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Trends in trapped ion mobility – Mass spectrometry instrumentation

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ABSTRACT

Trapped Ion Mobility Spectrometry (TIMS) is a recently developed form of ion mobility spectrometry (IMS) which is flexible in its operation and readily hybridized with mass spectrometry (MS). Prototype TIMS-MS instruments are applicable to a wide range of analytical problems including separation of isobars and isomers, the study of analyte conformation and unfolding, general separation of complex mixtures, and omics. Hybridization of TIMS with high performance mass analyzers such as ion cyclotron resonance (ICR) allows for the more effective analysis of highly complex samples. Adding trapping ahead of TIMS has enabled technologies such as Parallel Accumulation Serial Fragmentation (PASEF) for improved shotgun proteomics. Finally, tandem TIMS (tTIMS) adds flexibility, especially in top down proteomics. Here we highlight recent advances in TIMS-MS and their analytical applications.

Abbreviations

CBD	Cannabidiol
CC	Collision Cell
CCS	Collision Cross Section
CID	Collision Induced Dissociation
CIU	Collision Induced Unfolding
ESI	Electrospray Ionization
FAIMS	Field Asymmetric Ion Mobility Spectrometry
ICR	Fourier Transform Ion Cyclotron Resonance Mass Spectrometry
IMMS	Ion Mobility Mass Spectrum
IMS	Ion Mobility Spectrometry
LC	Liquid Chromatography
MALDI	Matrix Assisted Laser Desorption Ionization
MS	Mass Spectrometry
PASEF	Parallel Accumulation Serial Fragmentation
PTM	Post Translational Modification
Q	Quadrupole
SRFA	Suwannee River bottom Fulvic Acids
THC	Tetrahydrocannabinol
TIMS	Trapped Ion Mobility Spectrometry
TOF	Time-of-Flight Mass Spectrometry
tTIMS	Tandem TIMS

1. Introduction

Ion mobility spectrometry – mass spectrometry (IMS-MS) is a well-established analytical technique that separates ions based on differences in their shapes (IMS) and mass (MS) [1–4]. A significant shift in the use and perceived value of these technologies began to occur in the 1980s with the discovery of new ionization methods – matrix assisted laser desorption ionization (MALDI) [5] and electrospray ionization (ESI) [6] – which are gentle enough to produce intact gas phase molecular ions from essentially any polar biomolecule. This initiated a renewed interest in the development and application of improved mass analyzers, and more recently, ion mobility analyzers in combination with MS, to study biological problems. However, the first IMS-MS instruments with sufficient resolving power were relatively large (2–3 m long and 2–3 m high) and complex to use. Thus, significant effort in recent years has been expended towards developing IMS analyzers which are convenient, flexible, easily hybridized with MS, and therefore, readily disseminated.

Trapped IMS (TIMS) – the focus of this review - is just such an analyzer [7,8]. TIMS retains the fundamental principle of conventional drift cell IMS analyzers of separating ions by dragging them through a gas using a constant DC electric field [9–11]. However, TIMS essentially inverts this experiment. While ions are dragged through the gas in the center-of-mass reference frame, in TIMS they are stationary and the gas is flowing past them in the laboratory reference frame. A DC electric field is used to hold the ions in place – i.e. to trap the ions - against this moving gas. Because the physical extent of the TIMS trap and associated DC electric field is only a few centimeters (as opposed to a meter or more), only a few hundred (as opposed to a few thousand) volts are needed to produce an analytical field strength of up to ~70 V/cm. Nonetheless, the effective length of the column of gas which flows past the ions during the course of a TIMS analysis is typically much larger than the physical length of the TIMS trap. This combination of a high analytical field strength and a long column of gas, results in a high (~100) mobility resolution [12,13].

Ions are eluted from the TIMS analyzer, under operator control, by reducing the analytical field strength over time. TIMS is more flexible than previous analyzers as the analytical field is a readily ac-

