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Dual-Pressure Linear Ion Trap Mass Spectrometer Improving the Analysis of Complex Protein Mixtures

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The considerable progress in high-throughput proteomics analysis via liquid chromatography–electrospray ionization–tandem mass spectrometry over the past decade has been fueled to a large degree by continuous improvements in instrumentation. High-throughput identification experiments are based on peptide sequencing and are largely accomplished through the use of tandem mass spectrometry, with ion trap and trap-based instruments having become broadly adopted analytical platforms. To satisfy increasingly demanding requirements for depth of characterization and throughput, we present a newly developed dual-pressure linear ion trap mass spectrometer (LTQ Velos) that features increased sensitivity, afforded by a new source design, and demonstrates practical cycle times 2 times shorter than that of an LTQ XL, while improving or maintaining spectral quality for MS/MS fragmentation spectra. These improvements resulted in a substantial increase in the detection and identification of both proteins and unique peptides from the complex proteome of *Caenorhabditis elegans*, as compared to existing platforms. The greatly increased ion flux into the mass spectrometer in combination with improved isolation of low-abundance precursor ions resulted in increased detection of low-abundance peptides. These improvements cumulatively resulted in a substantially greater penetration into the baker's yeast (*Saccharomyces cerevisiae*) proteome compared to LTQ XL. Alternatively, faster cycle times on the new instrument allowed for higher throughput for a given depth of proteome analysis, with more peptides and proteins identified in 60 min using an LTQ Velos than in 180 min using an LTQ XL. When mass analysis was carried out with resolution in excess of 25 000 full width at half-maximum (fwhm), it became possible to isotopically resolve a small intact protein and its fragments, opening possibilities for top down experiments.

In the past decade, mass spectrometry has become a powerful tool for high-throughput analysis of complex proteomes.^{1–4} Liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI-MS/MS) has become the technology of choice in many proteomics laboratories for high-throughput peptide and protein identification and characterization. Linear ion traps and trap-based hybrid mass spectrometers have become popular for the analysis of protein digests because of their robustness, fast scan speed, and MS/MS sensitivity. Recent advances in complex proteome characterization using these systems are impressive with thousands of proteins identified and their modification sites elucidated in single experiments.^{5–8}

Routine comprehensive analysis of complex proteomes, however, is still hindered by a mismatch between the complexity of the mixture and the MS/MS acquisition speed and the analytical dynamic range of the tandem mass spectrometer.⁹ A single spectrum in an LC–MS analysis of a complex protein digest can contain hundreds of different molecular species, of which only a fraction are selected for data-dependent MS/MS during their elution. Consequently, the sampling and identification of low-abundance peptides from complex protein mixtures still poses a substantial analytical challenge. Undiscovered components within a proteome frequently include static or dynamically modified protein forms, which are of particular biological interest, for

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example, due to their common involvement in regulatory networks. The analytical challenge is further complicated by the fact that mid- and low-abundance cellular proteins are more diverse than high-abundance species; consequently digestion of a proteome yields many more peptides at low concentration levels than at high. Therefore, development of more sophisticated mass spectrometers featuring greater sensitivity, selectivity, acquisition rate, and resolution is essential to improve our ability to comprehensively characterize complex proteomics samples.

The majority of commercially available ESI tandem mass spectrometers used for high-throughput peptide identification are capable of acquiring mass spectra in excess of 10 Hz in MS and MS/MS modes. However, practical acquisition rates rarely exceed 3 Hz for both ion trap and time-of-flight (TOF) based instruments.^{10,11} The complete cycle time for a single MS/MS scan in ion trap instruments is limited by the combination of the rate with which ions are injected into the mass analyzer (injection time), the precursor isolation time, the dissociation time, and mass analysis scan time, i.e., how fast the ions are scanned out of the analyzer to the detectors.

LC-ESI presents the mass spectrometer with a highly dynamic ion population. Total and relative ion abundances may change by many orders of magnitude on a scale of seconds. Ideally, the mass spectrometer should be sensitive and selective enough to produce identifiable MS/MS spectra from the lowest detectable components but also fast enough to target most or all new components detected in every MS scan. With ion trap instruments, precursor ions can be isolated and accumulated for a period of time inversely proportional to their intensity, up to a user-defined maximum. This process is known as automatic gain control (AGC) and allows the instrument to dynamically exchange cycle time for effective sensitivity to accommodate varying precursor abundance. When precursor abundances are so low that accumulation times always reach the defined maximum, then sensitivity becomes proportional to the brightness of the ion beam. Increasing the efficiency of ion transmission to the ion trap mass analyzer will benefit performance in two main ways. First, ion injection times will be reduced proportionately to the increased ion transmission, leading to improved cycle time. Second, lower-abundance precursors can be successfully targeted without reaching the maximum injection time, translating to improved sensitivity.

The novel dual-pressure linear ion trap mass spectrometer described here features a newly designed ion source that provides increased ion transmission and a two-chamber ion trap mass analyzer featuring differential pressure regulation. The latter allows for more efficient isolation and dissociation, greater resolution, and much faster scanning rates than the basis linear ion trap instrument (LTQ XL). An additional benefit is high rf voltage ion injection, which allows for more selective ion trap filling. As a result, the new ion trap instrument is more sensitive and capable of higher acquisition rates and more efficient isolation of low-abundance components, especially in the presence of abundant interferences.

In this study we used enzymatic digests of soluble proteins from the multicellular nematode *Caenorhabditis elegans* (*C. elegans*) and the single celled fungi *Saccharomyces cerevisiae* to assess the performance of the new dual-pressure linear ion trap mass spectrometer. We also demonstrate the high-resolution improvements for top-down experiments using horse heart myoglobin.

