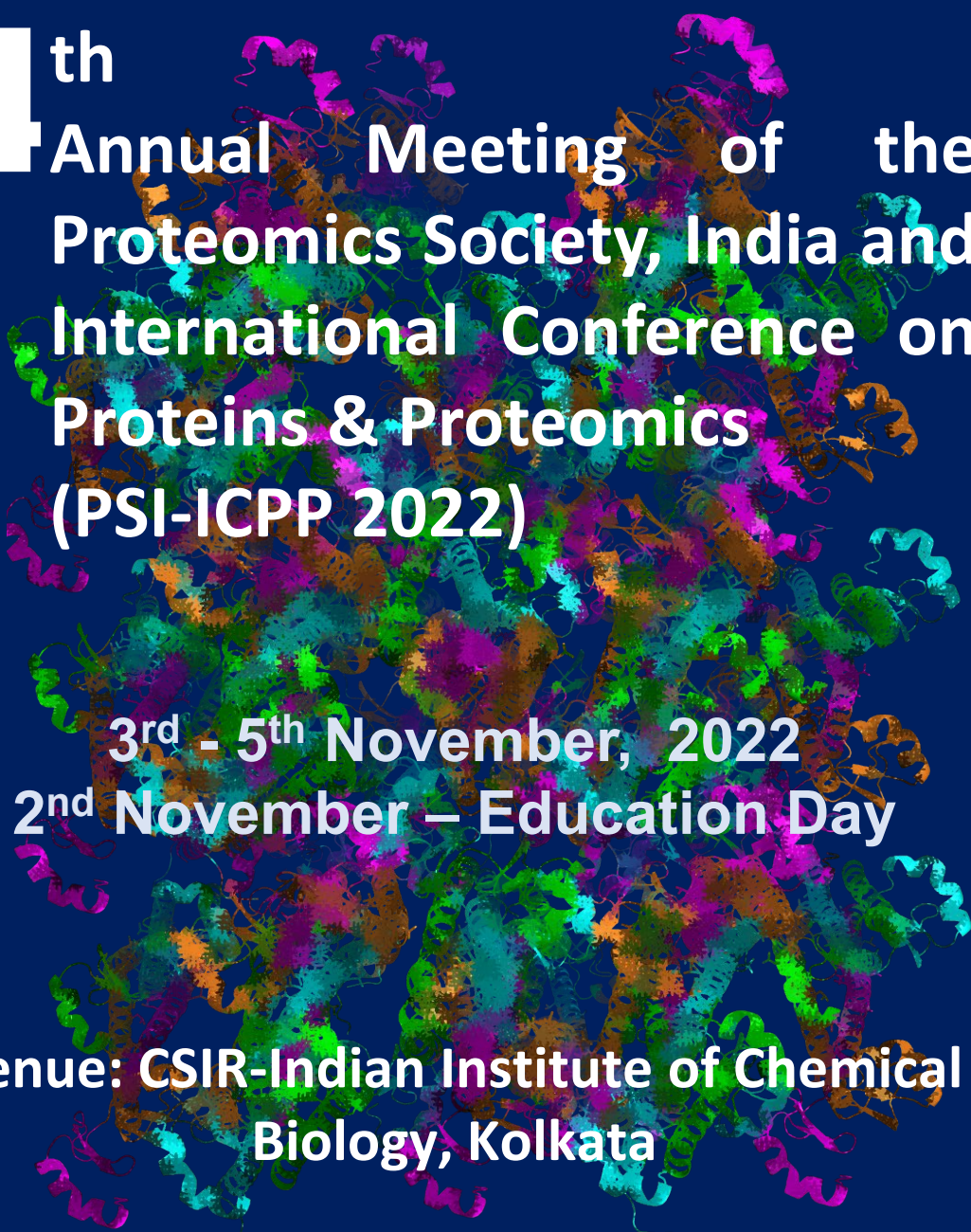
PSI

PSI-ICPP 2022

CSIR-IICB Kolkata

CSIR-IICB

14th Annual Meeting of the Proteomics Society, India and International Conference on Proteins & Proteomics (PSI-ICPP 2022)



3rd - 5th November, 2022
2nd November – Education Day

Venue: CSIR-Indian Institute of Chemical Biology, Kolkata

Organizing partners

NIPER, Kolkata

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IASST, Guwahati

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14th Annual Meeting of the Proteomics Society, India and International Conference on Proteins & Proteomics (PSI-ICPP 2022)

3rd - 5th November, 2022

2nd November – Education Day

Venue: CSIR-Indian Institute of Chemical Biology, Kolkata

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PSI-ICPP 2022

CSIR-IICB Kolkata

PSI CSIR-IICB

14th Annual Meeting of the Proteomics Society, India and International Conference on Proteins & Proteomics (PSI-ICPP 2022)

[3rd - 5th November 2022]

PSI commits to its responsibility to inspire the future proteome-ers, genom-ers promote proteo-genomic education to the young students, researchers, and college & university teachers. Thus, "Education Day", a knowledge initiative, is an integral part of the PSI Annual Meeting. It aims to comprehensively disseminate and teach basic and advanced genomics, proteomics and allied research tools.

Education Day: Proteins and Proteomics

2nd November 2022
(9:30 AM – 5:00 PM)

Venue: CSIR-Indian Institute of Chemical Biology (IICB), Kolkata

Eligibility: Research Scholars & Students, Ph.D., M.Sc. or B.Sc.
Last date of registration: October 30, 2022 (Register online)
Registration Fee: Rs. 500/- (Includes Lunch)

Co-ordinators

Dr. Sucheta Tripathy
CSIR-Indian Institute of Chemical Biology, Kolkata

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

Dr. Amit Yadav
Translational Health Science and Institute, Faridabad

Dr. Santanu Sengupta
CSIR-Institute of Genomics and Integrative Biology, Delhi

http://psi-icpp2022.proteomicssociety.in/

For further details please contact

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Message from President, Proteomics Society, India

It is my great pleasure to extend a warm welcome on behalf of the Proteomics Society, India Executive Council and my own to all the attendees including scientists, academicians, young researchers, delegates and students to the 14th Annual Meeting of Proteomics Society, India (PSI) and an International Conference on Proteins & Proteomics (PSI-ICPP 2022) being organized at CSIR-IICB, Kolkata, India from 2nd to 5th November 2022.

India has a distinguished history in the Protein Science. Proteomics science offers researchers with cutting edge tools to understand the genome function and over the years it has been used in health, food and nutrition research. Starting from its inception in 2009, Proteomics Society, India Annual Conferences are now very well established and the Society has been recognized globally. The common interest of the Society is in the interdisciplinary study of proteins using proteomics and other omics and sharing knowledge and developing interactions with national and international community in the field. PSI also conducts workshops every year as one of its education drives for the new proteomers in the field and to promote technology sharing. Many of the PSI-Executive Council members have served and serving the international bodies: Dr. Ravi Sirdeshmukh, Dr. Subhra Chakraborty and Dr. Sanjeeva Srivastava at HUPO, Dr. Ravi Sirdeshmukh and Dr. Shantanu Sengupta at AOHUPO, Dr. Niranjan Chakraborty at INPPO, Dr. Subhra Chakraborty at AOAPO. PSI celebrates March 18th as the "Proteomics Day" in India from 2016. Journal of Proteins and Proteomics (JPP), administered by PSI is serving the scientific fraternity to publish and read new findings in the area of protein science and proteomics. I am glad to tell that JPP is on the UGC CARE list and some of the published papers are in Pubmed with Pubmed ID as well.

I am sure everyone at PSI family and other colleagues started enjoying their own research work and excited about the new developments in the field after almost two years of global pandemic and challenging time. As was intended, PSI 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 organize its annual meeting in different corners of the country by joining hands with national laboratories and universities to facilitate the participation of the scientific community, in particular the students and teachers in the area.and endeavour to come up with select themes for the Conference focused upon contemporary issues in the field. Recent years have shown increased frequency of high complexity diseases worldwide. Despite significant advances in our understanding of the molecular basis of diseases, gaps remain in terms of disease pathogenesis as well as diagnosis and treatment. On the other hand, Impending changes in the global climate coupled with rapidly growing population have resulted in challenges related to food and nutrition.

The theme of this Conference is contemporary and relevant. I am sure the deliberations will give us newer insights into the emerging dynamics of Proteins and Proteomics in the area of human diseases, clinical study and agriculture. A well thought out and engaging academic programme has been planned for all, which will include Education Day program, Invited lectures, Young Scientist Talks, Industry talks, Poster sessions and other opportunities to Interact. In addition, selected students are also given an opportunity to present their research work as lightening short talks. I am confident that the Conference would provide an opportunity to explore new innovations in the areas, meeting & interaction with the leaders in the field, network with friend and colleagues. Furthermore, the conference will help evolving a roadmap for Proteomics Community to overcome challenges faced by the agricultural sector and health Research.

Kolkata, the City of Joy has taken pride in welcoming guests from all over the world since the ancient times, and has been a seat of education, literature, art and music. Kolkata has many institutions and

universities engaging in the ongoing research in the area of proteins and proteomics. The organizing institute, Indian Institute of Chemical Biology, is one of the premier institutes for higher education and research in India. I hope that the participants will have time to visit the laboratories of CSIR-IICB and enjoy the hospitality as well as the city and Its surroundings.

Before I close, I would like to encourage on more and more participation of young researchers 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. Look forward to such cooperation in future and work together to understand the human, animal, plant, micro-organisms and environmental biology with the help of the great proteome science.

Finally, I congratulate Dr. Arun Bandyopadhyay and Dr. Nakul C. Maity and the organizing committee to put together a very intense and intellectually stimulating program. I thank the Organizing Committee, Executive Councill of PSI, Industry partners, the invited speakers, young researchers and students for attending this conference and bringing your expertise as it would allow significant brainstorming, and help evolve a blueprint for future proteomic endeavors. I believe with your participation, we will make this fascinating scientific event successful, remarkable and memorable!

With warm regards

Dr. Subhra Chakraborty
President, Proteomics Society, India
Director, National Institute of Plant Genome Research

Message from the President, PSI-ICPP 2022 and Director, CSIR-IICB Kolkata

It is really gratifying to host 14th Annual Meeting of the Proteomic Society (I) from November 3-5, 2022 at our Institute which is being preceded by Education day on 2nd November. We appreciate the society for giving us the opportunity to organize this annual meeting and symposium which is first time occurring in normal mode post pandemic. Faculties and students of CSIR-IICB has been associated with the Proteomic Society since its inception. This institute is equipped with the facilities for proteomic research including high resolution mass spectrometers and expertise and tools computational analysis. Since "Chemical Biology" happens to be at the core of our research focus a significant number of researchers are participating in this seminar. A large number of Ph.D. students from our Institute are presenting their research findings and attending lectures during these 3 days conference. Active participation of the Ph.D. students from the host institute along with students from other institutions would make this 3 days meeting a more vibrant and interactive.

Organizing the conference of this stature obviously requires involvement of enormous human resources from the host organization. The faculties, students and staffs of CSIR-IICB have been tirelessly working for the last few months to make this event a grand success. I convey my deep sense of appreciation to all of them. I hope that the guests attending this meeting will feel comfortable and carry a good memory for the meeting as well as our organization.

I sincerely hope that these 3 days event will be a great scientific feast, help building network and collaborations with the proteomic experts across the nation which will be very useful for supporting our research endeavour in disease biology.

With warm regards

Dr. Arun Bandyopadhyay
President, PSI-ICPP 2022
Director, CSIR-IICB

Message from the Organizing Secretary, PSI-ICPP 2022

Warm greetings to all of you! As the secretary of the organizing committee and on behalf of each one there, It is indeed a very great pleasure for me to welcome you to 14th Annual Meeting of the Proteomics Society, India and International Conference on Proteins & Proteomics (PSI-ICPP 2022), Nov 03-05, 2022 at CSIR-Indian Institute of Chemical Biology. Our institute, CSIR-IICB, enthusiastically hosts the off-line mode event at the institute in charming weather of sunny November in the 'City of Joy' with great partnership with NIPER-Kolkata, NIPER- Hajipur and IASST-Guwahati.

Keeping with the goal and perspectives of the Proteomics Society, India, the major theme of the symposium is Proteins and Proteomics. The scientific sessions have been carefully planned that will cover a wide spectrum of topics related to proteomics and molecular approaches in understanding the diseases biology, disease diagnostics and, application of proteomics in animal husbandry and agriculture. The organizing committee has planned a feast of programs which will incorporate presentations from a number of respectable International & national faculties, young scientists, talented research scholars' and expert from industrial fraternities. It is consist of oral and poster presentations from young research scholars as well as senior and established scientists in the field, The experts from different countries will also speak on latest innovations and technical developments on aspects of mass spectrometry and proteomics.

Prior to the conference, on 2nd November 2022, we organize a "Education Day" program a knowledge initiative and it is an integral part of the proteomics society annual meeting. It will inspire the young students, researchers, and college & university teachers. Thus, experts from the fields comprehensively disseminate and teach basic and advanced genomics, proteomics and allied research tools in one full-day program. This proteo-genomic education will motivate future generation proteome-ers, genom-ers. This year several students, postdoctoral fellows, researchers, college and university teachers and those seeking to incorporate proteomics into research or academic programs are attending the day long program.

The PSI-ICPP 2022 organizing committee is pleased to announce that this year we are awarding poster prizes in recognition of outstanding research initiatives reflected in the abstract. Also there will be award for oral presentations by young research scholars. Proteomics society also provides travel awards to some of the PSI-member students who are selected for poster presentations at the PSI-ICPP 2022.

The organizing committee members are trying their best to ensure that your time and stay in Kolkata during the conference be one of the most memorable one and you go back with rich information and as a proud stakeholder of PSI-ICPP 2022. Kolkata is considered to be a renowned place of intellectual activities and is enriched with cultural heritage. Integral mind setup for scientific enrichment will start with inaugural season with lightening of the lamp and welcome song by the students of the institute. You will also be refreshed with the rhythm of the musical tone in the cultural night at the end of long scientific seasons and the refreshment will be served at the best of its level. I welcome all of you again to this wonderful gathering and make the maximum out of it. I sincerely thank each and every one bringing your expertise and contributing to the success of this four day program. My personal admiration and gratitude goes out to all of you.

With warm regards

Dr. Nakul C. Maiti
Secretary, PSI-ICPP 2022
Senior Principal Scientist, CSIR-IICB

Content

PSI-ICPP 2022 Schedule

Day 1: November 3, 2022		
09:00-09:10 AM	**Inaugural song and Lighting the Lamp**	
09:10-09:20 AM	**Welcome address by Dr. Arun Bandyopadhyay, Director CSIR-IICB**	
09:20-09:30 AM	**Address by the President PSI, Dr. Subhra Chakraborty, Director, NIPGR**	
09:30-09:45 AM	**Address by the Chief Guest, Prof. Samir Bhattacharya, Former Director, CSIR-IICB**	
09:45-09:55 AM	**Address by the Guest of Honour, Prof. V. Ravichandiran, Director, NIPER-Kolkata and NIPER-Hajipur**	
09:55-10:05 AM	**Vote of Thanks, Dr. Nakul C. Maiti, Secretary, PSI-ICPP 2022**	
SCIENTIFIC SESSION 1: Proteomics and molecular approaches in understanding diseases Chairperson: Dr. Niranjan Chakraborty and Prof. V. Ravichandiran		
10:10-10:30 AM	Dr. G.R. Chandak	The plasma proteome of sickle cell Anaemia in Indians: Insights from exploratory proteomic profiling
10:30-10:50 AM	Dr. Shashank Deep	A mechanistic insight into SOD1 misfolding, aggregation and inhibition
10:50-11:20 AM	Dr. Vinay Pawar	High-throughput proteomics as a keystone in multi-omic approaches to biomarker discovery & precision medicine **(Premas Life Sciences Pvt. Ltd.)**
11:20-11:45 AM	**High-Tea**	
SCIENTIFIC SESSION 2: Proteomics in disease diagnostics Chairperson: Dr. Shelley Bhattacharya and Dr. Mahesh J. Kulkarni		
11:45 AM -12:05 AM	Dr. Shibdas Banerjee	Ambient mass spectrometry imaging for protein conformational study and biomedical applications
12:05-12:25 PM	Dr. Srikanth Rapole	Identification and functional characterization of potential targets and biomarkers for multiple myeloma using global proteomic analysis and molecular approaches
12:25-12:45 PM	Dr. Tiannan Guo	Advances in clinical proteomics and applications in diagnosing thyroid nodules
SCIENTIFIC SESSION 3: Implication of proteins in metabolomics disorders Chairperson: Dr. R. Sirdeshmukh and Dr. S. Swarnakar		
12:50-01:10 PM	Dr. Partha Chakrabarti	Tempering proteotoxicity in nonalcoholic fatty liver disease (NAFLD)
01:10-01:30 PM	Dr. Trayambak Basak	Post-translational modifications of collagens at the interface of extracellular matrix (ECM) remodelling during fibrosis
01:30-01:40 PM	Dr. Sanjay K. Banerjee	Pregestational diabetes alters cardiac structure and function of neonatal rats: Understanding the molecular defects through omics approaches

01:40-03:00 PM	**Lunch Break**	
03:00-05:00 PM	**POSTER SESSION 1**	
05:00-05:30 PM	**Tea break**	
05:00-06:00 PM	**GB Meeting**	
06:00-07:30 PM	**Cultural Program**	
07:30-09:00 PM	**Dinner (Venue: CSIR-IICB, Kolkata)**	

Day 2: November 4, 2022

SCIENTIFIC SESSION 4: Mechanism of cancer: How the proteomic approaches help? Chairperson: Dr. G Narahari Sastry and Dr. Ramesh Ummanni		
09:00-09:20 AM	Dr. Poonam Gautam	Tumor tissue proteome driling of gallbladder cancer
09:20-09:40 AM	Dr. Soumen Manna	Evolution of metabolic machinery in response to nutritional and pharmacological stress in cancer cells
09:40-10:00 AM	Dr. Arun K. Trivedi	Proteomics based identification of myeloid differentiation regulators in AML
SCIENTIFIC SESSION 5: Sponsor's Talk Chairperson: Dr. P. Jaisankar and Dr. Umesh Prasad Singh		
10:05-10:25 AM	Dr. Jean-Baptiste Vincendet	Leveraging the power of Zeno trap and EAD for highly sensitive protein identification and quantitation **(Sciex)**
10:25-10:45 AM	Dr. Saravanan Kumar	Single cell proteomics workflow to interrogate the granularity of biological systems **(Thermo Fisher Scientific)**
10:45-10:55 AM	Mr. Venkatesh Sankarasetty	Latest Innovations in Mass Spec for 4-D OMICS **(Bruker)**
10:55-11:10 AM	**Tea Break**	
SCIENTIFIC SESSION 6: Proteins and Enzymes: Implication in disease biology research Chairperson: Dr. Chandrima Shaha and Dr. Suman Kundu		
11:10-11:30 AM	Dr. Surajit Bhattacharyya	Structural Basis of Regulation of Beta 2 Integrins in Leucocytes
11:30-11:50 AM	Dr. Apurba Sau	Underlying basis for stimulated GMP formation in human large GTPases and its effect on antiviral activity
11:50 AM -12:10 PM	Dr. Vishal Rai	Chemical technologies for precision engineering of proteins and antibodies

SCIENTIFIC SESSION 7: Infections, inflammation and proteins Chairperson: Dr. Rukhsana Chowdhury and Dr. Nahid Ali		
12:10-12:30 PM	Dr. Alka Rao	Bacterial business of cysteine glycosylation!
12:30-12:50 PM	Dr. Suparna Sanyal	A cold look at the protein synthesis machinery of a protozoan parasite *Giardia intestinalis*
12:50-01:10 PM	Dr. Arvind Korwar	Selenium-dependent metabolic reprogramming during inflammation and resolution
01:10-02:10 PM	**Lunch**	
SCIENTIFIC SESSION 8: Application of proteomics in animal husbandry and agriculture Chairperson: Dr. Ashok Mohanty and Dr. Jayati Sengupta		
02:10-02:30 PM	Dr. Maya Zachut	Integrating proteomics and phosphoproteomics of adipose tissue in research of dairy cow physiology
02:30-02:50 PM	Dr. S. K. Ambatipudi	Bovine milk fat globules: a source of hidden treasure
02:50-03:10 PM	Dr. Subhra Chakraborty	System level understanding of organeller control of multihost resistance in fungal disease
SCIENTIFIC SESSION 9: Proteomics in neurodegenerative diseases Chairperson: Dr. Swasti Raychaudhury and Dr. K.N. Chattopadhyay		
03:15-03:35 PM	Dr. Sandipan Ray	Multiplexed quantitative proteomics for understanding circadian rhythms and pharmacological modulators of circadian clocks
03:35-03:55 PM	Dr. Amol R. Suryavanshi	Delineation of altered brain proteins associated with rabies virus infection by quantitative proteomics
03:55-04:15 PM	Prof. Ashis K. Mukherjee	Unveiling the neuritogenesis mechanism of a snake venom nerve growth factor
04:15-04:30 PM	**Tea Break**	
04:30-06:30 PM	**POSTER SESSION 2**	
06:30-09:00 PM	**Banquet Dinner (Venue: The Tollygunge Club 120, Deshapran Sasmal Road, Kolkata-700 033, India)**	

Day 3: November 5, 2022

SCIENTIFIC SESSION 10: Implication of proteins in disease biology Chairperson: Dr. T. K. Maiti and Dr. S. N. Bhattacharyya		
10:00-10:15 AM	Dr. Shilpak Chatterjee	Intracellular acetyl CoA improves the immunotherapeutic efficacy of anti-tumor CD8+ T cells in cancer
10:15-10:30 AM	Dr. Kaushik K. Dey	Proteomic landscape: novel insights into pathogenesis and toward biomarker discovery for Alzheimer's disease

10:30-10:45 AM	Dr. S. Arumugam	Preclinical scientific validation of a traditional medicine for NASH
10:45-11:00 AM	Dr. Amit K. Mandal	Structural construct of glycated hemoglobin in a patient with poorly controlled Diabetes Mellitus
11:00-11:20 AM	**Tea Break**	
SCIENTIFIC SESSION 11: Research Scholar Presentation Chairperson: Dr. Jaya Bandyopadhyay and Dr. Dipyaman Ganguly		
11:20 AM -12:20 PM	Research scholar presentation	
SCIENTIFIC SESSION 12: Young Scientist Talk Chairperson: Dr. Syamal Roy and Dr. Abhijit Chakrabarti		
12:20-12:30 PM	Dr. Uttam Pal	Non-invasive perturbations to study loop dynamics and conformational flexibility in enzyme activity
12:30-12:40 PM	Dr. Narasimha Kumar Karanam	Harnessing tumor treating fields-induced dna damage and replication stress for novel cancer therapy options
12:40-12:50 PM	Dr. Kamalika Mukherjee	Probing the molecular mechanism of HuR mediated extracellular export of miRNAs
12:50-01:00 PM	Dr. Rajiv Kumar	Exploring novel bioactive peptide of *Picrorhiza kurroa* and its potential therapeutic implications
01:00-01:10 PM	Dr. Sudipta Das	Denaturation resistant P2 tetramer is required to import fatty acids into intraerythrocytic Plasmodium falciparum
01:10-01:20 PM	Dr. Debabrata Mandal	Metallic nanoparticle & exosome-based novel delivery of Amphotericin B for reduced toxicity and a possible oral formulation
01:20-01:30 PM	Dr. Shuvadeep Maity	Identification of the complex molecular signatures behind the differential vulnerability between ALS-resistant and sensitive motor neurons — a multi-omics approach
Valedictory Session		
01:30-02:00 PM	**Chairpersons:** Dr. Arun Bandyopadhyay, Dr. Nakul C. Maiti **Guest of honour:** Dr. G. Narahari Sastry, Dr. V. Ravichandiran, Dr. Ashis K. Mukherjee	
02:00-03:00 PM	**Lunch**	

Meeting of the EC members and the Organizing committee members 5 PM onwards

Abstracts

Invited Speakers

IS-1

The plasma proteome of sickle cell Anaemia in Indians: Insights from exploratory proteomic profiling

Anushri U, Richa Singh, Sravani Polepally, Shoma Naskar, PSDB Punyashri, Dipty Jain, Swasti Raychaudhuri, Giriraj Ratan Chandak

1. Genomic Research on Complex diseases Group (GRC-Group), CSIR-Centre for Cellular and Molecular Biology, Hyderabad. INDIA

2. Cellular Biochemistry and Proteomics Group, CSIR-Centre for Cellular and Molecular Biology, Hyderabad. INDIA

3. Shivram Mahatme Memorial Eye Welfare Charitable Trust, Nagpur. INDIA

Identification of disease-specific biomarkers has been a major challenge in clinical medicine. Plasma proteomics can be a powerful tool given its ability to measure relative abundance of hundreds of proteins from a single sample which can further be compared to identify relevant biomarkers. Plasma proteomics has previously been used to identify biomarkers of non-communicable diseases like cancer, cardiovascular disease and neurological disorder. Sickle cell anaemia (SCA) is a global monogenic disorder, yet has complex and diverse clinical manifestations, especially In India. In this study, we conducted exploratory proteomic profiling in SCA patients with a goal to identify biomarkers.

We used plasma samples from well-phenotyped and genotypically confirmed SCA patients to first explore different strategies of shotgun proteomics. We established a deep plasma proteomics workflow which use UHPLC-based fractionation of tryptic digested plasma peptides followed by optimized fraction-pooling based on charge and hydrophobicity that provided the best possible proteome depth needed for identifying biomarkers. This protocol was used to generate deep plasma proteomic data on 8 SCA patients and 1 healthy volunteer, using which, a sample size of 30 cases and controls each (80% and 95% confidence) was decided. We present preliminary results from differential protein expression analysis of 20 SCA patients and 5 normal subjects. A total of 1239 proteins were quantified with 1% false detection rate stringency. Normal samples had a lower median count of proteins than SCA patients. Quality control analysis involved removal of potential contaminants from the sample protocols, variance stabilization normalization to remove the batch effect. The clean dataset was was used to impute the not quantified proteins using MinDet. The principle component analysis plot of top variable proteins showed two distinct clusters representing SCA patients and healthy volunteers. Using a fold change cut-off of 1 and p-value 0.05, 16 differentially expressed proteins were identified. Reducing the stringency of statistical analysis led to identification of 12 more differentially abundant proteins in SCA patients. These proteins can be the potential candidates for biomarkers in sickle cell anemia and will be verified using targeted proteomics approach.

IS-2

A mechanistic insight into SOD1 misfolding, aggregation and inhibition

Shashank Deep
Indian Institute of Technology Delhi

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease linked to superoxide dismutase 1 (SOD1) misfold and aggregation. Unfortunately, the mechanism of SOD1 aggregation is still not completely understood and effective therapeutics against this disease are still elusive. Aggregation process is a complex process and consists of various events such as formation of aggregation competent species, nucleation, elongation and secondary nucleation. A prospective drug candidate may affect the various event in a different way and to different extent. With a mix of computational and experimental techniques, we gained a lot of insight into the post-translation modifications, oligomer formation, elongation and stability of the fibrils during SOD1 aggregation and their modulation in the presence of polyphenols. The results will help in shaping our choice of a drug candidate.

IS-3

Ambient Mass Spectrometry Imaging for Protein Conformational Study and Biomedical Applications

Shibdas Banerjee
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Mass spectrometry imaging (MSI) is a label-free analytical approach that allows untargeted and multiplex mapping of thousands of biomolecules in a biological specimen. MSI technologies have evolved remarkably for clinical applications in the last two decades. This presentation will highlight our current innovation, excitement, and challenges with a new MSI technique based on electrospray ionization (ESI) coupled to high field asymmetric waveform ion mobility spectrometry (FAIMS) developed in our laboratory. This ESI-FAIMS imaging approach has enabled us to track and visualize the routes of protein unfolding by capturing and quantitating a wide variety of co-populated intermediate conformers in solution, which are otherwise challenging to probe. A study with human blood indicated the existence of multiple conformers of hemoglobin subunits. This talk will also demonstrate our recent exploitations with ambient ionization mass spectrometry imaging in understanding the molecular (lipids/metabolites) aberration in the human hippocampus during temporal lobe epilepsy, evaluating nephrotic syndrome, and developments in cancer diagnostics.

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IS-4

Identification and functional characterization of potential targets and biomarkers for Multiple Myeloma using global proteomic analysis and molecular approaches

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Multiple myeloma (MM) is a plasma cell associated cancer and the second most common hematological malignancy worldwide. It is associated with lower prognosis and a higher risk of relapse which usually culminates into metastasis. The major challenge remains the identification of better diagnostic, prognostic, therapeutic and chemoresistance markers for MM. In this work, we have applied quantitative proteomic approaches and identified differentially regulated proteins associated with MM from human MM serum, Bone marrow interstitial fluid (BMIF), BM mono nuclear cells and respective controls. Integration of BMIF and serum quantitative data evaluation led to the identification of a panel of five proteins viz., haptoglobin, kininogen 1, transferrin, and apolipoprotein A1 along with albumin that was validated using ELISA in a larger cohort of serum samples. This panel of proteins could serve as a useful tool in the diagnosis and understanding of the pathophysiology of MM in the future. Quantitative proteomics data of MM plasma cells against controls yielded marginal zone B and B1 cell specific protein (MZB1) and voltage dependant anion channel 3 (VDAC3) as potential promising targets which are unexplored in MM. Validation results were in accordance with the exploratory quantitative proteomics data as evident from western blot and MRM approaches. Knock down of MZB1 protein and over expression of VDA3 in the RPMI 8226 cell line proves that these proteins could be better targets for MM pathophysiology. It is well known that chemoresistance is a major hurdle for the treatment of MM, we tried to explore the cellular response of MM cell lines to bortezomib, a first-class drug used in MM treatment. We established the RPMI 8226R (MM chemoresistance cell line) using bortezomib and carried out iTRAQ and label free quantitative experiments for identification of chemoresistance markers. Quantitative proteomics analysis identified 112 significant proteins using both the labelled (iTRAQ) and label free approaches. Among these candidate proteins, Exportin 1 (XPO1) plays crucial role in emerging bortezomib resistance using

functional studies like cell count assay, flow cytometry assay and soft agar assay. XPO1 could be a potential therapeutic target for MM and development of inhibitors of XPO1 might help to cure MM.

IS-5

Advances in clinical proteomics and applications in diagnosing thyroid nodules

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In recent years, the throughput of proteomics has improved by at least ten times, while the input materials required for a proteomic analysis have reduced by over ten times. The number of samples that can be effectively analyzed in a single project goes beyond 1000, with almost doubled proteomic depth. These advances have driven rapid proteomics analysis of minute amounts of clinical specimens from multi-center cohorts, offering practical guidance to precision medicine. In this talk, I will discuss about the latest advances of AI-empowered proteomic big data technologies and their applications in diagnosing thyroid nodules.

IS-6

Tempering proteotoxicity in Nonalcoholic fatty liver disease (NAFLD)

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NAFLD is a spectrum of progressive metabolic disease associated with excessive hepatic lipid accumulation which may be accompanied by sterile inflammation and fibrosis. Although the predominant metabolic insult in NAFLD is lipotoxicity, concomitant accumulation of intracellular proteinaceous inclusion bodies composed of ubiquitylated proteins has been well documented. However, the role of deranged protein metabolism and proteotoxicity in NAFLD is not known. We find significant hepatic proteasomal dysfunction in high fat diet (HFD) induced NAFLD mice model, a phenomenon that leads to production of proteotoxic reactive oxygen species (ROS) which in turn initiates ASK1 dependent liver injury. Conversely, failure of PPARγ mediated antioxidant program further accentuates proteotoxicity. Pharmacological targeting of ASK1 and PPARγ synergistically attenuates pathological hallmarks of NAFLD and provide a novel intervention strategy for NAFLD.

