Enhanced Separation and Ion Prefiltering using a High Performance Ion Mobility Device Coupled with the LTQ series of Mass Spectrometers

Adam Graichen, Robert Jackson, Ching Wu, and Mark Osgood;
Excellims Corporation, Acton, MA USA
Dirk Nolting and Alexander Makarov; Thermo Fisher Scientific, Bremen, Germany

This poster describes active research to interface IMS to the Thermo Scientific™ Orbitrap™ mass spectrometers, and results from a commercial IMS source which is sold and supported by Excellims Corporation.

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Fully Integrated IMS-MS

When coupled with mass spectrometry (MS), ion mobility spectrometry (IMS) adds tremendous value in the analysis of species that were either previously not resolved by MS or those that may lead to undesirable and highly complex spectra. As ion movement within a drift tube is governed by size/cross-sectional area, effective separation of molecules with even slight structural differences such as conformers, isobars, and isomers can be achieved. In order to maximize the advantages of this technology, we have developed, evaluated, and introduce an ambient pressure IMS device (**Fig. 1**) that readily integrates with a variety of Thermo Scientific mass spectrometers.

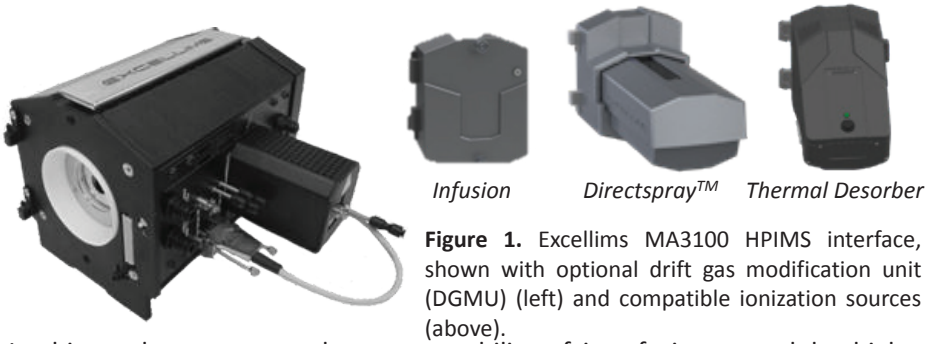


Figure 1. Excellims MA3100 HPIMS interface, shown with optional drift gas modification unit (DGMU) (left) and compatible ionization sources (above).

In this work we present the new capability of interfacing a modular high performance ion mobility spectrometer (HPIMS) with Thermo Scientific mass spectrometers that are designed to accept an atmospheric pressure ionization (API) source. Our device is available as a powerful add-on feature for existing customer instrumentation already in current use, with embedded IMS control directly in Thermo Fisher Scientific's Tune Plus software for Thermo Scientific™ Exactive™ Orbitrap MS and Thermo Scientific™ Q Exactive™ MS models. Beyond accomplishing rapid high resolution separation, the mobility selection afforded by our interface facilitates the removal or inclusion of specific ions for subsequent MS and MS/MS analysis; thereby reducing spectral complexity/congestion, enriching desirable ion populations, delivering added confidence in compound identification, and providing the opportunity to gain insight into the behavior of gas-phase ions not possible from mass spectra alone.

Requiring no additional hardware changes or modifications, the IMS device can be mounted or removed in minutes, with no break in system vacuum. A second Bradbury-Nielsen exit gate is located directly at the end of the 10.85 cm drift tube, effectively allowing only a user-defined portion of separated ions to pass (**Fig. 2**).

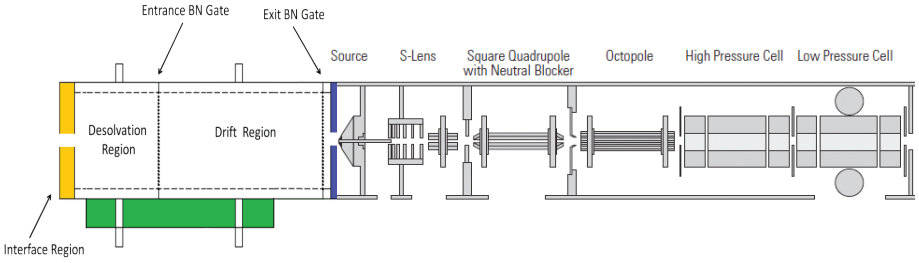


Figure 2. Representation of the dual gate ion mobility module interfaced with a Thermo Scientific™ LTQ Orbitrap Velos™ mass spectrometer indicating ionization interface region (shown with no source), desolvation region, ion gate #1, drift region, ion gate #2, and focusing lens of the IMS-MS interface.

