

In-Depth Analysis of the *A. thaliana* Proteome Using a Novel Hybrid Linear Trap-Orbitrap Mass Spectrometer

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Overview

Purpose: Maximize protein identification and sequence coverage for the analysis of the water-soluble proteome of *Arabidopsis thaliana* using a novel hybrid linear ion trap mass spectrometer.

Introduction

Enormous improvements in mass spectrometry technology over the last few years have increased the success in analyzing complex proteomes. However, many low-abundance proteins remain undetected in most large-scale proteomic analyses due to the limits of the acquisition rate and quality of MS/MS spectra. To improve the speed and depth of analyzing complex protein mixtures, we developed a new hybrid linear ion trap-Orbitrap™ mass spectrometer that provides faster MS/MS scanning at low and high resolution and mass accuracy.

The availability of both a complete genome sequence and high-quality genome annotation makes *Arabidopsis thaliana* an ideal model system to assess new proteomics technologies. Furthermore, *Arabidopsis thaliana* proteomics research may serve as a paradigm for other plant proteomics research projects and the advancement of plant proteomics in general. Due to the complexity of a dynamic proteome, different approaches have to be combined to measure protein expression and dynamics, stress and developmental responses, post-translational protein modifications, and protein interaction. So far, about 700 proteins from isolated *Arabidopsis thaliana* chloroplasts have been identified by mass spectrometry whereas the predicted number of proteins targeted to the plastid is approximately 3000.

Methods

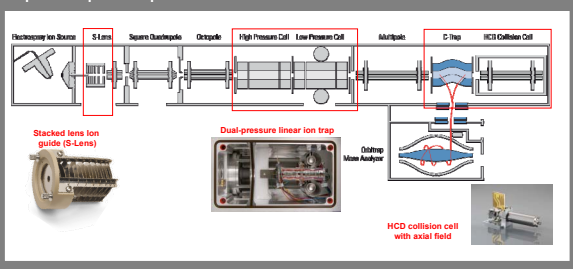
Leaves from 5-week-old *Arabidopsis thaliana* plants were harvested, frozen in liquid nitrogen, and ground until homogenized. The material was centrifuged and the soluble proteins from the supernatant were purified via a 1D SDS-gel. Electrophoresis was performed only for a short time until all proteins have migrated into the gel by about 2 cm. After staining, the colored portion of the gel lane was cut out and reduced to small pieces of about 1x1 mm. Gel pieces were destained, reduced with DTT, alkylated with iodoacetamide and subsequently digested with a Serine protease cleaving C-terminal to Lys and Arg. Peptides were separated by on-line chromatography on a reversed-phase column, followed by mass spectrometric analysis performed on the new hybrid linear ion trap / Orbitrap Velos mass spectrometer.

LC/MS: HPLC System: Thermo Scientific Surveyor MS pump with a flow splitter; Autosampler: MicroAS; Columns: C18 analytical integrifit column (3µm particles, 0.1 mm x 10 cm) with C18 trapping column 20 mm x 0.1 mm; Mobile Phase: A: Water/0.1% formic acid; B: Acetonitrile/0.1% formic acid; Flow Rate: 250 nL/min post split; gradients: 90 min resp. 120 min 0-40% B; Mass Spectrometer: Thermo Scientific LTQ Orbitrap Velos hybrid

Spray Voltage: 1.6 kV; Capillary Temp: 200 °C; Resolution settings: Full MS 60k, MS2 HCD 7.500; Target settings: Full MS 1e6, MS2: CID (LTQ) 5000, HCD 5e4 (predictive AGC on); Dynamic Exclusion™: Repeat count 1, exclusion list size 500, exclusion duration 60 s.

Data Processing: Thermo Scientific Proteome Discoverer software using SEQUEST® and Mascot™ database search algorithms. An *Arabidopsis* sub-database was created from the NCBI nr database. Search parameters were peptide size 700-10k, mass widths Full MS 10 ppm, MS2 0.8 Da for IT-CID MS2 spectra and 20 mmu for HCD MS2 spectra. Searches were performed using false discovery rates of <0.25% (stringent) and <5% (less stringent). Search results were filtered to consider unique proteins and peptides with high confidence and <1% FDR.

