

## CONTENTS

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Messages and Motivation .....MM 1-MM 6

### MOVING TRENDS AND TIPS IN PROTEOMICS

India and the Quest Towards Human Proteome ..... MTT 1-MTT 5  
*Ravi Sirdeshmukh*

The Field of Plant Proteomics: Challenges, Limitations and Future prospects ..... MTT 6-MTT 8  
*Aarti Pandey, Subhra Chakraborty and  
Niranjan Chakraborty*

Doing Proteomics - A Beginner's guide .....MTT 9-MTT 10  
*K. Dharmalingam*

### ARTICLES

Alteration in *Plasmodium falciparum* proteome upon treatment with various ..... 1-17  
anti-malarial drugs  
*Deepa Jha, Mohsin Raza, Tridibes Adak and  
Pralad C. Ghosh*

Targeting the Semen Derived Amyloids to Control HIV Transmission: ..... 19-27  
Perspectives and Challenges  
*Shalini Gour, Vibha Kaushik, Vijay Kumar  
Bharti Gaharwar and Jay Kant Yadav*

Recognition Dynamics of *Escherichia coli* Thioredoxin Probed using ..... 29-45  
Molecular Dynamics and Binding Free Energy Calculations  
*M. S. Shahul Hameed*

Statistical Insight into the Binding Regions in Disordered Human Proteome ..... 47-60  
*Uttam Pal, Mritunjoy Maity, Nitin Khot, Swagata Das  
Supriya Das, Sandip Dolui and Nakul C Maiti*

Domain Interaction Phage Panning (DIPP): A phage display based method ..... 61-72  
for mapping and characterizing protein-protein interaction interface  
*Shweta Shah and A. Gururaj Rao*

Technical Notes ..... 71-82

## GENERAL INFORMATION

**The Journal:** Journal of Proteins and Proteomics (JPP), with its genesis in India, is a peer reviewed journal envisaged to serve the world wide community of researchers and teachers dealing with the challenges of proteins and proteomics resulting in an improved understanding of protein science in general. Published bi-annually, the aim is also to supplement the regular issues with special issues in selected, relevant topics of protein science. The journal has an online presence at <http://www.jpp.org.in>.

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## **WELCOME TO THE FIRST ISSUE FROM PROTEOMICS SOCIETY, INDIA (PSI) (NINETEENTH ISSUE OVERALL)**

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More often than not, dreams do come true and they bring along unprecedented euphoria, aspirations, positivism, energy and even more dreams. I am happy and proud to announce that Journal of Proteins and Proteomics (JPP) is now an official journal of the Proteomics Society, India (PSI); a dream fulfilled. Since the end of 2012, we had an unofficial association with the Society which has now strengthened into a bond that promises to walk the path to glory together, that aspires to serve the community of protein science and proteomics hand in hand. The PSI-JPP association has the potential to achieve plenty.

PSI and JPP came into existence almost simultaneously, the former in 2009 and the latter in 2010. They were created independently without the knowledge of the existence of each other. However, they both nurtured and worked tirelessly towards a similar goal, that of furthering the cause of proteins and proteomics research and education. It is no surprise then that their paths were destined to merge and ultimately become one. It is also interesting to note that while PSI was born in South India, JPP was born in the North; the union thus becoming a national event. PSI-JPP has now become one. I cannot thank the society members enough for this dream being a reality. The office bearers of the society, in particular, exhibited never-ending zeal and enthusiasm to sort out all logistics to charter the path forward. They were supported by other members of the society, especially the pioneers of proteomics research in the country and the founder members of the society along with other emerging researchers and society members.

The newly formed association demanded lot of changes and streamlining, which is happening in the background, and which will be evident with time. The editorial board has seen an expansion as will be evident in this issue. A wonderful team with plenty of diverse expertise and experience has been assembled and I thank all the board members for their prompt and kind acceptance of the responsibility that lies ahead. The first issue of JPP under the comforting umbrella of PSI will be launched on March 18, 2016, which also is Proteomics Day in India.

The JPP-PSI association promises to bring in more meaningful changes in the way the journal is published, with a view to improve quality and outreach. We could thus have separate sections for protein science and proteomics research per se. We could include new sections like news and views or opinions, a section dedicated to PSI activities and announcements, articles for research education and so on and so forth. We could publish theme based issues and augment student participation. We welcome everyone associated with the journal to send in their ideas and thoughts.

Some new ideas have already been ushered in. We thus have a Messages and Motivation section now for you to tune in with any message, criticism, appreciation or a word of motivation and letters to editors. In this particular issue, Dr. Surekha Zingde (President), Dr. Abhijit Chakrabarti (Vice-President) and Dr. Shantanu Sengupta (Secretary) inspired us. Dr. M.A. Vijayalakshi (EC member of PSI) also expressed her heartfelt wishes. This is followed by another new section named Moving Trends and Tips in Proteomics where experts in the field provided glimpse of the changing trends in proteomics research and advice on how to go about conducting proteomics based investigations. In this issue, three pioneers, pillars and early practitioners of proteomics research in India, Dr. Ravi Sirdeshmukh, Dr. K. Dhamalingam and Dr. Niranjana Chakraborty adorned the section with their

insight. We cannot thank them enough. We invite all proteomics researchers to keep this section alive with their inputs as well, time to time. We could include methods and standard operating protocols in this section as well. In addition we have also included a section on Technical Notes in this issue, which will provide insight into proteomics tools and technologies, especially the ones that are emerging. We hope our counterparts in industries would fill us in and contribute generously in this section.

