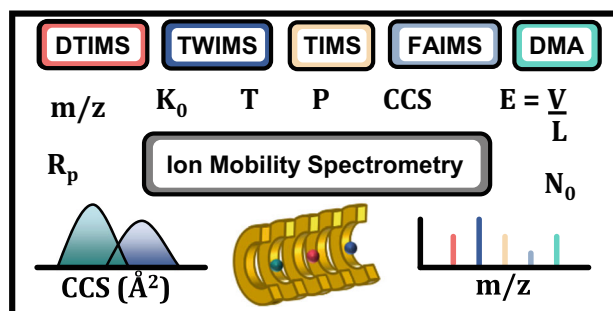


Ion Mobility Spectrometry: Fundamental Concepts, Instrumentation, Applications, and the Road Ahead

James N. Dodds, Erin S. Baker 

Department of Chemistry, North Carolina State University, Raleigh, NC 27695, USA



Abstract. Ion mobility spectrometry (IMS) is a rapid separation technique that has experienced exponential growth as a field of study. Interfacing IMS with mass spectrometry (IMS-MS) provides additional analytical power as complementary separations from each technique enable multidimensional characterization of detected analytes. IMS separations occur on a millisecond time-scale, and therefore can be readily nested into traditional GC and LC/MS workflows. However,

the continual development of novel IMS methods has generated some level of confusion regarding the advantages and disadvantages of each. In this critical insight, we aim to clarify some common misconceptions for new users in the community pertaining to the fundamental concepts of the various IMS instrumental platforms (i.e., DTIMS, TWIMS, TIMS, FAIMS, and DMA), while addressing the strengths and shortcomings associated with each. Common IMS-MS applications are also discussed in this review, such as separating isomeric species, performing signal filtering for MS, and incorporating collision cross-section (CCS) values into both targeted and untargeted omics-based workflows as additional ion descriptors for chemical annotation. Although many challenges must be addressed by the IMS community before mobility information is collected in a routine fashion, the future is bright with possibilities.

Keywords: Ion mobility spectrometry, IMS, Untargeted metabolomics, Mass spectrometry

Abbreviations: IMS, Ion mobility spectrometry; MS, Mass spectrometry; DTIMS, Drift tube ion mobility spectrometry; TWIMS, Traveling wave ion mobility spectrometry; TIMS, Trapped ion mobility spectrometry; FAIMS, Field asymmetric waveform ion mobility spectrometry; CCS, Collision cross-section

Received: 12 March 2019/Revised: 8 July 2019/Accepted: 15 July 2019/Published Online: 6 September 2019

Introduction

Ion mobility spectrometry (IMS) is the study of how ions move in gases under the influence of an electric field, or in other words, the electrophoretic mobility of ions in buffer gases. Interestingly, while some may view IMS as a newer technique, its historical origins date back to 1896 in Thomson and Rutherford's seminal work investigating the relationship between electrical conductivity and gaseous media [1]. Due to its fast screening capabilities and high sensitivity, IMS experienced rapid growth during the 1960s and into the subsequent decades as an atmospheric pressure device which could rapidly screen chemical vapors for trace quantities of hazardous mate-

rial [2, 3]. Over the past century, advances in instrumental design further pushed the popularity of IMS forward by enhancing its sensitivity and selectivity. The resulting portable IMS devices continue to be utilized for routine detection of explosives and chemical warfare agents in military operations, sporting events, and airports [2, 4].

While standalone IMS devices are very powerful, interfacing IMS with mass spectrometry (IMS-MS) has provided even more resolution of chemical space as the complementary separations in both the mobility and mass dimensions enable exceptional levels of selectivity and sensitivity [5–7]. Early IMS-MS instrumentation was generally associated with home-built instruments housed in academic settings [8, 9]. Routine adoption of IMS-MS as an analytical tool began in 2006 with the commercial introduction of the Waters Synapt HDMS, the first widely

Correspondence to: Erin Baker; e-mail: ebaker@ncsu.edu

marketed IMS-MS platform [10–12]. After generating considerable interest in both academic and industrial perspectives with the Synapt HDMS, many instrument developers quickly followed suit and developed their own IMS-MS platforms based on unique mobility separations, thereby adding IMS selectivity to MS for challenging systems in complex mixtures. While the flourishing growth of the IMS-MS field is exciting, a significant amount of confusion has developed among scientists new to the field in terms of understanding the subtle differences between different methods. Since each IMS technique possesses pros and cons in terms of specific applications, knowledge of the underlying fundamentals for each is helpful for designing experiments. In this critical insight, we aim to succinctly describe the fundamentals of each IMS technique and their respective advantages and disadvantages. For further readings on each technique, we direct the readers to more extensive review articles which describe each method in greater detail [5, 13–16].

Core Principles of IMS Devices

The core principle of IMS instrumentation is to separate ions in an inert gas (commonly termed “buffer gas”) under the influence of an electric field [17]. The applied electric field (E) forces ions to migrate through the buffer gas with a velocity (v_d) correlated to the specific analyte’s mobility (K), measured by Eq. 1.

$$K = \frac{v_d}{E} \quad (1)$$

In a given IMS experiment, the ions are separated by their differences in mobility through either space or time based on the particular IMS method used [13]. Smaller, more mobile ions travel faster (higher v_d) in a specific electric field strength than larger, less mobile ions (smaller K). Mobility for each ion, K , is measured as a function of the experimental parameters (i.e., temperature and pressure), which are often normalized to standard conditions in order to calculate the reduced mobility, K_0 . For simplicity, we use K to denote the mobility, which is interchangeable with K_0 as shown below:

$$K_0 = K \frac{p}{p_0} \frac{T_0}{T} \quad (2)$$

While the primary measurement in IMS analyses is the mobility [16], for many analytical applications, it has become routine to convert the measured mobility into the calculated collision cross-section value (CCS or Ω). The Mason-Schamp equation (Eq. 3) is often used to calculate the analyte’s CCS from the measured mobility, where Ω is the momentum transfer collision integral which describes the collision between the ion and the buffer gas and gives

direct information about the conformation of the ion traveling through the drift region [18, 19].

$$\Omega = \frac{3}{16} \left(\frac{2\pi}{\mu k_b T} \right)^{\frac{1}{2}} z e \quad (3)$$

The parameters of this equation are as follows: e , charge of an electron; z , ion charge; N_0 , buffer gas density; μ , reduced mass of the collision partners; k_b , Boltzmann’s constant; and T , the drift region temperature. The specifics of gas composition, operational pressure, temperature in the mobility region, analyte migration path, and applied field strength vary for each respective IMS platform (and depend heavily on experimental objectives). These parameters are represented graphically in Figure 1 [7]. From a simplified perspective, the CCS is a normalized measure of gas phase size, typically denoted in units of square Angströms (\AA^2) [19, 20]. While the Mason-Schamp equation is not universally accepted, currently it is the central equation used to calculate CCS in the community, and is utilized here accordingly. Although there are several IMS methods to conduct mobility separations, each unique instrument platform has distinct advantages and disadvantages. These differences are of importance when considering specific applications for each method (e.g., isomer separation, signal filtering and deconvolution, or CCS fingerprinting). In the following section, we describe the specific attributes for each IMS method in order to clarify common misconceptions and highlight the strengths and shortcomings of each technique.

