



The Protein Sequence Tag (PST®) Technology: a gel-free proteomic approach for broad application

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Overview

- The Protein Sequence Tag (PST®) technology is a gel-free proteomic approach based on a specific sample preparation, amino group labelling and the reduction of the sample complexity.
- The PST® approach is unbiased against proteins with different biochemical properties as pI, MW, hydrophobicity and, thus, is applicable to all kinds of protein samples.
- The PST® technology is currently expanded to obtain also quantitative proteomics data.

Introduction

Gel-free proteomic technologies are more and more employed in investigations concerning the regulation and interaction of proteins.^[1] Commonly in such approaches, the protein identification is achieved by MS/MS sequencing of corresponding peptides and the relative quantification is achieved through the incorporation of stable isotopes. However, such technologies may differ in the manner how they perform any labelling (e.g. metabolic or *in vitro*) and if and how they perform a reduction of sample complexity prior to the MS-based analysis.

We have recently shown the effectivity and the robustness of the PST® technology by the application on total mitochondria of *S. cerevisiae*.^[2] All the steps performed in the PST® approach are based on effective and robust chemical manipulations.

With respect to the development of a quantitative PST® approach, the steps were arranged in such a way, that the combination of different labelled probes can be achieved as early as possible.

The aim of the poster presented here is to show that the PST® technology addresses all kinds of protein samples, making this approach broadly applicable. Comparisons to other proteomic approaches were carried out and are presented, too.

The workflow of the PST® technology is illustrated in the following figure.

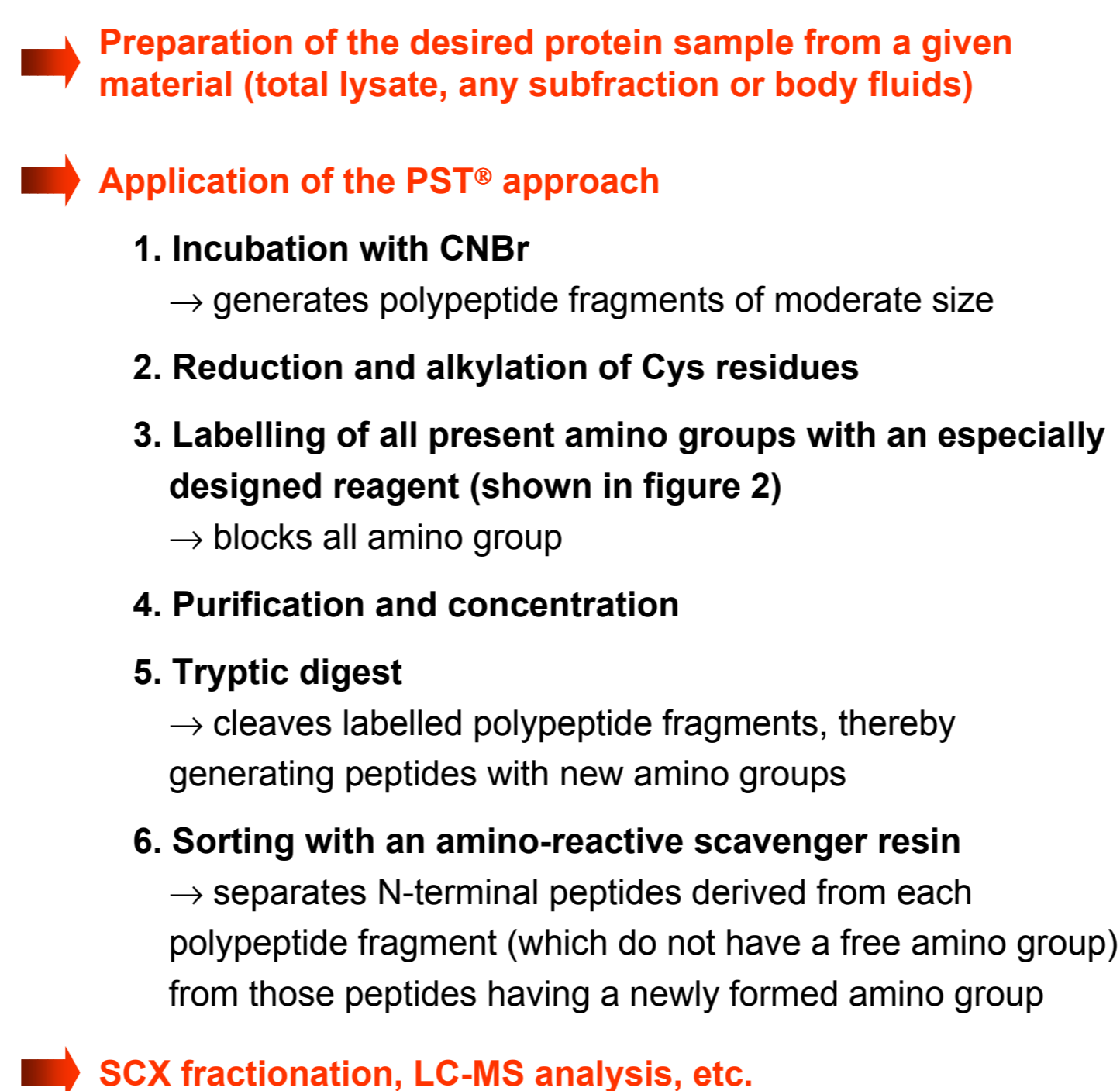
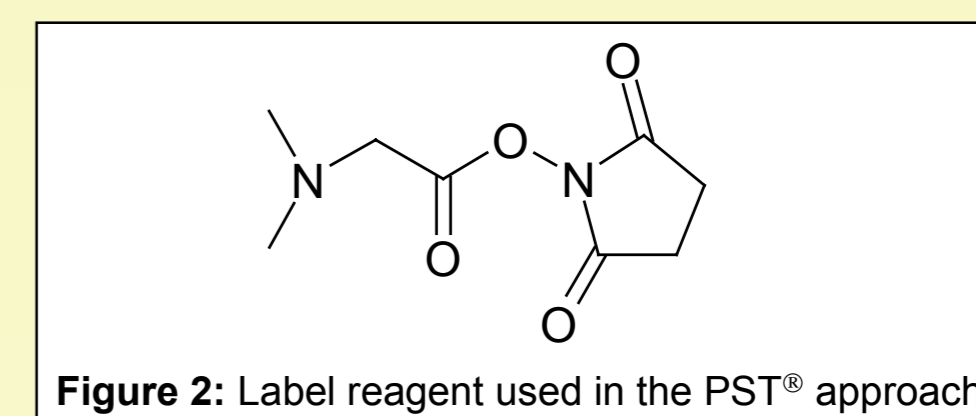


