



EVALUATION OF THE QUANTITATIVE PROTEIN SEQUENCE TAGS (qPST™) FOR COMPARATIVE PROTEOMIC ANALYSES OF COMPLEX BIOLOGICAL SAMPLES

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Overview

- The quantitative Protein Sequence Tags (qPST™) technology is a proprietary, gel-free proteomic approach targeted towards the differential quantitative protein analysis of complex proteomes of cells and body fluids.
- The qPST™ technology relies on stable isotope labelling using basic mass tags to allow for the differential quantitative analysis of peptide pairs in MS mode.
- These tags, that label amino groups, are designed to maintain typical peptide properties for SCX fractionation and purification and to ensure efficient MS analyses (concerning both identification and quantification).
- The robustness of the qPST™ approach is being demonstrated by the detection and confirmation of quantitative protein regulations in a well established yeast model grown on different carbon sources.
- Currently qPST™ is being applied to profile differential protein expression in human plasma samples from Alzheimer's disease (AD) patients compared to plasma from healthy volunteers. (*)

Principle of the qPST™ technology

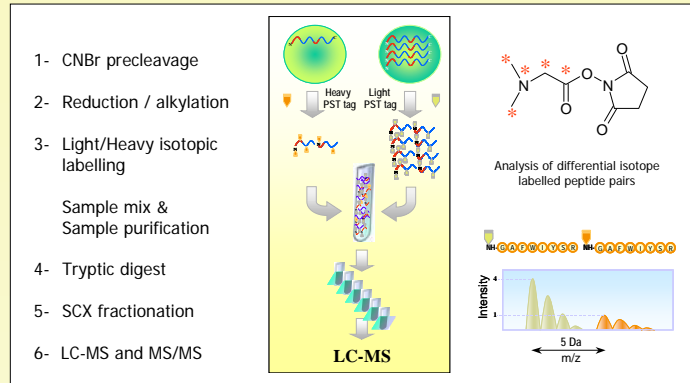


Figure 1. Schematic representation of the qPST™ technology. CNBr cleavage generates polypeptide fragments of moderate size. The labelling step uses the light and heavy isotope reagents, which block all amino group. After pooling of the samples (ratio 1/1), trypsin cleaves labelled polypeptide fragments, thereby generating ArgC peptides. The resulting peptide mixture is then fractionated using SCX column and each fraction is analyzed by LC-MS.

Introduction

Comparative proteome profiling using stable isotope peptide labelling and mass spectrometry has emerged as a promising strategy. Recently, we introduced the Protein Sequence Tag Technology (PST®) as a powerful gel-free tool for the analysis of a wide range of biological samples and fractions thereof such as nuclear and membrane extracts [1-3]. Here we present the results obtained with the quantitative Protein Sequence Tag (qPST™) technology when applied to differential quantitative analysis of complex proteomes.

The qPST™ approach is based on differential isotope labelled derivatisation tags, LC-MS and MS/MS analyses and integrated software modules for accurate differential quantitative analyses of peptide pairs.

After having assessed the qPST™ principle with simple protein mixtures [3], the performance of the qPST was evaluated with *S. cerevisiae*, grown on either galactose or ethanol as carbon source, to demonstrate the robustness of the approach in detecting quantitative changes in complex proteomes. Subsequently, qPST™ was applied to the investigations of human plasma samples of Alzheimer's disease vs. healthy patients in order to discover biologically relevant biomarkers in complex proteomes of body fluids.

Description of the qPST™ process

The qPST™ procedure consists of a series of integrated steps to prepare a complex protein mixture for subsequent MS-based analysis. All the steps performed in this approach are based on effective and robust chemical manipulations. The individual steps are arranged in such a way that the differently labelled probes can be combined as early as possible in the process as described in Figure 1.

Briefly, the initial CNBr mediated protein cleavage provides several beneficial effects, including enhancing accessibility of the functional groups for further manipulations, and increasing the number of labelling sites. Moreover, the CNBr step is highly effective to achieve solubilisation of membrane proteins, even if they have several membrane-spanning domains.

In the second step, a specific labelling of the amino groups is achieved with light or heavy tags, *N,N*-dimethylglycine *N*'-hydroxysuccinimide ester (DMG). This labelling reagent is designed to maintain intrinsic peptide properties such as their chromatographic behaviour in the SCX fractionation and their fragmentation behaviour during MS/MS. Moreover, the qPST tags introduce a minimal mass modification on peptides and the isotope pair presents a mass difference of 5 amu, which allows an unambiguous separation of the two isotopic patterns of the labelled peptides pair for relative quantitation as shown in Figure 1.