As for articles, I would say that the issue represents diversity in protein science and proteomics research in India and showcases the breadth of investigations. To honor the society, the *first article* to be presented in the issue is a review on malaria related proteomics research. The authors outline how anti-malarial drugs alter the proteome of *Plasmodium falciparum*. It is an easy-read and summarizes scope of research in this area. The *second article* focuses on another deadly human disease, HIV, and reviews how the semen derived amyloids can be targeted to curb HIV infectivity. The basic concepts and knowledge in the area have been aptly laid down and the challenges outlined. The *third article* is an *in silico* investigation of the recognition dynamics of *E. coli* thioredoxin, which interacts and regulates numerous cellular processes. This provides wonderful insight into molecular recognition by proteins. The *fourth article*, which won the cover page, is a bioinformatics analysis of the human disordered proteome. It provides a statistical insight into the regions of disordered proteins that are useful in binding other proteins. The *fifth article* is a research methods paper. This is the first time that JPP carries a research methods paper. This article describes a new phage display method that will be highly beneficial to the community to map and characterize protein-protein interaction interface.

The Cover page selection this time was through opinion of the entire editorial board. Everyone participated enthusiastically and two such entries got the nod finally – of which the one that won majority got to the front cover, while the other got to the back cover.

Endeavour of this sort, where we bring articles to readers and authors at no cost or insignificant price, cannot sustain without financial charity. We thank Abhishek Glass Industrial Corporation for sponsoring this issue and their generosity in the recent past as well.

We are happy to present to you the first issue of JPP from the Proteomics Society, India and the nineteenth issue overall. We hope you will like our effort and flood us with your suggestions, opinions, warm wishes and above all lots and lots of articles. We hope with the society association, the journal grows in popularity and status and quality production of scientific deliberation.

*Editor in Chief*  
**Dr. Suman Kundu**

## **JOURNAL OF PROTEINS AND PROTEOMICS AND THE PROTEOMICS SOCIETY, INDIA BEGIN A NEW JOURNEY TOGETHER.**

Protein science has come a long way from the days when proteins were identified for their role in cellular function through several milestones which include the biochemistry of the protein, its localization in the cell, its amino acid sequence, post translational modifications, secondary, tertiary and quaternary structures and then crystal structures. All these have been supported by technology developments to address the changing focus of the inquiries from cellular to molecular and from use of gram/microgram to nano amounts of the material required for the investigations. In parallel with the advent of the human genome sequence, the genes coding for the proteins have been identified and there is still an ongoing debate on the number of protein coding genes. The search is on for the "missing proteins". The alterations in the nucleotide sequences and in turn the protein sequences and post translational modifications have led to altered folding and function. The knowledge that cellular functions are regulated through the actions of multiple proteins necessitated the science of proteomics. Information about the association of the proteins either directly or indirectly for the functions has evolved into pathways. The pathways are now moving towards integrated circuits and systems biology, molecular pathology, molecular physiology.....and back full circle to the molecular cellular biology of the 'organome'. The emerging data is so complex that it necessitated its organization into databases for easy retrieval and analysis so as to understand the unknown and contribute effectively to societal needs.

Protein science has been receiving attention in India for decades. Scientific contributions from India have kept pace with the changing protein and proteomics revolution globally. Study of protein/s is now targeted to unravelling the complexity of several biological systems and studying the biome at large. Several of the investigations are of major and specific relevance to the country. These investigations are being undertaken from University colleges to Research Institutes. The Journal of Proteins and Proteomics (JPP) was started in 2010 with an aim to provide a platform to publish such scientific information and make it available to the scientific community without a cost (<http://jpp.org.in/>). The focus of the journal so far has been on proteins, their isolation and characterization and structure-function relationships. A few of the recent publications include use of proteomic tools. The journal has effectively supported several Society meetings with publications of the proceedings. It has also brought to attention the work of Indian scientists who are recognized nationally and globally for their contributions to Protein science.

The Proteomics Society, India (PSI; [www.psindia.org](http://www.psindia.org)) was established in 2009 by a group of senior scientists, whose mandate was to provide a platform to nurture the then emerging field of Proteomics. Since its inception, the Society has held seven annual meetings and conducted workshops for providing focussed proteomic information and networking. It has organized an Education day and pre-conference workshops on proteomic technologies at each of its Annual meetings for the benefit of college students and teachers and researchers considering the use of proteomics tools for their biological queries. It has also nurtured proteomics in India through national and international technology exchange with support from its experienced members and the international proteomics community. The Society and several of its prominent senior members are recognized globally for their contributions to proteomic science (1-5).

At this point in its journey to promote proteomic science in India, the Society has now taken on board the Journal of Proteins and Proteomics as its official journal. The Jan-March 2016 issue will be

the first issue of the Journal arising from the association of JPP and PSI. Through this interaction, JPP will now bring the science of Proteins and that of Proteomics to the community.

