

Report on the conference: "Mass Spectrometry and Proteome Analysis"

Joint congress of the French Mass Spectrometry Society and French Society of Electrophoresis and Proteome Analysis, Toulouse, 2003, September 16-19

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The French Mass Spectrometry Society ([SFMS](#)) and the French Society for Electrophoresis and Proteome Analysis ([SFEAP](#)) held their annual conference as a joint meeting in the wonderful city of Toulouse, from the 16th to the 19th of September. Besides the many charms of the typical old city of Toulouse and an impressive organization, the congress provided the participants with a state-of-the-art in proteome analysis methodologies, with an emphasis on mass spectrometry progress. With seventeen plenary lectures, 43 oral contributions, 188 poster communications, three roundtables and more than 500 participants, the congress gave a good overview of the current analytical problems and challenges in proteome analysis. The congress was deliberately oriented to multidisciplinary research, including participants from very different fields, including fundamentals of mass spectrometry, organic and inorganic mass spectrometry, proteomics, structural biology, cell biology...

¹ The content of this report only reflects the views and understandings of its author.

Mass spectrometry: an old and booming field

Fundamentals of mass spectrometry

Several contributions reviewed the current knowledge in fundamentals of mass spectrometry: [Jean-Claude Tabet](#) (Université Pierre et Marie Curie, Paris) summarized the current knowledge in ionization processes in MALDI and ESI ionization, with emphasis on the ionization pathways that result in multicharged ions and multimer ions. Two other talks from A.J. Stace (University of Sussex, UK) and Gilles Ohanessian (Ecole Polytechnique, Paris) were devoted to the characterization of metal complexes and sodium / peptides complexes in the gas phase.

Instrumental developments and new methodologies

Several major talks emphasized the recent developments in mass spectrometric tools for proteome analysis: [Brian T. Chait](#) (The Rockefeller University, New York) presented a new methodology called "Hypothesis-driven proteomics": due to developments in instrumentation, complex mixtures can be interrogated for the absence or presence of a predicted peptide. Thanks to multistage MS, the queried mass is isolated (one MS stage is thus used as a mass filter only) and fragmented to testify for the absence or presence of the expected peptides. The predicted masses are indeed used as signatures for a given protein, as would be the case for a spot in a Western blot experiment. About one hundred of masses can be interrogated in a single experiment, and examples of this methodology were provided for the detection of secreted peptides from a cell culture supernatant, with higher sensitivity compared to single MS stage. A.L. Burlingame (University of California SF, USA) emphasized the importance of the latest developments in mass spectrometry, such as MALDI axial TOF/TOF, Qqoa TOF-MS, ESI-Qtrap for protein post-translational modification elucidation. [P. Vouros](#) (Northeastern University, Boston, USA) resumed two decades of LC-MS developments with examples of DNA adducts in cancer research, and stressed that despite tremendous developments in capillary and nano

LC-MS, HPLC coupled to microESI-MS has still a great role to play due to its loading capacity, and possibility of multiple characterization through flow splitting. Finally, [Joseph Zaia](#) (Boston University, USA) presented the recent developments in glycosaminoglycans sequencing by ESI-MS/MS strategies.

Recent developments in FT-ICR-MS

A whole session was devoted to Fourier Transform-Ion Cyclotron Resonance- Mass Spectrometry (FT-ICR-MS). After a thorough tutorial on the basic physics and main features of FT-ICR-MS instruments by Jean-Francois Muller (Metz University, France), Buko Lindner (Research Center Borstel, Germany) introduced the use of FT-ICR instruments in everyday biomolecule characterization, with examples from glycolipids structure elucidation where FT-ICR-MS provided evidences that would have been totally misinterpreted without ultrahigh resolution combined to MS/MS capabilities. Denis Loyaux from Sanofi-Synthelabo clearly established the need for such instruments in the context of proteomic research: in a typical experiment of nanoLC-MS/MS, a QTOF instrument identified one protein within two minutes of chromatographic eluent from a protein mixture digest, whereas the FT-ICR identified ten more proteins during the same two minutes. However, the QTOF was still used in parallel to the FT-ICR instrument due to its fast MS/MS acquisition rate, whereas the FT-ICR was used basically for ultra-high resolution peptide mass measurements. Two contributions from Thermo Electron and Bruker gave an overview of commercially available instruments. Developments in the last two years were clearly devoted to build user-friendly instruments, exemplified by the adoption of a Windows platform by Bruker, as well as compatibility with database mining software developed for other instruments, and compactness of the Thermo Electron instrument. Both suppliers claimed that mass accuracy standard was clearly below 2 ppm, associated with resolution in the range of 100000 and acquisition rate of the order of 1 spectrum per second in full scan range. However, the last contribution of this session from [Guillaume van der Rest](#) (Ecole Polytechnique, Paris) gave a realistic view of the operation of an FT-ICR-MS: despite very good results on the analysis of crude snake venoms as well as study of multiprotein complexes, the everyday operation

was said to be much more tricky than claimed by the supplier. In particular, the adjunction of an ECD source for MS/MS in the ICR cell was found to be very delicate, especially to reach reproducibility. At the occasion of this last talk, it was mentioned by the chairman (Christian Rolando, Lille University, France) that the last talk was the only example of an FT-ICR-MS instrument devoted to life sciences in the academic research in France. This reflects the poor level of investments in instrumentation for proteomics in France, compared to Germany for example.

