Improved Identification, Quantification Accuracy, and Workflow Efficiency using a Modified Quadrupole Orbitrap Mass Spectrometer and Tandem Mass Tags (TMT) Approach

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ABSTRACT

Multiplexed quantitation strategies using Thermo Scientific™ Tandem Mass Tags™ (TMT™) enable precise measurement of peptide or protein abundance from multiple samples into a single high-resolution LC-MS analysis. Increasingly, various biological experiments demand higher quantitation accuracy and proteome coverage. Here, we demonstrate increased quantitative performance and throughput efficiency on a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer combined with a Thermo Scientific™ FAIMS Pro™ interface.

INTRODUCTION

Quantitative proteomics is a powerful tool to study how proteins function or interact with each other in a biological system. Isobaric tagging strategies using Tandem Mass Tags (TMT) allow up to 11 samples to be multiplexing in a single high resolution LC-MS experiment while enabling precise measurement of protein abundance. Isobaric tagging strategies can suppress ratio quantification and thereby accurate ratio determination in greater than TMT6plex experiments which can reduce the frequency of acquisition. Additionally, co-isolated ion interference can suppress ratio quantification and thereby mask true differences in protein abundance across samples. Here we evaluated the improvements of a modified quadrupole Orbitrap™ mass spectrometer in combination with a Thermo Scientific™ FAIMS Pro™ interface to reduce the above stated limitations on TMT quantitative experiments.

MATERIALS AND METHODS

To assess the sensitivity of the modified quadrupole Orbitrap mass spectrometer for TMT based quantitation, we utilized the Thermo Scientific™ Pierce™ TMT11plex yeast digest standard. This standardized sample provides users a tool to measure the accuracy, precision, and proteome depth of TMT methods across different instrumentation. For liquid chromatography (LC) conditions, we used an analytical gradient from 8-32% acetonitrile (vol/vol) with 0.1% (vol/vol) formic acid in 50min with a column heater set to 45°C, unless otherwise indicated. Experiments were run with a Thermo Scientific™ EASY-nLC™ 1200 HPLC system in combination with a Thermo Scientific™ EASY-Spray™ C18 50cm column coupled to a Thermo Scientific™ EASY-Spray™ ion source. Samples were analyzed on a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer with or without a FAIMS Pro interface. Settings for Orbitrap Exploris Series Instrument Control Software Version 1.0 follow below (Table 1). Raw data files were processed using Thermo Scientific™ Proteome Discoverer™ 2.3 software using the SEQUEST® HT search engine with a 10ppm MS1 and 0.02 Da MS2 mass tolerance, TMT6plex (229.163 Da) set as a static modification, and a 1% false discovery rate. All reagents were purchased from Thermo Fisher Scientific.

Table 1. Mass spectrometer data acquisition settings.

Acquisition Settings	MS2	FAIMS MS2
Ion transfer tube temperature	325°C	325°C
Positive spray voltage	2000 V	2400 V
FAIMS Pro CV	off	-45 / -60 / -75 CV
Top Speed (sec)	2.5	1.25 per CV
APD	off	on
RF lens	40%	40%
Orbitrap MS1 resolution	120000	120000
Scan range (m/z)	400-1400	400-1400
Standardized MS1 AGC target	50%	50%
MS1 max IT (mode)	Auto	Auto
MIPS (mode)	Peptide	Peptide
Precursor Fit	off	70% with 0.7 m/z
Charge State	2-5	2-5
Dynamic exclusion (sec)	45	45
MS2 resolution	45000	30000
TurboTMT (on ΦSDM / off eFT)	off	on
First mass MS2	110 m/z	110 m/z
MS2 Isolation Window	0.7 m/z	0.7 m/z
Standardized MS2 AGC target	300%	300%
MS2 max IT (mode)	Auto	Auto
MS2 HCD NCE%	36	36
MS2 Intensity threshold	5e3	5e3