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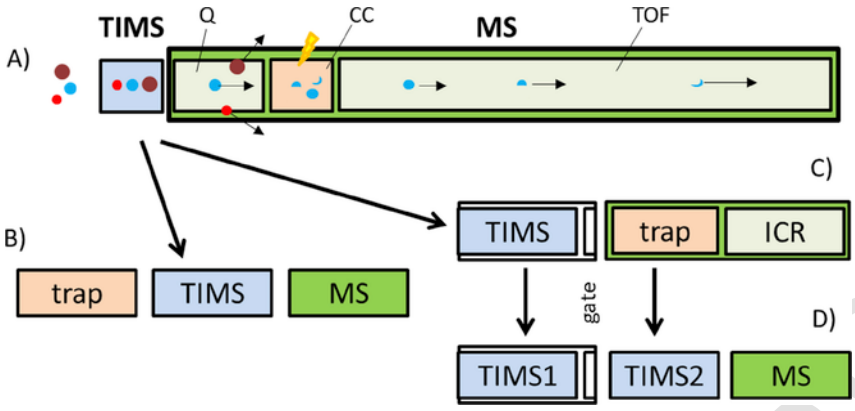

Fig. 1. A simplified representation of the four existing TIMS-MS configurations: (A) TIMS in rough proportion to a mass analyzer which, in this case, includes a quadrupole analyzer (Q) a collision cell (CC), and a time-of-flight mass analyzer (TOF); (B) a trap before TIMS for parallel accumulation; (C) Gated TIMS hybridized with FTICR MS; and (D) tandem TIMS – MS.

cessible and programmable parameter. One can, for example, select the mobility range to be analyzed and the speed of the analysis by selecting the range and rate over which the analytical field is scanned [14]. This means the TIMS experiment can be readily adapted to the application to be performed.

Thus, a TIMS device is a physically small mobility analyzer requiring potentials of only a few hundred volts which is readily hybridized with mass spectrometry to produce a flexible and user friendly instrument. TIMS is still a relatively new concept and practitioners are still exploring various ways of configuring, hybridizing, and applying this technology and many recent publications still reference prototype instruments. Two commercial products, timsTOF and timsTOF pro (Bruker Inc., Billerica, MA), have now been introduced, dramatically increasing the user base. We recently reviewed the basics of TIMS [15], thus we here focus on the latest advances in TIMS-MS instrumentation and the applications advantages they bring.

2. TIMS configurations

At present, four types of TIMS-MS instruments have been built (Fig. 1). Panel A highlights the relative size of the TIMS analyzer (~0.1 m) relative to the rest of the instrument (~2 m depending on the MS used). Clearly, the TIMS analyzer is sufficiently small and simple that it can be integrated with a mass spectrometer without significant impact on the instrument's size or its intrinsic performance. Though many instrument components are not represented it is worth noting that all four configurations include an analytical quadrupole (Q), for ion selection based on mass, and a collision cell (CC) for ion fragmentation, conditioning, and storage. In most instruments to date, TIMS is coupled with a time-of-flight (TOF) mass analyzer because of its principle advantage of speed. A typical TIMS mobility peak has a width of about one millisecond, whereas a single TOF analysis requires only about 200 microseconds. Thus, it's possible to produce several mass spectra per mobility peak and thereby adequately follow the progress of the mobility analysis.

2.1. Basic operation

Analyte ions are produced at atmospheric pressure - typically via ESI in the front of the instrument. These ions are entrained in nitrogen gas and introduced into the instrument's first pumping region where the TIMS resides. As originally conceived, the TIMS is a tube-like structure creating a cylindrically symmetric gas flow. Typically, the TIMS analyzer operates at about 3 mbar at its entrance end and as low as 1 mbar at its exit. This generates a gas flow through the TIMS analyzer. An RF field radially confines ions in the tube-like structure where, during TIMS operation, a DC field prevents the ions from moving downstream. The ions thus become trapped by the action of the gas flow and the RF and DC fields. The TIMS experiment consists of two fundamental steps - ion accumulation and ion elution. During accumulation, the DC field is kept at a fixed value, sufficient to retain ions of the lowest mobility of interest trapped against the gas flow roughly halfway down the length of the analyzer. Ions of higher mobility than this minimum will be accumulated as well with trapping position a function of mobility along the length of the TIMS trap. After a selected period of time, usually tens of milliseconds, further ions are blocked from entering the TIMS trap and ions are eluted from the TIMS trap by decreasing the strength of the DC field linearly over time. Eventually, as its strength decreases, the electric field will no longer suffice to offset the drag force due to the gas flowing through the analyzer and the ions will be pushed out of the TIMS trap according to mobility. As the lowest mobility ions experience the greatest drag force due to the gas flow, these will be the first to elute followed by successively higher mobility ions. Ions which elute from the TIMS are transmitted downstream to the mass spectrometer where they are mass analyzed to form an ion mobility mass spectrum (IMMS). More details on TIMS construction and operation can be found in the literature [9–11,15].

