Received: 3 November 2023 | Revised: 12 February 2024 | Accepted: 14 February 2024

DOI: 10.1002/pmic.202200436

REVIEW

Proteomics
Proteomics and Systems Biology

Computational tools and algorithms for ion mobility spectrometry-mass spectrometry

Dylan H. Ross[1] | Harsh Bhotika[2] | Xueyun Zheng[1] | Richard D. Smith[1] | Kristin E. Burnum-Johnson[2] | Aivett Bilbao[2]

[1]Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Washington, USA

[2]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington, USA

Correspondence
Aivett Bilbao, Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99354, USA.
Email: Aivett.Bilbao@pnnl.gov

Funding information
National Institute of General Medical Sciences, Grant/Award Number: P41 GM103493; U.S. Department of Energy (DOE), Office of Science, Biological and Environmental Research (BER) program; Early Career Research Program Award; Environmental Molecular Sciences Laboratory (EMSL); Biological and Environmental Research program, Grant/Award Number: DE-AC05-76RL01830; Department of Energy (DOE), Office of Science, Biological and Environmental Research (BER) program, Early Career Research Program Award; Environmental Molecular Sciences Laboratory (EMSL), a DOE Office of Science User Facility sponsored by the Biological and Environmental Research program

Abstract

Ion mobility spectrometry-mass spectrometry (IMS-MS or IM-MS) is a powerful analytical technique that combines the gas-phase separation capabilities of IM with the identification and quantification capabilities of MS. IM-MS can differentiate molecules with indistinguishable masses but different structures (e.g., isomers, isobars, molecular classes, and contaminant ions). The importance of this analytical technique is reflected by a staged increase in the number of applications for molecular characterization across a variety of fields, from different MS-based omics (proteomics, metabolomics, lipidomics, etc.) to the structural characterization of glycans, organic matter, proteins, and macromolecular complexes. With the increasing application of IM-MS there is a pressing need for effective and accessible computational tools. This article presents an overview of the most recent free and open-source software tools specifically tailored for the analysis and interpretation of data derived from IM-MS instrumentation. This review enumerates these tools and outlines their main algorithmic approaches, while highlighting representative applications across different fields. Finally, a discussion of current limitations and expectable improvements is presented.

KEYWORDS
algorithm, collision-cross section, computational tool, data processing, ion mobility spectrometry-mass spectrometry, lipidomics, metabolomics, proteomics, software

Abbreviations: AI, artificial intelligence; AT, arrival time; BSA, bovine serum albumin; CCS, collision-cross section; CE, capillary electrophoresis; CIU, collision induced unfolding; CZE, capillary zone electrophoresis; DDA, data-dependent acquisition; DHS, diffuse hard-sphere scattering; DIA, data-independent acquisition; DL, deep learning; DMA, differential mobility analyzer; DMS, differential mobility spectrometry; DT, drift time; DTIMS, drift tube IMS; DTM, discovery and targeted monitoring; EHS, exact hard-sphere scattering; FAIMS, field asymmetric IMS; FDR, false discovery rate; GC, gas chromatography; GUI, graphical user interface; IMS or IM, ion mobility spectrometry; LC, liquid chromatography; ML, machine learning; MS, mass spectrometry; MS/MS, tandem mass spectra; MSI, mass spectrometry imaging; PA, projection approximation; PCA, principal component analysis; QRAI, quadrupole resolved all ions; Rp, resolving power; RT, retention time; SFC, supercritical fluid chromatography; SLIM, structures for lossless ion manipulations; SPE, solid-phase extraction; TIFF, transferring identification based on FAIMS filtering; TIMS, trapped IMS; TM, trajectory method; TOF, time-of-flight; TWIMS, traveling wave IMS; UDMSE, TWIMS-DIA-MS ultra-definition MSE.

1 | INTRODUCTION

Mass spectrometry (MS) is an indispensable tool for the interrogation of complex mixtures, such as biological and environmental samples. However, the presence of isobaric species and wide dynamic range of concentration levels can limit the extent of useful information that can be obtained using MS alone. It is therefore common to employ various enrichment, fractionation, and/or separation techniques prior to MS analysis, in order to reduce the potential for ambiguity and enhance signals from the analytes or molecules of interest in a given study. Among these front-end separations, each having their own sets of strengths and limitations, ion mobility spectrometry (IMS, or IM for conciseness) is seeing increased application in MS-based omics analyses (i.e., proteomics, lipidomics, metabolomics, etc.) [1] and structural characterization of analytes across diverse fields of science [2]. IM is a gas-phase separation technique in which ions are subjected to an electric field that drives them through a cell filled with a low-pressure neutral buffer gas, where separation of the ions is achieved on the basis of their differential interactions with the buffer gas causing them to travel at different rates through the cell. Molecules of different sizes, shapes, and charges, are separated as they travel through the buffer gas in the IM cell, and their measured mobilities, referred to as arrival time (AT) or drift time (DT), can be used to infer the molecular property collision-cross section (CCS), which is partially orthogonal to m/z, and therefore allows for the separation of isomeric analytes that are indistinguishable by MS alone. The fast speed of IM (separation occurs on the order of milliseconds) makes it ideal to combine it with liquid chromatography (LC) as front-end separations (i.e., LC-IM-MS) for greater depth of characterization of complex mixtures over LC-MS, or it is possible to even omit LC entirely in applications where analytical throughput is most important while still providing better sensitivity and selectivity than in MS-only experiments.

Like in MS in general, software tools and algorithms have been paramount components in development and application of IM-MS. The most important consideration with respect to software is the determination of CCS values from raw measurements, which in some instruments may be derived directly while others require some form of calibration (discussed in the following sections). CCS is highly reproducible across different instruments/methods and reflects structural characteristics of compounds, making it an excellent property for use in annotation of unknowns. Despite the many benefits of IM and several available software tools, a primary factor that impedes the realization of its full potential in omics and related applications is a lack of robust and comprehensive software support, both in terms of low-level data extraction/processing and higher-level informatics algorithms [3]. In this review, we provide an overview of software tools that are currently available for the analysis of IM-MS data across multiple instrumentations and disciplines, highlighting their specific algorithmic approaches and available functionality, and provide insights into future developments and opportunities for improvement in this area.

2 | ION MOBILITY SPECTROMETRY-MASS SPECTROMETRY INSTRUMENTATION

IM has experienced great advancement in both fundamental technology development and applications in the past several decades. IM separates ions with differences in size or shape based on the balance of two forces that impact the ion movement, namely, the electric field and the drag force from the collision with buffer gas molecules [4], and provides insights into the structure. There are multiple variations of IM devices among hyphenated IM-MS instruments in current use, and these are primarily distinguished by the geometry of the mobility cell, the nature of the electric fields used, and how ions are moved through the device.

Different variations of the electric field and stationary state of the buffer gas have given rise to multiple IM-based platforms such as drift tube IMS (DTIMS) [5–10], traveling wave IMS (TWIMS) [11], trapped IMS (TIMS) [12], field asymmetric IMS (FAIMS) or Differential mobility spectrometry (DMS) [13–15], and differential mobility analyzer (DMA) [16]. While IM itself is very performant, when coupled with MS, IM-MS provides a powerful hybrid analytical tool. With the exception of FAIMS, which can be found with Orbitrap-based instruments, most IM-MS platforms employ time-of-flight (TOF) mass analyzers. The first commercial IM-MS instrument based on traveling wave was introduced by Waters in 2006 (Waters Synapt) [11], and soon became a widely used platform in academic and industrial fields. Since then, many instrument vendors followed and developed their own IM-MS platforms, notably trapped IM-MS by Bruker in 2009 [17] and commercial drift tube IM-MS by Agilent in 2014 [9, 10]. FAIMS was made available with Thermo-fisher instruments and DMS is available on Sciex instruments. The hyphenated IM-MS platform provides high IM resolving power (Rp), high sensitivity, and high throughput sampling, and has become a powerful and popular tool for applications broadly [18–22].

More recently, developments in IM technology continued to achieve higher Rp and improved separations [23]. Notably, a new approach using structures for lossless ion manipulations (SLIM) was recently utilized to enable long path TWIMS separations followed by MS analyses [24–26]. This approach allowed the construction of a 13 m serpentine TWIMS drift path providing previously unachieved IM Rp for biomolecules, more effective ion utilization, and significantly improved characterization of very small sample sizes. These results demonstrated the potential for high resolution proteomics, lipidomics, glycomics, and metabolomics IM-MS measurements. The single path SLIM IMS platform was soon made commercial available by MOBILion [27]. Similarly, Waters announced its high resolution platform, the cyclic TWIMS instrument with 1-m single path that allows multi-pass separation [28]. More developments are ongoing including tandem IM [29], and incorporation of IM with other analytical techniques such as collision-induced dissociation and cryo-infrared spectroscopy for complete structure elucidation [30]. A brief summary of commercial IM-MS instruments is listed in Table 1, and more details and corresponding publications can be found in recent reviews [2, 31–34].

TABLE 1 Ion mobility spectrometry-mass spectrometry instrumentation.

Acronym	Full name	Applied field	Separation principle	Typical readout	CCS calibration function	Resolving power (max. reported [31–34])	Vendor
DTIMS	Drift tube ion mobility spectrometry	Static	Temporal dispersive	Time	Linear	50–60 (low pressure) 200 (AP)	Agilent, TofWerk
TIMS	Trapped ion mobility spectrometry	Static	Trapping and release	Time	Linear	200–400	Bruker
TWIMS	Traveling wave ion mobility spectrometry	Oscillating	Temporal dispersive	Time	Nonlinear	30–40	Waters
Cyclic TWIMS	Cyclic Traveling wave ion mobility spectrometry	Oscillating	Temporal dispersive	Time	Nonlinear	60–80 (one pass) 750 (>100 passes)	Waters
SLIM TWIMS	Structures for lossless ion manipulations	Oscillating	Temporal dispersive	Time	Nonlinear	200–1500	MOBILion
FAIMS	Field asymmetric ion mobility spectrometry	Oscillating	Spatial dispersive	Compensation voltage	NA[a]	<30	Thermo, Sciex
DMA	Differential mobility analyzer	Static	Spatial dispersive	Voltage	Linear or nonlinear		SEADM, TSI

CCS, collision-cross section; DMA, differential mobility analyzer; DTIMS, drift tube ion mobility spectrometry; FAIMS, field asymmetric ion mobility spectrometry; SLIM, structures for lossless ion manipulations; TIMS, trapped ion mobility spectrometry; TWIMS, traveling wave ion mobility spectrometry.
[a]FAIMS does not support determination of CCS values.

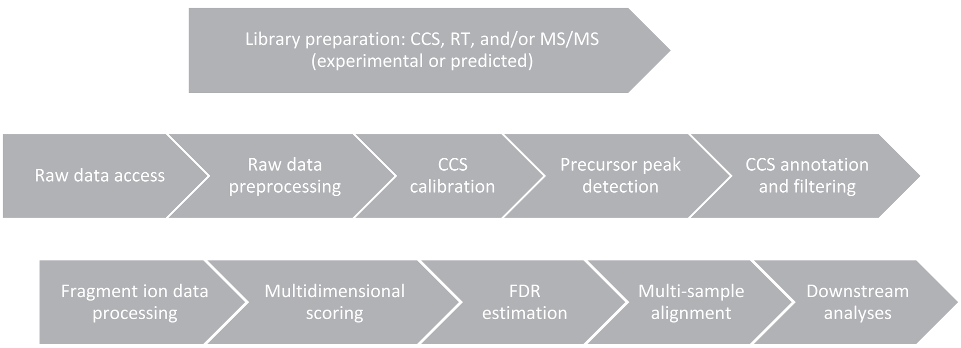

FIGURE 1 General steps in ion mobility spectrometry-mass spectrometry (IM-MS) processing workflows.

3 | COMPUTATIONAL APPROACHES FOR IM-MS

The number of software tools available for processing IM-MS data is limited compared to LC-MS. While the first choice of software has been traditionally proprietary tools offered by instrumentation vendors, over the years several free and open-source tools have been developed or adapted to support IM-MS. The general workflow steps are represented in Figure 1 and include: library preparation with CCS, retention time (RT), and/or tandem mass spectra (MS/MS) from predicted or experimental in-house or public databases, to be used as reference for molecular annotation), raw data access, raw data preprocessing, CCS (or IM) calibration, peak detection, CCS (or IM) annotation and filtering, fragment ion data processing, multidimensional scoring, false discovery rate (FDR) estimation, multi-sample alignment, and downstream analyses. Some of these steps can be combined, omitted, or replaced, depending on the specific instrumentation and application. Table 2 describe freely available software tools for IM-MS featuring a graphical user interface (GUI).

3.1 | Raw data access

Raw IM-MS spectra from commercial instruments are stored by the instrument acquisition software in proprietary formats. Proprietary formats are optimized according to the vendor hardware and these files can only be accessed using the vendor's provided software libraries.

IM-MS software tools either integrate the vendor libraries to support their format directly, or support open formats, typically mzML [35] files generated using the ProteoWizard toolkit [36], which supports

TABLE 2 Freely available GUI-based software tools for processing IM-MS data.

Software	Reference	Processing/peak detection type	Omics/molecular type	Input MS format	Programming language	Website
PNNL-PreProcessor	Bilbao et al., 2022 [44]	Raw signal enhancements	Any	Agilent, UIMF	C#	pnnl-comp-mass-spec.github.io/PNNL-PreProcessor
Skyline	MacLean et al., 2018 [66]	Targeted data extraction	Proteomics, Lipidomics, Metabolomics	mzML, Agilent, Thermo, Waters, SCIEX, Bruker	C#, open source	www.skyline.ms
MS-DIAL	Tsugawa et al., 2020 [67]	Untargeted feature detection	Lipidomics, Metabolomics	mzML, Agilent, Thermo, Waters, SCIEX, Bruker	C#	prime.psc.riken.jp/compms/msdial/main.html
MZmine 3	Schmid et al., 2023 [68]	Untargeted feature detection	Lipidomics, Metabolomics	mzML, Thermo, Waters, Agilent, NetCDF, mzData	Java, open source	mzmine.github.io
LipidOz	Ross et al., 2023 [42]	Targeted data extraction	Lipid carbon–carbon double bond positions	mzML, mza, Agilent	Python, open source	github.com/PNNL-m-q/lipidoz
CIUSuite 2	Polasky et al., 2019 [69]	Untargeted feature detection	Protein unfolding	Waters, Agilent	Python	sites.lsa.umich.edu/ruotolo/software/ciusuite-2
UniDec	Marty et al., 2015 [70]	Untargeted feature detection	Protein assemblies and lipoprotein nanodiscs	mzML, Agilent, Waters, Thermo	Python, C, open source	github.com/michaelmarty/UniDec
MaxQuant	Prianichnikov et al., 2020 [71]	Untargeted feature detection	Proteomics	Thermo, Bruker	C#	maxquant.org
FragPipe	Yu et al., 2020 [72]	Untargeted feature detection	Proteomics	mzML, Agilent, Bruker, Thermo	Java	fragpipe.nesvilab.org
DIA-NN	Demichev et al., 2022 [73]	Targeted data extraction	Proteomics	mzML, Bruker, Thermo	C++, C#	github.com/vdemichev/DiaNN

DIA, data-independent acquisition; GUI, graphical user interface; IM-MS, ion mobility spectrometry-mass spectrometry.

conversion from most instrument vendors. However, specific support to convert IM-MS data to mzML including all relevant IM metadata is often limited. Furthermore, the mzML format is inefficient for storing and accessing large amounts of spectra, particularly problematic for IM-MS combined with other separations [37].