RESULTS

To increase the scan acquisition rates for TMT11plex or TMT10plex experiments, we evaluated how a new feature call TurboTMT, powered by the ΦSDM algorithm, might effect the number of protein and peptide identifications. ΦSDM is a computationally intensive approach to increase resolution. Here we applied ΦSDM specifically to the TMT reporter ions. This increased the resolution sufficient to baseline resolve TMT isotopologues even when using 30000 or 15000 resolution for the MS2 scan, and increases the number of identifications by 10%. Furthermore, we evaluated how gas-phase fractionation could be used to increase quantification accuracy of the TMT MS2 approach using the FAIMS Pro Interface. Gas-phase fractionation decreased PSM co-isolation interference, improving TMT MS2 quantitation. Overall, the modified hybrid quadrupole-Orbitrap mass spectrometer includes new features such as a Precursor Fit filter, TurboTMT, and the ability to utilized a FAIMS Pro Interface that specifically benefit TMT MS2 based quantitation accuracy, increase identification rates, and enhance workflow efficiency.

A MODIFIED QUADRUPOLE ORBITRAP MASS SPECTROMETER

Figure 1. (1A.) Schematic representation of Orbitrap Exploris 480 mass spectrometer.
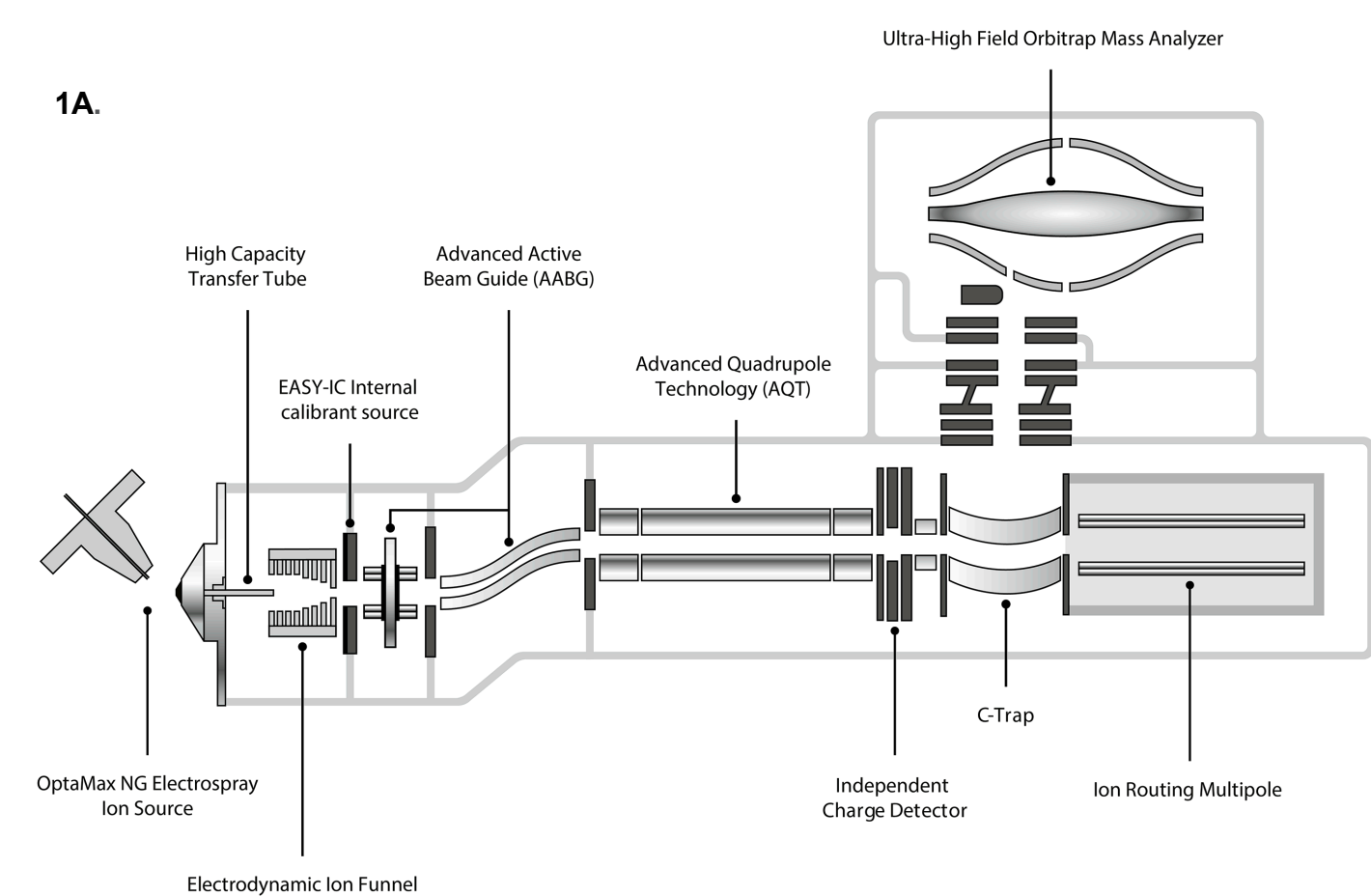