2.2. Applications

TIMS has already been used with mass spectrometry for a wide variety of applications, for instance in the separation of isomeric post translationally modified (PTM) peptides [16], the study of a lasso peptide and its isotopolog [17], the study of binding of AT Hook-3 peptides to DNA [18], the separation of parallel and antiparallel DNA duplexes [19], the analysis of growth hormone-releasing hormone peptide analogs [20], and the study of non-symbiotic hemoglobin [21] and nicotinamide adenine dinucleotide (NAD) [22]. Both TIMS and FAIMS (Field Asymmetric Ion Mobility Spectrometry) [23] were used for the analysis of variants of several complete histone tails in a study which showed that TIMS and FAIMS are about 50% orthogonal separation methods for these compounds and that hybridizing the two should produce a 2D peak capacity of several hundred [24].

As one might expect based on prior works using drift tube IMS [25,26], cationization of the analyte and the use of “modifiers” – small molecule additives to the drift gas - can have a significant effect on TIMS and its ability to separate analytes. Coordination of lasso peptide ions with metal cations can have a significant effect on

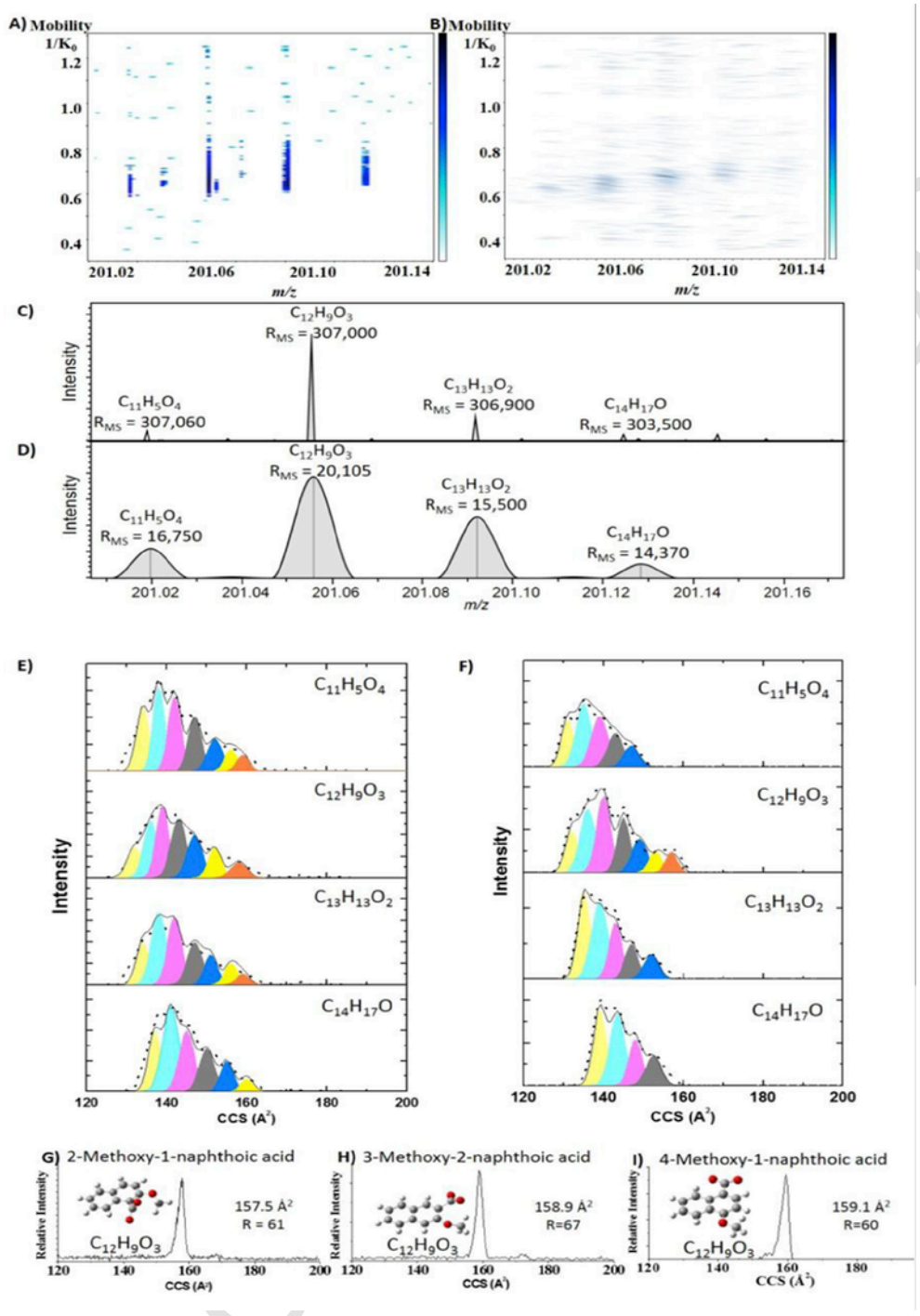

Fig. 2. (A) IMMS 2D plots of SFRA acquired using TIMS-FTICR and (B) TIMS-TOF. (C) Demonstration of Mass resolving power at *m/z* 201 for TIMS-FTICR and (D) TIMS-TOF. (E) Deconvoluted ion mobilograms for selected chemical formulas acquired on TIMS-FTICR and (F) TIMS-TOF. Figures (G, H, and I) show the mobility spectra of three standards analyzed by Gated TIMS-ICR. Reprinted with permission from Ref. [36]. Copyright 2018 John Wiley and Sons.

