High throughput single-shot proteomics on the timsTOF Pro 2

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Fig. 1 timsTOF Pro 2 for high throughput 4-D proteomics

Introduction

High throughput proteomics applications like clinical and personalized medicine proteomics require the combination of a maximum depth of proteome coverage in combination with quantitative accuracy, sample turnover and robustness. .

The timsTOF Pro 2 platform (Figure 1) featuring trapped ion mobility spectrometry (TIMS) allows for space and time focusing of peptide ions boosting sensitivity and allowing for efficient precursor scheduling and MS/MS acquisition rates of >100 Hz. Together with the separation of mobility offset but mass aligned (MOMA) isomeric species these features make the timsTOF Pro 2 an established standard in 4-D proteomics [1,2]. In this study we demonstrate the benefits of the timsTOF Pro 2 instrument in a label free proteomics study on a cell line and on short LC gradients.

Methods

200 ng and 20 ng of in-house prepared tryptic digests of Human embryonic kidney cell line (HEK) were analysed using a nanoElute UHPLC (Bruker Daltonics GmbH & Co. KG, Germany, Figure 2A) equipped with a 25 cm Aurora (75 µm ID, ionOpticks, Australia) column. Gradient times were 70 min and 35 min for 200 ng and 20 ng sample load, respectively (Figures 2B and 2C)

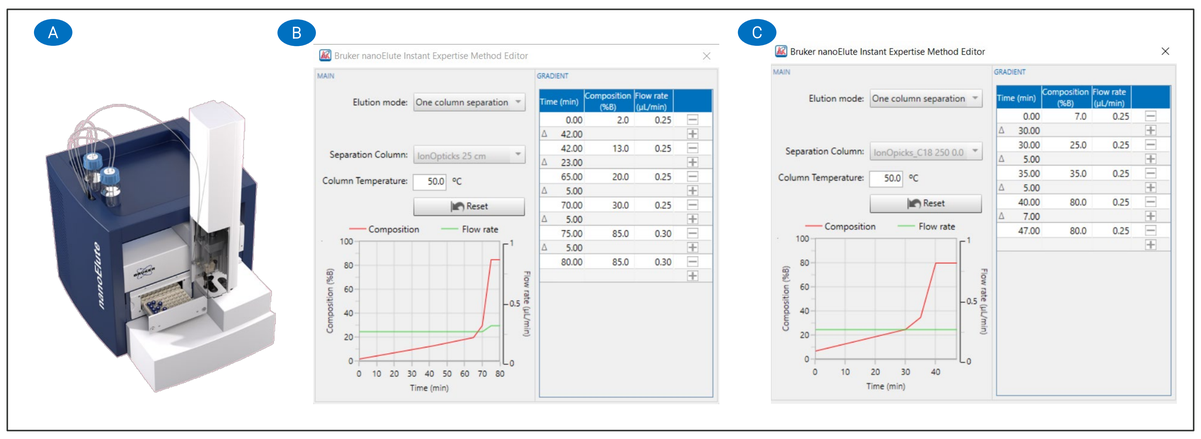

Fig. 2 chromatographic conditions to separate HEK peptides
A: Bruker nanoElute B: 70 min gradient conditions C: 35 min gradient conditions

Data were acquired in dda-PASEF and in dia-PASEF® mode and data processing of dda-PASEF data was performed in PaSER 1.0 and MaxQuant 1.6.17.0. dia-PASEF data were analysed in Spectronaut 14 (Biognosis, Switzerland) using a hybrid library of 24 fractions of HeLa cell digest and the current dia-PASEF data (Figure 3).

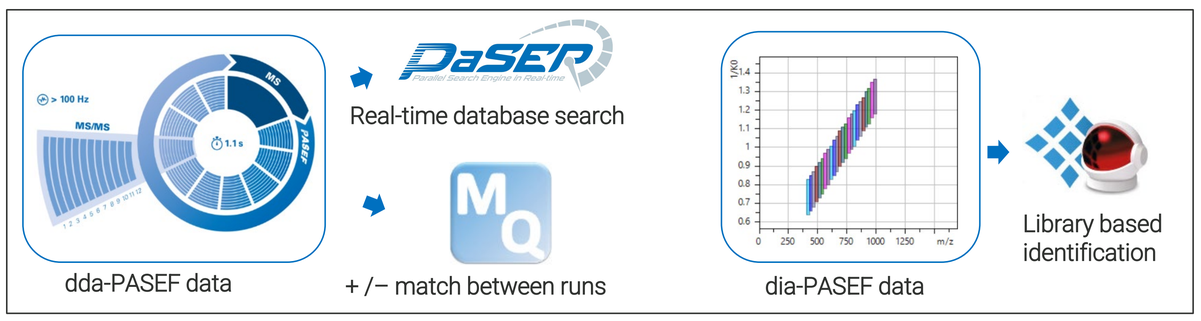

Fig. 3 Data processing for dda-PASEF and dia-PASEF data

References

[1] Meier F et al. (2015). J. Proteome Res.

[2] Meier F et al. (2018). Molecular & Cellular Proteomics

Results

- On the timsTOF Pro 2 from single dda-PASEF injections of 200 ng HEK peptides separated on a 70 min gradient > 7,000 protein groups and 60,000 peptide sequences can be identified with close to 90% data completeness (figure 4).