EXPERIMENTAL SECTION

Sample Preparation. The soluble fraction of mixed stage cryopreserved *C. elegans* homogenates was digested using a previously described protocol.¹² The digest was acidified and stored at -80°C . Samples were diluted in 0.1% formic acid prior to analysis by reverse phase LC-MS/MS. Yeast (*Saccharomyces cerevisiae*, strain BY4741) were grown to an OD₆₀₀ of ~ 0.7 , and the cells were centrifuged at 3000g, 4°C , for 5 min. The supernatant was resuspended in 1 mL of cold 50 mM ammonium bicarbonate buffer (pH 7.8), mixed with 1 mL of 0.5 mm glass beads, and lysed with a mini-beadbeater (Biospec Products Inc., Bartlesville, OK) for three 1 min repeats. The suspension was kept on ice for 1 min, then centrifuged at 2000g at 4°C for 10 min to separate the supernatant from beads and debris and then centrifuged at 14 000g at 4°C for 10 min to remove insoluble material. Digestion of the ammonium bicarbonate soluble fraction was performed as for the *C. elegans* sample.

LC-MS/MS Analysis. The samples were analyzed on a Thermo Scientific LTQ XL linear ion trap and on LTQ Velos dual-pressure linear ion trap mass spectrometers. Proteolytic digests of *C. elegans* and yeast were separated by reverse phase chromatography using a Thermo Scientific Surveyor MS pump delivering 300 nL/min of 2–25% acetonitrile in 0.1% formic acid gradient to a packed tip column (Magic C18AQ 5 μm particle, 250 \AA pore size, 150 mm \times 75 μm i.d., with a 15 μm tip opening, Michrom BioResources, Auburn, CA). Gradient lengths of 60 and 180 min were used. For data-dependent acquisition, the method was set to automatically analyze the top 10 most intense ions observed in the MS scan. An ESI voltage of 2.0 kV was applied directly to the HPLC buffer distal to the chromatography column using a microtee. The ion transfer tube temperature on the LTQ Velos ion trap was set to 250°C , 50 degrees higher than that used on the LTQ XL ion trap to compensate for a shorter path length. The S-lens on the LTQ Velos was set to 40%, and the tube lens in the LTQ XL to 100 V. The AGC target values (the approximate number of accumulated ions) for full MS and MSⁿ were 30 000 and 10 000, respectively. Normalized collision energy was set to 30% with activation times of 10 ms for the LTQ Velos and 30 ms for the LTQ XL. The MS/MS triggering threshold was set to 10 000 for the LTQ Velos and 1 000 for the LTQ XL, with a default charge state of 3+. Dynamic exclusion parameters were optimized according to expected chromatographic peak widths, resulting in a 15 s exclusion duration for the 60 min gradient and 30 s duration for the 180 min gradient. In both cases, a single repeat count and an exclusion list size of 500 were used. Because of the greater ion transmission of the ion source and the improved trapping efficiency on the LTQ Velos ion trap, the full MS maximum injection time (IT) was lowered to 10

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ms, as opposed to 50 ms for the LTQ XL. A maximum IT setting of 100 ms was used on both instruments for MS/MS scans. For the LTQ Velos, active rejection of singly charged precursors was used; this was made possible by the increased resolution in full MS, relative to the LTQ XL. A minimum of three replicates were acquired for each gradient on each instrument.

Database Searching and Protein Annotation. The tandem mass spectrometry data were extracted from the Thermo Fisher RAW files and placed in a compressed MS2 file format¹³ using the MakeMS2 program available from <http://proteome.gs.washington.edu>. The resulting MS/MS spectra were then searched twice; once against the target fasta sequences and a second time against a shuffled decoy database using SEQUEST. The *C. elegans* data were searched against a combination of the proteins in Wormpep 160, the NCBI *E. coli* annotations, and common contaminants. The *S. cerevisiae* data were searched against a combination of the predicted open reading frames from the *Saccharomyces* Genome Database and common contaminants. The output of the target and decoy database search was processed using the postprocessor Percolator.¹⁴ The data was then filtered at a q -value ≤ 0.01 ,^{15–17} corresponding to 1% false discovery rate (FDR) on the spectral level and assembled into parsimonious protein lists using a modification to the IDPicker algorithm.¹⁸ All reported values are an average of three replicate analyses with error bars indicating standard deviation.

Analysis of Instrument Performance Data. Instrument performance data were derived from RAW format data files using custom software. To examine the contributions to instrument cycle time (Figure 3), the mean values and standard deviations of time-variable components of each scan (injection time, MS/MS scan range) were calculated. To measure MS peak depth sampling (Figure 4B), each selected precursor in the data file was compared to the preceding centroided MS1 spectrum from which it was selected and its ranked intensity recorded; the intensity rank was then plotted versus retention time. To evaluate the distribution of selected precursors as a function of approximate local signal-to-noise ratio (Figure 4C), each selected precursor was compared to the preceding centroided MS1 spectrum and evaluated as follows: Local noise was estimated by taking the mean intensity of the four peaks preceding the precursor and the four peaks following the precursor (skipping the peak immediately following the precursor). Any contributing peak displaying intensity greater than 1.5 times the mean was then excluded, and the mean was recalculated. The ratio of the intensity of the precursor to this local mean intensity was recorded. The few precursors at the extreme edges of the MS1 window that could not be evaluated in this manner were disregarded. To evaluate the ion counts achieved

in MS/MS spectra at low sample load levels (Figure 6B), each MS/MS event was examined and the count of ions in the spectrum was reconstructed by multiplying the recorded total ion chromatogram (TIC, ions per second) and IT (ionization times, converted to seconds from milliseconds) values.

