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High Definition Data Directed Analysis: The Application of Quadrupole Ion Mobility Time-of-Flight Mass Spectrometry for Untargeted Proteomics Studies

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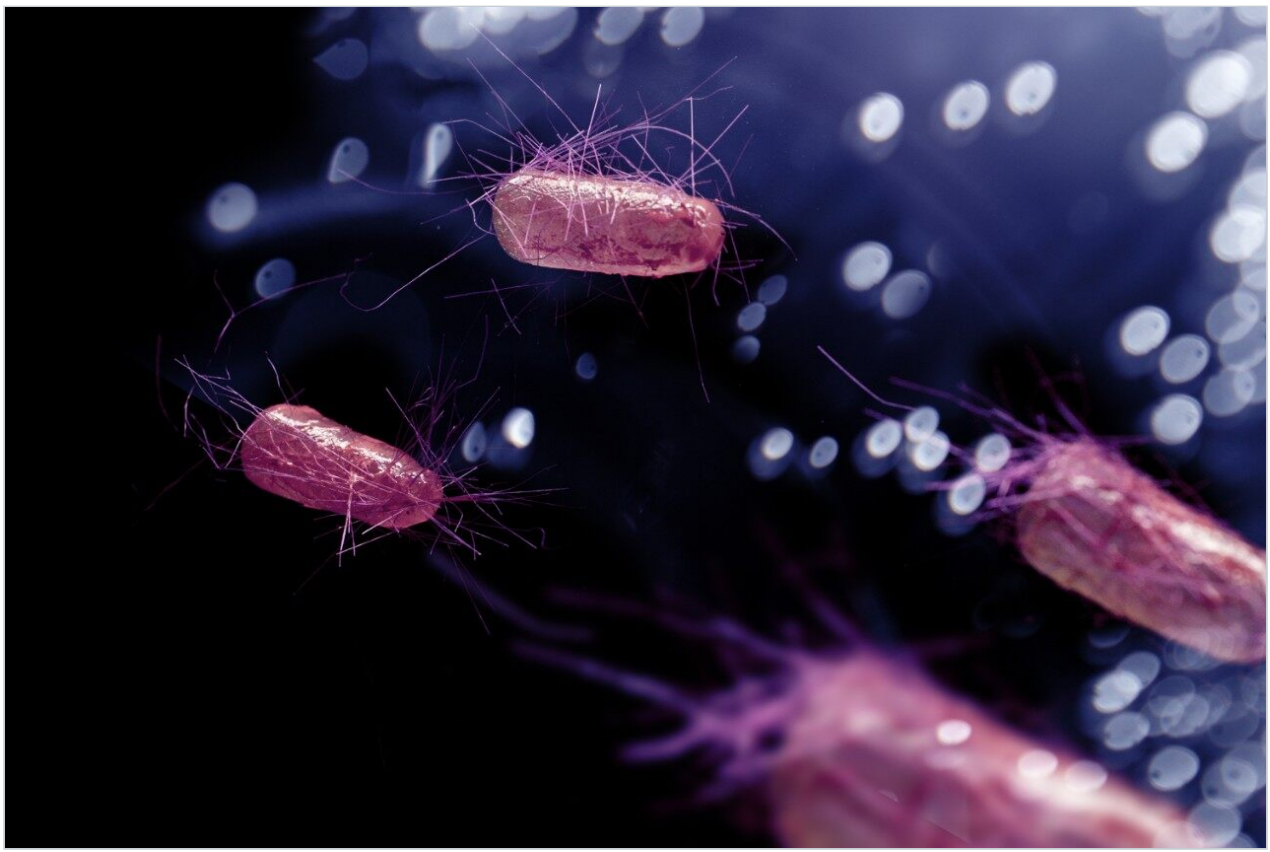

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Abstract

In this study, instrumental and application benefits are demonstrated for the identification of proteins and peptides in a new high definition data directed analysis (HD-DDA) mode, where ion mobility spectrometry is incorporated into a quadrupole time-of-flight mass spectrometer. HD-DDA uses a high duty cycle mode and enhanced decision making to provide a highly sensitive and selective experiment.

nanoACQUITY UPLC applications readily transfer to the ACQUITY UPLC M-Class System

Benefits

- Increasing protein identification and proteome coverage
- Confident identification at the lowest concentrations
- More efficient LC-MS/MS through faster decision making

Introduction

The increasing complexity of bottom-up proteomics challenges the capabilities of mass spectrometers to generate more and more detailed information. Instrument speed, sensitivity, and mass accuracy have all increased significantly over recent years, thereby affording better quality data, improved peptide sequence annotation, and more accurate identification results.

