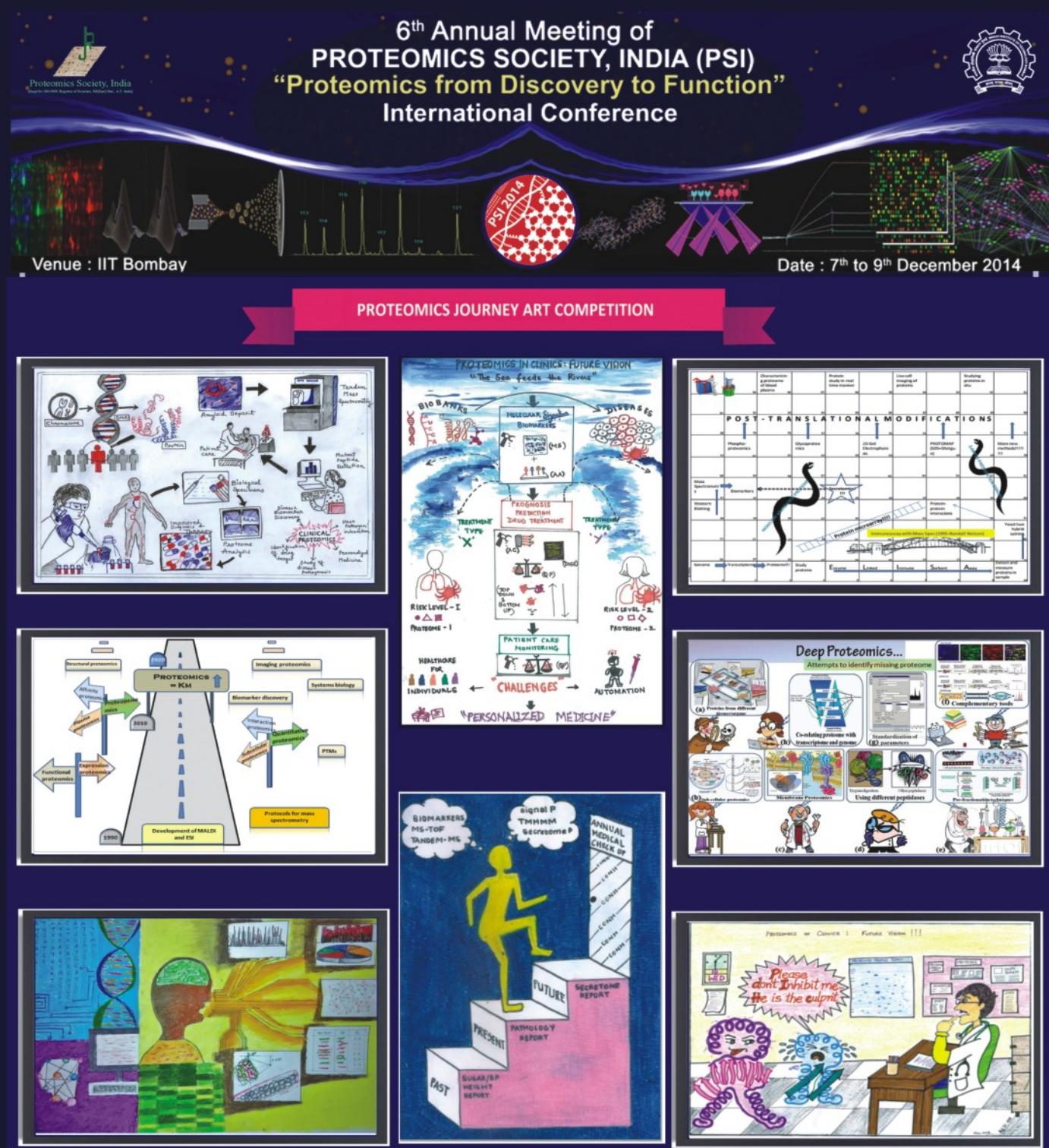


Journal of PROTEINS AND PROTEOMICS



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Volume 5 Number 3 2014



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6th Annual Meeting of
PROTEOMICS SOCIETY, INDIA (PSI)
"Proteomics from Discovery to Function"
International Conference

Proteomics Society, India
Society for the Advancement of Proteomics Research, IIT Bombay

Journal of Proteins and Proteomics

IIT Bombay

India

2014

7th to 9th December 2014

India

2014

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SPECIAL ISSUE ON

PROTEOMICS: FROM DISCOVERY TO FUNCTION

Guest Editor

DR. SANJEEVA SRIVASTAVA

Indian Institute of Technology Bombay, Mumbai

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Welcome Message by Convener and Guest Editor

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WELCOME TO THE THIRD SPECIAL ISSUE OF JPP AND FIRST SPECIAL ISSUE ON “PROTEOMICS”

The landmark (5th) year for JPP had begun well and is expected to end better. We had promised to bring out four issues this year (unlike 2-3 in previous years) and it is our pleasure to present to you here the third, with fourth in preparation. The joy and euphoria is overwhelming since this issue is the “**first special issue**” of **JPP on “Proteomics”** (the previous two were on protein folding and structural biology) and is being hosted on behalf of the esteemed Proteomics Society of India (PSI) for their 6th Annual Meeting in IIT Mumbai from December 7th to 9th, 2014.

We cannot thank PSI enough for this special issue. The president of PSI Dr. Surekha Zingde deserves special mention for this special moment for her benevolent help. Dr. Shantanu Sengupta, the secretary of PSI, had constantly encouraged us and went all out to bring in sponsors to print this issue. The moral support and the encouragement from all executive and life members of PSI has initiated an association between JPP and PSI, which we wish to see shape up into a fruitful long term one for further development and advance of the exciting art and science of proteomics. However, all this would have not been possible without the vision, dynamism, untiring efforts, active participation and magnificence of our Guest Editor of this issue, Dr. Sanjeeva Srivastava, who is also the organizer of the extravagant and all inclusive scientific feast (annual meeting 2014) of PSI in IIT Mumbai. It is his brain child and he has given shape to this special issue in more ways than I can mention.

Sponsors who partly helped meet our publications costs like ABSciex, Thermo-Fisher Scientific and Agilent are thankfully acknowledged. Dr. Sanjeeva Srivastava deserves special accolades for providing the remaining publication costs.

The special has been laid down in three parts. Part I consists of the details of the conference scheduling and programme. Part II hosts all abstracts of invited talks and posters, which is a wealth of information and an indicator of the progress proteomics has made over the last several years and a glimpse of the even brighter future that lies ahead of us. Part III of the special issue is a treasure for us to behold since it epitomizes history of proteomics in India through short and succinct articles – describing the beginning of PSI and proteomics research in India, its achievements in the recent past, the present and future activities and aspirations.

We hope you enjoy this special issue. We also seek to initiate a separate “Proteomics” section in the journal, given its significance to understanding biology in the current scenario, and wish to invite prominent PSI members and proteomics researchers to be a part of this initiative. We hope that the Indian scientific community of protein science and proteomics can look upto this journal with pride and belonging.

*Editor in Chief
Suman Kundu*

WELCOME TO THE 6TH ANNUAL MEETING OF PSI AND INTERNATIONAL PROTEOMICS CONFERENCE

Dear Colleagues,

The Proteomics Society, India (PSI) has been annually bringing together the proteomics community to encourage exchange of ideas, increase collaborations and enhance innovations at the National and International level. We are delighted to host the 6th Annual Meeting of PS(I) and International Proteomics Conference on “*Proteomics from Discovery to Function*” at the Indian Institute of Technology (IIT) Bombay from 7th – 9th Dec 2014. The Pre-conference event is scheduled on 6th Dec and post-conference workshops are scheduled on 10th – 11th Dec 2014.

The PSI-2014 Annual meeting and International proteomics conference is featuring many distinguished speakers, including Dr. Pierre Legrain (HUPO President), Dr. Mark Baker (HUPO President-elect), Dr. Gilbert Omenn (Ex-US HUPO President), Dr. Samir Hanash (Ex-HUPO President), Dr. Catherine Costello (Ex-HUPO President), Dr. Catherine Fenselau (Ex-President US-HUPO, ASMS), Dr. John Yates, Dr. Sudhir Srivastava, Dr. Brenda Andrews, Dr. Philip Andrew, Dr. Chung Ching Ming Maxey, Dr. Andrew Link, Dr. Robert L. Moritz, Dr. Juan Calvete and many distinguished scientists who will address the large gatherings.

Following the tradition of Proteomics Society, India to give an opportunity to the younger PSI members, Dr. Rapole Srikanth, Dr. Harsha Gowda and several others will give talks during the conference, and participants will also get an opportunity to listen to the PSI senior members Dr. Surekha Zingde, Dr. Ravi Sirdeshmukh, Dr. M. A. Vijayalakshmi, Dr. K. Dharmalingam, Dr. Akhilesh Pandey, Dr. Abhijit Chakrabarti, Dr. Rakesh Mishra, Dr. Utpal Tatu, Dr. Shantanu Sengupta and Dr. Mahesh Kulkarni among others, during the meeting.

The PSI-2014 in Mumbai will provide a unique opportunity to share your proteomics research with wide scientific community and a forum for the in-depth analysis of the challenges involved in studying the dynamic proteome. In addition to the highly stimulating scientific talks; the interactive sessions, panel discussions and Mumbai sightseeing activities are also scheduled in the pleasant weather of Mumbai in December. Your visit to Mumbai will provide a plethora of information on latest research in proteomics and the opportunity to network with fellow academicians, industrial researchers and editorial board members of prestigious journals. Have a stimulating, enjoyable and unique experience of this proteomics journey in 6th PSI conference.

Journal of Proteins & Proteomics is appreciated for publishing a special issue on “Proteomics” to present the conference proceeding and other details about PSI.

We are looking forward to welcome you to the beautiful campus of IIT Bombay!

Sincerely yours,
Dr. Sanjeeva Srivastava
Convenor, 6th PSI Meeting & International Conference
sanjeeva@iitb.ac.in
IIT Bombay, Mumbai
&
Guest Editor
Special Issue, Journal of Proteins and Proteomics

PREAMBLE- PROTEOMICS AND THE PROTEOMICS SOCIETY, INDIA

The advent of recombinant DNA technology in the early 70's moved the attention from protein science to genes and genomes. In early 2002, after a slumber of near three decades, Protein and Proteomic Research re-emerged as a Science requiring attention. This received impetus after the completion of the human genome sequence in April 2003. Since then, there are worldwide efforts to develop technologies for purification, identification, characterization, quantification, functional annotation of proteins, global protein profiling and generating a human proteome map. These include developments for mass spectrometry, bioinformatics and antibodies, which are now considered the three pillars of Proteomics.

In India too, the scientific community used global protein profiling for biological investigations since the late 1970s. The instrumentation used was however very primitive and the bioinformatic tools limiting or non-existent. The first consolidated efforts towards sophisticated proteomic research started in 2001-2002 with government funding bodies financing proteomic projects. These projects were then multi institutional to ensure that the state-of-art equipment established in major Institutes were made available to several academic centers. This ensured that the technical knowledge required to run the sophisticated instrumentation was available and the equipment were used optimally.

In 2009, several senior scientists felt the need to bring together the upcoming Proteomics community in India. The Proteomics Society, India (PSI) was registered in 2009 to provide the required forum. The Society emerged from the initiatives of Dr. Ravi Sirdeshmukh, Dr. M.A. Vijayalakshmi, Dr. Shantanu Sengupta and several others. Over the last five years we have succeeded in bringing together scientists, teachers and students from across the country through our Annual meetings, focused conferences, teacher training programs and workshops. Individual Scientists in laboratories equipped with the required instrumentation have provided the necessary expertise to colleagues across India in addressing their biological queries requiring proteomic tools.

These laboratories are also training centers for the younger upcoming proteomic scientist. The articles on PSI, the past Annual meetings and the meeting to come in 2015 provide a glimpse of the activities of the Society and the Proteomics research in India. The PSI Annual meeting scheduled for 2016 is to be a joint meeting with the Asia Oceania Agricultural Proteomics Organization (AOAPO) and it will be held in New Delhi. Our efforts have been to hold the meetings across India so as to increase participation from the local teachers and students and to expose them to the latest developments in Proteomics research. Each of the meetings has hosted pre-conference workshops in addition to those which are conducted independent of the Annual meetings.

It is apparent that the Society is acting as a node for international collaborations as well as an important forum for updating proteomics among the student and teacher community.

Proteomics has now emerged as an important tool for varied biological investigations in the country. Proteomics for Toxicology and Chemoprevention are the focus of investigations in the laboratories in Lucknow. The major hub for Plant and Microbial proteomics is in New Delhi and Agra. Serum, plasma, other biological fluids and tissue proteomics for several clinical conditions including cancer are receiving attention in New Delhi, Kolkata, Mumbai, Bengaluru, Madurai, Chennai and Pune. Sophisticated bioinformatic tools for proteomics are being developed in New Delhi and Bengaluru and separation technologies in Vellore. The IIT Bombay team has initiated the virtual proteomics forum for promoting proteomics through the internet. The multi-institutional efforts of the Institute of Bioinformatics group in Bengaluru have provided the first draft of the human proteome. Interestingly, proteomics is now a tool for dairy and veterinary research too. In parallel with proteomics, metabolomics is receiving immense attention in several laboratories. In many of the major Institutions, instrumentation and expertise near matches those in the West and Asia-Pacific. India's contribution to Proteomics is evident as we have representatives on the councils of the Human Proteome Organization (HUPO),

JPP 2

Asia Oceania Human Proteome Organization (AOHupo), International Plant Proteomics Organization (INPPO) and Asia Oceania Agricultural Proteomics Organization (AOAPO).

The abstracts of the invited lectures, oral and poster presentations at this meeting will provide a bird's eye view of Proteomics Research in India and abroad and the efforts of the Society in promoting its mandate as a forum for networking, exchange of expertise, teaching and technology transfer.

The 6th annual meeting of the PSI and the International Conference on Discovery to Function, at IITB has been possible with the concerted and persistent efforts of Dr. Sanjeeda Srivastava, the Convener and his team. The members of PSI Executive Council have provided mentorship to ensure that the meeting brings together the who's who in Proteomic Sciences worldwide.

Young scientists who are attending a PSI meeting for the first time will be happy to know that we are privileged to have the presence of the HUPO President and senior scientists who are involved in decision making for Proteomic programs globally. Their lectures will provide a view of the Proteomic scenario and the inter-institutional and inter-continental plans for deciphering the identity, function, location of the proteins coded for by the genome through the Biology/Disease Human Proteome Project, the Chromosome Centric Proteome Project and the Protein Atlas Project. The lectures during the conference are divided into related themed areas with presentations from our colleague scientists in India and those from abroad. The areas covered are targeted towards clinical and translational proteomics so that the title of Discovery to Function is aptly covered. Attention has also been given to advanced technologies which provide the tools for proteomic research. Increasing interest from industry is reflected in the inclusion of a full day session on Proteomics and Biosimilars; technological developments and regulatory aspects.

I take this opportunity to welcome you to this meeting on behalf of my colleagues on the Executive Council and all the members of PSI.

We hope that the interactions and discussions will lead to fruitful collaborations among the established scientists, while the younger members learn about the recent developments in Proteomics so that they can apply them to their research.

Dr. Surekha M. Zingde
President, Proteomics Society, India
[www.psindia.org;](http://www.psindia.org)
surekha.zingde@gmail.com

ORGANIZING COMMITTEE

Dr. Sanjeeva Srivastava (Convener, IIT Bombay, India), Dr. Surekha M. Zingde (President, Proteomics Society of India), Dr. Rapole Srikanth (NCCS, India), Dr. Bipin Nair (Amrita Vishwa Vidyapeetham, India), Dr. Rukmini Govekar (ACTREC, India)

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PROTEOMICS SOCIETY, INDIA (PSI)

Proteomics is taking an increasingly key position in biology and biomedical research. In India, many institutes and research groups have been using proteomics approaches in their research efforts and now we have a critical mass of proteomics scientists in the country. Over the last two decades, several new independent laboratories working in the area of proteomics have been established in Academic and National Institutes in India. As the research efforts expand and technologies evolve, it is important to facilitate interactions among the Proteomics community and help them share knowledge for the growth of this field. "**Proteomics Society, India**", is thus formed to provide a forum to pursue an agenda that will meet this objective. The Proteomics Society, India (PSI) has a mandate to promote and advance proteomics research through meetings and workshops to provide state of the art information to the proteomics community. PSI also meets the needs of the larger community of college teachers and students who teach and are considering use of proteomics by conducting focused workshops and seminars. The PSI is strongly dedicated to education and research in Proteomics. Even though it is still in its infancy, a lot of effort is being put in the direction of making this endeavour successful and useful.

The Executive Council of the society includes protein scientists from quite a few national labs and institutes already engaged in Proteomic research and we are further inducting representation from universities, industries and other organizations in the council. We encourage individual scientists, technologists, clinicians as well as corporate bodies to join the PSI as members and it is our desire to make the society most useful forum for techno-scientific and educational activities accessible to PhD students and scientists working in both academia and industry.

The Council invites all Proteomics researchers to join the society as members. For details of the Membership and benefits and more information please visit the PSI website: <http://www.psindia.org/index.html>

President: Dr. Surekha M. Zingde (Ex-ACTREC, India)

Vice President: Dr. Abhijit Chakrabarti (Saha Institute of Nuclear Physics, India)

Dr. Utpal S. Patra (Indian Institute of Science, India)

Secretary : Dr. Shantanu Sengupta (IGIB, New Delhi, India)

Treasurer: Dr. Rakesh Mishra (CCMB, India)



Proteomics Society, India

6TH ANNUAL MEETING OF PROTEOMICS SOCIETY, INDIA (PSI)

“PROTEOMICS FROM DISCOVERY TO FUNCTION”- INTERNATIONAL PROTEOMICS CONFERENCE

PRE-CONFERENCE: DEC 6, 2014 (SAT) PARALLEL SESSIONS

EDUCATION DAY

9:00 AM – 6:30 PM *Venue: Lecture Hall 21, Second Floor, Victor Menezes Convention Centre (VMCC)*

INDO-US BILATERAL WORKSHOP-“PROTEOMICS FOR TRANSLATIONAL RESEARCH”

8:00 AM – 6:30 PM *Venue: Board Room, Fourth Floor, VMCC*

CONFERENCE: DEC 7, 2014 (SUN)

7:30 AM – 8:00 PM *Venue: Auditorium, VMCC*

CONFERENCE: DEC 8, 2014 (MON) PARALLEL SESSIONS

PROTEOMICS SESSION

8:30 AM – 7:00 PM *Venue: Auditorium, VMCC*

BIOSIMILARS SESSION

8:30 AM – 7:00 PM *Venue: Lecture Hall 21, Second Floor, VMCC*

CONFERENCE: DEC 9, 2014 (TUE) PARALLEL SESSIONS

PROTEOMICS SESSION

8:30 AM – 5:00 PM *Venue: Auditorium, VMCC*

WORKSHOP BIOSIMILARS CHARACTERIZATION

8:30 AM – 7:00 PM *Venue: Seminar Hall 11, VMCC & CRNTS*

POST CONFERENCE WORKSHOPS: DEC 10 & 11, 2014 (WED & THU) PARALLEL SESSIONS

WORKSHOP 1: GEL-BASED PROTEOMICS *Venue: Seminar Hall 03, Ground Floor, VMCC*

WORKSHOP 2: MASS SPECTROMETRY *Venue: Seminar Hall 12, First Floor, VMCC*

WORKSHOP 3: TARGETED PROTEOMICS *Venue: Seminar Hall 13, First Floor, VMCC*

WORKSHOP 4: PROTEIN MICROARRAYS *Venue: Seminar Hall 14, First Floor, VMCC*

WORKSHOP 5: SURFACE PLASMON RESONANCE *Venue: Seminar Hall 11, First Floor, VMCC*

Indo-US Science and Technology Forum (IUSSTF) Workshop

6th DECEMBER, 2014

VENUE : INDIAN INSTITUTE OF TECHNOLOGY (IIT) BOMBAY, MUMBAI

Indo-US Science and Technology Forum

Proteomics for Translational Research

Indo-US Workshop



Agenda

Biomarker discovery prospects & challenges

Technological advancements & data analysis

Development of validation assays & clinical applications

Speakers:

Dr. Akhilesh Pandey
John Hopkins University

Dr. Andrew Link
Vanderbilt-Ingram Cancer Centre

Mr. Brendan MacLean
University of Washington

Dr. Catherine Fenselau
University of Maryland

Dr. David C. Muddiman
North Carolina State University

Dr. Gilbert Omenn
University of Michigan

Dr. John Yates
The Scripps Research Institute

Dr. Robert L. Moritz
Institute for Systems Biology

Dr. Samir Hanash
M.D Anderson Cancer Research Center

Dr. Sarath Chandra Janga
Indiana University School of Medicine

Dr. Sixue Chen
University of Florida

Dr. Sudhir Srivastava
National Cancer Institute

Dr. Yetrib Hathout
Children's Research Institute,
Center for Genetic Medicine Research

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Amrita University

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Institute of Bioinformatics

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Dr. Rapole Srikanth
National Centre for Cell Science

Dr. Ravi Sirdeshmukh
Institute of Bioinformatics

Dr. Shantanu Sengupta
Institute of Genomics and Integrative Biology

Dr. Suman Thakur
Centre for Cellular and Molecular Biology

Dr. Surekha M. Zingde
Ex-ACTREC

Dr. Utpal S. Tatu
Indian Institute of Science



INDO-US BILATERAL WORKSHOP
"PROTEOMICS FOR TRANSLATIONAL RESEARCH"

Venue: Board Room, Fourth Floor, VMCC

Indian Coordinator	Dr. Sanjeeva Srivastava, <i>Indian Institute of Technology Bombay, India</i>
US Coordinator	Dr. Philip Andrews, <i>University of Michigan, USA</i>
8:00 – 8:15 AM	Registration
8:15 – 8:30 AM	Welcome and Introductory speech about the workshop by the coordinators
8:30 – 1:00 PM	Brief talk by US and Indian speakers: Specific research interests and new avenues for Indo-US collaborative research
1:00 – 2:00 PM	Lunch Break
2:00 – 3:30 PM	Parallel break-out and panel discussion sessions

PANEL I: BIOMARKER DISCOVERY PROSPECTS AND CHALLENGES

Venue: Board Room, Fourth Floor, VMCC

Chairs: Dr. Abhijit Chakrabarti (*Saha Institute of Nuclear Physics, India*) &
Dr. Sudhir Srivastava (*National Cancer Institute, USA*)

Panelist: Dr. Gilbert Omenn (*University of Michigan, USA*), Dr. Kumaravel Somasundaram (*Indian Institute of Science, India*), Dr. Samir Hanash (*MD Anderson Cancer Center, USA*), Dr. Sanjeeva Srivastava (*Indian Institute of Technology Bombay, India*), Dr. Shantanu Sengupta (*Institute of Genomics and Integrative Biology, India*), Dr. Surekha Zingde (*Ex-Advanced Centre for Treatment, Research and Education in Cancer, India*), Dr. Yetrib Hathout (*Children's Research Institute, USA*)

PANEL II: TECHNOLOGICAL ADVANCEMENTS IN PROTEOMICS AND DATA ANALYSIS

Venue: Conference Room, Fourth Floor, VMCC

Chairs: Dr. David C. Muddiman (*North Carolina State University, USA*) &
Dr. Mahesh Kulkarni (*National Chemical Laboratory, India*)

Panelist: Dr. Andrew Link (*Vanderbilt University School of Medicine, USA*), Dr. Arun Trivedi (*Central Drug Research Institute, India*), Mr. Brendan MacLean (*University of Washington, USA*), Dr. Philip Andrews (*University of Michigan, USA*), Dr. Rapole Srikanth (*National Centre for Cell Science, India*), Dr. Ravi Sirdeshmukh (*Institute of Bioinformatics, India*), Dr. Robert L. Moritz (*Institute for Systems Biology, USA*), Dr. Sarah Chandra Janga (*Indiana University and Purdue University, USA*), Dr. Suman S. Thakur (*Centre for Cellular and Molecular Biology, India*)

PANEL III: DEVELOPMENT OF VALIDATION ASSAYS AND CLINICAL APPLICATIONS OF PROTEOMICS

Venue: Seminar Hall 11, First Floor, VMCC

Chairs: Dr. John Yates (*The Scripps Research Institute, USA*) &
Dr. Utpal S. Tatu (*Indian Institute of Science, Bangalore, India*)

Panelist: Dr. A. Srinivasan (*All India Institute of Medical Sciences, India*), Dr. Akhilesh Pandey (*John Hopkins University, USA*), Dr. Bipin Nair (*Amrita University, India*), Dr. Catherine Fenselau (*University of Maryland, USA*), Dr. Harsha Gowda (*Institute of Bioinformatics, India*), Dr. K. Dharmalingam (*Aravind Medical Research Foundation, India*), Dr. M. A. Vijayalakshmi (*VIT University, India*), Dr. Rakesh Mishra (*Centre for Cellular and Molecular Biology, India*), Dr. Sixue Chen (*University of Florida, USA*)

3:30 – 4:30 PM	Preparation of presentations by individual panels
4:30 – 4:45 PM	Tea break
4:45 – 5:30 PM	Presentations by individual panels
5:30 – 6:00 PM	Recommendations and conclusive remarks

Pre-Conference Event: Education Day

6th Annual Meeting of Proteomics Society, India

“Proteomics from Discovery to Function”

Welcome Address



Dr. Surekha Zingde
(President PSI)

Proteomics Society, India (PSI)
6th Annual Meeting
EDUCATION DAY
6th December, 2014



IIT Bombay

Coordinators

Introduction to Education Day



Dr. Ravi Sirdeshmukh
(Institute of Bioinformatics)

GEL-BASED PROTEOMICS



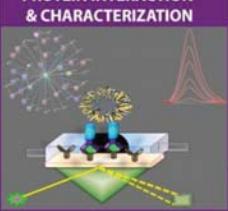
Dr. Jens Coorsen (University of Western Sydney)	Dr. Niranjan Chakraborty (National Institute of Plant Genome Research)	Dr. Subhra Chakraborty (National Institute of Plant Genome Research)
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MASS SPECTROMETRY



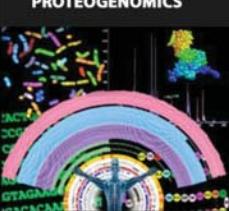
Dr. P. Babu (National Centre for Biological Sciences)	Dr. Ravi Krovidi (Agilent Technologies)
--	--

PROTEIN INTERACTION & CHARACTERIZATION



Dr. Ed Nice (Monash University)	Protein purification & characterization Demonstration
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PROTEOGENOMICS



Dr. Debasish Das (Institute of Genomics and Integrative Biology)	Dr. Keshav Prasad (Institute of Bioinformatics)
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Discussion and Interaction Session



Dr. Sanjeeva Srivastava
(IIT Bombay)

Vote of thanks

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**Pre-Conference Event: Education Day | 6th Annual Meeting of
Proteomics Society, India “Proteomics from Discovery to Function”**

Venue: Lecture Hall 21, Second Floor, Victor Menezes Convention Centre (VMCC), Indian Institute of Technology Bombay, Powai, Mumbai 400 076, India

Coordinators	Dr. Niranjan Chakraborty & Dr. Subhra Chakraborty (<i>National Institute of Plant Genome Research</i>)
9:00 – 9:20 AM	Registration
9:20 – 9:30 AM	Welcome and Introductory speech about the workshop by PSI President
9:30 – 9:40 AM	Introduction to Education Day by Dr. Ravi Sirdeshmukh

SESSION I: GEL BASED PROTEOMICS

9:40 – 12:10 PM	Talk by Dr. Jens Coorssen (<i>Quantitative top-down proteomics: A high resolution 2D gel-based approach</i>) Talk by Dr. Niranjan Chakraborty (<i>Application of nuclear proteomics: Current status and future prospects</i>) Talk by Dr. Subhra Chakraborty (<i>Analysis of extracellular matrix proteome and phosphoproteome: extending its function</i>)
11.10-11.40 PM	Tea Break

SESSION II : MASS SPECTROMETRY

12:10– 3:30 PM	Talk by Dr. P. Babu (<i>Evaluation & Basics of MS</i>) Talk by Dr. Ravi Krovidi (<i>Targeted phosphoproteomics analysis of Immunoaffinity enriched tyrosine phosphorylation in mouse tissues</i>) Parallel Hands-on and Demo Sessions for Gel Based and Mass Spectrometry based Proteomics (Venue: CRNTS, IIT Bombay)
1.30-2.15 PM	Lunch Break

SESSION III: PROTEIN INTERACTIONS AND LABEL FREE

3:30 – 4:45 PM	Talk by Dr. Ed Nice (<i>Protein-Protein interactions and their characterization</i>) Demo Session by BIA Separations (<i>Process analytical technology aided isolation of IgG from Cohn (I+II+III) paste</i>)
4.45-5.05 PM	Tea Break

SESSION IV: PROTEOGENOMICS

5:05 –6:05 PM	Talk by Dr. Debasis Dash (<i>Estimating the false discovery rate: applications in proteogenomics</i>) Talk by Dr. Keshava Prasad (<i>Integrating transcriptomic & proteomic data for accurate assembly and annotation of genomes</i>)
6:05 – 6:25 PM	Vote of Thanks by Dr. Sanjeeva Srivastava
6:25 PM	Discussion and interaction

** The detailed program for Education Day is provided in a separate booklet.

**6th Annual Meeting of
PROTEOMICS SOCIETY, INDIA (PSI)
“Proteomics from Discovery to Function”
International Proteomics Conference**

Venue : IIT Bombay **Date : 7th to 9th December 2014**

FINAL ANNOUNCEMENT

<p>KEYNOTE ADDRESS</p> <p>Dr. Pierre Legrain HUPO President</p> <p>Dr. Surekha Zingde President, PS(I), India</p>	<p>PLENARY SPEAKERS</p> <p>Main Conference</p> <p>Dr. Catherine Costello Ex-HUPO President-Boston University, USA</p> <p>Dr. Catherine Fenselau University of Maryland, USA</p> <p>Dr. Gilbert Omenn University of Michigan, USA</p> <p>Dr. John Yates The Scripps Research Institute, USA</p> <p>Dr. Mark S. Baker HUPO President-Elect Macquarie University, Australia</p> <p>Dr. Samir Hanash M.D. Anderson Cancer Center, USA</p>	<p>Biosimilars</p> <p>Dr. Anurag Rathore Indian Institute of Technology, Delhi, India</p> <p>Dr. Gary Kruppa MRM Proteomics Inc, Canada</p> <p>Dr. Mike Boyne US-FDA</p>
 <p>Dr. Sanjeeva Srivastava Convener</p>		
<i>Welcome to Mumbai</i>		

INVITED SPEAKERS

Dr. Abhijit Chakrabarti
Saha Institute of Nuclear Physics, India

Dr. Andrew Link
Vanderbilt-Ingram Cancer Center, USA

Dr. Anita Chugh
Syngene International, India

Dr. Arun Trivedi
Central Drug Research Institute, India

Dr. Brenda J. Andrews
University of Toronto, Canada

Mr. Brendan Maclean
University of Washington, USA

Dr. Christina Ludwig
ETH Zurich, Switzerland

Dr. Chung Ching Ming Maxey
National University of Singapore, Singapore

Dr. David Muddiman
North Carolina State University, USA

Dr. Debasis Dash
Institute of Genomics and Integrative Biology, India

Dr. Deepa Bisht
National JALMA Institute, India

Dr. Harsha Gowda
Institute of Bioinformatics, India

Dr. H. V. Thulasiram
National Chemical Laboratory, India

Dr. Himanshu Gadgil
Intas Pharma, India

Dr. Juan J. Calvete
Editor in chief, Journal of Proteomics, Spain

Dr. Kanury V. S. Rao
International Centre for Genetic Engineering & Biotechnology, India

Dr. Manuel Fuentes
University of Salamanca, Spain

Dr. Mark McDowell
Waters Corporation, UK

Dr. Nat Kav
University of Alberta, Canada

Dr. Peter Nilsson
KTH-Royal Institute of Technology, Sweden

Dr. Philip Andrews
University of Michigan, USA

Dr. Prasanna Venkatraman
TMC-Advanced Centre for Treatment, Research and Education in Cancer, India

Dr. Renu Deswal
University of Delhi, India

Dr. Sanjay Navani
Lab Surg Path, Mumbai, India

Dr. Pushkar Sharma
National Institute of Immunology, India

Dr. Ranjan Chakrabarti
United States Pharmacopeia, India

Dr. Rapole Srikanth
National Centre for Cell Science, India

Dr. Ravi Sirdesmukh
Institute of Bioinformatics, India

Dr. Robert Karlsson
GE Healthcare Lifesciences, Sweden

Dr. Robert L. Moritz
Institute of Systems Biology, USA

Dr. Rudolf Grimm
Agilent Technologies, USA

Dr. Rustom Mody
Lupin Biotech, India

Dr. Sanjay Gupta
TMC-Advanced Centre for Treatment, Research and Education in Cancer, India

Dr. Sudhir Srivastava
National Cancer Institute, USA

Dr. Utpal S. Tatu
Indian Institute of Science, India

Dr. Vijaya Pattabiraman
ETH Zurich, Switzerland

Dr. Yetrib Hathout
Children's National Medical Center, USA

Dr. Sarah Chandra Janga
Indiana University, USA

Dr. Shantanu Sengupta
Institute of Genomics and Integrative Biology, India

Dr. Sixue Chen
University of Florida, USA

Dr. Sriram Kumaraswamy
Pall ForteBio LLC

Dr. Sudhir Srivastava
National Cancer Institute, USA

Dr. Utpal S. Tatu
Indian Institute of Science, India

Dr. Vijaya Pattabiraman
ETH Zurich, Switzerland

Dr. Yetrib Hathout
Children's National Medical Center, USA

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CONFERENCE - DAY 1 (7TH DEC 2014- SUN)

Venue: Auditorium, VMCC, IIT Bombay

7:30 - 8:15 AM Registration

8:15 - 8:25 AM Invocation

PSI AND HUPO

8:25 - 8:55 AM Welcome by Convener Dr. Sanjeeva Srivastava & Inauguration

8:55 - 9:20 AM Opening address: PSI and proteomics in India

Speaker Dr. Surekha Zingde

Ex-Advanced Centre for Treatment, Research and Education in Cancer, Navi Mumbai, India

9:20 - 9:45 AM Keynote address: HUPO and its future for human biology

Speaker Dr. Pierre Legrain, *Institut Pasteur, France*

9:45 - 10:05 AM Tea

SESSION I: CANCER BIOMARKERS

Chairs Dr. Girish Maru (*Advanced Centre for Treatment, Research and Education in Cancer, India*) & Dr. Ravi Sirdeshmukh (*Institute of Bioinformatics, India*)

10:05 - 10:45 AM Plenary Lecture-1:

Proteomic and RNA-Seq analyses of cancers: Splice isoforms as a new class of cancer biomarker candidates

Speaker Dr. Gilbert Omenn

University of Michigan, USA

10:45 - 11:10 AM Invited Lecture-1:

Clinical applications of proteomics in cancer detection, diagnosis and prognosis

Speaker Dr. Sudhir Srivastava

National Cancer Institute, USA

11:10 - 11:35 AM Invited Lecture-2:

Proteomic identification of C/EBPalpha-p30 target proteins in acute myeloid leukemia

Speaker Dr. Arun Trivedi

Central Drug Research Institute, India

11:35 - 12:00 NOON Invited Lecture-3:

Searching for biomarkers involved in colorectal cancer metastasis from the proteomes and secretomes of two isogenic colorectal cancer cell lines

Speaker Dr. Chung Ching Ming Maxey

National University of Singapore, Singapore

12:00 - 1:00 PM Poster Session 1Cancer Biomarkers & Proteomics of Biofluids

Poster No. D-1-01 to D-1-60

1:00 - 2:00 PM Lunch & Poster Session

SESSION II: PROTEOMICS OF BIOFLUIDS

Chairs Dr. K. Dharmalingam (*Aravind Medical Research Foundation, India*) &
Dr. Kumaravel Somasundaram (*Indian Institute of Science, India*)

2:00 - 2:40 PM	Plenary Lecture-2: Integrating biomarkers into lung cancer screening
Speaker	Dr. Samir Hanash <i>The University of Texas MD Anderson Cancer Center, USA</i>
2:40 - 3:05 PM	Invited Lecture-4: Serum protein biomarkers discovery in duchenne muscular dystrophy using mass spectrometry and aptamers technology
Speaker	Dr. Yetrib Hathout <i>Children's Research Institute, USA</i>
3:05 - 3:30 PM	Invited Lecture-5: Clinical proteomics of hemolysates in hemoglobinopathy
Speaker	Dr. Abhijit Chakrabarti <i>Saha Institute of Nuclear Physics, India</i>
3:30 - 3:50 PM	Invited Company Lecture-1: A paleo-metabolomics study of the stomach content of oldest human ice mummy, the 5300 y old Tyrolean iceman or Oetzi
Speaker	Dr. Rudolf Grimm <i>Agilent Technologies Inc., USA</i>
3:50 - 4:10 PM	Tea
4:10 - 5:10 PM	PANEL DISCUSSIONS (Parallel Session)

Panel Discussion I: Quest of human proteome and missing proteins

Venue: Auditorium, VMCC, IIT Bombay

Moderator

Dr. Akhilesh Pandey, *John Hopkins University, USA*

Panelist

Dr. Gilbert Omenn, *University of Michigan, USA*

Dr. Mark Baker, *Macquarie University, Australia*

Dr. Robert L. Moritz, *Institute for Systems Biology, USA*

Dr. Sudhir Srivastava, *National Cancer Institute, USA*

Panel Discussion II: Metabolomics and its integration in system biology

Venue: Lecture Hall 21, Second Floor, VMCC, IIT Bombay

Moderators

Dr. Abhijit Mitra, *International Institute of Information Technology Hyderabad, India*

&

Dr. Kanury V. S. Rao, *International Centre for Genetic Engineering and Biotechnology, India*

Panelist

Dr. Harsha Gowda, *Institute of Bioinformatics, India*

Dr. Mahesh Kulkarni, *National Chemical Laboratory, India*

Dr. Rapole Srikanth, *National Centre for Cell Science, India*

Dr. Rudolf Grimm, *Agilent Technologies, USA*

SESSION III: PROTEOMICS & SYSTEMS BIOLOGY

Chairs Dr. K. V. Venkatesh (*Indian Institute of Technology Bombay, India*) &
Dr. Rakesh Mishra (*Centre for Cellular and Molecular Biology, India*)

5:10 - 5:35 PM	Invited Lecture-6: Global protein abundance and localization maps define dynamic subcellular flux networks in yeast
Speaker	Dr. Brenda Andrews <i>University of Toronto, Canada</i>
5:35 - 6:00 PM	Invited Lecture-7: Systems analysis of cell-specific immune responses to adjuvanted influenza vaccines
Speaker	Dr. Andrew Link <i>Vanderbilt University School of Medicine, USA</i>
6:00 - 6:25 PM	Invited Lecture-8: Uncovering the regulatory networks at post-transcriptional level in health and disease using genomic and systems approaches
Speaker	Dr. Sarath Chandra Janga <i>Indiana University School of medicine, USA</i>
6:25 - 6:50 PM	Invited Lecture-9: Computational proteomics pipeline for discovery of novel proteins in biological systems
Speaker	Dr. Debasis Dash <i>Institute of Genomics and Integrative Biology, India</i>
6:50 - 7:50 PM	Meet your Editors “Omics” and Publishers & PSI EC Meeting

Meet your Editors and Publishers

Moderator

Dr. Juan J. Calvete, Instituto de Biomedicina de Valencia, Spain

Panelist

Dr. Akhilesh Pandey, *John Hopkins University, USA*

Dr. Chung Ching Ming Maxey, *National University of Singapore, Singapore*

Dr. John Yates, *The Scripps Research Institute, USA*

Dr. Suman Kundu, *University of Delhi, India*

PSI EC Meeting : Venue: Conference Room, Fourth Floor, VMCC, IIT Bombay

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CONFERENCE - DAY 2 (8TH DEC 2014- MON) PARALLEL SESSIONS

PROTEOMICS SESSION

Venue: Auditorium, VMCC, IIT Bombay

SESSION IV: MASS SPECTROMETRY & PTM

Chairs	Dr. Keshava Prasad (<i>Institute of Bioinformatics, India</i>) & Dr. M. A. Vijayalakshmi (<i>VIT University, India</i>)
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8:30 - 9:10 AM	Plenary Lecture-3: Using mass spectrometry to understand cystic fibrosis as a protein misfolding disease Speaker Dr. John Yates <i>The Scripps Research Institute, USA</i>
9:10 - 9:35 AM	Invited Lecture-10: An innovative atmospheric pressure ionization source for mass spectrometry imaging/ INLIGHT strategy for quantitative glycomics Speaker Dr. David Muddiman <i>North Carolina State University, USA</i>
9:35 - 10:00 AM	Invited Lecture-11: Differential incorporation of histone H2A isoforms: adding higher level of complexity in chromatin organization during development and cancer Speaker Dr. Sanjay Gupta <i>Advanced Centre for Treatment Research and Education in Cancer, India</i>
10:00 - 10:20 AM	Tea

SESSION V: NEW MS APPROACHES

Chairs	Dr. Akhilesh Pandey (<i>Johns Hopkins University School of Medicine, USA</i>) & Dr. Philip Andrews (<i>University of Michigan, USA</i>)
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10:20 - 11:00 AM	Plenary Lecture-4: Advances in mass spectrometry for protein analysis Speaker Dr. Catherine Fenselau <i>University of Maryland, USA</i>
11:00 - 11:25 AM	Invited Lecture-12: Structures of protein complexes by chemical cross-linking mass spectrometry (CXL-MS) and CXL-ion mobility-MS Speaker Dr. Philip Andrews <i>University of Michigan, USA</i>
11:25 - 11:50 AM	Invited Lecture-13: Skyline: building an ecosystem for targeted quantitative proteomics Speaker Mr. Brendan MacLean <i>University of Washington, USA</i>
11:50 - 12:15 PM	Invited Lecture-14: Understanding mechanism of cysteine toxicity in yeast using a proteomics approach

JPP 15

Speaker Dr. Shantanu Sengupta
Institute of Genomics and Integrative Biology, India

12:15 - 1:05 PM	Poster Session 2 Proteomics and Systems Biology Mass Spectrometry & PTM New MS Approaches Interactomics and Multi-Omics Analysis Human Proteome Project MS and Metabolomics Poster No. D-2-01 to D-2-60
1:05 - 2:00 PM	Lunch & Poster Session
2:00 - 2:20 PM	Invited Company Lecture-2: A Multi-omic data independent investigation of drug mitigated obesity within a mouse model Speaker Dr. Mark McDowall <i>Waters Corporation, UK</i>

SESSION VI: INTERACTOMICS AND MULTI-OMICS ANALYSIS

Chair Dr. Sharmistha Dey (*All India Institute of Medical Sciences, India*) &
Dr. Utpal Tatu (*Indian Institute of Science, India*)

2:20 - 2:45 PM	Invited Lecture-15: Identification of altered cell signaling pathways in B- lymphocytic chronic leukemia (B-CLL) by functional proteomics approaches Speaker Dr. Manuel Fuentes <i>University of Salamanca, Spain</i>
2:45 - 3:10 PM	Invited Lecture-16: Structural interrogation of phosphoproteome identified by mass spectrometry reveals allowed and disallowed regions of phosphoconformation Speaker Dr. Prasanna Venkatraman <i>Advanced Centre for Treatment Research and Education in Cancer, India</i>
3:10 – 3:35 PM	Invited Lecture-17: Autoantibody profiling of gliomas and meningiomas to identify biomarkers using human proteome arrays Speaker Dr. Sanjeeva Srivastava <i>Indian Institute of Technology Bombay, India</i>
3:35 - 3:55 PM	Invited Company Lecture-3: Kinetic analysis of protein-protein interactions friend or foe? Speaker Dr. Robert Karlsson <i>GE Healthcare Life Sciences</i>
3:55 - 4:10 PM	Tea

SESSION VII: HUMAN PROTEOME PROJECT

Chair	Dr. Pierre Legrain (<i>Institut Pasteur, France</i>) & Dr. Surekha Zingde (<i>Ex-Advanced Centre for Treatment, Research and Education in Cancer, India</i>)
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4:10 - 4:50 PM	Plenary Lecture-5: Human proteome project keys to success: finding a shared language
Speaker	Dr. Mark Baker <i>Macquarie University, Australia</i>
4:50 - 5:15 PM	Invited Lecture-18: Advances in MS-based proteomics tools and resources
Speaker	Dr. Robert L. Moritz <i>Institute for Systems Biology, USA</i>
5:15 - 5:40 PM	Invited Lecture-19: Taking Indian proteomics to human proteome project
Speaker	Dr. Ravi Sirdeshmukh <i>Institute of Bioinformatics, India</i>
5:40 - 6:05 PM	Invited Lecture-20: Tissue-based map of the human proteome
Speaker	Dr. Sanjay Navani <i>Site Director, The Human Protein Atlas (HPA) Project, India</i>
6:05 - 6:45 PM	General Body Meeting Venue: VMCC Auditorium Parallel Session Author workshop – How to write for and get published in international scientific Journals Swati Meherishi, Springer Venue: Seminar Hall 11, First floor, VMCC
6:45 - 7:30 PM	Dinner
7:30 - 9:00 PM	Cultural Event: <i>Venue: Convocation Hall, IIT Bombay</i>



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NEW TRACK

PROTEOMICS PERSPECTIVE ON DEVELOPMENT AND CHARACTERIZATION OF BIOSIMILARS AND BIOTHERAPEUTICS

AGENDA

- Biosimilar Characterization: ICH Q6B and Analytical Challenges*
- Proteomic Solutions for Biosimilars/ Antibodies Characterization*
- Impurity Profiling, Purity and Contaminants*
- Structural and Functional Characterization*

Day-1: 8th Dec 2014
Lectures & Demo

09:00 - 09:40	Plenary Lecture
09:40 - 10:10	Invited Lecture
10:30 - 10:50	Speaker-1
10:50 - 11:10	Speaker-2
11:10 - 11:30	Speaker-3
11:30 - 12:00	Speaker-4-MS application
12:00 - 01:00	LC-HRMS Demo For Biosimilar Characterization
02:00 - 02:40	Plenary Lecture
02:40 - 03:00	Speaker-5
03:00 - 03:20	Speaker-6
03:20 - 03:50	Speaker-7-Label-free application
04:10 - 05:00	Label-free Interaction Demo For Biosimilar Characterization
05:00 - 06:00	Panel discussion (Indian Pharma, US-FDA, DCGI and others)

Standards for Indian Biosimilar Industry: Present status & Future Prospects

Day-2: 9th Dec 2014
Hands-on Workshop

Time	HRMS characterization	Targeted mAb analysis	SPR and Biosensors	Gel-based (DIGE)
09:00 - 11:00	Group I	Group II	Group III	Group IV
11:00 - 01:00	Group II	Group I	Group IV	Group III
01:00 - 02:00	Luncheon Interactions			
02:00 - 04:00	Group III	Group IV	Group I	Group II
04:00 - 06:00	Group IV	Group III	Group II	Group I

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BIOSIMILARS SESSION

Venue: Lecture Hall 21, Second Floor, IIT Bombay

8:30-9:00 AM Registration

SESSION I: BIOSIMILAR AND BIOTHERAPEUTICS IN INDIAN BIOPHARMA

Chair	Dr. Bipin Nair (<i>Amrita School of Biotechnology, India</i>)
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9:00–9:30 AM	Plenary Lecture-1 Indian biopharma: challenges and opportunities Speaker Dr. Anurag Rathore <i>Indian Institute of Technology Delhi, India</i>
9:30–9:50 AM	Keynote address: Biosimilar development and characterization Speaker Dr. Sudhir Sahasrabudhe <i>University of Utah, USA</i>
9:50–10:08 AM	Invited Lecture-1 Analytics in biosimilar development: characterization and comparability Speaker Dr. Rustom Mody <i>Lupin Biotech, India</i>
10:08 – 10:28 AM	Tea Break

SESSION II: CHALLENGES AND REGULATORY ASPECTS FOR BIOSIMILAR DEVELOPMENT & CHARACTERIZATION

Chairs	Dr. Kumar Prabhash (<i>Tata Memorial Hospital, India</i>) & Dr. Vidhyashankar Ramamurthy (<i>Syngene International, India</i>)
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10:30–11:00 AM	Plenary Lecture-2 Applications of mass spectrometry for characterizing biopharmaceuticals in the regulatory setting Speaker Dr. Mike Boyne <i>US-FDA</i>
11:00– 11:20 AM	Keynote address: Six blind men and a beast: co-operative analytics holds the key for product driven process development Speaker Dr. Himanshu Gadgil <i>Intas Pharma, India</i>
11:20– 11:38 AM	Invited Lecture-2 Emerging biotherapeutics: challenges in their characterization Speaker Dr. Anita Chugh <i>Syngene International, India</i>
11:38– 11:56 AM	Invited Lecture-3 Standards for biotherapeutics – USP approach Speaker Dr. Ranjan Chakrabarti <i>United States Pharmacopeia-India</i>
11:56 – 12:14 PM	Invited Lecture-4 Regulatory role of biophysics in the biopharma industry: perspectives from application to biosimilars Speaker Dr. Amarnath Chatterjee <i>Biocon Research Limited, India</i>
12:15 –1:05 PM	PANEL DISCUSSIONS

Moderator

Dr. Uma Raghuram, Spinco Biotech Pvt. Ltd.

Panel Discussion I: Regulatory views

Dr. Anita Chugh; Syngene International, India

Dr. Anurag Rathore; Indian Institute of Technology (IIT), India

Dr. Himanshu Gadgil; Intas Pharma, India

Dr. Kumar Prabhash; Tata Memorial Hospital, India

Dr. Mike Boyne; US-FDA

Dr. Ranjan Chakrabarti; United States Pharmacopeia, India

Dr. Rustom Mody; Lupin Biotech, India, Indian DCGI and FDA views

Panel Discussion II: Proteomics technology

Dr. Anette Persson; GE Healthcare, Sweden

Dr. Bipin Nair; Amrita School of Biotechnology, India

Dr. Bob Galvin; Bruker Daltonics

Dr. Dinesh Palanivelu; Biocon Research Limited, India

Dr. M. A. Vijayalakshmi; VIT University, India

Dr. P. Babu; C-CAMP, India

1:05 – 2:00 PM Lunch

**SESSION III: ADVANCES IN MASS SPECTROMETRY FOR
BIOSIMILAR CHARACTERIZATION**

Chair: Dr. M. A. Vijayalakshmi (*VIT University, Vellore, India*)

2:00 - 2:30 PM	Plenary Lecture-3 Structural characterization of biosimilars by HDX & top-down analysis using ECD/ ETD-FTMS
Speaker	Dr. Gary Kruppa <i>MRM Proteomics Inc, Canada</i>
2:30– 2:50 PM	Keynote address: Whole proteome resources: Access and sharing of data through the peptide and SRMAtlas proteomics suites
Speaker	Dr. Robert L. Moritz <i>Institute for Systems Biology, USA</i>
2:50–3:08 PM	Invited Lecture-5 Application of mass spectrometry in the characterization of biosimilars
Speaker	Dr. Taegen Clary <i>Agilent Technologies, USA</i>
3:08–3:26 PM	Invited Lecture-6 Targeted Proteomics –Advantages and challenges using mass spectrometric techniques like SRM and SWATH-MS
Speaker	Dr. Christina Ludwig <i>ETH Zurich, Switzerland</i>
3:26–3:44 PM	Invited Lecture-7 High Definition MS for the characterization of biosimilars
Speaker	Dr. Mark McDowall <i>Waters Corporation, UK</i>
3:45–4:00 PM	Tea Break

SESSION IV: FUNCTIONAL CHARACTERIZATION OF BIOLOGICS

Chair Dr. Uma Sinha Datta (*GE Healthcare Life Sciences*)

4:00 – 4:20 PM	Keynote address: Label-free binding assays in the processing and analysis of biopharmaceuticals
Speaker	Dr. Sriram Kumaraswamy <i>Pall ForteBio LLC, USA</i>
4:20 – 4:38 PM	Invited Lecture-8 Enabling chemical technologies for protein biotherapeutics development
Speaker	Dr. Vijaya Pattabiraman <i>ETH Zurich, Switzerland</i>
4:38 – 4:56 PM	Invited Lecture-9 Monitoring high order structure in biotherapeutic development using SPR, DSC and 2-D DIGE
Speaker	Dr. Anette Persson <i>GE Healthcare, Sweden</i>
4:56 – 5:25 PM	Label-free interaction studies: Demo and analysis for biosimilar characterization
5:25 – 6:45 PM	LC-HRMS talk & Demo for biosimilar data analysis
6:45 – 7:30 PM	Networking Dinner
7:30 – 9:00 PM	Cultural Event: <i>Venue: Convocation Hall, Indian Institute of Technology Bombay</i>



CONFERENCE - DAY 3 (9TH DEC 2014- TUE) PARALLEL SESSIONS**PROTEOMICS SESSION***Venue: Auditorium, VMCC, IIT Bombay*

SESSION VIII: MS AND METABOLOMICS	
Chairs	Dr. Abhijit Mitra (<i>International Institute of Information Technology Hyderabad, India</i>) & Dr. Shantanu Sengupta (<i>Institute of Genomics and Integrative Biology, India</i>)
8:30 - 9:10 AM	Plenary Lecture-6: Applications of novel mass spectrometry-based approaches for the definition of disease-related glycomes, lipidomes and peptidomes
Speaker	Dr. Catherine Costello <i>Boston University, USA</i>
9:10 - 9:35 AM	Invited Lecture-21: Pathogenicity of mycobacterium tuberculosis is expressed by regulating metabolic thresholds of the host macrophage
Speaker	Dr. Kanury V. S. Rao <i>International Centre for Genetic Engineering and Biotechnology, India</i>
9:35 - 10:00 AM	Invited Lecture-22: Metabolomic and lipidomic profiling towards novel theragnostic markers for breast cancer
Speaker	Dr. Rapole Srikanth <i>National Centre for Cell Science, India</i>
10:00 – 10:25 AM	Invited Lecture-23: Metabolomics of esophageal squamous cell carcinoma
Speaker	Dr. Harsha Gowda <i>Institute of Bioinformatics, India</i>
10:25 – 10:45 AM	Tea
SESSION VIII: MS AND METABOLOMICS (SESSION CONTINUES...)	
Chair	Dr. Mahesh Kulkarni (<i>National Chemical Laboratory, India</i>)
10:45 - 11:10 AM	Invited Lecture-24: Next generation snake venomics
Speaker	Dr. Juan J. Calvete <i>Instituto de Biomedicina de Valencia, Spain</i>
11:10 - 11:25 AM	Invited Short Talk-1: Role of novel glycan-receptor interactions in hydra regeneration
Speaker	Dr. P. Babu <i>C-CAMP, NCBS, India</i>
11:25 - 11:40 AM	Invited Short Talk-2: Development of an electrodynamic ion funnel with electrospray ionization source for proteomics studies using reflectron-ToF mass spectrometry
Speaker	Dr. K. Chandrashekhar Rao <i>Bhabha Atomic Research Centre, India</i>

11:40 - 11:55 AM	Invited Short Talk-3: Application of quantitative proteomics using mass spectrometry and systems biology
Speaker	Dr. Bhaswati Chatterjee <i>National Institute of Pharmaceutical Education and Research, India</i>
12:00 - 1:00 PM	Poster Session 3 Microbial Proteomics Animal and Plant proteomics Poster No. D-3-01 to D-3-60
1:00 – 2:00 PM	Lunch & Poster Session

SESSION IX AND SESSION X – PARALLEL SESSIONS

SESSION IX: MICROBIAL PROTEOMICS

Venue: Auditorium, VMCC, IIT Bombay

Chairs	Dr. Kanury V. S. Rao (<i>International Centre for Genetic Engineering and Biotechnology, India</i>) & Dr. Swati Patankar (<i>Indian Institute of Technology Bombay, India</i>)
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2:00 - 2:25 PM	Invited Lecture-25: Unfolded protein response in malaria: a transcript, protein and metabolite flux
Speaker	Dr. Utpal Tatu <i>Indian Institute of Science, India</i>
2:25 - 2:50 PM	Invited Lecture-26: Affinity proteomics reveals elevated muscle proteins in plasma of children with cerebral malaria
Speaker	Dr. Peter Nilsson <i>KTH-Royal Institute of Technology, Sweden</i>
2:50 - 3:15 PM	Invited Lecture-27: Identification of potentially involved proteins in aminoglycoside resistance in <i>Mycobacterium tuberculosis</i>
Speaker	Dr. Deepa Bisht <i>National JALMA Institute, India</i>
3:15 - 3:40 PM	Invited Lecture-28: Role and regulation of malarial calcium dependent kinases
Speaker	Dr. Pushkar Sharma <i>National Institute of Immunology, India</i>
3:40 - 3:55 PM	Invited Short Talk-4: Unique and differential protein signatures for HIV-1 & HCV mono-infection versus co-infection as determined by multiplex iTRAQ quantitative proteomics
Speaker	Dr. Pooja Jain <i>Drexel University College of Medicine, USA</i>
3:55 - 4:10 PM	Invited Short Talk-5: Proteomic perspective of extreme gamma radiation resistance in <i>D. radiodurans</i>
Speaker	Dr. Bhakti Basu <i>Bhabha Atomic Research Centre, India</i>

SESSION X: ANIMAL AND PLANT PROTEOMICS

Venue: Lecture Hall 21, Second Floor, VMCC, IIT Bombay

Chairs **Dr. Subhra Chakraborty (National Institute of Plant Genome Research, India) & Dr. Nat Kav (University of Alberta)**

2:00 - 2:25 PM	Invited Lecture-29: Proteomics and immunotechnology in agriculture
Speaker	Dr. Nat Kav <i>University of Alberta, Canada</i>
2:25 - 2:50 PM	Invited Lecture-30: Ambushing the Himalayan gold bush for antifreeze proteins with potential biotechnological applications
Speaker	Dr. Renu Deswal <i>University of Delhi, India</i>
2:50 - 3:15 PM	Invited Lecture-31: Redox and phosphorylation regulated proteins in plant stomatal signaling
Speaker	Dr. Sixue Chen <i>University of Florida, USA</i>
3:15 - 3:40 PM	Invited Lecture-32: Metabolomics: Terpene profiling of selected plants
Speaker	Dr. H. V. Thulasiram <i>National Chemical Laboratory, India</i>
3:40 - 3:55 PM	Invited Short Talk-6: Proteome analysis of functionally differentiated mammary epithelial cells and lactating udder tissue reveals protein signatures associated with lactation persistency and milk yield
Speaker	Dr. Ashok Mohanty <i>National Dairy Research Institute, India</i>
3:55 - 4:10 PM	Invited Short Talk-7: Dissecting the host gene interaction network in PBMCs infected with PPR vaccine virus uncovered transcription factors modulating immune regulatory pathways
Speaker	Dr. Ravi Kumar Gandham <i>Indian Veterinary Research Institute, India</i>
4:10 - 5:00 PM	Valedictory Function

5:00 PM onwards – Mumbai City Tour and Gala Dinner

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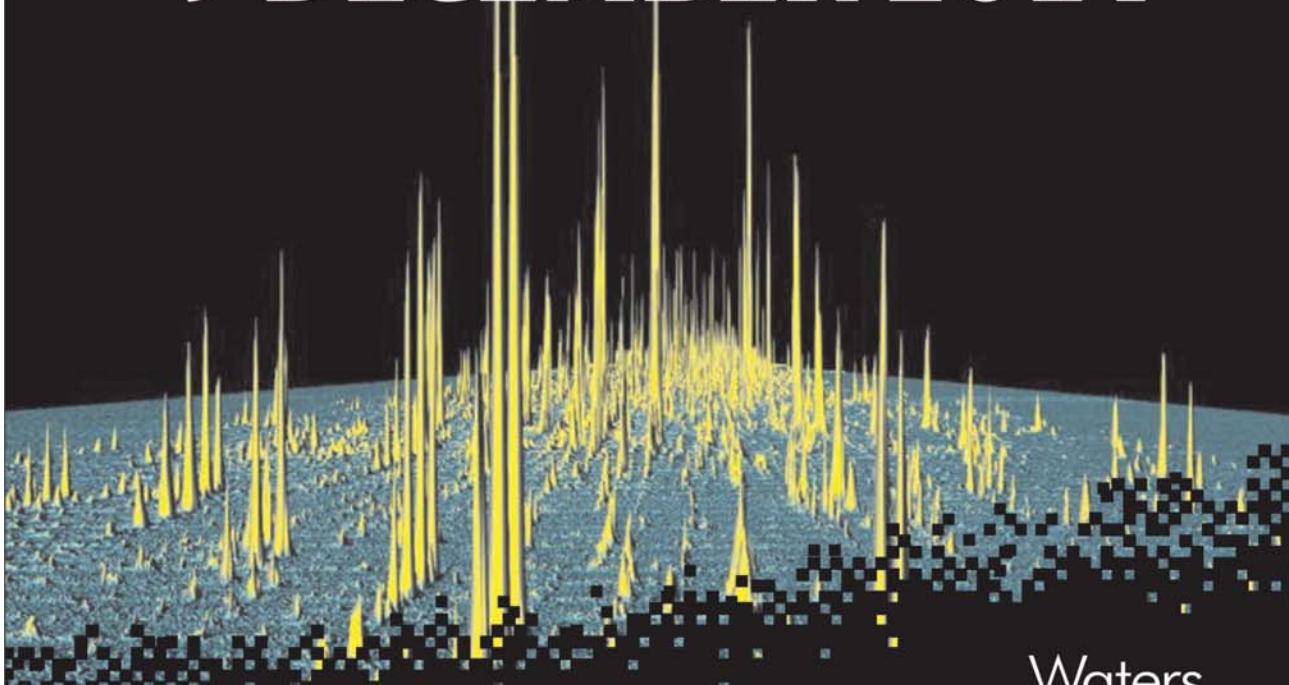
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HANDS ON WORKSHOPS

(9th-11th Dec 2014)

BIOSIMILARS WORKSHOP- 9TH DEC 2014

Venue: CRNTS, IIT Bombay

**WORKSHOP 1: HIGH RESOLUTION MASS SPECTROMETRY BASED BIOSIMILAR CHARACTERIZATION
(DISCOVERY PROTEOMICS)**

Venue: CRNTS, IIT Bombay

WORKSHOP 2: TARGETED PROTEOMICS USING TRIPLE QUAD MASS SPECTROMETRY

Venue: CRNTS, IIT Bombay

WORKSHOP 3: LABEL-FREE INTERACTION STUDIES USING USING DIFFERENT BIOSENSORS

Venue: Seminar Hall 11 & CRNTS, IIT Bombay

WORKSHOP 4: GEL-BASED (DIGE) ANALYSIS

Venue: CRNTS, IIT Bombay

POST CONFERENCE WORKSHOPS (10TH AND 11TH DEC 2014) - PARALLEL SESSIONS

WORKSHOP 1: GEL-BASED PROTEOMICS

Venue: Seminar Room 3, Ground Floor, VMCC, IIT Bombay

WORKSHOP 2: MASS SPECTROMETRY

Venue: Seminar Room 12, First Floor, VMCC, IIT Bombay

WORKSHOP 3: TARGETED PROTEOMICS

Venue: Seminar Room 13, First Floor, VMCC, IIT Bombay

WORKSHOP 4: PROTEIN MICROARRAYS

Venue: Seminar Room 14, First Floor, VMCC, IIT Bombay

WORKSHOP 5: LABEL FREE DETECTION

Venue: Seminar Room 11, First Floor, VMCC, IIT Bombay

Proteomics Society, India PS(I)
6th Annual Meeting
PROTEOMICS WORKSHOPS

10th -11th DECEMBER, 2014
 VENUE: INDIAN INSTITUTE OF TECHNOLOGY (IIT) BOMBAY, MUMBAI

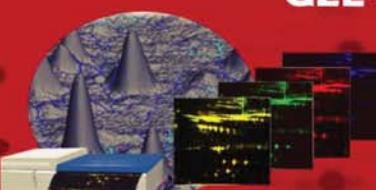
Dr. Sanjeeva Srivastava
Convenor

GEL-BASED PROTEOMICS
Learning Objectives

- Introduction to gel-based proteomics
- 2-DE workflow theory and hands-on session
- 2D-DIGE labeling and workflow
- Training in 2-DE data analysis
- Training in 2D-DIGE data analysis
- New advancements in gel-based proteomics
- Group discussions

MASS SPECTROMETRY
Learning Objectives

- Introduction to mass spectrometry
- Sample preparation-in-gel and in-solution digestion
- MALDI-TOF/TOF analysis for PMF and MS/MS
- LC-MS/MS analysis
- Quantitative proteomic analysis
- MS data analysis
- New advancements in mass spectrometry
- Group discussions

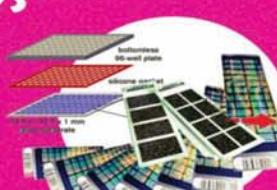


TARGETED PROTEOMICS
Learning Objectives

- Introduction to hypothesis driven proteomics measurement for validation
- Enhance understanding from exploratory proteomics to targeted
- Basic knowledge of skyline to implement targeted experiments
- Introduction to key concepts of targeted proteomics experiments
- Creating & using libraries of prior empirical measurements
- Absolute qualification versus detecting differences
- Statistics of study design & avoiding statistical pitfalls
- Basic understanding of MS stats

PROTEIN MICROARRAYS
Learning Objectives

- Introduction to protein microarrays
- Different types of protein microarray platforms
- Application of protein microarrays for protein interactions autoantibody screening
- Hands-on sessions for printing arrays
- Hands-on sessions for microarray assays
- Training in data analysis & interpretation
- New advancements in microarray technology
- Group discussions



LABEL-FREE DETECTION
Learning Objectives

- Introduction to Surface Plasmon Resonance & its applications
- Sensor surface properties & surface preparation strategies
- Interaction analysis for Protein-protein & Protein-small molecule studies
- Hands-on sessions for immobilization, binding & kinetics assays
- Training in data analysis & interpretation
- Group discussions

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7TH DECEMBER 2014

OPENING AND KEYNOTE ADDRESS

PROTEOMICS SOCIETY, INDIA (PSI)	Opening address	HUMAN PROTEOME ORGANIZATION (HUPO)	Keynote address
	 Dr. Surekha Zingde <i>(President, PSI)</i>	 HUMAN PROTEOME ORGANIZATION (HUPO) <i>Human Proteome Organization</i>	 Dr. Pierre Legrain <i>(HUPO President)</i>

Dr. Surekha M. Zingde*President, PS(I), EX-ACTREC, India.*

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 33y 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 is 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 69 peer reviewed papers, several book chapters, a monograph and 2 patent applications and a patent to her credit.

Abstract: After the completion of the human genome sequence in April 2003, protein and proteomic research re-emerged as a science requiring attention. Since then, there are worldwide efforts to develop technologies for purification, identification, characterization, quantification, functional annotation of proteins, global protein profiling and generating a human proteome map. In India, the first consolidated efforts towards sophisticated proteomic research started in 2001-2002 with government funding bodies financing multi-institutional proteomic projects. This ensured that the technical knowledge required to run the sophisticated instrumentation was available and the equipment were used optimally.

In 2009, several senior scientists felt the need to bring together the upcoming Proteomics community in India. The Proteomics Society, India (PSI) was registered in 2009 to provide the required forum. Over the last five years we have succeeded in bringing together scientists, teachers and students from across the country through our Annual meetings, focused conferences, teacher training programs and workshops. The Society is also a forum for international collaborations.

Proteomics has now emerged as an important tool for varied biological investigations in the country. There are major hubs

of focused proteomic studies from the North to the South and West to East of India as is evident from the publications in journals and the presentations at this the 6th Annual meeting of the Society. India's contribution to Proteomics is also acknowledged globally as we have representatives on the councils of the major Proteomic Organizations in the world. The future of Proteomics research in India is definitely very promising.

Dr. Pierre Legrain*President HUPO, Institut Pasteur, France.*

Pierre Legrain was trained as an immunologist and a cell biologist at the Pasteur Institute where he defended his PhD in 1981. After a two year period at Brandeis University (Mass, USA) where he studied pre-messenger RNAs splicing and nuclear export, he came back at the Pasteur Institute, and focused his research on protein-protein interactions. His methodology was successfully applied to many organisms, including Human. In 1998, he founded a biotech, Hybrigenics, where he served as scientific director until 2003. In 2003, he joined the Commissariat à l'Energie Atomique as director of a research department, then as Director of Life sciences. In 2011, he joined the Ecole Polytechnique where he currently serves as the Dean of the graduate School. He is a member of the Human Proteome Organisation (HUPO) council since its inception in 2001. He successively served as secretary general (2009-2010), Vice-President (2011-2012) and now as President (2013-2014). In 2009 he was appointed project manager for the Human Proteome Project (HPP) and contributed to the launch of the HPP consortium in 2011. His major scientific interest turns on system's biology and he is currently developing new hypotheses for inheritance of complex traits.

Abstract: For more than one decade, HUPO has served the proteomics scientific community, by gathering experts for different fields: mass spectrometry, antibodies and other affinity reagents for proteins, 2D-gel electrophoresis, enzymology, etc. The Human Proteome Project was launched in 2010. It is now well structured and it starts to deliver key results on human proteins identification and characterization. In several years from now, most if not all proteins encoded in the genome will be detected in tissue or fluid samples. In many cases, the amount of them will be known for normal and diseased contexts.

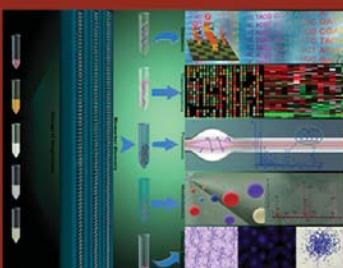
So, what should be the next challenge and what could be new avenues of research for proteomics scientists?

My personal bias is oriented towards systems biology. Understanding the contribution of hundreds of proteins for a

complex biological function, such as those sustained by the brain, will be a huge challenge. Issues such as organ localization, time expression, and half-life in dedicated cells will be encountered. And, even if those issues were mainly solved, a general frame describing a well-defined “biological function” will remain a major hurdle before modeling could be proposed and mechanistic description with predictive value could be achieved.

In those perspectives, I welcome any attempt to reinforce interdisciplinary efforts, especially with physicists, mathematicians and computer scientists, also for interactions with human sciences. A cross-fertilization of various scientific fields will be needed for those achievements and ultimately, for delivering better scientific and technological tools for medicine in particular.

SESSION I: CANCER BIOMARKERS

CANCER BIOMARKER	Plenary Talk	Invited talks		
	 Dr. Gilbert S. Omenn <i>Ex-US HUPO President (Director, CCMB, USA)</i>	 Dr. Sudhir Srivastava <i>(National Cancer Institute, USA)</i>	 Dr. Arun Trivedi <i>(Central Drug Research Institute, India)</i>	 Dr. Chung Ching Ming Maxey <i>(NUS, Singapore)</i>

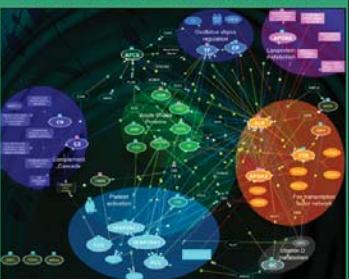
In addition to clinical symptoms and histopathological investigation, protein biomarkers have potential to be considered as promising candidates for tumor classification. The challenges and promises of “Cancer Biomarker” discovery will be discussed in this session.

SESSION II: PROTEOMICS OF BIOFLUIDS

PROTEOMICS OF BIOFLUIDS	Plenary Talk	Invited talks	
	 Dr. Samir Hanash <i>(EX-HUPO President) M.D. Anderson Cancer Center, USA</i>	 Dr. Yetrib Hathout <i>(Center for Genetic Medicine Research, USA)</i>	 Dr. Abhijit Chakrabarti <i>(Saha Institute of Nuclear Physics, India)</i>

Analysis of biofluids provides a less invasive approach for identification of biomarkers; however, pose a great challenge due to its low abundance in biofluids. The session will discuss sensitive and specific detection of surrogate protein markers from biofluids using high-throughput techniques.

