Rapid and Sensitive Phosphotyrosine Detection from Limited Sample Amounts

Matthew Willetts[1], Diego Assis[1], Elizabeth Gordon[1], Barry Zee[2], Alissa Nelson[2], Hayley Peckham[2], and Matthew Stokes[2]

[1]Bruker Daltonics, Billerica, USA, [2]Cell Signalling Technology Inc, Danvers, MA, USA

Introduction

Phosphorylation 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. Compared to proteins phosphorylated at serine and threonine, the abundance of tyrosine-phosphorylated (pY) proteins in total cell lysate is low, with pY constituting an estimated 1% of all phosphorylated residues. Improvements in enrichment strategies, instrument sensitivity, and post acquisition data processing are required to address this issue. Here we combine an improved immuno-enrichment methodology with a trapped ion mobility mass spectrometer and unique TIMScore for the detection of pY proteins from human cells.

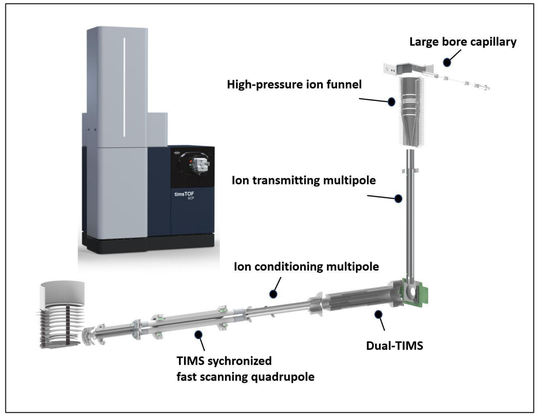

Fig. 1 The timsTOF SCP features new ion source geometry with additional higher pressure vacuum stage. This results in a 4-5 times increase in ion current while maintaining the industry leading robustness of the timsTOF Pro 2

Methods

Peptides were prepared using trypsin digestion of proteins derived from human gastric cancer HCT116 cells and subjected to PTMScan® HS Phospho-Tyrosine (P-Tyr-1000) kits (Cell Signaling Technology), to enrich for pY peptides independently of flanking amino acid sequences. Enriched peptides were desalted using in-house constructed C18-STAGE-Tips. The resulting extracts were reconstituted in 20uL of 0.1% formic acid in water and 5uL were separated by nano HPLC (nanoElute, Bruker) on 250 mm x 75 µm, 1.6 µm (IonOpticks, Australia). 30 min gradients were analyzed on a trapped ion mobility Q-TOF (timsTOF SCP, Bruker Daltonics) operating in PASEF (Parallel Accumulation and Serial Fragmentation) mode. Data were processed in real time using PaSER software (Bruker Daltonics). The MSMS spectra were searched against the Uniprot Human reviewed database with and without TIMScore.

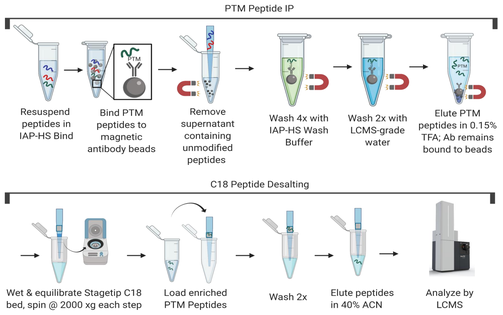

Fig. 2 . PTMScan® HS workflow SDS lysis followed by S-Trap™ digestion & cleanup efficiently extracts peptides for immunoaffinity enrichment using HS magnetic beads, which allows for single desalting step prior to LCMS.

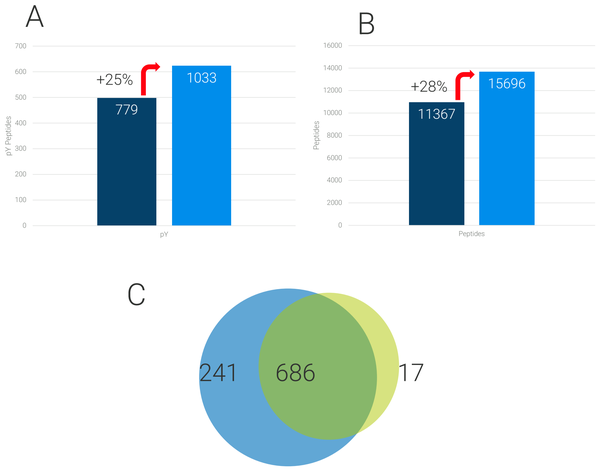

Fig. 4 A and B)) A total of 1033 unique phosphotyrosine peptides and 15696 unique peptides in overall were identified using TIMScore in PaSER. C) The search using TIMScore identified 127 unique phosphotyrosine compared with 19 in the search without TIMScore.

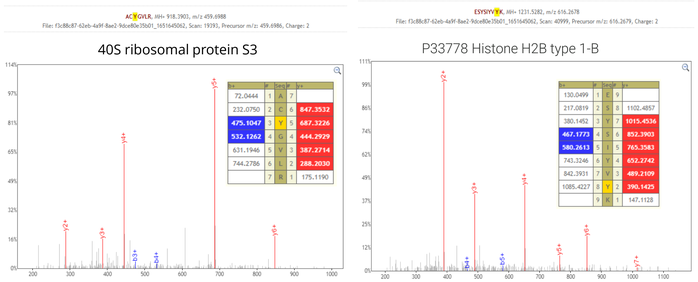

Fig. 5. Examples of two peptides which were only identified using TIMScore

Results

- timsTOF SCP (Figure 1) features a new transfer capillary with a later internal diameter and an addition higher pressure vacuum stage which results in a 4-5 times increase in ion current making it ideally suited to applications where sample amounts are limited such as phosphotyrosine analysis.
- New PTMScan® HS antibody enrichment workflow (CST) (Figure 2), significantly improves sensitivity and specificity for phosphorylated peptides.
- 1033 phosphotyrosine peptides were identified from enriched HCT116 cells.
- TIMScore™ utilizes this CCS value in a database search algorithm boosting the number of peptide spectrum matches (PSMs), peptides and proteins by utilizing a machine learning model to predict CCS and calculate how well the measured CCS fits the prediction. This resulted in a 28% increase in the number of identified peptides and a 25% increase in the number of phosphotyrosine peptides.

Conclusion

- 1033 phosphotyrosine peptides were identified from 1mg of HCT116 cells
- PTMScan HS Phospho-Tyrosine kits streamline the process of enrichment from small amounts of starting material
- Real-time database search in PaSER gives virtually instantaneous results as soon as the run is finished.
- TIMScore identifies 25 % more phosphotyrosine peptides.

Technology