Fast and Sensitive Proteomic Analysis of Formalin-Fixed Paraffin-Embedded Tissue Using a Trapped Ion Mobility Q-TOF

ASMS 2019, Poster MP762

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Introduction

Formalin-fixed paraffin-embedded (FFPE) tissue is the standard method of specimen preservation used in pathology departments around the world and as such represents a significant resource for retrospective analysis. Recent advances in sample preparation combined with modern, sensitive LCMS technologies have made such samples available to biomarker research. Sample specimens extracted from tissues are often very small and large sample cohorts demand high sensitivity and rapid sample throughput.

Here we present the use of a QTOF coupled with Trapped Ion Mobility for the highly sensitive and rapid analysis of proteins extracted from laser capture micro dissected human tissue isolates.

Methods

Samples of FFPE human kidney tissue from glomerulus and tubule were deparaffinized with three changes of octane, followed by three changes of 200 proof ethanol, single changes in 90 and 70% ethanol, and two changes of H2O. Proteins were extracted by boiling the tissue extracts for 20 min, followed by a 2 h incubation in a 60 °C water bath for protein recovery.

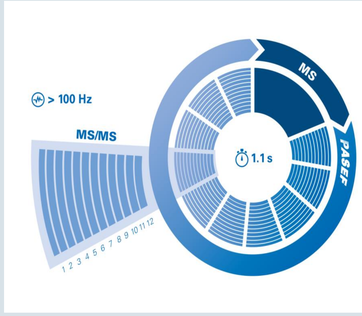

Fig. 1 The PASEF acquisition method: A PASEF cycle (total: 1.1 s) consists of 1 TIMS MS scan (100 ms) and 10 PASEF MS/MS scans (100 ms each) for shotgun proteomics experiments. Per PASEF scan on average 12 different precursors are selected for MS/MS resulting in a sequencing speed of > 120 Hz without a loss in resolution (> 40,000).

Proteins were digested with trypsin at 37 deg C overnight. 500ng of the resulting digested proteins were separated by nano HPLC (nanoElute, Bruker) on 250 mm x 75 µm, 1.6 µm (IonOpticks, Australia). 30 and 90 min gradients at 400nL/min were analyzed on an ion mobility equipped Q-TOF (timsTOF Pro, Bruker Daltonics) operating in PASEF mode at >100Hz (Figure 1). Data were processed in PEAKS X software (Bioinformatics Solutions Inc).

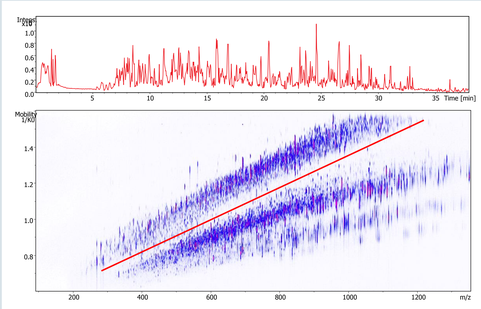

Fig. 2. The base peak chromatogram and TIMS heatmap are shown above. TIMS separates the singly charged background ions from the multiply charged peptides. The acquisition software allows the user to select where precursor selection should occur. Here only ions below the red line are selected for MSMS.

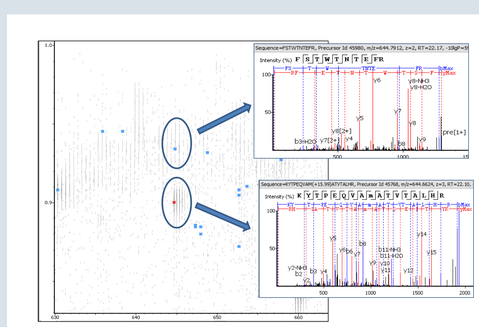

Fig. 3. TIMS separates ions with isobaric or overlapping precursor isotope envelopes. Here two different peptides that differ by 0.13 Da and also coelute are separated based on their CCS values allowing clean MSMS spectra to be acquired for each peptide.

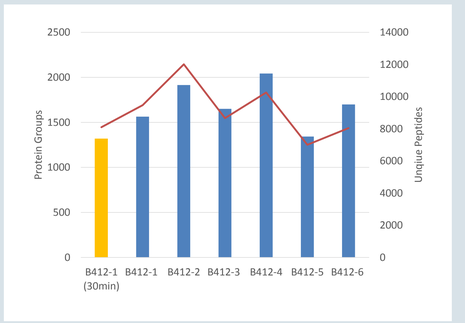

Fig. 4. Protein groups and unique peptides identified across the tissue sections. Blue bars represent data acquired from 90 minute gradients. The yellow bar data was acquired using a 30 min gradient. Reducing the gradient time by two thirds results in a decrease in protein groups and peptides of only 15%.

Results

Trapped Ion Mobility (TIMS) and the PASEF acquisition method on the timsTOF Pro significantly increases speed and sensitivity. The ion mobility separation provided by TIMS clearly separates the singly charged background from multiply charged peptides (Figure 2). This separation allows significantly less precursor overlap, resulting in cleaner more interpretable MSMS spectra. Figure 3 shows the resolution of coeluted, nearly isobaric peptides and the resulting distinct MSMS spectra identified as two unique peptides.

Using a 90 min gradient we identified more than 2000 protein groups and 10,000 unique peptides from FFPE human kidney isolates. We also ran one of the samples using a short 30 min gradient. In this case we identified 85 % of the protein and peptides compared to the same sample on a 90 min gradient. This highlights the capability of the timsTOF Pro to maintain high id levels even when using much shorter gradients. This represents a potential for significantly higher throughput compared with other technology, essential for studying large sample cohorts.

Conclusions

- The outstanding peak capacity and sensitivity of the timsTOF Pro make it very well suited for the identification of proteins from low sample amounts.
- Very high sequencing speeds enable gradient lengths to be significantly reduced while maintaining ID numbers.

timsTOF Pro