Relative quantification using TMT11plex on a modified Q Exactive HF mass spectrometer

Authors

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ABSTRACT

Purpose: To benchmark a new hybrid quadrupole-Orbitrap™ mass spectrometer for relative quantification.

Methods: HeLa cell lysate was labeled with Thermo Scientific™ TMT11plex™ reagents. Aliquots from all 11 channels were mixed in equimolar ratios and analyzed on the novel Thermo Scientific™ Q Exactive™ HF-X mass spectrometer.

Results: Up to 18% more peptides\proteins were identified and quantified in a relative quantitative study using TMT11plex compared to the predecessor Thermo Scientific™ Q Exactive™ HF mass spectrometer.

INTRODUCTION

To have a deeper understanding of how proteins function or interact with each other in a biological system, their protein expressions at different states have to be studied. There are many different approaches to study protein interactions. Using mass spectrometry, a common way of evaluating these changes is to determine the relative abundance of particular proteins by means of isobaric mass tagging (e.g., using TMT™ reagents) [1]. Today as many as 10 different cell conditions (using TMT10plex) can be analyzed in a single high resolution LC-MS experiment. Higher resolution MS/MS scanning is necessary for accurate ratio determination in increased multiplex TMT experiments (TMT10plex or TMT11plex). However, using higher resolution in MS/MS scans tends to reduce the frequency of acquisition. Here we evaluate the benefits of the novel Q Exactive HF-X mass spectrometer for relative quantification using the TMT method.

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MATERIALS AND METHODS

Sample Preparation

Digested HeLa was labeled with either TMT10plex or TMT11plex reagents according to the manufacturer's instructions. TMT0 and aliquots from all 11 channels were mixed in equal ratios and separated using the Thermo Scientific™ EASY-nLC™ 1200 HPLC system on a 25 cm Thermo Scientific™ EASY-Spray™ PepMap™ RSLC C18 column. The gradient length was 85 min (total run time of 120 min) at a flow rate of 300 nL/min. Labeled samples were analyzed on Q Exactive HF MS and Q Exactive HF-X mass spectrometer. Raw data files were processed using Thermo Scientific™ Proteome Discoverer™ 2.1 software. The peptides/proteins were identified using the SEQUEST® HT search engine. False-discovery rate (FDR) was set at 1%. Quantification was based only on unique peptides and peptides where all the reporter ions were present at average S/N > 10.

Mass Spectrometry

The Q Exactive HF MS and the Q Exactive HF-X MS were operated in the data-dependent acquisition (DDA) mode selecting the top most intense 20 precursors from each scan for fragmentation. A summary of the MS parameters is shown in Table 1.

Table 1. MS and MS/MS parameters for Q Exactive HF MS and Q Exactive HF-X MS

Parameter	Setting
Full MS parameters	
Resolution settings (FWHM at m/z 200)	120,000 (120k)
Full MS mass range (m/z)	350–1400
Target value	3e6
Max. injection time (ms)	50
MS/MS parameters	
Resolution settings (FWHM at m/z 200)	30,000 (30k), 45,000 (45k), 60,000 (60k)
Target value	1e5
Max. injection time (ms)	86, 120
Isolation width	0.7, 1.0, 1.2 Da
Collision energy (HCD)	32
Loop count	20
Charge state recognition	2–6
Peptide match	Preferred
Dynamic exclusion (s)	45 s
Intensity threshold	1e4

Liquid Chromatography

Table 2. LC gradient

Chromatography	Settings
LC	Easy-nLC1200
Mobile Phases	A: 0.1% FA in Water; B: 0.1% FA in 80% Acetonitrile
Gradients*	6–12% B in 3 min; 85 min to 32% B; 20 min to 78% B; 5 min to 95% B; 5 min at 95% B.
Flow Rate	300 nL/min
Separation Column	Thermo Scientific™ Acclaim ™ PepMap™ C18, 75 µm 25 cm, 2 µm, 100 Å

Data Analysis

Proteome Discoverer software version 2.1 was used to search MS/MS spectra against the IPI-human database using the SEQUEST HT search engine. Static modifications included carbamidomethylation (C) and TMT 6plex (peptide N-terminus; K). Dynamic modifications included methionine oxidation and deamidation (N; Q). Peptide groups were filtered for maximum 1% FDR using Percolator with Qvality [2]. The TMT reporter ion quantification method within Proteome Discoverer software was used to calculate the reporter ratios with mass tolerance ±20 ppm. Isotopic correction factors were applied according to the pertaining CoA. Only confidently identified peptides containing all reporter ions were designated as "quantifiable spectra". Protein ratio was expressed as a median value of the ratios for all quantifiable spectra of the unique peptides pertaining to that protein group. The processing and consensus workflow settings for "Peptide and Protein Quantifier" node are shown in Figure 1.

