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MALDI-HDMS^E: A Novel 'Data Independent' Acquisition Method for the Enhanced Analysis of 2D-Gel Tryptic Peptide Digests

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APPLICATION BENEFITS

- Delivers increased confidence in protein identification without increasing analysis time
- Provides a parallel fragmentation approach that enables sequencing from all tryptic peptides within the sample
- Allows for the re-investigation of data at alternate times in order to retrieve or obtain additional information

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SYNAPT® G2-S HDMS,™
DriftScope™ Software

KEY WORDS

PMF, MASCOT, Increase confidence, MS^E Data Viewer

INTRODUCTION

Peptide Mass Fingerprinting (PMF) is an unbiased protein identification technique that offers limited sequence information for identification purposes due to the reliance on the *m/z* measurement of proteolytic peptides.

An alternative technique commonly used conducts MS/MS sequencing experiments by fragmenting the tryptic peptides through sequential selection using the first analyzer of a mass spectrometer. This MS/MS approach has the following limitations:

- Only the most intense peptides are fragmented
- The user must choose the peptide to fragment
- Information is limited, as peptides can be missed if the sample is depleted
- After the sample is discarded, regaining knowledge from the sample is not feasible
- Potentially more time consuming than PMF alone

In this application note, we propose a novel method of acquiring data called High Definition MS^E (HDMS^E). This method maintains the independent nature of PMF but extends its utility by providing molecular and MS/MS-like peptide sequence information in a simple, generic fashion. Unlike traditional MS/MS analyses, where selection of the precursor ions by the first mass analyser is used, ion mobility separation (IMS) is utilized as an orthogonal separation of peptides in the gas phase, followed by simultaneous fragmentation and high resolution mass analysis. Peptides are associated with their fragments for protein identification using ion mobility (drift time) alignment.

EXPERIMENTAL

Sample preparation

Protein extracted from normal kidney cortex was separated by 2D PAGE. Gels were stained with ProteoSilver Silver Stain, and 23 spots were excised and destained. Gel pieces underwent a classic tryptic digestion for 4 hours at 37 °C. Supernatants, with two further extractions, were pooled together and dried. These were reconstituted in 10 µL of 0.1% trifluoroacid aqueous solution. Samples were mixed with 5 mg/mL of α–cyano-4-hydroxycinnamic acid CHCA matrix and spotted onto a MALDI target.

MS conditions

MS system:	MALDI SYNAPT G2 HDMS
Ionization mode:	Positive
Mass range:	100 to 3,000 Da
Low energy transfer collision voltage:	4 eV
Elevated energy transfer collision voltage:	30 to 199 eV

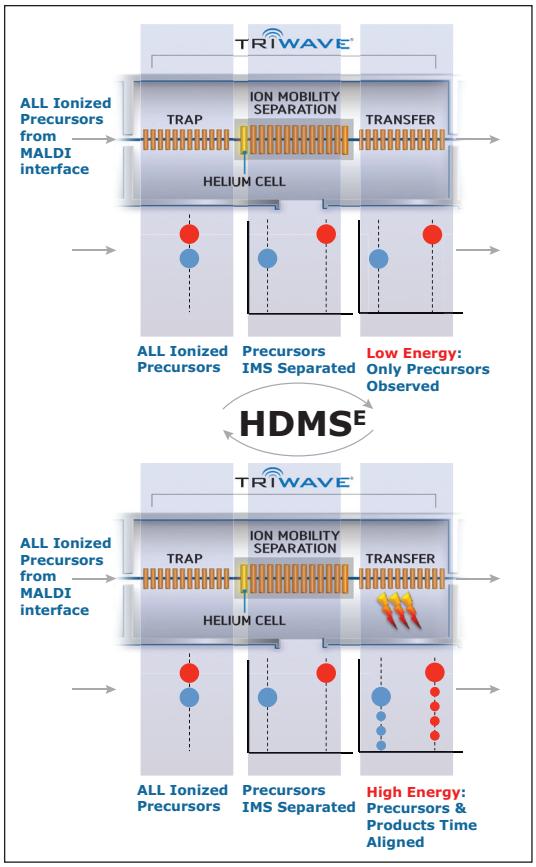

Figure 1. Schematic of a MALDI-HDMS^E experiment.

Data management

The first function was manually processed as a Peptide Mass Fingerprint (PMF) with only the *m/z* and intensities of the peptides submitted for a MASCOT database search.