Differences Between IMS Instrumentation

DTIMS

Drift tube ion mobility spectrometry (DTIMS) is often described as the classic IMS model and provides simplicity, relative ease of operation, and the ability to measure mobility (and calculate CCS) as a primary method [19, 21]. These attributes have enabled the DTIMS platform to be readily adopted by many commercial vendors for IMS-MS research (e.g., Agilent, Tofwerk, and Excellims) [22, 23]. The key component of DTIMS is the uniform electric field that propagates through the drift region. The drift region is a defined separation space in which the buffer gas has no directional flow, and the analytes traverse this pressurized region under the influence of a uniformly applied weak electric field (typically tens of V/cm). This uniform field enables DTIMS to measure K as a primary method, and hence calculate the corresponding CCS values for analytes from the Mason-Schamp equation. The ability to calculate CCS from first principles is perhaps the most significant advantage of DTIMS. Other IMS platforms (i.e., TWIMS) require calibrant ions with well-characterized CCS values previously obtained on DTIMS

Variations of Ion Mobility Platforms

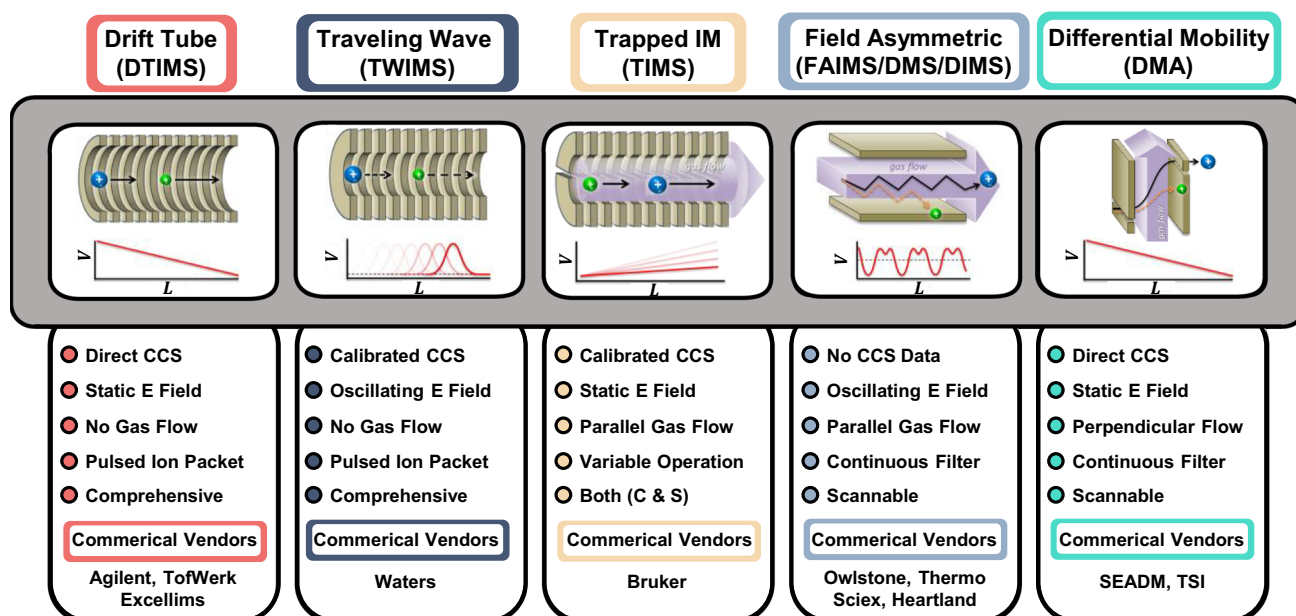


Figure 1. Variations of IMS technology with representative descriptions of applied field and gas dynamics. Bullet points describe the relative parameters of each IMS platform with key attributes reflecting the ability to measure CCS information, ion packet distribution, instrument footprint, and modularity among other relevant descriptors. Also included are the main instrument manufacturers which currently market each IMS method. Instrument diagrams have been adapted/reprinted from Ref. 7 “Lipid analysis and lipidomics by structurally selective ion mobility-mass spectrometry” 1811, Kliman, M. et. al. 935–945. 2011, with permission from Elsevier

instruments to create a calibration curve for calculating CCS of unknown analytes [24]. It also should be noted that many DTIMS experiments are also performed in a CCS-calibrated mode (often referred to as the single-field method) in order to maintain an analytical timescale which can be incorporated into the chromatographic timescale. The single-field method has been shown to provide highly reproducible CCS values, and further detail on it and other modes of acquisition for DTIMS are provided in the recent work by Stow et al. [25]

Additionally, many commercial DTIMS instruments do not require RF confinement to contain ion diffusion in the IMS experiment, though recent findings from Allen and Bush suggest ion heating effects noted from RF confinement are minor [26]. An additional advantage of DTIMS includes comprehensive ion collection, wherein all analyte mobilities are collected in a single pulsed experiment (as opposed to scanning instruments, e.g., FAIMS and DMA). However, because DTIMS analyzes ion pulses, its duty cycle is decreased in comparison to continuous IMS methods. For example, many experiments only trap the ions for 4 ms and then allow them to be separated in the drift region for 60 ms. This results in a duty cycle of 6.7 % (4 ms/60 ms), and all other ion signal is lost during the 54 ms waiting for the previous ion packet to hit the detector. In an effort to address this loss, many instrument vendors are utilizing multiplexing strategies in the pulsing sequence, wherein multiple packets are pulsed into the drift region at defined times as the first packet travels.

Knowledge of the pulsing times allows the subsequent signals to be deconvoluted to their correct arrival times using schemes such as the Hadamard Transformation [27, 28]. Recent studies have even shown that this approach can provide up to 50 % duty cycle, which is a great increase over the standard 6.7 % [29, 30].

An additional challenge of DTIMS systems is how to increase the resolving power of these devices. This is accomplished by increasing the voltage drop across the drift cell and decreasing temperature [31–33]. For precise DTIMS measurement, it is essential to keep the ions in the low field limit, therefore to increase the voltage drop either the length of the drift cell or pressure is increased [34, 35]. However, without careful focusing, an increase in both of these parameters increases ion diffusion and can cause extensive peak broadening and ion losses. There are both commercial low- and high-pressure DTIMS platforms, typically operated at ca. 4 Torr and 760 Torr [19, 23]. Low pressure systems are often used because ion focusing and obtaining higher sensitivity is easier at lower pressures, however, fewer collisions with the buffer gas decrease separation capacity. High-pressure systems often suffer from ion losses at the higher pressures due to difficulties in ion focusing, so they may not possess the sensitivity of lower pressure systems. However, continual advancements in DTIMS design and further increasing the pressure of the mobility region (e.g., atmospheric pressure and greater) and the resolving power for some platforms to between 100 and 250 ($t/\Delta t$) or even greater [23, 36].

TWIMS

Traveling wave ion mobility spectrometry (TWIMS) led to the widespread popularity of IMS-MS when it was first commercialized in 2006 with the Synapt HDMS and its successors (Synapt G2 in 2011 and Synapt G2-Si in 2013) by Waters Corporation. [10, 37–39] The drift region of TWIMS is very similar schematically to the DTIMS platform, where a stacked set of ring electrodes provides an applied voltage, driving ion motion through the drift region at reduced pressure (ca. 2–4 Torr). However, while DTIMS applies a uniform electric field to induce analyte migration, TWIMS utilizes an oscillating electric field to produce a set of voltage waves that push the ions through the drift gas towards the mass analyzer. A description of this oscillating electric field has been explained in detail in several publications [37, 40]. Also, TWIMS utilizes RF confinement to focus the ion packet while it migrates through the drift region, which provides increased analyte signal resulting from decreased ion diffusion [41]. Again, the ion heating resulting from RF confinement is thought to be a minor contributor, as work from Morsa and coworkers has noted that other ion heating in TWIMS resulting from variations in wave height, wave speed, and changes in pressure may be of more importance [42, 43]. A caveat of TWIMS devices is that each instrument must be calibrated with ions of known mobility prior to calculating CCS values of unknowns (analogous to the single-field method in DTIMS). Historically, polyalanine has been utilized for CCS calibration in TWIMS, although the specific compounds best suited for this purpose are still heavily debated in the field. For example, instrument calibration to obtain CCS values using peptide ions in lipid analyses have been shown to introduce significant error [44–46].

Many advantages and disadvantages of TWIMS are also shared with DTIMS, such as the pulsed ion packet delivery by ion gating and comprehensive analyte detection. Two key advantages of TWIMS are (1) low voltage requirements due to constant wave heights and (2) the ability to manipulate ion motion into long path length separations without significant ion loss. These long path length structures effectively enhance mobility separation by increasing the number of interactions between the analyte and drift gas [47]. The low voltage requirement for TWIMS has been essential in the design of two extremely long path length platforms (e.g., tens to hundreds of meters in length): the circular IMS device recently released by Waters Corporation [48] and the Structures for Lossless Ion Manipulations (SLIM) platform currently undergoing development in the Smith group at Pacific Northwest National Laboratory (PNNL, Richland, WA) [49, 50]. In a recent publication, the SLIM device used a path length of ca. 1 km to perform ion separations [51]. In this case, if a DTIMS platform was utilized that required a uniform voltage drop of approximately 12 V/cm, a power supply of > 120,000 V would be needed to supply the voltage at the beginning of the drift region. Due to safety concerns, this is impractical. However, TWIMS enables these long path length analyses with power supplies providing wave

height around 30 V. Furthermore, these long path length devices are showing enormous possibilities as demonstrated by their extremely high IMS resolving power (i.e., > 400 R_p) and separation of ions that have previously not been possible with the lower resolving power of DTIMS [48, 51].

