Enhanced peptide mapping with a PASEF enabled ultra high resolution QTOF

CASSS AT Europe 2018, Poster P-127-T

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Overview

Application of PASEF scans to peptide maps enhances sensitivity and data quality.

Introduction

Part of the regulatory requirements when developing a biological drug involve the in-depth characterization of low level heterogeneities, all the way down to part per millions of the drug. This is usually carried out with a peptide map and MS/MS driven by data dependent acquisition (DDA) for the identification of the peptides.

In this work we describe how the PASEF (Parallel Accumulation and Serial Fragmentation) acquisition method recently implemented on the timsTOF Pro QTOF (Fig. 1) can be applied to peptide mapping experiments. The implementation as done to achieve the goal of sensitive detection together with enhanced speed and data quality.

Methods

Sample

The NISTmAb Reference Material 8671 was reduced using DTT in TFE solution and alkylated with iodacetamide prior to overnight digestion with trypsin (Promega). 2 % acetonitrile and 0.1 % formic acid was added prior to UHPLC-MS.

LCMS Analysis

The tryptic peptides were separated using an Elute UHPLC (Bruker) equipped with an Intensity Solo 2 1.8 µm C18 100 x 2.1 mm column (Bruker). For peptide mapping a 5 minute gradient (2-10 % B in 30 seconds, 10 - 40 % B in 4.5 minutes at 100 µL/min) was used with a total runtime of 14 minutes. For HCPs a non-linear gradient from 2 - 40 % B in 150 minutes at 50 µL/min was used. PASEF spectra were acquired using a timsTOF Pro Q-TOF mass spectrometer (Bruker).

Discussion

PASEF acquisition method

Trapped Ion Mobility Spectrometry (TIMS) makes it possible to separate ions based on collisional cross section. With 2 TIMS cells in series, this can be done without ion loss (Fig.2). This additional dimension of separation make it possible to focus precursor ions in a small window in time and space.

Combining this property with fast electronics, it is possible to select precursors with a specific m/z and collisional cross section using the PASEF scan mode. The focusing effect of the TIMS cell improve the ion utilization rate (up to 100%) while increasing the duty cycle of MS/MS experiments. A high sequencing speed of over 100 Hz is reached in PASEF experiments.

In addition to the speed and sensitivity gains, the additional dimension of separation reduces the risk of acquiring chimeric spectra and provides optimal MS/MS data for the protein search engine and facilitate manual validation.

Accelerate peptide maps

Peptide mapping is one of the preferred techniques to assess primary sequence of a biomolecule. This assay is performed in high volumes and reducing the length of the measurement and data processing can afford substantial gains in productivity.

Using the PASEF technique for peptide mapping makes it possible to obtain extensive MS/MS sequence coverage even with very short LC gradients. As the precursor selection selectivity is controlled by the drift time and the quadrupole, this approach affords a great degree of selectivity and generate high quality MS/MS data.

Near 100% sequence coverage of NISTmab is easily achieved in 5 min (Fig. 3) while maintaining over 90% MS/MS coverage ensuring reliable sequence verification.

Improved host cell proteins (HCP) identification

Peptide mapping is also used to determine the presence of low level heterogeneities such as host cell proteins (HCPs). In the case of HCPs, this requires reliable identification of proteins present in a ratio of a 1 to 100 ppm of the therapeutic protein. This expansive dynamic range usually requires special approaches such as 2D-LC, libraries or extreme sample loads.

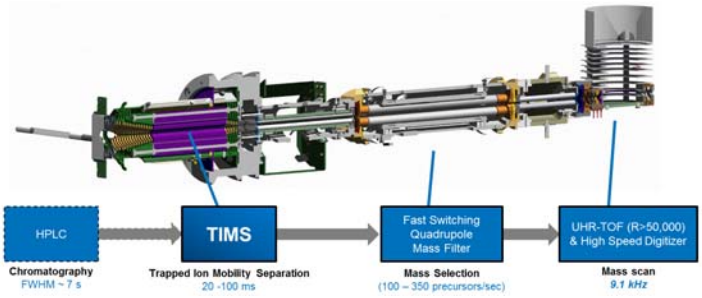

Fig. 1: Scheme of timsTOF Pro

Protein	MW [kDa]	pI	No. of Peptides	SC [%]
Fructose-bisphosphate aldolase A	39.3	8.3	15	53.0
Fructose-bisphosphate aldolase C	39.4	6.7	11	40.5
Glucose-6-phosphate isomerase	62.7	8.1	10	34.8
Protein disulfide-isomerase A6	48.1	5.0	4	13.4
Low affinity immunoglobulin gamma Fc region receptor II	36.7	6.2	3	17.6
Beta-2-microglobulin	13.8	8.6	3	22.7

Fig. 4: Example of proteins identified in NISTmab with a 1D-LC PASEF experiment with at least 3 unique peptides

Thanks to their ability to focus precursor ions, PASEF scans makes it possible to reliably identify very low abundant proteins.

