Absolute quantification of low abundance proteins by shotgun proteomics

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Proteome Factory

www.proteomefactory.com

In cooperation with:

Max-Planck-Institut
für Molekulare Pflanzenphysiologie

Stable isotope labelled peptides:

ThermoFisher SCIENTIFIC

Biopolymers, Ulm, Germany

Introduction

Multiple Reaction Monitoring (MRM)

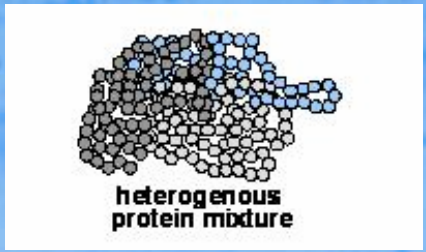
- General procedure
- Complex samples and in solution digestion
- Complex samples and in gel digestion

=> MASS WESTERN

General procedure:

1. Signature Peptide
2. Synthesis (Standard peptides)
3. Tuning (SRM or MRM)
4. Analyses

A practical approach



multidimensional fractionation

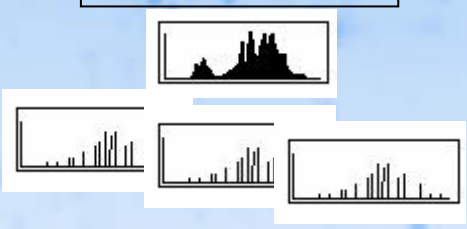
FPLC

digestion

2D HPLC

SCX/RP

analysis



MS

MS / MS

data base search

peptide identification → signature peptide list

labelling

stable isotope labelled standard peptides

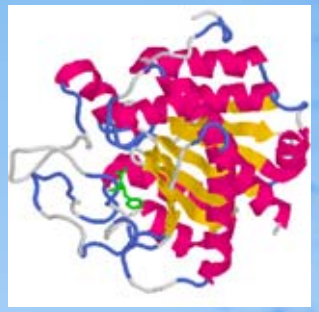
B theoretical approach

Protein sequence information from database

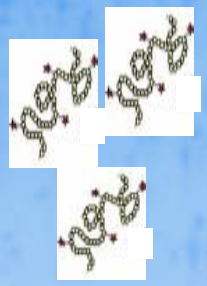
theoretical digestion

Wienkoop et al. 2004, RCM 18, 643-650

Absolute quantification procedure



+



sample + labelled standard peptides

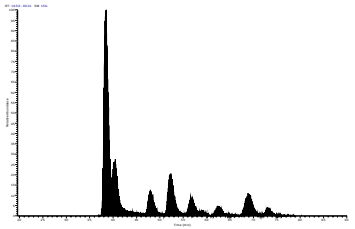
↓

digest:

- **in gel**
- **in solution**

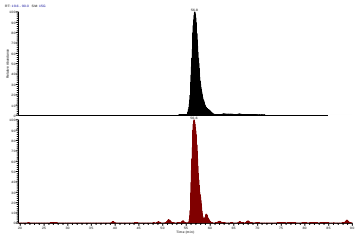
→

MS



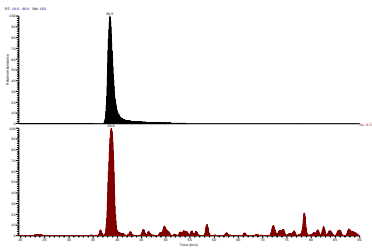
TIC

MRM



standard peptide x

native peptide x



standard peptide y

native peptide y

in solution vs in gel digestion

Advantage:

proteins of different sizes (physiological pathway)

=> faster

Disadvantage:

less sensitive

Complex sample and in solution digestion

-Pathway Analysis-

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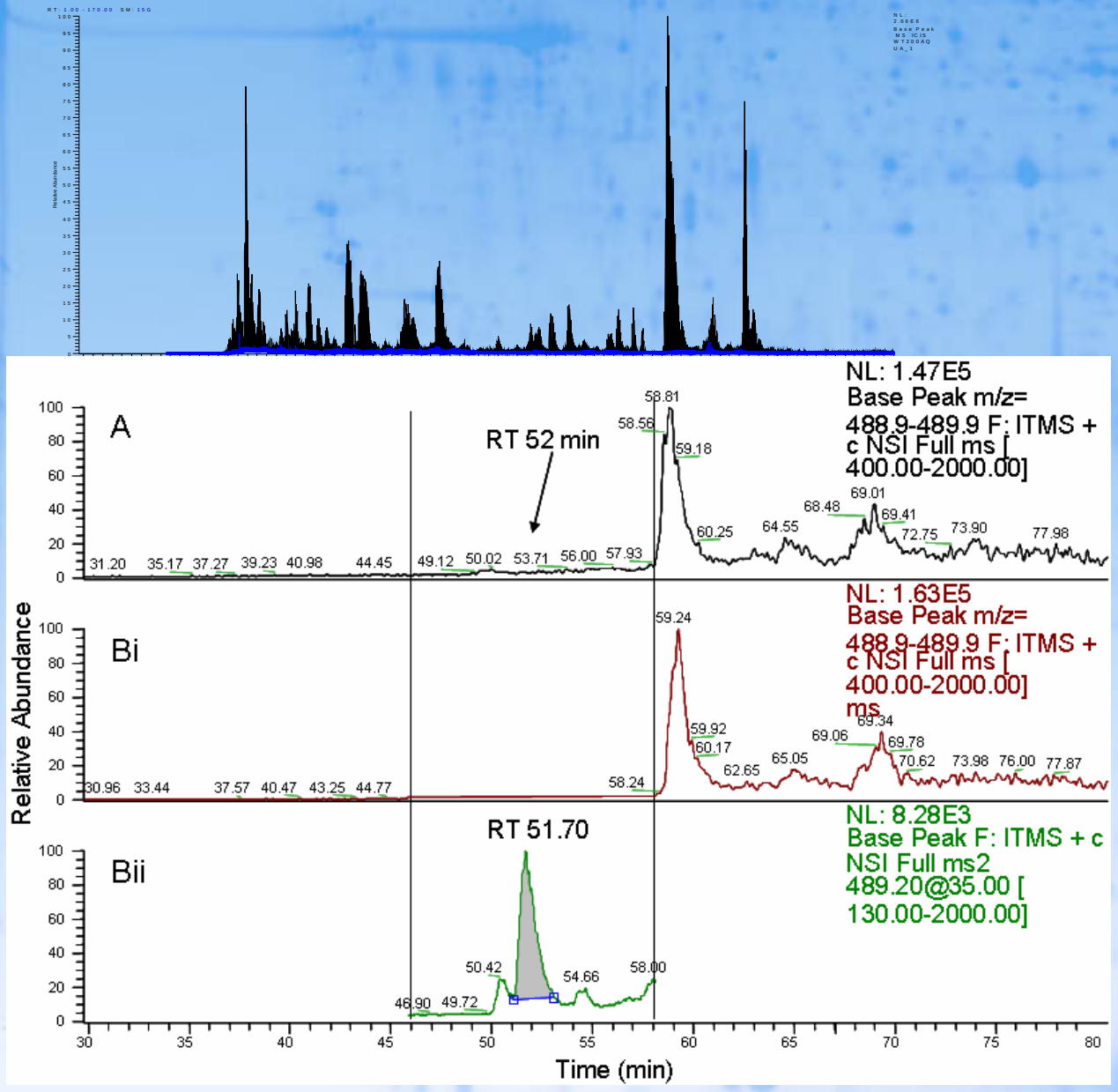
LTQ: sequential precursor isolation and fragmentation



dependent MSMS standard analysis

Method according to:

