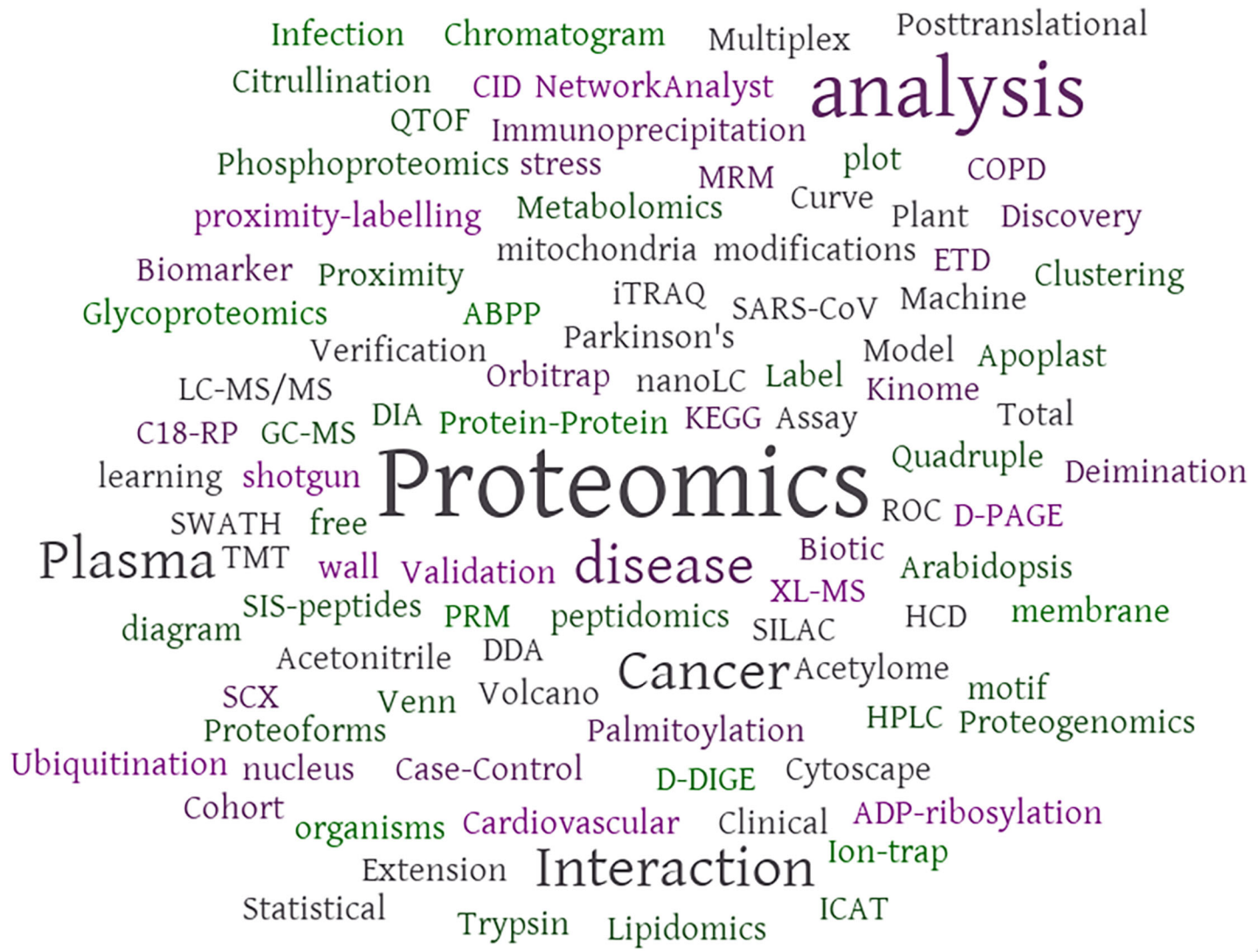
PSI Newsletter Vol. 9/No.1/JULY, 2021

PROTEOMICS SOCIETY, INDIA (PSI)



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Editor's Message

Dear Members of PSI,

During these very challenging times, through this Newsletter we bring you the interesting research that our members have been conducting.

The new initiative of PSI monthly webinars, was started to provide a platform on which members could meet up to listen to research addressing different biological queries using Proteomic tools. We have completed five. webinars during January to May 2021. We will continue the series from July 2021.

Members have conducted Proteomics day in Alagapa University, Karaikudi and at the Narendrapur campus of the Ramakrishna Mission Vivekananda Educational and Research Institute near Kolkata. These programs ensured that PSI could reached out to students and faculty in different parts of the country.

The Research Desk provides abstracts from the publications of our members. You will note that Proteomics has been used to study model systems, COVID, cancer, plant biology and pregnancy. What a range of areas of investigations!!.

Members are encouraged to contact the authors if they are interested in the published work, wish to collaborate with the authors or learn specific techniques in their laboratories. Do take advantage of this information.

We will continue to bring you the excitement of Proteomic Research in India. Do write to us with your views and suggestions.

With Regards,

Surekha Zingde, Tushar Maiti

Message from President, PSI

Dear Members of the Proteomics Society, India,

Hope you all are well and I would like to wish everyone a much safer days and cleaner environment ahead during this very difficult time that the world is facing.

This issue of the Newsletter testifies the major progress made by PSI even during the most challenging time. As was intended, the Proteomics Society, India, has continued to be devoted and dedicated to provide an excellent platform for its members and all the proteomers from India and abroad. As promised, PSI held webinars in the field and will continue with more such events throughout the Year. I am sure those who have attended the webinars must have enjoyed and I invite everyone to keep themselves updated and join the future events too. Also the "Proteomics day" celebrated at the Narendrapur campus of the Ramakrishna Mission Vivekananda Educational and Research Institute and Alagapa University, Karaikudi provided such platforms for academic interaction, particularly for young students and researchers.

Each year, the Society endeavour to come up with select themes for its annual meeting focused upon contemporary issues in the field. I have the honour and privilege to invite you to attend the Virtual 13th Annual meeting of PSI 2021 and International conference on "OMICS in Redefining Modern Biology" to be held during October 20th -23rd, which will bring together the leading scientists and scholars in the area to discuss the practical challenges, concerns and strategies in the field, promote collaborations and information exchange and share the new findings. Furthermore, if you have not done so already, it is time to visit https://hupo2021.org/ and PSI website to know more about this year HUPO ReCONNECT meeting and the annual meeting of PSI.

Journal of Proteins and Proteomics, administered by PSI will continue to serve the scientific fraternity to publish and read new findings in the area of protein science.

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

I wish to thank all PSI members for the success and invite to contribute to the Newsletter and future endeavours of the Society. At the end, I congratulate the Editors of this Newsletter for their excellent work to bring out this issue.

Once again, I wish the entire PSI Family a safe and healthy time ahead and an enjoyable reading of the newsletter.

With best wishes for a successful 2021,

Dr. Subhra Chakraborty

President, PSI

Monthly Webinar: A PSI new initiative

Dr. Subhra Chakraborty

Dr. Surekha Zingde

Dr. Sanjeeva Srivastava

Dr. Mahesh Kulkarni

PSI decided to initiate monthly webinars from January 2021 with the primary aim of providing a platform wherefrom PSI members could meet on a regular basis and also be informed how proteomic tools along with other OMICS technology are being used to address biological queries. The webinars were targeted to scientists who were considering introducing proteomics in their investigations, students and faculty who could update their knowledge and senior scientists who were interested in learning how proteomics was being used beyond their own focus areas of research.. The Webinars were conducted on the last Friday of each month. This Newsletter brings you the abstracts of the lectures which were conducted during January to May 2021. We take this opportunity to thank the Scientists who delivered the lectures and those who Moderated the proceedings.

WEBINAR-1: 29th January 2021

Dr. Srinivas Ambutipudi,
Associate Professor
Department of
Biotechnology,
IIT, Roorkee

SPEAKER

Dr. Ashok Mohanty,
NDRI, Karnal
(SESSION MODERATOR)

Indicators of Internal Ecological Factors of Shifting from Bovine Mammary Symbiosis to Dysbiosis: How Early? and How Fast?

Bovine milk contains different components with nutritional and immunological benefits and reflects animal pathophysiology. One such disease is mastitis, a prototypic emerging and reemerging bacterial disease that results in cut-by-cut torture to public health, animals, and the global economy. The complex interaction between pathogens and milk components that create a menace during mastitis, affecting animal and human health, has remained relatively unexplored. Of different milk components, the milk fat globule membrane (MFGM) influences the human intestine's microbial colonization. A comparative analysis between fresh and mastitic globules showed preferential adhesion of *Lactobacillus fermentum* to fresh MFGM, highlighting their potential application as a probiotic delivery vehicle and altering intestinal microbial consortia. Similarly, a systematic profiling of proteins and lipids in healthy cows and buffaloes showed seasons and breed-specific changes, indicating the dynamic nature of milk and provides a foundation for future studies linked to diseases specific to seasons and breeds. Subsequently, to gain more knowledge and identify possible gaps in milk physiology to maximize health-promoting effects, we developed a manually curated, an open online database of bovine milk proteome, BoMiProt (http://bomiprot.org), constituting over 3100 proteins identified by researchers to data, highlighting crucial insights.

