Large Scale Targeted Protein Quantification using HR/AM Selected Ion Monitoring with MS/MS Confirmation on the Orbitrap Fusion Tribrid MS

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Overview

Purpose: Highly sensitive and selective data independent acquisition (DIA) workflow for large-scale targeted protein quantification.

Methods: The Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer was set up for data independent acquisition. Three high-resolution, accurate-mass (HR/AM) selected ion monitoring (SIM) scans (240,000 FWHM) with wide isolation windows (200 amu) were used to cover all precursor ions of 400 – 1000 *m/z*. In parallel with each SIM scan, 17 sequential ion trap MS/MS with 12 amu isolation windows were acquired to cover the associated 200 amu SIM mass range. Quantitative information for all precursor ions detected in three sequential SIM scans is recorded in a single run. Plus, all MS/MS fragment information over the mass range of 400 – 1000 *m/z* is recorded for sequence confirmation of any peptide of interest by querying specific fragment ions in the spectral library. The quantitative performances and throughput of this novel approach were evaluated using various samples.

Results: The data collected from SIM scans with high resolving power provided unambiguous detection and quantitation of targeted peptide peaks by efficiently separating matrix interferences. LODs of 10 amol and 4 orders of linear dynamic range were observed with good precision.

Introduction

Recently, several data independent acquisition approaches[1,2] have been explored to increase quantitative reproducibility and comprehensiveness. These approaches use targeted extracted fragment ions from HR/AM MS/MS data collected with a wide isolation window, such as 25 Da, for quantification. The quantitative performance is often compromised by interfering fragments from co-eluted background compounds[3].

In the Orbitrap Fusion Tribrid MS (Figure 1), the Orbitrap detector can collect data with resolving power of 240,000 in less than 0.5 second. The linear ion trap can collect more than 20 CID MS/MS data in 1 second.

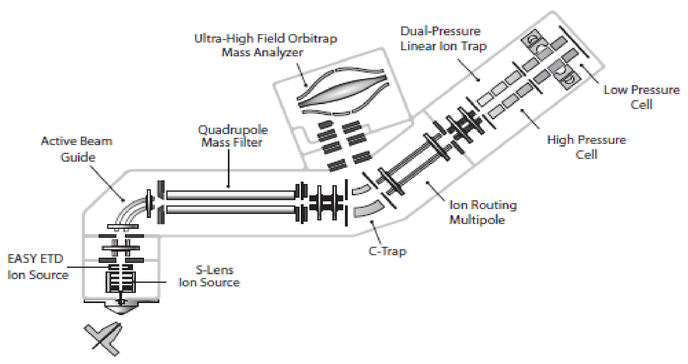


FIGURE 1. Instrument layout of Orbitrap Fusion MS

We developed a new DIA workflow which collects HR/AM SIM and collision-induced dissociation (CID) MS/MS in parallel (Figure 2). The quantification is carried out using HR/AM SIM data and the simultaneous peptide sequence confirmations are carried out using CID MS/MS at the expected RT relying on a spectral library (Figure 2).

