a leading cause of death irrespective of socioeconomic status, age and sex in India. Cardiomyopathy is majorly classified

P47

Comparative protein profiling of diseased tissue in rheumatoid arthritis

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Introduction: Rheumatoid arthritis (RA) is a debilitating autoimmune condition that often affects small joints of hand and feet in a symmetric fashion. RA is a systemic disorder where inflammatory cells primarily attacks synovial tissue of the joints and alters its protective function. Aggressive pannus tissue forms as a result of chronic synovial inflammation that eventually results in bone and cartilage destruction. The etiology and molecular mechanism behind the disease

progression largely remains unknown. In addition, its high prevalence rate and ineffective treatment suggests for the development of new therapies.

Methodology: In the present work, mass spectrometry based Isobaric tags for relative and absolute quantitation (iTRAQ) labelling technique was utilised in order to screen the proteins with significant differential expression followed by their bioinformatic studies.

Results and Discussion: A total of 365 proteins (1% Global FDR; 2 unique peptide) were identified using iTRAQ based mass spectrometry amongst which several proteins were shown to have altered expression. Bioinformatics analysis predicts few key pathways involved in rheumatoid arthritis.

Conclusion: Identified differentially expressed proteins may serve as potential diagnostic and prognostic markers. Further, targeting these deregulated proteins could help in the betterment of human health.

P48

Quantitative proteomic analysis towards new targets and biomarkers for multiple myeloma.

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Introduction: Multiple myeloma (MM) is a heterogeneous disease and accounts for 1% of all cancers and 14% of all hematological malignancies. MM is a malignant tumour of plasma cells, the major challenge remains the identification of better diagnosis and prognostic biomarkers. Our main aim of this study is to identify potential targets and biomarkers using multipronged proteomic approaches like 2D-DIGE, iTRAQ and label free analysis.

Materials and methods: In this work we used MM serum, Bone marrow (BM) plasma and BM mono nuclear cells and respective controls. Serum and plasma proteins were extracted and differential proteomic analysis was performed using 2D-DIGE and 4-plex iTRAQ labeling experiments. We also performed label free SWATH analysis of BM mono nuclear cells to identify differentially expressed proteins in MM.

Results and discussion: In the study of serum proteome alterations in MM, our quantitative proteomic analysis using DIGE and iTRAQ resulted 61 differentially expressed proteins in which 30 proteins showed increased expression and 31 proteins showed decreased expression. In case of bone marrow plasmastudy, we identified 35 differentially expressed proteins out of which 21 proteins were found to be up-regulated and 14 proteins were down-regulated. Further, proteomic analysis of mono nuclear cells yielded a total 892 proteins using SWATH analysis in which 222 proteins were found to be statistically differentially expressed. Bioinformatics data suggest that DNA replication, angiogenesis, apoptosis, integrin, WNT, CCKR signalling pathways were altered in MM. Our validation data in a different cohort of samples using western blot and LC-MRM-MS/MS are consistent with experimental data.

Conclusions: In this study, we employed complementary proteomic approaches to identify the protein targets and potential biomarker for MM. These protein signatures are not only helpful as diagnostic and prognostic markers but also provide insight disease pathogenesis information of MM.

P49

Proteomic changes in *Caenorhabditis elegans* exposed to *Shigella flexneri* revealed modulation of Reproductive System proteins.

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Introduction: Host-pathogen interaction studies are essential to understand the mechanism of altered biological interaction between the host and pathogen that mainly includes host defense and cellular mechanisms. By using *Caenorhabditis elegans*, a soil nematode was used as a model organism to study the impact of bacterial infection in cellular mechanism of the host. Analyzing the proteomic changes will shed more light on the altered cellular mechanism in the host during bacterial infection.

Methodology: *Caenorhabditis elegans* WT adult worms total proteins were isolated after *Shigella flexneri* infection for 24 h. *E. coli* OP50 was used as the control food source. 2D-gel electrophoresis followed by MALDI-MS analysis were performed to analyze the host regulated proteins during *S. flexneri* infection. Gene ontology (GO) analysis was used to categorize the biological functions of identified regulated proteins.

Results & Discussions: 2D-gel electrophoresis of *C. elegans* proteome revealed 455 spots were regulated with a threshold of >1.5 fold during *S. flexneri* infection for 24 h. By using MALDI-MS analysis, we identified 42 protein spots among the differentially regulated spots. GO analysis revealed 7 proteins, which are important for spermatogenesis; egg formation and yolk proteins are modulated. Among the 7 reproductive system associated proteins, regulation of VIT-6 and SPE-26 at the mRNA level were further validated by qPCR analysis. These results suggest that *S. flexneri* infection may modulate proteins, which are involved in reproductive system of the infected host.

Conclusions: This study expands our understanding on the impact of *S. flexneri* infection in a host at the proteome level.

P50

Proteomic Analysis of *Plasmodium falciparum* Patients from Different Endemic Regions of India to Understand

Malaria Pathogenesis and Identify Surrogate Markers of Severity.

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Plasmodium falciparum is major causative organism associated with malaria related morbidity. This study aims to identify alterations in the human serum proteome as a consequence of non-severe and severe infections by the malaria parasite *Plasmodium falciparum* to identify markers related to disease severity and to obtain mechanistic insights about disease pathogenesis and host immune responses. In discovery phase of the study, a comprehensive quantitative proteomics analysis was performed on three biological replicates of healthy controls (n=90), non-severe falciparum malaria (n=45) and severe falciparum malaria (n=30) patients using gel-based (SyproRuby and 2D-DIGE) and gel-free (iTRAQ) techniques on two independent mass spectrometry platforms (ESI-Q-TOF and Q-Exactive mass spectrometry), and some targets were validated on individual patients. Proteins showing altered serum abundance in falciparum malaria patients revealed the modulation of different physiological pathways including chemokine and cytokine signaling, IL-12 signaling and production in macrophages, complement cascades, blood coagulation and protein ubiquitination pathways. Some cell adhesion protein like galectin-3-binding proteins were found to be deregulated in severe malaria patients. Carbonic anhydrase 1 found to be upregulated in severe as compared to non-severe case. It may facilitate the survival of parasite into erythrocytes. Identified proteins including C-reactive protein, Plasma protease C1 inhibitor, alpha 1-antichymotrypsin and haptoglobin, which exhibited alterations in their serum abundance in different severity levels of malaria, could serve as potential prognostic markers for disease severity and to understand in malaria pathogenesis. The first comprehensive analysis revealed serum proteomic alternation in severe falciparum infected patients. This study has been done on pooled samples and 7 individuals. The same study has to perform on large cohorts to strength the potential biomarkers which can monitor severity of disease.

P51

Proteomics in biomarker discovery for early detection of pregnancy in Bovine: Current status and future direction

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Introduction: An early and precise pregnancy diagnosis is one of the most important requirements for better reproductive management in livestock such as cows and buffaloes. Early pregnancy diagnosis is crucial to shorten the calving interval to identify pregnancy at the earliest opportunity. Dairy farmers need to recognize pregnancy at the earliest opportunity so as to rebreed the dam at the very next opportunity if the animal is not pregnant. After fertilization and implantation, biomarkers such as progesterone, pregnancy associated glycoproteins

(PAG) interferon tau (IFN tau) and early pregnancy factor are expressed in serum. However none of them qualifies as ideal early pregnancy markers. Recently urine and saliva have become important sources, which are non-invasive in nature and easy to collect. The advancement in proteomics has opened up opportunities to look for pregnancy biomarkers in farm animals preferably before 21 days.

Methodology: We have used a series of proteomics techniques for identification of early pregnancy biomarkers in serum, urine and saliva. Techniques such as shotgun proteomics, 2D-GE, 2D DIGE, label free quantitation, iTRAQ and TMT etc have been used to identify potential biomarkers for early pregnancy diagnosis. The proteins from the serum, urine and saliva have been extracted and in-gel and in-solution tryptic digestion was performed. The tryptic peptides were subjected to Nano-LC-ESI-qTOF. The proteins were identified and quantified using Proteome discoverer, ProteinScape and Maxquant software.

Results and Discussion: We have identified potential biomarkers in serum, urine and saliva by following various proteomics and Mass Spectrometry strategies. Some of the proteins which have very high potential to be called as biomarkers for early pregnancy diagnosis (before 21 days after AI) in cows include proteins and enzymes. We have profiled a total of 1571 proteins in cow urine of for the first time (Bathla et al., 2015). Differential proteome analysis revealed more than 20 proteins which are associated with early pregnancy. Some non-enzyme proteins (non-enzyme) are Fibulin-2, GRP-27, Trefoil factor 3, IGFBP2, SPLNECAP, A2HSP, Uteroglobin, Uromodulin, sex hormone-binding globulin, Atrial natriuretic peptide receptor. More than 10 upregulated enzymes were also identified during pregnancy namely MBLSP2, GPCL, Antileukoproteinase-1-PIP-4,5-bisphosphate, phosphodiesterase, α -N-acetylgalactosaminidase, peptidyl peptidase, α -1-antiproteinase, etc. The Uromodulin, Gastrin RP, trefoil factor 3 etc. are detectable in urine and their role in pregnancy is known.

P52

Quantitative proteomics of cerebrospinal fluid from Tuberculosis meningitis co-infected with HIV

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Introduction: Along with its primary manifestation Tuberculosis (TB), *Mycobacterium tuberculosis* is also accountable for the mortality and neuromorbidity associated with Tuberculous Meningitis (TBM). HIV patients are frequently found to be co-infected with TBM. The present study focuses on the identification of potential biomarkers in the cerebrospinal fluid (CSF) that will aid in distinguishing the pathogenesis of TBM from TBM-HIV co-infection.

Methodology: Three CSF samples each from TBM subjects, demographically-matched TBM-HIV and control subjects were used in the study. Equal amount of proteins from each case was digested, labeled with iTRAQ reagent and fractionated. iTRAQ-based quantitative protein expression profiling and LC-MS/MS analysis of SCX fractionated peptides obtained from CSF samples was carried out on Orbitrap Fusion Tribrid mass spectrometer interfaced with Proxeon Easy-nLC 1000 system (Thermo Scientific, Bremen, Germany).

Results and Discussions: A total of 2,132 proteins were identified. Among the differential proteins identified, 87 were down-regulated and 629 proteins were up-regulated in TBM against the control, 266 down-regulated and 459 up-regulated in TBM-HIV against the control and 432 down-regulated and 98 up-regulated in TBM against TBM-HIV. Several differentially expressed proteins identified in our study were found to be associated with the pathogenesis of TBM and TBM-HIV but not reported earlier. Subset of differentially expressed proteins were found to be involved in regulation of immune system processes, protein binding, neuro-inflammation, neural cell growth and differentiation.

Conclusions: Identification of altered protein expression upon TBM and TBM-HIV infection added to the current knowledge of the host responses towards these infections. Further studies can unravel the utility of the differentially expressed proteins which in turn might aid in early diagnosis, prognosis as well as in the evaluation of disease progression of TBM and TBM-HIV

P53

Proteomic analysis of CVS-11 virus infected and uninfected Neuro N2a cells and BHK-21 cell line

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Introduction: Rabies is a fatal form of progressive encephalitis cause by a RNA virus belonging to family, *Rhabdoviridae*. Rabies virus has a broad host range that includes almost all mammals and dogs being a widespread primary vector. Around 55,000 human rabies deaths are estimated each year, making rabies one of the most fatal diseases. However, the molecular mechanism behind its neurological manifestation is still unclear and needs to be investigated. In this study, we tried to study the host responses upon Rabies virus infection. Mouse Neuro2a cells and baby kidney hamster (BHK) cell line (BHK-21) were infected with CVS-11 viral strain and the altered proteome was investigated. Neuro N2a and BHK-21 infection with CVS-11 was used to mimic the infection *in vivo*, which will lead to better understanding of rabies pathogenesis. Altered protein expression correlates with the rabies virus adaptability and pathogenicity in Neuro N2a cells and BHK-21 cell line.

Methodology: Proteins were extracted from CVS-11 infected and uninfected Neuro2a cells and BHK-21 cell lines followed by trypsin digestion. Peptides were then labeled with 8plex iTRAQ (isobaric tags for relative and absolute quantitation), followed by fractionation. Each fraction was then analyzed on Orbitrap Fusion at high resolution.

Results and Discussions: A total of 3,500 proteins were identified in this study, a subset of which were found to be differentially expressed upon CVS-11 infection. These proteins were found to be involved in different biological processes such as cell communication, signal transduction, metabolism, cytoskeleton rearrangement and transport.