For imaging IM-MS data, the imzML format [38] is used. Raw data converted to imzML is divided in two files, experimental details are stored in an XML file based on mzML and spectra are stored in a binary file in order to allow efficient storage. The Python pyimzML (https://github.com/alexandrovteam/pyimzML) package can be used to access data as lists and dictionaries, as described in a recent single-cell lipidomics study [39].

AlphaTims [40] is a tool for indexing LC-TIMS-MS data which enables fast access and multidimensional selections from raw data. The tool is freely available, implemented in Python and saves the indexed data in an HDF5 format for efficient access.

Recently, MZA [37] has been proposed to address limitations in accessing large MS files in general and facilitate software development and artificial intelligence (AI) research in multidimensional MS measurement, including IM-MS and data-independent acquisition (DIA) spectra. MZA is composed by a data conversion tool and a simple file structure based on the HDF5 format, providing easy, cross-platform and cross-programming language access to raw IM-MS-data, and enabling fast development of new tools in data science programming languages such as Python and R. It currently supports conversion from Agilent MassHunter ".d" and Thermo ".raw" formats, as well as mzML. While IM-MS data in MZA files can be directly read in any programming language using generic HDF5 libraries, the Python package mzapy [41] is available to further facilitate development of Python tools, offering standard MS utilities for efficient extraction and processing, such as mass and CCS calibration, signal processing, peak finding, and generating visualization plots. LipidOz [42] is an example tool using MZA and mzapy for automated elucidation of lipid carbon–carbon double bond positions from ozone-induced dissociation experiments.

3.2 | Raw data preprocessing

Several algorithms have been reported for preprocessing of raw IM-MS data. To enable more effective spectrum summation for improving

Rp and detection of low-intensity species, the command line DTW tool [43] employs single value alignment and nonlinear dynamic time warping to correct for variations in AT between IM separations, such as due to pressure fluctuations. The tool was demonstrated that for SLIM IM-MS increased Rp by as much as 115% for measurements in which 50 IM separations over 2 s were summed. It is publicly available for use with the universal ion mobility format (.UIMF) and text (.txt) files.

A widely used tool for DTIMS-MS and SLIM-MS is the PNNL-PreProcessor [44], a freely available, standalone, and user-friendly software, housing various algorithmic implementations to generate new files with enhanced signal quality and in the same instrument format. The algorithms effectively extend measurement dynamic range, while reducing file sizes for faster and memory-efficient downstream processing. Specifically, multidimensional smoothing improves peak shapes for low-abundance analyte signals (i.e., poorly defined), and saturation repair reconstructs the intensity profile of high-abundance peaks from various analyte types (improving over a previous algorithm [45]). Other functionalities are data compression and interpolation, IM demultiplexing (improving over a previous version [46]), noise filtering by low intensity threshold and spike removal, and exporting of acquisition metadata. An increase of 19.4% in lipid annotations and a two-times faster processing of LC-DTIMS-DIA-MS data from a complex lipid extract were reported in the original publication. The PNNL-PreProcessor is actively maintained and expanded. The most recent additions are algorithms to transform the raw IM-MS data directly to the CCS/z space (CCS calibration is discussed in the following section) to facilitate data interpretation and standardization, and conversion from IM-DIA-MS to pseudo-data dependent acquisition (DDA) files to enable and leverage utilization of existing LC-MS tools. Figure 2 shows an example of the CCS/z conversion, with visualizations of the original and CCS/z converted raw data for DTIMS-MS and SLIM-MS analyses of the Agilent tune mix. Using this functionality, raw files that were acquired with different IM methods can be analyzed together and, rather than comparing CCS values from centroids, the full CCS distributions from total (or mass extracted) ion intensities in the raw data can be visualized directly and easily compared (Figure 2E), for example, enabling direct determination of the Rp in CCS space. Furthermore, by charge normalizing, ions appear ordered in CCS/z versus *m/z* space as they would in AT versus *m/z* space [47].

In terms of improving IM Rp or separation post data acquisition, two algorithms have been reported. The HRdm is a proprietary GUI-based tool [48] that improves the sensitivity and resolution of multiplexed DTIMS-MS measurements. It performs simultaneous extended Hadamard demultiplexing and deconvolution by incorporating knowledge of the instrumental modulation defects. Results indicated that HRdm performs with an effective Rp of between 180 and 250 for the highest deconvolution settings. The DWSA-PSO algorithm [49], implemented in MATLAB, was reported for separating overlapping IM peaks of glycan isomers in TIMS-MS data using second-order differentiation combined with simulated annealing particle swarm algorithm based on sine adaptive weights.

3.3 | CCS calibration

IM does not measure molecular structures or surfaces directly, but rather the effect the structure has on the mobility of the gas-phase ion's structure in an electric field. A mathematical model of the physics of the measurement is used to translate the mobility value or AT observed with IM-MS instruments into the corresponding molecular ion's CCS [2]. DTIMS is the only platform that is accepted to provide direct CCS determination, that is, independently of reference ions with known CCS, by employing the "stepped-field method" (also called multi-field method). Typically for stepped field, IM-MS data is collected in a series of measurements at seven different stepwise-increased field values. CCS is determined by performing a linear regression of detected peak mobilities or AT centroids, and considering multiple instrument parameters (i.e., electric field, buffer gas, pressure, temperature, and drift-tube effective length). The relationship between CCS, mobility (K), and ion charge state (z) is given by the Mason–Schamp equation, where CCS is seen to be proportional to z/K. Rather than using AT from peak centroids, the FWHMstep method was reported to reconstruct the full CCS distribution from the AT distribution in stepped-field data, correcting the AT peak broadening caused by the ion's diffusion and other instrumental contributions to characterize the analyte's conformational diversity [50]. However, utilization of the stepped-field method has been limited in practice due to several reasons: (1) it is generally not compatible with LC due to the increased acquisition time needed to collect data in multiple fields, (2) it can be sensitive to variations in instrument parameters which requires manual adjustments, and (3) the data processing is laborious; therefore, it becomes impractical for analysis of complex mixtures and for generating very large CCS databases.

Secondary or empirical methods are nowadays preferred for CCS determination, where the CCS of analytes or unknowns is performed by calibration against 'known' CCS values (e.g., from literature or stepped-field experiments) of calibrant species. A calibration curve allows CCS determination of other analytes from their AT measured under the same IM conditions as the calibrants. DTIMS and TIMS data can be CCS-calibrated employing a linear regression, referred to as the "single-field method" in DTIMS. In both platforms, the Agilent Tune Mix solution ions are commonly used as reference for various molecular types, including peptides, metabolites, lipids, and other small molecules. For DTIMS, a separate infusion run of the calibrant ions is collected daily. For TIMS, the calibrant ions are typically infused for a few seconds at the beginning of each sample run.

For TWIMS-based platforms, nonlinear regression methods are typically required for CCS calibration, such as polynomial regression or linearized power regression (equations and details can be found in previous works [2, 51]). Linearized power regression could work best for analytes which are outside of the mass range of the calibrants. There is not an accepted universal set of calibrants for TWIMS, as the choice of calibrants could depend on the type of molecules, and the charge and mass range of the analytes to be calibrated. A series of singly charged polyalanine ions is often used to calibrate the CCS

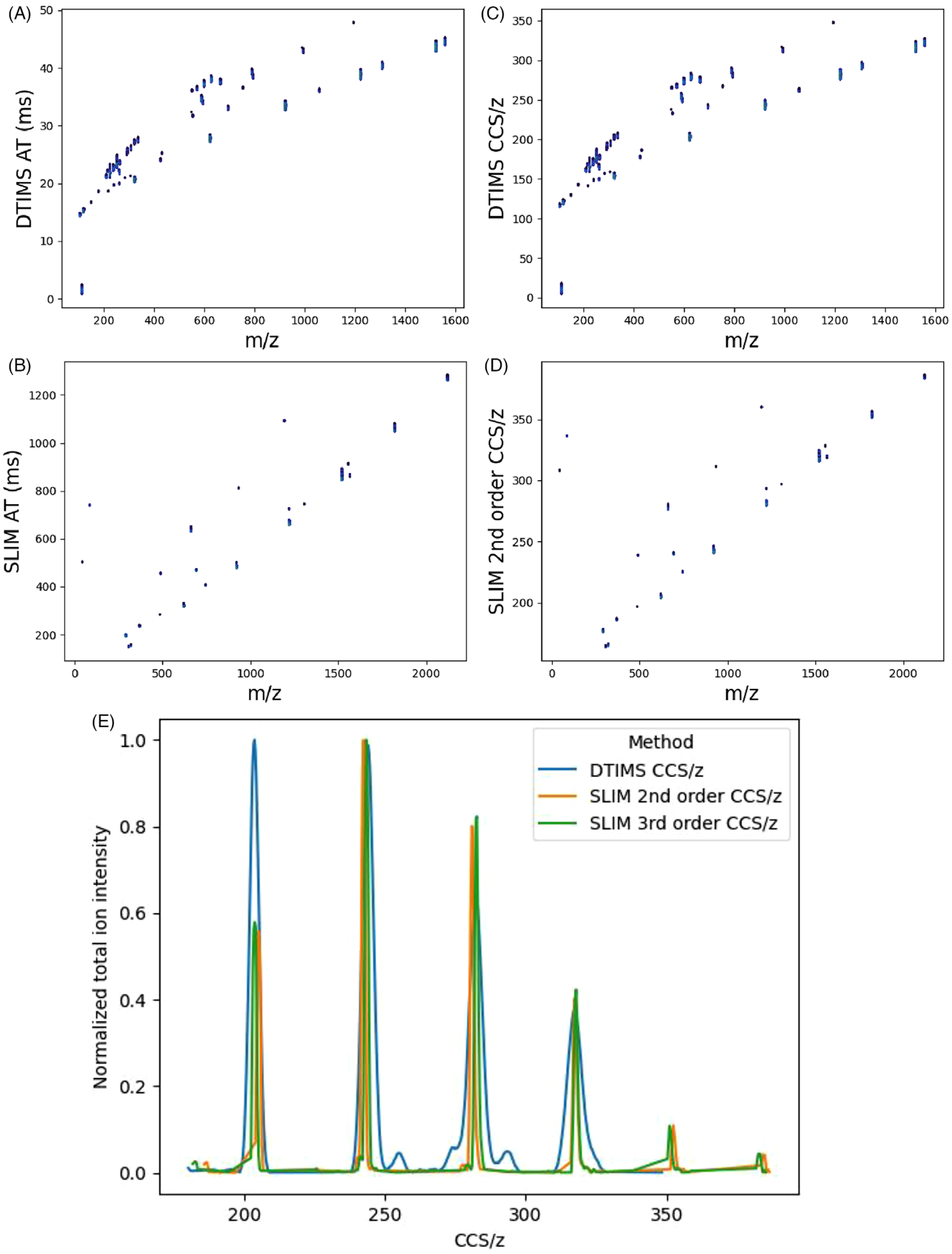

FIGURE 2 Examples of the CCS/z conversion functionality in the PNNL-PreProcessor. Original raw data for DTIMS-MS (A) and SLIM-MS (B) analyses of the Agilent tune mix solution with positive electrospray ionization, and their respective CCS/z converted raw data (C: DTIMS-MS, and D: SLIM-MS). Overlaid total ion intensities (E) from the CCS/z converted raw data allows direct comparison and visualization of the full CCS

for peptides, but limited performance has been reported for small molecules, for example drug-like compounds [52]. The evaluation and optimization of different calibrants and experimental conditions is an active area of research for TWIMS. Similarly, comparability and consolidation of CCS values across different IM platforms is continuously being investigated, since the applied calibration strategy including the selection of calibrant ions has been reported to influence deviations, and strategies for calibration and correction have been proposed [53].

DMA could be used as a primary method for CCS determination given precise knowledge of the applied voltage, DMA dimensions, and fluid flow rates. However, the uncertainty associated with such approaches in development has not yet been fully evaluated, and in many instances calibrant species are employed for CCS determination [2]. DMA is typically used to analyze large ions and charged particles, such as aerosol particles, antibodies, and viruses, and not peptides, lipids, or metabolites [32].

To perform the CCS calibration, the corresponding instrument vendor software is typically used, which stores in the data file the function coefficients determined by the regression, and those can be accessed by downstream processing software to annotate detected features. Example publications using vendor software for CCS calibration include Agilent IM-MS Browser for DTIMS [54] and SLIM [55], Bruker Metaboscape for TIMS [56], and Waters DriftScope for TWIMS [57]. Besides, in numerous instances of proof-of-principle demonstrations the CCS calibration is performed semi-manually in spreadsheets or with custom computer scripts. Alternatively, open-source tools addressing limitations in vendor software have been developed, most notably for automation needed in large-scale analysis. PIXiE [58] allows CCS calculation for the DTIMS stepped-field method from UIMF files. More recently developed, AutoCCS [51] supports CCS calibration for DTIMS and TWIMS-based methods from mzML files, mzapy [41] supports CCS calibration for single-field DTIMS and TWIMS-based methods from MZA files, and MOCCal [59] automates the assignment of unknown features to the power equation for CCS calibration in multiomics mixtures from a spreadsheet of features or mzML files.

3.4 | Peak detection

There are two general approaches to detect peaks from AT distributions and *m/z* values in raw IM-MS data. The first, referred to as untargeted feature detection, attempts to find all possible peaks present in the data and is implemented in most omics workflows. An earlier example tool is the LC-IMS-MS Feature Finder [60], a command line software that clusters deisotoped peaks with similar monoisotopic mass, charge state, RT, and AT values. The second strategy is targeted data extraction, where a list of targets ions of interest or a library is required to selectively extract signals from the raw data. While untargeted feature detection does not require prior knowledge and can allow investigation of unexpected molecules, targeted data extraction can be more sensitive and accurate in detecting noisy or low-abundance ions. In both strategies, common MS methods to detect and centroid values are applied, including local maxima search, wavelet transform, derivative-based detection, or gaussian peak fitting.

