Unveiling the performance of a novel high-resolution accurate mass platform for proteomics applications

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ABSTRACT

Purpose: Evaluate the performance of the Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer for single shot bottom-up proteomics.

Method: 1) Data independent acquisition (DIA) using narrow isolation windows (2 Th) and data dependent acquisition, 2) chromatographic separation using a 50 cm Thermo Scientific™ µPAC™ Neo UHPLC column connected to a Thermo Scientific™ Vanquish™ Neo UHPLC system, operated in direction injection mode, (3) data processing with Thermo Scientific™ Proteome Discoverer™ software using Chimerys™ algorithm.

Results: We identified >4,400 protein groups from yeast and >8,700 from a human cell line using a 19.9 min LC gradient, and 10,000 protein groups from the same human cell line using twice longer gradient. Also, >19,000 protein groups were identified from a Gut Microbiome standard using a 90 min gradient.

INTRODUCTION

LC-MS-based proteomics has proven to be a powerful tool for the deep profiling of proteins in biological samples. Despite its advances, obtaining comprehensive protein profiles remains challenging due to the complexity and wide dynamic range of proteomes. Here we investigate the capabilities of a novel HRAM mass spectrometer for the qualitative and quantitative single-shot based LC-MS analysis of different proteome samples with different complexity and dynamic range such as yeast cells, mammalian cells or microbiome.

MATERIALS AND METHODS

Sample preparation

Yeast digest (100 ug, Promega) was resuspended in 100 µl 0.1 % Formic acid. The vial was then sonicated for 5 min, followed by multiple sample aspiration and release cycles with a pipette to assure complete resuspension. The stock solution of 1,000 ng/µl was further diluted to a final concentration of 100 ng/µl.
HAP1 digest (500 ng/µl, prepared by the Coon Group) was injected either undiluted or diluted with 0.1% FA solution to cover a dilution series from 5-500 ng.
The ZymoBIOMICS® Gut Microbiome Standard was purchased from Zymo Research Corporation (Catalog No. D6331). It was then defrosted before adding lysis buffer. After a quick vortex, samples were transferred to bead beater compatible tubes and approximately 350 ul worth of 0.1mm glass beads and 0.5mm zirconium oxide beads were added to each sample. The bead beater was set at the speed of 10 for 5 minutes. The sample were then centrifuged, and the supernatant was transferred to a new tube, cleaned using 0.5 mL Pierce™ Detergent Removal Spin Column (product number: 87777) and dried down in SpeedVac and resuspended in 50 µl of lysis buffer in preparation for reduction, alkylation, digestion and clean-up step. Samples were reduced and alkylated followed by digestion with Lys-C and trypsin. Peptides were later cleaned up using SPE columns. Peptide concentration was measured by UV and the digest was then dried down in a SpeedVac. Dried samples were resuspended in 0.1% FA in water to have a final concentration of 0.5 µg/µl.

Liquid Chromatography Parameters

The peptides were separated on a 50 cm µPAC Neo UHPLC column using a Vanquish Neo UHPLC system. The UHPLC system was operated in a direct injection configuration. Flow rate were varied between 750 to 250 nl/ min within the gradient to maximize the elution profile. The Yeast and Human cell line digest were separated using either 19.9 (22.8 min total run time) or 39.9 min (42.8 min total run time) gradients, the Microbiome Gut standard was separated with a much longer gradient, 90 min (100 min total run time). The column was maintained at 50° C and connected to a Thermo Scientific™ EASY-Spray™ Nano & EvoSep emitter (10µ ID), which goes to the EASY-Spray™ Sources.

Mass Spectrometry Methods

Data was acquired on an Orbitrap Astral mass spectrometer (see figure 1 for instrument schematic) either in a data independent acquisition (DIA) using narrow isolation width of 2 Th, or a data dependent acquisition (DDA) mode. The DIA and DDA methods are shown in Table 1.

