

Volume 7

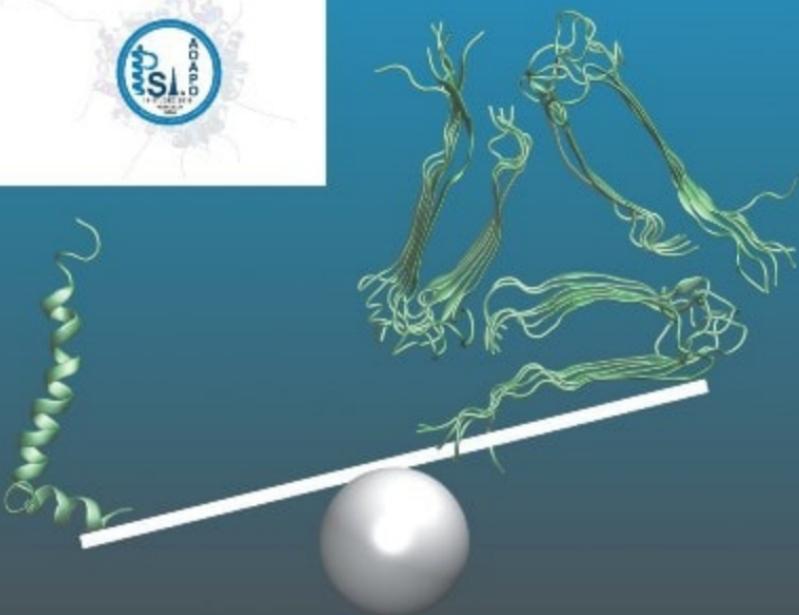
Number 4

2016

*Journal of*  
**PROTEINS AND PROTEOMICS**

Special Issue on

**ADVANCES IN STRUCTURAL, FUNCTIONAL AND INTERACTION PROTEOMICS IN FOOD AND HEALTH**



A Journal of the  
**PROTEOMICS SOCIETY, INDIA**

**ISP** International Science Press

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**Cover page figure :** 'Origin of Aggregation. A lot of proteins are amyloidogenic but generally does not aggregate. When a disordered or a structured protein shall aggregate, is governed by various intrinsic and extrinsic factors such as pH, hydrophobicity etc. The cover here shows the balance between amyloid beta peptide and its aggregate. When such a balance tips in favour of aggregate is determined by various factor analyzed in an article on page 259'



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## **GENERAL INFORMATION, GUIDELINES AND POLICIES**

**The Journal:** Journal of Proteins and Proteomics (JPP), administered by Proteomics Society, India (PSI), is a peer reviewed international journal envisaged to serve the world wide community of researchers and teachers dealing with the challenges of proteins and proteomics research resulting in an improved understanding of protein science in general. Published quarterly, the aim is also to supplement the regular issues with special issues annually in selected, relevant topics of protein science. The journal has an online presence at <http://www.jpp.org.in>. The journal publishes wide array of articles at no cost, whatsoever, to authors and provides free access to all articles through its website. Hard copies of the journal are available at nominal subscription charges.

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**Special Issue:** "Advances in Structural, Functional and Interaction Proteomics in Food and Health"*Guest Editors*

Dr. Renu Deswal, Delhi University  
Dr. Sanjeeva Srivastava, IIT Bombay  
Dr. Subhra Chakraborty, NIPGR

**8<sup>th</sup> Annual meeting of Proteomics Society, India (PSI)**  
**3<sup>rd</sup> Meeting of Asia Oceania Agricultural Proteomic Organization (AOAPO)**  
**International Conference on "Functional & Interaction Proteomics:**  
**Application in Food & Health"**  
**New Delhi, India**

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## WELCOME TO THE SPECIAL ISSUE FROM PROTEOMICS SOCIETY, INDIA (TWENTY SECOND ISSUE OVERALL)

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It is a pleasure to bring to you a special issue of Journal of Proteins and Proteomics (JPP), the official journal of the Proteomics Society, India (PSI). This special issue is being released during the international conference on "Functional & Interaction Proteomics: Application in Food and Health", in New Delhi, December 14-17, 2016 to coincide with the 8<sup>th</sup> Annual Meeting of the Proteomics Society, India and 3<sup>rd</sup> Meeting of Asia Oceania Agricultural Proteomic Organization (AOAPO) This **special issue** on "**Advances in Structural, Functional and Interaction Proteomics in Food and Health**" is indeed special in that, unlike the previous two special issues by the society, this issue is comprehensive with reviews and research articles in addition to the society's annual meeting abstracts, programme schedule, summary of conference related events, perspectives on education day (a flagship event of the society) and an interview based special article. This issue has been guest edited by Dr. Subhra Chakraborty (NIPGR, New Delhi), Prof. Renu Deswal (University of Delhi) and Dr. Sanjeeva Srivastava (IITB, Mumbai).

The scientific extravaganza, painstakingly convened by Dr. Subhra Chakraborty, will be an enriching experience for scientists across the globe. In addition to the conference, several other pre- and post-conference events will indeed be an academic exercise of the highest order. PSI led "Education Day" will initiate beginners in the art of proteomics, while several thoughtfully planned workshops will help researchers with advanced knowledge in the sub-areas of proteomics.

JPP, since its inception in 2010, has witnessed an upward trajectory in proteomics related research and activities in India. This special issue is a testimony to the expanding research activities in the area of proteomics and metabolomics across the country. An unprecedented large number of abstracts have been received for publication in the journal, which are included in the first section (conference proceedings) of this issue. The meeting will see several tens of posters being presented. For the article section, JPP has also witnessed large number of entries and not all of them could be included. The theme of the conference and the special issue emphasizing the use of proteomics research in food and health, especially resonates with the nutritional and therapeutic requirement of the earth, and the research articles are evidence that we are taking the right step in this direction. I would orient you to the editorial by Dr. Sanjeeva Srivastava for a snapshot of the contents of the research article section of this special issue, with one review and seven research articles. What gives me pleasure is the large number of articles that the journal is seeing now in the area of plant and agricultural proteomics, which were rarity in the recent past.

Two additional features also brighten this issue. One is the perspective on education day penned by Dr. Ravi Sirdeshmukh and Dr. Sanjeeva Srivastava. This brief article reflects the activities of education day and aptly echoes its purpose – that of educating the scientific community in the art of proteomics research. The journal will help carry the message across the globe motivating others to organize similar activity that is warranted of an active society. The other highlight of the issue is an article put together by Dr. Sanjeeva Srivastava and his team based on interviews of prominent proteomics researchers of the country. This article provides a unique perspective on the past, present and the future of proteomics research in India.

It is indeed a happy time for the journal with enhanced activities. Our readers, authors and well wishers would be elated to know that the journal has now been accepted for indexing in DOAJ

(Directory of Open Access Journals), a reputed database. This will enhance our visibility and acceptance in the community.

We are thankful to NIPGR (conference organizer), PSI and AOPAO for allowing the journal the opportunity to be part of this scientific exercise and experience. We especially thank all the office bearers and executive council of PSI for leading the journal to glory. The hard work by the editorial team, the conference organizing team, the publisher and press, the benevolent individuals behind the curtain and sponsors have made this issue an outright success. We take the opportunity to thank the sponsors who supported printing and associated costs through advertisements in this issue that helps the journal to be freely accessible and publish articles without any charge to authors. We thank all our reviewers for timely evaluation. We especially thank Dr. Subhra Chakraborty, the convener of the conference, for her dynamism and exceptional hard work.

With this issue, Journal of Proteins and Proteomics (JPP) completes seven years of its uninterrupted existence – a significant milestone in the development of a journal. We hope you will appreciate our efforts and provide us with suggestions for improvement. Constructive criticism, suggestions and help of any kind, as always, are most welcome.

**Suman Kundu**  
*Editor-in-Chief*

## OVERVIEW OF THE CONFERENCE AND ASSOCIATED SCIENTIFIC EVENTS

### *Conference*

8<sup>th</sup> Annual meeting of Proteomics Society, India (PSI)

3<sup>rd</sup> Meeting of Asia Oceania Agricultural Proteomic Organization (AOAPO)

International Conference on “Functional & Interaction Proteomics: Application in Food & Health”

Date: December 14 - 17, 2016

Venue: The Grand Hotel, New Delhi

### Pre-Conference Events

“Education Day” – PSI 2016

Date: December 12 - 13, 2016

Venue: NIPGR, New Delhi

Workshop: Targeted Proteomics and Data analysis

Date: December 9 - 13, 2016

Venue: Sciex facility, Gurgoan

### Post-Conference Events

Workshop: Gel-based and Gel-free Proteomics

Date: December 18 - 20, 2016

Venue: NIPGR, New Delhi

Workshop: Metabolomics

Date: December 18 - 20, 2016

Venue: AIRF, JNU, New Delhi

Workshop: Structural and Interaction Proteomics

Date: December 18 - 20, 2016

Venue: RCB, NCR Biotech-Science Cluster, Faridabad

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## MESSAGE



On behalf of members of the Executive Council of the Proteomics Society, India, I extend a warm welcome to all the delegates to the 8<sup>th</sup> Annual Meeting of the Proteomics Society which is organized along with the 3<sup>rd</sup> Meeting of the Asia Oceania Agricultural Proteomics organization (AOAPO).

Proteomics Society, India was established in 2009. In these past 9 years it has grown to be a Society recognized by the global proteomic community. Some of our Executive Council members represent India at the HUPO-Dr. Ravi Sirdeshmukh and Dr. Sanjeeva Srivastava; AOHUPO- Dr.Ravi Sirdeshmukh; Asia Coordinator for International Plant Proteomics Organization, INPPO- Dr. Niranjan Chakraborty and AOAPO- Dr. Subhra Chakraborty. We are a relatively small society, but a very active one. In these past years, since inception, we have conducted our regular annual meetings and a number of workshops each year with the mandate to provide education in state of the art Proteomics, a platform for networking between the experienced Proteomic Scientist and newcomers to the field and in addition to promote technology sharing and transfer. The details of our workshops and meetings are available on the PSI website [www.psindia.org](http://www.psindia.org).

On 18<sup>th</sup> March 2016, which PSI has decided to celebrate as Proteomics Day in India, the society has taken on the Journal of Proteins and Proteomics (JPP), as the official journal of the Society. The proceedings of this meeting are being brought out in JPP in its Dec 2016 issue.

Each of the Annual meetings of PSI has been organized in different parts of the country to facilitate the participation of the scientific community, in particular the college students and teachers in that area. The theme chosen for each of the meetings coincides with the broader research interest of the host institute so that the invited delegates have a chance to interact with the local scientists with the intent of potential long term scientific collaborations.

It is therefore appropriate that PSI joined hands with National Institute of Plant Genome Research (NIPGR) which is a premier Institution for plant genomic research and plant biology in India and the Asia Oceania Agricultural Proteomics Organization which has a mandate to further cooperation in agricultural proteomics in Asia Oceania region, to host this the 8<sup>th</sup> Annual Meeting of the Proteomics Society, India. I am confident that this cooperation between NIPGR, AOAPO and PSI will promote and stimulate plant, agricultural and animal proteomics in the Asia Oceanic region.

The choice of Delhi as the site for the 8<sup>th</sup> Annual Meeting of PSI was also due to the fact that this city has several institutions with active ongoing research in proteomics. These institutions are: NIPGR, National Institute of Immunology, NII; Regional Centre for Biotechnology, RCB; Delhi University, Institute for Genomics and Integrated Biology, IGIB; International Centre for Genetic Engineering and Biotechnology, ICGB.

The Convener of the 8<sup>th</sup> Annual Meeting of PSI, Dr. Subhra Chakraborty, who is an Executive Council member of PSI, and a Senior Scientist at the NIPGR, has along with the members of the local organizing committee in New Delhi put together a very intense and intellectually stimulating program which encompasses several areas of plant and agricultural biology, health and disease biology, structural and functional biology and systems biology. This is in addition to the pre and post conference workshops and the Education Day. I am sure you will enjoy the lectures from eminent and experienced scientists and oral and poster presentations from younger delegates.

I wish all the delegates fruitful deliberations and a Very Happy New Year 2017.

With Best Wishes

**Dr. Surekha M. Zingde**  
President, Proteomics Society, India  
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## MESSAGE



On behalf of the National Institute of Plant Genome Research (NIPGR), New Delhi, I am pleased to welcome all the attendees including scientists, academicians, young researchers, delegates and students to the 8<sup>th</sup> Annual meeting of Proteomics Society, India (PSI) in concurrence with the 3<sup>rd</sup> meeting of Asia Oceania Agricultural Proteomics Organization (AOAPO) and the International Conference on “Functional and Interaction Proteomics: Application in Food and Health” from 14-17<sup>th</sup> December, 2016.

The conference, composed of the lecture sessions as well as interactive poster sessions besides pre- and post-conference workshops, tackles important topics on agricultural and clinical applications. Since this conference covers the global aspects on “Food and Health” from fundamental issue to practical application of proteomics, it would benefit everyone interested in future progress of proteomics research. The event makes the perfect platform for global networking as it brings together renowned speakers and scientists from across the globe. I hope that this international conference will provide the participants with a truly enthralling experience and offer a variety of perspectives through which we can contribute to global food security and human health.

I would like to thank each of you for attending this conference and bringing your expertise as it would allow significant brainstorming, and help evolve a blueprint for future proteomic endeavors. I look forward to a robust exchange of ideas and wish the conference great success.

**Dr. Niranjan Chakraborty**

*Director, National Institute of Plant Genome Research  
New Delhi*

## MESSAGE



It is my great pleasure and honor to extend a warm welcome to you to the 8<sup>th</sup> Annual Meeting of Proteomics Society, India (PSI) in conjunction with the 3<sup>rd</sup> Meeting of Asia Oceania Agricultural Proteomics Organization (AOAPO) and an International Conference on “Functional and Interaction Proteomics: An Application in Food and Health” being organized at Hotel “The Grand”, New Delhi, India from 14<sup>th</sup> to 17<sup>th</sup> December 2016. This is a special occasion for National Institute of Plant Genome Research (NIPGR) and the Indian Proteomics Society in hosting the Asia Oceania Meeting for the first time in India.

The theme of the Congress is contemporary and relevant. I am sure the deliberations will give us newer and newer insights into the emerging dynamics of Proteomics and System Biology. A well thought out and engaging academic programme has been planned for all, which will include Education Day program, Pre- and Post-conference Workshops, Plenary lectures, keynote lectures, Invited lectures, Concurrent sessions, Poster sessions and other opportunities to interact. In addition, selected students are also given an opportunity to present their research work as lightening short talks. Recent years have shown increased frequency of high complexity diseases worldwide. Despite significant advances in our understanding of the molecular basis of diseases, gaps remain in terms of disease pathogenesis as well as diagnosis and treatment. On the other hand, impending changes in the global climate coupled with rapidly growing population have resulted in challenges related to food nutrition and crop productivity. Food processing and safety are also very important and essential disciplines.

I am confident that the Conference would provide an opportunity to explore new innovations in the areas, meeting & interaction with the world leaders in the field, discuss and network with friend and colleagues. Furthermore, the conference will help evolving a roadmap for Proteomics Community to overcome challenges faced by the agricultural sector and health research in order to provide food, health and nutritional security.

Delhi, the capital of India has taken pride in welcoming guests from all over the world since the ancient times. It has been a seat of literature, art, music, and architecture. In recent times, Delhi has fast become a favorite city for hosting international scientific meetings owing to its infrastructure, ease of entry, and a rich cultural experience that it offers. The organizing institute, National Institute of Plant Genome Research (NIPGR), is one of the premier institutes for higher education and research in India. We are assisted in this endeavor by the staff and students of NIPGR, who have worked tirelessly to make this event a success. I hope that the participants will have time to visit the laboratories of NIPGR and enjoy our hospitality as well as the city and its surroundings.

Finally, I thank the National, International and Local Organizing Committee, Executive Council of PSI and AOAPO, Indian National Science Academy, Indo-US Science and Technology Forum, Jawaharlal Nehru University, Regional Centre for Biotechnology, Industry partners, the invited speakers across the world and researchers for their cooperation and participation. Our thanks are due to DG-ICAR and VC-JNU for accepting our invitation to grace the occasion. I believe with your support and participation, we will make this fascinating scientific event successful, remarkable and memorable!

With warm regards

**Dr. Subhra Chakraborty**  
*Convener, PSI-AOAPO 2016*  
*Guest Editor, JPP 7(4) 2016*  
*Staff Scientist & Professor*  
*NIPGR, New Delhi*



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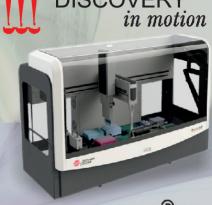
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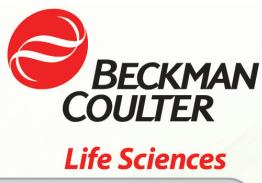


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## EDITORIAL

### Dr. Sanjeeva Srivastava

Guest Editor, JPP 7(4) 2016

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Ever since the birth of Proteomics Society, India (PSI), proteomics research in India has accelerated rapidly. Proteomics is currently at the forefront of biological research where many scientists are now turning towards quantitative analysis to answer the scientific query in hand. In contrast to the Human Genome Project, many scientists from the Indian subcontinent have made pivotal contributions to various proteome projects all around the globe. Two dedicated special issues on Proteomics Research in India published by Nature India [1] and Journal of Proteomics [2] have documented the rapid progress in proteomics research in India.

The field of proteomics has evolved tremendously from the traditional gel-based techniques to various high-throughput mass spectrometry based techniques. However, there are still many caveats to be overcome, one of them being the unambiguous detection of low abundant proteins. This stumbling block in proteomics research led to the birth of Multiple Reaction Monitoring (MRM)-based targeted approaches that are capable of perceiving varying concentrations of peptides across a dynamic range. Targeted proteomics, which was also chosen as the 2013 Nature Method of the year, is envisaged to play a pronounced role in translation of proteomics research to high-end clinical diagnosis.

Taking into account these ground-breaking developments, the need of the hour lies in bridging the gap between the vastly experienced global proteomics community and the enthusiastic growing Indian proteomics community by exposing them to the latest technological developments in proteomics. Emphasis should be given on strong educational foundation by organizing extensive seminars and hands-on workshops with a long-term vision of encouraging a healthy competitive atmosphere among Indian researchers in the global scenario. Towards this goal, we conducted the “Targeted Proteomics Workshop and International Symposium 2015”, at the Indian Institute of Technology Bombay. Alongside an impactful symposium, the event featured workshops on traditional as well as upcoming proteomic technologies. A specialized Global Initiative of Academic Networks (GIAN) course excelled in providing students with the best training and exposure on Targeted Proteomics – Skyline and Trans-Proteomic Pipeline [3]. Similarly, the 8<sup>th</sup> Annual Meeting of Proteomics Society, India scheduled to be hosted in 2016 would witness similar educational initiatives, including hands-on workshop and an Education Day designed for students and researchers new to the field of proteomics.

Additionally, e-learning outreach initiatives should also be advocated in order to engage the broad student community. Institutes like IITs and IISc are playing a paramount role in such activities to offer a wide array of online NPTEL and MOOC courses that provide the participants an interactive platform for extensive networking and an opportunity to enlighten themselves even in geographically inaccessible regions. Proteomics virtual labs that include various experimental demonstrations, are gaining momentum as well because many students explore such forums to extrapolate the knowledge amassed to their own research. These educational pursuits with a thoroughly workable structure have come a long way from the regular classroom lectures and textbook proficiency, and are received well by students from all spheres of research. Apart from these mainstream educational ventures, other web-based activities are, too, a great boon for young, budding talents who are yet to build their expertise in the proteomics domain. A paragon example would include the research-based documentary; “Proteomics: Translating the code of life” which was instrumental in sharing the perspective of several venerable scientists with the masses. However, with the success of such initiatives, many more workshops, educational and outreach activities should be encouraged to disseminate the knowledge of latest technologies in the proteomics research.

Proteomics is burgeoning field with numerous applications. The food and healthcare industry focusing on biomedical research are two areas which have harnessed the power of proteomics to catapult scientific

innovations. The “International Conference on Functional & Interactional Proteomics: Application in Food and Health” will provide a platform for several distinguished and budding scientists to present the recent advances in the field along with the current impediments to the scientific progress. In light of the central theme of this conference, this special issue focuses on the various developments in the food and health sector with respect to structural, functional and interaction proteomics. It comprises of editorials, research articles, education day workshop perspective, and an article based on the interview of few Proteomics Society, India members. As many as five research articles deal with plant-based proteomics studies, for example, characterization of phosphoproteins from *Hippophae rhamnoides* and 11S globulin from Kutaj by Dr. Deswal and Dr. Kumar, respectively. Another noteworthy work involves proteomics approaches to study the activation of reactive oxygen species signalling during seed germination and growth in barley plant by Dr. Kim. Apart from these, protein extraction protocols were compared for enhanced proteomic analysis from *Rorippa indica* by Dr. Sikdar. Another study by Dr. Sabareesh provided insight into arginine modification by different compounds in various medium using LC-ESI-MS. The other part of the special issue is devoted to proteomics studies aimed at reforming the healthcare sector. This comprises of an interesting work done by Dr. Maiti and group that studied the effect of sequence composition and physicochemical parameters on the stability/aggregation pattern of amyloidogenic and non-amyloidogenic human proteins. Other articles include the application of the enzyme Dihydrofolate Reductase in healthcare and drug designing by Dr. Chaudhari and the practicability of phosphoproteomics studies using biopsy-scale protein amounts by Dr. Gowda.

In conclusion, I would like to express my sincere gratitude to all the authors and reviewers for their invaluable participation in this special issue, which primarily delineates the sudden leap of proteomics research in the broad realms of food, health and industrial advancements. Journal of Proteins and Proteomics (JPP), a peer reviewed journal managed by the PSI, is instrumental in bringing out the topical trends in Indian proteomics research [4-5]. This international journal always strives for excellence and provides a suitable platform to aspiring young proteomics researchers to showcase their scientific quest. As a guest editor, I enjoyed interacting with authors and contributors in various capacity and hope that readers will appreciate this special issue, which is a compendium of several inspiring proteomics studies, perspective articles and displaying the breadth and depth of rapidly growing Indian proteomics community.

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# PSI-AOAPO 2016

**8<sup>th</sup> Annual Meeting of Proteomics Society, India  
3<sup>rd</sup> Meeting of Asia Oceania Agricultural Proteomics  
Organization**

*International Conference on Functional & Interaction Proteomics:  
Application in Food and Health*

The Grand Hotel, New Delhi, December 14-17, 2016

## Programme

Wednesday, December 14, 2016

Opening Ceremony : 8:30 – 9:45hr

Morning Plenary Talks : **Mike Snyder (9:45-10:20hr)**  
Precision Medicine: Using Omics  
and Big Data to Manage Health

**Kanury Rao (10:20-10:55hr)**  
Interrogating the Proteo-Metabolome Network  
To dissect Host-Pathogen Crosstalk

## GROUP PHOTO

Exhibition & Tea/Coffee : 10:55-11:25hr

**SESSION I: INTEGRATIVE OMICS & SYSTEM BIOLOGY (11:25 -12:30HR)**

<i>Abstract No.</i>	<i>Time</i>	<i>Speakers</i>	<i>Talk Title</i>
K001	11:25-11:50	Lloyd Sumner	Integrated Metabolomics for Deciphering Metabolic Pathways and Emerging Solutions for Addressing the Grand Challenges of Metabolomics
K002	11:50-12:10	Subhra Chakraborty	Understanding immune signaling in plant: a system biology approach
I001	12:10-12:25	Jin Koh	Integrative omics of Tragopogon polyploidy and evolution
OP01	12:25-12:30	Divya Rathi	Remodeling of the transcriptome, proteome and metabolome of Grasspea: The molecular basis of dehydration tolerance

**Poster session : 12:30 -13:10 hr****Lunch : 13:10 -13:55hr****SESSION II: MODEL/UNIQUE ORGANISM PROTEOMICS (13:55– 16:35HR)**

<i>Abstract No.</i>	<i>Time</i>	<i>Speakers</i>	<i>Talk Title</i>
K003	13:55-14:20	Paul Haynes	Label free and TMT proteomic analysis of two different rice varieties with contrasting drought tolerance phenotypes
K004	14:20-14:45	Emøke Bendixen	Host genetics influence the virulence of <i>E.coli</i> pathogens by altering glycan anchors in the gut epithelium.
K005	14:45-15:05	Uemura Matsuo	Complex responses of glycosylphosphatidylinositol (GPI)-anchored proteins in association with plant cold acclimation
K006	15:05-15:25	Renu Deswal	Proteomics of a Himalayan shrub a step towards Translational Research
K007	15:25-15:45	L.C. Rai	Decoding the role of diazotrophic cyanobacterial AhpC in abiotic stress management using proteomics and genomics approaches
K055	15:45-16:05	Shree Kumar Apte	Proteomic approaches unveil some secrets of the extreme radiation resistance in <i>Deinococcus radiodurans</i>
K008	16:05-16:25	Henning Hermjakob	The Omics Discovery Index
I002	16:25-16:40	Krishnaswamy Balamurugan	Proteomic analysis in <i>Caenorhabditis elegans</i> challenges with <i>Klebsiella pneumoniae</i> revealed the regulation of TOR pathway against infection
OP02	16:40-16:50	M.V. Jagannadham	Proteomic analysis of the outer membrane Vesicles (Omvs) of an Archebacterium <i>Natronicoccus Sp.</i> , whose genome sequences is not known.
OP03	16:50-16:55	Roshan Kumar	Global S-nitrosoproteome identification links UCHL1 to Parkinson disease pathology

**Exhibition & Tea/Coffee: 16:55-17:00hr**

**SESSION III: INTERACTION PROTEOMICS & CELLULAR NETWORK (16:50- 18:15HR)**

Abstract No	Time	Speakers	Talk Title
K009	16:50-17:15	Philip Andrews	Chemical crosslinking mass spectrometry of protein complexes using cleavable crosslinkers: Establishing quantitative connectivity diagrams.
K010	17:15-17:40	Marc Wilkins	The Role of Interaction Codes in Protein Interaction Networks
K011	17:40-18:00	Gaurisankar Sa	Integrating the role of FOXP3-interactomics in IL10 transcription in tumor T-regulatory Cells
C01	18:00-18:15	Aman Tyagi (Bio-Rad Laboratories)	Activation of p53 in Down Syndrome and in the Ts65Dn mouse brain is associated with a pro-apoptotic phenotype

Evening Plenary Talk : **Akhilesh Pandey (18:15-18:50hr)**  
Data-independent acquisition (DIA) mass spectrometry for comprehensive profiling of proteomes

**Cultural Programme & Gala Dinner: 19:30 hr onward**

**Thursday, December 15, 2016**

Morning Plenary Talk : **John Yates (9:00-9:35 hr)**  
New Strategies to analyze Protein-Protein Interactions

**SESSION IV: IMMUNITY, DISEASE PROTEOMICS IN ANIMAL & PLANT HEALTHCARE (9:35 -12:15 HR)**

Abstract No.	Time	Speakers	Talk Title
K012	9:35-10:00	Bonghee Lee	Direct conversion of stem cells by changing single protein dynamics
K013	10:00-10:25	K Dharmalingam	Comparative proteomics of human fungal pathogens
K014	10:25-10:45	Pawan Malhotra	Proteomics Study of Protein Methylation in <i>Plasmodium falciparum</i> at asexual blood stages

**Exhibition & Tea/Coffee : 10:45 -11:15hr**

K015	11:15-11:35	Robert Moritz	Proteogenomic approaches for biomarker discovery
K016	11:35-11:55	Sixue Chen	Development of redox proteomics technologies and application in stomatal guard cell immunity research
K017	11:55-12:15	Abhijit Chakraborty	Platelet proteomics in blood disorder
OP04	12:15-12:20	Sudip Ghosh	Proteometabolomic study illustrates dual role of oxalic acid in anti-nutrient signaling and non-host resistance
OP05	12:20-12:25	Pooja	Plasma proteome signatures for short-term and long-term acclimatization at high altitude

**Poster session : 12:25 – 13:05hr**

**Lunch : 13:05-13:45hr**

**SESSION V: GLOBAL & TARGETED PROTEOMICS FOR PRECISION MEDICINE (13:45-15:55HR)**

Abstract No.	Time	Speakers	Talk Title
K018	13:45-14:10	Stephen Pennington	Discovery and Development of Multiplexed Protein Biomarker Tests to Support Clinical Decision Making
K019	14:10-14:35	Jennifer Van Eyk	Changing medicine: Proteome centric precision health
K020	14:35-14:55	Richard Lipscomb	Applying proteomics to develop a precise, predictive test for diabetic kidney disease
I003	14:55-15:10	Srikant Rapole	Quantification and validation of potential biosignatures for intrinsic subtypes of breast cancer
I004	15:10-15:25	Sanjeeva Srivastava	Targeted Proteomics for the validation of biomarkers in Brain Tumors
I005	15:25-15:40	Satya Saxena	Proteomic Analysis of Treatment-Naïve Early RA Serum Identifies a Unique Protein Signature and Reveals Upregulation of Platelet Activation and Degranulation Pathways
I006	15:40-15:55	Mukul Midha	Industrialization of quantitative proteomics measurements by SWATH-MS

**Exhibition & Tea/Coffee: 15:55- 16:25hr**

**SESSION VI: PROTEOGENOMICS & BIG DATA ANALYSIS (16:25- 17:20 HR)**

Abstract No.	Time	Speakers	Talk Title
K021	16:25-16:45	Michael Hoopmann	Rapid analysis of Chemical cross linking data with Kojak
C02	16:45-17:00	Jim Thorn (SCIEX)	Analysis of Post Translational Modifications using CESI-MS
C03	17:00-17:15	Thomas Klemm (Illumina)	Unlocking the Power of the Genome with Multi-Omics data and the Illumina Correlation Engine
OP06	17:15-17:20	Puneet K. Kadimi	A Browser-based Interactive Dashboard for Exploratory Analysis of MS-based Proteomics data

Evening Plenary Talk : **John Aitchison (17:20– 17:55hr)**  
Host-pathogen interaction revealed by quantitative proteomics

**Proteomics Society of India – Governing Body Meeting (18:00 – 19:00 hr)**

**Friday, December 16, 2016**

Morning Plenary Talk : **Joshua LaBaer (9:00 -9:35 hr)**  
High throughput protein microarrays for biomarker and target discovery

**SESSION VII: STRUCTURAL PROTEOMICS (9:35 -11:25 HR)**

Abstract No.	Time	Speakers	Talk Title
K022	9:35-10:00	David Wemmer	Bacterial Transcriptional Activators: Protein Mechanics of their Activity
K023	10:00-10:25	Dinakar Salunke	Structural proteomics of plant seeds

**Exhibition & Tea/Coffee : 10:25 -10:45hr**

K024	10:45-11:05	Joseph Jez	From Proteins to Climate Change: Redox Proteomics in Plant Metabolism
K025	11:05-11:25	Ganesh Anand	Temperature-specific expansion of Dengue virus by mass spectrometry uncovers capsid quaternary interactions

**SESSION VIII: ORGAN & ORGANELLER PROTEOMICS (11:25 -12:30HR)**

Abstract No.	Time	Speakers	Talk Title
K026	11:25-11:50	Dominique Job	Plant proteomics, with a special focus on seeds
K027	11:50-12:10	Joshua Hazelwood	Profiling glycopeptides by HCD and ETD to characterize the plant Golgi localized UDP-GlcNAc transporter
K028	12:10-12:30	Niranjan Chakraborty	Proteomic analysis of the plant extracellular matrix: turning protein repertoire into application for crop improvement

Poster session : 12:30 – 13:00

**Lunch : 13:00-13:30hr**

K029	13:30-13:50	Klaas Van Wijk	Chloroplast Protein Homeostasis; protein maturation and degradation
K030	13:50-14:10	Rakesh Misra	Dynamics of proteome during development and ageing
K031	14:10-14:30	Chin Chiew Foon	The quest for protein markers in oil palm tissue culture
K032	14:30-14:50	Pao-Chi Liao	Discovery and validation of protein biomarkers for lung cancer metastasis using a cell secretome model and clinical specimens
I007	14:50-15:05	Lei Li	Protein degradation rate in Arabidopsis thaliana leaf growth and development

**Exhibition & Tea/Coffee : 15:05-15:25hr**

**SESSION VIII: METABOLOMICS & METABOLIC DISORDERS (15:25 -17:45HR)**

<i>Abstract No.</i>	<i>Time</i>	<i>Speakers</i>	<i>Talk Title</i>
K033	15:25-15:50	Susan Sumner	Metabolomics in maternal and child health
K034	15:50-16:10	Autar Mattoo	Metabolomics in a fix: Tomato genotypes reveal ecosystem-dependent and hormone-specific fluidity
K035	16:10-16:30	Shantanu Sengupta	Identifying potential markers for Coronary Artery Disease using Proteomics and Metabolomics approach
K036	16:30-16:50	Utpal Tatu	Global metabolomic profiling reveal triggers for sexual stageconversion in Malaria
K037	16:50-17:10	Ranjan Nanda	Deregulated metabolic phenotype of pulmonary tuberculosis patients
I008	17:10-17:25	Bingxian Yang	Omics' analysis of leaf from <i>Clematis terniflora</i> DC. under binary stress
C04	17:25-17:40	Lee Gethings (Waters)	Unbiased quantitative and qualitative metabolic profiling for translational medicine
OP07	17:40-17:45	T. More	Comprehensive quantitative metabolomics approach to investigate metabolic alteration in invasive Ductal Carcinoma of the breast

**AOAPO - Council Meeting (18:00 – 19:00hr)****Saturday, December 17, 2016**

Morning Plenary Talk : **Setsuko Komatsu (9:00 -9:35 hr)**  
 Nuclear phosphoproteomics to identify the abiotic stress tolerant mechanism in crop

**SESSION X: FOOD, NUTRITION AND STRESS OMICS (9:35 -13:20HR)**

<i>Abstract No.</i>	<i>Time</i>	<i>Speakers</i>	<i>Talk Title</i>
K038	9:35-10:00	Hosseini Salekdeh	TBA
K039	10:00-10:25	Taishi Umezawa	Quest for phosphosignaling pathways in ABA response in plants
K040	10:25-10:45	Nicolas Taylor	Harnessing the Wheat Proteome to Enhance Yield, Salinity Tolerance and Thermal Tolerance

**Exhibition & Tea / Coffee : 10:45 -11:15hr**

**JPP 19**

K041	11:15-11:35	Shaojun Dai	Salinity-Induced Palmella Formation Mechanism in <i>Dunaliellasalina</i> Revealed from Quantitative Proteomics and Phosphoproteomics Analysis
K042	11:35-11:55	Wei Ji	Quantitative proteomics reveals an important role of GsCBRLK in salt stress response of soybean
K043	11:55-12:15	Sharmila Chattopadhyay	Mechanistic insight into the involvement of glutathione in plant defence: a proteomic approach
K044	12:15-12:35	Bongani Ndimba	Application of Proteomics in Agricultural Research & Development: A South African Perspective
K045	12:35-12:55	A. K. Mohanty	Discovery of protein biomarkers in biofluids of bovine for early detection of pregnancy for sustained productivity
I009	12:55-13:10	Hariprasad G	Differential protein expression profiling as means to explain beneficial advantages of co-administering growth factor cocktail with cardiac stem cells for regenerative therapy
OP08	13:10-13:15	Swati Varshney	Effect of vitamin B <sub>12</sub> restriction on Wistar rat: A global proteomic profiling
OP09	13:15-13:20	Shivam Dubey	iTRAQ based comparative proteome profiling of A1A1, A2A2 and A1A2 beta casein variants of cow milk

Lunch: 13:20 – 14:00 hr

**SESSION XI: PTM PROTEOMICS & REGULATION BIOLOGY (14:00– 16:30HR)**

Abstract No.	Time	Speakers	Talk Title
K046	14:00-14:25	Hanno Steen	Clinical Proteomics – Let's not Forget the PTMs
K047	14:25-14:45	S Gopalan Sampathkumar	Glycoproteomics analysis enabled by hexosamine analogues through metabolic glycan engineering
K048	14:45-15:05	Mahesh J Kulkarni	Glycation inhibitors reduce protein glycation and extend chronological life span of <i>Saccharomyces cerevisiae</i> and <i>Caenorhabditis elegans</i>
K049	15:05-15:25	Chunaram Chowdhury	Proteomic exploration of lysine acetylation and ubiquitylation
K050	15:25-15:45	Harsha Gowda	Mapping molecular changes associated with tobacco induced cellular transformation by global and phosphoproteomic profiling
K051	15:45-16:05	Bing Yu	Quantitative proteomics and phosphoproteomics of sugar beet monosomic addition line M14 in response to salt stress
I010	16:05-16:20	Tianyi Ma	Characterization of SnRK2.6 and its phosphorylation substrates
OP10	16:20-16:25	Vandita Dwivedi	Glycoproteomics based analysis of site occupancy and microheterogeneity of Mucin-type-O-Glycans on CD43

**SESSION XII: HUMAN PROTEOME PROJECT INITIATIVES (16:25– 17:30HR)**

<i>Abstract No.</i>	<i>Time</i>	<i>Speakers</i>	<i>Talk Title</i>
K052	16:25-16:50	Eric Deutsch	The HPP Knowledge Base Pillar: Achieving a high-confidence snapshot of the human proteome observed thus far
K053	16:50-17:10	Ravi Sirdeshmukh	Chromosome Centric Human Proteome Project and the Indian Efforts
K054	17:10-17:30	Debashish Dash	Probing the missing Proteome: a computational approach

Closing program (17:30– 18.00hr)

**High Tea/Coffee (18:00-18:30hr)**

## EDUCATION DAY - PSI-2016

(12 – 13 December, 2016)

**Coordinators : Ravi Sirdeshmukh<sup>1,2</sup> and Sanjeeva Srivastava<sup>3</sup>**

<sup>1</sup>Institute of Bioinformatics (IOB), Bangalore

<sup>2</sup>Mazumdar Shaw Center for Translational Research (MSCTR), Bangalore

<sup>3</sup>Indian Institute of Technology Bombay (IITB), Mumbai

### SCHEDULE

**Monday, 12<sup>th</sup> December, 2016**

07:30-08:30 AM: Registration

Time	Speaker	Title of the talk
08:30 - 08:40 AM	<b>Subhra Chakraborty</b>	Introductory Remarks
08:40 – 08:50 AM	Surekha Zingde	Opening address
08:50 – 09:30 AM	Ravi Sirdeshmukh	Perspective of the Education Day Workshop

#### **MODULE-I: GEL-BASED AND GEL-FREE PROTEOMICS USING MASS SPECTROMETRY** **Chair: Ravi Sirdeshmukh**

09:30 – 10:00 AM	K. Dharmalingam	Fundamentals of Quantitative Proteomics using 2D and 2D DIGE
10:00 – 10:30 AM	Renu Deswal	Applications of Gel based Proteomics in Plants – Investigating Nitric oxide Signaling (Nitrosylation) in <i>B.junccea</i>
10:30 – 11:00 AM	<b>Tea break</b>	
11:00 – 11:30 AM	Debasis Dash	Mass Spectrometry and Protein Identification
11:30–12:00 PM	Dipankar Malakar	Mass Spectrometry-based Protein Quantitation Analysis

#### **MODULE-II: METABOLOMICS****Chair: Sixue Chen**

12:05-12:35 PM	Harsha Gowda	Introduction to mass spectrometry based metabolomics
12:35– 1:05 PM	Sixue Chen	Quantitative Metabolomics Application for Plants using MS
1:05 – 2:15 PM	<b>Lunch break</b>	
2:15 – 3:30 PM	Amol Suryavanshi	Demo Session: 2-DE Demo
3:30 – 4:00 PM	<b>Tea break</b>	
4:00 – 5:00 PM	Dipankar Malakar	Demo Session: MS Data Analysis and Quantitation

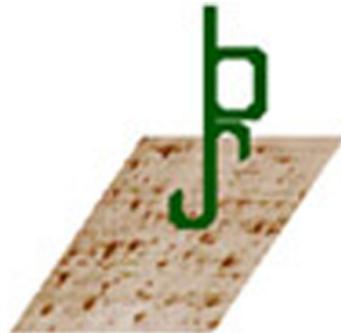
Tuesday, 13<sup>th</sup> December 2016

**MODULE-III: TARGETED PROTEOMICS**Chair : Robert Moritz

9:00 – 9:30 AM	Robert Moritz	Targeted Proteomics: TPP & SRM Atlas
9:30 – 10:00 AM	Srikanth Rapole	Targeted proteomics for clinical applications
10:00 – 10:20AM	Mahesh Kulkarni	Targeted Proteomics & PTMs
10:20 – 10:40 AM	Suman Thakur	From Target to Validation: Proteomics in Clinical Research
10:40 – 11:00 AM	<b>Tea break</b>	

**MODULE-IV: STRUCTURAL PROTEOMICS & INTERACTOMICS**  
Chair : MA Vijayalakshmi

11:00 – 11:30 AM	Henning Hermjakob	Interactions, Complexes, Pathways: Network context for molecular expression data
11:30 – 12:00PM	Discussion	Studying Interaction Using Proteomic Methods
12:00 – 12:30PM	Tushar K. Maiti	Chemical cross linking mass spectrometry in protein structure and molecular interaction
12:30 – 1:00PM	M A Vijaya Lakshmi	Separation technologies and role in proteomic analysis
1:00 – 2:15 PM	<b>Lunch break</b>	
2:15– 2:45 PM	Jason Chen	Functional and Interaction Proteomics on a chip
2:45–3:15 PM	Sanjeeva Srivastava	Interactomics: Protein Microarrays and Surface Plasmon Resonance
3:15 – 3:45 PM	<b>Tea break</b>	
3:45 – 5:15 PM	Interaction and discussion: Session moderated by Dr. Ravi Sirdeshmukh	
5:15 – 5:20 PM	Sanjeeva Srivastava	Vote of thanks!



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## PROTEOMICS EDUCATION DAY WORKSHOP – PERSPECTIVE

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**Ravi Sirdeshmukh<sup>1,2</sup> and Sanjeeva Srivastava<sup>3</sup>**

<sup>1</sup>*Institute of Bioinformatics (IOB), Bangalore, India*

<sup>2</sup>*Mazumdar Shaw Center for Translational Research (MSCTR), Bangalore, India*

<sup>3</sup>*Indian Institute of Technology Bombay (IITB), Mumbai, India*

E-mail: ravisirdeshmukh@gmail.com, sanjeeva@iitb.ac.in

Proteomics approaches have taken the centre stage in bio-medical research and are being increasingly applied to study all biological systems ranging from microbes to humans. Although scientists are yet to achieve full potential of proteomics research in clinical practice due to biological and analytical complexity, the prospects are strong and the progress is persistent. The Proteomics Education Day workshop (Dec 12-13, 2016) is designed for research students and young investigators who have initiated or plan to initiate application of proteomics in their research projects or college teachers who want to get an overview of the field, so that they use the information in their teaching modules. The purpose is not to go in depth but to keep to the essentials. Attention is paid to reflect current methods in practice with a touch on emerging trends. We have included few illustrative application talks but unable to include many different application talks. However, the conference that follows will have many of these application talks and we hope that the workshop attendees will certainly find them well connected when they sit through conference sessions. One thing to remember, this is the start and each area will need to be understood further, evaluated for its applicability in your research and then incorporated.

When Proteomics efforts were initiated in the post Genomic era, it was all 2D Gel based separation of proteins combined with mass spectrometry and bioinformatics methods to identify proteins. However, there were some limitations such as, 1. gel reproducibility, 2. limited throughput, and 3. access to only high abundant proteins. Developments in mass spectrometry (MS) have revolutionized the field and emergence of alternative, gel free LC- MS/MS based approaches for analysing total cellular proteins with or without chromatographic pre-fractionation are increasingly being used. **Module 1** of the workshop will cover basics of 2 D gel approach, some of its advantages as well as limitations involved, followed by the LC MS workflow and the methods of quantitation of protein expressions in this approach. Some of the limitations of traditional 2D gel-based proteomics were overcome by 2D-DIGE, which provided a strong basis for gel-based quantitative proteomics (Figure 1). The basics of mass spectrometry based proteomics are illustrated in Figure 2.

While LC MS/MS approach permits access to proteins of very low abundance and greater dynamic range of concentrations, it primarily analyzes peptides of the protein mixtures, thus losing direct link with the original individual proteins, a feature which is protected in 2 D Gel based approach. Identification of peptides based on the sequence information gathered from MS data and converting that into protein identifications requires extensive effort utilizing bioinformatics tools and databases. How this is done will be covered in the second talk in this Module. Methods like gel-free LC MS/MS approaches to understand changes in cellular protein expressions and levels in different physiological conditions including disease conditions will be discussed in this module.

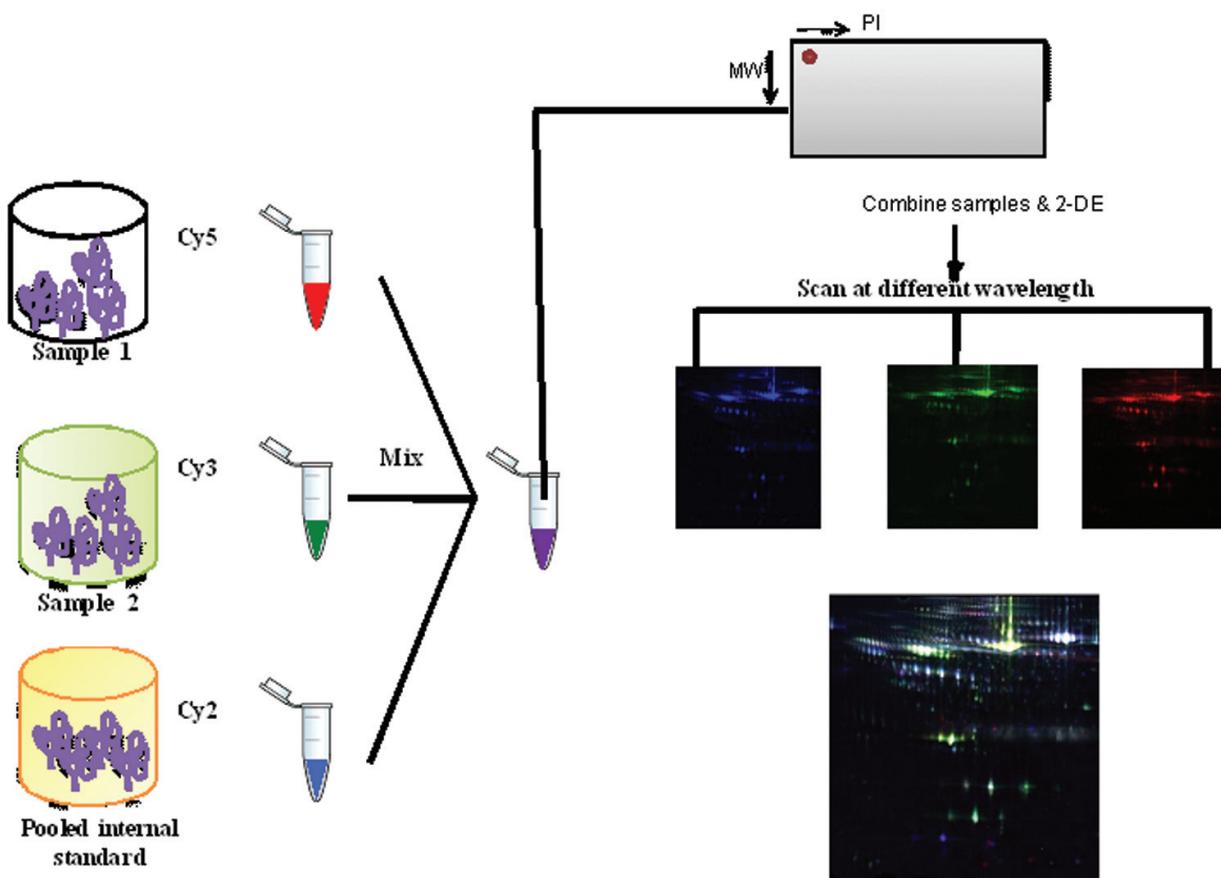
Proteins are functional entities of the genes in all cells and tissues. Many proteins are themselves enzymes while others influence enzyme activities. Alterations of such proteins involved in the metabolic pathways will naturally cascade into causing changes in metabolites. Thus in recent years, determination of large scale changes in metabolites has emerged as a valuable extension in omics domain. However, methods for metabolite analysis, their identification and quantitation are yet to evolve for their optimal

use and applications. Mass spectrometry again plays a key role here too. **Module 2** of the workshop is dedicated to discuss the evolving metabolite analysis platforms. A presentation on plant metabolite analysis will illustrate application of metabolomics to enhance understanding of plant biology.

