Proteomic Society – India (PSI)

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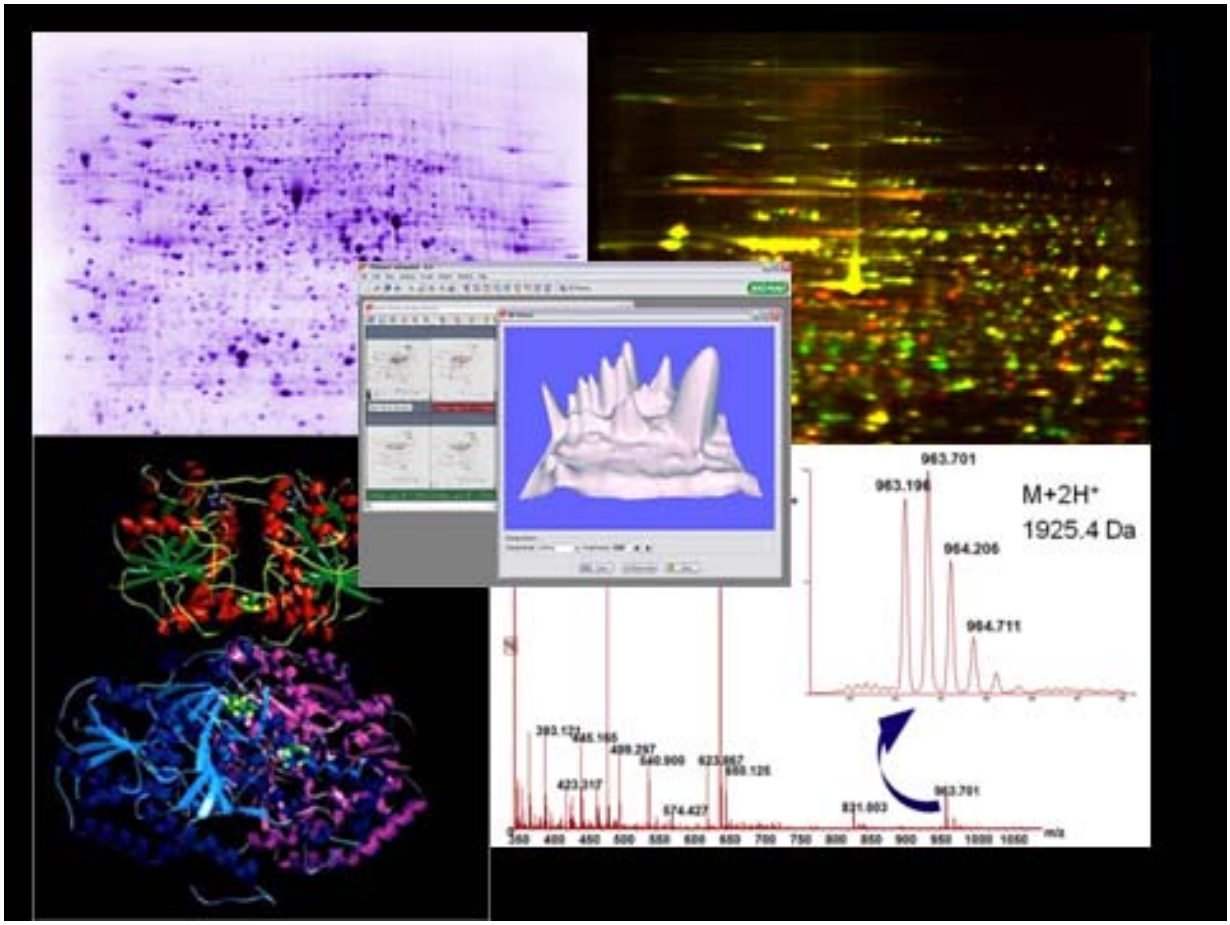
Editor: Abhijit Chakrabarti



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From Editor's Desk

Happy New Year to you all. I am happy to see that we now have our PSI News Letter, Volume 1, No. 1 beginning the year 2011 but let me confess that this inaugural News Letter of PSI was actually created by my graduate students, Suchismita, Shilpita and Avik – like my research. We would like to stay with the content-format that is reflected on the cover, but suggestions are welcome from the members. This time we have been somewhat liberal with regard to the length of the articles, however, for future we shall be attempting to make it more concise. Being the first News Letter, Ravi has contributed the most to this. I am sure it would be easy to bring it out at least thrice a year for which it is of utmost importance that all PSI members contribute providing articles of broad significance, news, technical opinions/views and of course, suggestions on this great adventure of Proteomics which is here to stay….

Abhijit Chakrabarti

Saha Institute of Nuclear Physics

From the council

Message from the President

As we all know, Proteomics is taking an increasingly key position in Biology and BioMedical research. India has a strong legacy in protein research. The pioneering work of late G. N. Ramachandran on protein structure is widely recognized and has a place in text books of biochemistry. Scholarly work on the structure and biochemistry of proteins and peptides has continued for many years in several Indian labs and institutes. Proteomics activities in which MS-based approaches are applied to biological questions or to the study of protein dynamics in health and disease are, however, recent. These activities and efforts in the country evolved around the turn of the century and today many Institutes and groups have been using proteomics approaches in their research efforts. The Council of Scientific and Industrial Research (CSIR) supported the first Proteomics program on cancer in 2001–2002 under the New Millennium Initiative at the Center for Cellular and Molecular Biology (CCMB) in Hyderabad**.** Subsequently a number of other centers in the country have initiated Proteomics programs funded by other national research agencies such as DBT, DAE, ICMR and others. The first Proteomics Conference in India convened at the CCMB, Hyderabad in 2003 was attended by students and scientists, and most of them were initial learners in Proteomics. A meeting in February, 2008 on Current Trends in Proteomics again organized at the same venue under the support of CSIR Network for International Collaborations was attended by close to 200 senior investigators and students, and all of them were already running Proteomics projects. A recent Meeting – 5th AOHUPO Proteomics Congress held in Feb. 2010 was a demonstration of the novel contributions in the field from the Indian Proteomics Community. The critical mass of proteomics scientists has thus gradually grown in the country. As the research efforts expand and technologies evolve, it is important to facilitate interactions among the Proteomics community and help them share knowledge for the growth of this field. Thus, meetings and interactions are going to be important for the group to build its capability further.. Proteomics group that had assembled at the 2008 meeting underscored the HUPO philosophy of cooperation, collaboration, and education among the fraternity. To facilitate these objectives, a consortium of the Indian proteomics scientists, **Proteomic Society – India** (PSI), has been formed in September 2009, to provide a forum to pursue an agenda that will meet the above objective. The Society is strongly **dedicated to education for research in Proteomics** through Seminar Series for students and college teachers, theme workshops for researchers and Annual Conferences to share their knowledge

