BRUKER AT ASMS 2021 - ADVANCES IN 4D-PROTEOMICS™

High throughput single-shot proteomics on the timsTOF Pro 2

Verena Tellstroem [1], Kristina Marx [1], Dirk Wunderlich [1], Gary Kruppa [2], Nagarjuna Nagaraj [1]

[1] Bruker Daltonics GmbH & Co. KG, Bremen, Germany

[2] Bruker S.R.O., Brno, Czech Republic

Introduction

- Successful proteomics research requires the combination of maximum depth of proteome coverage and quantitative accuracy with sample throughput becoming increasingly important in applications like clinical and personalized medicine proteomics.
- TIMS ion mobility separation delivers space and time focusing of ions hereby providing the basis for speed and sensitivity coupled with mobility offset but mass aligned (MOMA) isomeric species separation.
- The timsTOF Pro platform powered by PASEF® technology has become a standard in 4D-Proteomics™ delivering accurate in-depth information in a robust fashion and on fast gradients [1,2].
- The new timsTOF Pro 2 advances this well-established instrument platform with even better sensitivity, capacity and robustness, delivering high coverage and quantitative precision on even shorter gradients hence, increasing sample throughput.
- 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.

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

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

Methods

- 200 ng and 20 ng of in-house prepared tryptic digests of Human embryonic kidney cell line (HEK) were analysed
- Peptide separation was carried out using a nanoElute UHPLC (Bruker Daltonics GmbH & Co. KG, Germany, Figure 1A) 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 1B and 1C)

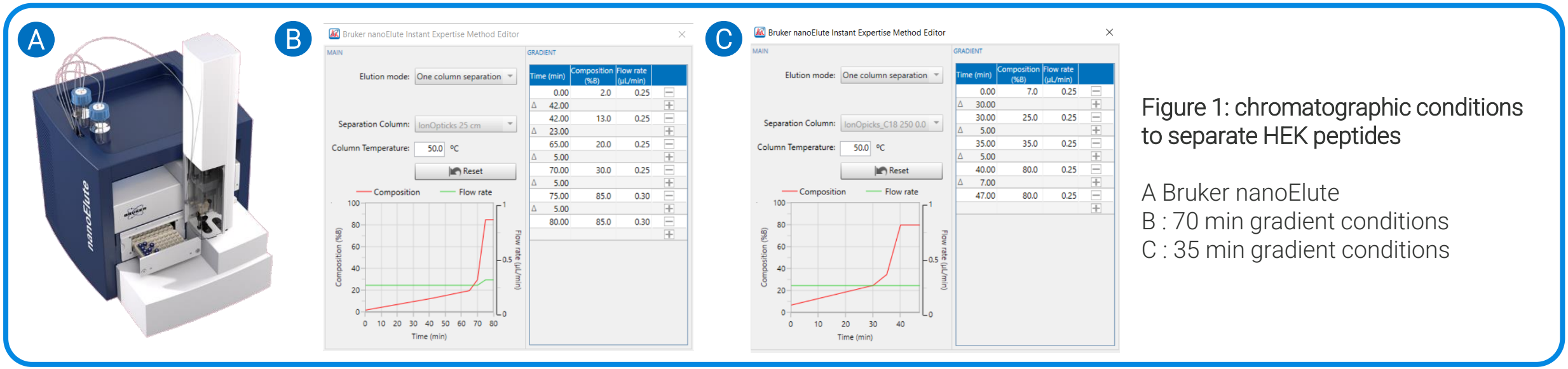

Figure 1: chromatographic conditions to separate HEK peptides

A Bruker nanoElute
B : 70 min gradient conditions
C : 35 min gradient conditions

Methods

- The LC was coupled to a timsTOF Pro 2 mass spectrometer (Bruker) via the Captive Spray source (Bruker)
- Data were acquired in dda-PASEF and in dia-PASEF® mode with methods optimized for the respective chromatographic conditions and sample load (Table 1 and Figure 2)

	70 min	35 min
MS settings:		
Mass Range	100 – 1,700 m/z	100 – 1,700 m/z
Ion Mobility Range	0.85 - 1.35 V-s/cm2	0.85 - 1.35 V-s/cm2
Ion Mobility Scan Time	100 msec	166 msec
MS/MS settings:		
Number of MS/MS Ramps	10 PASEF scan à 100 ms	10 PASEF scan à 166 ms
MS/MS Threshold	1750	1000
Target Intensity	14,500	14,500
High sensitivity mode	Disabled	Enabled

