Achieving the Highest Performance for Protein Identification and SILAC Relative Quantitation on a Benchtop Quadrupole Ultra-high-field Orbitrap Mass Spectrometer

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Overview

Purpose: The performance of the novel Thermo Scientific™ Q Exactive™ HF hybrid quadrupole-Orbitrap mass spectrometer is evaluated and compared with the Thermo Scientific™ Q Exactive Plus™ Orbitrap mass spectrometer for both routine peptide identification and SILAC relative quantitation.

Methods: The peptide identification capability was investigated through in-depth analysis of the HeLa cell lylsate proteome, under different gradient conditions. The relative quantitation accuracy and precision were evaluated using triplex SILAC samples.

Results: With higher resolution and faster scan speed, the Q Exactive HF MS identifies over 40% more proteins and peptides compared to a Q Exactive Plus MS using the same LC gradient. The Q Exactive HF MS also enables the quantitation of more proteins and provides with better quantitation precision.

Introduction

The Q Exactive Plus mass spectrometer combines high-performance quadrupole precursor selection with high-resolution, accurate mass (HR/AM) Orbitrap™ detection. Its unparalleled performance for proteomic applications resulting from its fast scan speed and high resolving power, has made it the choice for many proteomic researchers. However, to understand biological systems, there is a need to not only explore the proteome in more depth, but to monitor protein dynamics more accurately. In this study, the new benchtop quadrupole high-field Q Exactive HF mass spectrometer that allows faster scan speed (>1.8 times) and higher resolution (>1.8 times) was evaluated for proteomic applications and benchmarked against the Q Exactive Plus MS.

Methods

Sample Preparation

Thermo Scientific™ Pierce™ HeLa Protein Digest Standard was dissolved in 0.1% formic acid to a final concentration of 500 ng/µl. For a set of quantitative comparison, A549 cells were labeled in light/medium/heavy SILAC medium. Harvested cells were lysed, digested and mixed to a ratio of 1:1:1 and 2:1:10 for analysis. The mixing accuracy has been verified in a different experiment.

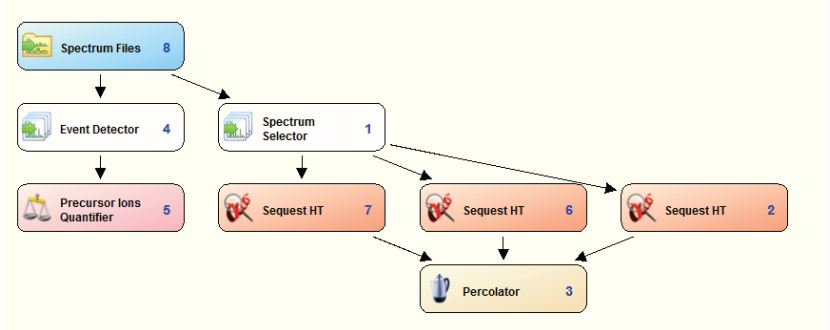
Liquid Chromatography

A Thermo Scientific™ EASY-nLC™ 1000 UPLC system and Thermo Scientific™ EASY-Spray™ Source with 50 cm EASY-Spray Column was used to separate peptides with 30% acetonitrile over 30 min and 60 min gradient, at a flow rate of 300 nl/min. The labeled samples were separated using a 120 minute gradient.

Mass Spectrometry

The HeLa lysate standards (1µg) were analyzed using a top 20 higher-energy C-trap dissociation (HCD) data-dependent tandem MS method on both a Q Exactive Plus and a Q Exactive HF mass spectrometer. The detailed instrument settings are in Table 1. Underfill ratios were adjusted to generate similar intensity threshold on two instruments. The SILAC labeled samples (1 µg) were evaluated using the same parameters, except using top 10 data dependent MS2 and higher MS1 resolutions (120K and 240K for Q Exactive HF MS, 70K and 140K for Q Exactive Plus MS, all resolution settings are provided at FWHM at *m/z* 200).

FIGURE 1. Thermo Scientific™ Proteome Discoverer™ workflow used to process data for relative quantitation.



