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A Dual Source Ion Mobility–Mass Spectrometer for Direct Comparison of ESI and MALDI Collision Cross Section Measurements

Sevugarajan Sundarapandian[†], Jody C. May[†], and John A. McLean^{*}

Department of Chemistry, Vanderbilt Institute of Chemical Biology, Vanderbilt Institute for Integrative Biosystems Research and Education, Vanderbilt University, Nashville, TN 37235

Abstract

In this report, we describe a dual ionization source ion mobility–mass spectrometer (IM–MS) instrument platform for investigations that critically compare ion mobility collision cross section (CCS) measurements obtained from different ionization methods. The instrument incorporates both matrix-assisted laser desorption ionization (MALDI) and nano-electrospray ionization (nESI) sources. The nESI source incorporates a keyhole geometry ion funnel design which facilitates axial ion focusing, accumulation, and generation of short duration (10–30 μ s) ion pulses for use with the IM–MS. The IM–MS instrument operation is independent of which ionization source is used. This allows comparisons of collision cross section measurements to be made between both ion sources with minimal differences in the instrumental arrangement. The performance of the nESI ion source is evaluated by measuring the collision cross section values of the charge states of equine cytochrome *c* ($z = 9$ to 16) and values are in good agreement (<2% deviation) with those previously reported in the literature. Several charge states ($z = 8$ to 11) of cytochrome *c* exhibit multiple cross sectional features in the ion mobility analysis. An analysis of the tryptic peptides of cytochrome *c* formed by both ESI and MALDI demonstrate that on average, +1 MALDI ions are similar in CCS to +1 ESI ions and are smaller than +2 ESI ions. The ion mobility resolving power with ESI (30–35) is comparable to that obtained by using MALDI (35–40), which suggests that both sources produce sufficiently narrow ion pulses for the measurement to be predominately diffusion rather than gate pulse width limited.

INTRODUCTION

Hybrid analytical instrumentation based on ion mobility coupled with mass spectrometry (IM–MS) is emerging as a powerful and promising technique for protein identification and characterization.¹ While IM–MS instruments have been used by analytical chemists since the early 1970's,^{2,3} much of the renewed biological interest in IM–MS stems from the incorporation of MALDI⁴ and ESI⁵ ionization methods, which extends the analysis capabilities to large, nonvolatile molecules. Fundamentally, the IM–MS method has not

^{*}To whom correspondence should be addressed: john.a.mclean@vanderbilt.edu.

[†]These authors have contributed equally to this work

changed significantly since it was first developed to explore low energy ion-molecule reactions in the 1960's,^{6,7} with the exception that technological advances have greatly improved instrument sensitivity and resolution in both analysis dimensions. IM-MS instruments rely first on the formation of gas-phase analyte ions and the subsequent tandem characterization of these ions by both the size-to-charge dependant ionic mobilities and the inherent mass-to-charge (m/z) ratio.^{8–11} For large molecular weight ions (greater than *ca.* 500 amu), the low field ion mobility depends primarily on the ion-neutral collision cross section (CCS)¹² with compact ion structures possessing higher measured mobilities (*i.e.*, shorter drift times) as compared with elongated ion structures. Thus, IM-MS instruments provide complimentary measurements of ion size and ion mass. The combined 2D analysis offers a unique partition of data into molecular class specific correlations which are indicative of the average gas-phase packing efficiency of their atomic constituents.^{1,13,14}

One of the longstanding issues in IM-MS research is to what extent the measured CCS depends on the ionization technique. This issue in turn is directly relevant to the physiological significance (or lack thereof) of measured ion mobility values.^{15,16} To date the majority of IM-MS measurements on biomolecular ions have been obtained using ESI as the ionization source. Recent studies have underscored the dependence of protein structure on charge state distributions measured from ESI,¹⁷ which can change considerably depending on both spray and solvent conditions.¹⁸ In the IM-MS analysis of ESI generated ions, there is a clear correlation between the ion charge state and measured CCS.^{19,20} In general, the CCS increases with the number of charges, because the low dielectric environment of the drift cell results in significant electrostatic repulsion and consequently denaturation of the protein.

