

Increased Proteome Definition Exploiting Performance Enhancements of a New Linear Ion Trap—LTQ Velos™

Tonya Pekar Second, Justin Blethrow, Jae Schwartz, Zhiqi Hao, and Vlad Zabrouskov

Thermo Fisher Scientific, San Jose, CA, U.S.A

Thermo
SCIENTIFIC

Overview

Purpose: To ascertain the advantages afforded by the LTQ Velos mass spectrometer versus existing technology for description of the proteome.

Methods: Single dimension reverse phase separation of complex peptide digests, or top down MS/MS of small proteins with higher resolution scanning.

Results: Significant increases in the number of identified proteins/unique peptides were observed for complex peptide digests, with largest increases detected for proteins of lower abundance. Increased resolution allows for resolution and top down analysis of small proteins.

Introduction

Comprehensive analysis of a proteome in the discovery stage is a necessary foundation for subsequent investigations of proteome dynamics. Effective means of characterizing a complement of proteins should be as exhaustive as possible, efficient, and reliable. Such analytical needs have been largely addressed by the use of tandem mass spectrometry for peptide and protein identification (ID) as technology continues to evolve, where linear ion trap and trap-based hybrid instruments have become the dominant solutions. The preference for these technologies is as much attributable to robustness and ease of use as it is to superior MS and, especially, MS/MS performance. Undiscovered components within a proteome often include dynamically modified forms which exist at abundance levels often undetectable by most instrumentation, requiring more exhaustive penetration into the complex proteome, made increasingly possible by more sophisticated instrumentation.

Here, we describe an innovative instrumental design that builds on the demonstrated performance benefits of linear ion traps in terms of speed, sensitivity, and dynamic range for MS/MS-based peptide identification. To satisfy increasingly demanding requirements for both throughput and exhaustive characterization of a complex peptide mixtures, the new ion trap demonstrates greatly increased ion flux powered by a brighter ion guide source, increased isolation efficiency, practical scan rates approaching 10 Hz, and enhanced resolution of greater than 20,000 ($m/\Delta m 50\%$). This results in superior ion transmission to the linear ion trap mass analyzer, better trapping and fragmentation efficiency, improved accumulation for low abundance precursors, dramatically improved duty cycle, and resolution capabilities beyond that of any commercial quadrupole ion trap demonstrated to date.

Methods

Bottom-Up

Complex peptide digests of un-fractionated *Caenorhabditis elegans* or *Saccharomyces cerevisiae* (yeast) were subjected to a single dimension of reverse phase separation on a 75 μm x 15 cm packed tip column (Magic C18AQ, Michrom). A Surveyor MS pump with a double split configuration was used to produce a flow rate of ~300 nL/min at the tip at 50% organic. A gradient of 2-25% acetonitrile in 0.1% formic acid was generated over 60 or 180 minutes. Samples were run on both the LTQ XL and LTQ Velos using data-dependent acquisition with predictive AGC enabled on the LTQ Velos and CID activation for both. Data were searched using Proteome Discoverer 1.0TM software with the MASCOTT search engine. All data were filtered to a 1% false discovery rate (FDR), as determined from a reverse database search.

Top Down

A solution of 300 fmol/ μL myoglobin in 50:50:0.1 water:acetonitrile:formic acid was infused directly into the mass spectrometer. ETD and PTR were used for fragmentation, with a 5 msec ETD activation time (fluoranthene), and 25 msec PTR, facilitated by benzoic acid.



FIGURE 1. Instrumental schematic of LTQ Velos mass spectrometer. The instrument features a dual pressure linear ion trap, and S lens source. The S lens produces a 5-10x increase in ion flux to result in increased sensitivity and reduced cycle times.

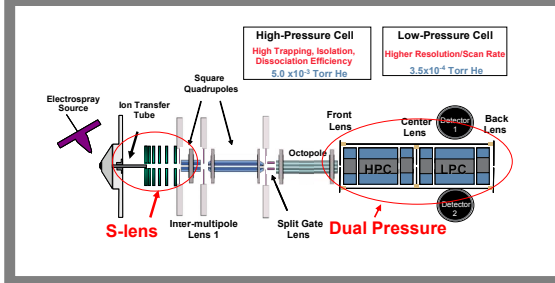


FIGURE 2a. Figures below illustrate the increased efficiency of trapping and fragmentation in the higher pressure cell of the LTQ Velos, and increased resolution obtainable in the lower pressure cell.