Data Analysis
The LCMS data for protein identification were analyzed by Thermo Scientific Proteome Discoverer software v.1.4. with the SEQUEST HT search engine. Modifications include oxidation (M) and carbamidomethylation (C). For SILAC labeled samples, the searching workflow is shown in Figure 1. The methionine oxidation was used across three SEQUEST HT nodes for dynamic modification. Static modifications and other workflow settings are listed in Table 2. Resulting peptide hits were filtered for maximum 1% FDR using the Percolator algorithm.

TABLE 1. Parameter settings used for comparing peptide identification performance of the Q Exactive HF and Q Exactive Plus mass spectrometers.

Parameters	Q Exactive HF MS	Q Exactive Plus MS
Full MS		
Scan range (*m/z*)	375-1500	375-1500
Resolution	60K	70K
Target value	3e6	3e6
Max injection time (*ms*)	50	50
Data dependent MS/MS		
Resolution	15K	17.5K
Target value	2e5	2e5
MS2 injection time (*ms*)	19	45
Top N	20	20
Isolation width (*Da*)	1.4	1.4
NCE	27	27
Underfill ratio	0.5%	1%
Intensity threshold	5.3e4	4.4e4

TABLE 2. Database search parameter settings for relative quantitation in Proteome Discoverer software.

Node	Parameters	Settings
Event Detector	Mass Precision	2ppm
Precursor Ions Quantifier	RT Tolerance of Isotope Pattern	0.2min
Sequest HT	Static modification	Carbamidomethyl (C)
Sequest HT	Static modification	Carbamidomehtyl (C) Label: +4.025 (K) Lable: +6.020 (R)
Sequest HT	Static modification	Carbamidomehtyl (C) Label: +8.014 (K) Lable: +10.008 (R)

Results

Comparison of Q Exactive HF and Q Exactive Plus mass spectrometers for protein and peptide identification.

The Q Exactive HF MS has a high-field Orbitrap™ mass analyzer implemented, rendering faster MS2 scan speed up to 18 Hz. As shown in Figure 2, a cycle including 1 full MS scan at 60K and 20 HCD MS/MS scans at 15K resolution on Q Exactive HF MS is completed in 1.13 sec, 37% shorter compared to the cycle including1 full MS scan at 70K and 20 HCD MS/MS scans at 17.5K on Q Exactive Plus MS. The acquisition of 20 HCD MS/MS spectra on the new instrument can be finished within a little over 1 second.

To determine whether the improvement in cycle time would translate into higher numbers of peptide/protein identifications, we perform a head-to-head comparison of using HeLa cell lysate (Figure 3). Under 30min gradient, we observe ~17,000 unique peptides and over 3200 protein groups on Q Exactive HF MS, with an increase of 44% and 43% in peptide and protein identifications over Q Exactive Plus. When comparing a 30 min gradient on Q Exactive HF MS to 60 min gradient on Q Exactive Plus MS, we only see 7.5% difference in protein identifications, demonstrating similar performance is achievable using only half of the time on the new instrument. For 30 min gradient, the MS/MS success rate (percentage of MS/MS scans that results in confident and unique identifications) for both instruments are approximately 55% to 60%. The increase in the HCD acquisition rate on the improved Orbitrap mass spectrometer does not compromise the quality of the MS/MS spectra.

FIGURE 2. Practical duty cycle of different instruments for peptide identification. A and B: stick view of scan events showing time window of a complete cycle of one full scan and 20 HCD scans on (A) Q Exactive Plus MS and (B) Q Exactive HF MS. (C) Enlarged view of 20 HCD scans on Q Exactive HF MS that can be achieved a little over 1 second.

