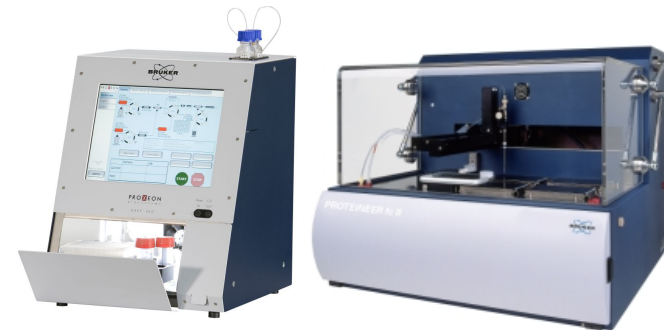


# ultrafleXtreme TOF/TOF

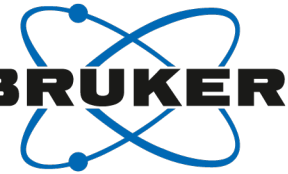
Re-defining MALDI-TOF/TOF performance in  
**proteomics & protein characterization**



sample  
proteinscape  
show



## News at Bruker Corporation And News at Bruker s.r.o.



- **Bruker Daltonics s.r.o. -> Bruker s.r.o. (1.9.2011)**
- **Bruker Daltonics LifeScience/CAM/CBRN**
- **Bruker s.r.o. = Bruker Daltonics Life Science only (CZ/SK)**  
2x sales, 3-4x service and application, 2x admin
- **newly also = Bruker AXS (XRF, XRD, SCXRD, uXRD) (CZ only)**  
1x sales, 1x service
- **MALDI Biotyper** – 18 units in the CZ, 2x in SK  
Extended service and application support needed
- **Bruker Daltonik GmbH in 2011**
  - purchase of Michrom nanoHPLC/CaptiveSpray
  - maxis 4G and maxis impact & amazon speed (ETD),
  - SCION GC-MS/MS
  - PRIME complete proteomics solution

# Bruker Daltonics

## Life Science Mass Spectrometry



- MALDI-TOF and -TOF/TOF Mass Spectrometry
  - ESI-(Q)TOF Mass Spectrometry
  - ESI- UHR-TOF Mass Spectrometry
  - ESI-Ion Trap Mass Spectrometry
  - ESI/MALDI-Q-FTMS
  - ESI-QqQ (from 2012)
- 
- Unique **Mass Spectrometry Solutions** for
    - **Proteomics** /Biomarker Analysis
    - MALDI Molecular Imaging
    - **Small Molecules** / **Metabolite** Studies
    - Food, Forensic & Environmental **Screening**
    - Functional Genomics/SNP Genotyping
    - Microorganism Identification and Classification



# Bruker Daltonics Product Lines



## flex series

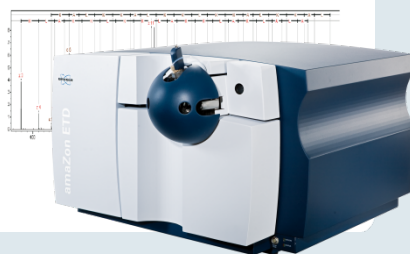
- Expression Proteomics
- MALDI Imaging
- Biomarker Discovery
- Microorganism ID



**MALDI-TOF(/TOF)**

## amaZon series

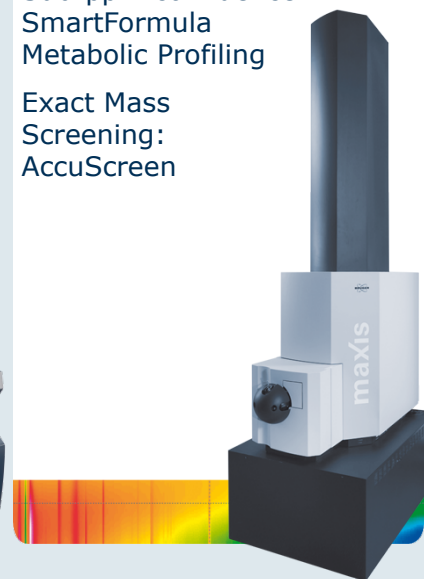
- PTM Discovery
- Expression Proteomics
- Metabolite ID
- MS<sup>n</sup> capabilities
- Top-Down sequencing with PTR



**ESI-LC/MS<sup>n</sup>-Ion trap**

## micrOTOF/maXis series

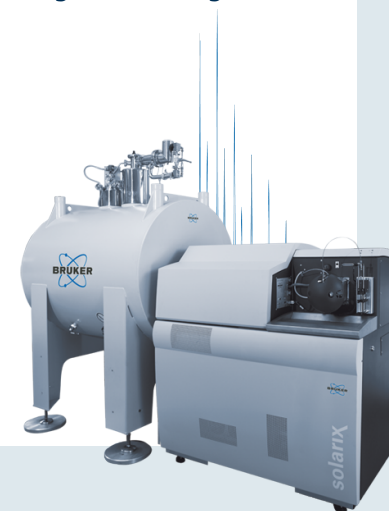
- Accurate Mass Determination  
"The Formula Machine"
- Sub-ppm confidence:  
SmartFormula  
Metabolic Profiling
- Exact Mass Screening:  
AccuScreen



**ESI-(Q)TOF/UHR-TOF**

## solarix

- The ultimate in Accurate Mass Determination
- Ultra-high resolution
- Top-Down & Bottom-Up proteomics
- Low maintenance, refrigerated magnets



**FTMS**



**prime –**

Proteomics through Integrated MALDI and ESI



Comprehensive  
Knowledge Output  
for Next Generation  
Proteomics



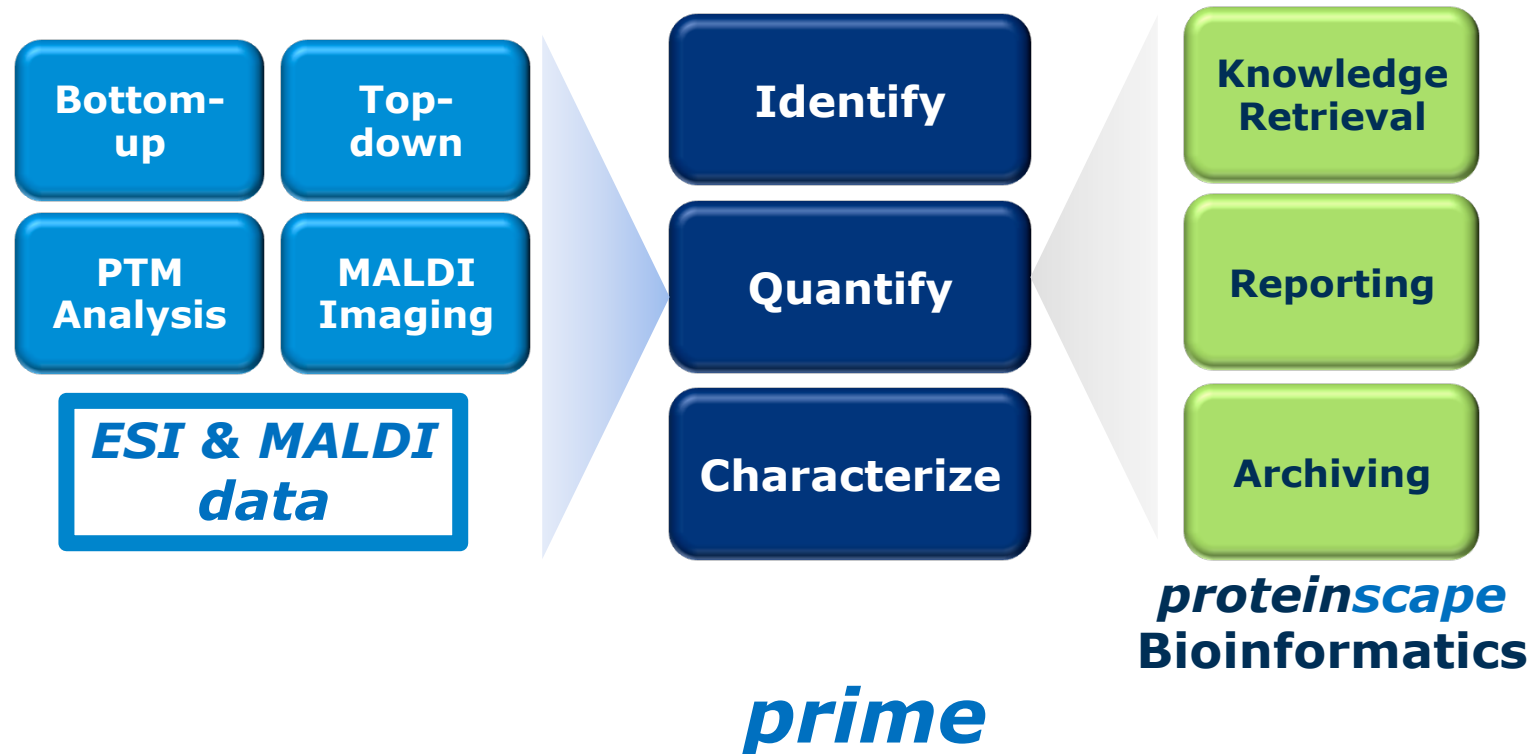
# Full Coverage of Technologies and Bioinformatics to Reveal the Proteome



