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EXPERT REVIEWS

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HUPO 2008 7th World Congress
Amsterdam, The Netherlands, 16–20 August 2008

The annual world congress of Human Proteome Organisation (HUPO) is one of the premier meetings in proteomics. Rotating between Europe, North America and Asia/Oceana, this year's host city was Amsterdam, The Netherlands. Proteomics still being a rapidly evolving field, HUPO meetings provide a platform for technical advancements in protein purification and separation techniques, innovations in mass spectrometry and applications in bioinformatics and computational biology. A special focus of this year's meeting was on proteome biology, indicating that the state of technology has progressed to a level permitting interrogation of biological systems in a meaningful way.

Over 2000 participants gathered in Amsterdam, The Netherlands, for the HUPO Annual World Congress. The organizers Albert Heck, Anne-Claude Gavin and Ruedi Aebersold put together a packed program with five concurrent sessions under the theme 'Proteome Biology', intentionally indicating that the status of the field has progressed beyond the point of mere technology development, now allowing us to focus on the 'real thing' by interrogating biological systems. Although programmed as a 3-day meeting, the main program was preceded by a 2-day pre-conference in a proven format. Educational sessions aimed to update those who were new to the field with the various technologies used in proteomics. The clinical day provided a platform for academia and industry to discuss technology and applications for the discovery of biomarkers in clinical samples. Finally, various workshops were organized over these 2 days by the HUPO initiatives. These initiatives focus on proteomic application in defined areas including several human organs (e.g., brain, kidney and urine, liver, and plasma proteomics), but also glycomics and stem cell proteomics. The success of these initiatives indicates that the scope of proteomics diversifies, but at the same time it can also be concluded that such international efforts are still needed to access highly complex proteomes in sufficient depth. The Proteomics Standards Initiative (PSI) deserves special mention, since they have paved the road for standardization of data formats over the past few years. The recent implementation of mzML and the expected introduction of analysisXML will greatly enhance accessibility and exchange of data obtained from a wide variety of experimental platforms.

The notion that proteomics has matured to a state that we can now address biological problems does not indicate that technology development in proteomics is over. Several bottlenecks prohibit getting to the ultimate goal where we can analyze all cellular proteins, including their modified forms, follow their creation and demise over time, and track their interactions with proteins and metabolites. The program of the conference was centered on a number of these bottlenecks, as well as on prime examples of the questions that can be addressed when these are overcome.

In order to move proteomics away from cataloguing protein parts lists in cells and tissue, the introduction of quantitative mass spectrometry has been essential. Initiated in the late 1990s with the introduction of the isotope-coded affinity tag (ICAT), *in vivo* labeling of cells with stable isotope-coded amino acids (SILAC) has become the method of choice wherever possible. This was pioneered by Matthias Mann

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(Max Planck Institute, Martinsried), who gave a plenary lecture with applications not only following dynamics of protein expression over time, but also using the label as a means to discriminate between differentially treated samples and identify relevant proteins in a subtractive manner. For instance, this includes a screen for proteins binding to H3K4-methylated histone tails, as presented by Michiel Vermeulen, where non-modified peptides were used as a control. The power of this approach emanates from the capability of extracting a small number of proteins out of a pool of hundreds that specifically bind to modified histones and that were identified based on a ratio deviating from 1. Software to analyze such data was developed by Jurgen Cox, who showed the versatility of Maxquant, which cannot only quantify SILAC-labeled peptides, but also performs recalibrations of precursor masses and retention times for enhanced accuracy and inter-run comparisons. Moreover, this can be used effectively to quantify peptides without the use of isotope labels. This was shown in an experiment where *Escherichia coli* proteins were mixed into a human cell lysate in various ratios, resulting in a separation of proteins from both organisms based on peak intensity. Another powerful application of SILAC was shown by Matthias Selbach (Max Delbrueck Center for Molecular Medicine, Berlin, Germany) identifying targets of miRNAs. The challenge here was to find proteins whose expression is only mildly regulated by miRNAs.

Although SILAC finds an increasing number of applications, alternatives need to be sought for tissues or organisms that cannot be grown under defined culture conditions. Shu-Hui Chen (National Cheng Kung University, Tainan, Taiwan) and Paul Boersema (Utrecht University, Utrecht, The Netherlands) showed how deuterium-labeled formaldehyde can be used to label α- and ε-amino groups in protein digests. The reaction by reductive methylation is fast and complete, introducing a 4-Da difference for each reacted amine. Multiplexing is possible by the use of D2–13C-labeled formaldehyde permitting a three-way comparison in a single experiment.