SESSION III: PROTEOMICS & SYSTEMS BIOLOGY

PROTEOMICS AND SYSTEMS BIOLOGY		Invited talks			
					
Dr. Brenda J. Andrews <i>(University of Toronto, Canada)</i>	Dr. Sarath Chandra Janga <i>(Indiana University & Purdue University, USA)</i>	Dr. Debasis Dash <i>(CSIR-IIGB, India)</i>	Dr. Andrew J. Link <i>(Vanderbilt University School of Medicine, USA)</i>		

Systems biology deals with integrated multi-omics approaches for better understanding of biological processes and disease pathobiology. Discussions in this session will be towards enhancing our understanding of dynamic operation of cellular, genetic and post-transcriptional networks.

INVITED COMPANY LECTURE

MULTI-OMICS APPROACHES	Invited Company Talk
	
Dr. Rudolf Grimm <i>(Agilent Technologies, USA)</i>	



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SESSION I: CANCER BIOMARKERS

PLENARY LECTURE-1

Speaker: Dr. Gilbert S. Omenn

University of Michigan, USA

Biography: Gilbert Omenn is Professor of Internal Medicine, Human Genetics, and Public Health and Director of the Center for Computational Medicine & Bioinformatics at the University of Michigan. He served as Executive Vice President for Medical Affairs and as Chief Executive Officer of the University of Michigan Health System from 1997 to 2002. He was Dean of the School of Public Health, and Professor of Medicine and Environmental Health, University of Washington, Seattle, 1982-1997. His research interests include cancer proteomics, chemoprevention of cancers, public health genetics, science-based risk analysis, and health policy. He was principal investigator of the beta-Carotene and Retinol Efficacy Trial (CARET) of preventive agents against lung cancers and heart disease; director, Center for Health Promotion in Older Adults; and creator of a university-wide initiative on Public Health Genetics in Ethical, Legal, and Policy Context while at the University of Washington and Fred Hutchinson Cancer Research Center. He leads the Human Proteome Project for the international Human Proteome Organization. He was president of the American Association for the Advancement of Science (AAAS) in 2006. Omenn is the author of 548 research papers and scientific reviews and author/editor of 18 books. He serves on the Scientific Management Review Board of the NIH. He received the John W. Gardner Legacy of Leadership Award from the White House Fellows Association in 2004; the Walsh McDermott Medal from the Institute of Medicine in 2008 for long-term contributions to the IOM and the National Academy of Sciences; and in 2013 the David E. Rogers Award from AAMC for major contributions to health and health care in America. Omenn received his M.D. magna cum laude from Harvard Medical School, and Ph.D. in genetics from the University of Washington.

Talk title: Proteomic and RNA-Seq analyses of cancers: Splice isoforms as a new class of cancer biomarker candidates

Gilbert S. Omenn¹, Yuanfang Guan¹, Yang Zhang¹, Rajasree Menon¹

¹*Department for Computational Medicine and Bioinformatics, University of Michigan, United States*

Alternative splicing is a remarkable evolutionary development that increases protein diversity from multi-exonic genes without requiring expansion of the genome. It is no longer sufficient to report the up- or down-expression of genes and proteins without dissecting the complexity due to alternative splicing. Combined RNA-Seq and proteomics analyses reveal striking differential expression of splice isoforms of key proteins in important cancer pathways and networks. Primary tumor cell lines from histologically similar inflammatory breast cancers show striking differences in hormone receptor-negative cell lines that are ERBB2 (HER2/neu)-amplified versus ERBB1 (EGFR) over-expressed with low ERBB2 activity. We have related these findings to protein-protein interaction networks, signaling and metabolic pathways, and predictions of functional differences between variants using multiple instance learning or I-TASSER-

based protein folding algorithms. Understanding the upstream ligands and regulators and the downstream pathways and interaction networks for ERBB receptors is certain to be important for explanation and prediction of the variable levels of expression and therapeutic responses of ERBB+ tumors in the breast and in other organ sites.

INVITED LECTURE-1

Speaker: Dr. Sudhir Srivastava

National Cancer Institute, USA

Biography: Dr. Srivastava received his Ph.D. in Biological Science from Banaras Hindu University in 1977. Subsequently, he received his M.S. in Computer Science from the Virginia Commonwealth University in 1987 and a M.P.H from the Johns Hopkins University in 1997. He did postdoctoral work at the University of Osaka, Japan; the University of California at San Francisco; and the University of Arizona, Tucson. Dr. Srivastava is Chief of the Cancer Biomarkers Research Group in the Division of Cancer Prevention, National Cancer Institute. He joined the National Cancer Institute in 1988. Since 1990, he has served as the Program Director in the Division of Cancer Prevention and focused his responsibility in developing molecular signatures of cancer cells for cancer detection research programs with primary emphasis on cancer screening, early detection, risk assessment and informatics. Dr. Srivastava is an internationally recognized leader in cancer biomarker research. Dr. Srivastava is best known for his work on developing medical guidelines on the diagnosis of Hereditary Non-polyposis Colorectal Cancer (HNPCC). He played a pivotal role in the development of the Bethesda Guidelines for diagnosing HNPCC, which is in clinical practice worldwide. He has received several honors and awards and is a member of a number of scientific committees worldwide. In 1995, he was elected to the American Joint Committee on Cancer (AJCC) which is responsible for developing staging criteria for cancers for worldwide use and currently serves on the AJCC Executive Committee. He was featured in *Wired Magazine* in August 2003 for his leadership in cancer diagnostics. He has been a visiting professor at several medical and academic institutions and has delivered several inaugural and keynote addresses. He is Editor-in Chief of the journal, *Disease Markers* and a sister journal *Cancer Biomarkers*, published by the IOS press and serves as Associate Editors and reviewers for several internally known journals. He has published more than 170 research papers, review articles and commentaries in peer reviewed journals. He has edited several monographs and book chapters: *Early Detection of Cancer: Molecular Markers*, published by the Futura Publishing Company in 1995 and *Molecular Pathology of Cancer*, published by IOS Press, Amsterdam in 1999. Recently, he edited two books on Informatics in Proteomics (2005) published by Francis and Taylor, New York and *Translational Pathology of Early Cancer* (2012), published by the IOS Press.

Talk title: Clinical applications of proteomics in cancer detection, diagnosis and prognosis

The success of development of new biomarkers for early cancer detection requires a complex, dedicated infrastructure that facilitates the coordination, management and collaboration among many institutions, both from academia and industry, with

the involvement of scientists and clinicians with diverse expertise. The Early Detection Research Network (EDRN) has fulfilled these expectations by establishing an infrastructure and a process for biomarker development using a multidisciplinary and multi-institutional approach. Since its inception in 2000, the main focus of the EDRN has been to bring new biomarkers to clinical validation. Integrated genomic and proteomic technologies are yielding a highly innovative strategy for identifying candidate biomarkers for early detection that draws upon the multiple disciplines represented within EDRN (i.e., clinical and basic science, technology development, biostatistics and bioinformatics). An efficient and cost effective way to rapidly verify potential candidate biomarkers developed by EDRN researchers and further refine a biomarker panel in pre-clinical validation studies is provided by employing highly sensitive targeted mass spectrometry-based technologies, such as SRM and PRISM-SRM, before further investment in the development of expensive, clinical-grade immunoassays.

In the context of the EDRN, Pacific Northwest National Laboratory was tasked with demonstrating the utility of MS-based proteomics as an alternative to the development and use of ELISA assays, particularly in conducting early verification and preclinical validation studies of candidate biomarkers derived from genomic and/or transcriptomic experiments. Initial proof-of-concept experiments were done using PSA as the target. It was discovered that the differential use of IgY14 and IgY14+SuperMix immunoaffinity depletion columns could separate total PSA (IgY14 flow through) from Free PSA (SuperMix flow through). In collaboration with the Johns Hopkins University (PI: Dan Chan), PNNL conducted a blinded study of 33 clinical serum samples of PSA comparing SRM to CLIA-approved ELISA assays. The PNNL SRM-MS assay matched the CLIA ELISA performance in analytical sensitivity and CV, and the correlation between the two different assays was better than 0.93.

The MS-based approach can thus obviate the need to generate peptide or protein-specific antibodies, and also provides a quality control check, peptide identification, and specificity of the assay. The technology has significant potential to change the routine pipeline for biomarker verification and prevalidation, thus conserving resources for a smaller set of more stringently tested candidate biomarkers that can be developed for ELISA-based tests of highly valuable samples.

EDRN is developing a secretome-array platform approach to discover new serum markers. This secretome array construction has three phases: (1) Gather secreted protein/gene information; (2) Map to Affymetrix probe set; and (3) Assemble and remove redundancy. The results obtained from these studies validated the ability of the secretome platform in identifying the two known ovarian cancer biomarkers (CA125 and HE-4), as well as other markers associated with ovarian cancer. Further analysis yielded potential early detection/diagnostic markers, as well as potential novel therapeutic targets. One such discovered marker, FGF-18, has shown promise in distinguishing normal from cancer in ELISA assays.

INVITED LECTURE-2

Speaker: Dr. Arun Trivedi

Central Drug Research Institute, Lucknow, India

Biography: Dr. Arun Trivedi is a senior scientist at CSIR-Central Drug Research Institute (CDRI). He has completed M.Sc. (Biotechnology) from BHU Varanasi. Dr. Arun Trivedi received his Ph.D. in Human Biology from Ludwig Maximilians University, Munich, Germany in 2006 and received his post-

doctoral training at the State Center for cell and gene therapy. His research has focused on identifying Interacting proteins of C/EBP α that may negatively regulate its functions leading to block in differentiation, culminating to acute myeloid Leukemia (AML). Recently, his research group is also working on regulation of E3 Ubiquitin Ligases in pathogenesis of cancer and their potential as therapeutic targets. Major objective has been to identify deregulated E3 ubiquitin ligases and their substrates which may have implications in cancer pathogenesis. He has received CSIR Young Scientist Award in Biological Sciences-2013 and Indian National Science Academy (INSA) Medal for Young Scientist 2013. He has published about 25 papers in peer reviewed international Journals till date.

Talk title: Proteomic identification of C/EBPalpha-p30 target proteins in acute myeloid leukemia

CCAAT/enhancer-binding protein alpha(C/EBP α) is a tumor suppressor and a critical regulator for early myeloid differentiation; it acts upon GCSFr promoter to promote granulopoiesis in particular. Owing to its role in myeloid differentiation, mutations in C/EBP α gene leading to block in myeloid differentiation and thus myeloid leukemia has very often been reported in several AML patients. N-terminal mutation leading to translation from an internal translational initiation site leads to the expression of a shorter 30-kDa dominant negative isoform C/EBP α -p30). Recent reports suggest that C/EBP α -p30 imparts its dominant negative effects over wild type C/EBP α via binding to target genes of C/EBP α . In addition, it has also been suggested that C/EBP α -p30 may target completely different genes that may have negative impact on myeloid differentiation. We therefore, sought to apply a global proteomics approach to identify the target proteins of C/EBP α -p30 using E2-inducible C/EBP α -p30 stable cell line, we show that Ubc9, an E2-conjugating enzyme essential for sumoylation is increased in its expression when C/EBP α -p30 is induced. We validated the increased expression of Ubc9 in patients with AML having C/EBP α -p30 mutations compared with other subtypes. We further confirmed that the increase of Ubc9 expression was mediated through increased transcription. Furthermore, we show that Ubc9-mediated enhanced sumoylation of C/EBP α -p42 decreases the transactivation capacity on a minimal C/EBP α promoter. Importantly, over expression of C/EBP α -p30 in granulocyte colony-stimulating factor (G-CSF)-stimulated human CD34 $^+$ cells led to a differentiation block, which was overcome by the siRNA-mediated silencing of Ubc9. In nutshell, our data indicates that Ubc9 is an important C/EBP α -p30 target through which C/EBP α -p30 enhances the sumoylation of C/EBP α -p42 to inhibit granulocytic differentiation.

INVITED LECTURE-3

Speaker: Dr. Chung Ching Maxey

National University of Singapore, Singapore

Biography: Maxey C.M. Chung, PhD is from the Department of Biochemistry, Yong Loo Lin School of Medicine, and who also holds a joint appointment with the Department of Biological Sciences, Faculty of Science, at the National University of Singapore, Singapore. He is presently the Secretary General of Human Proteome Organization (HUPO), and was previously Chair of the HUPO Awards Committee, a post he had held from 2009 – 2012. He has also been an active council member of Asian Oceanian Human Proteome Organization (AOHupo) since its inception, and was its Secretary General from 2007- 2012. He is currently serving as Senior Editor for *Proteomics* and *Proteomics*

- *Clinical Applications*, as well as being a member of the Editorial Board of *Journal Proteome Research*, *Journal of Proteomics*, and *Translational Proteomics*. He has co-edited a Special Issue of Proteomics titled “*Membrane Proteomics*” in 2008, and more recently on a special issue on “*Subcellular Proteomics*” that was published in November, 2010. In addition he is also a regular reviewer for several leading biochemical and proteomics journals. His laboratory has been focusing on the applications of proteomics in the study of human diseases, especially in cancer biomarker discovery. Specifically, his area of focus has been on gastrointestinal cancers, viz., gastric, liver and colon cancers. In recent years, his laboratory has also worked on the identification and elucidation of the proteins and the pathways involved in cancer metastasis using functional proteomics approaches. For this latter part of the work, he collaborates extensively with clinical colleagues from the hospitals in Singapore.

Talk title: Searching for biomarkers involved in colorectal cancer metastasis from the proteomes and secretomes of two isogenic colorectal cancer cell lines

Wei Wu¹, Qifeng Lin¹, Hwee Tong Tan¹ and Maxey C.M. Chung^{1, 2}

¹Department of Biochemistry, Yong Loo Lin School of Medicine,

²Department of Biological Sciences, Faculty of Science, National University of Singapore, Singapore

Colorectal cancer is the fourth most common cause of cancer deaths and the high mortality rate is mostly ascribed to liver

metastasis. To address the clinical needs in colorectal cancer metastasis management, we applied proteomics to identify intracellular and secreted proteins differentially expressed in the colon adenocarcinoma cell line HCT-116 and its metastatic derivative, E1. From this investigation, several proteins including stathmin-1 (STMN1) and drebrin (DBN1) were shown to be up-regulated in the metastatic cell line^{1,2}. Subsequent validation in clinical samples by immuno-histochemistry (IHC) and tissue microarray further confirmed STMN1 up-regulation as a shared hallmark of CRC progression and poor 5-year survival. Functional investigations *in vitro* also demonstrated that high STMN1 expression promotes a range of metastatic processes including migration, invasion, loss of adhesion and anchorage-independent colony formation. IHC analyses on patient tissue samples additionally showed that up-regulation of DBN1 could be observed in lymph node and liver metastases, indicating that DBN1 could have a clinically relevant role in colorectal cancer metastasis.

Subsequently, we also analysed the HCT-116 and E1 secretomes to search for novel biomarkers for prognosis and disease monitoring. After enriching for secreted glycoproteins using multi-lectin affinity chromatography, we observed the up-regulation of Laminin subunit beta-1 (LAMB1) in the E1 secretome. Interestingly, the level of LAMB1 was shown to be significantly higher in patient sera as compared to control sera. Based on the ELISA analysis of 92 serum samples, LAMB1 has the potential as a biomarker to discriminate between colorectal cancer patients from the controls, with an improved diagnostic performance over the current gold standard, carcinoembryonic antigen (CEA).

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SESSION II: PROTEOMICS OF BIOFLUIDS

PLENARY LECTURE-2

Speaker: Dr. Samir Hanash

The University of Texas MD Anderson Cancer Center, USA

Biography: Samir Hanash is one of the founding members of Human Proteome organization. Dr. Hanash has expertise in development and application of integrated approaches for the molecular profiling of cancer. He acquired his PhD degree from University of Michigan in field of human genetics and clinical pediatric oncology, and has been the principal investigator for the projects funded by National Cancer Institute. Presently he is head of the newly developed program in molecular diagnostics at Fred Hutchinson Cancer Research Center.

Talk title: Integrating biomarkers into lung cancer screening

Lung cancer is the leading cause of cancer death worldwide. Lung cancer screening with low-dose computed tomography (LDCT) has been shown to reduce mortality by 20%, although there are concerns including high false positivity, cost, and radiation exposure. Of note, the false positive rate of lung cancer screening with LDCT alone was 96.4% in the National Lung Cancer Screening Trial. Although false positives may be reduced with stricter nodule criteria, blood-based markers are a promising and attractive approach to complement LDCT because of the potential to identify those subjects that may be at increased risk of developing lung cancer, or that may be harboring early and potentially curable lung cancer, or that need to undergo further work-up for their indeterminate nodules. We have identified through a series of discovery and initial validation studies, a set of protein and autoantibody biomarker candidates with potential for early detection. Our goal is to develop a blood-based biomarker panel and test it in a screening setting to validate its utility to reduce unnecessary and invasive work-up by determining among screened subjects those individuals with lung nodules who would need or would not need further diagnostic work-up, and consequently to reduce mortality associated with lung cancer through more effective screening strategies. The progress made to date will be presented.

INVITED LECTURE-4

Speaker: Dr. Yetrib Hathout

Children's Research Institute, USA

Biography: Dr. Hathout obtained his PhD in Biochemistry and Molecular Cellular Biology in 1992 at the University of Burgundy in Dijon (France). After his PhD Dr. Hathout joined the laboratory of Biochemical Mass Spectrometry at the University of Maryland Baltimore County to work with Dr. Catherine Fenselau as postdoctoral fellow on drug protein interactions using mass spectrometry. In 1998 Dr. Hathout moved to the University of Maryland and continued to work on proteomics applications to study mechanisms of drug resistance in breast cancer cells. Dr. Hathout joined the Center for Genetic Medicine at Children's National Medical Center in 2003 to work on different type of pediatric diseases. Currently Dr. Hathout holds an associate professor position at the Center. At children's hospital Dr.

Hathout worked at the interface between clinicians and basic research scientists on proteomics application to study molecular mechanisms of pediatrics disease including brain tumors, neurodegenerative diseases and more recently muscular diseases. His laboratory is currently developing novel and innovative biomarker work for muscular dystrophies, the goal is to define less invasive serum and urine biomarkers to monitor muscular dystrophy disease progression and response to therapies. This will aid current and future clinical trials. Dr. Hathout has published more than 64 articles in the field of proteomics and biomedical applications.

Talk title: Serum protein biomarkers discovery in Duchenne muscular dystrophy using mass spectrometry and aptamers technology

Yetrib Hathout, Ramya L. Marathi, Sree Rayavarapu, Aiping Zhang, Kristy J. Brown, Haeri Seol, Heather Gordish-Dressman, Sebahattin Cirak, Luca Bello, Kanneboyina Nagaraju, Terry Partridge, Eric P. Hoffman, Shin'ichi Takeda, Jean K. Mah, Erik Henricson and Craig McDonald.

¹Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC20010, USA, ²Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Ogawa-higashi, Kodaira Tokyo 187-0031, Japan, ³Department of Pediatrics, Alberta Children's Hospital, Calgary, AB, Canada T3B 6A8 and ⁴Department of Physical Medicine and Rehabilitation, University of California, Davis School of Medicine, Davis, CA 95618, USA

Duchenne muscular dystrophy (DMD) is one of the most common and severe form of childhood muscular dystrophies affecting about 1 in 5,000 boys. The need to define surrogate biomarkers to monitor DMD progression and response to therapies is becoming crucial as promising treatment strategies are entering phase II and III clinical trials for this disease. The most commonly used surrogate endpoint for DMD to date, is the 6 minute walk test. However, this test seems to be not sensitive enough for clinical trials often conducted in a short period of time and also impractical for DMD patients who lost ambulation. In this study we implemented different proteome profiling strategies to systematically define blood circulating proteins associated with muscular dystrophy. In the first method we used SILAC mouse strategy in combination with mass spectrometry to accurately identify 29 serum protein biomarkers associated with DMD progression using two dystrophin deficient mouse models mdx-23 and mdx-52. Elevated proteins were mostly of muscle origin and included myofibrillar proteins, glycolytic enzymes, transport proteins and other muscle specific proteins. Decreased proteins were mostly of extracellular origin and included growth factors. Analysis of sera sampled from 1 week to 7 months old mdx mice revealed age-dependent changes in the level of these biomarkers with most biomarkers acutely elevated at 3 weeks of age. Serum analysis of DMD patients, with ages ranging from 4 to 15 years old, confirmed elevation of 20 of the murine biomarkers in DMD, with similar age related changes. In the second method we implemented high throughput SomaScan (aptamer) technology to screen levels of 1129 proteins in sera samples of DMD patients (n = 51) and age matched healthy volunteers (n = 17). This analysis validated previous mass spectrometry discovered biomarkers but also identified additional biomarkers overlooked

by mass spectrometry technology. Aptamer technology was found highly sensitive and reproducible over a wide dynamic range (up to 5 logs) enabling identification of 91 potential biomarkers. Most validated biomarkers were elevated in DMD sera (80%), and a minority decreased relative to volunteers (20%). The large majority of biomarkers showed a log-linear correlation with DMD disease progression, where they were at highest levels in the youngest patients, and declined with advancing age. This study provides a panel of biomarkers that reflect muscle activity and pathogenesis and should prove valuable tool to complement natural history studies and to monitor treatment efficacy in future clinical trials.

INVITED LECTURE-5

Speaker: Dr. Abhijit Chakrabarti

Saha Institute of Nuclear Physics, Kolkata, India

Biography: Abhijit Chakrabarti joined Saha Institute of Nuclear Physics, Kolkata in 1994 as a Lecturer after a postdoc in the Department of Biology of The Johns Hopkins University, Baltimore, USA (1990-1994). He did his PhD from Department of Biochemistry, Indian Institute of Science before that in 1990. Currently he is a Professor and Head of the Crystallography & Molecular Biology Division with research interest is in spectrin-based network of red blood cell, membrane asymmetry, assisted folding of proteins, hemoglobin variants, their implications in hemoglobinopathy and spectroscopic studies on structure-function relation of proteins. Since last 12 years his lab is actively involved in proteomic studies of blood disorders e.g. leukemia and thalassemia. He has published about 80 papers in peer reviewed international Journals till date.

Talk title: Clinical proteomics of hemolysates in hemoglobinopathy

Sickle cell disease (SCD) and HbE β-thalassemia, when HbE interacts with β-thalassemia, produce clinical manifestation of varying severity. Our efforts have been to study changes in protein levels of erythrocyte cytosol isolated from SCD & HbE β-thalassemic patients compared to normal individuals since clinical manifestations of both the diseases are quite heterogeneous. We have used 2D Gel, 2D fluorescence difference gel electrophoresis (DIGE) and MALDI-MS/MS-based techniques to investigate the differential proteome profiling of Hb-depleted fraction of cytosolic proteins of erythrocytes isolated from the peripheral blood samples of SCD and HbE β-thalassemia patients and normal volunteers. Our study showed that redox regulators such as peroxiredoxin 2, Cu-Zn superoxide dismutase and thioredoxin and chaperones such as α-hemoglobin stabilizing protein and HSP-70 were up-regulated in both SCD and HbE-β-thalassemia. Particularly in SCD, proteasomal subunits are found to be up regulated and phospho-Catalase level altered. Peroxiredoxin-2 shows significant dimerization in the SCD patients, a hallmark of oxidative stress inside erythrocytes. Our observations on the changes in the proteomic levels of redox regulators and chaperones in hemoglobinopathy could be important in understanding the role of HbE in disease progression and pathophysiology. Severe oxidative stress inside erythrocyte is evident in both the hemoglobinopathies and most

of the differentially regulated proteins are also common in both the hemoglobinopathies. Proteomics studies of hemolysates from other diseases e.g. hereditary spherocytosis, other body fluids e.g. serum in thalassemia and urine in urothelial neoplasm have also been initiated.

INVITED COMPANY LECTURE-1

Speaker: Dr. Rudolf Grimm

Agilent Technologies Inc., USA

Biography: Rudolf Grimm received his Ph.D. in Biology from the University of Munich/Germany. After completing a post-doc time at the University of Freiburg/Germany and the Riken Institute in Tokyo/Japan he joined Hewlett-Packard as a senior life science application chemist in 1991. In 1998 he became the head of protein chemistry at the Munich based and world's first start-up proteomics company Toplab. In June 1999 he joined Hexal Pharma (now part of Sandoz/Novartis) to establish the Biotech Laboratories for the successful development of generic recombinant protein drugs (Biosimilars). In September 2002 he re-joined Agilent Technologies as the worldwide proteomics and metabolomics market development manager. In January 2009 he became a Director of Science and Technology at Agilent Technologies. In September 2010 Rudi became an Adjunct Professor at the University of California in Davis and in August 2011 an Adjunct Professor at Chungnam National University in Daejeon/South-Korea. In 2012 he was appointed as Associate Director of Analytical Innovation at the Food for Health Institute of UC Davis. He is currently author and co-author of 130 scientific publications.

Talk title: A paleo-multiomics study of the stomach content of oldest human ice mummy the 5300y old Tyrolean Iceman or Ötzi

Rudolf Grimm^{1,2,3}, Hyun Joo An^{2,3}, Grace Ro², Sureyya Ozcan², Carlito Lebrilla², Oliver Fiehn², John Meissen², Rob Moritz⁴, Michael Hoopman⁴, Holly Ganz², Bart Weimer², David Bishop⁵, Philip Doble⁵, Amaury Gassiot⁶, Markus Wenk⁶, Frank Maixner⁷ and Albert Zink⁷

¹*Agilent Technologies, Santa Clara, USA*

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³*Chungnam National University, Daejeon, South-Korea*

⁴*ISB, Seattle, USA*

⁵*University of Technology Sydney Australia*

⁶*National University of Singapore, Singapore*

⁷*Institute for Mummies and the Iceman, EURAC, Bolzano, Italy*

Ötzi the Iceman is a well-preserved natural mummy of a man who lived about 5300 years ago. The mummy was found in September 1991 in the Ötztal Alps, hence Ötzi, near the Similaun Mountain and Hauslabjoch on the border between Austria and Italy. He is the oldest known natural human ice mummy. We performed the first paleo- multiomics study of this famous mummy including genomics, proteomics, glycomics, metabolomics, lipidomics and metallomics approaches. Complex multiomics data will be presented from samples taking out of his stomach. Interesting insights will be given in his last meal(s) and health conditions before he got killed.

SESSION III: PROTEOMICS & SYSTEMS BIOLOGY

INVITED LECTURE-6

Speaker: Dr. Brenda Andrews

University of Toronto, Canada

Biography: Dr. Brenda Andrews completed her PhD from the University of Toronto in Medical Biophysics in 1986. She then carried out her post doctoral work at The University of California, San Francisco. She is a Professor and Chair of the Banting & Best Department of Medical Research within the Faculty of Medicine at the University of Toronto. She is also Director of the Terrence Donnelly Center for Cellular and Biomolecular Research, a new interdisciplinary research institute with the mandate to create a research environment that encourages integration of biology, computer science, engineering and chemistry and that spans leading areas of biomedical research.

Talk title: Global protein abundance and localization maps define dynamic subcellular flux networks in yeast

A fundamental goal of cell biology is to define the dynamic properties of proteins in the context of different cellular compartments and environments. To evaluate protein abundance and localization on a proteome-scale in budding yeast, we developed a combined experimental and computational method to automatically generate global abundance-localization maps (ALMs) derived from measurements acquired at single-cell resolution. Our method combines array-based yeast genetics and automated microscopy for systematic and quantitative cell biological screens or phenomics. We exploit the yeast GFP collection, a unique resource consisting of thousands of strains with different genes uniquely tagged with GFP. Using this pipeline and the GFP collection, we generated an ALM of wild-type cells which includes ~3000 proteins (nodes) connected to one or more of 16 localization classes (hubs). To study proteome dynamics, we used a computational approach to map flux networks derived from ALMs produced following chemical or genetic perturbations. Our analysis revealed that the proteome is regulated broadly at the level of protein abundance changes with localization changes, including mass translocation of protein complexes such as the exosome, occurring primarily in response to specific perturbations. Flux networks provide a systems-level view and novel insight into global protein turnover and movement in eukaryotic cells. Our general approach, in particular our network analysis and visualization methods, is readily extensible to other systems.

INVITED LECTURE-7

Speaker: Dr. Andrew Link

Vanderbilt University School of Medicine, USA

Biography: Andrew J. Link, Ph.D. is an Associate Professor in the Department of Microbiology, Immunology and Pathology at Vanderbilt University School of Medicine in Nashville, TN. He received his B.S. in chemical engineering and B.A. and M.A. degrees in biology from Washington University in St. Louis, MO. He did his Master's dissertation project in the laboratory of Dr. Maynard Olson at Washington University School of Medicine. Dr. Link earned his Ph.D. in Genetics from Harvard University

where he did his dissertation work in Dr. George Church's laboratory. Dr. Link did his postdoctoral work in Dr. John Yates' laboratory at the University of Washington in Seattle, WA. As a postdoctoral fellow, Dr. Link pioneered the development of multidimensional chromatography coupled with tandem mass spectrometry for the shotgun proteomics analysis of whole proteomes and macromolecular protein complexes. Dr. Link's laboratory couples affinity purification approaches with proteomics technologies to identify novel protein interactions and posttranslational modifications in both eukaryotic transcription and translation complexes. Recently, his group has developed cell-specific system biology approaches to model the human immune response to vaccines. His lab applies protein profiling by multidimensional LC/MS/MS, label-free and isotopic quantification approaches, novel computational algorithms and data visualization strategies, and the integration of transcriptomic and proteomic data to model complex cellular processes and responses. Dr. Link is an associate editor for the journal *Proteomics* and serves on the editorial board of several journals. Dr. Link was a principal instructor at the CSHL Proteomics course for 10 years.

Talk title: Systems analysis of cell-specific immune responses to adjuvanted influenza vaccines

Systems biology is an approach to comprehensively study complex interactions within a biological system. Most systems vaccinology studies have utilized whole blood or peripheral blood mononuclear cells (PBMC) to monitor the immune response after vaccination. Because human blood is comprised of multiple hematopoietic cell types, the potential for masking responses of under-represented cell types is likely increased when analyzing whole blood or PBMC. To investigate the contribution of individual cell types to the immune response after vaccination, we established a rapid and efficient method to purify human T and B cells, natural killer (NK) cells, myeloid dendritic cells (mDC), monocytes, and neutrophils from fresh venous blood. Purified cells were fractionated and processed in a single day. Quantitative shotgun proteomics and RNA- were performed to determine expression profiles for each cell type prior to and after influenza vaccination. The results show that proteomic and transcriptomic profiles generated from purified immune cells differ significantly from PBMC. Differential expression analysis for each immune cell type during the early response after vaccination show unique proteomic and transcriptomic expression profiles and biological networks. This cell type-specific information provides a comprehensive systems biological approach to monitor vaccine responses.

INVITED LECTURE-8:

Speaker: Dr. Sarath Chandra Janga

Indiana University and Purdue University, USA

Biography: Dr. Sarath is an Assistant Professor in the School of Informatics and Computing at IUPUI with joint appointments in the Center for Computational Biology and Bioinformatics, Department of Medical and Molecular Genetics as well as Division of Gastroenterology/Hepatology in the Department of Medicine at the Indiana University School of Medicine. He obtained his PhD from University of Cambridge in Molecular and Systems Biology at the MRC Laboratory of Molecular Biology

and was a Post-doctoral fellow (Institute for Genomic Biology) at the University of Illinois in Urbana-Champaign. His team develops computational approaches to mine complex and heterogeneous data sources publicly available in biomedical sciences, with the goal of understanding how the regulation, structure and dynamics of biological systems shape the phenotypic landscape of an organism and its relevance to disease conditions. To achieve this overarching goal they exploit diverse sources of data, resulting from the ever increasing number of high-throughput technologies, to convert it into knowledge.

Talk title: Uncovering the regulatory networks at post-transcriptional level in health and disease using genomic and systems approaches

An important notion that is emerging in post-genomic biology is that cellular components can be visualized as a network of interactions between different molecules like proteins, RNA, DNA, metabolites and small molecules. This has led to the application of network theory to a wide range of biological problems including understanding regulation of gene expression, function prediction, biomarker identification and drug discovery settings. While my lab has been employing these approaches in a number of different contexts, in this seminar I will focus on regulatory networks controlled by Transcription Factors (TFs), RNA-binding proteins (RBPs) and regulatory RNAs. For instance, in post-transcriptional networks, typically trans-acting elements like RBPs form one set of nodes and their target transcripts/genes, of which they control the activity, form the other set of nodes. The links between them which have directionality from the trans-acting elements to their target genes, controlled by their cis-regulatory elements, form a complex and directional network of interactions. In the first half of my talk, I will focus on our recent understanding of the structure of the post-transcriptional regulatory networks in eukaryotic organisms. I will then present recent studies to uncover unconventional RBPs in various model organisms. In the second half, I will present our efforts to systematically dissect the expression dynamics of RNA-binding proteins (RBPs) in post-transcriptional networks (formed by RBPs and their target RNAs) and how RBPs are dysregulated across human cancers. Our analysis shows that RBPs generally exhibit high protein stability, translational efficiency and protein abundance but their encoding transcripts tend to have low half-life. Analysis of the RBP-RNA interaction network revealed that the number of distinct targets bound by an RBP (connectivity) is strongly correlated with its protein stability, translational efficiency and abundance. Finally, I will present our recent results demonstrating that RBPs are consistently and significantly highly expressed compared to other classes of genes (non-RBPs) as well as in comparison to well-documented groups of regulatory factors like Transcription Factors (TFs), miRNAs and long non-coding RNAs (lncRNAs) across all the human tissues examined. Our analysis also revealed the existence of a unique signature of ~30 RBPs that are very highly expressed across at least two-thirds of the nine cancers profiled in this study, and could be labeled as strongly upregulated (SUR) set of RBPs.

INVITED LECTURE-9

Speaker: Dr. Debasis Dash

CSIR-Institute of Genomics and Integrative Biology, New Delhi, India

Biography: Dr. Debasis Dash is a scientist at the CSIR-Institute of Genomics and Integrative Biology, New Delhi, India. His

research group is interested in developing algorithms and tools in the area of computational biology. His specific interests are developing novel approaches to annotate proteomes, analyse exomes, metagenomes and understand how variations at molecular level contribute to systemic level through ayurgenomics. His group has developed several tools and algorithms such as GenoCluster - for protein coding gene identification, PLHost - for Protein function assignment, machine learning tool "Pro-Gyan" for protein classification, "MassWiz", "Genosuite" and "Proteostat" for proteomics etc. Through collaboration, he works on understanding Ayurgenomics and deciphering signatures of extra-pulmonary tuberculosis. He has 2 patents under himself. 1) A computer based method for identifying peptides useful as drug targets and 2) A computer based method for predicting protein coding DNA sequences useful as drug targets.

Talk title: Computational proteomics pipeline for discovery of novel proteins in biological systems

Proteins are major components of molecular machinery within cellular systems. Development of methods for the discovery and identification of these proteomic landscapes are crucial in understanding any biological system. Proteomics experiments enabled by next-generation mass spectrometers assist in this but spew out more data per second that can be analyzed manually. The burden is eased by computational algorithms that automatically analyze these large amounts of datasets to fish-out known and novel proteins which help in understanding the biological phenomenon at molecular level in exquisite detail and throughput. We developed a novel intensity based algorithm MassWiz that enhances peptide identification and better segregated true peptides as compared to Mascot, Sequest, X!Tandem and OMSSA across diverse data sets and instrument types. The FlexiFDR re-scorer enhanced the resolution of true-vs-false hits further by exploiting the mass and charge state of identified peptides. Better statistical treatment using target-decoy approach is made easy by the ProteoStats library which makes FDR calculation and data evaluation easy with several available methods for comparison. The ProteomeAnalyst suite integrated the results from multiple algorithms and helps in visual analytics of the results and enhances sensitivity and specificity of peptide identifications and is supplemented with Protein Assembler algorithm for protein inference. Complete genome annotation of protein coding genes is a prerequisite for defining components of the biological system. Our integrated suite of software, Genosuite performs this task with the help of mass spectrometry data using four database search algorithms including MassWiz. The automatic pipeline aided discovery of novel gene products in the proteome of model organisms like *Bradyrhizobium japonicum* USDA110, *Methylobacterium extorquens* AM1 and pathogens like *Shigella flexneri*, suggesting that even well studied organism proteomes are not completely annotated.

Proteogenomic approaches for eukaryotes need more complex solutions owing to alternative splicing and integration using RNASeq and proteomics as implemented in EuGenosuite software helps in carving out the novel gene products in eukaryotes. Applying the pipeline on a shotgun proteomics data set, we could re-annotate *Rattus norvegicus*, another model organism close to humans. Dissecting out the genetic architecture with complete annotations is a big step and serves a springboard for transnational research. These advances were not possible without the unique software tools available at our disposal and such method developments need to go hand in hand for large scale systems biology projects.

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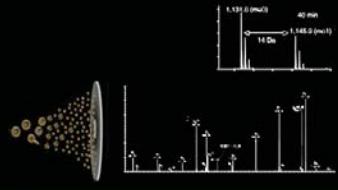


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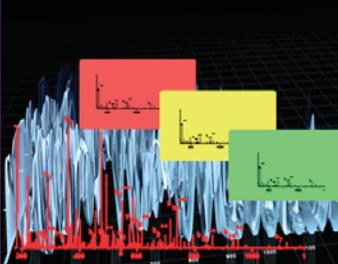
8TH DECEMBER 2014

SESSION IV: MASS SPECTROMETRY & PTM

Mass Spectrometry and PTM	Plenary Talk	Invited talks	
	 Dr. John R. Yates (The Scripps Research Institute)	 Dr. David C. Muddiman (North Carolina State University, USA)	 Dr. Sanjay Gupta (ACTREC, India)

The hydrophilic nature of PTM makes the sample handling and purification extremely challenging prior to MS analysis. Successful strategies for PTM analysis using mass spectrometry and specific PTM studies will be discussed in this session.

SESSION V: NEW MS APPROACHES

NEW MS APPROACHES	Plenary Talk	Invited talks		
	 Dr. Catherine Fenselau (Ex-President US-HUPO, ASMS Ex-Vice President -HUPO University of Maryland, USA)	 Mr. Brendan MacLean (University of Washington, USA)	 Dr. Shantanu Sengupta (Institute of Genomics and Integrative Biology, India)	 Dr. Philip Andrews (University of Michigan, USA)

High-resolution mass spectrometry is one of the most attractive choices for large-scale quantitative proteomic analysis. The technological challenges associated with both at the instrumentation and data-analysis levels have emerged in last few years, which will be discussed in this session.

SESSION VI: INTERACTOMICS, MULTI-OMICS ANALYSIS

INTERACTOMICS, MICROARRAYS & LABEL-FREE	Invited talks		
	 Dr. Manuel Fuentes (University of Salamanca, Spain)	 Dr. Prasanna Venkatraman (ACTREC, India)	 Dr. Sanjeeva Srivastava (IIT Bombay, India)

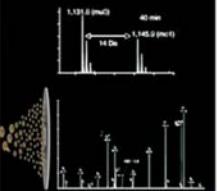
Protein/antibody microarrays and label-free proteomics are still at an early stage of development but its promises for the high-throughput proteomics applications will be discussed in this session.

SESSION VII: HUMAN PROTEOME PROJECT

HPP & PROTEOMICS	Plenary Talk	Invited talks		
	 Dr. Mark S. Baker <i>(HUPO President-Elect) Macquarie University, Australia</i>	 Dr. Robert Moritz <i>(Institute for Systems Biology, USA)</i>	 Dr. Ravi Sirdesmukh <i>(Institute of Bioinformatics, India)</i>	 Dr. Sanjay Navani <i>(Lab SurgPath, India)</i>

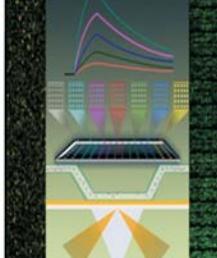
This session would discuss various HUPO initiatives, including HPP and various educational and training programs. This will be a stepping stone towards modern diagnostics, prognostics, therapeutics and preventive medical applications.

INVITED COMPANY LECTURES

NEW MS APPROACHES	Invited Company Talk
	 Dr. Mark McDowell <i>(Waters Corporation, UK)</i>

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INTERACTOMICS, MULTI-OMICS ANALYSIS	Invited Company Talk
	 Dr. Robert Karlsson <i>(GE Healthcare Life Sciences)</i>

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SESSION IV: MASS SPECTROMETRY & PTM

PLENARY LECTURE-3

Speaker: Dr. John Yates

The Scripps Research Institute, USA

Biography: John R. Yates is the Ernest W. Hahn Professor in the Department of Chemical Physiology and Molecular and Cellular Neurobiology at The Scripps Research Institute. His research interests include development of integrated methods for tandem mass spectrometry analysis of protein mixtures, bioinformatics using mass spectrometry data, and biological studies involving proteomics. He is the lead inventor of the SEQUEST software for correlating tandem mass spectrometry data to sequences in the database and developer of the shotgun proteomics technique for the analysis of protein mixtures. His laboratory has developed the use of proteomic techniques to analyze protein complexes, posttranslational modifications, organelles and quantitative analysis of protein expression for the discovery of new biology. Many proteomic approaches developed by Yates have become a national and international resource to many investigators in the scientific community. He has received the American Society for Mass Spectrometry research award, the Pehr Edman Award in Protein Chemistry, the American Society for Mass Spectrometry Biemann Medal, the HUPO Distinguished Achievement Award in Proteomics, Herbert Sober Award from the ASBMB, and the Christian Anfinsen Award from The Protein Society, the 2015 ACS Analytical Chemistry award and 2015 The Ralph N. Adams Award in Bioanalytical Chemistry. He was ranked by Citation Impact, Science Watch as one of the Top 100 Chemists for the decade, 2000-2010. He was #1 on a List of Most Influential in Analytical Chemistry compiled by *The Analytical Scientist* 10/30/2013 and is on the List Of Most Highly Influential Biomedical Researchers, 1996-2011, *European J. Clinical Investigation* 2013, 43, 1339-1365. He has published over 750 scientific articles with ~60,000 citations, and an H index 122.

Talk title: Using mass spectrometry to understand cystic fibrosis as a protein misfolding disease

John R. Yates, Sandra Pankow, Casimir Bamberger

A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. Several major technologies, but especially mass spectrometry, have benefited from large-scale genome sequencing of organisms. The sequence data produced by these efforts can be used to interpret mass spectrometry data of proteins and thus enables rapid and large-scale analysis of protein data from experiments. Advances in multi-dimensional separations as well as mass spectrometry have improved the scale of experiments for protein identification. This has improved the analysis of protein complexes, and more complicated protein mixtures. Quantitative mass spectrometry can be used to study biological processes such as protein-protein interactions, development or the effects of gene mutations on pathways. Recent studies on the loss of function mutant form of the Cystic Fibrosis Transport Regulator (?F508) as it progresses through the folding pathway will be presented. Through the study of protein-protein interactions and modifications that regulate maturation of CFTR, we are beginning to understand the critical interactions regulating pathways for export or destruction.

INVITED LECTURE-10

Speaker: Dr. David C. Muddiman

North Carolina State University, USA

Biography: David C. Muddiman is currently a Distinguished Professor of Chemistry and Founder and Director of the W.M. Keck FT-ICR Mass Spectrometry Laboratory at North Carolina State University. Prior to this, David was a Professor of Biochemistry and Molecular Biology and Founder and Director of the Mayo Proteomics Research Center at the Mayo Clinic College of Medicine in Rochester, MN. Prior to his appointment at the Mayo Clinic, he was an Associate Professor of Chemistry at Virginia Commonwealth University where he began his academic career as an Assistant professor in 1997 with an adjunct appointment in the Department of Biochemistry and Molecular Biology. David received his Ph.D. in Analytical Chemistry from the University of Pittsburgh in 1995 under the auspices of David M. Hercules. He then was a Department of Energy Postdoctoral Fellow at Pacific Northwest National Laboratory in the Environmental Molecular Sciences Laboratory working with Richard D. Smith from 1995-1997. Importantly, David had gained significant experience in the area of surface science during his graduate work with Professor Hercules and then electrospray ionization and Fourier transform ion cyclotron resonance mass spectrometry with Dr. Smith. He currently serves on the Editorial Advisory Board of *Mass Spectrometry Reviews*, *Molecular and Cellular Proteomics*, *Rapid Communications in Mass Spectrometry* and the *Journal of Chromatography B*. David is also an Associate Editor for the *Encyclopedia of Analytical Chemistry*. He also serves on the advisory board of the NIH Funded Complex Carbohydrate Research Center, University of Georgia, National Science Foundation FT-ICR Mass Spectrometry Laboratory, National High Magnetic Field Laboratory, Florida State University and the Yale/NIDA Neuroproteomics Center, Yale University. He is currently a member of the ASMS Board of Directors (elected) and is the Treasurer and member of Executive Board of US-HUPO (elected). His group has presented over 500 invited lectures and presentations at national and international meetings include 17 plenary/keynote lectures. His group has published over 200 peer-reviewed papers and has received four US patents. He is the recipient of the 2010 Biemann Medal, American Society for Mass Spectrometry, 2009 NCSU Alumni Outstanding Research Award, the 2004 ACS Arthur F. Findeis Award, the 1999 American Society for Mass Spectrometry Research Award, and the 1990-91 Safford Award, University of Pittsburgh, for Excellence in Teaching.

Talk title: An innovative atmospheric pressure ionization source for mass spectrometry imaging/ INLIGHT strategy for quantitative glycomics

Mass-spectrometry has the ability to elucidate new diagnostic, prognostic, and therapeutic biomarkers and translate them to the clinic. However, there are numerous challenges that exist that must be overcome. This presentation will discuss the challenges specific to the study of epithelial ovarian cancer (EOC) in humans and how these challenges have directed our thinking in terms of the development of mass spectrometry-based bioanalytical strategies. First, to augment the human model, we have developed a chicken model of spontaneous EOC which

allows us to control the environment and genetic background, a model which is characterized by rapid onset and progression of disease, and allows us to conduct longitudinal sampling. Second, we have developed hydrophobic tagging reagents to increase the electrospray response of *N*-linked glycans which has the major added benefit of being able to incorporate a stable-isotope label for use in relative quantification experiments. Third, we have developed a novel ionization technique that can be used for direct analysis and tissue imaging; the latter being a fruitful arena for biomarker discovery and understanding drug distribution and metabolism.

INVITED LECTURE-11

Speaker: Dr. Sanjay Gupta

Advanced Centre for Treatment Research and Education in Cancer, Navi Mumbai, India

Biography: Dr. Sanjay Gupta is a principal investigator at the TMC-Advanced Centre for Treatment Research and Education in Cancer, Navi Mumbai, India. A major research interest in his lab is to understand the epigenetic changes that take place at the molecular level during different stages of malignant transformation and DNA repair per se. Recently; we have uncovered an alteration of histone variants in initiation and progression of hepatocarcinogenesis. Current goals of the lab are to understand (1) how different alteration in histones in specific cell cycle stage affects their role in DNA damage response that escort repair machinery proteins in and out of the chromatin (2) transcriptional regulation of histone variants, variant-specific PTMs and their binding partners during carcinogenesis (3) how structure and function of chromatin fiber is altered when the canonical histone is replaced by histone variant and (4) how all these alterations can be used for better treatment modalities.

Talk title: Differential incorporation of histone H2A isoforms: Adding higher level of complexity in chromatin organization during development and cancer

¹Saikat Bhattacharyya, ¹Divya V Reddy, ¹Monica Tyagi and ¹Sanjay Gupta

¹Epigenetics and Chromatin Biology Group, Gupta Laboratory, Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Kharghar, Navi Mumbai, 410210, India.

Histone variants are emerging as key players in shaping chromatin structure, and, thus, in regulating fundamental cellular processes such as chromosome organization and gene expression. The expression is regulated in part through covalent post-translational modifications of histones, their variants and altered nucleosomal structure. Recent studies suggests role of histone variants in tumor development and progression, and the cancer-associated alteration in H2A variants are rapidly being uncovered. Earlier studies from our lab have identified and shown the differential expression of two major H2A isoforms, H2A.1 and H2A.2, both in hepato-cellular carcinoma and liver development. Despite the fact that the phenotypic association of H2A isoform has been elucidated in different physiological conditions nothing is known about their function at the molecular level.

We will present our recent work on understanding the regulation of H2A.1 and H2A.2 variants, their effects on nucleosomal dynamics and impact on cellular proliferation. Collectively, our findings implicate histone variants as potential players for cancer development via altering the structure and dynamics of the local chromatin fiber.

SESSION V: NEW MS APPROACHES

PLENARY LECTURE-4

Speaker: Dr. Catherine Fenselau

University of Maryland, USA

Biography: Catherine Clarke Fenselau is an American scientist who was the first trained mass spectrometrist on the faculty of an American medical school; she joined Johns Hopkins School of Medicine in 1968. She specializes in biomedical applications of mass spectrometry. She has been recognized as an outstanding scientist in the field of bioanalytical chemistry because of her work using mass spectrometry to study biomolecules.

Talk title: Advances in mass spectrometry for protein analysis

Interest has grown in the analysis of intact proteins by mass spectrometry. Major applications include quality control in the production of therapeutic antibodies, where 'top-down' analysis is faster than 'bottom-up' analysis and eliminates the possibility of modification during tryptic digestion. A more fundamental application is the identification of cell surface antigens, which are potential targets for small molecule and protein therapeutics. Advances in critical aspects of mass spectrometers have made possible molecular mass determinations of many intact proteins. These include effective ionization methods, e.g., MALDI and electrospray; sufficient resolution to allow assignment of charge states as well as mass; and adequate sensitivity in transmission and detection of heavy ions. In recent years the application of tandem mass spectrometry to intact proteins has been initiated. This requires activation sufficient to promote fragmentation of heavy ions, and novel bioinformatics programs to identify proteins based on fragment ions. Activation still has the potential for significant improvement. Chromatographic fractionation is also not adequate for automatic analysis of large protein mixtures.

INVITED LECTURE-12

Speaker: Dr. Philip Andrews

University of Michigan, USA

Biography: Dr. Philip Andrews is a professor at Medical School Department of Biological Chemistry, University of Michigan. My laboratory has worked on protein structure by mass spectrometry computational methods extensively over the past 20 years and we have developed a number of new techniques for protein analysis with our main emphases being on post-translational modifications and protein interactions. I was trained in protein chemistry and enzymology at Purdue University and during a subsequent post-doctoral stint I applied my training to the application of mass spectrometry to protein structure. At the University of Michigan I expanded this experience to the large scale analysis of proteins by mass spectrometry. Computational and Bioinformatics research has always been a major component of my laboratory and we have extensive experience and infrastructure in the analysis and management of large proteomics data sets, in the management of complex projects, and development of online resources. My laboratory is primarily focused on the analysis of protein interactions of membrane

proteins and the interactions of large complexes. We also work on phosphoproteome, general PTM analysis and pathway mapping. Many of our projects are collaborative in nature, underscoring our experience in bringing complex collaborative projects to successful conclusions. My laboratory has collaborated on a broad range of projects that involve identification of protein interactions and quantification of their changes. I also have extensive experience in proteomics, bioinformatics, and the management, analysis, and dissemination of large data sets in multi-institutional projects.

Talk title: Structures of protein complexes by chemical crosslinking mass spectrometry (CXL-MS) and CXL-ion mobility-MS.

The recent development of CID-cleavable crosslinker is having an impact on the analysis of protein complexes in structural mass spectrometry. The current generations of crosslinkers developed in our lab (DC4 and related structures) have standard linker arms with reactive NHS esters but a central connecting domain containing one or more quaternary cyclic amines. The central domain gives these crosslinkers their unique physical characteristics including: CID cleavable, membrane permeability, high solubility, and high reactivity. The last characteristic results in high stoichiometry of reaction with lysyl residues. Importantly, these crosslinkers maintain the surface charge balance of crosslinked proteins which stabilizes the structures and has advantages for top-down analysis. The unique MS₂ pattern of crosslinked peptides allows them to be easily identified and subsequent MS₃ identifies the component peptides.

Application of this class of crosslinkers to solution-phase protein complexes provides structural constraints that complement other methods. DC4-crosslinked *E. coli* cell extracts exhibited identical ribosome profiles to those of uncrosslinked extracts on sucrose gradients while Western blots of key ribosomal proteins exhibited discretely laddered bands for the crosslinked complexes. We identified specific sites of crosslinking consistent with the known crystal structure of ribosomes. *In vivo* crosslinking in *E. coli* also demonstrated that the Tsr chemoreceptor, an intrinsic inner membrane protein was crosslinked by DC4 to dimer and higher order complexes. Effective crosslinking of both soluble and membrane complexes has also been demonstrated in a number of mammalian cell lines both for whole cell lysates and intact cells.

We demonstrated that reconstituted protein complexes, Hsp70 dimer, the E3 ligase CHIP dimer, and the Hsp70/CHIP heterotetramer, crosslink with DC4 with greater efficacy than observed with some traditional crosslinkers. Oversampling of crosslink sites identified a number of intra-subunit and inter-subunit crosslinks that were consistent with the heterotetramer structure constructed from EM and partial crystal structures. Known flexible regions of Hsp70 exhibited patterns of crosslinking consistent with long distance interactions. Substantially different patterns of crosslinked peptides and dead-end peptides were observed between the three complexes, providing additional validation of structure. Top-down analysis of crosslinked protein complexes by ion mobility tandem mass spectrometry provides additional structural information and the use of crosslinkers containing intrinsic positive charges partially compensates for some of the current limitations of top-down analysis of complexes.

INVITED LECTURE-13

Speaker: Mr. Brendan MacLean

University of Washington, USA

Biography: Mr. Brendan worked at Microsoft for 8 years in the 1990s where he was a lead developer and development manager for the Visual C++/Developer Studio Project. Since leaving Microsoft, Brendan has been the Vice President of Engineering for Westside Corporation, Director of Engineering for BEA Systems, Inc., Sr. Software Engineer at the Fred Hutchinson Cancer Research Center, and a founding partner of LabKey Software. In this last position he was one of the key programmers responsible for the Computational Proteomics Analysis System (CPAS), made significant contributions to the development of X!Tandem and the Trans Proteomic Pipeline, and created the LabKey Enterprise Pipeline. Since August, 2008 he has worked as a Sr. Software Engineer within the MacCoss lab and been responsible for all aspects of design, development and support in creating the Skyline Targeted Proteomics Environment and its growing worldwide user community.

Talk title: Skyline: building an ecosystem for targeted quantitative proteomics

The Skyline project started in August, 2008 as a 2-year effort to bring better SRM/MRM software tools to the United States, National Cancer Institute's CPTAC Verification Working Group that could support the variety of mass spectrometers used by participating laboratories. Over 6 years later, the Skyline project is a thriving proteomics community open-source collaboration supporting 6 mass spec instrument vendors, with thousands of users worldwide and many thousands of instances started each week. In 2010, development began on extracting chromatograms from full-spectrum data types, with Skyline today providing the broadest available support of data independent acquisition (DIA/SWATH), parallel reaction monitoring (PRM) and MS1 filtering from data independent acquisition (DDA) data. Nature Methods named Targeted Proteomics its method of the year for 2012, noting the importance of Skyline in making this possible. Since then, Skyline has added a framework for integrating with external tools – with its own Tool Store, a web site repository named Panorama for managing and analyzing large collections of data processed in Skyline and direct integration with a cloud data storage framework named Chorus with fast remote chromatogram extraction for full-spectrum data types. This presentation will focus on recent developments and future directions in the Skyline ecosystem for targeted quantitative proteomics.

INVITED LECTURE-14

Speaker: Dr. Shantanu Sengupta

Institute of Genomics and Integrative Biology, New Delhi, India

Biography: Shantanu Sengupta, a Scientist at the CSIR-Institute of Genomics and Integrative Biology, Delhi, works in the broad area of Genomics and Proteomics. He completed his PhD from CSIR -Indian Institute of Chemical Biology. He then did his postdoctoral work from National Institute of Immunology, New Delhi and the Cleveland Clinic Foundation, Ohio, USA. At IGIB, he is working towards understanding the genetic, epigenetic and proteomic basis of cardiovascular diseases with a focus to understand the role of vitamin B₁₂ deficiency and elevated levels of homocysteine and cysteine in coronary artery

disease (CAD). He has shown that in India, CAD is significantly associated with deficiency of vitamin B₁₂, a micronutrient, deficiency of which leads to high levels of homocysteine and cysteine, also that the DNA methylation is significantly higher in CAD. Shantanu has been instrumental in setting up the proteomics facility at IGIB and is currently working towards identifying markers for CAD and other cardiovascular disorders using proteomics as a tool. He is currently the Secretary of the Proteomics Society (India). He has published several papers in reputed International journals and is an Associate Editor of *Journal of Genetics* and Academic Editor of *PLoS One*. He is also the Associate Dean of the Academy of Scientific and Innovative Research (AcSIR). He has received the DBT Biosciences award in 2011

Talk title: Understanding mechanism of cysteine toxicity in yeast using a proteomics approach

Biological thiols are the most important antioxidants that protect the cell from oxidative damage. Perturbation in the concentration of these thiols leads to disease states. Among the biologically important thiols, elevated levels of homocysteine are known to be associated with various diseases and are considered to be an independent risk factor for cardiovascular disease. One of the mechanisms proposed for homocysteine-induced vascular dysfunction involves the generation of reactive oxygen species during transition metal-catalyzed oxidation of circulating homocysteine resulting in the formation of hydrogen peroxide. However, if this is true then cysteine with pKa of 8.3 which is much lower than that of homocysteine (9.5) should generate greater amount of ROS than homocysteine and thus should also be toxic. Infact we had earlier shown that cysteine induced growth defect in yeast was more severe than homocysteine (Kumar *et al.* 2006). Subsequently we have also reported that high levels of cysteine and not homocysteine are associated with Coronary artery disease patients in India. (Kumar *et al.* 2009) which is also consistent with reports from other parts of the world (El-Khairi *et al.* 2003). However, despite numerous reports about the toxicity and the association of cysteine with various diseases, the underlying mechanism of cysteine induced toxicity remains poorly defined.

In the present study we focused to understand the detailed molecular mechanism of cysteine induced toxicity using yeast (*Saccharomyces cerevisiae*) as a model system. Using both labeled (iTRAQ) and unlabeled (SWATH-MS) based quantitative proteomics approach we have identified the proteins that are differentially expressed in yeast in the presence of cysteine. Although the number of proteins identified in iTRAQ and SWATH were comparable, SWATH could quantitate proteins of lower abundance as compared to iTRAQ. Using these techniques we found that cysteine alters the expression of many proteins involved in amino acid metabolism. With the help of amino acid screening we found that addition of Leucine could abrogate the growth defect induced by cysteine. Detailed studies are currently underway to characterize the cellular response which is required for survival during high level of cysteine and to understand how the supplementation of leucine can revert back the effect of cysteine.

INVITED COMPANY LECTURE-2

Speaker: Dr. Mark McDowell

Waters Corporation, UK

Biography: Dr. Mark McDowell obtained his BSc in Chemistry (1980) and his PhD in biological mass spectrometry (1984) from the University of Wales working on the development and

application of ‘primitive’ LC/MS technologies to the analysis of peptides and pharmaceutical products. He obtained a post-doctoral fellowship from the Royal Society (of Great Britain) in 1984 to research the mechanism of thermospray ionisation at the University of Bonn (Germany). Mark joined the LC/MS development group of VG Instruments in 1985 and was involved in the mass spectrometry operations and management of the company for over 29 years – throughout its evolution to Micromass and finally Waters Corporation. Prior to his retirement in February 2014, Mark was the Senior Manager responsible for strategic development of the company’s Pharmaceutical Discovery & Life Science business – focusing on mass spectrometry for the health & life sciences world-wide. Dr McDowall served two terms of office on Waters Corporation’s Scientific Advisory Board and was a member of the company’s Centres of Innovation Program that maintains strategic collaborations with many of the World’s premier research institutions. Today Dr. McDowall continues his career-long association with Waters Corporation as a business development consultant focusing on mass spectrometry in India.

Talk title: A multi-omic data independent investigation of drug mitigated obesity within a mouse model

Mark McDowall¹, Gertjan Kramer², Nicholas Dekker², Lee A. Gethings¹, John P. Shockcor¹, Victoria Lee³, Robert J. Beynon³, James I. Langridge¹, Johannes P.C. Vissers¹, Johannes M.F.G. Aerts²

¹Waters Corporation, Wilmslow, Cheshire, UK

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Introduction: Obesity is associated with metabolic syndrome, causing excess body fat to be accumulated, adversely affecting health and life expectancy. It has previously been shown that

glucosylceramides play a crucial part in such metabolic syndromes. The manipulation of glucosylceramides with small molecule drugs, in mouse models, has shown that symptoms can be negated. Regulation of the proteome, metabolome and lipidome during such drug manipulation is currently not fully understood.

Methods: Lipid and protein extracts of liver tissue from 3 control and 3 obese mice models were analysed. Protein extracts were proteolysed with trypsin and the resulting peptides separated over a 90-minute reversed-phase nanoscale UPLC gradient. Lipid extracts were prepared using 500 µL IPA/water (50:50) and separated over a 20-minute reversed-phase UPLC gradient. Data were acquired by data independent acquisition (LC/MS^E) in which the Collision Induced Dissociation (CID) energy was switched between low and elevated energy in alternately acquired spectra. For protein profiling Ion Mobility Separation (IMS) was integrated on-line (*i.e.* LC/IMS/MS^E) to increase the peak capacity of the analytical system. The acquired data were processed and searched with Progenesis QI software providing both identification and normalized, label-free, quantification of lipids/proteins.

Preliminary Results: Randomized proteomic samples (100 ng on-column) were analysed in triplicate to reveal 1200 highly curated proteins across all technical replicates and biological conditions. Over 300 proteins exhibited a significant fold change greater than 2. Unique peptides were used for relative label-free quantitation with median abundance normalization performed across all samples. Randomized lipid extracts (2 µL on-column) were analysed in triplicate. A lipid QC sample, comprised of aliquots of all samples in equal amounts, was injected after every 5 injections. Interrogation of the resulting data revealed over 500 lipid identifications (phosphatidylcholines, sphingomyelins, triglycerides and lysophosphatidylcholines). Unsupervised multivariate analyses showed clear distinction between obese and control groups in both proteomic and lipidomic experiments. Pathway analysis was employed to provide an insight to the underlying biology of the differentially expressed proteins and lipids.

SESSION VI: INTERACTOMICS, MULTI-OMICS ANALYSIS

INVITED LECTURE-15

Speaker: Dr. Manuel Fuentes

University of Salamanca, Spain

Biography: Manuel Fuentes graduated in Chemistry and Biochemistry from the University of Salamanca (Spain). After his MS in Biotechnology at University of Bielefeld (Germany), he joined at Biocatalysis Department at National Spanish Research Council (Madrid, Spain) for his PhD Thesis, entitled “Design and development of conjugation and immobilization methods of biomolecules for diagnostic methods useful in Genomics and Proteomics”. Afterwards, he moved to Harvard Institute of Proteomics at Harvard Medical School (Boston, EE.UU.) During almost 6 years, when he was working on biomarker and drug discovery in tumor and autoimmune pathologies by using a combination of high-throughput label free proteomics approaches. In 2009, he joined as a scientist at Cancer Research Center at University of Salamanca, where his research was focused on biomarker and drug discovery in hematological diseases, mainly for personalized medicine. He is also co-author of 80 peer-reviewed papers (ISI web of Knowledge) in international journals, 9 licensed international patents, 10 book chapters, and more than 50 invited lectures in national and international meetings.

Talk title: Identification of altered cell signaling pathways in B- lymphocytic chronic leukemia (B-CLL) by functional proteomics approaches

In post-genome era having sequence the human genome, one of the most important pursuits is to understand the function of gene-expressed proteins. The overwhelming size and complexity of human proteome requires very high-throughput techniques for rapid analysis. Despite significant advancements in molecular biology and genetic tools, this demand has not been satisfied and only a small fraction of the proteome has been understood at the biochemical level. Systems Biology and Proteomics strive to create detailed predictive models for molecular pathways based upon quantitative behavior of proteins. Understanding these dynamics networks provides clues into the consequence of aberrant interactions and why they lead to B-chronic lymphocytic leukemia. Historically, methods capable of collecting quantitative data on biochemical interactions could only be used for one or a few proteins at the time. Protein microarrays allow hundreds to thousands of proteins to be analyzed simultaneously, providing an attractive option for high-throughput studies such as protein-protein interaction, differential protein profiles. A novel bead suspension array systems based on color coded beads which are compatible with flow cytometry, allows measuring many proteins simultaneously because this novel approach offers the advantage that hundreds of different proteins or antibodies can be codified in specific color combination; in addition, it is combining with size resolution chromatography and subcellular fractionation, by this way, it is possible to determine protein complexes and/or specific protein identification. We will present preliminary differential protein profiles (BCL2, SOS, and LYN among others) obtained from normal B cells and aberrant B-cells from chronic lymphoid leukemia (with different cytogenetic alterations and well-characterized immunophenotype).

INVITED LECTURE-16

Speaker: Dr. Prasanna Venkatraman

Advanced Centre for Treatment Research and Education in Cancer, Navi Mumbai, India

Biography: We are interested in the general understanding of the mechanism of cellular homoeostasis both in health and in disease. In the lab we are interested in developing a model system to navigate through the various steps involved in proteasomal degradation. The emphasis is on the sequence and structural requirements for initiating recognition and the rate limiting steps involved at the primary level of communication between the substrate and the proteasome. We would like to understand how this communication then translates into down stream events like unwinding of the polypeptide chain and its subsequent degradation. We are also interested in the passive and active role played by the 19S regulatory subunits in these processes and those unrelated to degradation. Emphasis is to understand the molecular details of protein-protein interactions and deciphering the code buried in the language of communication between the proteasome and the vast and diverse family of proteins within the cell. Our research now includes a new addition- a molecule from the cell signaling family - 14-3-3 zeta. We have been drawn to this molecule due to its surprising role in functioning as a molecular chaperone. The structural requirement of this protein to function as a chaperone and the identification of a novel ATP binding motif responsible for this function is being investigated. We are keen to explore if this is a unifying principle across the protein family with different isoforms.

Talk title: Structural interrogation of phosphoproteome identified by mass spectrometry reveals allowed and disallowed regions of phosphoconformation

Background: High-throughput mass spectrometric (HT-MS) study is the method of choice for monitoring global changes in proteome. Data derived from these studies are meant for further validation and experimentation to discover novel biological insights. Here we evaluate use of relative solvent accessible Surface area (rSASA) and DEPTH as indices to assess experimentally determined phosphorylation events deposited in PhosphoSitePlus.

Results: Based on accessibility, we map these identifications on allowed (accessible) or disallowed (inaccessible) regions of phosphoconformation. Surprisingly a striking number of HT-MS/MS derived events (1461/5947 sites or 24.6%) are present in the disallowed region of conformation. By considering the protein dynamics, autophosphorylation events and/or the sequence specificity of kinases, 13.8% of these phosphosites can be moved to the allowed region of conformation. We also demonstrate that rSASA values can be used to increase the confidence of identification of phosphorylation sites within an ambiguous MS dataset.

Conclusion: While MS is a stand-alone technique for the identification of vast majority of phosphorylation events, identifications within disallowed region of conformation will benefit from techniques that independently probe for phosphorylation and protein dynamics. Our studies also imply

that trapping alternate protein conformations may be a viable alternative to the design of inhibitors against mutation prone drug resistance kinases.

INVITED LECTURE-17

Speaker: Dr. Sanjeeva Srivastava

Biography: Dr. Sanjeeva Srivastava is Associate Professor and Group Leader of Proteomics Laboratory at the Indian Institute of Technology Bombay India, a guest professor at the Central South University, China and a Visiting Scientist at the Biodesign Institute, Arizona. He obtained his Ph.D. from the University of Alberta and post-doc from the Harvard Medical School in the area of proteomics, and has specialized expertise in applications of data enabled sciences in global health, developing country and resource limited settings. Current research in this group centers on using high throughput proteomics for biomarker discovery in cancer and other diseases, to study protein-protein interactions and drug target discovery. Additionally, multi-dimensional Omics data are employed for *in silico* studies and models. The group has developed E-learning resources such as Virtual Laboratory as a community resource and is collaborating actively both across India and internationally to advance this knowledge frontier for the benefit of global health. Dr. Srivastava is recipient of several awards including the National Young Scientist Award (Canada), Young Scientist Awards (India) and the Apple Research Technology Support Award (UK). Recently he was awarded the excellence in Teaching Award – 2014 at IIT Bombay. He serves as Deputy Editor-in-Chief for CPPM and Associate Editor for several other international journals.

Talk title: Autoantibody profiling of glioma and meningioma to identify biomarkers using human proteome array

The heterogeneity and poor prognosis associated with brain tumors like glioma and meningioma, makes biomarker identification imperative. Neoplasms evoke the immune response by production of autoantibodies against autoantigens or tumour associated antigens (TAAs) which can be used for the early detection of cancers. Protein microarrays have made a substantial impact in the field of functional proteomics and has proven its potential as an excellent tool to screen such autoantibody responses by its ability to analyze hundreds of proteins in large number of clinical samples in parallel, overcoming the limitations of assay sensitivity and sample capacity. Autoantibody signatures across various grades of gliomas, sub-categories of glioblastoma multiforme (GBM) and meningioma was studied using human proteome chips containing ~17000 full-length human proteins. The deduced sets of classifier proteins helped to distinguish Grade II, III and IV glioma samples from the healthy subjects with 88, 89 and 94% sensitivity and 87, 100 and 73% specificity. Proteins namely, SNX1, EYA1, PQBP1 and IGHG1 showed dysregulation across various grades of glioma while IGHG4 was one protein that showed dysregulation across all grades of meningioma. NEDD9 was identified as an interesting protein with probable prognostic value to counter the heterogeneity presented by GBMs based on its proximity to the sub-ventricular zone (SVZ). Pathway analysis of differentially expressed proteins in these two diseases reveal many interesting pathways like enrichment of immunoregulatory, cytoskeletal remodeling pathways, neurotrophin signaling pathway, signaling by neural growth

actor and platelet derived growth factor (PDGF) etc. in addition to commonly associated pathways to tumorigenesis, which gives us an insight into the biochemical alterations arising due to autoimmune responses in glioma and meningioma.

INVITED COMPANY LECTURE-3

Speaker: Dr. Robert Karlsson

GE Healthcare Lifesciences, Sweden

Biography: Robert Karlsson is a senior staff scientist in the Protein Analysis Applications team in GE Healthcare Life Sciences and previous Research Director at GE Healthcare Life Sciences and Biacore AB. Robert graduated from the University of Stockholm in 1976. He started his professional career as an Analytical Chemist in Pharmacia later the same year. In the 1980ies he was recruited to a project in Pharmacia to develop an SPR biosensor. This resulted in the launch of the first BiacoreTM system in 1990. He published the original paper on kinetic analysis using Surface Plasmon Resonance in 1991 and has since published close to 40 papers describing Biacore applications. Prior to being a staff scientist Robert held positions as senior scientist, group manager and Research director at Biacore AB and GE Healthcare Life Sciences. As research director Robert was responsible for biochemistry, systems, applications and scientific market support teams. Robert was nominated European Inventor of the Year in 2006, and in recognition for bringing surface plasmon resonance to the screening field he received the Polypops award from SBS in 2007. Today Robert is involved with development of label free and bio molecular imaging technologies and applications, including having the patent on Calibration free active concentration in his name.

Talk title: Kinetic analysis of protein-protein interactions friend or foe?

Robert Karlsson¹, Åsa Frostell-Karlsson¹, Jerrard Hayes² and Pauline Rudd³

¹Bio-Analysis Systems R&D, GE Healthcare Life Sciences

²School of Biochemistry & Immunology, Trinity Biomedical Sciences Institute, Trinity College, Dublin, ³NIBRT-Glycoscience Group, NIBRT-The National Institute for Bioprocessing, Research and Training, Dublin, Ireland.

Protein –protein interactions are typically characterized in terms of their stoichiometry, binding site specificity and affinity. The kinetics of the interaction is also commonly considered as this provides information on the time that is required to shift from one state to another. Kinetic data also discriminate between interactions of the same affinity, and as more data points are considered in analysis, it is easier to observe deviations from a proposed binding mechanism. Kinetic data can therefore be extremely beneficial for analysis of protein-protein interactions, but the detailed information has at the same time the potential to reveal complexities that may be difficult to fully explain from a binding mechanism perspective.

