Proteomics in India: A Report on a Brainstorming Meeting at Hyderabad, India

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The Centre for Cellular and Molecular Biology, Hyderabad, India, was host for an international forum, or "brainstorming meeting," on proteomics held in November 2014, which provided the opportunity to showcase proteomic science in India and to allow discussions between Indian scientists and students and several international visitors. This provided an amalgamation of speakers and participants whose interests lay mainly in developing and using mass-spectrometry-based proteomics to advance their research work. A week-long workshop with hands-on training in proteomic methodology followed the meeting. ***Molecular & Cellular Proteomics 15: 10.1074/mcp.O115.055020, 2229–2235, 2016.***

Proteomics is one of the emergent technologies used worldwide that offers broad capabilities to scientists who want to characterize samples of different types from a variety of sources, ranging from humans to microorganisms. The development of robust, highly reproducible techniques, based in mass spectrometry, holds the key for fulfilling these aspirations. The aim of the meeting was to bring together some of the pioneers in this area with a large user group to discuss these technologies and their applications. The brainstorming meeting in Hyderabad* strove to provide an opportunity to not only make current proteomic users in India more competitive but also to attract other scientists to use proteomic tools for their research. Thus, this meeting greatly benefited Indian students and scientists—from both academia and industry—that were using proteomics or were planning to do so in their own investigations. The focus was on making proteomic techniques understandable and on explaining their applicability to answering biological problems in widely different fields of biology. This meeting also provided a substantial opportunity to initiate international and national collaborations.

One of the most stimulating things about the symposium was the question–answer sessions. Both senior scientists and especially young students had many thoughtful questions, leading to lively discussions. The interaction between participants and speakers continued even during the breaks for tea, lunch, high tea, and dinner for three consecutive days. The poster session provided another opportunity for exchange; all of the students explained their posters with enthusiasm and skill, and the ten best posters were selected by a panel composed of the overseas visitors.

Report/Meeting—The theme of the meeting was "Proteomics: Present and Future" and was held at the Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India, November 22–24, 2014. It was intended to cover many different applications of proteomic science, particularly issues important to India. More than 300 participants attended, with 82 scientific abstracts presented orally or in poster format.

The meeting began with an opening ceremony featuring a symbolic lighting of candles by Prof. Alexander Makarov and Prof. Ralph Bradshaw, representing the overseas visitors, and Prof. Padmanabhan Balaram, Prof. Dorairajan Balasubramaniam, and Dr. Chintalagiri Mohan Rao, representing the host country (Fig. 1*A*) and with welcoming remarks by the convener, Dr. Suman S. Thakur (Fig. 1*B*).

Day 1, Session 1: Mass Spectrometry and Its Applications–The inaugural address was delivered by Dr. Chintalagiri Mohan Rao, director of the CCMB, who talked about the past accomplishments and visions of the CCMB as a center of

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Brainstorming meeting (Nov. 22–24, 2014) and workshop (Nov. 25–Dec. 1, 2014) on Proteomics: Present and Future at the Centre for Cellular and Molecular Biology, Hyderabad, India, (Convener: Suman S. Thakur).

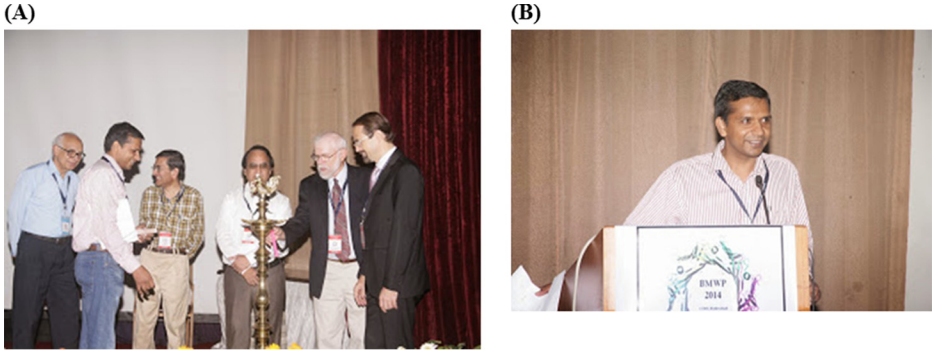

FIG. 1. (*A*) Inauguration of the brainstorming meeting by the lightning of the lamp: (from left) Prof. Dorairajan Balasubramaniam, Dr. Suman S. Thakur, Prof. Padmanabhan Balaram, Dr. Chintalagiri Mohan Rao, Prof. Ralph Bradshaw, and Prof. Alexander Makarov. (*B*) Welcome remarks of the brainstorming meeting delivered by convener Dr. Suman S. Thakur.

