Comparison of Targeted Proteomics Approaches on a TIMS-Q-TOF

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Introduction

Targeted quantitative acquisition method aims at accurately quantify protein abundances in large set of samples without missing values. It is the method of choice to verify and validate protein biomarker candidates in large sample cohorts.

We evaluated the potential of the Trapped Ion Mobility Separation (TIMS) – QTOF platform for targeted proteomics. We compared the PASEF (Parallel Accumulation Serial Fragmentation) acquisition method, which allows the acquisition of data dependent MS/MS spectra at very high speed (> 100 Hz) with new experimental targeted acquisition TIMS-PRM mode.

Methods

Samples were prepared and measured as described in (Fig.1). 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 was operated in PASEF and TIMS-PRM mode as described in (Fig.2). Post-processing analysis was performed with Data Analysis™, PeakXTM and Skyline-daily™.

Results

The benefit of the ion mobility trapping and separation has been clearly established with TIMS-PRM acquisition (Fig.3), 110 of the 214 heavy AQUA peptides could be quantified at the 31amol level and 168 at the 125amol level. In addition, the ion mobility separation can resolve isobaric and co-eluted interferences (Fig.4). The overall sensitivity of the instrument can be improved by increasing the trapping (Fig.5) and collision energies (not shown). TIMS-PRM also greatly benefits of physical ion trapping and time focusing effect of the TIMS cell for an improved sensitivity to compare with a standard Q-TOF operation (fig.6).

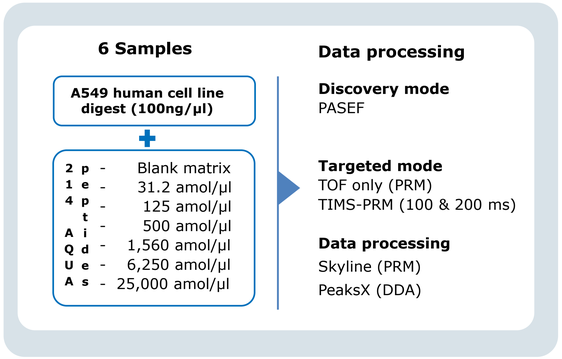

Fig. 1: Experimental setup.
The 6 samples have been acquired with the PRM (tims off), and tims-PRM (PASEF on) acquisition strategies

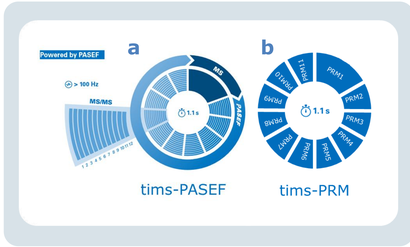

Fig. 2: Acquisition strategies.

a) Standard **PASEF** acquisition strategy. Untargeted identification & quantification (discovery mode) could be performed from these acquisition.
b) TIMS-PRM: in this prototype acquisition mode, only one mass is targeted for each tims event. The length of the cycle varies with the number of targets, which was comprised between 2 (200ms cycles) and 23 (2,3 sec cycles) for this experiment. The effects of 100 and 200 ms tims trapping time have been evaluated.

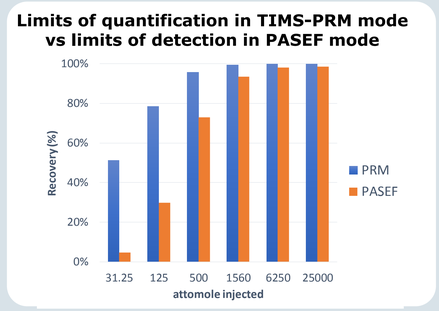

Fig. 3: PASEF and TIMS-PRM sensitivity comparison: >110 peptides are quantified at the 31 amol level with an accuracy error below 20% and a minimum of 3 transitions observed.
At that concentration, only 10 are identified using a standard PASEF Discovery run.

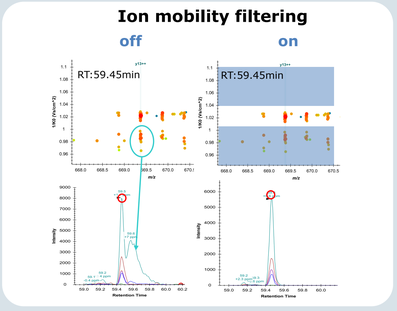

Fig. 4: Increased selectivity as a benefit of the ion mobility separation:it is obtained with all PASEF and tims modes. Illustrated on the heavy peptide TLLSDPTYR.

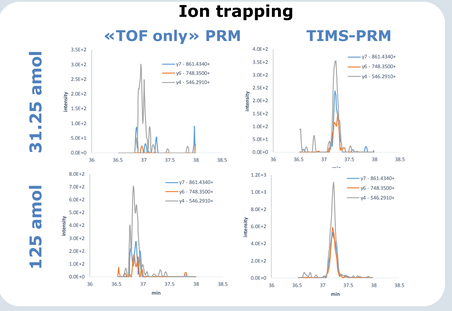

Fig. 6: Increase of sensitivity as a benefit of tims separation
Illustration of the sensitivity improvement in TIMS-PRM mode versus "standard Q-TOF" PRM for the peptide TLLSDPTYR.
Precursor ions are physically accumulated for 100ms and then eluted in 3-4 ms width peaks into the Q-TOF. It improves sensitivity when compared to the signal summation used with a standard Q-TOF setup.

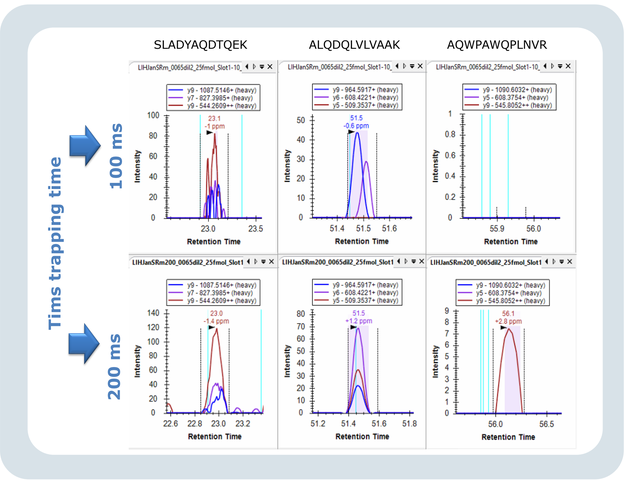

Fig. 5: Improvement of TIMS-PRM sensitivity by increasing trapping time
Limit of detection at 31 amol (in a 100 ng A549 digest) for three example peptides can be improved in TIMS-PRM by increasing the TIMS accumulation time from 100 to 200 ms, despite the non-specific nature of the accumulation.
The parallel accumulation allowed by the dual TIMS configuration keep the duty cycle (close to 100%)

Conclusions

- TIMS-Q-TOF have a strong potential for target proteomics due to an exclusive combination of selectivity, sensitivity and multiplexing.
- The variety of complementary acquisition modes, provides solutions to a variety of analytical challenges
- The great selectivity and sensitivity obtained by tims-PRM can further be improved by optimizing the acquisition parameters (trapping, collision)
- Enabling PASEF-PRM will allow targeting 10X more peptides in the same time frame

timsTOF PRO