Application of a library-free dia-PASEF approach for high throughput and high sensitivity proteomics

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Introduction

Data-independent acquisition (DIA) promises reproducible and accurate protein identification and quantification across large sample cohorts by using wide selection windows, rather than selecting individual peptides in DDA, to ensure that all precursor ions are fragmented in every sample. dia-PASEF takes advantage of the additional dimension of separation provided by trapped ion mobility (TIMS, Figure 1). The combination of DIA with PASEF allows to compensate for the traditional DIA pitfalls: by using a pattern of m/z isolation windows within consecutive TIMS events, the percentage of ions used can be greatly increased. The dia-PASEF cycle time can be reduced to make it compatible with short gradient separation while preserving a high selectivity. Here, we evaluate benefits of dia-PASEF for very short gradients enabling ultra-high sample throughput proteomics. Moreover, we demonstrate the performance of dia-PASEF on low sample loads down to 125 pg.

Methods

K562 tryptic digests (Promega) and inhouse prepared digests from HEK cell lines were used for benchmark measurements by coupling either a nanoElute (Bruker) with an Aurora-25 cm column (Ion Opticks) or EVOSEP One (EVOSEP) to a trapped ion mobility spectrometry – quadrupole time of flight mass spectrometer. timsTOF Pro 2 was used for short gradients and timsTOF SCP for low sample loads operating with optimized dia-PASEF schemes (Figure 2). Data was processed using library-free approaches with DIA-NN 1.8 (Demichev et.al, 2021) and Spectronaut 15 (Biognosys) using default settings.

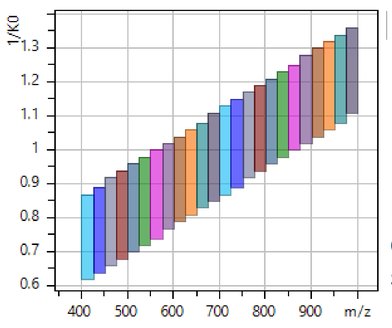

Fig. 2 dia-PASEF scheme

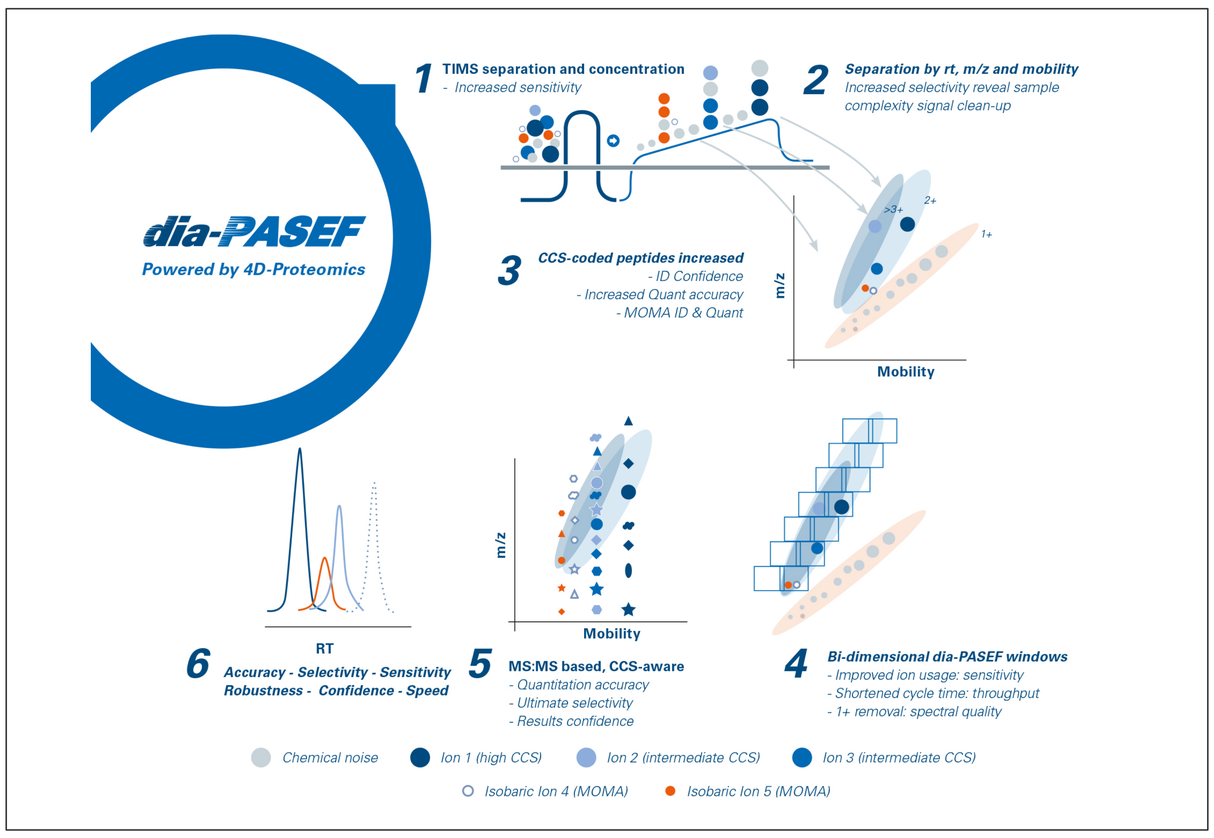

Fig. 1 dia-PASEF scheme of operation and advantages.