and experience to discuss the international Trends. PSI is still in its infancy but a lot of effort is being put in the direction of making its objectives successful and useful. I am happy that several experienced and well known investigators have enthusiastically joined this endeavour. The first Executive Council of the Society thus includes Protein scientists from some of the premier national labs / Institutes and universities already engaged in Proteomic research and we are further inducting representation from Industries and other organizations in the council. The Society has already initiated its Educational activity through **seminar series** at different locations in the country which included basic technology to their biological and clinical applications to discussing experimental challenges and trouble shoots. It has already conducted Hands-on Training Courses. As the educational agenda of the Society grows further, it is heartening that the vision of PSI has already made an impact on the international forums such as AOHUPO which would like similar education agenda to be extended to the AO region.

Thus I would like to request all the members particularly the Council members that in addition to our own research activity, we need to contribute to further the efforts of our other colleagues such that we enhance our own learning and that of others and large investments in Proteomics that are going on at present yield fruitful outcomes to eventually benefit the Indian community. Proteomics in clinical perspective is a multidisciplinary endeavour, so, I on behalf of my council members would like to encourage and invite individual scientists, technologists, clinicians as well as corporate bodies to join us so as to make the society most useful forum for exchanging techno-scientific and educational ideas for the benefit of Ph D students, scientists, clinicians and Industry as well.

Thank you - Ravi Sirdeshmukh

Research highlights

Proteomics in India : An Overview

Ravi Sirdeshmukh

(Adapted and updated from Mol. Cell. Proteomics (2008) 7, 1407).

Protein research has been a forte of Indian biological research for many years, and it is with this scientific background, Proteomics activities and efforts in the country evolved around the turn of the century. The Council of Scientific and Industrial Research (CSIR) supported the first Proteomics program on cancer in 2001–2002 under a new millennium initiative at the Center for Cellular and Molecular Biology (CCMB) in Hyderabad. Subsequently a number of other centers in the country have initiated Proteomics programs funded by other national research agencies and the trend seems to be gradually increasing. Proteomics of Brain and oral cancers represented the first Proteomics effort from India. Brain tumors, gliomas have low incidence but are highly significant in terms of cancer-related deaths. The CCMB group has been pursuing the Proteomics of these complex tumors (1). Now, with the help of clinical neurology groups and the Indian site of the Swedish Human Proteome Resource Center and HUPO antibody initiative, CCMB is organizing a nucleus for collaborations on antibody-based proteomics of gliomas. There are also several other groups now who are engaged in the study of gliomas and include a DBT Centre of Excellence at the Institute of Science, Bangalore or Antibody Proteomics lab at the Indian Institute of Technology, Mumbai. Cancers of the oral cavity occupy a high volume in India and have been the obvious initial targets for Proteomic investigations. The work from the Advanced Center for Treatment, Research, and Education in Cancer (ACTREC) in Mumbai in collaboration with CCMB has produced excellent work on tumor proteomics (2, 3). Some Infectious diseases are more relevant in the Indian context, malaria and leprosy being two among them. A group at the Indian Institute of Science, Bangalore has been working on protein-protein interactions involving molecular chaperones and is looking into the clinical proteome of human malaria parasites Plasmodium falciparum and Plasmodium vivax to understand their specific roles in the parasite growth and provide rational basis for anti-malarial drug development (4). Work from Madurai Kamraj University, Madurai has revealed changes in haptoglobin isoforms in the sera of leprosy patients (5), reflecting disease-related changes in a member of the abundant plasma proteins and demonstrating the importance of “abundant” plasma proteins in

differential proteomics of some diseases. Specific haptoglobin isoform association is also shown recently with Glioblastoma multiforrme (6), in a study from the IISc group. A gram negative bacterium A. baumanni is one of the highly drug-resistant microbes responsible for hospital infections. Scientists at All India Institute of Medical Sciences, New Delhi have been studying DIGE analysis of outer and inner membrane proteins of β-lactum resistant and native strain of the organisms which reveal the proteins associated with resistance and which could be explored further as drug targets (7). Visceral leishmaniasis is a major public health issues with the number cases increasing every year. Again, drug resistance of the parasite associated with immune suppression in the host demands effective markers for early diagnosis. Studies on the disease–associated, acetylated sialoglycoproteins present on erythrocytes from the patients are being carried out at the Institute of Chemical Biology, Kolkata. These proteins and modifications could serve as useful biomarkers. There are newer initiatives coming up from Institute of Genome and Integrative Biology and National Institute of Immunology, New Delhi, National Chemical Laboratory, Pune and Indian Institute of Chemical Biology, Kolkata. On the bioinformatics front, there are number of Indian groups who have made significant contributions; however, the efforts concentrated more on the analysis of the dynamics of protein structure and conformations. Work on protein databases or bioinformatics tools for processing proteomic data has been rather limiting. It is known that large part of the genome of various organisms including humans still remains to be "predicted". Implementation of Proteogenomic approaches in which high resolution LC MS data could be directly used to validate known as well as predicted genes. Institute of Bioinformatics (IOB), Bangalore has been exploring several systems from pathogenic organisms to plants to human genome to illustrate corrections in existing gene annotations as well as annotating expression of genes which are still predicted. Work on Mouse Embryonic Stem cell Proteome from CCMB also reveals several proteins corresponding to the predicted genes including putative transcription factors. IOB is also engaged in large scale Bioinformatics programs. The Human Protein Reference Database developed by IOB is one of the most widely used human protein database, whereas the most recent contribution of the IOB has been the human Proteinpedia (8), which is found to be a very valuable resource for the community. A group in Kolkata at Saha Institute of Nuclear Physics is involved in clinical proteomic studies of red blood cells implicated in blood disorders. They have come up with a novel hemoglobin-depletion technique to study disease-regulated expressions of