## The Proteome:

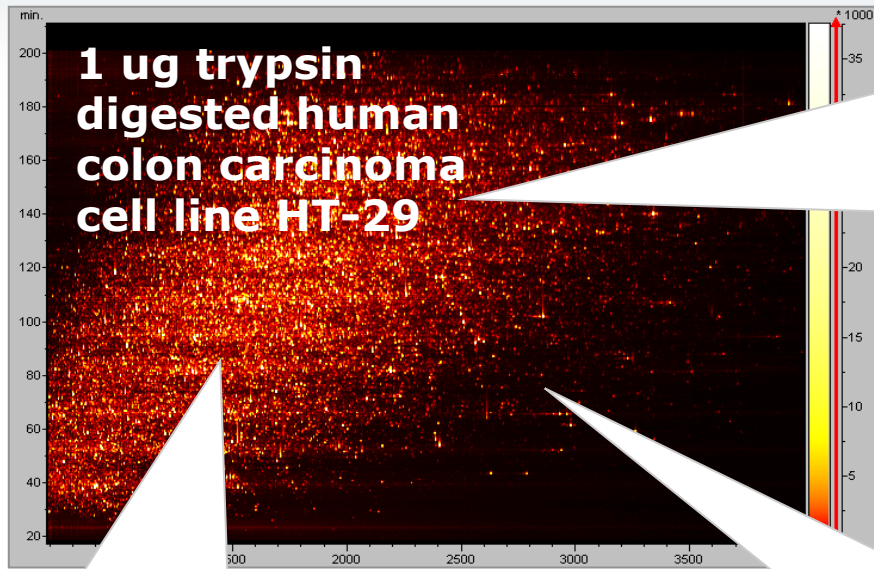
- far more complex than was ever expected
- highly dynamic in time, space and concentration
- highly variable due to modifications and mutations

**requires** complementary methods to generate reliable and complete information:



# Bottom-up Protein Identification

Bruker's MS enable greater proteome coverage in bottom-up proteome analyses



1 ug trypsin  
digested human  
colon carcinoma  
cell line HT-29

**maXis Impact:**  
**2,059**  
protein IDs

**9,873**  
unique  
peptides



**ultrafleXtreme:**

**1,506**  
protein IDs

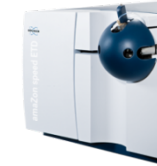
**5,550**  
unique peptides



**amaZon Speed:**

**1,516**  
protein IDs

**6,682**  
unique peptides



Highly increased  
speed: 2x MS/MS  
spectra/time → more  
peptides sequenced  
and id'ed

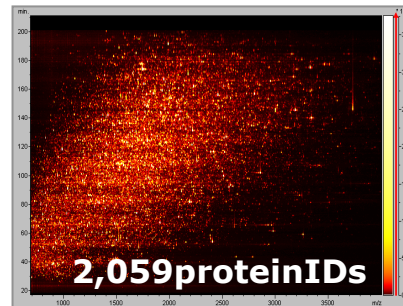
All protein numbers with FDR < 1%.

# Bottom-up Protein Identification

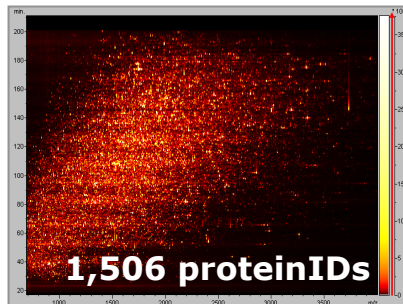
Clear benefit provided by ESI/MALDI complementarity



**maxis  
Impact**



**ultraflextreme**



*prime*

**Protein  
Extractor  
(proteinscape)**

**ESI+MALDI:**

**2,425**

**non-redundant  
protein IDs**

**(added ~20% by  
combining ESI and  
MALDI)**

**Takes 3  
mouseticks  
in proteinscape**

ultrafleXtreme:

Re-defining MALDI-TOF/TOF performance in proteomics & protein characterization



**smartbeam™-II laser:**

**True kHz acquisition speed  
in MS and MSMS**

**Perpetual™ ion source:**

**Laser based self-cleaning  
within 15min**

**PAN/FlashDetector™:**

**Unrivaled broadband  
resolution up to >40,000  
Low amol sensitivity**

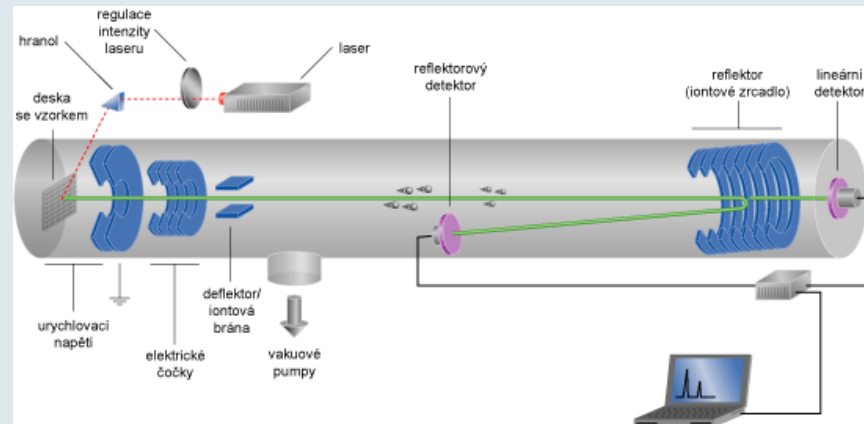
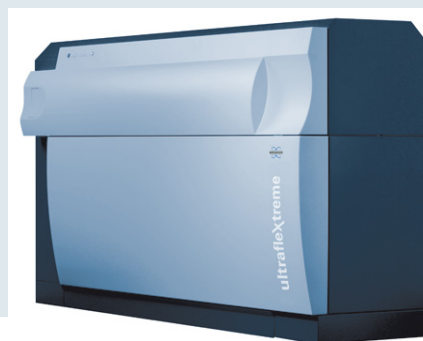
**4GHz digitizer:**

