Application of PASEF MS/MS scans to monoclonal antibody peptide mapping

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Introduction

Part of the regulatory requirements when developing a biological drug involves the verification of the primary sequence and in-depth characterization of low level heterogeneities. This is usually carried out with a peptide map and data dependent analysis MS/MS.

Here we describe how PASEF scans (parallel accumulation and serial fragmentation) are implemented on the timsTOF PRO QTOF and how they can be applied to peptide mapping, improving sensitivity, speed and selectivity.

Methods

Digestion

The NISTmAb Reference Material 8671 was reduced and alkylated with prior to tryptic digestion.

15 minute LC/MS Analysis

The peptides were separated using an Elute UHPLC (Bruker) equipped with an Intensity Solo 2 1.8µm C18 100 x 2.1 mm column (Bruker). A 5µg injection and 10 min gradient (2-40 %B) at 100 µL/min) were used.

PASEF MS/MS spectra were acquired using a timsTOF Pro Q-TOF mass spectrometer (Bruker) with 100 ms mobility frames and up to 12 precursors per frame.

Data was searched in Byonic (Protein Metrics), with common modifications including the most common N-glycan.

PASEF scans

Trapped Ion Mobility Spectrometry (TIMS) makes it possible to accumulate, separate and select precursors based on collisional cross section without ion loss.

This capability enables a novel MS/MS scan mode (PASEF, Fig.1a) which utilizes the focusing properties of TIMS to increase MS/MS speed selectivity and sensitivity at the same time.

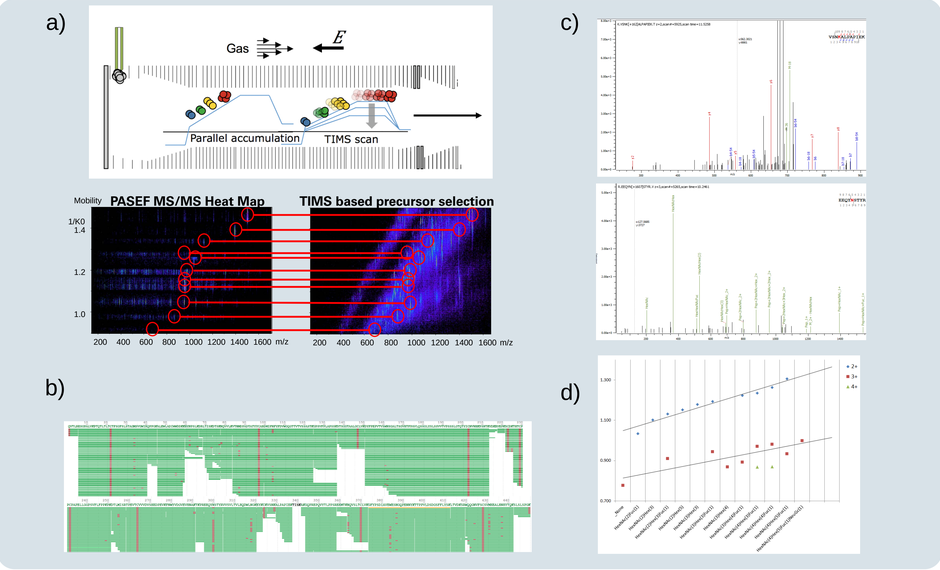

Fig.1 a) Schematic of PASEF based workflow - b) Heavy chain coverage map
c) MS/MS spectra for glycated K4 and G1F glycopeptide - d) Mobility of major glycans at N300

Results

The rapid 10 min gradient was sufficient to fully confirm the sample primary sequence (Fig.1b).

PASEF scans affords up to 120 fragmentation events per second. This allows the identification of low level modifications such as oxidation, deamidation and glycation (Fig.1c) with good signal to noise despite the relatively short gradient time.

Moreover the selectivity of TIMS based on ions collisional cross section makes it possible to improve spectral quality by reducing the risks of chimeric spectra (Fig.2). For example the glycation on the N-terminal peptide of the heavy chain can be determined despite the presence of 2 other peptides in a +/- 0.7 Da window.

Aside from acting as tool to filter out noise and improve the precursor picking, mobility also informs on the shape of the ion, with the mobility term increasing for ions of larger size or lower charge. Fig.1d shows the mobility of the main forms of the heavy chain peptide containing the conserved glycosylation typical of IgG (N300). As the glycan modification grows in molecular weight, so does the mobility term. This offers an additional parameter to validate glycopeptide identifications.

Since many glycopeptides coelute using reverse phase HPLC conditions, the PASEF method of concentrating and serially fragmenting ions shows orthogonal separations such as TIMS is a strong asset for the characterization of glycoproteins.

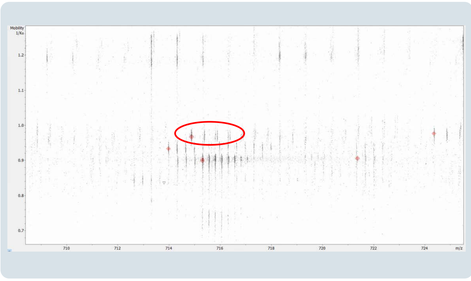

Fig.2 Mobility based separation of low intensity glycated peptide (K4) from coeluting peptides in same m/z range (2+ and 3+)

Conclusions

- Full mAb sequence coverage under rapid gradient (10 min) conditions
- Sensitive detection of highly distributed modifications such as lysine glycation
- Separation of chimeric MS/MS spectra using TIMS as a precursor selection tool
- PASEF improves glycopeptides sequencing rate and quality

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