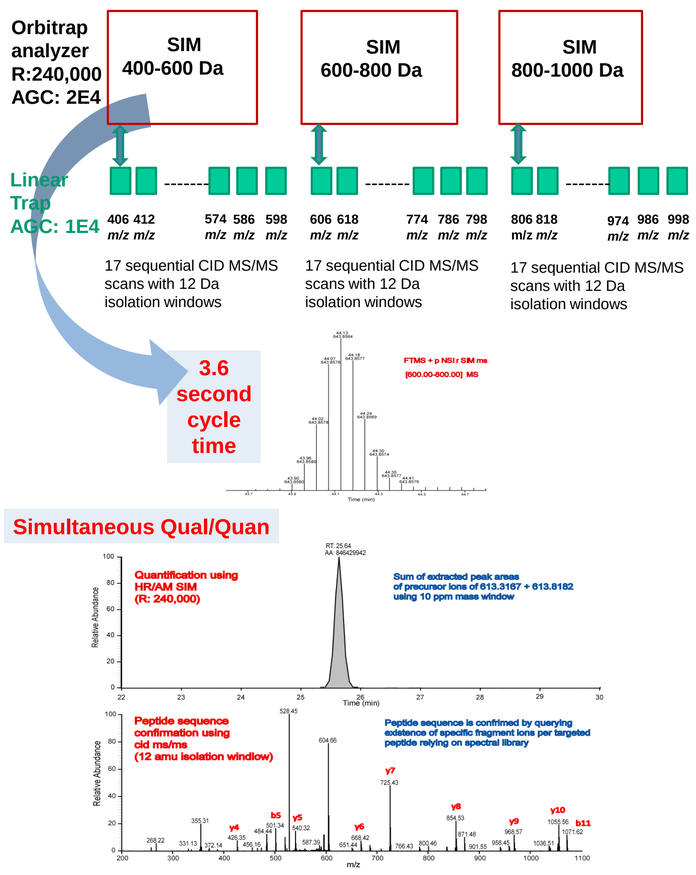


FIGURE 2. DIA workflow to collect HR/AM SIM at 240k resolution and 17 sequential CID MS/MS of 12 Da isolation width in parallel.

Methods

Sample 1: A mixture of seven isotopically labeled yeast peptides was spiked into either BSA or *E. coli* digests (500 ng/µL) at five different concentrations (0.01, 0.1, 1, 10 and 100 fmol/µL).

Sample 2: A standard mixture of 6 protein digests which covers five orders of magnitude concentration range (0.01, 0.1, 1, 10, 100 and 1000 fmol/µL) was spiked into an *E. coli* digest (500 ng/µL).

Nano-LC
System:ThermoScientific EASY-nLC™ 1000
Column: Thermo Scientific EASY-Spray™ PepMap C18 (2 µm, 75 µm x 50 cm); flow rate: 300 nL/min; buffer A: 0.1% FA/H_2O; buffer B: 0.1% FA/ACN; gradient: 5% to 25% B in 100 min, 25% to 35% B in 20 min.
Sample loaded directly on column; injection amount: 1 µL.

MS
Orbitrap Fusion MS equipped with a Thermo Scientific EASY-Spray source is used for all experiments. Capillary temp: 275 ºC; spray voltage: 1800 V.
FT SIM: resolution: 240,000; AGC target: 2+E04; isolation width: 200 amu; using trap for isolation.
CID MS/MS: rapid CID MS/MS, AGC target: 1+E04; isolation width: 12 amu; using Q for isolation.
Six scan events: three SIM scan events (scan 1, 3 and 5). Each SIM scan experiment is followed by one tMSn scan experiment which carries out 17 consecutive CID MS/MS events using predefined precursor ion inclusion list (Figure 2).

Data Processing
A spectral library was established using previous discovery data collected on the Orbitrap Fusion MS. Using Thermo Scientific Pinpoint™ v 1.3 sw, the XICs (5 ppm tolerance) of isotope C^{12} and C^{13} precursor ions per targeted peptide were used for quantification. Eight most intense fragment ions detected from discovery data are used for confirmation through spectral library match. A peptide with a P-value of less than 0.1 was considered high confidence by the spectral library match (Figure 3).

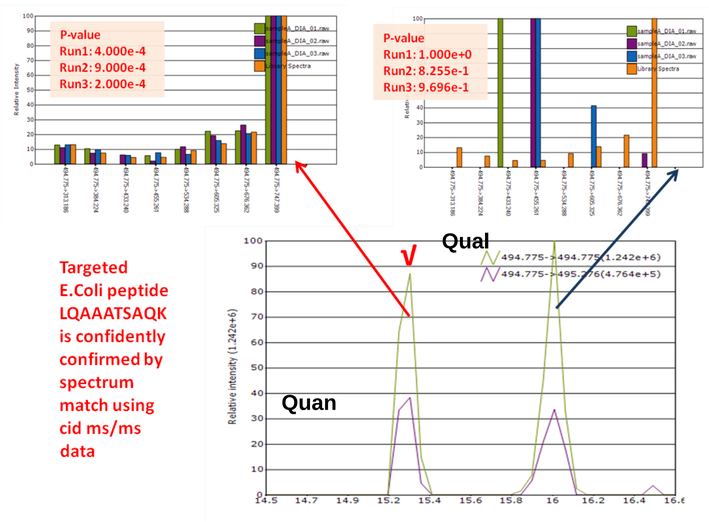


FIGURE 3. Simultaneous Qual/Quan using Pinpoint 1.3 software indicating low p-values and the correct target peptide at RT 15.3 min in the presence of a similar contaminant at RT 16.0 min.