Full scan	
Orbitrap Resolution	240000
Scan Range (m/z)	380-980
AGC Target (%)	500
Max Injection Time (ms)	3
DIA	
Precursor Mass Range (m/z)	380-980
Isolation Width (m/z)	2
HCD Collision Energy (%)	25
Detector Type	Astral
Scan Range (m/z)	150-2000
RF Lens (%)	40
Normal AGC Target (%)	300
Max Injection Time (ms)	3.5
Loop control	Time
Time (sec)	0.6

Full scan	
Orbitrap Resolution	120000
Scan Range (m/z)	350-1350
AGC Target (%)	300
RF Lens (%)	40
Max Injection Time (ms)	10
DDA	
Charge state	2-6
Minimum intensity	5000
Precursor Mass Range (m/z)	380-980
Isolation Width (m/z)	2
HCD Collision Energy (%)	27
Detector Type	Astral
Scan Range (m/z)	150-2000
Normal AGC Target (%)	100
Max Injection Time (ms)	10
Data-dependent mode	Cycle Time
Time (sec)	3

Table 1. Orbitrap Astral MS DIA and DDA parameters

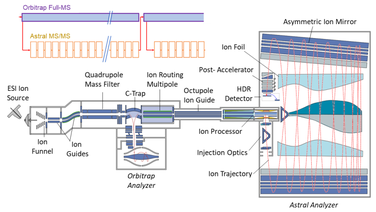

Figure 1. Schematic of the Orbitrap Astral mass spectrometer

Data Analysis

The raw files were processed with Proteome Discoverer software version 3.1 using CHIMERYS algorithm against UniProt protein database from yeast (7,311 sequences), human (20,528 sequences) and for the microbiome standard, a custom fasta files containing 66,250 sequences were used. The results were filtered to <1% peptide FDR and <=1% protein FDR.

RESULTS

Orbitrap Astral mass spectrometer

The Orbitrap Astral mass spectrometer is designed to deliver high resolution and accurate mass measurements with high sensitivity at extremely high acquisition rates of up to 200 Hz. We evaluated the performance of the novel HRAM platform for single-shot bottom-up proteomics using 3 samples types, classified as: (1) low complexity (yeast digest), (2) medium complexity (mammalian cell line digest), and (3) high complexity Gut microbiome digest.

Low complexity, Yeast digest

Yeast digest was injected in 4 different amounts (20, 50, 100 and 200 ng). The eluting peptides were analyzed on the Orbitrap Astral mass spectrometer, operated either in DDA or DIA mode. Figure 2 A&B shows the average number of protein and peptides groups identified from 3 technical replicates using a 19.9 min gradient. Over 4,500 protein groups and 45,000 peptide groups were identified for both DIA and DDA. Almost the whole Yeast proteome was covered with just a 19.9 min gradient. These numbers are comparable to previously published results[1], however, they are achieved using much lower sample amount and 3 times faster throughput.
A quick comparison of DIA to DDA for the yeast proteome on the Orbitrap Astral shows only a 5% difference in protein groups and 14% in unique peptides.

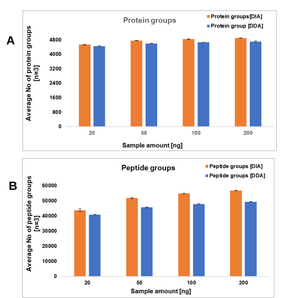

Figure 2. Average protein groups and peptide groups identified from yeast proteome

We also evaluated the uniqueness/complementary nature of each acquisition method as shown in figure 3 A&B. While the protein groups are very similar (almost whole yeast proteome identified with as low as 20 ng), over 12,000 peptide groups were uniquely identified in the DIA experiment and 5,000 in the DDA. We also looked at the repeatability of measurements, using 20 and 50ng yeast digest. As shown on figure 4 A-D, the results are consistent for both DDA and DIA.

