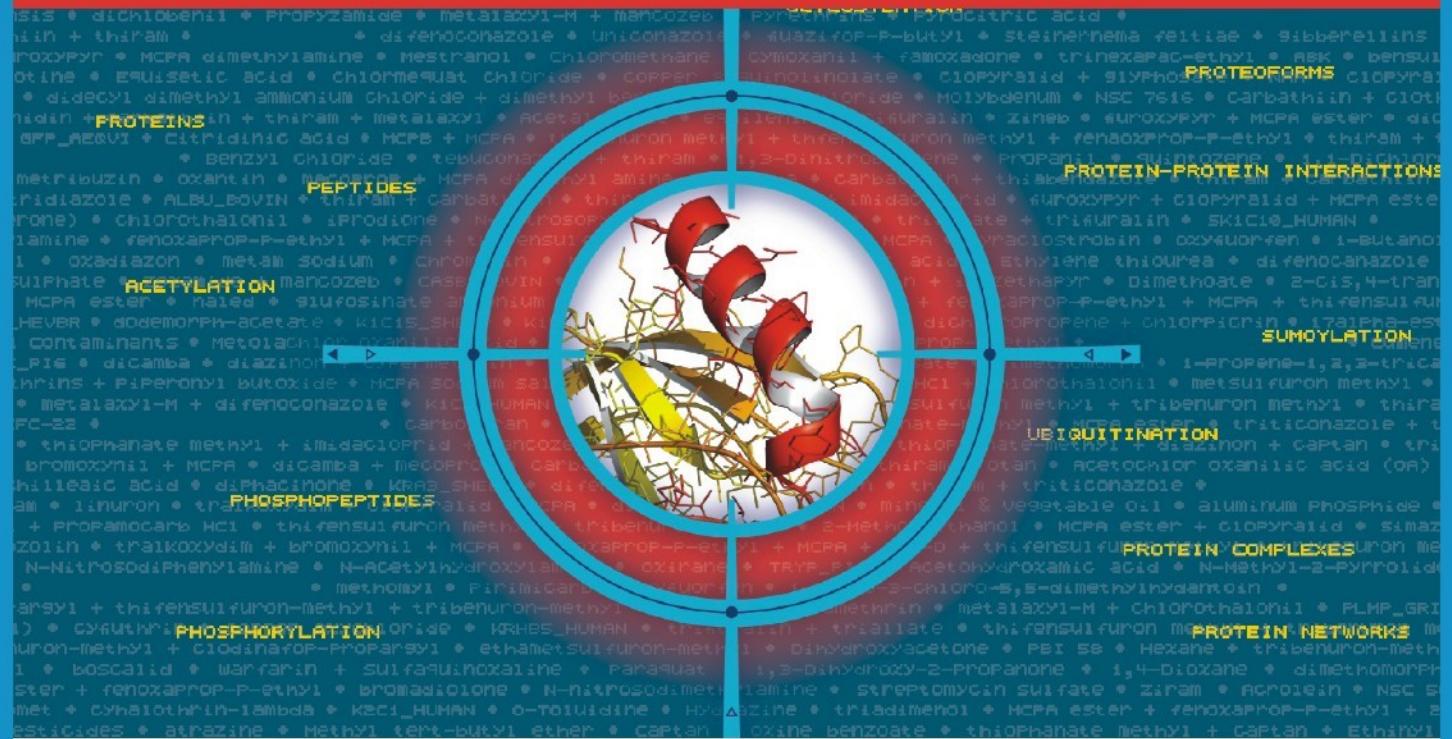


thermoscientific



More Proteins. More accurately.
Faster than ever.

Quantitation transformed

Biology is complex and understanding it is a big challenge. Identify and quantify more proteins and complexities such as PTMs faster and more accurately with our new portfolio of LC-MS instruments, sample prep solutions and software. HRAM solution using Thermo Scientific™ Orbitrap™ MS quantifies all detectable proteins and peptides with high specificity and fewer false positives, while triple quadrupole MS delivers SRM sensitivity and speed to detect targeted proteins more quickly. Join us in meeting today's challenges. Together we'll transform proteomics.



Thermo Scientific™ Orbitrap Fusion™ Tribrid MS
Unprecedented depth of analysis and
throughput for biological discovery



Thermo Scientific™ Q Exactive™ HF Orbitrap LC-MS/MS System
Screen and quantify known and unknown targets with HRAM Orbitrap technology



**Thermo Scientific™ TSQ Quantiva™
Triple-Stage Quadrupole MS**
Leading SRM sensitivity and speed
in a triple quadrupole MS/MS

For more information please contact:

Thermo Fisher Scientific India Pvt. Ltd.
102, 104, C Wing, Deloh, Iitanandani Business Park Powai, Mumbai 400076 India.
Tel: +91-22-6742 9494 • Email: analyze.in@thermofisher.com

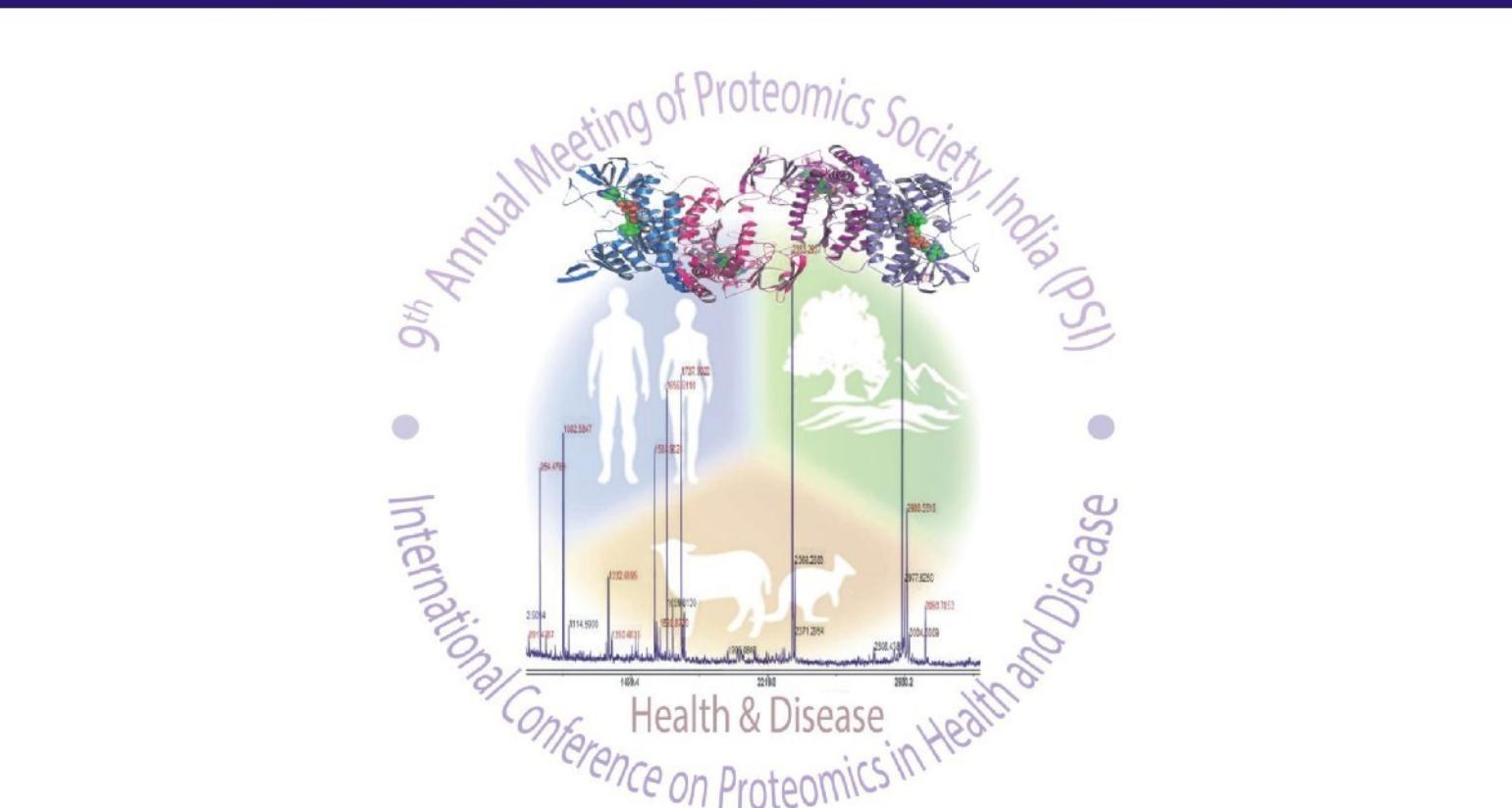
ThermoFisher
SCIENTIFIC

Journal of Proteins and Proteomics

Volume 8 Supplementary 2017

Journal of
PROTEINS AND PROTEOMICS

Conference Proceeding for 9th Annual Meeting of Proteomics Society, India (PSI)



International Conference on Proteomics in Health and Disease

Institute of Life Sciences, Bhubaneswar, India



A Journal of the
PROTEOMICS SOCIETY, INDIA

ISP International Science Press

Editorial Board — Journal of Proteins and Proteomics (JPP)

A Journal of the Proteomics Society, India (PSI)

EDITOR-IN-CHIEF

Suman Kundu

Department of Biochemistry, University of Delhi
South Campus, New Delhi, India

E-mail : jppindia@gmail.com

EDITORS FOR PROTEINS SECTION

Alo Nag

Department of Biochemistry,
University of Delhi (DU), South Campus
New Delhi, India

Amal Kanti Bera

Department of Biotechnology, Indian Institute of
Technology Madras (IITM), Chennai, India

Arvind M. Kayastha

School of Biotechnology, Banaras Hindu
University (BHU), Varanasi, India

Debashis Mukhopadhyay

Biophysics and Structural Genomics Division,
Saha Institute of Nuclear Physics (SINP), India

Gulsah Sanli

Department of Chemistry, Izmir Institute of
Technology, Izmir, Turkey

Himangshu S. Bose

Mercer University School of Medicine &
Memorial Health University, Medical Center,
Savannah, USA

Michael Blaber

Department of Biomedical Sciences,
Florida State University, Tallahassee, USA

Monica Sundd

National Institute of Immunology (NII),
New Delhi, India

N. Srinivasan

Molecular Biophysics Unit, Indian Institute of
Science (IISc), Bangalore, India

Rizwan Hasan Khan

Interdisciplinary Biotechnology Unit, Aligarh
Muslim University (AMU), Aligarh, India

Vikash Kumar Dubey

Department of Biosciences and Bioengineering,
Indian Institute of Technology Guwahati (IITG),
Guwahati, India

EDITORS FOR PROTEOMICS/BIOINFORMATICS SECTION

Abhijit Chakrabarti

Crystallography and Molecular Biology Division,
Saha Institute of Nuclear Physics (SINP),
Kolkata, India

Debasish Dash

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

Harsha Gowda

Institute of Bioinformatics (IOB), Bangalore;
Centre for Systems Biology and Molecular
Medicine, Yenepoya University, Mangalore, India

K Dharmalingam

Aravind Medical Research Foundation (AMRF),
Madurai, India

Kalpana Bhargava

Defence Institute of Physiology & Allied Sciences
(DIPAS), DRDO, Delhi, India

T. S. Keshava Prasad

Institute of Bioinformatics (IOB), Bangalore;
Centre for Systems Biology and Molecular
Medicine, Yenepoya University, Mangalore, India

Mahesh J Kulkarni

CSIR-National Chemical Laboratory (NCL),
Pune, India

Niranjan Chakraborty

National Institute of Plant Genome Research
(NIPGR), New Delhi, India

Rakesh K Mishra

CSIR-Centre for Cellular and Molecular Biology
(CCMB), Hyderabad, India

Ravi Sirdeshmukh

Institute of Bioinformatics (IOB); Mazumdar
Shaw Medical Foundation and Centre for
Translational Research, Bangalore, India

INTERNATIONAL ADVISORY BOARD

M.A. Vijayalakshmi

VIT University, Vellore, India

Maurizio Brunori

Sapienza- University of Rome, Rome, Italy

Michael I. Oshtrakh

Ural Federal University, Russian Federation

Rajiv Bhat

Jawaharlal Nehru University, New Delhi, India

Renu Deswal

Department of Botany, University of Delhi (DU),
Delhi, India

Sanjeeva Srivastava

Department of Biosciences and Bioengineering,
Indian Institute of Technology Bombay (IITB),
Mumbai, India.

Shantanu Sengupta

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

Srikanth Rapole

National Centre for Cell Science (NCCS), Pune,
India

Subhra Chakraborty

National Institute of Plant Genome Research
(NIPGR), New Delhi, India

Surekha M. Zingde

Formerly from Advanced Centre for Treatment,
Research and Education in Cancer (ACTREC),
Mumbai, India

Utpal Tatu

Department of Biochemistry, Indian Institute of
Science (IISc), Bangalore, India

ASSOCIATE MEMBERS

G. Hariprasad

Department of Biophysics, All India Institute of
Medical Sciences (AIIMS), New Delhi, India

K. Balamurugan

Department of Biotechnology, Alagappa
University (AU), Karaikudi, India

Lipi Thukral

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

Md. Imtiyaz Hassan

Centre for Interdisciplinary Research in Basic
Sciences, Jamia Millia Islamia (JMI), New Delhi,
India

Neel Sarovar Bhavesh

International Centre for Genetic Engineering and
Biotechnology (ICGEB), New Delhi, India



INTERNATIONAL SCIENCE PRESS

F-2562, Ansal's Palam Vihar, Gurgaon, Haryana
INDIA, Phone: 91-124-2365193
E-mail: internationalsciencespress@gmail.com



PROTEOMICS SOCIETY, INDIA

c/o CSIR-CCMB, Uppal Road, Hyderabad
Telengana 500007, E-mail: proteomicsociety@comb.res.in
www.psindia.org; www.jpp.org.in

GENERAL INFORMATION, GUIDELINES AND POLICIES

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

Copyright: Journal Articles by JPP is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License (<https://creativecommons.org>). Under the CC BY-SA license, JPP allows free access to its publications and one can copy, use, analyze, perform and present information publicly and produce and distribute derivative literature in any digital medium for any reasonable purposes, subject to appropriate acknowledgement of the authors and the journal with a link to the license. The journal allows rights to re-produce printed copies in accordance to the Creative Commons policies. If any content of the journal is re-mixed, transformed or built upon then it must be distributed with the same license as the original. Submission of an article implies that the authors agree to the copyright laws and principle followed by the journal in this regard.

Permissions: Please write to jppindia@gmail.com for information on how to request permissions to reproduce articles or any other information from the journal.

Disclaimer: The information and opinions presented in this journal reflect the views of the authors and not of the Proteomics Society or journal or its editors or international advisors or publisher and does not constitute endorsement by the journal or the society in any way. The journal or society does not assume any liability or responsibility for authenticity, correctness, accuracy, completeness or usefulness of the information published here and is the sole responsibility of the authors.

Plagiarism: The authors must ensure that they shun plagiarism in any form, whether in text material or data presented. Authors must thoroughly check their articles for plagiarism using standard, international tools and available practice and the journal assumes no responsibility for plagiarism committed by authors. Articles will be rejected or withdrawn if ever found guilty of plagiarism.

Ethical Issues, Rights: Authors are requested to conform to their institutional and country specific ethical guidelines and policies with respect to any biological sample. The society, journal or publisher carries no responsibility of any ethical mis-conduct. Proper ethical clearances must be obtained by the authors from appropriate authorities and the same must be declared in the published articles along with reference number and date of the clearance certificates. For human subjects and patient samples informed consent must be duly obtained by the authors as per regulations of the concerned authority and a statement to this effect should be included in the manuscript. Human and animal rights should not be violated and a statement to this effect must be included in the manuscript as well. All documents related to ethical issues must be readily available with the authors and must be produced on demand.

Conflict of interest: The authors must declare conflict of interests, if any.

Advertising Guidelines: JPP does accept classified advertisements from legal and well established agencies to promote the journal, as long as they conform to set policies of the journal and are related to the subject matter of the journal publications. Inquiries may be directed to jppindia@gmail.com. Advertisements do not however suggest that the journal endorses any of the products.

Supplementary Issue**9th Annual Meeting on Proteomics Society, India &
International Conference on Proteomics in Health and Disease****GUEST EDITOR: DR. AMOL R. SURYAWANSHI****CONTENTS**

Conference details, Committees

Message from President, PSI

Message from Director, ILS

Message from Convenor of Conference

Scientific Programme

Education Day Programme

Abstracts for Plenary Lectures	JPP 1-3
Abstracts for Keynote Lectures	JPP 4-11
Abstracts for Invited Speakers	JPP 12-16
Abstracts for Industry Lectures	JPP 17-18
Abstracts for Oral Presentations	JPP 19-24
Abstracts for Poster Presentations	JPP 25-51



WE LIVE **EFFICIENCY**

NEW **PURIFICATION TEAM**

Introducing the new Agilent InfinityLab LC Purification Solutions! A complete and scalable portfolio based on a single platform gives you the choice to tailor a system to meet your lab's current and future needs. Team up with Agilent and get on top of your analytical and preparative-scale LC purification challenges.

Live efficiency. Live purification.

www.agilent.com/chem/livepreplic
#WeLiveEfficiency #EfficientUHPLC



© Agilent Technologies, Inc. 2017

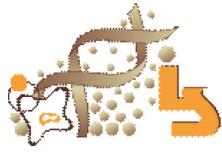


Agilent Technologies

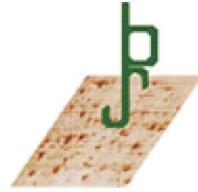


Institute of Life Sciences, Bhubaneswar

Nov. 29-Dec. 3, 2017



9th Annual Meeting of Proteomics Society, India (PSI)
International Conference on Proteomics in Health and Disease
Institute of Life Sciences, Bhubaneswar
November 30-December 02, 2017



OVERVIEW OF CONFERENCE AND ASSOCIATED SCIENTIFIC EVENT

9th Annual Meeting of Proteomics Society, India (PSI)
International Conference on Proteomics in Health and Disease
Institute of Life Sciences, Bhubaneswar

Date: November 30 - December 2, 2017

Venue: ILS Auditorium, Institute of Life Sciences, Bhubaneswar

Day 1 - Thursday, November 30, 2017,

- ☞ Conference - 9.00 AM to 6.45 PM
- ☞ PSI General Body meeting - 6.45 PM to 7.45 PM

Day 2 - Friday, December 01, 2017

- ☞ Conference - 9.00 AM to 7.00 PM

Day 3 - Saturday, December 02, 2017

- ☞ Conference - 9.00 AM to 12.00 Noon
- ☞ Valedictory function -12.05 PM to 01.00 PM

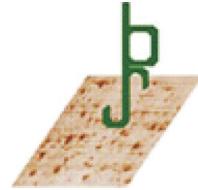
Pre-conference event

**Education day themed on
“Proteomics and Its Applications for Biological Research”**

Date: Wednesday, 29th November, 2017

Venue: ILS Seminar Hall, Institute of Life Sciences, Bhubaneswar

- ☞ Education day - 9.00 AM to 6.00 PM
- ☞ PSI Executive Council Meeting - 6.30 PM to 7.30 PM



9th Annual Meeting of Proteomics Society, India (PSI)
International Conference on Proteomics in Health and Disease
Institute of Life Sciences, Bhubaneswar
November 30-December 02, 2017

PSI 2017 Committees

Executive Council Members of Proteomics Society, India

Dr. Utpal S Tatu, President,

Professor, Indian Institute of Science, Bangalore

Dr. Subhra Chakraborty, Vice President,

Staff Scientist VII, National Institute of Plant Genome Research, New Delhi

Dr. Arun Bandyopadhyay, Vice President,

Principal Scientist, CSIR-Indian Institute of Chemical Biology, Kolkata

Dr. Mahesh J Kulkarni, Gen. Secretary,

Scientist, CSIR-National Chemical Laboratory, Pune

Dr. M.V Jagannadham, Treasurer,

Sr. Principal Scientist, CSIR-Centre for Cellular and Molecular Biology, Hyderabad

Dr. Sanjeeva Srivastava, Jt Secretary,

Associate Professor, Indian Institute of Technology Bombay, Mumbai

Dr. Ravi Sirdeshmukh,

Distinguished Scientist & Associate Director, Institute of Bioinformatics, Bangalore

Member and Past President of PSI,

Director, CSIR-Centre for Cellular and Molecular Biology, Hyderabad

Dr. Rakesh K Mishra, Member,

Professor, Saha institute of Nuclear Physics, Kolkata

Dr. Abhijit Chakrabarti, Member,

Principal Scientist, CSIR-Institute of Genomics & Integrative Biology, New Delhi

Dr. Shantanu Sengupta, Member,

Professor and Director - Research, Aravind Medical Research Foundation, Madurai

Dr. K Dharmalingam, Member,

Scientist F, Defence Institute of Physiology & Allied Sciences, Delhi

Dr. Kalpana Bhargava, Member,

Staff Scientist VI, National Institute of Plant Genome Research, New Delhi

Dr. Niranjan Chakraborty, Member,

Sr. Scientist, Institute of Life Sciences, Bhubaneswar

Dr. Amol R Suryawanshi, Member,

Sr. Scientist, CSIR-Centre for Cellular and Molecular Biology, Hyderabad

Dr. Suman S. Thakur, Member,

Wellcome Trust/DBT India Alliance Early Career Fellow, Institute of Bio-informatics, Bangalore

Dr. Harsha Gowda, Member,

Sr. Scientist, National Center for Cell Science, Pune

Dr. Srikanth Rapole, Member,

Professor, Department of Biochemistry, University of Delhi South Campus, New Delhi

Dr. Suman Kundu, Member,

Principal Scientist, National Dairy Research Institute, Karnal

Dr. Ashok Kumar Mohanty, Member,

Principal Scientist, CSIR-Indian Institute of Chemical Biology, Kolkata

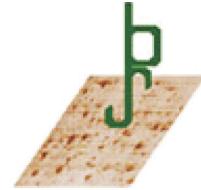
Dr. Sharmila Chattopadhyay, Member,

Scientist, CSIR-Institute of Genomics & Integrative Biology, New Delhi

Dr. Debasis Dash, Member,

Prof. and Ex - Deputy.Director, Advanced Centre for Training, Research and Education in Cancer, Mumbai

Dr. Surekha Zingde, Immediate Past President,



9th Annual Meeting of Proteomics Society, India (PSI)
International Conference on Proteomics in Health and Disease
Institute of Life Sciences, Bhubaneswar
November 30-December 02, 2017

PSI-2017 Conference Committees

PATRONS

Dr. K Vijay Raghavan, Secretary, Department of Biotechnology, India

CHAIRMAN

Dr. Ajay Parida, Director, Institute of Life Sciences, Bhubaneswar, India

CONVENER & CO-CONVENOR

Dr. Amol R. Suryawanshi, Institute of Life Sciences, Bhubaneswar, India

Dr. Soma Chatopadhyay, Institute of Life Sciences, Bhubaneswar, India

SCIENTIFIC ADVISORY COMMITTEE

Dr. Akhilesh Pandey, Johns Hopkins University, USA

Dr. D. R. Mani, Broad Institute, USA

Dr. Debasis Dash, CSIR-Institute of Genomics & Integrative Biology, New Delhi

Dr. Mahesh J Kulkarni, CSIR-National Chemical Laboratory (NCL), Pune

Dr. Ravi Sirdeshmukh, Institute of Bioinformatics, Bangalore

Dr. Shantanu Sengupta, CSIR-Institute of Genomics & Integrative Biology, New Delhi

Dr. Subhra Chakraborty, National Institute of Plant Genome Research, India

Dr. Surekha Zingde, Ex-President, PSI, Mumbai

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

LOCAL ORGANIZING COMMITTEE

Dr. Amol R. Suryawanshi, Institute of Life Sciences, Bhubaneswar

Dr. Soma Chatopadhyay, Institute of Life Sciences, Bhubaneswar

Dr. P. V. Ramchandar, Institute of Life Sciences, Bhubaneswar

Dr. T. K. Beuria, Institute of Life Sciences, Bhubaneswar

Dr. Dileep Vasudevan, Institute of Life Sciences, Bhubaneswar

Dr. Jagannath Dandapat, Utkal University, Bhubaneswar

Dr. Avinash Sonawane, KIIT-Biotechnology, Bhubaneswar

Dr. Palok Aich, NISER, Bhubaneswar

Dr. Pankaj Alone, NISER, Bhubaneswar

Dr. Jyotirmaya Mohanty, ICAR-CIFA, Bhubaneswar

Dr. D. Pradhan, Institute of Life Sciences, Bhubaneswar

Dr. Rajendra K. Behera, Institute of Life Sciences, Bhubaneswar

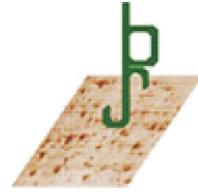
Dr. Prakash. K. Sahoo, Institute of Life Sciences, Bhubaneswar



Institute of Life Sciences, Bhubaneswar
Nov. 29-Dec. 3, 2017



9th Annual Meeting of Proteomics Society, India (PSI)
International Conference on Proteomics in Health and Disease
Institute of Life Sciences, Bhubaneswar
November 30-December 02, 2017



Message from President, PSI

Dear fellow delegates of proteomic science,

I take great pleasure in welcoming you to the 9th annual meeting of the Proteomic Society of India at ILS Bhubaneshwar located in the state of Odisha, India.

The ancient state of Odisha is well known for its beautiful sculptures, monuments and religious sites such as Konark, Jagannath and Rajarani temples. While you may be aware of the religious significance associated with these temples, it is possible that you may have missed the scientific significance behind these famous, religious sites.

It is true that the Sun temple that dates back to 13th century, houses the first ever engineering concept of an instrument to measure time!

Yes, the wheels of the famous chariot located at this site were designed to monitor time, based on the position of sun, to an accuracy of less than a minute. It is heart warming to see that our fore fathers designed the first ever concept to measure and monitor time and laid the foundation to the modern watch.

Over the centuries, however, tables have turned and we have transformed from a nation known for original scientific observations to followers of scientific advancement. This is particularly true for proteomic research in the country. We take pride in talking about expensive MS instruments acquired from companies all over the world and status of an institute is often judged by how expensive a machine does the institution possess.

While it is true that advance MS are useful in fast and accurate identifications of proteins, I must emphasize to the student community in the country that you can make some wonderful and original observations without using expensive equipment. Proteins don't necessarily require expensive equipments to share their mysteries. Proteins will talk to you if you have the patience to purify them, denature them, renature them, chemically modify them and measure their function. None of the above requires fancy equipments.

Let us all get inspired by the pioneering work that we witness in the beautiful temples located in the state of Odisha to remind us that the ability to make original scientific observations is inherent within our culture and we must make an effort to revitalize this quality within us.

I hope you will have an excellent learning experience during this conference at ILS.

With best wishes from all my fellow EC members of PSI,



Utpal Tatu
President, PSI and Professor, IISc, Bengaluru

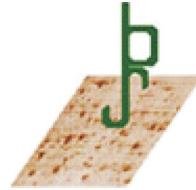


Institute of Life Sciences, Bhubaneswar

Nov. 29-Dec. 3, 2017



9th Annual Meeting of Proteomics Society, India (PSI)
International Conference on Proteomics in Health and Disease
Institute of Life Sciences, Bhubaneswar
November 30-December 02, 2017



Message from Director, ILS

I am extremely happy to welcome the delegated and invited guests for the International Conference on "Proteomics in Health and Disease" being organized at the Institute of Life Sciences, Bhubaneswar. We are extremely grateful to the Proteomics Society, India (PSI) for choosing ILS for their 9th Annual meeting coinciding with this conference.

Proteomics is a rapidly growing field of molecular biology that is concerned with the systematic, high-throughput approach to protein expression analysis of a cell or an organism. Advances in proteomics research during the last few years have made significant contribution to deepening our insight into the nature of the relationship between genes and proteins. With a series of innovations in the core technologies of two-dimensional electrophoresis and mass spectrometry, and a diversity of productive research programmes, the usefulness of this approach have had demonstrated success. Well annotated proteomics databases are now emerging in a number of fields to provide a platform for systematic research. Protein-sequence data are now available for many microorganisms, providing us with tools for understanding their resistance to antimicrobial drugs and for identifying novel agents for treating drug-resistant disease.



The results of genomics and proteomics increasingly promise the potential for future widespread adoption in medicine, health, agriculture and basic biology. Taking advantage of the progress made in other 'omics' approach and particularly the genomic data available today, the field of proteomics has potential to grow into the leading arena for the identification and characterization of cellular gene products (*i.e.* proteins) that are present, absent, or altered under a certain environmental, physiological and pathophysiological conditions. These will have significant contribution in developing research programmes aimed at addressing issues related to health and disease management in human, plants and animals and will contribute to improved health status as well as enhanced agriculture productivity.

I am sure the discussions and celebrations in this three day conference will be intellectually stimulating and scientifically rewarding. The bottom line of our research is to focus on transferring the scientific knowledge for addressing the societal issues. Hence, I am sure the discussions will provide pathways to developing strategies for taking research leads from "bench to bedside" as well as from "farm to fork".

I wish the conference a great success.

Ajay Parida
Director
Institute of Life Sciences, Bhubaneswar

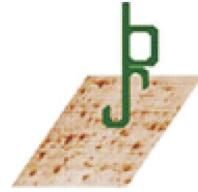


Institute of Life Sciences, Bhubaneswar

Nov. 29-Dec. 3, 2017



9th Annual Meeting of Proteomics Society, India (PSI)
International Conference on Proteomics in Health and Disease
Institute of Life Sciences, Bhubaneswar
November 30-December 02, 2017



Message from Convener, Conference

On behalf of the organising committee, it is my great pleasure and honour to extend a warm welcome to you to the 9th Annual Meeting of Proteomics Society, India (PSI) and an International Conference on Proteomics in Health and Disease (PSI2017) being organized at Institute of Life Sciences (ILS), Bhubaneswar, India from November 30 - December 02, 2017.

The PSI-2017 congress brings together national and international eminent scientists working in the field of proteomics for Health and Diseases covering human, animal and plant biology. I am sure that deliberations will give us newer and newer insights into the emerging dynamics of Proteomics in Health and Disease. A well thought out and engaging academic programme has been planned for all, which will include Education Day, Plenary lectures, Keynote Lectures, Invited Lectures, Panel Discussion, Poster Sessions and other opportunities to interact.



PSI2017 conference features many distinguished scientists from abroad including Dr. Stephan Pennington (HUPO President-elect), Dr. Hanno Steen, Dr. Spiros D. Garbis, Dr. Judith Steen, Dr. D. R. Mani, Dr. Akhilesh Pandey and many distinguished scientists from the country who will address the congress delegates.