IS-7

Post-translational modifications of Collagens at the interface of Extracellular matrix (ECM) remodelling during fibrosis

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Cardiac fibrosis-mediated heart failure (HF) is one of the major forms of end-stage cardiovascular diseases (CVDs). Cardiac fibrosis is an adaptive response of the myocardium upon any insult/injury. Excessive deposition of collagen molecules in the extracellular matrix (ECM) is the hallmark of fibrosis. This fibrotic response initially protects the myocardium from ventricular rupture. Although in mammals this fibrotic response progresses towards scar-tissue formation leading to HF, some fishes and urodeles have mastered the art of cardiac regeneration following injury-mediated fibrotic response. Zebrafish have a unique capability to regenerate the myocardium after post-amputation injury. Following post-amputation, the ECM of the zebrafish heart undergoes extensive remodeling and deposition of collagen. Being the most abundant protein of ECM, collagen plays important role in the assembly and cell-matrix interactions. However, the mechanism of ECM remodeling is not well understood. Collagen molecules undergo heavy post-translational modifications (PTMs) mainly hydroxylation of proline, lysine, and glycosylation of lysine during biosynthesis. The critical roles of these PTMs are emerging in several diseases, embryonic development, cell behaviour regulation, and cell-matrix interactions. The site-specific identification of these collagen PTMs in zebrafish heart ECM is not known. As these highly modified peptides are not amenable to mass spectrometry (MS), the site-specific identification of these collagen PTMs is challenging. Here, we have implemented our in-house proteomics analytical pipeline to analyse two ECM proteomics datasets (PXD011627, PXD010092) of the zebrafish heart during regeneration (post-amputation). We report the first comprehensive site-specific collagen PTM map of zebrafish heart ECM. We have identified a total of 36 collagen chains (19 are reported for the first time here) harbouring a total of 95 prolyl-3-hydroxylation, 108 hydroxylysine, 29 galactosyl-hydroxylysine, and 128 glucosylgalactosyl-hydroxylysine sites. Furthermore, we comprehensively map the three chains (COL1A1a, COL1A1b, and COL1A2) of collagen I, the most abundant protein in zebrafish heart ECM. We achieved more than 95% sequence coverage for all the three chains of collagen I. Our analysis also revealed the dynamics of prolyl-3- hydroxylation occupancy oscillations during heart regeneration at these sites. Moreover, quantitative site-specific analysis of lysine-O-glycosylation microheterogeneity during heart regeneration revealed a significant ($p < 0.05$) elevation of site-specific (K^{1017}) glucosylgalactosyl-hydroxylysine on the col1a1a chain. Taken together, these site-specific PTM maps and the dynamic changes of site-specific collagen PTMs in ECM during heart regeneration will open up new avenues to decode ECM remodeling and may lay the foundation to tinker the cardiac regeneration process with new approaches.

IS-8

Pregestational diabetes alters cardiac structure and function of neonatal rats: Understanding the molecular defects through omics approaches

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Pregestational diabetes (PGDM) lead to developmental impairment, especially cardiac dysfunction, in their offspring. However, the molecular mechanism of the developmental defects in the heart due to PGDM remains unclear. We hypothesized that the altered expression of several proteins and their signaling pathways due to hyperglycaemia during foetal development might be responsible for molecular defects and phenotypic changes in the heart. To understand the molecular defects in the seven-days old neonatal rats, streptozotocin-induced diabetic female rats were bred with healthy male rats. We collected seven-day-old hearts from the neonates and identified the molecular basis for phenotypic changes. Neonates from diabetic mothers showed altered electrocardiography and echocardiography parameters. Proteomics profiling revealed that high percentage of the proteins in the biological processes were involved in the heart development, cardiac muscle fiber development and response to calcium ion. Similarly, ontology analysis of the proteins showed that the down regulated proteins were involved in the response to calcium ion, adult heart development, cardiac muscle fiber development, mitochondrial matrix, extracellular exosome, poly(A) RNA binding, autophagy, oxidoreductase activity, and cadherin binding involved in cell-cell adhesion. Additionally, proteins associated with fibroblast migration, retinol metabolic process, heart development and calcium dependent protein binding were up-regulated. Thus, our results provide a comprehensive map of the cellular events and molecular pathways perturbed in the neonatal heart during PGDM. All of the molecular and structural changes lead to developmental plasticity in neonatal rat hearts and develop cardiac anomalies in their early life.

IS-9

Tumor tissue proteome profiling of gallbladder cancer

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Gallbladder cancer (GBC) is an aggressive malignancy of the gastrointestinal tract with a poor prognosis. It is important to understand the molecular processes associated with the pathogenesis of GBC and identify proteins useful for diagnostic and therapeutic strategies. Here, we have carried out an iTRAQ-based quantitative proteomic analysis of tumor tissues from GBC cases (n=19) and gallstone disease (GSD) as non-tumor control (n=12). We identified 432 differentially expressed proteins (DEPs) based on ≥ 2 unique peptides and ≥ 2 fold change with p value < 0.05. A total of 357 proteins were

detected in early stage GBC (stage I and II) and 182 proteins in advanced stage GBC (stage III). Of these, a total of 107 proteins were common in both early and advanced stage GBC. We analyzed the proteins associated with early stage and progression of the disease. We found 'neutrophil degranulation' (MPO, PRTN3, S100A8) and 'ECM organization' proteins (BGN, DCN, collagens) to be associated with early stage GBC and 'homotypic cell-cell adhesion' proteins (CEACAM5, HSPB1, FGB, FLNA) to be associated with the progression of the disease. Some of the proteins associated with these processes are verified by Western blot analysis. These and other proteins may be further explored for their potential in early detection the disease and as therapeutic targets in GBC. Overall, the study presents a protein dataset associated with GBC.

IS-10

Evolution of metabolic machinery in response to nutritional and pharmacological stress in cancer cells

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During the course of the disease, cancer cells experience varying degree of nutritional, redox and therapeutic stress. The ability of cancer cells to effectively respond and adapt to these stresses play an important role in determining disease progression and outcome. Metabolic reprogramming has been found to be a hallmark of neoplastic transformation. However, the role of metabolic reprogramming in the response of cancer cells to the aforementioned stresses remains poorly understood. There have been very few studies that investigated the temporal evolution of biochemical landscape during stress response. This talk will share results of our ongoing investigations into the nature of metabolic reprogramming associated with exposure to nutritional and pharmacological stress in cancer cells. In addition to characterization of temporal evolution of the metabolic machinery, it will also examine reversibility of these changes in order to assess the robustness of association between metabotype and phenotype. It will further describe identification of pathways and regulators associated with outcome of nutritional and pharmacological perturbations by combining gene and protein expression with metabolomics analysis. It will also discuss the translational potential of combination of nutritional and pharmacological interventions to control the disease.

IS-11

Proteomics based identification of myeloid differentiation regulators in AML

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Acute myeloid leukemia (AML) is heterogeneous disease characterized by surge in blast cells (>20%). Differentiation arrest in one of the major reason for this surge in blast cells. Therefore, understanding

mechanisms underlying differentiation arrest could pave the way for better targeting of AML. Perturbed stability of regulatory proteins and myeloid transcription factors such as C/EBPα and PU.1 could be one of the underlying mechanism. In line with this, we have recently focused on identifying E3 ligases involved in differentiation arrest by targeting such factors. In recent years we have extensively worked with E6-associated protein (E6AP), an E3 ubiquitin ligase and showed that it may potentially inhibit myeloid differentiation by targeting C/EBPα and GCSFR. In an attempt to identify, other protein substrates of E6AP having implication in myeloid differentiation, we applied pull-down based proteomics approach to identify interacting proteins of E6AP and identified MAX binding protein (MNT) as a potential substrate. MNT is a member of the Myc/Max/ Mad network of transcription factor that regulates cell proliferation, differentiation, cellular transformation and tumorigenesis. We further showed that E6AP indeed interacts with MNT and negatively regulates granulocytic differentiation by targeting MNT for degradation which is required for growth arrest and subsequent myeloid differentiation by various differentiation inducing agents.

IS-12

Structural Basis of Regulation of Beta 2 Integrins in Leucocytes

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Integrins are pivotal protein receptors involved in cell adhesion and migration. Integrins can transmit signal in a bi-directional fashion, inside-out and outside-in. Heterodimeric integrins are composed of two sub-units α and β with a single pass transmembrane domain in each sub-unit. The resting integrin undergoes a sequence of conformational changes upon activation. The four members (αLβ2, αMβ2, αXβ2 and αDβ2) of β2 integrins are exclusively express in leucocytes which are necessary for functional immune system. Dysfunctional β2 integrins are disease causing and show defect in many adhesion dependent processes, including chemotaxis, phagocytosis and homotypic aggregation. The cytosolic tails of integrins interact with a plethora of regulatory proteins facilitating bi-directional signaling cascades. We are investigating cytosolic protein complexes that are regulating outside-in signaling of β2 integrins using NMR, biophysical and functional analyses. In this talk, I will present structural analyses of β2 integrins multiprotein complexes that unravel important steps toward the understanding of sequential events leading to integrin's inside-out activation and outside-in signaling.

IS-13

Underlying basis for stimulated GMP formation in human large GTPases and its effect on antiviral activity

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Interferon-gamma-inducible human large GTPases, hGBP1 and hGBP2 have a distinctive feature of hydrolyzing GTP to GDP and GMP through successive phosphate cleavages. In hGBP1, GMP is the major product, whereas hGBP2 produces significantly less GMP, despite sharing a high sequence identity (78%). Using biochemical, biophysical, and *in silico* experiments, we studied the underlying basis for assembly-stimulated GMP formation by hGBP1 and its role in immunity. We found that hGBP1 forms a tetramer that is crucial for enhanced GMP formation. The W79A mutation in hGBP1 showed significantly reduced GMP formation. After the gamma-phosphate cleavage, the W79-containing region undergoes a conformational change, which helps to reposition the active site for the next cleavage step. This occurs through a stable H-bonding contact between the indole moiety of W79 (present near the active site) and the main chain carbonyl of K76 (located in the catalytic loop), essential for stimulated GMP formation. The study also showed that enhanced GMP formation in hGBP1 is crucial for anti-HCV activity. Similar to hGBP1, hGBP2 also forms a tetramer, but it has no role in GMP formation. We observed that unlike in hGBP1, the helical domain of hGBP2 has an insignificant role in the regulation of GTP hydrolysis, suggesting that the differences in GMP formation between hGBP2 and hGBP1 arise from differences in their GTP-binding domains. Our study suggests that sequence variation near the active site in these two close homologs leads to differential second phosphate cleavage.

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IS-14

Chemical technologies for precision engineering of proteins and antibodies

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The chemical toolbox for investigating biological systems or enabling biologics requires the precise covalent attachment of tags to the proteins. In this perspective, we have been leading the efforts toward chemical technologies to enable precise control over the site of bioconjugation. The critical

barrier involves the simultaneous deconvolution of multiple challenges associated with reactivity and selectivity. In this perspective, we have developed a DisINtegrate or DIN theory that allows us to create new reactivity landscapes on a protein's surface. It enabled the development of methods for targeting reactivity hotspots,[1,2] N-Gly residue-specific labelling (Gly-Tag®),[3] and modular Linchpin-Directed Modification (LDM®) platform.[4] Our state-of-the-art platform offers homogeneous antibody-drug conjugates (ADCs) for directed cancer chemotherapeutics and fluorophore conjugates (AFCs) for imaging-guided tumour surgery.[4,5] Besides, our findings create a hope that we will make precision therapeutics with small molecules possible one day. *The talk would highlight the philosophical connection between humans and proteins' behaviour and how it inspired the precision engineering of proteins and antibodies.*

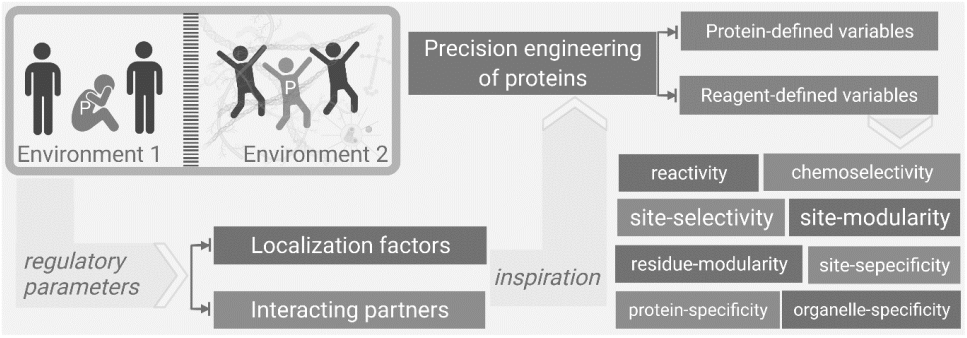

Figure. Regulatory parameters of human behavior inspired the precision engineering of proteins.

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IS-15

Bacterial business of cysteine glycosylation!

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Glycosylation is a widespread and universal post-translational modification of proteins. Glycoproteins play several important roles in biological systems. Glycans often contribute to the strength and stability of glycoproteins and are also involved in cell-cell interactions, signaling, adhesions, protein

localization, and the immune response in cells or organisms. Based on protein-glycan linkages, most biological glycoproteins could be Asp(N)-linked or Ser/Thr(O)-linked. In Firmicutes, Donk et al. (1) discovered a new class of ribosomally synthesized antimicrobial peptides harbouring Cys (S)-linked glycans. These glycoactive peptides were later named glycocins (the glycosylated bacteriocins). The enzyme that transfers glycan to the cysteine residue of the peptide is known as S-glycosyltransferase. The first S-glycosyltransferase was characterized in *Bacillus subtilis* (SunS) in 2011, followed by another in *Bacillus thuringiensis* (ThuS) in 2013 (2). Subsequently, our group at CSIR-IMTECH characterized an S-diglycosyltransferase, namely EntS from *Enterococcus faecalis* TX104 (3, 4 & 5) and an S/O-HexNAc/Hextransferase named SvGT from Actinobacteria *Streptomyces venezuelae* ATCC 15439 (6 & 7). EntS is unique in its function and can directly and sequentially transfer two hexose units to the acceptor peptide enterocin 96 at two chemovariant glycosites (Ser/Thr and Cys). Enterocin 96 has known antimicrobial activity against the foodborne pathogen *Listeria monocytogenes*. We have also observed that the number and type of glycans are directly correlated with the bioactivity of glycosylated enterocin 96 against *L. monocytogenes*. Next, using EntS, we developed an *Escherichia coli*-based microbial system (SELECTGLYCOCIN) suitable for directed evolution of O- and S-linked glycocines and discovery research on S-glycosyltransferases (8 & 9). The SELECT GLYCOCIN system was successfully used to identify and characterize an S/O-HexNAc inverting transferase (SvGT) and its substrate SvC encoded by ORF AQF52_3101 and ORF2AQF52_3099 of *S. venezuelae*, respectively. SvGT differs from EntS in its donor and acceptor specificity. Through various *in vitro* and *in vivo* assays, we further identified the shortest (five residues long) sequon suitable for glycosylation in SvC. Thus, the sequence Y(G/A/K/Q/EG)(C/S/T Y/N)(G/A P/Q)G is defined as the minimal acceptor sequence of SvGT. Although UDP-GlcNAc served as a donor in the cellular environment, SvGT was able to use UDP-Glc and UDP-GalNAc as donors *in vitro*. Our studies provide the first evidence that an anti-O-GlcNAc antibody (CTD110.6) crossreacts with S-GlcNAc. Therefore, CTD110.6 may be useful in the direct detection of SGlcNAcylated glycoconjugates. We also show that S-linked peptides resist glycosidases while equivalent O-like peptides do not. Knowing the enzyme specificities, we next used SvGT to screen two proof-of-concept neoglycocins against *L. monocytogenes* (6 & 7). S/O-linked antimicrobial peptides such as Glyocin F, Subblancin 168, and Enterocin 96 are stable over a wide pH and temperature range and exhibit antimicrobial activities against various microbes, including pathogenic and food spoilage bacteria. For potential applications of glycocines in food, agriculture, and healthcare products, Sglycosyltransferases are valuable tools for custom glycoengineering. The SELECT GLYCOCIN and Assay methods are generic. Therefore, they can be adapted for use with known or yet to be discovered S-linked glycocines. *The work was supported by CSIR.*

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IS-16

A cold look at the protein synthesis machinery of a protozoan parasite *Giardia intestinalis*

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Recent advancements in single-particle cryo-Electron Microscopy have revolutionized the entire field of protein sciences. It has strong implications in the field of protein synthesis as it enables visualization of almost all steps of protein synthesis with molecular details. Cryo-EM has been used to decipher the mechanism of 'elongation' of protein synthesis - both in bacteria (e.g. *Escherichia coli*) and higher eukaryotes (e.g. human). However, little has been studied so far about protein synthesis mechanism in lower eukaryotes. Here, using cryo-EM and fast kinetics, we have explored the molecular mechanism of elongation of protein synthesis in *Giardia intestinalis,* which is a protozoan parasite that causes diarrhea in humans. We have determined high-resolution structures of six naturally populated elongation-intermediate states of the Giardia ribosome, with tRNAs and eEF2 bound to it in various conformations. We have also followed ribosome dynamics that allows translocation to happen efficiently in the presence of eEF2. Our results not only reveal the specialities of translation elongation in the model lower eukaryotes, but also lead to vital understanding of the role of ribosomal dynamics that guide the process. Finally, we could also gain insights about antibiotic sensitivity of the protein synthesis machinery of lower eukaryotes.

IS-17

Selenium-dependent metabolic reprogramming during inflammation and resolution

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Trace element selenium (Se) is incorporated as the 21st amino acid, selenocysteine, into selenoproteins through tRNA[Ser]Sec. Selenoproteins act as gatekeepers of redox homeostasis and modulate immune function to effect antiinflammation and resolution. Bacterial endotoxin lipopolysaccharide (LPS) activation of murine bone marrow–derived macrophages cultured in the presence or absence of Se (as selenite) was used to examine temporal changes in the proteome and metabolome by multiplexed tandem mass tag–quantitative proteomics, metabolomics, and machine-learning approaches. Kinetic deltagram and clustering analysis indicated that addition of Se led to extensive reprogramming of cellular metabolism upon stimulation with LPS, to aid in the phenotypic transition toward alternatively activated macrophages, synonymous with resolution of inflammation. Se-dependent modulation of pathways mainly involving succinate dehydrogenase, pyruvate kinase and sedoheptulokinase predisposed bone marrow–derived macrophages to preferentially increase oxidative phosphorylation to efficiently regulate inflammation and its timely resolution. The use of

macrophages lacking selenoproteins indicated that all three metabolic nodes were sensitive to selenoproteome expression. Furthermore, inhibition of succinate dehydrogenase complex with dimethylmalonate affected the proresolving effects of Se by increasing the resolution interval in a murine peritonitis model. In summary, our studies provide novel insights into the role of cellular Se via metabolic reprograming to facilitate antiinflammation and proresolution.

IS-18

Integrating proteomics and phosphoproteomics of adipose tissue in research of dairy cow physiology

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During the periparturient period, lipolysis in adipose tissue (AT) mobilizes fatty acid reserves to meet high energy needs of dairy cows. This physiological response is accompanied by the synthesis and secretion of proteins (adipokines) that modulate metabolic functions, and generates free radicals that lead to a remodeling process characterized by an inflammatory response. Proteomic analysis explores the repertoire of tissue proteins at a given state, while cutting edge phospho-proteomics quantifies the abundance of specific phosphopeptides within the tissue. Together, these omics analysis provide a plethora of information on the main canonical pathways and networks that are enriched in the tissue, which can be used to provide insight on effects of different treatments and physiological states on the AT. Proteomics of subcutaneous 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. 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 affect inflammation. Therefore, increased oxidative stress in AT, associated with lipolysis and/or heat stress, could increase AT inflammation, as reflected by the AT proteome. 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. Dietary supplementation of conjugated linoleic acid (CLA) has enriched lipid metabolism pathways in AT of dairy cows, according to novel phospho-proteomic analysis of subcutaneous and abdominal AT. Proteomics of AT from highly efficient or non-efficient dairy cows discovered biomarkers of high efficiency. In conclusion, biotic and abiotic stressors, anti-inflammatory agents, dietary treatments and physiological state affect the abundance of proteins and phospho-peptides in

AT of dairy cows. Proteomics and phospho-proteomics of AT improves our understanding of AT physiology, and adds information on novel proteins and phosphopeptides in AT of dairy cows.
Keywords: adipose, proteomics, phospho-proteomics, peripartum, inflammation

IS-19

Bovine Milk Fat Globules: A Source of Hidden Treasure

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Milk is an essential part of the human diet and is widely accepted as a nutrient-rich source in developing countries. Milk fat globule (MFG), the spherical droplet consisting of triglycerides at its core, is surrounded by tri-layer containing glycerophospholipids, cholesterol, sphingolipids, glycoproteins, and glycolipids and, therefore, is considered a promising milk component for the development of functional food. Surprisingly, despite being nutritionally significant, there is limited information on the extent of natural variation in MFG lipid and protein composition and its microstructural properties across different ruminants that could be used as milk sources for a targeted supplement to improve human health. Hence, polar and neutral lipid content variation in MFG between widely consumed animal species, including cow, goat, and buffalo, was studied using high-performance thin layer chromatography (HPTLC). This quick, sensitive and cost-effective technique successfully separated, identified, and quantified five major polar (PL) and three neutral lipids (NL) from the MFG of cow, goat, and buffalo. After optimizing the parameters, a highly sensitive method was developed with lower detectable and quantification limits. The theory of correlation of globule size corresponding to its lipid content is well established in the literature, thus emphasizing the need to explore MFG microstructure, particularly its size. Fat globule membrane proteins are another essential macronutrient in fat globules; however, little is known about changes in their abundance across species and lactation stages. Hence, a comprehensive protein profiling by mass spectrometry-based proteomics collectively identified 453 MFG proteins in HF cow and Murrah buffalo across the three lactation stages in each animal. Of 366 non-redundant proteins in HF, 214 were common among all the lactation stages, whereas, in the case of Murrah buffalo, 246 proteins were found to be common out of 434. ANOVA analysis within the species identified 108 proteins in HF and 130 in Murrah, thus identifying MFG proteins playing a crucial role in human health. The variation in lipid and protein quantity among different animal species suggests more research to support their selection as a suitable source for developing functional food to impact human health positively.

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IS-20

System Level Understanding of Organeller Control of Multihost Resistance in Fungal Disease

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Cell compartmentalization into different subcellular organelles is an attribute conserved in eukaryotes, including plants. Nucleus, the regulatory hub is a dynamic system encompassing genetic information essential for the regulated expression of proteins and a repository of macromolecules those serve as the modulators of various signalling networks in response to extracellular stimuli, including pathogen stress. Growing evidences suggest that nuclear localization of pathogen effectors, R proteins, transcription factors (TFs) and regulators are vital for plant immunity. Spatial and temporal regulation of such immune regulators by nuclear proteins provides an important mechanism for fine-tuning of innate immune responses and downstream signalling events. Vascular wilt, caused by the multi-host fungal pathogen, *Fusarium oxysporum f.sp. ciceri* accounts to severe yield loss in the productivity of the second most important food legume chickpea, while Fusarium-mediated killing of worm and neuronal stress in humans have also been described. Despite the reports of *Fusarium* pathogenicity in different host systems the cellular pathways and mechanistic details that might serve as the functional basis of *Fusarium* associated disease or immune state during multi-host response remains to be explored. Further, the role of nucleus in developing such resistance response during vascular wilt in chickpea and how it imparts immunity by transcriptional and translational/post-translational reprogramming is still not well understood. Our study for the first time provides novel insights on host-specific immune signaling, biomolecular pathways and key regulators that impinge upon the surveillance mechanism of innate immunity in multi-host pathogen response.

IS-21

Multiplexed Quantitative Proteomics for Understanding Circadian Rhythms and Pharmacological Modulators of Circadian Clocks

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Circadian (approx. 24 hour) clocks exist in almost all living organisms and play a key role in regulating daily physiological and behavioral processes. 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 therapeutics. Several cellular targets involved in drug binding, transport, and metabolism show variations in their expression and activity throughout the day-night cycles. We investigated the molecular rhythmicity in a mammalian system lacking the core clock machinery using TMT-based multiplexed quantitive proteomics. In the same vein, we have demonstrated metabolic oscillations in *Drosophila* cells (*Schneider 2* cells) lacking clock genes and circadian rhythms of

metabolic flux in non-nucleated human red blood cells, i.e., in the absolute absence of any TTFL mechanisms. In this study, we 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). Kinome profiling indicated inhibition of CKId, ERK1/2, CDK2/7, TNIK, and MST4 kinases as a common mechanism of action for these compounds. 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. This study defines novel clock effectors that could inform precise therapeutic targeting of the circadian system in humans, which is critical to modulate daily rhythms for therapeutic benefit.

IS-22

Delineation of altered brain proteins associated with rabies virus infection by quantitative proteomics

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Rabies, a zoonotic viral disease is caused by the rabies virus (RABV). It 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. Post symptoms, majorly death occurs 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 total 2,188 brain proteins significantly, 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 majority of these proteins are novel or reported first time in rabies virus infection. In addition, it showed that most of these proteins have human homologues. Analysis with GO annotation and IPA 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 disorders. Total 34 proteins were successfully validated by qRT-PCR and three 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.

IS-23

Unveiling the Neuritogenesis Mechanism of a Snake Venom Nerve Growth Factor

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Snake venom is rich in several enzymatic and non-enzymatic toxins. Proteomic analyses have demonstrated that nerve growth factor (NGF) is a low molecular mass and a relatively low-proportion component of snake venoms. However, the exact mechanism of snake venom NGF to induce neuritogenesis was unknown. We studied the signal transduction mechanism of an NGF from Indian Russell's viper (*Daboia russelli*) venom to cause neuritogenesis in PC-12 neuronal cells. The in-sillico computational analysis predicted a strong binding of snake venom NGF to the TrkA receptor expressed in PC-12 cells. The transcriptomic and quantitative proteomic studies in unison have shown the differential expression of unique genes and intracellular proteins in snake venom NGF- treated PC-12 cells compared to control (untreated) PC-12 cells. It was noted that the cellular proteins are accountable for neuritogenesis and cell survival due to the upregulation of anti-apoptotic proteins expression and downregulation of pro-apoptotic proteins by snake venom NGF. The transcriptomic and proteomics analyses, as well as the Inhibition of cellular signalling pathways by pathway-specific small inhibitors in unanimity, suggested that snake venom NGF activates extracellular mitogen-activated protein kinase-1 (MAPK1) and protein kinase C (PKC) as significant and minor signalling pathways, respectively for inducing neuritogenesis. Interestingly, snake venom NGF does not activate the Jun N-terminal kinase (JNK) pathway to induce neuritogenesis.

IS-24

Intracellular Acetyl CoA Improves the Immunotherapeutic Efficacy of Anti-tumor $CD8^+$ T cells in cancer

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Immune checkpoint blockade and adoptive T cell therapy have yielded remarkable clinical successes. However, the efficacy is restricted hitherto to a small fraction of patients with malignancies. The major impediment to effective T cell-based immunotherapy of cancer is the dysfunctionality of T cells in the tumor microenvironment (TME). Amongst various immunosuppressive signals present in TME, nutrient limitation is considered as an important barrier to invoke anti-tumor response by T cells. Therefore, to elicit a robust anti-tumor immune response, T cells must acquire metabolic traits enabling sustained effector function at the tumor site. Here, we report that IL-12-stimulated $CD8^+$ T cells have elevated intracellular acetyl CoA levels and can maintain IFNγ levels in nutrient-deprived, tumour-conditioned media (TCM). Pharmacological and metabolic analyses demonstrated an active glucose-citrate-acetyl CoA circuit in IL-12-stimulated $CD8^+$ T cells supporting an intracellular pool of acetyl CoA in an ATP-citrate lyase (ACLY)-dependent manner. Intracellular acetyl CoA levels enhanced histone acetylation, lipid synthesis, and IFNγ production, improving the metabolic and functional fitness of $CD8^+$ T cells in tumors. Pharmacological inhibition or genetic knockdown of ACLY severely impaired IFNγ production and viability of $CD8^+$ T cells in nutrient-restricted conditions. Furthermore, $CD8^+$ T cells cultured in high pyruvate-containing media *in vitro* acquired critical metabolic features of IL-12-stimulated $CD8^+$ T cells and displayed improved anti-tumor potential upon adoptive transfer in murine lymphoma and melanoma models. Overall, this study delineates the metabolic configuration of $CD8^+$ T cells required for stable effector function in tumors and presents an affordable approach to promote the efficacy of $CD8^+$ T cells for adoptive T cell therapy.

IS-25

Proteomic landscape: novel insights into pathogenesis and toward biomarker discovery for Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disease, the most common cause of dementia in elderly persons. Accumulation of amyloid plaques in the brain is a characteristic of AD. The requirements for a biomarker include the ability to measure a pathologic process, predict outcome, distinguish disease or measure a pharmacological response to a drug treatment or therapeutic intervention. However, there are no reliable biomarkers for AD. Based on amyloid cascade and tau

hypotheses, protein biomarkers of different Aβ and tau species in cerebrospinal fluid (CSF) and blood/plasma/serum have been examined to correlate with brain pathology. Recently, unbiased proteomic profiling of these human samples has been initiated to identify many novel AD biomarker candidates, but it is challenging to define reliable candidates for subsequent large-scale validation. We present a comprehensive strategy to identify biomarker candidates of high confidence by integrating multiple proteomes in AD, including cortex, CSF, and serum. The proteomes were analyzed by the multiplexed tandem-mass-tag (TMT) method, extensive liquid chromatography (LC) fractionation and high-resolution tandem mass spectrometry (MS/MS) for ultra-deep coverage. Finally, candidate biomarkers identified by the MS discovery were validated by the enzyme-linked immunosorbent (ELISA) and TOMAHAQ targeted MS assays. We quantified 13,833, 5941, and 4826 proteins from human cortex, CSF and serum, respectively. Compared to other studies, we analyzed a total of 10 proteomic datasets, covering 17,541 proteins (13,216 genes) in 365 AD, mild cognitive impairment (MCI) and control cases. In summary, 37 proteins emerged as potential AD signatures across cortex, CSF and serum, and strikingly, 59% of these were mitochondria proteins, emphasizing mitochondrial dysfunction in AD. Finally, we prioritized the most promising AD signature proteins including SMOC1, TAU, GFAP, SUCLG2, PRDX3, and NTN1 by integrating all proteomic datasets. Our results demonstrate that novel AD biomarker candidates are identified and confirmed by proteomic studies of brain tissue and biofluids, providing a rich resource for large-scale biomarker validation for the AD community.

IS-26

Preclinical scientific validation of a traditional medicine for NASH

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Nonalcoholic steatohepatitis (NASH) is gradually reaching global epidemic proportions. Ayurveda, the traditional system of Indian medicine, uses polyherbal formulations to treat various diseases. However, their scientific rationale and mechanisms remain largely unexplored. *Vasaguduchyadi Kwatha* (VK) is a polyherbal Ayurvedic formulation, indicated for the treatment of liver diseases. In this study, the identification and quantification of the constituents of VK were done with the help of HPLC using external reference standards. Network pharmacology was performed to find the targets based on the information from databases and liquid chromatography and mass spectrometry (LC-MS) analysis. An *in silico* study confirmed the interaction between phytoconstituents and molecular targets. The effects of VK on NASH were confirmed using a high-fat diet-induced animal model. Biochemical analysis confirmed the significant effect on biomarker levels. Histological analysis

revealed a significant reduction in steatosis, lobular inflammation, and hepatitis after treatment, and These findings were supported by the decrease in the levels of inflammatory cytokines. Insulin resistance (IR) is calculated using the homeostasis model assessment (HOMA) index, which showed significant decrease in IR after treatment. Transcriptomics analysis has shown inflammatory and lipogenic genes' downregulation and lipolytic genes' upregulation, which were further validated and supported by the results of RTPCR analysis. Immunofluorescence analysis of tissues has shown a decreased expression of lipogenic and inflammatory markers and an increase in lipolytic markers. Overall findings from *in vivo* analysis support the network pharmacology data, showing that the Ayurvedic polyherbal formulation VK could be a potential treatment for NASH, acting through the PPAR signaling, inflammatory, and fatty acid synthesis pathways.