Proteomic analysis is a multistep process involving sample preparation, mass spectrometry and identification of the analytes through integration of the experimental output and databases in the public domain. Demonstration of these aspects using representative experimental examples will be performed in the **Post Lunch Demo sessions**.

The mass spectrometric signals of peptides depend upon several factors including its abundance, size, chemistry and of course, the instrument capability. Some peptides are consistently detected in the mass spectrometer, others are not. Such unique peptides could be considered as good representation of the presence of protein in the sample. Libraries of such peptides called Proteotypic peptides and their mass spectra generated by the community have been developed over years (SRM Atlas). Investigators are generating lists of proteins that are associated with specific clinical conditions which can be validated in multiple samples using their proteotypic peptides from SRM Atlas and using Selective or Multiple Reaction Monitoring (SRM/MMR) assays (Figure 3). **Module 3** will include resources for such peptides and their targeted analysis for detection and quantitation of proteins in the sample. A talk will illustrate application of this in a clinical study. Another talk will reflect how such an approach can be developed to study and follow post transcriptional modifications of a clinically important protein.

The large data generated through proteomics experiments have to be viewed and interpreted to permit biological inferences. Data deposition, mapping to networks, pathways and collation of the global data is an important bioinformatics component. There are basic pipelines and tools available for this and new ones could be developed to adapt to specific needs. An overview of this will start in **Module 4** (Figure 4).



*Figure 1: Gel-based Proteomics: 2D-DIGE*

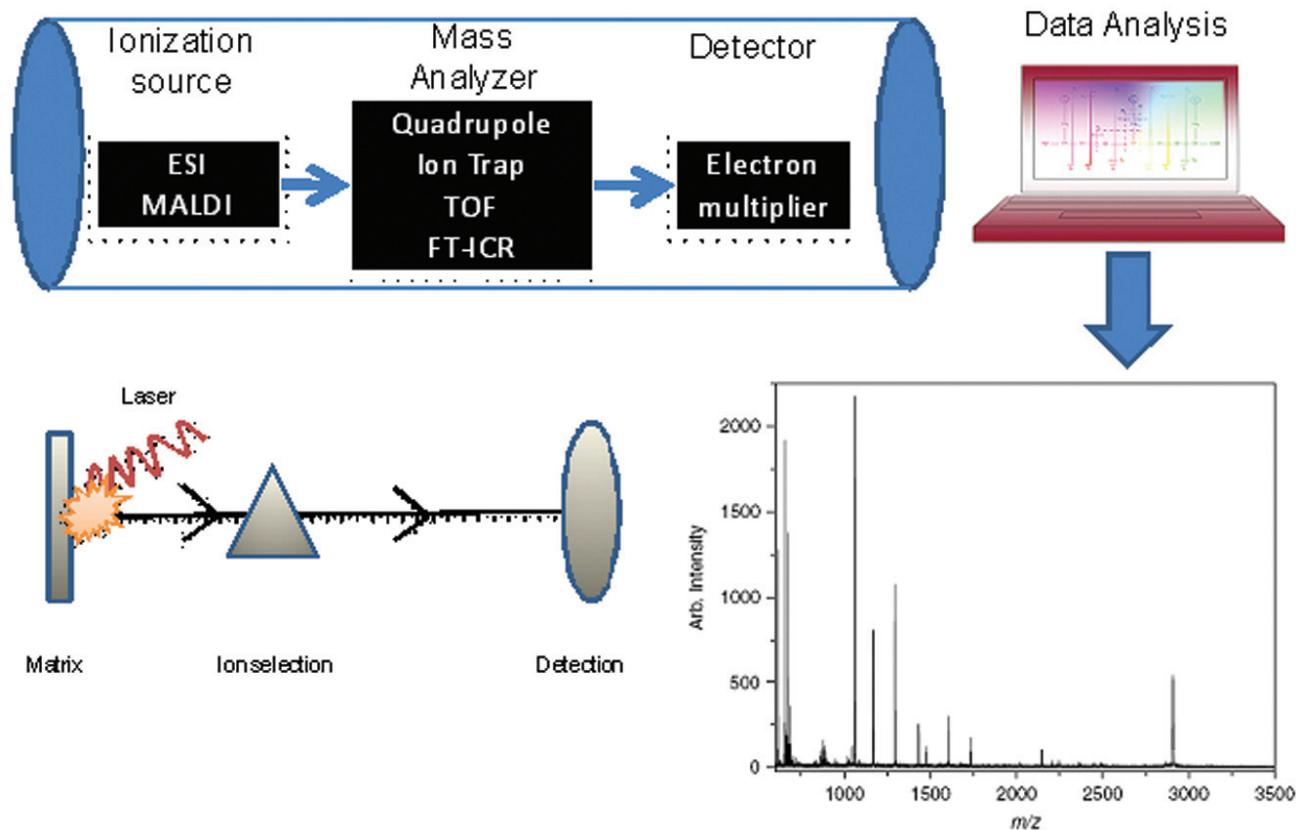


Figure 2: Gel-free Proteomics: Mass Spectrometry

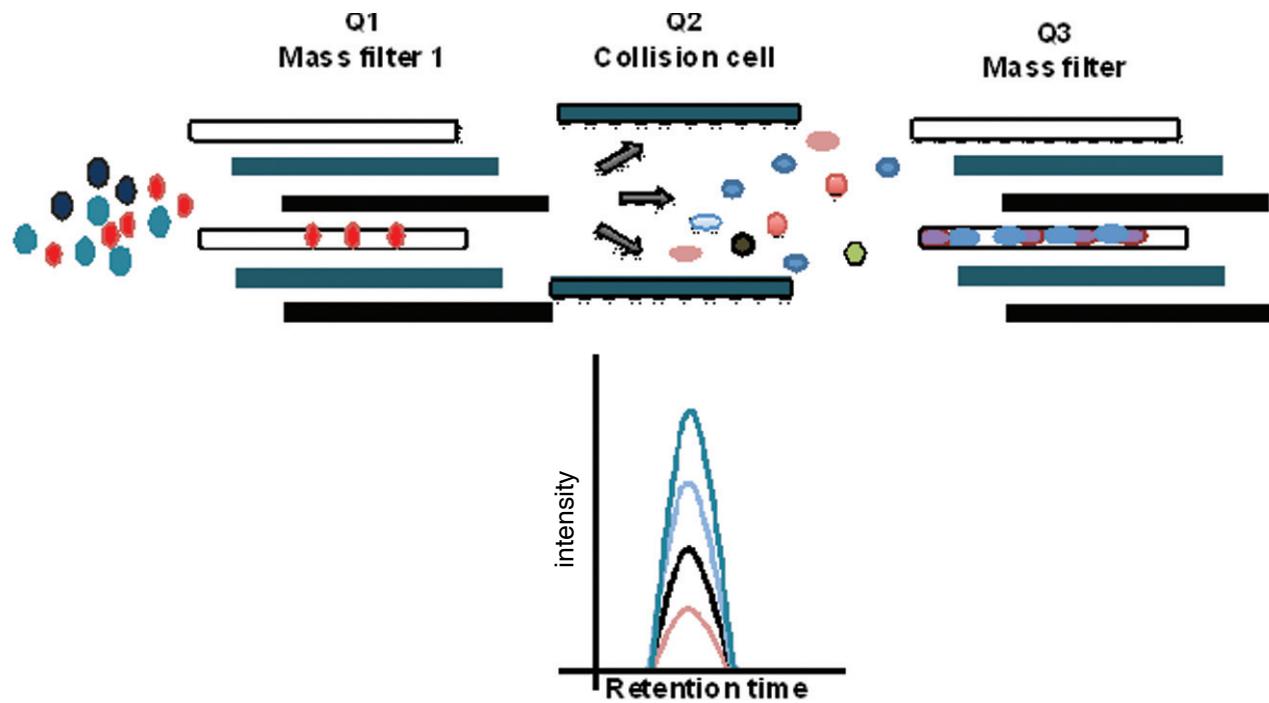


Figure 3: SRM/MRM analysis using targeted proteomics

Further, most of the key biological functions of proteins in a cell occur through interactions with other proteins, nucleic acids or small molecules. Identifying such molecular networks and complexes is key to draw biological inferences of the proteins identified under physiological or clinical conditions under study. While there could be experimental methods for capturing and directly detecting relatively strong interactions, others could be inferred using bioinformatics approaches developed based on wide range of direct and indirect data available to support the interactions. **Module 4** will also cover this subject. It is also to be noted that when it comes to detecting and understanding interactions, there are methods other than mass spectrometry, such as protein arrays (Figure 5). Two talks in these Modules



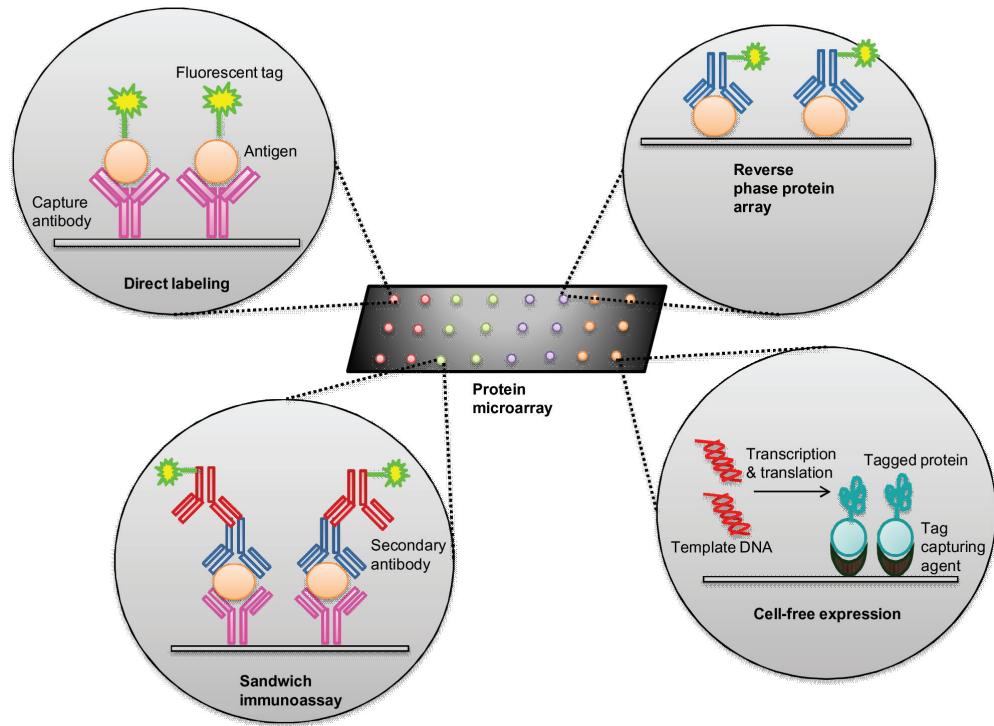
We work with the best scientists and engineers on research endeavours of all sizes, and provide the infrastructure needed to share data openly in the life sciences.

We develop databases, tools and software that make it possible to align, verify and visualise the diverse data produced in publicly funded research, and make that information freely available to all. But tracking and measuring the impact of these efforts can be a challenge.

Here, we provide examples of how or users in academia and industry perceive our value to their work, and report on our progress in meeting the needs of researchers throughout the world.

**“**We simply could not function without the core, reliably maintained data collections and world-leading expertise of EMBL-EBI for the organisation and analysis of biological data.

**Figure 4:** EBI view



**Figure 5:** Protein Microarrays

will cover these subjects. Biological interactions and affinities can also be exploited to develop protein capturing or separation methods. The basics and potential application of such methods in proteomics workflows will also be discussed.

Finally, no workshop will be comprehensive enough to cover all points where old as well as new questions are always possible to emerge and should be addressed. The workshop participants will always be thinking how each one of the methods or approach could be utilized in the context of their research endeavour. The interactive session at the end is reserved for hearing about some of the topics not discussed in details, unanswered questions, some specific details and anything else that is relevant in the context of proteomics methods and their applications that is likely to change our overall outlook and understanding of biological processes and their regulation or deregulation.



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## ABSTRACTS FOR PLENARY TALKS

December 14, 2016, 9.45 am

### Managing Health and Disease Using Big Data

**Michael Snyder, Kevin Contreipois, Brian Piening, Wenyu Zhou, Dalia Perelman, Gucci Gu, Denis Salins, Shana Leopold, Jessica Sibal, Tejas Mishra, Liang Liang, VarshaRao, NastaranHeidari, Reza Sailani, Lihua Jiang, Colleen Craig, Candice Allistar, Erica Weinstock, Justin Sonnenburg, George Weinstock, Tracy MacLaughlin,**

*Department of Genetics, Stanford University, USA*

Understanding health and disease requires a detailed analysis of both our DNA and the molecular events that determine human physiology. We performed an integrated Personal Omics Profiling (iPOP) of 100 healthy and prediabetic participants over three years including periods of viral infection as well as during controlled weight gain and loss. Our iPOP integrates multiomics information from the host (genomics, epigenomics, transcriptomics, proteomics and metabolomics) and from the gut microbiome. Longitudinal multiomics profiling reveals extensive dynamic biomolecular changes occur during times of perturbation, and the different perturbations have distinct effects on different biological pathways. Overall, our results demonstrate a global and system-wide level of biochemical and cellular changes occur during environment exposures and omics profiling can be used to manage health.

December 14, 2016, 10.20 am

### Interrogating the Proteo-Metabolic Network to Dissect Host-Pathogen Crosstalk

**Kanury V. S. Rao**

*THSTI-National Chair & Head, Drug Discovery Research Centre, Translational Health Science and Technology Institute, Faridabad, India*

Despite decades of intervention programs, *Mycobacterium tuberculosis* (Mtb) persists as an enduring pathogen in the human population. Infection is initiated through inhalation of the pathogen as an aerosol, following which Mtb enters the lung and infects alveolar macrophages. Although macrophages constitute the primary defense against microbial invasion, the pathogen *Mycobacterium tuberculosis* (Mtb) has evolved effective mechanisms to attenuate or inhibit the diverse anti-microbial pathways initiated by the host cell. This attenuation is mediated through active engagement with several biochemical pathways of the host cell. Resolution of these interactions is important for the development of more effective strategies for TB control. Our own work in this direction indicates that Mtb achieves a comprehensive control over the host cellular machinery through selective targeting of the central metabolic pathways. By modulating the rate of glucose uptake, and then 'fine-tuning' the distribution of flux between the glycolytic and pentose phosphate pathways, the pathogen redefines the output to ensure that the intracellular environment is adapted to its own needs. Key to this is the shunting of the pyruvic acid-derived acetyl-CoA into the production of ketone bodies, which then cause feedback activation of a GPCR to induce an accumulation of neutral lipids in the host cell. In addition to serving as a nutrient source for the bacilli, the perturbation in lipid homeostasis also aids in the inhibition of bactericidal pathways such as autophagy, lysosome acidification, ROI/RNI production, and mediators of the inflammatory response. As a result, a privileged niche is thus generated which then also allows the mycobacteria to slow down their replication, with a concomitant increase in the drug-sensitivity threshold. Thus through strategic re-calibration of the metabolic machinery, Mtb successfully transforms a hostile phagocytic cell into a secure haven that now actively supports bacillary survival and persistence.

December 14, 2016, 18.15 pm

## A novel data-independent acquisition (DIA) mass spectrometry approach integrated with RNA-seq for deep proteogenomic profiling

Akhilesh Pandey<sup>1,2,3</sup>

<sup>1</sup>McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore MD, USA

<sup>2</sup>Department of Biological Chemistry, <sup>54</sup>Pathology, <sup>6</sup>Oncology, Johns Hopkins University School of Medicine, Baltimore MD, USA

<sup>3</sup>Institute of Bioinformatics, International Technology Park, Bangalore, India

We have recently initiated integrated analysis of mRNAs with high resolution mass spectrometry for proteogenomic analysis of individual cell types. The availability of corresponding RNA-seq data provides a unique opportunity to search a database of peptides encoded only by mRNAs expressed in the individual cell types - i.e. intra-exonic as well as junctional (encoded by exon-exon junctions) peptides. This strategy also allowed us to directly interrogate the non-synonymous SNPs that were translated into proteins. However, one problem of current data-dependent approaches is that the more abundant peptides are readily identified while those that are less abundant (e.g. regulatory proteins such as transcription factors, certain alternative protein isoforms, proteins with alternative start sites, peptides from translation of microORFs) are not sampled. Data-independent acquisition (DIA) strategies provide an opportunity to sample peptides that would otherwise not be selected for fragmentation. However, the current implementation of such DIA approaches relies on very large  $m/z$  windows, which precludes identification of low abundance peptides. We have developed a novel pipeline to carry out DIA analysis on an Orbitrap Fusion Lumos mass spectrometer using very small  $m/z$  windows for fragmentation. We analyzed primary human umbilical vein endothelial cell (HUVEC) lysates by scanning sequentially through 150 Th windows in each LC-MS/MS run using the advanced quadrupole mass filter followed by fragmentation of peptide ions in the HCD cell in small  $m/z$  windows and ultimate detection of fragment ions in the Orbitrap mass analyzer. Several runs were carried out to cover the entire (350-1,450  $m/z$ ) mass range on an Orbitrap Fusion Lumos mass spectrometer. Our strategy allowed us to "enrich" several low abundance proteins and peptides, including post-translationally identified peptides, which would otherwise be missed by conventional strategies. The expression of these low abundance proteins was corroborated with the expression of transcripts in RNA-seq data. Overall, this approach can be coupled to any upstream fractionation/enrichment method for a more comprehensive characterization of the proteome.

December 15, 2016, 9.00 am

## Mapping Dynamic Protein Interaction Landscapes in *Saccharomyces cerevisiae* using a Novel Whole Network Enrichment Approach

John R. Yates, III, Stein, Benjamin D., Calzolari, Diego, Lavallée-Adam, Mathieu

Department of Chemical Physiology, The Scripps Research Institute, USA

We have developed and applied methods to annotate the architecture and dynamics of protein complex networks by utilizing a novel whole-network affinity pull-down method coupled to novel computational analysis tools. This method involves generation of TAP-tagged node proteins, which are expressed, pooled and affinity-purified in parallel. This approach allows for efficient system-wide identification and quantification of protein complex members and novel interactors in response to perturbation in a highly efficient manner. This proteomic platform has been enabled through development and application of biochemical and proteomic methodologies to study network response to known perturbations in established protein networks. Specifically, as a proof-of-principle, the developed platform has been employed to study changes in nutrient sensing protein complexes in response to rapamycin treatment in *Saccharomyces cerevisiae*.

This concept has been extended to the DNA damage response as well as energy homeostasis networks in yeast, and the latter correlated to a homologous mammalian network for investigation of metabolic dysregulation in disease. By measuring the dynamics of protein networks, we have identified novel associations within and between components that have otherwise been missed in traditional protein-by-protein Affinity Purification-Mass Spectrometry (AP-MS) methodologies, and this is the first proteomic platform to enable dynamic interaction measurements at a network-wide scale.

December 15, 2016, 17.20 pm

### **Systems Biology of Infectious Disease**

**John Aitchison**

*Professor, Institute for Systems Biology, Professor and Chief Science Officer, Center for Infectious Disease Research, USA*

With a focus on revealing and understanding the complex interplay between a pathogen and its host, systems biology holds promise for diagnosing, predicting and preventing infectious diseases. This presentation will discuss ways in which systems biology approaches can lead to better understanding of host responses to infection, pathogen biology, and interactions between hosts and pathogens.

December 16, 2016, 9.00 am

### **High Throughput Protein Microarrays for Biomarker and Target Discovery**

**Joshua LaBaer**

*The Biodesign Institute, Arizona State University, USA*

Self-assembling protein microarrays arrays can be used to study protein-protein interactions, protein-drug interactions, search for enzyme substrates, and as tools to search for disease biomarkers. In particular, recent experiments have focused on using these protein microarrays to search for autoantibody responses in cancer patients. These experiments show promise in finding antibody responses that appear in only cancer patients. New methods using click chemistry-based reagents also allow the application of these arrays for discovering new substrates of post translational modification.

December 17, 2016, 9.00 am

### **Nuclear Phosphoproteomics to Identify the Abiotic Stress Tolerant Mechanism in Crop**

**Setsuko Komatsu**

*Institute of Crop Science, National Agriculture and Food Research Organization, Japan*

Climate change is considered a major threat to world agriculture and food security. To improve the agricultural productivity and sustainability, the development of high-yielding stress-tolerant and climate-resilient crops is essential. Of the abiotic stresses, flooding stress is a serious hazard because it reduces growth and yield of crop. Proteomic analyses indicate that the effects of flooding stress are not limited to oxygen deprivation, but include many other factors. Although many flooding response mechanisms have been reported, flooding tolerance mechanisms have not been fully clarified for soybean. To identify the mechanism of flooding tolerance in soybean, proteomic technologies are used. Flooding-tolerant mutant

and other materials of soybean were used. The upstream event controlling the regulation of many kinds of flooding-responsive proteins was elucidated based on proteomic analyses of nuclear proteins and phosphoproteins. In soybean, initial flooding stress was perceived and transmitted into nucleus and led to the decrease of mRNA transport/boxCDsnoRNAs and histone variants related proteins. The decrease of nuclear proteins suppressed mRNA export/pre-ribosomal biogenesis and chromatin structure. The changes happened in nucleus continuously regulated cytoplasmic events including inhibition of protein synthesis, energy metabolism, and cell wall formation through phosphorylation. Ethylene weakened flooding tolerance of soybean through promoting seedling weight and energy consume; however, ABA enhanced flooding tolerance of soybean through retarding plant growth and controlling energy conservation. This work will expedite transgenic or marker-assisted genetic enhancement studies in crop for developing stress-tolerant lines or varieties under abiotic stress.

## ABSTRACTS FOR KEYNOTE TALKS (K001-K055)

December 14, 2016

K001

### **Integrated Metabolomics for Deciphering Metabolic Pathways and Emerging Solutions for Addressing the Grand Challenges of Metabolomics.**

**Feng Qiu, Dennis Fine, Aiko Barsch, Daniel Wherritt, Zhentian Lei, Bonnie Watson, VeredTzin, Daniel Wherritt, Dong Sik Yang, John H. Snyder, David V. Huhman, Stacy Allen, Yuhong Tang, Derek Nedveck, John Stanton-Geddes, Peter Tiffin, Nevin Young, and Lloyd W. Sumner**

*Professor Christopher S. Bond Life Sciences Center, University of Missouri Columbia, USA*

The research focus of the Sumner lab is multi-disciplinary in that we seek to develop novel metabolomics technologies and then use these technologies for discoveries and to better understand plant specialized metabolism. This presentation will first illustrate the application of integrated metabolomics for gene discoveries in plant specialized metabolism. The presentation will then focus on the development of computational and empirical solutions to address the number one grand challenge of metabolomics which is the large-scale and confident metabolite identifications.

This presentation will describe our integrated metabolomics platform and its use for novel gene discoveries associated with triterpenesaponin biosynthesis in the model legume, *Medicago truncatula*. Triterpenesaponins are structurally diverse specialized metabolites found in many plant families, including the Leguminosae. They possess a broad spectrum of bioactivities including allelopathic, anticancer, antifungal, antibacterial, anti-insect and anti-nutritive properties. In spite of their functional importance, many steps in the triterpenesaponin biosynthetic remain uncharacterized. We are using integrated metabolomics, correlated gene expression profiling and genome wide association studies (GWAS) for the discovery, prioritization, and characterization of novel saponin biosynthetic genes in the model

legume *Medicago truncatula* which is known to accumulate a large variety of differentially glycosylated saponins.

Large-scale and confident metabolite identifications is the number one grand challenge faced in modern metabolomics, and we are building both computational and empirical solutions to address this challenge. This presentation will describe a new Plant Metabolite Annotation Toolbox (PlantMAT) that includes methods for first predicting the metabolic potential of a plant system using informed phytochemistry and then searching the predicted and public databases with experimental LC-MS/MS data for metabolite identification prediction. This presentation will also describe a sophisticated UHPLC-MS-SPE-NMR approach that we are developing for higher-throughput empirical metabolite identification. The combination of computational and empirical identification has been very successful in the identification of special metabolites in *M. truncatula*.

K002

### **Understanding Immune Signaling in Plant: A System Biology Approach**

**Subhra Chakraborty**

*National Institute of Plant Genome Research, JNU Campus, ArunaAsaf Ali Marg, New Delhi-110067, India*

Plant health and agricultural productivity is one of the key issue to the sustainable food production worldwide. Patho-stress constraints plant growth and development and thus crop productivity. Immune-response in plant is a complex phenomenon and the exact physiological relevance and functional modification caused as a result of disease is poorly understood. Extracellular signals perceived at cell surface, ECM in particular trigger signaling pathways that branch to several biological responses. Eukaryotic nucleus is the regulatory hub and a dynamic repository for various biomolecules that dictate cell signaling processes. It not only hosts the genome, but also administers its transcription and the regulated expression of proteins, thereby playing critical role in cellular functions. In an

attempt to dissect the molecular circuitry of host-specific innate immunity in plants, we have applied a system approach to analyze vascular wilt responsive comparative organ-specific transcriptome and organeller proteomes in a food legume, chickpea. Network modeling based on our datasets illustrates immune responsive regulatory hubs and functional modules towards species-specific adaptation for cell survival. Critical analysis identified a bHLH domain containing hub protein of unknown function designated as immune-responsive factor 1, IRF1. Expression analysis of *IRF1* revealed its involvement in multivariate stress response. Protein-DNA interaction prediction indicates a sequence specific DNA-binding property for IRF1. Overexpression of IRF1 conferred wilt resistance in transgenic arabidopsis while functional complementation of the *Arabidopsis* mutant could rescue its diseased phenotype. Taken together, these results suggest IRF1 to be a major determinant of innate immunity. These findings might expedite the functional determination of the immunity related proteins and their prioritization as potential molecular targets for better adaptation and pathway bioengineering for healthier crops with enhanced yield.

K003

### **Label free and TMT proteomic analysis of two different rice varieties with contrasting drought tolerance phenotypes**

**Yunqi Wu, Mehdi Mirzaei, Dana Pascovici, Joel M. Chick, Brian J. Atwell and Paul A. Haynes**

*Dept of Chemistry & Biomolecular Sciences, Macquarie University, Australia*

We have undertaken a detailed quantitative proteomics analysis of two different rice varieties exposed to a time course drought stress regime. The two rice cultivars have contrasting genetic backgrounds, with Nipponbare a widely planted commercial variety and IAC 1131 known to be more tolerant to stress. Four-week-old seedlings of both varieties were exposed to moderate and extreme drought stress for seven days, followed by three days of re-watering. Leaves were harvested, proteins were extracted, and quantitative proteomic analysis was performed using both Tandem Mass Tag (TMT) labelling and label free quantitative

proteomics using normalised spectral abundance factors (NSAF). The results show that IAC 1131 seems to be able to cope with stressful conditions by introducing expression of a range of stress response and defence response related proteins. One such protein of particular interest was the ClpD1 protease, which has been correlated with stress tolerance in a range of biological systems. Nippon bare, on the other hand, appears to initiate a substantial reduction in chlorophyll biosynthesis in response to stress, in an apparent attempt to preserve metabolic resources.

K004

### **Host genetics influence the virulence of *E.coli* pathogens by altering glycan anchors in the gut epithelium**

**Emøke Bendixen**

*Institute for Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark*

Human health is closely linked to the health of animals, plants and microbes. This has become very clear with the rapid spread of microbial resistance to antibiotics (AMR), which currently poses a major threat to global human health. AMR is a direct consequence of a massive use of antibiotics both for humans and farm animals. With *E. coli* infections in pigs consuming 50 % of the total global antibiotics market, there is an urgent need to understand the host-pathogen interactions of *E. coli* and pigs.

We have studied a natural gene variant in the pigs FUT1 gene, that protects pigs from *E. coli* F18 strains. This model provides a unique opportunity to study host pathogen interactions that influence *E. coli* virulence.

We used mass spectrometry to characterize glycoproteins in the pigs gut epithelia, and found that FUT1 gene variants influence the glycan structures that are essential for adhesion of both pathogens and commensal bacteria in the pig gut. This talk will present our studies of how pig gene variants influence health, growth, microbiomes and glycan structures in the pig.

Our research is directly aimed to reduce the need for antibiotics in pig production, but the knowledge we create is essential also for developing pig models that are useful for human biomedical

research. Because of the close physiological and genetic similarities between humans and pigs, these models can support future studies of metagenomics, nutrigenomics and host-pathogen interactions in both pig and man. This talk will also present examples of relevant pig model models we have studied in the recent years.

K005

### **Complex responses of glycosylphosphatidylinositol-anchored proteins in association with plant cold acclimation**

**Daisuke Takahashi, Yukio Kawamura and Matsuo Uemura\***

*Department of Plant-biosciences and Cryobiofrontier Research Center, Iwate University, Morioka 020-8550, Japan*

Cold acclimation in plants results in alterations of plasma membrane protein composition, which is critical to increase in freezing tolerance. Although many studies have reported cold-acclimation-responsive plasma membrane integral and peripheral proteins, lipid-associated proteins in the plasma membrane including glycosylphosphatidylinositol-anchored proteins (GPI-APs) have not yet been characterized because of their low abundance. GPI-APs are considered to localize in the plasma membrane including microdomains that are regions enriched in specific lipids and proteins within the plasma membrane. Previously, in animal cells, GPI-AP functions associated with plasma membrane microdomains have been extensively discussed and some GPI-APs are considered to be released by endogenous phospholipase C activity from plasma membrane surface and transferred to extracellular matrix. In this study, thus, we aimed to investigate the responsiveness of GPI-APs to cold acclimation treatment in *Arabidopsis*. Label-free quantitative shotgun proteomics combined with a GPI-AP enrichment protocol by endogenous phospholipase C identified a number of GPI-APs (163 proteins). Some GPI-APs such as fasciclin-like arabinogalactan proteins and glycerophosphoryldiester phosphodiesterase-like proteins predominantly increased in plasma membrane and GPI-AP-enriched fractions while the changes of GPI-APs in the plasma membrane microdomains (and

apoplast) were small. These proteins are thought to be associated with cell wall structure and properties. We further analyzed aGPI-AP(At3g04010) that encodes a putative plasmodesmal callose degrading enzyme, beta-1,3-glucanase. mRNA expression of At3g04010 was transiently upregulated in vascular bundles during cold acclimation. At3g04010 knockdown mutants showed impaired freezing tolerance increase after cold acclimation and abnormal callose deposition during cold acclimation. Fluorescence of mCherry-tagged At3g04010 protein distributed similarly as callose in vascular tissues and plasmodesmata, implying the involvement of At3g04010 protein in carbohydrate distribution in tissues/areas associated with substance transport. This study suggests that responses of GPI-APs to cold are associated with readjustment of functions associated with the plasma membrane towards acquisition of freezing tolerance.

K006

### **Proteomics of a Himalayan Shrub, A Step Towards Translational Research.**

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Himalayan vegetation grows under harsh conditions like cold/freezing temperature, drought, hypoxia and high UV radiation. Seabuckthorn (*H.rhamnoides*), a Himalayan shrub is freezing tolerant and has pharmaceutical and nutraceutical properties. Therefore, it was used to decipher cold/freeze stress signaling following proteomics approach. Antifreeze proteins (AFPs) accumulate during freezing in apoplast as a defence mechanism and have applications in medicine and food industry.

2-DGE with nLC-MS/MS detected cold/freeze modulated proteins by differential profiling of cold/freeze treated laboratory grown seedlings. AFP activity was analysed (ice crystal morphology, TH) by Nanoliter osmometer coupled with phase contrast microscope and (Ice recrystallisation inhibition) by Splat assay. AFPs were purified by (Ice and chitin affinity chromatography). RBCs cryopreservation was analyzed by Harboe

spectrophotometrically. Green gold nanoparticles (GNPs) synthesized using leaf and berry aqueous extracts were analyzed by UV-Visible spectroscopy, Transmission electron microscope (TEM), Fourier transform infrared spectroscopy (FTIR) and X-ray powder diffraction (XRD). Antimicrobial and Nanocatalytic potential of GNPs was investigated using Agar diffusion method and 4-Nitroaniline (NA) reduction.

Differential display of cold/freeze modulated proteins identified 61 candidates from the reproducible repertoire (245 spots) including dual specific AFPs like chitinases, thaumatin like proteins and  $\alpha$ -1,3 glucanases besides stress signaling proteins Calmodulin, WRKY, Glyoxylase and GTPases. Out of three AFPs (two Class I chitinases and PGIP) purified, HRCHT1a showed maximum protection and cell survival against freeze-thaw induced haemoglobin leakage of rat RBCs at 40  $\mu$ g/ml. TEM and EDAX analysis confirmed monoisotropic spherical GNPs ( $27 \pm 3.2$  nm) by leaf and anisotropic ( $55 \pm 4.5$  nm, spherical, truncated triangles, hexagon) GNPs using berry extract. XRD showed diffraction peaks of gold at (111), (200), (220) and (311). FTIR analysis showed hydroxyl, carboxyls and amide groups confirming reductive and stabilizing properties of phenols, flavonoids and amino acids. GNPs exhibited antimicrobial and nanocatalytic activity by rapid (20-50 mins) reduction of 4-NA, a water contaminant.

Seabuckthorn AFPs can be used as RBCs cryopreservative. GNPs antimicrobial and nanocatalytic activity, may have applications in biomedicine and water decontamination.

K007

### **Decoding the role of diazotrophic cyanobacterial AhpC in abiotic stress management using proteomics and genomics approaches**

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AhpC, 1-cys peroxiredoxin, distributed across living organisms, is an antioxidant protein offering protection from reactive oxygen species. Its

biochemical and molecular characterization following heterologous expression in *E.coli* demonstrate cross tolerance to a host of abiotic stress. Its transgression in the cyanobacterium produced enormous change in the proteome. A comparative proteomics of ahpC-overexpressing (AnFPNahpC), ahpC mutant ( $\Delta$ ahpC) and wild type control *Anabaena* PCC 7120 unveiled AhpC-triggered two major events : (i) fold increase in proteins of metabolically most significant variables e.g. nitrogen fixation (1.6), photosynthesis (PSI, 1.08; PSII, 2.137), respiration (5.66) in AnFPNahpC and their (nitrogen fixation, PSI) PSII, respiration) down regulation in  $\Delta$ ahpC as compared to control cells, and (ii) appreciable upregulation of antioxidant defense proteins viz., and their subsequent down regulation in  $\Delta$ ahpC indicates the regulatory function of ahpC which was further attested by string network. where ahpC specifically interacts with upregulated GroEL (2.04) and SODA (1.32 fold) of AnFPNahpC. These proteins were down regulated in the  $\Delta$ ahpC as compared to the wild type. In view of enhance nitrogen fixation, photosynthesis and tolerance to a host of abiotic stress the transgenic *Anabaena* holds potential for application in rice fields in years to come.

K008

### **Omics Discovery Index - Discovering and Linking Public 'Omics' Datasets**

**Henning Hermjakob**

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Biomedical data, in particular omics datasets, are being generated at an unprecedented rate. As a result, the number of deposited datasets in public repositories originating from various omics approaches has increased dramatically in recent years. However, this also means that discovery of all relevant datasets for a given scientific question is non-trivial. Here, we introduce the Omics Discovery Index (OmicsDI - <http://omicsdi.org>), an integrated and open source platform facilitating the access and dissemination of omics datasets. OmicsDI provides a unique infrastructure to integrate datasets coming from multiple omics studies, including at present transcriptomics, proteomics, genomics and metabolomics, as a globally distributed resource.

Building on the experience of the ProteomeXchange consortium<sup>1</sup>, we have developed a lightweight central metadata-based portal for the efficient discovery of Omics datasets. Each participating omics data resource provides a minimal metadata description for each relevant dataset, and these are then indexed by Lucene, an industry standard document indexing system. The resulting resource can be accessed for search and browsing through a web interface, as well as through computational web services. As only data descriptions (metadata) are indexed, OmicsDI is resource-efficient and relatively easy to maintain up to date. For actual data access, all dataset descriptions reference the source repository, avoiding the replication of often very large raw data sets.

As of June 2016, OmicsDI provides a lightweight discovery tool including more than 70,000 omics datasets from ten different repositories, three different omics types, and three continents. In the interest of sustainability, the responsibility for provision of a well-formatted metadata records lies with the original data providers, similarly to the concept of publisher data provision to PubMed or EuroPMC.

OmicsDI supports full text search as well as ontology-based search extensions. In addition, we use the concept of biological dataset similarity, based on the number of shared biological entities, for example protein identifications, among datasets. This allows us to suggest potential relationships among datasets even if they don't share sufficient metadata annotation.

In conclusion, OmicsDI provides a central, lightweight index for currently more than 70,000 omics datasets from ten different repositories, supporting one-stop discovery of relevant, potentially multi-omics, datasets. OmicsDI is accessible at <http://www.ebi.ac.uk/Tools/omicsdi/>.

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<sup>1</sup> Vizcaíno JA, et al. ProteomeXchange provides globally coordinated proteomics data submission and dissemination. Nat Biotechnol. 2014 Mar 10;32(3):223-6.

## Chemical crosslinking mass spectrometry of protein complexes using cleavable crosslinkers: Establishing quantitative connectivity diagrams

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Chemical crosslinking as a method to probe protein interactions and provide distance constraints for protein solution structures has been one of the significant challenges in structural mass spectrometry despite the fact that chemical crosslinkers have been widely used in Biochemistry for several decades. New crosslinking reagents developed by several laboratories and with a variety of positive properties (the most useful of which is high lability to collision-induced dissociation) have made chemical crosslinking to probe complex structures much more practical. Our laboratory has developed several highly reactive CID-labile crosslinkers that have a number of other useful features, including maintenance of surface charge distribution, high stoichiometry, and high solubility. One of these, DC4, that targets Lys residues, has been used to map several protein complexes (model and unknown) and contributed to determination of their solution structures. Quantification is as important for crosslinking analyses as it has been for most proteomics applications but is much more challenging to implement. We have examined the use of label-free methods for the analysis of crosslink data and have defined the limits of this approach as well as its advantages. We have also used relative quantification using heavy and light isotope forms of DC4 to characterize protein complexes. Chemical crosslinking has proven to be a sensitive probe of changes in the solution structures of protein complexes in our hands.

K010

## Interaction Codes in Protein Interaction Networks

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Methylation of proteins occurs predominantly on arginine and lysine residues in the eukaryotic cell. Until recently, its predominance was unknown and its role obscure. Here we describe the construction of the first 'methylproteome network' for a eukaryotic cell and present evidence that methylation modulates protein-protein interactions in this network. We also report that arginine methylation can exist in crosstalk with phosphorylation in an SRGG motif found on many proteins. Strikingly, this can modulate the interaction specificity of proteins with many interaction partners, forming a specific 'interaction code'.

The yeast proteome was analysed for methylated proteins and to determine precise sites. Targeted data acquisition - electron transfer dissociation LC-MS/MS was used, as were yeast proteome arrays (containing 4,400 chips spotted onto microscope slides). To build the intracellular methylation network, all known and putative methyltransferases in yeast were knocked out and the methylproteome re-analysed to determine which enzymes were responsible for which methylation events on which sites. Enzyme-substrate links were further investigated by the LC-MS/MS analysis of recombinant substrate proteins methylated by recombinant enzymes, by *in vivo* methylation assays and/or the incubation of proteome arrays with recombinant enzymes. Validated enzyme-substrate links were integrated with the yeast interactome to generate the 'methylproteome network'. Kinase-substrate interactions were also co-mapped onto this network, highlighting proteins that are potentially subject to modification crosstalk. A new 'conditional two-hybrid' (C2H) system was constructed to test whether modifications can modulate protein-protein interactions.

Our analyses, together, showed that protein methylation is widespread in the eukaryotic cell. We discovered three new eukaryotic protein

methyltransferases, elongation factor methyltransferases 2, 3 and 7. Mammalian orthologs were also discovered for a number of these. Our integrated methyltransferase-substrate protein and protein-protein interaction network suggested that methylation might be a major modulator of protein-protein interactions. This was proven by our 'conditional two hybrid' system, in that half of the protein pairs involving arginine methylation showed a significant increase in interaction on methylation. Where phosphorylation was found adjacent to methylation, in a conserved but repeatedly used SRGG motif, we found that this can interfere with methylation and decrease certain protein-protein interactions. This raised the prospect of this and other modification-associated 'interaction codes' that exist inside protein interaction networks. Our general hypothesis of the protein 'interaction code' will be discussed.

K011

## Integrating the role of FOXP3-interactomics in IL10 transcription in tumor T-regulatory Cells

Gaurisankar Sa

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**Introduction:** The regulatory T (Treg) cell lineage is indispensable for induction of T cell tolerance, which happens to be one of the mechanisms of cancer immune evasion in tumor condition. FOXP3, a lineage-specification factor required for Treg cell differentiation and function, executes its multiple activities mostly through transcriptional regulation of its target genes. The molecular bases underlying the phenotypic and functional diversity of FOXP3<sup>+</sup> Treg cells remain obscure.

**Experimental Procedures:** Various biochemical, interactomic, proteomic, structural biology, micro-imaging, promoter assay as well as immunological methods were employed to unveil the mechanisms of *IL10* gene transcription in Treg cells in breast cancer model.

**Results:** Adding to the knowledge of abundant T cell plasticity in terms of cytokine production our study identified a population of FOXP3-expressing IL10-producing adaptive Treg cells, a subtype

distinct from FOXP3-negative IL10-producing class-1 Treg cells that contributes to IL10-dependent type-2 cytokine bias in breast cancer patients. An in-depth analysis reveals that FOXP3, in association with HAT1 modify *IL10* promoter epigenetically, making a space for pocketing STAT3-FOXP3 complex. As soon as HAT1 modify *IL10* promoter the FOXP3 dimer forms complex with dimeric STAT3. The interaction between FOXP3 and STAT3 induces certain conformational change which drives them to bind with chromatin DNA. A high-throughput docking module with target-receptor specificity and exon deletion mutation study showed that STAT3 dimer binds specifically to the exon-2 b-sheet region of FOXP3 through its N-terminal floppy domain to form STAT3-FOXP3 complex. Such activity of FOXP3 is extended to other STAT3 target genes, e.g., *IL6*, *VEGF*, *c-MYC*, *BCLxL*, *CCND1*, that lack FOXP3-binding site.

**Conclusion:** These results suggest a novel function of FoxP3 where failing to achieve direct promoter-occupancy, FOXP3 promotes transcription in association with a locus-specific transcription factor, STAT3, in T<sub>reg</sub> cells.

December 15, 2016

K012

## Direct conversion of stem cells by changing single protein dynamics

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Recently, stem cell therapy emerged as one of the promising ways for treatment of intractable diseases in human, however, the satisfactory results have not been reported yet in many diseases including stroke, acute myocardial infarction, AD, or PD, etc.

**Stem cell conversion:** We developed the novel algorithm for predicting condition-specific subcellular locations of the gene coding proteins at genome-wide level using only limited and condition-unspecified known locations. With systems biological mRNAs analysis of human stem cells using this method, the key target genes and their coding proteins which involved in maintaining pluripotency and differentiation process were

identified. Molecular biological manipulation of these proteins induced direct conversion of stem cells.

**Niche targeting:** Here we report that the lesions induced the activation and accumulation of macrophages, and the activated macrophages synthesize and secrete AGE-albumin which is critical for host cell death. Combined injection of hBD-MSC and the AGE-albumin inhibitors enhanced both the survival of hBD-MSC and the angiogenesis, and reduced the degeneration significantly in animal mouse models. Our data revealed that AGE-albumin from activated macrophages is critical for both host muscle cell death and hBD-MSC death.

**In conclusion:** Therefore, the combination of stem cells conversion and niche targeting could be one of the successful therapeutic strategies in stem cell treatment for intractable diseases.

K013

## Comparative proteomics of human fungal pathogens

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*Aspergillus flavus* and *Fusarium* infect the human eye leading to keratitis. Extracellular proteins, the earliest proteins that come in contact with the host and virulence related exoproteins, were identified in the fungus isolated from infected cornea. Virulence of the corneal isolates was tested in the *Galleria mellonella* larvae model and those isolates showing higher virulence were taken for subsequent exoproteome analysis. High-resolution two-dimensional electrophoresis and mass spectrometry were used to generate *A. flavus* exoproteome reference map as well as to profile most of the exoproteins. Analysis of the identified proteins clearly shows the major biological processes that they are involved in. Nearly 50% of the exoproteins possess catalytic activity and one of these, an alkaline serine protease (Alp1) is present in high abundance as well as multiple proteoforms. Many proteins in the *A. flavus* exoproteome have been shown to be virulence factors in other pathogens indicating the probable role for these

proteins in the corneal infection as well. Interestingly, the majority of the exoproteins do not have secretory signal indicating that they are secreted through the non-classical pathway. Thus, this study provides a clue to the early strategies employed by the pathogen to establish an infection in an immunocompetent host. Comparison of proteome profile of *Fusarium* will be discussed.

K014

### **Proteomics Study of Protein Methylation in *Plasmodium falciparum* at asexual blood stages**

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*Plasmodium spp.* are obligate intraerythrocytic protozoans parasites that undergo a number of developmental stages in the vertebrate host and mosquito vectors. Despite continuous efforts to eradicate malaria for over 4 decades, it still remains a major health problem mainly due to spread of drug resistance to multiple drugs, due to resistance of mosquitoes towards insecticidal agents and also because of complex life cycle of the parasite. There is thus a need to understand the biology of the parasite, importantly the gene regulatory processes to identify new drug targets. Evidence are emerging to suggest that Post-translational modifications (PTMs) play important role in regulating the fundamental aspects of *Plasmodium* growth and enzymes involved in PTMs are important targets for the development of new anti-malarials.

In recent years, methylation of proteins has been ranked as fourth common post-translational modification. In *Plasmodium*, we and others have shown the presence of enzymes involved in protein methylation as well as its role in parasite development. Here, we describe the proteome-wide analysis to know the extent of arginine and lysine methylation of *Plasmodium* proteins at asexual blood stages of the parasite. To identify the *Plasmodium* “**Methylome**”, we first tested the reactivity of anti methyl lysine as well as anti-methyl arginine antibodies with the asexual stage *P. falciparum* parasites. These antibodies recognized well the

*Plasmodium* proteins. We next prepared the parasite lysates and immunoprecipitated the parasite extracts from three asexual blood stages; ring, trophozoite and schizont. The immunoprecipitated samples were subjected to mass spectrometry analysis after limited trypsin/chymotrypsin digestion. Using LC-MS/MS analysis, we could identify 605 lysine methylated sites within *Plasmodium* 422 proteins and in case of arginine methylation, we could identify 843 *Plasmodium* proteins. Motif analysis revealed lysine and arginine methylation associated with GK, MK, GRx/RGx, RxG, GxxR motifs. Many of the *Plasmodium* methylated proteins have homologs known to be methylated in *Trypanosome*, human and yeast. Functional classification of methylated proteins revealed that these proteins are mainly involved in chromatin organization, trafficking and homeostatic processes and protein folding. Overall, our findings reveal that protein methylation is widespread in *Plasmodium* and plays an important regulatory role in diverse set the parasite pathways.

K015

### **Proteogenomic approaches for biomarker discovery**

**Robert L. Moritz**

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Proteomics technology continues to advance in providing unprecedented levels of identification and quantitation of proteins across a multitude of organisms. Coupled with these new technologies, new methods and bioinformatic approaches have now enabled the biologist to understand the dynamic proteome landscape beyond the static genomic blueprint. To capitalize on these advances, we and others have made available democratized resources to enable the quantitative interrogation of biologically meaningful data. Given these advances, we now find ourselves in an era of exciting capabilities in revealing comprehensive quantitative proteome measurements to advance our studies in biological systems and targeted biomarker studies at greater depth and speed than ever before. Our goal is to apply our quantitative tools to identify specific biomarkers to disease such as chronic kidney disease and provide capabilities in delivering tangible results in healthcare.

