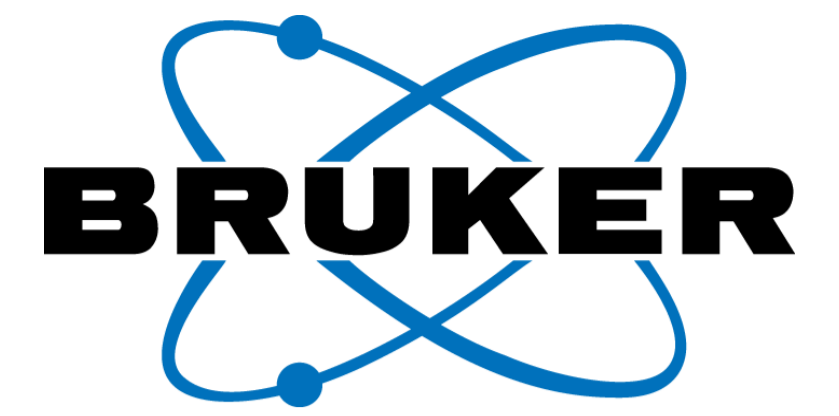


TMT Quantification for Shotgun Proteomics on a Trapped-Ion-Mobility quadrupole-Time-Of-Flight mass spectrometer (TIMS-QTOF) powered by Parallel-Accumulation and Serial-Fragmentation (PASEF)



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Introduction

Quantitative proteomics by isobaric labelling of peptides with TMT utilizes isotope-coded reporter-ions in the fragment spectra and enables the multiplexed relative quantification of several samples within a single data-dependent auto-MSMS run. Here we evaluate the performance of a unique dual trapped ion mobility spectrometer (TIMS) coupled to a QTOF (timsTOF Pro) for the analysis of isobaric labeling experiments. TIMS provides an additional dimension of separation and higher peak capacity which, when operated with the Parallel Accumulation Serial Fragmentation (PASEF) method, allows for operation at nearly 100 % duty cycle with very high sequencing speeds.

Methods

To investigate the performance of the timsTOF Pro for isobaric labelling experiments Flag-HA-ERCC8 was tandem affinity purified, aliquoted in ratios 10:4:1:1:4:10, and TMT labeled. To provide a background, mock Flag IP eluates from HeLa were aliquoted in equal ratios and labeled with TMT 126, 127, and 128 (Figure 1.)

ERCC8 IP peptides and mock Flag IP peptides were combined, desalted, and dried. Peptides were 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). 200 ng of sample were separated on 90 min, gradient (2-30% ACN). A PASEF cycle of 2.2 s was used equating to a 100 ms MS scan followed by ten 100 ms MSMS cycles each fragmenting up to 12 precursors. Feature extraction, database searching and quantitation were performed in PEAKS 8.5 (Bioinformatics Solutions Inc.)

