Optimized ion transfer settings for high sensitivity class I immunopeptidomics analysis on timsUltra AIP with Evosep One

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Introduction

- Identifying peptide antigens presented by the major histocompatibility complex is vital for understanding diseases and developing immunotherapies.
- Advances in mass spectrometry and nanoflow LC-MS have improved HLA class I detection, with the Evosep One offering high robustness and low sample carryover.
- Comparison of Whisper Zoom 20 method with an alternative nanoLC system.
- Use of optimized ion transfer on the timsTOF Ultra AIP to enhance immunopeptidome detection.

Methods

Samples:

- A375 human melanoma cell line (sample load = 5e5, 1e6 and 5e6).

LC (triplicate injections, Aurora Elite):

- nanoElute 2 (direct sample load or on a PepMap™ Neo Trap-Cartridge column, 54 min active gradient).
- Evosep One (Whisper Zoom 20 SPD). For separation.
- CaptiveSpray Ultra ionization source

timsTOF Ultra 2:

- Ion mobility range:1/k0=0.64-1.7; accu time: 166 ms; 8 PASEF ramps; optimized collision energies: 1/k0 0.7 → 20 eV, 1/k0 1.06 → 30 eV, 1/k0 1.1 → 40 eV, 1/k0 1.34 → 40 eV, 1/k0 1.68 → 70 eV; intensity threshold: 500; target intensity: 20,000, charge states: 1-3 precursor selection includes multiply charged precursors from 300-800 m/z and singly charged precursors from 700-1,300 m/z; 300 Hz enabled. Deflection 0 delta (70 V) and Δt6 (60 V) have been optimized for improved ion transfer.

timsUltra AIP:

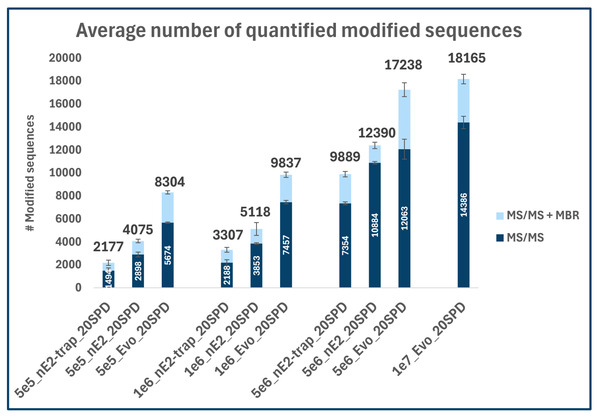
- Evaluation of amplitude, slope and storage base time of the AIP. The most promising settings are shown.

Processing:

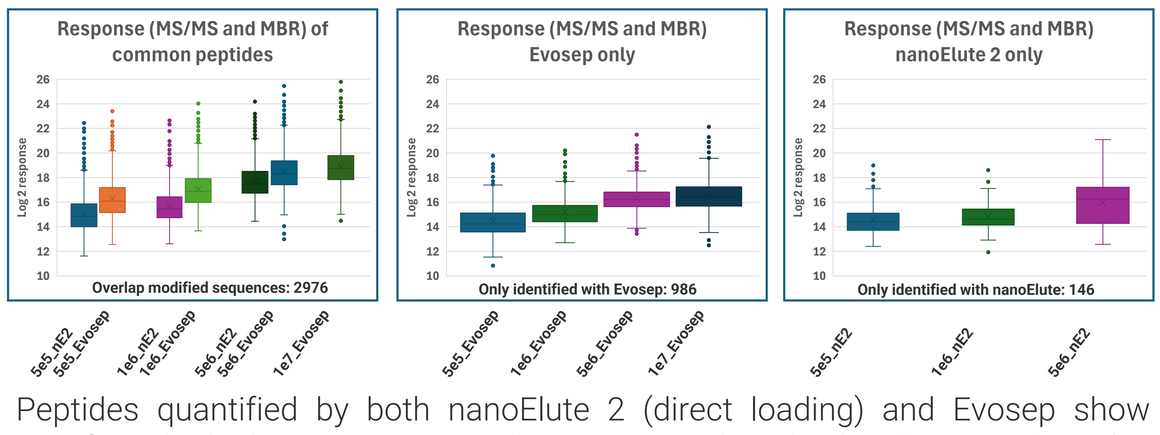
- MSFragger (4.0) using an HLA workflow in FragPipe (21.1). Rescoring with MSBooster and IonQuant was applied. General search parameters: Human uniprot database with 20,606 reviewed entries, 15 ppm precursor m/z tolerance; 30 ppm fragment m/z tolerance; enzyme: nonspecific; peptide length 7-20 amino acids; variable modifications: oxidation (M). Peptides were reported at a peptide and PSM FDR of <1%.