Bovine mastitis caused by *Staphylococcus aureus* is a significant impediment to milk production, causing enormous economic loss to the dairy industry worldwide. Of the different mastitis stages, subclinical mastitis (SCM) is the asymptomatic form causing significant loss economically as symptoms typically precede by many days and weeks before a diagnosis could be established. In contrast, clinical mastitis (CM) is easily detected through clinical symptoms. Considering the paucity of markers to detect SCM and the progression to CM, milk samples were collected based on histological confirmation and somatic cell counts (SCC) for proteomic analysis. Several proteins were collectively identified and quantitated with crucial insights into whey proteome dynamics and signature patterns indicative of disease progression. Taken together, results highlight the complexity of mastitis, including the interplay between host immunity, mammary ecology, and the shift from symbiosis to dysbiosis.

WEBINAR-2: 26thFebruary 2021

Dr. Manas Kumar Santra,
Scientist F,
National Centre for Cell Sciences,
Pune
(SPEAKER)

Dr. Ravi Sirdeshmukh,
Distinguished Scientist & Assoc. Director, IOB;
Principal Advisor, MSCTR, Bengaluru
(SESSION MODERATOR)

Reaching the drop through the ocean: Proteomics to capture dynamic interactions to understand cancer

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

SCF (SKP1, Cullin1 and F-box protein) ubiquitin ligase complexes catalyze the ubiquitination of most of the cellular proteins and responsible for degradation of 70% of cellular proteins. F-box proteins (have conserved F-box motif) function as substrate receptor in the complex and determine the substrate to be ubiquitinated. Deregulation of protein ubiquitination process by SCF complex leads to many diseases including cancer. Human genome encodes genes for 69 F-box proteins. We are working on F-box protein FBXO31. It functions as a tumor suppressor and is a dedicated DNA damage checkpoint protein. Using proteomic approach, we identified the cellular substrates of FBXO31 under DNA damage condition. We have discovered the molecular mechanism of regulation of key factors associated with the genomic stability by FBXO31. Details of the work will be discussed in the presentation.

WEBINAR-3: 26thMarch 2021

Dr. Ashok P Giri
Senior Principal Scientist
CSIR- NCL, Pune.
(SPEAKER)

Dr. Niranjan Chakraborty,
Professor of Eminence, NIPGR
New Delhi
(SESSION MODERATOR)

Approaches to unravel Molecular dynamics of Plant-Insect Interactions

Discovery, design, development and successful application of biomolecule(s) for agriculture and allied areas are in demand. Plant have inherent strengths to combat against insect pests and pathogens. In particular, natural defense of plants against insect pests exits at multiple levels and operated in a sophisticated manner. We have employed omics approaches to obtain molecular insights in to this dynamic plant-insect interactions. For example, molecular complexity exits in plant protease inhibitors (PPIs) and alpha-amylase inhibitors that interfere with insects' digestive enzymes. Depending upon cocktail of digestive enzymes in target insects, plants are capable of synthesizing variety of inhibitors at constitutively or upon insect attack. Here several such examples will be provided as outcome. Among them are serine PPI which are wound inducible and possess multiple inhibitory repeat domains. Upon exposure to recombinant PIs a dynamic transition in insect protease gene expression is evident. Transcriptomic, proteomic and metabolic analysis corroborate protease accumulation with additional several small molecules that might have significant role in insect physiology. In continuation to develop small molecules for crop protection, we have derived peptides from reactive center loops (RCLs) of natural serine PPIs. Both linear and bicyclic RCLs exhibited feeding deterrent and growth reduction activity against lepidopteran insect pests. Our results show that RCLs are potent inhibitors of insect proteases which could be used as pest control molecules in agriculture. This provides basis to develop next generation bio-inspired cyclic peptides for agriculture or medicinal applications.

WEBINAR-4: 23rd April 2021

Dr. H.A. Nagarajaram
Professor & Head
Department of Systems and Computational Biology,
University of Hyderabad,
Hyderabad
(SPEAKER)

Dr. Debasis Dash,
Chief Scientist and Head
Informatics and Big Data Unit
CSIR –IGIB, New Delhi
(SESSION MODERATOR)

Structural and Network-based Insights into Protein-Protein Interactions

One of the focusses of proteomics-based studies has been to elucidate proteome-wide protein-protein interactions under given spatio-temporal conditions. In order to understand condition specific influence (for example, known disease variants, infections caused by pathogens etc.), on protein interactome it is pertinent to have an understanding of the molecular features that form basis for intra as well as inter protein interactions. Detailed structural analyses of experimentally derived 3D structures of protein complexes have yielded invaluable information on specific molecular features that include the nature of binding interfaces as well as the associated physical forces that stabilize protein-protein binding. Using these one could discern, as for example, possible impacts of mutations at individual protein level as well as at the level of inter protein interactions. In addition, availability of proteome-wide data on inter protein interactions has enabled construction of protein interaction networks which show striking similarity to the other real-world networks as social networks, www etc., in their design principles. Furthermore, network representations of protein interactomes enable one to decipher the so-called centrality values of proteins which in turn relate to their essentiality in living cells. In this talk, I shall be discussing some of the interesting and essential aspects of inter-protein interactions at the molecular level as well as at the network level and possible impacts of variations in proteins, as discerned by some of the studies including those from my group.

WEBINAR-5: 28th May 2021

Dr. Pawan Malhotra
Group Leader-
Malaria Biology
ICGEB, New Delhi
(SPEAKER)

Dr. K. Dharmalingam,
Director- Research, AMRF, Madurai
(SESSION MODERATOR)

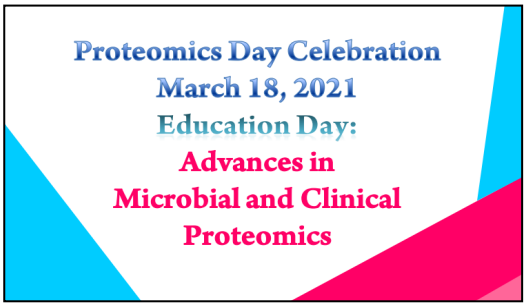
Revelation of novel secretory proteins, PTMs, protein complexes and drug targets using proteomic approaches on *Plasmodium falciparum* blood stages

P*lasmodium spp*. are obligate intra-erythrocytic protozoans parasites that undergo a number of developmental stages in the vertebrate host and mosquito vectors. Despite continuous efforts to eradicate malaria for over 5 decades, it still remains a major health problem mainly due to poor understanding of malaria biology. With the development of latest transcriptomic and proteomic tools, recently a great amount of information has been generated with respect to *Plasmodium* biology resulting in identification of new vaccine candidate antigens and drug targets. We have applied proteomic approaches over the past decade on asexual blood stages of parasite to understand the processes involved in parasite invasion and parasite development with an aim to identify novel vaccine/drug candidate antigens.

In the year 2009, a detailed proteome study was carried out in our laboratory to illustrate the parasite secretome. Twenty seven novel secretory proteins were identified. *In silico* computational analysis and subsequent characterization studies confirmed the secretory nature of these proteins. Further work characterized two of these secretory proteins; PfDegP and PfCCp1 for their role(s) in combating oxidative and thermal stresses and for inhibiting the complement system activation (Singh, M et al., 2009 **Mol. Cell Proteomics**, Sharma, S et al., 2014 **FEBS J** & 2018 **BJ**). In the next step, proteomic approaches were applied to identify protein complexes, in particular, the complexes involved in merozoite invasion of human RBCs as well as markers associated with severe malaria/cytadherence. One of the important complexes that was identified was the haemoglobin degradation & Hz formation complex. This complex has been characterized and is being targeted for new drug discovery (Chugh, M et al 2013, **PNAS, USA.,** Gupta, P. et al., 2017. **J Med Chem).** In a recent study, we successfully carried out proteome-wide analysis to evaluate the extent of arginine and lysine methylation of *Plasmodium* proteins at asexual blood stages of the parasite. To identify the *Plasmodium* "**Methylome**", the reactivity of anti methyl lysine as well as anti-methyl arginine antibodies was tested with the asexual stage *P. falciparum* parasites. Using LC-MS/MS analysis, 605 lysine methylated sites were identified within *Plasmodium* 422 proteins and in case of arginine methylation, 843 *Plasmodium* proteins were found to be methylated. Motif analysis revealed lysine and arginine methylation associated with GK, MK, GRx/RGx, RxG, GxxR motifs. Many of the *Plasmodium* methylated proteins have homologs known to be methylated in *Trypanosome*, human and yeast. Functional classification of methylated proteins revealed that these proteins are mainly involved in chromatin organization, trafficking and homeostatic processes and protein folding (Zeeshan, M et al. 2017. **J Prot Res;** Kaur, I et al., 2016. **Sci Report**).Overall, proteomic approaches have helped to illustrate novel secretory proteins, drug targets, novel markers and also the methylome of the parasite.