IS-27

Structural construct of glycated hemoglobin in a patient with poorly controlled Diabetes Mellitus

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Glycated hemoglobin (HbA_{1c}) is the molecular marker of long-term glycemic index of an individual. HbA_{1c} is formed via covalent modification of N-terminal α-amino group of β globin chain of hemoglobin with glucose via Amadori rearrangement. Exploiting surface charge differences between HbA_{1c} and native tetrameric hemoglobin (HbA_0) in cation exchange chromatography, quantification of HbA_{1c} is performed in a diagnostic laboratory. However, aforementioned glucose condensation is specific to primary amino groups in a protein. Therefore, structural characterization of glycated hemoglobin synthesized *in vivo* is essential as multiple glycations may interfere with HbA_{1c} assessment. The stoichiometric composition of different glycated hemoglobin from a diabetic patient with 19% HbA_{1c} was deduced using native mass spectrometry. We observed a comparable population of α and β glycated tetramers for mono-glycated hemoglobin. Surprisingly, doubly and triply glycated hemoglobin showed the presence of mono-glycated α and β globin chains only. Thus, we propose that glycation of human hemoglobin (HbA_0) occurs symmetrically across α and β globin chains with preference to unmodified globin first. Correlation between HbA_{1c} and mass spectrometry-based quantification of glycated hemoglobin showed a reliable assessment of the glycemic index of patients even with poorly controlled Diabetes Mellitus from HbA_{1c} measurement only.

Abstracts

Young Scientist

YS-1

Non-invasive Perturbations to Study Loop Dynamics and Conformational Flexibility in Enzyme Activity

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The enzymes are one of the prime targets for drug development efforts. Catalytic activity of an enzyme is regulated by several extrinsic (pH, temperature, solvent etc.) as well as intrinsic (loop motions) factors. Specific correlated motions within the loop regions of an enzyme can control its enzymatic activity.[1,2] Identification of regulatory loop regions attached to the active site of an enzyme can help researchers to understand the complexity of the enzyme to develop effective inhibitors. Our study focuses on exploring original non-invasive perturbation techniques to elucidate the loop motions responsible for enzyme activity and this is achieved by controlled modulation of the extrinsic factors mentioned above. In a recent study, we have identified the regulatory loops of dengue protease by solvent exchange and thermal perturbations.[3] Unlike mutation based approaches, the non-invasive perturbation methods can be used to study important disease related proteins without modifying their native structure. Such non-invasive perturbation methods can also be deployed to uncover the triggers for aggregations of intrinsically disordered proteins (β-amyloid, α-synuclein etc.), where the wild type protein undergoes aggregations due to unknown reasons.

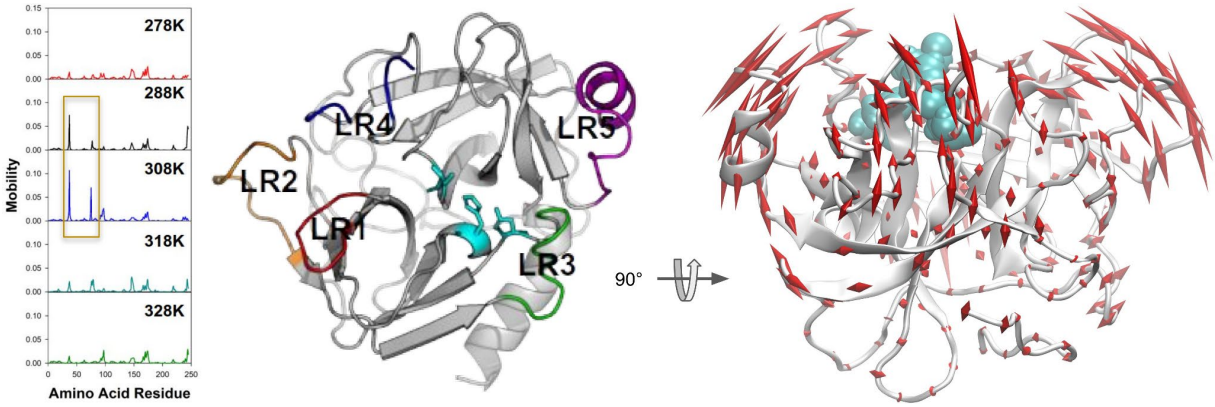

Figure 1: Thermal perturbation of loop dynamics in α-chymotrypsin.[2] Mobility plot (left) shows two prominent modes of vibrations around the optimum temperature. These motions are mapped to the loop regions (LR1–5) surrounding the catalytic triad (cyan). Range of motions (right).

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YS-2

Harnessing Tumor Treating Fields-induced DNA damage and replication stress for novel cancer therapy options

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A new physical cancer treatment modality called Tumor Treating Fields (TTFields) has demonstrated effectiveness in the treatment of solid tumors in vitro and in vivo. TTFields therapy is a non-invasive cancer treatment modality that delivers low intensity (1-3 V/cm), intermediate frequency (100-300 kHz) alternating electric fields to the tumor. The TTFields delivery device called Optune (NovoCure), has been approved for recurrent and newly diagnosed glioblastoma, unresectable locally advanced or metastatic malignant pleural mesothelioma (MPM) and clinical trials are ongoing for other cancers. The primary mechanism of TTFields action is thought to be interference with mitosis. To capture the whole picture of biological processes in a non-targeted and un-biased manner transcriptomics was employed in a series of non-small cell lung cancer (NSCLC) cells to understand global gene expression changes upon TTFields exposure. In line with previous findings, we found that most differentially expressed genes were involved in cell cycle and proliferation pathways. Interestingly we found that the expression of BRCA1 DNA damage repair pathway genes were significantly downregulated ($P < 0.05$) upon TTField treatment. However, the exact cause of the downregulation of DNA repair and cell cycle checkpoint genes has been elusive. To that end, we employed relative quantitative proteomic analysis using tandem mass tags (TMT). Samples were digested with trypsin and labeled with different TMT reagents, and then combined mixtures were analyzed on an Orbitrap Fusion mass spectrometer. The peptides were quantified by comparing the intensities of the TMT reporter ions. In the STRING DB analysis of differentially expressed proteins, there were interaction networks that included cell cycle, DNA damage repair and replication, transcription, and translation, which is consistent with transcriptomics data. Upstream analysis of key genes associated with cell cycle checkpoint and DNA repair identified reduced expression of the transcriptional activators E2F1 and E2F2 and increased expression of the transcriptional repressor E2F6, suggesting that TTFields affects the CDK–RB–E2F axis. Furthermore, the combination of TT Fields with various chemotherapy agents that induce replication stress may be an effective way to harness replication stress induced by TT Fields. These novel combinations deserve further exploration in clinical trials.

YS-3

Probing the molecular mechanism of **HuR mediated extracellular export of miRNAs**

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miRNAs, the 22 nucleotide long non-coding RNAs, form miRNP complexes with Argonaute proteins and regulate gene expression by imperfect base pairing to the 3'UTR of target messages. Human ELAV protein HuR is a RNA-binding protein which has strong affinity for AU rich elements (ARE) in the 3'UTR of target mRNAs. It binds with target mRNAs by replacing the miRNPs, stabilizes the mRNAs and facilitates their translation. Therefore, HuR is a negative regulator of miRNA function as it relieves the mRNAs from miRNA-mediated repression.

Exosomes are Extracellular Vesicles (EVs) of 30-90 nm and are secreted by a wide range of cells and contain proteins, RNAs and miRNAs. They help in cell-to-cell communication, by transporting various proteins, mRNAs and miRNAs. HuR can accelerates this Extracellular Vesicles (EVs) mediated export of miRNAs in hepatic and non-hepatic cells. In mammalian cells, HuR replaces miRNPs from target messages, reversibly binds miRNAs and replaces them from Ago2 on endoplasmic reticulum (ER) and subsequently, itself gets freed from respective miRNAs upon ubiquitination on multivesicular bodies (MVBs)[1]. This HuR mediated miRNA export is an important process in controlling metabolic pathways in not only hepatic cells but also found to affect cancer cell proliferation[1, 2]. Interestingly HuR-unloaded miRNAs get exported out via EVs in activated immune cells also and control inflammation[3]. Our recent work has suggested a dual role of HuR in controlling anti-inflammatory cytokine IL-10 expression in M2 polarized macrophages in the infection niche of *Leishmania donovani* infection[4].

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YS-4

Exploring Novel Bioactive Peptide of *Picrorhiza Kurroa* and Its Potential Therapeutic Implications

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Bioactive peptides are low-molecular-weight protein fragments of 2-20 amino acids (<3 kDa) having various pharmaceutical/nutraceutical benefits. In the recent past, bioactive peptides with multifunctional health benefits are the demand of the health sectors. Strikingly, the potential of plant-based peptides has been largely ignored or undervalued. Here, we identify one novel bioactive peptide (BP1) of 13 amino acids from *Picrorhiza kurroa* water-soluble, non-toxic, and non-allergenic. Sequence analysis suggests this peptide has antioxidant potential, angiotensin-converting enzyme, and dipeptidyl peptidase-IV inhibitory activity, which was further validated using molecular docking studies. BP1 significantly reduces the H_2O_2-induced accumulation of intracellular reactive oxygen species and malondialdehyde and activates the intrinsic antioxidant defense system in HEK293 cells. Further, BP1 restores the mitochondrial membrane potential and reverses the apoptotic effect of H_2O_2 in HEK293 Cells. Therefore, BP1 has antioxidant potential, angiotensin-converting enzyme, and dipeptidyl peptidase-IV inhibitory activities that could be used for peptide-based formulation(s) in pharmaceuticals to treat diabetes, cardiovascular and other diseases associated with reactive oxygen species.

YS-5

Denaturation resistant P2 tetramer is required to import fatty acids into intraerythrocytic Plasmodium falciparum

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The initiation of asymmetric karyokinesis of intraerythrocytic Plasmodium falciparum (Pf) begins without dismantling the nuclear envelope showing the hallmark feature of closed mitosis. In Pf,

karyokinesis precedes cytokinesis and cell body formation. Regulation at the beginning of nu- clear division either through checkpoints or by importing serum components was largely unknown. At the trophozoite stage, PfP2 tetramer trafficked to the infected erythrocyte (IE) surface and the inaccessibility of IE surface PfP2 to its bonafide ligand led to the arrest of nuclear division. Here we show that PfP2 tetramer localization on the IE surface and the beginning of nuclear division are concomitant in nature. Synthetically induced denaturation resistant PfP2 tetramer interacts with human serum fatty acids and phospholipids for its import into IEs at the beginning of karyokinesis. In the natively folded denaturation resistant PfP2 tetramer cage, the Cys-Cys redox switch regulates the bind- ing and subsequent release of fatty acids on the IE surface. This mechanistic insight of fatty acids import inside IEs using synthetically induced denaturation resistant PfP2 tetramer provides a unique drug screening platform for novel small molecule development against malaria.

YS-6

Metallic nanoparticle & exosome-based novel delivery of Amphotericin B for reduced toxicity and a possible oral formulation

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Objectives: Amphotericin B (AmB) is the gold standard for treatment of fungal and parasitic diseases but nephrotoxicity and complications related to intravenous injections is unavoidable. The objective of this study is to deliver AmB using conjugated gold naoparticles (GNP) for reduced toxicity and with entrapped milk exosomes for possible oral delivery.

Methods: Citrate-reduced GNPs (~39 nm) were functionalized with lipoic acid (LA), and the product GNP-LA (GL ~46 nm) was covalently conjugated with AmB using carboxyl-to-amine coupling chemistry to produce GNP-LA-AmB (GL-AmB ~48 nm). The nanoparticles were characterized by DLS, TEM, XRD and spectroscopic (ultraviolet-visible and infrared) methods. Experiments on AmB uptake of macrophages, ergosterol depletion of drug-treated parasites, cytokine ELISA, fluorescence anisotropy, flow cytometry, and gene expression studies established efficacy of GL-AmB over standard AmB. Further, we isolated exosomes from fresh milk by differential centrifugation. The exosomal proteins present in pellet were confirmed by SDS-PAGE and LC-MS analysis. Drug entrapment was done with ~3 mg of AmB against ~1 mg of exosomal protein at 37^0C. The size of AmB-loaded exosome is ~70 nm with a zeta potential of -17 mV. Approximately 35% drug release was observed within 12 h in blood plasma and *in vitro* efficacy was compared with standard AmB.

Results: Covalent conjugation AmB with GNP and entrapment with exosome reduced the cytotoxicity and increased the aqueous solubility. Efficiency of AmB conjugation in GL-AmB was ~78% and incubation in serum for 72 h showed <7% AmB release, indicating high stability of conjugate. GL-AmB with AmB equivalents showed ~5-fold enhanced antileishmanial activity compared with AmB against parasite-infected macrophages *ex vivo*. Macrophages treated with GL-AmB showed increased immunostimulatory Th1 (IL-12 and interferon-γ) response compared with standard AmB. In parallel,

AmB uptake was ~5.5 and ~3.7-fold higher for GL-AmB-treated macrophages.The ergosterol content in GL-AmB-treated parasites was ~2-fold reduced compared with AmB-treated parasites. For exosomes, the LC-MS analysis showed the major proteins as lactotransferrin, butyrophilin, annexin and tumor susceptibility factor 101. Approximately ~82% AmB entrapment was observed in AmB-exosome and *in vitro* killing efficacy was ~2 fold higher than AmB alone. Both GL-AmB and AmB-exosome are less cytotoxic and haemolytic than standard AmB.

Conclusion: Metallic nanoparticle and exosome based delivery provides reduced toxicity than standard AmB and cheaper formulation than costly liposomal AmB. Enhanced stability of milk exosome in mucous membrane and gastric juice may provide an oral route of delivery which is an urgent necessity for AmB.

Key words- AmB, GL-AmB, T_h1, Exosome, Oral delivery, ergosterol

YS-7

Identification of the complex molecular signatures behind the differential vulnerability between ALS-resistant and sensitive motor neurons — a multi-omics approach

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Amyotrophic Lateral Sclerosis (ALS) is an adult-onset fatal neurodegenerative disease without a known cure. Only a small fraction of the ALS cases are familial, and no single-gene cause has been identified. ALS is marked by selective loss of spinal motor neurons and denervation of skeletal muscles, eventually leading to paralysis of lateral organs. In contrast, a subset of cranial motor neurons (oculomotor, trochlear, and abducens motor neurons) are resistant allowing patients to retain eye movement until the late stages of the disease. The inability of the cells to respond appropriately to endoplasmic reticulum (ER) stress is thought to be one of the major reasons for ALS progression. To understand the molecular differences between cranial and spinal motor neurons that underlie ALS-sensitivity, we exploited a unique platform that generates highly pure populations of induced cranial and spinal motor neurons (iCrMN and iSpMN). Exposing both iCrMNs and iSpMNs to proteotoxic stress, we monitored transcriptome and proteome changes over 36 hours and identified a superior proteostasis capability of iCrMNs. Moreover, previously unidentified balance of proteasome system has been observed in the two different motoneuron population. The difference, in part, explains the complex behaviour of the two motor neurons during a diseased condiation.

Abstracts

Oral Presentation

OP-1

Epigenetic Regulation of Endothelial Metabolism: Implications in Understanding Metabolic Memory in Type 2 Diabetes

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Despite of lifestyle modifications and anti-diabetic medications, subjects with Type 2 diabetes (T2D) are always at risk of sustained vascular injury and overt to micro and macro vascular diseases which is referred to as 'metabolic memory'. The innermost lining of the vasculature is made up of a monolayer of endothelial cells which play a role in vascular homeostasis and is subjected to metabolic insults during T2D leading to endothelial dysfunction. This vascular damage results in the transition of the quiescent endothelial cells into a proliferative state which may be due to the underlying DNA methylation. Epigenetic mechanisms such as DNA methylation, histone modifications and micro RNAs are associated with T2D pathogenesis and vascular anomalies. DNA methylation is catalysed by a set of DNA methyltransferase isoforms (DNMT1, DNMT3A and DNMT3B). We hypothesize a crosstalk between DNA methylation mediated gene expression and altered metabolism in T2D, which may lead to metabolic memory. Hence, in the present study, we aim to understand the influence of DNA methyltransferases on endothelial metabolism. In response to glucose, we observed significant modulations of DNMT isoforms in endothelial cells. Further, we performed high throughput metabolomics (LC-MS/MS) analysis of the conditioned medium obtained from the DNMTs overexpressing endothelial cells. Data revealed that intermediates of the nucleotide metabolism were elevated in response to DNMT overexpression. Our data indicated that one carbon metabolism was also altered due to high glucose treatment in endothelial cells. Taken together, our data demonstrates a significant influence of overexpression of DNMT isoforms on endothelial metabolism.

OP-2

Quantitative Proteomic Profiling of *Naja Kaouthia* Venom from North-East India by Two Different Analytical Workflows and Assessment of Potency of Commercial Antivenom

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The Indian monocled cobra (*Naja kaouthia*), a category I medically significant snake, belongs to the Eelapidae family. This snake is responsible for a substantial level of morbidity and mortality in North East India (NEI). The quantification of venom proteome of *Naja kaouthia* from NEI was done by computing the relative abundance of toxin families by two different proteomic workflows: (i) 1D SDS-PAGE coupled to label-free quantification, and (ii) RP-HPLC followed by SDS-PAGE analysis to quantification based on area under RP-HPLC curve. The proteomics data obtained from both methods were compared. A total of 32 proteins (toxins) distributed over 10-14 snake venom protein families were identified by proteomic analyses from both workflows. The results of the proteomics analysis obtained from both the venom de-complexation and quantification methods displayed a fair correlation with densitometry band intensity of the crude venom, reinforcing the accuracy of both analytical workflows. Low molecular mass (<15kDa) toxins like three-finger toxins (3FTx) (58.5-64.2%) and phospholipase A_2 (PLA_2) (13.13- 16.0%) comprised the most abundant non-enzymatic and enzymatic proteins identified in NEI NkV. Venom-antivenom cross-reactivity determined by ELISA and Western blotting demonstrated the poor efficacy of the commercial PAVs in recognizing the low molecular weight (<15 kDa) toxins. Spectrofluorometric titration reflected the NEI NkV-specific antibodies in PAVs at a higher level than that previously reported for geographically distant eastern India NkV.

OP-3

Exploring the Translational Protein Expressions using Model Organism *Caenorhabditis Elegans* in Context to Neuro-Immune Response During Bacterial Infection

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The emerging opportunistic foodborne pathogen *Cronobacter sakazakii* causes life-threatening infections of meningitis, necrotizing enterocolitis, sepsis, and meningo-encephalitis, most predominantly in neonates, infants, and immunocompromised patients, with a relatively high mortality rate. In our study, *C. elegans* infected with the above pathogen have been analyzed primarily at the translational level, especially profiling the differentially regulated proteins involved in neuro-immune signaling pathways. The LC-MS/MS analysis of control and infected *C. elegans* protein samples helped us to identify a total of 784 proteins and they were analyzed using the STRING tool for understanding the protein-protein interaction from both control and infected proteins. Two abundantly expressed proteins (*let-363* and *acox-1.4*) were identified through KEGG pathway analysis (Human orthologue). *C. sakazakii* induced neuronal signaling pathway proteins in host, such as mTOR/ Axon Regeneration (*let-363),* Calcium signaling (*mlck-1*) and Longevity regulating pathways (*ddl-2*). Functional bioinformatics analysis revealed that identified proteins were mostly involved in cellular process, translation, autophagy, metabolic process, nucleic acid binding, and regulation of the actin cytoskeleton. Meanwhile, Peroxisome (*acox-1.4*) pathway protein was also upregulated, which plays a key role in redox signaling and neuronal inflammation. The Real Time qPCR analysis confirmed the elevated mRNA levels of *let-363, acox-1.4, mlck-1*, and *ddl-2* during the infection. DNA damage due to *C. sakazakii* infection in host performed with Propidium Iodide staining that suggested the involvement of the mTOR pathway led apoptosis. The loss of function of *let-363* showed the impact of the pathogen virulence in VC2312 (*let-363*) and BQ1 (*akt-1*) mutants through Lifespan, pharyngeal pumping and egg laying assays. Also, the *daf-2* (an upstream activator to *let-363*) regulated the expression of the axon developmental process was assessed with the using otIs117 [*unc-33*p::GFP+ *unc-4*(+)] IV transgenic strain. The present study found that the *C. sakazakii* infection impacted host neuro-immune signaling pathways that may have altered neuronal signaling and regulated several immune response proteins during the pathogen interaction.

Keywords: Host-Pathogen Interaction; Cronobacter sakazakii; Neuro-immune signalling pathways.

OP-4

Interaction of Cyclophilin with α-Synuclein

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Cyclophilin A. belonging to the immunophilin family, is known for its Peptide-Proline isomerase (PPIase) activities (catalysing cis-trans isomerization of Prolin)[1]. The protein also plays a key role in various neurodegenerative diseases including Alzheimer's disease, ALS and Parkinson's disease[2]. Previous studies showed that CyclophilinA interacts with the prolin-rich C-terminal end of the α-synuclein[4]. NMR investigation alsofound that Cyclophilin A (CypA) catalyses isomerization of proline 128 in the C-terminal domain of α-synuclein. Our study mainly focuses on interaction of Cyclophilin with α-synuclein and with its two other mutants S129A and S129W. Our previous studies have shown both S129A (that essentially replaces the polar side chain with a nonpolar methyl group) and S129W (tryptophan being a hydrophobic, helix promoting residue) shows reduced aggregation propensity and reduction in cytotoxicity when compared to wild α-synuclein. This study focuses on the effect of Serine 129 mutations on Cyclophilin protein's interaction with the Prolin in 128th position of α-synuclein. The S129 in alpha synuclein is known for its post transcriptional modifications and change in that may affect the interaction of the protein with Cyclophilin. We have primarily focused on multiple computational methods to understand the interaction. Molecular docking and molecular dynamics simulation are done to study the interactions and structural flexibility. The effect on the rate of fibrilization is also studied via computational studies. Biophysical studies including ITC, ThT assay and Circular spectroscopy are done to confirm these findings.

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OP-5

Identification and Functional Characterization of Candidate Biomarkers for Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is a malignant clonal disorder characterized by alterations and accumulations of immature cells known as blasts in bone marrow, blood and other tissues. This condition leads to reduction in number of mature blood cells which severely affects its normal functioning. Cytogenetic markers are the only means for diagnosis and assessing risk classification and treatment of AML. Our study aims to identify candidate biomarkers for AML progression and chemoresistance from clinical samples and cell line models by performing multipronged quantitative proteomic analysis and functional assays through various molecular and cell biology approaches. In our current study we performed label free quantitative proteomic analysis of bio fluids such as bone marrow interstitial fluid and serum, and bone marrow mononuclear cells from AML and respected control clinical samples. The quantitative proteomics of biological fluids like serum and bone marrow interstitial fluid led to the identification of candidate biomarkers viz. SAA1, FGG, CD44, APOB, APOE, and PF4 which can be used as potential diagnostic biomarkers for AML. In addition, label free quantitative proteomics of bone marrow mononuclear cells identified new targets namely CDC5L, ILF2, SUB1, CALM3 and C1QBP which can be novel targets for AML. Further, we identified potential therapeutic targets associated with AML Cytarabine resistance namely CALM3, C1QBP and DBI by comparing proteome changes in drug resistance and sensitive AML cell lines. In conclusion, the identified candidate markers/targets will be not only useful for diagnosis and therapeutics but also helpful in deep understanding of the molecular insights of AML.

OP-6

Plasma proteomic study reveals involvement of GALECTIN3-DECTIN1 mediated activation of inflammation in atherosclerosis

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Non ST-segment Myocardial Infarction (NSTEMI) patients, lacking the declarative ECG, like STEMI patients, comprise >70% of all Acute Coronary Syndrome. In major two third of the cases, the

pathophysiology includes, disruption of atherosclerotic plaque, with myocardial ischemia. Remaining one third is caused by superficial plaque erosion resulting in platelet activation and thrombus formation. Plasma profiling of NSTEMI patients revealed >800 proteins expressed uniquely, in compared to control samples, including GALECTIN3. Using pathway enrichment analysis of these leads, several relevant pathways have been identified. Among these, DECTIN1 mediated signaling pathway involving GALECTIN3 shows a high z score and low p value (<0.005). In vitro and in silico studies demonstrated that GALECTIN3-DECTIN1 interact and activate Ras/Raf1/NFκβ pathway to secret proinflammatory cytokine (TNFα). The current study provides an insight about how a chronic state of inflammation is maintained in NSTEMI patients that promotes the disease progression.

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OP-7

Exosomal Protein Marker for Early Prediction of Dengue Severity in the Pediatric Population

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Dengue virus is a positively-sensed RNA virus transmitted by *Aedes aegypti* and *Aedes albopictus* causing dengue infections in humans. There are four serotypes of dengue virus differing in their antigenicity. Based on WHO classification, it is classified as dengue without warning sign, dengue with warning sign, and severe dengue. Dengue infection affects the lungs, liver, and other organs. Approximately, 230 million cases of dengue are reported annually across the globe. In 2020, around 2 lakh cases of dengue with a 1-5% mortality rate were reported in India. Early prediction of dengue severity may help in reducing the mortality rate. To date, there are no biomarkers available for assessing dengue severity. Therefore, the present study aims to identify the exosomal protein alteration in the different dengue categories to find biomarkers for early prediction of dengue severity. The study includes exosomal proteomics as exosomes play a major role in intracellular communication, furthermore transport parent cell cargo to neighboring cells. We have collected 6 plasma samples from each dengue category as well as from healthy individuals. Exosomes were isolated and characterized using various molecular biology techniques. After characterization, proteins were isolated and labeled-free quantitative proteomics was performed using mass spectrometry. Comparative data analysis within groups was performed which resulted in 14 upregulated and 9 downregulated proteins having a 2-fold change in terms of their expression. The present study suggested that the identified proteins could be used as potential molecular biomarkers for early prediction of dengue severity in the pediatric population.

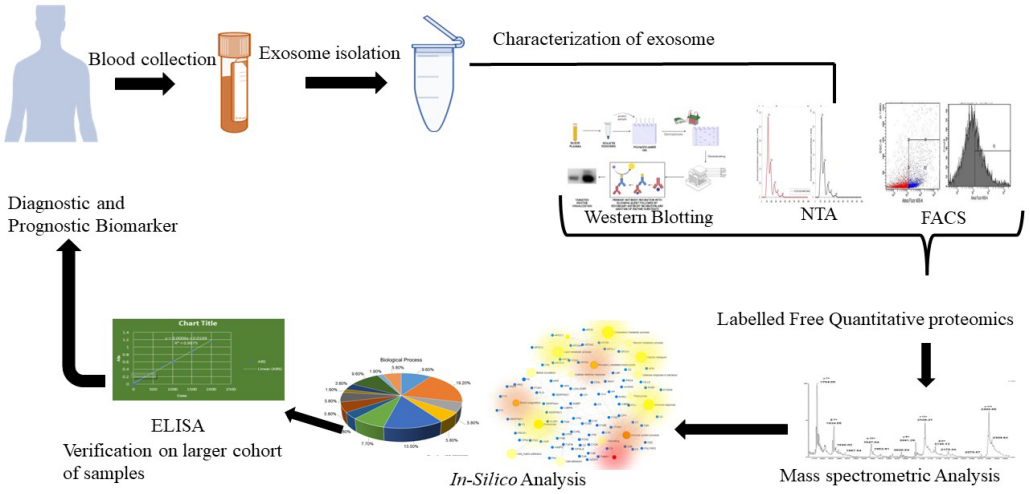

Figure 1: Graphical abstract of the overview of the methodology

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OP-8

A reliable scoring method to control false positives in variant proteogenomics

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The importance of single nucleotide polymorphisms (SNPs) is well known in diseases but we understand little in terms of their translation to form single amino acid polymorphism (SAP or variant) since all SNPs are not observed as SAPs in the corresponding proteins. Identification of novel and variant peptides using proteogenomics has paved the way to understand their unique and diverse

phenotypic relationships in health and disease. Proteogenomic studies on cancer, neurological and cardiovascular diseases have revealed many variants of clinical importance. However, a major challenge in proteogenomics is to segregate the true from false variant hits. Some approaches have been suggested to improve their identification, such as group-specific FDR estimation and workflows for better sensitivity. Implementation of these methods and workflows on identified results is quite complex and requires advanced bioinformatics skills. We analyzed many PSM descriptors capable of distinguishing true variants from false hits in MS/MS identifications. These descriptors pertain to spectral quality, mass spectrometry features, and search scores. We used a high-quality mass spectrometry dataset from the PRIDE database (PXD004010) containing 1.7 million spectra to test the features and develop an empirical-scoring which can control the false positives. We manually annotated the spectra to validate our scoring method and found that our method was able to detect the false hits and provide a better segregation of PSMs. We have also observed that in any variant proteogenomic mass spectrometry search, the rate of true variant identification may be around or less than 50%. Our scoring approach can reliably help to validate variant identifications and provide researchers with confidence to prioritize these variants for further studies.

OP-9

Paternal vitamin B12 status and sex-specific cardiometabolic health disparities in offspring

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Vitamin B12 is a micronutrient, available to us only from dietary sources. Maternal deficiency of vitamin B12 in-utero is known to increase the risk of cardiometabolic diseases in the next generation through epigenetic mechanisms. However, effect of paternal nutritional status on offspring have often been ignored. Paternal diet prior to mating also has the potential to influence offspring health through genomic imprinting. Thus, it is important to understand its implication in next generations. We developed a Wistar rat model with paternal B12 deficiency and studied the intergenerational effects of this deficiency employing biochemical, proteomic and metabolomic approaches. Male rodents were fed B12 restricted diet for 3 months, after confirming the vitamin deficiency (~78%) they were mated with females on control diet. The anthropometric and biochemical parameters were measured in the offspring at 3 and 12 months in F1 generations. Furthermore, to understand the altered processes and pathways due to deficiency, SWATH-MS based proteomics and untargeted metabolomics studies were performed in liver and skeletal muscle tissue of the offspring. Offspring born to B12 deficient male show altered plasma lipid and biochemical profile. Liver and skeletal muscle proteomics and metabolomics data indicates towards a sex-specific alterations in offspring where metabolic pathways like steroid hormone biosynthesis, amino acids, lipid and glucose metabolism are

dysregulated in F1 males while female offspring show no such changes. PPAR expression and signaling pathways were altered in a sex-specific fashion providing a probable explanation of sex-specific omics and phenotypic changes predisposing males to cardiometabolic risk in F1 generation.

OP-10

Integrated multi-omics approach decipher the role of extracellular matrix-nucleus signalling in rice blast disease

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Counting on an immune system comes with a high energetic cost for plants therefore defense responses are highly regulated and fine-tuned. At the organellar level, signalling is essential for coordinating the activities of different organelles in mounting defense response. The extracellular matrix (ECM) and nucleus both independently play a pertinent role in regulating plant defense. ECM acts as a physical scaffold that allows recognition and prevents the entry of phytopathogens while nucleus is the regulatory hub. Rice blast caused by *Magnaporthe oryzae*, is one of the most devastating diseases that adversely affect rice productivity. The role of inter-organellar signalling in response to *M. oryzae* remains unknown. Temporal ECM and nuclear proteome and phosphoproteome analysis of blast-resistant rice genotypes were carried out using isobaric labeling and TiO_2-based phosphopeptide enrichment followed by reverse phase and SCX fractionation and MS/MS analysis. In total, 315 ECM localized and 363 nuclear immune responsive proteins (IRPs) were identified. Of which 20 IRPs were found to be involved in calcium, oxylipin, eATP, hormone, and kinase signaling, which might play a putative role in inter-organellar signalling. The phosphorylation status of ECM and nuclear proteins depicted that 25 and 17 phosphoproteins were expressed differentially, respectively. Further, GC-MS-based metabolite profiling identified 109 metabolites with altered abundance. Data highlighted that phenyl propanoid, pinitol, lysine catabolism, and unsaturated fatty acid pathways might play a link between the two organelles. Proteoform and metabolome data were interrogated using correlation network analysis that identified significant functional modules pointing toward inter-organellar signalling during *Magnaporthe* infection.