Assignment of Cellular Copy Number to Proteins and Peptides Identified from *S. cerevisiae*. To evaluate depth of penetration into the yeast proteome, the *S. cerevisiae* database search results were processed and annotated as follows: The list of identified protein groups for each analytical run was compared to a database of cellular protein expression levels,¹⁹ and each protein was annotated with its corresponding copy number value, if present (approximately 62% of sequences in the published yeast database had experimentally determined copy number values). Protein groups containing more than one member (an indistinguishable set) were assigned the copy number of the most abundant member of the group. Unique identified peptides were assigned the copy number value of their parent protein; when a peptide could be attributed to multiple possible parents (e.g., homologues), the most abundant parent was assigned.

Intact Protein Analysis. Horse heart myoglobin (Sigma, St. Louis, MO) was dissolved in 50% acetonitrile with 1% formic acid to 0.5 pmol/ μ L and was infused directly into the mass spectrometer via static nanospray needle (New Objective, Woburn, MA). Single charge states were isolated over a 10 m/z window. MS/MS analysis of myoglobin was performed using the same fragmentation parameters as for peptide MS/MS. Individual fragments were isolated and analyzed by ultrazoom scan, using variable targets of 50–3000 ions and averaging 100–300 scans. The resulting spectra were manually deconvoluted, and intact protein and fragment masses were searched using 500 and 2 Da tolerances, respectively, against the human IPI database (version 3.52, November 2008, 66 600 basic sequences) containing the horse heart myoglobin sequence, using ProSight PC 1.0 (Thermo Fisher Scientific, Waltham, MA).

RESULTS AND DISCUSSION

Instrument Innovations. The new linear ion trap mass spectrometer described here (Thermo Scientific LTQ Velos) includes a high-pressure stacked ring ion optics system (“S-lens”) and a novel dual-pressure linear ion trap (Figure 1). Relative to the LTQ, the S-lens replaces the tube lens and skimmer.^{20–22} To date, the best known realization of this ion transmission technology in LC–MS instruments is the ion funnel, pioneered by Smith and co-workers.²³

The S-lens consists of a series of flat ring electrodes to which radio frequency (rf) voltages are applied, with opposite phases applied to even and odd numbered electrodes. The spacing between the electrodes progressively increases, and thereby the

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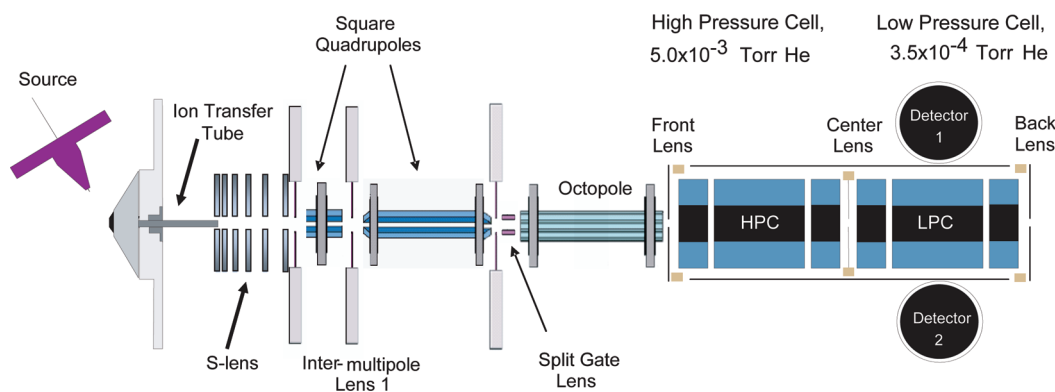


Figure 1. Schematic representation of the Thermo Scientific LTQ Velos mass spectrometer, containing a stacked-ring ion guide (S-lens) source and a dual-pressure ion trap with differential pressure regulation.

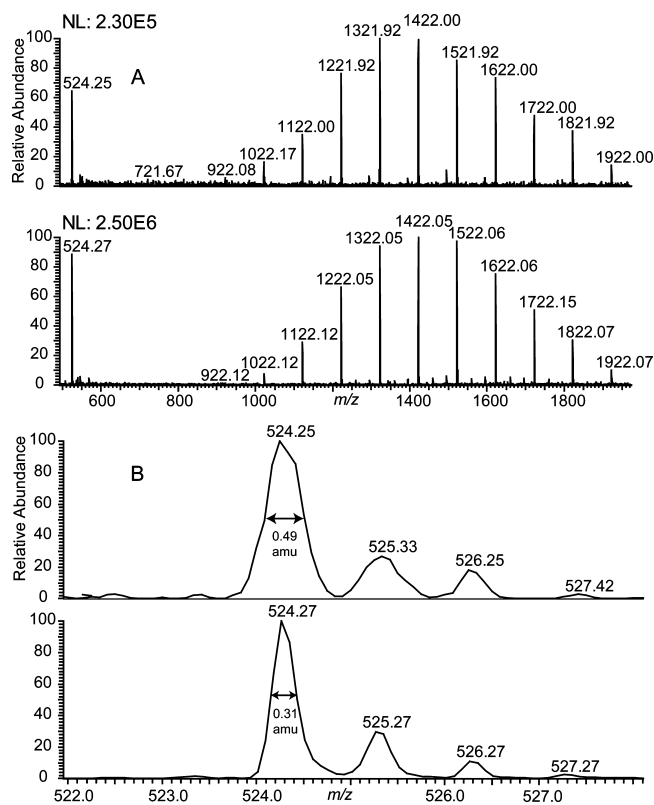


Figure 2. (A) Full MS scan from LTQ Velos (top) and LTQ XL (bottom) indicating greater than 10 \times improvement in sensitivity for the new instrument. The average observed improvement in sensitivity is 5-fold. (B) Normal scan resolution for LTQ XL (top) and LTQ Velos (bottom) in full MS achieved at scan rates of 16 000 and 33 000 amu/s, respectively.

confining rf electric field also increases, which focuses the expanding ion cloud as it travels through the device. The electrode openings were chosen to be large enough to capture the entire ion cloud emanating from the ion transfer tube while also as small as possible to minimize the rf voltage required to obtain the necessary focusing field strength.