Using the MS^E Data Viewer software, all peaks in each function were detected using the Apex3D algorithm and aligned to correlate fragments to their precursor based on their drift time.

A peak list (.pkl) file was automatically created for each sample by combining all the precursors and their respective fragments.

The .pkl file was then submitted to a Mascot MS/MS ion search. Results were displayed using Protein Summary, where MS and MS/MS information contribute to the overall identification score.

RESULTS AND DISCUSSION

Figure 2 shows an example plot of *m/z* versus drift time using DriftScope Software that displays both low and elevated functions in the case of the 2D-gel spot matching to ACTB_HUMAN.

In the elevated energy plot, it is clearly seen that the fragments have similar drift time to their associated low-energy precursor ions.

Figure 3 shows the MASCOT scores for both PMF and MALDI-HDMS^E analysis where identification was made in common.

Out of the 23 2D-gel samples analyzed, 21 samples were identified by both PMF and HDMS^E method, with just two that could not be identified by either method. For the remaining 21 samples, three samples were not identified above the 95% confidence level (in MASCOT) by PMF, whereas MALDI-HDMS^E successfully identified these components above the confidence threshold. MALDI-HDMS^E affords significant improvements to MASCOT identification scores for 20 out of the 21 identified samples.

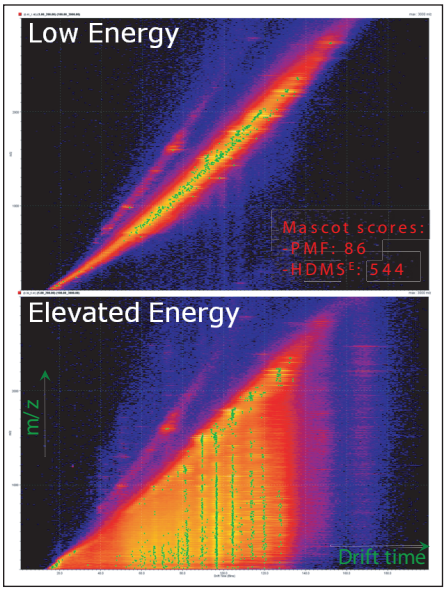

Figure 2. Display of the low and elevated energy MALDI-HDMS^E functions using DriftScope Software for 2D gel identified as ACTB_HUMAN.

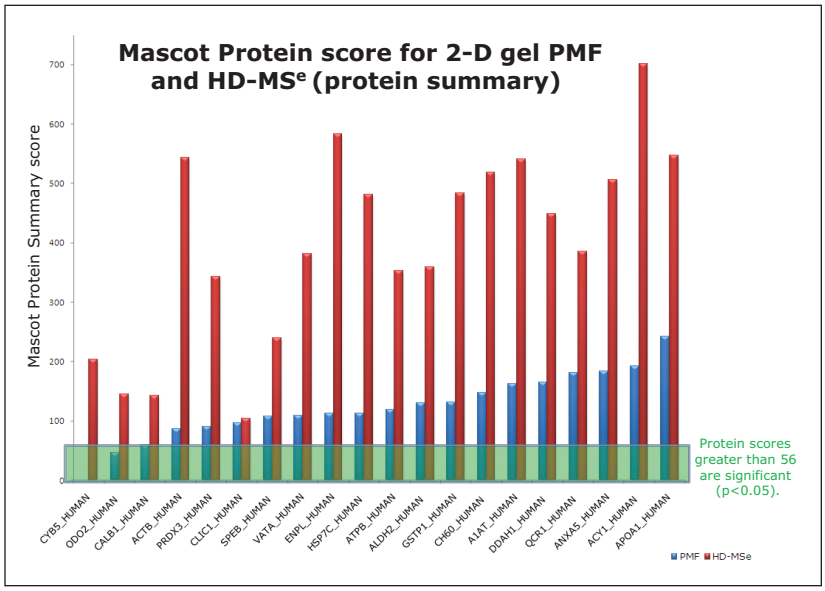

Figure 3. Results summary comparing PMF (blue) and MALDI-HDMS^E (red) MASCOT Protein Summary (www.matrixscience.com) identification scores.

Figure 4 displays the MS^E Data Viewer data from the tryptic digest sample containing ACTB_HUMAN, with window B showing the low energy spectrum for the selected precursor, $[M+H]^+$1790.8, within the drift time window.

