Increasing the depth of single shot proteomics with enhanced data acquisition and processing strategies using a new Orbitrap Tribrid MS

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ABSTRACT

Purpose: Single-shot proteomics depth of coverage has been enhanced by multiple advancements. Here we focus on the use of a new Orbitrap Tribrid MS and the Thermo Scientific™ Proteome Discoverer™ software with the CHIMERYS™ intelligent search algorithm paring with wide window acquisition to optimize the depth of proteome coverage in single-shot proteomics data.

Methods: We performed comparative analyses of standard runs using various gradient lengths, data-dependent acquisition MS^2 isolation widths and dynamic exclusion lengths, as well as a data-independent acquisition method. Data were acquired using a Thermo Scientific™ Orbtrap Eclipse™ Tribrid™ mass spectrometer or a Thermo Scientific™ Orbtrap Ascend™ Tribrid™ mass spectrometer. Data were processed using Proteome Discoverer 3.1 software.

Results: Increasing the precursor isolation width for data-dependent acquisition in tandem with processing using the CHIMERYS intelligent search algorithm results in improved protein and unique peptide identifications at all gradient lengths. These results are unique to the CHIMERYS intelligent search algorithm, as processing with Sequest™ HT using the same data does not provide substantial improvements.

INTRODUCTION

Advances in the online separation of complex proteomics samples, including ultra-high performance liquid chromatography (UHPLC), ultra high-resolution separation columns, as well as a new Orbitrap Tribrid MS with increased sensitivity and FTMS^2 scan rates enable deeper mining of the proteome with single-shot methods. In addition to the improvements described above, the CHIMERYS™ intelligent search algorithm unlocks the ability to deconvolute the chimeric spectra that still arise from the co-isolation and fragmentation of multiple peptides in tandem. Here we employ all these strategies together in a single-shot proteomics workflow for improved speed, sensitivity, and depth of coverage compared to current acquisition methods and previous search strategies.

MATERIALS AND METHODS

Sample Preparation and Liquid Chromatography

Thermo Scientific™ Pierce™ HeLa Digest Standard (20 µg/vial) was reconstituted by adding 20 µL of 5% ACN in 0.1% formic acid in water. Sample was aspired and pipetted approximately 10 times and then transferred to an autosampler vial for injection onto a Thermo Scientific™ µPac™ Neo column. Samples were separated at 300-800 nL/min using a Thermo Scientific™ Vanquish™ Neo UHPLC system. Gradient lengths between 60 minutes and 14.4 minutes were used to evaluate single-shot proteomics performance

Mass Spectrometry Methods

Data were collected using an Orbitrap Ascend Tribrid mass spectrometer in data-dependent acquisition and data-independent acquisition modes with full scan data collection using Orbitrap detection and fragmentation data collection also using Orbitrap (OT) detection. For data-dependent data collection the full scan was collected with 60,000 resolving power, and fragmentation data was collected at 15,000 resolving power for 60 minutes gradients, or 7,500 resolving power for shorter gradients. Quadrupole isolation widths for MS^2 acquisition were varied between 0.4 Th and 20 Th. The scan range used was 375-1500 m/z. Dynamic exclusion was set to 10 ppm with a duration varying from 20 to 60 seconds. Fragmentation was performed using HCD with a fixed collision energy of 25. For data-independent acquisition, a dilution series of HeLa was acquired from 1-100 ng using a 30 minutes gradient. MS^1 scans were collected at 60,000 resolving power and a scan range of 400-900 *m/z*. DIA scans were acquired at 30,000 resolution, a scan range of 145-1450 *m/z*, and an isolation window of 7 Th with 1 Th overlap between windows,

Data Analysis

The acquired raw data files were processed with Proteome Discoverer 3.1 software using the default SequestHT_Percolator, INFERYS_Rescoring_SequestHT_Percolator, and CHIMERYS_Percolator workflows paired with a standard consensus workflow. DIA raw files were processed with Proteome Discoverer 3.1.

RESULTS

Optimization of isolation window for data-dependent acquisition

To optimize acquisition strategies for use with the CHIMERYS intelligent search algorithm, we investigated the impact of isolation window on OT/OT acquisition method with an Orbitrap Eclipse Tribrid mass spectrometer with a FAIMS Pro Duo Interface using -50 and -70 CV. These results demonstrated a clear benefit to increasing isolation windows for both acquisition strategies with an isolation window of 1.5 Th providing the best results for OT/IT acquisition and between 2-4 Th providing the best results for OT/OT acquisition.

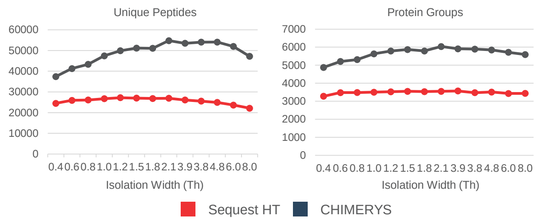

Figure 1. Comparison of the number of unique peptides and protein groups identified on average from OT/OT data-dependent acquisition with 3 replicates of HeLa cell lysate digest with a 1-hour gradient and variable isolation widths using different search strategies in the Proteome Discoverer software framework demonstrates an increase in unique peptides and protein groups for isolation widths between 2-4 Th when using the CHIMERYS intelligent search algorithm.

