2021

13th ANNUAL MEETING OF PROTEOMICS SOCIETY, INDIA & INTERNATIONAL CONFERENCE ON

"OMICS IN REDEFINING MODERN BIOLOGY"

20-23rd OCTOBER 2021

ABSTRACT BOOK

ORGANIZED BY

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ABOUT PSI

The Proteomics Society, India (PSI) is a non-profit body of scientists and academicians affiliated to top ranked Institutions and Universities in India. The goal of the society is to disseminate knowledge, training, and awareness amongst all interest groups in the broad area of proteomics research and its application. The society organizes conferences, seminars and workshops by soliciting the participation of internationally renowned scientists and researchers.

ABOUT CCMB

The Centre for Cellular & Molecular Biology (CCMB) is a premier research organization in frontier areas of modern biology. The objectives of the Centre are to conduct high quality basic research and training in frontier areas of modern biology, and promote centralized national facilities for new and modern techniques in the inter-disciplinary areas of biology.

CCMB was set up initially as a semi-autonomous Centre on April 1, 1977 with the Biochemistry Division of the then Regional Research Laboratory (presently, Indian Institute of Chemical Technology, IICT) Hyderabad forming its nucleus and Dr P M Bhargava heading the new Centre. Earlier, the Governing Board of the Council of Scientific and Industrial Research (CSIR) New Delhi, the apex body which constituted 44 research institutions in the country, approved the proposal in 1976 to establish such a Centre in view of the importance of research in the frontier and multi-disciplinary areas of modern biology. During 1981-82, CCMB was accorded status of a full-fledged national laboratory with its own Executive Committee and Scientific Advisory Council. With major expansion plans, it was decided to relocate the Centre to a spacious campus.

ABOUT CCMB SCIENCE FOUNDATION

On January 25, 2016, CCMB Science Foundation (CCMB-SF) was registered under Telangana Societies Registration Act, with the following aims and objectives:

- Conduct seminars/ lectures by scientists who have made pioneering and outstanding contributions to science.
- Sensitize institutions of teaching and school children about the excitement in scientific enquiry and learning scientific pursuits/ methods in the field of science in general and specifically in Biology/ biological sciences.
- Initiate and organize events, to further the advancement of excellence in science.
- Support/ organizing outreach programs for the general public to encourage scientific temper and bring about awareness about the talent developments and advances in science and technology.
- To organize Distinguished Lecture Series in the area of biology/ biological sciences and interface areas.

CONTENTS

ORGANIZING COMMITTEE

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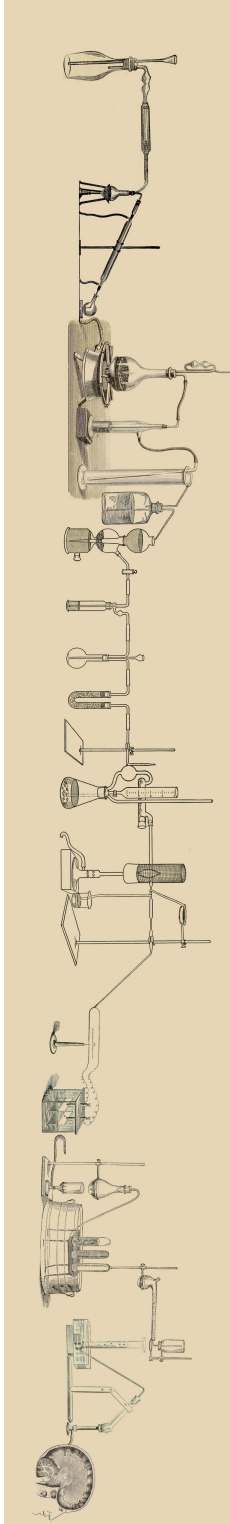

20th October, 2021 (Wednesday) - Education Day

Time	Talk No.	Speaker	Topic
09:00 - 09:15	Inauguration	Subhra Chakraborty, NIPGR, New Delhi, President-PSI	
09:15 - 09:45	EL-01	Srikanth Rapole, NCCS, Pune	Mass Spectrometry based Proteomics
09:45 - 10:15	EL-02	Ramesh Ummani, CSIR-IICT, Hyderabad	Protein microarrays and its applications
10:15 - 10:45	EL-03	Soumen Kanti Manna, SINP, Kolkata	Metabolomics in Cell Biology
10:45 - 11:00	Break		
11:00 - 11:30	EL-04	Niranjan Chakraborty, NIPGR, New Delhi	Plant Proteomics
11:30 - 12:00	EL-05	Ashok Mohanty, IVRI, Mukteswar	Animal Proteomics
12:00 - 12:30	EL-06	Poonam Goutam, National Institute of Pathology, New Delhi	Clinical Proteomics
12:30 - 13:00	EL-07	Mahesh J Kulkarni, CSIR-NCL, Pune	Targeted Proteomics
13:00 - 14:00	Lunch		
14:00 - 15:30	EL-08	Swasti Raychaudhuri, CSIR-CCMB, Hyderabad	Sample preparation for mass spectrometry based proteomics
15:30 - 15:45	Break		
15:45 - 17:15	EL-09	Amit Kumar Yadav, THSTI, New Delhi	Proteomics data analysis

17:15 - 17:45		Interaction/Q & A/Quiz/Closing remarks	
17:45 - 18:00	Break		
18:00 - 20:00		PSI EC meeting	

21st October, 2021 (Thursday) - International Conference, Day 1

INAUGURATION				
Chair	**Time**	**Talk No.**	**Speaker**	**Topic**
	09:00 - 09:05		Welcome Remarks by Convener, Omics-2021 - Swasti Raychaudhuri, CSIR-CCMB, Hyderabad	
	09:05 - 09:15		Remarks by Director, CSIR CCMB - Vinay K Nandicoori	
	09:15 - 09:25		Remarks by President, PSI - Subhra Chakraborty, NIPGR-New Delhi	
PLENARY SESSION - I				
Ravi Sirdeshmukh	**09:30 - 10:15**	PL-01	Michael P. Snyder, Stanford University School of Medicine, USA	Big data, health and COVID-19
Break				
SESSION - I : OMICS IN COVID-19				
R. Nagaraj & R Sankarnarayanan	**10:30 - 10:50**	IL-01	Tiannan Guo, Westlake University, Hangzhou, China	Proteomic and metabolomic investigation of host responses in COVID-19 patients

	10:55 - 11:15	IL-02	Dipyaman Ganguly, CSIR-IICB, Kolkata	Clinical and immunological outcomes of a RCT on convalescent plasma therapy in severe COVID-19: patho-physiological insights from plasma proteomic studies
Break				
SESSION - II : OMICS IN BASIC BIOLOGY (I)				
R. Nagaraj & R Sankarnarayanan	**11:30 - 11:50**	IL-03	Rakesh Mishra, CSIR-CCMB, Hyderabad	Proteomic analysis of nuclear matrix: evolution of nuclear architecture and structural basis of cellular memory
	11:55 - 12:15	IL-04	Kausik Chakraborty, CSIR-IGIB, New Delhi	Metabolism and its role in cellular proteostasis
	12:20 - 12:40	IL-05	Siddhesh Kamat, IISER, Pune	Mapping sphingolipid pathways during phagosomal maturation
Lunch				
PLENARY SESSION - II				
Subhra Chakraborty	**14:00 - 14:45**	PL-02	Matthias Mann, Max Planck Institute of Biochemistry, Germany	Ultra-high sensitive and computational workflows for single cell and deep visual proteomics
Break				
SESSION - III : OMICS IN HEALTHCARE				

Surekha Zingde & Krishnan Venkataraman	**15:00 - 15:20**	IL-06	Shantanu Sengupta, CSIR-IGIB, New Delhi	Proteomics in Clinical Practice: Bridging the gap from discovery to Application
	15:25 - 15:45	IL-07	Alka Rao, CSIR-IMTech, Chandigarh	Protein Glycosylation in *Actinobacteria*: How Sweet !
	15:50 - 16:10	IL-08	Inderjeet Kaur, L V Prasad Eye Institute, Hyderabad	Identification of predictive marker(s) for an early diagnosis of a blinding disorder in premature children using a multiOMICS approach
Break				
SESSION - IV : LIGHTNING TALKS - I (A, B & C)				
Santosh Kumar & Saikat Chowdhury	**16:45 - 17:45**	Parallel sessions	ROOM A - LT 1 to 9	
Md. Idris & Regalla Kumaraswami			ROOM B - LT-10 to 18	
P Chandra Shekar & Mukesh Lodha			ROOM C - LT 19 to 27	
Break				
	18:00 - 20:00		PSI GB meeting	

22nd October, 2021 (Friday) - International Conference, Day 2

SESSION-V : OMICS IN DISEASE BIOLOGY (I)				
Chair	**Time**	**Talk No.**	**Speaker**	**Topic**
Manjula Reddy & Geetanjali Sachdeva	**09:30 - 09:50**	IL-09	Vinay K Nandicoori, CSIR-CCMB, Hyderabad	*Mycobacterium tuberculosis* virulence and survival & the role of phosphorylation
	09:55 - 10:15	IL-10	Arun Bandyopadhyay, CSIR-IICB, Kolkata	Proteomic approaches for understanding molecular basis of inflammation in Atherosclerosis
	10:20 - 10:40	IL-11	Sanjeeva Srivastava, IIT, Mumbai	Mass spectrometry based proteomics and Fourier transform infrared spectroscopy for diagnosis and prognosis of COVID-19 infection
	10:45 - 11:05	IL-12	Dhanasekaran Shanmugam, CSIR-NCL, Pune	Characterizing evolutionarily distinct and highly diverged proteins with conserved functions from the parasite *Toxoplasma gondii.*
	11:10 - 11:25	IL-13	Amol Suryawanshi, ISL, Bhubaneswar	Quantitative proteomics approaches leads to identify differentially expressed brain proteins involved in furious rabies virus infection

Break				
SESSION - VI : LIGHTNING TALKS - II (A, B & C)				
Sonal Nagarkar Jaiswal & Venkat R. Chalamcharla	**11:45 - 12:45**	Parallel sessions	Room A - LT 28 to 36	
Md. Idris & Regalla Kumaraswami			Room B - LT 37 to 45	
Santosh Kumar & Saikat Chowdhury			Room C - LT 46 to 54	
Break				
SESSION - VII: ADVANCED TECHNOLOGIES IN OMICS				
Srikanth Rapole	**12:45 - 13:05**	TL-01	Nick Morrice, Sciex	Powerful new proteomic workflows enabled by the SCIEX ZenoTOF system
Lunch				
PLENARY SESSION - III				
Jyotsna Dhawan	**14:30 - 15:15**	PL-03	Agnus Lamond, University of Dundee, UK	Proteomic analyses of human stem cells
Break				
SESSION - VIII : DECODING OMICS - PROTEOGENOMICS, METAPROTEOMICS (I)				
Rakesh Mishra	**15:40 - 16:10**	IL-14	Juergen Cox, Max Planck Institute of Biochemistry, Germany	MaxDIA enables library-based and library-free data-independent acquisition proteomics

	16:15 - 16:45	IL-15	Jyoti Chaudhary, The Institute of Cancer Research, UK	Integrative Proteogenomics: Deconvoluting genetic determinants of protein abundance variation
Break				
SESSION - IX: LIGHTNING TALKS - III (A & B)				
P Chandra Shekar & Mukesh Lodha	**17:00 - 18:00**	Parallel sessions	Room A - LT 55 to 64	
Sonal Nagarkar Jaiswal & Venkat R. Chalamcharla			Room B - LT 65 to 75	
Break				
SESSION - X : DECODING OMICS - PROTEOGENOMICS, METAPROTEOMICS (II)				
Amitabha Chattopadhyay	**18:30 - 18:50**	IL-16	Jagannath Swaminathan, University of Texas at Austin and Erisyon Inc, USA	Sample preparation for single molecule protein sequencing technology - Fluorosequencing
	19:00 - 19:20	IL-17	Pratik Jagtap, University of Minnesota, USA	Metaproteomics: Promoting functional analysis of microbiome through online educational resources via the galaxy platform
	19:30 - 19:50	IL-18	Shankha Satpathy, Broad Institute of MIT and Harvard, USA	Dissecting proteogenomic vulnerabilities in cancers

23rd October, 2021 (Saturday) - International Conference, Day 3

PLENARY SESSION - IV				
Chair	**Time**	**Talk No.**	**Speaker**	**Topic**
Suman Kundu	**09:00 - 09:45**	PL-04	John Yates III, The Scripps Research Institute LA, USA	Proteome Analysis using Combined Single Neuron Patch-Clamp / Mass Spectrometry Analysis (PatchC-MS)
Break				
SESSION - XI : OMICS IN AGRICULTURE				
Subhra Chakraborty & Niranjan Chakraborty	**10:00 - 10:20**	IL-19	Paul A. Haynes, Macquarie University, Australia	Proteomic analysis of different varieties and species of rice under various stress conditions
	10:25 - 10:45	IL-20	Pengcheng Wang, Chinese Academy of Sciences, Shanghai, China	Study RAF-SnRK2 cascade in Arabidopsis by proteomic strategies
	10:50 - 11:10	IL-21	Paul E. Verslues, Academia Sinica, Taipei, Taiwan	Type 2C Protein phosphatases and their target proteins that regulate plant growth during drought stress
Break				
SESSION - XII : OMICS IN DISEASE BIOLOGY (II)				
Tushar Vaidya & K. Balamurugan	**11:30 - 11:50**	IL-22	Utpal tatu, IISC, Bangalore	Novel post-translational modifications on flagellar tubulin regulate motilities

				in neglected infectious disease caused by *Trichomonas vaginalis* and *Giardia lamblia*
	11:55 - 12:15	TL-02	Yue Xuan, Thermo Fisher Scientific	Trends in Life Science OMICS Research
	12:20 - 12:35	IL-23	Sandipan Ray, IIT-Hyderabad	Multiplexed quantitative proteomics for mechanistic study of pharmacological modulators of circadian time-keeping machinery
Lunch				
SESSION - XIII : OMICS IN ANIMAL BIOTECHNOLOGY				
Ashok Mohanty	**14:00 - 14:20**	IL-24	Maya Zachut, Institute of Animal Science ARO, Israel	A proteomics approach to unravel adipose tissue inflammatory responses in peripartum cows
	14:25 - 14:45	IL-25	Ashish Mukherjee, IASST, Guwahati	The Application of Mass Spectrometry and Other Analytical Techniques for the Quality Assessment of Commercial AntivenomD
	14:50 - 15:10	IL-26	Srinivas Kiran Ambatipudi, IIT, Roorkee	The Dynamics and Power of the Bovine Milk Components in Health and Disease
Break				

SESSION - XIV : OMICS IN BASIC BIOLOGY (II)				
Mandar V Deshmukh	**15:30 - 15:50**	IL-27	Amit Kumar Mandal, IISER, Kolkata	Structural analysis of post-translationally modified human hemoglobin: A native mass spectrometry based approach
	15:55 - 16:15	IL-28	Veena Patil, NII, New Delhi	Human CD4+ T cell memory subsets in infectious diseases: Lessons from multi-omics analysis
	16:20 - 16:40	IL-29	Manas Santra, NCCS, Pune	Reaching the drop through the ocean: Proteomic study to capture dynamic interactions for understanding cancer pathogenesis and treatment
Break				
SESSION - XV : PANEL DISCUSSION - "ETHICS IN COMMUNICATING SCIENCE"				
Moderator: Debasis Dash, CSIR-IGIB, New Delhi	**17:00 - 18:00**		D. Balasubramanian, LVPEI Surekha Zingde, PSI Subhash C Lakhotia, BHU, Varanasi	Ethics in communicating Science

VALEDICTORY				
	18:00 - 18:30		Subhra Chakraborty, Srikanth Rapole, Shantanu Sengupta, Arun Bandyopadhyay	

SPEAKERS

BIOSKETCHES, TITLES & ABSTRACTS

EDUCATION DAY

EL-01

Dr. Srikanth Rapole

National Centre for Cell Science, Pune

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Dr. Rapole obtained his PhD from the Indian Institute of Chemical technology, Hyderabad. He continued his Postdoctoral research from the University of Massachusetts, USA. He is presently at the National Centre for Cell Science, Pune. His main research interest is to quantitatively identify the protein signatures involved in human diseases including various cancers with state-of-the-art and highly sensitive mass spectrometry-based proteomic approaches. In addition, his group is also working to identify and quantify key metabolites and lipids that alters in cancer.

He has been awarded the Eminent Mass Spectrometrist award by the Indian Society for Mass Spectrometry (ISMAS). He served as the Director of proteomics and mass spectrometry lab at the University of Connecticut, USA.

His talk will be focused on Mass-Spectrometry based Proteomics.

EL-02

Dr. Ramesh Ummani

CSIR- Indian Institute of Chemical Technology, Hyderabad

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Dr. Ummanni holds a PhD degree from the Institute for Medical Biochemistry and Molecular Biology, University of Greifswald, Germany. He carried out his Postdoctoral research at the University of Hamburg, Germany. Dr. Ummanni's lab at the CSIR-Indian Institute of Chemical Technology, Hyderabad focuses on identifying new potential biomarkers and understanding cell-signalling mechanisms driven by de-regulated proteins specifically in prostate cancer. His work includes differential as well as functional proteomics. He is involved in identifying new chemical and molecular entities with anti-cancer, anti-tubercular, antiviral potential using cell based and target based screening of small molecule libraries.

His talk will be focused on protein microarrays and its application.

EL-03

Dr. Soumen Kanti Manna

Saha Institute of Nuclear Physics, Kolkata

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Dr. Manna obtained his PhD degree from the Tata Institute of Fundamental Research, Mumbai. Currently, he works at the Saha Institute of Nuclear Physics, Kolkata. His group mainly focuses on characterization of changes in metabolomic and proteomic (especially, post-translational modifications related to metabolism) signatures associated with gene-environment interaction and pathogenesis using mass-spectrometry. His group is also working on mass spectrometry-based imaging methods to study spatio-temporal changes in biochemical landscape that could be used to replace or supplement traditional histology for accurate diagnosis and prognosis.

His talk will be focused on Metabolomics in Cell Biology.

EL-04

Dr. Niranjan Chakraborty

National Institute of Plant Genome Research, New Delhi
nchkraborty@nipgr.ac.in

Dr. Chakraborty earned his PhD degree from the Jawaharlal Nehru University (JNU), New Delhi. Currently he works at the National Institute of Plant Genome Research (NIPGR), New Delhi. His group has been exploring the stress-responsive organelle proteomes of agriculturally important crops as a strategy to understand the molecular mechanisms of plant stress tolerance, with the ultimate goal of enhanced agricultural productivity.

Dr. Chakraborty is a fellow of the Indian National Science Academy (India), the National Academy of Sciences (India), and the National Academy of Agricultural Sciences (India). He was awarded with ICCR Commonwealth Scholarship and Fellowship; Biotechnology Overseas Associateship by DBT, GoI. He served at the Jawaharlal Nehru University, New Delhi as Research Scientist, University of California, Riverside, USA as Research Associate, Tezpur University, Assam and Presidency University, West Bengal as Guest Faculty.

His talk will be focused on Plant Proteomics.

Dr. Ashok Mohanty

ICAR- Indian Veterinary Research Institute, Mukteshwar

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Dr. Mohanty is a Joint Director of the ICAR- Indian Veterinary Research Institute, Mukteshwar. His area of research includes veterinary proteomics for discovery of biomarkers and diagnostic, genetic engineering of animal and viral proteins. His work had made a significant contribution in veterinary science such as developing the first buffalo specific cell line, urine based pregnancy detection method for livestock animals, development of microfluidic method for enrichment of live and motile spermatozoa in cattle, etc.

He also served at the CSIR-National Dairy Research Institute, Karnal.

His talk will be focused on Animal Proteomics.

EL-06

Dr. Poonam Gautam

National Institute of Pathology, New Delhi

gautamp@icmr.org.in

Dr. Gautam received her PhD from the Institute of Genomics and Integrative Biology, New Delhi and the University of Pune. She worked as a Research Associate at the Proteomics Research Facility in CSIR- Centre for Cellular and Molecular Biology before joining ICMR-National Institute of Pathology, New Delhi as a Scientists and Proteomics Facility In-charge. Her research interest lies in identification of biomarkers for early diagnosis and post treatment surveillance of gallbladder carcinoma (GBC) and glioma. Her research also includes understanding the molecular pathophysiology of cancer. She analyzes plasma-derived EVs with an aim to identify protein and miRNA-based molecular signatures as circulatory biomarkers for early detection of GBC.

She is a member of the Human Proteome Organization (HUPO).

Her talk will be focused on Clinical Proteomics.

EL-07

Dr. Mahesh J Kulkarni

CSIR-National Chemical Laboratory, Pune

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Dr. Kulkarni earned his PhD degree from the University of Agricultural Sciences, Bangalore. Currently he is appointed at the CSIR-National Chemical Laboratory, Pune as well as Associate Dean (Biological Science) of Academy of Scientific and Innovative Research. His research areas of interest include Mass spectrometry-based proteomics and metabolomics, protein glycation in diabetes, AGE-RAGE signaling, Chemical Biology, Post-translational modifications.

He was awarded by the Chellaram Diabetes Institute as a Special Recognition at the International Diabetes Summit and elected as the Fellow of Maharashtra Academy of Sciences.

His talk will be focused on Targeted Proteomics.

EL-08

Dr. Swasti Raychaudhuri

CSIR- Centre for Cellular and Molecular Biology, Hyderabad

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Dr. Raychaudhuri received his PhD degree from the Saha Institute of Nuclear Physics, Kolkata. Later he moved to the Max Planck Institute of Biochemistry for his Postdoctoral research. Currently, he is at the CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad. His lab is interested in studying protein-aggregation in age-related proteostasis stress models, especially to identify the response of the cellular proteome against the newly triggered aggregates due to various stresses. Simultaneously, his group also investigates how components of the proteostasis network, including molecular chaperones, degradation machinery and others collaborate to maintain the integrity of the proteome in the face of protein-aggregation stresses.

He served as the Head of Max Planck Partner Group at the CSIR-CCMB.

His talk will be focused on sample preparation for Mass-Spectrometry based proteomics.

EL-09

Dr. Amit Kumar Yadav

Translational Health Science and Technology Institute, Faridabad

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Dr. Yadav received his PhD degree from the CSIR-Institute of Genomic and Integrative Biology, Delhi. Currently, he is at THSTI, Faridabad. His interest is focused on studying post-translational modifications using Mass-spectrometry. He develops computational methodologies for the analysis of complex multi-dimensional mass spectrometry data and employ systems based approaches for data analysis and interpretation. He has developed several computational algorithms for isobaric quantitation data (iTRAQ and TMT), and devised HyperQuant tool for analysis of complex multiplexed (18-plex) proteomics data, along with tools for large-scale automated identification of PTMs in blind mode for metabolic diseases.

He has been awarded with the Innovative Young Biotechnologist Award (IYBA) 2013, Innovators Under 35, 2013 (TR35) in India by MIT Technology Review and Young Scientist Travel Award from DST in 2011. He is also a member of the Human Proteome Organization (HUPO).

His talk will be focused on Proteomics Data Analysis.

INTERNATIONAL CONFERENCE

PL-01

Prof. Michael P. Snyder

Professor & Chair
Department of Genetics
Director, Center for Genomics and
Personalized Medicine, Stanford University, United states
mpsnyder@stanford.edu

Prof. Snyder received his PhD degree from the California Institute of Technology, USA and carried out Postdoctoral training at the Stanford University, USA. Currently, he is a Professor and Chair of Genetics and the Director of the Center of Genomics and Personalized Medicine at Stanford University. He is a leader in the field of functional genomics and proteomics, and one of the major participants of the ENCODE project. His lab was the first to perform a large-scale functional genomics project in any organism, and has launched many technologies in genomics and proteomics. These includes the development of proteome chips, high resolution tiling arrays for the entire human genome, methods for global mapping of transcription factor binding sites (ChIP-seq), paired end sequencing for mapping of structural variation in eukaryotes, *de novo* genome sequencing of genomes using high throughput technologies and RNA-Seq. He has also combined different state-of-the-art "omics" technologies to perform the first longitudinal detailed integrative personal omics profile (iPOP) of a person and used this to assess disease risk and monitor disease states for personalized medicine.

He is the cofounder of several biotechnology companies, including Protometrix (now part of Life Tehcnologies), Affomix (now part of Illumina), Excelix, and Personalis, and he presently serves on the board of a number of companies.

Big data, health and COVID-19

Recent technological advances as well as longitudinal monitoring not only have the potential to improve the treatment of disease (Precision Medicine) but also empower people to stay healthy (Precision Health). We have been using advanced multiomics technologies (genomics, immunomics, transcriptomics, proteomics, metabolomics, microbiomics) as well as wearables for monitoring health in 109 individuals for up to 11 years and made numerous major health discoveries covering cardiovascular disease, oncology, metabolic health and infectious disease. We have found that individuals have distinct aging patterns that can be measured in an actionable period of time as well as seasonal patterns of health markers. Finally, we have used wearable devices for early detection of infectious disease, including COVID-19 and built an alerting system for detecting health stressors that is scaleable to the entire planet. We believe that advanced technologies have the potential to transform healthcare.

IL-01

Dr. Tiannan Guo

Westlake University, China

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Dr. Guo received his PhD degree from the Nanyang Technological University, Singapore. He conducted his Postdoctoral research at ETH-Zürich, Switzerland. He currently works at the Westlake University, China. His lab is interested in developing new technologies for generation of proteomics data with higher throughput, reproducibility and cost-effectiveness. They are also working towards stratifying intermediate prostate cancers and developing new diagnostic tools to detect thyroid cancer using different proteomics approaches. Their long-term research goal is to build digital biobanks containing proteome information from clinical specimens.

Proteomic and metabolomic investigation of host responses in COVID-19 patients

Host responses to SARS-CoV-2 are dynamic and complex. Here I will present applications of advanced proteomics technologies to interrogate host responses in the sera, urine and tissue specimens from COVID-19 patients. By proteomic comparison of immune responses between severe and non-severe cases in sera and urine specimens, we showed the feasibility to develop protein-based machine learning models to classify severe cases. We also identified characteristic serological immune responses of COVID-19 patients with prolonged disease course and extraordinary IgM positivity. Our proteomic analyses of COVID-19 specimens nominated potential intervention strategies that may be exploited to facilitate therapeutics against COVID-19.

Dr. Dipyaman Ganguly

CSIR-Indian Institute of Chemical Biology, Kolkata

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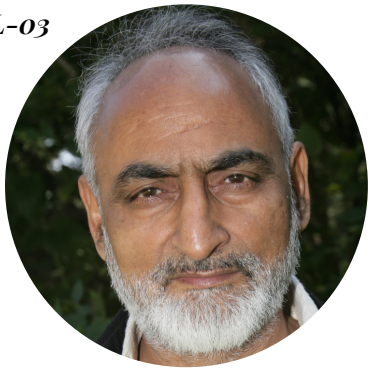
Dr. Ganguly received his first PhD degree from IICB, Kolkata and moved to Texas where he received another PhD. from University of Texas Health Science Centre at Houston. Later, he joined Columbia University Medical Centre for his Postdoctoral research. He works at the Indian Institute of Chemical Biology, Kolkata. His lab focuses on exploring the role of dendritic cells in autoreactive inflammatory contexts, deciphering molecular regulation of innate immune response and exploring the role of mechanical cues in modulating function in different immune cells.

Dr. Ganguly received many honors such as NASI Scopus Young Scientist Award in Medicine from National Academy of Science, India and Elsevier, National Bioscience Award for Career Development from DBT, GoI, CDRI Award for Excellence in Drug Research in Life Science from CDRI, India, Merck Young Scientist Award in Life Sciences from Merck, India.

