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Orbitrap Astral MS

Enhancing Immunopeptide Profiling with Orbitrap Astral Mass Spectrometer for Unbiased Discovery of Neoantigens

Fernanda Salvato, Lilian Heil, Ellen Casavant, Amirmansoor Hakimi, Tonya Pekar Hart

Thermo Fisher Scientific, San Jose, CA, USA

Abstract

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

Methods: A label-free DDA-based method is demonstrated with low input MHC peptides from HCT 116 cells.

Results: Our results show that Orbitrap Astral MS coupled to the Vanquish Neo UHPLC system has sensitivity to enable immunopeptidomics studies.

Introduction

Immunopeptidomics is the study of the peptides presented by major histocompatibility complex (MHC) molecules on the surface of cells. These MHC peptides have major implications for many areas of research, including immunotherapy and personalized medicine. For example, many studies in this field aim to identify low-level tumor specific antigens (TSAs) with the goal of developing personalized immunotherapies to target cancerous cells with a high degree of specificity. Mass spectrometry (MS) allows for direct immunopeptidomics analysis, enabling simultaneous identification and quantification of thousands of MHC peptides in a single run. The recently developed Orbitrap Astral mass spectrometer has enabled new levels of sensitivity and selectivity to provide deeper insights into the immunopeptidome. In this study, we utilized the Orbitrap Astral mass spectrometer to characterize the immunopeptidome extracted from HCT 116 cells to support the detection and annotation of potential neoantigens.

Materials and methods

Sample preparation

Class I MHC peptides were obtained by immunocapture with a pan-specific MHC class I antibody, 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. Samples were diluted to represent the equivalent of E+4 to E+6 cells of extracted IMP.

LC-MS/MS method

Peptides were separated on a Vanquish Neo UHPLC System in trap and elute configuration. Thermo Scientific™ PepMap™ Neo Trap cartridge and Aurora Ultimate™ column (25 cmx 75µm) were used. 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 Mass Spectrometer.

Data processing

The data analysis was performed using PEAKS Studio software (ver. 11) with the DeepNovo Peptidome workflow for database search and de novo peptides identification. Spectra were searched against the UniProt human database (20,607 sequences) with the no-enzyme option.

Search Engine Name: PEAKS
Parent Mass Error Tolerance: 10.0 ppm
Fragment Mass Error Tolerance: 0.02 Da
Enzyme: None
Peptide Length Range: 6 - 45
Database: Human_UniProt
Taxon: all species
Searched Entries: 20607
Deep Novo Score: 70.00%
Deep Novo Protein Association Tag Sharing: 5
Confident Amino Acid Threshold: 2.00%

Figure 1. PEAKS DeepNovo workflow settings.

Figure 2. Experimental workflow from sample preparation to data analysis.

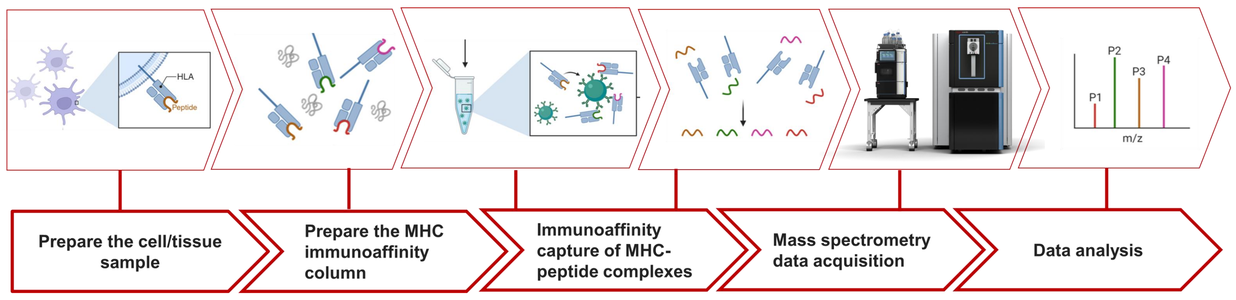


Figure 3. Experimental set-up for immunopeptidomics analysis. This workflow combines the high sensitivity and throughput offered by the Vanquish Neo UHPLC system with low flow with the speed of Orbitrap Astral MS associated with FAIMS interface for higher sensitivity. 00