Conclusion: In this study, we observed altered proteome after infection with CVS-11 in mouse nerve tissue derived cell line in control condition free from external stimulus. We found a significant increase in cytoskeletal proteins and stress response related proteins. This suggests host cytoskeletal protein modification might be required for viral assembly and release. Also, over-expression of antioxidants and stress-related proteins might be having protective role against rabies virus infection.

P54

Proteomic profiling of brain regions reveals complex biological basis for schizophrenia

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Introduction: Schizophrenia is a severely debilitating psychiatric disorder affecting about 30 million subjects worldwide, with onset typically occurring in adolescence or early adulthood. It is characterized by a diverse range of symptoms, including hallucination, delusions, reduction in affect and behavior, and disorganization of thought and language. Despite extensive research, etiology, biological process and molecular pathogenesis in different regions of human brain, which contribute to schizophrenia has not been well understood. A high-resolution mass spectrometry based quantitative proteomic analysis of different parts of brain can provide insight into altered signaling pathways and protein expression patterns.

Methodology: We employed mass spectrometry-based proteomic analysis to investigate the molecular alterations in schizophrenia. Autopsy samples of anterior cingulate cortex, dorsolateral prefrontal lobe, hippocampus and thalamus from three schizophrenic and three non-schizophrenic subjects with neurotrauma as controls were analyzed by 10 plex-TMT labeling and high-resolution mass spectrometry.

Results and Discussions: TMT-based quantitative proteomic analysis of selected brain regions from schizophrenia and control cases resulted in the identification of 230 proteins with altered expression among the pool of 5,351 proteins identified. Altered proteins have already been known to be involved in inflammatory immune response, oxidative stress pathways, neuronal architecture and mitochondrial dysfunction. Our analysis also leads to identification of differentially expressed proteins mapping to retinoic acid signaling, serine/threonine kinase signaling, extracellular matrix stability and energy metabolism which have been previously reported to be associated with schizophrenia. We also identified several novel proteins, which have not been reported previously in the context of neuronal diseases including schizophrenia.

Conclusion: This study reports the first quantitative proteomics analysis of anterior cingulate cortex, dorsolateral prefrontal lobe, hippocampus and thalamus regions in schizophrenia cases. The findings will significantly enhance the understanding of schizophrenia pathophysiology and signaling pathways and may serve as a baseline data for biomarker identification and treatment strategies.

P55

Phosphoproteomic analysis of gallbladder cancer

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Introduction: Gallbladder cancer (GBC) is the predominant form of biliary tract cancer. It often manifests at an advanced and unresectable stage. The malignancy is aggressive and the prognosis is dismal with a survival of less than 5 years in 90% of the cases. Early detection is incidental with surgery being the only curative option. Currently, neither are there reliable biomarkers for early diagnosis nor targeted therapy for the disease. This highlights the need to identify potential therapeutic targets to improve treatment options and disease outcome. We employed a phosphoproteomic strategy to identify signaling alterations in GBC using GBC cell lines.

Methodology: Phosphoproteomic analysis of five GBC cell lines based on the invasive property (non-invasive to highly invasive) was carried out using the tandem mass tags (TMT) labeling strategy. Phosphoserine/threonine containing peptides were enriched using TiO₂-based strategy after basic pH reverse phase liquid chromatography and analyzed using a high resolution Fourier transform LTQ-Orbitrap Velos mass spectrometer.

Results and Discussion: This phosphoproteomic study led to the identification of 2,847 unique phosphopeptides containing 2,460 unique phosphosites corresponding to 1,440 proteins. Among the hyperphosphorylated proteins, AKT1S1, FOXO3 and RPS6 were found to be hyperphosphorylated in this data set. AKT1S1 was found to be hyperphosphorylated at T246. AKT1S1 is a known substrate of AKT and a 14-3-3 binding protein. AKT1S1 binds to RPTOR and exhibits its inhibitory effect on mTOR signaling. PIM1, a serine/threonine kinase, is also known to phosphorylate AKT1S1 at T246 independent of AKT and thereby regulate mTOR activity.

Conclusion: This study will help in the identification of activated kinases involved in driving tumor progression in gallbladder cancer which, in turn, will assist in identifying therapeutic targets for gallbladder cancer. Studies are ongoing to understand the role of AKT1S1 signaling in GBC tumor progression.

P56

Identification of host response in cerebral malaria brain proteome using quantitative proteomic analysis

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Introduction: Malaria, caused by *Plasmodium* species is one of the most common infection observed globally with around 198 million cases reported annually. According to recent study around 1 million cases were observed in India. Cerebral malaria is result of severe neurological complication caused by *Plasmodium falciparum* infection with high mortality rate. Patients surviving cerebral malaria infection show long term cognitive impairment. Pathophysiology of cerebral malaria is still not completely understood. In this study, we have made an attempt to study altered brain proteome from individual with cerebral malaria. It will result in better understanding of host response in cerebral malaria brain proteome.

Methodology: Three autopsied frontal lobe brain samples from cerebral malaria patients and control individuals were used for study. Extracted proteins were digested and peptides were labeled using TMT-labeling technique and fractionated using basic pH reverse-phase liquid chromatography. Total 12 fractions were obtained that were further analyzed on Orbitrap Fusion Tribrid mass spectrometer

Results and Discussions: We identified total 3,686 proteins of which 78 proteins were differentially expressed in frontal lobe brain sample of cerebral malaria as compared to control. Proteins involved in processes such as immune response, myelination, cytoskeleton organization, coagulation were found to be deregulated. Pathway analysis showed overexpression of acute phase response protein pathway while 14-3-3 pathway was found to be downregulated.

Conclusion: In this study, we have explored pathogenesis and associated processes such as innate immune response, axonal injury, deregulation of coagulation and myelination in brain proteome of individuals with cerebral malaria. It suggests severe neuronal injury and heightened immune response potentially explaining the cerebral malaria clinical outcome.

P57

Cheung tobacco and cigarette smoke-induced molecular alterations in oral cancer

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Introduction: Tobacco, both in its smoked and smokeless forms, has been a causative agent for oral cancer. It is intuitive to consider that the effect of chewing and smoking tobacco would be similar, but pathobiology of oral cancer resulting from chewing tobacco may vary from cigarette smoking due to the variation in the composition of the pro-carcinogens and carcinogens. Besides, the method of intake for the two forms also varies greatly. The aim of this study was to compare the proteomic alterations in oral keratinocytes in response to cigarette smoke and chewing tobacco.

Methodology: Normal oral keratinocytes, OKF6/TERT1, were chronically treated with either chewing tobacco or cigarette smoke. Chronic exposure to both chewing tobacco and cigarette smoke resulted in an increase in cellular proliferation and induced invasive ability in the non-invasive oral keratinocytes. To understand the molecular alterations specific to each insult, we carried out quantitative proteomic analysis of OKF6/TERT1 chronically treated with chewing tobacco or cigarette smoke compared to the untreated cells.

Results and Discussion: To identify molecular alterations in response to chewing and smoking tobacco we carried out proteomic analysis of OKF6/TERT1 chronically treated with chewing tobacco or cigarette smoke using tandem mass tag (TMT) labeling approach and LC-MS/MS analysis. Analysis of the OKF6/TERT1 tobacco treated cells led to the identification of 3,636 proteins. Among these, 40 proteins were overexpressed and 70 proteins were downregulated with a fold value of ≥ 2 . Analysis of the OKF6/TERT1 smoke treated cells led to the identification of 5,794 proteins out of which expression of 181 proteins were differentially expressed with a fold value of ≥ 2 . Amongst the over expressed proteins between the two groups, 38 proteins were found to be overexpressed exclusively in the tobacco treated cells and 104 were overexpressed exclusively in the cigarette smoke treated cells. Similarly comparing the proteins that were downregulated between the two treatments revealed that only 3 molecules were common between the two. This data reveals that though both chewing and smoking tobacco causes oral cancer, the molecular alterations brought about by each insult is different.

Conclusions: This study strives to delineate the differences in the molecular alterations arising due to exposure to chewing tobacco and cigarette smoke. Elucidating these differences could eventually lead to discovery of potential early detection biomarkers and therapeutic targets in oral cancer patients based on their tobacco usage habits.

P58

Serum-based Biomarker Discovery for Human Fatty Liver disease

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Introduction: Fatty liver disease (FLD) is a state where triglyceride Fats get accumulated in hepatocytes. FLD is of two types: FLD that is linked to excessive intake of alcohol (alcoholic fatty liver disease - AFLD) and the FLD whose cause is linked to factors other than alcohol (non-alcoholic fatty liver disease - NAFLD). Currently, staging for FLD is performed by indirect examination of liver enzymes; ultra sound-based imaging technique, and invasive biopsy-based histological examination. Hence, identification of blood-based biomarkers is essential for the diagnosis of different stages of FLD ranging from steatosis, steatohepatitis, and cirrhosis. Interrogating blood from patients with different stages of fatty liver disease and also the samples from control subjects is envisioned to provide data that would be stowus with biomarker candidates for FLD.

Methodology: Serum samples were obtained from different stages of FLD patients. Control serum samples were also obtained from normal (healthy) individuals. After depleting albumin from the serum samples, protein expression pattern was analyzed using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as well as two-dimensional gel electrophoresis (2DE). After separation of the proteins, the gel was stained with either coomassie brilliant blue or silver stain and differentially expressed proteins were identified.

Results and Discussions: From the above analyses, protein bands specific to disease conditions were identified. Further work is being performed to get the identity of the protein using mass-spectrometry. Additional experiments are underway towards this line to process multiple samples and identify putative biomarkers.

Conclusion: The above work flow with proteomics coupled to mass-spectrometry of serum samples will be beneficial to obtain potential blood-based biomarkers for fatty liver disease.

P59

Identification of differential host response to infections in cryptococcal meningitis, toxoplasma encephalitis and tuberculous meningitis coinfecting with HIV

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Introduction: Neuroinfection is one of the factors which contribute to increased mortality rate in individuals infected with HIV. Cryptococcal meningitis, toxoplasma encephalitis and tuberculous meningitis are the most prevalent nervous system infection which is observed in HIV individuals. Though proteomic investigation has been carried out for these three neuroinfections independently, but there has been no previous attempt to compare differentially expressed proteins among these infections. Here, we have attempted to analyze

the altered proteome from frontal lobe brain tissue samples of individuals with these three conditions using 4-plex iTRAQ quantitative proteomics and high-resolution mass spectrometry.

Methodology: Autopsy tissue samples from brain were collected from five CM, TE, TBM patients co-infected with HIV-1 and control individuals. Equal amount of proteins from each case was further digested and labeled with iTRAQ reagent. Samples were then fractionated and analyzed using high resolution mass spectrometry.

Results and Discussions: Quantitative proteomic analysis was carried out of frontal lobe brain tissues from cryptococcal meningitis, toxoplasma encephalitis and tuberculous meningitis patients; co infected with HIV and control individuals using iTRAQ labeling followed by SCX fractionation and high-resolution Fourier transform mass spectrometry. Search using SEQUEST and Mascot algorithm against RefSeq protein database led to identification of 3,541 proteins, of which, 639 were found to be differentially expressed in CM while 735 and 820 were found to be differentially expressed in TE and TBM, respectively ($1.5 \geq$ fold).

Conclusions: Mechanism of pathogenesis in chronic meningitis in immune-compromised individuals is not well elucidated. Quantitative iTRAQ based proteomics and high resolution mass spectrometry have identified 3,541 proteins of which 639, 735 and 820 proteins were found to be differentially expressed in CM, TE and TBM respectively. We have validated the expression of three proteins MHC-1, PRELP and TF in brain tissue sample using immunohistochemistry. This study will help in addressing host response process and also in development of potential biomarkers that can help in differential diagnosis of TE, CM and TBM in HIV individuals.

P60

SILAC-based proteomic analysis to delineate mechanisms underlying erlotinib resistance in head and neck squamous cell carcinoma

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Introduction: Epidermal growth factor receptor (EGFR) plays an important role in the pathogenesis of Head and neck squamous cell carcinoma (HNSCC) and its overexpression is observed in about 90% of HNSCC cases. Tyrosine kinase inhibitors (TKIs) such as erlotinib and gefitinib among several other drugs are currently under Phase III investigations. However, most of these TKIs have shown a modest in clinical

trials. Development of intrinsic mechanisms to circumvent blockade of EGFR signaling is one of the major reasons of acquired resistance. Elucidating the molecular mechanism of resistance to EGFR-targeted therapies is therefore essential to identifying potential therapeutic targets.

