Accurate mass LC/MS as tool for label free quantitative proteome studies

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Introduction

For elucidation of biological processes different gel and MS-based techniques like e.g. two-dimensional difference gel electrophoresis (2D-DIGE), ICAT, ITRAQ, ICPL and in vivo isotope labeling have been used since quite a while, mainly for biomarker discovery.

Recent studies showed that label-free approaches can be applied very successfully.

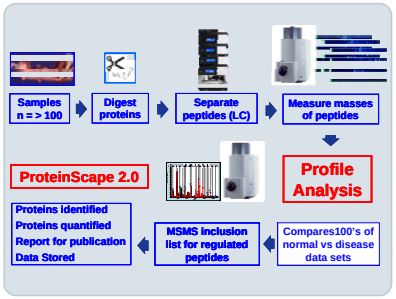

Fig. 1 Workflow for label-free quantitation

Methods

We have developed a new approach for label-free quantitation allowing both, biomarker discovery and validation. We have evaluated the MS based approach of label-free proteomics in respect of reproducibility and sensitivity.

The workflow for biomarker discovery is shown in figure 1. For the experimental setup we use nano-HPLC (Ultimate 3000, Dionex), ESI-QTOF-MS (micrOTOF-Q, Bruker Daltonics) and MS analysis software prototype for label-free proteomics analysis. Whole cell lysates of the human lung carcinoma cell line A549 obtained from the same cell culture samples were digested with trypsin. 500 ng of the trypsin digest were analyzed applying a 2 hour LC gradient.

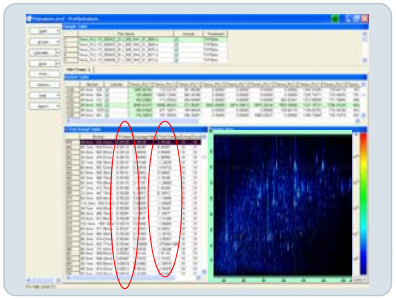

Fig. 2 ProfileAnalysis 2.0: Statistical analysis including all necessary processing steps

Statistical analysis including extracting molecular features, RT alignment, matching features, normalization, and quantitation based on a t-test or ANOVA is performed by ProfileAnalysis (fig. 2). In a second step we conduct targeted AutoMS/MS with a scheduled precursor list. Then identification and quantitation on a protein level is performed in ProteinScape (fig. 3).

To test the quality of our approach for validation purposes, 4 proteins at different concentrations were spiked into the cell lysate at different concentrations.

Figure 4 shows the high selectivity of our approach with high resolution extracted ion chromatograms (hrEIC's).

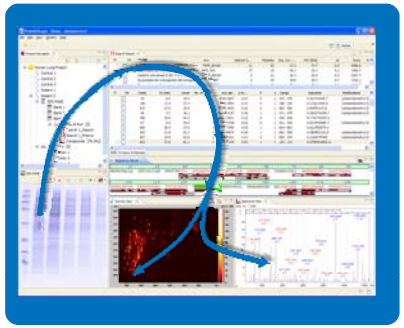

Fig. 3 Identification results displayed in ProteinScape 2.0

The actual quantitation results are in very good agreement with the real relative amount of protein in the cell lysate.

The quantitation results of the human lung carcinoma cell line upon TGF-beta treatment are discussed on a second poster (V12-T)

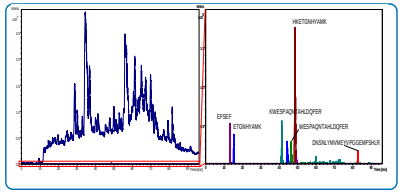

Fig. 4 High selectivity for validation of target peptides from very complex mixture
Note: the intensity scale of the right chromatogram is extended by more than a factor 100x

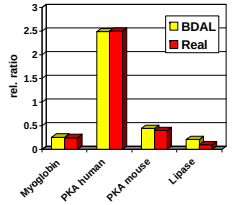

Fig. 5 Results from targeted quantitation. With the exception of Lipase average quantitation error <5%

Summary

• Label-free quantitative solution for discovery, identification, and targeting of regulated proteins, all on a single platform

High intra-spectra dynamic range of more than 4 orders of magnitude important for quantitation and validation

• High specificity because of better than 2-3 ppm mass accuracy

• Capability to re-evaluate already acquired data with hrEICs for validation purposes

Conclusions

Label free accurate mass LC-MS workflow for quantitation allowed to:

• Obtain high selectivity due to high resolution extracted ion chromatograms

• Obtain high reproducibility because no labeling chemistry is involved and the quantitation based on MS spectra

• Obtain high sensitivity in very complex samples due to the sensitivity of the micrOTOF-Q

ESI-QTOF