Evaluation of prmPASEF for multiplexed targeted proteomics.

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Introduction

prm-PASEF is a new targeted acquisition method that fully exploits the multiplexing capability and the high resolution of the TIMS-TOF mass spectrometer. Multiple peptides can be sequentially measured from a single ion mobility scan without compromising the sensitivity. We evaluated parameters including sensitivity, reproducibility, accuracy and dynamic range using AQUA peptides spiked in a Hela cell lysate. Finally, we applied the method to quantify the mutations and isoforms of the Ras oncoproteins family in cancer cell lines.

Methods

Samples were prepared as described in Fig.1 and 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. The target's retention time and Ion mobility values have been previously determined with a standard PASEF acquisition performed on the 16 amol/µl concentration point. Data were processed with Data Analysis™, PeakXTM and Skyline-daily™. Skyline daily was used for Collision Cross Section aware quantitation operations (Fig.2).

Results

In a prm-PASEF acquisition, several peptides ions can be targeted from a single (100ms) ion mobility separation (PASEF) event. As these ions are separated in the ion mobility dimension prior to fragmentation ,It enables high-multiplexed targeted acquisition without compromising the quantification performance and number of data points per LC peaks .Considering our evaluation sample, the time windows for addressing the 216 targeted peptides where heavily overlapping (Fig.3D). The ion mobility separation allowed to drastically reduce this complexity (Fig.3A). When several coeluting targets have overlapping isolation windows are overlapping, they are distributed in distinct prm-PASEF events, leaving the sensitivity untouched. In this case, only the number of points/chromatographic peaks is slightly reduced. In our experiment, an average of 4 targets were simultaneously acquired in each prm-PASEF event (Fig.3B) and the number of prm-PASEF events to acquire all coeluting targets did never exceed 10 (Fig.3C), resulting in optimal peak sampling and reproducibility (Fig.3E&F).The use of a 50ms accumulation time was sufficient to obtain a good linearity and LOQ, while the use of an internal standard allowed to further improve those values (Fig.4 A,B&C). The results obtained in the Hela cell digest background could be reproduced while using a pooled depleted serum digest background (Fig.4 D&E).

Once the analytical capabilities of prm-PASEF have been established, we used the approach to quantify disease-relevant RAS mutation in cancer cell lines, using a short 10 min gradient. The approach allowed to quantify those variants successfully using a very short injection to injection method (Fig.5). Note that we had not been successful in generating such results with untargeted approaches.

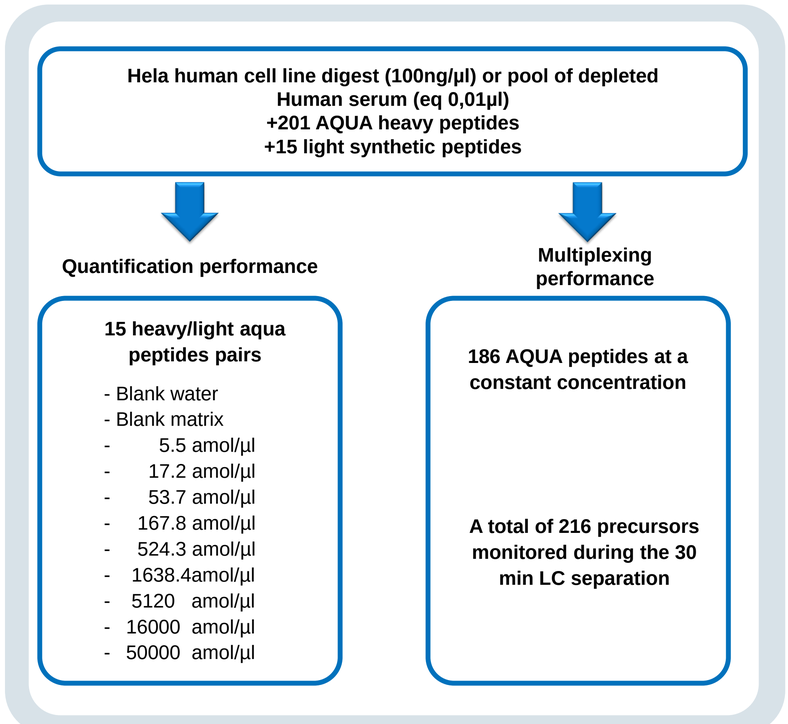


Fig. 1: Experimental setup.
The samples have been acquired in triplicate with the prm-PASEF acquisition mode.