Methodology: Drug resistant cell lines (SCC-R) were generated via a process of slowly escalating exposure of UM-SCC1 cells to erlotinib. SCC-S is used to designate the parental UM-SCC1 cells exposed to DMSO. SILAC-based global quantitative proteomic analysis was carried out to gain insights into the mechanism of erlotinib resistance in these cell lines. SCC-S cells were grown in heavy SILAC media whereas SCC-R cells were grown in light SILAC media. The lysates were mixed in equal amounts and subjected to in-solution tryptic digestion and fractionated. The fractions were analyzed on high resolution Fourier transform Orbitrap Fusion Tribrid mass spectrometer.

Results and Discussion: SILAC-based quantitative proteomics experiment led to identification of 5,427 proteins of which 509 proteins were overexpressed and 504 proteins were down regulated by more than 2 fold in SCC-R cells with respect to SCC-S cells. We observed dysregulation of several signaling molecules downstream of EGFR. In addition, we also observed overexpression of AXL which has been previously implicated in mediating erlotinib resistance in HNSCC. Furthermore, we identified 6.4 fold overexpression of Cub-domain containing protein 1(CDCP1), a transmembrane protein involved in cell adhesion and cell matrix association in SCC-R cells implicating its role in erlotinib resistance. SiRNA mediated knockdown of CDCP1 resulted in increased sensitivity of SCC-R cells to erlotinib.

Conclusion: Together, these data suggests an important role of CDCP1 in erlotinib resistance and epithelial to mesenchymal transition in HNSCC. It also demonstrates the therapeutic potential of CDCP1 in combination with EGFR TKIs in this disease.

P61

Oxidized HDL-ApoA1 as a biomarker of human cardiovascular risks and development of novel diagnostics for high throughput analysis

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Introduction: ApoA1 is the major component of HDL and it is believed that increase in the HDL levels in humans reduces the CVD risks. Latest findings show that quality of HDL is more important than quantity, as a modification due to oxidation renders them pro-inflammatory promoting atherosclerosis. Oxidized ApoA1 serves as an indicator of dysfunctional HDL and these modifications are mainly seen at specific amino acid residues such as tryptophan, tyrosine, methionine and lysine. The use of mass spectrometric methods for characterizing these modifications in each CVD sample is time consuming and expensive. Thus we focused on developing monoclonal antibodies which are highly specific to oxidized ApoA1 and evaluate whether highly specific monoclonal antibodies could be used in highthroughput

assays and validate them along with mass spectrometry as a proof principle to see whether oxidized ApoA1 serves as an early biomarker of CVD.

Methodology: Oxidation of Apo A1:Apo A1 was chlorinated chemically using sodium hypochlorite. The chlorination of Tyr residue at position 192 was confirmed by mass spectrometry. BALB/c mice were immunized with this antigen for generating monoclonal antibodies for high throughput screening.

Results and discussion: Both native and oxidized ApoA1 (specifically at Tyr192) were analyzed by mass spectrometry and the results indicated that the addition of chlorine atom in tyr192 residue subsequently chlorinated ApoA1 which was used as an antigen for immunization to generate the monoclonal antibody which is highly specific to chlorinated tyr192.

Conclusion: Proteomics approach via mass spectrometry seems to be a potential tool for analyzing different human diseases, particularly CVD. Moreover, this approach will provide information on early biomarker detection for risk assessment of CVD. However, for high throughput analysis of patient samples, there is a need for development of monoclonal antibodies for specific markers. We were successful in developing the monoclonal antibody against Chl-tyr-192 of ApoA1 and currently screening patient samples for this modification by both conventional immunotechnology and immunoproteomics based approaches.

Keywords: Apolipoprotein A1 (apoA1), High Density Lipoprotein (HDL), Cardiovascular disease (CVD), Tyrosine 192, Atherosclerosis.

P62

Identification of biomarkers of hepatotoxicity and chronic liver disease by plasma proteomic analysis of arsenic-exposed carp

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Introduction: Arsenicosis, caused by chronic ingestion of arsenic through contaminated water and food-chain, is a major public health problem. It is manifested through multisystem disorder including cancers of different organs. Identification of biomarkers for early detection of arsenic toxicity would be useful in preventing and/or disease management. Plasma, an important biological material that is easily accessible, consists of vast assemblies of proteins that reflect the physiologic or pathologic state of cells, tissues, and organs. The present study was carried out to investigate plasma proteome changes in arsenic-exposed carp (*Labeo rohita*), with the aim of identifying biomarkers for arsenicosis.

Methodology: Labeo rohitafingerlings were exposed to arsenic, at varying concentrations for 12 days. Plasma proteome changes were investigated using the gel based proteomics; 2-D gel electrophoresis, image analysis, MALDI-TOF/TOF MS, LC MS/MS and validation by transcript analysis.

Results and Discussions: Out of the 14 unique spots analysed, apolipoprotein-A1 (Apo-A1) (6/14), α -2 macroglobulin-like protein (A2ML) (2/14), transferrin (3/14) and warm-temperature acclimation related 65kDa protein (Wap65) (1/14) increased in abundance and interestingly all these proteins are liver specific. Transcript analysis validated the proteomic results. An interesting observation was made in case of Apo-A1 transcript analysis; two transcripts i.e. Apo-A1-1 and Apo-A1-2a were downregulated and another variant of Apo-A1-2 (named Apo-A1-2b) was 3 fold upregulated. Apo-A1-1 has been reported as a constitutive form, whereas Apo-A1-2 is selectively increased in hepatocellular carcinoma. In this backdrop, the variants Apo-A1-2a and Apo-A1-2b merit further investigation to find out if there is a specific linkage to arsenicosis.

Conclusions: As plasma is one of the easily available body fluids for clinical investigations, combination of the proteins Apo-A1, A2ML, Wap65 and transferrin could be used as biomarkers of hepatotoxicity and liver damage.

Keywords: Arsenicosis, Labeo rohita, Plasma proteome, RT-PCR, Biomarkers of hepatotoxicity.

P63

Preliminary studies to identify potential therapeutic targets in the blast crisis stage of chronic myeloid leukemia using proteomics approach

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Introduction: Chronic myeloid leukemia (CML) is associated with a specific chromosomal abnormality: the Philadelphia chromosome (Ph), originating from a reciprocal translocation between the long arms of chromosomes 9 and 22. This translocation produces the oncogenic fusion protein Bcr-Abl, with a constitutive tyrosine kinase activity with maintained autophosphorylation and substrate activation. The presence and activity of Bcr-Abl is necessary and sufficient to determine neoplastic transformation of hematopoietic multipotent cells. Specific drugs with anti-phosphotyrosine kinase activity especially, Imatinib mesylate, causes selective suppression of Bcr-Abl positive cell proliferation. Patients in chronic phase CML have a good response to imatinib (95% complete remission), but patients in the acute phase of CML (blast crisis, BC) do not respond as well to the tyrosine kinase inhibitors. Therefore the present study is aimed at identifying alternate therapeutic targets for CML-BC using proteomics approach.

Methodology: Response of three pairs of imatinib sensitive and resistant cell lines from CML-BC patients has been

investigated. Viability has been checked by trypan blue staining and MTT assay. Apoptotic population has been detected flow cytometry following staining with Annexin V-FITC. Cell cycle analysis has been carried out by flow cytometric analysis of cells stained with PI. Proteomic profiles of these cell lines have been generated pre and post imatinib treatment using nanoLC-MS-MS analysis.

Results and discussion: We have identified more than 2000 proteins in each of the cell lines. Similarities in the profiles of all sensitive and resistant cell lines as well as differences between the two groups have been identified. Pathway analysis of the differentiators has been done to identify the key pathways altered in all cell lines.

Conclusion: Proteins from the pathways found to be altered in resistant cells could be potential therapeutic targets for CML-BC.

P64

Identification of differentially expressed proteins involved in Nasopharyngeal carcinoma using High throughput proteomics approach

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Introduction: NPC is a common disease in South East Asia, some parts of Africa and the Arctic, in comparison to the Western world. NPC is more prevalent and eighth most common cancer in north eastern states of India particularly Nagaland, Manipur, and Mizoram. National Cancer Registry has reported the prevalence rate to be 1.82% amongst all cancers in this region. Proteomics is an effective platform to detect and characterize proteins globally and comparative proteomic approach is the main strategy to identify the differentially expressed proteins involved in the disease progression. In this study, our interest is to identify and validate the differentially expressed protein from plasma sample of NPC cases and Controls from north eastern states of India.

Methodology: Plasma samples collected from cases were classified according to the disease stage. Top 14 abundant proteins from this sample were depleted using the Multi affinity removal spin cartridge Hu-14. These depleted sample were separated by 2D electrophoresis using 7cm, pH 4-7 IPG strip and then on 12% SDS PAGE. Scan imaged were analyzed in Image master 2D platinum software. Further, spots of interest were identified with MALDI-TOF-MS.

Results and Discussion: In 2D analysis, we could identify as many as 408 and 428 spots on NPC and control plasma proteome profile. In comparison, 22 differentially expressed spots including 9 down regulated and 13 up regulated spots were identified which were subjected to MALDI-TOF-MS analysis. GO annotation for cellular component indicated that identified protein were mostly located at different part

of cell organelles. GO annotation for molecular function showed that these proteins were majorly involved in binding and transporter activity. GO annotation for biological process indicated that these were distributed among various process such as cellular, single organism, biological regulation and metabolic. Further, validation of these proteins is under way.

Conclusion: Using differential proteomics, we could identify few differentially expressed protein probably involved in NPC pathogenesis. These proteins might be useful as diagnosis tool or disease progression marker, however further studies are needed.

P65

Label Free Approaches for Identification of Biomarkers for Early Detection of Pregnancy in Cow Urine

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Introduction: An early and precise pregnancy diagnosis is an important criterion for better reproductive management in livestock like cows and buffaloes. Early pregnancy diagnosis in bovine is crucial to shorten the calving interval through enabling the farmer to identify open animals so as to treat and/ or rebreed them at the earliest opportunity. On conception, numerous signals are produced during bovine early pregnancy. Many of these hormones and proteins are of fetal-placental origin rather than of maternal origin. They are required for the successful establishment of pregnancy and the proliferation of normal and neoplastic cells. Many methods of pregnancy diagnosis, both direct and indirect, are being practiced in bovine species yet none qualifies as the ideal pregnancy diagnosis method. The advancement of proteomics in animal science research has facilitated discovery of pregnancy biomarkers in these animals. For pregnancy diagnosis urine is a desirable material because of the convenience of its collection.

Method: Urine sample from non-pregnant and pregnant (n=6) cows were collected on different days of pregnancy (0, 16, 22, 35), centrifuged and subjected to dia-filtration (in PBS buffer pH 7.4) using 3 kDa hollow fibre cartridge. 20 μg Ammonium Sulphate precipitated proteins were subjected to in-solution tryptic digestion followed by desalting. Peptides were fractionated in Nano LC, eluted at 400 nL/min over 135 gradient followed by MS/MS and MS(3) scans for identification and quantitation using Protein Scape3 and Maxquant 1.5.2.8 Software against IPI database using stringent parameters. Gene ontology of differential expressed proteins was done using Panther4.0.

Results and discussion: Total 407 proteins were identified using Proteinscape out of which 183 differentially expressed proteins were up regulated having fold change ≥ 1.5 . Sixty three differentially expressed proteins were identified through Maxquant. Out of 32 proteins were up-regulated having fold change ≥ 1.5 . Few proteins which are common in both analysis such as fibulin, GRP, IGF, sex hormone binding

protein, alpha 2HS glycoprotein were considered to be potential biomarker for early pregnancy detection. Protein-protein interaction study through String software revealed that these proteins were involved in blastocyst formation, implantation and embryo growth during pregnancy. Majority of proteins were involved in catalytic activity (36.4%) followed by binding (22.7%), enzyme regulator activity (22.7%), receptor activity (13.6%) and transporter activity (4.5%) revealed by GO study using Panther.

P66

Understanding the response of *Caenorhabditis elegans* against whole bacterial Proteome of *Salmonella Typhi*

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Abstract

The nematode *Caenorhabditis elegans* represents a quality model for studying various aspects of bacterial pathogenesis. Virulent factor of pathogen was reportedly sufficient for killing *Caenorhabditis elegans* and the factor(s) vary from effector molecules to toxic proteins. Many Gram positive and Gram negative bacteria are found to be pathogenic to *C. elegans* and the mode of infection in many of these bacteria is due to colonization or infection in the intestine. Bacterial infections leading to activation of immune cells by bacterial products resulting in enhanced release of mediators of inflammation. Endotoxin (LPS) is a major component of the outer membrane of Gram negative bacteria and is a critical factor in pathogenesis. The aim of the present study is to understand role of toxic/virulent protein(s) present in a pathogen (*Salmonella Typhi*) which probably promotes the apoptosis against the interacting host system (*C. elegans*). Experiments on *C. elegans* have demonstrated that both *S. Typhi* and its isolated protein fractions effectively killed the host, extensively damaged the gut and affected the reproduction as well as embryo growth. SDS-PAGE based protein analysis was performed for the protein samples collected at different time points from the unchallenged control and *S. Typhi* total protein treated *C. elegans*. The host proteins that showed differential regulation in their expression level against *S. Typhi* protein were selected based on their fold change of intensity on gels and were identified using MALDI/TOF-TOF analysis. The results obtained are being validated using specific *C. elegans* mutants and Western blotting for selective regulatory protein(s).

