



# ICPL™-Quadruplex – Isotopic Labeling of Intact Proteins

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## Introduction

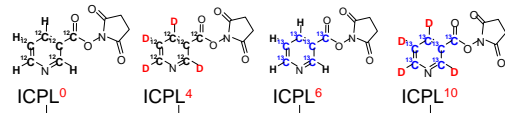
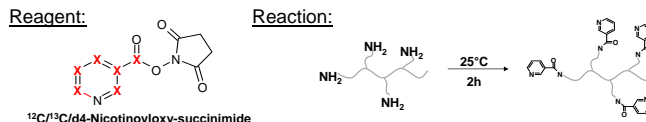
In-depth analysis of proteomes is challenged by the extreme dynamic range of protein abundance and vast complexity. To enable the quantitative analysis of even low abundant proteins, it is indispensable to reduce complexity on the level of proteins by several fractionation steps. However fractionation is time consuming and leads to loss and dilution of proteins. To compensate for this time consuming steps and to avoid non reproducible loss of protein species, the well established ICPL technology is introduced in its Quadruplex version (Serva GmbH).

The ICPL technology has been introduced in 2005 by Schmidt *et al.* It has been proved to be a highly sensitive method to selectively label primary amino groups in proteins, and to relatively quantify these proteins based on ICPL labels with different masses. Yet, ICPL Duplex and Triplex have been introduced allowing to compare two or three different samples simultaneously. Here we demonstrate, with a set of spiked proteins and using the new software tool ICPLQuant, how to accurately quantify four samples in one experiment.

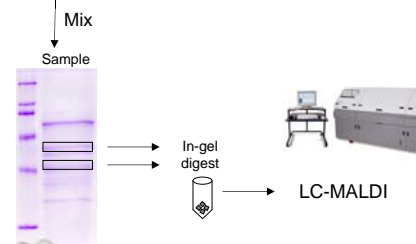
The efficiency (e.g. dynamic range, sensitivity, speed) of the method is demonstrated by the comparative analysis of four different mixtures of 3 proteins (Ovalbumin, Horseradish Peroxidase and Glycerokinase) spiked into a protein background of 8 standard proteins (Serva GmbH, DGPF Proteome Marker) separated by SDS-PAGE. Subsequently 2 gel slices were digested in a double digest with trypsin and endoproteinase GluC. Peptides were eluted, separated by nano-reversed phase HPLC and directly spotted onto MALDI targets for mass spectrometric analysis. (ABI 4700 Proteomics Analyzer). To identify regulated proteins, MALDI-MSMS of selected labeled peptides was performed. The quantification of the quadruplets was done using a new software, ICPLQuant (see Poster Brunner *et al.*).

## Methods

### Labeling of the proteins by the ICPL method and (LC)-MALDI-MS



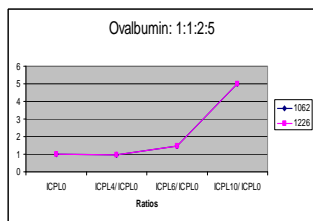
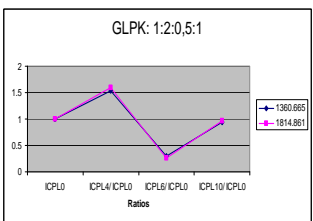
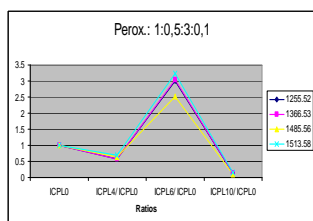
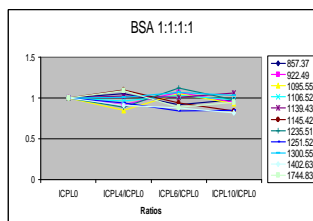
Protein	Ratios			
	ICPL <sup>0</sup>	ICPL <sup>4</sup> /ICPL <sup>0</sup>	ICPL <sup>6</sup> /ICPL <sup>0</sup>	ICPL <sup>10</sup> /ICPL <sup>0</sup>
Serva DGPF Proteome Marker (including BSA)	1	1	1	1
Peroxidase	1	0.75	3	0.1
Glycerokinase	1	2	0.5	1
Ovalbumin	1	1	2	5



## Results

### Quantification of ICPL quadruplexed proteins by ICPLQuant

The interpretation of the mass spectrometric data was done using ICPLQuant. The software package includes pattern recognition of typical ICPL peptides, their relative quantification and selection of at least one peptide for MS/MS analysis and identification. The program also compensates for the isotopic shift of the medium ICPL-derivative during reversed phase HPLC. Identification of regulated proteins, even during a large number of experiments, is thereby always done on the level of MS data by recognition of the typical ICPL Quadruplex pattern. Only those peptides have to be identified, which show a significant regulation pattern in a defined number of experiments.