After pooling the labelled mixtures, the following tryptic digestion of the labelled polypeptides results in an ArgC-like protein digest pattern due to the loss of lysine as cleavage site upon labelling. Consequently, a lower number of peptides than normally expected with trypsin digestion is generated, thereby resulting in a relative reduction of sample complexity. The peptide mixture is then fractionated by SCX chromatography and each fraction is further analysed by LC-MS as described in Figure 2.

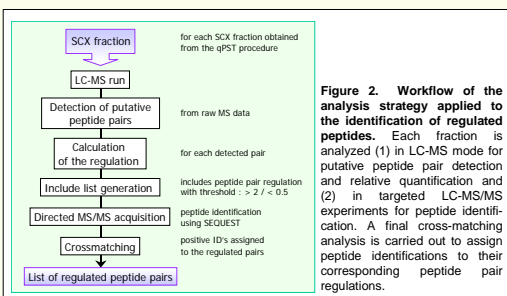


Figure 2. Workflow of the analysis strategy applied to the identification of regulated peptides. Each fraction is analyzed (1) in LC-MS mode for putative peptide pair detection and relative quantification and (2) in targeted LC-MS/MS experiments for peptide identification. A final cross-matching analysis is carried out to assign peptide identifications to their corresponding peptide pair regulations.

Results of the yeast study

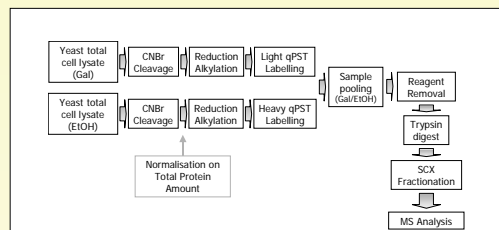


Figure 3. Workflow of the qPST™ application to a yeast glucose repression scheme. Yeast cells were grown in YP-rich medium with either 2% galactose (Gal) or 2% ethanol (EtOH) as a sole carbon source [4]. Total cell lysate of both samples were isolated and then analysed as described below. The comparative Gal/EtOH experiment was run in triplicate, starting from the sample preparation step to the MS analysis. Additionally, two control experiments using Gal/Gal and EtOH/EtOH comparisons were performed in order to assess the false positive rate.

	Gal/EtOH	Gal/Gal	EtOH/EtOH	Gal/Gal
no. of total pairs	1666	1671	1264	768
no. of regulated pairs	292	211	159	8
% of all pairs	12%	13%	13%	<1%

	Gal/EtOH	Gal/Gal	EtOH/EtOH
no. of matched peptide sequences	52	66	54
highest observed peptide regulation	6.32	6.55	6.62
lowest observed peptide regulation	0.15	0.09	0.05
no. of proteins with matched peptide coverage	35	38	38
	(1.44)	(1.64)	(1.36)

