

Operating, maintaining, and troubleshooting the sensitivity and robustness of timsTOF platforms for proteomics studies

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Introduction

Over the last few years, proteomics applications going from sample preparation to data analysis have been significantly improved with respect to the sensitivity and robustness. The development of the Parallel Accumulation Serial Fragmentation (PASEF) technology increases peak capacity, sensitivity, and acquisition speed for confident identification and quantification, and developing epiproteomics workflows for post-translational modifications (PTM) studies with maximum duty cycle (Figure 1). However, high throughput sensitive proteomics analysis requires a reliable quality control (QC) approach to maintaining the best performance and minimize down time. Here we study the factors that affect instrument performance and methods to monitor for these effects.

Conclusion

- Identification results ≠ LC/MS performance !
- Visualizing specific precursors (chromatograms, mobilograms,...) in addition to peptides & proteins identification is key towards meaningful proteomics data quality.
- This approach allows for more universal monitoring of the performance of the timsTOF instruments to significantly minimize customer down time.

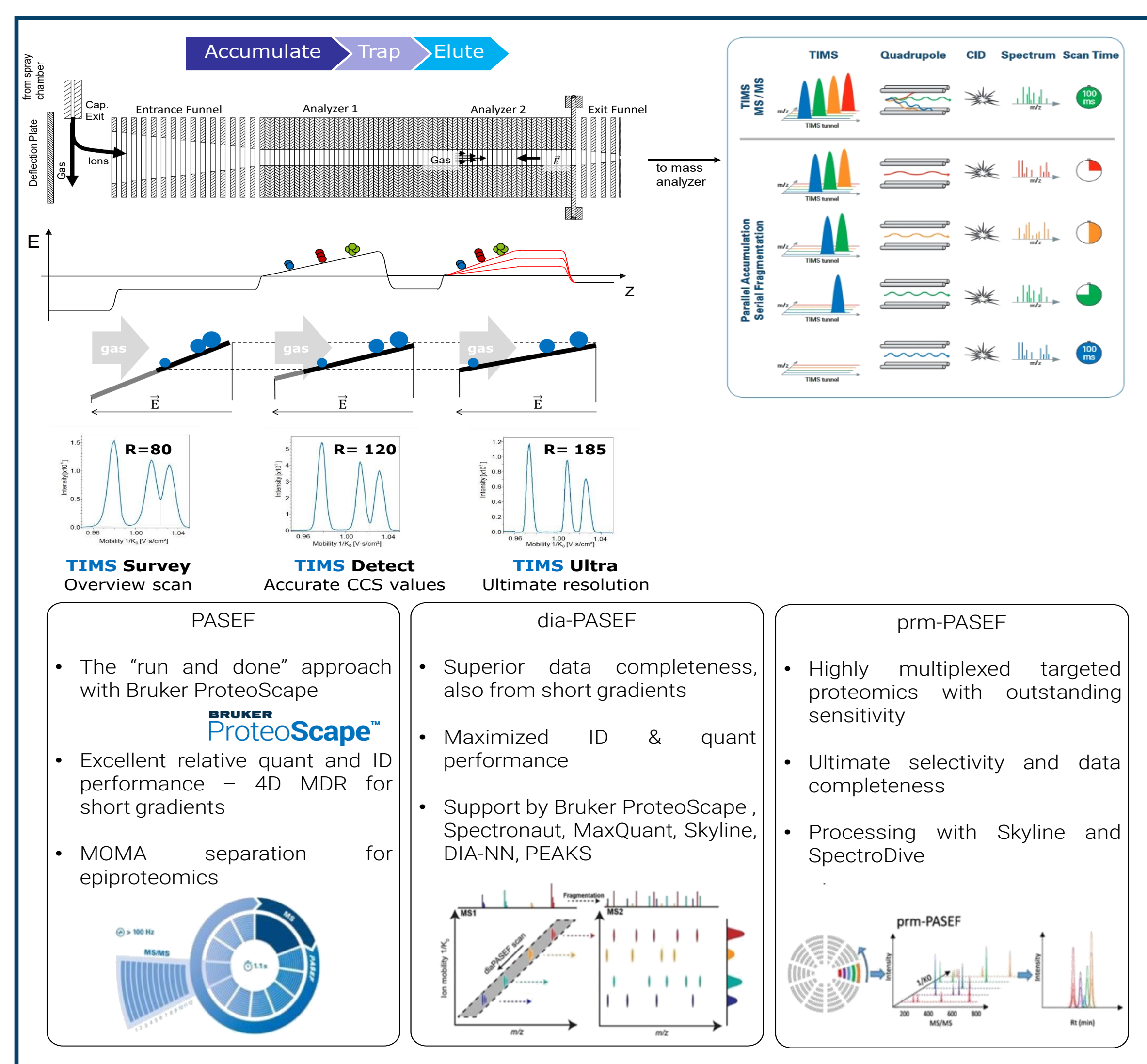


Fig. 1 timsTOF mass spectrometer equipped with the PASEF technology (above). All acquisition modes are demonstrated in the panels (below).