Figure 1: Workflow of the PST® technology.

Description of the PST® approach

- The initial CNBr cleavage provides several beneficial effects: (1) it makes the functional groups more accessible for further manipulations, (2) it increases the number of labelling sites which is favourable in the quantitative approach (3) it is highly effective to solubilise membrane proteins, even if they have several membrane-spanning domains.
- The labelling of the amino groups is optimised to be practically complete and to be highly specific towards amino groups. The label itself (for the structure of the label reagent, see figure 2) is designed to maintain intrinsic peptide properties concerning their chromatographic behaviour in the SCX fractionation and their fragmentation behaviour during MS/MS.



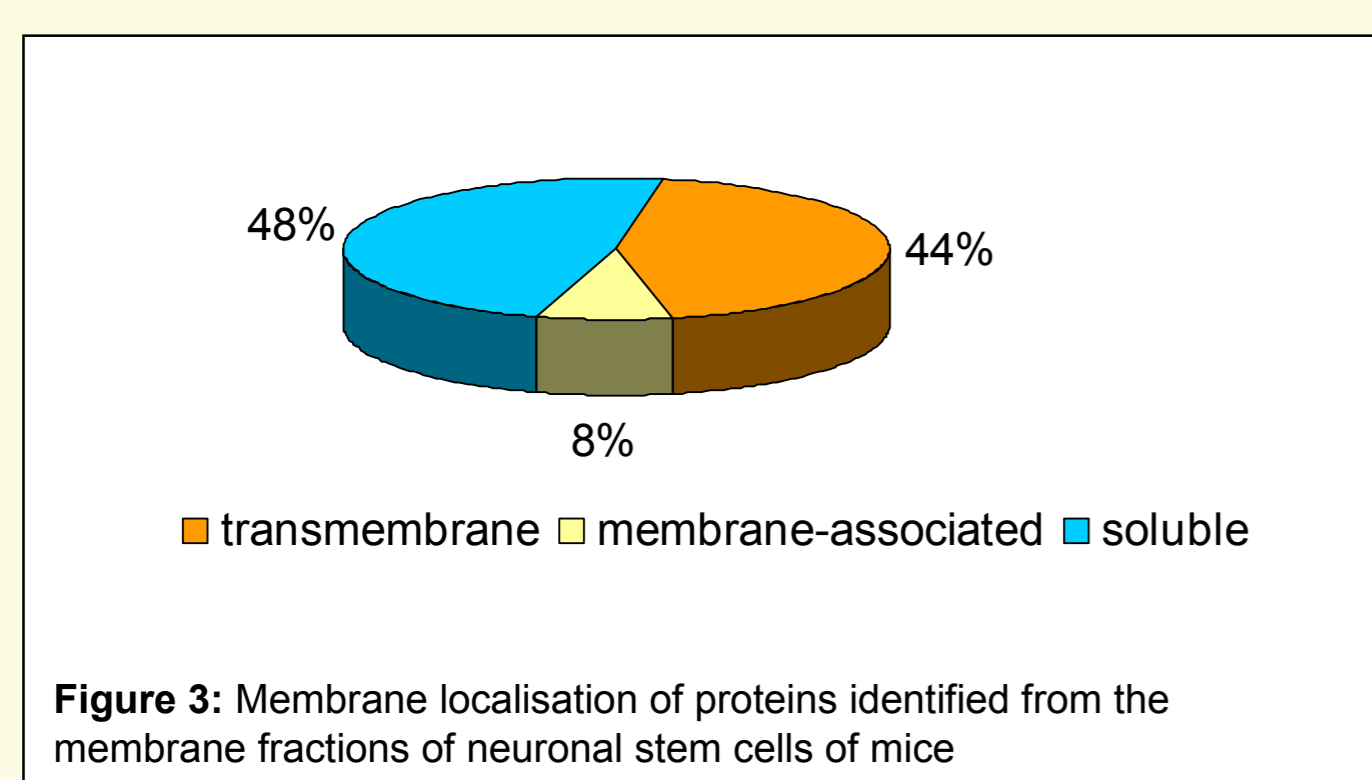
- In the quantitative PST® (qPST™) approach, ¹³C and ¹⁵N are incorporated into the label to achieve a mass difference of 5amu per label introduced into the peptide.
- Labelling of Lys residues causes the loss of Lys as cleavage site for trypsin, which results in ArgC-like digest pattern. Consequently, a lower number of peptides are generated by the tryptic digest, thereby delivering a reduction of the complexity.
- The sorting step is an optional step to further reduce the sample complexity. Such a further complexity reduction can lead to a higher number of pure protein identifications, but a certain degree of redundancy (= more than 1 identified peptide per protein) increases also the reliability of both the identifications and the regulation data.

Applications

1. Membrane fractions

We investigated the membrane fractions of neuronal stem cells of mice from both the BD03 and the BD08 cell lines. Beside to assess the technical performance of the PST® approach when working with membrane fractions, the aim of this study was to identify hydrophobic proteins related to the Alzheimer disease.

After the isolation of the membrane fractions, which was achieved by cell lysis, ultracentrifugation and a subsequent alkaline sodium carbonate treatment, we performed the PST® approach as illustrated in figure 1. A thorough analysis of the SEQUEST results obtained from the MS/MS data yielded in the identification of 200 proteins. Figure 3 shows the distribution of these proteins concerning their membrane localisation.



More than half of the found proteins were identified as transmembrane or as membrane associated proteins. Among them, we could identify G-protein coupled receptors and other proteins with more than 5 transmembrane domains.

About half of the remaining soluble proteins identified in that study had an isoelectric point >10. Those proteins are known to be hard to remove from membrane fractions by the alkaline sodium carbonate treatment.

