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

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Introduction

dia-PASEF (Meier et.al., 2019) takes advantage of the additional dimension of separation provided by trapped ion mobility (TIMS). The dia-PASEF cycle time can be reduced to make it compatible with short gradient separation while preserving high selectivity. This combination of DIA and PASEF compensates for the traditional DIA pitfalls: by using a pattern of m/z isolation windows within consecutive TIMS events, the percentage of ions used in dia-PASEF can be greatly increased. Here, we evaluate the benefits of dia-PASEF including library-free data processing for very short gradients, enabling ultra-high sample throughput proteomics. We also demonstrate the performance of dia-PASEF on low sample amounts and high throughput.

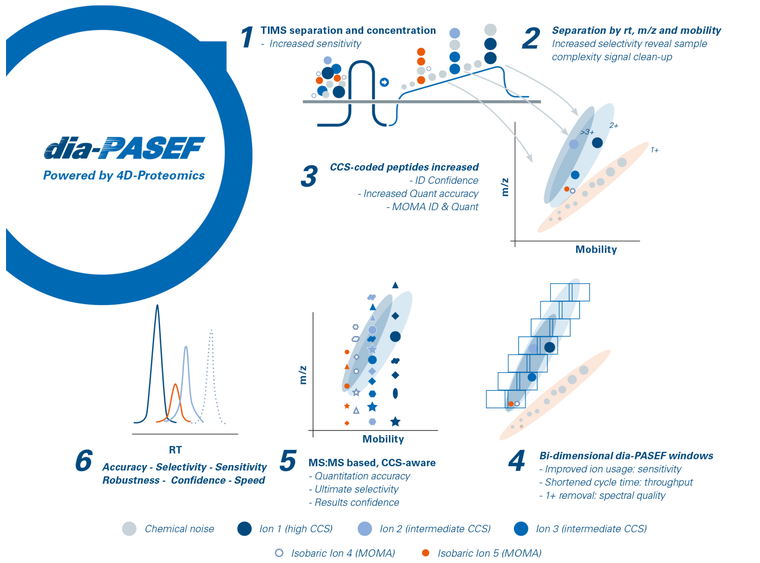

Figure 1: dia-PASEF scheme of operation and advantages

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. Data was processed using both library or library-free approach with DIA-NN 1.8 (Demichev et.al, 2021) incorporated into the PaSER software (Bruker) and Spectronaut 15 (Biognosys) using default settings.

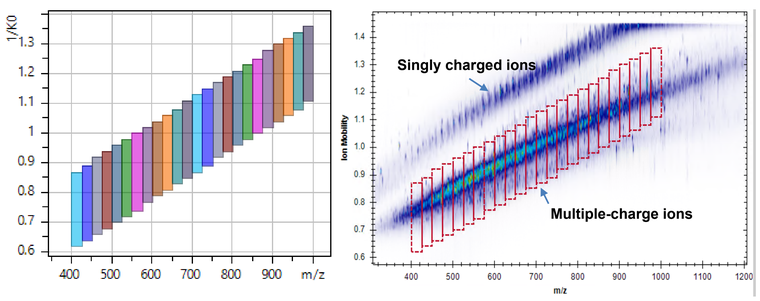

Figure 2: dia-PASEF scheme (left) and m/z – mobility heat maps from Spectronaut (right) showing different charge state distributions.

Figure 3: timsTOF Pro 2 and timsTOF SCP (Single Cell Proteomics) were combined to nanoElute or EVOSEP to evaluate high throughput and high sensitivity proteomics in dia-PASEF mode.

Results

Acquisition with the dia-PASEF MS method from 200 ng on column identified 6,866 protein groups on DIA-NN 1.8 software using an inhouse library. When library-free approach was evaluated, same acquisition mode could identify 6,300 protein groups using Spectronaut 15 and 7,300 PG using DIA-NN with a gradient of 70 minutes (15 SPD) (Figure 4). Benefits of dia-PASEF are even more pronounced when shorter gradients are compared. As also shown in figure 3, between 3,300 and 3,600 protein groups were identified from a 300 SPD method (4.8 min run time) using this in-house spectral library and library free approach, respectively. On average, comparing longest versus shortest gradients, acquisition time decreased by a factor of 20 while the number of identifications only dropped by a factor of 2.

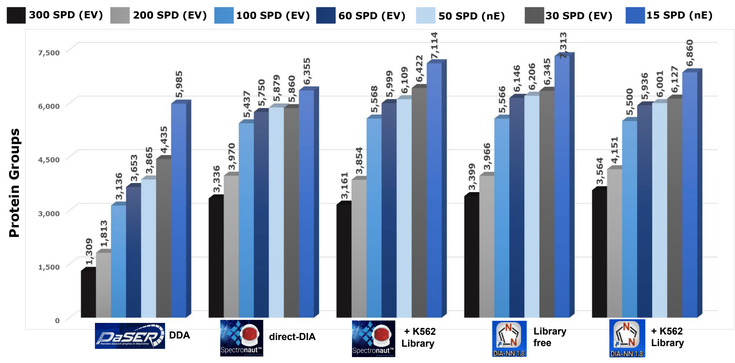

Figure 4: Number of identified protein groups with different gradients, approaches and software. SPD means samples per day.

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 25 cm column, a gradient of 35 minutes and flow of 250 nL/min, dia-PASEF mode identified from an average of 4,100 protein groups and around 30,000 peptides from only 20 ng of HEK peptides, with a data completeness of 96% and a CV of 5.3 % (Figure 5).

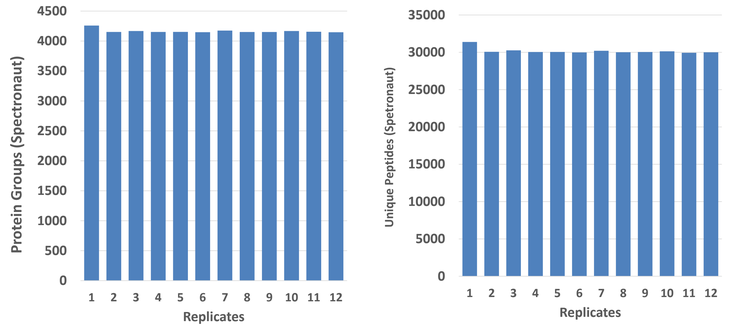

Figure 5: dia-PASEF results for 20 ng of a HEK cell line digest separated using a 35 min gradient. A) Protein group IDs @ 1% protein FDR and B) Peptide IDs @ 1% FDR.

To further investigate sensitivity of the timsTOF SCP, 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: High Sensitivity Proteomics Results from timsTOF SCP.

Chromatograph	Amount on column	Gradient time	Acquisition Mode	Protein Groups
nanoElute	20 ng	35 min	dia-PASEF	4,100
EvoSep One	125 pg	28 min (40SPD)	dia-PASEF	1,249

Conclusions

- **High depth proteomics from timsTOF SCP with sample amounts as low as 20 ng and 125 pg enable applications where sample amounts are limited, e.g. single cell protoemics, immunopeptidomics, tissue profiling or PTM enrichment experiments.**
- **The timsTOF Pro 2 allows time-consuming fractionation experiments can be replaced by single-shot injections boosting sample turnover and enabling high throughput proteomics in the clinical research as in cohort studies or personalized medicine.**
- **Data presented demonstrates the benefits of using dia-PASEF acquisition for high-throughput, deep proteome studies, using library-free approach.**

Meier F. et al. (2019) doi: https://doi.org/10.1101/656207

timsTOF Pro / timsTOF SCP