their conformation [27]. Similarly, coordination with cations was found to improve the TIMS mobility separation of some peptide isomers and certain drug isobars [28]. In one case, the protonated molecular ions of isobaric, cannabis derived compounds, tetrahydrocannabinol (THC) and cannabidiol (CBD), were inseparable by TIMS-MS, however, coordination with lithium, cesium, or silver cations allowed the two to be distinguished [29]. The mobility of analyte ions can be shifted by adding modifiers to the gas used in the TIMS analysis. Adding acetone as a modifier was also found to consistently change the IMS profile of AT-Hook-3 peptide ions [30].

Finally, TIMS has been found to be useful in the analysis of complex samples and in cases where liquid chromatography (LC) has traditionally be used as an additional separation method ahead of MS. In the discovery and monitoring of target compounds in biological systems, one study demonstrated the potential of TIMS-TOF for the analysis of peptide biomarkers in tumor tissue [31]. Another used LC

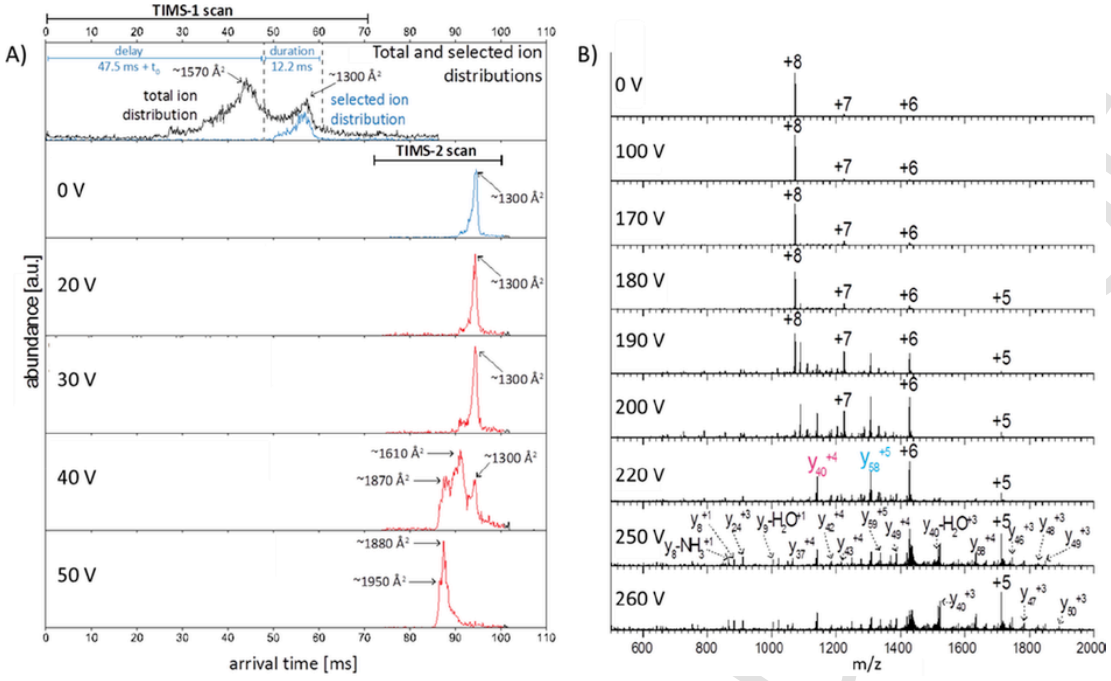

Fig. 3. (A) Ion mobility spectra resulting from the selection of the compact conformer of ubiquitin 7+ (top panel) and subsequent CIU at between 0 and 50 V,; and (B) fragment ion mass spectra resulting from the mobility selection of ubiquitin 8+ ions and their subsequent CID at between 0 and 260 V. Reproduced from Ref. [37] with permission from The Royal Society of Chemistry.