Table 1: dda-PASEF acquisition parameters

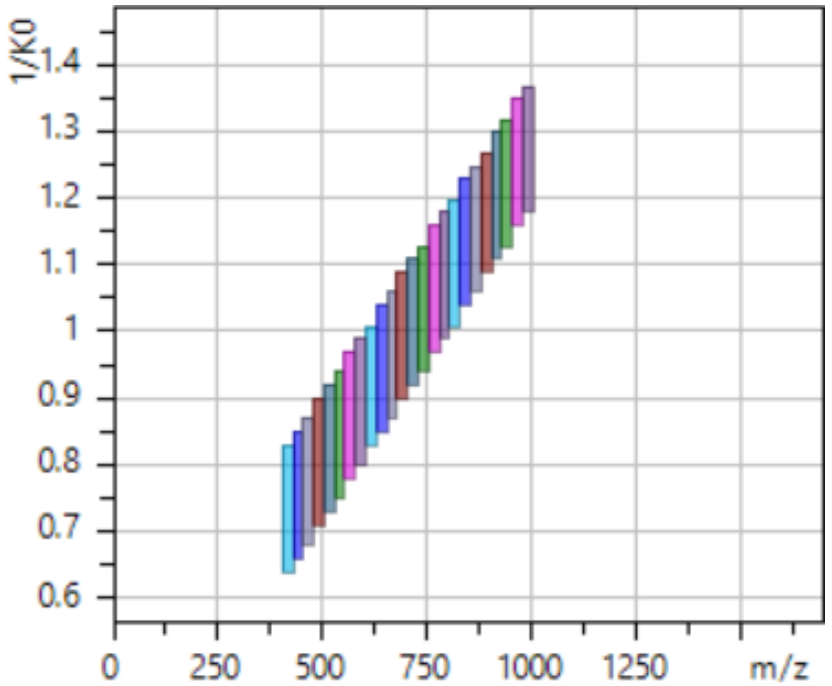

Figure 2: dia-PASEF acquisition scheme

Methods

- 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).