Reports on Proteomics Day

Proteomics Day Celebration 2021, Alagappa University, Karaikudi



The Proteomics Day was celebrated at the Department of Biotechnology, Science campus, Alagappa University on March 18th, 2021 with a focal theme "**Advances in Microbial and Clinical Proteomics"**. Ninety five participants from it host institute comprising Faculty members, Research Scholars and PG Students actively participated in the celebration. In addition to that, 15 participants comprising of Scientists and Research scholars from Aravind Medical Research Foundation, Madurai attended the meeting in deference to the invitation extended. The meeting was organized with an objective to educate advances in microbial and clinical proteomics and also to train 2D- Gel Electrophoresis & MALDI TOF/TOF. Updates on the latest developments in the area of Proteomics to the young Research Scholars and PG Students were explained.

The participants were formally welcomed for the Proteomics Day Lectures by **Prof. K. Balamurugan**, Executive Committee member, Proteomics Society of India and Professor in Department of Biotechnology, Alagappa University. In his Welcome and Thematic address, he explained about the genesis of Proteomics Society of India (PSI), activities of PSI in the recent past and current scenario. He also emphasized the importance of celebrating Proteomics Day and mentioned that understanding the crucial roles of Protein and modern day proteomics tools are more important for the scientific community. It also provides a chance to understand the needs of the biological system, to entangle any problems associated with microbial infections now and in near future.

Dr. S. Karutha Pandian, Senior Professor and Head of the Department of Biotechnology, Alagappa University gave a lecture on the scientific achievement made by his group in the field of proteomics under the topic "Advances in Microbial Proteomics". He dwelled on the novel therapeutics developed by his group for microbial infections. Furthermore, he also explained the basic details related to 2D Gel electrophoresis and MALDI-TOF technologies to the participants. He also highlighted the advances in microbial proteomics and its implications. He also encouraged the young upcoming scientists to embrace proteomics technology for their scientific research.

Reports on Proteomics Day

Prof. K. Dharmalingam, an eminent scientist working in the area of clinical proteomics, delivered the Proteomics Day lecture on "Leaderless proteomics of platelet dust". He emphasized the importance of leader sequences associated with secreted proteins and also the roles of multivesicular bodies. He has introduced the importance of extracellular vesicles, in particular on exosome proteins which are involved in cell-cell communications and cargo tools. He also explained the parameters required to analyze proteins using advanced Mass spectrometry tools. He further described the connectivity between clinical diseases, in particular with exosomal proteins as therapeutic targets.

After a short break for high-tea, Research Scholars working in the area of Clinical Proteomics presented and discussed their work as poster presentations. In total, 13 numbers of posters were displayed in the area of advanced proteomics. The Poster Session paved way for PG students to directly interact with research scholars on their work.

Afternoon interaction session was held in the mini-conference hall with **Prof. K. Dharmalingam** and Reasearch Scholars working in clinical proteomics and microbial proteomics.




Finally, **Prof. K. Balamurugan** proposed a formal vote of thanks wherein he profusely thanked Prof. K. Dharmalingam who is an authority in proteomics for having shared his team's rich experience and success stories to the budding proteomics scientists. Further, he appreciated the Scholars who have made poster presentations which paved the way for getting critical reviews/comments from experts. He profusely thanked the PSI for having provided funds for celebrating the Proteomics Day Celebrations at Alagappa University in a befitting manner.

Proteomics Day Webinar at Ramakrishna Mission Vivekananda Educational and Research Institute

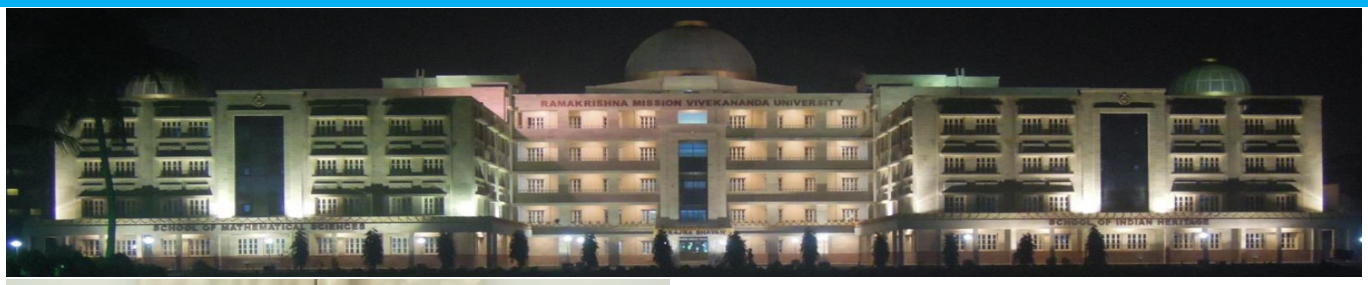
Professor Abhijit Chakrabarti, Saha Institute of Nuclear Physics, Kolkata
Dr. Kishor Kumar, Ramakrishna Mission Vivekananda Educational and Research Institute (RKMVERI)

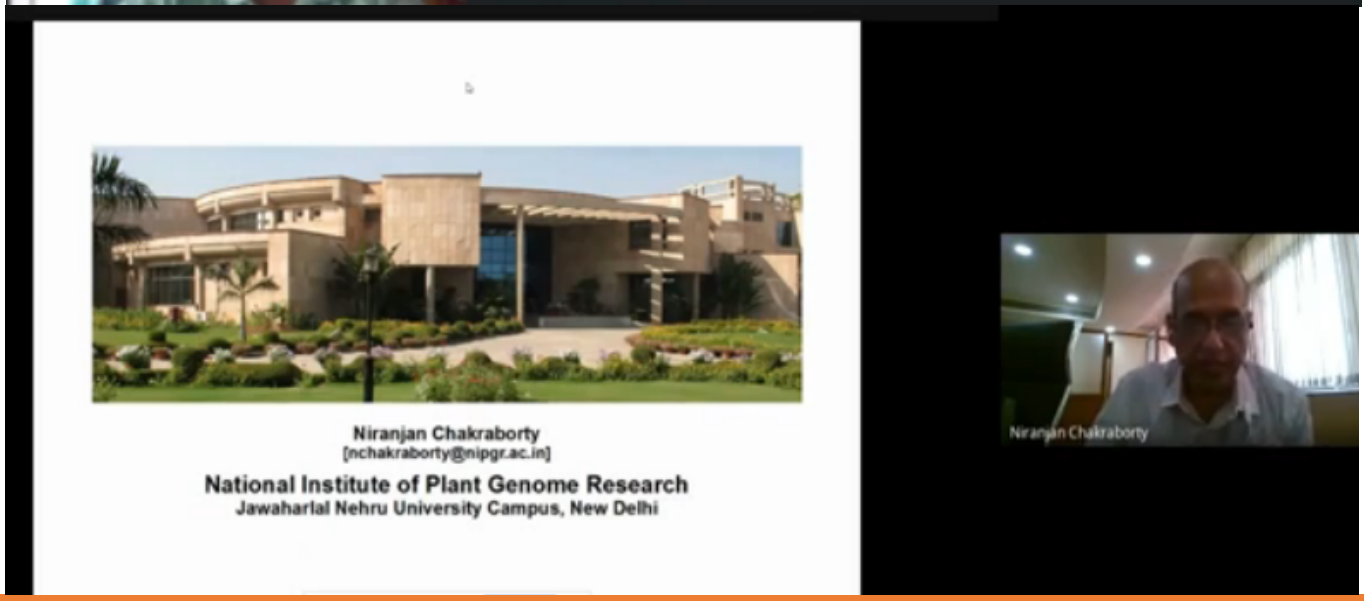
Proteomics day was celebrated through a Webinar entitled **"Proteomics in our daily life: from Agriculture to Health"** in Ramakrishna Mission Vivekananda Educational and Research Institute (RKMVERI) with the support of Proteomics Society, India (PSI) on 18 March 2021. Established in 2005, RKMVERI is an institution deemed-to-be university as declared by the Ministry of Human Resource Development, Govt. of India. At present, it operates through four campuses situated at Belur Math near Kolkata (main campus and headquarters), Coimbatore, Narendrapur (Kolkata) and Ranchi. The School of Biological Sciences of RKMVERI assumes a unique distinction with its already existing programs in Agricultural Biotechnology and subsequent addition of a new MSc program in Medical Biotechnology at the Narendrapur off campus in collaboration with the RKM Seva Pratishthan from October 2020. The day long Webinar was aimed particularly for the young researchers and students of both the disciplines, giving exposure to the area of proteomics and its applications in agriculture and health. The program was organized at the Integrated Rural Development and Management (IRDM) centre at the Narendrapur campus adhering strictly to the Covid-19 guideline.

Three eminent scientists in the field gave excellent lectures of 45 minutes duration, attended by 110 participants. About 60 of them were students attending from IRDM laboratories and classrooms, and about 50 more M.Sc students, research scholars, members of the faculty of neighbouring universities, research institutes and from different campuses of RKMVERI. Publicity of the webinar were done through circulation of e-posters and e-flyers among them.

