Advantages of a Dynamic Polygon for MHC Class I and II Immunopeptides

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Introduction

MHC-associated peptides modulate T cell immunity and play a critical role in generating effective anti-tumor immune responses[1]. Characterization of these peptides helps to generate therapeutic treatments and gain information on T cell mediated biomarkers. These peptides are challenging to characterize due to similar length, sequence conservation and lacking a defined termini when compared to peptides generated upon enzymatic digestion. To overcome these challenges, use of PASEF (Parallel Accumulation and Serial Fragmentation) enables to generate high quality peptide spectra and resolve coeluting and isobaric peptides. Moreover, the capability to easily tailor the mobility space enables preferential detection of groups and sub-groups of relevant peptides.

Methods

MHC class I peptides were separated on a 90 minutes gradient by nanoElute UPLC (Bruker Daltonics) on a 25 cm pulled emitter column (IonOpticks) and analyzed on a high resolution TIMS enabled QTOF instrument using the PASEF method (timsTOF Pro, Bruker Daltonics). An amount equal to 200 ng of estimated peptides were injected on column. For MHC class II peptides separation was performed on an EvoSep system equipped with an 8 cm performace column (a 60 samples per day method – 21 min gradient) and analyzed on the same instrument as previously described. An amount equal to 25 ng of estimated peptides was injected on the system. Data analysis was performed with PMI Byonic.

References

1) Lill et al.; Proteomics 2017, Volume 17, Issue 1-2: 1770010

MHC class I peptides results

(a)

MHC class I (n=3)		
PSMs (± %CV)	Unique Peptides (± %CV)	Proteins Groups (± %CV)
33825 (4.2)	15315 (4.8)	3762 (3.5)

(b)

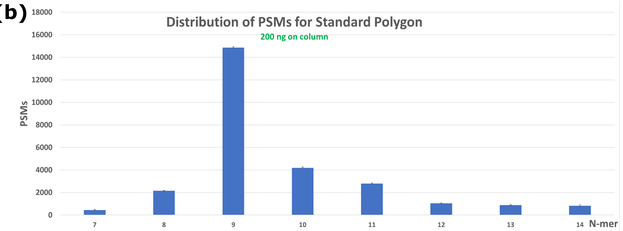


Fig. 1. (a) The sequencing speed of the timsTOF Pro and PASEF enable detection of approx. 16,000 peptides and 4,000 protein groups (PG). (b) N-mer PSMs distribution, matching expectations from literature

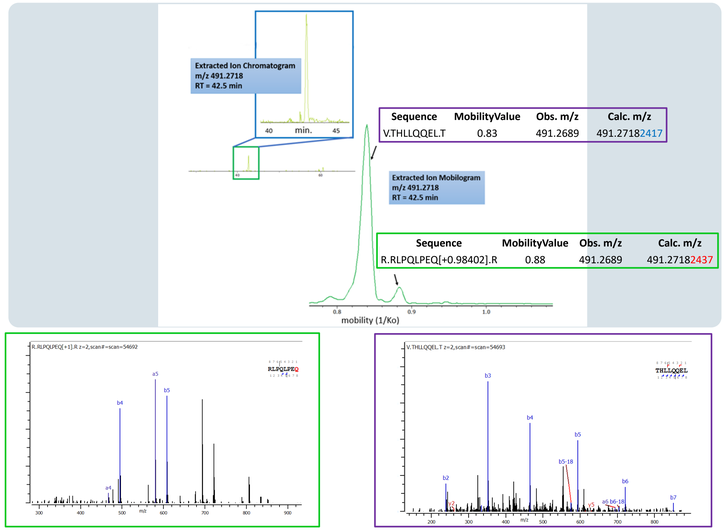


Fig. 2. Coeluting isobaric peptides at 42.56 min, are separated in the ion mobility dimension

MHC class II peptides results

60 SPD method – (amount injected on column 25 ng)						
	Polygon 200-1400 m/z		Polygon 250-950 m/z		Polygon 400-1300 m/z	
	Peptides	Proteins Groups	Peptides	Protein Groups	Peptides	Protein Groups
Rep_1	1902	457	2196	514	1704	433
Rep_2	1986	467	2559	558	1090	313
Rep_3	1431	379	2280	528	1925	464

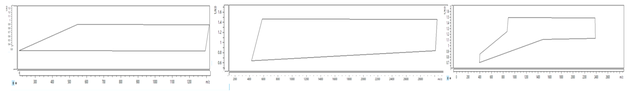
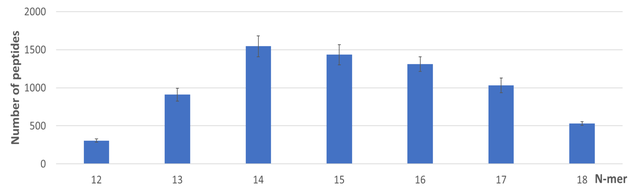


Fig. 3. Different polygon shapes and mass ranges used to maximize detection of peptides and PG; middle polygon generates best results

Graph and Table of major MHC II N-mer distribution



N-mer	Rep_1	Rep_2	Rep_3	Average	StDEV	%CV
12	307	325	284	305.33	20.55	6.73
13	849	1003	875	909.00	82.44	9.07
14	1443	1702	1493	1546.00	137.39	8.89
15	1317	1580	1404	1433.67	133.99	9.35
16	1231	1419	1279	1309.67	97.68	7.46
17	997	1138	955	1030.00	95.86	9.31
18	505	547	541	531.00	22.72	4.28

Fig. 4. N-mer distribution for middle polygon and mass range from figure 3 show N-mer varying from 14 to 16 are the most abundant species in the samples

Results

Analysis of MHC class I samples (figure 1) shows detection of a high number of peptides, with the unsurpassed sequencing speed and sensitivity of the timsTOFPro

Co-eluting peptides that are isobaric or have overlapping precursor ion isotope envelopes are resolved using Trapped Ion Mobility Spectrometry resulting in clean MS/MS spectra (figure 2)

Analysis of MHC class II samples with different settings (figure 3) shows dynamic parameters to maximize detection of peptides and PGs

Conclusions

- More than 16000 peptides were confidently identified in a 90 min gradient, with high repeatability for MHC class I peptides.
- PASEF on the timsTOF Pro with EvoSep separation identified more than 500 protein groups from a 21 min gradient separation, with just 25 ng on column (MHC class II peptides)
- Extra dimension of separation provided by TIMS allowed resolution of co-eluting, isobaric peptides

timsTOF Pro