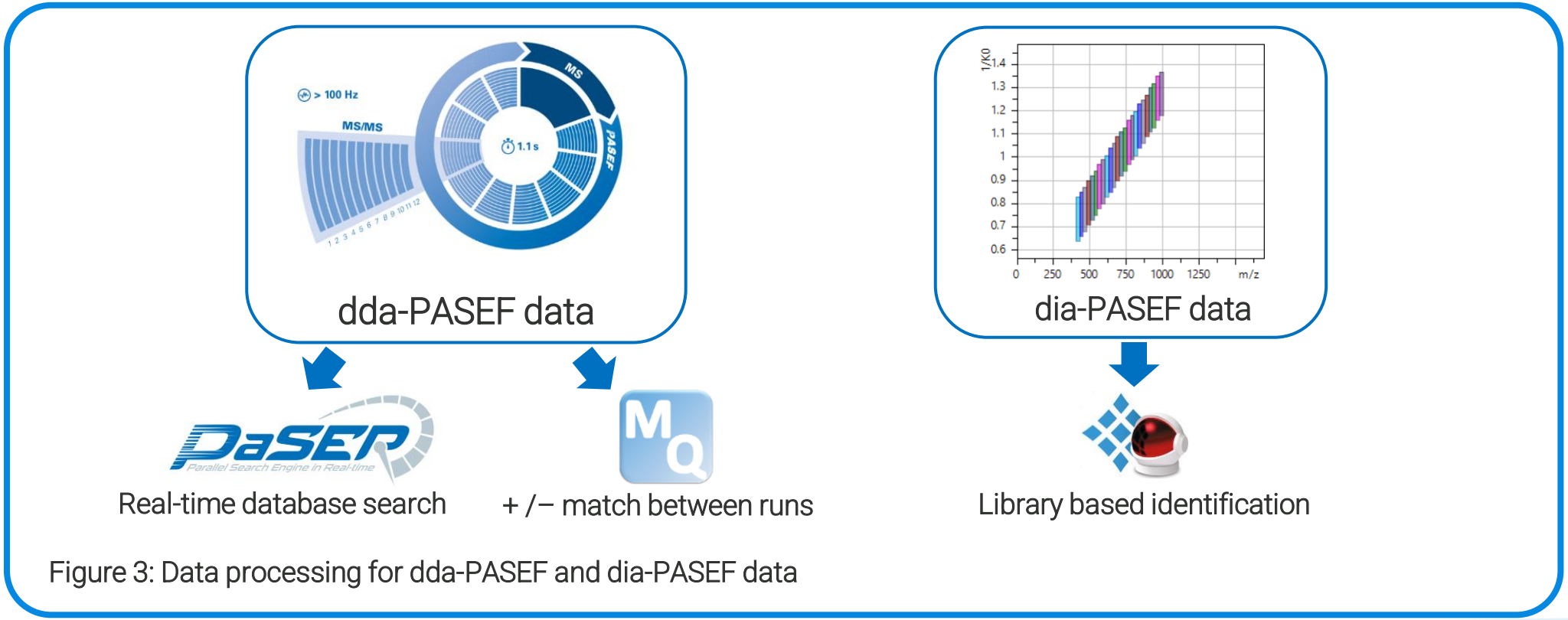

Figure 3: Data processing for dda-PASEF and dia-PASEF data

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).

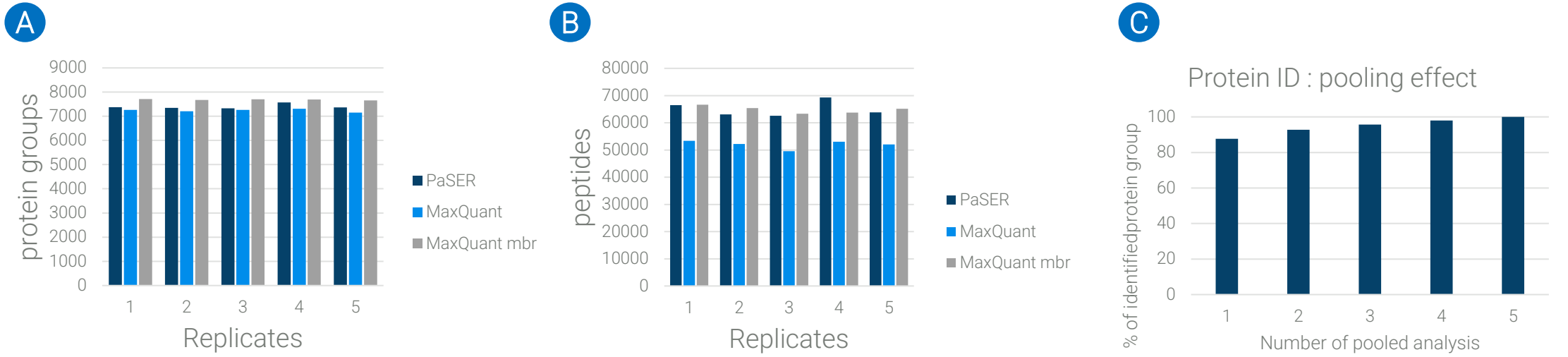

Figure 4: dda-PASEF results for 200 ng of a HEK cell line digest separated using a 70 min gradient.
A: Protein group IDs @ 1% protein FDR.
B: Peptide IDs @ 1% FDR.
C: Data completeness

Results

- dia-PASEF injections 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).