Overview of LC separation techniques

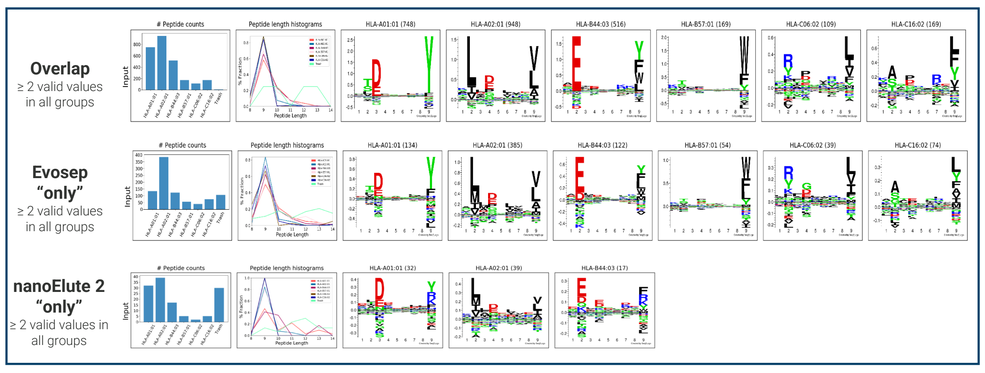
Comparison of different sample loading amounts on two LC systems measured on a timsTOF Ultra 2Overall I would try and reduce text



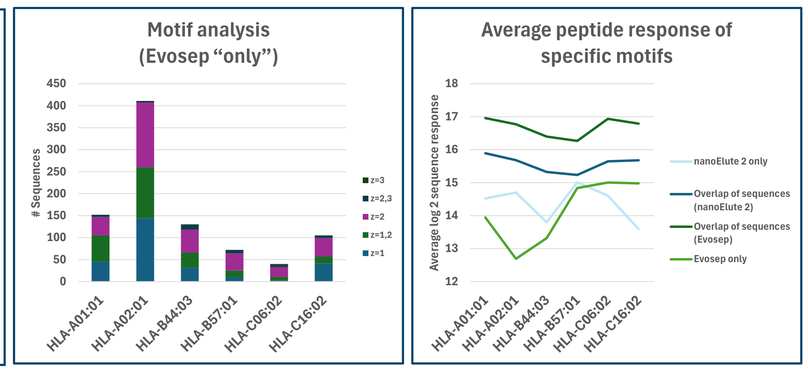
Across all sample amounts, the Evosep One consistently yields higher peptide identification rates.



Peptides quantified by both nanoElute 2 (direct loading) and Evosep show significantly higher responses when measured with the Evosep. Peptides uniquely identified by Evosep come from low abundant precursors. They exhibit a length distribution consistent with MHC I binders



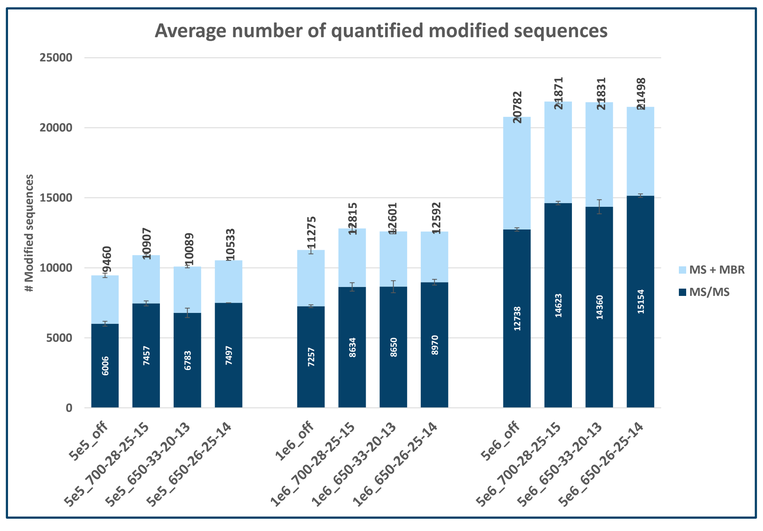
Cell line-specific allele distribution: Peptides uniquely identified by the Evosep system exhibit similar sequence motifs to those shared between nanoElute 2 and Evosep.