IgG-Fc γ receptor interactions are highly illustrative of the advantages and challenges of a kinetic approach for understanding protein-protein interactions. These interactions can be both simple and complex depending on the type of receptors and their state of glycosylation, and the focus of this talk will be on dissecting the complexity of these interactions by combining kinetic experimental design with the use of both glycosylated and non-glycosylated receptors.

SESSION VII: HUMAN PROTEOME PROJECT

PLENARY LECTURE-5

Speaker: Dr. Mark S. Baker

Macquarie University, Australia

Biography: Prof. Mark S. Baker (Bakes) has built a dynamic medium sized (~10) research team that focuses on molecular cell proteomics. Mark has 22 years senior management experience driving programs across academic, industrial and clinical cancer discovery settings alike, ~85 peer-reviewed publications, over 20 PhD student completions, a number of patents and he currently holds many competitive grants. His research interests include: novel proteomic biomarker discovery technologies, the biology of cancer metastasis, how proteins interact to drive biological outcome, proteases receptors and their inhibitors. His most recent work has focused on the development of solutions to the major problem of achieving comprehensive membrane proteome coverage and how this can be used to understand the mechanisms by which ovarian, colon, breast and prostate cancer develop. Mark is a passionate advocate for the commercialisation of Australia biotechnology and works closely with Commonwealth and State governments on roadmapping Australia's future investment in research infrastructure. Mark is a member of the HUPO Council and Chairs their Nominations Committee. Mark led the team that recently won the bid for the HUPO World Congress to be held in Sydney from 19-24th September, 2010.

Talk title: Human proteome project keys to success: Finding a shared language

Mark S. Baker, David Cantor, Harish Cheruku, Abidali Mohamedali, Sadia Mahoboob, Fei Liu, Shoba Ranganathan, Ed Nice, Lydie Lane, Bill Hancock, Young-Ki Paik, Emma Lunberg, Mark Connors, Pierre Legrain, Peter Karuso, Charlie Ahn, Gil Omenn

HUPO HPP Missing Proteins Workshop, Sydney 30th June 2014

Using pipelines developed under the Austral-New Zealand Chromosome 7 effort and collaboration with Chromosome 17 (USA) as an exemplar of the C-HPP efforts and applying these to all the human proteome, we discuss the so called "missing proteins" from the perspectives of both HUPO and other non-HUPO scientists who contributed to the HPP Missing Proteins Workshop held in Sydney on 30th June, 2014.

This workshop raised issues around the need for; (i) common languages, (ii) agreed metrics, (iii) analysis of appropriate steps by which data should be re-analyzed and (iv) the regular nature of HUPO reporting of integrated HPP outcomes.

In addition, the workshop identified the need for a new language that allows HUPO to explain the different "bins" into which all proteins expressed by the human genome can be carefully characterised, and that has now been recently proposed by Eric Deutsch at the Madrid HUPO Congress in October 2014.

Comments will be made by the workshop chair regarding the largest protein classes of "missing proteins" and some comments about possible biochemical characteristics that may contribute to these proteins being labeled as "missing".

We call for a global approach to finding these missing proteins and suggest that a single Wikipedia page listing all missing proteins by gene name and clickable to a compendium of

accessible and modifiable Wiki pages, one for each of the "missing proteins" may be appropriate. These pages would allow motivated global scientists to deposit/accumulate all types of "omics" and other scientific data in one place that may act as a series of clues as to where, where, why, how and with who these proteins are expressed in the normal, stressed and diseased human body. Using such a tool, HUPO scientists might be able to confirm the existence/expression of current "missing human proteins" and return them to the identified list.

INVITED LECTURE-18

Speaker: Dr. Robert L. Moritz

Institute for Systems Biology, USA

Biography: Dr. Robert Moritz, a native of Australia, joined the ISB faculty in mid 2008 as Associate Professor and Director of Proteomics. Dr. Moritz began his work in 1983 in the Joint Protein Structure laboratory of Prof. Richard J. Simpson (JPSL-Ludwig Institute for Cancer Research, and The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia). During his 25 years at JPSL, Dr. Moritz designed and implemented a number of technologies currently used in many proteomics laboratories across the globe. Examples include technologies such as the development of micro-chromatography for proteomics from the late 1980's to its current day implementation, a micro-fractionation technique widely used by many laboratories worldwide. His collaborative research into cytokine biochemistry, protein-receptor chemistry and cellular biochemistry culminated in the novel identification of a number of proteins (e.g., IL-6, IL-9 A33 ligand, DIABLO, as well as several others), their interacting partners, and 3-dimensional structures of their cell surface receptors important in human health concerns such as cancer and inflammation. During his time at JPSL, Dr. Moritz progressed through the ranks whilst obtaining his Bachelor's degree in Biochemistry with first-class Honors, and his Ph.D., from the University of Melbourne. Dr. Moritz has brought wide-ranging skills and expertise to ISB, much of it drawn from his Australian experience. There, in 2005, he conceptualized a shared proteomics high-performance computing system, organized a consortium of proteomic scientists from all states in Australia, and proposed a computational system specifically for proteomics data analysis for all Australian researchers to access. For this work, in 2006, he was awarded an enabling grant from the Australian National Health and Medical Research Council worth AUS\$2M. With that award, he established a bioinformatics center in Australia that enabled proteomic researchers anywhere in the country to analyze mass spectrometry data. It was the first effort on a national scale to bring proteomic data analysis and algorithms to any researcher in the whole country without the need for them to build their own bioinformatics group. In late 2006, the Australian Proteomics Computational Facility (APCF, www.apcf.edu.au) was inaugurated, and Dr. Moritz remains as Director of the APCF. The dedicated proteomics data analysis facility is equipped with a 1000 CPU high-performance computing cluster, and full-time software engineers for the continued development of proteomics algorithms and data validation. This facility serves all researchers in Australia and others regardless of their global geographical location. He is continuing that work at ISB by expanding the ISB proteomics centre into a national facility with online tools for data analysis.

Talk title: Advances in MS-based proteomics tools and resources

A major goal of the human proteome project is to advance the characterization of all coding gene products to complete the human proteome and to enable the application and dissemination of proteomic technologies that will support the comprehensive identification and quantitation of all the proteins, their isoforms, and post-translational modifications contained within the human proteome. With recent advances in comprehensive resources in technology, informatics and reagents, the ability to measure proteins in all its forms is now realized. These developments of new technology for multiplexed quantitative protein measurements, with a throughput consistent with the needs of iterative measurements of perturbed systems, has enabled the building and sharing of tools that are generally applicable to all proteomic-based studies. The advancements in proteomic informatics provides highly curated databases that enable standardization across workflows and form a solid basis for the widespread deployment of multiplexed assays and reporting of their results in a community based manner. This sharing and re-use of data and results provides confidence in the field of proteomics. I will discuss tools that are capable of generating complete, reproducible and quantitatively accurate datasets of entire proteomes.

INVITED LECTURE-19:

Speaker: Dr. Ravi Sirdeshmukh

Institute of Bioinformatics, Bangalore, India

Biography: Ravi Sirdeshmukh did his PhD in Biochemistry from the Centre for Cellular and Molecular Biology (CCMB), Hyderabad - a premier national laboratory and was post-doctoral visiting associate at the Washington University Medical School, St Louis, MO and a visiting Scientist at the National Institutes of Health, USA. He worked on Protein and RNA biochemistry for nearly 2 decades at the CCMB, where he later started the first Proteomics Facility and first Proteomics Program in the country. He joined the Institute of Bioinformatics, Bangalore as Asso. Director in 2010 and now holds a joint appointment as Principal Advisor, at Mazumdar Shaw Medical Center and Center for Translational Research, Bangalore. His current research interests have been in the areas of Proteomics and Multiomics Approaches for studying brain tumors and head and neck cancers. He had been the nodal scientist in CSIR network programs in Proteomics and also served as the Consortium Manager in CSIR Proteomics Network for International Collaborations. A co-author of the Draft Map of Human Proteome, he is a Co-PI in the multinational Chromosome 12 team of the ongoing Chromosome Centric Human Proteome Project initiated (C-HPP) of HUPO. Ravi Sirdeshmukh is the Founder President of the Proteomics Society, India and has been member of the council of HUPO and AOHUPO.

Talk title: Taking Indian proteomics to Human Proteome project

The Draft Map of Human Proteome published recently in two separate papers in Nature (one largely authored by the Indian group) not only projects the power of mass spectrometry to explore the complex challenge of the human proteome but also underscores the contributions of the Indian proteomics efforts in this endeavour. However, the 17,000-and more proteins profiled in the Nature papers showing their tissue wise or chromosome wise distribution or a comparable number of proteins studied using antibodies by the Human Protein Atlas is not the end of the quest. While the estimated number of

protein coding genes today is about 19000, the number of variants is increasing with the present number hovering around 39000. Human Proteome Organization (HUPO) has launched a systematic study of each human protein and its variants as a Global Consortium Project – the Chromosome Centric Human Proteome Project (C-HPP) which brings together all countries each focussed on one or more chromosomes to study the proteins in depth. In a consortium group comprising of 5 Asian countries including India (others being Thailand, Taiwan, Hong Kong and Singapore), chromosome 12 has been chosen as the focus of study. Together, the first series of 3 papers giving the overall vision and the initial contributions have just been published in the Journal of Proteome Research (JPR) – a journal dedicated to the C-HPP efforts. These papers describe the entire protein complement of chromosome 12 - the parts list including as many as 89 “missing proteins” and also the disease association of these proteins to Gliomas and major neurological disorders - Alzheimer’s and Parkinson’s disease. The talk will cover the potential of mass spectrometry based proteomic experimentation and bioinformatics data analysis that mark the usefulness of understanding proteins in the chromosome context. It is just the right opportunity to contribute to this global endeavour – the Human Proteome Project and be part of the exciting time ahead to unravel human biology.

INVITED LECTURE-20

Speaker: Dr. Sanjay Navani

Site Director, The Human Protein Atlas (HPA) Project, India

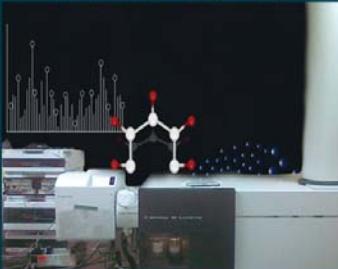
Biography: The Swedish Human Protein Atlas (HPA) program has been set up to allow for a systematic exploration of the human proteome using Antibody-Based Proteomics. This is accomplished by combining high-throughput generation of affinity-purified (mono-specific) antibodies with protein profiling in a multitude of tissues/celltypes assembled in tissue microarrays. The vision is to systematically generate quality assured antibodies to all non-redundant human proteins, and to use these reagents to functionally explore human proteins, protein variants and protein interactions. The data is publically available and presented as high resolution images of immunohistochemically stained tissues and cell lines (www.proteinatlas.org). Available proteins can be reached through searches for specific genes or by browsing individual chromosomes.

Talk title: Tissue-Based Map of the Human Proteome

Resolving the details of proteome variation in the diverse parts of the human body on the molecular level will contribute significantly to increase our knowledge of human biology and disease. Here, we present a map of the human tissue proteome based on an integrated omics approach employing quantitative transcriptomics on a tissue and organ level, followed by tissue microarray-based immunohistochemistry for precise spatial localization down to the single cell level. Our tissue-specific analysis shows expression of more than 90% of the putative protein-coding genes, and we used this to analyze the tissue-specific proteome, the human secretome, the membrane proteome, the druggable proteome, the cancer proteome and the metabolic functions in 32 tissues and organs. To facilitate biomedical research of human health and disease we integrated all the data and immunohistochemical images in an interactive web-based database (www.proteinatlas.org) that allows exploration of individual proteins as well as navigation of global expression patterns in all major tissues and organs in the human body.

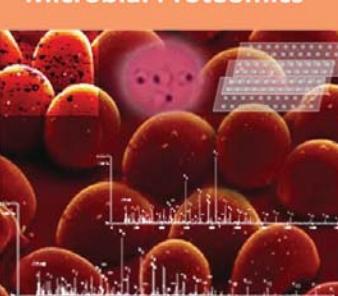
9TH DECEMBER 2014

SESSION VIII: MS AND METABOLOMICS

MS APPLICATIONS & METABOLOMICS	Plenary Talk	Invited talks			
	 Dr. Catherine E. Costello Ex-HUPO President (Boston University, USA)	 Dr. Kanury V. S. Rao (ICGEB, India)	 Dr. Juan J. Calvete (CSIS, Spain) Editor-in-chief, <i>Journal of Proteomics</i>	 Dr. Rapole Srikanth (National Centre for Cell Science, India)	 Dr. Harsha Gowda (Institute of Bioinformatics, India)

Metabolomic profiling combined with high-throughput technologies provides fast and accurate screening of thousands of biomolecules that are attractive for the identification of next-generation biomarkers and potential drug/vaccine targets. The session would feature some of the current developments in this steadily developing field of research.

SESSION IX: MICROBIAL PROTEOMICS

Microbial Proteomics	Invited talks			
	 Prof. Utpal S. Tatu (Indian Institute of Science, India)	 Dr. Deepa Bisht (National JALMA Institute for leprosy and Other Mycobacterial Diseases, India)	 Dr. Peter Nilsson (KTH-Royal Institute of Technology Stockholm, Sweden)	 Dr. Pushkar Sharma (National Institute of Immunology India)

This session will highlight the impact of Microbial Proteomics/Metabolomics studies in neglected tropical diseases. The other aspect of this theme is its enormous potential to be utilized as mini-factories to synthesize complex organic biochemicals. This session would also summarize the latest on such interesting prospects.

SESSION X: ANIMAL AND PLANT PROTEOMICS

Animal & Plant Proteomics	Invited talks			
	 Dr. Nat Kav (University of Alberta, Canada)	 Dr. Sixue Chen (University of Florida, USA)	 Dr. Renu Deswal University of Delhi, India	 Dr. H. V. Thulasiram Ncl, Pune

Plant proteomics have helped to characterize and validate novel genes for crop improvement, to study plant responses to different abiotic and biotic stress conditions, signaling and metabolic mechanisms underlying plant growth, development and interaction with the environment. The session based on plant proteomics would highlight certain interesting upcoming studies in this light.

SESSION VIII: MS AND METABOLOMICS

PLENARY LECTURE-6

Speaker: Dr. Catherine E. Costello

Boston University, USA

Biography: Catherine E. Costello is a William Fairfield Warren Distinguished University Professor at Boston Univ. (BU), with appointments in Biochemistry, Biophysics and Chemistry. She earned her AB at Emmanuel College, Boston, and her MS and PhD at Georgetown Univ., Washington, DC and was a postdoctoral fellow and Senior Research Scientist at the Mass. Inst. of Technology. She is the Founding Director of the BU School of Medicine Center for Biomedical Mass Spectrometry. Her research addresses the development and application of MS-based methods for investigation of protein post-translational modifications, protein folding disorders, cardiovascular and infectious diseases, glycobiology and bioactive lipids. She has authored more than 320 scientific papers. She is Vice President of the International MS Foundation (IMSF) and a member of the Board of the US-Human Proteome Organization (HUPO). She is an American Chemical Society (ACS) Fellow, an ACS Councilor and current chair of its Northeastern Section. She has been President of the International HUPO and the American Society for MS. She serves on several editorial and advisory boards. She has received the Award for Discovery in Proteomics (HUPO), the Thomson Medal (IMSF) and the Field and Franklin Award (ACS).

Talk title: Applications of novel mass spectrometry-based approaches for the definition of disease-related glycomes, lipidomes and peptidomes

The biochemical phenomena that underlie many human diseases are at best only poorly understood. Advances in analytical methodology, coupled with the rapidly growing power of bioinformatics, now make the exploration of these phenomena more approachable. With respect to the diagnosis and treatment of sporadic and chronic conditions, mass spectrometry developments in both pre- and post-ionization separations and in dissociation methods are providing critical tools that facilitate investigation of diseases at the molecular level, resulting in knowledge of subtleties that were previously difficult to discern, e.g., post-translational modifications, differentiation of isomers, intermolecular interactions and turnover. Examples from ongoing technology developments that impact studies of infectious and parasitic diseases will be presented.

INVITED LECTURE-21

Speaker: Dr. Kanury Venkata Subba Rao

International Centre for Genetic Engineering and Biotechnology, New Delhi, India

Biography: Area of Research @ ICGEB: Our group has worked for many years in the area of B-cell immunology and vaccine development with a special focus on understanding mechanisms guiding activation, selection, maturation, and differentiation of antigen-specific B-lymphocytes. Our more recent focus of research is to use the tools of Systems and High-throughput biology to decipher the dynamics of host-pathogen interactions in human macrophages infected with *Mycobacterium tuberculosis*.

Research @ DDRC: The DDRC is a multi-disciplinary research center that integrates basic with translational research in the field of drug discovery. The overall mission of the center is to combine multiple disciplines in order to generate a robust and versatile pipeline for drug discovery research. It functions as a technology-intensive base that enables drug discovery and development activity both in India and in interested laboratories elsewhere. It provides platforms and expertise for target/activity validation, *in silico* inhibitor design, high-content screening, structure-activity optimization of lead molecules, chemical synthesis of template structures, and pharmacological evaluation of the lead compounds. In addition, it also has its own active core programs that integrate experimental approaches with the tools of systems biology to further the goal of phenotypic drug discovery.

Talk title: Pathogenicity of *Mycobacterium tuberculosis* is expressed by regulating metabolic thresholds of the host macrophage

¹Parul Mehrotra, ¹Shilpa V. Jamwal, ¹Najmuddin Md Saquib, ¹Neeraj Sinha, ¹Zaved

Siddiqui, ¹Venkatasamy Manivel, ¹Samrat Chatterjee, and ¹Kanury V.S. Rao

¹International Centre for Genetic Engineering and Biotechnology

The success of *Mycobacterium tuberculosis* as a pathogen derives from its facile adaptation to the intracellular milieu of human macrophages. To explore this process, we asked whether adaptation also required interference with the metabolic machinery of the host cell. Temporal profiling of the metabolic flux, in cells infected with differently virulent mycobacterial strains, confirmed that this was indeed the case. Subsequent analysis identified the core subset of host reactions that were targeted. It also elucidated that the goal of regulation was to integrate pathways facilitating macrophage survival, with those promoting mycobacterial sustenance. Intriguingly this synthesis then provided an axis where both host- and pathogen-derived factors converged, to define determinants of pathogenicity. Consequently, whereas the requirement for macrophage survival sensitized TB susceptibility to the glycemic status of the individual, mediation by pathogen ensured that the virulence properties of the infecting strain also contributed towards the resulting pathology.

INVITED LECTURE-22

Speaker: Dr. Rapole Srikanth

National Centre for Cell Science, Pune, India

Biography: Dr. Srikanth Rapole did his PhD in analytical chemistry from IICT, Hyderabad where he developed new mass spectrometry methods for analysing beta-carbo-peptides. He did his post-doc from University of Massachusetts 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 2 years at University of Connecticut. Currently, he is working as a scientist at NCCS, Pune. His main research interest is to quantitatively identify the protein signatures involving in human diseases including cancer using mass spectrometry-based proteomic approaches. In addition, his group is also working to

identify and quantify key metabolites and lipids associated with human diseases using mass spectrometry and bioinformatics. He has been published more than 40 publications in reputed international journals. He has received best paper award from CSIR in physical sciences. Recently, he received the DBT-Rapid grant for young investigators award.

Talk title: Metabolomic and lipidomic profiling towards novel theragnostic markers for breast cancer

Breast cancer is the most common malignancy and the leading cause of cancer deaths in women worldwide. Metabolomics is the study of the metabolites and how they are affected by specific cellular processes. Metabolites and lipids are important players in biological systems. Hence, the comparative analyses of the metabolites and lipids of normal, benign and malignant serum samples can provide a better understanding of the molecular events involved in tumour development, and are essential for early detection and diagnosis of the disease. In this study, we analysed unbiased metabolomic profile of 75 serum samples of benign, malignant and age-matched healthy controls using three platforms LC-MS, GC-MS and NMR. LC-MS/MS based targeted quantitative metabolite analysis was performed in positive and negative modes using AB Sciex 4000 QTRAP. NMR based untargeted metabolomics profiling was carried out using 700 MHz Bruker Avance AV III NMR. GC-MS based untargeted metabolomic profiling was studied using Agilent 7890GC-5977A MSD. For phospholipid profiling, lipids were analysed by in-house MRM based platform using AB Sciex 4000 QTRAP LC-MS/MS. Expression of differential metabolites and lipids in breast cancer samples was analyzed by multivariate statistical analysis using MarkerView and Simca software. Functional network pathway analysis was performed using KEGG and oncomine concept maps (OCM) databases to investigate the biological context of the identified metabolites. Several amino acids, fatty acids, phospholipids, and organic molecules were observed to be differentially expressed in breast cancer when compared with benign and healthy controls. Bioanalytical data were subjected to multivariate statistics in order to visualize clusters of cases and to detect the metabolites that are able to differentiate breast cancer patients from healthy individuals. Very good discrimination within breast cancer and control groups was achieved. The results obtained from this study will be presented.

INVITED LECTURE-23

Speaker: Dr. Harsha Gowda

Institute of Bioinformatics, Bangalore, India

Biography: 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 a reviewer for several international journals including *Journal of Proteome Research*, *Proteomics*, *Journal of Proteomics*, *Molecular Biosystems* and *International Journal of Cancer* and an Editorial Board member of *Journal of Proteomics*. At IOB, he is employing cutting-edge technologies in genomics, proteomics and metabolomics to investigate biomarkers and therapeutic targets for various cancers.

Talk title: Metabolomic profiling of esophageal squamous cell carcinoma

Metabolic perturbations are well documented in cancers. Cancer associated metabolic changes are known to support the energetic and biosynthetic requirements of proliferating cells. Identifying dependency of cancer cells on specific metabolic pathways should provide novel avenues for therapeutic intervention. Gene expression profiling and quantitative proteomics studies in esophageal squamous cell carcinoma (ESCC) have revealed dysregulation of several metabolic enzymes. We have now carried out mass spectrometry based untargeted metabolomic profiling of esophageal squamous cell carcinoma (ESCC) using surgically resected tissue specimen as well as serum. Several metabolic features were found to be dysregulated in ESCC. Phosphatidylcholines were identified as one of the major class of dysregulated metabolites in ESCC. In addition to mono and diacylphosphatidylcholines, several ether phospholipids were also found to be dysregulated. These studies provide mechanistic insights into metabolic reprogramming that is commonly observed in ESCC.

INVITED LECTURE-24

Speaker: Dr. Juan J. Calvete

Instituto de Biomedicina de Valencia, Spain

Biography: Dr. Juan J. Calvete (Valencia, Spain, 1957) is Research Professor of the Spanish Research Council (CSIC), Professor of PEDECIBA (Uruguay), and Head of the Structural and Functional Venomics Laboratory at the Instituto de Biomedicina de Valencia. He studied Biology at the University of Valencia, and earned his Ph.D. degree in Biochemistry from Complutense University (Madrid, 1985). Dr. Calvete completed post-doctoral training in Protein Chemistry and Structural Biology at the Banting Institute (Toronto, Canada) (1987), the Max-Planck-Institute für Biochemie (Martinsried, Germany) (1988-92), and the Institut für Reproduktionsmedizin (Hannover, Germany) (1993-98) prior to assuming his current position in the Instituto de Biomedicina de Valencia in 1998. Dr. Calvete has coauthored more than 360 scientific publications in peer-reviewed journals and monographic books, which have received over 11500 citations according to the Google Scholar database. His current research focuses on structural and functional proteomics of snake venoms, having developed proteomics-based tool ("venomics" and "antivenomics") for exploring the evolution, composition, interactions with antivenoms, and biotechnological applications of venoms and toxins. Since 2011, Dr. Calvete serves as President of the European Section of the International Society on Toxicology. Ranked in position 63 in the field of Proteomics. Dr. Juan J. Calvete has served as first President of the Spanish Proteomics Society (SEProt). Currently, he is member of the Congress & Communication Committee of the European Proteomics Association, Editorial Board Member of Toxicon, Journal of Venom Research, Toxins, and Editor-in-Chief of the Journal of Proteomics.

Talk title: Next generation snake venomics

Venoms represent an adaptive trait and an example of both divergent and convergent evolution. The ecological advantages conferred by the possession of a venom system are evident from the extraordinarily diverse range of animals that have evolved venom for hunting, defense efficiency or competitor dissuasion. Despite venoms provide model systems for investigating (i) predator-prey interactions, (ii) the molecular bases for evolutionary and ecological adaptations, (iii) the generation of chemical and pharmacological novelty, and (iv) strategies for

the knowledge-based design of antivenoms to reduce the burden of the neglected pathology of snake bite envenoming that kills or maims thousands of healthy individuals every year, only recently advances in instrumentation and high-throughput methodologies have fueled an expansion of the scope of biological studies and strategies for assessing the toxin composition of snake venoms ("snake venomics"), directly (through proteomics-centered approaches) or indirectly (via venom gland transcriptomics and bioinformatic analysis) in a relatively rapid and cost-effective manner. The proteomics characterization of the chemical space, natural history, and immunoreactivity of snake venoms with homologous and heterologous antivenoms ("antivenomics"), offers a framework for clustering venoms based on within- and between-species shared traits and trends. Basic and applied aspects of venom research represent two sides of the same coin: understanding the principles governing the evolution of venomous systems is not only of relevance for understanding the molecular basis of adaptive venom phenotypic variations, but it is also of outmost importance for learning how to use deadly toxins as therapeutic agents, as well as for improving current antivenoms.

INVITED SHORT TALK-1

Speaker: Dr. P. Babu

Centre for Cellular and Molecular Platforms, NCBS-TIFR, India.

Talk title: Role of novel glycan-receptor interactions in hydra regeneration

Abstract: Diversity of glycans attached to glycoconjugates drives many of the processes involved in development, homeostasis and diseases. Structural characterization of the glycans is foremost step in understating their functions. Cell-cell communications, cell-matrix interactions and cell migrations play a major role during regeneration of Hydra. However, little is known about the molecular players involved in these critical events, especially the cell surface glycans. This presentation will discuss about discovery of novel polyfucosylated glycans attached to proteins and the role of specific glycan-receptor interactions in the regenerative process in *Hydra magnipapillata*.

INVITED SHORT TALK-2

Speaker: Dr. K. Chandrashekhar Rao

Bhabha Atomic Research Centre, Mumbai, India

Talk title: Development of an electrodynamic ion funnel with electrospray ionization source for Proteomics studies using reflectron-ToF Mass Spectrometry

K C Rao*, U S Sule, R K Pratap and K G Bhushan

Technical Physics Division, Bhabha Atomic Research Centre, Mumbai 400085.

Introduction: Electrospray Ionization (ESI) is a well established technique for the production of large molecular ions from liquid phase. However, ESI being an atmospheric ionization technique, a major challenge is to increase sensitivity since the transmission efficiency is very small at the first differential pumping stage. In this paper, we present a novel technique for increasing the sensitivity by using an indigenously developed Electrodynamic Ion Funnel. The ESI source is coupled to the Ion Funnel consisting of 52 electrodes followed by Octopole/Quadrupole -

Octopole assembly and a set of Einzel Lens. High Resolution Mass Spectrometry is performed on the bio-molecular ions using an orthogonal Reflectron ToF-Mass Spectrometer.

Instrumentation: The experimental setup consists of an ESI source with a syringe and an SS needle of internal diameter around 50 μm which is biased to ± 3000 V. A syringe pump controls the volumetric flow rate of the analyte through the needle in $\mu\text{l}/\text{hr}$, nL/min , etc. A counter electrode (usually grounded) with a small aperture of about 25 microns positioned a few mm away from the tip of the needle creates a strong electric field for the flow of ions from the tip of the needle. Ions formed from the 'Taylor Cone' due to ESI enter the Electrodynamic Ion funnel in the vacuum chamber through the aperture in the counter electrode. In the ion funnel, the internal diameters of the first 14 electrodes are kept constant at 25 mm to enhance the collection of diverging ions and thereafter till the 50th (and 51st) electrode the diameter progressively decreases to 3 mm. The last electrode has a 45° (half angle) conical aperture of diameter 1mm, which enhances the bunching of ion beam coming out of the ion funnel. The spacing between the electrodes and thickness of each electrode is 1.5 mm. A specially fabricated combined DC and RF power supply is used to drive the ions through the ion funnel. Ions exiting the Ion funnel are then transported with the help of octopole guides to an orthogonal pusher eventually focusing the ions into a reflectron TOF mass spectrometer.

Results and Discussions: Ion-simulation software SIMION 8.0 has been used to study and analyse the electrodynamic properties of Ion Funnel. The transmission efficiency of the Ion funnel has been studied for different pressures of 0.1 Torr, 0.5 Torr, 1 Torr and 5 Torr, for a range of frequencies (200 kHz – 2MHz) and amplitudes ($20 \text{ V}_{\text{pp}} – 80 \text{ V}_{\text{bp}}$) of RF signal. Different charge states from +1 to +12 have been tested for mass range 500 – 12000 amu. These studies have shown nearly unit transmission efficiency. Ion funnel was operated with the above mentioned parameters. As a test case, ion current from streptomycin sulphate (mass 1457.38 amu) was produced using ESI. Ion currents in excess of 1 nA have been measured at different electrode locations along the ion funnel. It is found that the ion currents are sensitive to both the frequency and the amplitude of the applied RF, indicating the presence of multiply charged states in the total ion current. Further studies are underway to mass analyze the ions using the reflectron TOF mass spectrometer.

Conclusion: Ion current measurements have been carried out for molecular ions of different masses and at different pressures inside the chamber, along the Ion Funnel. Ion currents upto 3 nA has been measured along the Ion Funnel for Streptomycin Sulphate of mass 1457.38 amu.

INVITED SHORT TALK-3

Speaker: Dr. Bhaswati Chaterjee

National Institute of Pharmaceutical Education and Research, Hyderabad, India

Talk title: Application of quantitative proteomics using mass spectrometry and system biology

Mass spectrometry based proteomics have become an important tool in analysing large numbers of proteins and its modification. Here I would be discussing about some applications of mass spectrometry based proteomics in combination with system biology approach. Mapping of Human protein-protein interaction will be useful for scientific community. Protein-protein interaction plays an important role in all cellular processes. Here we are mapping the protein interactome in cancerous HeLa cell

line and embryonic stem cells by using bacterial artificial chromosome (BAC) TransgeneOomics with quantitative interaction proteomics that is called as quantitative BAC–green fluorescent protein interactomics (QUBIC) which allows specific and highly sensitive detection of interactions. I will show the importance of single run in quantitative analysis of proteome

and post-translational modification especially phosphorylation. By using single run analysis I got more than 2200 proteins in E.Coli and by using interaction network and system biology approach, observed how the E.Coli proteins are regulating different processes.

SESSION IX: MICROBIAL PROTEOMICS

INVITED LECTURE-25

Speaker: Dr. Utpal S. Tatu

Indian Institute of Science, Bangalore, India

Biography: Utpal Tatu is a Professor at the Department of Biochemistry, Indian Institute of Science, Bangalore. His research focuses on biology of molecular chaperones with an emphasis on neglected diseases. Prof. Tatu's studies have highlighted the potential of exploiting chaperones as drug targets against infectious diseases such as malaria and trypanosomiasis. His lab is also credited with the discovery of a novel trans-splicing event in the expression of heat shock protein 90 gene from Giardia lamblia. His Ph.D. thesis at Indian Institute of Science received the best Ph.D. thesis award and a gold medal. Prof. Tatu did his post doctoral research at Yale University examining roles of molecular chaperones in protein folding, assembly and secretion. His study on the folding of Influenza virus hemagglutinin in the endoplasmic reticulum of mammalian cells has been cited in Molecular Cell Biology book edited by Harvey Lodish. His research contributions have been covered by editorials in Science as well as Nature Medicine and one of his research publications in JBC was selected as the best paper.

Talk title: Unfolded protein response in malaria: A transcript, protein and metabolite flux

My laboratory is interested in examining the role of molecular chaperones in the evolution of parasitism. By exploring parasites such as *Babesia*, *Candida*, *Entamoeba*, *Giardia*, *Plasmodium* and *Trypanosoma* our lab has shown an important link between parasite chaperones and the adaptation of these parasites to the host cell environments. Our studies show a critical role played by chaperones in the growth of the above infectious disease causing organisms in their respective hosts. Among other evolutionary forces, demands of the daunting, host cell milieu may have played an important role in shaping the biochemical activities, interactomes as well as cellular localizations of parasite chaperones. Chaperones, in turn, may have influenced not only the virulence of these parasites but also their speciation. It appears that evolution of molecular chaperones and parasitism may have progressed hand-in-hand. In my talk I will particularly focus on our recent efforts at understanding how the malarial parasite copes with challenges to its secretory capacity. By examining transcript, protein and metabolite flux we find that malarial parasite has evolved a novel Unfolded Protein Response Pathway that culminates into a stage switch.

INVITED LECTURE-26

Speaker: Dr. Peter Nilsson

KTH-Royal Institute of Technology, Stockholm, Sweden

Biography: PN is Professor in Proteomics at the *SciLifeLab Stockholm, KTH – Royal Institute of Technology*. He has since 2002 been heading the Protein Microarray Technology group within the Human Protein Atlas project. He is the platform director of Affinity Proteomics at SciLifeLab Stockholm and the site director of the Human Protein Atlas at SciLifeLab Stockholm. He is also the vice dean of the School of Biotechnology at KTH.

The main research focus is within development and utilization of various protein microarray technologies for antigen and antibody based proteomic profiling and biomarker discovery.

Talk title: Affinity proteomics reveals elevated muscle proteins in plasma of children with cerebral malaria

Systemic inflammation and sequestration of parasitized erythrocytes are central processes in the pathophysiology of severe *Plasmodium falciparum* childhood malaria. However, it is still not understood why some children are more at risks to develop malaria complications than others. To identify human proteins in plasma related to childhood malaria syndromes, multiplex antibody suspension bead arrays were employed. Out of the 1,015 proteins analyzed in plasma from more than 700 children, 41 differed between malaria infected children and community controls, whereas 13 discriminated uncomplicated malaria from severe malaria syndromes. Markers of oxidative stress were found related to severe malaria anemia while markers of endothelial activation, platelet adhesion and muscular damage were identified in relation to children with cerebral malaria. These findings suggest the presence of generalized vascular inflammation, vascular wall modulations, activation of endothelium and unbalanced glucose metabolism in severe malaria. The increased levels of specific muscle proteins in plasma implicate potential muscle damage and microvasculature lesions during the course of cerebral malaria.

INVITED LECTURE-27

Speaker: Dr. Deepa Bisht

National JALMA Institute for Leprosy & Other MycoBacterial Diseases, Agra, India

Biography: Dr. Deepa Bisht is a Research Officer at National JALMA Institute for Leprosy & Other MycoBacterial Diseases at Agra, India. She has a PhD from IMTECH, Chandigarh, India. She has 3 years of experience pertaining to mycobacterial research and has 5 papers to her credit. She is presently engaged in conducting a DST funded project on intracellular expression on proteins with mycobacteria using proteomic analysis. Her research interests are in biochemical and immunological characterization of mycobacterial proteins and targeted delivery of drug/antigen.

Talk title: Identification of potentially involved proteins in aminoglycoside resistance in *Mycobacterium tuberculosis*

Deepa Bisht, Prashant Sharma, Bhavnesh Kumar, Divakar Sharma, Neelja Singhal, Neetu Verma, V. M. Katoch* and K. Venkatesan

Department of Biochemistry, National JALMA Institute for Leprosy & Other Mycobacterial Diseases (ICMR), Tajganj, AGRA-282004

Mycobacterium tuberculosis continues to be a significant health burden. Emergence of drug resistant strains and a higher incidence of tuberculosis in people with HIV/AIDS have further worsened the situation. Aminoglycosides are important second line antibiotics used to treat patients with MDR-TB and resistance to them severely affects the options for treatment. In

this context, it is essential to increase the understanding of resistance mechanisms in order to develop drugs with potential activity against the pathogen. To fill the multiple gaps that remain for understanding resistance, in recent years proteomic tools have achieved significant progress in the characterization of proteins involved in the mechanism of resistance. In this study a proteome comparison was carried out between the aminoglycoside resistant and susceptible isolates of *M. tuberculosis* using two dimensional gel electrophoresis, matrix assisted laser desorption ionization time-of-flight and bioinformatic approaches. Intensities of some protein spots were found to be consistently increased in resistant isolates which will be discussed. In-depth study of these proteins will give an insight into probable sites of drug action other than established primary sites and hence may help in search of novel chemotherapeutic agents at these new sites as inhibitors.

INVITED LECTURE-28

Speaker: Dr. Pushkar Sharma

National Institute of Immunology, New Delhi

Biography: Dr. Pushkar Sharma is a staff scientist at the National Institute of Immunology, New Delhi, India. He completed his PhD from All India Institute of Medical Sciences (AIIMS), New Delhi, India. He is recipient of several awards and honours like Senior Research Fellow, The Wellcome Trust, UK; Swarnajayanti Fellowship, DST, India; National Bioscience Award; Fellow of the National Academy of Science India; Shanti Swarup Bhatnagar Award. His research interests include Interplay between signal transduction pathways regulates fate, survival and development of almost all cells. His research group is interested in dissection of signal transduction pathways in systems as diverse as apicomplexan parasites like *Plasmodium falciparum* and *Toxoplasma gondii* and mammalian cells like neurons. He is a member of important international forums and consortiums, which includes the malERA consultative group on Basic Science and Enabling technologies. As part of the malERA group, which was composed of researchers working in diverse areas of parasite biology, he was involved in providing recommendations on the future direction of basic malaria research, which would facilitate effective diagnosis and eradication of disease (*PLOS Medicine* 2011). He is reviewer for grants for international funding agencies like NIH, The Wellcome Trust, UK, DBT-Wellcome Alliance, MRC, UK, MRC, Australia. He is also a regular reviewer for journals like Plos Pathogens, Cellular Microbiology, Molecular Microbiology, etc.

Talk title: Role and regulation of malarial calcium dependent kinases

We are interested in signaling and trafficking events in malaria parasite, which are mediated by second messengers like calcium and phosphoinositides (PIPs). Calcium Dependent Protein Kinases (CDPKs) are major effectors of calcium signaling in the parasite. Our recent studies on PfCDPKs suggest that these kinases may regulate important parasitic processes like RBC invasion, intraerythrocytic development and parasite division. We demonstrate that PfCDPK1 may play a role in host cell invasion. This kinase is regulated by Phospholipase C (PLC) mediated calcium release and undergoes phosphorylation at several sites, which is critical for its activation. We have identified novel substrates of this kinase via which it may regulate the process of invasion. In contrast to PfCDPK1, PfCDPK7 is an atypical member of this group, which lacks the classical CDPK domain organization. PfCDPK7 has a PH domain via which it interacts with PI (4, 5) P2. PfCDPK7 transiently

localizes to the endoplasmic reticulum (ER) and vesicular compartments in the parasite and its interaction may guide its subcellular localization. *PfCDPK7*-disruption resulted in an interesting phenotype; it abrogates the growth of parasite in blood stages and impaired parasite division indicating that *PfCDPK7* is important for *P. falciparum* development. The analysis of *PfCDPK7*-KO parasites suggested that metabolism and/or trafficking of lipids may be altered, which may affect parasite growth. Comparative metabolomic studies are in progress, which may shed light on the role of this kinase in lipid metabolism. These and other studies provide novel insights into the role of PIPs and calcium in the parasite life cycle.

INVITED SHORT TALK-4

Speaker: Dr. Pooja Jain

Drexel University College of Medicine, USA

Talk title: Unique and differential protein signatures for HIV-1 & HCV mono-infection versus co-infection as determined by multiplex iTRAQ quantitative proteomics

Nawal M. Boukli^a, Zafar Kamal Khan, Vivekananda Shetty^b, Luis Cubano^a, Martha Ricaurte^a, Jordana Coelho-dos-Reis^c, Zacharie Nickens^b, Punit Shah^b, Andrew H. Talal^d, Ramila Philip^b, and Pooja Jain^{*}

^aUniversidad Central del Caribe School of Medicine, Biomedical Proteomics Facility Department of Microbiology and Immunology, Bayamon, Puerto Rico

^bImmunotope, Inc., Pennsylvania Biotechnology Center, Doylestown, PA

^cDepartment of Microbiology and Immunology, and the Drexel Institute for Biotechnology and Virology Research, Drexel University College of Medicine, Doylestown, PA

^dCenter for the Study of Hepatitis C, Weill Cornell Medical College, New York, NY

Pathogenesis of liver damage in patients with HIV and HCV co-infection is complex and multifactorial. Although global awareness regarding HIV-1/HCV co-infection is increasing little is known about the pathophysiology that mediates the rapid progression to hepatic disease in the co-infected individuals. In this study, we investigated the proteome profiles of peripheral blood mononuclear cells from HIV-1 mono-, HCV mono-, and HIV-1/HCV co-infected patients. The results of high-resolution 2D gel electrophoresis and PD quest software quantitative analysis revealed that several proteins were differentially expressed in HIV-1, HCV, and HIV-1/HCV co-infection. Liquid chromatography-mass spectrometry and Mascot database matching (LC-MS/MS analysis) successfully identified 29 unique and differentially expressed proteins. These included cytoskeletal proteins (tropomyosin, gelsolin, DYPLSL3, DYPLSL4 and profilin-1), chaperones and co-chaperones (HSP90-beta and stress-induced phosphoprotein), metabolic and pre-apoptotic proteins (guanosine triphosphate [GTP]-binding nuclear protein Ran, the detoxifying enzyme glutathione S-transferase (GST) and Rho GDP-dissociation inhibitor (Rho-GDI), proteins involved in cell prosurvival mechanism, and those involved in matrix synthesis (collagen binding protein 2 [CBP2])). The six most significant and relevant proteins were further validated in a group of mono- and co-infected patients (n=20) at the transcriptional levels. The specific pro and anti-apoptotic protein signatures revealed in this study could facilitate the understanding of apoptotic and protective immune-mediated mechanisms underlying HIV-1 and HCV co-infection and their implications on liver disease progression in co-infected patients.

INVITED SHORT TALK-5**Speaker: Dr. Bhakti Basu***Bhabha Atomic Research Centre, Mumbai, India***Talk title: Proteomic perspective of extreme gamma radiation resistance in *D. radiodurans*****Bhakti Basu* and Shree Kumar Apte***Molecular Biology Division, Bhabha Atomic Research Centre, Mumbai-400085, India*

Introduction: An extremophile, *Deinococcus radiodurans*, resists very high doses of a variety of clastogenic agents such as ionizing radiations, UV, desiccation or mutagenic chemicals as it repairs its damaged DNA with absolute fidelity. Its genome displays acquisition of eukaryotic DNA repair pathways such as strand annealing (SA) and non-homologous end joining (NHEJ) and deletion of universal prokaryotic DNA repair pathways such as RecBCD mediated HR, photo-reactivation and SOS response. Yet, it's the most DNA repair efficient organism known today.

Materials and Methods: The respective proteome profiles were resolved by 2D electrophoresis and the 2D gels were analyzed

for gamma radiation responsive differentially expressed proteins. The proteins of interest were identified by MALDI mass spectrometry.

Results and Discussions: Gamma irradiation exposure sets in immediate growth arrest phase during which the organism is envisaged to reassembles its shattered genome and recycles the radiation-damaged biomolecules. A proteomic investigation of this phase revealed selective upregulation of DNA repair proteins involved in strand annealing, nucleotide excision repair, non-homologous end joining and homologous recombination pathways. The level of a number of GTPases was upregulated while that of GTP utilizing proteins was downregulated. The expression of metabolic enzymes, by large, was unaffected by gamma irradiation. Oxidative stress alleviation machinery, which was constitutively present in abundance, displayed minor modulation.

Conclusions: The proteome kinetics during post-irradiation recovery, when correlated with the transcriptomic, knockout mutagenesis and biochemical evidences, emphasize focused sequential DNA repair and requirement of induced as well as constitutively expressed proteins, for the extreme radioresistance of *D. radiodurans*.



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SESSION X: ANIMAL AND PLANT PROTEOMICS

INVITED LECTURE-29

Speaker: Dr. Nat Kav

University in Alberta, Canada

Biography: Dr. Nat Kav is a Professor in the Department of Agricultural, Food and Nutritional Science (AFNS) and Associate Dean (Academic) for the Faculty of Agricultural, Life & Environmental Sciences (ALES) at the University of Alberta. He is responsible for all the undergraduate academic programs in the Faculty of ALES and for undergraduate student-related matters including experiential learning as well as other aspects of student engagement. Consistent with the University of Alberta's mandate, Dr. Kav also works with the Dean of ALES to further collaborative relationships of the Faculty of ALES with institutions in India. In addition to his role as Associate Dean, Dr. Kav has and continues to serve his Department, Faculty and the University on various committees. His research interests include the application of proteomics- and genomics-based discovery techniques to identify, characterize and validate novel genes for application in agriculture and human health. Another, related area of interest is in the area of immunochemical technology with a specific thrust in the development of exquisitely specific recombinant antibodies for diverse application in agriculture and medicine.

Talk title: Proteomics and Immunotechnology in agriculture

This lecture will focus on the application of discovery proteomics and immunotechnology in agriculture. We will first look at an example where two-dimensional electrophoresis and Mass Spectrometry were used to identify proteins secreted by the phytopathogen *Sclerotinia sclerotiorum*. This fungal pathogen is capable of causing devastating crop losses in canola which has significant economic impact on Alberta's and Canada's economies. We have successfully demonstrated that the virulence of this pathogen can be reduced by knocking out one of the virulence factors identified by 2DE-MS/MS. We will then look at an example of the application of recombinant antibody technology in the engineering of durable resistance against *S. sclerotiorum* in *Brassica napus* Canola. We will conclude this talk with our most recent work on the application of immunotechnology in the characterization of prion proteins, the causative agent of Bovine Spongiform Encephalopathy, an example from the animal side of agriculture.

INVITED LECTURE-30

Speaker: Dr. Renu Deswal

University of Delhi, India

Biography: Dr. Renu Deswal is an Associate Professor in the Department of Botany, University of Delhi. She received her M. Phil in Life Sciences in 1989 and PhD in Biochemistry in 1994, both from Jawaharlal Nehru University, New Delhi. Her research interests are Molecular Plant Physiology, Nitric oxide and cold stress signaling, Functional Genomics (Proteomics) in particular. Her research group is dissecting nitric oxide and cold stress signaling in Brassica (Indian mustard) and seabuckthorn

(a Himalayan shrub). Mostly proteomics tools are being employed for this analysis. She has been awarded with the DST's Young scientist's research project award. She is Member of International Plant Growth Substances Association (2004-07), European Federation of Biotechnology (EFB) and Society of Biological chemist, (SBC), India.

Talk title: Ambushing the Himalayan gold bush for antifreeze proteins with potential biotechnological applications

Ravi Gupta and Renu Deswal*

Introduction: Seabuckthorn (*Hippophae rhamnoides*) is a cold hardy bush, which grows in Himalayan region. It can withstand freezing temperatures (up to -40 °C) therefore can be a good resource to understand cold tolerance and also for antifreeze proteins (AFPs) which, are secreted in the apoplast and have application in medicine and cryopreservation of food.

Methodology: A simple procedure to germinate seedlings under laboratory conditions was developed. For identifying cold modulated proteins a gel based proteomics approach with nLC-MS/MS identification was used to resolve extracellular proteins from non-acclimated, cold acclimated and subzero acclimated seedlings. Spot analysis was done using Image master software. AFP activity was measured using nanolitre osmometer combined with phase contrast microscopy and splat assay. Secondary conformation was analysed by circular dichroism (CD). AFPs were purified by ice affinity chromatography (IAC) and Chitin affinity chromatography (CAC).

Results and Discussion: In all 61 cold/freezing modulated spots were observed out of 245 reproducible spots. Several putative antifreeze proteins (AFPs) like chitinase, thaumatin-like protein, β -1,4-glucanase and WRKY were identified which was supported with detection of antifreeze activity in extracellular proteins. Three antifreeze proteins 31, 34 and 41 kDa were purified from the apoplast of cold/freezing treated seedlings. A 41 kDa polygalacturonase inhibitor protein (PGIP) was purified using IAC and two 31 and 34 kDa class I chitinases (HrCHT1a and HrCHT1b) were purified by CAC. Out of the three AFPs, PGIP and HrCHT1b were glycoproteins. De-glycosylated PGIP and HrCHT1b retained the antifreeze activity, suggesting that glycosylation is not required for the antifreeze activity.

Conclusions: Circular dichroism (CD) analysis showed both the chitinases to have a predominantly β -stranded conformation (36-42%) and the β -strand content increased (~11%) during cold acclimation and with calcium. Preliminary results indicate that Chitinase could enhance the shelf life of mice RBCs in cold. This is the first report of cold/freezing stress induced extracellular proteome analysis in plants using a 2-DE-MS approach and detection of antifreeze activity in seabuckthorn.

INVITED LECTURE-31

Speaker: Dr. Sixue Chen

University of Florida, USA

Biography: Dr. Sixue Chen's areas of expertise fall in Biochemistry, Plant Metabolism, Functional Genomics, Proteomics, Metabolomics, and Mass Spectrometry. He learned mass spectrometry when he was collaborating with a chemist at

the Danish Royal Veterinary and Agricultural University 16 years ago. Dr. Chen carried out a lot of small molecule work at that time. Since joining University of Pennsylvania in 2001, he has worked on many different projects using proteomics and mass spectrometry. Dr. Chen has accumulated experience with different separation and fractionation technologies (e.g., 2-DE), different HPLC instruments including the nanoflow ultra performance LC, as well as mass spectrometers. During his tenure as the Proteomics Facility Director at the Danforth Center in Missouri, USA, Dr. Chen has developed a high throughput protein sequencing technology using mass spectrometry. Dr. Chen is aware of the importance of collaboration and implementing powerful proteomics technologies in solving challenging scientific problems. At University of Florida, Dr. Chen has established three major research projects; all were funded by the US National Science Foundation. Plant guard cell hormone signaling and glucosinolate metabolism were used as model systems. Based on findings from large-scale "omics" studies, many novel and testable hypotheses have been derived. Another major component of Dr. Chen's research program has been hypothesis driven and testing, i.e., characterizing molecular, biochemical and physiological functions of specific genes and proteins. The integration of hypothesis generation and hypothesis driven research will ultimately lead to a holistic view of cellular networks and processes in higher organisms.

Talk title: Redox and phosphorylation regulated proteins in plant stomatal signaling

Redox proteomics technologies are powerful in discovering novel redox regulated proteins. Using stomatal guard cells as experimental materials, an IPMDH and a SNRK2.4 were identified as redox-sensitive proteins employing 2D-DIGE and ICAT of guard cell proteins. The IPMDH is involved in glucosinolate biosynthesis. Our experiments have shown the involvement of glucosinolate metabolism in guard cell function. The SNRK2.4 is a member of subclass I of the SNRK2 family of protein kinases. In ABA-treated guard cells, SNRK2.4 showed increased oxidation of cysteine relative to controls. In vitro protein kinase activity assays of recombinant SNRK2.4 showed that both autophosphorylation and peptide substrate phosphorylation were inhibited by pretreatment with the oxidants H₂O₂, S-nitrosoglutathione (GSNO), and oxidized glutathione (GSSG). Pretreatment with oxidant followed by treatment with DTT prior to the assay reversed the inhibitory effect of oxidation. These data indicate that the activity of BnSNRK2.4 is regulated by redox in guard cells. The potential crosstalk between redox and phosphorylation is under investigation.

INVITED LECTURE-32

Speaker: Dr. H.V. Thulasiram

National Chemical Laboratory, India

Biography: Dr. Thulasiram completed his PhD from Indian Institute of Science, India (2001). He carried out his post doctoral research work at The University of Iowa, USA and The University of Utah, USA. His research group is interested in elucidation of the isoprenoid biosynthetic pathways with special emphasis on establishing the mechanisms of the enzyme-catalyzed transformations and how the enzymes promote the reactions. The isoprenoid biosynthetic pathways provide intermediates for the synthesis of a vast variety of structurally and chemically diverse natural products that serve numerous biochemical functions in living systems. Another area of interest is production of biologically important small molecules by using microbes or enzymes or through metabolic engineering.

Talk title: Metabolomics: Terpene profiling of selected plants

Plants produce vast array of structurally and functionally diverse secondary metabolites including isoprenoids. These compounds have functional roles in plants and many are useful in industry, agriculture, and medicine. As a result there are intense research efforts under way to characterize metabolites (targeted or untargeted) in line with genomics and proteomics. As our group is interested in isoprenoids with special emphasis on biosynthesis, biocatalysis and their biological activities, we are actively involved in targeted metabolomics of Neem, Sandalwood, and other plants, which are known to possess biologically and commercially important isoprenoids. Isoprenoid compounds constitute a large diverse class of "small molecule" natural products with over 70,000 individual known metabolites and these compounds are ultimately derived from two fundamental isoprenoid building blocks, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Geranyl diphosphate (GPP) is synthesized from IPP and DMAPP catalyzed by Geranyl diphosphate synthase (GDS) whereas darnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP) synthases catalyzes the consecutive condensation of IPP with DMAPP/GPP/FPP to give FPP and GGPP respectively. The first committed step in isoprenoids biosynthesis is cyclization of prenyl diphosphate (GPP/FPP/GGPP) and oxidosqualene by terpene synthases to yield mono-, sesqui-, di- or tri-terpene terpene skeletons, which will be converted into final isoprenoid products catalyzed by downstream enzymes including cytochrome P-450 systems.

INVITED SHORT TALK-6

Speaker: Dr. Ashok Mohanty

National Dairy Research Institute, Karnal, India

Talk title: Proteome analysis of functionally differentiated mammary epithelial cells and lactating udder tissue reveals protein signatures associated with lactation persistency and milk yield

Mohanty AK*, Janjanam J, Jena MK., Kumar S and Jaswal S

Animal Biotechnology Centre, National Dairy Research Institute, Karnal, India-132001

Introduction: Mammary gland is made up of a branching network of ducts that end with alveoli which surrounds the lumen. These alveolar mammary epithelial cells (MEC) are milk-secreting cells that secrete milk proteins during lactation which reflect the milk producing ability of farm animals. Little is known about the expression profile of proteins in the metabolically active MECs and tissues during lactation or their functional role in the lactation process. We have deciphered the proteome map of MECs and udder tissues in lactating cows and buffaloes in different stages of lactation to understand mammary biology.

Methodology: MECs from milk of 6 lactating Sahiwal cows (in their peak lactation) were isolated from milk using immunomagnetic beads and confirmed by RT-PCR and Western blotting. Proteins were extracted from MECs and subjected to 2DE MALDI-TOF/TOF MS and 1D-Gel-LC-MS/MS. We have performed the 2D DIGE technique to identify differentially expressed proteins (DEPs) during early, peak and late lactation in MECs and in buffalo udder tissue. Ingenuity pathway analysis was performed to correlate the role of DEPs in lactation. Identified proteins were subjected to extensive bioinformatics analysis.

Results and Discussion: Global proteome analysis of MECs revealed 497 unique proteins. Pathway analysis revealed 28 pathways ($p < 0.05$) providing evidence for involvement of various proteins in lactation function. We identified 41 differentially expressed proteins during lactation stages and 22 proteins in high and low milk yielding cows. Bioinformatics analysis showed that a majority of the differentially expressed proteins are associated in metabolic process, catalytic and binding activity. Up-regulated proteins in late lactation are associated with NF- κ B stress induced signalling pathways whereas Akt, PI3K and p38/MAPK signalling pathways are associated with high milk production. 12 new proteins including EEF1D, HSPA5, TSTA3, HSPD1 and PRDX6 during lactation were observed.

Conclusion: The data generated further provide a set of bovine MEC and udder specific proteins that will help the researchers to understand the molecular events taking place during lactation. New protein signatures associated with lactation were identified.

INVITED SHORT TALK-7

Speaker: Dr. Ravi Kumar Gandham

Indian Veterinary Research Institute, Uttar Pradesh, India

Talk title: Dissecting the host gene interaction network in PBMCs infected with PPR vaccine virus uncovered transcription factors modulating immune regulatory pathways

S. Manjunath¹, B. P. Mishra², A.P.Sahoo¹, Bina Mishra³, A.K.Tiwari⁴, Shikha saxena¹, Rajak Kaushal⁵, B.H.M Patel⁶, Amit Ranjan Sahoo¹, Sarath Chandra Janga⁷ and Ravi Kumar Gandham^{1*}

¹Computational Biology and Genomics Facility, Division of Veterinary Biotechnology, IVRI, Izatnagar (U.P), ² Directorate of Research, IVRI, Izatnagar (U.P), ³Division of Biological Production, IVRI, Izatnagar (U.P), ⁴ Division of Biological Standardization, IVRI, Izatnagar (U.P), ⁵Division of Virology, IVRI, Mukteshwar, ⁶Division of Livestock Production and Management, IVRI, Izatnagar (U.P), ⁷School of Informatics and Computing, IUPUI, Indianapolis, USA

Introduction: Peste des petits ruminants (PPR), is an acute transboundary viral disease of economic importance, effecting goats and sheep. Live attenuated vaccine of Sungri 96 is widely

used in Northern India against PPR. This vaccine virus, isolated from goat works efficiently both in sheep and goat. The molecular mechanism of this vaccine virus induced protection has not been understood till date. Therefore, in this study we investigated the host-vaccine virus interactions by infecting the peripheral blood monocellular cells (PBMCs) isolated from goat with Sungri 96 vaccine virus.

Methodology: The transcriptome data generated from infected goat PBMCs using Illumina Hiseq-2000 resulted in 120 million high quality 100-base paired-end reads. To improve the accuracy of prediction of differentially expressed (DE) genes we estimated global gene expression using both RSEM and cufflinks. The expression data generated from RSEM and cufflinks was analyzed using DESeq2, edgeR, and EBSeq; and cuffdiff2, respectively, to identify 4149 DE genes under PPRV infection. Initially, a cutoff of 2 fold was taken to select 2000 genes for gene ontology enrichment analysis using DAVID and G-profiler. These genes were extrapolated on the available comprehensive resource of protein–protein and genetic interactions database (BioGRID) and connectivity within them was established. Finally, 163 differentially expressed highly connected genes (DEHC - connectivity e⁻⁵ and fold change e⁻³) were represented in a network using Cytoscape 3.0.2. Transcription factors regulating these DEHC genes were identified using MEME and TOMTOM. The gene expression signatures were validated by Real time PCR.

Results and Discussion: On GO enrichment analysis of all the DE genes, a statistically significant number represented - immune signaling pathways. Antigen processing and presentation, spliceosome, chemokine and Jak-STAT signaling pathways, MAPkinase cascade, and apoptotic processes. Network analysis revealed that the protein - protein interaction network among differentially expressed genes is significantly disrupted in infected state. Several genes encoding TFs that govern immune regulatory pathways were identified to co-regulate the differentially expressed genes. TLR3 which is specific in recognizing single stranded RNA, IRF3 - the key transcriptional factor for establishment of antiviral state in infected cells, IFIT3 that induces IFN- γ antiviral response, CD3D - involved in T-cell development and GNAI3 - critical for normal B cell function were all upregulated reasserting the involvement of the immune response regulatory pathways.

Conclusions: This study provided insights into the host - PPRV vaccine virus interactome for the first time suggesting dysregulation of immune regulatory pathways and genes encoding TFs that govern these pathways.

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“Proteomics Perspective on Development and Characterization of Biosimilars & Biotherapeutics”

in

The 6th Annual Meeting of Proteomics Society, India (PSI), and International Proteomics Conference at IIT Bombay

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PREAMBLE

Therapeutic proteins are emerging as a promising candidate for prevention and treatment of various life-threatening diseases.¹ Biosimilars represents a class of drugs that are intended to offer analogous therapeutic efficacy and safety compared to their original counterparts that are no longer under intellectual property coverage. According to recent study, it is estimated that several patents including commercially popular brands worth 67 billion dollars will be expiring before 2020, providing an exciting possibility for biosimilar drugs to gain significant market share.² Biosimilar versions of numerous drugs (i.e., Eprex, Neupogen, and Genotropin) have already been developed and approved by regulatory agencies for clinical applications. By 2010, approximately \$172 million of revenue in the biosimilars market has already been recorded and it is estimated that it will increase over \$19 billion by 2014.^{3,4}

The structural complexity, complex manufacturing processes and increased risk of immunogenicity provides key challenge in preparing biological therapeutics compared to small molecular drugs. Changes in protein modifications (i.e. glycosylation, phosphorylation, methylation and hydroxylation) can impact therapeutic activity of the biosimilars. Downstream processes and related impurities can impact the native structure of proteins, increasing potential risk of immunogenicity.

Powerful analytical tools provide a possibility of comparing biosimilars with counterpart protein biopharmaceuticals, which is critical in addressing above-mentioned difficulties associated with the preparation of biological therapeutics. Therefore, Proteomics Society, India (PSI) invites all the participants across the globe to attend the 6th International Conference and Workshop on “Proteomics Perspective on Development and Characterization of Biosimilars & Biotherapeutics” on December 8-9, 2014 at IIT Bombay, India. This will provide essential platform to discuss the current state of the art in analytical technologies to assess the key characteristics of protein biopharmaceuticals that regulatory authorities have identified as being important in development strategies for biosimilars such as post-translational modifications, three-dimensional structures, and protein aggregation.

1. H. Schellekens, *Clinical therapeutics*, 2002, 24, 1720-1740
2. US\$67 billion worth of Biosimilar patents expiring before 2020, <http://www.gabionline.net/Biosimilars>, 2014
3. S. Aggarwal, *Nature biotechnology*, 2007, 25, 1097-1104
4. G. Dranitsaris, K. Dorward, E. Hatzimichael and E. Amir, *Investigational new drugs*, 2013, 31, 479-487

SESSIONS AND SPEAKER DETAILS

Session 1: Biosimilar and Biotherapeutics Scenario in Indian Biopharma

Session 2: Advances in Mass Spectrometry for Biosimilar Characterization

Session 3: Challenges and Regulatory aspects for Biosimilar Development & Characterization

Session 4: Functional Characterization of Biologics

Speakers from FDA and Regulatory

1. Dr. Michael Boyne, US-FDA
2. Dr. Ranjan Chakrabarti, United States Pharmacopeia, (India)

Speakers from Industry

1. Dr. Rustom Mody; *Lupin Biotech , Pune, India*
2. Dr. Anita Chugh; *Syngene International, Bangalore, India*
3. Dr. Gary Kruppa; *MRM Proteomics Inc, Canada*
4. Dr. Himanshu Gadgil; *Intas Pharma, Ahmadabad, India*
5. Dr. Sriram Kumaraswamy; *Pall ForteBio, USA*
6. Dr. Anette Persson; *GE Healthcare, Sweden*
7. Dr. Amarnath Chatterjee; *Biocon Research Limited, Bangalore, India*
8. Dr. Taegen Clary; *Agilent Technologies, USA*
9. Dr. Mark McDowell; *Waters Corporation, UK*

Speakers from Academia

1. Dr. Robert Moritz; *Institute for Systems Biology, USA*
2. Prof. Anurag Rathore; *Indian Institute of Technology Delhi, India*
3. Dr. Christina Ludwig; *ETH, Zurich, Switzerland*
4. Dr. Vijaya Pattabiraman; *ETH Zurich, Switzerland*
5. Dr. Sudhir Sahasrabudhe; *Medicinal Chemistry, University of Utah, USA*

Chairs

1. Dr. Bipin Nair; *Amrita School of Biotechnology, Kollam, India*
2. Dr. Vidhyashankar Ramamurthy, *Syngene International, Bangalore, India*
3. Dr. M. A. Vijayalaxmi; *VIT University, Vellore, India*
4. Dr. Uma Sinha Datta, *GE Healthcare, India*
5. Dr. Kumar Prabhakar, *Tata Memorial Hospital, Mumbai, India*

8th DECEMBER 2014

SESSION I: BIOSIMILAR AND BIOTHERAPEUTICS IN INDIAN BIOPHARMA

PLENARY SPEAKER



Dr. Anurag Rathore
IIT Delhi, India

KEYNOTE ADDRESS



Dr. Sudhir Sahasrabuddhe
University of Utah, USA

INVITED TALK



Dr. Rustom Mody
Lupin Biotech, India

SESSION II: BIOSIMILARS- CHALLENGES AND REGULATORY ASPECTS

PLENARY TALK



Dr. Mike Boyne
US-FDA

KEYNOTE ADDRESS



Dr. Himanshu Gadgil
Intas Biopharma

INVITED TALKS



Dr. Anita Chugh
Syngene International



Dr. Ranjan Chakrabarti
US-Pharmacopeia



Dr. Amarnath Chatterjee
Biocon Research Ltd.

SESSION III: ADVANCES IN MASS SPECTROMETRY FOR BIOSIMILAR CHARACTERIZATION

PLENARY SPEAKER



Dr. Gary Kruppa
MRM Proteomics, Canada

KEYNOTE ADDRESS



Dr. Robert Moritz
Institute of Systems Biology,
USA

INVITED TALKS



Dr. Taegan Clary
Agilent Technologies, USA



Dr. Christina Ludwig
ETH Zurich, Switzerland



Dr. Mark McDowall
Waters Corporation, UK

SESSION IV: FUNCTIONAL CHARACTERIZATION OF BIOLOGICS

KEYNOTE ADDRESS



Dr. Sriram Kumaraswamy
Pall Forte Bio, USA

INVITED TALKS



Dr. Vijaya Patabiraman
ETH Zurich, Switzerland



Dr. Anette Persson
GE Healthcare, Sweden

PANEL DISCUSSION**Regulatory views & Proteomics technology**

8th Dec 2014 Time: 17:30 – 18:30

Therapeutic proteins are next-generation drugs in the prevention and treatment of diseases, in particular human critical illness. Biosimilars are a new class of drugs intended to offer comparable safety and efficacy (or clinical equivalence) to their original reference products. India is one of the leading contributors in the world biosimilar market and is the third-largest in the Asia-Pacific region. India has demonstrated high acceptance of biosimilars, however, biosimilars are set to become an important part of the future medicines market. Therapeutic development of lower cost biosimilars will inevitably enter the drug market in the near future, increasing the market competition and patients' access to the more cost-effective therapies. This discussion will address the issues of concern with the use of biosimilars and need of appropriate regulations for their approval.

Moderator**Dr. Uma Raghuram;** *Spinco Biotech Pvt. Ltd.***Panel Discussion I: Regulatory views****Panelist****Dr. Michael Boyne;** *US-FDA***Dr. Anurag Rathore;** *Indian Institute of Technology (IIT), Delhi, India***Dr. Ranjan Chakrabarti;** *United States Pharmacopeia, India***Dr. Rustom Mody;** *Lupin Biotech, Pune, India***Dr. Himanshu Gadgil;** *Intas Pharma, Ahmedabad, India***Dr. Anita Chugh;** *Syngene International, Bangalore, India***Dr. Kumar Prabhush,** *Tata Memorial Hospital, Mumbai, India*

Indian DCGI and FDA views

Panel Discussion II: Proteomics technology**Panelist****Dr. M. A. Vijayalakshmi;** *VIT University, India***Dr. Bipin Nair;** *Amrita School of Biotechnology, India***Dr. Anette Persson;** *GE Healthcare, Sweden***Dr. Dinesh V Palanivelu;** *Biocon Research Limited, Bangalore, India*

SESSION I: BIOSIMILAR AND BIOTHERAPEUTICS IN INDIAN BIOPHARMA

PLENARY LECTURE-1

Speaker: Dr. Anurag S. Rathore

Professor, Indian Institute of Technology Delhi, India

Biography: Dr. Rathore is a Professor at the Department of Chemical Engineering, Indian Institute of Technology Delhi, India. He is also a consultant of Biotech CMC Issues. His previous roles included management positions at Amgen Inc., Thousand Oaks, California and Pharmacia Corp., St. Louis, Missouri. His areas of interest include process development, scale-up, technology transfer, process validation, process analytical technology and quality by design. He has authored more than 300 publications and presentations in these areas. He is presently serving as the Editor-in-Chief of Preparative Biochemistry and Biotechnology and Associate Editor for Journal of Chemical Technology and Biotechnology and PDA Journal of Science and Technology. He also serves on the Editorial Advisory Boards for Biotechnology Progress, BioPharm International, Pharmaceutical Technology Europe and Separation and Purification Reviews. Dr. Rathore has edited books titled Quality by Design for Biopharmaceuticals: Perspectives and Case Studies (2009), Elements of Biopharmaceutical Production (2007), Process Validation (2005), Electrokinetic Phenomena (2004) and Scale-up and Optimization in Preparative Chromatography (2003). He has a Ph.D. in Chemical Engineering from Yale University.

Title: Indian Biopharma: Challenges and opportunities

Abstract: The Indian pharmaceutical industry has seen remarkable growth over the past decade (from less than 5 billion \$ in 2004 to 25 billion \$ in 2014). India has established itself as a global producer of economical, safe and efficacious pharmaceutical products. What remains to be seen is if this success can be repeated in the next decade in production of biotech therapeutics? This talk will address three topics. First, the economic drivers for the biosimilars will be discussed. The unique requirements of the emerging markets will be highlighted. Next, the challenges that are faced during commercialization of biosimilars will be presented. While the central issue that has and will continue to dominate is our limited understanding of how the different quality attributes of a protein product impact its safety and efficacy, progress has been made via development of new tools that elucidate this relationship. Gaps that currently exist include a lack of standardization in which process and clinical data is collected, analyzed and reported; accessibility of data across molecules and sponsors; complexity of protein products with respect to the numerous quality attributes, structural heterogeneities and molecule to molecule differences in behavior; limitations associated with the ability of non-clinical tools in predicting clinical safety and efficacy; and complexity of the processes that are used to manufacture these protein products along with the raw materials that they use. Finally, technology drivers that can alleviate the above mentioned gaps will be discussed. They

include high throughput process development (HTPD), computational fluid dynamics (CFD), multivariate data analysis (MVDA), quality by design (QbD), and process analytical technology (PAT).

INVITED LECTURE-1

Speaker: Dr. Rustom Sorab Mody

Senior Vice President & Head of R&D, Lupin Ltd. (Biotech Division), Pune, India

Biography: A Biotechnologist with over 19 years of extensive Biopharmaceutical industry experience. Handled wide-ranging operations in the capacity of Head, Technical (R&D, Manufacturing & Quality), Techno-Commercial (identification of in-licensing & out-licensing opportunities, management of R&D alliances, selection & development of marketable products, technologies and services, management of business (partners/clients) and Facility Management (expansion of infrastructure & capabilities in sync with commercial interests & opportunities). A past history of hands-on experience in developing 6 biosimilar products from concept to commercialization. Currently handling 10 products in development, 2 of which are for Regulated Markets. Proven track record in directing multiple projects on a fast track with technical problem-solving capability, building strong intellectual property and designing the best course for project development, team building, and enhancement of quality and manufacturing efficiency. An out-of-box thinker with a strong understanding of biogeneric business in India and other semi-regulated and regulated markets.

Title: Analytics in biosimilar development: Characterization and comparability

Abstract: In the past two decades, biosimilar industry has made significant progress within the biotherapeutics industry as patent exclusivities have created an unprecedented rush among biosimilar companies to develop and market off-patented biotherapeutics. Because of the inherent complexity of biologics in terms of structure and the structure-function relationship, any change in the molecular structure, has the potential to adversely affect the quality, safety, or efficacy of a biotherapeutic. Therefore, it is well recognized that "Process is the Product". It is imperative for biopharmaceutical or biosimilar manufacturer to demonstrate the comparability of their product to the previously made product before the process change is introduced. Likewise, biosimilar manufacturers, who make copies of the innovator's product using a different process, are required to thoroughly evaluate their product's equivalency to the innovator's product. Using a variety of test methodologies a biosimilar developer can derive in-depth understanding of the molecular structure, heterogeneity, higher order structure, quality attributes, and functional behavior. In the presentation, regulatory requirements and challenges faced by biosimilar developers are being discussed.