Recently, singly charged peptide CCS values formed by MALDI were compared with the same charge states obtained from ESI and the majority of measured CCS values were similar across both ionization methods.²¹ However, it was noted that several peptides did deviate considerably in CCS (from 3 to 10%) between MALDI and ESI values reported in the literature.²² Although the cause and statistical significance of this deviation was unclear, it is important to note that different instrument platforms and sample preparation conditions were used. While differences in operational parameters (*e.g.*, pressure, temperature, and electric field) can be corrected to some degree through standardizing measured drift times to changes in gas number density (*i.e.*, reduced mobility values), this approximation loses precision with increasing disparity between experimental parameters.²³

In this report, we present the design of a modular MALDI and nESI source that was developed for rapid interchangeability on a drift cell IM-MS instrument platform. The MALDI source is based on a contemporary translational plate design, whereby ion gating into the drift cell is inherently short owing to the short duration of the laser pulse (<5 ns). Because the nESI is a continuous ion source which operates under ambient pressure, the source design incorporates ion transfer from atmosphere to vacuum (1–5 torr), ion accumulation, and gating ions as discrete packets into the IM-MS. To achieve optimal performance parameters, the ESI source utilizes an electrodynamic ion funnel²⁴ in a keyhole geometry for axial

focusing, trapping, and pulsing of ions. The keyhole geometry is conceptually similar to the hourglass design described by Smith and coworkers²⁵ in that it incorporates a second diverging-converging funnel region for ion accumulation. Ion trapping and pulse generation is achieved through the use of a single stop potential applied to the final exit aperture plate which is supplied through the same power supply electronics and software used with the existing MALDI configuration. This report describes the design considerations of the dual source IM-MS instrument and provides initial data in the comparison of CCS values obtained on the common platform for both MALDI and nESI generated biomolecular ions.

EXPERIMENTAL

The instrument used in this work is a prototype IM-MS configuration constructed in collaboration with Ionwerks, Inc. (Houston, TX) that was initially designed for MALDI-IM-MS²⁶ and imaging MALDI-IM-MS²⁷ capabilities. The IM-MS instrument incorporates a ~14 cm drift cell fabricated from stacked stainless steel plate electrodes and Viton[®] o-rings (DuPont Performance Elastomers, Wilmington, DE) sandwiched between interlocking Delrin[®] (DuPont) spacers to create an isolated supporting atmosphere of drift gas. Electrode dimensions are 3.18 mm inner diameter, which cause ions to traverse regions of relatively higher and lower electric fields.²⁸ Note that under both conditions the ions remain under the low-field condition.^{23,29} This design results in the continuous refocusing of ions to the center axis of the drift cell which greatly improves instrument sensitivity.³⁰ Ions exit the drift cell through a conductance limiting aperture (1.6 mm diameter) and pass through a differential pumping interface consisting of a gas skimmer and electrostatic ion optics that focus and shape the ion beam into a thin sheet. The ion distributions are mass analyzed by an orthogonally configured time-of-flight mass spectrometer (TOFMS) utilizing a 2-stage reflectron which results in improved mass resolution (>2000) across a wide m/z range. Ions are detected using a dual 40 mm microchannel plate assembly (5 micron pore size, Photonis, Sturbridge, MA) in a chevron configuration. The ion signal is digitized into time bins (time-to-digital converter, Ionwerks, Inc., Houston, TX) which are correlated to the ion introduction and time-of-flight analysis pulses of the experiment. Independent time resolution is defined in both the ion mobility and TOFMS dimensions through using a time interleaving data acquisition strategy²⁶. Custom data acquisition and visualization software (Ionwerks, Inc.) is used for data workup.

MALDI Source

The prototype IM-MS instrument with MALDI ionization capabilities is shown schematically in Figure 1A. The MALDI source consists of a translational XY sample stage (MC-5B, National Apertures, Inc., Salem, NH) that is capable of supporting a variety of commercial and custom sample plate designs. The sample support assembly is mounted within a vacuum chamber and incorporates a set of linear motion micrometers to adjust the XY tilt and overall distance of the support with respect to the drift region entrance. The sample plate surface is positioned ~9 mm from the ion sampling aperture of the mobility drift cell. Sample plates are introduced to the source chamber via a load-lock system incorporating a magnetically coupled linear motion arm (Huntington Mechanical Laboratories, Inc.,