In line with improved hardware features, novel LC-MS acquisition schemas and fragmentation mechanisms have been introduced, including parent ion discovery (PID) methods, data independent acquisitions (DIA), ion mobility (IM) assisted methods, and electron transfer dissociation (ETD). To date, IM has been mainly employed for the cross sectional and structural analysis of various analyte types,[1] and enhancing the specificity of DIA acquisitions such as HDMS^E.[2]

In this study, instrumental and application benefits are demonstrated for the identification of proteins and peptides in a new high definition data directed analysis (HD-DDA) mode, where ion mobility spectrometry is incorporated into a quadrupole time-of-flight mass spectrometer. HD-DDA uses a high duty cycle mode and enhanced decision making to provide a highly sensitive and selective experiment.

Experimental

The cytosolic content of *E.coli* and hela cells were digested using trypsin. Lysates were injected on a nanoACQUITY UPLC System equipped with an ACQUITY UPLC BEH 1.7 µm, 15 cm x 75 µm column coupled to a SYNAPT G2-*Si* Mass Spectrometer. Data were processed and searched with ProteinLynx Global SERVER and/or Mascot.[3]

Results and Discussion

HD-DDA enhancements include full support for Wideband Enhancement,[4] which affords a signal increase of five- to ten-fold as well as enhanced decision making logic when switching between MS and MS/MS modes. Wideband Enhancement utilizes ion mobility separation of product ions of a single charge state in combination with pusher synchronization to achieve nearly 100% duty cycle, as shown in Figure 1.

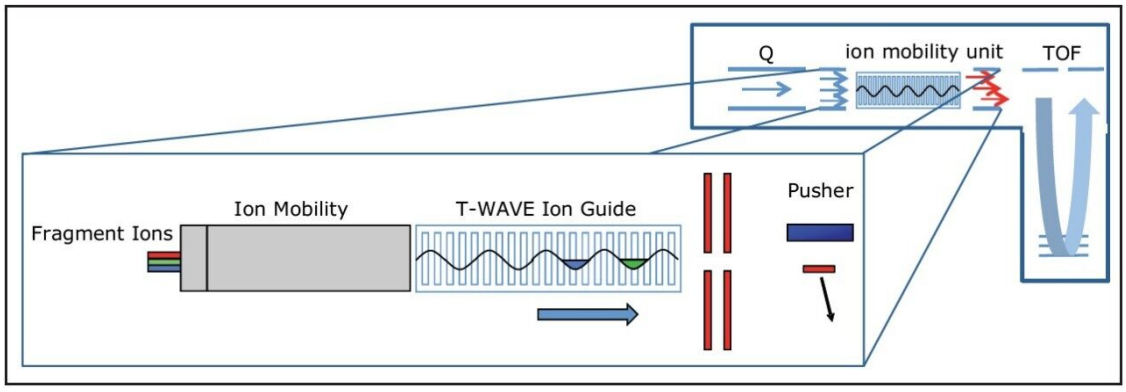

Figure 1. Experiment principle High Definition Data Directed Analysis acquisition. The product ions for a given charge state are separated and focused by ion mobility and the pusher duty cycle synchronized to the mobility (drift) times of the fragments.

HD-DDA acquisitions are typically performed in an untargeted mode and can be complemented with unlimited include and/or exclude lists. Collision energies are stepped, ramped, or determined in real-time based on *m/z* and charge state. Data can be processed and searched with either ProteinLynx Global SERVER or vendor-neutral search algorithms and validation tools such as Mascot and Scaffold,[5] respectively.

