Trapped Ion Mobility Spectrometry and PASEF Enables In-depth Characterization of Protein Ubiquitination

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Introduction

Ubiquitination is a post-translational modification (PTM) that is essential for regulating numerous biological processes, including protein degradation, immune response, signal transduction and DNA repair. Mass spectrometry (MS) based platforms have proven to be a powerful tool enabled proteome-wide profiling of PTMs at site-specific level. However, the characterization of global ubiquitination is still challenging owing to the sub-stoichiometric abundance of this PTM and co-elution of ubiquitinated peptides only differing in the position of the modified site. Here, we performed an in-depth characterization of enriched ubiquitinated peptides from human cells by trapped ion mobility (TIMS) and PASEF. Strikingly, over 17000 ubiquitinated sites and 5093 ubiquitinated proteins were identified from two cell lines.

Methods

Ubiquitinated peptides were enriched from 2 mg cell lysates with enrichment kit (PTM Biolabs, Cat: PTM-1104). Samples were analyzed by LC-MS/MS using a nanoElute LC (Bruker Daltonics) coupled to a trapped ion mobility equipped Q-TOF mass spectrometer (timsTOF Pro). Enriched peptides were separated in a C18 column (25 cm, 100 µm i.d., 1.9 µm) packed in house using a 50-minute gradient ranging from 2% to 37% acetonitrile (0.1% FA) at 450 nL/min. The column temperature was kept at 50°C. MS data were searched against the Human SwissProt database (20422 entries) using MaxQuant software version 1.6.6.0. The minimal modified peptide ion score was set to 40, and a false discovery rates (FDR) of 1% was used for peptide spectrum matches and protein identifications.

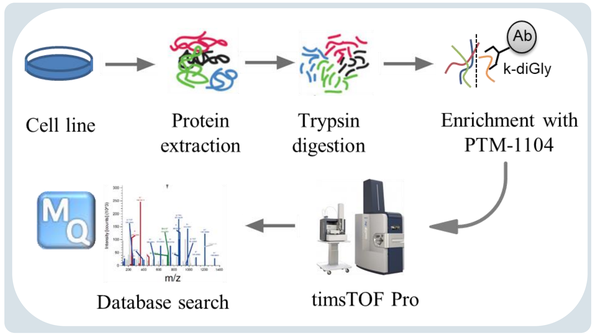

Fig. 1. The workflow of in-depth characterization of protein ubiquitination. Ubiquitinated peptides enriched from cell lysates with antibody were analyzed by timsTOF Pro, the sites of which were identified using MaxQuant.

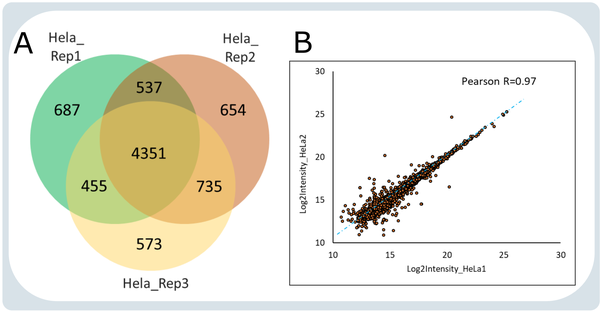

Fig. 2. The reproducibility of ubiquitinated peptides identified by timsTOF Pro. A) Overlap of HeLa ubiquitinated peptides identified from three injections. B) Scatter plot of LFQ intensities of ubiquitinated peptides between two technical replicates (Pearson correlation R=0.97).

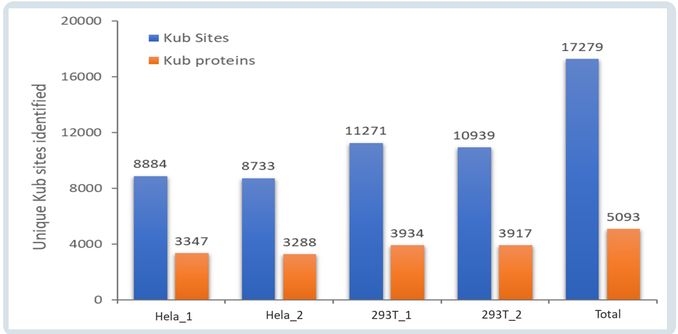

Fig. 3. The identification of ubiquitinated sites and proteins from different human cell lines with 50 min gradient. For each cell line, two replicates were performed.

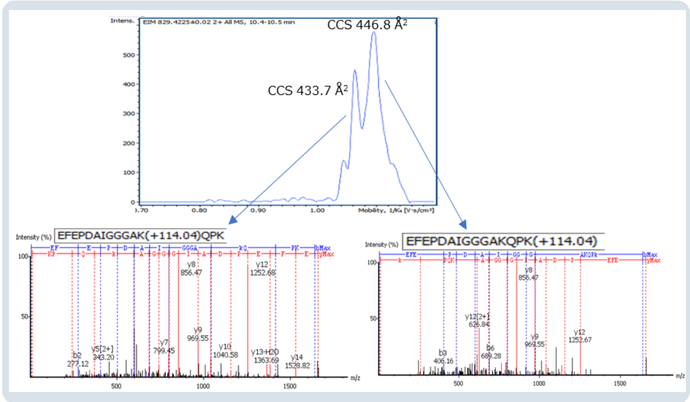

Fig. 4. The separation of the two co-eluting isobaric ubiquitinated peptides with different modification sites by ion mobility. A) The extracted ion mobilogram for 829.4115 *m/z* at 10.45 min. The isomers can be resolved by CCS value. B) Non-chimeric MS/MS spectra of the two site-specific ubiquitinated peptides.

Results

Benefiting from the high affinity enrichment kit and the high sensitivity timsTOF Pro mass spectrometer with ion mobility separation ability, in-depth profiling of protein ubiquitination was available. In the benchmark study using HeLa lysates, highly reproducible qualitative and quantitative identification of ubiquitination (R= 0.97, Fig. 2) was achieved. After enrichment optimization from 2 mg of initial material, above 8700 and 10000 ubiquitination sites can be identified from HeLa and 293T cell lines, respectively. In total, more than 17000 ubiquitination sites were identified from 4 samples with 50-min gradient only, corresponding to 5093 ubiquitinated proteins (Fig. 3). Additionally, the TIMS provides one more dimension of separation that enables to distinguish and quantification of co-eluting isobaric peptides with different modification sites (Fig. 4).

References

(1) He et al., Plant J. 2020, 101(6):1430-1447

(2) Meier F et al., Mol Cell Proteomics. 2018, 17(12):2534-2545

Conclusions

- High performance provided by TIMS and PASEF enables in-depth characterization of protein ubiquitination even with short LC run.
- High reproducibility of identification and label free quantification can be achieved combining high affinity enrichment kit and high sensitivity timsTOF Pro.
- TIMS with the separation ability at one more dimension, ion mobility, allows site-specific isomer identification.

timsTOF Pro