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REVIEW

2014 ASMS Fall Workshop: Ion Mobility Mass Spectrometry

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The 2014 ASMS Fall Workshop on ion mobility spectrometry-mass spectrometry (IMS/MS) was organized by Matthew F. Bush (University of Washington) and Alexandre A. Shvartsburg (Wichita State University), and held on November 6–7 at the Crowne Plaza Hotel in Seattle, WA. The objective was to convey a broad and balanced perspective on recent advances, current capabilities, near-term prospects, and outstanding challenges in IMS/MS technology and its analytical applications. Twelve tutorial talks were given by nine experts in the field to 80 attendees from academic, government, and industrial sectors of nine countries. The participation of ~30 graduate students, in part enabled by generous ASMS travel stipends, was the largest in the history of these workshops. The talks were grouped into four thematic half-day sessions: (1) Fundamentals and Instrumentation, (2) Targeted Separations and Detection, (3) Proteomics and System Biology, and (4) Structural Mass Spectrometry. Each session featured three talks of 50 min each and culminated in a 30-min discussion led by the speakers.

The choice of this year's agenda reflected a dramatic expansion in the volume and diversity of IMS/MS research and growth and maturation of associated community over the past decade. This has largely been precipitated by the introduction of various IMS/MS instruments by major vendors, including the IM/time-of-flight products by Waters and Agilent, and differential or field asymmetric waveform IMS (FAIMS) devices (coupled to various MS platforms) by Thermo, AB Sciex, and Owlstone. These systems have allowed a wide range of users without the expertise and scope to design and build their own custom hardware to experiment with and adopt IMS/MS tools in their work, with applications in new areas, including structural biology, glycobiology, and petroleomics. Involvement of scientists from the pertinent companies and their interaction with other attendees added much value to the workshop.

The workshop was opened by Professor Herbert H. Hill (Washington State University) who was active in the field since its earliest days in the 1970s and has been a consistent advocate for the technique and tireless builder of the IMS community. To set the framework for the workshop, he reviewed the principles, operational parameters, and performance metrics of IMS separations. He then introduced and compared the instrumental branches of IMS, such as drift-tube (DT) IMS, traveling-wave (TW) IMS, differential mobility analyzers (DMA), and FAIMS, particularly in the context of coupling to MS platforms. He also laid out the foundational concepts, such as the relationship of ion mobility to the drift velocity and orientationally-averaged ion–molecule collision cross section, the effect of gas composition and field intensity, the resolving power and its dependence on the instrumental parameters, and sensitivity and its enhancement by means of ion trapping and gate multiplexing. The talk concluded with his discussion of IMS selectivity and avenues to modify it, such as the metal cationization and addition of vapors to the buffer gas.

Professor David E. Clemmer (Indiana University) focused his presentation on the use of conventional (linear) IMS to study the conformations and folding of peptides and proteins. He reviewed the evolution of IMS/MS instruments from single-stage IMS systems that captured static pictures of isolated biomolecular ions to platforms comprising ion traps and/or two or three IMS stages, which can follow the morphologies of ions in time and track their structural inter-conversions upon collisional activation of selected conformers. The critical question in biological IMS/MS (and MS in general) is the extent of retention of solution conformations during ionization. Strong dependence of the IMS spectra for exemplary proteins such as ubiquitin on the solution composition indicate a strong memory of the tertiary structure in solution persisting through the electrospray ionization (ESI) process and IMS separation. Professor Clemmer convincingly showed how the sophisticated capabilities of multi-stage IMS allow detailed mapping of the conformational transitions for macromolecules and investigations of the associated thermodynamics and kinetics.

Alexandre A. Shvartsburg (Wichita State University) broadly covered FAIMS—an IMS approach based on the difference of mobilities in strong and weak fields elicited in an asymmetric waveform. As this difference is generally less well

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correlated to the ion mass compared with absolute mobility, FAIMS is substantially more orthogonal to MS than linear IMS, and thus tends to deliver higher resolution at equal nominal resolving power. Dr. Shvartsburg summarized the fundamentals of FAIMS, highlighting the factors that control the resolution and its enhancement using gas buffers rich in helium and hydrogen. He discussed selected examples showing exceptional resolution feasible with those buffers, such as the separation of isomeric amino acids, lipid diacylglycerol regioisomers with fatty acids attached at different sites, and isotopomers with different locations of labeled atoms. He also described the newest technique of ultra-FAIMS employing multichannel microchips, where gaps of <100 μm permit extreme fields up to ~150 kV/cm without electrical breakdown and thus useful separations on the microsecond timescale. The talk was concluded with practical advice for the choice of FAIMS instrumentation guided by the relative priorities of resolution, sensitivity, and speed in specific applications.