TIMS

Trapped ion mobility spectrometry (TIMS) is one of the newest IMS methods and was recently commercialized by Bruker Daltonics. The TIMS analyzer is comprised of a set of electrodes that form three regions: the entrance funnel, TIMS mobility region (ion mobility analyzer), and exit funnel. Both the entrance and exit control ion deflection and focusing, while the TIMS mobility region is utilized to accumulate, trap, and elute ions of interest as a result of the interplay between a parallel gas flow and an opposing electric field [52, 53]. In the previously described DTIMS and TWIMS methods, the gas flow is essentially stationary, but TIMS differs as it has a unidirectional buffer gas flow towards the MS detector. In effect, TIMS operates similar to DTIMS in reverse, wherein ion motion is directed towards the MS by gas flow in opposition to the applied electric field strength (ca. 70 V/cm) [53, 54]. The field strength in a TIMS experiment is slowly decreased to eject ions of specific mobilities from the mobility region for structural analysis. One main difference between TIMS and the previously described DTIMS and TWIMS techniques is its scanning operation. In DTIMS and TWIMS, all ions can be observed utilizing the same experimental conditions. TIMS requires changes to the experimental parameters to see all ions. Thus, it is only able to analyze each molecule as ejected. This property can be beneficial though causing TIMS to be highly selective in terms of separation efficiency (resolving power, ca. 200–400 $K/\Delta K$) [54, 55]. Selectivity is coupled with the instrument duty cycle and can be tuned based on experimental needs. For example, the rate at which the electric field is scanned determines the selectivity of an experiment. Slower scans are more selective and are more capable of separating analytes with similar mobilities than faster scans, yet faster scanning may be necessary when TIMS is coupled with LC [56]. Thus, the duty cycle of TIMS dictates the level of resolving power possible for each experiment and enables tunable levels of selectivity which can be modulated based on the application (i.e., selective vs. untargeted modes). As with DTIMS and TWIMS, TIMS utilizes a pulse of ions for separation, and also experiences some losses in duty cycle due to this pulsing. In addition, while TIMS utilizes RF confinement in the mobility region, there is no axial component to this applied frequency and the application of RF is not thought to affect ion structure or mobility [54]. Although recent literature suggests that TIMS devices can also measure K as a primary method (and hence CCS values), most publications calibrate TIMS with analytes of known mobility prior to analysis (in a similar fashion to TWIMS) [54, 57]. Another distinct advantage of TIMS is its compactness, ca. 5–10 cm. This small size is extremely advantageous in creating smaller instrument footprints or easily modifying standalone MS

platforms to gain TIMS capabilities. As a final note, recently, the ability of chaining multiple TIMS analyzers (TIMS-TIMS) has been described. These linkages enable versatile experimental design where different components can be placed between the multiple TIMS separations for more specific characterization of each ion detected (e.g., IMS-CID-IMS-MS) [58].

FAIMS/DMS/DIMS

Field asymmetric waveform ion mobility spectrometry (FAIMS), differential mobility spectrometry (DMS), and differential ion mobility spectrometry (DIMS) are atmospheric pressure IMS techniques typically interfaced directly behind the ion source and prior to entering the vacuum region of the mass spectrometer. These devices are extremely small, usually just a few square centimeters in surface area, and can be fabricated in different geometries such as cylindrical, planar, and chips. They are easily implemented on existing MS platforms and have a small aperture to maintain the vacuum of the MS system they are coupled to. FAIMS, DMS, and DIMS all operate under the same mechanism from an electronics perspective and only differ in the geometry of their respective electrodes; hence, these techniques are grouped in this manuscript [59, 60]. FAIMS/DMS/DIMS operate as mobility filters, wherein a periodic waveform is applied to separate ions under a parallel gas flow [61]. The voltage application alternates between high and low electric field strengths (ca. alternating polarity, with field strength often several kV/cm) [62], a process that filters for a particular analyte's change in mobility with field strength as opposed to absolute mobility. In effect, due to the application of this asymmetric waveform, FAIMS/DMS/DIMS devices cannot provide CCS values. In addition, the ion structure itself may change during the oscillation from low to high field strengths. Varying mobility behavior in FAIMS may result from dipole alignment and the clustering and declustering of the ions, and is described in greater detail in the literature [61, 63, 64]. However, due to the differences in separation characteristics, FAIMS/DMS/DIMS devices are able to provide a high degree of selectivity that may not be possible in other low field-only methods (i.e., <100 V/cm). Additionally, FAIMS/DMS/DIMS are filtering devices which can be scanned, wherein only analytes with a specific response to the changing electric field and those that match the applied compensation voltage (CV) are able to traverse the drift region and exit through the aperture. In this manner, FAIMS/DMS/DIMS operate in an analogous fashion to quadrupole mass analyzers, utilizing CV scans to transmit ions with various responses to change in mobility over a set period. Furthermore, these devices do not pulse ions into the mobility region like DTIMS, TWIMS, and TIMS; yet, they acquire continuous mobility data without loss in duty cycle for molecules capable of exiting the device under the specific parameters applied. This continuous collection of targeted spectra enables FAIMS/

DMS/DIMS devices to increase the signal-to-noise ratio for the ion(s) of interest by greatly removing unwanted chemical noise in MS spectra [65]. FAIMS/DMS/DIMS separations can also be enhanced by changing the gas composition in the mobility region [66, 67]. Currently, FAIMS/DMS/DIMS devices are marketed from Thermo Fisher Scientific, Owlstone Medical, Sciex, and Heartland MS.

DMA

Differential mobility analyzers (DMA) operate in a similar fashion to DTIMS in that both systems utilize a constant electric field and are able to measure K as a primary method. Three of the main differences are that DMA devices operate at ambient pressure, have a well-characterized unidirectional gas flow, and are scanned for the detection of the molecule of choice. DMA is capable of performing measurements not possible with DTIMS as it is typically utilized to detect very large analytes, such as aerosol particles [68], antibodies [69], viruses, and other macromolecules (ca. tens to hundreds of nm²) [70], and is not heavily applied in small molecule screening applications (e.g., lipids, metabolites). Recently, Fernández-García and coworkers have measured the mobilities of liquid nanodrops in air with DMA [71]. In a similar fashion, Ouyang and coworkers utilized DMA to calculate CCS of large metal iodide clusters for comparison with computational modeling approaches [72]. DMA therefore provides an important mobility device for measuring extremely large molecules not possible with other IMS-based methods. Because DMA is typically used to analyze macromolecules and not peptides, lipids, or metabolites, most CCS values for calibration of other IMS techniques are first collected on DTIMS, as opposed to DMA. DMA devices are currently marketed by SEADM and TSI.

Applications of IMS-MS

IMS methods are typically conducted in three principle application settings: isomer separations, signal filtering, and annotation of untargeted features via CCS database matching. In this section, we describe each of these applications of IMS and highlight specific examples in recent literature.

Isomer Separations

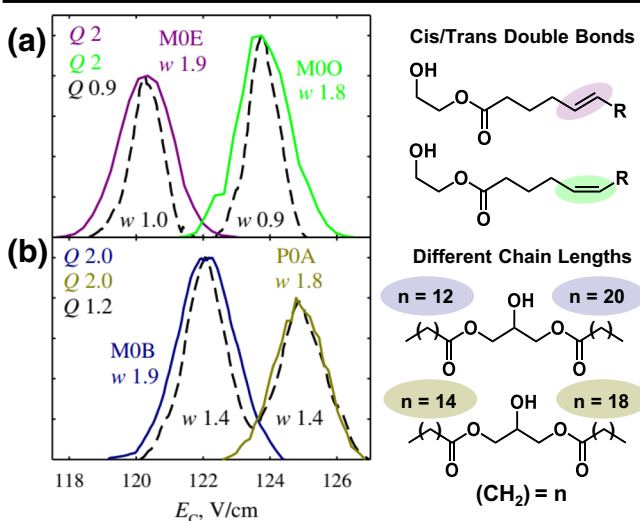
First and foremost, while mass spectrometers are very selective in terms of separating and identifying analytes with different chemical formulas, distinction of isomeric species in complex samples requires fragmentation methods, or chromatographic techniques in addition to the MS measurements. For structurally similar isomers such as lipids [73], carbohydrates [74], or amino acids [75], fragmentation spectra are often very similar and may fail to provide diagnostic ions of each species; hence, alternative methods of separation for these analytes prior to MS analysis is