To obtain an S-lens transmission efficiency that is uniform across the mass range for a full MS experiment, the ion injection time window of the ion trap is split into multiple periods; during each period the S-lens is operated at a different rf level.²⁴ To

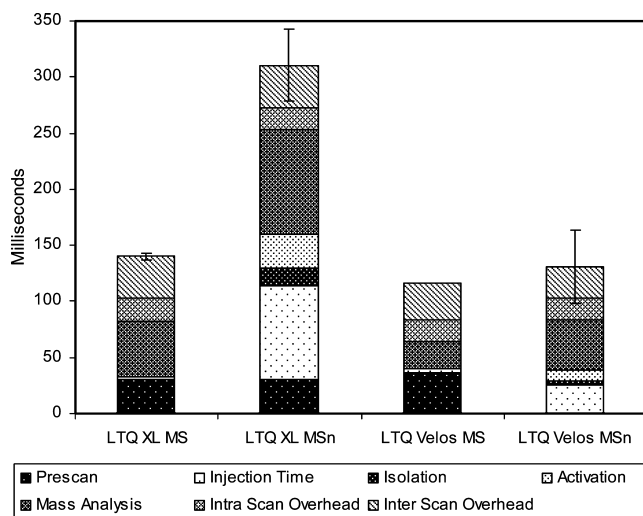


Figure 3. Instrument scan lengths as composites of several events in MS and MS' modes on LTQ XL and LTQ Velos, calculated from a single top 10 60 min LC-MS/MS run using 1 μ g of *C. elegans* digest. Out of seven events, five (six for MS scan) have constant duration and two (injection time and MS/MS mass analysis time) have variable durations. The error bars represent the standard deviation and reflect injection time variability for MS and MS/MS events and mass analysis time variability for MS/MS events.

prevent neutral species, droplets, and clusters from entering the linear ion trap, the line of sight between the atmospheric pressure ion source and the trap is interrupted by the longitudinally curved rods of an ion transfer quadrupole. A comparison of the ion intensities delivered by the standard tube lens/skimmer optics found in the LTQ versus the S-lens system for various compounds revealed an average 5-fold increase in intensity with the S-lens (Figure 2A).

The dual-pressure linear ion trap consists of two trapping cells separated by a single aperture lens to allow differential pumping between the cells. To improve ion trapping, isolation, and fragmentation efficiencies, the first cell is held at a higher pressure ($\sim 5 \times 10^{-3}$ Torr) than in previous linear ion trap systems. The increased isolation efficiency is traded for a 4-fold decrease in the time required for precursor ion isolation (16–4 ms) while still maintaining LTQ equivalent isolation efficiency and selectivity. Similarly, the greater inherent fragmentation efficiency (i.e., conversion of precursor ion to product ion) can be used to reduce the required collision induced dissociation (CID)

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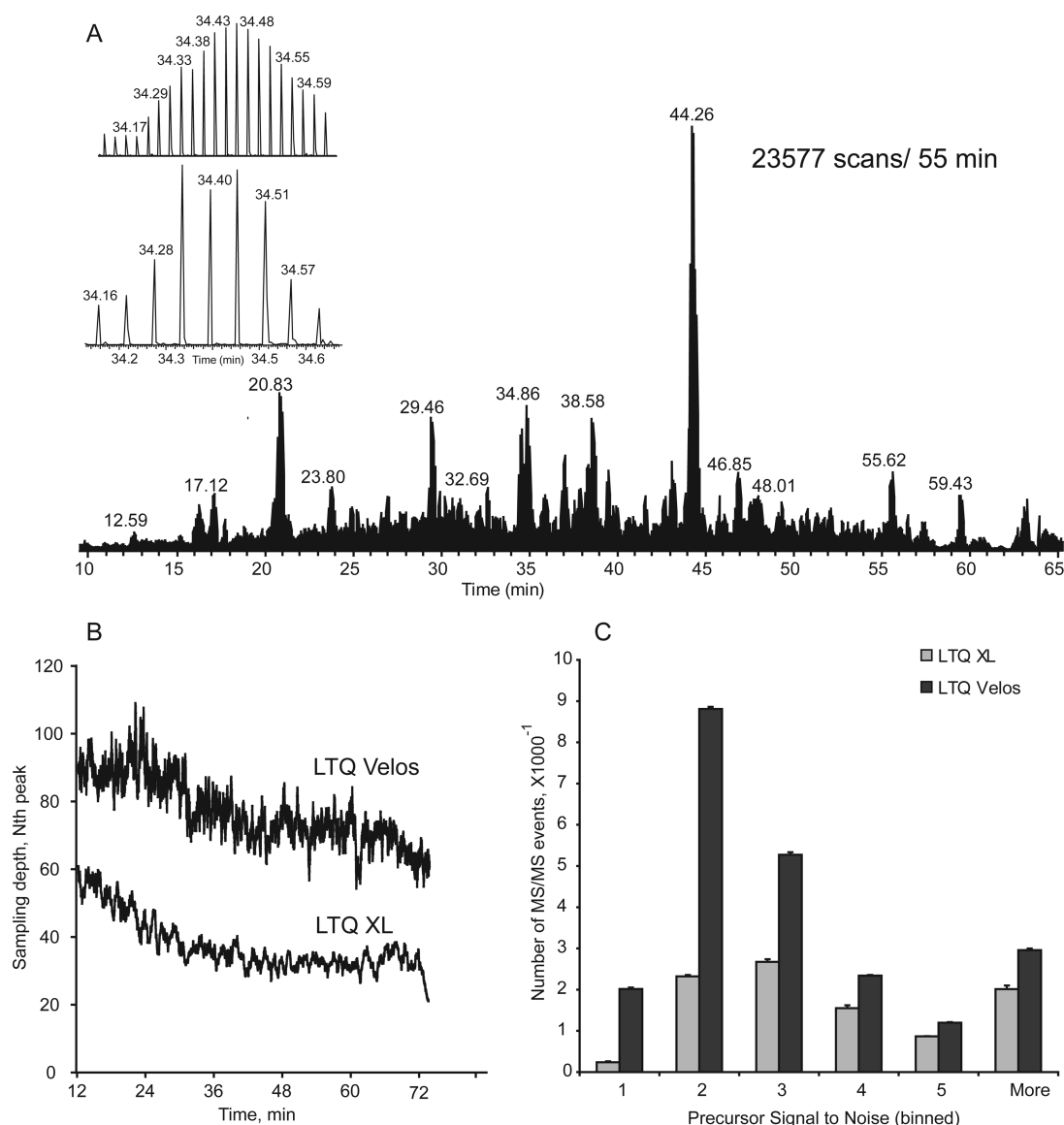