A TMT11PLEX YEAST DIGEST STANDARD

Figure 2. Schematic representation of Pierce TMT11plex Yeast Digest Standard. (2A.) The standard is composed of four Saccharomyces cerevisiae strains: three lines respectively lacking the non-essential proteins Met6, His4, or Ura2, and the parental strain BY4741 for reference channels. Modified from Schweppe et al. 2019.
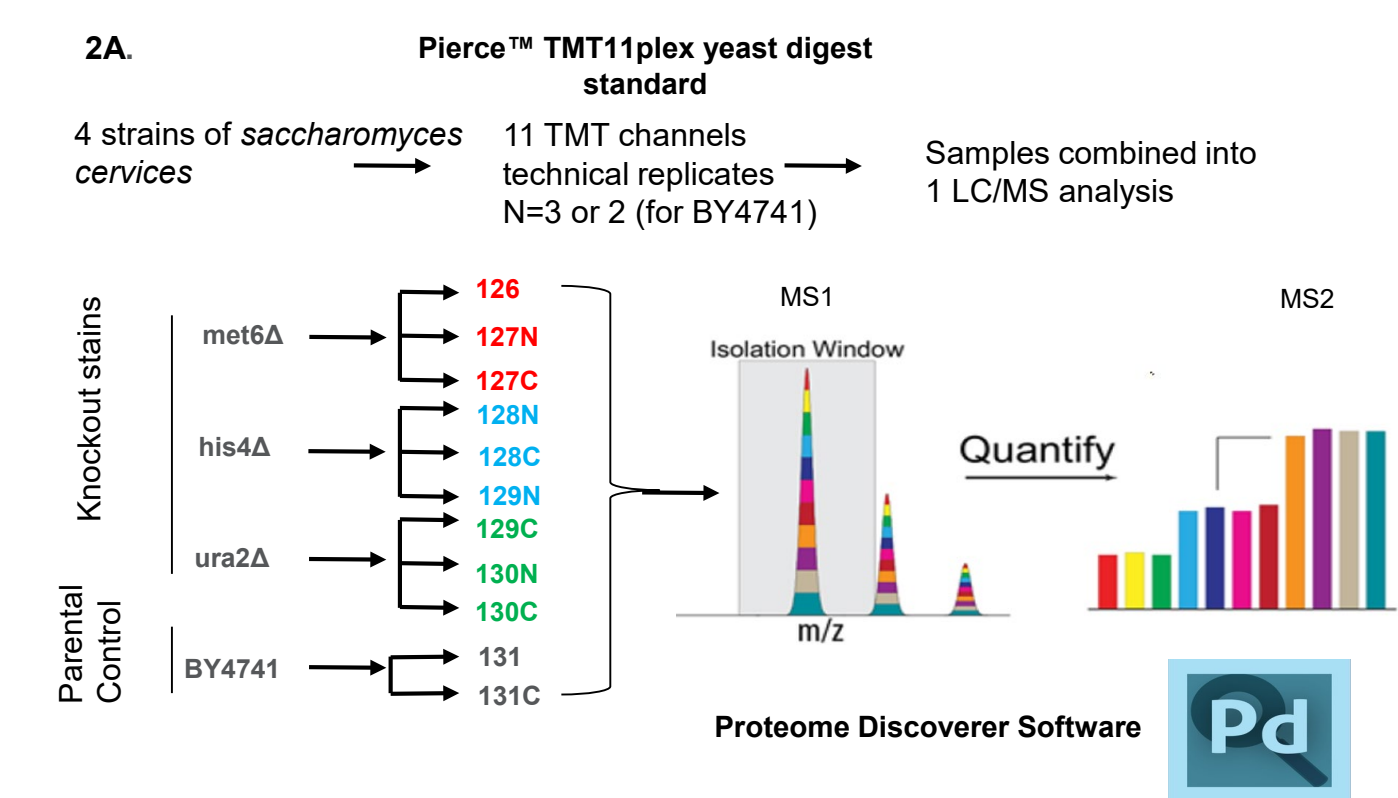