Methods

Commercial LC/MS-compatible Human protein extracts (HeLa & K562; Thermo Scientific and Promega, respectively) were analyzed by coupling either the nanoElute I (Bruker) or Evosep One (Evosep) to 3 different MS instruments (timsTOF Pro2, timsTOF SCP, and timsTOF HT). Several LC columns (PepSep and IonOpticks), quantities ranging from 60 pg to 1 µg on column, separation methods, and MS methods were evaluated. Raw data were processed with Bruker ProteoScope (BPS), DIA-NN, or FragPipe and analyzed using DataAnalysis and Skyline to extract 12 "target peptides" based on their retention time, CCS values, MS & MS/MS spectra, identified peptide sequence and protein (Figure 2). "Target peptides" were detected in K562 and HeLa digest across the whole gradient and ion mobility range on all LC/MS platforms used in this study.

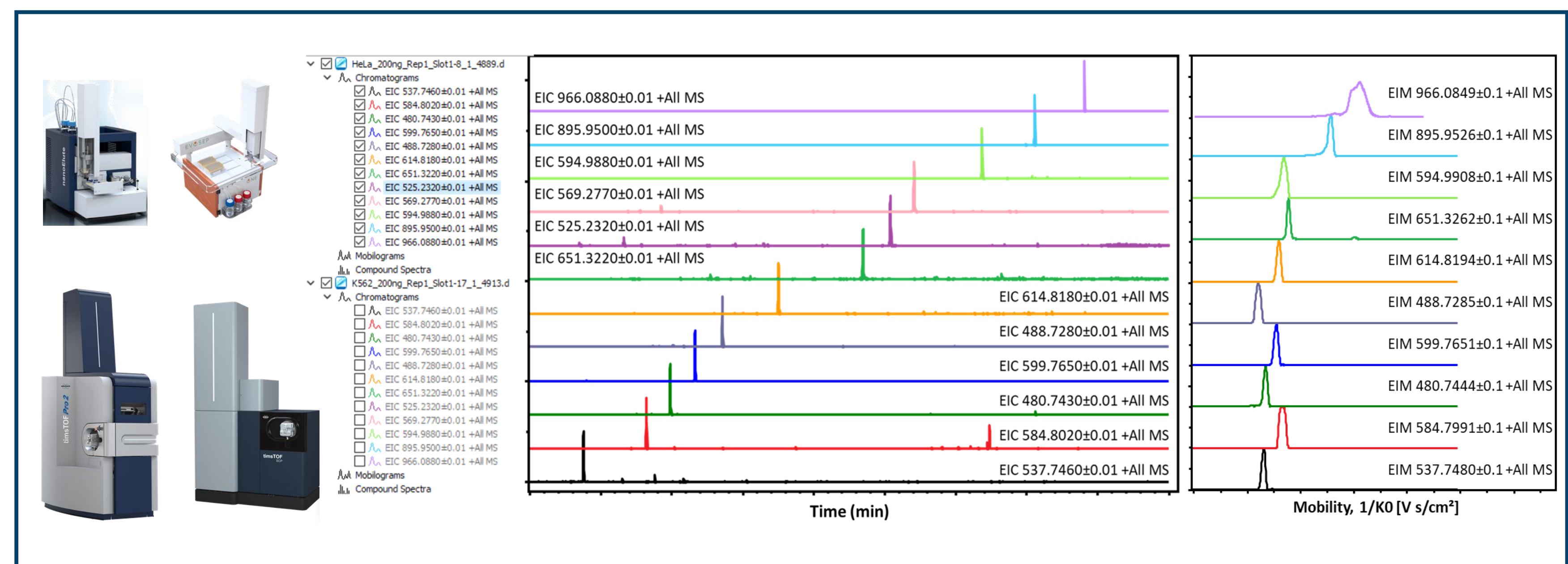


Fig. 2 Extracted Ion Chromatograms (EIC) and Extracted Ion Mobilograms (EIM) of peptides detected in both HeLa and K562 using the nanoElute I coupled to timsTOF Pro2. These "target peptides" were used to evaluate the performance of the LC/MS instrumentation platform.