proteomic research in India. He also presented studies from his laboratory concerning retinopathy of prematurity. It is a neonatal disease in the retina of premature babies, especially with low birth weights and short gestation periods, that results in blindness. Quantitative proteomic analyses have revealed that complement and coagulation components are up-regulated and crystallins and superoxide dismutase are down-regulated. These finding suggest that these proteins can serve as biomarkers for this disease. He was followed by Prof. Padmanabhan Balaram (Indian Institute of Science, (IISc), Bengaluru), who discussed the *de novo* sequencing of peptides from *Conus* venom. He emphasized how the integration of next-generation sequencing and mass spectrometry will be useful for the elucidation of unknown sequences of substances of potential value as therapeutics. He also presented in detail how disulfide pairing can be established using mass spectrometry, an important consideration in studies with *Conus* venom peptides. The session ended with Prof. Alexander Makarov (Thermo Fisher Scientific, Bremen, Germany; Utrecht University, Utrecht, The Netherlands), who delivered an entertaining and inspiring lecture about the development of Orbitrap instrumentation for proteomic studies and the extension of Orbitrap capabilities by integrating them with different modes of fragmentation (higher-energy collision dissociation, electron transfer dissociation, electron-transfer and higher-energy collision dissociation), field asymmetric ion mobility spectrometer, and matrix-assisted laser desorption/ionization (MALDI) ionization. His talk focused on both technical solutions, which will improve quantitative analyses, and on future trends and perspectives for new developments in Orbitrap mass spectrometry.

Day 1, Session 2: From Fundamental Biology to Applications—In this session, research with more direct translational applications was discussed. Dr. Roop Mallik (Tata Institute of Fundamental Research, Mumbai) described the movement of the phagosome, which matures to degrade pathogens, and how motor proteins play an important role in the transport of these organelles. He also elaborated on the transport of phagosomes by counting motor protein numbers on individual active phagosomes. Dr. Utpal Tatu (IISc, Bengaluru) discussed the response of unfolded proteins in the malaria parasite, highlighting the role of mass spectrometry in elucidating the clinically relevant proteomes of *Plasmodium falciparum* and *Trypanosoma evansi* along with the identification of antigens for animal trypanosomosis and the trans-splicing-based expression of Hsp90 in *Giardia lamblia*. Dr. Sathees C. Raghavan (IISc, Bengaluru) discussed chromosomal fragility caused by formation of altered DNA structures like G-quadruplexes, triplexes, and others. He also described the identification of an inhibitor of nonhomologous end joining, SCR7, that is affected by radio and chemotherapeutic agents, illustrating its potential as a cancer therapeutic.

Day 1, Session 3: Interaction Proteomics—The third session, which dealt with protein–protein interactions, started with Dr. Sanjeeva Srivastava (Indian Institutes of Technology, Mumbai), who discussed tools for translational research, especially the identification of candidate proteins as potential biomarkers for malaria and brain tumors. Dr. Bhaswati Chatterjee (National Institute of Pharmaceutical Education and Research, Hyderabad) described protein interaction networks using quantitative bacterial artificial chromosome—green fluorescent protein interactomics and the utility of single run analyses using liquid chromatography coupled to high-resolution mass spectrometry in detecting proteins and posttranslational modifications. Dr. Prasanna Venkatraman (Advanced Centre for Treatment Research and Education in Cancer, Tata Memorial Centre, Navi Mumbai) presented studies on the ubiquitin proteasome pathway, focusing on domain motif interactions. She used bioinformatics tools for determining the interaction partners of Proteasome Macropain non-ATPase subunit 9 and Proteasome Macropain non-ATPase subunit 10 and confirmed her results using recombinant proteins, emphasizing the potential of these entities as drug targets.