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P67

Proteomic analysis of 4-Hydroxy-2-Nonenal (4 HNE) induced insulin resistance in insulin sensitive cell lines.

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Introduction: Type II diabetes is characterized by high plasma glucose levels due to insulin resistance in tissues that rely on insulin for glucose uptake. Persistent high levels of glucose, by-products of aerobic metabolism or reduction in anti oxidant mechanisms due to aging, cause oxidative stress which is a major contributing factor to diabetic complications. One of the many detrimental effects of the oxidative stress is breakdown of membrane lipids through an oxidative chain reaction leading to production of smaller aldehydes, one of which is 4-Hydroxy-2-Nonenal (4 HNE). 4HNE itself causes oxidative stress, acts as a signalling molecule and chemically modifies proteins. We wish to explore whether 4HNE has a role to play in insulin resistance and if dietary anti oxidants can provide any protection against the harmful effects of 4HNE.

Methodology: The effect of HNE on glucose uptake was studied in rat muscle cells using fluorescent labelled glucose. Effect on GLUT4 translocation was studied by using CHO cells which stably over express GFP tagged GLUT4. Effects of dietary antioxidants namely resveratrol, carvacrol, estragole, baicalein, capsaicin and mangiferrin on glucose uptake in presence of HNE was studied. Proteomic techniques were used to determine the differential expression of proteins and protein modified by HNE treatment.

Results: Treatment of 4HNE to muscle cells reduced the glucose uptake and inhibited the GLUT4 translocation while the antioxidant resveratrol prevented this effect. Protein extraction and tryptic digestion of HNE treated and untreated 3T3 adipocyte cell lysates was carried out for LCMS analysis. Several proteins were identified to be upregulated and downregulated of which some are involved in insulin signalling. Studies to identify HNE modified proteins are underway. Similar studies will be carried out with prior treatment of anti oxidants in muscle cells and adipocytes.

Conclusion: HNE can possibly play a role in insulin resistance and dietary anti oxidants can prove to be potential therapeutics.

P68

Plasma proteomic profiling of high fat diet induced wistar rats treated with Curcumin and Dracaena cinnabari for anti-hyperlipidemic activity.

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Introduction: Dracaena cinnabari resin (Dragon's blood) and

Curcumin possessing anti-microbial, anti-oxidant, anti-viral and anti-inflammatory properties are used as a traditional medicine since ancient times. In the present study we have compared the therapeutic potential of both the plants against coronary artery diseases (CAD)/ atherosclerosis.

Methodology: CAD model was generated using Wistar rat by feeding high fat diet to two groups (G2 and G3) and normal diet was given to control group (G1). Plant extract (300 mg/kg) of Curcumin and Dragon's blood was used as drugs and given to rats. Drug was administered orally to G2 (Curcumin treated) and G3 (Dragon's blood treated) groups after symptoms of disease were developed. The activity was measured by assessing total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-C) and high density lipoprotein cholesterol (HDL-C).

Results and Discussion: A significant LDL level decrease (14.6%) was observed in Dragon's blood treated rats (group G3) compared to Curcumin treated (53.38%) group (G2). Similarly HDL levels was observed to be less (9.33%) in G3 (Dragon's blood) group compared to G2 (Curcumin treated) group (15.31%). Further protein profiling of rat plasma was carried out by running 2D gel electrophoresis of G1 (Control), G2 (Curcumin treated) and G3 (Dragon's blood treated) rats. Altered Protein spots were cut and proteins were identified by MALDI-TOF analysis.

Conclusions: Dracaena cinnabari found to be more effective than curcumin pure compound. Thus the study demonstrated that both the extracts exhibit a potent lipid lowering activity in diet induced hyperlipidemia.

P69

Biophysical characterization methods for biotherapeutic development

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Abstract

Efficiency of protein based biotherapeutics relies mainly on the physicochemical properties of the molecule including chemical and structural composition. Chemical and structural stability of biotherapeutics are rigorously assessed during the life time of the drug from discovery stage to product development and production. Liquid chromatography/mass spectrometry (LCMS) and spectroscopic methods are increasingly being used for assessment of molecular integrity and stability. Characterization of biotherapeutics' primary, secondary and tertiary structure using LCMS and spectrometric methods are presented here.

P70

Mass spectrometric analysis of glycation induced amyloid beta aggregation

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Introduction: Alzheimer's disease (AD), also termed as type III diabetes is a progressive neurodegenerative disease which is marked by memory loss, cognitive dysfunction and dementia. Formation of insoluble Amyloid beta (A β) plaques is the hallmark of AD. Hyperglycemic condition in diabetes promotes glycation of proteins including A β . Glycated A β shows increased aggregation propensity and neurotoxicity. This study describes the evaluation of antiglycating drugs on glycation and aggregation of A β by using mass spectrometry and biophysical approaches.

Methodology: We have used a unique technique, ion mobility mass spectrometry (IMMS), which is dependent upon the drift time of the aggregates, to evaluate the effect of aminoguanidine (AMG), isoprenylidine (IPN) on aggregation of A β . In addition to IMMS, AGE fluorescence, thioflavin T assay, light scattering and in-vitro neuronal cell culture techniques were used to validate the results.

Results and Discussions: We have found that both the drugs have reduced glycation and aggregation of A β . Similar effect was observed in neuronal cells.

Conclusion: We found AMG as a potential drug to prevent glycation induced A β aggregation and found to restore the A β caused neurodegeneration than IPN.

P71

ADME modelling of Ginsenosides and characterisation of Ginsenosides Rg1 phytosome: An approach towards discovery of potential drug lead for SOD1 and TARDBP targets of ALS

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Introduction: Amyotrophic lateral sclerosis (ALS) is devastating, rapidly progressing neurodegenerative disease that attacks healthy adults, leaving patients paralyzed and unable to breathe. Currently, there is no known cure or effective treatment available for ALS. But with the advances in technology, there has been enormous increase in the volume of the genetic data produced. There have been several newer tools available for analysis and interpretation of this data to obtain valid conclusions. The present study is aimed at synthesising and characterising ginsenoside phytosome based on the insilico studies.

Methodology: The selection of the targets, SOD1 and TARDBP is based on the reports and the frequency as well as the types of mutations occurring in the population under study. The structure of the targets was modelled using SWISSMODEL. One of the herbal drug molecule's, ginsenoside and their derivatives obtained from Pubchem

compound database has been used to test against the selected targets of ALS. Based on the insilico studies ginsenoside Rg1 was selected for synthesis of nano-phytosomes using L- α phosphatidylcholine by employing a salting out procedure. For SEM, phytosomes were sputter coated with 10 nm thickness of gold and visualization was done on FEG-SEM (SIRION). The FTIR analysis was carried out using Thermo Nicolet 6700 spectrophotometer by KBr disc method under transmission mode in the wavenumber range between 4000cm⁻¹ to 400cm⁻¹.

Results & Discussion: The potential drug leads have been predicted and modelled via ADME tools. The computationally predicted docking results and ADME data showed that Rg1 isomorph of ginsenoside can be a potential lead. The ginsenoside Rg1 was thus selected for synthesis of nano-phytosomes. Size of phytosomes obtained was in the range of 180nm to 195nm and surface morphology indicated the spherical nature of phytosome. The absence of a phenol group represented by a C-O stretching in the phytosomes suggests the interaction of ginsenoside Rg1 with the water soluble choline head group via a C-O end in its structure.

Conclusion: The integration of ADME data and docking studies aid in compound selection for synthesis of phytosome which is a boon to the earlier phases of drug discovery for ALS. The application of nano-drug increases the bioavailability and thus could be used for the treatment of chronic diseases like ALS, Parkinson's Diseases.

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P72

Differential proteomics of *Streptococcus pyogenes* upon treatment with 3-furancarboxaldehyde – An anti-biofilm agent

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Introduction: *Streptococcus pyogenes*, a multi-virulent, muscle licking and exclusive human pathogen is at the helm of various invasive and non invasive diseases. The ability of the pathogen to form biofilm is considered as one of the important determinants for its multidrug resistance development. In our previous study, we identified 3-furancarboxaldehyde (3FCA) as a potent and novel antibiofilm agent against *S. pyogenes*.

Objective: Differential proteome analysis of *S. pyogenes* grown in the presence and absence of 3FCA and to decipher its mode of action by identifying key protein targets.

Methodology: Protein profiles of 3FCA treated and untreated *S. pyogenes* were compared using two-dimensional gel electrophoresis and the differentially expressed proteins were identified using matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

Results: 3FCA was found to alter the protein expression

pattern of *S. pyogenes*. A total of 335 protein spots were found to be matched between control and treated gels. Among the 335 spots, 15 spots were found to be differentially expressed (11 down regulated and 4 up regulated) by more than 2 fold. The differentially expressed proteins were found to have significant role in amino acid biosynthesis, peptidoglycan synthesis and membrane transportation activity; all of which play a crucial role in cell wall biogenesis.

Conclusion: The protein expression of *S. pyogenes* was found to be altered upon treatment with 3FCA, an antibiofilm agent. The results suggest that 3FCA targets cellwallbiogenesis for its antibiofilm activity. Further validation using Western blot and real time gene expression analysis of the differentially expressed proteins is expected to throw more light on the Streptococcal protein targets of 3FCA.

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P73

Proteomic responses of *Pseudomonas aeruginosa* PAO1 to quorum sensing inhibitor curcumin

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Key words: *P. aeruginosa*; quorum sensing inhibition; curcumin; proteomics; iron homeostasis

Introduction: QS inhibitory potential of curcumin has been analysed at virulence and gene expression level. However, the effect of curcumin on the proteome of *P. aeruginosa* remains uncharted. Hence, it is necessary to analyse the effect of QS inhibition on the human opportunistic pathogen *P. aeruginosa* PAO1 proteome to find novel targets.

Methodology: Each 600 μ g of intracellular proteins of *P. aeruginosa* PAO1 grown in the absence and presence of 5 μ g mL⁻¹ of QSI were subjected to isoelectric focusing using immobilized pH gradient (IPG) strips (18 cm, pH 3-10 NL) at using standard parameters and the second dimensional electrophoresis was performed on 12 to 15% gradient SDS-PAGE. Protein spots were visualized by colloidal CBB G-250 and analysed using ImageMaster 2D Platinum 7 (GE Healthcare). More than two fold differentially regulated protein spots were selected for in-gel trypsin digestion and analysed using MALDI TOF/TOF Mass Spectrometer (AXIMA Performance, Shimadzu.Biotech). The proteins were identified by peptide mass fingerprinting using MASCOT and MS-Fit.

Result and Discussion: Among the detected spots (613), 48 (7.8%) spots are downregulated and 31 (5%) spots are upregulated by more than two fold. Based on statistical significance (ANOVA 0.05), seventeen downregulated and five upregulated protein spots were selected for MALDI TOF/TOF MS analysis. Downregulated proteins are involved in iron transport, transcriptional regulation, antibiotic resistance and chemotaxis. Curcumin treatment has upregulated the

expression of histidine kinase, ABC-transporter protein. Since iron transport proteins, two-component response regulators and sensor are involved in the activation of protease, exotoxin A and pyoverdin biosynthesis proteins in *P. aeruginosa* PAO1, downregulation of above said proteins by curcumin could be a major event in attenuation of virulence factor production.

Conclusion: Curcumin inhibits the virulence factor production of *P. aeruginosa* PAO1 by targeting the iron homeostasis, pyoveridine and pyochelin biosynthesis pathways.

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P74

Host Specific Variation in Proteins of Semi Parasitic Plant (Mistletoe) with Anti-Cancer Potentials

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Introduction: Mistletoe is semi-parasitic plant that has been extensively used in cancer treatment. Mistletoe therapy is widely accepted phytotherapy that provide first line of treatment for cancer by stimulating the immune system and improves quality of life. Mistletoe is a source of diverse phytochemicals (proteins) which have tremendous potential to serve as anti-inflammatory agents. These Proteins are identified as the active principle of mistletoe therapy and likely to act as anti-cancer agents for curative and adjuvant remedy.

