Bruker Daltonics

Technical Note # TN-27

ProteomeQuant – A Highly efficient Solution for Biomarker Discovery and label-free Identification of Regulated Proteins in Biological Systems

Abstract

A label-free solution for quantitative proteomics is presented which is based on a highly stable LC-MS/MS system offering high mass resolution, accuracy and sensitivity. Combining this system with powerful bioinformatics analysis and data-warehousing software, allows for comprehensive high-throughput quantitative studies. This is demonstrated using a human lung carcinoma cell line as model system.

Introduction

Quantification of changes in biological systems in response to certain treatments is an important but challenging task in proteomics [1]. So far no single quantitative workflow is considered to be the gold standard for this task. Classical methods use labeling with dyes or isotope enriched chemicals to generate internal standards for quantification. Label-free methods aim to compare direct mass spectrometric signal intensity across multiple LC runs without an internal standard. The main advantages of the label-free approach are reduced sample consumption and cost due to the elimination of labeling steps and a simple biochemical workflow.
In order to achieve quantification accuracies comparable to labeling methods, high reproducibility and stability of the LC-MS system in regard to protein/peptide mass, retention time, and intensity are mandatory. Sophisticated algorithms for data analysis enable label-free methods for high-throughput applications, such as clinical research studies for biomarker discovery and validation. ProteomeQuant is a flexible solution integrating accurate mass LC-MS/MS instruments with a bioinformatics platform for proteomics analysis.

ProteomeQuant workflow

The starting point of the ProteomeQuant workflow is a substantial number of biological samples including technical and biological replicates (Figure 1). The samples belong to different states of the biological system, e.g. healthy or diseased state. The workflow includes the following steps:

- For each digested sample one LC-MS run is performed on a high resolution, sensitive, and accurate LC-MS system like micrOTOF-QII ESI-Q-TOF or maXis™ UHR-TOF.
- Signals which originate from the same peptide across all LC-MS runs are elucidated and automatically assigned to a molecular feature.
- Using statistical methods, those peptides are determined which show significantly different intensities in different states.
- The determined regulated peptides are targeted for identification in subsequent LC-MS/MS runs.

- All MS/MS spectra are combined and searched against a protein database.
- Identified peptides are assigned to corresponding molecular features and quantification from the LC-MS runs.
- Protein regulations are calculated from peptide regulations.

Workflow properties

Identification and quantification of peptides are performed in separate LC runs of the sample. This has several advantages:

- The mass spectrometer is operated with optimal conditions for quantification. For accurate quantification the number of MS spectra per chromatographic peak should be as high as possible.
- Separating MS and MS/MS measurements in different LC runs allows for simpler method development and superb results in both MS and MS/MS runs. If desired, MS and MS/MS measurements are performed on different mass spectrometers. Intensity information obtained from MS runs is used in subsequent targeted MS/MS runs.
- In the targeted approach, MS/MS spectra are acquired only of significantly regulated peptides which can be at low abundance and would therefore be missed in data-dependant AutoMS(n) LC-MS/MS runs.
- Targeting significantly regulated peptides saves acquisition and analysis time as well as hard disk space. Therefore, the workflow is very efficient.
- Due to the measurement of replicates on a highly reproducible LC-MS system, protein quantification is as accurate and precise as in labeling approaches.
- Additional targeted, hypothesis-driven LC-MS/MS runs are made to validate protein identification and quantification.

ProteomeQuant instrumentation and software

- A highly stable nano HPLC (e.g. Dionex Ultimate 3000 or Bruker EASY-nLC™) and a mass spectrometer with high mass resolution, high sensitivity, and high dynamic intensity range for MS measurements as well as high sensitivity for MS/MS measurements. The micrOTOF-Q II™, maXis, or ultraflex III TOF/TOF mass spectrometers fulfill these requirements.
- ProteinScape™ as an integrated bioinformatics base package for LC and gel based proteomics workflows with full support for label-free quantification.