Day 1, Session 4: Brain and Blood Proteomics—The fourth session on cellular and clinical proteomics was opened by Prof. Ralph A. Bradshaw (University of California–San Francisco, San Francisco, CA), who discussed the phosphoproteome induced by nerve growth factor via the Trk receptor

and its similarity to that induced by the epidermal growth factor receptor. This neurotrophic factor and/or its precursor, pro nerve growth factor, are overexpressed in many human breast and prostate cancers and can stimulate tumor growth and development. In prostate cancer, its levels correlate with high Gleason scores, suggesting that it may be a good biomarker for identifying aggressive tumors. He also presented comparative transcriptomic, proteomic, and miRNA analyses of two breast cancer cell lines with different metastatic responses. Dr. Abhay R. Moghekar (John Hopkins University, Baltimore, MD) talked about changes in cerebrospinal fluid biomarkers that will aid in the prediction of which presymptomatic individuals will go on to develop Alzheimer's disease. This is of importance for the design of prevention trials since treatments of Alzheimer's disease, once symptoms have manifested, have largely been ineffective. Dr. Rakesh K. Mishra (CCMB, Hyderabad) discussed nuclear architecture and preservation of the proteome during the conversion of interphase nuclear matrix to metaphase mitotic chromosome scaffolds in drosophila cells. Notably, many of the proteins are derived from the nuclear matrix proteome. Finally, Dr. Abhijit Chakrabarti (Saha Institute of Nuclear Physics, Kolkata) discussed therapeutic biomarkers and mechanism of cellular transformation in platelets and malignant B-cells. Proteomic studies of B lymphocytes from B-cell acute lymphoblastic leukemia have determined 79 differentially regulated proteins participating in proteostasis, cytoskeletal organization, and leukemiogenesis. Further, proteomic studies of platelets from asymptomatic constitutional macrothrombocytopenia, HbEβ, and β thalassemia revealed that cytoskeletal changes necessary to maintain the structural and functional integrity of macrothrombocytes in asymptomatic constitutional macrothrombocytopenia, and the differential regulation of heat shock proteins and translation initiation factors are observed in both forms of thalassemia.

Day 2, Session 1: Plant Proteomics—Prof. Niranjan Chakraborty (National Institute of Plant Genome Research, New Delhi) opened the second day. He talked about the identification of CaFer1, a secretory ferritin that increases stress tolerance and growth in the extracellular matrix of the chickpea, further defining its role in the maintenance of iron homeostasis and iron-buffering. Dr. Yellamaraju Sreelakshmi (University of Hyderabad) discussed different networks that control carotenogenesis in the tomato. She used a systems biology approach at the level of the carotenoid proteome and transcriptome to understand the molecular basis of diversity in this process. Prof. Renee M. Borges (IISc, Bengaluru) discussed the brood-site pollination system involving figs and fig wasps, along with volatile organic compounds, which attract fig wasps, and the unique cuticular hydrocarbon profile of each of fig wasp species.

Day 2, Session 2: Microbial Proteomics—This session on microbial proteomics had four speakers who were all from Bengaluru. Prof. Kalappagowda Muniyappa (IISc, Bengaluru) spoke about his findings on the structure–function relationships of Hop1 that help to understand its role in meiotic chromosome synapsis and recombination. Prof. Dipankar Chatterji (IISc, Bengaluru) discussed the use of synthetic glycolipids by mimicking naturally occurring lipo arabinomannan and mycoloylarabinogalactams to monitor the components of the cell wall of *Mycobacteria*; he also explained about the synthesis of novel oligoarabinomannans as inhibitors of cell wall synthesis. Prof. Umesh Varshney (IISc, Bengaluru) provided insight into the mechanism of tRNA selection in ribosomal P-site in *Escherichia coli*. Based on experimental evidence, he proposed that 3 Guanine-Cytosine pairs play a critical role in $tRNA^{fMet}$ retention in the ribosome during conformational changes that help the transition of the 30S preinitiation complex into an elongation competent 70S complex. Prof. Hemalatha Balaram (Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru) described the role of posttranslational modifications in structural and thermal stability of enzymes from thermophiles and hyperthermophiles that are resistant to unfolding at high temperature. She explained the functional role of unusually stable succinimides in glutamine amidotransferase from hyperthermophile *Methanocaldococcus jannaschi*.