Mountain View, CA). Three DN100 viewports mounted to the sample chamber allow a variety of ionization lasers to be utilized with the MALDI source. For experiments described here, a high repetition rate (up to 5 kHz) frequency tripled Nd:YLF solid state laser (wavelength = 349 nm, Explorer 349, Spectra Physics, Mountain View, CA) is used for sample desorption/ionization. The fluence is attenuated with a continuously variable neutral density filter and reflected off of a mirror mounted opposite the sample and near to the ion beam axis, resulting in a beam profile on the sample plate that is very close to circular. Desorption/ionization occurs in a supporting atmosphere of 1–5 Torr helium which is also utilized as the drift gas for the ion mobility experiment. Performing MALDI at these elevated pressures results in the rapid thermalization of laser desorbed species through collisions with the helium drift gas.³¹ It was previously found that *ca.* 1 Torr is optimal for collisional cooling of MALDI generated ions while minimizing matrix adduct formation,³² which provides the impetus for conducting MALDI at these intermediate pressures. Furthermore, performing IM at these intermediate pressures allows for rapid drift times necessary when performing imaging IM-MS experiments. Timing of the experiment is synchronized to the pulse rate of the laser which generates ions in temporally-discrete packets (<5 ns) necessary for time-dispersive measurements.

nESI Source

The nESI ionization source is designed to couple directly onto the existing IM-MS instrument as a modular component which replaces the MALDI ion source as indicated in Figure 1B. Samples were introduced to the nESI source via direct infusion from a pump-driven microsyringe at typical solution flow rates of 500 nL min⁻¹. The nESI spray emitters are fabricated in house by pulling 100 μ m borosilicate glass capillary tubes to a final tip inner diameter of *ca.* 20 μ m. A conductive wire is inserted directly into the sample flow inlet at the spray needle via a T-junction PEEK union to supply the high potential (2–2.5 kV referenced to the inlet) necessary to generate the electrospray. The emitter assembly is mounted onto an XYZ translational stage (OptoSigma Co., Santa Ana, CA) for positioning with respect to the counter electrode inlet. The spray inlet to the spectrometer is at ground potential and consists of a metal capillary (0.5 mm inner diameter $\times$ 15 cm length) inserted into a stainless steel block which is heated with two cartridge style resistive elements (Omega Engineering, Inc., Stamford, CT). A closed-loop thermostat system (Omega) controls the temperature (400 K desolvation) which is monitored by a K-type thermocouple. Alternately, the temperature of the inlet can be raised to study thermally induced changes in the ion structure.³³ Ions which are entrained into the heated capillary exit into a differentially pumped region (1–5 Torr) which houses the keyhole ion funnel assembly.

Keyhole Ion Funnel

The keyhole ion funnel is comprised of 96 electrodes with various inner diameters machined from 0.5 mm thick brass plates. Total outer dimensions of each square plate is 50.8 mm. Electrodes are separated and electrically isolated by 0.78 mm thick Delrin spacers. As illustrated in Figure 2, the keyhole design consists of five sections: (i) ions enter a 20 electrode segment of the same inner diameter (25.4 mm) which allows free expansion of the ion/neutral jet emerging from the capillary; (ii) 20 electrodes taper in inner

diameter from 25.4 mm to 2.0 mm to focus ions on the central axis; (iii) 20 electrodes expand in inner diameter from 2.0 mm to 20.0 mm to create a high space charge trapping region; (iv) 32 electrodes taper to a 4.0 mm aperture where ion accumulation and trapping is accomplished; finally (v) four electrodes of 4.0 mm inner diameter maintain axial focus of ions as they are introduced into the drift region. The last electrode is supplied with the DC-only stopping potential and is fitted with a grid (300 lpi nickel, Precision Eforming, Cortland, NY) to improve the trapping efficiency of the device. It should be noted here that a recent high pressure ion funnel trap developed by Smith and colleagues for ion trapping and gating into an IM-MS utilizes multiple grids in its design to mitigate undesirable field penetration of the stop potential into the funnel region.³⁴ A multiple grid design applied to the keyhole ion funnel described here is also expected to improve the overall performance in terms of charge capacity and pulse response times.