Abstracts

Poster Presentation

PP-1

Fap65 is a Ciliary A-Kinase Anchoring Protein from *Chlamydomonas Reinhardtii* with a Role in Primary Ciliary Dyskinesia

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Motility is one of the most important biological phenomena observed in organisms that have cilia or flagella. This is an ATP-driven process; however, the mechanism of motility remains elusive. Several laboratories have been investigating the role of signaling/scaffold proteins towards dissecting this phenomenon. We have used MycBP-1 orthologue (FAP174) from the green chlorophyte, *C. reinhardtii*, as bait to pull down several protein molecules that are hypothesized to play a direct role in motility. These protein molecules localize in two projections of the central pair apparatus (C2a and C1b) of the cilia [1]. In order to characterize each of these protein partners we have investigated the direct binding domains between FAP174 and an A-kinase anchoring protein (FAP65) with two amphipathic helices (CrFAP65-AH1) and (CrFAP65-AH2). In order to study the binding kinetics between these two protein partners we have cloned the genes, overexpressed, and purified them to homogeneity. However, due to inclusion body formation, the amphipathic helices posed a serious problem during purification. Hence, a systematic approach to purify these inclusion body proteins from *E. coli* cells was developed. Interactions using overlay and SPR were studied which helped us not only to ascertain the binding but also the affinity by determining its equilibrium dissociation constants. In conclusion, the interaction studies have shown that the N-terminus of FAP174 binds to the amphipathic helices of an AKAP (FAP65) which also displays microtubule binding property. This indicates that FAP65 is an important signaling scaffold present in the central pair of *C. reinhardtii* axoneme.

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PP-2

In silico Analysis to Identify Novel lncRNA-miRNA-mRNA Regulatory Axes Associated with Gallbladder Cancer

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Competitive endogenous RNA (ceRNA) networks are reported to play a crucial role in regulating the cancer associated genes in cancer. However, there is limited information available on these networks in gallbladder cancer (GBC). Identification of novel ceRNA networks in GBC may improve the understanding of its pathogenesis and might serve as diagnostic markers or useful for therapeutic applications. For this, a literature survey was done to identify differentially expressed lncRNAs (DELs), miRNAs (DEMs), mRNAs (DEGs) and proteins (DEPs) in GBC. Ingenuity pathway analysis (IPA) using DEMs, DEGs and DEPs in GBC identified 242 experimentally observed miRNA-mRNA interactions with 183 targets. Pathway analysis using these targets revealed p53 signaling among the top pathway. Protein-protein interaction (PPI) analysis using STRING database and cytoHubba plug-in of Cytoscape software revealed 5 hub molecules, of which 3 of them (TP53, CCND1 and CTNNB1) were associated with p53 signaling pathway. Further, using Diana tools and Cytoscape software, we found novel lncRNA-miRNA-mRNA regulatory networks involving TP53, CCND1, CTNNB1. These regulatory networks may be experimentally validated in GBC and explored for diagnostic and therapeutic applications.

Keywords: Gallbladder cancer, lncRNA-miRNA-mRNA regulatory axes, p53

PP-3

Quantitative Tissue Proteome analysis of Lymph Node Metastatic Gallbladder Carcinoma

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Lymph node (LN) metastasis is the earliest sign of metastatic spread and an established predictor of poor outcome in gallbladder cancer (GBC). Patients with LN positive GBC have a significantly worse survival than patients with LN negative disease in spite of standard treatment which includes extended surgery followed by chemotherapy, radiotherapy and targeted therapy. Therefore, effective targeted therapy for LN positive GBC is an urgent need for the improved survival of these patients. In the present study, we used iTRAQ-based quantitative proteomic analysis using tissue cohort comprising of primary tumor of LN negative GBC, LN positive GBC and controls (Gallstone disease), to identify proteins associated with lymph node metastasis. A total of 58 differentially expressed proteins (DEPs) were found to be specifically associated with LN positive GBC based on the criteria of p value ≤ 0.05, fold change ≥ 2 and unique peptides ≥ 2. These include the cytoskeleton and associated proteins such as keratin, type II cytoskeletal 7 (KRT7), keratin type I cytoskeletal 19 (KRT19), vimentin (VIM), sorcin (SRI) and nuclear proteins such as nucleophosmin Isoform 1 (NPM1), heterogeneous nuclear ribonucleoproteins A2/B1 isoform X1 (HNRNPA2B1), which are already been reported to be involved in promoting invasion and metastasis. Bioinformatics analysis of the deregulated proteins in LN positive GBC using STRING database identified 'neutrophil degranulation' and 'HIF1 activation' to be among the top deregulated pathways. Western blot analysis further confirmed the overexpression of KRT7, KRT19, SRI and NPM1 in LN positive GBC in comparison to LN negative GBC. These proteins may be further analyzed in large cohort of samples and explored for their therapeutic applications in LN positive GBC.

PP-4

Metabolic Reprogramming in Type 2 Diabetes Associated Infections

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Immune cell functioning and metabolism are inter-dependently regulated and form an immuno-metabolic axis. Metabolic disorders including Type 2 Diabetes (T2D) display chronic inflammatory milieu characterized by constitutively activated innate immune cells including neutrophils and elevated pro-inflammatory mediators. This leads to significant alterations in immuno-metabolic axis in T2D resulting in malfunctioning of immune cells (example: neutrophils) and consequently leading to recurrent infections. Earlier studies in our lab have demonstrated hyperglycaemia in T2D subjects induced metabolic reprograming in neutrophils which led to insufficient pools of NADPH and resulted in weaker response to infections. Hence, in this study using rodent models of T2D and sepsis, we aimed to understand dynamics and cross talk between a) inflammatory mediators responsible for granulopoiesis and b) systemic metabolism. We developed T2D rodent models upon feeding high fat diet for 4 months and sepsis was induced by cecal ligation and puncture. In these mouse models we observed significant elevation of G-CSF, IL-6, TNF-, IL-13 and CXCL2 in sepsis conditions and substantial reduction in T2D with sepsis. Untargeted LC-MS/MS data revealed significant separation of study groups by principal component analysis indicating differential metabolism in T2D and infection (sepsis). Further analysis revealed lipid metabolism was significantly modulated in T2D and Sepsis models. Our data provides preliminary findings to understand cross talk between inflammation and metabolism.

PP-5

Neutrophils Respond Uniquely to Various Pathological Stimuli by Activating Distinct Signaling Pathways

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Neutrophils are the most profuse cell types of innate immunity and exhibit significant functional adaptivity and phenotypic heterogeneity in response to a variety of physiological and pathological stimuli. In addition to their active role in eliminating pathogens, exacerbated functioning of neutrophils leads to several tissue damage and thrombosis and contributes to sterile inflammation in several diseases including Type 2 diabetes (T2D). In T2D subjects, altered immuno-metabolic axis due to hyperglycemia and increased homocysteine level leads to functional impairment of neutrophils contributing to recurrent infections and activating platelets resulting in thrombotic events. Hence, in the context of T2D, inhibiting constitutive NETs formation and restoring neutrophil functions may serve as a therapeutic approach to prevent recurrent infection in diabetic subjects. This instigated us to explore phosphoproteome of activated neutrophils by distinct stimuli such as high glucose, LPS and homocysteine representing hyperglycemia, infection and thrombosis persuaders respectively. Quantitative phosphoproteomics by LC-MS/MS revealed that high glucose activated EGFR, SYK, and CSNK2A kinases associated with adaptive immune receptor signaling. Whereas homocysteine induced AKT, LRRK2 and SRC are involved in phagocytosis and production of cytokines. NTRK1, CDK1and MAPK3 kinases activated by LPS induction are concerned with platelet aggregation and cytokine Signaling. Further, we validated the phosphorylation level of C-JUN Kinase, ERK and AKT in neutrophils activated by these inducers by immunoblotting and observed varied effects of inducers on the phosphorylation levels of these kinases. In conclusion, our study might facilitate targeting neutrophil function/pathways to restore its functions and enables a better response to infection in T2D.

PP-6

An Insight into Exosome Proteome in Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a life-threatening condition with a high rate of morbidity and mortality, with progression of PAH regulated at a cellular level. Recently, the role of nano-sized exosome has been found to be important mediators of intercellular and extracellular communication for various physiological processes, responsible for the progression of PAH. Exercise plays an important role in improving function in pulmonary hypertension. Although the signaling pathways that regulate cardio-protection through exercise have not been fully understood, the positive impact of exercise on the various physiological systems are well established. The release of exosome into the circulation with exercise, may contribute to exercise-related adaptive systemic signaling. Specific changes in circulating exosome content could be used as exercise efficacy biomarkers. Exosomes are released more readily because of exercise than because of changes in vesicle size. The preliminary work by our group found clinical benefits with exercise training in PAH. Hence in this study we created monocrotaline treated rodent model (6 male rats for each 4 groups, age 8-12 weeks and Body weight range at 216.0 g to 295.0 g) to understand the effects of exercise on changes in the exosome proteome composition in an animal model of PAH. Mass spectrometry based quantitative proteomics analysis for exosome indicated modulation of VCAM1, APOE, GSN, and SERPINA1 in PAH. These proteins also participate in crucial pathways such as mTORC1 signaling, angiogenesis, coagulation, and epithelial-mesenchymal transition. In conclusion our study shows exosome proteome changes in PAH due to exercise.

PP-7

Protein Homocysteinylation: A Potent Mechanism for Immune Activation and Implications in Pathogenesis of Stroke

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Homocysteine (Hcy), an intermediate metabolite of the methionine pathway with clinical significance in the development of vascular and neurodegenerative disorders. The excess level of homocysteine termed hyperhomocysteinemia enhances oxidative stress, induction of protein modification, formation of reactive oxidation species, and extracellular matrix alteration. N-protein homocysteinylation an error editing mechanism of Hcy plays a pivotal role in the pathogenesis of cerebro -cardiovascular diseases with protein damage, aggregation, and autoimmune response. Neutrophils play a central role in innate immune response and are involved actively in the immune regulation, and pathogen clearance with the web-like chromatin structures called extracellular traps (NETs). A previous study from our lab showed the bidirectional activation of neutrophils and platelets in response to homocysteine. In the current study, we aimed to understand the effect of homocysteine and homocystienylated proteins on neutrophil activation using *in vitro, in vivo*, and clinically characterized ischemic stroke subjects. Significant formation of NETs by the homocysteinylated proteins was observed by fluorescence microscopy and spectrometric analysis. These NETs induced platelet aggregation. *In-vivo* hyper-homocysteine mice model established by high methionine diet showed elevated NETs components and platelet aggregation. Mass spectrometry and HPLC analysis revealed elevated homocysteine, homocysteinylation of albumin and autoantibodies against homocysteine in the clinically characterized stroke samples which correlated to neutrophil elastase and cell free DNA, indicating influence of homocysteine on neutrophil functioning in stroke pathogenesis.

PP-8

Identification of molecular players maintaining stemness in Ovarian cancer

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Serum is an inevitable component for the maintenance of proliferating cells. However, it is an artifact as far as cells' in-situ behaviour is considered, where cells are not proliferating constantly. Moreover,

serum free media is a well-established approach to maintain stem cells in-vitro and influence cellular plasticity, invasiveness etc in several cancers. Here we have subjected HGSC cell lines to serum starved condition as a surrogate for CSCs' maintenance. MaxQuant-Label free quantification (LFQ) was used in a panel of HGSC cell lines grown in the presence (+S) and absence (SS) of serum hoping that the candidates could be studied further for targeted therapies for CSCs in pan-ovarian cancer. The panel of HGSC cell lines used were representing Epithelial (E), intermediate Epithelial (iE), Epithelial/Mesenchymal hybrid (E/M), intermediate Mesenchymal (iM) and Mesenchymal (M) phenotypes which were stratified according to Tcf21/Slug expression earlier in the lab. Interestingly, LFQ based analysis showed a significant enrichment of CSC related molecular pathways in SS samples over the +S controls. Surprisingly, despite of the molecular architecture differences that they possess, all the HGSC SS samples showed an enrichment of mitochondria related pathways and proteins, in SS samples and we could experimentally validate the involvement of mitochondria under the serum starvation. It is interesting that recent studies support our findings and the putative role of mitochondria in CSC maintenance. Further, we are trying to hamper the mitochondrial function using antibiotics and ETC inhibitors to target CSCs and to rule out the chances of relapse.

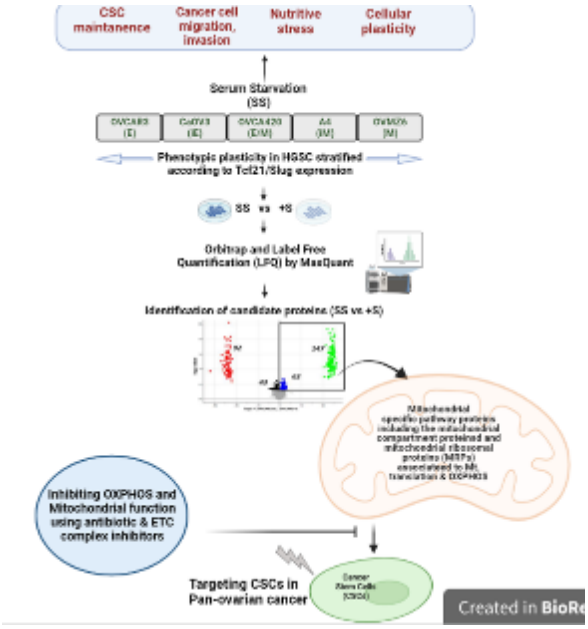

Figure 1: Graphical abstract

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PP-9

Comprehensive Mapping and Dynamics of Site-Specific Prolyl-Hydroxylation, Lysyl-Hydroxylation and Lysyl O-Glycosylation of Collagens Deposited in ECM During Zebrafish Heart Regeneration

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Cardiac fibrosis underlies the major forms of cardiovascular diseases. Cardiac fibrosis results from dysregulated extracellular matrix (ECM) remodeling. ECM remodeling is an important healing response, and it protects the myocardium from ventricular rupture. However, in mammals, the fibrotic response progresses toward heart failure instead of regeneration. Interestingly, zebrafish have a unique capability to regenerate the myocardium following an injury through extensive ECM remodeling. Collagens, the most abundant components of ECM play a significant role in the assembly and cell-matrix interaction. However, the exact mechanism of ECM remodeling leading to zebrafish heart regeneration is not well understood. Collagens are heavily modified post-translationally modified and their role has been implicated in several diseases. The specific sites of collagen PTMs and their dynamics during heart regeneration were not known. We utilized an in-house pipeline to analyse ECM proteomics datasets (PXD011627, PXD010092) of the zebrafish heart during regeneration (post-amputation). We have identified a total of 36 collagen chains (19 are reported for the first time here) harbouring a total of 95 prolyl-3-hydroxylation, 108 hydroxylysine, 29 galactosyl-hydroxylysine, and 128 glucosylgalactosyl-hydroxylysine sites. Furthermore, we comprehensively map the three chains (COL1A1a, COL1A1b, and COL1A2) of collagen I, the most abundant protein in zebrafish heart ECM. We detected oscillations in the dynamics of prolyl-3-hydroxylation occupancy and lysine-O-glycosylation microheterogeneity during heart regeneration. Moreover, we also detected a significant ($p < 0.05$) elevation of site-specific (K^{1017}) glucosylgalactosyl-hydroxylysine on the COL1A1a chain. These results of the alteration of dynamics of collagen PTMs during heart regeneration will open up new avenues to decode ECM remodeling and may lay the foundation to tinker the cardiac regeneration process with new approaches [1].

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PP-10

Comprehensive Organ-specific Proteome, Phosphoproteome, and Kinome Maps of *Drosophila*

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Drosophila, a simple multicellular model system, is an attractive choice for studying human diseases and drug effects due to its low maintenance costs, short generation time, and easy genetic manipulations. Notably, the *Drosophila* genome is 60% homologous to humans, and about 75% of the genes responsible for human diseases have homologs in fruit flies [1]. The diversity of protein expression and functions accounts for the multidimensional biological intricacy observed in most multicellular organisms, including *Drosophila* [2]. Kinase activities and signaling networks stimulate various disease-related pathways [3,4]. There is a dearth of information regarding the phosphoproteome patterns, signaling networks, and kinases in *Drosophila*. In this study, we inclusively searched gel-based and shotgun proteomics literature on *Drosophila* to build comprehensive organ (tissue)- and cell-type specific proteome and phosphoproteome maps (Fig. 1). Combining information from numerous articles and prominent databases, we cataloged 29399 proteins and 14809 phosphoproteins in *Drosophila*. The identified proteome and phosphoproteome were segregated further in an organ or tissue-specific manner along with different developmental stages and cell lines. We identified 1332 kinases and 444 phosphatases in *Drosophila,* which involve in diverse vital physiological pathways. Interactions among the identified kinases and phosphatases were further analyzed using various bioinformatics platforms, including STRING and NetworKIN tools. Additionally, orthologs for these identified kinases and phosphatases were searched in humans and mice. We anticipate that this study will help to understand the proteome organization and signaling regulations in *Drosophila* more pronouncedly.

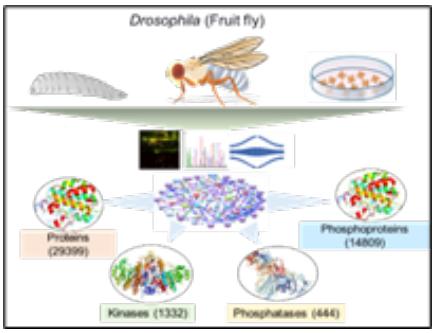

Figure 1: Comprehensive proteome map of *Drosophila* constructed from published literature

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PP-11

Systems-level Analysis of Aging Markers and Rhythmicity of Anti-aging Drug Targets for Potential Health and Therapeutic Benefits

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Circadian clocks have pronounced impacts on human health as they play a cardinal role in regulating our daily physiological and behavioral processes. Aging causes circadian disruptions and may predispose to many chronic diseases. Therefore, it is crucial to investigate the molecular mechanism and markers of aging and its effects on circadian dysregulations. In this study, we performed an *in-silico* analysis with a circadian perspective to identify aging markers and pharmacological targets for aging attenuation (Fig. 1). We constructed a comprehensive map of aging markers in humans and their homologs in mice and *Drosophila* from multiple databases (Human Ageing Genomic Resources, Aging Atlas, and Digital Aging Atlas)[1–3]. Using DAVID, we conducted a functional analysis of the aging genes and pharmacological targets to map the enriched pathways (FDR < 0.01). The most statistically significant and enriched pathways for aging-related genes include tyrosine receptor signaling, cytokine signaling, platelet activation, FOXO mediated transcription, signaling by leptin, fatty acid and lipid metabolism (Fig. 1). We investigated the anti-aging drugs and their targets using information from DrugAge and Aging Atlas databases. We investigated the rhythmic expression patterns of the drug targets in CircaDB at the transcriptome level[4]. We found 141 and 94 drug targets with robust rhythmic

expression patterns (Period 24±3 hr, JTKQ < 0.1) in mice and humans, respectively. The time-customized treatments point toward the possibilities of chrono-pharmacological approaches in geriatric medicine. This study will enrich our understanding of the critical connections between daily rhythms and aging.

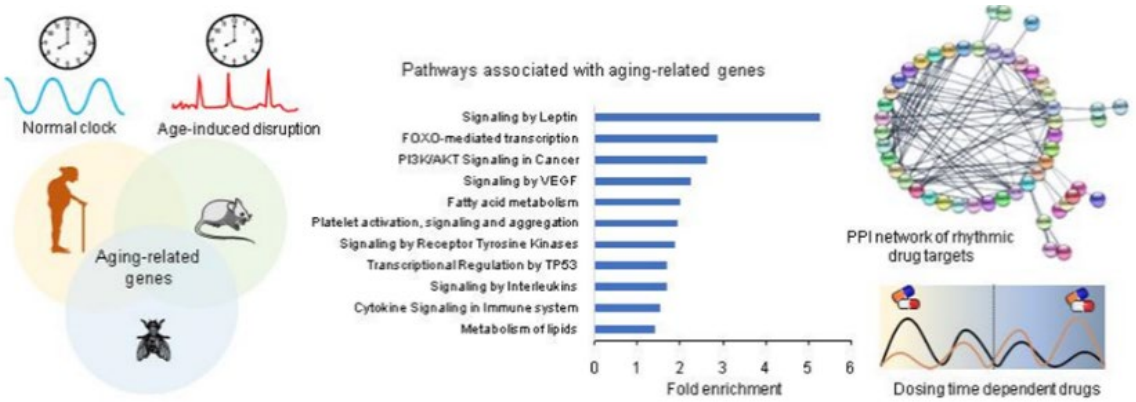

Figure 1. Systems-level analysis to study circadian associations with anti-aging drug targets and aging markers

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PP-12

Genome Mining Unravels Genome Streamlining and Natural Products Biosynthesis Diversity on Hapalosiphonaceae Family

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In hotspring microbial mats, thermophilic cyanobacteria carry out primary production and nitrogen fixation. Though studies have been done on how these thermophiles have evolved and adapted to high temperatures, notably in the unicellular *Synechococcus* species, there is little knowledge on the multicellular filamentous cyanobacteria. Hotspring organisms made up the majority of the species in the true branching cyanobacterial family Hapalosiphonaceae, whereas only a handful were terrestrial. Here, the present study is focussed on *Mastigocladus laminosus* UU774, an Indian thermophile isolated from Eastern state Orissa, whose genome is compared to the genomes of other Hapalosiphonaceae members. Comparative genomics investigation revealed the categorization of 44 strains into two distinct groups: one group is formed by the small-sized hyperthermophiles *Fischerella thermalis* species (ANI and AAI >90%) emanating from hot springs in USA, New Zealand and Japan, while the other group consisted of larger genome-sized moderate thermophiles and terrestrial organisms from various genera of *Mastigocladus*, *Hapalosiphon*, *Fischerella* and *Westiellopsis* (ANI and AAI >80% to <97%). According to the results of our pan-genome mining for genes encoding secondary metabolites, the distribution of biosynthetic gene clusters (BGCs) follows species phylogeny. *Fischerella thermalis* strains that are more thermophilic have fewer secondary metabolite gene clusters than mesophilic strains, which have a greater diversity of chemicals. These findings shed light on the genomic adaptability and evolution of Hapalosiphonaceae strains, where higher temperature organisms undergo genome streamlining, including genome size reduction and the evolution of metabolite genes.

PP-13

Reversal of Paraquat-Induced Apoptosis, Neurodegeneration, and Alteration of Metabolic Pathway Genes in the Rat Pheochromocytoma PC-12 Cell by Custom Peptides Developed from the Nerve Growth Factors from Snake Venoms

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Neurodegenerative disorders (ND), associated with the progressive loss of neurons, oxidative stress-mediated production of reactive oxygen species (ROS), and mitochondrial dysfunction, are significant health concerns. In recent years, synthetic biomaterials, for example, peptides possessing innate neurotrophic effects and enhanced neuroprotective activity, have found a profound interest as drug prototypes for the treatment of ND. We developed two small synthetic peptides from the nerve growth factors from snake venoms for treating ND. Computational analysis predicted significant interaction of peptides with the human TrkA receptor (TrkA), which was verified by *in vitro* binding study with rat pheochromocytoma PC-12 cells TrkA receptor to induce neuritogenesis. The pre-treatment of PC-12 cells with peptides significantly reduced paraquat (PT)-induced cellular toxicity and intracellular ROS production, prevented alteration of mitochondrial transmembrane potential (ΔΨm) and ATP production, and inhibited cellular apoptosis. These peptides lack adverse pharmacological effects in in vitro conditions. Functional proteomics analyses demonstrated the reversal of PT-induced upregulated and downregulated metabolic pathway genes in PC-12 cells pre-treated with these peptides, which was also validated by qRT-PCR analysis of critical pro-apoptotic and anti-apoptotic genes. Our study also deciphered the metabolic pathways regulated by these peptides to induce neuritogenesis and counteract the PT-induced neuronal damage in PC-12 cells. A network of gene expression profiles was proposed to understand the molecular interactions among the regulatory proteins to salvage the PT-induced damage by HNP. In a nutshell, the neuroprotective peptides developed in this study hold ample opportunity for creating neuroprotective drugs.

Keywords: Neurodegeneration; Parkinson's disease model; Peptide drug prototype; Proteomic analysis

PP-14

A Comparative Study of Proteome Composition of Indian Red Scorpion Venom with Other Scorpion Venom Proteomes: Failure of Commercial Antivenom to Immunerecognize the Abundance of Low Molecular Mass Toxins of Venom

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The Indian red scorpion (*Mesobuthus tamulus*), with its life-threatening sting, is the world's most dangerous scorpion species. The toxinome composition of *M. tamulus* venom (MTV) was determined by tandem mass spectrometry (MS) analysis of venom protein bands separated by SDS-PAGE. A total of 110 venom toxins were identified from searching the MS data against the Buthidae family (taxid: 6855) of toxin entries in non-redundant protein databases. The most abundant toxins of MTV are the Na^+ and K^+ ion channel toxins (76.7%), together giving rise to the neurotoxic nature of this venom. The other minor toxin classes in MTV 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%). Notably, the venom proteome composition of MTV was compared the proteome composition of *M.martensii* venom, which is the most prevalent scorpion species in eastern Asian countries. MTV does not show enzymatic activity (phospholipase A_2, L-amino acid oxidase, adenosine tri-, di-, and monophosphatase, hyaluronidase, metalloproteinase, and fibrinogenolytic), in vitro hemolytic activity, interference with blood coagulation, or platelet modulation properties. The abundant low molecular mass toxins (3−15 kDa) of MTV responsible for exerting the major pharmacological effects are poorly immune-recognized by commercial scorpion antivenom. This is a major concern for the development of effective antivenom therapy against scorpion stings.

PP-15

Crystal Structure Guided Extensive Study of Allosteric Ligand Binding Pocket Mutants of *Vibrio cholerae* HapR Leading to Rational Design and Computational Analysis for Probable Inhibitors

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Vibrio choleare HapR, a member of the TetR family of regulatory proteins, has been given the status of high cell density master regulator among the quorum sensing regulatory proteins (Ball et al., 2017). Being a master regulator, HapR controls a plethora of disparate cellular events in Vibrios. Despite being similar in its structure and function with other LuxR homologous in Vibrio species, HapR possess several differences in its putative ligand binding pocket (De Silva et al., 2007). As a preventive measure, these changes in the residues made the protein escape the effect of several inhibitors, specific for that allosteric site. However, these inhibitors, such as thiophenesulfonamide derivatives, worked efficiently against several other TetR family proteins (Kim et al., 2013). To decipher this inertness of HapR, step wise mutation of different pocket residues of the native HapR was done and their crystal structure was solved to do extensive structural analysis. Mimicking these pocket residues to be more conserved in nature also made the HapR ligand binding pocket able to accept the inhibitor molecules which allosterically inactivate the protein. This has been shown in crystals of HapR Quadruple Mutant with 2 newly synthesized inhibitor molecules VC111 and VC-212. Upon inactivation of the protein, "spreading leg" like conformational change was also noticed at the N-terminal DNA Binding domain. Further depending on these structural data new probable inhibitors for the allosteric site HapR was designed and their efficacy and efficiencies were evaluated with computational docking and molecular dynamics program.

Keywords: Master regulator, HapR, Quadruple mutant, Crystal Structure, Inhibitors

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PP-16

Geographical Variation in the Proteome Composition of the *Echis Carinatus* Venom from India and Sri Lanka: An Urgent Need for the Development of Sri Lanka-Specific Antivenom

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Snakebite envenomation is a global public health problem of high impact, particularly in the developing world. The saw-scaled viper (*Echis carinatus*) is a major venomous snake in several regions of the Indian subcontinent, the Middle East, and Africa. Because the clinical symptoms and pathophysiological manifestations following envenomation may vary depending on the geographical origin of the snakes, unveiling the complex venom proteome of a snake from a particular locale is extremely important for correlating venom proteome composition with pharmacological properties and the pathophysiology of envenomation. The proteome composition of *Echis carinatus* venom from southern India (SI ECV) and Sri Lanka (SL ECV) was studied for the first time by tandem mass spectrometry analysis. 90 and 42 enzymatic and non-enzymatic proteins belonging to 15 and 12 snake venom protein families were identified in SI ECV and SL ECV, respectively. When the venom proteome composition of SL ECV was compared to the SI ECV, 16 proteins were found to be in common; however, the relative abundances of some toxin families differ significantly. The proteome composition of ECV was well correlated with its enzymatic activities and pharmacological properties, and clinical manifestations observed in *Echis* envenomed patients. Immunological profiling unequivocally pointed to the poor recognition of < 20 kDa ECV proteins, such as PLA_2, subunits of snaclec, and disintegrin by commercial PAVs, and inadequate neutralization of PLA_2 enzyme, one of the major components of venoms, is a significant concern. All the PAVs showed a better immunological cross-reactivity and *in vitro* neutralization potency against SI ECV compared to SL ECV advocating the urgent need for the development of SL-specific PAV.

PP-17

Proteomic Analysis of Placental Tissue Depicts Differential Signatures In Spontaneous Pre-term Birth

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Placenta is an important transient organ associated with maintenance of pregnancy and child's development (1). It is an important organ for hormonal biosynthesis, chemical metabolism, transport and oxygenation of fetus. Insufficiency in placental growth can result in multi-dimensional outcomes such as spontaneous pre-term birth (sPTB), pre-eclampsia and inter uterine growth restriction (IUGR) (2). Pre-term birth is a global health issue and Indian subcontinent alone contributes around 23.4% to the tally (3). This study aims to assess the proteome of sPTB and term birth human placenta in order to identify the molecular factors associated with placental dysfunction. Mothers selected for this study had live, singleton pregnancy with no comorbidities and fetal anomalies. The cryo-homogenized case (POG: 32-36 weeks) and control (POG: 37-41 weeks) placental tissue samples (N=20) were analyzed using Label free Quantitation (LFQ) approach via MaxQuant. Comprehensive analysis of LFQ intensity led to identification of 1105 placental proteins. Out of these, expression level of 23 proteins were found to be significantly altered in Pre-term birth as compared to term birth. Our results demonstrate the differential expression of placental proteins in sPTB and functional association of these proteins with regulation of translation initiation and elongation, and alteration of protein degradation system. This study provides an opportunity for potential biomarker identification in placenta-related adverse pregnancy outcomes, and further refinement of diagnosis, and development of more efficient predictive models.

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PP-18

Plasma protein signature in spontaneous preterm birth

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Preterm birth (PTB) is a major public health problem and its effect, extend beyond the early infancy with substantial long-term consequences in late childhood and adult life. Spontaneous preterm birth includes preterm labor, preterm spontaneous rupture of membranes, preterm premature rupture of membranes (PPROM) and cervical weakness. Herein the present study we sought to identify changes in plasma proteome. SWATH-MS based label-free quantitative proteomics was performed to profile plasma proteome. Analysis of IDA data resulted in the identification of peptides, which corresponds to 1180 proteins with at least two unique peptides at FDR < 1%. This plasma-specific spectral library was used for quantitative SWATH-MS analysis. Upon evaluation of differential expression of the protein at Preterm compared to Term, we detected 23 proteins and 12 proteins differentially modulated (>1.5-fold) in POG at (18-20 weeks) and POG at (26-28 weeks) condition respectively. To validate a few of the differentially expressed proteins which shows an important role in pregnancy, we performed a MRM-based relative quantification of these proteins in the same subset of plasma samples from our cohort with similar clinical conditions. To verify the SWATH-MS quantitation, MRM-based relative quantitation of 28 proteins has been carried out. We observed a few proteins have shown a good difference between term and preterm with statistical significance. Our discovery analysis provides a signature for PTB which will be further validated in the GARBH-INI Cohort.