Table 1a

Table 1b

#	protein name	no. of peptides	protein regulation (Gal/EtOH)	standard deviation	CV
1	ADH1_YEAST	2	0.58	0.04	12%
2	ADH2_YEAST	2	0.58	0.04	12%
3	ADH3_YEAST	2	0.58	0.04	12%
4	ADH4_YEAST	2	0.58	0.04	12%
5	ADH5_YEAST	2	0.58	0.04	12%
6	ADH6_YEAST	2	0.58	0.04	12%
7	ADH7_YEAST	2	0.58	0.04	12%
8	ADH8_YEAST	2	0.58	0.04	12%
9	ADH9_YEAST	2	0.58	0.04	12%
10	ADH10_YEAST	2	0.58	0.04	12%
11	ADH11_YEAST	2	0.58	0.04	12%
12	ADH12_YEAST	2	0.58	0.04	12%
13	ADH13_YEAST	2	0.58	0.04	12%
14	ADH14_YEAST	2	0.58	0.04	12%
15	ADH15_YEAST	2	0.58	0.04	12%
16	ADH16_YEAST	2	0.58	0.04	12%
17	ADH17_YEAST	2	0.58	0.04	12%
18	ADH18_YEAST	2	0.58	0.04	12%
19	ADH19_YEAST	2	0.58	0.04	12%
20	ADH20_YEAST	2	0.58	0.04	12%
21	ADH21_YEAST	2	0.58	0.04	12%
22	ADH22_YEAST	2	0.58	0.04	12%
23	ADH23_YEAST	2	0.58	0.04	12%
24	ADH24_YEAST	2	0.58	0.04	12%
25	ADH25_YEAST	2	0.58	0.04	12%
26	ADH26_YEAST	2	0.58	0.04	12%
27	ADH27_YEAST	2	0.58	0.04	12%
28	ADH28_YEAST	2	0.58	0.04	12%
29	ADH29_YEAST	2	0.58	0.04	12%
30	ADH30_YEAST	2	0.58	0.04	12%
31	ADH31_YEAST	2	0.58	0.04	12%
32	ADH32_YEAST	2	0.58	0.04	12%
33	ADH33_YEAST	2	0.58	0.04	12%
34	ADH34_YEAST	2	0.58	0.04	12%
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36	ADH36_YEAST	2	0.58	0.04	12%
37	ADH37_YEAST	2	0.58	0.04	12%
38	ADH38_YEAST	2	0.58	0.04	12%
39	ADH39_YEAST	2	0.58	0.04	12%
40	ADH40_YEAST	2	0.58	0.04	12%
41	ADH41_YEAST	2	0.58	0.04	12%
42	ADH42_YEAST	2	0.58	0.04	12%
43	ADH43_YEAST	2	0.58	0.04	12%
44	ADH44_YEAST	2	0.58	0.04	12%
45	ADH45_YEAST	2	0.58	0.04	12%
46	ADH46_YEAST	2	0.58	0.04	12%
47	ADH47_YEAST	2	0.58	0.04	12%
48	ADH48_YEAST	2	0.58	0.04	12%
49	ADH49_YEAST	2	0.58	0.04	12%
50	ADH50_YEAST	2	0.58	0.04	12%
51	ADH51_YEAST	2	0.58	0.04	12%
52	ADH52_YEAST	2	0.58	0.04	12%
53	ADH53_YEAST	2	0.58	0.04	12%
54	ADH54_YEAST	2	0.58	0.04	12%
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60	ADH60_YEAST	2	0.58	0.04	12%
61	ADH61_YEAST	2	0.58	0.04	12%
62	ADH62_YEAST	2	0.58	0.04	12%
63	ADH63_YEAST	2	0.58	0.04	12%
64	ADH64_YEAST	2	0.58	0.04	12%
65	ADH65_YEAST	2	0.58	0.04	12%
66	ADH66_YEAST	2	0.58	0.04	12%
67	ADH67_YEAST	2	0.58	0.04	12%
68	ADH68_YEAST	2	0.58	0.04	12%
69	ADH69_YEAST	2	0.58	0.04	12%
70	ADH70_YEAST	2	0.58	0.04	12%
71	ADH71_YEAST	2	0.58	0.04	12%
72	ADH72_YEAST	2	0.58	0.04	12%
73	ADH73_YEAST	2	0.58	0.04	12%
74	ADH74_YEAST	2	0.58	0.04	12%
75	ADH75_YEAST	2	0.58	0.04	12%
76	ADH76_YEAST	2	0.58	0.04	12%
77	ADH77_YEAST	2	0.58	0.04	12%
78	ADH78_YEAST	2	0.58	0.04	12%
79	ADH79_YEAST	2	0.58	0.04	12%
80	ADH80_YEAST	2	0.58	0.04	12%
81	ADH81_YEAST	2	0.58	0.04	12%
82	ADH82_YEAST	2	0.58	0.04	12%
83	ADH83_YEAST	2	0.58	0.04	12%
84	ADH84_YEAST	2	0.58	0.04	12%
85	ADH85_YEAST	2	0.58	0.04	12%
86	ADH86_YEAST	2	0.58	0.04	12%
87	ADH87_YEAST	2	0.58	0.04	12%
88	ADH88_YEAST	2	0.58	0.04	12%
89	ADH89_YEAST	2	0.58	0.04	12%
90	ADH90_YEAST	2	0.58	0.04	12%

Table 1c

Table 1c: List of regulated proteins identified within the yeast study. Table 1a: The raw MS data of each SCX fraction were first analysed for putative peptide pairs based on accurate retention time and m/z . Only pairs showing a regulation >2.0 or <0.5 with intensity >25 cts were taken into account for directed MS/MS experiments in order to identify their peptide sequences. Results of the control study (Gal/Gal and EtOH/EtOH) show a minimal false positive rate in the qPST approach as only 1% or less of the detected pairs was detected as regulated peptide pairs. Table 1b: In total, the three experiments identified 90 different peptide sequences for 56 proteins, making an average protein coverage of 1.61 peptides per protein. Reproducibility of peptide regulations in the 3 repeats showed a CV of around 6%. Table 1c: List of regulated proteins identified within the yeast study.

Protein	no. of peptides	qPST protein regulation (Gal/EtOH)	standard deviation	CV	ICAT	literature data database knowledge
ADH1_YEAST	2	2.78	0.04	12%	<0.01	induced by ethanol
ADH2_YEAST	2	2.78	0.04	12%	<0.01	induced by ethanol
ADH3_YEAST	2	2.78	0.04	12%	<0.01	induced by ethanol
ADH4_YEAST	2	2.78	0.04	12%	<0.01	induced by ethanol
ADH5_YEAST	2	2.78	0.04	12%	<0.01	induced by ethanol
ADH6_YEAST	2	2.78	0.04	12%	<0.01	induced by ethanol
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ADH8_YEAST	2	2.78	0.04	12%	<0.01	induced by ethanol
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