Immunopeptidomics

A semi-targeted Orbitrap Tribrid method for simultaneous quantification and discovery of immunopeptides

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Abstract

Purpose: Showcasing a Thermo Scientific™ Orbitrap Tribrid™ mass spectrometer method template utilizing both ion trap and Orbitrap for Simultaneous Quantitation and Discovery (SQUAD) analysis of immunopeptides.

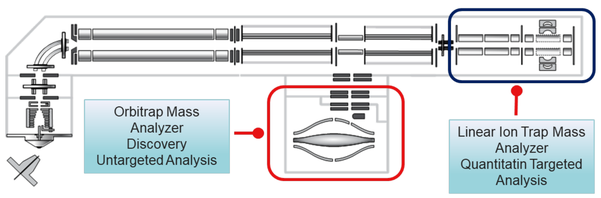
Methods: Class I MHC peptides were spiked with synthetic, heavy-labeled AQUA peptide standards of common HLA peptides and analyzed using a semi-targeted approach. The Orbitrap was employed for data-dependent MS2 data collection, while the ion trap was utilized for targeted analysis via parallel reaction monitoring (PRM).

Results: A method that integrates Orbitrap and linear ion trap scans allows for both discovery analysis and targeted peptide quantitation in a single run with minimal impact on peptide identifications. The use of linear ion trap quantitation doubles sensitivity, facilitating precise quantitation.

Introduction

Immunopeptidomics is a rapidly evolving field that aims to identify and quantify naturally occurring peptides presented by the major histocompatibility complex molecules to the immune system. Many experiments focus on both the discovery of unknown peptides, or the quantification of a set of peptides of interest, requiring enough sample for at least two injections. Utilizing a Thermo Scientific™ Orbitrap™ Ascend MultiOmics Tribrid™ mass spectrometer which combines both a high-resolution Orbitrap and a highly sensitive ion trap, a data-dependent experiment for untargeted analysis can be combined with highly sensitive quantitative data in the same analysis using a single injection. Here, we present a new method utilizing the high-resolution Orbitrap detector for discovery, while simultaneously collecting quantitative data in parallel with the ion trap detector.

Figure 1. Orbitrap Ascend MultiOmics mass spectrometer instrument view



Materials and methods

Sample Preparation

Class I MHC peptides were obtained by immunocapture with W6/32-conjugated resin on 100 million HCT-116 cells. After cleanup on StageTips, the starting material was diluted 100x with 0.1% formic acid. A dilution series was prepared by spiking synthetic heavy labeled AQUA peptide standards into the MHC peptide sample at concentrations from 100 amol to 10 fmol.

LC-MS/MS Method

Samples were analyzed using an IonOpticks Aurora Ultimate TS 25cm column connected to a Thermo Scientific™ Vanquish™ Neo UHPLC System and Orbitrap Ascend MultiOmics MS with a Thermo Scientific™ FAIMS Pro Duo interface. A gradient length of 72 minutes was used. The semi-targeted method used the Orbitrap analyzer to collect data-dependent MS2 data while the ion trap was used for targeted PRM analysis.

Data Analysis

The acquired raw data files were processed using Thermo Scientific™ Proteome Discoverer™ 3.1 software, PEAKS® Studio 11, and Skyline software.

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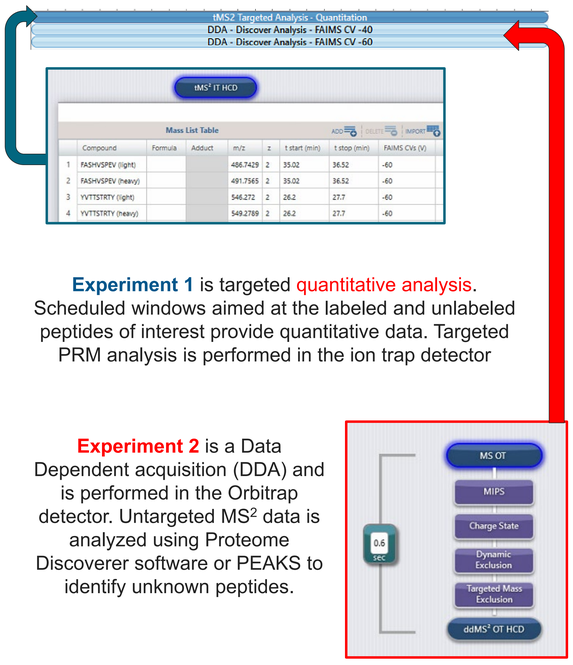
Figure 2. Method editor set up



