PSI News Letter Vol.7/No.2/September, 2019

PROTEOMICS SOCIETY, INDIA (PSI)

EDITORS DR. TUSHAR KANTI MAITI DR. SUREKHA M. ZINGDE

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EDITORS' MESSAGE

Dear PSI Members,

We take over the responsibility of presenting the PSI Newsletter from Dr. Sanjeeva Srivastava who has very efficiently brought to you the activities of the PSI for the last two years.

This newsletter is the second issue of 2019. We bring to you the Proteomics Day activity held in Mumbai, organized by Dr. Geetanjali Sachdeva, National Institute of Research in Reproductive Health. This program was a Proteomics Day event which we conduct at different Institutions to celebrate the day (18th March 2009) when the Proteomics Society, India was registered. We look forward to more PSI members coming forward to host this event in 2020.

The Research Section brings you recent articles the laboratories of Dr. Mahesh Kulkarni, Dr. M. S. Inamdar and Dr. Shantanu Sengupta. Dr. A.K. Yadav updates us on the new trends in Proteomics.

Dr. Ashok Mohanty invites you to Karnal for the 11th Annual PSI meeting. Do check out the details and ensure you will be there to meet other PSI Members and listen to the latest in different areas in Proteomics.

Dr. Debashish Mukhopadhya and Dr. Soumen Kanti Manna announce a Symposium on Advances in Biomedical Mass Spectrometry.

We look forward to meeting you in Karnal during 2nd- 4th December 2019.

Best wishes,

Dr. Tushar Kanti Maiti tkmaiti@rcb.res.in Dr. Surekha M. Zingde <u>surekha.zingde@gmail.com</u>

Dear Members of the Proteomics Society, India,

It is a great pleasure for me to write this President's message as the new Executive council is getting ready to take over the administrative functioning of the PSI at the upcoming annual meeting in Karnal in December 2019. I will officially hand over charge to the incoming President Dr. Subra Chakraborty in the month of December. Needless to say, all the council members will continue to support the society in whatever way it is possible.

On behalf of my Executive Council comprising of Dr. Mahesh Kulkarni, Dr. Subhra Chakaraborty, Dr. Arun Bandopadhyay as well as Dr. Sanjeeva Srivastava, I thank you all for giving us an opportunity to serve the society in the last term. I also want to place on record our sincere thanks to past council members including Dr. Ravi Sirdeshmukh, Dr. Surekha Zingde, Dr. Shantanu Sengupta and all the members of the EC committee for their kind cooperation.

In my last President's message of the PSI NL I am also delighted to share with you that HUPO council has chosen India as the venue for the HUPO Congress in 2022! This is a great achievement for all of us as this will be the first time that HUPO Congress will come to India! I want to place on record all the efforts made by the Core Committee, including Dr. Subhra, Dr. Ravi, Dr. Shantanu as well as Dr. Sanjeeva in making this happen. We have decided to hold this important event in Hyderabad and I am sure you will hear more about the developments in the due course.

As you will see new members of the EC, namely Dr Tushar Maiti has taken over the responsibility as the Editor of the PSI NL and our past President, Dr. Surekha Zingde has offered to support him in this effort.

As you know the upcoming annual meeting of the Society in December 2019 will take place under the leadership of Dr Ashok Mohanty at ICAR, Karnal. I am excited to share with you that he has chosen 'One health' as the main theme for this annual meeting that will bring experts from India and abroad to deliberate on this relevant theme. I hope to see you at the meeting in Karnal in December.

Thank you for your support and cooperation.