PP-19

Identification and Functional Characterization of Therapeutic Targets Associated with Breast Cancer Resistance

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Acquired drug resistance during chemotherapy leads to tumor recurrence in breast cancer patients and is a significant cause of mortality. Chemotherapy and radiotherapy induce enhanced DNA damage

and DNA damage response pathways promoting resistance and creating a barrier for further improvement of cancer patient survival. The mechanism of resistance has been associated with altering the cellular function of selective proteins in cancer cells. It would be challenging to identify specific protein networks implicated in chemo-radio resistance and decipher their mechanism of action in resistance. Doxorubicin induces cell death by inhibiting the α-subunit of topoisomerase II (TOP2-A) causing double-stranded breaks, henceforth preventing cell survival. Despite its effective use in breast cancer, doxorubicin resistance is the major limitation of its use. In our study, protein targets associated with doxorubicin and ionizing radiation resistance were uncovered using mass-spectrometry-based proteomics. Label-free quantitative proteomics of doxorubicin-resistant cells identified top differentially regulated proteins like LACTB, FN1, FDXR, F11R, and TRAF2 with fold change ≥ 2.0 for upregulated and ≤ 0.5 for downregulated proteins. Bioinformatics analysis of ionizing radiation resistance revealed proteins related to altered biological functions like protein ubiquitination, DNA replication, chromatin remodeling, and negative regulation of apoptotic signaling pathways. Subsequently, their interactome networks should be further investigated to emphasize their role in crucial resistance pathways. Overall, we identified therapeutic targets that could also predict the disease progression, treatment response, and recurrence of the disease.

PP-20

Snakebite Diagnosis: An Insight on the Current Aspects of Development of Tools and Bio-analytical Methods for Snake Venom Detection to Resolve the Age-Old Dilemma

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Snakebite, a neglected medical emergency resulting in numerous fatalities and long-term disabilities, is a source of global concern. The treatment of this medical crisis solely depends on the administration of mono or bi, or polyvalent antivenom, whose effectiveness relies on the unambiguous identification of the bitten species of snake. However, the classical methods of snake identification used in clinics have several drawbacks, which has inspired scientists worldwide to address this issue by developing species-specific snake venom diagnostic kits as an alternative. Recently the scientific community has made considerable strides in creating quite a few simple, inexpensive, rapid, specific, and sensitive snake venom detection kits. Despite all these efforts snake venom detection kit for identifying snake species of Australia is the lone diagnostic kit that has been commercialized which is a severe concern in efficacious snakebite therapy. This study discusses the key issues about the rapid diagnosis of snake envenomation, tools, and techniques developed and/or invented recently to detect snakebites. Successful commercialization of the designed diagnostic kits, particularly in the rural and underdeveloped areas of tropical countries, warrants much more intensive studies in terms of

efficacy, affordability, storage stability, and usability, in addition to standardization of techniques for use in clinics.

PP-21

Understanding The role of MZB1 in Multiple Myelomagenesis and Malignancy Using Proteomic and Molecular Approaches

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Multiple myeloma (MM) is a plasma cell-associated cancer and it accounts for 13% among all haematological malignancies worldwide. MM still remains as an incurable haematological malignant disease due to its poor prognosis. Therefore, discovering novel markers and targets for diagnosis and therapeutics of MM is an essential unmet need. In our previous study, we have carried out quantitative proteomic analysis of patient-derived MM mononuclear cells (MNCs) and identified marginal zone B and B1 cell specific protein (MZB1) as a candidate protein for MM. Our preliminary data indicate that higher expression of MZB1 is closely associated with progression of MM pathogenesis and could be a potential target for MM. However, how MZB1 promotes MM malignancy is remain elusive. Therefore, understanding the role of MZB1 in MM malignancy might help to develop effective therapeutics for MM. In this study, we are elucidating the molecular mechanism of MZB1-mediated MM malignancy using proteomic and molecular biology approaches. We carry out MZB1 immunoprecipitation followed by mass spectrometry (IP-MS) and found the MZB1 interacting partner viz. WTAP having crucial role in MM progression. Further, label free quantitative (LFQ) proteomics of MZB1 KD MM cells against wild type MM cell lines revealed the putative targets associated with MZB1 mediated multiple myeloma. This study will not only be useful to understand the significant role of MZB1 in MM but also could be helpful to establish it as a potential target to develop therapy for MM.

PP-22

Characterization and Understanding of the Structure-Function Properties of *Bacillus* Spp. Fibrinolytic Enzymes by Computational Analysis

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The fibrinolytic enzymes produced by *Bacillus sp.* have found significant therapeutic efficacy in preventing thrombosis, a potentially fatal cardiovascular disease brought on by an aberrant pathophysiological condition of blood clotting in vessels. However, thorough computational studies on the amino acid composition, basic physiological properties, presence of functional domain and motifs, and secondary and tertiary structure analysis of these enzymes can lead to the development of a specific enzyme with improved catalytic activity and other properties to increase their therapeutic potential. The protein sequences of sixty fibrinolytic serine protease enzymes produced by *Bacillus* species, with molecular mass ranging from 12-86 kDa, were retrieved from NCBI databases. The multiple sequence alignment showed that 49 possess a conserved domain with a catalytic triad of Asp196, His242, and Ser569. The *in silico* analysis predicted the instability index of sequences, except DQ997813.1 from *Bacillus subtilis,* in the range of 1.94-37.77, and their aliphatic index is in the range of 68.9-93.41, indicating high thermostability of these proteins. The random coil means the value of 36.52% suggested the predominance of this secondary structure in these proteases. A set of 50 amino acid residues representing motif 3 signifies the Peptidase S8/S53 domain that was invariably observed in 56 sequences. A more precise secondary structure prediction showed seven distinct motif maps and topological diagrams. The majority of sequences lack disulfide bridges, and they pose 25 enzyme cleavage sites. Additionally, 28 of the overall sequences have transmembrane helices, with two sequences having the most disordered areas. This structure-function analysis may also help develop enzymes with desirable properties for use as potential therapeutic targets.

PP-23

Insight Into the Interaction of Conjugated Gold Nanoparticle on Non-Enzymatically Modified Lysozyme Protein

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Protein glycation is a prevalent non enzymatic protein post translational modification that alters the protein structures due to complex reactions between the free amino group of protein and the carbonyl moiety of sugar residues. This causes production of Advanced glycation end products (AGEs) over time. Glycation affects the native protein in a detrimental way and leads to misfolding and protein aggregation. As a result, these protein aggregates trigger the onset of neurodegenerative diseases. Nanoparticles have recently gained more prominence as having therapeutic potential in biological fields and other medical areas. In this study, gold nanoparticle conjugated with polyvinylpyrrolidone polymer was synthesized and characterized by biophysical techniques. The current study focuses on the anti-glycating effect of conjugated gold nanoparticle on glycated Hen Egg White Lysozyme at physiological condition. The amyloidogenic protein human lysozyme and the hen egg white lysozyme share a substantial structural similarity, which makes the latter an ideal model protein. Glycation induced protein aggregation was monitored by various techniques such as fluorescence spectroscopy, scattering intensity measurement, thioflavin T and Congo red. The results

elucidate that gold nanoparticle conjugated with polyvinylpyrrolidone polymer has an inhibitory effect on protein glycation and glycation induced aggregation. This study will further help in providing deeper insights on gold nanoparticle as a promising therapeutic approach to understand protein post translational modifications as well as protein folding and aggregation.

PP-24

Rapid Modulation of Alpha-Synuclein Conformation in Presence of Membrane Mimetic Microenvironment

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Intracellular deposition of Lewy bodies in dopaminergic neuronal cells is pathological hall mark of Parkinson's Disease (PD). Alpha-Synuclein (α-Syn) is found to be the main component of this Lewy bodies.[1] The protein lacks structure and in solution it exists as highly dynamic polypeptide chain. During interaction it gets stabilize and forms a partially folded structure.[1] The effect of short chain alcohol on the secondary structural conformation of α-Syn has been studied to mimic the effect of lipid membrane. Far UV CD showed simple alcohol methanol changed the structure to helical conformation and then beta conformation from random structure. This phenomenon was observed at lower percentage methanol, whereas in presence if higher percentage of methanol the structure changed to beta conformation and maintained its structure throughout the incubation period. For the native α-Syn in the aqueous system, the RMSD value for simulation is somewhat higher with a sharp increase of around 12 ns to a maximum of 3.1 nm followed by stabilization at the saturated value of 2.4 nm. The RMSD value for simulation α-Syn at lower percentage of methanol remains low. Conformational illustrations were also imaged under AFM. Oligomers formed at lower percentage of methanol showed height of (1.5-2) nm, with gradual incubation it formed proto fibrillar structure with a height of ~3.5 nm. In higher percentage of methanol spheroidal oligomeric species of (0.5-2) nm in height were formed. However, no predominant fibrillar conformation was detected with longer incubation period. Hence characterization of these species has potency to open new arena in amyloid biology.

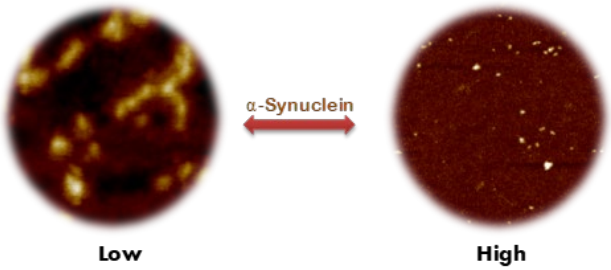

Figure 1: Conformation of alpha-Synuclein in presence of lower and higher percentage of methanol.

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PP-25

Arachidonic acid causes reversal of hyperglycaemia-induced skeletal muscle atrophy by reducing ROS production and inflammation

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Skeletal muscle accounts for 40% of the total body weight and is a predominant site for diverse metabolic activities including carbohydrate, protein and lipid metabolism. On the contrary, excess amount of these metabolites causes toxicity to the muscles and one such conditional phenomenon is hyperglycaemia or excess glucose in the blood[1]. The condition of hyperglycaemia can be triggered by disorders like diabetes mellitus[2] and Cushing's syndrome[3]. This translates to a cascade of signals in skeletal muscle cells which primarily leads to the increased expression of inflammatory cytokines and increased ROS production. Additionally, it alters the expression of certain muscle specific genes responsible for the maintenance of the skeletal muscle like *MyHC1* and *MyoG*. Due to this, the skeletal muscle loses its integrity in a phenomenon referred to as skeletal muscle atrophy or muscle wasting. Over the years quite a number of bioactive compounds have been used to reverse this phenomenon including bioactive lipids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which possess anti-inflammatory activities[4]. Arachidonic acid, an n-6 polyunsaturated fatty acid (n-6 PUFA) has been observed to have muscle regeneration activities which is a concentration dependent process[5] and requires the conversion to its metabolites like lipoxins and epi-lipoxins. In this study, we try to elucidate the reversal effect of arachidonic acid on hyperglycaemia induced skeletal muscle atrophy thereby reducing the inflammatory pathways leading to the restoration of homeostasis in the skeletal muscle cells.

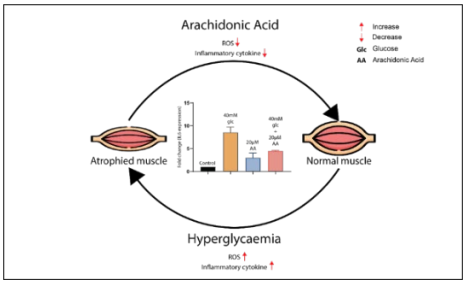

Figure 1: Arachidonic acid causes reversal of the muscle atrophy via restoration of ROS production and inflammatory cytokines

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PP-26

Quantitative Tissue Proteome Profile Reveals Neutrophil Degranulation and Remodeling of Extracellular Matrix Proteins in Early Stage Gallbladder Cancer

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Gallbladder cancer (GBC) is an aggressive malignancy of the gastrointestinal tract with a poor prognosis. It is important to understand the molecular processes associated with the pathogenesis of early stage GBC and identify proteins useful for diagnostic and therapeutic strategies. Here, we have carried out an iTRAQ-based quantitative proteomic analysis of tumor tissues from early stage GBC cases (stage I, n=7 and stage II, n=5) and gallstone disease (GSD) as non-tumor control (n=6). We identified 357 differentially expressed proteins (DEPs) based on ≥ 2 unique peptides and ≥ 2 fold change with p value < 0.05. Pathway analysis using the STRING database showed, 'neutrophil degranulation' to be the major upregulated pathway that include proteins such as MPO, PRTN3, S100A8, MMP9, DEFA1, AZU and 'ECM organization' to be the major downregulated pathway that include proteins such as COL14A1, COL1A2, COL6A1, COL6A2, COL6A3, BGN, DCN. Western blot analysis confirmed the elevated expression of MPO, PRTN3 and S100A8 in early stage of the disease. Based on the above results, we hypothesize that there is an increased neutrophil infiltration in tumor

tissue and neutrophil degranulation leading to degradation of extracellular matrix (ECM) proteins promoting cancer cell invasion in the early stage GBC. Some of the proteins (MPO, MMP9, DEFA1) associated with 'neutrophil degranulation' showed the presence of 'signal sequence' suggesting their potential as circulatory markers for early detection of GBC. Overall, the study presents a protein dataset associated with early stage GBC.

PP-27

Requirement of ChaC family of γ-glutamyl cyclotransferases in *Leishmania* parasite for switching to its slow growth state and long-term survival

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Glutathione is an important redox molecule throughout all Kingdoms, its synthesis as well as degradation plays a key role for maintaining redox homeostasis. Although glutathione synthesis is well studied in trypanosomatids, glutathione degradation pathways are still missing in parasites. Here we report two ChaC proteins (LmChaC$_{2a}$ and LmChaC$_{2b}$) in unicellular protozoan parasite *Leishmania*, which specifically degrade reduced glutathione (GSH), but no other γ-glutamyl peptides, trypanothione or oxidized glutathione. Interestingly, activity measurements reveal that recombinant LmChaC$_{2b}$ (38 kDa) shows ~17 times more catalytic efficiency than LmChaC$_{2a}$ (28 kDa) toward GSH. Quantitative experiments suggest that LmChaC$_{2b}$ expression is controlled by sulphur stress, whereas LmChaC$_{2a}$ is constitutively expressed in the parasites. To understand its exact physiological role in *Leishmania major*, we have created overexpressed, knockout and complement cell lines. Flow cytometric analysis suggests that null mutants have more reductive environment due to the presence of higher amount of GSH and lower amount of reactive oxygen species. LmChaC2-expressing cells can easily grow in GSH-containing sulphur depleted media but null mutants fail to grow, suggesting that LmChaC2 plays a pivotal role for cell survival when GSH is used as solely sulphur-source. The growth analysis showed that null mutants enter the stationary phase quickly but displayed impaired long time survival demonstrating that LmChaC2-aided GSH degradation pathway is essential for long-term survival. Furthermore, *in-vivo* studies indicate that LmChaC-dependent controlled GSH degradation is crucial for intracellular survival of *Leishmania* following infection in macrophage or mice. This work clarifies that LmChaC2 plays crucial role in GSH homeostasis in *Leishmania* [1].

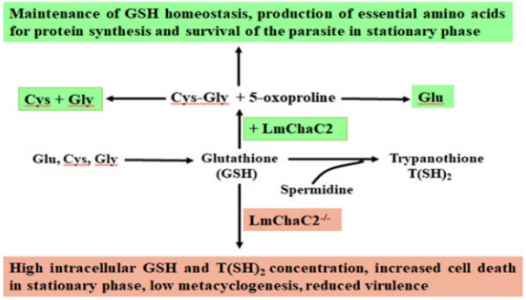

Figure 1: Schematic diagram depicts the importance of LmChaC2 proteins in the parasites

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PP-28

Effect of Single Point Mutations on α-Synuclein Structure and Plasticity

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Involvement of α-synuclein and its mutations[1] is well established in the Parkinson's Disease, the second most common neurodegenerative disease. Pathological aggregation of α-synuclein results formation of soluble oligomers which are suspected to be even more toxic than mature fibrils. Multiple factors including interaction with phospholipid membranes[2] can accelerate or slow down the rate of this aggregation and phase separation. We showed conformational changes as a consequence of different point mutations and interaction pattern of α-synuclein with different solvent condition[3]. Among the three domains forming the α-synuclein, the C-terminal domain[3] is postulated to be responsible for the stabilization of non-fibrillar monomer forms of protein. In our current investigation we showed two different point mutations, S129W and S129A on α-synuclein, to check the changes in the conformational or structural plasticity of protein mainly associated with the C-terminal region. In order to decipher that, interaction of α-synuclein with different phospholipids based on charge were investigated and compared with wild type α-synuclein. Different phospholipid membranes interacted differently with each type of protein which further indicated the significance of those mentioned point mutations on C-terminal region on the structural stability as well the aggregation kinetics.

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PP-29

Proteomic approach for understanding Diabetes mellitus using *Caenorhabditis elegans* as a model organism

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Type 2 Diabetes Mellitus (T2DM), one of the most prevalent metabolic disorders, is caused by either defective insulin secretion by pancreatic beta cells or the inability of insulin-sensitive tissues to respond. Proteomics is a promising technique for identifying key regulatory players responsible for the metabolic disturbances that can be used for early disease diagnosis and identification of therapeutic response prediction. The nematode, *Caenorhabditis elegans* has been used as an attractive model for understanding metabolic disorders. In this regard, the present study was undertaken to assess the key regulatory players involved in the intermediate pathway of glucose metabolism through LCMS/MS analysis. The proteomic approach has revealed a unique perspective on circulating biomarkers and specific targets of T2DM. A total of 268 proteins were identified; the protein profile suggests extensive reorganization of core intermediary metabolic pathway upon supplementation of 100mM glucose to *C. elegans*. The results provided that there is a modification in molecular response as well as in physiological condition when compared to wild type. The LCMS/MS analysis revealed that the differentially regulated proteins were mainly involved in lipid synthesis, fatty acid β-oxidation and glycolysis process, etc during the T2DM condition.

Keywords: Diabetes mellitus, Proteomics, LCMS/MS and *Caenorhabditis elegans*

PP-30

$CD34^+/45^+$ proteins: Roles, Interacting Proteins and Beyond

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The enigmatic molecule $CD34^+$ and its differentiated progenitor exist together in the hematopoietic milieu. However, $CD34^+$, the transmembrane glycoprotein makes its existence in cells present in

various organs such as eye, muscle etc. Interestingly, the cells from the neuroectodermal lineage named keratocytes in eye express CD34. However, conventional cells expressing CD34 are from the blood lineage-mesoderm. Reports from other labs have indicated an increased expression of CD34 upon transplantation of rat bone-marrow MSCs in injured rabbit corneas. Moreover, keratocytes are involved in corneal wound healing via fibrosis, development of myofibroblasts and then apoptosis of myofibroblasts post wound healing. Similarly, irradiated bone marrow also exhibits fibrosis via myofibroblasts formation. Here, we have found a strong interaction between CD34, TGFβ, collagen, laminin, heparan sulphate, proteoglycans, nidogen 1,2, PDGF, Thrombospondin 2, Matrilin 2, 4 and Fibronectin.

In this work, we have tried deciphering the flexibility of CD34 protein in eye and also the hematological lineage explaining the versatility of its function. Moreover, we have also deciphered the respective interacting partners of CD34 molecule present in eye and blood. The technology used is various bioinformatics software like Cytoscape, String Network, Molecular Docking and other various database tools.

Keywords: Hematopoietic stem cells, CD34+, Keratocyte, Bone Marrow, Bioinformatics

PP-31

Understanding the Principles Underlying Proteomic Remodelling in Defining the Role of CD24 in Self-Renewal and Differentiation

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Background: Cancer is a complex disease that results from somatic genome instability with dynamic changes in proteome leading to dysregulation of signaling pathways, enabling cancer cells to evade normal functioning of the cell. Oral cancer is one of the most prevalent cancers worldwide and ranks second in incidence and mortality in India. Epithelial tumors contain cancer stem-like cells, which possess a unique property of self-renewal and differentiation. Cancer cell surface biomarkers CD24 and CD44 play notable roles in regulating cancer stem cell properties, and therefore the growth and progression of Oral Squamous Cell Carcinoma (OSCC). The role of CD44 is well studied in different cancer; however, explicit studies have not been done in the field of CD24. In the current study, two cell lines, SCC032 [$CD44^{positive}$ $CD24^{negative}$] and SCC084 [$CD44^{positive}$ $CD24^{positive}$] were used for proteomic analysis.

Objective: Proteomic profiling of $CD44^{positive}$ $CD24^{positive}$ and $CD44^{positive}$ $CD24^{negative}$ tumor derived cell lines to understand the principles underlying proteomic remodelling in defining the role of CD24 in self-renewal and differentiation.

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PP-32

Integrated nuclear and mitochondrial turnover defines the Complex I stoichiometry

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Mitochondria is a unique organelle, in terms that it possesses its own genetic material, along with its very own transcription & translation machinery. Despite this, majority of the mitochondrial proteins are nuclear encoded and then imported into the organelle, facilitated by the presence of a Mitochondrial Targeting Signal (MTS). The compartmentalization of genes and involvement of both cytosolic and mitochondrial ribosomes in protein synthesis, warrants a synchronicity in both the nuclear encoded and mitochondrial encoded proteins. Such a synchronicity becomes a cardinal factor in the biogenesis and functioning of mitochondrial multiprotein complexes such as Respiratory Complex I (CI). CI is the largest enzyme (~ 1 MDa) catalysing the first step of the Oxidative Phosphorylation. It is made up of 44 subunits, of which 7 are encoded by the mitochondrial DNA whereas the remaining 37 subunits are encoded by the nuclear DNA. Surprisingly, MTS is present in only 20 of the 37 nuclear encoded CI subunits. Therefore, CI assembly requires a coordinated cytosolic and mitochondrial translation, import of nuclear encoded subunits with and without MTS, stoichiometric interaction of the nuclear encoded and mitochondrial encoded subunits and their balanced degradation inside and outside mitochondria. Here we report the turnover rates of CI subunits determined using a SILAC Pulse-Chase Strategy. We believe that further substantiation of this data with the import rates of CI nuclear encoded subunits will provide a clear insight into how this equilibrium between the nuclear encoded and mitochondrial encoded subunits is maintained within the cell.

PP-33

The peptide NLS-p53(380-386, 3Ac) encapsulated in phosphatidylcholine-stearylamine (PC-SA) liposome enhanced doxorubicin sensitivity of adenocarcinoma cells carrying p53^{R273H} hotspot mutation through induction of apoptosis

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Cancer is a disease with the highest mortality rate worldwide. Development of drug resistance is one of the major causes of cancer-associated death. Inactivation of p53 tumor suppressor functions, often through point mutations, is essential for carcinogenesis to proceed. A sub-class of such p53 point mutations gains new functions, including drug resistance and enhanced proliferation, in addition to the loss of function. We previously reported a synthetic peptide that binds tightly to human transcriptional positive cofactor 4 (PC4) and disrupts its interaction with a Gain-of-Function-mutant-p53 and reversed MDR1-mediated drug resistance. For improving pharmacokinetic properties and delivery, we entrap the peptide into cationic phosphatidylcholine-stearylamine (PC-SA) liposome, which has its own unique anti-tumor properties against specific cell lines. Herein we report p53 peptide entrapped in PC-SA liposome enhances the chemosensitivity of doxorubicin in cell lines bearing p53 mutation (R273H). The drug-induced cell killing effect was significantly higher in comparison to standalone therapy with free peptide or liposome with enhanced chemosensitivity, downregulation of MDR1 and PCNA, and induction of apoptosis. The liposome-encapsulated peptide could be the first step towards developing therapies targeting tumors bearing Gain-of-Function mutant-p53 protein that uses PC4 as a partner.

PP-34

Metal Ion Cofactor Modulates Liquid-Liquid Phase Separation of Superoxide Dismutase 1

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The misfolding and aggregation of Cu/Zn superoxide dismutase (SOD1) are believed to be associated with Amyotrophic lateral sclerosis (ALS)[1]. SOD1 is also involved with stress granules (SGs)[2], a type of membraneless organelle supposedly form via liquid-liquid phase separation (LLPS) of proteins containing low-complexity, disordered regions. Using experiments and computer simulations, we report here that structural disorder in two loop regions of SOD1 induced by the absence of metal cofactor - Zn, triggers its LLPS, eventually leading to fibrillar aggregates upon prolonged incubation. The addition of exogenous Zn to immature, metal-free SOD1 and the severe ALS mutant - I113T, stabilized the loops and restored the folded structure, thereby inhibiting LLPS and aggregation. In contrast, the Zn-induced inhibition was found to be partial in another severe ALS-associated mutant - G85R, which exhibits reduced Zn-binding. Moreover, a less-severe ALS mutant - G37R with perturbed Cu binding did not undergo LLPS. In conclusion, our work establishes a role for Zn-dependent modulation of SOD1 disorder and LLPS as a precursor phenomenon leading to the formation of toxic amyloids.

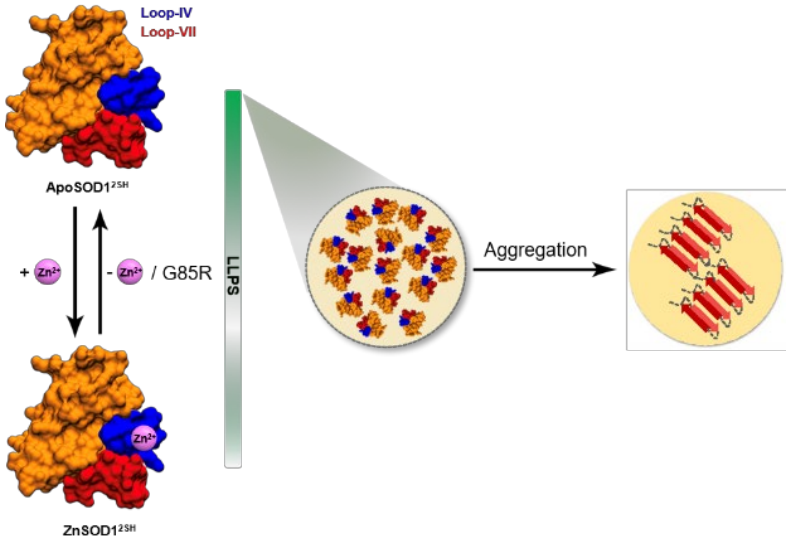

Figure 1: A schematic describing the mechanism by which Zinc regulates LLPS and aggregation of SOD1.

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PP-35

Metabolites from Marine Cyanobacteria *Oscillatoria Salina* having Anti-Cancerous and Anti-Microbial Properties

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Cyanobacteria (also known as blue-green algae) are an ancient group of gram negative photosynthetic bacteria that occur abundantly in fresh, brackish, and marine waters, and terrestrial environments. *Oscillatoria salina* is a non-branched, filamentous, non heterocystous, cyanobacteria abundantly present on both fresh water and marine environments and has been widely studied for production of a number of medicinal and pharmaceutically important secondary metabolites, cyanotoxins. In the present study, *O.salina* obtained from National Facility for Marine Cyanobacteria, was grown in ASN media for 16:8 hours of light and dark phases. The genome of this organism was sequenced using both Illumina and Oxford Nanopore platform and the assembly was generated using SPAdes genome Assembler. Like most Cyanobacteria, O. salina also displayed pH neutralizing properties. AntiSMASH analysis of *O.salina* genome showed biosynthetic gene clusters for various anticancer and antimicrobial metabolites. To validate this result, the metabolites were extracted from *O. salina* using 1:1 methanol chloroform. MTT assay showed significant dose dependent cytotoxicity of the crude extract on A549 lung cancer cells. Moreover, treatment of A549 cells with this extract significantly altered the cell morphology. The methanol chloroform extract also showed significant spheroid disintegration property in A549 cells corroborating with the in-silico prediction. Furthermore, antibacterial assay performed with the crude 1:1 methanol chloroform extract showed that 50 mg/ml of the extract showed significant antibacterial activity against the gram positive bacteria *S.aureus*.

PP-36

Influences of extraction methods on physicochemical characteristics and oxidative stability of omega-3 fatty acid-rich edible oils

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Dietary fats and oils are directly related to inflammatory response, and exploring the potential of edible oils for maintaining normal health and preventing chronic diseases is emerging as a promising strategy. Polyunsaturated fatty acids (PUFA) or essential fatty acids (ω3 fatty acids) play a vital role in human health. Fish oil and Marine algae are primary sources of PUFA. However, vegan sources are getting more attention due to their safety awareness. Further, PUFAs are highly unstable. Hence, the extraction method plays a crucial role in maintaining the stability of oil and retaining the essential compounds. The present study assessed the impact of cold-press (CP) and solvent extraction (SE) on oil quality from underutilized PUFA-rich oil seeds such as *Chia, Ocimum, Perilla,* and *Sacha inchi*. PUFA content of the seeds was 71.4 to 83.7 %, particularly α-linolenic acid, the most abundant fatty acid. Free fatty acid, peroxide, and iodine values were higher in SE oils as compared to corresponding CP oils. Saponification and unsaponofication value was also higher for SE oils. The highest oxidative stability and extended shelf life were observed with CP *Sacha inchi* oil as compared with SE oil. The enhanced oxidative stability of CP is due to retaining the tocopherols in the extracted oils. The samples were analyzed for thermal behavior by Differential scanning calorimetry and no differences in the FTIR spectra. The observed results suggest that the cold press is a suitable method for extracting oil from PUFA-rich oilseeds.

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PP-37

A Multi-omics Analysis of The Diversity of Lanthipeptides in Cyanobacteria

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Cyanobacteria produce a variety of secondary metabolites with a wide range of bioactivities and structural diversity. The production of those natural compounds involves various biosynthetic pathways. The genes that code for the enzymes used in those pathways are typically found in co-regulated clusters called "biosynthetic gene clusters" (BGCs). BGCs come in a variety of forms depending on the biosynthetic machinery they use, such as terpenoids, NRPS, PKS, RiPPs, and hybrids of NRPS and PKS. Genome mining techniques reveal the presence of a wide variety of BGCs throughout the cyanobacteria genome, a treasure trove of natural products. We have analyzed 1920 high quality cyanobacterial genomes from the NCBI Genbank, and used antiSMASH 6.0.1 to predict the BGCs in order to investigate this goldmine. The sequence similarity network was then created by doing a large-scale comparison analysis of anticipated BGCs, and homologous BGCs with comparable domain organization were then clustered into GCFs (Gene Cluster Families). When looking into family of RiPPs, Lanthipeptides, which are characterized by the presence of several lanthionine (Lan) or (methyl-) lanthionine rings to form thioether linkages, are discovered to be the most common class. There are five different classes of lanthipeptides, and we made an effort to determine how common each class was among cyanobacteria. The class II and V are the most prevalent. Then we began investigating these two classes. Then, as a key enzyme in the biosynthesis of class II lanthipeptides, we attempted to conduct an evolutionary investigation of LanM lanthionine synthetase. The other lanthipeptide classes need to be studied further.

PP-38

Extrapolation of Energy Producing Genes from Metagenomics Environmental Samples

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Recent advancements in computational biology have shown various aspects to metagenomics, it deciphers microbial genomes & their taxonomic classification with functional annotation. It refers various environments such as freshwater lakes, global oceans, gut microbiomes and soil microbiome etc. Recently, development of metagenome is highly optimized & memory efficient by using various computational tools. Microbial composition secretes several organic compounds, nitrogen fixation in soil environment. In this study, soil microbes within metagenome that was isolated from eastern part of our country, India that had shiny substances floating on the surface resembling oil. Our metagenomics analysis suggests bacteria is the predominant kingdom present in the sample. Among the bacterial phyla Proteobacteria & bacteroidota were the most abundant phyla that accounts for almost 90% of the total population. Soil bacteria have huge potential to evolve in response to new environmental stress. On the other hand, it shows very much interesting characteristics such as it release oil like shiny substances. In order to understand the chemical composition of the substance, we have used several chemical approaches to unravel the biochemical characterization of secreted compounds. We have characterized samples from one season when oil secretion happens. Now, we will collect the soil sample from the same location in a different season when oil like substances are not secreted. We would analyze all the metagenome samples along with chemical characterization to come up with a conclusion.