Methods

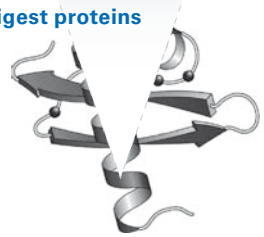
We demonstrate the successful application of ProteomeQuant in a proteomics study of a cell culture model, human lung carcinoma cell line A549. The treatment of these cells using TGF-beta is a model for lung fibrosis. We investigated the system in a 2D-DIGE study and the described label-free approach. In the DIGE experiment 50 µg protein from whole cell lysate were labeled with the minimal dyes and separated by carrier ampholine based 2-DE. The performance of label-free proteomics was investigated by a setup comprising nano-HPLC (Ultimate 3000, Dionex), ESI-Q-TOF, micrOTOF-Q II and MS analysis software for label-free proteomics analysis based on the ProteinScape bioinformatics package. Each LC-MS run used 500 ng of the trypsin digest with a 2 hour LC gradient.

Results

By considering 10 biological replicates per state we could reveal 104 differentially regulated proteins (fold change 1.5, $p < 0.001$) in the DIGE study. So far we identified 74 of these 104 regulated proteins by using MALDI-TOF/TOF-MS (ultraflex, Bruker Daltonics). In the label-free approach we detected 667 differentially regulated peptides. The differentially determined peptide masses were used to construct an inclusion list for targeted MS/MS analysis in subsequent LC-MS/MS runs. From these runs, 333 peptide MS/MS spectra were matched to 148 regulated proteins from database searches (Figure 2). The comparison of the identified proteins between 2D-DIGE study and label-free proteomics results in 16 proteins found in both experiments. However, most of the proteins were found only in one approach, indicating that a combination of results from both techniques is very promising for comprehensive quantification. Results from the different workflows are collected and compared in the ProteinScape database. Further details of the extensive study will be published elsewhere [2].

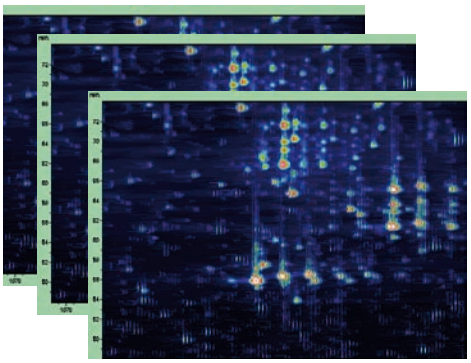
Samples n ≥ 20

Digest proteins



Compares100's of normal vs disease data sets

Measure masses of peptides



Protein identification and quantification in ProteinScape

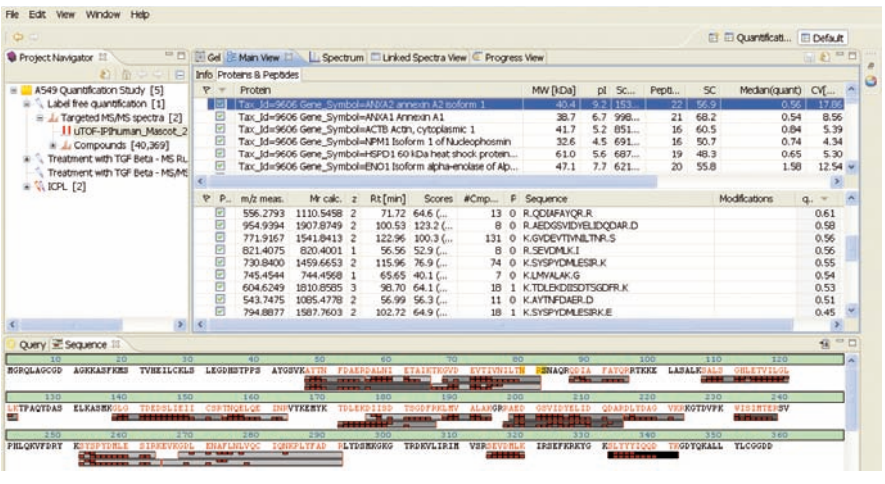


Fig. 1: The ProteomeQuant Workflow. A number of samples which belong to different states of the biological system are digested. For each sample an LC-MS run is performed. Analysis software elucidates peptides showing a significant intensity difference across the different biological states. MS/MS spectra of these regulated peptides are recorded in additional targeted LC-MS/MS runs and identified by using ProteinScape. After identification, protein quantifications are calculated and reports generated.