required. IMS provides complementary separation of isomers by utilizing structural differences in mobility to resolve these analytes. Isomeric compounds have been separated by various IMS methods across a wide scope of biological classes including nucleic acids [76], carbohydrates [77, 78], lipids [79], and peptides [10, 37]. For example, Figure 2a, b describe a recent separation of diglyceride (DG) isomers from Bowman and coworkers who utilized FAIMS to separate isomeric lipids which differed in their double bond orientation (cis/trans isomers, Figure 2a) and chain length (Figure 2b) [80]. Another recent study from Hofmann and coworkers demonstrated the ability of TWIMS to separate linkage and stereoisomers in simple carbohydrates resulting in baseline resolution [77]. Prototype IMS devices such as the SLIM platform at Pacific Northwest National Lab (PNNL) have demonstrated that even enantiomers can be separated by IMS when complexed with other selective ions such as various cyclodextrins [81]. Enantiomeric mixtures have also been separated in IMS using copper-complexation strategies and diastereomeric adduction [82, 83]. Protein conformers have been recently studied by both TIMS and DTIMS [55, 84]. Thus, while isomeric separations still remain very challenging in the analytical community, recent advances in chromatography and IMS are beginning to provide the necessary selectivity to separate and characterize these compounds, which can then in turn aid in elucidating the role of isomers in biological systems.

Signal Filtering by IMS-MS

Another application of IMS focuses on reducing the complexity of mass spectra, particularly in situations where background ions are in high relative abundance compared to the ion of interest. Though all IMS methods which can significantly increase the signal-to-noise ratio for specific ions and decrease background noise, such as DMS, FAIMS, DIMS, or DMA, are well suited for this purpose because they can operate as intrinsic mobility filters. For example, Levin and coworkers utilized DMS to simplify complex spectra of oligosaccharides (Figure 2c) [85]. Using the mobility filtering characteristics of DMS, the MS signal of the target pentasaccharide dramatically increased in comparison to the abundant background ions noted in full scan without IMS (Figure 2d). Mobility filtering is also particularly advantageous in standalone IMS systems deployed in the field for vapor analysis [86]. Although not described in detail in this review, overtone mobility spectrometry (OMS) also acts as a mobility filter, wherein only analytes with an intrinsic mobility matching the resonate frequency applied in OMS are transmitted towards the mass analyzer [87]. The fundamentals of OMS and potential applications have been thoroughly explored in previous publications [87–89]. IMS methods have also been used to separate contaminant ions from signals of interest in order to acquire higher quality spectra. FAIMS devices have shown utility in separating 1^+ contaminant

Separation of Isomeric Analytes



Signal Filtering in Mass Spectrometry

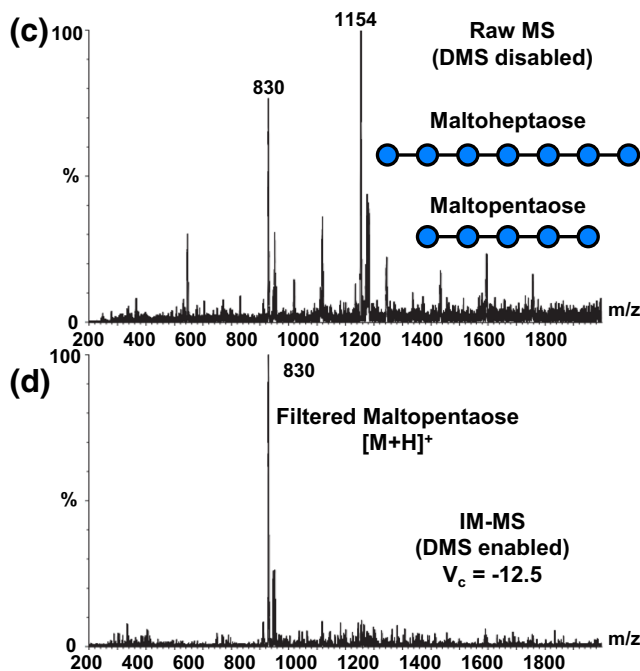


Figure 2. Illustrations of common applications of IMS-MS from recent publications. (a) Separation of lipid isomers with variations in cis/trans double bonds and variations in chain length adapted/reprinted from reference [80] with permission from Springer, Journal of the American Society for Mass Spectrometry. Bowman, A. P. et. al. Copyright, 2017. (b) Illustration of signal filtering by IMS for simplification of MS signals in targeted workflows. Adapted/reprinted from reference [85] with permission from Springer, Journal of the American Society for Mass Spectrometry. Levin, D. S. et. al. Copyright, 2007

ions from higher charge state proteins and peptides of interest [90]. This capability has been extremely important in avoiding biases in trap-based mass analyzers, which require automated gain control (AGC) [91]. It should be

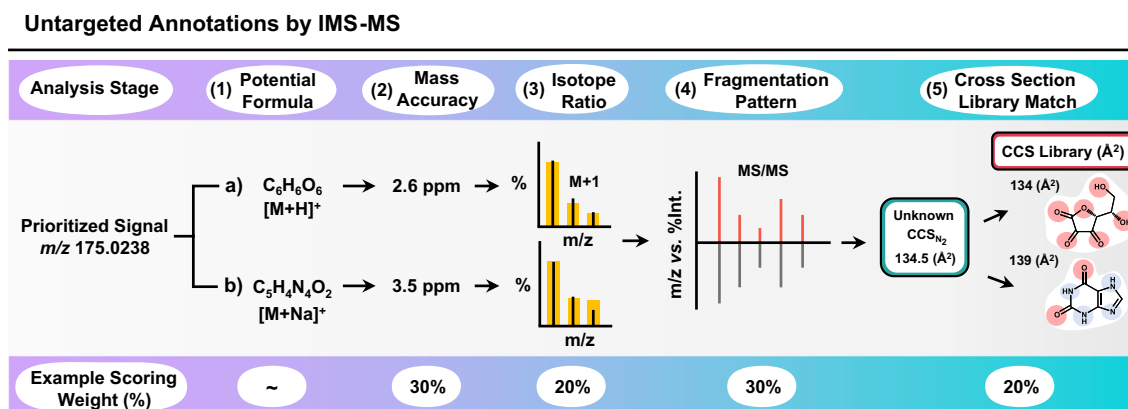


Figure 3. Illustrative workflow of untargeted identifications by IMS-MS with incorporation of additional confidence gained with subsequent chemical information acquired from each analytical technique

noted that while FAIMS and DMA are physical mobility filters, the mobility dimension of other instruments can be used to provide post-acquisition filtering in data processing for specific analytes of interest. For example, DTIMS and TWIMS have also been extremely important in the proteomic and metabolomic analyses of complex samples such as water, soil, and plant material samples, all of which possess a diverse range of molecular contaminants [92]. By separating peptides of interest to different mobility regions away from the high concentrations of organic material (e.g., humic acid substances in soil and polyphenols in plants), natural contaminants (e.g., abundant salts or polymers), and detergents [92, 93], the proteome coverage of environmental samples can be greatly improved.

Untargeted Annotations by IMS-MS

The last major application of IMS discussed in this insight focuses on incorporating mobility information into both targeted and untargeted MS workflows [20]. Because IMS separates ions on a millisecond timescale, these separations can be easily nested into pre-existing LC/GC-MS approaches. In global analyses, spectral features are prioritized by statistical analysis (e.g., volcano plots, PCA) and are

subsequently annotated through a combination of analytical descriptors, including (but not limited to) retention time alignment, accurate mass measurement, isotope ratio analysis, and fragmentation pattern matching [94, 95]. For isomeric compounds, these molecular descriptors are often shared between species, specific isomer identification remains challenging. Incorporating mobility information (more specifically, K_0 or CCS values) as additional ion descriptors can alleviate some of these challenges in untargeted approaches and provide additional confidence that the molecule is accurately annotated, Figure 3 illustrates the untargeted annotation process, wherein a prioritized feature is noted at m/z 175.0238. Given a mass error tolerance of 10 ppm for this singly charged m/z , there are 73 possible entries noted in METLIN which are comprised of 9 unique molecular formulae. To obtain further structural specificity, additional methods such as isotope ratio analysis, fragmentation data, retention time separation, and CCS database matching (provided a certain tolerance in CCS value) can be used to increase structural confidence in annotation of the prioritized feature. We should note that incorporating CCS values for untargeted methods in this manner would currently be called “known-unknowns” analysis, wherein the analyte being annotated would have been