3.5 | CCS matching and filtering

CCS values can be used in addition to other molecular identifiers, such as RT and accurate mass, to increase the specificity of the compound identification and feature annotation, and are particularly relevant for distinguishing isomeric species. Typically, experimental CCS values are matched to a library of experimental or predicted CCS values using an error tolerance according to the instrumentation. Representative publications using CCS are referred next.

Silveira et al. performed a conformational analysis in terms of measured IM Rp and CCS of several model peptides by TIMS [61]. The results provided evidence that at least six conformations (four compact and two elongated) comprised the gas phase distribution of a triple-charged analyzed ion.

Leyva et al. employed TIMS coupled to Fourier transform ion cyclotron resonance MS and tandem MS for the structural characterization of dissolved organic matter [62]. Raw data was processed by vendor software for assignment of chemical formulas, afforded by the ultra-high mass Rp. Spectra for each molecular formula were processed using the custom-built Software Assisted Molecular Elucidation (SAME) Python package. Candidate structures were obtained from the chemical formulas by in-silico fragmentation using the MetFrag CL tool [63] and the PubChem database (pubchem.ncbi.nlm.nih.gov), and experimental CCS were compared to theoretical CCS generated using the trajectory method (TM) with the IMoS software.

Bianco et al. used DMA to separate environmentally relevant constitutional (catechol, resorcinol, and hydroquinone) and configurational (cyclohexanediols and fatty acids) isomers [64]. Computational CCS predictions with the Ion Mobility Software (IMoS) could reproduce the experimental order of the ion mobilities between the isomers, confirming the isomer identification.

Rose et al. demonstrated a data processing workflow that combines CCS matching with regression analysis and chemical class prediction to improve confidence in DTIMS-MS lipid annotations [65]. After

distributions of experiments acquired with different IM platforms or acquisition methods, or converted using different CCS calibration functions. Raw data was CCS calibrated using the Agilent IM-MS Browser (linear calibration function was applied to DT, and polynomial of 2nd and 3ed order to SLIM). The PNNL-PreProcessor automatically detects the calibration coefficients for the function and the buffer gas, and then maps each raw data point (*m/z*, AT) to the CCS/z space (*m/z*, CCS) by applying the regression function and scaling to the reduced mass term. Raw files were converted to MZA format. Standard Python packages were used to read the raw data from MZA files and to make the plots. Corresponding data and example Python and R scripts can be found at https://github.com/EMSL-Computing/CCSz-demo. AT, arrival time; CCS, collision-cross section; DT, drift time; DTIMS, drift tube ion mobility spectrometry; MS, mass spectrometry; SLIM, structures for lossless ion manipulations.

untargeted feature detection, filtering candidate compound identifications on the basis of their measured CCS increased the confidence of the annotations of 883 compounds by narrowing their candidate lists by over 50% on average. Using the SIFTER machine learning (ML) algorithm to predict the classification of unidentified features provided insight into the likely classes of 192 additional compounds.

Kedia et al. evaluated the power of SLIM-MS for identifying lipid isomers in total brain and liver lipid extracts by spiking standards at different concentrations [55]. Isomeric resolution and measured AT and CCS values were preserved independently of matrix effects.

4 | CCS DETERMINATION METHODS

The determination of CCS, either directly or using calibration, enables confident identification and structural characterization of compounds including small molecules [74], lipids [75], and even macromolecules like proteins [33]. This is especially useful in the context of omics experiments in which thousands of features could be detected, and the ability to confidently distinguish between closely related compounds or isomers can have significant impact on the ultimate biological interpretation of the results. A primary factor limiting the utility of CCS for compound identification is the availability of reference values (i.e., "known" calibrants) and comprehensive databases to compare against when identifying unknowns. While the measurement and curation of large-scale CCS databases to aid in identification of unknowns is an ongoing effort in the IM field [47, 76–78], due to limited availability of chemical standards, time, and expense constraints, these approaches will never produce coverage across more than a tiny fraction of the vast and diverse small molecule chemical space. An alternative means of identifying unknowns by CCS involves the use of computational methods for predicting CCS values, effectively expanding the identifiable chemical universe far beyond compounds for which reference measurements exist (i.e., a "reference-free" compound identification paradigm). CCS prediction methods can be broadly categorized into one of two general approaches, theory-driven (calculation) or data-driven (prediction), their advantages and tradeoffs are next discussed.

4.1 | Theory-driven CCS calculation

The theory-driven calculation methods rely on understanding and modeling the molecular interactions that underly the IM separation and simulating them to ultimately derive a calculated CCS value. These methods have a long history, and the complexity of the calculations have evolved alongside advancements in the theoretical and practical understanding of the technology itself. The general workflow for theoretical CCS calculation involves generation of low-energy 3D conformers for analytes, followed by calculation of CCS using some representation of the molecular interactions underlying the IM separation. Generation of physically relevant low-energy conformers of analyte ions is a subject deserving much more in-depth discussion than can be offered here [79], but for the purposes of this review it suffices to point out that generally the more complex modeling methods are capable of producing more relevant (i.e., accurate) structures, albeit at the cost of lower computational speed and greater system requirements. As with molecular modeling, there are many methods of calculating CCS that differ primarily in the level of detail with which they simulate the molecular interactions that occur in an IM separation, and the accuracy of calculations generally increases with this complexity.

MOBCAL was the first program developed for calculating theoretical CCS, and used three methods of increasing complexity (projection approximation [PA] [80]; exact hard-sphere scattering [EHS] [81]; TM [82]) as the theoretical basis. The TM, which models momentum transfer between the analyte and drift gas molecules from both direct collisions and long-range interactions, represents the most complete model of IM interactions and thus has served as the gold standard for calculation of accurate CCS values up to the present. The original MOBCAL was only capable of modeling with He as the drift gas; however, it was later modified to use N_2 (the norm for modern IMS instruments) [83]. Later implementations of the TM, including Collidoscope [84], HPCCS [85], and MobCal-MPI [86] were developed with multiprocessing and other program optimizations for greater computational efficiency, but relying on essentially the same or similar theoretical model as used in the original MOBCAL. An implementation of the TM has recently been presented [87], which includes additional complexity in the model that allows for the calculation of CCS values at a level of precision suitable for application to ultra-high-resolution IM separations. The PA and EHS methods are less complex (and generally less accurate) than the TM; however, their significantly faster computation time make them suited for calculating CCS for very large numbers of compounds or much larger compounds that have very high atom count. These approximation methods are especially valuable when high precision or absolute accuracy of the predicted CCS values are not the top priority. Multiple improvements upon the PA/EHS approximation methods implemented in the original MOBCAL have been presented in recent years, including: a projection superposition approximation [88], a diffuse hard-sphere scattering (DHS) model implemented in the tool IMoS [89, 90], and a PA method optimized for very large molecules in the IMPACT tool [91]. The landscape of tools for theoretical CCS calculation is complicated, but broadly speaking the level of complexity of the CCS calculation (and upstream molecular modeling) method is selected to balance the desired level of precision and accuracy against computational time and effort. An added benefit of theory-driven CCS calculation comes from the direct relationship between calculated CCS and the model representing the molecular interactions in IM separations, allowing for interpretation and rationalization of calculation results in chemical/structural terms.

4.2 | Data-driven CCS prediction

In recent years, the accumulation of a critical mass of experimental CCS values available to the IM community has enabled the use of data-driven methods for CCS prediction. These data-driven methods rely on connecting experimentally observed CCS values with various chemical and/or structural properties, increasingly using some manner of AI/ML to learn this complex relationship for large numbers of compounds. CCS values can then be efficiently and accurately predicted for other compounds without experimental CCS values, provided that their properties are similar enough to the compounds used to train the predictive model. The use of data-driven methods for prediction of CCS has been reviewed in-depth elsewhere [76]. Briefly, use of these methods has been enabled by the accumulation in recent years of experimental CCS values for large compound collections. Data-driven CCS prediction uses structural trends among large collections of compounds to predict CCS for similar compounds without reference CCS values. While specific details vary between individual prediction models, the approach can generally be broken into three steps: data curation, feature engineering, and model training. Training data typically consists of large collections of CCS values, either measured experimentally or gathered from published data. In the feature engineering step, a numerical representation of the compounds in the training dataset is chosen, typically consisting of some set of molecular descriptors that reflect the structural characteristics of the compounds. Finally, a model is selected, with the most popular models for this task been support vector machines (SVM), and trained using a proportion of the selected dataset, with the remaining data excluded during training for later use in validating the model's performance. The ultimate accuracy of the trained CCS prediction model is dependent primarily on four key factors stemming primarily from the general steps described above: (1) chemical space of the training data, (2) selection of molecular descriptors, (3) model selection, and (4) the accuracy of the measurements that make up the training data. These prediction models derive their predictions from trends in the data they are trained on, so accurate predictions can only be made for compounds that share structural characteristics with the training data, and that accuracy is bounded by the accuracy of the measurements from which the training data was generated. CCS is a property that reflects the structural characteristics of a molecule, so in order to accurately predict it the strategy for numerically representing molecules in the training data (i.e., feature engineering) must sufficiently reflect those structural characteristics. Model selection is slightly less important than the other factors since the nature of the training data (and features) will to some degree restrict the selection of models for a given task. MetCCS was the first published ML model for prediction of CCS for small molecule metabolites [92]. Multiple other CCS prediction models have been presented in the years since, including DeepCCS [93], AllCCS [77, 94], CCSbase [78, 95], CCS Predictor 2.0 [96], and SigmaCCS [97]. Each of these tools are trained on different sets of compounds and using different models, but they are all generally capable of producing accurate CCS predictions, provided sufficient similarity between the training compounds and those used for CCS prediction. As it has been reported for metabolomics, improvements in ML prediction of CCS values through better training sets will also likely reduce sources of variance [98].

5 | MULTIDIMENSIONAL WORKFLOWS COMBINING IM-MS AND FRONT-END SEPARATIONS

Although IM-MS alone has a wide range of applications, its coupling with front-end separations, such as LC, gas chromatography (GC), supercritical fluid chromatography (SFC), solid-phase extraction (SPE), and capillary electrophoresis (CE), is extremely beneficial for analyses of complex mixtures with increased sensitivity, peak capacity, and the scope of molecular information [99]. Multiple disease screening and environmental evaluations have illustrated that the IM-based multidimensional separations extract information that cannot be acquired with each technique individually. Furthermore, most IM-MS platforms can be operated in DIA mode, which allows systematic collection of multiplexed fragment-ion spectra from all detectable precursors within a wide m/z range and in a single chromatographic run, independently of their intensities [100].

In terms of data processing, peak detection is significantly more challenging than in traditional MS due to the additional dimension of separation. Thus, many algorithms commonly process one dimension at a time in a stepwise fashion (i.e., m/z centroiding or peak picking, followed by RT peak detection, and finally IM peak detection), and some tools adapted from LC-MS workflows simply ignore the IM dimension (completely or partially). Workflows often combine strategies including steps like noise reduction, baseline correction, and multi-sample alignment. To perform fragment ion data processing of DIA spectra, similarly to precursor peak detection, algorithms may employ targeted data extraction or untargeted feature detection followed by fragment ion deconvolution to generate pseudo-DDA MS/MS[100]. For MS/MS annotation in proteomics and lipidomics workflows, prediction of theoretical fragment ions or diagnostic ions is often applied, which is supported by well-established (more in proteomics [72] than in lipidomics [67]) fragmentation rules. These rules are grounded in the fundamental understanding of the chemical properties and structural characteristics of peptides and lipids, and the type of instrumentation and fragmentation energy employed, allowing implementation of algorithms with the specific cleavage patterns and fragmentation pathways that occur within these biomolecular classes. Conversely, in metabolomics, the approach of spectra library matching is widely used for MS/MS annotation due to the absence of universally applicable fragmentation rules. Small molecules encompass a vast array of chemical structures and functional groups, resulting in diverse fragmentation behaviors that are often unpredictable. Additionally, they can undergo multiple fragmentation pathways depending on experimental conditions and instrument parameters. The spectral library matching strategy capitalizes on the wealth of annotated spectra accumulated in spectral libraries, offering a pragmatic solution for metabolite identification despite the inherent complexities of metabolite fragmentation. Moreover, the greater availability of public spectral libraries and modern AI methods are enabling prediction of spectral libraries with increased accuracy for both proteomics and metabolomics applications, offering an alternative to database incompleteness due to unavailability of standard compounds and allowing annotation of unknowns. Finally, rule-based, or ML-based multidimensional scoring algorithms can be utilized to combine the various sub-scores from each dimension (accurate mass, RT, CCS, MS/MS). These processing strategies and software used for different omics studies are next discussed with representative publications.

5.1 | Proteomics

Xia et al. created a set of software tools in R to convert TWIMS data generated from analysis of fragment ions into a variety of formats used in proteomics, including MGF [101]. The authors demonstrated that IM can be used to calculate the charge state of a fragment ion to improve peptide identification and provided preliminary evidence of structural differences between b and y ions for certain peptide sequences.

Distler et al. presented the TWIMS-DIA-MS ultra-definition MSE (UDMSE) method and the Java software package ISOQuant [102]. This approach utilized IM-specific collision-energy profiles to enhance precursor fragmentation efficiency. Results provided high reproducibility and substantially improved proteome coverage of the HeLa cell proteome compared to previous implementations of MS^E. ISOQuant is an open-source software tool for processing label-free quantitative UDMSE from results after preprocessing and database searching with the Waters vendor software ProteinLynx Global SERVER.

Burnum-Johnson et al. described the discovery and targeted monitoring (DTM) approach using LC-DTIMS-MS [103]. In DTM, heavy labeled target peptides are spiked into tryptic digests and both the labeled and unlabeled peptides are detected using LC-IM-MS instrumentation. Compared with the broad LC-MS discovery measurements, DTM yielded greater peptide/protein coverage and detected lower abundance species and with similar limits to selected reaction monitoring (SRM). In this workflow, the authors collected LC-MS/MS data on a Orbitrap platform and performed databased search to build an AMT tag database with accurate masses and RTs. In-house C# algorithms were used for LC-IM-MS feature definition, alignment, and visualization.

Skyline was adapted to analyze LC-IM-DIA-MS data from numerous instrument vendor formats [66]. For the initial evaluation, a tryptic digest of bovine serum albumin (BSA) was spiked into a yeast protein digest at seven different concentrations, and Skyline was able to rapidly analyze LC-DTIMS-DIA-MS data for 38 of the BSA peptides. Chouinard et al. showcased the potential of LC-SLIM-MS for improving the sensitivity, separation, and throughput of phosphoproteomics analyses [104]. Data from a mixture of 51 heavy-labeled phosphopeptides, including positional isomers, were analyzed using Skyline and demonstrated limits of detection as low as 50–100 pM (50–100 amol/μL) for several phosphopeptides. Other example publications using Skyline include Van Puyvelde et al. [105] for TIMS-MS and Rojas Echeverri et al. [57] for TWIMS-MS.

