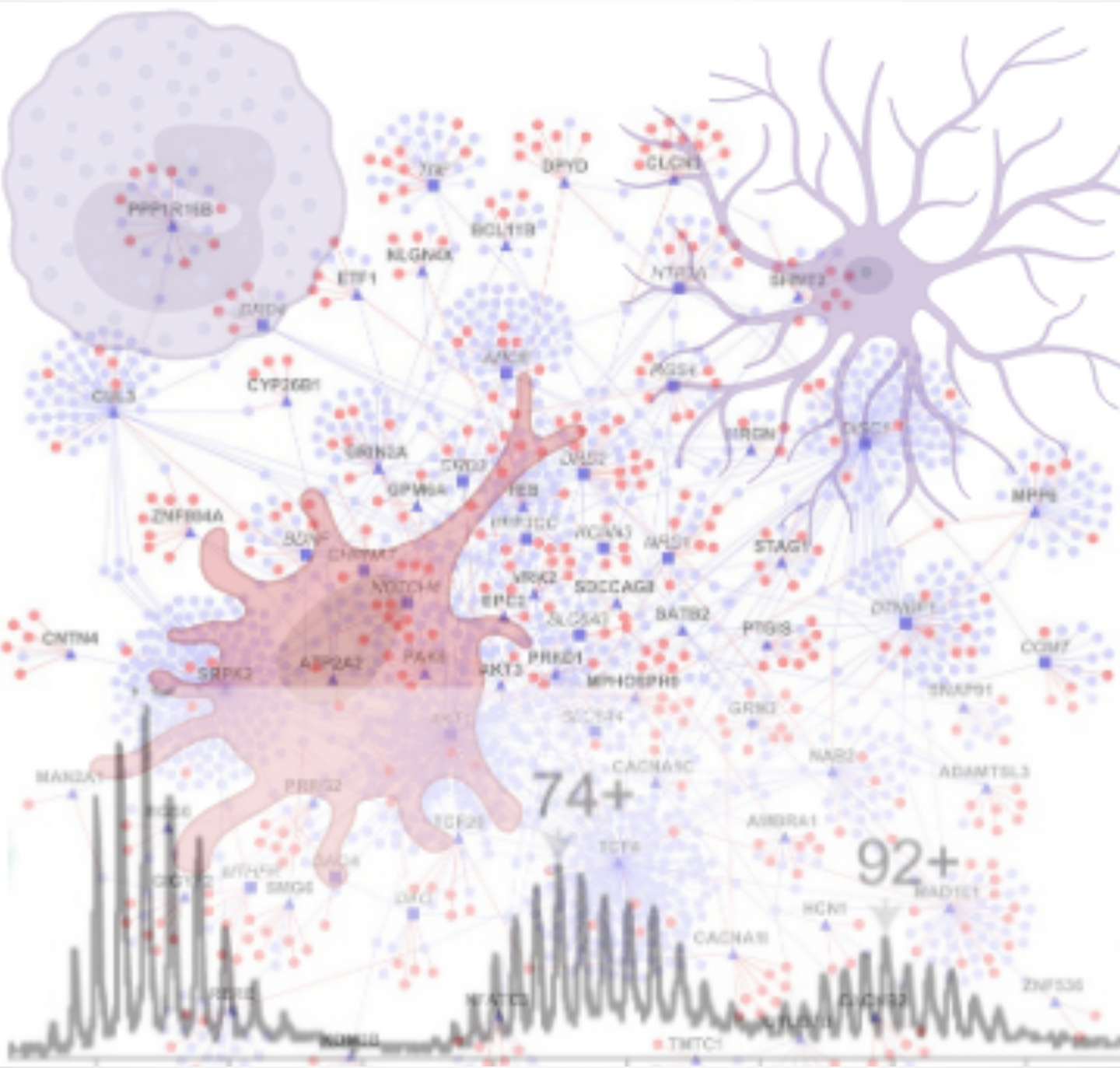
PSI Newsletter Vol. 8/No.2/December, 2020

PROTEOMICS SOCIETY, INDIA (PSI)



EDITORS
Dr. Tushar K. Maiti & Dr. Surekha Zingde

PSI Executive Committee

PRESIDENT
Dr. Subhra Chakraborty

VICE PRESIDENT
Dr. Shantanu Sengupta
Dr. Arun Bandyopadhyay

GEN. SECRETARY
Dr. Srikanth Rapole

TREASURER
Dr. Swasti Raychaudhuri

JT. SECRETARY
Dr. Ashok Kr Mohanty

PAST PRESIDENT OF PSI
Dr. Ravi Sirdeshmukh
(Founder President)
Dr. Surekha Zingde
Dr. Utpal Tatu

MEMBER

Dr. Utpal Tatu

Dr. Ravi Sirdeshmukh

Dr. Rakesh K Mishra

Dr. Abhijit Chakrabarti

Dr. Mahesh J Kulkarni

Dr. K Dharmalingam

Dr. Niranjan Chakraborty

Dr. Debasis Dash

Dr. Sanjeeva Srivastava

Dr. Suman Kundu

Dr. Tushar Kanti Maiti

Dr. Krishnan Venkataraman

Dr. Ramesh Ummanni

Dr. K. Balamurugan

Dr. Geetanjali Sachdeva

Editor's Message

Dear PSI Members,

The past few months have been very challenging for all of us.

However, PSI went ahead with its planned activities for the year using the Virtual mode.

This issue of the PSI Newsletter brings you a summary of the most sought after events, the Education Day and the Annual PSI meeting with a themed International Symposium.

Dr. Sanjeeva Srivastava and Dr. Srikanth Rapole have summarized the Education Day program succinctly. The delegates both new comers to proteomics and those who wanted to update themselves benefitted immensely.

This was followed by the three day Annual meeting and Symposium packed with information on the state of the art proteomics research in different areas of Health and Agriculture. Our Conveners, Dr. Ashok Giri, Dr. Mahesh Kulkarni and Dr. Dhanashekaran overcame all odds and brought together luminaries in the field to update our delegates.

This issue brings to you research from different laboratories across India, on a variety of subjects. It is apparent that proteomic tools are being widely used to address biological queries.

Members are requested to send us information about their research using proteomics, information they may require to facilitate their research and proteomics related programs that they are planning to conduct at their Institutions.

With Best Wishes for the NEW YEAR 2021,
Take care, Stay safe

Dr. Tushar Kanti Maiti
tkmaiti@rcb.res.in

Dr. Surekha M. Zingde
surekha.zingde@gmail.com

Message from President, PSI

Dear Members of the Proteomics Society, India,

Happy New Year!

On behalf of the PSI-EC and my own, I would like to wish all the Proteomics Society, India members a very happy and healthy year ahead during this unprecedented topsy-turvy situation of COVID-19 pandemic. Goodbye to 2020, the most disruptive and stressful year; hello to a good vaccine and setting for a new beginning in 2021. We are not only stepping to the First day of the New Year, 2021, but the start of the First year of a New Decade. Let the New Year remove the fear of the deadly creature and usher a blessed new Era of health, happiness with new aspirations in a cleaner environment.

COVID-19 pandemic has turned us physically apart and it is not yet over. Nonetheless, it made us learn many things and united the world as "One World". To circumvent this exceptional time we needed a well thought exceptional actions. PSI charted a path forward keeping in mind the depth and duration and some bit of uncertainties at every front. As was intended, the Proteomics Society, India, has continued to be devoted and dedicated to provide an excellent platform for its members and all the proteomers from India and abroad. Each year, the Society endeavour to come up with select themes for its annual meeting focused upon contemporary issues in the field. Recent years have shown increased frequency of high complexity diseases worldwide, including the deadly COVID-19 pandemic. The main objective of 12th Annual meeting of PSI 2020 and International virtual symposium on "Integrated omics approaches in health and agriculture" was to bring together the leading scientists and scholars in the area to discuss the practical challenges, concerns and strategies in the field, promote collaborations and information exchange and share the new findings. The first ever virtual annual meeting of PSI with the pre-conference Education Day happened in December 2020, was an enriching experience and a great success. PSI would continue webinars in the field throughout the New Year and I invite everyone to keep themselves updated and join the future events.

