The PASEF method on a TIMS-QTOF mass spectrometer for High Sensitivity Phosphoproteomics

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Introduction

Developments in high resolution mass spectrometers and specific enrichment of phosphorylated peptides tailored for the global analysis of protein phosphorylation serve as a powerful tool for cellular biologists studying signal transduction pathways. However, the abundance differences in protein phosphorylation span several orders of magnitude, pushing the need for instruments with higher sensitivity and increased peak capacities. Here we evaluate the performance of a unique dual trapped ion mobility spectrometer (TIMS) coupled to a QTOF that provides an additional dimension of separation and higher peak capacity. When operated with the Parallel Accumulation Serial Fragmentation (PASEF) method, it also allows for operation at nearly 100 % duty cycle with very high sequencing speeds. Additionally the TIMS separation allows resolution of co-eluting isobaric species including peptides that only differ by the site of phosphorylation.

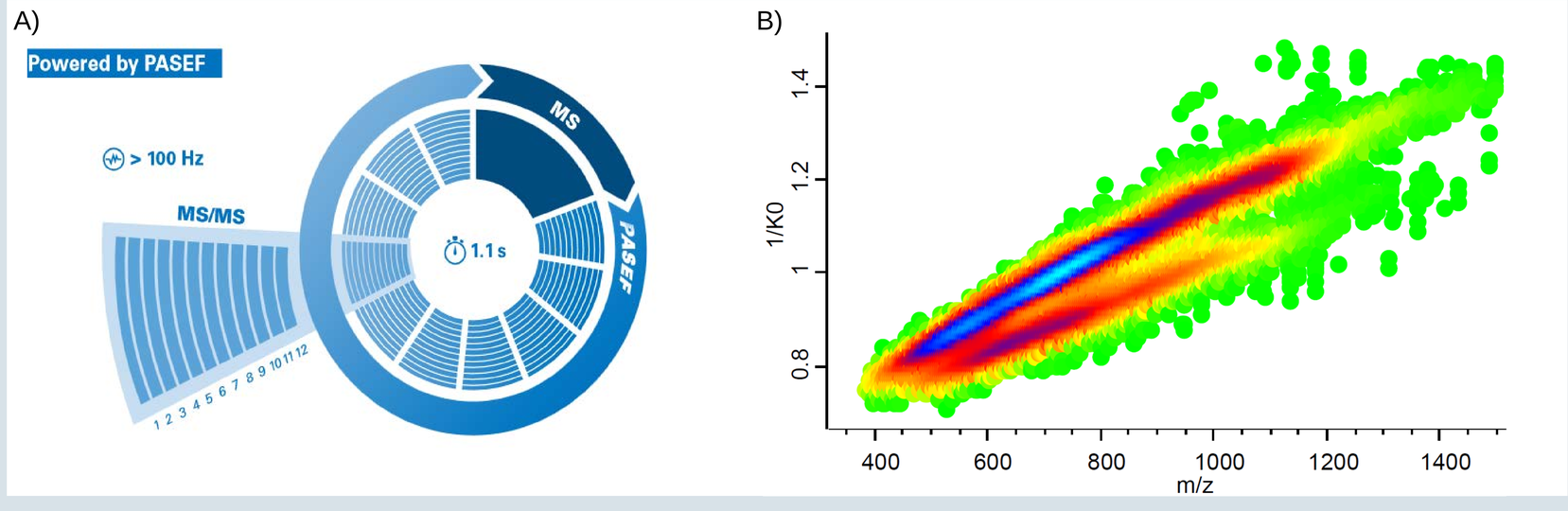

Fig.1 A) PASEF acquisition scheme for sequencing speeds >100 Hz. B) Density heatmap of identified phosphopeptide features eluting from the nano-LC separation over the 90 min gradient in the 1/K0 vs m/z space

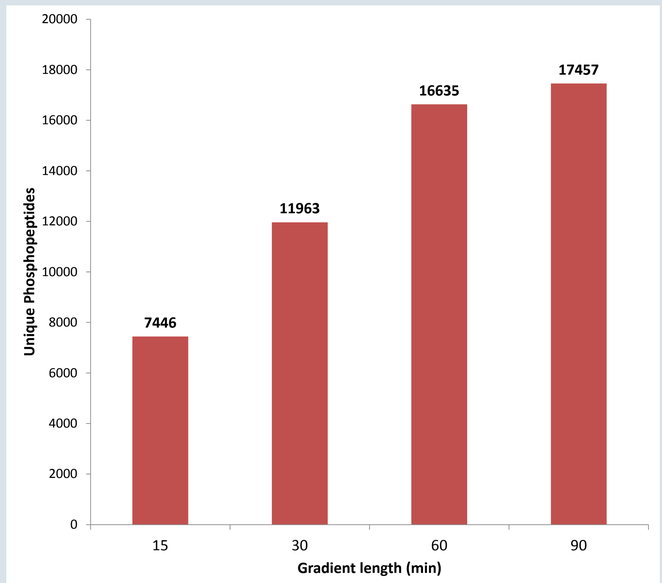

Fig. 2.Unique phosphopeptides (1% FDR) identified from enriched HeLa cell digests using 15, 30, 60 and 90 min gradients.