Methodology: To identify host specific variation in proteins, one-dimensional gel electrophoresis was performed to analyze differential protein expression in two different host plants of mistletoe. The study was performed by separating the total proteins by SDS PAGEand gels were developed using silver stain. Differentially expressed proteins were identified by mass spectrometry analysis. The differential expression of selected protein was further validated using Western blot analyses.

Results and Discussion: In mistletoe, eleven differentially expressed protein spots are recognized. The protein spots are identified using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF/MS-MS). Densitometry analysis revealed that out of 11 differentially expressed protein spots, 9 protein spots has been found to be up regulated in host *flacourtie indica* as compared to host *oleo dioica*. The differential expression of selected protein was further validated using Western blotting. Detailed analyses of functioning of these differentially expressed proteins would help in better understanding of their role in therapeutics.

Conclusions: This study would be extremely useful in understanding the role of host in improving efficacy of phyto-medicines in cancer therapeutics.

P75

Computational Approach on Tamoxifen Resistance Estrogen-Dependent Breast Cancer

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Introduction: Around 70% of patients who are having breast cancer are estrogen dependent or estrogen positive. Estrogen upon binding to the estrogen receptor (ER) ligand binding domain triggers a cascade mechanism for uncontrolled cancer cell growth and proliferation. Hence, tamoxifen was designed and used as an anti-estrogen compound in the treatment of breast cancer. Recently patients are showing acquired resistance towards this drug. Hence in this study we tried to understand the influence of mutations on acquired resistance of ER towards tamoxifen using insilico methods.

Methodology: To understand the acquired resistance of ER, we have chosen mutations from literature and evaluated for their effect using SIFT and Polyphen. The deleterious mutations obtained were homology modelled with crystal structure of ER using modeller9v7. The protein structures were validated in SaVES and stabilized by molecular dynamic simulation using GROMACS 4.5.5. Molecular docking was performed for the wild-type ER and mutant ER with tamoxifen to understand the resistance mechanism based on structural interaction.

Results and Discussion: The results of molecular dynamic simulation for stabilizing the protein has shown the structural variation among the wild-type and mutant ER proteins that remains the major reason for tamoxifen resistance. This was further confirmed by the docking results that enumerates the wild-type ER-tamoxifen complex showing high binding affinity comparing the mutant ER-tamoxifen complexes.

Conclusion: The current study enlightens the fact that mutations might be the major cause for acquired resistance of ER towards tamoxifen in patients who develop breast cancer. Hence we suggest that design and synthesis of new anti-estrogen analogues that can target potential biomarkers like ER and ER interacting protein will give a future on breast cancer treatment.

P76

Proteome analysis of biotic and abiotic stress in rice (*Oryza sativa L.*) reveals a cross-talking chitinase for developing multiple stress tolerance through amiRNA technology

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In natural field conditions, crop plants are exposed to more than one stress and have evolved with specific mechanisms to tolerate and survive under these stress conditions. Research to understand the molecular mechanism of stress tolerance

through omics technologies have led to the observation of many cross-talking genes and their protein products in multiple stresses. With the objective of identifying such overlapping proteins during biotic and abiotic stress, rice varieties ASD 16 and White Ponni were subjected to salt, drought, heavy metal and temperature (abiotic stress) as well as fungal and bacterial pathogen infection (biotic). Leaf proteomes were analysed by 2-dimensional electrophoresis in 3-10 IPG strips in the first dimension followed by 12% SDS-PAGE in second dimension. Several proteins that differentially expressed in individual stress compared to control plants were identified by LC-MS/MS Mass Spectrometry. MASCOT search results of the peptide sequences have shown acidic endochitinase (locus ID: Os01g64100 of Rice Annotation Project Data Base) which is expressing high during drought and fungal pathogen infection in ASD 16 rice variety. As an alternative to the transgenic approach to over-express chitinase towards developing multiple stress tolerant rice crop, through genome-wide bioinformatics analysis, miRNA (miRf12014-akr) specific to chitinase mRNA was identified as target for amiRNA technology. Artificial antisense miRNA expressed in rice cells is expected to suppress the miRNA and thus allow the chitinase mRNA to express, which is otherwise expressing based on induction under natural conditions. Transgenic plants that will be developed through this strategy is expected to have tolerance to both drought and fugal disease tolerance throughout the crop period since the cross-talking chitinase will be constitutively expressing.

P77

Mapping protein coding regions in the human genome

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Abstract

Introduction: Protein coding genes in the human genome have been annotated using a combination of transcript evidence and prediction programs that take advantage of conservation across species. However, large scale proteomics studies have now revealed several novel proteins encoded by the human genome that were missed by previous annotation pipelines. Here we have explored a novel framework for mapping potential protein coding regions in the human genome that leverages the availability of large scale transcriptomic and proteomic datasets.

Methodology: Average length of coding exons in human is ~160 bps. We scanned the human genome for conserved regions of length ≥ 100 nucleotides using in-house PERL script. Three pass searches of unassigned MS/MS spectra were carried out against following custom protein databases - translated conserved non-genic regions with transcript support, conserved non-genic region without transcript support and less conserved region with transcript support. The searches were performed using SEQUEST and MASCOT through Proteome Discoverer (Version 1.4) software suite (Thermo Scientific, Bremen, Germany).

Results and discussion: Human genome has 385,530

conserved blocks of length ≥ 100 (3% of the genome). Known protein coding genes span ~66% of these conserved blocks and the remaining 33% of the conserved blocks are intergenic. We identified novel proteins encoded by these intergenic regions that are not represented in public repositories. This suggests existence of several as yet unidentified proteins encoded by the human genome. Our studies highlight the need for revisiting parameters that are currently used for annotating protein coding regions in the genome.

Conclusions: Large scale datasets from high-throughput platforms can be utilized for more comprehensive and unbiased annotation of genomes. We propose a novel framework for systemic identification of protein coding potential of conserved as well as less conserved loci.



Rapid generation of accurate information on proteoform distribution and relative abundance by UHR-QTOF MS.

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Introduction

Mass spectrometry and bioinformatics are rapidly becoming the main tools for proteome analysis. In addition to the identification of proteins, their post-translational modifications, and their relative abundance, it is also important to obtain quantitative information on the distribution of proteoforms. This is particularly true for proteoforms that are generated by proteolytic enzymes or by post-translational modifications. These proteoforms can be analyzed by mass spectrometry, but the analysis is often time-consuming and requires significant expertise. We have developed a new methodology for rapid generation of proteoform distribution and relative abundance data using a UHR-QTOF MS.

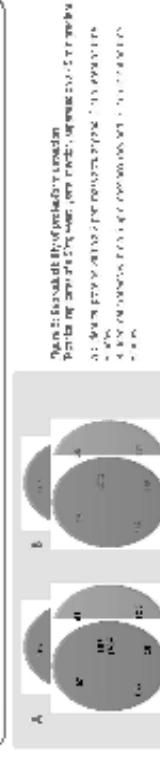
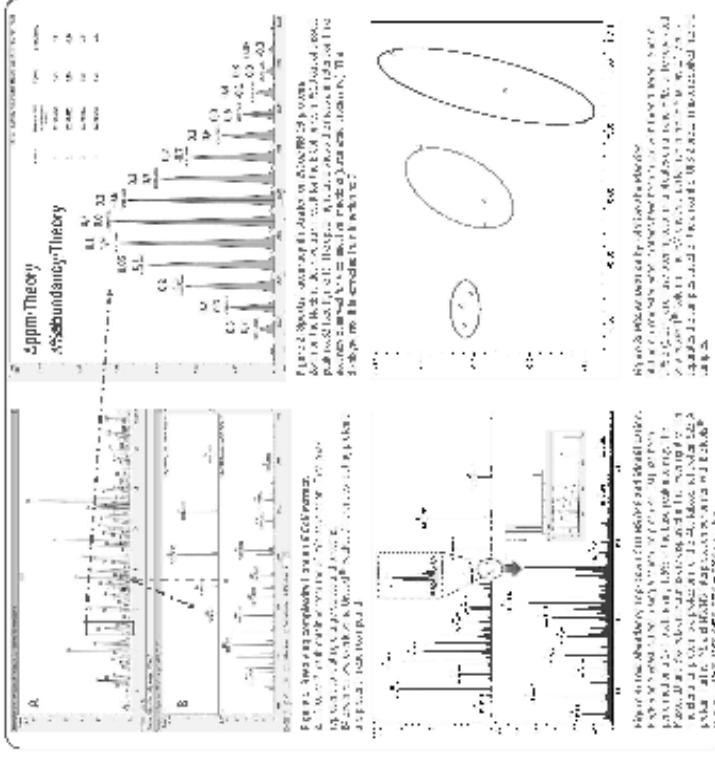
Methods

Proteoform distribution data were generated using a Bruker Daltonics Q-ToF Premier XE mass spectrometer equipped with a nano-electrospray ionization source. The samples were analyzed in triplicate. The relative abundance of each proteoform was calculated as the ratio of the peak intensity of the proteoform to the total peak intensity of all proteoforms. The distribution of proteoforms was then analyzed using a statistical method called "Supporting Abundance Theory".

The results show that the relative abundance of each proteoform can be accurately determined using this method. The distribution of proteoforms is shown in Figure 1. The distribution is highly skewed, with a few major peaks and many minor peaks. The relative abundance of each proteoform is shown in Figure 2. The distribution is highly skewed, with a few major peaks and many minor peaks. The relative abundance of each proteoform is shown in Figure 2. The distribution is highly skewed, with a few major peaks and many minor peaks.

Results

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Supportive Abundance Theory
The relative abundance of each proteoform is calculated as the ratio of the peak intensity of the proteoform to the total peak intensity of all proteoforms. The distribution of proteoforms is then analyzed using a statistical method called "Supportive Abundance Theory".
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Protein Profiling

Supportive Abundance Theory
The relative abundance of each proteoform is calculated as the ratio of the peak intensity of the proteoform to the total peak intensity of all proteoforms. The distribution of proteoforms is then analyzed using a statistical method called "Supportive Abundance Theory".
The results show that the relative abundance of each proteoform can be accurately determined using this method. The distribution of proteoforms is shown in Figure 1. The distribution is highly skewed, with a few major peaks and many minor peaks. The relative abundance of each proteoform is shown in Figure 2. The distribution is highly skewed, with a few major peaks and many minor peaks.

Identification of a novel lipid family from leukemia cell line

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Introduction: Lipids are recognized vital for immune system, nutrition and signal transduction. Lipidomic study became important to identify aberrant lipid signatures in disease conditions. Latest advancements in mass spectrometry and related softwares facilitated lipidomic research in the identification of novel lipids and in the better understanding of metabolism.

Methodology: THP-1 cells were extracted with a water, methanol and chloroform (1:1:2 v/v). The lower organic layer has been carefully taken out and the aqueous layer was re-extracted with 1- butanol. The extracts from organic and aqueous layers were evaporated to dryness and were loaded

onto Bond Elut aminopropyl cartridges and eluted with suitable combination of solvents to elute specific lipid classes. Ten different fractions were collected each containing a different lipid class. The fractions obtained were analyzed on an Eclipse C18 column connected to iFunnel Q-ToF instrument.

Results & Discussion: Among 10 fractions, Phosphatidic acids were eluted from the SPE cartridge with acidic methanol. Negative mode LC-MS/MS of phosphatidic acids showed an ion at m/z 97 as a characteristic feature. But in the same fraction, some lipids gave ion m/z 111 instead of 97 in the CID spectrum hinting a novel or modified head group. Positive mode MS/MS analysis of these compounds showed a base peak resulting from a loss of 112 Da from the precursor ion. Phospholipids show characteristics ions at m/z 173 and 155 in positive mode MS/MS spectra. Instead, the analytes of interest showed ions 187 and 169 in their CID spectra. Since these fragment ions represent head group of the lipid it can be confirmed that the lipids of interest have novel or modified head group.

Conclusion: A shift of 14 Da in the fragment ion mass indicated the presence of a methylated phosphate group. Thus a new lipid family containing modification in the phosphate head has been identified.

High-Throughput Screening MALDI-TOF Assays beyond 100,000 Samples per Day



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Introduction

Automated protein sequencing by mass spectrometry (MS) has become one of the most common methods for protein characterization. In addition to protein sequencing, automated peptide mapping and differential proteomics are also becoming increasingly popular. These techniques are based on the principle of separating proteins into individual peptides and then identifying them by mass spectrometry. The major advantage of this technique is that it can be used to analyze complex mixtures of proteins, such as those found in whole cells or tissues. This allows for the simultaneous analysis of many different proteins, which makes it a powerful tool for studying biological systems.