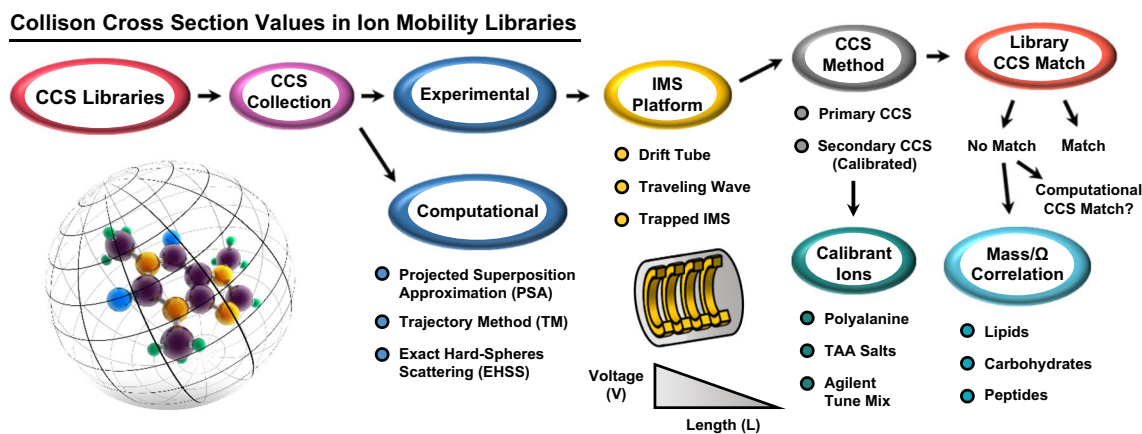


Figure 4. Workflows for generation and interpretation of CCS values in IMS-MS experiments

characterized by a previous mobility experiment on a standard and the subsequent data uploaded into a CCS database. Characterization of “unknown-unknowns” is much more challenging, wherein no database match for the CCS and m/z exists. In this scenario, using the ratio of mass to mobility (often termed “mass-mobility trendlines”) can also be useful for characterizing unknowns into a potential biological class (e.g., peptides, carbohydrates, lipids) [19, 96]. These trendlines are established by previously calculated CCS values, and pre-existing relationships are extrapolated to characterize unknown-unknowns. Computational approaches may also be utilized in these analyses, but currently, there is no centrally accepted workflow for *in silico* approaches (e.g., projection super-approximation, trajectory method, or exact hard-spheres scattering), and a great amount of research is being dedicated to using molecular dynamics and machine learning to reduce the error between experimental and theoretical CCS values [97–100].

From a small molecule perspective, a generation of high confidence mobility measurements for library inclusion remains a key challenge in the IMS community [101]. The schematic workflow for developing unified workflows and interpreting the corresponding data is represented in Figure 4. In order to generate highly reproducible CCS values (ca. < 0.5 % RSD) for database matching, many standardization challenges persist in the IMS community, such as unified protocols for instrument calibration, preferred calibrant ions, and many more. A recent work by Gabelica et al. has demonstrated that addressing many of these challenging questions requires communal consensus between both academic and industrial investigators to advance the study and application of IMS-MS technology [16]. To generate highly reproducible mobility data (and corresponding CCS values), we direct our readers further towards the communal knowledge developed in that publication for detailed information regarding specific IMS platform guidelines (e.g., calibration protocols, instrument settings, and data reporting).

Future Directions and Conclusions

IMS is experiencing continual innovation through novel instrument developments, new methods of acquiring and filtering data, and continually developing computational strategies, all of which provide increasing confidence in mobility information acquired both experimentally and *in silico* [98]. The resolving power of IMS has increased by an order of magnitude in less than a decade [102], opening up new research opportunities to separate and identify previously indistinguishable chemical isomers and isobars (see, Figure 3). Recent developments have even interfaced IMS to ultra-high resolution mass analyzers such as the Orbitrap MS through modulation of ion pulsing [103, 104]. As a research tool in both academia and industry, continual advances in IMS-MS technology are attracting new scientists to the community daily, and the potential applications of these analytical strategies are still being

discovered. While many challenges remain for routine incorporation of mobility analysis and CCS information into untargeted workflows, the future of IMS is bright and its role in separation science is only expected to keep climbing.

Acknowledgements

This research was supported by the NIH National Institute of Environmental Health Sciences (P42 ES027704) and by startup funds from North Carolina State University.

References

1. Thomson, J.J., Rutherford, E.: On the passage of electricity through gases exposed to Röntgen rays. *Philos. Mag.* **42**(258), 392–407 (1896)
2. Mäkinen, M.A., Anttalainen, O.A., Sillanpää, M.E.T.: Ion mobility spectrometry and its applications in detection of chemical warfare agents. *Anal. Chem.* **82**(23), 9594–9600 (2010)
3. Ewing, R.G., Atkinson, D.A., Eiceman, G.A., Ewing, G.J.: A critical review of ion mobility spectrometry for the detection of explosives and explosive related compounds. *Talanta.* **54**(3), 515–529 (2001)
4. Eiceman, G.A., Stone, J.A.: Peer reviewed: ion mobility spectrometers in national defense. *Anal. Chem.* **76**(21), 390 A–397 A (2004)
5. Kanu, A.B., Dwivedi, P., Tam, M., Matz, L., Hill, H.H.: Ion mobility–mass spectrometry. *J. Mass Spectrom.* **43**(1), 1–22 (2008)
6. McLean, J.A., Ruotolo, B.T., Gillig, K.J., Russell, D.H.: Ion mobility–mass spectrometry: a new paradigm for proteomics. *Int. J. Mass Spectrom.* **240**(3), 301–315 (2005)
7. Kliman, M., May, J.C., McLean, J.A.: Lipid analysis and lipidomics by structurally selective ion mobility–mass spectrometry. *Biochim. Biophys. Acta.* **1811**(11), 935–945 (2011)
8. Wyttenbach, T., Kemper, P.R., Bowers, M.T.: Design of a new electrospray ion mobility mass spectrometer. *Int. J. Mass Spectrom.* **212**(1), 13–23 (2001)
9. Wu, C., Siems, W.F., Asbury, G.R., Hill, H.H.: Electrospray ionization high-resolution ion mobility spectrometry–mass spectrometry. *Anal. Chem.* **70**(23), 4929–4938 (1998)
10. Pringle, S.D., Giles, K., Wildgoose, J.L., Williams, J.P., Slade, S.E., Thalassinou, K., Bateman, R.H., Bowers, M.T., Scrivens, J.H.: An investigation of the mobility separation of some peptide and protein ions using a new hybrid quadrupole/travelling wave IMS/oa-ToF instrument. *Int. J. Mass Spectrom.* **261**(1), 1–12 (2007)
11. Thalassinou, K., Grabenauer, M., Slade, S.E., Hilton, G.R., Bowers, M.T., Scrivens, J.H.: Characterization of phosphorylated peptides using travelling wave-based and drift cell ion mobility mass spectrometry. *Anal. Chem.* **81**(1), 248–254 (2009)
12. Chan, Y.-T., Li, X., Soler, M., Wang, J.-L., Wesdemiotis, C., Newkome, G.R.: Self-assembly and traveling wave ion mobility mass spectrometry analysis of hexacadmium macrocycles. *J. Am. Chem. Soc.* **131**(45), 16395–16397 (2009)
13. May, J.C., McLean, J.A.: Ion mobility–mass spectrometry: time-dispersive instrumentation. *Anal. Chem.* **87**(3), 1422–1436 (2015)
14. Cumeras, R., Figueras, E., Davis, C.E., Baumbach, J.I., Gràcia, I.: Review on ion mobility spectrometry. Part 1: current instrumentation. *Analyst.* **140**(5), 1376–1390 (2015)
15. Cumeras, R., Figueras, E., Davis, C.E., Baumbach, J.I., Gràcia, I.: Review on ion mobility spectrometry. Part 2: hyphenated methods and effects of experimental parameters. *Analyst.* **140**(5), 1391–1410 (2015)
16. Gabelica, V., Shvartsburg, A. A., Afonso, C., Barran, P. E., Benesch, J. L. P., Bleiholder, C., Bowers, M. T., Bilbao, A., Bush, M. F., Campbell, J. L., Campuzano, I. D. G., Causon, T. J., Clowers, B. H., Creaser, C., De Pauw, E., Far, J., Fernandez-Lima, F., Fjeldsted, J. C., Giles, K., Groessl, M., Hogan, Jr., C. J., Hann, S., Kim, H. I., Kurulugama, R. T., May, J. C., McLean, J. A., Pagel, K., Richardson, K., Ridgeway, M. E., Rosu, F., Sobott, F., Thalassinou, K., Valentine, S. J., Wyttenbach, T., Recommendations for reporting ion mobility mass spectrometry measurements. *Mass. Spec. Rev.* **38**, 291–320 (2019)