SESSION II: CHALLENGES AND REGULATORY ASPECTS

PLENARY LECTURE-2

Speaker: Dr. Michael T. Boyne II

US-FDA

Biography: Dr. Boyne received his Ph.D. in Chemistry from the University of Illinois Urbana-Champaign for his work on the development and application of next generation mass spectrometry for protein analysis at high-resolution. After an American Cancer Society/Canary Foundation Postdoctoral Fellowship at Washington University School of Medicine in Saint Louis where he worked with the NIH/NIGMS Biomedical Mass Spectrometry Resource and clinical oncologists developing tumor progression biomarkers, he joined the FDA in early 2010. As a research chemist, he serves as an expert for the comparative analysis of complex drug products, designs and validates bioanalytical methods for preclinical and clinical studies evaluating aspects of pharmacology, toxicology, and drug exposure, and has established a research program for physicochemical characterization of protein therapeutics and monoclonal antibodies. He has received a number of research awards in his time at FDA including the FDA Scientific Achievement Award for Excellence in Laboratory Science as part of the OBP/OTR bioprocess research team and the CDER Regulatory Science Excellence Award for work on a complex drug product and its characterization. Michael T. Boyne II is a research chemist in the Division of Pharmaceutical Analysis within the Center for Drug Evaluation and Research at the US Food and Drug Administration. Specializing in bioanalytical chemistry. He works on the comparison and characterization of complex drugs, including protein therapeutics, monoclonal antibodies and natural products, and he serves as a subject matter expert for the implementation of modern analytical technologies into the regulatory review process.

Title: Applications of mass spectrometry for characterizing biopharmaceuticals in the regulatory setting

Abstract: The scope and purpose of this seminar is to discuss and assess the application of mass spectrometry methods as an analytical tool for characterizing therapeutic proteins and monoclonal antibodies. Mass spectrometry constitutes an essential tool for the characterization of protein therapeutics and monoclonal antibodies, for analytical comparability and similarity, as described in ICH Q5E and the FDA draft Guidance for Industry “Quality Considerations in Demonstrating biosimilarity to a Reference Protein Product”. Specific case examples will be presented to convey our current understanding of the issues involved with reproducible sample processing, the range of appropriate chromatography tools, and the different techniques for mass spectral acquisition. Efforts in development of reference materials and data libraries for high quality data interpretation, structural characterization, and their value to the regulatory process.

KEYNOTE ADDRESS

Speaker: Dr. Himanshu Gadgil

Sr. Vice President, Intas Biopharmaceuticals Ltd., Ahmedabad, India

Biography: Himanshu has pursued PhD in Biochemistry from University of Tennessee, Memphis, USA. He is currently working as a Sr. Vice president Biotech Division for Intas Pharmaceuticals Ltd. Under his leadership the company has launched 4 biosimilar products in India and 1 biosimilar product in EU. Prior to Intas he has over 10 years combined experience in Waters/Micromass and Amgen in various leadership position and gained wide range of experience from pre-IND to post commercialization of therapeutic proteins. Himanshu has broad expertise in Process development, Formulation Development, Protein characterization, Mass Spectrometry with over 40 publications and patents in these fields.

Title: Six blind men and a beast: Co-operative analytics holds the key for product driven process development

Abstract: Recent advancements in analytical technologies have revamped CMC and clinical development of biotherapeutics. The focus has shifted from a rigid manufacturing process supported by limited testing to a comprehensive physicochemical understanding of a product which guides optimal process design space. This talk will touch upon advances in the analytical toolbox for chemical, structural and functional characterization of proteins. A CQA based process and product development approach will be discussed with specific examples and case studies of how comprehensive CQA profiling can guide the development of biologics and biosimilars.

INVITED LECTURE-2

Speaker: Dr. Anita Chugh

Head Biology, Syngene International, Bangalore, India

Biography: An outstanding academic background and a progressive operational track record in the arena of Discovery Biology. Gained her M.Sc. and Ph.D., from premier Biomedical Research Institute of India (AIIMS), and did Post-Doctoral Fellowship at McMaster’s University in Ontario, Canada, before returning to India in 1995. Since then, she has been working operationally for 18+ years in discovery biology, spanning both empirical and contract service roles. Has expertise and contemporary understanding of Biology, across spectrum of disciplines including discovery and development of small and large molecule therapeutics. Currently, Research Director, Biology at Syngene International leading a group of 150+ scientists.

Title: Emerging biotherapeutics: Challenges in their characterization

Abstract: Monoclonal Antibodies have been and continue to be the dominant category in biotherapeutics. Characterization tools for these molecules are established and proteomics plays an important role in their characterization. Not too far behind in

the pipeline are candidate biotherapeutic molecules that are either antibody fragments or are made up of non-antibody scaffolds, modified in multiple ways to enhance desired properties. These novel molecules pose unique challenges in terms of their characterization. While proteomic tools continue to improve, it is imperative that these tools are effectively leveraged and seamlessly integrated with other available and emerging technologies in order to overcome these challenges.

INVITED LECTURE-3

Speaker: Dr. Ranjan Chakrabarti

Vice President-Biologics, United States Pharmacopeia-India

Biography: Dr. Ranjan Chakrabarti is currently Vice President – Biologics and Biotechnology at United States Pharmacopeia Convention at their India office. He has over 19 years of experience in Pharmaceutical and biopharma industries. Before joining to Industry, he worked in Academics at SUNY, Buffalo and University of Massachusetts Medical Centre at USA and successfully coordinated research projects in Cancer Cell Biology and Diabetes. He has guided several Ph. D. students. Before joining USP, Dr. Ranjan was leading the Biology Group at Dr. Reddy's Drug discovery and also served at key management position in GVK Biosciences. He has worked with several National and International companies for discovery and development of both chemical and biological molecules. Dr. Ranjan is the Co-Inventor of 32 US Patents; published 55 papers in peer reviewed International Journals and presented 65 lectures in International and National Conferences.

Title: Standards for biotherapeutics – USP approach

Abstract: United States Pharmacopeia (USP) is the oldest Pharmacopeia in the world involved in Standard setting process for both Chemical and Biological Medicines. Standards for biological medicines in the USP go back more than 80 years and today are comprised of more than 100 monographs for articles licensed in the US. The monographs are supported by a portfolio of close to 40 General Chapters as well as Biological Reference Materials. Biological Standards work is guided by Expert Committees with members from different countries, which in turn is supported by Expert Panels. Standard setting process for Biologics is based on the Quality Attributes of the specific drugs. USP is developing standards in the area of Therapeutic Proteins, Monoclonal Antibodies, Blood Products, Peptides and Vaccines. Monoclonal antibodies (MAbs) have become the single most important product class in the area of modern recombinant therapeutics. Procedural standards for the analysis of MAbs can

be an important tool for the creation of consistent analytical expectations and sound technical approaches. USP has developed a General Chapter <129> that collects several of the most critical analytical procedures for MAbs and will be supported by USP System Suitability Standards. The talk will focus on the present activities of USP in developing standards for biological medicines.

INVITED LECTURE-4

Speaker: Dr. Amarnath Chatterjee

Biocon Research Ltd., Bangalore, India

Biography: He has done his doctorate in Biophysics from Tata Institute of Fundamental Research, Mumbai, India followed by post-doc from The Scripps Research Institute, La Jolla, CA, USA. His present role at Biocon as Scientific Manager, he lead the molecular characterization group, includes scientists (constituted by scientists with Masters and PhD degrees) for all Insulin analogues produced by Biocon. He is involved in designing high-throughput as well as detailed strategies for the physicochemical characterization of biologics. Analytics and characterization SME for partnered projects (partners include Mylan Inc., Fuji Film Pharma and so on). He is involved in strategy designs for novel anti-diabetic products.

Title: Regulatory role of biophysics in the biopharma industry: Perspectives from application to biosimilars

Abstract: Bio-pharmaceutics in the recent years has seen remarkable growth essentially due to the usage of recombinant proteins as a therapeutic agent. This is more so due to the ease of designing recombinant proteins so as to have custom function and long circulation half-life. Further production of recombinant proteins is cost effective when compared with pharma drugs that require multi-step synthetic reactions. However, proteins have their limitations in terms of post translational modifications (PTMs) and high susceptibility to a variety of enzymatic and non-enzymatic degradation during manufacturing, formulation and storage, which in effect lead to the loss of their structure and biological function. Hence it becomes imperative that the degradation pathways are monitored and deciphered well in advance of human use. Regulatory authorities the world over are hence highly critical when it comes to Chemistry, Manufacturing and Controls (CMC) data on such drugs. The talk will focus on the usage of various biophysical tools in obtaining insights into some of the CMC parameters. The talk will also show how biophysics and structural biology helps in regulating problems associated with the production of bio-pharma drugs using examples from on-going projects in our laboratory.

SESSION III: ADVANCES IN MASS SPECTROMETRY

PLENARY LECTURE-3

Speaker: Dr. Gary Kruppa

MRM Proteomics, Canada

Biography: Gary Kruppa is currently CEO and President of MRM Proteomics, with over 30 years of experience in mass spectrometry and over 20 years of experience in sales, marketing, business development, management, and M&A activity in the life science and diagnostic applications areas of mass spectrometry. He previously served as the Vice President (Business Development) from 2004 to July 2014 at Bruker Daltonics. From 2001 to 2004, he was a Principal Member (Technical Staff) at Sandia National Laboratories. At Sandia, he authored several publications on the use of mass spectrometry to study protein structures, and developed key collaborations. He had previously been associated with Bruker since 1991 in various roles such as Product Manager and Vice President (Fourier Transform Mass Spectrometry). Dr. Kruppa completed his B.S. at the University of Delaware and completed his Ph.D. at the California Institute of Technology.

Title: Structural characterization of biosimilars by HDX and top-down analysis using ECD/ETD-FTMS

Abstract: Rapid developments in biopharmaceuticals have put high demands on analytical tools that can provide accurate and comprehensive characterization of therapeutic proteins, including biosimilars. Amide hydrogen/deuterium exchange (HDX), monitored by mass spectrometry (MS), is now a powerful technique for measuring protein higher-order structural changes. Although the enzyme digestion-based “bottom-up” approach is most commonly used, it only gives peptide-level information, often with incomplete sequence coverage, and suffers from high level of back-exchange (typically 10-50%). The “top-down” approach, which fragments intact proteins directly using electron-based dissociation, is becoming an important alternative and has several advantages. However, the commonly used top-down strategies are direct-infusion based, and thus can only be used with volatile buffers. This has meant that the “top-down” approach could not be used for studying proteins under physiological conditions — the very conditions which are often very important for preserving a protein’s native structure and function. In this presentation, we show how top-down ECD/ETD, HDX, and subzero temperature HPLC can be combined and used to overcome these limitations and characterize the higher-order structure of protein biosimilars, including interferons and monoclonal antibodies. This method keeps the back-exchange level as low as 2%, and has no limitations in terms of protein type and sample solution conditions. The differences in higher-order structure between protein variants, as well as the impact of PTMs and ligand binding on antibody’s conformation were determined, with close to single-residue resolution.

KEYNOTE ADDRESS

Speaker: Dr. Robert Moritz

Institute for Systems Biology, USA

Biography: Dr. Robert Moritz, a native of Australia, joined the ISB faculty in mid-2008 as Associate Professor and Director of

Proteomics. Dr. Moritz began his work in 1983 in the Joint Protein Structure laboratory of Prof. Richard J. Simpson (JPSL-Ludwig Institute for Cancer Research, and The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia). During his 25 years at JPSL, Dr. Moritz designed and implemented a number of technologies currently used in many proteomics laboratories across the globe. Examples include technologies such as the development of micro-chromatography for proteomics from the late 1980’s to its current day implementation, a micro-fractionation technique widely used by many laboratories worldwide. His collaborative research into cytokine biochemistry, protein-receptor chemistry and cellular biochemistry culminated in the novel identification of a number of proteins (e.g., IL-6, IL-9 A33 ligand, DIABLO, as well as several others), their interacting partners, and 3-dimensional structures of their cell surface receptors important in human health concerns such as cancer and inflammation. During his time at JPSL, Dr. Moritz progressed through the ranks whilst obtaining his Bachelor’s degree in Biochemistry with first-class Honors, and his Ph.D., from the University of Melbourne. Dr. Moritz has brought wide-ranging skills and expertise to ISB, much of it drawn from his Australian experience. There, in 2005, he conceptualized a shared proteomics high-performance computing system, organized a consortium of proteomic scientists from all states in Australia, and proposed a computational system specifically for proteomics data analysis for all Australian researchers to access. For this work, in 2006, he was awarded an enabling grant from the Australian National Health and Medical Research Council worth AUS\$2M. With that award, he established a bioinformatics center in Australia that enabled proteomic researchers anywhere in the country to analyze mass spectrometry data. It was the first effort on a national scale to bring proteomic data analysis and algorithms to any researcher in the whole country without the need for them to build their own bioinformatics group. In late 2006, the Australian Proteomics Computational Facility (APCF, www.apcf.edu.au) was inaugurated, and Dr. Moritz remains as Director of the APCF. The dedicated proteomics data analysis facility is equipped with a 1000 CPU high-performance computing cluster, and full-time software engineers for the continued development of proteomics algorithms and data validation. This facility serves all researchers in Australia and others regardless of their global geographical location. He is continuing that work at ISB by expanding the ISB proteomics centre into a national facility with online tools for data analysis.

Title: Whole proteome resources: Access and sharing of data through the peptide and SRM Atlas proteomics suites

Technology advances have provided unprecedented levels of protein identification and quantitation across a multitude of organisms, including human. To capitalize on these advances in comprehensive proteome interrogation, we have developed new tools, methods and bioinformatics approaches to propel these advances to broad and routine usage. The democratization of these resources has enabled these tools to be routinely used and enable biologically meaningful data to be collected, analyzed and shared. To support broad dissemination of these enabling tools and resources, we have developed new systems and repositories and integrated these developments into our existing and new suites of bioinformatics software and repositories. I will discuss these tools that are capable of generating complete, reproducible

and quantitatively accurate proteome datasets. Examples of these include our developments in targeted proteomics utilizing SRM and SWATH-MS and applications to quantitative biomarker discovery.

INVITED LECTURE-5

Speaker: Dr. Taegen Clary

Life Sciences and Diagnostics Group, Agilent Technologies, USA

Biography: Taegen Clary is currently the Director of Marketing for the Pharmaceutical Segment at Agilent Technologies, managing global segment management teams and global and regional marketing program managers focused on the pharma and biopharma markets. Taegen joined Agilent in 2007 as a product manager in the Chemistry and Supplies Division, managing the bio-chromatography portfolio. In 2010, Taegen joined Life Sciences Group Segment Marketing organization as the Biopharmaceutical Segment Manager. Prior to Agilent, Taegen worked at Bio-Rad Laboratories, managing the laboratory scale bio-chromatography portfolio. He also worked in the pharmacology department at Berlex Biosciences, now a part of Bayer Healthcare, analyzing both small molecule drugs and biomarkers using LC/MS. Taegen has a Bachelor's of Science in Biotechnology from UC Davis and an MBA from St. Mary's College of California.

Title: Application of mass spectrometry in the characterization of biosimilars

Abstract: As the number of biosimilars in development continues to grow rapidly, the analytical requirements for approvals are also increasing. Mass spectrometry has been a staple in the analysis of small molecule drugs and has rapidly become a routinely used tool for the characterization of biologics. Increases in the mass range and accuracy at high masses has made mass spectrometer the ideal tool for confirming protein masses, peptide sequences and identification of modifications. This talk will cover the three most commonly used LC/MS techniques for characterizing therapeutic proteins and will demonstrate their utility for confirming comparability of biosimilars. The focus will be on intact mass measurements including reduced forms, peptide mapping including identification of post translational modifications and glycan analysis. Relevant new methods and technologies in each of these areas will be covered in detail.

INVITED LECTURE-6

Speaker: Dr. Christina Ludwig

Institute of Molecular Systems Biology, ETH Zürich, Switzerland

Biography: Christina Ludwig completed her PhD from the Technical University Dortmund, Germany in 2008. She is doing her post-doctoral research at Institute of Molecular Systems Biology, ETH Zürich, Switzerland. She is the recipient of Young investigator award at the conference "Micromethods in protein chemistry" in Dortmund, Germany. She has publication in various peer reviewed journals like *Journal of Biological Chemistry* and *Biochemistry*. She has been invited as a speaker in several workshops on targeted proteomics.

Title: Targeted Proteomics -Advantages and challenges using mass spectrometric techniques like SRM and SWATH-MS

In recent years targeted proteomic workflows, including techniques like Selected Reaction Monitoring (SRM) and

SWATH-MS, have gained in acceptance and are now frequently used by the proteomic community to investigate protein abundances as well as post-translational modifications (PTMs). The major advantage of targeted workflows compared to discovery-driven techniques lies in better quantitative data quality resulting from improvements in reproducibility of data acquisition, quantitative precision and accuracy. However, the drawback of any targeted technique is the requirement of prior knowledge about all targeted analytes of interest in form of a mass spectrometric assay that includes information like: i) which peptides to target per protein, ii) which specific PTM at which specific position is of interest iii) in which dominant charge state the peptides ionize iv) how the peptides' fragmentation pattern look like and iv) what are the expected peptides' retention times on the current LC system. In this presentation I will review the basic principles of SRM and SWATH-MS and highlight their specific advantages and disadvantages based on actual research projects ongoing in our laboratory. I will demonstrate the reproducibility of SWATH-MS in an inter-laboratory study, show how we aim for generating high-quality genome-wide mass spectrometric assay libraries for various organisms, including *Mycobacterium tuberculosis*, *Saccharomyces cerevisiae* and human, and highlight how targeted proteomics can improve the quantitative analysis of protein phosphorylations.

INVITED LECTURE-7

Speaker: Dr. Mark A. McDowell

Waters Corporation, United Kingdom

Biography: Dr. Mark McDowell obtained his B. Sc in Chemistry (1980) and his PhD in biological mass spectrometry (1984) from the University of Wales working on the development and application of 'primitive' LC/MS technologies to the analysis of peptides and pharmaceutical products. He obtained a post-doctoral fellowship from the Royal Society (of Great Britain) in 1984 to research the mechanism of thermospray ionisation at the University of Bonn (Germany). Mark joined the LC/MS development group of VG Instruments in 1985 and was involved in the mass spectrometry operations and management of the company for over 29 years – throughout its evolution to Micromass and finally Waters Corporation. Prior to his retirement in February 2014, Mark was the Senior Manager responsible for strategic development of the company's Pharmaceutical Discovery & Life Science business – focusing on mass spectrometry for the health & life sciences world-wide. Dr. McDowell served two terms of office on Waters Corporation's Scientific Advisory Board and was a member of the company's Centres of Innovation Program that maintains strategic collaborations with many of the World's premier research institutions. Today Dr McDowell continues his career-long association with Waters Corporation as a business development consultant focusing on mass spectrometry in India.

Title: High definition MS strategies for the development of novel biopharmaceuticals & characterization of biosimilars

High Definition Mass Spectrometry (HDMS) is defined as the on-line combination of Ion Mobility (IM) separation and high resolution Time-Of-Flight (TOF) Mass Spectrometry (MS). Ion Mobility separates ionized molecules (e.g. Proteins/peptides) on the basis of their size-to-charge ratio. Conceptually IM separation can thus be likened to gas phase electrophoresis. Moreover IM separation is very fast, typically taking only 10-100 milliseconds per analysis, and is thus readily performed in concert with on-line HPLC separation. After calibration (e.g. with well-

characterized peptide/protein standards) an HDMS instrument is able to determine the Collision Cross-Section (CCS), in square Angstroms, of any protein in its native (or modified) state. CCS is a reproducible and precise physicochemical property of an ionized protein that can be used diagnostically. CCS data provide an insight to tertiary protein structure that complements Hydrogen/Deuterium Exchange (H/DX) MS data. The CCS of a

biopharmaceutical can provide an orthogonal metric for product characterization and/or the detection of protein misfolding. IM separation is compatible with both Collision Induced Dissociation (CID) and Electron Transfer Dissociation (ETD). We have successfully combined these techniques for the top/middle down characterization of biopharmaceuticals and Biosimilars.

SESSION IV: FUNCTIONAL CHARACTERIZATION OF BIOLOGICS

KEYNOTE ADDRESS

Speaker: Dr. Sriram Kumaraswamy

Senior Director, Pall ForteBio LLC, USA

Biography: Sriram Kumaraswamy is senior director, strategic marketing and applications for Pall ForteBio LLC. The research scientist turned marketing professional leads the development and implementation of commercial strategy to grow Pall's process analytical technology portfolio with a cross-functional team of marketers and scientists. Sriram has responsibility for product management, outbound marketing, applications development and technical support functions at Pall ForteBio. Previously, he conducted research and development at start-up technology ventures to successfully develop and commercialize label-free and fluorescence-based instrument and assay platforms that supported drug discovery research. Sriram has four patents and several publications on fluorescent assay techniques and has written book chapters on label-free biolayer interferometry. He conducted post-doctoral research on semi-conducting materials at the University of Arizona, and doctoral research in synthetic and physical organic chemistry at the University of California at Berkeley. Sriram is passionate about technical innovation and commercialization.

Title: Label-free binding assays in the processing and analysis of biopharmaceuticals

Biopharmaceuticals have complex structures that provide great analytical challenges in the successful development of drugs. Binding assays play a crucial role in drug development, enabling analysis of drug-target binding affinity and specificity. They are in routine use from early discovery to measurement of consistency between batches of drug produced, and equivalency between a biosimilar and its original product. Bio-layer interferometry (BLI) technology has gained prominence for its broad application along various stages of development from target identification through lead selection, pre-clinical testing, and bioprocess development to quality control in manufacturing of antibody and recombinant protein therapeutics, vaccine and small molecule drugs. This presentation will describe BLI technology, and its applications in biopharma for lead screening, Fc receptor binding assays, cell culture and quality control, and in small molecule analysis.

INVITED LECTURE-8

Speaker: Dr. Vijaya R. Pattabiraman

Laboratory of organic chemistry, ETH Zurich, Switzerland

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

methods for the preparation of synthetic proteins and the development of associated technology.

Title: Enabling chemical technologies for protein biotherapeutics development

The completion of the Human genome project more than a decade ago has opened up opportunities for seemingly endless research projects and research fields. This is ushering in an era of personalized medicine primarily driven by molecular level understanding of bimolecular interactions. Great advances in the field of proteomics and related technologies have enabled the identification and study of several disease related proteins. This has facilitated the development of several small molecule drugs that interact with these proteins. However, in the past years protein based drugs have globally dominated the new drugs market. By 2020, more than 50% of top-selling drugs are expected to be protein-based drugs. The tremendous growth in the biopharmaceutical sector also necessitates the advent of new methods for producing protein-based drugs, which is currently dominated by recombinant technology. Landmark progress in chemical methods has now enabled the routine preparation of synthetic proteins in both post-translationally modified and unmodified forms. Moreover, chemical protein synthesis allows precise incorporation of any unnatural amino acid, reporters, probes etc. to study protein structure and function. This lecture will focus on the frontier chemical methods for accessing synthetic proteins highlight few successful synthetic clinical protein candidates and elucidate the advantages of the new chemical technologies over conventional methods for the development of biotherapeutics.

INVITED LECTURE-9

Speaker: Dr. Anette Persson

GE Healthcare, Sweden

Biography: Anette Persson has worked with label-free interactions since the earliest years. With a background in biochemistry and clinical chemistry at Uppsala University, she came to Pharmacia Biosensor in late 1980's, a few years before the commercial launch of the first Biacore instrument. After 10 years in the system department, working with application development with a focus on the integration of sensor surfaces, flow system and application needs in the first biosensor instruments, Anette moved to a position as European application specialist with a supporting role for both existing users and sales to new customers. Since 1997 Anette has held a position of manager in the Knowledge and Training group with responsibility for developing and providing training courses and application support.

Title: Monitoring high order structure in biotherapeutic development using SPR, DSC and 2-D DIGE

Several attributes, including among others activity, concentration, purity and stability, are relevant to process control and quality assessment of biotherapeutic product quality. This presentation highlights contributions from surface plasmon resonance (SPR), differential scanning calorimetry (DSC) and 2-D fluorescence difference gel electrophoresis (2-D DIGE) to quality assessment of antibody biotherapeutics, using stress-related changes as a model system. The results indicate that the combination of these orthogonal techniques is a powerful tool in quality assessment.



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Poster no: D-1-01**A Multi-Pronged Approach for Molecular Profiling of Gastric Adenocarcinoma**

Raja R.^{1*}, Subbannayya Y.¹, Syed N.¹, Pinto S. M.¹, Manda S.S.¹, Renuse S.¹, Sahasrabuddhe N. A.¹, Marimuthu A.¹, De Wijn R.³, Srivastava M. K.⁴, Veerendra Kumar K. V.², Vijayakumar M.², Prasad T. S. K.¹, Kumar R. V.², Pandey A.^{5,6,7} Chatterjee A.¹ and Gowda H.¹

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Introduction: Gastric cancer is an aggressive disease generally diagnosed at an advanced stage as patients present with non-specific symptoms. Molecular profiling of gastric cancer through multiple approaches is likely to provide potential biomarkers for early detection and molecules for targeted therapeutic intervention.

Methodology: We employed an iTRAQ-based quantitative proteomic approach to identify differentially expressed proteins in gastric cancer tissues compared to adjacent non-neoplastic gastric tissue. A similar strategy was employed to identify differentially expressed proteins in the sera of patients with gastric cancer. Kinome profiling was also performed using tyrosine kinase PamChip peptide array platform.

Results and discussion: iTRAQ-based quantitative proteomic analysis led to the identification of 22 proteins that were overexpressed and 17 proteins that were downregulated in gastric tumor tissues compared to the adjacent normal tissue. Among the proteins identified, Calcium/calmodulin-dependent protein kinase kinase 2 (CAMKK2) was found to be 7-fold overexpressed in the gastric tumor tissue. siRNA based approaches revealed a crucial role for CAMKK2 in gastric cancer cell proliferation and invasion. Serum protein profiling led to the identification of 643 proteins, of which 62 proteins were differentially expressed in serum of gastric cancer patients when compared to the controls (51 proteins were in higher abundance and 11 in low abundance). We identified several novel secreted proteins associated with gastric cancer such as inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4), leucine-rich repeat protein 1 (LRR1) and sex hormone-binding globulin (SHBG). ITIH4 was further validated across gastric cancer sera using multiple reaction monitoring (MRM). Through kinase profiling, we identified platelet-derived growth factor receptor, beta polypeptide (PDGFRB) and paxillin (PAXN) to be hyperphosphorylated in 4 of the 6 patient tumor tissues when compared to the adjacent non-neoplastic tissue. Furthermore, we observed activation of several kinases including IGF1R, INSR and Ephrin A family of receptor tyrosine kinases.

Conclusion: Taken together, our integrated approach has identified several candidate molecules which could serve as potential early detection biomarkers or therapeutic targets for gastric cancer.

Poster no: D-1-02**Apolipoprotein A1 as a potential biomarker in the ascitic fluid for the differentiation of advanced ovarian cancers**

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Introduction: Ovarian cancers are classified as primary, if it arises in the ovary and secondary or metastatic, if the origin is from other parts of the body. The clinical manifestations of these cancers in the advanced stage are very similar making it difficult to distinguish clinically, histopathologically and radiologically. The therapeutics and management of the primary and secondary malignancies are completely different. While, the advanced primary malignancies are treated by cytoreduction followed by chemotherapy, the metastatic tumors are treated mainly with palliative chemotherapy. The prognosis is better for primary than secondary ovarian cancer making their diagnosis very crucial for patient care.

Methodology: Gel based proteomic approaches were used to study the differentially expressed proteins in the ascitic fluid of fourteen patients with advanced ovarian cancer. The relative ratios of the protein expression were estimated by densitometric analysis. The spots with more than three fold difference were subjected to in-gel trypsin digestion and identified by mass spectrometric analysis. The differential expression of one of the proteins was further validated by western blot experiments and ELISA.

Results and Discussion: Programmed Cell Death 1-Ligand 2 and apolipoprotein A1 were seen to be up regulated in the advanced primary ovarian cancer while apolipoprotein A4, and chain L, humanized version of the anti-human fas antibody Hfe7a were seen to be up regulated in the metastatic variant. Validation for the expression of apolipoprotein A1 shows that a 61.8ng/ml cut off is ideal to differentiate the primary and secondary advanced ovarian cancers. The assay has 100% sensitivity, 75% specificity and a positive predictive value of 90.9%.

Conclusions: There are proteins which are differentially expressed in the ascitic fluid of patients with primary and secondary ovarian cancer. Apolipoprotein A1 is a potential biomarker that can be used to differentiate the closely mimicking clinical scenarios of advanced ovarian cancer.

Poster no: D-1-03**Quantitative proteomic analysis of different stages of rat lingual carcinogenesis**

Bihari Lal Soni¹, Arivusudar Marimuthu², Harsh Pawar², Sharada S. Sawant¹, Anita Borges³, Ranganathan Kannan⁴, Akhilesh Pandey^{5,6,7,8}, Arvind D. Ingle⁹, Hindahally Chandregowda Harsha², Milind Murlidhar Vaidya¹.

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Introduction: Oral squamous cell carcinoma (OSCC) remains a major cancer in the Indian subcontinent, comprising more than

30% of all cancers. Precancerous lesions like leukoplakia and sub mucous fibrosis are also prevalent in India due chewing and smoking habits. In patients, the molecular analysis of multiple steps is hampered by the unavailability of biopsies of all the stages of carcinogenesis. Amongst the animal models of carcinogenesis, 4 Nitro-quinoline 1 oxide (4NQO) induced rat model of carcinogenesis remains the preferred model for studies related to oral carcinogenesis because of its similar changes observed in humans. Proteomics is a powerful tool for biomarker discovery used to understand the differences between protein expression profiles of normal vs. diseased samples.

Methodology: Approximately 5-6 weeks old Sprague Dawley rats (SD rats) were fed with 4NQO in drinking water at a concentration of 30 ppm. Rats were divided in three groups and each group was treated for 120, 160 and 200 days respectively. Four plex iTRAQ LC-MS proteomics analysis was applied at each stage of tongue tumorigenesis. We validated some known and novel proteins either by IHC or real time PCR.

Results and Discussion: We observed Hyperplasia, papilloma and squamous cell carcinoma in 120, 160 and 200 days of 4NQO treatment respectively. iTRAQ study led to identification of 2,223 proteins. Some up or downregulated known (Vimentin, fascin etc.) and novel proteins (Tenascin N, Thrombospondin2 etc.) were validated by either IHC or real Time PCR. Further novel proteins like TNN and Coronin 1a were also validated in human tissues.

Conclusions: This is the most extensive quantitative proteomic study in rat model of 4NQO-induced oral carcinogenesis carried out until date. We are able to extrapolate our rat model data to human system indicating the fact that this model has potential to be used for biomarker discovery in human oral cancer.

Poster no: D-1-04

Identification of new targets and biomarkers for hematological malignancies using secretome proteomic analysis

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Introduction: Hematological malignancies (HM) are one of the major cancers leading to death. There is an urgent need for identifying more sensitive and effective biomarkers and therapeutic agents for HM. Secretomics is a study of proteins secreted by cell lines is a powerful approach to identify novel targets and biomarkers in cancer. Our main aim of this study is to identify secreted proteins using several HM cell lines to discover novel diagnostic and prognostic biomarkers. Further, compare these secreted proteins with differentially expressed serum proteins in clinical samples of HM.

Materials and methods: Cell lines of HM were grown in serum free medium up to 24 hours in conditioned medium. Proteins were extracted from conditioned medium using conventional proteomic approaches. Secretory proteins were fractionated using SDS and each fraction was separated using the reverse phase Nano-LC and analyzed using LC-MALDI-TOF/TOF. The Levels of cytosolic proteins like beta-actin and beta-tubulin were used to check the quality control of secretome. In case of serum, proteins were extracted after depletion of two most abundant proteins and subjected to iTRAQ quantitative proteomic approach to identify differentially expressed proteins in different HM.

Results and discussion: Preliminary secretomics data identified 40 non-redundant proteins including alpha fetoprotein, kaliocin, hornerin, alpha enolase, elongation factor-2,

glutathione S-transferase, peroxiredoxin-6, apolipoprotein A1, and alpha-2-HS-glycoprotein. Some of these proteins are also identified in our preliminary serum proteomic analysis of HM. Bioinformatic analysis indicate that majority of proteins involve in metabolic processes, cellular processes and developmental process. We are in the process of confirming these preliminary results with more number of cell lines and clinical samples to come up with the novel targets and biomarkers for HM.

Conclusion: This secretome study provides an initial data resource of hematological malignancies for further study in serum and bone marrow samples from HM patients.

Poster no: D-1-05

Mode of action of cisplatin in acute and chronic leukaemia

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Introduction: Leukaemia involves irregular function of white blood cells, bone marrow and lymphatic system. Till date, the exact cause of the disease is unknown. Acute leukaemia is very aggressive and mostly affects immature abnormal blood cell blasts. Chronic leukaemia grows gradually and targets mature blood cells. Treatment of acute leukaemia is very difficult, while attempts have been made to treat chronic leukaemia. In this current scenario, finding an efficient drug for leukaemia will be of great help.

Methodology: In the present study, we have tried to decipher the mode of action of cisplatin in both acute and chronic leukaemia using cell biology, molecular biology and mass spectrometry based quantitative proteomics.

Results and Discussions: Effect of Cisplatin has been studied on human acute myelogenous leukaemia cells (THP-1), acute lymphogenous leukaemia cells (Jurkat), and chronic myelogenous Leukaemia cells (K-562). It seems that Cisplatin induces apoptosis in a dose and time dependent manner. Notably, IC₅₀ value in these three cell lines has been observed to differ after treatment with Cisplatin. The cytotoxic effect of Cisplatin was not prominent in 24 hours in Jurkat cell line. Notably, the effect on DNA fragmentation in K562 and Jurkat was very prominent. Proteomic study reveals that Cisplatin significantly effects the expression of oncogenes, DNA topoisomerase, CDK6, glucose 6 phosphate isomerase, heat shock proteins, elongation factors, tumour suppressors and immunity related proteins.

Conclusions: The study thereby reveals that in the leukaemia cell lines THP-1, K562 and Jurkat, the toxicity assay of Cisplatin might not be directly related with its effect on DNA. Proteomic study also suggests that mode of action of Cisplatin is not similar in the three types of leukaemia.

Poster no: D-1-06

Differential Proteome reveals major role of metabolic pathways in conferring radio resistance to recurrent Glioblastoma

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Introduction: Radioresistance is a major cause of recurrence in Glioblastoma. However, targeting radiation escapers (RE) have

not been possible due to incomplete understanding of survival mechanism in these cells. Since proteins are the ultimate product of biological processes, we are analyzing the total proteome of RE to gain insights into the molecular mechanism of radioresistant Glioblastoma.

Methodology: To capture the radiation escapers that are not visible/accessible in the patients, we developed an *in vitro* radiation resistance model from GBM cell lines (U87 and SF268) and short term cultures from primary patient samples. In this model <10% cells survive lethal dose of radiation (radiation escapers) and resume growth (recurrent cells) like parent population. As a preliminary experiment, differential proteomic analysis was performed for the parent, RE and recurrent cells of SF268 using iTRAQ.

Results and discussion: 2720 proteins were identified by iTRAQ across parent, RE and recurrent cells. 31% proteins were differentially expressed in RE and 32 % proteins were differentially expressed in recurrent cells compared to the parent population. Pathway analysis of all the differential proteins in each comparison with the parent population shows six pathways uniquely upregulated in both RE and recurrent cells. 8 pathways are uniquely downregulated in RE and 12 pathways are uniquely downregulated in recurrent cells. Interestingly, two major deregulated pathways: (a) Proteasome and ubiquitin mediated degradation pathways (b) TCA cycle and oxidative phosphorylation show inverse relationship in the RE and recurrent cells. Proteasome and ubiquitin mediated degradation pathway is upregulated in RE and downregulated in the recurrent cells whereas TCA Cycle and oxidative phosphorylation is upregulated in the recurrent cells and downregulated in radiation escapers. Functional studies are ongoing to understand the biological significance of this deregulation.

Conclusion: Based on our data we hypothesize that reprogramming of metabolic state in radiation escapers and recurrent cells gives them a survival advantage against radiation.

Poster no: D-1-07

Differential Proteomic Profile in HPV Positive Retinoblastoma Using Two-Dimensional Difference Gel Electrophoresis (2D-DIGE): A Pilot Study

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Introduction: Retinoblastoma is a rapidly developing pediatric ocular neoplasm, with an increased prevalence in developing countries. The risk factors for sporadic retinoblastoma are poorly understood. There are reports of the association of HPV in retinoblastoma tumor tissues but its differential proteomic profile has not been reported till date. Our study aims to study the differential proteomic profiling of positive and negative retinoblastoma.

Methodology: 42 retinoblastoma and 42 normal retinal tissues from donor eyes were recruited. DNA isolation was followed by quantitation and α -actin gene amplification. Screening of different HPV subtypes was carried out using HPV genoarray kit (Hybribio Ltd, Hongkong) that utilizes L1 consensus primers to simultaneously amplify 21 HPV genotypes. Differential proteome analysis of the tissue from eight retinoblastoma tumors

(4 each of HPV positive and negative cases) was compared to controls (n=4). 2D-DIGE coupled MALDI-TOF-TOF mass spectrometry was employed. Analysis was done with Decyder 7.0 software. BVA module was used to detect spots and to match all 18 spot maps from 6 gels. Differentially expressed spots were identified ($p < 0.05$, ± 1.5 folds, consistent in all subjects) and picked. Following in-gel digestion, peptide mass fingerprints were obtained.

Results and Discussions: 10 (25.6%) retinoblastoma tested positive for HPV subtype 16, none of the controls tested positive. Among the three groups (HPV infected, uninfected and control), 102 differentially regulated protein spots were identified which were constituted by 43 unique proteins determined by mass spectrometric analysis. Out of these, 12 proteins were significantly upregulated in HPV positive patients vis-a-vis HPV negative cases. HPV positive tumor exhibited significant upregulation of 12 proteins, and HPV negative cases showed upregulation of 8 proteins when compared to controls. The proteins which were highly regulated include GFAP, RBP3, RLBP1, SERPINA3, CKB, SAG, TF and CRABP1.

Conclusions: Our study has highlighted some interesting proteins like GFAP that have prospects of being used as potential candidates for biomarkers and therapeutics. Although, this needs further validation.

Poster no: D-1-08

Volatile Organic Compounds as Potential Biomarkers for Breast Cancer Diagnosis: A Non-Invasive Metabolomics Approach

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Introduction: Breast cancer (BC) is the most common cancer diagnosed in women with a high mortality rate if not diagnosed and treated at early stage. The major reason for this high mortality rate is lack of awareness and late diagnosis. The present diagnostic techniques involve methods which are expensive and invasive. This restricts a majority of women to avail them. Therefore, there is an urgent need to develop diagnostic methods which would be less expensive and non-invasive in nature. The use of urine and saliva as samples for disease monitoring and diagnosis is a promising option in early diagnosis. Here, we propose a very simple and non-invasive methodology using HS-SPME followed by GC-MS analysis capable of identification of breast cancer specific volatile organic compounds (VOCs) which could easily discriminate between BC subjects and healthy controls.

Methodology: The processed urine (4ml) and saliva (2ml) were subjected to VOCs isolation by HS-SPME technique and analyzed via GC-MS. The VOCs identified were subjected to advanced statistical analysis in order to discriminate between BC and healthy control subjects.

Results and discussions: In our preliminary data of urine, 12 VOCs are found to be up-regulated and 8 VOCs are down-regulated in BC. In case of Saliva, the abundance of 8 VOCs is increased and 2 VOCs abundance is decreased. Bioanalytical data were subjected to multivariate statistics in order to visualize clusters of cases and to detect the VOCs that are able to differentiate breast cancer patients from healthy individuals. Very good discrimination within breast cancer and control groups

was achieved. We are in the process of confirming these preliminary results with more number of samples to come up with a panel of VOCs specific to BC.

Conclusion: Volatile metabolomics data of urine and saliva can be used as non-invasive biosignatures to differentiate between BC and healthy volunteers.

Poster no: D-1-09

Grade specific tissue proteomic analysis of human gliomas

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Introduction: Gliomas are brain tumors originating from glial cells and are classified into four different grades by the WHO, with grade-I & II gliomas grouped under low grade gliomas and grade-III & IV as high grade gliomas. There is however a need for grade specific markers in glioma tissues for better identification of various grades of gliomas.

Methodology: Tissue proteome was extracted using Trizol method. Using proteomic approaches like In 2D-DIGE, gels were scanned and analyzed using DeCyder 2D software version 7.0. Significantly altered protein spots were subjected to in-gel digestion followed by MALDI-TOF/TOF analysis. For iTRAQ analysis, the tissue proteome was buffer exchanged with a LC-MS compatible buffer (0.5M TEAB), followed by in-solution digestion and labeling with iTRAQ reagents. Further the labeled peptide samples were pre-fractionated using offgel fractionator, followed by the data acquisition using LC-MS/MS. Obtained data was analyzed using SpectrumMill software.

Results and Discussion: Proteins associated with glycolysis, gluconeogenesis, pentose phosphate pathway, aminoacid metabolism, fatty acid metabolism was found to be altered in gliomas. Most of the proteins associated with glycolysis/gluconeogenesis, pentose phosphate pathway were found to be down regulated in grade-IV gliomas (GBM). Proteins like complement C3, nicotinamide phosphoribosyltransferase, fibrinogen, alpha-1-antitrypsin, alpha-2-macroglobulin etc. showed positive correlation with increase in the grade of the tumor, while brain acid soluble protein, neurogranin, myelin basic protein, synapsin-1 showed negative correlation with increase in grade of the tumor.

Conclusion: In this comprehensive study protein signature of potential candidates was identified for different grades of gliomas. We also identified few pathways relevant to different grades of the tumor. Validation of the identified markers on a larger cohort of glioma patients would affirm their role as grade specific markers.

Poster no: D-1-10

Proteomic and transcriptomic profiling of *in vitro* established radiation resistant oral cancer cells for identification of radioresistance related biomarkers

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Introduction: In India, oral cancer is the most common cancer in males and ranks third among females. Radiotherapy is an

integral part of oral cancer treatment, either alone or in combination with surgery. Often, during radiotherapy, oral tumours of a subset of patients develop radioresistance that creates major obstruction towards its efficacy. Thus, the aim of our study was to establish radioresistant cell lines from different oral subsites using clinically admissible low dose radiation and their profiling by proteomic and transcriptomic approaches to identify proteins associated with radioresistance in oral cancer.

Methodology: We have established three radioresistant oral cancer cell lines; 70Gy-AW13516, AW8507 and 70Gy-SCC029B. Radiation was given in 2Gy dose by ⁶⁰Co- γ linear accelerator (Bhabhatron-2) upto a total dose of 70Gy to each of the parental cell line and radioresistant character was determined by clonogenic assay. Proteomic profiling of parental/resistant cells was done by 2D-gel electrophoresis. Gels were analysed by PD-Quest software and differential spot identities revealed by MALDI-TOF/TOF. To explore changes at transcript level; cDNA microarray was performed by Affymetrix Gene Chip array and analysed by Gene Spring GX-12.5 software.

Results & Discussion: MS identified 106 differentially expressed proteins among three parental/radioresistant cell lines with significant MS/MS score. Panel of 8 common proteins across three sets; GRP78, STIP1, PGP, PKM2, GRP94, PDIA3, HSP70-1A/B and HSPA8 were selected and validated by Real time-PCR. Status of these markers in radiotherapy failed oral tumours by Immuno-histochemistry is currently ongoing. Similarly, statistically significant genes (>3 fold) from radioresistant cells of different subsites were mapped by David & Panther functional tools. Pathway analysis revealed genes related to cell survival pathways like; PI3, P38, Wnt and Apoptosis signalling.

Conclusions: Proteomic and transcriptomic profiling of established oral radioresistant cells revealed some radioresistance related biomarkers that may provide new insight in understanding mechanism underlying clinical radioresistance.

Poster no: D-1-11

Non-coding DNA derived Anti-Cancer Peptides tagged to DARPins: Novel Immuno-modulatory Scaffold therapeutics for effective treatment of Breast Cancer

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Introduction: Breast cancer, a deadly disease of breast tissue, contributes significantly to cancers impacting women worldwide. The outcome is a relatively high number of cancer deaths particularly in the advanced nations. This cancer can be dissected in detail based on various molecular subtypes, risk factors, clinical behavior and response to treatment. Scarcity of early screening measures and lack of proper awareness results in more deaths due to this cancer in India. However, early detection and combative programs in developed nations results in better endurance rates for women. Hence, diverse studies identifying novel small molecules involved in the progression of breast cancer hold the key to the advancement in therapy. We have isolated a number of anti-cancer peptides from non-coding DNA. We are currently analyzing their role in Breast cancer. We intend to conjugate the novel peptides to DARPins (designed ankyrin repeat proteins) which are a novel class of binding molecules capable of conquering restrictions imposed by monoclonal antibodies.

Methodology: Our current focus is to perform sequence similarity searches and narrow down to a breast cancer subset.

In this study, novel anti-cancer peptides generated from non-coding DNA will be tested for their role in regulating cell migration and angiogenesis in Breast Cancer Cells. *DARPins* (*Designed Ankyrin Repeat Protein*), the non-antibody based low cost small proteins are made up of four or five repeats. The variable region of the antibody has been engineered for target binding and they result in alternative design solutions for antibodies. Therefore, *DARPins* molecules isolated from non-coding DNA will be conjugated to the anti-cancer peptides and Nanomedicine molecules will be generated. The small size and high binding affinity of this Peptide-*DARPins* conjugate will culminate in deep drilling into solid tumors.

Results and Discussions: The novel Peptide-*DARPins* conjugate therapeutic molecules have been generated. These molecules are being tested in MCF7 Breast Cancer cells for their role in VEGF induced angiogenesis, capital event in the development of metastases. Breast cancer prognosis and treatment can benefit a lot from this new drug format based therapeutic Nanomolecules.

Conclusions: We hope to give a new direction to immunomodulatory methods of anti-Breast cancer therapeutics and perceive the Peptide-*DARPins* conjugate from non-coding DNA as next generation of protein therapeutics.

Poster no: D-1-12

Metabolomic and Lipidomic Profiling using Mass Spectrometry based Approaches towards Detection of Early Disease Markers for Breast Cancer

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Introduction: Breast cancer is the most common malignancy and the second most common cause of cancer related mortality. Metabolites and lipids are important players in biological systems. Hence, the comparative analyses of the metabolites and lipids of normal, benign and malignant serum samples can provide a better understanding of the molecular events involved in tumour development, and are essential for early detection and diagnosis of the disease.

Methodology: We have analysed 25 serum samples of each, benign, malignant and age- matched healthy controls. LC-MS/MS based targeted quantitative metabolite analysis was performed in positive and negative modes using AB Sciex 4000 QTRAP. NMR based metabolomics profiling was carried out using 700 MHz BrukerAvance AV III NMR. For lipid profiling, lipids were extracted using MTBE method and analysed using in house MRM based platform for Phosphatidylcholines (PC), Phosphatidylethanolamine (PE), phosphatidylserine (PS), and Phosphatidylinositol (PI) using AB Sciex 4000 QTRAP LC-MS/MS. Expression of differential metabolites and lipids in breast cancer samples was analyzed by multivariate statistical analysis using MarkerView and Simca software.

Results and Discussions: In this work, we applied mass spectrometry and NMR to identify the differentially expressed metabolites in breast cancer. In addition, we also used quantitative MRM based MS approach to identify phospholipids involved in breast cancer. Exploratory PCA of identified metabolites and lipids were employed to detect intrinsic clustering and possible outliers. PLS-DA, OPLS-DA further maximized the group separation, based on which a statistical model was built. These statistical models showed good

discrimination between breast cancer patients and healthy controls.

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

Poster no: D-1-13

Testican-1 and Prosaposin - Potential Biomarkers in the Early Diagnosis of Chewing Tobacco-Induced Oral Cancer

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Introduction: Secreted proteins from cancer cells or tissue are an important class of molecules as they are involved in multiple biological processes related to cancer metastasis and progression. Secretome of cancer cells serves as a rich reservoir of potential biomarkers. Cell line-based models are suitable for such studies as difference in the abundance of the secreted proteins between cancer and normal cells can be easily quantified. The aim of this study was to find secreted markers that can be used in the early diagnosis of oral cancer in response to chewing tobacco.

Methodology: Normal oral keratinocytes, OKF6/TERT1, were chronically treated with chewing tobacco. Chronic exposure to chewing tobacco led to increased cellular proliferation and induced invasive ability in the non-invasive oral keratinocytes. To understand the molecular mechanism of the insult imparted by chewing tobacco, we compared the secretome of the OKF6/TERT1 cells treated with chewing tobacco and the parental cells using quantitative proteomic approach.

Results and discussion: Isobaric tags for relative and absolute quantitation (iTRAQ)-based mass spectrometry-based analysis of the secretome led to the identification of a total of 2,873 proteins, amongst which 320 proteins were over-abundant and 176 proteins were downregulated (≥ 2 -fold) in the OKF6/TERT1 cells treated with chewing tobacco. Among the over-abundant proteins, sparc/osteonectin, cwcw and kazal-like domains proteoglycan (testican) 1 (SPOCK1) (5.5-fold) and Prosaposin (PSAP) (3-fold) were found to be over abundant in the chewing tobacco treated cells. Immunohistochemistry-based validation using oral cancer tissue microarrays revealed overexpression of these proteins in tumor tissues compared to normal oral tissues.

Conclusions: This data suggests that SPOCK1 and PSAP may serve as potential early detection biomarker in oral cancer especially in tobacco users. Further, validation in a larger cohort using proximal body fluid like saliva is needed before being applied in a clinical setting.

Poster no: D-1-14

Silencing of high-mobility group box 2 (HMGB2) modulates cisplatin and 5-fluorouracil sensitivity in head and neck squamous cell carcinoma

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide with a poor prognosis. Despite significant improvement in treatment methods, the 5 year survival rate remains 40-50% due to lack of early detection biomarkers and chemotherapeutic targets. This highlights the need for biomarkers that can be used for early diagnosis of HNSCC or can serve as prognostic markers.

Methodology: We used 8plex iTRAQ (isobaric tags for relative and absolute quantitation)-based quantitative proteomic approach followed by liquid chromatography and high resolution tandem mass spectrometry (LC-MS/MS) of a panel of HNSCC cells, one non-neoplastic (OKF6/TERT1) and three neoplastic cells (JHU-O11, JHU-O22 and JHU-O29). We assessed the role of nuclear protein HMGB2 in the sensitivity of HNSCC cells to cisplatin and 5-FU. Immunohistochemical (IHC) staining of HMGB2 was done to validate the overexpression in HNSCC tissues using TMA.

Results and discussion: LC-MS/MS analysis resulted in the identification of 3,284 proteins, amongst which 37 proteins were overexpressed (>2 fold) while 77 proteins were downregulated in the HNSCC cells. The proteins that were upregulated include DNA binding protein high-mobility group box 2 (HMGB2) Ezrin (EZR), Midkine (MDK) and Calpastatin (CAST). HMGB2, was one of the novel proteins identified by us in this study was overexpressed in all the HNSCC cell lines. Immunohistochemical labeling of HMGB2 in a panel of HNSCC tumors using tissue microarrays revealed overexpression in 86% of tumors. The HMGB proteins are known to bind to DNA structure resulting from cisplatin-DNA adducts and affect the chemosensitivity of cells. We observed that siRNA-mediated silencing of HMGB2 increased the sensitivity of the HNSCC cell lines to cisplatin and 5-FU.

Conclusion: Our results indicate that HMGB2 plays an essential role in HNSCC tumor resistance and provides new insights into the role of HMGB2 in chemo resistance in HNSCC.

Poster no: D-1-15

Identification of potential biomarkers and therapeutic targets in head and neck squamous cell carcinoma using high resolution mass spectrometry

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is an epithelial malignancy with poor prognosis due to its high metastatic nature. Although small molecule inhibitors and monoclonal antibodies such as cetuximab specific for EGFR are in clinics, the efficacy of molecular targeted therapy is limited in HNSCC. Protein kinases not only play a central role in cell signaling networks but are also serve as excellent therapeutic targets. Phosphoproteome profiling to identify activated kinase pathways is an established approach to identify novel therapeutic target in cancer.

Methodology: We performed a quantitative phosphotyrosine profiling of a panel of HNSCC cell lines and non-neoplastic cell line OKF6/TERT1 using mass spectrometry-based phosphoproteomics. We carried out a tandem mass tag labeling coupled with antiphosphotyrosine antibody-based approach to identify activated molecules in HNSCC. We validated the expression levels of candidates in HNSCC primary tissue using tissue microarrays.

Results and Discussion: We identified a total of 61 phosphosites in 47 proteins in the HNSCC cells compared to OKF6/TERT1. We identified the hyperphosphorylation of proteins, including protein tyrosine phosphatase, non-receptor type 11 (PTPN11), myelin protein zero-like 1 (MPZL1) and tyrosine kinases such as LYN proto-oncogene (LYN), EPH receptor A2 (EPHA2) and DYRK1A. We observed the hyperphosphorylation of DYRK1A (Tyr-322) in all the HNSCC cell lines (>1.5 fold) compared to OKF6/TERT1. DYRK1A is a member of the conserved family of DYRKs that autophosphorylate a critical tyrosine in their activation loop. Recent studies have indicated the role of DYRKs in regulation of mitotic transition and apoptosis induced by DNA damage. DYRK1A stimulates EGFR degradation and reduces EGFR dependent growth in glioblastoma. Immunohistochemical validation using tissue microarrays, revealed DYRK1A to be overexpressed in 90% of HNSCC cases.

Conclusions: Our result suggests that DYRK1A may serve as a potential biomarker or therapeutic target in HSNCC. The precise role of the protein in HNSCC tumor progression needs further evaluation.

Poster no: D-1-16**Macrophage migration inhibitory factor - a therapeutic target in gallbladder cancer**

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Introduction: Gallbladder cancer (GBC) is a malignancy of the biliary tract and the fifth most common cancer of the gastrointestinal tract. It is often detected at an advanced and unresectable stage. The prognosis is dismal with a survival of less than 5 years in 90% of the cases. Poor prognosis in GBC is mainly due to late presentation of the disease and lack of reliable biomarkers for early diagnosis. Targeted therapy in GBC is also not very well explored. This highlights the need to identify potential therapeutic targets in addition to early diagnostic markers to improve treatment options and disease outcome.

Methodology: Proteomic analysis of four gallbladder cancer cell lines based on the invasive property (non-invasive to highly invasive) was carried out using the isobaric tags for relative and absolute quantitation (iTRAQ) labeling technology. *In vitro* cellular assays were carried out on the gallbladder cancer cell lines using siRNA-mediated silencing or MIF inhibitors, ISO-1 and 4-IPP.

Results and Discussion: The proteomic study led to the identification of 3,183 proteins amongst which 627 were overexpressed and 327 were downregulated (2-fold). Among the overexpressed proteins, macrophage migration inhibitory factor (MIF), a pro-inflammatory cytokine, was found to be 3-fold overexpressed. Functional inhibition of MIF using 4-IPP in a panel of gallbladder cancer cell lines led to a significant decrease in their cell viability, colony forming ability and invasive property. Inhibition of MIF using 4-IPP was found to be 5 times more potent than the prototypic MIF antagonist, ISO-1. Similar results were obtained on silencing of MIF using its specific siRNA.

Conclusions: Our data suggests that MIF plays an important role in tumor aggressiveness of gallbladder cancer and targeting MIF should be a useful strategy in gallbladder cancer.

Poster no: D-1-17**Investigation of proteomic alterations associated with STAT3 Inhibitors; molecular mechanism of LLL12 and S3I201**

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Introduction: STAT3 interacts with several other cellular proteins and together these proteins regulate cellular processes

like cell cycle regulation, proliferation, development and other functions in response to activation via growth hormone and various cytokines. STAT3 remains constitutively active in several cancers including gliomas and plays an important role in progression, poor prognosis and recurrence of gliomas. Therefore, several STAT3 inhibitors have been identified through various approaches which inhibit growth of glioma. The aim of this study was to understand the role of STAT3 in glioma and to examine effect of LLL12 and S3I201, which are inhibitors of STAT3, on proteome of glioblastoma cells using proteomics approaches.

Methodology: Multiple proteomic techniques such as DIGE and iTRAQ were used to examine the proteomic alterations associated with STAT3 inhibitors using U87 glioma cells. Triose phosphate isomerase 1 (TPI1) and phosphoglycerate mutase 1 (PGAM1) were further investigated using ELISA assay and western blotting.

Results: Proteomic analysis identified several differentially expressed proteins which have functional role in cellular metabolism, apoptosis, and cytoskeleton. Several proteins like Apoptosis-inducing factor 1, Proliferation-associated protein 2G4 etc. involved in apoptosis, invasion, and functions as transcription factors were identified; showed altered expression in response to LLL12 and S3I201 treatment indicates their contribution towards anti-tumorigenic functions of these inhibitors. TPI1 and PGAM1 were further studied to investigate their functional role in disease progression. LLL12 was found to be more potential anti-glioma agent than S3I201. Here, we demonstrate STAT3 inhibition as a potential approach for therapeutic interventions in glioma and also support that metabolic interruption in glioma cells could be a more potent approach for their growth inhibition.

Conclusion: This study revealed effect of STAT3 inhibitors on whole proteome of U87 glioma cells and provides a better understanding of function of STAT3 as well as mechanism of action of LLL12 and S3I201. This analysis helps us in the identification of proteins, which may serve as suitable drug targets for glioma treatment.

Poster no: D-1-18**Proteomic analysis of IDH1 mutations in gliomas**

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Introduction: Gliomas are tumors arising from the glial cells of the brain and have been reported to harbor a number of gene mutations with wide range of effects. IDH1 gene mutations in grade-II and grade III glioma tumors have been shown to be responsible for increased survival of patients over the wild type gene containing glioma patients. However, there is no evidence available for the proteomic differences in the two subpopulations of glioma and we have hence carried out a comprehensive proteomic analysis using two different platforms to gain a better insight into the molecular differences existing due to this mutation.

Methodology: Glioma tissue samples were confirmed for IDH mutations using IHC and gene sequencing. Tissues with G395A point mutation (R132H) were considered as IDH1 mutant (IDH1 +ve) while the ones without the point mutation was considered IDH1 wild type (IDH1 -ve). Whole tissue proteomic analysis of

IDH1 + and IDH- gliomas across grades 2, 3 and 4 was carried out using gel-based and gel-free techniques. In 2D-DIGE, tissue proteomes were compared between individuals with IDH1 +ve and IDH1 -ve across the same grade of glioma. Further, quantitative proteomic analysis of individual and pooled glioma tissue protein samples was performed using LC-MS/MS.

Results and Discussion: Proteomic analysis of IDH1+ve and IDH1-ve yielded interesting results with differences in pathways like Glycolysis, Pentose phosphate pathway, TCA cycle and Glutamine-Glutamate conversion pathway all of which are involved in glucose metabolism required for the growth and survival of tumor cells. In addition to this, proteins with roles in oxidative stress response, Ras oncogene pathway, VEGF signaling pathway and ATP synthesis were found to have significantly altered expression levels among the two subpopulations across all grades of gliomas.

Conclusion: Validation of a few candidate proteins involved in angiogenesis, VEGF signaling, Glutamine-glutamate conversion pathway could help in better understanding the major differences between IDH1 +ve and IDH1 -ve gliomas.

Poster no: D-1-19

Multipronged Quantitative Proteomic Analyses Indicate Modulation of Various Signal Transduction Pathways in Human Meningiomas

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Introduction: Meningiomas constitute approximately 30% of all intracranial tumors and possess diverse clinical manifestations and histological characteristics. This study aims to investigate alterations in the human serum and tissue proteome in different grades of human meningiomas to obtain insights about disease pathogenesis and identify grade specific surrogate protein markers.

Methodology: Comparative proteomic analysis of healthy controls (n = 45) and different grades of meningiomas; grade I (n = 15), grade II (n = 5) and grade III (n = 1) were performed by using three complementary quantitative proteomic approaches; 2D-DIGE, iTRAQ-based and label-free quantitative proteomics in combination with ESI-Q-TOF LC/MS-MS or Q-Exactive mass spectrometry. The differentially expressed serum and tissue proteins (fold change > 1.5; p < 0.05) identified in different grades of meningiomas were subjected to functional pathway analysis using PANTHER and DAVID functional annotation tools for better understanding of the biological context of the identified proteins and their association with disease pathophysiology.

Results and Discussion: A few identified proteins from serum like Thrombospondin-1, Carbonic anhydrase 1, Leucine-rich alpha-2-glycoprotein, Afamin etc. exhibited high levels of differential expressions in the benign meningioma (grade I) can act as early detection markers. Quite a few candidates were identified from tissue proteome study, like protein S-100A6, AHNAK, caveolin showed sequential increase in low and high-grade meningiomas, whereas differential expressions of collagen alpha-1 (VI), protein S100-A9, etc. transgelin-2 were found to be grade specific. Validation of four differentially expressed targets; Hpx, CP, RBP4 and Apo A-I was performed using ELISA. Several

differentially expressed proteins were found to be associated with diverse signaling pathways including Integrin, Wnt, Ras, EGFR, EIK and FGR signaling.

Conclusions: Our findings provide comprehensive overview and new insights regarding the association of various signal transduction pathways in meningioma pathophysiology and may introduce new opportunities for the detection and prognosis of meningiomas.

Poster no: D-1-20

Phosphotyrosine profiling of curcumin induced signaling in head and neck cancer

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Introduction: Curcumin is a naturally occurring anti-cancer agent and has been shown to inhibit cell growth in a number of tumor cell lines and animal models. Recent studies have shown the anti-cancer effects of curcumin in head and neck squamous cell carcinoma (HNSCC). Better understanding of the mechanistic details of curcumin in HNSCC will enable the design of more targeted strategies. Phosphoproteome profiling to identify activated kinase pathways is an established approach to identify signaling mechanism for any anti-neoplastic compound.

Methodology: We performed quantitative phosphotyrosine profiling of HNSCC cell line (CAL-27) in response to curcumin using Fourier transform mass spectrometry. For this, we employed stable isotope labeling by amino acids in cell culture (SILAC) technology. Phosphopeptides were enriched using anti-phosphotyrosine antibody-based approach and analyzed on an Orbitrap Fusion™ Tribrid™ mass spectrometer.

Results and Discussion: A total of 516 phosphotyrosine sites were identified that mapped to 339 proteins. Of these, 118 proteins were differentially phosphorylated upon curcumin treatment. These included multiple kinases including tyrosine kinase, non-receptor, 2 (TNK2), fyn-related Src family tyrosine kinase (FRK), EPH receptor A2 (EPHA2), mitogen-activated protein kinase 3 (MAPK3) which were hypophosphorylated and YES proto-oncogene 1, Src family tyrosine kinase (YES1), protein kinase C, delta (PRKCD), mitogen-activated protein kinase 12 (MAPK12) were hyperphosphorylated. This data provides insight about the potential signaling pathways altered by curcumin in HNSCC.

Conclusions: This study provides novel information about the curcumin induced signaling pathways in HNSCC. In depth study of some of the novel identified kinases is required to understand their role in curcumin induced signaling.

Poster no: D-1-21**Phosphoproteomic analysis of cigarette smoke induced signaling in lung cancer**

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Introduction: Lung cancer is one of the leading causes of cancer related deaths worldwide. Smoking is a major risk factor for multiple types of cancers including lung cancer. Exposure to second hand smoke or even just smoking in the past can also increase the chances for the offspring to develop lung cancer.

Methodology: To investigate the effect of chronic exposure to cigarette smoke on lung cells, we developed a cell line model where H358 (bronchioalveolar lung cancer cells) were chronically treated with cigarette smoke condensate (CSC) for 12 months. We carried out stable isotope labeling by amino acids in cell culture (SILAC) - based quantitative phosphoproteomic analysis. For phospho-serine and threonine analysis, we employed affinity enrichment using TiO₂ for enrichment of phosphopeptides and carried out high resolution mass spectrometry analysis to quantitate relative abundance of phosphosites in cigarette smoke exposed cells.

Results: We have identified a total of 1,324 phosphosites. Amongst these, 232 phosphosites were found to be hyperphosphorylated and 130 phosphosites were found to be hypophosphorylated in cigarette smoke exposed cells. We identified several kinases involved in cell survival, proliferation and migration to be hyperphosphorylated in the cigarette smoke exposed cells which include epidermal growth factor receptor (EGFR), ephrin receptor type A (EPHA2), mitogen activated protein kinases (MAPKs) and p21 activated kinase (PAK). Activation of aryl hydrocarbon receptor (AHR) affects various downstream signaling pathways that control the initiation and promotion of lung tumorigenesis. We identified various downstream targets of AHR such as members of aldehyde dehydrogenase family, aryl hydrocarbon receptor nuclear translocator like (ARNT) and cytochrome p450 family members in cigarette smoke treated cells.

Conclusion: Using quantitative phosphoproteomics approach we have identified molecular pathways and potential therapeutic targets associated with cigarette smoking in lung cancer.

Poster no: D-1-22**SILAC-based quantitative proteomics reveals molecular alterations in human skin keratinocytes upon chronic exposure to arsenic**

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Introduction: Chronic exposure to arsenic has been associated with multiple dermatological disorders including skin, bladder and lungs cancers. Although, arsenic is cleared from these sites and excreted through urine, a substantial amount of the residual arsenic is left in keratin-rich tissues such as skin, hair and nails. The major hallmarks of arsenicosis include hyper- and hypopigmentation of skin, hyperkeratosis and hyperplasia predominantly on soles and palms. Majority of this knowledge source is from epidemiological studies. The underlying molecular mechanism of arsenic poisoning is not clearly understood. To understand the molecular alterations in human skin in response to arsenic, we developed a cell line model, using the non-neoplastic human keratinocyte HaCaT, where the cells were chronically exposed to 100nM arsenic for six months.

Methodology: LC-MS/MS of twelve basic pH RPLC fractions was carried out in replicates on LTQ-Orbitrap Velos mass spectrometer. Triple quadrupole-based multiple reaction monitoring (MRM) was employed for validation and relative quantification of candidate proteins. Western blotting was carried out for anti-apoptotic markers. ROS were measured using 2', 7'-dichlorofluorescein diacetate assay.

Results and discussions: SILAC-based quantitative proteomics resulted in identification and quantitation of 2,193 proteins amongst which 39 proteins were overexpressed (≥ 2 -fold) and 52 downregulated (≥ 2 -fold) upon chronic arsenic exposure. We observed overexpression of aldo-keto reductases (AKR1C2 and AKR1C3), glutamate-cysteine ligase catalytic subunit (GCLC) and NAD (P) H dehydrogenase [quinone] 1 (NQO1) that are induced upon oxidative stress. Several members of the plakin family including periplakin (PPL), envoplakin (EVPL) and involucrin (IVL) that are critical for terminal differentiation of keratinocytes were found to be downregulated. Calprotectin, a dimeric protein complex of S100A8/S100A9 that confers epithelial resistance to the invading bacteria was also found to be downregulated upon chronic arsenic exposure. In addition, we also observed an increase in the basal ROS levels in arsenic treated cells along with increase in the levels of multiple anti-apoptotic proteins. MRM was employed as a complementary approach to validate some of the differentially expressed proteins. AKR1C2 and PPL were confirmed to be overexpressed and downregulated respectively in arsenic treated cells using MRM based validation.

Conclusions: Our approach provides comprehensive insights into the proteome level changes upon chronic arsenic exposure of non-neoplastic human skin keratinocytes. We observed concordance between SILAC-based quantitation and MRM results for candidate proteins.

Poster no: D-1-23**HSP27: A new kid on the block!**

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Introduction: Osteoporosis is a potentially crippling disease, characterized by low bone mineral density (BMD) and increased susceptibility for fragility fractures. The central pathological mechanism is increased bone resorption compared with formation, mostly stemming from estrogen deficiency at menopause. Peripheral monocytes are precursors of osteoclasts (bone resorbing cells) and thus proteins important to early stages of osteoclastogenesis and commitment to the osteoclast lineage can be unravelled in monocytes. Hence, with the purpose of identifying such proteins we compared monocyte proteomes in pre and postmenopausal women with low versus high BMD.

Methodology: 4 plex iTRAQ coupled to LC-MS/MS was employed for quantification of monocyte proteins from (1) premenopausal women with low BMD (2) premenopausal women with high BMD (3) postmenopausal women with low BMD and (4) postmenopausal women with high BMD. Total and phosphorylated heat shock protein 27 (HSP27) was estimated using intracellular ELISA in iTRAQ samples (n=10 in each of the four categories) and in additional samples for validation (n=10 in each of the four categories). Transwell assays were performed to analyze effect of recombinant HSP27 (rHSP27) on monocyte chemotaxis.

Results and Discussion: Tandem mass spectrometry revealed a total of 1801 proteins and 80 proteins were differentially expressed in various combinations belonging to several gene ontology categories. Notably, HSP27 was distinctly upregulated in low BMD condition in both pre and postmenopausal women. This was confirmed in individual samples for total ($p<0.02$) as well as phosphorylated HSP27 ($p<0.05$). Also, phosphorylated HSP27 exhibited a statistically significant predictive value for low BMD further strengthening our findings ($p<0.0001$). Interestingly, monocyte chemotaxis increased with the addition of rHSP27.

Conclusions: HSP27 may be a prospective marker for low BMD in premenopausal and postmenopausal Indian women; and we suggest a novel role for HSP27 in early stages of osteoclastogenesis.

Poster no: D-1-24**Identification of non-invasive biomarkers of coordinate metabolic reprogramming associated with colorectal cancer**

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Background & Aims: Colorectal cancer is a leading cause of cancer mortality worldwide. Currently there is a lack of robust noninvasive methods for screening and diagnosis of the disease,

which has been shown to improve therapeutic outcome and reduce mortality. This study sought to identify mechanistically-associated diagnostic signatures of colorectal carcinogenesis.

Methods: Electrospray ionization mass spectrometry coupled with hydrophilic interaction liquid chromatography was used for global and targeted analysis of urine and tissue metabolome in wild-type and Apc Min/+mice (C57BL/6J) as well as in mice bearing azoxymethane (AOM)-induced tumors (129P3/J) and saline-treated healthy controls. Gene expression analysis was also performed in normal colon and colon tumor tissue. Tissue metabolomics and gene expression analysis using Apc CDXERT2 mice, which gets acute colon-specific Apc disruption on tamoxifen treatment, were performed to examine causal association between oncogenic signaling and metabolic derangements. Finally, metabolic signature of human colon tumor tissues was analyzed.

Results: This is the first report on the longitudinal effect of colorectal carcinogenesis on the urine metabolome. The creatinine-normalized urinary excretion of thirteen metabolites associated with amino acid metabolism, urea cycle and polyamine metabolism, nucleic acid metabolism and methylation were found to be elevated in tumor-bearing (Apc Min+) mice compared to their healthy (wild-type) counterparts. Combination of metabolites according to their pathway of origin significantly improved diagnostic potential with those associated with nucleic acid metabolism and methylation showing 100% accuracy. Tissue metabolomics and gene expression analysis revealed that these biomarkers were, indeed, reflection of dysregulation of aforementioned metabolic pathways in colorectal tumors. Notably, the expression of both type-I and type-II protein arginine methyl transferases (PRMT), which produce symmetric- and asymmetricdimethylarginine, respectively, as well as other genes related to methylation machinery were found to be elevated in tumor tissue. Elevation in urinary excretion of dimethylarginines as signatures of aberrant methylation in tumor tissue is novel in the context of colorectal carcinogenesis. Protein Interaction Network Analysis using changes in gene expression signature, revealed that observed metabolic changes, especially those in urea cycle and polyamine metabolism, nucleic acid metabolism and methylation are coordinated. Combination of metabolites related to these pathways resulted in accurate identification of mice at very early asymptomatic stages of tumorigenesis. Colon-specific acute disruption of Apc expression showed that such coordinate metabolic reprogramming including that of methylation machinery is an early event in pathogenesis of colorectal cancer. Similar metabolic derangements were also observed in AOM -induced colorectal carcinogenesis in mice. It was interesting to note that the patterns of rearrangements were conserved in spite of difference in etiology of colorectal carcinogenesis as well as the genetic background in mice. Thus, starting from unbiased global metabolic fingerprinting, this study not only revealed novel signatures of aberrant methylation and wide-spread coordinate metabolic reprogramming, but also identified mechanistically-associated noninvasive metabolic signature that could be used for screening and early diagnosis. Analysis of paired normal (adjacent non-tumor) and tumor tissue from patients, indeed, showed stage-dependent progressive elevation of twelve common metabolites related to aforementioned metabolic pathways in human colorectal tumors. Notably, ten metabolites (proline, threonine, glutamic acid, arginine, N1-acetylspermidine, xanthine, uracil, betaine, symmetric-dimethylarginine, and asymmetricdimethylarginine) that were elevated in urine of tumor-bearing mice were also elevated in human colorectal tumors.

