

New prm-PASEF® for highly multiplexed targeted acquisition in clinical samples.

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Introduction

We developed a prm-PASEF targeted acquisition method that fully exploits the multiplexing capability of the TIMS-TOF Pro, allowing multiple peptides to be sequentially measured from a single ion mobility scan with no sensitivity loss. We evaluated the performance of this prm-PASEF method using AQUA peptides spiked in a HeLa cell lysate sample. The testing of this method on clinical research samples has been delayed due to the COVID-19 situation.

Methods

Samples were separated by nano-HPLC (nanoElute, Bruker Daltonics) on 250 mm pulled emitter columns (IonOpticks, Australia) with a 30 min gradient. Peptides were analyzed on a timsTOF Pro instrument (Bruker Daltonics) operated in prm-PASEF acquisition mode (prototype). Data were processed with Skyline-daily.

HeLa human cell line digest (100ng/μl)
+201 AQUA heavy peptides
+14 light synthetic peptides

Quantification performance

14 heavy/light aqua peptides pairs

- Blank water
- Blank matrix
- 5.5 amol/μl
- 17.2 amol/μl
- 53.7 amol/μl
- 167.8 amol/μl
- 524.3 amol/μl
- 1638.4 amol/μl
- 5120 amol/μl
- 16000 amol/μl
- 50000 amol/μl