CONSISTENCY AND PRECISION

Figure 3. Achieving reproducible TMT quantitative performance on the Orbitrap Exploris 480 MS. To assess the reproducibility of the new instrumentation, we employed the TMT11plex yeast digest standard by analyzing 500ng with a 50min gradient and standard MS2 conditions. We monitored (3A.) quantified proteins (3B.) quantified peptides, (3C.) Interference Free Index, and (3D.), the precision of TMT MS2 quantitation across replicate channels. The TMT QC was run once per day during the week.
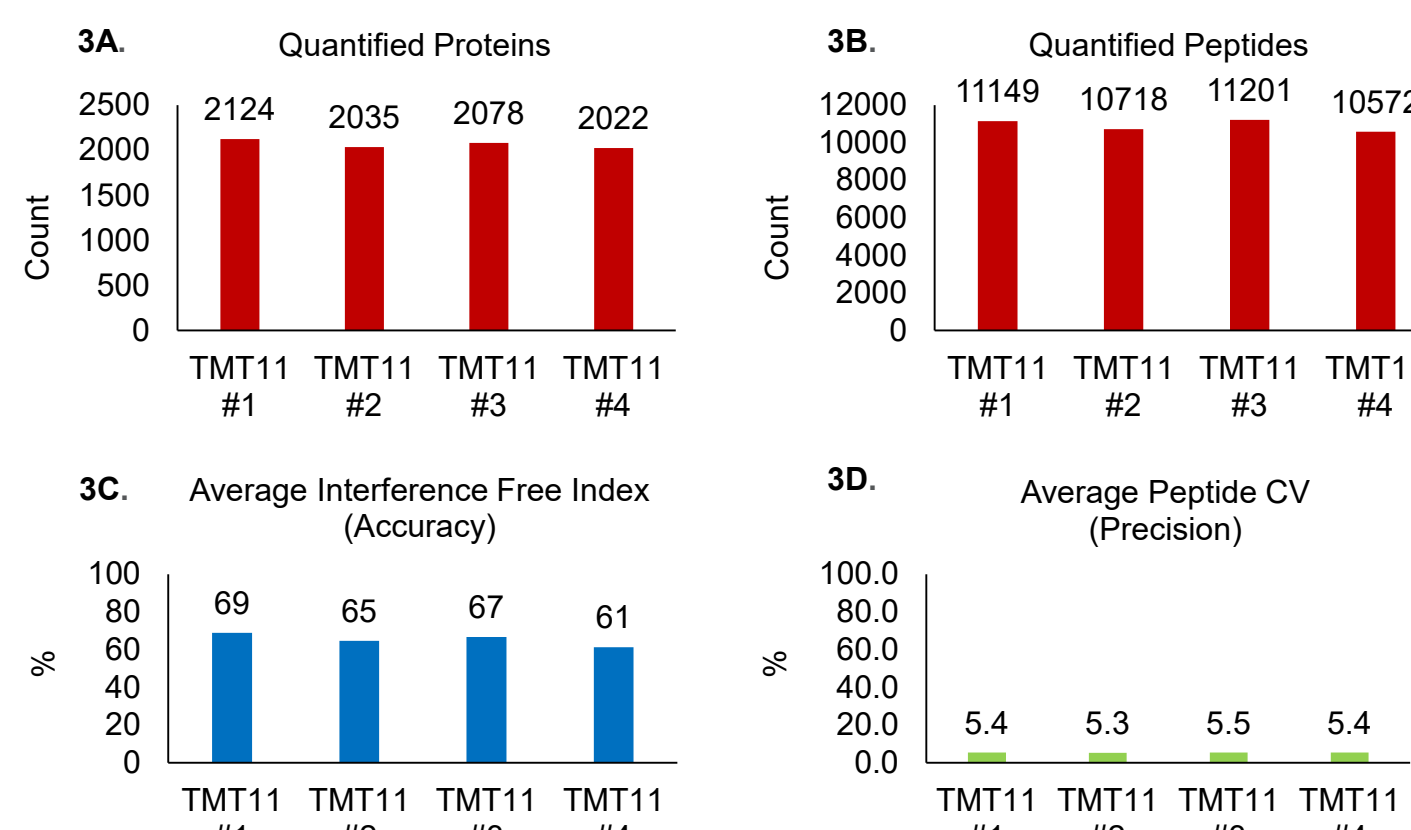