The benefit of Wideband Enhancement is demonstrated in Figure 2. Here, an *E.coli* tryptic digest was analyzed by means of nanoscale LC-MS/MS. Data were acquired by normal DDA and HD-DDA. In both cases, 0.1 seconds of MS/MS data were taken at the same moment of time within the LC peak. For this particular experiment, on average across the complete MS/MS spectrum, a five-fold signal increase was observed.

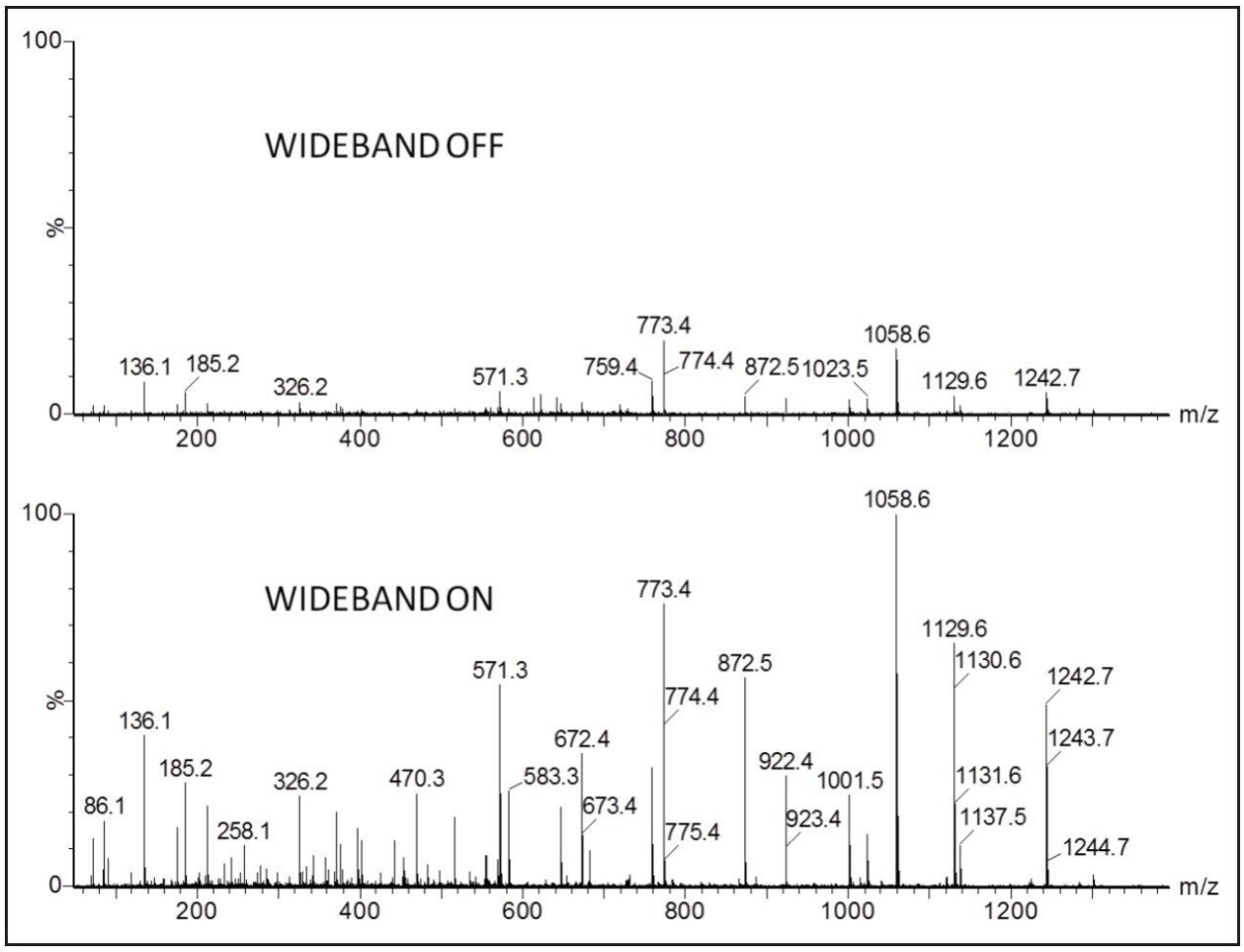

Figure 2. Effect of Wideband Enhancement on MS/MS signal-to-noise for VILAGEVTTPVTVR when switched at the same point within the LC peak. Vertical axes are linked.