Removal of contaminants

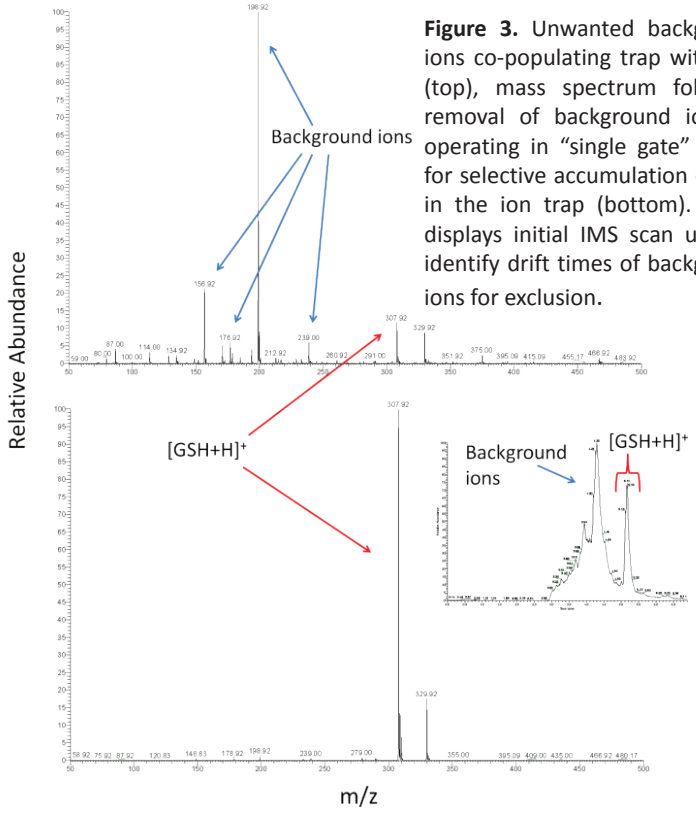


Figure 3. Unwanted background ions co-populating trap with GSH (top), mass spectrum following removal of background ions by operating in "single gate" mode, for selective accumulation of GSH in the ion trap (bottom). Inset displays initial IMS scan used to identify drift times of background ions for exclusion.

Eliminating isobaric interferences

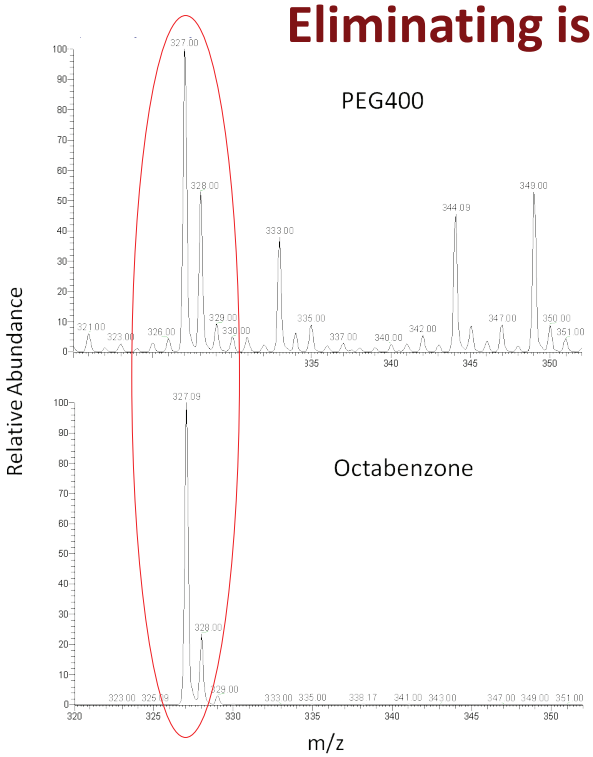
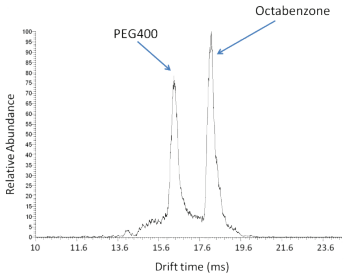


Figure 4. Mass spectra of PEG400 & Octabenzone showing spectral overlap at *m/z* 327 (left). Reconstructed IMS chromatogram showing separation of these nominally isobaric species (below). As PEG can be a widely encountered contamination in MS, the presence of this interference at many m/z values may dominate the spectrum and limit the observation of specific analytes of interest. Pre-MS cleanup methods can be implemented to remove salts, detergents, and other interferences, however, such purification procedures are generally time consuming, low throughput, and risk the loss of precious sample.