Conclusions: Biomarkers associated with coordinated metabolic reprogramming can serve as early noninvasive signature of colorectal carcinogenesis.

Poster no: D-1-25**iTRAQ - based Quantitative Proteomic Analysis of Follicular Fluid Reveals the Deregulation of Cumulus Oocyte Complex Matrix in Women with PCOS**

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Introduction: Polycystic ovary syndrome (PCOS) is a complex heterogenous disorder in women. The follicular development is arrested at preantral stage, resulting in the formation of multiple cysts and anovulation. To gain insights into the aberrant follicle development in PCOS, the proteome of follicular fluid (FF) from control and PCOS women was compared to using quantitative proteomics approach to identify differentially regulated proteins. Several proteins essential for follicle development, including components of cumulus oocyte complex (COC) matrix; TNFAIP6, FBLN1, ITI, SDC4, AMBP, PTX3 and AREG were found to be down regulated in PCOS. COC matrix is a hyaluronan rich matrix that surrounds the oocyte and cumulus granulosa cells, whose formation, expansion and mucification is critical for ovulation and fertilization. The EGF like growth factors predominantly AREG, traverse LH signal from mural to cumulus granulosa cells, and induces transcription of several genes of COC matrix, required for its expansion.

Methodology: FF samples from healthy and PCOS women were immunodepleted of high abundance proteins and flow-through fractions were subjected to iTRAQ labeling followed by LC-MS/MS analysis. Differential expressions two COC matrix proteins were validated in FF. The gene level differences of COC matrix components was studied in cumulus granulosa cells of control and PCOS women, by real time PCR. Further studies are ongoing to understand down-stream mechanism of deregulated COC matrix expansion in PCOS.

Results: AREG and FBLN1 in FF of PCOS women were observed to be downregulated by ELISA and Western blotting experiments. Several genes of COC matrix including AREG, TNFAIP6, HAS2 and PTX3 have been found to be deregulated in the cumulus granulosa cells of women with PCOS, confirming the results of proteomic analysis of FF obtained from these women.

Conclusion: Decreased expression of AREG observed in PCOS women may deregulate several COC matrix gene expressions. This may be a probable contributor of improper organization and expansion of COC matrix, which may affect oocyte development and ovulation in these women.

Poster no: D-1-26**Identification of Urinary Proteomic Biomarkers in Breast Cancer using Complementary Gel-based and Gel-free Quantitative Proteomic Approaches**

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Introduction: Breast cancer (BC) is the most frequent malignancy of women in the world as whole. Survival rates of

patients are high when the BC is detected at a very early stage. The limited available methods for diagnosis involve painful and invasive methods 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. Biofluids such as urine may serve as an excellent non-invasive source of easily available protein markers for disease diagnosis. In the present study we aim to investigate such protein markers in early grades of BC urine.

Methodology: In this work, we applied mass spectrometry based iTRAQ labeling technique and complementary 2D-DIGE to check for the differentially expressed proteins in BC and benign urine samples against those present in healthy individuals. Comparisons were made between proteins extracted from pooled urine of 8 breast cancers, benign and normal individuals each. The statistically significant differentially expressed proteins were subjected to bioinformatics analysis to understand the biological context of these proteins.

Results and Discussion: The differentially expressed proteins found using 2D-DIGE and iTRAQ were compared to get significantly expressing proteins using both the techniques. Interestingly, several proteins identified in this study were not reported previously in the context of BC. Bioinformatic analysis predicts that some of key pathways involving in breast cancer. Results from complementary proteomic approaches and bioinformatics analysis will be presented.

Conclusion: These differentially expressed proteins secreted in the urine of BC patients may serve as promising early diagnostic and prognostic markers. In addition urinary proteins may provide excellent non-invasive platform to create a panel of biomarkers that will be easily available for diagnostic purpose.

Poster no: D-1-27**Isolation and Identification of Buffalo (*Bubalus bubalis*) Milk Lipids**

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Introduction: Bovine milk is a significant part of human diet of all age groups as it provides protein, fat, carbohydrate, calcium, potassium and vitamins. More recently, there have been reports unraveling an ever-accumulating range of bioactives with beneficial effects on human health. For example, certain group of lipids (e.g. conjugated linoleic acid), carbohydrates (e.g. lactulose) and proteins (e.g. immunoglobulins) influence human health in a positive way. In contrast to proteins and carbohydrates, there is very limited information available on lipid composition and its variation due to extraneous factors (e.g. breed, season and stage of lactation)

Methodology: Milk was collected from Murray buffalo, a high yielding breed of buffalo, used in dairy industry. Lipids were extracted from milk by dichloromethane-ethanol solvent system. Subsequently, lipids were identified and characterized using mass spectrometry (MS).

Results and discussion: A total of 65 compounds were confidently identified. Lipid identifications were possible by matching identical peptide sequences within the bovine database using Simlipid program. Sequence identities were only deemed acceptable if more than two peptides were identified.

Conclusion: This report of lipidomic analysis represents a first step in determining the identity of lipids in Murray buffalo enabling future quantitative studies with other high yielding dairy cattle to increase a certain group of milk lipids for betterment of human health.

Poster no: D-1-28**Serum proteomic analysis for early prediction of gestational hypertension**

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Introduction: Gestational hypertension (GH) is the development of hypertension in pregnant women after 20 weeks gestation without the presence of protein in urine. There are no reliable tests available for early prediction of GH. As severe GH is associated with maternal and fetal morbidity, there is a need for the development of biomarkers for early prediction of the disease.

Methodology: Blood samples were collected from women with GH (n=31) and normotensive women (controls; n=40) during three different stages of gestation: (a) 8-12 weeks (T1) (b) 18-20 weeks (T2) and (c) after 21 weeks (T3). A comprehensive proteomic analysis of serum samples from all groups of GH and controls was performed using 2DE. Differential expression of spots between T1 of GH and T1 of controls was analyzed. These differently expressed spots were further investigated in T2 and T3 of GH. Finally, all spots differently expressed in T1, T2 and T3 of GH cases were excised, digested with trypsin and identified using MALDI TOF/TOF MS.

Results and discussion: 25 protein spots were differently expressed in T1 of GH as compared to T1 of controls. Out of these, 17 spots were found to have comparable expression in both T2 and T3 of GH. 8 spots showed down-regulation in T2 and T3. It seems logical to presume that these 8 spots are involved in GH progression and can be explored as early predictive markers for the disease. On MALDI TOF/TOF MS analysis, these 8 spots corresponded to 7 proteins including apolipoprotein-A1, α -1-antitrypsin, vitronectin, serum albumin, serotransferrin, α -2-macroglobulin and hemopexin. 5 amongst these showed interaction with 90% confidence interval in STRING analysis.

Conclusion: Identification of differently expressed proteins during early phase of gestation in GH is likely to contribute towards development of serum marker(s) for GH. Presently, iTRAQ experiments are underway to complement the findings.

Poster no: D-1-29**An integrated gel-based and gel-free approach for identification of serum protein markers for endometriosis diagnosis**

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Introduction: Endometriosis is a common benign gynecological disease, characterized by the proliferation of functional

endometrial glands and stroma outside the uterine cavity. Early detection is crucial for timely diagnosis and treatment of the disease. Laparoscopy, a surgical procedure, followed by biopsy is presently the gold standard for diagnosis of endometriosis. The need for a less invasive test which can effectively screen and accurately diagnose the disease is well recognized.

Methodology: Blood samples were collected from women with endometriosis (n=232) and controls (n=91). A comprehensive proteomic analysis of serum samples from patients with early (Stage I-II/minimal-mild) and late (Stage III-IV/moderate-severe) endometriosis using 2DE, 2D-DIGE and iTRAQ was performed. Various statistical filters were applied to identify the most relevant protein spots. Further variable reduction was performed using multivariate statistics. Functionally relevant protein spots with maximum prediction accuracy were identified using marker selection tool with partial least squares as search method. Further, an advanced supervised statistical analysis was performed using orthogonal partial least squares discriminant analysis (OPLS-DA) and the most significant protein spots responsible for class discrimination identified. Expressions of these identified proteins were validated for all stages of endometriosis (Stage I, II, III and IV) in a separate cohort of patients.

Results and Discussion: The most significant protein spots identified from V-plot (VIP score vs. correlation coefficient) of OPLS-DA analysis corroborated the findings from marker selection tool. When further validated with western blotting analysis, it was evident that haptoglobin (HP), Ig kappa chain C region (IGKC), alpha-1B-glycoprotein (A1BG) can be effectively used for diagnosis of stage II, III and IV endometriosis. For diagnosis of Stage I, only IGKC and HP seemed promising.

Conclusions: Our study provides a tool for the differentiation of different stages of endometriosis, which is expected to have significant clinical relevance in terms of clinical assay development and thus, diagnosis.

Poster no: D-1-30**Differential plasma proteomics profile of Rats after Sarin exposure**

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Introduction: Sarin is highly toxic organophosphorus nerve agent. It inhibits neural enzyme acetyl cholinesterase (AChE) that leads to accumulation of acetylcholine at the synaptic cleft. It causes a wide range of toxic effect due to hyper activation of nicotinic and muscarinic AChE receptors leading to excessive secretions, muscle fasciculation, nausea, vomiting, respiratory distress and neurological effect. Inhibition of plasma cholinesterase is taken as the biomarker of exposure of organophosphorus compounds. Long term psychomotor function deficiency and reduced learning and memory functions have been observed several years post exposure of sarin among survivors. Plasma proteomics was carried out to study changes reflected at blood level due to sarin exposure.

Methodology: Male Wistar rats (100-120gm) were exposed with sarin and plasma was isolated from treated and control blood. After AChE estimation, albumin depleted plasma samples were separated by two-dimensional gel electrophoresis (2DE). After staining, the gels were imaged and differential expression of spots was analyzed using PD Quest. Spots of interest were then digested with trypsin and the proteins identified using MALDI-

TOF and selected peptide was sequenced by MALDI-TOF/TOF mass spectrometry followed by MASCOT data base search. Expression profile of major proteins was validated by western blot.

Result and Discussion: After exposure to 0.5 LD₅₀ of sarin inhibition of AChE persisted after one week of exposure. There were 10 plasma proteins identified with significant changes in expression (>1.5-fold) which included preproheptoglobin, heptoglobin, α -1-macroglobin on 1st day and altered preproheptoglobin and proapolipoprotein A1, Vitamin D binding protein peptide after one week of exposure. Out of these altered expression profile of apolipoprotein and haptoglobin has been implicated in neuro degenerative disorders. Apart from these, changes in expression of certain immunoglobulins were also observed.

Conclusion: Altered plasma proteomic profile after sarin exposure can help identify possible biomarkers of neuro degenerative changes at early time points.

Poster no: D-1-31

Conjoint Methacrylate Monolithic IMAC: Plasma Proteome

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Introduction: One of the major challenges in the Bottom-Up proteomics is in the analysis of complex peptide mixture generated by digestion of proteome. In this approach, identification of proteins is achieved through identification of proteolytic peptides. Prior to mass spectrometric experiments the complexity of the proteome needs to be reduced which can be achieved by Liquid Chromatographic (LC) methods. The present study demonstrates use of polymethacrylate monolithic immobilized metal affinity chromatography (IMAC) in conjoint fashion for protein fractionation. IMAC exploits the ability of immobilized transition metal ions to bind to surface exposed histidine residues on proteins/peptides through coordinate bond formation. Different metal ions (Cu, Ni, Zn & Co) chelated to IDA (Iminodiacetic acid) have differential retention of proteins with the above transition metals depending on the accessibility, number and loci of histidyl residues on the protein surface. Thus proteins can be fractionated in their native state from complex biological samples using IMAC with above listed metals in sequential manner in a conjoint mode. An added advantage of this approach is the use of a similar buffer system for binding/elution of the proteins.

Methodology: CIM-IDA monolithic disk of 0.34ml dimension were obtained from BIA Separations, Slovenia. Four different CIM-IDA disks were charged with metal ions were placed in the order Co (II), Zn (II), Ni (II), and Cu (II) respectively in a single housing. Binding of plasma proteins was carried out using 25mM MMA pH 7.4 (binding buffer), and the retained proteins were eluted using 500mM Imidazole in binding buffer. Both non retained and eluted fractions were reduced, alkylated and digested with trypsin and subjected to LC-MS analysis (ESI-QTOF, Agilent Tech., USA) and annotated using Spectrum Mill.

Results and Discussions: Total plasma was subjected to fractionation on a CIM-IDA metal conjoint setup. The total numbers of proteins identified after fractionation was found to be 3 fold higher than total plasma without fractionation (170 vs. 60 proteins). A similar observation was also noted for Albumin-IgG Depleted Plasma (180 vs. 70 proteins). These data infer that depletion is not a pre-requisite for conjoint system and

subsequently reduces the complexity. Further this approach minimizes sample loss and fractionation can be achieved within short time.

Conclusions: The experimental results using plasma samples clearly indicate the potential of this novel conjoint IMAC strategy as an efficient tool for proteomic applications.

Poster no: D-1-32

A proteomic analysis of low grade vs high grade gliomas using cerebrospinal fluid

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Introduction: WHO categorizes Gliomas into four different grades, where grade-I & II gliomas are grouped under low grade gliomas while grade-III & IV gliomas are clustered as high grade gliomas. Cerebrospinal fluid (CSF), the protective fluid surrounding the central nervous system (CNS) is a reservoir of transported biological substances, the waste and toxic excretion of the brain. Hence, composition of CSF reflects any alteration in the CNS functioning making it a potential candidate for biomarker discovery.

Materials & Methods: CSF samples were subjected to acetone precipitation followed by desalting to remove salts and non-proteinaceous impurities. 2D-DIGE and iTRAQ were used to identify proteomic alterations amongst different grades of gliomas. For 2D-DIGE labeled protein samples were subjected to isoelectric focusing followed by SDS-PAGE. The gels were analyzed using DeCyder 2D software and the differentially expressed significant protein spots were further subjected to In-gel digestion and MALDI-TOF/TOF analysis. For iTRAQ analysis, prior to labeling, buffer exchange was done with a LC-MS compatible buffer, followed by in-solution digestion. These labeled peptides were pre-fractionated using offgel fractionator, each fraction was subjected to LC-MS/MS.

Results and Discussion: Proteins associated with integrin signaling pathway, cytoskeletal regulation, glycolysis, gluconeogenesis, were found to be altered in gliomas. Proteins like vimentin, profilin, macrophage-capping protein, selenium-binding protein, protein disulfide-isomerase, peptidyl-prolyl cis-trans isomerase, etc. were increased with increase in tumor grades, while vitronectin, apolipoprotein C-III, synapsin-1, superoxide dismutase (Cu-Zn) etc. showed a negative correlation with increase in grade of the tumor.

Conclusion: CSF proteomic analysis in gliomas holds immense potential as it gives a mechanistic insight into glioma pathogenesis. A panel of proteins selected from our study may act as potential biomarker candidates for early diagnosis of gliomas using a comparatively less invasive method. However, there is a need for further validation of the proteins on larger clinical cohorts.

Poster no: D-1-33**Identification of potential biomarkers in bovine urine for early detection of pregnancy**

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Introduction: An early and precise pregnancy diagnosis is an important criterion for better reproductive management in livestock like cows and buffaloes. Progress in the fields of protein separation and identification technologies has accelerated research into bio fluids proteomics for protein biomarker discovery. Urine has become an ideal and rich source of biomarkers in proteomics to analyze the differential expression of urinary proteins in pregnant and non-pregnant cows by 2D and DIGE.

Method: Urine sample from pregnant and non-pregnant (n=6) cows were collected on different days of pregnancy (0, 7, 16, 22, 35), centrifuged and subjected to dia-filtration. Protein precipitation was done by using Ammonium Sulphate. Precipitated proteins were redissolved, dialyzed, cleaned up and quantified. 350µg (each sample) protein were subjected to 2D-GE (pH 4-7) followed by CBB staining. Destained gel was scanned by Labscan and Image Master Platinum software for detection of differentially expressed proteins. Further confirmation of this experiment was done by DIGE. 15µg proteins were minimally labeled with 200 pmol Cy3, Cy5 and an internal standard Cy2 fluorescent dye in dark. The proteins were subjected to 2D GE (pH 4-7). The gels were scanned and visualized with a Typhoon variable Mode Imager and analysis was performed with DeCyder 6 software.

Results: Nine (9) differential expressed proteins were observed in 2D gel by IMP analysis. In DIGE experiment based on a threshold of a greater than 1.2-fold change, 6 spots were detected. Identification of these proteins was done by MALDI-TOF. Few potential biomarkers for early pregnancy were detected such as: Mannan-binding lectin serine peptidase 2, Alpha-1-microglobulin, Glutaminyl-peptide cyclotransferase precursor and Hemopexin.

Conclusion: These results may provide a better understanding of the pregnancy and help in the development of diagnostic kit for early detection of pregnancy in cows.

Poster no: D-1-34**Identification of biomarkers for risk prediction and disease progression in Retinopathy of Prematurity (ROP): a potentially blinding disorder**

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Introduction: Retinopathy of prematurity (ROP) is a common blinding disease caused by the abnormal growth of blood vessels in the retina of premature babies of low gestation age and low birth weight. As the vitreous humor is in contact with the retina, protein components in the vitreous are affected by the physiological and pathological conditions of the retina. We are performing high throughput proteomics on vitreous humor

samples to identify potential biomarker/s in the progression of ROP.

Methodology: We have depleted 14 high abundant proteins from the individual vitreous samples of ROP patients (n=11) and age-matched congenital cataract patients (considered as controls; n=7). We have analyzed the high- and low-abundant protein fractions by on-line liquid chromatography electrospray mass spectrometry (LC-MS/MS). MS/MS data was searched from the International Protein Index database (human IPI v3.60) and PEAKS Studio software (v5.3) was used employing a 1% FDR cut-off to identify proteins and perform label-free quantification. Some of the highly up- and down-regulated proteins were validated by western blotting.

Results and Discussions: We have identified a total of 597 proteins in the vitreous humor of ROP patients (n=11) and 335 proteins in controls (n=7). About 55% proteins are common between both ROP and controls. Label-free quantification suggests that ~265 proteins are differentially expressed in ROP patients. Pathway analysis indicates the over-expression of the "complement and coagulation cascade" in the vitreous humor of ROP patients. Western blot results indicate that plasminogen, histidine-rich glycoprotein, factor XII, fibrinogen γ-chain and complement C3 are significantly up-regulated, whereas αA- and βB-crystallin are significantly down-regulated in ROP patients.

Conclusion: Our study shows that the levels of proteins involved in complement pathway, coagulation cascade and angiogenesis are elevated, whereas inhibitors of angiogenesis and coagulation are down-regulated in ROP patients. Thus, our study identifies potential biomarkers in the progression of ROP.

Poster no: D-1-35**Proteomics of induced Arthritis in Female Lewis rats by M1 type of Group A. Streptococcus (GAS)**

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Introduction: Rheumatoid arthritis (RA) is an autoimmune disease characterized by pronounced synovial inflammation. Despite significant therapeutic advances, a new targeted approach is needed. Our objective in this work was to investigate the proteomics of blood and liver tissue of lewis rats arthritis induced by M1 type of Group A *Streptococcus* strain.

Methodology: Arthritis was induced in 8-week-old Lewis rats by giving intraperitoneal injection of sonicated cells of heat-killed *Streptococcus* strain M1 into female Lewis rats. Effects on disease progression were assessed daily for paw swelling by using digital Plethysmometer. Protein profile of plasma and liver tissues were assayed by two dimensional gel electrophoresis followed by identification by Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF).

Results and Discussion: Inflammation was observed on 12th day of injection and it gets disappeared on 16th day. The paw volume, weight, degree of erythema & swelling were measured daily. Rats were sacrificed on 17th day. Blood & liver tissues were collected from each group of control, health & diseased rats. Protein profiling was done & significant results were obtained.

Conclusions: Arthritis like symptoms was observed (increase in paw volume, weight, degree of erythema & swelling) in lewis rats 1st time when induced with M1 type of Group A *Streptococcus* strain.

Protein profiling showed alteration in the expression of many proteins in Arthritis induced rats.

Poster no: D-1-36**Comparative profiling of bovine urinary proteins using various extraction methods reveals more than 1000 proteins**

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Introduction: Urine is an important source to assess the health status of human being and animals. Different research groups worldwide have used urine as a non-invasive source for detection of pregnancy and various other diseases in human being. Pregnancy in human being is detected by detecting Human Chorionic Gonadotropin (HCG) hormone in urine. However this hormone is absent in bovine urine. Therefore till date early pregnancy detection in bovine has not been possible using bovine urine as a source material. In the absence of HCG analogue in bovine urine during pregnancy, we expect that different pregnancy associated proteins may be present in bovine urine. In the present investigation we standardized the protein extraction methods from bovine urine and have followed shotgun proteomics approaches for profiling total number of proteins using LC/MS/MS in non-pregnant cows.

Methodology: Urine sample from 3 non-pregnant cows were collected aseptically. Three different protein extraction methods such as dia-filtration (in PBS buffer pH 7.4) using 3 kDa hollow fibre cartridge, Ammonium Sulphate precipitation and Proteo-Spin column (Norgen) were followed and comparative proteome analysis of the isolated proteins was performed by preparing tryptic peptides in solution and In-Gel (1D SDS-PAGE). The tryptic peptides were identified by shotgun proteomic approaches using LC-MS/MS (Maxis-HD, Bruker) at 0.05 ppm using Proteinscape software which uses Mascot Search algorithm.

Results and Discussion: We have till date identified 1070 proteins which included proteins involved in various metabolic and signalling pathways. A good number of proteins seemed to be membrane and extracellular proteins.

Conclusion: The present report is the first exclusive study on the bovine urine proteins to have been able to report a huge number of proteins catalogued till date which provides a useful reference for comparing datasets obtained using different methodologies. The urinary proteome may prove useful in biomarker discovery in the near future.

Poster no: D-1-37**Serum lipidomics study in women with polycystic ovarian syndrome (PCOS) using mass spectrometry**

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Introduction: Polycystic ovarian syndrome (PCOS) is the most common endocrine and metabolic disorder in women of reproductive age. PCOS is linked with dyslipidemia and other metabolic dysregulations. The need for development of suitable diagnostic markers for PCOS is well recognized. Furthermore,

the detailed pathophysiology of PCOS is not completely resolved. Comparative analysis of lipid expression may improve this situation.

Methodology: Serum samples were collected from 18 infertile women (9 PCOS and 9 tubal factor infertility cases as controls). PCOS was diagnosed by the Rotterdam 2003 criteria and subjects randomly recruited. Briefly, serum lipids were extracted using methyl tert-butyl ether (MTBE) method. Quantitative MRM based MS approach was used to acquire targeted MS data for specific lipid moieties in the positive and negative ion modes using a 4000 QTRAP MS equipped with a HPLC containing C4-300 column. All the acquired spectra were then subjected to statistical analysis such as PCA, PLS-DA and OPLS-DA.

Results and Discussion: In this study, we have identified differently expressed serum lipids using LC-MS/MS in serum of women with PCOS. Univariate analyses indicate that several lipid moieties including different types of phosphatidylcholine, lysophosphatidylcholine, sphingomyelin, phosphatidylethanolamine and triglycerides were identified to have altered expression in PCOS as compared to controls. PCA detected intrinsic clustering based on differential lipid expression between the 2 groups. The PLS-DA and OPLS-DA analyses have shown further discrimination of PCOS from controls and statistical model was built with sufficient sensitivity and specificity.

Conclusion: Alteration in the levels of specific lipids identified by LC-MS/MS in PCOS is expected to contribute towards identification of lipid based marker and improve understanding of the complex pathogenesis of the disease. Metabolomics and proteomics studies are also underway to integrate with lipidomics findings using systems biology approach for improved understanding pathophysiology of PCOS.

Poster no: D-1-38**Functional analysis of SNPs in Dopamine- α -Hydroxylase gene by genotype-phenotype correlations**

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Introduction: Dopamine- α -Hydroxylase (DBH, E.C 1.14.17.1) is an oxidoreductase that converts dopamine to norepinephrine. It is a candidate gene and a drug target for neuropsychiatric and cardiovascular disorders. Variants in this gene may affect susceptibility to some of these complex disorders. However information of critical variants in this gene is rudimentary. Through this study we have tried decipher critical variants from a subset of prioritised variants.

Methodology: Nonsynonymous (ns) SNPs in this gene were prioritised based on SIFT algorithm. The prioritised SNPs were made in synthetic constructs using site directed mutagenesis and transfected into HEK293 cells followed by stable cellline generation. While the enzyme activity from the spent medium was analysed on a UPLC, the quantities were assayed using MRM^{HR} on a TripleTOF 5600. The ratio of activity to quantity was calculated for the wildtype and each of the mutants and compared.

Results and discussion: Of the five SNPs studied, one SNP was not secreted. Three other SNPs were found to be differentially expressed leading to a change in activity, and one matched the wildtype. None of the mutants contributed to an innate activity difference.

Conclusions: Variants in this gene may cause secretory defects leading to a quantitative change in the protein. The DBH locus is also considered to be Quantitative trait loci (QTL) for DBH

activity. The differential levels of protein may manifest as an increased susceptibility to complex disorders or differential drug response. These findings are of relevance in case of Schizophrenia characterized by high levels of dopamine and Parkinson's disease with low dopamine levels. Characterisation of other variants in this gene or genes such as DBH may contribute to risk predictions which may pave way for personalised medicine.

Poster no: D-1-39

Identification of biomarkers for Proliferative Diabetic Retinopathy (PDR) in the human vitreous humor

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Introduction: Diabetes is characterized by metabolic abnormalities and long-term complications involving multiple organs. Diabetic retinopathy (DR) is caused when the retina is involved. DR can be either non-proliferative (NPDR) or proliferative (PDR). The pathology of PDR includes capillary occlusion, neovascularisation and breakdown of the blood-retinal barrier, which will eventually result in the loss of vision. Although the prevalence of diabetic retinopathy among self-reported diabetics is 26.8%, in most cases diagnosis is achieved only at an advanced stage. Biomarkers for early detection of PDR will tremendously help in the management of this disease. As the pathological conditions of the retina in PDR will affect the protein components of the vitreous, we performed a quantitative proteomic analysis of vitreous to identify the differentially regulated proteins in PDR vitreous.

Methodology: Vitreous samples of PDR patients and non-diabetic subjects were used. The abundant proteins in the samples were depleted using a Human 14 Multiple Affinity Removal System. The iTRAQ labelled samples were fractionated using SCX chromatography and analysed on a LTQ-Orbitrap Velos mass spectrometer. The mass spectrometry data was searched using Sequest through Proteome Discoverer software suite. Four of the differentially regulated proteins were validated using Multiple Reaction Monitoring (MRM).

Results and Discussions: We identified a total of 677 proteins, out of which 63 were found to be more abundant while 76 were found to be less abundant in PDR vitreous when compared to normal vitreous. Several proteins that play a role in angiogenesis and vascular permeability were identified. Overabundance of FGG, A2M, ABCB9 and AMBP proteins in PDR vitreous was validated in twelve PDR vitreous samples using LC-MRM.

Conclusions: This study provides a number of proteins, which are found to be overabundant in the vitreous of PDR patients. Further, validation of these differentially regulated molecules in an easily accessible fluid such as tear will lead to the development of biomarkers for early detection of diabetic retinopathy.

Poster no: D-1-40

Proteomic analysis of Immune complexes in Diabetes

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

Methodology: Immune complexes from control and diabetic plasma were isolated using Protein-G sepharose columns, followed by in solution digestion and identification, quantification and characterization by nano LC MS/MS.

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

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

Poster no: D-1-41

Comparative Proteomic Analysis of Extremely Low and Moderate Parasitemic and Longitudinal Cohorts of *Vivax* Malaria Patients for Identification of Potential Prognostic Serum Biomarkers

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Introduction: *Plasmodium vivax* has the most extensive global distribution and its diagnosis, treatment, and control is even more challenging than the *falciparum* malaria, since *P. vivax* can cause severe and fatal manifestations even at a very low parasite biomass and there is no precise, systematic global assessment of endemicity for *vivax* malaria.

Methodology: The present study has been conducted to perform an in-depth comparative analysis of serum proteome profiles of

extremely low [LPVM (parasite count < 200/ μ L): n = 23] and moderate parasitemic [MPVM (parasite count > 2000/ μ L): n = 38] *vivax* malaria patients and healthy controls [HC: n = 40] to identify early diagnostic and prognostic protein markers. Additionally, for longitudinal analysis, samples (n = 10) were collected during the early febrile, defervescence, and convalescence stages of the disease. Different hematological and liver function parameters including haemoglobin, WBC, RBC and platelet counts, ESR, total bilirubin, SGOT, SGPT and AP were analyzed. Comparative serum proteome profiling was performed by using 2D-DIGE and iTRAQ-based quantitative proteomic approaches and results were validated by ELISA.

Results and Discussion: A few identified proteins like Ceruloplasmin and Apo A-1 exhibited equal levels of differential expressions in LPVM and MPVM can act as early diagnostic markers; while the proteins like SSA and Haptoglobin, which shown sequential alterations with respect to the parasite load can be considered as potential disease monitoring/prognostic markers. Indeed, hypohaptoglobinemia is considered as an epidemiological and clinical indicator for malaria. Interestingly, analysis of longitudinal cohorts indicated cyclic alterations in the expression levels of Haptoglobin, RBP4, ApoE and Apo-A1 with the different stages of the infection, which could serve as indicators of the disease progression.

Conclusions: Our findings may open up new opportunities for the early detection and prognosis of *vivax* malaria as well as could provide better understanding of pathogenesis of *P. vivax* infection.

Poster no: D-1-42

Deciphering the signaling network of Interleukin-33 by SILAC-based quantitative phosphoproteomic analysis

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Introduction: Interleukin-33, a member of the IL-1 family of cytokines plays diverse roles in the regulation of immune responses. IL-33 exerts its effects by binding to a heterodimeric receptor complex -Interleukin-1 receptor like 1 and an accessory receptor protein IL-1RAcP. Activation of the receptor complex leads to the activation of NF- κ B and MAP kinase pathways resulting in the production and release of pro-inflammatory cytokines from hematopoietic cells contributing to the development of immune related diseases. However, a detailed understanding of the signaling pathways activated by IL-33 remains elusive.

Methodology: SILAC-based global quantitative phosphoproteomic analysis was carried out to gain insights into the IL-33 mediated signaling mechanisms. The cells grown in heavy SILAC media were stimulated with IL-33 whereas cells grown in light SILAC media were left unstimulated. The lysates were mixed in equal amounts and subjected to in-solution tryptic digestion followed by enrichment of phosphopeptides. The

enriched samples were analyzed on high resolution Fourier transform mass spectrometer.

Results and Discussion: SILAC-based global quantitative phosphoproteomic analysis of IL-33 mediated signaling resulted in the identification of 7,191 phosphorylation sites derived from 2,746 proteins of which, 1,050 sites corresponding to 672 proteins were regulated by IL-33. We report the identification of several known downstream effectors including ERK1/2 and p38 and novel molecules that have not been reported previously. Our analysis also revealed enrichment of proteins involved in cytoskeleton reorganization and actin binding that has not been previously linked to IL-33 signaling suggesting its role in macrophage activation. Integration of the data along with literature-based cataloging of molecular reactions enabled generation of a large signaling network governed by IL-33. 575 novel proteins were added to this signaling pathway as opposed to the 36 known molecules from 200 studies.

Conclusions: This study reports the first quantitative analysis of IL-33-regulated phosphoproteome. The findings will significantly expand the understanding of IL-33 mediated signaling events and have the potential to provide novel therapeutic targets pertaining to immune related diseases where dysregulation of IL-33 is observed.

Poster no: D-1-43

Bioavailability of Nano Ferric Pyrophosphate salt and its effect on plasma proteins invivo

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Introduction: Food fortification with water soluble iron salt is considered to be an effective way to combat Iron-deficiency anemia, although associated organoleptic changes still remains a challenge. We have addressed those issues by using nano form of poorly water soluble and creamish white ferric pyrophosphate [$Fe_4(P_2O_7)_3$] salt in rat model. However, the penetration of nanoparticles across paracellular pathway and subsequent interactions with proteins of biological matrix might lead to alter proteomics profile. The present plasma proteomics study provided valuable information to gain insights about the protein-nanoparticle interaction invivo.

Methodology: The relative bioavailability of $Fe_4(P_2O_7)_3$ nanoparticles with respect to $FeSO_4$ was calculated using hemoglobin regeneration efficiency method in anemic rats. 2D Nano-LC/MS based plasma proteomics was performed for normal rats in two groups, fed with nano FePP and $FeSO_4$ separately.

Results and Discussion: The relative bioavailability of nano $Fe_4(P_2O_7)_3$ was found to be 96.2%, while that of micro $Fe_4(P_2O_7)_3$ was reported to be 59%. Thus reducing particle size to nanoscale improved the bioavailability of $Fe_4(P_2O_7)_3$. The salt was found to be non-toxic in rats. The quantitative proteomics experiment showed that the protein Fetuin-B decreased with increase in dose level in $Fe_4(P_2O_7)_3$ groups whereas it remained unchanged in $FeSO_4$ group. Fetuin-B being a negative acute phase protein might be expected to show dose level response to foreign nanoparticles. Gelsolin, Kininogen-1 and Haptoglobin were identified exclusively in $Fe_4(P_2O_7)_3$ group. With the increase in dose level of nanoparticles, Gelsolin and Kininogen-1 were observed to decrease whereas Haptoglobin was increased in its expression level.

Conclusion: $Fe_4(P_2O_7)_3$ in its nano form could be a promising candidate for food fortification in Iron-deficiency anemia. Mass

spectrometry based proteomics could be one of the reliable techniques to monitor the interaction between nanoparticles and proteins invivo, which is necessary as a safety measure for the use of nanoparticles.

Poster no: D-1-44

Identification of Selective Sodium Channel blocker from *Conus tessulatus* and *Conus monile*

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Introduction: Cone snails are a group of marine gastropods have well developed toxin machinery for prey capture and self defense by inhibiting several ion channels and excitatory cells of neurons and muscle fibers. Earlier studies on conopeptides show that chief venom machinery has evolved to target key cell surface signaling components of ion channels and receptors. Hence in this study we focus on the isolation and characterization of individual conopeptides from *Conus tessulatus* and *Conus monile* which could be a selective sodium channel blocker.

Results: Sequential extraction of about 10 venom ducts from each cone snail yielded a mixture of conopeptides which were confirmed by SDS PAGE analysis and the peptides were found to be less than 3KDa. Further the peptide mixture is subjected to RP-HPLC profiling, which yielded 5 and 6 major fractions in *C. monile* and *C. tessulatus* respectively. Patch Clamping analysis of each fraction on Cardiac Sodium Channel (Na_v 1.5) showed different responses. Of the five fraction collected from *C. monile*, two showed mild block rest three were found to be inactive on Na_v 1.5. Whereas in *C. tessulatus* out of six fractions one fraction was found to efficiently block Na_v 1.5 and two fractions had the ability to block the channel partially and one fraction inhibited the inactivation gate of the channel whereas other two remained inert.

Discussion: From the results it is evident that conopeptide fractions have activity on Na_v 1.5. This could be due to the presence of single or many small peptides in the fractions. Hence techniques like FPLC and UHPLC will give us the separate individual peptides from the fraction mixture and sequencing will give an insight view on the peptides obtained from the fractions of *C. monile* and *C. tessulatus*.

Poster no: D-1-45

Proteomic Approaches to Understand Ovarian Physiology and Pathophysiology: Emphasis on Polycystic Ovary Syndrome

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Introduction: Folliculogenesis is the process of development, maturation and release of oocytes from the ovary. Follicular fluid

(FF), which surrounds the ovum, is a vital and dynamic element of a growing ovarian follicle, comprised of various proteins which modulate the oocyte maturation and ovulation. Our lab is focused on investigating the molecular mechanisms of folliculogenesis, especially in an ovulatory dysfunction like polycystic ovary syndrome (PCOS). In-depth proteomic analysis of FF may serve as an alternative for ovarian biopsies to study ovarian function. We mapped the proteome of FF obtained from normo-ovulatory healthy women undergoing in vitro fertilization for better understanding of ovarian physiology. By using quantitative proteomic approach we compared the FF proteome of controls with PCOS, which is a major cause of anovulatory infertility, to delineate underlying molecular defects.

Methods: To characterize the proteome of normal follicular fluid, a multipronged approach of protein-peptide separation followed by LC-MS/MS analysis was carried out. The differentially regulated proteins from control and PCOS women were identified using a highly sensitive quantitative proteomics approach of iTRAQ labeling followed by LC-MS/MS.

Results: 480 proteins of diverse functional category were identified in normal FF, of which many are known to be essential for folliculogenesis. Additionally, we report presence of several novel proteins in FF, not previously known to be present in ovarian microenvironment. Quantitative proteomics of FF from women with PCOS lead to identification of 770 proteins, the largest number of proteins identified in FF till date. Of these, 186 were found to be differentially expressed in these women (99 up and 87 down).

Conclusions: The catalog of normal FF generated can be used as a resource for oocyte biomarker development. Several vital proteins indispensable for the follicular growth including extracellular matrix, complement coagulation cascade, angiogenesis, metabolism etc. were found to be altered in PCOS, which may partially explain the aberrant folliculogenesis observed in these women.

Poster no: D-1-46

Identification of signature proteins for early detection of Noise Induced Hearing Loss in the mine workers: A proteomic approach

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Introduction: Noise exposure is one of the most persistent health hazards in mining. Mine workers are exposed to high level of noise, responsible for Noise Induced Hearing Loss (NIHL). Worldwide 16% of the disabling hearing loss in adults results from excessive exposure to noise in the workplace. Noise damages key molecules like proteins present in the micro-machinery of the ear required for the mechano-electric transduction of sound waves. Inner ear is crucial organ of hearing. There are key proteins present in tectorial membrane, Inner Hair Cells (IHC'S) and Outer Hair Cells (OHC'S) and Stereocilia in the cochlea. Most studies related to cochlear protein expression/loss have been conducted in animal models. However, with regard to humans, relatively little is known about the cochlear protein expression in NIHL with special reference to presence in blood. The aim of the proposed study, to identify protein biomarkers associated with the occurrence of NIHL in serum samples of mine workers, will be performed by using proteomic approach.

Methodology: The serum samples will be subjected to 1D and 2D Electrophoresis for the evaluation of differential expression of proteins. Multiplex ELISA will be performed for the determination of ear cell protein concentration. Also, Maldi Toff,

LC-MS will be included accordingly to identify the differentially expressed protein.

Results and Discussion: The concentration and the stage of the loss or over expression of protein will be the target result of this study. The differentially expressed protein might be used as a signature protein for the early detection of NIHL.

Conclusion: Based on the results, conclusion will be drawn whether the differentially expressed protein could be a biomarker for the early detection of NIHL.

Poster no: D-1-47

Human saliva: Body's mirror in health and disease?

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Introduction: Human saliva is a protein-rich, easily accessible source of potential local and systemic markers to monitor changes that occur under pathological conditions. However, little is known about the changes in abundance associated with normal physiology for subsequent identification of disease specific markers. Thus, initially a comprehensive proteomic profiling of pooled saliva collected from the parotid glands of healthy female subjects divided into two age groups was performed followed by characterization of saliva collected from primary Sjögren's syndrome (pSS) subjects, a disease primarily effecting females.

Method: Parotid saliva collected from healthy subjects were subjected to hydrophobic charge interaction chromatography to separate high- from low-abundance proteins prior to characterization by multidimensional protein identification technology (MudPIT). In SS, MudPIT was performed followed by reversed-phase (RP) analysis.

Results and Discussion: Collectively, 532 proteins were identified in the two groups of healthy female subjects. Of these proteins, 266 were identified exclusively in one age group, while 266 proteins were common to both groups. Selected representative MS findings (e.g. amylase) were validated by Western blots.

In SS subjects, MudPIT analysis led to confident identification of 1246 proteins. Of these proteins, 529 were only detected in either the pSS or HC sample, while 206 of these proteins were differentially expressed. In parallel, the same samples were quantified using reversed-phase (RP) MS. Fifty eight of 71 proteins identified by RP overlapped with MudPIT results. Five proteins (e.g. lysozyme) were further analyzed by targeted label-free quantification to confirm the similar relative differential expression observed by RP and MudPIT approaches.

Conclusion: Results of these studies encourage using saliva as an alternate diagnostic body fluid as well as support the use of MS for global discovery and validation of marker proteins for improved and early diagnosis of diseases.

Poster no: D-1-48

Ultra-Sensitive High Performance Liquid Chromatography- Laser Induced Fluorescence (HPLC-LIF) based Proteomics for Clinical Applications

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Introduction: In recent years, proteomics techniques have advanced tremendously in life sciences and medical sciences. It comprises various technologies dedicated for the detection and identification of proteins in body fluids, tissue homogenates and cellular samples to understand biochemical mechanisms leading to different diseases [1, 2]. The methods include techniques like High Performance Liquid Chromatography, 2D-PAGE, MALDI-TOF-MS, SELDI-TOF-MS, CE-MS and LC-MS techniques etc. We have developed High Performance Liquid Chromatography- Laser Induced Fluorescence (HPLC-LIF) based proteomics method to separate and detect proteins in body fluids for clinical applications.

Methodology: Details of HPLC-LIF instrumentation are available elsewhere [3-8]. It consists of a HPLC system with a reversed phase biphenyl column for the separation of proteins. A rigidly fixed capillary flow cell associated with the system was fabricated in our laboratory for the detection and collection of the eluting proteins. The sample in the flow cell is excited with frequency doubled Ar+ laser beam with wavelength 257nm. Protein fluorescence is collected and focused onto the slit of the monochromator set at 340 nm by using collection optics. The fluorescence signal detected by a photomultiplier tube is sent to the Lock-in amplifier interfaced with the computer generates protein profile of the sample of interest. The method is extremely sensitive to detect proteins with limit of detection of the order of femto-moles [8].

Results and Discussions: Protein profiles of different clinical samples (serum, saliva, tissue homogenates and cellular samples) from volunteers with different disease conditions were recorded by using HPLC-LIF set-up. More than 300 protein profiles of body fluids were recorded using this technique. The protein profile data were analysed using Principal Component Analysis (PCA) for objective classification and detection of malignant, premalignant and healthy conditions. The technique has been established by our group for routine screening and objective diagnosis of cervical [4, 5] and oral [6, 7] cancers.

Conclusions: Analysis of protein profiles of body fluids using HPLC-LIF technique has shown that the technique is powerful tool for screening, early detection and staging of various type cancers.

Poster no: D-1-49**Intermittent hypoxia facilitates human acclimatization to high altitude: Evidences for plasma proteomics studies**

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Abstract: Exponential decrease in partial pressure of inspired oxygen with increase in altitude results in hypobaric hypoxia. This extreme environmental condition challenges human performance, survival and may lead to a number of life-threatening clinical manifestations. Increasing number of population inflow to high altitudes for work, sport, pilgrimage and leisure warrants a better understanding of high altitude related disorders. In an attempt to understand molecular events associated with human acclimatization to high altitude, we exposed healthy volunteers (age: 27 ± 4 years, weight: 58 ± 5 kg) to intermittent hypoxia (IHT, 12% FiO₂, 4 hrs/day for 4 consecutive days) at sea level. Following IHT exposure, the subjects were airlifted to Leh (11200 ft) along with control subjects. Physiological parameters like acute mountain sickness (AMS), SpO₂, BP, HR and heart rate variability (HRV) of all the subjects were monitored for 1, 3 and 6 days respectively and plasma from fasting blood samples were collected both at Delhi and Leh. Using iTRAQ-based proteomics studies, we have identified 199 proteins for IHT treated subjects. Interestingly, we have identified hemoglobin and associated proteins, apolipoproteins, complement and coagulation factors for IHT treated subjects. Comparison of protein profiles revealed that IHT treatment at sea level elicits faster response of hypoxia adaptive proteins upon exposure to high altitude as compared to non-IHT treated subjects. Pathway analysis revealed that complement and coagulation cascade as well as PPAR signaling pathway are the major signaling pathways. The activation of PPAR signaling pathway highlights the essential roles of lipid and glucose metabolism contributing to energy homeostasis as key pathways for hypoxic adaptation. Our cumulative results demonstrate that IHT exposure at sea level facilitates rapid human acclimatization to high altitude.

Poster no: D-1-50**Proteomic Analysis of Human Plasma in Rheumatic Heart Disease**

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Introduction: Rheumatic fever in childhood is the most common cause of Mitral Stenosis in developing countries. The disease is characterized by damaged and deformed mitral valves predisposing them to scarring and narrowing (stenosis) that

results in left atrial hypertrophy followed by heart failure. Despite the high prevalence and increased morbidity, no biochemical indicators are available for prediction, diagnosis and management of the disease. Adopting a proteomic approach to study Rheumatic Mitral Stenosis may therefore throw some light in this direction.

Methodology: Six plasma samples, three each from the control and patient groups were pooled and subjected to low abundance protein enrichment. Pooled plasma samples (crude and equalized) were then subjected to in-solution trypsin digestion separately. Digests were analyzed using nano LC-MS^E. Data was acquired with the Protein Lynx Global Server v2.5.2 software and searches made against reviewed *Homo sapiens* database (UniProtKB) for protein identification. Label-free protein quantification was performed in crude plasma only.

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

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

Poster no: D-1-51**Comparative serum proteomic profiling in active vs. remitting granulomatosis with polyangiitis using two dimensional gel electrophoresis along with MALDI-TOF-MS**

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Introduction: Granulomatosis with Polyangiitis (GPA), an autoimmune vasculitis, in its limited form, poses no major threat to patient life/organs involved, but the systemic form runs a relentless relapsing and remitting course, inspite of treatment. We designed this study to find proteins which could differentiate limited from systemic GPA and the active state from its remission state of GPA.

Methodology: Eighteen patients of GPA fulfilling ACR and CHCC in active as well as in remitting state and 4 healthy controls (HC) were included in the study. Patients were categorized into two major groups consisting 7 patients with limited GPA and 11 patients with systemic GPA. For proteomics analysis, two-dimensional gel electrophoresis along with Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry was done. A total of 14 gels were run from pooled patients' sera from active GPA and remission as well as pooled HC serum.

Results: There was significant differential expression of proteins in limited vs. systemic GPA and between active systemic vs. remitting patients of systemic disease. We identified nine maximally differentially expressed and five proteins which were not detected in HC. Among nine proteins, one (pro-low density lipoprotein receptor-related protein 1) was down regulated & four proteins (Haptoglobin Hp, Hp2, Vitamin D binding protein, Killer cell lectin-like receptor subfamily F member 2), were up-regulated in both limited and systemic active disease, two

proteins like Ig gamma-4 chain C region protein & serum albumin were up-regulated in limited active GPA and two proteins i.e. cysteine rich secretory protein LCCL domain-containing 2 precursor & Serine-threonine-protein kinase A-Raf were up-regulated in systemic active disease. Levels of interleukin 17 (IL-17) and VDBP by ELISA could distinctly demarcate active disease vs. remission.

Conclusion: Our study provides potential protein markers of active disease vs. remission in GPA.

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Poster no: D-2-01**Role of GTP binding protein mlaF in bacterial membrane permeability**

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Multidrug resistance (MDR) is a significant challenge in the treatment of infectious diseases and cancer. Members of ABC superfamily are ubiquitous among all the kingdoms of life and form a sub-family of membrane proteins characterized by homologous ATP-binding and large multi-spanning transmembrane domains. In Gram-negative bacteria, the outer membrane is chemically asymmetric unlike inner membrane where the bilayer membranes are made up of almost equal amount of phospholipids. The asymmetric compositions of phospholipids in outer membrane of these bacteria are maintained by different alternative mechanisms such as PldA, PagP and Mla ABC transport pathway in normal as well as in stress conditions.

In Mla ABC protein complex, MlaF is an essential nucleotide binding protein of an unknown function in Gram-negative bacteria. On the basis of its sequence similarities to other nucleotide binding proteins, it has been revealed that MlaF is a classic ABC transport nucleotide binding component and displaying a typical nucleotide binding motif including Walker motif, Q-loop and H-loop. To investigate the role of GTP-binding motif of MlaF, we generated a series of Alanine-substitution mutations at the positions that are highly conserved among homologous proteins. Biochemical and biophysical data have shown that nucleotide binding motif in MlaF is important for Mla transport pathway which modulate membrane permeability in Gram-negative bacterial system.

Poster no: D-2-02**Identification, sequencing & Homology modelling of Cytochrome P450 from *Rhipicephalus (Boophilus) annulatus***

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Introduction: *Rhipicephalus annulatus* are considered as the most important group of arthropods to transfer disease pathogens from one animal to another. The resistance of ticks to acaricides is threatening problem and results in decreased production and restricted traffic of livestock. CytochromeP450 monooxygenases are known to play a major role in insecticide resistance, allowing resistant insects to metabolize insecticides at a higher rate. However, studies on the Cytochromep450 dependent drug resistance in *R.annulatus* have not been reported so far. The current study focuses on identification, sequencing and protein modelling of *CYP4W1*. This can aid in identifying the drug interaction sites of *CYP4W1* and thus revealing their mechanism behind drug resistance.

Methodology: *CYP4W1* gene was identified in *Boophilus annulatus* and sequenced using Sanger method. Sequence analysis and Phylogenetic analysis was done by MEGA 5. Translation of the sequence was done using translate tool from

Expasy. The 3-dimensional structure of the sequence was predicted using Homology modelling techniques from Swiss-Model and the model quality was assessed using the GMQE, QMEAN4 and Ramachandran plot.

Results & discussion: For the first time CYP450 gene from *R. annulatus* was identified and sequenced. Certain regions in the sequence such as signature motif, FxxGxxxCxG; and ExxR (saltbridge) in the mature CYP450 are highly conserved. Although the signature motif of P450 is present, a number of mutations which includes L55>S70, T59>A74, I111>L126, V217>L249 and S273>A305, S301>P333 have been identified in these regions. The predicted homology model constructed from CYP450 gene sequence was of reasonable quality.

Conclusion: The newly identified sequence of the Cytochrome p450 from *R. annulatus* contains highly conserved P450 motif in addition to various mutations which may be important for the drug resistance. Further analysis of the binding site, the predicted model will help in the investigation of insecticide resistance and may serve as a lead in the development of candidate P450 inhibitors.

Poster no: D-2-03**Anticipating “Panomics” With Human Systems Biology approach Investigation of the complex Insilco structural-activity relationships of novel Neurexin (NRXN) and Neuroligin (NLGN) proteins derivatives targeting epidermal growth factor-like (EGF-like) sequences and laminin G domains**

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Background: The Neuroligin-Neurexin (NRXN-NLGN) complex is a heterothallic adhesion system at neuronal synapses. Both Neuroligin and Neurexin have synaptogenic activities that organize pre- and postsynaptic compartments in a bidirectional manner. Multiple genes, alternative promoters, and extensive alternative splicing generate Neuroligin and Neurexin variants that may underlie synapse-specific functions. Mutations in Neuroligin and Neurexin genes are associated with neurodevelopmental disorders, highlighting the importance of these proteins for cognitive function. Panomics is a term used to refer to the range of Molecular biology technologies including genomics, Proteomics, Metabolomics, Transcriptomics, Epigenomics and so forth. That focuses on the, high quality databases, the analyses of data that are generated in Human systems biology and network medicine approaches and the generation of testable hypothesis. Human Systems Biology is the pertinence of computational biology to Human health and disease.

Introduction: Neurexin and Neuroligin function in the vertebrate nervous system cell adhesion molecules and receptors. Most transcripts use the upstream promoter and encode alpha-Neurexin isoforms; Alpha-Neurexin contains epidermal growth factor-like (EGF-like) sequences and laminin G domains, and they interact with neurexophilins. In humans, alterations in genes encoding Neurexin and Neuroligin are implicated in Autism and other cognitive diseases, such as Tourette syndrome and Schizophrenia.

Methodology: The first objective function in the study is to analyse the processing of NRXN-NLGN pathway was carried using Cell Designer 4.3, a structured diagram editor for drawing gene-regulatory and biochemical networks and are stored using the Systems Biology Markup Language (SBML), a standard for

representing models of biochemical and gene-regulatory networks.

Phase I: Structural and attributes based NRXN and NLGN modeling as proteomics approach. To be identifying complex Insilco structural-activity relationships of novel Neurexin (NRXN) and Neuroligin (NLGN) proteins derivatives targeting epidermal growth factor-like (EGF-like) sequences and laminin G domains.

Phase II: Panomics software infernoRDN and Sipper software can be used to aspects enabling the detection and quantitation of multiple RNA or protein targets simultaneously(<http://www.progenetix.org/cgi-bin/score.cgi>) the array plot view is usually accessed through a sample or series detail link, and allows for the visualization of existing probe level plots and the creation of variants.

Poster no: D-2-04

In silico identification of high interacting proteins in *Mycobacterium tuberculosis H37Rv*

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Introduction: It is well known that proteins interact to carryout various biochemical functions. Alteration in protein interactions can cause diseases. Cascade of protein-protein interactions (PPIs) can change the proteome condition from a healthy state to a diseased state. In order to understand the disease process well, study at proteome level is more important. PPIs make an interaction network and it is more likely that any of the high degree nodes in the network are involved in the cascade of PPIs that can lead to the diseased conditions. Therefore, these nodes can be the potential targets for the effective control of the diseases. Tuberculosis is one of the leading infectious diseases in the world. Computational methods to identify PPIs of its causative agent are the easier, quicker and more cost effective.

Methodology: Computational methods to predict PPIs are Gene Neighborhood, Gene Fusion, Phylogenetic Profiles, Gene Coevolution, etc. Offline BLAST is performed across 62 proteome sets of *M. tuberculosis H37Rv* and 3904 orthologs were predicted from it. These orthologs are used to predict PPI using the above computational methods. The predicted PPIs are used build a PPI network and its hubs are identified.

Results and Discussions: We have identified 3,500 PPIs using Gene Neighborhood, 166 using Gene Fusion, 20,114 using Phylogenetic Profile and 54,005 using Gene Coevolution methods. These PPIs are used to build an interaction network for *M. tuberculosis H37Rv*. It has 76032 edges connected across 3891 nodes. Correlation coefficient for the network is found to be 0.84 indicating it's a good network. Highest degree of 437 is predicted for "pth" and "recA" proteins.

Conclusions: "pth" and "recA" are the most highly interacting nodes in the PPI network of *M. tuberculosis H37Rv*.

Poster no: D-2-05

Salivary Gland Proteins of Indian *P. argentipes*: probable role in protection against visceral leishmaniasis

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Visceral Leishmaniasis, a vector borne disease caused by *Leishmania donovani* is transmitted through the bite of infected

female sand fly (*Phlebotomus argentipes*) in India. Pre-exposure to uninfected fly bite or pre-immunization with saliva gland proteins (SGHs) decreased/ prevented from disease progression in host. Protection against the disease may be due to the production of antibody against SGHs by host and that may neutralize disease promoting molecules /components present in the saliva which is not yet identified. Some data suggested the protection through cell-mediated immune response and a delayed-type hypersensitivity to the salivary antigens. In *P. papatasi* vector of *Leishmania major*, a 15 kda saliva protein named as SP15 was reported which provide protection against *L. major* in mice model but protective role of saliva proteins of Indian *P. argentipes* is yet to be reported. In our study, *P. argentipes* saliva proteins were separated on 15% SDS-PAGE followed by western blotting using patient sera. Western blotting showed anti saliva antibody formation in VL patients but not in non- endemic region healthy serum which suggests that *P. argentipes* saliva proteins may have immunomodulatory role against VL. It was also found that SGH of *P. argentipes* has crucial effect on cytokine production by human monocytes. FACS analysis revealed an increased IFN- α and decreased IL-10 production by SGH stimulated monocytes. Therefore, the saliva of Indian *P. argentipes* contains some proteins which may have protective roles against VL and if so, these salivary proteins may be used as a candidate for future vaccine development.

Poster no: D-2-06

Nanny Model for Intrinsically Disordered Proteins

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Introduction: Intrinsically disordered proteins (IDPs) and ID regions commonly occur in eukaryotic cells and are strongly implicated in protein function. It was found that IDPs are inherently unstable and undergo proteasomal degradation by default. It has been proposed that they can escape from degradation by interacting with a protective protein partner, called a "nanny". Here we test the hypothesis that nannies control the levels of their ID clients thereby regulating their function using the AP-1 transcription complex as a modelsystem in which ID containing protein, c-Fos interacts with c-Jun.

Methodology: c-Fos, c-Jun and their mutants are fused to EGFP and mRFP and expressed in bacteria. Dynamic freedom of their disordered tails is assessed from the spread of the FRET intensity distribution between the fluorescent tags. Interaction strength is determined using surface Plasmon resonance. The transcriptional efficiency of the complex is measured in an *in vitro* transcription/translation assay and protein half life is determined in HeLa cells by translation shut-off. c-Fos is carried through *in vitro* evolution on clones of a random cDNA library generated by error-prone PCR. Structural characteristics are determined with the softwares, IUPred and PONDR VSL1.

Results & Discussion: Clones expressing AP-1 containing mutant c-Fos will be selected based on highest transcriptional efficiency. The *c-fos* will be sequenced, the mutations identified mapped on the complex, their spatial distribution and interactions will be analysed and their effect on protein structure, on protein-protein and protein-DNA interactions of the complex will be characterized. We are establishing here direct relationships between nanny interactions, ID stability and half-life.

Conclusion: If the hypothesis holds and human nannies influence the level of their partners by masking their ID regions then they are potential therapeutic targets and regulating their level or interaction with clients can be sites of pharmacological intervention.

Poster no: D-2-07

Unraveling the function of novel yeast proteins

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Introduction: Owing to increasing structural genomics projects such as the Protein Structure Initiative (PSI), there has been an exponential rise in the number of protein structures being deposited in the public domain. Quite a few of these proteins are uncharacterized with respect to their function. Experimental investigations to annotate protein function are expensive and laborious, which makes computational methods for predicting protein function very attractive. One of the recent strategies to unravel the function of a protein is by predicting its small molecule binding partners. The prerequisite being that it must be predicted with confidence *a priori* whether the protein is an enzyme. We developed a consensus prediction method which combined the strengths of five various enzyme prediction methods and maximized the number of correct predictions.

Methodology: Five protein function prediction methods, that are publicly available were used, namely, SVMProt, Profun, Profunc, Evolutionary Trace Annotation server (ETA), and Extended Similarity Group (ESG). A dataset of 200 enzymes and non-enzymes was submitted to the above mentioned servers using automation scripts and predictions were retrieved. These predictions were then used to build a random forest model for consensus predictions.

Results and Discussions: The consensus method performs better as compared to individual prediction methods, when tested on the training set. Further fine tuning of the model and rigorous cross validation is ongoing.

Conclusions: The consensus method serves as an aid for promoting protein function annotation (enzyme/non-enzyme) for novel proteins.

Future work: This method would be further used to annotate hypothetical/novel proteins of *Saccharomyces cerevisiae*. The proteins that are predicted as enzymes will be subjected to virtual screening, to predict small molecule binding partners. Further, these enzymes will be expressed and purified and their interaction with small molecule metabolites will be validated by experimental methods.

Poster no: D-2-08

Using ANS as a tool for investigating conformational dynamics in Concanavalin A

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Introduction: Concanavalin A (ConA) is a tetrameric lectin which is synthesized in the cotyledons of developing jack bean seeds and accumulates in the protein bodies of parenchyma cells. It is a homotetramer, each subunit having binding sites for divalent metal ions (Mn^{2+} and Ca^{2+}). ConA has found its applications in the field of cancer because of its property to

agglutination in many types of cells hence causing apoptosis. Proper fold of the lectin binding protein is needed to induce apoptosis. Thus our study is to elucidate the structural dynamics and thermodynamics of ConA in different chemical environment.

Methodology: ConA has been isolated from proteome of jack bean seed using salting out salt, ammonium sulfate. Precipitates were further purified by affinity chromatography using Sephadex S100 beads. The purity of the purified protein was further confirmed using LC-ESI-MS. Thermodynamics and conformational dynamics of native Con A is being studied in different conditions like pH values, salt concentrations, chaotropes and nanoparticle concentrations using different biophysical techniques like UV-Vis spectrophotometer, FT-IR spectroscope, CD spectropolarimeter, Isothermal Titration Calorimeter etc.

Results and Discussion: ConA has been successfully purified from its natural source i.e. jack bean. Preliminary study shows that the native conformation of ConA is having predominantly α -sheet conformation with melting temperature $74^{\circ}C$ at pH 7. However, the conformation undergoes drastic conformational change even with slight changes in pH.

Conclusions: Although, Con A is an all α -sheet protein there are considerable changes in its conformation and stability at different pH, chaotropic and salt concentration.

Poster no: D-2-09

Characterizing the role of Cas3 helicase domain in CRISPR-Cas interference

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Introduction: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and Cas proteins represent the adaptive immune system in the prokaryotes against the invading viruses and plasmids. Cas3 which has an N-terminal HD phosphohydrolase and C-terminal Superfamily-2 helicase domain (DExD/H-box helicase) plays an important role in the CRISPR RNA (crRNA) guided interference of the invading DNA. Structural and functional aspects of helicase domain of Cas3 are poorly understood and its importance in target cleavage is yet to be addressed.

Methodology: Conserved motifs and domains in Cas3 were identified using *in silico* analysis and helicase domain and null mutants of Cas3 from *Bacillus halodurans* were created. Nucleic acid duplex unwinding by Cas3 helicase was tested. ATP hydrolysis and binding were studied using colorimetric and fluorescence assays.

Results and Discussions: Cas3 is able to unwind the duplex DNA and as predicted the polarity of the unwinding is primarily in the 3' to 5' direction. Biochemical characterization of wild type and mutant version hints towards the importance of selected residue in ATPase activity. A stimulation of ATP hydrolysis was observed in presence of nucleic acids.

Conclusions: The presence of helicase domain with its characteristic motifs suggests that Cas3 has ATP binding motif and shows an ATPase activity. Stimulation of ATPase activity in the presence of nucleic acid suggests some coordination between target binding and ATPase activity. In parallel to the ATPase activity, Cas3 is able to unwind the duplex DNA in the 3' to 5' direction which is dependent on its concentration, metal ions and the presence of ATP.

Poster no: D-2-10**Cyclosporine A- and MnTMPyP-induced changes in the mitochondrial proteome profile of cypermethrin-induced nigrostriatal dopaminergic neurodegeneration****Agrawal S*, Singh M. P**

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Introduction: Cypermethrin induces nigrostriatal dopaminergic neurodegeneration leading to PD phenotype in rats. Defects in the mitochondrial function and alteration of mitochondrial proteins are reported in PD. Cyclosporine A, a mitochondrial permeability transition pore opening inhibitor and MnTMPyP, SOD/catalase mimetic could be expected to offer neuroprotection against cypermethrin-induced PD phenotype. The objective of this study is to evaluate the effect of cyclosporine A and MnTMPyP on the mitochondrial proteome in cypermethrin-induced nigrostriatal dopaminergic neurodegeneration.

Methodology: Wistar rats were intraperitoneally treated with standard dose of cypermethrin, cyclosporine A or MnTMPyP along with their respective controls. Two-dimensional polyacrylamide gel electrophoresis and matrix assisted laser desorption/ionization-time of flight/ time of flight were employed to identify the differentially expressed proteins in the substantia nigra and striatum of rat brain. Mitochondrial membrane potential and complex I activity were measured by spectrophotometrically/spectrofluorimetrically, however, number of tyrosine hydroxylase-positive neurons and striatal dopamine content were examined by immunohistochemistry and high performance liquid chromatography, respectively.

Results and Discussions: A total of nineteen mitochondrial proteins related with oxidative phosphorylation system, anti-oxidation, cell signalling and neurogenesis were found to be dysregulated in cypermethrin-treated rats as compared to control animal. Cyclosporine A and MnTMPyP respectively, modulated the expressions of three and four striatal and five and six nigral proteins. Cyclosporine A or MnTMPyP significantly restored the cypermethrin mediated increased nigral dopaminergic neurodegeneration and reduced mitochondrial membrane potential and complex I activity towards the normalcy.

Conclusions: The result obtained thus demonstrate that of the study demonstrate that cyclosporine A and MnTMPyP offer neuroprotection against cypermethrin-induced nigrostriatal dopaminergic neurodegeneration.

Poster no: D-2-11**Differential protein expression of cancer cells MDA-MB-231 on treatment with *Choerospondias axillaris*****Mann Sonia^{1,2}, Sarkar Ashish¹, Gupta Rajinder K² & Biswas Sagarika^{1*}**

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Introduction: Compounds derived naturally from plants and microbes have provided a number of useful cancer chemotherapeutic drugs. Plants, in particular, have played a dominant role in the development of sophisticated traditional medicine systems. Natural compounds may prove useful in prevention and treatment of cancer. The current study thus focuses on the anti-cancer properties of *Choerospondias axillaris* fruits on cancer cell line. *Choerospondias axillaris* belongs to family Anacardiaceae, commonly known as Lapsi/Lupsi. Studies

have shown the fruit is rich in essential amino acids and its properties signify its importance in human health.

Methodology: Healthy and Cancerous cell line (MDA-MB-231) was exposed to methanolic extract of *Choerospondias axillaris* at 5mg/ml concentrations for 18h. Treated and control cells retrieved after drug-treatment and proteome profile were compared after separation by 2-D gel electrophoresis followed my mass spectrometry analysis.

Results and discussion: In vitro selective cytotoxic effects were observed of methanolic extract on cancer cell line through MTT cell viability assay whereas same was not observed for healthy cell line. Analysis of 2-D gel electrophoresis showed 11 differentially expressed protein spots that may have role in anti-cancer or in maintaining normal cellular functions.

Conclusions: The *in vitro* effect of methanolic extract of *Choerospondias axillaris* fruits reveals significant cytotoxicity effect on cancer cells indicating a potential usefulness in the prevention and treatment of cancer.

Poster no: D-2-12**Elucidating the Functional Genetic Elements in Emu(*Dromaius novaehollandiae*) through Homology Modelling, Phylogenetic Analysis, Transcriptome and Microbiome Profiling****Thashi Bharadwaj^{1*}, Amrittha A¹, Deepa A.V¹, Shalini K¹, Joby Pulikkan¹, Abdul Munee², Tony Gracie¹**

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Introduction: Emu (*Dromaius novaehollandiae*), a member of ratite family of flightless birds, are native to Australia but found widely dispersed across varying habitats in all but one continent. Successful wild and captive breeding capacity of emus is attributed to its ability to tolerate wide range of temperature owing to effective thermoregulation and varied diet patterns. Though morphological variation among emu is very less, very little data is available on their genetic variability. Peculiar feeding habits and digestibility of wide variety of food suggests the presence of either specific gastric enzymes or a plethora of microorganisms assisting in digestion. Furthermore, very little is known about the anti microbial peptides of these organisms given the medicinal value associated with emu meat and oil. The current study aims to explore these areas in detail to identify novel and atypical functional genomic elements in Emu and to identify the anti microbial peptides present in this species. In this preliminary work we have identified and sequenced genes responsible for regulating secondary sexual characteristics (*SOX9*) and the microbial defense gene, Avian betadefensin (*AvBD*). We have also profiled the cecal microbiome of Emu.

Methodology: Microbial profiling was done by amplifying and analyzing the bacterial 16S rRNA. Sequence analysis and Phylogenetic analysis was done by MEGA 5. Translation of the sequence was done using translate tool from Expsay. The 3-dimensional structure of the sequence was predicted using Homology modelling techniques from Swiss-Model and the model quality was assessed using GMQE, QMEAN4 and Ramachandran plots.

Results and Discussion: The most prevalent phylum in the cecal microbiota of the emu is Bacteroidetes, a phylum known for fermentative metabolism and degradation of plant polysaccharides. The *AvBD* gene and predicted protein is phylogenetically very similar to ostrich which is also a member of ratite family. The study has also revealed effects of

environmental variations on the development of sexual differentiation in these birds.

Conclusions: The characterization of the antimicrobial peptides in Emu may help in explaining and understanding its strong immunity and also can be used for the development of new medicines. More studies into various genes involved in development of secondary sexual characteristics can provide more insights into the environmental effects on development.

Poster no: D-2-13

Regulation of Aging in Yeast by Glycation inhibitors

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Introduction: Yeast (*Saccharomyces cerevisiae*) is an excellent model system to examine the eukaryotic aging as it has highly conserved cellular signaling pathways and organelles. It has been well established from yeast to higher organisms, that calorie restriction retards the aging process and increases the lifespan, while high caloric diet accelerates the aging process and decreases the life span. Various pathways mediate the extension of lifespan in caloric restriction including reduced oxidative stress, TOR signaling and Sir2 mediated pathways. However, the precise mechanisms by which these pathways are inactivated are not elucidated. Here we propose that in high caloric /glucose condition, the cellular proteins are modified by glycation leading to altered function. We studied the protein glycation and differential gene expression in varying glucose concentration and correlated with chronological lifespan (CLS) of yeast.

Methodology: Yeast CLS was studied in varying concentration of glucose. Protein glycation, differential protein expression analysis was performed by using LC-MS/MS. Involvement of glycation was studied by using glycation inhibitor.

Results and Discussion: In this study, we have used two concentrations of glucose 2% (high) and 0.5% (low). High glucose decreased the yeast CLS. This was accompanied with increased ROS levels and protein oxidation. Proteomic analysis revealed modification of several proteins involved in glycolysis. Further SWATH based differential protein analysis suggested that down regulation of proteins involved in mitochondrial respiration. Glycation inhibitor suggested involvement of protein glycation in differential protein expression and CLS.

Conclusion: This study reveals the role for protein glycation in decreased CLS under high glucose condition.

Poster no: D-2-14

Oxidative stress and Translational attenuation involved in age dependent Myocardial infarction

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Introduction: Myocardial infarction (MI) is caused by the prolonged starvation of cardiac cell because of reduced oxygen and nutrient supply leading to cardiac arrest. However, early

diagnosis and timely management of MI is important in patients of different age groups since ageing could be a significant player in the onset of the disease. We have used murine model of MI for identifying novel age specific proteomic markers to elucidate the major pathways affected leading to onset of the disease in the different age groups.

Methods: 9 weeks and 70 weeks old male Wister rats (n=10 in each group), were used to generate MI by occlusion of left anterior descending coronary artery (LAD), and the animals were euthanized after 10 days of ischemic period. iTRAQ based quantitative LC-MS proteomic workflow was undertaken to identify the differentially expressed proteins.

Results and Discussions: We have identified a total of 1671, 1116 & 1163 proteins in three different (includes three biological replicates for each group) experiments at 1% FDR. A total of 137 and 179 differentially expressed proteins were identified in 9 weeks MI and 70 weeks MI model compared to their respective controls, out of which 72 were common and were involved in protein folding, hypoxia and proteostasis imbalance. Up regulation of RAC1 and down regulation of TRAP1 in 70 week MI indicates ROS overload in higher age group. Further, a total of 6 proteins were unregulated exclusively in 9 week MI compared to respective control and their expressions were also greater than 70 week control. These 6 proteins of Early age MI play role in maintaining the cellular integrity by enhancing cytoskeleton architecture, cell growth and differentiation. Moreover, the ribosomal proteins machinery and protein degradation machinery is unregulated to counteract the effect of translational attenuation in late age MI.

Conclusion: This indicates that increased oxidative stress and translation attenuation leads to cell death mediated MI. Further the study would help us to identify novel age specific markers of MI.

Poster no: D-2-15

A Proteomic analysis of salivary glands of female *Aedes aegypti* Linn

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Introduction: *Aedes aegypti*, a major vector for dengue, chikungunya and yellow fever has been completely sequenced in 2007. However, large majority of predicted protein coding genes remained hypothetical. In this study, we describe a proteomic analysis of salivary glands of *Aedes aegypti* using high resolution Fourier transform mass spectrometry. The mass spectrometry data was searched against protein database of *Aedes aegypti* and related species.