I am delighted to share with you that India would be hosting the HUPO 2023 Congress at Hyderabad. We shall be updating you time to time the progress and new happenings towards holding the meeting. Journal of Proteins and Proteomics, administered by PSI will continue to serve the scientific fraternity to publish and read new findings in the area of protein science. I am so glad to tell you that JPP is on the UGC CARE list and some of the published papers are in Pubmed with Pubmed ID as well.

It gives me great pleasure to bid farewell to a challenging yet exciting 2020, and we look forward to even more eventful and challenging year ahead. I am sure everyone at PSI family is enjoying their own research work and excited about the new developments in the field.

I would like to close by encouraging more and more participation of young members to keep the spirit high of the Society and request all to make maximum use of the proteomic science in answering research questions, collaborate and continuing as an active team. New vision and aspiration from all are most welcome. Let us know how PSI-EC can be of any help to its members.

Once again, I wish the entire PSI Family a very satisfying, successful, and rewarding year ahead and an enjoyable reading of the newsletter.

Stay united, Stay safe and Stay protected with Mask.

With best wishes for a successful 2021,
Dr. Subhra Chakraborty
President, PSI

A report on

12th Annual Meeting of the Proteomics Society, India (PSI) and international online symposium "Integrated Omics Approaches in Health and Agriculture

Organized by

CSIR - National Chemical Laboratory, Pune - 411 008, India
from November 21-24, 2020

PSI – Education Day – 21st Nov 2020
MS-based Proteomics: Concepts, Data analysis and Visualization

Scientists from around the world met and deliberated over the advances made in the field of proteomics in an International Virtual Symposium on "Integrated Omics Approaches in Health and Agriculture" under the aegis of Proteomics Society, India (PSI). The 12th annual meeting of Proteomics Society, India was held on 22-24th November 2020.

Before the symposium was held online due to the pandemic, the PSI conducted a day-long program, Education Day, on "MS-based Proteomics: concepts, Data Analysis and Visualisation" on 21st November 2020 to promote proteomics' science far and wide. The Education Day program was conducted by Dr. Srikanth Rapole, from NCCS, Pune, and Prof. Sanjeeva Srivastava from IIT Bombay. Over 128 students and faculties from India and abroad attended Education Day. It was a culmination of a week-long short lecture series where participants were appraised of the new tools and techniques in the world of proteome research. Participants were provided with pre-requisite materials, session, software downloads before the Education Day. This was aimed for the participants' orientation, many of whom had no prior knowledge of proteomics sciences and data analysis pipeline. Let us have a brief overview of what all students were exposed to during this week-long extravaganza of learning.

Prof. Sanjeeva Srivastava and his team of TAs from IIT, Bombay, namely Mr. Saicharan Ghantasala, Mr. Deeptarup Biswas, Ms. Susmita Ghosh, Ms. Medha Gayatri J Pai, Ms. Shalini Aggarwal, Mr. Nirjhar Banerjee from Proteomics Lab, Department of Biosciences and Bioengineering, IIT Bombay conducted regular evening sessions on the basics of both discovery and targeted proteomics. They were acclimatized with proteomics sample preparation. Further, primary data analysis software platforms like Mascot, MaxQuant, Skyline were explained. Also, big data analysis platforms like Metaboanalyst, Reactome were described. The participants actively engaged in the learning process, which was further accentuated by interactive quiz sessions.

Ready with a primer to the brave new world of proteomics, the participants were more than prepared to attend the intensive day-long session with leading scientists from around the globe.

Dr. Subhra Chakraborty, President, Proteomics Society, India started the introductory session to introduce participants to Proteomics and Journey of PSI. The lecture session started with a talk on "Mass Spectrometry based Proteomics" by Dr. Srikanth Rapole. He briefed the participants on the various mass spectrometry-based technologies used to understand the dynamic proteome. This was followed by an enlightening session by Prof. Sanjeeva Srivastava. He shifted the spotlight on innovations in the world of proteomics in his lecture entitled "Recent Innovation in Proteomics." The participants were surprised to know how proteomics technologies have helped scientists to understand the dynamic proteome. These sessions were followed by Q&A sessions which helped the inquisitive learners clear doubts. Both the mentors provided the participants with answers to their satisfaction. The over-exuberant Q&A sessions proved that the mentees were indeed enjoying the sessions. The day rolled on to some hands-on sessions. Mr. Deepatrup conducted a session on MaxQuant for MS-Data Analysis. This was followed by a lecture from none other than the inventor of a highly cited data analysis tool "Reactome".

PSI Annual meeting, 2020

Dr. Henning Hermjakob, Head of Molecular Systems EMBL-EBI, joined from the United Kingdom to enlighten the participants about Reactome's application. The participants learned the utility of Reactome and how it aids the scientists to make sense of the biology of the data obtained via Big Data approaches like mass spectrometry.

The following session was hands-on. It was hosted by Mr. Saicharan and Ms. Medha. They explained in detail two applications, namely Skyline & Panorama, and its use for SRM Assays. Being a hands-on session gave the participants a flavor of conducting a real-life experiment. Dr. Samridhi Sharma from Macquarie University, Australia, joined the session and helped participants explore a novel field of targeted proteomics in her lecture "Targeted MS Assays For Immunotherapeutic Applications." The final lecture of the day was by Prof. Graham Ball from Nottingham Trent University, UK. Prof. Graham Ball enthralled the participants with a very informative talk entitled "Omics Data: AI & Machine Learning." He explained how biological Big Data needs to be handled and how artificial intelligence-driven analysis of this Big Data can help scientists unearth hitherto unknown facets of experimental data.

The final session was a quiz which was conducted by Ms. Susmita Ghosh and TAs. Award winners picture is included below. The program was finally concluded with the thanksgiving note by Dr. Srikanth Rapole and Professor Sanjeeva Srivastava.

12th Annual Meeting of the Proteomics Society, India (PSI) and international online symposium "Integrated omics approaches in health and agriculture"

The 12th Annual Meeting of the Proteomics Society, India (PSI) and the accompanying symposium "Integrated Omics Approaches in Health and Agriculture " was hosted by CSIR-National Chemical Laboratory, Pune.

Dr. Ashok Giri, Dr. Dhanasekaran Shanmugam, and Dr. Mahesh Kulkarni were the conveners of the symposium. This year considering the ongoing COVID-19 pandemic, the event was held online from Nov 22-24 using the CISCO-Webex Platform. The focus of this year's meeting was to provide a platform to discuss discoveries, latest developments, and applications in health and agricultural sciences.

The inaugural session of the symposium was held on the afternoon of November 22. Dr. Dhanasekran Shanmugam gave the opening remarks and Prof. Ashwini Kumar Nangia, Director, CSIR-NCL gave the welcome remarks of the symposium. Dr. Subhra Chakraborty (NIPGR, New Delhi), PSI President, gave the inaugural remarks and overview of PSI and its activities. This was followed by two Keynote lectures chaired by Dr. Ravi Sirdeshmukh, founder PSI President and Dr. Srikanth Rapole (NCCS, Pune), current PSI General Secretary. The first lecture was delivered by Prof. Stephen Pennington, HUPO President titled Delivery of Advanced Protein Tests for Precision Medicine. The second lecture was delivered by Dr. Subhra Chakraborty and it was titled as System-Level Understanding of Nutrient Dynamics in Developing Seed.

Following the inaugural session, Session-1 of the symposium was chaired by Dr. Surekha Zingde, former PSI president, and Dr. Arun Bandyopadhyay (IICB, Kolkata), PSI Vice President. The speakers of this session included Dr. Amit Kumar Yadav (THSTI, Faridabad), Dr. Swasti Raychaudhuri (CCMB, Hyderabad), Dr. Adbul Jaleel (RGCB, Thiruvananthapuram), and Dr. Sheon Mary (University of Glasgow, UK). Dr. Yadav discussed bioinformatics approaches for the identification of PTMs, Dr. Raychaudhuri spoke on profiling of respiratory complexes using mass spectrometric approaches, Dr. Jaleel presented on metabolomics profiling of healthy individuals with a risk of type 2 diabetes and Dr. Mary spoke about the role of Uromodulin in cardiovascular diseases.

