

An elegant, gel-free strategy for quantitative protein profiling using isotope labelled PST tags (qPST)

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Overview

- Gel-free quantitative Proteomics techniques deal with the analysis of high complex mixtures.
- The gel-free PST (Protein Sequence Tag) technology reduces the complexity for MS analysis [2].
- Quantitation in PST (qPST) is done by stable isotope labelling.
- The labelling and the mixing of samples can be performed at an early stage of the process.
- Here we present results of the qPST quantitation on protein level and first results for a yeast digest.

The PST-Process

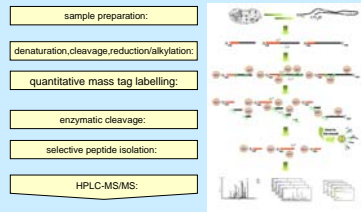


Figure 1. Protein Sequence Tag sample procedure.

Methods

HPLC ESI-MS/MS and data analysis.

For the quantitative PST procedure (qPST) we have tested the original PST tag and two different isotopic labeled tags, one containing four ¹³C Atoms, the other one containing four ¹³C atoms and one ¹⁵N atom, resulting in a mass difference of 4 or 5 Da respectively. The labeled digests of BSA, Glutamate Dehydrogenase and Yeast were analyzed using defined molar ratios (e.g. 1:2; 1:1; 2:1). All the MS measurement were done on a Q-ToF 2 Instrument equipped with a Cap LC HPLC system. Samples were trapped onto a 0,3 mm i.d. x 5 mm precolumn and eluted through a 75 µm i.d. RP18 column to the ESI ion source. The MS results were interpreted manually, peak areas of the isotopic clusters were compared.

Introduction

For quantitative differential protein analysis gel-free profiling strategies are of high interest. The PST (Protein Sequence Tag) technology deals with the isolation and MS/MS based identification of one N-terminal peptide from each polypeptide fragment generated by cyanogen bromide cleavage of a mixture of proteins. The PST technology has excellent performance in the analysis of membrane proteins [2]. Stable isotope coding is nowadays a well established MS based quantification method in proteomics [3]. For an alternative quantitation in MS/MS mode see [4]. Here we describe the PST technology for differential protein quantitation by using stable isotope labeled tags. The labelling and the mixing is done at an early stage of the process.

The quantitative PST-Process

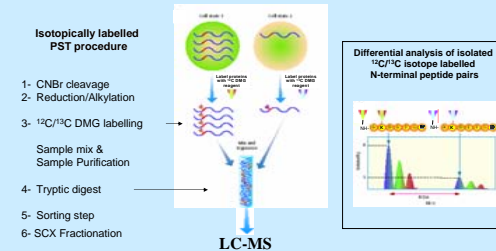


Figure 2. Quantitation in the Protein Sequence Tag sample procedure.

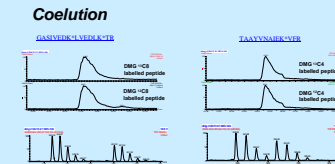


Figure 3. Coelution shown in elution profiles and the mass spectra of the singly and doubly labeled pairs.

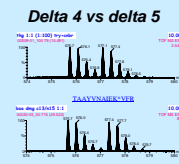


Figure 4. Comparison of ESI-MS spectra of the singly and doubly labeled pairs. 5Da mass difference in a pair.

Results

Quantitation for different ratios

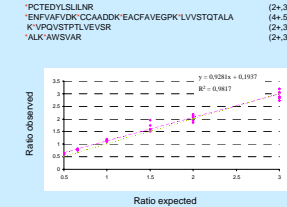


Figure 5. Validation of the accuracy of the quantitation for different abundance ratios of BSA peptides.

Quantitation for different repeats

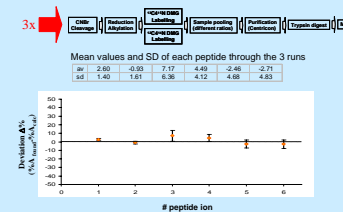


Figure 6. Reproducibility of three independent experiments of the quantitation steps for a BSA digest with an expected ratio of 1:1.

First results for a yeast digest

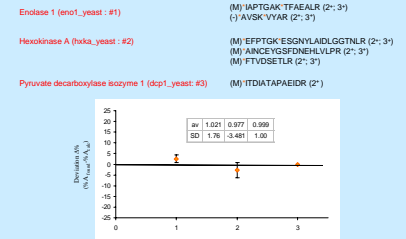


Figure 7. Accuracy of the quantitation on a yeast digest on protein level (expected ratio 1,0).

Conclusions & Perspectives

- The proprietary PST technology is expanded for quantitation.
- A mass difference of 5 Da is better than 4 Da.
- The labelling for the quantitation is done early in the process.
- qPST has an accurate and reproducible quantitation process.
- The qPST technology represents a powerful tool for expression studies of soluble and hydrophobic proteins.

References

- [1] Washburn, M.P., D. Wolters, et al. "Large-scale analysis of the yeast proteome by multidimensional protein identification technology" *Nature Biotechnology* **2001**, 19 (3): 242-247
- [2] Kuhn, K., Hamon, C. et al. "Isolation of N-Terminal Protein Sequence Tags from Cyanogen Bromide Cleaved Proteins as a novel Approach to Investigate Hydrophobic Proteins" *J. of Proteome Research* **2003**, 2: 599-609
- [3] Gygi, S.P., Aebersold, R. et al. "Quantitative analysis of complex protein mixtures using isotope-coded affinity tags." *Nat. Biotechnol.* **1999**, 17: 994-999
- [4] Thompson, A., Schäfer, J., Kuhn, K., Schwarz, J., Hamon C. et al. "TMT Mass Tags - A Novel quantitation Strategy for Comparative Analysis of Complex Protein Mixtures by MS/MS" *Anal. Chem.* **2003**, 75 (8):1895 - 1904

- The qPST quantitation uses a pair of stable isotope labeled tags. Tagging before the digest enhances the accuracy of the quantitation.
- Both species coelute in a RP HPLC chromatogram.
- A mass difference of 5 Da in comparison to 4 Da has benefits for the accuracy of the quantitation of singly labeled peptides.
- The quantitation is accurate for different abundance ratios.
- The qPST quantitation has a good reproducibility.
- First results of the accuracy for qPST for a yeast digest are shown.