The entire ion funnel assembly is electrically connected using 4 zero insertion force sockets (Digi-key Corp., Thief River Falls, MN), two on either side for the capacitive and resistive networks. A direct current (DC) potential (50–80 V) is applied across the length of the ion funnel to direct ions through the device. The sine wave from a waveform generator (20 MHz function generator, Wavetek, San Diego, CA) is fed into a custom built radiofrequency (RF) oscillator circuit where it is amplified (30–80 V amplitude) and split into two 180 degree off-phase outputs. Each of these two outputs is supplied to every other electrode in the funnel to create the phase offset radial electric field necessary for ion focusing. The nESI source ion exit aperture is mounted to coincide with the position of the MALDI sample plate surface, such that ions are introduced to the drift cell at the same spatial position using both ion sources. The experimental starting trigger is applied to the laser for MALDI mode, or to a pulser which switches between an ion transmission and ion blocking potential on the last funnel electrode when in nESI mode. In this way, the source configuration used (whether MALDI or nESI) is transparent to the operation and data acquisition of the IM-MS instrument.

Modeling of the Keyhole Ion Funnel

The keyhole ion funnel operational parameters were evaluated using SIMION[®] 8.0 ion optics simulator incorporating the hard sphere collision model (*collision_hs1.lua*, Scientific Instrument Services, Inc., Ringoes, NJ).³⁵ The hard sphere collision model incorporates a variable mean free path calculation which is determined from the field and kinetic energy dependant ion velocity. The ion's position is readjusted through a step integration process with time steps sufficiently small to account for all collision events. Upon a collision, the ion's new velocity and kinetic energy are calculated, weighed against a temperature and number density-dependant Maxwell-Boltzmann distribution. A random collision angle is assigned based on an elastic interaction and the ion's position in the electric field is then determined based upon the results of the Laplace calculations. This iterative process is repeated many times over the course of the simulation, which results in continuous adjustments to the electric field and diffusion-dependant position of the ion. Since collision frequency is gas density dependant, the hard sphere model becomes computationally

expensive as the gas pressure is increased and is not utilized in this work for pressures exceeding *ca.* 10 Torr.

The hard sphere collision model is incorporated into an electrodynamic field program developed in house for RF ion optics. This RF program superimposes a time-dependant potential on top of a linearly decreasing DC potential across the length of the device. The RF potentials are applied 180 degrees out of phase to every other electrode in the funnel. The RF phase start is randomized at each ion's time-of-birth to avoid ion synchronization effects. Ion-ion interactions are not taken into account in the modeling, which may be significant for high ion densities and/or small geometry drift regions.³⁶ This may be especially important for RF field confinement of a large number of ion space charges. However, these calculations are computationally expensive in that the simulations described in this work for 100 ions at 3 Torr requires *ca.* 8 hrs when performed on a 2.4 GHz quad-core processor computer (PowerEdge 840 server, Dell Corporation, Round Rock, TX). Experiments were performed by varying the trapping time (data not shown) to confirm that at the low ion currents used in this work, space-charge effects can largely be neglected, provided a sufficiently high trapping potential is used to confine the ions.

Samples and Preparation

Equine cytochrome *c* and human hemoglobin were purchased from Sigma-Aldrich (St. Louis, MO) and used without any further purification. Sequencing grade modified trypsin was purchased from Promega (Madison, WI). The protein was digested using methods described elsewhere.³⁷ Briefly, the lyophilized protein was dissolved in 50 mM ammonium acetate solution and was thermally denatured at 90°C for 15 minutes. The samples were then proteolytically digested with trypsin (*ca.* 20:1 wt of substrate per wt of trypsin) at 37°C for 24 hrs. For MALDI analyses, samples were mixed with α -cyano-4-hydroxycinnamic acid (in methanol) in a molar ratio of *ca.* 1:1000 analyte to matrix, then spotted onto the sample stage and allowed to dry. This step was repeated two additional times for the same sample spot. For nESI analyses, the sample was dissolved in methanol with 2% acetic acid and infused directly into the spray emitter.