For this particular experiment, 15 concurrent MS/MS experiments were conducted per survey scan. The results, shown in Figure 3, illustrate the increase in MS/MS total ion current (TIC) as a function of the MS/MS channel when contrasting DDA with HD-DDA. The average increase per function was 420%, which is consistent with the results shown in Figure 2. The inset is an example MS/MS from the 15th channel, illustrating that MS/MS data with good signal-to-noise can be readily obtained from the lower abundant

peptides present within the sample.

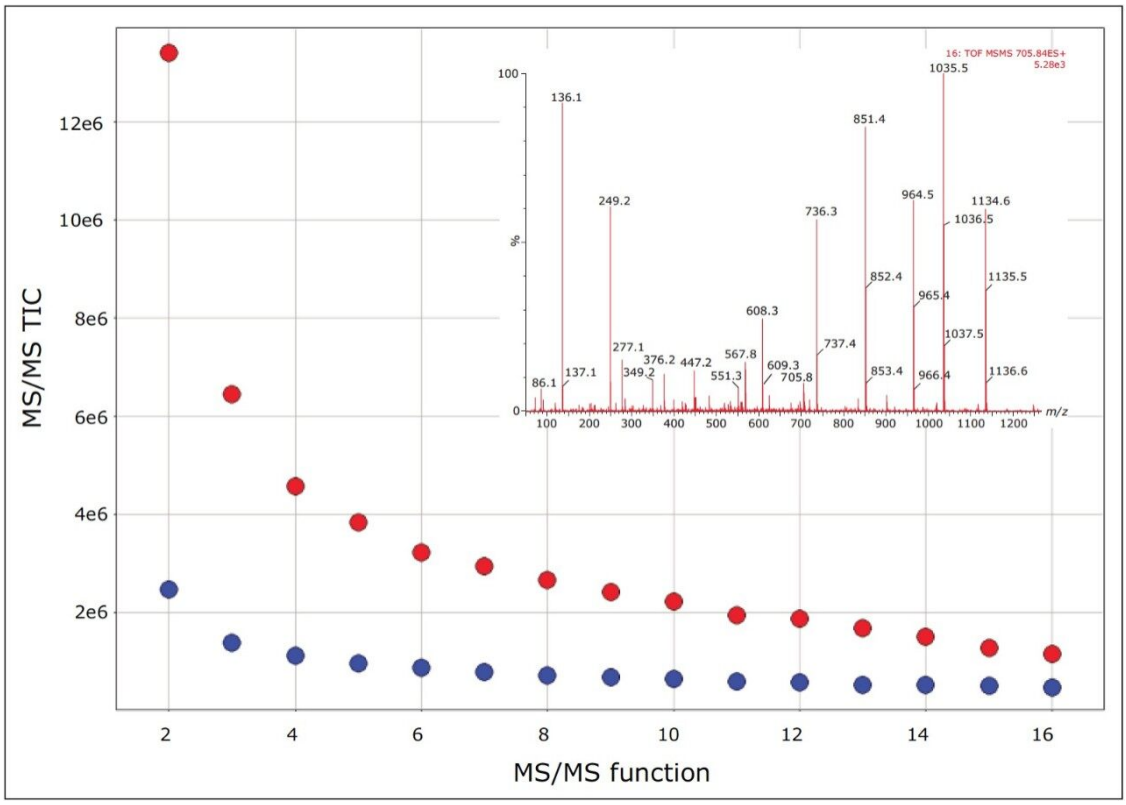

Figure 3. Effect of Wideband Enhancement on MS/MS TIC (red = Wideband Enhancement on; blue = Wideband Enhancement off). Shown inset is an example MS/MS spectrum from the 15th MS/MS function.