Since this symposium was online, instead of posters, lightning talks by the Ph.D. student and early career researchers was organized in Session-2 and Session-7 on November 22 and November 23 respectively. The Chairs for the lightning talks were Dr. Amol Suryawanshi (ILS, Bhubaneswar), Dr. Alka Rao (IMTECH, Chandigarh), Dr. Dhanasekaran Shanmugam (NCL, Pune), Dr. Debasis Dash (IGIB, New Delhi), Dr. Mahesh Dharne (NCL, Pune), Dr. Mahesh Kulkarni (NCL, Pune), Dr. Rakesh Joshi (NCL, Pune), Dr. Subashchandrabose Chinnathambi (NCL, Pune), Dr. Syed Dastager (NCL, Pune) and Dr. Vasudevan Seshadri (NCCS, Pune). The lightning talk session was highly appreciated and was the main takeaway from this symposium. Based on the presentation, the best five lightning talks were awarded to Ms. Prachi Agnihotri (IGIB, New Delhi), Dr. Hemangi S. Bhonsale (TIFR, Mumbai), Ms. Barnali Deb (IOB, Bangalore), Ms. Divya Arunachalam (AMRF, Madurai), Ms. Shakuntala Bai (NCL, Pune).

Session-3 was chaired by Prof. Utpal Tatu (IISc, Bangalore), former PSI President, and Dr. Manoj Bhat, Director, NCCS, Pune. The speaker of this session was Prof. Akhilesh Pandey (Mayo Clinic, USA) Prof. Jennifer Van Eyk Cedars-Sinai Medical Center, USA), and Prof. Michael Snyder (Stanford University School of Medicine, USA).

Prof. Pandey spoke on the development of mass spectrometry-based targeted assay for direct detection of novel SARS-CoV-2 coronavirus from clinical specimens, Prof. Eyk spoke on the role of automated high content proteomics for personalized medicine, and Prof. Snyder discussed the analysis of Big Data and Health.

On day 2 of the meeting, Session-4 in the morning was chaired by Dr. Vidya Gupta (NCL, Pune) and Dr. Mahesh Kulkarni. Speakers in the session included Dr. Prabhod Kumar Trivedi (CIMAP, Lucknow), who spoke on the role of small peptides in plant growth and flavonoid biosynthesis. Dr. Soumein Kanti Manna (SINP, Kolkata), who spoke about his proteomics work on diabetic nephropathy, and Dr. Ranjan Nanda (ICGEB, New Delhi), who discussed serum extracellular vesicle proteome in tuberculosis. This was followed by a Thermofisher Sponsored lecture delivered by Dr. Sarvanan Kumar (Thermo) on Dual/ Hybrid Fragmentation Principles in Mass Spectrometry.

Session-5 had five lectures and was chaired by Dr. Ashok Giri and Dr. Geetanjali Sachdev (NIRRH, Mumbai). In this session, Prof. David Greening (Baker Heart and Diabetes Institute, Australia) shared his work on extracellular vesicles from human cardiac stem cells, Dr. Samuel Bacobza (Agricultural Research Organization -Volcani Center, Israel) spoke on high throughout multi-omic characterization of a Solanum Introgression Population, Dr. Suhua Li (Max Planck Institute for Chemical Ecology, Germany) delivered a lecture on Strigolactone signaling induced metabolism in tobacco stems, Prof. Asaph Aharoni (Weizmann Institute of Science, Israel) delivered a lecture on root shoot signaling. One of the lectures in this session also included a Nanostring Sponsored Talk by Dr. Marshall Feterl who discussed a morphology-driven high-plex spatial analysis of tissue microenvironments.

The main attraction of this symposium was the plenary lecture by Prof. Ruedi Aebersold (Institute of Molecular Systems Biology, Switzerland) in Session-6, which was chaired by Dr. Subhra Chakraborty and Prof. Suman Kundu (Delhi University, New Delhi). Prof. Aebersold discussed the modular proteome and its clinical significance. Following this, Prof. Ross Waller (University of Cambridge, UK) delivered his lecture on whole-cell spatial proteomics of apicomplexans. Dr. Arun Bandopadhyay spoke on proteomic analysis of compromised inflammation-resolution and reverse cholesterol transport in coronary artery disease. Prof. Riitta Lahesmaa (University of Turku, Finland) presented his lecture on the regulation of human T helper cell differentiation. This session concluded with a Sigma sponsored presentation.

Session 8 had two lectures and was chaired by Dr. Niranjan Chakraborty (NIPGR, New Delhi) and Dr. Jomon Joseph (NCCS Pune). The first lecture of this session was delivered by Prof. Arun Sreekumar (Baylor College of Medicine, USA) on biological insights from metabolic re-wiring in African American prostate cancer. The second given by Prof. Dinesh Kumar (University of California, USA), was on TurboID-based proximity labeling for in planta identification of protein interaction networks. Session-9 to Session-12 were held on day 3 of the symposium.

Session 9 was shared by Dr. Rakesh Mishra (CCMB, Hyderabad) and Dr. Tushar Kanti Maiti (RCB, Faridabad). There were three speakers in this session, starting with Dr. Prashant Kumar (IOB, Bangalore) who spoke on extracellular matrix organization as a novel therapeutic target in bladder carcinoma. Prof. Anurag Rathore (IIT Delhi) delivered a lecture on the analytical characterization of biotherapeutic products and Dr. Anil Koul (Johnson & Johnson, Belgium) delivered a lecture on the discovery of bedaquiline - a novel MDR-TB drug.

In Session-10, which was chaired by Dr. Dhanasekaran Shanmugam (NCL, Pune) and Dr. Ashok Mohanty (NDRI, Karnal), Dr. Markus Hartl (Max Perutz Labs, Austria) delivered his lecture on the use of mass spectrometry to understand inosine decoding. Prof. Teck yew Low (Universiti Kebangsaan, Malaysia and General Secretary, AOHUPO) present his lecture on protein interaction networks in Wnt Signalling. This was followed by Sciex sponsored lecture by Dr. Markus Ralser (Crick Institute, UK) who delivered a lecture on ultra-high proteomics with scanning SWATH and its application for functional proteome annotation.

Session-11 was chaired by Dr. Anu Raghunathan (NCL, Pune) and Dr. Ramesh Ummani (IICT, Hyderabad), in which Dr. Therese Koal (Biocrates, Innsbruck, Austria) discussed how metabolic pathways can be studied using Biocrates platforms. This was followed by two lectures on Covid-19. Dr. Shantanu Sengupta (IGIB, Delhi) delivered a talk titled COVID 19: From detection to surveillance and Prof. Sanjeeva Srivastava (IIT, Mumbai) delivered his lecture on the deep omics-based investigation of COVID-19 samples for identification of key molecular networks of disease severity.

Session 12 was the final session of the symposium and was dedicated to popular lectures Covid-19 delivered by four eminent speakers; Dr. Anurag Agrawal (IGIB, New Delhi), Dr. Amit Sharma (NIMR, New Delhi), Dr. Dhruv Chaudhury (PGIMS, Rohtak), and Dr. Rakesh Mishra, (CCMB, Hyderabad). The symposium closed with a valedictory function in which the president of PSI Dr. Subra Chakraborty announced the winners of lightning talk presentations and quiz in here closing remarks and congratulated the organizers for successful conducting of the meeting. The symposium concluded with the vote of thanks given by Dr. Mahesh Kulkarni.

