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MEETING REPORT

MPSA 2006

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The 16th Methods in Protein Structure Analysis Conference (MPSA 2006) was held on the campus of the Université of Lille, Lille, France, August 29–September 2, 2006, and was attended by more than 200 researchers from Europe, the Americas, and Asia. Lying on the main high-speed train line leading north from Paris, well connected with England via the Eurostar, and home to the Université de Lille as well as a branch of the Institute Pasteur, Lille provides an excellent venue for scientific meetings. The Polytech'Lille, in the Cité Scientific, a few kilometers south of the city center, is easily reached by an automated metro system. The modernized medieval city center offers tourists a wealth of opportunities and excellent restaurants. Indeed, as the meeting ended, Lille was preparing for the ''Braderie,'' the largest flea market in Europe. In addition, the local organizers thoughtfully provided a well-guided tour of nearby Bruges, Belgium, where two dozen of the meeting participants who stayed through the end admired the lace and sampled Belgian beer and chocolates.

The MPSA conferences began in 1974 with a small workshop in Boston, 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 MPSA 2000, they have been organized by the International Association for Protein Structure Analysis and Proteomics (IAPSAP, http://www.iapsap.bnl.gov), a non-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 characterization have developed, the focus of the conferences has remained on providing a venue in which new techniques for protein analysis are shared among researchers, students, and the manufacturers of equipment or providers of services. The goal of MPSA 2006 was to explore new experimental approaches for the analysis of protein post-translational modifications and their roles in regulating biological function from chromatin structure to the control of neuronal signaling. Time only permitted a sampling of examples in which new methodologies in mass spectrometry and imaging are contributing to an understanding of the many ways in which such modifications as phosphorylation, acetylation, methylation, glycosylation, ubiquitination/sumoylation, lipidation, and proline isomerization orchestrate biological function. But conference attendees were rewarded with a truly exciting set of presentations. The program and abstracts are available on the IAPSAP website at http://www.bnl.gov/iapsap/meeting.asp. It is anticipated that selected talks presented at MPSA2006 will form the basis for several mini-review series, as was the case for MPSA 2004 (Appella and Anderson 2005a,b). As with the past two MPSA Conferences, on the day preceding the conference, the organizers provided an optional, two-session course on identifying post-translational modifications with mass spectrometry and mass spectrometry database-searching

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techniques, taught by Nicholas Sherman (University of Virginia, Charlottesville) and David Creasy (Matrix Science, London).

The conference opened with a keynote address, ''Beyond the Double Helix: Reading and Writing the Histone Code,'' by C. David Allis (Rockefeller University, New York). After reminding the audience that histones, including their highly modified tail segments, are the most conserved proteins in nature, Allis elaborated on how the three major mechanisms that modulate the structure of chromatin fibers—covalent histone modifications, chromatin remodeling by ATP-dependent complexes, and the utilization of histone variants—combine to regulate gene expression and other important processes using protein motifs, such as the bromodomain and chromodomain, which recognize acetylated lysine and methylated lysine, respectively. Recent studies from several laboratories, including Allis', have shown how the plant homeodomain (PHD), although structurally unrelated to the chromodomain, recognizes trimethyl lysine with high specificity. An emerging theme is how several domains that recognize post-translationally modified sites create a network of interactions that orchestrate chromatin use, a theme echoed by several speakers and summarized in the final lecture of the meeting by Tony Paulson. The keynote address was followed by an IAP-SAP-sponsored reception in the lobby of the Polytech'-Lille, Cité Scientifique, which featured regional food and games.

