Evaluation of iTraq™ Quantitation with Higher Energy Collisional Dissociation on a Hybrid LTQ Orbitrap™ XL Mass Spectrometer

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Overview

Purpose: Evaluating different parameter set for iTraq™ quantitation by means of HCD fragmentation in a LTQ Orbitrap XL

Methods: Protein mixture labeled in different ratios. Nano HPLC coupled to a LTQ Orbitrap XL. Experiments in parallel mode (survey @60.000 resolution→6 data dependent ion trap MS/MS ("Top 6"), or in sequential mode (survey 30.000→3 dd HCD. Nano-HPLC 60 min. gradient, C_{18} column (150mm x 75µm)

Results: Quantification by means of iTRAQ chemistry is well supported by the new LTQ Orbitrap XL with HCD fragmentation.

Introduction

The introduction of the LTQ Orbitrap XL including a multipole collision cell offers an additional collisional activation method on the system. Besides CID in the linear ion trap, the collision cell allows for Higher Energy Collisional Dissociation (HCD) experiments, which provides access to the low molecular mass range in MS/MS experiments with high efficiency. An important application within the proteomics field is the discovery of potential differences reflecting certain disease states. One approach of looking for differences are isotopic labeling techniques such as iTRAQ. Fragmenting iTRAQ labeled peptides within this collision cell enables accessing reporter ions, while having enough information for peptide identification in the higher mass region.

FIGURE 1. High energy collisional dissociation (HCD) fragment spectrum of a tryptic peptide. Depicted is the sequence coverage for b- (green) and y- ions (red) and the reporter ion region (inset). Intensities for reporter ions and fragment ions are similar .

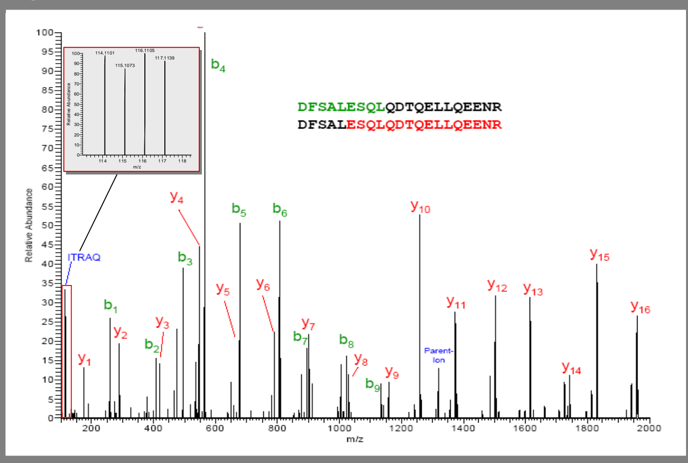


Table 1. Protein Mixture and their ratios used for the experiment

	Reporter ion ratio			
Protein	**114**	**115**	**116**	**117**
LACB	1	2	2	1
PYGM	1	0.2	0.05	0.01
ALBU	1	5	20	100
CYC	1	3	3	1
CAH2	1	0.5	0.2	0.03
CASB	1	2	2	4

Methods

A six protein mixture labeled and mixed in different ratios (Table1) was used to perform the evaluation. Measurements were performed at different AGC (1e5, 5e5 and 1e6) target values and at two different normalized collision energies (35 and 45) on a LTQ Orbitrap XL. A parallel experiment, performing one Orbitrap full scan @ 60.000 resolution, and 6 dd ion trap MS/MS (Top6) was performed for comparison of the identification efficiency of HCD fragmentation. For evaluation the MASCOT™ search engine version 2.2.01 (Matrix Science, London, UK) was used.

FIGURE 2. Various settings for collision energies and target values for HCD yield similar results in terms of number of IDs, sequence coverage and Mascot Protein score for all settings. These results were also obtained, if the LTQ Orbitrap worked in parallel mode (top 6) depicted in medium blue.

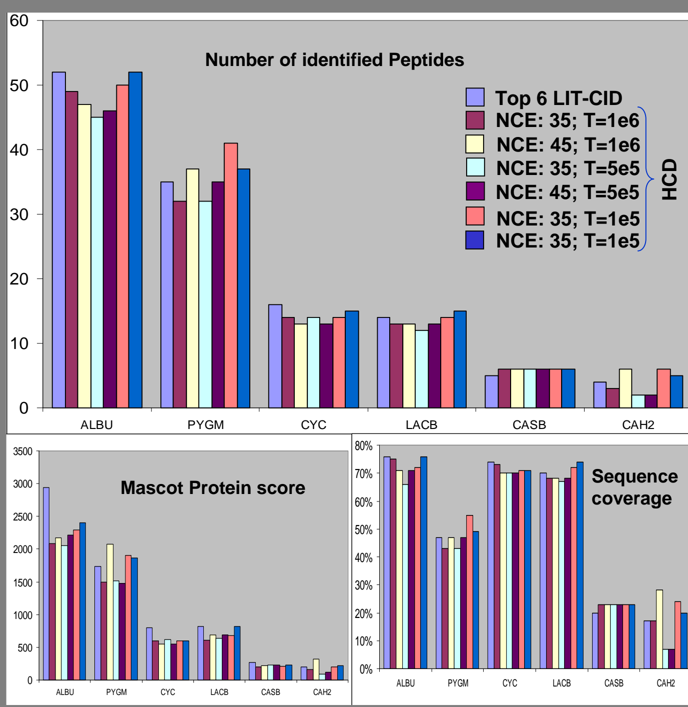
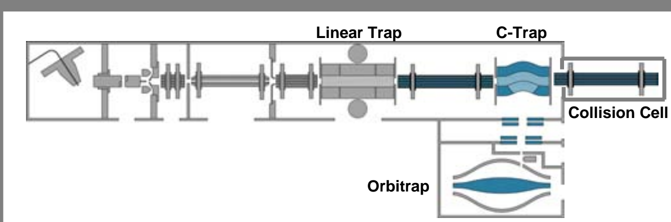