PP-39

Assessing *Apiospora* Species for the Production of Industrially Important Enzymes Using Reverse Genetics and Genetic Engineering

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Apiospora malaysiana is an ascomycetes endophytic fungus belonging to *Apiospora* species, which is known for the production of various bioactive and industrial important compounds. It was isolated in our lab from *Termitomyces* cultures and identified by 18s ITS sequencing. We sequenced the genomes of *A. malaysiana* using Illumina as well as Oxford Nanopore long read sequencing. The total sequencing depth was 250X with Illumina and 1.08X with Oxford Nanopore. After cleaning, 44370409 X 2 illumina reads (38,509,379 X 2 paired end; 5,861,030 X 2 mate pair reads) and 29,721 error corrected nanopore reads were used for assembly using *De-novo* SPAdes genome assembler (v3.11.1) gives 91 contigs, which were further super scaffolded in 43 scaffolds using BOSS. The genome assembly was 46 MB with N50 of 24, 29,633 bp (The k-mer analysis suggested a size of 45,286,039 bases). Augustus was trained twice with the transcript sequences to predict 12056 gene models, which was again annotated using funannotate (v1.6.0) resulting in prediction of 13200 genes. The expression of Diamond blast enhancer against nr database was used for primary annotation. We are also doing RNA seq analysis to get the transcript level expression of genes. At least 60% of the genes were novel (with no known identity in nr database) and plethora of other very niche specific genes such as cellulases, hydrolase, ABC transporters are found in the genome. We are now functionally characterizing the genes.

PP-40

Computational study on deciphering the protein-protein interaction involved in replication and proofreading mechanism of coronaviruses: A comparison between SARS-CoV-2, SARS-CoV and MERS-CoV

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Most of the proteins associate with other proteins, by forming protein-protein-interaction (PPI) complexes and networks that are central to almost all physiological processes. There are various possibilities of disturbing the natural PPI network due to genetic mutation, viral or bacterial infections, environmental factors etc., which may leads to various diseases. Despite the increasing knowledge of PPI through network pharmacology, there is a lacuna in understanding the PPI at atomistic detail. Here we highlighted some of the selected PPI of coronaviruses (CoVs) involved in viral replication-transcription and RNA proofreading activity. We explored the molecular mechanism of RNA polymerase (NSP12) interaction with its co factors NSP7 and two NSP8, the main toolbox for RNA replication and transcription of all CoVs. Additionally we analyzed the mechanism of interaction of Exoribonuclease NSP14 with its co-factors NSP10, crucial for high-fidelity proofreading activity to ensure replication proficiency of CoVs. Extensive computational study performed for the selected PPI complexes of SARS-CoV-2 and compared the results with SARS-CoV and MERS-CoV so as to ascertain the infectivity and transmissibility. The interaction profiling (interface area, hotspot residues, nature of bonds and energies between NSPs) provides valuable insight in detecting hither to identify hotspot residues and potential insights on alanine scanning mutagenesis.

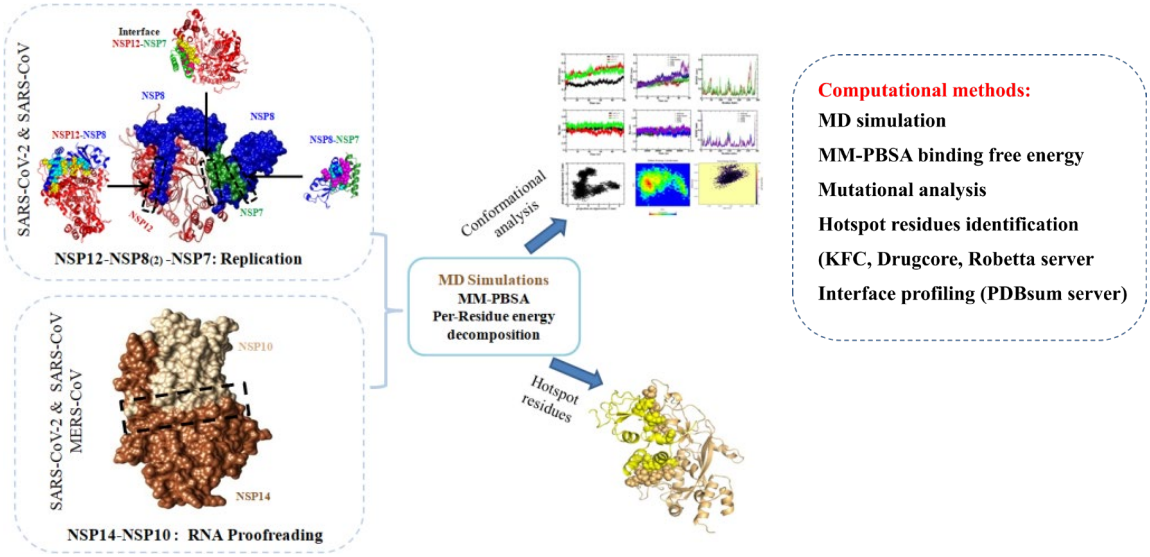

Figure: Representation of replication (NSP12- NSP8$_2$-NSP7) and RNA proofreading (NSP14-NSP10) complexes subjected to molecular modelling techniques for conformational analysis and deciphering the key hotspot residues at PPI interface

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PP-41

Oncogene-mediated Glycolytic Axis Promotes Lactate-induced Epigenetic Alterations to Facilitate Ovarian Cancer Cell Proliferation

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Homeobox gene families are associated with embryonic development and organogenesis. PITX2, one of the members of this family is involved in oncogenic regulation apart from its different development regulatory functions[1]. PITX2 has been earlier shown to induce ovarian cancer cell proliferation through the activation of different signalling cascades[2]. Increased cancer cell proliferation requires a constant supply of nutrients for both ATP and biomass synthesis which is facilitated by altered glycolytic rate[3]. This present study highlights the involvement of PITX2 in enhancing the cellular glycolysis pathway in ovarian cancer cells through AKT-phosphorylation. PITX2 expression correlates positively with that of the glycolytic rate-determining enzyme, Lactate dehydrogenase-A (LDHA), in both high-grade serous ovarian cancer tissues and common ovarian cancer cell lines. Interestingly, transient localization of enzymatically active LDHA in the nucleus was observed in PITX2-overexpressed ovarian cancer cells. This nuclear LDHA produces higher concentrations of the glycolytic end product, lactate which accumulates in the nuclear compartment resulting in decreased histone deacetylase (HDAC1/2) expression and increased histone-H3/H4 acetylation. Blocking lactate production by silencing LDHA reduced cancer cell proliferation. Taken together, this is the first report of its kind to show that the developmental regulatory homeobox gene PITX2 could enhance oncogenesis through enhanced glycolysis of tumor cells followed by epigenetic modifications.

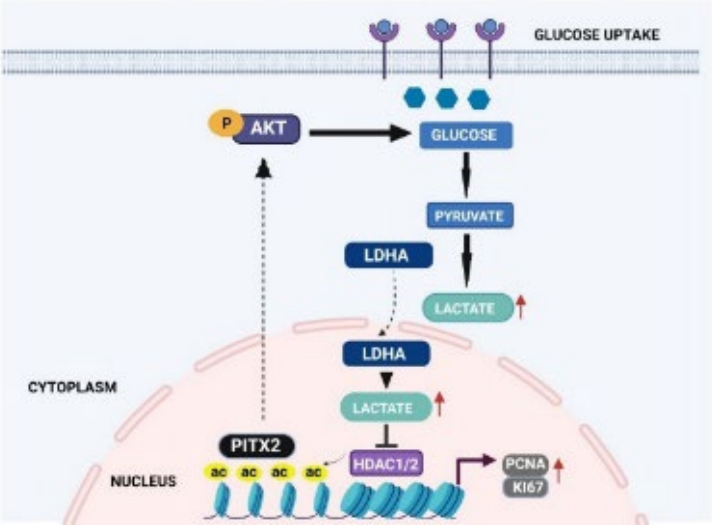

Figure 1: PITX2 regulates lactate-mediated epigenetic alterations to promote oncogenesis

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PP-42

TGFβ1-PITX2A/B Signaling Axis Potentiates Stemness And Chemoresistance In Ovarian Cancer Cell

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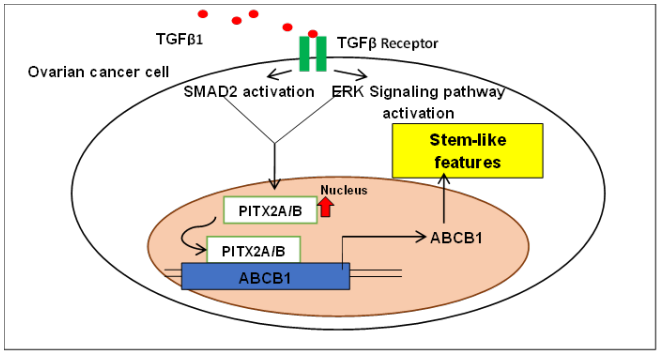
Ovarian cancer (OC) is one of the deadliest gynecological malignancies due to its asymptomatic nature, chemoresistance and recurrence, culminating into a very high mortality rate. However, there is not adequate knowledge about the mechanisms behind these phenomena. The presence of chemoresistant cancer stem cells in a tumor mass potentiate the risks of cancer recurrence. We aimed to decipher the molecular mechanism behind stemness and chemoresistance in OC. Earlier studies

suggested that different isoforms of PITX2, a homeobox transcription factor are associated with OC progression by regulating different signaling cascades. Moreover, they are shown to regulate the expression of drug efflux transporters in colon and kidney cancers, rendering chemoresistance properties in the tumor cell. Considering these backgrounds, we decided to seek the role of PITX2 isoforms in promoting stemness and chemoresistance in OC cells. In the present study, PITX2A/B has been found to control stemness by directly regulating the transcription of ABCB1, a drug efflux transporter. To further explore the regulatory mechanism of PITX2 gene expression, we found that PITX2A/B expression is augmented by TGFβ1 through both SMAD and non-SMAD signaling pathways. Collectively, we conclude that TGFβ1-activated PITX2A/B induces stem-like features and chemoresistance properties in the OC cells.



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PP-43

Unraveling the dynamics and implications of Ets1-DAXX protein-protein interaction in ovarian cancer

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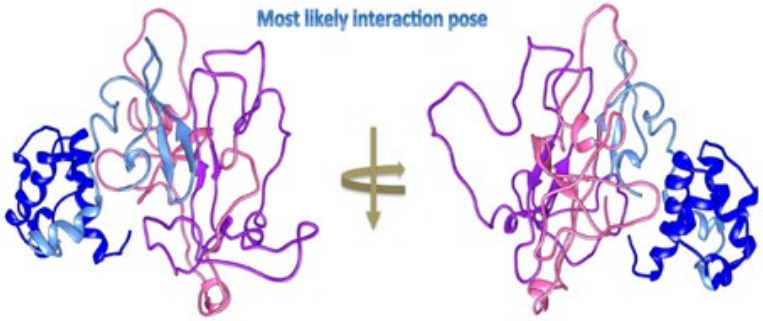
Over the last decade extensive reports have emerged indicating a pivotal role of transcription factors and their aberrant expressions in inducing oncogenic transformations. Ets1, a proto-oncoprotein belonging to the Ets family of transcription factors has been established as an indispensable candidate player contributing to oncogenesis, regulating different facades of cancer progression including hematopoietic development, angiogenesis, invasion, apoptosis etc. (1). Literature reports suggest that Ets1 undergoes a host of protein-protein interactions (PPI), often exerting regulatory implications on its functioning. One such significant interaction partner for Ets1 is DAXX (Death Domain Associated

Protein 6), reported to repress Ets1's transcriptional activity through PPI (2). Recent advancements have established DAXX as an important oncogenic player and a potent therapeutic target in ovarian cancer (OC) (3). In this view, we have validated a strong PPI between Ets1 and DAXX proteins in both OC cell lines and patient tissue samples. Further, in silico approaches identified the N-terminal of Ets1 DAXX interacting domain (DID) and C-terminal of DAXX Ets1 binding domain to be involved at the interaction interface, subsequently validated through in vitro pull-down assays of deletion constructs (DEL) with respective wildtype (WT) interaction partners. Ets1-DAXX PPI resulted in an EMT suppression by repressing Ets1 transcriptional activity on DAXX WT overexpression, subsequently rescued on DAXX silencing in OC cell lines. Furthermore, this repression by WT DAXX was abrogated on DAXX DEL overexpression. Cumulatively, our studies put forth ETS1-DAXX PPI as an important conjuncture of therapeutic intervention in ovarian cancer progression.



Most likely interaction pose for Ets1 and DAXX PPI

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PP-44

ETS1 drives EGF-induced Warburg effect and metastasis in ovarian cancer cells

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Epithelial ovarian cancer (EOC), the most abundant and lethal type of ovarian cancer (OC), is often encountered with alterations in Epidermal Growth Factor (EGF) mediated pathways [1]. The malignant cells show the 'Warburg effect' where most of the glucose entering the cells is utilized to form lactate even in the presence of oxygen [2]. Since, growth factors play immense role in guiding almost every

step of cancer development, our prime focus was to unravel the mechanistic details of EGF induced Warburg effect and subsequent cancer progression in EOC cells. In order to investigate the mechanism behind EGF mediated oncogenesis, we looked for the protein having highest co-relation with EGFR in ovarian cancer (TCGA-OV) tissues using RPPA (Reverse Phase Protein Array) filter. ETS1 emerged as the topmost candidate with a Pearson correlation co-efficient of 0.51. ETS1 is a proto-oncogene over-expressed in various malignancies including ovarian cancer and is known to play crucial role as key glycolytic modulator and metastasis inducer [3]. EGF treatment on cell lines SKOV3 and OAW-42 increases ETS1 via the Extracellular Signal-Regulated Kinase1/2 (ERK1/2) pathway. ETS1, in turn, increases migratory properties in cancer cells by increasing glycolysis, lactate production. ETS1 was found to be transcriptionally regulating two crucial players of the Warburg effect namely HK2 and MCT4. Dual blockage of HK2 and MCT4, reduces cell migration without causing much cytotoxicity. Overall, our findings suggest EGF mediated Warburg effect in EOC cells is mediated by ETS1 (Figure1) favoring cancer advancement by increasing metastasis and opens a new avenue for therapeutic intervention.

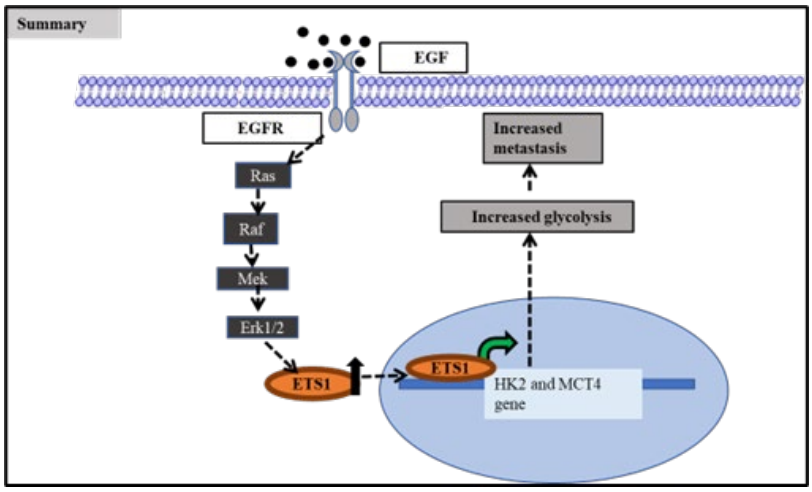

Figure1: EGF by activating EGFR receptor, increases ETS1 via ERK1/2 pathway to increase the transcript levels of HK2 and MCT4 resulting in overall increment of glycolysis and metastasis in EOC cells.

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PP-45

Clinical Significance of Cytochrome C in the Diagnosis and Treatment of Glioma

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Gliomas are the most common type of aggressive and deadly brain cancer. Similar to other types of cancer, disruption of the processes that control apoptosis takes place in the case of gliomas too. Knowing apoptosis and other related processes are believed to be essential for getting the causes of malignant tumors and developing anti-cancerous medications for therapy. This study was designed to identify a potential apoptotic protein/s target that is responsible for apoptosis in low to high-grade gliomas. To achieve the goal, a protein array was performed using different grades of glioma tissue sample (n=6 for each grade) and further validated using Flowcytometry and Immunocytochemistry. This shows a considerable alteration in the expression of the five apoptotic proteins Clusterin, HSP27, Catalase, Cytochrome C, and SMAC. On the basis of expression level and metabolic processes, the intricate enzyme Cytochrome C, one of the five significantly changed proteins, was further considered for validation. The findings showed that glioma tissues express Cytochrome C at lower levels than healthy tissues. Even more noteworthy is the fact that the increase in glioma grades resulted in a decrease in Cytochrome C level. The finding suggests that Cytochrome C might be a potential target to increase the apoptosis in Glioma. Further, we have performed a bioactivity assay using PubChem and suggested five potential drug molecules, that will be agonists for Cytochrome C expression, which may further lead to initiation of the apoptosis in glioma tissue.

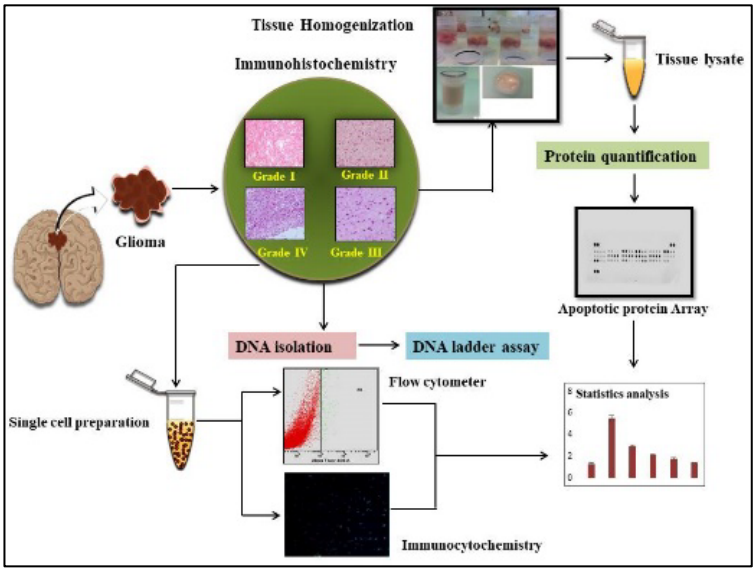

Figure: Graphical abstract of the overview of the methodology

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PP-46

Isolation and characterization of bioactive peptides from *Picrorhiza kurroa*

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Picrorhiza kurroa Royle ex Benth. is one of the well-known herbs of the Himalayan region which has immense medicinal value. However, its medicinal value at the peptide level is still elusive which restricts the development of peptide-based therapeutics. In this study, we have identified 65 peptides from *P. kurroa* hydrolysate. Among these, one novel bioactive peptide, ASGLCPEEAVPRR (BP1) was identified having antioxidant potential and showed angiotensin-converting enzyme (ACE) and dipeptidyl peptidase-IV (DPP-IV) inhibitory activities. The molecular docking results indicated that BP1 has a strong binding affinity toward the active pockets of ACE and DPP-IV.BP1 also showed its protective effect against H_2O_2-induced oxidative damage by inhibiting intracellular reactive oxygen species (ROS) and malondialdehyde accumulation and triggering the intrinsic antioxidant defense system in HEK-293 cells. Phase-contrast microscopy revealed that before exposure to H_2O_2, pre-treatment with BP1 retains the normal morphology of HEK-293 cells and helps in blocking apoptosis. Besides, it also suppresses ROS-induced mitochondrial apoptosis *via* restoring the mitochondrial membrane potential (ΔΨm) and inhibiting caspase 3/7 activity. It was concluded that antioxidant potential and ACE and DPP-IV inhibitory activities of BP1 could be utilized for concocting peptide-based formulation(s) in pharmaceuticals to ameliorate diabetes, cardiovascular diseases, and other ROS-associated diseases.

Keywords: *Picrorhiza kurroa*, bioactive peptide, antioxidant activity, ACE, DPP-IV, molecular docking

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PP-47

Platform Integration for High Through-put Multi Omics Data Analysis And Text Processing

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With recent advancement in Bioinformatics, a huge amount of multi omics data has been generated worldwide as well as by researchers working all over India. One of the next challenges in biology is to annotate and analyse the sequences deposited in the databases. However, in India there is no such Bioinformatics work-flow management system. We propose to create a data integration and data analysis and publishing platform that makes computational biology accessible to research scientists without much knowledge of computer programming or systems administration experience. Additionally, it is the need of the hour to provide a platform to all the genomes published from India to be deposited in one place.

We are primarily aiming to develop tools for Bioinformatics and big data analysis for omics data both from plants, animals and microbial sources. We host omics and protein-interaction data as well as novel web-analysis platforms using our existing methods that are optimized for specific purposes. We also carry out functional profiling of microbes from different sources. Currently, there is a huge lacuna in this area.

In addition, there will be a specialized repository for genomes originated and sequenced from India. This will serve a very useful resource for looking at genetic diversity, population genetics and gene flow between our sub-continent. We will also create a platform for analyzing image data originating from hospitals. The analysis will be based on Artificial Intelligence from trained data-sets. We handle sequence data, image data, metabolic data, diagnosis data and data from other sources. This will be a leading edge, trendsetting, and timely effort to unify the multi omics data into an integrated platform for better visibility and access.

PP-48

Plasma Protein Carbonyl Modification Signature in Spontaneous Preterm Birth

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Preterm birth is considered as a condition of neonatal birth before completion of 37weeks and preterm birth contributes almost 1 million deaths of neonates per year globally. This mortality can be reduced upto 75% with proper management and care of neonates, which is possible only in the case of early prediction. One of the major tools for the early prediction is the timely assessment of mother's blood for various changes. Though till now there is no specific blood related marker is in practice. During the pregnancy large number of changes occur in the mother's body including enhanced metabolic rate which in turn increases the load of oxidative molecules. The level of these oxidative stress and changes induced may act as a promising approach to predict the delivery-time. One of the major stress molecules is carbonyl, which has been already shown to be associated with preterm, targets the plasma proteins and leads to production of carbonylated adducts. In this study we aim to study the changes in the carbonylated proteome during the healthy pregnancy and in pre-term condition. Here, we have chosen two-time point of POG at 18-20 weeks and POG at 26-28 weeks in both the term and pre-term conditions and identified the carbonylated proteins in the plasma using mass spectrometry. We have observed the decrease in carbonylated proteins in preterm-birth conditions as compared to term birth. The preterm mother may have compromised metabolic activity. This signature may be useful for predictive analysis along with other biochemical and clinical parameters.

PP-49

Leishmania donovani Repressor of Differentiation Kinase 2 (RDK2): A promising diagnostic target against visceral leishmaniasis

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Visceral leishmaniasis (VL), a major global health concerns due to its morbidity and mortality. Until recently, no diagnostic technology has provided a highly precise and cost-effective way of detection for VL worldwide. As a result, the development of strong, specific, and commercially translatable diagnostic assays is critical. We are currently working to investigate the diagnostic potential of a novel parasite antigen. The serine/threonine kinase Repressor of Differentiation Kinase 2 (RDK2) plays a variety of roles in parasite life cycle progression. Its potential as a VL diagnostic candidate, however, hasn't been examined. We cloned, overexpressed LdRDK2 and used serum and urine samples to test the recombinant RDK2 for the diagnosis of human VL. According to silico research, RDK2 is conserved among *Leishmania* species, with the least conservation in humans. In the immunoblot assay, RDK2 generated immune-reactive bands with antibodies seen in the sera of VL patients with no cross-reactivity. Moreover, when compared to healthy controls and other disorders, antigen showed strong reactivity with IgG antibodies from VL sera, with 78 percent sensitivity and 86 percent specificity. Its utility tested for non-invasive VL diagnosis using urine samples from patients obtained 94 percent sensitivity and 86 percent specificity. RDK2 was found to have higher sensitivity and treatment response in urine samples than in serum samples in ELISA analysis, indicating its potential as a point of care (POC) antigen. In conclusion, we looked into the role of RDK2 as a viable diagnostic marker for VL in both invasive and non-invasive techniques, as well as its potential as a promising POC antigen in cases of treatment response.

PP-50

Proteomic Analysis for Identification of the Molecular Players for Oxidoreductive Adaptation in Metabolic Syndrome

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The association of clinical conditions like hypertension, diabetes, atherogenic dyslipidemia (AD) and obesity with increased risk of developing atherosclerotic cardiovascular disorders (CVD) is well established. Hence for the ease of identification of such patients, clinicians and researchers have clustered the above mentioned factors under the common terminology of 'Metabolic Syndrome'. A better understanding of the relationships between different components of Metabolic Syndrome is

critical in comprehending the pathophysiology linking them to CVD. Atherogenic Dyslipidemia, a component of Metabolic Syndrome, is characterised by elevated levels of low-density lipoproteins (LDL), serum triglycerides (TG) and decreased levels of high-density lipoproteins (HDL). Oxidative modification of LDL by ROS, forming oxLDL, represents the initial onset of atherosclerosis. To understand the oxidoreductive pathways playing a key role in AD, proteome analysis of normal and dyslipidemic Apoe $^{-/-}$ mice liver was performed. Mice in separate groups were fed with either normal chow diet (NCD) or high cholesterol diet (HCD) and high resolution mass spectrometry was conducted for the identification of the proteins in the liver tissue which might be critical for oxidoreductase adaptation in atherogenesis. Ingenuity Pathway Analysis (IPA) reveals that the glutathione metabolism pathways enriched when mice were fed with HCD. Preliminary studies reveals differential expression of GST isoforms in HCD fed mice liver suggesting that an increase in expression of Glutathione-s-transferase pi (GSTpi) and GST m1 might act as a possible protective adaptation to combat the increased cholesterol-induced oxidative stress. Therefore, downregulation of these isoforms may be responsible for increased CVD risk in AD.

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PP-51

Mycobacterial Methionine Aminopeptidase Type1c Binds Small Ribosomal Subunit and Thereby Acts as an Anti-association Factor

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The early events of nascent polypeptide chain synthesis on the ribosome include enzymatic processing and chaperone-assisted folding. The nascent chain emerging out of the ribosomal tunnel encounters protein factors at the tunnel exit for enzymatic processing. Methionine aminopeptidase catalyses the N-terminal methionine excision. Prokaryotes have only one class of MetAP (type1), whereas eukaryotes, including humans have 2 classes (type1 and 2). *E.coli* possesses a single sub-type of type 1 MetAP, MetAP1a, which is sufficient for its growth and survival. On the other hand, mycobacteria possess both 1a and 1c (1c possesses an additional ~40 amino acid N-terminal extension). There are several contrasting evidences suggesting either 1a or 1c as the predominant MetAP in mycobacteria. However, the exact reason for maintaining the two types of MetAP remains elusive. We aimed to understand the temporal regulation between these two types of MetAP in mycobacteria with the principal focus on deciphering any functional differences. MetAP1c showed higher expression during late log and stationary phase of cell growth along with high binding affinity to 30S ribosomal subunit. Subsequently, re-association assay and light scattering studies highlighted the anti-association activity of MetAP1c. Deleting the N-terminal extension nullified the anti-association property which prompted us to hypothesise a novel 30S interaction coupled anti-association mediated by the N-terminal

extension of MetAP1c. Unlike E.coli, mycobacteria cannot form 100S hibernating ribosomes so this anti-association by MetAP1c might be a pivotal mechanism by which the translation rate is regulated during stationary phase.

PP-52

Structural elucidation of a serotonin receptor (GPCR) with intact intracellular loop regions using high-resolution cryo-electron microscopy

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The serotonin 2A receptor (5-HT_{2A}R) is a member of G-protein coupled receptor (GPCR) family. Mental disorders including schizophrenia, depression and anxiety is caused due to impairment of 5-HT_{2A}R. Structural studies of the 5-HT_{2A}R can provide an important insight in understanding the downstream signalling and regulation mechanisms. The general structure of the 5-HT_{2A}R consists of an extracellular N-terminus, followed by seven transmembrane alpha helices domain (TM 1-7),which are connected by three intracellular (ICL 1-3) and three extracellular (ECL 1-3) loops, an amphipathic helix (H8) and an intracellular C-terminus. With the recent advancement of structural biology techniques such as crystallography and cryogenic electron microscopy, have elucidated distinct structural states of the 5-HT_{2A}R. Significant allosteric modulation occurs in the intracellular loop region of the receptor upon ligand binding. However, none of the available structures (truncated) provides information regarding the role of the intracellular loops, especially the third intracellular loop (ICL3), which is an important site for the binding of G-protein, as they are missing from the structures. Hence in order to perform structural studies of the native receptor containing the loop regions using cryo-electron microscopy, we have cloned, overexpressed and purified the full length, native form of the 5-HT_{2A}R using the baculoviral expression system in Sf9 (*spodoptera frugiperda*) insect cell line. The full length structure of the 5-HT_{2A}R with the intact ICL3 loop will help us to provide more information about the functioning of the receptor.

PP-53

Escherichia coli protein YchF halts translation initiation of leaderless mRNA on the 70S ribosome by locking P-site tRNA at its position

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E.coli protein YchF is a member of the Obg-GTPase family, a family of proteins which classically are ribosome-associated GTPases. However, YchF is reported to be an ATPase, and a negative regulator of H_2O_2 hypersensitive response in bacterial cells. YchF binds to the ribosome, and its binding is enhanced in the presence of ATP. Furthermore, the 70S ribosome stimulates the ATPase activity of the protein. Importantly, YchF expression is reduced during stress, as the protein acts as an inhibitor of the translation of leaderless mRNA, thus aiding in survival of the cell during stress.

We aimed to elucidate the binding of YchF to the ribosome structurally, using cryo-electron microscopy and also to understand its associated function. Using binding assays, we determined a critical role of transfer RNA (tRNA) in the binding of the protein. 3D structure determination of the *E.coli* 70S ribosome in complex with YchF protein, in the presence or absence of tRNAs, helped us to identify the binding site of the protein in the 70S ribosome. The 70S-YchF map reveals that binding of the protein apparently interferes with P-site tRNA binding, which is the first step for translation initiation of leaderless mRNA on 70S ribosomes. The 70S-tRNA-YchF map clearly shows that YchF locks tRNA in a distorted conformation at the P-site, effectively making the ribosome incapable of translation initiation. We postulate that, using this unique mechanism, YchF prevents translation of leaderless mRNA, thereby aiding in its regulation.

PP-54

Intra and Extracellular Metabolite Profiling of *Acinetobacter baumannii* DS002

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The metabolite profile of *Acinetobacter baumannii* was determined by extracting both the intracellular and extracellular metabolites. Nucleotides, amino acids, and their derivatives such as orotidine 5 monophosphate, adenylosuccinate, S-adenosyl-methionine, (S)-dihydroorotate etc. were

identified among 26 intracellular metabolites. In addition to these amino acid and nucleotide derivatives fatty acids like palmitic acid (play a role in antibacterial), oleic acids, icosanoic acid, myristic acid were also found as part of intracellular metabolites. Nucleotides and secondary metabolites like-prodigiosin, l-citrulline, and antibiotics precursors like L-2,3-diaminopropionic acid and cycloserine were identified among the extracellular metabolites. Presence of antibiotics such as cycloserine in extracellular metabolites is expected to contribute for the adaptive potential of the strain, especially in a polymicrobial environment.

PP-55

Multivalent Interactions Induces Phase Separation of A-Synuclein and Forms More Toxic Aggregates in a Yeast Model of Parkinson's Disease

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Neurodegenerative diseases were known to occur through aggregation of the misfolded and intrinsically disordered proteins in the brain tissues [*1*]. However this field has been extensively studied, our current understanding about these aggregation mechanism are limited. This is also a similar case with the second most prevalent neurodegenerative disease; Parkinson's, which is due to the aggregation of α-synuclein in the presynaptic vesicles in neuronal cells. But recently this aggregation is shown to occur through a process called liquid-liquid phase separation, which opens up a whole area for exploring the biophysical phenomenon governing this action [*2*]. In this study, we tried to show the liquid-liquid phase separation of α-synuclein inside yeast cells (*Saccharomyces cerevisiae*) using a positively charged polymer; polyethyleneimine and our supporting *in vitro* results suggests that, this act of phase separation due to the positively charged polymer is because of its binding with the negatively charged C-terminal domain of α-synuclein which modulates the long-range electrostatic interactions between the monomers and eventually driven by the short-range hydrophobic interactions which stabilizes the droplets formed. Moreover our finding also highlights that the aggregates formed via LLPS pathway are more toxic than the aggregates formed via non-LLPS pathway. Our study will serve as a model to induce phase separation of α-synuclein inside yeast and it tries to describe the possible mechanism which mediates this process.