I look forward to the members of the PSI along with the experienced members of the Editorial Board and the contributions from the scientific community towards strengthening the scientific impact of the journal and taking it to greater heights of excellence.

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## **PROTEINS AND PROTEOMICS....**

Indian scientists have traditionally excelled in theoretical science, mathematics, statistics and medium level computation as a community of practicing scientists. On the other hand, in experimental science, we might have not met expectations with notable exceptions of conventional Biochemistry and Protein Chemistry. In these areas, we have long been generating quality work, training manpower and developing related infrastructure for competitive research. Till date, purification of individual target proteins either from natural sources or through recombinant DNA technology, followed by their biochemical, biophysical and structural characterization either in the native state or during the process of unfolding/refolding and biomolecular interactions, have been one of the major areas of research in protein science practiced in Universities and Research Institutes of India. It then goes without saying that we ought to produce a quality peer-reviewed Journal dedicated for Proteins that would attract scientists of the globe to publish in India as well. The fact remains that there are many Indian Journals not doing well in this context. However, there are few which are performing decently and have earned respect in the global scientific community, measured by their rise in the Impact Factors. In particular, Indian Journals those have earned visibilities deal with manuscripts in broader areas of biological sciences or in areas of classical origin, viz Genetics. It is essential that a journal dedicated to Protein Science from India follow in the footsteps of its successful predecessors. This is where “Journal of Proteins and Proteomics” gains significance and relevance.

In my opinion, Journal of Proteins and Proteomics is going to gain grounds due to its inherent ambition of trying to combine both the conventional Protein Chemistry with the modern areas of Proteomics. Proteomics research, the science of investigation of sets of proteins, have begun to excel in India and will complement conventional Protein Chemistry. It would thus augur well for the large number of conventional protein scientists and those in proteomics to work in unison, with this journal providing a platform for the happy union. All that is needed for a new Journal to shine is to ensure that a manuscript gets its due from the peers and the knowledge or data in the manuscript is disseminated as fast as possible. It is thus important for Indian scientists to realize that there are growing needs for the merger of huge numbers of conventional protein chemists with the comparatively fewer proteomics scientists so that a large body of scientists could contribute to this particular Journal.

Proteomics is gaining importance with each passing day in Biology and particularly in Biomedical research. India has a long legacy in protein research. The pioneering work of G. N. Ramachandran is widely recognized and has rightfully occupied an immortal place in the text books of Biochemistry. Scholarly work on the structure and biochemistry of diverse proteins and peptides has been continuing for many years in several Indian laboratories and institutes. Proteomics activities, in which MS-based approaches are applied to solve biological problems or study protein dynamics in health and diseases, are however very recent with several institutes and groups incorporating the proteomics approach at the turn of the century. India now has a critical mass of Proteomics scientists. Post Y2K, many of us had embarked on the journey into “Omics” biology to unearth possibilities of bigger discovery and catching up with the “frontiers” of global science. We started searching for biomarkers or new therapeutic proteins and pumped money for quick benefits. However, the entire Omics community, particularly those in Proteomics and Metabolomics, have realized that research on conventional

protein science also has to continue simultaneously. It is my dream to see all individual Protein Societies in India to join hands with Proteomics Societies and vice versa and literally flood Journal of Proteins and Proteomics with quality articles on Proteins and Proteomics. In the era of Open Access Journals and increasing costs of publication in the journals of the West, I am afraid we have to make our claims as early as possible and publish at a lower cost. It would not be wise for us to depend all the time for our publication needs on traditional publication powerhouses. We do have a very large protein science community in this country for which a journal like Journal of Proteins and Proteomics need to be supported and has to live long and healthy!!

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## **LET THE SOCIETY AND THE JOURNAL GROW TOGETHER**

Proteomics Society is currently one of the most active academic societies in India, which in only a very short span of 7 years, has plenty of achievements to be proud of. The Society engages in various activities round the year, which includes conducting scientific meetings, workshops and education & training programs for students, college teachers and young scientists. The Society provides opportunities for networking and collaborations while spreading the cause of proteomics research nationwide.

A new feather in the cap of this growing academic body is “The Journal of Proteins and Proteomics”, a journal with genesis in India and one which is making a mark of its own. This journal provides the society a platform that can amplify all its ongoing activities. It helps the society serve the scientific community in multi-dimensional ways. While the journal will publish articles in the areas of protein science and proteomics, it will also help showcase the achievements of Indian scientists in this field. It will include technical notes and collate information on meetings, workshops and other activities. I am sure there are plenty of other activities that can be brought under the ambit of the journal for which I solicit your constructive suggestions.

The journal also allows the society to interact with the world in a more meaningful manner. It allows us to leave our own footprints in the proteomics research arena and bring up and solve issues that will benefit the entire community at large. It will help improve the quality of research and publications. Since there are no journals dedicated to proteomics research in India, we take it as a challenge to develop one. It is for us to take this journal forward with passion.