Clinical and immunological outcomes of a RCT on convalescent plasma therapy in severe COVID-19: patho-physiological insights from plasma proteomic studies

A randomised control trial on use of convalescent plasma therapy (CPT) in a small Indian cohort of severe COVID-19 patients was performed. While the primary outcome analyses revealed no significant clinical benefit secured by the patients on CPT, exploratory subclass analyses identified a group of responsive patients. Proteomic characterization of convalescent plasma identified a number of anti-inflammatory proteins which contributed to a prominent anti-inflammatory effect of CPT in the responsive patients, apart from the neutralizing antibodies. Further exploratory analyses in the same cohort of patients identified circulating level of soluble urokinase-like plasminogen activator receptor (suPAR) to be a plausible mechanistic link between the systemic hyper-inflammation and the hyper-coagulable state encountered in these patients and accordingly had a predictive value for clinical outcomes. Proteomic studies on plasma from convalescent as well as active patients with COVID-19 thus offered critical insights into the disease biology.

IL-03



Dr. Rakesh Mishra

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Dr. Mishra obtained his PhD from the University of Allahabad and then moved to Indian Institute of Science, Bangalore; University of Bordeaux, France; Saint Louis University, USA; and University of Geneva, Switzerland for his Postdoctoral research. He is currently the Director of the Tata Institute of Genetics and Society. His research interest are comparative genomics of non-coding DNA in the context of evolution of complexity, genetic basis of anterior-posterior body axis formation in animals and role of epigenetic regulation in development.

He served as the Director of CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad. Under his leadership, the Atal Incubation Centre was established at CCMB, which provides life science entrepreneurs the infrastructure and mentorship needed to encourage innovation. Dr. Mishra is an elected fellow of the major science academies in India and a recipient of several honors and awards, including the JC Bose National Fellowship.

Proteomic analysis of nuclear matrix: evolution of nuclear architecture and structural basis of cellular memory

The nucleus is a highly structured organelle and contains many functional compartments. A major component of this organization is likely to be the non-chromatin scaffolding called the nuclear matrix (NuMat). Experimental evidence over the past decades indicates that most of the nuclear functions are at least transiently associated with the NuMat. Earlier, we reported NuMat proteome analysis from Drosophila melanogaster embryos which revealed its links with nuclear architecture and functions.

We also discovered, from a comparative analysis of the NuMat proteomes of different stages of embryos that 65% of the NuMat proteome is dynamic during development. Further, we showed that cultured skeletal muscle cells in three morphologically and functionally distinct cellular states—proliferating myoblasts (MBs), terminally differentiated myotubes (MTs), and mitotically quiescent (G0) myoblasts have ~40% of the proteins common in the NuMat proteome. Like in different stages of Drosophila embryos, in mammalian system, about two third of the NuMat is dynamic. This NuMat dynamics suggest a possible functional link between NuMat and embryonic development.

In order to explore the origin and evolutionary conservation of NuMat components we used Drosophila melanogaster and Danio rerio embryos to identify core NuMat proteins that are conserved between the two organisms. We further compared our results with Mus musculus NuMat dataset and Homo sapiens cellular localization dataset to define the core homologous NuMat proteins across the evolutionary lineage that consists of 252 proteins out of which 86 have originated from the pre-existing proteins in prokaryotes. Our analysis paves the way to understand the evolution of the complex internal nuclear architecture and its functions.

Finally, we ask how such a complex and dynamic architecture which has functional implication is preserved and re-established during mitosis. We compared the proteome of NuMat and an equivalent biochemical fraction in the mitotic chromosome known as mitotic chromosome scaffold (MiCS). Our study elucidates that as much as 67% of the NuMat proteins are retained in the MiCS indicating that the features of nuclear architecture in interphase nucleus are retained on the mitotic chromosomes. We propose that the structural context of NuMat, are retained in MiCS and possibly play key role in retaining cellular memory during cell division.

IL-04

Dr. Kausik Chakraborty

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Dr. Chakraborty earned his PhD degree from the Indian Institute of Science, Bangalore. Currently he works at the CSIR-Institute of Genomics and Integrative Biology, New Delhi. His lab is majorly interested in metabolic regulation of protein folding. They are dissecting the phenomenon of metabolic homeostasis and its role in governing protein folding *in vivo*, understanding cellular sensors of protein misfolding along with identifying the pathways that regulate proteostasis to understand their mechanism as well as designing small molecules to assist intracellular protein folding.

He was awarded the INSA medal for Young Scientists in 2012 and S. Ramachandran - National Bioscience Award for Career Development in 2019.

Metabolism and its role in cellular proteostasis

Cellular proteostasis is maintained by a plethora of molecular chaperones and proteins that participate in Quality control. Metabolites in addition to these large protein macromolecules also play a role in cellular proteostasis; we have started appreciating this fact only recently. Our work is aimed at understanding the contribution of metabolites to proteostasis and the role of metabolic perturbations in proteotoxicity. Here we will aim to provide evidence on how we use metabolomics/proteomics/genomics to study this connection.

Dr. Siddhesh Kamat

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Dr. Kamat received his PhD degree from Texas A&M University, USA. Later he joined the Scripps Research Institute for his Postdoctoral research. Currently, he works at the Indian Institute of Science Education and Research (IISER), Pune. His lab uses advanced Mass- spectrometry based metabolomics and proteomics techniques to study lipid signaling and metabolism in the nervous and immune system.

He has earned a number of awards and fellowships like Merck Young Scientist Award in Biological Sciences (2019), Indian National Science Academy (INSA) Young Scientist Medal (2019), DST-SERB India, Early Career Research Award (April 2017 - March 2020). He is also an adjunct faculty at the Tata Institute of Fundamental Research (TIFR), Mumbai.

Mapping Sphingolipid Pathways During Phagosomal Maturation

Phagocytosis is an important physiological process, which, in higher organisms is a means of fighting infections and clearing cellular debris. During phagocytosis, detrimental foreign particles (e.g. pathogens, apoptotic cells) are engulfed by phagocytes (e.g. macrophages), enclosed in membrane-bound vesicles called phagosomes, and transported to the lysosome for eventual detoxification. During this well-choreographed process, the nascent phagosome (also called early phagosome, EP) undergoes a series of spatiotemporally regulated changes in its protein and lipid composition, and matures into a late phagosome (LP), that subsequently fuses with the lysosomal membrane to form the phagolysosome. While several elegant proteomics studies have identified the role of unique proteins during phagosomal maturation, corresponding lipidomics studies are sparse. In this talk, I will present our LC-MS/MS based targeted lipidomics and proteomics approaches in mapping diverse sphingolipid pathways, and possible implications of this lipid class during phagosomal maturation.
nnection.

PL-02

Prof. Dr. Matthias Mann

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Prof. Mann received his PhD from Yale University. He carried out his Postdoctoral research at the University of Southern Denmark, Odense. He currently heads the department of Proteomics and Signal Transduction at the Max Planck Institute of Biochemistry, Germany. He is also the Director of the proteomics program at the Novo Nordisk Foundation Center for Protein Research (NNF-CPR) in Copenhagen where he also leads the Clinical Proteomics group. His prominent research works includes the development of peptide sequence tags, which was one of the first methods used to identify peptides on mass spectra. Prof Mann is the pioneer of a metabolic labeling technique called SILAC (stable isotope labeling with amino acids in cell culture) which is now widely used in quantitative proteomics. Currently his lab focuses on furthering technological advancements in mass spectrometry and its downstream computational analysis.

From his research group in Munich, originated PreOmics - a company commercializing sample prep sets, and EVOSEP - a company commercializing protein analysis equipment. He has received numerous distinctions, particularly the Lundbeck and the Novo Nordisk Research Prizes, the Meyenburg Cancer Research Award, the Schelling and the Leibniz Prizes. Prof. Mann has served at the European Molecular Biology Laboratory, Heidelberg as Group Leader. He has authored over 700 peer-reviewed publications and is one of the most widely cited researchers in the world.

Ultra-high sensitive and computational workflows for single cell and deep visual proteomics

Mass spectrometry (MS)-based proteomics field has made tremendous technological improvements in the last decade. Recently, new instrument types based on time of flight (TOF) technology have gained interest. In this lecture, I will describe the trapped ion mobility (tims) – Parallel accumulation serial fragmentation (PASEF) technology developed in my department. When coupled with low flow chromatography and an improved ion source, timsPASEF enables analysis of single cells and accurately describe their heterogeneity. We have used this technology for Deep Visual Proteomics (DVP), where we combine high resolution microscopy, automated image recognition by AI and ultrasensitive timsPASEF analysis in data independent mode (diaPASEF) to dissect cell type-specific proteome change under normal and pathological-states. All these workflows are embedded in our software suites called AlphaPept and Clinical Knowledge Graph. These efforts bring us closer towards precision oncology for the future.

Dr. Shantanu Sengupta.

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Dr. Sengupta received his PhD degree from the National Institute of Immunology, New Delhi. For his Postdoctoral training he moved to University of Pennsylvania School of Medicine, Philadelphia. He currently works at the CSIR-Institute of Genomics and Integrative Biology, New Delhi. His lab is interested in identifying pre-diagnostic markers for cardiovascular diseases using genetic, epigenetic and proteomic approaches. Research from his lab had shown that vitamin B12 deficiency is associated with Coronary Artery Disease in the Indian population. They have also developed a map of mitochondrial methylomes and showed that there are distinct patterns of epigenetic regulation in mitochondria.

He received the National Bioscience Award for Career Development in 2011.

Proteomics in Clinical Practice: Bridging the gap from discovery to Application

Mass spectrometry-based proteomics is a powerful technique that provides comprehensive insight into protein changes during the course of disease. Application of the knowledge gained from proteomic analysis in clinical settings can aid in disease diagnosis and prognosis. From markers that could aid in diagnosis and/or prognosis to developing quantitative assays could be done using mass spectrometry-based proteomics. In one such study, we have identified proteins which could potentially be used as markers for celiac disease, a chronic digestive disorder resulting from an immune reaction to gliadin, a gluten protein found in wheat, barley, rye, and sometimes oats. Initially a SWATH-MS based quantitative proteomics was performed in duodenal biopsy tissues from celiac disease patients, patients with other enteropathies and controls (patients with gastrointestinal reflux disorder). We identified several proteins that were upregulated in celiac disease. We are currently validating these proteins using MRM based assay. Apart from diagnosis, proteomics can aid in prognosis of diseases. For instance, we were able to identify proteins that are altered at 3 to 7 days post COVID-19 infection which could throw some light on the etiology of the disease. Proteomics can increase the predictive accuracy of diseases if done in a longitudinal prospective cohort. Towards this, we propose to develop a Pan- India long term prospective longitudinal cohort and develop MRM based assays that could be the future of Clinical Proteomics.

Dr. Alka Rao

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Dr. Alka Rao works at the CSIR- Institute of Microbial Technology, Chandigarh. Her group works is interested on elucidating the post-translational modifications of proteins namely glycosylation and N- α acetylation, in bacteria. They have successfully developed the enzymatic method for S-diglycosylation of proteins and peptides using bacterial enzymes which proves helpful in improving food preservation, antibiotic resistance management, pharmaceutical and industrial enzyme production. They have also developed growth -promoting products for crop application in collaboration with Criyagen Agri and Biotech Pvt. Ltd.

She was recently appointed as a Member of the National Biodiversity Authority by the Ministry of Environment, Forest and Climate Change, Govt of India in 2020. Apart from this, she is also a member of Navodaya Vidya Samiti (NVS) and Executive Committee of NVS, MHRD, Govt of India, member of Scientific panel on food additives, FSSAI, MoHFW, Govt of India, Member of Department of Biotechnology (DBT).

Protein Glycosylation in *Actinobacteria*: How Sweet !

Actinobacteria are industrially important bacteria and are the largest producer of a variety of macrolide antibiotics. *Actinobacteria* also include human and plant pathogens. As early as in 1971, a phytotoxin harboring a threonine-linked glycan was discovered in *Corynebacterium*. Since then, several biologically important glycoproteins have been observed in the culture filtrates of different *Actinobacteria*. Similar to eukaryotes, an O-mannosylation mechanism and a membrane-associated O-mannosyl transferase were characterized in *Mycobacterium tuberculosis* in 2005. It was soon followed by the identification of the protein O-mannosylation pathway in *Corynebacteria* and *Streptomyces*. Now in 2021, our group at CSIR IMTECH reports a rather rare form of glycosylation, namely S-glycosylation, in *Streptomyces venezuelae* ATCC 15439. This talk will focus on sharing the traditional understanding and new experimental evidence about protein glycosylation in actinobacteria.

Dr. Inderjeet Kaur

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Dr. Inderjeet Kaur obtained her PhD degree from the Guru Nanak Dev University (GNDU), Amritsar. She served as a lecturer at GNDU before joining L V Prasad Eye Institute to continue her research on molecular genetics, with a focus on the genetics of eye disease. Her research focuses on understanding the molecular mechanisms in complex eye diseases, age-related macular degeneration (AMD), myopia and glaucoma.

She received the Young Investigator (Merit) Award (Basic Sciences) from the Association on Research in Vision and Ophthalmology (ARVO) at the SERI-ARVO 2007 meeting, Singapore, and the Amjad Rahi Prize (Indian Eye Research Group, Hyderabad, 2005). She is also the recipient of the B M Birla Science Medal, 2011. She was a visiting Scientist at Massachusetts Eye and Ear Infirmary, USA.

Identification of predictive marker(s)for an early diagnosis of a blinding disorder in premature children using a multiOMICS approach

Retinopathy of prematurity (ROP) is a neurovascular complication of preterm birth that causes severe visual impairment in children worldwide and has a high prevalence (35-40%) in India. Preterm babies born with extremely low gestational ages and birth weights are likely to develop ROP, but its progression is highly variable and unpredictable. Our initial analysis using targeted microarray revealed a potential role for genes involved in angiogenesis, development of fetal retina, trans-endothelial migration, oxidative stress, inflammation, cholesterol metabolism and neurodegenerative processes in ROP pathogenesis. Global transcriptomics and proteomics of blood and vitreous humor corroborated these findings and revealed that activated microglial cells in the retina under hypoxia expressed complement C3, VEGF, MMP2 & 9 and IL-1β resulting in abnormal blood vessel proliferation. Induction of hypoxic stress to microglial cells led to the downregulation of MAPK, WNT and NOTCH1 signaling, which could be rescued via inhibition of MMP activity with doxycycline. We also evaluated the possibility of MMP and C3 levels in tears as potential markers for predictive testing of preterm babies in the community. A severity dependent activation of MMPs was seen in ROP tears. Since metabolic pathways were found associated with ROP based on both genomics and global transcriptome analysis, further exploration of metabolic activity in vitreous humor of ROP was undertaken through a global metabolome profiling of ROP, which indicated several vital pathways with a significant upregulation of metabolites in energy, fatty acid, amino acid and nucleotide degradation. In summary, MMP9 activation correlated with ROP and can be a predictive marker for disease progression. Our multi-OMICS approach for understanding ROP pathogenesis demonstrated the potential involvement of several deregulated signaling pathways including WNT, MAPK, NOTCH1 and metabolism of nucleotide degradation, amino acids and lipids in ROP pathogenesis.

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Dr. Nandicoori received his PhD degree from the Indian Institute of Sciences , Bangalore and Postdoctoral training from the A & M University, Texas, USA and University of Virginia, USA. Later he joined National Institute of Immunology, New Delhi as scientist. Currently, he is the Director of CSIR- Centre for Cellular and Molecular Biology, Hyderabad. His research interest lies in understanding kinase-mediated signaling networks in *Mycobacterium tuberculosis* and their role in regulating cellular events of the pathogen and host-pathogen interactions.

He has been awarded with the NASI-Scopus Young Scientist Award in 2009, the National Bioscience Award for Career Development in 2010 and is an elected member of Guha Research Conference, India (GRC). He is also a fellow of The National Academy of Sciences (India), Allahabad since 2014.

Mycobacterium tuberculosis virulence and survival & the role of phosphorylation

Tuberculosis has been a long-standing problem in INDIA and due to the emergence of drug-resistant strains, the search for new drug targets continues. We have been working on delineating phosphorylation based signaling cascades modulated by Eukaryotic like Serine/Threonine Protein Kinases and phosphatase in Mtb. With the help of conditional mutants, we established that kinases PknA, PknB and phosphatase PstP are essential for both in vitro and in vivo growth. With the help of high throughput phospho-proteomics, we established that abrogation of PknB ligand-binding is linked to activation loop hyperphosphorylation and indiscriminate hyper-phosphorylation of PknB substrates as well as other proteins, ultimately causing loss of homeostasis and cell death.

Virulence effectors secreted by *Mycobacterium tuberculosis* help subvert host immune mechanisms and therefore are critical for the establishment of infection and pathogenesis. However, knowledge in terms of signaling mechanisms that modulate the secretion of virulence factors is sparse. Using high-throughput Mass-spectrometric analysis of mycobacterial secretome, phosphoproteome and phospho-secretome combined with empirical validations, we showed fascinating regulation of mycobacterial secretion via protein phosphorylation. I would like to discuss our approach and results in the above two studies.

IL-10

Dr. Arun Bandyopadhyay

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Dr. Bandyopadhyay is the Director of the CSIR- Indian Institute of Chemical Biology, Kolkata. His research mainly deals with identification of protein biomarkers for risk assessment of cardiovascular diseases. His lab also works on understanding the biochemical basis of compromised reverse cholesterol transport in humans and inflammation resolution in plaque stability and implications in atherosclerosis. His lab is interested in unveiling mitochondrial dynamics in cardiac hypertrophy, miRNA mediated molecular regulation of mitochondrial dysfunction in cardiac hypertrophy and identification of small molecules for the management of respiratory disorders.

He is a fellow of The National Academy of Sciences (India).

Proteomic approaches for understanding molecular basis of inflammation in atherosclerosis

The current advancement in proteomic technologies helps studying global protein expression changes associated with human disease processes. The detection, identification and characterization of variations in the proteome occurring during the course of heart disease provide insight into the underlying molecular mechanisms and potential cardiac specific biomarkers for regular, systematic observation and assessment of cardiac status. Cholesterol is a vital component of the cell, and its homeostasis is one of the critically regulated process. Although Reverse cholesterol transport (RCT) plays a critical role in removing cholesterol from the arterial wall very few reports directly relate chronic inflammation and RCT with atherosclerosis. Mass spectrometric analysis of the human plasma identified about 2500 proteins in subjects with myocardial infarction. Computational study indicated that most of the identified proteins were related to chronic inflammation, atherosclerosis and RCT. To understand the pathophysiological significance of the identified proteins, macrophage derived foam cells were utilized for their critical role in RCT which indicated the imbalance of RCT via the interaction of AZGP1 with CD36. We also found that ABCA1, the primary cholesterol transporter was downregulated in hyper-cholesterol conditions in macrophages, which might be responsible for compromised reverse cholesterol transport (RCT) and hyperlipidemia. Surprisingly, ABCA5, a lesser known family member was upregulated to maintain cholesterol efflux. We established ABCA5 as the primary efflux mediator under high cholesterol load. These observations were further validated in-vivo using mice models of atherosclerosis (ApoE-/-) and hyperlipidemia (PPARα-/-) in response to high cholesterol diet. Computational analysis revealed a unique conformation of ABCA5 proposing a favored route for cholesterol loading onto HDLs for reverse cholesterol transport especially in case of hyperlipidaemia. In overall, the present study demonstrates a biochemical basis for compromised reverse cholesterol transport in the local milieu of the luminal wall of the artery which are critical for atheroinflammation and atheroprogression.

Dr. Sanjeeva Srivastava

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Dr. Srivastava obtained his PhD degree from the University of Alberta, Canada and carried out his Postdoctoral research at the Harvard Medical School, USA. Currently, he is the Group Head of proteomics laboratory at the Indian Institute of Technology, Bombay. His lab focuses on discovery of biomarkers and drug targets and deciphering the protein interaction networks in complex human diseases (gliomas) and infectious diseases (malaria) using high throughput proteomics, protein microarrays and mass spectrometry.

Dr. Srivastava serves on the Executive Council of Human Proteome Organization (HUPO). One of his special contributions has been the development of e-learning resources (MOOC - mass spectrometry and interactomics courses; Virtual Proteomics Laboratory). He has made first ever proteomics documentaries - "Proteomics: Translating the Code of Life" and "Human Proteome Project (HPP)". He has directed the HUPO "Perspective in Proteomics" video interview series, which is hosted on HUPO website.

Mass spectrometry based proteomics and Fourier transform infrared spectroscopy for diagnosis and prognosis of COVID-19 infection

During the past year, the understanding of COVID-19 severity has strengthened substantially. Using Mass-spectrometry based proteomics, metabolomics & ML approaches, we discovered classifiers of COVID-19 severity such as AGT, FGG, APOB and SERPINA3 and also developed targeted Selected Reaction Monitoring assays for clinical translation. The altered plasma proteome of COVID-19 severe patients revealed dysregulation of peptidase activity, regulated exocytosis and myeloid leukocyte activation pathways. This study revealed that mass spectrometry-based peptide tests can be used by the clinicians for diagnosis as well as identified pathways/ markers as the predictors of the disease progression. Further, we also investigated the potential of attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy as a rapid blood test for classification of COVID-19 disease severity using a cohort of 160 COVID-19 patients. In summary, this study demonstrates the potential of ATR-FTIR spectroscopy as a rapid, low-cost COVID-19 severity triage tool to facilitate COVID-19 patient management during an outbreak.

Dr. Dhanasekaran Shanmugam

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Dr. Shanmugan obtained his PhD degree from the Indian Institute of Science, Bangalore followed by his Postdoctoral research at the University of Pennsylvania, USA. He is currently serving at the CSIR- National Chemical Laboratory, Pune. His research focuses on studying various aspects of parasite metabolism, especially those that are unique to the parasite or distinct from host. His lab studies various aspects of metabolic adaptation evolved by these parasites to sustain a parasitic life style using a combination of genomic, biochemical, genetic and metabolomic techniques.

Characterizing evolutionarily distinct and highly diverged proteins with conserved functions from the parasite *Toxoplasma gondii*

Parasitic protozoa possess many evolutionarily distinct and divergent proteins in comparison to their human and animal hosts. These proteins are considered as attractive drug targets as many of them appear to perform parasite specific functions essential for survival. Our work on metabolism and sub-cellular protein targeting in the parasite *Toxoplasma gondii* has resulted in the identification and characterization of many novel parasite proteins with important functions. Bulk ATP synthesis in the parasite mitochondrion is accomplished by the F-type ATP synthase (Complex V) which is a multi-protein complex. The enzyme complex consists of two distinct parts; the F1 sector, which has highly conserved subunit composition, and the FO sector, which is missing most of its subunits. We have isolated the intact native F-type ATP synthase from enriched *T. gondii* mitochondria and identified at least 20 novel subunit components which are critical for the functional and structural integrity of the *T. gondii* F-type ATP synthase. Tail anchoring of proteins in the ER, and likely in other sub cellular membranes, is achieved by the coordinated action of five different GET chaperones. Although *T. gondii* encodes a definite set of tail anchored proteins, whether a functional GET pathway exists in this parasite was not clear since three out of five canonical GET chaperones could not be readily identified by sequence. Using proximity labelling technique we could successfully identify two novel GET chaperones and confirm the existence of a functional GET pathway in *T. gondii*. The evolutionary origin of these divergent proteins and the implications of their conservation within a parasitic phylum will be discussed.

IL-13

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Dr. Suryawanshi received his PhD from the National Institute for Research in Reproductive Health (ICMR), University of Mumbai. He is currently working in the field of Clinical Proteomics and Proteoinformatics at the Institute of Life Sciences, Bhubaneshwar. His major research focus is on Disease Proteome Mapping, Bio-marker discovery, and identification of post-translational modifications in proteins with respect to their role in disease pathogenesis. He also investigates Cancer and viral diseases particularly Rabies, CHIKV, Dengue, SARS-CoV-2 etc.

He is a member of the Human Proteome Organization (HUPO). He is also a life member of the Laboratory Animals Scientist Association, India (LASA), Society of Biological Chemists, India (SBC), and the Indian Society for Mass Spectrometry (ISMAS).

Quantitative proteomics approaches leads to identify differentially expressed brain proteins involved in furious rabies virus infection

Rabies, a neglected zoonotic viral disease caused by the rabies virus (RABV) has the highest fatality rate among all infectious diseases. Despite the existence of control measures, dog-transmitted human rabies accounts for 56,000 annual deaths world-wide with 60% deaths being reported in India with approximately three times more occurrence of furious form of rabies than the paralytic form. Currently, there is no suitable diagnostic tool for rabies before the onset of clinical symptoms and once symptoms appear; death is ultimate within a short period due to unavailability of therapeutics. Therefore, identification of host proteins altered due to RABV infection may provide some insight into the molecular pathophysiology of rabies. In this study, we aimed to identify and characterize the differentially expressed proteins (DEPs) involved in rabies virus infection using multiple quantitative proteomic approaches. First, iTRAQ coupled LC-MALDI MS/MS approach was performed using rabies-infected and control dog brain tissue samples and 477 proteins including 19 DEPs were identified. In another approach iTRAQ-8plex coupled with HRMS could identify a significantly total 2,188 brain proteins, including 140 DEPs in furious rabies-infected cases compared to controls. Furthermore, the statistical analysis showed that 26 proteins were down-regulated and 14 proteins were up-regulated significantly in the furious rabies-infected cases. Our analysis showed that some of these molecules are novel. In addition, it showed that most of these proteins have human homologues. Analysis with GO annotation and IPA showed that proteins associated with calcium signaling and calcium transport pathway were most affected due to RABV infection along with efficient neuronal function proteins and metabolic pathway associated proteins.

Further, neurological disease and psychological disorders were identified as top diseases and disorders. Some of these proteins were successfully validated by qRT-PCR and two proteins were successfully validated by western blot. This study provides the list of altered proteins and their probable role in RABV infection. However further studies are needed to confirm their role and to understand their utility in rabies pathogenesis which is currently in progress.

TL-01

Dr. Nick Morrice

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Nick Morrice obtained his PhD from the University of London, UK and after 7 years at the University of Melbourne in Australia, joined the MRC Protein Phosphorylation Unit at the University of Dundee, UK. Here he set up the proteomics facility for both the University of Dundee and the MRC Unit, specializing in protein phosphorylation site analysis using a combination of mass spectrometry and Edman sequencing of radiolabelled phosphopeptides. He became a group leader in 2002 running both a research group and core facility before moving to the Beatson Institute for Cancer Research In 2010 as Head of Proteomics and Group Leader. After setting up a successful proteomics and metabolomics facility he joined Sciex in 2014 as a senior support specialist for proteomics. His main focus with the company is developing microflow applications with the TripleTOF and zenoTOF mass spectrometers to improve throughput of both DDA and Swath analyses of complex biological samples.

Nick has published over 200 papers in the field of signal transduction and proteomics and has been a member of a number of scientific societies such as The Biochemical Society and the ABRF.

His talk will be focused on Powerful new proteomic workflows enabled by the SCIEX ZenoTOF system

PL-03

Prof. Angus. I Lamond

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Prof. Lamond received his PhD degree from the MRC laboratory of Molecular Biology, UK and carried out his Postdoctoral training at the Massachusetts Institute of Technology, USA. Currently he is a Professor of Biochemistry at the University of Dundee, UK. His lab's interest lies in studying various biological processes such as RNA processing, nuclear organization, protein turnover kinetics and post-translational protein modifications. His group his also involved in studying organellar proteome and gene regulatory mechanisms in major cellular processes like cell cycle progression and oncogenic progression. He has pioneered various new approaches, combining quantitative proteomics and novel computational strategies for the system-wide analysis of cellular responses. To name a few are PepTracker, a tool for quantitative mass-spec data management and visualization, and Encyclopaedia of protein dynamics, a collection of multi-dimensional proteome database.

He has received numerous distinctions such as Colwarth medal and Novartis medal by British Biochemical Society, Elected fellow of The Royal Society of Edinburgh (1996), Elected fellow of The Royal Society (2010), Elected fellow of The Academy of Medical Sciences (2014). Prof. Lamond, also served at EMBL, Heidelberg, as a Group leader. He has authored over 250 peer-reviewed publications.