APP, nicastrin and neprilysin, which are described to be involved in the Alzheimer disease^[3], could be identified.

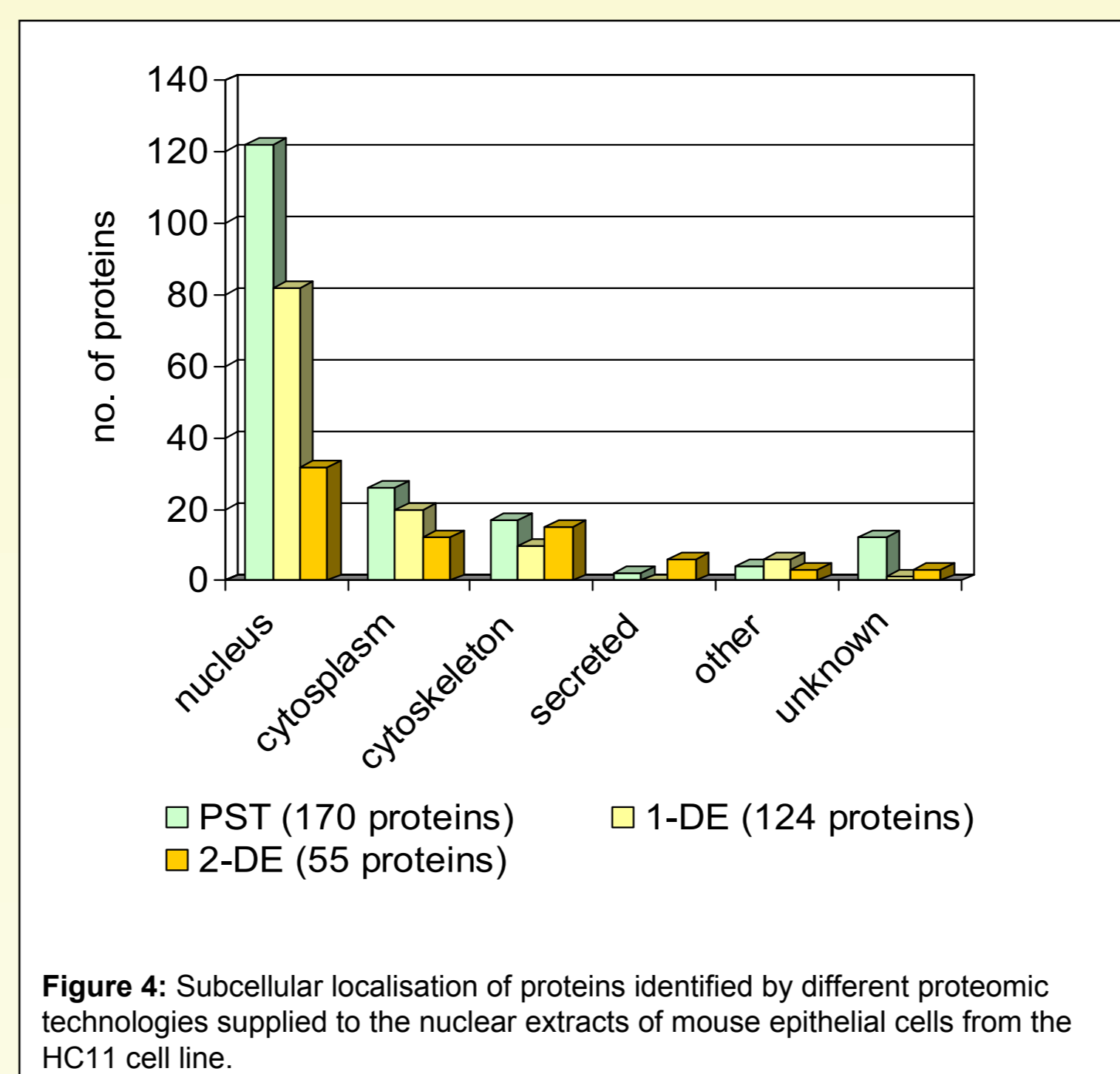
Another example for the investigation of a membrane fraction, namely the membrane fraction of crude mitochondria from yeast, is described in very detail at the poster entitled „Analysis of low abundant membrane proteins using the gel-free Protein Sequence Tag (PST®) Technology“.