redox regulators and chaperones in the red cell cytosol (9, 10). IOB is now being supported by DBT (Centre of excellence jointly with NIMHANS, Bangalore) for studying neurological disorders such as epilepsy and stroke. It is also planning to set up a major Program on Translational research. MassWiz is a new tool developed by the Bioinformaticians at the Institute of Genomics and Integrative Biology, Delhi, based on custom peptide library of length 5-35 for protein identifications from LC / MS-MS data. In another novel approach, scientists from ACTREC Mumbai, has developed strategy to assign multiple phosphorylation events in a protein based on phosphopeptide information from MS databases and structural information of a protein derived from PDB. Moving away from the disease proteomics, there efforts to study biological regulation such as development and differentiation – Nuclear Proteome of Drosophila, proteome of Zebra Fish and the Proteome of embryonic stem cells (11,12; CCMB, Hyderabad). Wonderful plant proteomics work came up from National Institute of Plant Genome Research, New Delhi on profiling of proteins involved in seed desiccation identifying dehydration responsive nuclear proteins from chickpea (13, 14). N-terminal sequence represents a unique signature of a protein and is normally determined by conventional chemical approach developed by Sanger. There are also efforts to determine N-terminal sequences using mass spectrometry, in which the N-terminal of a protein is chemically modified and thee peptides are either affinity purified and sequenced or sequenced directly using Mass Spectrometry. A scientist from Institute of Microbial Technology, Chandigarh has developed a new chemical reaction to achieve this (15). Now, there are also private centers that offer Proteomics services, which are helpful to research groups that do not maintain their own infrastructure. Some of the vendor companies have also set up their Research and Development labs or are planning their Research and Development programs in collaboration with Proteomics groups affiliated to universities or research institutes.

Many of these results were presented at a very recent International Proteomics Conference, the 5th AOHUPO in February 2010. They reflected fully the growing Indian Proteomics interests and were a demonstration of a quantum progress and innovative insights coming up from the Indian Proteomics community.

Under the concept of cooperation, collaboration, and education among the fraternity, Proteomic Society – India (PSI), has been formed in 2009 with a strong agenda of Proteomics education in the country through training, theme workshops, and scientific meetings etc. and is actively pursuing its efforts to achieve this objective effectively.

References

1. Chumbalkar, V. C., Subhashini, C., Dhople, V. M., Sundaram, C. S., Jagannadham, M. V., Kumar, K. N., Srinivas, P. N., Mythili, R., Rao, M. K., Kulkarni, M. J., Hegde, S., Hegde, A. S., Samual, C., Santosh, V., Singh, L., and Sirdeshmukh, R. (2005) Differential protein expression in human gliomas and molecular insights. Proteomics 5, 1167–1177

2. Rukmini B. Govekar, Anil K. D'Cruz, K. Alok Pathak, Jaiprakash Agarwal, Ketayun A. Dinshaw, Roshan F. Chinoy, Nikhil Gadewal, SadhanaKannan, Ravi Sirdeshmukh, Curam S. Sundaram, Siddhi A. Malgundkar, Shubhada V. Kane, Surekha M. Zingde. (2009). Proteomic profiling of cancer of the gingivo-buccal complex: Identification of new differentially expressed markers. Proteomics – Clinical Applications 3, 1451-1462.

3. Sanjeev Shukla, Rukmini B Govekar, Ravi Sirdeshmukh, Curam S Sundaram, Anil D'Cruz, K AlokPathak, Shubhada V Kane, Surekha M Zingde (2007) Tumor antigens eliciting autoantibody response in cancer of gingivo-buccal complex. Proteomics – Clinical Applications, 1 , 1592-1604.

4. PragyanAcharya, Rani Pallavi, SyamaChandran, Harshini Chakravarti, SheetalMiddha, JyotiAcharya, Sanjay Kochar, DhanpatKochar, AmitSubudhi, Arun P. Boopathi, ShilpiGarg, Ashis Das, Utpal Tatu. (2009). A glimpse into the clinical proteome of human malaria parasites Plasmodium falciparum and Plasmodium vivax.Proteomics – Clinical Applications 3, 1314-1325.

5. Gupta, N., Shankernarayan, N. P., and Dharmalingam, K. (2007) Serum proteome of leprosy patients undergoing erythema nodosumleprosum reaction: Regulation of expression of the isoforms of haptoglobin. J. Proteome Res. 6, 3669–3679.

6. Kumar,DM, BThota ‡ SV Shinde, K. V. Prasanna, A. S. Hegde, A. Arivazhagan,| B. A. Chandramouli,| Vani Santosh,‡ and Kumaravel Somasundaram (2010) Proteomic Identification of Haptoglobin r2 as a Glioblastoma Serum Biomarker: Implications in Cancer Cell Migration and Tumor Growth.J Prot. Res.9,5557.

7. Vashist J, Tiwari V, Kapil A, Rajeswari MR (2010) Quantitative profiling and identification of outer membrane proteins of beta-lactam resistant strain of Acinetobacter baumannii. .J Proteome Res. 9, 1121.

8. Mathivanan, S. et al. (2008) Human proteinpedia enables sharing of human protein data. Nat. Biotechnol. 26, 164–167.

9. Bhattacharyya, D., Mukhopadhyay, D. and Chakrabarti, A. (2007). Hemoglobin depletion from red blood cell cytosol reveals new proteins in 2D gel based proteomics study. (2007). Proteomics – Clinical Applications 1, 561-564.

10. Dipankar Bhattacharya, Sutapa Saha, Sumanta Basu, Sudipa Chakravarty, Amit Chakravarty, Debashis Banerjee & Abhijit Chakrabarti. (2010). Differential regulation of redox proteins and chaperones in erythrocyte proteomes in HbEβ-thalassemia. (2010). Proteomics – Clinical applications 4, 480-488.

11. Kallappagoudar S, Varma P, Pathak RU, Senthilkumar R, Mishra RK.(2010) Nuclear matrix proteome analysis of Drosophila melanogaster. Mol Cell Proteomics. 9:2005.