Proteomic analyses of human stem cells

Deep mining of proteomes, using Mass spectrometry (MS) based technology, can provide invaluable insights, at a systems level, into cell physiology and disease phenotypes. A further challenge concerns how to analyze and integrate these proteomic data with other parallel 'omics' and cell phenotypic data and how to manage the large resulting volumes of complex information. I will describe our progress in using quantitative proteomics for the large-scale analysis of human induced pluripotent stem cells (iPSCs), including recent detailed comparisons between independent iPSC and human ESC lines. We have generated a deep proteome of human iPS cells and characterized the major determinants affecting proteome variation across multiple human iPSC lines from healthy donors. These data identified >700 human iPSC protein quantitative trait loci (pQTLs), for which we mapped trans regulatory effects. We also identified the impact on the proteome of loss of X chromosome inactivation in iPSC lines derived from healthy female donors. Finally, I will discuss computational approaches for visualizing, sharing and interactively exploring large, poly-omics data sets.

Dr. Juergen Cox

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Dr. Cox received his PhD in Physics from the Massachusetts Institute of Technology, USA. He worked as a Scientific Consultant and Algorithm Developer at the Genedata, Martinsried, Germany before starting his Postdoctoral research at the Technical University of Munich, Institute for Genome-Oriented Bioinformatics, Freising, Germany. Dr. Cox is currently a Group Leader at the Max Planck Institute of Biochemistry. His lab develops algorithms and tools for analyzing the vast amounts of spectral data that are produced in modern proteomics experiments. While working at the Max Planck Institute of Biochemistry, he developed MaxQuant and Perseus software, a worldwide popular platform for computer-based proteomics data analysis. He served as an Honorary Professor of Proteomics at the University of Copenhagen, Denmark.

He received Mass Spectrometry in the Life Sciences Award from the German Society for Mass Spectrometry and Gilbert S. Omenn Computational Proteomics Award from US HUPO.

MaxDIA enables library-based and library-free data-independent acquisition proteomics

MaxDIA is a universal platform for analyzing data-independent acquisition proteomics data within the MaxQuant software environment. Using spectral libraries, MaxDIA achieves cutting-edge proteome coverage with significantly better coefficients of variation in protein quantification than other software. MaxDIA is equipped with accurate false discovery rate estimates on both library-to-DIA match and protein levels, also when using whole-proteome predicted spectral libraries. This is the foundation of discovery DIA - a framework for the hypothesis-free analysis of DIA samples without library and with reliable FDR control. MaxDIA performs three- or four-dimensional feature detection of fragment data and scoring of matches is augmented by machine learning on the features of an identification. MaxDIA's novel bootstrap-DIA workflow performs multiple rounds of matching with increasing quality of recalibration and stringency of matching to the library. Combining MaxDIA with two new technologies, BoxCar acquisition and trapped ion mobility spectrometry, both lead to deep and accurate proteome quantification.

Prof. Jyoti Choudhary

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Prof. Choudhary received her PhD and Postdoctoral training in Biochemistry from the Imperial College, London. She is currently a Professor and head of the Proteomics Core Facility and Career Faculty Leader of the Functional Proteomics Research Group at The Institute of Cancer Research, England. Her research focuses on the development and implementation of novel Mass-spectrometry and data analysis approaches for proteome discovery. Her lab is mainly involved in understanding how the organization and dynamics of protein networks underpin cancer progression and resistance. Her group is extensively engaged in developing bioinformatics tools and techniques for improved protein identification and its modification through mass spectrometry.

She was one of the founders and head of Mass-spectrometry facility at Cellzome, Chemoproteomics technology, which monitors the interaction of small molecules with their protein targets to profile the effect of drugs in different cell and tissue proteomes. She is one of the contributors of GENCODE consortium to provide proteomics data to assist the complete annotation of human and mouse protein coding genes.

Her talk will be focused on Integrative Proteogenomics: Deconvoluting genetic determinants of protein abundance variation

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Dr. Swaminathan pursued his PhD degree from the Edward Marcotte lab in Center for Systems and Synthetic Biology, The University of Texas at Austin. He continued as a Postdoctoral fellow at the same university. In 2018, he co-founded Erisyon Inc. with Talli Somekh. The company is doing pioneering work in single molecule protein sequencing.

Sample preparation for Single molecule protein sequencing technology - Fluorosequencing

Mass-spectrometry is the primary instrumental technology for identifying proteins and understanding the complexity and dynamics of proteomes. However, current techniques are limited in digital quantitation and sensitivity, especially in the identification of less abundant proteins. We overcame these limitations through a single molecule method for identifying peptides in a highly parallel fashion, through selective labeling of multiple amino acid residues with fluorophores and observing the patterns of fluorescence changes on individual peptide molecules through cycles of Total Internal Reflection Fluorescence microscopy (TIRF) imaging and and in-place Edman degradation. The mapping of the partial fluorescence sequence (fluorosequence) to reference protein databases identifies the source protein. Here, we describe the unique sample preparation process for the technology, which includes selective labeling of amino acid side chains with fluorophores as well as targeted coupling of peptide's N and C-terminal amino acids. The method incorporates solid-phase attachment of peptides, which not only improves the performance of multiple chemical labeling steps, but also stabilizes them for transport. Automation of the process is described for enhancing the utility of such single molecule protein sequencing technology.

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Dr. Jagtap recieved his PhD degree from the Centre for Cellular and Molecular Biology, Hyderabad. Later he moved to Max-Planck Institute for Developmental biology, Germany and University of Michigan, USA for his Postdoctoral research. Currently, he is a Research Assistant Professor at the Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota. His research interests include developing analytical workflows for the analysis of complex data, with particular emphasis on MS-based proteomics applications in metaproteomics, proteogenomics, and Data Independent acquisition (DIA) data analysis.

He has helped to manage the Galaxy-P project from its inception. He also served as Managing Director of the Center for Mass Spectrometry and Proteomics at the University of Minnesota.

Metaproteomics: Promoting Functional Analysis of Microbiome through online educational resources via the Galaxy Platform

Microbiomes play a critical role in health and disease in human hosts and in environmental ecosystems. Characterizing their functional role requires analysis of multi-omics data such as metaproteomics - the large-scale characterization of the entire proteome of environmental microbiota at a given point in time.

Functional microbiome analysis using metaproteomics methods offers advantages over traditional metagenomics methods in that it can help in understanding functions expressed by the microbiome along with taxonomic composition.

However, the implementation of the software tools in a workflow to a researcher is not trivial. To facilitate this, we have incorporated bioinformatics workflows within the Galaxy framework. The Galaxy for Proteomics (Galaxy-P) team has been conducting workshops at various annual research conferences and via novel online resources for the last four years.

The talk will present our work on the use of metaproteomics workflows within Galaxy framework to analyze the taxonomic and functional state of microbiomes and generate outputs useful for biological interpretation.

Dr. Shankha Satpathy

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Dr. Satpathy received his PhD degree from the University of Copenhagen, Denmark. After finishing his Postdoctoral research in Broad Institute of MIT and Harvard, USA he joined there as a Research Scientist. Currently, he holds a Group Leader position at the Proteomics Platform of Broad Institute. His research area intersects multiple disciplines involving both basic scientists and clinicians. His lab leads many clinical proteomics and proteogenomic projects. He is involved in the comprehensive proteogenomic characterization of lung adenocarcinoma and lung squamous cell carcinoma.

Dr. Satpathy also contributes to industry and academic collaborative projects using mass spectrometry and other proteomics tools to investigate biological questions with potential translational impact.

Dissecting proteogenomic vulnerabilities in cancers

Genomic analyses in cancer have been enormously impactful, leading to the identification of driver mutations and development of targeted therapies. But the functions of the vast majority of somatic mutations and copy number variants in tumors remain unknown, and the causes of resistance to targeted therapies and methods to overcome them are poorly defined. Recent improvements in mass spectrometry-based proteomics now enable the ability to look directly at the consequences of genomic aberrations, providing deep and quantitative analyses of tumor tissues. Integration of proteins and their post-translational modifications identified by proteomics with genomic, epigenomic, and transcriptomic data constitutes the new field of proteogenomics, and it is already leading to new biological and diagnostic knowledge with potential to improve our understanding of malignant transformation and therapeutic outcomes. I will describe recent developments and key findings obtained using proteogenomics to analyze Lung and Breast Cancer (1-2), the most dominant cancers world-wide, and describe proteogenomic methods (3) being developed to address clinical questions.
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PL-04

Prof. John Yates III

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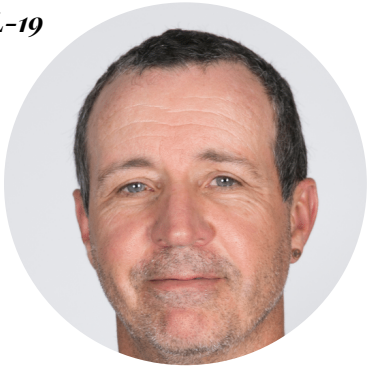
Prof. Yates completed his PhD from the University of Virginia, USA. He is currently a professor of Chemical Biology at the Molecular Medicine Department, Scripps Research Institute, USA. His lab focuses on development and application of mass spectrometry-based proteomics techniques to answer biological questions. They have played instrumental role in the evolution of proteomic field with the introduction of new tools like Multidimensional Protein Identification Technology (MudPIT), pulse Azidohomoalanine labelling in mammals (PALM) to quantify the newly synthesized protein, capillary electrophoresis followed by mass-spectrometry to characterize protein complexes. His lab uses proteomics study to unravel molecular mechanisms involved in Cystic Fibrosis, investigations into affective disorders of the brain, including schizophrenia and depression.

He has been honored with multiple awards including American Society for Mass Spectrometry Research Award in 1996, Pehr Edman Award in 1998, HUPO Distinguished Achievement Award in Proteomics, Germany in 2005, ACS award in analytical chemistry in 2015. He has authored over 700 peer-reviewed publications and is one of the most widely cited researchers in the world.

Combined single neuron Patch-Clamp/Mass-Spectrometry analyses (PatchC-MS)

As interest in single-cell analysis increases, performing single cell MS still remains a challenge. Herein we demonstrate patch-clamp electrophysiological recordings of single human iPSC-derived neurons followed by mass spectrometry analysis of the same cell. Human induced pluripotent stem cell (hiPSC)-derived cerebrocortical neurons are evaluated electrophysiologically by whole-cell recordings with a patch electrode capillary. The neuron is then aspirated into the capillary and expelled into a microtube .A simple digestion protocol is performed, and samples are analyzed by mass spectrometry. The single-cell digests are separated by nanoflow UPLC coupled to a Bruker timsTOF or a Thermo Eclipse, both operating in data dependent modes. Whole-cell recordings were performed on Alzheimer's disease (AD) and isogenic, gene-corrected control (wild-type/WT) hiPSC-derived cerebrocortical neurons. WT neurons of interest were chosen based on their ability to fire action potentials, manifest voltage-gated sodium and potassium currents, and neurotransmitter-mediated postsynaptic currents. We have previously published that AD hiPSC neurons, like those in human AD brain, exhibit enhanced spontaneous action potential frequency, increased voltage gated sodium currents, and increased excitatory postsynaptic current frequency compared to WT neurons (Ghatak et al., eLIFE, 2019). We selected these AD neurons to compare to WT controls for further proteomic analysis.MS data analysis was performed with ProLuCID, Byonic and MSFragger. When injecting half of the contents of a single digested neuron, we were able to identify between 400-2000 proteins per sample. We performed single-cell patch-clamp electrophysiology combined with mass spectrometry proteomic analysis.



Prof. Paul A. Haynes

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Dr. Haynes graduated from the Macquarie University, Australia with a PhD in chemistry. He continued his Postdoctoral research at the Rockefeller University, USA, and the University of Washington, USA. Currently, he is a Professor in the Department of Molecular Science at the Macquarie University, Australia. His specializations are in plant and environmental proteomics. Research in his lab focuses on how cells from different organisms respond to the imposition of external stresses. His research laboratory has also branched out in recent years to include an active program in bioarchaeological proteomics.

He is President of the Asia Oceania Agricultural Proteomics Organisation (AOAPO) and a member of the 2020 ARC College of Experts. He also served at the Torrey Mesa Research Institute, San Diego as Principal Scientist and the University of Arizona, Tucson as Associate Professor.

Proteomic analysis of different varieties and species of rice under various stress conditions

Rice is one of the most important food crops in the world, and the productivity of rice crops is threatened by a number of different environmental stresses. We have investigated the proteomic response of rice varieties and species with different genetic backgrounds, when exposed to a range of different abiotic stresses, including drought, high and low temperatures, and salt.

Physiological parameters including leaf water potential and plant growth rates were measured. Proteins from tissues of young rice plants were extracted, peptides were separated using reversed phase nanoLC, and identified and quantified using high resolution orbitrap mass spectrometry, followed by peptide to spectrum matching.

This presentation integrates results from a number of different rice stress response studies performed in our laboratory in recent years. In one study, plants from 8 different rice varieties were subjected to drought stress and recovery. Proteins involved in proteolytic processing pathways were significantly increased in abundance, while many proteins significantly reduced in abundance in stress conditions were involved in photosynthesis. Some proteins were uniquely expressed in specific genotypes, while 8 proteins were up-regulated in response to drought stress in all genotypes, including actin-depolymerizing factor 3 (ADF-3) and GSH-dependent dehydroascorbate reductase 1. In a separate study, three different species of rice were exposed to drought stress: O. sativa cv. Nipponbare; Oryza australiensis; and Oryza glaberrima cv. CG14. The O. australiensis was able to retain more water in leaf cells, than the other two species. A majority of proteins increased in abundance in stress conditions in O. australiensis were associated with photosynthesis and carbohydrate biosynthesis.

A third study focused on phosphoproteomic analysis of O. sativa plants grown under control and drought stress conditions. Extensive changes were seen in proteins involved in membrane transport, including aquaporins, and also in proteins involved with carbohydrate metabolism and RNA processing.

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Dr. Wang received his PhD in Plant Biology from the Henan University, China in 2010. He continued his Postdoctoral research at the Purdue University, USA. He is currently Principal Investigator at the Shanghai Center for Plant Stress Biology, Chinese Academy of Sciences, China. His lab performs cutting-edge phosphoproteomics to identify the substrates of the important protein kinases in abiotic stress responses and build up the protein kinase-substrate networks in plants. By biochemical, genetic, and physiological approaches, they aim to identify novel components in abiotic stress signaling pathways to improve stress tolerance in agriculture crops.

Study RAF-SnRK2 cascade in Arabidopsis by proteomic strategies

The SNF1-regulated protein kinase 2s (SnRK2s) are key components in osmotic stress and ABA receptor coupled core signaling. Recently, we performed quantitative phosphoproteomics analysis on osmotic-responsive kinase phosphorylation and found that the B subgroup of Raf-like protein kinases (RAFs) are quickly activated by osmotic stress. The B2, B3, and B4 RAFs are quickly activated by osmotic stress and are required for phosphorylation and activation of SnRK2s. B4 RAFs phosphorylate and activate ABA-independent SnRK2s. The B2 and B3 RAFs directly phosphorylate and activate ABA-activated SnRK2s up osmotic stress. However, ABA treatment does not activate B2 and B3 RAFs, but the basal level of B2 and B3 RAF activity is essential for SnRK2 activation. The activated SnRK2s then intermolecularly trans-phosphorylate other SnRK2s that are not yet activated to amplify the response. By profiling the ABI1-associated proteins by proximity labeling, we found that RAFs are direct targets of ABI1. ABI1-mediated dephosphorylation on this site strongly promotes the kinase activity of most of B2 and B3 RAFs. ABI1 has dual functions in ABA signaling by dephosphorylating and inhibiting SnRK2 to prevent SnRK2 activation in an unstressed condition, while dephosphorylating some B2 and B3 subgroup RAFs to keep their basal kinase activity. We also set up a cell-sorting-based nano-scale pipeline to study cell-type-specific proteomics. We reveal that a unique RAF-SnRK2 cascade exists in the guard cell. Our findings reveal that proteomics could be an efficient strategy for studying the signaling pathway in plants.

Dr. Paul E. Verslues

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Dr. Verslues received his PhD from the University of California, USA. Currently, he is a faculty at the Institute of Plant and Microbial Biology, Academia Sinica, Taiwan. His group is engaged in deciphering the drought sensing and signaling mechanisms as well as in better understanding metabolic changes associated with drought acclimation, drought signaling. His team has identified a membrane-associated protein AFL1 and its phosphorylation that promotes growth during drought. Now they are focusing on understanding the mechanism of ALF1 signaling by phosphoproteomics. His group is also working on proline accumulation in drought and how its metabolism contributes in drought resistance. They are also trying to understand the role of abscisic acid (ABA) accumulation during stress adaptation.

Type 2C Protein phosphatases and their target proteins that regulate plant growth during drought stress

Plants have a large number of Type 2C protein phosphatases (PP2Cs) compared to other organisms. Most of these phosphatases are still of unclear function. The Clade A PP2C Highly ABA-Induced 1 (HAI1) and the Clade E Growth-Regulating (EGR) PP2Cs regulate plant growth during drought stress. Quantitative phosphoproteomics of hai1-2 and egr1-1egr2-1 mutants was used to identify phosphorylation sites regulated by these PP2Cs. The proteomics analysis was performed on plants acclimated to drought treatment to induce HAI1 and EGR expression. This analysis found that HAI1 primarily affected phosphorylation of nuclear-localized proteins, consistent with the predominantly nuclear localization of HAI1. Of the HAI1-regulated phospho-proteins we identified, AT-Hook Like 10 (AHL10) was found to affect stress-responsive gene expression and plant growth in a manner dependent upon the HAI1-regulated phosphorylation site (Wong et al., 2019). Phosphoproteomics of egr1-1egr2-1found that EGRs primarily regulated plasma membrane and cytoskeleton-associated proteins, consistent with EGR localization along the plasma membrane. An EGR-regulated phosphorylation site on Microtubule-Associated Stress Protein 1 (MASP1) is critical for plant growth and microtubule stability during drought stress (Bhaskara et al., 2017). Ongoing work in our laboratory has identified additional HAI1 and EGR-regulated phosphoproteins important for drought resistance.

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Prof. Tatu secured his PhD degree from the Indian Institute of Science (IISc), Bangalore and moved to Yale University, USA for his Postdoctoral research. He is currently a Professor of Biochemistry at IISc, Bangalore. His lab focuses on understanding the disease pathogenesis and role of heat shock protein 90 (Hsp90) in growth and virulence of various disease-causing organisms namely *Plasmodium*, *Giardia*, *Trichomonas*, *Entamoeba*, *Trypanosoma*, *Babesia* and *Theileria*. The lab uses quantitative proteomics and was among the first to publish clinical proteome of *Plasmodium falciparum* and *Plasmodium vivax* trans-splicing based expression of Hsp90 in *Giardia lamblia*.

Prof. Tatu is an elected fellow of the Indian Academy of Sciences. DBT, GoI awarded him the National Bioscience Award for Career Development in 2008.

Novel post-translational modifications on flagellar tubulin regulate motilities in neglected infectious disease caused by *Trichomonas vaginalis* and *Giardia lamblia*

Protozoan pathogens are responsible for infections that are highly prevalent, especially in developing and under-developed countries. Flagellar motility is exhibited by a variety of organisms ranging from bacteria to certain cell types in mammals. In the context of pathogens, flagellar motility plays a significant role in the establishment of infection in the host. Understanding the regulation of motility in protozoan parasites would highly enhance our understanding of pathogenesis in these organisms. All eukaryotic flagella are made of microtubules and driven by dynein motor proteins. However, every organism is unique in terms of its flagellar waveform, beat frequency and its general motility pattern. With recent research, it is becoming clear that despite overall conservation in flagellar structure, the pattern of tubulin post-translational modifications within the flagella are diverse and may contribute to variations in their patterns of motility. Microtubules are subjected to post-translational modifications known as glycylation and glutamylation. In this study, using global, untargeted mass spectrometry, we have analysed the tubulin post-translational modification in enriched flagella of protozoan parasites Giardia lamblia and *Trichomonas vaginalis*. Using MS/MS, we were able to identify the previously unknown sites of monoglycylation and glutamylation in tubulin in *G.lamblia*. and T.vaginalis. Using isolated flagella, we also characterized the previously unknown flagellar proteome in *G.lamblia* and *T.vaginalis* indicating specific proteins which could play a role in regulating the motility of the parasites. Altogether, the flagellar proteomes as well as the sites of tubulin PTMs in these organisms, reveals potential mechanisms in regulating flagellar motilities in these neglected protozoan parasites.

TL-02

Dr. Yue Xuan

Thermo Fisher Scientific

Dr. Yue Xuan has a doctorate degree in analytical chemistry from the Technical University of Dortmund. She is a Senior Global Product Marketing Manager in the Chromatography and Mass Spectrometry division of Thermo Fisher Scientific. Specializing in innovative life science technologies to create unique solutions for research and clinical use in the area of Precision Medicine. In her existing role, she initiates and leads global strategic collaborations partnering with top institutions, research centers and industry stakeholders worldwide. She is also an inventor with patents filed in the UK, Germany, the United States and China for the novel methodologies and workflows on the orbitrap-based mass spectrometer platform.

Her contributions have been recognized as she has been selected through a peer-review process to chair sessions at prominent scientific conferences such as Analytica Munich, and she is a regular speaker at international conferences and symposiums.

Trends in Life Science OMICS Research

Cutting edge Omics research delivers biological knowledge that can revolutionize human health. LC-MS-based proteomics analysis is a powerful analytical tool for identification and quantification of thousands of proteins in complex biological samples, leading to more advanced understanding in biochemistry and delivering the promise of precision medicine. Our collaborations with trusted, globally renowned partners and experts in OMICS research enable us to develop workflow and technology advancements that drive life science research. As a result, we provide you with continuously more productive and reliable solutions to discover, identify, and quantitate biomolecules in complex biological systems. In this presentation, we are going to share with you the cutting-edge proteomics technologies and innovations to advanced science.

Dr. Sandipan Ray

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Dr. Ray obtained his PhD degree from the Indian Institute of Technology, Bombay. He completed his Postdoctoral training from the MRC Institute of Metabolic Science, University of Cambridge, UK. He currently works at the Indian Institute of Technology, Hyderabad, where he conducts research in the fields of circadian rhythms, signaling networks and infectious diseases.

Dr. Ray is a recipient of the prestigious Thermo Scientific Annual Tandem Mass Tag Research Award in 2018. He is associated with multiple leading scientific organizations including the Human Proteome Organization (HUPO), US-Human Proteome Organization (US-HUPO), and Society of Biological Chemists (SBC), India. He is elected to the prestigious Royal Society of Biology, UK in 2020. He has also worked at various universities and research institutes including the University of Pennsylvania, USA; the University College London, UK and the Francis Crick Institute, UK.

Multiplexed quantitative proteomics for mechanistic study of pharmacological modulators of circadian time-keeping machinery

Many human diseases such as mood and mental disorders, cancers, diabetes, and cardiovascular diseases are associated with circadian misalignments and dysregulation. Targeting or modulating the components of the clock machinery is now emerging as a new avenue in pharmacological research. This study systematically deciphered the cellular effects and molecular targets of drugs/drug-like compounds that can alter the circadian period length or phase. We investigated the molecular targets of circadian period modulating compounds in the human osteosarcoma cells using an integrated multi-dimensional quantitative proteomics workflow combining global proteome, phosphoproteome and kinome mapping, and thermal proteome profiling (TPP).We have demonstrated changes in phosphorylation levels and activity of several proteins and kinases involved in essential signaling pathways, including MAPK, BCR, AMPK, and mTOR signaling by the circadian clock modulating compounds. TPP analysis revealed the direct binding of some of these drugs with clock-regulatory kinases and their modulators. Since the effectiveness of drugs with shorter half-lives is consistently influenced by their dosing time, we are also investigating the impact of dosing time on the efficacy of these compounds. In sum, to develop highly effective and less toxic new drug molecules, we need a detailed mechanistic understanding of how the circadian clock modulators exhibit their effects to target those aspects of cell functions and physiological processes precisely. We anticipate that this comprehensive study will contribute towards a better understanding of the mechanism of action of these pharmacological modulators of circadian clocks, which is critical in clearly defining molecular targets to control daily rhythms for therapeutic benefit.

Dr. Maya Zachut

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Dr. Zachut earned her PhD degree from The Hebrew University, Jerusalem. She carried out her Postdoctoral research at the Hebrew University and Volcani Center in collaboration with the Weizmann Institute of Science, Israel. Currently, she is the Principal Investigator at the Department of Ruminant Sciences, ARO Volcani Center, Israel. Her lab studies adipose tissue function, metabolic stress response and diversity between cows in early lactation. They use advanced proteomics analysis and bioinformatics to examine the effects of metabolic and environmental stressors on adipose tissue function, metabolism and performance in dairy cows.

A proteomics approach to unravel adipose tissue inflammatory responses in peripartum cows

Proteomic analysis explores the repertoire of proteins at a given state. In recent years, proteomics of subcutaneous adipose tissue (AT) revealed numerous inflammatory proteins in AT from peripartum (PP) dairy cows, highlighting the presence of complement and acute phase proteins in AT. Bioinformatics analyses pointed to the key role of inflammatory pathways in AT of PP cows. Proteomics of AT from cows with a high degree of metabolic stress, represented by increased AT lipolysis postpartum, showed differential abundance of complement and acute-phase proteins in AT compared to cows with a low degree of metabolic stress. In cows that had an insulin-resistant (IR) AT, the top differential function was the inflammatory response; and inflammatory signals are known to induce IR. Hence, proteomics of AT demonstrate that metabolic stress and lipolysis enrich AT with inflammatory proteins, possibly contributing to subacute inflammation in PP cows. Proteomics of AT from heat-stressed late pregnant cows revealed enrichment of the Nrf2-mediated oxidative stress response and acute-phase response. PP cows suffer from oxidative stress related to AT lipolysis, and both oxidative stress and lipolysis affecting inflammation. Therefore, increased oxidative stress in AT, associated with lipolysis and/or heat stress, could increase AT inflammation, as reflected by the AT proteome. In ketotic cows, proteomic analysis showed a downregulation of key innate immune response proteins in AT, suggesting that the health status affects the proteome and AT inflammation. Systemic treatment of postpartum cows with anti-inflammatory sodium salicylate unexpectedly enriched the AT proteome with inflammatory pathways of the complement system, cytokine signaling, and acute phase response, perhaps due to immune cell recruitment. In conclusion, biotic and abiotic stressors, the health status and treatment with anti-inflammatory agents affect the abundance of inflammatory proteins in AT. Proteomics of AT improves our understanding of AT inflammation, and adds information on novel proteins in AT of PP cows.

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Dr. Mukherjee earned his PhD degree from the Burdwan Medical College of Burdwan University, West Bengal. He also received a D.Sc. degree from the University of Calcutta. Currently he is appointed as the Director of Institute of Advanced Study in Science and Technology, Guwahati. His area of research includes snake venom biochemistry, environmental biotechnology , industrial microbiology, drug discovery from natural resources. His lab focuses on traditional and modern drug discovery and disease diagnosis, biodiversity and ecosystem research.

He received many honors like Young Scientist Award from various organizations such as the Indian Science Congress Association, the Association of Medical Biochemists of India, the Zoological Society of India, and the Physiological Society of India, Best Researcher Award from the Tezpur University and National Bioscience Award for Career Development etc. He served at various prestigious universities like the University of Connecticut Health Center, USA; the University of Northern Colorado, USA and the Tezpur University, Assam.

The application of Mass-Spectrometry and other analytical techniques for the quality assessment of commercial antivenom

Snake bite envenomation is an occupational health problem, particularly for the rural communities of the developing world, the Indian subcontinent included. Commercial antivenom, used for snakebite treatment, is mainly produced in horses. It contains whole or pepsin-digested immunoglobulin G (IgG), and the accomplishment of antivenom therapy depends upon its ability to abrogate or reduce envenomation's local and systemic toxicity. In addition, antivenom administration must be safe for the patients. Therefore, antivenom manufacturers must ensure that these products are effective and safe in treating envenomations, and there should not be batch to batch variation in the quality of commercial antivenom. The physicochemical characteristics of antivenom formulations determine its efficacy and safety, purity of the immunoglobulin fragments and antibodies, presence of protein aggregates, endotoxin burden, preservative load, and batch to batch variation, as well as on the ability to neutralize the most critical toxins of the venoms against which the antivenom is designed. Recent studies from our laboratory have demonstrated that mass spectrometry and laboratory-based simple analytical techniques can be applied to assess antivenom quality, safety, stability, and efficacy. It may be recommended that these tests be applied to screen the quality of commercial antivenoms before their pre-clinical and clinical assessment.