2. Nuclear fractions

This kind of protein sample was investigated by the PST® technology as well as by the 1-DE/PMF and the classical 2-DE approaches. The global goal of that study was to identify nuclear proteins that are involved in the differentiation of epithelial cells by using a set of different approaches. However, here in this presentation, we focussed on the comparison of the protein identification rate of different proteomic technologies when applied to nuclear fractions.

Cultured mouse cells from the HC11 cell line at different stages of development were employed in these investigations. The nuclear extracts were obtained by a smooth disintegration of the cells which leaves the nuclei intact, spinning down of these nuclei and of unbroken cells by slow centrifugation and a directed lysis of the nuclei only with an appropriate buffer. Subsequently, the three above mentioned methodologies were supplied to the same samples each.

Figure 4 shows the outcome of these approaches after a thorough analysis of all the obtained MS data.



The PST® technology identified with 170 proteins more proteins than both gel-based approaches. Both the PST® and the 1DE/PMF approach identified nearly 70% of nuclear proteins, which shows the successful enrichment of this subfraction.

Interestingly, the overlap between the PST® and the gel-based technologies concerning the protein identities was found to be around 50%, the overlap between 1-DE and 2-DE was even lower. This shows the complementary of different proteomic approaches. Therefore, a combined application will increase not only the reliability of the found data but also the total outcome.

