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™ Astrea™ mass spectrometer for single-shot bottom-up proteomics.

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

Results: We identified >4,400 protein groups from yeast and >700 from a human cell line using a 18-0 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 HRM mass spectrometer for the qualitative and quantitative single-shot bottom-up LC-MS analysis of different proteome samples with different complexity and dynamic range such as yeast cells, human cells, and a Gut microbiome sample.

MATERIALS AND METHODS

Sample preparation

Yeast digest (100 ng). Prototrophs was reconstituted in 100 µl 0.1% Formic acid. The vacu 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 1,200 µg was further diluted to a final concentration of 100 ng. HAP1 digest (500 ng), prepared by the Coon Group, was injected undiluted or diluted 1:2 with water.

Peptide preparation

The HAP1 digest was created and each load was analyzed in triplicate. After a quick vortex, samples were transferred to bead beater compatible tubes and stored at the speed of 10 for 5 minutes. The sample was then centrifuged, and the supernatant was transferred to a new tube, cleaned using 0.5 mL Pierce™ High Performance Tip. It was then defrosted before adding lysis buffer.

Data Analysis

The raw files were processed with Proteome Discoverer software version 3.1 using CHIMERE algorithm against UniProt protein database from yeast (7,371 sequences), human (20,528 sequences), and for the microbiome standard, a custom fasta files containing 60,265 sequences were used. The results were filtered to >1% peptide FDR and >1% protein FDR.

RESULTS

Orbitrap Astrea mass spectrometer

The Orbitrap Astrea 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 HRM-platform for single-shot bottom-up proteomics using 3 sample 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 eulating peptides were analyzed on the Orbitrap mass spectrometer, operated either in DDA or DIA mode. Figure 2A shows the average number of protein and peptide groups identified from 3 technical replicates with a 19.9 min gradient. Over 4,500 protein groups and 40,000 peptide groups were identified in 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.

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 using a 90-minute gradient, we identified 19,650 protein groups and 113,429 peptide groups from 3 technical replicates. This is an increase of over 40% protein groups and about 20% peptide groups compared to the Orbitrap Exomes 480 (see figure 5).

We also evaluated the uniqueness/completeness nature of each acquisition method as shown in figure 3.ABB. 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 50 ng yeast digest. As shown on figure 4.A-B, the results are comparable for both DIA and DDA.

CONCLUSIONS

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

With the novel Orbitrap Astrea mass spectrometer we were able to identify almost the whole yeast proteome 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 DIA experiment using HAP1 digest, we could identify 5,011 protein groups and 72,834 peptide groups from 5 ng and 7,271 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.

With a Gut Microbiome standard, the Orbitrap Astrea MS identified 45% more protein groups and 25% more peptide groups than Orbitrap Exomes 480.

We also showed that DIA and DDA on the Orbitrap Astrea MS generate similar results, with slightly higher numbers of identifications for DDA compared to DIA.

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TRADEMARKS/LICENSEING

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