Conclusion

- ProteomeQuant consists of an integrated instrumentation- and software platform for performing label-free quantification of significantly regulated proteins in biological systems.
- Due to lower sample consumption, elimination of labeling steps, simple biochemical workflows, and targeted MS/MS measurements of only regulated peptides, ProteomeQuant is suitable for high throughput studies such as biomarker discovery and validation.
- ProteomeQuant gives complementary results compared to other quantification techniques like DIGE. The powerful bioinformatics platform integrated in ProteomeQuant allows for merging proteomics data from different sources for a comprehensive description of changes in biological systems.

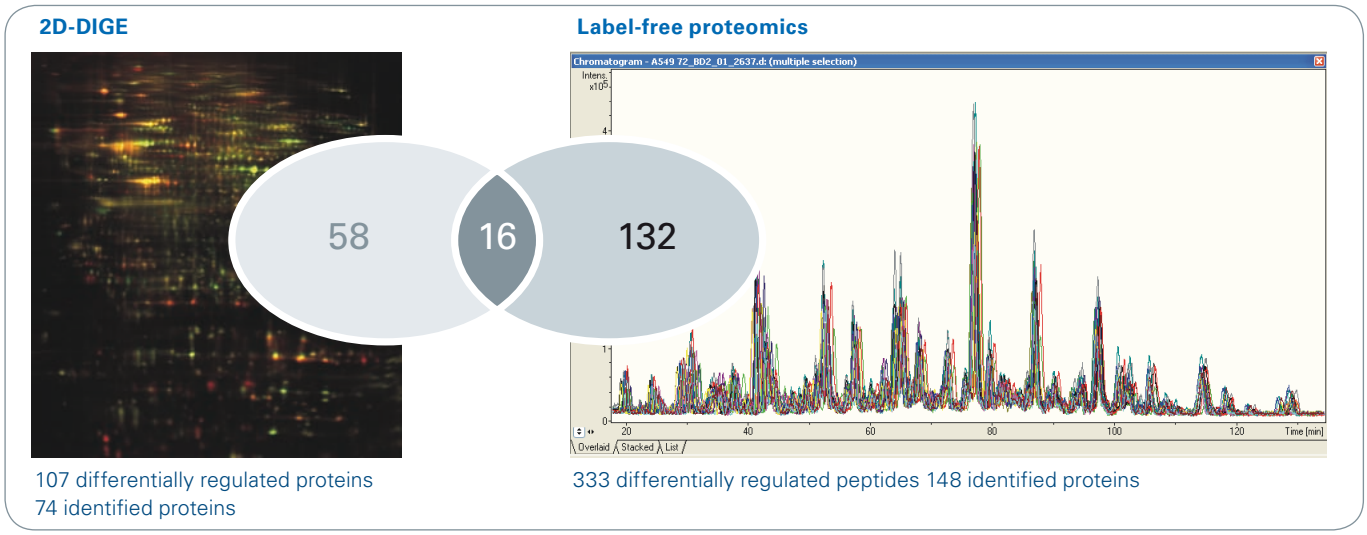


Fig. 2: DIGE study and label-free methods give complementary results regarding identified regulated proteins. Proteomics data of different workflows are merged in ProteinScape for a comprehensive description of changes in biological systems.

References

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Instrumentation & Software
maXis
micrOTOF-Q II
ultraflex III
EASY-nLC
ProteinScape

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