12. Singh SK, Rakesh KS, Ramamoorthy K, Pardha Saradhi AV, Idris MM (2010) Proteome Profile of Zebrafish Brain Based on Gel LC-ESI MS/MS Analysis. J Proteomics Bioinform 3: 135-142

13. Choudhary MK, Basu D, Datta A, Chakraborty N, Chakraborty S. (2009) Dehydration-responsive nuclear proteome of rice (Oryzasativa L.) illustrates protein network, novel regulators of cellular adaptation, and evolutionary perspective. Mol Cell.Protmics. 8,1579.

14. Pandey A, Rajamani U, Verma J, Subba P, Chakraborty N, Datta A, Chakraborty S, (2010) Identification of extracellular matrix proteins of rice (Oryzasativa L.) involved in dehydration-responsive network: a proteomic approach. J Proteome Res.9,3443.

15.Dhaunta N, Fatima U, Guptasarma P. (2011) N-Terminal sequencing by mass spectrometry through specific fluorescamine labeling of α-amino groups before tryptic digestion. Anal Biochem. 408:263.

Technical notes

Host Cell Protein Profiling: GE Healthcare Life Sciences, Uppsala, Sweden

There are strong demands from the FDA and EMEA to assure the safety of therapeutic products. Thus, it is becoming increasingly important for biopharmaceutical manufacturers to obtain a deeper understanding of how process conditions affect the quality of the final product with regard to impurities. Impurities can be related to the process, product, or host cell and may include host-cell proteins (HCP), DNA, viruses, or IgG aggregates. For biopharmaceuticals manufactured using recombinant DNA technology, the majority of impurities encountered are host-cell proteins. Because HCP can be immunogenic and possibly cause anaphylactic reactions in patients, routine HCP determination is compulsory for all recombinant therapeutics including enzymes/proteins and hormones. ELISA is currently the standard approach for HCP determination, although it is usually performed in combination with other methods such as 1-D SDS-PAGE or IEF-PAGE to give more information about the proteins present. In this study we used a model system to illustrate the possibilities of using 2-D DIGE (2-D Difference Fluorescence Gel Electrophoresis) to improve the understanding of process conditions in the production of biopharmaceuticals. The 2-D DIGE method is proven to have high sensitivity and specificity, and can be used as a stand-alone method for characterization of HCP profiles without the need for antibodies.

Although ELISA, Coomassie-stained SDS-PAGE, and IEF-PAGE are the methods currently used as standard for HCP determination, they each present problems with regard to HCP analysis. While immunoassays such as ELISA have the advantage of specifically detecting immunoreactive proteins, they do not detect weakly reactive proteins or non-immunoreactive proteins. Hence, the FDA is expressing concerns that these assays might not be sufficient to give a complete picture of contamination with HCP.

Immunologic methods are not always easy to work with, as they are arduous to develop, and specific antibodies must be acquired. False negatives may be detected by antibody-based assays due to sample denaturation or steric hindrance causing epitopes to be missed, and false positives may also be detected due to cross-reactivity. In

addition, although immunoassays detect the presence of immunoreactive HCP and quantify the total amount, complimentary methods are needed in order to elucidate HCP patterns.

The HCP pattern can be observed and quantitated with 1-D SDS-PAGE or IEF-PAGE, but the quantitation level varies depending on the equipment (e.g., software) available, both resolution and sensitivity are limited, and IEFPAGE data is difficult to correlate to SDS-PAGE data. Also, these methods lack reliable internal markers for accurate quantitation.

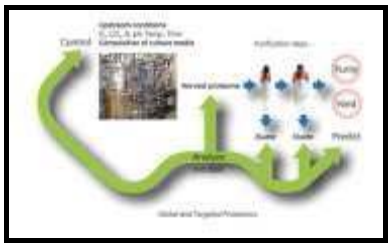
2-D electrophoresis is an established method, commonly used in proteomic research for analyzing complex protein mixtures. 2-D DIGE was developed with the aim of simplifying the process and improving quantitation. 2-D DIGE is an electrophoretic method that allows accurate quantitation of small differences in protein abundance between different samples with high statistical confidence.

The Ettan™ DIGE technology from GE Healthcare allows for the simultaneous co-separation of two different samples, plus an internal standard, on an individual gel. The samples and internal standard are each fluorescently labeled with a different CyDye™ DIGE Fluor dye. Because two samples are multiplexed using the same internal standard on each gel, and each protein spot has its own corresponding internal standard spot used for normalization, all gels can be directly compared, reducing the number of gels required for each experiment. In contrast to immunoassays, 2-D DIGE detects all proteins, independent of their immunogenic response. Using DeCyder™ 2-D software, 2-D DIGE can detect the smallest possible real differences in protein expression, quantitatively and with statistical certainty. These advantages address the need for quantitative and sensitive HCP profiling.

Reduction of Impurities

Identification and quantification of impurities early in the drug development process is crucial, and an improper investigation of this can lead to serious consequences. Therefore, it is important to investigate manufacturability at an early stage in the process by varying upstream conditions and analyzing the effects on downstream

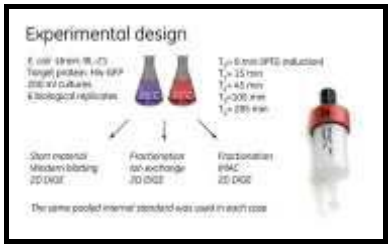
processing (i.e., purification). As shown in *Figure 1* there are several upstream conditions (e.g., cell cloning and growth conditions) that may have quantitative and qualitative effects on HCP content in the final product.



In controlled experiments, upstream conditions can be modified, and the downstream effects on critical impurities can be measured. By analyzing the proteome, upstream conditions can be altered to optimize bioprocessing and predict the yield and purity of the target protein. This type of analysis could also be performed on the transcriptome or peptidome to gain an even deeper understanding about the effects of a process change. Using a systematic approach, the acquired knowledge can be used to control bioprocessing on an entirely new level.

Selecting Optimal Conditions

We cultured E. coli cells expressing histidine-tagged green fluorescent protein (GFP) at 37°C. Some of these samples were cooled to 20°C before IP TG induction and then cultured at 20°C to test different upstream conditions. After induction, six biological replicate samples were taken at each time point and temperature (Figure 2).



