#### **Bruker Daltonics**

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

S. Hahner<sup>1</sup>, S. Steckbeck<sup>2</sup>, W. Evers<sup>1</sup>, A. Resemann<sup>1</sup>, Ben Owens<sup>3</sup> and D. Suckau<sup>1</sup> <sup>1</sup>Bruker Daltonics, Bremen, Germany <sup>2</sup>Univ. of Applied Sciences, Bremerhaven, Germany <sup>3</sup>Bruker Daltonics, Billerica, MA, USA

#### Introduction

Stable isotope protein labeling with ICPL (isotopecoded 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 4plex is demonstrated for artificial protein samples with controlled concentrations.

Ouantification 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<sup>™</sup> 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<sup>™</sup> 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





determination of labeled peptide pairs (MS data)

• calculation of intensity ratios of the regulated peptides

• integration of the peptide lists of all fractions into an overall protein list

• statistic overview of the quantification data of all peptides/protein

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### 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  $\uparrow \psi$  in the peptide list (*Figs.* 1, 2).
- An increased protein guantification error (CV > 10%) is obtained if <5 regulated peptide 4-plets are detected.

Protein	theor. Ratios					
	ICPL <sup>0</sup> / ICPL <sup>6</sup>	ICPL <sup>®</sup> / ICPL <sup>4</sup>	ICPL <sup>4</sup> / ICPL <sup>10</sup>	ICPL <sup>6</sup> / ICPL <sup>10</sup>		
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 <sup>0</sup> /	CV(%)	ICPL <sup>9</sup> /	CV(%)	ICPL <sup>4</sup> /	CV(%)	10			
	ICPL°		ICPL <sup>4</sup>		ICPL <sup>10</sup>		- 10			
Phosphorylase B	1.10	7.93	0.34	7.34	1.07	7.83	-			
Lactoperoxidase	1.04	4.96	4.12		1.03	8.22				
GAPDH	1.07	1.90	0.27	2.81	0.98	3.74				
Serotransferrin	1.07	1.41	4.31	13.15	1.10	10.63				
Peroxidase	1.04	1.03	1.02	5.50	1.08	2.33				
BSA	1.03	4.16	<b>^</b>		1.02	2.59				
Ribonuklease	1.15	5.51	0.27	11.95	1.07	2.58				

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

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



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#### Data evaluation in proteinscape:

- Reliable determination of outliers by Protein-Scape 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
  - Ouantification across multiple LC-runs
  - Decov validation of a single nonredundant, quantitative protein list
  - Interactive, visual validation tools
  - HUPO/PSI reporting

MALDI-TOF