17. Viehland, L.A., Mason, E.A.: Gaseous ion mobility in electric fields of arbitrary strength. *Ann. Phys.* **91**(2), 499–533 (1975)
18. Revercomb, H.E., Mason, E.A.: Theory of plasma chromatography/gaseous electrophoresis. *Review. Anal. Chem.* **47**(7), 970–983 (1975)
19. May, J.C., Goodwin, C.R., Lareau, N.M., Leaprot, K.L., Morris, C.B., Kurulugama, R.T., Mordehai, A., Klein, C., Barry, W., Darland, E., Overney, G., Imatani, K., Stafford, G.C., Fjeldsted, J.C., McLean, J.A.: Conformational ordering of biomolecules in the gas phase: nitrogen collision cross sections measured on a prototype high resolution drift tube ion mobility-mass spectrometer. *Anal. Chem.* **86**(4), 2107–2116 (2014)
20. Mairinger, T., Causon, T.J., Hann, S.: The potential of ion mobility-mass spectrometry for non-targeted metabolomics. *Curr. Opin. Chem. Biol.* **42**, 9–15 (2018)
21. Jurneczko, E., Barran, P.E.: How useful is ion mobility mass spectrometry for structural biology? The relationship between protein crystal structures and their collision cross sections in the gas phase. *Analyst.* **136**(1), 20–28 (2011)
22. May, J.C., McLean, J.A.: A uniform field ion mobility study of melittin and implications of low-field mobility for resolving fine cross-sectional detail in peptide and protein experiments. *Proteomics.* **15**(16), 2862–2871 (2015)
23. Groessl, M., Graf, S., Knochenmuss, R.: High resolution ion mobility-mass spectrometry for separation and identification of isomeric lipids. *Analyst.* **140**(20), 6904–6911 (2015)
24. Ahmed, A., Cho, Y.J., No, M.-h., Koh, J., Tomczyk, N., Giles, K., Yoo, J.S., Kim, S.: Application of the Mason-Schamp equation and ion mobility mass spectrometry to identify structurally related compounds in crude oil. *Anal. Chem.* **83**(1), 77–83 (2011)
25. Stow, S.M., Causon, T.J., Zheng, X., Kurulugama, R.T., Mairinger, T., May, J.C., Rennie, E.E., Baker, E.S., Smith, R.D., McLean, J.A., Hann, S., Fjeldsted, J.C.: An interlaboratory evaluation of drift tube ion mobility-mass spectrometry collision cross section measurements. *Anal. Chem.* **89**(17), 9048–9055 (2017)
26. Allen, S.J., Bush, M.F.: Radio-frequency (rf) confinement in ion mobility spectrometry: apparent mobilities and effective temperatures. *J. Am. Soc. Mass Spectrom.* **27**(12), 2054–2063 (2016)
27. Clowers, B.H., Siems, W.F., Hill, H.H., Massick, S.M.: Hadamard transform ion mobility spectrometry. *Anal. Chem.* **78**(1), 44–51 (2006)
28. Prost, S.A., Crowell, K.L., Baker, E.S., Ibrahim, Y.M., Clowers, B.H., Monroe, M.E., Anderson, G.A., Smith, R.D., Payne, S.H.: Detecting and removing data artifacts in Hadamard transform ion mobility-mass spectrometry measurements. *J. Am. Soc. Mass Spectrom.* **25**(12), 2020–2027 (2014)
29. Belov, M.E., Buschbach, M.A., Prior, D.C., Tang, K., Smith, R.D.: Multiplexed ion mobility spectrometry-orthogonal time-of-flight mass spectrometry. *Anal. Chem.* **79**(6), 2451–2462 (2007)
30. Morrison, K.A., Siems, W.F., Clowers, B.H.: Augmenting ion trap mass spectrometers using a frequency modulated drift tube ion mobility spectrometer. *Anal. Chem.* **88**(6), 3121–3129 (2016)
31. Kanu, A.B., Gribb, M.M., Hill, H.H.: Predicting optimal resolving power for ambient pressure ion mobility spectrometry. *Anal. Chem.* **80**(17), 6610–6619 (2008)
32. Siems, W.F., Wu, C., Tarver, E.E., Hill Jr., H.H., Larsen, P.R., McMinn, D.G.: Measuring the resolving power of ion mobility spectrometers. *Anal. Chem.* **66**(23), 4195–4201 (1994)
33. Tabrizchi, M.: Temperature effects on resolution in ion mobility spectrometry. *Talanta.* **62**(1), 65–70 (2004)
34. Kemper, P.R., Dupuis, N.F., Bowers, M.T.: A new, higher resolution, ion mobility mass spectrometer. *Int. J. Mass Spectrom.* **287**(1), 46–57 (2009)
35. Baker, E.S., Clowers, B.H., Li, F., Tang, K., Tolmachev, A.V., Prior, D.C., Belov, M.E., Smith, R.D.: Ion mobility spectrometry-mass spectrometry performance using electrodynamic ion funnels and elevated drift gas pressures. *J. Am. Soc. Mass Spectrom.* **18**(7), 1176–1187 (2007)
36. Kirk, A.T., Raddatz, C.-R., Zimmermann, S.: Separation of isotopologues in ultra-high-resolution ion mobility spectrometry. *Anal. Chem.* **89**(3), 1509–1515 (2017)
37. Giles, K., Williams, J.P., Campuzano, I.: Enhancements in travelling wave ion mobility resolution. *Rapid Commun. Mass Spectrom.* **25**(11), 1559–1566 (2011)
38. Ansari, D., Andersson, R., Bauden, M.P., Andersson, B., Connolly, J.B., Welinder, C., Sasor, A., Marko-Varga, G.: Protein deep sequencing applied to biobank samples from patients with pancreatic cancer. *J. Cancer Res. Clin. Oncol.* **141**(2), 369–380 (2015)
39. Struwe, W.B., Benesch, J.L., Harvey, D.J., Pagel, K.: Collision cross sections of high-mannose N-glycans in commonly observed adduct states – identification of gas-phase conformers unique to [M – H][–] ions. *Analyst.* **140**(20), 6799–6803 (2015)
40. Shvartsburg, A.A., Smith, R.D.: Fundamentals of traveling wave ion mobility spectrometry. *Anal. Chem.* **80**(24), 9689–9699 (2008)
41. Morsa, D., Gabelica, V., De Pauw, E.: Fragmentation and isomerization due to field heating in traveling wave ion mobility spectrometry. *J. Am. Soc. Mass Spectrom.* **25**(8), 1384–1393 (2014)
42. Morsa, D., Gabelica, V., De Pauw, E.: Effective temperature of ions in traveling wave ion mobility spectrometry. *Anal. Chem.* **83**(14), 5775–5782 (2011)
43. Merenbloom, S.I., Flick, T.G., Williams, E.R.: How hot are your ions in TWAVE ion mobility spectrometry? *J. Am. Soc. Mass Spectrom.* **23**(3), 553–562 (2012)
44. Forsythe, J.G., Petrov, A.S., Walker, C.A., Allen, S.J., Pellissier, J.S., Bush, M.F., Hud, N.V., Fernández, F.M.: Collision cross section calibrants for negative ion mode traveling wave ion mobility-mass spectrometry. *Analyst.* **140**(20), 6853–6861 (2015)
45. Bush, M.F., Campuzano, I.D.G., Robinson, C.V.: Ion mobility mass spectrometry of peptide ions: effects of drift gas and calibration strategies. *Anal. Chem.* **84**(16), 7124–7130 (2012)
46. Gelb, A.S., Jarratt, R.E., Huang, Y., Dodds, E.D.: A study of calibrant selection in measurement of carbohydrate and peptide ion-neutral collision cross sections by traveling wave ion mobility spectrometry. *Anal. Chem.* **86**(22), 11396–11402 (2014)
47. Garimella, S.V.B., Ibrahim, Y.M., Webb, I.K., Ipsen, A.B., Chen, T.-C., Tolmachev, A.V., Baker, E.S., Anderson, G.A., Smith, R.D.: Ion manipulations in structures for lossless ion manipulations (SLIM): computational evaluation of a 90° turn and a switch. *Analyst.* **140**(20), 6845–6852 (2015)
48. Giles, K., Ujma, J., Wildgoose, J., Pringle, S., Richardson, K., Langridge, D., Green, M.: A cyclic ion mobility-mass spectrometry system. *Anal. Chem.* (2019)
49. Deng, L., Ibrahim, Y.M., Baker, E.S., Aly, N.A., Hamid, A.M., Zhang, X., Zheng, X., Garimella, S.V.B., Webb, I.K., Prost, S.A., Sandoval, J.A., Norheim, R.V., Anderson, G.A., Tolmachev, A.V., Smith, R.D.: Ion mobility separations of isomers based upon long path length structures for lossless ion manipulations combined with mass spectrometry. *ChemistrySelect.* **1**(10), 2396–2399 (2016)
50. Deng, L., Garimella, S.V.B., Hamid, A.M., Webb, I.K., Attah, I.K., Norheim, R.V., Prost, S.A., Zheng, X., Sandoval, J.A., Baker, E.S., Ibrahim, Y.M., Smith, R.D.: Compression ratio ion mobility programming (CRIMP) accumulation and compression of billions of ions for ion mobility-mass spectrometry using traveling waves in structures for lossless ion manipulations (SLIM). *Anal. Chem.* **89**(12), 6432–6439 (2017)
51. Deng, L., Webb, I.K., Garimella, S.V.B., Hamid, A.M., Zheng, X., Norheim, R.V., Prost, S.A., Anderson, G.A., Sandoval, J.A., Baker, E.S., Ibrahim, Y.M., Smith, R.D.: Serpentine ultralong path with extended routing (SUPER) high resolution traveling wave ion mobility-MS using structures for lossless ion manipulations. *Anal. Chem.* **89**(8), 4628–4634 (2017)
52. Michelmann, K., Silveira, J.A., Ridgeway, M.E., Park, M.A.: Fundamentals of trapped ion mobility spectrometry. *J. Am. Soc. Mass Spectrom.* **26**(1), 14–24 (2015)
53. Hernandez, D.R., DeBord, J.D., Ridgeway, M.E., Kaplan, D.A., Park, M.A., Fernandez-Lima, F.: Ion dynamics in a trapped ion mobility spectrometer. *Analyst.* **139**(8), 1913–1921 (2014)
54. Ridgeway, M.E., Lubeck, M., Jordens, J., Mann, M., Park, M.A.: Trapped ion mobility spectrometry: a short review. *Int. J. Mass Spectrom.* **425**, 22–35 (2018)
55. Silveira, J.A., Ridgeway, M.E., Park, M.A.: High resolution trapped ion mobility spectrometry of peptides. *Anal. Chem.* **86**(12), 5624–5627 (2014)
56. Pu, Y., Ridgeway, M.E., Glaskin, R.S., Park, M.A., Costello, C.E., Lin, C.: Separation and identification of isomeric glycans by selected accumulation-trapped ion mobility spectrometry-electron activated dissociation tandem mass spectrometry. *Anal. Chem.* **88**(7), 3440–3443 (2016)
57. Chai, M., Young, M.N., Liu, F.C., Bleiholder, C.: A transferable, sample-independent calibration procedure for trapped ion mobility spectrometry (TIMS). *Anal. Chem.* **90**(15), 9040–9047 (2018)
58. Liu, F.C., Ridgeway, M.E., Park, M.A., Bleiholder, C.: Tandem trapped ion mobility spectrometry. *Analyst.* **143**(10), 2249–2258 (2018)