A rapid and sensitive method to detect SARS-CoV-2 virus using targeted-mass spectrometry

Praveen Singh[1,2], Rahul Chakraborty[1,2], Robin Marwal[3], V. S. Radhakrishan[3], Akash Kumar Bhaskar[1,2], Himanshu Vashisht[3], Mahesh S. Dhar[3], Shalini Pradhan[1], Gyan Ranjan[1,2], Mohamed Imran[1,2], Anurag Raj[1,2], Uma Sharma[3], Priyanka Singh[3], Hemlata Lall[3], Meena Dutta[3], Parth Garg[4], Arjun Ray[4], Debasis Dash[1,2], Sridhar Sivasubbu[1,2], Hema Gogia[3], Preeti Madan[3], Sandhya Kabra[3], Sujeet K. Singh[3], Anurag Agrawal[1,2], Partha Rakshit[3], Pramod Kumar[3], Shantanu Sengupta[1,2]

[1]CSIR-Institute of Genomics and Integrative Biology, Mathura Road, New Delhi 110025, India

[2]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India

[3]National Center for Disease Control, New Delhi 110054, India

[4]Department of Computational Biology, Indraprastha Institute of Information Technology, Okhla Industrial Estate, Phase III, New Delhi 110020, India

We developed a simple, sensitive and rapid method to directly detect the presence of SARS-CoV-2 virus from naso-oropharyngeal swabs using a targeted mass spectrometric approach. This will complement techniques like RT-PCR for rapid diagnosis and isolation of infected individuals in the current pandemic conditions where reagents and resources are limited.

Initially, we identified peptides specific to SARS-CoV-2 specific proteins from naso-oropharyngeal swab samples obtained in viral transport media (VTM), using a high resolution mass spectrometer. These peptides were then subjected to triple quadrupole mass spectrometery and using a sMRM method with a short gradient of 2.3 minutes we could consistently identify two peptides in the samples from individuals infected with SARS-CoV-2. Naso-oropharyngeal swab samples in VTM were inactivated by incubation with a lysis solution containing 25% guanidinium thiocyanate and 5% SDS. Protein precipitation was performed from the solution using trichloroacetic acid (TCA), followed by reduction, alkylation and digestion of protein using trypsin. Using high-resolution mass spectrometry (HRMS) we could identify 22 peptides from 4 proteins specific to SARS-CoV-2. Of these, three structural proteins-spike glycoprotein (spike), nucleoprotein (NP) and envelope small membrane protein (ENV) and a non-structural protein Replicase polyprotein 1ab (Rep). Among these, eight peptides from 3 proteins (Rep, Spike and NP), were unique and un-modified and were selected for generation of an in-house sMRM method. Peptide uniqueness and sequence variability analysis using NCBI blasp suite and screening through reported strains in GISAID database respectively showed that 7 of the 8 peptides were unique to SARS-CoV-2 and conserved among 54,000 strains of SARS-CoV-2 (as on 1st July, 2020). Based on the consistency and quality of data we selected two peptides-AIVSTIQRKYK from Rep and QIAPGQTGK from spike for the sMRM method. We also including a peptide AEFAEVSK from human serum albumin as an indicator for protein amount. We analyzed 103 samples using the sMRM method developed in two different sample sets. The set 1 consisted of 20 samples (including follow up) from 14 patients for whom we had follow-up RT-PCR data. Interestingly, although most of the patients had recovered as was evident from their being RT-PCR negative and being free of symptoms, we found that they continue to retain the peptides even after

14 days of initial infection. In 2 of the patients, RT-PCR was found to be negative while the patients had symptoms. One of these patients was found to be negative on the 1st day but was found positive on the second day while we found peptide peaks to be present on both the days, clearly indicating the sensitivity of the method. In set 2, we analysed 82 case-control samples. Among these 63 were RT-PCR positive and considered as patients. For controls, we considered 19 individuals who tested negative for RT-PCR and also did not have antibodies against SARS-CoV-2 (as evident from serology test indicating the absence of IgM and IgG against SARS-CoV-2). Mass spectrometric analysis revealed that in all the 19 negative samples, SARS-CoV-2 specific peptides were not detected. Of the 63 positive samples, 57 were found to be positive using our method. Thus, our method shows a sensitivity of 90.4% and specificity of 100% with respect to RT-PCR positive samples and true controls (serologically negative, RT-PCR negative).

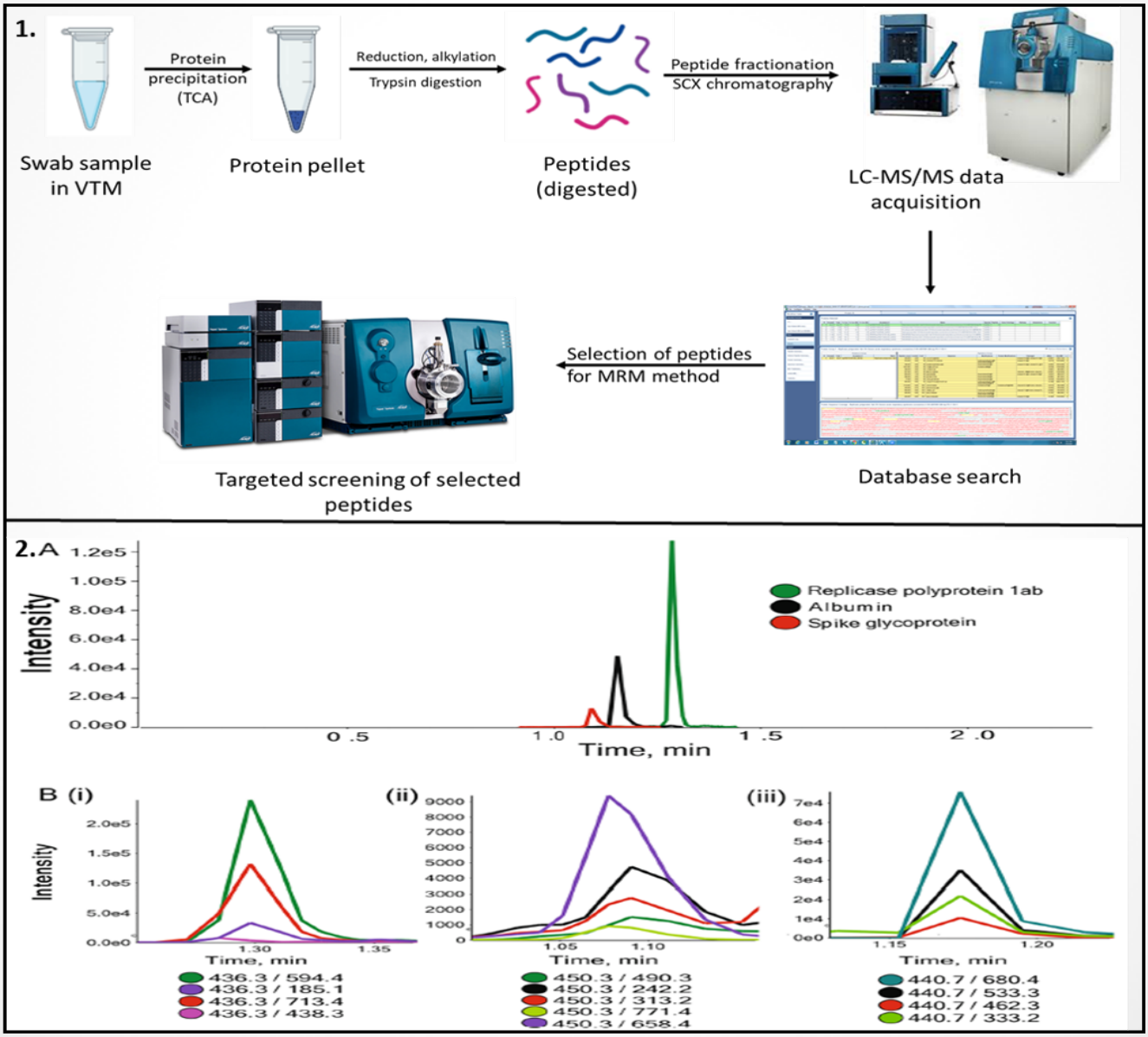


Figure: *1. Pipeline followed for SARS-CoV-2 specific protein identification and generation of sMRM method. 2.(A) Chromatographic separation of the three selected peptides from naso-oropharyngeal protein digest. (B) Exacted ion chromatogram from (i) AIVSTIQRKYK (Replicase polyprotein 1 ab), (ii) QIAPGQTGK (Spike glycoprotein) and (iii) AEFAEVSK (human serum albumin).*