## **Development of redox proteomics technologies and application in stomatal guard cell immunity research**

**Sixue Chen**

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Plant pathogens have caused serious crop losses, and they gain entry into leaves through natural stomatal pores on leaf surface. Stomatal innate immunity responses are fast processes that take place within the first few minutes of pathogen exposure. One objective of my lab research is to identify molecular switches that regulate the fast processes of stomatal movement in response to pathogen invasion. A novel redox proteomics method was developed to identify protein redox switches. The method was called cysTMTRAQ that combines two types of isobaric tags, cysteine tandem mass tags and isobaric tag for relative and absolute quantification, in one experiment. The method not only enables simultaneous analysis of cysteine redox changes and total protein level changes, but also allows the determination of bona fide redox modified cysteines in proteins through the correction of protein turnover. Using this powerful method, we were able to create an inventory of previously unknown potential redox regulated proteins, and highlight some potential regulatory mechanisms in stomatal guard cell innate immunity. Among these proteins, we identified a lipid transfer protein-II (LTP-II) undergoing oxidation in response to flg22 during stomatal closure. LTPs are small, basic proteins present in higher plants. They are known to be involved in key cellular processes such as stabilization of membranes, cell wall organization, and signal transduction. LTPs are also known to play important role in response to biotic and abiotic stresses, and in plant growth and development. Using reverse genetics, we have conducted functional studies of the LTP-II in stomatal guard cells. Here a potential mechanism by which LTP-II functions in stomatal guard cell defense response will be discussed.

## **Platelet Proteomics in Blood Disorders**

**Abhijit Chakrabarti**

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Platelets play important roles in thrombosis, inflammation, wound repair and stroke. They are characteristically small and discoid in resting state and normally circulate in blood at levels of approximately  $150\text{-}400 \times 10^9/\text{L}$ . Clinical proteomics studies are done for profiling of platelet proteins in healthy versus pathological states to discover specific differentially regulated proteins using 2D gel based electrophoretic separation (2DGE) followed by tandem MALDI mass spectrometry for identification and characterization. Densitometry results obtained from 2DGE are further validated by immunoblotting. Purity and morphology of the platelets were checked by flow cytometry using CD41a-FITC antibody and TEM analysis respectively. Nearly 800 spots were identified using PDquest software and 100s of proteins annotated by combined searches (MS+MS/MS).

Differential regulations of cytoskeletal proteins e.g. actin binding proteins - myosin regulatory light chain6, coactosin, actin related protein 2/3 complex, redox regulator peroxiredoxin2 and pathway specific proteins transgelin2, transthyretin and protein disulfide isomerase (PDI) have been observed in Harris Platelet Syndrome, an asymptomatic constitutional macrothrombocytopenia (ACMT) and autosomal dominant platelet disorder characterized by mild-to-severe thrombocytopenia and presence of giant platelets. Numerous cases of thalassemia have been reported with thromboembolic complications due to the hypercoagulable state, the mechanism of which is not well understood. Our study revealed elevated levels of chaperones like HSP70, PDI, peroxiredoxin2 and superoxide dismutase1 along with high ROS levels. Upregulation of translation initiation factor 5a is also observed in thalassemia, playing a protective role toward cell survival under oxidative stress. Thrombohaemorrhagic complications are found in chronic myeloid leukemia. Elevated levels of enzymes like GST, LDH, calcium binding proteins, calreticulin and regulatory protein 14-3-3  $\epsilon$  along with chaperones

and regulatory proteins indicate towards regulation of Integrin binding and platelet activation.

K019

K018

## **Discovery and Development of Multiplexed Protein Biomarker Tests to Support Clinical Decision Making**

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Despite a few decades of proteomics research and the apparent discovery of multiple protein biomarkers to support the diagnosis and treatment of patients, the number of biomarkers that have been developed to the stage of being used routinely is disappointingly low. So, it's clear that whilst there's huge interest in the development of new biomarkers and major drivers for this to happen, including the pharmaceutical industry's need to better stratify patients for effective treatment, as yet biomarker development and delivery is proving very challenging. This presentation will cover:

*The problem.* For many diseases, developing biomarkers to support the decisions of which patients to treat, when to treat them and with what treatment, remains challenging.

*Biomarkers of Utility.* The use of proteomics for new protein biomarker discovery has not yielded many clinically used biomarker tests - why is this?

*A strategy.* A pragmatic, 'real world' and patient-centric approach for the discovery development and delivery of biomarkers of potential clinical utility will be introduced.

*Some solutions.* The application of this strategy for the development of biomarker tests to support clinical decision in *prostate cancer* and *psoriatic arthritis* will be described.

*Delivery.* Potential strategies for the delivery and implementation of multiplexed protein biomarker tests will be explored.

## **Changing medicine: Proteome centric precision health**

**Justyna Fert-Bober<sup>1</sup>, Irene van den Broek<sup>1</sup>, Erin Crowgey<sup>1</sup>, Vidya Venkatraman<sup>1</sup>, Ronald Holewinski, Qin Fu, Jennifer E. Van Eyk**

Cedars-Sinai Medical Center, Los Angeles, CA, USA

Precision health requires success in two intertwined aspects: precision therapy and personalized medicine. Precision therapy is effectively treating the right disease, to have therapies that target for the correct pathological pathways. Personalized medicine requires diagnosing a specific individual's disease based on accurate assessment their complex health and pathological status. Our underlying premise is that an individual's baseline proteome reflects their past and present and thus, will dictate their future health and disease. We have combined tracking citrullinated proteins, an irreversible post-translational modification that can drive disease dysfunction as well as induce autoantibodies. The ability to quantify citrullinated peptides by mass spectrometry has been improved by combing searches against a library comprised of the maximal number of citrullinated peptides, data independent acquisition and downstream analytes. We have been able to identify and precisely quantify proteins and their modified forms in 5 target organs in 4 different diseases. As well, we are determining which are acting as autoantigens which could compound their role in disease over time. This work has led us to consider the need for continuous patient-centric health screening. We have developed technical pipelines for patient screening. This has required development of microsampling devices, point of service devices, pathways for client data return and specific clinical grade assays. We have begun down this path with production of system suitability and quality control measures, assays and volumetric sampling device and will discuss the remaining challenges and requirements involved.

(<sup>1</sup>Equal contribution)

K020

## Applying proteomics to develop a precise, predictive test for diabetic kidney disease

**Richard Lipscombe<sup>1</sup>, Scott Bringans<sup>1</sup>,  
Kirsten Peters<sup>1,2</sup>, Jun Ito<sup>1</sup>, Kaye Winfield<sup>1</sup>,  
Thomas Stoll<sup>1</sup>, Wendy Davis<sup>2</sup>, Timothy Davis<sup>2</sup>**

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Diabetes is becoming the greatest health challenge of the 21st century. Globally there are 415 million people with diabetes, of which 69 million are in India. Chronic kidney disease is a significant complication of diabetes that affects 35% of patients, leading to dialysis or kidney transplant, and in 15% of diabetics, death from kidney failure.

Current measures of kidney disease (measurement of albuminuria and/or estimated glomerular filtration rate (eGFR)) cannot predict future decline in kidney function. Clinicians require better tools to deliver more effective diagnosis and monitor treatment to improve health outcomes.

Global and targeted proteomics was applied to discover and validate a panel of novel plasma biomarkers of rapid decline in kidney function in type 2 diabetics. To achieve this a longitudinal clinical study was conducted following 500+ individuals with diabetes over a four year period.

Based on trajectory analysis, 10% of the cohort were defined as 'rapid decliners' in their eGFR. The biomarker panel, termed Promarker D, was able to predict these individuals with 87% accuracy.

K021

## Rapid analysis of chemical cross-linking data with Kojak

**Michael Hoopmann**

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Chemical cross-linking and mass spectrometry have enabled the analysis of protein-protein interactions and protein topologies. In particular, the availability of diverse cross-linking reagents, coupled with advancements in mass analyzer

resolution and throughput, has provided researchers with a formidable arsenal when studying protein interactions. This rapid expansion and diversification of resources has also created significant challenges for the analysis of these data, particularly in computational throughput and statistical validation. I will discuss these challenges and demonstrate solutions we have implemented in the development of our software tools for the identification and validation of cross-linked peptides identified from mass spectra.

December 16, 2016

K022

## Bacterial Transcriptional Activators: Protein Mechanics of their Activity

**David Wemmer**

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Bacterial RNA polymerase with a sigma54 subunit has the unusual property that it can bind to promoter DNA but will not initiate transcription until acted upon by another protein, termed a transcriptional activator. Such activators generally have three domains affecting different functions: regulation, interaction with polymerase and activating it, and binding DNA sequence specifically. The basis for the activity in each of these domains will be discussed. We have studied several different transcriptional regulators and have shown that they can have two distinct regulatory mechanisms, even when acting through the same triggering mechanism (phosphorylation). In contrast we also found that two distinct regulatory domains, with different triggering mechanisms (phosphorylation vs. ligand binding) can act with the same basic mechanism. We determined the structure of the oligomerization-activation domain, which suggests its mechanism of action. The DNA binding domains usually have helix-turn-helix motifs that recognize DNA from the major groove, and may also serve as a dimerization motif. The comparison of structures allows general themes about regulation to be identified, which likely carry over to other members of this protein family.

K023

## Structural proteomics of plant seeds

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Plant seeds are a rich source of proteins. It is now apparent that while these proteins would have critical physiological roles, many of them are causative agents for allergies. Comparative structural proteomics involving different related seeds, in addition to defining possible determinants of allergy, would provide functional insights in the context of each protein. Analytical separation of the proteins from seed extracts facilitated identification of the dominant proteins by quick sequencing methods. Proteins were subjected to the functional screens through homology searches and complete crystallographic analyses were carried out in case of the relevant proteins. We discovered that a protein purified from cowpea (*Vigna unguiculata*) and the corresponding protein from grass pea (*Lathyrus sativus*) belonged to haemopexin fold which apparently works as a molecular switch regulated by oxidative stress. Two proteins, a vicilin and a non-specific lipid transfer protein, were similarly characterized from egg plant (*Solanum melongena*) seeds. While the vicilin was associated with cofactors linked to critical metabolic processes, the non-specific lipid transfer protein, with lipid bound at a non-canonical site on the surface, provided structural insights concerning allergenicity. Another protein from velvet bean (*Mucuna pruriens*) seeds exhibited antibody-mediated anti-snake venom activity without any obvious structural similarity with venom proteins.

K024

## From Proteins to Climate Change: Redox Proteomics in Plant Metabolism

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The regulation of plant thiol metabolism highlights nature's ability to engineer pathways that respond to multiple inputs and cellular demands using

mechanisms that range from the simple to the elaborate. The role of redox environment as a regulatory factor in plant biochemistry is only beginning to be examined. For example, in plant sulfur metabolism, thiol-based redox control mechanisms govern the biochemical activity of different steps in the pathway. Combining x-ray crystallography, protein chemistry, and mass spectrometry revealed the molecular basis of how thiol-based switches alter protein activity. Building on these insights, we used redox proteomics to examine how oxidative environmental stresses alter protein function across metabolism. Ozone is the most important pollutant with respect to agricultural losses, especially for crops like soybean. Using plants grown in field conditions under ambient and high ozone, redox proteomics reveals extensive changes in oxidation state and/or total expression levels for proteins across a range of metabolic systems. This redox-response of proteins is a major source of metabolic regulation not directly observable by standard 'omic' methods. Mapping metabolic redox networks that respond to environmental stresses will provide insights for modifying plants to meet the challenges of maintaining crop production during climate change.

K025

## Temperature-specific expansion of Dengue virus by mass spectrometry uncovers capsid quaternary interactions

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Protein dynamics is key for correlation of structure with function. Amide hydrogen/deuterium exchange mass spectrometry (HDXMS) and structural mass spectrometry in general are powerful probes for monitoring dynamics of proteins and macromolecular complexes in solution. Here, we describe dynamics of dengue viral particles in solution and map effects of human host-specific perturbations. Dengue serotype 2 (DENV2) has been shown to undergo a large expansion in viral capsid size at the human host temperature of 37 °C by cryo-EM. In contrast, temperature-induced capsid expansion was not observed in the other three serotypes at 37 °C. The

low resolution cryo-EM structures of expanded DENV2 offer limited molecular details of the changes in the viral capsid associated with expansion and also precluded other protein components underlying the capsid surface. In order to further comprehend serotype-specific differences associated with temperature shifts, we have applied amide hydrogen/deuterium exchange mass spectrometry (HDXMS) to monitor dynamics of whole DENV1 and 2 particles in solution and to capture temperature-specific changes at peptide resolution at 37 °C and further at 40 °C (mimicking high fever). Our results indicate that DENV2 show high intrinsic and non-uniform dynamics across the C, E and M-proteins compared to DENV1 at 28 °C. At 37 °C, DENV2 shows temperature-specific changes with the biggest change at the E-intradimeric interface while DENV1 did not show any temperature specific-changes consistent with cryo-EM. However, at 40 °C, a different set of temperature-specific loci were detected in DENV1 while no further temperature-specific changes from 37 °C in DENV2 were detected. These changes are due to specific assemblies of E-protein on the viral particle rather than isolated unassembled E-proteins in solution and underscore the importance of protein quaternary contacts, packing of lipid bilayer and RNA genome. The increased dynamics and temperature-dependent expansion highlight the potential for whole virus HDXMS in exposing hidden linear epitopes for targeted antibody discovery.

K026

### **Plant proteomics, with a special focus on seeds**

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Studies on cellular proteomes started in the years 95, coinciding with the tremendous progress in the identification of proteins by mass spectrometry. By this time the plant scientists recognized the importance of these new approaches, particularly in the case of plant species for which we began to dispose of genomic sequencing data, e.g., Rice and *Arabidopsis*. To date more than 18,000 papers have been published on plant proteomics. Since this initial period, the plant proteomic scientific

community structured, leading to important initiatives as the establishment of international organizations such as the International Plant Proteomics Organization (INPPO) and the Oceania Agricultural Proteomics Organization (AOAPO), the organization of international meetings, the publication special issues devoted to plants in large-audience scientific journals (eg, Proteomics) or the publication of a new journal dedicated to this plant research topic named *Frontiers in plant Proteomics*.

Since 1998 our laboratory has focused on the characterization of seed development and their vigor by using proteomics. In particular, we showed that germination was strictly dependent on the proteins stored in the mature seeds and on the neo synthesized proteins from mRNAs also stored in the mature seeds, thus justifying the use of a proteomic approach. I will describe the main results achieved by our team and by many other authors regarding the mechanisms governing the accumulation of seed storage proteins rich in essential amino acids (eg, lysine, methionine) and whose importance is considerable for animal feed and human, the mechanisms governing the restart metabolism during germination, the mobilization of seed storage proteins during this process, the impact of the environment on seed vigor, the mechanisms of seed dormancy exit, or the mechanisms accounting for the exceptional survival of embryo in a dry state. These studies highlight individual proteins or PQLs (Protein Quantity Loci) that can be used as biomarkers of the seed quality in selection schemes. They are also useful to characterize the mechanisms of plant and seed evolution (e.g., Amborella Genome Project, 2013).

K027

### **Profiling glycopeptides by HCD and ETD to characterize the plant Golgi localized UDP-GlcNAc transporter**

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Glycosylation reactions require activated glycosyl donors in the form of nucleotide sugars to drive

processes such as post-translational modifications and polysaccharide biosynthesis. Many of these reactions often occur in the Golgi from cytosolic-derived nucleotide sugars, which are actively transferred into the Golgi lumen by nucleotide sugar transporters (NSTs). We have established a yeast proteoliposome transport assay coupled to LC-MS designed to screen substrates of plant encoded NSTs. Using this approach, we recently identified a plant UDP-GlcNAc transporter responsible for the delivery of substrate for the maturation of *N*-glycans. To ascertain the biochemical phenotype of UDP-GlcNAc transporter loss-of-function mutants, we have been using HILIC to enrich glycopeptides from plant samples then characterized glycopeptides using complementary fragmentation approaches. This involves the generation of an ETD spectrum based on the presence of an oxonium ion derived from the glycan (e.g. HexNAc, 204.09 m/z) in the HCD spectra. This approach has enabled us to profile thousands of glycopeptides from plant samples. The analysis of glycopeptides from the loss-of-function UDP-GlcNAc transporter mutant indicated that peptides containing complex glycans (e.g. GlcNAc) only comprised about 5% of the population compared to wild-type plants which contained over 35% of the population. Significantly, over 50% of the glycopeptide population of the loss-of-function UDP-GlcNAc transporter mutant contained high mannose structures, namely HexNAC(2)Hex(5), which represents the structure prior to the addition of the first GlcNAc in the Golgi lumen. We have also commenced sphingolipid profiling of the loss-of-function mutants which has revealed that the biosynthesis of GlcNAc containing sphingolipids in *Arabidopsis* is also reliant on this UDP-GlcNAc transporter. Our findings indicate that the reference plant *Arabidopsis* contains a single UDP-GlcNAc transporter responsible for the maturation of complex *N*-glycans and sphingolipids in the Golgi lumen.

## Proteomic analysis of the plant extracellular matrix: turning protein repertoire into application for crop improvement

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Proteomic analysis of the chickpea extracellular matrix led to the identification of an array of stress-responsive proteins. Further investigation on the proteome profiling revealed the stress-induced differential expression of phytoferritins. Ferritins, in general, store and sequester iron, but little is known about their function in plants, particularly in stress responses. We screened the chickpea cDNA library and cloned the stress-responsive phytoferritin, designated CaFer1, and assessed its role in stress tolerance. The *CaFer1* transcripts are upregulated under dehydration and high salinity, and also by treatment with abscisic acid (ABA), suggesting that its stress-responsive function might be associated with ABA-dependent network. Additionally, it was found to have role in the defense against *Fusarium* wilt disease. Functional complementation of the yeast frataxin-deficient mutant  $\Delta yfh1$  indicates that CaFer1 functions in enhanced tolerance to oxidative stress. The compartmentalization of CaFer1 to the extracellular space, besides chloroplast, establishes its inimitable nature from that of other phytoferritins. Overexpression of CaFer1 in *Arabidopsis* led to altered expression of iron-responsive genes, and improved growth and development. CaFer1 interacts with the iron transporter IRT1, suggesting a distinct role in maintenance of iron homeostasis. This study demonstrates that characterization of the extracellular matrix *in vitro* can be used to identify novel proteins, which would provide the basis for effective engineering strategies for crop improvement program.

## **Chloroplast Protein Homeostasis; protein maturation and degradation**

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Intra-plastid proteolysis is essential in plastid biogenesis, differentiation and protein homeostasis (proteostasis) and feedback mechanisms between proteostasis and nuclear gene expression through retrograde signaling must exist. Determinants of chloroplast protein life-time and stability are poorly understood, even if this is of critical importance for chloroplast proteostasis. Protein N-termini are prone to modifications and are major determinants of protein stability in bacteria, eukaryotes, and perhaps also in chloroplasts. Most chloroplast proteins undergo N-terminal maturation, but this is poorly understood due to insufficient experimental information. This motivated an extensive characterization of chloroplast protein N-termini using terminal amine isotopic labeling of substrates (TAILS) and mass spectrometry, generating nearly 14,000 MS/MS spectra matching to protein N-termini. Many nuclear-encoded plastid proteins accumulated with two or three different N-termini; we evaluated the significance of these different proteoforms. Ala, Val, Thr (often in N- $\alpha$  acetylated form) and Ser were by far the most observed N-terminal residues, even after normalization for their frequency in the plastid proteome, while other residues were absent or highly under-represented. Plastid-encoded proteins showed a comparable distribution of N-terminal residues, but with a higher frequency of Met. Infrequent residues (*e.g.* Ile, Arg, Cys, Pro, Asp, Glu) were observed for several abundant proteins (*e.g.* HSP70/90, RBCL, Fd-GOGAT) likely reflecting functional regulation through their N-termini. In contrast, the thylakoid luminal proteome showed a wide diversity of N-terminal residues, including those typically associated with instability (Asp, Glu, Leu, Phe). We propose that after cleavage of the chloroplast transit peptide by stromal processing peptidase, additional processing by unidentified peptidases occurs to avoid unstable or otherwise unfavorable N-terminal residues. The possibility of a chloroplast N-end rule is discussed also in the context of our recent discovery of a binary ClpS-ClpF adaptor system.

## **The quest for protein markers in oil palm tissue culture**

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Tissue culture is an important technology in oil palm industry. Due to the single meristem shoot, the only way to mass clonal propagate oil palm in order to capture maximum agronomic potential is through tissue culture. Many commercial oil palm tissue culture laboratories therefore have been established to produce elite clonal planting materials. This strategy would help to mitigate the need to encroach to limited agriculture land available for oil palm plantation. However, there were many challenges in the production of oil palm plantlets through tissue culture. One of the main challenges is the low conversion rate of the explants to the final embryo stage which will subsequently develop into plantlets. In this study, proteomic approach was used to elucidate the fundamental mechanisms underlying initiation and proliferation of oil palm cultures at the cellular and molecular levels. Differential proteins from oil palms with records of high and low proliferations were isolated and identified using 2D-PAGE coupled with MALDI TOF-TOF mass spectrometry. The protein has been found to be mostly associated with metabolism. Further characterization of these proteins using qPCR indicated three protein species which were correlated in abundance with mRNA levels. The proteins are triosephosphate isomerase, L-ascorbate peroxidase and superoxide dismutase. In the mRNA expression study, these proteins were significantly different in both protein and mRNA levels between high and low proliferation groups (*P*-value < 0.05) suggesting the potential of developing the three proteins into biomarkers for early selection of high proliferate oil palm tissue culture samples.

K032

**Discovery and validation of protein biomarkers for lung cancer metastasis using a cell secretome model and clinical specimens**

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As the leading cause of cancer death worldwide, lung cancer lacks effective diagnosis tools and treatments to prevent its metastasis. Metastasis is a major obstacle that must be overcome for the successful treatment of lung cancer. Proteins secreted by cancer cells may facilitate the progression of metastasis, particularly within the phases of migration and invasion. To discover metastasis-promoting secretory proteins within cancer cells, we used a hollow fiber culture (HFC) system and mass spectrometry-based label-free quantitative proteomics approach to compare the secretomes from the lung adenocarcinoma cell lines CL1-0 and CL1-5, which exhibit low and high metastatic properties, respectively. Among the 703 proteins quantified, 50 of which were expressed at different levels between the two cell lines and considered to be potentially useful biomarkers for lung cancer metastasis. Several of those candidates for useful protein markers, such as COL6A1, A1AT, PARK7, ACTN4, and TIMP-1, were further examined and validated using cell functional assays and clinical specimens for their possible applications in lung cancer prevention and treatment.

K034

**Metabolomics in a fix: Tomato genotypes reveal ecosystem-dependent and hormone-specific fluidity**

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Metabolomics is 'omics' approach to define metabolic patterns during growth, development

and senescence in biological systems and how these change in response to genetic events, environment and disease. The effects of the field environment (ecosystem services) on plant hormone-specific metabolite profiles are largely unknown. Nuclear Magnetic Resonance (NMR) spectroscopy was used for quantifying metabolites that are central to primary metabolism in tomato fruit during ripening. Changes in primary metabolites of genotypes mutated for the deficiency of ethylene or methyl jasmonate were unique under all growth conditions and opposite of high polyamine genotype results. The high polyamine trait was found to dominate the low ethylene and low jasmonate mutations under field conditions. Generally, research investigating metabolomic patterns have focused on the first (statistical) moment, i.e. changes in mean values, whereby observed metabolic mean values and differences between mean values are presented to describe the metabolomic state or change in state of the organism under study in response to an exogenous organetic manipulation. When we used PCA and WCNA for discerning the patterns of eigen metabolites over treatments, we found that these approaches did not adequately capture how metabolites responded to genotype and treatment changes. The WCNA approach is based on the assumption that the correlation structure of the data does not vary across treatment effects, though means may be affected by treatments. Instead, we found that the observed relationships among the metabolite pairs often changed substantially across the different genotypes and mulching systems in our experiments. To discern how the networks of metabolites changed as conditions varied, we used an approach based on characterizing changes in correlations between metabolite pairs among subsets of data. This particular approach is novel and was necessary because our experimental material yielded highly variable metabolic responses that could not be easily understood using the traditional analytical approaches for first moment statistics. We show here that the networks defined by metabolite relationships in the tomato fruit metabolome are hormone and environment specific, demonstrating the highly plastic nature of the fruit primary metabolome.

K035

## **Identifying potential markers for Coronary Artery Disease using Proteomics and Metabolomics approach**

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The silent epidemic of non-communicable diseases especially those due to impaired metabolism is fast becoming a significant public health problem in developing countries like India. Among these diseases, Cardiovascular Diseases (CVD) has emerged as the largest cause of mortality and morbidity worldwide especially in developing countries. For instance, it is expected that by 2030 about 60% of the world's Coronary Artery Disease (CAD) patients will be Indians. The cost of managing such complex disorders is astronomical and it is increasingly affecting the working populace. Thus, early detection of these diseases is of utmost importance to reduce the disease associated morbidity. Since CAD is a complex disorder where both genetic and lifestyle (including dietary habits) factors contribute significantly, finding new potential markers specific to Indian population holds its own clinical importance specifically in early detection and efficient management of the disease. In recent years, several studies focused to identify genetic markers that could be associated with CAD. However, most of these markers have low discriminative accuracy. Similar is the case with the classical biochemical markers used for CAD. Thus, identification of newer markers is necessary to increase the predictive accuracy. To this end, both proteomics and metabolomic studies hold tremendous potential since gene-environment interaction could lead to modulation of various proteins and metabolic pathways. Thus, identifying differentially expressed proteins and altered metabolite levels could aid in deciphering pathways that could be involved in the pathophysiology of the disease. The systematic analysis of metabolites (e.g., sugars, amino acids, organic acids, lipids, etc.) in biologic specimens reflects the body's metabolic state and is influenced by environmental factors such as diet, medications, and the gut microbiome. We used a quantitative proteomics approach and an

untargeted metabolomic approach to identify proteins and metabolites that are altered in CAD patients. Using iTRAQ method followed by rigorous statistical analysis we identified 4 proteins that along with hypertension and diabetes could account for greater than 88% of CAD cases. Using reverse phase and HILIC based chromatography followed by mass spectrometry we identified a total of 32metabolites ( $\geq 2$  fold;  $p < 0.05$ ) in plasma whose levels were significantly altered in CAD samples. Further, we validated the discriminative ability of these metabolites in an independent set of CAD and control samples using multivariate PLS-DA analysis. Interestingly, several metabolites including Lyso PC (18:0), Cortisol, Lyso PC (P-17:0), and glycerophosphocholine had significantly different levels in CAD patients. After rigorous statistical analysis we identified about 15 metabolites that could act as top discriminators for CAD. Most importantly alterations in the levels of some of these metabolites could be explained due to the altered expression of proteins in CAD patients.

K036

## **Global metabolomic profiling reveal triggers for sexual stageconversion in Malaria**

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The malaria parasite heavily relies on secretory functions for itspathogenesis. Is the parasite equipped with machinery to tackleperturbations to its secretory pathway? In my talk I will describeour study revealing a complete absence of genes involved in the canonicalunfolded protein response pathway in *Plasmodium falciparum*. Accordingly,the parasite was unable to up-regulate endoplasmic reticulum (ER)chaperones or ER-associated degradation in response to ER stress. Globalprofiling of gene expression, proteomic and metabolomic analysis uponredox stress revealed a network of AP2 transcription factors, theirtargets andspecific metabolites being activated and/or upregulated. The overalloutcome was an upregulation of genes involved in protein export and

the sexual stage of the parasite life cycle, culminating in gametocytogenesis. Our results suggest that the malaria parasite uses ER stress as a cue to switch to the transmissible, sexual stage.

December 17, 2016

K039

K037

## Deregulated metabolic phenotype of pulmonary tuberculosis patients

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Due to tuberculosis, two persons every three minutes are lost in India. In terms of loss to society, approximately 100 million productive working days are lost due to illness associated with tuberculosis and 1.3 billion days due to tuberculosis deaths. In tuberculosis infection, the causative organism induces shift in host metabolic pathways that may favor for its successful proliferation for further transmission. Understanding these critical host-pathogen interactions at molecular levels is important to develop appropriate personalized therapeutic interventions. Host genetics also play critical role in effectiveness of therapeutic interventions and it play critical role in the clinical outcomes. In this presentation, we will share some of our laboratory findings on urine metabolite analysis of freshly diagnosed active- and non-tuberculosis patients and their acetylation status. We observed a deregulated tyrosine-phenylalanine metabolism in active tuberculosis patients. We also determined the acetylation status of a tuberculosis patient's population from north eastern states of India by monitoring the tuberculosis drugs and their intermediates. Majority of this patient population are found to be slow acetylators. We will highlight the importance of biofluids metabolic phenotyping to understand tuberculosis disease which might be useful to develop appropriate personalized therapeutic interventions at population level.

## Quest for phosphosignaling pathways in ABA response in plants

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Abscisic acid (ABA) is a phytohormone that is critical for stress responses or seed development. The major ABA signaling pathway consists of three core components; receptor, protein phosphatase (PP2C) and protein kinase (SnRK2), indicating the importance of phosphosignaling in this pathway. Recently, we are trying to investigate ABA-dependent phosphosignaling pathways by several experimental approaches. The one is phosphoproteomics, which has successfully identified a lot of phosphoproteins in Arabidopsis, including a number of potential SnRK2 substrates. Among them, SnRK2 substrate 1 (SNS1) is phosphorylated by SnRK2, and it negatively regulates seed germination of Arabidopsis. Now we are applying a phosphoproteomic approach to some other plants, for example Physcomitrella patens or barley, to consider evolutionary conserved parts of ABA signaling components in land plants. The other experiment is a protein-protein interaction (PPI) analysis. We are using a high-throughput PPI screening system based on in vitro translation and AlphaScreen technology. This helps us to confirm SnRK2 substrates predicted from phosphoproteomic data, and also to identify novel SnRK2-interacting proteins. Recently we identified a novel SnRK2-interacting protein which negatively regulates ABA responses. We believe that such approaches will bring some new insights of ABA signaling in plants.

K040

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

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Bread wheat (*Triticum aestivum*) is an important crop plant, both nutritionally and economically.

Worldwide it is grown on more land than any other commercial crop and is the most important food grain for humans, providing 20% of total calories consumed. Annually ~700 million tonnes is produced with and export value of >USD\$46 billion. The amount of land available for crop production is in decline, but the demand for food is increasing. In fact, the Food and Agriculture Organization of the United Nations estimates that the global demand for food will double by 2050, thereby intensifying the need to improve wheat yields and to breed varieties that can utilise low quality agricultural land.

To meet this challenge, we have been developing a wheat database, that is analogous to the human SRMAtlas, which has just been released publically at [www.wheatproteome.org](http://www.wheatproteome.org). This database allows us to accurately quantify large numbers of proteins that we select, using a mass spectrometry based approach. During its development we have been able to leverage this database to study the wheat proteome response to both salinity and cold exposure. From these studies we have been able to identify novel responses of wheat by mapping both proteomic and metabolite changes on to metabolic networks to identify bottlenecks and investigate these using classical plant biochemistry. At the same time, as part of an International Wheat Yield Partnership (IWYP) project we have begun to use this approach to examine the impact of the 3 fold variation in respiratory rate seen in global cultivars on yield production and to identify key control points in metabolism involved in this variation. The detailed knowledge we are building of the molecular processes that underpin environmental responses and yield production of wheat may enable the breeding of improved varieties, with higher yields or improved stress tolerance.

## Salinity-Induced Palmella Formation Mechanism in *Dunaliella salina* Revealed from Quantitative Proteomics and Phosphoproteomics Analysis

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Palmella stage is crucial in the life cycle of some unicellular algae to survive in various extreme environment conditions. The halotolerant algae *Dunaliella salina* is a good single-cell model for studying plant adaptation to high salinity. To clarify the molecular adaptation mechanism in salinity shock-induced palmella formation, we performed a comprehensive physiological, proteomics and phosphoproteomics analysis upon palmella formation of *D. salina*. We found 151 salinity-responsive proteins and 35 salinity-responsive phosphoproteins were involved in multiple signaling and metabolic pathways upon palmella formation. Taken together with photosynthetic parameters and enzyme activity analysis, the patterns of protein accumulation and post-translational modification highlighted that the dynamics of cytoskeleton and cell membrane curvature, the accumulation and transportation of exopolysaccharides, photosynthesis and energy supplying (*i.e.* PSII stability and activity, cyclic electron transport, photorespiration, and C4 pathway), nuclear/chloroplastic gene expression regulation and protein processing, ROS homeostasis, and salt signaling transduction were all sophisticated modulated upon palmella formation. To our knowledge, this study is the first close investigation of protein complement and PTM regulation for understanding the mechanism of algae palmella formation.

**Quantitative proteomics reveals an important role of GsCBRLK in salt stress response of soybean**

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Salinity severely threatens land use capability and crop yields worldwide. Understanding the mechanisms that protect soybeans from salt stress will help in the development of salt-stress tolerant leguminous plants. We have previously reported that *GsCBRLK* functions as a positive regulator of plant tolerance to salt stress. In order to investigate the physiological and molecular mechanisms underlying the salinity tolerance regulated by *GsCBRLK*, the gene was overexpressed in soybean plants. Here we examined the salt-responsive proteomes of the *GsCBRLK* overexpression soybean and wild type plants using iTRAQ-based proteomic approach to investigate the global effects and potential downstream targets of *GsCBRLK*. A total of 941 proteins showed significant changes in protein abundance in soybean leaves, and 574 of the NaCl-regulated proteins were *GsCBRLK*-dependent. Among the identified proteins, four protein changes in the two genotypes after NaCl treatment were validated using Western blot analysis, at the same time, 10 proteins transcriptional levels were detected using qRT-PCR analysis. Identification of the salt-responsive proteins has revealed the involvement of *GsCBRLK* protein in the enhancement of ROS scavenging and photosynthesis capacity in soybean, which was corroborated with the physiological effects of *GsCBRLK* overexpression. More importantly, the proteomic data has suggested the regulatory function of *GsCBRLK* in salt signal transduction pathway mediated by Ca<sup>2+</sup>/CaM. These findings have contributed to our knowledge of plant *GsCBRLK* mediated salt tolerance mechanisms.

**Mechanistic Insight Into the Involvement of Glutathione in Plant Defense:  
A Proteomic Approach**

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Glutathione, the master antioxidant, is a nearly ubiquitous non-protein tripeptide thiol found in both prokaryotes and eukaryotes except for some organisms which use other thiol cofactors. This molecule helps to prevent or even reduce the effect of certain human diseases which are of major concern in today's world including cancer, inflammation, kwashiorkor, Alzheimer's disease, Parkinson's disease, sickle cell anaemia, liver disease, cystic fibrosis, HIV, AIDS, infection, heart attack, stroke and diabetes. In plants, this molecule is a major player in redox chemistry, heavy metals and electrophilic xenobiotics elimination, serving as electron donor for biochemical reactions, long-distance transport of reduced sulfur, stress defense gene expression, posttranslational modifications through glutathionylation, role in biotic and abiotic stresses and so on. Previously we reported the crosstalk of GSH with other established signalling molecules viz. SA, ET, ABA etc. to combat environmental stress conditions. For that, transgenic plants viz. *Nicotiana tabaccum* and *Arabidopsis thaliana* have been developed by the constitutive overexpression of γ-ECS, the rate limiting enzyme of GSH biosynthetic pathway. Here, with a view to identify and characterize the proteins regulated by GSH to combat environmental stress, comparative proteomic profiling of treated transgenic (viz. *NtGB 9*, *NtGP 11*, *AtECS1*) and non-treated transgenic control lines along with the wild type plants were performed. Interestingly, several stress responsive proteins viz. HSPs, NBS-LRR, LRR-RK, GST, GRP etc. were identified with increased accumulation suggesting the active involvement of GSH in the efficient activation of these proteins. Furthermore, the down-regulation of some SA, ABA and ethylene biosynthetic/signalling pathway proteins in stress treated transgenic/mutant lines suggests the dynamic role of GSH in the activation of these stress modulating compounds to combat stress. QRT-PCR

analysis of selected transcripts was performed for further validation. Together, the involvement of GSH in plant defense and its mechanism will be discussed.

perspective of Agricultural Proteomics with examples and particular attention to applications in South African Research and Development.

K045

K044

## **Application of Proteomics in Agricultural Research & Development: A South African Perspective**

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Majority of people in Africa and Asia rely entirely on primary agriculture for their livelihood. Agricultural products are generally used for food/meat, animal feed, biofuels, clothing, fibre, medicine and building materials. Crops therefore, remain the main artery/vein for the survival, productivity, growth and development of the world's food, health and economic systems. As such, agricultural products are a central pillar of modern bioeconomy. For example, in Europe, the knowledge-based bioeconomy has an estimated worth of more than 2 trillion Euros, an industry that supports more than 21 million families. Agriculture, a cornerstone of human survival and development in less industrialized countries, particularly in Africa and Asia, with the majority of the developing world's human populations dependent exclusively on subsistence farming. Proteomics, which is generally defined as the simultaneous and high throughput study of protein expression profiles in cells, tissues, organs and organisms, is now recognized as one of the most important tools used in the identification and characterization of proteins (and genes) of biological and biotechnological interest. Over the past 15 years, the agricultural proteomics field has grown notably, contributing positively to global biotechnology research, particularly in new knowledge generation, medical and agricultural applications. This talk will give an African

## **Discovery of protein biomarkers in biofluids of bovine for early detection of pregnancy for sustained productivity**

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An early and precise pregnancy diagnosis is an important criterion for better reproductive management in livestock such as cows and buffaloes. Currently different methods (direct and indirect) are in use for diagnosis of pregnancy. The direct methods include per rectal palpation and ultrasonography. However, their application is limited in terms of accurate detection by day 45th and 30th day respectively. The indirect methods include immunological based assay for detection and quantitation of target proteins (Pregnancy Associated Glycoprotein: PAG) and hormones such as progesterone (P4), pregnadiol, interferon tau related to pregnancy. However, these methods have inherent limitations of specificity and false positive results in ELISA. However, for sustained productivity, detection of pregnancy by 21<sup>st</sup> day of pregnancy is necessary. Worldwide, different research groups have used serum, urine and saliva as sources for detection of pregnancy and various other diseases in human being. Pregnancy in human being is currently detected by presence of human chorionic gonadotropin (HCG) in urine. However, this hormone is absent in bovine urine. Therefore, Pregnancy diagnosis (PD) in dairy animals has remained elusive. Urine and saliva are considered as ideal source of biological material for biomarker discovery as it is non-invasive in comparison to other body fluids. After conception, numerous biomolecules such as steroids, prostaglandins and proteins are expressed during early pregnancy. Many of these hormones and proteins are of fetal-placental origin rather than of maternal origin. Our group is working intensively on the potential of biofluids such as urine, saliva and serum in cow as a resource for pregnancy diagnosis. In one of our findings we have reported that cow urine contains

more than 1550 proteins. These proteins have diverse origin, many of which have been found to be associated with early pregnancy. We have further investigated the differences in proteome of urine at different stages of pregnancy in comparison to non-pregnancy. We have also identified a number of proteins in urine and serum which are either up regulated or down regulated in pregnancy by following high-throughput proteomic approaches involving 2D-DIGE, Label free and labelled quantitation. Some of these proteins are associated with pregnancy and potential biomarkers. We have cloned and expressed few selected proteins for their future use in pregnancy diagnosis. Currently our focus has been to validate these potential biomarkers using targeted proteomics followed by immune screening.

K046

### Clinical Proteomics – Let's not Forget the PTMs

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The main focus of proteomics-based biomarker discovery lies on the detection of protein abundances and changes thereof in different body fluids and/or tissue specimens. This is achieved by either direct measurements at the protein level or indirect measurements at the transcript level (although protein and transcript are arguably two different entities that do not necessarily correlate). However, even directly measuring protein abundances by ELISA and/or MS often ignores the importance of post-translational modification (PTM) on the disease relevance of a protein; in other words it is not always the abundance of a protein that determines its potential as a biomarker but the abundance of one or more PTMs. As an example, we will discuss in this lecture the potential of PTM profiling on the protein Tau as a biomarker for the differentiation between diverse forms tauopathy-associated dementias.

### Glycoproteomics analysis enabled by hexosamine analogues through metabolic glycan engineering

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Glycocalyx, defined as the dense layer of carbohydrates found attached to proteins and lipids on mammalian cell surface, serves as the first-point of contact for transduction of stimuli from environment in the form of growth factors, cell-cell, cell-extracellular matrix, and cell-pathogen interactions. Almost all membrane proteins, including CD molecules, are post-translationally modified with glycans (both N- and O-linked). Owing to their non-template driven biosynthesis, glycoproteins display microheterogeneity in site occupancy as well as the size, nature, and structure of glycans in a cell and cell-state dependent manner. The physiological and spatio-temporal functions of various glycoforms of a given protein are not clearly understood. One of the major goals of glycoproteomics is to characterize, catalogue, and quantify the various glycoforms found in complex biological mixtures and their differential analysis between normal and disease conditions. Unlike genomics and proteomics, which are template driven and have attained sufficient levels of sophistication for use by larger research community, technologies for the study of metabolism driven glycoproteomics are still in infancy. In this presentation, we will illustrate results from our recent efforts towards the application of metabolic glycan engineering (MGE) for glycoproteomics.

Firstly, exhaustive site mapping analysis of mucin-type O-glycosylation (MTOG) on CD antigens, particularly CD43 (leukosialin/sialophorin) from Jurkat cells would be discussed. CD43 is estimated to carry 80-90 sites of MTOG and previous studies using galactoglycoprotein, a secreted form of CD43, were able to characterize 25 occupied sites. Using high energy collision dissociation - product triggered - electron transfer dissociation (HCD-PD-ETD) technique on nano-LC-ESI-MS/MS, we were able to identify and assign a total of 57 (including 32 new sites) out of 91

potential sites on CD43. Additionally, 21 glycopeptides from seven tryptic peptides corresponding to micro-heterogeneous glycoforms and degenerate site occupancy were characterized. Pre-treatment with a thiol-carrying analogue of *N*-acetyl-D-galactosamine (GalNAc) resulted in inhibition of MTOG reducing the occupied sites from an average of 60 to 30 sites. Secondly, the design and development of carbohydrate-neuroactive hybrid (CNH) molecules for MGE of CNS tissues across blood-brain barrier (BBB) will be discussed. Unlike corresponding non-hybrids, CNH molecules display facile ability to reach and effect MGE across BBB. A profile of the engineered sialo-glycoproteome of mouse brain was obtained using click-chemistry based enrichment. Sialo-glycoproteins carrying *N*-azidoacetyl-D-neuraminic acid (NeuAz) were captured on alkyne-beads, trypsinized, and analyzed by nano-LC-ESI-MS/MS. The CNH strategy opens up exciting avenues for modulation of glycosylation in brain for differential analysis of neurodegenerative disorders and brain-mapping projects.

K048

### **Glycation inhibitors reduce protein glycation and extend chronological life span of *Saccharomyces cerevisiae* and *Caenorhabditis elegans***

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Advanced Glycation End products (AGEs) are implicated in the aging process. Thus, reducing AGEs by using glycation inhibitors may help in attenuating aging process. To study the influence of glycation inhibitors in regulation of aging, we have used two model systems *Saccharomyces cerevisiae* and *Caenorhabditis elegans*, a worm. In the yeast system, we show that Aminoguanidine (AMG), a well-known glycation inhibitor, decreases the AGE modification of proteins in NR (2% glucose) and extends CLS (Chronological lifespan) similar to that of CR condition (0.5% glucose). Proteomic analysis revealed that AMG back regulates the expression of differentially expressed proteins especially those involved in mitochondrial respiration in NR condition, suggesting that it

switches metabolism from fermentation to respiration, mimicking CR. AMG induced back regulation of differentially expressed proteins could possibly due to its chemical effect or indirectly by glycation inhibition. To delineate this, Metformin (MET), a structural analog of AMG and a mild glycation inhibitor and Hydralazine (HYD), another potent glycation inhibitor but not structural analog of AMG were used. HYD was more effective than MET in mimicking AMG suggesting that glycation inhibition was responsible for restoration of differentially expressed proteins. Thus glycation inhibitors particularly AMG, HYD and MET extend yeast CLS by reducing AGEs, as well as by modulating the expression of proteins involved in mitochondrial respiration.

Whereas, in *C elegans*, Rifampicin, a well known antibiotic for tuberculosis, extends the lifespan by reducing *in vivo* protein glycation. It was able to overcome glucose toxicity, reduce AGEs and increase life span under hyperglycaemic conditions also. An analog of Rifampicin, Rifamycin SV possesses similar properties. For its effect on longevity, Rifampicin requires DAF-18 (nematode PTEN) as well as JNK-1 and activates DAF-16, the FOXO homolog. Interestingly, the drug treatment modulates transcription of a different subset of DAF-16 target genes, those not controlled by the conserved Insulin-IGF-1-like signalling cascade. Together, the dual ability to reduce glycation *in vivo* and activate DAF-16 makes Rifampicin and its analogs effective life span extending interventions.

K049

### **Proteomic exploration of lysine acetylation and ubiquitylation**

**Chunaram Chowdhury**

*University of Copenhagen, Denmark*

A great majority of proteins in eukaryotic cells are modified by different posttranslational modification (PTMs). PTMs are key constituents of cell signaling networks, comprising some of the most sophisticated control switches in the cell. Given the vast complexity and their all essential role in controlling biology of living cells, analysis of proteins and PTMs is one of the most exiting areas of research in biology.

Recent developments in high-resolution mass spectrometry (MS), computational proteomics, and optimized PTM-enrichment strategies have greatly facilitated unbiased (non-hypothesis driven) analysis of posttranslational modifications. We have applied these approaches to investigate the dynamics of the two major lysine PTMs - acetylation and ubiquitylation. Large-scale analysis of these PTMs provided systems view of signaling networks, and indicated involvement in diverse biological processes. I will discuss these recent developments and highlight applications of high resolution MS for analysis of lysine acetylation and ubiquitylation dynamics in response to different cellular perturbations.

K050

### **Mapping molecular changes associated with tobacco induced cellular transformation by global and phosphoproteomic profiling**

**Harsha Gowda**

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Tobacco consumption in both smoking and chewing form are risk factors for several diseases including cancers. Mechanisms by which cigarette smoke causes cancer is extensively studied and is attributed to several carcinogenic compounds that exist in tobacco and some that are produced during combustion. These studies have been mainly carried out using acute treatment models. However, molecular mechanisms underlying chewing tobacco induced cellular transformation is not well understood. We have developed chronic treatment models by treating non-neoplastic esophageal and oral keratinocyte cell lines with condensed cigarette smoke and aqueous extract of tobacco over a period of 12 months. Both smoke treated and chewing tobacco treated cells acquired oncogenic phenotype in 6-8 months and showed increased proliferation and invasion capability. Deep proteomic profiling studies revealed distinct changes in cigarette smoke treated and chewing tobacco treated cell models providing insights into mechanisms associated with tobacco induced cellular transformation. These insights would be valuable to identify biomarkers

relevant for risk assessment and to develop therapeutic intervention strategies for cancers associated with tobacco usage.

K051

### **The quantitative phosphoproteomics of sugar beet M14 and function of phosphorylation sites of one serine/threonine protein kinase in sugar beet M14 in response to salt stress**

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Understanding how plants respond to and tolerate salt stress is important for engineering and breeding effort to boost plant productivity and bioenergy in an ever challenging environment. Sugar beets (*Beta vulgaris*) are classified as salt-tolerant crops. Scientists have studied the interspecific crossing of cultivated sugar beet (*Beta vulgaris* L. 2n = 19) and *B. corolliflora* Zoss. for decades. In our lab, Guo et al. obtained the sugar beet monosomic addition line M14, which contains the *Beta vulgaris* L. genome with the addition of No.9 chromosome of *B. corolliflora* Zoss. Sugar beet M14 line is a unique germplasm that contains genetic materials from *Beta vulgaris* L. and *Beta corolliflora* Zoss, and it exhibits tolerance to salt stress. Our previous studies have shown that M14 can grow in 500 mM NaCl treatment for 7 days without losing viability, making it an interesting material for studying salt stress tolerance.