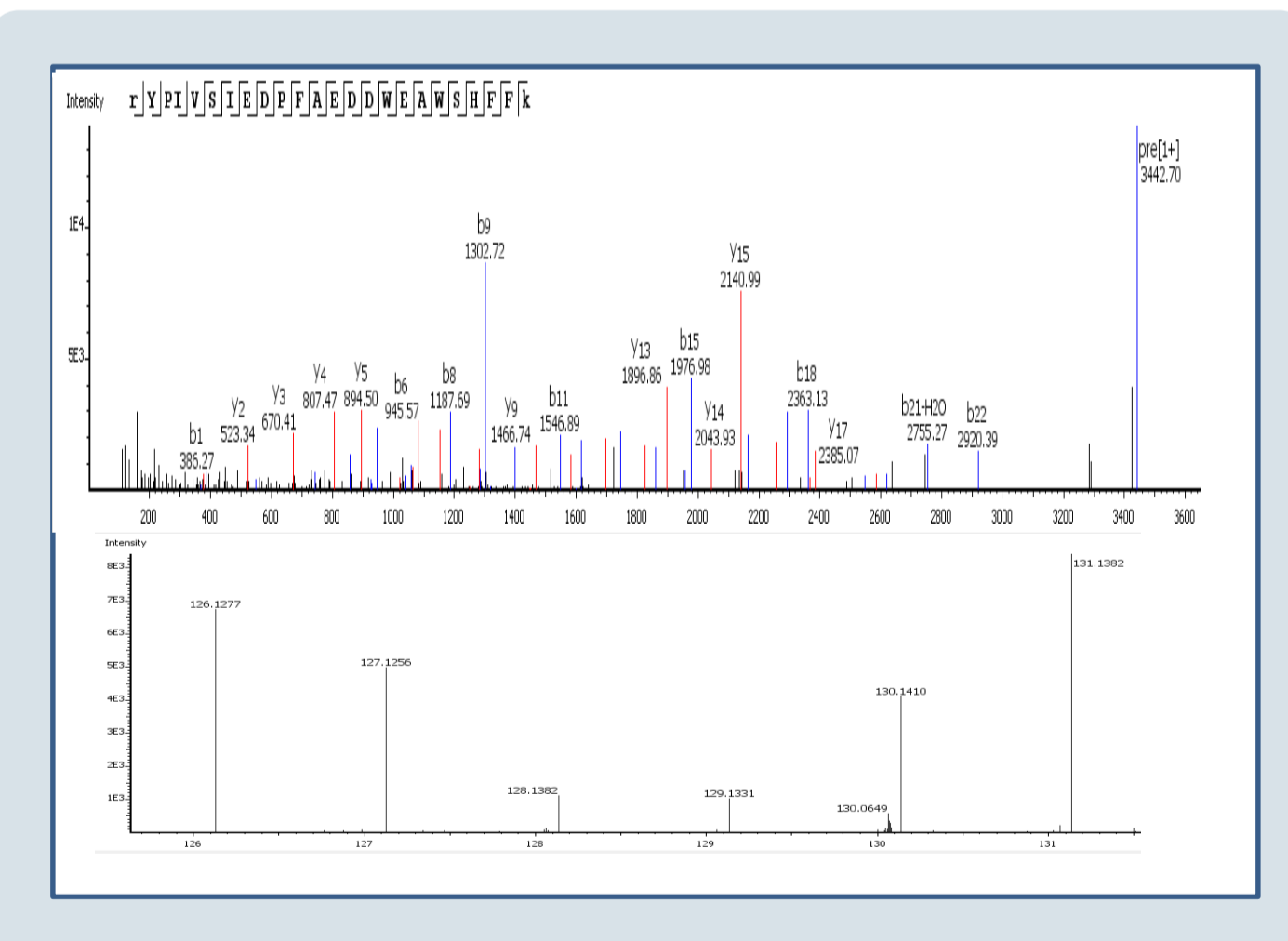


Fig. 2. The stepped MSMS method acquires an optimized MSMS spectrum for sequence ions (top spectrum) and a second optimized for the reporter ion region (bottom spectrum). The two spectra are automatically combined in the instrument software.

