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Meeting Report*

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The 15th Methods in Protein Structural Analysis Conference (MPSA2004) was held on the campus of the University of Washington, Seattle, Washington, August 29 - September 2, 2004 and was attended by 220 researchers from more than a dozen countries. The MPSA conferences began in 1974 with a small workshop in Boston, MA organized by Richard A. Laursen, Boston University, for the purpose of exchanging information on the then newly developed instruments and chemistry for sequencing proteins. MPSA conferences are held every two years and have usually alternated between Europe and the U.S. Since MPSA2000, they have been organized by the International Association for Protein Structure Analysis and Proteomics (IAPSAP, http://www.iapsap.bnl.gov), a not-for-profit organization established in 1999 to promote the discovery and exchange of new methods and techniques for the analysis of protein structure (Appella et al., 2001). Although the scope of MPSA conferences has expanded as new techniques for protein analysis have been developed, the focus of the conferences has remained on providing a venue in which new techniques for protein analysis are shared between cutting edge researchers, students, and the manufacturers of equipment or providers of services. The rationale for MPSA2004 was to explore new experimental and computational approaches for protein structural analysis and protein interactions that extend from primary sequence analysis through advanced techniques for imaging protein machines in living and near life-like circumstances, i.e. a "systems" approach to understanding protein structure and function.

The conference opened with a keynote address, "Protein Interactions", by Stan Fields, Howard Hughes Medical Institute, Department of Genome Sciences and Medicine, University of Washington, Seattle, WA. After describing how technology development goes through three phases, invention, scale-up, and data analysis, Fields discussed computational approaches to validating high throughput determination of protein-protein interactions using yeast two-hybrid-based methods. Illustrating the value of such methods through two projects, the use of the split ubiquitin assay to identify interactions among membrane proteins in the yeast *Saccharomyces cservisiae*, and a two-hybrid approach to identify interacting proteins in the malaria parasite *Plasmodium falciparum*, Fields showed how data on co-regulation, interactions with paralogues, probabilistic networks, and computational methods all could be used to assess confidence as to which interactions are meaningful. He concluded, however, by saying that new techniques are needed to determine where and when protein-protein interactions occur, to quantify the strength of the interactions, and to determine their consequences. Furthermore, techniques are needed to extend these methods for the analysis of single cells. The address was followed by

an IAPSAP-sponsored reception on the terrace of McHahon Hall, while darkness fell and the view of Mt. Rainer faded as a full moon rose over the participants.

The remainder of the scientific program, which was held in the HUB auditorium, was divided into ten sessions. Leading off with a continuation of "The Interactome", Michael Snyder, Yale University, USA, discussed how global analyses of biochemical activities could be performed to identify protein targets of small molecules, novel DNA binding activities, and to create in vitro maps of protein phosphorylation using protein chips containing most yeast proteins. Particularly revealing were assays of specific mono- and polyclonal antibodies that exhibited specificities from one to 1770 targets and yeast kinases that ranged from one or two to a few hundred targets. Additional "Analytical Strategies in Proteomics" were discussed by three speakers. Ulf Landegren, Uppsala University, Sweden, described the analysis of individual and interacting proteins using proximity ligation reactions. This newly developed technology uses proteins (including interacting proteins) to mediate the ligation of short linear DNAs or to create DNA circles that then may be amplified for precise, highly sensitive detection, by hybridization in homogeneous or solid-phase assays. Tomas Rejar, Northeastern University, USA, then described new approaches to maximize the information content of proteomic samples using LC-MALDI-TOF/TOF MS analysis as well as ESI MS at high sensitivity. As illustrated through analysis of the 4,524 proteins encoded by *Methanosarcina acetivorans*, which has multiple redundant protein families, multiplexed columns were used to overcome the liquid chromatography bottleneck while a 2 kHz repetition laser was developed to increase throughput 10-fold by MALDI compared to current commercial machines. Richard Smith, Pacific Northwest National Laboratories, USA, then described advances in quantitative ultra-high-efficiency proteomics based on nano-liquid chromatography coupled with tandem mass spectrometry emphasizing an achieved dynamic range of greater than eight orders of magnitude in an analysis of the human plasma proteome. More than 1600 human proteins were identified including low-level cytokines. These presentations were complemented by four short talks selected from submitted abstracts. Two afternoon workshop sessions featured presentations by Don Hunt, sponsored by Thermo Electron; Phillip Ross, Applied Biosystems; Jeff Silva, Waters Corporation; and Gary Kuppa, Bruker Daltonics. These were followed by a poster session to round out the first full day of the conference. Hunt offered an impressive description of the automated identification of proteins in complex mixtures with attamole sensitivity, which he illustrated with several examples including protein-protein interactions that regulate a human phosphatase, antigens presented on class II MHC molecules, and proteins involved in the acquisition long term memory.

