

# Detailed Annotation of Qualitative Differences in Recombinant Protein Samples – A QC Exercise

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## Introduction

We analyzed the sample set from „Qualitative Proteomic Study“ of **ABRF-PRG08** using top-down LC-MALDI-TOF-MS. Reflector-In-Source-Decay (reISD) allows obtaining sequence tags from undigested, isolated proteins, which can be used for

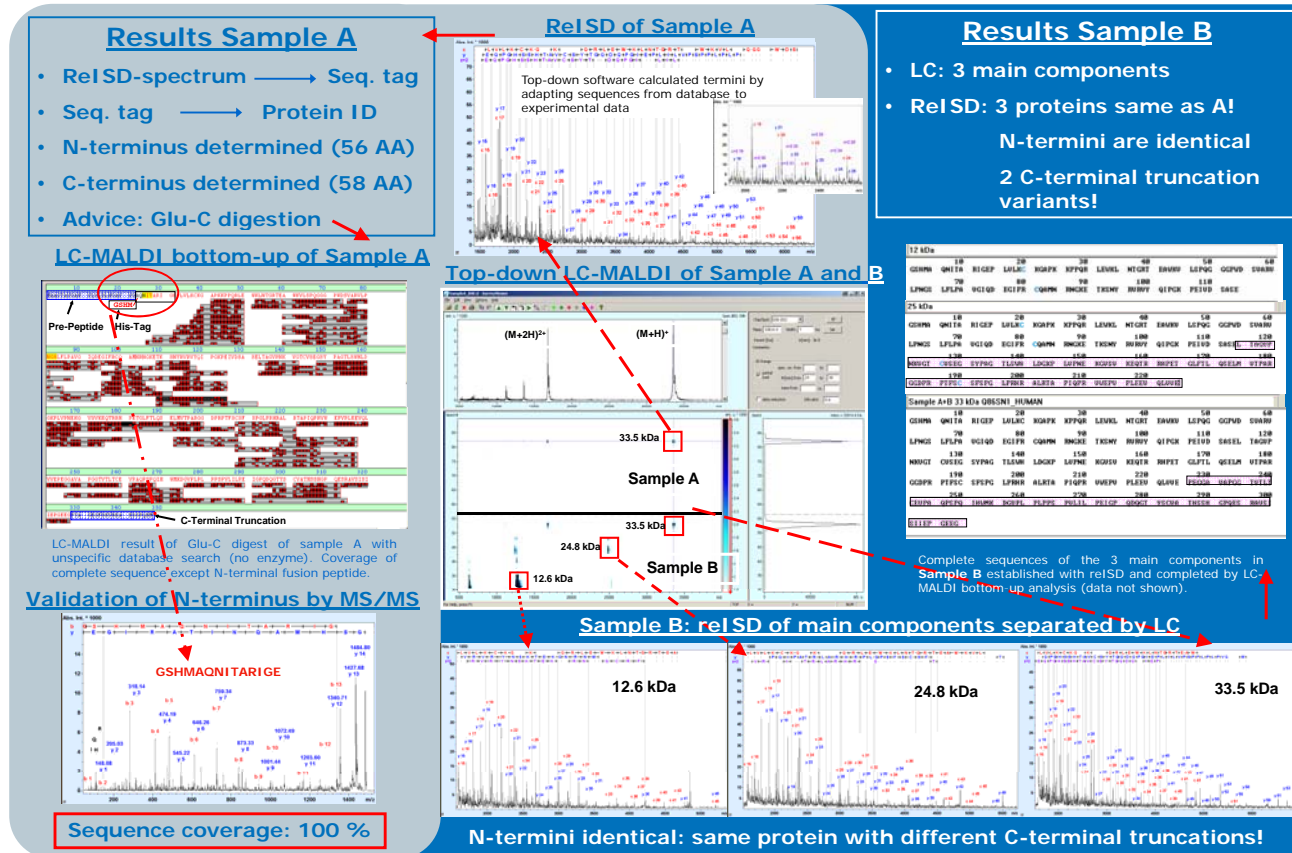
- protein identification
- sequence verification
- N- and C-terminus assignment

reISD is an easy and fast tool for quality control of recombinant proteins.

## Methods

Samples A and B of PRG2008 represent 2 batches of a human protein expressed in *E. coli*. Reduced and alkylated samples were LC separated, fractions were collected on Anchor-Chip targets (Bruker) using sinapinic acid matrix. Linear mode spectra were automatically acquired, reISD spectra manually on an Ultraflex III MALDI-TOF/TOF-MS (Bruker). GluC digest LC-MALDI analysis of sample A provided increased sequence coverage of previously IDed protein.

Top-down sequence data were analyzed by BioTools 3.1. Database searches: Mascot 2.2 (Matrix Science) and MS-BLAST (EMBL/Harvard).



## Summary

- Two samples were compared that contained the same 33 kDa protein. In addition, sample B contained 2 truncated forms of that protein.
- LC-MALDI-reISD top-down sequencing strategy clearly detected the different forms and allowed assigning C-terminal truncation variants.
- Top-down sequence data suggested Glu-C as proper protease.
- Glu-C LC-MALDI-MS/MS provided 100% sequence coverage due to additional peptide matches from the central part of the protein.

## Conclusions

Top-down LC-reISD-MALDI is a new powerful approach for protein QC simultaneously providing:

- Contaminant detection
- Protein ID
- N/C-termini assignment
- Truncation variants
- Modifications/location

Protein digests alone don't provide this as terminal and modification infos are unrelated to each other.

MALDI-TOF/TOF