Exploring the power of the timsTOF Ultra to investigate the HLA immunopeptidome with high sensitivity and accuracy, allowing for smaller sample sizes while maintaining a robust immunopeptidome

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Introduction

Immunopeptidomics is a rapidly growing field of proteomics that plays a vital role in identifying and quantifying immunopeptides presented by major histocompatibility complex (MHC) molecules on the cell surface to the immune system.

The timsTOF Ultra combines trapped ion mobility with modified source design enabling increase in ion current providing very high sensitivity and increased dynamic range.

By leveraging the new optimized workflow to identify HLA Class I peptides (immunopeptidome) from cell extracts, the ability to maintain high sensitivity and accuracy allows for smaller sample sizes (biopsy) while maintaining a robust immunopeptidome.

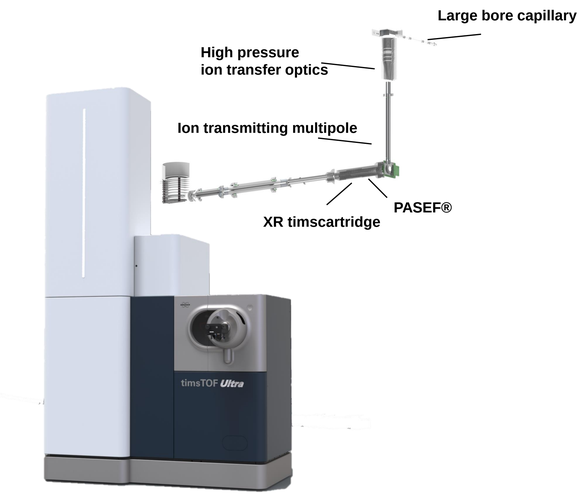


Figure 1: timsTOF Ultra features new ion source geometry with additional higher pressure vacuum stage

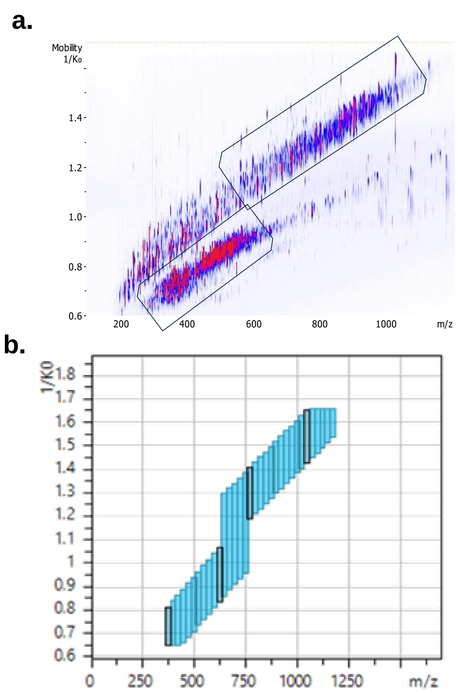


Figure 3: dda- and dia-pasef acquisition modes. a) the dda-pasef mobility ranged from 0.6 to 1.7 with a 100ms PASEF ramp time. Two polygons were designed to specifically select the singly and doubly charged precursors for fragmentation The total cycle time was 1.18 seconds.
b) dia-pasef window placement scheme. The ramp time was set to 70 ms with a total cycle time of 0.92 second.