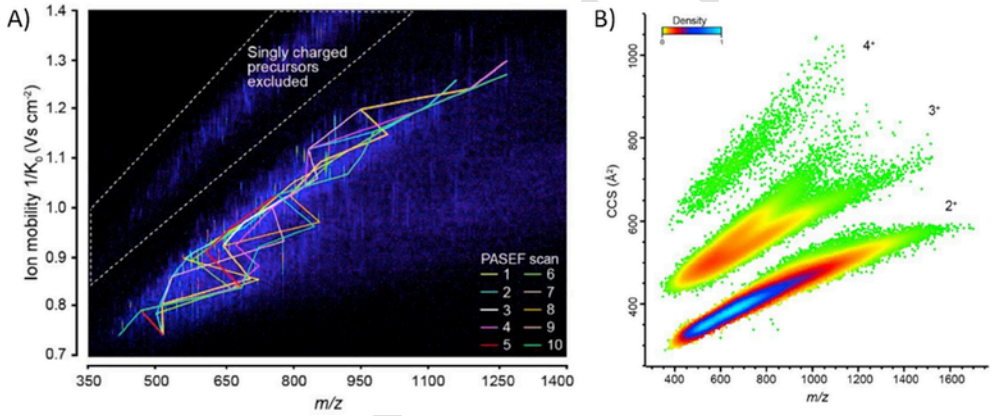

Fig. 4. (A) An example, out of many, IMMS spectra acquired during the course of an LC-TIMS-MS analysis of a HeLa digest. The lines connect the mobility, *m/z* coordinates of precursor ions selected during the ten subsequent PASEF scans. (B) A plot of the distribution of CCS vs. *m/z* values for over 100,000 species from a human tryptic peptide sample. This research was originally published in Molecular & Cellular Proteomics [40]. Copyright the Authors.

coupled with TIMS-TOF to detect and quantitate hydroxylated metabolites of polychlorinated biphenyls in blood plasma without the need for labor-intensive sample preparation [32]. Furthermore, LC-TIMS-MS was compared to two dimensional LC-MS lipid analysis – i.e. lipidomics [33]. The authors concluded that no single, untargeted method is sufficient for all lipidomics applications, and that these two techniques, LC-TIMS-MS and LCxLC-MS, may be used in a complimentary manner.

3. Expanding TIMS capabilities

Once the basics of TIMS were established, additional instrument configurations have been explored. Because TIMS itself is a trap, it is readily hyphenated with other ion traps. This leads to other TIMS-MS instrument configurations distinguished by the position and construction of the added trapping device (Fig. 1B–D). As discussed further below, a trap before the TIMS (Fig. 1B) efficiently handles incoming ions and relieves the TIMS of the task of accumulating the analyte ions. Placing the trap after the TIMS (Fig. 1C) enables the hybridization of TIMS with slow mass analyzers. And configuring the trap after the TIMS as another TIMS (Fig. 1D) allows for the tandem mobility analysis of analyte ions.

Alternate ion optical geometries and RF drive frequencies have also been explored. By lowering the RF drive frequency used to confined ions radially by about a factor of two in combination with a new, convex electrode geometry, ions of more than 950 kDa in MW could be TIMS-MS analyzed due to a significant increase in the pseudo potential well for high *m/z* ions [34,35].

3.1. Gated TIMS – ICR & complex samples

"Gated TIMS" – i.e. TIMS followed by a selecting gate [36,37] - has been hybridized to a Fourier transform ion cyclotron resonance (ICR) mass spectrometer [38] (Fig. 1C). In this configuration, the collision cell of the ICR mass spectrometer acts as an ion trap downstream of the TIMS. Because the ICR produces mass spectra at a rate of one per second, it is not fast enough sample TIMS scans – hence the use of Gated TIMS. As ions are eluted from the TIMS analyzer, the gate is timed to only admit ions of a specific mobility. Gated ions of the same mobility from several TIMS scans can be accumulated in the trap before injecting them into the ICR mass analyzer. Thus, while the ICR mass spectrometer mass analyzes group of ions, the Gated TIMS + trap efficiently mobility analyzes and accumulates a next group of ions. If each group of ions, and corresponding mass spectrum, are of a successively higher mobility, then combining the resulting mass spectra produces an IMMS spectrum equivalent to that which would be obtained from a TIMS-TOF, but with a higher mass resolution [38].