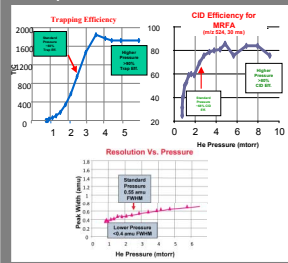
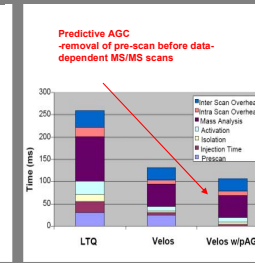


FIGURE 2b. Figure demonstrating decreased time required for individual scan functions for data-dependent MS/MS scans in the LTQ Velos versus the LTQ XL.



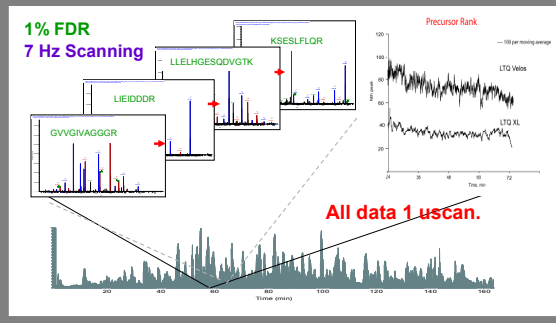
Data Analysis

Data were searched against a database with Proteome Discoverer software with the MASCOT search engine and filtered to a 1% FDR report. Data were also analyzed with custom software to facilitate extraction of relative raw information and interpretation.

Result

The Thermo Scientific LTQ Velos mass spectrometer consists of a dual pressure linear ion trap and a stacked ring ion guide source (S lens) (Figure 1). The new ion source is a radio frequency (RF) ion guide that promotes significant increases in the transmission of ions into the mass analyzer, thus reducing the time required to inject the desired ion population into the linear ion trap. The first trap is held at a higher pressure (~5 x 10⁻³ Torr) to allow improved efficiency in trapping, isolation, and fragmentation. As such, a 65% decrease (30 msec → 10 msec) in the required CID activation time is achieved while maintaining the same efficiency of fragmentation, and a 4-fold decrease in the time required for precursor ion isolation is achieved at higher pressure. The second trap, held at a lower pressure of approximately 0.4 mTorr, allows for a faster ion scan out with increased resolution (Figure 2a). Additionally, the LTQ Velos provides a significant improvement in isolation efficiency in the higher pressure trap. As a result, a great increase is achieved in the selectivity and efficiency of accumulation for low abundance precursors in the presence of abundant interfering ions, permitting improved dynamic range for MS/MS analysis. In conjunction with new hardware, a novel implementation for controlling the ion population in the ion trap produces a dramatic increase in the practical scan rate achieved in a typical data-dependent tandem MS experiment. This function, termed "predictive AGC", eliminates scans conducted prior to each tandem MS scan (known as pre-scans) by predicting the injection time required for the specified precursor ion from the full scan (Figure 2b).

FIGURE 3. Base peak chromatogram from a 1 μg injection of a yeast digest. Shown are 4 sequential scans acquired at a rate of 7 Hz, identified at a 1% false discovery rate (FDR). The inset at right shows the average rank of the precursor selected for MS/MS with data-dependent acquisition for the LTQ Velos (average 80th peak) versus the LTQ XL (average 40th peak).



LTQ Velos interrogates the complex proteome to a greater depth

The instrument produced sequential scans of identifiable spectral quality at a rate of >7 Hz for 1 μg of a complex yeast proteome digest separated over 180 minutes, a rate more than twice that of the LTQ XL (Figure 3a). Because of its higher scan rate, the LTQ Velos sampled precursor species to twice the depth seen with the LTQ XL instrument, as seen in figure 3b. Figure 3

FIGURE 4a. Increase in identification of proteins and unique peptides for a 1 μg injection of *C. elegans* tryptic digest. Relative standard deviation of <10% for triplicate injections. The LTQ Velos identified more proteins in a 60 minute gradient than the LTQ in 180 minutes to allow increased throughput.