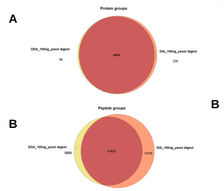

Figure 3. Comparison of protein and peptide groups identified using DIA and DDA

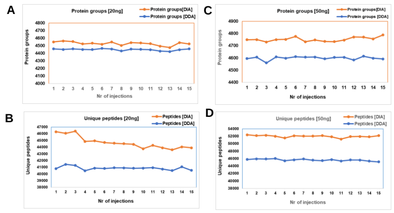

Figure 4. Repeated injections of 20 and 50 ng yeast digest

Medium complexity mammalian cell line digest

In a second experiment, we analyzed a Human cell line digest (HAP1) using both DIA and DDA. First, a dilution series of HAP1 was created and each load was analyzed in triplicate using data dependent acquisition. In the 19.9 min gradient, 5,921 protein groups and 72,834 peptide groups were identified from 5 ng and 8,721 protein groups and 153,420 peptide groups from 500 ng sample load (see figure 5 A&B). We further analyzed 500 ng HAP1 digest in both DIA and DDA modes using 39.9- and 61.9-min gradients and observed that the difference between protein and peptide groups for DIA and DDA did not differ very much, in both cases exceeding 10,000 protein groups (see figure 6 A&B).

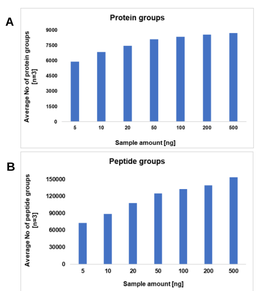

Figure 5. Protein groups and peptide groups identified from HAP1 protein digest in a DDA experiment

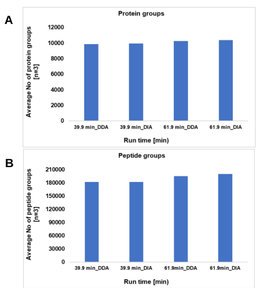

Figure 6. Comparison between DIA and DDA on the Orbitrap Astral MS using HAP1 protein digest

High complexity Gut microbiome digest

The complexity and wide dynamic range of microbiome samples has often been a challenge for LC-MS analysis. The Gut Microbiome standard was created to mimic a microbiome sample. It contains different organisms, mixed in different ratios. Analyzing 500 ng of the Gut microbiome standard digest on the Orbitrap Astral MS using a 90-minute LC gradient, we identified an average of 19,659 protein groups and 113,439 peptide groups from 3 technical replicates. This is an increase of over 40% protein groups and about 25% peptide groups compared to the Orbitrap Exploris 480 (see figure 7).

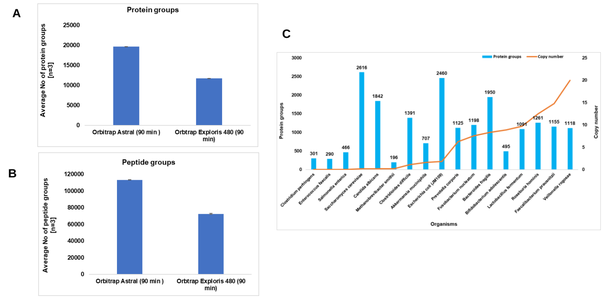

Figure 7: Protein groups (A) and peptide groups (B) identifications from Gut Microbiome standard on the Orbitrap Astral MS and Orbitrap Exploris 480 MS using a 90 min gradient, and (C) Histogram showing the number of protein groups and peptide groups identified per species in the Gut microbiome sample.

CONCLUSIONS

We evaluated the performance of the Orbitrap Astral mass spectrometer for single-shot proteomics using three different sample types of different complexity.

- With the novel Orbitrap Astral mass spectrometer we were able to identify almost the whole yeast protein using 20 ng sample and 19.9 min LC gradient. These results were obtained at three times higher throughput and with much lower sample load compared to previously published results.
- In a DDA experiment using HAP1 digest, we could identify 5,921 protein groups and 72,834 peptides from 5 ng and 8,721 protein groups and 153,420 peptide groups from 500 ng using 19.9 min LC gradient. With a longer gradient, the number of protein groups identified exceeded 10,000.
- From a Gut Microbiome standard, the Orbitrap Astral MS identified 45% more protein groups and 25% more peptide groups than Orbitrap Exploris 480.
- We also showed that DIA and DDA on the Orbitrap Astral MS generate similar results, with slightly higher numbers of identifications for DIA compared to DDA.

REFERENCES

1. Hebert AS, Richards AL, Bailey DJ, Ulbrich A, Coughlin EE, Westphall MS, Coon JJ. The One Hour Yeast Proteome, Mol Cell Proteomics. 2014 Jan; 13(1): 339–347

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TRADEMARKS/LICENSING