Methods

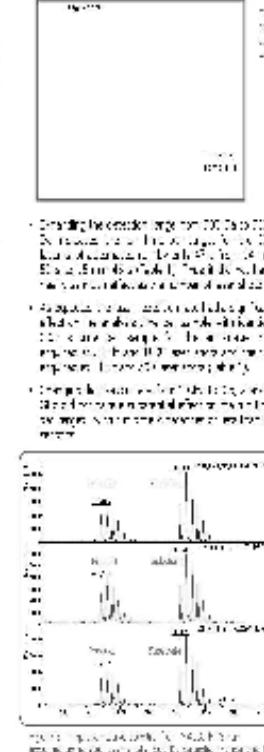
The workflow for high-throughput screening of peptides is as follows: 1) Protein extraction and fractionation: Cells are lysed and the resulting mixture is centrifuged to remove cellular debris. The supernatant is then loaded onto a column for protein separation. 2) Digestion: Proteins are digested with trypsin or chymotrypsin to release individual peptides. 3) Sample preparation: Peptides are extracted from the digest and purified using solid-phase extraction (SPE) cartridges. 4) MALDI-TOF analysis: The purified peptides are analyzed using a Bruker MALDI-TOF mass spectrometer. The instrument uses a pulsed nitrogen laser to ionize the peptides and a reflectron to focus the ions onto the detector. The mass spectrum is recorded and analyzed using Bruker's DataAnalysis software.

Results

• Protein extraction and fractionation: A total of 400 samples were analyzed using this method. The results showed that the method is reliable and reproducible across all samples. • Digestion: A total of 400 samples were digested using this method. The results showed that the method is reliable and reproducible across all samples. • Sample preparation: A total of 400 samples were prepared using this method. The results showed that the method is reliable and reproducible across all samples. • MALDI-TOF analysis: A total of 400 samples were analyzed using this method. The results showed that the method is reliable and reproducible across all samples.

Table 1: High-throughput screening of peptides using MALDI-TOF mass spectrometry.

	Total	Uniq.	Mean	Std Dev.	Median	Range	Mean	Std Dev.
1	400	300	200	100	180	100-300	180	100
2	400	300	200	100	180	100-300	180	100
3	400	300	200	100	180	100-300	180	100
4	400	300	200	100	180	100-300	180	100
5	400	300	200	100	180	100-300	180	100
6	400	300	200	100	180	100-300	180	100
7	400	300	200	100	180	100-300	180	100
8	400	300	200	100	180	100-300	180	100
9	400	300	200	100	180	100-300	180	100
10	400	300	200	100	180	100-300	180	100
11	400	300	200	100	180	100-300	180	100
12	400	300	200	100	180	100-300	180	100
13	400	300	200	100	180	100-300	180	100
14	400	300	200	100	180	100-300	180	100
15	400	300	200	100	180	100-300	180	100
16	400	300	200	100	180	100-300	180	100
17	400	300	200	100	180	100-300	180	100
18	400	300	200	100	180	100-300	180	100
19	400	300	200	100	180	100-300	180	100
20	400	300	200	100	180	100-300	180	100
21	400	300	200	100	180	100-300	180	100
22	400	300	200	100	180	100-300	180	100
23	400	300	200	100	180	100-300	180	100
24	400	300	200	100	180	100-300	180	100
25	400	300	200	100	180	100-300	180	100
26	400	300	200	100	180	100-300	180	100
27	400	300	200	100	180	100-300	180	100
28	400	300	200	100	180	100-300	180	100
29	400	300	200	100	180	100-300	180	100
30	400	300	200	100	180	100-300	180	100
31	400	300	200	100	180	100-300	180	100
32	400	300	200	100	180	100-300	180	100
33	400	300	200	100	180	100-300	180	100
34	400	300	200	100	180	100-300	180	100
35	400	300	200	100	180	100-300	180	100
36	400	300	200	100	180	100-300	180	100
37	400	300	200	100	180	100-300	180	100
38	400	300	200	100	180	100-300	180	100
39	400	300	200	100	180	100-300	180	100
40	400	300	200	100	180	100-300	180	100
41	400	300	200	100	180	100-300	180	100
42	400	300	200	100	180	100-300	180	100
43	400	300	200	100	180	100-300	180	100
44	400	300	200	100	180	100-300	180	100
45	400	300	200	100	180	100-300	180	100
46	400	300	200	100	180	100-300	180	100
47	400	300	200	100	180	100-300	180	100
48	400	300	200	100	180	100-300	180	100
49	400	300	200	100	180	100-300	180	100
50	400	300	200	100	180	100-300	180	100
51	400	300	200	100	180	100-300	180	100
52	400	300	200	100	180	100-300	180	100
53	400	300	200	100	180	100-300	180	100
54	400	300	200	100	180	100-300	180	100
55	400	300	200	100	180	100-300	180	100
56	400	300	200	100	180	100-300	180	100
57	400	300	200	100	180	100-300	180	100
58	400	300	200	100	180	100-300	180	100
59	400	300	200	100	180	100-300	180	100
60	400	300	200	100	180	100-300	180	100
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63	400	300	200	100	180	100-300	180	100
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65	400	300	200	100	180	100-300	180	100
66	400	300	200	100	180	100-300	180	100
67	400	300	200	100	180	100-300	180	100
68	400	300	200	100	180	100-300	180	100
69	400	300	200	100	180	100-300	180	100
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75	400	300	200	100	180	100-300	180	100
76	400	300	200	100	180	100-300	180	100
77	400	300	200	100	180	100-300	180	100
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79	400	300	200	100	180	100-300	180	100
80	400	300	200	100	180	100-300	180	100
81	400	300	200	100	180	100-300	180	100
82	400	300	200	100	180	100-300	180	100
83	400	300	200	100	180	100-300	180	100
84	400	300	200	100	180	100-300	180	100
85	400	300	200	100	180	100-300	180	100
86	400	300	200	100	180	100-300	180	100
87	400	300	200	100	180	100-300	180	100
88	400	300	200	100	180	100-300	180	100
89	400	300	200	100	180	100-300	180	100
90	400	300	200	100	180	100-300	180	100
91	400	300	200	100	180	100-300	180	100
92	400	300	200	100	180	100-300	180	100
93	400	300	200	100	180	100-300	180	100
94	400	300	200	100	180	100-300	180	100
95	400	300	200	100	180	100-300	180	100
96	400	300	200	100	180	100-300	180	100
97	400	300	200	100	180	100-300	180	100
98	400	300	200	100	180	100-300	180	100
99	400	300	200	100	180	100-300	180	100
100	400	300	200	100	180	100-300	180	100



Conclusions

• MALDI-TOF mass spectrometry is capable of processing up to 100,000 samples per day. • MALDI-TOF mass spectrometry is able to identify unique peptides in a timely manner. • MALDI-TOF mass spectrometry is a reliable and accurate method for high-throughput screening of peptides.

MALDI-HTS

P80

Metabolomics of vitreous humour from retinoblastoma patients

Seetaramanjaneyulu Gundimeda^{1*}, Syed Salman Lateef¹, Nilanjan Guha¹, Deepak SA¹, Arunkumar Padmanaban¹, Ashwin Mallipatna², Arkasubhra Ghosh².

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Introduction: Retinoblastoma (Rb) is the most common malignant tumor of the eye in children. Inactivation of both copies of the RB1 gene in a child's retina is known to be the cause of cancer. Gene expression studies on patients' cancer tissue samples revealed several pathways e.g. cytokine-cytokine receptor interaction, oxidative phosphorylation, phototransduction pathways to be significantly perturbed in patients compared to controls. In this study, metabolomics of vitreous humor samples were performed to discover differential metabolites in Rb patients that can provide a direct or indirect link to the pathways found in cancerous tissue.

Methodology: The samples were extracted using methanol: ethanol (1:1 v/v). 9 patient and 2 controls samples were used. The extracted samples were subjected to LC/QTOF-MS and GC/QTOF-MS analysis. For LC-QTOF analysis, C18 and HILIC columns were used. Molecular features were searched against METLIN database and confirmed by METLIN library. For GC-QTOF analysis, data was acquired using EI source on a DB-5ms column. The results were searched against Fiehn RTL library. The metabolomics and gene microarray results were combined and analyzed using pathway centric approach.

Results and Discussion: Wide variety of compounds ranging from amino acids, carbohydrates, nucleobases and lipids were identified from GC/MS and LC/MS. Among lipids, Phosphatidyl cholines (PC), ether linked phosphatidyl ethanolamines (PE), ceramides, sphingomyelins and sphinganines were identified. Lipids, especially PCs and ether linked PEs were found to be up regulated in patient samples. Few ether lipids found to be 5 folds more in disease. Carnitines and free fatty acids were also up regulated in patient samples. As the biosynthesis of ether lipids starts in peroxisomes, this study suggests an altered peroxisomal metabolism in these patients. Among the other metabolites, squalene and cholastane triol reflecting cholesterol metabolism, were also dysregulated.

Conclusions: Fluids surrounding cancer tissues were used to understand the pathway changes in Rb and further extended to find biomarkers

P81

High Resolution Mass Spectrometry for the Comprehensive Characterization of Low Molecular Weight Heparins

Annu Uppal, Dipankar Malakar, Faraz Rashid and

Manoj Pillai

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Low molecular weight heparin (LMWH) is class of antithrombotic drug known for their increased bioavailability and pharmacodynamics. The comprehensive characterization of LMWHs is important from the drug quality and safety aspects because of high risk of introduction of structurally related impurities due to the incomplete reactions or side reactions.

Liquid Chromatography Mass spectrometric methods are the preferred methods for LMWH compositional analysis but are always challenged due to the presence of hundreds of peaks of broad molecular weights along with large number of structural isomers at each chain length having similar retention times. In addition, lack of suitable bioinformatics tools for fast and efficient spectral interpretation and detailed structure information on individual chains, makes it further challenging.

The high quality TOFMS data with TripleTOF® in combination with chromatographic resolution resulted in the identification of enoxaparin oligosaccharides ranging from 2 saccharide units to 20 saccharide units, with or without acetylation, variable sulfation degrees and high mass accuracy of less than 20 ppm. Data processing involved the automated LCMS reconstruction tool and then matching the experimental MWs to the theoretical MWs derived from the database containing all possible LMWH structures. In addition to the oligosaccharides having the defined enoxaparin structures, some minor components such as odd number oligosaccharides and saturated enoxaparin, were also detected. High performance fast scanning TripleTOF® system with high resolution chromatography provides in depth characterization of the oligosaccharide composition of LMWH.

P82

Improved peptide identification by ProteinPilotTM v5.0 software using chimeric spectra detection

Dipankar Malakar, Faraz Rashid, and Manoj Pillai
SCIEX, 121 Udyog Vihar, Phase IV, Gurgaon, Haryana, India

Abstract:

A complicating factor for protein identification within complex mixtures by LC-MS/MS is the problem of "chimera" spectra, where two or more precursor ions with similar mass and retention time are co-sequenced by MS/MS. In the present study hela cell lysate showed 14.5% more protein and 19.7% more peptide identification when analysed using 60 minute gradient by using multi-precursor search option in ProteinPilotTM 5.0. The trend was more prominent when chromatographic gradient was narrowed down to 40 minute. Similar study conducted on proteome of cytoplasmic fraction of sertoli cells in rat. It identifies 13.6% and 11.2% more proteins run at 40 min and 60 min gradient respectively when multi-precursor search option was turned on in ProteinPilotTM 5.0. Although in plasma sample multi-precursor search option showed relatively less significant effect in terms of proteins/peptides identification. Hence multi-precursor search option in ProteinPilotTM 5.0 significantly increases protein identification in complex sample.