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Dr. Ambatipudi received his PhD degree from the Macquarie University, Australia. He continued his Postdoctoral research at the University of Rochester, USA. Currently, he works at the Indian Institute of Technology, Roorkee. His lab's interest lies in understanding the role of milk proteins in the pathophysiology of animal disease, chemoresistance and breast cancer progression. His group performs comprehensive Mass-spectrometry based lipidomics to characterize fat globules in bovine milk.

He has a patent on the process of isolation and enrichment of fat globules. He has also authored a book on veterinary sciences and book chapters in multiple books.

The dynamics and power of the bovine milk components in health and disease

Bovine milk is an important biological fluid due to its nutritional and immunological benefits. It is an easily accessible and rich source of potential markers (e.g., proteins, lipids), and any changes in the expression of these constituents due to exogenous (e.g., seasons) and endogenous (e.g., breed) factors would alter normal functional properties of milk; such changes would be expected to reflect local or systemic illness. The changes in whey proteome abundance in two conventional milch breeds, Holstein Friesian (HF) cow and Murrah (Mu) buffalo were performed across summer and winter. Collectively, 490 proteins were identified with extensive changes between the two animal groups, with proteins showing breed and season-specific variations. Subsequently, an attempt to understand the dynamics of whey proteome in response to S. aureus infection, whey protein collected from healthy, subclinical mastitis (SCM), and clinical mastitic (CM) in HF and Mu were investigated. A total of 1479 proteins were identified, of which 128 and 163 had signature pattern in each stage indicative of the progression of the disease. Another constituent is milk fat globules (MFG), and its lipid distribution by exogenous phospholipids using microscopy showed higher phospholipid content in fresh compared to mastitic MFG Membrane. Xanthine oxidase assay indicated membrane impairment, with lower activity in mastitic samples compared to fresh globules. Influence of globule membrane on the interaction with L. fermentum demonstrated preferential adhesion of bacteria to fresh compared to mastitic globules, including the enhanced extent of binding. Collectively, the results of the studies have provided deeper insights into the complexity, dynamic nature, and functional significance of different milk constituents and laid the foundation to understand better milk that will ultimately help facilitate early therapeutic and husbandry-based intervention to improve animal health and milk quality.

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Dr. Mandal received his PhD degree from the Bose Institute, Kolkata. He was a Postdoctoral fellow at the University of Texas Health Science Center, USA followed by another Postdoc at the Indian Institute of Science, Bangalore. Currently, he works at the Indian Institute of Science Education and Research, Kolkata. His research interest lies in molecular medicine where he studies structure of proteins involved in human diseases. He uses clinical proteomics approaches using patient samples to study hemoglobinopathies, iron deficient anemia, mental disorders, prostate cancer and multiple sclerosis.

He received the Centers of Innovation (COI) award from WATERS, USA in 2013. He served as a Professor at the St. John's Research Institute, St. John's National Academy of Health Sciences, Bangalore.

Structural analysis of post-translationally modified human hemoglobin: A native Mass-Spectrometry based approach

Two important post-translationally modified of human hemoglobin are glycated and glutathionylated hemoglobin. Glycated hemoglobin (GHb) is used as a biomarker for the long-term glycemic index of an individual and it is clinically used to diagnose diabetes mellitus. GHb is formed via irreversible covalent modification of N-terminal α-amino group of β globin chain with glucose via Amadori rearrangement. The differential surface charges between GHb and native hemoglobin (HbA0) is used for their separation and quantification through cation exchange chromatography. However, glucose condensation is specific to primary amino groups. Therefore, structural characterization of GHb, synthesized in vivo, is essential as multiple glycations may interfere with GHb assessment. While analyzing the stoichiometric composition of glucose bound to α and β globin chains in tetrameric hemoglobin molecule across singly, doubly and triply glycated hemoglobin molecules, we observed that glycation of human hemoglobin occurs symmetrically across α and β globin chains with a preference to glycate the unmodified globin chains. Correlation between the conventional and mass spectrometry-based quantification of GHb showed a reliable estimation of the glycemic index of individuals carrying HbA0. Mutant globin chain with a change in the amino acid on the surface of tetrameric hemoglobin molecule might have different retention time than HbA0. Thus, glycated analog of the variant hemoglobin might elute at different retention time compared to GHb. Thus, mass spectrometry based quantification of GHb, which is free from any such interference, might be a reliable method for assessing the glycemic index.

Glutathionylation of hemoglobin is an example of reversible post-translation modification where free and most accessible cysteine residues of the β globin chain, Cys93,undergoes thiol-disulfide exchange with oxidized glutathione (GSSG) to form Glutathionyl hemoglobin (GSHb). In general, glutathionylation occurs under the condition of elevated oxidative stress in vivo. GSHb was reported to act as a biomarker of oxidative stress under several clinical conditions such as chronic renal failure, iron deficiency anemia, hyperlipidemia, diabetes mellitus, Friedreich's ataxia, atherosclerosis. The functional abnormality associated with six-fold tighter oxygen binding of GSHb might be attributed to the conformational transition of the deoxy state of GSHb towards oxy hemoglobin like conformation. Here we report the structural integrity and overall architecture of the quaternary structure of GSHb using native mass spectrometry platform. The dissociation equilibrium constants of both tetramer/dimer (Kd1) and dimer/monomer equilibrium (Kd2) were observed to increase by 1.91 and 3.64 folds respectively.

Cigarette smoking is known to produce hypoxia, a state of inadequate oxygen supply to the tissues. Hypoxia plays a pivotal role in the development of chronic obstructive pulmonary disease. Smoking during pregnancy imposes risk for the unborn child. Besides carbon monoxide, the irreversible covalent conjugation of para-benzoquinone (pBQ), derived from cigarette smoke, with human hemoglobin was reported to contribute in hypoxia. The functional assay of Hb-pBQ, performed through the oxygen dissociation equilibrium curve, showed a significant decrease in both P50 and cooperativity. The structural integrity of Hb-pBQ measured through Kd indicated that compared to HbA0, Kd1 of tetramer/dimer and Kd2 of dimer/monomer equilibria were increased by 4.98 and 64.3folds respectively. We proposed that the significant destabilization of the functionally active conformation of hemoglobin upon conjugation with pBQ results in tighter oxygen binding which eventually leads to hypoxia.

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Dr. Patil received her PhD from the Temasek Life Sciences Laboratory, Singapore. Later, she carried out her Postdoctoral trainings at the Sanford Burnham Prebys Medical Discovery Institute, USA; University of California San Diego, USA and La Jolla Institute for Immunology, USA. Currently, she is a Faculty Member at the National Institute of Immunology, New Delhi.

Her research interest lies in understanding the development and memory in immune response upon infection and immune response against neonatal sepsis. Special focus of the lab is to understand the mechanism of maintaining long term memory in CD4+ cytotoxic memory T-cells post infection.

Human CD4+ T cell memory subsets in infectious diseases: lessons from multi-omics analysis

The acquisition of immunological memory to infections is a hallmark of protective immunity and hence forms the basis for vaccinations. During the evolutionarily conserved process of T cell immunological memory development, the naive T cells that have not previously encountered antigen, differentiate during the primary infection into memory T cells that have specialized functions in immune defense to a later infection with the same pathogen. Each pathogen elicits a specialized memory subset and once formed this specialized T cell memory can provide life-long immunity to the same pathogen. Two of such specialized memory subsets we study are CD4+ cytotoxic memory T cells formed in response to viral infections (dengue virus, cytomegalovirus) and CD4+ T helper memory subsets (TH1, TH17 andTH1/17) formed in response to bacterial infection (Mycobacterium tuberculosis). The single-cell and bulk multi-omics analysis of CD4+ T cell memory subsets in humans revealed heterogeneity and identified new subsets with long-term memory features. Understanding the biology of such specialized long-term memory subsets will pave the way for developing strategies to boost durable immune responses following vaccination against these pathogens.

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Dr. Santra pursued his PhD from the Indian Institute of Technology, Bombay. He carried out his Postdoctoral research at the University of Massachusetts Medical College, USA. He currently works at the National Centre for Cell Science, Pune. His lab works on deciphering the role of F-box proteins in development and diseases.

He has recently been awarded the National Bioscience Award for Career development by the Department of Biotechnology, Government of India for his contributions to bioscience.

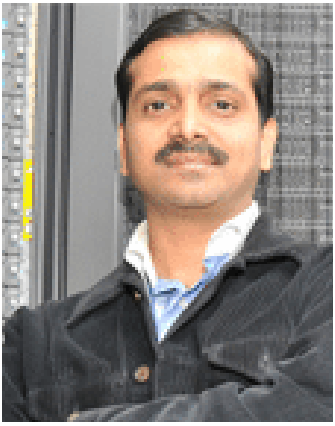
Reaching the drop through the ocean: Proteomic study to capture dynamic interactions for understanding cancer pathogenesis and treatment

Ubiquitination is a post-translational modification of proteins, where proteins are covalently conjugated with small protein ubiquitin (76 amino acids). Ubiquitination of proteins plays important role in determining their function in many biological processes including cell cycle progression, cellular signaling, cell death and DNA damage/repair. Proteins may be mono-ubiquitinated, multi-monoubiquitinated or polyubiquitinated. During polyubiquitination, ubiquitin moieties conjugate each other through utilizing one of the 7 lysine residues (K6, K11, K27, K29, K33, K48, K63) of the preceding ubiquitin moiety and the carboxylic group of last amino acid (Glycine) of adjacent ubiquitin moiety. Among the different types of polyubiquitination, proteins with K11/K48 linked polyubiquitination are marked for degradation through proteasome. It is a very dynamic process and mass spectrometry is most useful tool to detect the polyubiquitinated proteins.

SCF (SKP1, Cullin1 and F-box protein) ubiquitin ligase complexes catalyze the ubiquitination of most of the cellular proteins and responsible for degradation of 70% of cellular proteins. F-box proteins (have conserved F-box motif) function as substrate receptor in the complex and determine the substrate to be ubiquitinated. Deregulation of protein ubiquitination process by SCF complex leads to many diseases including cancer. Human genome encodes genes for 69 F-box proteins. However, cellular targets of most the F-box proteins remain elusive. Identification of targets is very important to understand their role in cancer pathogenesis. Using proteomic approaches, we have deciphered the importance of several F-box proteins in cancer pathogenesis through identification of their interactomes. Details of the work will be discussed during my presentation.

PANEL DISCUSSION

"Ethics in Communicating Science"



Dr. Debasis Dash
CSIR-IGIB,
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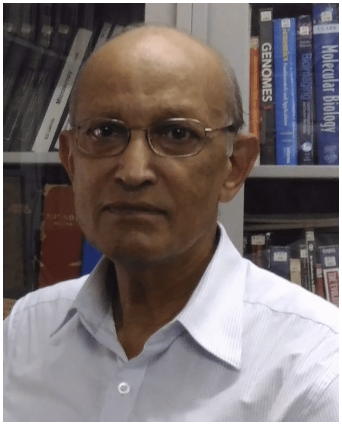
Ex Dy. Director
ACTREC, Mumbai
PANELIST



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LIGHTNING TALKS

LT-1

GELFrEE Fractionation and MALDI-TOF MS identification of Buffalo Meat and Pork Specific Carbonic Anhydrase-3 Peptides for Meat Species Authentication.

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Present study evaluated the novel GELFrEE protein fractionation as an effective in-solution separation tool for total proteins isolated from buffalo meat and pork and further characterization using mass spectrometry.

Authentic buffalo (*Bubalus bubalis)* meat and pork (*Sus scrofa*) samples were obtained from retail shops. Extraction of proteins was carried out with phosphate buffered saline and SDS by simple trituration and filtration method. GELFrEE fractionation was performed as suggested by Expedeon Ltd. using 5% cartridge with a fractionation range from 3.5 to 500 kDa resulting in protein separation into 12 fractions within 2 hours. The GELFrEE fractions was subjected to SDS-PAGE for validation with midi-gel electrophoresis apparatus using 12% gel followed by in-gel trypsin digestion and analysis in linear, positive ion mode on the MALDI-TOF ULTRAFLEX III mass spectrometer. NCBI database was searched to find amino acid sequences of the detected proteins and differences in species-specific peptide sequence was compared using Clustal O(1.2.4) multiple sequence alignment.

Mass spectrometric identification of proteins from the selected SDS-PAGE bands obtained through GELFrEE fractions confirmed the presence of buffalo meat and pork specific carbonic anhydrase-3. Percent identity score for pairs of carbonic-anhydrase-3 sequences from buffalo meat and pork calculated using Clustal 1.2revealed that the amino acid sequences between buffalo meat and pork differed by 7%. Current study successfully detected buffalo meat-specific GGPLAAPYR, GEFQLLLDALDK, EPITVSSDQIAK and pork-specific GGPLTAAYR, GEFQLVLDALDK and EPITVSSDQMAK derived from carbonic anhydrase-3 with 100% confidence in raw as well as cooked meat samples.

These results confirmed the robustness of GELFrEE fractionation as an efficient and rapid fractionation tool for low molecular weight proteins and can be successfully explored in the development of a proteomic based approach for meat authentication.

LT-2

ModLocator: a universal modification site localization tool

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Post translational modifications (PTMs) are the small chemical functional groups that aid in expanding a protein's functional repertoire in the cell. They are known to regulate all major cellular processes like signaling, transport, protein interactions, subcellular localization, degradation and enzyme activation. Despite the global attention and innovations in

identifying protein modifications from shotgun proteomics data, the identification rates for PTMs is slow. This is due to the absence of adequate software for confident site localization in database search algorithms. The contemporary tools are limited to specific modification or do not utilize complete MS/MS information. Here, we designed ModLocator, a software algorithm that can facilitate accurate and precise site localization for any arbitrary number of modifications from virtually any database search engine results in tabular format. In current implementation, it directly supports modification search results (MSGF+, X!Tandem, MassWizetc) or open search results (ModA and MSFragger). The algorithm rescores the identified peptide to accurately localize the PTM site(s) based on probabilistic and heuristic scoring systems. Apart from implementing scores like Ascore, Delta Scores, PhosphoRS (PTMScore), we also calculate dot product based score and LocScore (based on MassWiz score utilizing intensity and mass errors). We tested the accuracy of the algorithm on the phosphopeptides synthetic library dataset from Ferries et al 2016. This is a next-generation localization tool that can also be interfaced with modification annotation using UniMod annotations and tackles the unwieldy problem of combination of multiple modification masses that remain unannotated.

LT-3

Can the Jigsaw Puzzle Model of Protein FoldingRe-assemble a Hydrophobic Core?

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According to the 'jigsaw puzzle' model of protein folding, the isomorphism between sequence and structure is substantially determined by the specific geometry of side-chains interactions, within the protein interior. In this work, we have attempted to predict the hydrophobic core of cyclophilin (LdCyp) from *Leishmania donovani*, utilizing a surface complementarity function, which selects for high goodness of fit between hydrophobic side-chain surfaces, rather in the manner of assembling a three-dimensional jigsaw puzzle. The computational core prediction method implemented here has been tried on two distinct scenarios, (1) on the LdCyp polypeptide chain with native non-core residues and all core residues initially set to alanine, (2) on a poly-glycine polypeptide chain. Molecular dynamics simulations appeared to indicate partial destabilization of the two designed sequences. However, experimental characterization of the designed sequences by circular dichroism (CD) spectroscopy and denaturant (GdmCl) induced unfolding, demonstrated disordered proteins. Stepwise reconstruction of the designed cores by cumulative sequential mutations identified the specific mutation (M122L) as primarily responsible for fold collapse and all design objectives were achieved upon rectifying this mutation. In summary, the study demonstrates regions of the core to contain highly specific (jigsaw puzzle like) interactions sensitive to any perturbations and a predictive algorithm to identify such regions. A mutation within the core has been identified which exercises an inordinate influence on the global fold, reminiscent of metamorphic proteins. In addition, the computational procedure could predict substantial regions of the core (given main-chain coordinates) without any reference to non-core residues.

LT-4

The CC′ loop of IgV domains of the immune checkpoint receptors, plays a key role in receptor:ligand affinity modulation

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Antibodies targeting negative regulators of immune checkpoints have shown unprecedented and durable response against variety of malignancies. While the concept of blocking the negative regulators of the immune checkpoints using mAbs appears to be an outstanding approach, their limited effect and several drawbacks, calls for the rational design of next generation of therapeutics. Soluble isoforms of the negative regulators of immune checkpoint pathways are expressed naturally and regulate immune responses. This suggests, affinity-modified versions of these self-molecules could be effective lead molecules for immunotherapy. To obtain better insights on the hotspot regions for modification, we have analysed structures of 18 immune receptor:ligand complexes containing the IgV domain. Interestingly, this analysis reveals that the CC' loop of IgV domain, a loop which is distinct from CDRs of antibodies, plays a pivotal role in affinity modulation, which was previously not highlighted. It is noteworthy that a ~5-residue long CC' loop in a ~120 residue protein makes significant number of hydrophobic and polar interactions with its cognate ligand. The post-interaction movement of CC' loop to accommodate the incoming ligands, seems to provide additional affinity to the interactions. *In silico* replacement of the CC' loop of TIGIT (one of the inhibitory immune checkpoint receptors) with that of its ligands Nectin-2 and PVR followed by

protein docking trials suggests a key role of the CC' loop in affinity modulation in the TIGIT/Nectin pathway. The CC' loop appears to be a hotspot for the affinity modification without affecting the specificity to their cognate receptors.

LT-5

In silico analytics on Methane monooxygenase enzymes: a promising bio-remedial candidate for Hydrocarbons

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(AIB Communication No. AIB/RA/2021/405)
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Hydrocarbons released from traffic fuel have long been known to pollute our environment and a certain subgroup called the volatile organic compounds (VOCs), including compounds like 1,3-butadiene, and benzene are infamous for their carcinogenic potential. In recent years, Bioremediation has emerged as a universally adopted approach for degrading or transforming hazardous pollutants into less or even non-toxic compounds, using enzymatic digestion property of microorganisms. In the current study, we explore the structural properties and protein-protein interaction of one such enzyme. Methane mono-oxygenase belongs to the class of oxidoreductase class of enzymes and is able to catalyse the hydroxylation of all kinds of hydrocarbons including, alkanes, alkenes, cyclic, and aromatic compounds. Methane monooxygenase hydrolase (MMH) and methane monooxygenase hydroxylase (MMHx) enzymes from *Methylocella silvestris* and *Mycobacterium rhodesiae,* respectively were

assessed using online tools for protein-protein interaction, Ramachandran plotting and Swiss modelling. The network stats of MMH shows 11 number of nodes, 55 number of edges, an average node degree of 10 and average local clustering coefficient-1 with PFAM protein domains in Methane/Phenol/Toluene hydroxylase, while that of MMHx shows number of 11 nodes, 37 number of edges, an average node degree-6.73 and an average local clustering coefficient of 0.863 with PFAM protein domains in Methane/Phenol/Toluene hydroxylase, Oxidoreductase FAD binding and NAD binding domain, Alcohol and Zinc binding dehydrogenase. Clash score obtained using Swiss modelling was 0.98 for MMH and 2.08 for MMHx. Ramachandran favoured for MMH was 95.49% with bad angles 40/5858 and 96.27 % with bad angles 50/5858 for MMHx. The results obtained from the study may propose the potential leads in discovering novel enzymatic pathways that can be employed in bio remedial prospects.

LT-6

G-quadruplex (G4) Structure in the Promoter Region Regulates EFHC1 Gene Expression

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Background

DNA sequence and structure play a key role in the regulation of gene expression. Recent studies provide strong evidence that specific G-quadruplex structures can be formed naturally by the G-rich DNA sequence at many human promoter regions, thereby raising the possibility of

transcriptional regulation of these genes by the G-quadruplex structures. In this study, we provide evidence for the presence of G-quadruplex structures at the promoter region of *EFHC1* (EF-hand containing) gene, which has a diverse physiological role and is also implicated in Juvenile myoclonic epilepsy.

Methods

Three putative G-quadruplex (G4) forming sequences in the promoter region of *EFHC1* gene were bioinformatically ascertained and verified the formation of G-quadruplex structure in vitro using fluorescence spectrometry. The putative G4 sequences were treated with 100 mM KCl along with three control sample DNA (ssDNA, calf thymus DNA and plasmid DNA). The fluorescence readings were measured at 476nm (excitation) and 645nm(emission), respectively, using Ru-OL as the fluorescence emitting ligand. For biological validation, these three putative G4 sequences were cloned at the end of the SV-40 promoter of the Firefly luciferase reporter plasmid pGL4.13 (luc/2) and transfected into Hela cells along with the reporter plasmid without the G4 sequences and PIS2 (Renilla luciferase reporter plasmid) as an internal control. Firefly and Renilla luciferase activities were assayed using the Dual Luciferase Reporter Assay System (Promega).

Results and Conclusion

In this study we have identified three putative G-Quadruplex forming sequences in the promoter region of *EFHC1* gene named as EFHC1-GQ1, EFHC1-GQ2 and EFHC1-GQ3 and further verified the formation of the G-quadruplex secondary structure formation. Among these three sequences, the strong evidence for forming the G-quadruplex secondary structure was shown by EFHC1-GQ1 motif as shown by its highest-fold increase in fluorescence intensity and decrease in the luminescence activity during fluorescence spectrometry and dual-luciferase assay respectively. Identifying tissue specific G-quadruplex unwinding helicases in future will enhance our understanding on the differential expression of EFHC1 in the brain.

LT-7

In Silico approach towards identification of potential inhibitors of Helicobacter pylori phosphatase, HppA

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Helicobacter pylori is a gastric mucosal pathogen and is associated with diseases like peptic ulcer and gastric cancer. To combat *H. pylori* infection, there is an urgent need for new class of antibiotics due to emergence of drug-resistant strains. A 28kDa enzyme, HppA of class C acid phosphatase with phosphomonoesterase activity, play crucial role in electron transport chain. It is also involved in cation-flux as well as pH regulation on the cell envelope. There is no alternative pathway for the pyruvate conversion in *H. pylori*; hence, the inhibitors targeting the bacterial enzyme may have selective toxicity. In this work, we report a 3D model structure of *H. pylori* HppA, which consisted of monomeric α+β model generated by utilizing MODELLER software. Next, in attempts to identify inhibitors of this protein, high throughput *in-silico* screening and molecular docking procedures were performed. Various knowledge-based inhibitors and small molecules from databases such as DrugBank and BindingDB were screened on the basis of good docking score and docking energy. Detailed analysis of non-covalent interactions within the active site was assessed. Finally, ten potent molecules were proposed as potential inhibitors based on the investigation of molecular interactions map and protein-ligand fingerprint analyses. To ensure the efficiency of the selected compounds, binding free energies

were calculated using MM-GBSA as well as their ADMET properties were predicted. Our *in-silico* studies have identified lead molecules that may act as potential molecules for the development of effective drugs targeting HppA protein subjected to further experimental validation.

LT-8

α-Synuclein filaments are benign protein homeostatic compartments with an associated risk of nuclear injury

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Amyloidogenic proteins form fibrillar aggregates or inclusion bodies which are hallmark of many age-related neurological diseases. The pathological role of amyloid fibrils has obscure views, from them being the cause of the pathogenesis to them being the inert-end products of aggregation. During the course of biogenesis of these inclusion bodies, amyloid proteins are proposed to interact and sequester many cellular proteins. Loss of function of these proteins due to their precipitation may contribute to the disease pathogenesis. Another hypothesis in the field suggests that sequestration of oligomers into large insoluble deposits is protective to avoid these promiscuous interactions and thereby reduce toxicity. Intriguingly, the abovementioned mechanisms have been worked out using mainly a single amyloidogenic protein i.e. mutant huntingtin.

A-Synuclein is also one amyloidogenic protein associated with many synucleopathies including Parkinson's disease. Proteome interaction with α-Synuclein filamentous aggregates is poorly studied so far due to lack of convenient model systems. In our lab, we have prepared α-Synuclein aggregation model system in mammalian cell line HEK293T. This model recapitulates the slow aggregation kinetics of α-Synuclein allowing us to investigate interacting proteome during the course of aggregation and its impact on cellular metabolism. Using quantitative Mass Spectrometric analysis of soluble, insoluble and total proteome fractions, we observed no significant alteration in the levels and solubility of the cellular proteome except certain nuclear and mitochondrial proteins. Analysing nuclear proteome, we found enrichment of mitochondrial proteins into the nuclear fraction suggesting nuclear leakiness. Microscopy images confirmed nuclear envelope injuries in presence of large filamentous aggregates that hampered nucleo-cytoplasmic compartmentalization of proteins. Overall, we hypothesize that formation of α-Synuclein filaments is a cellular strategy to store this amyloidogenic protein into benign and inert compartments with an associated risk of nuclear injury.

LT-9

Systematic analysis of high temperature stress-responsive nuclear proteins provides global view of thermotolerance

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Elevation in temperature above optimum level drastically reduces crop yield. Due to the complexity of high temperature stress (HTS) responses, understanding the molecular basis of thermotolerance has remained a major challenge. Chickpea, being a winter crop, is hypersensitive to HTS and its growth is hampered when temperature exceeds 35ºC. To delineate thermotolerance responses, seedlings of several chickpea cultivars were subjected to HTS, and stress-induced physicochemical changes were evaluated. The changes in osmotic potential, photosynthetic pigments, electrolyte leakage and lipid peroxidation, besides accumulation of phenolics and flavonoids were examined. We also investigated differential expression of stress-responsive genes, particularly those coding for heat shock proteins (HSPs) and antioxidant enzymes. The results of morpho-physicochemical and gene expression analysis facilitated the identification of HTS- tolerant cultivar. Next, we developed the HTS-responsive nuclear proteome of the most tolerant cultivar, ICC 1205. The proteomic analysis identified 2705 non-redundant set of proteins, which constitutes a complex network of proteins involved in multivariate cellular processes. Comparative analysis of the proteome landscape led to the identification of 414 HTS-responsive proteins that appears to have key roles in HTS-adaptation. More significantly, screening and characterization of the proteome recognized an upregulated HTS- responsive protein, associated with root phototropism henceforth designated CaRPT-2. The transcript of *CaRPT2* was also found to be highly upregulated under HTS. We further investigated the transcriptional regulation of *CaRPT2* in chickpea exposed to multivariate biotic and abiotic stresses. To demystify its precise role in thermotolerance we generated stable overexpression lines of *CaRPT2* in chickpea and currently characterizing their physiological and stress responsive behavior. In conclusion, this combinatorial approach correlating the differential morpho-physicochemical attributes and proteome profiling in genotype-specific manner may unearth the intrinsic mechanism of thermotolerance extendible to crop improvement.

LT-10

Serum Proteome Profiling of Lymphatic Filariasis Patients for Identification and Characterization of Biomarkers

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Lymphatic Filariasis (LF) affects more than 120 million people in tropical and subtropical areas of the world and has drastic social and economic impact causing high morbidity and long illnesses leading to social exclusion and loss of wages. WHO is recommending a combination of Ivermectin, Diethylcarbamazine citrate and Albendazole (IDA) to accelerate the global elimination of Lymphatic Filariasis (LF). Year 2030 is the projected filarial eradication year hence monitoring of filarial infection in sentinel population is essential to assess the outcome of GPELF and to re-evaluate and formulate further strategies for success of GPELF. Parasite infection leads to host immune dysregulation which is responsible for filarial disease progression. Filarial parasites are known to cause host immune dysregulation, therefor it is imperative to examine the serum proteome of LF patient. In this study, we have performed a proteomic analysis of serum samples from healthy control, asymptomatic, acute and chronic LF patient. To obtain an insight of host immune dysregulation, immunoelectrophoresis combined with SDS-PAGE revealed 19 differentially expressed serum protein in LF cases. The differentially expressed protein spots were subjected to MALDI-MS/MS analysis for protein identification. Functional analysis suggested the involvement of differentially expressed proteins in complement and coagulation cascades and hemostasis. This is the first

report of analysis of comparative human serum proteome alterations in different category LF patients, which revealed several differentially expressed proteins, including albumin, c-reactive protein, α-1-antitrypsin, which have not been reported in context of Lymphatic Filariasis previously. Differentially expressed proteins identified in this study may further be investigated as diagnostic or prognostic serum biomarkers for Lymphatic Filariasis.