Venable JD *et al.*

(2004) Nature Methods

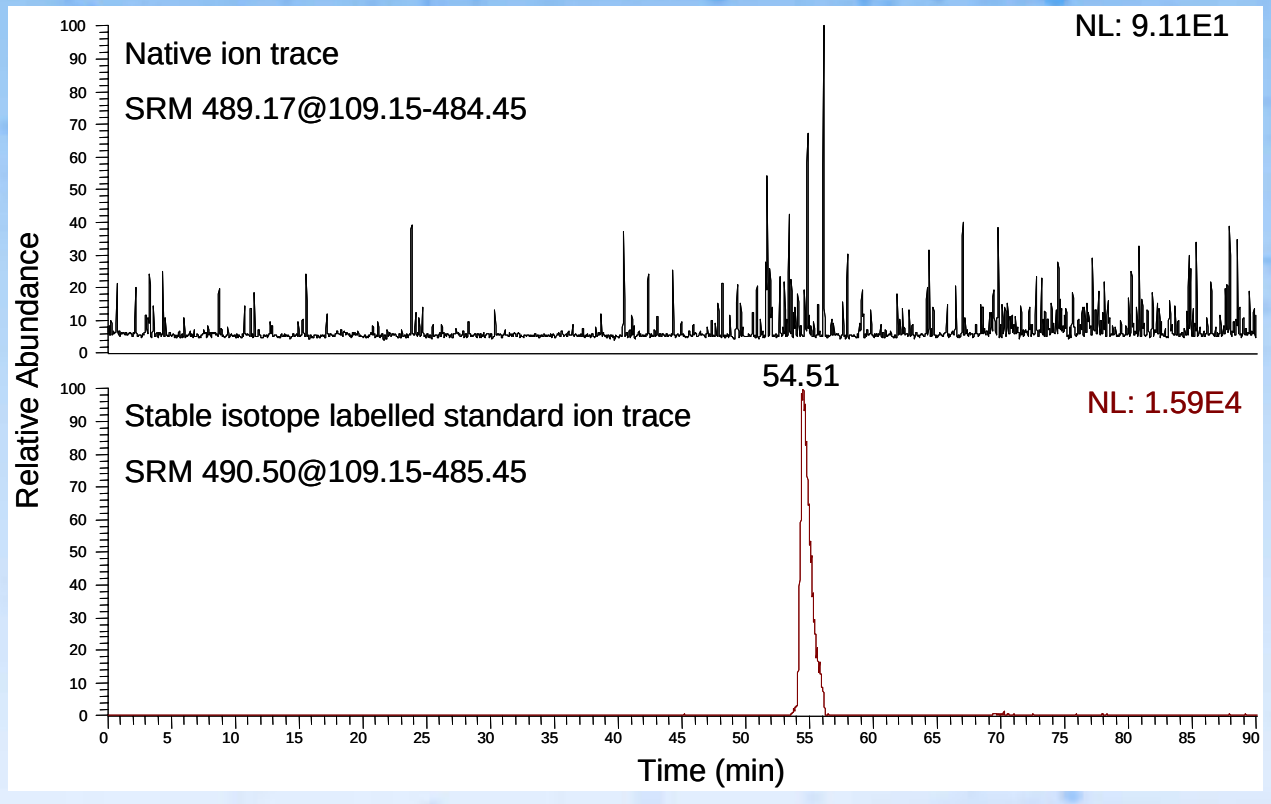
Wienkoop and Weckwerth 2006, JExB

TSQ vs. LTQ

TSQ higher sensitivity for targeted analyses using MRM.