Day 2, Session 3: Mechanistic Proteomics—The session on mechanistic proteomics featured remarks by Dr. Judith Steen (Boston Children's hospital, Boston, MA), who talked about E3 ligase that offers the potential to understand basic mechanisms of survival of motor neuron stability and the degradation of survival of motor neuron, which is dependent on a phosphorylation site formed by GSK-3-beta in addition to HuD, a neuron-specific RNA-binding protein that interacts with survival of motor neuron. Dr. Ragampeta Srinivas (Indian Institute of Chemical Technology, Hyderabad) discussed the differentiation of isomeric unnatural amino-acid-containing peptides by electrospray ionization–tandem mass spectrometry (ESI-MS/MS). Nonnatural amino acids, especially β-amino acids and their hybrids formed with natural L-amino acids, have importance in pharmaceutical and foldamer chemistry. Dr. Ravi Sirdeshmukh (Institute of Bioinformatics, Mazumdar Shaw Centre for Translational Research, Bengaluru) closed this session with a discussion of the proteomics of glioma. He stressed the integration of omics technologies to develop clinical applications relevant to these central nervous system tumors.

Day 2, Session 4: CCMB Foundation Day Talk—A special session featuring the CCMB foundation day talk entitled "From Neuron to Brain" was delivered by Prof. Krishnaswamy Vijayraghavan (Department of Biotechnology, Department of Science and Technology, Department of Scientific and Industrial Research, Government of India). He discussed how stem cells shape circuit properties in the brain of drosophila. He also emphasized in detail about wiring the brain with stem cell progeny and the way the stem cell lineages make up the brain. He also described the transformed lineage that makes func-

tional synapses in the antennal lobe and orthodenticle, which controls the identity of stem cell lineages, especially the lateral accessory lobe ventral 1 lineage.

Day 3, Session 1: Mass Spectrometry and Biomarker Discovery—The last day began with a session on mass spectrometry and biomarker discovery. Prof. Jayant Udgaonkar (National Centre for Biological Sciences, Bengaluru) discussed studies on prion protein aggregation by using hydrogen deuterium exchange–mass spectrometry with the observation that amyloid fibrils are more stable to hydrogen–deuterium exchange than the native monomer, thereby allowing both forms to be quantified. Prof. David E. Clemmer (Indiana University, Bloomington, IN) then discussed two model systems, bradykinin and polyproline, that undergo structural transitions in solution and the determination of their conformation in the gas phase. His approach was to vary the composition of the solution from which the ions are formed and electrosprayed, thus proving the ion mobility spectrometry–mass spectrometry technique was a powerful mode of studying structural transitions. Finally Prof. Hanno Steen (Boston Children's Hospital, Harvard Medical School, Boston, MA) discussed the recent advances in technology (filter-aided sample preparation/filter-aided sample preparation-like workflows and state-of-the-art instrumentation) in combination with data-independent acquisition routines that allow the discovery of urinary biomarkers on a large scale, even when there is limited availability of urine samples in pediatric diseases.

Day 3, Session 2: Mass Spectrometry and DNA—The session on mass spectrometry and DNA was opened by Prof. Kumar Somasundaram (IISc, Bengaluru), who described his recent work on the identification and validation of serum cytokine signatures for distinguishing sera of glioma patients from that of normal healthy individuals; his results suggested tumor secreted cytokines act on tumor macrophages to induce a factor that is responsible for tumor angiogenesis. Prof. Valakunja Nagaraja (IISc, Bengaluru) explained the role of DNA gyrase and topoisomerase 1 in chromosome dynamics and gene expression. Different experiments, especially genome-wide occupancy profiles of these enzymes, along with RNA polymerase, were reported. He discussed *in vivo* topoisomerase traffic on transcription units and also the working of two supercoiling domains during *in vivo* transcription. Prof. Desirazu Narasimha Rao (IISc, Bengaluru) discussed the DNA binding protein, DNA processing protein A, in *Helicobacter pylori* that has two functional interactions with the *H. pylori* restriction–modification system by inhibiting restriction enzymes and stimulating methyltransferases. Prof. Siddhartha P. Sarma (IISc, Bengaluru) discussed new peptide sequences and their conformation from the venom of Indian cone snails. Bioactive peptides from the venom of these animals are cysteine rich, and the multiple disulfide bonds, which reinforce the stability, can be used as structural scaffolds.

