ASMS Conference Workshop:
Ion Mobility: How to Interpret the Data?
Ion Mobility MS Interest Group
Presiding: Erin Baker, Stephen Valentine, and Valerie Gabelica

At this year's ASMS conference, the presentation of ion mobility content commenced with the ion mobility-mass spectrometry short course titled "Ion Mobility Mass Spectrometry: An Introduction to Instrumentation, Applications, and Data Analysis". Originally a 2-day course, the new course format followed that established in the preceding year where it was shortened to 1 day (Sunday, 9 am to 4 pm). The course was taught by Prof. Brian Clowers (Washington State University), Prof. John McLean (Vanderbilt University) and Dr. Erin Baker (PNNL) and had ~29 participants. With the growing popularity of IMS-MS instrumentation and methods, the short course presents an initial/expanded exposure to individuals wishing to utilize such technology. Similar to last year, the short course was divided into 6 sections: (1) Introduction of IMS, (2) IMS Theory, (3) Drift Tube IMS, (4) Traveling Wave IMS, (5) FAIMS, and (6) Future Directions & Discussion. Due to the sizeable attendance and diverse interactions, the organizers intend to offer this short course again next year.

The interest group was very pleased to see the continued inclusion of three ion mobility focused oral sessions for the 2016 conference: "Ion Mobility: Small Molecules, Pharmaceuticals, and DMPK" (chaired by Cris Lapthorn, attended by 450); "Ion Mobility, FAIMS, & DMS: New Developments and Applications" (chaired by George Stafford, attended by 550); and, "Ion Mobility: Structure" (chaired by Kevin Pagel, attended by 350).

Beyond the three dedicated sessions, there were at least 18 additional IMS related presentations during the conference featuring ion mobility techniques, data, or instrumentation. Of particular note is that these presentations were included in a diverse range of fundamental and applied sessions, including "Instrumentation: FTMS"; "Metabolomics: Untargeted Profiling"; "Membrane Protein MS"; "Energy, Petroleum, and Biofuels: Instrumentation and Method Development"; Fundamentals: Metal ion Cationization, Metal-Ligand Interactions and Catalysis"; "HRMS for Quantitation in Drug Discovery, Development and Beyond"; "Instrumentation: non-FT based Analyzers"; "Lipids and Profiling"; "Native MS in Structural Biology"; "Metabolomics: New Technologies and Applications"; "Protein-Ligand Interactions"'; "Macromolecular Complexes"; "Carbohydrates"; "Fundamentals: Molecular Modeling and Quantum Mechanical Calculations in IM and MS"; and, "Lipidomics: New MS Technologies and Applications". Particularly encouraging was the "Fundamentals: Molecular Modeling and Quantum Mechanical Calculations in IM and MS" session which had multiple talks describing IM-MS data. This again shows the increasing popularity of IM techniques.

The IMS workshop, entitled "Ion Mobility: How to Interpret the Data?", was held from 5:45 to 7:00 pm on Tuesday, June 7th. The workshop was well attended with more than 300 participants. The focus this year was on data interpretation. To place all the audience including newcomers on the same page, a tutorial lecture of 15 min by Kevin Pagel (Freie Universität Berlin) summarized the general principles of ion mobility measurement, how to extract collision cross sections directly from drift tube (DT) measurements or after calibration from travelling wave (TW) measurements, and how to interpret collision cross sections (CCS). The comparison of the CCS values of the protomers of benzocaine measured in helium and nitrogen illustrates that the collision cross section is a property of the ion-gas colliding pair: the two protomers have almost identical collision cross sections in helium, but the collision cross sections differ dramatically in nitrogen because the polarizable gas interacts differently with each protomer. This is a first example of tricky data

interpretation: (1) one molecule can be electrosprayed as two coexisting protomers, and (2) observing one or two peaks in the arrival time distribution is gas-dependent.

The rest of the workshop was organized with 9 flash presentations (three series of three consecutive flash talks, each followed by a general discussion with the audience).