Methodology: 850 salivary glands were dissected from lab-reared *Aedes aegypti*. Approximately 100 µg of proteins derived from salivary gland was resolved on SDS-PAGE gel. In-gel digestion of proteins was carried out using trypsin. LC-MS/MS analyses of the in-gel fractions were carried out on LTQ-Orbitrap VELOS. High resolution MS/MS spectra were searched against the protein database of *Aedes aegypti* using Sequest search engine in Proteome discoverer software.

Results and Discussion: A total of 944 proteins were identified and catalogued using LC/MS-MS based approach. This is by far the largest catalogue of organ specific proteins of *Aedes aegypti* and contains a large opportunity for further studies and research on vector biology, physiology and transmission potential.

The maximum number of blast hits was from *Aedes aegypti* followed by *Anopheles gambiae*, *Drosophila melanogaster* and *Culex quinquefasciatus* indicating the high degree of sequence similarities between these species. The similarity mean ranged 48.05% to 100%. The sequence length varied from 56 amino acids to upto a maximum of 11328 amino acids.

Gene Ontology was used to describe protein function. The sequences were ascribed GO terms ranging from one functional term to upto 34 terms for a single sequence indicating the multiple functional roles of certain proteins in the salivary glands.

At least 198 proteins in the LC-MS/MS derived dataset were hypothetical proteins with no function assigned to them. These are the priority candidates for functional analysis and expression studies. We have given a probable predictive functional analysis based on two methods. Firstly by assigning a KOG/COG classification to them using STRING9.1 network. Secondly, these sequences were run through a BLASTp search against non redundant database and homologs were identified. A GO term was then assigned to them using blast2go portal.

Out of the total 944 sequences, 291 (30.84%) proteins are found to be involved in one or more metabolic pathways. 44 such known pathways operating in *Aedes aegypti* have their representation in our dataset which is specific to salivary glands of the organism. In addition to these, 25 immunogenic proteins have been identified in salivary glands which are high potential candidates for future experimental studies.

Conclusion: This study can provide a benchmark for unbiased deep proteomics analyses of a vector mosquito, findings of which will enable future discoveries in molecular associations of host-vector-pathogen interactions.

Poster no: D-2-16

Proteomic analysis and protein carbonylation profile in the cerebral cortex of swim trained rats

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Cerebral cortex is an important region which play important role in learning and memory. Studies have suggested that exercise promotes neuroplasticity and improves cognitive functions. However, the molecular mechanisms underlying these effects are limited. Using proteomics, we have analyzed the changes in protein expression and carbonylation in the cerebral cortex of sedentary and swim trained animals. Male Wistar rats of 12 months old were swim trained in rectangular glass tank (77cm x38cm x39cm) filled with water to a height of 26 cm with a load of 3% of their body weight tied to their tails. Initially, they were made to exercise for 5 min/day with a progressive increase to 30 minutes/day for a total training period of 4 weeks with 6 days/week. Sedentary controls were restricted to cage activity. Image analysis revealed that 9 spots were significantly expressed in the trained animals and the proteins indentified were related to functions associated with plasticity and metabolism. Carbonylation is one of the most common oxidative modifications of proteins and a marker of oxidative stress was identified using redox proteomic approach. The Hspa 8 and Tubalb were identified as significantly less oxidized proteins in the trained animals. The identification of these proteins provides

new insights into the mechanisms associated with training and in treating age-related neurological disorders.

Poster no: D-2-17

Phosphoproteome and proteomic analyses of extracellular matrix reveal interplay of predicted and unexpected components of cell wall signaling in plant

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Introduction: Cell wall is responsible for orchestrating complex regulatory mechanism. However, little is known about this modulation in plants. Reversible protein phosphorylation, a substochiometric PTM is known to regulate protein function and signaling pathways. Phosphorylation events in cell wall are triggered by a variety of stimuli leading to diverse outcomes, including mechanosensing and stress response.

Methodology: The proteome and phosphoproteome was developed with ECM enriched fraction using combination of 1 DE, 2 DE and subsequent staining with Pro-Q Diamond, SCX, IMAC and MOAC. Proteins were identified using MALDI-TOF/TOF, ESI-MS/MS, and Triple TOF/MS. To validate 2-DE immunoblot analysis was performed for hydroxy amino acid phosphorylation. Further, a protein-interactome model was developed.

Results and discussion: To provide novel imminent in cell wall dynamics, we have developed ECM phosphoproteome and proteome map of a food legume chickpea (*Cicer arietinum* L.). MS analysis led to the identification of around 1483 phosphoproteins and 2913 proteins, presumably involved in a variety of biological functions viz. cell wall remodeling, mechanical signaling, innate defense, redox homeostasis, protein folding and degradation. *In silico* prediction and mass spectrometric identification of site-specific phosphorylation of amino acid residues indicated their possible effect on ECM signaling network and dynamics at system level, which include both the regulatory as well as the functional proteins. Further, we interrogated the dataset using cluster and network analyses that identified significant protein modules and small correlation groups. A model network was constructed that integrate the phosphoproteins and proteins involved in transduction of stimulus and their outputs in the molecular machinery of the wall.

Conclusions: Together, these analyses enhance understanding of how cell wall reorganize and usurp signaling networks to facilitate sensing of pathogen. To our knowledge, this is the first report on the comprehensive understanding of the complex phosphoprotein network operating in plant extracellular matrix.

Poster no: D-2-18

Metabolic Pathway Re-engineering using Multi-Omics Datasets

Presenter: Prof. Vishwesh Kulkarni (University of Warwick)

* (Note: This work is in collaboration with Prof. Jean-Loup Faulon (CNRS) and Prof. Karthik Raman (IIT Madras))

Abstract: Metabolic circuits are a promising alternative to other conventional genetic circuits as modular parts implementing functionalities required for synthetic biology applications. So far, these have primarily focused on production circuits. But, emergent applications such as smart therapeutics require circuits

that enable sensing and regulation. So, we present an automated pipeline for embedded metabolic circuits that explores the circuit design space from a given set of specifications and selects the best circuits to implement based on desired constraints. Here, we show how to incorporate transcriptomic data, proteomic data, and metabolomic data. Synthetic biology circuits embedded in a chassis organism that is capable of controlling the production, processing, sensing, and the release of specific molecules were enumerated in the metabolic space through a standard procedure. In that way, design and implementation of applications such as therapeutic circuits that autonomously diagnose and treat disease, are enabled, and their optimization is streamlined.

Poster no: D-2-19

Comparative Proteomic Study Illustrates Cross Talk of Diverse Cellular Pathways and Novel Protein Network in Disease Progression

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Introduction: Fruit is an assimilator of signals and metabolites, thus considered potential target for pathogen attack. In response to patho-stress plants reorganize their proteome and reconfigure their physiology. These remodeling are coordinated by a poorly understood network of signal transduction, hormonal cascades, and metabolic reallocation.

Methodology: To explore plant susceptibility towards necrotrophic fungal attack, we conducted time-series protein profiling of *Sclerotinia rolfsii* invaded tomato (*Solanum lycopersicum*) fruit. Equivalent-sized red-ripe tomato fruits were infected at the stylar region with *Sclerotinia rolfsii* and incubated at 22 °C in sterile condition. Tissues were collected after removal of fungal mycelium, harvested, and stored at -80°C. Soluble proteins were isolated and separated by 2-DGE, scanned and analyzed with PDQuest (Bio-Rad). One-way ANOVA and PCA were carried out for statistical significance of protein abundance. Significantly altered protein spots were trypsin-digested and identified by LC-MS/MS and MALDI-TOF/TOF. Pathostress-responsive protein correlation network was visualized in Cytoscape.

Results and Discussions: The differential display of proteome revealed 340 pathostress-responsive protein spots (PSRPs) that change their intensities for more than 2.5-fold. Mass spectrometric analyses led to the identification of 164 PSRPs involved in disease progression; regulating diverse functions. We validated the dynamic changes in the expression of regulatory and structural proteins using immunoblot analysis. Further, to unravel the transition switches of the molecular cascades, we interrogated the proteome data using network analysis that identified five significant protein modules pointing towards the onset and context of disease signaling and metabolic pathway activations.

Conclusions: This study reports for the first-time kinetically controlled patho-stress responsive protein-network and cellular pathways during post-harvest storage in a sink tissue, particularly fruit. The network representation may facilitate the prioritization of candidate proteins for future quality improvement program.

Poster no: D-2-20

Identification of the differentially localized proteins in nuclear and cytoplasmic fractions of Sertoli cell using quantitative proteomics approach

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Sertoli cell (Sc) is an important cell type of testis, also called the nursing cells of germ cells. It not only provides architectural support to the germ cell division and differentiation but also provides milieu for spermatogenesis leading to sperm production. Although, the hormonal milieu comprising of *Follicle stimulating hormone (FSH)* and *Testosterone (T)* are same in 5 days old and 12 days old rats, 5 days old Sc are immature and incapable of supporting spermatogenesis. Whereas, Sc become mature by 12 days of age and allows proper transduction of the hormone mediated signalling which is associated with onset of active spermatogenesis. This suggests that Sc becomes mature at or before 12 days of age, thereby responding to hormonal signalling causing induction of robust spermatogenesis. Any defect or perturbation in acquirement of this ability would interfere with normal spermatogenesis leading to infertility. It may be feasible to diagnose and treat such cases of infertility by understanding the molecular basis of Sc maturation.

Proteome profile of a cell type defines its identity and functionality. Proteome profile is in turn regulated by the transcriptome profile of the cell. Nucleus being the centre of RNA synthesis, dictates the identity of a cell type. However, the transcriptional machinery which ultimately controls the transcriptome profile of the cell, is governed by the specific set of proteins present within the nucleus. Some of these proteins may directly bind to DNA to regulate gene expression and others may be involved in protein-proteins interactions for co-ordinated gene expression. Thus, it would be reasonable to assume that the proteome profile of nucleus predominantly governs the functions and identify of a cell type.

In this study, we used quantitative proteomics approach using SWATH workflow to identify the differentially nuclear localized proteins in infant and pubertal Sc. Infant and pubertal Sc of rat were cultured and their nuclear and cytoplasmic protein fractions were isolated. The nuclear and cytoplasmic protein fractions of infant and pubertal Sc were subjected for IDA (Information Dependent Acquisition) analysis to identify the proteins in each sample. The IDA data of all the protein fractions were clubbed together to build a comprehensive peptide ion library. Also, SWATH acquisition was performed for all the protein fractions. The SWATH data of each protein fraction was then interrogated against the comprehensive peptide ion library to quantify the proteins present in each sample and nuclear vs cytoplasmic abundance was calculated for each protein independently in infant and pubertal Sc. Upon analysis (*fold change > 2*), it was found that 93 proteins were common to the nuclei of infant as well as pubertal Sc. Notably, we found that 35 proteins were exclusively localized in the nucleus of infant Sc whereas 82 were specific to the nucleus of pubertal Sc. The age specific nuclear localization of certain proteins suggested their role in designating the functionality and identity to these cells. [We thank Trayambak Basak of IGIB, New Delhi for his valuable help]

Poster no: D-2-21**Molecular Investigations of Protriptyline as a Multi-Target Directed Ligand in Alzheimer's Disease**

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Introduction: Alzheimer's disease (AD) is a complex neurodegenerative disorder involving multiple cellular and molecular processes. The discovery of drug molecules capable of targeting multiple factors involved in AD pathogenesis would greatly facilitate in improving therapeutic strategies. The repositioning of existing non-toxic drugs could dramatically reduce the time and costs involved in developmental and clinical trial stages. In this study, preliminary screening of 140 FDA approved nervous system drugs by docking suggested the viability of the tricyclic group of antidepressants against three major AD targets, viz. Acetylcholinesterase (AChE), β -secretase (BACE-1), and amyloid β ($A\beta$) aggregation, with one member, protriptyline, showing highest inhibitory activity.

Methodology: Detailed biophysical assays, together with isothermal calorimetry, fluorescence quenching experiments, kinetic studies and atomic force microscopy established the strong inhibitory activity of protriptyline against all three major targets. The molecular basis of inhibition was supported with comprehensive molecular dynamics simulations.

Results and Discussions: In this study, preliminary screening of 140 FDA approved nervous system drugs by docking suggested the viability of the tricyclic group of antidepressants against three major AD targets, viz. Acetylcholinesterase (AChE), β -secretase (BACE-1), and amyloid β ($A\beta$) aggregation, with one member, protriptyline, showing highest inhibitory activity.

Conclusions: The multifactorial nature of AD makes its treatment complex and unmanageable. In this study we investigated the efficacy of the tricyclic antidepressant, protriptyline, against important AD targets. Our in vitro and in silico investigations established the inhibitory effects of the drug on AChE, amyloid aggregation, BACE-1 and glycation. Protriptyline was able to inhibit AChE and β -secretase by binding at the active site and causing conformational changes. In addition, it strongly prevented self-assembly of $A\beta$ and glycated $A\beta$. It is a FDA approved drug for the treatment of depression, narcolepsy, Attention Deficit Hyperactivity Disorder (ADHD) and headaches and its ability to cross blood brain barrier (BBB) is an additional advantage, which is a crucial requirement of molecules used for intra-cranial diseases. To the best of our knowledge, this is the first study in which an anti-depressant drug has been shown to inhibit multiple targets of AD. Our results strongly ratify protriptyline as a promising candidate for AD therapy, and its further evaluation in animal and clinical studies.

Poster no: D-2-22**Characterization of amyloid formation by glucagon-like peptides: role of basic residues in heparin-mediated aggregation**

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Introduction: Protein folding and its aggregation into amyloid is one of the important process of post-translational modification

of protein. Glycosaminoglycans (GAGs) have been reported to play a significant role in amyloid formation of a wide range of proteins/peptides either associated with diseases or native biological functions. The exact mechanism by which GAGs influence amyloid formation is not clearly understood.

Methodology: To study the fibril formation by GLP1 and GLP2, peptides were dissolved in 5% D-mannitol, 0.01% sodium azide and seed-free solutions were prepared using ultracentrifugation. Peptides were incubated at 37°C in presence and absence of the representative GAG heparin, and amyloid formation was monitored at regular time intervals by ThT binding, CD spectropolarimetry and EM studies.

Results and Discussions: We show that the aggregation and amyloid formation by these peptides follow distinct mechanisms; GLP1 follows nucleation-dependent aggregation, while GLP2 forms amyloids without any significant lag-time. Investigating the role of heparin, we also find that heparin interacts with GLP1, accelerates its aggregation and gets incorporated within its amyloid fibrils. In contrast, heparin neither affects the aggregation kinetics of GLP2 nor gets embedded within its fibrils. Furthermore, heparin preferentially influences the stability of the GLP1 fibrils over GLP2 fibrils. Our *in silico* results show that the 'Basic—non basic—Basic' (B-X-B) motif of GLP1 (K28-G29-R30) facilitates the interaction between heparin and peptide monomers. However, the absence of such a motif in GLP2 could be the reason for a significantly lower strength of interaction between GLP2 and heparin.

Conclusions: The present study helps to understand the role of heparin in protein aggregation and provides an insight into the nature of the heparin-protein interaction. Our study will significantly help to understand the mechanism(s) of heparin-driven protein aggregation not only related to amyloid formation in secretory granules, but also amyloids associated with diseases.

Poster no: D-2-23**Dynamics of chickpea phosphorylome reveals shared and distinct proteins, and identifies CaDREPP1 that functions in stress adaptation**

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Introduction: Posttranslational modifications of proteins, particularly phosphorylation, control multivariate cellular processes but its functional connectivity are still elusive. To gain better understanding of the role of phosphorylation in stress response, we developed a differential phosphorylome in chickpea.

Methodology: A gradual water-deficit condition was imposed on 3-week-old seedlings of chickpea (*Cicer arietinum* L. cv. JG-62) and tissues were harvested on the basis of temporal changes in MAPK activity. The phosphorylome was characterized by classical 2-DEcoupled with LC-ESI-MS/MS analysis. Among the dehydration-responsive phosphoproteins (DRPPs), there was a plant-specific DREPP family protein, designated CaDREPP1 (Developmentally Regulated Plasma membrane Protein). The complete sequence of *CaDREPP1*, its genomic organization, and possible function/s in plant were investigated.

Results and Discussions: Proteomic analysis led to the identification of 91 DRPPs out of which 41 proteins were found to be downregulated, 34 were upregulated, while 16 displayed mixed patterns of expression. The identified DRPPs were classified into 11 major functional categories. SOTA analysis yielded 11 expression clusters. CaDREPP1 was found to be upregulated during the course of dehydration with maximum accumulation at 24 hours. *CaDREPP1* encoding a protein with

molecular weight of 22.8kDa and pI4.8 was cloned. Confocal microscopy showed the localization of CaDREPP1 protein in the plasma membrane. The transcripts were induced by both abiotic and biotic stresses suggesting its key role in stress adaptation.

Conclusion: The stress-responsive phosphorylome identified a number of components that are associated with signal transduction network. The results suggest that an orchestrated modulation of phosphorylome may play a significant role in stress adaptation, although the exact contribution remains to be investigated.

Poster no: D-2-24

Regulation of Glucose Uptake in Mammalian Cells by AGEs

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Introduction: The epidemics of type two diabetes characterized by insulin resistance is affecting world at an alarming rate. Primary manifestation of diabetes is hyperglycemia, which leads to non-enzymatic glycation of proteins to form Advanced Glycation End products (AGEs). But even well before occurrence of insulin resistance factors of modern life style like an unbalanced diet rich in carbohydrates and saturated fats, obesity and lack of physical activity lead to formation of AGEs. These AGEs can modify proteome and further damage cellular structures via a number of mechanisms, including the formation of cross-links between key molecules in the basement membrane of the extracellular matrix (ECM) and the interaction of AGEs with RAGEs on cell surfaces, thus altering cellular functions. So it becomes important to test whether AGEs promote insulin resistance.

Methodology: This study is carried out in insulin sensitive cells such as muscle cells (L6) and Cho-GLUT4-GFP cells. Effect of Methylglyoxal (MG) is evaluated on cell viability, apoptosis, ROS, glucose uptake and glut4 translocation and glycogen synthesis. Immunofluorescence staining is done for glut1 and glut4 expression. LC MS analysis of total cell lysate and insulin receptor pull down was done to study differential protein expression and AGE modifications of proteins induced by MG.

Results and Discussions: In this study, we have used methylglyoxal, highly reactive dicarbonyl AGE precursor and evaluated its effect on glucose uptake and GLUT4 translocation. Methylglyoxal induces differential protein expression and glycation modifications

Conclusions: This study reveals effect of AGEs on glucose uptake and will give better insights to molecular mechanism leading to insulin resistance

Poster no: D-2-25

Enhancing functional repertoire of Eukaryotic RNA Polymerase

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Introduction: Eukaryotic transcription by RNA polymerase II (Pol II) is accomplished by a fine-tuned interplay with chromatin remodeling complexes, transcription factors and RNA processing

factors. The regulatory platform for the coupling of these nuclear events of RNA biogenesis and RNA maturation is thought to be the carboxy terminal domain (CTD) of the largest subunit of Pol II. The CTD is a highly conserved unusual domain consisting of a repeating heptapeptide consensus sequence Tyr₁Ser₂Pro₃Thr₄Ser₅Pro₆Ser₇ with potential epigenetic modifications (phosphorylations) sites. The changes in CTD phosphorylation observed in response to cell cycle regulation events and to extracellular stimuli raise the intriguing possibility that the Pol II CTD serves to integrate multiple signaling pathways and associate with proteins that globally affect gene regulation.

Methodology :(I) Genetic manipulation of CTD (incorporation of tag and protease sites): A PCR based homologous recombination is used for the incorporation of His-Biotin-His and Tev site at the C-terminus and N-terminus of CTD in the *Saccharomyces cerevisiae*.

(II) Identification of the broader range of transcription regulatory proteins associated with CTD: The cells will be grown in various synchronized condition and crosslinked using formaldehyde *in vivo*. The isolation and purification of uncrosslinked and crosslinked CTD samples will be carried out and the sample/s will be run on 2D electrophoresis followed by the MS/MS analysis to identify the associated proteins.

Results: We have incorporated a His-biotin-his tag at the C-terminus and a Tev protease site at the N-terminus of CTD heptads by PCR based homologous recombination. The observed growth of the wild type and the modified strains are almost similar. We are optimizing the purification of proteins in budding yeast as well as in animal cells.

Discussion: Here, we propose to implement a strategy to functionally characterize the epigenetic modifications of CTD which would establish the mechanistic link between transcription and post transcription events which are seamlessly integrated.

Conclusion: Regulation of transcription is one of the most important steps that control cell growth and differentiation. Understanding the extent of gene regulation at the epigenetic level would help address and control the abnormal cellular behavior in various diseases and will eventually contribute towards more targeted treatment strategies.

Poster no: D-2-26

Compartment specific PTM study of endogenous PKC's using mass spectrometry in human breast cancer cell line

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Introduction: Protein kinase C's are distributed in different compartment and found at the nexus of the many signaling pathways. PKCs regulate many vital pathways required for cell survival, proliferation and metabolism. Any aberrant alteration in the PKC structure/sequence/chemical modification leads to manifestation of many diseases ranging from inflammation to cancer. Recently, the compartment specific functions of these PKC isoforms are attributed to the post-translational modifications (PTM) at specific sites. PTM plays an important role in regulating their activation and translocation in different compartments of the cell. Most of the study of PKC PTM is done in non-endogenous conditions. Here we are trying to isolate endogenous PKC using membrane preparation and cell surface labeling methods. Mass spectrometry has evolved dramatically, and is now considered a key technology for site mapping, quantification of chemical modifications (PTM) and even for detecting protein-protein interactions. The right combination of MS method with maximum enrichment is crucial for mapping of proteins to study their PTMs in specific cell compartment.

Methodology: Cell surface labeling helps to separate the cell surface protein by labeling the surface protein with biotin nonspecifically followed by affinity purification using a neutravidin column. Membrane preparation will enrich for membrane proteins including membrane associated protein by using ultracentrifugation. The combination of these two methods will isolate and enrich form membrane associated proteins by enriching for the cell surface proteins first by labeling, followed by membrane protein partition by ultracentrifugation. MS will then be used to analyze the PTMs in membrane associated and cytoplasmic PKCs.

Result and Discussion: The investigations for developing a protocol that combines cell surface labeling and membrane protein preparation will be presented with preliminary PTM analysis by MS.

Conclusion: Methods that can isolate endogenous PKC activities and PTMs to different cell compartments will allow further understanding of context specific PKC signaling in the regulation of cell processes.

Poster no: D-2-27

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

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Introduction: Tuberculosis is one of the leading causes of morbidity and mortality worldwide. H37Ra and H37Rv are two closely related strains of *Mycobacterium tuberculosis*, which are avirulent and virulent, respectively. The two strains show genetic differences in terms of single nucleotide variations, insertions and deletions. However, a comparative proteomic analysis of virulent strain versus avirulent strain will provide an insight into altered biological networks responsible for virulence in *Mycobacterium tuberculosis*.

Methodology: In this study, LC-MS/MS analysis of H37Ra and H37Rv strains was carried out on a Fourier transform mass spectrometer. The two strains were grown in Middlebrook 7H9 media with ADC supplement to log phase. We used *in vitro* labeling by tandem mass tags (TMT) for quantitative proteomic analysis of H37Ra and H37Rv to identify proteomic differences between the two strains.

Results and Discussion: We identified 3,220 proteins, of which 31 were differentially expressed in H37Ra with respect to H37Rv. EspA secretion-associated protein (Rv3616c), EspC secretion-associated protein (Rv3615c) and Pilin (Rv3312A) which are involved in virulence and pathogenesis were found to be downregulated in H37Ra. Our study also revealed downregulation of Rv3824c in H37Ra which could be due to mutation in PhoP. We also identified Peptide synthetase Nrp (Rv0101) which in H37Ra has an N-terminus mutation where tryptophan is replaced by stop codon TAG. This results in 2,420 amino acid truncation and affects the synthesis of phthiocerol dimycocerosate (PDIM) rendering the bacilli attenuated. While

this proteome comparison validates the genomic and phenotypic differences known between H37Rv and H37Ra, it also provides novel pathways that could be involved in pathogenesis.

Conclusions: Proteomic differences between the virulent and avirulent strains of *Mycobacterium tuberculosis* would provide a better insight to its virulence and pathogenesis.

Poster no: D-2-28

A Plant Glycoside Hydrolase to Be Used In the Post-translational Modification of O-GlcNAc Modified Proteins for Therapeutic Purpose of Glycosylation Disorders

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Introduction: O-linked beta N-acetyl glucosamine (O-GlcNAC) is both an abundant and dynamic post translational modification similar to phosphorylation that occurs on serine and threonine residues and its anomaly is implicated to disease conditions.

Methodology and results: Our research envisages the isolation of a novel plant protein to be classified as a glycoside hydrolase GH84 family and shows striking properties of the pancreatic beta cell human O-GlcNAcase. The plant enzyme specifically cleaves beta 1,4 O-linked GlcNAc specific sugar cores, has an optimum pH 5.7 – 7.0 and retains significant activity at pH 7-8 like the O-GlcNAcase. Furthermore it displays Michaelis-Menten kinetics and has a Km of 1.6mM. This is in good agreement with the studies that have measured the kinetics of enriched fractions of O-GlcNAcase. In contrast to the lysosomal hexosaminidases, it has neutral pH optima and is heat labile.

Discussion: Interestingly this plant protein migrates at an apparent molecular weight of 85 KDa, very much similar to the 75 KDa splice variant nuclear protein O-GlcNAcase. The isolated protein exhibits both O-GlcNAcase activity (cleaves beta 1, 4 GlcNAc) as well as serine phosphate activity (O-PO4). This is in line with the enzyme calcineurin in the pancreatic beta cell O-GlcNAcase containing complex exhibiting phosphate activity. Therefore this enzyme may find promising role in the participation of signal transduction pathway in human pancreatic beta cell, where inhibition of O-GlcNAcase activity has been implicated in type II diabetes.

Conclusion: Presently there is no enzyme which can cleave beta 1, 4 linked GlcNAc from ser/thr residue except the pancreatic beta cell O-GlcNAcase. Also cloned enzyme of O-GlcNAcase has not been up to the mark. Therefore this plant therapeutic protein with its unique characteristic can unravel new findings using latest technology in O-GlcNAc modification related disorders, more particularly diabetes.

Poster no: D-2-29**Unique and differential protein signatures for HIV-1 &HCV mono-infection versus co-infection as determined by multiplex iTRAQ quantitative proteomics**

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Pathogenesis of liver damage in patients with HIV and HCV co-infection is complex and multifactorial. Although global awareness regarding HIV-1/HCV co-infection is increasing little is known about the pathophysiology that mediates the rapid progression to hepatic disease in the co-infected individuals. In this study, we investigated the proteome profiles of peripheral blood mononuclear cells from HIV-1 mono-, HCV mono-, and HIV-1/HCV co-infected patients. The results of high-resolution 2D gel electrophoresis and PD quest software quantitative analysis revealed that several proteins were differentially expressed in HIV-1, HCV, and HIV-1/HCV co-infection. Liquid chromatography-mass spectrometry and Mascot database matching (LC-MS/MS analysis) successfully identified 29 unique and differentially expressed proteins. These included cytoskeletal proteins (tropomyosin, gelsolin, DYPLSL3, DYPLSL4 and profilin-1), chaperones and co-chaperones (HSP90-beta and stress-induced phosphoprotein), metabolic and pre-apoptotic proteins (guanosine triphosphate [GTP]-binding nuclear protein Ran, the detoxifying enzyme glutathione S-transferase (GST) and Rho GDP-dissociation inhibitor (Rho-GDI), proteins involved in cell prosurvival mechanism, and those involved in matrix synthesis (collagen binding protein 2 [CBP2]). The six most significant and relevant proteins were further validated in a group of mono- and co-infected patients ($n=20$) at the transcriptional levels. The specific pro and anti-apoptotic protein signatures revealed in this study could facilitate the understanding of apoptotic and protective immune-mediated mechanisms underlying HIV-1 and HCV co-infection and their implications on liver disease progression in co-infected patients.

Poster no: D-2-30**Investigations into the role of amphiphilicity in $\text{A}\beta_{16-22}$ self-assembly**

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Introduction: $\text{A}\beta_{16-22}$ constitutes the amyloidogenic core of the β -amyloid peptide. $\text{A}\beta_{16-22}$ (Ac-KLVFFAE-am) readily forms fibers having in-register, antiparallel β -sheets in aqueous solutions at neutral pH. At acidic pH (pH ~2), however, the peptide self-assembles with one-residue shift in the strand registry; assembly with such a shift in registry results in highly ordered nanotubes. At pH 2 the aspartate side chain becomes protonated imparting the peptide an amphiphilic character that favors out-of register assembly. We are studying the various parameters directing $\text{A}\beta_{16-22}$ self-assembly.

Methodology: $\text{A}\beta_{16-22}$ and its analogs are synthesized to study the role of amphiphilicity and electrostatic interactions in the $\text{A}\beta_{16-22}$ self-assembly. The peptides are synthesized using solid phase peptide synthesis employing Fmoc chemistry and characterized with HPLC and ESI-MS. Aggregation is studied in aqueous solutions at different pH as well as in the low dielectric solvents to probe the electrostatic interactions.

Results and Discussions: Replacement of lysine (a small charged group, NH_3^+) with arginine (a bulkier charged group, guanidinium group) confers higher amyloidogenicity to the peptide allowing its rapid assembly into straight, rod-like fibers. Solvents with very low dielectric slow down the assembly suggesting that electrostatic interactions alone can't efficiently drive the self-assembly. Methanol, a solvent with moderate dielectric constant, however, results in long fibers suggesting a balance between the hydrophobic and electrostatic interactions in $\text{A}\beta_{16-22}$ assembly. The superstructures obtained under various conditions differ considerably from each other in terms on their morphology and dimensions.

Conclusions: The change in the size of head group and charge distribution may change the electrostatic interactions which may alter the registry thereby affecting the self-assembly of $\text{A}\beta_{16-22}$ and its analogs. To investigate the factors determining the morphology of the self-assembled structures, we plan to investigate the registry of the peptides in the fibrils using H/D exchange. The H/D exchanged fibrils will be dissolved to obtain monomeric peptides and analyzed using MS with and without fragmentation. An out-of-register residue is expected to exchange its amide protons with deuterium more readily allowing their identification.

Poster no: D-2-31**Achieving the Highest Performance for Protein Identification and SILAC Relative Quantitation on the New Q Exactive Platform**

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Introduction: To understand biological systems, there is a need to not only explore in more depth the proteome, but to monitored protein dynamics more accurately. In this study, a Thermo Scientific Q Exactive hybrid quadrupole-Orbitrap mass spectrometer with a new high field orbitrap that allows faster scan speed (>1.5 times) and higher resolution (>1.5 times) was evaluated for proteomic applications and benchmarked against the current Q Exactive plus.

Methods: A tryptic yeast and Hela standard was used for peptide and protein identification comparison on the new and current Q Exactive platforms. A Thermo Scientific EASY-nLC 1000 UPLC system and EASY-Spray Source with 50cm Easy-Spray Column was used to separate peptides with a 30% acetonitrile over different gradient lengths at 300 nL/min. For a set of quantitative comparison between the instruments, A549 cells were labeled in light/medium/heavy SILAC medium and mixed to a ratio of 1:1:1 and 2:1:10 followed by digestion. The samples were analyzed using data dependent MSMS. Raw files were analyzed by Proteome Discoverer software.

Preliminary data: We observed an increase of 10 -20% peptide identifications in Hela lysate on the new QE, due to faster scan speed of the orbitrap. The peptide identification rate was over 1.5x higher than before. The MS/MS success rate for both instruments was approximately 50%. The increase in the HCD acquisition rate on the new QE did not compromise the quality of the MS/MS spectra.

While assessing the quantitative accuracy of the instruments, it was found that the main factor contributing to the quantitative inaccuracy in SILAC type sample is the background ion/impurity interference. However, with the higher resolution on the new orbitrap, we observed the improvement in both quantitative accuracy and precision.

Conclusion: The new orbitrap technology improves both the protein/peptide identification and the accuracy in the SILAC quantitation

Poster no: D-2-32

TMT10-Tails Workflow for Identification and Quantification of neo N-terminal Proteolitic Peptides

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Introduction: Proteolysis is an essential posttranslational modification significantly impacting the fate of a protein and those it interacts with. Consequently, proteases and their substrates are an increasingly important domain of study in proteomics and drug research. Detecting, validating and quantifying the sites and specificities of proteases, many of which are only vaguely categorized, presents a substantial challenge. Presented herein is a complete workflow for this analysis employing TMT10plex with accurate Synchronous Precursor Selection (SPS) MS³ quantitation and a new comprehensive pipeline, Proteome Discoverer 2.0, featuring the Byonic search engine.

Methodology: The extracted proteome of control and protease deficient B-cells were labeled with TMT10plex, followed by reduction/alkylation, digestion and N-termini enrichment. LC/MS data were collected on an Orbitrap Fusion using the SPS-MS³ method followed by ion trap CID. The top ten most abundant CID fragments were coisolated and subjected to MS3 HCD fragmentation followed by FTMS detection of reporter ions. Additional data was collected using ion trap Electron Transfer Dissociation employing charge dependent precursor selection logic. Data were searched using the Byonic node in Proteome Discoverer 2.0 to identify and quantify neo-N-Termini and determine protease candidates and cleavage site specificity.

Results: The high performance of the Orbitrap Fusion allowed for the identification of nearly 3000 protein and 18000 unique peptides before enrichment and 2500 and 5500 respectively following enrichment. In the enriched sample, over 2700 peptides acetylated at the N-terminus were identified. Significantly, 800 TMT10plex N-terminally labeled peptides were detected constituting very high confidence sites of protease activity. Overall, the combination of negative N-terminal peptide enrichment coupled with higher multiplexing TMT, high resolution SPS MS³, and an advanced data analysis pipeline enabled deeper global profiling of the N-termini proteome.

Conclusions: Highly multiplexed terminomics quantitation combined with MS3 quantitation can dramatically facilitate the monitoring of proteases and a better understanding of diseases.

Poster no: D-2-33

Effective use of novel data independent analysis methods to obtain comprehensive and reproducible characterization of a biological pathway

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Introduction: Data Independent Analysis (DIA) bridges protein identification and targeted quantitation by acting as a screening technique to rapidly and thoroughly survey a sample and direct future targeted analysis. In this work, we have built high performance methods on the Orbitrap Fusion and Q Exactive HF to take advantage of analyzer parallelization with WiSIM and precursor multiplexing with msxDIA and applied them to investigate drug resistance in cancer cells.

Methods: Treated and control non-small-cell-lung-cancer cells were lysate and trypsinized. An aliquot was used to build a protein/peptide library. And the rest was analyzed using DIA. Orbitrap Fusion and Q Exactive HF mass spectrometers were used. A Thermo Scientific EASY-nLC 1000 UPLC system and EASY-Spray Source with 50cm Easy-Spray Column was used to perform the LCMS analysis. Raw files were analyzed by Proteome Discoverer, Pinpoint and Skyline software.

Results: A high quality library consisting in over 3000 proteins was built. Special emphasis was put in deciphering the effect of drug resistant in MAPK pathway. It is well known that a mutated version of BRAF plays a critical role in certain subtypes of cancers. This has led to the development of a family of drugs that target this isoform. However, those drugs have not improved the mortality rates due to drug resistance. Developing drugs that target other members of the MAPK pathway is critical. And therapies that combine these new drugs in conjunction with BRAF inhibitors could counteract the mechanisms of resistance. Using both WiSIM and precursor multiplexing with msxDIA we were able to reveal important expression changes in the pathway. Additionally, these methods allow going back in time retrospectively interrogate data for new target peptides and proteins.

Conclusion: The new orbitrap technology enhances the sensitivity of DIA allowing its use as a satisfactory alternative to targeted methods.

Poster no: D-2-34

PknI, a serine/threonine protein kinase interacts with Rv2159c of *Mycobacterium tuberculosis*

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Introduction: Tuberculosis, a deadly disease caused by *Mycobacterium tuberculosis*, infects one-third of the world's population. Reversible phosphorylation of proteins mediated by protein kinases and phosphatases play a cardinal role in regulating cellular physiology in response to extracellular cues. *M. tuberculosis* genome encodes 11 eukaryote-like Serine/Threonine protein kinases (STPK) and their physiological roles are being investigated. Diverse essential molecular processes inside the cell are carried out by protein-protein interactions. In this study, we have used protein-protein interaction to identify the substrates of PknI and to understand their role in cell division.

Methodology: The search for proteins interacting with PknI was performed using an *in vitro* assay, Far-western blot. The proteins that interacted with PknI were identified using Mass spectrometry (MALDI-TOF/MS). One of the interacting partners thus identified was cloned, overexpressed and confirmed using GST Pull down assay. *In silico* analysis using molecular modelling, molecular dynamics and docking were performed to identify the amino acids that are involved in the interaction.

Results & Discussion: The interacting proteins for PknI were identified as Rv2159c and Rv0148. The PknI-Rv2159c interaction pair was further studied for the critical amino acid residues responsible for the interaction. Rv2159c, a member from Carboxymuconolactone decarboxylase (CMD) family, predicted to be an antioxidant with alkylhydroperoxidase activity, appears to contain two cysteine residues Cys-X-X-Cys, responsible for peroxidase activity. Rv2159c was shown to be selectively induced in virulent strains and repressed in attenuated strains of *M. tuberculosis*. It is hypothesized that, interaction with Rv2159c is important for PknI to maintain the cell homeostasis.

Conclusion: Our studies indicated that, PknI interacts with Rv2159c, a peroxidase protein of *M. tuberculosis*. Thus by protein interaction, a newer protein namely Rv2159c has been identified as an interacting partner of PknI.

Poster no: D-2-35

Attributes of linear peptides mediating interactions with Proteins, Antibodies and MHC complexes

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Introduction: Linear stretches of protein sequences have been shown to be important in mediating interactions with proteins, antibodies and MHC class I & class II complexes. Here, we made an attempt to dissect the physico-chemical properties of different classes of peptides mediating the above-mentioned interactions and their role in modulating the biological function.

Methodology: Eleven physico-chemical properties of 8000 linear peptide sequences comprising of 2000 each from 4 classes: (i) Linear motifs (LMs) mediating protein-protein interactions (PPIs), (ii) Continuous B-cell epitopes and (iii) & (iv) T-cell epitopes belonging to MHC class I & II, respectively, were computed. These linear peptides were clustered using Hierarchical Clustering based on the physico-chemical properties of their amino acid residues. Significant differences in the physico-chemical composition of the four different classes of linear peptides were identified using One-way ANNOVA. Furthermore, the average values of the percentage compositions of these four classes of peptides were compared with those of the peptides from non-immunogenic food proteins and disordered regions of proteins.

Results and Discussions: The physico-chemical composition of different classes of linear peptides were segregated into 9 separate clusters, and within these clusters each class of peptide were grouped together, thus forming 4 different sub clusters. Percentage composition of polar and tiny amino acids was found to be significantly different across the four classes of peptides. Composition of non-immunogenic food peptides indicated a marked difference from the immunogenic B-cell and T-cell epitopes but similarity to the non-immunogenic LMs. In addition, composition of disordered peptides also showed similarity with LMs but were distinct from B-cell epitopes generally derived from ordered regions of proteins.

Conclusions: Overall, the study inferred significant difference in the physico-chemical composition of peptide sequences having different physiological functions and showed that the LMs

mediating PPIs were more closely associated with peptides from disordered regions of proteins, as compared to B-cell epitopes.

Poster no: D-2-36

Structural basis for K⁺ dependent hydrolysis and domain crosstalk in a highly conserved GTPase Era

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Introduction: Era is an essential GTPase that participates in the 30S ribosome assembly. Era possesses a GTP binding domain and a KH domain that binds RNA. Unlike classical GTPases like Ras wherein an arginine residue supplied in Trans by a GTPase activator protein (GAP) to promote GTP hydrolysis, Era employs K⁺ ions as activator element to hydrolyze GTP. It is noted that RNA binding at the KH domain promotes GTP hydrolysis at the GTP binding domain and *vice versa*. The structural basis for K⁺ dependent hydrolysis, the RNA dependent GTPase stimulation and their importance in assembly of the 30S subunit remains unaddressed.

Methodology: Using sequence and structural analysis a series of residues involved in K⁺ dependent hydrolysis have been identified in *Aquifex aeolicus* Era. A graph theory based approach has been employed to identify the residues involved in the cross talk between the KH and GTPase domain. The identified residues are probed for their importance using site directed mutagenesis followed by biochemical analysis.

Results: We identify that residues located in the G1 and switch-II region, when mutated, abrogates K⁺ dependent GTP hydrolysis and thus suggesting their involvement in catalysis. Deletion of T212 that is part of a residue-residue communication pathway between the KH and GTPase domains too reduces the GTPase activity.

Conclusion: The abrogation of GTPase activity on disrupting K⁺ and active water stabilizing residues establishes their importance in the hydrolysis reaction. Disruption of residue-residue interaction pathway also affects inherent GTPase activity suggesting towards a finer co-ordination between the KH and GTPase domains.

Poster no: D-2-37

In-silico structural and functional characterization of Inter alpha trypsin inhibitor heavy chain 4

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Introduction: Inter alpha trypsin inhibitor heavy chain (ITIH4) is an acute phase, anti-apoptotic and matrix stabilizing protein that is functional as a protein complex in association with Hyaluronic acid (HA). The protein complex includes two heavy chains and one light chain linked to each other through non-disulfide covalent linkage. Our previous study showed the increased expression of ITIH4 during the inflammation of Rheumatoid Arthritis. Analysing the ITIH4 would be crucial to understand the regulation and localisation of Serum derived Hyaluronic acid Associated Protein (SHAP) complex in acute phased inflamed conditions.

Methodology: Three dimensional (3D) model of ITIH4 was constructed by utilizing the protein sequence (UniProt ID: Q14624). Active sites and ligand binding sites were predicted through CASTp and 3DLigandSite respectively. Antigenic peptides of the protein that elicits an antibody response were

identified by antibody epitope prediction server. Physical and functional protein-protein interactions of this protein were revealed through STRING server.

Results: Model was constructed and validated through ramachandran plot indicating all the residues in the allowed region. Five potential active sites were identified in the structure. The STRING database revealed total 7 proteins as the partners of ITIH4. Ginzu server analysed three functional domains as domain A having transglutaminase activity suggesting the self catalysed loading of bikunin onto the domain B. Domain B and domain C respectively exhibits divalent cation dependent adhesion and ABC transporter like properties that are possibly involved in energy dependent translocation of the inhibition complex.

Conclusion: Findings indicate that inhibition of serine proteases by bikunin is regulated through the loading of bikunin to the heavy chain complex and the ATP dependent translocation of functional inhibition complex in ECM.

Poster no: D-2-38

Does phosphorylated sites on the disordered interface signatures the interacting behavior of proteins? A comparative mapping of phosphorylation propensities on disordered interfaces of interactome and negatome

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Introduction: Protein-Protein interaction (PPI) forms the core of interactomics system and unsurprisingly, aberrant PPIs are the basis of diseases, such as Alzheimer's and cancers. Studies showed that hub proteins in interaction networks in signaling pathways have more disordered residues than non-hubs and since the signaling mechanisms involving PPI are regulated by phosphorylation, disordered interfaces could be thought to be extremely phosphorylated. In the present study we sought to map the phosphorylation sites onto disordered regions on interacting proteins-Interactomes and non-interacting proteins-Negatomes.

Methodology: Dataset of non-interacting protein included 784 proteins retrieved from Negatome database 2.0. 2252 interacting proteins were retrieved from “GeneMania” – a database for protein interaction networks. Intrinsically disordered regions were predicted with “Disopred” program. The binding interfaces were defined by “PDBePISA” server, while, phosphorylation sites were derived from “NetPhos” program. All phosphorylation sites were mapped onto protein structures using alignments calculated by the MUSCLE program.

Results and Discussion: As anticipated, the extent of phosphorylation in interactomes were significantly higher in disordered regions to its ordered counter parts ($p=0.014$). Insights revealed that the disordered regions in negatome were sparse in comparison to those in interactomes ($p=0.0024$). Sparse Phosphorylated sites were observed in negatomes, however these sites did not have significant distribution in ordered or disordered regions ($p=0.884$). Since, Gly-X-Gly tripeptides confers flexibility to the protein backbone, number of these tripeptides were higher in disordered regions than ordered regions ($p<0.0001$), Gly-X-Gly tripeptides were present in negatomes but comparatively less in number relative to interactomes ($p<0.0001$)

Conclusion: The widespread non-flexible and ordered regions in the negatomes confer the non interacting nature of the protein

in turn makes it poor participant in signal transduction that involves phosphorylation. Our study sheds light on the importance of phosphorylated sites on disordered regions as a mark to decide whether protein would possibly interact or not.

Poster no: D-2-39

Accurate assembly and annotation of genomes by integrating transcriptomic and proteomic data

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Introduction: Whole genome sequencing efforts of new organism aims to provide accurate assembly and annotation of protein-coding genes in the genome. It involves a multistep process where genome sequencing and assembly are often disconnected from systematic annotation of genes. Gene prediction programs aided by transcriptome data and manual curation are being used to annotate genomes. Surprisingly, proteome data is almost always missing from these annotation efforts although the technologies are now sufficiently evolved to complement these efforts. We demonstrate here, how complementing genome sequencing with transcriptome and proteome data would provide near complete assembly and annotation of newly sequenced genomes.

Methodology: We analyzed transcriptome and proteome of multiple organs of *Anopheles stephensi* to achieve broad coverage of all protein-coding genes in this important malaria vector in India. We identified several novel protein-coding regions in *Anopheles stephensi* by following proteogenomic methodologies, which resulted in the correction of errors in both assembly and gene annotation in this vector. Using several custom designed bioinformatics approaches, we compared this novel dataset onto 15 newly sequenced Anopheline genomes and rectified thousands of errors in these genomes.

Results and Discussions: By carrying out comprehensive proteome profiling, we presented protein coding evidence for 82% of all the genes annotated in *Anopheles stephensi* genome. Transcriptome and proteome data together provided evidence for more than 400 novel genes in the genome that are missing in current genome annotation. This dataset also resulted in revision of 955 gene structures that were annotated using prediction programs. We extended this analysis to 15 recently sequenced Anopheline genomes and discovered 6,000 of such assembly and annotation errors across these genomes and corrected them using transcriptomic and proteomic data.

Conclusions: Integrated transcriptomic and proteomic data could be effectively used to improve both assembly and annotation of genomes.

Poster no: D-2-40**Eukaryotic genome re-annotation using multi-omics data**

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Introduction: Genome annotation is a continuous yet challenging process. It forms the basis for the experimental studies involving genes, transcripts or proteins from single to system wide scale. *Rattus norvegicus* (Norway Rat) is a model organism to study various human diseases. Despite its application, rat genome annotation lags behind similar efforts in mouse and human. As reported, splicing patterns in multiple tissues are species specific. Thus mouse annotations should not be used as surrogates for rat genome annotation. Experimental detection of transcripts and proteins can be used to annotate genes and should be used complementary to *in silico* predictions.

Methodology: In this work we utilized publically available high throughput RNA-seq transcriptomic and mass spec proteomic data to re-annotate rat genome. We developed a multi-algorithmic analysis pipeline EuGenoSuite to analyze large scale proteomic data against a transcriptome database. A rat brain microglia proteomic dataset was searched against the transcript database created from RNA-seq reads from rat brain.

Results and Discussion: A total of 276 unique mapping novel peptides were discovered. Intergenic novel peptides were mostly un-annotated part of genes as inferred by the transcript observation and gene conservation. Peptides from non-coding loci highlighted translation of eight annotated pseudogenes. We also detected peptides from annotated non-coding exons in various important genes e.g. mcm5: a minichromosome maintenance complex protein.

Conclusion: In summary, our analyses improved the current knowledge of annotated protein coding regions and transcripts from rat genome. Similar proteogenomic re-annotation efforts should enable a better reference for human disease studies in this model organism.

Poster no: D-2-41**Interaction proteomics reveals brain region-specific AMPA receptor sub-complexes**

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Introduction: AMPA receptor is one of the most well studied excitatory ionotropic glutamate receptor present in the brain and is involved in fast synaptic transmission. Its regulation and surface expression is widely believed to be the molecular basis of learning and memory and several neuropsychiatric disorders. Recent studies have revealed a substantial number of AMPA receptor auxiliary proteins, which potentially contribute to the regulation of AMPA receptor trafficking, subcellular receptor localization and receptor gating properties. However, A multitude of AMPA receptor interacting proteins have been identified for which the specific interaction rules are largely unknown.

Methodology: We examined the AMPA receptor interactomes from cortex, hippocampus and cerebellum by comprehensive multidimensional Immunoprecipitation based proteomics strategy to tease out brain region specific complexes of the AMPAR.

Results and Discussions: AMPA receptor interacting proteins are part of overlapping and distinct sub-complexes in cortex, hippocampus and cerebellum and can be part of non-AMPA receptor protein assemblies. We observe novel distinct non-overlapping complexes in different brain regions, which are not necessarily correlated with expression differences.

Conclusions: The brain region-specific differences in AMPA receptor complexes suggest distinct regulatory mechanisms of AMPA receptor properties in different brain regions. AMPA receptor function is likely far more complexly regulated than currently anticipated and a distinct role of these interacting proteins in different brain regions needs to be carefully examined.

Poster no: D-2-42**Discovery of Interacting partners of gankyrin, an oncogenic non-ATPase subunit of the proteasome-Evidence for common hotspot sites of interaction**

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Introduction: Protein-protein interactions create highly dynamic, spatio-temporal functional networks. Many oncogenes and tumor suppressors act as hubs in the functional network and are capable of rewiring the network leading to an altered phenotype. Since rewired networks are unique to cancer cells, these specific interaction sites become very important drug targets. Therefore, we aim to identify the physiological relevant direct interacting partners of gankyrin which is known to be overexpressed in several cancers. Based on the principles of sequence conservation and the role of key residues in dictating the binding affinity at protein interfaces, we predicted novel putative interacting partners of gankyrin.

Methodology: Predicted interactions were validated in gankyrin overexpressing HEK293 cells and other cancerous cell lines using immunoprecipitation. Gankyrin-CLIC1 interaction was studied in detail. Recombinant proteins and their corresponding single/triple mutants were purified and GST pull down assay, Isothermal titration calorimetry, ELISA, Surface plasma resonance experiments were performed to test the effect of predicted residues in interaction. Circular dichroism, tryptophan fluorescence studies were done to test the effect of structural changes upon mutations in CLIC1. Functional significance of the interaction was deduced using knockdown and rescue experiments.

Results and Discussion: Interactions of gankyrin with eight proteins were validated inside the cellular milieu. Interacting partners form the complex with gankyrin which is stabilized by interactions through linear short stretch of four amino acids (EEVD). Mutations in the tetrapeptide sequence abolish complex formation. Mutations in CLIC1 affect their secondary structure but not the tertiary structure. Hence, the sequence and secondary structural changes might regulate the binding affinity of gankyrin-CLIC1. Gankyrin interaction with CLIC1 through E, E and D results in EEVD enhanced migratory properties of MDA-MB-231 cells.

Conclusions: A tetrapeptide sequence is a hotspot site of interaction in the gankyrin network. Functionally important interaction present only in cancerous cells can be targeted by small molecules.

Poster no: D-2-43**Identification of the protein network involved in biogenesis of tunneling nanotubes (TNTs)****Pergu, R* and Mylavarapu, S[#]***Laboratory of Cellular Dynamics, Regional Centre for Biotechnology, 180, UdyogVihar Phase I, Gurgaon*

Introduction: Cell-to-cell communication plays an important role in physiological processes of multi-cellular organisms. A novel mechanism for intercellular communication named TunnelingNanoTubes (TNTs) recently discovered in several cell types is essential for several crucial functions in health and disease. However, the molecular mechanism(s) of TNT formation and function are not well understood. Recently, a protein MSec was reported essential for TNT formation. Here we used MSec as bait in a genome-wide proteomic approach to identify its proteininteractome to understand the molecular mechanism of TNT formation and/or function.

Methodology: MSec was cloned into a mammalian expression vector containing localization and affinity purification (LAP) tag. The tag contains a tandem affinity purification (TAP) tag of 6x Histidine, FLAG and Streptavidin Binding Protein (SBP). The LAP-tagged MSec construct was transfected into mammalian cells and stably expressing (MSec-LAP-tag) cells were generated. Cell lysates of stable cell lines were subjected to affinity purification and the eluent collected, trypsinized and analyzed by mass spectrometry for the identification of the MSec interactome.

Results and Discussion: We identified six interacting proteins of MSechthat consistently appeared in multiple experiments with significant scores. We are validating these interactions in terms of TNT formation and/or function by various biochemical, biophysical, and cell biological assays like, Western blotting, immunofluorescence colocalization by high-resolution optical microscopy, gel exclusion chromatography and si-RNA mediated knock down.

Conclusions: Our studies will elucidate the biochemical networks and mechanisms that drive TNT formation and/or function, and thus help illuminate their role in vital cellular processes.

Poster no: D-2-44**Screening of inhibitors for mushroom tyrosinase using Surface Plasmon Resonance****Jyoti Jadhav^{1*}, Sushama Patil¹**

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Introduction: Tyrosinase inhibitors have been used as whitening or antihyperpigment agents because of their ability to suppress dermal-melanin production. In this present study, screening and kinetic evaluation of different small molecules on mushroom tyrosinase was studied using surface plasmon resonance.

Material and method: Mushroom tyrosinase dissolved sodium acetate buffer was immobilized to a CM5 chip using amine coupling. Kinetics was performed with various concentrations of all compounds in a single cycle kinetics or multi-cycle kinetics mode.

Result and discussion: The binding constant K_D (M) values obtained for tannic acid, phloroglucinol, saffron, catechol and pyrogallol are 6.716×10^{-7} , 7.835×10^{-8} , 4.316×10^{-10} , 1.553×10^{-8} and 5.638×10^{-13} M respectively. Pyrogallol has been found to

display high affinity for mushroom tyrosinase whereas catechol, saffron and phloroglucinol have been found to bind with low affinity. The study reveals the Biacore/SPR sensor's ability for screening and detection of inhibitors for mushroom tyrosinase. Native and incubated mushroom tyrosinase with above inhibitors was also investigated from structural point of view by circular dichroism (CD) spectroscopy.

Conclusion: Analysis (or Methodology) described here can be used to rapidly screen and optimize various leads compounds for other enzymes and elucidate structure function inter-relationships between various enzymes.

Poster no: D-2-45**Piwi-Interacting RNAs: New Players of Cardiac Hypertrophy****S. Ramasamy, K. Shanmuga Rajan, G. Velmurugan**

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The cardiac hypertrophy is as an adaptive strategy in response to stress. Pathological cardiac hypertrophy (PHy) induced during onset of diseases like hypertension is irreversible and leads to heart failure while, physiological hypertrophy (PhHy) induced during chronic exercise is reversible and not associated with cardiomyocyte death. PhHy was considered as a natural tactic to prevent cardiac dysfunction and failure. In recent years, the role of miRNAs in cardiomyocytes is well established but even the expression of piwi-interacting RNAs (piRNAs) in cardiac tissue is not largely explored. We report the expression of piRNAs in the cardiac system in both *in vivo* and *in vitro* model system. The expression of piRNAs in *in vivo* -- H9c2 cardiomyoblasts indicates the presence of innate piRNA synthesis machinery in cardiomyocytes. Annotation of reads to different repetitive DNA sequences revealed large representation of retrotransposons (RT), which are post transcriptionally silenced by piRNAs. The piRNAs and predicted piRNA-like molecules exhibited significant upregulation during cardiac hypertrophy induction in rats by chronic swimming and in H9c2 cells by alpha-2 macroglobulin treatment. These piRNAs and piRNA-like molecules targeted a wide range of RT in the rat genome.

The present study is the first to show the simultaneous abundant expression of RT and piRNAs and its differential expression during cardiac hypertrophy. This suggests the foreseeable role of RT and piRNAs in cardiac physiology and pathology. Therefore, elucidating the role of piRNAs in combination with RT will pave a new path to understanding of the molecular etiology and management of cardiovascular diseases.

Poster no: D-2-46**MASS-1: A New Sensitive &High-Throughput SPR Platform for Label-Free Characterization of Biomolecular Interactions****Vishal Kamat***

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Introduction: Recent advancements in the field of biopharmaceuticals have led to an increased demand for the development of sensitive instruments to characterize therapeutic drugs. In the last decade, a number of different instruments able to measure real-time, label-free protein-protein interactions with high throughput capability have entered the marketplace.

Recently, Sierra Sensors launched a surface plasmon resonance (SPR) based high-throughput, real-time, label-free analysis system called the Molecular Affinity Screening System (MASS-1). The results of assays performed to evaluate the utility of MASS-1 in Regeneron's drug discovery workflow will be presented.

Methodology: Performance of MASS-1 instrument was tested by characterizing a large panel of antibodies binding to different molecular weight antigens under diverse assay formats. Binding sensitivity and reliability of the instrument was assessed using proteins and peptide binding to different density antibody surfaces. In order to evaluate the throughput capability and reliability of the data generated on MASS-1 instrument, comparability study comprising of approximately 150 monoclonal antibodies was performed and kinetics of antibody binding were compared with those measured on a BIACore platform.

Results and Discussions: No significant difference in binding kinetics parameters was observed when same antibody was captured across different channels of MASS-1. Binding kinetics parameters measured using the high-throughput single injection kinetics assay was comparable to the parameters calculated using standard multi-cycle binding kinetics assay. Small molecular weight peptide binding studies demonstrated that MASS-1 is a very sensitive instrument with a high signal to noise ratio. Moreover, the quality of the observed data was not affected by the background noise of the instrument. Finally, comparability study revealed that the kinetic parameter values of antibody binding to antigen measured on MASS-1 were comparable to those generated using BIACore.

Conclusion: The data presented in this study suggest that MASS-1 is a robust, sensitive and high-throughput instrument and the binding kinetics parameters measured using MASS-1 are comparable to BIACore.

Poster no: D-2-47

Homology Modeling and Docking Studies of Human Chitotriosidase with Its Natural Inhibitors

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Introduction: A number of chitinase or chitinase-like proteins (C/CLP) are ubiquitously expressed in the air and intestinal tracts from insects to mammals. In general, chitinases provide protection to the host against exogenous chitin-containing pathogens. Recent studies show that C/CLP has a role in the development and progression of allergic inflammation and tissue remodeling. In humans, two types of chitinolytic enzymes (chitotriosidases (CHT1) and Acidic Mammalian Chitinase (AMCase)) have been detected. Consequently, chitinase inhibitors were suggested to have anti-inflammatory potential against asthma and allergic diseases, including atopic dermatitis and allergic rhinitis.

Methodology: In this study various naturally occurring chitinase inhibitors of microbial origin were studied with the help of protein-ligand docking against human chitinase (chitotriosidases). The structure of CHT1 was modelled by homology modelling technique with MODELLER 9.1.2 and validated with the help of various tools from SAVES server. Following validation, secondary structure, active sites and solvent accessibility of the protein was analysed with various computational tools. Molecular Dynamic (MD) simulation studies were conducted by GROMACS simulation package to study the stability of the structure. This was further followed by docking studies with natural inhibitors like allosamidine, argifin and argadin against CHT1 by MOLEGRO docking software.

Result and Discussion: The inhibitor giving the best docking results was used for further study.

Poster no: D-2-48

Proteogenomics Toolkit: A workbench for integrated proteomic, transcriptomic and genomic data analysis

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Introduction: Advances in the sequencing technology led to the enormous deposition of genomic sequences of several organisms in the public repositories. The protein coding regions of these genomes were predicted using automated gene prediction algorithms. Mass spectrometry based proteomic data are being used since a decade to provide experimental evidence and to improve these annotations for several organisms. Additionally, next generation sequencing of disease related genomes is revolutionizing to identify clinically relevant variants such as single nucleotide variants, RNA-edits, alternatively spliced forms etc. Recently proteomic evidence of these variants gained interest among the community to elucidate the functional importance at the protein level.

Methodology: The toolkit was developed using Python programming language. The individual scripts were written as an interactive tool to provide various optional parameters. These programs run on Linux as well as Windows environment.

Results and Discussion: We developed a sophisticated toolkit to analyze proteomic data in combination with the genomic and transcriptomic data to improve the annotations of protein coding regions of any organism of interest. The functionality of the toolkit includes, to create custom made databases to search against mass spectrometer data, confirming existing annotations of protein coding regions, correction of gene and exon boundaries, confirmation and correction of frame of translation, identifying novel genes, novel splice forms and providing protein evidence for the variants derived from next generation sequencing technologies.

Conclusions: This toolkit would play an important role in the annotation of protein coding regions of several organisms. It would serve as a template for providing protein level evidence for variants derived from disease genome analysis.

Poster no: D-2-49

Human proteome map portal as a resource of high-quality mass-spectrometry derived data to study the expression of proteins across human tissues

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Introduction: Human genome sequencing has made tremendous impact on the study of human system and disorders.

While genome sequence is essentially same across all cell types, studying the whole transcriptome and proteome explain their specific roles. Though whole transcriptome analysis of different cell types gives a clue on set of genes being expressed there exists no direct proof of translation under the prevailing dynamics of post-transcriptional and translational regulation. Though the community has been talking about creating a comprehensive draft of human proteome since a while, "A draft map of the human proteome" (Kim *et al.*, 2014) is one of the first independent initiative taken up by our group.

Methodology: An in-depth proteomic analysis of 17 adult tissues, 7 fetal tissues and 6 primary hematopoietic cells has resulted in identification of proteins encoded by 17,294 genes, which accounts for 84% of total annotated protein-coding genes in human. The resulting data from our study is converted into relational table structure and made available to the community through an interactive web resource. Human Proteome Map (HPM) portal is developed using the 3-tier web architecture using MySQL for persistence, PHP, HTML and AJAX for dynamic visualization.

Results and Discussions: Human Proteome Map (HPM) portal which is available at <http://www.humanproteomemap.org> enables the user to study the relative expression of genes visualized as heatmap across human tissues and cell types studied. An intuitive molecule page lists the known isoforms of a gene with respective coverage by mass-spectrometry derived peptides both in a graphical and formatted text. Peptide and spectral level information is organized to facilitate pick the right peptide candidate for MS based targeted proteomics (MRM) using the relative identification across human tissues and best observed transition state(s) of product ions.

Conclusions: HPM will stand as the primary platform with rich visualization capabilities of representing protein and peptide level information in carrying out future human proteomics experiments.

Poster no: D-2-50

Multilevel qualitative LC"MS metabolomic approach to study the urinary responses of *Tinospora cordifolia* treatment in hyperlipidemic patients

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Introduction: *Tinospora cordifolia* (willd.) miers juice (TCJ) is known to contain number of metabolites with antihyperlipidemic properties but its mechanism is not known well. A multicompartmental nontargeted LC"MS metabolomics approach was used to study the metabolic responses in urine of hyperlipidemic patients after treatment with aqueous extract of *Tinospora cordifolia* (Willd) Miers.

Methodology: 20 individuals underwent TCJ treatment (100ml decoction/individual/day), of these 8 were hyperlipidemic. Urine samples obtained from healthy, hyperlipidemic and post TCJ treated hyperlipidemic individuals were processed and analyzed in a standardized UPLC-QTOFMS instrument. To study the metabolic responses, a supervised multivariate data analyses, involving PCA and PLS-DA statistical models were performed for pattern recognition. The method was validated by analyzing several known and unknown, abundant and minute metabolites in human urine samples from normal, hyperlipidemic patients and the hyperlipidemic patients treated with TCJ.

Results and Discussion: PCA and PLS-DA which revealed marked effects of the drug on urine metabolic profiles. The effect of treatment with *Tinospora* aqueous extract was observed across several metabolic pathways. These include cholesterol metabolism, nucleotide synthesis, heme synthesis, steroids synthesis, neurotransmitter synthesis and generation of antioxidative responses. It has positive effect on Dopamine, Adrenaline and DOPEG neurotransmitters synthesis (FC>2.0). It boosts heme synthesis by upregulating the heme precursor Protoporphyrin IX (FC>2.0). It upregulates Progesterone, Testosterone and DHEA hormones and has lipidostatic effect on cholesterol and triglyceraldehyde. Uric acid and histamine were found down-regulated. The neuroprotectant CDP-choline and inosine monophosphate nucleotide (IMP) synthesis were upregulated as a result of TCJ treatment (FC>2.0). Sulfatides a type of sulfolipid and Sphinganine were other metabolites whose levels were elevated after treatment in hyperlipidemic individuals.

Conclusion: The LC ESI-QTOF MS/MS method in positive as well as Negative mode of ion polarity helps to study the urinary non-targeted qualitative metabolic responses of TCJ treatment in hyperlipidemic patients without sample derivatization. Several unknown metabolites obtained in this study would assist in finding newer urinary biomarkers of hyperlipidemia.

Poster no: D-2-51

LC-MS-based serum metabolomic analysis reveals dysregulation of phosphatidylcholines in esophageal squamous cell carcinoma

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Introduction: Metabolic perturbations are widely understood to be critical in oncogenesis. Metabolomics, which provides a global view of cellular biochemical changes, is an appropriate tool for the discovery of diagnostic or therapeutic biomarkers in cancer biology. We employed an LC-MS-based metabolomic profiling approach to study biochemical changes in the serum of ESCC patients.

Methodology: Methanol-based metabolite extraction was carried out on serum samples from 40 ESCC patients and 10 healthy controls. Liquid chromatography quadrupole time-of-flight (LC-QTOF) mass spectrometry-based approach was employed to analyse each sample in triplicates. Pre-processing of raw data using a Molecular Feature Extractor (MFE) algorithm generated a unique list of molecular features. Mass Profiler Professional (Agilent Technologies) was used for filtering, statistical analysis and visualization of the data. Filtration criteria of log₁₀ transformed fold change ≥ 5 and p-value ≤ 0.01 yielded a list of 652 dysregulated features, including 100 phosphatidylcholines (PCs). Accurate mass library searches were employed for tentative identification of selected molecular features. Tandem mass spectrometry using collision induced dissociation was carried out in positive and negative modes for further characterization and confirmation of select metabolites.

Results and Discussion: Numerous metabolite classes, notably lipids such as phosphatidylcholines (PC), glycerophosphoethanolamines (PE), sphingomyelins and acyl carnitines were identified in this study. In concurrence with previous studies on ESCC, our study has identified a number of dysregulated PCs. Structural elucidations of certain key PCs required positive mode analysis to identify diagnostic ions such as m/z 184 and negative mode analysis to identify associated acyl chains (m/z 255, 253 and 281). Using this strategy, we have elucidated the structures for seven PCs, including PC (20:4/0:0), a lysophosphatidylcholine and PC (16:0/h 18:2), a PC with a hydroxylated fatty acyl chain. PC downregulation may lead to decrease in plasmalogens, a lipid class that protect against free radical attack and oxidative damage.

Conclusions: Our results provide novel insights into the dysregulation of PCs and associated lipid metabolism in ESCC as compared to healthy subjects.

Poster no: D-2-52

Identification of metabolic markers in CAD using an untargeted LC-MS based metabolomics approach

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Introduction: Coronary artery disease (CAD), a complex metabolic disorder is one of the largest causes of death worldwide. Herein we have used metabolomics as a tool to identify metabolites that could be used as potential markers for coronary artery disease.

Methodology: Blood samples from 18 angiographically proven CAD cases and 18 controls were analyzed in this study. 100 µl of plasma was precipitated using 200 µl of acetonitrile. The supernatant was lyophilized, redissolved and subsequently fractionated by reverse-phase and HILIC based methods followed by high resolution ESI-MS.

Results and Discussions: The MS/MS spectral data (.wiff files) were imported in Peak View (ABSciex) and quantitative analysis was performed by Marker View (ABSciex). In the reverse phase 160 peaks and in HILIC based LC-MS, 155 peaks and 175 peaks were found significant (p -value < 0.05 with 2 fold up and down regulation) in the negative and positive mode respectively. To identify the metabolites we used HMDB library and METLIN database with a tolerance of 30 ppm (mass) and \pm 2 minutes of retention time and validated each of them by matching the MS/MS fragmentation pattern. Using this approach we have identified 25 differentially regulated metabolites some of which have been previously reported to be associated with cardiovascular events (like Lyso PC, PE) We have also identified some metabolites that might be playing significant role in various processes that are associated with cardiovascular diseases.

Conclusions: Herein, we report that an unbiased metabolomics study have the potential to identify newer markers which are involved in several important biological pathways like beta oxidation, lipid metabolism etc. which in turn are implicated in CAD.

Poster no: D-2-53

Metabolomics Analysis of *Plasmodium vivax* and *Plasmodium falciparum* Induced Alterations in Humans to Decipher Pathogenesis

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Introduction: Investigation of parasite induced alterations in host metabolome can enhance our understanding of disease pathogenesis and host-parasite interactions in malaria. Hitherto there is no published literature describing the metabolomic alterations in humans in vivax malaria and its comparison with *P. falciparum* infection.

Methodology: In this study 8 independent biological replicates from control and malaria patients [Healthy controls (HC), non-severe vivax (NSVM), severe vivax (SVM), non-severe falciparum malaria (NSFM), and severe falciparum malaria (SFM)] were analyzed, and the ion intensity for each molecular ion was averaged across the replicates. Classification of severe and non-severe malaria was defined following the standard guidelines provided by WHO. Metabolite separation was performed on an Agilent HRLC system and Agilent MassHunter TM software was used for untargeted MS data acquisition. The list of compounds and their associated formulas was derived from the METLIN data base. Analysis was performed on the basis of molecular feature using the mass profiler professional (MPP) software.

Results and Discussion: Using LC-MS coupled with multivariate statistical data analysis approaches over 3000 serum metabolites were screened among which 145 and 157 were found to be differentially expressed (p < 0.05 and fold-change $>$ 2) in falciparum and vivax malaria, respectively. Among the altered metabolites, 52 were common in both the plasmodial infections. Quite a few metabolites like adrenaline, arachidonylserine, ginsenoside Rh2, which exhibited sequential altered levels in non-severe and severe malaria can be considered as potential candidates for monitoring disease progression. Identified metabolites were found to be associated with various vital physiological pathways including amino acid metabolism, glucose homeostasis, and antioxidant pathways.

Conclusions: The foremost intend of this study is to investigate disease pathogenesis of malaria, but it also proves to be applicable in the development of diagnostic surrogates for the disease; and in the discrimination of the two types of plasmodial infections.

Poster no: D-2-54

Lithium Response in Bipolar Disorder: New Insights from Serum Metabolomics

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Introduction: Bipolar disorder (BD), also known as manic-depressive illness, is a severe mood disorder affecting about 1% of the world population, characterized by alternating episodes of hyperactive mania and severe depression with several cognitive and behavioral abnormalities. Lithium is extensively

used as mood stabilizing drug and considered as primary drug for acute and maintenance treatment of BD. However BD population exhibits a differential response to lithium. In this study we intend to identify the key metabolites and metabolic pathways altered in BD and also make an effort to reveal metabolomic basis for differential response for lithium in BD patients.

Methodology: Patients attending Kasturba Hospital, Manipal were screened for BD, who were treated with lithium carbonate for at least two years and grouped as responders and non-responders following established criteria. The metabolites extracted using cold methanol was analyzed in LC-MS using Agilent 6520 Q-TOF. Data was extracted by Mass Hunter workstation, statistical analysis was performed in mass profiler professional and metabolites were identified using Metlin and HMDB databases. Significantly altered metabolites were validated by multiple reaction monitoring.

Results and Discussions: We identified 5094 features across 54 clinical samples (control n=18, responders n=18, non-responders n=18). Total of 942 compounds were identified as metabolites and we observed significant changes in 48 metabolites that were mapped to amino acid, lipid and nucleotide metabolism. PLSDA analysis resulted in 99.07% accuracy indicating accurate classification of the data. Amino acids such as Proline, Valine and Glutamate were significantly downregulated in bipolar disorder. Metabolite set enrichment analysis resulted in variations in fatty acid biosynthesis, sphingolipid and a-linoleic metabolic pathways in BD subjects. We observed changes in neurotransmitter levels between responders and non-responders.

Conclusions: Our study provides first evidence for metabolic differences in BD patients exhibiting differential response for lithium.