Multiplexing performance

187 AQUA peptides at a constant concentration

A total of 215 precursors monitored during the 30 min LC separation

Results

Fig. 1: prm-PASEF, a new highly parallelized acquisition method

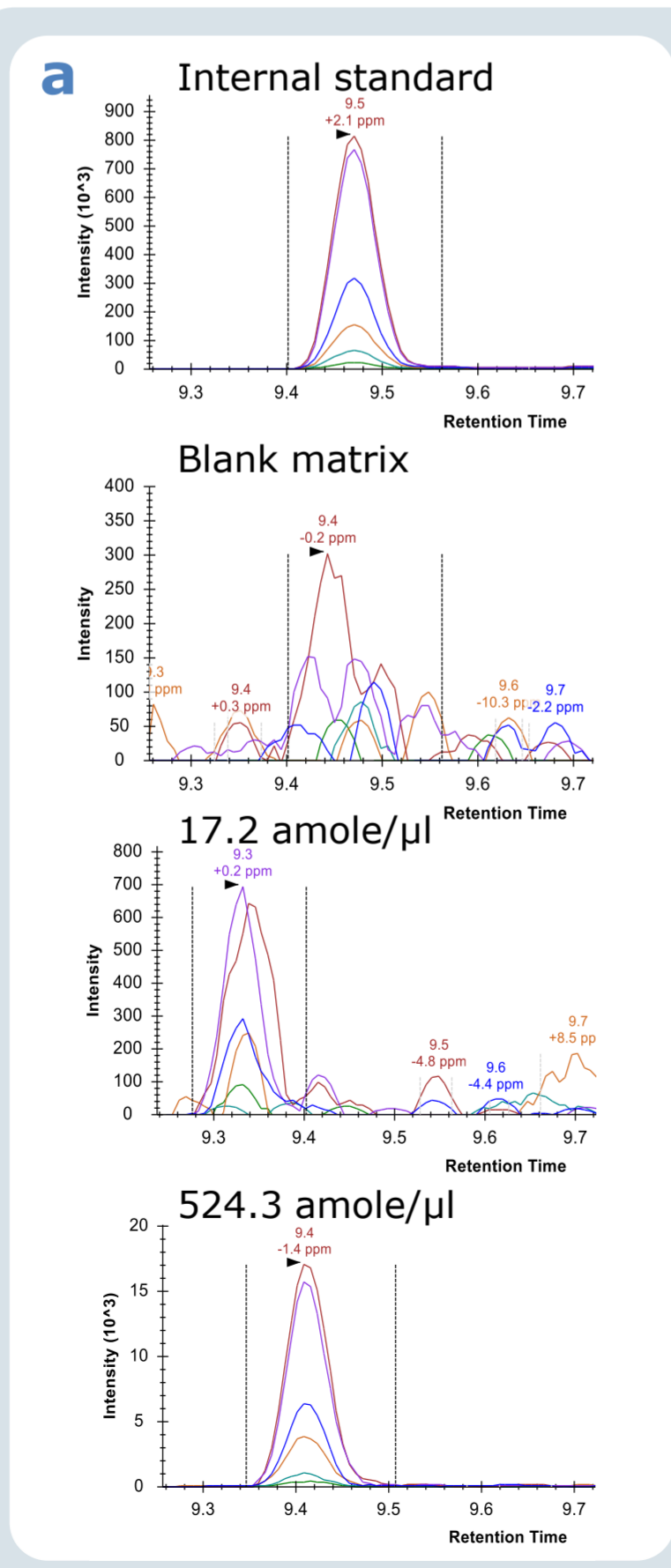
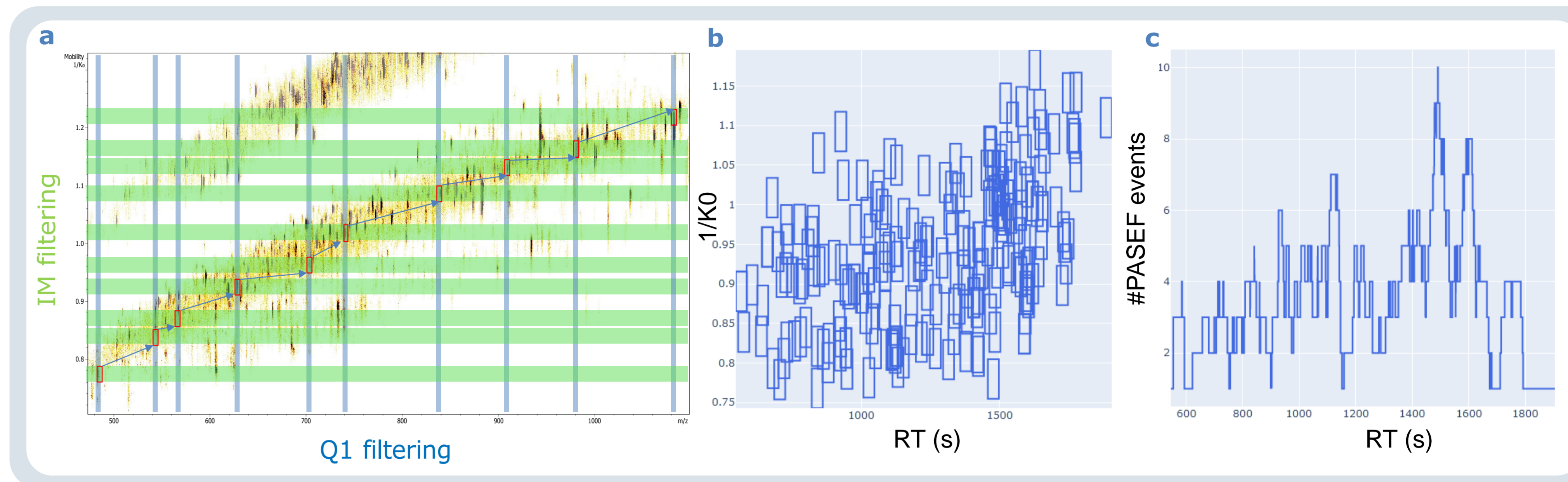
a) Selection of the targeted peptide precursor ions during a single PRM-PASEF event.

Up to 11 precursors can be selected within a 100ms IMS scan and consecutively fragmented in the collision cell.

High selectivity in the precursor's selection is obtained by combining the ion mobility (green bars) with the quadrupole isolation windows (blue bars)

b) Representation of PASEF-PRM isolation windows in the ion mobility (1/K0) and chromatography retention time (s) dimensions

c) Visualization of the MS cycle time across the chromatography separation (1 frames = 100ms)



amole	Calibration curves				Quality controls	
	replicate #1	replicate #2	replicate #3	RSD (%)	replicate #1	replicate #2
ATVVYQGER standard						
17.2	94%	92%	96%	2.0%	81%	81%
53.7	113%	119%	107%	5.2%	118%	104%
167.8	114%	113%	114%	0.6%	115%	114%
524.3	112%	111%	110%	1.1%	114%	112%
1638.4	101%	102%	103%	0.9%	104%	105%
5120	94%	93%	94%	0.9%	95%	96%
16000	89%	89%	94%	3.3%	91%	96%
50000	83%	82%	83%	0.9%	84%	85%

Fig. 2: Quantification performance

a) Representative prm-PASEF traces of the peptide ATVVYQGER. Peak area ratios (heavy/light) were calculated to normalize the MS signal.

b) Three replicates of the calibration curve were acquired, the accuracy of each calculated concentration level and the relative standard deviation were calculated.