The next session on Targeted Separations and Detection was launched by Professor Facundo M. Fernandez (Georgia Tech) who presented on the topic of ambient IMS for pharmaceutical forensics. Counterfeiting of high-volume drugs against common infectious diseases (antibiotics and antimalarials) and contraceptives is a persistent public health challenge in many lower-income nations with weak regulatory and law enforcement systems. The problem also affects developed countries through the growing importation of medicines, increasing travel between nations, and the emergence of global drug-resistant pathogens facilitated by the use of adulterated drugs containing the correct active ingredient in substandard doses. Despite security measures such as sophisticated labeling, testing the drug content is indispensable, and portable and relatively inexpensive drift time IMS (DTIMS) systems are a good solution for quick field analyses in this context. Professor Fernandez showed multiple examples where the combination of direct analysis in real time (DART) ion sources with DTIMS developed in his lab rapidly and effectively distinguished proper medicines from typical adulterated drugs that are sold across the world.

Professor Hill then returned to talk about the environmental, metabolomic, and security applications of IMS. Food supply safety has been in the news recently, with cases of tampering or accidental contamination resulting in multiple fatalities and raising public concern. The speaker demonstrated the impressive capabilities of IMS/MS, in conjunction with liquid chromatography (LC) or gas chromatography (GC), to detect substances such as melamine and illicit dyes in various foods. He then moved to metabolomic applications, showing the value of the IMS dimension in chemometric profiling of markers for diabetes, drug addiction, Parkinson's disease, and cancer. The original application of IMS, and still an active area of R&D, was in the security arena. The use of IMS to detect explosives, chemical warfare agents, and environmental signatures of nuclear reprocessing was also surveyed in this talk.

The day was closed by Dr. Shvartsburg speaking on the use of IMS to localize post-translational modifications (PTMs) and sequence inversions in proteomics. Many proteins and, thus, proteolytic peptides incorporate the same PTM at different sites, creating multiple localization variants with different biological activities that coexist within cells. Determination of this variation is crucial to understand and eventually control the molecular mechanisms underlying many diseases. Current LC/MS protocols are challenged by such isomers, which often co-elute during LC and/or yield non-unique fragments when using tandem mass spectrometry (MS/MS) methods. Dr. Shvartsburg showed examples of the separation of residue sequence isomers and localization variants using FAIMS, even for large peptides such as histone tails and the smallest PTMs such as methylation. He showed that the resolution of variants does not degrade with increasing peptide size, suggesting the potential applicability of this method to yet larger peptides and even intact proteins. Some variants can also be resolved by linear IMS, but less effectively because of correlation to the MS dimension. A near-perfect orthogonality between FAIMS and linear IMS for these species means that 2-D FAIMS/IMS separations would be even more effective.

On the second day of the workshop, the session on Proteomics and Structural Biology was started by Professor Clemmer's lecture on high-throughput separation of proteolytic digests. Such separations followed by MS are central to bottom-up proteomics and traditionally involve chromatography or electrophoresis. As molecules move faster in gases than in liquids by orders of magnitude, IMS separates digests in milliseconds (rather than minutes to hours for condensed-phase methods), leading to extraordinary throughput. The orthogonality of LC to IMS allows LC/IMS to provide excellent separations while reducing the charge competition during ionization. The talk covered the key benefits of IMS/MS: better sensitivity and, thus, sequence coverage attributable to reduction of chemical noise and augmentation of overall peak capacity, simultaneous monitoring of multiple fragmentation channels in parallel MS/MS processes, and the ability to finely differentiate peptides using consecutive IMS stages. The use of novel "combing" technique and "shift reagents" (additives to the buffer gas that preferentially bind certain residues and thus select the peptides comprising them) rounded up the talk.

Professor Helen Cooper (University of Birmingham, UK) then discussed the utilization of FAIMS in real-world LC/MS-

based proteomic workflows. This effort employed a commercial cylindrical-gap device that, compared with planar analogs, offers a moderate resolving power but better sensitivity performance often wanted in practice. As FAIMS is a filtering technique, global analyses require stepping through a sequence of compensation voltages (CVs). Operationally, stepping can be internal (within a peak eluting from LC) or external (with a designated LC run for each CV). The statistics of peptide and protein identifications revealed the advantage of external stepping, possibly because of a higher duty cycle without frequent CV switching. FAIMS separation was shown to be superior to strong-cation exchange fractionation prior to LC, but the methods are largely complementary and would be best deployed in parallel.