The onus is on us to nurture the journal; to see it grow to greater heights and to serve the society through this platform in addition to the numerous other activities that the society already engages in. All we need to do is to think of this journal as our very own, contribute in whatever way we can and sustain it with passion. A major reason why most of the Indian journals do not make a mark, is the reluctance of researchers to publish their high quality work in these journals because of their low “impact factor”. Interestingly, the “impact factor” of these journals is low due to the lack of quality publications which do not attract citations. I sincerely believe that it is high time we try and circumvent this chicken-or-egg-first problem. If we all take a decision to publish at least one quality work in our journal per year, I am confident that within a short time, India can boast of a journal that has created an impact in the scientific community.

Let us all work together to achieve this glory. I pledge on my part to give it all that I can.

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## **WISHES FOR JOURNAL OF PROTEINS AND PROTEOMICS**

I am very pleased to be a part of the Journal of Proteins and Proteomics (JPP), which has the potential to act as a window for bridging Proteomics research in India and the world, both in academic and industrial segments. Proteomics is still an emerging field in India. Nevertheless, this growth is exponential, as has been reflected in the recent Nature India Special Issue themed on Proteomics in India. I am sure very soon this aspect will be reiterated in the pages of JPP on a regular basis. The journal will have an opportunity to aptly showcase the achievements of the Indian proteomics community, while integrating research work from across the globe.

Since the inception of the Proteomics Society, India (PSI) in 2009, Indian scientists actively pursuing research in the domains of proteomics have had a platform to share their expertise through annual meetings and have also been actively involved in global proteomics meetings, which has in turn been very well appreciated by the international community. The present adoption of the JPP by the PSI is yet another forward move, which will take proteomics research in India to even higher levels.

Although proteomics has shown enormous potential for clinical applications, their real-life applications have been limited owing to the inability to distinguish structural differences between proteins and their variants. In order to overcome these challenges, separation science is now being hyphenated with proteomics. Bio-separation science and technology is the system that exploits molecular recognition or bio-recognition, which becomes a very crucial tool in studying proteins, even before proteomics can be exploited to its best. Separation science includes conventional tools as well as cutting-edge technologies with new matrices and ligands (both biospecific and pseudobiospecific). Particularly, some of the systems, which have been proposed by some experts, can discriminate between structural variants. Thus, development of integrated systems hyphenating them with proteomics analysis will provide solutions to many of the current challenges in the field. Having represented and contributed for years in the area of separation technology, I sincerely hope that JPP will take up these issues in its fold as well. I am eager to help the journal in focusing in the field of separation technology along with various advancements in other technologies in proteomics.

With these new advances, I sincerely hope and believe that JPP will give an opportunity for Indian industries and MNCs to look into emerging technologies, so that the Industry-Academia partnership can be strengthened using JPP as a window in addition to all its purely academic endeavour. I wish JPP to become a lighthouse system for portraying our capacities and make our international standing very strong. We will all work together to make this dream come true.

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## **INDIA AND THE QUEST TOWARDS HUMAN PROTEOME**

**Ravi Sirdeshmukh**

Given the diversity of proteins in terms of their chemistry, biological functions and dynamics, deciphering human proteome and its function is one of the highly complex biological challenges ever encountered by the scientific community. "Missing Proteins" - lacking clear or adequate protein level experimental evidence for their existence, multi-functionality of proteins and the existence of undiscovered splice variants of the proteins are some major contributors to the challenge. To unravel the repertoire of proteins and their multiple proteoforms originating from about 20,000 protein-coding human genes and to do it in a comprehensive manner in physico-chemical and functional terms is thus a task to be handled by the entire proteomics community and not one group or the other.

### **Mass spectrometry - the work horse**

Large endeavour like *The Human Genome Sequence* could not have been implemented without the technological development and improvement in analytical platforms for DNA sequencing over the past decades. Their miniaturization and enhanced sensitivity and computational pipelines helped in achieving the speed and throughput. On a parallel line, development and application of mass spectrometry has revolutionized the scope of protein analysis. The instrumentation capabilities and the analysis pipelines have improved on all fronts - the throughput, sensitivity, resolution, accuracy and user-friendly formats. All this has enabled a gradual transition from the conventional style of analysing one protein-at-a time, to a few hundred proteins by 2-dimensional gel based separation combined with peptide mass finger printing of individual proteins, to application of shot-gun proteomic strategies capable of deeper access to a few thousand proteins in the form of their peptides using liquid

chromatography-mass spectrometry (LC-MS/MS) approach. With that too having some limitations and constraints for deep-analysis of multiple clinical samples, recent years have seen the emergence of targeted analysis of the proteome - *the data independent analysis*, which uses prior information of proteins and peptides and analyzes pre-selected, large number of peptides in a sample in targeted manner. A wide range of biological databases and bioinformatics tools for querying data as well as innovative peptide tagging-chemistries for quantitative purpose have added further strength to the mass spectrometry applications. Top-down proteomics of intact proteins using mass spectrometry or tissue imaging using mass spectrometry is not common and routinely implemented but is potentially promising for application. Emergence of metabolomics and strategies for integration of the data originating from different omics studies, now in place, allow integrative biology in general. Protein research has been in the fore front in India since many years and served as the foundation for imbibing all the above developments for taking our research efforts in the large scale Proteomics mode.

