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

Benoit Fatou[1], Conor Mullens[1], Elizabeth Gordon[1], Diego Assis[1], Sebastian Vaca[2], Michael Krawitzky[2], Matthew Willetts[1]

[1]Bruker Daltonics Inc., 40 Manning Road, Manning Park, Billerica, MA 01821, USA

[2]Bruker Daltonics Inc., 101 Daggett Dr, San Jose, CA 95134, USA

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.

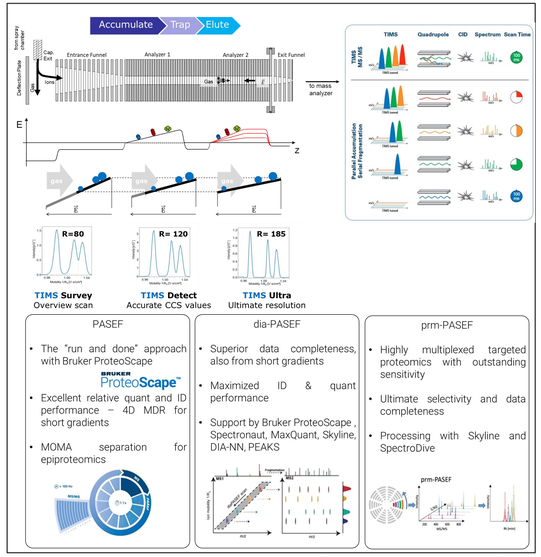

Fig. 1 timsTOF mass spectrometer equipped with the PASEF technology (above). All acquisition modes are demonstrated in the panels (below).

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 ProteoScape (BPS), DIA-NN, or FragPipe and analyzed using DataAnalysis and Skyline to extract "target peptides" based on their retention time, CCS values, MS & MS/MS spectra, identified peptide sequence and protein (Figure 2).

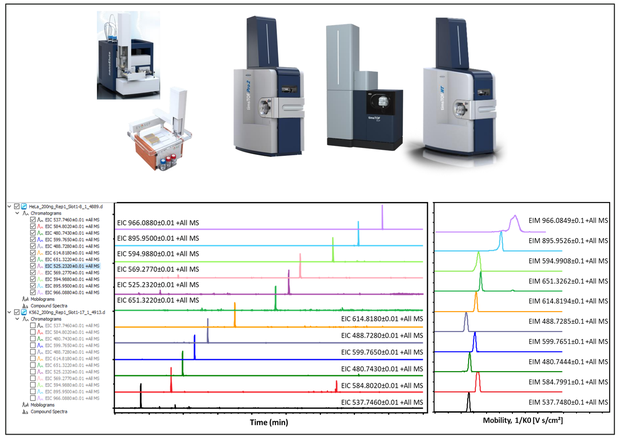

Fig. 2 Extracted Ion Chromatograms (EIC) and Extracted Ion Mobilograms (EIM) of peptides detected in both HeLa and k562 using the nanoElute I coupled to timsTOF Pro2. These "target peptides" will be used to evaluate the performance of the LC/MS instrumentation platform.

Results

- A set of 12 tryptic peptides were selected across the entire gradient time (Figure 2).
- Peptide detection independent of the samples (HeLa or k562) and LC/MS platforms.
- "Target peptides" list demonstrated specific retention times & ion mobility values that can be useful to monitor the LC/MS performance in addition to the number of precursors/peptides/proteins identified.

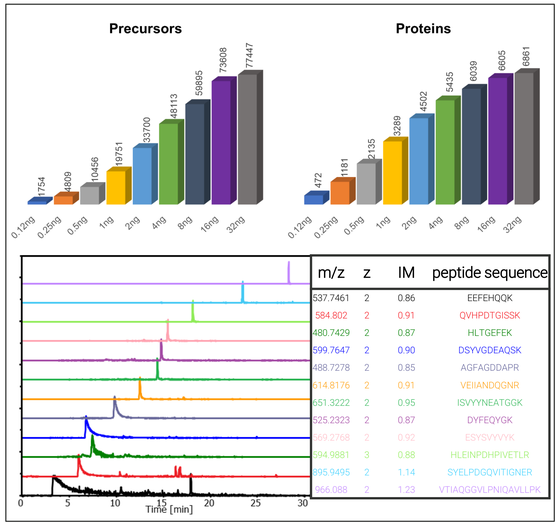

Fig. 3 Dilution series of k562 digests using the Evosep coupled to timsTOF SCP mass spectrometer (DIA; Whisper 40SPD)

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 4ng 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) or increasing (n=20) 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.

NanoElute1-timsTOF SCP	Method settings	Protein (MSFragger)	Peptide (MSFragger)	% change compared to reference method	
Reference Method (5ng; DDA; 85min)		4753	47305		
k562_Method1	# PASEF ramps 10 -> 4	4539	39088	↘ 4.5 %	↘ 17.4 %
k562_Method2	Target Intensity 20000 -> 1000	4738	40838	↘ 0.3 %	↘ 13.7 %
k562_Method3	Intensity Threshold 500 -> 100	4605	38845	↘ 3.1 %	↘ 17.9 %
k562_Method4	Ramp Time 166 -> 100ms	4289	36817	↘ 9.8 %	↘ 22.2 %
k562_Method5	Duty Cycle 100 -> 6%	1675	11238	↘ 64.8 %	↘ 76.2 %
k562_Method6	# PASEF ramps 10 -> 5 MS repetitions 1x -> 2x	4460	36583	↘ 6.2 %	↘ 22.7 %
k562_Method7	# PASEF ramps 10 -> 20	4322	32982	↘ 9.1 %	↘ 30.3 %
k562_Method8	TIMS settings Custom -> Detect (see Figure 1)	3688	18238	↘ 22.4 %	↘ 61.4 %

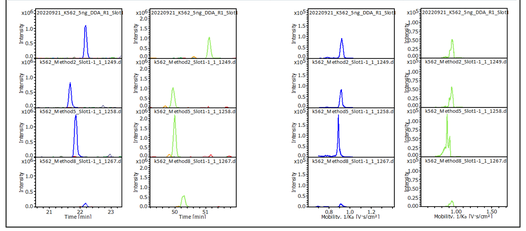

Fig. 4 MS settings evaluation using the nanoElute I coupled to timsTOF SCP mass spectrometer (DDA; 85 min). Comparison of the number of precursors and proteins and evaluation of the EIC and EIM of m/z599.7650 (blue) and 594.9880 (green).

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.