The webinar was conducted via the Google meet platform using screen sharing and arranged in two sessions: pre-lunch and post-lunch session. The program took off with the inaugural speech and word of blessings from Swami Atmapriyananda, Pro-Chancellor, RKMVERI followed by Swami Sarvottamananda, Vice- Chancellor, RKMVERI and Swami Sarvlokananda, Secretary, Ramakrishna Mission Ashrama, Narendrapur. The pre-lunch session was moderated by Prof. Rukhsana Chowdhury, RKMVERI. The first speaker, Dr. Niranjan Chakraborty of National Institute of Plant Genome Research (NIPGR) spoke on “Understanding mitochondrial proteome dynamics and defense response: turning knowledge into application” and the second talk was delivered by Dr. Shantanu Sengupta, of CSIR Institute of Genome and Integrative Biology (CSIR-IGIB) on “Clinical Proteomics for Disease Diagnosis and Prognosis”. After each talk, there were short discussions, where students and other participants took active interest and interacted with the speakers. After a short lunch break, Dr. Arun Bandyopadhyay of CSIR Indian Institute of Chemical Biology (CSIR-IICB) spoke on “Proteomic Analysis for Understanding Pathophysiology of Coronary Artery Disease”. The session was moderated by Prof. Subrata Banerjee of RKMVERI. The webinar ended with the vote of thanks by Dr. Kishor Kumar, the Convenor of the webinar.

Reports on Proteomics Day





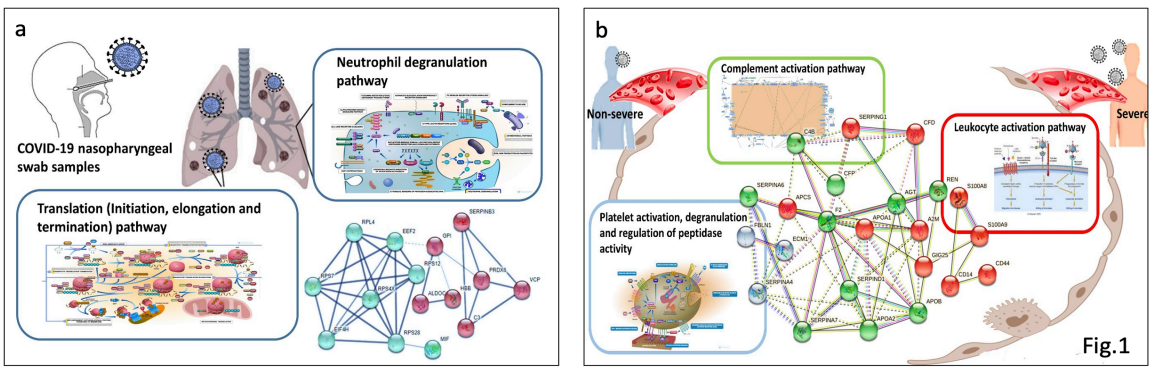
A multipronged deep omics-based investigation of COVID-19 samples identifies key molecular networks of disease severity progression

Kruthi Suvarna[1], Deeptarup Biswas[1], Sachee Agrawal[2], Om Shrivastav[2], Jayanthi Shastri[2] and Sanjeeva Srivastava[1]

[1] *Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai, India.*

[2]*Kasturba Hospital for Infectious Diseases, Chinchpokli, Mumbai, Maharashtra 400034.*

The altered molecular proteins and pathways in response to COVID-19 infection are still unclear. Here, we performed a comprehensive proteomics-based investigation of nasopharyngeal swab samples from patients with COVID-19 to identify the viral peptides and study the host response by employing simple extraction strategies (**Pat #202021034687**). Few of the host proteins such as Interleukin-6, L-lactate dehydrogenase, C-reactive protein, Ferritin, and aspartate aminotransferase were found to be upregulated only in COVID-19-positive patients using targeted multiple reaction monitoring studies. The most important pathways identified by enrichment analysis were neutrophil degranulation, interleukin-12 signaling pathways, and mRNA translation of proteins thus providing the detailed investigation of host response in COVID-19 infection (**iScience, 2021**). Furthermore, considering that the blood samples are most suitable for rapid clinical translation, we also performed the label-free quantitative proteomics of plasma samples from a cohort of 71 patients (20 COVID-19 negative, 18 COVID-19 non-severe, and 33 severe). Of the 1200 proteins detected in the patient plasma, 38 proteins were identified to be differentially expressed between non-severe and severe groups. The altered plasma proteome revealed significant dysregulation in the pathways related to peptidase activity, regulated exocytosis, blood coagulation, complement activation, leukocyte activation involved in immune response, and response to glucocorticoid biological processes in severe cases of SARS-CoV-2 infection. Furthermore, we employed supervised machine learning (ML) approaches using a linear support vector machine model to identify the classifiers of patients with non-severe and severe COVID-19 **(Front. Physiol., 2021)**. Putative biomarkers such as angiotensinogen and SERPING1 and ML-derived classifiers including the apolipoprotein B, SERPINA3, and fibrinogen gamma chain were validated by targeted mass spectrometry-based multiple reaction monitoring (MRM) assays **(Pat # 202023054753)**. Thus, we conclude that mass spectrometry-detected host proteins have a potential for disease severity progression; however, suitable validation strategies should be deployed for the clinical translation. Furthermore, the *in silico* docking of potential drugs with host proteins involved in the dysregulated pathway might aid in COVID-19 therapeutic interventions



Bankar *et. al* (2021) ***iScience***, 24:102135; Suvarna *et al*. (2021) ***Frontiers in Physiology***, 12:652799

Proteomic Alterations in Multiple Myeloma: A Comprehensive Study Using Bone Marrow Interstitial Fluid and Serum Samples

Venkatesh Chanukuppa[1], Ravindra Taware[1], Khushman Taunk[1], Tathagat Chatterjee[2], Sanjeevan Sharma[3], Venkatesan Somasundaram[3], Faraz Rashid[4], Dipankar Malakar[4], Manas K. Santra[1] and Srikanth Rapole[1],*

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[2]*Army Hospital (Research and Referral), Dhaula Kuan, New Delhi, Delhi 110010*
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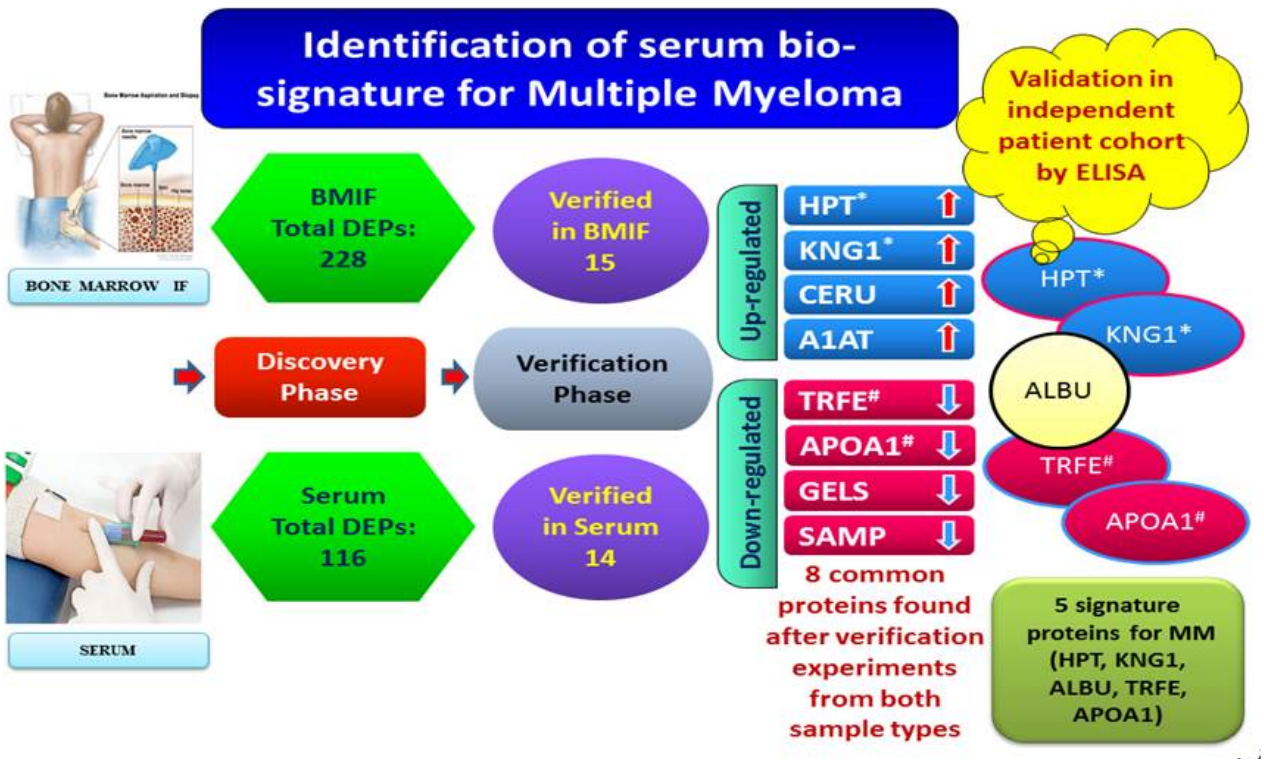
Multiple myeloma (MM) is a plasma cell-associated cancer and exists as the second most common hematological malignancy worldwide. Although researchers have been working on MM, a comprehensive quantitative bone marrow interstitial fluid (BMIF) and serum proteomic analysis from the same patients' samples is not yet reported. The present study involves the investigation of alterations in the BMIF and serum proteome of MM patients compared to controls using multipronged quantitative proteomic approaches viz., 2D-DIGE, iTRAQ, and SWATH-MS. A total of 279 non-redundant statistically significant differentially abundant proteins were identified by the combination of three proteomic approaches in MM BMIF, while in the case of serum 116 such differentially abundant proteins were identified. Verification experiments were performed in a fresh independent cohort of samples using immunoblotting and MS based SRM assays. Thorough 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