Experiment 1 is targeted quantitative analysis. Scheduled windows aimed at the labeled and unlabeled peptides of interest provide quantitative data. Targeted PRM analysis is performed in the ion trap detector

Experiment 2 is a Data Dependent acquisition (DDA) and is performed in the Orbitrap detector. Untargeted MS^2 data is analyzed using Proteome Discoverer software or PEAKS to identify unknown peptides.

Figure 3. Complete SQUAD workflow

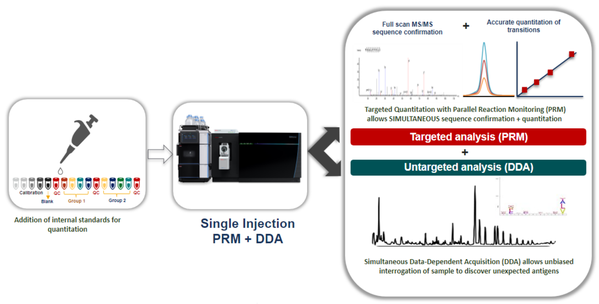
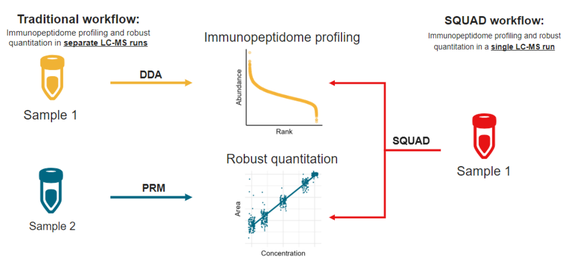


Figure 4. Comparison of traditional and SQUAD workflows



Results

The addition of targeted ion trap scans for quantitation in parallel with the Orbitrap discovery analysis has minimal impact on peptide identifications while providing a significant increase in sensitivity. The SQUAD method maintains a wide dynamic range and large overlap of peptide identifications with the traditional DDA method. Linear ion trap scans showed accurate quantitation across three orders of magnitude with a 2x average improvement in sensitivity.

Figure 5. Peptide identifications from single shot analysis of 1e6 cell equivalents of HCT 116 cells. Data acquired using SQUAD with DDA in 72-min run and searched with PEAKS 11 DeepNovo peptidome workflow. Over 5600 peptides identified per run.

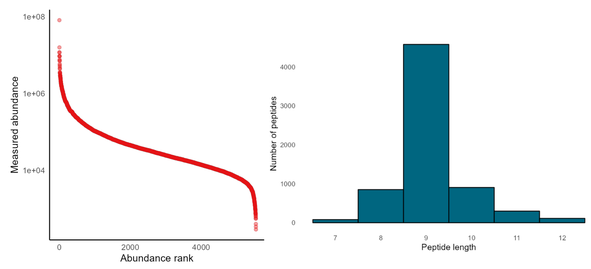
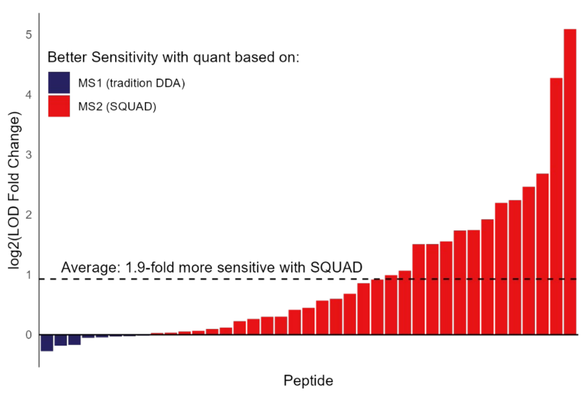


Figure 6. Peptide identifications from 1 replicate injection of 1e6 cell equivalents of HCT 116 cells. Data acquired using traditional DDA or SQUAD with DDA in 72-min run and searched with PEAKS 11 DeepNovo peptidome workflow.