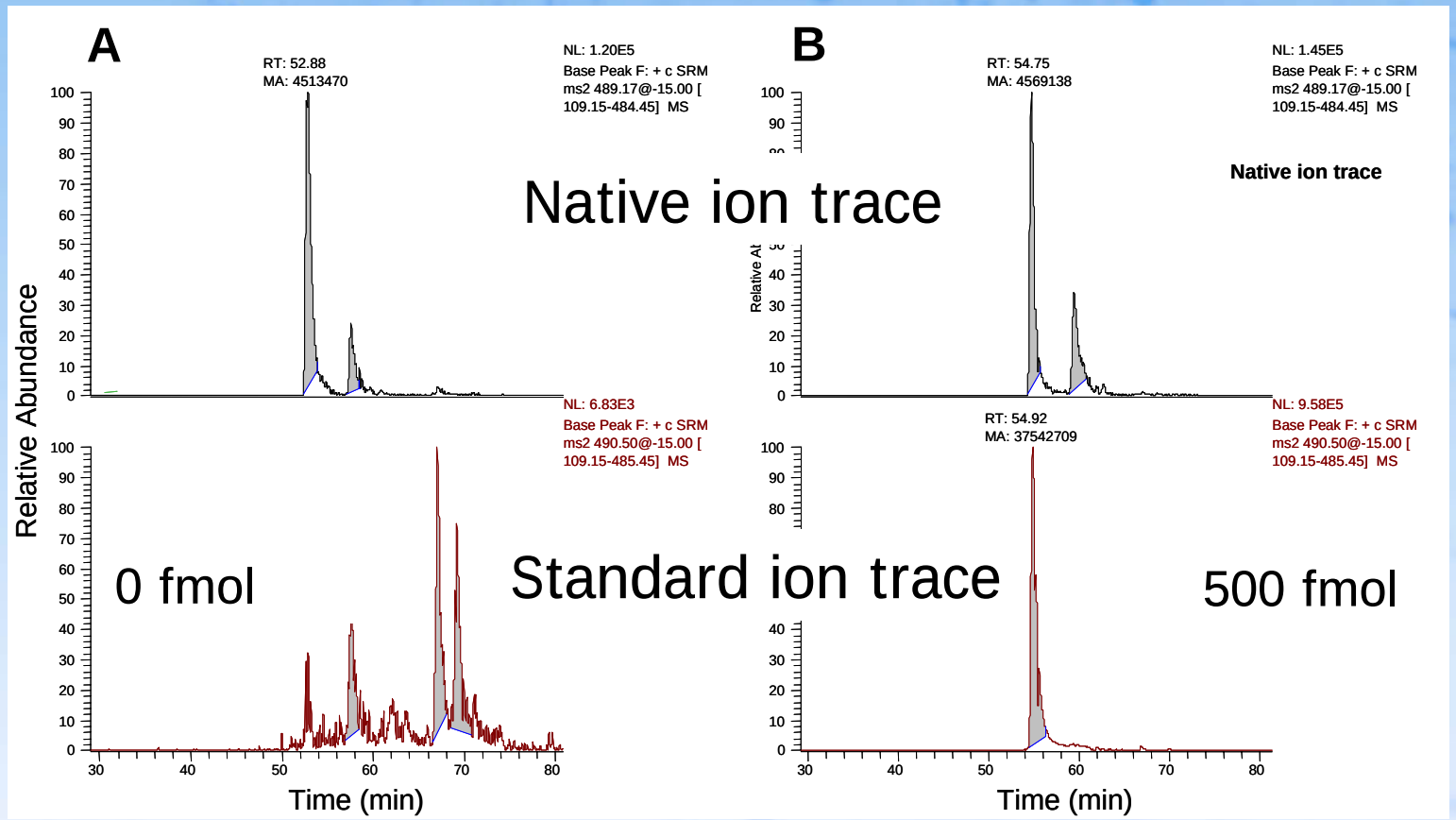
Absolute quantification.