Figure 4. (A) A typical base peak LC–MS trace from 1 μg of *C. elegans* digest recorded on a LTQ Velos. Inset shows that the sampling frequency (inversely proportional to the distance between the two adjacent full MS base peaks) is ~ 2.3 times higher for LTQ Velos (inset top) than for LTQ XL (inset bottom, equivalent run). (B) Running averages (100 points) for MS peak depth sampling for LTQ Velos and LTQ XL. (C) Number of MS/MS events vs estimated local signal-to-noise ratios of precursor ions. LTQ Velos spent most of the $2\times$ extra scans sampling lower abundance precursors than LTQ XL.

activation time by 67%, while maintaining the same benchmark fragmentation efficiency. Unlike previous linear ion traps, the new dual-pressure ion trap has a fully symmetric geometry with ejection slots in all four rods. This design provides fully symmetrical rf fields within the ion traps and allows ion injection to occur at higher rf voltages, with simultaneous application of isolation waveforms.^{25,26} This application of isolation waveforms improves the analysis of low-abundance precursors in complex matrixes, as it allows the trap to be filled with only the m/z of the target analyte. Without the use of isolation waveforms, the trap needs to be filled with a fairly wide window of masses or even an unfiltered ion population prior to isolation of the minority component of interest. For very low-abundance

precursors, this can present complications due to space charge effects from the excess ion population. These complications are greatly reduced with the symmetric dual-pressure design, resulting in improved sensitivity and dynamic range for MS/MS analysis of low-abundance components.

In contrast to the first cell, the second cell of the dual-pressure ion trap is held at a lower pressure ($\sim 4 \times 10^{-4}$ Torr) than in previous linear ion traps. This lower pressure allows attainment of higher mass resolution for a given scan rate. Some of the gains in resolution can be sacrificed to achieve an increase in the m/z scan rate. With the LTQ Velos a 2-fold increase in scan rate can be obtained while achieving the same, or superior, resolution than the LTQ XL (Figure 2B).

In combination with new hardware, a novel method for controlling the ion population in the ion trap was implemented. Termed “predictive AGC”, this approach eliminates the “prescans”

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typically conducted prior to each tandem MS scan, saving up to 30 ms per data-dependent MS/MS spectrum. These prescans are normally used to evaluate the momentary intensity of the selected precursor to calculate an appropriate injection time for the tandem MS scan. The predictive AGC approach instead refers to the preceding full MS scan data to evaluate the intensity of the selected precursor. Under typical acquisition conditions, the median MSⁿ event occurs well under a second after the full MS used to predict parent ion injection time.

Cumulatively, these improvements resulted in a decrease in typical full MS scan duration from 140 (LTQ XL) to 116 ms (LTQ Velos) and a decrease in typical MS/MS duration from 310 (LTQ XL) to 131 ms (LTQ Velos), while maintaining or improving the quality of the MS/MS spectra (Figure 3).

Instrument Performance with Complex Peptides Mixtures. First, we evaluated the performance of the new ion trap mass spectrometer for analysis of an enzymatic digest from the multicellular organism *C. elegans*. In this sample, the peptides originate from worms at all developmental stages and from all cell and tissue types. This highly heterogeneous mixture results in a highly complex mixture with an enormous dynamic range given that many proteins will be expressed in only a subset of the cell types and in one developmental stage, while others will be expressed ubiquitously. Given the dynamic range of protein abundance in yeast¹⁹ and the known dynamic range of mRNA transcripts in both *C. elegans*²⁷ and yeast, we estimate the dynamic range between the least and most abundant protein in this sample to be >10⁸.