### **Infrastructure and research focus**

The Council of Scientific and Industrial Research (CSIR) supported the first Proteomics program and the first Proteomics Facility in the country at the Center for Cellular and Molecular Biology (CCMB) Hyderabad, in 2001-2002. Today a large number of public and private institutions have high end proteomics platforms to conduct proteomics research using one or the other approaches described above. Some of the national facilities of particular mention are the facilities at the CSIR-Institute of Genomics and Integrative Biology (IGIB), International Center for Genetic

Engineering and BioTechnology (ICGEB), National Institute of Plant Genome Research (NIPGR), all at New Delhi; National Chemical Laboratory (NCL) and National Cell Science Centre (NCCS), Pune. A private not-for-profit organization, the Institute of Bioinformatics (IOB) in Bangalore earlier engaged in large scale Bioinformatics programs set up its mass spectrometry lab with support from the Department of Biotechnology (DBT), Government of India. IOB is also instrumental in setting up state-of-the-art proteomics facility and coordinating proteomics research at two other Centers, namely, NIMHANS, Bangalore and Yenepoya University at Mangalore. Another private research centre under Aravind Medical Research Foundation (AMRF), Madurai runs a DBT-supported facility for its research. These centres have been actively working on a spectrum of research areas. If one makes a literature search since the time of the first proteomics publication from India, on human brain tumors, in 2005 [1] from the author's group, then at CCMB-Hyderabad, one could see important contributions from the Indian Proteomics scientists – touching every area listed above – quantitative proteomics, protein databases and bioinformatics tools, integrated data analysis and now targeted proteomics and others [2-4]. Here, a few of them may be mentioned. The NIPGR Group, through series of publications has demonstrated how even 2D-MS approach with somewhat limited capability can be intelligently applied to study plant proteomics. IOB, through its large number of publications arising from both in-house projects as well as collaborations, has shown how mass spectrometry can be exploited maximally to study proteins quantitatively for their clinical relevance. The NCL group not only identified the role of a protein modification in the context of diabetes but also formulated the basis for developing an assay for clinical application. The IGIB group works on cardiac disorders using animal models applying proteomics and bioinformatics approaches. The ICGEB group has focused the mass spectrometry-based studies on a very targeted question of *Mycobacterium tuberculosis* infection to explore host proteins and metabolites. The AMRF group has been working on eye-infections and other conditions utilizing

mass spectrometry. (Only overall interest areas of the groups are mentioned; no specific references are therefore cited. Also, there are important contributions from other groups that are not listed.)

### **Draft Maps and now - chromosome centric Human Proteome Project**

Above activities represent the areas of primary research interest of the individual research groups, many of them having the potential to be developed and incorporated in the perspective of building the Human Proteome. However, two seminal publications, the mass spectrometry and the antibody based human proteome maps [5,6] majorly contributed by the Indian groups (IOB, Bangalore and Labsurgpath, Mumbai), are exemplary efforts that represent fully dedicated large scale works and the first staples in the mission towards the Human Proteome.

The Draft Map of Human Proteome contributed almost entirely by the IOB group [5], provides a catalogue of proteins, their tissue-distribution and developmentally regulated expression based on large scale mass spectrometry data. The IOB work not only describes the known components of the human proteome but also provides evidence for the "missing proteins", novel proteins, expressed pseudogenes, novel frames and novel start sites for proteins. A publication by Human Protein Atlas team in Sweden, describing Tissue-Based Human Proteome Map with major Indian contribution from the pathology team of Labsurgpath, Mumbai (an official site of Human Protein Atlas, Sweden), is a study of expression and distribution of human proteins across tissues and cells using specific antibodies [6]. The antibody based proteomic map provides tools and analysis of protein expression and their tumor-related alterations in human tissues. In a way it expands the huge potential and scope of immunohistochemistry in clinical context. The scientific details of the two publications have been reviewed earlier [7]. However, it may be noted here that the IOB effort demonstrates the power and capability of mass spectrometry as well as the grit and teamwork of the young group to achieve a task of this magnitude; the second

publication reveals the impacting hard work of a group of surgical pathologists inspired by the new science of proteomics.

The goal of understanding each protein and its variants and relating them to functions does not stop here but is an ongoing quest. An extended and deeper study of the biology of a protein and its regulation is still open. The Chromosome centric Human Proteome Project (C-HPP) and Biology and Disease centric HPP (B/D-HPP) initiated by the Human Proteome Organization (HUPO) are the two arms of the global effort to pursue this endeavour. Using experimental or bioinformatics approaches, the two arms of HPP (C-HPP and B/D-HPP) aim to catalogue and comprehensively study human proteins and their variants in terms of biology and disease, in a chromosome centric manner. The task is humongous, for the reason that although the number of protein-coding genes and their primary protein products has stayed around 20,000, the number of protein variants has increased and is more than 40,000 as per the current estimate and may expand even further. The project therefore brings together research teams from various countries (more than 20), each focussed on a chosen chromosome. India is also a part of this international effort. A consortium formed among five Asian countries - Hong Kong, India, Singapore, Taiwan and Thailand, currently led and coordinated by the author aims to study Chromosome 12 encoded proteins and their disease context as a part of this initiative. The group launched the effort with the first series of papers giving the overall vision and initial contributions [8-10] which have been reviewed by the author [7]. Subsequently there have been other contributions in the 2015 Special Issue of Journal of Proteome Research dedicated to the project [11,12] and some expected to appear in the 2016 special issue of the journal. Another independent publication by an Indian group, also appeared in 2015 [13], that describes an approach and identification of “missing” proteins in general.