Protein	Observed Mass (Light)	Ratios			
		ICPL <sup>0</sup>	ICPL <sup>4</sup> /ICPL <sup>0</sup>	ICPL <sup>6</sup> /ICPL <sup>0</sup>	ICPL <sup>10</sup> /ICPL <sup>0</sup>
BSA	857.37	1	1.05	0.92	0.98
BSA	922.49	1	0.92	1.07	0.95
BSA	1095.55	1	0.85	1.05	0.93
BSA	1106.52	1	0.96	1.03	0.98
BSA	1139.43	1	1.02	1.01	1.06
BSA	1145.42	1	1.1	0.94	0.84
BSA	1235.51	1	0.89	1.12	0.98
BSA	1251.52	1	0.94	0.84	0.85
BSA	1300.55	1	1.01	1.07	1.03
BSA	1402.63	1	0.91	0.88	0.82
BSA	1744.83	1	1.09	0.89	0.95
Median		1	0.96	1.01	0.95
SD		0	0.08	0.09	0.07
CV (%)		0	8.66	9.22	8.15

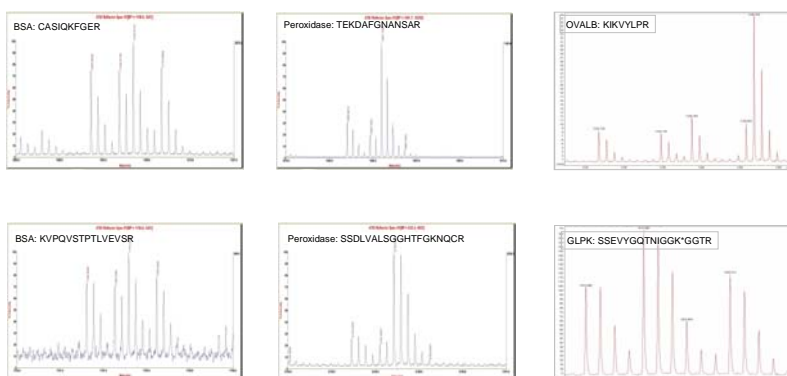
Protein	Observed Mass (Light)	Ratios			
		ICPL <sup>0</sup>	ICPL <sup>4</sup> /ICPL <sup>0</sup>	ICPL <sup>6</sup> /ICPL <sup>0</sup>	ICPL <sup>10</sup> /ICPL <sup>0</sup>
Perox	1255.52	1	0.58	3	0.13
Perox	1366.53	1	0.58	3.04	0.13
Perox	1485.56	1	0.63	2.52	0.1
Perox	1513.58	1	0.7	3.23	0.16
Median		1	0.62	2.95	0.13
SD		0	0.06	0.30	0.02
CV (%)		0	9.12	10.25	18.84

Protein	Observed Mass (Light)	Ratios			
		ICPL <sup>0</sup>	ICPL <sup>4</sup> /ICPL <sup>0</sup>	ICPL <sup>6</sup> /ICPL <sup>0</sup>	ICPL <sup>10</sup> /ICPL <sup>0</sup>
Ovalb.	1062.59	1	0.92	1.30	4.59
Ovalb.	1226.68	1	0.95	1.47	5.01
Median		1	0.94	1.38	4.80
SD		0	0.02	0.12	0.30
CV (%)		0	1.85	8.69	6.21

Protein	Observed Mass (Light)	Ratios			
		ICPL <sup>0</sup>	ICPL <sup>4</sup> /ICPL <sup>0</sup>	ICPL <sup>6</sup> /ICPL <sup>0</sup>	ICPL <sup>10</sup> /ICPL <sup>0</sup>
GLPK	1360.665	1	1.54	0.30	0.94
GLPK	1814.861	1	1.60	0.27	0.96
Median		1	1.57	0.28	0.95
SD		0	0.04	0.02	0.01
CV (%)		0	2.78	8.13	1.52

## MALDI-MS spectra of ICPL Quadruplets

Examples for ICPL Quadruplets of spiked proteins as well as from one background protein (albumin):



## Summary + Conclusion

- 3 standard proteins were spiked into a protein background with high dynamic range. Their different concentrations were compared using ICPL Quadruplex. Following SDS-PAGE, double digest and LC-MALDI, ratios were calculated.
- Protein identification was done by MALDI-MSMS. With ICPL MSMS identification is needed only of regulated peptides, which is a fast and low cost approach.
- Detection and quantification of Quadruplets were done using the ICPLQuant software. ICPLQuant provides an excellent tool for the automated detection and quantification of multiplexed ICPL peptides.
- The ICPL Quadruplex technology proved to be highly accurate and useful for the identification and quantification of spiked proteins within a complex protein background using appropriate pre-fractionation steps. Average CV < 9%.