

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

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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 quantitation 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 qPST-Process

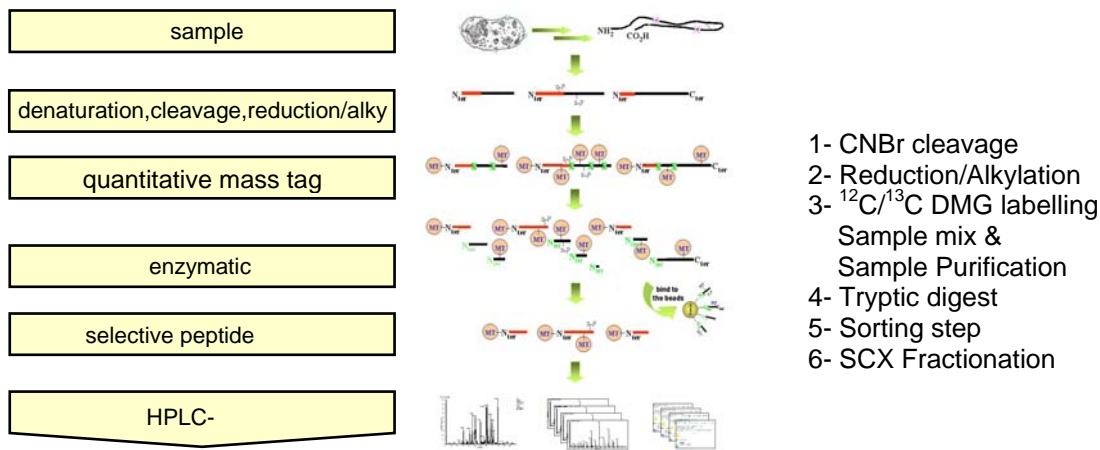


Figure 1: Protein Sequence Tag sample procedure and the steps for quantitation.

Methods

For the quantitative PST procedure (qPST) we have tested the original PST tag and two different isotopic labeled tags, one containing four ^{13}C Atoms, the other one containing four ^{13}C atoms and one ^{15}N 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.

Results

Quantitation for different repeats



Mean values and SD of each peptide through the 3 runs

av	2.60	-0.93	7.17	4.49	-2.46	-2.71
sd	1.40	1.61	6.36	4.12	4.68	4.83

Coelution

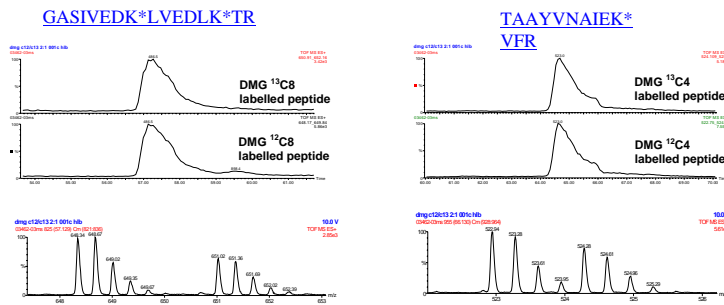


Figure 1: Coelution shown in elution profiles and the mass spectra of the singly and doubly labeled pairs.

Delta 4 vs delta 5

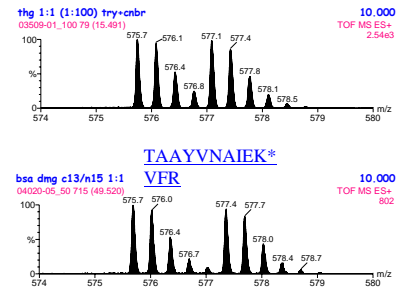


Figure 2: Comparison of ESI-MS spectra of using 4 Da vs. 5Da mass difference in a pair.

- 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 accuracy of the quantitation for different abundance ratios is shown.
- The qPST quantitation reproducibility is shown for three different repeats.
- First results of the accuracy for qPST for a yeast digest are shown.

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

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- [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: 598-609
- [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 MSMS", *Anal. Chem.* **2003**, 75 (8):1895-1904