Chasing “missing proteins” is one common goal of all chromosome teams and so that of Chromosome 12 team also. The publication by Manda et al. [9] provided new mass spectrometry

evidence for 89 “missing proteins” from Chromosome 12. After curation of the data from this and other publications, the present number of “missing proteins” in neXtProt was corrected to 142 as against 171 earlier. In future efforts, the IOB group is exploring deep, sub-site centric proteome of the human brain to detect additional missing proteins that may be very low abundant or expressed only in functionally specific areas of the brain. The Singapore group from National University of Singapore and from the Bioprocessing Technology Centre, Singapore plan to look for missing proteins in induced pluripotent cell line data. Apart from building the “part list” of chromosome 12 through data mining or experimental approaches, the analysis so far set initial leads to open the following biology and disease-driven objectives.

1. Extract a subset of proteins which may have secretory potential from among the cancer related genes/proteins mapping to chromosome 12. This will be a step towards building a chromosome and cancer centric resource of priority circulatory proteins and their proteotypic peptides for targeted studies.
2. Develop novel molecular insights to understand in depth co-expression, co-regulation/de-regulation and co-localization of cancer related genes and proteins. It has been observed that many differentially over expressed tumor (glioma) proteins mapped to chromosomal loci including a well characterized amplicon on Chromosome 12 [10,11]. There are also instances where key protein members altered in tumors (oral malignancies) have the genes for their subunit components localized on different chromosomes. Clustering of altered genes at key loci such as amplicons or presence of protein sub unit component genes localized on two different chromosomes open new biological questions in terms of intra- or inter-chromosomal cross talks and regulation of genes.
3. Use chromosome centric omics data on diseases and integrate it with medical

terms used in the context of diseases and associated range of clinical conditions and develop an integrated network – a “Diseasome Map” that would display differentially regulated molecular entities and inter-related clinical phenotypes and reveal new entities from this relational context for clinical application [11].

4. Integration of expression data at transcriptomic and proteomic level would provide an additional tag to the results that emerge from the above two exercises and may generate new regulatory leads [12]. The integration of transcriptomic and proteomics data also reveals new alternately spliced variants of proteins, etc. thus expanding the part list further.

### Prospective plan and future

The above lead objectives have emerged from two separate analyses that was carried out with experimental data from primary brain tumors (gliomas) and tumors of the oral cavity [refs. 10-12,14]. This underscores the possibility that the chromosome centric protein analysis work can be effectively embedded into the other work areas of the investigator lab. Continuing this effort in the next phase of the B/D-HPP effort, the approach will be extended to other diseases and clinical conditions of interest to individual investigator groups and include GI tract tumors (IOB, Bangalore and NUS, Singapore), Diabetes and associated disorders (NCL, Pune) and cardiac disorders (IGIB, New Delhi). While this is being done, efforts are necessary to engage basic cellular and molecular biologists, clinical geneticists and others working on chromosome structure and function to develop a working hypothesis on regulatory aspects in the context of co-expression and co-localization of genes and develop experimental designs to study them. India being a centre with many expert scientists in these areas, there are high prospects of pursuing this goal.

Meetings and interactions are thus going to be important for the group to build broader capability and the proteomics community in India is well geared for this. The Proteomics interest group in India has been active since many years. Inter-institutional collaborations, educational

workshops and conferences have been happening since the initial days of proteomics activity. During the initial years of Proteomics activity in the country, they were mostly coordinated from CSIR-CCMB, Hyderabad. Proteomics Society, India (PSI) was formed during later years and formally launched its activity in 2010 with its first event, the 5<sup>th</sup> AOHUPO Congress. PSI supports and continues the philosophy of education and collaboration through its annual conferences, educational workshops and training courses has developed an identity over the years on the national and international forum. The group can move on with the research effort with enhanced connectivity among the spectrum of scientist with varied expertise and pursue a well-integrated approach to the global mission of exploring the Human Proteome. The initiative of adopting the Journal of Proteins and Proteomics will add an extra element in this mission and hopefully the journal will develop into an effective medium to project the efforts of the Indian Proteomics Scientists as well as those from other regions in this collective human endeavour.

The author thanks the HUPO fraternity for interactions on the Human Proteome Project. The author is the Founder President of PSI and draws inspiration from the societal commitment of PSI leadership and its members. The scientific ideas and leads discussed in the article have largely emerged from the work of Mr. Manoj Kumar Gupta, Ms. Savita Jayaram from the author's group.

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## **THE FIELD OF PLANT PROTEOMICS: CHALLENGES, LIMITATIONS AND FUTURE PROSPECTS**

**Aarti Pandey, Subhra Chakraborty and Niranjana Chakraborty\***

According to the latest United Nations estimates, the world population is expected to reach 8.2 billion in 2025. Assuming that no significant changes in per capita grain consumption occur, the global requirement of food grains in that year would be about 3050 million tonnes including food, feed and industrial use. This ever-increasing population growth and food requirement coupled with decline in arable land as well as changing global climate has made it crucial to ensure efficient crop yields and sustainable food production. To achieve this objective, novel tools are required for protecting crops against biotic and abiotic stresses and for unraveling the mechanisms underlying the development and vitality of seeds. The headway made in effecting the sequencing of whole genomes across the plant kingdom has ushered in the post-genomic era where proteomics has become an increasingly important technique that offers unique insight into the cellular function at the level of cell, organelles, tissues, organs and whole organisms. In fact, plant biology has now whole-heartedly embraced high-throughput 'omics' technologies to accelerate the process of discovering the gene functions in a systematic manner.