The remainder of the scientific program, which was held at the Polytech'Lille, was divided into 11 sessions, punctuated by two poster sessions and sponsored presentations by Agilent and Applied Biosystems, Inc. After an opening welcome on Wednesday morning from Guy Lippens (CNRS/Université de Lille 2), Chair of the MPSA 2006 organizing committees, Kung Ping Lu (Harvard Medical School, Boston) described how prolyl isomerization modulates serine/threonine phosphorylation and dephosphorylation to effect cell signaling that is important for Alzheimer's disease and cancer. Indeed, the Pin1 isomerase is overexpressed in many prostate cancers. Titia Sixma (Netherlands Cancer Institute, Amsterdam) then described how the catalytic efficiency of the E2 conjugating enzymes is affected by the structural and enzymatic effects of SUMO modification. Session 2 began with an exploration of the keratinocyte ''SUMOeome'' by Van Wilson (Texas A&M University, College Station). Two-dimensional gel analysis revealed extensive and dynamic changes in the SUMOsome during keratinocyte differentiation, and methods for identifying new SUMO-modified targets were described. Short presentations then followed. Isabelle Landrieu (Université de Lille), explored the molecular function of the Pin1 prolyl *cis*/*trans* isomerase using nuclear magnetic resonance, and Heinz Nika (Albert Einstein School of Medicine, Bronx), described a solid-phase strategy for improved phosphopeptide detection and phosphorylation site determination. Following the lunchtime seminar sponsored by Agilent, on a new, solution-based, isoelectric focusing technique for efficient protein and peptide isoform fractionation and a high-resolution column-based method that even allows the separation of intact membrane proteins, the afternoon session began with a presentation by Alma Burlingame (University of California, San Francisco) on the challenges for mass spectrometry to meet the complexity of protein covalent modifications, especially multisite phosphorylations. Peter Roepstorff (University of Southern Denmark, Odense) then spoke about the selective isolation of peptides containing acidic modifications using nanocolumns containing titanium dioxide, which his group has used to characterize the role of post-translational modifications in protein localization. Kris Gevart (Ghent University) then discussed peptide-centric tools for studying protein processing. His laboratory has developed a technology, termed ''combined fractional diagonal chromatography'' (COFRADIC), to isolate predefined sets of peptides for further characterization by LC-MS/MS and has applied it to the proteomic analysis of apoptotic Jurkat T-lymphocytes. Maurine Linder (Washington University, St. Louis) described the mechanism and consequences of protein palmitoylation, which facilitates membrane interactions and regulates protein trafficking. Christopher Grangeasse (University of Lyon) wrapped up the afternoon with a description of protein tyrosine phosphorylation in bacteria. Surprisingly, bacterial tyrosine kinases share no sequence homology with their eukaryotic counterparts. The Day 2 presentations were followed by a poster session and buffet.

Day 3 began with a return to the topic of chromatin modifications from Neil Kelleher (University of Illinois, Urbana-Champaign) who described the detection of discrete patterns of post-translational modifications in the human nucleus by high-resolution mass spectrometry. Using a ''top-down'' approach (see below), his group in collaboration with Craig Mizzen (University of Western Ontario, London) has established a reference set of expressed histone forms for each of the five histones in human cells. The use of ion/ion chemistry and tandem mass spectrometry to accomplish a comparative analysis of post-translationally modified proteins and peptides was described by Don Hunt (University of Virginia, Charlottesville). Using these technologies to obtain the sequence of 15–60 amino acids from both the N and C termini, together with the intact protein mass, unknown proteins can be identified. High-precision measurements, combined with other techniques were applied to studies of cell migration, the histone code and cancer vaccine development. Cécile Rochette-Egly then explained how phosphorylation, in cooperation with the ligand, coordinates

and fine-tunes the activity of nuclear retinoic acid receptors. In the first of two shorter talks, Jürgen Dieker (IBMC/CNRS, Strasbourg) spoke about post-translational modifications on the human lupus autoantigens during apoptosis. Solange Serrano (Institute Butantan, São Paolo) then described the use of surface plasmon resonance to characterize the interactions of the cysteine-rich domains of snake venom metalloproteinases with FACIT collagens XII and XIV and matrilins. Such interactions destabilize the extracellular matrix, which leads to hemorrhage. After the lunchtime seminar sponsored by Applied Biosystems on selective detection, sequencing, and quantitation of post-translational modifications using the Q-TRAP LC/MS/MS system, Donald Kirkpatrick (Harvard Medical School, Boston) explained the use of the recently developed ubiquitin-AQUA method and mass spectrometry for the direct analysis of ubiquitin chain linkages regulating proteasomal degradation and proteasome independent signaling. Leonie Waanders (Max Planck Institute of Biochemistry, Martinsreid) then described the analysis of post-translational modifications of murine myelin basic protein using the LTQ-FT and LTQ-Orbitrap mass spectrometers.