TurboTMT

Figure 4. TurboTMT powered by the ΦSDM algorithm, increases protein and peptide identifications. ΦSDM is an advanced spectra processing algorithm that increases resolution within a range of the spectrum without requiring a longer transient. (4A.) Here it is applied to 0.22 Da windows sufficient to baseline resolve TMT isotopologues. (4B.) This approach increases the number of identifications at 50min (4C.) with the largest improvement for gradients less than 120min.
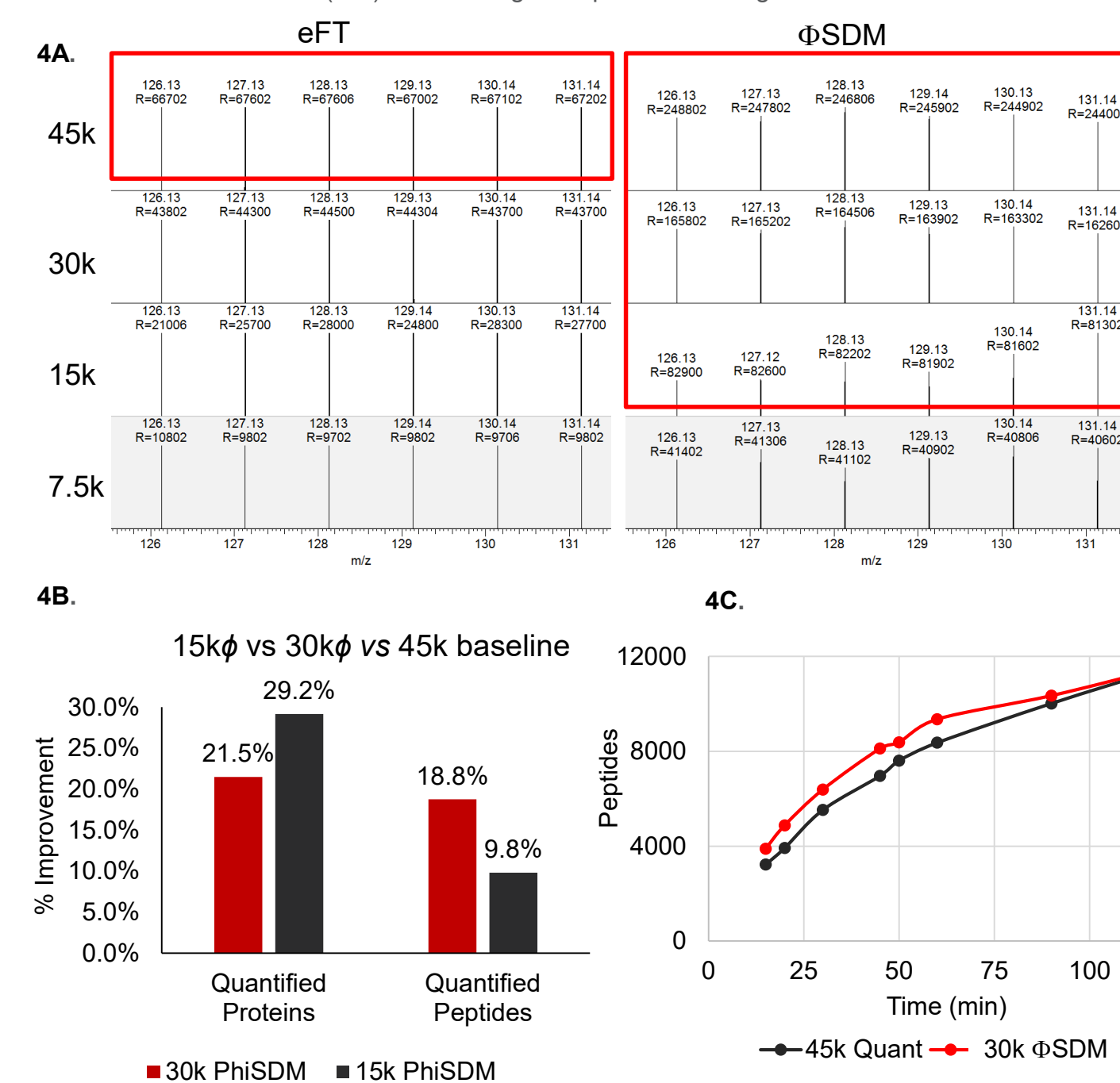