Meier et al. demonstrated the use of the correlation of molecular weight and IM in LC-TIMS-DIA-MS, termed diaPASEF [106]. The authors developed Mobi-DIK software, an extension of the established targeted data extraction OpenSWATH workflow by inclusion of the IM dimension for both signal extraction and scoring. Data acquired from whole proteome digests and mixed organism samples demonstrate deep proteome coverage and a high degree of reproducibility as well as quantitative accuracy, even from 10 ng sample amounts.

Prianichnikov et al. introduced the IM-enhanced MaxQuant software, offering an end-to-end computational workflow for LC-TIMS-DDA-MS shotgun proteomics data [71]. The workflow includes highly parallelizable 4D feature detection and de-isotoping, non-linear multidimensional mass recalibration, a new matching between runs algorithm that utilizes aligned peptide CCS, and MS1 level label-free quantification. Woo et al. described an acquisition and peptide identification method using MaxQuant, called transferring identification based on FAIMS filtering (TIFF), to improve the sensitivity and accuracy of label-free single-cell proteomics [107].

Yu et al. adapted the fast search engine MSFragger for LC-TIMS-DDA-MS data and developed IonQuant for accurate quantification, as a workflow integrated into the FragPipe GUI [72].

Demichev et al. reported the adaptation of the software DIANN for LC-TIMS-DIA-MS data and generation of optimized spectral libraries using FragPipe, as well as the advantage of neural network-based scoring [73]. A 2D peak-picking algorithm detects local maxima (1/K0 or the inverse ion mobility) in the mobility versus m/z space, followed by chromatogram extraction of precursor and fragment ions. The DIANN ion mobility module is also integrated into FragPipe to provide the full workflow within a single GUI.

Reilly et al. developed an integrated pipeline streamlining a fully automated sample preparation platform with FAIMS-DIA-MS and an optimized library-free DIA database search strategy using the proprietary Spectronaut software [108].

Xu et al. demonstrated how Capillary Zone Electrophoresis combined with FAIMS (CZE-FAIMS-MS/MS) can improve top-down proteomics, as FAIMS can efficiently fractionate proteoforms by their size [109]. CZE-FAIMS-MS/MS processed by open-source top-down software boosted the number of proteoform identifications from a yeast sample by nearly three-fold relative to CZE-MS/MS alone.

5.2 | Lipidomics

Paglia et al. presented a review about the state-of-the-art in IM-MS technologies and approaches utilized to support lipidomics and metabolomics applications, and assessed the challenges and opportunities in this growing field [110]. Moran-Garrido et al. summarized the latest developments in IM-MS analyses for lipidomics, focusing on the current acquisition modes and the approaches for pre-treatment and data analysis [111].

LipidIMMS Analyzer is a software to support the accurate identification of lipids in IM-MS, incorporating a large-scale database covering over 260,000 lipids and four-dimensional structural information for each lipid (i.e. m/z, RT, CCS, and MS/MS) [112]. This tool accepts as input the text files defining features and MS/MS (e.g., csv and MGF) generated by various vendor software (Agilent, Bruker, Waters, Sciex).

Two widely used software tools for lipidomics and metabolomics are MS-DIAL and Skyline. MS-DIAL 4 is the upgraded version of MS-DIAL supporting LC-IM-MS data from various instrument platforms [67]. The authors reported a comprehensive lipidome atlas with RT, CCS, and MS/MS and annotated 8051 lipids using human, murine, algal and plant biological samples. Kirkwood et al. reported a protocol about using Skyline for lipidomics [113]. This protocol describes Skyline's expanded capabilities, including small-molecule MS/MS libraries, indexed RT and CCS filtering, and provides a step-by-step description for importing data, predicting RT, validating lipid annotations, exporting results, and editing a manually validated 500+ lipid library. Ross et al. presented a survey of software tools available for the analysis of IM-MS-based lipidomics data and evaluated lipid identifications produced by Skyline and MS-DIAL on open-access data [114].

Rose et al. presented a lipidomics data processing workflow to increase confidence in molecular class annotations based on CCS values [65]. This approach uses class-specific regression models built from a standardized CCS repository (the Unified CCS Compendium) in a parallel scheme that combines a new annotation filtering approach with an ML class prediction strategy.

Ross et al. developed LipidOz, an open-source Python tool for the automated determination of lipid double bond positions from Ozone-induced dissociation DTIMS-MS data, combining traditional automation and deep learning (DL) approaches [42]. The results demonstrate the ability of LipidOz to robustly assign double bond positions for lipid standard mixtures and complex lipid extracts, enabling practical application of OzID in lipidomics studies.

Zhang et al. utilized TIMS-MS coupled with dual-polarity ionization imaging to enable high-throughput in situ profiling of the single-cell lipidome [39]. The authors developed the MSI Parser software for automated data analysis (https://github.com/lingjunli-research/Automatic-MSI-Spectra-Extraction).

5.3 | Metabolomics

Feuerstein et al. reported a DTIMS-MS acquisition method termed quadrupole resolved all ions (IM-QRAI) which allows a DT-directed sequential isolation of precursor ions using mass windows of up to 100 Da, instead of the typical All Ions method that fragments the complete precursor mass range [115]. The authors used MS-DIAL, MS-Finder, and Skyline to process the LC-DTIMS-QRAI-MS data.

Wang et al. reported a study using a 2D-LC-TWIMS-DIA-MS platform for analyzing plant metabolites [116]. The authors used AllCCS for CCS prediction to annotate and distinguish isomeric compounds.

DEIMoS is a Data Extraction for Integrated Multidimensional Spectrometry Python software, demonstrated with LC-DTIMS-DIA-MS data [117]. The tool offers an application programming interface and command-line access for high-dimensional MS data analysis workflows, with functionality for feature detection, sample alignment, CCS calibration, isotope detection, and MS/MS spectral deconvolution.

PeakDecoder is an ML-based algorithm that learns to distinguish true co-elution and co-mobility from raw data and calculates metabolite identification error rates, which was demonstrated with LC-DTIMS-DIA-MS data [118]. The workflow combines MS-DIAL and Skyline with custom R scripts for target-decoy generation and FDR estimation, training, and inference, based on an SVM.

Met4DX is a mass spectrum-oriented bottom-up assembly algorithm for IM-resolved metabolomics, demonstrated with LC-TIMS-DDA-MS data [119]. Rather than detecting the LC-IM-MS features first, the workflow assembles features in a reverse order of multidimensional separation, i.e., from the MS/MS.

Ramtanon et al. described a method for LC-TIMS-DDA-MS for the identification of enzymatic reactivity descriptors in 22 industrial biomass samples, called hydrolysates [120]. The authors performed multivariate data analysis through principal component analysis (PCA) to view data trends and pinpoint potential hydrolysate chemical descriptors.

MZmine 3 is the newest MZmine version adapted to process LC-IM-MS data to perform feature detection and alignment, resolving peak shapes in both the RT and IM dimension in LC-IM-MS and extracting IM-resolved ion image features with spatial distributions in IM-MS imaging demonstrated with LC-TIMS-MS data [68].

Asef et al. presented a workflow for LC-TWIMS-MS incorporating de novo molecular formula annotation and predicted MS/MS structure elucidation using SIRIUS 4 with ML CCS predictions to identify differential unknown metabolites in mutant strains of *Caenorhabditis elegans* [98]. Predicted MS/MS were matched to Orbitrap runs with same LC conditions.

Wen et al. developed a structural similarity networking assisted CCS prediction interval filtering (SSN-CCSPIF) strategy for compound identification in complex matrices, implemented in Python and demonstrated with LC-DTIMS-MS data [121].

Lu et al. utilized LC-DTIMS-MS for the characterization of riverine and coastal dissolved organic matter [122]. The authors employed vendor software for processing their data and annotation using ChemSpider and the METLIN Metabolites database.

Finally, a handful of tools have been reported for GC-IM-MS workflows. The GCIMS R package includes denoising, baseline correction, spectral and chromatographic alignment, peak detection, integration, and peak clustering [123]. Other tools for GC-IM include a Python package [124] and a workflow in MATLAB [125]. Calle et al. reported the utilization of the IM summed spectrum combined with ML algorithms for the characterization of fruit juices from GC-IM-MS data [126].

6 | MASS SPECTROMETRY IMAGING COMBINED WITH IM-MS

Mass spectrometry imaging (MSI) enables determination of the spatial distribution of analytes in an untargeted fashion and from a wide variety of samples [127]. In MSI, small regions ("pixels") of a planar sample are ionized and analyzed by MS, and by scanning these small areas across the *x* and *y* dimensions of the sample an "image" can be built up with mass spectra taking the place of light intensity in an optical image. Due to the typical configurations which involve directly ionizing analytes from the surface of the sample, MSI is usually incompatible with front-end separations such as LC, which can contribute to issues such as ion suppression or identification uncertainty due to presence of isomers. Analysis time of front-end separations is also a contributing factor since many hundreds or even thousands of pixels may need to be analyzed in order to build up an image of sufficient detail to be useful. However, since IM is performed in the gas phase after ionization, and separations occur on a millisecond timescale, hyphenated IM-MSI techniques are seeing increased usage as the IM separation enhances sensitivity and identification confidence without significantly reducing the analytical throughput.

The landscape of software tools for processing MSI data has been reviewed recently [128] but information specific to IM-MSI is limited. Searching the literature for published studies including IM-MSI revealed surprisingly few tools in regular use. The majority of published IM-MSI data were analyzed using vendor-provided software: High Definition Imaging (https://www.waters.com/waters/en_US/High-Definition-Imaging-(HDI)-Software/nav.htm?cid = 134833914) for data acquired on TWIMS-MS systems or SCiLS Lab (https://www.bruker.com/en/products-and-solutions/mass-spectrometry/ms-software/scils-lab.html) for data acquired on TIMS-MS systems. A smaller but notable proportion of studies used custom software tools, written in MATLAB [129–134] or Python [135–140], for analysis of their IM-MSI data. Another tool, IM-MSIC, was developed as a set of scripts that coordinate between Skyline and vendor software (Agilent Mass Hunter/Mass Profiler) to produce ion images from IM-MSI data acquired on DTIMS-MS systems [141]. At a high level, the software tools that are available for analysis of IM-MSI data are highly vendor-specific and dependent on the experimental platform used to acquire the data. In cases where data are acquired on a non-standard setup or the vendor-specific software does not adequately accommodate the experimental design/data analysis procedure, custom scripting, and/or software tools are necessary.

7 | MACROMOLECULES AND MACROMOLECULAR COMPLEXES CHARACTERIZATION BY IM-MS

Over the past two decades, the investigation of macromolecular biomolecules with IM-MS techniques has provided substantial insights into the field of structural biology. An IM-MS workflow applied to a given target analyte provides mass, charge, and conformation information, which can be used to discern structural information [33]. An overview and introduction to the software tools available for native IM-MS are included in the perspectives by Allison et al. about computational tools [142] and related challenges [143]. Specific examples of tools and applications are next described.

Stojko et al. reported an approach combining characteristics obtained by native MS with TWIMS-MS conformational screening, using vendor software [144]. The authors concluded that the method was valuable in characterizing extremely subtle dynamic conformational changes induced when ligands bind to protein assemblies.

Collision induced unfolding (CIU) has emerged as a valuable technique for distinguishing protein and protein complexes through their distinct unfolding pathways in the gas phase. The CIU fingerprint is a 3D contour plot of ion intensity as a function of activation voltage and AT. The most popular software tools used for analyzing CIU data include CIUSuite and ORIGAMI. CIUSuite 2[69] and its predecessor, CIUSuite [145], are Python packages for TWIMS-MS and DTIMS-MS. CIUSuite 2 includes preprocessing, feature detection, and ML classification methods. The ORIGAMI software [146] is for TWIMS-MS applied to multimeric protein assemblies, which allows the user to visualize the effect of activation on the mobility of the parent ion, as well as on any fragment ion. The user can export the data in the form of heat maps, waterfall, or wire plots as well as interactive and shareable webpages.

UniDec, the Universal Deconvolution software, provides a rapid, robust, and flexible deconvolution of IM-MS data for protein assemblies and lipoprotein Nanodiscs with minimal user intervention [70]. Incorporation of the charge-state distribution in Bayesian prior probabilities provides separation of the *m/z* dimension into its physical mass and charge components. The tool has been demonstrated with data from TWIMS-MS, FAIMS-MS, and DTIMS-MS, and recently incorporated updates for batch processing [147].

Baird et al. showcased the use of FAIMS-MS and MS/MS analyses to study histone tail variants representative of proteoforms in the middle–down range [148].

Cole et al. examined early stages of insulin fibrillogenesis by an in-house modified DTIMS-MS instrument [149]. Molecular modeling (MOBCAL software implementing the TM) was performed to identify and characterize oligomers formed during the "lag" phase that precedes fibril growth.

Degiacomi et al. reported EM∩IM, a Python software that allows both the calculation of a CCS from an electron density map, and the exploitation of IM-MS data to augment the interpretation of electron microscopy data [150]. EM∩IM thereby facilitates the use of data obtained in the gas phase within structural biology studies employing diverse experimental methodologies.

8 | CONCLUDING REMARKS

Software is as important as hardware and the combination of IM-MS empowered by current software tools is revolutionizing numerous and diverse fields of molecular characterization. While its strength in distinguishing molecular analytes with indistinguishable masses is evident, challenges in data processing remain. The very size of IM-MS data, particularly when combined with front-end separations such as LC, has posed challenges to bioinformaticians and researchers, underscoring the necessity for robust and efficient computational tools. The sheer volume of data necessitates solutions, including raw data formats, that can handle vast datasets with efficiency, accuracy, and speed. The proposed data conversion tool and format MZA [37] is addressing these limitations, providing easy, cross-platform, and cross-programming language access to raw IM-MS-data, and enabling fast development of new tools.

Instrumentation in IM-MS is increasingly on an evolutionary trajectory. Continuous advancements are anticipated that would not only enhance data acquisition rates but also the quality and Rp of the data. As these improvements manifest, there will be a growing need for computational tools to evolve in tandem. This is particularly the case for advancements in data acquisition rates and Rp as these will further increase IM-MS data file sizes, which already poses a challenge for software development. The co-evolution of both instrumentation and software tools will be crucial in harnessing the full potential of IM-MS, ensuring its effective application in analysis of complex samples such as MS-based omics.

