Maximizing sensitivity and number of targets: evaluation of prm-PASEF with live retention time correction on a single cell-capable ESI-TIMS-Q-TOF platform.

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introduction

New acquisition strategies based on the use of Parallel Accumulation Serial Fragmentation (PASEF®) or dia-PASEF® approaches have improved the sensitivity and data completeness performance of untargeted proteomics strategies. Despite this development, targeted proteomics approaches are still reference methodologies for the verification of biomarker candidates in large sample cohorts. Targeted approaches increase the sensitivity and also alleviate the problem of quantitative missing values between samples. prm-PASEF® has therefore been developed to translate the advantages of the parallel accumulation serial fragmentation (PASEF) acquisition method to the targeted proteomics field. In comparison with standard SRM or PRM methods, the prm-PASEF method increases the number of peptides that can be targeted in a single acquisition LC gradient of a given length, without compromising the selectivity or the sensitivity. In this communication, we evaluate the potential of this method when used in combination with an instrument designed for ultimate sensitivity

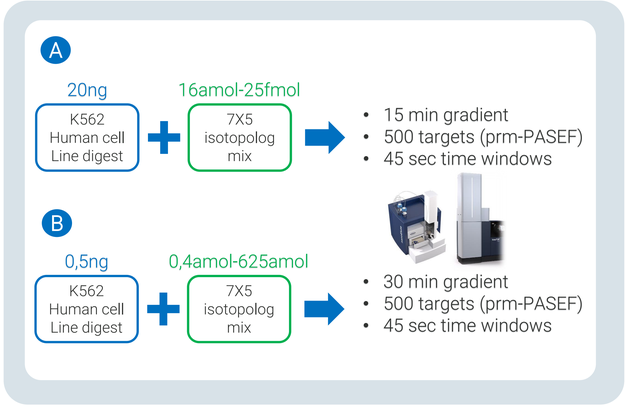


Fig. 1: Experimental setup.
The samples have been acquired in triplicate with both prm-PASEF and dia-PASEF acquisition modes.

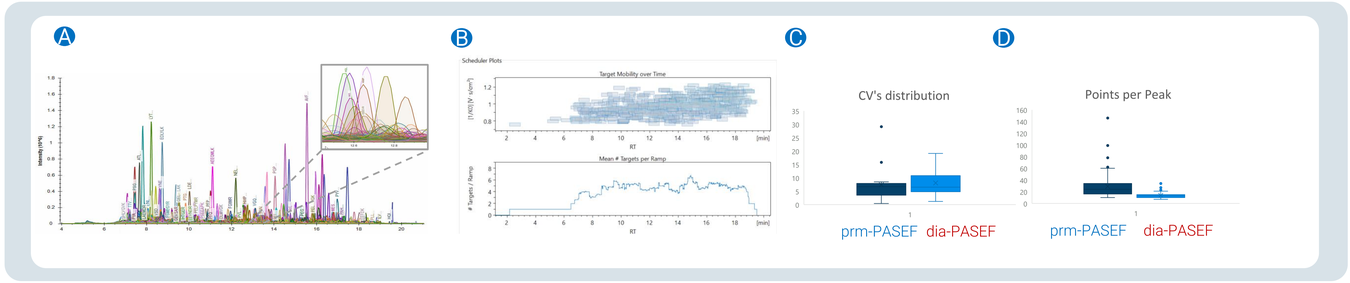


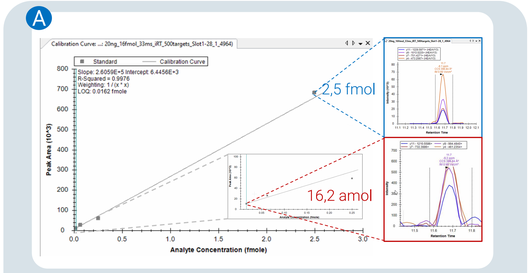
Fig. 2 : high target density acquisitions with prm-PASEF and dia-PASEF
A)- Extracted Ion Chromatograms the 500 targets traces on a 15 min prm-PASEF acquisition of sample A. **B)-** Corresponding retention time/mobility isolation boxes display (upper graph) together with the number of targets addressed in each prm-PASEF cycle (50 ms, lower graph). **C)-** CV distributions for all targets meeting the integration criteria in prm-PASEF (blue) and dia-PASEF (red). **D)-** number of data points / chomatographic peaks for all targets meeting the integration criteria.