The increased sensitivity benefits afforded by HD-DDA for a bottom-up LC-MS proteomics experiment for the same *E.coli* sample are shown in Figure 4. Panel A shows the increase in number of peptides sequence matches. The Venn intersection in panel B contrasts the protein identifications. A significant increase in number of identified peptides (34.8%) and proteins (42.8%) was observed.

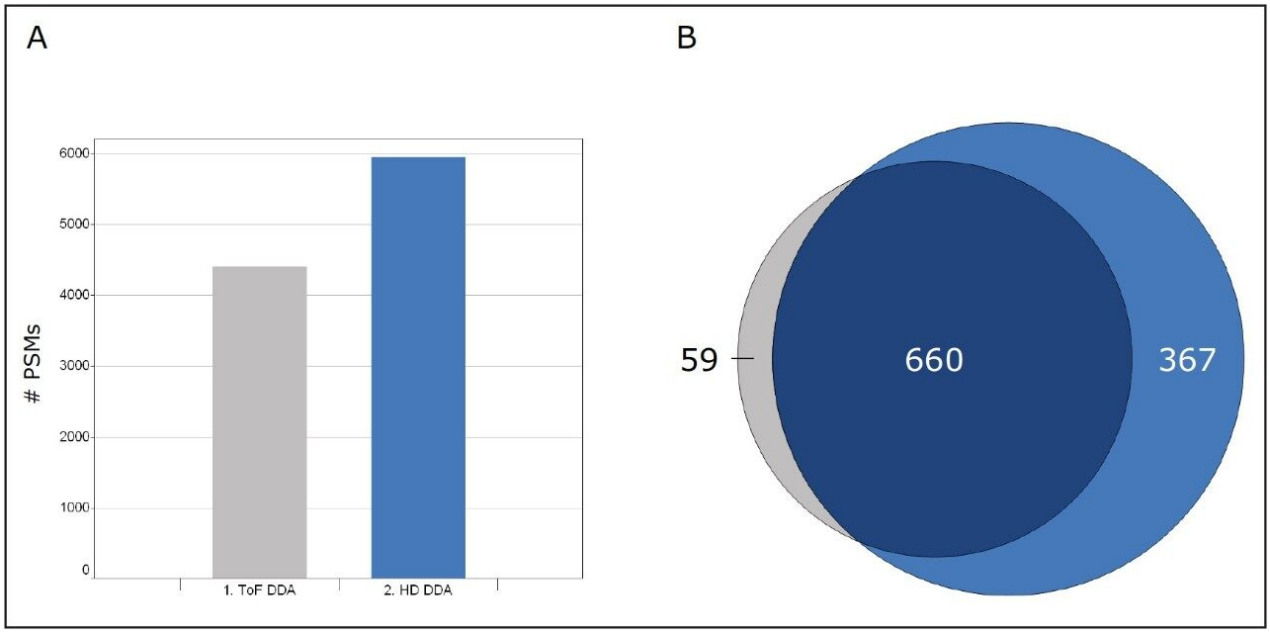

Figure 4. Number of identified E.coli peptides (A) and protein identifications Venn intersections (B) for using Tof DDA (gray) and HD-DDA (blue).

The search results from a more challenging sample are shown in Figure 5. Here, the PLGS search results are summarized for the analysis of a HeLA tryptic digest. A total of more than 2200 proteins were identified that passed a 95% identification confidence threshold. In this particular experiment, the spectrum identification rate was equal to 38%.