Conversely, the accessibility and usability of computational tools is as vital as their analytical capabilities. Open-source software is highly encouraged, as it is crucial for reproducible science and promotes a large and active community of developers and users who provide support and help to improve the software. At the same time, recognizing that many end-users of IM-MS may not be experts in programming or data science, there is a compelling need for software tools to feature user-friendly GUIs. Tools should be both, scalable for large-scale processing, and user-friendly for end-users. Tools with intuitive GUIs empower a wider audience, including those without a technical computational background, to engage in data analysis seamlessly. The incorporation of GUIs simplifies complex procedures and reduces data analysis efforts, making the software more accessible, and thereby broadening its application, robustness, reliability, and therefore widespread adoption by the IM-MS community. The advancement in IM-MS should not only be in the realms of hardware and algorithmic prowess but equally in ensuring that these tools are democratized for all users. Similarly, rather than a burden, these software tools should be easy to deploy or install. A simple and functional GUI should be preferred over a fancy one which is difficult to deploy, use, and maintain. It is important to acknowledge that these software engineering tasks require time and resources, especially when aiming to develop code that is robust, well-documented, tested, and performant, and this effort should be supported. An emphasis on creating software that is scalable, easy to deploy, and integrates complex functionalities beneath an easy-to-navigate interface will be paramount to the widespread adoption and success of IM-MS analysis across all fields.

In terms of algorithms, there is a discernible shift toward the adoption of AI/ML in data analysis. For instance, PeakDecoder learning from raw data and using an SVM for scoring annotations and FDR analysis in metabolomics LC-IM-DIA-MS, and LipidOz applying DL to assign double-bond positions in lipids from raw LC-IM-MS data. The inherent capability of modern AI methods to learn from and make predictions on very complex data makes them valuable allies in discerning complex patterns and understanding the intricacies of IM-MS data. Likewise, while most computational MS tools are tailored to a specific omics domain, the community could glean insights from diverse omics disciplines and merge concepts across fields rather than operating in isolation. Leveraging modern AI techniques offers an exciting opportunity to reimagine multiomics solutions, enabling algorithms to operate seamlessly from the raw data level to identify, quantify, and integrate information from different types of molecules. In the foreseeable future, one can expect a surge in tools that integrate AI/ML, not only to achieve fully automated molecular annotation and interpretation of results, but to enhance Rp and predict and optimize experimental conditions.

In conclusion, while the current landscape of computational tools for IM-MS analysis is promising, there are panoramas yet to be explored. Challenges remain, but the synergistic confluence of advanced instrumentation and AI/ML points toward an optimistic and bright future for IM-MS data analysis. We expect that this review will serve as a reference for both novice and established researchers, to learn and improve their IM-MS-based molecular investigations, as well as to get inspiration to develop innovative software tools and algorithms. As research in this arena progresses, innovative solutions are expected that will further democratize and amplify the capabilities of IM-MS across varied applications to derive new molecular knowledge.

AUTHOR CONTRIBUTIONS

Aivett Bilbao conceived and supervised this work, and wrote the manuscript with input from all authors. Dylan H. Ross wrote the Introduction, CCS determination methods, and MSI sections, and drafted the manuscript revision. Harsh Bhotika contributed to the tables, literature survey, and Python and R scripts for Figure 2. Xueyun Zheng wrote the Instrumentation section. Richard D. Smith and Kristin E. Burnum-Johnson acquired funding. All authors reviewed and approved the manuscript.

ACKNOWLEDGMENTS

The authors thank the contributions for the implementation of the CCS/z conversion functionality in the PNNL-PreProcessor from Dr. John C. Fjeldsted and Dr. Sarah M. Stow (conceptualization and evaluation), and Bryson C. Gibbons (code refinements). This work was funded by the National Institute of General Medical Sciences grant P41 GM103493, and the U.S. Department of Energy (DOE), Office of Science, Biological and Environmental Research (BER) program, Early Career Research Program Award to K.E.B.-J. Portions of this work were supported by the Environmental Molecular Sciences Laboratory (EMSL), a DOE Office of Science User Facility sponsored by the Biological and Environmental Research program under Contract No. DE-AC05-76RL01830.

CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

None.

ORCID

Dylan H. Ross https://orcid.org/0009-0005-2943-2282
Xueyun Zheng https://orcid.org/0000-0001-9782-4521
Aivett Bilbao https://orcid.org/0000-0003-2985-8249

REFERENCES

1. Burnum-Johnson, K. E., Zheng, X., Dodds, J. N., Ash, J., Fourches, D., Nicora, C. D., Wendler, J. P., Metz, T. O., Waters, K. M., Jansson, J. K., Smith, R. D., & Baker, E. S. (2019). Ion mobility spectrometry and the omics: Distinguishing isomers, molecular classes and contaminant ions in complex samples. *Trends in Analytical Chemistry*, *116*, 292–299. http://doi.org/10.1016/j.trac.2019.04.022
2. Gabelica, V., Shvartsburg, A. A., Afonso, C., Barran, P., Benesch, J. L. P., Bleiholder, C., Bowers, M. T., Bilbao, A., Bush, M. F., Campbell, J. L., Campuzano, I. D. G., Causon, T., Clowers, B. H., Creaer, C. S., Pauw, E. D., Far, J., Fernandez-Lima, F., Fjeldsted, J. C., Giles, K., … Wyttenbach, T. (2019). Recommendations for reporting ion mobility Mass Spectrometry measurements. *Mass Spectrometry Reviews*, *38*(3), 291–320. http://doi.org/10.1002/mas.21585
3. May, J. C., & McLean, J. A. (2022). Integrating ion mobility into comprehensive multidimensional metabolomics workflows: Critical considerations. *Metabolomics*, *18*(12), 104. http://doi.org/10.1007/s11306-022-01961-0
4. Mason, E., & McDaniel, E. (1998). *Transport properites of ions in gases*. Wiley.
5. Cohen, M. J., & Karasek, F. W. (1970). Plasma Chromatography™—A new dimension for gas chromatography and mass spectrometry. *Journal of Chromatographic Science*, *8*(6), 330–337. http://doi.org/10.1093/chromsci/8.6.330
6. Hoaglund, C. S., Valentine, S. J., Sporleder, C. R., Reilly, J. P., & Clemmer, D. E. (1998). Three-dimensional ion mobility/TOFMS analysis of electrosprayed biomolecules. *Analytical Chemistry*, *70*(11), 2236–2242. http://doi.org/10.1021/ac980059c
7. Wyttenbach, T., Kemper, P. R., & Bowers, M. T. (2001). Design of a new electrospray ion mobility mass spectrometer. *International Journal of Mass Spectrometry*, *212*(1–3), 13–23. https://doi.org/10.1016/S1387-3806(01)00517-6
8. Tang, K., Shvartsburg, A. A., Lee, H. N., Prior, D. C., Buschbach, M. A., Li, F., Tolmachev, A. V., Anderson, G. A., & Smith, R. D. (2005). High-sensitivity ion mobility spectrometry/mass spectrometry using electrodynamic ion funnel interfaces. *Analytical Chemistry*, *77*(10), 3330–3339. http://doi.org/10.1021/ac048315a
9. Ibrahim, Y. M., Baker, E. S., Danielson, W. F., Norheim, R. V., Prior, D. C., Anderson, G. A., Belov, M. E., & Smith, R. D. (2015). Development of a new ion mobility time-of-flight mass spectrometer. *International Journal of Mass Spectrometry*, *377*, 655–662. https://doi.org/10.1016/j.ijms.2014.07.034
10. May, J. C., Goodwin, C. R., Lareau, N. M., Leaptrot, 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. (2014). Conformational ordering of biomolecules in the gas phase: Nitrogen collision cross sections measured on a prototype high resolution drift tube ion mobility-mass spectrometer. *Analytical Chemistry*, *86*(4), 2107–2116. http://doi.org/10.1021/ac4038448
11. Pringle, S. D., Giles, K., Wildgoose, J. L., Williams, J. P., Slade, S. E., Thalassinos, K., Bateman, R. H., Bowers, M. T., & Scrivens, J. H. (2007). An investigation of the mobility separation of some peptide and protein ions using a new hybrid quadrupole/travelling wave IMS/oa-ToF instrument. *International Journal of Mass Spectrometry*, *261*(1), 1–12. https://doi.org/10.1016/j.ijms.2006.07.021
12. Michelmann, K., Silveira, J. A., Ridgeway, M. E., & Park, M. A. (2015). Fundamentals of trapped ion mobility spectrometry. *Journal of the American Society for Mass Spectrometry*, *26*(1), 14–24. http://doi.org/10.1007/s13361-014-0999-4
13. Brown, L. J., & Creaser, C. S. F (2013). Asymmetric waveform ion mobility spectrometry analysis of proteins and peptides: A review. *Current Analytical Chemistry*, *9*(2), 192–198.
14. Guevremont, R. (2004). High-field asymmetric waveform ion mobility spectrometry: A new tool for mass spectrometry. *Journal of Chromatography A*, *1058*(1-2), 3–19. http://doi.org/10.1016/j.chroma.2004.08.119
15. Kolakowski, B. M., & Mester, Z. (2007). Review of applications of high-field asymmetric waveform ion mobility spectrometry (FAIMS) and differential mobility spectrometry (DMS). *Analyst*, *132*(9), 842–864. http://doi.org/10.1039/b706039d
16. Rus, J., Moro, D., Sillero, J. A., Royuela, J., Casado, A., Estevez-Molinero, F., & Fernández de la Mora, J. (2010). IMS–MS studies based on coupling a differential mobility analyzer (DMA) to commercial API–MS systems. *International Journal of Mass Spectrometry*, *298*(1–3), 30–40. https://doi.org/10.1016/j.ijms.2010.05.008
17. Fernandez-Lima, F., Kaplan, D. A., Suetering, J., & Park, M. A. (2011). Gas-phase separation using a trapped ion mobility spectrometer. *International Journal for Ion Mobility Spectrometry*, *14*(2), 93–98. http://doi.org/10.1007/s12127-011-0067-8
18. Mäkinen, M. A., Anttalainen, O. A., & Sillanpää, M. E. T. (2010). Ion mobility spectrometry and its applications in detection of chemical warfare agents. *Analytical Chemistry*, *82*(23), 9594–9600. http://doi.org/10.1021/ac100931n
19. Armenta, S., Alcala, M., & Blanco, M. (2011). A review of recent, unconventional applications of ion mobility spectrometry (IMS). *Analytica Chimica Acta*, *703*(2), 114–123. https://doi.org/10.1016/j.aca.2011.07.021
20. Lapthorn, C., Pullen, F., & Chowdhry, B. Z. (2013). Ion mobility spectrometry-mass spectrometry (IMS-MS) of small molecules: Separating and assigning structures to ions. *Mass Spectrometry Reviews*, *32*(1), 43–71. http://doi.org/10.1002/mas.21349
21. Lanucara, F., Holman, S. W., Gray, C. J., & Eyers, C. E. (2014). The power of ion mobility-mass spectrometry for structural characterization and the study of conformational dynamics. *Nature Chemistry*, *6*(4), 281–294. http://doi.org/10.1038/nchem.1889
22. Chouinard, C. D., Wei, M. S., Beekman, C. R., Kemperman, R. H. J., & Yost, R. A. (2016). Ion mobility in clinical analysis: Current progress and future perspectives. *Clinical Chemistry*, *62*(1), 124–133. http://doi.org/10.1373/clinchem.2015.238840
23. Ibrahim, Y. M., Hamid, A. M., Deng, L. L., Garimella, S. V. B., Webb, I. K., Baker, E. S., & Smith, R. D. (2017). New frontiers for mass spectrometry based upon structures for lossless ion manipulations. *Analyst*, *142*(7), 1010–1021. http://doi.org/10.1039/c7an00031f
24. 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., Norherim, R. V., Anderson, G. A., Tolmachev, A. V., & Smith, R. D. (2016). Ion mobility separations of isomers based upon long path length structures for lossless ion manipulations combined with mass spectrometry. *ChemistrySelect*, *1*(10), 2396–2399. http://doi.org/10.1002/slct.201600460
25. Deng, L., Ibrahim, Y. M., Hamid, A. M., Garimella, S. V. B., Webb, I. K., Zheng, X., Prost, S. A., Sandoval, J. A., Norheim, R. V., Anderson, G. A., Tolmachev, A. V., Baker, E. S., & Smith, R. D. (2016). Ultra-high resolution ion mobility separations utilizing traveling waves in a 13 m serpentine path length structures for lossless ion manipulations module. *Analytical Chemistry*, *88*(18), 8957–8964. http://doi.org/10.1021/acs.analchem.6b01915
26. Ibrahim, Y. M., Hamid, A. M., Deng, L., Garimella, S. V. B., Webb, I. K., Baker, E. S., & Smith, R. D. (2017). New frontiers for mass spectrometry based upon structures for lossless ion manipulations. *Analyst*, *142*(7), 1010–1021. http://doi.org/10.1039/C7AN00031F
27. May, J. C., Leaptrot, K. L., Rose, B. S., Moser, K. L. W., Deng, L., Maxon, L., DeBord, D., & McLean, J. A. (2021). Resolving power and collision cross section measurement accuracy of a prototype high-resolution ion mobility platform incorporating structures for lossless ion manipulation. *Journal of the American Society for Mass Spectrometry*, *32*(4), 1126–1137. http://doi.org/10.1021/jasms.1c00056
28. Giles, K., Ujma, J., Wildgoose, J., Pringle, S., Richardson, K., Langridge, D., & Green, M. (2019). A cyclic ion mobility-mass spectrometry

