Orbitrap MS

Leveraging advanced mass spectrometry technology with Orbitrap Astral Zoom MS for in depth immunopeptidome profiling

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Abstract

Purpose: Demonstrate the performance of Thermo Scientific™ Orbitrap ™ Astral ™ Zoom mass spectrometer (MS) and Thermo Scientific™ Vanquish ™ Neo UHPLC system in the analysis of major histocompatibility complex (MHC) peptides.

Methods: A label-free DDA-based method is demonstrated to identify singly and multiply charged ions derived from major histocompatibility complex MHC class-I peptides.

Results: Our results show that the Orbitrap Astral Zoom MS coupled to the Vanquish Neo UHPLC system has sensitivity, dynamic range and selectivity to enable immunopeptidomics studies.

Introduction

Immunopeptidomics is the study of the peptides presented by MHC molecules on the surface of cells. These MHC peptides have major implications for many areas of research, including immunotherapy and personalized medicine. Mass spectrometry allows for direct immunopeptidomics analysis, enabling simultaneous identification and quantification of thousands of MHC peptides in a single run. The new Orbitrap Astral Zoom MS has enabled new levels of sensitivity and selectivity to provide deeper insights into the immunopeptidome.

Materials and methods

Sample Preparation

Class I MHC peptide complexes were enriched from IM-9 human B-lymphocyte cells, starting with a culture of 1e8 cells, using the pan-specific MHC class I antibody (W6/32 clone). HLA-associated peptides were isolated using the AssayMap Bravo automated system (Agilent Technologies) after lysing the cells with NP-40 lysis buffer. The peptides were then extracted from the antibodies with 30% acetonitrile. The samples were dried and resuspended in a solution of 3% acetonitrile and 5% formic acid in water. To ensure cell equivalence, samples were injected into the LC-MS system at different volumes to represent peptide loads equivalent to 1e5, 1e6, 1e7, and 1e8 cells.

LC-MS/MS method

Peptides were separated on a Vanquish Neo UHPLC System using Aurora Ultimate™ column (25 cmx 75µm). Total run time was 72 min. Thermo Scientific™ EASY-Spray™ Ion Source was used coupled to the Thermo Scientific™ FAIMS Pro Duo interface. Peptides were analyzed by the Orbitrap Astral Zoom MS.

Data Processing

The data analysis was performed using PEAKS Studio 12 with DeepNovo Peptidome workflow for database search and de novo peptides identification. Spectra were searched against UniProt human database (20,607 sequences) with no-enzyme option. The sequence motif and binding properties of 9-mer peptides were analyzed using MHCMotifDecon 1.2 and NetMHCpan 4.0.

Figure 1. Experimental set-up for immunopeptidomics analysis.

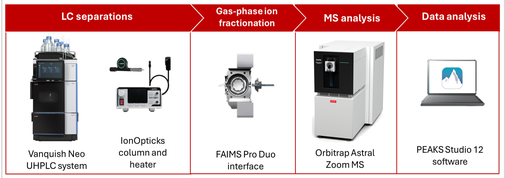


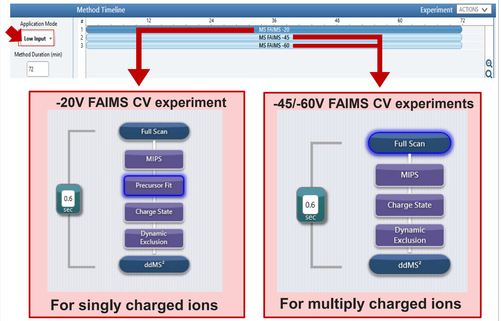
Table 1. LC-MS settings.

Time (min)	Duration (min)	%B	Flow rate (µL/min)
Run			
0.0	0.0	2.0	0.5
1.0	1.0	5.0	0.5
1.1	0.1	5.0	0.2
61.1	60	35.0	0.2
Column wash			
63.1	2.0	70.0	0.2
67.1	4.0	99.0	0.2
67.2	0.1	99.0	0.5
72.0	4.8	99.0	0.5

Table 3. MS parameters for method that includes both singly and multiply charged ions.

Source parameters	For -45 and -60V FAIMS CV experiments	Only for -20V FAIMS CV experiment
Spray voltage (kV)	2.0	
Ion transfer tube temperature (°C)	280	
FAIMS Mode	User defined	
FAIMS inner electrode temp. (°C)	100	
FAIMS outer electrode temp. (°C)	90	
Total carrier gas flow (L/min)	3.5	
Orbitrap MS full scan parameters		
FAIMS CV	-45 and -60	-20
Resolution	120,000	120,000
Scan Range (m/z)	350-1500	700-1500
RF lens (%)	45	45
AGC Target (%)	300	300
Maximum Injection Time (ms)	100	100
MIPS Properties		
Monoisotopic peak determination	Peptide	Peptide
Precursor Fit		
Fit threshold (%)	Does not contain this filter	15
Fit window (m/z)	Does not contain this filter	2
Charge State		
Include charge states	2-4	1
Dynamic Exclusion		
Exclude after n times	1	1
Exclusion duration (s)	15	15
Mass tolerance (ppm)	Low: 5 High: 5	Low: 5 High: 5
Exclude isotopes	yes	yes
Perform dependent scan on single charge state per precursor only	yes	yes
Astral MS2 scan parameters		
Isolation Window (m/z)	1.5	2
HCD collision energy (%)	28	28
Scan Range (m/z)	110-1500	110-1500
Pre-accumulation	ON	ON
Normalized AGC target (%)	30	30
Maximum injection time (ms)	100	100
Microscans	1	1
Data Dependent Properties		
Data dependent mode	Cycle time	Cycle time
Time between master scans (s)	0.6	0.6