High resolution mobility analysis

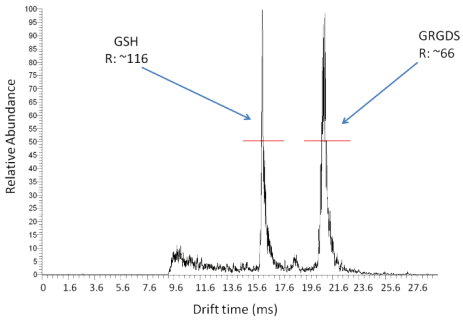


Figure 5. A resolution over 100 is possible in the separation of two peptides (GSH and GRGDS). Routinely operating with a resolving power of over 60, there exists the potential for the IMS-MS system described herein to accomplish the required separation and detection found when utilizing LC-MS. Unlike LC-MS, HPIMS-MS would involve a profoundly simplified method and rapid screening.

Targeted accumulation & Identification confidence

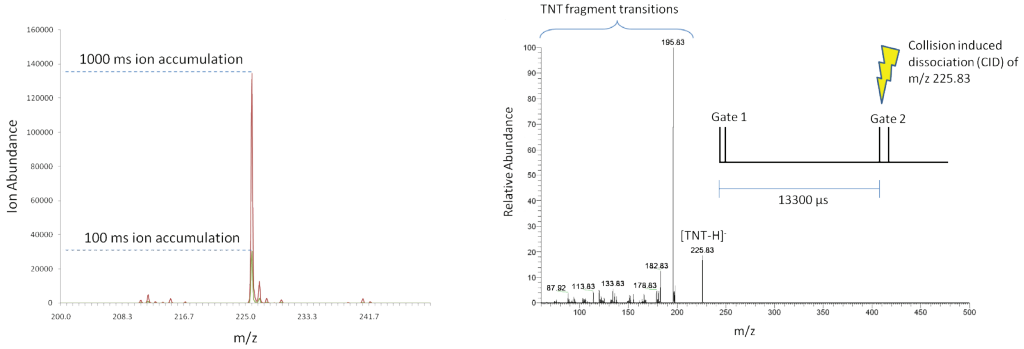


Figure 6. Extended ion accumulation times possible for improved detection sensitivity $[TNT-H]^-$ (left); IMS coupled parallel reaction monitoring (PRM) of TNT for highly specific analyte confirmation (right).

Selective charge state enrichment

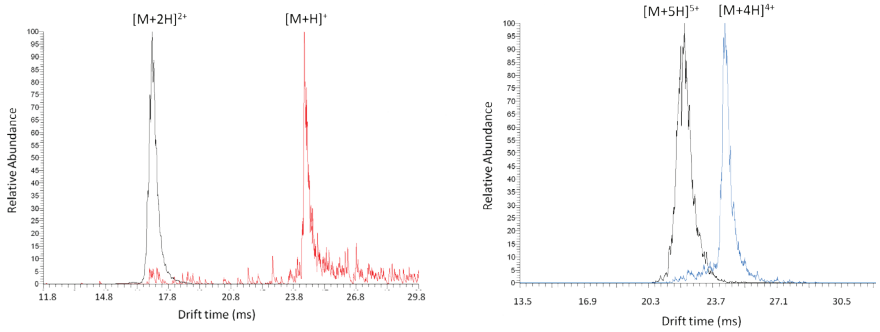


Figure 7. Separation of 1+ & 2+ Bradykinin (left), and 4+ & 5+ insulin (right).