**Superior mass accuracy  
<5ppm (external cal.)  
<1ppm (internal cal.)**

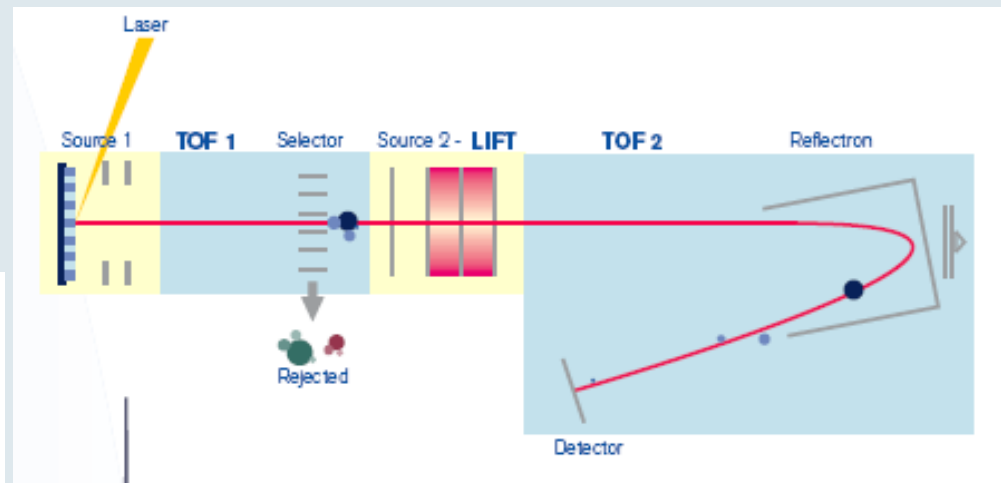
# MALDI – based MS Detection



**MALDI** = Matrix Assisted Laser Desorption-Ionization



- Time of Flight (TOF) – reflex IV



- TOF/TOF - ultraflex Xtreme



# MALDI = Matrix Assisted Laser Desorption-Ionization

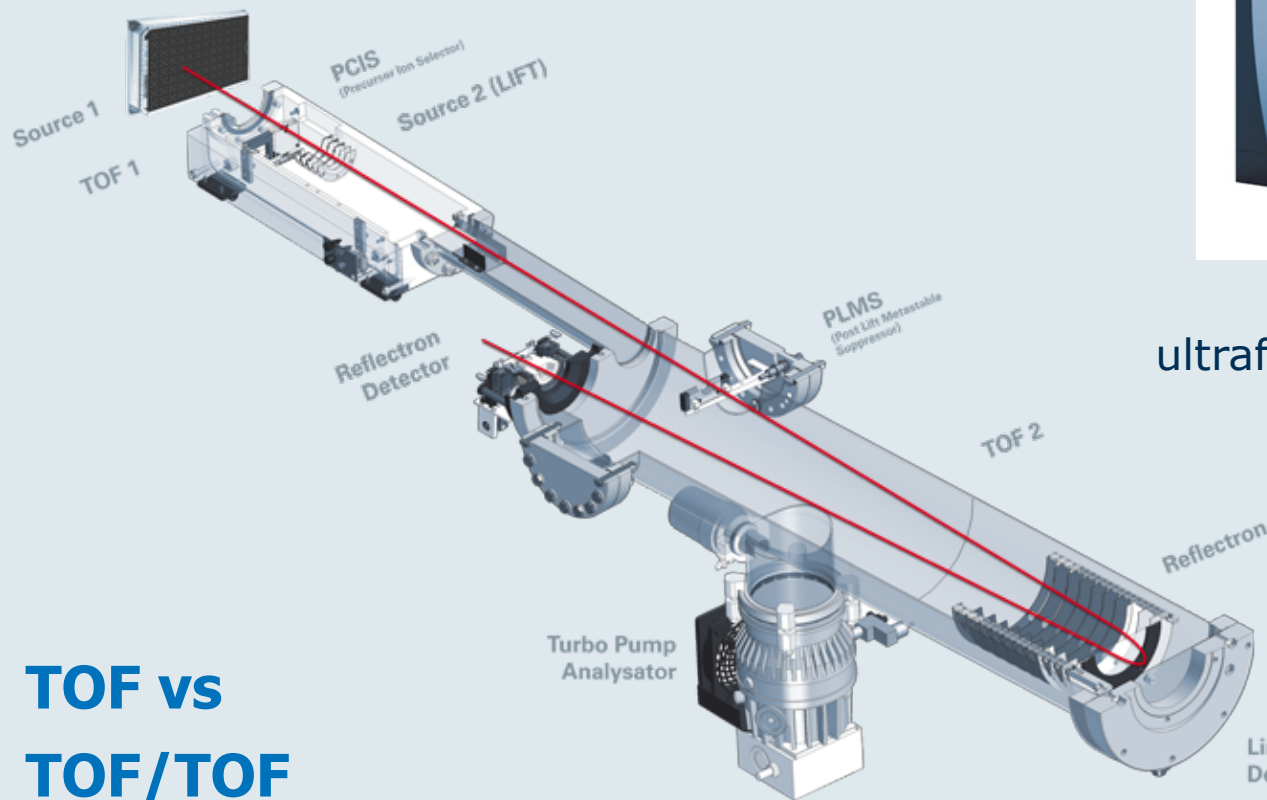


## MALDI

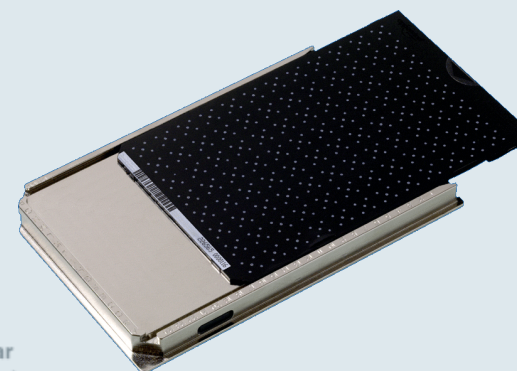
MALDI type of ionization produces mainly ions with low charges, thus mass range of detector must be wider