The third day's scientific sessions concluded with presentations of the Edman Awards and IAPSAP Young Investigator Award (see http://www.bnl.gov/iapsap/meeting.asp). To commemorate its origins and to honor Pehr Edman, in 1988 MPSA initiated the Edman Award to recognize individuals whose efforts have significantly advanced the field (Wittmann-Liebold 1989). This year's Edman awardees were Fred McLafferty (Cornell University, Ithaca) and Darryl Pappin (Applied Biosystems). Dr. McLafferty was honored for his pioneering work in mass spectrometry including gaseous ion reactions (McLafferty rearrangement), instrumentation (GC/MS, LC/MS, MS/MS), fragmentation techniques, and computer data acquisition, reduction, and peptide identification. Darryl Pappin was honored for his contributions to the development of instruments and membrane-based supports for solid-phase Edman sequencing and the development of the MASCOT search program. In 2000, IAPSAP added a Distinguished Young Investigator Award. This year's Award went to Ben Davis (Oxford University) for the development of new methods for controlling the reactivity, selectivity, and specificity of oligosaccharide formation and the addition of oligosaccharides onto proteins. The Edman Awards and the Distinguished Young Investigator prizes were sponsored by Applied Biosystems, Inc. and Agilent Technologies. In his award address, McLafferty described the extension of "top-down" mass spectrometry to proteins from ~50 kDa to >200 kDa by using electrospray additives, heated vaporization, and separate noncovalent and covalent bond dissociation (Han et al. 2006). Ben Davis then discussed how precisely glycosylated enzymes and proteins could be constructed by using highly selective synthetic chemistry for use in drug delivery, in vivo sensing, and biocatalysis, and how new classes of glycoconjugate, glycodendriprotein, and glycoviruses could be used as powerful inhibitors of bacteria or for gene delivery (Doores et al. 2006). Although Darryl Pappin was not able to attend the meeting, his recent work on iTRAQ isobaric peptide labels for quantifying proteins using mass spectrometry was presented as a poster (Ross et al. 2004). That evening, participants enjoyed a gala conference dinner in the Couvent des Minimes, a seventeenth-century convent in the city center that has been renovated as a four-star hotel and restaurant.

The final day of scientific sessions began with a discussion by Anne Dell (Imperial College, London) of mass spectrometric strategies for the analysis of oligosaccharides. MALDI mapping and ES-MS/MS sequencing of permethylated *N*- and *O*-glycans were used to define the glycomes of individual glycoproteins, cells, tissues, and organs. Jasna Peter-Katalinic (University of Münster) explored the complexity of protein *O*-glycosylation by mass spectrometry after on-line capillary electrophoresis or automated chip-based sample admission. The use of DNA sequencers for high-throughput glycan analysis was presented by Nico Callewaert of Ghent University. Naoyuki Taniguchi (Osaka University) described novel approaches for functional glycomics. Using sugar remodeling techniques based on the manipulation of glycotransferase genes, he showed that branched structures of *N*-glycans are important for many physiological and pathological processes. Following an excellent lunch, participants were both educated and entertained by Baldomero Olivera's (University of Utah, Salt Lake City) description of post-translational modifications of peptide toxins from *Conus* snail venoms. These venoms, which are used by the snails to catch fish but also are toxic to humans, consist of 100–200 peptide toxins of 10–30 amino acids with multiple disulfide cross-links that are modified by hydroxylation, epimerization, and glycosylation of various amino acids, sulfation of tyrosine, bromination of tryptophan, γ-carboxylation of glutamate, and more. His spectacular movies certainly put the audience in a state of near nirvana without the need for neurotoxins. Lloyd Ruddock (University of Oulu, Finland) then explained how intradomain conformational flexibility contributes to catalyzing disulfide–dithiol exchange during oxidative protein folding by the 17 human protein disulfide isomerases, few of which, although discovered 40 years ago, have been well characterized. Predrag Radivojac (Indiana University, Bloomington) followed with the description of a method for predicting post-translational modification sites and its utility in mass spectrometry experiments. In the plenary closing lecture, Tony Pawson (Samuel Lunenfeld

Research Institute, Toronto) then summarized how a dedicated family of modular domains, through their ability to recognize post-translational modifications, mediate protein–protein interactions that orchestrate dynamic cellular organization. This thesis was illustrated with three systems: the binding properties of the 14-3-3 proteins; the PDZ-, BAR-, and SH3-domain-based interactions; and the SH2/SH3-containing Nck adaptor proteins that couple specific phosphotyrosine signals to the actin cytoskeleton.

This meeting truly lived up to the IAPSAP mission to promote the discovery and exchange of new methods for protein structure analysis and to facilitate their application in the pursuit of solutions to biological problems. The meeting closed with the announcement that MPSA 2008 would be held in Sapporo, Japan, in the fall of 2008. Further information will be posted on the IAPSAP website (www.iapsap.bnl.gov) as it becomes available.

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