Chanukuppa *et al*. (2021) ***Frontiers in Oncology,*** 10:566804

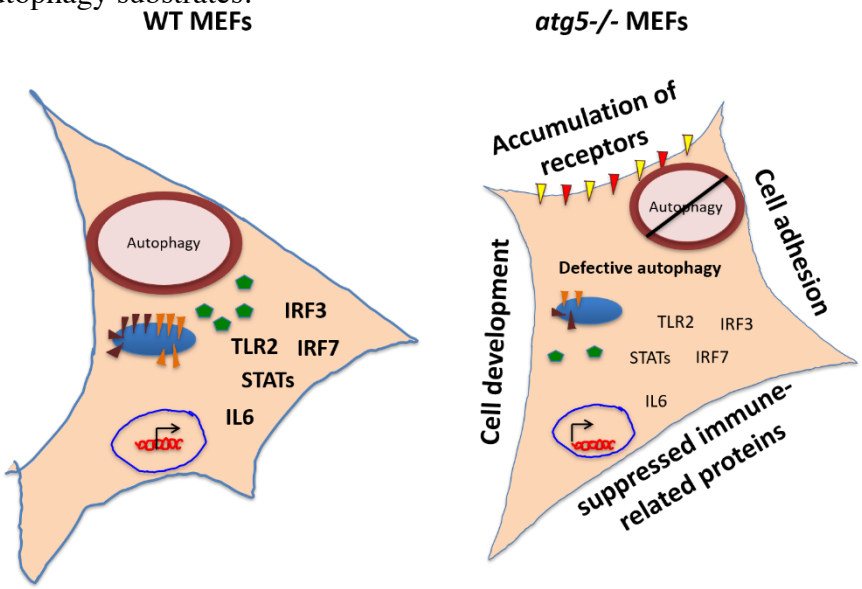
Characterization of autophagy modulated cellular pathways through a quantitative proteomics approach

Kiran Bala Sharma and Manjula Kalia

Regional Centre for Biotechnology, NCR Biotech Science Cluster, Faridabad, Haryana, India

Autophagy is a crucial cellular degradative pathway that maintains cellular homeostasis by controlling protein turnover and removal of damaged organelles and aggregated proteins. It regulates protein levels under both basal and stress conditions such as starvation, infection, cancer and neurodegeneration. Genetic or pharmacological perturbation of autophagy results in massive changes in the cellular proteome and impacts nearly every biological process.

Quantitative mass spectrometry (MS) based proteomics has emerged as a powerful technique to quantitate protein abundances, and can generate comprehensive comparative maps of global proteome/biological changes under diverse conditions. Tandem mass tag (TMT)-based MS proteomics is one such robust quantitative technique that can examine relative protein abundances in multiple samples (parallel multiplexing). We examined the impact of basal autophagy on the proteome of mouse embryonic fibroblasts (MEFs) through TMT-based quantitative mass spectrometry analysis of WT (autophagy competent) and *atg5-/-* MEFs (autophagy deficient) cells[1]. At a 1% false discovery rate, our study identified 151,284 peptides that corresponded to 10,032 proteins. After exclusion of proteins with less than two unique peptides, a total of 7795 were short-listed for further analysis. Functional enrichment analysis was applied to proteins showing differential expression. We observed that absence of autophagy remodels nearly 14% of the total identified proteome. The cellular pathways that were impacted most were development, adhesion, transport, signal transduction, metabolism, immune and inflammatory responses. Several of the upregulated proteins were receptors involved in TGFβ signalling, JAK-STAT signalling, junction adhesion, and interferon/cytokine-receptor interaction, and were validated as autophagy substrates. This established the constitutive role of autophagy in receptor turnover and potential to impact the associated signalling. Several crucial innate immune sensors and effectors were downregulated in the autophagy deficient condition. The study provides a comprehensive overview of the autophagy-regulated biological processes, protein-protein interaction networks, and identified novel autophagy substrates.



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Proteomic Approach To Understand The Host-Pathogen Interactions For Future Therapeutics: Gain From A Miniature Nematode Model, *C. elegans*

Balamurugan K

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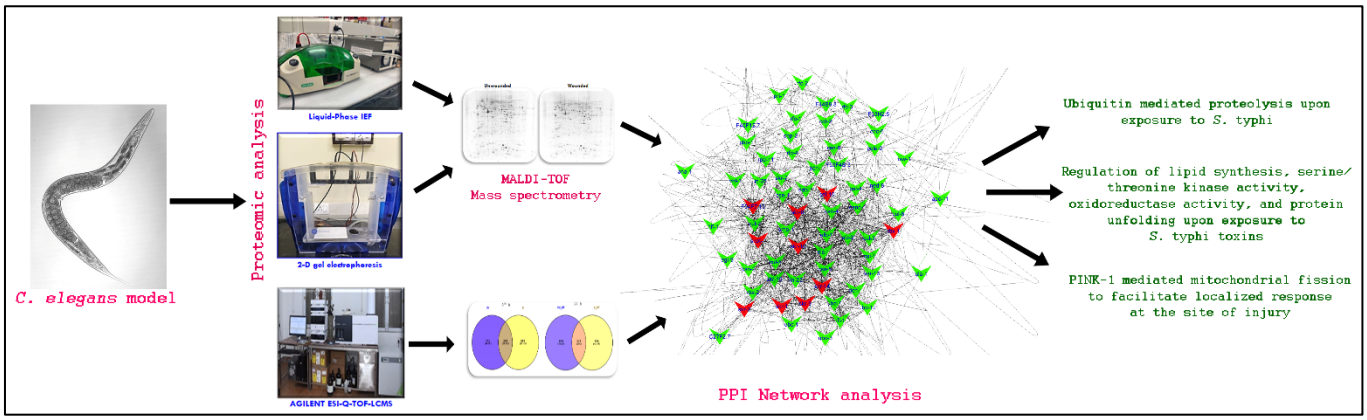
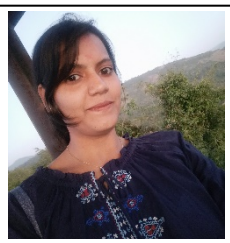
Host Pathogen Interactions (HPI) are crucial for the manifestation and following development of infection/disease in the host system where understanding them at molecular level is critical for prevention/ diagnosis/ treatment of the same. In this context, the advanced molecular fields including proteomics and metabolomics plays an inevitable role in which countless findings are reported every year that helps in improving our understating of HPI. In this context, few of our recent findings (in 2019-20) using a popular model system, *C. elegans*, a tiny nematode are provided below.

As many as 123 proteins that are orthologous to humans, are identified during *C. elegans* – *Salmonella enterica* Serovar Typhi interaction using the standard proteomic tools including liquid phase IEF and MALDI-ToF-Mass Spectrometry. Following bioinformatics based analysis of the identified proteins has uncovered the crucial involvement of ubiquitination pathway upon exposure to *S.* typhi (1).
Furthermore, 150 regulatory proteins were identified during *C. elegans* – toxic proteins of S. typhi interaction using MALDI-ToF-Mass Spectrometry following two-dimensional gel electrophoresis (2-D GE). Through which the crucial regulatory events (lipid synthesis, serine/threonine kinase activity, oxidoreductase activity, and protein unfolding) that are regulated upon exposure to bacterial toxins were uncovered (2). In addition to the findings from HPI, crucial proteins involved in wound repair process was also uncovered using MALDI-ToF-Mass Spectrometry following 2-D GE and LC-MS/MS analysis. Among the identified players, PINK-1, a mitochondrial Serine/threonine-protein kinase which is known to regulate mitochondrial dynamics, was found to be the central player in facilitating the mitochondrial fission to mediate localized response at the site of injury.