Here the recent studies of two aspects about phosphoproteomics of sugar beet M14 and function of phosphorylation sites of BvM14-STPK (one serine/threonine protein kinase in sugar beet M14) were introduced.

About phosphoproteomics of sugar beet M14, we report the characteristics of the M14 plants under 0, 200, and 400 mM NaCl using label-free quantitative proteomics approaches. Protein samples were subjected to total proteome profiling using LC-MS/MS and phosphopeptide enrichment to identify phosphopeptides and phosphoproteins. A total of 2182 proteins were

identified and 114 proteins showed differential levels under salt stress. Interestingly, 189 phosphoproteins exhibited significant changes at the phosphorylation level under salt stress. Several signaling components associated with salt stress were found, e.g. 14-3-3 and mitogen-activated protein kinases (MAPK). Fifteen differential phosphoproteins and proteins involved in signal transduction were tested at the transcriptional level. The results revealed the short-term salt responsive mechanisms of the special sugar beet M14 line using label-free quantitative phosphoproteomics.

About function of phosphorylation sites of BvM14-STPK, based on the transcriptome database of M14 under salt stress, the full-length cDNA named BvM14-STPK was obtained by PCR. Sequence analysis showed that BvM14-STPK had a ser/thr binding domain, which belonged to the family of Protein Kinase C Family. The Real-Time PCR result showed that BvM14-STPK gene was responsive to salt stress. BvM14-STPK fusion protein was expressed in prokaryotic expression system and identified by Western blot. After gel digestion, desalting, enrichment, the peptides of BvM14-STPK were identified by mass spectrometry. 31 phosphorylation sites were identified, including 19 serine, 9 threonine and 3 tyrosine. In order to reveal the function of phosphorylation sites in BvM14-STPK, 8 key serine phosphorylation sites were divided into five segments to research. By site-directed mutagenesis technology, each of five serinephosphorylation sites were mutated into aspartic acid in BvM14-STPK, which showed that each of five serinephosphorylation sites became sustained phosphorylated. Simultaneously, each of five serine phosphorylation sites were mutated into phenylalanine in BvM14-STPK, which showed that each of five serine phosphorylation sites became non-phosphorylated. The function of phosphorylation sites of BvM14-STPK were further clarified in transgenic tobacco.

Protein phosphorylation on serine, threonine and tyrosine residues is one of the most widespread post-translational modifications (PTMs) in cellsignaling and can regulate diverse properties of proteins. Plants respond to salt stress by triggering phosphorylation cascades. Analysis of the M14 phosphoproteome and function of phosphorylation sites of BvM14-STPK under salt stress has provided insight into specific response mechanisms underlying salt stress tolerance. The knowledge gained can be potentially applied to improving crop salt tolerance.

## The HPP Knowledge Base Pillar: Achieving a high-confidence snapshot of the human proteome observed thus far

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The Human Proteome Organization's Human Proteome Project (HPP) Knowledge Base Pillar is tasked with synthesizing the resources and data generated by the many groups of the HPP into an integrated view of the human proteome. This includes information generated via mass spectrometry and presented in PeptideAtlas, antibody affinity information generated and stored in the Human Protein Atlas, and other knowledge gleaned from the literature, all integrated into the neXtProt Knowledge Base. In order to ensure the highest quality for HPP results, the HPP Mass Spectrometry Data Interpretation Guidelines have been developed in broad consultation with the community. Together, these resources enable us to achieve a high-confidence snapshot of the human proteome observed thus far.

## Chromosome Centric Human Proteome Project and the Indian Efforts

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The chromosome Centric Human Proteome Project (C-HPP) is being steered by many national and regional teams each of them being focussed on one specific chromosome. The Indian effort is targeted on Chromosome 12, along with group members from Singapore, Taiwan, Hong Kong and Thailand. At present, the focus has been on the following objectives: 1. To explore missing proteins and protein variants through already available datasets as well as new; 2. To develop a well annotated priority list of proteins and proteotypic peptides which can be explored for clinical applications in a targeted manner; 3. To identify differentially regulated gene clusters in clinical conditions to

understand mechanisms of intra - and inter-chromosomal regulatory cross talks; 4. To integrate transcriptomic and proteomic data to develop integrated view of biological processes and diseases (not restricted to Chromosome 12).

In the Biology and Disease angle (B/D HPP), the present Indian effort is targeted on CNS tumors, other cancers and neurological disorders and the prospective efforts includes diabetic nephropathy, cardiac disorders and infectious diseases. The contributions and the potential insights from this effort will be discussed.

these identifications could be attributed to differences in reference proteome databases, exemplifying use of a single standard database for human protein detection from MS data. Our results suggest that search strategies with modified parameters can be rewarding alternatives for extensive profiling of missing proteins. We conclude that using complementary spectral data searches incorporating different parameters like PTMs, against a comprehensive and compact search database, might lead to discoveries of the proteins attributed so far as the missing human proteome.

K055

K054

## Probing the Missing Human Proteome: A Computational Perspective

**Debasis Dash**

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The Chromosome-Centric Human Proteome Project (C-HPP) aims at the identification and cataloguing of the proteome with respect to chromosomes in the neXtProt knowledgebase. The missing human proteome comprises of predicted protein-coding genes with no credible protein level evidence detected so far. These missing proteins may unearth important biological functions, hitherto unknown, as many of these are membrane receptors, in particular, the olfactory receptors. This knowledge can also impart a better understanding of the complex mechanisms in tissues such as the brain that have a high probability of expressing these missing proteins. In the present study, a multi-step data analysis approach was implemented. We explored various computational parameters, crucial during protein searches from tandem mass spectrometry (MS) data, for their impact on missing protein identification. Variables such as differences in search database composition, shared peptides, semitryptic searches, post-translational modifications (PTMs), and transcriptome guided proteogenomic searches are taken into consideration. We used in house developed Genosuite - a multi-algorithmic approach for protein detection from publicly available mass spectra from recent studies covering diverse human tissues and cell types. Using the aforementioned approaches, we successfully detected 24 missing proteins (22-PE2, 1-PE4, and 1-PE5). Maximum of

## Proteomic approaches unveil some secrets of the extreme Radiation resistance in *Deinococcus radiodurans*

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The Gram-positive, non-pathogenic bacterium *Deinococcusradiodurans* finds mention in the Guinness Book of World Records as "world's most radiation resistant organism". Complete genome sequencing has not revealed much about its extraordinary ability to repair extreme DNA damage caused by ionising radiations and other genotoxic stresses. Proteomic investigations have revealed the following unusual feature during the post-irradiation recovery (PIR) of this microbe : (1) proteome appears nearly intact following exposure to  $\alpha$ -ray doses as high as 6kGy (2) radiation activates protease activity of certain proteases (3) the activated proteases selectively degrade many (radiation damaged?) proteins, followed by their rapid resynthesis - a phenomenon described by us earlier as "protein recycling" (4) chaperones, oxidative stress alleviators and energy metabolism players are among the first proteins to be resynthesized during PIR (5) degradation of the DdrO repressor by the PprI protease acts as a molecular switch that induces/enhances synthesis of several DNA repair genes that are part of the DNA damage regulon (DDR) in this bacterium (6) detailed analysis of the proteome modulation during the lag preceding the post-irradiation growth resumption reveal the chronological sequence of synthesis and activity of DNA repair proteins, which facilitate rapid repair and restoration of a functional genome. Nearly one-third of this organism's genome has no functional identity, but many of these are radiation-responsive genes and proteins. Elucidation of mechanistic aspects of radiation-responsive gene expression has also led to isolation of radiation-inducible gene promoters which can be exploited for applications in high radiation environment.

**ABSTRACTS FOR INVITED TALKS (I001-I010)**

December 14, 2016

I002

I001

**Comparative proteomics of the recently formed allopolyploid *Tragopogon mirus* and *T. miscellus* (Asteraceae)**

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Polyplody (whole-genome duplication) is recognized as an important evolutionary process in speciation and genome evolution of diverse organisms, particularly plants. However, much of our current understanding of polyplody is based on analyses of crop species. Here, we examined the proteomes of naturally occurring allopolyploids (*Tragopogon mirus* and *T. miscellus*) and its diploid parents (*T. mirus* = *T. porrifolius* X *T. dubius*, *T. miscellus* = *T. pratensis* X *T. dubius*). Using label-free proteomics techniques, we analyzed the differential protein expression levels between the allopolyploids and their diploid parents. A total of 8,642 proteins were identified from young leaf tissues of diploid and polyploid *Tragopogon* species. This study will provide valuable insights into the proteomes of recently formed allopolyploids. In addition, the proteomics data set generated here provides the most comprehensive resource for the *Tragopogon* polyploid system, which enables further studies of gene and protein expression patterns in different tissues and under different conditions. Therefore, the results have improved our understanding of plant genome evolution and adaptation to the environment.

I002

**Proteomic Analysis in *Caenorhabditis elegans* Challenges with *Klebsiella pneumoniae* Revealed the Regulation of TOR Pathway against Infection**

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Host-pathogen interaction studies at the protein level attract greater interest by providing profound knowledge on different dimensions of interface between host and pathogen. The alarming increase of epidemic infections by several drug-resistant bacteria renowned the necessity of insightful knowledge on microbial pathogenicity and host immune defense at protein level. The recent advent in proteomic tools advances our perspectives on host-pathogen interaction by deciphering the possible molecular mechanism(s) at both ends. *Klebsiella pneumoniae* is a potent Gram-negative bacterium belongs to the family of *Enterobacteriaceae* causes several acute to chronic dreadful infections in human. Hitherto, the lack of understanding on host defense mechanism and scanty availability of suitable model organisms make the researchers unsuccessful to come out with appropriate therapy. *Caenorhabditis elegans* is a well established model system to study the host-pathogen interactions at both transcriptional and translational level against different bacterial infections. Earlier, our lab has analyzed the changes in host proteome against diverse Gram-negative bacterial pathogens such as *Salmonella Typhi*, *Vibrio alginolyticus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Shigella* Spp. The results suggested that *C. elegans* exhibited a specific innate immune response against different pathogens by regulating the proteins like PDI-2, DAF-21 and EEF-2 against *V. alginolyticus*, *P. mirabilis* and *P. aeruginosa* respectively. Since the experimental out-comes from *C. elegans* were highly comparable to higher animals/mammals, the present study was carried out to understand the

alterations in host proteome against *K. pneumoniae* infection. Comparison of protein profiling by MALDI-MS/MS and LC-MS/MS of nematodes challenged with *K. pneumoniae* identified many (~200) host encoded regulatory proteins. Functional bioinformatics analyses of identified proteins suggested that the metabolic and dauer formation pathways were over-represented. The increased level of intestinal atrophy and ROS generation indicated that the regulation of metabolic pathway was mediated through TOR. The downregulation of upstream regulators TOR pathway in immunoblotting analysis confirmed the involvement of this pathway against *K. pneumoniae* infection. Furthermore, the susceptibility of TOR pathway specific *C. elegans* mutants towards *K. pneumoniae* validated the involvement of TOR pathway in host defense against *K. pneumoniae*.

December 15, 2016

I003

### **Quantification and validation of potential biosignatures for intrinsic subtypes of breast cancer**

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Breast cancer (BC) is the most frequent malignancy observed in women throughout the world. Survival rates of the patients significantly increases, provided the BC is detected at an early stage. The limited available methods for diagnosis involve painful and invasive procedures such as biopsy. There is an urgent need to discover novel biomarkers for BC which will not only be helpful in detecting the disease at an early stage, but also help in monitoring disease progression and treatment efficacy. BC is highly heterogeneous cancer and includes four principal intrinsic subtypes, namely luminal A, luminal B, HER2 enriched and triple negative breast cancer. In the present study, we aim to identify potential biosignatures for breast cancer on human samples using multipronged proteomic approaches at subtype level. Multivariate statistical analysis was carried out to extract statistically significant differentially expressed proteins belonging to specific subtypes. The panel of differentially

regulated proteins was subjected to various bioinformatics tools to elucidate the various pathways involved in four intrinsic subtypes of breast cancer. A few important statistically significant candidate proteins were validated using the conventional western blotting method. Another validation strategy was adopted using the mass spectrometry based MRM assays. The MRM assays appear promising approach to validate potential biosignatures for intrinsic subtypes of breast cancer.

I004

### **Targeted Proteomics for the validation of biomarkers in Brain Tumors**

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Brain tumors are rare cancers, which account for 30% and 20% cancer related mortality in children and young adults, respectively. Tumors are classified into benign and malignant cancers. Most of the gliomas and medulloblastomas are malignant brain tumors, while meningiomas are benign tumors. An integrated iTRAQ-based quantitative tissue proteomic analysis of glioblastomas, medulloblastomas and meningiomas was performed using different controls, including colon adenocarcinomas as disease control to identify differentially expressed proteins in different groups of brain tumors. Tumor tissue proteins were digested using trypsin followed by iTRAQ labeling. Labeled peptides were pooled and subjected to off-gel fractionation followed by LC-MS/MS analysis. Various metabolic pathways including sugar metabolism, ECM-receptor interaction, complement and coagulation pathways were found to be altered in brain tumors. Fructose & mannose metabolism, spliceosome and aminoacid metabolism were found to be altered in GBM, medulloblastoma and meningiomas respectively. Proteins like CRYAB, GFAP, BASP1 and SNCA were found to be significantly altered in gliomas, whereas VIM, RABP1, ANXA2 and SBP1 showed differential

expression in meningiomas. Various ribosomal and histone proteins were found to be significantly altered in medulloblastomas. Serum autoantibody screening revealed presence of antibodies against SBP1, TPD52L2, GSTP1 and FABP5 in meningioma patients. Few of the identified targets were further validated using MRM-based quantitative approach, where the digested proteins from the tissue lysates were run on triple quadrupole mass spectrometry. The data files obtained were analyzed using Skyline. For each protein a minimum of 3 peptides with at least 3 corresponding transitions were used for quantification. This comprehensive quantitative and targeted proteome profiling of brain tumors identified few potential markers and revealed altered pathways specific to different brain tumors. This study offers insights into tumor pathophysiology and would help in better understanding of tumor biology.

*(equal contribution)*

I005

### Proteomic Analysis of Treatment-Naïve Early RA Serum Identifies a Unique Protein Signature and Reveals Upregulation of Platelet Activation and Degranulation Pathways

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**Background:** Rheumatoid arthritis (RA) is an inflammatory disease of joints with an autoimmune etiology that is characterized by synovial hyperplasia, infiltration of immune cells into the synovium and cartilage/bone erosions. There is increasing evidence of a “window of opportunity” of 8-12 weeks during which treatment initiation may result in better efficacy. In addition, it is becoming clear that the pathogenesis of RA begins

many years before the development of the disease. For successful disease interception, at-risk individuals that will develop the disease in a period of 12-18 months need to be successfully identified. Therefore, we initiated serum proteomics efforts to identify diagnostic and prognostic biomarkers of early RA. These biomarkers will eventually be studied in longitudinal pre-RA to RA samples for their potential utility in the identification of at-risk individuals.

**Methods:** Serum samples from seropositive (ACPA+RF+) and seronegative (ACPA-RF-) early RA (<12 months of disease duration), and healthy controls were analyzed using Data Independent Acquisition (DIA), a next-generation high-resolution mass spectrometry-based global proteomics platform for robust quantification of virtually all detectable peptides and proteins in a complex sample. Analysis of neat and depleted (top 20 abundant proteins) serum samples was carried out to identify unique protein signatures. Top discriminatory proteins from these workflows were validated by SomaScan targeted proteomics assay and a high-throughput Western blotting technology integrated in ProteinSimple’s Peggy-Sue platform.

**Results:** Mass spectroscopy-based DIA proteomics platform identified 49 proteins in ACPA+RF+ and 46 proteins in ACPA-RF- early RA patients that were differentially induced (>2-folds; p<0.01) in comparison to healthy serum samples. GO annotation of these proteins identified up-regulation of platelet activation and degranulation pathways in early RA patients. Comparison of neat and depleted serum samples revealed a panel of seven upregulated proteins present in both datasets. The up-regulation of these proteins in early RA serum samples was confirmed by orthogonal SomaScan and ProteinSimple platforms.

**Conclusion:** Our results provide comprehensive proteomic characterization of early RA serum samples and demonstrate upregulation of platelet activation and degranulation pathways in early RA.

I006

## Industrialization of quantitative proteomics measurements by SWATH-MS

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Although SWATH-MS or data independent acquisition (DIA) strategies coupled with nanoflow rate chromatography can provide a higher sensitivity and minimal sample usage, quantitative proteomics applications up to now have been restricted by low throughput, low data reproducibility and low robustness. Here we standardized a micro flow rate chromatography system to be used in combination with SWATH-MS acquisition to assess the overall comparative performance by exploring its analytical capabilities, reproducibility, stability, robustness and ease of use. We show that micro LC-SWATH-MS is able to precisely quantify more than 5000 proteins even with one dimensional high-resolution separation capabilities with small particle columns which ultimately provide high depth of coverage. This micro-LC setup can perform large scale quantitative proteomic experiments over hundreds of samples with robust operation, reproducibility across different complex matrices. Attaining high accuracy and signal stability in label free proteomics, micro LC-SWATH-MS workflows enabled processing many hundreds of samples in an automated unattended operation with equivalent or superior higher performance characteristics. Steps to achieve these types of results and operational specifics will be discussed.

December 16, 2016

I007

## Protein degradation rate in *Arabidopsis thaliana* leaf growth and development

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Protein homeostasis is a balance between degradation and synthesis. In order to characterize protein turnover to plant growth and development,

we have applied  $^{15}\text{N}$  labelling approaches in leaves of the *Arabidopsis* rosette. By progressively labelling new peptides with  $^{15}\text{N}$  and measuring the decrease in the abundance of over 60,000 peptides with natural isotope abundance profiles we determined the degradation rate of 1228 proteins. The exponential constant of the decay rate ( $K_D$ ) for each protein during growth showed a wide distribution, ranging from 0 to 2 per day. This showed *Arabidopsis* protein half-lives vary from several hours to several months. We found new rapidly degrading subunits in a variety of protein complexes in plastids, identified the set of plant proteins whose degradation rate changes in different leaves of the rosette and correlated with leaf growth rate, and calculated the protein turnover energy costs in different leaves and their key determinants within the proteome. The role of degradation in keeping protein homeostasis during development was discussed.

I008

## 'Omics' analysis of leaf from *Clematis terniflora* DC. under binary stress

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*Clematis terniflora* DC. is a valuable resource possessing potential pharmaceutical value and UV-B irradiation can change this effect by influencing the accumulation of secondary metabolites. Proteomic, transcriptomic and metabolomic analyses of leaf of *C. terniflora* were performed to investigate the systematic response mechanisms to UV-B irradiation. Leaves were collected from UV-B irradiated plants without or with dark treatment. Proteins related to stress, cell wall, and amino acid metabolism; metabolites related to carbohydrates, fatty acids, and amino acids; genes related to amino acid metabolism, tricarboxylic acid cycle, and mitochondrial electron transport chains were gradually increased in response to high-level UV-B irradiation with dark treatment. With the integration of these data and confirmative results, the omics analysis suggests that high-level UV-B

irradiation with dark treatment induces the enhancement of reactive oxygen species scavenging system and tricarboxylic acid cycle in leaf of *C. terniflora*. Furthermore, the abundance of dihydrolipoyl dehydrogenase/glutamate dehydrogenase, content of gamma-aminobutyric acid, and genes related to biosynthesis of lignins and flavonoids/isoflavonoids were significantly increased/upregulated following high-level UV-B irradiation with dark treatment, indicating the induction of gamma-aminobutyric acid shunt pathway and the secondary metabolism pathway in leaf of *C. terniflora* under the binary stress, which may revealed the mechanism of enhanced medicinal potential in *C. terniflora* exposed to high-level UV-B irradiation with dark treatment.

December 17, 2016

I009

## **Differential protein expression profiling as means to explain beneficial advantages of co-administering growth factor cocktail with cardiac stem cells for regenerative therapy**

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**Background of the study:** Cardiac stem cells trigger paracrine mechanisms mediated by cytokines that repair infarcted tissue and certain cytokines in turn bestow stem cells with effective regenerative functions. We have sought to study the effect of growth factor cocktail on cardiac progenitor stem cells, and the translational value of administering growth factors along side cardiac stem cells in animal myocardial infarction models.

**Methods and Results:** Cardiac stem cells were isolated from the hearts of twelve laboratory-bred female *Wistar* albino rats. Stem cell progeny was

confirmed by flowcytometry studies and cardiac progeny was established by Immuno-electro fluorescence studies using troponin antibody. Cultured stem cells grown in the presence and absence of growth factor cocktail was compared by inverted phase contrast microscopy for cellular morphology and MTT assay for proliferative functions. Animal experiments included injecting saline, growth factors, stem cells, and combination of stem cell and growth factor cocktail in four groups of myocardial infarction rat models. Myocardial infarction was established by magnetic resonance imaging, and post-intervention follow up studies was done for four weeks by two-dimensional M-mode echocardiography. Cells grown with growth factors were seen to have distinct cardiac morphology showing elongated cells with branching fibers and centrally placed nucleus; functionally these showed 30% increase viability, and had over expression of proteins which are known to confer cardiac protection and counter oxidative stress. Rats that received combination therapy showed significant improvement in cardiac function as compared to rats that received individual therapy of saline, stem cells or growth factors alone. DIGE coupled with mass spectrometric experiments was done to delineate possible cellular pathways to explain the advantages bestowed by growth factors on cardiac stem cells. Interestingly, aldehyde dehydrogenase, guanine deaminase, alpha 2 macroglobulin and G6PD that play a role in cardio protection, development, cytokine regulation and negate oxidative stress are over expressed in cells primed with growth factors.

**Conclusions:** Cytokine growth factors confer distinct advantages are in terms of growth, differentiation, proliferation and cardiac function. DIGE based proteomic experiments are an ideal platform to explain the above observational outcomes.

**Characterization of Thiol Redox Modifications of SnRK2.6-2C from *Brassica napus***

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Sucrose non-fermenting 1-related protein kinase 2.6 (SnRK2.6), aka Open Stomata 1 (OST1) in *Arabidopsis thaliana* plays a pivotal role in abscisic acid (ABA) mediated stomatal closure. Four SnRK2.6 orthologs were identified in *Brassica napus* genome in our previous work. In this study, one ortholog BnSnRK2.6-2C, which was highly induced by ABA treatment in stomatal guard cells at the transcription level, showed redox-regulated autophosphorylation activity *in vitro*. Autophosphorylation of BnSnRK2.6-2C was inhibited by oxidants S-nitrosoglutathione (GSNO)

I010 and oxidized glutathione (GSSG) in a dose-dependent manner, and the inhibition could be reversed by nonspecific or specific reductants. Phosphorylation sites on serine and threonine residues in the protein including the activation loop were mapped. Monobromobimane (mBBr) labeling was used for detecting and identifying redox modifications of cysteine residues in the BnSnRK2.6-2C. Interestingly, modifications were also found to be dose-dependent by GSNO but not GSSG. Mass spectrometer (MS) analysis revealed the unexpected mBBr labeling on tyrosine residues and the utility of mBBr as a stable thiol-label for evaluation of cysteine redox status. In this study, more cysteine modifications were identified than previously reported in literature. For example, an irreversible modification sulfonic acid (trioxidation) was identified with high confidence, so was the cysteine glutathionylation, which was rarely found in plant proteins. Among all the six cysteine residues in BnSnRK2.6-2C, C159 was identified in all the treatments with different modifications, suggesting it has high redox sensitivity and reactivity. Since it is the only cysteine in the activation loop of this kinase, redox regulation of C159 of the BnSnRK2.6-2C in the physiological context of stomatal functions will be further studied.

## ABSTRACTS FOR INDUSTRY TALKS (C01-CO4)

December 14, 2016

December 15, 2016

C01

C02

**Activation of p53 in down syndrome and in the Ts65Dn mouse brain associated with a pro-apoptotic phenotype**

**Aman Tyagi** on behalf of Antonella Tramutola,  
**Gilda Pupo, Fabio Di Domenico, Eugenio Barone, Andrea Arena,**  
**Chiara Lanzillotta, Diede Broekaart,**  
**Carla Blarzino, Elizabeth Head,**  
**D Allan Butterfield, and Marzia Perluigi\***

*Bio-Rad Laboratories*

Down Syndrome (DS) is the most common genetic cause of intellectual disability resulting from trisomy of chromosome 21. The main feature of DS neuropathology includes early onset of Alzheimer's disease, with deposition of senile plaques and tangles. We hypothesized that apoptosis may be activated in the presence of AD neuropathology in DS, thus we measured proteins associated with upstream and downstream pathways of p53 in the frontal cortex from DS cases with and without AD pathology and from Ts65Dn mice, at different ages. We observed increased acetylation and phosphorylation of p53, coupled to reduced MDM2-mediated ubiquitination and lower levels of SIRT1. Activation of p53 was associated with a number of down-stream targets (bax, PARP1, caspase-3, heat shock proteins and PGC1 $\alpha$ ) that were modulated in both DS and DS/AD compared with age-matched controls. In particular, the most relevant changes (increased p-p53, acetyl-p53 and reduced formation of MDM2/p53 complex) were found to be modified only in the presence of AD pathology in DS. In addition, a similar pattern of alterations in the p53 pathway were found in Ts65Dn mice. These results suggest that p53 may integrate different signals, which can result in a pro-apoptotic-phenotype contributing to AD neuropathology in people with DS.

**Analysis of Post Translational Modifications using CESI-MS**

**Jim Thorn**

*Sciex, USA*

Despite recent progress in top-down mass spectrometry (MS), most studies are performed by infusion of highly purified proteins and protein complexes. This is mainly due to the lack of high efficiency front-end separation tools that would allow for direct analysis of these molecules. Capillary electrophoresis (CE) is an established and powerful technique for intact protein analysis, particularly in the characterization of biologics both native and denaturing. The integration of CE and ESI into one dynamic process, within the same device (deemed CESI) provides the means to perform highly efficient protein separation and ionization in the ultra-low nanoflow regime (>30 nL/min) simply using an open capillary tube. Thus, CESI-MS is ideally suited for top-down proteomics, native MS and analysis of post translational modifications. In this presentation, results from top down and bottom-up approaches to study post translational modifications using CESI-MS will be reviewed.

C03

**Unlocking the Power of the Genome with Multi-Omics data and the Illumina Correlation Engine**

**Thomas Patrick Klemm**

*Sr. Sales Specialist, Illumina Singapore*

Integrated multi omics research data is a striking buzz word in today's scientific and business discussions. Even more amazing is the outreach it has already shown into the daily news and people's personal lives, by changing the outcome of infectious pandemics, pregnancies, cancer therapies

and helping newborns and young children with inherited diseases to overcome previously unsurmountable hurdles in human history. With the genomics field evolving at light speed, innovation is key to keep this development alive. Illumina is constantly improving their products and widening the portfolio to give researchers and clinicians access to new information in a multitude of applications.

It is crucial to stay updated on new releases and developments to achieve the best results in studies looking at genetic content and variation. Here we will discuss how cutting edge research is using proteomics data, Genome Wide Association Studies (GWAS), epigenetic research and large scale population studies, using next generation sequencing (NGS) and array technology, to discover new findings and therefore paving the way to change in how we will practice medicine in the future. One way to achieve such success in uncovering the unknown can be done using the new software tool from Illumina called Correlation Engine (formerly known as NextBio). After an overview of the technology we will also showcase a few scenarios in which it can be applied with multi omics data.

Illumina is playing a crucial role in transforming the way we will be looking at patients in the future, being the market leader in genomics and following a single vision: To improve human health by unlocking the power of the genome!

## Qualitative and Quantitative Characterization of a Novel Scanning Quadrupole DIA Method for Omics Analysis

Keith Richardson; Christopher J Hughes;  
Lee A Gethings; Martin Green;  
Richard Chapman; Jason Wildgoose;  
Arkadiusz Grzyb; Praveen Harapanahalli;  
Kirsten Craven; Johannes PC Vissers\*;  
James I Langridge and Ian J Edwards

*Waters Corporation, United Kingdom*

Targeted LCMSbased assays are increasingly applied in the postdiscovery omics area with emphasis on validation, the first of many phases in translational analyses, or in studies that are aimed at gaining the understanding of biological systems, drug development and treatment. Context is driving current omics experiments, thereby driving the development of LCMS acquisition methods that can provide both qualitative and quantitative information in a single experiment. An alternative DIA mode operation, whereby a resolving quadrupole is scanned during highresolution precursor and production acquisitions, was applied for targeted lipidomics and proteomics quantitation experiments using transition extraction list and compound library based approaches.

## ABSTRACTS FOR ORAL PRESENTATIONS (OP01 – OP10)

December 14, 2016

OP01

### **Remodeling of the Transcriptome, Proteome and Metabolome of Grasspea: The Molecular Basis of Dehydration Tolerance**

**Divya Rathi, Akanksha Pareek, Deepika, Subhra Chakraborty and Niranjan Chakraborty\***

*National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi 110067, India*

It is unlikely that the molecular basis of stress tolerance in crops, particularly in a large legume family Fabaceae, comprising 946 genera, can be accurately unraveled in a few species. Despite their hardiness, the major constraint to global legume production have been the multivariate environmental stresses. Grasspea is one of the best examples of an orphan legume, which has thus far received little research attention. Grasspea cultivars are capable of withstanding a myriad of environmental stresses, making it a model system to study stress tolerance. We therefore investigated molecular basis of stress tolerance in grasspea by subjecting 3-week-old seedlings to dehydration for duration of 144 h. There were no visible changes in the seedlings till 48 h of dehydration and the leaflets curled after 60 h and displayed chlorosis. Effects of stress caused alterations in relative water content, photosynthetic pigments, increased accumulation of proline and membrane disruption. The temporal effects of dehydration were evaluated at the transcriptomic, proteomic and metabolomic levels using *de novo* RNA-seq, 2-D gel electrophoresis and GC-MS, respectively. The 2-DE analysis revealed 163 proteins that were differentially regulated in a qualitative as well as quantitative manner. Conversely, approximately hundred-fold transcripts were found to be dehydration-responsive when compared to the proteins. Furthermore, dehydration-responsive metabolome profile displayed significant alteration in 30 metabolites associated with global metabolic pathways. The combined analysis of the multi-

omics landscape of grasspea would not only provide useful insights into the underlying mechanism of stress tolerance, but also enlist the novel biomarkers that could be used for targeted genetic manipulation for crop improvement.

OP02

### **Proteomic Analysis of the Outer Membrane Vesicles (Omvs) of an Archebacterium *Natrinococcus* Sp., Whose Genome Sequence Is Not Known**

**M. V. Jagannadham\*, P. Gayathri, C.V.B.Swamy, D.B. Kameswari**

*CSIR-Centre for Cellular and Molecular Biology, Uppal road, Tarnaka, Hyderabad-500007*

The outer membrane vesicles (OMVs) from an archaea *Natrinococcus* sp. obtained from the sambhar lake of Rajasthan were isolated and characterized for their molecular components. The pink pigments present in the archaea were found to be segregated to the OMVs. The proteins from the outer membrane vesicles of arches *Natrinococcus* sp., whose genome sequence is not known were identified using a database prepared from the genome sequences of some other *Natrinococcus* sp. The peptide sequences were validated by acetylating the tryptic peptides followed by determination of the *de novo* sequence of the peptides. In this method, the acetyl ammonium ions confirm the N-terminal aminoacid of the peptide, where as the b-ion and y-ion series leads to the sequence determination of the peptides. It was shown that b1-ions of the amino acids were identified, representing the N-terminal amino acids of the tryptic peptides. The relative intensity of the b-ions increased in the acetylated peptides as compared to the un-acetylated peptides generated by trypsin digestion. An increase in the occurrence of b-ions, and a relative increase in the intensity of b-ions followed by the identification of b1-ions of different amino acids improved the *de novo* sequencing efficiency of the peptides. The MS/MS spectra were analyzed by SEQUEST, PEAKS and the proteins were identified using stringent criteria. The proteins from the OMVs were identified by

comparing the *de novo* sequence of the peptides determined using PEAKS algorithm. This approach was useful in the identification and also validation of the identified proteins.

OP03

## Global S-Nitrosoproteome Identification Links UCHL1 to Parkinson Disease Pathology

**Roshan Kumar<sup>1,4</sup>, Deepak K Jangir<sup>1</sup>,  
Garima Verma<sup>2</sup>, Shashi Shekhar<sup>3</sup>,  
Pranita Hanpude<sup>1,4</sup>, Sanjay Kumar<sup>1,4</sup>,  
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Parkinson disease is characterised by excessive serum nitric oxide level and upregulated nitric oxide synthase activity. Aberrant level of nitric oxide leads to the covalent binding of NO moiety to protein cysteine residues and it is one of the main mechanisms of nitric oxide signalling. Cysteine thiols act as redox switch and regulate various physiological process. Using a combination of the alkylating biotin switch assay and LC-MS/MS analysis, we have revealed the S-nitrosoproteome of rotenone induced Parkinson's disease mouse model brain and rotenone treated SH-SY5Y cells. A total of 49 and 249 nitrosylated proteins identified in Parkinson's mice model brain and rotenone treated SH-SY5Y cells respectively. We have identified Ubiquitin C-terminal hydrolase 1(UCHL1) as one of the protein which undergoes nitrosative modification. Cysteine 90,152 and 220 of UCHL1 gets modified under nitrosative stress condition which regulate its deubiquitinating activity and ubiquitin binding. We have shown by employing various biophysical approaches that S-nitrosylation of UCHL1 alters its conformation leading to the formation of amorphous aggregates and constituting one of the component of Lewy body. Our findings provide a new link between

UCHL1-nitrosylation and Parkinson's disease pathology.

December 15, 2016

OP04

## Proteometabolomic Study Illustrates Dual Role of Oxalic Acid in Anti-Nutrient Signaling and Non-Host Resistance

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Fruit is an assimilator of metabolites, nutrients, and signaling molecules, thus considered as potential target for pathogen attack. Oxalic acid (OA), an ubiquitously present metabolite plays dual role in fungal pathogenicity in a concentration dependent manner. While at higher concentration it induces programmed cell death leading to fungal invasion, low oxalate build resistance in plant. Although OA has been identified as a virulence determinant for rot disease caused by *Sclerotinia* sp., our understanding of how oxalate downregulation impart host immunity is limited. We conducted time-series protein and metabolite profiling of *Sclerotinia rolfsii* invaded wild-type and E8.2-OXDC tomato (*Solanum lycopersicum*) fruit using 2-DE coupled LC-MS/MS and GC-MS analysis. The differential display of *Sclerotinia* infected tomato fruit proteome revealed 105 patho-stress responsive proteins (PSRPs), while network analysis identified major protein hubs pointing towards the onset and context of disease signaling and metabolic pathway activations. A step further, to investigate the role of OA and the metabolic consequences of oxalate down-regulation, we generated transgenic tomato (E8.2-OXDC) expressing oxalate degrading enzyme from the fungus *Flammulina velutipes* (*FvOXDC*) specifically in the fruit. Our data showed that ectopic expression of *FvOXDC* specifically degrades OA in tomato. A comparative proteomics approach in E8.2-OXDC tomato fruit displaying fungal resistance. Mass spectrometric analyses identified 92 OXDC-responsive immunity related protein spots (ORIRPs) presumably associated with acid metabolism, defense signaling and endoplasmic reticulum stress. Metabolome study indicated

differential abundance of some of the organic acids paralleling the proteomic analysis. Further, we interrogated the proteome data using network analysis that identified modules enriched in known and novel disease- and immunity-related prognostic proteins. This study reports, for the first-time, kinetically controlled concentration dependent oxalate responsive protein network during post-harvest storage in a sink tissue, particularly fruit and constitute the basis towards understanding the onset and context of disease and immune signaling.

OP05

### **Plasma Proteome Signatures for Short-Term and Long-Term Acclimatization at High Altitude**

**Pooja, Manish Sharma, Rajkumar Tulsawani, Kalpana Bhargava and Niroj Kumar Sethy\***

*Peptide and Proteomics Division, Defence Institute of Physiology and Allied Sciences, Defence Research and Development Organisation, Lucknow Road, Timarpur, Delhi – 110054, India*

Exposure to high altitude and thus hypobaric hypoxia is a pervasive physiological stimulus and has broad implications for the pathophysiology of diseases correlated to tissue hypoxia, e.g. heart failure, severe obesity and obstructive sleep apnea syndrome. More than 140 million people live permanently or travel to high altitudes ( $> 2500$  m) for work, sports and leisure around the world including Asia, East Africa, North, Central and South America. With an increasing number of people moving to high altitude, the study of physiological acclimatization to hypoxia and related diseases is growing in importance. In the present study, using iTRAQ-based plasma proteomes approach we have identified 318 proteins (1% FDR) for healthy high altitude sojourners during acute (7 days) and prolonged (120 days) hypobaric hypoxia exposure at Leh (3520 m), India. Pathway analysis revealed that prolonged exposure to hypobaric hypoxia down regulated platelet activation, complement activation, cholesterol transport and inflammatory response pathways which were further validated with ELISA and western blot analysis. HPLC-based estimation of plasma amino acids revealed differential activation of Nitric oxide pathway during high altitude

acclimatization. We have also compared the plasma proteomes of high altitude sojourners with Ladakhi natives to decipher the similarities between high altitude acclimatization and adaptation. Our results highlight the differential activation of energy generating, vasodilatation and inflammatory pathways during acute and chronic high altitude exposure.

OP06

### **A Browser-based Interactive Dashboard for Exploratory Analysis of MS-based Proteomics data**

**Puneet Kumar Kadimi, Suruchi Aggarwal, Amit Kumar Yadav\***

*Drug Discovery Research Center, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, 3rd Milestone, Gurgaon- Faridabad Expressway, Faridabad, Haryana-121001*

Mass Spectrometry-based proteomics has become the main approach for analysing and understanding the role of proteins in a biological system. Yet visualization of the large amounts of proteomics data in interactive ways is still challenging. In a Mass Spectrometry based experiment large amount of data are generated at different stages, which makes in-depth analysis of specific proteins tedious and unwieldy. Here we present an Interactive web-based dashboard application for exploratory analysis of data obtained via Mass Spectrometry based experiments. This application accepts raw Spectrum in MGF, mzML, Peptide-Spectrum matches and protein inference in pepXML, mzIdentML or TSV to visualize them in Interactive apps for exploratory analysis. It has been developed using JavaScript and can be viewed using any modern web browser. For example, this application displays a protein list found in an experiment (from mzIdentML file), displays sequence coverage, peptide quality and spectral counts information visually in an interactive manner. Inference results are compiled and grouped in single or multi-peptide hits which can be drilled down to explore further. Individual protein features like Post-translational modification, Secondary structure information in tracks can be visualized with details on sites and modification types available from uniprot database. Peptides found in experiment can be visualized in a sequence track mapped over their

Protein sequence location to display protein coverage. This interactive application makes data analysis easier and helps a researcher devote time to understand biology rather than parsing files or ogling at dull spreadsheets.

December 16, 2016

OP07

### **Comprehensive Quantitative Metabolomic Approach to Investigate Metabolic Alterations in Invasive Ductal Carcinoma of the Breast**

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Invasive ductal carcinoma (IDC) is a type of breast cancer that accounts for 70% of all breast cancer cases. Metabolites are important players in biological systems and identifying IDC specific metabolic alterations can provide a better understanding of the molecular events involved in IDC progression. In this study, metabolic profile from the serum of 75 IDC, 33 benign and 33 healthy controls were acquired using targeted LC-MRM/MS and untargeted GC-MS approach. Further, metabolic profiles of 25 tissue samples of each IDC, benign and normal samples were also acquired to correlate the findings from serum samples of IDC. Targeted LC-MS/MS based quantitative metabolite analysis was performed in positive (HILIC) and negative (T3) modes using MRM mode on AB Sciex 4000 QTRAP MS. Untargeted metabolic analysis was performed using Agilent 5977 mass selective detector. In total, we were able to detect 101 metabolites in targeted and 218 metabolites in the untargeted analysis. IDC specific metabolic alterations were identified by employing multivariate and univariate statistical analysis using SIMCA software. Exploratory PCA was employed to detect intrinsic clustering and possible outliers. PLS-DA, OPLS-DA were further maximized the group separation, based on which a statistical model was built. These statistical models were clearly able to discriminate between IDC samples from benign and healthy controls. Statistically significant, 36

metabolites in targeted and 53 metabolites in untargeted approach were identified. These metabolites were further submitted for metabolic pathway analysis using metaboanalyst. The top seven pathways observed as Purine metabolism, Pyrimidine metabolism, Butanoate metabolism, Glycine, serine and threonine metabolism, D-Glutamine and D-glutamate metabolism, Ascorbate and aldarate metabolism and Glutathione metabolism. Overall in this study, we demonstrate that metabolic profiles are altered in IDC and these findings are not only helpful in understanding the progression of IDC subtype of breast cancer but also useful as potential therapeutic targets.

December 17, 2016

OP08

### **Effect of Vitamin B<sub>12</sub> Restriction on Wistar Rats: A Global Proteomic Profiling**

**Swati Varshney<sup>1,2</sup>, Arunachal Chatterjee<sup>1</sup>, Ajay Bhat<sup>1,2</sup>, Usha Sree R<sup>3</sup>, Lovejeet Kaur<sup>3</sup>, Vislavath Jyothi<sup>3</sup>, Pujitha Kommineni<sup>4</sup>, Rakesh Mishra<sup>3</sup>, GR Chandak<sup>3</sup>, M Raghunath<sup>4</sup>, Shantanu Sengupta<sup>1,2\*</sup>**

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Deficiency of vitamin B<sub>12</sub> is common in Indian population especially in pregnant mothers. In-utero Vitamin B<sub>12</sub> deficiency (which regulates one carbon metabolism including DNA methylation) during early pregnancy may rewire the proteome and methylome of the fetus in a way that programs development of complex disorders like cardiovascular diseases in later stages of life. We are interested in understanding whether these changes are transmitted multi-generationally to set the permanent change in physiology of target organs such as skeletal muscles and liver.

To assess the transgenerational effect of maternal B<sub>12</sub> deficiency, we developed the control and B<sub>12</sub> deficient Wistar female rat model (F<sub>0</sub>) and mated them with normal male rats. We measured biochemical and lipid levels for F<sub>1</sub> (3-12 months) and

$F_2$  (3-12 months) pups born to  $B_{12}$  restricted mothers. We generated the proteome profile of 3-month old  $F_1$  pups. Four-plex iTRAQ experiments were performed in triplicates for liver, kidney and skeletal muscle to generate differential proteome of male and female tissues separately. In each 4plex, tissues from control group (C), vitamin  $B_{12}$  restricted (VR), rehabilitated at conception (RC), and rehabilitated at parturition (RP) were included.

We identified a total of 2279, 2212, 2449 and 2199, 2182, 2379 proteins in three different biological replicates experiments at 1% FDR in male and female liver respectively. We found 239 proteins to be differentially expressed (129 up and 110 down-regulated) in male liver and 317 proteins (195 up and 122 down-regulated) in female livers. Of these, 18 up-regulated and 11 down-regulated proteins were common. We also found tissue-specific changes in protein expression in kidney and skeletal muscle as well. We observe distinct differences in protein expression among males and females suggesting sex-specific effects of maternal vitamin  $B_{12}$  restriction. This study will provide a comprehensive understanding of the effect of vitamin  $B_{12}$  deficiency on different tissue types.

OP09

### iTRAQ Based Comparative Proteome Profiling of A1A1, A2A2 and A1A2 Beta Casein Variants of Cow Milk

Preeti Rawat<sup>1</sup>, Shivam Dubey<sup>1</sup>, Shveta Bathla<sup>1</sup>, Alka Chopra<sup>1</sup>, Sudarshan Kumar<sup>1</sup>, Manishi Mukesh<sup>2</sup>, Monika Sodhi<sup>2</sup>, Ashok Kumar Mohanty<sup>1\*</sup>

<sup>1</sup>National Dairy Research Institute, Karnal

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Cow milk is a wholesome food which contains about 3.5% proteins, of which approximately 80% are caseins (alpha, beta and kappa) and 20% are whey proteins. Milk whey contains numerous minor proteins which play important role in imparting positive health attributes. Beta casein is also a regulatory molecule which regulates lactogenesis. Milk has been categorized into 3 different genotypic variants namely A1A1, A1A2 and A2A2. The A1A1 and A2A2 betacasein variants differ at amino acid position 67 with histidine in A1 and proline in A2 milk. During gastrointestinal

proteolytic digestion, A1 releases 7 amino acid "opioid" bioactive peptide BCM 7 and A2 releases BCM 9. Epidemiological evidences suggest that consumption of A1 milk can cause type I diabetes mellitus, coronary heart diseases, sudden infant death syndrome, Schizophrenia and autism. The A1/A2 hypothesis therefore is important for public health. Till now no information is available on the protein composition of milk of these 3 genotypic variants. In the present investigation we have identified comparative proteome of milk from A1A1, A1A2 and A1A2 Beta casein variants. Rawmilk sample were collected individually from 30 Karan fries cows, 10 each from A1A1, A1A2 and A1A2. Defatted milk samples were subjected to ultra-centrifugation for whey preparation. Tryptic peptides were prepared from whey proteins and iTRAQ labeling was done. Labeled peptides were pooled and fractionated into 96 fractions using bRPLC over 80 min gradient. The 96 fractions were separately pooled to 12 fractions and were subjected to nLC-ESI-MS. Identification of the peptide spectra using Mascot search revealed 1049 out of which were 117 differentially expressed. Twenty one proteins were up regulated and twenty two proteins were down regulated having fold change >1.5 and <0.6 respectively ( $p < 0.05$ ). The gene ontology studies using Panther software revealed that majority of proteins are actively involved in catalytic activities (49%), binding (29%), receptor (8%), structural molecular activities (5%) followed by transporter (2%) and antioxidant activities (1%). These results may provide a better understanding of their significance in human health on consumption of milk having A1A1, A1A2 and A2A2 betacasein variants.

OP10

## Glycoproteomics Based Analysis of Site Occupancy and Microheterogeneity of Mucin-Type O-Glycans on CD43

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Among various post-translational modifications naturally found on proteins, glycosylation is unique due to its inherent complexity and diversity. Particularly, mucin-type O-glycosylation (MTOG) is initiated by addition of alpha-N-acetyl-D-galactosamine (GalNAc) to Ser/Thr in the ER and Golgi. Efforts to exhaustively characterize MTOG involve gene editing and glycoproteomics based nano-LC-ESI-MS/MS methods. CD43 (leukosialin/sialophorin) is found abundantly on surface of T-cells and is known to negatively regulate immune activation. We have recently shown that Ac<sub>5</sub>GalNTGc, a non-natural analogue of GalNAc,

induced drastic inhibition of MTOG levels on CD43 in a thiol-dependent manner through metabolic incorporation. CD43 is predicted to carry 80-90 O-glycans on an average and is found to express in two major glycoforms 115 and 130 kDa depending on cell type and status of activation. Herein, we report our results on the exhaustive site occupancy analysis of extracellular domain of CD43 using high energy collision dissociation - product dependent - electron-transfer dissociation (HCD-PD-ETD) methodology. Soluble CD43-Fc-His chimera from lentivirally transduced Jurkat cells was subjected to digestion with trypsin and Glu-C. HCD-PD-ETD analysis of the glycopeptides revealed a total of 57-60 sites to be occupied by GalNAc. Of the 57 sites 32 sites are newly identified, 16 are identified in an earlier report, and nine were identified by both the studies. Jurkat cells treated with Ac<sub>5</sub>GalNTGc showed a drastic reduction in site occupancy from an average of 60 sites to 30 sites in multiple runs and analysis. Currently, efforts are underway to obtain a mass spectrometry based evidence for the presence of GalNTGc on CD43 and specific sites that are susceptible to decoration by the non-natural analogue. Our results illustrate the utility of MTOG inhibitors as enabling tools for the investigation of site occupancy and micro-heterogeneity in mucin-like glycoproteins and mucins, which are known to support evasion from immune surveillance in various diseases.