59. Kolakowski, B.M., Mester, Z.: Review of applications of high-field asymmetric waveform ion mobility spectrometry (FAIMS) and differential mobility spectrometry (DMS). *Analyst*. **132**(9), 842–864 (2007)
60. Guevremont, R.: High-field asymmetric waveform ion mobility spectrometry: a new tool for mass spectrometry. *J. Chromatogr. A*. **1058**(1), 3–19 (2004)
61. Cooper, H.J.: To what extent is FAIMS beneficial in the analysis of proteins? *J. Am. Soc. Mass Spectrom.* **27**(4), 566–577 (2016)
62. Guevremont, R., Purves, R.W.: Atmospheric pressure ion focusing in a high-field asymmetric waveform ion mobility spectrometer. *Rev. Sci. Instrum.* **70**(2), 1370–1383 (1999)
63. Barnett, D.A., Belford, M., Dunyach, J.-J., Purves, R.W.: Characterization of a temperature-controlled FAIMS system. *J. Am. Soc. Mass Spectrom.* **18**(9), 1653–1663 (2007)
64. Krylova, N., Krylov, E., Eiceman, G.A., Stone, J.A.: Effect of moisture on the field dependence of mobility for gas-phase ions of organophosphorus compounds at atmospheric pressure with field asymmetric ion mobility spectrometry. *J. Phys. Chem. A*. **107**(19), 3648–3654 (2003)
65. Hatsis, P., Kapron, J.T.: A review on the application of high-field asymmetric waveform ion mobility spectrometry (FAIMS) in drug discovery. *Rapid Commun. Mass Spectrom.* **22**(5), 735–738 (2008)
66. Barnett, D.A., Ells, B., Guevremont, R., Purves, R.W.: Separation of leucine and isoleucine by electrospray ionization–high field asymmetric waveform ion mobility spectrometry–mass spectrometry. *J. Am. Soc. Mass Spectrom.* **10**(12), 1279–1284 (1999)
67. Kaszycki, J.L., Bowman, A.P., Shvartsburg, A.A.: Ion mobility separation of peptide isotopomers. *J. Am. Soc. Mass Spectrom.* **27**(5), 795–799 (2016)
68. de la Mora, J.F., de Juan, L., Eichler, T., Rosell, J.: Differential mobility analysis of molecular ions and nanometer particles. *TrAC Trends Anal. Chem.* **17**(6), 328–339 (1998)
69. Pease, L.F., Elliott, J.T., Tsai, D.-H., Zachariah, M.R., Tarlov, M.J.: Determination of protein aggregation with differential mobility analysis: application to IgG antibody. *Biotechnol. Bioeng.* **101**(6), 1214–1222 (2008)
70. Hogan, C.J., de la Mora, J.F.: Ion mobility measurements of non-denatured 12–150 kDa proteins and protein multimers by tandem differential mobility analysis–mass spectrometry (DMA-MS). *J. Am. Soc. Mass Spectrom.* **22**(1), 158–172 (2011)
71. Fernández-García, J., Fernández de la Mora, J.: Measuring the effect of ion-induced drift-gas polarization on the electrical mobilities of multiply-charged ionic liquid nanodrops in air. *J. Am. Soc. Mass Spectrom.* **24**(12), 1872–1889 (2013)
72. Ouyang, H., Larriba-Andaluz, C., Oberreit, D.R., Hogan, C.J.: The collision cross sections of iodide salt cluster ions in air via differential mobility analysis–mass spectrometry. *J. Am. Soc. Mass Spectrom.* **24**(12), 1833–1847 (2013)
73. Morrison, L.J., Parker, W.R., Holden, D.D., Henderson, J.C., Boll, J.M., Trent, M.S., Brodbelt, J.S.: UVliPiD: a UVPD-based hierarchical approach for de novo characterization of lipid structures. *Anal. Chem.* **88**(3), 1812–1820 (2016)
74. Lareau, N.M., May, J.C., McLean, J.A.: Non-derivatized glycan analysis by reverse phase liquid chromatography and ion mobility–mass spectrometry. *Analyst*. **140**(10), 3335–3338 (2015)
75. Dodds, J.N., May, J.C., McLean, J.A.: Investigation of the complete suite of the leucine and isoleucine isomers: toward prediction of ion mobility separation capabilities. *Anal. Chem.* **89**(1), 952–959 (2017)
76. Lippens, J.L., Ranganathan, S.V., D'Esposito, R.J., Fabris, D.: Modular calibrant sets for the structural analysis of nucleic acids by ion mobility spectrometry mass spectrometry. *Analyst*. **141**(13), 4084–4099 (2016)
77. Hofmann, J., Hahm, H.S., Seeberger, P.H., Pagel, K.: Identification of carbohydrate anomers using ion mobility–mass spectrometry. *Nature*. **526**, 241 (2015)
78. Pagel, K., Harvey, D.J.: Ion mobility–mass spectrometry of complex carbohydrates: collision cross sections of sodiated N-linked glycans. *Anal. Chem.* **85**(10), 5138–5145 (2013)
79. Kyle, J.E., Zhang, X., Weitz, K.K., Monroe, M.E., Ibrahim, Y.M., Moore, R.J., Cha, J., Sun, X., Lovelace, E.S., Wagoner, J., Polyak, S.J., Metz, T.O., Dey, S.K., Smith, R.D., Burnum-Johnson, K.E., Baker, E.S.: Uncovering biologically significant lipid isomers with liquid chromatography, ion mobility spectrometry and mass spectrometry. *Analyst*. **141**(5), 1649–1659 (2016)
80. Bowman, A.P., Abzalimov, R.R., Shvartsburg, A.A.: Broad separation of isomeric lipids by high-resolution differential ion mobility spectrometry with tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.* **28**(8), 1552–1561 (2017)
81. Nagy, G., Chouinard, C.D., Attah, I.K., Webb, I.K., Garimella, S. V.B., Ibrahim, Y.M., Baker, E.S., Smith, R.D.: Distinguishing enantiomeric amino acids with chiral cyclodextrin adducts and structures for lossless ion manipulations. *Electrophoresis*. **39**, 3148–3155 (2018)
82. Domalain, V., Hubert-Roux, M., Tognetti, V., Joubert, L., Lange, C.M., Rouden, J., Afonso, C.: Enantiomeric differentiation of aromatic amino acids using traveling wave ion mobility–mass spectrometry. *Chem. Sci.* **5**(8), 3234–3239 (2014)
83. Zhang, J.D., Mohibul Kabir, K.M., Lee, H.E., Donald, W.A.: Chiral recognition of amino acid enantiomers using high-definition differential ion mobility mass spectrometry. *Int. J. Mass Spectrom.* **428**, 1–7 (2018)
84. May, J.C., Jurneczko, E., Stow, S.M., Kratochvil, I., Kalkhof, S., McLean, J.A.: Conformational landscapes of ubiquitin, cytochrome c, and myoglobin: uniform field ion mobility measurements in helium and nitrogen drift gas. *Int. J. Mass Spectrom.* **427**, 79–90 (2018)
85. Levin, D.S., Vouros, P., Miller, R.A., Nazarov, E.G.: Using a nanoelectrospray–differential mobility spectrometer–mass spectrometer system for the analysis of oligosaccharides with solvent selected control over ESI aggregate ion formation. *J. Am. Soc. Mass Spectrom.* **18**(3), 502–511 (2007)
86. Miller, R.A., Nazarov, E.G., Eiceman, G.A., Thomas King, A.: A MEMS radio-frequency ion mobility spectrometer for chemical vapor detection. *Sensors Actuators A Phys.* **91**(3), 301–312 (2001)
87. Kurulugama, R.T., Nachtigall, F.M., Lee, S., Valentine, S.J., Clemmer, D.E.: Overtone mobility spectrometry: Part 1. Experimental observations. *J. Am. Soc. Mass Spectrom.* **20**(5), 729–737 (2009)
88. Valentine, S.J., Stokes, S.T., Kurulugama, R.T., Nachtigall, F.M., Clemmer, D.E.: Overtone mobility spectrometry: Part 2. Theoretical considerations of resolving power. *J. Am. Soc. Mass Spectrom.* **20**(5), 738–750 (2009)
89. Valentine, S.J., Kurulugama, R.T., Clemmer, D.E.: Overtone mobility spectrometry: Part 3. On the origin of peaks. *J. Am. Soc. Mass Spectrom.* **22**(5), 804–816 (2011)
90. Bagag, A., Giuliani, A., Canon, F., Réfrégiers, M., Naour, F.L.: Separation of peptides from detergents using ion mobility spectrometry. *Rapid Commun. Mass Spectrom.* **25**(22), 3436–3440 (2011)
91. Hebert, A.S., Prasad, S., Belford, M.W., Bailey, D.J., McAlister, G.C., Abbatiello, S.E., Huguet, R., Wouters, E.R., Dunyach, J.-J., Brademan, D.R., Westphall, M.S., Coon, J.J.: Comprehensive single-shot proteomics with FAIMS on a hybrid orbitrap mass spectrometer. *Anal. Chem.* **90**(15), 9529–9537 (2018)
92. Baker, E.S., Burnum-Johnson, K.E., Ibrahim, Y.M., Orton, D.J., Monroe, M.E., Kelly, R.T., Moore, R.J., Zhang, X., Theberge, R., Costello, C.E., Smith, R.D.: Enhancing bottom-up and top-down proteomic measurements with ion mobility separations. *Proteomics*. **15**(16), 2766–2776 (2015)
93. Hengel, S.M., Floyd, E., Baker, E.S., Zhao, R., Wu, S., Pasa-Tolic, L.: Evaluation of SDS depletion using an affinity spin column and IMS-MS detection. *Proteomics*. **12**(21), 3138–3142 (2012)
94. Gibson, C.L., Codreanu, S.G., Schrimpe-Rutledge, A.C., Retzlaff, C.L., Wright, J., Mortlock, D.P., Sherrod, S.D., McLean, J.A., Blakely, R.D.: Global untargeted serum metabolomic analyses nominate metabolic pathways responsive to loss of expression of the orphan metallo β -lactamase, MBLAC1. *Mol. Omics*. **14**(3), 142–155 (2018)
95. Liang, Q., Liu, H., Zhang, T., Jiang, Y., Zhang, A.-H.: Untargeted lipidomics study of coronary artery disease by FULPC-Q-TOF-MS. *Anal. Methods*. **8**(6), 1229–1234 (2016)
96. Hines, K.M., Ross, D.H., Davidson, K.L., Bush, M.F., Xu, L.: Large-scale structural characterization of drug and drug-like compounds by high-throughput ion mobility–mass spectrometry. *Anal. Chem.* **89**(17), 9023–9030 (2017)
97. Zhou, Z., Xiong, X., Zhu, Z.-J.: MetCCS predictor: a web server for predicting collision cross-section values of metabolites in ion mobility–mass spectrometry based metabolomics. *Bioinformatics*. **33**(14), 2235–2237 (2017)
98. Zhou, Z., Tu, J., Xiong, X., Shen, X., Zhu, Z.-J.: LipidCCS: prediction of collision cross-section values for lipids with high precision to support ion mobility–mass spectrometry-based lipidomics. *Anal. Chem.* **89**(17), 9559–9566 (2017)
99. Soper-Hopper, M.T., Petrov, A.S., Howard, J.N., Yu, S.S., Forsythe, J.G., Grover, M.A., Fernández, F.M.: Collision cross section predictions using