Window C is the elevated energy spectrum for the selected precursor that shows the associated fragment ions, more intuitive facilitation, and interactive results reviewing.

Window D shows the high number of fragments that have been used for scoring by MASCOT during protein database identification. The quality of the sudo MS/MS is clearly visible.

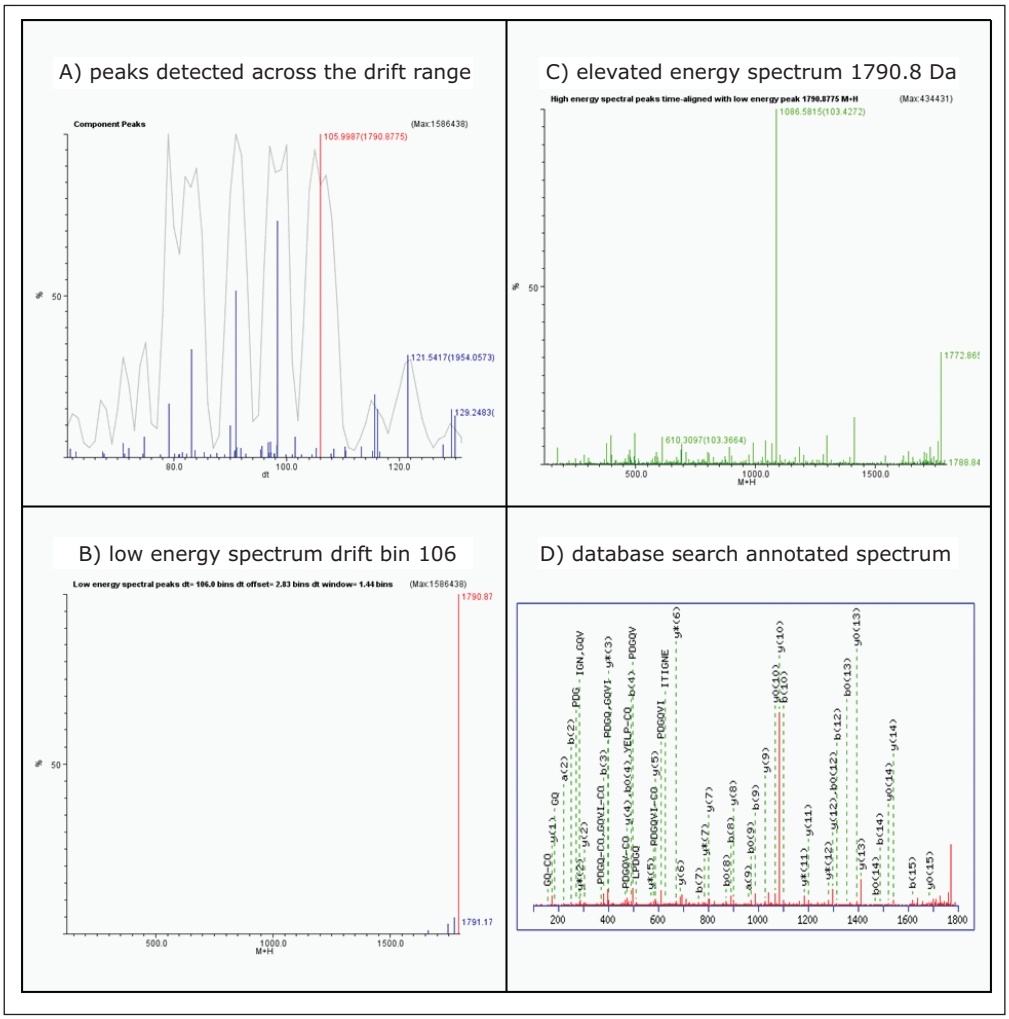

Figure 4. MS^E Data Viewer browser displaying the Elevated spectrum (sudo MS/MS) of $[M+H]^+$ 1790.8 from the ACTB_HUMAN 2D-gel sample with the MASCOT MS/MS peptide identification.

CONCLUSIONS

- This novel data acquisition method shows a clear improvement in protein identification compared to Peptide Mass Fingerprint (PMF) analysis, with concurrently significant increases in protein identification scores.
- The information that can be obtained from the HDMS^E spectra is similar to traditional MS/MS spectra generated on a MALDI SYNAPT G2 Mass Spectrometer.
- MS^E Data Viewer is a powerful software tool that correlates the low and elevated datasets and creates .pkl files for MASCOT database search.

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