LT-11

Comparative Transcriptome Profile Analysis of Parathyroid Adenoma with Cardiovascular Disease and Diabetes Mellitus to Determine the Gene-Disease Relationship

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Primary hyperparathyroidism (pHPT), caused by solitary parathyroid adenomas (PA) in most cases (Approx. 85%), and it has been previously reported that parathyroid adenomas are associated with Cardiovascular disease (CVD) and Diabetes Mellitus (DM). To understand the molecular basis of the Parathyroid adenomas, we have investigated the genetic association among PA, CVD, and DM through an integrative network-based approach and observed a remarkable resemblance. Herein, transcriptome analysis was performed to recognize the differentially expressed genes (DEGs)and compared the PA-associated genes with overlapping genes of

CVD and DM to determine the gene-disease relationship. The disease-gene network revealed 12 highly clustered modules/Sub-modules comprising 43 hub genes largely involved in the mRNA metabolic process, RNA binding, macromolecule catabolic process, intracellular protein transport, nucleic acid metabolic process, RNA splicing, etc. This study attempted to create a robust workflow taking parathyroid disorder-associated diseases (PC, DM, and CVD) into consideration and emphasize the need for an hour to re-think and re-devise therapies and therapeutic management (from a strategic point of view). The findings of the current study also provide us an opportunity to focus on the untouched aspects of any disease, in particular with their distant related gene-sets (means genes associated with other diseases), and to work in synergy to have a collective physiological effect on one's pathological phenotype. Our network-based analysis opens a new horizon for more personalized treatment, drug-repurposing opportunities, investigates new targets, multidrug treatment, and may be helpful in further analysis of the mechanisms underlying parathyroid adenoma and associated diseases.

LT-12

Diverse effect of Atorvastatin treatment normalizes the angiogenesis and increases the drug concentration at granuloma site (Controlling the dissemination of *M. tb* infection)

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Background: Statins, 3-hydroxy 3-methyl glutaryl coenzyme A reductase inhibitors reduces the cholesterol level in the macrophages and aids to prevent the entry of *Mycobacterium tuberculosis (M. tb)* pathogen into the macrophages and potentiate the host response against the pathogen. However the studies could not assess the effect of statins on drug penetration which can be elevated through reduction in cholesterol and vascularity. Chemokine and chemokine receptors are important mediators of angiogenesis so we hypothesized that statins could affect the vasculature.
Methods: We investigated the effect of atorvastatin on *M. tb* induced angiogenic chemokines and vascular endothelial growth factor (VEGFA). Anti-angiogenic effects at high concentrations were associated with decreased endothelial release of vascular endothelial growth factor, increased endothelial apoptosis. In guinea pig models, inflammation induced angiogenesis was significantly inhibited with high concentrations of atorvastatin (5mg/kg). In this study we detected the accumulation of drug at the peripheral sites of lungs and impaired drug distribution to diseased site. Efficacy of rifampicin and isoniazid along with atorvastatin on viability of *M*. *tb* was demonstrated.
Results: High dose of statins were able to create phenotypic and normal granuloma vasculature and shows the additive effect with rifampicin. Our

findings demonstrate that statins reduces the spread of infection to other organs and lower the bacterial load at the granuloma sites by promoting normal vascularization

Conclusion: Our data demonstrate that miliary tuberculosis is associated with elevated levels of angiogenic factors. Anti-VEGF therapy using statins has the potential to enhance treatment in patients with TB.

LT-13

Human organic cation transporter-1 (OCT-1/SLC22A1) and capricious response to metformin in patients with type 2 diabetes.

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Background: Organic cation transporter 1 (OCT1/SLC22A1) primarily governs the action of metformin, one of the most commonly used drugs for the treatment of type 2 diabetes mellitus, in liver. There are considerable inter individual variations in metformin response, with about 35% of

patients failing to achieve initial glycemic control. In light of this, it is crucial to obtain a greater understanding of the influence of OCT1 expression or polymorphism in the context of variable responses elicited by metformin treatment. Our study group involved responders and non-responders to metformin therapy.

Methods: Reverse transcription PCR and Real time PCR were used to analyse the isoform variation and mRNA levels of OCT-1 in our subjects. Further molecular docking was carried out to investigate the interaction of metformin with OCT-1. This was followed by polymorphic analysis and sequencing of samples. Finally invitro functional assays were carried out on transfected cells to assess the effect of the changes in OCT-1 on metformin transport by quantification of activated AMPK.

Results: We observed that the variable response to metformin in the two groups is independent of isoform variation and mRNA expression of OCT-1. Further, molecular docking provided us an insight into the hotspot regions of OCT-1 for metformin binding. Genotyping of these regions revealed1222A>G, 181C>T and 1201G>A changes that were further found to affect metformin transport invitro which was illustrated by their effect on the activation of AMPK, the marker for metformin activity.

Conclusion: Taken together, our results corroborate the role of OCT-1 in the transport of metformin and also point at OCT1 genetic variations possibly affecting the transport of metformin into the cells and hence its subsequent action in responders and non-responders.

LT-14

Detailed investigation on the role of lipid metabolizing enzymes in retinopathy of prematurity pathogenesis

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Purpose:
Extremely preterm infants are at risk of developing retinopathy of prematurity (ROP) that causes impaired vision or blindness. ROP progression is characterized by neovascularization and neuroinflammation in the retina. Lipid metabolism is significantly altered in neovascularization, inflammation and neurodegeneration however its role in regulation of ROP has not been explored much. We therefore, aimed to explore the contributions of altered lipid metabolism in ROP pathogenesis.

Methods:
Blood, vitreous humor (VH) and fibrovascular membrane (FVM) samples were collected from preterm infants with ROP and No-ROP. Quantitative PCR was performed for comparing gene expression of lipid metabolizing

enzymes, angiogenesis and apoptotic genes among cases and controls. Activity for lipid metabolizing enzymes was assessed by measuring their metabolites in VH by LC-MS/MS. Further confirmation of significantly deregulated lipid metabolizing enzyme was performed in FVM by immunohistochemistry.

Results:

Most of the lipid metabolizing enzymes genes such as *CYP1B1, CYP2C8, COX2,* and *ALOX15* were upregulated while *EPHX2*responsible for conversion of epoxy fatty acids into diol fatty acids was significantly (<0.05) down regulated. The metabolites derived from abundantly expressed enzymes (blood) were found to be upregulated while metabolite from *EPHX2* did not show any change. Moreover, Ephx2 was not seen in the glial cells present in FVM of ROP subjects. Angiogenic gene *VEGF165, VEGF189*, *Notch1* and *APH1B*, apoptotic genes *Caspase3, Caspase8* were also significantly (<0.05) upregulated.

Conclusions:

Our result suggests that lipid metabolism has a potential role in ROP pathogenesis. A reduced*EPHX2* activity and expression seems to cause abnormal angiogenesis via *Notch1* upregulation.

LT-15

Intervention of Ayurvedic drug Tinospora cordifolia attenuates the metabolic alterations in hypertriglyceridemia: A pilot clinical trial

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Purpose: Hypertriglyceridemia (HG) is an independent risk factor with more prevalence than hypercholesterolemia and its attributes to cardiovascular disease (CVD) and pancreatitis. Hence, it becomes imperative to search for new triglyceride (TG) lowering agents. Tinospora cordifolia (TC) is a well-known Ayurvedic drug and a rich source of protoberberine alkaloids hence can contribute to TG lowering without side effects. Hence, to explore the therapeutic efficacy of T. cordifolia and its effects on biochemistry and metabolome in the patients of hyper-triglyceridemia, clinical trials were conducted.

Methods: Patients (n=24) with hypertriglyceridemia were randomized into two groups to receive T. cordifolia extract (TCE) (3.0gm/per day) and metformin (850mg/day) for 14 days having>300mg/dl triglyceride level and cholesterol in the range of 130-230mg/dl. Lipid profiles of blood samples were analyzed. Urine samples were subjected to HPLC-QTOF-MS to quantify oxidative damage and abnormal metabolic regulation.

Results: Intervention with TCE reduced the triglyceride, LDL, and VLDL levels to 380.45±17.44, 133.25±3.18, and 31.85±5.88 mg/d L and increased the HDL to 47.50±9.05 mg/dL significantly ($p<0.05$) in the HG patients after 14 days treatment. TCE dosage potently suppressed the inflammatory and oxidative stress marker's i.e., levels of isoprostanes significantly ($p<0.01$). Qualitative metabolomics approach i.e., PCA and PLS-DA showed significant alterations ($p<0.05$) in the levels of 40 metabolites in the urine samples from different groups.

Conclusion: TCE administration depleted the levels of markers of HG i.e., VLDL, TG, and LDL significantly. Metabolomics studies established that the anti-HG activity of TCE was due to its anti oxidative potential and modulation of the biopterin, butanoate, amino acid and vitamin metabolism.

LT-16

Expression of mitophagy genes in human senescence and their association with cancer- an Omics approach

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Mitochondria, the organelle primarily responsible for energy production, is involved in essential metabolic processes. Mitophagy clears dysfunctional mitochondria, and through this process it maintains cellular homeostasis. The dynamics of mitophagy decrease substantially with ageing, resulting in accretion of the dysfunctional mitochondria. This turns out to be a big risk factor in various cancer types as well as inflammatory diseases, diabetes, and neurodegeneration. We have tried to link the interplay between mitophagy, senescence and cancer, by making use of the network analysis software, Cytoscape. The enrichment analysis show the involvement of cell communication, protein metabolism, transcription factor activity and ubiquitin-specific protease activity. The major hub genes identified are ATG5, ATG7, ATG12, ATG14, BECN1, GABARAP, ULK1, and RB1CC1. The association with cancer shows the upregulated expression of has-miR-214-5p microRNA. Possible therapeutic strategy could involve the use of drugs such as estradiol and rapamycin.

LT-17

Association of human senescence genes with cancer using omics approach

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MicroRNA has been proven to be an indispensable marker in the carcinogenesis, owing to the fast-growing molecular technology in the past few years. There is an exponential increase in the number of human miRNA candidates being reported daily, and there are 2300 mature miRNA, out of which 48% are annotated in the miRBase V22 database. However, a comprehensive study on the miRNA mediated pathways in the cancer is still underway. As there are vast number of miRNA candidates being predicted to be involved in the carcinogenesis, a computational approach of molecular profiling is needed to understand the disease progression in depth. Cancer is a highly faceted disease where multiple cellular and metabolic pathways have a direct role in cancer development and suppression, Cellular senescence (CS) is one of them. CS is a highly preserved phenotype in mammals which is predicted to supress cancer. The antagonistic pleiotropy theory suggests that these tumour suppressing genes becomes detrimental with age. Interestingly, Evolutionary theory proposes that the beneficial and negative effects link can be broken. By developing a systems biology approach to study, the genes involved in cancer and senescence could narrow down to a potentially important pathway. The OMICS approach could help us identify accurate targets for diagnosis and prognosis. Our work focuses on analysing the gene and miRNA list from OMICS data, determining the statistically enriched

pathways, visualizing and interpreting them. Our initial studies have identified two important genes - RAD51 and RPS27A and an oncogenic potential miRNA cluster – miR-17-92 (oncomiR-1). These genes are mainly involved in the DNA repair, nuclear-transcribed mRNA catabolic process and nonsense-mediated decay. Further computational studies would reveal promising targets involving these genes and miRNA cluster.

LT-18

Analysis of Osteoarthritis genes in human senescence using *in silico* tools

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Osteoarthritis is a degeneration of cartilage in hands, hips and knees resulting in stiffness and pain on those parts. The deterioration of cartilage which cushions the joints might be due to mutation in cartilage producing genes. Similarly, human senescence causes silencing of various genes leading to many diseases and osteoarthritis is one of them related to aging. The disease being usually manifested at elderly people, we try to identify the osteoarthritis genes that are differentially expressed especially in aging using the "SeneQuest" database. Then, we have used Cytoscape to visualize the network and identified 'hub genes' with Cytohubba. String enrichment analysis had shown the following GO Biological Process, Molecular function: collagen trimmer and collagen containing extracellular matrix. We find out the signature gene COL2A1responsible for osteoarthritis and next studied

its involvement in cancer using “DepmapPortal”. Head and Neck Cancer, and Skin Cancer were found to have the highest score in cancer dependency with the signature gene where the COL2A1 protein had mutation in 7 and 95 different positions respectively. While analyzing the post translational modification (PTM) on “PhosphoSitePlus,” we got that acetylation to be major PTM for the gene. While looking for a therapeutic approach, we observed the “Sprifermin” (NCT01919164) drug for treating knee osteoarthritis to be in Phase II clinical trials[1].

1. Zeng, N., et al., *Efficacy and safety of sprifermin injection for knee osteoarthritis treatment: a meta-analysis.* Arthritis Res Ther, 2021. **23**(1): p. 107.

LT-19

Association of glutathione S-transferase M1 and T1 gene polymorphisms with the severity of COVID-19

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Aim and objective:

COVID-19 is caused by SARS-CoV-2 infection and ranges from asymptomatic to fatal respiratory diseases. Virus induced oxidative stress plays an

important role in the regulation of the host immune system and an important factor for COVID-19 severity due to fragility of the antioxidant system. Glutathione-S-transferase (GST) enzyme provides cellular protection against oxidative damage. So, in this study we investigated the role of GSTM1 and GSTT1 gene polymorphism with COVID-19 susceptibility, as well as its outcome.

Material and methods:

The study included 269 RT-PCR confirmed COVID-19 patients with mild (n=149) and severe (n=120) conditions. All subjects were genotyped for GSTM1 and GSTT1 by multiplex polymerase chain reaction (mPCR) followed by statistical analysis (SPSS-21).

Results:

Frequency of GSTM1 null, GSTT1 null and GSTM1 null/GSTT1 null combination was higher in severe COVID-19 patients as compare to mild patients but did not observed significant association. In cox hazard model, death was significantly 2.28-fold higher in patients with GSTT1 null genotype (T1) (P = 0.047). In combination, patients having GSTM1 present (M1+) and GSTT1 null (T1-) genotypes showed poor survival rate (P = 0.02).

Conclusion:

Our results suggest that severity of COVID-19 was higher in individuals having GSTT1 null genotype. This study may provide predictive marker for COVID-19 severity.

LT-20

Lipidomics of Retinoblastoma

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Purpose: To identify dysregulated pathways in Retinoblastoma (RB)
Method: Lipids were extracted from three cell lines MIOM1 (control), WERI-RB (Non aggressive) and NCC (Aggressive) using chloroform methanol in 2:1. Total lipid extract was injected into LC coupled to QExactive plus mass spectrometer. Accurate mass data was acquired in both positive mode and negative mode. Statistical analysis was done using Compound Discoverer and dysregulated species were identified.

Results: More than 1000 lipids were found to be dysregulated between control and cancer samples. Among all the glycerophospholipids, phosphatidyl choline (PC)s and Phosphatidyl ethanlomines (PE)s were found more in number. From the positive mode data, we found monoacyl PC (22:0/0:0) and PC(24:1/0:0) were down regulated in cancer samples compared to control. These molecules were confirmed by their characteristic ion at m/z 104 in their MS/MS spectra. We found dihydroceramides, ceramides, hexosyl ceramides, lactosyl ceramide and complex glycosphingolipids in positive mode. We confirmed dihydroceramides by their characteristic ion at m/z 266 and ceramides at m/z 264 glycoceramides by the neutral loss of 162 or 180. Out of 50 identified dysregulated compounds more than 30 molecules belong to the class of sphingolipids. Log2 fold change of these molecules varied from -9.41 to 9.24.

Three phosphatidyl inositol (PI) lipids were identified from negative mode data and all were up regulated in NCC. The ratio of cancer cell Vs control cell was found to be 7.53, 2.36 and 2.59. Surprisingly abundance of two of the three PI molecules in WERI-RB are comparable to control. All these molecules are confirmed by the diagnostic ion at m/z 241 in their MS/MS spectra.

Conclusion: Down regulation of apoptotic ceramides may be the reason for cancer cell survival and thus ceramide pathway can be a potential drug target for the treatment of RB.

LT-21

Understanding the role of DEPTOR in the progression and metastasis of lung cancer

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Bronchoalveolar Lavage fluid (BALF) contains all the proteins secreted by lung epithelial cells and includes many cellular components such as immune cells. Therefore, a change in the secretory pattern of these proteins such as their altered expression may be identified and reflected as an indicator of a lung specific pathology. In this study, we devised a proteomic approach to identify the lung cancer specific protein markers from bronchoalveolar lavage fluid. A combination of Two-dimensional gel electrophoresis and MALDI TOF MS/MS analysis was used to analyse the BALF protein composition of patients suffering from different subtypes of lung cancer using non-cancerous inflammatory disease BALF samples as controls. Deptor was found to be differentially overexpressed in lung adenocarcinoma when compared to its matched inflammatory disease control. Deptor is an endogenous inhibitor of mTORC1 and mTORC2 that plays a crucial role in the development and progression of human cancers. The biological role of Deptor in lung cancer metastasis, as well as the underlying molecular processes, remain unknown. To validate our proteomic findings ELISA and immunohistochemistry were employed to examine Deptor expression in lung carcinoma BALF and lung carcinoma tissues. The expression levels of Deptor in lung carcinoma BALF and lung carcinoma tissues were found to be significantly higher than inflammatory

diseases controls. Functional experiments demonstrated that Deptor overexpression promoted the migration and invasion of human adenocarcinoma cells and induced EMT by upregulating the expression of vimentin while down regulating E-cadherin. Moreover, we employed a top-down approach for finding three novel molecules which may prove effective in disrupting Deptor-mTOR interaction. Following Deptor modelling and validation we performed grid-directed structure based virtual screening by specifying the residues of Deptor known to interact with mTOR. A library of 10,000 protein-protein disrupting molecules was screened against the defined region of Deptor. For atomistic insights regarding Deptor-topmost hit interactions molecular dynamics simulation was performed for 1000 ns. These molecules after rigorous wet lab studies may prove as promising candidates for anticancer therapy.

LT-22

Studying protein modifications innon-alcoholic fatty liver disease by mining public proteomics data

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Various posttranslational modifications (PTMs) like acylations, phosphorylation, and methylation are known to be important in liver pathophysiology and measuring these changes in depth, at PTM sites level will guide the next generation of biomarkers. The re-analysis of public data

and clinical samples can reflect upon the pathways that are perturbed at various stages of the disease, which can help in clinical decision making.

We applied the PTM algorithms to a public dataset for mining the modifications in liver disease. To achieve this, we downloaded clinical mass spectrometry proteomics RAW data (PXD011839) for comparing healthy, NAFLD and cirrhosis patients in a comprehensive reanalysis. The original study generated a plasma proteome dataset that analyzed protein expression using mass spectrometry but modifications were not analyzed and a reanalysis can discover hidden information in the large-scale dataset. The data was converted into MGF format using the msconvert program from ProteoWizard, which contained 399 files with ~6.2 million spectra. This dataset was searched with phosphorylation, methylation, acetylation along with common artefacts of methionine oxidation and deamidation as variable modifications, as well as carbamidomethyl as fixed modification using the MSGF+ search engine. This output was processed using the PTM tools developed in-house, to analyze the proteins and PTMs.

A large fraction of proteome was found to be overlapping between the comparison groups (control vs disease) and these were best suited for differential mod-form analysis. We have quantitatively mapped the peptides for the data, and analyzed the proteins to calculate their mod-form distribution, and will investigate the molecular signatures to differentiate the conditions based on their mod-forms in an independent cohort. This will help in development of potential candidate biomarkers that can differentiate between the healthy, NAFL and cirrhosis conditions.

LT-23

Sperm-originated long noncoding RNAsand chromatin imprints in organismal development and cancer.

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Sperm has been considered as transcriptionally incompetent and a passive vehicle that transmits merely paternal genome to the oocyte during fertilization. This assumption is due to their compact nuclei and minimal cytoplasm. With the current advancement in next-generation sequencing approaches, some studies have shown traces of coding as well as noncoding RNAs in sperm and additionally, they reported highly structured and organized chromatin states. There is also a subset of long noncoding RNAs (lncRNAs) that have been shown to have an important role during development. Therefore, there is a need for genome-wide and transcriptome-wide approaches to investigate sperm lncRNAs, their chromatin profiles, and their role in development and disease. This study took advantage of the available high-throughput sequencing datasets to investigate sperm-originated lncRNAs and sperm-established chromatin states. Surprisingly, sperm-originated lncRNAs carry distinct chromatin

marks correlating with their transcript levels. We found that most sperm-specific lncRNAs to be active during zygotic genome activation (ZGA)which is later stages of preimplantation development. The lncRNAs that are common to both sperm and oocyte were found active in the early stages of preimplantation development, pre-ZGA. During post-implantation stages of development and in somatic tissues sperm-specific lncRNAs were found to be silent whereas common lncRNAs were active. Throughout these developmental stages, the chromatin states were well correlated with the levels of transcripts. We observed that sperm-specific lncRNAs that were silent post-implantation getting aberrantly activated in multiple cancers. Additionally, we show that protein-coding genes having bivalent promoters that were silent in sperm getting activated throughout the development process by losing their bivalency and gaining active chromatin marks. Overall, we found sperm-specific lncRNAs with well-defined chromatin profiles to be active in ZGA, silent in later stages of development and somatic tissues but seen aberrantly active in cancers from multiple tissues of origin

LT-24

Mitochondrial targeted curcumin inhibits glutathione reductase and modulates mitochondrial redox: Novel strategy for treatment of therapy resistant NSCLC

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Non-small cell lung cancer (NSCLC) is the most common type of lung cancer and contributes 85% of total lung cancer. Mitochondria play a vital role in cell survival and are also the primary source of ROS generation that regulates the cellular redox homeostasis. However, excess production of ROS can trigger apoptotic cell death. Hence, targeting mitochondria can be a good strategy for killing cancer cells. The dedicated thioredoxin and glutathione redox systems are the central antioxidant defense mechanisms by which mitochondria neutralize the excess ROS. In cancer these anti-oxidant systems get upregulated to cope with oxidative stress insult caused due to dysfunctional mitochondria. This upregulated antioxidant system leads to drug resistance in lung cancer. Mitocurcumin (MiC) is the derivative of curcumin that contains triphenylphosphonium moiety, which can be selectively targeted to the mitochondria.

Our study described that mitocurcumin inhibits recombinant glutathione reductase *in vitro* in cell free and cell based A549 cells. Mitocurcumin acts on glutathione reductase (GR) independently of NADPH which serves as the cofactor in enzyme catalysis. The type of inhibition mitocurcumin exhibited on glutathione reductase was mixed-II typewhere Km and Vmax both decreased. The secondary plot portrayed the affinity of mitocurcumin for the free enzyme was weaker than that for enzyme-substrate complex. The inhibition of GR affected mitochondrial and cellular GSH pool by increasing both mitochondrial and cellular ROS in a dose and time dependent manner. *In silico* docking studies revealed that mitocurcumin binds to allosteric site of GR and the affinity of mitocurcumin towards GR was more as compared to curcumin. Altogether, this study concluded that mitocurcumin modulates mitochondrial glutathione system that leads to ROS dependent apoptosis in A549 cells.

LT-25

Augmenting breast cancer multigene panels with pattern recognition receptors

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Breast cancer is a complex, multifactorial disease with environmental, lifestyle and genetic factors modulating disease pathogenesis. Understanding molecular and biochemical mechanisms of disease progression have impacted its detection, surveillance and treatment strategies. Mutations have been lately recognised as the fundamental lesions driving cancer. The effect of mutations on the cell survival is dependent on state of competing cells and microenvironment, certain mutations may provide an adaptive advantage. Alongwith mutations, focusing on immunomodulatory molecules, which influence the microenvironment, can help us understand the disease in a holistic manner. The recent advancements in DNA sequencing technologies have led to the availability of various breast cancer multigene panels. These panels target various genes involved in bypassing the cellular checkpoints and creating the tumour microenvironment, favouring neoplastic transformations. The

genes covered in these panels extensively measure the major hallmarks of cancer-related biology. However, the effect of immune system on the microenvironment is underappreciated in these panels. Elucidating enabling characteristics such as immune evasion, inflammation and aberrant glycosylation may supplement to a better understanding into the landscape of breast cancer progression.

Inflammation and aberrant glycosylation have been acknowledged in favouring cancer progression. Considering these enabling characteristics, pattern recognition receptors (PRRs) emerge as a contender to establish a better perspective of the microenvironment. PRRs recognise molecular structures on pathogens and altered host cells, thus activating downstream signalling, that influences the microenvironment. These receptors link innate and adaptive immune system, furthermore manifesting an integrated response in restoring microenvironment balance. Many animal lectins including galectins, mannose binding lectin, selectins *etc*. serve as PRRs. They have been reported to modulate inflammation and target aberrant glycosylation in various cancers. These proteins, through their interactions with glycan moieties, influence inflammation, thereby, modulating the microenvironment and cancer progression. Thus, after systematic and comprehensive studies, inclusion of these molecules as a component of multigene panels, may provide a better perception into the disease landscape and be pivotal in determining plausible therapeutic resolutions.

LT-26

A peek into the key secretory proteins involved in progression and metastasis of Human Colo-rectal cancer

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Numerous cancer types, remain poorly understood and conventional chemotherapy are till-date connected to their severe and deadly side-effects. There is a pressing need to discover biomarkers which are novel and highly sensitive, that will apply to a large patient pool. Cancers are ca heterotypic tumours which are ecosystems of dysfunctional tumour-epithelia with a vast array of other cell types referred collectively as stromal cells. These populaces are highly representative of innate immune cells, amongst which the populace of macrophages are the most dominant. The macrophages usually referred to, in the tumour microenvironment as tumour associated macrophages come from a circulating monocytic pool playing a key part in the planning and promotion of tumour. Macrophages are based on a complex interaction with tumour cells to obtain tumorigenic properties. In this research study, co-culture studies have shown that these macrophage characteristics are dictated by tumour-derived secretory signals that support their tumour-promoting phenotypes. When human monocytes were co-cultured with human colon carcinoma cells, it allowed monocytic cells to expulse tumour-promoting factors, thereby enhancing colon carcinoma cell proliferation, migration, aggregation and invasiveness. Overall, the research offered an opportunity to understand the precise protein/s secreted by each individual cell types (in monoculture as well as

co-culture scenarios) by using SILAC-based technique and various in silico tools, thereby establishing an interaction framework amongst colon carcinoma and the monocytes, revealing the establishment and promotion of pro-inflammatory and pro-metastatic tumour microenvironment, due to surplus of excretory proteins in the cellular secretions, be it classical, non-classical or otherwise.

LT-27

Cytotoxic mechanism of *Choerospondias axillaris* fruit extract by regulating the expression of SNCAIP and SNCA on MDA-MB-231 cells

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Background: Cancer is a huge problem of disease globally. Today, the percentage of people die from cancer is more than a combination of various diseases. In females, most common types of malignancies that occur are breast and cervical. The present focus has been shifted on medicinal plants as a form of therapy and there is a constant need to identify new therapeutic agents. *Choerospondias axillaris*, Lupsi/Lapsi, is an underutilized and edible fruit of family Anacardiaceae possessing many health benefits and has been used in the remedy of various diseases.
Objective: In the present communication, we evaluated the molecular mechanism of *C. axillaris* extract in regulating cell death in human breast cancer cells (MDA-MB-231).
Method: Methanol extract of *C. axillaris* was prepared and compounds were screened by Gas chromatography-mass spectrometry. Protein

profiling study of *C. axillaris* was performed by two-dimensional gel electrophoresis followed by identification of differential proteins by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS/MS) analysis. The effect of fruit extract was determined on MDA-MB-231 cells by MTT ((3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay followed by protein-protein interaction using online bioinformatics tools.