A typical LC/MS base peak chromatogram from this sample is shown in Figure 4A. Over the course of a 60 min gradient, analyzing a sample load of 1 μg, the LTQ Velos acquired 23 557 scans over 55 min at an average acquisition rate of 7.2 Hz, more than twice as fast as the LTQ XL for the same conditions (Figure 4A, inset). Because of its higher scan rate, the LTQ Velos sampled precursor species to twice the depth seen with the LTQ XL instrument (Figure 4B). Notably, because of the brighter ion beam, fewer than 4% of MS/MS events used the maximum injection time, compared to the LTQ XL where 66% of all MS/MS scans used the maximum injection time. This was despite the fact that the majority of additional MS/MS scans performed by the LTQ Velos relative to the LTQ XL targeted low-abundance precursors (Figure 4C). Analysis of the resulting data revealed that the LTQ Velos identified a substantially larger number of unique peptides and proteins from this sample. With the use of equivalent sample amounts of 1 μg and 60 min long separations, the LTQ Velos ion trap identified 95% more unique peptides at 1% FDR than did the LTQ XL (Figure 5A). Remarkably, the identification success rate (number of assigned MS/MS spectra/total number of MS/MS spectra) was 34% for LTQ Velos runs, equivalent to that of the LTQ XL, despite that most of the additional scans targeted low-abundance precursors.

We examined the overlap between the proteins identified with the two instruments (Figure 5B). For a 60 min long separation, the LTQ Velos ion trap identified on average 90% of the proteins identified by the LTQ XL ion trap. Therefore, the LTQ XL ion trap identified a subset of the proteins identified by the LTQ Velos

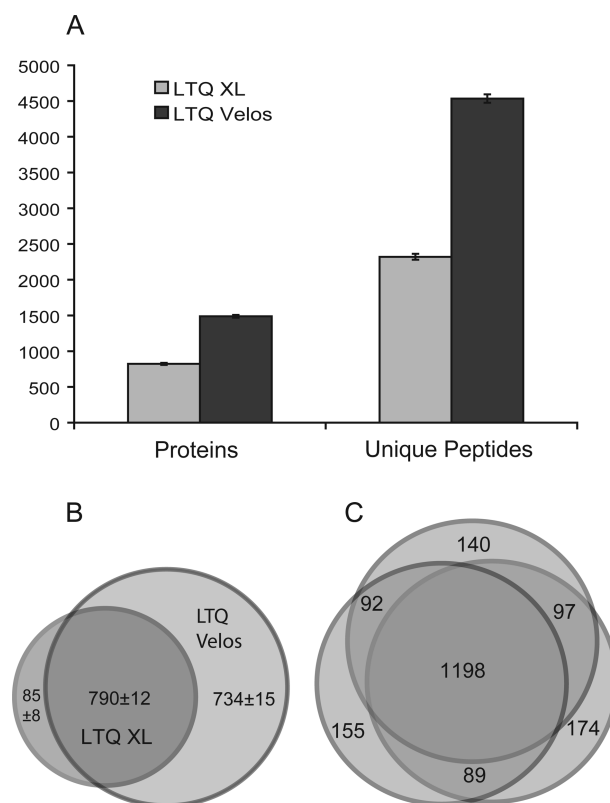


Figure 5. (A) Identified unique peptides and proteins from 1 μg of *C. elegans* digest LC-MS runs reported at <1% FDR (spectral level). (B) Venn diagram illustrating the overlap in proteins identified between the two instruments for a 60 min reverse phase separation of 1 μg of *C. elegans* digest. (C) Venn diagram illustrating the overlap in proteins identified in three replicated 60 min separations of 1 μg of *C. elegans* digest on LTQ Velos.

ion trap, rather than accessing a complementary population of proteins. Within replicate runs on the LTQ Velos, overlap of the identified proteins was greater than 78%, where 89% of proteins were identified in two out of three runs on average, indicating high reproducibility (Figure 5C).

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 128% greater than that achieved by the LTQ (Figure 6A). With such a low sample load, over 95% of the MS/MS events used 100 ms (maximum) injection times on both instruments. Figure 6B 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 7 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. As a result, the success rate for matching an MS/MS spectrum to a peptide sequence was 25% on LTQ Velos vs 14% on the LTQ XL for these runs. The low level runs on the LTQ Velos achieved scan rates of 4.2 Hz on average, significantly lower than the runs with the higher sample amount, as the 100 ms MS/MS injection times increased the cycle time substantially. Nonetheless, the LTQ Velos achieved a scan

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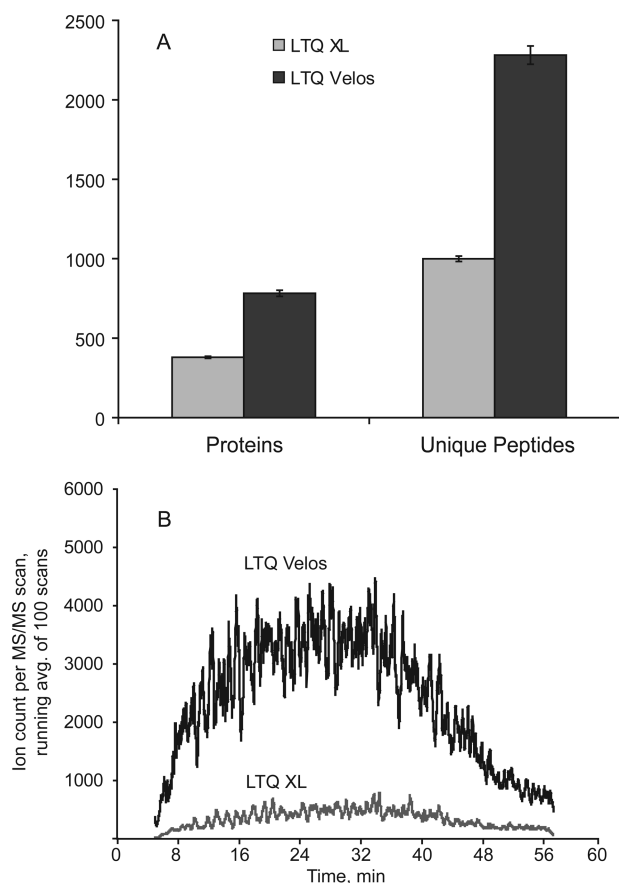


Figure 6. (A) Identified unique peptides and proteins from 20 ng of *C. elegans* digest LC–MS runs reported at <1% FDR. (B) Running average (100 per) of the number of ions recorded in MS/MS scans from these runs (one run per instrument shown). Under injection time limited conditions (as in this experiment), this is proportional to ion beam brightness.

rate 44% higher than that of the LTQ XL (2.9 Hz), while almost doubling the per-scan success rate.