Figure 1. Proteome Discoverer 2.1 software Data Processing workflow and Consensus workflow
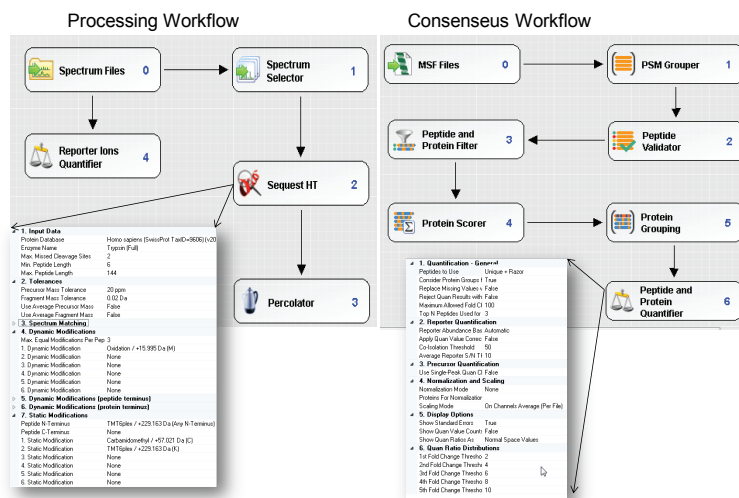

Results

Accumulating enough ions at a given time in TMT experiments, especially when running multiplexed TMT (TMT10, TMT11) samples, is a prerequisite for accurate quantification. Usually widening the isolation window would increase the influx of ions getting to the detector, but the downside is the increase in co-isolation which decreases quantitation accuracy. The novel Q Exactive HF-X mass spectrometer with a High Capacity Transfer Tube (HCTT) and an Electrodynamic Ion Funnel can be used to address this, as its brighter source affords more ion flux, at a given time, into the instrument than the round-bore capillary/S-lens combination on the Q Exactive HF MS. With more ions getting into the instrument, it is possible to use narrower isolation width, a procedure normally applied in relative quantitation using TMT, to reduce isolation interferences. To demonstrate the gain in sensitivity, HeLa digest was labeled with TMT0 and diluted to different concentrations (10–1000ng). The dilution series was measured on both a Q Exactive HF MS and a Q Exactive HF-X MS, using the same MS and MS/MS parameters except for the S-Lens/Funnel RF level, which was 50 on the Q Exactive HF MS (S-Lens) but was set to 40 on the Q Exactive HF-X MS (electrodynamic ion funnel). The results in figure 2 show that more peptides and protein groups were identified with the Q Exactive HF-X MS. Furthermore, as the dilution factor increased, so did the difference in the number of identified/quantified peptides and proteins between both instruments.

Figure 2. Performance evaluation of the Q Exactive HF-X MS compared to the Q Exactive HF MS. The same parameters were used on both instruments : AGC MS:3e6, Max IT MS/MS:120ms, MS/MS Resolution: 30k, Loop count: 20, Isolation width: 0.7, AGC target MS/MS:1e5. Results are averages from duplicate experiments.
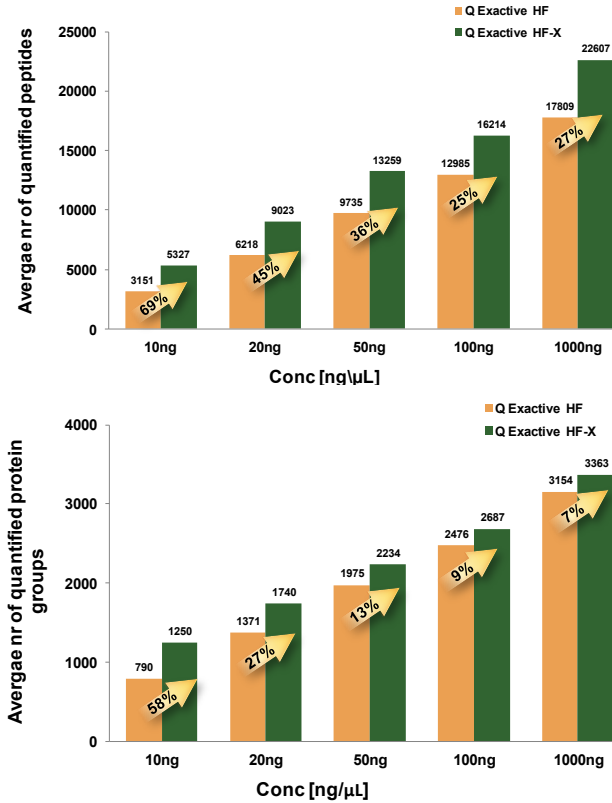

