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Advancing Low Input Chromatography: Developing a novel Column for Improved Immunopeptidomics IDs

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Introduction

The timsTOF Ultra leverages trapped ion mobility and a modified source design, enhancing ion current for exceptional sensitivity and an extended dynamic range. This cutting-edge technology finds application in immunopeptidomics, an expanding field crucial for uncovering profound insights into the immune system. By identifying and quantifying immunopeptides presented by major histocompatibility complex (MHC) molecules on cell surfaces, immunopeptidomics contributes significantly to understanding immune responses. This knowledge not only advances our comprehension of immune processes but also holds promise for enhancing immunotherapies and the development of vaccines, showcasing the pivotal role of timsTOF Ultra in pushing the boundaries of proteomic research for immunological advancements.

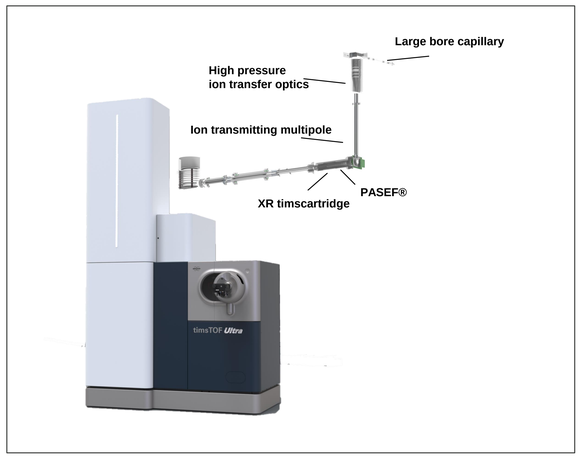

Fig. 1: timsTOF Ultra features new ion source geometry with additional higher pressure vacuum stage

Methods

This study explores the application of timsTOF Ultra in immunopeptidomics using IM9 B lymphocyte cells. Extracted peptides underwent chromatographic separation with 11-44 min gradients using nanoElute 2, analyzed via PASEF on timsTOF Ultra. Data were searched using MSFragger and Peaks Online software for comprehensive insights.

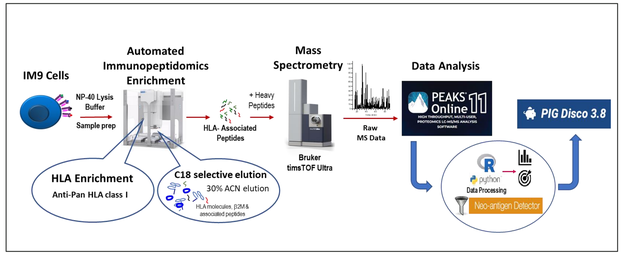

Fig. 2: Immunopeptidomics workflow. HLA enrichment following by selective elution of the HLA-associated peptides were performed using a liquid handling robot. The dda-pasef data files were searched using FragPipe v21.1 or PEAKS 11 online software.

The aim of this study is to evaluate the performance of using monolithic column separation for immunopeptidomics applications. HLA-associated peptides were directly injected in a monolithic reverse phase analytical column (200 cm x 75 µm ID) and separated using different gradient times. A volume of 1 µl/injection, equivalent to 10 millions cells from the original samples was selected. The timsTOF Ultra was configured to acquire the data in dda-pasef mode employing HLA-tailored isolation polygons, a 50 or 100 ms TIMS ramp, five MS2 frames/cycle, one cycle overlap, using the high-sensitivity mode.

Table 1: Separation methods created for the Mosaic column.

Gradient time	Total time* (*from sample pickup to sample pickup)	Flow rate (µl/min)
11 minutes	30.3 minutes	1.85
33 minutes	55.5 minutes	1.25
44 minutes	66.5 minutes	1.25