Another topic that relates to many biological applications in health and disease is the systematic analysis of post-translational modifications in general, and particularly phosphorylation. Ole Jensen (University of Southern Denmark, Odense, Denmark) gave an overview of the various techniques that have emerged over the past few years for capturing phosphopeptides from complex protein mixtures, notably metal affinity strategies. These approaches are now adopted by researchers for large-scale analyses uncovering profiles of protein phosphorylation in cells or tissue. The challenge is in the identification of hundreds of phosphopeptides while maintaining acceptable levels of false-positive identifications. Another complication is in calling the exact phosphorylation site in a given peptide. The combination of quantitative proteomics focusing on phosphorylation events was shown in a number of studies, looking at dynamics during the cell cyclus (Jesper Olsen, Max Planck Institute, Martinsried, Germany), differentiation of embryonic stem cells (Jeroen Krijgsveld, Utrecht, The Netherlands) and of mesenchymal stem cells (Tine Thingholm, University of Southern Denmark).

A crucial question in phosphoproteomics is not only to trace modified peptides, but also to identify the kinase–substrate relationships. This can be done in a direct way by comparing the phosphorylation profiles of wild-type and kinase knockout cells. This was employed by Nick Morrice (University of Dundee, UK) for the identification of AMPK substrates. Bernd Bodenmiller (ETH Zurich, Switzerland) took a broader approach by knocking out individual kinases and phosphatases in yeast to determine kinase–substrate networks. An alternative approach was proposed by Rune Linding (Institute of Cancer Research, London, UK) who developed a computational framework to infer these relationships from phosphorylation motifs in target proteins and the network context of both kinases and substrates. Several of these predicted relationships could be verified experimentally. This indicates the power of this approach, but also shows the importance of correct assignment of phosphorylation events in (large-scale) proteomic efforts, and the characterization of protein networks in general.

Several contributions focused on protein interactions and networks, covered extensively in a plenary lecture by Anne-Claude Gavin (EMBL, Heidelberg, Germany). By introducing the term of 'social networks' of a cell she indicates both stable interactions in protein complexes, as well as transient ones in cellular pathways. Such wiring diagrams are being created for yeast and *Mycoplasma* by TAP-tagging technology. Analysis of the latter organism with just 689 open reading frames is to provide a minimal protein interactome, as shown by Sebastian Kuehner (EMBL).

Another way of looking at protein networks is by direct interrogation of cellular systems for known components in a targeted approach. A technique that is gaining strength is selected reaction monitoring, which tracks peptides in complex mixtures based on the specific combination of precursor and fragment masses that can be selected in triple quad mass spectrometers. Although such transitions can be predicted *a priori*, it is typically based on prior knowledge and previous observations of these peptides. In their lectures, Ruedi Aebersold and Bruno Domon (ETH Zurich) stressed the importance of collecting this information in a 'peptide atlas' for the design of targeted analyses of multiple peptides in a multiplexed experiment. A glimpse of how an application could look like was provided by Paola Picotti (ETH Zurich) who designed selected reaction monitoring experiments mapping metabolic pathways in yeast in a single experiment. By growing cells under a variety of conditions and through the use of stable isotope labeling, expression levels of multiple enzymes could be monitored. Importantly, proteins spanning the entire range of abundance (between 1E6 and 50 copies per cell) could be detected and quantified.

Many more large-scale analyses were shown than can be described here. Yet, it was reassuring to see multiple contributions focusing on improvements in workflows that are at the basis of any application. In particular, among the approximately 1500 posters were many examples for improved isolation, extraction, separation, digestion and ionization of proteins and peptides. To mention one example, Scott Ficarro (Harvard Medical School, Boston, USA) showed a miniaturized version of a liquid

chromatography mass spectrometry configuration, where he used analytical columns with 10 µm ID, packed beds of 1.7-µm particles, and an electrospray ionization emitter opening of under 1 µm, allowing to drop flow rates to 1 nl/min. Despite the deterioration in chromatographic performance, compensation by electrospray ionization efficiency resulted in unsurpassed sensitivity. Although the focus of this meeting was on biology, it did not miss out on giving ample space to improvements like these in the details of proteomics. It will be interesting to see how these continue to energize the field, and how they will permit us to look closer, deeper and broader into biology, next year, when HUPO gathers in Toronto, Canada.

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