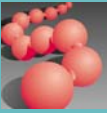


# ICPL (Isotope-Coded Protein Labeling) QUADRUPLEX Technology for quantitative Proteomics



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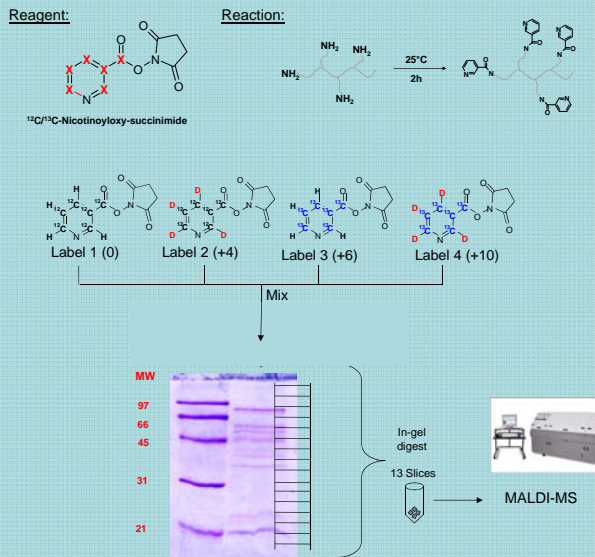
## 1. Introduction

The ICPL technology has been introduced in 2005 by Schmidt et al. It has been shown 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 report about a new, fourth ICPL label leading to the QUADRUPLEX technology. It is now possible to quantify four samples in one experiment.

Protein labeling has the advantage that different species of a protein (protein isoforms, degradation products and posttranslational modified species) can be recognized and separated on the protein level by applying any protein separation method like SDS-PAGE, 2D gel electrophoresis, free flow electrophoresis, RP or ion exchange chromatography, etc.

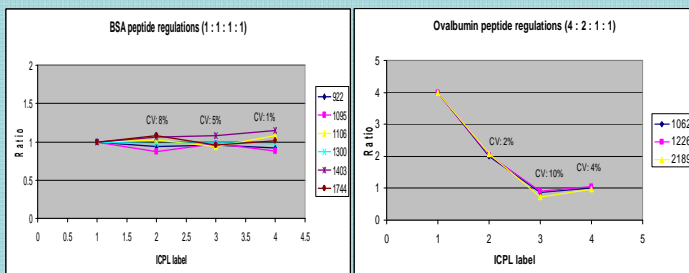
To demonstrate the quantitative capabilities of the ICPL QUADRUPLEX technology, four different mixtures of 3 standard proteins (Ovalbumin, Horseradish peroxidase and Glycerokinase (GLPK)) were spiked into a complex protein background. After multiplexing, the proteins were separated by SDS-PAGE, digested by trypsin and analysed by MALDI-MS (ABI 4700 Proteomics Analyser). To identify regulated proteins, MALDI-MS/MS of selected labeled peptides was performed. The quantification of the quadruplets was done using a new software, ICPLQuant (see Poster P-Tue 143).

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



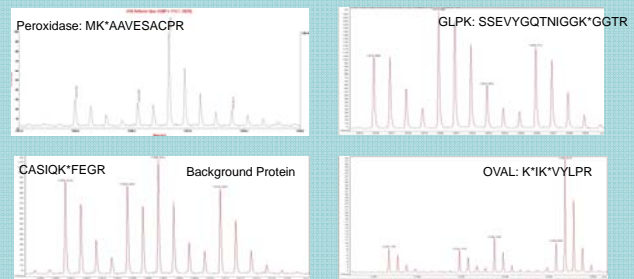
## 3. ICPL QUADRUPLEX labeling of two standard proteins

BSA in a ratio 1 : 1 : 1 : 1 and ovalbumine in a ratio 4 : 2 : 1 : 1 was mixed, ICPL labeled, and separated by SDS-PAGE. After cleavage by trypsin, peptides were further separated and analysed by MALDI-LC/MS.

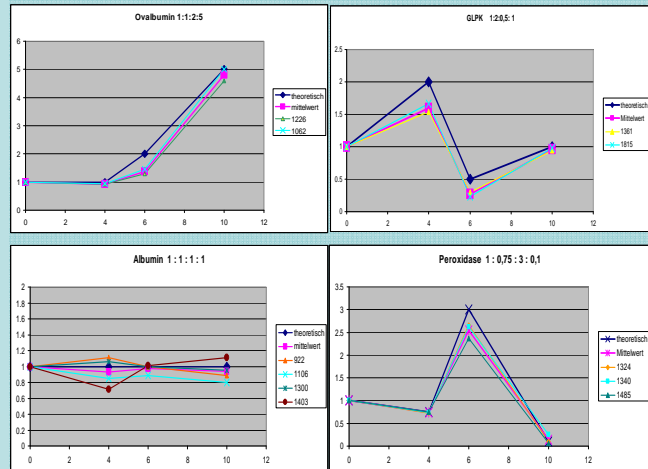


## 4. MALDI-MS spectra of ICPL QUADRUPLETS

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



## 5. Quantification of ICPL quadruplexed proteins by ICPLQuant



## 6. Summary

- 2 standard proteins containing different ratios were compared after SDS-PAGE, slicing, digest and MALDI-MS. The CV is smaller 10%!
- 3 reference proteins (OVA, HRP, GLPK) were spiked in different ratios into a complex protein background. 4-plexed samples containing different ratios of these spiked proteins were compared after SDS-PAGE, slicing, tryptic digest, and MALDI-MS.
- Detection and quantification of the QUADRUPLETS were done using the ICPLQuant software using MALDI-MS spectra deisotoped by the Mascot Distiller software.
- All three proteins could be quantified by several peptides
- Protein identification was done by MALDI-MS/MS of selected peptides

## 7. Conclusions

- The ICPL QUADRUPLEX technology proved to be highly sensitive and useful for the identification and quantification of spiked proteins within a complex protein background using an appropriate prefractionation step
- Overlapping signals in MS due to complexity presents the major difficulty in accurate quantification. Prefractionation steps preferentially on the protein level are necessary to reduce complexity.
- ICPLQuant provides an excellent tool for the automated detection and quantification of multiplexed ICPL peptides.

## 8. References

- Schmidt, A. et al., Proteomics 2005, 5(1), 4-15
- Brunner, A. et al. Poster HUPO 2008 P-Tue 143