With warm regards,

Utpal Tatu

Proteomics Society, India (PSI) in association with Indian Council of Medical Research-National Institute for Research in Reproductive Health (ICMR- NIRRH) organized a one-day workshop entitled "A Primer on Proteomics" on 23rd April 2019 at ICMR-NIRRH, Parel, Mumbai. The objective of the workshop was to introduce graduate and postgraduate students of Mumbai and neighboring cities to the basic principles of various techniques employed in proteomics and also to appraise them of data collected using proteomics-based tools. The workshop included seven invited talks and four demonstration sessions. The faculty included Dr. Surekha Zingde, Past President of Proteomic Society, India; Dr. Ajit Datar, Advisor. Shimadzu Analytical (India) Pvt. Ltd, Mumbai; Dr. Sanjeeva Srivastava, Professor, Department of Biosciences and Bioengineering, IIT, Bombay, Mumbai; Dr. Shubha Chakraborty, Professor, National Institute of Plant Genome Research, New Delhi; Dr. Rukmani Govekar, Scientific Officer, Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Navi Mumbai, Dr. Rahul Gajbhiye, Wellcome trust DBT India Alliance Intermediate fellow and Scientist 'D' ICMR-NIRRH and Dr Srabani Mukherjee, Scientist F, ICMR-NIRRH. Demonstration sessions were conducted by Dr. Geetanjali Sachdeva, Dr. Priyabka Parte, Dr Susan Thomas, and Dr Dhanshree Jagtap.



Dr Smita D Mahale, Director, ICMR-NIRRH welcomed the participants and faculty. She highlighted the relevance of proteins and proteomics in biomedical research. Dr Geetanjali Sachdeva presented the objectives of the workshop i.e. to orient graduate and postgraduate students to proteomics and its applications in biomedical research. Dr. Surekha Zingde, the first speaker in the pre-lunch session, presented a comprehensive overview of the proteomics field, starting from its genesis to the recent advances. Her presentation covered the wide spectrum of proteomic applications in biological sciences.

She also introduced the participants to the milestones achieved by the Human Proteome Organization (HUPO). Towards the end of her talk, Dr Zingde spoke on the mandate of PSI, its membership and activities and also on the journal published by PSI, the Journal of Proteins and Proteomics.



Dr. Ajit Datar introduced the participants to the basic principles of Mass Spectrometer. His talk briefed the audience about the basic components of a Mass Spectrometer such as Chromatography System different devices for ionization analyzer and detector. He also spoke on different types of mass analyzers and recent advances in Mass Spectrometry. Dr. Datar's talk was followed by an interactive talk on gel free quantitative proteomics by Dr. Sanjeeva Srivastava. Dr. Srivastava elaborated on the need to comprehensively understand multilayered molecular networks and thereby gain a wholesome overview of various biological processes. His talk provided an overview of gel-free proteomic tools such as iTRAQ/TMT, MRM, DIA, and label-free proteomics. Dr. Srivastava also spoke on the application of proteomics in stratifying brain tumors at the molecular level.







Dr. Subhra Chakraborty delivered a very interesting talk on "Proteome to Interactome". She elaborated on the use of proteomics to dissect anti-nutrient and immune response in plants. She briefed the audience about how her proteomics based investigations led to the identification of key regulatory biomarkers, which can serve as a valuable resource for developing strategies towards stress adaptation, improved productivity and nutritional quality. The post lunch session included three talks on applications of proteomics in biomedical research.

Dr. Rukmani Govekar shared her research observations on proteomic analysis of Chronic Myeloid Leukemia (CML) to understand the mechanism of chemoresistance (to tyrosine kinase inhibitors imatinib). SWATH-MS label free quantification and iTRAQ analysis of imatinib treated and sensitive CML cells were used to identify novel components of the transforming gene (BCR/ABL) pathway and alternative pathways.



Dr. Rahul Gajbhiye delivered a talk on "Proteomics in Endometriosis". Novel autoimmune makers were identified in the serum of women with endometriosis, using 2D proteomics. He also spoke on the use of these markers in early diagnosis of endometriosis



The last talk was delivered by Dr. Srabani Mukherjee on follicular fluid proteomics in women with Polycystic Ovarian Syndrome, a pathology associated with follicular growth arrest. She presented her research on the proteome of follicular fluid from control and PCOS women, which revealed that many proteins involved in development significantly oocvte are downregulated in women with PCOS. Dr. Mukherjee stated that the protein dataset of follicular fluid from women with PCOS generated using iTRAQ is a useful resource for screening the biomarkers for oocyte and embryo quality.