Poster no: D-2-55

Use of MALDI in studying metal binding with protein and protein dimerization

Atul Gajanan Thawari, and Chebrolu Pulla Rao*

Nanoparticle and nanocrystal of mercury belongs to new class of nano-materials. Understanding the structural aspect of these nanocomposites is central to understanding their properties. For the first time protein (α -Lactalbumin) bound mercury nanoparticles was prepared and characterized by the matrix-assisted laser desorption and ionization mass spectrometry (MALDI MS), ITC, FEG-TEM and Molecular Dynamic (MD) studies. The number of mercury in nanocrystal was determined by the MALDI. These nanocrystals found to have application in the photonics and sensing. MALDI was also successfully used for the studying protein dimerization and aggregation. (β -LG) was found to form the nanofibrils in presence of CuII. This CuII mediated fiber has role in material science and nanotechnology and biology. CuII found to enhance the dimer formation in the α -LG which was pH dependant. The nature of dimer was confirmed by the SDS and Reducing-SDS PAGE.

Poster no: D-2-56

Biomarkers and Bioscavengers for Organophosphorous compounds exposure

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Introduction: 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) by selectively binding the active site serine hydroxyl moiety leading to accumulation of acetylcholine at the synaptic cleft. This binding is supported by Glutamic acid and histidine environment there by forming a trio complex. Inhibition of plasma cholinesterase is taken as the biomarker of exposure of OP compounds. We have detected the active centre peptide after OP exposure using MALDI-TOF/MS. They also form non-aged adduct at tyrosine residue in albumin when plasma is incubated with nerve agents. Proteomic methods have been developed to verify the OP exposure. It was reported that 411 tyrosine residue selectively phosphorylated by OP out of other 18 tyrosine residues due to its unusual low pKa over the surface of albumin.

Methodology: Different experiments were carried out by taking control and OP treated AChE, BChE samples (OP concentration 0.178mM & 1.78mM). Samples were subjected to SDS-PAGE (10%), after analysis corresponding bands were cut and subjected to In-gel digestions as per our protocols. Serum/Plasma samples are spiked with OP at (10-100 μ M) concentration and kept at 37 °C for 24 hrs for complete reaction. Protease Digestions were performed using Trypsin (20 μ g/100 μ l), It was dissolved in 50 mM ammonium bicarbonate and the ratio employed for in solution digestion was 1:50 and 1:30 for in-gel digestion. Pepsin was dissolved in cold 10mM HCl (4mg/ml). All the trypsin digestions were performed in ammonium bicarbonate solution (pH-8.0) and Pepsin digestions were carried out in 10 mM HCl (pH -2.5) for 3-6 hrs at 37 °C. The respective proteins are then digested with trypsin, pepsin and the peptides identified using MALDI-TOF/MS followed by MASCOT data base search.

Result and Discussion: OP mediated inhibition of cholinesterases resulted in phosphorylated enzyme complex. Aging was also observed, organophosphate-ChE(OP-ChE) conjugate undergoes dephosphorylation of ChE to generate active enzyme and dealkylation of phosphorylated-ChE. Tyrosine - 411 has been identified as novel phosphorylation site upon exposure of Albumin with OP. Albumin was subjected to both trypsin and pepsin digestions after its isolation both in vitro & in-vivo. Identification of these phosphorylated peptides will not only serve as excellent biomarkers but forms the basis for selection of Haptens/linear epitopes in immunological detection Conclusion: Identification of Active centered peptides (ACP) of the cholinesterase enzymes in plasma proteome after OP exposure will help as biomarkers and Prophylactic treatment of BChE may serve as Bioscavangers before OP exposure.

Poster no: D-2-57

High resolution MRM based quantitation of transgenes expressed in genetically modified *Brassica juncea*

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To assess the safety of the transgenes introduced in genetically modified (GM) crops rigorous evaluations are carried out to study the food, feed and environmental safety of the inserted protein. The quantification of the transgene expression in different parts of the plant is one of the important parameter required for the release and the approval for the use of GM crop. In the present study, MRMHR methodology was developed and employed successfully for absolute quantification of three proteins bar, barnase and barstar in genetically modified crop of *Brassica*

junccea. The tryptic digest of purified recombinant proteins for bar, barstar and barnase were analysed for the identification of unique peptides and MRMHR method was developed. Two specific and unique AQUA peptides for each protein were synthesised and used as internal standards. The data showed a good linearity over 3.5 orders of magnitude in case of all the peptides with coefficient correlation >0.995. The quantification was done in various tissues of the plant like leaf, stem, bud, anthers, seed and seedling using a high resolution accurate mass platform. The result demonstrated that the *bar* gene which is driven by constitutive promoter showed protein expression in all the tissues whereas for *barnase* and *barstar* (under the tapetum specific promoter) very low expression was seen only in the buds, as expected. We showed that the high resolution MRM quantitation has the advantage of removal of matrix interferences and accurate quantification of very low abundant proteins like *barnase* and *barstar* that are spatially and temporally regulated.

Poster no: D-2-58

Exploring Impact of Dynamic Accumulation for Improving MS/MS Quality of QqTOF Data

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Recent innovations in QqTOF instrumentation has resulted in a large increase in MS and MS/MS acquisition speed providing deeper coverage of complex proteomes. In data dependent acquisition, selection and timing of MS/MS acquisition is triggered when a precursor mass surpasses a minimum threshold. At constant speed, this can create a dataset with a range of MS/MS spectral quality. In this study it was shown

that by dynamically adjusting the acquisition time for MS/MS on analytes derived from biological samples can provide improved MS/MS data quality throughout the data dependent acquisition experiment. To understand and optimize the dynamic accumulation (DA) workflow, a range of acquisition rates and precursor intensity combinations were explored using standard proteome samples. Applications of such workflow showed improvement in SCIEX iTRAQ® reagent quantitation, peptide mapping sequence coverage, more confident identification of low abundant peptides.

Poster no: D-2-59

Improved data quality using variable Q1 window widths in SWATH™ acquisition - Data independent acquisition on the TripleTOF® 6600 and 5600+ systems

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The goal of quantitative proteomics is to both identify and quantify a broad range of proteins and peptides. The extreme complexity and dynamic range of proteins challenges traditional data dependent workflows to reproducibly and deeply interrogate the sample.

The data independent acquisition strategy, MS/MS^{ALL} with SWATH™ acquisition, provides a comprehensive quantitative analysis of complex proteomes, providing full scan high resolution MS/MS on all detectable peptides eluting off the column. These data can then be re-interrogated over and over again for different proteins and peptides as new hypotheses emerge. In this study, the SWATH™ acquisition was further optimized for proteomic analysis by balancing Q1 window width, the number of Q1 windows and accumulation time which showed increase in depth of coverage without compromising reproducibility in the data sets obtained from E coli proteome. By narrowing Q1 windows allowed to provide improved peak group detection, signal/noise of analytes for quantitation.

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Poster no: D-3-01**Identification of Preferentially expressed Proteins in Early Stage of Lactogenic Differentiation in Mammary Epithelial Cells**

Jaswal S¹, Jena MK¹, Anand V¹, Verma AK¹, Singh S¹, Kumar S¹, Reddy TJ², Malakar D¹, Mukesh M³, Sodhi M³, Kaushik JK¹ and Mohanty AK¹

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Introduction: The mechanism governing differentiation of mammary epithelial cells is yet to be elucidated in molecular details. Dome formation is a first phenotypic change, when the proliferating cells get differentiated. The study was aimed at identifying preferentially expressed proteins which may be playing important role in early stage of lactogenic differentiation in mammary epithelial cells. We used a buffalo mammary epithelial cell line (BuMEC) which was established in our lab.

Method: The present study was performed on *in vitro* cultured buffalo (*Bubalus bubalis*) mammary epithelial cells (BuMECs) which were in their 25th–30th passage. Proliferating cells were induced for differentiation by lactogenic hormones (insulin, prolactin and cortisol). Sub cellular fractions such as cytosolic and mitochondrial fractions were collected from proliferating and differentiated BuMECs. Subsequently, differentially expressed proteins were captured by 2D-DIGE (Difference Gel Electrophoresis) and identified by MALDI-TOF mass spectrometry. Bioinformatics analyses of the identified proteins were performed and the pathways involved were identified. The results were validated by Real-Time PCR analysis of selected proteins.

Results and Discussion: A total of 21 proteins were differentially expressed (16 cytosolic and 5 mitochondrial). Annexin – I, II, V and the S100 calcium-binding proteins (S100A4, S100A2 and S100A11) which were differentially expressed and may have potential role in formation of domes in BuMECs representing the early stage of lactogenic differentiation. The experimental findings along with the bioinformatics analyses reveal the proteins responsible for dome formation, and ultimately the differentiation process.

Conclusion: The identified proteins may be involved in the early stages of mammary epithelial cell differentiation occurring during late pregnancy and early lactation in buffalo mammary gland in particular and ruminants in general.

Poster no: D-3-02**Active site plasticity empowers the Microbial Proteome: Insight into the crRNA processor and its interaction partners in CRISPR-Cas type I-C system**

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Introduction: Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) in association with CRISPR associated (Cas) proteins constitutes a formidable defense system against mobile genetic elements in prokaryotes. In type I-C, the ribonucleoproteins surveillance complex comprises only three Cas proteins, namely, Cas5d, Csd1 and Csd2. We report the promiscuous DNase activity of Cas5d in presence of divalent metals in addition to its metal independent crRNA processing. The active site that renders RNA hydrolysis may be tuned by metal to act on DNA substrates too. Although Csd2 seems to be inert, Csd1 too exhibits RNase and metal-dependent DNase activity.

Methodology: The binding site in Cas5d was probed using intrinsic tryptophan fluorescence. Activity assays were performed for Cas5d, Csd1 and Csd2 in presence and absence of metal. Active site residues were identified using site directed mutagenesis.

Results and Discussions: Tryptophan fluorescence quenching with increasing amounts of Mg²⁺ suggested conformational changes. Scatchard and Klotz plot analysis negated the presence of multiple classes of metal binding, while the Hill analysis hinted the possibility of negative cooperativity in binding the metal in Cas5d. In the absence of DNA, Cas5d affinity towards metal is weak (kd = 35.79 mM). However, when DNA is present, the affinity is strong (kd = 1.33 μM), suggesting that the DNA too contributes to metal binding. Csd1 in *B. halodurans* showed RNase and DNase activity while Csd2 was inert and exerted regulatory role in complex.

Conclusions: The various roles of Cas proteins enhances the outreach of the bacterial defense strategy by countering the phages that escapes the defense with reduced restriction sites and/or modification of phage genome.

Poster no: D-3-03**Cadmium toxicity responses allay with heat pretreatment in cyanobacterium *Anabaena* sp. PCC 7120**

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Introduction: Anthropogenic activities alarmingly influence all spheres of life which is mainly manifested with increase in average temperature on earth. However, the fate of hazardous elements such as heavy metals in cultivated lands is also a matter of global concern. Cadmium is one of the most abundant, active at concentration much lower than other heavy metals and readily taken up by the crop plants and cyanobacteria. Thus, combined effect of temperature and cadmium on living entities draws significant attention for thorough investigation. Present study explored the possible role of heat pretreatment in palliating cadmium toxicity in cyanobacterium *Anabaena* sp. PCC7120.

Methodology: Exponentially growing *Anabaena* sp. PCC7120 was subjected to five experimental conditions: (i) only heat treatment at 39°C, (ii) at 45 °C, (iii) preheat treatment at 39 °C then subjected to 10μM cadmium chloride, (iv) preheat treatment at temperature 45 °C then subjected to 10μM cadmium chloride, (v) only 10μM CdCl₂ treatment. Alteration in growth behaviour, pigment content, physiological parameters such as size of the ATP pool, NADPH/NADH level, nitrogenase activity and biochemical parameters such as glutathione, thiol, and phytochelation content, GST and GR assay measured was investigated for one, three, five, seven and nine day of treatment for all above five experimental setups. Proteomics study for the same is under process with two dimensional gel electrophoresis method for further exploring the protein profile involved in stress responses.

Results and Discussions: A comparison of all five experimental setups with control showed day's dependent altered responses for all parameters measured. Their performance in response to stress suggests that although only heat treatment setups were performing best with better growth behavior, greater pigment and antioxidant content and higher enzymatic activity assay than preheat treatment setups, but the later one showed highest phytochelatin content along with better responses in all other parameters measured than only cadmium treatment.

Conclusions: This preliminary experiment concludes that preheat treatment alleviated cadmium toxicity in cyanobacterium *Anabaena* sp. PCC7120.

Poster no: D-3-04

Proteomic analysis for secretome of *C.botulinum* type B and Identification of Immunogenic proteins for potential vaccine/ diagnostic candidate molecule for Botulism

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Introduction: *C.botulinum* is an obligate anaerobic bacterium that causes infant, wound and food borne botulism. Secretory proteins of *C. botulinum* are known to be involved in virulence and may mediate important host-pathogen interactions. Numerous secretory proteins produced by *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Helicobacter pylori* have been previously identified as immunogenic proteins from the respective secretome using 2D gel electrophoresis (2-DE) and mass spectrometry. Identifying immunogens are important for development of diagnostics, vaccines, passive immunotherapy and understanding of the humoral immune response. The aim of the present study was to identify and characterize immunogenic proteins of *C.botulinum* type B secretome for the development of potential vaccine / diagnostic molecule against botulism.

Methodology: Immunoproteomic was used to screen the extracellular immunogenic proteins of *C. botulinum* type B. In the present study, secretory proteins of *C.botulinum* type B which were expressed in both tryptone peptone glucose yeast extract (TPGY) and cooked meat media (CMM) were separated using two-dimensional gel electrophoresis and proteins spots were identified by MS-MS analysis. The polyclonal antibody was raised in mice against secreted protein in TPGY medium and CMM medium and the sera were used for Western blot analysis to identify the immunogenic proteins.

Result and Discussion: Elucidated the proteomic profiles of the secretome of *C. botulinum* type B in TPGY medium and CMM medium. In TPGY media, 85 proteins were predominantly observed in the extracellular milieu where as in CMM media 65 proteins were observed. All these proteins were identified by MS-MS analysis. Among this 14 proteins were immunogenic in TPGY media and 09 proteins were immunogenic in CMM media.

Conclusion: Proteomic profile of extracellular proteome of *botulinum* type B was elucidated. We identified approximately 23 immunogenic proteins, which can be further validated for the potential as Vaccine / diagnostic candidate molecule against botulism.

Poster no: D-3-05

Early accumulated proteins are crucial for butachlor stress management in *Anabaena*

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Introduction: Cyanobacteria are indispensable components of paddy soil often appended with heavy herbicide load. Butachlor is one such extensively used rice field herbicide which negatively affects the cyanobacterial proliferation, yet the molecular

mechanism underlying its toxicity in diazotrophic cyanobacteria is largely unknown. The present study focuses on the comparative proteomics to decode the molecular basis of butachlor toxicity/tolerance in three *Anabaena* species e.g. *Anabaena* sp. PCC 7120, *A. doliolum* and *Anabaena* L31.

Methodology: All three *Anabaena* spp. were grown under standard laboratory conditions. The LC₅₀ concentration of butachlor was determined by the plate colony count method. Various physiological parameters and 2D protein profiles of each *Anabaena* spp. were compared at control, 1, 3, 5 and 7 days of butachlor treatment.

Results and discussion: 75 differentially expressed proteins from each *Anabaena* sp. included those involved in photosynthesis, C, N and protein metabolism, redox homeostasis, and signal transduction. While early accumulated proteins related to photosynthesis (atpA, atpB), carbon metabolism (fbp, fba and prk), protein folding (groEL, PPIase), regulation (orrA) and other function (OR, akr) appeared crucial for tolerance of *Anabaena* L31, the late accumulated proteins in *Anabaena* 7120 presumably offer acclimation during prolonged exposure to butachlor. Contrary to above, a multitude of down-accumulated proteins vis-a-vis metabolisms augment sensitivity of *A. doliolum* to butachlor. A cluster of high abundant proteins (atpA, groEL, OR, AGTase, Alr0803, Alr0806, Alr3090, Alr3199, All4050 and All4051) common across the three species may be taken as markers for butachlor tolerance and deserve exploitation for stress management and transgenic development.

Conclusion: Comparative proteomics revealed the existence of species specific protein dynamics in the test organisms. It was tempting to recommend application of a mixed population of *Anabaena* L31 and *Anabaena* PCC7120 as an efficient biofertilizer in paddy fields receiving butachlor treatment.

Poster no: D-3-06

Differential proteome analysis of blood stage of *Plasmodium falciparum* after treatment with anti-malarials

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Introduction: Owing to the emergence of resistance in malaria parasites against most anti malarial compounds has highlighted the urgency to develop either new combination therapies using available drugs or develop new drugs to replace failing ones.

There is a need to understand the mode of action of proposed anti-malarials & mechanism of resistance with existing drugs so that we can develop new anti malarial drugs and simultaneously delay the resistance of parasite against currently used drugs.

Mechanism of drug action can be better understood with proteome studies of malarial parasite post drug treatment.

Methodology

- In vitro synchronized culture of *P. falciparum* 3D7 was maintained in human RBCs and RPMI-1640 media.
- Trophozoite stage *P. falciparum* culture was treated with various concentrations of artemisinin and monensin separately to obtain the IC₅₀ of both drugs.
- Isolation of parasite protein was done after treatment of trophozoite stage culture with 2xIC₅₀ of artemisinin and monensin separately for 24 hrs.
- Analysis of differentially expressed proteins was done using SDS-PAGE followed by 2D gel electrophoresis in drug treated and untreated parasite.

- Further identification of differentially expressed proteins was done using MALDI-TOF.

Results and Discussions: The IC₅₀ of artemisinin and monensin was 41ng/ml and 2.1ng/ml respectively. In SDS PAGE gel, one band was up regulated and one was down-regulated after artemisinin treatment however after monensin treatment, one band was up regulated and four bands were down regulated. In 2D gel electrophoresis 9 spots were down regulated and 2 were up regulated after monensin treatment.

Further two down regulated proteins in monensin treated parasites were identified as heat shock proteins using MALDI-TOF.

Conclusions: The study revealed that monensin is very effective against *P. falciparum* growth and its mechanism of action is different than artemisinin. Hence it would be a better partner in artemisinin combination therapies against drug resistant malaria.

Poster no: D-3-07

Study of *Leishmania donovani* antigen from VL subjects for diagnosis

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Visceral Leishmaniasis (VL) or Kala-azar, caused by intracellular protozoan parasite *Leishmania donovani*, is a serious health problem in North Eastern part of India. The disease is lethal if left untreated. Chemotherapy is the only mode to control the disease. For this, satisfactory diagnosis is essentially required. The conventional diagnostic test for VL is the demonstration of amastigotes stage of *Leishmania* parasite in bone marrow or splenic aspirates under microscope or detection of antigen or parasite DNA in PCR based assay. The overall sensitivity of these detection systems appears to be highly unsatisfactory, if splenic aspiration is not considered. The two antibody detection tests, DAT and rapid immunochromatographic tests (rK-39) are identified with field adaptability and with acceptable sensitivity and specificity. Since these tests are based on antibody detection in human blood the reliability is not assured due to some false positive results. It cannot discriminate between active and past infection as the longevity of antibodies are very long (>2 years). In this study, we have identified antigen present in the body fluid through two dimensional gel electrophoresis with immobilized pH gradients combined with protein identification by mass spectrometry. The identified antigen of *Leishmania* will be used for diagnosis as well as prognosis. Detection of antigens is comparatively more convincing as it is eliminated quickly from body system as soon as the cure of disease. This diagnostic tool not only will be able to discriminate the past infection but also it will be helpful in diagnosis of VL in HIV positive cases.

Poster no: D-3-08

Roles of glycosylphosphatidylinositol (GPI)-linked aspartyl proteases in cellular physiology of *Candida glabrata*

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Introduction: *Candida spp.* are the leading nosocomial fungal pathogens. Among *Candida spp.*, *C. glabrata* is second most

common cause of invasive candidiasis. The key virulence factors of *C. glabrata* include biofilm formation, survival in phagocytes, colony switching and regulated expression of families of cell wall-associated adhesins and aspartyl proteases. *C. glabrata* possesses a family of eleven putative glycosylphosphatidylinositol (GPI)-linked aspartyl proteases, named yapsins (encoded by *CgYPS1-11* genes), which are required for its virulence, cell wall maintenance, adherence to mammalian cells and survival in macrophages.

Methodology: We have used a combined approach of genetic, cellular biology and biochemical techniques to investigate the role of *C. glabrata* yapsins in vacuole homeostasis.

Results: We show that a *C. glabrata* mutant lacking eleven yapsins, *CgYPS1-11Δ*, possesses an enlarged vacuole, and displays vma⁻ (vacuolar membrane ATPase)-like phenotypes with elevated metal ion susceptibility in alkaline pH medium and diminished Vma activity. Additionally, increased CPY secretion and reduced cellular CPY activity in the *CgYPS1-11Δ* mutant indicate a pivotal role for yapsins in vacuole functions and physiology. We also demonstrate a perturbed cell wall homeostasis in the mutant as rich-medium grown-*CgYPS1-11Δ* cells had constitutively activated protein kinase C (PKC)-mediated cell wall integrity signaling pathway, and displayed cell surface abnormalities.

Discussion: Disruption of *CgYPS1-11* genes results in chronic stress viz., constitutive cell wall defects, high ROS levels, reduced energy and stress metabolite levels, metal ion susceptibility and diminished V-ATPase activity.

Conclusion: We report new roles for yapsins in *C. glabrata* physiology and implicate them for the first time in sorting of the vacuolar-lumenal enzyme carboxypeptidase Y (CPY).

Poster no: D-3-09

In-silico analysis of Nitrogen as simulation pathway of *Arthrosphaera platensis*

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Introduction: Protein content of a cell is a result of cumulative effect of various processes/pathways that starts from transcription of a gene and ends at the final protein synthesis. Nitrogen assimilation pathway is an important pathway which supplies the cell with nitrogen requirements. In this study, we try to understand the interplay between nitrogen assimilation pathway and total protein content of *Arthrosphaera (Spirulina) platensis*.

Materials and Methods: Using sequence and structure analysis tools, we compared sequences and structures of following proteins and their respective genes of Nitrogen assimilation pathway; Glutamine Synthetase (GS), Glutamate Synthase (GOGAT), Nitrate Reductase (NR), Nitrite Reductase (NiR) and Membrane Nitrate transporter (MNT). These proteins and genes from *Spirulina* are examined with that of *Synechocystis*, *Escherichia coli*, *Salmonella typhimurium*, *Saccharomyces cerevisiae*, *Arabidopsis thaliana* and *Oryza sativa* to identify the key differences at sequence and structure level which can be attributed to functional difference of this pathway.

Results and Discussion: GS sequence of selected species was subjected to MSA followed by phylogenetic analysis. The phylogenetic tree was well in accordance with the rRNA phylogenetic tree. Further a PSA of GS of *Spirulina* with *Synechocystis* was done to find a C-terminal domain in *Spirulina*. In case of NR different domains were found in case of prokaryotes (MopB nitrate-R Nap A, MopB CT nitrate R Nap A 1) and eukaryotes (Eukary NR moco (SO family moco), Cyt b5, Cyt b5

reductase like). NiR of *Spirulina* was surprisingly closer to *Arabidopsis* (42.8% identity) and Rice (42.6% identity) than to Yeast (9.1% identity).

Conclusion: Our results suggest that in an additional domain exists within GS of *Spirulina*, which was not reported previously (Aldolase class-I). Different domains of NR throw light on evolutionary aspect of the protein. Nearness of NiR of *Spirulina* with *Arabidopsis* and Rice reveals the interplay between photosynthesis and nitrogen assimilation.

Poster no: D-3-10

Role of an oxidative-stress regulator (OxyR) in organic peroxide-induced oxidative stress in *Azospirillum brasiliense* Sp7

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Introduction: Molecular oxygen acts as premier electron acceptor and serves a vital role in fundamental cellular functions including aerobic respiration. Electron transfer to oxygen can produce reactive oxygen species (ROS) *viz* superoxide, hydrogen peroxide and hydroxyl radicals, that can damage important biomolecules in the cell. To counter the deleterious effects of ROS, organisms have evolved strategies to detoxify these ROS. Antioxidative enzymes play major role in overcoming the oxidative stress, which is regulated by global regulator, OxyR. *Azospirillum brasiliense* Sp7, a plant growth-promoting rhizobacterium, contains two paralogs of *oxyR* (designated as *oxyR1* and *oxyR2*, respectively). The two *oxyR* paralogs (*oxyR1* and *oxyR2*) are organized divergently to the genes encoding alkyl hydroperoxidase genes and catalase, respectively. The objective of this work was to study the role of OxyR1 regulator under peroxide induced oxidative stress.

Methodology: In order to understand the role of OxyR1 regulator during peroxide-induced oxidative stress, we inactivated *oxyR1* gene by inserting a kanamycin resistance gene cassette (Km). Sensitivity of the parent strain and mutant strain to the peroxide stress was compared by zone of inhibition on 0.8% agar plate. To identify the regulatory *cis*-acting elements and to study the regulation of *ahpC* gene, a series of deletion derivatives were constructed from 5' end to construct *ahpC-lacZ* fusions, and recombinant plasmids were conjugatively mobilized in to the parent and mutant strain (*oxyR1::Km*).

Result and Discussion: The *oxyR1::Km* mutant strain was more tolerant to cumene hydroperoxide and tert-butylhydroperoxide as compared to the parent. Study with *ahpC-lacZ* fusion revealed that expression of the *ahpC* gene was five times upregulated in the mutant in comparison to that in the parent, indicating that OxyR1 acts as a negative regulator for *ahpC* gene expression. This also explains why *oxyR1::Km* mutant tolerates higher levels of organic peroxide. Further, the study with a series of *ahpC* upstream deletions showed that a characteristic structural motif containing TN₁₁A region is important for full activation via OxyR1 binding. Using electrophoretic mobility shift assay we have shown that the purified recombinant OxyR1 protein binds to the 262bp region located between *oxyR* and *ahpC*.

Conclusion: OxyR1 negatively regulates expression of *ahpC* gene in *A. brasiliense*. Under reducing condition, it binds to the *ahpC* promoter, but exposure to organic peroxides derepresses the expression from *ahpC* promoter via a change in the conformation of OxyR1.

Poster no: D-3-11

Comparative proteome analysis of pathogenic and non-pathogenic mycobacterium ÄsigF mutant and isogenic wild type strains

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Introduction: SigF has been characterized as a general stress, stationary phase sigma factor involved in the virulence of the mycobacterium. Interestingly, the SigF was reported to be absent in fast-growing nontuberculous mycobacteria until we reported its presence in few non-pathogenic fast-growing mycobacterial species followed by reports in database (Singh and Singh, 2008). SigF homologs from pathogenic and non-pathogenic mycobacteria tend to cluster separately, which implies that the SigF addresses different regulon in patho-physiologically distinct mycobacterial species. The presence of SigF across the mycobacterium genus suggests a wider but distinct role of this alternative sigma factor in mycobacterial physiology apart from its alleged role in regulation of the virulence genes expression in pathogenic mycobacteria.

Methodology: *M. smegmatis* and *M. bovis* ÄsigF mutants and their isogenic wt strains were cultured in 7H9 medium. Log and stationary phase cultures were used for conventional 2DE and DIGE analysis. Gels were analysed by Image master platinum and Decyder softwares, and differentially expressed proteins spots were identified by matrix assisted LASER desorption/ionization time of flight mass spectrometry (MALDI TOF/MS).

Results and Discussion: Comparative proteome analysis of the isolates showed forty five proteins in *M. smegmatis* and forty four proteins in *M. bovis* to be differentially expressed in mutants. These proteins were identified by MALDI TOF MS/MS, and based on database analysis, they were found to have roles in different metabolic pathways, stress response and intracellular survival of mycobacteria. Unlike *M. Bovis* BCG where sigF is expressed predominantly in stationary phase, we observed continued expression of sigF in *M. smegmatis* throughout the growth of bacterium at a level comparable to sigA expression.

Conclusions: The SigF regulon in *M. bovis* comprised genes which explained the phenotypes exhibited by the mutant and highlighted the importance of this sigma factor in adaptation to stationary phase and oxidative stress response in mycobacteria.

Poster no: D-3-12

Molecular characterization of CRISPR adaptation in *Thermotogamaritima* MSB8

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Introduction: CRISPR-Cas (Clustered regularly interspaced short palindromic repeats – CRISPR associated proteins) systems confer adaptive immunity to bacteria against invading phages and plasmids. This mechanism is categorized into three stages, where adaptation is the prime stage involving recognition and uptake of short nucleic acid fragments derived from invading genetic elements. This stage of CRISPR-Cas system is the least studied. Cas1, Cas2 and Cas4 are the proteins predicted to be involved in this stage of adaptive immunity. Present study is centered on molecular characterization of Cas1, Cas2 and Cas4 from *Thermotogamaritima* MSB8.

Methodology: Heterologously expressed Cas1, Cas2 and Cas4 were purified using affinity chromatography and were tested for nuclease activity against various DNA substrates viz. dsDNA (linear, and circular), ssDNA (linear and circular).

Results: It was found that all the purified proteins degrade both linear and circular dsDNA substrates in presence of divalent metal ions.

Conclusion: Activity of these proteins concludes that they act as divalent metal dependent, non-specific endonucleases. We are yet in a process of evaluating the role of these nonspecific nucleases in a sequence specific integration process (adaptation stage).

Poster no: D-3-13

Proteomic analysis for understanding the role of porins in the organic solvent-tolerance of *Pseudomonas aeruginosa* PseA

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Introduction: Solvent-tolerant microbes have the unique ability to thrive in presence of organic solvents. The present study describes the effect of increasing hydrophobicity ($\log P_{ow}$ values) of organic solvents on the proteome of the solvent-tolerant *Pseudomonas aeruginosa* PseA cells. The cells grown in presence of hydrophobic solvents were analysed for variation in their proteomic profile induced by solvents of varying hydrophobicity.

Methodology: Proteins from outer membrane fractions as well as the secretome were extracted from the late log phase cells and the changes in their expression studied by 2-D gel electrophoresis. The protein spots showing significant changes in their expression were analysed by LC-ESI MS/MS.

Result and Discussions: Over 17 outer membrane protein spots and 67 protein spots from the secretome of the solvent exposed cells were significantly affected. Some of them showed differential expression, yet others were absent from the solvent-exposed cells altogether. The identity of the proteins from the outer membrane proteome matched with various known porins of *Pseudomonas* reported previously. The highlights of the results are: (i) the detection of intracellular proteins in the secretome of cells exposed to lower $\log P_{ow}$ solvents, indicating membrane damage induced by these solvents; (ii) a significant down-regulation of A-type flagellin in the outer membrane protein component, indicating a tendency to reduce the cell surface area, a phenomenon reported previously in some unicellular algae exposed to solvents and (iii) up-regulation of OprE, an anaerobically-induced porin.

Conclusions: Organic solvents of lower $\log P_{ow}$ values significantly affected the integrity of the cells resulting in membrane damage and release of cytoplasmic proteins into the external environment. A-type flagellin was significantly reduced whereas OprE was up-regulated in presence of solvents.

Poster no: D-3-14

Proteomic analysis of cytosolic fraction of kanamycin and amikacin resistant *Mycobacterium tuberculosis* isolates

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Introduction: Kanamycin (KM) and amikacin (AK) are second line anti-tuberculosis drugs used to treat tuberculosis (TB) and

resistance to them rigorously affects the treatment strategy. Many explanations have been anticipated for KM & AK drug resistance but still resistance mechanisms are puzzling. Cytosolic proteins have an anticipated role in the biological processes & pathogenesis; so these are potential targets for the development of new diagnostics, vaccine & therapeutics. Therefore, analysis of mycobacterial proteome in relation to drug resistance is urgently required.

Methodology: *M. tuberculosis* sensitive and resistant clinical isolates were cultured in Sauton's medium and after four weeks cells were harvested by centrifugation. After sonication, whole cell lysates were ultra centrifuged to remove the membrane contaminant and cytosolic fraction (supernatant) were precipitated using SDS-TCA precipitation method. Cytosolic proteins were resolved by two dimensional gel electrophoresis and differentially expressed proteins (more than two fold) were selected for identification by matrix assisted LASER desorption/ionization time of flight mass spectrometry (MALDI-TOF/MS) and characterization by bioinformatic tools like BLASTP, InterProScan, KEGG motif scan and molecular docking.

Results and Discussions: Consistently ten over-expressed proteins were identified in resistant isolates were belonged to the category of virulence, detoxification, adaptation, intermediary metabolism & respiration, regulatory proteins, and conserved hypothetical. Molecular docking shows that both drugs bind into the central cavity /functional domain of hypothetical proteins. Besides their predefined roles, these proteins might be involved in drug resistance and survival of mycobacteria.

Conclusions: We assume that these proteins might be playing some crucial role in contributing resistance to AK and KM. These findings need further research for the development of newer therapeutic agents or molecular markers which can directly be targeted to a gene/protein responsible for resistance.

Poster no: D-3-15

Molecular Characterization of a immune reactive protein from *Rhizopus oryzae*: an allergenic and pathogenic mold causing asthma and mucormycosis

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Introduction: Fungal infection and allergy have reached a pandemic dimension worldwide, including tropical countries. Mold allergen triggered asthma and pulmonary Mucormycosis, caused by opportunist pathogen *Rhizopus oryzae* (RO) is present in immunocompromised individuals. To understand the molecular basis of the disease, it is important to characterize the antigen at molecular level. In this study we have adopted immunoproteomics, recombinant DNA technology and structural studies to characterize this immune-reactive antigen.

Methodology: Spore mycelia 2D proteome was confronted with patient sera and the immunoreactive proteins were identified via MALDI-TOF/TOF and N-terminal sequencing. This glycoprotein antigen was identified as a 44kDa aspartic endopeptidase. Allergen was purified via column chromatography. Recombinant form of this antigen was prepared by cloning and expressing a 1206 bp cDNA ORF in *E. coli* (BL21) and purified by Ni-column. Both the native and recombinant antigens were further tested for immunoreactivity. Structural modeling predicted a refined 3D conformation of this antigen with 3 potential B-cell epitopes. The critical residue(s) in one such epitope was converted by site-directed mutagenesis and the mutant forms were tested for sero-reactivity.

Results and Discussion: The purified native and recombinant antigens were found to have retained immunoreactivity. Periodate modification test confirmed that sugar-moiety was not responsible for immune recognition. The mutant form of the antigen was not able to bind with IgE and IgG as revealed by western blot.

Conclusion: This is the first potent immune reactive antigen reported from this Mucormycosis agent (RO). The 3D conformation of the antigen has a potential immunodominant surface epitope, which upon mutation, has lost its ability to act as an immunodeterminant. This wild and hypoantigenic forms, can be further studied in great detail for its potential use as infection biomarker and vaccination agent respectively.

Poster no: D-3-16

Phenol extraction-based protein preparation method for 2D gel electrophoresis of bacterial proteomes

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Introduction: Good sample preparation is crucial for high-resolution separation of complex protein mixtures by 2D gel electrophoresis (2DE). Our model bacterium *Tetrathiotobacter kashmirensis* being found refractory to the conventional TCA/acetone precipitation-based method (TCA method), we have improvised a phenol extraction-based method (PE method) for the bacterium. Subsequently, to validate its general applicability, the method was evaluated against the TCA method for two other bacteria, *Escherichia coli* and *Mycobacterium smegmatis*.

Methodology: Cultures grown upto mid-log phase were harvested and lysed in an extraction buffer (EB) or in EB containing 30% sucrose (EBS). In TCA method, proteins were precipitated from EB with TCA and acetone. In PE method, proteins from EBS were extracted with phenol and finally precipitated with ammonium acetate dissolved in methanol. Precipitates were then washed with ice-cold methanol and acetone and subjected to 2DE. Some protein spots from *T. kashmirensis* gel, obtained after PE method, were excised, trypsin digested, and subjected to MALDI MS-based identification.

Results and Discussion: For *T. kashmirensis*, TCA method gave only ~413 poorly resolved protein spots that too with excessive streaking and smearing of proteins. In contrast, the PE method gave a far better result with about 874 intense and highly resolved spots without any streaking or smearing. In case of *E. coli*, the PE method gave better resolution with ~941 spots in comparison to 769 spots of TCA method. For *M. smegmatis*, the PE method gave 786 highly resolved protein spots in comparison to 504 poorly resolved spots in TCA method. Four proteins each from the outer membrane, periplasm, and cytoplasm of *T. kashmirensis* were identified by MALDI-MS.

Conclusions: Our work has standardized a MALDI compatible, phenol extraction-based method of sample preparation for 2DE of bacterial total cellular proteome, which is efficient in extracting proteins from all the cellular compartments of a bacterial cell.

Poster no: D-3-17

Exploring trafficking mechanisms of Ribosomal protein P0 in *Toxoplasma gondii*

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Introduction: As a part of the lateral stalk of 60S subunit of eukaryotic ribosomes, P0 phosphoprotein plays an essential role

in the elongation step of translation. Earlier we have demonstrated extra-ribosomal role of P0 on the surface of infective forms of *Plasmodium falciparum* as well as *Toxoplasma gondii*, implicating P0 in host cell invasion. Surprisingly, P0 does not have any known classical signal sequence or transmembrane domains. In this study we are trying to understand the trafficking mechanism of P0 in Apicomplexans using *T. gondii* as a model. This would enhance our knowledge of protein trafficking in malaria parasite, which could be exploited as potential drug and vaccine targets.

Methodology: *T. gondii* were cultured using Human foreskin fibroblast cells. Parasite proteins were resolved on 2D-PAGE and spots were analysed by staining and immunoblotting and mass spectrometric analysis was done to explore the post-translational modifications (PTMs). Deletion constructs of P0 were made with HA tag, parasites were transfected with these and the localization of tagged-P0 was evaluated.

Results and discussions: Western blots of 2D-SDS PAGE probed with specific antibodies revealed the presence of multiple isoforms of TgP0. Mass-spectrometric analysis showed the presence of various PTMs such as phosphorylation, glycosylation and methylation. Expression of P0 deletion constructs revealed that extreme C terminal deletions of P0 did not affect its transport to the surface.

Conclusions: P0 is documented to be a protective protein, since anti-TgP0 antibodies blocked parasite invasion. In this study we found that surface localization was not affected when 40 amino acids at the C-terminal of P0 were deleted. We have also detected certain PTMs of P0. The effect of the PTMs (through specific mutations), as also that of N-terminal deletions of P0, would help us understand the importance of these amino acid residues in the transport of P0 to the surface of infective parasites.

Poster no: D-3-18

Carbohydrate recognizing protein with soluble glycan pools against enteric infections to prevent diarrheal episodes and resulting inflammation

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Introduction: Aberrant glycosylation can directly lead to increased susceptibility to immune associated pathologies resulting in acute infections and the resulting inflammation. As human intestinal epithelial layer is extensively and exclusively associated with O-glycans and GalNAc – ser/thr O-linked oligosaccharides comprise more than 50% of the mucin molecule by weight, mucinal O-glycosylation plays a vital role in immune associated pathologies.

Methodology and Results: We selected a natural Glycoprotein source containing rich O-GalNAc sugars attached to serine residues of the protein. The carbohydrate binding protein (CBP) has been stripped of its sugar moieties using enzymatic digestion to enable the shielding effect of internal residues of O-glycan chains in this source for defensive interventions. This is as much as the terminal structure of glycans in the human intestinal epithelial layer with unknown significance and yet unidentified due to their masking effects. The resulting O-GalNAc specific CBP with multi glycosylation sites have shown significant anti bacterial activity against enteric pathogens namely *klebsiella pneumonia*, *salmonella spp*, *shigella flexineri* and *E-Coli*. Interestingly the above pathogens invade through the

O-GalNAc moiety despite their varied forms of infection and repercussions.

The major challenge with the above modification of specific O-glycans is that the process involves both low stoichiometries of modification as well as low occupancy rate (15%) of the glycosylation pathway at a particular site making their detection very difficult. The recovery of CBP including the cleaved glycans has been 0.5-1% by weight.

Discussion: This explains the fact that despite the reduction in the respective O-glycan core of the intestinal epithelial layer by as much as 40 % of native levels during enteric invasions, infections were not progressive highlighting the role of quality glycans with unique specificities in enteric pathogenesis. Only a novel class of CBP with immune modulatory protein acting as host defence and keeping enteric pathogens at bay will provide an excellent opportunity to contain diarrheal infections and inflammation.

Poster no: D-3-19

Molecular evaluation of RNase from *Aspergillus niger* and Phytol of *Nymphaea pubescens* as cytoskeletal targeting elements in cancer

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Introduction: Noncommunicable diseases, like cancer, heart disease and cerebrovascular disease, remain the leading causes of death, together accounting for 58% of all deaths. Cancer is a leading cause of death worldwide, it accounted for 7.9 million deaths (around 13% of all deaths) in each year and projected to continue rising, with an estimated 17 million deaths in 2030. About 72% of all cancer deaths in 2007 occurred in low- and middle-income countries. We focused our research work is on the molecular action of Ribonucleases of *Aspergillus niger* and Phytol from *Nymphaea pubescens* on Actin of Homosapiens involved in the cancer development.

Methodology: MDA-MB-231 human breast carcinoma cell line was maintained in tissue culture medium (Leibovitz-15 medium supplemented with 10 % heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1x nonessential amino acids, 50 IU/mL penicillin, and 50 IU/mL streptomycin) at 37 °C and 5 % of CO₂. Isolation and identification of bioactive compound phytol from *N. pubescens*. The obtained *invitro* results were analyzed further through *insilico* analysis (Docking and MD Simulations studies). To gain better relaxation and accurate arrangement of atoms, refinement was done on the human actin and *A. niger* RNase and Phytol of *N. pubescens* by energy minimization (EM) and molecular dynamics (MD) simulations using 43A2 force field of Gromacs96 implemented in the Gromacs 4.0.5 package, finally the interaction energies were calculated by protein-protein, protein-ligand docking using the HEX and autodock respectively.

Results and Discussion: We found that RNase (*A. niger* RNase) and Phytol significantly and dose dependently inhibited invasiveness of breast cancer cell line MDA MB 231 by 55 % (P<0.01) at 1 μM concentration. At a concentration of 2 iM, the anti invasive effect of the enzyme increased to 90 % (P<0.002).

Conclusions: These in-vitro and in-silico structural studies prove the effective inhibition of actin activity by *A. niger* RNase Phytol of *Nymphaea pubescens* in neoplastic cells and thereby provide new insights for the development of novel anti cancer drugs.

Poster no: D-3-20

Proteomic approach of adaptive response to arsenic stress in *K. pneumoniae*

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Introduction: Proteomic approach has become a powerful tool which is used extensively to investigate the protein expression pattern and characterization of proteins in cells when exposed to different environmental stress conditions. Rather than targeting a particular family of proteins a comparison of protein profiles from control and stressed conditions allow the identification of adaptive proteins and provides an insight of metabolic pathways and unveils the molecular mechanism of stress response in various microorganisms. Proteomics approach has been used to study the effect of arsenic on bacteria.

Methodology: Comparative proteomic analysis using two-dimensional gel electrophoresis (2-DGE) and matrix assisted laser desorption ionization-time of flight-time of flight (MALDI-TOF-TOF) was used to monitor the proteins undergoing changes in expression levels under arsenic stress [200 mM arsenate As(V) and 2.5 mM arsenite As(III)]. Also the alteration in antioxidant enzymes activity in presence of As(V) and As(III) were determined by spectrophotometric assay as well as activity staining.

Results and Discussions: Under arsenic stress, most intense and differentially expressed protein spots present over all triplicate gels were excised from both control and test [As(V) and As(III)] gels. Among them, 62 in As(V) stress whereas 45 in As(III) stress were successfully identified by MALDI-TOF-TOF with a significant mascot score >74(p-value < 0.05). In this study, differentially expressed proteins are categorized into functional classes and their role in protecting cell from arsenic stress is discussed.

Conclusions: The results obtained in this study suggest multiple factors play an important protective role during arsenic stress.

Poster no: D-3-21

The CI repressor protein of *Staphylococcus aureus* temperate phage Ph11 has DNA binding properties

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Introduction: Temperate bacteriophages can have two developmental pathways, namely, the lytic mode of development and the lysogenic mode of development. The lysogenic pathway requires the action of the CI repressor protein while the lytic pathway requires the Cro protein. Bacteriophage φ11, a *Staphylococcus aureus* temperate phage also harbors the cl and cro genes in the divergent orientation. Further, the cl-cro intergenic region harbors 3 putative operators (designated O1, O2, and O3). Thus far, a number of *Staphylococcus aureus* bacteriophages have been discovered; however the regulatory elements involved in their developmental pathways have not been studied in detail at the molecular level. Studies on the interaction

between the CI protein and its cognate operators will not only enrich our knowledge about the molecular biology of the bacteriophage, but will also go a long way in the construction of tightly regulated expression vectors.

Method: *S. aureus* RN4220 and *E. coli* BL21 (DE3) cells were routinely grown in Trypticase soy broth and LB, respectively. Molecular biological techniques including plasmid DNA isolation, digestion of DNA by restriction enzymes, ligation of DNA fragments, transformation and polymerase chain reaction were carried out according to the standard procedures. Protein estimation, native and SDS-PAGE, and staining of polyacrylamide gel were carried out by the standard procedures. His-CI was purified from *E. coli* BL21 (DE3). Partial proteolysis was performed as described. Circular Dichroism spectroscopy (200–260 nm) was carried out according to a standard method. Analytical gel filtration chromatography was done as described. Equilibrium binding of CI to 0.1 nm P-labeled DNAs was investigated by the standard gel shift assay. DNase I and DMS foot printing were performed according to the standard procedures.

Results and Discussions: Partial proteolysis of His-CI indicates that the protein has a two-domain structure and carries two flexible regions: one at the N-terminal end and another almost at the middle of the molecule. Circular Dichroism spectroscopy results indicate that His-CI is mostly composed of α -helix and β -sheet at room temperature. DNA binding experiments indicated that His-CI binds specifically to its cognate operators O1 and O2 and the interacting bases of the cognate operators have also been determined.

Conclusions: The present studies reveal that CI repressor of Phi11 possesses two domains. His-CI contains a-helix substantially and exists as a dimer in solution. CI binds to 15 bp homologous operator DNAs. CI binds to O1 and O2 operator DNAs in the *cI-cro* intergenic region. The data together suggest that CI or Cro-mediated gene regulation in phage x11 is different from that of phage λ . Additionally; CI binds to operator DNA as a dimer.

Poster no: D-3-22

Proteome analysis of putative probiotic *Lactobacillus fermentum* BIF 19 strain

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Introduction: Certain *Lactobacillus fermentum* strains are commercially marketed as probiotics based on their putative or proven health promoting effects. Tolerance to bile salt has often been used as the most important probiotic selection criteria. Several proteins are involved in bile tolerance process that needs to be identified by proteomic methods by determining differential proteome of an organism. In India no report is available till date on the differential proteome analysis of putative probiotic *Lactobacillus fermentum* strains which will shed light on bacterial adaptation factors to GI tract conditions. This will also help to identify key proteins which may be used to screen new strains.

Methodology: The cytosolic proteins from *L. fermentum* BIF 19 in 1.2 % bile salts were extracted by lysozyme treatment. Proteins were subjected to IEF (7 cm with linear range of 4-7, GE healthcare). The gels were scanned by Labscan and analysed in Image master Platinum 7 software. The spots in control and bile treated samples were compared. Differentially expressed protein spots were selected, digested and subjected to MALDI TOF/TOF (AB SCIEX TOF/TOF 5800). The spectrum of tryptic peptides obtained was subjected to database search (Mascot search) and the identified proteins in various cellular processes

were assessed by PANTHER 9.0, MAS 3.0 and DAVID Bioinformatics resources 6.7.

Results and Discussion: Total of 185 common and 8 new spots were obtained in control and treated samples (1.2% bile salt) of BIF-19. Among these, 26 up-regulated, 20 down-regulated and 4 new spots of BIF-19 were selected and identified. The proteins involved in regulation of homeostasis, ABC transporter, carbohydrates and lipid metabolism were highly activated while those involved in the reproductive functions were down-regulated during the bile stress. The new spots identified were found to be the isoforms of the existing proteins.

Conclusions: Bile stress changed the protein profile of *L. fermentum* BIF-19 in which some pathways were activated whereas others were suppressed. The metabolic pathway becomes highly activated as the cells require more energy to survive in bile stressed condition. Nucleotide synthesis and cell division were suppressed under bile stress in order to balance the internal environment of the organism.

Poster no: D-3-23

Diguanylate cyclase of *Vibrio cholerae*: The importance of the GGEEF Domain

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Introduction: In between cholera epidemics, *Vibrio cholerae* survives in aquatic environments by forming surface biofilms, which are resistant to external environmental stress like chlorine and antibiotics. The bacterium has been known to secrete an external exopolysaccharide (EPS) matrix. Proteins with a conserved GGD (E) EF domain have been implicated in the regulation of EPS production in *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Yersinia pestis*, etc. The regulation of EPS production and hence, biofilm formation is performed by cyclic diguanylate (c-di-GMP), which is synthesized by GGD (E) EF domain proteins from other systems as well. Thus the signature domain of GGD (E) EF in conjunction with another sensory domain has been earmarked as ‘diguanylate cyclase’.

Methodology: Putative GGD (E) EF protein VCO395_300 from *Vibrio cholerae* was cloned and expressed in an *E. coli* system as a GST-tagged fusion protein. The protein was purified by multiple chromatographic methods including affinity and gel filtration. Site-directed mutagenesis at three central positions of the GGEEF signature sequence using primer method was achieved generating mutants having a different amino acid for each site. Oligomeric status of mutant as well as wild type proteins was determined using both HPLC and gel-filtration. The unfolding dynamics, determination of free sulphydryl groups, and other biophysical characterization was performed for all the above as well.

Results and Discussion: The wild type and the mutant proteins at the three central positions, however, show similar functionality as observed from the cyclic diguanylate assay. The oligomeric status, quantity of free sulphydryls and the MALDI mass do not show major discrepancies as well. The unfolding dynamics as observed from the fluorescence of free tryptophan residues do not indicate any structural changes either. This is indicative of the fact that mutations at the three sites may not produce any significant structural difference in the domain.

Conclusion: The effect of mutagenesis on the wild type GGEEF protein from *Vibrio cholerae* which has diguanylate cyclase activity (presumed to be essential for the colony formation) with respect to the GEE positions do not primarily reveal any difference in activity or major structural shift. It can be tentatively concluded that mutations in these positions do not affect the functionality of the domain.

Poster no: D-3-24**Stress responsive proteome dynamics in the Cyanobacterium *Anabaena* sp. strain PCC7120**

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Introduction: *Anabaena* PCC7120, a native of Indian paddy fields, is known to tolerate several abiotic stresses. Oxidative stress is central to all the biotic and abiotic stresses and results in accumulation of reactive oxygen species (ROS). The weedicide paraquat (or methyl viologen) is an important stressor which generates ROS, directly through Mehler reaction. The present study investigated the proteome dynamics of *Anabaena* 7120 at LD₅₀ dose (2 iM MV for 6 h) and the mechanism of adaptation to the stress.

Methodology: At LD₅₀ dose, the proteome dynamics was studied using iso-electric focussing (17 cm IPG strip with 3-10 pI) followed by 14% SDS-PAGE. Differentially expressed proteins were identified by MALDI mass spectrometry based peptide mass fingerprinting.

Results and Discussion: In response to MV, proteome of *Anabaena* 7120 displayed increased or decreased abundance of 42 or 32 proteins, respectively. MV elicited a strong oxidative stress alleviation response in *Anabaena* as elevated levels of ROS detoxifying enzymes (Prx, NTR, Dps, PetH, FMNr, PAPr, Alr4404 and Alr7524) were observed. Cellular damage by MV was also well managed by increased abundance of DnaK, GroEL, PNPase, RP-L9, ArgS, EF-G, EF-Tu and EF-Ts proteins. Levels of proteins involved in photosynthesis were drastically reduced along with those of enzymes involved in carbon fixation and central metabolism was also modulated following MV exposure.

Conclusion: The major targets of MV sensitivity in *Anabaena* 7120 are phycobilisomes of PSII. MV hampers the production of reducing power and ATP which further limits carbon fixation. The organism counters MV stress by (a) restoring energy by redirecting metabolic flux through OPP, (b) enhancing ROS scavenging abilities and (c) restoring cellular homeostasis.

Poster no: D-3-25**Proteomic analysis of drug treated *Klebsiella pneumoniae*: Exploring potential targets for future drug designing**

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Increasing antibiotic resistance is a very serious problem being faced by microbiologists and physicians worldwide. The problem is getting worse since resistance against “last resort” carbapenems has emerged. Bacteria generally counter the effect of antimicrobials through their cleavage/modification/efflux or over expression/modification of targets. Antimicrobials initiate a whole cascade of mechanisms that involve different proteins besides beta-lactamases. It is the combined effect of many proteins that confer resistance to bacterial cells and these may be attractive targets for developing drugs, immunodiagnostics or therapeutics. Our goal in this study was to identify proteins other than carbapenemase (KPC), involved in conferring resistance to bacteria. In this study, Two-dimensional gel electrophoresis (2-DE), matrix assisted laser desorption/

ionization time of flight (MALDI-TOF) and *in silico* methods were employed to study the whole cell proteome of a clinical *Klebsiella pneumoniae* strain NP6 harboring bla_{KPC-2} in presence of a carbapenem drug meropenem at its sub-MIC concentration and in its absence. On comparing 2-DE patterns, 16 spots with differential expression of ≥ 2 folds were selected for analysis by MALDI-TOF. Of these, 11 proteins were consistently over expressed in the treated condition with antibiotic whereas 5 proteins appeared only in the protein profile of the treated Bacteria.

Poster no: D-3-26**Genomic analysis of *Colletotrichum gloeosporioides*, an endophytic fungi, Lifestyle transition in host**

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Introduction: Endophytic fungi are source of valuable compounds, useful in pharmaceutical/agricultural industry eg. Taxol. Our aim was to isolate the true endophytic fungi, from the endangered forest tree *Cynometra travancorica* and analyse the organism genetically.

Methodology: Isolation of true endophytic fungi was on Oat meal agar. The sample was collected from 4 different forest areas of Kerala. Genomic DNA was isolated using standard procedures and partial sequencing was carried out. The D1/D2 region of LSU (Large subunit 28S rDNA) gene sequence was used to carry out BLAST with the nr database of NCBI genbank database.

Results and discussion: *Colletotrichum gloeosporioides* was identified as true endophyte from *Cynometra travancorica*, possessing dispensable, potential pathogenicity – associated chromosomes that can be horizontally transferred between compatible strains. The partial sequencing result of the organism was 100% matching in BLAST study with earlier reports. Six scaffolds from the fungi sequenced earlier were similar to our result. The gene densities are below the genome average. Only two of the scaffolds have GC contents of below 50%. Majority of genes identified on these scaffolds encode protein with no homology to known sequence in NCBI non redundant database which are characters of fungal effectors, proteins that have important role in disabling the host defense system. Evidence of molecular mimicry for the production of subtilisin by horizontal gene transfer from the plants have been reported. The genus *Colletotrichum* consists of 29 to 700 species. *C. gloeosporioides* has a long history as a model pathogen for fundamental, biochemical, physiological and genetic studies. Their biotrophic life strategies contribute their prominence as symptomless endophytes of living plant tissues..

Conclusion: *Colletotrichum* exhibit different forms in their life cycle even within a single species like Biotrophy → Necrotrophy → Endophytic → Latency. It's a unique opportunity for comparative proteomic analysis of their association with plants. Our ongoing study is on the same.

Poster no: D-3-27

CaTLP1 promotes vegetative growth in plants and enhances stress tolerance through ABA-dependent signalling

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Introduction: Tubby-like proteins (TLPs) feature a characteristic “Tub domain” and are found in all multicellular organisms. While a wide array of cellular functions of TLPs have been established in animals, their role in plants is still elusive. We screened a stress-responsive extracellular proteome of chickpea and identified a novel TLP, designated CaTLP1. We functionally characterized CaTLP1 and explored the possibilities for the targeted genetic manipulation in crop plants for stress tolerance.

Methodology: The function of CaTLP1 was investigated by complementation in *S. cerevisiae* and *Arabidopsis* mutants followed by overexpressed seedlings. *CaTLP1* regulatory sequence was analyzed for tissue-specific and stress-responsive expression. Quantitative transcript abundance of CaTLP1 in wild-type, mutant and complemented *Arabidopsis* seedlings were quantified by QRT-PCR. The proteomes of the seedlings were characterized by classical 2-DE coupled with LC-ESI-MS/MS. Interactome network was determined by pulldown and direct immunoprecipitation.

Results and Discussion: The expression analysis revealed that *CaTLP1* is involved in multivariate stress response. The stress induced expression of CaTLP1 is regulated through ABA-dependent pathway. Overexpression of CaTLP1 in transgenic plants conferred stress tolerance along with improved shoot and root architecture. Functional complementation of the yeast mutants established that CaTLP1 also functions in oxidative stress tolerance. Proteomics analysis revealed 96 differentially expressed proteins which might be involved in maintaining physiochemical process under dehydration.

Conclusions: Molecular genetic analysis displayed differential expression of CaTLP1 under stress conditions, and preferential expression in the nucleus suggest its correlative association with enhanced stress tolerance. Analysis of the interactome and identification of differential protein profile could lead to the new insight into the stress-responsive protein(s) in altered cellular condition.

Poster no: D-3-28

Secretome analysis of chickpea reveals dynamic extracellular remodeling and identifies CaRRP1, a leaderless Bet v 1-like protein that participates in stress response

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Introduction: Secreted proteins maintain cell structure and biogenesis besides acting in signaling events that are crucial for cellular homeostasis during stress adaptation. Plant secretomes usually contain significantly more leaderless secretory proteins (LSPs) that participate in stress response.

Methodology: The cell suspension culture of chickpea were prepared in liquid MS media and inflicted with dehydration.

The purity of the secretome was established by enzymatic assay and immunoblot analysis. Secreted proteins were characterized by 1- and 2-DE coupled with LC-ESI-MS/MS. The cDNA encoding a LSP, designated CaRRP1, was cloned and the gene structure and genomic organization were investigated. Stress regulated expression of *CaRRP1* was determined by QRT-PCR and functional activity was examined in yeast mutant *YJL036w*.

Results and Discussion: The cell viability was found to be maintained until 96 h, but gradually declined at later stages of dehydration. There was a steady increase in RWC during the early stages which could be positively correlated with increased proline content, causing osmotic adjustment. Proteomic analysis led to the identification of 789 proteins presumably involved in multivariate cellular processes. The dynamic range of PTMs in the secretome implies the systemic regulation of protein secretion. One-third of the secreted proteins displayed no N-terminal secretion signals suggesting a nonclassical secretion route. Screening of the secretome identified a novel leaderless protein, CaRRP1. Functional complementation of CaRRP1 could rescue the growth defects in *YJL036w* indicating its stress-responsive role.

Conclusions: This represents the first comprehensive analysis of dehydration-responsive plant secretome and the complex metabolic network operating in the extracellular space, which remained largely uninvestigated.

Poster no: D-3-29

Proteomic analysis of elicitation of Downy Mildew disease resistance in Pearl Millet by seed priming with β -aminobutyric acid and *Pseudomonas fluorescens*

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Introduction: Pearl millet is the seventh most important crop globally. It is one of the most drought tolerant crops which serve as staple crop for poorer sections of Asia and Africa. However, the commercial production of the crop is affected to about 50% by an obligate biotroph- *Sclerospora graminicola*. In our study, we used proteomic approach to investigate the elicitation of downy mildew disease resistance in pearl millet by biotic and abiotic elicitors.

Methodology: Initially, pearl millet seeds primed with elicitors (BABA and *P. fluorescens*) were challenge inoculated with the biotrophic pathogen before being germinated for two days. Protein extracted from seedlings of different treatments were fractionated by 2DE and the differentially accumulated proteins were further analyzed by nano-LC/MS/MS, database search, univariate and multivariate statistics and classified based on their functional process, sub-cellular localization. Gene enrichment tools like DAVID and STRING were employed for pathway analysis.

Results and discussion: Among the 63 differentially accumulated proteins detected by 2DE experiment, 50 were identified by mass spectrometric analysis. Univariate statistics revealed that significant difference were observed between treatments based on the protein expression profile. Multivariate statistics revealed clustering of samples between treatments based on pathogen inoculation and not on elicitor treatment types. Most of these proteins belonged to energy metabolism and defense category. Gene enrichment analysis and protein abundance profile revealed that elicitors induced PR-protein and

proteins of carbohydrate metabolism might contribute to plant defense and development.

Conclusion: Seed priming with elicitors, indeed had a major impact on dynamics of protein abundance in pearl millet seedling proteome by providing protection against the plant pathogen. Thus, these elicitors could be tried as an alternative strategy for disease management. However, output from this study needs to be further validated by experiments to obtain much better picture on the rescue effect provided by these elicitors.

Poster no: D-3-30

Study of enzyme induction during decolourization of Acid blue 158 using a novel bacterial consortium SDG

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Introduction: Decolourization of dyes involves a biochemical process utilizing enzymatic machinery of the bacteria itself. Use of enzyme became an alternative to conventional physico-chemical treatment with regards to decolourization and degradation of dye containing wastes. Various different oxidoreductive enzymes are studied for their involvement in cleavage of dye molecules. In this study, presence of some dye degradative enzymes and their induction in the presence of Acid blue 158 was investigated.

Methodology: Decolourization of Acid blue 158 was studied at different time interval using nutrient broth containing 50 ppm of dye. Induction of Azoreductase, Laccase, Tyrosinase, Lignin peroxidase and DCIP - reductase were studied using earlier developed methods. Enzyme activity was further confirmed by extraction of intracellular protein fraction followed by its native PAGE and gel zymography using Acid blue 158 as substrate.

Results and Discussion: Decolourization profile of Acid blue 158 by SDG consortium showed > 85% decolourization in just 12 h and ~94% decolourization after 36 h of incubation. Significant induction was found in the activity of intracellular DCIP reductase and azoreductase which was 146% and 132%, respectively. Intracellular tyrosinase was induced by 133.33%, whereas 111.53% intracellular laccase activity was recorded. Enzyme activity was not recorded in case of laccase, tyrosinase, DCIP reductase and azoreductase in the extracellular enzyme fraction. It is interesting to know that lignin peroxidase activity was found constant in intracellular as well as extracellular enzyme fraction. Appearance of decolourized gel band confirmed the presence of dye decolourizing enzymes in the protein sample.

Conclusions: Consortium SDG can potentially decolourize Acid blue 158. Presence of dye was found to induce enzymes. Induction of azoreductase and NADH-DCIP reductase showed involvement in dye decolourization. Results of zymography supported the findings.

Poster no: D-3-31

Purification assay and production optimization of antifungal protein from *Aspergillus giganteus* (MTCC No: 8408) Using Taguchi statistical approach

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Introduction: *Aspergillus giganteus*, an imperfect ascomycete, has been found to secrete protein molecules of low molecular mass, may be cationic or anionic character and a high content of cysteine residues stabilized by disulfide bridges (Nakaya, K., et

al., 1990) and potent inhibitors against the germination and growth of filamentous Ascomycetes (Olson, B. H., et al., 1965).

Methodology: The producing strain *Aspergillus giganteus* (MTCC No: 8408) was cultivated in modified nutrient media containing soluble starch, beef extract, peptone, NaCl, in presence of variable concentration of K_2HPO_4 , $MgSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$ and pH. Isolation and purification of proteins were done by using a combinatorial approach of $(NH_4)_2SO_4$ precipitation followed by Carboxy methyl Cellulose chromatography (CMC). Assay (fungicidal/fungistatic) against a model organism, *Aspergillus niger* and optical density was measured at 600 nm. Production optimization was done in accordance with Taguchi's L 9 orthogonal array, four factors at three levels of variations. The effect of these factors was identified using the Analysis Of Variance (ANOVA) based on Signal to Noise (S/N) ratio.

Results and Discussions: $FeSO_4$ and K_2HPO_4 were shown to have strongest effect in the tested range (main effect plot). $MgSO_4$ and pH does not show a considerable effect. Antifungal activities of proteins (AFP) based on the inhibition of proliferation of *A. niger*, were shown in 70-90% fraction [$(NH_4)_2SO_4$ precipitation] after subsequent CMC chromatography and found to possess fungistatic property (20-30 $\mu g/ml$, MIC_{50}). The levels optimized are shown below: K_2HPO_4 : 4 g/lit, $MgSO_4$: 0.5g/lit, $FeSO_4$: 0.004 g/lit, pH: 5.75.

Conclusions: AFP has been found basic in character. The closeness of the actual and predicted values determined the validity of the model and hence Taguchi's design proved to be powerful tool in optimizing production in our study. Further developments of a novel purification strategy are under process to have more potent activity of AFP.

Poster no: D-3-32

Global proteome analysis of *Bacillus subtilis* under a Plant-derived Naphthoquinone: Plumbagin

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Introduction: Infectious diseases are prime threat for humankind mostly due to the increasing antibiotic resistance among the diverse microbes. Bacterial cell division is an attractive antibacterial target. Plumbagin is a plant-derived naphthoquinone, a natural product which exhibits antibacterial activity against the gram-positive bacteria by targeting the cell division.

Methodology: In the present study, we investigated the effect of plumbagin on bacterial cell division by monitoring the morphological changes using fluorescence microscopy. We performed quantitative proteome analysis of *Bacillus subtilis* using 2DE and iTRAQ using LTQ-Orbitrap and Q-TOF mass spectrometry. Further, the gene expression analysis, metabolic activity assay and molecular docking were performed to validate a few interesting proteins.

Results and Discussions: Plumbagin treatment leads to the filamentous morphology of the bacteria with multiple nucleoids and global quantitative proteome analysis indicated the differential expression of 230 proteins (1% FDR, ≥ 1.5 fold-change and ≥ 2 peptide). Most of these proteins were found to be involved in heme biosynthesis, TCA cycle, protein synthesis, fatty acid synthesis and cell division. TCA cycle was found to be repressed significantly, which suggests a defect in electron transport chain to regenerate the NAD required for crucial enzymatic reactions. Moreover, the molecular docking analysis of NADH dehydrogenase in *B. subtilis* and the previous reports on *E. coli*

has pointed out that plumbagin blocks the action of this enzyme required for the respiratory activity.

Conclusion: We anticipate that plumbagin blocks the cell division by altering the membrane permeability required for energy generation. This is the first report, to the best of our knowledge, providing new insights at a proteome level, which suggests putative mode(s) of action of plumbagin and attendant cellular targets in *B. subtilis*. The findings also suggest new ways forward for the modern omics-guided drug target discovery, building on traditional plant medicine.

Poster no: D-3-33

Proteomics investigation of mitosis and meiosis in budding yeast

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Introduction: Mitosis and meiosis are two different types of cell division cycles with entirely different and definite biological consequences for almost all the eukaryotic species. In presence of fermentable carbon source and plenty of nitrogen, budding yeast undergo rapid mitosis while in absence of fermentable carbon and in absence of nitrogen, cells switches to meiotic mode. Owing to mechanistic difference associated with chromosome segregation during mitosis and meiosis and recent genomics studies it is anticipated that proteome of cell undergoing mitosis and meiosis varies.

Methodology: We performed 2-DE as well as 2D-DIGE to investigate the differential expression of proteins in mitosis and meiosis. Proteomics data was verified by western blot analysis. Differential phosphorylation analysis was done by gel based phosphoproteomics using Pro-Q Diamond stain specific for phosphoproteins.

Result and Discussion: 2-DE and 2D-DIGE identified 26 unique proteins, which showed differential abundance in mitosis and meiosis. Western blot analysis verified our proteomics data and protein stability assay suggests that increased abundance of proteins in meiosis is a result of increased protein stability. Phosphospecific staining of 2-DE gels showed that increased abundance in meiosis may be due to post-translation modification of proteins in the form of increased phosphorylation in meiosis. Further iTRAQ based experiment support our gel based experiments. Proteins identified in gel based and iTRAQ experiment follows same trends. Cell biological experiment also suggests that yeast Bmh1 and Bmh2 have overlapping role in meiosis. Deletions of either BMH1 or BMH2 alone have no effect on sporulation efficiency and spore viability. Our present work shows that yeast Bmh1 and Bmh2 shows increased abundance and phosphorylation in meiosis and both share overlapping function in meiotic cell cycle.

Conclusion: Using gel based proteomics we identified several interesting proteins, including BMH1, which shows differential expression in mitosis and meiosis. Further increased abundance of proteins in meiosis may be due to the increased protein stability or post-translation modification.

Poster no: D-3-34

Exploring the membrane proteome of diazotropic cyanobacterium *Anabaena* PCC7120 through combined proteomics and *in silico* approaches

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Introduction: Membrane proteins are implicated in key cellular functions such as small molecules transport, cell-cell and/or cell-pathogen and/or cell substrate recognition and interaction, cell communication and signalling. Therefore, identification and characterization of these proteins is necessarily required to elucidate cellular mechanisms. In present study diazotrophic *Anabaena* sp. PCC7120 is used as model organism due to its agriculture significance as biofertilizer, close resemblance with higher plant chloroplast and availability of full genome sequence. While the membrane proteome of *Synechocystis* sp. PCC 6803 have been extensively studied only a few reports dealing with the outer membrane proteins of *Anabaena* were reported although total membrane proteome of this organism have remained unexplored as yet.

Methodology: Membrane proteins were extracted by employing a protocol described by Hall *et al.*, 2011.

Results and Discussion: In total 62 different proteins were identified from 2D gels by MALDI-TOF MS/MS analysis. Of these protein 14, 2 and 5 proteins posses Sec, TAT and lipoprotein signal respectively. 23 proteins were predicted to be integral membrane proteins having atleast one trans-membrane segment, including 7 unique proteins namely protochlorophyllide oxido-reductase, Na⁺/H⁺-exchanging protein, Alr1355, Alr2055, Alr3514, Alr2903 and Alr2751 as new entries to the proteome of *Anabaena*. The membrane protein profile was quite distinct from the cytosolic one containing proteins of outer membrane, cell envelope, periplasm, plasma and thylakoid membrane. Identified proteins includes the most abundant outer membrane porins i.e. Alr0834, Alr4499 and Alr4550; cell envelope proteins i.e. chloroplast membrane-associated 30 kD protein (All2342) and chloroplastic outer envelope protein homolog TolC (Alr2269); transport and binding proteins i.e. ABC transporter ATP binding protein (Alr2372), Na⁺/H⁺-exchanging protein (Alr2264), phosphate ABC transporter (All4575) and bicarbonate transport ATP-binding protein (Alr2880) and components of photosynthetic machinery.

Conclusion: This study provides a valuable reference for future membrane proteins studies in N₂-fixing cyanobacteria.

Poster no: D-3-35

Molecular mechanism of cysteine induced toxicity using *Saccharomyces cerevisiae* as a model system

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Introduction: Cysteine, a proteinogenic amino acid synthesized via the trans-sulfuration pathway is important for many vital processes in a cell. However, besides being biologically important, several studies have shown that excess cysteine is toxic in many organisms. An elevated level of cysteine has also been associated with neurological and cardiovascular diseases. However, the

mechanism of cysteine's toxicity is not well understood. This study is focused on delineating the mechanism of cysteine induced toxicity using *Saccharomyces cerevisiae* as a model system.

Methodology

Proteomics Approach: iTRAQ-4plex (isobaric tag for relative and absolute quantitation) and SWATH-MS (Sequential windowed acquisition of the theoretical masses) based proteomics quantification was used to study the proteome change induced due to cysteine treatment.

Genome wide deletion screen: Yeast deletion library in 96 wells format was used, cells were grown with and without cysteine for 12 hrs and relative cell density was measured using multimode reader.

Results and Discussion: Cysteine induces growth defect in yeast strain BY4741 (MATa his3⁰ leu2⁰ met15⁰ ura3⁰) in a dose dependent manner. Using both SWATH and iTRAQ based approach we quantified 1368 proteins, among which 146 proteins were up regulated and 256 were down regulated. Cysteine treatment altered the expression of the proteins involved in amino acid metabolism. With the help of amino acid screening we found that leucine reverted the growth inhibition induced by cysteine. We also show that cysteine induced growth defect is accentuated if the transcription factor (leu3) required for branched chain amino acid synthesis is deleted.

From the genetic screen of around 2000 non-essential genes we found that deletion of genes involved in vacuolar-ATPase assembly imparts resistance to cysteine toxicity.