FIGURE 3. *Schematics of LTQ Orbitrap XL. The additional octapole collision cell is placed behind the C-trap. For High Energy Collisional Dissociation (HCD) ions are transferred to the octapole and, after fragmentation, they re-enter the C-trap before being ejected into the orbitrap.*



Results and Discussion

Protein ID

Results for database search are displayed in Figure 2. In all cases we identified the proteins with high confidence and high sequence coverage. We obtained similar results for all of our experiments in terms of identification efficiency. To best of our knowledge and from our experience, increasing the complexity of the sample pushes the balance in favour of high-speed, parallel detection (data not shown).

Quantitation

The second most important prerequisite for iTRAQ quantitation is quality and reliability of the reporter ion detection. For this reason we performed quantitation for the different parameters settings described above using MASCOT. The results are displayed in Figure 4. All proteins were quantified in good agreement with their theoretical ratios. In some cases (especially the high dynamic range proteins) we measured consistently lower ratios, indicating a systematic error, possibly a result of incomplete labeling during sample preparation.

FIGURE 4. Quantitation results for the 6 protein mixture compare favorably with theoretical values. All ratios are normalized to the reporter ion at m/z 114.

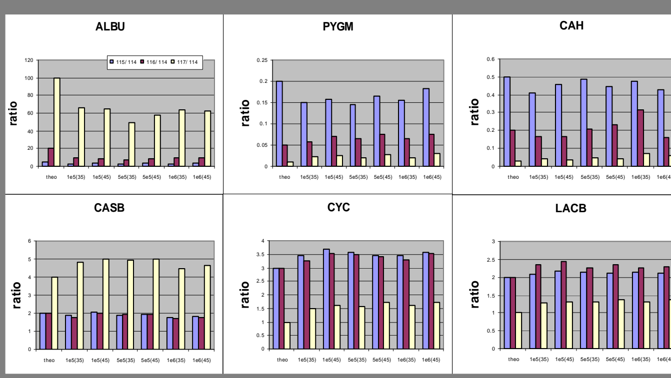
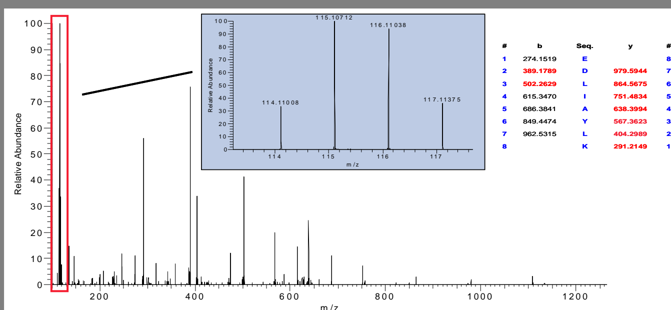


Figure 5 shows one example for a spectrum obtained from a Cytochrome C tryptic peptide. It shows a 1:3:3:1 ratio as expected and 100% sequence coverage.

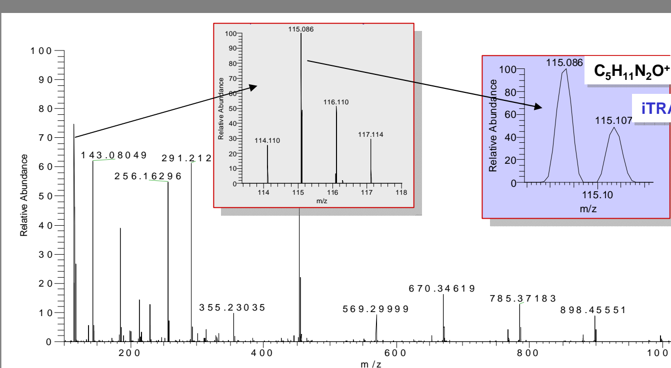
FIGURE 5. Example of MS/MS spectrum of a Cytochrome C peptide. The Mascot result shows 100 % sequence coverage, while the reporter ion region shows a 1:3:3:1 ratio for the four reporter ions.



Interferences

Besides high reproducibility of the reporter ions, we want to point out the usefulness of high resolution measurements to resolve reporter ions from interferences originating from amino acid fragments, in particular, in the low mass range (Figure 6).

FIGURE 6. Interference originating from Arginine. Such fragments could lead to false positive quantitation results, and the high resolution power is of major benefit to perform the quantification reliably.



Conclusions

The new LTQ Orbitrap XL system with its octapole collision cell is well suited for performing iTRAQ quantitation work. All measurements showed high sequence coverage, a sufficient number of identified peptides and reliable quantitation results. In addition, we established the importance of high resolution measurements in the low mass range, where the presence of unresolved, interfering ions may lead to incorrect quantitation results.

We demonstrated consistent quantitation results for high and low abundance proteins with ratios up to 1:100. More relevant, however, we demonstrated highly precise quantitation results for minor, "regulatory" proteins with ratios between 1:2 and 1:3. In the future we plan to evaluate the behaviour of iTRAQ quantitation in complex samples and biological matrices.

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