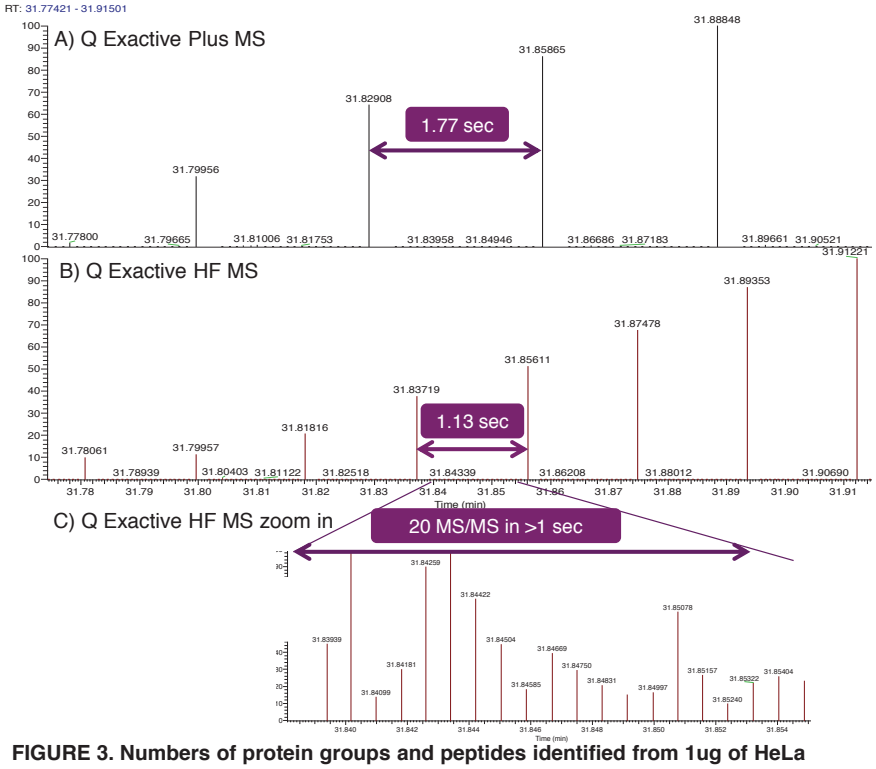
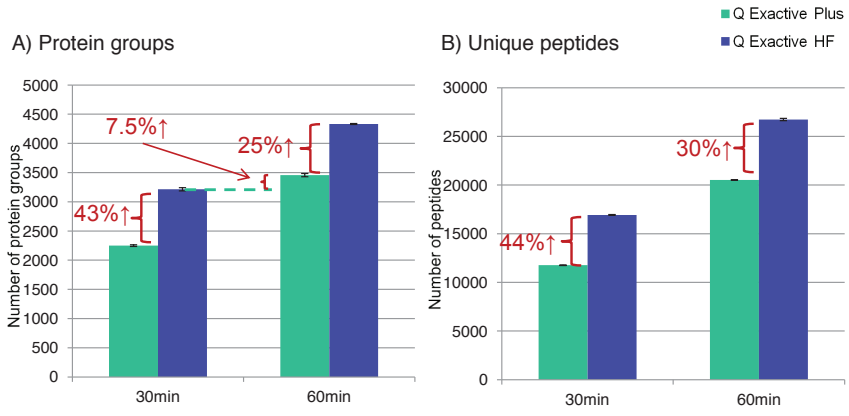


FIGURE 3. Numbers of protein groups and peptides identified from 1ug of HeLa using 30 min and 60 min gradients on Q Exactive Plus and Q Exactive HF mass spectrometers. Results represent an average of two replicate runs on each instrument.



Comparison of Q Exactive HF and Q Exactive Plus mass spectrometers for Quantitative Performance on SILAC labeled samples

In addition to the peptide and protein identifications, we also evaluate the quantitation performance of the new instrument for triplex SILAC samples. The triplex SILAC samples generate spectra which are three times more complex than the regular ones, and therefore high resolution is needed to distinguish the co-eluting species. As shown in Figure 4, a resolution of 240K on Q Exactive HF MS could easily resolve two peaks which cannot be resolved by 70K resolution on Q Exactive Plus MS. Separation of the merged peaks assures the accurate quantitation of the dominant peaks, and also facilitates the identification and quantitation of the additional peaks.

FIGURE 4. The improvement of resolution from Q Exactive Plus MS to Q Exactive HF MS ensures accurate assignment of the precursor ion and therefore accurate quantitation.