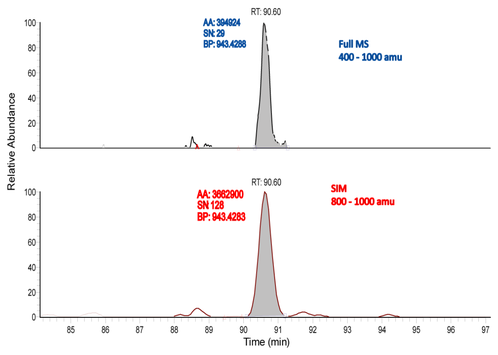
Results

Evaluation of Detection Limits and Linear Dynamic Range of the DIA Workflow

The detection limits and the quantitative dynamic range of this DIA workflow were evaluated using a mixture of seven isotopically labeled yeast peptides spiked into *E. coli* digests (500 ng/µL) at five different concentrations (0.01, 0.1, 1, 10 and 100 fmol/µL).

Unlike full MS, which isolates all precursor ions of the full mass range, the SIM scan with 200 amu isolation windows effectively “enriches” all precursor ions in that window while excluding all other ions outside the mass range of interest. This resulted in a lower limit of quantification in much the same way that selectively collecting peptides on a trapping column would. As a result, the SIM scan, even with a wide isolation window, can provide much higher sensitivity for low concentration peptides compared to full MS (Figure 4).

FIGURE 4. Several-fold increased sensitivity using SIM compared to using Full MS.



The seven isotopically labeled peptides spiked into 500 ng *E. coli* digests were detected at the lowest 10 amol concentration on column level with four orders of linear dynamic range.

Detecting and Quantifying Low-abundance and High-abundance Spiked Proteins with the DIA Workflow in a Single Experiment

Six bovine protein digests at dynamic abundance levels (BSA to β-lactoglobulin covering concentration range 10 amol to 1 pmol on column) were spiked into a 500 ng *E. coli* matrix. The HR/AM SIM provided high sensitivity, high selectivity and wide dynamic range to detect all six spiked proteins reproducibly over five orders of magnitude dynamic range (Table 1, results of triplicate analysis). Ninety percent of quantified peptides gave %CVs less than 10%.

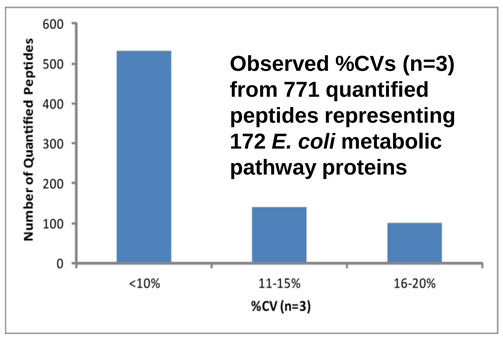
Table 1. Quantifying six bovine proteins spiked in 500 ng *E. coli* matrix at five orders of dynamic concentrations in a single experiment.