## ABSTRACT FOR POSTER PRESENTATION

December 14, 2016

### Session I- Integrative Omics and System Biology

AIOSB1

#### Proteomic Investigations on Multimetal Uptake and Pesticide Degradation by *A. fumigatus*

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Indian rivers are often contaminated with a mixture of heavy metals and pesticide. These contaminants enter the food chain and thus pose significant health risks. Microorganisms such as fungus offer a lucrative option of remediating wastewater *ex-situ*. Therefore in this study a newly isolated fungal strain *A.fumigatus* was assessed for its multimetal uptake potential in the composite salts medium using glucose and lindane as the carbon and energy source. This strain was able to grow in the presence of 1% glucose, 30 mg/L lindane and 30 mg/L multimetal (5 mg/L each of Cd<sup>2+</sup>, Cr<sup>6+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup> and Zn<sup>2+</sup>). The cube root growth kinetic constant (k) of the strain was obtained as 0.0211 g<sup>1/3</sup> L<sup>-1/3</sup> h<sup>-1</sup> in batch study. Also proteomic studies were conducted towards the elucidation of mechanisms of metal removal by the fungus at molecular level. Briefly, the extracted total fungal cellular proteins were subjected to tryptic digestion and the resultant peptides were analysed by LC-MS/MS. The differentially regulated proteins such as of enzymes serine carboxypeptidases, lysophospholipase, glycogen phosphorylase related to post translation

modification, transport and carbohydrate metabolism were particularly down regulated. The enzymes which were significantly up regulated were RNA polymerase II, phosphoserine aminotransferase, cysteine dysulfurase NFSI, and superoxide dismutase pertaining to transcription, coenzyme transport, amino acid transport and antioxidant production respectively. Therefore the current study was instrumental in revealing the various inherent mechanism of metal accumulation by this isolated filamentous fungus at molecular level.

AIOSB3

#### Integrated Organ Specific Transcriptome And Nuclear Proteome Decipher Regulatory Pathways Underlying Plant Immunity

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Disease incidence is the most crucial factor that negatively influences plant growth and limits productivity worldwide. Plants have various innate mechanisms that confer the capability to perceive the signal and translate the perception into specific response to particular pathogen. One of the big gaps in our understanding of plant immunity is in the regulatory and signaling pathways that operate immediately after pathogen recognition. These molecular events involve gene expression reprogramming, differential transcript and protein accumulation, signal transduction dependent activation or repression of target factors, and various post-translational events. To understand the immune response during vascular wilt, we performed RNA-seq analysis of *Fusarium oxysporum* inoculated and mock treated chickpea roots. A total of 407 million paired-end reads were generated from pathostress-responsive root libraries which were assembled into ~64,500 transcripts. Together a total of ~5580 host transcripts were differentially expressed during root infection, including several canonical and non-canonical transcription factors.

Considering the complexity of the wilt disease, significantly more genes and proteins are likely to be involved in defense. To identify the protein regulatory network involved in disease response, we developed the nuclear proteome and phosphoproteome that led to the identification of 1380 proteins and 627 phosphoproteins, which were categorized further into nucleus specific functions. Co-expression networks were generated to highlight the functional modules associated with stress response. Deregulated expression of several nucleus related transcripts and proteins suggest the ability of the plant to evoke parallel regulatory signaling pathways.

AIOSB4

### **Molecular Analysis of Methyl Jasmonate Treated *Podophyllum hexandrum* and Identification of CAD Isoforms Relevant To Podophyllotoxin Biosynthesis**

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Podophyllotoxin (ptox) is a therapeutically important lignan derived from *Podophyllum hexandrum* and is used as a precursor for the synthesis of anticancer drugs etoposide, teniposide and etopophose. The aim of this study is to investigate changes in the *P. hexandrum* cell proteome and transcript potentially related to ptox accumulation in response to methyl jasmonate (MeJA) elicitation. High-resolution two-dimensional gel electrophoresis (2-DE) followed by colloidal Coomassie staining and mass spectrometric analysis were used to detect statistically significant changes in cell's proteins from control and MeJA treated cell culture of *P. hexandrum*. Data were subjected to functional annotation from a biological point of view through KEGG. The phenylpropanoid and monolignol pathway enzymes were identified, amongst these, chalcone synthase, polyphenol oxidase, caffeoyl CoA 3-O-methyltransferase, S-adenosyl-L-methionine-dependent methyltransferases, caffec acid-O-methyl transferase etc. are noted as important. Changes in transcript level were explored by comparison of whole transcriptome

analysis of control and treated cell culture and it was revealed that a CAD (cinnamyl alcohol dehydrogenase) was most significantly up regulated after the treatment. CAD is involved in both lignin and lignan production. So, we target to isolate ptox specific CAD isoforms. To isolate the CAD favoring ptox, we deduced full length cDNA sequences of four CAD isoforms: *PhCAD1*, *PhCAD2*, *PhCAD3* and *PhCAD4* from the contigs of the transcriptome data and expressed in *E. coli*. In vitro, in vivo and in silico study of these four PhCADs isoforms revealed that *PhCAD3* is specifically involved in ptox accumulation whereas *PhCAD1* favoured ptox and lignin biosynthesis equally. It provides a useful resource for future research not only on the ptox biosynthetic pathway, but an overall molecular understanding on *P. hexandrum*, an endangered medicinal herb with immense therapeutic importance.

AIOSB5

### **Analysis of Gene Co-Expression Network to Identify Candidate Stress-Responsive Genes in Potato (*Solanum tuberosum*)**

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Plants growing in natural conditions are exposed to various environmental stresses that negatively impact their growth and productivity. Adaptations and responses of plants to these stresses are highly complex and include changes at the molecular and cellular level. To counter the challenges posed by different stress conditions, plants have evolved numerous strategies and adaptive measures including changes mediated by modulation of gene expression, which enable them to survive. Thus, it is of prime importance to identify regulatory genes that have essential roles in environmental stress tolerance. Driven by the advantages of systems biology, the objective of this study was to identify potential stress-responsive genes in potato (*Solanum tuberosum*), including cold, heat, and salt stress. For this purpose, a gene co-expression network was constructed and sets of gene modules correlated to stress conditions were obtained. For further prioritizing the candidate genes, analysis was further restricted to genes with high topological

centrality values and significant association with stress condition. Gene ontology analysis of significant modules suggested important regulatory mechanisms involved during stress conditions. The analysis provided various stress responsive genes in Potato, which may be further useful to generate novel hypotheses for improving our understanding and mechanisms of plant's response against different environmental stresses.

### Session III- Interaction Proteomics and Cellular Network

AIPCN1

#### **Analysis of the Differential Protein Expression Pattern Caused By the Translation Initiation Fidelity Defective Eukaryotic Initiation Factor 5 (Eif5<sup>G31R</sup>) Mutant**

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In the eukaryotic system recognition of the AUG start codon and selection of the Open Reading Frame (ORF) on the mRNA is accomplished by the scanning 40S ribosome-initiation factor complex. Non-AUG start codon such as UUG, CUG and GUG are also reported to be used by translation initiation machinery to translate protein is less well understood. The factor eIF5 protein plays an important role in translation start site selection by providing the GAP (GTPase activating protein) function. However, in yeast translation initiation fidelity defective eIF5<sup>G31R</sup> mutant causes preferential utilization of UUG as initiation codon and termed as Suppressor of initiation codon (Sui) phenotype and provide an important tool to understand the selection of non-AUG codon as a start codon. Bioinformatics analysis of yeast genome suggests more than 10% of the mRNA has in-frame or out-of-frame UUG codon at the 5'UTR region. We are hypothesizing that the dominant negative eIF5<sup>G31R</sup> mutant alters the translation of these genes and affects the cellular physiology. In order to understand the effect of eIF5<sup>G31R</sup> mutation on differential protein expression, we cultured

*Saccharomyces cerevisiae* wild type and eIF5<sup>G31R</sup> mutant cells. The total cell lysate from these cells was subjected to 2D gel electrophoresis. The 2D proteome profiles were compared with Image master 2D platinum software and observed that 35 protein spots were significantly altered in between these profiles. On sequencing using MALDI-TOF/ TOF, ten proteins were identified which includes one up-regulated and rest nine down-regulated. Some of these proteins are predicted to be involved in glycolysis pathway, amino acid biosynthesis pathways, mitochondrial nucleoid, organelle function and antioxidant activity. This study opens up new vista to study the defects in translation initiation pathways and its adverse secondary effects on different cellular process.

AIPCN2

#### **Intermittent Normobaric Hypoxia Training Before Altitude Ascent Elicits Hypoxic Signaling Pathways**

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Intermittent hypoxia (IH) is acute hypoxic exposure achieved by reducing oxygen fraction from the inhaled air. Proven benefits of IH training during physical exercise have increasingly created interest in studying the benefits during acute mountain experiences or hypobaric hypoxia. Recent studies have indicated the clinical implication of IH. However, despite the emerging trend of studies on IH associated benefits the molecular mechanism behind the effect of IH remains poorly understood. We therefore, used a quantitative proteomics approach to understand the changes that occur in plasma proteome of age matched human subjects on rapid ascent to high altitude after Intermittent hypoxia exposure at sea level. We introduced the healthy human subjects (n=10) per group and collected plasma samples before and immediately after IH exposure (SL and Post IHT-SL), 4 days after the stay at altitude (IHT-HAD4) and 7 days after the stay at high altitude (IHT-HAD7) and subjected the isolated plasma to iTRAQ based quantitative proteomics. Data analysis using network analysis

tools revealed a drastic shift in the key metabolic pathways including lipid metabolism, inflammatory signalling and ROS generating events. Although the trend of each of the metabolic or disease pathways is not conclusive yet, but in relation to previously published studies on non-IHT samples from the author's lab revealed a pre-activation of hypoxia signalling. In conclusion a time dependent proteomics analysis of plasma of human subjects exposed to IH prior to altitude ascent showed temporal impairment in proteins associated with lipid and inflammatory pathways.

AIPCN3

combinatorial phosphorylation mutants of LIC1. We find through high resolution microscopic analysis that distinct phosphorylation events of LIC1 affect discrete stages of early and late mitosis. Our data suggests a tight temporal regulation of LIC1 phosphorylation that dictates key functional outcomes in mitosis. We are using genome-wide interactomic analyses to uncover the specific molecular interactions mediated by these phosphorylation events. In conjunction with various other approaches, our study will illuminate the underlying mechanism of mitotic functional diversity of dynein imparted by phosphorylation of the LIC1 subunit of cytoplasmic dynein.

AIPCN4

### **Regulation of Discrete Mitotic Functions of the Dynein Motor by Site-Specific Phosphorylation**

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The multi-subunit molecular motor cytoplasmic dynein plays an indispensable role as the vehicle to transport cargoes towards the negative end of microtubules within cells. Dyneins are known to play distinct roles during the cell cycle owing to their ability to switch between diverse sets of cargoes. The most dramatic biochemical change imparted on dynein is the phosphorylation of its Light Intermediate Chain subunits (LICs) during the interphase to mitosis transition. LICs are the only subunits of the dynein complex that undergo major cell cycle dependent phosphorylation and incorporate ~12 fold more phosphate during mitosis, including multi-site phosphorylation by the master mitotic kinase cdk1. The mitotic phosphorylation of LICs triggers detachment of dynein from membranes/ organelles during interphase with concomitant binding to other mitotic cargoes. However, it is not known how the phosphorylation of LICs during mitosis by cdk1 at four sites allows dynein to bind new cargoes and perform distinct functions. We aim to decipher the mechanism by which specific phosphorylation events on LIC1 control different mitotic cargo binding to dynein which in turn regulates its diverse mitotic functions, using a series of single and

### **RuvB Family of Proteins from *Oryza sativa*: Silent Players Come to Limelight**

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RuvBL proteins, members of AAA+ superfamily are essential, highly conserved, and multifunctional in nature. Previous studies suggest the involvement of RuvBL proteins in cellular functions such as DNA damage repair, mitotic assembly, switching of histone variants and assembly of telomerase core complex etc. These cellular functions indicate the role of these proteins in cell viability and mutations in these proteins is lethal. Similarly, in *Arabidopsis* RuvBL proteins are essential for the viability of sporophyte and gametophyte. Being such an important protein family it has been scarcely studied in rice. In this study we have characterized two important members of this protein family- RuvBL1a and RuvBL2a in *Oryza sativa* using the in silico methods, biochemical and protein-protein interaction methods. In silico analysis lead to the identification of four characteristic motifs - WALKER A, WALKER B, SENSOR I and SENSOR II involved in ATP hydrolysis and nucleic acid unwinding activity. Structural modelling of these proteins also showed their identity with the protein structures of the homologs from other organisms. The transcript level of both the proteins OsRuvBL1a

and OsRuvBL2a was found to be upregulated under abiotic stress (salinity and cold). The biochemical characterization showed that recombinant OsRuvBL1a and OsRuvBL2a exhibit nucleic acid independent ATPase and 3' to 5' DNA helicase activities. Yeast two hybrid analysis was performed to find out the interacting partners of these proteins to elucidate their functional pathways in cell. Y2H study showed the interaction between OsRuvBL1a and OsRuvBL2a also. Y2H analysis identified that OsRuvBL1a and OsRuvBL2a interact with proteins such as NBS-LRR, Zinc Finger, histone H3, core histone domain containing protein, NAC domain containing protein etc. Using all these results we have hypothesized a model to explain the regulation of cellular functions. This study is significant to understand the various cellular mechanisms including the stress tolerance in plants.

#### AIPCN5

### **Novel Insights into the Molecular Mechanisms of Formation of Intercellular Nanoconduits in Mammalian Cells**

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Intercellular communication is a vital requirement to harmonize the behaviour of cells in a community and helps to achieve tissue homeostasis and maintenance of multicellular organisms. Intercellular nanoconduits help in intercellular communication over long distances and allow for bi- or uni-directional transport of cellular cargo between cells. Research has shown the functional importance of these cytoplasmic conduits in several physiological processes such as signal transduction, embryonic development, immune responses, apoptosis, cancer, neurodegenerative diseases initiation, intercellular pathogen transfer and nanoparticle delivery between cells. Regardless of their established implications in health and disease, there is rudimentary mechanistic understanding of the biogenesis and function of intercellular nanoconduits. Recently, the protein B94 was reported to be essential for nanoconduit formation, in complicity with other key proteins. We

hypothesize that B94 interacts with key interaction partners and localizes at specific subcellular regions to mediate nanoconduit biogenesis. We also aim to biochemically and biophysically characterize the interaction of B94 with these partners. We have explored the morphometrics of nanoconduits with an emphasis on the subcellular localization of B94 in mammalian cells. In addition, we have identified the global protein interactome of B94, with a view to understanding the functional importance of these interactions towards nanoconduit formation. Finally, we have biophysically characterized B94 as an obligate helical dimer. Present efforts are focused on understanding the functional importance and biochemical characterization of the homotypic and heterotypic interactions of B94 towards nanoconduit formation.

AIPCN6

### **Proteome-Wide Effect of Lactadherin in Mammary Epithelial Cells for Regulation of Cell Growth**

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Mammary gland is an exocrine and modified sebaceous gland, which is made up of a branching network of ducts that end in alveoli. It is an ideal system to study the molecular mechanisms associated with cell proliferation, differentiation and oncogenesis. The glycoprotein lactadherin also known as MFG-E8 mediates phagocytic clearance of apoptotic cells and influences the physiological cyclic changes taking place in mammary gland. Recent study shows that MFG-E8 is the part of MFGM and plays a major role in mammary gland development. In our current work we have shown that lactadherin isoform MFG-E8 along with GSN controls the cell growth process of epithelial cells. We have successfully down regulated the MFG-E8 protein through stable transfection of MFG-E8 shRNA. Mass spectrometry based (Q-TOF) proteome analysis by Label Free Quantification (LFQ) approach identified 151 down-regulated and 53 up-regulated proteins in stably transfected silenced MFG-E8 Buffalo mammary epithelial cell line. A total of 540 proteins were identified through MaxQuant 1.5.2.8 using NCBI *Bubalus bubalis*

database. Bioinformatics analysis performed through online software tool String 10.0 connected with offline software cytoscape 3.2.1 shows that MFGE 8 makes direct protein-protein interaction with GSN, RALGAPA2, CD9, ITGB5 and ALB. DAVID analysis shows that these proteins are involved in diverse biological processes, cellular functions, molecular functions and pathways. Furthermore, with the help of Reactome and KEGG pathway database we identified that MFGE 8 and GSN together responsible for regulation of proliferation. These *in silico* results were later confirmed by qRT-PCR. Furthermore, BrdU and MTT assay validated that stably transfected cells has more life span compared to normal counterpart. Overall this study supports that MFGE8 together with GSN regulates the proliferation of cells. Till date to the best of our knowledge, this is the first study that shows the direct interaction of MFGE8 with GSN and their role in regulation of cell growth.

December 15, 2016

#### **Session IV- Disease Proteomics in Plant, Animal and Human Health**

BDPPAH1

#### **Identification of Single Mutations in Hemoglobin Variants Using Modified Sample Preparation and Digestion Method and Separation Power of LC Coupled with MALDI MS/MS**

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Around 7% of the global population carries an abnormal hemoglobin gene. Over 330,000 infants are born annually with hemoglobinopathies and it is the major cause of morbidity and mortality in early childhood. The treatments rely heavily on the diagnosis of hemoglobin variants. The routine/conventional techniques used for the identification of mutation in hemoglobin variants have their own limitations like co-migration of variants in electrophoresis and co-elution in HPLC. The WHO (2002) report on Genomics and Health has emphasized on the development of precise molecular techniques for screening of hemoglobin

disorders. A sensitive, robust and reproducible method was thus developed to identify single substitution mutations in the hemoglobin disorders from sequence of the entire globin chains. The method was MS compatible and dealt with certain limitations like difficulty in getting complete sequence coverage. Using a specific organic solvent, digestion with a combination of proteases, treating the digestion mixture with 10% acetonitrile prior to incubation and combining the separation power of LC coupled with MALDI MS/MS resulted in 100% sequence coverage in the  $\alpha$  chains and very high sequence coverage in the  $\beta$  chains. A hemoglobin variant database was created to specify the search and reduce the search time. All the mutations were thus identified using a non-targeted approach. This method could be used in future for regular screening of any single mutation in hemoglobin variants.

BDPPAH2

#### **Identification of Proteins Associated with Myocardial Infarction in Different Age Group**

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Myocardial infarction (MI) due to prolonged ischemia has been critical for optimal recognition and timely management in patients of different age groups since ageing itself contributes as a significant risk factor in this disease. Very few studies have been conducted so far, which are implicated in age specific alterations in heart with respect to MI and hence potential age specific MI markers are yet to be identified. Hence, we embarked on this study to identify novel age specific proteomic markers in a murine model of MI and elucidate major pathways that are affected exclusively in different age group of MI model.

Male Wister rats of 9 and 70 weeks old were used to develop MI by occlusion of left anterior descending coronary artery (LAD) for 10 days. Age and sex matched rats were sham operated to use as controls. iTRAQ based quantitative LC-MS proteomic workflow was undertaken to identify the differentially expressed proteins.

We have identified a total of 1160 proteins in four different groups (9 weeks and 70 weeks MI and control) at 1% FDR. A total of 174 and 205 differentially expressed proteins were identified in 9 weeks MI and 70 weeks MI tissue respectively when compared with their respective age and sex matched controls. A total of 78 and 109 proteins were altered exclusively in 9 weeks and 70 weeks MI groups respectively compared to their age-matched controls. Biological pathway analysis revealed enrichment of different pathways in these two different age group of MI model which suggests that recovery from stress due to MI at early age are more conspicuous than from late age MI.

This study would help us to identify novel age specific markers of MI and explore the major specific pathways which are affected exclusively in each age group.

BDPPAH3

### **Understanding Mechanism of Action of Drug Resistance Reversal Potential of Usnic Acid by Comparative Proteomics Approach**

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Antibiotic resistance is an overwhelming problem worldwide to combat the infectious diseases. *Staphylococcus aureus* is an uncompromised human pathogen which causes wide range of infections such as sepsis, toxic shock syndrome, endocarditis or osteomyelitis. Controlling this pathogen is highly complicated has due to emergence of MRSA, VRSA and MDR strains. Over past few years, researchers around the globe putting serious efforts for searching novel antibiotics against multi drug resistant (MDR) microbes. In recent years, the herbal medicine has attained popularity due to its advantageous features. Moreover, many natural compounds such as berberine, totarol, curcumin,

khusimol, gallic acid etc has been reported in recently against quit a few MDR strains.

Usnic acid is a natural dibenzofuran compound extracted from several species of Lichen. It has been used in medicines, beauty products, perfumes, cosmetics and it also has antiviral, antimicrobial, antiprotozoal, anti-inflammatory, anti-growth and so on. Though recent studies showed its antibacterial against gram positive organism such as *S. aureus* but its mechanism of action is still unclear. In current study, we used state-of-the-art proteomic technology such as two dimensional electrophoresis coupled with Matrix assisted laser desorption and ionization-time of flight (MALDI-TOF/TOF) mass spectrometry to understand the proteome alteration in *Staphylococcus aureus* (MRSA2071). Our proteomics analysis has highlighted alteration of 25 protein spots (18 protein spots were down regulated and 7 protein spots were up regulated) with respect to untreated control. The altered proteins were involved membrane biosynthesis, metabolic processes and electron transport chain pathways which are essential for the survival of the bacteria. To the best of our knowledge, this is the first report describing the effect of Usnic acid on *S. aureus* and we are doing validation experiments to get insight into the mechanism of action.

BDPPAH4

### **Proteomic Analysis of Plasma from Mice Model Following Pulmonary Infection with *Francisella Tularensis* LVS**

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*Francisella tularensis*, one of the most virulent bacterial pathogens causes the zoonotic disease tularemia in humans by multiple routes. It is a facultative intracellular bacterial pathogen. The subspecies *tularensis* is especially virulent for humans when inhaled and respiratory tularemia is associated with high mortality if treatment is delayed. In this work, a comparative proteomic study of plasma proteins was undertaken using a mouse model of pulmonary tularemia, induced by *Francisella tularensis* LVS to understand the pulmonary host response to virulent *F. tularensis*.

In this study, a 2-dimensional electrophoresis proteomic approach was utilized to characterize protein changes in plasma from mice exposed to *F. tularensis* LVS, intranasally. The composition of plasma proteins was altered following infection, including proteins involved in neutrophil activation, oxidative stress and inflammatory response. Components of the innate immune response were induced including the acute phase response, however the timing of their induction varied. Finally, this study identifies several candidate biomarkers, including decreased level of transthyretin and alpha 1-antitrypsin and upregulation of haptoglobin that are associated with *F. tularensis* LVS infection. Similarly, significant differences were also noticed in the expression levels of IFN- $\alpha$  and TNF- $\alpha$  in plasma of infected mice.

BDPPAH5

### **Proteomics Approach Leads to Identify Differentially Expressed Proteins Involved In Nasopharyngeal Carcinoma**

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Nasopharyngeal carcinoma (NPC) is a rare form of malignancy having remarkable geographic and racial distribution. Worldwide prevalence of NPC is mostly in East and Southeast Asia. In India, NPC is also rare except North eastern states and reported prevalence rate of NPC is 1.82% amongst all cancers in this region. Proteomics approach has become a most promising approach for identification of differentially expressed proteins and further checks their ability to use as potential biomarker in various diseases including cancer. In this study, we aim to identify and validate the differentially expressed protein from plasma sample of NPC cases and Controls from north eastern states of India.

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.

In 2D analysis, we could identify as many as 394 and 423 spots on NPC and control plasma proteome profiles respectively. In comparison, 23 differentially expressed spots including 16 spots highly expressed in NPC and 7 spots highly expressed in Control were seen. These spots were subjected to MALDI-TOF-MS analysis and total 15 proteins were identified. GO annotation for molecular function showed that proteins were majorly involved in binding activity and biological process indicated that these were distributed among various process such as single organism processes, biological regulation and response to stimulus.

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.

BDPPAH6

### **Redox Homeostasis & Dys-Regulation of Bio-Energetics: Activation of Systemic Redox Stress Attenuation Mechanisms at Very High Altitude Leads to Survival at Extreme Altitude.**

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High-altitude areas comprise 1/5<sup>th</sup> of the globe's landmass and have a population of about 140 million apart from the estimated millions of annual tourists. There are three zones in these areas: High Altitude zone (5,000-11,500 ft); Very High Altitude zone (11,500 ft-18,000 ft) & Extreme Altitude zone (>18,000 ft). Hypobaric hypoxia is the prime health concern in these areas as it is the causal factor for patho-physiological events like HAPE, HACE & AMS. The studies of hypobaric hypoxia have been restricted to temporal scale studies with no regards

to the variation in severity as a function of altitude. We present the effects of acute exposure to different altitudes on the lung and plasma proteomes in conjunction with various biochemical and immunological parameters as well as oxidative stress specific transcripts. We exposed male SD rats (210-230 g) to varying degrees of hypobaric hypoxia (High, Very High & Extreme altitude zones) or normoxia for 24 h. For extreme altitude, there were two groups, one was exposed to extreme altitude directly (25k) and the other group was given a short pre-exposure at very high altitude zone (25k'). We observed 100% mortality in 25k group but 100% survival in 25k' group. Proteomic analysis (LC-MS/MS & MALDI) alongwith various other parameters, e.g.- levels of TBARS, glutathione, thioredoxin, other biochemical and immune parameters and PCR arrays for oxidative stress specific transcripts revealed activation of global redox stress attenuation mechanisms due to immense dysregulation of bio-energetics' machinery and imbalance in redox homeostasis, particularly in lung tissue, upon exposure to very high altitude. All these processes are further restored in the 25k' group (pre-exposed) leading to 100% survival. We thereby conclude that during acute exposure, systemic redox stress attenuation mechanisms are activated upon failure of lung specific redox homeostasis mechanisms at very high altitude and pre-exposure at very high altitude has beneficial effects upon induction to extreme altitude.

BDPPAH7

### **Molecular Mechanism Reveal That Sarin Exposure Leads To CNS Injury in Rats**

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Sarin is an organophosphorus compound which irreversibly inhibits acetylcholine esterase (AChE). Acute effects of exposure include lacrimation, salivation, tremors, bulging of eye balls, paralysis, while the chronic effect causes neurological and neurobehavioral abnormalities. In this study, proteomic changes after a single 0.5 LD<sub>50</sub> dose of

sarin exposure was performed to investigate some milestone changes related to long term CNS injury. Altogether, this study established the major molecular mechanism associated with toxic insult.

Plasma and brain choline esterase activities were estimated to determine AChE inhibition. We used 2DE- MS approach to identify the differentially expressed proteins, which was further characterized by western blot and immunohistochemistry. Results were further validated by histology.

The results revealed that most of the proteins related to chaperone, cytoskeleton function, energy and carbohydrate metabolism as well as signal transduction pathways had altered expression levels. Moreover, by interactome predictions, we observed Parkinson's disease related proteins showed at the top, with least false discovery rate. Thus we tried to trace out major mechanisms associated with sarin exposure and observed mitochondrial dysfunction, ER stress and defect in synaptic plasticity just after post sarin exposure. For long term changes, defects in protein folding and the accumulation of some protein related to neurodegenerative diseases were observed. Long term sarin exposure was related to defects in cognitive function and it caused astrocytes gliosis, activation of microglia which leads to neurodegenerative condition.

This is the first proteomic study of surviving animals (Wistar rats) after sarin exposure. The significant findings of this work will serve as an initial framework which will give greater insight in the major mechanisms associated with toxin exposure leading to CNS injury. Therefore, this study opens new avenues for elucidation of therapeutic targets after sarin exposure.

BDPPAH8

### **AB105- Study of Patho-Stress Responsive Nuclear Proteome in Rice (*Oryza Sativa* L.): A Functional Approach**

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Molecular dissection of disease state has received considerable interest because of its biological and

physiological significance in crop production. To better understand the regulatory mechanisms underlying disease response, we report here the comprehensive analysis of the nuclear proteome in rice (*Oryza sativa* L.) challenged with *Magnaporthe grisea*. Disease incidence is one of the most important factors that greatly influences plant growth, development and sustainability of most crops. Understanding translational remodelling in response to disease is of great interest. Nuclear proteins constitute a highly organized, complex network that plays diverse roles during cell development, other physiological processes and environmental adaptations. To explore the regulatory mechanism operated during patho-stress, nuclear proteome was developed in rice. Three leaf stage plants were infected with *Magnaporthe grisea* and tissue collected at eight different hpi. Isolated nuclear proteins were subjected to iTRAQ and LC-MS/MS to evaluate changes in nuclear compartment. We reproducibly identified 550 proteins in response to patho-stress in three biological and four technical replicates. Dysregulated protein expression was observed in early and later stages of invasion. Classification of the nuclear proteins according to GO annotation showed that most proteins were part of ribonucleic acid biogenesis and DNA binding activity respectively. Our study enhances our understanding of biological processes under patho-stress and uncover novel nuclear proteins playing important role in rice susceptibility towards disease. These findings contribute in understanding the regulatory mechanism of disease susceptibility.

BDPPAH10

### **Proteome and Phosphoproteome Analysis of Potato Cell Wall during *Verticillium Dahliae* Stress.**

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Potato (*Solanum tuberosum* L.) is the fourth most important crop as a human food after corn, rice and wheat, it's grown all over the world under different climatic conditions. *Verticillium dahliae* is one of the

most influential soilborne disease that cause vascular wilt diseases in wide range of important crop plants including potato and cause yield losses. This pathogen can survive in soil and infected plant debris. The pathogen invades the potato plant by penetrates the roots and interferes with the transportation of water, the infected tubers show vascular discoloration in rings near the end of the stem and reduction in tuber size as well as quality. To study the mechanisms associated with defense responses in potato (*Solanum tuberosum* L.) upon *V. dahliae* infection, a comparative proteomic and phosphoproteomic analysis of infected potato susceptible cultivar Kufri Chipsona-1 was performed by 2-DE combined with MS/MS analysis. A total 65 proteins and 60 phosphoproteins were identified, these proteins are mainly involved in stress responses and defense, signalling, wall hydration, and wall architecture and remodelling that would lead to the understanding of the diverse and complex mechanisms, biological processes operating in ECM towards crop specific adaptation during patho-stress.

BDPPAH11

### **Proteomic & Bioinformatics Analysis of Molecular Mechanisms of Radioprotection Offered By Radioresistant Bacterial Metabolite RKIP-006**

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Planned (medical interventions) or unplanned (Nuclear accidents) ionizing radiation exposure elicits a set of complex biological responses including protein expression and altered signaling pathway that manifest to radiation injury. Few prospective radiation countermeasures are still under developmental stage globally. However so far, not a single radiation countermeasure agent is approved for human application.

In the present study, molecular mechanism of radioprotection offered by radioresistant bacterial metabolite RKIP006 was explored using proteomic and bioinformatics tools and techniques.

Radioprotective efficacy of RK-IP-006 was evaluated using HEPG<sub>2</sub> and human PBMCs cells. Proteomics analysis based on iTRAQ technology was performed to determine the protein profile changes in human PBMC cells treated by gamma radiation (3Gy) and bacterial radioprotector RKIP-006. LC-MS/MS data was analyzed and grouped under Gene Ontology categories Cellular Localization, Molecular Function and Biological Process. Protein-protein interaction analysis was done through STRING tools. Further, radiation and bacterial metabolite RKIP006 induced differential protein expression study was carried out in HEPG2 through 2D-PAGE & immunoblotting.

Bacterial metabolite RKIP006 showed significant radioprotective efficacy in human PBMC and HEPG2 cells. iTRAQ analysis demonstrated that out of 178 total differentially expressed proteins, 67 protein were found up-regulated and 111 down regulated in treated human PBMC. iTRAQ data were further subjected to gene ontology analysis. Gene ontology analysis showed the differentially expressed proteins were mainly associated with ATP metabolic, Purine nucleoside metabolic and immune system biological process. Protein interaction network and KEGG pathway analysis revealed altered metabolic pathways, glycolysis/gluconeogenesis, biosynthesis of amino acids and antioxidant pathways. Moreover, Bcl2 and HSP 70 expression level were increased while Bax and p53 expressions reduced in RK-IP-006 treated plus irradiated HEPG2 cells as compared to only irradiated cells. All together, it can be concluded that bacterial metabolite RKIP006 pretreatment overcome radiation induced injury by improving mitochondrial response, antioxidant and regulation of signal transduction.

BDPPAH12

### Analyzing the Mutational Impact on Apolipoprotein A1 Function and Stability

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Coronary artery diseases (CAD) are leading cause of death worldwide that occurs when deposited on

the artery wall as a plaque. In our previous study, we have identified and validated Apolipoprotein A-I (apoA-I) as down regulated protein in CAD. Functional analysis of this protein indicated its anti-inflammatory and cardioprotective role that promotes the reverse transportation of cholesterol from peripheral tissues to HDL that converts nascent HDL to mature HDL. Apo-A1 activates the conversion of cholesterol to cholesterol esters by inducing lecithin cholesterol acyl transferase (LCAT) enzyme which helps in recognition of HDL by liver HDL. Mutational analysis indicated the presence of A164S single amino acid substitutions in LCAT binding site that may interfere in the cholesterol metabolism and may enhance the risk of heart diseases without lowering the HDL cholesterol level. In order to investigate the effect of this mutation on the function and the stability of the protein, we have carried out a comparative study of wild type (WT) protein as well as its mutant. Crystallographic structure of ApoA-I was taken from protein data bank (PDB) and mutated with the help of swiss PDB viewer (SPDBV). Effect of this mutation on protein behaviour was analysed with DUET, Folding RaCe, I-Mutant, MuPro, SIFT, and SDM servers. This analysis indicated that A164S mutation that considerably affect the stability and function of ApoA-I and may play an important role during CAD.

BDPPAH13

### iTRAQ- Based Proteomic Profiling of Rat Serum In Atherosclerosis Induced by High Fat Diet.

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The pathogenesis of atherosclerosis is complex owing to molecular heterogeneity in the afflicted population. High-fat diet (HFD)-induced, increases the chances of developing cardiovascular disease. To study the mechanism(s) by which a HFD increase the risk of cardiovascular disease, we followed quantitative proteomic strategies and used iTRAQ (isobaric tagging for relative and absolute

quantification) labeling to plasma protein of HFD induced rat for 3 months. iTRAQ yielded sensible exactness while Protein co-migration could bargain the evaluation in 2D DIGE. iTRAQ methodology is most fragile but it is subjected to impedance associated with the arranged molecule selector assurance of the MALDI-TOF/TOF instrument. Within 2 months of starting the diet, the rat plasma cholesterol profile shows positive sign of atherosclerosis. Rats were treated with herbal extract, pure compound and standard drug for 1 month. Plasma was then collected from each group and labeled with iTRAQ reagent. On the basis of iTRAQ analysis differentially expressed protein which shows high significance in the four study groups were studied. For this study, the samples were collected from control, treated with herbal extract, pure compound and standard drug. Further validation data sets were made and analyzed which gives some significantly differentially expressed proteins followed by western blot and ELISA analysis validation. These putative biomarkers may be clinically useful for the development of novel therapeutics targeting the specific pathology.

BDPPAH14

### **Proteomic profiling of Rheumatoid Arthritis for identification of specific markers**

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Rheumatoid arthritis (RA) is a systemic autoimmune disease leading it to inflammation of joints and surrounding tissues. Etiology of this disease is not completely understood. Rheumatoid Factor (RF) and anti-citrullinated protein antibody (ACPA) are the only diagnostic markers for RA but the specificity and sensitivity is still debatable. Thus, the identification of novel marker is essential for better understanding of pathogenesis of this disease. We used proteomic strategy to identify differentially expressed proteins by enriching phosphoproteins and glycoproteins from blood, synovial fluid and tissues of RA patients and compared it with healthy control sample. For this study, synovial fluid and tissues of osteoarthritis patients have been taken as control. The study was carried out by running 2 dimensional gel

electrophoresis, phosphostaining of gels and identification of differential spot by MALDI TOF MS/MS. Further, identified proteins have been validated by Western blot, ELISA using recombinant pure protein, Immunohistochemistry of synovium and Immuno-electron microscopy. In addition to this, we detected autoantibodies in RA plasma by immunoprecipitation and western blot analysis. *In-silico* studies have also been carried out in order to functionally annotate the proteins. Predicted proteins may be used as a serum diagnostic marker along with other biochemical parameters and clinical symptoms for screening and diagnosis of RA.

BDPPAH15

### **Identification and Localization of Coronary Artery Disease (CAD) Specific Marker to Reveal Its Functional Implications**

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Coronary artery disorder (CAD) has been persisted as a lethal cardiac disease claiming massive mortality and morbidity rate all through the world. Despite, advances in proteomics evaluation, identification of several significant differential protein as a marker, few are remain characterized because of lack of their cellular assessment. Since there is no specific and sensitive marker for CAD, identification and measurement of the newer marker in the plasma through expression level elevation/depletion mechanism that specify CAD at the initial stage may be an effective approach. Thus, this study was aimed to evaluate novel marker at a preliminary stage in stable CAD individual relative to healthy. We made an attempt to do proteomics analysis by two-dimensional gel electrophoresis (2-DE) and MALDI-TOF MS/MS analysis. Further, to assert affirmation and validation, western blotting, ELISA and flow Cytometry has been carried out. Moreover, to examine the cellular localization and their expression pattern depletion mechanism in CAD patient's, isolated peripheral blood cells were tested

using immunofluorescence (IF) analysis. Our experimental validation and statistics confirmed our results and observed to be significantly associated in CAD patients with a statistical difference ( $p<0.05$ ). In conclusion, studying the cellular protein expression pattern of marker and their preventive measurement level helps us in understanding its varied functions in disease promotions like misfolding, aggregation, deposition etc. at disease prone cells and tissues underlying in CAD.

BDPPAH16

### **Understanding Rheumatoid Arthritis Pathology Using Cellular and Proteomic Approach**

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Rheumatoid arthritis (RA) is a common autoimmune disorder with unknown etiology. The disease is systemic in nature and predominantly affects bones and cartilage of synovial joints in symmetric fashion. Synovial tissue is the primary target site for inflammation where infiltrated immune cells significantly modify its protective function. Various immune mediators secreted from resident cells of synovial tissue and infiltrated inflammatory cells eventually result into joint destruction. Besides, insufficient knowledge behind disease progression, unavailability of disease marker and treatment inefficacy necessitates for further research in drug development. Determining the cellular behavior of isolated synovial cells from diseased tissue in combination with high throughput quantitative proteomics analysis could provide an in-depth understanding in disease biology. Present study was carried out utilizing detailed microscopic studies of cultured synovial cells along with mass spectrometry based quantitative proteomics technique in order to screen for potential markers and therapeutic targets followed by their bioinformatics studies. Our results indicated abnormal behavior of RA cells under

different experimental conditions. Additionally, our previous proteomic study identified several differentially expressed proteins. Further, their Bioinformatics analysis has highlighted the major altered pathways that play crucial role in disease pathogenesis. Our findings explain the major pathological and histological changes that occur in RA joint and could lead to the discovery of potential therapeutic targets.

BDPPAH17

### **Butyrylcholinesterase (Bche): A Novel Bioscavenger for Op Nerveagents Toxicity**

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Nerve agents belong to the class of Organophosphorus (OP) compounds which are highly toxic in nature. Sarin(GB), Soman (GD) and VX inhibits cholinesterase enzymes (AChE & BChE) leads to accumulation of acetylcholine at the synaptic cleft by selectively binding the active site serine hydroxyl moiety. This binding is supported by Glutamic acid and histidine environment thereby forming a trio complex. Inhibition of plasma cholinesterase is taken as the biomarker of exposure of OP compounds We have identified the active centre peptide after OP exposure using MALDI-TOF/MS. Aged adducts at Serine residue have also been identified in cholinesterase enzymes when incubated with plasma. Proteomic methods have been developed to verify the OP exposure.

We have developed methods to identify protein modifications resulting from exposure to OP nerve agents. These poisons make adducts not only on AChE and BChE, but also on albumin. The information gained from studying protein adducts is useful for proving exposure and for identifying the type of poison. Low doses that cause no symptoms can nevertheless be detected by analyzing blood samples. BChE, pseudocholinesterase which is found in human plasma, is a bioscavenger and when it finds nerve agent in the blood, it deactivates by selective binding over serine residue at its 225<sup>th</sup> position. 50 ml Plasma was collected from pooled blood of Rabbits and BuChE was purified by affinity & ion exchange chromatography using procainamide

sepharose columns. The subunit was found to be 72.18 kDa and the corresponding band was cut from the SDS-Gel electrophoresis. PMF gave 17 peptides with sequence coverage 33% after in-gel digestion and MALDI-TOF/MS analysis. The active centre peptide identified was SVTLFGES\* AGAASVSLHLLSPR with m/z 2199.56. Aged peptides identified after the loss of alkylgroup.

Our aim was to develop a method to rapidly diagnose exposure to nerve agents, by treating blood with soman, sarin and analyzing samples in the mass spectrometer. The method will look for peptides of BChE-nerve agent adducts. Detoxification of OP nerve agents can be achieved by additional supply of fresh frozen plasma/BChE before the exposure prophylactically. This enzyme has a broad spectrum of efficacy against OP compounds, which makes it a prime candidate for the role of stoichiometric bioscavenger.

BDPPAH18

using syngeneic CT26 tumor model in Balb/c mice show that combination of CEL and DOX significantly decreases the tumor burden with increase in cancer cell apoptosis through downregulation and upregulation of intermediate proteins of various pathways such as metastatic, proliferation, apoptosis pathways. For that we have used iTRAQ labeling LC-MS/MS studies to investigate the regulation of those proteins in CT-26 tumors on treatment with combination of DOX and CEL. A total of 1542 proteins were identified. Differentially expressed proteins were subjected to pathway analyses using online tools. We confirmed the pathways which were responsible for apoptosis and decrease proliferation in colon cancer *in vivo*. These studies conclude that combination of natural products like CEL in combination with first line chemotherapy drugs help in reducing their toxic dose with increase in therapeutic effect by modulate the expression of distinct proteins in colon cancer.

BDPPAH19

### **Probing the In Vivo Synergistic Anticancer Potential of Celastrol and Doxorubicin Combination using Proteomic Studies**

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Drug combination is an effective strategy to overcome the challenges such as drug toxicity and drug resistance; thereby increasing the therapeutic effects that are limited in single drug therapy. Additionally use of nanoparticles is efficient method to transfer drugs to target and enhance drug availability at tumor site using Enhance Permeability and Retention (EPR) effect. In our study, we provide sufficient evidence that drug combination of two bioactive molecules; doxorubicin (DOX) and celastrol (CEL) work synergistically in inhibiting the colon cancer cell proliferation. We will show that selective combination ratios of CEL and DOX show increase in cancer cells apoptosis, and decrease in mitochondrial membrane potential. *In vivo* studies

### **Comparative Proteomic Profiling of *Alternaria Alternata* Infected Transgenic and Wild Type *Mentha Arvensis***

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*Mentha arvensis* is susceptible to a variety of fungal diseases, which impose significant production constraints that affect both the yield and overall quality of mint oils. A proteomic approach was used to study the changes in the leaf proteome profile of *Mentha* infected with a necrotrophic fungus, *Alternaria alternata*. High-resolution two-dimensional gel electrophoresis (2-DE) and mass spectrometric analysis was used to identify highly abundant proteins differentially expressed in response to fungal infection. From a total of 210 reproducibly detected and analyzed spots, the intensity of sixty-seven spots was altered, and forty-five of them were successfully identified by matrix assisted laser desorption/ionization time of flight-mass spectrometry (MALDI TOF/TOF MS/MS). Another approach was undertaken to raise

transgenic *Mentha* over-expressing GSH whose role in plant abiotic stress tolerance is well known. A differential proteomic analysis was performed to focus the altered abundance of functionally important protein species in control and infected transgenic *Mentha*. Results showed a significant variation in the protein profile of the infected transgenic plant as compared to the wild/control transgenic counterpart. In addition to protein species related to stress and defense, redox regulation, transcription factors and energy & metabolism, protein species related to signalling and gene regulation as well as cell division also showed differential accumulation in infected transgenic. Hence, proteomics can be used as a tool to decipher the mechanism of action of GSH in providing tolerance against a necrotrophic fungus, *A. alternata* in transgenic *Mentha*.

BDPPAH21

### **Proteomic Profiling of Stacked Transgenes Reveals the Role of Various Pathways to Understand the Mechanism of Disease Resistance against *Magnaporthe-Oryzae***

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Rice blast is one of the most serious biotic stresses caused by a fungal pathogen *Magnaporthe oryzae* (*M. oryzae*). Blast resistance genes *Pi54* and *Pi54rh* have earlier been cloned and individually validated in susceptible variety of rice. However, highly variable nature of *M. oryzae* could break the resistance. Therefore, gene stacking is an emerging method which provides stable resistance to fungal pathogen. In the present study transgenic rice line was developed by stacking of blast resistance genes *Pi54* and *Pi54rh* together in a single vector cassette under the control of different promoters. Differential proteomics analysis was performed to understand the effect of stacked transgene in rice defense against *M. oryzae*.

Total protein was extracted from leaves of the transgenic rice lines having stacked resistance genes *Pi54* and *Pi54rh* together, transgenic rice lines

having single resistance genes *Pi54* and *Pi54rh* separately as well as wild type Taipei 309 (TP309) rice line. Seven days old seedlings were inoculated with highly virulent *M. oryzae* spore suspension. Protein isolated from all the four rice lines were processed simultaneously for 8-Plex iTRAQ labelling and fractionated with SCX chromatography. Quantitative analysis was done using Protein Pliot v4.0 software.

Phenotypic results of fungal inoculation in transgenic rice line showed that gene stacking (*Pi54+Pi54rh*) plants showed high degree of resistance to *M. oryzae* as compared to single blast resistant gene rice line. Proteomics analysis identified 296 (in transgenic rice line having *Pi54rh*), 314 (in transgenic rice line having *Pi54*) and 369 (in transgenic rice line having stacked *Pi54+Pi54rh*) differentially expressed proteins (1% FDR and 1.2 fold). Pathway analysis of differentially expressed protein identified multiple pathways associated with response to defense and stress.

In conclusion, different secondary metabolites, stress and defense related pathways which might work in a network were found highly enriched in stacked transgenic rice line as compared to the transgenic lines containing single blast resistance genes. These pathways may insight new aspects to understand the resistance spectrum against *M. oryzae*.

BDPPAH22

### **Proteome Profiling of *Pseudomonas Syringae* Infected *Pad2-1*, A Glutathione Depleted *Arabidopsis* Mutant, Confirms the Dynamic Role of Glutathione in Regulating Pathogen Defense in Plants**

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Pathogen-mediated yield loss of plant products is a serious concern in today's world, making it necessary to explore how plants defend themselves to pathogens. Till last decade, GSH is gaining importance to play a significant role in controlling pathogen invasion. However, the molecular

mechanism how GSH provides this support is largely unknown. In this investigation, for the first time, we applied comparative proteomics approach to identify the protein-species altered under GSH depleted condition during infection. To this end, the GSH depleted *pad2-1* mutant and wild-type Col-0 leaves were infected with *Pseudomonas syringae*. Changes in the proteome profiles of *pad2-1* and Col-0 after infection were studied. The *pad2-1* mutant displayed severe susceptibility to *P. syringae* infection compared to the wild-type Col-0 plants thus re-establishing a fundamental role of GSH in defence. Apart from general up-accumulation of energy metabolism-related protein-species in both infected Col-0 and *pad2-1*, several crucial defence-related protein-species were identified to be differentially accumulated. Leucine-rich repeat-receptor kinase (LRR-RK) and nucleotide-binding site-leucine-rich repeat resistance protein (NBS-LRR), known to play a pioneering role against pathogen attack, were only weakly up-accumulated in *pad2-1* after infection. Transcriptional and post-transcriptional regulators like MYB-P1 and glycine-rich repeat RNA-binding protein (GRP) and several other stress-related protein-species like heat shock protein 17 (HSP17) and glutathione-S-transferase (GST) were also identified to be differentially regulated in *pad2-1* and Col-0 in response to infection. Together, the present investigation reveals that the optimum GSH level is essential for the efficient activation of plant defence signaling cascades thus conferring resistance to pathogen invasion. This knowledge will further help to develop novel strategies for stress management, thus minimizing pathogen-mediated damages in plants.