The first reported large-scale plant proteomics study was carried out in *Arabidopsis* (Kamo et al., 1995; Rossignol, 2001). The first such study in India was published almost a decade later (Bhushan et al., 2006; Narula et al., 2015). Since then plant proteomics studies have expanded progressively and are now being carried out in various institutions/universities spread across the country. Currently, various proteomic approaches that are being employed successfully for elucidating plant processes include gel-based and mass spectrometry-based methods that involve both label-based and label-free protein

quantification. In recent few years, the focus seems to have shifted in favor of non-gel based methods. Even in this changed scenario, complete protein extraction from different plant organs or subcellular organelles remains the greatest perplexing aspect of proteome analysis. Protein-protein interactions and post-translational modifications add another dimension into protein molecular function. The data generated through proteomics can substantially contribute to the understanding and monitoring of physiological events and development of biotechnological strategies. Having said that, despite the recent significant advancements in proteomics techniques, there is still scope for improvement.

The peculiarities of the plant kingdom have forced the researcher to adjust their proteomic strategies so as to improve efficiency and accuracy of results. The key challenges for a successful proteomics experiment include low protein concentration, difficulties in protein extraction, genome multiploidy, high Rubisco abundance in green tissue, and/or an absence of well-annotated and complete genome sequence (Bindschedler and Cramer, 2011). Despite decreasing sequencing costs, increasing sequencing speed and improvements in gene annotation and modeling, complete and well-annotated genomes for all crop plants seems only a distant reality. Further, multiploidy among the plant genomes adds to this limitation. However, the situation is now improving with the completion of several whole-genome sequencing projects for model and non-model plants. A fruitful proteomics workflow is dependent on both the starting material and its complexity and may require sequential solubilization, depletion (to eliminate major proteins such as RubisCO), or subfractionation in order to obtain maximum



protein coverage. Plant cells are considered more recalcitrant than animal, prokaryote, or unicellular eukaryote cells owing to their low relative protein content; the presence of the cell wall and vacuoles that account for the majority of the cell mass; and the accumulation of large quantities of polysaccharides, lipids, phenolics, and other secondary metabolites that reduces the protein solubilization, modifies the protein chemical composition, or interferes with downstream steps in the proteomics workflow (Jorin-Novo *et al.*, 2015). Apart from the above, there are also differences in the protein and non-protein content across the organs and this variability is also dependent on the developmental stages as well as the health of tissues being studied. For example, time-point studies for biotic and abiotic stresses become particularly laborious due to extremely low amount of protein that can be extracted from the tissues at later stages. The organ under consideration also affects the choice of a protocol for maximum protein coverage. Even though leaves have considerably higher protein content than roots and stems, high concentration of phenolics as well as presence of Rubisco makes it a difficult organ to work with. Similarly, seeds have a high concentration of sugars as well as lipids, which again add to various challenges. Nevertheless, new strategies are forever evolving to deal with these issues. A series of reviews dealing with the development of plant proteomics is available in literature (Canovas *et al.*, 2004; Rossignol *et al.*, 2006; Jorin-Novo *et al.*, 2007; Jorin-Novo *et al.*, 2009; Jorin-Novo, 2014; Jorin-Novo *et al.*, 2015).

While most studies have focused on profiling the proteomes and protein modifications in model species at whole plant, organ and/or organellar level, proteomics has also been widely used to investigate protein dynamics during growth and development as well as sensitivity to adverse environmental conditions, thus contributing to the development of next generation crops. Now that we have generated a plethora of information in this new age of plant proteomics, coupled with other 'omics' technologies, the ensuing accepted phase will be the amalgamation of all this data following a systems biology approach. The integration of proteomics with other data, ranging

from genomics, transcriptomics, metabolomics and phenomics, will provide cross-validation of the obtained results thereby increasing the biological significance. The systems biology approach will not only aid in elucidating the complex signaling and metabolic network underlying plant growth, development and interaction with the environment, but will also serve as a valuable resource to enable predictable engineering of plant processes towards improved crop yield, nutrition and defense. The development of databases catering to plant research community will be an added feature. An incessant upgrading of proteomic methodologies is also needed to address the issues of tomorrow. Better strategies for protein quantification as well as identification will in turn lead to a deeper proteome coverage and enhanced understanding of plant processes. Last but not the least, the proteomics technology needs to be made accessible to non-specialized groups as this will greatly benefit the plant research community worldwide.