In addition to the brighter source on the Q Exactive HF-X MS, further steps have been undertaken to reduce the overhead between scans. This allows for longer ion accumulation (injection time) for every detection time, thus facilitating the implementation of lower resolution settings. The Q Exactive HF-X MS has a lowest resolution of 7500 @ m/z 200 and runs at this resolution with an acquisition speed of 40 Hz. This makes the novel Q Exactive HF-X MS mass spectrometer 2 times faster than the Q Exactive HF MS. Furthermore, a new resolution setting of 45,000 (45k, 96msec transient) was implemented. This resolution is especially relevant for PTM experiments, e.g ,phosphorylation and relative quantitation using multiplex TMT reporter ion, such as TMT10-/TMT11plex. The 45k resolution is sufficient to baseline resolve all the reporter ions of the TMT11plex as shown on figure 3A and B.

Figure 3. Reporter ion region for a HeLa peptide acquired with resolution setting 45k. The isobaric channels are all baseline resolved. (B) an overlay of 2 reporter ions, measured with 45k (black) and 60k (red).
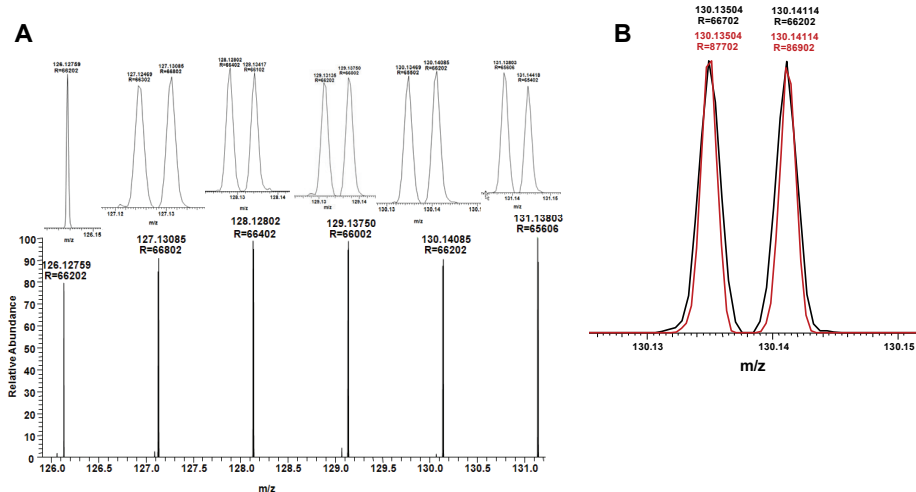
Reducing the resolution does not affect the resolving power for the isobaric tags but afford an improvement in scan speed. At 60k resolution setting, the Q Exactive HF MS runs at a maximum acquisition speed of approximately 7 Hz; using the 45k setting the instrument can run at about 10 Hz, which is a ca 25% increase in acquisition speed. Figure 4 shows a comparison of the cycle time for scan events consisting of a full scan (120k) and 20 MS/MS (45 or 60k) scans, for a selected region of the chromatogram. The Q Exactive HF-X MS achieves overall cycle times of about 2.3 s, 16% improvement compared to the Q Exactive HF MS.

Figure 4. Scan cycle comparison between 60k resolution setting on the Q Exactive HF MS and 45k on the Q Exactive HF-X MS.
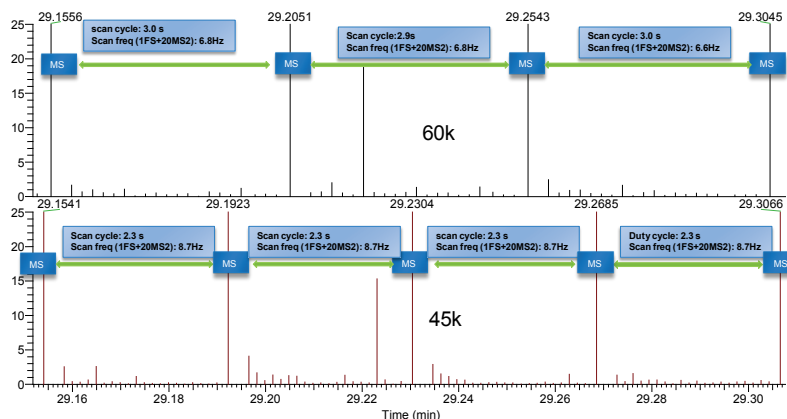
To evaluate the gain in performance, HeLa digested labeled with TMT11plex, mixed in an equimolar ratio was analyzed on both the Q Exactive HF MS using 60k resolution and the Q Exactive HF-X MS using 45k resolution in MS/MS. The maximum MS/MS injection time were set so that both instruments should run in optimum parallelization (120 ms for the Q Exactive HF and 86 ms for the Q Exactive HF-X MS). The Q Exactive HF MS was operated at S-lens RF level 50; the Q Exactive HF-X MS contains an electrodynamic ion funnel, for which the funnel RF level was set to 40. All other MS and LC parameters, including solvents, column and sample were kept the same. To minimize isolation interference, an isolation width of 0.7 Da was used. Compared to the Q Exactive HF MS, the average number of identified/quantified peptides increased by 18% and the protein groups by 11% on the Q Exactive HF-X MS, as shown in figure 5 A and B.