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. In light of this, we were interested in quantitatively assessing the ability of the instrument to identify low-abundance cellular proteins *within* complex digests; i.e., to assess proteome penetration depth. To do so, we analyzed whole-cell digests of baker's yeast (*Saccharomyces cerevisiae*), taking advantage of the documented expression levels for most yeast proteins.¹⁹ Analytical runs were performed in triplicate on the LTQ XL and LTQ Velos, using 180 min LC gradients and 1 μ g of digest per run. The results from three replicate runs were aggregated for each instrument, and the identified proteins were annotated with their cellular expressions levels. The results were visualized at the level of unique proteins, and of unique peptides, where expression levels for peptides are derived from their parent proteins (Figure 7A,B). 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).

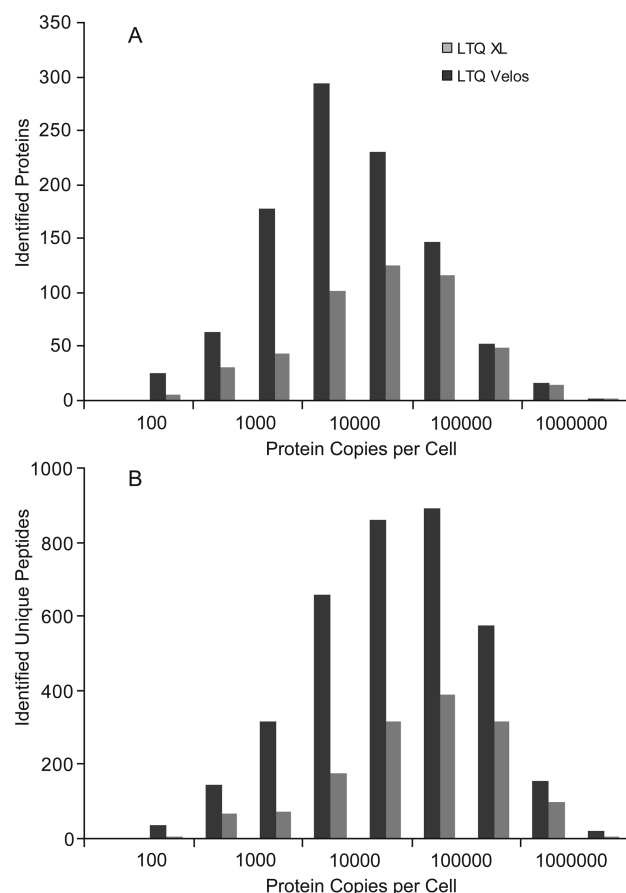


Figure 7. Identified proteins (A) and unique peptides (B) as a function of their cellular expression levels in whole-cell digests of baker's yeast (*Saccharomyces cerevisiae*). Analytical runs were performed in triplicate on LTQ XL and LTQ Velos, using 180 min LC gradients and 1 μ g of digest per run. The results from replicate runs were aggregated for each instrument, and the identified proteins were annotated with their cellular expressions levels.¹⁹

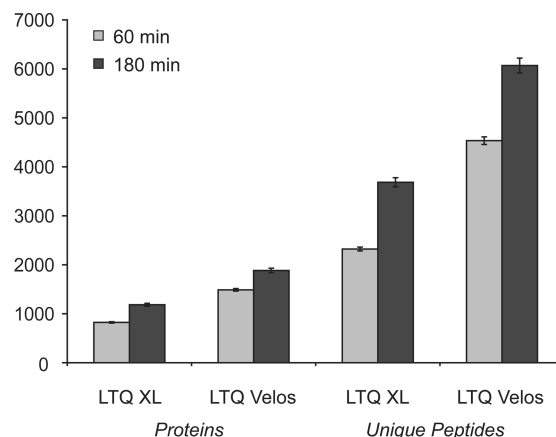


Figure 8. High throughput as a function of improved instrument performance. The LTQ Velos identified more unique peptides and proteins in a 60 min analysis than did the LTQ XL in a 180 min analysis of the same complex mixture (1 μ g of *C. elegans* digest).

LTQ Velos Improves Experimental Throughput. A faster scan rate not only improves duty cycle but 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 min analysis than did the LTQ XL in a 180 min