#### **BDPPAH23**

### **iTRAQ-Based Quantitative Proteomics Analysis Revealed Putative Serological Biomarker for Early Detection of Pregnancy in Cows**

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Pregnancy is a complex process which includes series of events such as fertilization, formation of

blastocyst, implantation of embryo, placental formation and development of fetus. The success of these events depends on various interaction which is synchronized by endocrine interaction between a receptive dam and competent embryo. These interaction leads to change in expression of hormones and proteins. But till date no protein biomarker is available which can be used to detect successful completion of these events. We employed quantitative proteomics approach to develop putative serological biomarker which have diagnostic applicability for early detection of pregnancy in cows. For this study, sera was collected from control (non-pregnant, n=6) and pregnant animals on successive days of pregnancy (7, 19, 45, n=6). The sera was subjected to depletion for removal of albumin using Norgen depletion kit. The tryptic peptides were labeled with iTRAQ. The peptides were pooled and fractionated using bRPLC over 80 min gradient. Then 12 fractions were injected to nLC for identification and quantitation in DDA mode using ESI. Identification using Mascot search revealed 2056 proteins out of which 352 proteins were differentially expressed. Twenty proteins were upregulated and twelve proteins were down regulated with fold change >1.5 and <0.6 respectively (p < 0.05). The gene ontology studies of DEPs using Panther software revealed that majority of proteins are actively involved in catalytic activities, binding and enzyme regulatory activities. The DEP'S such as NF2, MAPK, GRIPI, UGT1A1, PARP, CD68 were further subjected to pathway analysis using KEGG and Cytoscape plugin cluego that showed involvement of proteins in successful implantation, maintenance of pluripotency, regulation of luteal function, differentiation of endometrial macrophages, protection from oxidative stress and developmental pathways such as Hippo. Further efforts are continuing for targeted proteomics, western blot to validate potential biomarkers and development of diagnostic kit for early pregnancy diagnosis in cows.

BDPPAH24

proliferative and differentiation inducing effect of LIF.

BDPPAH25

## **Quantitative proteomics analysis uncovers the molecular mechanism of Leukemia Inhibitory Factor (LIF) for differentiation-inducing effect**

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Leukemia inhibitory factor (LIF) is a pleiotropic glycoproteins synthesized and secreted in various body tissues. LIF is a molecule that was initially identified through its ability to induce macrophage differentiation in the murine M1 myeloid leukemic cell line. We successfully overexpressed the buffalo LIF (BuLIF) protein through transfection of the pAcGFP-N1 vector into COS-1 cell line to examine the antiproliferative/differentiation inducing effect of LIF. To establish a stably transfected cell line expressing BuLIF, several rounds of selection in the presence of G418 and eventually, single cell clonal expansion was performed. The strong expression of BuLIF was observed at 30<sup>th</sup> passage and cells could grow in the absence of selection pressure without losing GFP signal. Confirmation of genomic integration of BuLIF was performed through PCR amplification on genomic DNA and sequencing (at 150<sup>th</sup> passage). The SDS-PAGE and Western blot revealed the identification of transgenic LIF around 65-70 kDa. Mass spectrometry-based (Q-TOF) Label-Free Quantification (LFQ) approach was used for the functional annotation of antiproliferative effect of LIF. Total of 2083 protein were identified with the overall PSM (peptide spectrum match) as 14278 with the corresponding 5973 peptides as the best spectra match through MaxQuant 1.5.2.8. Bioinformatics analysis through STRING and Cytoscape showed the interaction of LIF with LIFR, OSM, OSMR, CLCF1, CSF3, EGK04900 and KCNJ1 proteins. Gene ontology analysis using DAVID and Cluego showed the involvement of LIF interacting partners in differentiation. To confirm the anti-proliferative effect of LIF on cancerous kidney cells (SV-40 transformed COS-1 cells), BrdU and MTT assay was carried out, further validation was performed with the help of RT-qPCR. Till date to the best of our knowledge, this is the first study that has been performed on COS-1 cells for the anti-

## **Functional Adjustment in the Soluble Proteome of Neuro2a Cells During Proteasome Inhibition By Mg132**

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Solubility is an important determinant of protein-function. Large-number of proteins deviates from normal function due to insolubility during aging or in neurodegenerative diseases. Simultaneous malfunction of proteasome mediated protein-degradation is also observed. However, whether proteasome-dysfunction is a cause or consequence of protein-aggregation is not clear. We blocked protein-degradation in cell culture by treating Neuro2A cells with MG132; a specific, potent, reversible, and cell-permeable proteasome inhibitor. In addition to the known proteostasis-dependent protein FlucDM-EGFP, this treatment resulted in insolubility of several mitochondrial inner membrane/matrix proteins as measured by SILAC-based quantitative mass spectrometry. These mitochondrial proteins are physiochemically distinct - low molecular weight, less hydrophobic and highly basic. Further experiments identified loss of mitochondrial complex-I activity as one of the first toxic events upon proteasome dysfunction. We did not observe appreciable increase of heat-shock chaperones at protein-level upon MG132 treatment but significant transcriptional upregulation was noticed. Increased abundance of histone proteins and their modifiers in the soluble proteome suggested potential readjustment of chromatin assembly facilitating such transcription. Follow-up experiments indicated that proteasome-inhibition mediated transcriptional alteration is a programmed event controlled by Histone H3 trimethylation (H3K4me3) and this activation mechanism is consistent during other proteotoxic events like heat stress.

**Session V- Global and Targeted Proteomics for Precision Medicine and Food**

BGTPMF1

**Comparative Proteomic Analysis between Maize F<sub>1</sub> Hybrid and Its Parental Lines during Early Seed Germination**

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Heterosis describes the phenomenon wherein F<sub>1</sub> heterozygous hybrid generally exhibits improved agronomic performance compared to genetically diverse homozygous parental inbred lines. Maize (*Zea mays L.*) is an excellent model system for understanding the molecular mechanisms of heterosis because it exhibits high levels of phenotypic, DNA sequence, transcriptional and translational variation. In this study, comparative proteome analysis of dry and 24 h water-imbibed seeds of F<sub>1</sub> hybrid (DHM 117) and parental inbreds (BML 6 and BML 7) was performed using two-dimensional gel electrophoresis in combination with protein matrix-assisted laser desorption ionization-time of flight-mass spectrometric analysis. A total of 196 and 262 proteins were differentially expressed out of 853 and 1077 protein spots detected in dry and water-imbibed seeds, respectively in F<sub>1</sub> hybrid and its parents. Among the differentially expressed proteins, 49% of proteins in dry seeds (96/196) and 39.7% of proteins in water-imbibed seeds (104/262) were expressed non-additively. Twenty nine protein spots that showed differential accumulation in dry seeds and water-imbibed seeds of F<sub>1</sub> hybrid and parents were identified using mass spectrometric analysis and functionally annotated. The differentially expressed proteins between F<sub>1</sub> hybrid and parents were classified into seven classes including storage proteins, metabolism and energy, defense and stress, transcription and translation, cell cycle, hormone biosynthesis and unknown. The largest class (31%) of differentially expressed proteins were related to metabolism and energy involved in several biological processes such as Glycolysis, ATP synthesis and tricarboxylic acid cycle. Interestingly,

two proteins viz., ABA-inducible protein Rab 28 and indoleacetaldehyde oxidase of auxin biosynthesis showed differential accumulation in F<sub>1</sub> hybrid as compared to parents. These results indicated that the differential expression of proteins particularly those involved in metabolic and energy processes as well as hormone biosynthesis in F<sub>1</sub> hybrid as compared to the parents might be responsible for rapid seed germination in F<sub>1</sub> hybrid.

BGTPMF2

**Differential Proteomic Analysis of Multiple Myeloma Towards New Targets and Biomarkers**

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Multiple myeloma (MM) is a heterogeneous disease and accounts for 1% of all cancers and 14% of all hematological malignancies. In MM, Malignant plasma cells expand and accumulate in the bone marrow, leading to bone resorption and over production of antibodies. The major challenges remains in MM are identification of better prognostic and therapeutic biomarkers. In this work we used MM serum, Bone marrow (BM) plasma, BM mono nuclear cells and respected controls. Serum and BM plasma proteins were extracted and differential proteomic analysis was performed using 2D-DIGE and 4-plex iTRAQ experiments. We also performed SWATH analysis of BM mono nuclear cells to identify differentially expressed proteins in MM. In the study of serum proteome alterations in MM, our quantitative proteomic analysis using DIGE and iTRAQ resulted 140 differentially expressed proteins in which 51 proteins showed increased expression and 89 proteins showed decreased expression. In case of BM plasma study, we identified 184 differentially expressed proteins out of which 101 proteins were found to be up-regulated and 83 proteins were down-regulated. Further, proteomic analysis of BM mono nuclear cells yielded a total 892 proteins using SWATH analysis in which 222 proteins were found to be statistically differentially expressed. Bioinformatics analysis of the proteomics 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 discovery data. Proteins like LMNA, MANF, RPN1, VDAC3, PGK1, ANXA2 were showing similar differential expression pattern in MM serum, BM plasma and BM mono nuclear cells in both discovery phase as well as validation phase. These protein signatures are not only helpful as MM diagnostic and prognostic markers but also provide insight disease pathogenesis information and could be potential targets for targeted MM treatment.

BGTPMF3

### **Quantitative Proteomic Analysis to Access the Modulation of Cellular Response to Palmitic Acid Induced Lipotoxicity in Hepg2 Cell Line**

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\*Equally contributed

It has been proposed that nutrient surplus and a consequent free fatty acid accumulation causes the hepatosteatosis, a fundamental cause of lipotoxicity. This activates several signaling pathways which leads to aggravation of the metabolic imbalance, oxidative and ER stress, and hepatocyte cell death. Moreover, growing evidences indicate that lipotoxicity altered several cellular kinases in hepatocytes. Despite of several studies, the detailed mechanism of lipotoxicity and subsequent cell death is not well established. In the present study, we use high-resolution mass spectrometry to perform an in-depth analysis of lipotoxicity to elucidate a comprehensive mechanistic network between metabolic alterations, oxidative and ER stress, and apoptosis. With cutting-edge quantitative proteomics, both labeled (iTRAQ) and label free (SWATH) methods, we have shown the modulation of cellular proteins and its association with lipotoxicity in Palmitic acid treated HepG2 cells with different time intervals. In addition, the study of post-translational modification (PTM) allow us to

captured predominantly low abundant signal proteins. The integrated analysis of quantitative proteomics and PTM showed improved coverage of key protein networks involved in lipotoxicity. We identified significant alterations of proteins associated with metabolic load that may facilitate increase in ATP production, oxidative and ER stress. Interestingly, the Apoptosis inducing factor (AIF) is increased significantly and might be involved in oxidative stress mediated apoptosis. Thus, our findings provide a new insight into the mechanism of lipotoxicity mediated cellular response.

BGTPMF4

### **Targeted quantification of glycated peptides of hemoglobin by mass spectrometry: Carboxymethylation, not deoxyfructosylation, is the predominant modification of N-1 $\beta$ -valine**

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**Background:** N-1-(deoxyfructosyl) valine (DFV)  $\beta$ -hemoglobin ( $\beta$ -Hb), commonly referred as HbA1c, is widely used diagnostic marker in diabetes, believed to provide glycemic status of preceding 90-120 days. However, the turnover of hemoglobin is about 120 days, the DFV- $\beta$ -Hb, an early and reversible glycation product eventually may undergo irreversible advanced glycation modifications such as carboxymethylation or carboxyethylation. Hence quantification of N-1-(carboxymethyl) valine (CMV) and N-1-(carboxyethyl) valine (CEV) peptides of  $\beta$ -Hb would be useful in assessing actual glycemic status.

**Results:** Fragment ion library for synthetically glycated peptides of hemoglobin was generated by using High Resolution-Accurate Mass Spectrometry (HR/AM). Using parallel reaction monitoring (PRM), deoxyfructosylated, carboxymethylated and carboxyethylated peptides of hemoglobin were quantified in clinical samples from healthy control, pre-diabetes, diabetes and poorly controlled diabetes. For the first time, we report mass spectrometric quantification of CMV and CEV peptides of  $\beta$ -hemoglobin. Carboxymethylation was found to be the most abundant modification of N1-

$\alpha$ -Valine. Both CMV- $\alpha$ -Hb and CEV- $\alpha$ -Hb peptides showed better correlation with severity of diabetes in terms of fasting glucose, postprandial glucose and microalbuminuria.

**Conclusions:** This study reports carboxymethylation as a predominant modification of N- $\beta$ -Valine of Hb, and quantification of CMV- $\alpha$ -Hb and CEV- $\beta$ -Hb could be useful parameter for assessing the severity of diabetes.

## Session VI- Proteogenomics & big data analysis

BPBDA1

### Next-Generation Spatio-Temporal Proteomics Data Analysis And Visualization

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Mass Spectrometry-based proteomics has infiltrated molecular biological labs and system-wide analysis of proteomes is rapidly becoming commonplace. Instead of qualitative protein profiling, cutting-edge technologies now enable quantitative proteomics measuring the spatio-temporal dynamics of biological systems in high-throughput manner at unparalleled scale. We have developed several tools to enable the quantitative analysis of such complex and voluminous data. We have developed QuantWiz<sup>IQ</sup> tool that enables large scale isobaric quantitation from iTRAQ (upto 8-plex) or TMT (upto 10plex) data and supporting tool HyperQuant that can integrate identification and quantification results from hyperplexing experiment (18plex). Large datasets are cumbersome to analyse in static charts or spreadsheets and development of interactive visualization facilitates facile analysis using modern web technologies and JavaScript based applications. Standardized file format interoperability and support for most HUPO-PSI standard formats makes these apps compatible with most proteomics analysis workflows and our rich biological data can really "come to life" wherein specific biological questions of interest can be

quickly answered and newer hypotheses posed easily.

December 16, 2016

## Sesion VII- Structural Proteomics

CSP1

### Protein Modelling and Molecular Dynamics Simulation of CIPK Protein from Stress Tolerant *Oryza sativa*

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**Background:** The CBL (Calcineurin B like protein) - CIPK (Calcineurin B- like interacting protein kinase) signalling system is a newly emerging plant - specific and  $Ca^{2+}$  dependent network mediating abiotic stress tolerance system in plants. Understanding the interaction of CIPK protein/ ligands in plant system is of utmost importance to develop plants for biotic and abiotic tolerance.

The CIPK is a type of protein kinase that gets activated when CBL protein interacts with it.

**Methodology:** The CIPK5 3D structure of protein was predicted by computational homology modelling. The structure model reported through MODELLER was saved as .pdb and analysed. The resulted model was verified and validated by using various softwares such as PROCHECK, Verify3D, ERRAT and ProSA. To study the properties and stability of CIPK, a structure refinement by MD simulation is done using GROMACS 4.6 package and a protein-protein interaction shows the closely interacted protein of CIPK and also the signalling behaviour of the protein was performed by using STRING database.

**Results:** The behaviour of the predicted structure was well-simulated and analysed through RMSD and RMSF of the protein. The current research provides the modeled 3D structure of CIPK that will help in understanding the proper mechanism of gene regulation by *in silico* studies.

**Conclusion:** CIPK5 is a important signalling molecule in *Oryza sativa* and the structure of which is unknown. By analyzing different structural and physiological parameters of CIPK5 protein of *Oryza sativa* it was concluded that the protein plays an important role in abiotic and biotic stress.

CSP2

## Mapping the Refolding Pathway of a Large Multi Domain Protein using Mass Spectrometry

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Understanding the protein folding pathway, its mechanism and the role of folding intermediates has always been a fundamental area in the field of biological research. Various approaches have been applied to increase our understanding of the intermediates and the transition states of proteins from their unfolded to the near native or native states. Multi domain proteins comprise a major percentage of the total cellular proteome and little is known about their folding pathways.

In this study we have optimized a protocol to study the refolding event of a model multi domain protein, malate synthase G (MSG) at the molecular level and tried to answer some fundamental questions regarding complexity of protein folding. We have applied limited proteolysis approach with LC-MS/MS to get a molecular level resolution of these intermediates. The different refolding intermediates of MSG were captured and subjected to limited proteolysis using trypsin. The peptides thus generated were separated on a C18 column using nanoLC and subjected to ESI-MS. With the use of softwares; HyStar 3.2, DataAnalysis, BioTools and Pattern Lab we identified certain regions on the protein that are differentially sensitive to enzymatic cleavage, region that constitutes the folding nucleus and characterized the transition states at the molecular level. Using a simple approach along with MS we have tried to get a better understanding of the dynamic conformation of the refolding intermediates and map the refolding pathway of a large protein. Our results demonstrate the utility of mass spectrometry for characterization of protein

folding intermediates of large multi domain proteins that are a challenge to study.

## Session VIII- Organ, Organelle and Single-cell Proteomics

COOSCP1

### Comparative Disease-Responsive Extracellular Matrix Proteome and Phosphoproteome of Resistant Chickpea under Vascular Wilt

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Patho-stress is the most significant factor that adversely affects plant growth, development and productivity affecting agriculture and geographical distribution of crop plants worldwide. Vascular wilt, caused by *Fusarium oxysporum* f. sp. *ciceris*, is the most important root disease of chickpea. It is widespread in occurrence and causes significant economic losses every year worldwide. ECM or cell wall in plant is a dynamic system that represent as a first line defence to restrict entry of potential pathogen and mediating cell signalling to perceive and transmit extra- and intercellular signals in many pathways the molecular interaction between microbial pathogen and host occurs in the extracellular space, thus ECM plays an important role in cell fate decision under various patho-stresses. In this study, we have developed a comprehensive ECM proteome and phosphoproteome maps by 2-DE coupled with ESI-MS/MS for resistant chickpea cultivar WR-315 during *F. oxysporum* f. sp. *ciceris*.vascular wilt to understand the underlying mechanism that enables this variety to withstand disease conditions and to provide new insight into the underlying mechanisms of patho-stress tolerance. The analysis of the wilt-responsive proteome and phosphoproteome revealed 1472 differentially expressed protein spots of which a total of 211 were identified and 450 differentially expressed phosphoprotein spots of which a total of 130 were identified respectively. The identified proteins were

involved in a variety of functions that *might help in understanding the plant pathogen interaction and enhancing our understanding on the physiological and molecular mechanisms underlying patho-stress response.*

COOSCP2

## **Integrated Gel-Based and Gel-Free Search for Plasma Membrane Proteins in Chickpea (*Cicer Arietinum L.*): An Augmentation of Membrane Protein Repertoire**

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Plasma membrane (PM) encompasses total cellular contents and serves as semi-porous barrier to cell exterior. It serves as a physical boundary of the cell and bestows upon it a number of important functions viz., cell-cell interaction, transport, signal perception and its transduction, endocytosis, cell wall biosynthesis, and responses to stress factors. Most of the essential tasks of PMs are carried out by their proteinaceous components, which make the PM protein repertoire to be diverse and dynamic. Here, we report the systematic analysis of PM proteome of a food legume, chickpea and develop a PM proteome reference map. Proteins were extracted from highly enriched PM fraction of four-week-old seedlings using aqueous two-phase partitioning. To address a population of PM proteins that is as comprehensive as possible, both gel-based and gel-free approaches were employed, which led to the identification of a set of 2732 non-redundant proteins. These included both integral proteins having bilayer spanning domains as well as peripheral proteins associated with PMs through posttranslational modifications or protein-protein interactions. Further, the proteins were subjected to various *in-silico* analyses and functionally classified based on their gene ontology. Finally, an inventory of the complete set of PM proteins, identified in several monocot and dicot species, was created for comparative study with the generated PM protein dataset of chickpea.

COOSCP3

## **Global Analysis of the Dehydration-Responsive Mitochondrial Proteome in Rice (*Oryza Sativa L.*)**

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Rice is the most important crop, representing the staple food for more than half the world's population. The rice crop requires larger amount of water throughout its life cycle when compared to other crop species. Water-deficit or dehydration causes severe threat to rice production in the rainfed areas across the world. Dehydration response in plants is a complex phenomenon, and is poorly understood. Plants exposed to dehydration greatly rely on the protection of cellular integrity to prevent physiological injury by orchestrating mitochondrial energy metabolism. A detailed understanding of global analysis of protein expression in mitochondria may lead to cellular adaptation against water-deficit conditions. To dissect the molecular mechanism, four-week-old rice seedlings were subjected to progressive dehydration by withdrawing water, and the stress severity was assessed by physicochemical reactions and organellar architecture. The comparative analysis of the mitochondrial proteome using iTRAQ led to the identification of differentially expressed proteins with diverse cellular functions including energy production, cellular metabolism, cell signaling and translation. The metabolite identification and quantification, based on gas chromatography (GC)-MS analysis, indicated the pathways involved and the network topology of dehydration response in rice. Additionally, several of the identified differentially expressed proteins, previously not known to be involved in dehydration response, were validated by transcript analysis using qPCR and their localization to the mitochondria was confirmed by transient expression in *Nicotiana benthamiana*. Further molecular analysis will provide new insight into the

underlying mechanism of dehydration response and involvement of mitochondrial proteins for better adaptation against water-deficit conditions.

COOSCP4

### **Nutrient-Responsive Seed-Specific Proteome and Phosphoproteome in Developing Chickpea (*Cicer Arietinum L.*) Seeds**

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Nutritional quality of crop plants is one of the key issues to sustainable food production worldwide. Storage organs display diverse nutritional quality and complex multistep development and act as sinks in plants. Seeds represent a major source of nutrients for human and animal livestock diets. Leguminous seeds constitute a remarkable source of nourishment and energy for the human body and chickpea is known to have highest nutrient composition. The major events associated with nutritional status in seed are a complex process, and are majorly divided into four main steps: pre-storage, synthesis, accumulation and utilization of nutrients. Differential display of nutrient-responsive sub-cellular seed-specific proteome and phosphoproteome was developed with emphasis on cytosolic fraction in developing chickpea (*Cicer arietinum L.*) seeds using 2-DE. Reversible protein phosphorylation, PTM is known to regulate protein function and signaling pathways. Proteins were identified using LC-MS/MS analysis. MS analysis led to the identification of around 194 proteins and 93 phosphoproteins, presumably involved in a variety of biological functions viz. storage and biogenesis, followed by protein folding and degradation, carbohydrate metabolism and amino acid metabolism, while some were involved in secondary metabolism. Other assigned category consisted of proteins involved in redox homeostasis and signaling. Further, we interrogated the dataset using cluster and network analyses that identified significant protein modules and small correlation groups heat map is generated for the identified proteins. Proteins involved in storage and biogenesis such as vicilin, globulin, legumin showed increased

expression across the different stages of seed development. Together, these analyses enhance understanding of how nutrients are being synthesized at globular and early heart stage, accumulated at cotyledon and mature stage which are being further utilized during germination stage. Thus, seed development involves highly dynamic processes of cell division, differentiation, growth, pattern formation and macromolecule production elucidating the underlying mechanisms which will provide insight into the complex system coordinating plant development and metabolism. Proteomics provides more powerful tool to understand the complex protein dynamics and the underlying regulatory mechanisms during seed development.

COOSCP5

### **Proteomic Reference Map of Vetiver Root (*Chrysopogon Zizanioides L. Roberty*) and Its Comparison with Contrasting Root Architectural Variety Illustrates Pathway Abundance Proteins**

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Khus or Vetiver [*Vetiveria zizanioides* (L.) Nash., syn. *Chrysopogon zizanioides* (L.) Roberty; family Poaceae] is a perennial, wildly growing aromatic grass occurring all across India in variable agro-climatic conditions, markedly in foothill wetlands, river banks and marshy soil. India is considered to be the center of origin of vetiver. A higher degree of genetic diversity including ecological and geographic adaptation, morphological and reproductive traits, and concentration and composition of essential oil is widely distributed in the Indian subcontinent. To unravel and understand the complexity of root-protein system (thick and smooth) of perennial *khus* oil yielding vetiver grass two-dimensional gel electrophoresis (2-DE) was used to develop a proteome reference map with detection of about 400 protein spots by PDQuest analysis. Of which, 160 proteins have been

identified by MALDI-MS/MS analyses implicated in a variety of cellular functions. The identified proteins, involved in bioenergy and metabolism, were in highest number (30%), while 26% belong to protein biogenesis and storage. Vetiver root proteome indicates the presence of few new cytosolic proteins of unknown functions along with many previously known resident proteins. To the best of our knowledge, this is the first report of a root proteome of an un-sequenced vetiver grass. Moreover, a comparative root proteome with another vetiver of contrasting root architecture (thin and hairy) was also carried out which revealed the upregulation of several proteins associated with bioenergy and metabolism in vetiver-A as compared to vetiver-B. Furthermore, multivariate analysis of the resulting protein patterns suggests the cultivar-specific protein expression. The developed reference map may be exploited to dissect biochemical pathways encompassed by the identified proteins in any associated condition. The obtained proteins information by comparative proteomics study, responsible for better root architecture, may be used in genetic engineering as well as breeding programs for the benefit of farmers practicing vetiver.

COOSCP6

### **Proteomic Study of Embryogenic Competence Acquisition and Expression in *Nothapodytes Nimmoniana* (J. Graham) Mabberly**

**Tasiu Isah**

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Somatic embryogenesis (SE) is the most suitable biotechnological tool for clonal propagation of endangered woody plants but, many bottlenecks limit its applicability with many forest trees. *Nothapodytes nimmoniana* is an endangered forest tree most convenient source for the large scale isolation of anticancer alkaloid camptothecin. The in vitro cultures of *N. nimmoniana* showed poor response to the production of Embryogenic Calli (EC) and Somatic Embryogenesis (SE) and produced embryos exhibited a lack of maturation under maturation culture conditions. Two-dimensional electrophoresis (2DE) and mass

spectrometry were employed to study changes in proteomic expression during embryogenic competence acquisition and expression in the in vitro cultures of the tree. Calli were induced from mature seed embryos, various explants of 3-4 weeks old axenic seedlings, and embryogenic callus obtained from the calli at very low frequency. Globular embryos were induced from the EC, and embryos from seed embryo-derived calli during developmental stages harvested from cultures for protein extraction and resolution on 2DE. Analysis of the resolved proteins in the gels showed increased accumulation across embryogenic competence acquisition and developmental stages of the SE. The mass spectrometric analysis with database search aided identification of a low number of the selected resolved protein spots. Obtained results showed higher metabolic and physiological processes involved in the acquisition of embryogenic competence in *N. nimmoniana* calli; higher stress, energy metabolism, protein synthesis and other metabolic processes played a role in the upregulated expression of the associated proteins in the EC over Non-Embryogenic calli (NEC). The expressed proteins during SE, by their cellular role, are involved in stress responses, energy metabolism, carbon fixation, secondary metabolism and other metabolic functions while three proteins are of unknown cellular role. The putative role of the expressed proteins during embryogenic competence acquisition and expression provided insight into the importance of stress and metabolism during acquisition of embryogenic competence and physiology of SE in *N. nimmoniana*. Molecular studies on the role of stress in embryogenic competence acquisition in the callus cultures of *N. nimmoniana* could open further possibilities for understanding the metabolism and physiological processes involved in enhanced production of EC with application in optimizing culture conditions to improve SE in *N. nimmoniana* and application to scale-up production of CPT to mitigate overharvest of the endangered natural population.

## Protein Degradation Rate in Arabidopsis Thaliana Leaf Growth and Development

Lei Li

In order to characterize plant protein degradation rates and understand their determinants as they relate to plant growth rate, we have applied  $^{15}\text{N}$  labelling approaches in leaves of the Arabidopsis rosette. We found a series of leaves in Arabidopsis plants for which the proteome was stable over time and degradation rates of individual proteins could thus be measured by considering the dilution of total protein abundance through growth. By progressively labelling new peptides with  $^{15}\text{N}$  and measuring the decrease in the abundance of over 60,000 peptides with natural isotope abundance profiles we determined the degradation rate of 1228 proteins. The exponential constant of the decay rate ( $K_{\text{D}}$ ) for each protein calculated from the relative isotope abundance of each peptide and the fold change in protein abundance during growth showed a wide distribution, ranging from 0 to 2 per day. This showed Arabidopsis protein half-lives vary from several hours to several months. In assessing intrinsic factors to explain these protein degradation rates, little effect of the N-end amino acid of proteins or of protein aggregation propensity were found, however protein complex membership and specific protein domains were strong predictors of degradation rate. We found new rapidly degrading subunits in a variety of protein complexes in plastids, identified the set of plant proteins whose degradation rate changes in different leaves of the rosette and correlated with leaf growth rate, and calculated the protein turnover energy costs in different leaves and their key determinants within the proteome.

## A Comprehensive Analysis of Lipids in Plasma Using Liquid Chromatography-Mass Spectrometry

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Lipids play an important role in cellular homeostasis, signaling and are used as disease biomarkers. Lipid compositions of biofluids such as plasma are complex, reflecting a wide range of diversity and concentration of different lipid classes. Their varying degree of chemical complexity makes their elution from liquid chromatography challenging. Also, several lipid species are present as isobars making their interpretation in mass spectrometry difficult.

Here, we performed targeted liquid chromatography-mass spectrometry (LC-MS) with multiple reaction monitoring (MRM) for 212 plasma lipid species in positive mode. We have compared two different LC protocols: (A) Methanol/water and (B) IPA/ACN/water. Mass spectrometry parameters such as declustering potential (DP), entrance potential (EP), collision cell exit potential (CXP) and collision energy (CE) were optimized for each lipid class. High resolution MS/MS was generated to identify the isobaric peaks by fragmenting the two most intense peaks with trap fill time of 10 ms and Q0 trapping turned on. Isobaric peaks were identified based on neutral loss of fatty acyl groups from the parent ion. Finally, we have assessed the stability of lipids and their degree of variation across different runs spread across different days over a month's time period. Technical variation was assessed using triplicate measurements, while biological variation was measured with three different healthy control samples.

Our results indicate that though IPA has relatively poorer ionization efficiency than methanol, IPA/ACN/water protocol provides

better peak shape. Further, both neutral and phospholipids can be detected in a single LC run. Also, internal standards show relatively poorer linearity with increasing concentration in protocol A than in protocol B indicating that the latter helps in better elution of lipids from the column. Our study with the use of technical and biological replicates highlight the importance of the range of biological and technical variation observed in plasma lipids analysis.

CMMD2

### **Understanding the Mechanism of Growth Inhibition by Metabolites in One Carbon Metabolism Pathway (Homocysteine, Cysteine and SAH)**

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One carbon metabolism (OCM) involves transfer of a single carbon unit in the form of methyl group. In OCM, dietary Methionine is converted to S-adenosyl methionine (SAM) by S-adenosyl methionine synthase. SAM acts as a universal methyl group donor and carries out several transmethylation reactions including methylation of biomolecules DNA, RNA and protein. In turn, SAM is converted to S-adenosyl homocysteine (SAH) which is further hydrolyzed to homocysteine and adenosine by the action of SAH hydrolase. The homocysteine thus formed can be converted to its precursor SAH again, it can re-methylate to form methionine or can undergo transsulfuration for the biosynthesis of cysteine and glutathione. Among different metabolites which are part of OCM, excess levels of SAH, homocysteine and cysteine has been shown to be toxic in different model organisms. All three of these toxic metabolites are also associated with various diseases. However it is still unclear if the mechanism of toxicity for metabolites overlap and does cell responds to these toxic metabolites in the similar way. This study is focused on in-depth comparison of the toxicity of cysteine, homocysteine and SAH using the eukaryotic model system *Saccharomyces cerevisiae*.

Excess of Cysteine has been shown to cause an amino acid imbalance (Unpublished data). To compare the toxicity of cysteine with homocysteine and SAH, we performed an amino acid screen and found that Leucine can rescue both cysteine and homocysteine toxicity but not that of SAH. SAH toxicity has been reported to be alleviated by SAM (Kumar et al 2011), however we found that SAM can only partially rescue cysteine toxicity and has no effect on homocysteine toxicity. Thus to further do a detailed comparison of cysteine, homocysteine and S-adenosyl Homocysteine induced toxicity we used an iTRAQ based LC-MS approach and found various proteins to be differentially expressed. Detailed analysis is underway.

CMMD 3

### **Change in redox balance couples with redistribution of metabolic flux to protect *Gclm*-knockout mice from alcoholic liver disease**

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Alcoholic liver disease (ALD) is one of the leading causes of mortalities associated with chronic alcohol consumption. The disease is initiated with lipid accumulation (steatosis) in liver, which is reversible in early stages. In some cases the disease may progress to cause irreversible liver damage leading to steatohepatitis, fibrosis and cirrhosis. However, key players and events responsible for progression of the disease are not fully understood and therapeutic options are limited for the advanced stages of the disease. Understanding of molecular events associated with pathogenesis is essential to develop novel therapeutic strategies. Since oxidative stress is known to play an important role in disease progression, the effect of knocking out the *Gclm* gene, which is involved in biosynthesis of natural anti-oxidant glutathione, on ALD pathogenesis was examined in this study.

Surprisingly, *Gclm*-null mice were found to be protected from alcoholic steatosis compared to the wild-type. In order to investigate the mechanism responsible for protection, the reorganization of metabolic pathways was examined. Liver samples from wild-type and *Gclm*-null mice either treated with alcoholic or calorie-compensated non-alcoholic control diet were extracted and global metabolic profiling was carried out using mass spectrometry. Changes in metabolic signatures were identified and quantified through multiple reaction monitoring. Expression levels of key genes involved in respective metabolic pathways were also examined. Results showed widespread changes in amino acid, nucleic acid and amino sugar metabolic pathways to be associated with impairment of glutathione metabolism. It was found that acetyl CoA is siphoned towards a new metabolic sink rather than *de novo* lipid biosynthesis in alcohol-treated knockout mice. Taken together, the analysis revealed that shift in redox balance due to attenuation of GSH levels in *Gclm*-null mice is coupled together with redistribution of amino acid and acetyl CoA flux to protect them from alcoholic steatosis.

this study. 12 different samples each with 4 biological replicates were studied. 25mg of finely powdered stem samples of these plants were used for metabolite extraction. Ion-pair chromatography on Agilent Eclipse plus C18 was used to profile 21 amino acids, 12 sugars, 9 phenolic compounds and 4 nucleobases. Agilent Poroshell Pentafluro phenyl propyl (PFP) column was used to profile 24 organic acids. LC-MS data was acquired in triplicates on Agilent 6550 iFunnel Q TOF instrument. Genespring was used for the statistical analysis. Differential features between samples were identified by ANOVA. Metabolome at different conditions was compared with metabolome at 20 °C. Effect of Carbon dioxide at 20 °C found to be minimal. However, significant effect of CO<sub>2</sub> on the metabolome was observed at 30 °C. Phenyl pyruvic acid, glyoxylic acid, Caffeic acid and Raffinose found to be dysregulated between these two conditions. UDP-glucose that play a vital role in cellulose bio-synthesis remained unaltered. The plant looks to increase its tolerance in all low temperature conditions by enhancing flavonoids like Myricetin. Pathway analysis and integrating with transcriptomics is underway.

CMMD4

CMMD5

### **Effect of Stress on Eucalyptus Plant Metabolome**

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Wood pulp with higher amounts of lignin is a matter of concern for paper industry. Lignin has to be removed from cellulose for industrial applications and this is expensive and not environment friendly. Searching for factors that inhibit lignin biosynthesis and enhance cellulose biosynthesis has been a topical interest for plant scientists. We studied Eucalyptus plant metabolome to understand the effect of environmental factors such as temperature and Carbon dioxide on plant metabolome in turn effecting lignin biosynthetic pathway. Plants were exposed to temperatures 10°C, 20°C and 30°C with CO<sub>2</sub> levels 0 ppm and 760 ppm. Two different plant species, *Eucalyptus globulus* and *Eucalyptus grandis* were chosen for

### **Isolation of Herbivore Associated Molecular Patterns from Oral Secretion of *Spodoptera Litura* and Its Effect on Defense Related Metabolome of *Arabidopsis Thaliana***

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About half a million insect species inhabit the planet, of which majority feed on plants. During herbivory, plants activate direct defense mechanisms via phytohormones and secondary metabolites. Plants have different mechanisms to distinguish mechanical wounding and herbivory mediated wounding, and they respond in different ways to defend them with diverse signaling and secondary metabolites biosynthesis. To perceive potential insect herbivores, plants identify different organic molecules in the oral secretions or

oviposition fluids of the insects and commence molecular sensing to induce their defense mechanism. These herbivore derived agents are known as Herbivore Associated Molecular Patterns (HAMPs). We use *Arabidopsis* and its herbivore pest *Spodoptera litura* (cutworm), as model system to study early and rapidly active defense pathways that help in herbivory recognition. The identity of HAMPs, is unknown in our system and we use HAMP induced cytosolic  $\text{Ca}^{2+}$  elevation and secondary metabolites as marker for its identification. We demonstrate a liquid chromatography based partial purification of HAMP like molecule from oral secretion of *Spodoptera litura*, which act as elicitor to induce the herbivore derived early defense responses in *Arabidopsis thaliana*. The present work also reports the effect of wounding as well as direct *Spodoptera litura* insect feeding on defense related secondary metabolites, viz. glucosinolates, anthocyanins, flavonoids and phenolics in major signaling pathway mutants of *Arabidopsis thaliana*.

CMMD6

### **Evaluation of MALDI-MS Platform for Peptidomic, Lipidomic and Metabolomic Imaging Studies in the Context of Non-Alcoholic Fatty Liver Disease**

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Non-alcoholic fatty liver disease (NAFLD) is one of the most common non-infectious aetiology responsible for liver degeneration. It is significantly associated with insulin resistance and may progressively lead to fibrosis and cirrhosis. Early and accurate diagnosis is of key importance to limit fatal consequences. However, it remains challenging due not only to asymptomatic nature of the disease in early stages but also to significant overlap between histological features associated with alcoholic liver disease (ALD) and NAFLD. However, given the distinct aetiology, the molecular signatures and their spatial distribution associated with these two diseases are likely to be distinct. Such differences in molecular signatures could be examined by imaging mass spectrometry to

distinguish between ALD and NAFLD liver samples even in absence of information on alcohol consumption. MALDI mass spectrometry has been shown to be a promising technique for tissue imaging. However, the response and behaviour of different types of analytes (such as, metabolites, lipids, peptides, proteins) in MALDI imaging is far from being well-understood and uniform. In this study, we evaluated the quantitative potential of MALDI-based analysis of representative metabolites, lipids and peptides. The effect of laser power, sub-spectra, averaging and normalization on limit of detection (LoD), limit of quantitation (LoQ) and dynamic range were examined on a SCIEX MALDI TOF/TOF 5800 instrument. Manually-curated analyte distribution patterns on 384-well MALDI plate were imaged. Effect of laser power, sub-spectra, averaging and normalization on surface roughness and surface trueness parameters were analyzed. All data deconvolution, alignment, binning, and image reconstruction and surface analysis were performed using a set of in-house 'R'-based algorithms. Results showed that experimental parameters for optimization of LoD, LoQ and dynamic range are dependent on the nature of the analyte. It also showed that internal standard normalization significantly helps to improve the quantitative imaging potential using MALDI.

CMMD8

### **Effect of Homocysteine on Lipid Profile of Human Hepatocyte**

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Homocysteine, an important component of one carbon metabolism pathway, is a sulfur-containing amino acid and is related to altered levels of metabolites within one carbon metabolism. Also, in cardiovascular disease (CVD) patients, higher levels of plasma homocysteine are associated with the altered lipid profile. These studies suggest that altered levels of different lipid species may have mechanistic role in CVD. Thus, for an in-depth understanding of the role of homocysteine in regulating the lipid metabolism, we have performed

a targeted liquid chromatography-Mass spectrometry (LC-MS) with multiple reactions monitoring (MRM) in positive mode for more than 250 lipid species in human hepatocyte (Hep G2).

In this study, we have treated the Hep G2 cells with 1mM and 5mM homocysteine for 24 hours. Cell viability assay using ALAMAR-Blue shows that homocysteine causes 3% and 15 % death in 1mM and 5mM concentrations, respectively in comparison to control cells. Further we performed an LC-MS based lipid profile at different concentrations of homocysteine with stringent cut-off {(downregulated)  $0.5 \geq \text{Fold change} \geq 2$  (upregulated) and ( $p \leq 0.05$ )}. We found that at lower concentrations of homocysteine (1mM), only one lipid species was significantly altered with respect to control cells. In higher concentrations (5mM) of homocysteine, 10 lipid species were found to be differentially altered. The levels of lipid species altered in 5mM homocysteine treated cells belongs to the class dihydroceramide (dhCer), alkylphosphatidylcholine [PC(O)], phosphatidylethanolamine (PE), phosphatidylethanolamine plasmalogen [PE(P)], phosphatidylserine (PS), cholesterol ester (CE), and Diacylglycerol (DG).

**Conclusion:** Homocysteine alters the lipid profile in Human hepatocytes.

CMMD9

### Role of Neem Leaf Extract In Treating Cancer

Mr Ankit Kumar

*Azadirachta indica* (Neem) is a tree in mahogany family **Meliaceae**. Neem is widely grow in India. Neem is used by social communities as since ancient time as clearing of teeth, microorganism and others insects. Neem leaf extract is being used to treat-**Murine carcinoma**, a protein from neem leaf have been isolated, which is **neem leaf glycoprotein(NLGP)** . Generated anti-NLGP antibody (primarily IgG2a0) was tested for its anti-tumor active in **murine carcinoma**(EC, CT-26),sarcoma (S180) and melanoma (B16Mel) tumor models. Tumor models revealed high expression of **CEA(Carcinoembryonic antigen)** like protein on the surface of CT-26 tumor. subsequent examination of the cross-reactivity of anti-NLGP antibody with

purified or cell bound CEA revealed prominent recognition of CEA by anti-NLGP antibody, as detected by ELISA, Western Blotting and immunohistochemistry. This recognition seems to be responsible for anti-tumor function of anti-NLGP antibody only on CEA-like protein expressing CT-26 tumor models ,as confirmed by ADCCreaction in CEA+ tumor system where dependency to anti-NLGP antibody is equivalent to anti-CEA antibody.

CMMD10

### 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 may lead 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.

December 17, 2016

## Session X- Food, Nutrition and Stress Proteomics

DFNSP1

### Physicochemical Analysis and Proteome Profiling Of High Temperature Stress in Bread Wheat (*Triticum Aestivum L.*): Dissecting the Dynamics of Thermotolerance

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The IPCC's (Intergovernmental Panel on Climate Change) Report in 2014 suggested an approximate increase in temperature of 4-5°C by the end of 21<sup>st</sup> century. Increase in earth surface temperature is one of the most important factors which affect plant growth and development, and crop yield. The high temperature stress (HTS) greatly limits wheat cultivation and productivity worldwide. It is suggested that HTS-responsive changes in expression of proteins may contribute to cellular adaptation against such stress. Most of the molecular understanding of HTS-responsive cellular adaptation in wheat has been evolved from transcriptome analyses. The HTS differentially affects the integrity of cell membranes, cytoskeleton structures and stability of proteins causing metabolic imbalance; however, HTS-responsive comparative proteome profiling, particularly proteins in the subcellular fraction, is rare. We screened the status of thermotolerance of nine wheat varieties implying different physicochemical

and molecular indexes, and selected relatively tolerant cv. Unnat Halna and sensitive cv. PBW343. Further analyses of morphological architecture, histochemical status and cellular antioxidant defence confirmed the contrasting adaptation of cv. Unnat Halna and PBW343 under HTS. To better understand the molecular basis of HTS tolerance mechanism, we isolated the cytoplasmic fraction from the tolerant cv. Unnat Halna and developed differential cytoplasmic proteome map under HTS. The proteomic analysis revealed 105 differentially expressed proteins presumably involved in cellular adaptation and impinge on the molecular mechanism of thermotolerance in crop species.

DFNSP2

### Exploring the Role of Calcium Ion in Regulation of Metabolic Processes via Ntca (Global Transcriptional Regulator) In *Anabaena* Sp. PCC 7120

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Calcium is an important macronutrient for both prokaryotes and eukaryotes. It acts as an important second messenger mediating rapid response to environmental changes. The present investigation deals with proteome profiling of *Anabaena* 7120 and its derivative *ntcA* mutant in response to varied calcium doses (0, 1, and 10 mM CaCl<sub>2</sub>). Concentration of 1 mM CaCl<sub>2</sub> salt was the optimum concentration whereas 10 mM CaCl<sub>2</sub> was the inhibitory concentration for both the wild type and mutant strains. The results showed that highly significant alteration were evident in terms of protein abundance and differential response related to key processes of photosynthesis, energy and metabolism, nitrogen metabolism, oxidative and antioxidative stress, transport and signaling and fatty acid metabolism. Some hypothetical proteins were also realized during proteome analysis. The study discusses the key processes of *Anabaena* 7120 affected by calcium and the pathway of interaction of calcium with NtcA in regulation of metabolic processes using 2DE technique followed by protein characterization through MALDI TOF MS/MS.

DFNSP4

**Metabolomics, Ionomics, Antioxidant Activity and Proximate Analysis of the Fruit Extract of the Halophyte *Salvadora Persica* L., Untangle A Potential Source of Non-Conventional Plant Food**

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*Salvadora persica* L. is a medicinally important plant predominantly used in oral hygiene. However, little attention has been given towards the nutritional composition of this plant. This study encloses the proximate and mineral ions profiling, amino acid composition, metabolite profiling and antioxidant potential of *S. persica* fruit. The ripe fruit contained substantial amount of sugars, mineral nutrients, carotenoids, polyphenols and flavonoids. The metabolomics of the fruit extract through GC-MS analysis identified of 22 metabolites comprising of sugars, sugar alcohols, organic acids, organic base and aromatic silica compounds. The identified metabolites have been previously reported to have potential antioxidant, antimicrobial, antitumor and anti-hyperglycaemic properties. The GC-MS analysis indicated high glucose and glucopyranose contents in fruit of *S. persica*. The antioxidant activity of the fruit extract was also remarkably high along with high mineral ions and essential amino acids. HPLC analysis revealed presence of essential and non-essential amino acids required for healthy body metabolism. The cysteine was found to be in highest amount among all amino acids quantified. As compared to other medicinal plants, previously reported as a source of non-conventional food and with some of the commercially important fruits, *S. persica* fruit appears to be a potential source of essential mineral nutrients, amino acids, vitamins (ascorbic acid and carotenoids) and pharmaceutically important metabolites. The data presented here proposed that *S. persica* fruit may

be used as a source of non-conventional food or reinvigorating ingredient for processed food to reduce deficiency of nutrients among the vulnerable population group.

DFNSP5

**Seed Proteomics Approach for Identifying of Sorghum Genotypes by Using 2-dimensional Gel Electrophoresis and MALDI-TOF-MASS Spectrometry**

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In the last years, proteomics analyses in cereals have significantly increased but few studies have been performed in sorghum. Sorghum seed proteomics investigation not only potential markers for identifying of among genotypes but also their nutritional aspects and utilizing in the breeding programs. However, we reported here a proteomics approach for different sorghum genotypes by using two-dimensional gel electrophoresis (2-DGE) and Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). A comprehensive proteomics analysis were therefore attempted using mature seed of six sorghum genotypes i.e., IS 3477, IS 33095, IS 7005 (non pigmented), IS 2898, IS 7155, IS 1202 (pigmented). The six high resolution gels of 2-Dimensional gel electrophoresis (2-DGE) stained with coomassie brilliant blue (CBB) which were enabled a total of 600 above protein spots including the pigmented lines were shown below 100 whereas non pigmented lines were exhibited above 100 protein spots. The protein spots of each genotype were well separated based on their molecular weight and pI values which ranges between 12-90 kDa and 4-9.5 respectively. A total of fourteen identical protein spots were selected from the six genotypes pertaining 4 common spots and 10 specific spots, showing a notable change, were sequenced by MALDI-TOFMS analyzer. Unfortunately, out of the seed responsive fourteen protein spots, eight protein spots annotated to sorghum and orthologs from other monocots such as maize, rice, and barley.

Most of the identified proteins belongs to seed storage protein, Heat shock protein, RNA binding & zinc finger proteins, carbohydrate metabolism and hypothetical proteins. This study would be of interest to use these proteins to develop quick test for seed quality as well as gene annotation.

DFNSP6

### **Metabolic Stress and Translational Arrest: Fate of Excess Cysteine**

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**Background:** Nutritional stress is generally related to insufficient supply of nutrients, however cell also experience stress due to excess of nutrients. How cell responds to excess nutrients is poorly understood. Among various nutrients, excess of cysteine-a thiol containing amino acid has been known to be toxic in many organisms. Elevated levels of cysteine have also been associated with neurological and cardiovascular diseases. However, despite the fact that cysteine can cause toxicity and is associated with many diseases the mechanism of how excessive cysteine causes toxicity is not known. Using *Saccharomyces cerevisiae* as a model system in this study is focused on delineating the detailed molecular mechanism behind toxicity of excess cysteine.