The post-tea session was followed by four demonstration sessions. The participants were divided into three groups and each group was introduced to the following activities:

- a) Identification of the proteins of relevance using a gel- based approach. Towards this, the participants were introduced to 1D and 2D gel electrophoresis systems and their applications in addressing specific research queries. Excision of protein spots from a 2-D gel was also shown to them.
- b) In the second exercise, participants were briefed about various in silico tools to identify protein ids, sites of posttranslational modifications and interaction partners. They were also given instructions on how to use Protein Atlas.
- c) Participants were introduced to Nanofluidic immunoassay (NIA) for detecting posttranslational modifications of a protein.
- d) This was followed by a demonstration on Surface Plasmon Resonance and its applications in validating protein-protein interactions.







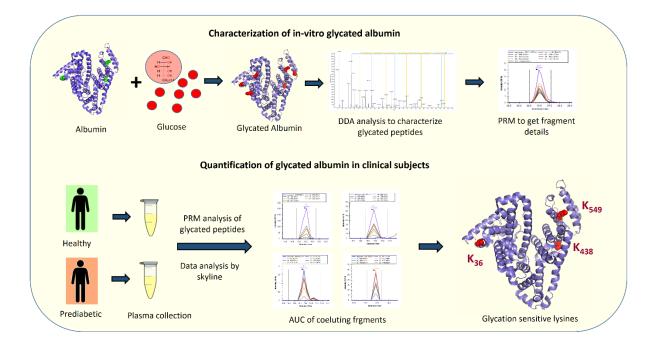
The workshop was concluded with a vote of thanks by Dr. Uddhav Chaudhari, Co-Convenor of the workshop and distribution of participation certificates.

<u>Glycation of glucose sensitive lysine residues K36, K438 and K549 of albumin is</u> associated with prediabetes

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Prediabetes is a condition which reflects intermediate glycemic status between normal and diabetes. If prediabetes is not controlled in time then it can lead to development of diabetes and microvascular complications. The number of prediabetic cases is growing substantially and it has been estimated that it will affect 482 million people worldwide by 2040. The annual conversion rate of prediabetes to diabetes is about 5 - 10%. However, the timely diagnosis of prediabetes and intervention in lifestyle changes can reverse the prediabetic population to normoglycemic at a similar rate. Currently, the glycemic status is determined by OGTT, FPG or HbA1c, despite the utility of these standard diagnostic tests, many times prediabetes remains undiagnosed. Therefore, this study aimed at the identification of novel markers for efficient prediction of prediabetes. In this pursuit, we have evaluated the ability of glycated peptides of albumin in predicting prediabetes. This study reports targeted quantification of four glycated peptides of serum albumin particulary FK(CML)DLGEENFK, K(AML)VPQVSTPTLVEVSR, K(CML)VPQVSTPTLVEVSR, and K(AML)QTALVELVK, corresponding to 3 glucose sensitive lysine residues K36, K438 and K549 respectively in healthy and prediabetic subjects. These peptides showed significantly higher abundance in prediabetes than healthy subjects, and showed significant correlation with various clinical parameters including FBG, PPG, HbA1c, and altered lipid profile. Therefore, together these four peptides constitute a panel of markers that can be useful for prediction of prediabetes.



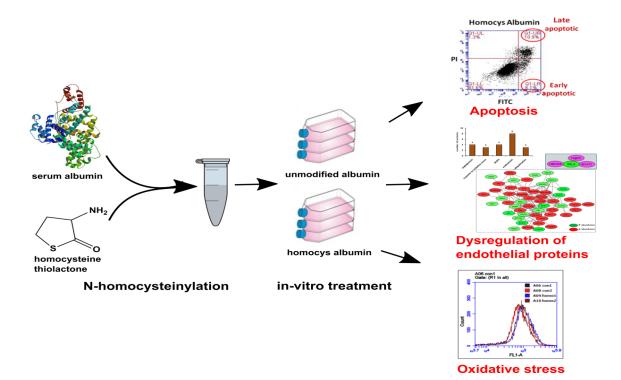
Reference:

Rathore R, Sonwane B, Jagadeeshaprasad MG, Kahar S, Santhakumari B, Unnikrsihnan AG, Kulkarni MJ*. Glycation of glucose sensitive lysine residues K36, K438 and K549 of albumin is associated with prediabetes. 2019 Journal of Proteomics. J Proteomics. 2019 Aug 5;208:103481.