Analysis using 2-D DIGE was performed on the samples, and the resulting 2-D protein spot maps of different gels were compared to the internal standard with DeCyder 2-D software. A ttest between the two temperatures showed 438 differentially expressed proteins (0.0001 level of significance). Of these, 130 proteins of interest were picked and identified.

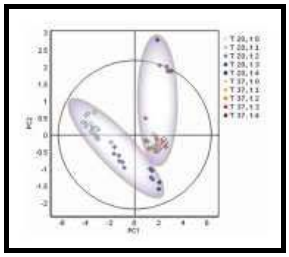
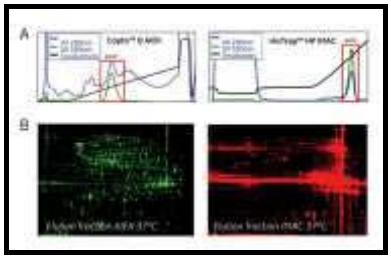


Figure 3: *DeCyder 2-D software analysis shows the principal component analysis*

The majority of proteins differentially expressed in E. coli were down-regulated at 20°C, possibly due to stress induced by reducing the temperature after culturing. At 37°C, most of the proteins were upregulated over time. The samples taken at 20°C and time point t 4 showed similarities to those taken at the early time point and 37°C (Figure 3). A possible explanation for this is that the cultures had adapted to the low temperature and started to produce similar proteins to the 37°C cultures.

Different downstream processing conditions were analyzed by fractionating the collected samples and using either a Capto™ Q anion exchange column or a HisTrap™ IMAC HP column. Different fractions were collected from each column and analyzed by 2-D DIGE. Chromatograms from the two purification methods are shown in Figure 4A. Two examples of protein spot maps of samples cultured at 37°C and fractionated using either ion exchange or affinity chromatography are shown in Figure 4B.



Because all gels in the experiment used the same internal standard, it was possible to link the spot maps of the fractionated samples back to the spot maps of the start material (cultured at 37°C or 20°C). The spot maps showed that several host-cell proteins were still present after IMAC purification. These were likely to be histidine- or tryptophan-containing proteins, which can also bind to the resin. Many variants/isoforms of the histidine-tagged target protein could also be separated and identified. Using information obtained from 2-D DIGE it is possible to reduce certain impurities in the eluate by selecting optimal upstream and downstream conditions.

Gaining control over purity and yield in biopharmaceutical production is becoming essential for regulatory approval of drugs. Organizations such as the FDA are calling for better process understanding. Systematic analysis is crucial for making improvements in upstream and downstream processes. The 2-D DIGE method is a promising tool that enables the analysis of optimal growth conditions and purification methods. The use of an internal standard enables the linking of analyses of upstream and downstream changes. As 2-D DIGE runs an in-gel standard with each sample, comparisons among samples are precise, thereby providing reliable detection and quantitation of changes in impurities resulting from process manipulations. 2-D DIGE is an easily validated method for HCP determination that delivers high-quality results.

ÅsaHagner-McWhirter, Ph.D
Maria Winkvist, Ph.D
LennartBjörkesten, Ph.D
Gunnar Malmquist, Ph.D

Proteomics Tools from Bio-Rad Laboratories

With a leadership position in Expression Proteomics, Bio-Rad has been at the forefront of providing important solutions to researchers enabling them to do proteomics research with ease. In order to enhance the knowledge of the Proteomics community and to update them about the latest happenings in the field of Proteomics, Bio-Rad organized a Workshop at the 5th AOHUPO Congress held at CCMB, Hyderabad, Feb 2010. Bio-Rad had invited two key opinion leaders viz- Prof. Pier George Righetti (Politecnico di Milano, Italy) & Maxey Chung (from National University of Singapore) to share their valuable experience in the field. Important Technical points and the possible solutions discussed at the Workshop are given below.

A most widely discussed area in proteomics is ***Differential Expression Proteomics*** where scientists look for variations in protein expression level across samples. Protein separation is a key step to study this in these experiments, one of the established approach being the 2-D Gel Electrophoresis. Contaminants such as salts, detergents, and ionic compounds in the samples could drastically interfere with the experiment. A careful sample preparation can result in high reproducibility of 2 Gels. "Fixing Proteomics Campaign" was an initiative conducted by BioRad along with Novartis Institutes for Biomedical Research and Nonlinear Dynamics in order to help the proteomics community's efforts to develop standards and validated protocols to ensure reproducibility in 2-D gel electrophoresis applications. The goal for the above campaign is to restore credibility to the field by pushing for greater reproducibility of 2-D gel experiments. Details were presented at the Congress. Almost 65% reproducibility was demonstrated in 2-D gels runs from various labs across the world, in which Dr. Ravi Sirdeshmukh's Lab (CCMB, India) was one of the participants. Bio-Rad has applied its expertise in solution chemistry, chromatography, and electrophoresis to develop the most comprehensive suite of products available for protein sample extraction, cleanup and fractionation helpful for the success of 2 D gels.

Presence of abundant proteins in the sample is another challenge in Proteomics experiments. The guest speakers shared their experiences regarding sample preparation by using the ProteoMiner^{TM} technology. They addressed these fundamental challenges in proteome analysis with regards to samples being often dominated by a relatively

small number of high-abundance proteins whose presence can obscure other less abundant proteins and limit the capacity and resolution of the separation technique employed. This is very apparent in serum and plasma, where some twenty proteins constitute more than 98% of the protein mass. It also applies to the proteome of leaves and other photosynthetic tissues, where over 50% of the protein typically consists of the single enzyme ribulosebisphosphate carboxylase (RuBisCo). **ProteoMiner™ technology** is an effective tool for enriching low-abundance proteins relative to high abundance proteins. It has been developed for biomarker discovery with serum samples, but has the potential to be applicable to a wide range of sample types. The technology is novel & powerful with the ability to eliminate the protein dynamic range problem present in complex biological samples, such as serum or plasma. This is accomplished through the use of a combinatorial library of hexapeptides bound to chromatographic beads. As a complex biological sample is added to the beads, proteins bind to their specific affinity ligands, high-abundance proteins saturate their specific ligands and excess protein is washed away. In contrast, medium- and low-abundance proteins continue to bind to their ligands and are therefore enriched during the process. The result is a reduction in the dynamic range which allows for the detection of medium- and low-abundance proteins. ProteoMiner™ technology is commercially available as ProteoMiner™ protein enrichment kits from Bio-Rad Laboratories, Inc.