ENHANCED TMT MS2 PERFORMANCE

Figure 5. Achieving improved proteome depth and accuracy for MS2 quantitation (5A.) Schematic representation of the TMT workflow on Orbitrap Exploris 480 mass spectrometer. Modified from Schweppe et al. 2019. (5B.) Precursor Fit increases isolation specificity and reduces PSM isolation interference when using APD. (5C.) We evaluated how combining new features including Precursor Fit filter, TurboTMT, and the ability to utilized a FAIMS Pro interface with -45,-60,-75,-90 CVs on 4 hour gradient with on the modified hybrid quadrupole-Orbitrap mass spectrometer influenced TMT identification rates and the interference free index at the (5D.) protein or (5E.) peptide level as visualized in TKOmics.com.
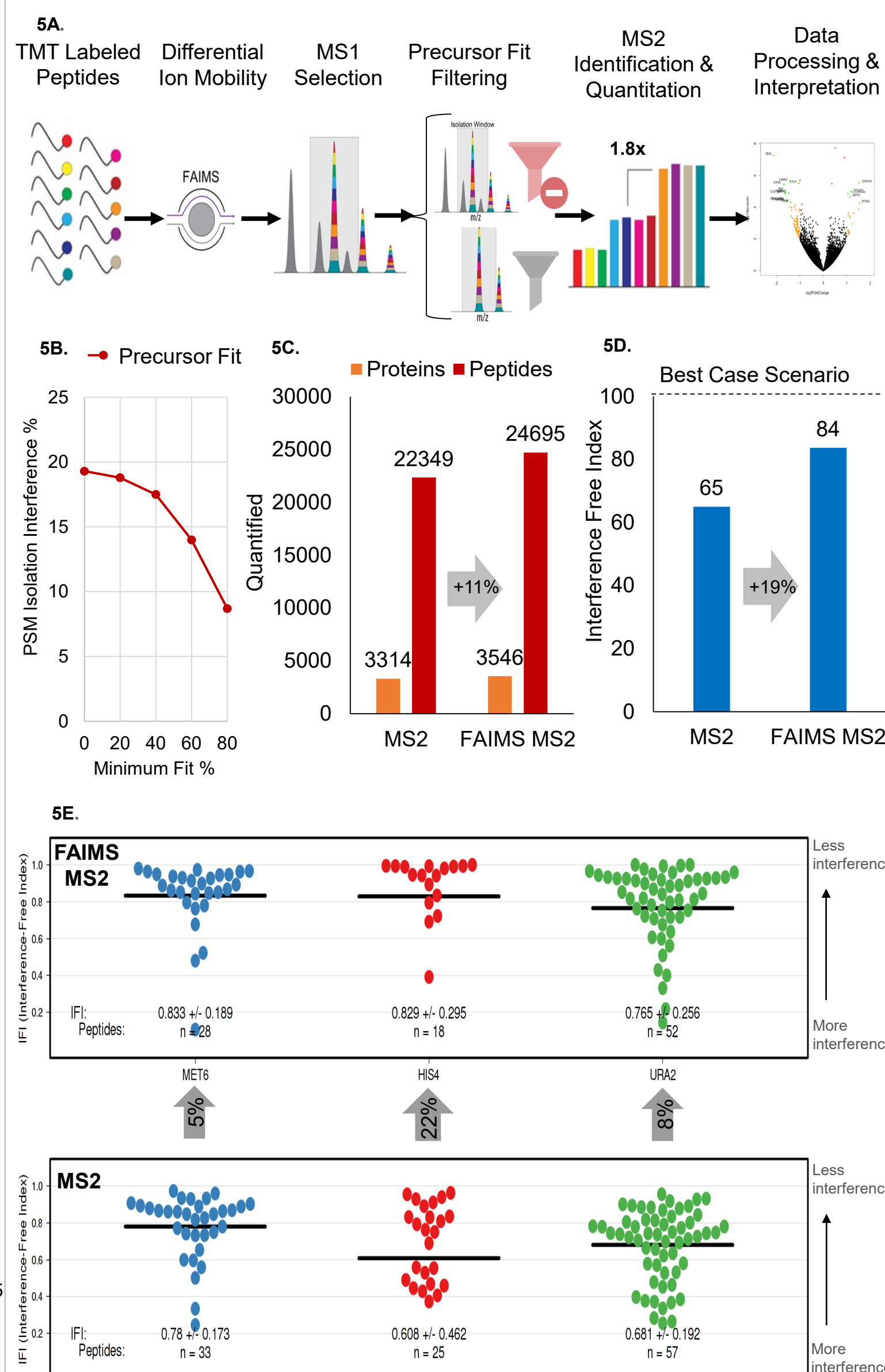
IMPROVED QUANTITATION ACCURACY