Results

Users mostly rely on the number of identified peptides and proteins to monitor the performance of the LC/MS platform. Using an Evosep One connected to a timsTOF SCP allows for the confident identification of more than 5,400 proteins and 48,000 peptides in dia-PASEF from 4 ng of K562 injected on an IonOpticks column. In addition, the expected number of peptides and proteins identified may sometimes be associated with bad chromatographic performance (e.g., wide peak width for hydrophilic peptides; Figure 3).

Then, we tested different MS settings to lower the QC performance compared to a reference method. For each method, we evaluated the total number of proteins and peptides but also the

quantitative effect from the "target peptides" (Figure 4). For example, decreasing (n=4; Method1) or increasing (n=20; Method7) the number of PASEF ramps allows for decreasing the number of peptides to respectively 17% and 30% compared to the reference method. In addition, setting a 6% duty cycle gave rise to the lowest number of peptides and proteins as expected. However, this method demonstrated the highest intensity of the ion chromatograms and mobilograms extracted from the "target peptides" compared to the reference method. This might be due to low number of precursors and higher accumulation time per Tims frame.

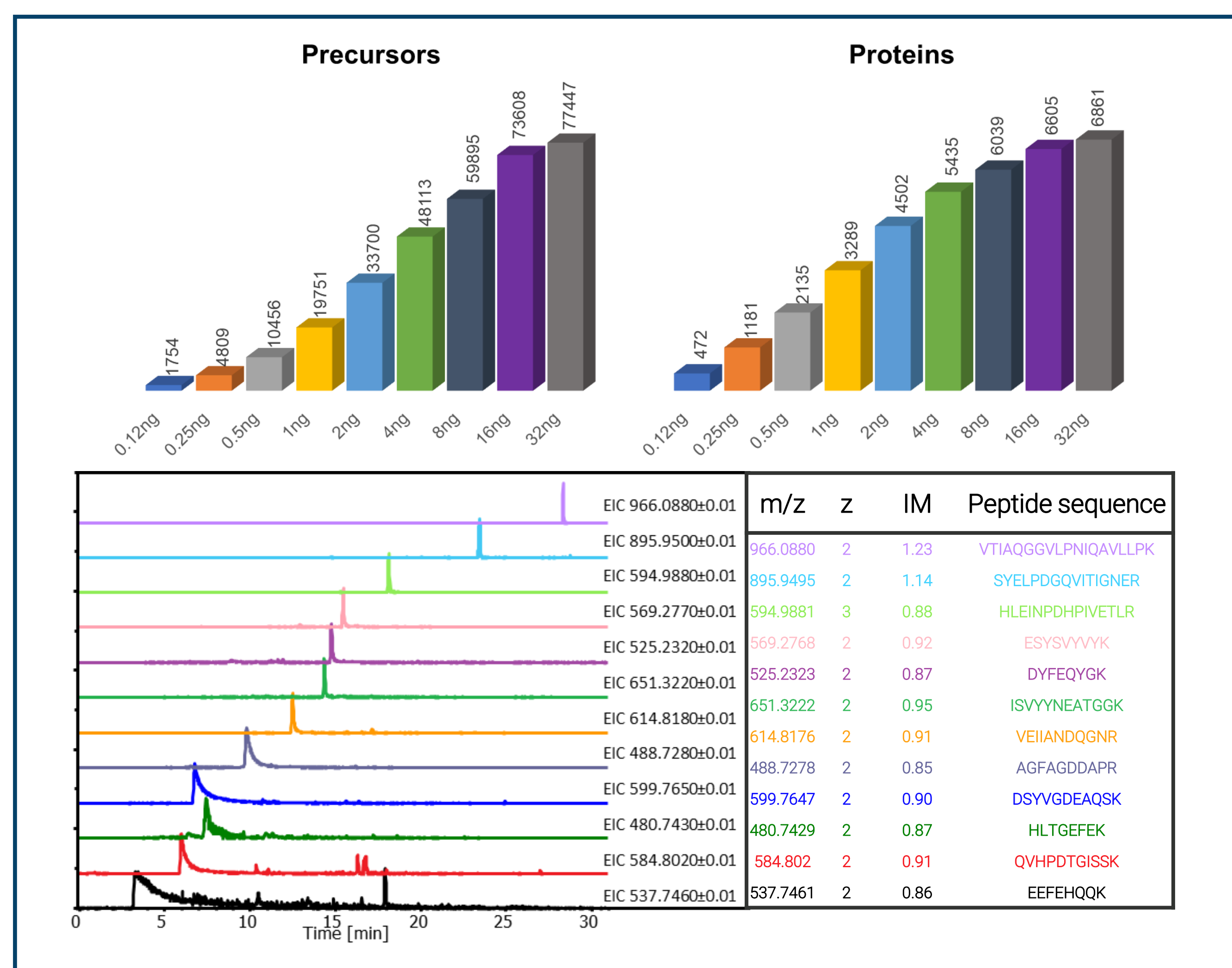


Fig. 3 Dilution series of K562 digests using the Evosep coupled to timsTOF SCP mass spectrometer (DIA; Whisper 40SPD) and EICs for 12 "target peptides"