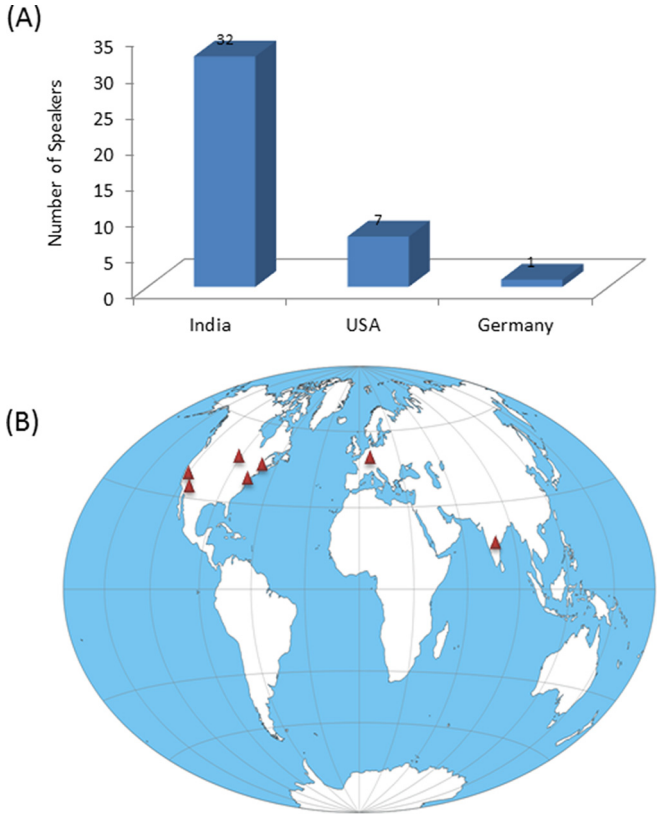

FIG. 2. (*A*) Number of speakers that participated from India, USA, and Germany (*B*) An overview of speakers that participated from: USA (San Francisco, Loma Linda, Bloomington, Boston, Baltimore), Germany (Bremen), and India. Map of world has been downloaded from http://www.freeworldmaps.net/printable/printable-world-map.gif.

Day 3, Session 3: Mass Spectrometry and Drug Discovery—The last session dealt with mass spectrometry and drug discovery. Dr. Arun Bandyopadhyay (Indian Institute of Chemical Biology, Kolkata) found that the protein profile of blood plasma in rheumatic mitral stenosis serves as an indicator for future mechanistic studies with the carboxyl-terminal propeptide of type I procollagen as a biomarker for diagnosis of the rheumatic heart disease. Dr. Wilson Aruni, (Loma Linda University, Loma Linda, CA) talked about the human microbiome and its transition from symbionts to pathobionts. Dr. Wendy Saffell-Clemmer (Baxter International, Bloomington, IN) talked about the development of stable protein formulations by a quality-by-design approach in pharmaceutical freeze drying. Notably, the result is a stable acceptable product using the fastest possible robust cycle. The last talk of the meeting was by Dr. Suman S. Thakur (CCMB, Hyderabad), who talked about prediabetic biomarkers and drug discovery in cancer using mass-spectrometry-based quantitative proteomics. He also described how developments in chromatography have made a great impact on the identification of proteins. Using long columns, small bead size, and extended LC-MS runs coupled with high resolution Orbitrap mass spectrometry led to the detection of about 75% of the yeast proteome and

