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

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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 abundances 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 TIMS TOF Pro mass spectrometer (Bruker Daltonics) equipped with a nanoEase UHPLC (Bruker Daltonics) and a home-packed 4F C18 column (25 cm, 100 μm I.D., 2.5 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 liver (Oryza sativa sequence) and Uniprot of Oryza sativa M463852 (83258 sequences). The false discovery rate (FDR) was set at below 1% for peptide spectrum matches and protein group identifications.

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 samples (Fig. 2), nearly 5000 acetylated lysine peptides can be identified with 1000 μg of starting materials. The Log2 LFQ intensity of acetylated lysine peptides shows a good reproducibility with R²>0.94 (Fig. 3).

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.