Result: A total 9 differentially expressed proteins were identified. Among 9 identified proteins, synphilin-1 protein was found to be significantly downregulated, validated by western blot and RT-qPCR analysis. Further, possible interacting partners of synphilin-1 (SNCAIP) were found to have their possible role in cancer.

Conclusion: Our data implicate that the presence of bioactive compound(s) in *C. axillaris* fruits might play an important role in inhibiting the proliferation of breast carcinoma cells and Synphilin-1 protein may play a role of apoptotic function.

LT-28

Potential peripheral blood gene biomarkers for *Taenia solium* infection of the brain

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Background: *Taenia solium* cyst infection of the brain, Neurocysticercosis (NCC), is a common cause of acquired epilepsy in India and often difficult to distinguish from other brain disorders with epilepsy clinically, on brain images and by serology. This necessitates brain biopsies to confirm diagnosis. Advanced next generation gene sequencing may permit identification of genetic blood biomarkers that distinguish NCC from other brain disorders with epilepsy.

Aim: To identify peripheral blood genes significantly associated with neurocysticercosis compared to other brain disorders with epilepsy.

Method: Peripheral blood (2.5ml) was collected from 26 NCC patients and 24 patients with other brain disorders with epilepsy in PAXgene RNA tubes, RNA extracted and cDNA libraries of RNA >6 RIN prepared using NEBNext Ultra Poly(A) mRNA Library Illumina Kit. Purified PCR products of the cDNA library were sequenced with Illumina Hi-Seq 2500 to a depth of 5595 million reads (paired end 2x100bp).

Transcripts were analysed with DESeq2 statistical software to determine those significantly associated with NCC compared to non-NCC disorders which were then subject to receiver operating characteristic (ROC) analysis and Gene Set Enrichment Analysis and Ingenuity Pathway Analysis.

Results: 1,74,166 transcripts were identified in RNA of whole blood with reference to the human reference genome, of which 40 were significantly upregulated and 120 down regulated with log2 fold change in NCC compared to non-NCC disorders. 70% of these transcripts are associated with cellular and molecular functions and involved in immune, cytokine and growth factor signalling.

On ROC analysis 34 upregulated transcripts were found to be 100% specific for NCC compared to non-NCC disorders. A combination of three of these transcripts PLEKHM1, MIA2 and S100PBP achieved 84.6% sensitivity with 100% specificity in distinguishing NCC from non-NCC disorders.
Conclusion: A combination of three peripheral blood genes show potential as biomarkers in distinguishing between epilepsies of NCC and non-NCC brain disorders.

LT-29

Tear proteome analysis to understand the etiopathogenesis in the ocular surface of chronic Stevens Johnson syndrome.

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Background: Stevens Johnson syndrome (SJS) is a drug induced and immune driven disease which affects cutaneous and mucous membranes of the body. The disease manifest from acute to chronic stage where the ocular surface is the most affected part. The damage occurred in the acute stage will cause progressive scaring including angiogenesis in ocular surface leading to trauma and visual impairment. SJS developmental pathways and its mechanism still needs to be further explored.

Aim: This study aims to understand the etiopathogenesisofocular surface in chronic SJS using tear proteomics.

Methods: Tear samples were collected from chronic SJS(n=6) with age-gender matched controls(n=6), followed by in-solution trypsin digestion to analyze in mass spectrometer to identify and quantify the tear proteome in SJS patients. Ingenuity pathway analysis (IPA) was performed to understand the significantly differentially regulating pathways in chronic SJS samples. Further validation of clinically correlated proteins was done by using multiplex ELISA method in tear samples of chronic SJS(n=24), severe dry eye disease (DED(n=14)) and age-gender matched controls(n=24).

Results: The total tear proteins identified were ∼2768 in which 249 proteins were significantly differentially regulated in chronic SJS tears. The IPA analysis, of the significantly differential regulated proteins revealedIL-8 signaling pathway (p-value 1.24E-06) and inflammatory response (p-value 2.64E-04) as major player in chronic SJS tears and could be correlated with disease condition. ELISA validation indicated thatCXCL-10 ($p \leq 0.044$) and IL-8 ($p \leq 0.009$) are significantly altered in SJS tears compared with healthy controls. In comparison with chronic SJS tears and DED tears, IL-8 ($p \leq 0.04$) and CXCL-10 ($p \leq 0.010$) were found to be differentially expressing.

Conclusion: IL-8 and CXCL-10 may play role in causing angiogenesis in chronic SJS patient ocular surface etiopathogenesis. Further validation and experimental proof is needed for confirmation.

LT-30

Deciphering extracellular-matrix collagen PTM-networks during neointima formationin post-stented coronary arteries

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Coronary artery disease (CAD) is one of the major causes of death worldwide. In CAD, myocardium gets deprived of oxygen due to impeded blood flow due to plaque formation. Coronary artery stenting is the gold-standard surgical measure for treating CAD patients. However, stenting could lead to the formation of neointima, an additional layer formed in arteries due to injury(stenting). Neointima results in restenosis of coronary arteries. In clinics, two types of stents namely bare metal stent(BMS) and drug-eluting stent(DES) are used. BMS and DES both may induce formation of neointima resulting in restenosis of the arteries. We hypothesize that remodeling of extracellular matrix(ECM) collagen(post-translational modification) PTM-network is the prime-mechanism of neointima formation. We utilized publicly available proteomic data set(#PXD005726) of BMS and DES induced neointima in the pig arteries. In-house MS analytical pipeline revealed significant elevation in the level of Collagen 1 alpha 1 (COL1A1) ($p<0.001$) in BMS induced neointima compared to DES. We also detected upregulation of 7 other chains of collagens including COL1A2, COL14A1 and COL12A1 ($p<0.05$) in BMS neointima. Importantly, for the first time we documented differential collagen PTM-network in the

neointima of BMS / DES. Significant upregulation in the occupancy of 3-hydroxyproline (P^{758}, P^{872}, P^{881}) sites in COL1A1 of DES induced neointima compared to BMS induced neointima was revealed. We also found that in COL1A1, G-Hyk^{573} (galactosyl-hydroxylysine) and GG-Hyk^{339}(glucosyl-galactosylhydroxylysine) levels were significantly lower ($p<0.01$) in BMS neointima than DES neointima. In addition, we also quantitated higher levels of hydroxylysine-pyridinoline (HyK-Pyr) mature cross-links (XLs) in COL1A1 in BMS induced neointima compared to DES induced neointima. Increased collagen XLs and altered collagen PTMs-network in the two different stents induced neointima formation may shed key insights in the pathogenesis of restenosis. These comprehensive collagen-network maps will lay the foundation to decipher ECM remodeling during BMS/DES induced neointima formation.

LT-31

Proteomic profiling of serum exosomes from HIV patients with and without tuberculosis co-infection

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Early diagnosis paired with appropriate treatment is important for the effective management of tuberculosis (TB) in individuals with HIV/TB co-infection. Presently available diagnostic measures for early diagnosis of TB in HIV positive patients offer sub-optimal diagnosis. With the aim of

identifying potential biomarkers for early diagnosis of TB in HIV positive patients, serum pool samples of HIV patients with and without TB co-infection (HIV positive and sputum smear positive individuals, HIV positive and Extra pulmonary TB patients, HIV positive and TB negative individuals, HIV negative and TB positive individuals),as well as from healthy humans (HIV negative and TB negative individuals), were processed to isolate exosomes followed by proteomic analysis using SWATH-MS method. The quantification of exosomes was performed by electron microscopy followed by SDS-PAGE and western blotting methods. A total of 111 proteins were identified at 1% FDR and were considered for protein lists and for visual comparative analysis. The gene symbols ID of the identified proteins were retrieved through Uniprot online tool. Out of 111 exosomal proteins, 76 differentially expressed proteins (DEPs) 54 upregulated (fold change variation >1.5) and 43 downregulated (fold change variation: >1.5) were found among the study groups. Out of 76 DEPs, 8 proteins {Hemoglobin alpha-1 globin chain (HBA1), Ig heavy chain V-I region (IGHVI-2), Serum amyloid A-1 protein (SAA1), Hemoglobin, beta (HBB), C-reactive protein (CRP), Vitronectin (VTN), Apolipoprotein A-II (APOA2) and Complement component C8 gamma chain (C8G)} were found commonly expressed among the study groups. The expression of two proteins (VTN and IGHVI-2) were found statistically significantly ($P<0.05$) expressed in the group of HIV positive and TB positive patients. In summary, this study identifies some crucial exosomal proteins which can be considered as diagnostic biomarker(s) for early diagnosis of TB among HIV positive patients after few validation studies.

LT-32

An *in silico* analysis of Single Nucleotide Polymorphisms of *LGALS4* along with Association of rs4802887 with Esophageal Cancer

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Rationale: Esophageal cancer is eighth most common cancer worldwide and fourth most common in developing countries. Altered glycosylation pattern of cell membrane molecules is a characteristic attribute of oncogenesis affecting cell-cell and cell-matrix interactions. Lectins are proteins, binding to specific carbohydrate patterns, thus suggested to affect cancer progression. Galectin-4, an animal lectin, has shown effect on cancer progression/metastasis in digestive system cancers. This role of galectin-4 can be attributed to variations in *LGALS4,* galectin-4 encoding gene. These variations can have multiple implications. Single nucleotide polymorphisms (SNP) are the most common genetic variations. So, the present study was

designed to *in silico* analyse SNPs in *LGALS4*followed by experimental validation of rs4802887 with susceptibility towards esophageal cancer.

Methodology: The SNP list of *LGALS4* was obtained from dbSNP. The validated SNPs with MAF ≥0.05 were further proceeded for their conservation status using Ensembl Genome Browser and conserved SNPs were analyzed for the study. The non-coding SNPs were further analyzed using SNPinfo for function prediction, RegulomeDB for prioritization of potentially regulatory variants, and Functional Analysis through Hidden Markov Models (FATHMM). Furthermore, rs4802887 (MAF=0.2708), was experimentally analyzed in esophageal cancer by Sanger sequencing in 63 patients and 53 healthy controls. MedCalc software was used for statistical analysis.

Results: The human *LGALS4* contains 3689 SNPs, out of which 25 were shortlisted. Human *LGALS4*sequence was found to be conserved with Bonobo, Chimpanzee, Gorilla, Orangutan, Vervet-AGM, and Olive baboon. Out of these 25 SNPs, one was present in 5' UTR region, two in 5' near gene, and 22 in intronic region. SNPinfo predicted two SNPs to affect the transcription factor binding site. RegulomeDB score predicted one SNP to be likely to affect binding and expression of a gene, and two SNPs to be likely to affect binding. FATHMM software predicted four SNPs to be deleterious. Genotypic analysis of rs4802887 showed higher frequency ofT allele and its homozygous genotype cases (43.65%; 22.22%) than controls (32.08%; 13.21%). Frequency of G allele and its homozygous genotype was lower in cases (56.35%; 34.92%) than controls (67.92%; 49.06%). Heterozygosity was marginally higher in cases (42.86%) than controls (37.74%), suggesting risk towards disease susceptibility (OR:1.481; p>0.05)

LT-33

Upregulation of LRG-1 in Osteoarthritis patients promotes inflammation and joint fibrosis: Proteomic study

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Osteoarthritis is most common inflammatory joint disorder and a major cause of suffering people at older age which associated with increased socioeconomic burden. Proteomics has emerged as robust technique for biomarker discovery in number of disease and elucidate their pathway involve. Thus, we have used SWATH analysis technique to identify differentially expressed protein in OA compared to healthy Controls. Leucine rich alpha-2-glycoprotein which is found to be the highest differentially expressed protein among the identified proteins has been further validated. We have shown that increase level of leucine rich alpha-2-glycoprotein lead to osteoarthritic joint fibrosis which further increase inflammation. Restrict joint movement along with cartilage degradation and synovial membrane inflammation is key factor involving in OA. Although the mechanism of OA is not known till date but, the key factors involving the joint fibrosis can be a critical target to understand the disease mechanism.

LT-34

Identification of key molecular players and their associated pathways involved in the tumor stage-specific progression of lung squamous cell carcinoma

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Lung cancer is a highly metastasizing and prevalent cancer with a mortality rate of 10 million as per GLOBOCON 2020[1]. The currently available diagnosis and treatment processes, including molecular targets and markers, are not adequate to tackle the disease progression, especially in SCLC, NSCLC, and adenocarcinoma tumors. Despite a considerable amount of data in lung cancer studies, there is still a lack of comprehensive knowledge in the molecular mechanism underlying its various types of lung cancer. Therefore, there is an immense need to find novel and stage-specific diagnostic and therapeutic strategies. Earlier studies in some cancers reported that noncoding RNA shows significant potential in early-stage detection of the disease [2][3].

In the current study, we have performed differential gene expression analysis on tumor stage-specific TCGA-LUSC RNA-seq transcriptome data. For the identified differentially expressed genes, we analyzed the protein-protein interaction network to find essential proteins in the network which might have a role in causing the disease.

We found that 2004 genes upregulated and 606 were downregulated in 2610 differentially expressed genes based on absolute logFC = 2 and adjusted P-value threshold <0.01. The PPI network of differentially

expressed genes was found to be having 445 proteins and 1029 interactions.

Network topological analysis reveals 34 proteins as hubs in the network based on degree centrality. 33 proteins were found to be bottlenecks based on betweenness centrality, and ten essential proteins were found to have both the properties of hubs and bottlenecks. Further, we performed functional enrichment analysis to identify significant GO terms and associated KEGG pathways. It results in 585 biological processes, sixty-one cellular processes, 115 molecular functions, and 24 pathways were associated with respective proteins. Further, we performed survival analysis to check the prognosis of these genes in the LUSC patients to propose stage-specific diagnostic therapies.

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LT-35

Peptidomic insight into urine to predict animal physiology

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The proteins in the body are under continuous turnover process resulting in the release of thousands of peptides. The unused and excess of such wastes are released into urine. Urine is a diagnostic sample which can be collected very easily and non-invasively. The dairy animals are reared under domesticated condition where the timely knowledge of physiological condition is very much desirable for the best management of the herd. In our study we targeted three physiological states of Sahiwalcows namely pre-puberty, pregnancy and lactation. Endogenous peptides were extracted from 30 individual cows belonging to three groups, each group comprising of ten animals (n = 10). nLC-MS/MS experiments revealed 5239, 4774, and 5466 peptides in the heifer, pregnant and lactating animals respectively. Urinary peptides of <10 kDa size were considered for the study. Peptides were extracted by 10 kDa MWCO filter. Sequences were identified by scanning the MS spectra ranging from 200 to 2200 m/z. The peptides exhibited diversity in sequences across different physiological states. In heifer and lactating animals' urine, low molecular weight peptides ranging from 1.4–1.5 kDa were more prevalent. In contrast, in pregnant animals' urine, peptides of relatively large size in the range of 1.8, 2.2, and 2.9 kDa were more prevalent. The amino acid composition indicated that alanine, glycine, leucine, proline, and serine (in decreasing order of abundance) were the most frequently occurring amino acids. Manual curation of 22

selected proteases resulted in the discovery of an average of 7215 protease sites. We determined the common protease activity in all three physiological conditions and found that 54 proteases out of 62 potential proteases (85.7%) were common. No protease enzyme could be uniquely associated with pregnancy. However, two unique proteases were reported in the heifer and lactating groups. The occurrence of Matrix Metalloproteases (MMPs) isoforms was wide spread across all the physiological states. The enzymatic degradation of target proteins during pregnancy was found somewhat slow and suppressed.

LT- 36

Biochemical and functional characterization of Guar *(Cyamopsis tetragonoloba)* korma proteins and its implications for phenyl ketonuria (PKU) die

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Phenylketonuria (PKU) is an autosomal recessive disorder caused by mutations in the phenylalanine hydroxylase (PAH) gene, results in the accumulation of phenylalanine (Phe), an essential amino acid mainly metabolized in the liver by the PAH system. Globally 0.45 million individuals have PKU, with global prevalence in screened populations is 1:23,930 and 1

in 18,300 in India. Guar meal is the combination of husk and germ portion of the guar seeds, a major by-product of guar gum processing and is utilized as cattle feed. Guar meal is a rich source of protein (38–50%). It is one of the cheaper raw materials available in large quantities, non-toxic, and environmentally friendly. Since it is cost-effective and has higher protein content, this material can be incorporated into human foods, mainly when protein insufficiency is a crucial nutritional issue. The present study aims to produce a cost-effective protein supplement devoid of phenylalanine (Phe) for the nutritional management of Phenylketonuria (PKU). We have investigated the biochemical, nutritional and functional properties of guar meal korma protein isolate and characterized the complete removal of phenylalanine (Phe) from the hydrolysate. Guar korma protein isolate was prepared by isoelectric pH precipitation method, and protein content of 90.81 % (yield-37.40 %) was achieved under optimal conditions. Amino acid analysis revealed that guar korma composed of all essential amino acids and met the minimum requirement for pre-school children as recommended by FAO/WHO/UNU. Further, Amino acid score (AAS) analysis revealed that valine (60.83 %), lysine (61.64 %) and threonine (61.85 %) are in limited amounts in guar korma. Protein digestibility-corrected amino acid score (PDCAAS) value of korma showed 50.30 %. *In vitro* protein digestibility (IVPD) study revealed that guar korma was completely hydrolyzed and showed (80.56 %) *in vitro* protein digestibility, indicating good digestibility. In addition, functional properties such as emulsifying activity (0.214±0.003), emulsion stability (20.53±2.22), foaming capacity (69±1.41%), foaming stability (81±6.53%), water holding capacity (1.06±0.12 ml), oil holding capacity (3.25±0.35 ml) and bulk density (0.254±0 g/ml) were analyzed and found to be comparable with soy protein isolate. Guar korma protein hydrolysates were prepared by acid and/or enzymatic hydrolysis (alcalase, pronase and papain). The phenylalanine was removed from the protein hydrolysate using activated charcoal (AC) treatment. The amino acid analysis of AC treated samples showed a significant reduction of Phe, and the complete removal was achieved after 2.5 % of AC treatment. The protein hydrolysate free of Phe can be used to develop formulations

combined with other foods to provide low cost, safer, nontoxic and natural food supplements for the PKU.

LT- 37

Omics analysis of Ageing genes associated with Sirtuins to target Lewy Body Dementia

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Dementia with Lewy bodies (DLB) is a neurodegenerative disorder characterised by the accumulation of aggregated α-synuclein. Although Alzheimer's, and Huntington's disease are linked with ageing, only very few studies conducted on Lewy Body Dementia (LBD). In our study, a total of 255 genes were identified in the aging human brain using Digital Ageing Atlas and visualised in Cytoscape v.3.8.2. The maximum confidence (score) cut-off 0.9 was applied to retrieve the String network with 111 genes. The top 10 ranking genes and the SNPs found in that list were identified using Cytohubba (MCC) and Panther. These *de novo* mutations in Guanine-nucleotide binding protein, beta 1 (GNB1) causes Global developmental delay. A further set of Sirtuin (SIRT) genes were associated with this network to get a combined network of 176 nodes. Next, the GEO dataset GSE20292 of LBD was filtered for differentially expressed genes and a network of 202 genes was created based on it. Merging these two networks provides us with a wide variety of motifs which were studied using MCODE.HDAC1 was identified as the most connected protein in this network. The major Gene Ontology (GO) terms with high significance and low FDR were identified and

listed as follows. GO process- nervous system development with 67 genes; GO component- cytoplasm (167 genes); Molecular function- protein binding (114 genes). Also we were able to identify transmission across chemical synapses and neuronal system as two significant pathways from the Reactome, and a further 14 pathways from the KEGG related to this network.

LT-38

Understanding the role of autophagy in human senescence using Enrichment analysis

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Cellular senescence is a process of irreversible cell cycle arrest that persists for a long time. Senescence is a continuous stress response that involves a variety of signaling pathways and the combination of which determines the morphological character. When autophagy is inhibited, the senescence phenotype, including senescence-associated secretion, is delayed. We have collected a sample of autophagy genes in human senescence that were differentially regulated. Using Cytohubba, we selected the top 10 genes and grouped them using MCODE and the results show MTOR and BECN1 in both categories based on MCC attributes. The top 10 genes (MTOR, BECN1, ATG7, ATG5, SQSTM1, ATG12, RB1CC1, GABARAPL1, PIK3C3, and ULK1) might be used as anti-ageing treatment options. We also focused on the important signaling pathways, molecular activities, and biological processes involving these autophagy regulating genes. Also we have used the drug

Everolimus for the upregulated gene set with MTOR, and Estradiol benzoate for the downregulated gene with BECN1 to see how they influenced the autophagy in relation to cancer.

LT-39

A comprehensive proteogenomics reference map of the human brain

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The identification of tissue-specific peptidoforms can reveal insights into protein expression patterns and their functional correlation. Understanding the proteomic landscape of brain can provide a means to decipher its role in health and disease. To achieve this goal, human brain proteome project was launched to identify proteins in the different regions of brain with major focus on neurological disorders. But, our knowledge about the presence of peptidoforms in different brain regions is still limited. Towards this, we have developed a method using proteogenomic approach employing multi-algorithm searches to identify peptidoforms and proteoforms. This method allows deep profiling of millions of spectra that are publically available in PRIDE database. We have built a comprehensive search database containing more than 34 million variants and isoform-specific tryptic peptides using amino acid polymorphisms from neXtProt

and genetic polymorphisms from GENCODE databases. This comprehensive search database is used to identify any peptide variant ever reported in the neXtProt or GENCODE database resources. We have identified about 20,000 peptidoforms from 19 PRIDE datasets belonging to healthy human brain tissues/regions like Cerebrum, Substantia Nigra, Pituitary, Temporal Lobe, Corpus Callosum and Hippocampus. The collective dataset involved 14.5 million spectra processed using the three search engines - MSGF+, X!Tandem and OMSSA. These peptidoforms show tissue-specific expression patterns, which can provide a reference map of healthy brain proteoforms and will act as a useful resource for comparative disease proteomics. The analyzed data from this study has been compiled as a publically available and user-friendly resource called HuBSProt. It is a dedicated MS-level data resource for finding and comparing proteoforms, as a ready reference for the brain proteomics community. HuBSProt can help users to evaluate search results and identify false hits in neurological disorders. It is the much needed reference map for brain specific tissue proteoforms to understand brain disorders in more detail.

LT-40

Expression of the fibrinogen alpha protein and its associated TLR-4 receptor complex in human osteoarthritis.

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Background- Osteoarthritis (OA) is a degenerative disease of cartilage loss that leads to joint deformity, urging the need for a better understanding of its aetiology and pathophysiological progression. A key feature of OA is a systemic, exaggerated inflammatory condition involving abnormal cytokines levels in the circulatory system and an altered level of proteins in the serum. In our previous studies, we found that the expression level of fibrinogen alpha protein (FBA) was up-regulated in plasma, and was detected in the synovial fluid of OA patients compared with healthy controls.

Aim -The mechanism and related key protein are still unknown how this heightened inflammatory condition manifests. The aim of the study is to investigate the expression and co-localization of FBA with its associated Toll like receptor (TLR-4) complex to understand the pathophysiology in OA.

Method- Peripheral mononuclear cells (PBMCs) and synovial tissue of healthy control and osteoarthritis patients were used to evaluate for expression and co-localization of FBA and TLR-4 by immunofluorescence. The distribution pattern of FBA in tissue and phenotype of cells expressing these proteins was monitored by immunohistochemistry.

Result – FBA protein was found to co-localize ($p<0.0014$) with TLR-4 receptor in OA PBMCs and synovial tissue compared to control. Expression of TLR-4 receptor ($p<0.04$) and FBA ($p<0.03$) were found significantly up-regulated in the PBMCs cells. The expression and distribution of FBA was observed in the lining and sublining layer of the OA synovium tissue and was found to be significantly up-regulated ($p<0.0095$). Further, the in-silico study also showed that FBA plays an important role in inflammation via TLR-4 receptor and activates NF-κB and MAPK signaling pathways.

Conclusion- Our findings suggest that the fibrinogen-TLR4 axis might play an important role in the atypical activation of PBMCs and synovial tissue in OA patients that may contribute to the exaggerated inflammatory condition.

LT-41

Signalling pathways that regulate cellular senescence

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A normal somatic cell undergoes many cycles of cell division, this brief process is riddled with barriers. As a consequence, it might cause irregularities in cellular activities in the form of oxidative stress, DNA damage, and oncogene activity. Also it might be responsible for irreversible process in the cells called replicative senescence. The strategy is to control the accumulation of abnormal cells, post-cell division and once the cells have entered senescence stage further divisions are terminated. Hayflick was the first scientist to observe cessation of cell division after a particular number of sub-culturing of cells. Following his finding, there are growing interests to find the cell signaling pathways and markers crucial for identifying cell senescence. Retinoblastoma protein (Rb) and p53 have been reported several times in association with senescence and tumor suppression. Telomeric shortening after each cell division lead to uncapping of chromosomal end, and this erosion/attrition of telomere serve as an indicator of senescence. Today the world is striving towards economically stable healthy societies, and countries that have achieved healthy-ageing population are focusing their attention towards “Age related diseases” (ARD) in their geriatric population. Therefore it is of paramount importance to understand senescence related abnormalities and apply the preventive measures in real time ageing related disorders. In this review we tried to

shed some light on the numerous researches done around "The Hallmarks of Cellular Senescence" and major cell signaling pathways involved in the cross-talk. Understanding the connection between the transcriptional activators and inhibitors regulating the senescence process might provide some therapeutic strategies in curbing age-related diseases at the earliest.

LT-42

Quantitative mass spectrometric approach to identify potential biomarkers in head and neck squamous cell carcinoma treated with radiotherapy

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Head and neck squamous cell carcinoma (HNSCC) is the second most prevalent cancer in the Indian male population. Radiotherapy (RT) with concomitant chemotherapy is the standard treatment for advanced HNSCC, which proves toxic to the patient. Early identification of patients with radio-resistant tumors is an important goal for scientists and clinicians. We have used a quantitative serum proteomics platform to study the differential expression of proteins with the progress of treatment.
Serum samples were collected from patients with HPV negative oropharyngeal and laryngeal tumors. Samples were collected before the start of RT (PreRT), 48 hours after RT (48hrsRT), and 1week after RT (1WeekRT). Patients were classified as "good responders" or "poor responders" based on their clinical outcome at follow-up. Relative

quantitation of serum was carried out by iTRAQ to identify the differentially expressed proteins. Twelve proteins showing more than 1.5-fold differential expression were chosen for targeted mass spectrometry validation.
A 1.5-2.5 fold pre-treatment upregulation of clusterin, gelsolin, extracellular matrix proteins, and proteins of the IGF pathway was observed in poor responders. A 2.0-5.0 fold upregulation of S100 proteins, clusterin, gelsolin, extracellular matrix proteins, IGF1, IGF2, and IGFBP3 was observed in poor responders within 48 hours to 1 week of starting RT.
The present results are the first report for a panel of twelve potential proteins which may facilitate the identification of patients who are most likely to develop resistance to radiotherapy. The significant and consistent upregulation of clusterin and gelsolin at PreRT and within 48 hours to 1 week of RT indicates their potential to act as early predictive and prognostic markers, respectively.

LT-43

Effect of estrogen in managing Rheumatoid arthritis pathophysiology

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Background

Rheumatoid Arthritis (RA), a persistent systemic disease linked with majorly joint inflammation leading to cartilage destruction is one such autoimmune disease with female prevalence (Female: male ratio 4-5:1 (age<50) and 2-3:1 in later ages). Several controversies regarding estrogen influence on RA raise an interest to search for the pathways regulated by estrogen in RA.

Objectives

This study aims to identify differentially expressed proteins in RA patients upon estrogen exposure leading to their in-depth study for understanding the mechanism of estrogen by *in vitro* and *in vivo* methods.

Methods

Expression level of inflammatory proteins like NF-KB, TRAF2 were measured before and after estrogen induction in primary RA fibroblast like synoviocytes normal SW982 synovial cell lines. Estrogen induction was given in CIA ovariectomized rat model to check the inflammatory parameters and other effects of estrogen.