Tuesday's scientific sessions emphasized "Computational Biology and Protein Families", and "Protein Posttranslational Modifications and Cell Signaling". Leading off, David Eisenberg, University of California at Los Angles, USA, returned to the analysis of protein interactions using two approaches. The first was the synthesis of information from fully sequenced genomes, e.g. *Micobacterium tuburculosis* and *Rhodopseudomonas palustris*, into knowledge about the network of functional interactions using microarray studies and computational approaches including Rosetta Stone, Phylogenetic Profile, and Gene Neighbor. Clustering methods were then used to interpret pathways (http://doe-mbio.ucla.edu/pronav). David Baker, University of Washington, USA, discussed recent advances in protein structure prediction and design. Issues included: How can one develop better energy functions? Are there protein structures that are stable but have not been sampled during evolution? Can one design new catalytic activities not found in nature? Anna Tramontano, University of Rome "La Sapienca", Italy, then discussed the current status and future of protein fold recognition using computational methods that attempt to infer protein three-dimensional structure from their amino acid sequences, a goal of the CASP competitions. Keith Dunker, Indiana University School of Medicine, USA, then explained that, in contrast to most enzymes, many proteins involved in signaling and regulation exhibit disordered regions, which facilitate the uncoupling of affinity and specificity, leading to readily reversible interactions. The morning sessions were completed by four additional short talks selected from abstracts.

The Tuesday afternoon session began with a presentation on biomarker discovery by proteomics and its role in drug and diagnostic development by Steven Carr, who recently joined the Broad Institute of MIT and Harvard. Although over 100 proteins and peptides have been approved by the FDA as biomarkers, the introduction of new disease and mechanism-based biomarkers has slowed over the last 10 years. Nevertheless, proteomics holds the promise of providing panels of multiple peptide and protein biomarkers for disease based on their detection in biofluids. Jeff Gorman, The University of Queensland, Australia, then described methods for detecting post-translational modifications of

transcription factors regulating the responses to environmental stimuli such as oxygen status. Such modifications, include the hydroxylation of a proline that regulates degradation of hypoxia inducible factor (HIF) and of an asparagine that prevents recruitment of the transcriptional coactivator CBP/p300. Gerald Hart then discussed the dynamic glycosylation by O-GlcNAc of factors that modulate transcription, signaling and stress in multicellular organisms. Current data suggest that O-GlcNAc, discovered only in 1983, occurs at levels similar to protein phosphorylation and serves, competitively with phosphorylation, as a major metabolic sensor that globally regulates cell signaling pathways as illustrated through several examples, including RB and p107. The presentations concluded with a talk by Doug Sheeley, National Center for Reserach Resources, NIH, USA, on programs and funding opportunities relevant to proteomics. At the evening poster session, participants also had an opportunity to meet with vendors and exhibitors displaying the latest technologies in protein structure analysis and proteomics.

Day 3 covered four scientific topics: “High Throughput Technologies for Protein Production” and Folding”, “Analysis of Macromolecular Complexes”, “Chemical Proteomics and Imaging Methods”, “Protein Engineering and Biotechnology”, and concluded with the Edman and Young Investigator Awards. Frank Collart described high throughput strategies for structural and functional genomics being developed in collaboration with the Roche Protein Expression Group (Indianapolis, USA) at the Argonne National Laboratory in Chicago, USA. During the past year, 4000 bacterial target proteins were cloned, 1000 of which were expressed in soluble form; complete structures were obtained from 80. Expression was with both *E. coli*-based in vivo and cell-free systems, which gave 70% concurrence in expression; however, only 68% of clones gave detectable polypeptides while 23% of the total yielded soluble proteins. Mark Fisher, University of Kansas Medical Center, USA, then explained how proteins could be refolded in a high throughput manner using a combination of the GroEL chaperonin and osmolytes. Combining both methods allowed the isolation of many difficult proteins in sufficient quantity for X-ray crystallography or NMR structural determinations and simplified the chaperonin reaction by eliminating the need for the co-chaperonin GroES. Jeff Ranish, Institute for Systems Biology, Seattle, WA, USA, described the study of macromolecular complexes by quantitative proteomics using stable isotope tagging and mass spectrometry, focusing on the analysis of transcription factor complexes from yeast and human cells. Positive, quantitative identifications of complex components were possible even with a high background of non-specific proteins. Min Li, Johns Hopkins University, USA, then discussed in vitro and cell-based systems for analyzing protein interactions using random peptide display and selection with desirable binding or functional properties. Jill Trewhella, University of Utah, described the use of neutrons, which have unique scattering properties that enable one to distinguish between components of complexes, for analyzing changes in protein complexes illustrated with data from the second messenger cAMP-dependent protein kinase and changes in muscle troponin complexes mediated by calcium.