Keeping tradition of Proteomics Society, India to give an opportunity to young PSI members and scientist, many young scientists will be presenting their research work and delegates will also get an opportunity to listen to senior PSI members and scientists. In addition, selected students are also given an opportunity to present their research work as lightening short talks.

PSI2017 congress in Bhubaneswar will provide unique opportunity to share your proteomics research with wide scientific community and a forum for in-depth analysis of the challenges involved in studying the dynamic proteome. In addition to highly motivating plenary, keynote and invited talks; panel discussion; you will enjoy pleasant weather in Bhubaneswar. Bhubaneswar is known as "The City of Temples" and is one of the most culturally effervescent cities in India. Further, Bhubaneswar also plays an important role as a regional gateway to the Golden Tourist Triangle of Puri, Konark, and Chilika Lake. I hope participants will have time to visit and enjoy the city and its surroundings.

Finally, I thank the Scientific Advisory and local Organising Committee, Executive council of PSI, Industry partners, Invited speakers and delegates for participation. I hope with your support and participation, we will make this fascinating scientific event successful and memorable.

With warm regards

Amol R. Suryawanshi
*Convener, PSI-2017 and
Guest Editor, Conference Proceedings, JPP*

Scientific Program

Day 1: Thursday 30th November 2017

Time	Title of the Talk and Speaker Name / Details
08.00 AM to 09.00 AM	Registration
09.00 AM to 09.10 AM	Welcome address

PLENARY & KEYNOTE SESSION 1

Session Chair

Dr. Ajay Parida, Director, ILS, Bhubaneswar
Dr. Utpal Tatu, President, PSI& IISc, Bangalore

09.10 AM to 09.50 AM	PL-01:	Discovery Proteomics may not translate easily to Clinical Proteomics <i>Dr. M. Radhakrishna Pillai, RGCB, Thiruvananthapuram</i>
09.50 AM to 10.30 AM	PL-02:	Clinical Proteomics: Are We Finally Ready for Biomarker Discovery? <i>Dr. Hanno Steen, Boston Children's Hospital, Boston, USA</i>
10.30 AM to 10.50 AM	KN-01:	Proteomic profiling of plasma membrane in chickpea reveals a novel negative regulator of dehydration tolerance <i>Dr. Niranjan Chakraborty, NIPGR, New Delhi</i>
10.50 AM to 11.10 AM	KN-02:	Addressing vitamin B12 deficiency- Need of the hour <i>Dr. Shantanu Sengupta, CSIR-IGIB, New Delhi</i>
11.10 AM to 11.30 AM	Break	Group Photo Exhibition & High Tea
11.30 AM to 12.30 PM		Inauguration

SESSION 1: GLOBAL AND TARGETED PROTEOMICS

Session Chair

Dr. Subhra Chakraborty, NIPGR, New Delhi
Dr. Judith Steen, Boston Children's Hospital, Boston, USA

12.30 PM to 12.50 PM	KN-03:	Endometrial Secretome and Its Modulation: From "Function" to "Dysfunction" <i>Dr. Gitanjali Sachdeva, ICMR-NIRRH, Mumbai</i>
12.50 PM to 01.10 PM	KN-04:	Proteome-wide effect of silencing of Milk Fat Globule Membrane Epithelial Growth Factor 8 (MFGE8) on lactocytes <i>Dr. Ashok K. Mohanty, ICAR-NDRI, Karnal</i>
01.10 PM to 03.00 PM	Break	Lunch and Poster session I
03.00 PM to 03.15 PM	IL-01:	Applications of proteomics in plant biology: with special reference to abiotic stress <i>Dr. Giridara-Kumar Surabhi, RPRC, Bhubaneswar</i>
03.15 PM to 03.30 PM	IL-02:	Proteomics in vaccine development against parasitic infections <i>Dr. Jyotirmaya Mohanty, ICAR-CIFA, Bhubaneswar</i>
03.30 PM to 03.45 PM	IL-03:	Defect in translation initiation fidelity alters differential protein expression pattern and cellular physiology in <i>Saccharomyces cerevisiae</i> <i>Dr. Pankaj Alone, NISER, Bhubaneswar</i>
03.45 PM to 03.55 PM	O-01:	Development of a LC-MS based method for typing of A1/A2 milk and their comparative proteome analysis using TMT based quantitation approach <i>Mr. Shivam Kumar Dubey, ICAR-NDRI, Karnal</i>

03.55 PM to 04.05 PM	O-02:	Differentially expressed proteins in hepatopancreas of freshwater prawn, <i>Macrobrachium rosenbergii</i> After <i>Vibrio harveyi</i> challenge <i>Mr. Mohan R. Badhe, ICAR-CIFA, Bhubaneswar</i>
04.05 PM to 04.20 PM	Break	Exhibition & High Tea

SESSION 2: DISEASE PROTEOMICS & BIOMARKER DISCOVERY

Session Chair

Dr. Sanghamitra Pati, RMRC, Bhubaneswar
Dr. B. S. Das, Bhubaneswar

04.20 PM to 04.40 PM	KN-05:	Heat shock protein 40 as a knob chaperone in <i>Plasmodium falciparum</i> infected human erythrocytes <i>Dr. Utpal Tatu, IISc, Bangalore</i>
04.40 PM to 05.00 PM	KN-06:	Plasma proteomics in Coronary Artery Disease <i>Dr. Arun Bandyopadhyay, CSIR-IICB, Kolkata</i>
05.00 PM to 05.15 PM	IL-04:	Proteomics and functional analysis of outer membrane vesicles of bacteria <i>Dr. M V Jagannadham, CSIR-CCMB, Hyderabad</i>
04.20 PM to 04.40 PM	Break	Exhibition & High Tea
05.15 PM to 05.30 PM	IL-05:	Saliva as a diagnostic fluid: Are we there yet? <i>Dr. Kiran S. Ambatipudi, IIT, Roorkee</i>
05.30 PM to 05.45 PM	IL-06:	Application of Quantitative high throughput Proteomics in health and disease <i>Dr. Suman S. Thakur, CSIR-CCMB, Hyderabad</i>
05.45 PM to 05.55 PM	O-03:	Multipronged proteomic analysis of multiple myeloma towards new targets and biomarkers <i>Mr. Chanukuppa Venkatesh, NCCS, Pune</i>
05.55 PM to 06.05 PM	O-04:	Identification of estrus associated proteins in saliva of water buffalo (<i>Bubalus bubalis</i>) using high throughput Mass Spectrometry <i>Ms. Rubina Kumari Baithalu, ICAR-NDRI, Karnal</i>

PLENARY SESSION 2

Session Chair

Dr. Mrutunjay Suar, Director, KIIT-Biotech, Bhubaneswar
Dr. Srikanth Rapole, NCCS, Pune

06.05 PM to 06.45 PM	PL-03:	Novel insight to the causal link between insulin signaling deregulation and its potential etiology of aggressive prostate cancer: Implications to the development of novel biomarkers and treatment targets <i>Dr. Spiros D. Garbis, University of Southampton, UK</i>
06.45 PM to 07.45 PM		PSI GB meeting (ILS Auditorium)
07.45 PM to 10.00 PM		Banquet Dinner

Day 2: Friday 1st December 2017

Time

Title of the Talk and Speaker Name

PLENARY SESSION 3

Session Chair

Dr. M. A. Vijayalakshmi, VIT, Vellore

09.00 AM to 09.40 AM	PL-04:	Proteogenomic Analysis of Breast Cancer <i>Dr. D. R. Mani, Broad Institute, USA</i>
----------------------	--------	----------------------------------------------------------------------------------------

SESSION 3: PROTEOGENOMICS AND BIG DATA ANALYSIS

Session Chair

Dr. Ravi Sirdeshmukh, IOB, Bangalore
Dr. Surekha Zingde, Ex-President PSI

09.40 AM to 10.00 AM	KN-07:	An integrative proteogenomics approach to unravel human proteoforms <i>Dr. Debasis Dash, CSIR-IGIB, New Delhi</i>
10.00 AM to 10.20 AM	IL-07:	Reverse Phase Protein Array (RPPA) for integrating cancer genomics and proteomics data to unwire lung cancer relevant signaling and drug resistance mechanisms <i>Dr. Ramesh Ummanni, CSIR-IICT, Hyderabad</i>
10.20 AM to 10.30 AM	O-05:	A comprehensive proteome map of Pashmina fibre by combining multiple protein extraction methods and proteogenomics approach <i>Ms. Sakshi Kaith, ICAR-NDRI, Karnal</i>
10.30 AM to 10.40 AM	O-06:	Data exploration and in-silico analysis of human proteome for development of web-based targeted proteomics framework <i>Ms. Manika Singh, Institute of Bioinformatics, Bangalore</i>
10.40 AM to 11.00 AM	Break	Exhibition & High Tea

SESSION 4: METABOLOMICS AND INTEGRATION OF OMICS

Session Chair

Dr. Shantanu Sengupta, CSIR-IGIB, New Delhi
Dr. Palok Aich, NISER, Bhubaneswar

11.00 AM to 11.20 AM	KN-08:	Integrative multiomics analysis of vivax malaria <i>Dr. Sanjeeda Srivastava, IIT, Mumbai</i>
11.20 AM to 11.40 AM	KN-09:	Integrated omics implied the endogenous protease inhibitors influence protease gene expression in <i>Helicoverpa armigera</i> Dr. Ashok Giri, CSIR-NCL, Pune
11.40 AM to 12.00 PM	KN-10:	Proteomic and metabolomic profiling towards candidate markers for Breast cancer <i>Dr. Srikanth Rapole, NCCS, Pune</i>
12.00 PM to 12.15 PM	IL-08:	Asthma-COPD overlap syndrome (ACOS) - a new disease or a variant of asthma or COPD? A (1H)-NMR based metabolomics study <i>Dr. Koel Chaudhary, IITK, Kharagpur</i>

12.15 PM to 12.30 PM	CL-01:	Integrated Biology Approach for comprehensive understanding of Biological systems: Tools and challenges <i>Dr. Saurabh Nagpal, Agilent Technologies, Manesar</i>
12.30 PM to 12.45 PM	CL-02:	SWATH acquisition: A robust methodology to address various biological questions <i>Sciex, Gurgaon, Haryana, India</i>
12.45 PM to 12.55 PM	O-07:	Integrated proteomic and miRNA profiling analyses in oral keratinocytes in response to cigarette smoke <i>Mr. Mohd Younis Bhat, Institute of Bioinformatics, Bangalore</i>
12.55 PM to 01.05 PM	O-08:	Plant responses and global significance: Elevated CO ₂ and temperature effects on Picrorhiza kurroa <i>Dr. Rajiv Kumar, CSIR-IHBT, Palampur</i>
01.05 PM to 03.00 PM	Break	Lunch and Poster session II

SESSION 5: POST-TRANSLATIONAL MODIFICATIONS (PTMS)

Session Chair

Dr. Akhilesh Pandey, Johns Hopkins University, USA

Dr. Abhijit Chakrabarti, SINP, Kolkata

03.00 PM to 03.20 PM	KN-11:	Using Proteomics to Understand Dementias associated with Tauopathy <i>Dr. Judith Steen, Boston Children's Hospital, Boston, USA</i>
03.20 PM to 03.40 PM	KN-12:	Abundance and glycation status of albumin determines hemoglobin glycation <i>Dr. Mahesh Kulkarni, CSIR-NCL, Pune</i>
03.40 PM to 04.00 PM	CL-03:	The tims TOF Pro with PASEF: Digging deeper into the proteome with record-breaking speed, sensitivity and robustness. <i>Dr. Lucy Ann Woods, Bruker Daltonik GmbH, Germany</i>
04.00 PM to 04.10 PM	O-09:	Complexity and diversity of mucin-type O-glycosylation of CD43 revealed by electron transfer dissociation mass spectrometry <i>Ms. Vandita Dwivedi, NII, New Delhi</i>
04.10 PM to 04.20 PM	O-10:	Phospho T693 EGFR: a prognostic marker of recurrent non-functioning pituitary adenoma <i>Mr. Ashutosh Rai, PGIMER, Chandigarh</i>
04.20 PM to 04.30 PM	Break	Exhibition & High Tea

SESSION 5: HUMAN PROTEOME PROJECT-CHPP/B-DHPP

Co-ordinator

Dr. Ravi Sirdeshmukh, IOB, Bangalore

04.30 PM to 06.00 PM	PD-1:	Panel Discussion: HPP and Future prospect Panellist: TBA
----------------------	-------	--------------------------------------------------------------------

PLENARY SESSION 4

Session Chair

Dr. D. R. Mani, Broad Institute, USA

06.00 PM to 06.40 PM	PL-5:	Proteomics for Patients 'From protein biomarker discovery to the development of diagnostic tests' <i>Dr. Stephan Pennington, UCD Conway Institute, Ireland</i>
07.30 PM to 08.30 PM		Cultural Program
08.30 PM to 10.00 PM		Dinner

Day 3: Saturday 2nd December 2017

Time

Title of the Talk and Speaker Name

PLENARY SESSION 5

Session Chair

Dr. Utpal Tatu, President, PSI & IISc, Bengaluru

09.00 AM to 09.40 AM	PL-6:	Dissecting interactomes using proximity-dependent biotinylation <i>Dr. Akhilesh Pandey, Johns Hopkins University, USA</i>
----------------------	-------	------------------------------------------------------------------------------------------------------------------------------

SESSION 7: INTERACTION PROTEOMICS & PROTEIN NETWORKS

Session Chair

Dr. Stephan Pennington, UCD Conway Institute, Ireland

09.40 AM to 10.00 AM	KN-13:	Structural Investigation of Plant Seed Proteome <i>Dr. Dinakar M. Salunke, ICGEB, New Delhi</i>
10.00 AM to 10.20 AM	KN-14:	Integrative Omics and Biomolecular Network Analysis Dissect Host-Specific Immune Dynamics in Fusarium disease <i>Dr. Subhra Chakraborty, NIPGR, New Delhi</i>
10.20 AM to 10.40 AM	KN-15:	Identifying Protein Networks to evade High Altitude Illnesses: A Proteomics Approach <i>Dr. Kalpana Bhargava, DIPAS, Delhi</i>
10.40 AM to 11.00 AM	Break	Exhibition & High Tea
11.00 AM to 11.15 AM	IL-09:	To understand the role of Mycobacteria glyconetwork in tuberculosis <i>Dr. Avinash Sonawane, KIIT, Bhubaneswar</i>
11.15 AM to 11.30 AM	IL-10:	Suppression of apoa1 2b in arsenic exposed carp Labeo rohita indicates efficacy of curcumin against arsenicosis <i>Dr. Bimal P. Mohanty, ICAR-CIFRI, Kolkata</i>
11.30AM to 11.45AM	IL-11	Oncogenic splicing switch and glucose metabolism in breast cancer <i>Dr. Sanjeev Shukla, IISER, Bhopal</i>
11.45 AM to 11.55 AM	O-11:	Dehydration induced mitochondrial defence response by catabolism of reactive oxygen species in rice <i>Dr. Dipak Gayen, NIPGR, New Delhi</i>
12.00 PM to 01.00 PM		Valedictory function
01.00 PM to 02.30 PM	Break	Lunch

Education Day Program

"Proteomics and Its Applications for Biological Research"

Education Day: Wednesday 29th November 2017

Time	Title of the Talk and Speaker Name
08.00 AM to 09.15 AM	Registration
09.15 AM to 09.30 AM	Inauguration & Welcome

Co-ordinators

Dr. Surekha Zingde, Ex-President, PSI
Dr. Srikanth Rapole, NCCS, Pune

SESSION I: TECHNOLOGY

Session Chairs

Dr. B. P. Shaw, ILS, Bhubaneswar
Dr. Palok Aich, NISER, Bhubaneswar

09.30 AM to 10.00 AM	EL-01:	Why and How of Proteomics for Biological Research <i>Dr. Surekha Zingde, Ex-President, PSI</i>
10.00 AM to 10.30 AM	EL-02:	Mass Spectrometry based Quantitative Proteomics <i>Dr. Srikanth Rapole, NCCS, Pune</i>
10.30 AM to 11.00 AM	EL-03:	Databases for Proteomics <i>Dr. Debasis Dash, IGIB, New Delhi</i>
11.00 AM to 11.10 AM	Break	Tea
11.10 AM to 11.40 PM	EL-04:	Chromatography for Proteomics <i>Dr. M. A. Vijayalakshmi, VIT, Vellore</i>
11.40 AM to 12.10 PM	EL-05:	Targeted quantification of glycated peptides of albumin in diabetes, <i>Dr. Mahesh Kulkarni, NCL, Pune</i>
12.10 PM to 12.40 PM	EL-06:	Gel based Quantitative Proteomics <i>Dr. Abhijit Chakrabarti, SINP, Kolkata</i>
12.40 PM to 01.10 PM	EL-07:	PTMs identification and characterization <i>Dr. Hanno Steen, Boston Children's Hospital, Boston, USA</i>
01.10 PM to 02.00 PM	Break	Group photo & Lunch

SESSION II: APPLICATIONS

Session Chair

Dr. J. Dandapat, Utkal University, Bhubaneswar

02.00 PM to 02.30 PM	EL-08	Proteomic profiling of Arabidopsis during <i>Pseudomonas syringae</i> infection and under altered glutathione conditions <i>Dr. Sharmila Chattopadhyay, IICB, Kolkata</i>
02.30 PM to 03.00 PM	EL-09	Proteomic Window for Insights into the Oddities of "Reproduction" <i>Dr. Geetanjali Sachdeva, NIRRH, Mumbai</i>
03.00 PM to 03.30 PM	EL-10	Proteomics in Dairy and Animal Science Research <i>Dr. Ashok Mohanty, NDRI, Karnal</i>

03.30 PM to 04.30 PM	Proteomics Dr. Amol Suryawanshi, co-ordinator facility visit
03.45 PM to 04.15 PM	Break High Tea
04.30 PM to 05.00 PM	EL-11 Statistics and Machine Learning Methods for Proteomics and Proteogenomics <i>Dr. D. R. Mani, Broad Institute.</i>
05.00 PM to 05.30 PM	EL-12 Protein Microarrays for biomarker validation <i>Dr.Sanjeeva Srivastava, IITB, Mumbai</i>
05.30 PM to 06.00 PM	Feedback and Concluding remarks

Acknowledgement

Sponsors

thermo
scientific

BIO-RAD

SCIEX

BRUKER

 **Agilent** | Trusted Answers

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Support

**CiNSERB
DIA**



ABSTRACTS FOR PLENARY, KEYNOTE, INVITED AND INDUSTRY LECTURES

PLENARY LECTURES

PL-01

Discovery Proteomics may not translate easily to Clinical Proteomics

M. Radhakrishna Pillai, FRCPath, PhD, FASc, FNASC,
FAMS, FNA

Professor of Disease Biology & Director, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram 695014, India

The success human genome project allowed a much more elaborate understanding of the molecular basis of diseases. However, to develop better understanding of the dynamic protein pathways involved in normal and disease states, and consequently elucidating response to medical treatment, is the challenge to meet if we are to effectively treat disease. While proteomics still remains a tool of choice for discovery research, a better innovation in proteomic technology is required to realize the potential for proteomic profiling to become standard practice in the clinical laboratory.

RGCB has had substantial success in the use of proteomics to understand disease biology. Proteomic analysis of chikungunya virus - infected Human Embryo Kidney cells (HEK293) helped us short-list 30 cellular proteins that would be potential candidates for targeted study of molecular interactions of the virus with host cells. Another study identified proteins that are uniquely expressed during reactivation of MTb bacteria from dormancy, which were suggested as potential targets for therapeutic intervention. The proteome profiles of the pandemic strain Vibrio parahaemolyticus during the planktonic and biofilm stages is another example of identification of proteins involved in resistance mechanisms. Proteome analysis has also opened new horizons to establish scientific basis for traditional Indian Ayurveda drugs. For example, proteome analysis on the effects of "Amalakirasayana" on cardiac mitochondria described how cardiac function is improved by the rasayana in hypertrophic conditions. Cell signaling pathways analyzed by proteomics demonstrated how Bis (3,5-diiodo-2,4,6-trihydroxyphenyl) squaraine, a drug that could be used for photodynamic therapy disrupts redox homeostasis and induce mitochondria-mediated apoptosis human breast cancer cells. An exploratory proteomic analysis helped us reveal a novel event of

β-hCG induced hemoglobin protein expression in protecting the BRCA1 mutated cancer cells from oxidative stress, making it resistant to drugs.

These are only small incremental discoveries in a small research institute, best defined as a collection of PhD theses. They have been larger and more networked efforts world over. There was genuine optimism and hope that based on huge advances in basic biology and technology, proteomics could revolutionize diagnostic sciences by creating novel disease biomarkers for diagnosis, monitoring, and prediction of therapy. However the dismal truth is that despite more than 15-year of efforts, use of proteomics has discovered no major disease biomarkers. There has been substantial financial investment from government funding agencies (including the NIH) and diagnostics companies. The complexities of vector host interactions in infections, multifactorial etiology of non-communicable diseases and lack of ample expertise available for big data analysis have posed substantial challenge in making clinical proteomics as routine tool in diagnostics and therapeutic applications.

PL-02

Clinical Proteomics: Are We Finally Ready for Biomarker Discovery?

Hanno Steen, Ph.D.

Associate Professor of Pathology, Depts. of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, MA, USA

For 2 decades proteomics has promised a revolution in biomarker discovery. While there have been several studies describing newly discovered biomarkers, few have become approved for diagnostics. Arguably instrumentation and methodology has only now become sufficiently advanced to realize proteomics-based biomarker discovery in practice. This talk describes a new clinical proteomics platform featuring novel methods in sample preparation and data acquisition to enable the throughput needed for biomarker discovery projects that overcome the interpersonal variability of body fluid and tissue composition. Example applications range from urine to plasma proteomics in the context of infectious, inflammatory and traumatic diseases.

Novel insight to the causal link between insulin signaling deregulation and its potential etiology of aggressive prostate cancer: Implications to the development of novel biomarkers and treatment targets

Spiros D. Garbis, PhD

Faculty of Medicine – University of Southampton, UK

The systems interrogation of human derived clinical specimens has now become requisite to any comprehensive biomarker discovery and its functional validation research program. It relies heavily on the analysis of high throughput functional genomic features at multiple levels of molecular biology events, namely gene expression at the transcript, protein and metabolite levels. Many of these biomolecular species are oftentimes manifested in exosomes and lipid microvesicles and exhibit both organotypic and organotropic properties. However, the clinical exploitation of such multi-omic observations, either in terms of gaining a mechanistic understanding of the molecules undergoing perturbations as they relate to specific disease processes, or how they effectively stratify disease down to the individual patient level (personalized medicine) regardless of their innate heterogeneity of presentation, has had limited success. To achieve this, we have developed a unique, high-precision LC-MS based, biomarker discovery and functional validation pipeline that can differentiate benign versus aggressive prostatic disease at the minimally invasive blood level. It was found that the progression to aggressive prostate cancer implicated the disruption of multiple biological pathways such as DNA damage response elements, autophagy, apoptosis, cell metabolism and their convergence to insulin signaling dysregulation. Hallmark proteins to these signatures included pyruvate kinase M1/M2 isoforms, p21, eEF isoforms 1-3, IGF BP isoforms 1-6, and MAP kinase isoforms 1 and 2 that could be used on a combinatorial basis for the early prediction of aggressive prostate cancer and its potential treatment.

References

- Larkin, *et al. Br J Cancer*, 2016.
- Galanos, *et al. Nature Cell Biol*, 2016.
- Garbis, SD, *et al. Anal Chem*, 2011.

Proteogenomic Analysis of Breast Cancer

D. R. Mani, Ph.D.

Broad Institute of MIT and Harvard, Cambridge, MA. USA

We present a large-scale proteogenomic analysis of human breast cancer, combining quantitative mass spectrometry-based proteomics and phosphoproteomics data and next generation sequencing-based genomics data. Integrated analysis of genomic and proteomic data provided insights into protein-mRNA correlation, effects of copy number changes on the proteome, and the consequences of mutations on cell signalling. Interrogation of the Library of Integrated Network-based Cellular Signatures (LINCS) database identified candidate genes with copy number alterations that are direct drivers of *trans*-effects at other gene loci. Global proteomic data confirmed a stromal-enriched group of tumors in addition to basal and luminal clusters, and pathway analysis of the phosphoproteome identified a G-protein-coupled receptor cluster that was not readily identified at the mRNA level. In addition, many highly phosphorylated kinases were identified using outlier analysis. The study demonstrates the utility of an integrated proteogenomic analysis of breast cancer, providing insights into breast cancer biology and identifying potential therapeutic targets.

Proteomics for Patients 'From protein biomarker discovery to the development of diagnostic tests'

Stephen R Pennington

School of Medicine, UCD Conway Institute, University College Dublin, Dublin, Ireland

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

1. The Clinical Problem: For many diseases, developing biomarkers to support the decisions of which patients to treat, when to treat them and with what treatment, remains challenging.
2. Biomarkers of Utility: The use of proteomics for new protein biomarker discovery has not yielded many clinically used biomarker tests - why?
3. A Strategy: A pragmatic, 'real world' and patient-centric approach for the discovery development and delivery of biomarkers of potential clinical utility.
4. Potential Solutions: The application of this strategy for the development of biomarker tests to support clinical decision in *prostate cancer* and *psoriatic arthritis*.
5. Biomarker Delivery: Potential strategies for the delivery and implementation of multiplexed protein biomarker tests.

PL-06

Dissecting interactomes using proximity-dependent biotinylation

Akhilesh Pandey

*Johns Hopkins University School of Medicine, Baltimore,
Maryland 21205, USA
E-mail: pandey@jhmi.edu*

Proteins rarely function in isolation as they often occur as components of multiprotein complexes.

Identification of protein-protein interactions is fraught with challenges regardless of the system utilized. Mass spectrometry-based proteomic approaches offer an opportunity to identify protein-protein interactions in an *in vivo* setting in a sensitive fashion. However, a shortcoming of such approaches is that a large number of non-specifically bound proteins can be identified in addition to the true interactors. We have previously developed SILAC as a quantitative proteomic approach that can reliably distinguish the true interactors from other proteins. Another major limitation of immunoprecipitation-based strategies for identifying protein interactors is that it is difficult to detect transient interactions as well as interactors that are present in low abundance in cells. To circumvent this, proximity-dependent biotinylation strategies (e.g. BioID and APEX) have been developed. In these approaches, the biotinylated proteins are generally identified by digesting proteins captured by streptavidin beads which leads to identification of proteins although biotinylated peptides are not directly identified. We have recently developed biotinylation site identification technology (BioSITe) as a method for direct capture of biotinylated peptides which increases both the sensitivity and specificity of experiments that employ this biotinylation-based approach. I will discuss our recent studies applying this method to study signalling complexes and subcellular proteomics in different contexts. This method is generally applicable and is likely to become a standard addition to proximity-dependent biotinylation strategies for a more comprehensive characterization of the interactome of any protein as well as for direct characterization of biotinylation sites such as in some click chemistry applications.

KEYNOTE LECTURES

KN-01

Proteomic profiling of plasma membrane in chickpea reveals a novel negative regulator of dehydration tolerance

Niranjan Chakraborty

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

Non-availability of water or dehydration is one of the major agricultural hurdles across the globe, which causes significant crop loss. Due to the complexity of dehydration response, understanding molecular basis of dehydration tolerance has remained a major challenge. It has put an impetus on the innovation-driven research for development of hardy crops and sustainable agriculture. To better understand the underlying mechanism of dehydration tolerance, we developed a reference as well as dehydration-responsive proteome map of plasma membrane (PM), which regulates cellular exchanges in a spatiotemporal fashion. We generated an inventory of 2732 proteins from a grain legume, chickpea, the largest in any crop species thus far, of which 163 proteins were identified as dehydration-responsive. An in-depth screening of the molecular signatures in the proteome landscape led to the identification of an integral PM protein harboring an EF-hand domain flanked by two $\text{Na}^+/\text{Ca}^{2+}$ exchanger domains. Interestingly, the putative transporter was found to be consistently downregulated at both proteome and transcript level, and confirmed to be bona fide PM resident. To probe its regulatory role, we generated overexpression (OE) lines of the candidate gene in chickpea, under RD-29 stress inducible promoter. Integration of the gene was confirmed by both gene and selection marker specific PCR analysis. When subjected to dehydration, the overexpression line showed significant loss of vigour as compared to unstressed control. Further physiological analyses including lipid peroxidation, contents of proline and photosynthetic pigments showed the OE lines to be hypersensitive to the subjected stress. Together these results suggest that the candidate membrane transporter might function as a negative regulator of dehydration response in chickpea in specific and plants in general.

KN-02

Addressing vitamin B12 deficiency- Need of the hour

Shantanu Sengupta

*CSIR-Institute of Genomics and Integrative Biology, Mathura Road,
New Delhi*

Vitamin B12 is a water soluble vitamin that acts as a cofactor for two important reactions. This micronutrient is synthesized by microorganisms and mammals have evolved pathways for absorption, transport and cellular uptake of this vitamin. Low intracellular vitamin B12 may be due to low intake (especially in individuals adhering to a strict vegetarian diet) or due to defects in absorption, transport or cellular uptake. Deficiency of this vitamin results in the elevation of two thiol amino acids, homocysteine and cysteine, which have been associated with various complex diseases and is considered to be an independent risk factor for cardiovascular disease. A significant proportion of Indian population have low vitamin B12 due to both low intake and mal absorption of this vitamin. We hypothesized that vitamin B12 deficiency could play a role at least in part to the incidences of cardiovascular disease in India. India is considered to be the cardiovascular disease capital of the world. It is projected that by 2030, 60% of world's Cardiovascular Disease patients will be Indians. Akin to most of the complex disorders, both genetic and environmental factors (including diet) contribute to the burden of CVD. Our results from genome wide association studies revealed that several single nucleotide polymorphisms (SNP) are associated with CAD, some of which are novel while some have already been reported. However, these SNPs could not account for more than 10% of the disease burden. This led us to focus on environmental factors (mainly diet) since it is increasingly believed that gene-environment interactions play an important role in the etiology of CAD. We had shown that low levels of vitamin B12 and its active fraction holotranscobalamin, is associated with CAD in Indian population. Further, using a maternal vitamin B12 deficient rat model, we show increased levels of triglyceride in the pups born to mothers fed with B12 deficient diet which could be mediated via differential expression of PPAR alpha and gamma. Interestingly, the promoter region of PPAR gamma was found to be hypomethylated while

that of PPAR alpha hypermethylated. Our plasma proteomics studies revealed that impairment of reverse cholesterol pathway may be an important factor in the context of CAD in Indian population. This was also supported from our metabolomics data. We thus believe that a systems biology approach with completely different layers of information (genome, epigenome, proteome and metabolome) is the only way to understand disease progression and complexities associated with it in a holistic manner.