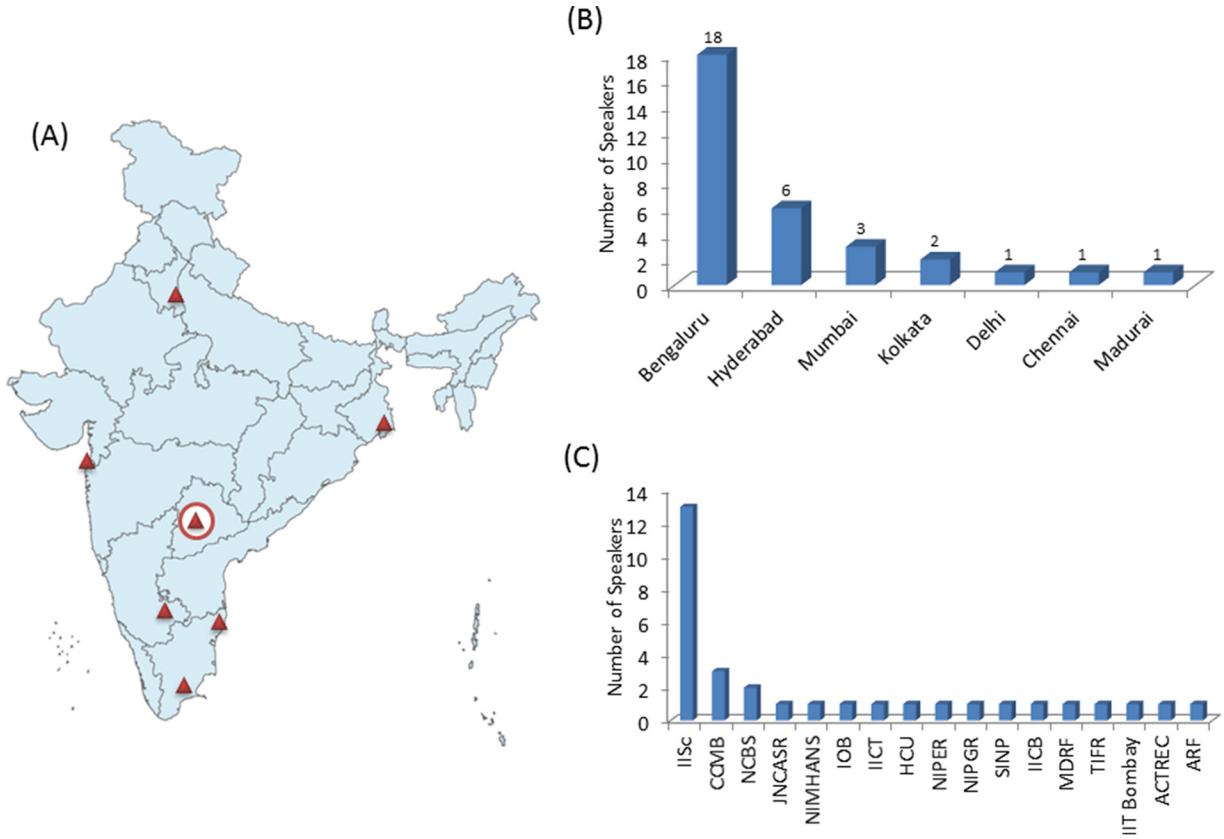

FIG. 3. (*A*) An overview of speakers that participated from India: Bengaluru, Hyderabad, Mumbai, Kolkata, Delhi, Chennai, and Madurai. The map of India has been downloaded from http://www.mea.gov.in/india-at-glance.htm. (*B*) Number of speakers that participated from different cities of India (*C*) Number of speakers that participated from institutes/universities of India: Indian Institute of Science (IISC), Bengaluru; Centre for Cellular and Molecular Biology (CCMB), Hyderabad; National Centre for Biological Sciences, Bengaluru; Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru; National Institute of Mental Health and Neuroscience, Bengaluru; Institute of Bioinformatics, Indian Institute of Chemical Technology; Hyderabad Central University, Hyderabad; National Institute of Pharmaceutical Education and Research, Hyderabad; National Institute of Plant Genome Research, Delhi; Saha Institute of Nuclear Physics, Kolkata; Indian Institute of Chemical Biology (IICB), Kolkata; Madras Diabetes Research Foundation, Chennai; Tata Institute of Fundamental Research, Mumbai; Indian Institutes of Technology, Bombay, Mumbai; Advanced Centre for Treatment, Research and Education in Cancer, Mumbai.

5000 proteins of a human cell line without prefractionation. Further, this technology along with prefractionation served as platform to detect more than 10,000 proteins in human embryonic stem cells. He emphasized how proteomics is coming closer to transcriptomics and genomics. He also described successful investigations of novel natural and synthetic metabolites that have been tested for anticancer properties on different human cancer cells, including retinoblastoma, leukemia, and melanoma. The mode of action and signaling mechanisms of these novel drugs have been successfully revealed by using quantitative proteomics with label-free, stable isotope labeling by amino acids in cell culture and Iisobaric tags for relative and absolute quantitation (iTRAQ) methods.