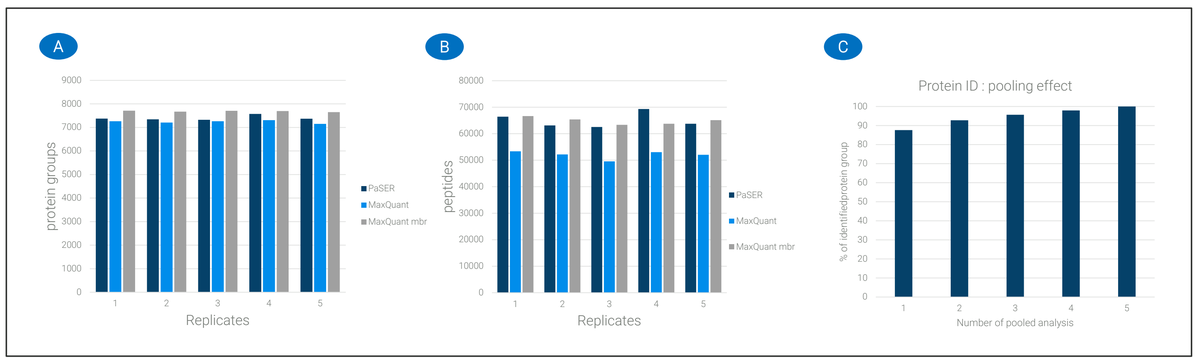

Fig. 4 dda-PASEF results for 200 ng of a HEK cell line analysed on a 70 min gradient.
A: Protein IDs @ 1% protein FDR B: Peptide IDs @ 1% FDR C: Data completeness

- dia-PASEF runs of 200 ng HEK peptides separated using a 70 min gradient result in the identification of > 7,800 protein groups and 77,000 peptide sequences with 7,493 protein groups reproducibly quantified (data completeness: 91.9%) (figure 5).

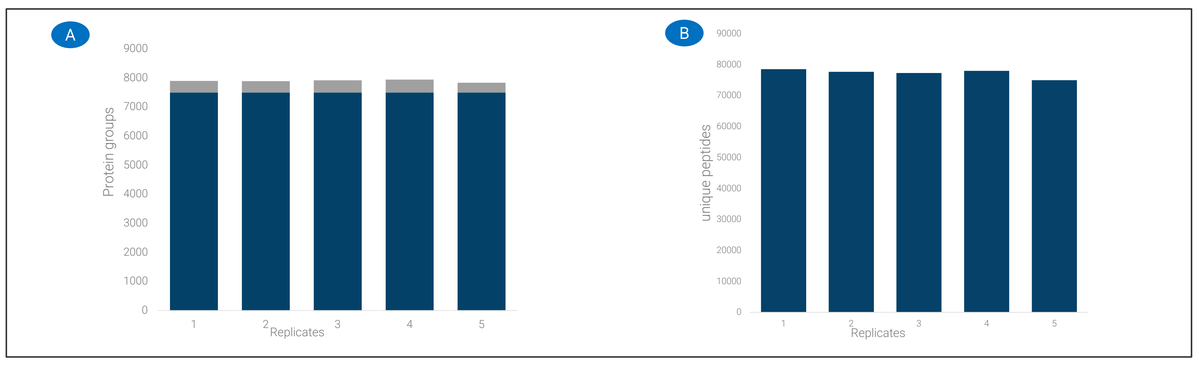

Fig. 5 dia-PASEF results for 200 ng of a HEK cell line analysed on a 70 min gradient.
A: Protein group IDs @ 1% FDR (light Grey) and proteins quantified in all samples (Blue) B: Peptide IDs @ 1% FDR

- In dia-PASEF mode from only 20 ng of HEK peptides separated on a 35 min gradient on average 4,100 protein groups and around 30,000 peptides can be identified with a data completeness of 96% and a CV of 5.3 % (figure 6).

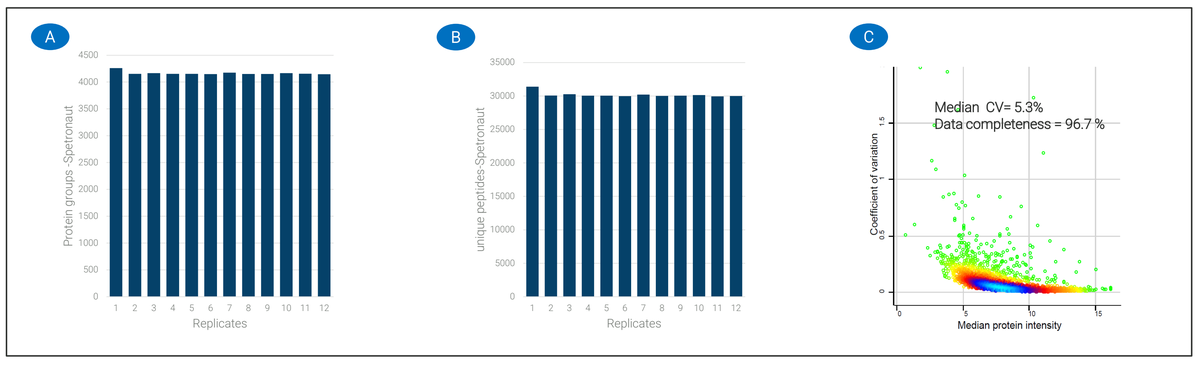

Fig. 6 dia-PASEF results for 20 ng of a HEK cell line analysed a 35 min gradient.
A: Protein group IDs @ 1% protein FDR. B: Peptide IDs @ 1% FDR. C: CV distribution

Conclusion

- The timsTOF Pro 2 identifies > 7,700 protein groups and 70,000 sequences from a single shot injection on a 70 min gradient.
- A low number of missing values and very low CVs demonstrate quantitative reliability in both, dda-PASEF and dia-PASEF mode.
- Results from sample amounts as low as 20 ng on 35 min gradients open up the possibility to advance into applications where sample amounts are limited, e.g. immunopeptidomics, tissue profiling or PTM enrichment experiments.
- With the 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.