Reducing Compression Effects and Expanding the Multiplex Capabilities on a timsTOF Pro with PASEF

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Introduction

- Multiplexing more channels has obvious advantages, but neutron encoded mass tags have a mass defect requiring >60,000 mass resolution at 100 m/z for baseline resolution.
- Here we demonstrate the multiplexing of 9 channels
- Accurate quantitation is afforded because baseline resolution of each reporter ion is achieved.
- We demonstrate different trapping times in a trapped ion mobility spectrometer (TIMS) operated in varying parallel accumulation serial fragmentation (PASEF) mitigates the compression effect common to isobaric mass tagging experiments.

Materials and Methods

- HeLa (Pierce), yeast and K562 (Promega) digests aliquoted at ratios of 4:4:1
- Data analysis was performed with PEAKS X+, with a peptide FDR of 1%.

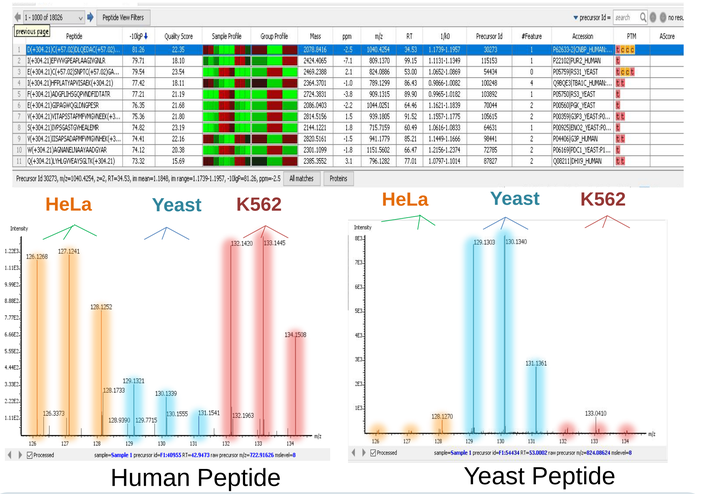

Figure 1. PEAKS X+ Quant analysis demonstrating multiplexing of 9 TMT channels. Pool sample was prepared and aliquoted at a ratio of 4:4:1.

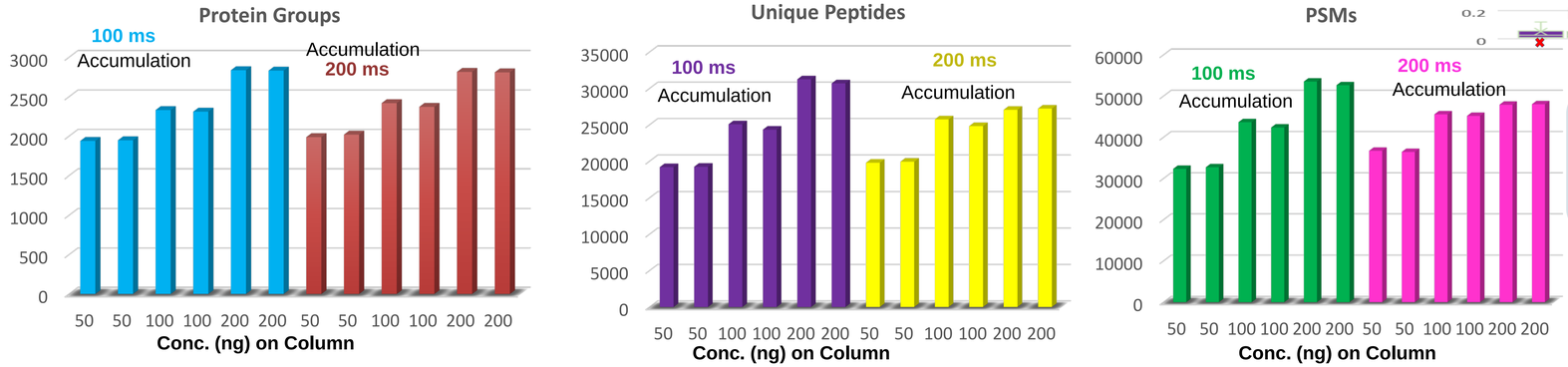

Figure 2. PEAKS X+ results of injecting different TMT sample concentrations ranging between 50 to 200 ng on column. Different colors represent different accumulation times. At 100 ms accumulation time, up to 10 PASEF MS/MS (2.21 s cycle time) scans were permitted. At 200 ms accumulation time, up to 5 PASEF MS/MS (2.25 s cycle time) scans were permitted.

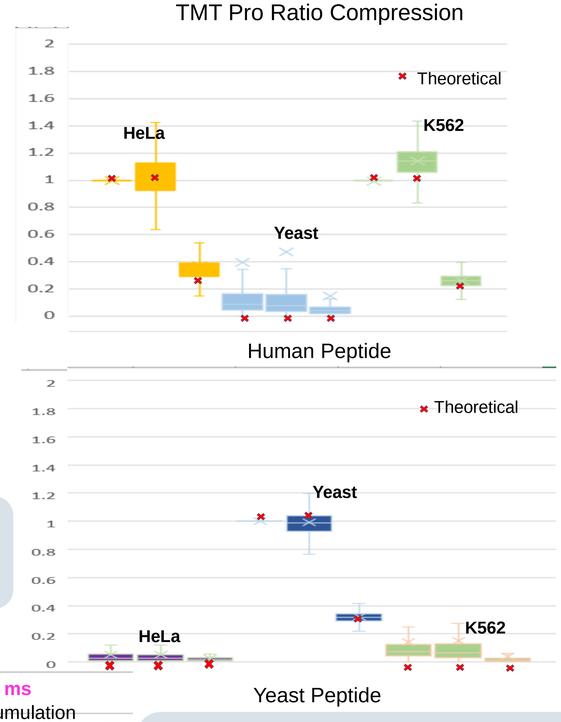

Figure 3. Theoretical vs Experimental ratio compression of TMT Pro (9 Channels) on the timsTOF Pro. Expected ratios are 1:1:0.25

Results

- We tested accumulation time within the TIMS device with the rationale that longer trapping times resulted in better IM resolution, id-based metrics and the lower the compression effect.
- Lower CV values, ranged between 14-25%.
- More than 19,000 (50 ng), 25,000 (100ng) and 27,000 (200 ng) unique peptides over a 90 min gradient.
- More than 32,000 (50ng), 43,000 (100ng) and 50,000 (200ng) PSMs (FDR 1%) were identified over a 90 min gradient

Conclusions

- In this experiment, we demonstrate the multiplexing ability of the timsTOF Pro using 9 channels from the TMT Pro Kit over a 90 min gradient.
- In the near future, we plan to demonstrate the systems speed and performance using shorter 20 min gradients.

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timsTOF Pro