The first series presented cases of tricky ion mobility peak interpretation. Yoko Ohashi (Riken, Japan) presented a case somewhat analogous to the protomers mentioned above, but in the framework of investigating fragmentation processes: the sodium cationizing agent could end up at two different locations on a given fragment, resulting in two peaks in IMS, yet corresponding to only one parent molecule. Johanna Hofmann (Fritz Haber Institute Berlin) illustrated in the framework of quantification studies how peak broadness depends on ion intensity, which could result in possible misinterpretation of a broad peak as multiple conformers. Interaction with the audience pointed to the effect of space charge on ion mobility peak width and limitations in IMS dynamic range. Finally, Eleanor Dickinson (Manchester Institute of Biotechnology) presented the challenges in representing complex collision cross section distributions of the different charge states of intrinsically disordered proteins. Echoing with the tutorial, she highlights that the collision gas in which the primary CCS measurements were carried out and the type of instrument on which the reported experiments are carried out should be explicitly mentioned when reporting collision cross sections. The proposed notation is: $^{TW/DT}CCS_{He/N2}$, for example $^{DT}CCS_{N2}$ for drift tube measurements carried out in nitrogen buffer gas, or $^{TW}CCS_{He}$ for travelling wave measurements re-calibrated using collision cross sections that had been measured for the calibrant in helium (despite that the TW measurements may be carried out in a mixture of helium and nitrogen).

The second series of flash talks illustrated data recorded on different instrumental platforms. Julia Kaszycki (Wichita State University) showed high-resolution FAIMS separation of isotopologues. This illustrates that even for a single molecule isomer, the non-monoisotopic mass peaks can lead to several ion mobility peaks because several isotopologues contribute to the population. The capability of FAIMS to separate these isotopologues is an opportunity for the analytical characterization of isotopically labeled compounds, but is also challenging because multiple peaks do not necessarily mean that molecules of different atomic connectivities were present in the sample. Coupling high-resolution MS to high-resolution IMS or FAIMS will be instrumental to help resolving and assigning isotopologues. Hélène Lavanant (Normandie Université) illustrated another case resulting in different IM peaks for a single mass and charge: on phosphoric acid anionic clusters, she found multiple peaks that were actually due to higher-order clusters drifting through the mobility cell but fragmenting after the cell: the detected ions therefore have the mass and charge of the fragment, but drifted with the collision cross section of the precursor ion. Studying the effect of tuning parameters on the IM peak distribution was crucial to reveal such effects. Abby Gelb (University of Nebraska) highlighted the persisting challenge in collision cross section calibration of travelling wave instruments. In the framework of her research on glycopeptides, she tested whether calibration accuracy was dependent on the class of molecule (carbohydrates vs. peptides) or on the charge state of the calibrant. In her case, biomolecule class mismatch resulted in smaller error (1-2%) than charge state mismatch (4-5% error on average). Charge state matching is therefore imperative, especially for the low charges (i.e., 1+ vs. 2+). This topic stirred discussion on class matching, as some other groups had found it very important for accuracy in their own datasets. A currently open question is the accuracy of the primary values used for the calibrants, and only interlaboratory studies will make it possible to establish consensus drift tube values for calibrant sets and then settle the problem of travelling wave calibration.

Finally, the third series of talks illustrated initiatives in automated data analysis for ion mobility data. We heard examples in three very different areas of application. Yuwei Tian (University of Michigan) presented automated data interpretation of collision-induced unfolding of proteins. The arrival time distributions typically contain multiple peaks, and the goal is to track how the positions, relative intensities, and collision energy dependence of these peaks vary among biomolecule variants, or in the presence of small molecule binders. CIU interpretation is therefore a relative measurement. Paolo Benigni (Florida International University) presented unsupervised data analysis of IMS-FTICRMS of small hydrocarbons, which included collision cross section matching criteria for structural assignment. The interpretation here relies on an absolute measurement and matching with theoretical values, and this is already challenging for hydrocarbons as small as $C_{10}H_8$. Finally, Xing Zhang (University of Colorado) showed recent developments for the automatic and global analysis of metabolomic and lipidomic samples, by detection and matching of spectral features including the IM space. Discussion ensued on the interpretation of the features detected automatically, in light of all previous examples showing that one molecule could give rise to several IM peaks and hence several features. The current peak assignment approach in metabolomics relies on reference IM-MS spectra recorded on reference compounds

In summary, many discussions in this workshop revolved around the peak interpretation, with several examples showing that it is often necessary to think beyond the “one molecule = one peak” framework. Future development in automated de novo structural assignments by IM-MS will need to feed on fundamental studies on the principles underlying ion mobility separation and understanding all factors affecting cross-platform comparisons (including gas nature, field strength, and calibration issues). These are certainly topics for further lively discussions at future ion mobility workshops!

We hope this gives insight into the IM-MS presence at ASMS. Respectfully yours,

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