- 2-dimensional molecular descriptors. *Chem. Commun.* **53**(54), 7624–7627 (2017)
100. Plante, P.-L., Francovic-Fontaine, É., May, J.C., McLean, J.A., Baker, E.S., Laviolette, F., Marchand, M., Corbeil, J.: Predicting ion mobility collision cross-sections using a deep neural network: deepCCS. *Anal. Chem.* **91**(8), 5191–5199 (2019)
101. Picache, J.A., Rose, B.S., Balinski, A., Leaptrot, K.L., Sherrod, S.D., May, J.C., McLean, J.A.: Collision cross section compendium to annotate and predict multi-omic compound identities. *Chem. Sci.* **10**(4), 983–993 (2019)
102. Dodds, J.N., May, J.C., McLean, J.A.: Correlating resolving power, resolution, and collision cross section: unifying cross-platform assessment of separation efficiency in ion mobility spectrometry. *Anal. Chem.* **89**(22), 12176–12184 (2017)
103. Poltash, M.L., McCabe, J.W., Shirzadeh, M., Laganowsky, A., Clowers, B.H., Russell, D.H.: Fourier transform-ion mobility-orbitrap mass spectrometer: a next-generation instrument for native mass spectrometry. *Anal. Chem.* **90**(17), 10472–10478 (2018)
104. Ibrahim, Y.M., Garimella, S.V.B., Prost, S.A., Wojcik, R., Norheim, R.V., Baker, E.S., Rusyn, I., Smith, R.D.: Development of an ion mobility spectrometry-orbitrap mass spectrometer platform. *Anal. Chem.* **88**(24), 12152–12160 (2016)