Workshop on microfluidic tools

A workshop on microfluidic devices for proteomics, with emphasis on the coupling with mass spectrometers was organized and chaired by Hubert Girault (EPFL, Switzerland). [Christian Bergaud](#) (LAAS-CNRS, Toulouse) presented a general introduction to printing technologies for microarrays, [Christian Rolando](#) (Université des Sciences et Technologies de Lille) and [Niels Lion](#) (EPFL, Switzerland) presented microfabricated nanospray and microspray emitters. Then several industrial representatives presented their technological platforms: Gary Williams ([Advion Biosciences](#), Norwich, UK) gave an overview of the ESI-Chip™ system as an automated, high-throughput sample delivery to the mass spectrometer; interestingly, the possibility that in the near future, liquid separation prior to sample introduction into the mass spectrometer may not be necessary or even desirable, due to progress in complex mass spectrometric methods, was discussed. Presentations from [Gyros](#), [Cypherger](#) and [Biacore](#) completed the scene with microfluidic devices coupled to MALDI-MS. In some extent, this workshop was a little bit disappointing due to the very small audience, reflecting the difficulties of these new technologies to penetrate the market. All industrial suppliers agreed on the fact that the spreading of such new technologies was very low, especially outside the USA. The only figures were provided by Ciphergen, who claims to have sold 600 SELDI systems (including their functionalized ProteinChips™, liquid handlers and SELDI-TOF-MS), most of them in the USA (one third in research labs, one third in industry and one third in clinical labs). So despite tremendous scientific progress in the development of

microanalytical tools for proteomics, end-users do not seem ready to operate this drastic change in technologies.

Proteomics: insufficient tools leading to mixed strategies

The overall picture given by the congress is that proteomicists are still struggling with analytical limitations. This was clearly stated by Thierry Rabilloud (CEA Grenoble, France) during the workshop on proteomics challenges (for an overview of Thierry Rabilloud's views on these problems, please see his [conference](#) given at the [NIH Proteome Interest Group](#)). In a fascinating lecture, [Hubert Hondermarck](#) (Université des Sciences et Technologies de Lille- CNRS, France) emphasized the difficulties in current 2D-GE- MALDI-MS approaches in the context of breast cancer differential proteomics from clinical samples. Only in-vitro cell lines gave robust and reproducible results and allowed the identification of two potential markers and targets. Hubert Hondermarck clearly showed that alliance of cell biology, molecular biology and proteomics technology was necessary to obtain biologically relevant information, and that brute force, large-scale approach can really miss pertinent information due to analytical limitations. Most of lectures concerning proteome analysis testified that 2D-GE remains the workhorse for research labs, with a great deal of work done on sample preparation for solubilization of membrane proteins. But it seems that 2D-GE is now reaching a more or less robust state, and appears to many end-users as a routine, state-of-the-art analytical technology, especially for quantification (probably due to the efforts by many instrument suppliers to provide robust image analysis tools). So despite clear analytical limitations in terms of sensitivity and dynamic range, 2D-GE remains the tool of choice for differential proteomics.

[Eric Forest](#) (Protein Mass Spectrometry Lab, CEA Grenoble, France) reviewed mass spectrometric techniques for the study of protein/protein complexes, among which direct

mass spectrometric studies, cross-linking experiments, hydrogen/deuterium exchange... which were further illustrated with typical examples in several oral contributions.

The only contribution really relevant to large-scale data acquisition was given by Laurence Florens from The Scripps Research Institute ([John Yates III's laboratory](#)) about the proteomics of *Plasmodium falciparum* at different stages of infection. The application of high-throughput MudPit (Multi Dimensional Protein Identification Technology) resulted in the identification of new proteins predicted from ORF databases (but not reported in protein databases) and clustering of genes at the protein level as well as subcellular localization at critical stages of parasite life cycle.

During the workshop on proteomics challenges, a discussion arose on the role of HUPO; Thierry Rabilloud exposed his views about the conflicts that rages among the organization: as to him, two groups confront one another, namely, the "organizers" and the "standardizers". The organizers tend to coordinate heterogeneous efforts and projects all over the world without putting any constraints on methodologies or standards to be used. At present their main effort aims at establishing a common database format for proteomics information gathering and publishing. On the contrary, the standardizers try to impose kinds of "good proteomics laboratory practices", methodologies and standards that would be the only one recognized at the international and collaborative level. This approach is modeled on the Human Genome Project, where standardized technologies and platforms allowed the distribution and gathering of work and information among different centers. The conflict lies on the recognition of the maturity of proteomic technologies: for organizers, technologies are still too heterogeneous to establish standards, for standardizers, homogeneity and cooperative work will be provided by standards. This schism roughly corresponds to a European / American separation, and no consensus between these two groups can be foreseen to date.

The overall impression given by proteomics contributions is that it still remains a very heterogeneous field: while proteomics facilities try to set-up high-throughput capabilities (with all associated budget limitations), mainly based on classical 2D-GE – MALDI-MS approaches, many potential end-users are just interested in observing very few proteins. One head of a proteomic service facility even reported that many researchers just want to

use proteomic technologies to observe the variations of less than five proteins, which is already enough to give several years of work of microcharacterization. On the contrary, some others like Hubert Hondermarck reported that large-scale brute force approach is still limited by analytical technologies, and usually misses pertinent results. His conclusion is that hypothesis-driven research (with good amounts of physiology) is needed to obtain pertinent results. Proteomics thus seems to balance between researchers that are not really interested in large-scale or system biology, and those who would like to do it, but still struggle with analytical problems.