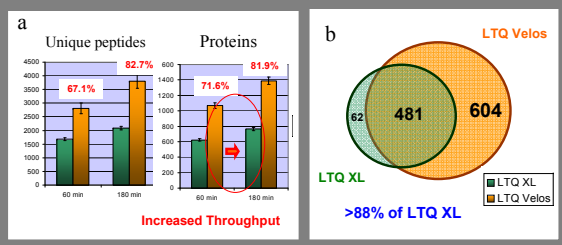


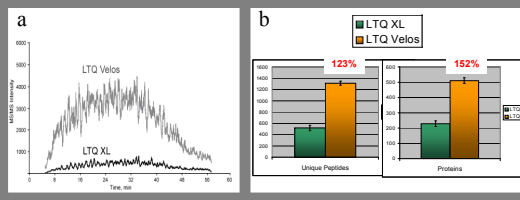
Figure 4b: Venn diagram illustrating the overlap in protein ID for a 1 μg injection of *C. elegans* tryptic digest. >88% of the proteins identified by the LTQ XL were identified by the LTQ Velos comparing single injections.

LTQ Velos identifies a greater number of unique peptides than LTQ XL

An increase in the number of identified unique peptides and proteins in a proteolytic digest of both *C. elegans* and *S. cerevisiae* was achieved using an LTQ Velos mass spectrometer when benchmarked against the LTQ XL. The LTQ Velos identified 67% ± 3.3 more unique peptides and 71% more proteins at 1% FDR than that of the LTQ XL (figure 4). It was also found that a significant overlap existed in the sets of proteins identified between instruments, where >88% of protein identified by the LTQ XL were also identified by the LTQ Velos for a typical 60 minute gradient (figure 4). Overlap within identical runs was >70%.

FIGURE 5a. Increase in the intensity of ion count for the MS/MS scans in the LTQ Velos vs the LTQ XL across a chromatographic run for 20 ng of a *C. elegans* tryptic digest, demonstrating a 6x increase in MS2 ion flux.

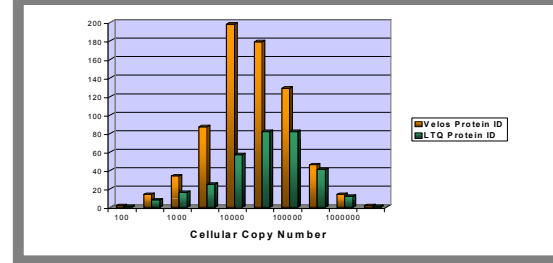
Figure 5b: Increase in identification of proteins and unique peptides for 20 ng of *C. elegans* tryptic digest.



LTQ Velos samples proteins over a greater dynamic range

To further assess the performance of the LTQ Velos for analysis of low-abundance components, the *C. elegans* sample was diluted 50-fold to 20 ng per injection and analyzed on both linear ion trap instruments. The number of proteins and unique peptides identified by the LTQ Velos was 123% and 152%, respectively, greater than that achieved by the LTQ XL (Fig. 5b). With such a low sample load, over 95% of the MS/MS events used 100 ms (maximum) injection times on both instruments. Figure 5a shows a running average of the total number of ions present in MS/MS scans where injection times reached 100 ms (maximum), for both instruments. On average, the number of ions in the MS/MS scans was almost seven times higher in the LTQ Velos, reflecting both the increased efficiency of ion transfer to the mass analyzer and the more efficient ion isolation and accumulation.

FIGURE 6. Caption



LTQ Velos samples proteins over a greater dynamic range

Our observations with the *C. elegans* digest indicated that the new instrument provided substantial benefits for the analysis of low-abundance and low signal-to-noise precursors. To quantitatively assess the ability of the instrument to identify low-abundance cellular proteins within complex digests; i.e., to assess proteome penetration depth, we analyzed whole-cell digests of yeast, taking advantage of the documented expression levels for most yeast proteins. The results from three replicate runs were aggregated for each instrument, and the identified proteins were annotated with their cellular expression levels. The results were visualized at the level of unique proteins. This analysis indicated that while both instruments identified about the same number of high-abundance proteins (more than 100,000 copies per cell), the LTQ Velos identified 2-4 times more medium-abundance unique peptides and proteins (10,000-100,000 copies) and also 2-4 times more low-abundance peptides and proteins (below 10,000 copies) (figure 6).