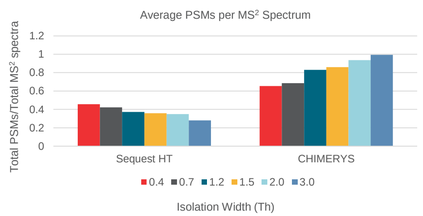

Figure 2. Comparison of the number of PSMs per MS^2 spectra identified with different isolation widths and search strategies for 1-hour acquisition OT/IT data for 1 µg of HeLa cell lysate digest. Wider isolation widths increase instrument utilization in combination with CHIMERYS processing by identifying more PSMs per spectrum.

Improvements in Orbitrap scan rates with new Tribrid architecture

The addition of a Front Ion Routing Multipole in the Orbitrap Ascend allows for an improved ion transfer routine that results in higher sensitivity and OT/OT scan rates. When utilizing the 7,500 resolving power setting, the Orbitrap Ascend has a theoretical max scan rate of 50 Hz, resulting in an ~30% increase in scan rates over previous instrumentation.

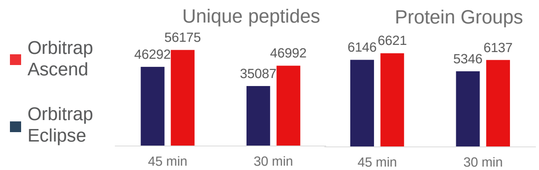

Figure 3. Comparison of the number of unique peptides and protein groups identified on average from data-dependent acquisition with 3 replicates of HeLa cell lysate digest with a 45- and 30-minutes gradient, 4 Th isolation width. and 7,500 resolving power OT MS^2 on the Orbitrap Ascend and Orbitrap Eclipse. The increased scan rate results in significantly improved identifications, especially at lower gradient lengths.

Optimization of isolation windows by gradient length

To determine how the optimal isolation window changes as the gradient length decreases, data was acquired at gradient lengths varying from 45 to 14.4 minutes. These results show that an optimum remains at 4 Th at all gradient lengths. Increasing the isolation width results in reduced performance as the number of peaks in the spectra increases.

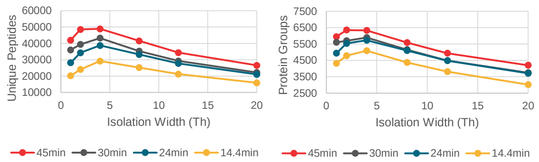

Figure 4. Comparison of the number of unique peptides and protein groups identified on average from OT/OT data-dependent acquisition with a variable isolation widths shows that an isolation width of 4 Th remains optimal at all gradient lengths

Optimization of Dynamic Exclusion length

To further determine the impact of other acquisition parameters that impact MS^2 precursor selection and acquisition we also tested modifications of the Dynamic Exclusion length. This determined that a decrease in the Dynamic Exclusion time resulted in minimal differences in the identification of unique peptides and protein groups. Although the scan rate increased with the increased number of precursors selected, this only resulted in a higher PSM/Unique peptide ratio.

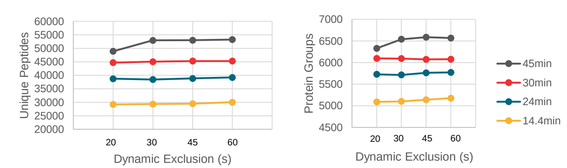

Figure 5. Comparison of the number of unique peptides and protein groups identified on average from OT/OT data-dependent acquisition with 2 replicates of HeLa cell lysate digest with a 30 minutes gradient, 4 Th isolation width, and variable Dynamic Exclusion. Decreases in the Dynamic Exclusion length resulted in a higher number of precursors selected from the MS^1 scans and an increase in the PSM/Unique Peptide ratio. However, it made minimal difference in the overall identification rate.

Data-independent Acquisition with CHIMERYS

New in Proteome Discoverer 3.1 is the capability to process DIA data with the CHIMERYS search algorithm. To demonstrate this functionality, a HeLa dilution was acquired with concentrations varying from 1-100ng on a 30 minutes gradient.

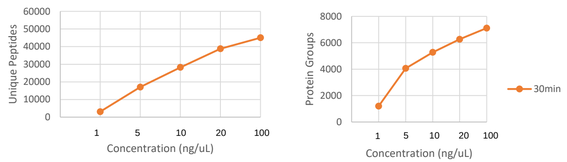

Figure 6. Comparison of the number of unique peptides and protein groups identified on average from data-independent acquisition with variable sample concentrations using the CHIMERYS intelligent search algorithm in Proteome Discoverer.

CONCLUSIONS

These results demonstrate that pairing optimized instrument acquisition strategies for OT/OT acquisition leveraging UHPLC, ultra-high resolution µPac Neo columns, and advanced processing strategies with Proteome Discoverer software and the CHIMERYS intelligent search algorithm can synergistically improve the depth of proteome coverage and increase throughput.

- Increasing the MS^2 isolation width for data-dependent acquisition to between 2-4 Th for OT/OT acquisition method along with processing using Proteome Discoverer software with the CHIMERYS intelligent search algorithm improves performance
- Wide window acquisition and data processing using Proteome Discoverer software with the CHIMERYS intelligent search algorithm improves performance across a wide range of protein loads and gradient lengths
- The CHIMERYS intelligent search algorithm can now process data-independent acquisition data

TRADEMARKS/LICENSING

PO2023-98EN