ultraflexXtreme



## TOF vs TOF/TOF

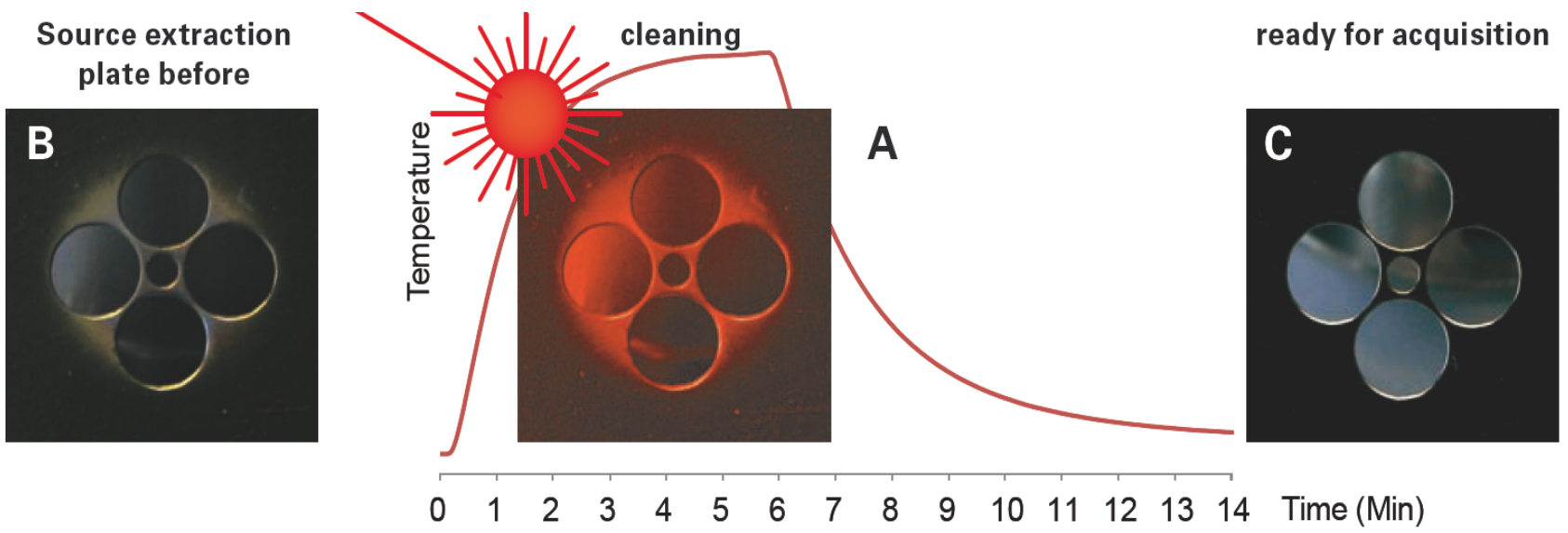


MTP format MALDI target

ultrafleXtreme:



Perpetual™ ion source: Laser based self-cleaning **within 15min** for **improved robustness & minimum instrument down time**

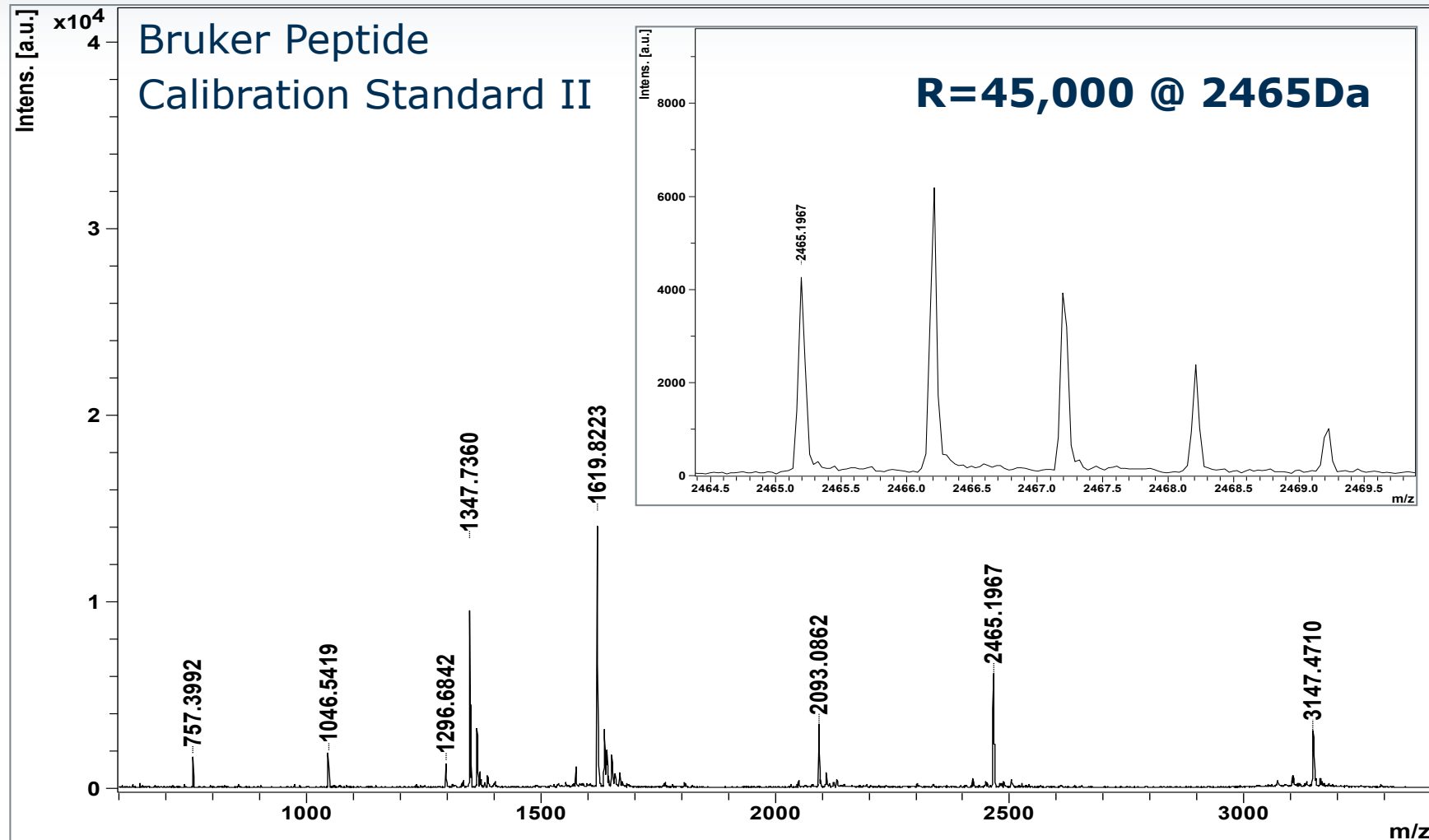




ultrafleXtreme:

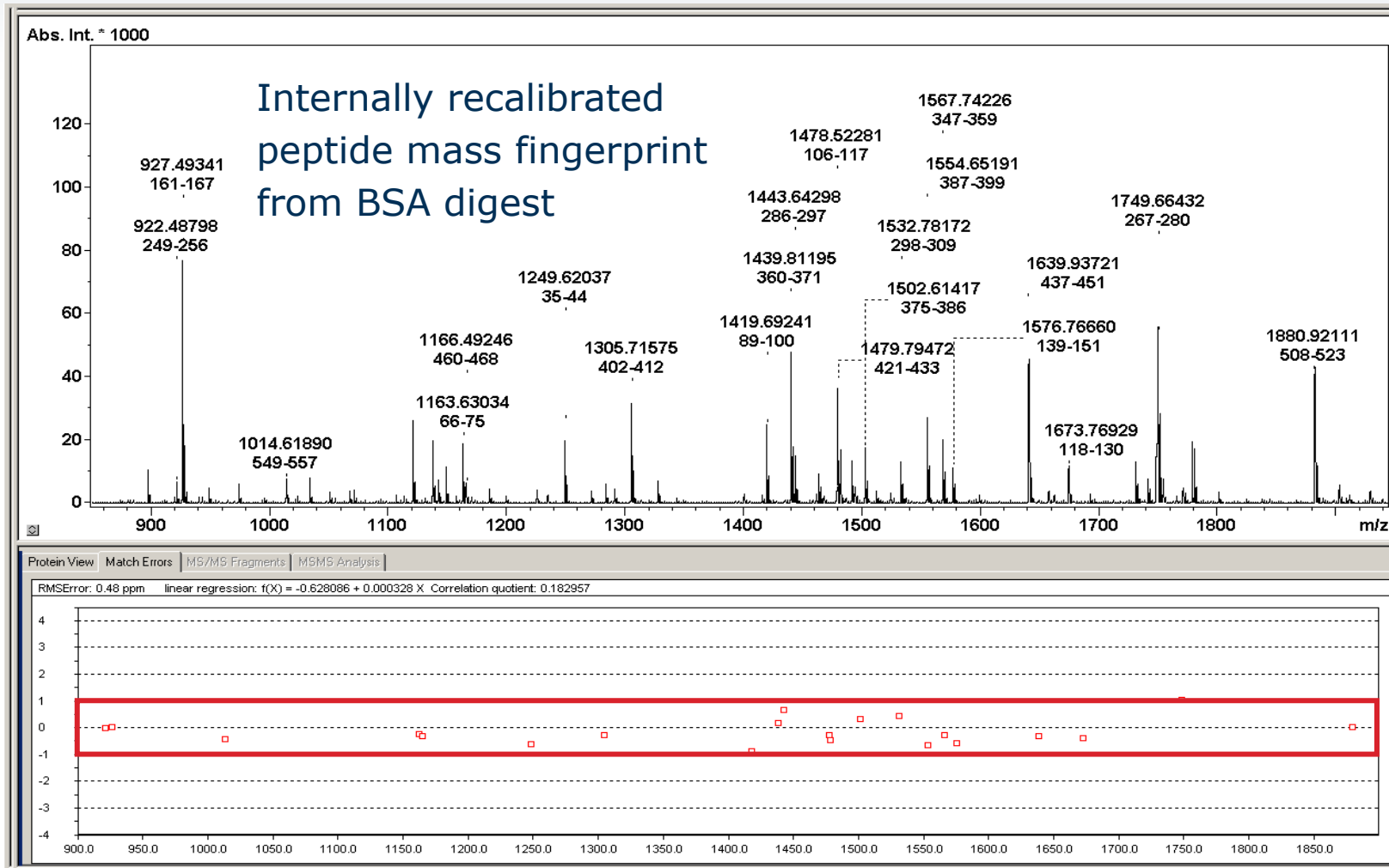
PAN/FlashDetector™: Unrivalled broadband resolution

up to >40,000



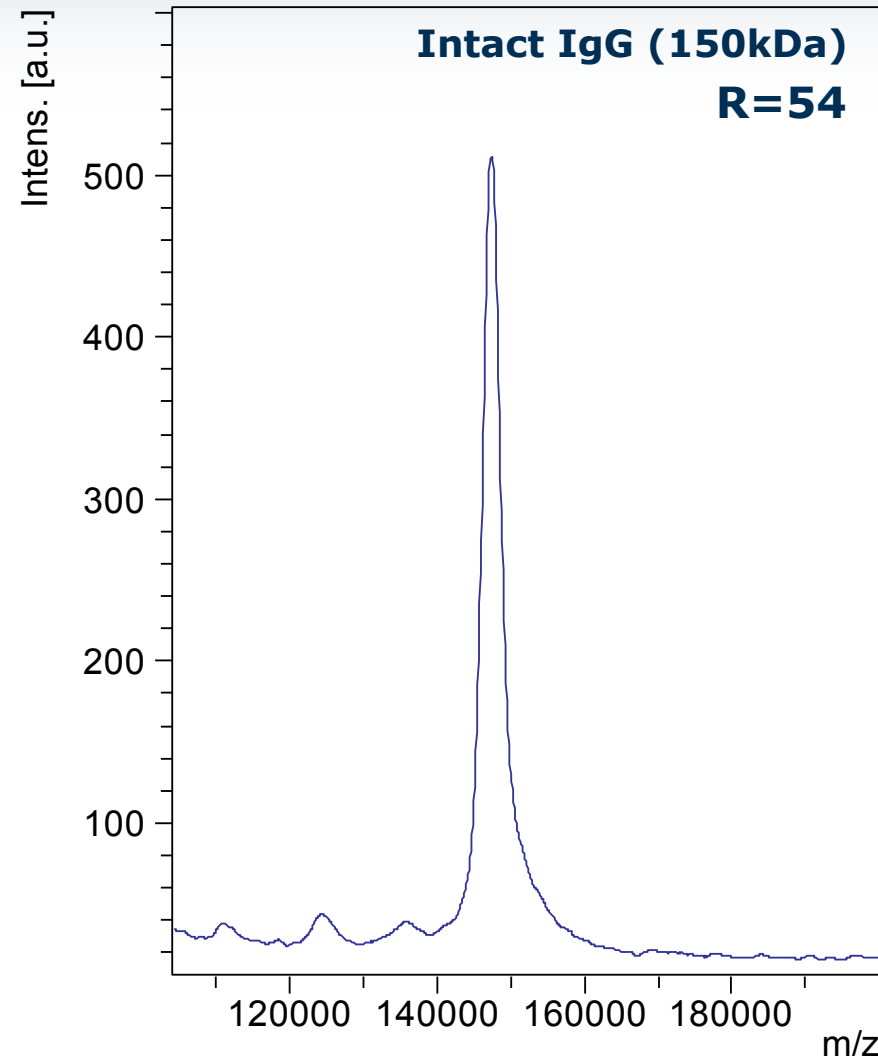
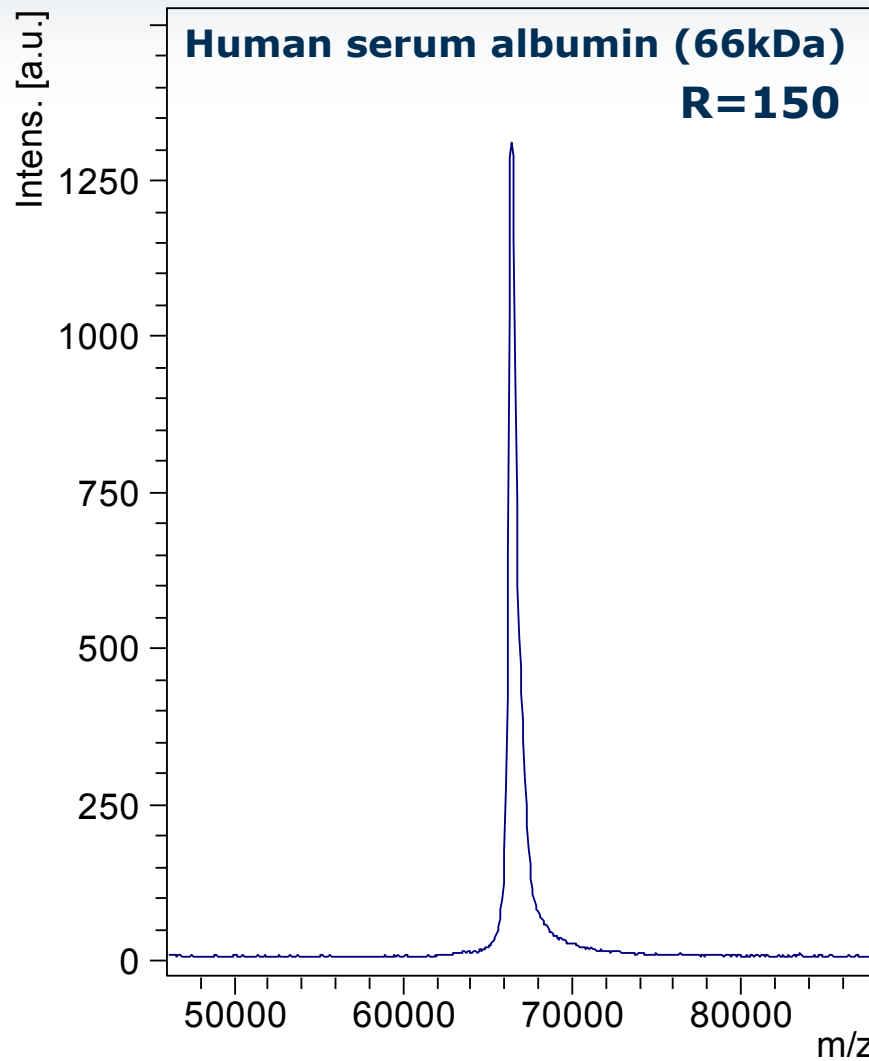
ultrafleXtreme:

4GS/s digitizer: Superior mass accuracy <5ppm (external)  
<1ppm (internal)



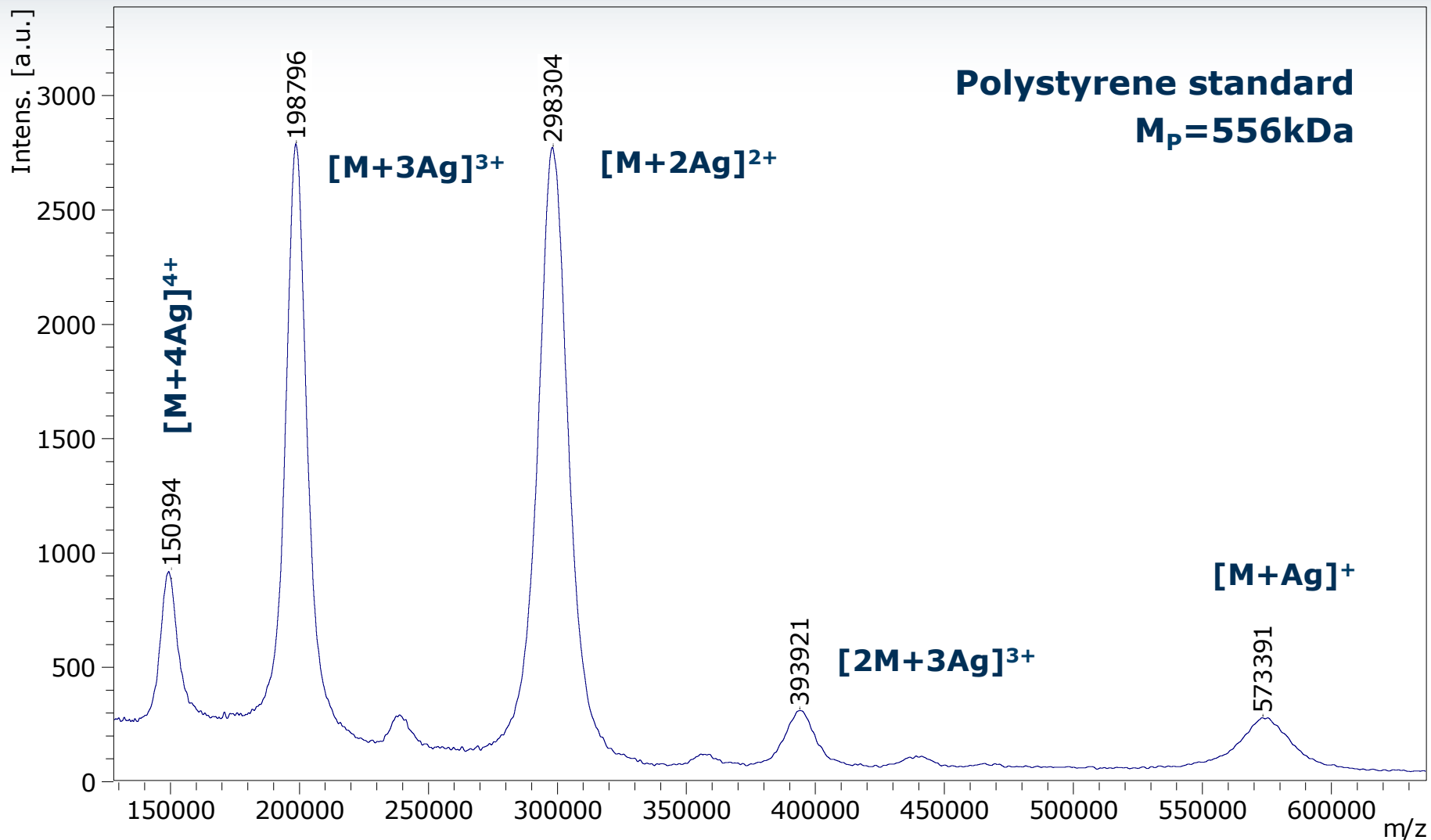
ultrafleXtreme:

On-axis linear detector: Outstanding performance in  
HMW measurement



ultrafleXtreme:

On-axis linear detector: Outstanding performance in  
HMW measurement



**ultrafleXtreme:**  
**Application examples**



**Proteomics:**

**Accelerated analysis of complex proteomic samples at improved resolution and mass accuracy:**

- LC-MALDI analysis of 500ng E.coli digest

**Protein characterization:**

**MALDI top-down terminal sequencing of intact proteins:**

- by-products in a recombinant TAU protein

**MALDI Imaging:**

**TLC-MALDI for Lipidomics**

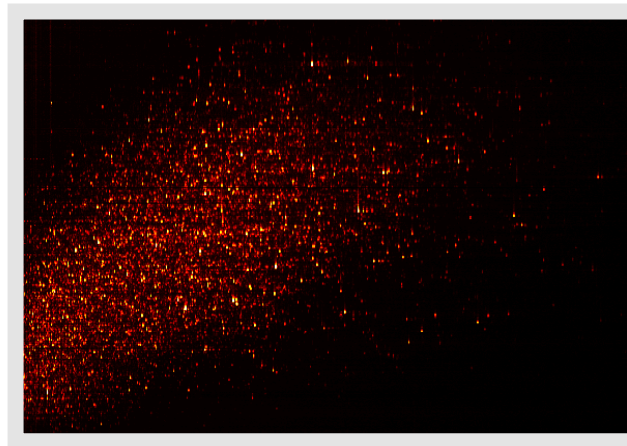


ultrafleXtreme:

Accelerated analysis of **complex proteomic samples**  
at improved resolution and mass accuracy

**Showcase:**

LC-MALDI analysis of 500ng E.coli digest



ultrafle**X**treme:

## Analysis of **complex proteomic samples**

LC-MALDI analysis of 500ng E.coli digest



### LC system: Bruker EASY-nLC

**Trap column:** Nanoseparations RP-18 capillary, 100 $\mu$ m x 2cm, 5 $\mu$ m particles

**Analytical column:** Pepmap 100, 75 $\mu$ m x 15cm, 3 $\mu$ m particles, 300nl/min

**LC gradient:** **Gradient I** (medium): 2 ... 45% B within 2 hours  
**Gradient II** (long): 2 ... 45% B within 3 hours  
(**A**: 0.05%TFA in H<sub>2</sub>O; **B**: 0.05%TFA in 90/10 ACN/H<sub>2</sub>O)

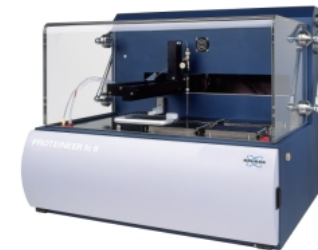
**MALDI spotting:** **Bruker PROTEINEER fc II**  
Gradient I: 2 x MTP AnchorChip 384TF; 768 fractions  
Gradient II: MTP AnchorChip 1536TF; 1152 fractions  
MALDI matrix HCCA