RESULTS AND DISCUSSION

Simulation Results

A geometric model of the keyhole ion funnel is depicted in Figure 2A. Ion trajectory and energy simulations were initiated with SIMION[®] in order to estimate the optimum experimental parameters necessary to operate the keyhole ion funnel. Because there are a wide range of possible combinations of operational conditions which will result in favorable performance of the device, several key parameters were initially chosen as a fixed starting point for simulations. Based on pumping conductance calculations of the instrument, it was determined that the funnel region could readily be evacuated to a pressure between 1–5 Torr, and so initial simulations were conducted at 3 Torr. Ions of 1000 *m/z* were selected for simulations as this is a reasonable value that targets proteolytically derived peptides. Finally, simulations were conducted at a fixed RF frequency of 500 kHz based on previously reported values in the literature [38]. Exploratory simulations

(data not shown) suggest that optimum ion focusing and transmission was achieved using 60 V DC across the funnel with an RF amplitude range of 50–80 V at 500 kHz. The stopping potential necessary to trap and accumulate ions in region (iv) is analyte mass dependant. For 1000 m/z ions, a minimum of 20 V was found to be sufficient for ion trapping. Simulated ion trajectories are shown in Figure 2B for an ensemble of 20 ions with mass-to-charge values of 1000 m/z using the optimized conditions. Figure 2C shows a 3-dimensional plot of ion position, time, and kinetic energy for the 20 ions of 1000 m/z using the above optimal conditions with 50 V RF amplitude and a 20 V stopping potential. The specific regions of the keyhole funnel are annotated on the position axis.

Simulation results suggest that no significant ion heating occurs during transit through the ion funnel, with the highest recorded kinetic energy values falling well below 1 eV. As would be expected the two areas where ions experience the most field induced kinetic energy increases are in the converging regions of the ion funnel, namely, between regions (ii) and (iii), and in region (v). This is an intuitive result given that ions in these regions are in close proximity to the electrodes, where electric fields are higher than what the ion would experience at the funnel axis.

Another observation that is made from the data projected in Figure 2C is that ions experience the highest residence times in the converging electrode regions (ii) and (iv). This effect has been previously noted in the literature pertaining to the early development of ion funnels and is a consequence of an unavoidable trapping potential that develops when the electrode inner diameter approaches the value of the electrode spacing.³⁹ This trapping field can be mitigated through the use of a DC-only “draw out” potential on the last funnel electrode. In contrast with using ion funnels at the end of the drift cell, where the effect of extended residence time in the funnel should be considered, this does not pose an issue in this work, since there is no temporal information that is encoded prior to the ion gating event.

Figure 3 illustrates dynamic mass range simulations which investigate the efficiency of the keyhole ion funnel for focusing and trapping ions over a wide range of m/z values. Ensembles of 20 ions with m/z values of 1000, 5000, 10000, 15000 and 20000 Da were modeled (Figure 3A) using the optimum conditions determined for the 1000 m/z ions (50 V RF amplitude, 60 V DC across funnel and 3 Torr helium). At increasingly higher m/z values, the RF electric field becomes less effective at focusing ions to the center axis of the funnel. Under these conditions, the ion funnel is ineffective for collecting ions greater than *ca.* 10000 m/z . The efficiency of the keyhole funnel for higher m/z ions can be improved by increasing the gas number density which will improve collisional dampening of the ion energy. This effect can be seen in the trajectory simulations in Figure 3B for 20000 m/z ions simulated at increasing gas pressures. At 5 Torr, the 20000 m/z ions are readily focused and trapped in the keyhole ion funnel. Experimentally, it is straightforward to constrict the pumping conductance of the ion funnel chamber to provide the higher pressure necessary for more efficient focusing of larger m/z ions.

Experimental Results

Early ion mobility studies of cytochrome *c* revealed that an ensemble of stable gas-phase conformations exist for many of the charge states formed from the ESI process [19]. In a series of benchmarking experiments, Figure 4A contains a 2-dimensional ion mobility-mass spectrum of cytochrome *c* analyzed using the nESI-IM-MS instrument. A distribution of charge states between +7 to +19 and centered around +11 is observed. In mobility space, cytochrome *c* exhibits gas-phase collision cross sections which strongly depend on the charge state. Extracted ion mobility drift time profiles for charge states +7 to +19 are shown in Figure 4B. These profiles exhibit non-Gaussian distributions, potentially suggesting that the measured profiles represent a composite of stable gas-phase conformations. The higher charge state species exhibit much narrower profiles than do lower charge states, with a transition in relative abundances occurring between extended and compact conformations at intermediate charge states between +9 and +11. Greater conformational diversity is observed at these intermediate charge states, which is consistent with other ion mobility measurements on the electrosprayed cytochrome *c* system.^{40,41}