Increased supra-organization is a dynamic multistep remodelling of respiratory complexes against proteostasis stress

Shivali Rawat, Suparna Ghosh, Debodyuti Mondal, Valpadashi Anusha and Swasti Raychaudhuri
CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad - 500007, India

One of the indispensable processes of life is to keep energy generation on in mitochondria utilizing the protein complexes called Respiratory Complexes (RCs). The RCs contain proteins encoded in the nuclei as well as mitochondria of cells. The nuclear-encoded proteins combine with mitochondrial ones in different steps to eventually form a functional RC. Different studies suggest that these complexes are plastic. Shivali Rawat et al from Swasti Raychaudhari's lab have developed a novel mass spectrometry based strategy to capture the dynamic plasticity of RC-organizations. They combine iBAQ (intensity based absolute quantification) and SILAC (stable isotope labelling by amino acids in cell culture) based mass spectrometry to establish a two-dimensional complexome profiling protocol. Using this novel protocol, they uncover the biphasic remodelling of RC-organizations to protect cellular respiration in stressed cells. They demonstrate that Complex-II (CII) and CV-subunits are increasingly incorporated into oligomers during various protein homeostasis stress conditions. CI, CIII and CIV-subunits are engaged into supercomplex formation. They unravel unique quinary-states of supercomplexes at early-stress that exhibit plasticity and inequivalence of constituent RCs. Core stoichiometry of CI and CIII is preserved whereas CIV-composition varies. These partially disintegrated supercomplexes remain functionally competent via conformational optimization. Subsequently, increased stepwise integration of RC-subunits into holocomplex and supercomplexes re-establish steady-state stoichiometry. Overall, they propose that the mechanism of increased supra-organization of RCs mimics the cooperative unfolding and folding pathways for protein-folding, restricted to RCs only and not observed for any other mitochondrial protein complexes.

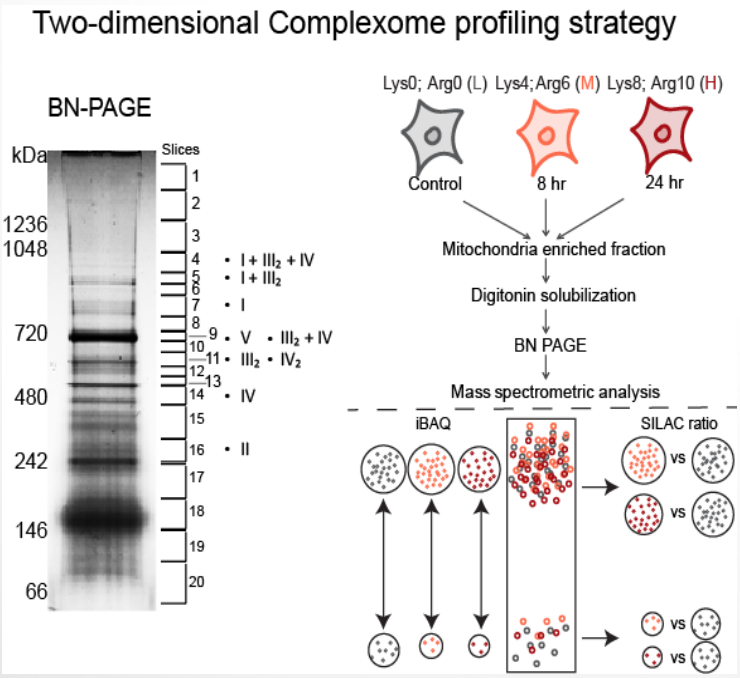


Figure Legend:
Left *- BN-PAGE stained with coomassie brilliant blue showing separation of mitochondrial protein complexes. The gel slices processed for mass spectrometry are marked and numbered. Dots indicate RCs identified in respective slices.* ***Right*** *- Flowchart describing iBAQ and SILAC-based two-dimensional complexome profiling strategy using BN-PAGE. Each dot represents one RC-subunit. Cells are stressed for indicated time-points. Colour-code for SILAC-labelled peptides.*

Correlation of venom toxinome composition of Indian red scorpion (*Mesobuthus tamulus*) with clinical manifestations of scorpion sting: Failure of commercial antivenom to immune-recognize the abundant of low molecular mass toxins of this venom

Bhabana Das[1], Aparup Patra[1] and Ashis Kumar Mukherjee[1]*
[1]*Microbial Biotechnology and Protein Research Laboratory, Department of Molecular Biology and Biotechnology, School of Sciences, Tezpur University, Tezpur- 784028, Assam, India*

The Indian red scorpion (Mesobuthus tamulus), with its life-threatening sting, is the world's most dangerous species of scorpion. It belongs to the family Buthidae and order scorpiones and spread across the Indian subcontinent. In particular, scorpiones from Buthidae family are reported to be more toxic than the scorpiones from other families, though geographical variation may also lead to the diversity of scorpion toxins (qualitative and quantitative differences) that can influence the severity of scorpion stings. However, the venom proteome composition of Indian red scorpion was not explored. Therfore, the toxinome composition of M. tamulus venom was determined by tandem mass spectrometry (MS) analysis of venom protein bands de-complexed by SDS-PAGE. A total of 110 venom toxins were identified from searching the MS data against Buthidae family (taxid: 6855) of toxin entries in non-redundant protein databases. The Na^+ and K^+ ion channel toxins taken together are the most abundant toxins (76.7%) giving rise to the neurotoxic nature of this venom. The other minor toxin classes in the M. tamulus venom proteome are serine protease-like protein(2.9%), serine protease inhibitor(2.2%), antimicrobial peptide (2.3%), hyaluronidase (2.2%), makatoxin (2.1%), lipolysis potentiating peptides(1.2%), neurotoxin affecting Cl^- channel (1%),parabutoporin (0.6%), Ca^{2+} channel toxins(0.8%), bradykinin potentiating peptides(0.2%), HMG CoA reductase inhibitor (0.1%) and other toxins with unknown pharmacological activity (7.7%).Several of these toxins have been shown to be promising drug candidates to treat several diseases and may also be developed as eco-friendly bio insecticides. The biochemical characterization reveals that M. tamulus venom is devoid of enzymatic activity in vitro haemolytic activity, interference with blood coagulation, or platelet modulation properties. Among 86 poisonous species of scorpions found in India, M. tamulus venom is reported to cause the most lethal causing fatalities in children and adults. Scorpion sting may also cause local reactions such as edema, intense pain, erythema, and itching. The systemic effects of sting include cardiovascular disturbances (bradycardia, tachycardia, hypertension, hypotension, and cardiogenic shock), pulmonary edema, systemic circulation (increased cytokines, nitric oxide/histamine release), hyperglycemia, and priapism in male children. The clinical manifestations post M. tamulus sting are well correlated with its venom proteome composition. An abundance of low molecular mass toxins (3-15kDa) are responsible for exerting the major pharmacological effects of M. tamulus venom though they are poorly immune-recognized by commercial scorpion antivenom. This is a major concern for the development of effective antivenom therapy against scorpion sting.