<u>Proteomic study of Endothelial Dysfunction in Response to Homocysteinylated</u> <u>Albumin</u>

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Homocysteine is a non-protein sulphur containing amino acid formed during the methionine cycle whose elevated plasma levels, also termed as hyperhomocystenemia, is reported to be involved in the progression of cardiovascular and neurological disorders. Although a number of studies have been carried out in this aspect, the exact mechanism is still not fully understood. In our study 'Proteomic study of endothelial dysfunction in response to homocysteinylated albumin' published in the Journal of Proteins and Proteomics this year, we have looked in to the possible role of homocysteine modified albumin in the development of endothelial dysfunction and consequent cardiovascular complications. Since protein linked homocystamide, and not free homocysteine, is the major circulating form in the blood we have first incubated serum albumin with homocysteine thiolactone, the cyclic analogue of homocysteine, to obtain N-homocysteinylated albumin. This modified serum albumin was then used to study endothelial response using Human Umbilical Vein Endothelial Cells (HUVEC) in culture. This study is the first total proteomic study in endothelial cells in response to N-homocysteinylated albumin and identified around 53 proteins showing alteration in abundance. It therefore indicates a possible mechanism involving interaction of the homocysteine modified protein with a receptor that can initiate cellular signaling cascade leading to endothelial dysfunction.



Reference:

 Banarjee R, Sharma A, Bai S, Deshmukh A, Kulkarni MJ*. Proteomic study of endothelial dysfunction in response to homocysteinylated albumin. Journal of Proteins and Proteomics. 2019. DOI 10.1007/s42485-019-00015-9

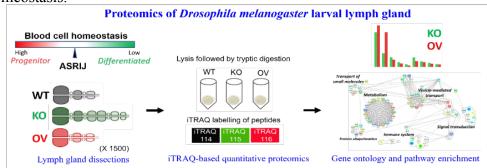
<u>Proteomics of Asrij perturbation in *Drosophila* lymph glands for identification of new regulators of hematopoiesis</u>

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Hematopoiesis is the complex and dynamic process of blood cell generation and maintenance. Aberrant hematopoiesis contributes to the origin of several blood cell disorders. Conserved molecular programs between insects and vertebrates are known to regulate blood cell precursor maintenance, proliferation, differentiation and lineage commitment. Studies on the Drosophila blood system have given important insights into vertebrate hematopoiesis and the causes of blood cell disorders. However, molecular mechanisms underlying blood cell precursor maintenance and differentiation are incompletely understood. Owing to simplicity and ease of genetic analysis, the Drosophila melanogaster larval lymph gland is a well-accepted and relevant model for studying molecular mechanisms underlying hematopoiesis. Despite its many advantages as a model, the microscopically small size (1.5-2 mm in length, made of ~1000-1500 cells), relatively transparent and fragile nature of the lymph gland are major roadblocks to obtaining sufficient tissue for molecular and biochemical analyses. While the lymph gland transcriptome has been reported, challenges associated with sample collection make mass spectrometry difficult due to which proteins expressed in this tissue remain largely unknown. Sinha et al undertook a proteomic analysis of the Drosophila larval lymph gland to identify changes accompanying hematopoiesis. Using genetically modified Drosophila lymph glands that maintain stemness (Asrij Overexpression: OV) or promote differentiation (asrij null: KO) of blood progenitors and comparing to wild type (WT), 1500 lymph glands per genotype were dissected and processed for iTRAQ-based quantitative proteomics. Through enrichment analyses of the proteomes thus generated and subsequent validation in vivo, they found that Asrij, a conserved and key regulator of Drosophila hematopoiesis and immunity, affects multiple protein clusters involved in metabolism, immune system, vesicle-mediated transport, transport of small molecules, signal transduction and protein ubiquitination. They identified additional regulators and effectors of blood cell homeostasis and generated a useful data resource. This study widens the scope and applicability of Drosophila lymph gland hematopoiesis as a tool for understanding mechanisms regulating vertebrate blood cell homeostasis.



Vitamin B12 insufficiency: An important mediator in the complex etiology of cardiovascular diseases

Shantanu Sengupta CSIR-Institute of Genomics and Integrative Biology, Mathura Road, Delhi-110020

Cardiovascular diseases are the largest cause of mortality worldwide especially in developing countries like India. It is projected that by 2030, almost 60% of world's Coronary Artery Disease (CAD) patients will be Indians. It is therefore, important that we look at these problems through the prism of our unique lifestyle, cultural habits and genetic composition.