Table 1 provides a list of measured collision cross section values for the observed charge states of cytochrome *c* generated from the electrospray. CCS values were obtained from experimental data by fitting the mobility distributions from Figure 4B to a Gaussian profile and converting the centroid drift time values to cross section using the Mason-Schamp relationship.⁴² Despite the presence of multiple peak features in some of the drift time spectra, the resulting CCS values obtained are in good agreement (<2% deviation) with previously reported values.²² Most of the measured CCS values are within 1% of literature. The greatest observed deviation in measured CCS occurs for the +11, +12, and +16 charge states (>1% deviation). For the +11 and +12 charge states, the mobility ATDs exhibit multiple conformational features which are not fully resolved and thus leads to some ambiguity in the fitting of the data to an ideal Gaussian distribution. Uncertainty in peak fitting becomes less of an issue with better mobility resolution, as is the case with charge states greater than +12, where mobility distributions are narrow.

A 2D plot of the IM-MS analysis of the tryptic peptides of cytochrome *c* is provided in Figure 4C. The majority of possible tryptic peptides are represented in the spectrum. Higher charge state peptide ions appear at lower drift times due to the force of the electric field scaling with the number of charges.⁴³ CCS values obtained from both MALDI and ESI generated ions were compared with one another in order to determine what collision cross section differences, if any, exist between ions generated from these two types of ion sources. Table 2 contains cross section values obtained from several tryptic peptides of hemoglobin. The CCS values are compared across both MALDI and ESI generated ions from this work and from CCS values reported previously in the literature. As with the intact cytochrome *c* CCS values (table 1), the tryptic hemoglobin peptide cross sections measured for ESI (+2) compare well to previously reported values, with differences of <2 %. Experimentally, the ESI literature values were obtained on an instrument utilizing an ion trap for ion accumulation and injection of ion packets into the drift cell,²² rather than an ion funnel like that used in this work. This suggests that provided minimal heating of the

ions and using similar trapping times may result in similar conformations, although the exact instrumental arrangement is different. An experimental comparison across the same charged ions was not possible due to the low abundance of +1 ions generated from the ESI in this work, and so +2 charge state ions were chosen for comparison with +1 MALDI ions. The +1 MALDI CCS values measured here are similar to those previously measured for +1 ESI,²² which may suggest that ions of similar cross section are formed by both ionization methods. Also of note is the general trend of +2 ions being larger in cross section than +1 ions from either MALDI or ESI, which conforms to the conventional thinking that the ion expands to accommodate the increased number of charges owing to Coulombic repulsion.

CONCLUSIONS

Collision cross section values were measured for the charge states $z=+10$ to $+20$ of intact cytochrome *c* as well several tryptic peptide ions of hemoglobin ($z=+1$ and $+2$) and compare favorably to previously reported ESI CCS literature values. MALDI generated ions are close in size to their +1 ESI analogues and the general trend of increasing CCS with increasing charge state is observed, lending support to the claim that ion size increases to accommodate an increased number of charges. Work is underway to expand the database of measured CCS values using the dual source IM-MS instrument and with a larger dataset, we will be able to more confidently draw comparisons between mobility measurements obtained with both MALDI and ESI. Such a comparison has important implications in the use of MALDI or ESI for making measurements in structural biology and in fundamental biophysics.

To facilitate such comparisons, a nESI ion source incorporating a keyhole geometry ion funnel was developed for IM-MS applications. Ion trajectory simulations were performed to establish the optimal operating parameters and to characterize the theoretical performance over a range of analytical conditions. Experimentally, the ion funnel performs with optimal voltage and pressure parameters that are very close to those predicted through simulations (500 kHz RF, 50 V amplitude at 3 Torr) with the notable exception that the experimental stopping potential used (60 V) is significantly more than what is found through simulations (20 V). This higher required stopping potential is likely a result of a high concentration of space charge causing ions to leak through the potential barrier, and indeed the SIMION® model utilized does not account for space charge effects. As reported previously, the performance of the electrodynamic ion funnel is found to be sensitive to the frequency and magnitude of the RF field as well as the operational base pressure of the device.