**Results & Discussion:** In order to characterize the cellular response during hyper-cysteinemia, an iTRAQ based quantitative proteomics approach was used. 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 unable to rescue in "leu3 strain (Leu3 is a transcription factor regulates genes involved in branched chain amino

acid biosynthesis) and leucine was not able to "ncl1 deleted strain (Ncl1 is a tRNA m5C-methyltransferase, methylates tRNALeu (CAA) at the anticodon wobble position). It clearly indicates that pyruvate and leucine can alleviate cysteine induced toxicity through Leu3 and Ncl1 respectively.

Intercellular amino acid measurement using o-Phtalaldehyde based derivatization revealed that hyper-cysteinemia causes an imbalance of other amino acids. Further by using s35 radiolabelling and polysome profiling we found that high levels of cysteine induces translational arrest.

**Conclusion:** Excess cysteine induces an amino acid imbalance and hence decreases protein translation.

DFNSP7

### **Proteome Atlas of Pigeonpea: Developmental proteins**

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Pigeonpea is the second most important food legume crop of India but its productivity has remained stagnated for the last five decades. There is need to improve its yield potential by changing the plant type and harvest index. In the present study we have developed complete proteome atlas of root and shoot of young seedlings of pigeonpea. As a first step we have identified the proteins of mature seeds and seedlings. A gel free LC-MS analysis were employed for identifying proteins of the tissues. The proteins were extracted from seeds, shoot and root after 10 days of germination. Total 78, 97 and 58 proteins were identified in seed, root and shoot, respectively. The maximum proteins were found for seed followed by root and shoot. Among them 45 proteins were common to all the 3 samples. The uncharacterized proteins were maximum for shoot tissues. Interestingly Dof family of proteins were significantly observed for all the samples. There are 38 Dof proteins are present in pigeonpea genome, among them 13 were reported for seed, root and shoot samples. DNA binding with One Finger (Dof) protein is a plant specific

transcription factor having diverse role in plant growth and development. An attempt to characterize CcDof gene family using various bioinformatic tools like protein motif analysis, phylogenetic analysis and secondary structure analysis has been made. The structural, functional and in-silico studies of Dof transcription factor attempted here exhibits great opportunity to analyse the pigeonpea genome in terms of number of Dof genes and shed light on evolution of legume crops. The multiple functions of Dof genes and their role and position in the metabolic pathways needs to be explored for crop improvement.

DFNSP8

### **Comparative Proteomic Analysis Reveals Different Adaptation Mechanisms in Traditional and Modern Rice Genotypes under Phosphorus Deficiency**

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Phosphorus (P) is one of the essential macronutrient required for plant growth and development. However, most of the soil P exists in the form of organic compounds or insoluble complexes, not readily available to root for uptake. Application of P-fertilizers can compensate for low P availability, but the global source of P, rock phosphate is non-renewable and will be exhausted in the next 50-100 years. P deficiency is therefore, a major problem for cultivation of important food crops like rice in many world soils. Most of the modern high yielding rice genotypes are sensitive to P deficiency whereas traditional rice cultivars are naturally compatible to low P ecosystems. These low P tolerant genotypes have evolved a number of adaptations that allow them to withstand low levels of P in soil. These mechanisms broadly include modifications in root system architecture for enhanced P uptake and improved P-use efficiency. However, the underlying genetic mechanism for low P tolerance in traditional low P tolerant genotypes remains largely unclear. To delineate the molecular mechanisms for low P tolerance, comparative proteomic analysis of two contrasting rice genotypes, Dular and PB1 was carried out by two-dimensional gel electrophoresis (2-DE). Both

genotypes were hydroponically-raised under low P ( $1 \mu\text{M NaH}_2\text{PO}_4$ ) and sufficient P conditions ( $320 \mu\text{M NaH}_2\text{PO}_4$ ) for 15 days, after which proteomic analysis of root and shoot tissues was performed. In total 1030 (root) and 1693 (shoot) spots were detected in both genotypes. Out of these, 400 and 283 spots were differentially expressed in root and shoots, respectively in both genotypes under low P condition. Most of the identified differentially regulated proteins were found to play important roles in metabolism, signaling and root system architecture modulation. Present study identified the key protein regulators involved in differential adaptation mechanisms of both genotypes under P deficient condition.

DFNSP9

### **Comparative Phosphoproteome Analysis of Rice Genotypes Revealed New Insights in Phosphorus Homeostasis under Phosphorus Deficiency**

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Phosphate (P) is one of the most critical mineral nutrients for plants growth and development. Nonetheless, total P concentration in soil system is abundant but it is not readily available to the plants. To maximize uptake of P from soil solution, plants have adopted various strategies like elongation of root hairs, lateral roots, increased density of root hairs and laterals. These strategies depend on the diverse genotypic variability of the plants. Modern rice genotypes have been greatly invested with high dose of phosphatic fertilizers to obtain higher yields. However, these elite genotypes fail to deter under severe P stress as they require high input of phosphatic fertilizers to sustain under low P stress. On the other hand, traditional genotypes which are P efficient in nature can grow better under low P conditions as compared to improved modern cultivars. P deficiency has serious implications on signaling cascades since phosphorylation and dephosphorylation are the key steps to regulate the signaling mechanisms. To understand the mechanism underlying the low P tolerance of traditional and modern genotypes, we performed phosphoproteome analysis of root and shoot tissues of a low P tolerant genotype, Dular and a low P sensitive genotype PB1 under low P conditions. For

this, rice seedlings were raised hydroponically for 15 days under low P ( $1 \mu\text{M NaH}_2\text{PO}_4$ ) and sufficient P conditions ( $320 \mu\text{M NaH}_2\text{PO}_4$ ) followed by two-dimensional gel electrophoresis (2-DE). Changes in the protein phosphorylation status in both genotypes was analysed by staining of 2-DE gels with phosphospecific fluorescent Pro-Q Diamond dye. Our analysis revealed immense decrease in protein phosphorylation status in PB1 as compared to Dular under P deficiency. Protein identification by mass spectrometric revealed several commonly and differentially expressed putative phosphoproteins involved in a variety of functions including photosynthesis, photorespiration, metabolism, signalling and cell defence.

DFNSP10

### **Proteometabolomic Characterization of Sweetpotato Illustrates Genotype-Specific Patterns for Defined Proteins and Metabolites in Nutrient Acquisition**

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Sweetpotato has long been acknowledged as a significant contributor of global caloric needs, which continues to be of remarkable economic value. It is an important staple and emergency food in many countries and its annual world production hovers to about 130 million tons. In recent years, sweetpotato research has received additional global attention. However, investigation on the traits that directly influence nutrient acquisition and availability of phytochemicals in sweetpotato remains largely unexplored. We therefore aimed at analyzing differential nutrient availability and phytochemicals, besides proteometabolic profiles of two contrasting genotypes of sweetpotato, an orange-fleshed sweetpotato (OFSP) and a white-fleshed sweetpotato (WFSP). Phytochemical screening displayed higher content of carbohydrate, reducing sugar and total phenolic content in WFSP, while OFSP showed augmented total protein, flavonoids, anthocyanins, and carotenoids. The kinetics of starch and cellulose degradation was observed to be lesser in OFSP during storage.

Proteomic analyses led to the identification of 1541 and 1201 proteins in orange fleshed and white fleshed sweetpotato genotypes, respectively, presumably associated with binding, followed by catalytic, transferase, hydrolase, kinase and transporter activities. Furthermore, metabolome profiling revealed 148 and 126 metabolites in cv. OFSP and WFSP, respectively. These results would not only form the basis for future comparative proteometabolomic efforts for sweetpotato, but also provide new insights into how genotype-specific traits are developed in crop species.

DFNSP11

### **Differential Proteomic Changes during Post-Harvest Storage of Wild-Type and OXDC Tomato Points towards Cross Talk of Signal Components and Metabolic Consequences**

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Fruits of angiosperms evolved intricate regulatory machinery for sensorial attributes and storage after harvesting. Organogenesis and maturation of storage organs forms the molecular and biochemical bases of organoleptic and nutritional qualities with metabolic specialization. Tomato fruit (*Solanum lycopersicum*) are highly perishable horticultural product and have distinctive feature to undergo a shift from heterotrophic metabolism to carbon assimilation partitioning. Storage effect in fruit is a genetically controlled process and has been correlated with ripening related processes. We have earlier shown metabolic consequences of oxalate down-regulation in fruit. Transgenic tomato plants expressing an oxalate decarboxylase (*FvOXDC*) in the fruit, leads to decrease in the oxalic acid content besides increase fungal tolerance and better storage effect. Here, we elucidate the post-harvest storage-regulated molecular determinants affecting organoleptic features, signalling and hormonal regulation. A comparative proteomics approach has been applied between wild-type and E8.2-OXDC tomato in temporal manner. The MS/MS analyses led to the identification of 32 and 39 differentially abundant proteins associated with primary and

secondary metabolism, assimilation, biogenesis, and development in wild-type and E8.2-OXDC tomatoes, respectively. Next, we interrogated the proteome data using correlation network analysis that identified significant functional hubs pointing toward storage related coinciding processes through a common mechanism of function and modulation. Furthermore, physiochemical analyses exhibited reduced oxalic acid content with concomitant increase in citric acid, lycopene and marginal decrease in malic acid in E8.2-OXDC fruit. Collectively, our study provides insights into temporal distribution of many classes of storage related proteins, identified regulatory module for pathway formulation and may provide a wide characteristics involved in maintaining fruit quality at the protein level for biological discovery.

DFNSP12

### **Secretome Profiling of Chickpea Identifies a Leaderless Bet V1-Like Protein That Participates In Stress Response**

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Secreted proteins maintain cell structure and biogenesis, besides acting in signaling events crucial for cellular homeostasis during stress adaptation. To better understand the underlying mechanism of stress-responsive secretion, the suspension-cultured cells of chickpea were subjected to water-deficit conditions. Cell viability of the suspension culture remained unaltered until 96 h, which gradually declined at later stages of dehydration. Proteomic analysis led to the identification of 215 differentially regulated proteins, involved in multivariate cellular processes that include metabolism, cell defence and signal transduction suggesting their concerted role in stress adaptation. One-third of the secreted proteins displayed no N-terminal secretion signals suggesting a nonclassical secretion route. Screening of the secretome identified a leaderless Bet v 1-like protein, designated CaRRP1, the export of which was inhibited by brefeldin A. We investigated the gene structure and genomic organization, and demonstrated that CaRRP1 may be involved in stress response. The transcript abundance of CaRRP1 was significantly and positively correlated

to stresses caused by both abiotic and biotic factors. Functional complementation of CaRRP1 could rescue the growth defects in yeast mutant, deficient in vesicular transport indicating a partial overlap of protein secretion and stress response. Our study provides the most comprehensive analysis of dehydration-responsive plant secretome and the complex metabolic network operating in plant extracellular space.

DFNSP13

### **Integrated Analysis of Nutrient-Associated Transcriptome and Metabolome of Developing Chickpea Seed**

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Storage organs in plants show remarkable diversity in reserve synthesis and accumulation that affect growth, development, and productivity. They display diverse nutritional quality and complex multistep development highly related to nutrient metabolism and transport as an intense sink activity in plants. The synthesis and accumulation of each class of storage component requires the coordination of many genes and proteins that encode the enzymes of the respective pathways. Metabolic state and feedback regulation of low molecular weight compounds influences the nutrient dynamics in developing seed. Chickpea, a legume, known to have high nutrient composition and is an assimilator of various metabolites. RNA-seq using Illumina Hi-seq 2000 paired-end sequencing technology was used to generate an extensive map of nutrient-associated chickpea seed transcriptome. Further, metabolites were extracted, derivatized, diluted and injected to GC-MS. The nutrient-responsive transcriptome revealed a total of 192.3 million reads assembled into 191,487 total numbers of contigs. Out of the total 60,584 transcripts, 6582 were found to be differentially expressed at one or more developmental stages which were further categorized based on their putative functions viz. metabolism, transcription, signaling, development and storage etc. Further, Gene ontology (GO) analysis of differentially expressed transcripts was done using Blast2GO. To complement the transcriptomic study, metabolome study revealed

identification of diverse compound ranging from non polar to polar metabolites including sugars, sugar phosphates, organic acids, amino acids. The parallel analysis between the quantification of metabolites and determination of transcripts aid in constructing a metabolic atlas summarizing the dynamics of nutrient synthesis, accumulation and utilization on central metabolic pathways in developing chickpea seeds.

DFNSP14

**Purification and Characterization of a Novel Myrosinase ( $\beta$ -Thioglucoside Glucohydrolase) from *Lepidium latifolium* L.**

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Glucosinolates and their hydrolysis products play crucial role in nutritional supplementation, chemoprevention and plant-insect interactions. Much of the biological activity of the hydrolysis products is governed by the quantitative and qualitative content of isothiocyanates. Apart from their spontaneous hydrolysis, myrosinase (EC 3.2.1.147) is mainly involved in the hydrolysis of glucosinolates to isothiocyanates, nitriles and thiocyanates and thus, is key for various health and ecological benefits. Myrosinase was investigated on various developmental stages in *Lepidium latifolium* L., a novel *phytofood* found in Ladakh Himalayas. All the expressed isozymes were characterized using LC-MS/MS and assessed for their redox regulations. Biotic interaction during those stages were also quantified to assess their relationship between the hydrolysis products. The constitutively expressed myrosinase was purified from the leaves to homogeneity in three step purification process. Purified enzyme exists as dimer in native form (~160 kDa) with a subunit size of ~70 kDa. The enzyme exhibited maximum activity at pH 6.0 and temperature 50 °C. With sinigrin as substrate, the enzyme showed  $K_m$  and  $V_{max}$  value of  $171 \pm 23 \mu\text{M}$  and  $0.302 \mu\text{moles min}^{-1} \text{mg}^{-1}$ . The enzyme was found to be redox regulated, with an increase in  $V_{max}$  and  $K_{cat}$  in the presence of GSH. Reduced forms of the enzyme were found to be more active. This thiol regulated kinetic behavior of myrosinase signifies

enzyme's strategy to finely tune its activity in different redox environments, thus, regulating its biological effects.

DFNSP15

**Proteomic Changes during Fruit Development and Ripening of Tomato Overexpressed with N-Glycan Processing Enzyme B-D-N-Acetylhexosaminidase**

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Fleshy fruit ripening is a coordinated developmental process that imparts nutritional and sensory qualities to fruits while altering their color, texture, aroma, and flavor. During ripening, degradation of cell wall N-glycoproteins and free N-glycan levels significantly influence softening in tomato.  $\beta$ -D-N-acetylhexosaminidase ( $\beta$ -Hex) is a cell wall located N-glycan processing enzyme and it breaks the glycosidic bonds between carbohydrates, as well as between carbohydrate and noncarbohydrate. The suppression of gene of this enzyme was resulted in enhanced shelf life of fruits without affecting plant growth, fruit development and yield. In order to further investigate the role of this enzyme, the over expression of  $\beta$ -Hex was carried out in tomato and transgenic plants were raised. The overexpression of  $\beta$ -Hex in tomato results in the reduction of fruit size, higher flowering and early ripening. The transgenic fruits were less harder and showed the sign of deterioration much early as compare to control. Further, identification and quantification of significant changes to the fruit proteome was carried out during developmental and ripening stages using high-throughput iTRAQ and high-resolution mass spectrometry. 1262 and 1082 proteins were differentially expressed during fruit development and ripening stages, respectively. During fruit development, 706 proteins were upregulated and 556 proteins were downregulated. In the fruit ripening stage, 583 proteins were upregulated and 515 were downregulated. The identified proteins are involved in hormonal

signaling, stress response, developmental process and ripening-related pathways including cell-wall metabolism, photosynthesis, oxidative phosphorylation, carbohydrate and fatty acid metabolism, protein synthesis, and processing. Moreover, affected protein levels were correlated with the corresponding gene transcript levels. The characterization of identified proteins and signaling pathways will help to elucidate the molecular mechanism during fruit development and ripening processes.

DFNSP16

### **Phytochemical and Proteomic Analysis of High Altitude Medicinal Mushroom: *C. Sinensis & G. Lucidum***

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High altitude medicinal mushrooms, *Cordyceps sinensis* (*C. sinensis*) popularly known as "Dong Chong Xia Cao" (winter worm summer grass) in China, India, Nepal and Bhutan and *Ganoderma lucidum* (*G. lucidum*), one of the most-prized herbs of Ayurveda also known as Bhu-Sanjivani. Is commonly known as "Lingzhi" in Chinese, "Reishi" in Japanese. Both the mushrooms have received considerable attention due to the presence of various biologically active compounds. Their medicinal effects such as immuno-enhancement, antioxidant, anti-aging, hepatoprotective, neurocardio protective effects etc. are well documented. Despite having reported health benefits and economic importance, the qualitative phytochemical analysis, proximate composition and proteome study of Indian isolates of these mushrooms have relatively a very little information available.

Here, for the first time we present the results of the proximate analysis of the crude powder of *C. sinensis* and *G. lucidum* in terms of various parameters such as moisture content, total ash,

protein, crude fat, crude fiber, carbohydrate, nitrogen and heavy metals etc. The preliminary qualitative analysis of crude powder as well as aqueous extract of *C. sinensis* and *G. lucidum* revealed the presence of alkaloids, carbohydrates, saponins, proteins, phenolic compounds, fat and gums. 1-D electrophoresis in crude powder as well as in the aqueous extracts indicated the presence of a wide range of proteins. Further, MALDI-TOF-TOF analysis showed the presence of characteristic proteins in both the medicinal mushrooms and Venn diagram demonstrated the distribution of proteins in these samples. In conclusion, the present study provides a comprehensive qualitative phytochemical analysis, proximate composition and proteome study on Himalayan isolates of *C. sinensis* and *G. lucidum* which could endorse its use as a functional food.

DFNSP17

### **Isolation of Oligopeptides from a High Altitude Medicinal Mushroom: *Ganoderma Lucidum***

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Natural sources including plants and mushrooms are repositories of metabolites such as glycolipids, terpenoids, flavonoids, polysaccharides and proteins. Such phytoconstituents are capable of imparting many beneficial effects like antiproliferative, antiinflammatory and nutraceutical effects. But these natural sources are known to contain certain other phytoconstituents too like peptides and nitrogenous compounds that have been lesser explored in terms of pharmacological action. In this study, an attempt to isolate and characterize bioactive oligopeptides from Himalayan variety of *Ganoderma lucidum* (GL), a medicinal mushroom prevalent in high altitudes of Asian regions was undertaken. GL has been known since the last many decades as an oriental medicinal herb bearing antioxidant, anticancer and performance enhancing actions.

A Sephadex G-25 based size exclusion chromatography procedure was followed for the separation of oligopeptides from GL. The

methodology was kept similar for GL fruiting body (GLF) and mycelium (GLM). Precisely, an extract of GL was prepared in Tris Cl buffer. This extract was loaded onto an appropriately equilibrated G-25 stationary phase. Elution was achieved using 0.1M NaCl buffer prepared in Tris Cl. The eluates were collected, pooled together basing on similar absorbance values as recorded at 220 nm and dried thereafter. Three unique fractions each for GLF and GLM were isolated. These fractions were tested for protein estimation. Characterization of all the above fractions by techniques like NMR and IR was done.

The presence of oligopeptides was found to be more predominant in GLM fractions compared to GLF. Lowry assay confirmed the maximal presence of peptides and smaller proteins in *Ganoderma lucidum* mycelium than fruiting body.

*Ganoderma lucidum*, well known for its medicinal attributes demonstrated richness in terms of oligopeptide contents. This study can be taken forward by directing the usage of GL peptides towards improvement of healthcare while using GLF and GLM fractions as potent nutraceuticals.

DFNSP18

### Pathogenes Related Class 10 Proteins: Molecular Insights and Potential Application in Abiotic Stress Tolerance

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Abiotic stresses such as salinity, drought, heat, cold and heavy metals are major constraints for sustainable agricultural production and food security. Studying plant responses to these adverse conditions can help in determining the strategies to combat them. A proteomic approach was used to compare protein expression between *Arachis hypogaea* callus cell lines adapted to salinity stress and control cell lines. A large number of

proteins were identified as pathogenesis related (PR) proteins belonging to class 10 and suggested that these differentially phosphorylated proteins play a crucial role in plant defense system against salinity stress. Full length PR10 was isolated (474 bp) from cell lines, and was named *AhSIPR10* (GenBank accession number: DQ813661)). Multiple sequence alignments pointed out phylogenetic affiliations of *AhSIPR10* with several other PR10 family members and presence of conserved class 10 specific protein motifs. Differential expression analysis of *AhSIPR10* using qPCR indicated rapid upregulation of *AhSIPR10* in peanut callus cell lines across salinity, heavy metal, cold and mannitol-induced drought stress environments. Likewise, *AhSIPR10* mRNA expression also responded to defense/stress signaling molecules, including salicylic acid (SA), abscisic acid (ABA) and methyl jasmonate. A functional validation of role of *AhSIPR10* protein in alleviating abiotic stress was carried through its over-expression in different transgenic systems. Overexpression of the *AhSIPR10* gene in transgenic tobacco and banana plants resulted in enhanced tolerance to salt and drought stress. These results indicated that the *AhSIPR10* protein played an important role in the defense mechanism of plants against multiple *abiotic stresses*. Further characterization of *AhSIPR10* gene and its regulation under other abiotic and biotic stress environments will enhance our understanding of the molecular cross-talk among various defense pathways.

DFNSP19

### Proteome Characterization of Copper Stress Responses in the Roots of Sorghum

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Copper (Cu), is an essential micronutrient for all living organisms, but at elevated concentrations, it

is extremely toxic to plants and can inactivate and disturb protein structures. To explore the molecular changes involved in copper stress response, the present study was conducted in the roots of sorghum seedlings. The morphological characteristics were reduced by Cu stress, and the most significant growth inhibition was observed in plants treated with a combination of the highest concentrations of Cu<sup>2+</sup> ions. High throughput two-dimensional polyacrylamide gel electrophoresis coupled with MALDI-TOF-TOF mass spectrometry was performed to explore the molecular responses of Cu-induced sorghum seedling roots. Two dimensional gels stained with silver staining, a total of 422 differential expressed proteins ( $\leq 1.5$ -fold) were identified using Progenesis SameSpot software. A total of 21 protein spots ( $\leq 1.5$ -fold) from Cu-induced sorghum roots were analyzed by mass spectrometry. Out of 21 differential expressed protein spots, a total of 10 proteins were up-regulated while 11 proteins were down-regulated. The abundance of most identified protein species from the roots, which function in stress response and metabolism was significantly enhanced while another protein species involved in transcription and regulation were severely reduced. Taken together, these studies indicate a good correlation among the morphological, ionic and proteome alterations in sorghum seedling roots exposed to excess copper.

DFNSP20

### Differential Protein Expression in Low-N Tolerant and Low-N Sensitive Maize Genotypes under Nitrogen-Deficiency Stress

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Nitrogen (N) is essential for proper plant growth and its application has proven to be critical for agricultural produce. However, for unavoidable economic and environmental problems associated with excessive use of N-fertilizers, it is an urgent demand to manage application of fertilizers.

Improving the N-use efficiency (NUE) of crop plants to sustain productivity even at low N levels is the possible solution. In the present investigation, contrasting low-N sensitive (HM-4) and low-N tolerant (PEHM-2) genotypes were identified and used for comparative proteome-profiling of leaves under optimum and low N as well as restoration of low N on 3<sup>rd</sup> (NR3) and 5<sup>th</sup> (NR5) days after re-supplying N. The analysis of differential expression pattern of proteins was performed by 2-D gel electrophoresis. Significant variations in the expression of proteins were observed under low N, which were genotype specific. In the leaf proteome, 25 spots were influenced by N treatment and 4 spots were different between the two genotypes. Most of the proteins that were differentially accumulated in response to N level and were involved in photosynthesis and metabolism, affirming the relationship between N and carbon metabolism. In addition to this, greater intensity of some defense proteins in the low N tolerant genotype was found that may have a possible role in imparting it tolerance under N starvation conditions. The new insights generated on maize proteome in response to N-starvation and restoration would be useful towards improvement of NUE in maize.

DFNSP22

### Global Proteome Profiling of Milkwheyin Indianzebu (Sahiwal)Cattle

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Milk is an important source of nutrition for human beings in their daily diet. Cow's milk is a major source of nutrition for humans composed of 3.5% protein consisting of 2.7% casein, 0.7% milk serum proteins and 0.1% MFGM protein. Milk proteins constitute high abundant proteins such as casein ( $\alpha$  s1-casein,  $\alpha$ -s2 casein,  $\beta$ -casein,  $\kappa$ -casein) and low abundant whey proteins. The whey proteins play important role as growth factors and in host defence mechanism against various pathogens. Sahiwal (*Bos indicus*) is a high milk producing breed of zebu cattle which are reared in north western region of India. The proteome of milk from *Bos taurus* has been

reported till date. However, there is no report on the milk proteome composition in Indian zebu cattle. Recent report suggests A1 variant in *Bos taurus* is found to be related to various allergies and other health ailments compared to A2 variant of  $\alpha$ -casein in zebu cattle. A more complete knowledge of the milk proteome of zebu cattle is an important consideration in dairy product manufacturing. Milk samples were collected from 10 indigenous Sahiwal cattle for profiling of whey proteins. Animals were selected from three stages of lactation viz. early, mid and late stages of lactation. Milk samples were defatted and whey was prepared by ultracentrifugation. Protein was precipitated using 10% TCA and 90% Acetone. A total of 500  $\mu$ g of protein was pooled from 10 whey samples from different stages of lactation followed by tryptic digestion and LC-ESI-MS analysis. MS spectra were searched in Mascot search engine for protein identification. A total of 1653 proteins were identified. The gene ontology analysis of identified proteins using Panther software revealed that majority of proteins are actively involved in catalytic, binding and structural molecular activities, cellular processes, localization, metabolic and immune system processes. Pathway analysis using KEGG and Cytoscape plugin in *clueGO* shows involvement of proteins in different metabolic pathways, antigen processing and presentation, complement and coagulation cascades. Some proteins were found to be related to apoptosis and viral carcinogenesis playing role in different signalling pathways such as PI3K-AKT, MAPK, AMPK/HIF-1 and HIPPO signalling pathway. Our future strategy involves identification of various host defence proteins of milk whey in different species of farm animals.

## Session XI- PTM Proteomics and Regulation Biology

DPPRB1

### Characterization of Thiol Redox Modifications of SnRK2.6-2C from *Brassica napus*

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Sucrose non-fermenting 1-related protein kinase 2.6 (SnRK2.6), aka Open Stomata 1 (OST1) in *Arabidopsis thaliana* plays a pivotal role in abscisic acid (ABA) mediated stomatal closure. Four SnRK2.6 orthologs were identified in *Brassica napus* genome in our previous work. In this study, one ortholog BnSnRK2.6-2C, which was highly induced by ABA treatment in stomatal guard cells at the transcription level, showed redox-regulated autophosphorylation activity *in vitro*. Autophosphorylation of BnSnRK2.6-2C was inhibited by oxidants S-nitrosoglutathione (GSNO) and oxidized glutathione (GSSG) in a dose-dependent manner, and the inhibition could be reversed by nonspecific or specific reductants. Phosphorylation sites on serine and threonine residues in the protein including the activation loop were mapped. Monobromobimane (mBBr) labeling was used for detecting and identifying redox modifications of cysteine residues in the BnSnRK2.6-2C. Interestingly, modifications were also found to be dose-dependent by GSNO but not GSSG. Mass spectrometer (MS) analysis revealed the unexpected mBBr labeling on tyrosine residues and the utility of mBBr as a stable thiol-label for evaluation of cysteine redox status. In this study, more cysteine modifications were identified than previously reported in literature. For examples, an irreversible modification sulfonic acid (trioxidation) was identified with high confidence, so was the cysteine glutathionylation, which was rarely found in plant proteins. Among all the six cysteine residues in BnSnRK2.6-2C, C159 was identified in

all the treatments with different modifications, suggesting it has high redox sensitivity and reactivity. Since it is the only cysteine in the activation loop of this kinase, redox regulation of C159 of the BnSnRK2.6-2C in the physiological context of stomatal functions will be further studied.

**Keywords:** BnSnRK2.6-2C, redox regulation, phosphorylation, thiol modification, monobromobimane, mass spectrometry

DPPR3

### Metabolic Engineering of Glycoproteins of Brain in Living Mice and Their Identification through Click Chemistry and Glycoproteomics.

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Among the four major classes of biological macromolecules namely, nucleic acids, proteins, lipids, and glycans, the study of glycoconjugates has been the most challenging due to inherent non-template driven biosynthesis, branching, complexity and diversity. In recent decades, metabolic glycan engineering (MGE), combined with bio-orthogonal ligation reactions, has emerged as a powerful methodology for identification, tagging, and imaging of glycoconjugates both *in vitro* and *in vivo*. Particularly, Bertozzi and coworkers have shown that peracetylated *N*-azidoacetyl-D-mannosamine (Ac<sub>4</sub>ManNAz, **1**) could be employed for the engineering of glycoconjugates in living animals. Treatment of mice with **1** resulted in the expression of *N*-azidoacetyl-D-neuraminic acid (NeuAz) on glycoproteins of various tissues such as heart, kidney, and liver. However, no expression was detectable in the brain possibly owing to the blood-brain barrier. Herein we show that a simple, yet powerful, strategy of carbohydrate-neuroactive hybrid (CNH) molecules was able to successfully access the central nervous system (CNS) across BBB and effect MGE of glycoproteins of the brain in

living mice. The CNH strategy facilitated the identification of brain glycoproteins susceptible to MGE through a click-chemistry based enrichment strategy. NeuAz-carrying sialoglycoproteins from mouse brain lysates were selectively reacted with agarose-beads carrying alkyne group using the copper (I) mediated alkyne-azide cycloaddition (Cu-AAC) reaction, along with appropriate controls. Glycoproteins proteins bound to the beads were trypsinized and the released peptides were analyzed by nano-LC-ESI-MS/MS for protein identification. We were able to identify 122 glycoproteins that are uniquely found in mice treated with CNH molecules compared to the controls. Further, treatment of the beads with PNGase-F resulted in the release of formerly *N*-glycosylated peptides. We believe that our CNH strategy might prove to be an enabling tool for the in-depth study of brain mapping as well as understanding the role of glycosylation in development and disorders of CNS.

DPPR4

### Integrated Nuclear Proteomic and Phosphoproteomic Reveals Chromatin Remodeling As a Reprogramming Modulator during *Magnaporthe* Invasion

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Reprogramming of plant translational machinery during patho-stress involves a dynamic rearrangement of the proteomic and phosphoproteomic landscape. To characterize this roadmap, we have performed nuclear protein and phosphoprotein profiling by gel based approach on unstressed and stressed three leaf-stage rice seedling invaded with *Magnaporthe griseae* at several time points. In total, 206 proteins and 132 phosphoproteins were identified using ESI-MS/MS. The study reported that subnuclear organelle involving chromatin, nuclear membrane and matrix were profusely affected during patho-stress. We identify an immune signature that define genotype specific response of fungus pathogen and encompasses proteins that function in nuclear morphogenesis, architecture formulation, transport and defense. Our data reflects cardinal features of

the immune response modulated by phosphorylation of key proteins during hemibiotropic fungus interaction. To characterize the mechanisms regulating immune or disease phenotype variation under patho-stress, we aimed to provide an integrated view of protein organization by co-expression network analysis. Our results showed the regulation of immune response in monocots is complex and regulated by the coordinated action of immunity related proteins and phosphoproteins. Remarkably, we note that a co-expression module corresponding to phosphorylation activation states is correlated with a differentially expressed functional protein module. These observations implicate that dysregulated immune responses leads to altered levels of phosphorylation state of proteins. Further, biological modules govern common or novel functionality for functional distinctions between disease or immunity. The identification of stress specific alterations will facilitate both the elucidation of the molecular pathophysiology and the development of improved diagnostic testing. These results reveal the crucial role that nucleus plays as a regulatory switch driving external perturbations. Further, our data defines novel molecular subgroups affected during invasion.

DPPRB5

### **Exploring Lysine methylome of *Plasmodium falciparum* to investigate molecular regulators of PTMs crosstalk at asexual blood stages**

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Despite continued efforts to control malaria; it still remains one of the leading causes of death worldwide. Sustained efforts are thus required to understand parasite biology in order to identify novel drug targets and develop new therapeutic

interventions against malaria. Emphasis has been emerging towards the post-transcriptional and post-translational regulation of the *Plasmodium* life cycle. Lysine methylation of histone proteins is well documented in different organisms, however in recent years; lysine methylation of proteins outside histone code is emerging as an important post-translational modification (PTM). We performed a global analysis of lysine methylation of *Plasmodium falciparum* proteins at asexual blood stages. Using anti-methyl lysine specific antibodies, immunoprecipitation was performed with parasite protein lysates at three blood stages. These antibodies extensively immunostained the parasites in a stage specific manner. Using LC-MS/MS analysis, a total of 570 lysine methylated proteins at three different asexual stages were identified with 605 lysine-methylated sites within 422 *Plasmodium* proteins. Functional classification of lysine-methylated proteins showed that these proteins represent diverse classes of cellular functions such as nucleotide metabolic processes, chromatin organization, transport, homeostatic processes and protein folding. Motif analysis of lysine-methylated peptides revealed the presence of new motifs in *Plasmodium* as compared to previously known motifs in other organisms. When compared with the existing dataset of phosphorylated and acetylated *P. falciparum* proteins; we found that 209 lysine-methylated proteins were also phosphorylated; 173 proteins were acetylated and 113 proteins possessed all three PTMs thus indicating extensive cross talks between various PTMs. Our findings suggest that the protein methylation at lysine residues is widespread in *Plasmodium* which along with other PTMs, seems to play important regulatory role in parasite development.

DPPRB6

### **A Parallel Pipeline for Mapping Post-Translational Modifications from Proteomics Data**

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Large scale identification of protein post-translational modifications (PTMs) from proteomics

mass spectrometry data is a huge computational challenge. The unanticipated modifications are searched using the unrestrictive (blind-PTM) searches, but these are time-consuming, need more compute-hardware and inherently have higher false positives and negatives in peptide identification and modification site localization. MODa is a fast tool for blind PTM searches but still needs massive time for searches. Execution becomes tedious with increasing data size. Using data-level parallelization to harness the power of multi-core processors, we have created a configurable, highly automated data-parallel pipeline, ModST, that reduces the runtimes for MODa significantly (1/5th to 1/12th) even on commodity work-stations, automatically parses, tabulates and integrates the results without requiring manual intervention. It performs parallelization on multiple-cores for any arbitrary number of files. It also integrates the results, calculates Å score and Å probability, performs modification mass correction which can correct for localization errors and provides tabulated results for ease of analyses. The fidelity of PTM identification increases manifold by these steps which help in removing false positive annotations. Veracity of pipeline was tested and confirmed by several experimental datasets. ModST is a versatile, portable and fast data-parallel blind-PTM pipeline that can harness even modest multicore computer hardware and is available at <https://sourceforge.net/projects/modst>

## The Disordered Human PTM-ome

**Pallavi Mahajan<sup>#</sup>, Suruchi Aggarwal<sup>#</sup>,**  
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(# equal contribution)

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Protein post-translational modifications (PTMs) are covalent modifications of protein residues that extend protein functions and regulate many cellular processes. Proteins with long disordered regions, referred to as intrinsically disordered regions (IDRs), have been recognized to be important in multiple roles owing to their flexibility in acquiring a structure based on biological context. Many proteins may be wholly disordered but still possess numerous biologically important functions. An association of these two widespread phenomenon has not been studied in detail. We downloaded the human proteome as per NextProt database (<https://www.nextprot.org/>), a curated human proteome set from Uniprot, and analysed this data for PTMs. In this study, we investigated the correlation between various PTMs and protein disorder. To associate intrinsic disorder in Nextprot database sequences with PTMs multiple disorder predictive tools DISOPRED, DISEMBL, IUPRED and GLOBPLOT were integrated and the combined results were compared with the DISPROT database. The PTMs were mapped on the defined disorder regions and categorized as disordered and non-disordered mapped PTM. Many PTM sites were found to occur in disordered regions. Further, results were also mapped for PTMs as per their presence in disorder-binding or non-binding regions for putative functional analysis. This analysis throws light on how disorder regions act as the incubation ground for PTM site evolution and rewiring of functional modification sites arise by selection.



## A PERSPECTIVE ON PROTEOMICS RESEARCH IN INDIA

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In this perspective article, we have tried to bring together the luminaries of Indian Proteomics community as well as emerging scholars who have started making an outstanding contribution. We sincerely appreciate all the scientists who have participated in this interview series and apologize for not being able to accommodate many of the other distinguished scientists in the Indian proteomics community to whom we could not contact due to the shortage of time while assembling this special issue. This article presents relevant aspects of steadily developing proteomics research in India and provides a glimpse of its promising future.



**Name:** Dr. Surekha M Zingde

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**Summary:** Dr. Surekha Zingde is President of the Proteomics Society, India. She retired as Dy. Director, Cancer Research Institute, ACTREC, Tata Memorial Centre, Kharghar, Navi Mumbai, in March 2013 after 33 years of service with the Centre. Her expertise is in Cancer Biology, with focus on oral cancer proteomics, chronic myeloid leukemia, membrane proteins and signal transduction. She has vast experience in setting up a research institute. She was Professor of Life Sciences of the HBNI. She is member of committees of the CSIR, DBT, BRNS and LTMT, reviewer for several journals and reviewer of thesis from different universities. Dr. Zingde is a visiting faculty at University of Mumbai-DAE Centre of Excellence in Basic Sciences, Mumbai, a member of the Research advisory board and Chairperson of the ethics committee of the National Burns Centre, Navi Mumbai and Chairperson of the Institutional committee for stem cell research of HiMedia Laboratories. She provides consultancy for life science research. Dr. Zingde is an Executive Committee member of Indian Women Scientists Association, Navi Mumbai, which has the mandate to take science to the public. She has 75 peer reviewed papers, several book chapters, a monograph and 4 patents to her credit.

### Interview

*Q1. What is the level of ease of using Proteomics over other approaches for answering key biological questions?*

Proteomics techniques have evolved over time. Initially simple biochemical techniques were employed to study and characterize proteins; now the basic principles of the simpler techniques have been amalgamated to generate several high end instruments which permit multiple analyses to be done either on the same sample or on different samples at one time thereby reducing variation in results arising from technical issues. While it is not exactly the ease that draws researchers to the various proteomics tools it is the fact that these tools now permit identification and characterization of a spectrum of proteins at one time and thereby provide an opportunity for global proteomic analysis of biological questions.

The study design for a biological inquiry actually dictates the choice of the proteomic tools to be used to address the question. Sample choice, the amount available, its processing and the technical parameters of the equipment necessary for the analysis would be some of the considerations which would determine the ease of using different proteomic tools. Availability of the equipment and proper training for their optimal use are other factors that determine the ease of using techniques like 2DE, DIGE (Gel based) and LC based mass spectrometric techniques (Gel free). To obtain useful results from any proteomics experiment, especially in the clinical scenario, one needs to ensure that a focused clinical question is addressed. Thus, the ease of integrating proteomic tools in biological research is dependent on the several aspects related to the overall experimental pipeline right from identifying a relevant biological question, sample collection to instrumentation and analysis.

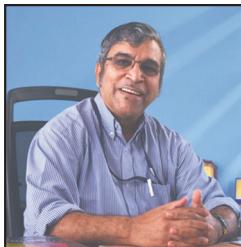
*Q2. What have been the major challenges in integrating Proteomics in your research over the years?*

The reason for integrating proteomics in my research on oral cancer was the underlying biological question to be addressed like risk assessment, diagnosis, prognosis, survival prediction, treatment response, etc and the need to identify and characterize a spectrum of proteins, instead of just one or two proteins. Further, the study design, which includes defining the type of sample to be obtained, amount of sample available for the study, sample processing techniques and availability of the appropriate instruments, all these determined the kind of proteomic approach to be used. The abundance of the protein(s) to be studied also played a major role in selecting the appropriate proteomic technique. Integrating proteomics into my research therefore depended largely on the availability of the right sample for the clinically relevant biological question of my interest, as well as the right proteomic tools and training to use these tools.

Investigations in proteomics of oral cancer initially addressed broader queries like differentiating between the normal and the diseased group, now they are being used to answer more complex and focused queries like understanding inter-grade variability, sub-site specificity, response to treatment, environmental factors, etc. The availability of highly specific and sensitive end point markers from proteomic studies is however still not significant. In spite of using the same approach, data emerging from different laboratories often do not match. The reason for this is majorly the variance in sample type, collection, storage and sample processing as well as the algorithms used for analysis of the large data sets. All these issues are now receiving attention so as to ensure that the emerging results are translatable in to the clinic.

*Q3. How has your association with PSI been over the years?*

I have been associated with the PSI since the time it came into existence since I am one of its founder members. My association has been in terms of contributing to the formulation of the guidelines of how it is going to run. I have seen it grow from a few 50 members when we started to more than 200 members from many institutions, at present. Each of the PSI meetings has now progressed to be large international meetings where PSI and its members are recognized by the global proteomic community. Some of the members of PSI Executive Council are also members of several other global organizations and it is through them that information about Indian proteomics research is spread worldwide in addition to the published literature. PSI members have been contributing to international proteomics projects as well, with Dr. Ravi Sirdesmukh on the International chromosome related proteomics project, Dr. Sanjeeva Srivastava on the proteomics of infectious diseases, Dr. Niranjan and Dr. Subhra Chakraborty on plant proteomics and the Institute of Bioinformatics group on the human proteome map. The platform that PSI has provided has not only helped members to interact with the international community, it has contributed to proteomics effort within the country as well. Many of the members have been organizing programs in and around their parent institutions thereby enabling transfer of knowledge about proteomics to the researcher interested in using proteomics tools or those seeking to learn state of the art high end methodologies which can provide a new dimension to their biological queries. PSI members have also facilitated proteomic inquiries in the country by providing access to their facilities to those who do not have them.



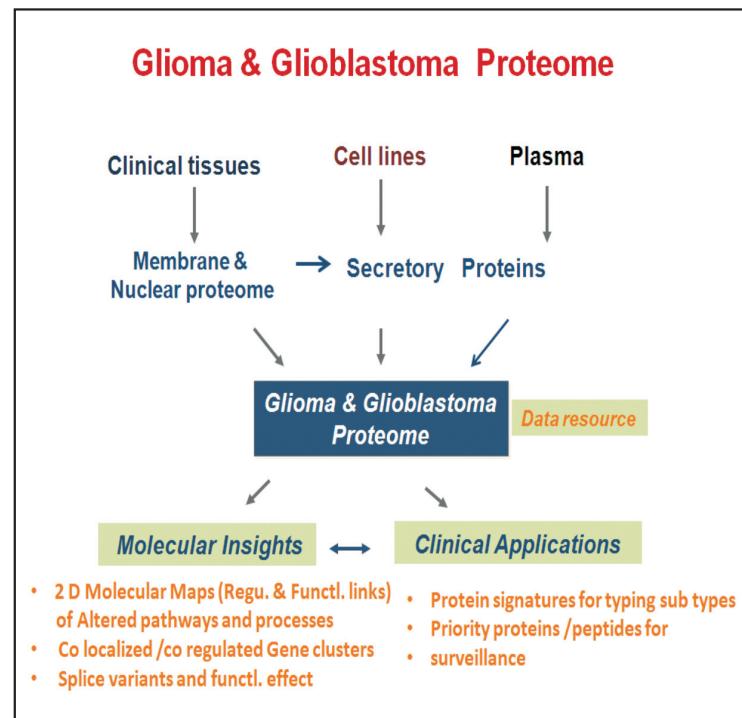
**Name:** Dr. Ravi Sirdeshmukh

**Designation and Institute:** Founder President, Proteomics Society (India);

Distinguished Scientist and Associate director, Institute of Bioinformatics, Bangalore; Principal Advisor, Mazumdar Shaw Medical Foundation and Center for Translational Research, Bangalore

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**Summary:** Dr. Ravi Sirdeshmukh received his Ph.D in Biochemistry from the Centre for Cellular and Molecular Biology (CCMB), Hyderabad where he later served as a Group Leader and Head, Proteomics Facility. His research interests span in the areas of protein and nucleic acid biochemistry. His earlier work on antitumor ribonucleases is one of the highly cited contributions in the field. He was responsible for establishing the first mass spectrometry-based Proteomics laboratory in the country at the CCMB, Hyderabad and initiated cancer proteomics in India. His current interest has been in the areas of Proteomics of gliomas – a major class of brain tumors and Head and Neck cancers. An expert reviewer of a number of Proteomic journals, member of the editorial boards and research review committees, Dr. Ravi Sirdeshmukh is the founder President of the Proteomics Society, India. He has been a council member of Human Proteome Organization (HUPO) and Asia Oceania HUPO for many years and currently leads Chromosome 12 Consortium of the Chromosome centric - Human Proteome Project (C-HPP) of HUPO.



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## Interview

*Q1. Over the years how has the field of Proteomics aided in the better understanding of Glioma pathobiology?*

Gliomas are the major primary tumors of the brain and glioblastoma is the most aggressive type, and one of the most lethal tumors known. They are also very complex tumors – both in terms of their pathophysiology and clinical management. The tumor has been analyzed at the genomic level to understand frequent mutations, copy number changes, complex rearrangements that may underlie the pathogenesis of this tumor. Emergence of high throughput technologies like RNA sequencing and mass spectrometry has permitted identification of differentially expressed genes at transcript and protein levels, respectively, using clinical specimens to delineate altered pathways, processes and interaction networks. The miRNA profiling has been done to understand the regulatory changes. In the past decade, these approaches have also been used with cerebrospinal fluid, and plasma from glioma patients to identify miRNA or protein signatures that may have diagnostic, prognostic and predictive potential. Our lab remains one of the major contributors of proteomics data to understand the pathobiology of these tumors and explore protein level changes for clinical applications. We have used clinical specimens to carry out in depth study of altered membrane and nuclear proteins of these tumors to identify altered molecular pathways and processes, major house-keeping proteins, metabolic enzymes as well as regulatory proteins such as transcription factors, splicing factors, receptors, etc. At least for glioblastoma, we have further supported this data with data from cell lines and plasma from patients. To enhance the strength and significance of the protein changes observed by putting them together in regulatory and functional dimensions, we have developed a pipeline to integrate gene expression data from transcript and protein levels with miRNAs which are key regulators of gene expression. With this we are now generating a snap shot of molecular changes connected in 2 Dimensions – longitudinal regulatory cascades linked in horizontal dimension to various cellular functions and anatomical / histological features that could be associated with the tumor. Thus we have developed a data resource of a large repertoire of proteins integrated to their regulatory links which enhances pathophysiological and molecular view of these tumors. Full clinical exploitation of this is, however, yet to happen. Clinical applications such as developing protein signatures for tumor sub typing or surveillance, identifying leads for new therapeutic strategies, are therefore being explored by us with high priority. In one such plan, with the potential plausibility of registering MRI signals to some of the anatomical features of the tumor and then molecular data to anatomical features, we hope to link molecular data with MRI and get the 3D view of the tumor in molecular terms which would be useful to develop novel strategies for pre or post- surgery treatment plan for the glioma patients.

Finally, a word on Glioma proteomics and C-HPP and B/D HPP Project – a global HUPO initiative. Chromosome-12 (Chr-12) is the target chromosome for India and the consortium of four other Asian countries. By mapping differentially altered protein genes to chr-12 loci, we could identify gene clusters that harbour in well characterized amplicon regions or chromosomal deletions. This implies co-regulation or de-regulation of genes in the cluster which are sometimes present on different chromosomes and opens up important questions about mechanism of intra or inter chromosomal regulatory cross talks and their modulations in the disease context.