Conclusion: Leucine plays an important role in survival during cysteine induced stress. Functional Vacuolar-ATPase is required for cysteine induced toxicity.

Poster no: D-3-36

Mass spectrometry based PTMs identification of covalently modified targets of β -lactam antibiotics in *Staphylococcus aureus*

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Introduction: Methicillin resistant *S. aureus*, abbreviated as MRSA is one the greatly feared strains of *S. aureus*, which has become resistant to most antibiotics. In this scenario *S. aureus* has become a paradigm for understanding the molecular mechanisms of multiple drug resistance and identify the novel targets. Many antibiotics including β -lactam act on bacteria by way of covalent modifying the target proteins. The β -lactams are by far the most widely used and efficacious of all antibiotic. The established target proteins for β -lactam are enzymes that catalyze late steps in murine biosynthesis, a bacteria-specific pathway. However, it is possible that β -lactam can modify other cytosolic proteins of the bacteria and cause cytotoxicity. Therefore, in this study we have attempted to identify β -lactam modified proteins, which could be potential new targets.

Methodology: *S. aureus* (MRSA) isolated from human fetal blood sample, 16S rDNA gene identification was performed to check the phylogenetic position of strain, sensitive *S. aureus* collected from NCIM, CSIR-NCL Pune, for *in vitro* and *in vivo* studies. The Minimum inhibitory concentration (MIC) of benzylpenicillin for *S. aureus* (MRSA) was established. The *in vitro* and *in vivo* samples were digested and data acquired by LC MSE approach (Synapt HDMS, Waters and Thermo Easy nLC1000-Q Exactive Orbitrap Mass Spectrometry). The *in silico* analysis preformed PatchDock online server <http://bioinfo3d.cs.tau.ac.il/PatchDock/>

Results and Discussion: The 16S rDNA gene sequence blast on NCBI showed 99% similarity with *S. aureus*. Totally 67 proteins were found to be *in vitro* modified by β -lactam, of which 7 and 3 proteins were found to modified *in vivo* in sensitive and resistant *S. aureus*. The *In silico* analysis showed that the benzylpenicillin (β -lactam) is in the proximity of the proteins.

Conclusion: This strategy resulted in identification of novel targets of β -lactam. The usefulness of these proteins as drug target needs to be evaluated.

Poster no: D-3-37

Identification of novel CDPK substrates in *Plasmodium falciparum* by mass spectrometry based proteomic, phosphoproteomic and metabolomic analyses

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Introduction: It is now clear that several effectors of second messenger signaling including CalciumDependent Protein Kinases (CDPKs), have emerged as key players in parasite development. Since CDPKs are absent from the host they are considered to be important drug targets. However, the parasitic substrates and targets of most CDPKs have not been identified, which has contributed significantly to the lack of understanding of their function. To achieve this goal, we carried out mass spectrometry based proteomic and phosphoproteomic analyses of *Plasmodium falciparum* strains in which key CDPKs were genetically manipulated. Since preliminary data suggested that some of the CDPKs may be involved in lipid metabolism, metabolic profiling was also carried out.

Methodology: Equal amount of protein was isolated from *Pl. falciparum* wild-type and CDPK deficient parasites. Proteins were reduced, alkylated and enzymatically digested using trypsin. Peptides from both experimental and control samples were labeled with iTRAQ and fractionated using basic Reverse Phase Liquid Chromatography. A fraction of the sample was analyzed on LTQ Orbitrap Velos mass spectrometer for comparative proteomic analysis and rest was processed for TiO₂-based enrichment of phosphopeptides. Metabolomic analyses of the samples were carried out on quadrupole time-of-flight mass spectrometer.

Results and Discussions: Phosphoproteomic analysis led to identification of more than 500 phosphopeptides, out of which, several phosphopeptides exhibited differential phosphorylation in CDPK-deficient strain. However, these proteins, which represented CDPK targets, showed no change in level of expression as revealed by comparative proteomic analysis. Some of these proteins were phosphorylated by recombinant CDPKs in *in vitro* kinase assays suggesting that these proteins may be putative substrates for CDPKs. Comparative metabolomic analyses revealed several metabolites, which were differentially regulated in CDPK-deficient strain.

Conclusions: Several putative CDPK substrates were identified by using high-throughput proteomic and phosphoproteomic analyses followed by validation using biochemical assays. Studies are in progress to ascertain the role of phosphorylation of the substrates in their parasitic function. Metabolomic studies revealed that some of the CDPKs may be involved directly or indirectly in lipid metabolism and studies directed at understanding the underlying mechanisms are in progress.

Poster no: D-3-38**Proteomic landscape of *Candida tropicalis* - an opportunistic pathogen**

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Introduction: *Candida tropicalis* is an opportunistic pathogen causing candidiasis in immunocompromised and immunosuppressed patients. Although whole genome sequence was made available in 2009, most proteins predicted have not been validated at level of proteome. Therefore, we carried out an in-depth proteomic analysis of cell lysate and conditioned media using high-resolution mass spectrometry. Further, we used this proteomic data to refine existing genome annotation using proteogenomic approach.

Methodology: *Candida tropicalis* (MTCC 184) was cultured in yeast nitrogen base media and proteins were obtained from cell pellet and conditioned media. Multiple fractionation techniques were employed followed by high-resolution mass spectrometry analysis (LC-MS/MS). The MS/MS data was searched against *Candida tropicalis* protein database and hypothetical six-frame translated genome database using SEQUEST and Mascot using Proteome Discoverer 1.4 software suite.

Results and Discussions: We identified 13,434 peptides corresponding to 2,316 proteins from a total of 125,499 MS/MS spectra. Literature survey and bioinformatics analysis of the proteins identified in conditioned media inferred 74 secreted proteins. Orthology-based approach revealed a majority of these proteins to be associated with virulence in *Candida albicans*. Using proteogenomic approach, we identified 516 unique genome search specific peptides (GSSPs) that resulted in identification of 70 novel genes, 11 novel exons and 20 other corrections of gene models.

Conclusions: In-depth proteomic analysis of *Candida tropicalis* provided experimental evidence for the predicted proteome. Use of proteogenomics strategy enabled identification of several novel protein-coding genes and refinements to the existing gene models.

Poster no: D-3-39**Functional characterization and kinetic studies of Chalcone synthase from *Emblica officinalis* Gaertn**

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Introduction: Flavonoids are important plant secondary metabolites that mediate diverse biological functions such as chemical messengers and antioxidants. One of the main key enzymes involved in the flavonoid biosynthesis is chalcone synthase (CHS). CHS catalyzes the condensation of p-coumaroyl-CoA with three C₂units from malonyl-CoA producing a naringenin chalcone from which diverse flavonoid end products are derived. *Emblica officinalis* Gaertn commonly known as gooseberry belonging to the family Euphorbiaceae is one of the

most important medicinal plant species found in South Asian subcontinent. Keeping in view the immense medicinal properties attributed to this plant, studies were initiated on understanding the molecular aspects of biosynthesis of secondary metabolites imparting these properties. Here we have functionally expressed EoCHS and have studied various kinetic parameters via Surface Plasmon resonance.

Methodology: Recombinant EoCHS was expressed as a fusion protein in pET23b-vector system along with Trx-His tag in *E.coli*BL21 (DE3) cells. EoCHS was purified by NTA – affinity chromatography. Reactions were carried out with 45 µg of protein and various substrates at 30 °C for 2 hours. Radio labeled products was resolved on silica gel TLC plates and analyzed by reverse phase HPLC. Various kinetic parameters including substrate concentration, pH and thermal stability of the recombinant EoCHS was analyzed by Surface Plasmon resonance (SPR).

Results and Discussion: The purified enzyme was of 62kDa with a yield of 1.4mg/L. The recombinant EoCHS showed functional divergence and was able to catalyze all substrates ranging from aliphatic to aromatic. The kinetic constants for each substrate were determined which resulted in differences in enzyme activity. The maximum catalytic activity of EoCHS was found at an optimum pH of 7 and temperature of 25°C.

Conclusion: The overall functional and kinetic studies of EoCHS provide novel strategies for combinatorial biosynthesis of unnatural pharmaceutically important polyketides.

Poster no: D-3-40**Proteomic identification of allergenic proteins from Date Palm (*Phoenix sylvestris*) pollen**

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Introduction: Allergy to date palm pollen is common in people inhabiting in suburban areas of West Bengal. An immunoproteomic approach has been employed to identify IgE reactive proteins from date palm pollen.

Methodology: 10 patients along with two controls participated in the experiment. Clinical tests such as skin prick test and specific IgE ELISA was done. Total protein was profiled on a 4-7 pI range IPG strip. One dimensional and two dimensional immunoblot with patient specific sera were performed. Glycoprotein staining to ascertain presence of glyco moiety in allergic protein and histamine release assay of allergic sera was done. MALDI TOF/ TOF was used to acquire MS/MS spectra from the IgE reactive spots. A proteomic workflow employing *de novo* sequencing was developed. Data generated from sequencing was thereafter used in homology based search to confirm the identification of the proteins.

Results and Discussions: Patients showed a positive correlation with skin prick test, Specific IgE ELISA, Histamine release and western blot data. 1D western blot showed a total of 16 IgE reactive bands. 80% of the patients showed allergic reactivity in the 43Kd region and hence may be termed as major allergen. A total of 170 spots were well resolved in the 2D gel using coomassie stain. 2D immunoblot showed a total of 15 allergic spots. Their intensities and concentrations were calculated with an internal standard. Using a layered proteomic workflow, all the 15 proteins were identified. Type 3 membrane protein of molecular weight 40Kd was found to be glycoprotein in nature and was of highest intensity.

Conclusions: Date Palm pollen is allergic in nature. *De novo* sequencing holds the promise of identifying proteins from

unsequenced genomes. Type 3 membrane protein, a glycoprotein may be termed as a major allergen and can be further studied in detail.

Poster no: D-3-41

Insights in to the protein- small molecule interaction of Quinolone synthase from *Aegle marmelos* Corr

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Introduction: Type III polyketide synthases (PKS) are fascinating group of enzymes responsible for the synthesis of an array of natural products known as polyketides with significant pharmacological properties. *Aegle marmelos* commonly known as Bael or wood apple is a tree native to India, present throughout Southeast Asia. Quinolone alkaloids, found abundantly in *A. marmelos* possess diverse biological activities and have recently gained attention as potential lead molecules for novel drug designing. Resmi *et al.*, (2013) already reported a novel Type III PKS named quinolone synthase (QNS), from bael that is involved in the biosynthesis of quinolone. In this study, we have used surface plasmon resonance (SPR) based high-throughput system to study and characterize protein “ small molecule interactions which may play an important role in regulatory biological processes.

Methodology: Recombinant QNS was functionally expressed in *E.coli* BL21 DE3 as an N-terminally Trx-His-tagged protein and was purified by Ni-NTA affinity chromatography. Further, affinity-purified fusion protein was immobilized on a sensor chip inserted into the flow chamber of a ProteOn™XPR36 Protein Interaction Array System (*BioRad*). Ligand molecules varying from small to bulkier CoAs were used in the flow-through analyte, as mobile phase.

Results and Discussions: The recombinant QNS showed interaction with many substrate molecules ranging from small aliphatic to bulkier aromatic CoA's. The receptor-ligand binding interactions, based on binding affinity, thermodynamics and ligand efficiency, were determined by SPR which will be accountable for the difference in enzyme activity. The resultant data showed that the QNS-substrate binding interactions were detected in a concentration dependent manner, signifying that the SPR-based studies can be a valuable tool to analyze protein-small molecule interactions where multiple ligands are involved.

Conclusions: The overall studies and findings will provide an insight into the reaction mechanism for the enzyme that can be used to generate novel pharmaceutically valuable product.

Poster no: D-3-42

Understanding mechanisms for rice yield under drought: Proteome and protein perspective

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Introduction: Rice is a critically important food crop directly related to alleviating hunger and poverty in Asia & Africa. Deciphering the molecular mechanisms that underlie the plants' response to drought are challenging due to complex interplay of genes, proteins & metabolites. Quantitative proteomic and

targeted metabolite analysis was conducted on parental and drought tolerant NILs for QTL *DTY*_{12.1} at the reproductive stage. The *OsNAM*_{12.1} a prime candidate gene was studied to understand its role in rice root architecture and yield under drought.

Methodology: Tandem mass tag (TMT) proteomic approach led to the identification of 991 proteins, which were further analysed using MapMan tool. Metabolic profiling using LC-MS/MS based analysis was used to study the content of sugars, sugar alcohols, starch, C-N and free amino acid content. *In vitro* biochemical assays and 2D western blots were performed to understand the SUMOylation status of *OsNAM*_{12.1}.

Results and Discussion: Drought stress caused changes in the expression of proteins involved in photosynthesis, carbohydrate metabolism and C-N acquisition/remobilization. This study mainly suggested that drought tolerant NILs showed better source and sink capacity with increased sucrose and starch content in their spikelets. Roots of these plants also suggested an interesting strategy of reserving sufficient carbon sources and energy for the growth of lateral root. The change in the *OsNAM12.1* 2D-immunodetection patterns suggested deSUMOylation in the NILs compared to the parents. An ubiquitin protease from the epistatic loci was demonstrated to act as a deSUMOylating protein.

Conclusions: The importance of differential drought-mediated deSUMOylation of *OsNAM12.1* and its potential role in explaining the epistasis noted for a functional *qDTY12.1* has been elucidated. This is the first comparative proteomic-metabolomic study on field proven drought tolerant NILs and presents an interesting overview of the mechanisms involved and the network of events that lead to increase yield under drought.

Poster no: D-3-43

Heterologous expression, characterization and *in silico* approach for structural analysis of phosphomannose isomerase (PMI) from *Cyamopsis tetragonoloba*

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Introduction: Phosphomannose isomerase (PMI) or Mannose-6-phosphate isomerase (EC 5.3.1.8) is an enzyme that catalyses interconversion of mannose-6-phosphate to fructose-6-phosphate. It plays a vital role in biosynthesis of GDP-mannose, an important precursor of many polysaccharides. PMI can be classified into three major types based on sequence similarity and domain organization. Type I PMIs are monofunctional enzymes found in all eukaryotes including yeast, *Candida albicans*, and certain bacteria including *Escherichia coli*, carrying out Man-6-P"!Fru-6-P interconversion. Type II PMIs are bifunctional enzymes having both PMI and GDP-mannose pyrophosphorylase activities, carrying out synthesis of capsular polysaccharides and D-mannose metabolism. PMI provides the only known metabolic link between glycolysis and GDP-mannose needed for glycoconjugate synthesis.

Methodology: The 3D modelled structure of PMI from *Cyamopsis tetragonoloba* was generated by homology modelling using 1PMI as template. The model having lowest DOPE Score was further evaluated using PROCHECK and ERRAT plot after energy minimization using Swiss-Pdb Viewer

4.01 (<http://spdbv.vital-it.ch/>). Secondary structure analysis was done by Sable 2. The biophysical techniques like Circular dichroism (CD), fluorescence spectroscopy and differential scattering calorimetry (DSC) were used to study properties like secondary structure, intrinsic fluorescence and melting temperature of purified protein for characterization of purified protein.

Results and Discussion: In this study, guar phosphomannose isomerase was purified by heterologous expression of its cDNA in *E. coli* Rosetta (DE3). CD analysis showed protein to be stable at pH range of 6 to 8 and temperature range of 25°C to 60°C. Cofactors like Zn²⁺ and Mg²⁺ provided stability to the protein. Further, melting temperature (Tm) was reconfirmed by DSC.

Conclusions: PMI is a typical metalloenzyme that has a cupin domain. The residues which were involved in interaction with Zn²⁺ were Gln117, His 282, His 119, and Glu 144. Isolation of pure recombinant protein will be further used for kinetic and structural studies.

Poster no: D-3-44

Partial purification and characterization of an intracellular mycelial lectin from *Fusarium compactum*

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Introduction: Lectins are non-immune carbohydrate binding proteins or glycoproteins with specific binding sites for certain glycoproteins. The omnipresence of lectins in biosphere plays a crucial role in diverse biological processes. Surprisingly due to their multifaceted properties, biological role of lectins is speculative and they are a subject of intensive investigations. Recent research showed a remarkable development in lectin science as their use in studying glycoprotein biosynthesis, mapping neuronal pathways, anti-cancer therapies and in mitogenic stimulation of lymphocytes is well documented.

Methodology: In the present study, an intracellular mycelial lectin has been partially purified and characterized from *Fusarium compactum* MTCC 2014. Biological action spectrum and carbohydrate inhibition studies of lectin were assayed by standard method (Singh *et al.* 2008). Partial purification of *Fusarium compactum* lectin was done by conventional purification techniques (Bollag *et al.*, 1996). Antimicrobial activity of lectin has also been evaluated against *Staphylococcus aureus* MTCC 3760, *Bacillus cereus* MTCC 430, *Escherichia coli* MTCC 1302, *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 281 by agar diffusion assay with slight modifications (Chumkhunthod *et al.* 2006).

Results and Discussions: *Fusarium compactum* lectin revealed agglutination of all human blood types (A, B, O, and AB) and rabbit erythrocytes. Carbohydrate inhibition studies showed affinity for D-fructose, D-mannitol, 2-deoxy-D-ribose, bovine submaxillary mucin, porcine stomach mucin, asialofeitin, inulin, dextran and γ -globulin. The purification protocol resulted in 21.3-fold purification with a yield of 80%. Optimum pH and temperature for lectin activity was observed to be 4.5-6.5 and 30-45°C, respectively. Lectin was thermostable and could withstand temperature up to 70°C. Lectin from *Fusarium compactum* showed significant antifungal activity against *Aspergillus niger*.

Conclusions: The unique carbohydrate specificity of *Fusarium compactum* lectin advocates their purification using lectin affinity chromatography. The antimicrobial activity of *Fusarium compactum* illustrates their significance in clinical applications.

Further, these findings could be used in the next generation medicines, if efficient research is contributed in their understanding.

Poster no: D-3-45

Bioremediation of reactive azo dye containing wastes: stimulation of oxido-reductive enzymes and zymography of induced reductive enzymes in novel bacterial consortium TSR-2

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Introduction: "Bioremediation" has become a key microbial tool to deal with different pollutants in the field of environmental science. Due to complexity of structure and electron withdrawing capacity of azo dyes, they are very difficult to biodegradation. Degradation of azo dyes and their wastes is mainly mediated by the oxido-reductive enzymes, such as lignin peroxidase (EC 1.11.1.14), laccase (E.C.1.10.3.2), tyrosinase (E.C. 1.14.18.1), NADH-DCIP reductase (EC 1.6.99.3) and azo reductase. According to reaction type, these oxido-reductase enzymes transfer electrons from a substrate to an acceptor (dye). Enzymatic treatment is most effective, when there is a high concentration of target contaminants and low concentration of interference.

Methodology: In this study, presence and induction of enzymes participate in degradation of dye and dyes contaminated wastes were evaluated. Tyrosinase, laccase, lignin peroxidase, NADH-DCIP reductase and azo reductase was assayed spectrophotometrically using reported standard methods. Zymography for NADH dependent and NADH independent azo reductase enzyme was carried out using azo dye as substrate.

Results and Discussions: The developed bacterial consortium was found to effectively decolorize 200 mg/L Reactive Acid orange 7 dye within just 30 min and during decolorization 308% Laccase, 615.80% azo reductase, 251.74% DCIP reductase and 154.63% lignin peroxidase enzymes were induced. While in case of mixed dyes containing simulated waste treatment, 145% intracellular and 776% extracellular tyrosinase, 160% intracellular and 482% extracellular lignin peroxidase were induced, along with induction in both reductive enzymes. Further, in case of real industrial dye effluent treatment, induction of intracellular azo reductase (107%) and NADH-DCIP reductase (128%) in addition to extracellular laccase (489%) indicate the vital role of the consortium in the degradation process. Zymogram showed colorless band in dye containing gel.

Conclusions: Significant induction in tyrosinase, lignin peroxidase and azo reductase in presence of DCIP reductase and laccase suggest their vital role in decolorization process.

Poster no: D-3-46

Biochemical characterization and structural analysis of a novel protein Bioremediase isolated from a thermophilic bacterium BKH1 significant to different biotechnological application

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Introduction: Elucidation of the integral biology of thermophilic micro-organism, manifold research works are going on their

enzymes due to their potential biotechnological applications. Thermostable enzymes are more promising to scientific development industrially. This study involves the isolation and characterization of such a novel thermostable protein (bioremediase) from a thermophilic bacterium (BKH1) which is significant not only in bio-concrete technology but also having a key role in silicon industry in the preparation of nanosilica.

Methodology: The morphological study of the bacterium BKH1 was analyzed by using Scanning Electron Microscopy, Atomic Force Microscopy and Transmission Electron Microscopy. The biochemical and thermo dynamical characterization of the protein was analyzed by using UV-spectroscopy, Fourier Transform Infrared Spectroscopy and Isothermal Titration Calorimetry. Mass spectroscopy was performed to identify sequence homology. The structural analysis was done by using Circular Dichroism and various Bioinformatics tool.

Results and Discussions: The morphological study of the Gram negative bacterium BKH1 shows long rod shaped structure. The protein bioremediase possess silica leaching attribute. From partial N-terminal sequence and mass spectrometry analysis, the protein bioremediase shows 78% homology to bovine carbonic anhydrase II (CA II). Like CA II, there is a zinc involvement in protein moiety which takes part in the nano-biosilicification mechanism. Interestingly, there is no functional similarity with CA II which confirms the uniqueness of the protein. With the help of Circular Dichroism spectral analysis the secondary structure of the protein shows 12.21% α helix, 14.84% anti-parallel sheet, 14.10% parallel sheet, 17.70% β turn and 41.15% random coil structure.

Conclusions: The microbial origin of biosilicification activity may be explored in various biotechnological processes that could have industrial importance.

Poster no: D-3-47

***Arabidopsis thaliana* under altered glutathione conditions- A Proteomic approach identified primarily defence related proteins**

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To obtain in depth insight into the role of glutathione (GSH) in defence signalling network, employing comparative proteomics approach, we have performed comparative proteome profiling of *Arabidopsis thaliana* leaves under altered GSH conditions viz. GSH-fed plants, control wild-type (Col-0) plants and *pad2.1* mutant (22% of GSH as present in wild-type plants). Results identified that in GSH-fed Col-0, majority identified proteins belong to signalling, stress and protein degradation, on top of related to redox, protein synthesis, RNA processing and enzyme families etc. Likewise in *pad2.1*, majority proteins identified were related to stress & defence and also related to redox etc. Our previous investigation also reported that GSH is operational through salicylic acid (SA)-mediated pathway to combat biotic stress. In continuation to this, we have analysed changes in leaf proteome profile of *A. thaliana* in response to SA and identified many defence related proteins like pathogenesis related protein 10a (PR10a), disease resistance-like protein, putative late blight-resistance protein, WRKY4, MYB4, etc. Together, present investigation established the function of GSH in plant defence in addition to provide further clues to unfold the intricate position of GSH in defence signalling network *in planta*.

Poster no: D-3-48

Targeting of recombinant human α_1 -proteinase inhibitor to ER enhances yield, biological activity and stability in transgenic tomato plants

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Introduction: Human α_1 -proteinase inhibitor (α_1 -PI) is the most abundant serine protease inhibitor in blood and heterologous expression of recombinant α_1 -PI has great potential for therapeutic applications. However, stability and functional efficacy of the recombinant protein expressed in alternate host are of major concern. In this study, the response of protein accumulation site on yield, biological activity and *in planta* stability was analyzed via targeting to different subcellular locations- endoplasmic reticulum (ER), apoplast, vacuole and cytosol in transgenic plants.

Methodology: Modified codon-optimized gene encoding recombinant α_1 -PI was employed for *Agrobacterium*-mediated transformation of tomato. Various signal sequences were used for differential subcellular targeting of the recombinant protein. Recombinant α_1 -PI protein was purified from transgenic plants by immunoaffinity chromatography. The purified protein was analyzed for yield, stability, biological activity and glycosylation profile using DAC-ELISA, elastase inhibition assay, mobility shift, enzymatic deglycosylation and lectin-binding assays. *In situ* localization of the recombinant α_1 -PI was confirmed by immunohistochemical staining using Confocal Microscopy. Pharmacokinetic evaluation of plant derived α_1 -PI was performed in rat model system.

Results and Discussions: Maximum accumulation of plant-derived α_1 -PI was achieved from 1.5 to 3.2% of TSP by retention in ER lumen, followed by vacuole and apoplast; whereas cytosolic targeting resulted into protein degradation. The ER-localized α_1 -PI variant showed maximum yield and inhibitory activity for elastase. The purified protein appeared as a single band of @48–50 kDa on SDS-PAGE with pI value ranging between 5.1 and 5.3. Results of mass spectrometry and optical spectroscopy revealed the structural integrity of the purified protein comparable to native serum α_1 -PI. Conformational and pharmacokinetic studies revealed relatively lower stability and half-life of the plant-derived α_1 -PI, compared to its serum counterpart.

Conclusions: Our data suggested significance of protein sorting to different subcellular locations in transgenic plants for the production of stable, glycosylated and biologically active recombinant proteins.

Poster no: D-3-49

Study of stress proteins induced by perchlorate stress in extremely halophilic archaea

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Introduction: Halophilic archaea are extremely resistant to Environmental stresses like high salt concentrations, change in temperature, radiations and chemical stress. These archaea induce the production of stress proteins when subjected to chemical and physical stress.

Methodology: *Haloarcula marismortui* and *Haloarchaea S2* were previously isolated from solar salterns of Mumbai. They were cultured in 250 ml shake flask in Sehgal and Gibbons medium containing 15 % NaCl and perchlorate for 5-7 days and their growth in terms of absorbance was monitored. *Haloarchaea S2* and *Haloarcula marismortui* were subjected to perchlorate stress (100-500 mM) and residual perchlorate was estimated using methylene blue colorimetric assay and the growth was observed spectrophotometrically at 600 nm. The generation time of the archaea in response to stress was studied and the protein profiling was performed using SDS-PAGE to study the various stress proteins produced due to stress. Simultaneously the total DNA and RNA was also extracted and assessed for the upregulation of chaperonin genes using universal type II chaperonin archael thermosome PCR primers.

Results and Discussions: *Har. marismortui* produced typical proteins in response to stress as observed by SDS-PAGE. These over expressed proteins may be attributed to the production of chaperonins that are oligomeric molecular chaperon proteins. *Hfx. volcanii* produces group II chaperonin proteins. These chaperonins (Hsp 60/Cpn 60) are coded by individually dispensable genes cct1-3 in *Hfx.volcanii*. The nucleic acids extracted were screened for the presence of cct genes using universal type II chaperonin primers and after multiple optimizations.

Conclusions: The effect of chemical stress on archaea was studied. In the current investigation, production of stress proteins was demonstrated. These organisms are subjected to multiple stresses in the environment and produce chaperonin proteins, heat shock proteins and many other stress proteins for protection against these factors.

Poster no: D-3-50

Proteomic investigation of *Caenorhabditis elegans* subjected to bacterial infection: Implication of unfolded protein response pathway

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Introduction: The fact that 60-80% gene sequences in aptly described genome of *C. elegans* are homologous to that of humans combined with its easy genetic screening and availability of mutant repository, well characterized developmental processes, ease of handling and short life cycle has rendered the free-living soil nematode a preferred animal model for wide range of investigations. It is also believed that, given the significant similarities between innate defense system of vertebrates and invertebrates, *C. elegans* is an ideal model system for investigation of bacterial pathogenesis and response to infection. In addition, in spite of the brief history of *C. elegans* proteomics (not much popular in India yet), the proteome-based studies are attracting attention in several areas of research. The present study extends the proteomics research to bacterial pathogenesis in *C. elegans* and initiates a new area of investigation in the country.

Methodology: The model animal was challenged with *Vibrio alginolyticus*, a highly pathogenic species of bacteria perceived

as a serious threat to human health and marine aquaculture. Subsequently, regulation of *C. elegans* proteome was explored employing standard proteomics tools of two-dimensional differential gel electrophoresis (2D-DIGE) and mass spectrometry. The differentially regulated proteins were identified using PMF and MALDI TOF/TOF analysis. The results thus obtained were validated using Western blots for candidate regulatory proteins and the corresponding transcriptional regulation using real-time PCR. The interaction network for candidate proteins was predicted using STRING and functional validation was performed using respective *C. elegans* mutant strains.

Results and Discussion: 21 proteins were found to be up-regulated while four were down-regulated. Up-regulated proteins included those involved in stress-response (PDI-2, HSP-6), immune-response (KIN-18, GST-8) and energy-production (ATP-2) while proteins involved in structural maintenance (IFB-2) and lipid metabolism (SODH-1) were down-regulated.

Conclusion: The investigation highlighted the significance of unfolded protein response (UPR) pathway during bacterial infection.

Poster no: D-3-51

Expression of antioxidant enzyme (protein) peroxidase during salt stress

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Plants growing under field conditions are exposed to various environmental factors. Any deviation in these factors from the optimum levels is deleterious to plants and leads to stress. Stress may be caused due to Abiotic factors like high temperature, cold, drought, salinity, heavy metals or Biotic factors like viruses, insects, nematodes, bacteria, fungi etc. however among these stresses drought, high salinity, high temperature, low temperature and metal stress are major environmental factors, that have an adverse effect on growth of plants and productivity of crops.

Plants have adapted to respond to these stresses at molecular, cellular and physiological levels. When plants are subjected to abiotic stress a number of genes are turned on and the products of these genes not only function in stress tolerance but also in regulation of gene expression and signal transduction in stress response. Because of their importance in crop protection, these traits have been extensively investigated in the last three decades and a great deal of progress has been achieved in production of plants tolerant to environmental stress.

Pas palumsrobiculam. L (kodomillet) grown in small pockets in the states of Andhra Pradesh, Karnataka, Tamil Nadu, Orissa, Bihar, Madhya Pradesh, Maharashtra and Gujarat. The crop is extremely resistant to drought and salt, which attracted our attention therefore, the present study was initiated to understand the mechanism of abiotic stress response in kodomillet. The summary of the investigation includes evaluation of biochemical parameters such as antioxidants, antioxidant enzymes and other stress specific factors. Salt stressed kodomillet showed the induction/ over expression of two isozymes with dual activity of antioxidant enzyme Peroxidase and Phosphatase. From the investigations, it is clear that the thekodomillet has an efficient antioxidant system to cope with abiotic stresses.

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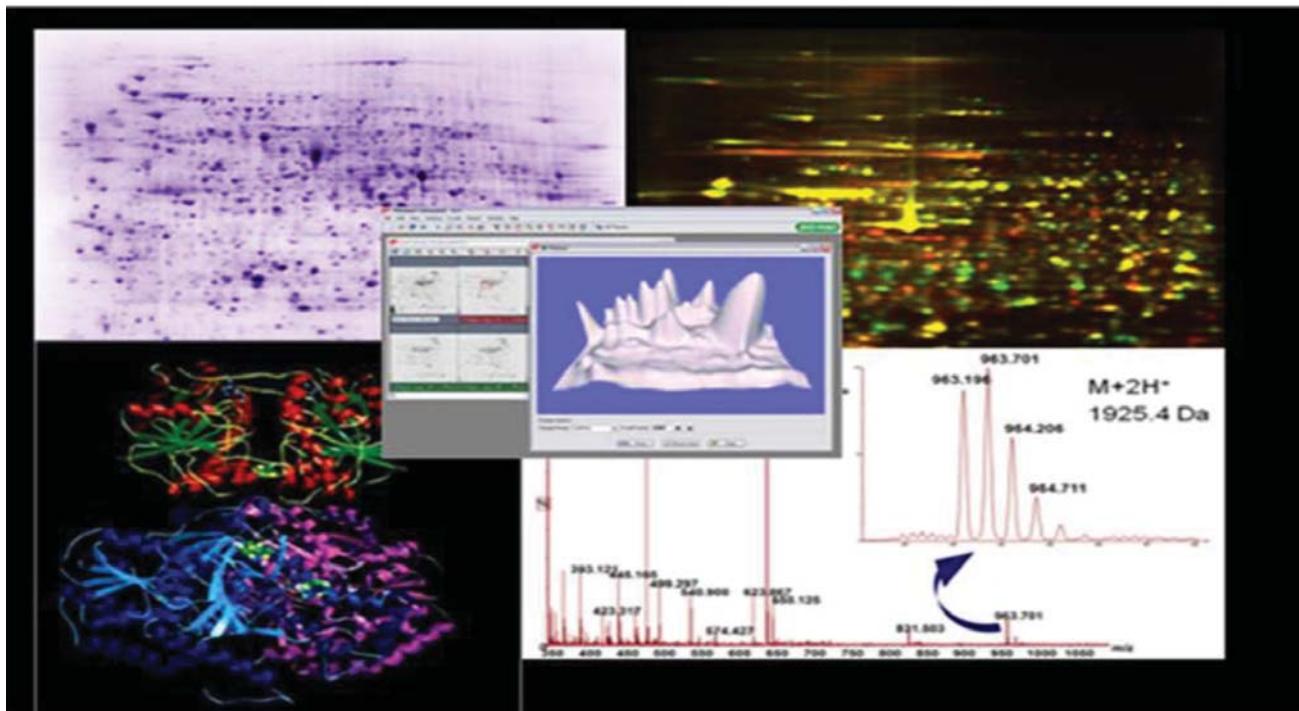
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Proceedings of the 4th Annual Meeting of the Proteomics Society, India

Dr. Mahesh Kulkarni and Dr. Ashok Giri
National Chemical Laboratory, Pune, India

The Fourth Annual Meeting of Proteomics Society, India (PSI) was held at CSIR-National Chemical Laboratory, Pune during November 22-24, 2012. As part of this event an International Symposium on "Proteomics beyond IDs." was organized. The objective of the symposium was to provide a platform to discuss new developments in proteomics, especially involving proteomic characterization including quantitation, post translational modifications, localization, protein-ligand interactions, etc. The symposium covered various themes; Quantitative and Functional Proteomics in human diseases, Activity based proteomics, Proteome mapping and Post translational modifications, Plant Proteomics and Proteogenomics, Chemical and Structural proteomics, and new developments in mass spectrometry and proteomic technologies. Each theme had about 4-5 speakers of international repute both from India and abroad.

The symposium started with welcome remarks by Dr. Sourav Pal, Director, CSIR-National Chemical Laboratory, Pune, Dr. Vidya Gupta Chair, Biochemical Sciences Division, Dr. K. N. Ganesh, Director IISER, Pune, Dr. S. Sivaram, former Director, CSIR-NCL, and Dr. Ravi Sirdeshmukh, Distinguished Scientist, Institute of Bioinformatics, Bangalore and President, PSI. This was followed by a keynote lecture on "Mass spectrometry of proteins and peptides" by Professor Balaram, Director, Indian Institute of Science, Bangalore. The session was concluded with acknowledgements by Dr. Surekha Zingde, Deputy Director, ACTREC, Navi Mumbai.

The first scientific session of the symposium was on "Quantitative and Functional proteomics in Human Diseases" where in Dr. Ravi Sirdeshmukh described integrated view of using various omics approaches in study of glioblastoma. Prof Ed. Nice, Director, Monash Antibody Technologies, Australia, spoke on discovery and validation of novel biomarkers using targeted proteomics approaches for colorectal cancer. Dr. Abhijit Chakrabarti, Saha Institute of Nuclear Physics, Kolkata, presented his work on differential protein expression in hemoglobinopathy and thalassemia. Dr. Don Jones, University of Leicester, UK, briefed about chemoprevention using quantitative proteomics in his lecture. Dr. Abdul Jaleel, Rajiv Gandhi Center for Biotechnology, Trivandrum, made a presentation on role of protein aging in type 2 diabetes.

The second session was on "Activity and chemistry based proteomics", where Dr. Rainer Bishoff, presented information on quantitative protein analysis of MMP-9 in broncho-alveolar lavage fluid of patients who have undergone lung transplantation. Dr. Shantanu Sengupta, Institute of Genomics and Integrative Biology, Delhi, discussed proteomic profiling in Vitamin B12 deficiency

condition. Dr. Sunil Adav from Nanyang Technological University, Singapore spoke on quantitative secretomic analysis of *Trichoderma reesei* strains. Prof. B.C. Harinath, Mahatma Gandhi Institute of Medical Sciences, Sevagram, presented his studies on Mycobacterial Secretory Proteome.

The second day of the symposium started with a session "Proteome mapping and Post translational modifications". The highlight of this session was a plenary lecture on "A draft map of Human proteome" by Prof. Akhilesh Pandey, from Johns Hopkins University, USA. This was followed by lectures from Prof. Newman Sze (Nanyang Technological University, Singapore); Prof. Karine Roch (University of California, Riverside, USA); and Dr. Kameshwar Rao (Defence Research and Development Establishment, Gwalior). Prof. Sze discussed post-translational modifications for diagnosis and elucidation of molecular mechanisms of disease. While Dr. Roch spoke on Global Mapping of Histone modifications in *Plasmodium falciparum* and Dr. Kameshwar Rao, discussed the post-translation modifications in Ricin.

The second session of the second day was on "Plant and Microbial Proteomics". The speakers of this session were Prof. Asaph Aharoni, Weizmann Institute of Science, Rehovot, Israel; Prof. Ales Svatos, Max Planck Institute for Chemical Ecology, Germany; Prof. Anil Kumar, Banaras Hindu University, Varanasi; and Prof. Renu Deswal, Delhi University, New Delhi. Prof. Aharoni elaborated the usefulness of proteomic and metabolomics technologies in chemical genetics screens, while Prof. Svatos discussed the quantitative and qualitative approaches in plant proteomics. Prof. Anil Kumar made a presentation on systems biology of a *Rhizobacterium* using, proteomic and phenotypic approaches and Prof. Deswal presented a lecture on nitric oxide (NO) based Post-translational modifications (PTM's) under cold stress.

The next session was on "New developments in mass spectrometry and proteomics" and the speakers of this session included mainly application scientists from Mass spectrometry companies. Dr. Ron Bonner, ABSCLIX, Canada briefed about SWATH Acquisition for quantitative proteomics, Dr. Mark McDowell, Waters MS Technologies, UK, discussed Traveling-Wave Stacked Ring Ion Guided technology for Electron Transfer Dissociation and Ion Mobility Separations, Prof. Markus Macht, Bruker, Germany presented Integrated proteomic approaches using MALDI and ESI, and Prof. Sham Nal, Agilent, Europe discussed High-Throughput Quantitation approach for Biomarkers discovery and Dr. Andreas Hummer, Thermo Fischer Scientific, Germany highlighted the Impact of High-Performance Mass Spectrometry on Biological Research.

The last day of the symposium began with a session on "Structural and chemical proteomics and protein interaction". Prof. Anthony Wilkins, University of York, UK, summarized the discovery of inhibitors against a malaria and leishmania target involving chemistry and

protein crystallography; Dr. Kaushik Chakraborty, IGIB, New Delhi, described the role of Chemical chaperone assisted intracellular folding to buffer mutational variations; Dr. Chaitanya Saxena, SHANTANI, Pune briefed about Incremental Innovations in Chemical Proteomics Space. This session was followed by a session on Protein interactions. Prof. Karl Anderson, Uppsala University, Sweden provided information on Real-time protein interaction assays on living cells; Prof. Desh Deepak Singh, Indian Institute of Advanced Research, Gandhinagar unravelled the systems to study interactome; Dr. R. Srikanth, NCCS, Pune, discussed the role of cooper in Microglobulin amyloid formation as studied by Mass Spectrometry.

The meeting was attended by 374 delegates. There were 93 poster presentations which were viewed between the main themed sessions. The last session comprised of presentations by Best poster Awardees, followed by distribution of Best poster and Travel Awards.

A pre-conference one day workshop on "Tutorial on proteomics workflow" was conducted on the November 2012. During this workshop the participants were given an overview of proteomics 21st and demonstrations for 2DE, DIGE, nano and CHIP LC, label free quantification, iTRAQ, MALDI imaging and post translational modifications by scientists and specialists from different companies for proteomic equipment.

Proceedings of the 5th Annual Meeting of the Proteomics Society, India

Dr. Utpal Tatu

Indian Institute of Science, Bengaluru, India

The 5th annual meeting of the Proteomics Society was held at IISc, Bengaluru during 28th-30th Nov. 2013. About 450 participants from various scientific disciplines including medical, pharmacy and engineering and biotech professionals attended the conference. They included lecturers and senior research scientists from academic as well as from biotech and pharmaceutical organizations. The theme of 5th Annual Meeting of Proteomics Society-India was Medical Proteomics. Each day of scientific sessions was dedicated to Nobel laureates namely - Prof. Frederick Sanger, Prof. James Rothman and Prof. Koichi Tanaka respectively. The conference was inaugurated by Prof. P. Balaram, Director, Indian Institute of Science, Bangalore. The scientific sessions were initiated by an outstanding talk by Prof. S. K. Brahmachari, Director General, CSIR on the use of Omics technology in improving healthcare.

The scientific sessions included 38 plenary talks by leading national and international scientists. They provided the irresearch views on topics ranging from proteomics, genomics, metabolomics and volatileomics. In addition there were presentations on new technology developments in mass spectrometry. A panel discussion entitled "Applications of Mass spectrometry in Medical Research" highlighted the concept of personalized medicine. The poster presentations were held for two days

(28-29th Nov. 2013) where in participants who were PhD students, post docs and young scientists from academia and industry were allowed to showcase their research. Over 75 entries were received and 68 posters were presented. The abstracts for all the entries were printed in a conference proceeding which was distributed amongst registered participants. The other highlights of the meeting were (a) Q&A, (b) Science & Sketch and (c) Science and society sessions. These informal sessions provided ample opportunity to exchange scientific ideas amongst the young scientific community. The above deliberations were aptly judged and best presentations were provided cash prizes.

On the second day of the meeting, a special scientific presentation with audio visual effects was organized by Thermo Scientific at The Lalit Ashok which was followed by dinner. The presentation by Thermo scientists included new development in orbitrap technology in mass spectrometry. Once again these sessions were followed by Q & A. These deliberations provided an opportunity for the delegates to network amongst themselves and facilitated exchange of scientific ideas. To balance the grilling scientific deliberation a cultural programme which comprised Indian classical music, Kathak, Bharatanatyam, Odissi and Rajasthani folk dance were organised. These cultural performances provided a good relief to young scientists after the heated scientific schedule. The success of the meeting can be seen from the testimonials which were provided by scientists from academia and industry who had participated in the event.

A Pre-Meeting Workshop on Mass Spectrometry and Metabolomics was held on 27th Nov 2013. C-CAMP (Centre for Cellular and Molecular Platforms, Bengaluru) organized this workshop in collaboration with the 5th Annual Meeting of Proteomics Society India in order to create a better understanding of the field of Metabolomics and its applications. A total of 39 participants attended the workshop. The workshop covered topics on introduction to Metabolomics, quantitative metabolomics and volatile organic compounds followed by hands on training session on quantitative metabolomics. The overall feedback from the participants was very positive and most of the participants found the workshop to be well structured and course material to be relevant.

Proteomics: Education, Awareness, Research and PS(I)

Dr. Abhijit Chakraborty¹ and Dr. Shantanu Sengupta²

¹Saha Institute of Nuclear Physics, Kolkata, ²CSIR- Institute of Genomics and Integrative Biology, New Delhi, India

The field of Proteomics has already grown considerably since when Dr. Peter James coined the word in his paper titled "Protein identification in the post-genome era: the rapid rise of proteomics" in 1997. The release of Human Genome Sequence data in 2004 highlighted the larger complexity of proteome in comparison to the genome. 19,599 genes encoding the proteins may result in 1 million or more proteins or protein fragments, upon considering

alternative RNA splicing the post-transitional modifications. The field is hugely driven with the prospects of discovering new protein markers for diagnostic purposes and of novel molecular targets for drug discovery. Proteomics, as a field is being given a huge thrust globally to mature and achieve aspirations of 21st century, in the field of biology and biomedical research. In India, although the first "Proteomics" conference in India was held in CCMB, Hyderabad in 2003, there were only a few groups in the country who were actively pursuing research in this field.

The interest in the field of proteomics in India gathered momentum in the later half of the decade when many Institutes and groups started using proteomic approaches in their research efforts. In 2007, the Council of Scientific and Industrial research undertook a large project in plasma proteomics which led to the networking of a few scientists in the CSIR system. It was then felt that since there was a critical mass of Scientists using Proteomics as a tool it is important to facilitate interactions among them to expand on the research efforts as the technology evolved. Thus, another conference was organized in CCMB in 2008 on the Current Trends in Proteomics. This congregation was attended by close to 200 senior investigators and students, underscoring the HUPO philosophy of co-operation, collaboration, and education among the fraternity. During this meeting the proposal of forming a Society was put forth which was whole heartedly supported by all the delegates. This led to the birth of Proteomics Society in India.

Proteomics Society was registered as a Society on the 18th March 2009 with the objective to promote and advance the field of proteomics and the professional interests of the Proteomics community in line with the global developments and competition. The Society is strongly dedicated to education for research in Proteomics through seminar series for students and college teachers, theme workshops for researchers and Annual Conferences to share their knowledge and experience to discuss the international trends. Besides, several hands-on trainings have been provided to students and young scientists.

The first annual meeting of the Society was held at CCMB, Hyderabad in Feb. 2010 along with the 5th AOHUPO Proteomics Congress displaying novel contributions in the field from the Indian Proteomics Community. Subsequently each year annual meetings are held at various locations in India where more than 350 students and scientists attend and exchange ideas (Table 1). The 6th Annual Meeting of the Proteomics Society is being held in Dec 2014 at IIT Bombay. This year's focus will be on "Proteomics from Discovery to Function". The vision of PSI has already made an impact on the international forums such as AOHUPO which would like similar education agenda to be extended to the Asia-Oceania region. Most importantly the establishment of Proteomics Society is associated with the steady rise in the number of publications in the field of proteomics in India (Figure 1).

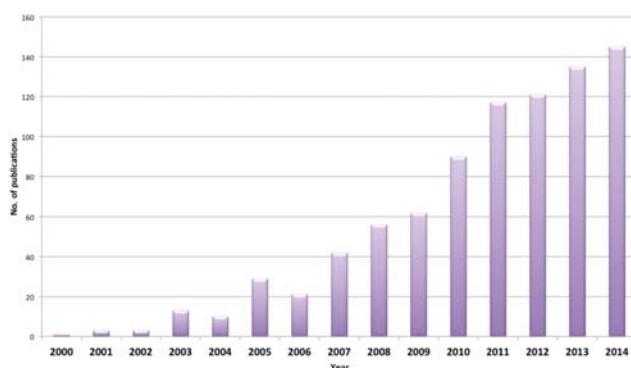


Figure 1: Rising trend of annual publications in proteomics from India (Total 848).

Table 1
Details for the Annual Proteomics Meetings held

Annual Proteomics Meeting	Year	Location
I	February 21-25, 2010	CCMB, Hyderabad
II	April 3-5, 2011	JNU, New Delhi
III	December 15-18, 2011	SINP, Kolkata
IV	November 22-24, 2012	NCL, Pune
V	November 28-30, 2013	IISc, Bengaluru
VI	December 7-9, 2014	IIT Bombay

We have been watching Proteomics flourishing well in India since the formation of PS (I) in the year 2009. Along with advancements in the MS based technologies, tools of bioinformatics and molecular biology, Indian scientists engaged in rigorous proteomics research have been able to make marks in the international arena. The rise in the number of publications and increase in the trained pool of Scientists, establishment of state-of-the-art laboratories across India catering to the ever increasing needs of proteomic science clearly indicate not only to the progress but has also established the fact that Proteomics has been a career choice for many budding scientists. Given the genetic diversity, renewed interests in biochemistry and metabolomics alongside and availability of ever expanding pools of clinical subjects, the future of Indian Proteomics is genuinely brighter.

Mapping the Human Proteome

Dr. Harsha Gowda¹ and Dr. Akhilesh Pandey²

¹Institute of Bioinformatics, Bangalore, ²McKusick-Nathans Institute of Genetic Medicine, ³Department of Biological Chemistry, ⁴Department of Oncology, ⁵Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, USA

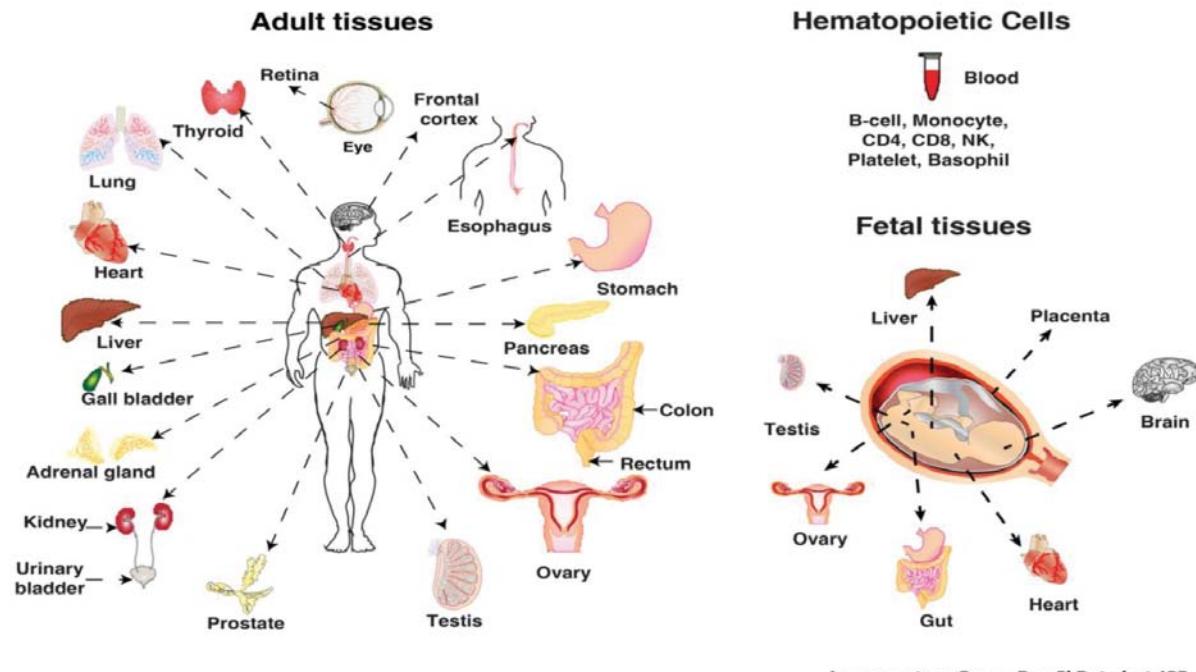
Successful completion of the Human Genome Project (HGP) in 2003 represented a quantum leap in biomedical research. It provided some of the early estimates of number of protein coding genes in the human genome.

Proteins serve as fundamental workhorses in every cell. We employed cutting-edge methods in mass spectrometry based proteomics and carried out an unbiased survey of human proteome across 17 adult tissues, 7 fetal tissues and 6 purified primary hematopoietic cells (*Nature* (2014) 509, 575–581).

Significant Outcomes of the Study

- Generated tandem mass spectra for proteins encoded by 17,294 genes (~84% of the total known) in the human genome

- Identified nearly 200 novel proteins using proteogenomics approach
- Established proteomic evidence for nearly 2/3rd of human proteins that were designated as “missing proteins”
- Demonstrated protein coding potential of several pseudogenes and non-coding RNAs
- Developed a web based resource (www.humanproteomemap.org) to enable scientific community to navigate across protein expression dataset across 30 human tissues/cell types



Future Outlook

This study upheld the need for systematic mapping of the human proteome from multiple anatomically distinct sites. Some of the missing proteins are likely to be expressed in specific tissues/cell types that have not been sampled in our study. Further sampling should enable us to identify more novel proteins and find the remaining missing proteins. Considering post-translational modifications play a pivotal role in regulating protein function, a similar mapping effort would prove beneficial. Differential protein expression pattern observed across various tissues can also serve as a basis for prioritizing candidate biomarkers and therapeutic targets for pathological conditions affecting these tissues/cell types. In addition, such datasets will also prove useful for fundamental discoveries in biology. Spectral libraries developed based on these experimental datasets will significantly improve MS/MS searches in the future and also prove useful in selection of proteotypic peptides for developing multiple reaction monitoring assays for the human proteome.

About Journal of Proteins and Proteomics

Dr. Suman Kundu
University of Delhi, India

Journal of Proteins and Proteomics (ISSN No. 0975-8151) is a protein-centric journal envisaged and established to serve the community of scientists worldwide who work in the area of protein science and proteomics. This non-profit journal was envisioned in 2010 to create a platform to share novel findings, to erect hypothesis for future introspection, to put together available knowledge in literature in a comprehensive manner in areas related to “protein science and proteomics”. It is published semi-annually from India by International Science Press, Serials Publications, Delhi. In addition to regular issues, an annual special issue is also published in specific area of protein science and proteomics. The journal publishes research articles, reviews, essays, commentaries, reports, letters, teaching material, in memoriam, recollections etc. with focus on Protein Science and Proteomics.

JPP publishes articles at absolutely no cost, whatsoever, to contributing authors. JPP allows free download of its

articles worldwide for readers. It only encourages subscription of its hard copy issues at a minimal price to support associated costs. JPP promotes academic charity for the cause of education, research activity and dissemination of knowledge for one and all. A selfless editorial team and several well wishers who work behind the curtains ensure that JPP serves the protein science community with no string attached.

Vision & Mission

The most enigmatic and dynamic macromolecule that define living systems, Proteins, occupy a central place in biology being the indomitable workhorses in cells. Understanding their structure, deciphering their function, unraveling their folding mechanism, defining their interacting partners – protein by protein or of the proteome – presents daunting challenges. Whether the objective is to image the novelty and beauty of structure-function relationship and pathways of bioactive conformation attainment for purely academic aesthetics or use them for therapeutic intervention, or whether it is essential to characterize the whole proteome, the challenges are manifold and deserve heightened attention. Rapid success in the areas of health and medicine, agriculture and manufacturing would depend heavily on our ability to exploit the riches of “protein mines”. For all those who dare to accept the challenges, who delve in the intricacies of protein mystery, who take pain to tame the wild, this journal is our tribute. This “protein-centric” research tabloid, the first of its kind in India, is to honour their timeless effort, is to create a platform to share their novel findings, to erect hypothesis for future introspection, to put together available knowledge in literature in a comprehensive manner and to build momentum to that monumental aspiration of being able to know all that there is to know about proteins. The aspiration may sound out of bounds in the current context, but why not atleast take the first step!

We foresee JPP to be a premiere journal in the area of proteins and proteomics in the next 4-5 years. Our vision, our goal, our untiring efforts, we believe, will ensure the rise of this dedicated journal. We cater to the needs of both authors and readers in a unique way – mainly by

ensuring flow of information and knowledge in a priceless (literally meaning) way. In general, to publish papers or to access them either the author or the reader has to pay a price, often exorbitant. In the open-access world, readers get the advantage while authors pay. In the classical mode, authors either do not pay or pay much less while readers have to subscribe journals or pay for a coveted article. We seek to strike a balance such that neither of the beneficiaries pays. Authors publish papers in JPP without having to spend a single penny whatsoever. At the same time, we make all articles freely available on our websites for interested readers so they do not have to pay either. Such a policy has seen authors working under limited resources getting an opportunity to share their findings with a wide viewership, with free access to both.

We also visualize wider inclusion of students and their engagements in our mission. We plan to involve their participation with responsibilities normally not assigned to them with regard to widely acclaimed journals of repute. We believe such a step would prepare students better for the tough world of “publish or perish” reality. We believe that it would help students mature faster into the scientific world and instil in them a sense of belonging, the pleasure of achievement and the urge to attain higher learning.

Our vision also includes efforts to popularize protein scientists, to immortalize their achievements and contributions through the pages of JPP in a section aptly entitled “Soldiers of Science”. Pioneering scientists whose tireless efforts have helped the field of protein science advance and reach great heights but often whose contributions go unnoticed will adorn the aforesaid section in all their glory. Short biographies written by a person who know the scientists best, would help inspiring, nourishing and motivating young, blooming minds. It would instil a sense of satisfaction in the scientist himself while his peers can cherish the memories.

JPP would also publish short, succinct, easy-flowing, informative and thought provoking reviews or articles that help students learn topics that are part of their degree curriculum. These would be written by teachers in a lucid language that promotes learning without associated complexities.

ANNOUNCEMENT FOR THE SPECIAL ISSUES

Post-conference special issue in JOURNAL OF PROTEOMICS

(Editor-in-chief Dr. Juan J. Calvete); (Guest editor: Dr. Sanjeeva Srivastava)

We are pleased to announce a special issue “PROTEOMICS IN INDIA” to be published in Journal of Proteomics (<http://www.journals.elsevier.com/journal-of-proteomics/>).

Time-line:

Nov 15, 2014: Expression of Interest – Abstract Submission

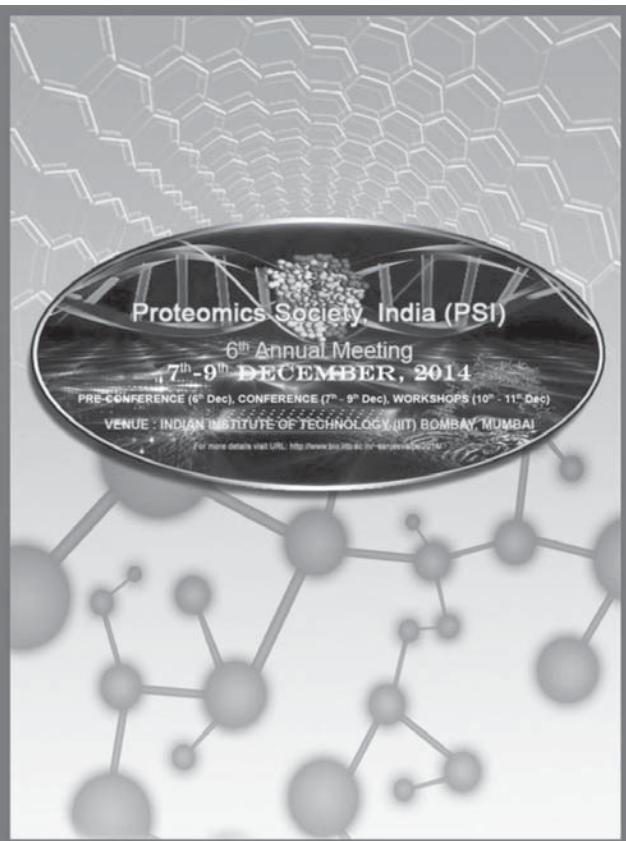
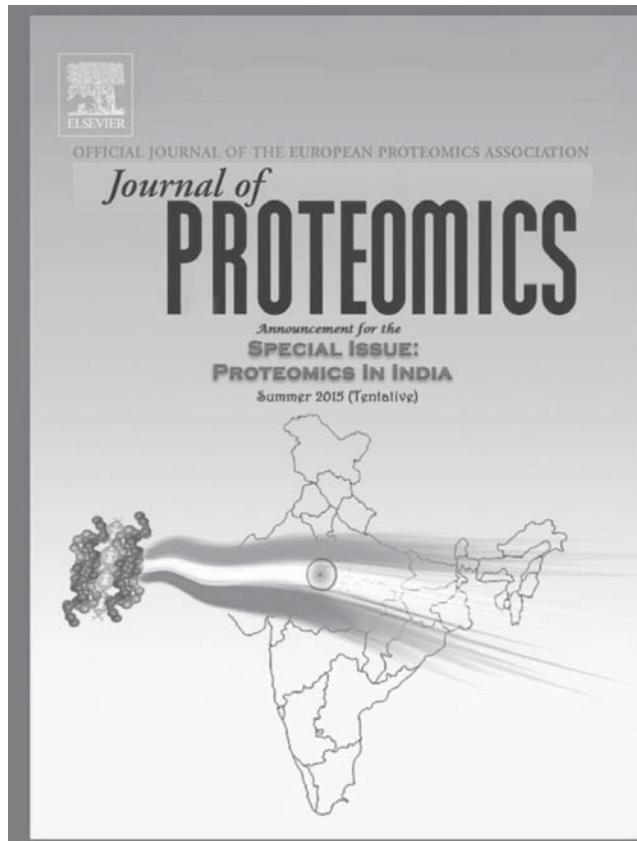
Dec 15, 2014: Announcement of selected abstracts to be invited for the full article

Jan 31, 2015: Submission of Manuscript

Feb – April, 2015: Review process (peer review and revision)

May-June 2015: Publication of Special Issue

- Reviews, Original Research Articles and Short Correspondence are now open.
- Proposals can be same which you have submitted for the conference or new work; however, for the consideration in special issue you need to submit fresh proposal in attached format by Nov 15, 2014. Please submit your proposal to JofP.India@gmail.com



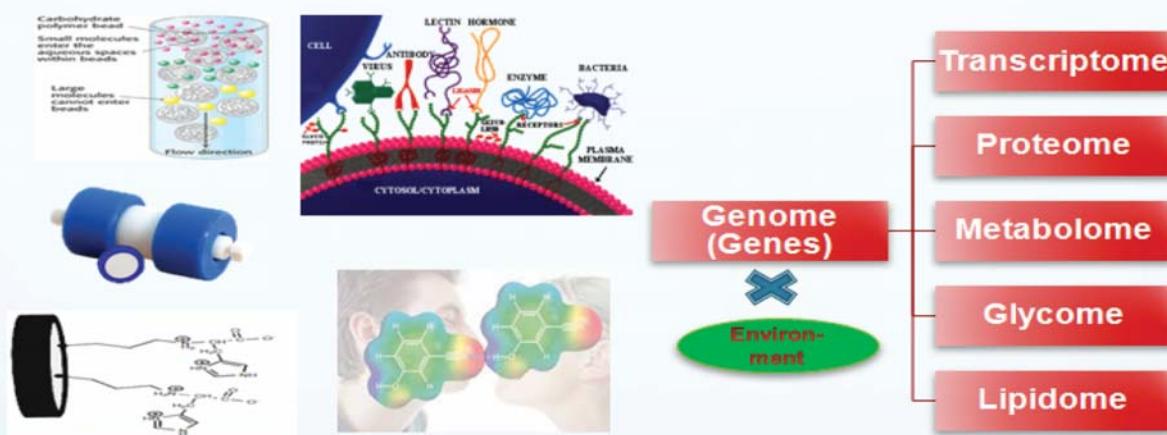
ANNOUNCEMENT FOR THE 7TH ANNUAL MEETING OF PROTEOMICS SOCIETY, INDIA

Organizers: Prof. M.A. Vijayalakshmi, Prof. Krishnan Venkataraman

Announcement

7th Annual Meeting of Proteomics Society–India (2015)

- Duration of conference: 3 days
 - Tentative dates: Between November 23, 2015- December 10, 2015
 - Venue: Centre for BioSeparation Technology, (CBST), VIT University, Vellore, INDIA. 632014.
- The seventh annual PSI meeting will be organized by Advanced Centre for BioSeparation Technology (CBST) at VIT University, Vellore, Tamil Nadu, India. This conference is themed on **Biochromatography, Molecular Recognition and Proteomics** and it aims to integrate cross disciplinary subjects for better understanding of complexity of proteome, metabolome, glycome, lipidomes of humans, plants, micro-organisms, etc for various applications.
- Themes of the meeting
 - Biochromatography, Molecular Recognition and Proteomics
 - Structural and Functional Proteomics
 - Glycomics, Lipidomics, Metabolomics & Metallomics
 - Biomarker Proteomics:- Diseases/Disorders
 - Applications of Proteomics in Biopharma/Bioindustries



Biochromatography

Organizers

Prof. M.A.Vijayalakshmi (Director); Convenor
 Prof. Krishnan Venkatraman; Organizer
 Advanced Centre for Bio-Separation
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Molecular Recognition

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- omics

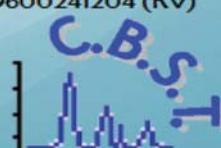
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URL: <http://www.isotope.com/>

GENERAL INFORMATION

IIT BOMBAY

Indian Institute of Technology (IIT) Bombay is known to be one of the premier institutes for the higher education and research in India. Over the years IIT Bombay has extended its abilities to contribute to quality teaching and research in almost all fields of science and technology through state-of-the-art facilities for academia and researchers. The Department of Biosciences and Bioengineering at IIT Bombay has put in balanced endeavors to be a part of basic science and applications in the field of biology, biomedical and bioengineering research.

Proteomics is an emerging field of research globally and IIT Bombay aims to keep-up the pace in this burgeoning field, which aims to identify proteins that are characteristic for disease formation & progression, and can potentially uncover diagnostic, prognostic markers or novel drug targets, and could help to understand the underlying mechanisms. IIT Bombay is emerging as a vibrant hub of bio-research and proteomics in India. At IIT Bombay currently research on proteomics and protein biochemistry is being pursued at various levels, e.g. Disease proteomics of glioblastoma, meningioma, malaria pathobiology, proteomics involving cell division of prokaryotic & eukaryotic cells, and recombinant proteins and protein structure analysis by developing and applying high end bioinformatics and statistical techniques.

To ensure high quality research, IIT has set its benchmark on par with some of the best institutes nationally and internationally. During the last two years IIT Bombay has set-up several high throughput platforms for the proteomics analysis such as GenePix4000B Microarray Scanner for Protein Microarray studies, OmniGrid Accent Microarrayer for printing of microarray slides, Autoflex MALDI TOF/TOF for mass spectrometry, 6550 iFunnel Q-TOF for mass spectrometry, Biacore T-200 for label free and protein interaction studies, Typhoon FLA 9500 scanner for 2D-DIGE experiments, Ettan IPG-phor 3 IEF System and Ettan DALI six electrophoresis units for 2D gel based experiments. Other facilities like High Performance Liquid Chromatography (HPLC), Fast Performance Liquid Chromatography (FPLC) and Protein sequencer are also available. For analyzing the sizable data generated through these high throughput techniques, IIT Bombay also boasts a list of sophisticated software dedicated to each of the above techniques such as Spectrum Mill, Mass Profiler Professional, Acuity, De cyder 2D Differential Analysis software, ImageMaster Platinum, BIAevaluation and Mascot are few amongst them.

Faculty expertise in the Department of Biosciences and Bioengineering, IIT Bombay ranges from the basic Biological Sciences to Biochemical Engineering and Computer Sciences and Engineering. This breadth and depth is necessary to meet the demands of Systems Biology and Omics research. Researchers at IIT Bombay look forward to contribute actively to the field of science with few groups dedicated to proteomics and systems biology research. Together, with the Proteomics Society, India, we share the vision to be innovative and pioneer, and contribute to cutting edge proteomics research to set benchmarks at the international level by interactions, collaborations and continued perseverance.

GROUND FLOOR PLAN (VMCC)

EXHIBITION FLOOR PLAN - VMCC Ground Floor

The plan shows a large circular VMCC AUDITORIUM at the center. To its left is a row of four CLASS ROOMS. To its right are two more CLASS ROOMS. Above the auditorium is a large area labeled 'PO' (POSTERS) with several green dots indicating poster locations. To the left of the auditorium is a yellow area labeled 'VWL' (VIP WAITING LOUNGE). Above the auditorium is a yellow area labeled 'CF' (CAFETERIA). A blue area labeled 'FC' (FOOD COUNTER) is located at the top right. A blue area labeled 'ST' (STAGE) is at the bottom left. An 'ENTRY' point is indicated at the bottom center. Stairs are shown on the right side.

EXHIBITION LIST

COMPANY	
Sandor	G - 01 S
HIMEDIA	G - 02 S
Allied Scientific Product	G - 03 S
MRM	G - 04 S
C - CAMP	G - 05 S
Proteomics Society of India	G - 07 S
Journal of Proteins and Proteomics	
Cambridge Isotope Laboratories	G - 08 M
Information Booth	G - 09 M
Control Room	G - 10 M

S - 3 x 2 mt.
M - 3 x 3 mt.

FIRST FLOOR PLAN (VMCC)

EXHIBITION FLOOR PLAN - VMCC First Floor

The plan shows a large circular VMCC AUDITORIUM at the center. To its left is a row of five CLASS ROOMS. To its right are three more CLASS ROOMS. A blue area labeled 'ST' (STAGE) is at the bottom left. Stairs are shown on the right side. Numbered purple boxes (1-11) indicate exhibitor locations around the auditorium.

EXHIBITION LIST

COMPANY	
Control Room	F-01S
Bio-Rad	F-02L
PALL Corporation	F-03L
BIA Separations	F-04L
GE Healthcare	F-05L
Agilent Technologies	F-06L
Waters	F-07L
Thermo	F-08L
AB SCIEX	F-09L
Bruker	F-10L
Biolinx	F-11L

S - 2X3 mt.
L - 3X4 mt.

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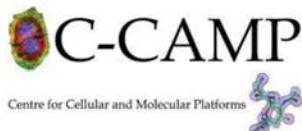
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By the time you read this page, I am sure you have thoroughly enjoyed the extensive and passionately laid down matter of the Special Issue concerning the PSI 6th Annual Meeting and International conference on Proteomics, the informative abstracts and activities of PSI, thanks to our Guest Editor, Dr. Sanjeeva Srivastava. In the following pages, I take pleasure in presenting to you research abstract, company profile and information about generous sponsors who had helped us partly meet the expenses of assembling and printing the entire issue.

The vision of JPP has been to publish articles at no cost whatsoever to authors or readers. We have been doing so over the last five years. Such an ambitious vision has been made successful by numerous charitable subscriptions from well wishers and mainly through sponsorships from big-hearted organizations like ABSciex, Agilent and Thermo, all of whom have helped us yet again with their generosity in meeting printing costs. Such act of kindness for an academic cause helps us survive and fulfill our dreams and aspirations. We also thank Dr. Shantanu Sengupta, Secretary, PSI profusely for mediating these sponsorships. We thank all the executive members of PSI and Dr. Sanjeeva Srivastava for their encouragement and meeting rest of the printing costs for this special issue.

Dr. Suman Kundu
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Effective use of novel data independent analysis methods to obtain comprehensive and reproducible characterization of a biological pathway

Michael A. Blank¹, Ryan Bomgarden¹, Neelu Puri², Rosa Viner¹, Daniel Lopez-Ferrer^{1*} and Andreas FR Hühmer¹

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Introduction

Data Independent Analysis (DIA) bridges protein identification and targeted quantitation by acting as a screening technique to rapidly and thoroughly survey a sample and direct future targeted analysis. In this work, we have built high performance methods on the Orbitrap Fusion and Q Exactive HF to take advantage of analyzer parallelization with WiSIM and precursor multiplexing with msxDIA and applied them to investigate drug resistance in cancer cells.

Methods

Treated and control non-small-cell-lung-cancer cells were lysate and trypsinized. An aliquot was used to build a protein/peptide library. And the rest was analyzed using DIA. Orbitrap Fusion and Q Exactive HF mass spectrometers were used. A Thermo Scientific EASY-nLC 1000 UPLC system and EASY-Spray Source with 50cm Easy-Spray Column was used to perform the LCMS analysis. Raw files were analyzed by Proteome Discoverer, Pinpoint and Skyline software.

Results

A high quality library consisting in over 3000 proteins was built. Special emphasis was put in deciphering the effect of drug resistant in MAPK pathway. It is well known that a mutated version of BRAF plays a critical role in certain subtypes of cancers. This has leaded to the development of a family of drugs that target this isoform. However, those drugs have not improved the mortality rates due to drug resistance. Developing drugs that target other members of the MAPK pathway is critical. And therapies that combine these new drugs in conjunction with BRAF inhibitors could counteract the mechanisms of resistance. Using both WiSIM and precursor multiplexing with msxDIA we were able to reveal important expression changes in the pathway. Additionally, these methods allow going back in time retrospectively interrogate data for new target peptides and proteins.

Conclusion

The new orbitrap technology enhances the sensitivity of DIA allowing its use as a satisfactory alternative to targeted methods.

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- It separates proteins or peptides according to their isoelectric points, whereby the separated components are recovered in liquid phase.

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GENERAL INFORMATION

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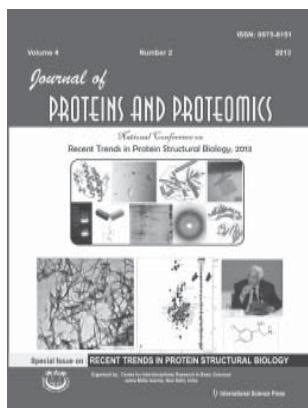
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