KN-03

Endometrial Secretome and Its Modulation: From “Function” to “Dysfunction”

S Bhutada¹, KashmiraBane¹, T Basak², Lalita Savardekar¹, RR Katkam¹, SM Metkari¹, UK Chaudhari¹, SD Kholkute¹, S Sengupta², Raj Sawant³, Neeta Warty³, Rahul Gajbhiye¹ and Geetanjali Sachdeva^{1*}

¹Indian Council of Medical Research-National Institute for Research in Reproductive Health (ICMR-NIRRH), Mumbai, India

²CSIR-Institute of Genomics and Integrative Biology, New Delhi, India
³Sanjeevani Clinic, Kandivali, Mumbai, India

Endometrium (inner mucosal layer of the uterus), though a natural host, extends its hospitality to the embryo only during a specific period termed “receptive phase or implantation window” in the menstrual cycle. Spatiotemporally regulated endocrine, autocrine, and paracrine events in different cellular compartments contribute to endometrial maturation and acquisition of secretory phenotype for receptivity. All these events qualitatively and quantitatively modify not only the molecular repertoire of endometrial cells but also of the uterine lumen. Considering that before making physical contact with endometrium, embryo spends considerable time in uterine cavity bathed with endometrial secretions (uterine fluid-UF), strides have been made by some investigators to assess the effect of specific uterine fluid components on fertility. However, a majority of these studies employed targeted or candidate-based experimental approaches with *a priori* supposition. We have undertaken a non-targeted proteomics-based approach to develop human uterine fluid proteome and study its modulation during the receptive phase. Isobaric Tag for Relative and Absolute Quantitation (iTRAQ) analysis of the uterine fluid samples from women in the pre-receptive and receptive phases revealed

identities of 127 proteins in the human uterine fluid and also modulation in the expression of 27 proteins during the receptive phase. Further investigations, were focused on High Mobility Group Binding Protein (HMGB1), one of the UF proteins that displayed lesser abundance in the receptive phase than in the pre-receptive phase. Cellular HMGB1 also revealed the same pattern. Endometrial and uterine fluid samples from rats also were found to have lower levels of HMGB1 in the receptive phase, than in non-receptive phase. A physiological requirement for HMGB1 levels to decline during the receptive phase was also reflected by its significant reduction on the day of implantation in pregnant rats. Further, recombinant HMGB1 when exogenously administered in excess caused implantation failure in rats. Rat uterine horns exposed to high levels of extracellular HMGB1 were found to have histological alterations in the endometrium as well as activation of inflammatory cascades. Collectively, these observations suggested that aberrantly high levels of HMGB1 in the receptive phase are not conducive for fertility. Next, we investigated whether endometriosis, an endometrial disorder associated with inflammation and infertility, also presents aberrations in the expression of HMGB1. Results suggest higher expression of endometrial HMGB1 in the women with endometriosis, compared with fertile women. Overall, our proteomics based investigations have provided significant clues about endometrial physiology and pathology. Also, these studies have provided a pool of targets which can be explored for their potential in fertility management.

Study Funding: Department of Biotechnology, Government of India and Indian Council of Medical Research (ICMR), Government of India

KN-04

Proteome-wide effect of silencing of Milk Fat Globule Membrane Epithelial Growth Factor 8 (MFGE8) on lactocytes

Ashok K. Mohanty^{}, Arvind K Verma and Syed Azmal Ali**

Animal Biotechnology Centre, National Dairy Research Institute, Karnal, Haryana, 132001
E-mail: *ashokmohanty1@gmail.com

Mammary gland is an exocrine and modified sebaceous gland, which is made up of a branching network of ducts that end in alveoli. It is an ideal system to study the molecular mechanisms associated with cell proliferation, differentiation and oncogenesis.

The glycoprotein lactadherin also known as MFG-E8 mediates phagocytic clearance of apoptotic cells and influences the physiological cyclic changes taking place in mammary gland. Recent study shows that MFG-E8 is the part of MFGM and plays a major role in mammary gland development. In our current work we have shown that lactadherin isoform MFG-E8 along with GSN controls the cell growth process of epithelial cells. We have successfully down regulated the MFG-E8 protein through stable transfection of MFG-E8 shRNA. Mass spectrometry based (Q-TOF) proteome analysis by iTRAQ approach identified total of 6040 proteins in stably transfected silenced MFG-E8 Buffalo mammary epithelial cell line through MaxQuant 1.5.2.8 using NCBI *Bubalus bubalis* database. Bioinformatics analysis performed through online software tool String 10.0 connected with offline software cytoscape 3.2.1 shows that MFG-E8 makes direct protein-protein interaction with GSN, RALGAPA2, CD9, ITGB5 and ALB. DAVID analysis shows that these proteins are involved in diverse biological processes, cellular functions, molecular functions and pathways. Furthermore, with the help of Reactome and KEGG pathway database we identified that MFG-E8 and GSN together responsible for regulation of proliferation. These *in silico* results were later confirmed by qRT-PCR. Furthermore, BrdU and MTT assay validated that stably transfected cells has more life span compared to normal counterpart. Overall this study supports that MFG-E8 together with GSN regulates the proliferation of cells. Till date to the best of our knowledge, this is the first study that shows the direct interaction of MFG-E8 with GSN and their role in regulation of cell growth.

KN-5

Heat shock protein 40 as a knob chaperone in *Plasmodium falciparum* infected human erythrocytes

**Shuba Varshini Alampalli^{1#}, Manish Grover^{1#},
Syama Chandran¹, Pragyan Acharya^{2*} and
Utpal Tatu¹**

¹Department of Biochemistry, Indian Institute of Science, CV Raman Avenue, Bangalore – 560012, India

²Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar East, New Delhi – 110029, India

Malaria pathogenesis, especially in the case of *Plasmodium falciparum* infections, relies upon the ability of the *Plasmodium* infected erythrocytes (iRBCs) to cytoadhere to endothelial cells in the microvasculature. This interaction between iRBCs and host endothelial cells is highly specific and is mediated by parasite

encoded antigenic proteins known as PfEMP1, which are present on iRBC specific membrane protrusions known as “knobs”. Knobs consist of a unique combination of parasite-encoded proteins that interact with host proteins to present multiple PfEMP1s into the extracellular space towards cytoadherence and antigenic variation. We have carried out proteomic profiling of detergent resistant fractions from the plasma membrane of infected erythrocytes and combined the outcomes with well established knob interactions from literature and structural insights from bioinformatics approaches. Based on these data we propose a structural model for organization of knob components which uncovers novel features of knob protein interactions and provide several points for potential therapeutic interventions.

KN-6

Plasma proteomics in Coronary Artery Disease

**Arun Bandyopadhyay¹, Devasmita Chakravarty,
Alipta Guha Roy, Apabrita Ayan Das,
Kamalika Roychoudhury, Somaditya Mukherjee,
Tanima Banerjee, ²Sudip Ghosh, ²Santanu Dutta,
³Prakash Chandra Mandal,
⁴Khawer Naveed Siddiqui**

¹Cell Biology and Physiology Division, CSIR-Indian Institute of Chemical Biology, Kolkata.

²Institute of post graduate Medical Education & Research (IPGMR), SSKM Hospital, Kolkata.

³Apollo Gleneagles Hospital, Kolkata.

⁴Ruby General Hospital, Kolkata.

E mail: arunb@iicb.res.in

Proteomics and bioinformatics are powerful tools to identify protein based biomarkers involved in a disease state. The current advancement in proteomic technologies helps studying global protein expression changes associated with human disease processes. One of the advantages of these proteomic studies is that new biomarkers (diagnostic and/or prognostic) can be discovered which will help provide a better framework for treatment of cardiovascular diseases. Thus, the detection, identification and characterization of variations in the proteome occurring during the course of heart disease will provide both (i) insight into the underlying molecular mechanisms and (ii) potential cardiac specific biomarkers for regular, systematic observation and assessment of cardiac status. The aim of this study was to provide a list of potential blood based protein markers for CAD. We utilized on-line label-free MS/MS using blood plasma as the source

material. On-line LC-ESI-MS is the method of choice because the initial LC separation step decreases the amount of analytes that can be simultaneously ionized. Thus, the possibility of ion suppression is reduced rendering the method quantitative in nature. Such label-free quantitative LC-MS approaches can compare innumerable samples. We have identified 2500 proteins in human plasma out of which about 1200 proteins are unique to each group i.e STEMI, NSTEMI and UA. Through Perseus and Ingenuity Pathway Analysis of uniquely identified proteins, we statistically analysed the distribution of identified protein and identified statistically enriched pathways. This led to the identification of significantly enriched pathways including scavenger receptor signalling, atherosclerosis signalling and pathways related to coronary artery disease. Further filtering based upon Z score values led to the identification of a specific list of protein candidates which may act as prospective markers and may have some implications in the development of the disease. One of the proteins, down regulated in CAD subjects was found to be correlated with the risk factors of atherosclerosis. Using Apoe knock out mice we demonstrate that this particular cell adhesion protein is regulated by cholesterol and high fat indicating a direct involvement in reverse cholesterol transport process. [This work is supported by CSIR grant no. MLP123 to AB].

KN-07

An integrative proteogenomics approach to unravel human proteoforms

Debasis Dash

G.N. Ramachandran Knowledge Centre for Genome Informatics,
CSIR-Institute of Genomics and Integrative Biology, Mathura Road,
Delhi, 110025, India.

Study of system wide variations and their crosstalk requires integrative analyses of multiple layer of information. Proteogenomic approaches demands such diverse layers of high-throughput omics data, e.g., genomics, transcriptomics, proteomics, etc. Application of proteogenomics to eukaryotic genomes still remains extremely challenging due to lack of high throughput open source tools.

We have developed an open source Integrated Transcriptomic Proteomic (ITP) pipeline comprising of two analysis modules, each for transcriptomics and proteomics data. While the first module aligns and assembles RNA-Seq reads into transcripts by utilizing the reference genome, the second one creates a

database by translating thus obtained transcripts and then searches mass spectra against this database using multiple search engines. The pipeline is easy to use and has several advantages.

In case of re-annotation and exon boundary identification, a definitive transcript model is required. ITP maps every novel peptides identified using multiple search engines and also indicates most probable protein coding isoform despite extensive peptide sharing among isoforms. It also aids in discovery of tissue specific proteoforms by creation of customized search databases.

Using proteogenomics pipelines (Genosuite, ITP, Peppy, Enosi, and ProteoAnnotator) on publicly available RNA-sequence and MS proteomics data, we discovered several protein coding genes. Using rat brain microglia data, 363 novel peptides representing novel proteoforms for 249 gene loci were identified. These novel peptides led to the discovery of novel exons, translation of annotated untranslated regions, pseudogenes, and splice variants for various loci; many of which have known disease associations, including neurological disorders like schizophrenia, amyotrophic lateral sclerosis, etc. Novel isoforms were also discovered for genes implicated in cardiovascular diseases and breast cancer for which rats are considered model organisms. Using only OMSSA and X!Tandem to search MS data we have successfully identified several missing human proteins. Further analysis using tissue specific data will be discussed.

Thus, ITP reduces time and space complexity while improving the accuracy in peptide discovery and aids in deciphering the invisible proteome. In summary, existing proteogenomic pipelines that are complementary in nature should be used in combination to maximize peptide discovery and proteogenomic inference.

Integrative multiomics analysis of vivax malaria

Sandip K. Patel¹, Apoorva Venkatesh¹, Gangadhar Chatterjee², Kunal Sehgal³, Arunansu Talukdar⁴, Sanjay K. Kochar⁵, Jayanthi Shastri⁶, Sheetal Bankar⁶, Urmila Thatte⁷, Swati Patankar¹ and Sanjeeva Srivastava^{1*}

¹ Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai 400076; ² Dept of Biochemistry, Grant Govt Medical College and Sir JJ Group of Hospitals, Byculla, Mumbai 400008; ³ PD Hinduja National Hospital & Medical Research Centre, Veer Savarkar Marg, Mahim, Mumbai 400016; ⁴ Medicine Department, Medical College Hospital Kolkata, 88, College Street, Kolkata 700073; ⁵ Department of Medicine, Malaria Research Center, S.P. Medical College, Bikaner 334003; ⁶ Department of Microbiology, T. N. Medical College and BYL Nair Hospital, Mumbai; ⁷ Departments of Clinical Pharmacology, Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

*Correspondence: Dr. Sanjeeva Srivastava, E-mail: sanjeeva@iitb.ac.in

Plasmodium vivax is responsible for an enormous proportion of the global malaria burden. However, mechanisms underlying its pathogenesis still remain obscure with limited knowledge on parasite biology. An integrative multiomics approach including proteomics and metabolomics of the host serum and proteomics of the parasite was used (i) to understand the underlying pathobiology, (ii) to identify signatures of disease severity and (iii) to discover biomarkers for rapid disease diagnosis. A quantitative serum proteomics analysis of severe and non-severe vivax malaria patients as compared to healthy controls provided evidences for the modulation of diverse physiological pathways including oxidative stress, cytoskeletal regulation, lipid metabolism and complement cascades in severe vivax malaria. The study also identified proteins such as Superoxide dismutase, Vitronectin, Titin, Apolipoprotein E, Serum amyloid A, and Haptoglobin as potential predictive markers for malaria severity. Metabolomics analysis confirmed modulation of pathways similar to those identified in our proteomics studies. Metabolites such as proline, 3-nitrotyrosine, dihydropteroic acid, harderoporphyrin, and others like homogentisic acid, indoleacetaldehyde, phenylacetic acid and alicyluric acid were up and down regulated in severe cases respectively suggesting their role as makers for severity.

A study of the proteome of *P. vivax* clinical isolates revealed highly abundant parasite proteins. While a major proportion of the *P. vivax* proteins were either hypothetical or involved in basic cellular activities, few

proteins such as such as tryptophan-rich antigen, Pv-fam-d protein, Plasmodium exportedprotein, Pvstp1 and a hypothetical protein were detected in more than 80% of the patients. Simultaneously, we also identified a few merozoite surface proteins, metabolic enzymes and a putative uncharacterized protein amongst others in the sera of vivax patients for evaluation as diagnostic antigens. Our findings provide new avenues to tackle the challenges of vivax malaria diagnosis and enhances our understanding of disease pathogenesis and severity.

Integrated omics implied the endogenous protease inhibitors influence protease gene expression in *Helicoverpa armigera*

Purushottam R. Lomate^{†1}, Veena Dewangan^{†1}, Neha S. Mahajan^{†1}, Yashwant Kumar¹, Abhijeet Kulkarni², Li Wang³, Smita Saxena², Vidya S. Gupta¹, and Ashok P. Giri¹

¹Plant Molecular Biology Unit, Division of Biochemical Sciences, CSIR-National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411 008; ²Department of Bioinformatics, Savitribai Phule Pune University, Pune 411007; ³Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames 50011, IA, USA

Mechanisms regulating protease gene expression in insects are largely enigmatic. Using *de novo* RNA sequencing and proteomic analysis, we examined changes in *Helicoverpa armigera* larvae fedon recombinant plant protease inhibitor (rPI) at different time intervals. Here we present evidence supporting a dynamic transition in *H. armigera* protease expression upon rPI feeding with general down-regulation of protease genes at early time points (0.5 to 6 h) and significant up-regulation of specific trypsin, chymotrypsin and aminopeptidase genes at later time points (12 to 48 h). Further, we provide evidence on link of endogenous *H. armigera* PIs in activation/deactivation of protease gene expression. Our results show differential expression and distinct isoform of endogenous PIs in rPI-fed larvae. Co-expression of endogenous PIs with digestive proteases is also evident. Finally, we propose potential mechanism of protease regulation in *H. armigera* and subsequent possible adaptation to cope with anti-nutritional components of plants.

Proteomic and metabolomic profiling towards candidate markers for Breast cancer

Srikanth Rapole*

*Proteomics Lab, National Centre for Cell Science, Ganeshkhind, Pune-411007, MH, India.

Breast cancer (BC) is the most common malignancy and the leading cause of cancer deaths in women worldwide. Early diagnosis of BC improves the likelihood of successful treatment and can save many lives. Thus early diagnostic biomarkers are critically important for detection, diagnosis, and monitoring disease progression in BC. There is an urgent need to discover new and better candidate biomarkers for BC that would be helpful in improving diagnosis, prognosis and treatment. Proteomics and metabolomics approaches are most powerful techniques which can discover novel biomarkers for different cancers. Proteomics provides information of protein-based biomarkers include alterations in the protein levels and posttranslational modifications of proteins found in tissues and bodily fluids. Metabolomics is the study of the metabolites and how they are affected by specific cellular processes. We employed multipronged quantitative proteomic approaches like 2D-DIGE, iTRAQ and SWATH-MS and identified differentially regulated proteins at subtype level in tissue, serum and urine samples. Further, some of the statistically significant differentially regulated proteins were verified and validated by immunoblotting and SRM assays. We have also performed a comparative analysis of metabolome of normal, benign and malignant serum and tissue samples for the understanding of the molecular events involved in tumor development that are essential for early detection and diagnosis. Very good discrimination within breast cancer and control groups was achieved. These results provide valuable insights into metabolic adaptations of breast cancer, which can help not only to identify diagnostic markers but also potential therapeutic targets.

Using Proteomics to Understand Dementias associated with Tauopathy

Judith A. Steen, Ph.D.

Associate Professor of Neurology, F. M. Kirby Center for Neuroscience Research/Boston Children's Hospital and Harvard Medical School, Boston, MA, USA

Tauopathies are a heterogeneous class of neurodegenerative disorders that exhibit a common pathological hallmark: the abnormal aggregation of tau protein inside neuronal and/or glial cells in the brain. To better understand the relationship between disease and tau Post-Translational Modifications (PTMs), we developed quantitative proteomics platform that provides global proteomic data and detailed information about tau using the FLEXITau assay. FLEXITau is a mass spectrometry (MS)-based targeted method that precisely measures the tau PTM profile and provides absolute quantification for tau. We analyzed a large set of post-mortem brain samples [BA39 region] of over 150 samples, covering various tauopathies including Alzheimer's disease (AD), Progressive Supranuclear Palsy (PSP), Pick's Disease (PiD), corticobasal degeneration (CBD) as well as age matched non-demented controls using both FLEXITau and unbiased global proteomics on various biochemical fractions. We previously reported that each condition possesses a unique molecular signature determined by its PTM state based on our FLEXITau assay and developed a classifier to stratify the patients and identify important regions for antibody development. Using the isoform database, we were now able to characterize isoform differences between the various tauopathies and validate some of the FLEXITau data via this orthogonal method. Furthermore, by cross-referencing our sarkosyl fractionation and global proteomics analysis, we were able to identify specific enzymes and modifiers that explain the PTMs. Our platform and approach is a powerful way to understand mechanisms and find biomarkers and therapeutic targets for disease.

Abundance and glycation status of albumin determines hemoglobin glycation

Mahesh J. Kulkarni

*Proteomics Facility, Division of Biochemical Sciences, CSIR-National Chemical Laboratory, Pune-411008, India.
E-Mail: mj.kulkarni@ncl.res.in*

Diagnosis and management of diabetes majorly depends upon the glycated hemoglobin (HbA1c) levels. Various factors influence HbA1c levels such as analytical methods, clinical conditions like anaemia or pregnancy, lifespan of erythrocytes. In this study, we unequivocally demonstrate that the serum albumin level and its glycation status influence hemoglobin glycation using erythrocyte culture. Erythrocytes maintained in low serum albumin concentration displayed increased hemoglobin glycation and vice versa, as measured by HbA1c, advanced glycation end product (AGE) modification monitored by fluorescence spectrometry, western blotting and mass spectrometry, which was associated with increased serum albumin glycation that perhaps decreased its ability to protect hemoglobin glycation. This was demonstrated by treatment of N(e)-(carboxymethyl)lysine (CML) modified serum albumin (CMSA) which failed to protect hemoglobin glycation; instead it increased hemoglobin glycation possibly by altering in erythrocyte structure and membrane permeability as observed by scanning electron microscopy and hemolysis respectively. The inability of CMSA to reduce hemoglobin glycation was due to lack of availability of free lysine residues, which was corroborated by using N(e)- (acetyl) lysine serum albumin (AcSA) and clinical diabetic plasma. This is the first study that demonstrates that modification of lysine residues of albumin impairs its ability to inhibit glycation. Furthermore, correlation studies between HbA1c and serum albumin or relative albumin fructosamine (RAF) from clinical subjects supported our experimental finding that albumin abundance and its glycation status influence hemoglobin glycation. Therefore, we propose serum albumin level and its glycation status to be quantified in conjunction with HbA1c for better management of diabetes.

Structural Investigation of Plant Seed Proteome

Dinakar M. Salunke

*International Centre for Genetic Engineering & Biotechnology,
New Delhi*

It is apparent that plant seedproteins participate in critical physiological activities particularly during germination. Comparative structural proteomics involving different related seeds would provide functional insights in the context of each protein. Analytical separation of the proteins from seed extracts facilitated identification of the dominant proteins by quick sequencing methods. Proteins were subjected to the functional screens through homology searches and complete crystallographic analyses were carried out in case of the relevant proteins. Considering that we purified all these proteins directly from plant source, they crystallized along with the co-factors naturally associated with them giving further insights in their physiological roles. We discovered that a protein purified from cowpea (*Vigna unguiculata*) and the corresponding protein from grass pea (*Lathyrus sativus*) belonged to haemopexin fold which apparently works as a molecular switch regulated by oxidative stress. Two proteins, a vicilin and a non-specific lipid transfer protein, were similarly characterized from egg plant (*Solanum melongena*) seeds. While the vicilin was associated with cofactors linked to critical metabolic processes, the non-specific lipid transfer protein, with lipid bound at a non-canonical site on the surface, provided structural insights concerning associated allergenicity. Another protein from velvet bean (*Mucunapruriens*) seeds exhibited antibody-mediated anti-snake venom activity without any obvious structural similarity with venom proteins.

Integrative Omics and Biomolecular Network Analysis Dissect Host-Specific Immune Dynamics in *Fusarium* disease

Subhra Chakraborty

*National Institute of Plant Genome Research, Aruna Asaf Ali Marg,
New Delhi-110067, India
Email: subhrac@hotmail.com, schakraborty@nipgr.ac.in*

Morbidity and mortality associated with fungal infections and emergence of resistant fungal strains

necessitate study of fungal pathogenesis and host innate immunity. Innate immune response is governed by conserved cellular events in phylogenetically diverse hosts. However, the underlying molecular mechanisms by which this process is regulated against multi-host pathogen remains unknown. *Fusarium oxysporum*, a medically and agronomically important multi-host pathogen is known to be associated with neuronal stress in humans and vascular wilt in plants, while *Fusarium*-mediated killing of worm has recently been described. To elucidate regulatory framework of *Fusarium*-associated disease and immune response, we analyzed the gene and protein expression during infection, integrated temporal expressions and network analysis with genetic inactivation data in worm and plant. Longitudinal spatiotemporal multiomics analyses and the derived biomolecular networks revealed organ and organelle function in diverse kingdoms during fungal invasion. Results indicate that a ubiquitous response occurs during *Fusarium* infection mediated by highly conserved regulatory components and pathways. Furthermore, our data identified disease responsive genes conserved and unique among animal and plant. Finally, this study for the first time provides novel insight on cross-species immune signaling that impinge upon the surveillance mechanism of innate immunity in multi-host pathogen response and may facilitate discovery of cellular therapeutic targets for *Fusarium*-associated disease.

KN-15

interventions are available for preventing high altitude illness, the protein networks that are affected by them or help evade them during hypobaric hypoxia exposure are not properly elucidated causing the known interventions to cause side-effects and require medical supervision. In a nutshell, these interventions provide symptomatic relief but can't confer acclimatization. Thus there is an urgent requirement to address the issue of hypobaric hypoxia at the proteome level and provide suitable interventions based on the protein networks involved in AMS, HAPE and HACE. Over many years, we have amalgamated considerable insight into the different networks affected by hypobaric hypoxia across humans (in plasma and recently, salivary proteome) and SD rats (in lung, brain and plasma). Based on it, we have been able to implicate Renin-angiotensin, eNOS-cGMP and kallikrein-bradykinin pathways; postulate putative biomarkers like sulfotransferase 1A1 to assess HAPE patients; identify novel protein markers in rat lung (regucalcin, Haao) responding to hypobaric hypoxia and common pathways in rat and human (Acute phase response signalling, LXR/RXR Signalling) to create an *in-silico* hybrid between the two and delivered dynamic side-effect free prophylactics like cerium nanoparticles, NAP (peptide derived from Activity-dependent neuro-protective peptide) and aqueous suspension of silymarin. In the near future, we aim to provide the defining trends in PTMs during hypobaric hypoxia exposure and deliver a diagnostic panel of plasma or saliva proteins to assess the acclimatization status at high-altitude.

Identifying Protein Networks to evade High Altitude Illnesses: A Proteomics Approach

Kalpana Bhargava* and **Yasmin Ahmad**

Defence Institute of Physiology & Allied Sciences (DIPAS),
Defence R&D Organization (DRDO), Lucknow Road, Timarpur
New Delhi-110054

*Email: kalpanab2006@gmail.com

High altitude exposes an organism to not only cold temperature, UV radiation and harsh terrain but also hypobaric hypoxia. Hypobaric hypoxia, basically decreased partial pressure of ambient oxygen, causes ample oxidative stress at appropriate altitude and duration to cause life threatening conditions like Acute mountain sickness (AMS), High altitude pulmonary edema (HAPE) and High altitude cerebral edema (HACE). These diseases potentially affect millions visiting high altitude areas every year. Although many

INVITED LECTURES

IL-01

Applications of proteomics in plant biology: with special reference to abiotic stress

Giridara-Kumar Surabhi

*Plant Molecular Biology and 'OMICS'-laboratory, Regional Plant Resource Centre, Bhubaneswar-751015, Odisha, India.
E-mail: surabhigk@gmail.com*

Crop plants are exposed to various biotic and abiotic stress factors under field conditions, and yield reduction caused by these stress can reach up to 50%. The plants have evolved highly complex but well coordinated adaptive responses operating at transcriptional and post-transcriptional, translational and post-translational levels, to alleviate the cellular damage caused by different stress factors. A comprehensive understanding of regulation either at all levels or one of these will provide better tools to improve plant's performance under stress. In order to unravel the molecular mechanism underlying stress tolerance in different crop/ plants (barley, foxtail millet, mulberry etc.), we performed combined two-dimensional gel electrophoresis with mass spectrometry-based protein identification and quantitative genetic approaches to identify candidate proteins responsible for stress tolerance. My talk will focus on current understanding of the proteomics in addressing abiotic stress tolerance in crop plants.

IL-02

Proteomics in Vaccine Development against Parasitic Infections

J. Mohanty* and P.K. Sahoo

*ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar
751002, India
Email: jmohantycifa@gmail.com

Vaccines have played important role in controlling several microbial infections in humans and animals. Developing vaccine against parasites are however, relatively much slower compared to bacterial or viral pathogens as the parasites contain a wide variety of antigens and also use several immune evasion strategies such as antigenic variation, molecular

mimicry, etc. Besides, they exhibit complex lifecycles and other biological characteristics, which further complicate the vaccine development process. Thus, the identification of efficient protective antigens as vaccine candidates is crucial to the successful development of a vaccine against parasites. Proteomics is a recent technique that can generate profiles of whole proteins in a biological sample. It yields information on protein identification, post-translational modifications, as well as quantification and localization of proteins. Central to proteomics study is the high-throughput and sensitive instrument, mass spectrometer for analysis of the proteome. Data obtained from mass-spectrometric analysis are interpreted using bioinformatics tools with the available genomic or transcriptomic databases. The accumulated gene and protein sequences of parasites over the last few years have provided a remarkable amount of data that can be useful in discovery of novel immunogens leading to vaccine development. The available technologies would help in shortlisting the candidate vaccines which then have to be developed and tested for protective efficacy. Another proteomics approach namely, immunoproteomics involving 2D electrophoresis followed by western blotting shows promise in deciphering immunoreactive proteins of the pathogensthat stand as potential vaccine antigens. This approach is now is being applied against a number of parasites both in humans and animals in quest of successful vaccine candidates. Besides discussing the current status and the latest improvements in proteomics towards vaccine development against parasitic infections, work done by our group towards vaccine development against fish ectoparasite, *Argulus siamensis* will be presented.

IL-03

Defect in translation initiation fidelity alters differential protein expression pattern and cellular physiology in *Saccharomyces cerevisiae*

Anup Kumar Ram¹, Amol R Suryawanshi² and Pankaj V Alone^{1*}

¹*School of Biological Science, National Institute of Science Education and Research Bhubaneswar*

²*Institute of Life Science Bhubaneswar*

The selection of the Open Reading Frame (ORF) on mRNA is first critical step in the decoding of genetic

code into a sequence of polypeptide chain in protein translation. In the eukaryotic system, this process is accomplished by assembly of translation initiation factors, tRNA_i and mRNA on 40S ribosome and scanning for AUG start codon from 5' to 3' direction. Non-AUG start codon such as UUG, CUG and GUG are also reported to be used by translation initiation machinery, however, their selection mechanism is less well understood. The initiation factor eIF5 plays an important role in translation start site selection by providing the GAP (GTPase activating protein) function. However, in yeast translation initiation fidelity defective eIF5^{G31R} mutant causes preferential utilization of UUG as initiation codon and termed as Suppressor of initiation codon (Sui⁻) phenotype and also shows severe slow growth phenotype. The strong Sui⁻ phenotype eIF5^{G31R} mutant causes Gcn⁻ phenotype by utilizing upUUG codon from the 5' upstream regulatory region of GCN4 transcript and down-regulates its translation. Bioinformatics analysis of yeast genome suggests more than ~10% of the mRNA has in-frame or out-of-frame UUG codon at the 5'UTR region. We are hypothesizing that the dominant negative eIF5^{G31R} mutant alters the translation of these genes and affects the cellular physiology. Differential protein expression pattern of the eIF5^{G31R} mutant was compared with its isogenic wild type strain using 2-Dimensional gel electrophoresis and iTRAQ technique followed by mass-spectrometric analysis. Out of approximately 1300 proteins identified to show differential protein expression pattern, 26 proteins showed significant up-regulation and some were shown to associate with DNA replication stress, micro-autophagy and protein degradation pathway; whereas, 7 proteins showed significant down-regulation and one of them associated with pyrimidine nucleotide biosynthesis. This altered protein expression pattern shown by eIF5^{G31R} mutant might be a reason for cellular stress and slow growth phenotype.