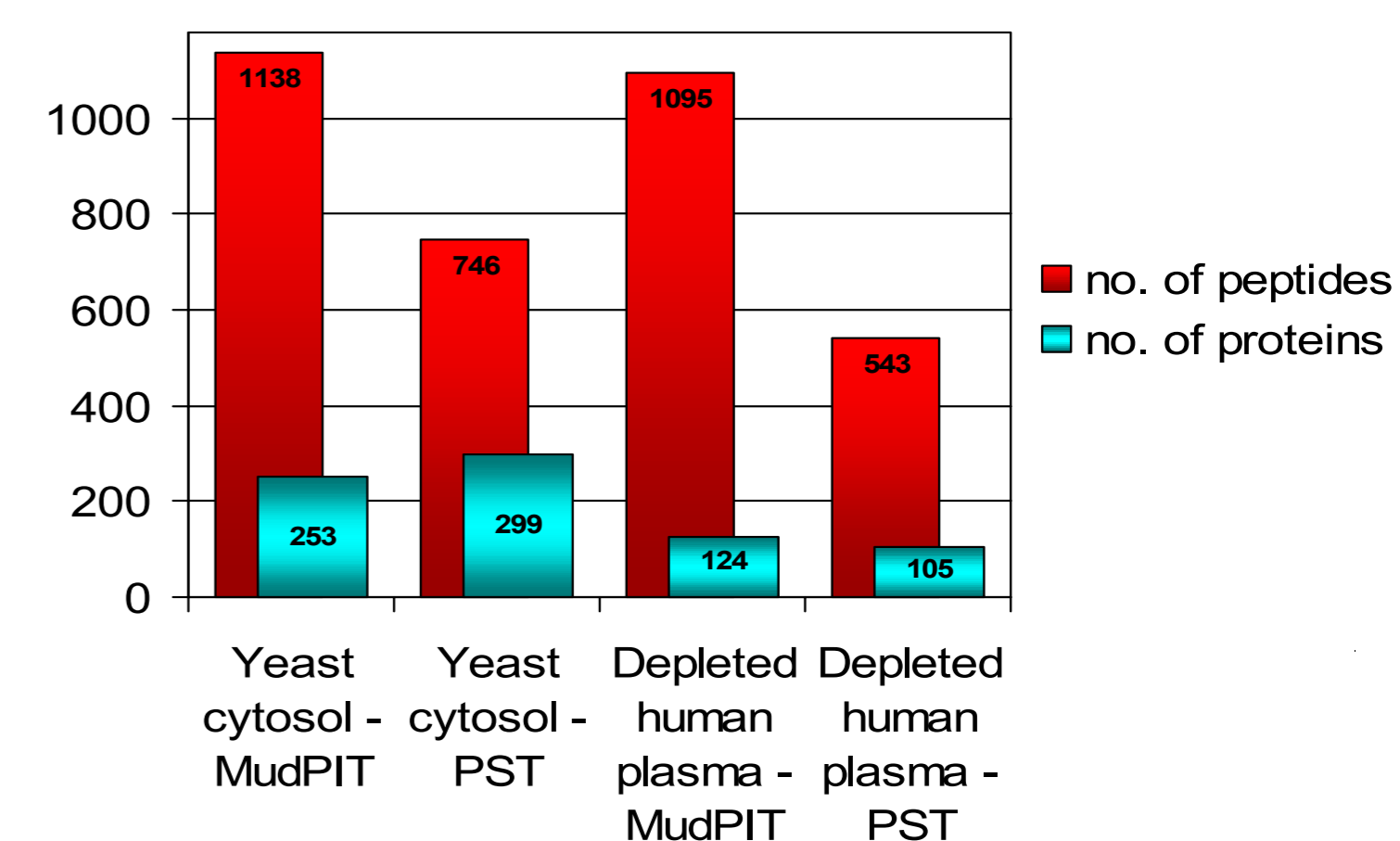
3. Soluble proteins

Very detailed investigations with this kind of samples were performed using either the cytosolic fraction of *S. cerevisiae* (obtained as a side fraction in the isolation of crude mitochondria according to reference [4]) or human plasma, depleted from the 6 most abundant proteins.

In parallel to the PST® approaches applied to these two soluble protein samples, we also performed MudPIT analyses^[5] with the same protein amounts and identical SCX and reversed phase separations. This study should allow us to assess the performance of our PST® technology in a direct comparison to an alternative gel-free approach.

With respect to development of a quantitative PST® approach, we skipped the final sorting step in these investigations to achieve a certain peptide coverage per protein. A moderate degree of redundancy increases the reliability of both the identifications and in particular the regulation data. When we performed the PST® approach including the sorting, as described in the other application examples, typically more than two-thirds of the proteins are identified by one peptide only.

Figure 5 shows the outcome of all methods performed on the two samples.



In both cases, MudPIT identified quite more peptides compared to the PST® approach (50% more for yeast cytosol and 100% more for depleted human plasma). But in spite of that, the number of represented proteins is even higher with PST® (in the case of the yeast cytosol) or slightly lower only (in the case of depleted human plasma). This shows the beneficial effect of the complexity reduction of the PST® approach which is caused by the labelling of the lysine residues and, thus, the loss of these residues as cleavage site for the trypsin.