The session was closed by Professor John A. McLean (Vanderbilt University), who spoke about the integration of IMS into systems, synthetic, and chemical biology strategies. A hallmark of IMS is its strong mass/mobility correlation for homologous species. This limits the available separation space, but also establishes the generation of trend lines in IMS/MS palettes that typify biomolecular classes such as lipids (including some sub classes, e.g., sphingomyelin and phospholipids), peptides, oligonucleotides, and carbohydrates. This capability can be integrated into systems biology applications, such as online monitoring of organs on a chip (for instance, a liver bioreactor) that promise to drastically cut drug discovery costs. Molecular imaging, normally performed using MALDI/MS, can also incorporate IMS to disentangle isobaric overlaps and focus on the desired classes (e.g., lipids that comprise markers of cancer cells).

The last session on Structural Mass Spectrometry was opened by Professor Matthew F. Bush (University of Washington), who discussed the importance of collision cross sections in biomolecular structural analysis. Information about ion structures is extracted from IMS data by matching the measured mobilities to those computed for candidate geometries, hence the utility of IMS as a structural tool hinges on the accuracy of its underlying theory. Professor Bush reviewed the approaches to ion mobility calculations, ranging from simple projection approximations to exact hard-sphere scattering models to molecular dynamics (MD) in realistic ion–molecule potentials with attractive wells. He then addressed the methods for evaluating mobilities in nitrogen, which has become topical with the proliferation of work on commercial instruments requiring N_2 buffers. Accelerated methods approximating rigorous but costly molecular dynamics (MD) simulations and a new paradigm that represents ions as electron density isosurfaces (not atomic coordinate sets) were also mentioned. Examples of these tools to elucidate the assembly of protein complexes were given to close the presentation.

Professor Michael T. Bowers (University of California, Santa Barbara) then gave an overview of his work exploring the pathways of peptide aggregation relevant to neurodegenerative diseases, including Alzheimer's and Parkinson's. Those conditions are associated with amyloid plaques made by peptide polymerization; however, the mechanism of this, and consequent tissue damage, has remained obscure. Most important here is the initial oligomerization step that could not previously be inspected. Professor Bowers showed the application of IMS/MS measurements to characterize the oligomer structures as a function of size, and pinpointed the transition from isotropic aggregates to new arrangements that mobility calculations suggest to be "cylindrins" and "corkscrews." Instead of proceeding to fibrils composed of pleated β-sheets, those structures may be off-pathway dead ends that are the actual neurotoxic agents of "protein misfolding disorders." Clarification of this matter is obviously crucial to begin developing therapies against these currently untreatable diseases.

The last talk of the workshop was given by Professor Catherine E. Costello (Boston University) on the determination of glycan and glycoconjugate structures. Glycosylation is the least understood PTM that, unlike others, produces not just linkage isomers (by latching onto different residues) but also branching isomers within the PTM. These species are not fully resolved by chromatography, and the limited efficiency and multiple pathways of MS/MS fragmentation impede their elucidation. Incorporation of DTIMS helps by distinguishing the oligosaccharide and glycoconjugate isomers that have differing mobilities and form distinct trend lines in IMS/MS space. The preferred MS/MS approach to elucidate PTMs is electron transfer dissociation and related radical dissociation methods, such as electron activated dissociation (EED), but these can require long acquisition times and thus present a challenge to implement after DTIMS without major resolution loss. An IMS/EED combination was instead enabled using selected-accumulation IMS (in an ion funnel) in front of an FTICR mass spectrometer. This capability was shown to allow isomers (e.g., permethylated sugars) to be distinguished where DTIMS/MS failed because of uninformative glycosidic cleavages upon ergodic collision-induced dissociation.

As always, ASMS surveyed the participants after the workshop about various scientific and organizational aspects of the meeting, and to solicit suggestions for improvement and for future workshop topics. Attendees uniformly liked the conference, with the highest score given for the speakers' knowledge of the topic. One attendee reported, "I've never attended an ASMS workshop, and I can tell you that now I will certainly

consider it again, and recommend it to others! This far surpassed my expectations. I really didn't know anything at all before I came, and now I feel that I have the tools to start working on my project and thinking creatively about how to approach it. Thank you so much!!!". Readers are reminded that anyone can submit a Fall Workshop topic suggestion to the ASMS Education committee by using the on-line form (http://www.asms.org/conferences/suggest-a-topic).