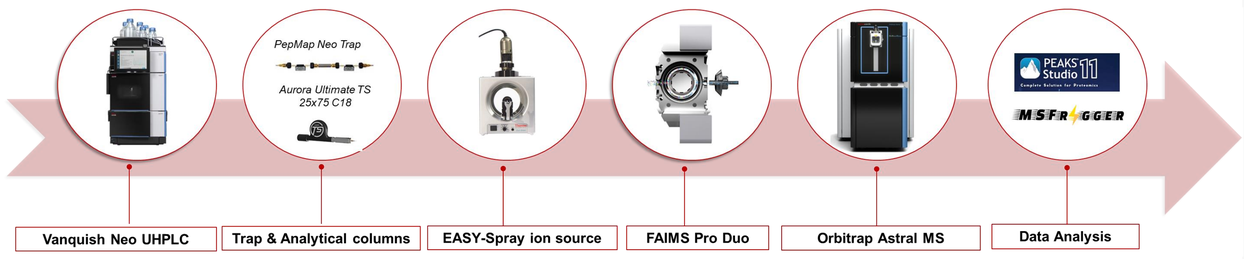


Table 1. LC-MS settings. Trap and Elute configuration was used.

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

Figure 4. Components of each experiment

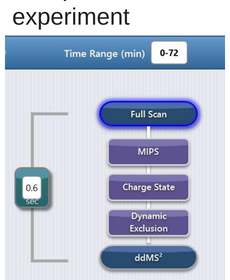


Table 2. MS settings

	Property	Setting
Source	Spray Voltage	1.9 KV
	Capillary Temp	275
	FAIMS CV	- 50 V and -70 V
	Total carrier gas flow (L/min)	3.5
Full MS	Scan Range (m/z)	300-1500
	Orbitrap Resolution	120000
	Max IT (ms)	100
	RF Lens (%)	45
	AGC Target (%)	300
DDA	Data dependent Mode	Cycle Time
	Time between Master scans	0.6 s
	Isolation window	1 m/z
	Detector Type	Astral
	HCD Collision Energies	29
	Scan Range	110-2000
	AGC Target (%)	30
	Max Injection Time (ms)	100

Results

Figure 5. FAIMS CV optimization using E+6 cell equivalent injections. Single CV method were run to evaluate the peptide yield from each CV with the aim to chose CV that provide better coverage

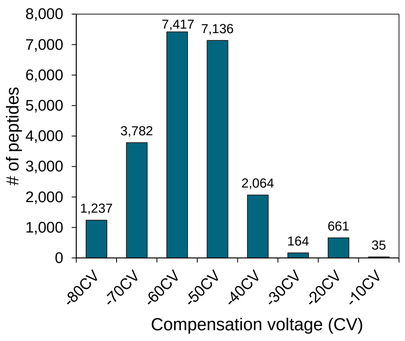


Figure 6. Peptide overlap between different CVs

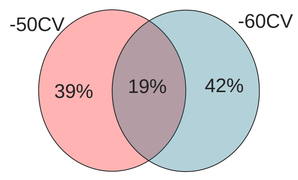


Figure 7. Sensitivity at low loads.MS2 spectrum of 465.76 *m/z,* peptide **AEAEKIKAA** from E+5 to E+7 cell equivalent loads.

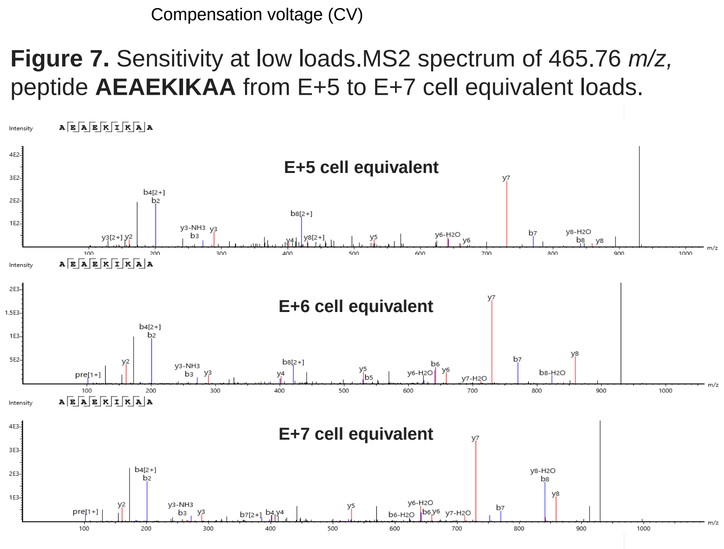


Figure 6. Isolation width experiment. Narrower width can result in higher PSM to MS2 ratio. The extend of this effect depends on the loading material. For higher loads is worth trying narrower wights.

