Improved Immuno-affinity Enrichment Combined with Trapped Ion Mobility Mass Spectrometry for Significantly Improved PTM Sensitivity

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Introduction

Post-translational modification of proteins represents an essential mechanism that regulates the function and abundance of proteins and is critical to a wide variety of cell processes such as signal transduction, cell development and mitosis. Post-translationally modified peptides are often present in low abundance and there is a need to characterize PTMs from limited starting material. Here we combine an improved immuno-enrichment methodology with high sensitivity trapped ion mobility mass spectrometry for improved sensitivity from less starting material.

Methods

Post-translationally modified peptides were prepared from human HCT116 cells protein tryptic digests using two different PTMScan® HS kits (Cell Signaling Technology), each enriching a specific class of PTM: acetyl-lysine and the ubiquitin remnant K-ε-GG from 1 mg starting material. The resulting extracts were separated by nano HPLC (nanoElute, Bruker) on 250 mm x 75 µm, 1.6 µm (IonOpticks, Australia). 80 min gradients were analyzed on a trapped ion mobility Q-TOF (timsTOF Pro 2, Bruker Daltonics) operating in PASEF (Parallel Accumulation and Serial Fragmentation) mode. Ions were accumulated in the first TIMS analyzer and eluted based on their mobility in the second analyzer using 166 ms ramp times. Data were searched against Human and Mouse fasta databases using PEAKS Online X build 1.4.2020-10-21 (Bioinformatics Solutions Inc, Waterloo, Canada).

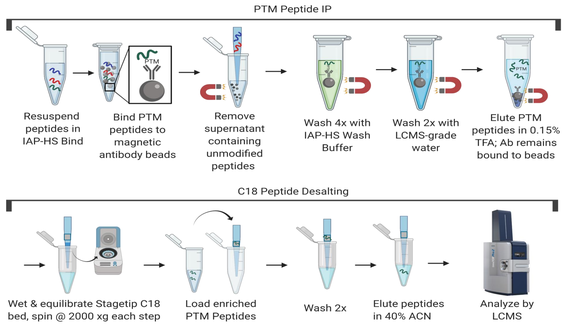

Figure 1. PTMScan® HS workflow SDS lysis followed by S-Trap™ digestion & cleanup efficiently extracts peptides for immunoaffinity enrichment using HS magnetic beads, which allows for single desalting step prior to LCMS.

Sample	Proteins	Peptides	Unique K-ε-GG Peptides	% Modified Peptides
KGG PTMScan Classic	2423	10796	2566	24%
KGG PTMScan HS_1	1328	4649	3250	70%
KGG PTMScan HS_2	1431	5227	3500	67%

Figure 2. An average of 3375 K-GG peptides were identified by the timsTOF Pro 2. PTMScan® HS workflow enriches more unique K-GG peptides compared to the PTMScan Classic. Selectivity is improved as shown by the percent of identified peptides that contain the KGG modification. A 65% improvement in selectivity was observed.

Sample	Peptides	Unique AcK Peptides	% Modified Peptides
Human AcK_1	7184	4291	60
Human AcK_2	7254	4447	61
Human AcK_3	7394	4338	59

Figure 3. Acetylated lysine modified peptides enriched from 1 mg of HCT116 cells.

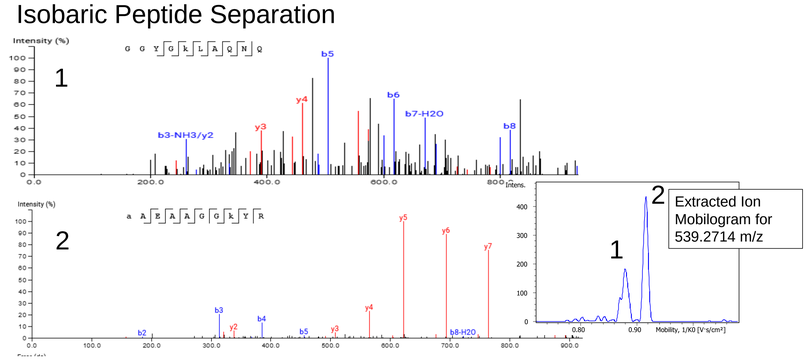

Figure 4. High resolution TIMS resolves coeluting, isobaric peptides. Here two peptide coeluting at 32.9 min and differing only by 0.003 Da are separated by the different CCS valves (inset).

Results

New PTMScan® HS antibody enrichment workflow (CST) (Figure 1), significantly improved sensitivity and specificity for ubiquitinated peptides. A 32 % improvement in the number of K-GG peptides enriched using the new workflow was observed (Figure 2). Additionally, a 65 % improvement in selectivity for K-GG modified peptides was obtained compared to the previous workflow.

The increased peak capacity from the extra dimension of separation provided by TIMS and increased sequencing speed of the PASEF method enables large numbers of PTM identifications from small sample amounts. Here an average of 3275 K-GG peptides and 4359 AcK peptides were identified from 1 mg of starting material (Figures 3 and 4)

The very high mobility resolution of the TIMS analyzer on the timsTOF Pro enabled separation of coeluting, isobaric peptides (Figure 4). This is achieved while maintaining high sensitivity and a very high duty cycle. Allowing isobaric peptide separation in complex samples.

Conclusions

- Optimized K-GG peptide immuno-affinity enrichment method improves sensitivity by requiring less sample input, specificity by increasing fraction of ubiquitinated peptides recovered, and simplicity by taking fewer days to complete.
- The outstanding peak capacity and sensitivity of the timsTOF Pro make it very well suited for the identification of low abundance post translational modifications from small sample amounts.

timsTOF Pro 2