Purity Control

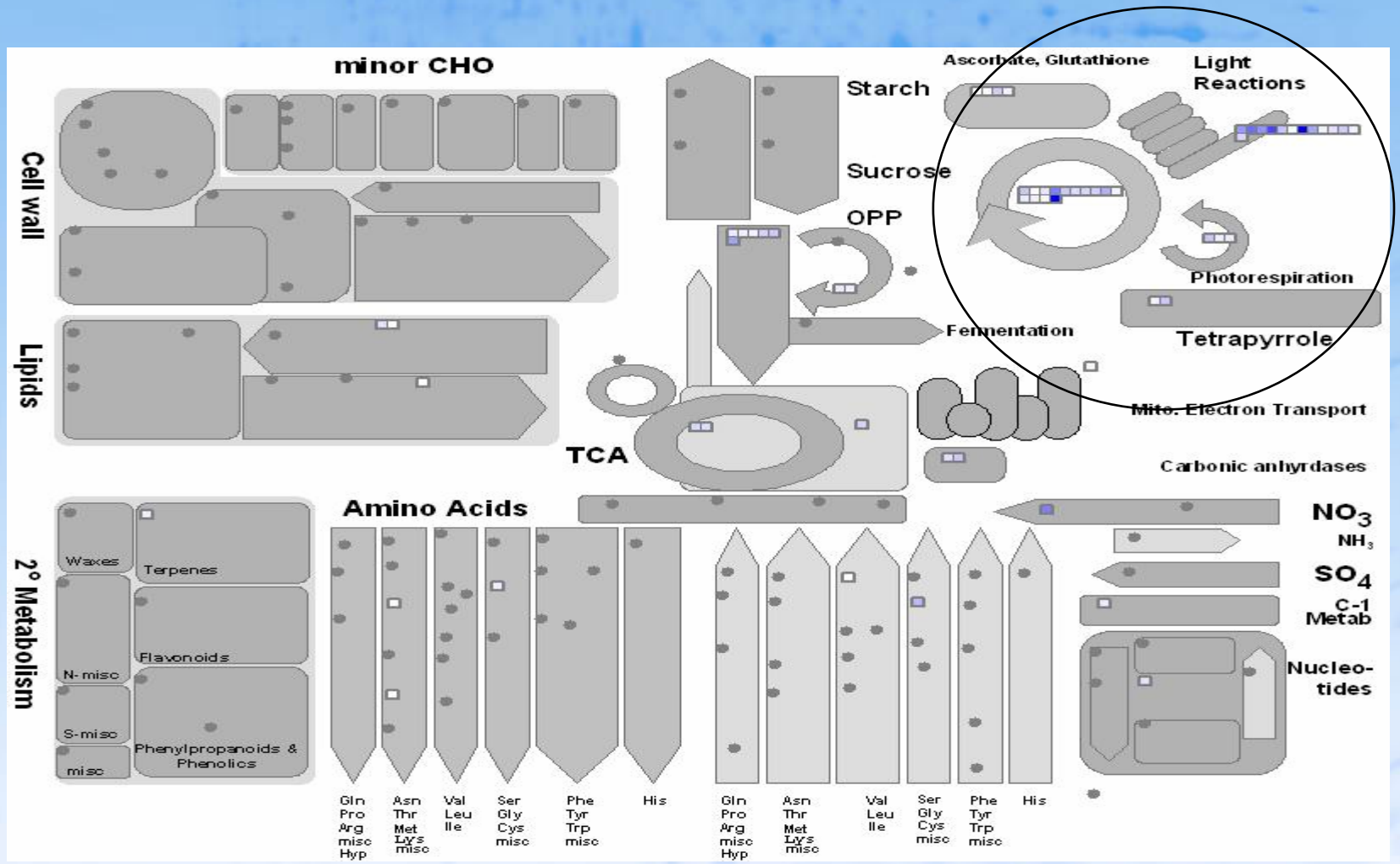


Sample without standard

Sample with standard



Automated protein pathway assignment: MAPMAN



Usadel *et al.* 2005, PlantPhys

Complex sample and in gel digestion

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Mass Western vs. Western Blot

Isoforms (specificity) ?

Quantification ?

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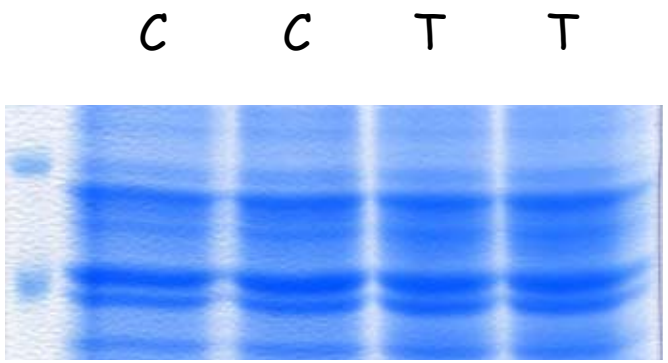
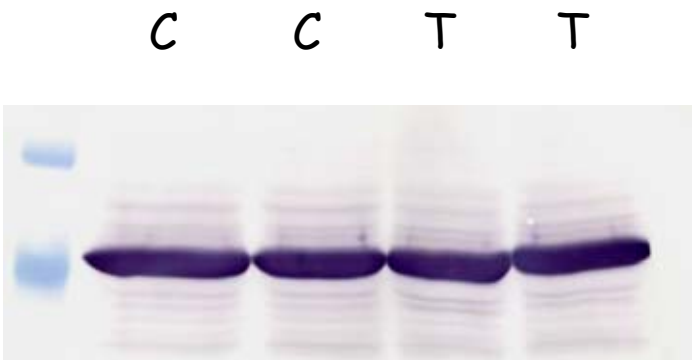
Traditional Western Blot Analysis