system. *Analytical Chemistry*, *91*(13), 8564–8573. http://doi.org/10.1021/acs.analchem.9b01838
29. Liu, F. C., Ridgeway, M. E., Park, M. A., & Bleiholder, C. (2022). Tandem-trapped ion mobility spectrometry/mass spectrometry (tTIMS/MS): A promising analytical method for investigating heterogenous samples. *Analyst*, *147*(11), 2317–2337. http://doi.org/10.1039/D2AN00335J
30. Pellegrinelli, R. P., Yue, L., Carrascosa, E., Ben Faleh, A., Warnke, S., Bansal, P., & Rizzo, T. R. (2022). A new strategy coupling ion-mobility-selective CID and cryogenic IR spectroscopy to identify glycan anomers. *Journal of the American Society for Mass Spectrometry*, *33*(5), 859–864. http://doi.org/10.1021/jasms.2c00043
31. Dodds, J. N., & Baker, E. S. (2019). Ion mobility spectrometry: Fundamental concepts, instrumentation, applications, and the road ahead. *Journal of the American Society for Mass Spectrometry*, *30*(11), 2185–2195. http://doi.org/10.1007/s13361-019-02288-2
32. Kartowikromo, K. Y., Olajide, O. E., & Hamid, A. M. (2023). Collision cross section measurement and prediction methods in omics. *Journal of Mass Spectrometry*, *58*(9), e4973. http://doi.org/10.1002/jms.4973
33. Christofi, E., & Barran, P. (2023). Ion mobility mass spectrometry (IM-MS) for structural biology: Insights gained by measuring mass, charge, and collision cross section. *Chemistry Review*, *123*(6), 2902–2949. http://doi.org/10.1021/acs.chemrev.2c00600
34. Delafield, D. G., Lu, G. Y., Kaminsky, C. J., & Li, L. J. (2022). High-end ion mobility mass spectrometry: A current review of analytical capacity in omics applications and structural investigations. *TrAC Trends in Analytical Chemistry*, *157*, 116761. http://doi.org/10.1016/j.trac.2022.116761
35. Deutsch, E. (2008). mzML: A single, unifying data format for mass spectrometer output. *Proteomics*, *8*(14), 2776–2777. http://doi.org/10.1002/pmic.200890049
36. Chambers, M. C., Maclean, B., Burke, R., Amodei, D., Ruderman, D. L., Neumann, S., Gatto, L., Fischer, B., Pratt, B., Egertson, J., Hoff, K., Kessner, D., Tasman, N., Shulman, N., Frewen, B., Baker, T. A., Brusniak, M.-Y., Paulse, C., Creasy, D., … Mallick, P. (2012). A cross-platform toolkit for mass spectrometry and proteomics. *Nature Biotechnology*, *30*(10), 918–920. http://doi.org/10.1038/nbt.2377
37. Bilbao, A., Ross, D. H., Lee, J. Y., Donor, M. T., Williams, S. M., Zhu, Y., Ibrahim, Y. M., Smith, R. D., & Zheng, X. (2023). MZA: A data conversion tool to facilitate software development and artificial intelligence research in multidimensional mass spectrometry. *Journal of Proteome Research*, *22*(2), 508–513. http://doi.org/10.1021/acs.jproteome.2c00313
38. Schramm, T., Hester, Z., Klinkert, I., Both, J. P., Heeren, R. M. A., Brunelle, A., Laprevote, O., Desbenoit, N., Robbe, M. F., Stoeckli, M., Spengler, B., & Römpp, A. (2012). imzML–A common data format for the flexible exchange and processing of mass spectrometry imaging data. *Journal of Proteomics*, *75*(16), 5106–5110. http://doi.org/10.1016/j.jprot.2012.07.026
39. Zhang, H., Liu, Y., Fields, L., Shi, X., Huang, P., Lu, H., Schneider, A. J., Tang, X., Puglielli, L., Welham, N. V., & Li, L. (2023). Single-cell lipidomics enabled by dual-polarity ionization and ion mobility-mass spectrometry imaging. *Nature Communications*, *14*(1), 5185. http://doi.org/10.1038/s41467-023-40512-6
40. Willems, S., Voytik, E., Skowronek, P., Strauss, M. T., & Mann, M. (2021). AlphaTims: Indexing trapped ion mobility spectrometry-TOF data for fast and easy accession and visualization. *Molecular & Cellular Proteomics*, *20*, 100149. http://doi.org/10.1016/j.mcpro.2021.100149
41. Ross, D., Bilbao, A., Lee, J. Y., & Zheng, X. (2023). mzapy: An open-source python library enabling efficient extraction and processing of ion mobility spectrometry-mass spectrometry data in the MZA file format. *Analytical Chemistry*, *95*(25), 9428–9431. http://doi.org/10.1021/acs.analchem.3c01653
42. Ross, D. H., Lee, J. Y., Bilbao, A., Orton, D. J., Eder, J. G., Burnet, M. C., Deatherage Kaiser, B. L., Kyle, J. E., & Zheng, X. (2023). LipidOz enables automated elucidation of lipid carbon-carbon double bond positions from ozone-induced dissociation mass spectrometry data. *Communications Chemistry*, *6*(1), 74. http://doi.org/10.1038/s42004-023-00867-9
43. Hollerbach, A. L., Conant, C. R., Nagy, G., Monroe, M. E., Gupta, K., Donor, M., Giberson, C. M., Garimella, S. V. B., Smith, R. D., & Ibrahim, Y. M. (2021). Dynamic time-warping correction for shifts in ultrahigh resolving power ion mobility spectrometry and structures for lossless ion manipulations. *Journal of the American Society for Mass Spectrometry*, *32*(4), 996–1007. http://doi.org/10.1021/jasms.1c00005
44. Bilbao, A., Gibbons, B. C., Stow, S. M., Kyle, J. E., Bloodsworth, K. J., Payne, S. H., Smith, R. D., Ibrahim, Y. M., Baker, E. S., & Fjeldsted, J. C. (2022). A preprocessing tool for enhanced ion mobility-mass spectrometry-based omics workflows. *Journal of Proteome Research*, *21*(3), 798–807. http://doi.org/10.1021/acs.jproteome.1c00425
45. Bilbao, A., Gibbons, B. C., Slysz, G. W., Crowell, K. L., Monroe, M. E., Ibrahim, Y. M., Smith, R. D., Payne, S. H., & Baker, E. S. (2018). An algorithm to correct saturated mass spectrometry ion abundances for enhanced quantitation and mass accuracy in omic studies. *International Journal of Mass Spectrometry*, *427*, 91–99. http://doi.org/10.1016/j.ijms.2017.11.003
46. 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. (2014). Detecting and removing data artifacts in Hadamard transform ion mobility-mass spectrometry measurements. *Journal of the American Society for Mass Spectrometry*, *25*(12), 2020–2027. http://doi.org/10.1007/s13361-014-0895-y
47. Picache, J. A., Rose, B. S., Balinski, A., Leaptrot, K. L., Sherrod, S. D., May, J. C, & McLean, J. A. (2019). Collision cross section compendium to annotate and predict multi-omic compound identities. *Chemical Science*, *10*(4), 983–993. http://doi.org/10.1039/c8sc04396e
48. May, J. C., Knochenmuss, R., Fjeldsted, J. C., & McLean, J. A. (2020). Resolution of isomeric mixtures in ion mobility using a combined demultiplexing and peak deconvolution technique. *Analytical Chemistry*, *92*(14), 9482–9492. http://doi.org/10.1021/acs.analchem.9b05718
49. Hu, X., Zhou, J., Li, J., Gao, W., Zhou, J., Yu, J., & Tang, K. (2023). An improved algorithm for resolving overlapping peaks in ion mobility spectrometry and its application to the separation of glycan isomers. *Analyst*, *148*, 5514–5524. http://doi.org/10.1039/d3an01042b
50. Marchand, A., Livet, S., Rosu, F., & Gabelica, V. (2017). Drift tube ion mobility: How to reconstruct collision cross section distributions from arrival time distributions? *Analytical Chemistry*, *89*(23), 12674–12681. http://doi.org/10.1021/acs.analchem.7b01736
51. Lee, J. Y., Bilbao, A., Conant, C. R., Bloodsworth, K. J., Orton, D. J., Zhou, M., Wilson, J. W., Zheng, X., Webb, I. K., Li, A., Hixson, K. K., Fjeldsted, J. C., Ibrahim, Y. M., Payne, S. H., Jansson, C., Smith, R. D., & Metz, T. O. (2021). AutoCCS: Automated collision cross-section calculation software for ion mobility spectrometry-mass spectrometry. *Bioinformatics*, *37*(22), 4193–4201. http://doi.org/10.1093/bioinformatics/btab429
52. Hines, K. M., Ross, D. H., Davidson, K. L., Bush, M. F., & Xu, L. (2017). Large-scale structural characterization of drug and drug-like compounds by high-throughput ion mobility-mass spectrometry. *Analytical Chemistry*, *89*(17), 9023–9030. http://doi.org/10.1021/acs.analchem.7b01709
53. Feuerstein, M. L., Hernandez-Mesa, M., Valadbeigi, Y., Le Bizec, B., Hann, S., Dervilly, G., & Causon, T. (2022). Critical evaluation of the role of external calibration strategies for IM-MS. *Analytical and Bioanalytical Chemistry*, *414*(25), 7483–7493. http://doi.org/10.1007/s00216-022-04263-5

54. Kurulugama, R. T., Darland, E., Kuhlmann, F., Stafford, G., & Fjeldsted, J. (2015). Evaluation of drift gas selection in complex sample analyses using a high performance drift tube ion mobility-QTOF mass spectrometer. *Analyst*, *140*(20), 6834–6844. http://doi.org/10.1039/c5an00991j
55. Kedia, K., Harris, R., Ekroos, K., Moser, K. W., DeBord, D., Tiberi, P., Goracci, L., Zhang, N. R., Wang, W., Spellman, D. S., & Bateman, K. (2023). Investigating performance of the SLIM-based high resolution ion mobility platform for separation of isomeric phosphatidylcholine species. *Journal of the American Society for Mass Spectrometry*, *34*(10), 2176–2186. http://doi.org/10.1021/jasms.3c00157
56. Schroeder, M., Meyer, S. W., Heyman, H. M., Barsch, A., & Sumner, L. W. (2019). Generation of a collision cross section library for multi-dimensional plant metabolomics using UHPLC-trapped ion mobility-MS/MS. *Metabolites*, 10(1). http://doi.org/10.3390/metabo10010013
57. Rojas Echeverri, J. C., Volke, D., Milkovska-Stamenova, S., & Hoffmann, R. (2022). Evaluating peptide fragment ion detection using traveling wave ion mobility spectrometry with signal-enhanced MS(E) (SEMS(E)). *Analytical Chemistry*, *94*(31), 10930–10941. http://doi.org/10.1021/acs.analchem.2c00461
58. Ma, J., Casey, C. P., Zheng, X., Ibrahim, Y. M., Wilkins, C. S., Renslow, R. S., Thomas, D. G., Payne, S. H., Monroe, M. E., Smith, R. D., Teeguarden, J. G., Baker, E. S., & Metz, T. O. (2017). PIXiE: An algorithm for automated ion mobility arrival time extraction and collision cross section calculation using global data association. *Bioinformatics*, *33*(17), 2715–2722. http://doi.org/10.1093/bioinformatics/btx305
59. Hynds, H. M., & Hines, K. M. (2024). MOCCal: A multiomic CCS calibrator for traveling wave ion mobility mass spectrometry. *Analytical Chemistry*, *96*(3), 1185–1194. http://doi.org/10.1021/acs.analchem.3c04290
60. Crowell, K. L., Slysz, G. W., Baker, E. S., LaMarche, B. L., Monroe, M. E., Ibrahim, Y. M., Payne, S. H., Anderson, G. A., & Smith, R. D. (2013). LC-IMS-MS feature finder: Detecting multidimensional liquid chromatography, ion mobility and mass spectrometry features in complex datasets. *Bioinformatics*, *29*(21), 2804–2805. http://doi.org/10.1093/bioinformatics/btt465
61. Silveira, J. A., Ridgeway, M. E., & Park, M. A. (2014). High resolution trapped ion mobility spectrometery of peptides. *Analytical Chemistry*, *86*(12), 5624–5627. http://doi.org/10.1021/ac501261h
62. Leyva, D., Jaffe, R., & Fernandez-Lima, F. (2020). Structural characterization of dissolved organic matter at the chemical formula level using TIMS-FT-ICR MS/MS. *Analytical Chemistry*, *92*(17), 11960–11966. http://doi.org/10.1021/acs.analchem.0c02347
63. Ruttkies, C., Schymanski, E. L., Wolf, S., Hollender, J., & Neumann, S. (2016). MetFrag relaunched: Incorporating strategies beyond in silico fragmentation. *Journal of Cheminformatics*, *8*, 3. http://doi.org/10.1186/s13321-016-0115-9
64. Bianco, A., Neefjes, I., Alfaouri, D., Vehkamaki, H., Kurten, T., Ahonen, L., Passananti, M., & Kangasluoma, J. (2022). Separation of isomers using a differential mobility analyser (DMA): Comparison of experimental vs modelled ion mobility. *Talanta*, *243*, 123339. http://doi.org/10.1016/j.talanta.2022.123339
65. Rose, B. S., May, J. C., Picache, J. A., Codreanu, S. G., Sherrod, S. D., & McLean, J. A. (2022). Improving confidence in lipidomic annotations by incorporating empirical ion mobility regression analysis and chemical class prediction. *Bioinformatics*, *38*(10), 2872–2879. http://doi.org/10.1093/bioinformatics/btac197
66. MacLean, B. X., Pratt, B. S., Egertson, J. D., MacCoss, M. J., Smith, R. D., & Baker, E. S. (2018). Using skyline to analyze data-containing liquid chromatography, ion mobility spectrometry, and mass spectrometry dimensions. *Journal of the American Society for Mass Spectrometry*, *29*(11), 2182–2188. http://doi.org/10.1007/s13361-018-2028-5
67. Tsugawa, H., Ikeda, K., Takahashi, M., Satoh, A., Mori, Y., Uchino, H., Okahashi, N., Yamada, Y., Tada, I., Bonini, P., Higashi, Y., Okazaki, Y., Zhou, Z., Zhu, Z.-J., Koelmel, J., Cajka, T., Fiehn, O., Saito, K., Arita, M., … Arita, M. (2020). A lipidome atlas in MS-DIAL 4. *Nature Biotechnology*, *38*(10), 1159–1163. http://doi.org/10.1038/s41587-020-0531-2
68. Schmid, R., Heuckeroth, S., Korf, A., Smirnov, A., Myers, O., Dyrlund, T. S., Bushuiev, R., Murray, K. J., Hoffmann, N., Lu, M., Sarvepalli, A., Zhang, Z., Fleischauer, M., Dührkop, K., Wesner, M., Hoogstra, S. J., Rudt, E., Mokshyna, O., Brungs, C., … Pluskal, T. (2023). Integrative analysis of multimodal mass spectrometry data in MZmine 3. *Nature Biotechnology*, *41*(4), 447–449. http://doi.org/10.1038/s41587-023-01690-2
69. Polasky, D. A., Dixit, S. M., Fantin, S. M., & Ruotolo, B. T. (2019). CIUSuite 2: Next-generation software for the analysis of gas-phase protein unfolding data. *Analytical Chemistry*, *91*(4), 3147–3155. http://doi.org/10.1021/acs.analchem.8b05762
70. Marty, M. T., Baldwin, A. J., Marklund, E. G., Hochberg, G. K., Benesch, J. L., & Robinson, C. V. (2015). Bayesian deconvolution of mass and ion mobility spectra: From binary interactions to polydisperse ensembles. *Analytical Chemistry*, *87*(8), 4370–4376. http://doi.org/10.1021/acs.analchem.5b00140
71. Prianichnikov, N., Koch, H., Koch, S., Lubeck, M., Heilig, R., Brehmer, S., Fischer, R., & Cox, J. (2020). MaxQuant software for ion mobility enhanced shotgun proteomics. *Molecular & Cellular Proteomics*, *19*(6), 1058–1069. http://doi.org/10.1074/mcp.tir119.001720
72. Yu, F., Haynes, S. E., Teo, G. C., Avtonomov, D. M., Polasky, D. A., & Nesvizhskii, A. I. (2020). Fast quantitative analysis of timsTOF PASEF data with MSFragger and IonQuant. *Molecular & Cellular Proteomics*, *19*(9), 1575–1585. http://doi.org/10.1074/mcp.tir120.002048
73. Demichev, V., Szyrwiel, L., Yu, F., Teo, G. C., Rosenberger, G., Niewienda, A., Ludwig, D., Decker, J., Kaspar-Schoenefeld, S., Lilley, K. S., Mülleder, M., Nesvizhskii, A. I., & Ralser, M. (2022). dia-PASEF data analysis using FragPipe and DIA-NN for deep proteomics of low sample amounts. *Nature Communications*, *13*(1), 3944. http://doi.org/10.1038/s41467-022-31492-0
74. Liu, L., Wang, Z., Zhang, Q., Mei, Y., Li, L., Liu, H., Wang, Z., & Yang, L. (2023). Ion mobility mass spectrometry for the separation and characterization of small molecules. *Analytical Chemistry*, *95*(1), 134–151. http://doi.org/10.1021/acs.analchem.2c02866
75. Tu, J., Zhou, Z. W., Li, T. Z, & Zhu, Z. J. (2019). The emerging role of ion mobility-mass spectrometry in lipidomics to facilitate lipid separation and identification. *TrAC Trends in Analytical Chemistry*, *116*, 332–339. http://doi.org/10.1016/j.trac.2019.03.017
76. Li, X., Wang, H., Jiang, M., Ding, M., Xu, X., Xu, B., Zou, Y., Yu, Y., & Yang, W. (2023). Collision cross section prediction based on machine learning. *Molecules (Basel, Switzerland)*, *28*(10). http://doi.org/10.3390/molecules28104050
77. Zhang, H., Luo, M., Wang, H., Ren, F., Yin, Y., & Zhu, Z. J. (2023). AllCCS2: Curation of ion mobility collision cross-section atlas for small molecules using comprehensive molecular representations. *Analytical Chemistry*, *95*(37), 13913–13921. http://doi.org/10.1021/acs.analchem.3c02267
78. Ross, D. H., Cho, J. H., & Xu, L. (2020). Breaking down structural diversity for comprehensive prediction of ion-neutral collision cross sections. *Analytical Chemistry*, *92*(6), 4548–4557. http://doi.org/10.1021/acs.analchem.9b05772
79. Aminpour, M., Montemagno, C., & Tuszynski, J. A. (2019). An overview of molecular modeling for drug discovery with specific illustrative examples of applications. *Molecules (Basel, Switzerland)*, *24*(9). http://doi.org/10.3390/molecules24091693
80. Von Helden, G., Hsu, M. T., Gotts, N., & Bowers, M. T. (1993). Carbon cluster cations with up to 84 atoms – structures, formation mechanism, and reactivity. *The Journal of Physical Chemistry*, *97*(31), 8182–8192. http://doi.org/10.1021/j100133a011
81. Shvartsburg, A. A, & Jarrold, M. F. (1996). An exact hard-spheres scattering model for the mobilities of polyatomic ions. *Chemi-*