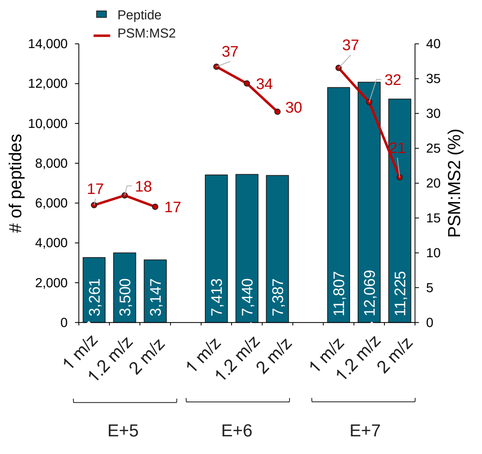


Figure 8. Base peak chromatograms of HCT116 sample injected at different loads using the method described above.

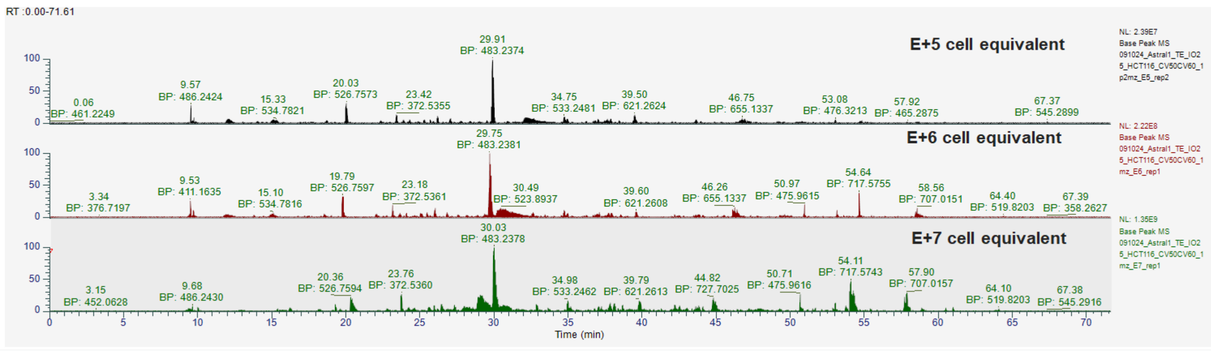
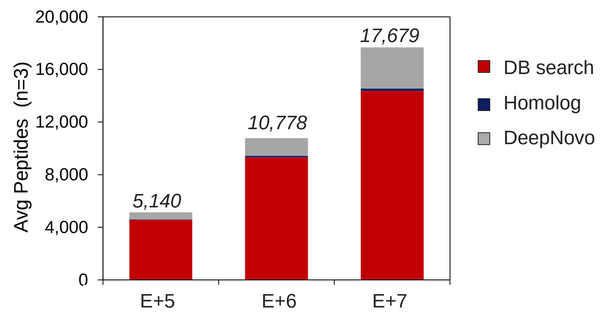
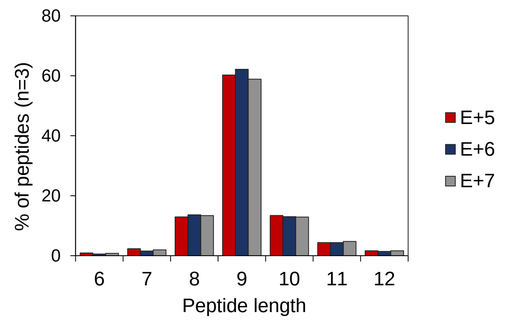


Figure 7. MHC peptides from HCT-116 cells identified using double FAIMs CV (-50 and -60) and PEAKS Studio 11

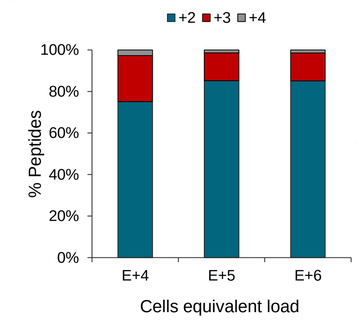
A) Number of peptides annotated by PEAKS The majority of hits are coming from DB search.



B) Peptide length distribution as expected for HLA-class I peptides. Same distribution is observed for all loads.



C) Charge state distribution observed for all loads.



High proportion of +2 peptides detected in all loads.

Conclusions

- Improved sensitivity of and dynamic range of detection for immunopeptide analysis with the Vanquish Neo UHPLC system coupled to an Orbitrap Astral mass spectrometer equipped with FAIMS Pro Duo interface selectivity enables deeper depth of coverage with higher throughput of analysis
- Optimization of FAIMS CV and isolation width can impact results
- Loading capacity is not limited by MS allowing analyses from ultra low (E+5) to high (E+7) loads of material.
- Increased sensitivity allows for compatibility with low levels of material equivalent to samples extracted from tissue biopsy samples

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