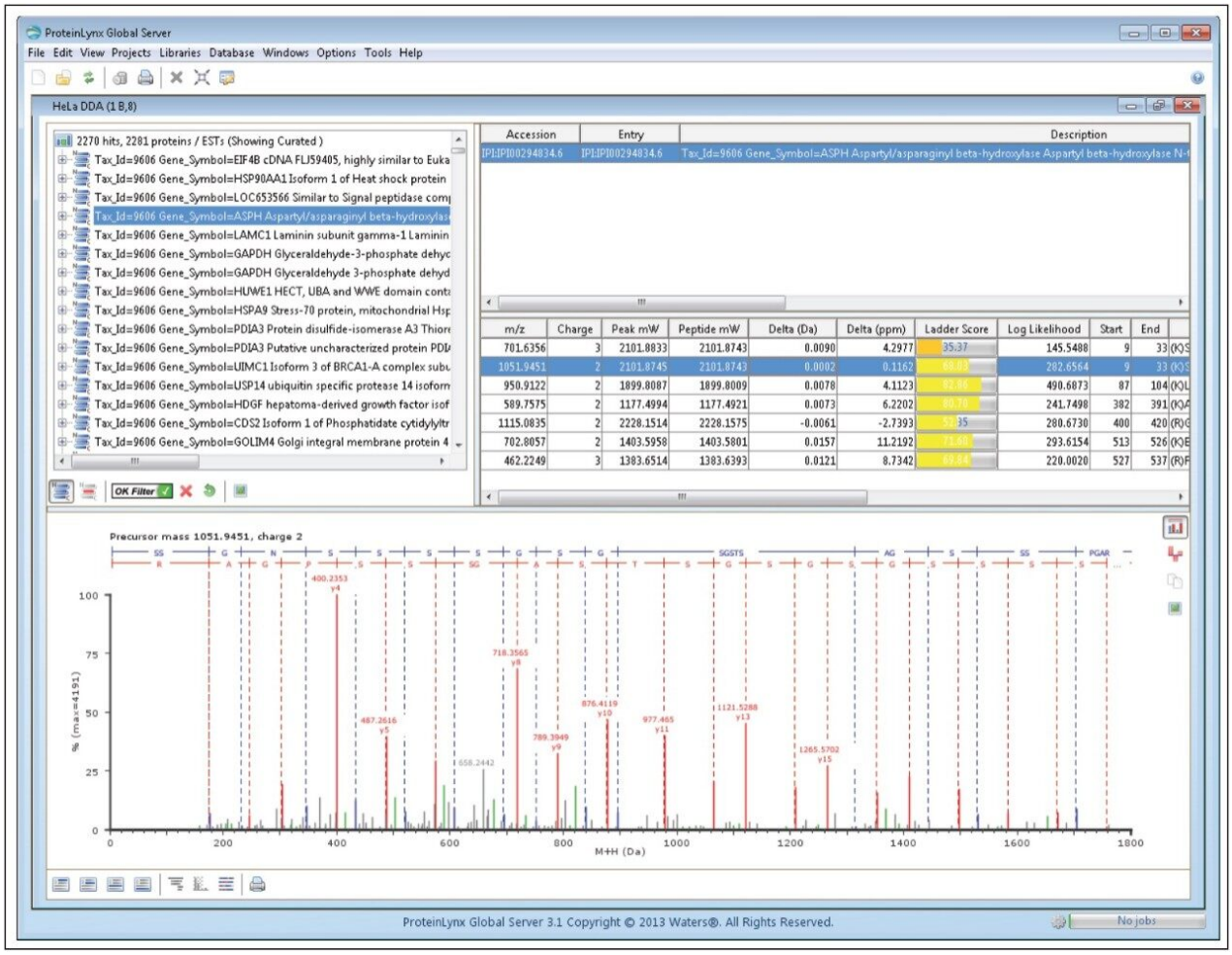

Figure 5. PLGS search identification example of a HeLa tryptic digest by means of HD-DDA that was analyzed with a 120-minute nanoscale LC reversed-phase gradient and IPI amino acid sequence database. The average amino acid coverage was equal to 14.8% and the average number of matched spectra per protein to four.

Conclusion

- HD-DDA Wideband Enhancement provides a typical signal increase of five- to ten-fold
- Spectral data quality for low abundance species/peptides is significantly increased
- The percentage of MS/MS spectra generating a positive match is dramatically increased
- Improved identification numbers of HD-DDA data is adirect result of the enhanced sensitivity and spectral

quality improvements

References

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