The data of Fig. 2 compare the performance of a prototype TIMS-TOF to a prototype Gated TIMS-ICR mass spectrometer in the analysis of a complex sample [38]. The analyte is a Suwannee river bottom fulvic acids (SRFA) standard – a reference material maintained by the International Humic Substances Society. Fig. 2A, B shows a limited region of the TIMS-MS spectra obtained using Gated TIMS-ICR and TIMS-TOF, respectively. The y-axis is given as inverted, reduced mobility, K_o, as measured with the TIMS, and the x-axis is the mass-to-charge ratio of the ions, m/z, as measured with either the ICR or TOF mass analyzer. Fig. 2C, D are the corresponding mass spectra obtained by collapsing the data of Fig. 2A, B respectively onto their mass axes. The main differences between the ICR and TOF is mass resolution - of the order of 300,000 in ICR and 20,000 in this TOF instrument and analysis time – 10 s of minutes for TIMS-ICR analysis and 100 s of ms for TIMS-TOF spectra. In the case of SRFA, the higher resolution of the ICR is important at an m/z of about 400 or greater. At such masses, the complexity of the sample makes it difficult or impossible for the TOF to resolve adjacent species. Because of this difference in mass resolution, the number of peaks from mass resolvable species (not considering mobility in either case) measured via the TIMS-TOF vs the TIMS-ICR was 4950 and 7760 respectively.

Fig. 2E, F illustrate that TIMS on TOF (Fig. 1A) performs, essentially the same as Gated TIMS on ICR (Fig. 1C). The panels of Fig. 2E, F shows the mobility spectrum for the four isobars in the corresponding mass spectra of Fig. 2C, D, respectively. As indicated by the colored Gaussian peaks, the mobilograms for each of the four isobars can be deconvoluted into several structural isomers. Such deconvolution is supported by the measurement of three individual standards, the mobility spectra for which are shown in Fig. 2G, H, I. The width and measured cross sections for the standards are consistent with the deconvoluted result. There were three to five isomers per chemical formula and the complete, deconvoluted TIMS-ICR data set yielded 22,300 features as compared to 7600 on the TIMS-TOF.

3.2. Tandem TIMS (tTIMS) & top-down proteomics

A tandem TIMS (tTIMS) analyzer is formed by placing a second TIMS analyzer downstream of a Gated TIMS analyzer (Fig. 1D) [39]. As discussed above, the pressure and pressure drop across each TIMS analyzer is important to its performance. Differential pumping in the interface region between the two TIMS analyzers allows for control of the entrance and exit pressures of TIMS-1 independently from those of TIMS-2. Hence, there are two different ways to set the pressures, given by the relative magnitude of the exit pressure of TIMS-1 and the entrance pressure of TIMS-2.

In "forward flow", the exit pressure of TIMS-1 is larger than the entrance pressure of TIMS-2. Ions are then passively transported through the interface region as they are dragged towards TIMS-2 by the flowing gas. Because the entrance pressure of TIMS-2 is lower in this setting, the resolving power of TIMS-2 is also reduced. Nevertheless, this setting appears most appropriate for applications in native mass spectrometry because of the gentle transport of ions through the interface.

In "reverse flow", the exit pressure of TIMS-1 is lower than the entrance pressure of TIMS-2. This means that the drag force in the interface region pushes ions back towards TIMS-1. An accelerating electric potential is thus needed to actively force ions through the interface region. This may activate ions due to energetic ion-neutral collisions in the interface. However, because the entrance pressure of TIMS-2 is higher in this setting, the achievable resolving power is also higher. This makes reverse flow the natural choice for proteomics or glycomics studies where high resolving powers are advantageous.