Index for Oral Presentation

Lecture No	Speaker	Abstract
PL1	Prof. Giulio Superti-Furga	Global analysis of genes and proteins that convey the action of drugs
PL2	Prof. Peter Nilsson	Human Protein Atlas enabled neuroproteomic profiling of body fluids
IL 1	Dr. Richard Kumaran Kandasamy	Proteomic approaches to study the dynamics of antiviral innate immune signaling
PL 3	Prof. Akhilesh Pandey	Quantitative proteomics to study oncogenic signalling pathways
PL 4	Dr. Sudhir Srivastava	Proteomics and Precision Health: A Phenotypic Approach to Precision Cancer Detection
L 1	Dr. Amit Kumar Yadav	Hyperplexing enables study of strain-specific temporal dynamics of host response to mycobacterial infection
YSL1	Ms. Suruchi Aggarwal	Emergent properties of post-translational modifications in the human disease proteome
PL 5	Prof. Alois Jungbauer	Proteomics analysis of recombinant antibodies purified by affinity chromatography or precipitation, are they different?
IL2	Dr. Andreas Huhmer	Protein Quantitation- Which LCMS method is right for my experiment?
IL3	Dr. Wilfrid Boireau	On-chip analysis and proteomics studies of blood microparticles with label free detection techniques
IL4	Dr. Suman S. Thakur	Importance of chromatography in quantitative proteomics and drug discovery
IL5	Dr. Kalpana Bhargava	A proteomic analysis of endurance augmenting activities of Cerium oxide nanoparticles
IL6	Dr. Mark McDowall	A Comparison of Peptide Quantification Using a Novel Integrated Microfluidics Device and a Nanoscale UPLC with MRM Detection
YSL2	Ms. Choudhury Manisha	Proteomic characterization of two elapid snake venoms: Indian cobra and common krait
IL7	Dr. Kali Kishore Reddy	Conjoint IMAC device (disk to microfluidics): a pre-fractionation approach for plasma proteomics
PL 6	Prof. Tony Futerman	So many sphingolipids — what do they all do?
PL 7	Prof. Robert Plumb	Understanding Human Health and Disease With LC/MS Based Metabolic Phenotyping
IL8	Prof. Abhijit Mitra	CCPM v3.4: Towards Collaborative Metabolomics
IL9	Dr. Jim Thorn	Proteomics and Metabolomics Analysis by CESI-MS: Application to Biologics Characterisation, Bio similarity Assessment and Biomarker Discovery
IL10	Dr. Mahesh J Kulkarni	Proteomic analysis of circulating immune complexes in diabetes: Targeted analysis of glycated albumin using fragment ion library
IL11	Dr. Jaran Jainhuknan	Glycoproteins Analysis – Challenges and Solutions
IL12	Prof. Niranjan Chakraborty	Nuclear envelope resident, CaSUN1, participates in dehydration signaling by modulating unfolded protein response

L 2	Dr. Sharmila Chattopadhyay	Proteomic analysis of <i>Podophyllum hexandrum</i>
L 3	Prof. Karutha Pandian Shunmugiah	Undersanding the Microbial Responces to Quorum Sensing and Biofilm Inhibitors –A Proteomic Approach
L 4	Prof. Krishnaswamy Balamurugan	Proteomic analysis in <i>Caenorhabditis elegans</i> to understand bacterial infections
PL 8	Prof. Mark S. Baker	TranslationalTissue and Plasma Biosignatures of Colorectal Cancer
IL13	Dr. Shannon Cornett	High-throughput Spatial Proteomics of Tissue using rapifleX MALDI Tissue types
IL14	Dr. Rakesh K Mishra	Proteome of Nuclear Architecture
L 5	Dr. Sagarika Biswas	Phosphoproteomic profiling for the identification of novel marker for coronary artery disease
IL15	Dr. Amol R Suryawanshi	Proteomics approach to identify host factors involved in Chikungunya virus infection
IL16	Dr. Arun Bandyopadhyay	Proteomic analysis of human plasma in Rheumatic heart disease
IL17	Dr. Chaitanya Saxena	Applications and Advances in Chemical-Proteomics for Drug Target Identification
IL18	Dr. Vijaya Pattabiraman.	Chemical ligation methods for preparation of homogeneous protein biomolecules
IL19	Dr. Jaran Jainhuknan	Biosimilars Characterization & Quantitation
PL9	Prof. Ales Podgornik	Chromatographic monoliths in PAT
PL 10	Dr. Matjaz Peterka	Analytics and PAT in Virus Particle Manufacturing
IL20	Dr. Anette Persson	Novel Comparability Approach and Innovative Quality Control
L 6	Dr. Sharmistha Dey	Evaluation of expression levels of a signaling protein, p38 β in pancreatic cancer and design of peptide inhibitors against the same
IL21	Dr. Vivek Halan	High Throughput Screening of therapeutic proteins in early stage development

Index for Poster Presentation

P1	Ms. Renu verma	Quantitative proteomic and phosphoproteomic analysis of H37Ra and H37Rv strains of Mycobacterium tuberculosis
P2	Mr. Kumar K.M.	Protein Quantitation Using Data Independent Acquisition (DIA) on Orbitrap Fusion Platform
P3	Ms. Soujanya Yelamanchi	Unraveling the proteome of human pineal glands
P4	Mr. Natrajan Pradeep	E-pharmacophore mapping, molecular docking and dynamics simulations to propose potential inhibitors of CDK5
P5	Ms. Shakuntala Bai	Identification of Methylglyoxal Responsive proteins in L6 Rat Muscle Cells
P6	Mr. Ashish Khaparde	Development of immobilized metal-ion (IMA) monolith multiplexed microfluidic device for plasma proteomics using LC-MS.
P7	Mr. Arun Govind G	Pre-fractionation and mass spectrometric characterization of HDL-APO A1 from human plasma
P8	G. Krishna Priya & George A	Green Fluorescent Protein: Eco-friendly dye for leather
P9	Ms. Jayshree Advani	Proteogenomic analysis of H37Ra strain of Mycobacterium tuberculosis using high resolution mass spectrometry
P10	Ms. K. Vanitha Shyamili	In-silico Analysis of Ubiquitin - Protein Interactions in Human
P11	Mr. Krishna pate	Pepdize: web-based comparative in-silico protein digestion tool
P12	Mr. Manish Kumar	Human Brain Proteome: molecular insights into regional heterogeneity and neurological disorders
P13	Ms. Praseeda Mol	Proteomic analysis of milk from early, mid and late lactation stage of Malnad Gidda breeds
P14	Mr. Vinuth N. Puttamarlesh	Rapid processing of biological samples for proteomic analysis using Pressure - cycling
P15	Dr. Ravikiran T	Differential expression and oxidative modification of proteins in the cerebral cortex of physically trained old rats.
P16	Ms. Sneha M. Pinto	A proteome map of the human eye
P17	Ms. Sougrakpam Yaiphabi Chanu	Cuticle proteome analysis of Brassica juncea leaves show presence of cuticle synthesis and stress signaling proteins.
P18	Ms. Shalini Jaswal	Shotgun Proteomics of Saliva in Indigenous Sahiwal Cow Reveals More Than 1400 Proteins
P19	Dr. Naveena BM	A Proteomic Based Approach to Differentiate Sheep Meat with Buffalo Meat
P20	Mr. Kiran Kumar Datta	Proteomic profiling to identify chewing tobacco-induced signalling in esophageal cells
P21	Shreya Ghosh	Understanding the mechanism behind alleviation of cysteine induced toxicity using <i>Saccharomyces cerevisiae</i> as a model system
P22	Ms. Reema Mohan Banarjee	PROTEOMICS OF ENDOTHELIAL DYSFUNCTION
P23	Swathi Varshney	Effect of vitamin B12 restriction on Wistar rats: A Global proteomic profiling
P24	Mr. Jagadeesha Prasad M.G.	AGE modified hemoglobin –A better marker for monitoring long-term glycemic status in diabetes.
P25	Ms. Boomathi Pandeswari P.	Attempts to understand the role of arginine in influencing electrospray ionization collision induced dissociation characteristics: A middle-down proteomic investigation
P26	Ms. Manu Kandpal	A pipeline for large scale mapping of post translational modifications from shotgun proteomics data

P27	Mr. Aafaque Khan	Cigarette smoke induced molecular alterations in esophageal cells
P28	Mr. Deepak Rawat	Proteomic Analysis of Halophilic Bacterium Efficient in Degrading Acid Orange 7 Dye
P29	Mr. B. Balasubramaniam	Emphasis of Phosphorylated Regulatory protein during <i>Shigella</i> spp. infection in model organism <i>Caenorhabditis elegans</i>
P30	Mr. Manoj kumar Gupta	Deregulated “miRNA and Target” cascades in GBM and their implications in transcriptomic and proteomic analysis
P31	Mr. Dehin Bhagat	In silico analysis of amino acid sequences of bacterial (D/L) hydantoinase and allantoinase for their substrate specificity and physicochemical characterization
P32	Ms. Pushpanjali Dasauni	Identification and Characterization of Hemoglobins Variants by Novel Methods for Simpler Diagnosis of Hemoglobinopathies
P33	Mr. Nitin Kishor	Mass spectrometric n-terminal sequencing of peptides using a bacterial aminopeptidase
P34	Mr. Shariq Qayyum	Proteomic analysis of enterococcal biofilms under quercetin stress
P35	Dr. R. Shyama Prasad Rao	Evolutionary dynamics of N-glycosylation sites in hemorrhagic fever viral envelope proteins
P36	Mr. Tushar H More	Investigation of phospholipid alterations in breast cancer using mass
P37	Dr. Kiran Ambatipudi	Mass-spectrometry based characterization of the composition of Murrah buffalo milk lipids
P38	Dr. K.K. Asha	Effect of fish oil supplementation on Acyl CoA Cholesterol Acyl Transferase expression in liver of experimental rats
P39	Ms. B.Keerthika	Extraction and Characterization of Steviol Glycosides from Stevia
P40	Dr. Ayesh Noor	Profiling of flavonoid-subclasses in <i>Myristica fragrans</i> and <i>Cordyline terminalis</i> by LC-ESI-MS
P41	Mr. Yeshwanth Subbannayya	Expression dynamics of protein kinases and phosphatases across human tissues
P42	Ms. Priya sivadasan	Chromosomal alterations, differentially expressed gene clusters and potential biomarkers in Oral Cancer: Insights from Proteomics
P43	Mr. Benvil Rodrigues	Quantitative proteomic analysis of cerebrospinal fluid from paralytic rabies and Guillain–Barré syndrome
P44	Rajubhai K Dhabi	Identification of subtype specific serum potential protein biomarkers in breast cancer using complementary gel-based and gel-free quantitative proteomic approaches
P45	Mr. Gourav Dey	Repertoire of differentially expressed proteins in cerebrospinal fluid of rabies survivor and non-survivor individuals
P46	Arunachal Chatterjee	Identification of novel markers of Dilated, Hypertrophic and Restrictive cardiomyopathy using Plasma Proteomics approach
P47	Ms. Pooja Kumari & Ms. Ankita Sharma	Comparative protein profiling of diseased tissue in rheumatoid arthritis
P48	Mr. Chanukuppa Venkatesh	Quantitative proteomic analysis towards new targets and biomarkers for multiple myeloma.
P49	Mr. S. Marudhu Pandiyan	Proteomic changes in <i>Caenorhabditis elegans</i> exposed to <i>Shigella flexneri</i> revealed modulation of Reproductive System proteins.
P50	Mr. Vipin Kumar	Proteomic Analysis of <i>Plasmodium falciparum</i> Patients from Different Endemic Regions of India to Understand Malaria Pathogenesis and Identify Surrogate Markers of Severity.
P51	Dr. Ashok Kumar Mohanty	Proteomics in biomarker discovery for early detection of pregnancy in Bovine: Current status and future direction
P52	Ms. Oishi Chatterjee	Quantitative proteomics of cerebrospinal fluid from Tuberculosis meningitis co-infected with HIV