Day 3, Session 4: Closing Remarks—The closing remarks of the meeting were given by Prof. Ralph A. Bradshaw, who summarized the many oral and posters presentations by noting that they spanned the gamut of proteomics from basic fundamental biology to translational clinical work. He briefly described the past 20 years of proteomics, beginning in 1993 when Dr. Marc Wilkins, then a student in Australia, coined the term proteomics, by emphasizing that it represented a paradigm shift driven by 2D gel electrophoresis, microarrays, advances in mass spectrometry (ESI and MALDI ionizations), and finally high-throughput genome analyses. Proteomics, he pointed out, strives to provide complete information about all the proteins that occur in an organism, tissue, or organelle, from humans to microbes, including elucidating protein–protein interactions and posttranslational modifications. Protein folding is another area where mass-spectrometry-based proteomics has useful applications. He noted that all of these areas were covered in this conference, thus nicely fulfilling the conference theme and illustrating why basic science and translational research should be carried out in a parallel and interactive manner. He emphasized that proteomics has a bright future but it is very hard to predict how proteomics will take shape in the next 5 years. There is a strong need to integrate the data from all the omics, especially genomics,

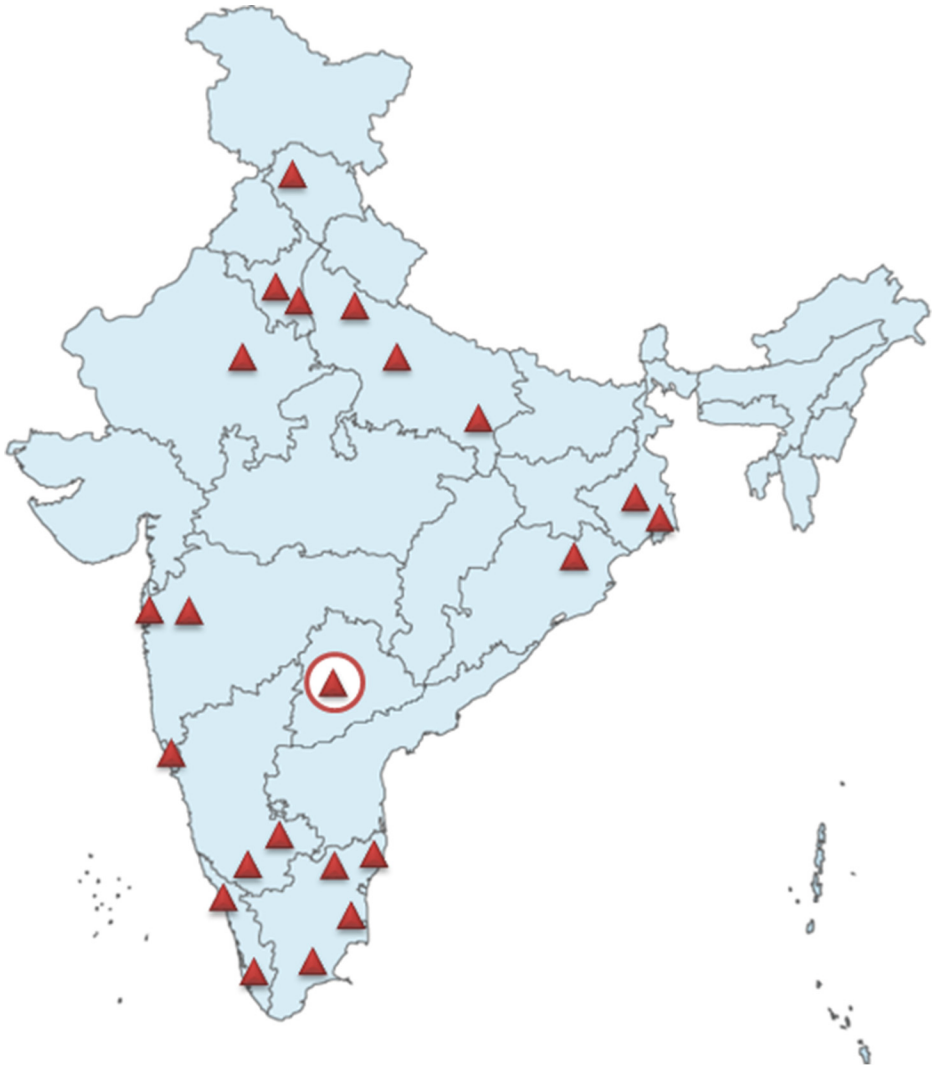

FIG. 4. **An overview of the distribution of participants from different cities of India: Palampur, Gurgaon, Jaipur, Delhi, Izzatnagar, Kanpur, Varanasi, Barrackpur, Kolkata, Sambalpur, Mumbai, Pune, Nagpur, Hyderabad (circled), Goa, Bengaluru, Mysuru, Chennai, Vellore, Pattambi, Thanjavaur, Madurai, and Thiruvanathapuram.**

transcriptomics, proteomics, and metabolomics, with the last two sharing a natural affiliation because they use the same mass spectrometric techniques. Finally, he remarked that he felt that proteomics will play an increasingly important role in human health and societal issues, including environmental research and food technology, all topics of great importance to all mankind, but particularly to India. Thus, he felt, this symposium had served its host very well.