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Identification of Evolutionarily Conserved Nuclear Matrix Proteins and Their Prokaryotic Origins

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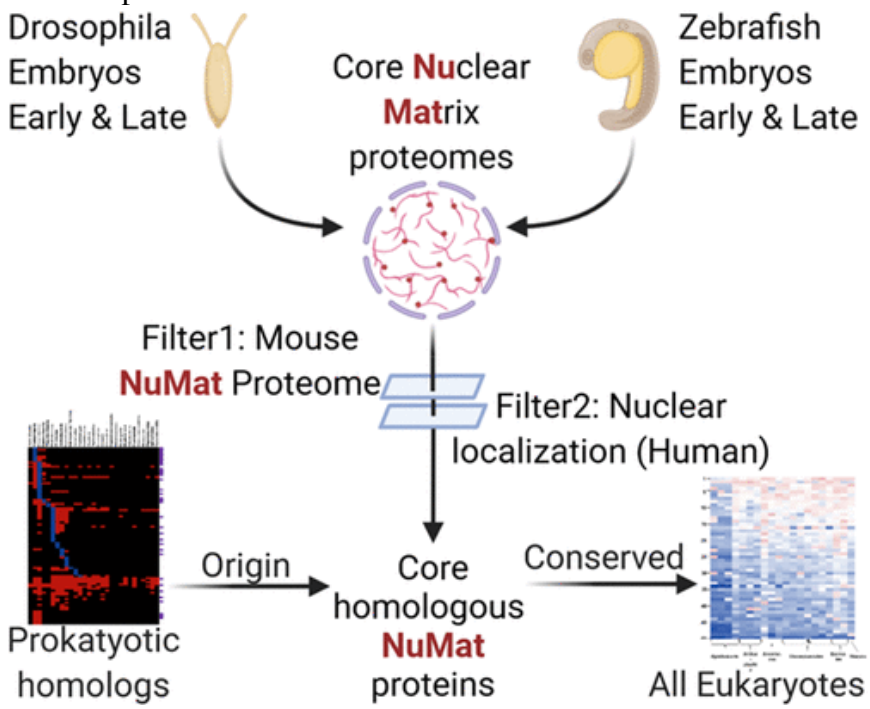
Compared to prokaryotic cells, a typical eukaryotic cell is much more complex along with its endomembrane system and membrane-bound organelles. Although the endosymbiosis theories convincingly explain the evolution of membrane-bound organelles such as mitochondria and chloroplasts, very little is understood about the evolutionary origins of the nucleus, the defining feature of eukaryotes. Most studies on nuclear evolution have not been able to take into consideration the underlying structural framework of the nucleus, attributed to the nuclear matrix (NuMat), a ribonucleoproteinaceous structure. This can largely be attributed to the lack of annotation of its core components. Since NuMat has been shown to provide a structural platform for facilitating a variety of nuclear functions such as replication, transcription, and splicing, it is important to identify its protein components to better understand these processes. In this study, we address this issue using the developing embryos of *Drosophila melanogaster* and *Danio rerio* and identify 362 core NuMat proteins that are conserved between the two organisms. We further compare our results with publicly available *Mus musculus* NuMat dataset and *Homo sapiens* cellular localization dataset to define the core homologous NuMat proteins consisting of 252 proteins. We find that of them, 86 protein groups have originated from pre-existing proteins in prokaryotes. While 36 were conserved across all eukaryotic supergroups, 14 new proteins evolved before the evolution of the last eukaryotic common ancestor and together, these 50 proteins out of the 252 core conserved NuMat proteins are conserved across all eukaryotes, indicating their indispensable nature for nuclear function for over 1.5 billion years of eukaryotic history. Our analysis paves the way to understand the evolution of the complex internal nuclear architecture and its functions.



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Dynamic Alteration in the Vaginal Secretory Proteome across the Early and Mid-Trimesters of Pregnancy

Bhoj Kumar[1], Amit Kumar Dey[1], Sandhini Saha[1], Abhishek Kumar Singh[1], Pallavi Kshetrapal[2], Nitya Wadhwa[2], Ramachandran Thiruvengadam[2], Bapu Koundinya Desiraju[2], Shinjini Bhatnagar[2], Dinakar M. Salunke[3]* Faraz Rashid[4], Dipankar Malakar[4], Tushar Kanti Maiti[1]*, and GARBH-Ini Study Group*

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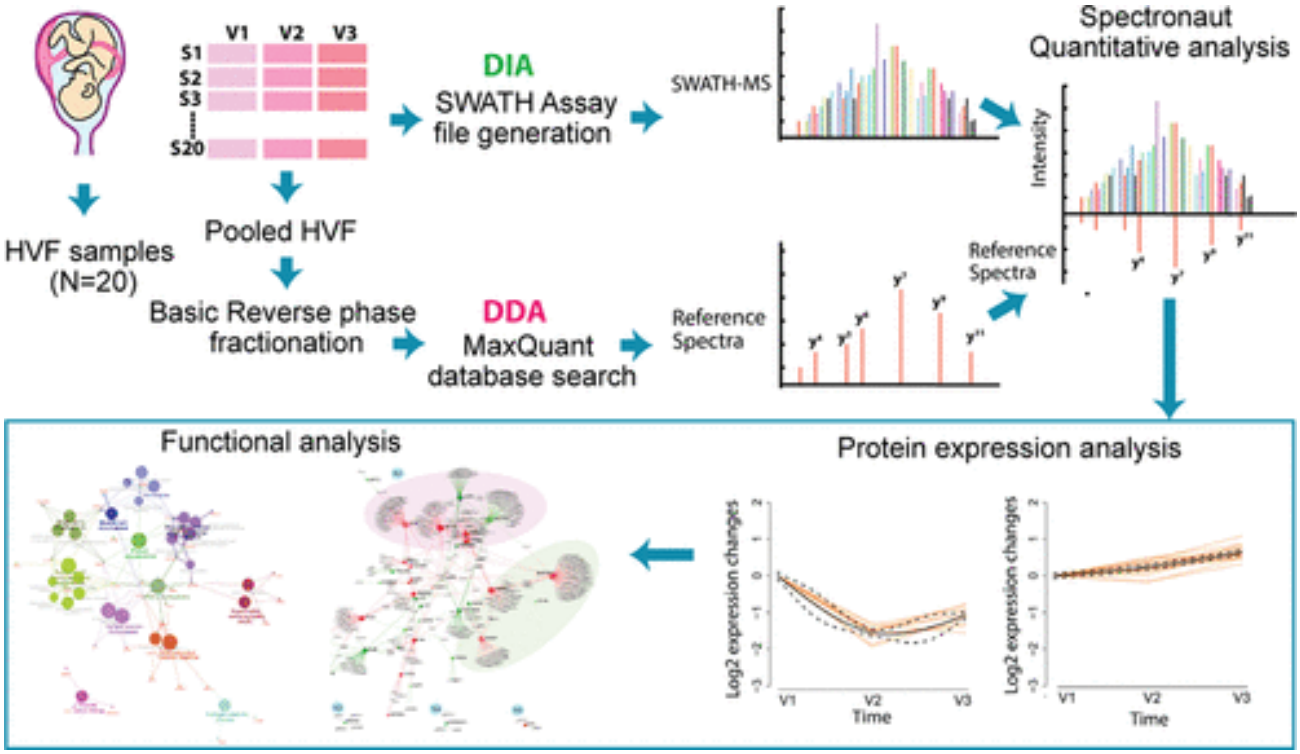
[4]Sciex Gurgaon, India

Pregnancy is characterized by intense physiological and structural alterations in the vagina, cervix, and overlying fetal membranes. High vaginal fluid (HVF) is a proximal fluid that covers the lower part of the female reproductive system and the severity of vaginal pathology often adversely affects pregnancy outcomes. To identify the correlation of vaginal fluid proteome dynamics and physiological changes during the progression of pregnancy, a longitudinal study was performed on 20 pregnant women who delivered a baby in >37 weeks without any complications. SWATH-MS-based label-free quantitative proteomics was performed to profile the HVF proteome at three time points defined as V1 (7–12 weeks), V2 (18–20 weeks), and V3 (26–28 weeks). Linear mixedeffect models were used to estimate protein abundance as a function of the period of gestational age. In this study, we identified 1015 HVF proteins and 61 of them were significantly altered until late second trimester. Our result demonstrates that the HVF proteins reveal gestational age-specific expression patterns and the function of these proteins is associated with tissue remodeling, organ development, and microbial defense. Our study provides an opportunity to monitor the underlying physiology of pregnancy that may be further probed for the biomarker identification in pregnancy-related adverse outcomes.