*Q2. What have been some of the most challenging aspects in your journey in the field of proteomics?*

In the post genomic era, Proteomics emerged as an attractive platform for implementation in biomedical research and I had the privilege and responsibility of setting up what was considered as the first major proteomics facility in the country at the Centre for Cellular and Molecular Biology, Hyderabad, under a New Millennium Initiative (NMITLI). What I am writing below is an historical account – the “journey” that also connects to the roots of Proteomics Society, India. In those days, while Proteomics was a new entry, even protein mass spectrometry itself was not the in-thing in Indian biological research except for a few select labs using it for studying specific peptides or small proteins. So, in the absence of adequate technical experience and help around, and without any formal training in mass spectrometry, there was a challenge and also some nervousness while taking decisions. But a scientific approach, serious desire to learn and understand and more than that the strength of basic protein biochemistry I had, all prevailed and the high investment first full-fledged proteomics facility was up in 2001, with one of the best possible platforms available at that time. The bigger responsibility was to make the facility functional and use for the purpose it was meant for – cancer proteomics for biomarker and target discovery. The expectations from the investments were very high as we went through the learning curve, paused but stood firm and got the program moving. We also ensured that in this journey, we involved other prospective proteomics researchers from universities and research institutes in the country, through seminars, workshops, discussion sessions and some collaborations so that they would be better prepared when they have to stand on their own. It is important to mention that I enjoyed inspiration and full institutional support for my efforts from the then Director, Dr. Lalji Singh. The first Proteomics Conference and a Training workshop was organized at CCMB, Hyderabad in 2003 and was attended by international figures like Dr. Sam Hanash, Founder President, HUPO and Prof. Richard Simpson, the then President, AOHUPO and others. Seeing the potential in India, Sam engineered my inclusion and brought me on board with HUPO and Richard invited me to AOHUPO. My association with HUPO and AOHUPO starting from 2003, was of immense help in later years. I had the opportunity of interacting with the global community and to be close to evolving thoughts and processes in proteomics. Whenever required, I could seek and get help which I always tried to share with others in the country.

Implementing proteomics in research programs itself was a new experience and then doing it in the context of cancer and that too for brain cancers added exponentially increasing complexity to the next challenge. In the initial phase, the concepts were different and proteomics was being looked at as a mere analytical tool for “testing” clinical (or other) samples and generating case-wise data that could be consistently repeated. The science component was in the back seat. I remember the review committee warning me to focus on analysis and technical validations rather than getting into biological interpretations. But the basic scientist in me was not ready to accept it (and I was right and proven so in later years). Finally we published the first cancer proteomics paper from the country that was reviewed as technically sound and carried useful biological insights. That was the step which gave us the required jump-start and motivated us to dig deep later and also advocate the ideas to others.

Capacity building was something always close to my heart right from the beginning. It was an important requirement in proteomics research labs that came up later at several other institutes in the country. But how to achieve it in a broader scale was a challenge. Recognizing this need and its importance, the Council of Scientific and Industrial Research (CSIR) came forward to start a program entitled CSIR-Proteomics Network for International collaborations with decent financial support. Almost a dozen groups from across the globe readily consented to be part of the network and help. I had the privilege of managing it along with some of my other CSIR colleagues and we tried to use it effectively by facilitating scientists

and students attend international meetings, short term fellowships for training in other labs overseas and organizing conferences and workshops. As it often happens in India an initiative started by one visionary need not necessarily enjoy continuing support from the subsequent leadership. In the same way this very useful program started in 2007 as a 5 year program met its silent death in just 2 years when there was change in Guard at Delhi. However, there is often a silver lining written somewhere. In one international meeting (the last event) organized under the same program in 2008, sensing the danger ahead I and others mooted the idea of an alternative forum with a broader base and there was a whole hearted support from the entire community including the international members gathered then. That was the emergence of the **Proteomics Society** – an independent body officialised in 2009 and which took the growing proteomics mood in the country to a larger platform and it is heartening to see the society is doing overwhelming service. Today, proteomics activities in India have spread over a much larger portfolio of students and investigators; the contributions touch upon multiple areas of plant and animal kingdom and from basic biology to clinical research. No doubt this broad-spectrum activity will enrich the community immensely for future growth of Proteomics in the country and on the international front.

*Q3. How do you foresee the growth of Proteomics in the coming years?*

If we look back in retrospect over the past 15 years, the answer for the growth of proteomics in the coming years is evident. Proteomics today has taken a discrete position in the broader area of protein science – as said above from basic biology to human health. Mass spectrometry instrumentation and capabilities witnessed rapid development in the past decade that has no parallel in its recent history. New workflows and experimental designs are being explored. Targeted proteomics is emerging as a powerful alternative to unbiased shot-gun proteomics that has been in use currently. A rich antibody resource is now available to the community. Multi omics integration pipelines are being developed and can be extended to metabolomics – metabolites being the ultimate reflections of human physiology. The question, “what has proteomics given to medicine”, may not have a clear answer and strong claims at present but given all these positive developments on the analytical front and keeping a mindset aligned to biomedical and other societal applications and the prospects of important contributions to human health and disease are undoubtedly very high. What is important is to use the present power of proteomics methods and approaches to pursue good science with a persistent goal of solving the challenges of human health and disease.



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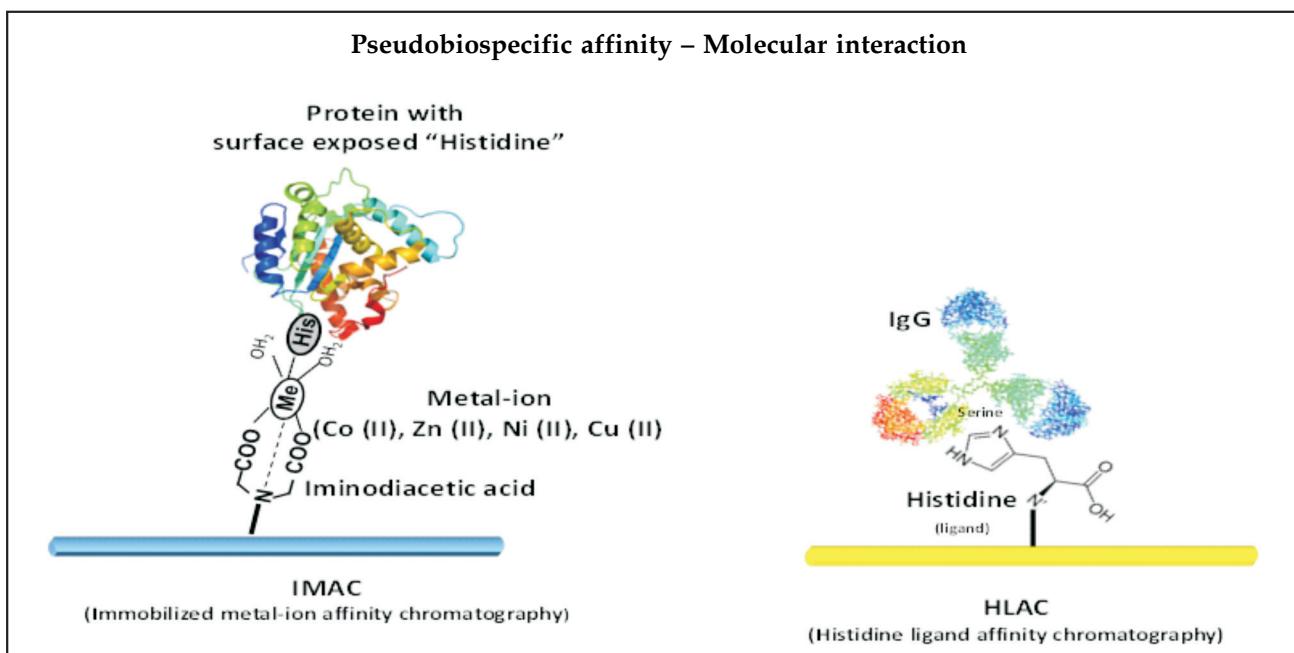
**Summary:** Prof. M.A. Vijayalakshmi with her basic formation in chemistry, from All India Institute of Chemistry, Calcutta in 1966, obtained a Ph.D from University de Bourgogne, Dijon, France in 1974 and then her D.Sc. from University de Technologie de Compiegne, France in 1980 combined with University of Uppsala, Sweden. She worked as a Research Engineer at CNRS, National Research Council Group, UTC, France from 1979 to 1990. She became a Full Professor in UTC in 1990, and in 2001 she became a Distinguished Professor in UTC (called Professor “Class Exceptional” in French) which is the highest level in the academic career in France. She is a Visiting Professor at University of Arizona, Tucson, USA (1991 to date) and Anna University, Chennai, India (1986 to date). She was also a Visiting Professor at Laval University, Quebec, Canada (1986 - 1987) and a Visiting Scientist at University of Wageningen, The Netherlands (1975) and at University of New South Wales, Sydney, Australia (1983). She was a Research Fellow at Uppsala University, Sweden, under a Franco-Swedish agreement for collaboration, from 1977 to 1983. She served as a Consultant in Biotechnology at VITU from 2001 to 2004. She is the base

for a collaborative agreement (MoU) signed between VITU and UTC covering all the fields and being implemented in a very active manner in the form of faculty exchanges. Her research interest lies in Biochromatography and Pseudo bioaffinity systems, Molecular Interactions, Biomedical application, Proteomics and Immunotechnology. Her list of achievements in this field with over 150 publications, 7 patents, is unparalleled. She also is a recipient of the prestigious "PIERCE AWARD" instituted by the International Society for Molecular Recognition (USA), in 1999 for outstanding achievements in the field of Affinity Technology and Bio-recognition at the International Level. She established the Centre for Bioseparation Technology, a high level R & D centre in the area of Separation Science and Technology. This centre is the first of its kind under "High Priority Research Area" related to industrial development set up in India in 2005 with the initiative of DST. In this context DST has solicited Prof. Vijayalakshmi to come back to set up this facility in India.

### **Profile and expertise of the laboratories in India and France which are established by her and she assumes the direction of.**

The Advanced Centre for BioSeparation Technology (CBST) is dedicated to the field of separation sciences and molecular interactions. It has a unique combination of being innovative and highly intellectual with keen interest in translation. This has been built as a policy and scientific culture.

This innovative research with high intellectual input has resulted in development of very original and simplified systems for both analytical and preparative aspects of proteins. These methods are complimentary/ competitive to conventional ones in efficiency and are being adopted by the industries both in India and abroad. This has made an important contribution for India both scientific and technological aspects to face the global challenges, resulting in products produced by Indian Industries contributing to its growth. This culture is successfully transferred to youngsters in India, in bringing a paradigm shift in the young researchers shaped at CBST.



### **Interview**

*Q1. How have several of the proteomic tools been paramount in analysis and separation of complex mixtures?*

Technological advances in the mass spectrometry field resulted in high sensitivity mass spectrometers like Thermo Fisher Orbitrap mass spectrometer which can detect even low abundant proteins, thus increasing the protein coverage. Understanding the molecular interactions is the key for success in pre-

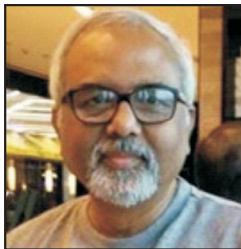
fractionation of molecules of interest. By introducing metal affinity (specific aminoacid residues like histidine in the peptides) separation method after trypsin digestion, the complexity of proteome can be reduced by trapping peptides with histidine residues and increases the protein coverage by analyzing both trapped and unbound peptides separately. This process also helps in the identification of low abundant proteins, as the complexity of the trypsin digested protein sample is reduced by ~15 fold. Phospho-peptide enriching can be done using metal affinity chromatography. Truncated, mutated and isoforms of the proteins can be separated based on their physicochemical characters. Prior information for targeted proteomics based work can be obtained from this approach. Thus, several advancement in the field have aided in separation technologies as well.

*Q2. What are the factors that you feel could have aided you in your pursuits in the field of bio separations?*

Development of Metal affinity chromatography methods for separation based on the different amino acids other than histidine would be also an interesting way to get more number of interactions. Another aspect that could have aided in answering key questions would be employing different strategies for digesting and denaturing proteins. Denaturation of proteins using their physicochemical properties rather than chemical reagents to improve the efficiency of protein digestion using proteases like trypsin could further improve the coverage. Availability of proteomics technologies was earlier an issue but now with almost every lab acquiring specialized mass spectrometric platforms we are in a much better position to do depth analysis of several critical biological queries.

*Q3. How has PSI as a promoter of Proteomics in India, evolved over the years?*

PSI has helped in identifying the potential scientists and collaborating with each other to share their expertise in the field of Proteomics. It also helped the scientific community in recognizing the importance of proteomics in understanding various biological questions/problems. Complementarities among the proteomics scientists and other scientific communities helped in the development of various technologies which resulted in the rapid growth of proteomics field.

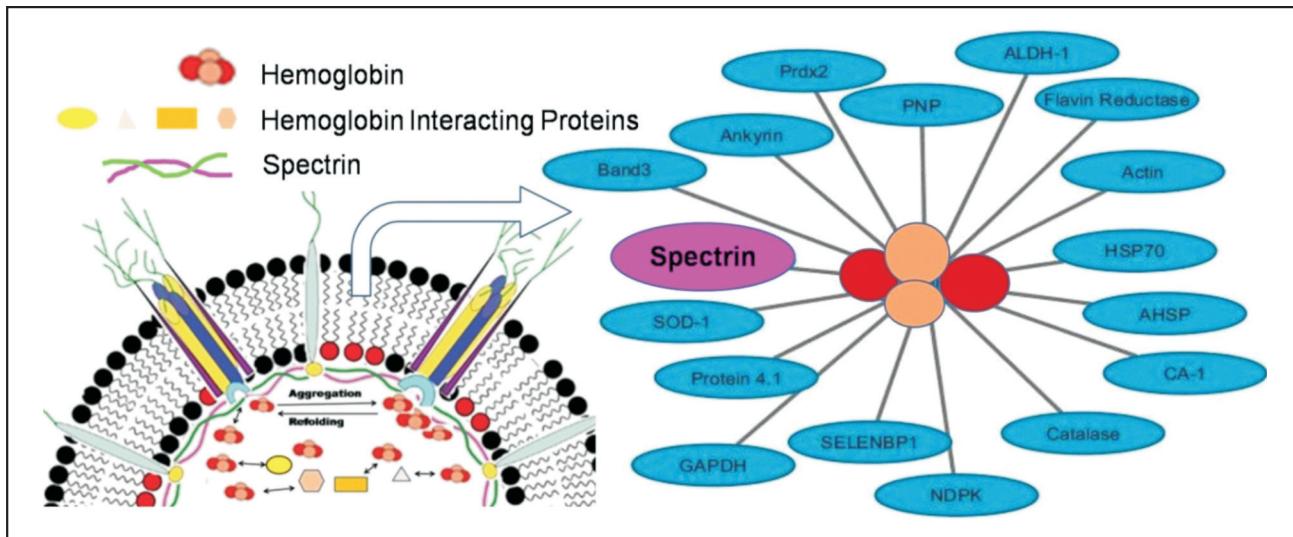


**Name:** Dr. Abhijit Chakrabarti

**Designation and Institute:** Senior professor and HoD of Crystallography & Molecular Biology Division, Saha Institute of Nuclear Physics, Kolkata.

**E-mail:** abhijit.chakrabarti@saha.ac.in

**Summary:** Dr. Abhijit Chakrabarti is currently working at Saha Institute of Nuclear Physics as a senior professor and Head of Department of Crystallography & Molecular Biology Division. Erythrocytes undergo ineffective erythropoiesis, hemolysis, and premature eryptosis in sickle cell disease and thalassemia. Abnormal hemoglobin variants associated with hemoglobinopathy lead to vesiculation, membrane instability, and loss of membrane asymmetry with exposal of phosphatidylserine. This potentiates thrombin generation resulting in activation of the coagulation cascade responsible for subclinical phenotypes. His work on the localization of aminophospholipid and anticancer drug binding site along with discovery of chaperone activity in erythroid and non-erythroid spectrin have been shown the involvement of membrane skeletal proteins, particularly spectrin in maintenance of membrane asymmetry and eryptosis. Clinical proteomics had proven to be an important field of research in studies of blood and blood diseases. Blood cells and its fluidic components have been proven to be easy systems for studying differential expressions of proteins in diseases e.g. hemoglobinopathies, different types of anemias, myeloproliferative disorders, and coagulopathies. Proteomic studies of erythrocytes and platelets reported from his group indicated redox regulators and chaperones implicated in the signaling networks.



## Selected Publications

1. Basu, A. and A. Chakrabarti (2015). "Hemoglobin interacting proteins and implications of spectrin hemoglobin interaction." *J Proteomics* 128: 469-475.
2. Basu, A., S. Harper, E. N. Pesciotta, K. D. Speicher, A. Chakrabarti and D. W. Speicher (2015). "Proteome analysis of the triton-insoluble erythrocyte membrane skeleton." *J Proteomics* 128: 298-305.
3. Chakrabarti, A., S. Halder and S. Karmakar (2016). "Erythrocyte and platelet proteomics in hematological disorders." *Proteomics Clin Appl* 10(4): 403-414.
4. Karmakar, S., D. Banerjee and A. Chakrabarti (2016). "Platelet proteomics in thalassemia: Factors responsible for hypercoagulation." *Proteomics Clin Appl* 10(3): 239-247.

## Interview

*Q1. How have the proteomics tools enabled better understanding of erythrocytes and haematological malignancies?*

Erythrocytes have been the focus of protein-based studies from the early eighties. The first major contribution using MS-based techniques came about in the year 2004 and then in 2006. Since then using latest proteomic techniques, the current erythrocyte protein count stands at about 2500. On one hand, the proteomic tools have helped better understanding of blood diseases providing robust high throughput data showing participation of numerous different interaction pathways. On the other hand, with faster advancement of MS-based technology, lots of protein-protein interaction networks have been drawn which had to be validated using conventional antibody-based techniques. As a consequence, proteomics could not stand as a discipline on its own as happened for genomics. I believe with increased sensitivity of detection, in future we could detect even very low copy number proteins and the list would expand.

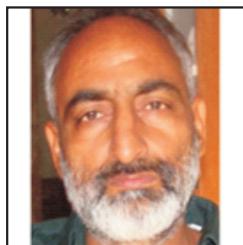
*Q2. What has been the most challenging aspect for integrating Proteomics in your research?*

Setting up of an advanced MS-based laboratory for proteomics in an Indian Institute or University is the most challenging. The second challenge comes in form of handling of the volume of data churned out by the modern mass spectrometers in the shortest period of time. It is also important to have a steady flow of supply of clean samples for mass spectrometry runs which in my opinion, not many labs are capable of producing. Companies who are selling mass spectrometers have to play an important role.

*Q3. How do you see proteomics impacting the current and future research community in India?*

I am not very hopeful with the prospect of proteomics as such, all by itself. However, proteomics has become an integral part of many labs which has no doubt increased the acceptability of MS-based omics techniques to biologists, in general. The proteomics labs have to maintain strong collaborations between themselves.

Not every protein labs should go for setting up of mass spec facilities. There has to be stronger collaborations between the conventional protein labs with those of proteomics labs. It does not look good when I see the impact factor of Molecular & Cellular Proteomics dropping down from 8.4 (in 2010) to 5.9 (in 2015).

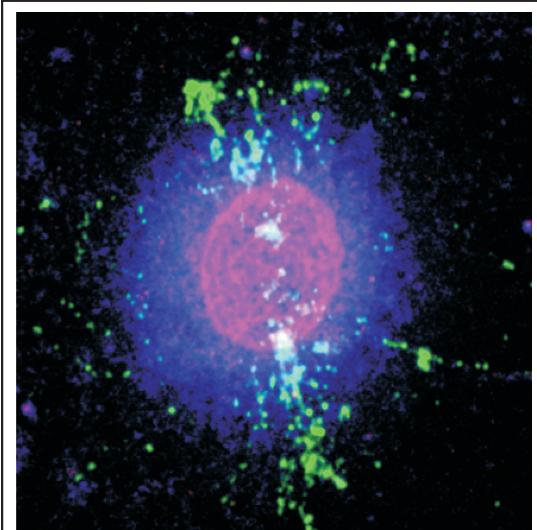


**Name:** Dr. Rakesh K Mishra

**Designation and Institute:** Director, CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad

**E-mail:** mishra@ccmb.res.in

**Summary:** Dr. Mishra received his Ph.D in Organic Chemistry in 1986 from University of Allahabad and started his carrier in biology by studying DNA structure at Molecular Biophysics Unit of the Indian Institute of Science, Bangalore, and, subsequently, effect of such structures on the regulatory functions at the Centre for Cellular and Molecular Biology, Hyderabad. He has studied antisense control of protozoan parasites at the university of Bordeaux, France, and mechanism of rRNA processing in frog eggs at Saint Louise University, USA. Studied body axis formation in fruit fly at the University of Geneva, Switzerland. He joined CCMB, Hyderabad in 2001 and studying how genetic information is stored and used in living systems using fruit fly and zebrafish as the experimental organisms. His current research interests are: how the genetic material, DNA, is packaged in the nucleus of the cells and how different cell types utilize genomic DNA in distinct manner. Dr Mishra is elected fellow of Indian Academy of Sciences, National Academy of Sciences, India, Andhra Pradesh Academy of Sciences, Indian National Science Academy and Telangana Academy of Sciences. Dr Mishra is also J C Bose National Fellow.



## Interview

*Q1. How has Proteomics been helpful for your studies on nuclear matrix proteins?*

Nuclear Matrix (NuMat) has been a biochemical preparation under scrutiny for some time. Like in many other areas of biology, NuMat field has also been hugely impacted by the technological revolution we are seeing in proteomics. In my lab, we have been able to explore the entire NuMat proteome in different cell types and in different experimental conditions. This has given a much needed confidence and boost to this very delicate biochemical preparation.

*Q2. How can Proteomics be integrated in mainstream molecular biology research for gaining deeper insight into several problems like that of epigenetics?*

Although not quite there, but we have been able to use proteomics approach to dynamics and evolutionarily conserved components of NuMat across the animal species, like we do using genomics approach. Although as efficient as genomics, but proteomics brings the additional value of functionality and mechanisms at molecular level in such high throughput comparative analyses. When we are able to compare differential proteome composition in different cell type of an organism or same cell type in different environmental context, it is actually the epigenetic consequence in a way. Large scale PTM analysis of chromatin constituents has greatly strengthened the field of epigenetics. More reliable high throughput

technology for proteomics and user friendly instrumentation will be great catalyst to take this line of research further.

*Q3. How do you see the involvement of India in pursuing various Proteomics based approaches?*

There are several centers where world class proteomics activity is already in place. Human genome variations that are exclusively from Indian population will provide fodder for proteomics mediated diagnostics/prognostics with major impact in medical field. In addition to this, I believe, India will contribute to the tool development and innovations in the area of proteomics.



**Name:** Dr. Harsha Gowda

**Designation and Institute:** Faculty Scientist, Institute of Bioinformatics, Bangalore and Centre for Systems Biology and Molecular Medicine, Yenepoya University, Mangalore.

**E-mail:** harsha@ibioinformatics.org

**Summary:** Dr. Harsha Gowda did his Ph.D at the Institute of Bioinformatics, Bangalore. During his Ph.D, he worked in Dr. Akhilesh Pandey's laboratory at Johns Hopkins University, USA on proteomic profiling of pancreatic cancers where his work involved proteomic approaches to study signaling pathways activated in pancreatic cancers and identification of novel biomarkers. In addition, he has worked as a visiting scientist in Dr. Gary Siuzdak's laboratory at Scripps Center for Metabolomics and Mass Spectrometry, USA. He is a recipient of Wellcome Trust-DBT Early Career Fellowship which is awarded for the most promising young researchers in India. He is also a recipient of Sir C.V. Raman young scientist award by the Karnataka state government. At IOB, he is employing cutting-edge technologies in genomics, proteomics and metabolomics to investigate biomarkers and therapeutic targets for various cancers. In addition, he is using proteogenomics methods to identify novel protein coding regions in the human genome. He has published more than 100 research articles in peer reviewed international journals. He is a reviewer for several international journals and an Editorial Board member of *Journal of Proteomics* and *Journal of Proteins and Proteomics*. He is a member of Indian Association for Cancer Research (IACR) and an elected executive council member of Proteomics Society of India (PSI).

## **Interview**

*Q1. How has several of the recent proteomic tools aided in your research in esophageal squamous cell carcinoma?*

Proteomics tools have tremendously impacted my research. I use quantitative proteomics tools extensively in my research. There is still room for significant developments in quantitative proteomics space and I look forward for those developments in the future.

*Q2. What have been the most challenging aspects of integrating proteomics in your research?*

Upgrading infrastructure to keep pace with the global developments in proteomics has been a challenge. As mass spectrometers are expensive, it can only be addressed if funding agencies are willing to allocate bigger amounts for this purpose.

*Q3. How would you like to see PSI expand to play a more prominent role in the Indian research community?*

There is a need for capacity building for proteomics in the country. This includes developing state-of-the-art infrastructure across a number of institutions in the country and trained human resource that is available for using and maintaining these facilities. PSI should work at the highest levels including funding bodies and ministries to work towards avenues that would enable these developments. A taskforce that looks into these aspects within funding agencies would be a good start. Access to proteomics tools and methods is still a major challenge within the country and it does not improve unless some immediate steps are taken towards these developments.



**Name:** Dr. Suman S. Thakur

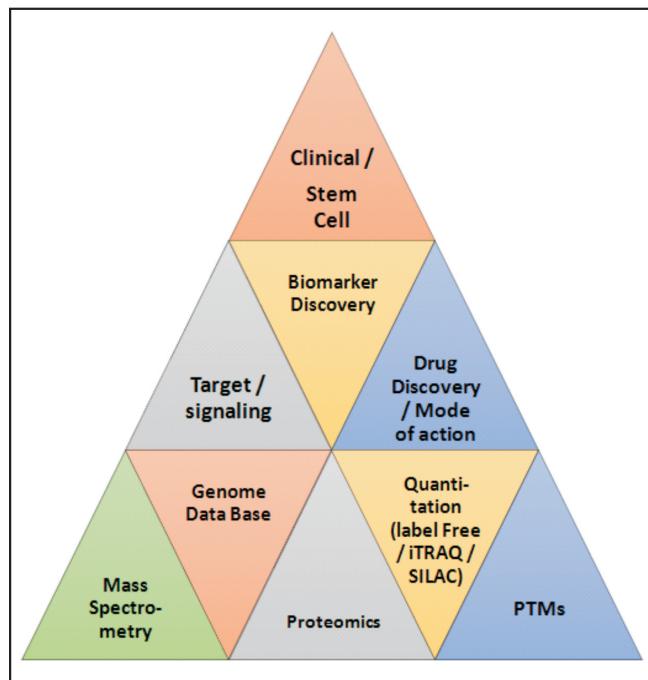
**Designation:** Senior Scientist

**Institute:** Centre for Cellular and Molecular Biology, Hyderabad, India

**E-mail:** sstccmb@yahoo.com

**Summary:** Dr. Suman S. Thakur has worked on Mass Spectrometry with Prof. P. Balaram at Molecular Biophysics Unit (MBU), Indian Institute of Science (IISc) Bangalore and on High throughput Quantitative Proteomics with Prof. Matthias Mann at Max Planck institute of Biochemistry (MPIB), Munich, Germany.

Currently, his lab at CCMB Hyderabad focuses on application of Mass Spectrometry in Health and Disease especially target to Drug Development. His group uses mass spectrometry based high throughput quantitative proteomics, natural product chemistry, cell biology, animal model, stem cell, and modern biology approach to find the target, develop novel drugs and elucidate its mode of action against cancer and other metabolic disorders such as diabetes. Cancer is a hyperplastic condition that leads to uncontrolled proliferation, invasion, metastasis, dysregulation of apoptosis and cell cycle. Cancer is the second leading cause of worldwide human mortality after cardiovascular diseases. They are investigating the efficacy of various natural metabolites on different human cancer cells like leukaemia, melanoma, and lung cancer. Further, the molecular basis for EGFR associated cancer progression, response and resistance to EGFR targeted therapy in lung cancer, and other cancers is also been investigated.



## Patent

1. Ravindranath V, Gupta A, Sehgal N, Jain SC, Thakur SS, Khanna P. WithaniaSomnifera plant extract and method of preparation thereof. United states patent, Patent No: US, 8,481,087 B2 9th July 2013

## Selected Publications

1. Chatterjee, B., A. Makarov, D. E. Clemmer, H. Steen, J. Steen, W. Saffell-Clemmer, A. R. Moghekar, C. Mohan Rao, R. A. Bradshaw and **S. S. Thakur** (2016). "Proteomics in India: A Report on a Brainstorming Meeting at Hyderabad, India." *Mol Cell Proteomics* 15(7): 2229-2235.
2. Chatterjee, B. and **S. S. Thakur** (2012). "Microbial profiling: extend ethnicity of human microbiome." *Nature* 487(7405): 39.
3. Das, G., D. K. Thotala, S. Kapoor, S. Karunanithi, **S. S. Thakur**, N. S. Singh and U. Varshney (2008). "Role of 16S ribosomal RNA methylations in translation initiation in *Escherichia coli*." *EMBO J* 27(6): 840-851.
4. Dokala, A. and **S. S. Thakur** (2016). "Extracellular region of epidermal growth factor receptor: a potential target for anti-EGFR drug discovery." *Oncogene*.
5. Gujjula, R., S. Veeraiah, K. Kumar, **S. S. Thakur**, K. Mishra and R. Kaur (2016). "Identification of Components of the SUMOylation Machinery in *Candida glabrata*: ROLE OF THE DESUMOYLATION PEPTIDASE CgUlp2 IN VIRULENCE." *J Biol Chem* 291(37): 19573-19589.

6. Halder, B., S. Singh and **S. S. Thakur** (2015). "Withania somnifera Root Extract Has Potent Cytotoxic Effect against Human Malignant Melanoma Cells." PLoS One 10(9): e0137498.
7. Mitra, S., G. S. Sameer Kumar, V. Tiwari, B. J. Lakshmi, **S. S. Thakur** and S. Kumar (2016). "Implication of Genetic Deletion of Wdr13 in Mice: Mild Anxiety, Better Performance in Spatial Memory Task, with Upregulation of Multiple Synaptic Proteins." Front Mol Neurosci 9: 73.
8. Sehgal, N., A. Gupta, R. K. Valli, S. D. Joshi, J. T. Mills, E. Hamel, P. Khanna, S. C. Jain, **S. S. Thakur** and V. Ravindranath (2012). "Withania somnifera reverses Alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver." Proc Natl Acad Sci U S A 109(9): 3510-3515.
9. **Thakur, S. S.**, T. Geiger, B. Chatterjee, P. Bandilla, F. Frohlich, J. Cox and M. Mann (2011). "Deep and highly sensitive proteome coverage by LC-MS/MS without prefractionation." Mol Cell Proteomics 10(8): M110 003699.

## Interview

*Q1. How has quantitative proteomics approach aided you in stem cell research?*

I am always curious to understand the programming especially how from one single cell to complete human body take the size and shape with such a great degree of perfection. In this era of development biology, stem cell is playing very important role especially having characteristic feature of pluripotency, self-renewal and differentiation ability. The quantitative proteomics has helped to understand the pluripotency in human embryonic stem cells (hESCs) especially level of expression of different proteins and transcription factor. This knowledge can be used as tool for therapeutics as regenerative medicine.

*Q2. What have been the biggest challenges in integrating proteomics in your research?*

Mass spectrometry is one of the best growing tools of this century. Unfortunately the high price of instrument is one of biggest challenges for not being used by common researcher. This is limiting proteomics to the big laboratories and Instrument(Central) facility in most of the institutes. The ample machine time is required for the success of many in-depth biological studies of clinical importance, and method development for contributing in the field of proteomics and mankind.

*Q3. How do you foresee the field of proteomics in next 10 years?*

Mass spectrometry based proteomics will be more popular and cost friendly thereby reaching to several biology lab including fundamental science, agriculture and clinical laboratories in next 10 years. The high resolution mass spectrometry would be more compact, with reduced size and with increase in resolution, speed and sensitivity. Mass spectrometry analysis has potential to replace antibody and other validation techniques but its success will depend upon low cost, low maintenance, robustness of the mass spectrometry instrument and its availability to common researcher.



**Name:** Dr. Srikanth Rapole

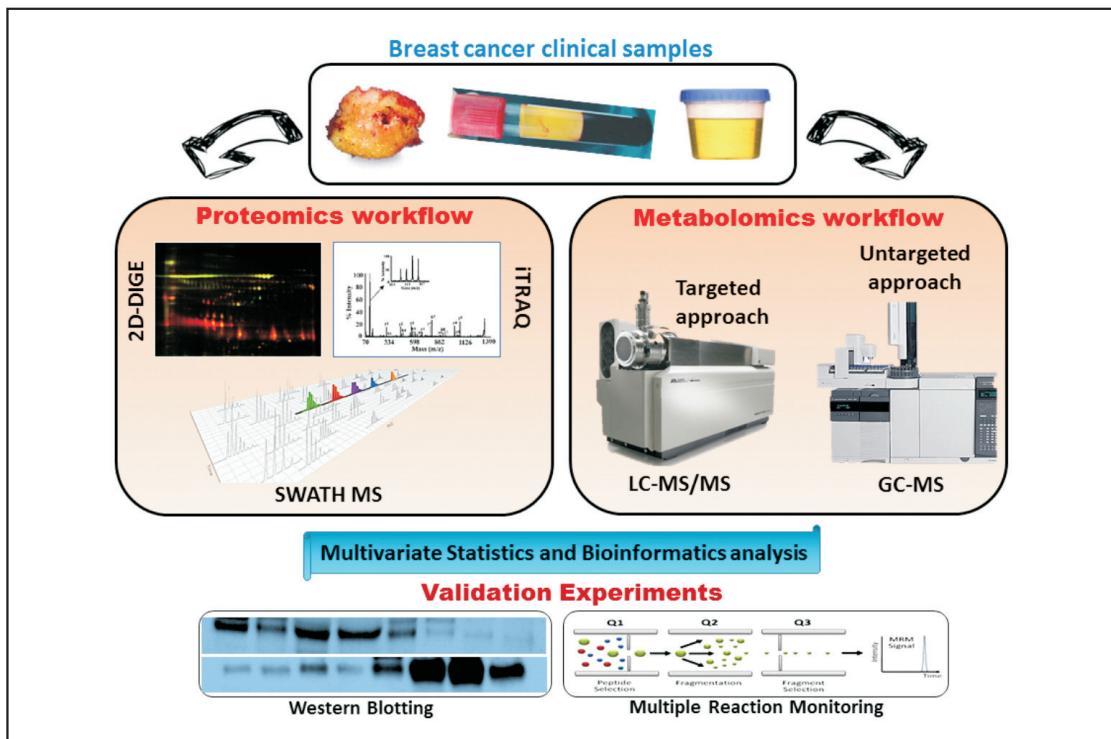
**Designation and Institute:** Faculty Scientist, National Centre for Cell Science, Pune.

**E-mail:** rsrikanth@nccs.res.in

**Summary:** Dr Srikanth Rapole completed his master's degree in organic chemistry from Devi Ahilya University. He did his PhD in analytical chemistry from Indian Institute of Chemical technology (IICT), Hyderabad where he developed new mass spectrometry methods for analysing beta-carbo-peptides. He did his post-doc from University of Massachusetts (UMASS) on metal protein interactions and protein-protein interactions to understand the mechanism of protein aggregation and amyloid formation. After that, he worked as proteomics and mass spectrometry lab director for two years at University of Connecticut. Currently, he is working as a scientist at NCCS, Pune. He is an active member of Indian

society for mass spectrometry, American society for mass spectrometry and proteomics society of India. He has published more than 50 publications in reputed international journals.

His main research interest is to investigate biomarkers and therapeutic targets for various cancers using state-of-the-art and highly sensitive mass spectrometry-based proteomic approaches. His group also investigating phosphoproteomic profiling and changes in phosphorylations for various cancers to understand phosphorylation mediated signaling pathways. In addition, his group is working to identify and quantify key metabolites and lipids associated with various cancers using mass spectrometry and bioinformatics.



## Selected publications

1. Gajbhiye, A., R. Dabhi, K. Taunk, G. Vannuruswamy, S. RoyChoudhury, R. Adhav, S. Seal, A. Mane, S. Bayatigeri, M. K. Santra, K. Chaudhury and **S. Rapole** (2016). "Urinary proteome alterations in HER2 enriched breast cancer revealed by multipronged quantitative proteomics." *Proteomics* 16(17): 2403-2418.
2. More, T., S. RoyChoudhury, K. Gollapalli, S. K. Patel, H. Gowda, K. Chaudhury and **S. Rapole** (2015). "Metabolomics and its integration with systems biology: PSI 2014 conference panel discussion report." *J Proteomics* 127(Pt A): 73-79.
3. More, T.H., M. Bagadi, S. RoyChoudhury, M. Dutta, A. Uppal, A. Mane, M. K. Santra, K. Chaudhury and **S. Rapole** (2016). "Comprehensive quantitative lipidomic approach to investigate serum phospholipid alterations in breast cancer." *Metabolomics* (In press).
4. Paul, D., V. Chanukuppa, P. J. Reddy, K. Taunk, R. Adhav, S. Srivastava, M. K. Santra and **S. Rapole** (2016). "Global proteomic profiling identifies etoposide chemoresistance markers in non-small cell lung carcinoma." *J Proteomics* 138: 95-105.
5. Pendharkar, N., A. Gajbhiye, K. Taunk, S. RoyChoudhury, S. Dhali, S. Seal, A. Mane, S. Abhang, M. K. Santra, K. Chaudhury and **S. Rapole** (2016). "Quantitative tissue proteomic investigation of invasive ductal carcinoma of breast with luminal B HER2 positive and HER2 enriched subtypes towards potential diagnostic and therapeutic biomarkers." *J Proteomics* 132: 112-130.

## Interview

*Q1. How would proteomics and metabolomics profiling of breast cancer patients help in obtaining diagnostically relevant markers?*

Breast cancer (BC) is one of the most heterogeneous types of cancer observed in women throughout the world. It has a tendency towards fair survival periods for patients, provided it has been diagnosed at an early stage of disease occurrence and the correct molecular subtype has been identified. Molecular biomarkers identified for specific subtypes of BC seem to provide a ray of hope in the early diagnosis as well as better treatment of BC. Towards this, proteomics and metabolomics based biomarker discovery studies have gained pace due to the technological advancements of mass spectrometry (MS) instrumentation. The marker proteins or metabolites are very low abundant in the samples to be analysed which could only be detected through high sensitive and ultra resolution MS instruments. The expression levels of these low abundant proteins and metabolites could enlighten our path towards finding biomarkers for early diagnosis of breast cancer. Various altered pathways due to BC can be determined through the powerful bioinformatics tools by subjecting the candidate proteins and metabolites obtained through advanced statistical treatments. This strategy could also be implemented towards the profiling of the onco-treatment response efficacy and the predictive capabilities of the treatment response for patients which is key step towards personalised medicine. To come up with diagnostically relevant markers for BC, the candidate proteins and metabolites signature should be validated in a large cohort of different BC sample sets. This is a huge challenge and we are working towards it with a hope that we can detect BC cancer early enough with precise subtype so that medical interventions could be implied on time and a life could be saved.

*Q2. What has been the most challenging aspects of keeping the proteomics facility functional at NCCS, Pune?*

The most challenging aspect of keeping a proteomics facility functional in any institute is the proper maintenance and handling of MS instruments which holds true for NCCS, Pune too. MS instruments are very sensitive to changes in environmental and physical parameters and thus these aspects should be maintained with strict adherence to the company recommended specifications. The maintenance and calibration of the instruments has to be performed by only well trained MS professionals. Another aspect is the proper operation of the instruments. In this regard, a SOP should be prescribed which has to be followed by the users. This enhances the operational hours and reproducibility of the experimental results. It is difficult to take comprehensive maintenance contact (CMC) for any institute every year due to the economic constraints. But it is necessary to have annual maintenance contract (AMC) every year which allows engineers to check performance and maintenance of the instrument twice a year. AMC is also helpful to replace spoiled consumables and tubings which increase the performance of mass spectrometer. Another key aspect is that MS technicians should work closely with application scientists and engineers to troubleshoot the problems and explore latest applications. These are the reasons for successful functioning of proteomics facility at NCCS. There are many more challenges which may seem very small issues but impact hugely in optimum function of a proteomics facility.

*Q3. How do you see Proteomics being integrated in clinical applications in future? What new approaches would you like to develop/incorporate in your current research to advance it further?*

Though proteomics has been there for a while at the research arena, it has evolved with tremendous potentials in the field of clinical applications in the last decade. This has been made possible with the developments and improvements of mass spectrometry technologies in terms of sensitivity, selectivity and resolution. One of the recent developments is targeted proteomics which allows validating candidate markers in a large cohort of samples. At present the future for proteomics being integrated in clinical applications looks a little bit cloudy, but with perseverance and a common goal of all the proteomics fraternity towards defeating deadly diseases is achievable in near future. Proteomics can play a significant role in early detection, planning and monitoring of treatment as well as personalised medicine for heterogeneous diseases like cancer.

More than 50% of breast cancer patients do not respond to chemotherapy and therefore the mortality rate increases. Our lab is currently working to identify drug resistant markers/targets for individual drug treatment and combination therapy. For that, we are focusing on modern proteomics approaches like SWATH based quantitative proteomics, SILAC based quantitative phospho-proteomics and targeted proteomics approaches to not only identify candidate biomarkers for multidrug resistance but also decipher the intriguing molecular mechanism of multidrug resistance.

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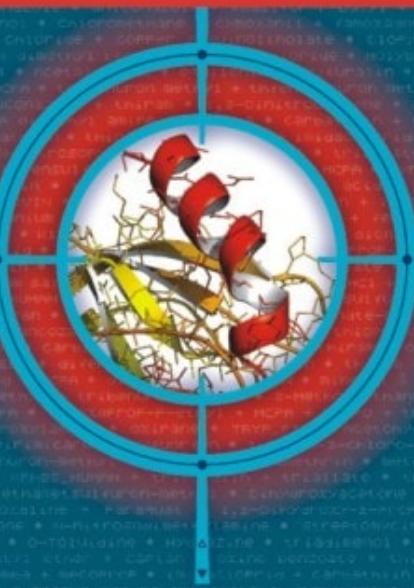


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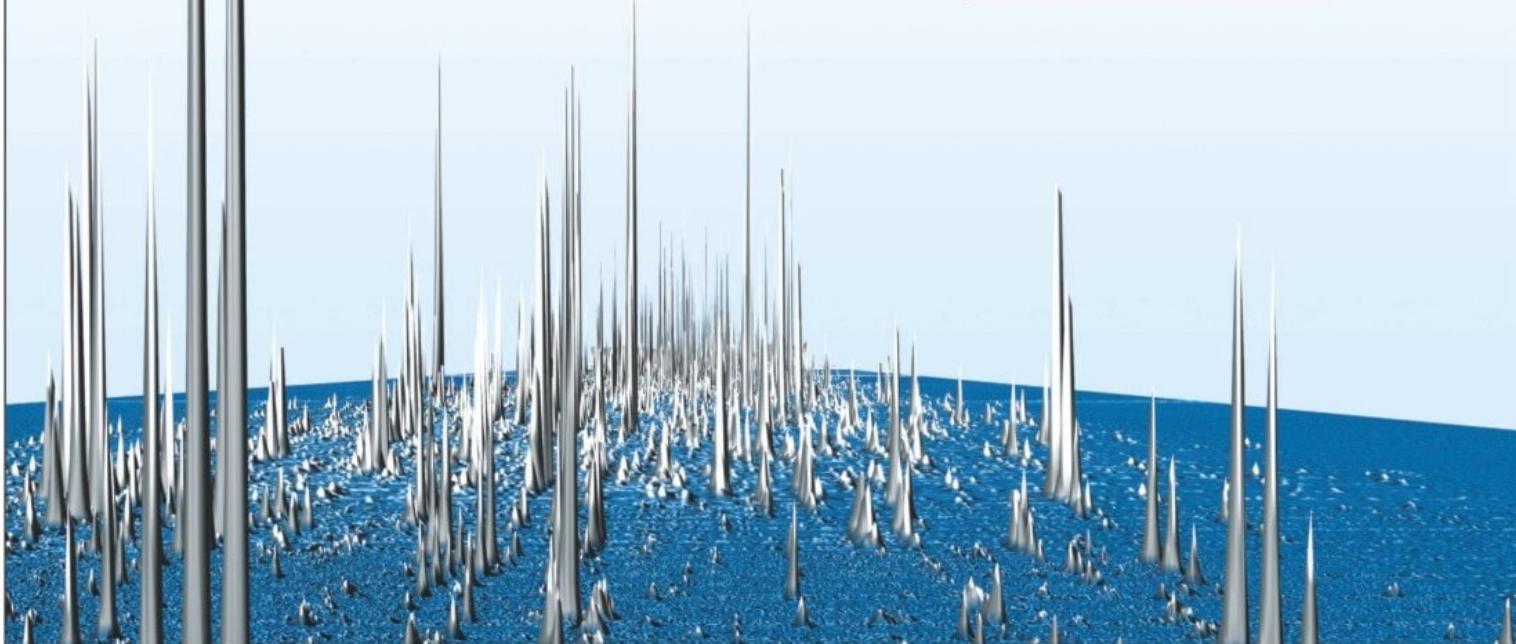
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# Proteomics International – your partner in Biomarker Discovery and Verification

## Biomarker Discovery Platform



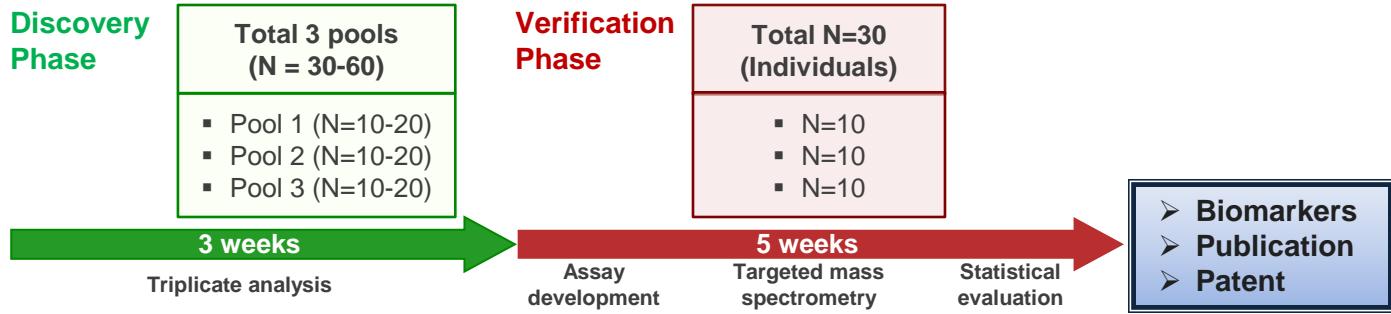
Proven, effective and proprietary platform

DIAGNOSTIC TEST OR MEDICINE

- The biomarkers market is vast. It is projected to grow from a value of **\$24.1 billion today to \$45.6 billion by 2020**
- Proteomics International (PI) has developed the **Promarker platform**, a proprietary mass spectrometry based technology platform for the **discovery and verification** of a panel of **diagnostic protein biomarkers**
- Using **Promarker platform**, PI has produced and validated a predictive test, **PromarkerD**, for the **diagnosis of diabetic kidney disease**<sup>1,2,3</sup>
- PI is vetting opportunities to employ its proven **Promarker platform** across other areas of unmet need to pursue **new diagnostic biomarkers**



## Biomarker Discovery and Verification Experiments



## Outcomes

- ✓ A panel of **diagnostic protein biomarkers**
- ✓ **Publication**
- ✓ **Patent application** for the diagnostic biomarkers
- ✓ Discovery and verification results → Advanced clinical study → **Diagnostic test**

## Sample requirement

- Plasma – Discovery phase: 50µL pooled sample (from 10-20 individuals; 2.5-5µL per individual)  
Verification phase: 50µL individual sample
- Other (e.g. cell lysate, plant, tissue) – Discovery phase: 100µg protein sample per replicate (3 x biological replicates)  
Verification phase: 100µg protein sample



Proteomics International

<sup>1</sup> Bringans *et al.*, (2012) AB Sciex Technote Publication number: 4250211-01.

<sup>2</sup> Patent "Biomarkers associated with pre-diabetes, diabetes and diabetes related conditions". Granted in Australia and USA. Accepted in Singapore. Pending in Brazil, Canada, China, Europe, India, Indonesia, Japan, Russia.

<sup>3</sup> Bringans *et al.*, (2015) Open Proteomics (Submitted).

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