Figure 6. FAIMS Pro™ interface and Precursor Fit filter decrease ratio compression. An equimolar mixture of Thermo Scientific™ Pierce 6 Protein Digest ( ) was labeled, mixed in various ratios, and spiked into overwhelming background of Thermo Scientific™ HeLa Protein Digest Standard ( ). 1ug of peptides were measure on a 120min analytical gradient using a modified hybrid quadrupole-Orbitrap™ mass spectrometer, with or without the FAIMS Pro interface and the Precursor Fit Filter. Data was analyzed and visualized in Proteome Discover 2.3.
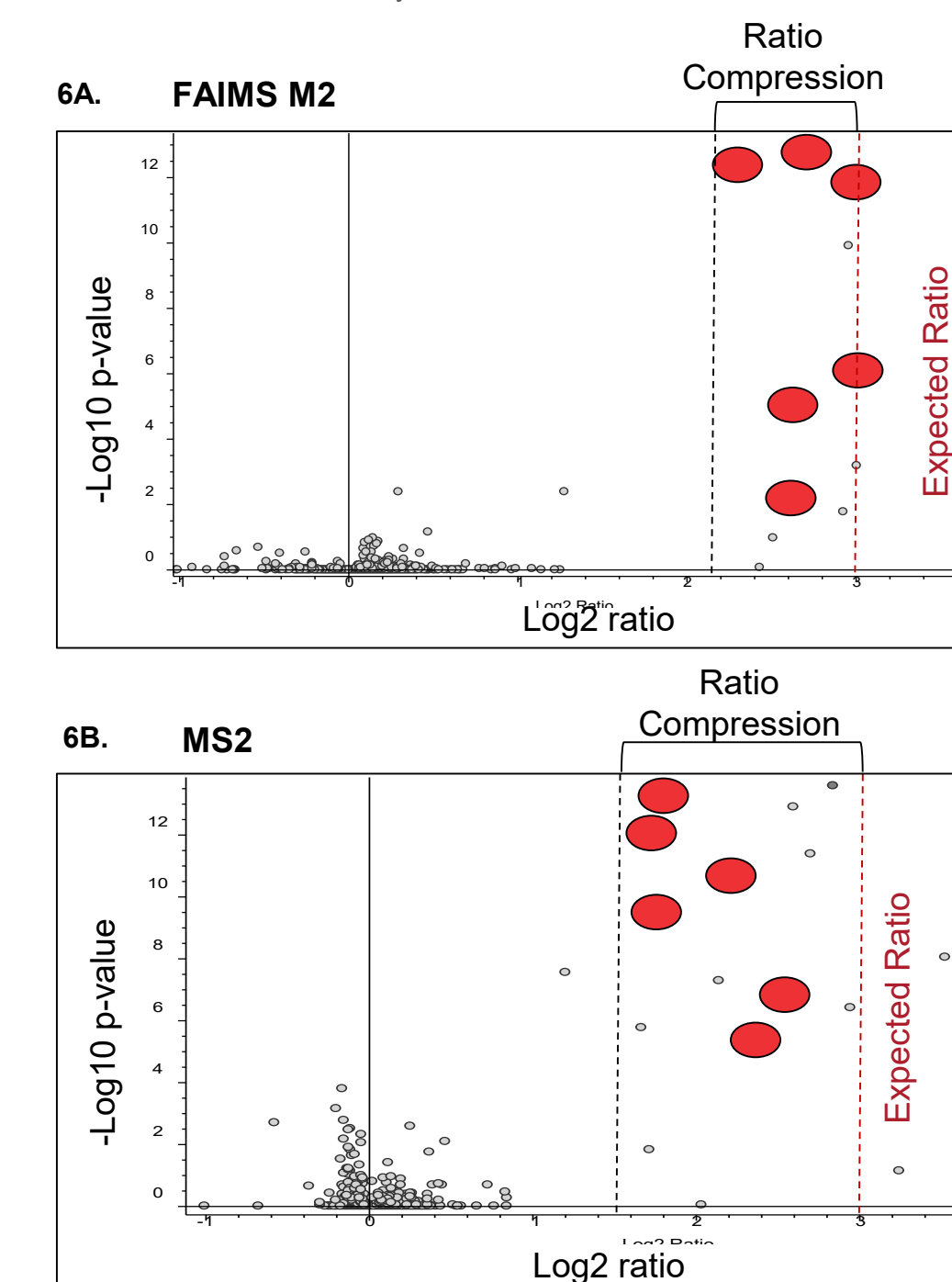
CONCLUSIONS

We present a modified quadrupole Orbitrap mass spectrometer combined with a FAIMS Pro interface that can improve identification rates and accuracy for TMT MS2 based quantitation.

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TRADEMARKS/LICENSING

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