FIGURE 1: Schematic of the newly developed LTQ Orbitrap Velos hybrid linear ion trap-Orbitrap mass spectrometer



Results

The technological improvements implemented in the new hybrid ion trap-Orbitrap mass spectrometer (Figure 1) are (a) a progressive stacked-ring ion guide providing a five-fold increased ion transmission¹; (b) a dual-cell differentially pumped ion trap with a higher pressure region for improved ion trapping, isolation, and CID efficiencies, and a lower pressure region for improved resolution and/or scan speed²; (c) predictive AGC for increased scan speed; (d) an HCD-collision cell with axial field for improved performance; (e) improved vacuum in the Orbitrap chamber for improved intact protein analysis.

NanoLC-MS/MS analysis of 200 ng of the highly complex protein digests from *Arabidopsis thaliana* was carried out on both the LTQ Orbitrap Velos™ and an Thermo Scientific LTQ Orbitrap XL system using multiple repeats of a medium length gradient of 90 min (fragmentation with Top 20 CID) and a long gradient of 150 min (fragmentation with Top10 HCD). Chromatographic results for each system were very reproducible and inter-instrument results were very similar.

Assessment of the spectra quality showed that the LTQ Orbitrap Velos produced significantly higher quality HCD MS2 spectra than the LTQ Orbitrap XL™. Low-resolution MS2 CID spectra were of comparable quality in both instruments. The quality of HCD spectra, as well as the significantly improved acquisition speed for all spectra types, were the main contributing factors for the improved success rate in protein identification achieved.

Figure 2 and Figure 3 demonstrate the acquisition speed using the Top20 ITMS CID and Top10 HCD method. In both cases high quality MS2 spectra were obtained leading to an improved identification rate (Figures 4 and 5).

FIGURE 2: NanoLC-MS/MS analysis of 200 ng *Arabidopsis thaliana* digest using TOP20 ITMS² method (8-9 Hz for ITMS2 CID scans, 5e3 ions)

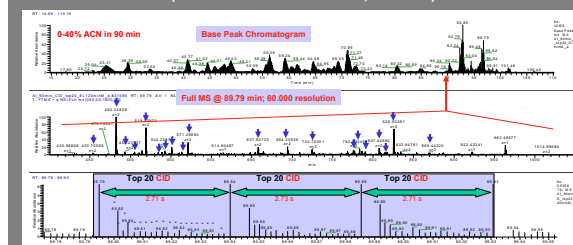


FIGURE 3: NanoLC-MS/MS analysis of 200 ng *Arabidopsis thaliana* digest using TOP10 HCD method (5-6Hz for HCD scans, 5e4 ions)

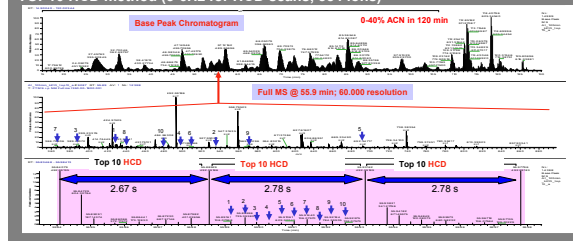


FIGURE 4: NanoLC-MS² analysis of 200 ng *Arabidopsis thaliana* digest using TOP20 ITMS² CID method: Assigned MS² spectra from one scan cycle @ 89.8 min. Identified sequences indicate b- and y-ions supporting the identification.

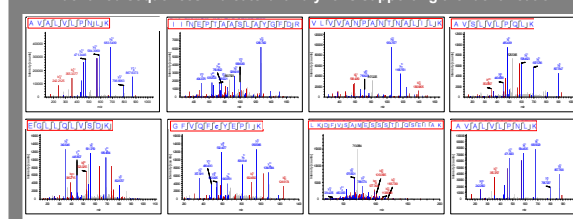
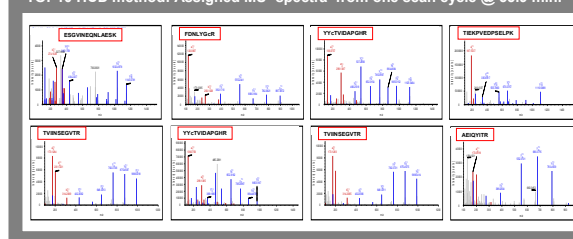


FIGURE 5: NanoLC-MS² analysis of 200 ng *Arabidopsis thaliana* digest using TOP10 HCD method: Assigned MS² spectra from one scan cycle @ 55.9 min.