cal Physics Letters, *261*(1-2), 86–91. http://doi.org/10.1016/0009-2614(96)00941-4
82. Mesleh, M. F., Hunter, J. M., Shvartsburg, A. A., Schatz, G. C., & Jarrold, M. F. (1996). Structural information from ion mobility measurements: Effects of the long-range potential. *The Journal of Physical Chemistry*, *100*(40), 16082–16086. http://doi.org/10.1021/jp961623v
83. Campuzano, I., Bush, M. F., Robinson, C. V., Beaumont, C., Richardson, K., Kim, H., & Kim, H. I. (2012). Structural characterization of drug-like compounds by ion mobility mass spectrometry: Comparison of theoretical and experimentally derived nitrogen collision cross sections. *Analytical Chemistry*, *84*(2), 1026–1033. http://doi.org/10.1021/ac202625t
84. Ewing, S. A., Donor, M. T., Wilson, J. W., & Prell, J. S. (2017). Collidoscope: An improved tool for computing collisional cross-sections with the trajectory method. *Journal of the American Society for Mass Spectrometry*, *28*(4), 587–596. http://doi.org/10.1007/s13361-017-1594-2
85. Zanotto, L., Heerdt, G., Souza, P. C. T., Araujo, G., & Skaf, M. S. (2018). High performance collision cross section calculation-HPCCS. *Journal of Computational Chemistry*, *39*(21), 1675–1681. http://doi.org/10.1002/jcc.25199
86. Ieritano, C., Crouse, J., Campbell, J. L., & Hopkins, W. S. (2019). A parallelized molecular collision cross section package with optimized accuracy and efficiency. *Analyst*, *144*(5), 1660–1670. http://doi.org/10.1039/c8an02150c
87. Harrilal, C. P., Garimella, S. V. B., Chun, J., Devanathan, N., Zheng, X., Ibrahim, Y. M., Larriba-Andaluz, C., Schenter, G., & Smith, R. D. (2023). The role of ion rotation in ion mobility: Ultrahigh-precision prediction of ion mobility dependence on ion mass distribution and translational to rotational energy transfer. *Journal of Physical Chemistry A*, *127*(25), 5458–5469. http://doi.org/10.1021/acs.jpca.3c01264
88. Bleiholder, C., Wyttenbach, T., & Bowers, M. T. (2011). A novel projection approximation algorithm for the fast and accurate computation of molecular collision cross sections (I). Method. *International Journal of Mass Spectrometry*, *308*(1), 1–10. http://doi.org/10.1016/j.ijms.2011.06.014
89. Larriba, C., & Hogan, C. J. Jr. (2013). Ion mobilities in diatomic gases: Measurement versus prediction with non-specular scattering models. *Journal of Physical Chemistry A*, *117*(19), 3887–3901. http://doi.org/10.1021/jp312432z
90. Larriba-Andaluz, C., & Hogan, C. J. Jr. (2014). Collision cross section calculations for polyatomic ions considering rotating diatomic/linear gas molecules. *Journal of Chemical Physics*, *141*(19), 194107. http://doi.org/10.1063/1.4901890
91. Marklund, E. G., Degiacomi, M. T., Robinson, C. V., Baldwin, A. J., & Benesch, J. L. (2015). Collision cross sections for structural proteomics. *Structure (London, England)*, *23*(4), 791–799. http://doi.org/10.1016/j.str.2015.02.010
92. Zhou, Z., Shen, X., Tu, J., & Zhu, Z. J. (2016). Large-scale prediction of collision cross-section values for metabolites in ion mobility-mass spectrometry. *Analytical Chemistry*, *88*(22), 11084–11091. http://doi.org/10.1021/acs.analchem.6b03091
93. Plante, P. L., Francovic-Fontaine, E., May, J. C., McLean, J. A., Baker, E. S., Laviolette, F., Marchand, M., & Corbeil, J. (2019). Predicting ion mobility collision cross-sections using a deep neural network: DeepCCS. *Analytical Chemistry*, *91*(8), 5191–5199. http://doi.org/10.1021/acs.analchem.8b05821
94. Zhou, Z., Luo, M., Chen, X., Yin, Y., Xiong, X., Wang, R., & Zhu, Z. J. (2020). Ion mobility collision cross-section atlas for known and unknown metabolite annotation in untargeted metabolomics. *Nature Communications*, *11*(1), 4334. http://doi.org/10.1038/s41467-020-18171-8
95. Ross, D. H., Seguin, R. P., Krinsky, A. M., & Xu, L. (2022). High-throughput measurement and machine learning-based prediction of collision cross sections for drugs and drug metabolites. *Journal of the American Society for Mass Spectrometry*, *33*(6), 1061–1072. http://doi.org/10.1021/jasms.2c00111
96. Rainey, M. A., Watson, C. A., Asef, C. K., Foster, M. R., Baker, E. S., & Fernandez, F. M. (2022). CCS Predictor 2.0: An open-source Jupyter notebook tool for filtering out false positives in metabolomics. *Analytical Chemistry*, *94*(50), 17456–17466. http://doi.org/10.1021/acs.analchem.2c03491
97. Guo, R., Zhang, Y., Liao, Y., Yang, Q., Xie, T., Fan, X., Lin, Z., Chen, Y., Lu, H., & Zhang, Z. (2023). Highly accurate and large-scale collision cross sections prediction with graph neural networks. *Commuications Chemistry*, *6*(1), 139. http://doi.org/10.1038/s42004-023-00939-w
98. Asef, C. K., Rainey, M. A., Garcia, B. M., Gouveia, G. J., Shaver, A. O., Leach, F. E. 3rd, Morse, A. M., Edison, A. S., McIntyre, L. M., & Fernandez, F. M. (2023). Unknown metabolite identification using machine learning collision cross-section prediction and tandem mass spectrometry. *Analytical Chemistry*, *95*(2), 1047–1056. http://doi.org/10.1021/acs.analchem.2c03749
99. Zheng, X., Wojcik, R., Zhang, X., Ibrahim, Y. M., Burnum-Johnson, K. E., Orton, D. J., Monroe, M. E., Moore, R. J., Smith, R. D., & Baker, E. S. (2017). Coupling front-end separations, ion mobility spectrometry, and mass spectrometry for enhanced multidimensional biological and environmental analyses. *Annual Review of Analytical Chemistry (Palo Alto Calif)*, *10*(1), 71–92. http://doi.org/10.1146/annurev-anchem-061516-045212
100. Bilbao, A., Varesio, E., Luban, J., Strambio-De-Castillia, C., Hopfgartner, G., Muller, M., & Lisacek, F. (2015). Processing strategies and software solutions for data-independent acquisition in mass spectrometry. *Proteomics*, *15*(5-6), 964–980. http://doi.org/10.1002/pmic.201400323
101. Xia, D., Ghali, F., Gaskell, S. J., O'Cualain, R., Sims, P. F., & Jones, A. R. (2012). Software for analysing ion mobility mass spectrometry data to improve peptide identification. *Proteomics*, *12*(12), 1912–1916. http://doi.org/10.1002/pmic.201200029
102. Distler, U., Kuharev, J., Navarro, P., Levin, Y., Schild, H., & Tenzer, S. (2014). Drift time-specific collision energies enable deep-coverage data-independent acquisition proteomics. *Nature Methods*, *11*(2), 167–170. http://doi.org/10.1038/nmeth.2767
103. Burnum-Johnson, K. E., Nie, S., Casey, C. P., Monroe, M. E., Orton, D. J., Ibrahim, Y. M., Gritsenko, M. A., Clauss, T. R. W., Shukla, A. K., Moore, R. J., Purvine, S. O., Shi, T., Qian, W., Liu, T., Baker, E. S., & Smith, R. D. (2016). Simultaneous proteomic discovery and targeted monitoring using liquid chromatography, ion mobility spectrometry, and mass spectrometry. *Molecular & Cellular Proteomics*, *15*(12), 3694–3705. http://doi.org/10.1074/mcp.M116.061143
104. Chouinard, C. D., Nagy, G., Webb, I. K., Shi, T., Baker, E. S., Prost, S. A., Liu, T., Ibrahim, Y. M., & Smith, R. D. (2018). Improved sensitivity and separations for phosphopeptides using online liquid chromotography coupled with structures for lossless ion manipulations ion mobility-mass spectrometry. *Analytical Chemistry*, *90*(18), 10889–10896. http://doi.org/10.1021/acs.analchem.8b02397
105. Van Puyvelde, B., Daled, S., Willems, S., Gabriels, R., Gonzalez de Peredo, A., Chaoui, K., Mouton-Barbosa, E., Bouyssie, D., Boonen, K., Hughes, C. J., Gethings, L. A., Perez-Riverol, Y., Bloomfield, N., Tate, S., Schiltz, O., Martens, L., Deforce, D., & Dhaenens, M. (2022). A comprehensive LFQ benchmark dataset on modern day acquisition strategies in proteomics. *Scientific Data*, *9*(1), 126. http://doi.org/10.1038/s41597-022-01216-6
106. Meier, F., Kohler, N. D., Brunner, A. D., Wanka, J. H., Voytik, E., Strauss, M. T., Theis, F. J., & Mann, M. (2021). Deep learning the collisional cross sections of the peptide universe from a million experimental values. *Nature Communications*, *12*(1), 1185. http://doi.org/10.1038/s41467-021-21352-8
107. Woo, J., Clair, G. C., Williams, S. M., Feng, S., Tsai, C. F., Moore, R. J., Chrisler, W. B., Smith, R. D., Kelly, R. T., Pasa-Tolic, L., Ansong, C., & Zhu, Y. (2022). Three-dimensional feature matching improves

coverage for single-cell proteomics based on ion mobility filtering. *Cell Systems*, *13*(5), 426–434.e4. http://doi.org/10.1016/j.cels.2022.02.003