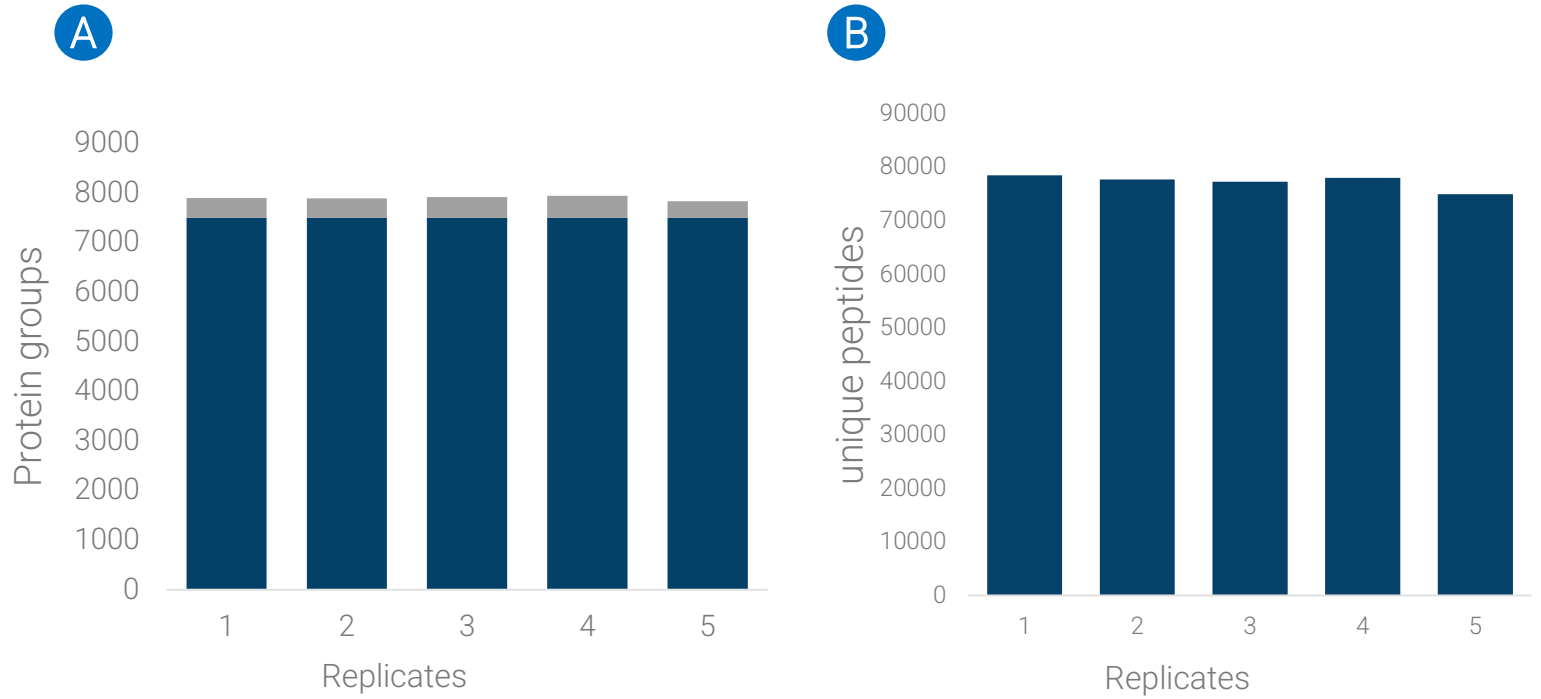

Figure 5: dia-PASEF results for 200 ng of a HEK cell line digest separated on a 70 min gradient.

A: Protein group IDs @ 1% FDR (light Grey) and proteins quantified in all samples (Blue)

B: Peptide IDs @ 1% FDR

Results

- Only 20 ng of HEK peptides separated on a 35 min gradient lead to the identification of nearly 4,500 protein groups and 30,000 peptide sequences in dda-PASEF mode (figure 6).