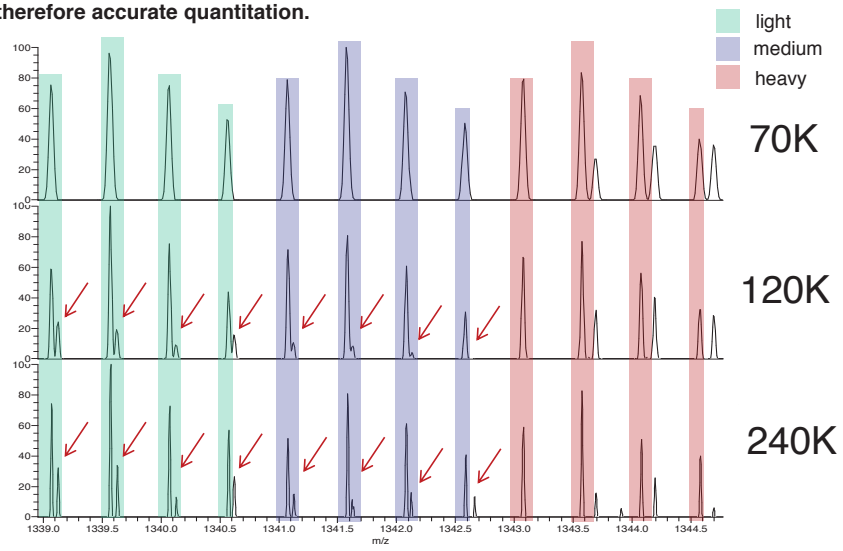
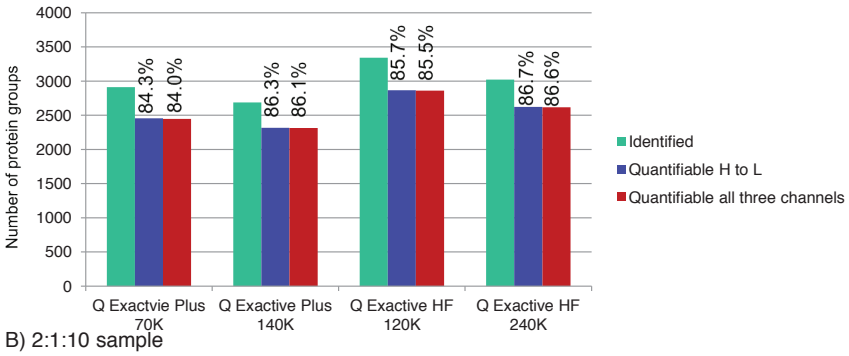


FIGURE 5. Comparison of identification and quantitation results for Q Exactive Plus MS and Q Exactive HF MS under different resolution. Numbers of protein groups identified, quantifiable through heavy to light channels, and quantifiable through all three precursor ion channels are shown. The percentage of quantifiable proteins compared to the identifiable proteins are also shown. Results represent an average of two replicate runs on each instrument.