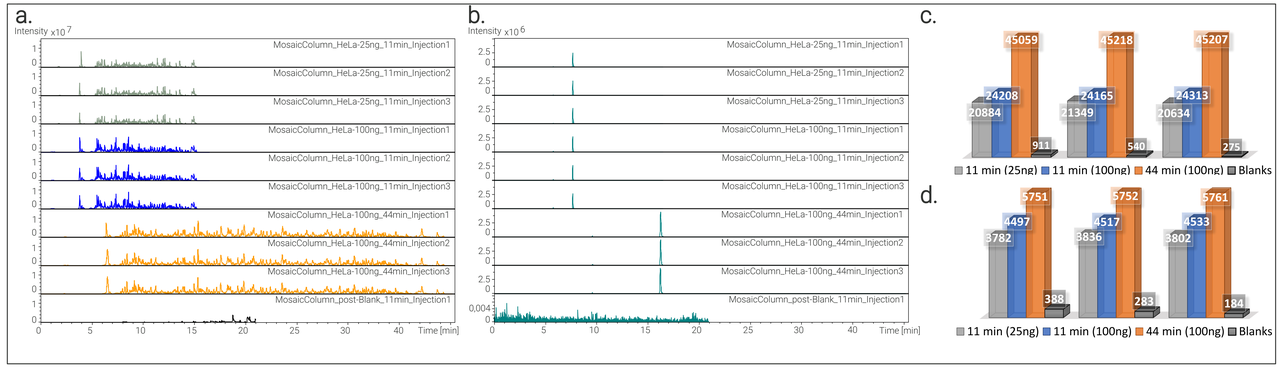

Fig. 3 Quantitative assessment of HeLa peptides and protein groups in dda-PASEF mode. a) Base peak chromatograms extracted from 25 and 100ng of HeLa standard using 11- and 44-min gradient times, respectively. b) Extracted ion chromatograms from precursor ion m/z 599.7647 +/- 0.005. c) Total number of identified peptides and d) protein groups using FragPipe v21.1 from 25 and 100ng of HeLa standard using 11- and 44-min gradient times, respectively.

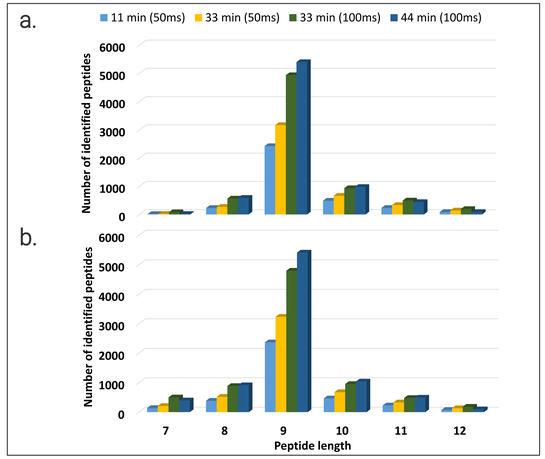

Fig. 4 Quantitative assessment of the peptide distribution (7-12 amino acids) identified using a) FragPipe v21.1 and b) PEAKS 11 online software. A number of 1e8 cells were processed using the immunopeptidomics workflow describing in Fig 2 and an estimated amount of 1e7 cells was injected onto the Mosaic column.

Results

- Three separation methods created for the Mosaic column (Table 1).
- Automated immunopeptidomics workflow to enrich HLA-associated peptides (Fig. 2).
- Benchmarking the LC/MS platform using HeLa standard (Fig. 3)
- Evaluate the Mosaic column to specifically identify HLA Class I peptides (Fig. 4).

Summary

- Possibility to design fast gradient time using the 200 cm Mosaic column.
- 25 ng of HeLa demonstrates ~ 21k peptides and ~ 3800 protein groups in dda-pasef mode.
- Only 2 % peptide carryover on the first blank after injecting 100ng HeLa on a 44-minutes gradient.
- High proportion of 9mer HLA Class I peptides enriched from IM9 cells.
- Increasing the TIMS ramp from 50 to 100 ms allows for a 2-fold increase in HLA Class I peptide identification.
- Up to 5000 9mer peptides identified using the 44-minutes gradient.

Conclusion

- Short gradients without compromising peptide identification and quantification.
- Mosaic column significantly reduces peptide carryover.
- Further experiments to evaluate different lengths and inner diameter to maximize sensitivity.
- Proper comparison with conventional C18 columns.

nanoElute 2 – timsTOF Ultra (immunopeptidomics)

Innovation with Integrity