IL-04

Proteomics and functional analysis of outer membrane vesicles of bacteria

M V Jagannadham

Senior Principal Scientist, CSIR-Centre for Cellular and Molecular Biology, Hyderabad-500007, India
E-mail: jagan@ccmb.res.in

Gram- negative bacteria release outer membrane vesicles (OMVs) of size ranging from 20 -250 nanometres. These vesicles were shown to contain phospholipids, lipopolysaccharides (LPS), proteins

and in some cases nucleic acids. The OMVs were shown to mediate antibiotic resistance in multiple ways. In some cases they serve as vehicle for the transport of antibiotic-inactivating enzyme. OMVs also help the producer organism in getting rid of toxic substances by transporting them out of the cell. In the present study, the role of OMVs prepared from *Pseudomonas syringae* Lz4W and *E. coli* and their mode of action were studied against some antibiotics. Studies in our laboratory revealed that outer membrane vesicles protected the producer bacterium and two other bacterial species from two membrane-active antibiotics by absorbing the antibiotics and not allowing them to reach the target cells. OMVs produced by *E. coli* could also degrade the antibacterial molecules. OMVs prepared from an Antarctic bacterium *Pseudomonas syringae* Lz 4W protected the bacterium against membrane active antibiotics. NPN uptake studies revealed that they protected by sequestering the antibacterial molecules. Proteomics studies of the outer membrane vesicles revealed the presence of several proteases and peptidases. These may be responsible for degrading the antibacterial molecules. Therapeutically useful antibiotics are losing efficacy and we are left with very few antibiotics for clinical management. The mechanisms of bacterial resistance to antibiotics are important to devise remedial measures. These studies helped in understanding the mechanism of antibiotic resistance and other ways to sensitise bacteria against existing antibiotics.

IL-05

Application of Quantitative high throughput Proteomics in health and disease

Suman S. Thakur

CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India

High throughput quantitative proteomics has great potential to uplift the human health care especially to diagnose the disease at early stages and help in the treatment of disease. We have developed a technique single-run analysis without any pre-fractionation to understand and quantify accurately the changes at the level of proteome especially it has great potential in early diagnosis of the disease where multiple biomarkers need to detect. In depth quantitative proteomics able to find the cause of disease especially narrowing down to particular gene and it is coming almost closer to transcriptomics and genomics. The

quantitation of proteins using stable isotope labelling with amino acids in cell culture (SILAC), Isobaric tags for relative and absolute quantitation (iTRAQ) and label free quantitation (LFQ) has been applied to find the biomarkers especially in infectious disease, metabolic disorder-diabetes and its complications and pre-eclampsia. Quantitative proteomics is very helpful to understand the mode of action of different known and unknown drug and also in understanding the host pathogen interaction including complex process of signalling mechanism. Our group is working to develop novel anti-cancer drug targeting Cell adhesion, AKT signalling and EGFR against different specific cancers such as retinoblastoma, leukaemia, lung cancer and melanoma using cell culture and mouse model along with the help of quantitative proteomics. Mode of action and signalling mechanism of these novel drugs has been revealed by using quantitative proteomics.

IL-06

Saliva as a diagnostic fluid: Are we there yet?

Srinivas K. Ambatipudi

*Department of Biotechnology, Indian Institute of Technology,
Roorkee, India*

Human saliva is a protein-rich fluid which performs multiple functions such as lubrication, digestion, maintenance of mucosal integrity, and maintenance of general oral health by interacting with a complex collection of oral microbiota. Differential expression of these proteins alters the normal functional properties of saliva including act as source of potential biomarkers. However, little is known about these changes with normal aging as well as in primary Sjögren's syndrome (pSS), an autoimmune disease commonly seen in perimenopausal women. Thus, a comprehensive proteomic profiling of pooled saliva collected from the parotid glands of healthy female subjects, divided into two age groups 1 and 2 (20-30 and 55-65 years old, respectively) was performed using multidimensional protein identification technology (MudPIT). Collectively, 532 proteins were identified in the two age groups. Of these proteins, 266 were identified exclusively in one age group, while 266 proteins were common to both groups. Subsequently, MudPIT was performed in pSS which led to identification of 1246 proteins, of which 477 did not change, 529 were only detected in either the pSS or HC sample, while 206 of these proteins were

significantly up-regulated ≥ twofold and 34 were down-regulated ≤ 0.5. Subsequently, specific salivary proteins that modulate *S. mutans* growth and their colonization of the tooth surface in the oral cavity was identified as common salivary protein-1 (CSP-1) by mass spectrometry (MS) approach. Recombinant CSP-1 when exposed to *S. Mutans* displayed enhanced adherence to experimental salivary pellicle and to glucans in the pellicle formed on hydroxyapatite surfaces demonstrating the influence of salivary protein in initial colonization of this pathogenic bacterium onto the tooth surface. Results of these studies support the use of MS for global discovery of markers including identification of specific salivary proteins that interact with microbes to increase our understanding of oral pathophysiology.

IL-07

Reverse Phase Protein Array (RPPA) for integrating cancer genomics and proteomics data to unwire lung cancer relevant signaling and drug resistance mechanisms

Ramesh Umanni

*Scientist, CSIR-Indian Institute of Chemical Technology,
Hyderabad, India*

In complex diseases such as cancers, changes in protein expression and their activation by posttranslational modification are involved in tumor initiation and its progression. Often these are not reflected by genome level alterations. Therefore, functional proteomics to understand altered protein functions associated with tumor development will provide insights in understanding the disease pathophysiology. In functional proteomics, Reverse Phase Protein Array (RPPA) technology is useful to analyze protein expression and activation in high-throughput assays on thousands of samples including tissue and cell lysates, serum, plasma or other body fluids simultaneously. RPPA can measure protein expression and its modifications such as phosphorylation. In RPPA, samples are printed on nitrocellulose coated glass slides as microspots to accommodate large number of samples and probed with validated antibodies to analyze target of interest. RPPA requires highly validated antibodies. Protein expression and activation data collected from limited quantity of sample allow the analysis of multiple activated signaling pathways directly and simultaneously. The power of RPPA can be useful for

protein profiling, screening and validation of diagnostic and prognostic biomarkers, monitoring of protein kinetics, drug target identification, clinical research and individualized medicine. We applied RPPA approach for a quantitative analysis of cancer relevant proteins and phospho proteins in 84 non-small cell lung cancer (NSCLC) cell lines. The RPPA data was integrated with transcriptomic data, genomic aberrations, and drug sensitivity of the cell lines. I will present about the RPPA technology and its applications in functional proteomics to understand signaling pathways associated with different genetic aberrations in NSCLC.

IL-08

Asthma-COPD overlap syndrome (ACOS) - a new disease or a variant of asthma or COPD? A (¹H)-NMR based metabolomics study

**Chaudhury K¹, Ghosh N¹, Choudhury P¹,
Subramani E¹, Samanta S², Joshi M³,
Banerjee R⁴, Bhattacharyya P²**

¹School of Medical Science and Technology, Indian Institute of Technology Kharagpur, West Bengal, India, Pin-721302

³National Facility for High-field NMR, Tata Institute of Fundamental Research, Mumbai, Maharashtra, India, Pin-400005

²Institute of Pulmocare and Research, Newtown, Kolkata, West Bengal, India, Pin-700156

⁴Department of Agricultural and Food Engineering, Indian Institute of Technology Kharagpur, West Bengal, India, Pin-721302
koel@smst.iitkgp.ernet.in

Asthma and chronic obstructive pulmonary disease (COPD) are two common obstructive lung diseases which have been extensively investigated. However, asthma-COPD overlap syndrome (ACOS), where patients exhibit features of both asthma and COPD, remains a weakly defined clinical entity. No specific biomarker exists to differentiate ACOS from asthma or COPD, thereby making management of the disease challenging. Metabolomics is being increasingly used for the understanding of new diagnostic and prognostic approaches of various lung diseases. Here, we test our hypothesis which proposes that metabolomic analysis of serum and exhaled breath condensates (EBC) will help classify ACOS better and ascertain whether this overlap syndrome has its own unique pathophysiology. Serum and EBC samples were collected from patients with asthma (n=32), COPD (n=32), ACOS (n=40) and healthy controls (n=33). Proton NMR spectra of paired samples were acquired using 800 MHz Bruker Avance III

spectrometer. Following spectra pre-processing, both multivariate and univariate analysis were applied. Using the OPLS-DA model, ACOS could be well discriminated on comparing with asthma, COPD and controls. Metabolites including glucose, pyruvate, valine, lysine, glutamine, phenylalanine and creatinine were found to be significantly dysregulated in serum of ACOS patients. Also metabolites such as propionate, pyruvate, valine, fatty acid, acetate and acetone followed a similar trend in EBC of ACOS subjects. A possible disruption in energy metabolism cycles in ACOS as compared to asthma, COPD and controls is suggested. In conclusion, the distinct metabolic signatures of ACOS are strongly suggestive of the disease having a distinct clinical identity, as compared to asthma and COPD. These findings pave way for further validation studies which could help unravel this complex overlap syndrome better.

IL-09

To understand the role of Mycobacteria glyconetwork in tuberculosis

Avinash Sonawane

*Professor, School of Biotechnology, KIIT University, Bhubaneswar
asonawane@kiitbiotech.ac.in*

Mycobacterium tuberculosis (*Mtb*), the etiological agent of tuberculosis (TB), is a leading cause of morbidity and mortality among the infectious diseases. *Mtb* employ various strategies to modulate the host immune responses to facilitate its persistence in macrophages. Mycobacteria replicate intracellularly, most commonly within the phagosomes of macrophages, therefore, the bacterial proteins essential for intracellular persistence are particularly attractive targets for intervention of new anti-mycobacterial drugs. *Mtb* cell wall contain large number of glycoproteins, however, the role of many of them in pathogenesis remain largely undefined. Here, we attempted to capture mannosylated glycoproteins from the *Mtb* cell wall using different lectins and subjected to LC-MS analysis. We identified several novel *Mtb* proteins responsible for modulation of host innate as well as adaptive immune responses. We have shown that these *Mtb* proteins are involved in host cell invasion, drug resistance, induction of genomic instability, autophagy inhibition, induction of hypermethylation, inhibition of antimicrobial peptide synthesis, and scavenging oxidative stress radicals through induction of peroxisomes. These modulatory activities were found to aid *M. tuberculosis* persistence

in human macrophages and zebra fish. Taken together, these studies have identified novel putative glycoproteins that act as virulence as well as immunomodulatory factor, and thus it may provide a novel drug target.

IL-10

Suppression of *apoA1 2b* in arsenic exposed carp *Labeorohita* indicates efficacy of curcumin against arsenicosis

Bimal Prasanna Mohanty, Arabinda Mahanty, Tandrima Mitra, Satabdi Ganguly, Basanta Kumar Das, Sasmita Mohanty, Santosh Kar

ICAR-Central Inland Fisheries Research Institute, Barrackpore,
Kolkata-700120, INDIA

KIIT School of Biotechnology, KIIT University, Bhubaneswar-751024,
INDIA
bimal.mohanty@icar.gov.in

Arsenic is a highly carcinogenic environmental contaminant and arsenic toxicity (arsenicosis) is a major public health concern in many countries. Arsenicosis leads to clinical manifestations like melanosis, keratosis and cancer of various organs in humans. Early diagnosis would help in better management of patients. To identify biomarkers of arsenicosis, we carried out plasma proteomic analysis of arsenic-exposed *Labeorohita* and identified ApoA1, A2ML, Wap65 and Transferin as potential biomarkers (J Hazard Mater 336:71-80.). To identify which isoform of the gene ApoA1 better responds to arsenic toxicity, we carried out gene expression analysis of three *ApoA1* variants viz. *ApoA1 1*, *ApoA1 2a* and *ApoA1 2b* and also investigated whether dietary supplementation of curcumin, a potential herbal drug/food supplement, has any ameliorative potential against arsenicosis. Fishes were divided into seven groups; three groups were fed with basal diet; two groups were fed a low-dose of curcumin-supplemented-basal diet and two were fed high-dose of curcumin-supplemented-basal diet, for seven days prior to arsenic (Sodium arsenate) exposure and this continued during the exposure period. One of the basal diet fed groups served as the control and the other two groups were exposed to 5- and 15-ppm arsenic, respectively. After the exposure period, fishes were sacrificed, liver tissues were taken out and gene expression analysis of ApoA1 variants was carried out. The *ApoA1 1* and *ApoA1 2a* variants were down-regulated in arsenic-exposed fishes;

however, their expression was found to be same as in control in the curcumin-supplemented-diet-fed fishes. Expression of *ApoA 2b* variant was only found in arsenic-exposed fishes but neither in the control nor curcumin-supplemented-diet-fed fishes. This study showed that *ApoA1 2b* responds well to arsenic toxicity and down-regulation of *ApoA1 2b* proves the efficacy of curcumin against arsenicosis, in a dose-dependent manner. *ApoA1 2b* appears to be a diagnostic marker of arsenicosis as well.

IL-11

Oncogenic splicing switch and glucose metabolism in breast cancer

Sanjeev Shukla

Department of Biological Sciences, Indian Institute of Science Education and Research Bhopal, MP, India

The cancer cells thrive on glucose by converting it to lactate at the end of glycolysis. The phenomenon is known as aerobic glycolysis or Warburg effect and promotes the growth of the cancer cells. The alternative spliced isoform Pyruvate kinase M2 (PKM2) contributes to the Warburg effect by promoting aerobic glycolysis whereas PKM1 isoform promotes oxidative phosphorylation. The *PKM* gene contains two mutually exclusive exons, exon 9 and 10 which are alternatively included in the final transcript to give rise to *PKM1* and *PKM2* isoform respectively. In this study, we report that the intragenic DNA methylation-mediated binding of BORIS (Brother of regulator of imprinted sites) at the alternative exon of *Pyruvate Kinase (PKM)* is associated with cancer-specific splicing that promotes Warburg effect and breast cancer progression. Interestingly, inhibition of DNA methylation or BORIS depletion or CRISPR/Cas9-mediated deletion of BORIS binding site leads to splicing switch from cancer-specific *PKM2* to normal *PKM1* isoform. This results in the reversal of Warburg effect and inhibition of breast cancer cell growth, which may serve as a useful approach to inhibit the growth of breast cancer cells. Importantly, our results show that in addition to *PKM* splicing, BORIS also regulates alternative splicing of several genes in a DNA methylation-dependent manner. Our findings highlight the role of intragenic DNA methylation and DNA binding protein, BORIS in cancer-specific splicing and its role in tumorigenesis.

INDUSTRY LECTURES

CL-01

Integrated Biology Approach for comprehensive understanding of Biological systems: Tools and challenges

Saurabh Nagpal

Agilent Technologies, Manesar, India

Due to inherent complexities of biological systems, researches require multi-pronged strategies to understand and study them. Traditionally these strategies were applied one after the other to validate the results and to address the cause-effect relationship between results. Increasing, we are witnessing simultaneous use of Genomics, Proteomics and Metabolomics; giving rise to a new inter-disciplinary approach referred to as Systems-Biology.

Mass-spectrometry is a widely accepted analytical tool in the field of and the hallmarks of mass-spectrometry based workflows are reproducibility and sensitivity. These very attributes make it well suited for use in various omics applications. However, some specialized tools and software are required for adapting mass-spectrometry for these “Omics” applications.

The presentation will highlight the Mass-spectrometry platforms available to the researchers today and optimal data analysis of the data generated on these instrument. The challenges associated with integration of various Omics approaches and ways to overcome them will also be presented.

CL-02

SWATH acquisition: A robust methodology to address various biological questions

Sciex, 121 Udyog Vihar, Gurgaon, Haryana, India

Quantitative proteomics employing mass spectrometry is an indispensable tool in life science research. Targeted proteomics has emerged as a powerful approach for reproducible quantification but is limited in the number of proteins quantified. SWATH-mass spectrometry consists of data-independent acquisition

and a targeted data analysis strategy that aims to maintain the favorable quantitative characteristics (accuracy, sensitivity, and selectivity) of targeted proteomics at large scale. Quantitation using SWATH method can be used to achieve lower limit of detection and quantitation in comparison to quantitation using survey data. Retrospective data interrogation strategy in SWATH data acquisition mode can be utilized to achieve unbiased identification of site of post translational modifications. SWATH data acquisition also can be used for protein-protein interaction studies. SWATH can be used for host cell protein analysis which has become an essential process for biosimilar drug characterization. Recent study revealed that the acquisition of reproducible quantitative proteomics data by multiple labs is achievable, and broadly serves to increase confidence in SWATH-mass spectrometry data acquisition as a reproducible method for large scale protein quantification.

CL-03

The timsTOF Pro with PASEF: Digging deeper into the proteome with record-breaking speed, sensitivity and robustness.

Dr. Lucy Ann Woods

Bruker Daltonik GmbH, Germany

Proteomics researchers require mass spectrometers with increased speed and sensitivity to address the complex mixture of peptides eluting from nano-flow LC columns in data dependent shotgun proteomics workflows. The previously introduced “Parallel Accumulation Serial Fragmentation” method (PASEF, Meier et al., JPR 2015, PMID: 26538118) for trapped ion mobility spectrometry (TIMS) quadrupole time of flight (QTOF) instruments, enabled five to ten times faster data dependent acquisition of fragment ion spectra. The potential demonstrated in 2015 has now been achieved, along with even more impressive gains in sensitivity due to the 100% duty cycle, and focusing of the ions in time and space, provided by the PASEF method.

In this talk the principles of TIMS and the PASEF method, and how they enable a simultaneous gain in speed and sensitivity, *at the same time*, will be explained. The speed of the PASEF method is only compatible with a very fast scanning instrument such

as a Q-TOF, and it will be shown how this combination results in full sensitivity resolution (FSR) with resolving power of 50,000 FWHM (at m/z 1222) for both MS and MS/MS data, with low ppm mass accuracy and true isotopic patterns (TIP) all at MS/

MS acquisition rates in excess of 100 Hz. The benefits of the PASEF method for identifying more proteins in less time and with less sample in data-dependent shotgun proteomics experiments will be demonstrated.

ABSTRACTS FOR ORAL AND POSTER PRESENTATIONS

ORAL PRESENTATIONS

O-01 / P-01

Development of a LC-MS based method for typing of A1/A2 milk and their comparative proteome analysis using TMT based quantitation approach

Shivam K. Dubey¹, Preeti Rawat¹, Shveta Bathla¹, Alka Chopra¹, Sumit kumar singh¹, Sudarshan Kumar¹, Manishi Mukesh², Monika Sodhi² and Ashok K. Mohanty^{1*}

¹ National Dairy Research Institute, Karnal, Haryana, 132001

² National Bureau of Animal Genetic Resources, Karnal, Haryana, 132001

*Correspondence- ashokmohanty1@gmail.com

Beta-casein in cow milk contains 209 amino acids and is expressed as two major variants namely A1 and A2. In A2 and A1 variant, Proline and Histidine are present at 67th position respectively. Consumption of A1 milk is reportedly associated with human health problems (diabetes, CVD etc.) due to generation of BCM-7 peptide in the GI tract which interacts with μ -opioid receptors in the nervous, endocrine and immune system. However, the suggested health problems are not full proof.

No protein based methods are till date available for detection of A1 and A2 milk and their differential protein compositions. In the present study we report novel strategies for i) Development of a UHPLC coupled MS based method for typing of A1 and A2 milk on the basis of 40 Da difference between Histidine and Proline at 67th position respectively; ii) Comparative proteome profiling of A1A1, A2A2 and A1A2 Betacasein variants of cow milk using TMT based method. To achieve this, casein was extracted from milk with a denaturing 8 M urea protocol, which breaks apart the micelles and solubilizes the proteins. Chromatography of the protein in the urea extract separated the various phosphorylated variants of alpha, beta and kappa forms of casein which were subjected to Mass Spectrometry in ESI-qTof. Peak identification was based on the protein masses in the deconvoluted spectra leading to a mass difference of 24,018 Da and 23,978 Da in A1 and A2 milk respectively. Subsequently, differentially expressed proteins in A1/A2 milk were identified by TMT based quantitative based method. A total of 2054 proteins were identified

out of which 212 were differentially expressed. The differentially expressed proteins were analyzed using various bioinformatics softwares e. g. Panther, Cytoscape, Clugo etc. to ascertain their association in various metabolic pathways and their relevance in human health.

O-02 / P-02

Differentially expressed proteins in hepatopancreas offreshwater prawn, *Macrobrachiumrosenbergii*after *Vibrio harveyi*challenge

J. Mohanty, M. R. Badhe, A. R. Suryawanshi^a, P. K. Sahoo, B. R. Pillai, P. Das, A. Mohapatra and J. K. Sundaray

ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar 751002, India

*^a Institute of Life Sciences, Bhubaneswar 751023, India
E-mail: mohanbadhe1212@gmail.com*

Among the commercial freshwater prawns in India, *Macrobrachiumrosenbergii* has emerged as one of the most important species owing to the advantages like faster growth rate and high consumer demand in both domestic and export market. However, poor management practices make the animals more susceptible to various infectious diseases. Hence, there is an urgent need to study the defencemechanism of this species against microbial infections that would help devising appropriate control strategies. The study thus aimed at identifying protein molecules involved in immunity of this speciesthrough proteomics approach.

Differentially expressed proteins in the hepatopancreas of *M. rosenbergii*juveniles in response to bacteria, *Vibrio harveyi*challenge was studied by 2D gel electrophoresis and mass spectrometry. The prawns (5-10g size) were injected intramuscularly at dose of 0.1 ml of live bacterial suspension (10^6 cfu/ml) and hepatopancreas were collected after 6 h exposure along with control. The protein samples from hepatopancreas were run in 2D gel electrophoresis and the gel images were analyzed by Imagemaster 2D Platinum 7.0 software. The differentially expressed proteins were detected based on a fold change value

of >2 and analysis of variance (ANOVA) at $p<0.05$. These protein spots were excised from the gels and subjected to MALDI-TOF-TOF mass spectrometry. The mass spectra (MS/MS) generated were analyzed against NCBIprot database (Taxonomy: other metazoa), and 20 differentially expressed proteins were identified. The study revealed that exposure of *M. rosenbergii* to bacteria could induce modulation in expression of various proteins, possibly playing roles in its immunity.

O-03 / P-20

Multipronged proteomic analysis of multiple myeloma towards new targets and biomarkers

Chanukuppa V¹, Chatterjee T², Sharma S², Jagadeeshaprasad MG³, Rapole S^{1,*}

¹ Proteomics Lab, National Centre for Cell Science, Ganeshkhind, Pune 411007, India

² Armed Forces Medical College, Pune 411040, India

³ National Chemical Laboratory, Pune 411008, India;

E-mail: venkatesh.ch53@gmail.com

Multiple myeloma (MM) is a heterogeneous disease and accounts for 1% of all cancers and 14% of all hematological malignancies. In MM, malignant plasma cells expand and accumulate in the bone marrow, leading to bone resorption and the over production of antibodies. The major challenge remains the identification of better prognostic and therapeutic biomarkers. In this work, we used MM serum, Bone marrow interstitial fluid (BMIF), BM mono nuclear cells and respected controls. Proteins from Serum, BMIF and BM Mono nuclear cells (MNCs) were extracted and differential proteomic analysis was performed using 2D-DIGE, 4-plex iTRAQ and SWATH. In the study of serum proteome alterations in MM, our quantitative proteomic analysis resulted 140 differentially expressed proteins in which 51 proteins showed increased expression and 89 proteins showed decreased expression. In BMIF study, we identified 184 differentially expressed proteins out of which 101 proteins were found to be up-regulated and 83 proteins were down-regulated. Further, proteomic analysis of BM MNCs yielded a total 892 proteins using SWATH analysis in which 222 proteins were found to be statistically differentially expressed. Bioinformatics data suggest that DNA replication, angiogenesis, apoptosis, integrin, WNT, CCKR signalling pathways were altered in MM. Validation results in a new cohort of samples using western blot and MRM are consistent with quantitative proteomics data. Based on the

literature, we selected three proteins viz. Plasma cell induced resident E.R protein (MZB1), Voltage dependent anion channel 3 (VDAC3), Cullin associated and neddylation dissociated 1 (CAND1) for further functional studies. MZB1 was found be up regulated while VDAC3 and CAND1 were found to be down regulated in our study. These protein signatures are not only helpful as diagnostic and prognostic markers but also provide insight disease pathogenesis information of MM.

O-04 / P-21

Identification of estrus associated proteins in saliva of water buffalo (*Bubalus bubalis*) using high throughput Mass Spectrometry

Shashikumar, N.G¹, Baithalu, R.K^{*1}, Bathla, S², Ali, S. A², Rawat, P², Kumaresan, A¹, Kumar, S², Jaiswal, L¹, Maharana, B.R³, Mohanty, T.K¹ and Mohanty, A.K²

¹ Molecular Reproduction cum Teaching Lab, Animal Reproduction, Gynaecology and Obstetrics, ICAR-National Dairy Research Institute, Karnal 132001, Haryana, India;

² Cell Biology and Proteomics Lab, Animal Biotechnology Center, ICAR- National Dairy Research Center, Karnal, Haryana, India,

³ Regional Research Center (LUVAS), Veterinary Subunit, Uchani, Karnal, Haryana, India,

*Corresponding Author: Dr. Rubina K. Baithalu, Scientist
E-mail address: rbaithalu@gmail.com

Accurate and efficient detection of estrus is one of the major causes of poor reproductive efficiency in buffalo due to poor manifestation and seasonal differences in expression of estrus signs; and higher incidences of silent estrus (29%). Till date estrus detection in buffalo is based on behavioural signs and its efficiency is 50%. Therefore, there is a need to identify suitable biomarker for on spot detection with high precision. Saliva is a non-invasive fluid which can be used for discovery of biomarkers associated with estrus. In the current study, we report on the identification of estrus associated proteins in saliva using high-throughput proteomic approaches. Saliva samples (n=8) were collected on proestrus, estrus, metestrus and diestrus, respectively and equal amount of salivary proteins was taken (total 500 μ g protein) and subjected to in-solution digestion followed by fractionation by b-RPLC and nLC-MS/MS (ESI-qTOF). The proteins were identified by Mascot search algorithm. Label free quantitation (LFQ) of the proteins among different stages revealed a total of 275, 371, 304 and 565 proteins with 2 peptides for proestrus, estrus, metestrus and diestrus stages respectively. A total of 81 proteins were common in

all stages. A total of 66, 81, 130 and 273 proteins were specific to proestrus, metestrus, estrus and diestrus respectively. LFQ data revealed 255 differentially expressed proteins (DEPs), out of which 34 proteins were up-regulated (fold change e 1.5) and 26 proteins were down-regulated (fold change d 0.6) at estrus stage as compared to metestrus and diestrus stage. Notable among few important proteins include Cullin-associated NEDD8-dissociated protein 1, thioredoxin, SRGAP3, Toll-like receptor 3, Heat shock 70kDa protein 1A, Inhibin beta A chain, testin etc which are involved in estrus physiology and can be considered as important candidates specific to estrus in buffalo which may have biomarker potential subject to further validation.

O-05 /P-47

A comprehensive proteome map of Pashmina fibre by combining multiple protein extraction methods and proteogenomics approach

Sakshi Kaith^a, Shalini Kalra^a, Shveta Bathla^a, Zaffar Iqbal^b, Basharat Bhat^b, F. D. Shiekh^c, Aadil Ayaaz^b, Nazir A. Ganai^b, Sudarshan Kumar^a, Ashok K. Mohanty^a, Jai K. Kaushik^{a*}

^a Animal Biotechnology Centre, National Dairy Research Institute, Karnal 132001 (INDIA),

^bAnimal Genetics & Breeding, Sher-e-Kashmir University of Agriculture, Science & Technology, Srinagar (INDIA)

^cHigh Mountain Arid Agriculture Research Institute, Leh (INDIA)

*Correspondence: jai.kaushik@icar.gov.in

To understand the fibre composition and development, proteome profiling of pashmina fibre was carried out. Proteome analysis of pashmina fibre is quite challenging due to its poor solubility and difficulty in protein extraction. 90-95% of fibre substance composed of protein components including two major proteins; Keratins (KRTs) and Keratin associated proteins (KAPs). In the present study, we tried to extract and identify as many proteins as possible, from hard to solubilise Pashmina fibre by using different protein extraction strategies. Methods included different combinations of detergents (SDS, CHAPS), denaturants (Urea, Thiourea) and reducing agents (DTT, DTE, 2-ME). ESI-QUAD-TOF (MS/MS) data were acquired and spectra generated were searched against Uniprot mammalian database and our pashmina RNA-Seq based protein database. Upto246 non redundant proteins based on e 2 peptides could be identified. These proteins included isoforms of KRTs and KAPs, desmoplakin, desmoglein,

plakoglobin, trichohylin, fillagrin, TGM3, profiling and others involved in structural maintenance, fibre development and fibre keratinization. Around 47 different KRTs and KAPs identified which could be involved in fibre development, cell and follicle cycle, intermediate filament organization, including other biological pathways analysed by STRING using *Bos taurus* database which is evolutionarily close to *Capra hircus*. Different methods resulted in identification of various proteins, however SDS based method resulted in maximal recovery of proteins, e.g. with 2% SDS + 5% 2-ME method we could identify 494 proteins of which 139 were common with those of 394 proteins identified in proteins extracted with 0.1% SDS + 25mM DTE. The present study suggested that use of different protein extraction methods is crucial for proteome profiling of difficult to solubilize cells/tissues or fibre. The discovery of pashmina specific new proteins was achieved by using RNA-Seq data on skin samples of pashmina goat (*Capra hircus*). This helped in increasing the accuracy and number of identified proteins.

O-06 / P-48

Data exploration and in-silico analysis of human proteome for development of web-based targeted proteomics framework

Manika Singh^{1,2}, Harsha Gowda^{1,3}

¹ Institute of Bioinformatics, International Technology Park, Bangalore, India, ² Amrita School of Biotechnology, Amrita Vishwa Vidyapeetham, Kollam, India, ³ YU-IOB Center for Systems Biology and Molecular Medicine, Yenepoya University, Mangalore, India.

Dr. Harsha Gowda: harsha@ibioinformatics.org;
Manika Singh: manika@ibioinformatics.org

Prerequisite for doing targeted proteomics experiment is identification of proteotypic peptides. Identifying the right set of proteotypic peptides for each protein is often challenging. Several resources have been developed to provide list of proteotypic peptides across various organisms. Most of these resources were initially developed by predicting proteotypic peptides predominantly based on physicochemical characteristics of the peptides. Over the years, several shotgun proteomics studies have been carried out using various mass spectrometry platforms. These datasets are being deposited to public repositories making the datasets easily accessible. Proteomics databases including Proteomics DB provide access to large scale proteomics datasets enabling determination of most frequently observed peptides for each protein. These experimentally observed peptides serve as a most reliable source to determine proteotypic peptides.