Figure 5. Comparison between the 60k resolution setting on the Q Exactive HF MS and 45k on the Q Exactive HF-X MS. Results are averages from duplicate experiments, 1 ug load.
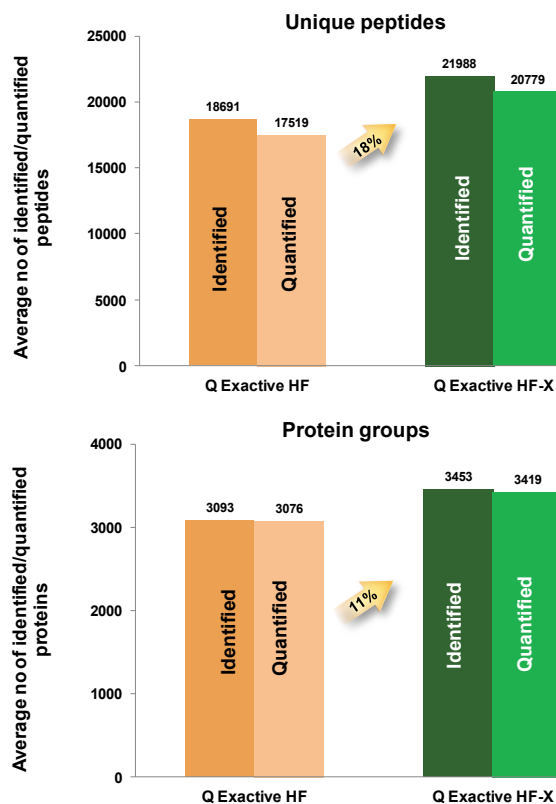
Figure 6 shows results obtained for different isolation widths on the Q Exactive HF-X MS. Minimal changes in the numbers of identified/quantified peptides and protein groups were observed. This is a merit of the improved ion influx through the brighter ion source. The percentages of peptides and proteins that were quantified in these experiments were in the same range (90–94%).

Figure 6. TMT11 HeLa quantification using and 45k on the Q Exactive HF-X MS at different isolation width.
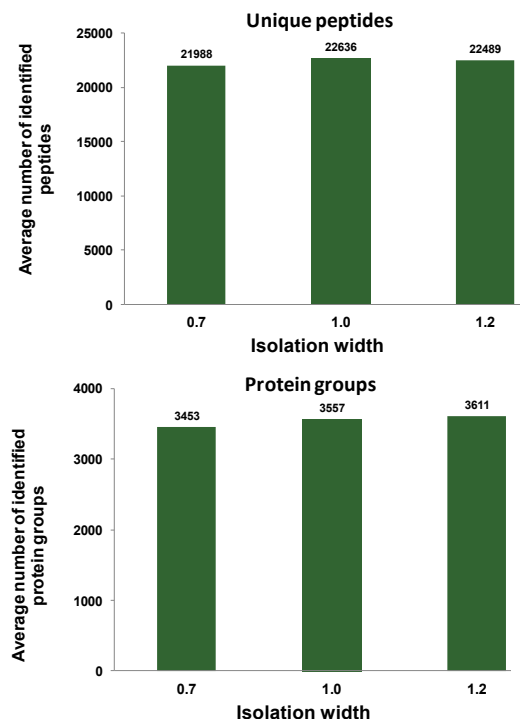
CONCLUSIONS

The Q Exactive HF-X MS was evaluated for relative quantitation using TMT11plex and the results show that:

▪ The increased sensitivity of the Q Exactive HF-X MS provides access to significantly more reproducibly quantified peptides and proteins (>50%) from diluted samples such as samples containing post-translationally modified peptides.

▪ The new resolution setting of 45k on the Q Exactive HF-X MS results in up to 25% increase in speed compared to the 60k setting on the Q Exactive HF MS.

▪ Using the 45k resolution setting for MS/MS on the Q Exactive HF-X MS, 18% more peptides and 11% more proteins were identified/quantified.

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