Each TIMS analyzer in tTIMS can be individually operated to transmit ions (without mobility-separation), to mobility-separate ions, or to mobility-separate as well as to trap ions. Additionally, the interface region can be set to simply transmit ions, to select ions with mobilities of interest, and/or to activate the ions traversing the interface. Hence, the ions may be manipulated in various ways as discussed in Ref. [39]. Generally, however, ions are mobility separated in TIMS-1, selected and activated in the interface, and are mobility-analysis in TIMS 2. This mode of operation allows collision-induced unfolding (CIU) and collision-induced dissociation (CID) experiments of mobility-selected ions.

CIU experiments measure how the momentum transfer cross sections of the ion changes when it is vibrationally activated by ion-neutral collisions. Such measurements are widely used to characterize structures of protein systems, because they characterize the relative height of energy barriers associated with breaking of non-covalent bonds as the ion unfolds due to collisional-activation [40].

Fig. 3A illustrates how a compact, folded conformation of ubiquitin charge state $[M+7H]^{7+}$ is mobility-selected from a distribution of conformations and then collisionally-activated to produce unfolded isomers by TIMS-CIU-TIMS. Notice that tTIMS achieves CIU of intact protein ions at fairly low activation potentials. A bias of 50 V between aperture-2 and deflector-2 energized $[M+7H]^{7+}$ ubiquitin enough to unfold compact ions into their unfolded isomers. Because up to 250 V are available as bias between aperture-2 and deflector-2, TIMS-CIU-TIMS experiments can be performed for significantly larger protein systems. This ability to perform mobility-selective CIU experiments is important when analyzing species when the initial protein species exists as a mixture of distinct (constitutional or conformational) isomers. Here, tTIMS is able to perform CIU experiments separately for the selected isomers, thereby characterizing their structural differences.

In CID experiments, a precursor ion is collisionally-activated to produce fragment ions, revealing the identity of the precursor ion. Such CID experiments are widely used in proteomics experiments to identify (partial) amino acid sequences and/or locate post-translational modifications by tandem-mass spectrometry. CID experiments can be performed in the interface region of tTIMS by simply increasing the activation voltage between aperture-2 and deflector-2. Fig. 3B illustrates TIMS-CIU-TIMS experiments for mobility-selected $[M+8H]^{8+}$ ions of the protein ubiquitin. Here $[M+8H]^{8+}$ ubiquitin ions dissociated at activation-voltages larger than ~190 V in the tTIMS interface region. Top-Down sequencing is possible, as shown

by the a_n, b_n, y_n fragment ions, including their neutral loss satellites, common in bottom-up and top-down. Sequence coverage increased with activation voltage up to the available ~250 V.

Being a new development, the benefits of mobility-selective CIU and CID experiments with tTIMS instrumentation are currently under investigation. The ability of tTIMS to mobility-separate and select ions in TIMS-1 prior to performing CIU or CID experiments is expected to be beneficial in a number of scenarios related to native mass spectrometry measurements. It was previously shown that TIMS [41] and tTIMS [39] instruments are gentle enough to peptide and protein systems that even the small protein ubiquitin is retained close to its native state. More recently [42], it was demonstrated that ubiquitin largely retains its native inter-residue contacts with an intact hydrophobic core when studied by tTIMS. Hence, tTIMS allows, for example, isomer-selective top-down sequencing experiments when distinct (conformational or constitutional) isomers co-exist for a protein system.

3.3. PASEF & shotgun proteomics

For improved ion handling, a TIMS-MS instrument can be configured with an ion trap in front of the TIMS analyzer (Fig. 1B) [43]. This trap – typically another TIMS device - accumulates ions from the source while the TIMS is simultaneously analyzing a previously accumulated group of ions. When TIMS analysis of the "previous" group is complete, the newly accumulated ions are rapidly dumped from the trap into the TIMS analyzer. While the TIMS analyzer is analyzing the new group of ions, the trap is accumulating the next group of ions from the source. Operating in this way, ions are continuously being collected from the source, offering 100% duty cycle in principle at least. Importantly, the ions are eluted from the TIMS in packets – i.e. mobility peaks – about 1 ms in wide. This leads to a compression of ions, for example from a 100 ms accumulation into a 1 ms peak and therefore a signal increase of more than an order of magnitude [43]. This together with the fact that the various species elute separately in time enables the technology of "Parallel Accumulation SErial Fragmentation" (PASEF) now used in shotgun proteomics [44,45].