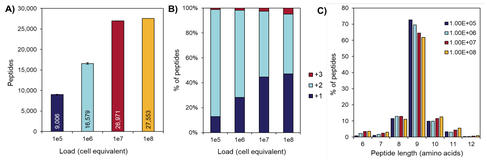
Figure 2: DDA method for analysis of singly and multiply charged ions. (A) Low input application mode is selected and three experiments with different FAIMS CVs are used: -20V experiment for singly charged ions, -45V and -60V FAIMS CV experiments for multiply charged ions.



Results

Sensitivity: detection of singly & multiply charged ions

Figure 3: MHC- class I peptides identified using low input mode on Orbitrap Astral Zoom MS. (A) Average number of identified peptides (n=3), (B) charge state of identified peptides and (C) peptide length distribution in the various loads (1e5 to 1e8 cell equivalents).



- Deep coverage and sensitivity demonstrated from low to high loads.
- Singly and multiply charged ions are detected using the 3 FAIMS CV method.
- Detected peptide exhibited expected length (8-10mers) of Class-I peptides.

Unique peptides detected as different charge states

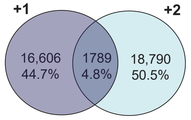
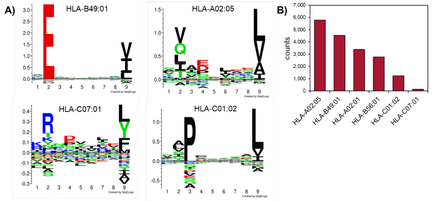


Figure 4: Venn diagram of peptides detected as singly (+1) or doubled charged (+2) ions. Most peptides are detected as one charge state (+1 or +2), highlighting the selectivity of FAIMS to enhance immunopeptidome coverage.

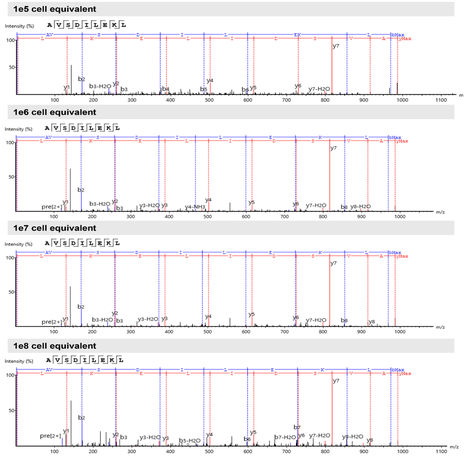
Figure 5: 9mers exhibit classical MHC-I peptide binding motifs. (A) Motifs were predicted using the web-based version of MHCMotifDecon 1.2. (B) Frequency of predicted HLA alleles to be present in the sample analyzed. Data visualization from 1e7 cell equivalent load.



Spectral quality for reliable ID and quantitation

Figure 6: Spectral quality is maintained from low to higher loads. Fragmentation of peptide AVSDILEKL at different sample loads (1e5 to 1e8 cell equivalent) is represented by b and y ion pairs. Data extracted from PEAKS Studio 12.

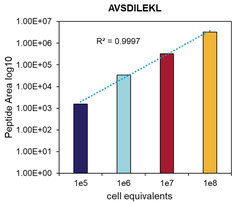
The spectral quality remained consistent across all loads, with b and y ion pairs extending over a wide mass range starting as low as *m/z* 150.



High quantitative linearity

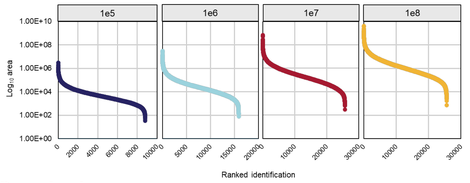
Figure 7: Quantitative linearity across different sample loads.

High quantitative linearity (R^2=0.99) was observed across all loads.



Wide dynamic range

Figure 5: Dynamic range spans up to seven orders of magnitude on Orbitrap Astral Zoom MS.



Conclusions

- Orbitrap Astral Zoom MS equipped with FAIMS Pro Duo interface and coupled to Vanquish Neo UHPLC system provides high sensitivity and the dynamic range necessary for deeper coverage of immunopeptidomic samples
- The low input application mode on Orbitrap Astral Zoom MS offers the sensitivity necessary to analyze low levels of material equivalent to samples ectracted from tissue biopsy samples
- Loading capacity is not limited by MS allowing analyses from ultra low (1e5) to high (1e8) loads of sample
- FAIMS enables the analysis of singly and multiply charged ions enhancing the immunopeptidome coverage
- AGC modulates injection times for each peptide, producing high quality spectra across a wide range of loading levels. This spectral quality is crucial for downstream analysis and confident identification and quantitation of neoantigens

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