Coomassie gel



Western blot

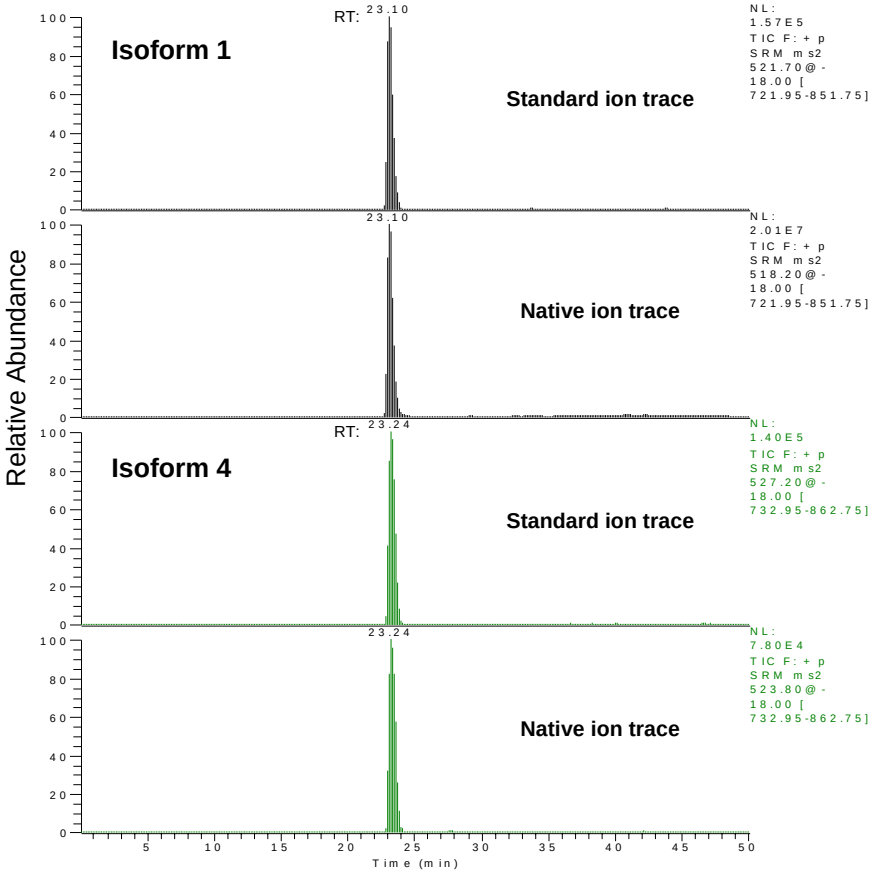
E. Larrainzar, Uni Navarra, Spain

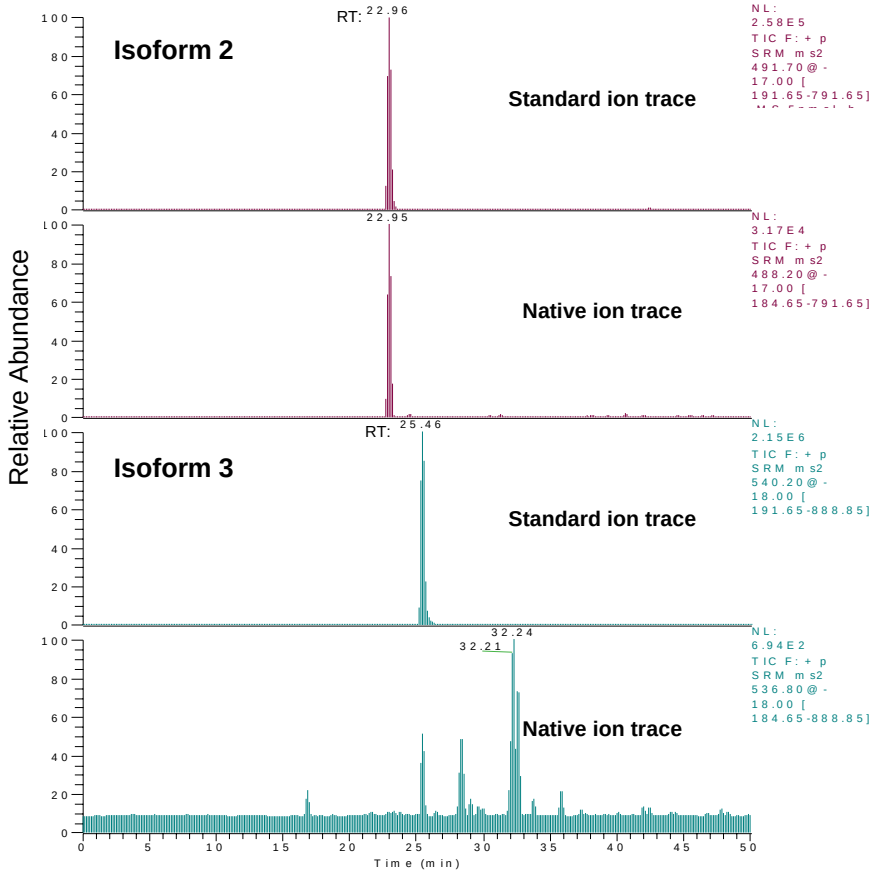
MASS WESTERN

Searching for Isoforms

(no original sequence)

```
Isoform 1    ....  GDVQYILDDVRXLFSEALSRIKKQGLDIIPRLQIITRLLTDEVGSTCGQRLFKVYGIEHC
Isoform 2    ....  GDVQYILDDVRXLFNEALRRIKQQGLDIKPRLQIITRLLTDEVGSTCGERLFKVYDIEHC
Isoform 3    ....  GDVQYILDDVRXLFEEALQKIELQGLNVKPQLQVVTRLITNEKGSTCNQELFPIIKIKHS
Isoform 4    ....  GDVQYILDDVRXLFNEALARIQKQGLDFTPRLQIVTRLITDEKGSTCNQRLFRVSGIDYT
```





Detection of low abundance isoforms (in gel)

Isoform 1	**12**	**pmol**
Isoform 2	22	fmol
Isoform 3	nd	
Isoform 4	171	fmol

Isoform Identification

- isoform identification and quantification of corresponding western blot signals
- isoform identification responsible for enzyme activities

Conclusion