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PP-56

Understanding the functional role of the conserved GTPase HflX from structural studies

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The functional impact of the heat stress-related protein HflX on translational machinery of *E. coli* has been revealed. Structural descriptions of the HflX-bound 70S ribosome and ribosomal subunits (50S and 30S) along with the functional assays reveal mechanisms of action of HflX during heat stress. HflX can bind with the 70S as well as ribosomal subunits (50S and 30S) and acts as an anti-association factor. We have found that binding sites of HflX on the 70S ribosome distinctly differs in the presence or absence of tRNAs. In the presence of tRNAs, when the E-site remains blocked, HflX approaches intersubunit space of the 70S ribosome from the A-site. HflX binding through the A-site of the ribosome promotes ratcheting of the ribosome resulting in movement of P-site tRNA to P/E hybrid state which may lead to release of the E-site tRNA. Whereas, HflX binds the vacant 70S ribosome through the E-site, disrupts the inter-subunit bridges due to steric clash (particularly B2a) and splits the 70S ribosome. We have also established that HflX displays an ATP dependent RNA helicase activity. The ND1 domain is the ATP-binding domain and the linker helical domain of HflX has a crucial role in RNA unwinding process. Interestingly, we have found that mycobacterial HflX seems to function differently as compared to *E. coli* HflX.

PP-57

Effect of Short-Term Use of FFP2 (N95) Mask on Salivary Metabolome of Young Healthy Volunteers

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Use of face mask has become a part of public life in the post-pandemic era. However, the understanding of the effect of wearing mask on physiology remains incomplete. This is the first report on the effect of wearing FFP2 mask on metabolic composition of saliva, a proximal matrix to breathe, along with cardiopulmonary parameters. In the first phase, un-induced saliva was collected from young (31.2±6.3 years) healthy volunteers (n =10) before and after wearing FFP2 (N95) mask for 30 minutes. In the second, saliva samples were collected similarly from 27 healthy volunteers (28.2±4.5

years). Metabolites were analyzed by GCMS. Results showed that such short-term mask use did not cause any significant change in heart rate, pulse rate and SpO_2. Three independent data normalization approaches were used to analyze changes in metabolomic signature. The individuality of overall salivary metabotype was found to be robust and minimally affected by mask use in most cases. In spite of significant inter-individual variability, increases in relative abundances of L-fucose, 5-aminovaleric acid, putrescine and phloretic acid were observed irrespective of the method of data normalization in both batches. Results revealed that even in absence of any change in cardiopulmonary parameters, N95 mask use was associated with correlated changes in metabolites plausibly originating from altered microbial metabolic activity. These might also explain change in odour perception that was reported to be associated with mask use. Potential implications of these changes on mucosal health and immunity warrants further investigations to evolve a more prudent mask use policy.

PP-58

Detection of Pearl Millet Lipases by Activity-Based Proteome Profiling (ABPP) Strategy

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Millets are referred to as “Nutricereals” due to their balanced nutritional compositions. Though the millets are nutritionally superior and the health benefits were scientifically proven, the commercial assessability of millet-based products is challenging due to the low shelf life. Lipases are responsible for the deterioration of product quality. Hence the complete inhibition of lipase activity is mandatory to enhance the stability. Various physiochemical treatments have been reported for the inactivation of lipase activity. However, the nutritional factors were compromised in the process with limited success. In the present study, we focused on identifying active lipase in pearl millet (PM) using activity-based protein profiling (ABPP). ABPP, a functional proteomic strategy, detects and identifies the enzymes irrespective of their abundance. PM was fractioned into an aleurone layer, embryo, and endosperm, and total protein was extracted to measure lipase activity. The lipase activity was observed in all the fractions, and it was the maximum in the embryo. The in-gel ABPP of PM fractions revealed the presence of active lipases, and a unique pattern was observed in each fraction. The maximum lipases were detected in 20 to 50 kDa region. Lipidome analysis revealed that the presence of linoleic acid (18:2) was significantly higher in triacylglycerol as compared to other lipids. Trilinoleoylglycerol was a predominant triacylglycerol, whereas 1-oleoyl-2-linoleoyl-sn-glycero-3-phosphocholine was in phospholipids. The present study provides the first level of evidence for the existence of different types of lipases in each layer of PM. Further gel-free ABPP is required for the identification of the lipases.

PP-59

Proteome-wide analysis of the rice cytoskeleton illustrates functional discovery in dehydration adaptation and protein-protein association networks

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The plant cytoskeletal proteins play a crucial role in regulating the cytoskeleton dynamics, supporting critical biological processes such cell wall morphogenesis, stomatal conductance and the buildup of abscisic acid in response to water-deficit stress or dehydration. However, it is still unknown which specific biochemical processes and regulatory mechanisms the cytoskeleton contributes to determine plants' ability to respond to dehydration. Thus, we developed the dehydration-responsive cytoskeletal proteome map of a resilient rice cultivar to better understand the molecular mechanisms behind dehydration tolerance in plant, and the function of the cytoskeleton. Initially, four-week-old rice plants were subjected to gradual dehydration and the strength of the compensatory physiological reactions caused by dehydration were observed using multivariate physicochemical indices. Next, we performed label-free quantitative proteome analysis and identified 1833 dehydration-responsive cytoskeletal proteins (DRCPs). This proteome map represents the biggest inventory of cytoskeleton and cytoskeleton-associated proteins and is, to our knowledge, the first report of a stress-responsive plant cytoskeletal proteome. Significantly, MapMan analysis of the DRCPs in rice revealed a variety of cellular systems for recognizing and managing stress responses. These findings point to a connection between the global regulation of cytoskeletal proteome and metabolic rewiring of adaptive responses that may confer dehydration tolerance, particularly in rice and other cereals.

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PP-60

Like a goddess with multiple roles- the aurora kinase from Leishmania donovani

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The causative organisms of Visceral leishmaniasis, the *Leishmania donovani* parasites, invade the visceral organs causing morbidity comparable to malaria. The treatment options being exorbidant and

prolonged, have the additional risk of reversion. Moreover, despite several ongoing trials in the immunopotentiating direction, involving functional characterization and safety assays, very few are in the pipeline to reach clinical trials anytime soon.

Our preliminary data have unearthed several possible roles for the aurora-like kinases from these parasites. They have been studied extensively for their role in cell-cycle and as potential anti-cancer targets. These are highly conserved (79-100%~ among kinetoplastids), and sufficiently different from their human homologs as confirmed by alignment, docking, and highly sensitive radioactive kinase assays using their natural H3 substrate. Moreover, they demonstrate parasite killing at an impressive IC_{50} of 105.9nM for promastigotes and 36.4nM for amastigotes, indicating a promising target for drug designing. The availability of inhibitors against their human analog makes drug-repurposing possible, thus saving enormous time and funds. Bioinformatics-based comparison of their immunologically active peptides to reported antigens of proven efficacy indicate comparable antigenicity in stimulating MHC-medicated pathways of peptide presentation and T-cell activation. Recent ELIZA-based assays have also demonstrated their potential as good diagnostic markers. Lastly, although remaining to be seen in *L. donovani,* genetic knock-outs have demonstrated drastically reduced virulence in the related protozoan parasites – *Plasmodium falciparum* and *Toxoplasma gondii.*

PP-61

Identification of Potential Protein Diagnostic Markers for Celiac Disease

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Celiac disease (CeD) is an autoimmune condition, characterized by certain serological and histologcal profiles triggered by ingestion of gluten based diet. Despite substantial increase in the number of CeD diagnoses over the years, many patients remain undiagnosed. There is a crucial need of non-invasive biomarker/s for the diagnosis and management of CeD and other enteropathic diseases. We therefore attempt to identify proteins that are strongly associated with CeD and other enteropathies (OE) through proteomics approaches using intestinal mucosal biopsies collected from a total of 30 patients with CeD, 22 OE and 25 controls. Data independent approach (DIA) based SWATH-MS method was employed to identify differentially expressed proteins among these groups. Furthermore, random forest based feature selection algorithm (BORUTA) was used to identify proteins that classify samples between the groups. Using SWATH-MS based proteomic analysis, we identified and relatively quantified 3424 proteins, of which 315 proteins were significantly differentially expressed (>1.5 folds) among 3 above mentioned groups. BORUTA analysis identified 37 proteins that could differentiate between these groups. Multivariate analyses was performed to identify potential protein markers. Identification of such markers that can discriminate between celiac disease and other enteropathies

could potentially alleviate the need for tissue biopsy both for the diagnosis and serial monitoring of the disease while they are on treatment.

PP-62

Acute heart failure: Towards identifying protein prognostic markers

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Acute heart failure (AHF) is defined as a syndrome with rapid onset or gradual increase in signs and symptoms of heart failure (HF). In presence of pre-existing cardiomyopathy or other cardiovascular factors leads to manifestation of AHF. It is associated with high mortality and hospital readmission rates. In first 3 months after hospitalization, readmission rates are as high as 30% and overall 1-year mortality rates are up to 23% in India. Existing treatments for AHF are mostly symptomatic, based on decongestive drugs. Poor management adds on to worsening of the condition. In order to identify proteomic prognostic markers, we categorized patients into 3 groups (recovered, sick and expired) based on baseline and 3 months' follow-up data of NT-pro BNP, ejection fraction (EF) and NYHA classification. "Recovered" showed improvement with symptoms, in "sick" symptoms were exacerbated on follow up while "expired" category included patients who could not survive by 3 months of follow up after their first hospital admission. We recruited 30 patients in each group and SWATH MS based label free quantitative plasma proteomics was performed on baseline and follow up samples. Boruta feature selection algorithm was used to analyse plasma proteins to identify proteins which can differentiate the 3 categories based on baseline data. Total of 215 proteins were quantified among all samples and feature selection tool identified 16 proteins to be potential prognostic marker associated with acute heart failure patients.

PP-63

Mechanistic insights into the endocytic pathway of N-acetylglucosamine transporter (Ngt1) in *Candida albicans*

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The amino sugar, N-acetylglucosamine (GlcNAc) has a unique role in Candida's development and virulence. It stimulates various signaling pathways including the expression of its catabolic genes in *Candida albicans*. N-acetylglucosamine transporter (Ngt1) has a pivotal role in GlcNAc signaling as well as in GlcNAc internalization. Earlier studies reported that the addition of unrelated carbon sources accelerated the endocytosis of proteins in eukaryotes, particularly for transporters, ion channels, and receptor proteins. But just after the endocytosis of membrane protein, they are either sent to the lysosome for degradation or are recycled back to the plasma membrane. Hence we were interested to identify the fate of this sugar transporter. Our studies revealed that it was dependent on a post-translationally modified form of the protein and this modification turned out to be phosphorylation-dependent ubiquitylation. Further, the role of Ras1 in ubiquitylation-mediated endocytic trafficking of Ngt1 and its subsequent turnover rate at the cell membrane was also elucidated. We have taken advantage of multifarious molecular biology techniques, in particular, fluorescence microscopy, Immuno-precipitation, and immunoblot analysis to understand the intricate molecular events involved in endosomal trafficking. Overall our studies establish that there exists sophisticated and subtle phosphorylation-dependent ubiquitylation machinery that is responsible for proper maintenance of the transporter level at the cell surface. This study also elucidates the role of the *UBI4* locus and Ras1 signaling component in Ngt1 ubiquitylation and its endocytosis.

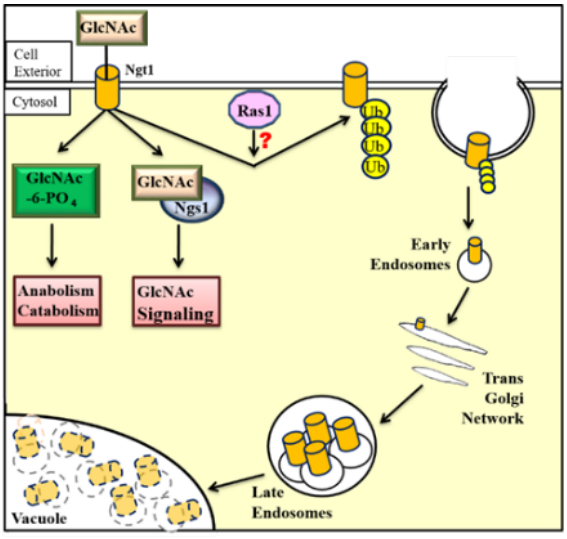

Figure 1: The working model figure represents the import of N-acetylglucosamine (GlcNAc) in *Candida albicans* through GlcNAc transporter (Ngt1) and the role of Ras1 in the endocytosis of Ngt1 and its turnover at the cell surface.

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PP-64

Analysis of Membrane Proteins of Streptomycin-Resistant *Mycobacterium Tuberculosis* Isolates

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Drug-resistant tuberculosis remains a health security threat and resistance to second-line drugs limits the options for treatment. Consequently, there is an utmost need for identifying and characterizing new biomarkers/drug targets of prime importance. Membrane proteins have an anticipated role in biological processes and could qualify as biomarkers/drug targets. Streptomycin (SM) is recommended as a second-line treatment regimen only when amikacin resistance has been confirmed. As extensively drug-resistant (XDR) isolates are frequently cross-resistant to second-line injectable drugs, an untapped potential for the continued use of SM has been suggested. The study aimed to analyze the membrane proteins overexpressed in SM resistant isolates of *Mycobacterium tuberculosis* using proteomics approaches. Membrane proteins were extracted employing sonication and ultracentrifugation. Two-dimensional gel electrophoresis (2DGE) of membrane proteins was performed and identification of proteins was done by liquid chromatography-mass spectrometry (LCMS) and bioinformatics tools. On analyzing the two-dimensional (2D) gels, five protein spots were found overexpressed in the membrane of SM resistant isolates. Docking analysis revealed that SM might bind to the conserved domain of overexpressed proteins and Group-based prediction system-prokaryotic ubiquitin like protein (GPS-PUP) predicted potential pupylation sites within them. These

proteins might be of diagnostic importance for detecting the cases early and for exploring effective control strategies against drug-resistant tuberculosis, particularly SM.

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PP-65

Characterization of different types of α-synuclein oligomer and their role in Parkinson's diseases

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α-Synuclein is a member of the intrinsically disordered protein family. It is a dominant component of the amyloid deposition in the substantia nigra region of the brain, a pathological signature of Parkinson's disease. Different studies have been performed earlier to determine the toxic form of alpha synuclein e.g. oligomer, mature fibrils.[1–3] Currently, it is well established that the oligomer forms of alpha synuclein is more toxic and various types of oligomer have been reported. Enormous attention has been drawn in this field till now. We expressed the wild type recombinant alpha synuclein, SDS-PAGE gel and Mass spectroscopic analysis are done and confirmed the molecular mass at 14.49 KD. Subsequently, we were successful in the preparation and separation of oligomers of different nature. We are in the process of understanding how the stability and electronic behaviours of sole tyrosine residue differs in different oligomeric state. We measured the fluorescence of different oligomers of alpha synuclein and found some changes in the peak positions. The CD analysis and Raman investigations are further performed to define the structure of the protein in different state. The cytotoxic effects of the oligomers in different cell lines will be investigated.

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PP-66

Efferocyte Derived Extracellular Vesicles Promote Inflammation Resolution

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Injury and the ensuing inflammation leads to cell death in the local tissue. The process of clearance of these dead cells by phagocytes is called efferocytosis and plays a key role in inflammation resolution and restoration of tissue homeostasis. Defective efferocytosis leads to unresolved inflammation and exacerbates several chronic inflammatory diseases including atherosclerosis. Inflammation resolution involves the coordinated regulation of multiple cells. Hence, intercellular communication between these diverse cells types plays a central. Many studies have demonstrated that small extracellular vesicles (sEVs) carrying selected proteins and non-coding RNAs play a crucial role in intercellular communication. However, whether efferocyte-derived sEVs regulate the resolution phase of inflammation is currently unknown. We show that efferocytes release sEVs with pro-resolving activity as demonstrated by their ability to enhance efferocytosis efficiency of naïve macrophages and skew them towards a pro-reparative M2 macrophage phenotype both in vitro and in vivo. SWATH-MS based proteomics analysis of the sEVs showed around 150 proteins differentially expressed in effero sEVs, out of these, two proteins which play regulatory roles in efferocytosis, HMGB1 and PSAP were also validated in *in vitro* conditions. We show that sEV-associated PSAP signals via a G-protein coupled receptor GPR37 on naïve macrophages to upregulate the expression of Tim4, a key efferocytosis receptor. More importantly, therapeutic administration of efferocyte-derived sEVs in a model of chronic non-resolving inflammation, namely atherosclerosis, led to increased macrophage efferocytosis, decreased plaque necrosis, decreased lesional inflammation, and improved lesional stability. These data provides the rationale for testing their role as a therapeutic agent for advanced atherosclerosis and other chronic inflammatory diseases.

PP-67

Effect of specific nanocomposite on protein structure and stability

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Nanoformulation Based disease treatment is gaining attention throughout the world. In our laboratory we prepared and characterized different metal nanoparticles and finding their effect on protein stability.[1–3] Here we showed the formulation of naringenin (NAR)-loaded gold nanoparticles (NAR-AuNP); Naringenin is a polyphenolic natural compound , acts as a reducing as well as a stabilizing agent in the synthesis of nanoparticles. Transmission electron microscopy revealed ~10 nm diameter particles, whereas the hydrodynamic diameter according to dynamic light scattering (DLS) was found to be ~24 nm. These nanoparticles showed a surface plasmon resonance peak at ~533 nm and did not show any fluorescence. Our NAR-AuNP showed the ability to interact with monomeric α-Synuclein protein resulting in a red shift of the SPR peak. From the thioflavin T assay we showed that in presence of nanoparticles ~3.5 times lower fibril is formed than the control. Circular dichroism (CD) spectra also indicated the absence of cross beta sheet structure, a hallmark of protein aggregation, when incubated with NAR-AuNP. Further, atomic force microscopy (AFM)-images analysis confirmed that the NAR-AuNP inhibits fibril formation. All the imaging and aggregation kinetics data, thus, confirmed that the nanocomposite hindered the nucleation step of the formation of α-Synuclein amyloid fibril formation. The nanosystems, therefore, may be explored further towards the development of treatment for Parkinson's and related diseases.

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PP-68

Structure Function Relationship of Dengue NS2b/NS3 Protease: Exploration Through Solvent Exchange and Temperature Perturbation

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Enzymes are biological catalysts that conduct certain biochemical reactions. They are also the workhorse behind specific microbial and viral diseases caused to mankind. Dengue, a mosquito-borne viral disease is a threat to every household in tropical and subtropical countries. In spite of its high infection and mortality rate, prevention and treatment against the virus remains cryptic. New drug discovery (*1*) against the virus requires a thorough understanding of the structural-functional relationships of the target enzymes. Loop motions and correlated structural dynamics is known to be associated with enzyme functionality (*2*, *3*). In this study, we have targeted Dengue NS2b/NS3 protease (Figure 1), which is conserved among different flaviviruses. We have identified the correlated motions in the all important regulatory loop regions located adjacent to the active site triad of the enzyme (Figure 1). These motions were perturbed using non-invasive solvent exchange and the resulting effects (uncoupling of bending and rocking motions of the loops) can be mapped to *in vitro* enzyme activity. We observed significant changes in terms of activity of the enzyme with increasing concentration (≥10% vol) of a cosolvent dimethyl sulfoxide (DMSO). Furthermore, the bending and rocking motions of the loops have altered to a large extent in the case of point mutations in non substrate binding residues that are known to abolish enzyme activity. Together these experiments highlighted the involvement of **β**-turn motions of regulatory loop regions in dengue serine protease activity.

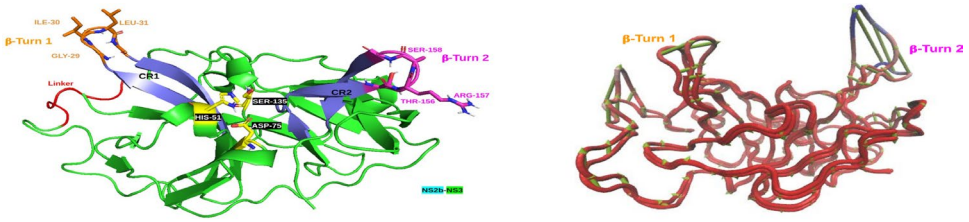

Figure 1: Structure of dengue NS2b/NS3 protease (Left). The turn 1 and turn 2 show correlated motions modulating enzymatic activity. Bending motion obtained from molecular dynamics simulation is shown on the right side. Green arrowheads indicate the direction of motions.

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PP-69

Leishmania Exports miR-146a from Infected to Naïve Macrophage: Non-Canonical Role of HuR in Anti-Inflammatory Response Control

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Leishmania donovani causes visceral leishmaniasis which exists as an intracellular protozoan parasite inside macrophage cells. The parasites residing within infected macrophages exhibits an elaborate cross-talk between the neighbouring naïve macrophages and the hepatocytes present in the liver tissue microenvironment. Extracellular vesicles (EVs) play a pivotal role in communication between cells. Mass spectrometric analysis of infected EVs were done to understand any specific protein candidate followed by exploring the small RNA population. *Leishmania* depolarizes mitochondria in the infected macrophages which prevents the entry of miR-122 containing EVs released from hepatocytes thereby preventing the creation of a pro-inflammatory niche. *Leishmania donovani* infected marcophages export out miR-146a via EVs which when uptaken by neighbouring hepatocytes attenuates the levels of miR-122 thereby fostering the survival of the parasite. In the naïve macrophages, miR-146a containing EVs get into a dialogue with the RNA binding protein, HuR and reduces the levels of inflammatory cytokines, TNFα and IL-1β. Interestingly, HuR plays a non-canonical role in regulating the anti-inflammatory milieu by releasing miR-21 through EVs thus increasing the levels of its target mRNA, IL-10. The opposing forces miR-146a and HuR acts in concert to create an IL-10 surplus environment inside the macrophages and skews it to M2 phenotype.

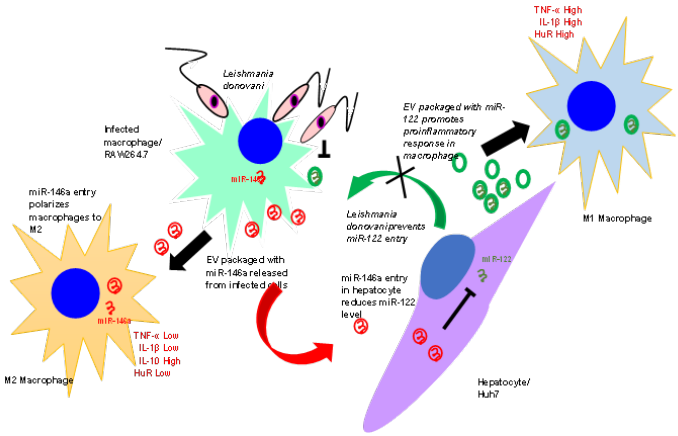

Figure 1: Graphical abstract, the proposed model of EV mediated cross-communication in *Leishmania donovani* infected macrophages with neighbouring hepatocytes and naïve macrophages.

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PP-70

Caprylic Acid Mediated Cell Death Target Mevalonate Kinase of Leishmania Donovani Parasite

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The protozoan parasite *Leishmania* causes a multitude of diseases with symptoms ranging from skin lesions to severe visceral organ damage. Due to drug resistance and high cost in AmBisome treatment safe, affordable drugs are required. In our study caprylic acid (CA), with a C8 chain, was found to be the most effective against *Leismania donovani*, the causative agent of visceral leishmaniasis(VL), among various saturated medium-chain fatty acids (FA, C8-C18).The anti-leishmanial activity of various CA analogues with C8 linear chain, but not longer, and carboxyl/ester group was comparable to CA. Reduction in ergosterol content was the main factor triggering CA-mediated cell death. Mevalonate kinase (MevK), a key enzyme of ergosterol biosynthesis pathway of parasite, was identified as the target by molecular docking and MD simulation experiments. Enzyme assay using purified recombinant MevK and CA/CA analogues showed competitive inhibition pattern. MevK have a strong binding interaction with CA/CA analogs as proven by isothermal calorimetry (ITC) and circular dichroism (CD) studies. Increased IC_{50} of CA in parasites, over-expressed with homologous MevK,

revealed that MevK expression is directly correlated with CA inhibition. MevK was downregulated in CA-treated cells in a dose-dependent manner as confirmed by LC-HRMS based proteomics studies. The mechanism of the antileishmanial effect of CA, a natural product, was established against VL where toxicity and drug resistance with current chemotherapeutics demand an alternative. The identification of an enzymatic target with kinetic parameters and mechanistic insights was reported, for the first time, against any microrganism for a natural medium-chain FA.

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PP-71

Evaluation of Anti-Depressant Like Effect of 4-Methyl Esculetin Through Inhibition of NLRP3 Inflammasome

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The pathophysiology of depression is heavily reliant on inflammation. Evidence suggested that etiology of depression is linked with the NLRP3 inflammasome-induced inflammation.Therefore, blocking the activation of the NLRP3 inflammasome may be beneficial for treating depression. Due to toxicity of currently available anti-depressant there is needed to develop novel, safe, and affordable drugs for the treatment of depression. 4-methyl Esculetin (4MECT) is a natural coumarin derivative that possessing anti-inflammatory activity. However, the antidepressant action of 4MECT has not been addressed. Therefore, we explored the antidepressant effects of 4MECT and its underlying mechanism. The Docking and Molecular dynamic simulation research revealed that the 4-MESC has greater potential towards NLRP3 PYD. Blood-brain barrier permeability confirmed by SwissADME Pharmacokinetic tool. Our results showed that High dose (50mg/kg) of 4MECT significantly decreased immobility time in TST and FST and do not affect locomotor activity of mice. 4MECT significantly reduced LPS-induced elevated levels of ROS, lipid peroxidation and enhance the SOD activity and glutathione level revealed its anti-oxidant potential against oxidative stress. 4-MECT also alleviate LPS induced pro-inflammatory cytokines including IL-6, and TNF-α in serum and brain tissue. 4MECT exert its neuroprotective action through elevation of BDNF and reduction in cortisol level. 4MECT decreased the expression levels of NF-κB, NLRP3, capase-1, IL-1β and cleaved gasdermin D in the hippocampus. These findings demonstrate that 4MECT exerts antidepressant-like effects, by inhibiting NLRP3 inflammasome and may be considered as anti-depressant therapeutic in future.

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PP-72

Fisetin Loaded Solid Lipid Nanoformulation Suppresses the Migration and Invasion in Human Colon Cancer Caco-2 Cells Through Modification of Epigenetics, Oxidative and Inflammatory Cascades

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Colorectal cancer is major cause of mortality and morbidity worldwide. The migration and invasion features, which is associated with epigenetic, oxidative and inflammatory alteration response, act as vital roles in the development of colon cancer. Fisetin, a bioflavonoid compound, is widely spreaded in vegetables and fruits. Although fisetin exerts antioxidant and anticancer activities, the molecular signaling pathways in human colon cancer cells remain unclear. Hence, the present study was conducted to investigate the effect of fisetin on migratory and invasive activity of colon cancer and the involvement of epigenetics cascades (DNMT-1, 5-MC, HDAC) and other underlying mechanism. In the present study, we aimed to explore the proliferation, migration, apoptosis and autophagy in caco-2 human colorectal adenocarcinoma cells. Caco-2 cells were treated with Fisetin (0, 0.01, 0.1, 1, 10, 100, 1000, and 1000 µM) for 24 and 48 h, respectively. Cells were also treated with 5-fluorouracil at a concentration of (4µg/ml) showed very a smaller number of viable cells (1e) (0, 1, 2, 3 and 4 µg/ml), and fisetin (10, 100 µM) combined with 5-fluorouracil (1, 2, 3 and 4 µg/ml) respectively, and cultured for 24 h after treatment. MTT assay was utilized to evaluate the effects of fisetin alone or fisetin combined with 5-fluorouracil on proliferation of caco-2 cells. Cell wound-scratch assay was used detect cell migration and epigenetic profiling done by using immunofluorescence at 48 h (100 µM) treatment. Fisetin inhibited the proliferation and migration, and induced apoptosis and autophagy of caco-2 cell lines human colon cancer cells.

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PP-73

Proteomic profile of diabetic nephropathy and promoting renal progenitors in diabetic mice

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Diabetes is one of the fastest growing diseases worldwide and has been recognized as one of the leading causes of death and disabilities worldwide. As of 2019, an estimated 463 million people had diabetes worldwide, and 80% of diabetic patient develop chronic diabetic complications such as retinopathy, nephropathy, hypertension, foot ulceration, and CNS dysfunction. Over long periods of time, insufficiently controlled blood sugar can lead to kidney damage. Hence, this study focuses on screening one of the key natural drug, Dehydrozingerone (DHZ) against diabetes-induced nephrotoxicity. Dehydrozingerone is a curcumin analog reported for inhibition of renal lipotoxicity. The present study focuses on multiple usage of Dehydrozingerone against various pathological conditions. Dehydrozingerone significantly enhanced the regeneration of glomerular podocytes and promotes renal regeneration in diabetic mice. We used a well-established mouse model of type 2 diabetes induced by High Fat Diet (HFD) and concurrent low doses of streptozotocin (STZ). We have identified several plasma protein biomarkers associated with diabetes associated kidney disease histopathology and adverse clinical outcomes. In this study we determined that Dehydrozingerone normalizes several plasma protein biomarkers such as the urine albumin, creatinine, bilirubin, and urea nitrogen contents in diabetic mice. Further, diabetic mice showed significant nephrotoxicity was evidenced by elevated level of plasma glucose, triglyceride, cholesterol and urea level, however, diabetic mice treated with DHZ showed improvement in the blood serum level of these markers($p < 0.05$). Interestedly, Dehydrozingerone significantly enhanced the renal regeneration of kidney podocyte cells, the primary glomerular epithelial cells that form a slit membrane and act as a barrier against proteinuria. Podocyte damage causes morphological changes, detachment, and apoptosis, leading to proteinuria, glomerulosclerosis and renal failure. Further, qPCR data supports that Dehydrozingerone enhances the expression of podocalyxin, WT1 and TRPC6 which are key genes in the development of Podocyte system ($p < 0.05$). Proteomic data also suggests the noticeable changes at cytoskeleton level through decrease expression of cytoskeleton protein like actinin, Tropomyosin, etc. Proteomic data further suggests that diabetic condition led to the downregulation of 60s ribosomal subunit dependent genes and ER resident protein ERp29 ($p<0.050$ at 99% confidence level) which are instrumental in insulin biosynthesis, and DHZ treatment reinstated the insulin biosynthesis. Similarly, diabetic mice treated with Dehydrozingerone reinstated serin/threonin dependent DNA Damage Repair (DDR) which supports the beneficial effect of Dehydrozingerone in diabetic nephrotoxicity via regulating genotoxicity. Collectively, our findings prove that Dehydrozingerone has a protective role against diabetes-induced nephrotoxicity in experimental mice.