On the national front, recent years have seen a tremendous growth in plant proteomics research in terms of publications in spite of limited funding, expensive proteomics setup and scarcity of trained workforce (Deswal *et al.*, 2013). Plant proteomics research in India is making a noticeable impact to the global proteomics scene with major focus on organellar proteomics, stress proteomics, plant development, food and nutrition, and post-translational modification/s (Narula *et al.*, 2015). Though the establishment of Proteomics Society, India (PSI) was a major achievement, the representation of plant proteomics was initially minimal. This situation is seemingly changing. In fact, Indian science and scientists have also made their presence felt at the International Plant Proteomics Organization (INPPO), which saw the establishment of an 'INPPO-India-Nepal Plant Proteomics Chapter (INPPC).

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## ***Moving Trends and Tips in Proteomics***

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### **DOING PROTEOMICS - A BEGINNER'S GUIDE**

**K. Dharmalingam**

Proteomics by nature invoke in many, both novice and experienced investigators alike, the awe of dealing with huge data. Associated with this large data are the misgivings in the mind of many, the problem of noise interfering in the interpretation of real data. Therefore one has to be extremely careful both in data generation and data interpretation. In this brief write up I will highlight some of the issues that could be of help to the beginner to plan properly before embarking on major proteomic studies.

Proteomic approaches will help only if one selects appropriate problem to explore. The availability and use of high-end equipment is not a guarantee that you will be able to answer important questions. Therefore utmost care is needed in the selection of the experimental study and the choice of the experimental system. In this phase, one also should understand the capability of the instrument to handle complex samples and its ability to deal with the depth of analysis needed for the specific study. Availability of the sample is a constraint that should be addressed in the early phase.

The selection of the problem as well as the sample goes hand in hand with correct sample handling procedures. At this stage make sure to get the advice of your clinician scientist about the samples if you dealing with clinical samples. In fact, it is important to keep the clinician informed about the methods and procedures you use all the time. Many times new questions arise and new avenues open up when you explain the approaches you are using in order to solve a problem to a medical practitioner. In proteomic studies many a time wrong sample processing leads to erroneous result. Extreme care should be taken to adhere meticulously to established procedures when dealing with biopsy materials

and body fluids, starting from collection to storage. More importantly adapt the method to your laboratory practice. Just because the sample is kept frozen at very low temperature does not mean there will not be any protein alteration! Particularly in biomarker study this step will lead to extra amount of time and effort if the procedures are not strictly followed.

Sample pre-fractionation is another step that will go a long way in obtaining a comprehensive picture of the proteome. One must fine-tune the pre-fractionation techniques to help overcome the limitations of sensitivity of the Mass spectrometer. One has to keep in mind the importance of good biochemical fractionation techniques that will help immensely in compensating the shortcomings of the sensitivity issues. In fact clear understanding of protein biochemistry will help you all along. Protein biochemistry and proteomics complement each other. However, care must be taken to prevent sample loss and biased redistribution of proteins due to fractionation. Many resort to shot-gun approach to examine the proteome and avoid fractionation totally. In practical terms it could be difficult. In such cases where this offline fractionation is incorporated, extra care should be given for the prevention of sample loss and bias. The amount of information one could get because of pre-fractionation makes this an essential step under many experimental approaches. In a recent workshop on "Experimental approaches to Proteomics" conducted at AMRF, Madurai, many young participants expressed their surprise when they were exposed to the various problems one encounters starting from protein estimation to peptide clean up. This aspect needs careful planning and execution for any proteomics experiment.

Today there are multitudes of mass spectrometry platforms available for the investigator. Consequently one has to choose the available instrument setup for the instrument in his or her lab. My advice is to understand fully the instrument and the capabilities of the particular mass spectrometer. Develop a standard operating procedure and make sure the instrument is tuned to its optimum performance. Regular diagnostic check using a complex defined protein mix prepared in your lab by the experimenter is a must. The vendor recommended standards are useful but they are not going to help you if the instrument could not perform well with your samples. I urge my senior colleagues to put up their standard operating procedures developed for their instrument platform, if possible, in the pages of this Journal.

Data analysis depends on either vendor specific software or open source softwares available freely. It is better to use the vendor-developed software if you are convinced the open source softwares are not as good. Vendors upgrade and fix the problems of the software regularly and some of the open source softwares are not updated regularly. In any case you might try the open source software to enhance your analysis. Apart from what software could do to analyze your data learn to examine the output manually.

Data interpretation and integration are the final goals of proteomics experiment. Therefore, one should pay close attention to this aspect. In many cases it is good to rope in a bioinformatician

at this stage, if you have an in-house expertise. It pays to get expert advice in extracting information from the data.

Quantitative proteomics is now a mature technology. A recent paper (Schmidt, A. et al., *Nature Biotechnology*, 2016. 34.104-112) describing the quantitative data for more than 2300 proteins of *E.coli*, (55% of *E.coli* proteome), grown under 22 different conditions is a good example of the power of quantitation at proteome wide scale. Ability to examine quantitatively the proteome rearrangement according to the life style of the organism is an exciting development.

We, in India have a distinct advantage of using proteomics fruitfully because of the diversity and availability of quality samples. All one has to do is to forge productive collaboration between proteome experts and other practitioners such as clinicians. Young investigators have a golden future ahead if they choose proteomics for their research.

Last but not the least, this new journal, *Journal of Proteins and Protein Biochemistry*, will go a long way in bringing Indian proteomics in particular and proteomics in general to a larger audience.

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