As a **quality control**, the concentration of two curves were back-calculated with the linear regression of the first curve (green tab). Limits of quantification (LOQ) were defined as lowest concentration point within 80% < accuracy < 120% associated to a signal higher than the mean(blanks) + 3 × SD(blanks)

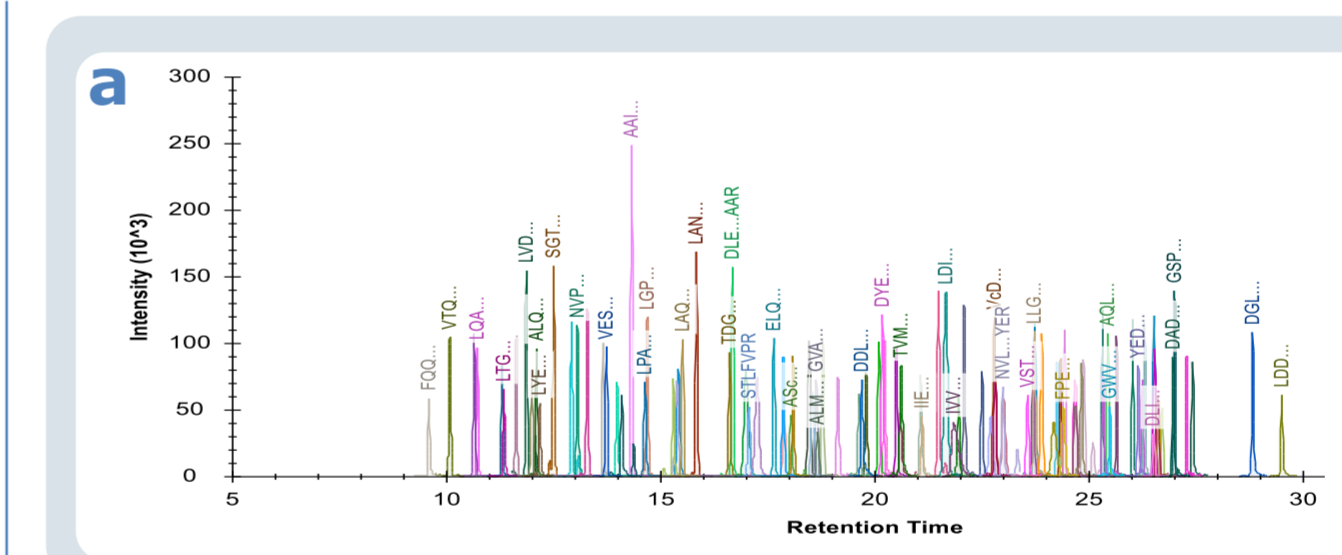


Fig. 3: Multiplexing performance

a) Representative prm-PASEF traces of the peptide ATVVYQGER. Peak area ratios (heavy/light) were calculated to normalize the MS signal.

b) RSD(%) of the peak areas of all peptides monitored in 30 prm-PASEF runs (label free data).

c) Number of data points across the chromatographic peaks.

Conclusions

- prm-PASEF takes advantage of the trapped ion mobility technology for the targeted proteomics analysis.
- The sensitivity and selectivity of the acquisition method is improved by the ion mobility filtering and time focusing effect that happens during a PASEF acquisition.
- The high multiplexing capacity of the prm-PASEF maximizes the number of peptides quantified in a single analysis while keeping an excellent chromatographic peak profiling.
- prm-PASEF delivers accurate quantitation for a large number of targets over sharp chromatographic peaks and is compatible with high-throughput chromatography for the screening of large clinical sample collections.