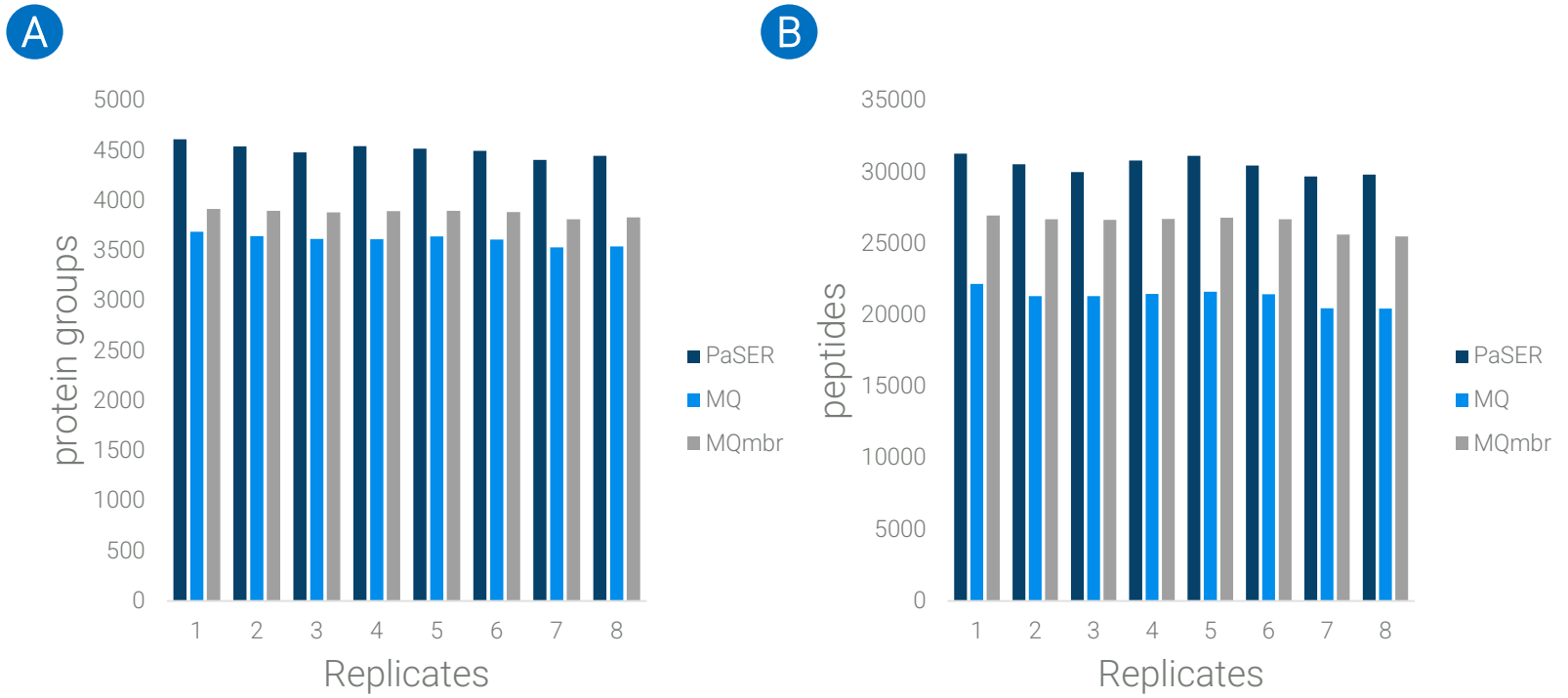

Figure 6: dda-PASEF results for 20 ng of a HEK cell line digest separated on a 35 min gradient.

A: Protein group IDs @ 1% FDR

B: Peptide IDs @ 1% FDR

Results

- 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 7).

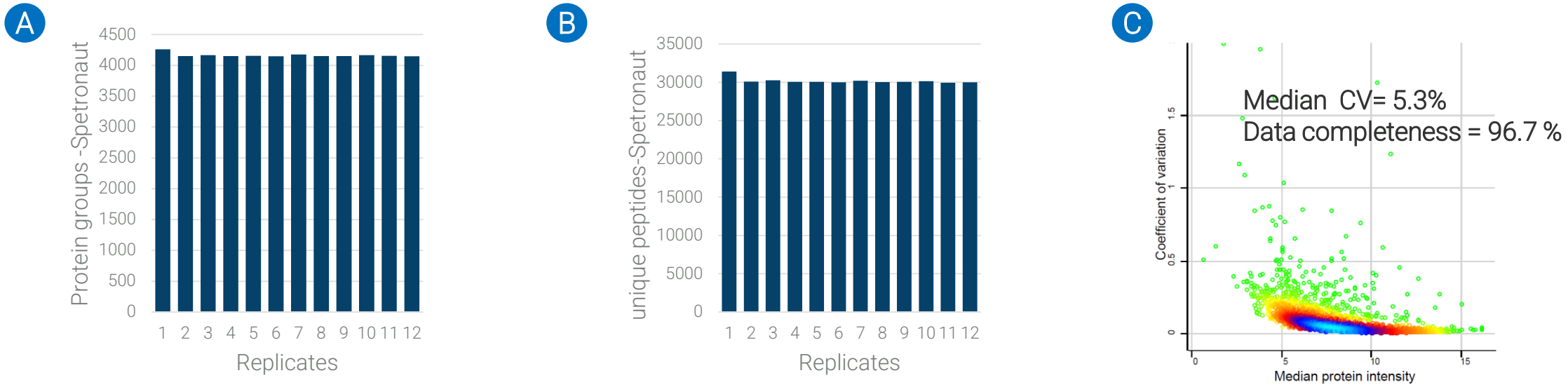

Figure 7: dia-PASEF results for 20 ng of a HEK cell line digest separated using a 35 min gradient.
A: Protein group IDs @ 1% protein FDR.
B: Peptide IDs @ 1% FDR.
C: CV distribution

Conclusion

- The timsTOF Pro has become the standard in 4D-Proteomics with its ability to deliver in-depth proteome coverage in single shot injections on short LC gradients.
- The timsTOF Pro 2 extends these capabilities even further with its ability to identify > 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 e.g. in cohort studies or personalized medicine.