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Figure 1.
A schematic diagram of the IM-MS with interchangeable (A) MALDI and (B) ESI ionization source modules.

Figure 2.

(A) A 3D model of the keyhole ion funnel and (B) ion trajectories through the funnel with the last electrode set for ion accumulation. The five regions of the ion funnel and simulation parameters are discussed in the text. (C) A multidimensional plot of calculated ion position, time and kinetic energy for 20 ions of 1000 m/z as they traverse the keyhole ion funnel.

Figure 3.

(A) Ion trajectories for 20 ions of various m/z values illustrating the loss in focusing abilities for increasingly larger m/z . At 20000 m/z and 3 torr, the electric field is ineffective at refocusing ions to the center of the device.

(B) Increasing the pressure from 3 to 5 torr effectively dampens the ion's kinetic energy such that its trajectory can be favorably altered. Simulation conditions are: 50 V RF, 60 V DC across funnel, 20 V stopping potential, helium background gas.

Figure 4.

(A) A 2-dimensional ion mobility-mass plot of ESI generated cytochrome *c* ion charge states. **(B)** The corresponding ion mobility arrival time distributions for each of the observed charge states of cytochrome *c*. **(C)** An IM-MS 2D plot of the tryptic peptides of cytochrome *c* obtained with ESI.

Table 1

Measured collision cross section values for the charge states 9–16 of cytochrome *c*. The calibrant ion, bradykinin +2, is also included.

Protein Charge State	m/z	Collision Cross Section Values Å ²		
		Avg. Measured Exp. Values	Literature Values *	% Difference
(bradykinin) ²⁺	530.8	242	242	0.00
(cytochrome c) ⁹⁺	1374.4	1980	1964	0.83
(cytochrome c) ¹⁰⁺	1237.1	2210	2226	0.72
(cytochrome c) ¹¹⁺	1124.7	2328	2303	1.07
(cytochrome c) ¹²⁺	1031.1	2305	2335	1.28
(cytochrome c) ¹³⁺	951.8	2400	2391	0.39
(cytochrome c) ¹⁴⁺	883.9	2451	2473	0.88
(cytochrome c) ¹⁵⁺	825.1	2564	2579	0.60
(cytochrome c) ¹⁶⁺	773.6	2642	2679	1.40

* Literature values for cytochrome c were obtained from reference [40]

Collision cross section values of the tryptic peptides of human hemoglobin measured as obtained from both MALDI ($z = +1$) and ESI ($z = +2$) ionization sources. Reported literature values are also provided for comparison.

Table 2

Tryptic Peptide	Peptide Mass [Da]	CCS Values from Literature [Å ²]		CCS Values Measured In This Work [Å ²]	
		ESI +2 ^a	ESI +1 ^a	ESI +2	MALDI +1
SAVTALWGK	932.09	229.36	231.09	228.9 ± 3	232.1 ± 1
VHLTPEEK	952.08	--	228.28	229.3 ± 2	225.1 ± 2
RPPGFSPFR (bradykinin)	1059.56	242.00	--	242.0 ± 1	242.0 ± 1
LHVDPENFR	1126.24	255.74	246.51	253.0 ± 3	246.9 ± 1
VVAGVANALAHK	1149.37	272.31	267.76	273.8 ± 2	268.3 ± 3
FLASVSTVLTSK	1252.48	289.60	298.80	287.4 ± 5	273.4 ± 2
LLVVPWTQR	1274.54	287.97	283.07	286.9 ± 2	275.3 ± 2
VNVDEVGGEALGR	1314.43	287.12	278.91	290.2 ± 3	280.7 ± 2
TGQAPGFTYTDANK	1470.60	297.61	--	296.7 ± 3	288.2 ± 1
EETLMEYLENPK	1495.67	312.71	309.03	309.5 ± 3	297.4 ± 1
VGAHAGEYGAEALER	1529.64	306.47	--	306.9 ± 4	285.9 ± 2
TYFPHFDSLHGSAQVK	1834.03	364.16	--	363.6 ± 3	341.2 ± 1
FFESFGDLSTPDVVMGNPK	2059.29	378.16	--	376.5 ± 3	381.3 ± 3

^aData from reference [22] for ESI generated ions.