Materials & Methods

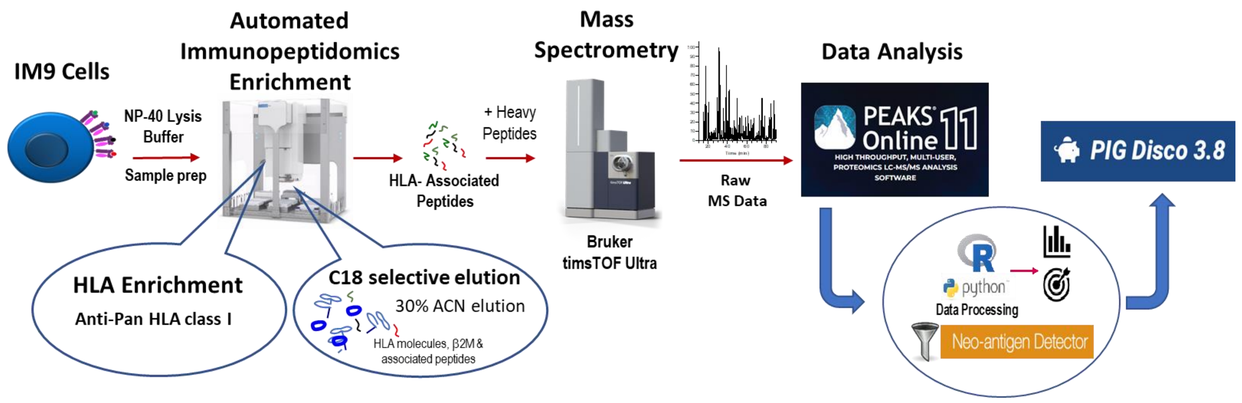


Figure 2: Immunopeptidomics workflow. Different numbers of IM9 cells (B lymphocyte cell line) ranging from 2e4 to 1e7 cells. HLA enrichment following by selective elution of the HLA-associated peptides were performed using a liquid handling robot. A custom list of heavy isotope labeled peptides were spiked into the samples prior to LC/MS analysis. The dda-pasef data files were then searched using PEAKS 11 software whereas the dia-pasef files were searched using DIA-NN and Spectronaut. Binding prediction of the HLA-associated peptides was realized using PIG Dicso 3.8

Results

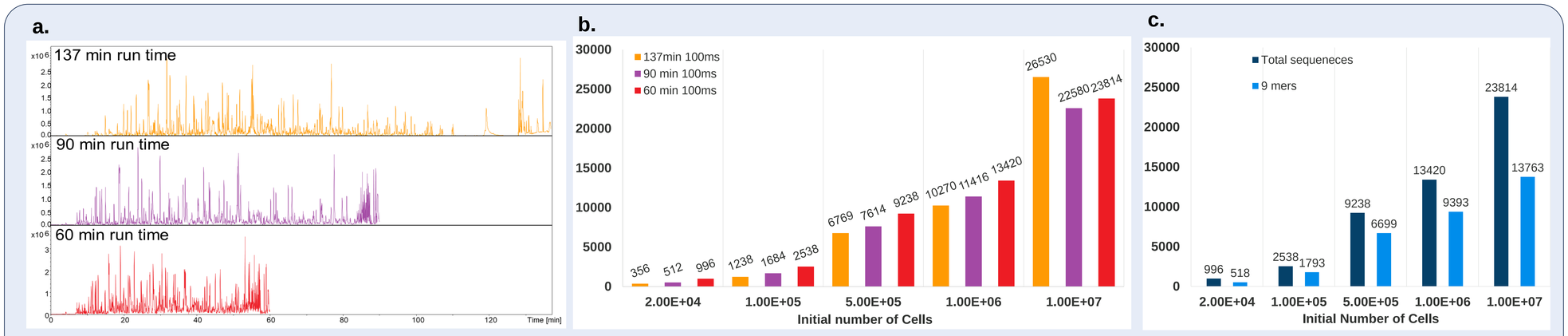


Figure 4: Quantitative assessment of HLA-associated peptides in dda-PASEF mode. a) Base peak chromatograms extracted from 1e6 IM9 cells using 137-, 90-, and 60-minutes run times, respectively. b) Total number of identified peptides using PEAKS 11 using different amounts of IM9 cells before the sample preparation using different run times. c) Comparison of the total number of identified peptides vs. the number of 9 amino acids peptide sequences at the different amounts of IM9 cells using the 137-run time separation method.

a.

Peptide Binding predictions for IM9 1.00E6 137min gradient					
Allele	**Total Binders**	**Strong Binders**	**Weak Binders**	**9mers**	**Percent Binders**
HLA-A02:01	3715	2805	910	7807	48
HLA-A02:05	4508	3610	898	7807	58
HLA-B49:01	2947	2479	468	7807	38
HLA-B56:01	1329	900	429	7807	17
HLA-C01:03	3106	1619	1487	7807	40
HLA-C07:01	2218	568	1650	7807	28

Peptide Binding predictions for IM9 1.00E6 90min gradient					
Allele	**Total Binders**	**Strong Binders**	**Weak Binders**	**9mers**	**Percent Binders**
HLA-A02:01	3846	2892	954	8232	47
HLA-A02:05	4667	3718	949	8232	57
HLA-B49:01	3116	2619	497	8232	38
HLA-B56:01	1429	1015	414	8232	17
HLA-C01:03	3177	1664	1513	8232	39
HLA-C07:01	2265	545	1720	8232	28

Peptide Binding predictions for IM9 1.00E6 60min gradient					
Allele	**Total Binders**	**Strong Binders**	**Weak Binders**	**9mers**	**Percent Binders**
HLA-A02:01	3938	2921	1017	8561	46
HLA-A02:05	4853	3792	1061	8561	57
HLA-B49:01	3202	2683	519	8561	37
HLA-B56:01	1519	1074	445	8561	18
HLA-C01:03	3295	1714	1581	8561	38
HLA-C07:01	2291	555	1736	8561	27

b.