We have developed a web based resource to provide a list of experimentally observed proteotypic peptides for the human proteome. The user can search any human protein of their interest to obtain list of proteotypic peptides and corresponding transitions.

O-07 / P-50

Integrated proteomic and miRNA profiling analyses in oral keratinocytes in response to cigarette smoke

Mohd Younis Bhat^{1,2#}, Jayshree Advani^{1,3#}, Pavithra Rajagopalan^{1,4}, Krishna Patel^{1,2}, Vishalakshi Nanjappa¹, Hitendra S. Solanki^{1,4}, Arun H. Patil^{1,4,5}, Firdous Bhat^{1,2}, P.P Mathur⁴, Bipin Nair², T.S. Keshava Prasad^{1,5}, David Sidransky⁶, Harsha Gowda^{1,*} and Aditi Chatterjee^{1,*}

¹ Institute of Bioinformatics, International Technology Park, Bangalore 560066, India

² Amrita School of Biotechnology, Amrita Vishwa Vidyapeetham, Kollam, India

³ Manipal University, Madhav Nagar, Manipal 576104, India.

⁴ School of Biotechnology, KIIT University, Bhubaneswar 751024, India

⁵ YU-IOB Center for Systems Biology and Molecular Medicine, Yenepoya University, Mangalore 575018, India

⁶ Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins University, School of Medicine, Baltimore, MD 21231, USA

These authors contributed equally;

*Corresponding author: younis@ibioinformatics.org

The major established etiologic risk factor for oral cancer is tobacco consumed in various forms such as smoking and chewing. Despite being one of the leading causes of oral cancer, the molecular alterations induced by cigarette smoke remains largely unclear. Carcinogenic effect of cigarette smoke is through chronic and not acute exposure. To understand the molecular alterations induced by cigarette smoke, we developed a cell line model where non-neoplastic oral keratinocytes (OKF6/TERT1) were chronically exposed to cigarette smoke for a period of 6 months. This resulted in increased cell scattering and invasive ability of normal oral keratinocytes. Using this cellular model, we studied the differential expression of miRNAs and proteins associated with cigarette smoke. miRNA sequencing resulted in the identification of 456 annotated miRNAs of which 06 were significantly dysregulated (e 4 fold; p-value d 0.05) in smoke exposed cells compared to parental cells. Integration of miRNA sequencing data with proteomic data resulted in identification of 16 proven protein targets which (e 1.5 fold; p-value d 0.05) showed inverse directional correlation with the significantly dysregulated miRNAs. These protein targets were

found to play an essential role in cell growth through chromatin remodeling and transcriptional regulation. In addition, we identified 18 novel miRNAs in cells exposed to cigarette smoke. Our study provides a framework to understand the oncogenic transformation induced by chronic exposure to cigarette smoke in normal oral keratinocytes.

O-08 / P-51

Plant responses and global significance: Elevated CO₂ and temperature effects on *Picrorhizakurroa*

Rajiv Kumar, Reema Thakur, Robin Joshi, Dinesh Kumar, Surender K. Vats, Sanjay Kumar

CSIR - Institute of Himalayan Bioresource Technology, Palampur, India

Email: rajiv@ihbt.res.in

Global warming leads to climate change is currently one of the most serious issue for ecology and ecological applications. Rising atmospheric CO₂ level and temperature have begun to impact on life cycles, distribution as well as yield of various crop and medicinal plant species. Yet, how medicinal plants respond to these changing environment is least understood. Therefore, in this study we investigated how elevated CO₂ level and temperature affect plant phenotype, metabolite and proteome in medicinal plant *Picrorhizakurroa*. In our experimental setup *P.kurroa* plants were grown in ambient CO₂ concentration or control, elevated CO₂ at 550±50 μmol mol⁻¹ in free air CO₂ enrichment (FACE) and elevated temperature 2.5 - 3.0°C higher than ambient in free air temperature increase (FATI) for 150 days. Based on phenotypic study *P. kurroa* plant modulates their root architecture and photosynthetic efficiency to enhance adaptation to FACE and FATI conditions. Gel based and gel free approach were used to detect 232 differentially expressed proteins presumably involved in metabolic process, biosynthetic process, protein modification along few proteins with unknown function that play an important role in plant adaptation. Furthermore, significant qualitative and quantitative differences were noticed in metabolites concentration. A total thirty one compounds were identified and twenty four of which has been quantified first time using NMR and clustered for discriminations in control, FACE and FATI samples using statistical heat plot. Our results suggested that *P. kurroa* plant utilized multiple strategies for improving their tolerance at elevated CO₂ level and temperature.

Complexity and diversity of mucin-type O-glycosylation of CD43 revealed by electron transfer dissociation mass spectrometry

Vandita Dwivedi¹, Asif shajahan¹, Gino stolfa², Monika Garg¹, Shanta Sen¹, Kanwaljeet Kaur³, Sriram Neelamegham², and Srinivasa-Gopalan Sampathkumar^{1*}

¹Laboratory of Chemical Glycobiology (CGB), National Institute of Immunology (NII), New Delhi ²Department of Chemical and Biological Engineering, NY State Center for Excellence in Bioinformatics and Life sciences, State University of New York (SUNY), Buffalo, NY, USA.

³ StructuralBiology Unit (SBU), National Institute of Immunology (NII), New Delhi
E-mail: gopalan@nii.ac.in

Mucin-type O-glycosylation (MTOG) is the most complex post-translational protein modification and is initiated by the addition of α -N-acetyl-D-galactosamine (α -GalNAc) to Ser/Thr on polypeptides. CD43 (leukosialin / sialophorin) is known to exhibit a bottle-brush like structure owing to abundance of MTOG and is considered as a negative regulator of T-cell activation. CD43 is found in 115 kDa and 130 kDa glycoforms in immune cells and is estimated to carry 80-90 mucin-type O-glycans. Previous studies by Schmid and co-workers on galactoglycoprotein (the secreted form of CD43) using Edman degradation have identified 25 occupied Ser/Thr sites.

Herein, we present our results on the exhaustive MTOG site mapping analysis of extracellular domain of CD43 (aa 20-253) using high energy collision induced dissociation – product dependent – electron transfer dissociation (HCD-PD-ETD) methodology on high resolution nano-LC-ESI-MS/MS. Soluble CD43-Fc-His recombinant protein was purified from lentivirally transduced Jurkat cells and enzymatically digested. Our analysis revealed a total of 69 sites out of potential 93 sites (46 Ser and 47 Thr) to be occupied by HexNAc. Of the 69 sites, 49 were newly identified sites and 18 sites were matched with previous report; seven sites reported earlier were not detected in our study, presumably due to limited proteolysis. CD43 peptides with micro-heterogeneity and isobaric combinatorial site-occupied glycoforms were identified. HCD-PD-ETD analysis of CD43-Fc-His, from Jurkat cells incubated with the Ac₅GalNTGc (a metabolic MTOG inhibitor), provided evidence for metabolic incorporation and reduction in site-occupancy as a result of MTOG inhibition.

Efforts to apply mass spectrometry for O-glycan site occupancy studies are in infancy. Similar studies on human and rabbit a-dystroglycan have identified 25 and 21 MTOG occupied sites, respectively. Our results illustrate the complexity of MTOG on CD43 and glycan modulations caused by Ac₅GalNTGc. Deciphering the MTOG diversity might provide insights into functional roles of CD43 glycosylation in immunological processes.

Phospho T693 EGFR: a prognostic marker of recurrent non-functioning pituitary adenoma

Ashutosh Rai^a, B D Radotra^b, Kanchan K Mukherjee^a, S K Gupta^a, Pinaki Dutta^c

^aDepartment of Neurosurgery^a, Histopathology^b, Endocrinology^c
Postgraduate of Institute of Medical Education and Research,
Chandigarh, India
Email: raiashutosh28@gmail.com

Non-functioning pituitary adenomas (NFPA) are the most frequent type of pituitary tumor. This benign tumor is usually presented as a macroadenoma larger than 10 mm. Analysis of its molecular and clinical characteristics has been complicated by histological and etiological heterogeneity. Because of the absence of a clear therapeutic target for recurrent NFPA, conventional radiotherapy is the only available systemic treatment option for these patients. The ability to predict the recurrence of a tumor at the time of the initial surgery would be helpful in deciding whether adjunctive therapy is necessary and decreasing morbidity. Aim of our study is to investigate expression pattern of phosphoproteins in recurrent NFPA.

Here, we describe a comprehensive phosphoproteomic evaluation of 20 non-functioning pituitary adenomas. Peptides from 20 tumor samples were enriched with TiO₂ beads and fractionated using bRPLC and subjected to high throughput LC-MS/MS-Orbitrap Fusion™ Tribrid™ Mass Spectrometer for analysis. Up to 5 precursor ions were chosen for MS/MS analysis. Data was analysed using MASCOT and SEQUEST. Using the bioinformatics pipeline (PhosphositePlus, Gene Ontology, DAVID, and KEGG) eight candidate phosphoproteins involved in cell proliferation and growth were selected for validation using immunohistochemistry (n=200) and western blotting (n=18).

In this study, 1345 phosphoprotein groups and 2233 unique phosphopeptides were identified. The EGFR, MEK, and STAT1/3, β -Catenin, BRAF, and HSP27 were significantly hyperphosphorylated in the recurrent group as compared to the non-recurrent NFPA in our mass spectrometry data. While validation on large cohort ($n=200$) using tissue microarray, we found that nuclear EGFR phosphorylated at Thr 693 was significantly associated with recurrence.

Identification of these phosphoproteins provides a roadmap for patient stratification, and prognostication for recurrence and trials for targeted therapy.

O-11 / P-63

Dehydration induced mitochondrial defence response by catabolism of reactive oxygen species in rice

Dipak Gayen^a, Pragya Barua^a, Nilesh Vikram Lande^a, Amit K Dey^b, Tushar Kanti Maiti^b, Subhra Chakraborty^a and Niranjan Chakraborty[§]

^aNational Institute of Plant Genome Research, Jawaharlal Nehru University Campus, Aruna Asaf Ali Marg, New Delhi-110067, India

^bRegional Centre for Biotechnology, NCR Biotech Science Cluster, 3rd Milestone Gurgaon-Faridabad Expressway, Faridabad, Haryana 121001, India.
gayen_dipak@yahoo.co.in

Decreasing water availability or dehydration limits crop yield more than any other environmental stress across the world, particularly in rain-fed areas. Despite

extensive research over past several decades, little is known about mitochondrial regulation and energy metabolism under water-deficit conditions. We, for the first time, mapped dehydration-induced global changes in mitochondrial proteome landscape to dissect the molecular mechanism underlying crop adaptation. Four-week-old rice seedlings were subjected to progressive dehydration and stress severity was assessed by analysing the morphophysiological traits. In-depth comparative proteomics analysis led to identification of an array of stress-responsive proteins, presumably involved in a variety of cellular functions that includes energy production, protein transportation and ROS detoxification, among others. Identification and quantification of intracellular metabolites, using GC-MS analysis, indicated pathways involved and network topology of dehydration response. Next, several of the identified differentially expressed proteins, previously not known to be involved in dehydration response, were validated by transcript analysis. Among the dehydration-responsive biomarkers, an uncharacterised protein harbouring a domain of unknown function, was markedly upregulated. Proteome-scale interactome networks coupled with yeast two-hybrid screening and co-immunoprecipitation analysis identified peroxiredoxin as an interacting protein, putatively involved in mitochondrial ROS catabolism. Overexpression of novel candidate in yeast demonstrated enhanced stress tolerance when compared to WT strain under dehydration indicating its functions in the positive regulation of peroxiredoxin. Taken together, these results impinge on the stress responsive novel biomarker contributing to ROS catabolism, preventing cell death.

POSTER PRESENTATIONS

Session-1 Global and Targeted Proteomics

P-01 (Please refer O-01) ; P-02 (Please refer O-02)

P-03

Pharmacoproteomics of multitargeting in antimycobacterial drug-target discovery**Satakshi Hazra, Sanjukta Patra**

*Department of Biotechnology, Indian Institute of Technology
Guwahati, Guwahati- 781039, Assam, India
sanjukta@iitg.ernet.in*

Upon infection, *Mycobacterium tuberculosis* (Mtb) deploys specialized proteins with the capacity to modulate a variety of host-cellular pathways. Thus, with combination drugs impacting multiple targets simultaneously, one approach is to focus on interactions between these disease-relevant pathways which will lead to novel target discovery and help in greater understanding of the "multi target: multi drug" model. As reported for the drug SQ109, multi target inhibition showed potent inhibition of tuberculosis cell growth, as well as very low rates of spontaneous drug resistance. Further, studies on ATP synthase inhibitor bedaquiline and the discovery of Q203, a candidate drug targeting the cytochrome bc1 complex, have opened avenues that the combination of drugs targeting elements of the oxidative phosphorylation pathway can lead to a completely new regimen for drug-susceptible and multidrug resistant tuberculosis as well as combat dormant or latent mycobacterial infection. This strategy in our present work intends at exploiting about Rv0183 and Rv3802c proteins essential for Mtb lipid metabolism and its inhibitors. In our quest to find synergistic multi-target biomarkers, we highlight several functional interacting partners like RNA polymerase factor sigma-70 sigG involved in host intracellular survival after infection for Rv0183 and polyketide synthase pks13 and transmembrane protein aftB involved in the biosynthesis of the arabinogalactan region of the mycobacterial cell wall for Rv3802c as viable multitargets which are largely unexplored but may be useful in order to achieve the overarching aim of shortening tuberculosis treatment. We also show screening of potent drug combinations targeting qcrB and qcrC genes which are essential components of the electron transport chain and thereafter link the key enzymes of the respiratory and lipid metabolism pathways to elucidate novel multi-targets.

P-04

Proteomic profiling of *Arabidopsis* exhibiting varied GSH content to understand its role in plant defense**Asma Sultana & Sharmila Chattopadhyay***

*Plant Biology Laboratory, Organic & Medicinal Chemistry Division,
CSIR-Indian Institute of Chemical Biology, 4, Raja S. C. Mullick
Road, Kolkata-700032, India
E-mail: asma.sultana44@gmail.com*

Plants are confronted with numerous environmental stresses throughout their life cycle. Consequently, they have evolved highly sophisticated defense strategies to respond to their ever-changing environment and ensure survival through integrating signaling networks, mediated by the interactions of salicylate (SA), jasmonate (JA), ethylene (ET), abscisic acid (ABA) as well as reactive oxygen species (ROS). Over the past decades the role of glutathione (GSH) to combat environment stress conditions is gradually getting established. However, the molecular mechanism is yet to be explored. A transgenic *Arabidopsis thaliana* line (*AtECS*), over-expressing the key enzyme of GSH biosynthesis, viz. γ -ECS (γ - glutamylcysteine synthetase) has been developed. HPLC analysis noted with enhanced GSH content of this transgenic line. Finally, a comparative proteomics analysis of *A. thaliana* exhibiting varied GSH content viz. Col-0 (wild type), and *AtECS*, has been performed. Functional classification of identified proteins revealed that mostly belonged to the stress and defense category, carbon and energy metabolism etc. Proteins identified by 2-DE MALDI- TOF-MS/MS, viz. RUBISCO ACTIVASE, ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, ATP synthase CF1 alpha subunit, ATP synthase CF1 beta subunit, HSP 70-7, ACC oxidase, HSP70 cognate, DHAR1, CA2, cold stress protein, etc. were noted up-regulated and mention-worthy indicating the dynamic role of GSH in plant defense.

Tandem mass tag (TMT) based comparative expression analysis of milk whey proteins in Livestock

Alka Chopra^a, Shveta Bathla^a, Preeti Rawat^a,
Shivam K. Dubey^a, Arvind Verma^a,
Sudarshan Kumar^a, Vikas Vohra^b, and
Ashok K Mohanty^a

^a ICAR-National Dairy Research Institute, Karnal - 132001 (Haryana) INDIA

^b ICAR-National Bureau of Animal Genetic Resources, Karnal - 132001 (Haryana) INDIA
E-mail: ashokmohanty1@gmail.com

Milk is an important nutritional source as well as a medium of transfer of host defense proteins from mother to offspring. Cattle and Buffalo milk accounts for major milk production, however, milk of non-traditional species such as goat, camel, sheep and donkey also have therapeutic value in human health. Different antimicrobial peptides related to host defense may be present in milk of different farm animals. Thus, the present study was conducted with the aim to explore species specific unique proteins as well as to analyze their comparative expression in Cattle, Buffalo and Goat milk. Raw milk samples from different lactation stages were pooled together, defatted and ultra-centrifuged for milk whey precipitation. Differential expression profiling of proteins in these milch species was done by labelling the proteins using *Tandem mass tag (TMT)* followed by their identification by LC-ESI-MS.

Differential proteome analysis in Cattle and Buffalo led to identification of a total of 3041 proteins and 295 differentially expressed proteins. 64 proteins were found to be up-regulated (> 1.5) and 76 down-regulated proteins (< 0.6). However, 37 proteins were found to be up-regulated and 76 down-regulated in Cattle vs Goat. Gene ontology using Panther software revealed involvement of major proteins in catalytic, binding and structural molecular activities. Differentially expressed proteins were found to play role in signaling pathways related to host defense, metabolism and stress tolerance such as Chemokine and Cytokine signaling pathway, Alzheimer disease-presenilin pathway, Cadherin signaling pathway, Heterotrimeric G-protein signaling pathway and Oxidative stress response. Thus, the information generated through this study will provide comparative evaluation in these milch species to ascertain suitability of milk from a particular species in human health promotion. The author acknowledge the financial

support for the study. Project was funded by DST-SERB under the N-PDF program [File No.-PDF/2015/000770].

A comparative proteomic analysis of whole saliva from individuals exposed to hypobaric hypoxia

Shikha Jain, Yasmin Ahmad and Kalpana Bhargava

Defence Institute of Physiology and Allied Sciences, Timarpur, Delhi-110054

Corresponding author: kalpanab2006@gmail.com

Hypobaric hypoxia is a pathophysiological condition which triggers oxidative stress to proteins, lipids, and DNA. Identifying the molecular variables playing key roles in this process would be of paramount importance to shed light on the mechanisms known to counteract the negative effects of oxygen lack. For the last few years, saliva has gained attention as a diagnostic fluid due to its various advantages over other biological fluids. Various studies focused on saliva flow and composition in response to hypobaric hypoxia but till date no study using proteomic approach has been investigated. The present study focuses on exploring the informative protein biomarkers in the human saliva proteome and their potential role in high altitude induced hypobaric hypoxia. Here, we have compared saliva samples from individuals exposed to high altitude for seven days (HAD7) with the individuals at sea level (Normoxia) using two-dimensional (2-D) gel electrophoresis followed by MALDI-TOF/TOF. By comparing the saliva proteins of exposed group with those of a control group, several proteins with a significant alteration were found. The up-regulated proteins were apoptosis inducing factor-2, cystatin S, cystatin SN and carbonic anhydrase 6. The down regulated proteins were polymeric immunoglobulin receptor, alpha-enolase and prolactin-inducible protein. Among these proteins, the alterations of alpha enolase, carbonic anhydrase 6, prolactin-inducible protein, cystatin S and cystatin SN were further confirmed by Western blotting. The expression patterns of the five selected proteins observed by Western blot were in agreement with 2-DE results, thus confirming the reliability of the proteomic analysis. Pathway analysis of the identified proteins revealed two major pathways such as Role of Akt in Hypoxia induced Hif1 activation and nitrogen metabolism that can play role in hypobaric hypoxia.

Taken together, this approach gives novel insights into alterations of salivary proteome exposed to hypobaric hypoxia.

P-07

Evaluation of *In vitro* antidiabetic and cytotoxic studies of a novel galactose specific lectin isolated from *Chrysophyl lumcainitoL*

Deepti Madayi, K K Elyas

University of Calicut, Malappuram, India
E-mail: deeptim@uoc.ac.in, kkelyas@yahoo.com

Lectins are glycoproteins of non-immune origin with different carbohydrate binding specificity and biochemical properties. A majority of research has been focused on the biochemical and structural aspects of lectins. In this particular investigation an effort has been undertaken to probe into the antidiabetic and cytotoxic properties of this lectin. *Chrysophyl lumcainito L*, commonly known as Star apple is an ornamental tree which bears edible fruits. The lectin purified from *Chrysophyl lumcaini* to leaves by Sephadex Gel filtration chromatography eluted as two peaks. Peak 2 which exhibited activity was found to be a 29kDa protein which exhibits specificity towards Galactose. Biochemical characterization of the lectin has already been performed which gave a clear indication of the various parameters which affect the stability of the lectin. Antifungal activity assays indicated the ability of the lectin to act against fungi such as *Rhizoctoniasolani* and *Phytophthora capsici*. In order to test the potential of the lectin in the area of therapeutics and herbal medicinethe following investigation was carried out. Previous research has demonstrated that the aqueous extract of *Chrysophyllumcainitoleaves* exerts a hypoglycemic activity at doses $\leq 20\text{g/l}$. Therefore, a study was conducted to analyse the antidiabetic activity of the purified lectin. Glucose uptake studies by means of flow cytometrywas conducted utilizing NBDG to perform glucose uptake studies and check for the levels of NBDG inside the cells in the presence of test and standard (Metformin). Cytotoxic studies were performed using the brine shrimp lethality test as a preliminary assay to determine the LC_{50} (lethality concentration). Anti-proliferative activity of the lectin against selected cell lines were conducted to determine the IC_{50} value. Due to their unique properties, lectins play an important role in amalgamating the fields of Proteomics and Glycomics.

Activation of Redox and Energy Homeostasis via STAT3-RXR-Nrf 2 upon Altitude Variation

Subhojit Paul, Anamika Gangwar,
Kalpana Bhargava, Yasmin Ahmad

Defence Institute of Physiology & Allied Sciences, Defence R&D Organization, Timarpur, New Delhi 110054
yasminchem@gmail.com

Hypobaric hypoxia elicits several patho-physiological manifestations, some of which are known to be lethal. Among various molecular mechanisms proposed, imbalance between radical generation and antioxidant defence is promising. Increasing altitude simply means a falling pO_2 gradient. This causes hypobaric hypoxia, which is dependent upon two factors- altitude and time. The effects of pO_2 gradient i.e. variation of altitude are yet unknown although it is a significant factor in context of acclimatization protocols. In present study, the focus was to understand and propose a model for rapid acclimatization of high altitude visitors to enhance their performance based on molecular changes. We used simulated hypobaric hypoxia at established thresholds of high altitude stratification based on known physiological effects. We observed the effects of acute (24 h) exposure to high (3049 m; pO_2 : 71 kPa), very high (4573 m; pO_2 : 59 kPa) and extreme altitude (7620 m; pO_2 : 40 kPa) zones on lung and plasma using semi-quantitative redox specific transcripts and quantitative proteo-bioinformatics workflow in conjunction with redox stress assays. Our findings indicate that very high altitude zone elicits systemic energy and redox homeostatic processes due to failure of lung energy and redox homeostasis by modulating the STAT3-RXR-NRF2 trio. We also document a rapid acclimatization protocol causing a shift from 0 to 100% survival at 7620 m in male SD rats upon rapid induction. This protocol was observed to facilitate subjugation of oxidative stress and cytoskeletal perturbations in lung at 7620 m. Finally, we posit the various processes downstream of STAT3-RXR-NRF2 and the plasma proteins that can be used to ascertain the acclimatization status of an individual.

Comparative proteomic evaluation of effect of ageing in Ladakhi and low lander females

**Pooja, Dishari Ghosh, Kalpana Bhargava,
Usha Panjwani, Bhuvnesh Kumar,
Niroj Kumar Sethy***

Defence Institute of Physiology and Allied Sciences,
Defence Research and Development Organisation,
Lucknow Road, Timarpur, Delhi – 110054, India
* Corresponding author: niroj@dipas.drdo.in

Advancing age and high altitude exposure has broad implications for vascular alterations that lead to wide range of pathophysiology of disorders including cardiovascular diseases, pulmonary hypertension, dyslipidemia and diabetes. Several researchers have investigated physiological variations and role of endothelium derived factors in elderly high altitude natives. But there is a lack of information for the underlying molecular processes. In the present study, we investigated the abundance of angiogenesis, protease and cytokine proteins in young (27 ± 3 years) and middle-aged (50 ± 2 years) Ladakhi females born and residing at Leh (Altitude 3,520 m) and compared them with age matched low land females (Delhi, Altitude 216 m). This resulted in identification of 46 angiogenesis related, 34 protease related and 5 cytokines to be differentially regulated among the young and aged sea level and native females. Pathway analysis using MetaCore revealed ECM remodeling, activation of HIF-1a targets and vascular endothelial cell damage as the topmost pathways in middle-aged females. Further analysis of oxidative stress parameters revealed significant higher levels of ROS, MDA and protein carbonyls in middle-aged Ladakhi and low land females. Our results, demonstrate that ageing promotes oxidative stress and vascular damage irrespective of altitude of stay. These findings will aid in better understanding of human response to environmental and pathological hypoxia.

Protein splicing regulation: A target for novel anti-tubercular drugs

**Sunita Panda¹, Ananya Nanda¹,
Dr. Sasmita Nayak^{1a}**

¹ School of Biotechnology, KIIT University, Bhubaneswar, 751024, Odisha, India

^a Kalinga Institute of Medical Science, Bhubaneswar 751024, Odisha, India

E-mail: sasmita.n@kiitbiotech.ac.in

Inteins are “intervening sequences” that interrupt the coding sequence of a gene. These are transcribed and translated along with the host protein and splice out post-translationally by a self-catalyzed process called protein splicing generating a functional host protein. Interestingly, inteins are present in regulatory and conserved proteins of pathogenic organisms such as in *Mycobacterium*, *Coxiellaburnetii*, and *Cryptococcus neoformans*. Tuberculosis (TB) stands out as the most widespread infectious disease in the world and is a major health problem in India. About 2 Billion of people are currently infected with *Mycobacterium tuberculosis* (*Mtu*) world wide leading to 3 million people death every year. Since multi drug resistance (MDR) TB is emerging as a new global health hazard, intein-splicing inhibitors targeting the essential proteins in mycobacteria would provide a novel platform for treating TB. Higher eukaryotes such as humans lack inteins, thus splicing inhibitors directed against essential proteins would provide a narrow spectrum drug(s) highly specific towards *Mtu* infection. *Mtugenome* has three intein containing genes; *recA*, *dnaB*, and *sufB*. While RecA and DnaB proteins play an important role in the DNA replication and repair respectively, SufB is a component of the Fe-S cluster assembly complex and is essential for growth and virulence of mycobacteria during periods of stress. Protein splicing involves highly coordinated interactions among critical residues including those near intein-extein splice junctions. Past works have shown splicing regulation in various inteins by changing pH values, temperatures and in presence metals. However, none of these studies have been done in mycobacterial SufBintein to date. Our preliminary works via *in vitro* splicing studies have shown splicing inhibition of *Mtu* Suf Bintein in presence of metals such as Zn and Pt. Further studies are going on to confirm the mechanistic roles of intein critical residues during splicing and cleavage reactions in *Mtu*SufB.

Key Words: Splicing, Suf B, Intein, *Mycobacterium tuberculosis*.

**Total Leaf Proteome Map of Pigeonpea
(*Cajanus cajan* (L.) Millspaugh))
under salt stress**

Bangaru Naidu Thaddi* and
Arunalakshmi Kommaraju

GIT, GITAM University, Visakhapatnam, Andhra Pradesh,
India-530045

E-mail: drbangarunaidu@gmail.com

Salinity is one of the most widespread agricultural problems in the entire world that makes fields unproductive. Identification of salt stress varieties to improve productivity is inevitable. The present study aims to establish the total leaf proteome map of pigeon pea varieties ICPL 87119 (salt stress tolerant) and ICPL 85063 (Salt stress sensitive) under salt stress of NaCl by using Two-dimensional Gel Electrophoresis (2-DGE). The plants were grown hydroponically in different salt stress concentrations of 0, 50mM, 100mM, 150mM & 200mM. The effect of salt stress on length and fresh weight of seedling was determined. Increasing salt stress level up to 200mM resulted in a reduction of plant growth, height and leaf necrosis. Leaf proteins extracted by TCA/Acetone buffer were focused after cup-loading onto IPG strips. Successful separation in the second dimension was achieved using gradient gels in a vertical SDS-PAGE system. The 2-D gels of the two genotypes were stained with Coomassie Brilliant Blue (CBB) and the expression level of proteins was evaluated by PDQuest software. Twenty-two proteins were visibly up-regulated and sixteen proteins down-regulated in response to 150mM NaCl treatment. MALDI-TOF-MS sequencing analysis of up and down regulated protein spots will be further analyzed. To the best of our knowledge, this is the first study reporting in the regulation of protein expression in pigeon pea varieties growing under salt stress.

Proteome alteration in human saliva and vaginal fluid with progression of pregnancy

**Bhoj Kumar^{1§}, Amit Kumar Dey^{1§}, Nitya Wadhwa²,
Pallavi Kshetrapal², Dinakar M Salunke³,
Shinjini Bhatnagar^{2*}, Preterm birth study group
and Tushar Kanti Maiti^{1*}**

¹ Functional Proteomics Laboratory, Regional Centre for Biotechnology, NCR Biotech Science Cluster, Faridabad, 121001, India

² Pediatric Biology Centre, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, Faridabad, 121001, India

³ International Centre for Genetic Engineering and Biotechnology Aruna Asaf Ali Marg, 110 067 New Delhi, India

*tkmaiti@rcb.res.in and shinjini.bhatnagar@thsti.res.in

§ Equal contribution and both are presenting authors

Pregnancy represents a dynamic state with numerous physiological transient changes. Saliva and human high-vaginal fluid (HVF) are the important body fluids which reflect physiological changes during pregnancy. Despite their importance, data on dynamic protein changes with progression of pregnancy in saliva and HVF are limited. To investigate proteomic alteration in maternal saliva and HVF during the progression of normal pregnancy, we have performed high throughput label free (SWATH) quantitative mass spectrometry. Maternal saliva and HVF sample with three POG time points (<14, 18-20, and 26-28 weeks of POG) were analyzed. The spectral library with unique 770 and 1104 proteins for saliva and HVF respectively have been made. Several proteins are differentially modulated ($p<0.05$) with advancement of gestation age. Cystatin's, clusterin, carbonic anhydrase 6, neutrophil defensin 1, lactotransferrin, lactoperoxidase in saliva and cornulin, calmodulin-like protein 5, prelamin-A/C, annexin A1, protein S100-A9, and actin cytoplasmic in HVF are significantly altered in progression of pregnancy. These proteins represent the major functional categories of defense mechanism like, innate immune system, neutrophil and platelet degranulation, platelet activation and its response to elevated cytosolic Ca^{++} , and hemostasis. This study may provide an insight into the adaptive mechanism involved in normal pregnancy, and associated risk factors including preterm birth by comparing proteome map of both term and preterm group across the pregnancy period.