The Proteomics workshop at AOHUPO was an excellent platform to discuss these tools for proteomics research & Bio Rad plans to introduce the Proteomics Technology for different types of biological samples for which workshops will be planned in collaboration with the Proteomics society to share national & international expertise and educate the fraternity of proteomics researchers with these important tools.

BrijeshPandey
Product Manager
Bio-Rad Laboratories

Opinion

2D or not 2D

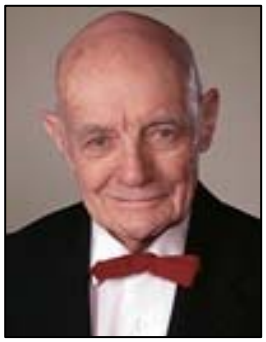
Recent years, gel free approaches comprising of LC- MS/MS analysis of total cellular proteins with or without pre-fractionation are getting increasing popular. Going back in the history of proteomics approaches, when Proteomics efforts were initiated in the post Genomic era, it was all 2D Gel based separation of proteins combined with mass spectrometry and bioinformatics tools. The LC MS approaches dominated during subsequent years on account of three main reasons. (1) Difficulties faced in gel reproducibility. (2) Lack of high throughput capability, and (3) access to cellular proteins with relatively higher abundance. True that LC MS approaches permit exploration of proteins of very low abundance and greater dynamic range of concentrations. A recent review (Zang et.al. J Prot. Res. ASAP, Dec., 2010) with plasma proteins demonstrates that one can reach out plasma proteins with a concentration range of over 7 orders of magnitude. Despite this tremendous advantage we have to remember that LC MS/Ms approach primarily analyzes peptides of the protein mixtures, thus losing direct link with the original individual proteins, a feature which is protected in 2 D Gel based approach. The discussion mentioned in the Technical Notes of this News Letter reveals that given the use of good reagents and sample preparation protocols, one can achieve appreciable degree of reproducibility in 2D gel runs carried out even across many labs. Further, large format gels permit analysis of more than 1000 protein spots of whole cell proteins and when combined with DIGE approach, can, in principle, allow good quantitative analysis of substantial number of cellular proteins.2D gel based proteome analysis still remains to be an important tool for microbiologists (Sá-Correia I, Teixeira MC.(2010) Expert Rev Proteomics.7:943). An Indian group has revealed important regulatory network involved in stress response in rice (Choudhury et al. Mol. Cell Prot. (2009) 8, 1579) by effectively analysing a sub proteome (nuclear in their study) by 2D gels. In immunoproteomics approach, immunoreactive proteins from cells or tissues are identified by immunoblotting and reacting them with sera from individual patients (shukla et al. (2007) Proteomics – Clinical Applications1, 1592). Protein isoforms constitute an important feature of protein functions. They may be associated with distinct cell types or different cellular conditions. They can originate from variable peptide sequences or post translational modifications in proteins. It is estimated that 15 % of the known mutations are a result of splicing defects generating variations in protein sequences. Many of them may be even

disease associated. Assignment of isoformic peptides or peptides originating from aberrant splicing events is a great challenge in LC MS/MS approaches. 2D gels have the capability of resolving isoforms based on even marginal charge differences which with other proteins. Gupta et al. (J. Prot. Res. (2007) 6, 3669) and more recently (Kumar et.al. J.prot. Res. (2010) 9, 5557) have shown association of specific isoforms of serum haptoglobin in leprosy and glioblastoma patients, respectively. Even with the current advances in Mass spectrometry technologies or instrumentation, we do not have reliable MS methods for direct analysis of protein complexes. On the other hand, we can today separate protein complexes on native 2D gels, recover proteins/peptides from the complexes and analyse them by MS. The message to learn is, every analytical approach and platform has its own merits and demerits. We have to make a proper evaluation of an approach in the context of the immediate and long term biological/clinical question and make an intelligent choice of a single or a combination of approaches for our experiments.

Ravi Sirdeshmukh,

General

Remembering John Fenn



John B. Fenn is best known for the development of electrospray as an ionization method for mass spectrometry (MS). In electrospray, an electric field disperses a solution into a fine mist of charged droplets. As the solvent evaporates, solute molecules are released as free gas-phase ions. The process is gentle enough that large biological molecules can be ionized without breaking apart. The ions are then detected by MS. Electrospray has become one of the most common MS ionization methods and has become the largest driver of the remarkable extensions of MS into the life sciences over the past two decades.

Fenn did the work for which he received the Nobel Prize while at Yale University, where he was a professor of applied science and chemistry from 1967 to 1980 and a professor of chemical engineering from 1981 until his retirement in 1987. After a few years as an emeritus professor, Fenn moved to Virginia Commonwealth University as a research professor in 1994. He shared the ***2002 Nobel Prize in Chemistry*** for the above work that allowed the application of mass spectrometric analysis to proteins and other large biological molecules. Today mass spectrometry has become an integral tool for Proteomics - large scale analysis of proteins and their post translational modifications in health and disease.

Born in New York in 1987, John Fenn died on Dec.10 2010, at the age of 93. Among friends and colleagues, John was considered to be a passionate scientist, an inspiring teacher and above all a warm human being with a strong sense of humility. Let us pay our tributes to this great personality.