Results

Inflammatory status of RA FLS by estrogen induction demonstrated reduction of inflammatory proteins related to NFKB pathway. *In vivo* study demonstrated decrease of inflammatory parameters along with reduction in physiologic characteristics in rat model linked with RA.

Conclusions

Expression changes of specific identified proteins can directly link estrogen mediated pathways in regulating disease pathogenesis. These proteins can become potential targets for therapy and can provide different ways of gender based treatments in RA.

LT-44

Differential Protein Transthyretin and Receptor for Advanced Glycation End Product's levels associated with Rheumatoid Arthritis pathogenesis

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Objective: Rheumatoid arthritis (RA) is a complex, chronic autoimmune, and inflammatory disease of joints. The identification of multifaceted etiologic changes at the protein level in RA remains an important need. We aimed to identify differential proteins (DPs) to uncover inflammatory indicators and their association to RA pathogenesis.
Methods: 2-DE and SWATH-MS were used to identify DPs from plasma of RA and healthy control(HC). Fluorescence phenylboronate gel electrophoresis (Flu-PAGE) combined with mass spectrometry was used for protein glycation detection in RA plasma. The disease specificity of identified DPs was confirmed by ELISA and Western blot analysis. The functional implication of glycated protein was determined and validated by *in-vitro* analysis in fibroblast-like synoviocytes.

Results: A total of 150 DPs (127 increased and 23 decreased) were identified by 2-DEand SWATH-MS analysis in RA plasma compared to HC. Nine proteins were identified as glycated by Flu-Page LC-MS/MS. Amongst, Transthyretin (TTR), serotransferrin, and apolipoprotein-A1(Apo-A1) were found to be differential and glycated. ELISA and western blot confirmed the disease-specific increased expression of TTR and RAGE in RA. The results signify the aberrant expression of TTR and RAGE associated with RA pathogenesis. Further, TTR-RAGE interactions via Co-immunoprecipitation were validated *in-vitro* using RA-FLS.
Conclusion: Our findings showed that the level of TTR was increased in RA plasma, along with an altered glycation rate. TTR and RAGE interaction in RA-FLS may have pathogenic/inflammatory significance.

LT-45

In silico expression of circadian genes in human senescence

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Circadian rhythms are controlled by a collection of clock genes that establish transcriptional feedback loops and produce a 24-hour cycle of circadian oscillation. Aging affects a wide range of physiological, hormonal, and behavioral cycles. Although new data shows that cellular ageing has a role in a variety of age-related illnesses, the consequences of cellular ageing on circadian rhythms have not been studied in detail. In this project, we aim to shed light on the effect of senescence on human circadian clock through a series of in silico tools and databases. We studied the differential

expression of circadian genes involved in human senescence. The studies showed that TP53 and SIRT1are the most important genes in both senescence and circadian rhythm. The functional enrichment analysis of the data also indicates a strong relationship between the circadian clock and the senescence genes being studied. These findings suggest that as cells age, their ability to transmit circadian signals to their clocks deteriorates. Thereby modulating the clock gene expression might be a possible treatment for age-related circadian rhythmicity deterioration.

LT-46

Identification and Analysis of Target proteins and Natural Compounds using Bioinformatics Approaches to treat Breast Cancer.

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Breast Cancer is the most common non-cutaneous malignancy in women, which has an estimated 268,600 new cases and 41,760 deaths in 2019 thus regarded as second leading cause of mortality grow up. Cancer occurs by a series of successive mutations in genes and these mutations alter the protein structure and ultimately protein functions. An accumulation of molecular mutants results in 277 types of different cancers. From evidences it is well known that the breast cancer subgroups shares similar gene activation or repression which alters common signaling pathways. These genes and signaling pathways are probably implicated in the tumorigenesis and progression of breast cancer. A deeper understanding of the metastatic

cascade in breast cancer will be critical for developing therapeutic interventions to combat breast cancer metastasis. Present study will focus on understanding mechanism of metastatic cascade and to find out better site of interaction on target protein where natural compounds can bind and may improve the disease stage and lead to overcome from the disease condition. As natural compounds have now been confirmed to have pharmacological function, with many of them capable of targeting cellular processes or deregulated genes that inhibit tumorigenesis. Thus, the present study will add one more idea in the existing treatments of breast cancer by identification and analysis of target proteins and natural compounds using bioinformatics approaches to treat breast cancer.

LT-47

Ameliorating effects of *Withania somnifera* in Rheumatoid arthritis through molecular docking analysis

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Background: Rheumatoid arthritis (RA) is a chronic joint inflammatory disorder characterized by repercussions of malfunctioning immune system resulting in synovial joint inflammation, synoviocytes proliferation and pannus formation leading to bone and cartilage destruction. The current therapeutics involves the widespread use of selective cyclooxygenase 2 (COX-2) inhibitors as primary drugs in controlling the clinical manifestation of RA. However, this inhibition has been validated in many studies and clinical trials to be associated with increased cardiovascular risk. Therefore, downstream inhibition of microsomal prostaglandin E synthase-1 (mPGES-

1) counters the therapeutic inefficiency of COX-2 inhibitors. mPGES-1 inhibition apart from rheumatoid arthritis has also been highlighted in other inflammatory diseases such as osteoarthritis, Alzheimer's disease and atherosclerosis.

Objective: The current study aimed to find the potent phytochemicals of *Withania somnifera* (WS) that targets against mPGES-1 enzyme. *Withania somnifera* has been included in the study due to its known anti-inflammatory properties and its potential in controlling the arthritic symptoms in arthritis rat model.

Methodology: The docking of the selected phytochemicals and the target protein mPGES-1 was carried out on AutoDock vina and the resulted interaction were analyzed using Discovery studio.

Results and conclusion: The obtained results reported that WS phytochemicals, Withanolide A, Withanolide B and Withanolide D exhibited better docked efficiency with binding energies at -7.6, -7.1 and -7.6 kcal/mol. WS phytochemicals, thus, may poses the potentials in ameliorating the effects of Rheumatoid arthritis inflammatory inhibitors and with further analysis developed into alternative herbal treatment for RA.

LT-48

Whole-genome sequencing unravels the novel genetic determinants associated with intravitreal triamcinolone acetonide-induced ocular hypertension

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Triamcinolone-acetonide-induced ocular hypertension (OHT) is reported in 30% of intravitreal steroid-treated Indian subjects, which when left unmonitored could lead to irreversible optic nerve head damage. Earlier genomics studies by target single nucleotide polymorphism (SNP) genotyping, and whole-genome sequencing (WGS) studies have failed to infer the variants and pathways associated with TA-OHT. Therefore, this study aimed to identify novel genetic determinants associated with TA-OHT among Indian subjects. The blood sample was collected from 52-TA treated subjects with informed consent, DNA was isolated. Intraocular pressure (IOP) values were monitored up to 6-months post-injection, and subjects were classified as steroid-responders (SR: IOP ≥ 21mmHg), and steroid non-responders (NR: IOP ≤ 20mmHg). Subsequently, WGS was performed, variants were filtered and prioritized based on their

pathogenicity, and disease association, followed by gene ontology and pathway enrichment analysis. Based on the IOP values, 25 subjects were identified to be SR, and 27 were NR to TA treatment. Variants identified from WGS unraveled 45 intronic, and 28 exonic SNPs were associated with TA-OHT progression. Among exonic variants, only 6 SNPs present in *CRPPA, PLOD1, SHARPIN, TIMELESS, CHD9,* and *ARHGAP1* genes were directly associated with TA-OHT progression. While, variants in *MYL10*, *OPTN, WDR36, TGF-β2* and its latent form, *TNF,* and *COL* family genes, were observed to be the major indirectly implicating genes aiding TA-OHT progression. The gene ontology reports decode that the prioritized variants have a vital role in eye, brain, and bone deformities. In addition, pathway enrichment analysis revealed that these genes were majorly involved in focal-adhesion, cardiomyopathy, extracellular matrix, and actin cytoskeleton re-organization signaling pathways, which on dysregulation could lead to TA-OHT progression among Indian subjects. Overall, appropriate use of the identified variants would benefit the ophthalmic community by analyzing these markers from blood samples before steroid treatments that would reduce the burden of secondary OHT.

LT-49

Cross kingdom regulation: A new approach in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a joint-destroying chronic inflammatory and autoimmune disease. Several research have been conducted to date that promise a cure, however the remedy has not yet reached adequate levels. Plant microRNAs (miRNAs) are becoming a popular strategy to treat diseases since they are naturally found in the food, have few or no side effects, and work in an energy-saving manner. Herbal approach to treat disease by a cross-kingdom mechanism via exogenous miRNA is an emerging trend to target associated genes with RA pathogenesis as a therapeutic potential. Exogenous miRNA influences signaling in other organisms by influencing their gene expression across species and kingdoms. The concept of acquired/exogenous miRNA into pathophysiological prospect provides an opportunity to explore inter-species kingdom like regulation of plant miRNAs (diet derived) on human health. The change in gene expression was attributed by a short (22-24) nucleotide long sequence that binds to its complementary region to suppress/silence the gene expression. This makes exogenous miRNA a novel approach for targeted therapy to treat complex chronic inflammatory diseases and can add new dimensions to herbal medicine. Our study provided the clues that *C.longa* derived miRNA has the potential to target human inflammatory gene expressions leading to reduce the symptoms of RA. Validation of above described miRNA transmission from plants to human (cross-kingdom transmission) may revolutionize the drug therapy in RA.

LT-50

A quantitative proteomics approach revealed alteration in dog brain proteome during furious rabies virus infection

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Rabies is a neglected zoonotic disease caused by rabies virus (RABV). Despite the existence of control measures, dog-transmitted human rabies accounts for 56,000 annual deaths world-wide with 60% deaths being reported in India with approximately three times more occurrence of furious form of rabies than the paralytic form. Currently, there is no suitable diagnostic tool for rabies before the onset of clinical symptoms and once symptoms appear; death is ultimate within a short period due to unavailability of therapeutics. Therefore, identification of host proteins altered due to RABV infection may provide some insight into the molecular pathophysiology of rabies. In this study, we aimed to identify differentially expressed proteins (DEPs) involved in furious form of RABV infection using 8-plex iTRAQ combined with High Resolution Mass Spectrometry. Out of 6952 identified dog brain proteins, 2188 proteins were statistically significant and among them, 140 proteins were differentially expressed in infected samples based on the fold change value. Further statistical analysis identified 40 DEPs including 26 down-regulated and 14 up-regulated proteins in infected samples compared to controls. Analysis with GO annotation and IPA software showed that proteins associated with calcium

signalling and calcium transport pathway were most affected due to RABV infection along with efficient neuronal function proteins and metabolic pathway associated proteins. Further, neurological disease and psychological disorders were identified as top diseases and disorder which are known as the typical symptoms of furious form of rabies. Some of these proteins were successfully validated by qRT-PCR and two proteins were successfully validated by western blot. This study provides the list of altered proteins and their probable role in RABV infection. However further studies are needed to confirm their role and to understand their utility in rabies pathogenesis which is currently in progress.

LT-51

Supramolecular reorganization of respiratory complexes is a unique mitochondrial proteome adaptation against proteostasis stress

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Cell adapts to different stress conditions through reorganizing existing proteome by undergoing conformational change, chemical modifications or by protein interactions. Supramolecular structures of multiple enzymes, also known as metabolons, represent one such kind of proteome adaptation. Metabolons are formed by dynamic non-covalent associations between enzymes catalyzing sequential reactions in a multistep metabolic pathway, thereby facilitating substrate channeling. Multiple supramolecular structures perform metabolic functions inside

mitochondria that include mitochondrial respiratory chain supercomplexes, TCA cycle metabolon, etc. We have done extensive quantitative mass spectrometry of mitochondrial proteome in proteasome inhibited cells. Two dimensional complexome profiling analysis revealed an increased abundance of mitochondrial respiratory supercomplexes during proteasome inhibition. In contrast, abundance of individual subunits was unchanged suggesting increased supra-assembly of respiratory complexes is a remodeling of the existing subunits without altering the overall subunit-pool. This proteostasis stress mediated reorganization is limited to Respiratory complexes only among the mitochondrial protein complexes and not observed for pyruvate dehydrogenase complex and TCA cycle metabolon. We hypothesize that oxidative phosphorylation is the major source of cellular energy supply during proteostasis stress and therefore cellular investment in remodeling respiratory complexes is preferred over other metabolic machineries during proteasome inhibition.

LT-52

Expression of Recombinant Silk Fibroin-Cecropin B Fusion Protein with Antibacterial and Antioxidant Properties

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For centuries, Silk has been used commercially for textile purposes. Recently, silk is emerging as an important biomaterial for biomedical applications. *Bombyx mori* silk consists of fibroin and sericin in which heavy

chain fibroin makes up the bulk of the *B. mori* silk fibre and it contributes to the physical properties of the silk. The outstanding mechanical properties of fibroin together with biocompatibility and slow biodegradation *in vivo* make this a novel protein. In the present proposal we focus on enriching the native fibroin with a potent antimicrobial and anticancer protein, Cecropin B to make a multifunctional silk protein with improved characteristics. Using the versatile yeast, *Pichia pastoris*, select part of GAGAGS repeat sequence from fibroin heavy chain gene is fused with full length cecropin B gene. The expressed fibroin and fibroin-cecropin B recombinant proteins were confirmed by SDS-PAGE, western blot followed by MALDI-TOF analyses. The antibacterial activity of the recombinant fibroin-cecropin B fusion protein was evidenced by zone of inhibition against both *Escherichia coli* and *Staphylococcus aureus*. The antioxidant and anti-UVB potential of recombinant proteins was verified by exposing recombinant protein treated HADF cells to UVB and H2O2. The protective effect of recombinant fusion protein was evidenced in terms of cell viability and significant reduction of LDH. Further, wound healing activity was analysed by *in vitro* scratch assay using HADF cells, where the recombinant fusion protein induced cell proliferation and cell migration towards the wound area. Based on these data, novel recombinant fibroin fusion protein will have applications in regeneration of damaged tissues- wound healing and cell culture applications.

LT-53

Bioprospecting Anti-Cancer Peptides (ACPs) from proteome of Muscle Tissue from Threatened Indian walking catfish, *Clariasmagur* (Hamilton 1822) by Mass spectrometry approach

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Clariasmagur (Hamilton, 1822), a freshwater walking catfish is one of the most popular aquaculture fishes in India and Asian subcontinents due to its nutritional value and better taste. The present study was undertaken to understand muscle proteome of magur. Muscle tissue is of significance in fishes due to presence of high protein content and poly unsaturated fatty acids (PUFA) proved to have medicinal and therapeutic value. Fishes procured were acclimatised and muscle tissue was studied. Briefly, muscle protein extract prepared from *C. magur,* by homogenizing muscle tissue in 50 mMTris buffer with protease inhibitor for downstream processing, digested with trypsin in solution, reduced with dithiothreitol (DTT) and alkylated with iodoacetamide (IAA) for LC/MS analysis. The peptides were separated on Waters Synapt G2 Q-TOF equipped with Electro-Spray Ionisation (ESI) for DATA independent acquisition for MS analysis. The raw data was processed by Protein Lynx Global Server (PLGS) software. Peptide tolerance limit was set at 50 ppm with minimum fragment match of 2 peptides for proteins. *In silico* approach was used to retrieve ACPs from muscle proteome derived from LC/MS by BIOPEP, Anti-CP and iDACP online servers. Out of a total of 468 peptides, 60 peptides showed anti-cancer peptide (ACPs) activity. Out of 19 non-allergenic peptidesas analysed by

AlerPred software, one peptide was toxic as revealed by ToxinPred software. The peptides were ranked with 0.9884 being highest and 0.0341 being the lowest by Peptide Ranker tool. This study reports *C. magur* derived anti-cancer bioactive peptides as a natural, less toxic anti-cancer therapeutic source exhibiting anti-tumor activity by activating apoptosis in mitochondria slaying tumor cells.

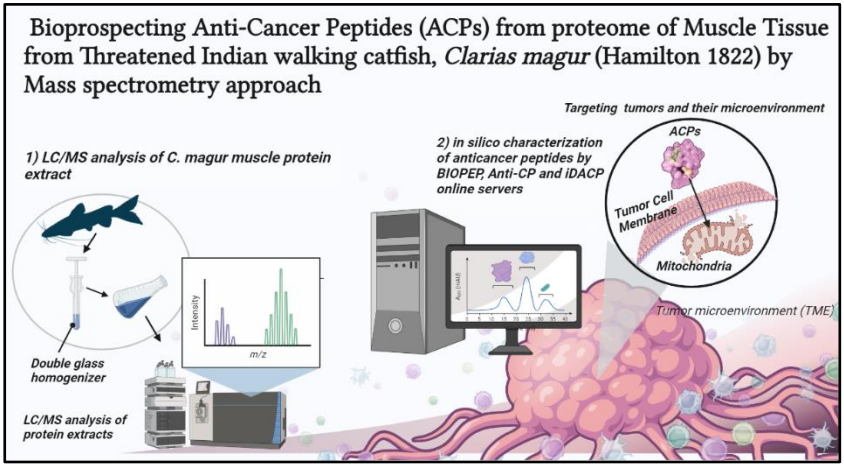

Figure depicting outline of workflow

LT-54

Extraction of Proteins from early-pregnancy Buffalo placentas and comparative efficacy of different methods for enrichment of Glycoproteins

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Ruminant placenta has several notable characteristics. They possess localized regions of villous chorionic 'cotyledons' that interdigitate with a glandular region in the maternal uterus to form 'placentomes'. Placentation in buffalo is of the synepitheliochorial type where placentome develops because of local interactions between fetal placenta and uterine epithelium. The scientific community has a perception about Pregnancy Associated Glycoproteins (PAGs) as only major glycoproteins in placenta. However, preliminary experiments suggest that there are several other than PAGs which are important glycoproteins. In an attempt to improve upon existing knowledge about cotyledonary glycoproteins, gravid uteri from pregnant (mid pregnancy 60days to 90days) water buffalo were collected from local slaughter house, immediately after retrieval fetal cotyledons were separated out, washed with saline thoroughly and processed further for protein extraction. Isolated proteins were then subjected for glycoproteins enrichment via affinity chromatography using three different lectins namely Glycoprotein Enrichment resin based on phenyl boronic acid, concanavalin A (ConA) and wheat gram albumin (WGA) immobilized on agarose beads. Eluates from these lectins and total cotyledonary proteins were further processed for profiling via Mass spectrometry (EASY-nLC 1200 system (Thermo Fisher Scientific). nLC-MS/MS data identified total 1742, 227, 565 and 649 proteins from cotyledonary protein sample, glycoprotein enrichment resin, ConA and WGA eluates respectively. Apart from different PAGs isoforms, several other glycoproteins were also enriched which were not examined before. Additionally, Glycoprotein confirmation was done by Periodic acid Schiff's base (PAS) staining. On Comparison of glycoproteins enrichment from three different lectins WGA was found to be more efficient. This study advocates the necessity for examination of pools of glycoproteins besides Pregnancy associated glycoproteins for their role in establishment of successful pregnancy in female water buffalo.

LT-55

Characterisation of toxic N-ethyl adducts on aa-tRNA recycled by archaeal DTD2 in land plants

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Reactive metabolites are an integral part of the biological systems as they fuel a plethora of elementary processes of life. Despite their critical significance, unwanted accumulation of these toxic metabolites causes genotoxicity, cancer, and cell death. Here, we identified the role of chiral proofreader DTD2 in protecting the plants from acetaldehyde, a toxic intermediate of anaerobic respiration. Using electrospray ionization mass spectrometry (ESI-MS), we discovered that acetaldehyde generates stable ethyl modification on aminoacyl-tRNAs (aa-tRNA). Tandem fragmentation studies (MS2) demonstrated that modification happens only on the amino acid part of aa-tRNA. L-aa-tRNAs remain protected by elongation factor thermo unstable while D-aa-tRNAs get modified. DTD2 protects plants from acetaldehyde by decoupling N-ethyl-D-amino acids from N-ethyl-D-aa-tRNAs (NEDATs). Overall, the research uncovers the chemical basis of acetaldehyde hypersensitivity in DTD2 knockout plants. We also discovered DTD2 gene transfer event from methanogenic archaea to the progenitor of land plants that played a crucial role in land plant emergence.

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LT-56

Understanding the molecular response of rice (*Oryza sativa* L.) to *Rhizoctonia solani* phytotoxin using untargeted metabolomics (Metabolomics analysis of rice during sheath blight development)

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A host-selective phytotoxin designated as *Rhizoctonia solani* toxin (RST) is identified to be a potential pathogenic factor of *Rhizoctonia solani*AG1 IA, causing sheath blight (ShB) of rice. To understand the mechanism of necrosis incited by RST, the metabolomic changes induced by the phytotoxic metabolite in a susceptible rice cultivar were elucidated by Gas Chromatography-Mass Spectrometry (GC-MS) analysis and compared with that of the pathogen to identify rice metabolites targeted by the phytotoxin. The profiles of about 29 metabolites with various physiological roles in rice plants have been identified worldwide. Unsupervised and supervised multivariate chemometrics (Principal Component Analysis, PCA and Partial Least Squares-Discriminant Analysis, PLS-DA) and cluster (Heat maps) analyses were used to compare the metabolites obtained from chemical profiles of the treatments with sterile distilled water (SDW) control. The results indicated that the rice plant expressed a greater

number of metabolites in response to the pathogen rather than the phytotoxin and was lowest in SDW control. The key metabolites expressed in rice in response to the treatments were investigated by the Variable Importance in Projection (VIP) analysis using $P< 0.05$ VIP >15. The analysis identified 7 and 11 upregulating metabolites in the phytotoxin and the pathogen treatments, respectively, compared to the untreated control. Among the phytotoxin-treated and the pathogen inoculated samples, the phytotoxin treated sample recorded upregulation of 6 metabolites, whereas 9 metabolites were upregulated in the pathogen inoculated samples. These upregulating metabolites are speculated for the necrotic symptoms characteristic to both the phytotoxin and pathogen. In this analysis, hexadecanoic acid and dotriacontane were found to be highly expressed metabolites specific to the phytotoxin and pathogen-treated samples, respectively. Besides upregulation, the metabolites also have a VIP score of >1.5 and hence fulfilled the criteria of classifying them as reliable potential biomarkers. In the pathway analysis, hexadecanoic acid and dotriacontane were identified to be involved in several important biosynthetic pathways of rice, such as the biosynthesis of saturated fatty acid and unsaturated fatty acids, cutin, suberin, and wax. When tested for inhibiting R. solani, hexadecanoic acid and dotriacontane had 75% and 80% inhibitory effects on the growth of the pathogen. The study concludes that these two compounds can be further studied as antifungal candidates aginst the pathogen.

LT-57

*Macrophomin*a secretome and mycelial proteome reveal potential effectors and virulence factors for disease establishment in host plant

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Secretome plays a key role in cell signaling, intracellular trafficking and migration of invasive weaponries, including effectors in phytopathogenic interactions. Extracellular weaponries secreted by pathogens are vital for increased virulence and disease establishment during plant-pathogen interaction. *Macrophomina phaseolina* represents a class of necrotrophic fungal pathogens that can infect more than 500 plant species. Fungal effectors can function within the plant apoplast or can translocate into plant cells where they target specific host proteins or enter subcellular compartments. Effector identification can enable disease control strategies. In search of fungal secretory virulence factors, we computationally predicted 986 secretory proteins from the genomic sequences of *Macrophomina* of which 303were predicted as effectors by using publicly available online tools. Gel-based *Macrophomina* secretome and mycelial proteome were developed by processing 171 bands in three biological replicates followed by LC-MS/MS analysis. We have identified 312 secretory proteins and 1704 mycelial protein extracted from axenic culture of which 70 and 346 were predicted as effectors, respectively. 41effectors were found to be common in both methods. Molecular masses of identified

secretory and mycelial proteins were distributed between 10 and 250 kDa, with majority of proteins exhibiting a molecular mass of 37-100 kDa. Functional categorization of the secretory proteome revealed their involvement in wall metabolism (34%), ion accumulation and signaling (30%), apoplast transport (11%), protein folding and degradation (9%), and defense (2%). In addition, gene ontology prediction of mycelial proteins showed overrepresentation of proteins involved in fungal development, signaling and disease establishment. Fungal protein-protein interaction network analysis identified major hub proteins related to fungal growth and progression, signaling events, fungal metabolism, degrading enzymes and protein turnover. Deciphering proteome-based *M. phaseolina* plant-pathogen interactions will play key insight in disease control strategies for crop improvement programs.

LT-58

S-nitrosoglutathione Reductase (GSNOR) in *Brassicajuncea* seems to be a multicopy gene

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S-nitrosylation, a Nitric oxide (NO) -based post-translational modification, is a reversible covalent attachment of the NO moiety to a cysteine thiol to form S-nitrosothiol (SNO) that regulates a wide range of physiological and biochemical processes by reprogramming of gene expression and altering the protein function. S-nitrosoglutathione (GSNO) acts as a stable NO reservoir that directly mediates transnitrosylation reaction. S-

nitrosoglutathione reductase (GSNOR), a master regulator of NO homeostasis, regulates GSNO turnover in cells. GSNOR mediated NO homeostasis is preeminent in developmental processes, metabolic programming, and a multitude of abiotic and biotic stress responses. The phylogenetic and sequence alignment analysis showed high homology of GSNORs among green plants and it is reported as a single copy gene in *Arabidopsis thaliana*. In-silico analysis showed 4 genomic sequences of GSNOR (1912bp, 2050 bp, 2053bp, and 2538 bp) in *Brassica juncea*. All four GSNOR genomic sequences were confirmed by Sanger sequencing. The genomic sequences code for 1140 bp (379 aa), and 1221 bp (413 aa) products. Gene structure analysis showed more than 94% homology in the exonic regions. Most of the variations lied in introns. The subcellular localization prediction using InterProScan, PSORT, and BUSCA (Bologna Unified Subcellular Component Annotator) suggested that BjGSNORs localize to the cytosol, Golgi apparatus, endoplasmic reticulum, and plasma membrane. Four immunospots at different pIs were confirmed by 2-D western blots. Overall, the study indicates GSNOR to be a multicopy gene localized at multiple sites. The regulation and roles of these multiple copies are being investigated.

LT-59

Proteome landscape of rice cytoskeleton revealed a novel nucleic acid binding protein, OsAlba1

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The highly dynamic nature of cytoskeleton is vital for all cellular process, whether it be cell division, cell activity, apoptosis, morphogenesis or signalling. Being a fundamental building block of cell structure and survival, cytoskeleton's function in stress resistance has been brought into question over the past decade. However, the correlation between cell metabolism and cytoskeletal networks are poorly understood. Therefore we developed the cytoskeletal proteome landscape of rice for better understanding of such events. Proteins were extracted from highly enriched cytoskeletal fraction of four-week-old rice seedlings, and the purity of the fraction was stringently supervised. A total of 2577 non-redundant proteins were identified using both gel-based and gel-free approaches. These included both microfilament and microtubule associated proteins and their binding proteins, consisting of hypothetical as well as novel cytoskeletal proteins. Further, various *in silico* analyses were performed, and the proteins were functionally classified on the basis of their gene ontology. Among the novel cytoskeletal components identified was OsAlba1, an Alba (acetylation lowers binding affinity) domain containing protein. Alba is controlled by acetylation and deacetylation, where acetylation at specific N-terminal lysine residue lowers its binding affinity toward dsDNA. Non-acetylated Alba protein has higher binding affinity towards ds DNA. We purified wild-type OsAlba1 through bacterial overexpression and carried out protein-DNA binding assay. We demonstrated that acetylated OsAlba1 is unable to bind to dsDNA, while non-acetylated one retains the binding affinity towards both dsDNA and ssDNA. Interestingly, OsAlba1 binds to DNA in sequence independent manner. Our results also show OsAlba1 binds to minor groove of the dsDNA. Altogether these findings unveil new insights of how cytoskeletons undergo dynamic remodelling and Osalba1 in genome organisation.