There is a relatively small impact to the number of IDs when targeting 78 precursors (39 light +39 heavy peptides). A wide dynamic range is maintained with SQUAD relative to traditional DDA.

Figure 7. PRM (MS^2) quantitation is 2x more sensitive than MS^1 quantitation



PRM (Targeted MS^2) scans in the linear ion trap have increased sensitivity over MS^1 quantitation using the Orbitrap detector.

Figure 8. Example dilution curve of heavy peptide in constant background. Selected chromatograms corresponding to different points on curve shown.

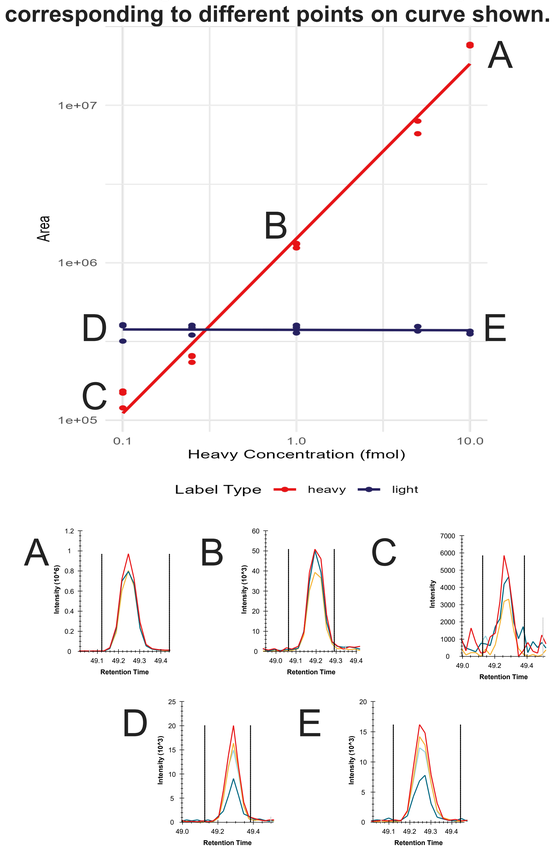
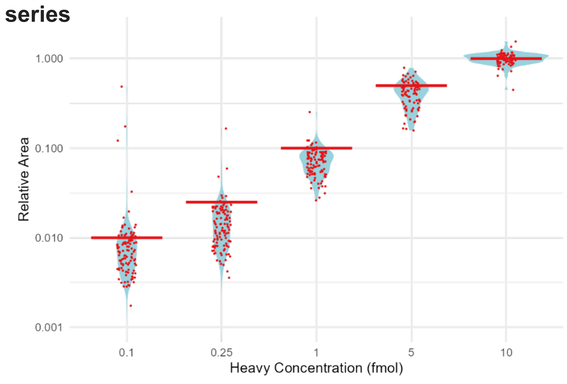


Figure 9. Accurate quantitation across dilution series



Conclusions

- Orbitrap DDA MS2 can be combined with highly sensitive ion trap PRM MS2 scans into a single method using the dual detectors of the Orbitrap Ascend MultiOmics MS.
- Combining discovery measurements in parallel with quantitation of known targets reduces the sample requirements and time needed to perform the analysis.
- The PRM quantitation in the linear ion trap is more sensitive and selective than MS^1 quantitation, enabling analytically rigorous measurements while maintaining discovery depth.
- Future improvements in sensitivity could be gained by utilizing Real Time Library Search to trigger quantitation scans with longer injection times

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