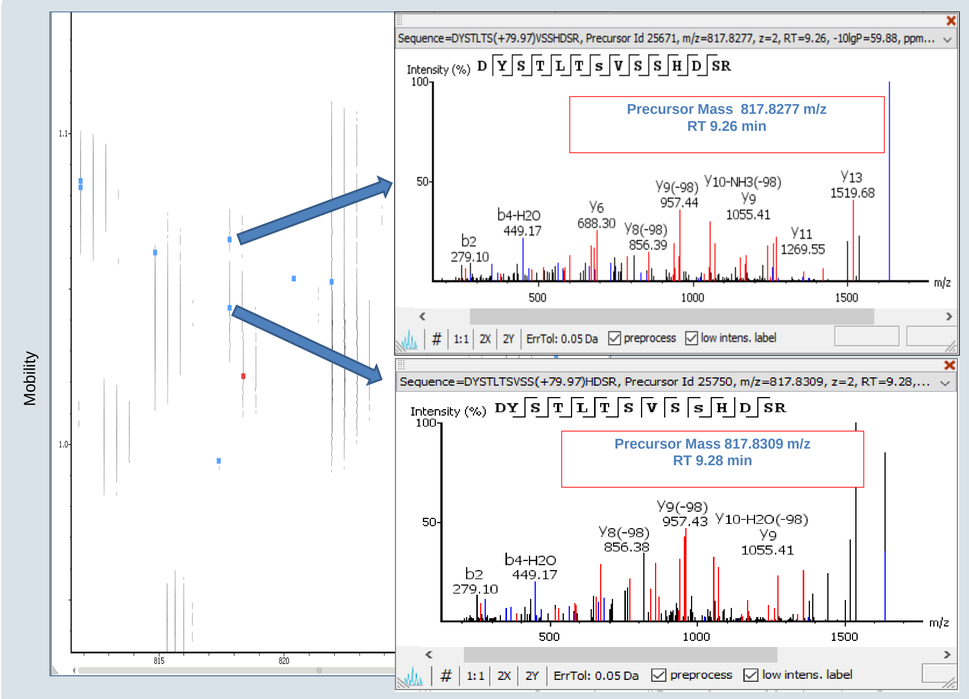

Fig. 3. Co-eluting phosphopeptides that differ only by the phosphorylation localization site are separated by TIMS, resulting in non-chimeric MS/MS spectra that enable localization of the different sites of phosphorylation.

Methods

Phosphopeptides from HeLa cell digests were IMAC enriched as previously described (Ruprecht et al., 2015), separated by nano-HPLC (nanoElute, Bruker Daltonics) on a 250 mm pulled emitter column (IonOpticks, Australia) and analyzed on a high resolution TIMS enabled QTOF instrument using the PASEF method (timsTOF Pro, Bruker Daltonics). Peptide amount corresponding to pre-enrichment ranged from 50-200 µg and were separated on 15 min, 30 min, 60 min and 90 min gradients (2-30% ACN). A PASEF cycle of 1.1 s was used equating to a 100 ms TIMS MS scan followed by ten 100 ms PASEF MS/MS cycles each fragmenting up to 12 precursors (Figure 1A). Feature extraction and database searching were performed in PEAKS 8.5 (Bioinformatics Solutions Inc.)

Results

Resolution of co-eluting isobaric peptides

Co-eluting peptides that are isobaric or have overlapping precursor ion isotope envelopes can be resolved using Trapped Ion Mobility Spectrometry resulting in clean MS/MS spectra. Figure 3 shows an example where two co-eluting peptides with same sequence but different sites of phosphorylation were separated based on their collisional cross sections enabling discrete MS/MS spectra which were confidently assigned to different phosphopeptides by PEAKS database search. This would not be possible on a conventional non-IMS mass spectrometer

Phosphopeptide ID with PASEF

The increased peak capacity from the extra dimension of separation provided by the TIMS and increased sequencing speed of the PASEF method enables very large numbers of phosphopeptide identifications from short gradients and low sample amounts. More than 17,400 unique phosphopeptides were identified from the 90 min separation (Figure 2). Approx. 7500 unique phosphopeptides were identified in the 15 min gradient from only 50 µg of total protein before enrichment.

Conclusions

- PASEF on the timsTOF Pro identified more than 17,400 phosphopeptides from a 90 min gradient.
- Almost 7500 unique phosphopeptides were confidently identified in a 15 min gradient.
- Extra dimension of separation provided by TIMS allowed resolution of co-eluting, isobaric peptides

timsTOF Pro