PP-74

Evaluation of Cytokines in Tuberculosis Patients Infected with COVID-19

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There is a growing evidence that COVID-19 and tuberculosis interact[1]. Immune dysregulation suggests a twofold danger posed by co-infection[2], which worsens COVID-19 severity and favours TB disease progression[3]. This study was undertaken to evaluate the changes in serum concentration of cytokines among tubercular patients infected with COVID-19. It was a cross-sectional study carried out with a sample size of 40 participants. Participants were divided into four different equal groups – (i) Healthy Control (TB & COVID-19 both negative), (ii) COVID Control (TB positive but COVID negative), (iii) TB Control (COVID-19 Positive but TB negative), and (iv) Test group (positive for TB & COVID-19 both). ELISA and Flow Cytometry were performed using standard protocol to estimate the serum levels of IL-6, TNF-α IFN-γ, IL-10. Results of the study indicated that the mean ± standard deviation value of IL-6 levels in blood serum was higher in test group (135.50±9.88) among all groups. TNF-α serum concentration was also higher in test group (135.70±19.88). Similarly, IFN-γ serum level was observed highest in test group (125.50±5.06), followed by TB control group (77.60±28.35). IL10 showed higher serum concentration in healthy control group (142±7.54) and lowest limit found in test group (100.66±5.13). The significant elevation in serum concentration of IL-6, TNF-α, IFN-γ among test group patients in comparison with healthy control group indicated hyper inflammation and it may have the potential to worsen COVID-19 severity in patients[4,5]. As IL10 maintains normal tissue homeostasis, decreased level of serum concentration in test group indicates tissue damage and favours TB disease progression.

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PP-75

Silencing of Carbohydrate Sulfotransferase 15 by Small Interfering RNA Cream in Ovalbumin-Induced Atopic Dermatitis

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Atopic dermatitis (AD) is a common, chronic, inflammatory skin disease. It is distinguished by dry eczematous skin with extreme pruritus, increased allergen-specific IgE serum antibodies, and inflammatory infiltrates causing proallergic cytokines. Carbohydrate sulfotransferase 15 (CHST15) is an enzyme responsible for producing chondroitin sulphate E. It produces proinflammatory cytokines such as IL-1 and IL-6 and promotes the release of TGF- β in AD. Small interfering RNA (siRNA) targeting CHST15 is a synthetic siRNA that contains sequences complementary to a portion of the CHST15 gene's mRNA sequence. We hypothesize that the cream containing CHST15 siRNA might be a potential therapy for AD. In the *in vivo* study, female balb/c mice were given intraperitoneal injections of chicken egg ovalbumin (OVA) (10 µg) with 4 mg of aluminium hydroxide on days 0, 7, and 14. On days 14-21, animals were sensitized epicutaneously with OVA patches. Following day 21, animals were given CHST15 siRNA cream (10 µg) twice a week after confirming the AD. From days 28 to 35, mice were provided with another OVA patch. Experiments after the animal sacrifice include Western blotting analysis, qRT-PCR, ELISA, histopathology, and immunofluorescence. The CHST15 siRNA cream ameliorated the OVA-induced skin pathology, as evident by the normalization of epidermal thickness and suppression of inflammatory cells in the epidermis. The expression of proinflammatory mediators like IL-1β and TGF-β, Th2 promoting cytokine (TSLP), and Th2 inflammatory cytokines were suppressed markedly. These results indicate that CHST15 siRNA cream could be a potential therapy for treating AD.

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PP-76

Evaluation of Safety and Efficacy of *Picrorhiza kurroa* plant Rhizome Extract on Diabetic Nephropathy

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Diabetic nephropathy, also known as diabetic kidney disease, is a serious diabetes-associated microvascular complication leading to end-stage renal disease worldwide. The aetiology of diabetic kidney disease is not completely understood. The vasculature, metabolic, and inflammatory pathways are the primary targets for treating diabetic kidney disease. The current treatment strategy relies on inhibiting the renin-angiotensin-aldosterone system (RAAS) and glycaemic control. *Picrorhiza kurroa*, an Ayurvedic herb, is well known for its antioxidant and anti-inflammatory activity. Thus, we aimed to determine the role of *Picrorhiza kurroa* extract on diabetic kidney disease in a rat model. *In vitro* studies on the extract using HEK cells exhibited a significant decrease in the protein expression levels of NLRP3, p47phox, p67phox, gp91phox, IL-1β, TGF-β1, and fibronectin via Western blot analysis. In

addition, the malondialdehyde (MDA) levels were reduced upon treatment. In *in vivo* studies, streptozotocin-induced Albino Wistar rats displayed an improvement in the glomerular morphology when observed at a microscopic level via haematoxylin and eosin staining. The NLRP3, p47phox, TGF-β1, and fibronectin proteins were significantly reduced upon treatment, which was further confirmed via an immunofluorescence study. A decline in MDA levels and an increase in the SOD levels were validated via ELISA. The biochemical analysis revealed a marked decrease in the creatinine levels, whereas no significant change in the glucose levels in the urine. This study concluded that the methanolic extract of *Picrorhiza kurroa* rhizomes possesses anti-inflammatory and antioxidant properties and exhibited a protective action in diabetic nephropathy.

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PP-77

ICAM-1 Inhibits ERK Pathway and neuroinflammation induced by Amyloid-β And Synaptic Degeneration In *5xFAD* Mice

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Gliosis is one of the key expositions of amyloid-β (Aβ) exposure in Alzheimer's disease (AD). Microglia, the immune cells of CNS, innately respond to Aβ and get contrastingly activated during disease course. However, molecular targets to manipulate this transformation towards the enrichment of anti-inflammatory subtype are yet to be uncovered. Here, we interrogated the kinetics of Soluble Intra Cellular Adhesion Molecule (sICAM-1) secreted from Aβ-treated microglia as well as astrocytes and its role in amelioration of neuroinflammation in AD. When we incorporated ICAM-1 intraperitonially in *5xFAD* transgenic mice, it reversed the $A\beta_{1-42}$ mediated microglial as well as astrocytic activation, reduced $A\beta_{1-42}$ mediated neuron loss and cleared Aβ plaqueas observed by immunostaining and western blotting. Moreover, ICAM-1 refurbished synaptic protein expressions and restored synaptic

integrity. ICAM-1 was found to inhibit Aβ mediated ERK activation in microglia as well as subsequent inflammation. Therefore, ICAM-1 plays a pivotal role in regulation of neuroinflammatory meshwork of AD and targeting ICAM-1 could be a good therapeutic strategy.

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PP-78

Cloning Expression and Purification Of Cas9 Protein from Lactic Acid Bacteria

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Lactic acid bacteria (LAB) received attention because of their food-grade status. Recently, there has been a surge in the interest in modulating the genome of LAB for applications in biomedicine and biomedical engineering to improve food quality and control intractable diseases: intestinal infections, obesity, hypertension, colon cancer, etc. One of the key factors in exploring LAB beyond the scope of traditional genetic engineering is intricately linked to the development of a food-grade CRISPR-Cas9 genome engineering tool. Commercial CRISPR-Cas9 is not food-grade; hence it is unsuitable for human application. To accomplish the aim of developing a food-grade CRISPR-Cas9 technology, we have cloned, expressed and purified Cas9 from *Lactobacillus fermentum M1*. We have started doing Bio-physical characterization and in vitro assays.

PP-79

Sortase A: Turning the Foe Into Friend

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In this decade, antimicrobial resistance (AMR) and shortage of new antibacterial medications are the major global public health threats that result in difficulties in treating bacterial infections. Microbes are known to form three-dimensional clusters on both natural and artificial surfaces called biofilms,

and they form a penetration barrier to antibiotic therapy. In finding alternative strategies, antimicrobial peptides (AMPs) captured much attention as an alternative to conventional antibiotics. AMPs have been utilized to kill microorganisms and prevent biofilm formation. Sortase A (Srt A), a transpeptidase enzyme produced by many Gram-positive bacteria, catalyzes a cell wall sorting reaction that results in the attachment of a surface protein with an LPXTG (X= any amino acid) peptide motif on the outer cell surface, thus facilitating the virulence of the bacteria. Many probiotics are Gram-positive bacteria, and sortase A is present on the cell surface. Previously, our research group observed the utility of the surface modifications using sortase A activity. Here we have designed two novel AMPs, CND-LPETG and RKKWFW-LPETG, that could be attached to various surfaces, including medical devices and Probiotic cell surfaces, using the protein stapler Sortase A. We further explored the ability of these peptides to inhibit biofilm formation in Gram-positive bacteria. CND-LPETG, when incubated with *Staphylococcus aureus*, exhibited 57% biofilm inhibition, and RKKWFW-LPETG showed 79% biofilm inhibition. We envisage that this could be an innovative strategy to modify the cell surface of the probiotic *Bacillus clausii* by incorporating these peptides to tackle gastrointestinal tract infections.

Keywords: Antimicrobial resistance, Antimicrobial peptide, Sortase A, Biofilm, Probiotics

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PP-80

Phenome India CSIR Health Cohort Knowledgebase (PI-CHeCK)- Apan Indialongitudinal cohort to develop clinically useful personalized risk prediction scoresfor cardiometabolic disorders in Indian Population

CSIR Cohort Consortium*

** To be presented by Viren Sardana, CSIR-Institute of Genomics and Integrative BiologyNew Delhi on behalf of CSIR-Cohort Consortium*

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Globally, prospective cohorts have been developed and monitored longitudinally to assess risk scores for metabolic and lifestyle disorders. However, India, though has witnessedseveralcohorts localized to districts or states and some being multicentric for metabolic disorders, a Pan Country cohort has been lacking to develop and predict risk scores for cardiometabolic disorders as true countrywide representation is difficultto be obtained. To address this gap and eyeing the future in personalized and precision medicine, CSIR, India has approved the establishment of Phenome India CSIR Health Cohort Knowledgebase (PI-CHeCK), a longitudinal prospective cohort. This cohort leverages the permanent employee base of CSIR including both; currently working and pensionersand their spouses for longitudinal monitoring. This cohort unlike other cohorts leverages the pan India presence of CSIR labs and is expected not to be affected by a high attrition rate. PI-CHeCK would enable collection of blood samples of planned 5000 participants at 3 time points over 5 years. These would be utilized for development of MRM panels for specific diseases and disorders through a proteomic and metabolomics approach. The cohort also plans to conduct objective organ-based assessment of liver, lung function, skin, heart, bone etc through non-invasive testing means.

PP-81

Drug Discovery against SARS-CoV-2 Cysteine Proteases: *In vitro* Enzyme Activity Assay and Structure-Function Relationship

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Rapid evolution of mutations giving rise to various sub-strains evading vaccine based acquired immunization (**1**) made it quite clear that drug based therapeutics are needed along with the vaccines to serve as effective solutions to the lingering problem of COVID19 pandemic. Besides, we still need potent drugs to counter the spread of viral infection within the body in the cases of affected people showing moderate to severe symptoms. Among some other potential drug development targets, we have zeroed in our focus on the two cysteine proteases of the virus; the 3CL-Protrease (mPro) and the Papayin like protease (PlPro) (**2**)**.** Both function as the titular characters in the generation of a functional viral replicase complex (**3**)**,** thus enabling the viral spread and also play essential roles in its

various attempts to outsmart the host's innate immune response triggering the viral host-immune escape (**4**)**.** Therefore, in an attempt to study the detailed structure-function relationships of the two enzymes and find probable inhibitors that can block their activity, we have purified both the recombinant enzymes and screened a library of natural products, known drug molecules and new chemical entities against them (Figure 1). The molecules showing nanomolar and low micromolar inhibitory constant (IC_{50}) can be studied further to develop potential drugs against COVID-19.

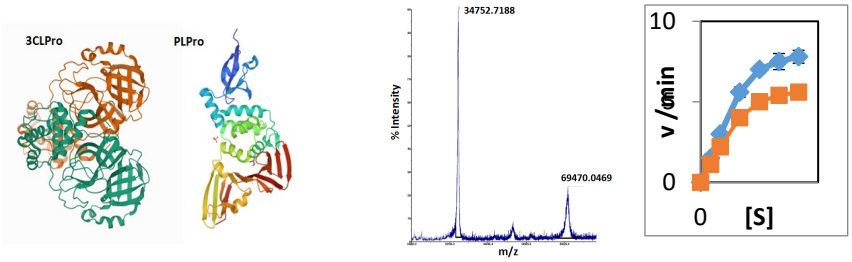

Figure 1: Structure of 3CL-Pro and PL-Pro (left). Mass spectra of recombinant 3CL-Pro. Schematic of enzyme activity assay with fluorogenic substrate in absence and presence of inhibitor (far right).

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PP-82

Naringenin Attenuates Oxaliplatin Induced Neuropathic Pain by Reducing Oxidative Stress and Inflammation: Focus on Mitochondrial Dysfunction

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Oxaliplatin has a great role against malignant colorectal cancer but suffers from dose-limiting side effects with clinical features like numbness, paresthesia, etc.[1] This study focused on the evaluating role of naringenin in maintaining oxidative stress and mitochondrial dysfunction in oxaliplatin-induced peripheral neuropathy (OIPN). The study was conducted in male SD rats treated with oxaliplatin (4mg/kg, IP) twice weekly for four weeks to develop neuropathy. Simultaneously, Naringenin

(25mg/kg and 50mg/kg, oral) was administered.[2] Neuropathic studies were assessed by evaluating mechanical and thermal hyperalgesia.[3] Behavioral, functional, and protein expression results showed that naringenin protects against oxaliplatin-induced peripheral neuropathy. Compared with the oxaliplatin group, naringenin significantly improved against thermal hyperalgesia and cold allodynia and improved nerve perfusion. Also, there was a significant decrease in the expression of PGC-1α, NrF2, TFAM, and Beclin in the sciatic nerve samples of oxaliplatin-induced animals, which suggested the development of mitochondrial dysfunction. The IHC studies in nerve microsections revealed elevated NF-κB, a marker of inflammation, which was reduced with naringenin treatment. Treatment with naringenin prevented mitochondrial dysfunction and oxidative stress. These results suggested that the neuroprotective of naringenin may be due to its antioxidant and anti-inflammatory effects, which might have improved mitochondrial function in OPIN.[4]

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PP-83

In-Vitro Anti-Inflammatory Activity of Extracts from *Wrightia tinctoria* Seedpod

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W. tinctoria, an Indian herb known as *Indrajao*, has been long acknowledged for having significant therapeutic potential in traditional medicine. Different parts of this plant is vastly used in treating wide

array of diseases like jaundice, dysentery, psoriasis, inflammatory bowel disease and type 2 diabetes which are well linked with chronic inflammation. Several studies have highlighted the anti-inflammatory potential of the leaves and bark of this plant, while the seed-pods, commonly used form to treat diabetes by the practitioners of traditional medicine in India, remains unexplored. We demonstrate significant anti-inflammatory potential of the hexane fraction (Fr-B) of ethyl acetate extract of the seedpods in reducing lipopolysaccharide and palmitate mediated inflammation in RAW264.7 macrophages and HepG2 cells respectively. GC-MS and NMR profiling of Fr-B revealed the existence of several poly unsaturated fatty acis and their esters like hexadecanoic acid, ethyl hexadecanoate, 9,12-octadecanoic acid, 9,12,15-octadecatrienoic acid, 9,12,15-octadecatrienoic acid ethyl ester, ethyl linoleate and octadecanoic acid ethyl esters.

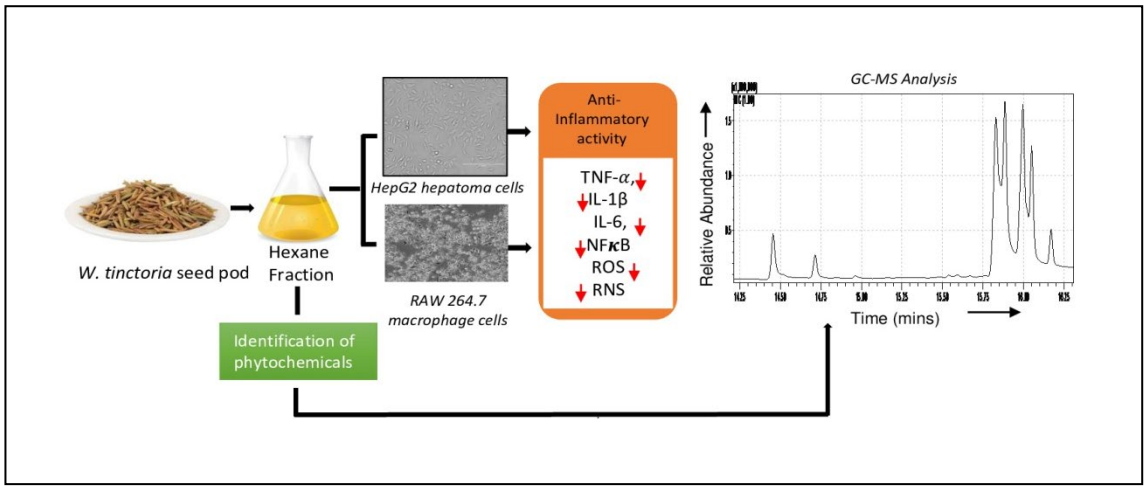

Figure 1: Hexane fraction (Fr-B) of ethyl acetate extract of the *W. tinctoria* seedpod reduces lipopolysaccharide and palmitate mediated inflammation in HepG2 and RAW264.7 cells

PP-84

Effect Of SH3PXD2B From Breast To Lung Cancer Migration And Metastasis

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In spite of advancements in Breast Cancer treatment, BC remains the most common cancer and the second largest leading cause of death among women worldwide (1). Interestingly, the Basal-like breast cancer (BLBC) displays a lung tropism of metastasis (2) causing Lung Cancer (LC) and one of the prime potentialities of metastatic BC is degradation, invasion and migration together through the extracellular matrix (ECM) where the degree of invasiveness can be tied in with the presence of dynamic actin-rich membrane structures called podosomes or invadopodia. SH3PXD2B (3) is a scaffold protein and critical for intravascular and extravascular invasion and metastasis of various types of tumors. The mutations of this gene are well-known for the causality of Frank-ter Haar syndrome (FTHS) and Borrone Dermato-Cardio-Skeletal (BDCS) syndrome is required for podosome formation and is involved in cell adhesion and migration of numerous cell types. Given the emphasis on

podosome/ invadopodia formation and its supportive role in BC migration and metastasis, the question arises as to how SH3PXD2B affects the promotion of metastasis through ECM degradation, imperative for breast cancer progression. We hypothesize that a significant alteration of BC metastasis and migration through molecular modifications of SH3PXD2B on *in vitro* (4) BC, Lung Metastatic BC (LM) and *in silico* BC and LC for the structural insights through molecular modeling as an integrative analysis with (non-small cell LC)-NSCLC. Also, further investigation is on to find out the interactome of SH3PXD2B using immunoprecipitation through Orbitrap-MS as an integrative study for sequence-based predictive models designed to identify binding partners of SH3PXD2B.

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PP-85

Differential adsorption of proteins on graphene oxide: A fluorescence quenching study

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Studying interactions of nano dimensional materials such as graphene oxides and nanoparticles with proteins has gained tremendous importance in recent times due to their potential application in the delivery of drugs, genes and other pharmaceutically important molecules inside cells. We have employed steady state fluorescence spectroscopy and Atomic Force Microscopy (AFM) for investigating the adsorption of three globular unrelated proteins of different size, the tetrameric proteins, alcohol dehydrogenase & Concanavalin A, the small monomeric lysozyme and a large, multidomain, membrane skeletal protein spectrin, on graphene oxide (GO) suspended in aqueous Tris buffer at pH 7.8. We have estimated the equilibrium binding constant (K_b), the changes in Gibbs free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) and found that the K_b associated with the proteins are of

the order of 10^{-4} M and the formation of the GO–Protein complex are spontaneous with ΔG in the range of - 6.5 – 7.0 kJ mol^{-1}. However, the adsorption is modestly endothermic, accompanied by the large increase in enthalpy (ΔH + 32.4 $kJ.mol^{-1}$ for ADH). Differential adsorptions of proteins were evaluated from GO induced quenching study of tryptophan fluorescence and the anisotropy measurements. Quenching of the proteins by GO was found to be biphasic in nature, clearly indicating a combination of both static and dynamic quenching processes. The steady state anisotropy of the protein tryptophans also showed similar trends of changes in GO-bound proteins, both in the native and denatured forms. The thermodynamic parameters indicate a predominantly enthalpy driven association of the proteins at three different temperatures 20, 30 and 40◦C with ADH showing the largest propensity in such interactions.

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PP-86

ModLocator – accurate modification localization tool allows next-generation mod-form mapping from proteomics data

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The protein post-translational modifications (PTMs) impart great diversity to the existing proteins by forming different mod-forms or proteo-forms which may be functionally different. To identify and localize these modforms and understand their connection with their phenotype, their accurate site localization and annotation is a big challenge. Even though identifying protein modifications from shotgun proteomics data has advanced tremendously, the PTM identification rates remain low. It is in part due to tools that are limited to specific fragmentation type, search engines, modification types, statistical models etc. and do not utilize the full MS/MS information. To address these challenges, we developed ModLocator, an algorithm that can facilitate accurate and precise site localization for any arbitrary number of modifications from virtually any database search engine results. It can read tabular format for search results and can directly support modification localization for MSGF+, X!Tandem, MassWiz database search engines that search variable modifications. It can also process open search results from ModA and MSFragger. The algorithm rescores peptides and accurately localizes the PTM site(s) based on probabilistic and heuristic scoring systems. Apart from standard localization scores such as Ascore and PhosphoRS (ptmScore), it can also calculate dot products. It also encompasses LocScore (derived from MassWiz score) that makes it the

only tool to utilize peak intensity to discriminate close mod-isomers. Using synthetic phosphopeptides library benchmark dataset, we benchmarked the accuracy of the algorithm. This is a next-generation localization tool that can handle diverse fragmentation types, search engine outputs, types of PTMs and search modes like variable modification search and open searches.

PP-87

Identification of potential lipid species associated with Coronary Artery Disease

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The identification of lipids in cells, tissues, and bio-fluids is difficult due to the high degree of complexity due to varying concentrations and structural diversity. Therefore, new techniques for in-depth lipid analysis are required. Using a variable retention time window and relative dwell time weightage, we developed a mass spectrometry-based lipidomics screening technique. Within 24 minutes, we were able to identify over a thousand unique lipid species with detection limit ranging between femto to nanomolar. 883 lipid species having a 30% CV could be identified belonging to 16 lipid classes. We also attempted to identify isomers with the TAG lipid class.

Lipidomics profiling is a strong approach for exploring new biomarkers and mechanisms for various pathological conditions. It could also help delineate the mechanisms associated with cardiovascular diseases since perturbation of lipid metabolism is prominent in CVD and is one of the main mechanisms that causes disease progression. In this regard developed multiple reaction monitoring (MRM) based mass spectrometric technique was used to identify lipid species that might be exploited as possible biomarkers for CAD. The study was conducted at CSIR-IGIB in collaboration with All India Institute of Medical Sciences (AIIMS), New Delhi. We analyzed 304 samples (153 control and 151 CAD) using our recently developed MRM based lipidomics approach where more than 1000 lipid species representing different types of lipid classes can be identified. MultiQuant 3.0.2 software was used for peak review and data processing. This approach led to the identification of several lipid species which were Significantly Differentially Expressed (SDE) in CAD samples (adjusted by BH and confirmed/tentative selection using Boruta random forest). We found ceramides species, CER.24.1, CER.18.0 were elevated in CAD and CER.24.0 were down compared to control. Plasma ceramides have been shown to be a promising marker in the identification of patients at risk of adverse cardiovascular events. We also show that triglycerides containing long chain fatty acids and not short and medium chain are associated with CAD risk in Indian population. Lipid biomarkers along with clinical predictors might be useful candidates in differentiating CAD from healthy individuals.

We have also used this method to understand the altered lipidome in cases of B12 deficiency.18 plasma lipids were found altered owing to vitamin B12 insufficiency. Lipid species containing ω-6 fatty acid chains were found to be considerably elevated in vitamin B12 deficient samples, whereas ω-3 fatty acid chain lipid species were found to be reduced.

This technology allows quick screening of a large number of lipid species in a single experiment, advancing our understanding of lipids in biological processes.

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Plasma proteomics unravels pathways associated with Acute-on-chronic liver failure and their outcomes in patients with cirrhosis

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Background: Progression from AD to ACLF impairs survival in patients with cirrhosis. To discern pathophysiology of disease progression and patients' outcomes, we employed untargeted plasma proteomics.

Patients & Methods: Hospitalized AD or ACLF patients and healthy controls (HC) were recruited. Plasma samples bio-banked, at recruitment, were depleted off the abundant proteins before LCMS proteomics. GO and pathway analysis was performed using overrepresentation approach.

Results: Proteomics was performed in 29 AD, 55 ACLF patients and 10 HCs**.** Patients were age and sex matched, age 43 and 40 years; 87.2 % and 93.1% males in ACLF & AD. ACLF patients had higher WBC count (14700 vs. 7300), procalcitonin (1.3 vs. 0.42), SIRS components (2 vs 1), CLIF-C-OF (12 vs 7) and mortality (74.5% vs. 17.2%) (p= 0.000) than AD.

Of 512 identified proteins, 30 were differentially expressed between AD and ACLF group ($p<0.05$); 85 and 2 exclusively expressed in ACLF and AD. Pathways of immune activation in response to stress and infections such as chemotaxis, neutrophil degranulation and leukocyte activation were upregulated in both AD and ACLF vs. HCs, a disease defining fingerprint. ACLF patients overexpressed immunoregulatory pathways and negative regulation of oxidative stress than AD group. Phagocytosis, complement activation, carbohydrate and lipid metabolism were significantly downregulated in ACLF than AD conferring immune-metabolic failure in ACLF. Highly compromised regulation of coagulation cascade was noted in cirrhosis, which progressed from AD to ACLF. Similarly, there was loss of positive regulation of protein processing machinery in cirrhotics and a stepwise increase in the proteolytic stress was

observed from AD to ACLF. 63 DEPs were noted in non-survivors versus survivors (adj. p<0.05).

Conclusion: Proteomics revealed aberrations in metabolic pathways, proteolysis, coagulation majorly associated with ACLF group. Increased proteolytic stress, and immunometabolic failure were majorly associated with conferring poor outcomes in patients with ACLF.

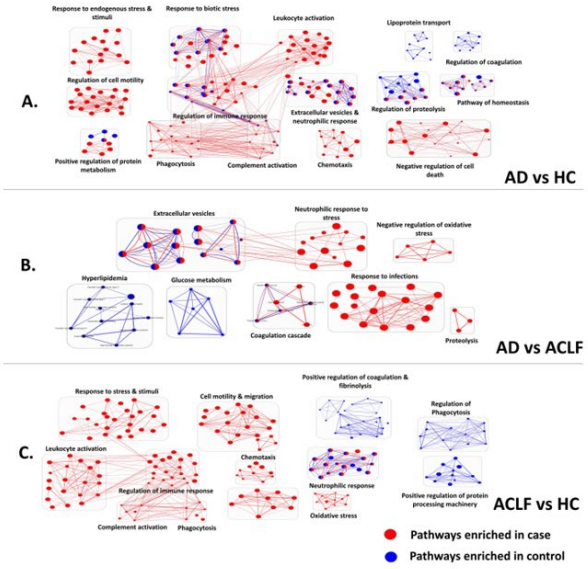

Figure 1: Pathway network map

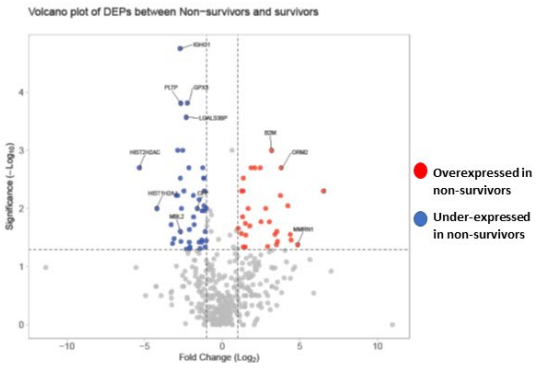

Figure 2: Volcano plot depicting DEPS between survivors and non-survivors

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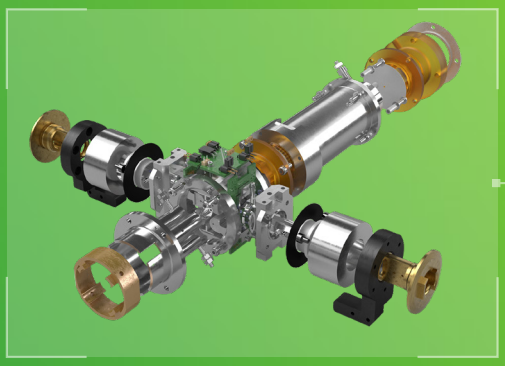

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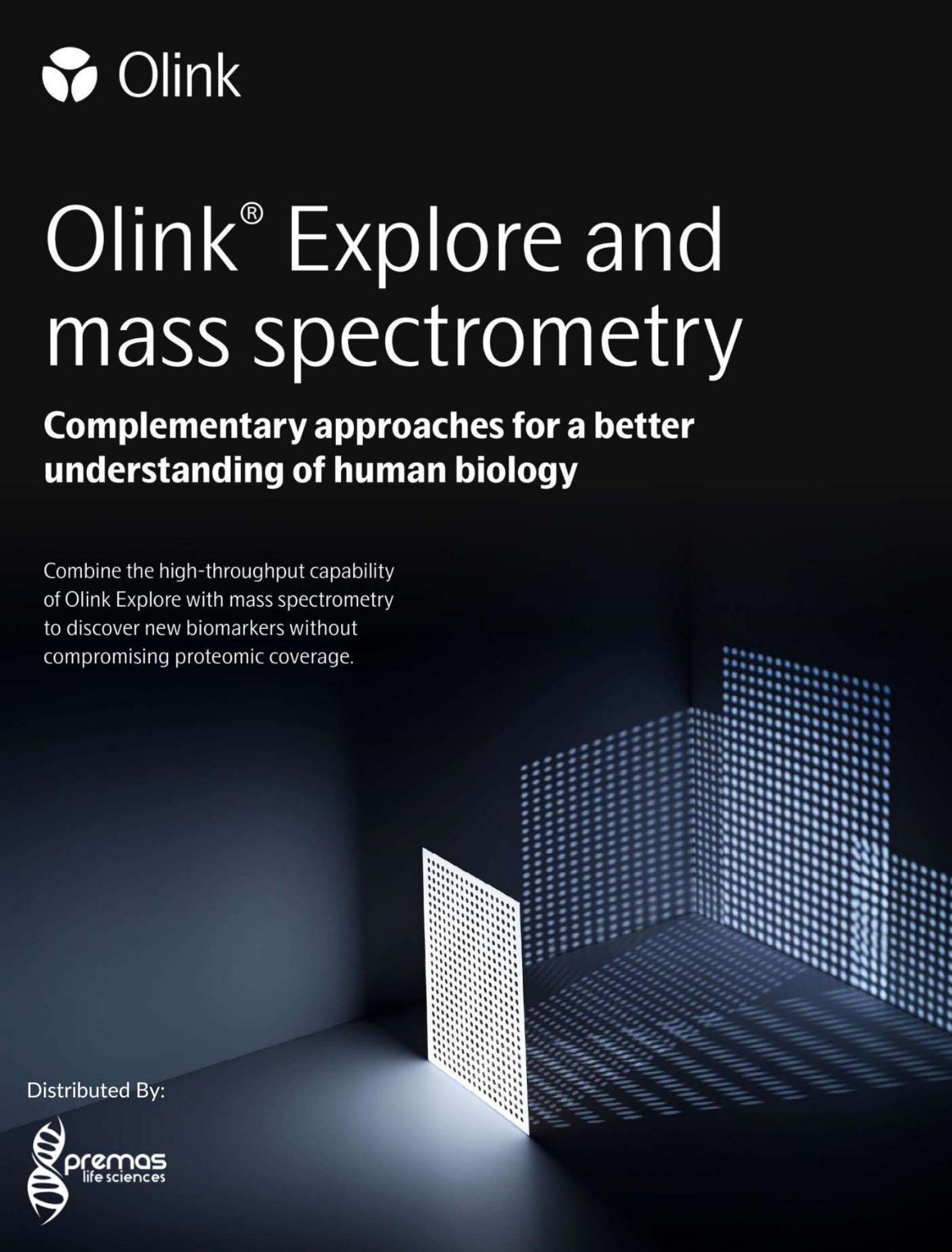

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