- Stable isotope dilution technique is highly sensitive for targeted absolut quantification especially also for low abundance proteins.
- Detailed and complex pathway analyses are possible.
- Mass Western approach gives more detailed information than traditionel western blot analyses.

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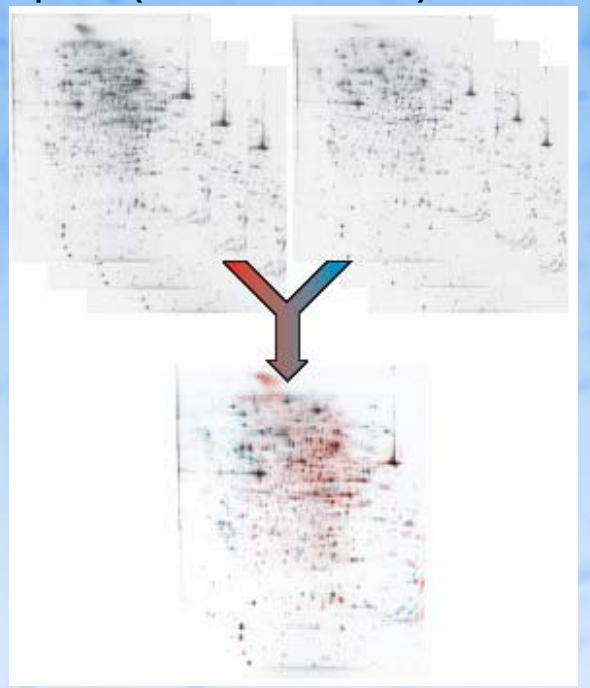
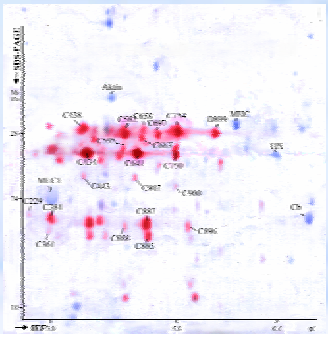
www.proteomefactory.com

Analysis of all kind of protein samples by extreme
high resolution 2DE - separation of up to 10,000 protein spots (40x30 cm 2DE)



- Target / Biomarker Identification
  - Differential Proteomics Studies
  - Plasma / CSF Proteomics Studies
    with depletion of high abundant proteins
- Pharmaco Proteomics Studies
- Immuno Proteomics
- Protein Separation / Western Blots



Thanks to

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