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Dioscorea alata Tuber Proteome Analysis Uncovers Differentially Regulated Growth Associated Pathways of Tuber Development

Shruti Sharma[1] and Renu Deswal[1]*

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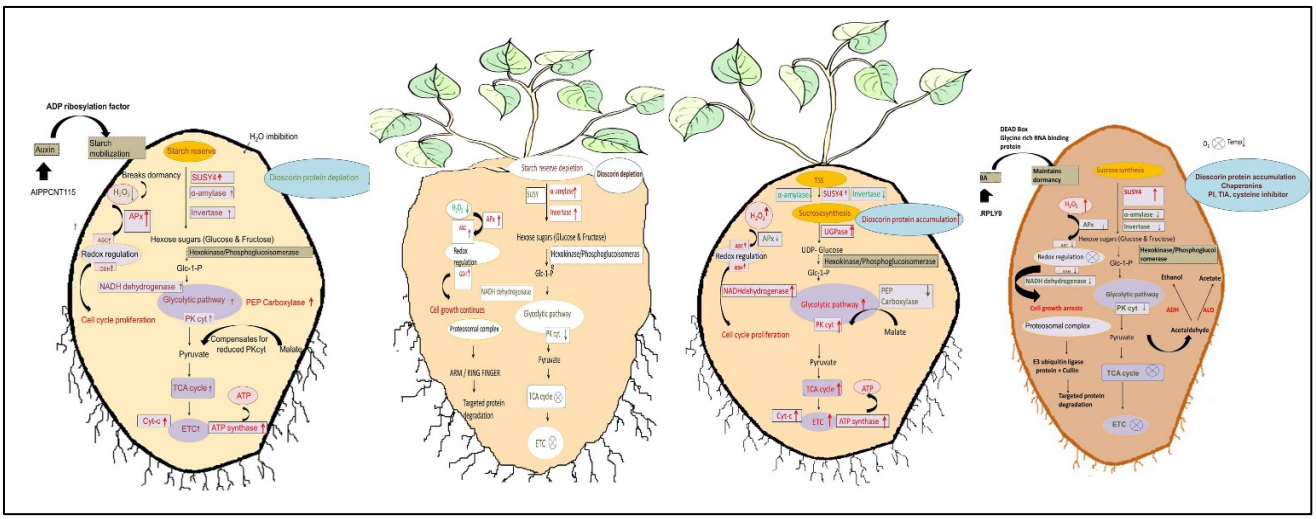
**Corresponding author-E-mail: rdeswal@botany.du.ac.in, Tel./Fax: 91-011-27662273.*

Tuberous and root crops are important staple crops for almost 40% of the population in the developing counties. One of these tuber crops that have gained popularity is *Dioscorea c*ommonly called yams. Despite being a good sources of carbohydrates, fibres, minerals (potassium), vitamins (Vitamin C, A, K1, folate and β carotene) and low in fatty acids and sugars in comparison with the staple crops, little or no information on *D. alata* tuber proteins is available. Also, the tuber life cycle represents an important model for studying the relationship between metabolism and development. To contemplate the metabolic changes associated with the tuber growth, a stage specific gel free proteome analysis of four distinct morphological stages namely germinating tuber (S1), degrading tuber (S2), new tuber formation (S3) and tuber maturation (S4) a comprehensive data set identifying 78.2% of the total 3,681 proteins was generated. Differential regulation of biochemical and physiological pathways revealing both expected (carbohydrate metabolism and redox regulation) and novel biological processes (transcription factors and hormonal regulatio) characteristic for each developmental stage was observed. The onset of tuber germination and new tuber formation,showed up-regulated the redox machinery, components of carbohydrate metabolism and Glycolysis. On the other hand carbohydrate machinery along with the proteosomal complex (ARM/RING FINGER) were active during the tuber degradation. On maturation an increase in the abundance of the components of ABA signaling along with the novel transcription factors (DEAD Box and Glycine rich RNA binding proteins) was observed, which are known to be involved in maintaining dormancy. Interestingly, the presence of components of ethylene biosynthesis during tuber formation hints towards its probable role in regulating its post-harvest shelf life. Based on the results and their validation the enzymes APx, MDHAR, INV and SUS can be regarded as markers for tuber growth. The availability of large-scale protein abundance data will enable researchers to address key questions concerning relationship between abundance patterns and identification of the fluxes responsible for protein accumulation for this tuber crop.



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Identification of active serine hydrolases during rice (Oryza sativa) seed germination by functional omics

Achintya Kumar Dolui[1,2] and Panneerselvam Vijayaraj[1,2*]

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Rice is the staple food for half of the world population, and the edible oil derived from rice bran is a repository of invigorating nutraceuticals and fatty acid composition. For decades, the overarching goal of research in rice bran is to effectively stabilize the bran using different physicochemical methods for enhanced oil extraction. However, due to the lack of knowledge on active lipases present in the bran, the commercial accessibility of the technologies were unsuccessful. Hence, the main objective of our study was to identify the active lipases and get insights into the catalytic properties of the enzymes using the functional omics approach. During seed germination, lipolytic enzymes are involved in mobilizing lipid reserves for proper seedling growth and development. Lipases and esterases, which belong to SH superfamily, play a crucial role during this reactivation of metabolism. Conventional genomic and proteomic approaches have provided enormous information about the presence and expressional profile of rice (*Oryza sativa*) enzymes. However, such studies fail to provide insights into the functional status of the identified enzymes. The major limitation of such abundance-based methods is that the abundance of mRNA is often weakly correlated with protein function due to post-transcriptional and post-translational modifications. To fill such gaps and advance our knowledge, we adopted activity-based protein profiling (ABPP), a powerful and revolutionary chemo-proteomics tool, to unlock the active players involved in rice seed germination on a global scale. ABPP uses an active site-directed probe to monitor the functional state of enzymes, and it enables the detection and affinity purification of the target enzymes. The application of ABPP, in combination with mass spectrometry, provides information about functional enzymes irrespective of their abundance. We have applied the ABPP approach to reveal the activity of lipases during seed germination in rice (*Oryza sativa*) and profile the active serine hydrolases. We successfully mapped the active sites of 43 SHs encompassing lipases/esterase, GDSL lipases, serine proteases, serine carboxypeptidases, ABHD protein, pectin acetylesterases, and other SHs. Each SHs are active at different time points of seed germination to support the required enzymatic process, revealing the dynamic nature of the active proteome (Activome). Cravatt and colleagues pioneered the ABPP, which they used to discover active enzymes and their inhibitors in the mammalian system. However, to the best of our knowledge, for the first time, we have reported the comprehensive profiling of active serine hydrolases in cereal crops, *Oryza sativa*. The mRNA expression levels of those genes encoding the identified SHs were further analyzed using microarray. The lipidome analysis by High-Resolution Mass Spectrometry (HRMS) revealed distinct patterns of molecular species distribution in individual lipid classes and shed light on the metabolic connections between lipid mobilization and rice seedling growth. Changes in the mobilization of storage lipids and their molecular species remodelling were correlated with the expression of the identified lipases and their activity in a time-dependent manner.

Further, we studied the physiological significance of the identified SHs and their importance under biotic stress with *Fusarium verticilliodes* infection, a seed-borne pathogen. The infection impairs lipolytic activity, eventually reducing the seedling growth. Together, our study unrevealed a much-needed portfolio of active enzymes mediating the process of germination. The data generated through these approaches will provide further stimuli for advancing fundamental research and deciphering the role of lipases in lipid homeostasis during rice storage and germination.