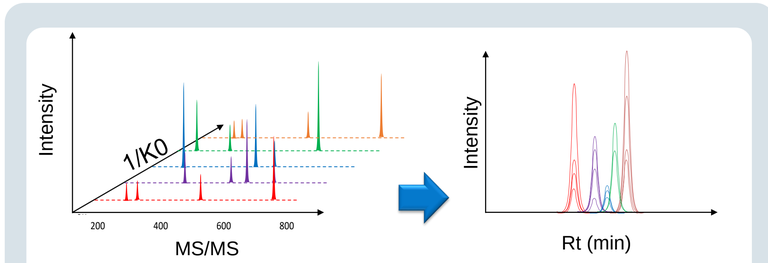
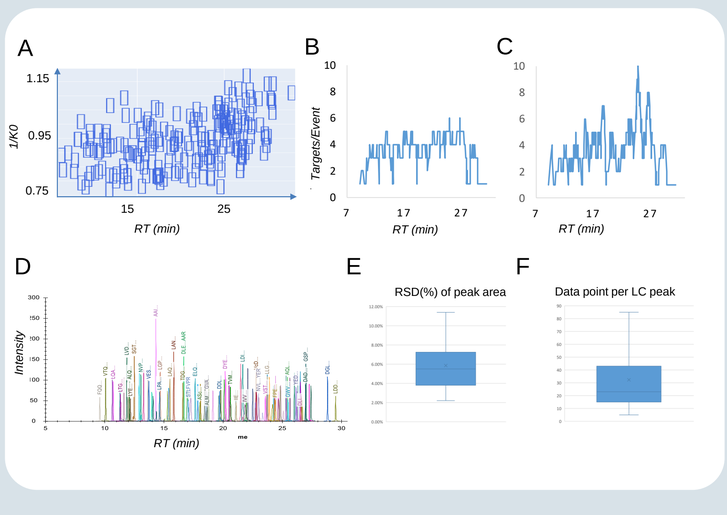
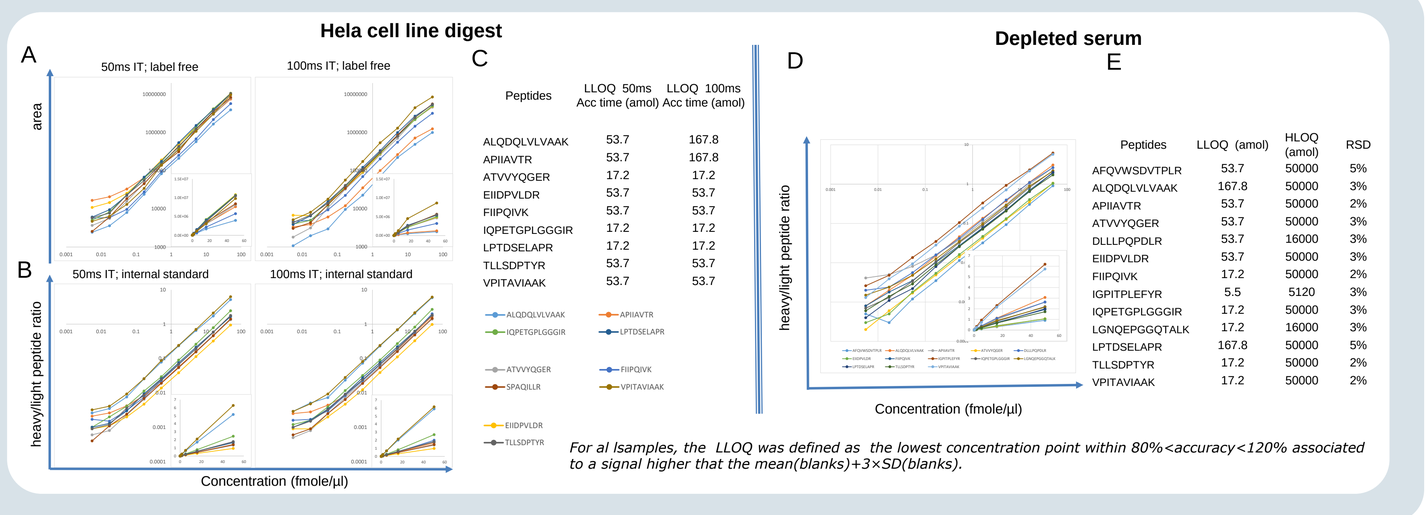