Using a standard 1D-UHPLC-MS setup with a 10 cm 2.1 mm ID column and a 30 µg load, a number of known NIST HCPs in the 1-100 ppm range could be identified with at least 3 unique peptides (Fig. 4). This allows for a confident identification, reduces the amount of necessary manual validation and makes it possible to apply the "Top 3" quantitation approach.

As already discussed in the peptide map section, good quality MS/MS spectra can be obtained despite the very low abundance of the precursor. For example, Fig. 5 shows the MS/MS spectra for 3 ß-microglobulin peptides. Despite being reported at levels in between 7 and 12 ppm in the literature, this HCP could be reliably identified using the standard Bruker peptide mapping setup.

Further evaluation is now ongoing to develop a more specialized assay and further increased HCP coverage.

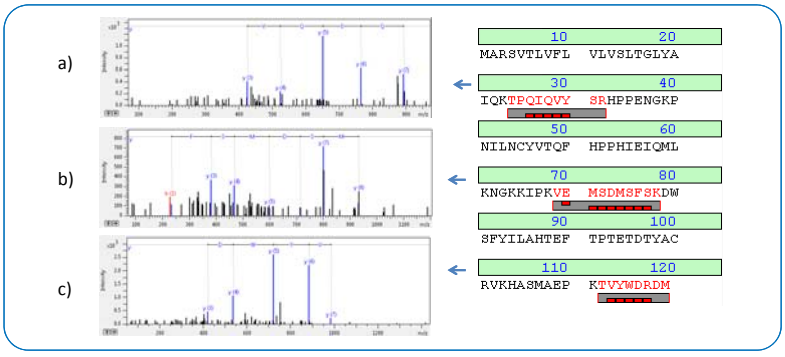

Fig. 5: Unique ß-microglobulin peptides identified by 1D-LC MS in the NISTmab reference material

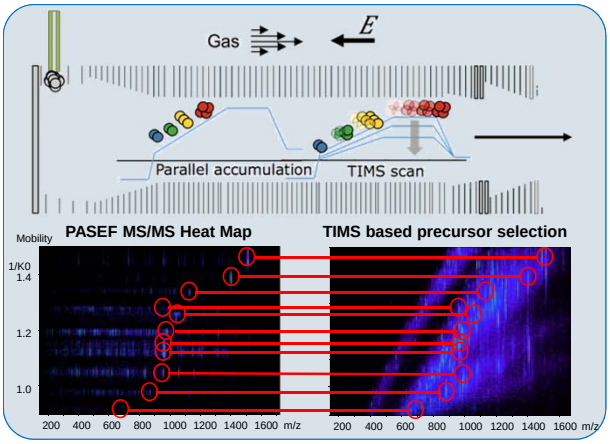

Fig. 2: Top - Accumulation and focusing of precursor ions in the TIMS cell. Bottom - PASEF scan allows 2 dimensional precursor isolation

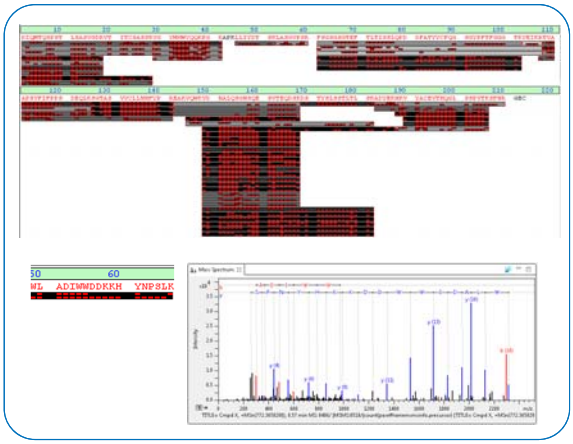

Fig. 3: Top – High MS/MS coverage of the NISTmab light chain in 5min Bottom – MS/MS spectrum for heavy chain peptide 49-66

Conclusions

- Proteomics application of PASEF scans demonstrate improved identification abilities
- The speed improvement afforded by the timsTOF PRO has the potential to significantly accelerate LC-MS based peptide mapping experiments
- The sensitivity of the PASEF based workflows is well suited to the analysis of low level heterogeneities such as HCPs

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