Indian red scorpion (Mesobuthus tamulus)

Proteomics analysis revealed the importance of inflammation mediated downstream pathways and the protective role of curcumin during bleomycin-induced pulmonary fibrosis in C57BL/6 mice

Mahesh Manjunath Gouda[1,2], Sindhu Priya ES[1,3#], Rex DAB[4#], Prashant Kumar Modi[4],Jaikanth Chanderasekaran[5], and Yashodhar Prabhakar Bhandary[1*]

[1]Yenepoya Research Centre, Yenepoya University, Deralakatte, Mangalore -575018, Karnataka, India

[2]Comprehenisve Pneumology Centre, Max-Lebsche-Platz-31,Helmholtz-Zentrum München-81377, Germany

[3]Yenepoya Pharmacy College and Research Center, Yenepoya (Deemed to be University), Deralakatte, Mangalore -575018, Karnataka, India

[4]Centre of Systems Biology and Molecular Medicine (CSBMM), [1]Yenepoya Research Centre, Yenepoya (Deemed to be University), Deralakatte, Mangalore -575018, Karnataka, India

[5]PSG College of Pharmacy, Peelamedu, Coimbatore – 641004, Tamil Nadu, India

Bleomycin (BLM)-induced pulmonary fibrosis is characterized by the inflammation in the alveoli and subsequent deposition of extracellular matrix (ECM), myofibroblasts and impaired fibrinolytic system. Here we describe high throughput hits of LC-MS analysis, major hematological changes, and the role of IL-17A mediated p53-fibrinolytic pathway during the progression of pulmonary fibrosis and the therapeutic potential of curcumin in regulation of progressive pulmonary fibrosis. C57BL/6 mice were exposed to BLM, followed by the curcumin intervention after 24 and 48 h. Mice were sacrificed after 7 days to validate the hematology parameters, molecular pathways, and LC-MS based proteomics. LC-MS analysis was done using Q-Orbitrap mass spectrometer. Schrödinger was used to perform the *In silico* molecular docking studies. BLM exposed mice were undergone gradual weight loss and altered lung phenotype, however, it was reversed by the curcumin treatment. The significant changes in the hematology parameters were confirmed the severity of BLM-exposure in the mice and IL-17A mediated p53-fibrinolytic system components expression and alveolar epithelial cells (AECs) apoptosis further confirmed the pathophysiology of pulmonary fibrosis. Differentially expressed proteins were characterized and mapped using proteomics approach. The hits from the proteomics were further validated using the molecular techniques such as qPCR, immune-fluorescent staining, and western blot analysis. The study revealed the strong interaction of curcumin with p53, uPA, and PAI-I proteins. Key role of IL-17A mediated inflammation in the impairment of the p53-fibrinolytic system, and AECs apoptosis was confirmed during BLM-induced pulmonary fibrosis. Therapeutic efficacy of curcumin exhibited a protective role against the progression of pulmonary fibrosis, which promises the potent therapeutic modality to target IL-17A mediated p53-fibrinolytic system to regulate the progressive pulmonary fibrosis.

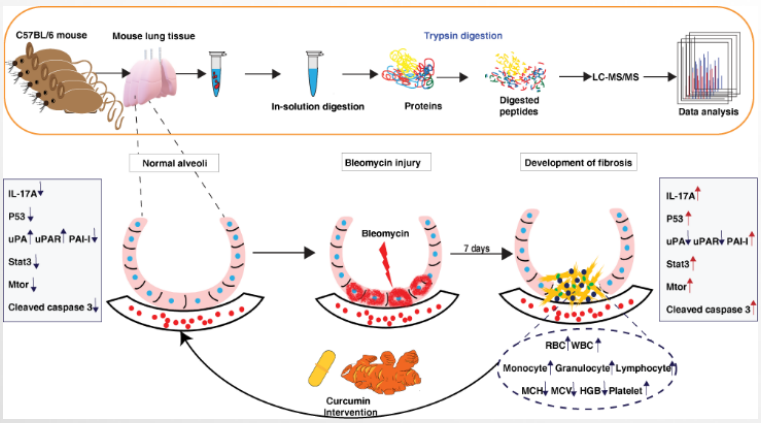


Figure legend: *The figure represents the schematics of bleomycin-induced lung injury, curcumin intervention in mouse, and the LC-MS based proteomics analysis to validate the molecular changes during the progressive pulmonary fibrosis.*

Clinical Proteomics Profiling for Biomarker Identification Among Patients Suffering with Indian Post Kala Azar Dermal Leishmaniasis

Priyank Jaiswal [1], Manab Ghosh[2], Goutam Patra[1], Bibhuti Saha[2] and Sumi Mukhopadhyay[1*]

[1]Department of Laboratory Medicine, School of Tropical Medicine, Kolkata, India,

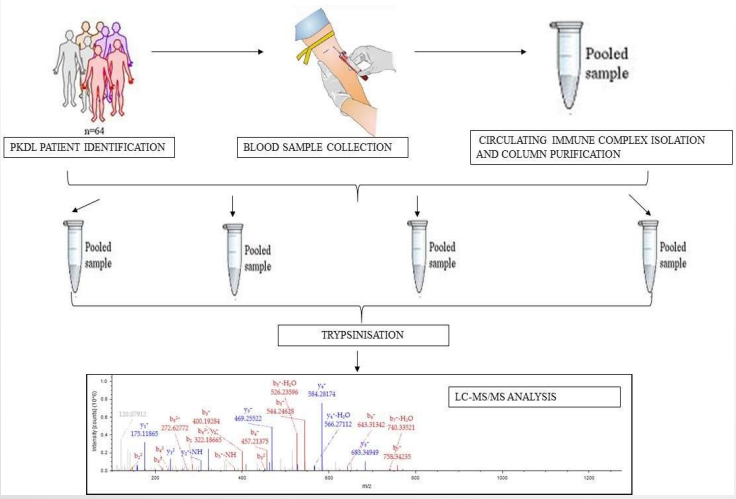
[2]Department of Tropical Medicine, School of Tropical Medicine, Department of Health & Family Welfare, Government of West Bengal, Kolkata, India

Post Kala Azar Dermal Leishmaniasis (PKDL), the dermal sequel of Visceral Leishmaniasis (VL) has shown its prevalence worldwide. Although non-fatal, but the PKDL individuals acts as reservoir of the *Leishmania donovani* parasite and are regarded as the potent source for VL transmission. Diagnostic and confirmatory test like punch biopsy are highly invasive, shows low sensitivity and specificity specially toward identifying macular (MAC) PKDL patients, due to low parasite load. PKDL patients residing in rural areas suffer mostly from improper diagnosis and in turn act as mobile parasite reservoir, transmitting VL among healthy individual (HI).

To assist in proper identification of PKDL patients, especially with low parasite load, we have utilized the powerful LC-MS/MS technology for biomarker identification expressed during early phase of infection even when the parasite load is very low. Peptides were analysed by electrospray ionization mass spectrometry using the Easy nano LC 1200 system coupled to a Q-Exactive Plus Orbitrap mass spectrometer. In this study we have characterized the glycated (Circulating Immune Complexes) CICs, among PKDL patients, suffering with MAC or polymorphic (POLY) forms, as compared with HI and Cured (CR) individuals. protein level alterations among all study groups were confirmed by Western blot and ELISA.

Among MAC PKDL patients 43, 60, 90 proteins were altered compared to POLY PKDL, HI, and CR groups, respectively. Filtering for the most significant proteins, Plasminogen (PLG) and Vitronectin (VTN) were identified which promisingly identified MAC PKDL cases. PLG levels were over expressed in MAC patients compared to POLY patients, HI and CR individuals. Further VTN levels were over expressed in POLY patients compared to MAC patients, HI and CR individuals

Active surveillance results from endemic districts of West Bengal revealed drastic rise of MAC PKDL cases, alarming the urgency for field adaptive efficient biomarker. Our findings suggest that PLG and VTN as novel diagnostic and prognostic protein biomarker for MAC and POLY PKDL efficient case management.