Over the last fifteen years one of our main focuses was to identify predictive markers for CAD using multi-omics approach. We identified several single nucleotide polymorphisms associated with CAD. However, these could account for only 10-15% of the disease burden. We also pioneered the view that DNA methylation in CAD is high *per se* and is further accentuated by elevated levels of homocysteine, an independent risk factor for CAD¹. One of the factors that elevate the levels of homocysteine is vitamin B12, a cofactor of methionine synthase that catalyzes the conversion of homocysteine to methione. It is known that a large proportion of the Indian population is deficient in vitamin B12 since this vitamin is completely absent in plants. Most importantly, we showed that vitamin B12 deficiency and elevated cysteine levels was associated with CAD in Indian population². This piqued our interest in trying to understand the role of cysteine and assess if Vitamin B12 contributes to the etiology of CAD.

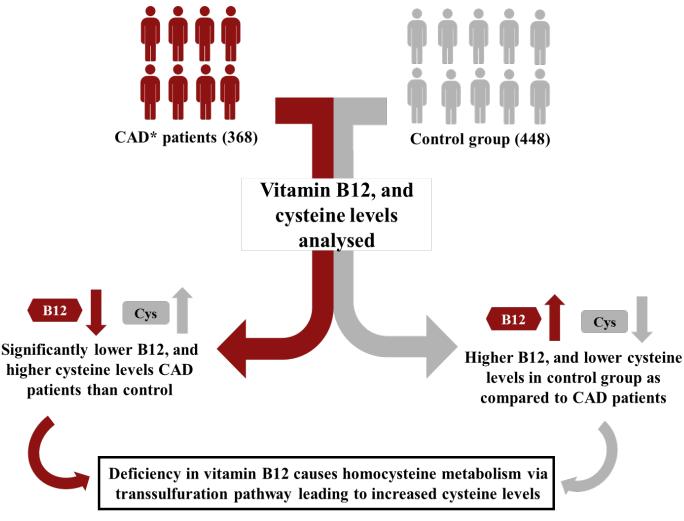
To understand the effect of vitamin B12, we developed a maternal vitamin B12 deficient rat model, in collaboration with CCMB and NIN, Hyderabad and studied its effect on the offsprings. Our observations highlighted that chronic maternal B12 deficiency is associated with increased body fat and insulin resistance in the offspring which were reverted following supplementation of mothers at conception confirming the causality³. Proteome profiling of liver of B12 deficient offspring highlighted the involvement of PPAR signaling, carbohydrate, amino acid metabolism and fatty acid oxidation pathways^{4,5}. We also confirmed the epigenetic basis of these effects.. Further, analysis of DNA methylation in young CAD patients also showed that genes belonging to cholesterol metabolism, hyperglycemia and endothelial dysfunction, are differentially methylated⁶. Interestingly, we observed that vitamin B12 has a gender and tissue specific effect, with male pups born to mothers fed with a vitamin B12 deficient diet developing atherogenic risk, with increased triglyceride and decreased HDL levels. The expression of Apolipoprotein A1 (Apo A1), the major constituent (70%) of HDL, was significantly low in these male offsprings. We also constructed the first complete ApoA1 monomer structure using large-scale multiple independent atomistic molecular dynamics trajectories, which will enable to decipher the molecular mechanisms of downstream reactions in reverse cholesterol pathway⁷. Interestingly, our plasma proteomic analysis of CAD patients in India identified 4 proteins in the reverse cholesterol pathway, including ApoA1 to be significantly low. These proteins along with hypertension and diabetes could account for 88% of CAD8. Our untargeted metabolomics study on CAD patients hinted at impaired phosphatidylcholine pathway in CAD patients⁹.

To assess the role of homocysteine and cysteine, we used a yeast model and demonstrated that homocysteine leads to ER and mitochondrial stress^{10,11}. Using proteomics approach, we identified the pathways that are altered in the presence of cysteine. We show that cysteine alters amino acid metabolism and inhibits protein translation¹². Interestingly, we had previously shown that there is a significant decrease in total protein concentration in CAD patients and cysteine levels positively correlated with albumin concentration in CAD patients.