Change in the redox status triggers tuber germination and showed the involvement of Asada –Halliwellpathway in germination

Shruti Sharma and Renu Deswal*

Department of Botany, University of Delhi

*Corresponding author-rdeswal@botany.du.ac.in

In *Dioscorea*, dioscorin (31 kDa) is the major storage protein constituting 85% of the total tuber proteins. An integrated proteomic and biochemical approach was used to understand the physiological role of dioscorin in *Dioscorea alata* tuber. SDS-PAGE and 2-DGE maps revealed dioscorin depletionon germination, accompanied with sugar mobilization as confirmed by HPLC analysis with differential regulation of enzymes involved in starch metabolism.In mature tuber 35 spots at 31 kD were observed, of which only 12 spots (identified as dioscorin isoforms) were observed after purification (using ion exchange and gel filtration chromatography). As there is hardly any information on other tuber proteins besides dioscorin, an attempt to search for such proteins was undertaken.Minor proteinsDREB 1A, caffeic acid 3-O-methyltransferase and Rab-1 small GTP binding protein were identified perhaps for the first time in the *Dioscorea* proteome (DEAE-cellulose unbound fractions). The *insilico* analysis using STRING revealed these to be involved in oxidative stress, carotenoid synthesis and vesicular transport respectively suggesting these physiological processes to be important during growth of the new tuber.Differential regulation of an operational Asada-Halliwell cycle was also established during the tuber growth with a 2.6 fold increase in ascorbate (AsA) content, 3.8 fold increase in glutathione reductase activity showing overall reductive status in germinating tuber and 5 fold increase in the Hydrogen peroxide (H_2O_2)due to 3 fold lower Ascorbate peroxidase (APx) activity indicating overall oxidative status in the mature tuber.These initial investigations have provided interesting leads for a detailed analysis of the *D. alata* tuber proteome. Thus, a proper staging of the tuber is underway for better understanding of the heterogeneity of the complex protein dioscorin.A systematic stage wise analysis is important prerequisite to manipulate its quality in future as an alternative food crop.

A metal/chelate poly-HEMA monolith capillarymicrocolumn for selective depletion of IgG from human plasma for proteomics

**Ashish Khaparde, Vijayalakshmi, M. A,
Kishore K. R. Tetala**

Advanced Centre for Bio-Separation Technology (CBST),

VIT University, Vellore-632014, Tamil Nadu, India

ashish2018@yahoo.com, kishore.tetala@gmail.com

Proteomic analysis of clinical samples is very essential for diagnosis, therapy and disease monitoring. However,in complex biological samples such as plasma; the practical hindrance lies in the identification of targets(low abundant proteins) which are predominantly masked by the presence of both high and middle abundant proteins.

Over the years, various chromatography based pre-fractionation approaches like size exclusion, reverse phase, affinity chromatography etc. are widely employed. However,the limitation lies in the disability to multiplex them into a single system using single buffer system.Recently, Karan et. al. (*J. Chromatogr. B*, 2017, 1052, 1-9) showed the potential of multiplexing immobilized metal/chelate affinity system as a new pre-fractionation approach for plasma proteomics. The limitation is sample dilution during pre-fractionation process indicating the need for a minianalyticalsystem of this approach.

Further in this progression, we aim to develop a minianalytical column. As a proof-of-concept, we developed a new poly HEMA (HEMA-*co*-DEGDA-*co*-DATD) monolith capillary microcolumn functionalized with "IDA-Cu (II) complex". Multi-step and 2-step chemical modification approaches were performed to immobilize chelator (i.e. iminodiacetic acid, IDA) followed by chelating with metal-ion (Cu (II)) to achieve adsorption of proteins. These monoliths are highly organized and interconnected large globular structures which are dominated by macropore region helping the hydrodynamic transport of molecules through the channels.

For the first time, a reaction of sec. amine ligand with aldehyde functional material was successfully reported. Overall, the Cu (II)-IDA monolith capillary microcolumn showed good permeability ($3.05 \times 10^{-13} m^2$), high IgG adsorption capacity and reusability(even after 5 consecutive adsorption-desorption cycles). The amount of protein (IgG/HSA)

adsorbed on Cu (II)-IDA monolith prepared via the two chemistries is almost similar. Using this affinity monolith capillary microcolumn, we selectively depleted ~ 95% of IgG from human plasma (dilution of 1:16). Currently, work is in progress towards preparing a multiplex minianalytical column for removal of high abundant proteins from plasma.

P-15

Global proteomic perspective and identification of abiotic stress responsive proteins in rice: An *in silico* approaches

**Pandiyar Muthuramalingam,
Shanmugaiah Karutha Pandian and
Manikandan Ramesh***

*Department of Biotechnology, Alagappa University, Karaikudi,
Tamil Nadu, India
mrbiotech.alu@gmail.com

Abiotic stressors like drought, salinity, oxidative, and cold stress can adversely affect the overall rice production. Understanding the proteomic response of rice when it is exposed with these stresses provides novel insights on biological response mechanisms that can hinder or regulate its survival. Recent trends and the availability of whole genome sequence and high throughput capabilities to regulate gene co-expression patterns has shifted the research focus from the study of single proteins or small complexes to the whole proteome. In this perspective, the search for unswerving methods for assigning protein function is primary importance. There are diverse computational approaches existing for deducing the function of proteins of unknown function using information derived from sequence similarity, gene ontology, clustering patterns of co - expressed genes, phylogenetic profiles, chromosomal colinearity, and protein - protein interactions, protein complexes. The present study focuses on retrieving the abiotic stress related rice proteins from available literatures and validating them to understand the potential candidates that drive the molecular cross talks during abiotic stress through multiple computational approaches. These findings will be useful to study the complex biochemical response, physiological function, signaling and molecular network of particular protein in rice crop against multiple abiotic stress conditions.

iTRAQ based proteomics profiling in Vitamin B₁₂ Wistar rats

**Swati Varshney^{1,2}, Arunachal Chatterjee¹,
Ajay Bhat^{1,2}, Usha Sree R³, Lovejeet Kaur³,
Vislavath Jyothi³, Pujitha Kommineni⁴,
Rakesh Mishra³, Giriraj Ratan Chandak³,
Manchala Raghunath⁴, Praveen Singh^{1,2},
Shantanu Sengupta^{1,2 *}**

¹ CSIR-Institute of Genomics and Integrative Biology,
New Delhi, India.

² Academy of Scientific Innovative Research, New Delhi, India

³ CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India

⁴ National Institute of Nutrition, Hyderabad, India

Vitamin B₁₂ is a water soluble vitamin and is an important cofactor integral to the one carbon metabolism and DNA methylation. Thus it is important for the cellular homeostasis. Deficiency of vitamin B₁₂ can have serious haematological and neurological consequences. Deficiency of vitamin B₁₂ is common in Indian subcontinent particularly in pregnant women show more than 70% deficiency. Therefore, in-utero vitamin B₁₂ deficiency may result in rewiring of the epigenetic patterns and hence the proteome in the foetus. This in turn may lead to establishment of complex disorders like cardiovascular diseases in later stages of life.

To evaluate the effect of maternal Vitamin B₁₂ deficiency we developed B₁₂ deficient wistar rat females and mated them with control males. We measured biochemical and lipid levels for the two generations (i.e. F₁ and F₂) of the pups born to B₁₂ restricted mothers following from 3-12 months. We generated the proteome profile of F₁ 3 and 12 month's old pups. The triplicate 4-plex iTRAQ experiment was performed for three tissues i.e. liver, kidney and skeletal muscle of male and female tissues separately. In each 4-plex, tissues from control group, vitamin B₁₂ restricted, rehabilitated at conception and rehabilitated at parturition were included.

We identified proteins at 1% FDR in males and female tissue. The replicate experiments of all the tissue are highly correlated. We observed 239 and 317 differential proteins in male and female liver experiments respectively of which 18 proteins are commonly up regulated and 11 are down regulated in F₁ 3 months' time point. Similar change in protein expression is also seen in kidney and skeletal muscle as well in F₁ 12 months' time point. We have found the tissue and sex specific changes in protein

expression in all the three tissues due to vitamin B₁₂ restriction.

P-17

Delineating the molecular mechanism of Cysteine induced toxicity

Rahul Chakraborty^{1,2}, Ajay Bhat^{1,2}, Kausik Chakraborty^{* 1,2}and ShantanuSengupta^{*1,2}

¹ CSIR-Institute of Genomics and Integrative Biology,
Mathura Road, New Delhi, India

² Academy of Scientific and Innovative Research, New Delhi, India

Cysteine, a thiol containing amino acid synthesized via the transsulfuration pathway is a rate limiting precursor for glutathione synthesis and is also utilized for protein synthesis. Among the biological thiols elevated levels of homocysteine is known to be associated with various diseases and is considered to be an independent risk factor for cardiovascular disease. Recent evidences actually support this fact and it has been shown that cysteine induced growth defect in yeast was more severe than homocysteine (Kumar *et al.* 2006). Reports also suggest that elevated levels of cysteine may be associated with cardiovascular disease (El-Khairy *et al.* 2003).

Using Yeast (*Saccharomyces cerevisiae*) as a model system in this study we have tried to find the mechanism of cysteine induced toxicity. To characterize the cellular response in presence of high level of cysteine we have performed a quantitative proteomics experiment. We found several proteins that were differentially expressed in presence of high level of cysteine. Out of which aminoacid metabolic proteins are mainly upregulated and glycolytic – TCA cycle proteins are downregulated. Intracellular aminoacid measurement by using o-phtalaldehyde reveals that cysteine causes aminoacid imbalance in the cells and by using s35 labeled methionine we also found that cysteine induces translational arrest. Further we have found that supplementation of high levels of leucine and pyruvate can rescue cysteine induced toxicity.

From the genetic screen of around 4800 non-essential genes, we found several genes which are sensitive to cysteine. Among which pyruvate was unable to rescue in Δ leu3 strain (Leu3 is a transcription factor regulates genes involved in branched chain amino acid biosynthesis) and leucine was not able to Δ ncl1 deleted strain (Ncl1 is a tRNA m5C-methyltransferase, methylatest RNAleu (CAA) at the anticodon wobble position). It clearly indicates that

pyruvate and leucine can alleviate cysteine induced toxicity through Leu3 and Ncl1 respectively.

P-18

MALDI-TOF/MS Analysis of DFP-Albumin Adduct

Jebin Jacob John, M. Kameswara Rao^{*} and R. Bhattacharya

Pharmacology & Toxicology Division, Defence R&D Establishment, (DRDE-DRDO) Gwalior -474002 (MP)
*mkrao@drde.drdo.in**

Organophosphorous (OP) compounds inhibit cholinesterase enzymes in the blood and it is responsible for its acute toxicity. Our aim is to develop biomarker assay to ascertain exposure of OP-class of nerve agent compounds. OP compounds also bind to other proteins in the blood and the albumin is a potential biomarker of OP exposure. The site of covalent binding was identified as Tyr (Y)-411 in the active centre peptide. Although there are 18 tyrosine (pKa~10) residues in the albumin but Y- 411 is the active site due to its unusual low pKa (7.9-8.3) and lies on the surface of the albumin. Our main objective is to study the characteristic ions for the various OP labels, along with the frequency at which each appears and the relative intensity of the signals. The second goal is to explore the environment of the labeled peptides in an attempt to establish factors that could promote the reaction of tyrosine with OP. We have found that tyrosines which are susceptible to reaction with OP frequently lie within 6 Å of a positively-charged groups (lysine, arginine, or histidine) in the three dimensional structure. This suggests that charge-charge, through-space ion-pairing may be lowering the pKa for these tyrosines, making them better nucleophiles that are more capable of reacting with OP. The third goal is to establish tyrosine as a site for reaction of OP with proteins. This is consistent with the wide distribution of proteins that contain tyrosines which react with OP. Human/mice plasma or albumin was treated with diisopropylfluorophosphate (DFP) at alkaline pH, digested with pepsin at pH 2.3 and analyzed by MALDI-TOF/MS. The sequence of two peptides labelled with DFP were identified as VRY⁻TKKVPQVSTPTL, m/z 1717.19 & LVRY⁻TKKVPQVSTPTL, m/z 1829.55. Modified peptides by DFP were observed at m/z 1881.09, 1994.11 respectively. None of this DFP-albumin adducts lost an alkoxy group leading to the conclusion that aging does not occur. The Presence of Y- 411 on

an exposed surface of albumin also suggest that antibody response could be generated against the albumin adducts. Plasma was depleted using blue sepharose columns for the purification of albumin and phosphopeptides were enriched with Ga⁺ columns. *In-vivo* experiments were carried out in mice by treating DFP subcutaneously at 1LD₅₀ dose. Plasma samples were collected after 1h, 4h, 24h, 7d & 14d. The MALDI-TOF/MS assay developed is simple, rapid and the DFP-albumin adducts serves as an excellent biomarker of exposure of OP compounds. These covalently bonded albumin adducts formed are highly stable and can be detected even at 30-40% of cholinesterase inhibition. The advantage of these adducts is that intact agent can be identified with structural conformity due to non-aging of the adducts when bound to albumin.

P-19

Limited proteolysis as a tool for elucidating protein conformations responsible for function

Dr. Paramita Chaudhuri Basu¹, Dr. Howard Young²

¹Department of Life Sciences, Presidency University, Kolkata, India;

²Department of Biochemistry, University of Alberta,
Edmonton, Canada.

paramita.dbs@presiuniv.ac.in

P-type ATPases are a special class of cation transporter membrane proteins which move through different conformation states during the transport of their specific ions. Important P type ATPase ion transporters include the calcium pump of the human heart, the plant PM H⁺ pump and the Na⁺K⁺ exchanger. These transporters are also called E1-E2 ATPases, based on the conformation state in which the protein exists when bound to its primary ion (E1) and the conformational state which binds the counter transported ion (E2). The general cycle for ion transport follows a Post-Albers scheme, in which E1 and E2 are connected in a cyclic format via several phosphate bound intermediate states. The two commonest intermediate states are E1-P and E2-P. These transporters utilize the energy derived from ATP hydrolysis to drive the ion transport against the concentration gradient, in the process getting phosphorylated (P-type).

Bacterial magnesium transporters are a special class of P-type ATPases, which are unique in having structural similarity to the human and plant transporters, despite their bacterial origin. These have long been debated to transport magnesium as the counter transported ion, while the primary ion remains

elusive. Using limited proteolysis on purified and reconstituted bacterial magnesium transporters showed for the first time that magnesium was the primary ion and bound the transporter in the E1 state. Protons were also identified as the putative counter transported ion. A model was also generated of the bacterial magnesium transporter during its transport cycle following the Post-Albers scheme. In the model, the magnesium bound E1 and the proton bound E2 conformational states has been highlighted. This work shows the application of limited proteolysis as a classical biochemical tool for elucidating the structure function dynamics of purified and properly folded active proteins.

Session-2 Disease Proteomics and Biomarker Discovery

P-20 (Please refer O-03); P-21 (Please refer O-04)

P-22

Identification of potential biomarkers of adrenocorticotrophic hormone (ACTH) secreting pituitary adenomas

**Tyagi A¹, Khan AA^{2,3}, Puttalamlesh VN^{2,4},
Datta KK¹, Pinto SM¹, Prasad TSK^{1,2}, Dutta P⁵,
Mukherjee KK⁶, Gowda H^{1,2}**

¹ YU-IOB Center for Systems Biology and Molecular Medicine,
Yenepoya University, Mangalore, India

² Institute of Bioinformatics, Bangalore, India

³ School of Biotechnology, KIIT University, Bhubaneswar,
Odisha, India

⁴ Amrita School of Biotechnology, Amrita University, Kollam, India

⁵ Department of Endocrinology, Postgraduate Institute of
Medical Education and Research, Chandigarh, India

⁶ Department of Neurosurgery, Postgraduate Institute of
Medical Education and Research, Chandigarh, India
ankur@yenepoya.edu.in; harsha@ibioinformatics.org

Cushing's disease (CD) is a rare condition caused by over secretion of adrenocorticotrophic hormone (ACTH) by pituitary adenoma. The over secretion of ACTH hormone ultimately causes endogenous hypercortisolism by stimulating the adrenal glands. Endogenous hypercortisolism is associated with an increased risk of cardiovascular and metabolic manifestations, as well as respiratory disorders, psychiatric complications, neurocognitive disorders, osteoporosis and infections, diabetes, leading to high rates of morbidity and mortality. The diagnosis of CD still remains a challenge due to the unclear pathobiology of disease. CD is often treated by surgery,

hormonal replacement therapy and steroids. Surgery is the most preferred choice of treatment (transsphenoidal surgery, or radiotherapy/radiosurgery) for ACTH secreting pituitary adenomas. However, majority of patients show recurrence of tumor within 5 to 10 years after surgery. There are no effective biomarkers for diagnosing and monitoring CD. We used iTRAQ based quantitative proteomics and identified several differentially expressed proteins in CD. The utility of candidate proteins as biomarkers and putative targets in CD should be further evaluated.

P-23

The Role of an environmentally stress-induced protein, dermcidin in platelet aggregation in acute myocardial infarction.

Sarbashri Bank¹, Smarajit Maiti¹, Asru K Sinha², Santanu Guha³

¹CMT Lab, Dept. of Biochemistry, Vidyasagar University, Midnapur

²Sinha Institute of Medical Science & Technology, Kolkata

³Dept. of Cardiology, Calcutta Medical College & Hospital, Kolkata
sbank.biochem@gmail.com

Acute myocardial infarction (AMI) is the devastating heart disease and one of the leading killer diseases in all over the world. Platelet aggregation is a normal physiological phenomenon but when it is massive due to atherosclerotic plaque rupture in the pericardial arteries and as a result call death is occurred as black patchy areas on the left ventricle of the heart surface. Aspirin is able to inhibit platelet aggregation in cardiovascular disease but it is the corner-stone therapy and fails to inhibit aggregation in AMI. Neither the use of aspirin nor the way to restore to restore the sensitivity of the platelets to AMI is currently available. We found a 11kDa environmentally stress-induced protein, dermcidin isoform-2 in the circulation of ACS and AMI. The concentration of protein was 40 folds higher in AMI than in normal individuals. It was found a heterogeneous binding sites population of the protein on the platelet surface. When 4μM of the dermcidin was incubated with normal PRP, aspirin was unable to inhibit platelet aggregation and it was found that dermcidin antibody was able to inhibit platelet aggregation in the presence of aspirin. It was also observed that the first dose of aspirin was able to produce nitric oxide (NO) but unable to inhibit platelet aggregation and the second dose of aspirin sensitized the platelets and was able to inhibit aggregation. Insulin and NO itself showed the same effect when they were used in the first dose instead of low dose aspirin. So, it might be said that a unique and specific

dose of aspirin could be able to inhibit dermcidin and we hypothesized that catalase, insulin and the low dose of aspirin might be able to neutralize the dermcidin effect and inhibit cell death in AMI and recurrence of the disease.

P-24

Identification of potential biomarkers in cow milk for detection of Sub-clinical mastitis using TMT based quantitative proteome analysis

Shveta Bathla^{1,2}, Anil Sindhu², Shivam K. Dubey¹, Preeti Rawat¹, Alka Chopra¹, Rubina Baithalu¹, Ajay Kumar Dang¹, Jai K. Kaushik¹, Sudarshan Kumar^{1*}, Ashok K. Mohanty^{1*}

¹National Dairy Research Institute, Karnal 132001, India

²Deenbandhu Chhotu Ram University of Science and Technology, Sonepat 131001, India

*ashokmohanty1@gmail.com, kumarsudarshan@gmail.com

Mastitis involves inflammation of the mammary gland primarily due to infection by pathogenic microbes that enter via the teat canal. It effects animal health that affects milk production causing huge economic losses to the farmers. Host-pathogen interaction leads to activation of immunological responses leading to altered expression of proteins in milk. Although, a number of proteins are known to be associated with sub-clinical mastitis but their applicability as diagnostics marker is elusive needing identification of unique protein biomarkers for early prediction of infection. In the present study, we have used TMT labeled MS based approaches to identify potential biomarkers of SCM.

To identify the relative changes in whey proteins between normal and affected cows at sub-clinical stage, milk was collected from individual quarters (n=98) of 25 Karan Fries cows. Conventional test such as CMT, SCC and EC was done to categorized milk into Normal, SCM and CM conditions. To identify the DEPs in whey, milk was defatted and ultra-centrifuged. Tryptic peptides were generated and labeled with TMT tags followed by their fractionation, desalting and n-LC coupled MS (ESI-qTOF) to acquire MS data in DDA mode. DEPs were identified by Protein Scape and further analyzed by Cytoscape software to look for specific molecular targets and pathways.

A total of 2,293 proteins were identified out of which 221 proteins were differentially expressed. Out of 221 DEPs, 83 proteins were significantly up-

regulated and 22 proteins were down regulated having fold change ≥ 1.5 and ≤ 0.8 respectively. Top 15 proteins (TRPM4, GCLC, PYGB, BMI1, CHI3L1, PCNT etc) with fold change (≥ 3) were selected as potential targets for their evaluation as a potential biomarkers. The PPI analysis of DEPs revealed major protein hubs which are actively involved in immune responses, regulation of anti-inflammatory pathways, phagocytosis and homeostatic responses for prolonged survival of host.

We report for the first time the highest number of proteins in milk whey. We have also identified novel targets for their further evaluation as potential biomarkers of SCM.

P-25

Elucidation of Protein Markers for Detection of Pathogenic Bacteria Using Tandem Mass Spectrometry

Sakshi Rajoria, Ravi Bhushan Kumar and Syed ImteyazAlam*

*Biotechnology Division, Defence Research and Development Establishment, Defence Research and Development Organization, Gwalior, Madhya Pradesh, India
Email: syimteyaz@gmail.com*

Some pathogens and toxins have the potential to be used as weapons of mass destruction and instigate population-based fear. In the prevailing scenario of bioterrorism, the use of biological agents can be through an overt or covert means to cause harm for ideological, political, or financial gain. Rapid, sensitive, and unambiguous identification of biological agents is of paramount importance for confirmation of the event and to mitigate the direct and indirect damages to public health and resources. The present investigation proposes methodologies for unambiguous detection using tandem mass spectrometry, in a biological emergency. Protein markers have been elucidated for specific detection of bacterial select agents in the taxonomic group of Alpha-Proteobacteria, Beta-Proteobacteria, and Firmicutes through literature mining and *in silico* tools. Proteotypic peptides form the selected candidate marker proteins were populated in the inclusion list for targeted search approach for pathogen identification employing MALDI-TOF-TOF instrument. Agents recovered from environmental matrices were shown to be amenable to detection by a downstream shot gun tandem mass spectrometric analysis. Applicability of the proposed methodology was demonstrated in validation experiments for the detection of toxin and bacterial agents. This novel

directed search approach using pathogen specific unique peptides alongside shot gun identification of proteins with sequence information is of immense value in unambiguous detection biological agents in biological emergencies and beyond.

P-26

Role of Proteomic studies in identification and characterization of *Mycobacterium tuberculosis* strains

Pranavi Sreeramoju¹, Malathi Jojula¹, Lavanya Poonem¹, Chakrapani Chatala², Surya Prakash²

¹ Dept of Microbiology Sri Shivani College of Pharmacy, Warangal, Telangana, pranisreeramoju@gmail.com

² Novita Health N Techs, Hyderabad, Telangana, chatlachakri@gmail.com

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is one of the important diseases in public health. According to World Health Organization in 2015, 10.4 million people developed TB and 1.8 million died from the disease, out of these 0.4 million people were co-infected with TB and HIV. It was considered that proteins, lipids, lipoglycans, peptidoglycans and enzymes of the cell wall are the signaling, effector molecules in the disease process. The aim of the study is to identify and characterize the *Mycobacterium tuberculosis* strains using proteomics. Clinical samples were collected from the suspects attending to District Tuberculosis Centre (DTC), Warangal attached to Mahatma Gandhi Memorial (MGM) hospital and identified based on smear microscopy and culture. Two-dimensional gel electrophoresis (2DE) along with mass spectrometry (MS) is a powerful and effective tool for identification and characterization of *Mycobacterium tuberculosis*. Two-Dimensional Gel Electrophoresis and Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry were used for diagnosis and comparison of proteins. 1 MTB strain, 1 MDR-TB strain and H37rv control were used for the study. MTB and MDR-TB strains were isolated from the patient's sputum sample and were detected, identified and characterized based on phenotypic and genotypic variations. We were able to identify more than 800 proteins by mass spectrometry of which some are new and novel proteins. Proteomic studies resulted in the identification of different proteins which was supporting for new strain detection.

A novel protein of *Mycobacterium tuberculosis* helps to develop biomarkers for early diagnosis of tuberculosis among diabetics

**Sireesha Tanniru¹, Asha Syed¹,
Divya Murahari² and Malathi Jojula^{2*}**

¹ Dept. of Bio-Technology, Vignan University, Vadlamudi, India

² Dept. of Microbiology, SSCP, Warangal, India
sai848@gmail.com; jojulamalathi@yahoo.co.in

Diabetes Mellitus (DM) is a chronic metabolic disorder; it is a risk factor for developing of Tuberculosis (TB). DM causes immune dysfunction and moderate suppression of the immune system. *Mycobacterium tuberculosis* is very important pathogen leads to the prevalence of drug resistance such as MDR & XDR. Protein is a *meta*-cleavage product (MCP) hydrolase that catalyzes the hydrolytic cleavage of a carbon-carbon bond in cholesterol metabolism. Aim of our study to develop new biomarkers for early diagnosis of TB among diabetes for the community. Study was carried out in Warangal district from 2013-2016 and diagnosed 55 TB cases among diabetes and reported based on smear microscopy, culture and DST. Primers were designed and sequence of the protein confirmed by protein blast (NCBI). Protein detection (QIAGEN Protein extraction kit)and protein identification was carried in SSCP lab.10 clinical isolates of mycobacterium tuberculosis identified based on the phenotypic and genotypic variations were used for plotting the growth curve and identification of different proteins released during the growth. Growth was reported periodically from the day of inoculation in liquid media that is 3rd, 5th, 9th, 15th and 21st and protein detection was followed as same using different protein detection kits, protein expression was more during the 15th and 21st day of inoculation. All 10 isolates were sequenced; Protein blast and protein identification was done. Novel cell wall proteins were identified by complete protein profiling which expressed during growth. New protein is a critical for Cholesterol metabolism which represents a novel target for anti-tuberculosis therapy among diabetic - TB co-infections.

iTRAQ based relative quantification of proteins in cow urine for identification of early pregnancy biomarker

**Arvind K Verma, Preeti Rawat, Shveta Bathla,
Munnalal Yadav, Suman Choudhary,
Syed Azmal Ali, Sudarshan Kumar,
Rubina Kumari, Jai K Kaushik, Tushar K Mohanty
and Ashok K Mohanty***

*Animal Biotechnology Centre
National Dairy Research Institute, Karnal
ashokmohanty1@gmail.com*

An early and precise pregnancy diagnosis is an important criterion for better reproductive management in livestock. Early pregnancy diagnosis in cow is crucial to shorten the calving interval by identification of non-pregnant animals at the earliest opportunity. For pregnancy diagnosis, urine is a desirable source because of its convenience for collection in large amount. On conception, numerous signals are produced in cow during early pregnancy leading to changes in expression of biomolecules such as hormones and proteins. These molecules may be of fetal-placental origin rather than of maternal origin which are essential for successful establishment of pregnancy. Till date no protein biomarker is known which can be used to detect early pregnancy in cattle. In the present report, we employed iTRAQ based quantitative proteomics approach to identify putative urinary biomarkers which may have diagnostic potential for early detection of pregnancy in cows. Urine was collected from control (non-pregnant=6) and pregnant animals (n=6) on successive days of pregnancy on days 0 (day of AI), 16, 22, 35 post AI. The urine was diafiltered and concentrated using 3 kDa hollow fiber cartridge. The tryptic peptides were prepared from diafiltered urine and labeled with iTRAQ tags, fractionated, desalting and subjected to nLC-ESI-qTOF. Proteins were identified using Mascot search algorithm that revealed 403 proteins out of which 33 were differentially expressed proteins (DEPs). The gene ontology studies of DEPs using Panther software revealed that a majority of proteins are actively involved in catalytic activities, binding and enzyme regulatory activities. Some of the DEPs including MBP, SERPIN, IGF, KNG1, ITIH4 etc. which were further subjected to ClueGo pathway analysis tool that showed involvement of proteins in successful embryo implantation, establishment and maintenance of pregnancy.

RNA-seq analysis revealed host-specific immune response during *Fusarium* infection

Pooja R. Aggarwal*, Sudip Ghosh*, Kanika Narula,
Rajul Tayal, Papri Nag, Niranjan Chakraborty,
Subhra Chakraborty*

*National Institute of Plant Genome Research, Aruna Asaf Ali Marg,
New Delhi-110067, India
aggarwal.pooja28@yahoo.com; sudip_ghosh@nipgr.ac.in*
*Both are presenting authors

Although precisely controlled innate immune response is governed by conserved cellular events in phylogenetically diverse hosts, the underlying molecular mechanisms by which this process is regulated against multi-host pathogen remains unknown. *Fusarium oxysporum* is known to be associated with neuronal stress in animals and vascular wilt in plants. We performed temporal transcriptome analysis of chickpea and worm infected with *Fusarium* to better understand gene expression switches in host specific disease/immune response. Integrative gene-regulatory network elucidated tangible insight into interaction coordinators leading to pathway determination governing distinct phenotypes. Global network analysis identified prognostic genes related to disease progression. These genes were exhibited diverse biological functions, including signal perception, cellular homeostasis, metabolic remodeling and stress response. Overall, our results indicate that a ubiquitous response occurs during *Fusarium* infection mediated by highly conserved regulatory components and pathways, which can be exploited further for the identification of disease responsive genes conserved among animal and plant. Finally, our study provides novel insight on cross-species immune signaling associated with fungal infection.