PASEF is typically used in conjunction with the LC-TIMS-MS/MS analysis of samples of protein digest. During the course of an LC run, many peptides elute from the LC at any given moment. The PASEF approach first analyzes the effluent to produce TIMS-MS spectra like the one shown in Fig. 4A. Such "survey" spectra are used to determine what species will be selected for MS/MS analysis in subsequent TIMS scans and to plan the precursor selection. PASEF synchronizes MS/MS precursor selection with TIMS separation thus increasing sequencing speed without any loss in sensitivity. Fig. 4A is one of many IMMS spectra acquired during the course of an LC-TIMS-MS analysis of a HeLa digest. The lines in the figure connect the mobility, *m*/*z* coordinates of precursor ions selected during the ten subsequent PASEF scans. Ions eluted from the TIMS analyzer are selectively transmitted based on their *m*/*z* by the analytical quadrupole (Q). Once selected on the basis of mobility and *m*/*z*, precursor ions are fragmented in the collision cell (CC) and mass analyzed in the TOF mass analyzer. The process of taking a survey scan followed by several PASEF scans is repeated for the course of the LC run. As the precursor ions are peptides formed by digestion of proteins, the fragment ion spectra provide a partial sequence of the original protein that can be used to identify the proteins in the original sample. Because all precursor ions are accumulated in parallel and eluted serially, the speed of producing MS/MS spectra and therefore peptide sequencing is increased an order of magnitude without sacrificing sensitivity. Notice in Fig. 4A that singly-charged species were readily excluded by their characteristic positions in the IMMS spectrum.

During a 120 min PASEF analysis of HeLa digest, an example of a single (~100 ms) survey scan resulted in the selection of about 50 precursor ions [45]. About 32 of these were low-abundance and were analyzed multiple times on subsequent PASEF scans for improved statistics. In this example, 118 MS/MS were acquired every second for the duration of the 120 min LC run, resulting in over 800,000 fragmentation spectra. Such a high MS/MS rate allows for "near exhaustive precursor selection … or re-sequencing weak precursors." Using the MaxQuant data analysis software, over 6400 proteins could be identified from the data produced in a single HeLa run with a high quantitative reproducibility ($R > 0.97$). In another example, 2900 proteins could be identified from the 30 min analysis of 10 ng of HeLa digest.

One of the trends in TIMS usage, especially with complex samples, is to use the additional dimension of mobility, or equivalently collision cross section (CCS), not just to separate analyte species but to improve specificity [31–33,38]. Adams et al. used an early form of TIMS installed on an Impact HD to perform LC-TIMS-MS to identify opioids and their metabolites in urine by taking advantage of known LC retention times, CCSs, and ion masses [46]. With precisions of less than 0.3% in retention time, 0.6% in CCS, and about 1 ppm in mass, the authors claimed a higher confidence in identification than traditional LC multiple reaction monitoring approaches. This trend toward using CCS information is further emphasized by recent works developing machine learning and prediction algorithms for the rapid prediction of CCSs for species relevant to lipidomics [47] and metabolomics [48].

More recently Meier et al. used a timsTOF pro to study the potential use of peptide CCS values in shotgun proteomics [45]. In one test, HeLa digest was separated into 24 fractions, each of which was analyzed via PASEF using a 2 h LC gradient. This resulted in 113,478 CCS values from 89,939 unique peptide sequences and about 9000 protein groups (proteins distinguishable from each other by their identified peptides). The density distribution plot of Fig. 4B shows characteristic charge state dependent trend lines. These CCS results were highly reproducible (0.1% median absolute deviation)- about ten times more reproducible than LC retention times even when the same column and gradient are used [45]. Importantly, they are reproducible under differing instrument conditions – for example different TIMS ramp rates. Thus CCS values have the potential for improving specificity and providing an avenue to improved proteomics methods.

4. Conclusions

TIMS is a small (few cm long) flexible (adjustable mobility range and speed) IMS analyzer which is readily hybridized with MS. TIMS-MS in its most basic configuration has already shown applicability to a wide range of analytical problems including PTM peptide analysis, isomer and conformer separation, analysis of drug isobars, histone tails, DNA duplexes, and lipids. Hybridization of TIMS with high performance analyzers such as ICR allows for more effective analysis of highly complex samples such as SRFA, containing as many as 20,000 species [38]. Adding trapping ahead of TIMS in addition to downstream MS has led to technologies such as PASEF for improved shotgun proteomics [45]. Finally, tandem TIMS brings additional analytical power, including the possibility of top down proteomics analyses [39]. The potential of TIMS-MS and its various instrument configurations is still being explored, but the trend is for its application space to dramatically expand over the next several years.

Acknowledgements

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