ICPL 4-plex: Isotopic Protein Labeling for Quantitative Protein Analysis by nano-LC-MALDI-TOF/TOF

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Introduction

Stable isotope protein labeling with ICPL (isotope-coded protein labeling) has proven to be a highly accurate method for protein quantification. With the new ICPL 4-plex technology (Fig. 1) it is possible to quantify four different proteome samples per experiment.

The efficiency of the MALDI-MS quantification approach of proteins labeled with the new ICPL 4-plex is demonstrated for artificial protein samples with controlled concentrations.

Quantification of the ICPL-labeled proteins was performed with the ProteinScape software.

Methods

Two protein samples consisting of 7 protein standards were labeled with the new SERVA ICPL™ 4-plex kit (Bruker) (***Fig. 1***).

After ICPL labeling, the protein samples were combined and digested by trypsin. The resulting peptides were separated by nano-reversed phase HPLC (EASY-nLC) and directly collected onto MTP AnchorChip™ 800/384 MALDI-targets (all Bruker) (***Fig. 1***).

Relative quantification of identified ICPL labeled peptides was performed by LC-MALDI analysis on the ultraflex-II MALDI-TOF/TOF using ProteinScape *2.0* (Bruker) (***Fig. 2***).

Workflow

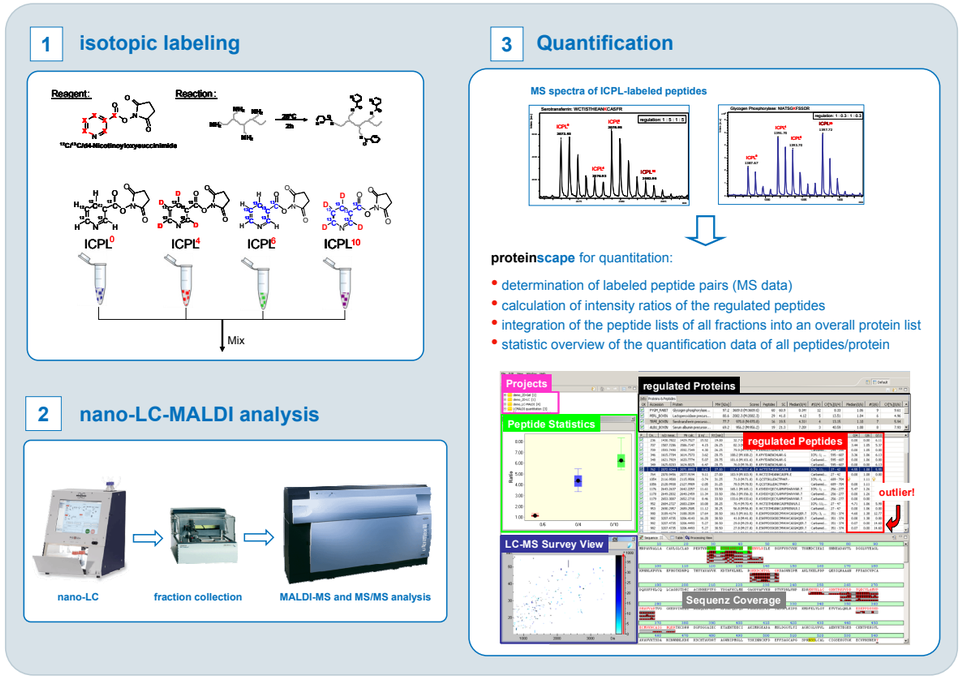

Fig. 1 ICPL 4-plex Quantification nano-LC-MALDI workflow using **proteinscape** for identification and quantification of proteins

Results

- The standard protein samples labeled with ICPL 4-plex were mixed at ***ratios from 0.25 to 30***.
- All proteins were identified after MS/MS analysis; quantification was typically in the ***CV<10 %*** range (***Fig. 2***).
- A non-redundant list of the identified proteins containing the quantification results is assembled in ProteinScape ***even across multiple LC-runs!***
- A regulation value of 1:30 (BSA) results in the detection of singlet peptide signals which is indicated with ↑↓ in the peptide list (***Figs. 1, 2***).
- An increased protein quantification error (CV > 10%) is obtained if <5 regulated peptide 4-plets are detected.

Protein	theor. Ratios			
	$ICPL^0/ICPL^6$	$ICPL^0/ICPL^4$	$ICPL^0/ICPL^{10}$	$ICPL^6/ICPL^{10}$
Phosphorylase B	1	0.33	1	0.33
Lactoperoxidase	1	4	1	4
GAPDH	1	0.25	1	0.25
Serotransferrin	1	5	1	5
Peroxidase	1	1	1	1
BSA	1	30	1	30
Ribonuklease	1	0.25	1	0.25

Protein	exp. Ratios (Median)							
	$ICPL^0/ICPL^6$	CV(%)	$ICPL^0/ICPL^4$	CV(%)	$ICPL^0/ICPL^{10}$	CV(%)	$ICPL^6/ICPL^{10}$	CV(%)
Phosphorylase B	1.10	7.93	0.34	7.34	1.07	7.83	0.34	9.49
Lactoperoxidase	1.04	4.96	4.12	13.51	1.03	8.22	4.10	6.93
GAPDH	1.07	1.90	0.27	2.81	0.98	3.74	0.27	16.53
Serotransferrin	1.07	1.41	4.31	13.15	1.10	10.63	5.55	7.97
Peroxidase	1.04	1.03	1.02	6.50	1.08	2.33	1.01	1.97
BSA	1.03	4.16	↑	-	1.02	2.59	↑	-
Ribonuklease	1.15	5.51	0.27	11.95	1.07	2.58	0.35	18.86

Fig. 2 Quantification results obtained in ProteinScape for the nano-LC-MALDI analysis of a protein sample labeled with the ICPL 4-plex.

***Data evaluation in* proteinscape:**

- Reliable determination of outliers by ProteinScape based on quartile analysis, which are not considered for protein quantification (***Fig. 1***).
- The Peptide Statistics View in ProteinScape provides a concise overview of the quantification data of all regulated peptides for a given protein (***Fig. 1***).
- The LC-MS Survey Viewer and the Sequence Viewer allow for the evaluation of the experimental nano-LC-MALDI data.

Conclusions

- The ***ICPL 4-plex*** technology provides for quantification of protein samples analyzed by nano-LC-MALDI at the ***10 % CV*** level with ***dynamic range > 5***
- **proteinscape** provides for
  - Outlyer detection
  - Quantification across multiple LC-runs
  - Decoy validation of a single nonredundant, quantitative protein list
  - Interactive, visual validation tools
  - HUPO/PSI reporting

MALDI-TOF