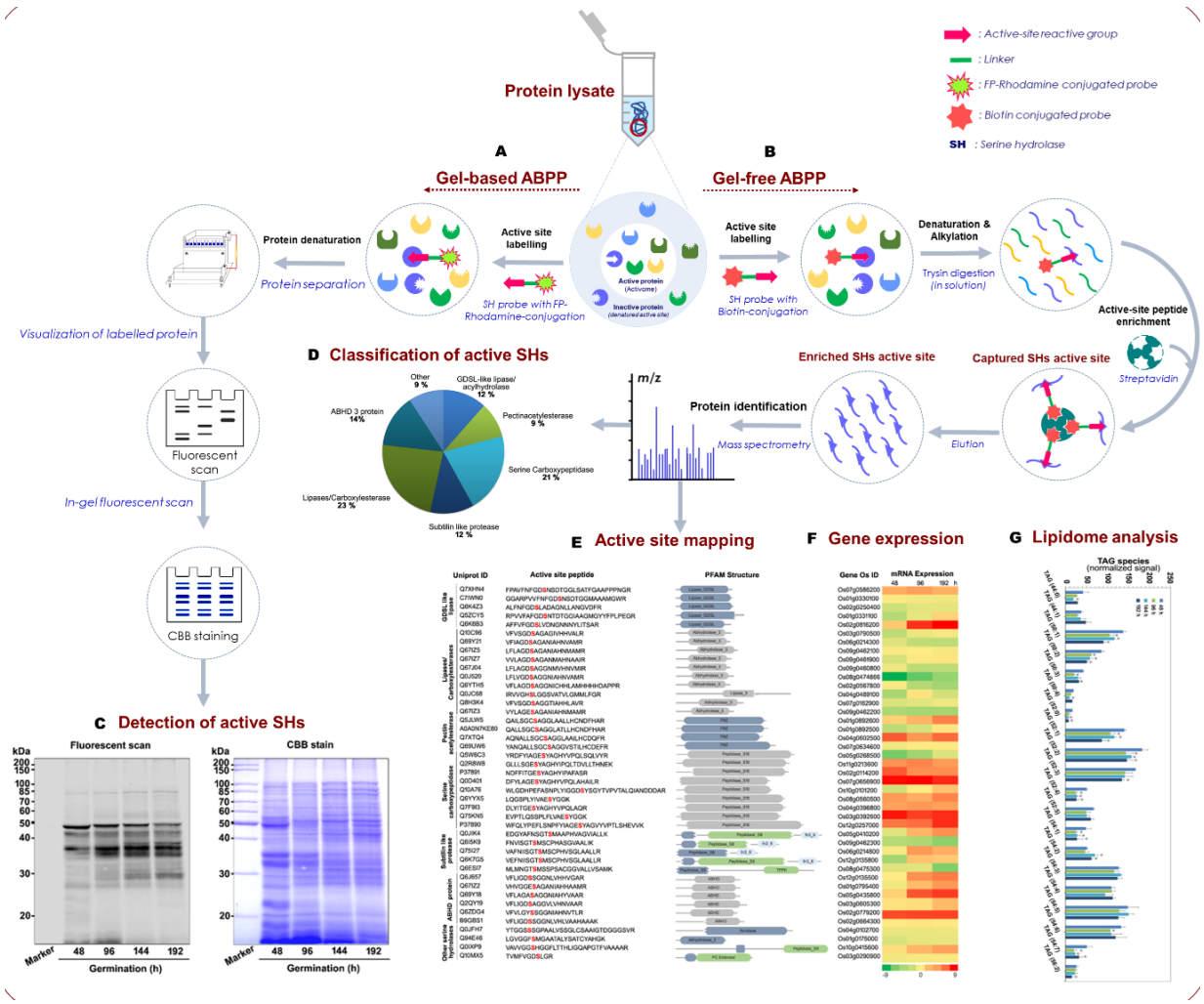

Figure 1. Identification of active serine hydrolases (SHs) by activity-based protein profiling (ABPP) and their expression and the impact on lipid mobilization during rice germination. *Schematic representation depicts the active-site peptide profiling workflow to detect and identify SHs present in the sample by gel-based* ***(A)*** *and gel-free* ***(B)*** *ABPP.* ***C,*** *Distribution of the 43 identified SHs under various classes.* ***D,*** *In-gel fluorescence labelling of rice SHs during germination.* ***E,*** *List of SHs identified by gel-free ABPP using Nano LC-MS/MS analysis and their corresponding active-site peptide sequences.* ***F,*** *Heat map displaying the level of mRNA expression of the genes encoding the identified SHs.* ***G,*** *Triacylglycerol (TAG) molecular species analysis of rice during germination by HRMS.*

Dolui *et al.(2020)* ***Plant Physiology*** *184: 693*

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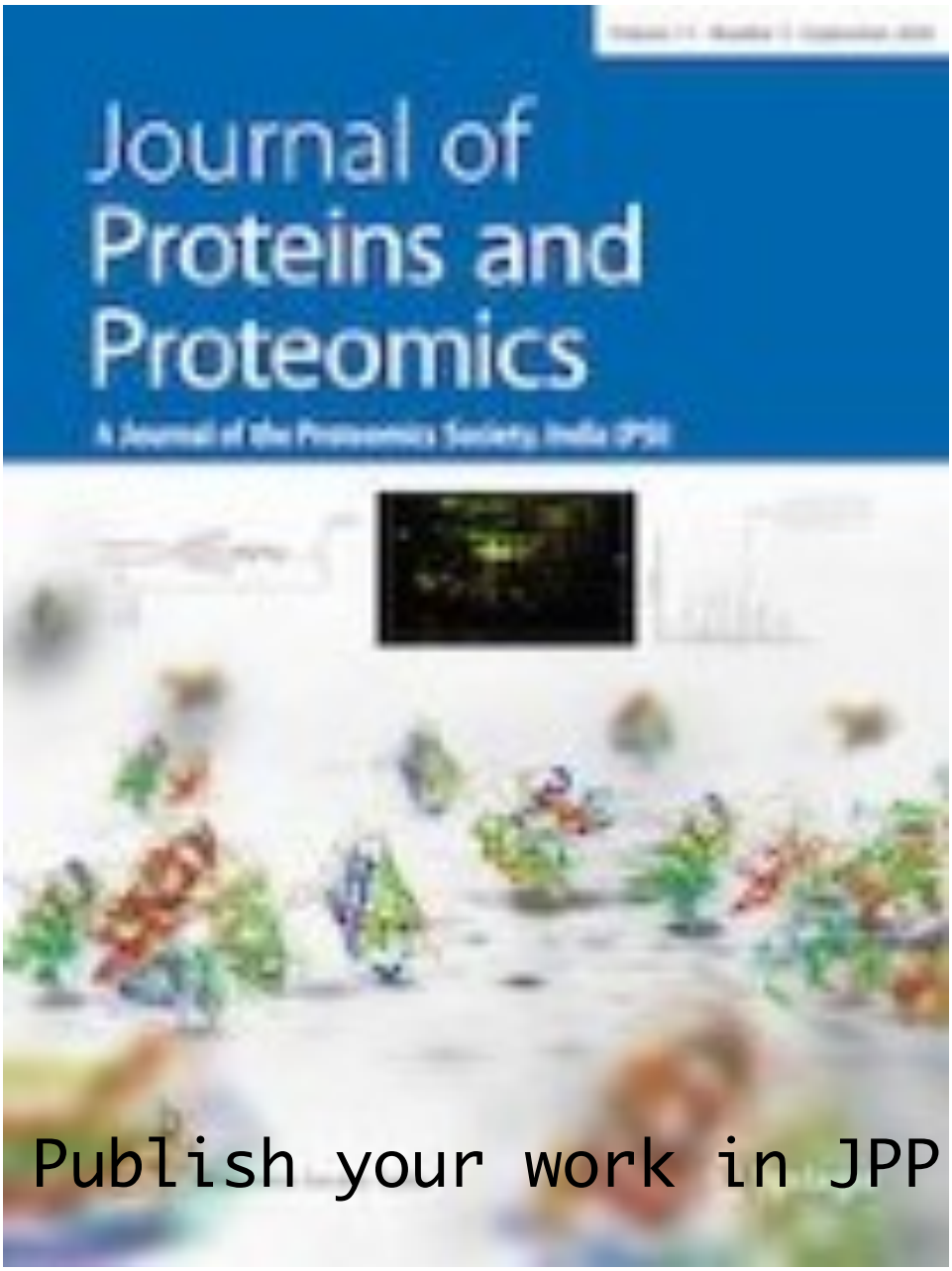

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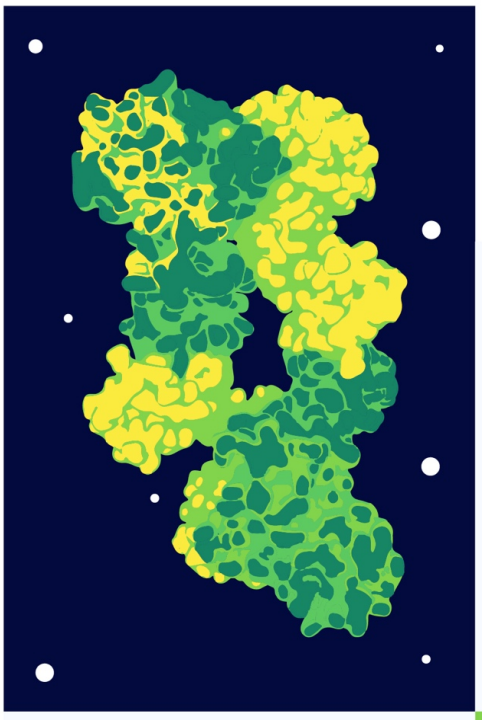

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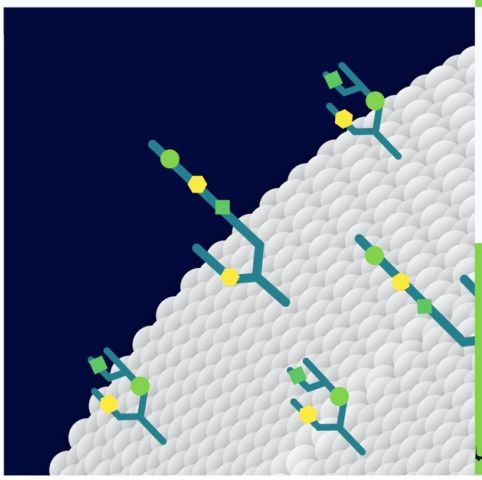

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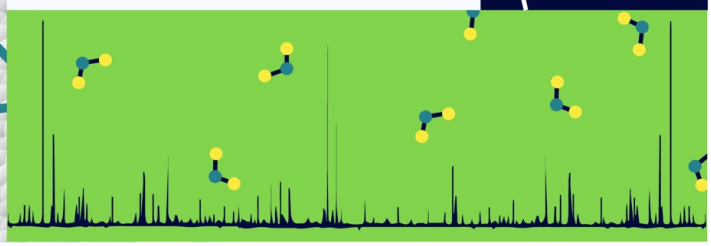

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