Following lunch, Ben Cravatt, The Scripps Research Institute, USA, discussed activity-based protein profiling using chemical approaches for functional proteomics. Serine hydroxylases, which represent one percent of the proteome, were selected using fluorophosphonates as a class-selective covalent inhibitor, and the selected enzymes could be used to distinguished breast cancer and melanoma cells. Secreted and membrane-bound activities were the most informative. Non-directed strategies, e.g. the use of sulfonate esters, which select about 10 different enzyme activities, also were described. Mark Ellisman, University of California, San Diego then discussed imaging of the nervous system at multiple length scales. Correlation of light and electron microscopic techniques through powerful computational methods, combined with recent progress in molecular biology, will now permit three-dimensional visualization of neurological structures and their protein constitutents, enabling a new understand of the brain. Focusing more sharply on the role of structure in human health, John Desjarlais, Xencor, Monrovia, CA, USA, then illustrated the rational engineering of protein drugs, such as antibodies or cytokines that have superior efficacy and reduced side-effects.

The day’s scientific sessions concluded with presentation of the Edman Awards and IAPSAP Young Investigator Award. To commemorate its origins and honor Pehr Edman, in 1988, MPSA began awarding the Edman Award to individuals whose efforts had significantly advanced the field (Wittmann-Liebold, 1989). This year’s Edman Awardees were Stephen Altschul, Computational Biology Branch, National Center for Biotechnology Information, National Institutes of Health, USA, and Dr. Amos Bairoch, Swiss Institute for Bioinformatics, Geneva, Switzerland, who were honored for their contributions to the development of bioinformatics methods and databases for the analysis of proteins and

their structures. In 2000, IAPSAP added a Distinguished Postdoctoral/Young Investigator Award. This year's Distinguished Young Investigator Award went to Dr. Niroshan Ramachandran, Harvard Institute of Proteomics, Boston, USA, for the development of novel self-assembling protein microarrays for the functional analysis of proteins. The Edman Awards and the Distinguished Young Investigator prizes were sponsored by PE Applied Biosystems. Information on this year's awards was published in *Protein Science* [**13:**, 2278-2279]; this information and an electronic copy of the MPSA2004 program book are available at the IAPSAP website (http://www.iapsap.bnl.gov).

The final half-day of the conference was composed of two sessions: "Cell Pathway Regulation and Metabolomics", and "Systems Biology". Trisha Davis led with an elegant description of the lattice structure of the yeast spindle pole body as probed by fluorescence resonance energy transfer (FRET). The spacial arrangement of the 5 core components of the yeast spindle pole body was accessed with FRET data, using a newly developed metric that facilitates a comparison of FRET values. The deduced arrangement suggested a mesh network of interlocking proteins that, when combined with cryo-electron tomography, led to a composite model that offers a novel view of the structure and assembly of the spindle pole body. Jeremy Nicholson, Imperial College, London, UK, then described how one's past, present and future could be foretold in two minutes through metabolomics, an analysis of the metabolites in different fluids of the body, primarily using NMR. Nicholson's description was a perfect segue into the presentation of Trey Ideker, University of California at San Diego, USA, who described the mapping, validating, and comparison of biological networks, which is the major challenge of today's systems biology. Ideker described how physical network models are constructed from a scaffold of protein-protein and protein-DNA interactions, integrated with expression profiles, to explain observed responses to DNA damage and other toxins. Automated procedures are then used to select gene knockouts for validation. Lee Hood, President, Institute for Systems Biology concluded the session with a description of the four elements of systems biology that provide insight into the emergent properties of the system and the way in which these properties arise. Systems approaches will provide the basis for predictive, preventive and personalized medicine, a goal for which, with the complete sequencing of the human genome and those of several model organisms, should soon be within reach. Ken Walsh, University of Washington, USA, organizer of MPSA1986, in his concluding remarks, then compared and contrasted the science presented at MPSA1986 and MPSA2004. While there is still much to learn, the fields of protein chemistry and structural biology have indeed come a long, long way in a mere 18 years.

Logistics of the meeting were amply and efficiently handled by SimpleMeetings, a Seattle-based organization. The organizers are please to acknowledge generous support from the Office of Biological and Environmental Sciences of the U.S. Department of Energy, the National Institutes of General Medical Sciences, NIH, Amgen, Inc., the Institute for Systems Biology, and from numerous company sponsors. While the densely packed schedule left little time to enjoy the delights of Seattle and the nearby attractions of Oregon, Washington State, and British Columbia, in keeping with the analytical traditions of MPSA meetings, participants enjoyed "times of flight" at the meeting banquet in the Museum of Flight, where they participated in a massively parallel proteomics analysis of Western salmon and chocolate torte. MPSA2004 closed with the announcement that the next MPSA conference, MPSA2006, will be held in Lille, France in the fall of 2006. Further information will be posted on the IAPSAP website (www.iapsap.bnl.gov) as it becomes available.

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