P53	Ms. Lathika Gopalakrishnan	Proteomic analysis of CVS-11 virus infected and uninfected Neuro N2a cells and BHK-21 cell line
P54	Mr. Manjunath. D	Proteomic profiling of brain regions reveals complex biological basis for schizophrenia
P55	Ms. Tejaswini Subbannayya	Phosphoproteomic analysis of gallbladder cancer
P56	Mr. Apeksha sahu	Identification of host response in cerebral malaria brain proteome using quantitative proteomic analysis
P57	Ms. Pavithra Rajagopalan	Chewing tobacco and cigarette smoke-induced molecular alterations in oral cancer
P58	Ms. V. Keerthana, Ms. Esther Jebarani, Ms. Pavithra Sellaperumal	Serum-based Biomarker Discovery for Human Fatty Liver disease
P59	Mr. Peeyush Prasad	Identification of differential host response to infections in cryptococcal meningitis, toxoplasma encephalitis and tuberculous meningitis coinfecting with HIV
P60	Mr. Ankit P. Jain	SILAC-based proteomic analysis to delineate mechanisms underlying erlotinib resistance in head and neck squamous cell carcinoma
P61	Mr. Lokeshwaran	Oxidized HDL-ApoA1 as a biomarker of human cardiovascular risks and development of novel diagnostics for high throughput analysis
P62	Ms. Sudeshna Banerjee	Identification of biomarkers of hepatotoxicity and chronic liver disease by plasma proteomic analysis of arsenic-exposed carp
P63	Ms. Mythrei Narasimhan	Preliminary studies to identify potential therapeutic targets in the blast crisis stage of chronic myeloid leukemia using proteomics approach
P64	Mr. Bijaya nanda Panigrahi	Identification of differentially expressed proteins involved in Nasopharyngeal carcinoma using High throughput proteomics approach
P65	Mr. Munna Lal Yadav	Label Free Approaches for Identification of Biomarkers for Early Detection of Pregnancy in Cow Urine
P66	Mr. Dilawar Ahmed Mir	Understanding the response of <i>Caenorhabditis elegans</i> against whole bacterial Proteome of <i>Salmonella Typhi</i>
P67	Ms. Rashmi K Godbole	Proteomic analysis of 4-Hydroxy-2-Nonenal (4 HNE) induced insulin resistance in insulin sensitive cell lines.
P68	Ms. Rupsi Kharb	Plasma proteomic profiling of high fat diet induced wistar rats treated with Curcumin and Dracaena cinnabari for anti-hyperlipidemic activity.
P69	Mr. Nimesh Kumar Sutariya & Mr. Phanikumar Nasika	Biophysical characterization methods for biotherapeutic development
P70	Ms. Patil Gowri Vijay	Mass Spec Analysis of Glycation induced amyloid beta aggregation
P71	Dr. Savithri Bhat	ADME modelling of Ginsenosides and characterisation of Ginsenosides Rg1 phytosome: An approach towards discovery of potential drug lead for SOD1 and TARDBP targets of ALS
P72	Mr. Ashwinkumar Subramenium .G	Differential proteomics of <i>Streptococcus pyogenes</i> upon treatment with 3-furancarboxaldehyde – An anti-biofilm agent
P73	Mr. S. Sethupathy	Proteomic responses of <i>Pseudomonas aeruginosa</i> PAO1 to quorum sensing inhibitor curcumin
P74	Mr. Saurabh Sharma	Host Specific Variation in Proteins of Semi Parasitic Plant (Mistletoe) with Anti-Cancer Potentials
P75	Divya G	Computational Approach on Tamoxifen Resistance Estrogen-Dependent Breast Cancer
P76	Ms. B. Sruthilakshmi	Proteome analysis of biotic and abiotic stress in rice (<i>Oryza sativa</i> L.) reveals a cross-talking chitinase for developing multiple stress tolerance through amiRNA technology
P77	Mr. Anubhav Kaphale	Mapping protein coding regions in the human genome

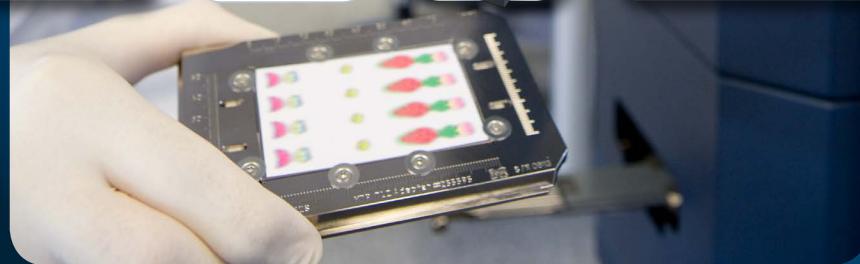
P78	Pierre-Olivier Schmit	Rapid generation of accurate information on proteoform distribution and relative abundance by UHR-QTOF MS.
P79	Sergei Dikler	High-Throughput Screening MALDI-TOF Assays beyond 100,000 Samples per Day
P80	Dr. Seetaramanjaneyalu Gundimeda	Identification of a novel lipid family from leukemia cell line
P81	Dr. Annu Uppal	High Resolution Mass Spectrometry for the Comprehensive Characterization of Low Molecular Weight Heparins
P82	Dipankar Malakar	Improved peptide identification by Protein PilotTM v5.0 software using chimeric spectra detection

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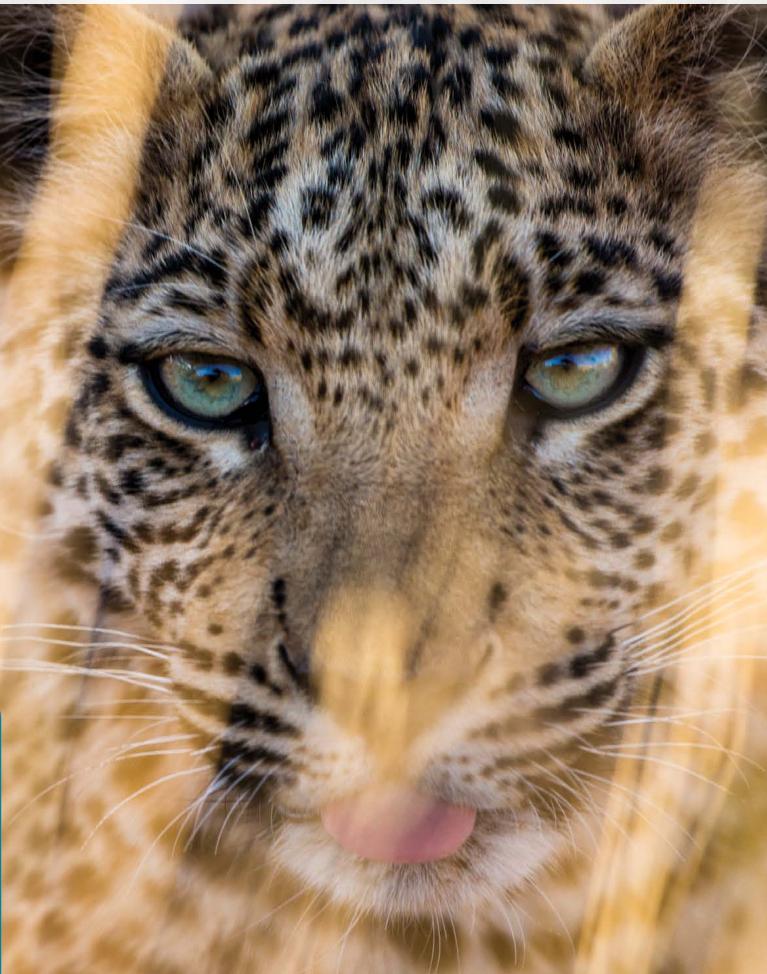
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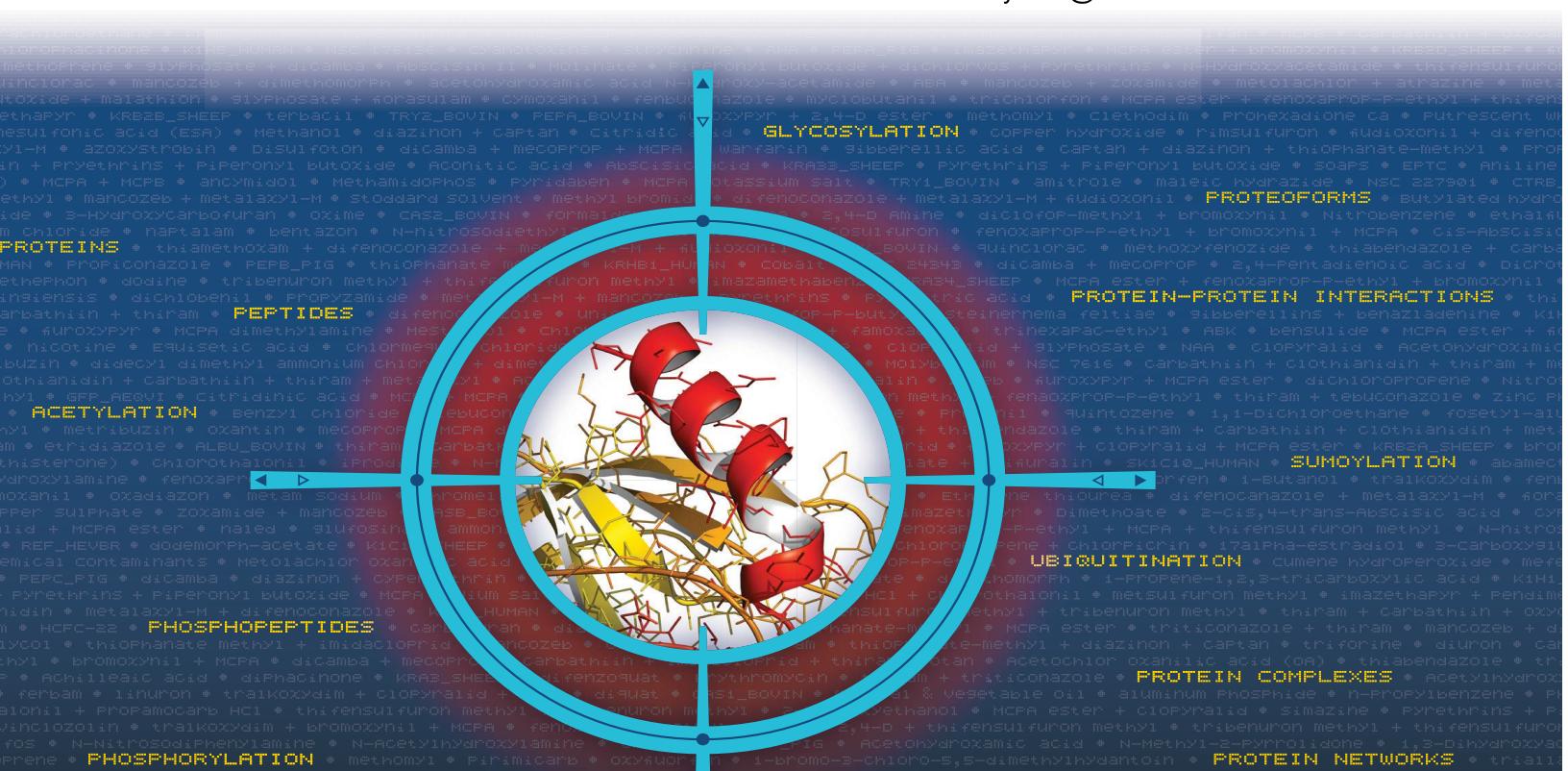
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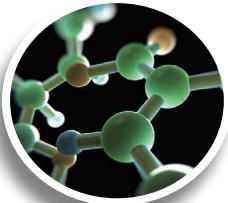


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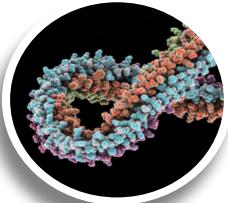
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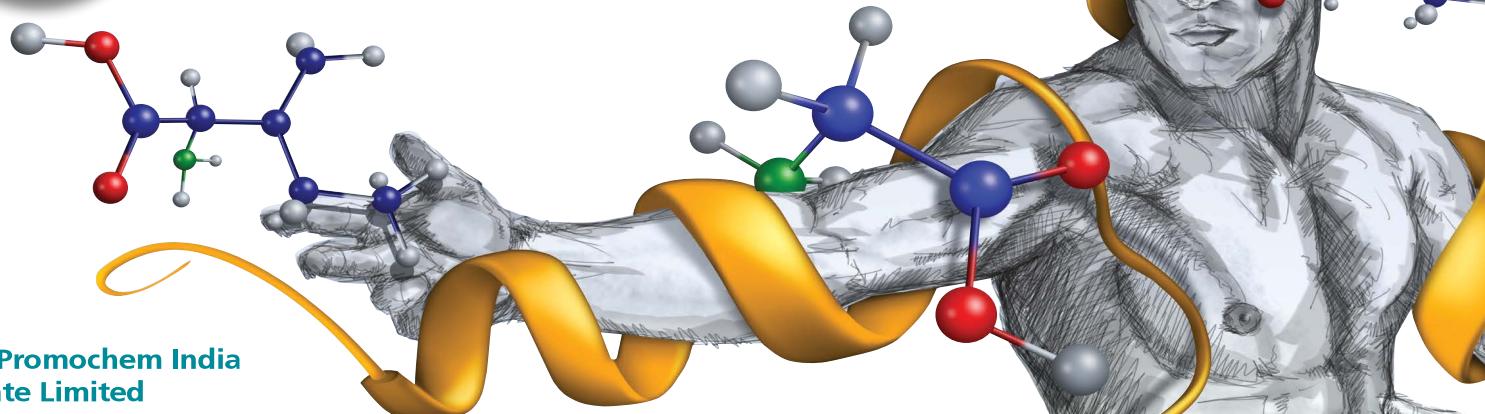
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