Separation of pseudo-isobaric species

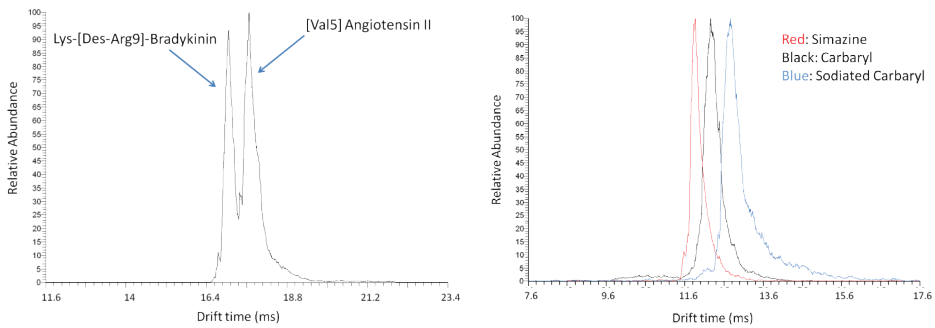
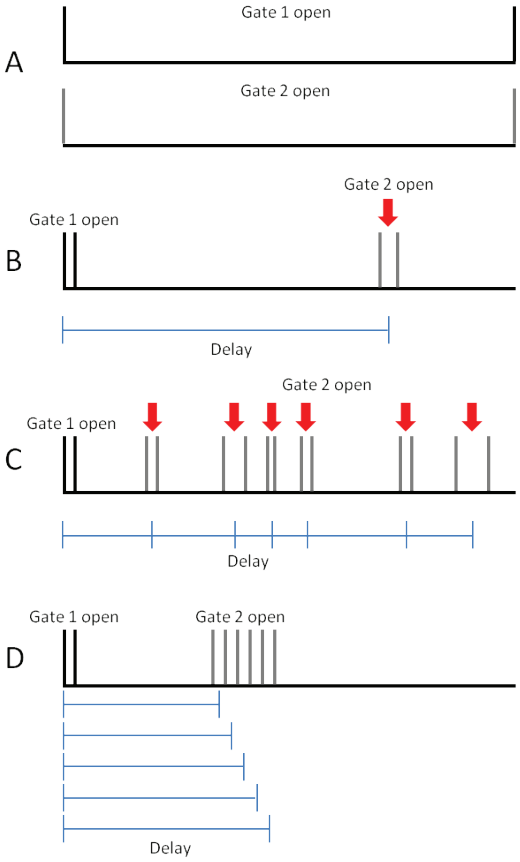


Figure 8. IMS "scan" mode for (left) Lys-[Des-Arg9]-Bradykinin + [Val5] Angiotensin II (m/z: 517) and (right) simazine + carbaryl (m/z: 202).

4 Modes of Operation



Open (A): Ions flow continuously through both ion gates unrestricted into the mass spectrometer. Although our IMS interface can rapidly be removed for reinstallation of the original API source, conventional mass spectral data can be obtained operating in this mode without the risk of a significant reduction in ion transmission efficiency.

Single Gate (B): Ions within a narrow specified ion mobility range can be selected and allowed to pass into the mass spectrometer.

Multi Gate (C): The selection and concurrent passage of multiple ion mobility windows into the mass spectrometer.

Scan (D): Scanning the delay in opening of the second ion gate after the first ion gate has been opened; sequentially stepping a window of variable drift width across the chosen drift time range. This mode is used if (A) the drift times of analyte species are unknown or (B) the user wishes to generate a comprehensive multi-dimensional IMS-MS plot from a complex mixture as in **Fig.9**.

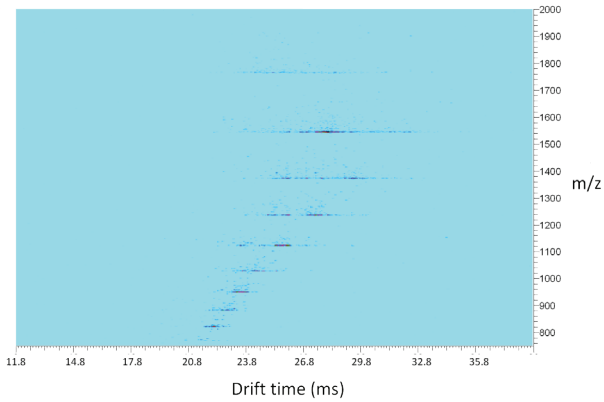


Figure 9. Multi-dimensional IMS-MS plot of intact cytochrome c showing drift time distributions corresponding to 7+ to 15+ charge states. As supported by the presence of distinct species associated with various charge states, IMS provides the unique opportunity to gain insight into protein systems where the difference in structure (conformational state) may be highly relevant for proper functionality.

Summary

(A) Removal or *prefiltering* of contaminants and spectral interferences
(B) Targeted ion accumulation for *improved sensitivity* and potentially *enhanced* MS^n *fragmentation*
(C) Ancillary confidence in analyte identification via IMS-PRM monitoring
(D) Multi gate mode enabling complete transmission for a series of ions
(E) Isolating and examining *conformational states*
(F) Optional DGMU and Faraday plate detector assembly expand the utility of this device and equip the unit to function as a stand-alone IMS instrument

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