Results

We used a 300 samples per day method (SPD, 4.8 min run time) to evaluate the performance of dia-PASEF for high throughput proteomics. In classical DIA data analysis approaches spectral libraries are inevitable, being the basis for data extraction. Nowadays, also library-free approaches exist, resulting in increased throughput as there is no need for extensive fractionation and data acquisition using DDA. DIA-NN and Spectronaut both include software modules which enable operating without a spectral library. Using this approach, we identified up to 3400 protein groups / 22,500 peptides in just 4.8 min run time from 200 ng sample load (Figure 3). These results clearly show that the timsTOF Pro 2 in combination with dia-PASEF can be used for high throughput data-independent single-shot acquisitions without time-consuming fractionation experiments.

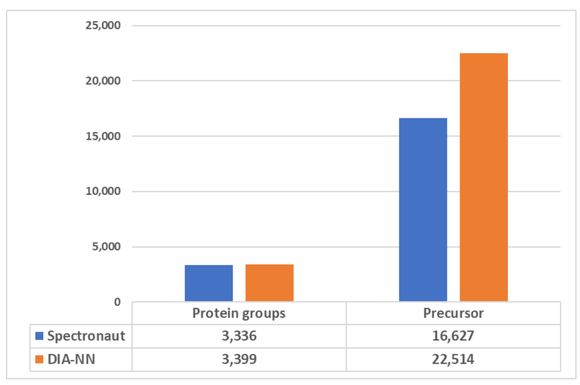

Fig. 3 Number of identified protein groups and precursors from library-free data processing using different software solutions

To demonstrate the potential of dia-PASEF for high sensitivity proteomics we used a timsTOF SCP mass spectrometer in combination with nanoElute and EVOSEP One. Using an Aurora column 25 cm (Ion Opticks) in a gradient of 35 minutes and flow of 250 nL/min, dia-PASEF mode identified from only 20 ng of HEK peptides an average of 4,100 protein groups and around 30,000 peptides, with a data completeness of 96% and a CV of 5.3 % (Figure 4).

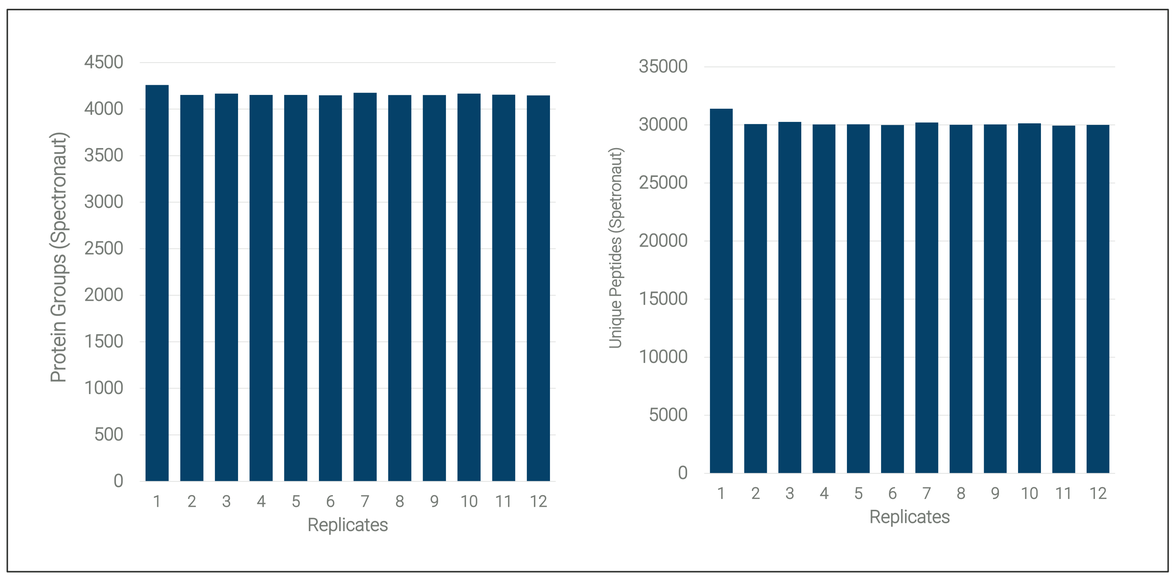

Figure 4: dia-PASEF results for 20 ng of a HEK cell line digest separated using a 35 min gradient. A) Protein group IDs @ 1% protein FDR and B) Peptide IDs @ 1% FDR.

To further investigate timsTOF SCP sensitivity, a low flow rate delivery from the Evosep system (Whisper100, 40 samples per day with gradient flow of 100nl/min) was coupled to this mass spectrometer and only 125 picograms of HEK was loaded on the Evotip. On this setup 1,249 (±123, n=6) protein groups could be reproducibly identified using dia-PASEF and library-free based approach (Table 1).

Table 1: High sensitivity proteomics results from timsTOF SCP.

LC system	Amount on column	Gradient time	Protein Groups
nanoElute	20 ng	35 min	4,100
EvoSep One	125 pg	28 min (40SPD)	1,249

Summary

- Using timsTOF Pro 2, time-consuming fractionation experiments can be replaced by single-shot injections boosting sample turnover and hence enabling high throughput proteomics in the clinical environment as in cohort studies or personalized medicine.
- High depth proteomics from timsTOF SCP with sample amounts as low as 20 ng and 125 pg open up the possibility to advance into applications where sample amounts are limited, e.g. single cell approach, immunopeptidomics, tissue profiling or PTM enrichment experiments.

Conclusion

- dia-PASEF makes use of the correlation of molecular weight and ion mobility in a trapped ion mobility mass spectrometer
- Single run analysis of whole proteome digests demonstrates deep proteome coverage and exceptional sensitivity for high sample throughput
- The timsTOF SCP in combination with dia-PASEF allowed identification of 1,249 protein groups from just 125 picograms of sample

timsTOF Pro 2 / timsTOF SCP