Extracellular matrix associated proteome changes during vascular wilt caused by *Fusariumoxysporum* in chickpea

Arunima Sinha^a, Eman Elagamey^{a,b},
Kanika Narula^a, Sudip Ghosh^a,
Magdi A.E. Abdellatef^{a,b}, Niranjan Chakraborty^a,
Subhra Chakraborty^a

^a*National Institute of Plant Genome Research,
Aruna Asaf Ali Marg, New Delhi-110067, India*
^b*Present Address: Plant Pathology Research Institute, Agricultural Research Center (ARC), 9 Gamaast., Giza 12619, Egypt
arunima35@gmail.com*

Extracellular matrix (ECM) is the unique organelle that perceives stress signals and reprograms molecular events of host cell during patho-stress. Vascular wilt caused by *Fusariumoxysporum* is a major impediment for global crop productivity. To elucidate the role of ECM proteins, the temporal changes of ECM proteome was studied in vascular wilt resistant chickpea cultivar upon *F. oxysporum* infection. The 2-DE protein profiling coupled with mass spectrometric analysis identified 166 differentially expressed proteins involved in variety of functions. Our data suggest that wall remodelling, protein translocation, stabilization and chitin triggered immunity are major players in ECM signalling during fungal attack. Furthermore, we interrogated the proteome data using network analysis that identified modules enriched in known and novel immunity-related prognostic proteins. This study for the first time provides an insight into the complex network operating in the ECM and impinges on the surveillance mechanism of innate immunity during patho-stress in crop plant.

Universal stress protein (Rv2005c) of *M. tuberculosis*: Potential involvement in virulence and aminoglycosides resistance

Divakar Sharma^{1,2*}, Manju Lata¹,
Mohammad Faheem², Asad Ullah Khan²,
Beenu Joshi³, Krishnamurthy Venkatesan¹,
Sangeeta Shukla⁴ and Deepa Bisht^{1*}

¹ Department of Biochemistry, National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Tajganj, Agra, 282004, India

² Interdisciplinary Biotechnology Unit, Aligarh Muslim University Aligarh, 202002, India

³ Department of Immunology, National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Tajganj, Agra, 282004, India

⁴ School of Studies (SOS) Zoology, Jiwaji University, Gwalior, India

*divakarsharma88@gmail.com

Tuberculosis is an airborne infectious disease caused by *Mycobacterium tuberculosis* which threatens the globe. Aminoglycosides {Amikacin (AK) & Kanamycin (KM)} are second line anti-TB drugs used against the treatment of drug resistant tuberculosis and targets the steps of protein translation machinery. Several mechanisms have been proposed to elucidate the phenomena of aminoglycosides resistance but still our knowledge is insufficient. Universal stress protein (Rv2005c) was found to be overexpressed in aminoglycosides resistant isolates. Universal stress protein (Rv2005c) in *M.tuberculosis* plays a crucial role in growth, virulence, stress, latency and drug resistance. To establish the relationship of universal stress protein Rv2005c with AK & KM resistance, Rv2005c was cloned, expressed in *E.coli* BL21 using pQE2 expression vector and antimicrobial drug susceptibility testing (DST) was carried out. DST showed that the minimum inhibitory concentration (MIC) of Rv2005c recombinant cells were five and four folds shifted with AK and KM E-strips respectively. Overexpression of Rv2005c leads to shift in MIC which might be signifying its involvement in the survival of mycobacteria by inhibiting/modulating the effects of AK & KM released from the E-strips. Interactome also suggested that Rv2005c and its interacting protein partners might be cumulatively involved in *M.tuberculosis* virulence, resistance, stress or latency.

Proteomic analysis of oral cancer patients delineated by tobacco usage habits

Firdous Ahmad Bhat^{1,2}, Sonali V Mohan^{1,3},
Keshav K Datta⁴, Kiran M Kumar^{1,2}
Vinuth N. Puttamallesh^{1,2},
Mohd Younis Bhat^{1,2}, Jayshree Advani^{1,3},
Pavithra Rajagopalan^{1,5}, Arnab Pal⁶,
Samapika Routray⁷, T.S. Keshava Prasad^{1,4},
Bipin Nair², Mandakulutur S. Ganesh⁸,
Jay Gopal Ray⁹, Harsha Gowda^{1,7} and
Aditi Chatterjee^{1,*}

¹ Institute of Bioinformatics, International Technology Park, Bangalore 560066, India; ² Amrita School of Biotechnology, Amrita University, Kollam 690 525, India; ³ Manipal University, Madhav Nagar, Manipal 576104, India; ⁴ YU-IOB Center for Systems Biology and Molecular Medicine, Yenepoya University, Mangalore 575018, India;

⁵ School of Biotechnology, KIIT University, Bhubaneswar 751024, India; ⁶ Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh, 160012, India; ⁷ Department of Dental Surgery, All India Institute of Medical Sciences, Bhubaneswar-751019, India; ⁸ Department of Surgical Oncology, Vydehi Institute of Oncology, Bangalore 560 066, India ⁹ Department of Oral Pathology, Dr. R. Ahmed Dental College & Hospital, Kolkata, 700 014, India

Tobacco usage is a known risk factor associated with development of oral cancer. It is mainly consumed in two different forms (smoking and chewing) that vary in their composition and methods of intake. India has one of the highest rates of oral cancer in the world. Despite being the leading cause of oral cancer, molecular alterations induced by tobacco are poorly understood. There are limited number of studies which have investigated the effects of chewing tobacco on oral epithelia. In this study we studied the proteomic alterations in oral cancer patients based on their tobacco using habits (tobacco chewers and those with no habits). Proteomic analysis of oral cancer primary tissue samples (from chewing tobacco users and those with no habits) was carried out using tandem mass tags (TMT) labelling strategy using high-resolution Fourier transform Orbitrap Fusion mass spectrometer. This led to the quantification of 3,139 proteins. 355 proteins were found dysregulated, of which 147 and 63 proteins were dysregulated exclusively in tobacco chewers and those with no habits respectively (≥ 1.5 fold and $p\text{-value} \leq 0.05$). Pathway analysis of the proteins which were overexpressed in chewers revealed that a large majority of them to be involved in cell cycle, protein synthesis, cellular growth and proliferation. Similarly, pathway analysis of the proteins which were down regulated revealed that they are involved in mitochondrial dysfunction and oxidative

phosphorylation. This study can serve as a scaffold to understand the molecular alterations in oral cancer based on tobacco usage habit of a patient and enable in the identification of early detection markers to identify high risk population.

P-33

Proteomic study of Endothelial Dysfunction in Response to Homocysteinylated Albumin

**Reema Banarjee, Akshay Sharma, Shakuntala Bai,
Arati Deshmukh, Mahesh Kulkarni***

*CSIR-National Chemical Laboratory, Pune
mj.kulkarni@ncl.res.in*

Homocysteine (Hcy) is a non-protein amino acid and a structural analogue of cysteine, formed as an intermediate during methionine biosynthesis. Elevated levels of homocysteine in blood or hyperhomocysteinemia, can result from a deficiency of folic acid, vitamin B12 or vitamin B6 and has been associated as a risk factor with cardiovascular and neurological diseases. It gets converted to its cyclic analogue, homocysteine thiolactone (Hct), by methionyl-tRNA synthetase during the process of translation. Hct is highly reactive and can react with the free amino groups of proteins forming an N-homocysteinylated product. The chemically modified protein has altered structure and function and can be deleterious to cells. Accumulation of such chemically modified proteins in plasma could be one of the possible mechanisms for the development of cardiovascular complications in patients with hyperhomocysteinemia. To investigate this, we synthesized homocysteinylated albumin *in vitro* via reaction of human serum albumin (HSA) with homocysteine thiolactone. Since endothelial dysfunction, or loss of regulatory functions of vascular endothelial cells, is one of the initial steps in the development of cardiovascular disease, we have studied the effect of homocysteinylated albumin on the viability and function of Human Umbilical Vein Endothelial Cells (HUVECs) in culture. Further, differential proteomic analysis in these cells was performed by SWATH. Treatment of HUVECs with homocysteinylated albumin for 24h negatively affected cell viability. Also, SWATH analysis showed the abundance of about 53 proteins, out of the total 1100 identified, to be altered in homocysteinylated HSA treated cells as compared to those treated with control unmodified HSA. Thus, homocysteinylated protein

can lead to dysfunction in vascular endothelial cells and can increase risk of cardiovascular complications in hyperhomocysteinemia.

P-34

Elucidation of molecular mechanisms of an active lead against *Candida albicans* yeast – hyphal transition assisted biofilm formation through proteomic approaches

**Krishnan Ganesh Prasath, Sivasamy Sethupathy,
Shunmugiah Karutha Pandian***

*Department of Biotechnology, Science Campus, Alagappa University,
Karaikudi-630003, India*

**sk_pandian@rediffmail.com; ganeshprasathk@gmail.com*

Candida spp. are small yeast like fungi that reproduce by budding through chlamydospores. They are commensals found in human gut flora, mucosal surface, skin, and genitals that turned to be an opportunistic pathogen in humans during immunocompromised conditions. Recent study amongst 150 species of *Candida* spp. revealed that 50 % to 70 % of invasive candidiasis was caused by *Candida albicans* (*C. albicans*) followed by non-albicans species such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*. *C. albicans* is dimorphic opportunistic pathogen that grows as unicellular budding yeast and filamentous hyphae or pseudohyphae cells. It has been reported that yeast form of *C. albicans* are avirulent in nature and their transition to hyphal form along with biofilm formation, sterol synthesis, secreted aspartyl protease, lipase formation are the key factors for *C. albicans* pathogenicity. An active lead from a plant extract was found to reduce the formation of *C. albicans* biofilm (82 %) without affecting the growth at 125 µg mL⁻¹ which is taken as Biofilm Inhibitory Concentration (BIC). Also, the BIC of active lead potentially inhibit the production of other virulence factors such as yeast-hyphal transition, sterol synthesis, mature biofilm, extracellular polymeric substance (EPS) and lipase formation. The intracellular proteome of untreated and active lead treated *C. albicans* was separated in gradient SDS-PAGE and it was found that differential expression was not perfectly visualized due to the cellular complexity of *C. albicans*. Hence, 2-D electrophoresis (2-DE) was done and differential expression of the proteins were observed. 801 protein spots were detected and spots with fold difference > 1.5 were picked for MALDI-TOF/TOF analysis for the identification of differentially regulated proteins. This analysis will decipher the molecular mechanisms

behind the anti-biofilm, anti-virulence activity of the active lead and their ability to prevent the yeast to hyphal transition.

P-35

Identification of the molecular mechanism of the anti-biofilm agent against the quorum sensing mediated biofilm formation and virulence factors of the nosocomial human pathogen *Acinetobacter baumannii* through proteomic approaches

**Anthonymuthu Selvaraj,
Shunmugiah Karutha Pandian***

Department of Biotechnology, Alagappa University, Science Campus, Karaikudi-630 003, Tamil Nadu, India.

* sk_pandian@rediffmail.com

Acinetobacter baumannii is an opportunistic human pathogen that causes nosocomial infections owing to multi-drug resistance towards the most conventional antibiotics. Quorum sensing (QS) mediated biofilm formation plays an important role in persistence and resistance of *A. baumannii*. The current study aims to analyze the QS mediated inhibition of biofilm formation and virulence factors of *A. baumannii* when treated with the monoterpene compound through proteomic approaches. 200 μ g ml⁻¹ of the monoterpene compound was found to effectively inhibit the biofilm formation of *A. baumannii* without affecting its growth and hence potentially limiting the possibility of the bacterium attaining resistance towards the anti-biofilm agent. Microscopic analysis and crystal violet assay revealed the anti-biofilm activity of the bioactive compound. The result of colony forming unit assay, growth curve and Alamar blue assay confirmed the non-antibacterial nature of bioactive compound. In addition, the bioactive compound reduced the production of extracellular polysaccharide, lipase, motility and cell surface hydrophobicity of *A. baumannii*. Furthermore, other QS based virulence factors such as catalase and superoxide dismutase production were also reduced by the bioactive compound, thereby making *A. baumannii* more susceptible to the healthy human blood and antibiotics. In order to identify the molecular mechanism of the anti-biofilm compound, total proteome was extracted from the control and treated bacterial cells. Differentially expressed proteins (N=108) of *A. baumannii* upon treatment with the bioactive compound were analyzed through 2D gel electrophoresis(2DE) and Image Master 2D Platinum 7 software (GE Health

care). Further, MALDI and Nano LC-MS analysis will throw more lights on the molecular mechanism of anti-biofilm agent.

P-36

Study of physiology and proteome alterations in *Caenorhabditis elegans* during *Salmonella enterica* Serovar Typhi exposure

**Boopathi Balasubramaniam^a and
Krishnaswamy Balamurugan^{a*}**

^a Department of Biotechnology, Science Campus, Alagappa University, Karaikudi, Tamil Nadu, India-630 003

*bsuryar@yahoo.com

Enteric fever persists as a significant prevalent bacterial infection caused by *Salmonella enterica* Serovar Typhi throughout the world. The mode of transmission is due to ingestion of contaminated water or food or urine of infected carriers. Among the existing model systems, *C. elegans* remains a much-admired eukaryotic model organism to study the host pathogen interactions at both genomic and proteomic levels. The objective of the current study is to explore the physiology and proteomic alterations in the host during *S. Typhi* infection. The liquid survival assay has been done which showed the complete mortality at 132h±2h in the *S. Typhi* treated worms. The microscopic analysis has evidenced the *in vivo* colonization of *S. Typhi* throughout the intestinal region of *C. elegans* during different time points. The pharyngeal pumping rates of *S. Typhi* infected worms were varied over the different time course of infection and completely arrested at 132 h. The chemotaxis assay results disclosed that *C. elegans* did not avoid *S. Typhi* in late hours after 48 h and moved easily without any interference over the NGM plates spotted with *S. Typhi* and *E. coli* OP50. Furthermore, SDS-PAGE profiling of control and treated protein samples from worms clearly revealed that a number of proteins were differentially regulated during various time points. In addition, the control and pathogen exposed *C. elegans* protein samples were fractionated using liquid phase IEF (Micrortofor) and they were subjected for SDS-PAGE analysis. The profiling of the liquid phase IEF fractionated samples have revealed that crucial proteins were differentially regulated based on their pI. Altogether, the data proposed that exposure of *S. Typhi* elicits infection in the host, *C. elegans* by affecting its normal physiological and also molecular parameters.

Pane of candidate genes, highlights an affirmative path into invasion and metastasis of Oral Cancer by means of an integrated approach!

Samapika Routray

All India Institute of Medical Sciences, Bhubaneswar, Odisha

Oral Cancer is well known for its 5 year survival rate and poor prognosis. The prediction of candidate genes to understand invasion and metastasis has become an important task in current biological research.

A preliminary search for candidate gene (emphasizing on their role in invasion and metastasis) from online oral cancer gene database (OrCGDB) was conducted and four genes were located. We used the database STRING ('Search Tool for the Retrieval of Interacting Genes/Proteins') in the current study to understand the interactions of our candidate proteins and Oncomine database for pathological and clinical data of these genes in different stages of oral cancer. A thorough pathway analysis and the scrutiny of the literature showed that these genes had no previous record of linking them together for aggressive behaviour of oral cancer. We further analysed archived FFPE blocks of various grades of oral cancer using iTRAQ-based mass spectrometry for its presence and role of the same. Validation of our pane of candidate genes using immunohistochemistry (IHC) finally established the outcome.

Results of these study expressed a strong communiqué and interrelationship between these candidate genes. A hypothetical pathway analysis led us to propose a role for the identified genes in invasion and metastasis in OSCC.

Our study focuses on the corroborative and affirmative path of a pane of candidate genes expression in various degrees of differentiation associating it to oral cancer aggression, hoping to further pave the way towards better outcome and less overtreatment.

Protein marker signatures in plasma, serum and urine for early diagnosis of epsilon toxin exposure

**Prabhakar Babele, Smarti Verma,
Ravi Bhushan Kumar, Bhoj Kumar,
Syed Imteyaz Alam***

*Biotechnology Division, Defence Research and Development Establishment, Defence Research and Development Organization, Gwalior, Madhya Pradesh, India
syimteyaz@gmail.com

Epsilon toxin (ETX) is an extremely potent pore-forming toxin produced by *Clostridium perfringens* and a category B biological agent. ETX is a major virulence determinant of toxinotypes B and D of this medically and militarily important pathogen and is implicated in pathogenesis of rapidly fatal pulpy kidney disease in lambs caused by toxinotype D. In the prevailing scenario of bioterrorism, early diagnosis of ETX exposure is of paramount importance for implementation of timely medical countermeasures. As the toxin is lethal and acts rapidly, it is hard to diagnose intoxication using classical sero-diagnostic tools. Owing to its low lethal dose and rapid binding with receptors in the target organs, the detection of circulating toxin is extremely difficult even with sensitive immunoassays. Host protein marker signatures in plasma, serum or urine can be the plausible answer for early diagnosis of ETX exposure. The present study describes a two dimensional gel electrophoresis - mass spectrometry (2DE/MS) approach to elucidate the host marker signatures following intravenous injection of toxin in mouse model. In total, 82 proteins were identified differentially expressed either in plasma, serum or urine. Fibrinogen, kininogen and apolipoprotein A1 are among the proteins showing significant alteration in abundance in all the three proximal fluids indicating perturbations in hemostasis and lipid transport. Differentially expressed proteins in plasma/serum also included those involved in blood coagulation, hemoglobin transport and oxidative stress while calcium and iron homeostasis related proteins exhibited differential abundance in the urine samples. To the best of our knowledge this study provides the first differential proteomic profile of host response in plasma, serum and urine against intravenous ETX exposure, thus providing clues to mechanism of toxicity and potential diagnostic tools.

Unveiling the Pleiotropy of Leukemia Inhibitory Factor (LIF) Through Label Free Quantitative Proteomics approach

Sudarshan Kumar*, Syed Azmal Ali,
Ashok Kumar Mohanty,

Proteomics and Cell Biology Lab, Animal Biotechnology Center,
National Dairy Research Institute, Haryana, India
kumarsudarshan@gmail.com

Leukemia inhibitory factor (LIF) is a ubiquitous, multifunctional, hyper glycosylated cytokine protein belonging to IL-6 class of interleukins. It has been implicated in a variety of functions in different organs including proliferation, differentiation and survival. For example, in stem cells it supports pluripotency, in uterus its helps in implantation, in leukemia cell, it helps in differentiation etc. The molecular mechanism behind all these diverse functions downstream to LIF is a poorly understood subject. In this work, we have attempted to examine the role of BuLIF in fibroblast cells and explain signalling pathways leading to its growth inhibitory effects. A stably transfected cell line COS-1_BuLIF_GFP was developed which over-expressed BuLIF_GFP in the secreted medium. The biological activity of BuLIF was examined as dome like formation in COS-1 cells which was found to be mediated through stat3 phosphorylation. Later, the BuLIF protein was purified to homogeneity by CNBr coupled antiGFP pull down assay and tested on M1 myeloid Leukemia Cells which turned out to be growth inhibitory. To unravel the active signalling pathways and determination of non-canonical pathways downstream to BuLIF, Label free quantitative Proteomics was performed in high-resolution nLC-MS/MS followed by deep bioinformatics analysis on Cytoscape. The MS/MS data identified 2083 proteins with 231 differentially expressed proteins which subsequently, revealed the LIF-mediated changes in the signalling molecules resulting in activation of multiple pathways including MEK/ERK, Ras, mTOR, Hippo, and RAP1 pathways apart from three well known PIP3, STAT3 and MAPK pathways. The functional validation of the DEGs has been confirmed by different assays such as BrdU,MTT, Caspase 3/7, western blot and qRT-PCR. Also, the molecular weight of LIF with and without glycosylation was identified as 58.99 kDa and 48.9 kDa respectively. The purified LIF showed maximum inhibition at 72 hours. Finally, we conclude that BuLIF is involved in perturbation of many signalling pathways eventually culminating into reduction in proliferation without any effect on

apoptosis. These identified effectors downstream to BuLIF could be important targets in various diseases such as neurodegenerative diseases, cancer and infertility in a context dependent manner.

Proteomic approach to decipher *In vivo* antidiabetic potential of an Ayurvedic formulation

**Sharad Pawar^{#1}, Amey Shirolkar¹,
Shridhar Chougule¹, Sharayu Rajadhyaksha²,
Sudesh Gaidhani³, Vasant Narke²**

¹Department of Pharmacology, Regional Ayurveda Institute for Fundamental Research (RAIFR), Kothrud, Pune-411038;

²TOXINIDA, Bibwewadi, Pune-412303

³Central Council for Research in Ayurvedic Sciences (CCRS)
Ministry of AYUSH, Govt. of India Janakpuri, New Delhi-110058
sd_pawar@yahoo.com

The LC-MS based qualitative bottom-up proteomic approach has incredible applicability in characterization at organismal proteome level. In this study, antidiabetic action of hydroalcoholic extract of an ayurvedic formulation was traced through proteomics profiling in an animal experimentation model. The study groups were composed of healthy control, vehicle control, Glibenclamide standard treated, extract 250mg/kg and 500mg/kg body weight orally treated categories. The blood samples were collected after 28 days of treatment and used to obtain serum. The serum proteins from rats were digested in-solution and subjected to mass spectrometric analysis. The multivariate statistical analysis for pre-post treatment comparison yielded 19 significant proteins. While, within above mentioned five groups comparison yielded 37 significant proteins. Highly confident R² and Q² values were observed in Partial Least square discriminant analysis (PLS-DA). The drug was found to impact Insulin based carbohydrate metabolism, oxidative stress, blood coagulation, haemoglobin metabolism, nervous and blood vessels development related proteins and pathways. DENN domain-containing protein 4C, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2, MAX gene-associated protein, probable E3 ubiquitin-protein ligase MYCBP2, receptor-type tyrosine-protein phosphatase F, nuclear receptor coactivator 6, alpha-2-HS-glycoprotein precursor and alpha-2-HS-glycoprotein precursor proteins which are closely associated with GLUT-4 based glucose transportation and insulin metabolism were observed. Serine-protein kinase ATM, polyribonucleotide nucleotidyltransferase 1,

mitochondrial and CLIP-associating protein 1 that are associated with oxidative-nitrosative stress and cellular senescence, were identified. Microtubule-actin cross-linking factor 1, Serotransferrin precursor, porphobilinogen deaminase, Phosphorylase b kinase regulatory subunit beta, A-kinase anchor protein 13, glutamine-fructose-6-phosphate transaminase 2 and axin-2 were some of other expressed proteins involved in significant functions.

P-41

Proteomic alterations in rat brain during chronic sarin intoxication: elucidation of major mechanism

Kalyani Chaubey*, Syed Imteyaz Alam*,
D. P. Nagar*, Chandra Kant Waghmare*,
S. C. Pant*, Lokendra Singh*, Nalini Srivastava†,
and Bijoy K. Bhattacharya*

* Defence Research & Development Establishment (DRDE), Jhansi Road, Gwalior (MP), 474002, India

† School of studies in Biochemistry, Jiwaji University, Gwalior (MP), 474002, India
bio.kalyani@gmail.com

Sarin is an organophosphorus (OP) chemical warfare agent which irreversibly inhibits acetylcholinesterase. Acute toxicity after sarin exposure is due to hyper activation of the nicotinic and muscarinic receptor leading to cholinergic symptoms like lacrimation, salivation, seizures, fasciculation, tremor and hypothermia. Survivors of acute toxicity often develop long term neuropathology referred as organophosphorus ester induced chronic neurotoxicity (OPICN). It may develop either by single large dose or by multiple small doses of OP compounds leading to chronic neurobehavioral alterations. Neuronal and neurobehavioral abnormalities were observed as a result of chronic effects among survivors. Pathogenic processes and mechanism of chronic neuro-toxicity is yet unknown and this is a standing goal of a researcher. For this, we studied proteomic and phosphoproteomic changes in rat brain regions after single 0.5 LD₅₀ dose of sarin and investigated some milestone changes associated with long term CNS injury. Plasma and brain cholinesterase activities were determined after sarin administration. We further used 2DE-MS approach to identify early proteomic and phosphoproteomic changes and traced expression of selected proteins for longer time points. This study showed changes in the proteins related to chaperone function, ER stress and cytoskeleton functions at an earlier stage. Predictive interactions analysis of all

differentially expressed proteins demonstrated the putative role of Parkinson's disease related proteins and ER stress related phosphoproteins in post sarin exposure. Some of the changes at an earlier stage, including those involved in neurodegeneration, movement and cognitive function, defects in chaperone function, cytoskeleton function, mitochondrial dysfunction and endoplasmic reticulum (ER) stress were shown to persist for a longer period. This is the first proteomic study among the survivors of sarin exposure in animal model. This study provides a preliminary framework for further validation of the major mechanisms of sarin toxicity suggested here, which may open new avenues for elucidation of therapeutic intervention.

P-42

Label free quantitative approach to identify possible novel therapeutic targets for PVR (Proliferative vitreoretinopathy).

Sumit Sharma^a, Maryada Sharma^a, Deeksha Katoch^b, Reema Bansal^b, Mangat R. Dogra^b, Manni Luthra Guptasarma^a

^a Department of Immunopathology, ^bDepartment of Ophthalmology Postgraduate Institute of Medical Education and Research, Chandigarh -160012.

^amguptasarma@yahoo.com ;
Sumit.pgimer@gmail.com

Proliferative vitreoretinopathy is an ocular fibrotic disorder that occurs in 5-10% of patients undergoing surgery for rhegmatogenous retinal detachment. Despite advancements in the surgical techniques, PVR remains a major obstacle to successful retinal reattachment surgery. Therefore, there is an urgent need to identify novel targets which can be of therapeutic importance to prevent this disorder.

Retinal pigment epithelial cells (ARPE 19) were treated with vitreous and sub retinal fluid samples derived either from patients undergoing retinal reattachment surgery or with vitreous derived from cadaver eyes and cultured for 12h at 37°C and 5% CO₂. Patient derived vitreous resulting in PVR-like morphological features, were labelled as Test PVS, while those resulting in non-PVR like features were labelled as Control PVS. ARPE 19 cells treated with cadaver-derived vitreous were labelled as CAD. Cells from all cultures were pelleted, followed by lysis, and subjected to reduction, alkylation and trypsinization. Sample processing was followed by identification of

whole cell proteome and comparisons between each of them was done using label free quantitative approach. All the experiments were done using EASY-nLC 1000 system coupled to Q-Exactive mass spectrometer equipped with nanoelectrospray ion source. Differentially expressed proteins were subjected to pathway enrichment using STRINGs and DAVID.

It was observed that Ca²⁺/calmodulin dependent protein kinase II or CaMKII was expressed 50-fold higher in TPVS-treated cells compared to cadaver vitreous treated cells; when TPVS and CPVS treated cells were compared, it was seen that the expression was 2.4-fold higher in TPVS vs CPVS treated cells.

Ca²⁺signaling may play an important role in the fibrotic phenotype in TPVS-treated cells, and it is therefore proposed as a possible therapeutic target in the context of PVR.

P-43

Molecular dissection of extracellular matrix proteome reveals discrete mechanism regulating *Verticilliumdahliae* triggered vascular wilt disease in potato

Eman Elagamey^{a,b}, Arunima Sinha^a, Kanika Narula^a, Magdi A.E. Abdellatef^{a,b}, Niranjan Chakraborty^a, Subhra Chakraborty^a

^a National Institute of Plant Genome Research,
Aruna Asaf Ali Marg, New Delhi-110067, India

^b Plant Pathology Research Institute, Agricultural Research Center (ARC), 9 Gamaast., Giza, 12619, Egypt
E-mail: emanelagamy@yahoo.com

Plants exposed to patho-stress mostly succumb due to disease by disruption of cellular integrity and changes in the composition of the extracellular matrix (ECM). Vascular wilt, caused by the soil borne hemibiotrophic filamentous fungus *Verticilliumdahliae*, is one of the most significant diseases that adversely affects plant growth and productivity. The virulence of the pathogen associated with the ECM-related susceptibility of the host plant is far from being understood. To better understand ECM-associated disease responses that allow the pathogen to suppress plant immunity, a temporal analysis of ECM proteome was carried out in vascular wilt susceptible potato cultivar upon *V. dahlia* infection. The proteome profiling led to the identification of 75 patho-stress responsive proteins (PSRPs), predominantly involved in wall hydration, architecture, and redox homeostasis.

Two novel clues regarding wilt disease of potato were gained from this study. First, wall crosslinking and salicylic acid signaling significantly altered during patho-stress. Second, generation of reactive oxygen species and scavenging proteins increased in abundance leading to cell death and necrosis of the host. We provide evidence for the first time that how fungal invasion affects the integrity of ECM components and host reprogramming for susceptibility may function at the cell surface by protein plasticity.

P-44

Identification of serum-based oral cancer biomarkers in tobacco chewers using high-resolution mass spectrometry

Mohanty V^a, Jain A^b, Pinto SM^a, Routray S^c, Mohanty N^d, Prasad TSK^{a,g}, Ganesh MS^e, Pal A^b, Ray JG^f, SubbannayyaY^a, Gowda H^{a,g}, Chatterjee A^{a,g}

^a Center for Systems Biology and Molecular Medicine, Mangalore, Karnataka 575018, India

^b Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh, 160 012, India

^c Department of Dental Surgery, All India Institute of Medical Sciences, Bhubaneswar- 751019, India

^d Department of Oral Pathology & Microbiology, Institute of Dental Sciences, Siksha O'Anusandhan University, Bhubaneswar-751 003, India

^e Department of Surgical Oncology, Vydehi Institute of Oncology, Bangalore 560 066, India

^f Department of Oral Pathology, Dr. R. Ahmed Dental College & Hospital, Kolkata, 700 014, India

^g Institute of Bioinformatics, International Tech Park, Bangalore, Karnataka 560066, India

varsham@yenepoya.edu.in; yashwanth@yenepoya.edu.in; aditi@bioinformatics.org

Oral cancer is one of the most common cancers occurring worldwide which is associated with significant mortality rate. It is ranked third among the most common cancers in India. The major risk factors for OSCC include consumption of alcohol, use of betel quid, chewing of tobacco, cigarette smoking and infection by human papilloma virus. The high incidence rate can be attributed to the use of smokeless or chewing tobacco mainly among the low socio-economic section of the society. Though it is intuitive to imagine that both smoking and chewing tobacco will have the same impact, these two forms vary in their composition of pro-carcinogenic and carcinogenic agents. Besides, the method of intake for the two forms also varies greatly. Serum-based biomarkers are of considerable importance in the early diagnosis of cancer as they are minimally-invasive in nature and

are commonly used for diagnostic purposes. In this study, we carried out TMT-based quantitative proteomic profiling where serum from oral cancer patients with chewing habits and healthy controls were analysed on Thermo Scientific Orbitrap Fusion Tribrid mass spectrometer resulting in identification about 500 proteins of which more than 50 proteins were differentially expressed. Validation in a large cohort of well classified oral cancer patient samples will result in identification of novel blood-based biomarkers for the same.