Events and Reports

1. 5th AOHUPO Congress

The 5th AOHUPO Congress was convened by Dr Ravi Sirdeshmukh and successfully organized at the centre for Cellular and Molecular Biology, Hyderabad, India, from February 21 to 25, 2010. The Congress was organized as a joint event overlapping with the 14th ADNAT (Association of Promotion of DNA Finger Printing and other DNA Technologies) Convention and the 1st International Conference of the Proteomics Society, India. It was held on the beautiful campus of the Centre for Cellular and Molecular Biology - a premier research institute in India, which provided a very subtle mix of academic ambiance and quiet friendly environs. The Congress was attended by over 350 registrants and presented an elaborate scientific program under the theme, "New Perspectives in Proteome Research". Spread over five days, starting with the **Education Day** followed by a day devoted to the Vendor Company **Workshops** and then three days of scientific **symposia**, the conference provided a learning opportunity for beginner students, a platform for young investigators to present their ideas and another occasion to the seasoned Proteomics experts to share their experience and expertise. A Two Week, post conference Hands-on Training Course in Quantitative Proteomics was an additional, valuable integration to help a small and select group of scientists who are at the initial stage of their Proteomics endeavours. The Education Day (21stFeb) was attended by over one hundred students and young faculty scientists. The resource faculty was drawn from various disciplines with the aim to cover various aspects of the Proteomics Technology. On 22nd February, six vendor companies (Agilent Tech., Bio-Rad, GE Life Sci, Pall Life Sci., Thermo Fisher Scientific and Waters) who were also major sponsors of the Congress conducted workshops aided by their R& D scientists as well as external collaborators and unravelled their respective portfolios of hardware, software and reagent tools. The evening of 22nd Feb, when most of the delegates had already arrived in Hyderabad, was highlighted by a Public Lecture by Sam Hanash on the Prospects and Challenges in Molecular Diagnostics and the traditional music by the folk group of the Indian desert land – Rajasthan. While Sam Hanash set the tone for the symposium to start on the

following day, the folk music was a mesmerizing experience for the delegates. The Scientific symposium which started on the 23rd Feb included key note lectures by some of the proteomics scientists who laid the foundation of current proteomics, invited talks from a range countries page 2Including India, selected oral presentations, a major Poster Session and two open discussions in the context of international initiatives in Proteomics. The topics covered were very varied and encompassed methods and experimental approaches, bioinformatics, clinical proteomics, proteomics of infectious organisms, proteomics in the study of development and differentiation in plants and animals and protein interactions.

To encourage young research students doing high-quality work, a total of four student abstracts were selected for oral presentations. A large number of abstracts were received and were presented in the Poster session organized as an evening session in the open environment, with natural tree surroundings. It offered a very lively environment for a free, informal discussion among the delegates. There were also two open discussions about large initiatives including AOHUPO membrane proteomics initiative in Human Embryonic Stem cells and Human Proteome Project. At the end, there was award ceremony for winner of Poster awards. The Poster award committee found it difficult to restrict to the original number of awards. In all, there were five awards given, one from ADNAT and four on behalf of AOHUPO. The conference ended with cheer and profuse acknowledgements to all involved. A two week Hands-on Training Course in Gel-based and Gel-free Quantitative Proteomics followed the main Conference and was attended by scientists from national Institutes and Industry.

Ravi Sirdeshmukh

2. *Hands on Training on Tissue Microarray*

A Hands on Training on Tissue Microarray convened by Dr.Sunita Saxena, Director, Institute of Pathology (ICMR), New Delhi was held on 8th-9th June, 2010 at Institute of Pathology (ICMR), Safdarjung Hospital Campus, New Delhi-110029, in collaboration with Alphelys SAS, France and Lucent Biomedical, New Delhi.

Tissue microarray (TMA) technology is a high-throughput research tool, which has greatly facilitated and accelerated tissue analyses by in-situ technologies.

TMA are amenable to every research method that can be applied on the standard whole sections yet making possible the analysis of hundreds of tissue specimens in a single experiment using a single gene, antibody or probe. It plays a central role in target verification of results from cDNA arrays, expression profiling of tumors and tissues, and is proving to be a powerful platform for proteomic research. In comparison to the standard histology sections TMA is advantageous as they give experimental uniformity with judicious use of precious tissue and antibodies available for research. Its capability to analyze a large number of samples simultaneously also results in improved precision of statistical analysis at enhanced speed.

The main objective of this workshop was to provide the pathologist and scientific fraternity a platform to get hands on training om tissue microarray, its application and to get up-to-date with latest in the field. The training was conducted by Mr. Pierre Chaumat, President & CEO, Alphelys SAS, France. The Scientific Programme comprised a series of lectures delivered by distinguished international and national level scientists followed by the hands on training on the TMA equipment provided by Alphelys SAS, France. The guest scientists included Mr. Pierre Chaumat, President & CEO, Alphelys SAS, France, Dr. Ravi Sirdeshmukh, Distinguished Scientist, Institute of Bioinformatics, Bangalore, India, Dr. Sanjay Navani, Surgical Pathologist and Immunohistochemist, Lab Surgpath, Site

Director, Human Protein Atlas (HPA), Project, Mumbai and Dr.Avninder Singh, Scientist, Institute of Pathology (ICMR), New Delhi.

Sunita Saxena

3. *PSI Seminar Series I on Indian Proteomics : User's Perspective*

PSI Seminar Series I was held in Saha Institute of Nuclear Physics (SINP), Kolkata during July 2-3, 2010. The meeting was organized by Abhijit Chakrabarti of SINP and Rukhsana Chowdhury of the Indian Institute of Chemical Biology (IICB) with active help from Drs. Debashis Mukhopadhyay of SINP (poster design and circulation), Arun Bandyopadhyay of IICB (Treasurer), Santasabuj Das of NICED (National institute of cholera and enteric diseases), Kalipada Das of Bose Institute and Koustubh Panda of Calcutta University (brochure design and publication and fund raising). There were 75 participants comprising of young PhD students (60, of which 40 were first year graduate students), 10 young faculty members (including 1 Clinician doctor) and research associates and 5 M.Sc students. Participants came from research Institutes of Kolkata with 15 each from IICB, SINP, Bose Institute and Calcutta University and rest from NICED, St. Xavier's College, IISER-Kol and NRS Medical College.

The feedback has been excellent. Majority said that it had been great opportunity for them to interact with Proteomics experts of our country which has been one of the key motivating factors. Every speaker scored very high with Dr.Niranjan Chakraborty and Prof. Dharmalingam scoring a shade better than the rest. All topics of the seminars have been well covered including the talk on separation techniques by Prof. Vijayalakhshmi. DIGE has been a superhit for the gel-based workers struggling to quantify protein spots. The entire seminar series was designed for research students where Drs.Surekha Zingde, Yogeswar Shukla and Shantanu Sengupta's presentations were very much appreciated. Needless to say that Dr. Ravi Sirdeshmukh set the tune of the senior series on "Indian Proteomics: User's Perspective" perfectly for the rest.