	Retention Time	CV% (n=3)	Run1	Run2	Run3
›spIP02769|ALBU_BOVIN Serum albumin (10 attomole on column)					
HLVDEPQNLIK	51.06	22	8.40E+05	1.32E+06	1.47E+06
›spIP02662|CASA1_BOVIN Alpha-S1-casein (100 attomole on column)					
FFVAPFPEVFGK	110.66	8	2.39E+06	2.39E+06	2.00E+06
›spIP00366|DHE3_BOVIN Glutamate dehydrogenase (1 fmol on column)					
LQHGTILGFPK	56.78	21	5.47E+06	5.55E+06	3.37E+06
GASIVEDK	21.11	6	1.02E+07	8.98E+06	9.11E+06
MVEGFFDR	68.04	7	3.46E+06	3.07E+06	2.94E+06
RDDGSVEVIEGYR	67.64	28	1.91E+06	3.46E+06	2.03E+06
HGGTIPIVPTAEFQDR	68.8	1	3.39E+08	3.43E+08	3.43E+08
C[Carboxymethyl]AVVDVPFGG	73.73	14	1.63E+07	1.60E+07	1.17E+07
YSTDVSVDEVK	46.35	10	2.11E+07	1.70E+07	1.77E+07
›spIP00921|CAH2_BOVIN Carbonic anhydrase (10 fmol on column)					
VLDALDSIK	65.52	4	2.39E+08	2.34E+08	2.15E+08
VGDANPALQK	23.66	7	1.89E+08	1.66E+08	1.64E+08
EPISVSSQQMLK	55.15	9	1.07E+07	8.92E+06	8.69E+06
AVVQDPALKPLALVYGEATSR	91.67	1	3.46E+07	3.54E+07	3.58E+07
YGGPLKAAQQPDGLAVVGVFI	113.84	10	3.60E+06	4.54E+06	4.26E+06
›spIP80025|PERL_BOVIN Lactoperoxidase (100 fmol on column)					
SPALGAANR	20.17	3	1.05E+09	9.81E+08	9.94E+08
LFQPTHK	19.72	11	5.30E+08	4.64E+08	4.09E+08
DGGIDPLVR	58.98	5	1.18E+09	1.09E+09	1.05E+09
IHGFDLAAINLQR	79.44	3	1.14E+09	1.13E+09	1.06E+09
GFC[Carboxymethyl]GLSQPK	49.46	9	1.57E+09	1.32E+09	1.29E+09
RSPALGAANR	14.27	13	5.65E+08	5.66E+08	4.24E+08
IVGYLDEEGYLDDQNR	76.67	1	3.49E+08	3.49E+08	3.39E+08
VPC[Carboxymethyl]FLAGDFR	88.27	6	1.73E+09	1.88E+09	1.63E+09
LIC[Carboxymethyl]DNTHITK	33.78	3	3.56E+08	3.78E+08	3.56E+08
DYLPIVLGSEMQK	94.67	7	1.11E+08	1.24E+08	1.32E+08
FWWENPGVFTEK	102.01	5	4.59E+08	4.84E+08	4.24E+08
AGFYC[Carboxymethyl]PTPPY	77.27	3	2.22E+09	2.24E+09	2.08E+09
›spIP02754|LAC3_BOVIN Beta-lactoglobulin (1 pmol on column)					
IDALNENK	25.32	11	9.15E+09	8.75E+09	6.98E+09
TPEVDDEALEKFDK	59.56	5	2.17E+10	2.13E+10	1.95E+10
VLVLDTDYKK	46.5	4	1.29E+09	1.20E+09	1.16E+09
TPEVDDEALEK	41.33	6	4.28E+10	3.72E+10	3.76E+10
TPEVDDEALEKFDK	59.77	5	2.17E+10	2.13E+10	1.95E+10
WENGEC[Carboxymethyl]AQK	22.78	5	3.64E+08	3.30E+08	3.30E+08
LSFNPTQLEEQC[Carboxymeth	89.46	2	4.13E+10	4.16E+10	4.00E+10

Detecting and Quantifying a Large Number of Proteins of Interest with the DIA Workflow in a Single DIA Experiment

The same DIA data files acquired in triplicate to quantify six spiked bovine proteins were used to additionally quantify 172 *E. coli* proteins which are involved with metabolic pathways based on annotation results by Thermo Scientific Protein Center™ software. 771 peptides were quantified representing these 172 proteins. Excellent analytical precision was observed (Figure 5). 69% of quantified peptides gave %CVs less than 10%. 88% of quantified peptides gave %CVs less than 15%. All quantified peptides gave CVs less than 20%.

FIGURE 5. Excellent analytical precision while quantifying large number of targeted proteins using the HR/AM SIM with the DIA workflow.



Conclusion

- A unique data independent acquisition workflow, which collects HR/AM SIM and rapid CID MS/MS in parallel on the new Orbitrap Fusion MS was developed. Any detected precursor ions in the HR/AM SIM can be quantified using XICs at narrow mass tolerance, and simultaneously confirmed using CID MS/MS by applying a post-acquisition targeted data extraction approach.
- Employing SIM scan with extremely high resolving power (240,000) separates most background interferences from analyte signal, resulting in highly selective and sensitive quantification. Ten (10) attomole on column LOD and 4 orders of linear dynamic range were observed with the developed DIA workflow.
- Over 5 orders of magnitude protein abundance for 6-protein mixture spiked into *E. coli* background could be detected and quantified with good analytical precision in a single experiment.
- 771 peptides representing 172 *E. coli* metabolic pathway proteins could be detected and quantified in the same experiment with good precision and accuracy.

References

1. Gillet, L.C. *et al. Mol Cell Proteomics, 2012, 11, O111.016717.*
2. Egertson,J.D. *et al.* Multiplexed Data Independent Acquisition for Comparative Proteomics. *ASMS poster, 2012*.
3. Gallien. S. *et al. J. Proteomics, 2012, 81, 148–158.*

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