The averaged peptide coverage per protein (2.49 for yeast cytosol and 5.17 for depleted human plasma, resp.) should still be sufficient to obtain a reasonable amount of redundant data when applying the quantitative PST® approach.

This study shows that by applying the PST® technology, we demonstrate a similar performance to MudPIT. However, when using our quantitative PST® approach (qPST™) we gain a considerable additional advantage by obtaining quantitative proteomics data through this particular *in vitro* isotopic labelling methodology.

Conclusion

- Both membrane and nuclear fractions, which are usually difficult to analyse in gel-based proteomic approaches, were investigated effectively by the presented PST® technology.
- In comparison to the well-established MudPIT approach, the PST® technology shows a comparable performance in respect to the amount of identified proteins.
- The current development of a quantitative PST® approach will strongly increase the value of this broadly applicable proteomic technology.

References

- [1] B. Doman, S. Broder, *J. Prot. Res.* **2004**, *3*, 253-260.
- [2] K. Kuhn, A. Thompson, T. Prinz, J. Müller, C. Baumann, G. Schmidt, T. Neumann, C. Hamon, *J. Prot. Res.* **2003**, *2*, 598-609.
- [3] S. S. Sisodia, P. H. St George-Hyslop, *Nature Rev. Neuroscience* **2002**, *3*, 281-290.
- [4] H. Martin, C. Eckerskorn, F. Gartner, J. Rassow, F. Lottspeich, N. Pfanner, *Anal. Biochem.* **1998**, *265*, 123-128.
- [5] M. Washburn, D. Wolters, J. Yates III., *Nature Biotech* **2001**, *19*, 242-247.