It was; however, felt that this design would only be good for research students oriented for proteomics work. For general awareness, PSI will consider more detailed classroom material , starting from basics of protein separations, folding & design, a bit of structure, post-translational modifications, 1D, 2D gel-electrophoresis, depletion techniques, bioinformatics and mass spectrometry.

Abhijit Chakrabarti

4. Workshop on 'Clinical proteomics: Technology and Beyond' ACTREC on 26th and 27th November, 2010

The workshop was organized by Dr Surekha Zingde and Rukmini Govekar. At ACTREC, clinical proteomics with special interest in cancer has been undertaken by several investigators. Since one of the mandates of ACTREC is education, it was thought appropriate to educate the college teachers about proteomics with special reference to clinical applications. Teachers can then disseminate this knowledge to a large number of students. Proteomics Society India is a group of scientists who have contributed immensely to development of proteomics in India and are keen on educating others. The concept of a meeting on clinical proteomics by both ACTREC and PSI was materialized due to the financial support by LTMT.

Twenty teachers from thirteen colleges in Mumbai and twenty-three students registered as participants in the meeting. The number had to be restricted due to the capacity of the rooms housing the workstations for proteomics. Lectures were kept open to all members of the Tata Memorial Centre.

The meeting included lectures and demonstrations. The lectures were held on 26th November 2010.

Dr. Surekha Zingde, Deputy Director, CRI-ACTREC, who conceptualized this programme, gave an overview of the field of proteomics technology and their applications in clinics. This lecture formed the basis for all the remaining lectures

The first session on 'Technological advances in proteomics included lectures on various technologies used in proteomics were given by experts from research institutions as well as from technical specialists from companies which manufacture these instruments. This session was chaired by Dr Surekha Zingde, Deputy Director, ACTREC.

Dr. Ravi Sirdeshmukh, CCMB, Hyderabad spoke on mass spectrometers and their clinical applications. He could not visit ACTREC due to personal reasons but gave the presentation on SKYPE. The lecture was very useful for teachers and students to understand the basics of mass spectrometry and the use of the same for various applications.

This was followed by a vibrant presentation on liquid chromatography by **Dr. M. A. Vijayalakshmi**, Director, CBST, Vellore Institute of Technology, Vellore.

Although the teachers know about liquid chromatography, this lecture contributed to developing better understanding of different separation matrices and their applications.

Dr. Sanjeeva Srivastava, IIT-B, Mumbai, took the audience through a more recent and an advanced technique of protein arrays. His systematic presentation made the difficult topic much simpler for the teachers and students.

After Dr Sirdeshmukh introduced the audience to mass spectrometry, **Dr Ian Sanders**, Bruker Daltonics Ltd, UK, explained the application of mass spectrometry in molecular histology- a technology known as MALDI imaging.

The first session provided sufficient information of technologies used for separation and identification of proteins. The **second session** on 'Proteomic tools to address clinical problems ' included talks by scientists who are using proteomics technologies to address several clinical queries ranging from glycation of proteins in diabetes by **Dr. Mahesh kulkarni**, NCL, Pune to unraveling the proteomic alterations which result in the senescence of erythrocytes from beta thalassemia by **Dr. Abhijit Chakrabarti**, SINP, Kolkata; from trying to seek answers for female infertility due to endometriosis by **Dr. Geetanjali Sachdeva**, NIRRH, Mumbai to deciphering the molecular pathology of human malarial parasite by **Dr. Utpal Tatu**, IISc, Bangalore. Dr Tatu gave a SKYPE presentation. This session was chaired by Dr Vijayalakshmi, VIT, Vellore.

These lectures generated a lot of questions in the minds of teachers and students. The speakers answered all of them with a lot of enthusiasm. Dr. Vijayalakshmi herself generated a lot of questions and made the discussion interesting.

Five of the student participants gave oral presentations and five presented their work in the poster session. The session on oral presentation by students was chaired by Dr Abhijit Chakrabarti, SINP, Kolkata.

On the second day of the meeting i.e. 27th November, 2010, representatives from companies whose instruments are installed in the proteomics facility of ACTREC, gave demonstrations of their instruments to the participants. Additionally, technical specialists from each company gave talk on their respective subject

Dr. Brijesh Pande from Bio-Rad gave a talk on 'Gel based separations and related techniques & instrumentation**'.** The talk helped the participants to assimilate the latest in 2DGE such as the use of IPG strips in the first dimensional separation, the newer versions of gel scanners which were shown during demonstrations.

Another technology for protein separation is liquid chromatography. In our workstation the LC from Agilent is coupled to the MALDI plate spotter. Separated proteins in each spot are then analyzed by mass spectrometry. **Mr B. V. Pendam** from Agilent technologies explained to the participants the advances in liquid chromatography such as separation at nano-liter scale and the CHIP technology.

Dr Marcus Macht from Bruker-Daltonics gave a talk on 'Mass spectrometry based techniques and instrumentation' and demonstrated the use of mass spectrometer for protein identification.

The meeting was concluded by distribution of certificates of attendance to the participants. The participants gave their feed-back about the meeting. They appreciated the organizing of lecture cum demonstrations on Proteomics. All the teachers expressed that the principles of several of the techniques used in Proteomics and their applications were much clearer now after they had attended the meeting. They also felt more confident about imparting this knowledge to the students. As organizers we feel that the mandate of the meeting was met.

Feedback was also taken from the faculty about the participants and they were requested to select three best from teachers who showed interest in the whole program. The teacher/s who could be considered for further support/training through the LTMT was She could be invited to the next meeting of the IAC of the LTMT for an interview.

Surekha Zingde

Upcoming Events

- ***Annual Proteomics Meeting, JNU Delhi, March, 2011***

  Seminar series, theme workshops 2011

- ***Quantitative 2D Gel Electrophoresis in Proteomics***

  School of Biotechnology, Madurai Kamraj University, Madurai

- ***International conference on Omics Meets Disease Biology***

  SINP, Kolkata, December 2011

(Tentative, Venues and dates will be announced later)

- ***Therapeutic protein characterization – Proteomics and Mass Spectrometry Approach***
- ***Plant proteomics- Experimental approaches and application***
- ***Glioma workshop- clinical questions, experimental approaches and new dimensions***