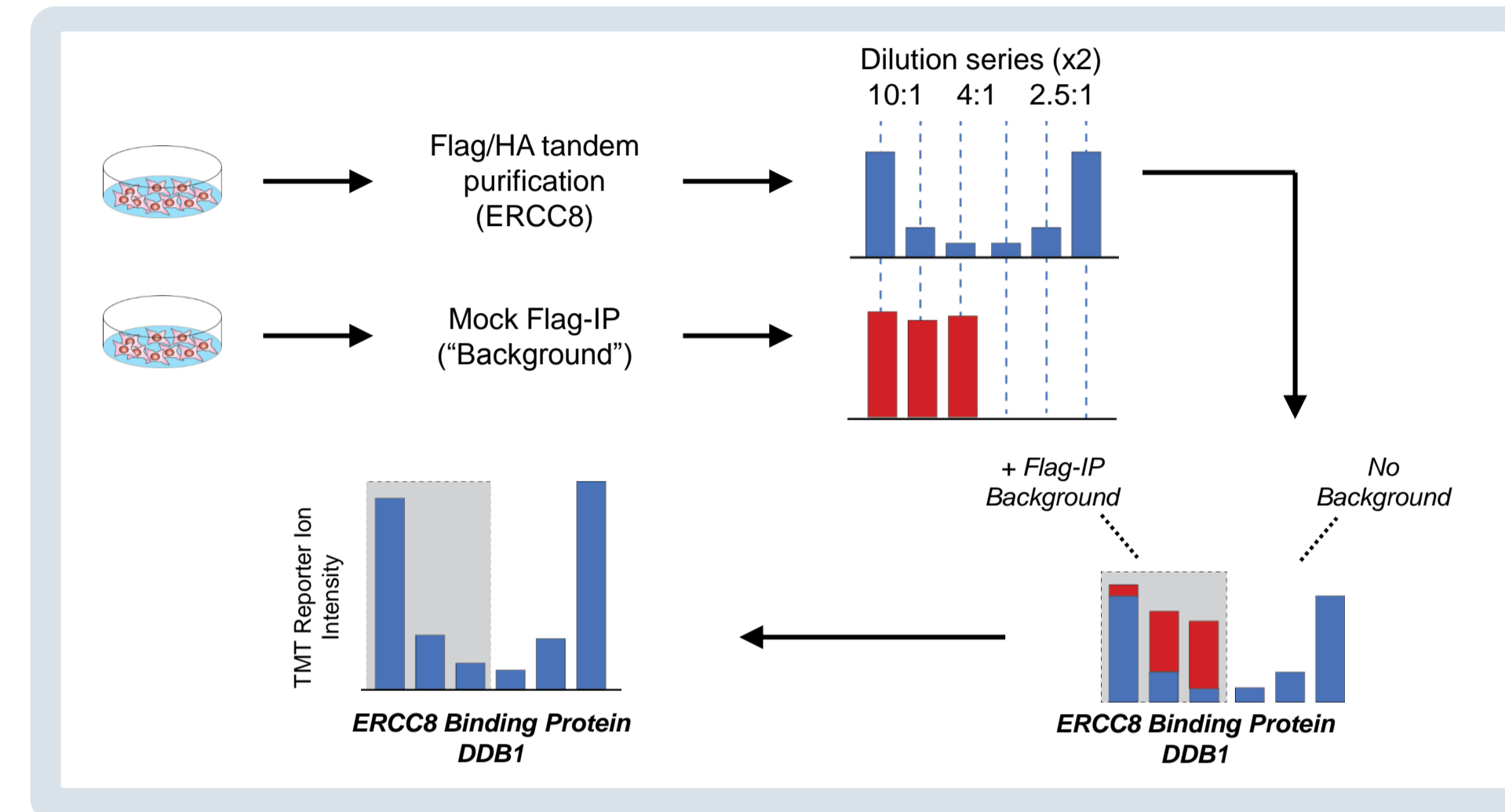


Fig. 1. Flag HA-ERCC8 tandem purified peptides were aliquoted in ratios 10:4:1:1:4:10 and labeled with TMT. These were mixed with 3 equal aliquots of mock Flag IP eluates from HeLa labeled with TMT 126, 127 and 128.