Through our various model, we have therefore unravelled a comprehensive contribution of genetics, epigenetics, proteomics, metabolomics, nutrition, and their interplay in the manifestation of CAD in India.

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Emerging and recent trends in Proteomics

Dr. Amit Kumar Yadav THSTI, NCR Biotech Science Cluster, Faridabad, Haryana-121001

These are exciting times to be in mass spectrometry based proteomics research with these methods foraging into newer areas and mining the proteomic information at an exceptional scale and depth. It is actively addressing biological questions aided by development of new tools and methods overcoming significant biological and technical challenges. This is pushing the proteomics community towards biomedical applications faster than envisioned before. Some of the exciting frontiers in proteomics are discussed below-

- *Single cell proteomics* the days of discerning proteins from cellular mRNA levels are over. Single cell proteomics is here. By labeling digested peptides with amino acid specific fluorophores, they are discerned via imaging after every Edman degradation cycle. This allows for assessment of proteomes of single cells to infer biological roles and implications of heterogeneity at single-cell level and its macro-level biological implications in developmental processes, health and disease.
- *Proteogenomics for mining proteoforms in diseases* the rise of proteogenomics has added a new dimension is large scale biological inference of alternate start sites, splice variants, polymorphisms and posttranslational modifications to facilitate the mining of proteoforms. Particularly in several cancers like colorectal and breast cancer, proteogenomics studies have revealed somatic mutations and polymorphisms that may have diagnostic power towards disease-stage stratification, disease subtype classification and early diagnosis.
- Intensity prediction using Deep Learning the rise of deep learning has infiltrated nearly all fields including biological mass spectrometry. It has now also made deep inroads in seemingly intractable problem of intensity prediction for tandem mass spectra, which can form the basis for better and faster protein identification. Using recurrent neural networks (RNN) trained to learn intensity from millions of available annotated spectra, these deep learning tools allowed accurate prediction of MS/MS ion intensities and are demonstrated to be useful as reference libraries for protein identification from DDA as well as DIA data. Tools like pDeep, Prosit alongwith DeepMass:Prism and winner can predict highly useful intensity based spectral libraries as reference to identify proteins.
- Glycomics using DIA A new method Glyco-DIA, allows the study of glycosylations- one of the most complex modifications that surface proteins usually harbor to increase their repertoire of functions. These play several important biological roles like imparting structural conformation, rigidity, signaling scaffold and thus important for driving the host pathogen interactions in bacterial and viral diseases. Using a DIA spectral library for identifying O-glycopeptides, the method makes it feasible to study glycoforms without enrichment, in which the identifications and site stoichiometry quantitation is boosted by the DIA O-glycopeptide libraries.
- *Microbial community metaproteomics* the attention to microbial proteomics in gut microbiome has been challenged by lack of useful methods and due to the complexity involved. But the role of microbiome in diseases is beginning to be investigated in detail using proteomics methods. There are several studies that connect the gut microbiome in pediatric inflammatory bowel disease, non-alcoholic fatty liver disease and colorectal cancers etc. This is the new emerging direction in disease proteomics that seeks to dissect out the role of gut microbiota in disease and explore pathogenesis in more detail.

INVITATION



11th Annual Meeting of Proteomics Society, India, 2019 and International Conference on Proteomics for System Integrated Bio-Omics, One Health and Food Safety, 2nd Dec to 4th Dec 2019 ICAR-NDRI, Karnal, INDIA

On behalf of PSI, National Dairy Research Institute (ICAR-NDRI), Karnal is organizing, 11th Annual Meeting of Proteomic Society, India, 2019 (PSI) and International Conference on "Proteomics for System Integrated Bio-Omics, One Health and Food Safety" (ICPBHF-2019) at Karnal, Haryana, India from December 2nd to 4th, 2019 preceded by pre-conference workshop (28th Nov – 1st Dec) and Education Day (1st Dec). It is our pleasure to invite the scientific fraternity community in Omic Research community to participate actively. NDRIPSI 2019 took this platform to invite the proteomics community from India and abroad for the upcoming event.

In the technological advanced society the high throughput data recording and big data analysis is in infancy. Henceforth, it is an immediate prerequisite for all the eminent scientist and researchers across the globe to gather and exchange the current knowledge. In this regard, we already got confirmation from excellent international speakers for plenary and keynote lectures. And also national prominent speakers are confirmed.