108. Reilly, L., Lara, E., Ramos, D., Li, Z., Pantazis, C. B., Stadler, J., Santiana, M., Roberts, J., Faghri, F., Hao, Y., Nalls, M. A., Narayan, P., Liu, Y., Singleton, A. B., Cookson, M. R., Ward, M. E., & Qi, Y. A. (2023). A fully automated FAIMS-DIA mass spectrometry-based proteomic pipeline. *Cell Reports Methods*, *3*(10), 100593. http://doi.org/10.1016/j.crmeth.2023.100593
109. Xu, T., Wang, Q., Wang, Q., & Sun, L. (2023). Coupling high-field asymmetric waveform ion mobility spectrometry with capillary zone electrophoresis-tandem mass spectrometry for top-down proteomics. *Analytical Chemistry*, *95*(25), 9497–9504. http://doi.org/10.1021/acs.analchem.3c00551
110. Paglia, G., Smith, A. J., & Astarita, G. (2022). Ion mobility mass spectrometry in the omics era: Challenges and opportunities for metabolomics and lipidomics. *Mass Spectrometry Reviews*, *41*(5), 722–765. http://doi.org/10.1002/mas.21686
111. Moran-Garrido, M., Camunas-Alberca, S. M., Gil-de-la Fuente, A., Mariscal, A., Gradillas, A., Barbas, C., & Saiz, J. (2022). Recent developments in data acquisition, treatment and analysis with ion mobility-mass spectrometry for lipidomics. *Proteomics*, *22*(15-16), e2100328. http://doi.org/10.1002/pmic.202100328
112. Zhou, Z., Shen, X., Chen, X., Tu, J., Xiong, X., & Zhu, Z. J. (2019). LipidIMMS analyzer: Integrating multi-dimensional information to support lipid identification in ion mobility-mass spectrometry based lipidomics. *Bioinformatics*, *35*(4), 698–700. http://doi.org/10.1093/bioinformatics/bty661
113. Kirkwood, K. I., Pratt, B. S., Shulman, N., Tamura, K., MacCoss, M. J., MacLean, B. X., & Baker, E. S. (2022). Utilizing Skyline to analyze lipidomics data containing liquid chromatography, ion mobility spectrometry and mass spectrometry dimensions. *Nature Protocols*, *17*(11), 2415–2430. http://doi.org/10.1038/s41596-022-00714-6
114. Ross, D. H., Guo, J., Bilbao, A., Huan, T., Smith, R. D., & Zheng, X. (2023). Evaluating software tools for lipid identification from ion mobility spectrometry-mass spectrometry lipidomics data. *Molecules*, *28*(8). http://doi.org/10.3390/molecules28083483
115. Feuerstein, M. L., Kurulugama, R. T., Hann, S., & Causon, T. (2021). Novel acquisition strategies for metabolomics using drift tube ion mobility-quadrupole resolved all ions time-of-flight mass spectrometry (IM-QRAI-TOFMS). *Analytica Chimica Acta*, *1163*, 338508. http://doi.org/10.1016/j.aca.2021.338508
116. Wang, M., Xu, X. Y., Wang, H. D., Wang, H. M., Liu, M. Y., Hu, W. D., Chen, B. X., Jiang, M. T., Qi, J., Li, X. H., Yang, W.-Z., & Gao, X.-M. (2022). A multi-dimensional liquid chromatography/high-resolution mass spectrometry approach combined with computational data processing for the comprehensive characterization of the multicomponents from Cuscuta chinensis. *Journal of Chromatography A*, *1675*, 463162. http://doi.org/10.1016/j.chroma.2022.463162
117. Colby, S. M., Chang, C. H., Bade, J. L., Nunez, J. R., Blumer, M. R., Orton, D. J., Bloodsworth, K. J., Nakayasu, E. S., Smith, R. D., Ibrahim, Y. M., Renslow, R. S., & Metz, T. O. (2022). DEIMoS: An open-source tool for processing high-dimensional mass spectrometry data. *Analytical Chemistry*, *94*(16), 6130–6138. http://doi.org/10.1021/acs.analchem.1c05017
118. Bilbao, A., Munoz, N., Kim, J., Orton, D. J., Gao, Y., Poorey, K., Pomraning, K. R., Weitz, K., Burnet, M., Nicora, C. D., Wilton, R., Deng, S., Dai, Z., Oksen, E., Gee, A., Fasani, R. A., Tsalenko, A., Tanjore, D., Gardner, J., … Burnum-Johnson, K. E. (2023). PeakDecoder enables machine learning-based metabolite annotation and accurate profiling in multidimensional mass spectrometry measurements. *Nature Communications*, *14*(1), 2461. http://doi.org/10.1038/s41467-023-37031-9
119. Luo, M., Yin, Y., Zhou, Z., Zhang, H., Chen, X., Wang, H., & Zhu, Z. J. (2023). A mass spectrum-oriented computational method for ion mobility-resolved untargeted metabolomics. *Nature Communications*, *14*(1), 1813. http://doi.org/10.1038/s41467-023-37539-0
120. Ramtanon, I., Berlioz-Barbier, A., Remy, S., Renault, J. H., & Masle, A. L. (2023). A combined liquid chromatography – trapped ion mobility – tandem high-resolution mass spectrometry and multivariate analysis approach for the determination of enzymatic reactivity descriptors in biomass hydrolysates. *Journal of Chromatography A*, *1706*, 464277. http://doi.org/10.1016/j.chroma.2023.464277
121. Wen, J. H., Guo, A. Q., Li, M. N., & Yang, H. (2023). A structural similarity networking assisted collision cross-section prediction interval filtering strategy for multi-compound identification of complex matrix by ion-mobility mass spectrometry. *Analytica Chimica Acta*, *1278*, 341720. http://doi.org/10.1016/j.aca.2023.341720
122. Lu, K., Gardner, W. S., & Liu, Z. (2018). Molecular structure characterization of riverine and coastal dissolved organic matter with ion mobility quadrupole time-of-flight LCMS (IM Q-TOF LCMS). *Environmental Science & Technology*, *52*(13), 7182–7191. http://doi.org/10.1021/acs.est.8b00999
123. Oller-Moreno, S., Mallafre-Muro, C., Fernandez, L., Caballero, E., Blanco, A., Guma, J., Marco, S., & Pardo, A. (2023). GCIMS: An R package for untargeted gas chromatography – Ion mobility spectrometry data processing. *Chemometrics and Inteligentl Laboratory Systems*, *241*, 104938. https://doi.org/10.1016/j.chemolab.2023.104938
124. Christmann, J., Rohn, S., & Weller, P. (2022). gc-ims-tools – A new Python package for chemometric analysis of GC-IMS data. *Food Chemistry*, *394*, 133476. http://doi.org/10.1016/j.foodchem.2022.133476
125. Freire, R., Fernandez, L., Mallafre-Muro, C., Martin-Gomez, A., Madrid-Gambin, F., Oliveira, L., Pardo, A., Arce, L., & Marco, S. (2021). Full workflows for the analysis of gas chromatography-ion mobility spectrometry in foodomics: Application to the analysis of Iberian Ham Aroma. *Sensors (Basel)*, *21*(18). http://doi.org/10.3390/s21186156
126. Calle, J. L. P., Vazquez-Espinosa, M., Barea-Sepulveda, M., Ruiz-Rodriguez, A., Ferreiro-Gonzalez, M., & Palma, M. (2021). Novel method based on ion mobility spectrometry combined with machine learning for the discrimination of fruit juices. *Foods*, *12*(13). http://doi.org/10.3390/foods12132536
127. Buchberger, A. R., DeLaney, K., Johnson, J., & Li, L. (2018). Mass spectrometry imaging: A review of emerging advancements and future insights. *Analytical Chemistry*, *90*(1), 240–265. http://doi.org/10.1021/acs.analchem.7b04733
128. Baquer, G., Semente, L., Mahamdi, T., Correig, X., Rafols, P., & Garcia-Altares, M. (2023). What are we imaging? Software tools and experimental strategies for annotation and identification of small molecules in mass spectrometry imaging. *Mass Spectrometry Reviews*, *42*(5), 1927–1964. http://doi.org/10.1002/mas.21794
129. Claes, B. S. R., Bowman, A. P., Poad, B. L. J., Heeren, R. M. A., Blanksby, S. J., & Ellis, S. R. (2023). Isomer-resolved mass spectrometry imaging of acidic phospholipids. *Journal of the American Society for Mass Spectrometry*, *34*(10), 2269–2277. http://doi.org/10.1021/jasms.3c00192
130. Chen, C. L., Kuo, T. H., Chung, H. H., Huang, P., Lin, L. E., & Hsu, C. C. (2021). Remodeling nanoDESI platform with ion mobility spectrometry to expand protein coverage in cancerous tissue. *Journal of the American Society for Mass Spectrometry*, *32*(3), 653–660. http://doi.org/10.1021/jasms.0c00354
131. Claes, B. S. R., Bowman, A. P., Poad, B. L. J., Young, R. S. E., Heeren, R. M. A., Blanksby, S. J., & Ellis, S. R. (2021). Mass spectrometry imaging of lipids with isomer resolution using high-pressure ozone-induced dissociation. *Analytical Chemistry*, *93*(28), 9826–9834. http://doi.org/10.1021/acs.analchem.1c01377
132. Kontunen, A., Tuominen, J., Karjalainen, M., Anttalainen, O., Tolonen, T., Kumpulainen, P., Lepomaki, M., Vehkaoja, A., Oksala, N., & Roine, A. (2020). Differential mobility spectrometry imaging for

pathological applications. *Experimental and Molecular Pathology*, *117*, 104526. http://doi.org/10.1016/j.yexmp.2020.104526

133. Michno, W., Wehrli, P. M., Koutarapu, S., Marsching, C., Minta, K., Ge, J., Meyer, S. W., Zetterberg, H., Blennow, K., Henkel, C., Oetjen, J., Hopf, C., & Hanrieder, J. (2022). Structural amyloid plaque polymorphism is associated with distinct lipid accumulations revealed by trapped ion mobility mass spectrometry imaging. *Journal of Neurochemistry*, *160*(4), 482–498. http://doi.org/10.1111/jnc.15557
134. Hale, O. J., Sisley, E. K., Griffiths, R. L., Styles, I. B., & Cooper, H. J. (2020). Native LESA TWIMS-MSI: Spatial, conformational, and mass analysis of proteins and protein complexes. *Journal of the American Society for Mass Spectrometry*, *31*(4), 873–879. http://doi.org/10.1021/jasms.9b00122
135. Jiang, L. X., Hernly, E., Hu, H., Hilger, R. T., Neuweger, H., Yang, M., & Laskin, J. (2023). Nanospray desorption electrospray ionization (Nano-DESI) mass spectrometry imaging with high ion mobility resolution. *Journal of the American Society for Mass Spectrometry*, *34*(8), 1798–1804. http://doi.org/10.1021/jasms.3c00199
136. Esselman, A. B., Patterson, N. H., Migas, L. G., Dufresne, M., Djambazova, K. V., Colley, M. E., Van de Plas, R., & Spraggins, J. M. (2023). Microscopy-directed imaging mass spectrometry for rapid high spatial resolution molecular imaging of glomeruli. *Journal of the American Society for Mass Spectrometry*, *34*(7), 1305–1314. http://doi.org/10.1021/jasms.3c00033
137. Schwaiger-Haber, M., Stancliffe, E., Anbukumar, D. S., Sells, B., Yi, J., Cho, K., Adkins-Travis, K., Chheda, M. G., Shriver, L. P., & Patti, G. J. (2023). Using mass spectrometry imaging to map fluxes quantitatively in the tumor ecosystem. *Nature Communications*, *14*(1), 2876. http://doi.org/10.1038/s41467-023-38403-x
138. Neumann, E. K., Migas, L. G., Allen, J. L., Caprioli, R. M., Van de Plas, R., & Spraggins, J. M. (2020). Spatial metabolomics of the human kidney using MALDI trapped ion mobility imaging mass spectrometry. *Analytical Chemistry*, *92*(19), 13084–13091. http://doi.org/10.1021/acs.analchem.0c02051
139. Djambazova, K. V., Klein, D. R., Migas, L. G., Neumann, E. K., Rivera, E. S., Van de Plas, R., Caprioli, R. M., & Spraggins, J. M. (2020). Resolving the complexity of spatial lipidomics using MALDI TIMS imaging mass spectrometry. *Analytical Chemistry*, *92*(19), 13290–13297. http://doi.org/10.1021/acs.analchem.0c02520
140. Spraggins, J. M., Djambazova, K. V., Rivera, E. S., Migas, L. G., Neumann, E. K., Fuetterer, A., Suetering, J., Goedecke, N., Ly, A., Van de Plas, R., & Caprioli, R. M. (2019). High-performance molecular imaging with MALDI trapped ion-mobility time-of-flight (timsTOF) mass spectrometry. *Analytical Chemistry*, *91*(22), 14552–14560. http://doi.org/10.1021/acs.analchem.9b03612
141. Mesa Sanchez, D., Creger, S., Singla, V., Kurulugama, R. T., Fjeldsted, J., & Laskin, J. (2020). Ion mobility-mass spectrometry imaging workflow. *Journal of the American Society for Mass Spectrometry*, *31*(12), 2437–2442. http://doi.org/10.1021/jasms.0c00142
142. Allison, T. M., Barran, P., Benesch, J. L. P., Cianferani, S., Degiacomi, M. T., Gabelica, V., Grandori, R., Marklund, E. G., Menneteau, T., Migas, L. G., Politis, A., Sharon, M., Sobott, F., & Thalassinos, K. (2020). Software requirements for the analysis and interpretation of native ion mobility mass spectrometry data. *Analytical Chemistry*, *92*(16), 10881–10890. http://doi.org/10.1021/acs.analchem.9b05792
143. Allison, T. M., Barran, P., Cianferani, S., Degiacomi, M. T., Gabelica, V., Grandori, R., Marklund, E. G., Menneteau, T., Migas, L. G., Politis, A., Sharon, M., Sobott, F., Thalassinos, K., & Benesch, J. L. P. (2020). Computational strategies and challenges for using native ion mobility mass spectrometry in biophysics and structural biology. *Analytical Chemistry*, *92*(16), 10872–10880. http://doi.org/10.1021/acs.analchem.9b05791
144. Stojko, J., Fieulaine, S., Petiot-Becard, S., Van Dorsselaer, A., Meinnel, T., Giglione, C., & Cianferani, S. (2015). Ion mobility coupled to native mass spectrometry as a relevant tool to investigate extremely small ligand-induced conformational changes. *Analyst*, *140*(21), 7234–7245. http://doi.org/10.1039/c5an01311a
145. Eschweiler, J. D., Rabuck-Gibbons, J. N., Tian, Y., & Ruotolo, B. T. (2015). CIUSuite: A quantitative analysis package for collision induced unfolding measurements of gas-phase protein ions. *Analytical Chemistry*, *87*(22), 11516–11522. http://doi.org/10.1021/acs.analchem.5b03292
146. Migas, L. G., France, A. P., Bellina, B., & Barran, P. E. (2018). ORIGAMI: A software suite for activated ion mobility mass spectrometry (aIM-MS) applied to multimeric protein assemblies. *International Journal of Mass Spectrometry*, *427*, 20–28. https://doi.org/10.1016/j.ijms.2017.08.014
147. Phung, W., Bakalarski, C. E., Hinkle, T. B., Sandoval, W., & Marty, M. T. (2023). UniDec processing pipeline for rapid analysis of biotherapeutic mass spectrometry data. *Analytical Chemistry*, *95*(30), 11491–11498. http://doi.org/10.1021/acs.analchem.3c02010
148. Baird, M. A., Anderson, G. A., Shliaha, P. V., Jensen, O. N., & Shvartsburg, A. A. (2019). Differential ion mobility separations/mass spectrometry with high resolution in both dimensions. *Analytical Chemistry*, *91*(2), 1479–1485. http://doi.org/10.1021/acs.analchem.8b04518
149. Cole, H., Porrini, M., Morris, R., Smith, T., Kalapothakis, J., Weidt, S., Mackay, C. L., MacPhee, C. E., & Barran, P. E. (2015). Early stages of insulin fibrillogenesis examined with ion mobility mass spectrometry and molecular modelling. *Analyst*, *140*(20), 7000–7011. http://doi.org/10.1039/c5an01253h
150. Degiacomi, M. T., & Benesch, J. L. (2016). EM intersectionIM: Software for relating ion mobility mass spectrometry and electron microscopy data. *Analyst*, *141*(1), 70–75. http://doi.org/10.1039/c5an01636c

How to cite this article: Ross, D. H., Bhotika, H., Zheng, X., Smith, R. D., Burnum-Johnson, K. E., & Bilbao, A. (2024). Computational tools and algorithms for ion mobility spectrometry-mass spectrometry. *Proteomics*, *24*, e2200436. https://doi.org/10.1002/pmic.202200436