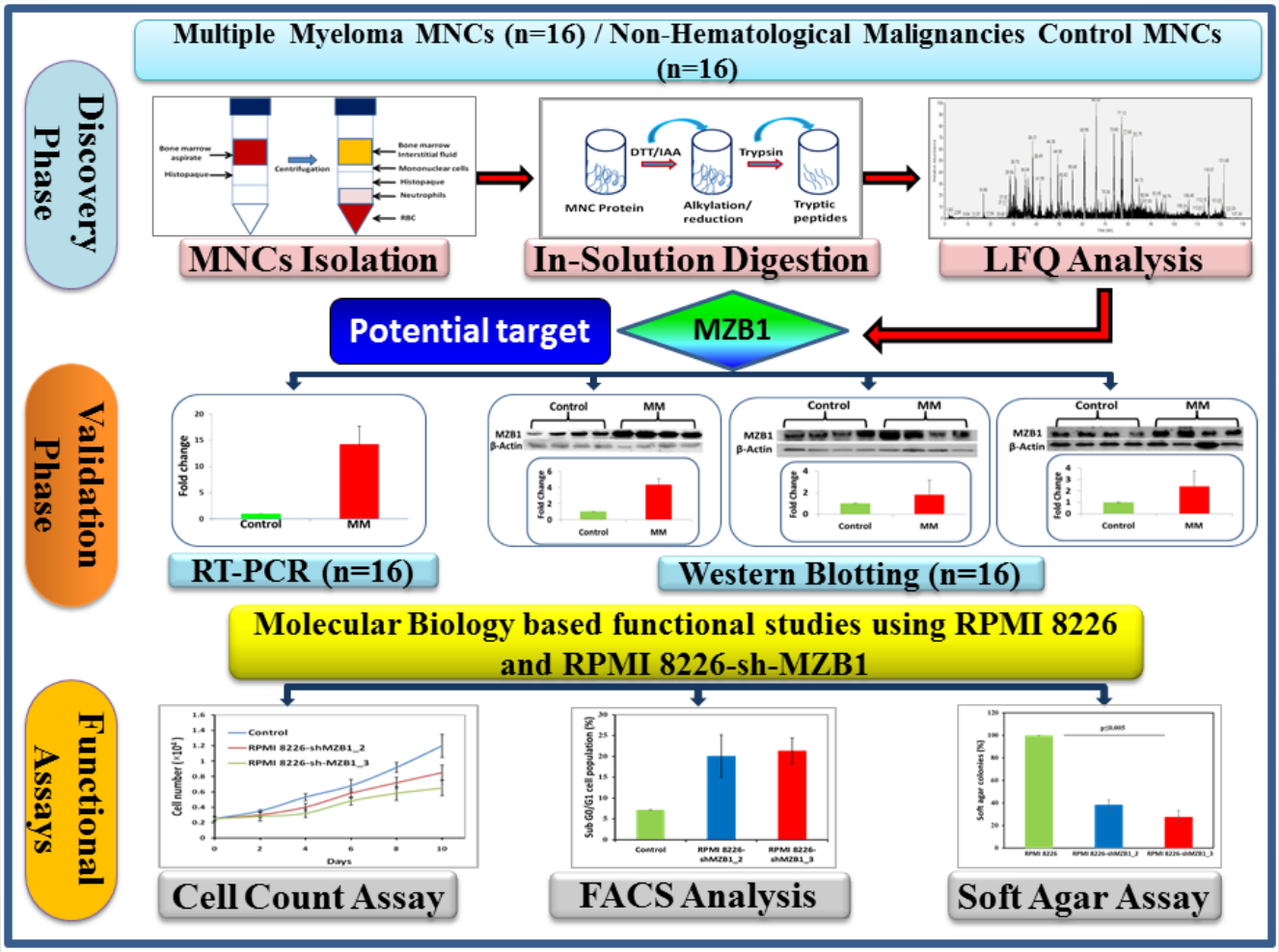
Schematic representation of the experimental study design. Sample selection strategy for the proteomics study; HI (n = 12), MAC (n = 20), POLY (n =20) and CR (n = 12). Patient population used for protein levels, belongs to the same patient population as that of the proteomics study. HI, Healthy Individuals; MAC, Macular PKDL patients; POLY, Polymorphic PKDL patients; CR, Cured Individuals

Proteomics and functional study reveal MZB1 as a potential target of Multiple Myeloma

Venkatesh Chanukuppa[1], Debasish Paul[2], Khushman Taunk[1], Tathagata Chatterjee[3], Sanjeevan Sharma[3], Amey Shirolkar[1], Manas K. Santra[2], Srikanth Rapole[1,*]

[1]Proteomics Lab, National Centre for Cell Science, Ganeshkhind, Pune-411007, MH, India. [2]Cancer Biology and Epigenetics Lab, National Centre for Cell Science, Ganeshkhind, Pune-411007, MH, India. [3]Armed Forces Medical College, Pune-411001, MH, India. ([*]E-mail: rsrikanth@nccs.res.in)

Multiple Myeloma (MM) is a cancer of the plasma cells that accounts for 13% of all the known hematological malignancies. MM still remains as an incurable malignancy with a poor prognosis due to lack of suitable markers. Therefore, this study aims to identify markers associated with MM malignancy using patient-derived MM mononuclear cells (MNCs). Label-free quantitative proteomics analysis revealed a total of 192 differentially regulated proteins in which 79 proteins were up-regulated and 113 proteins were found to be down-regulated in MM MNCs. Among these candidate proteins, marginal zone B and B1 cell specific protein (MZB1) was selected for further investigation as this protein was one of the significantly DE protein and its functions are associated with characteristic features of MM. Our functional studies revealed that higher expression of MZB1 is closely associated to promote the progression of MM pathogenesis and could be established as a potential target to develop therapy for MM in future.

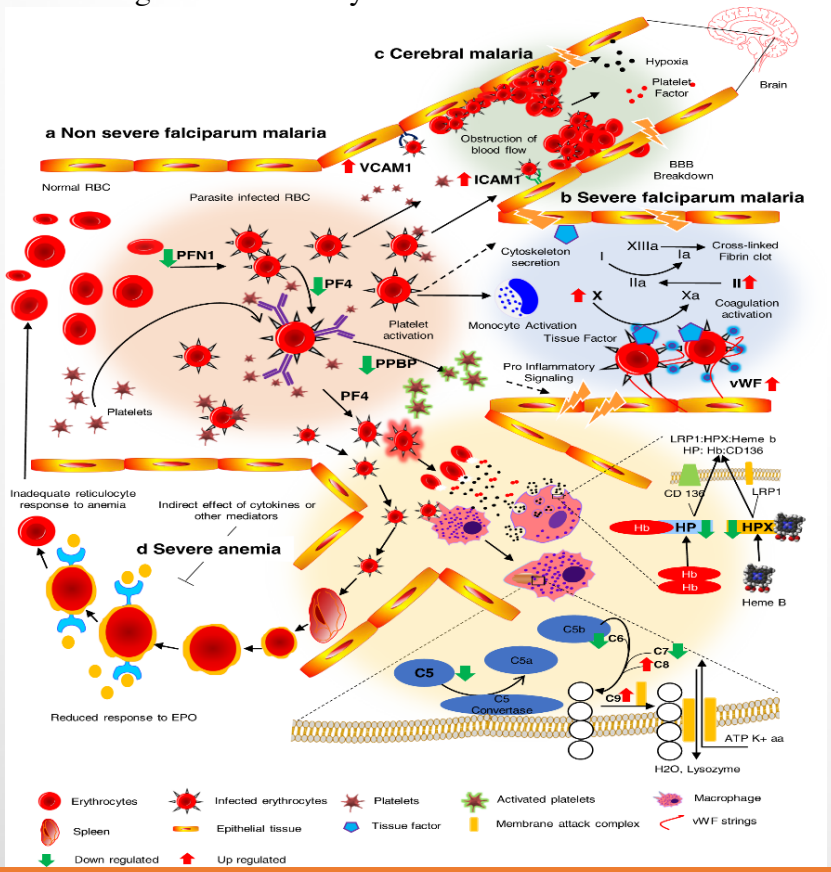


Multiplexed quantitative proteomics provides mechanistic cues for malaria severity and complexity

Vipin Kumar[1], Sandipan Ray [1,7,8], Shalini Aggarwal[1,8], Deeptarup Biswas[1], Manali Jadhav[1], Radha Yadav[2], Sanjeev V. Sabnis[2], Soumaditya Banerjee[3], Arunansu Talukdar[3], Sanjay K. Kochar[4], Suvin Shetty[5], Kunal Sehgal[6], Swati Patankar[1] & Sanjeeva Srivastava[1]