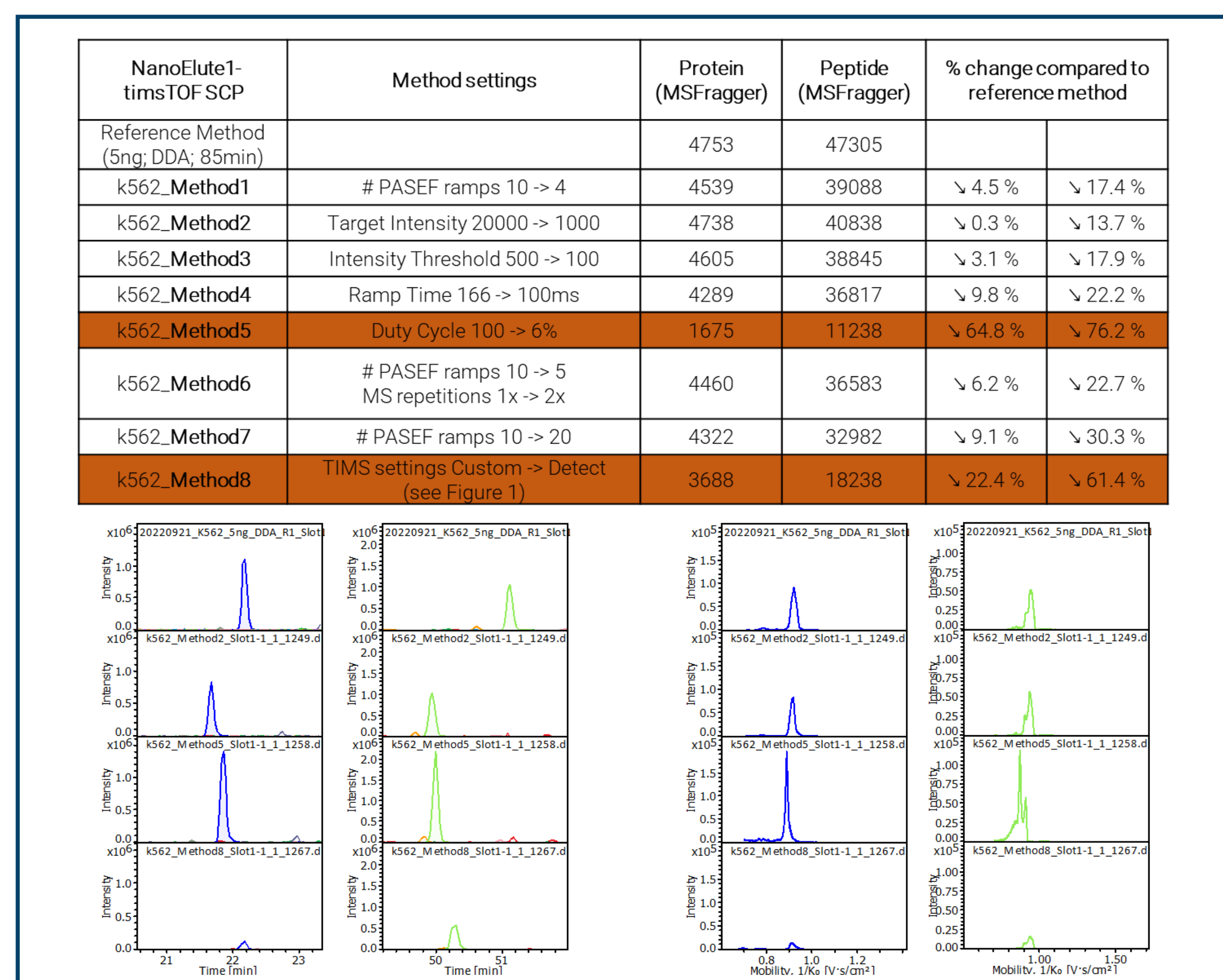


Fig. 4 MS settings evaluation using the nanoElute I coupled to timsTOF SCP mass spectrometer (DDA; 85 min). Comparison of the number of precursors and proteins and evaluation of the EIC and EIM of m/z 599.7650 (blue) and 594.9880 (green).