LTQ Velos improves experimental throughput

A faster scan rate improves duty cycle and also allows for the use of shorter chromatographic gradients to increase experimental throughput. The LTQ Velos identified more unique peptides and proteins in a 60-minute analysis than did the LTQ XL in a 180-minute analysis of the same complex mixture (figure 4a). A significant increase in throughput for analysis of simple mixtures with high dynamic range (i.e. gel bands) is, therefore, anticipated.

FIGURE 7. The increased resolution of UltraZoom scan allows resolution of small proteins, as seen for intact myoglobin (~16.5 kDa). An effective resolving power of >25,000 is demonstrated.

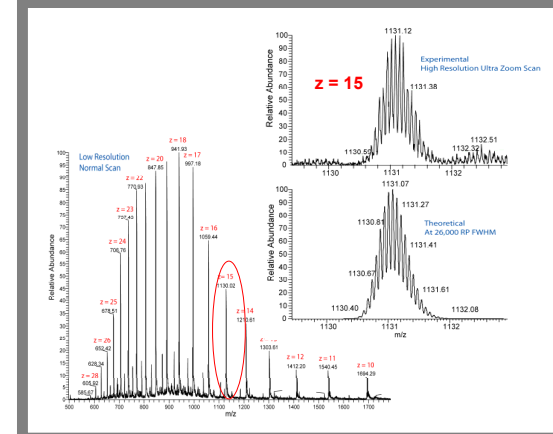
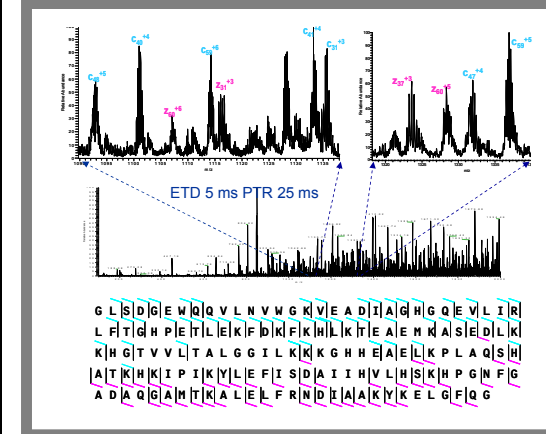


FIGURE 7. Top down ETD-PTR MS/MS spectrum of intact myoglobin using ZoomScan on the LTQ Velos, demonstrating resolution of 6+ fragments. Shown below is the fragment map of assigned ions for myoglobin obtained from above fragmentation spectrum.



Increased resolution for top down experiments

The increased resolution afforded by the LTQ Velos allows isotopic resolution of small intact proteins. Figure 9 (A, B) shows the isotopic resolution of intact myoglobin (15+ ion) acquired using the ultra-zoom scan mode of LTQ Velos, in comparison with a plot of the theoretical distribution, demonstrating >25,000 resolving power (figure 7). Electron transfer dissociation MS/MS coupled to proton transfer was performed on this precursor ion using the zoom and ultrazoom map method, and the resulting isotopically resolved fragments were manually deconvoluted and matched against the sequence for myoglobin. The spectrum and fragment map are shown in Figure 8.

Conclusions

- The LTQ Velos identified up to 81% more proteins than the existing state-of-the-art linear ion trap, the LTQ XL.
- A greater proportional increase in identification was observed for species of lower abundance or lower signal-to-noise, 152% increase in protein ID for low abundance injection of *C. elegans*. Greater coverage of lower abundance proteins in yeast digest permitted by the LTQ Velos.
- The LTQ Velos offers an increase in experimental throughput, demonstrating the ability to identify more proteins and peptides than the LTQ XL in 1/3 the gradient length for a complex sample.
- The LTQ Velos demonstrates a resolving power of >25,000 FWHM to achieve resolution of a 15+ charge state of intact myoglobin when targeting expected mass, and allowing top down analysis of proteins.

Acknowledgements

We would like to thank Gennifer Merrifew and Michael Maccoss from University of Washington for preparation of samples, Jason Russell, Danielle Swaney and Josh Coon from the University of Wisconsin, and Maurizio Splendore, Eloy Wouters, and Jean-Jacques Dunyach for influential support in source development.

Magic C18AQ is a trademark of Michrom Bioresources. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries. MASCOT is a trademark of matrix science, ltd.