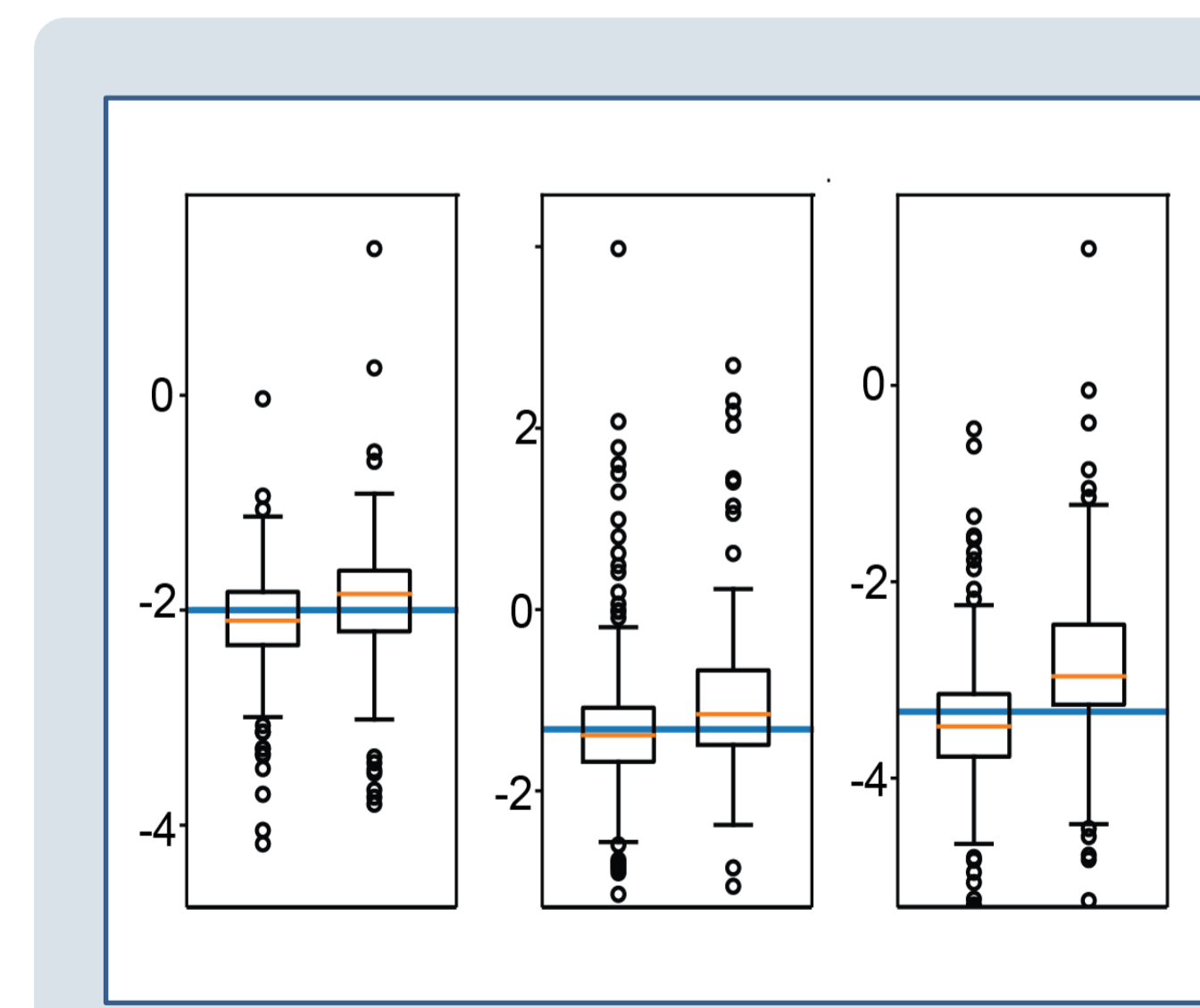


Fig. 3. Box plots showing calculated reporter ion ratios for ERCC8 Binding Protein DDB1 (126 channel as the denominator). PASEF reduces ratio distortion observed with isobaric labels.

Results

The unsurpassed sequencing speed of the timsTOF Pro and PASEF allow an innovative stepped MSMS method where two MSMS scans were acquired for each precursor. One aimed at gaining maximum quality sequence ion information and the second specifically optimized for the reporter ion region of the spectrum to ensure the best quality quantitative data (Figure 2). The two spectra are automatically combined. Despite acquiring 2 MSMS spectra for each selected precursor the speed of the PASEF method fragments more than 50 precursors per second.

The data acquired with TIMS activated showed reduced reporter ion compression compared with data acquired with TIMS Off (Figure 3) suggesting the potential for improved quantitation performance with isobaric labeling methodologies while maintaining the high sequencing speeds and sensitivity of the timsTOF Pro with PASEF.

Protein complex samples provided courtesy of Marto Lab, Dana-Farber Cancer Institute, Boston, MA.

Conclusions

- Accurate reporter ion ratios.
- The sequencing speed of the PASEF method allow optimized MSMS spectra for sequence ions and reporter ions while maintaining a sequencing speed of >50 Hz.
- The outstanding peak capacity and sensitivity of the timsTOF Pro make it very well suited to isobaric labeled quantitation experiments.

timsTOF Pro