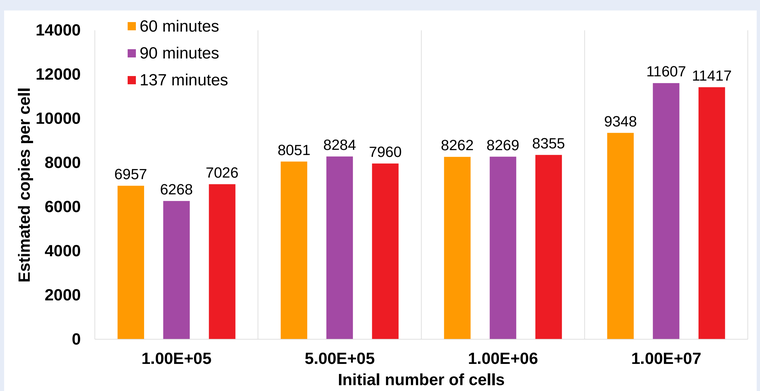
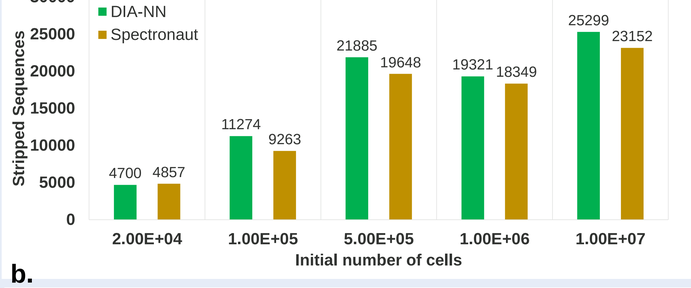


Figure 5: a) Peptide binding predictions for IM9 cells. b) Estimated number of HLA-associated peptides copies per cell.

a.



b.

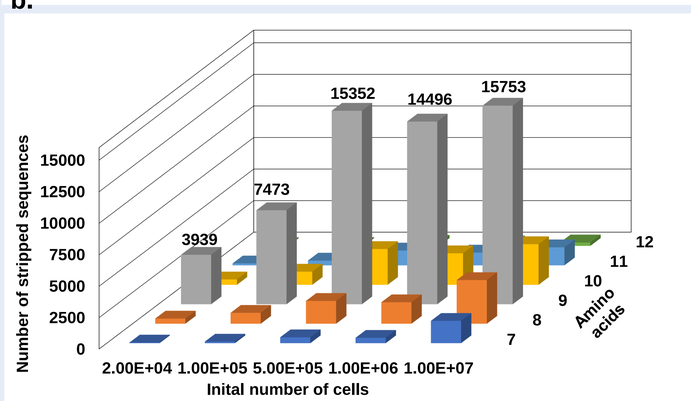


Figure 6: Power of dia-pasef analysis for ultrasensitive high throughput immunopeptidomics.
a) Total number of identified peptides using DIA-NN and Spectronaut using different amounts of IM9 cells before the sample preparation. The gradient time waste to 30 minutes.
b) Quantitative assessment of the peptide distribution (7-12 amino acids) associated with the initial number of cells. The dia-pasef data files were searched using DIA-NN and a spectral library was generated using previous dda-pasef data files.

Conclusions

- ✓ Ultrasensitive identification of more than 9,000 peptides from 5e5 IM9 cells using the 60 min gradient method.
- ✓ 72 % of them are HLA-associated peptides.
- ✓ Peptide binding predictions are strong for most of the HLA alleles.
- ✓ Significant number of HLA-associated peptides copies per cell.
- ✓ dia-pasef technology demonstrates unprecedent sensitivity by doubling the number of identified peptides for the same number of cells while decreasing by half the run time (**up to 21k peptides using dia-pasef vs. 9k peptides using dda-pasef @5e5 IM9 cells**).