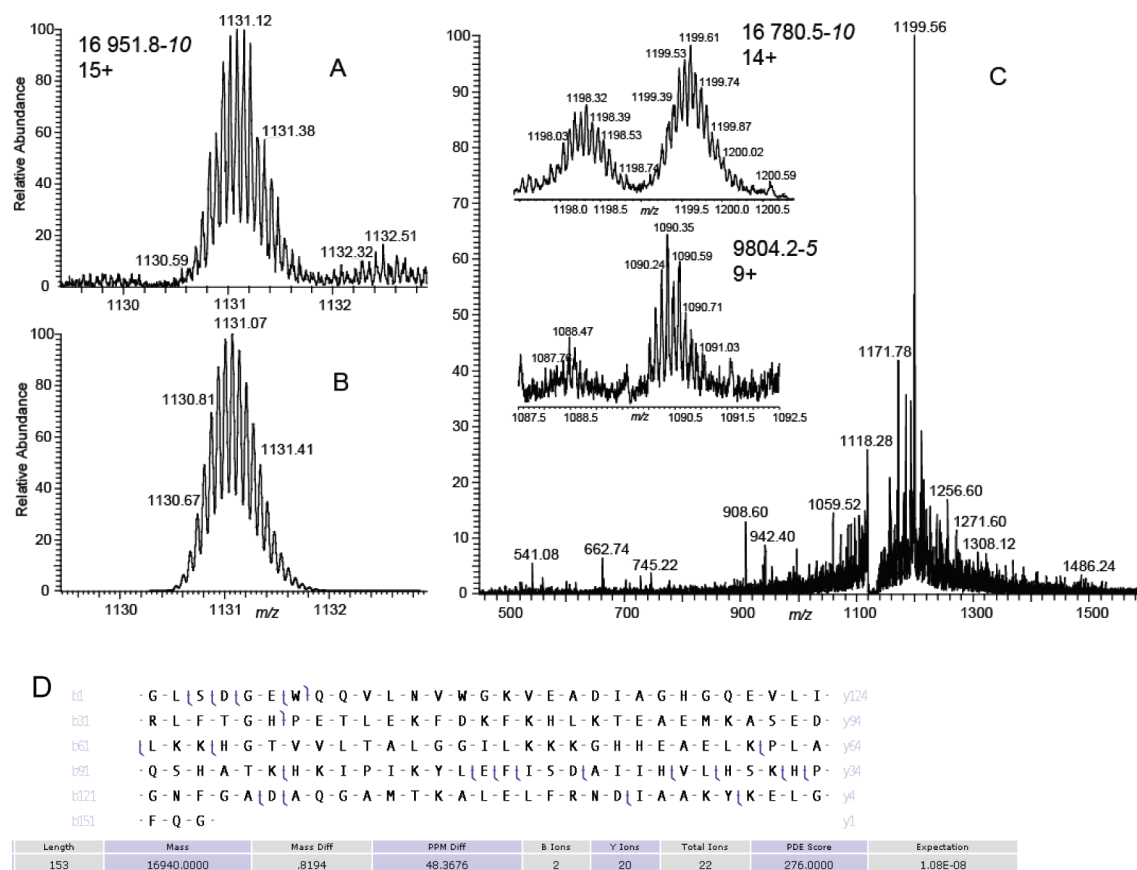


Figure 9. The increased resolution afforded by the LTQ Velos allows isotopic resolution of small intact proteins. (A) Resolution of the 15+ molecular ion of horse heart myoglobin using an ultra zoomscan. (B) Theoretical isotopic distribution for this ion at 25 000 fwhm. (C) MS/MS of the 15+ molecular ion recorded using a zoomscan with fragments up to 8 kDa isotopically resolved. Inset: Larger fragments are resolved using the ultra zoomscan. The mass difference (in units of 1.00235 Da) between the most abundant isotopic peak and the monoisotopic peak is denoted in italic after each M_r value. (D) Fragmentation map of horse heart myoglobin (from the output of ProSightPC), showing a 10^{-8} expectation score for this match.

analysis of the same complex mixture (Figure 8). A significant increase in throughput for analysis of simple mixtures with high dynamic range (i.e., gel bands) is, therefore, anticipated. Experiments requiring multiple runs/replicates would benefit from throughput, i.e., those requiring method development or biological and/or technical replicates. Quantitative experiments would also benefit from the increased scan rate as more scans are acquired across narrow chromatographic peaks (i.e., from UPLC).

Increased Resolution for Top Down Experiments. Traditionally, ion traps were limited to resolutions below about 10 000, sufficient to resolve isotopes in small to medium size peptide ions up to approximately 7 kDa. This limited the suitability of quadrupole ion traps for the analysis of larger peptide and protein ions. The increased resolution afforded by the LTQ Velos allows isotopic resolution and, thus, top down analysis of small intact proteins. Figure 9A,B shows the isotopic resolution of intact myoglobin (15+ ion) acquired using the ultra zoomscan mode of LTQ Velos, in comparison with a plot of the theoretical distribution, demonstrating >25 000 resolving power. The isotopic profile is the result of averaging 230 scans taken over a 10 m/z range with an AGC target of 500 ions per scan.

MS/MS analysis was performed on this precursor ion using the zoom and ultra zoom map method (Figure 9C), and the resulting isotopically resolved fragments were manually deconvoluted and searched against the human database with the horse

myoglobin sequence added to it using ProSightPC 1.0. Remarkably, myoglobin was the highest scoring protein out of the list of possible 23 candidate proteins and the only protein which had a P -score better than 10^{-4} . The fragment map is shown in the Figure 9D. The acquisition time scale achieved in this experiment would allow top down analysis with isotopic resolution of ions up to 17 kDa on a chromatographic time scale.

CONCLUSIONS

Sensitive detection and identification of components within a complex proteomic sample is crucial for the characterization and understanding of proteome dynamics. The technological advancements of the LTQ Velos ion trap, including increased speed of acquisition and improved precursor ion isolation and sensitivity, have resulted in substantial improvements in practical performance for the identification of peptides and proteins when compared to existing state-of-the-art technologies. Notably, considerable improvements in analytical performance for low-abundance species have demonstrably provided a greater depth of proteome characterization. In addition, a sizable increase in practically achievable resolution allows for the isotopic resolution of proteins up to 17 kDa, opening an avenue for top down experiments on a stand-alone ion trap.

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