A) 1:1:1 sample



B) 2:1:10 sample

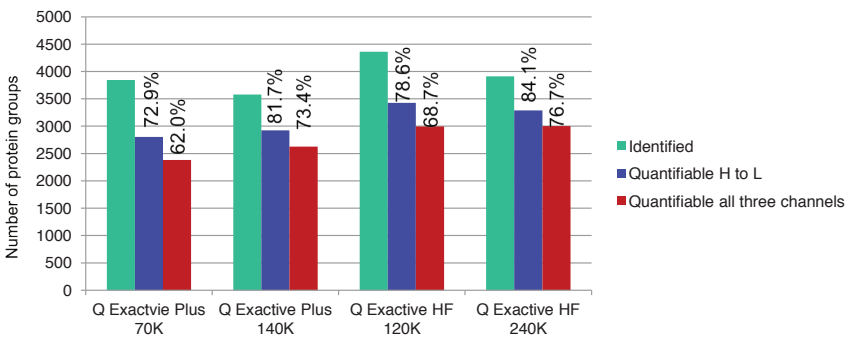
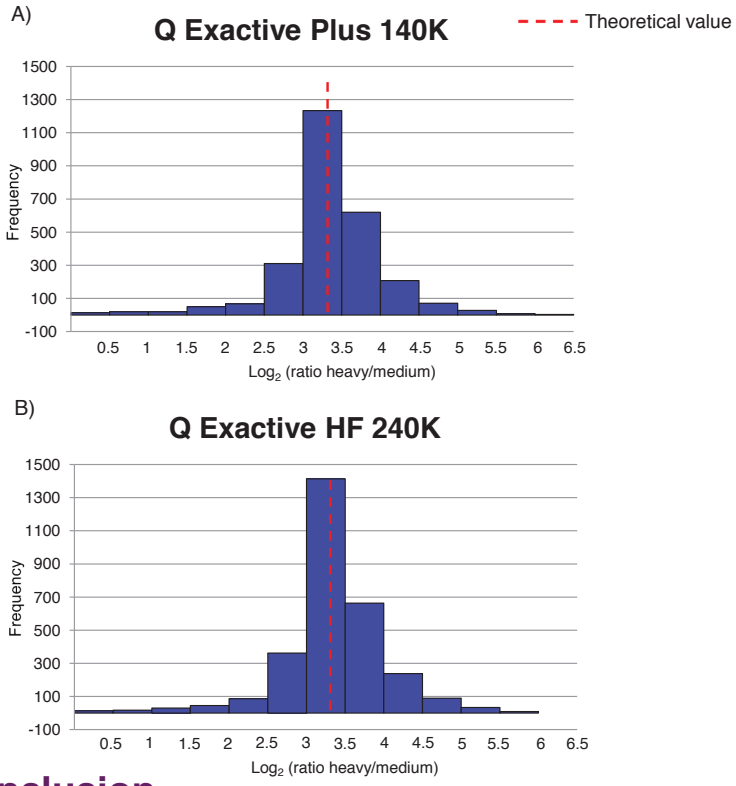


Figure 5 demonstrates the proteins identified, quantifiable through two precursor ion channels (heavy to light) and quantifiable through all three channels (all three precursor ions are present) for 1:1:1 and 2:1:10 mixed cell lysates. For both cases, Q Exactive HF MS not only identifies more proteins compared to the Q Exactive Plus MS , but also quantifies more proteins, as illustrated by the quantified protein numbers and the highlighted percentage. This can be attributed to the higher speed, which collects more spectra in a given time, and higher resolution/accurate mass, assisting the correct assignment of the precursor ion channels. Please note that in 2:1:10 high dynamic range sample, 120K resolution identifies the highest number of proteins, but 240K produces similar numbers of quantifiable proteins (red bar), demonstrating the power of high resolution for the relative quantitation.

Quantitative precision and accuracy is another parameter we assess. We challenge the instrument with high dynamic range triplex SILAC sample (light:medium:heavy=2:1:10). Figure 6 illustrates the histograms of protein ratio distributions on two instruments. On Q Exactive HF MS, there are larger portions of proteins whose ratios are closely distributed around the theoretical values, confirming the necessity of high resolution and accurate mass for precise quantitation.

FIGURE 6. Histograms of heavy to medium (10 to 1) protein ratio distributions on A) Q Exactive Plus MS and B) Q Exactive HF MS. Red line is the theoretical value ($log_2 10=3.322$). The frequency represents the number of proteins in each bin.



Conclusion

- The Q Exactive HF MS shows significant improvement in the number of protein and peptide identifications as a result of the higher scan speed.
- The Q Exactive HF MS generates similar numbers of peptide/protein identifications as Q Exactive Plus MS in half the gradient time. This will render the complete analysis of the human proteome (2D separation) achievable using much shorter instrument time.
- High resolution and accurate mass on the Q Exactive HF Orbitrap MS provides the most number of proteins quantified and the best quantitative precision.
- Faster scan speed and higher resolution of the new ultra-high-field Orbitrap MSassures the highest performance for protein identification and SILAC relative quantitation.

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