High sensitivity lysine acetylation profiling with Trapped Ion Mobility Spectrometry and PASEF

ASMS 2020, WP 507

Xianming Liu[1], Jun Zhu[2], Chen Bu[2], Lianqi Zhou[2], Ning Chen[3], Xiaoxian Du[1], Shourjo Ghose[4], Stephanie Kaspar-Schoenefeld[5], Heiner Koch[5].

[1]Bruker Daltonics, Shanghai, China
[2]PTM Biolabs Inc, Hangzhou, Zhejiang Province, China.
[3]Bruker Daltonics, Beijing, China
[4]Bruker Scientific LLC, Billerica, MA, US
[5]Bruker Daltonics, Bremen, Germany.

Introduction

Lysine acetylation plays important roles in living cells, including transcriptional regulation, cell cycle, apoptosis and so on. The comprehensive profiling of protein acetylation by MS-based proteomics technology remains a huge analytical challenge, which is mainly related to the low abundance and the high dynamic range of acetylation, as well as the deacetylation in various cellular contexts. Here, a high-affinity lysine acetylation enrichment kit was used for enrichment of lysine acetylated peptides. Afterwards, a QTOF equipped with trapped ion mobility using the PASEF acquisition mode was used for the profiling of lysine acetylation, and almost 18000 lysine acetylated sites were identified using a 50-min gradient in rice leaf samples.

Methods

Acetylated peptides were enriched from mouse liver and rice (*Oryza Sativa*) leaf samples with a lysine acetylation enrichment kit (CAT PTM-104, PTM-Biolabs). All the digests were analyzed with a timsTOF Pro mass spectrometer (Bruker Daltonics) equipped with a nanoElute UHPLC (Bruker Daltonics) and a home-packed RP C18 column (25 cm, 100 µm I.D., 2.0 µm). Separation was performed at a flow rate of 450 nL/min at 50°C. Elution gradient was increased from 5% to 22 % B within 50 min, acquired data were searched by MaxQuant (v 1.6.6.0) and Mascot (v2.5) against SwissProt database of mouse (17018 sequences) and Uniprot of Oryza_sativa_MH63RS2 (83258 sequences). The false discovery rate (FDR) was set at below 1% for peptide spectrum matches and protein group identifications.

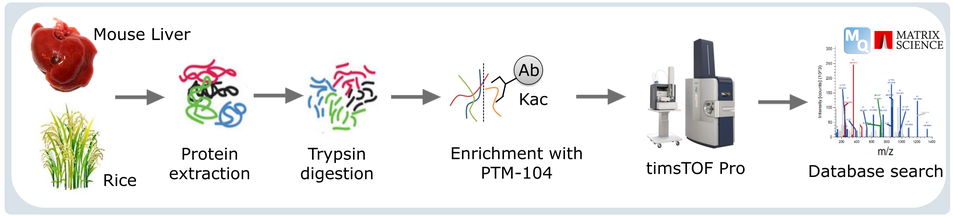

Fig. 1. Workflow for acetylation profiling. The resulting samples were analyzed by timsTOF Pro. Data were processed with MaxQuant and Mascot.

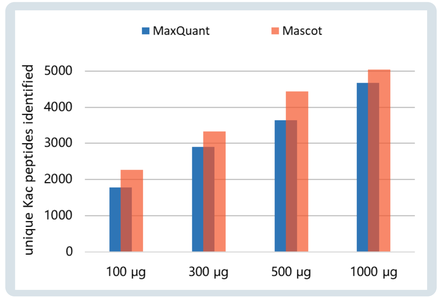

Fig. 2 Identification of the acetylated lysine peptides with four different starting amount (mouse liver).

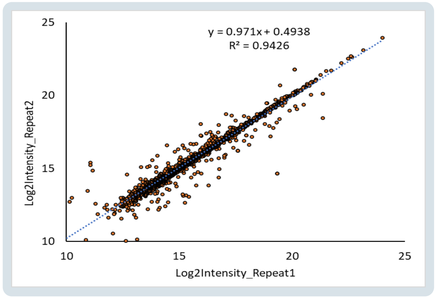

Fig. 3 Biological reproducibility of the acetylated lysine peptides (mouse liver)

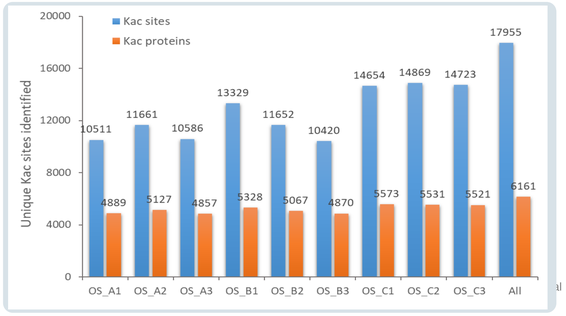

Fig. 4 Identification of acetylated sites and protein groups in 9 rice leaf samples

Results

PASEF as a sensitivity enhancer

The increased peak capacity encouraged from the extra separation by TIMS and increased sequencing speed by PASEF enable the in-depth analysis of lysine acetylation even with short gradient and low sample amount.

Together with the high affinity enrichment kit and the highly sensitive timsTOF Pro, over 1800 acetylated lysine peptides can be identified with 100 µg of starting materials and 50 min gradient in mouse liver sample (Fig. 2), nearly 5000 acetylated lysine peptides can be identified with 1000 ug of starting materials. The Log2 LFQ intensity of acetylated lysine peptides shows a good reproducibility with R^2> 0.94 (Fig. 3).

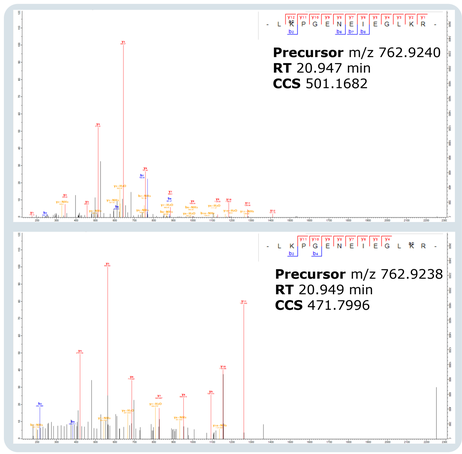

Fig. 5 Co-eluting of two acetylated lysine peptides. The two peptides have the same RT, but can be separated by TIMS, resulting in non-chimeric MS/MS spectra that enable the right localization.

PASEF for lysine acetylation profiling in rice

For rice leaf samples analysis (Fig. 4), MaxQuant was used for data processing and performed a strict filtration. Over 4800 protein groups, 10000 lysine acetylated sites were identified in a single shot (50 min gradient). Cumulatively, almost 18000 lysine acetylated sites, over 6100 protein groups were identified in nine rice leaf samples.

TIMS as an extra dimension of separation

TIMS provides an extra dimension of separation which enables more reliable qualitative and quantitative analysis of PTMs. Fig. 5 shows a good example of two co-eluting lysine acetylated peptides with the same m/z and RT can be separated by TIMS, leading to the right localization of acetylation site.

References

(1) Meier et al.; Journal of Proteomics Research 2015

Conclusions

- Combined with the enhancement of speed and sensitivity, TIMS-PASEF enables more identification of acetylation sites from lower sample amount with shorter LC gradient.
- The extra dimension of separation provided by TIMS enables to discern co-eluting positional isomer and get more specific MS/MS spectra.
- The advantages of TIMS-PASEF shown here make timsTOF Pro the ideal tool for the comprehensive characterization of PTMs.

timsTOF Pro