A comparison number of the unique proteins and peptides identified using HCD and CID spectra showed an overall increase of 40-50% for the LTQ Orbitrap Velos ion trap versus the LTQ Orbitrap XL ion trap, especially for runs with short- to medium-length gradients. Typically, a similar number of proteins were identified using the LTQ Orbitrap Velos with a gradient half as long as on the LTQ Orbitrap XL. Protein ID using HCD and 120-minute gradients yielded a protein ID number of ~600 in each single run which is comparable to 3 consecutive ITMS CID experiments with only 90 min gradient (both types acquired on the LTQ Orbitrap Velos). Consecutive runs improved coverage of high-abundance proteins and increased confidence in low-abundance proteins. Protein ID differences from consecutive runs resulted, in most cases, from either failure to trigger fragmentation of low-abundance peptides in all runs or differences in scores which resulted in only some of the runs passing filter criteria.

FIGURE 7: a) Stacked BPCs of 3 consecutive CID runs (90 min gradient); b) 3 portions of the protein ID table indicating differences in peptide IDs in the 3 consecutive runs. c) XIC of a low intensity peptide @ m/z 439.8, a peptide that was found in all 3 runs, but for run I the score was too low to pass the filter set.

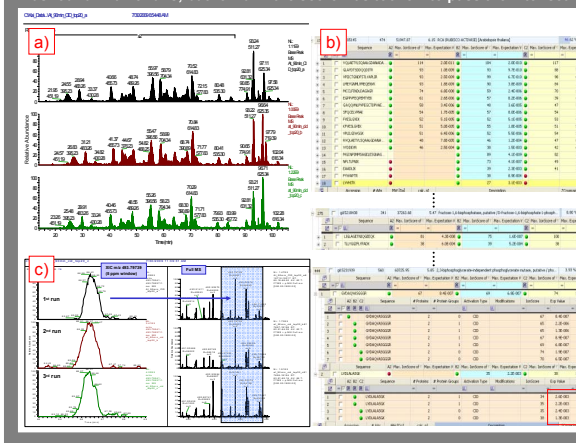
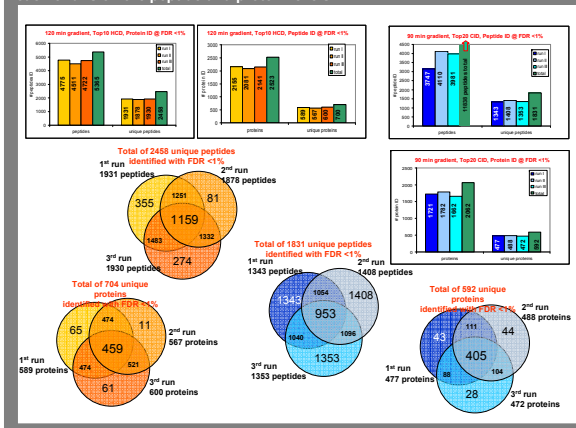


FIGURE 8: Unique peptides and proteins identified in 3 consecutive runs using a TOP10 HCD method (200 ng *A. thaliana* digest, 0-40% ACN in 120 min) versus a TOP20 CID method (0-40% ACN in 90 min). Venn diagrams indicate overlap between each 3 runs on the peptide and protein levels.



Conclusion

- The enhanced sensitivity and improved duty cycle of the LTQ Orbitrap Velos ion trap enable a significant increase in the number of unique peptides and proteins identified, with higher sequence coverage and confidence, compared to the LTQ Orbitrap XL ion trap.
- Consecutive runs show an overall overlap of ~60-70% on the peptide level and ~80-85% on the protein level.
- Protein ID rate for CID and HCD are of the same order of magnitude considering the different gradients used in this example.
- Quality and acquisition speed of HCD spectra are significantly improved, making this technique more amenable for standard protein ID of complex samples.

References

- E. R. Wouters, M. Splendore, M.W. Senko, J. E. P. Syka, J.-J. Dunnyach, "Design of a progressively spaced stacked ring ion guide for improved ion transmission at high pressure", ASMS 2008.
- J. C. Schwartz, J. E. P. Syka, P. M. Remes, S. T. Quarmby, "A New Dual Cell Linear Ion Trap Configuration for Improved Quadrupole Ion Trap Performance", ASMS 2008.
- Aronsson H, Jarvis P. FEBS Letters, 529, 215-220 (2002).
- Schwartz, J. et al., A New Dual Cell Linear Ion Trap Configuration for Improved Quadrupole Ion Trap Performance, ASMS (2008).

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