Pierre-Olivier Schmit
Bruker France SAS, France

PASEF & beyond: developing the potential of the TIMS-Q-TOF architecture for proteomics

Targeted proteomics approaches are now commonly used, either to perform targeted biomarker candidate discovery or to validate candidate biomarkers that had been pointed out by untargeted discovery proteomics approaches. In this work we evaluate the potential of the Trapped Ion Mobility Separation (TIMS) – QTOF design for targeted Proteomics. This architecture enables the PASEF (Parallel Accumulation Serial Fragmentation) acquisition strategy, which allows the acquisition of data dependent MS/MS spectra at very high speed (> 100 Hz) and already proved to deliver a combination of maximum proteome coverage and enhanced sensitivity. We compared the results extracted from a standard PASEF acquisition to the ones obtained with TIMS-PRM and TIMS-BroadBand CID fragmentation. The first insights of the DIA-PASEF acquisition mode will also be described.

An equimolar mixture of 214 quantified synthetic peptides labeled with stable isotopes (AQUA peptides) was diluted in a 100ng/µl of human cell line digest. The dilution series covered 6 concentration levels ranging from 31.25 amol/µl to 25 fmol/µl. All samples and controls were separated by nano-HPLC (nanoElute, Bruker Daltonics) on 250 mm pulled emitter columns (IonOpticks, Australia) with a 60 min gradient and analyzed on a timsTOF Pro instrument (Bruker Daltonics). The timsTOF Pro was operated in data dependent PASEF mode, and exploratory targeted TIMS-PRM or TIMS-broadband CID acquisition modes. For those two, TIMS trapping times of 100 and 200 ms have been used. Post-processing analysis was performed with Data Analysis™, PeakX™ and Skyline-daily™.

A Mascot search of data dependent acquisition with PASEF mode of cell lysate digest spiked with 25fmol, 6,25fmol, 1562.5amol, 500amol, 125amol and 31.25 amol of the AQUA mixture detected 214/214/214/180/74 and 7 of the original 214 AQUA peptides, respectively. The untargeted analysis of this dataset using a combination of accurate mass, retention time and ion mobility values (XIC and IM) as a filter typically allowed to detect the peptides one dilution step below their previous identification threshold and linearity was demonstrated down to the 125 or 31 amol for most of those peptides.

The early TIMS-PRM allowed to quantify 110 of the Aqua peptides at the 31 amol concentration level and 58 of them at the 125amol level. Those results and the possible improvements will be discussed.