PASEF on a TIMS-QTOF for reproducible, sensitive and high-throughput shotgun proteomics

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Introduction

In (DDA) data dependent acquisition experiments only 20% of eluting peptide features are targeted by current mass spectrometers. This is a result of limitations in sequencing speed, sensitivity and resolution. The previously introduced “Parallel Accumulation Serial Fragmentation” method (PASEF, Meier et al., JPR 2015) on a trapped ion mobility spectrometry quadrupole time of flight mass spectrometer has been shown to increase the sequencing speed and sensitivity of MS/MS scans at high resolution. Here, we demonstrate instrument performance on low sample amounts (< 200 ng), offering unprecedented possibilities to investigate samples at maximum sensitivity, throughput and reproducibility.

Methods

The nanoElute HPLC (Bruker Daltonics) was on-line coupled to a high-resolution TIMS-QTOF mass spectrometer (timsTOF Pro, Bruker Daltonics) with a CaptiveSpray ion source (Bruker Daltonics). The peptide mixture (< 200 ng) were loaded onto a 250 mm pulled emitter column (IonOpticks, Australia).

Chromatographic separation was carried out using a linear gradient of 5-30% buffer B (100% ACN and 0.1% FA) at a flow rate of 400 nl/min over 30-90 min. For all analyses we applied the PASEF method with a cycle time of 1.1 s including 1 TIMS MS scan (100 ms) and 10 PASEF MS/MS scans (100 ms each, > 120 MS/MS) (Figure 1). Data analysis was performed using Mascot 2.5.1 (MatrixScience), PEAKS (Bioinformatics solutions Inc.) or MaxQuant (Jürgen Cox, Max Planck Institute of Biochemistry) which were optimized for the analysis of PASEF data.

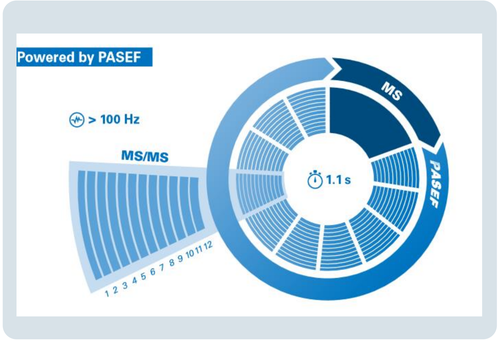

Fig. 1 The PASEF acquisition method: A PASEF cycle (total: 1.1 s) consists of 1 TIMS MS scan (100 ms) and 10 PASEF MS/MS scans (100 ms each) for shotgun proteomics experiments. For each PASEF scan on average 12 different precursors are selected for MS/MS resulting in a sequencing speed of > 120 Hz without a loss in resolution (1222 @ 50,000).

Results

To investigate the speed and sensitivity of the PASEF method for shotgun proteomics, we first analyzed a complex peptide mixture derived from a mammalian cell line. We separated 100 ng of peptide digest via HPLC with gradient lengths of 30, 60 and 90 min and performed triplicate analysis (Figure 2). We could identify ~ 4000 proteins using a 30 min gradient and ~ 5300 proteins with a 90 min gradient.

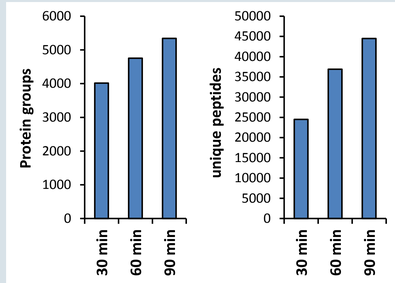

Fig. 2 High proteome coverage with low sample amounts (100 ng): Number of peptide and protein identifications using a 30, 60 and 90 min gradient.

Using 200 ng and a 90 min gradient we could identify more than 6000 proteins in each run covering a dynamic range of 5 orders of magnitudes (Figure 3A). Comparing protein identities between this three analyses we observed an overlap of more than 90% of the identified proteins. This indicates very high reproducibility with the PASEF method (Figure 3B).

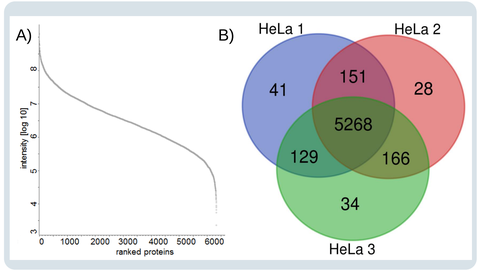

Fig. 3 High reproducibility over a wide dynamic range: A) Protein identifications of HeLa digest covering a dynamic range of 5 orders of magnitudes. B) Overlap of protein identifications of HeLa triplicate analysis using 200 ng sample amount and a 90 min gradient. Only proteins that were identified with at least 2 peptides are considered here.

Also, the reproducibility of the label-free intensities determined by MaxQuant is excellent with a $R^2 = 0.99$ (Figure 4A) for replicate analysis. To evaluate the accuracy of label-free quantification we spiked in Yeast and *E.coli* in two different concentrations (2:1 and 1:4) into the HeLa proteome and injected from each sample 150 ng on column. Good accuary in quantification could be achieved by applying PASEF. Both species could be nicely separated in the expected ratios of 2:1 and 1:4 (Figure 4B).

References

Meier F. et al., Journal of Proteomics Research 2015

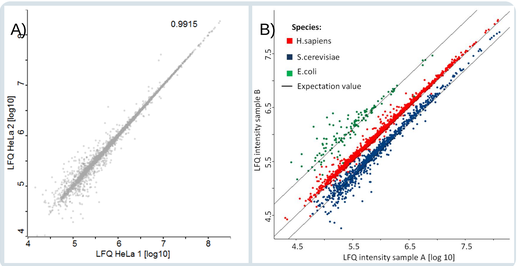

Fig. 4 Reproducible and accurate quantification: A) LFQ intensities of two HeLa replicates. B) Log-transformed ratios (sample A/sample B) of yeast, *E.coli* and Hela proteins plotted over LFQ intensities.

Conclusions

- High depth of proteome coverage with low sample amounts and short gradients
- High reproducibility in identification and label-free quantification
- Accurate label-free quantification
- PASEF is excellent for high-throughput deep shotgun proteomics analyses with limited sample amount

timsTOF Pro