P-45

An insight into the role of glutathione in plant defense by proteomic analysis of infected *Arabidopsis thaliana* under altered GSH conditions

Priyanka Boro, Riddhi Datta¹ and Sharmila Chattopadhyay*

*Plant Biology Laboratory, CSIR-Indian Institute of Chemical Biology,
4, Raja S. C. Mullick Road, Kolkata-700032, India¹*

¹ Present address: Department of Botany, Dr. APJ Abdul Kalam Govt.
College, New Town, Kolkata
priyankaboro.92@gmail.com

Glutathione (GSH) plays an important role in plant responses during biotic stress. However, the molecular mechanism conferring this tolerance remains to be explored. In this investigation a proteomic analysis of *Arabidopsis thaliana* Col-0 and *pad2-1*, a GSH depleted mutant, in response to *Pseudomonas syringae* and *Botrytis cinerea* infection has been performed to explore the intricate position of GSH in defence against biotrophic as well as necrotrophic pathogens. Morphological analysis of control and infected plants were performed. The *pad2-1* mutant displayed severe susceptibility under infected conditions compared to the wild-type Col-0 plants thus re-establishing a fundamental role of GSH in defence. Results were noted with the up-accumulation of energy metabolism-related protein-species in both infected Col-0 and *pad2-1*, however, several defence-related protein-species were identified to be differentially accumulated. Interestingly, some salicylic acid responsive as well as ethylene responsive protein-species were significantly up-accumulated in Col-0 in comparison to *pad2-1* under *P. syringae* and *B. cinerea* infection respectively. Several other stress-related protein-species viz. PR1, PR4, HSP17, GST, NBS-LRR, LRR-RK, etc. were identified to be differentially regulated in response to infection in addition to some ethylene pathway proteins like

ACC oxidase. Hence, present investigation further confirmed that GSH is essential for the efficient activation of plant defence.

P-46

Lactobacillus rhamnosus (MTCC 1408) protects neonatal mice from salmonella mediated pathogenesis through Phenylalanine, Tryptophan biosynthesis pathway

Aman Kumar Naik^a, Raktim Mukerjee^a, Uday Pandey^a, Sohini Mukhopadhyaya^a, Debashmita Das^a, Pratikshya Ray^a, Saurabh Chawala^a, Santibhusan Senapati^b & Palok Aich^a

^a School of Biological Sciences, National Institute of Science Education and Research Bhubaneswar (NISER), PO: Bhimpur-Padampur, Via: Jatni, Khurda 752050, Odisha, India; ^b Institute of Life Sciences, Nalco Square, Chandrasekharpur, Bhubaneswar, Odisha 751023, India
aman.naik@niser.ac.in

Infection persists as one of the major causes of death in newborn children. Increasing consumption of antibiotics for infection is a major burden for the society. Antibiotic consumption not only enforces selective evolution of microorganism but also cause several diseases in adulthood. Recent developments in microbiome research have shown that probiotics can be used to treat different infectious diseases. In this study we have screened *Lactobacillus rhamnosus* (LR) (MTCC 1408) for the treatment of salmonella mediated infection. We have shown that LR treatment to the neonates rescue 85% of the Salmonella infected mice. The histopathology, gene expression, and the protein level data suggest that LR treatment to the neonates modulate the immunity towards anti-inflammatory mode which reduces the salmonella mediated inflammation. We have also shown that LR decreases gut permeability by regulating junction protein gene expressions. Salmonella count in both culture based and qRT based method proved that the total Salmonella counts LR treated mice significantly decreased from non-treated mice. NMR based metabolomics of serum further suggested that Phenylalanine-Tryptophan biosynthesis pathway is having crucial role in inducing anti-inflammatory activity. This study provides major support towards clinical intervention of probiotics in infectious diseases.

Session-3 Proteogenomics and Big Data Analysis

P-47 (Please refer O-05); P-48 (Please refer O-06)

P-49

Understanding the role of Tissue Specific Proteoforms

Anurag Raj

G. N. Ramachandran Knowledge Centre for Genome Informatics,
CSIR-Institute of Genomics and Integrative Biology, South Campus,
Sukhdev Vihar, Mathura Road, Delhi 110025
anurag.raj@igib.res.in

There are around 20,300 genes predicted in human genome. Many different analyses like RNA sequencing or microarray experiments have shown that these genes are expressed in various different tissues and more than half of them give rise to alternative splice variants. There are many chemical modifications of the protein products like genetic polymorphism, alternative splicing, post-translational modifications (PTM) and proteolysis which occur during or after translation resulting in different molecular forms of protein that vary with time, location and physiological or disease conditions. These different molecular forms of proteins are called proteoforms. There are multiple protein structure alterations present in a complex proteoform. This gives rise to a huge difference between target proteoforms and their corresponding reference sequences. The advancement in proteomics technologies has allowed us to detect and quantify proteins and their modifications with a higher precision. However, prediction from genetic or transcriptomic analyses still leave out the detection of proteoforms as their expression is restricted to time and/or position.

Proteoforms resulting from different allelic variations (mutations, indels, SNPs), alternative splicing and other pre-translational mechanisms, post-translational modifications (PTMs), conformational dynamics, and functioning have different sizes, protein–protein interactions, and protein–ligand interactions, might dramatically increase the functionality of the encoded protein. Furthermore, the proteoforms arising from a single gene copy in a given cell affect its biological function. Rather than simply studying the different mutations in the gene, the knowledge of proteoforms can better explain the disease. Consequently, understanding the mechanisms underlying the dysregulation of proteoforms by

several genomic and non-genomic mechanisms in diseases is imperative.

Session-4 Metabolomics and Integration of omics

P-50 (Please refer O-07); P-51 (Please refer O-08)

P-52

Proteo-metabolomic study illustrates role of nucleus in rice blast resistance

Kanika Narula, Pooja Choudhary,
Niranjan Chakraborty, and Subhra Chakraborty

National Institute of Plant Genome Research, Aruna Asaf Ali Marg,
New Delhi-110067, India.
gayatrinarula@yahoo.com

Nucleus is a dynamic system that regulates protein expression and serves as modulator of signaling events dictating cell fate decisions during patho-stress. Blast disease caused by hemibiotrophic fungus *Magnaporthe griseae* is a major impediment for global crop productivity. To elucidate the role of nucleus and molecular mechanism associated with cellular immunity, the temporal changes of nuclear proteome and metabolome was studied in blast resistant rice (*Oryza sativa*) cultivar upon *M. griseae* infection. The differential display of *Magnaporthe* infected resistant rice cultivar proteome revealed 215 immune-responsive proteins (IRPs) presumably associated with nucleic acid biogenesis and chromatin remodeling. Furthermore, blast-responsive metabolome profile displayed significant alteration in 165 metabolites (IRMs) associated with global metabolic pathways, particularly sugar alcohol and organic acids paralleling the proteomic analysis. Network analysis identified major protein hubs enriched in known and novel disease- and immunity-related prognostic proteins pointing towards the onset and context of disease signaling and metabolic pathway activations. Multivariate and network-based analyses successfully revealed the difference between the covariance structures of the integrated data sets. Combined analyses of multi-omics landscape of rice not only provide useful insights into the underlying mechanism of blast resistance, but also enlist novel biomarkers for targeted genetic manipulation for food and nutrition security.

Proteometabolomic landscape influences cultivar-specific acquisition of phytochemicals and source of nutrients in sweetpotato

**Shubhendu Shekhar, Divya Mishra,
Subhra Chakraborty and Niranjan Chakraborty***

National Institute of Plant Genome Research, Jawaharlal Nehru University Campus, Aruna Asaf Ali Marg, New Delhi-110067, India
shubhendu.21@gmail.com

Sweetpotato is the seventh most important food crop across the globe, and has a significant contribution to the source of nutrition and phytochemicals. Tubers of sweetpotato are used mostly for human consumption, animal feeds and industrial raw materials. Despite its significance and promise, investigation on the regulation of nutrient acquisition and availability of phytochemicals in sweetpotato remains largely unexplored. To elucidate the molecular basis for differential nutrient availability and phytochemical source of nutrition and utilize the natural genetic diversity, a series of physicochemical and proteometabolomic experiments were performed using two contrasting cultivars, an orange-fleshed sweetpotato (OFSP) and a white-fleshed sweetpotato (WFSP). Physicochemical screening demonstrated higher carbohydrate, reducing sugar and total phenolic contents in cv. WFSP, but an augmented level of total protein, flavonoids, anthocyanins, and carotenoids was observed in OFSP. Comparative proteomic analysis by 1-DE coupled with mass spectrometry led to the identification of 1541 and 1201 proteins in cv. OFSP and WFSP, respectively, which might have a key role for the functional diversity leading to differential nutrient acquisition. The proteomic analysis further revealed cultivar-specific accumulation of proteins along with evolutionarily conserved proteins. Additionally, metabolome profiling displayed 148 and 126 metabolites in cv. OFSP and WFSP, respectively. Quantitative proteomic analysis using 2-DE revealed differential expression of 68 proteins in both cultivars, whereas 105 proteins were unique to cv. OFSP and 65 proteins to WFSP. These results may provide new insights into molecular basis for differential nutrient and phytochemical availability in tuber crops in specific and plants in general.

Remodeling of the transcriptome, proteome and metabolome of grasspea: the molecular basis of dehydration tolerance

**Akanksha Pareek, Divya Rathi, Saurabh Gayali,
Subhra Chakraborty, Niranjan Chakraborty**

National Institute of Plant Genome Research, Jawaharlal Nehru University Campus, Aruna Asaf Ali Marg, New Delhi 110067, India.
pareekakanksha1@gmail.com

Environmental stress remains a major constraint to global legume production worldwide despite their hardiness. It is least likely that the varied molecular basis of stress tolerance in members of legume family, Fabaceae, constituted by 946 genera, can be accurately unraveled in a few species and therefore, more species must be investigated. Grasspea (*Lathyrus sativus* L.) is one of the best examples of an orphan legume, which has thus far received little research attention. Grasspea cultivars are capable of withstanding a myriad of environmental stresses, making it one of the best systems for such studies. We investigated the molecular basis of stress tolerance in grasspea subjecting 3-week-old seedlings to dehydration for a duration of 144 h. Physicochemical analyses was conducted by parameters such as relative water content, membrane stability, and impact on accumulation of proline and photosynthetic pigments. The temporal effects of dehydration were evaluated at the transcriptomic, proteomic and metabolomic levels using *de novo* RNA-seq, and 2-D gel electrophoresis coupled with mass spectroscopy and GC-MS, respectively. The 2-DE analysis revealed 171 proteins that were differentially regulated in qualitative as well as quantitative manner. Furthermore, 5201 transcripts of various functional classes including transcription factors, cytoplasmic enzymes and structural cell wall proteins, among others were found to be dehydration-responsive. The metabolome profiling revealed 59 dehydration-responsive metabolites that include sugar alcohols and amino acids. Despite the lack of genome sequence information of grasspea, the time course of physicochemical and molecular responses suggest a synchronized stress response that activates cellular components and prevents cell death through modulation of various signaling pathways. We propose a hypothetical model that highlights novel biomarkers and explain their relevance in dehydration-response, which would facilitate targeted breeding and aid in commencing crop improvement efforts.

Proteomic and metabolomic analysis of *Nothapodytes nimmoniana* (J. Graham) extracts treatment on HeLa cell line

Shivani Gayakwad¹, Amey Shirolkar¹, Apurva Dixit¹, Shraddha Bharsakale¹, Shrikant Warkad¹, Sudesh Gaidhani², Sharad Pawar^{1*}

Department of Pharmacology, RAIFR, Pune – 411038, India.
*gayakwad.shivani@gmail.com ; *sd_pawar@yahoo.com*

Nothapodytesnimmoniana (J. Graham) is a natural source of camptothecin anticancer drug. Different extracts of *Nothapodytes* plant were studied for *in vitro* action on cervical cancer cell line, the HeLa. The proteins and metabolites from the treated and control samples were extracted. The processed samples were analyzed for differentially expressed proteins and metabolites on RRLC-ESI-QTOFMS. The results were composed of closely linked cancer proteins and still unidentified proteins. The CBFA2T1, cysteine-rich protein 2-binding protein, Zinc finger protein 788, transcription factor RFX3 and angiotonin-like protein 1 were significantly expressed proteins while 3-Hydroxysuberic acid, Indole-3-carboxylic acid, N8-Acetylspermidine, L-Octanoylcarnitine were significantly expressed metabolites. Aminoacyl-tRNA biosynthesis, purine metabolism and valine, leucine, arginine, proline metabolism were the most prominent pathways. The unidentified proteins have a great chance of becoming signature molecules of cervical cancer. The multi-omics approach has greatest metabolites and proteins profiling potential hence was applied here to find signatures of cervical cancer.

understand the pathophysiology of ischemia-reperfusion induced AKI and develop early biomarkers for effective clinical management. In the present study, using porcine AKI model, we have identified early metabolite changes in ischemia/reperfusion induced kidneys.

Female pigs (n=6; 50-70 kg) were randomized to an ischemic AKI protocol (20 to 60 mins bilateral renal artery clamping) and then reperfused for 2 hours. Kidney cortex tissue samples from both the ischemia/reperfused and sham-control group (n=6) were processed to extract metabolites and identified using mass spectrometer. Further analysis was done using SIMCA or Metaboanalyst to identify key metabolites associated with Ischemia or reperfusion.

Comparative analysis between control, ischemia and reperfusion kidneys revealed altered levels of various metabolites. Breakdown of several glucogenic and ketogenic amino acids was observed in our study. Breakdown products of tryptophan metabolism such as formylkynurenin and indole were detected in IR induced kidneys. Breakdown of other aromatic amino acids such as Tyrosine and Phenylalanine to hippuric acid was also observed. Recent proteomic and metabolomic studies by others also revealed breakdown of tryptophan in mouse AKI models.

This study provides large scale molecular information about the early metabolite changes in ischemia/reperfusion induced AKI. Amino acid degradation/catabolism was identified as one of the major early metabolic changes in ischemia/reperfusion induced AKI which was reported to be associated with tissue inflammation. Further analysis of these amino acids and their downstream products in urine or plasma samples may be beneficial for the development of early AKI biomarkers.

Metabolome of the Porcine Kidney after Ischemic Acute Kidney Injury

Ravindra Varma Polisetty¹, Cath Ortori², Hyun Kim², Dave Barret² and David S. Gardner²

¹ Sri Venkateswara College, University of Delhi, India;

² School of Pharmacy, University of Nottingham, UK;

³ School of Veterinary Medicine, University of Nottingham, UK.
ravindra@svc.ac.in

Acute kidney injury (AKI) is a common and serious complication in patients undergoing chronic treatment or kidney transplantation. There is a critical need to

**Session-5 Post-translational Modifications
(PTMs)**

P-57 (Please refer O-09); P-58 (Please refer O-10)

P-59

Role of phosphorylation on secretion in *Mycobacterium tuberculosis* and its impact on its survival

**Basanti Malakar^a, Komal Chauhan^b,
Haroon Kalam^b, Dhiraj Kumar^b and
Vinay Kumar Nandicoori^{a,*}**

^aNational Institute of Immunology, Aruna Asaf Ali Marg,
New Delhi – 110067

^bCellular Immunology Group, International Centre for Genetic
Engineering and Biotechnology, Aruna Asaf Ali Marg,
New Delhi 110067
^{*}vinaykn@nii.ac.in

Mycobacterium tuberculosis faces numerous environmental stresses upon entering the host. A common mode of regulation of the cell's response is through phosphorylation/ dephosphorylation of specific target proteins. One such mechanism is modulated through two component phosphosignaling pathways. In addition to the classical Two-Component system, *M. tuberculosis* is equipped with 11 eukaryotic like serine threonine protein kinases (STPKs) and one tyrosine kinase. Reports have suggested that these eukaryotic like STPKs regulate a wide array of functions inside the pathogen such as cellular metabolism, transcriptional regulation, cell wall biosynthesis and cell division. We are working towards the identification of novel substrates to better understand the effect of phosphorylation on overall cellular physiology and growth of pathogen. Towards this we have performed phosphoproteome and phosphosecretome analysis to identify novel targets and secretory proteins that are phosphorylated. We also used proteomics to identify the secretome of mycobacteria, which contributes enormously towards pathogenesis. We have identified two very important secretory proteins of Type VII Secretory System (T7SS) to be phosphorylated. We investigated the impact of phosphorylation on protein-protein interaction, secretion and eventual survival of pathogen in the host.

P-60

**Low abundance of albumin enhances its glycation and impairs its ability to protect against hemoglobin glycation:
A mechanistic study using erythrocyte culture**

**Mashanipalya G. Jagadeeshaprasad¹,
Vinashta Venkatasubramani¹,
Ambika G. Unnikrishnan², and
Mahesh J. Kulkarni^{1*}**

¹ Proteomics Facility, Division of Biochemical Sciences, CSIR-National Chemical Laboratory, Pune-411008, India

² Department of Diabetes and Endocrine Research, Chellaram Diabetes Institute, Pune, Maharashtra, India.
gj.prasad@ncl.res.in and *mj.kulkarni@ncl.res.in

Diabetes diagnosis and management majorly depends upon the glycated hemoglobin (HbA1c) levels. Various factors influence HbA1c levels such as analytical methods, clinical conditions like anaemia or pregnancy, lifespan of erythrocytes etc. In this study, we unequivocally demonstrate that the serum albumin level and its glycation status influence hemoglobin glycation using erythrocyte culture. Erythrocytes maintained in low serum albumin concentration displayed increased hemoglobin glycation and vice versa, as measured by HbA1c, advanced glycation end product (AGE) modification monitored by fluorescence spectrometry, western blotting and mass spectrometry, which was accompanied with increased serum albumin glycation, which perhaps decreased its ability to protect hemoglobin glycation. This was demonstrated by treatment of N(ε)-(carboxymethyl) lysine (CML) modified serum albumin (CMSA) which failed to protect hemoglobin glycation; instead it increased hemoglobin glycation possibly by altering in erythrocyte structure and membrane permeability. The inability of CMSA to reduce hemoglobin glycation was due to lack of availability of free lysine residues, which was corroborated by using N(ε)- (acetyl) lysine serum albumin (AcSA) and clinical diabetic plasma. This is the first study that demonstrates that modification of lysine residues of albumin impairs its ability to inhibit glycation. Furthermore, correlation studies between HbA1c and serum albumin or relative albumin fructosamine (RAF) from clinical subjects supported our experimental finding that albumin abundance and its glycation status influence hemoglobin glycation. Therefore, we propose serum albumin level and its glycation status to be quantified in conjunction with HbA1c for better management of diabetes.

The role of histone 3 lysine 4 methylationin the prognosis of clear cell renal cell carcinoma

Aman Kumar¹, Sharawan Kumar Singh², Nandita Kakkar³, Rajendra Prasad¹

¹ Department of Biochemistry, ²Department of Urology,
³Department of Histopathology, Postgraduate of Institute of Medical Education and Research, Chandigarh, India
aman.jain1424@gmail.com

Histone modifications play an important role in setting up the epigenetic profile of a cell during the process of tumorigenesis. Previous studies documented the role of several histone modifications in predicting clinical outcome in patients of various tumors. In the present study, we evaluated the prognostic potential of histone 3 lysine 4 mono-methylation (H3K4me1), -di-methylation (H3K4me2) and -tri-methylation (H3K4me3) in clear cell renal cell carcinoma (ccRCC). Histone 3 lysine 4 mono-methylation (H3K4me1), -di-methylation (H3K4me2) and -tri-methylation patterns were determined on isolated histones from 50 histopathological confirmed cases of ccRCC and adjacent normal renal tissues by ELISA assay. The global H3K4me levels were increased in ccRCC as compared to adjacent normal renal tissues. Further, lower cellular levels of all the three modifications viz H3K4me1, H3K4me2 and H3K4me3 were found to be associated with higher TNM stage and Fuhrman grade of the tumor. Also, there was a reduction of H3K4me levels with tumor metastasis. Interestingly, there was a successive increase of H3K4me levels with the increase in degree of methylation, H3K4me3 levels being the highest. Therefore, the present study provides the prognostic potential of global H3K4 methylation in ccRCC.

Comprehensive Analysis of Nuclear Phosphoproteome of Chickpea (*Cicer arietinum*)

Pooja Choudhary, Kanika Narula, Pooja R. Aggarwal, Niranjan Chakraborty, Subhra Chakraborty*

National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi-110067, India
poojachoudhary31@gmail.com

Nucleus, the regulatory hub is a dynamic system that orchestrates complex signaling events dictating cell fate decisions. Nuclear proteins play plethora of functions during cell development, different physiological processes and environmental adaptations. Posttranslational modifications (PTMs) modulate protein function and have a ubiquitous role in diverse range of cellular functions. Repercussions of phosphorylated nuclear proteins in context with cellular functions are largely unknown. To provide novel imminent in nuclear dynamics, we have developed a comprehensive nuclear phosphoproteome map of a food legume chickpea. MS analysis led to the identification of 627 phosphoproteins, presumably involved in a variety of biological functions. *In silico* prediction and MS identification of site specific phosphorylation of amino acid residues indicated their possible effect on nuclear signaling network and dynamics at system level. Our study illustrates the mechanism of nuclear dynamics and elucidated the phosphoprotein network that branches to several hormonal and signaling pathways. To our knowledge, this gives a comprehensive understanding of the complex phosphoprotein network in cellular signaling operating in plants.

Session-6 Interaction Proteomics & Protein Networks

P-63 (Please refer O-11)

P-64

Spectrin interacting proteins of erythrocytes

Dipayan Bose and Abhijit Chakrabarti*

Crystallography & Molecular Biology Division, Saha Institute of Nuclear Physics, HBNI, Kolkata 700064, India.
*abhijit.chakrabarti@saha.ac.in

Spectrin based protein network, known as the membrane skeleton have been found to be implicated in hematological disorders. It is established that spectrin interacts with intact haemoglobin and the globins and this interaction is modulated by phosphate metabolites such as ATP and DPG. Spectrin exhibits chaperone property and has moderate resemblance with α -hemoglobin stabilizing protein. It is thus hypothesized that it may interact with heme containing proteins in general. We investigate the binding of spectrin to hemoglobin variants, hemoglobin-derived globin chains, erythroid cytosolic catalase and mitochondria resident cytochrome C. The functional implication of these interactions, specifically the modulation of the peroxidase and catalase activities of these proteins, in presence and absence of spectrin, are investigated. The peroxidase activity of hemoglobin variants and globin chains increased in presence of spectrin while cytochrome C did not show as great of an increase; presence of ATP increased the enzyme activity in all cases. The activity of catalase too increased upon spectrin binding, being strongly influenced by ATP.

P-65

Suppression of protein nitrosylation causes increased oxidative damage and protein carbonylation in hypoxic cardiomyoblast: Inverse relation between carbonylation and nitrosylation

Anamika Gangwar, Subhojit Paul, Yasmin Ahmad, Kalpana Bhargava

*Defence Institute of Physiology & Allied Sciences, Defence R&D Organization, Timarpur, New Delhi-110054.
kalpanab2006@gmail.com*

Altitude associated hypoxia is known to cause severe patho-physiological outcomes like High altitude pulmonary/cerebral edema which can be life-threatening without immediate medical aid and rescue. Hypoxic insult causes impaired redox balance along with oxidative stress, protein carbonylation and instigation of apoptotic events. Protein nitrosylation, via NO mediated biochemical effects, is known to prevent the ill-effects of hypoxia. Although several routes and mechanisms of NO mediated cytoprotection are known during hypoxic insult, but no relationship between the two downstream events of oxidative stress, protein carbonylation and protein nitrosylation is known yet. In this study, we investigated the interaction or cross-talk between protein carbonylation and nitrosylation in hypoxia. Using standard NO inhibitor L-NAME and simulated hypoxic conditions in hypoxia sensitive cell line H9c2, we measured the levels of radicals, cell death and mitochondrial potential, glutathione content, protein nitrosylation and protein carbonylation. The results were then analyzed in light of NO bioavailability. Reduced NO availability and thus lower nitrosylated proteins during hypoxia not only caused an increase in cell death, but also increased the degree of carbonylation in proteins. Hence, proved an inverse relation between protein nitrosylation and carbonylation. This further opens new possibilities to explore the potential cross talk between nitrosylation and carbonylation, especially through some common antioxidant mediators such as Glutathione and Thioredoxin in the context of increased or decreased NO.



Bruker Daltonics

Committed to Your Success

Advanced Mass Spectrometry Solutions



- Ion Trap: amazon series
- ESI-(Q)-TOF: micrOTOF series
- UHR-TOF: maXis
- MALDI-TOF(/TOF): flex series
- FTMS: solariX series
- Triple Quad: GC-TQ/LC-TQ

Proteomics

- Top-down and Bottom-up Strategies
- Flexible Quantitation
- Detailed Intact Protein and PTM Analysis

Biomarker Analysis

- Full MALDI Imaging Solution
- Profiling via LC-MALDI and LC/ESI-MS
- MALDI Biotype Bacterial ID

Small Molecules/Metabolites

- Fastest Parallel Multitarget Screening
- Forensic Toxicology
- Pesticide and Food Analysis

Target Screening

- LC/MS Based Metabolic Profiling
- Complementary NMR Workflows
- Empirical Formula Determination
- Full Open Access Capability

Contact us for more details and system demonstration :

Bruker India Scientific Pvt. Ltd.

Shakti Towers, A2, Block-1, 7th Floor,

766, Anna Salai, Chennai - 600 002.

Tamil Nadu, INDIA.

Tel: +91-44-28414539

Fax: +91-44-28414571

Email: sales.bdal.in@bruker.com;

Web: www.bruker.com

Other Offices :

| Bangalore | Delhi | Kolkata | Mumbai

MASS SPECTROMETRY

think forward



DON'T BE LIMITED BY TRADITIONAL FLUORESCENCE

Three Targets + Total Protein Normalization

A Whole New World of Fluorescent Western Blotting

What do we mean by traditional fluorescence? It's fluorescent western blotting with only two colors. That is, detection of two proteins on a single blot. So, to detect more proteins, you need to strip and reprobe or cut your blot. But not anymore. With Bio-Rad's new fluorescent western blotting workflow you can move beyond two colors and use our stain-free technology for total protein normalization. Be limitless.

BIO-RAD

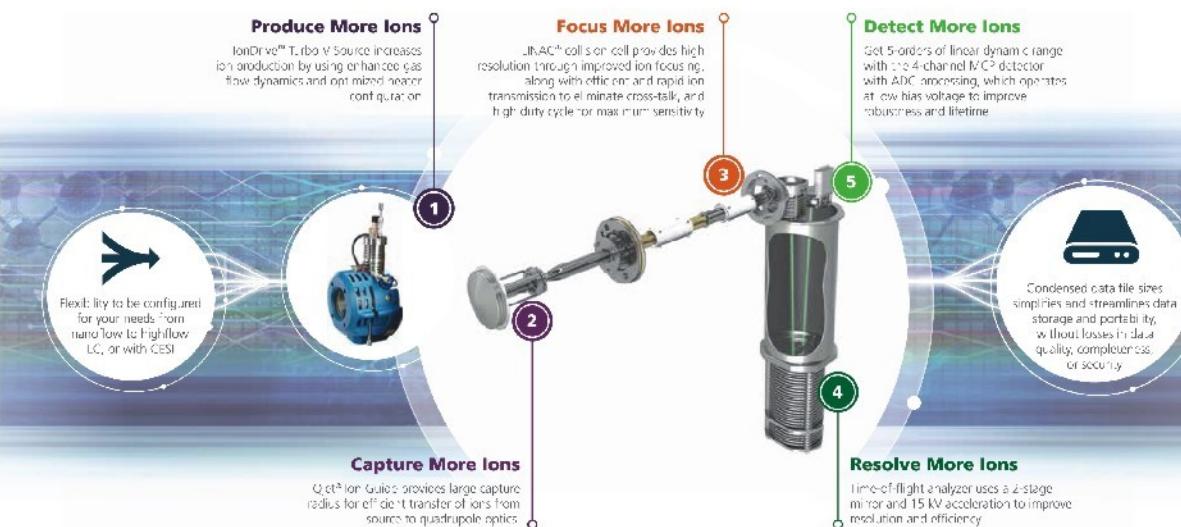
Learn more at bio-rad.com/info/TBD

TripleTOF® 6600: Powerful. Productive. Versatile.

Learn the Top 5 Technology

Advancements of the Accurate Mass TripleTOF 6600 System

From front to back, the TripleTOF 6600 accurate mass system can help you to analyze your samples faster, without compromising between speed, resolution, and sensitivity. Explore the technological advances that give this system the performance edge.



Take a Deeper Dive into Complex Samples with the TripleTOF 6600 High-Performance Sensitivity & Linear Dynamic Range

Maximal Versatility to Take on any Challenge

Discovery the benefits of the

TripleTOF® 6600 System Technology for these selected application areas

Empower your lab to take on new challenges and projects by choosing a platform that offers maximal versatility. Get us and running with new workflows quickly by leveraging a familiar system with the widest breadth of applications available.



Lipidomics

Broadly profile large numbers of lipids with the unique Infusion MS/MS/MS Workflow.

[Read More Here](#)



Metabolomics

Streamline your metabolomics with single injection SWATH® Acquisition and XCVPlus data processing.

[Read More Here](#)



Biologics Analysis

Sensitivity and large dynamic range enable comprehensive biopharmaceutical characterization as well as low-level host cell protein detection.

[Read More Here](#)



Food and Environmental

Perform research at the forefront of science. The power of SWATH® Acquisition enables you to get answers to regular workflows would miss.

[Read More Here](#)



Proteomics

Balance throughput and sensitivity for large-scale proteomics studies with Microflow SWATH® Acquisition. Dig deeper into your proteome with variable workflow SWATH® Acquisition.

[Read More Here](#)



Drug Metabolism

Be confident you're not missing low-level or toxic metabolites or metabolites in your sample by utilizing a single injection, comprehensive SWATH® Acquisition.

[Read More Here](#)



Targeted Quantitation

Whether targeting proteins, lipids, or small molecules, or metabolites, obtain high-quality quantitation with the XCV™ Workflow and 5-orders of linear dynamic range.

[Read More Here](#)



Forensics

Acquire all the analytes in your evidence and see a true profile of your sample. Only SWATH® Acquisition creates a digital archive of your sample so re-analysis is not subjected to sample degradation.

[Read More Here](#)

Take a Deeper Dive into Complex Samples with the TripleTOF 6600 High-Performance Sensitivity & Linear Dynamic Range