There are eight theme areas for the ICPBHF-2019 including 1-Omics Technologies for One Health and Productivity; 2- Clinical and Disease Proteomics in Human, Animal and Plant; 3-Biomarker Discovery, Translational Omics and Entrepreneurship; 4-Proteogenomics, Integrative omics and Systems Biology; 5- Structural Proteomics and Drug Targets; 6-Proteomics of Model Organisms, PTMs and Big Data Analysis; 7-Proteomics and Mass Spectrometry Applications in Food Safety and Allergy; 8-Metabolomics in Biological applications.

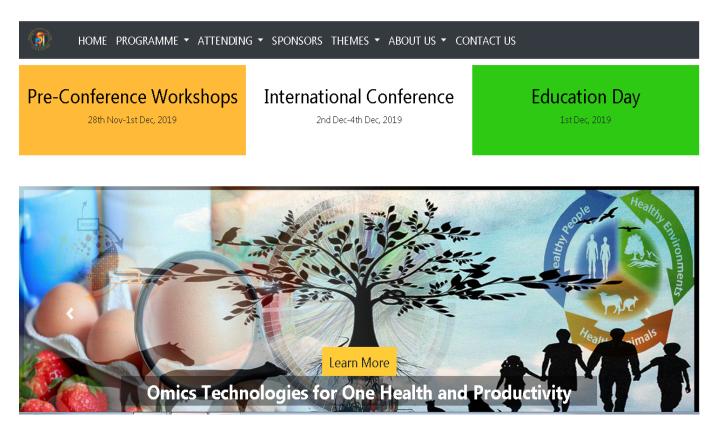
The ICPBHF-2019 website is completed with full fledged information of all kind (fig 1). All the speaker's details are provided in the speakers tab and workshop details are described with separate dedicated web pages for workshop. Out of three workshops, the plan of the two workshop is already included and very soon we will load the last one too. The registrations for the workshops/education day/conference have been started from 1st July 2019 onwards. The early bird conference registration will close on 30 Sep 2019. Till now we are successful to attract good number of participant to attract for the enrollment in the event. The conference is going to make the ensuing highly scientific and unique in many aspects.

For the first time, the 11th PSI meeting and international conference will be organized at ICAR-NDRI, Karnal Haryana. Karnal is located 125 kilometers north of Delhi on national highway no. 1 towards Chandigarh. The institute covers an area of more than 1000 areas of beautiful land scaping and is involved in research and teaching in contemporary areas of biological sciences involving Animals, Science, Dairy Technology and Engineering. ICAR-NDRI, Karnal has excellent infrastructure in terms of conducting meetings and seminars with availability of two auditoriums namely D. Sunderesan auditorium (900 capacity) and N. N. Dastur auditorium (160 capacity). Apart it has many small seminar rooms for holding small scale meetings. It has well furnished hostels and guest houses for hosting the guests. NDRI has a vibrant student community.

INVITATION

You can reach to us in the following contact and website details.

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Upcoming Proteomics Conferences/ Workshops

SINP School and Symposium on Advances in Biomedical Mass Spectrometry 2019

11-17 November, 2019 at Saha Institute of Nuclear Physics, Kolkata

Mass Spectrometry is one of the most powerful analytical tools. From relatively simple mixtures or purified analytes, it has evolved to enable analysis of complex mixture of biomolecules, large macromolecular assemblies and intact microbes. Recent advances has further enabled analysis of biomolecules *in situ* paving the way for analysis of single cells to real-time analysis of live tissue, breath and biofluids. Continuing with the annual school series (Epigenetics in 2018), we are delighted to announce a school (Nov 11-12) that will recapture the evolution of biomedical mass spectrometry till-date by leading international experts. It will be followed by a symposium (Nov 13-14) that will showcase latest advances in its applications. It will have brain-storming sessions to bring together academicians, clinicians and vendors to discuss challenges and opportunities in biomedical mass spectrometry. It aspires to create a platform for intimate dialogue and partnership between all stakeholders. A hands-on workshop on metabolomics (Nov 15-17) will conclude the event. We cordially invite you to be a part of this exciting event.



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SSABMS

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