Methods

Commercially available K562 cell line digest (Promega) was mixed with a mixture of isotopolog peptides (Pierce™ LC-MS/MS System Suitability Standard) containing 5 isotopologs for each of the 7 peptides in the mixture. Two solutions were prepared to cover a concentration range of 25 fmol to 16 amol isotopolog in a 20ng K562 digest background (Sample A) or a concentration range of 625 amol to 0,4 amol isotopolog in a 0,5ng K562 digest background (sample B).
Samples were injected in triplicates on a 25cm X 75 µm pulled emitter column (IonOptics) and separated using a 15 min (sample A) or a 30 min gradient (sample B) generated by a nanoElute nano-HPLC system (Bruker) coupled to a newly designed ultra-high sensitivity timsTOF SCP (Bruker) operated in dia-PASEF or prm-PASEF mode. Data were processed using Skyline daily. (Fig. 1)

Results

The prm-PASEF method was set up to target either the 35 isotopologs alone or in combination with 465 endogenous K562 peptides. The co-isolation of the 465 endogenous peptides (Fig.2A & 2B) did not influence the intensity measured for the 35 isotopologs (data not shown). In these conditions, more than 96% of the targeted peptides had a CV<20%, with a median value of 5,1% (Fig.2C). Despite the high number of targets the peaks were defined with 24 points on average in prm-PASEF and 13 points on average in dia-PASEF with no peaks being defined with less than 13 (prm-PASEF) or 7 (dia-PASEF) points (Fig.2D). Depending on the peptides, the Lower Limit of Quantitation (LLOQ) observed in prm-PASEF were between 16,2 and 250 amol for sample A



B

7X5 Sequence	20ng_16-25_prm LLOQ amol	HLOQ fmol	R2	% Accuracy @ LOQ
-.GISNEGQNASIK.I [1, 12]	16,2	2.5	0.93	87
K.IGDYAGIK.T [13, 20]	62	25	0.98	97
K.TASEFDSAIAQDK.E [21, 33]	16.2	2.5	0.97	93
K.ELGQSGVDTYLQTK.S [34, 47]	62	25	0.98	87
K.SFANQPLEVVYSK.L [48, 60]	62	25	0.95	98
K.LTILEELR.E [61, 68]	62	2.5	0.99	96
R.ELASGLSFPVGFK.- [69, 81]	62	0.25	0.99	100

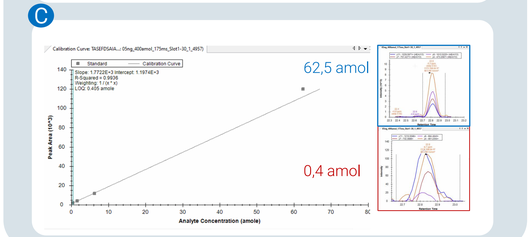


Fig. 3: prm-PASEF quantitation
A) – quantitation curve obtained for the TASEFDSAIAQDK peptide in a 15 min/500 targets prm-PASEF analysis of sample A. **B) -** Corresponding results table for all isotopologs in the sample. **C) -** quantitation curve obtained for the SFANQPLEVVYSK peptide from a 30 min prm-PASEF analysis of sample B.

and between 0,4 amol and 6,25 fmol for sample B (Fig.3). The accuracy measured at the lower limit of quantitation was higher than 90%, and LLOQ observed for the dia-PASEF analyses were slightly higher.

Conclusion

- The timsTOF SCP operated in prm-PASEF mode delivers a sub-attomole sensitivity from reduced sample load, even with a high number of targets
- The massive parallelization capacity of the prm-PASEF approach enables an increase in the number of targeted compounds without affecting sensitivity or selectivity.
- The high number of data points per peak should allow the use of real-time retention time correction, enabling work with even shorter gradients

timsTOF SCP