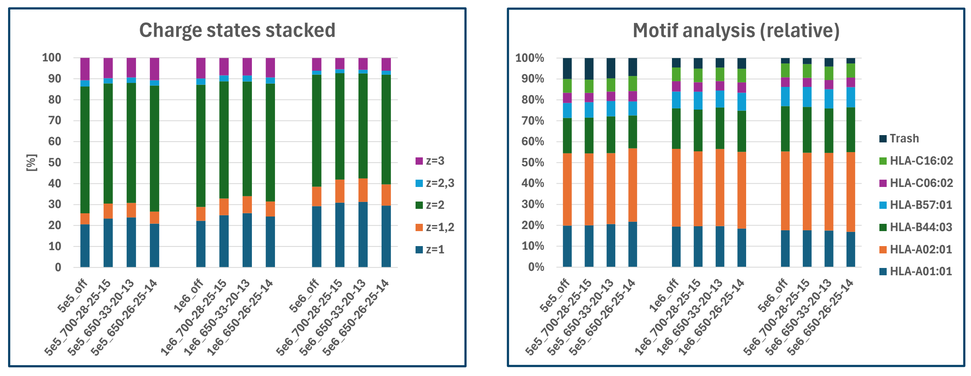
Enhanced identification of peptides associated with the *A02:01 allele with Evosep. Additional peptide identifications are of low abundance.

Improved ion transfer with broad m/z transmission

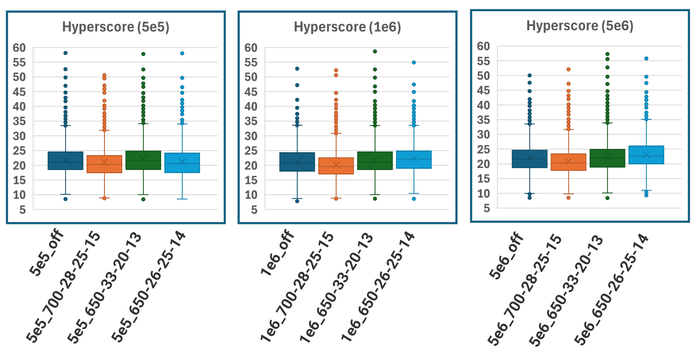
Impact of AIP on peptide identification across different sample loading amounts using Evosep One



Enabling AIP consistently increased MS/MS-level identifications by approximately 20% across all sample amounts.



Charge state distributions with 650 Vpp amplitude, 25 slope, 14 µs storage base time were like those with AIP off, suggesting consistent m/z transmission characteristics. Relative sequence motif analysis remained consistent across all ion transfer settings, indicating robustness of sequence-level features.



AIP settings (650 Vpp amplitude, 25 slope, 14 µs storage base time) yielded comparable or higher median hyperscores relative to AIP off, indicating improved identification confidence. Enhanced peptide responses were also observed (data not shown).

Conclusion

- The Evosep One yields excellent results in peptide identification and quantification, consistently yielding higher identification rates and stronger peptide responses across all sample loading amounts.
- Peptides uniquely identified by Evosep tend to have lower intensities but maintain similar length and sequence motif characteristics of A375 MHC I binders, with enhanced detection of low-abundance peptides associated with specific alleles such as *A02:01, *A01:01, and *B44:03.
- The AIP on the timsTOF Ultra platform improves MS/MS-level identifications by ~20% and enhances peptide confidence scores, without compromising charge distribution or sequence motif integrity.

Immunopeptidomics