[1]Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai 400076, India. [2] Department of Mathematics, Indian Institute of Technology Bombay, Mumbai 400076, India. [3] Medicine Department, Medical College Hospital Kolkata, 88, College Street, Kolkata 700073, India. [4] Department of Medicine, Malaria Research Centre, S.P. Medical College, Bikaner 334003, India. [5] Dr. L H Hiranandani Hospital, Mumbai 400076, India. [6] Sehgal Path Lab, Mumbai 400053, India. [7]Present address: Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania, Philadelphia, PA 19104, USA. [8]These authors contributed equally: Sandipan Ray, Shalini Aggarwal. email: sanjeeva@iitb.ac.in

Management of severe malaria remains a critical global challenge. In this study, using a multiplexed quantitative proteomics pipeline we systematically investigated the plasma proteome alterations in non-severe and severe malaria patients. We identified a few parasite proteins in severe malaria patients, which could be promising from a diagnostic perspective. Further, from host proteome analysis we observed substantial modulations in many crucial physiological pathways, including lipid metabolism, cytokine signaling, complement, and coagulation cascades in severe malaria. We propose that severe manifestations of malaria are possibly underpinned by modulations of the host physiology and defense machinery, which is evidently reflected in the plasma proteome alterations. Importantly, we identified multiple blood markers that can effectively define different complications of severe falci- parum malaria, including cerebral syndromes and severe anemia. The ability of our identified blood markers to distinguish different severe complications of malaria may aid in developing new clinical tests for monitoring malaria severity.



Possible molecular mechanisms driving the different complications of severe falciparum malaria

Relative and quantitative phosphoproteome analysis of macrophages in response to infection by virulent and avirulent mycobacteria revels a distinct role of the cytosolic RNA sensor RIG-I in *Mycobacterium tuberculosis* pathogenesis

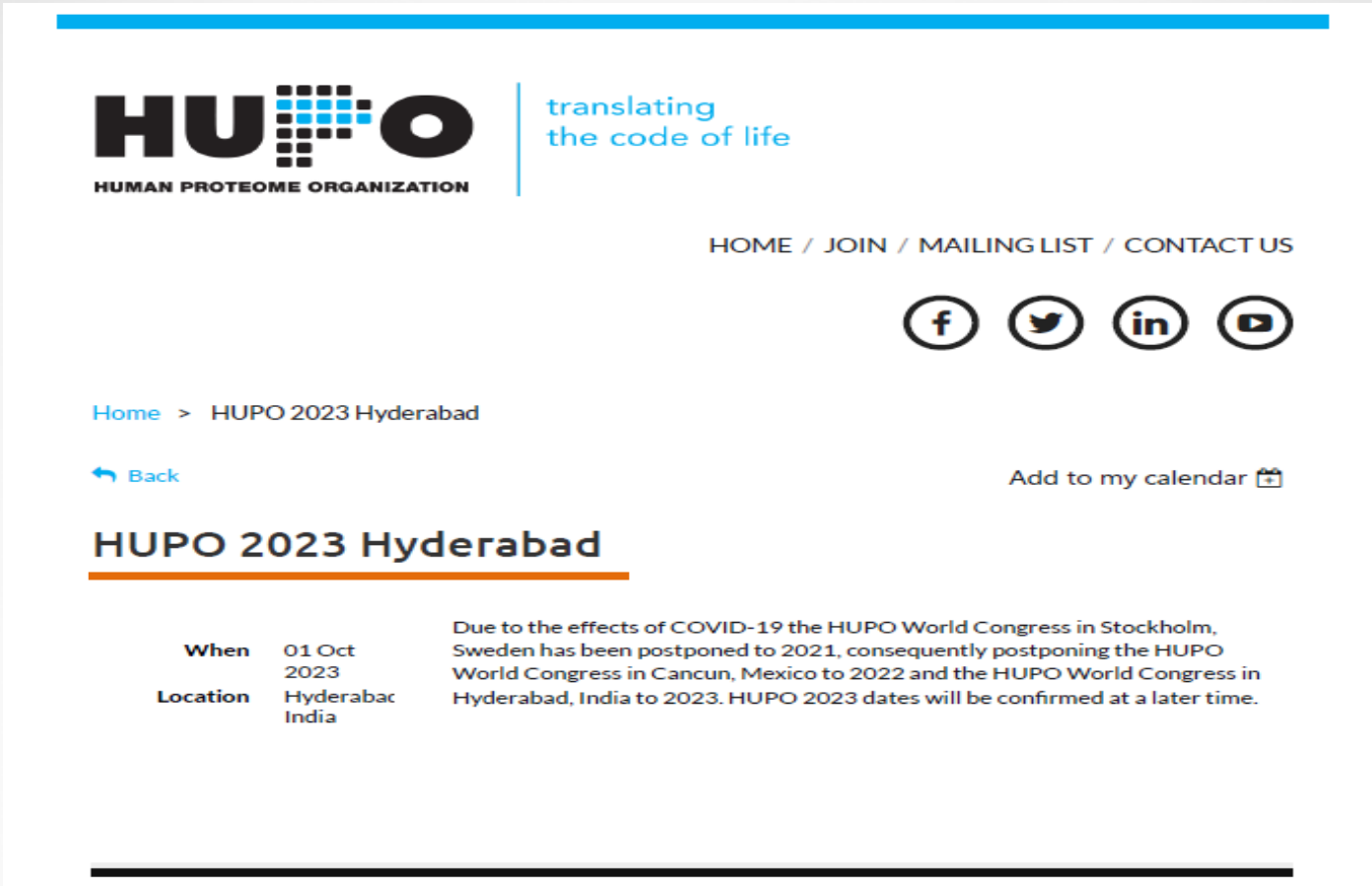
Eira Choudhary, C.Korin Bullen, Renu Goel, Alok Kumar Singh, Monali Praharaj, Preeti Thakur, Rohan Dhiman, William R. Bishai, and Nisheeth Agarwal*

**Laboratory of Mycobacterial Genetics, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, Faridabad 121001, Haryana, India*

The seminal information about the physiological alterations in the host signalling machinery by virulent mycobacteria features the discovery of various post-translational modification (PTMs), of which protein phosphorylation is the most abundant. The fact that the virulent and avirulent mycobacteria elicit varying host responses during infection, makes it an interesting facet to explore and to generate phosphoproteome database that will help us elucidate the specific effect of these closely related microorganisms on host kinases and phosphatases.

In the present study, we examined the temporal modulations in the phosphoproteome profile of macrophages during *Mycobacterium tuberculosis* (Mtb) and *M. bovis* BCG (BCG) infection, by using the tandem mass tag-based quantitative mass spectrometry. We observed global dephosphorylation of host proteins after infection with virulent mycobacteria in contrast to avirulent BCG-infected macrophages. Moreover, quantitative analysis revealed depletion of specific phosphopeptides in Mtb-infected macrophages whereas BCG infection resulted in their abundance, many of which correspond to various kinases. Multiple cellular pathways belonging to MAPK signalling, Immune responses, GTPase signalling, PI3K signalling etc. were found to be modulated and the corresponding proteins showed differential phosphorylation upon infection with virulent and avirulent mycobacteria. Besides, we also observed selective enrichment of phosphopeptides belonging to Interferon signalling and Calcium signalling pathways, exclusively upon Mtb infection. Based on our finding that Mtb infection elicits differential regulation of RIG-I dependent Interferon-Stimulated Gene proteins, we also determined the biological importance of cytosolic RNA sensing RIG-I/IFN-β signalling during Mtb infection. The results established increased Mtb survival due to evasion of innate immune response by RIG-I-mediated production of IFN-β and IL-1 and inhibition of autophagy, which further open future prospects of validating the therapeutic potential of RIG-I as host-directed therapy against Mtb infection.

Proteomics Society, India to host HUPO in 2023



Join Us
For Membership forms
Click on
www.proteomicssociety.in/membership

Proteomics Society, India

Registered under Andhra Pradesh Societies Registration Act. (35/2001) (Regd. No. 286/2009) proteomicsociety@ccmb.res.in
www.proteomicssociety.in