Fig. 2: prm-PASEF data extraction.
Extracted ion chromatograms (XICs) are generated from the signal of fragments ions. The quantification is based on the area under the peak

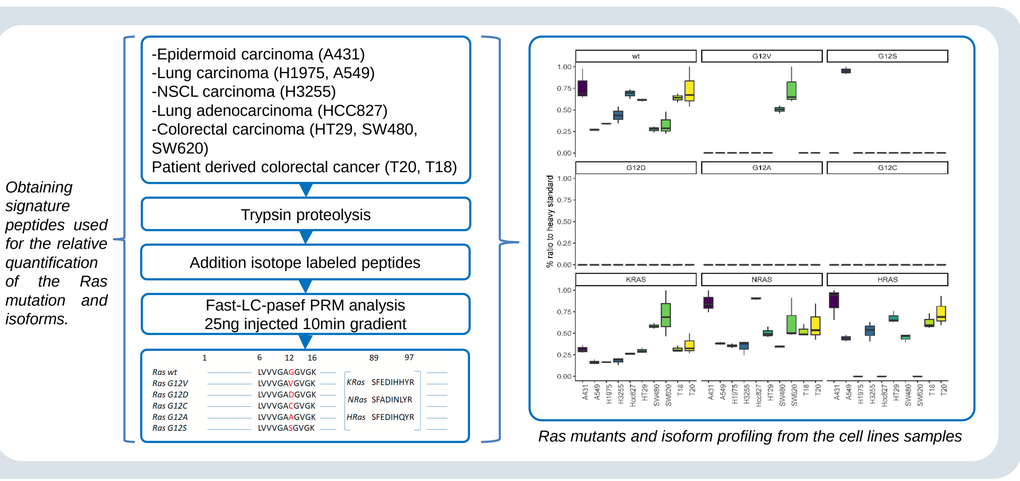




For al lsamples, the LLOQ was defined as the lowest concentration point within 80%<accuracy<120% associated to a signal higher that the mean(blanks)+3×SD(blanks).

Fig. 3: prm-PASEF parallelisation:
A) Representation of PASEF-PRM isolation boxes in the ion mobility (1/K0) and chromatography retention time (min) dimensions.
B) Visualization of the number of targets per PASEF event across the chromatography separation.
C) Visualization of the PASEF event per MS cycle across the chromatography separation.(1 PASEF event = 100ms)
D)Representative prm-PASEF traces of the targeted 216 precursors in all LC-(prm-PASEF) runs
E) RSD(%) of the peak areas of all peptides monitored in 30 prm-PASEF runs (label free data for the serum samples)
F) Number of data points across the chromatographic peaks (serum samples)

Fig. 4: prm-paSEF quantitation results
A) Label-free quantification results on the Hela cell samples with variable tims accumulation time
B) Internal Standard quantification results on the Hela cell samples with variable tims accumulation time
C) Lower Limits of Quantitation for the Hela Cell Digest samples
D) Internal Standard quantification results on the depleted serum samples (50ms tims accumulation time)
E) Lower Limits of Quantitation for the depleted serum samples



Ras mutants and isoform profiling from the cell lines samples

Fig. 5: Screening RAS mutants in cancer cell lines
Quantification of the Three RAS isoforms, including disease relevant variants, in total cell extracts. prm-PASEF was run with a with a 10 min gradient.

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 massive parallelization capacity of the prm-PASEF approach allow to increase the number of targeted compounds while leaving sensitivity untouched

timsTOF PRO