Report/Workshop—The workshop that followed the main meeting provided hands-on training in mass-spectrometry-based proteomics along with bioinformatics analyses and lectures to the participants, who will be using proteomics in their research work. The inaugural talk in the workshop was delivered by Prof. Ralph Bradshaw. Prof. David Clemmer, Prof. Hanno Steen, Prof. Judith Steen, and Dr. Suman S. Thakur, followed with lectures about different aspects of mass spectrometry and five invited talks by Prof. Ravi Kumar (National Institute of Mental Health and Neuroscience, Bengaluru). Prof. Raghvendra Vadrarajan (IISc, Bengaluru), Prof. Kuppamuthu Dharmalingam (Aravind Medical Research Foundation, Madurai), Dr. Muthuswamy Balasubramaniam (Madras Diabetic Research Foundation, Chennai), and Prof. Sandhya Viswasyariah (IISc, Bengaluru) filled out the workshop program. Prof. Kumar highlighted his research using mass-spectrometry-based proteomics to identify 259 proteins in cryptococcal-infected human brain, including those involved in movement through the blood brain barrier. Utilizing mutational sensitivity derived from deep sequencing data, Prof. Vadrarajan discussed protein model discrimination and structural analysis. He also talked about specific mutants that were purified and identified to gain an in-depth view into the mutational effects on the *in vivo* stability of proteins, their folding properties, and their correlation with those expressed *in vivo*. Dr. Balasubramaniam discussed the pres-

ent state of proteomics and metabolomics research in diabetes. The methods and application of quantitative proteomics were discussed by Prof. Dharmalingam. He highlighted proteoforms that have diversity in structure and distinct function. Prof. Viswasyariah summarized her group's finding about the evolutionary conservation and divergence in signaling mechanisms involving cAMP using *Mycobacteria* as a model system.

Speakers and Participants—Altogether, 40 international and national speakers spoke during the symposium and workshop (Fig. 2*A*). Seven speakers were from the United States of America (San Francisco, Loma Linda, Bloomington, Boston, and Baltimore) and one speaker was from Bremen, Germany (Fig. 2*B*). Thirty-two Indian scientists from different cities such as Bengaluru, Hyderabad, Mumbai, Kolkata, Delhi, Chennai, and Madurai participated (Fig. 3*A* and 3*B*). Interestingly, 13 speakers were from the Indian Institute of Science, Bengaluru, one of the top institutes in India. Other speakers were from many other well-known Indian institutes (Fig. 3*C*).

The participants were from various cities of India, including Palampur, Gurgaon, Jaipur, Delhi, Izatnagar, Kanpur, Varanasi, Barrackpur, Kolkata, Sambalpur, Mumbai, Pune, Nagpur, Hyderabad, Goa, Bengaluru, Mysuru, Chennai, Vellore, Pattambi, Thanjavaur, Madurai, and Thiruvananthpuram (Fig. 4). Most of the participants were Ph.D. students and some were postgraduate and postdoctoral researchers from India.

Industry was also represented by Prof. Alexander Makarov, who is associated with Thermo Fisher Scientific, Bremen, Germany. Four participants came from Indian industries (Himedia, Serum Institute of India and Biological E Ltd., India).

Summary—This meeting was a stimulating gathering of international and Indian experts in the area of proteomics. All attendees mutually benefited from listening to the speaker presentations and the subsequent informal discussions. Topics covered included the development and use of mass spectrometry, sample preparation, chromatography, fragmentation, posttranslational modification identification, and the analysis of the proteomes of organelles, plants, microbes, cancer cells, venoms, and neurons. As more and more proteomic analyses are used in different areas of science, these methods offer the potential of helping in solving problems for all mankind and especially the challenges India faces in agriculture and medicine. With time, genomic-related instruments have become cheaper, so it may be expected that in a similar fashion, proteomic-related instruments will also become cheaper. Thus, in the future, proteomics has the potential to play a greater role in fundamental, translational, and clinical research. It is the young scientists who were in attendance that will carry forth this work, and it was a pleasure to have such a diverse group of speakers at the Hyderabad meeting for them to interact with.

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