LT- 60

Combined spatio-temporal multiomics analyses of wilt immunome identifies regulatory hubs in vascular wilt disease

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Counter action strategies are pre-requisite for assault and defence against virulence factors of pathogen and innate immune system of plants. Modulation of plant immune system by host-specific determinants fine-tunes cellular components involving multiple organelles, particularly nucleus to mount resistance against pathogen attack. Vascular wilt caused by root pathogen *Fusarium* species is governed by host specific resistance in crop plants, including chickpea. In this study, we temporally developed nuclear proteome, metabolome and transcriptome to better understand gene expression switches in host specific resistance. Integrative analysis elucidated tangible insight into interaction coordinators leading to pathway determination governing immune state. Analyses identified hubs of known, novel and co-regulated genes and nuclear proteins which were appeared to be under metabolic control. At transcriptional and translational level, 216 immune responsive transcripts and ~30 nuclear proteins were identified, which were found to be associated with diverse nuclear and non-nuclear functions. Metabolite profiling detected 67 immune responsive metabolites of diverse chemical categories, particularly purines and nitrogenous bases. Functional enrichment revealed immunome containing three subnetworks involving CTI, PTI and ETI which likely represent key components that coordinate various biological processes favouring defence response. Our

robust statistical assessment captured known and unexpected nuclear protein, transcript and metabolite interaction, candidate novel regulators as future biomarkers. This study first time showed system-wide quantitative architecture corresponding to genotypic characteristics in wilt landscape. One of the candidate immune responsive factors, IRF 817was further characterized for its role in regulating immune status of chickpea against *Fusarium*. Interaction proteomics study identified 200 putative protein-protein interactors of IRF 817known to be associated with DNA replication, RNA synthesis, protein turn over, cell division, secondary carbohydrate degrading enzyme, transcription regulation, pathostress response and signalling.

LT-61

Comparative nuclear proteomics, phosphoproteomics and metabolomics analyses reveals mechanistic insights into disease vs immune signaling in riceblast

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Plant innate immunity is activated by microbe, or pathogen-associated molecular patterns in accurate manner determined by transcriptional, translational/post-translational and metabolic reprogramming. At organellar level, functional integrity of nucleus is constantly challenged by endogenous and exogenous factors regulating various cellular processes, including immune response. Rice blast, caused by hemibiotrophic fungus *Magnaportheoryzae*, is one of the most devastating disease that adversely

affect rice productivity. Role of nuclear proteoforms and their regulation in response to *M. oryzae* remains unknown. Temporal nuclear proteome and phosphoproteome analysis of blast-resistant and susceptible rice genotypes were carried out using iTRAQ and TiO_2-based phoshopeptide enrichment followed by SCX fractionation and MS/MS analysis. In total, 357 immune responsive proteins (IRPs) and 315 disease responsive proteins (DRPs) were identified. which ~55 nuclear proteins were found to be common and associated with chromatin remodelling, nuclear architecture, signalling, redox homeostasis and stress response. Phosphorylation status of nuclear proteins depicted that 25 and 22 phosphoproteins were expressed differentially in resistant and susceptible genotype, respectively linked to cell division, chromatin remodelling andsignaling. Further, GC-MS based metabolite profiling was conducted to identify 67 common disease/immune responsive metabolites with altered abundance during patho-stress. Data depicted how primary and secondary metabolite pool regulate chromatin and translational landscape during blast infection. Proteoform and metabolome data was interrogated using correlation network analysis that identified significant functional modules pointing toward immune/disease-related coinciding processes through common mechanism of remodelling and homeostasis. Novel clues regarding blast resistance included overrepresentation of nuclear architecture and chromatin remodelers, which provides evidence that coordination of nuclear function and reprogramming of host machinery regulate disease or resistance mechanism against blast disease. One of the immune/disease biomarkers, ALBA showed contrasting expression in resistant and susceptible rice cultivar during blast disease. Subcellular analysis revealed its dual localization in nucleus and cytosol. Further ALBA exhibited non-specific binding with ss-DNA, ds-DNA and RNA and might be a potential biomarker for blast disease thereby playing a key role in stress response.

LT-62

Phytochemical compounds as potential candidate to intervene the function of Sodium-Proton antiporters; *Ec*-NhaA.

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Sodium-Proton antiporter, NhaA is a ubiquitous protein found in cytoplasmic membranes of all the prokaryotic and eukaryotic systems. Theses antiporters have been widely studied in *E. coli* and their homologs are observed in human and found crucial for various pathophysiological conditions such as hypertension, heart diseases, blood pressure etc. NhaA are responsible for virulent properties of many pathogens like *V. cholerae, Yersinia pestis* etc. In present work we have exploited *In silico* approaches to find lead phytomolecules to interfere the activities of sodium-proton antiporters in *E. coli.* The plant based natural compounds database was used to screened 350 phytochemicals from various plant (using IMPPAT; a plant based natural compounds database.) as potential ligands for NhaA protein (PDB: 4ATV). Further blind docking was performed by Autodock vena, proposing 46 ligands with the binding energy ranging from -7.5 to -9.3 kcal/mol. Out of 46 ligands, ADME test has recommended 26 ligands which illustrated the non-BBB permeability, good GI absorption and solubility. The phytochemical luteolin has the highest binding affinity with NhaA showing the binding energy -9.3 kcal/mol. A derivative of steroid, Benzoyllineolone (-8.9 kcal/mol) was also observed as good candidate. Apigenin and 7-O-allylapigenin have similar binding affinity (-8.7 kcal/mol)

towards the target protein. Further Rhamnocitrin, abrectorinl and kaempferol were also observed as a promising candidate molecule with the binding energy of -8.6, -8.4 and -8.4 kcal/mol respectively. These phytomolecules may be proposed as good candidate molecule to interfere the activity of sodium-proton antiporters that may lead to affect survival of the various pathogenic bacteria. This study has established the NhaA as promising drug targets as well as screened phytocompounds as lead molecules for drug discovery. Further it may assist to find out effective therapeutic approach in associated human pathophysiological condition, especially heart diseases.

LT-63

Natural structural variation in homeologs of floral promoter *SOC1* and upstream regulator SVP present complex combinatorial interaction patterns in polyploid *Brassica juncea* for fine-modulation of flowering time

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Polyploid *Brassica juncea*, an economically important crop, suffers severe yield losses due to terminal heat stress, the impact of which will likely be more pronounced due to erratic weather patterns and global warming. Flowering time is a target trait for building climate resilience and enhancing productivity since early flowering mustard lines can evade multiple environmental stresses. As a key floral integrator, *SOC1*(*SUPPRESSOR of OVEREXPRESSION of CONSTANS*), is an interesting candidate to analyze

impact of combinatorial interaction patterns in regulatory modules within polyploid cytotypes. Several input pathways converge on homeologs of promoter elements of *SOC1,* of which repressor SVP (*SHORT VEGETATIVE PHASE*) is implied in ageing and temperature pathway. Delineating precise molecular interactions among various*SOC1*homeologs and SVP proteins can facilitate introduction of early flowering in *Brassica* by modification of binding motifs on promoters and/or critical amino-acid residues of upstream proteins. The present study was undertaken to identify the impact of polyploidy induced variation among homeologs of *SOC1* promoter and SVP proteins on their interaction pattern in *B. juncea*. Copy number and domain analysis in addition to phylogenetics revealed sequence diversification in both SVP proteins and*SOC1* promoter homeologs signifying structural variation. Structures were modelled for SVP proteins and *SOC1* promoters using I-TASSER/SWISS-MODEL and 3D-DART, respectively. Structural variants were observed for nine SVP proteins. Interactions were studied via *in silico* docking using HADDOCK server. SVP candidates with a stronger binding potential were identified. Critical HOTSPOTS as key amino acid residues were annotated in each DNA-protein complex. Our analyses revealed comparable binding potential for all except one SVP protein with *SOC1* promoters despite mutation in TFBS on two *SOC1* promoter homeologs (AALF and AAMF1). In summary, our study highlights quantitative contribution of diverse promoter and protein homologs for fine modulation of flowering.

LT-64

Integration of metabolomics and proteomics to unveil orchestration of photorespiration and carbon allocation in *Microchloropsis gaditana* NIES 2587

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Photosynthetic organisms have evolved and adapted strategies to overcome the limiting concentrations of CO_2. In this regard, the CO_2-concentrating mechanism (CCM) developed by microalgae implies an efficient machinery to acquire CO_2 in limiting environment. Inorganic carbon transporters channelize CO_2 towards Rubisco, however, there are significant differences in the CCM of some species and it is obscurely understood. In the present study, we performed qualitative metabolomics and proteomics on *Microchloropsis gaditana*, under the influence of very-low CO_2 (VLC; 300 ppm, or 0.03%) and high CO_2 (HC; 30,000 ppm, or 3% v/v) at the intervals of 0, 6, 12 and 24 hours. Our results demonstrate that HC supplementation channelizes the carbon flux towards enhancing the biomass yield, increasing up to 1.7-fold. Cyclic electron flow driven (CEF) by PSI confers energy to the cells in VLC in the initial acclimation stage. Our qualitative metabolomic analyses has identified nearly 35 essential metabolites among which significant fold-change was observed a photorespiratory by-product, glycolate, in VLC resulting in delayed growth and lower biomass. Whole cell proteomics study was performed in *M.*

gaditana in both VLC and HC conditions and a total of 998 proteins were identified. In VLC, cells undergo dynamic changes to activate biophysical CCM with the help of bicarbonate transporters. In conclusion, comprehensive changes occur inside the cell that consequently mediate the assimilation and regulation of carbon metabolic loadout such that it favours fatty acid biosynthesis in HC. In conclusion, our emphasis is to delineate carbon assimilation in *M. gaditana* with the help of advanced multi-omics tools and provide translational approach for the enhanced production of biofuels and biorenewables.

LT-65

The chloroplast genome of a resilient chlorophycean microalga *Asterarcys* sp.

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Asterarcys is a resilient microalgal species which can sustain high light intensities and have high biomass and lipid productivity under mixotrophy. These attributes make it a potential candidate to produce biodiesel and biofuels. However, no genetic information is currently available for this species. Here, we report the chloroplast genome sequence of *Asterarcys* sp. and compare it with the chloroplast genome of closely related chlorophycean microalgae. The chloroplast genome of *Asterarcys* sp. is present as a ~111 kb circular molecule with 88 genes and a coding density of 50.28%. The 111,776 bp genome shows the typical quadripartite structure with two 6297 bp long inverted repeats separating single copy

regions (SSC) of almost equal sizes. The small and compact genome of *Asterarcys* shows similarity in gene structure and organization with that of its closest relative *Scenedesmus obliquus*. It reinforces the compact nature of chloroplast genomes of Sphaeropleales as compared to that of inflated genomes in Chlamydomonadales. The genome shows a biased distribution of genes with 50 of the protein-coding genes encoded from one strand and 15 from the opposite strand. This biased distribution of genes is likewise to that of *Scenedesmus obliquus*. The phylogenetic tree based on protein-coding genes from chlorophycean species places *Asterarcys* close to *Scenedesmus*. The chloroplast genome information of *Asterarcys* will help understand the phylogeny of Sphaeropleales and Chlorophyceae. In the present study, we also report a simple and effective method for isolation of *Asterarcys* sp. chloroplast DNA of high quality and purity which is an essential prerequisite for efficient genome sequencing.

LT-66

Unveiling enhanced docosahexaenoic acid production upon glycerol uptake in indigenous strain *Aurantiochytrium*: an integrated omics perspective

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Aurantiochytrium sp.is a unicellular marine heterotroph with an excellent potential for producing polyunsaturated fatty acids (PUFAs) and other nutraceuticals. To this end, this heterotrophic microalga serves as an ideal

microbial cell factory for sustainable biorefinery processes. In the present study, we evaluated the regulatory mechanism behind the glycerol induced enhanced docosahexaenoic acid (DHA) production in the native isolate *Aurantiochytrium* sp. In this context, we employed multi-omics tools i.e., qualitative metabolomics and proteomics to elucidate the intricate cellular metabolism. Our metabolomics results identified ~32 metabolites comprising of amino acids, sugars and citric acid cycle intermediates. Furthermore, to illustrate the mechanism we tracked whole cell proteomics profile in *Aurantiochytrium* sp. following a time-course pattern identifying ~2000 proteins. Glycerol supplementation reveals upregulation of proteins involved in the pentose phosphate pathway (PPP) such as transaldolases/transketolases. Metabolomic profiling in the presence of glycerol identified upregulation of ribitol (intermediate of PPP) and amino acids such as valine, leucine and isoleucine. In addition, integration of metabolome and proteome unveils enhanced acetate concentration due to upregulation of proteins such as glycerol kinase and pyruvate dehydrogenase leading to enhanced biomass and DHA accumulation in glycerol. In conclusion, our integrated omics (*i*omics) approach revealed that glycerol can induce cell growth with improved fatty acid yields via., upregulation of pentose phosphate pathway.

LT-67

Bacterial bioremediation: Strategies adopted by microbial consortium for the sequestration of lead from the environment

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Exorbitant accretion of lead is a major solicitude for the environment because of its toxic nature which is associated with soil microbial heterogeneity, agronomical production as well as human wellbeing. Lead is one of the major components of pollution which makes air, water and soil contagious. Lead, being a hazardous product of waste cause high toxicity for plants, animals and micro-organisms. Various thermochemical techniques used for the rectification of lead from the pre-existing surroundings are highly expensive and also not productive because of the formation of different form of toxic compounds.

Bioremediation is regarded as the most effective technique for the sequestration of heavy metals from the environment with the help of plants and microbes. Micro-organisms are highly resistant in comparison to the eukaryotes which plays major role to attenuate the toxicity of lead. The absorption and accumulation of toxic heavy metals with the help of bacteria illustrate various metabolic associated and independent pathways. The most productive strategies exhibited by the microbial consortium to rectify the toxicity of lead from the significant sources such as soil, sludges and wastewater to purify the environment are biotransformation, biosorption, precipitation and encapsulation. Bacterial strains which are genetically improved show high efficiency and have various techniques of remediation from soil and different industrial wastes. Capable lead-resistant bacteria may be genetically improved for the excessive production of metallothionein, biosurfactants and proteins would lead to be good approaches to purify the environment from industrial effluents. In addition, this molecular technology acknowledges the production of strains with specific metal-binding properties through the expression of peptides which chelate proteins and metals. Although environmental bioprocessing has not investigated the different aspects of intercommunication between metals and microbes. Additional development and implementations are required to provide the non-toxic form of lead in the ecosystem.

LT-68

Global proteome analysis revealed metabolic remodeling during biofilm formation by *Mycobacterium fortuitum*

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Mycobacterium fortuitum, a nontuberculous mycobacterium (NTM)is present ubiquitously in the environment. It causes medical complications like joint infections, osteomyelitis, skin lesions, and infections concerning surgical sites. An important feature of its pathogenesis is its biofilm-forming potential. The biofilm formation trait contributes not only to the pathological consequences, rather facilitates the survival of the pathogen under diverse environmental conditions. The study provides the first description of the global proteome of *M. fortuitum* cells grown as biofilms, using a label-free mass spectrometry approach. Global proteome analysis helped in uncovering the metabolic pathways essential for *M. fortuitum* survival and persistence during biofilm growth state. A major follow-up revealing the induction of proteins related to energy generation was observed. Enzymes of the glycolytic cycle, the pentose phosphate pathway, anaerobic fermentation, and the TCA cycle showed significant overexpression in *M. fortuitum* biofilms in comparison to their expression in the planktonic growth state. In addition, the enzymes catalyzing oxidative phosphorylation also showed upregulation. Inhibition studies of the induced enzymes would help in combating the pathological consequences

of the *M. fortuitum* biofilm formation. The analysis would facilitate the discovery of protein entities as biomarkers that can be targeted for handling medical repercussions of *M. fortuitum* isolates. The study may also present remedies for combating other infections caused by biofilm-forming pathogenic microorganisms.

LT-69

Proteomic Approach to Explore the Effect of *Ocimum sanctum* on Filarial Parasite.

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Lymphatic filariasis (LF) is a vector borne disease very common in tropical and sub-tropical countries. 893 million people across the globe are threated by LF in 49 countries. Three nematodes are causative agents of this disease *viz*, *Wuchereriabancrofti, Brugiamalayi* and *Brugiatimori*. The filarial worms have long life span and remain for several years in the lymphatics of the host. Clinical pathology remarkably progresses by obstruction of lymphatic fluid and development of inflammatory responses against worms. The available anti-filarial drugs are Diethylcarbamazine, Albendazole and Ivermectin but these drugs have only microfilaricidal activity. All these drugs lack adulticidal activity and have numerous side effects too. Thus, there is a need of anti-filarial drugs which are adulticidal and with lower side effects. Traditional herbs and Ayurvedic medicines have been used for the treatment of filariasis in India since ancient times. *Ocimum* is a holy plant commonly in India and used since ancient times as Indian traditional drug. We have tried to explore the anti-filarial properties of *Ocimum* by studying

its effect on bovine filarial parasite *S. cervi*. Reactive oxygen species and DNA fragmentation was studied to evaluate the effect of Ocimum on adult female parasites. In 2-Dimensional electrophoresis more than more than 20 spots were differentially expressed in *Ocimum* treated worms as compared to control group. AMALDI-MS/MS analysis of some major differentially expressed spots of the treated parasites was done in which GST, Enolase, HSP 70, GAPDH were majorly affected proteins.

LT-70

Overexpression of an endogenous *Cecropin B* shows enhanced immunity to bacteria in transgenic silkworms, *Bombyx mori*

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Silkworms and other insects confer innate immunity by expressing antimicrobial peptides (AMP) through induction of Toll and IMD pathways. Cecropin B, an AMP from Bombyx mori has broad range antibacterial activity against both gram-positive and gram-negative bacteria. In an attempt to develop a silkworm strain expressing anti-bacterial properties, a transgenic vector, piggyBac overexpressing Cecropin B gene was constructed under its own promoter. The vector had GFP under the control of elongation factor alpha (ELFα) promoter as a marker for screening transgenic silkworms. Transgenic silkworms were generated by microinjecting vector along with the helper vector into the silkworm

eggs.The mRNA level of Cecropin B in the fat body of transgenic line was higher than non-transgenic line in response to E. coli and S. aureus. The mortality of transgenic line decreased as compared to non-transgenic line post bacterial infections. These results imply that overexpressing an endogenous AMP gene can enhance the resistance of silkworms against the bacteria.

LT-71

IL-13 regulates levels of IL-17 and Nitric Oxide in human VL

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Visceral leishmaniasis (VL) is a potentially fatal disease that is caused by *Leishmania donovani* parasite. The disease is established in presence of Th2 and suppression of Th1 cytokines but with no clear distinction between the two subsets. Further, Th17 responses are also dysregulated and have been reported as a risk for VL. Since IL-13 is a known regulator of Th17 responses in some inflammatory disease models, we investigated if IL-13 regulates Th17 responses in human VL. In the present study, levels of IL-17 and IL-13

were measured in pre and post treatment VL patients, endemic (EC) and non-endemic healthy controls (NEHC). Significantly raised levels of IL-13 were observed in VL patients when compared to endemic controls and the levels subsided after treatment. PBMCs isolated form healthy doors were incubated with culture containing 10% VL/ EC/NEHC plasma. The preconditioning experiments yielded induction of IL-17 from PBMCs incubated with control plasma but not with patient's plasma indicating presence of inhibitory factors in VL. Neutralizing IL-13 restored IL-17 to significant levels in VL plasma showing that elevated IL-13 levels may be linked to suppression of Th17 responses. We also investigated if IL-13 affects nitric oxide (NO) levels. The latter is an important innate defence against *Leishmania* parasite and its suboptimal production is frequently found in VL. NO levels were significantly raised in the endemic controls compared to patients and non-endemic healthy controls. Neutralisation of IL-13 in pre conditioning experiments partially restored NO levels in patient plasma indicating an important role of IL-13 in NO induction.

LT-72

A metabolomic approach to decipher the neuronal regulations of *Caenorhabditis elegans* during *Cronobactersakazakii* infections

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The small molecules determine the phenotype of an organism by playing a crucial role in activation of several regulatory mechanisms of the host. The alterations in the metabolome and subsequent host defence against the invaded pathogen are due to both primary and secondary metabolites synthesized during their interactions. The altered metabolites act as a factor for modulations in neuronal signals. *Caenorhabditis elegans* is a convenient model to study the neuronal regulations and neuronal players due to the availability of complete mapping of neuronal network. Neuronal signals such as neurotransmitters contribute extensively during actions of Gut-Microbiota-Brain axis. Numerous small molecules are involved in the biosynthesis and degradation of those neuronal signals. In the current study, the modifications at the physiological and biochemical levels were initially appraised to monitor the impact of candidate pathogen, *Cronobacter sakazakii* on the host *C. elegans*. In addition, the behavioural analyses indicated the changes at the specific neurons of the host system. In order to understand the small molecules which likely mediated neurotransmission, the whole metabolome of *C. elegans* during the infection of *C. sakazakii* was assessed using LC-MS/MS. Based on alterations in derivatives/interacting components of neurotransmitters obtained from metabolomics data, the small molecules involved in biosynthesis and transportation of candidate neurotransmitters were validated using qPCR analyses. MALDI-ToF/ToF technique also revealed the drastic modification in the regulation of specific neurotransmitters. In addition, the importance of short-chain fatty acids and lipid droplets accumulation in host neuronal regulations were studied. Overall the present study suggested the fatty acids mediated specific neuronal regulations in the host, *C. elegans* during *C. sakazakii* infections.

LT- 73

Impact of *Enterobacter aerogenes* on model host, *Caenorhabditis elegans*

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The popular model system, *Caenorhabditis elegans* has been explored for studying the impact of human pathogens in concern with pathology and mortality. Recently, a group of pathogens has been brought up for their significant contribution in hospital acquired nosocomical infection and emergency clinical gained contaminations in immunocompromised patients called as ESKAPE, a sort of pathogens such as *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacte rbaumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.* In fact, the *Enterobacter* spp. has reported under critical priority list as per WHO report, 2017. This study investigates the host responses against *Enterobacter aerogenes* (*EA*), which is a gram negative bacterium, reported as gut microbe, has the ability to cause urinary tract infections. We conducted a series of experiments to test whether colonization and transmission of EA in *C. elegans* could enable the behavioural changes upon pre-exposure. The pathogenicity of pathogen was assessed against *C. elegans* by lifespan assay which revealed the 50% mortality after 11 days in comparison with laboratory control, *E. coli* OP50. The effects of EA interaction was monitored with the feeding behaviour of *C. elegans* using three different assays: (I) Binary food preference, (II) Lawn avoidance, and (III) Lawn attraction assays. The feeding behaviour assays suggested that *C. elegans* appears to be attracted towards EA. Furthermore,

the impact of bacterial interaction was assessed through microscopic analysis of *C. elegans* along with CFU in different time points (24, 72 and 108 hr). We found defective egg production, accumulation of bacterial cell in post pharynx region and gonadal shirkage in worms exposed to *EA*. Then, the regulatory microRNA (miRNA) and their target gene interactions were identified through *in silico* analysis. To validate the *in silico* analysis, expression profile of candidate miRNAs and their target genes were analysed with qRT-PCR. The downregulation of target genes and upregulation of candidate miRNAs suggested that miRNA mediated regulation of immune response. This study reveals that miRNA dependent gene regulation which could pave a way for identification of new tools against pathogens.

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Development of a therapeutic cocktail for *pseudomonas aeruginosa* biofilm infections using engineered enzymatic quorum quenchers in combination with phages.

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Microbial infections are major havoc in medical setups. They are often treatable, but the prevalence of antimicrobial resistance and biofilm-forming abilities of microbes causes treatment ineffective. Around 2/3rd of microbial infections in medical setups are biofilm based. The nearly impermeable nature of biofilms results in poor drug penetration causing suboptimal effect and establishes the environment responsible for the

emergence of antibiotic resistance. Developing novel antimicrobials or using conventional antimicrobials at a higher dosage could be a solution but are not enough considering the associated side effects with a higher dosage of antimicrobials. The process of biofilm formation is a multi-step event involving a cascade of several regulatory processes often depicted through quorum sensing mechanisms. Research work suggests several microbial populations secrete small molecules and enzymes as quorum quenchers (QQs) downregulating the biofilm process. QuiP (Huang et al.; 2013), HacB (Wahjudi et al.;2013), QsdB (Tanniers et al.; 2013) are few examples of such enzymatic QQs found effective against microbial biofilms. In this work, we are planning to engineer chimeric QQs utilizing rational designing and engineering approaches, which have much more effectivity than parent molecules. We believe if combined with an active bacteriolytic particle, it will result in effective dispersal of biofilms and clearing up the microbial infection (Høyland-Kroghsbo et al., 2017) without stressing to the emergence of antimicrobial resistance, the infectivity of conventional therapies and minimize the ongoing novel antimicrobial searches.

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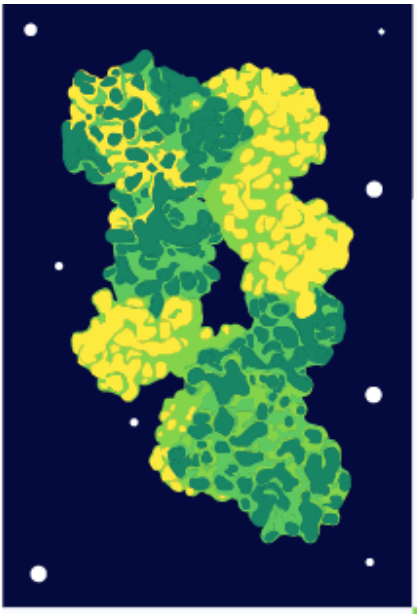

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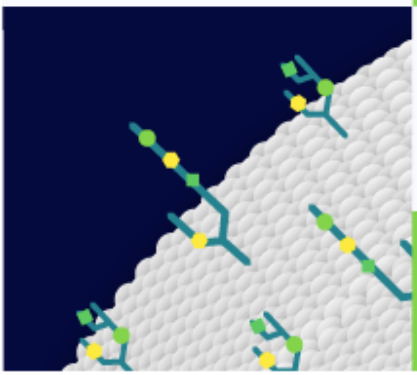

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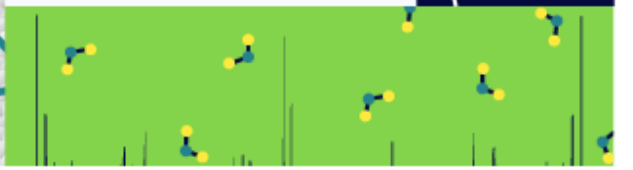

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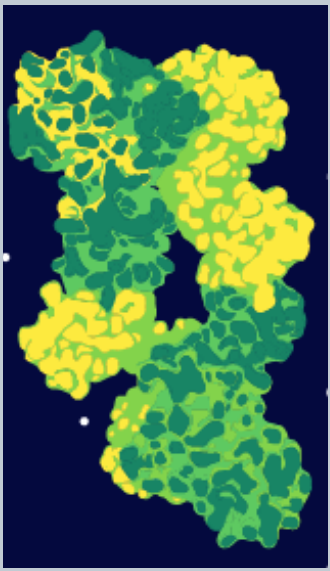
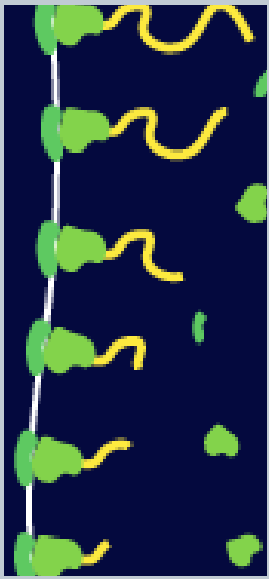
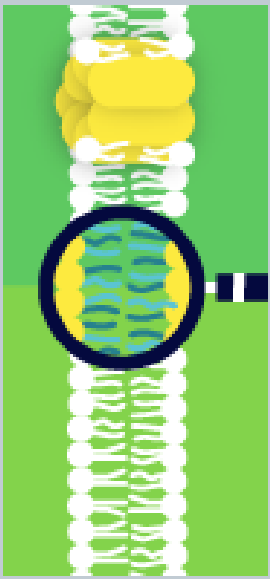

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