Novel MS based Strategies for Differential Quantitative Analysis of Complex Proteomes

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The growing demand for novel and tailored therapeutic targets and reliable biomarkers for the diagnosis, prognosis and monitoring of diseases continues to stimulate innovations in "omics" technologies. In the past decade, new insights into biochemical pathways disturbed by pathologies could be gained by differential profiling of proteins using approaches such as 2D-gelelectrophoresis [1] combined with mass spectrometry. A number of candidate targets and biomarkers have already been delivered to the drug discovery pipelines [2]. Intensive efforts have been made to develop alternative technologies for quantitative differential gel-independent proteome analysis. These techniques include promising concepts involving stable-isotope labeling combined with mass spectrometry. We have developed a gel-independent peptide/protein tags for differential quantitative proteome analysis including the quantitative Protein Sequence Tag® **(qPST™)** and the Tandem Mass Tag® (TMT®) technology.

The qPST™ is a further development of our previously published PST® procedure [3]. The method is based on N-terminal labelling of cyanogen bromide cleaved proteins using an unique class of stable isotope labels , the Basic Mass Tags (BMT). The qPST™ combines the features of relative protein quantification via isotopomeric peptides pairs and MS/MS based identification. As required for accurate performance, the corresponding qPST™-tag-labelled 'light' and 'heavy' peptides show identical chromatographic retention times. A key feature of the qPST™ approach is that the labeling can be performed at the protein or large peptide level and hence sample pairs can be combined at an early stage of the analysis process. Resulting advantages to other approaches are shown.

As a proof of concept study we have differentially analyzed the lysate of yeast grown on either ethanol or galactose as the only carbon source. The differential protein expression results obtained from this yeast qPST™ experiment, which will be shown in this presentation, are in agreement with the data available in the public domain.

Tandem Mass Tags® **(TMT®)**, a further tagging approach developed by our team introduces a novel and innovative concept for highly accurate and sensitive quantification of peptides/proteins in complex proteomes [4]. This is achieved by the special design of the TMT tag which consists of (i) reporter group, (ii) a mass balancing group and (iii) a reactive group. Consequently all TMT® labelled peptide pairs have the identical mass and the quantification is achieved by specific reporter ions at predicted m/z values generated in the MS/MS mode. The advantages of this approach are shown.

Literature

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