**MS calibration:** external calibration (Bruker Peptide standard II)

**Data analysis:** **Bruker ProteinScape**

**MASCOT search:** Database: Swissprot  
Taxonomy: E.coli  
MS tolerance: 15ppm  
MS/MS tolerance 0.5Da

**Result compilation:** ProteinExtractor



sample  
protein  
show  
protein  
scaffold  
proteinscape

ultrafleXtreme:

## Analysis of **complex proteomic samples**

LC-MALDI analysis of 500ng E.coli digest

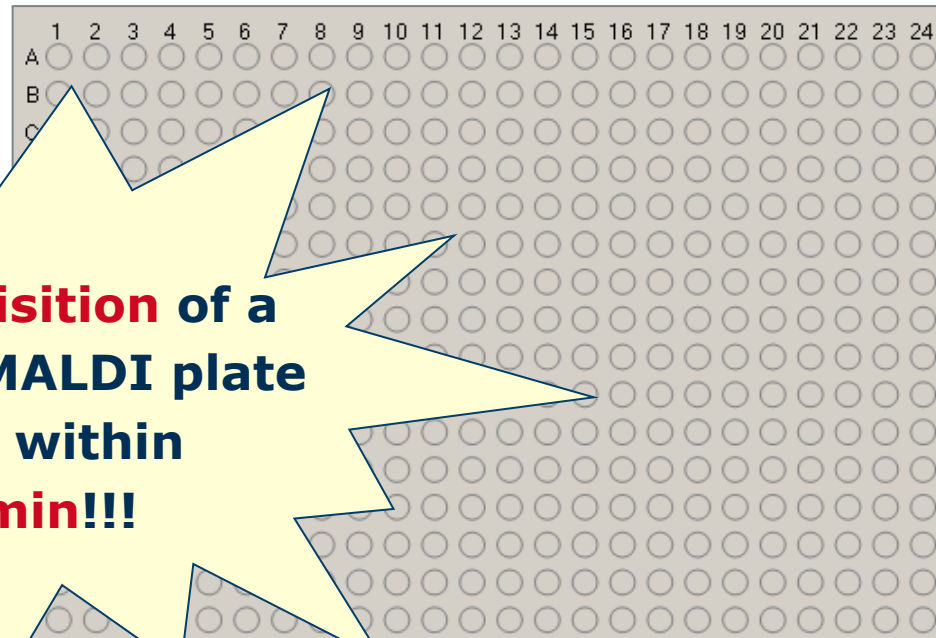


**1000 Hz acquisition speed in MS and MS/MS mode:**



≤ 3s per MS spectrum

≤ 5s per MS/MS spectrum



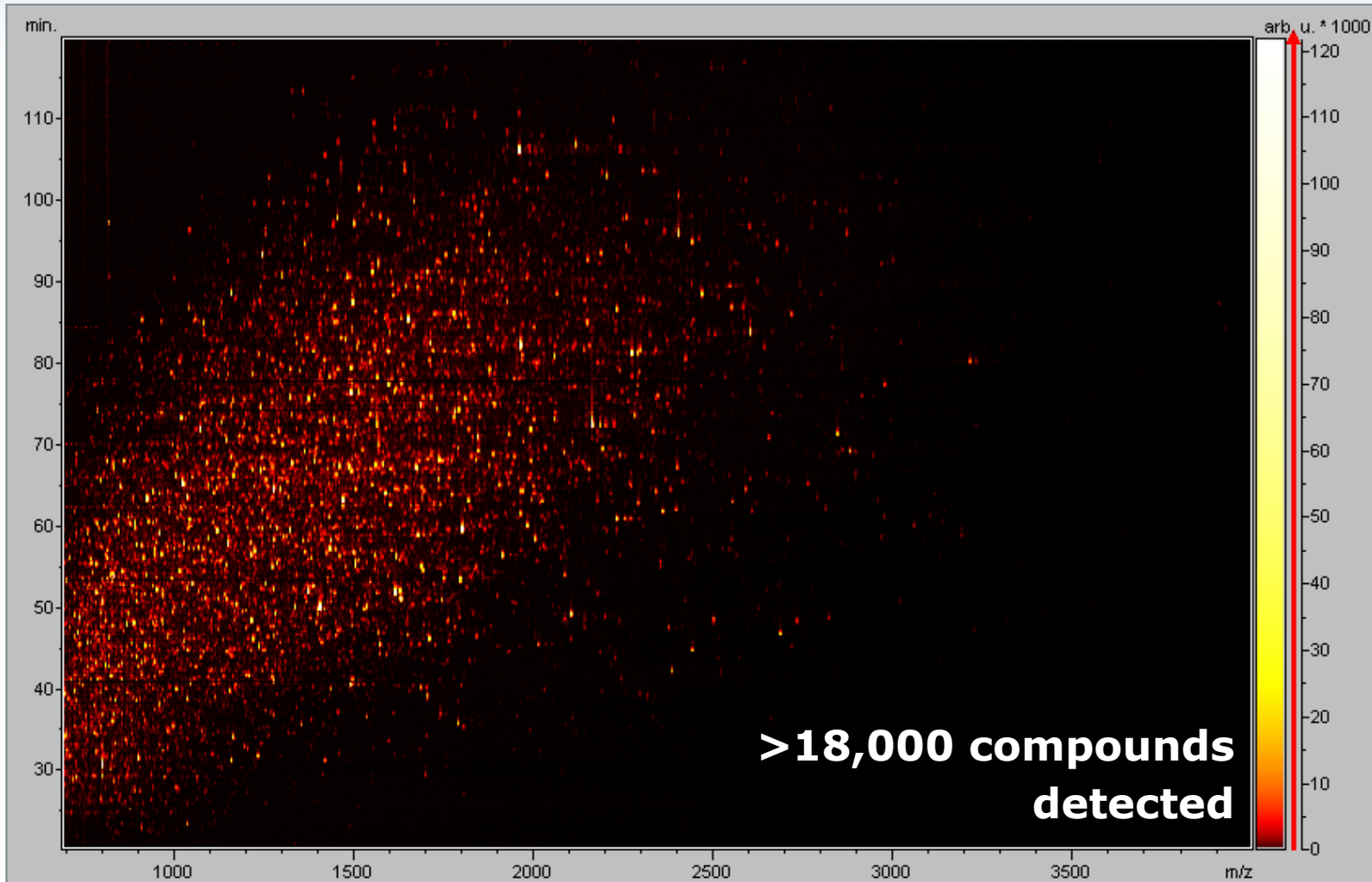
**MS acquisition of a  
384MTP MALDI plate  
done within  
20min!!!**



ultrafleXtreme:

## Analysis of **complex proteomic samples**

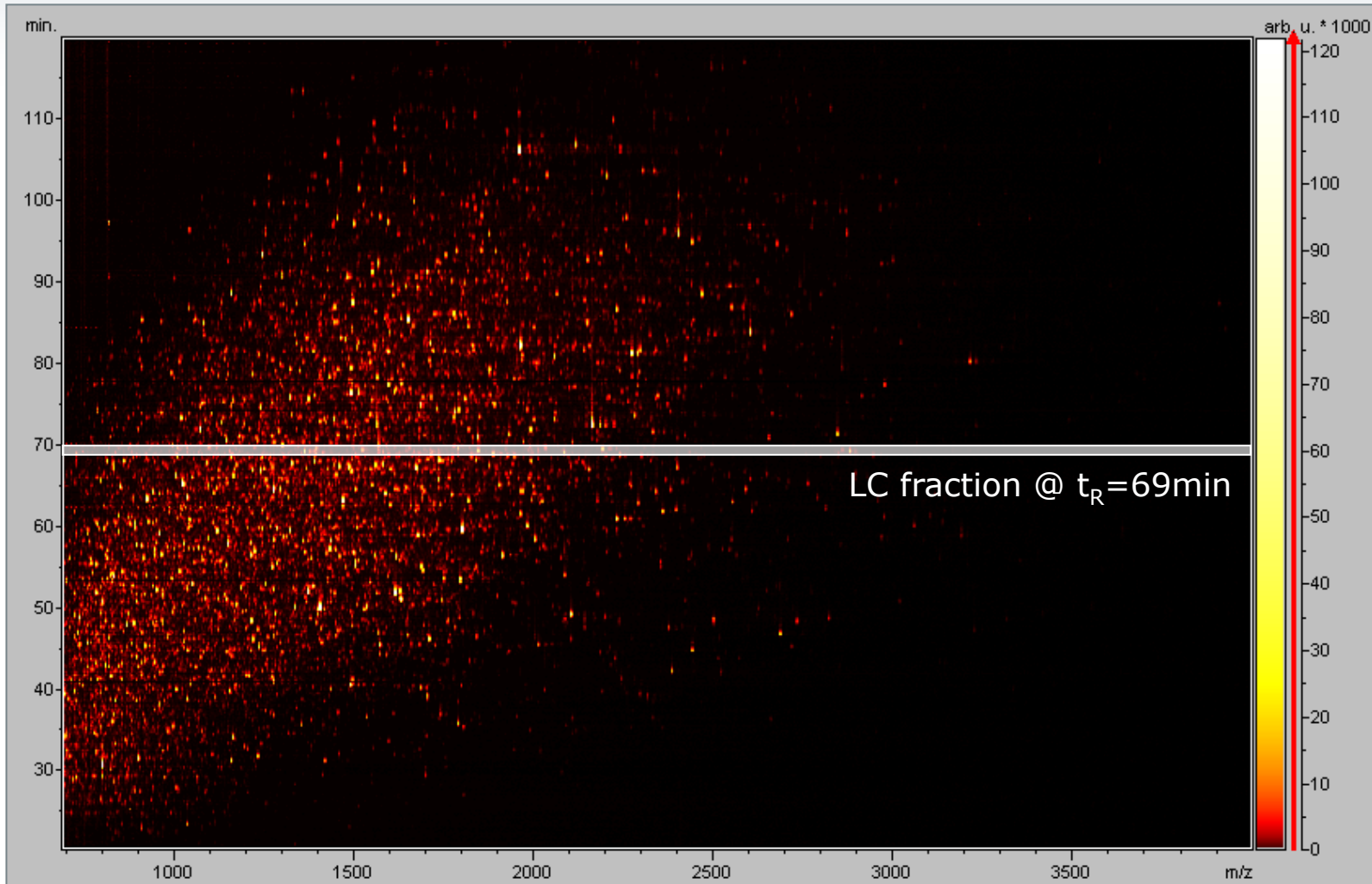
LC-MALDI analysis of 500ng E.coli digest (**gradient I**)



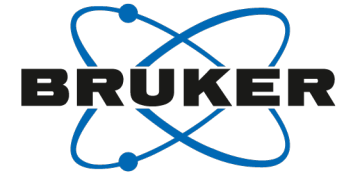
ultrafleXtreme:

# Analysis of complex proteomic samples

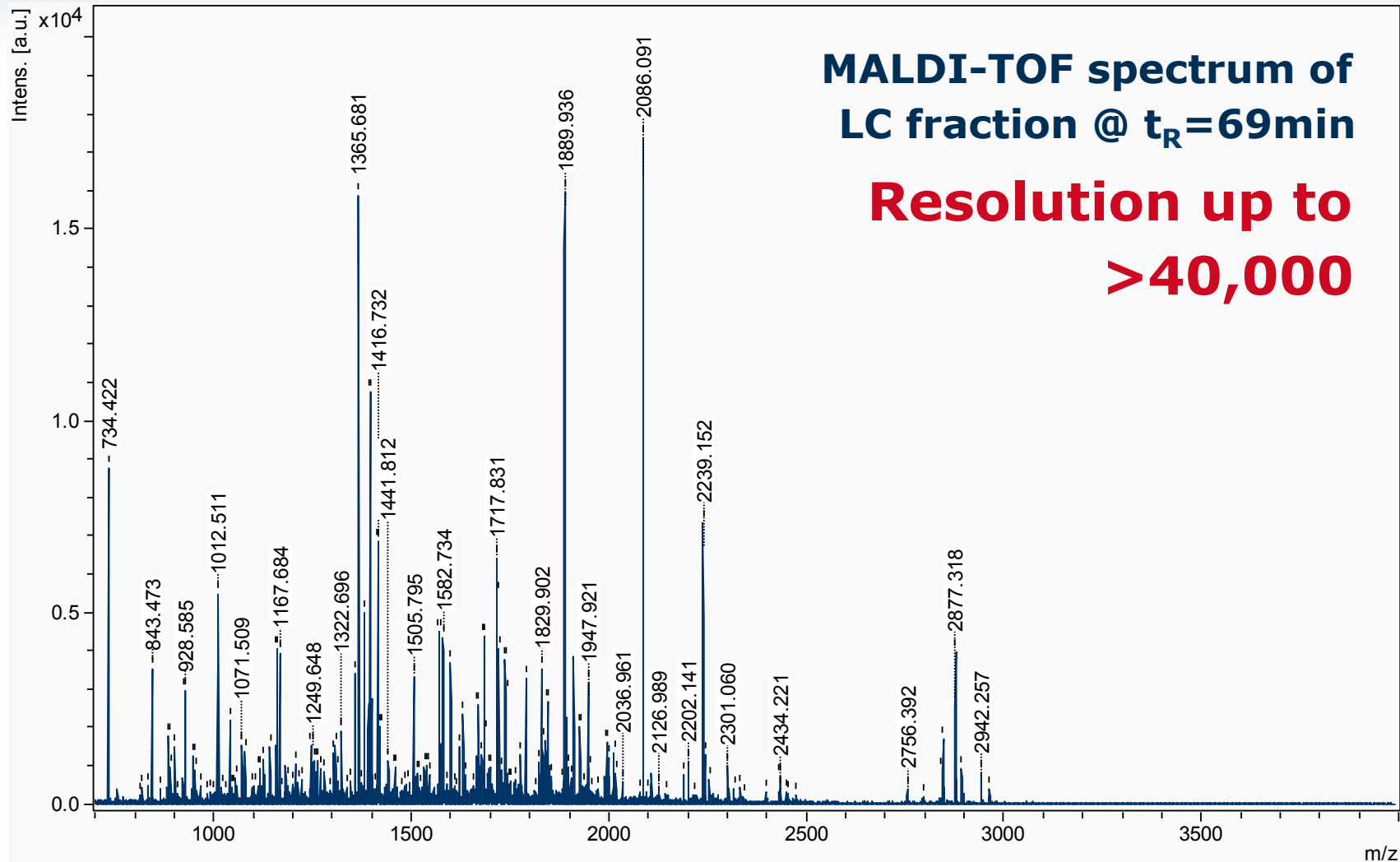
LC-MALDI analysis of 500ng E.coli digest (gradient I)



ultrafleXtreme:  
Analysis of **complex proteomic samples**



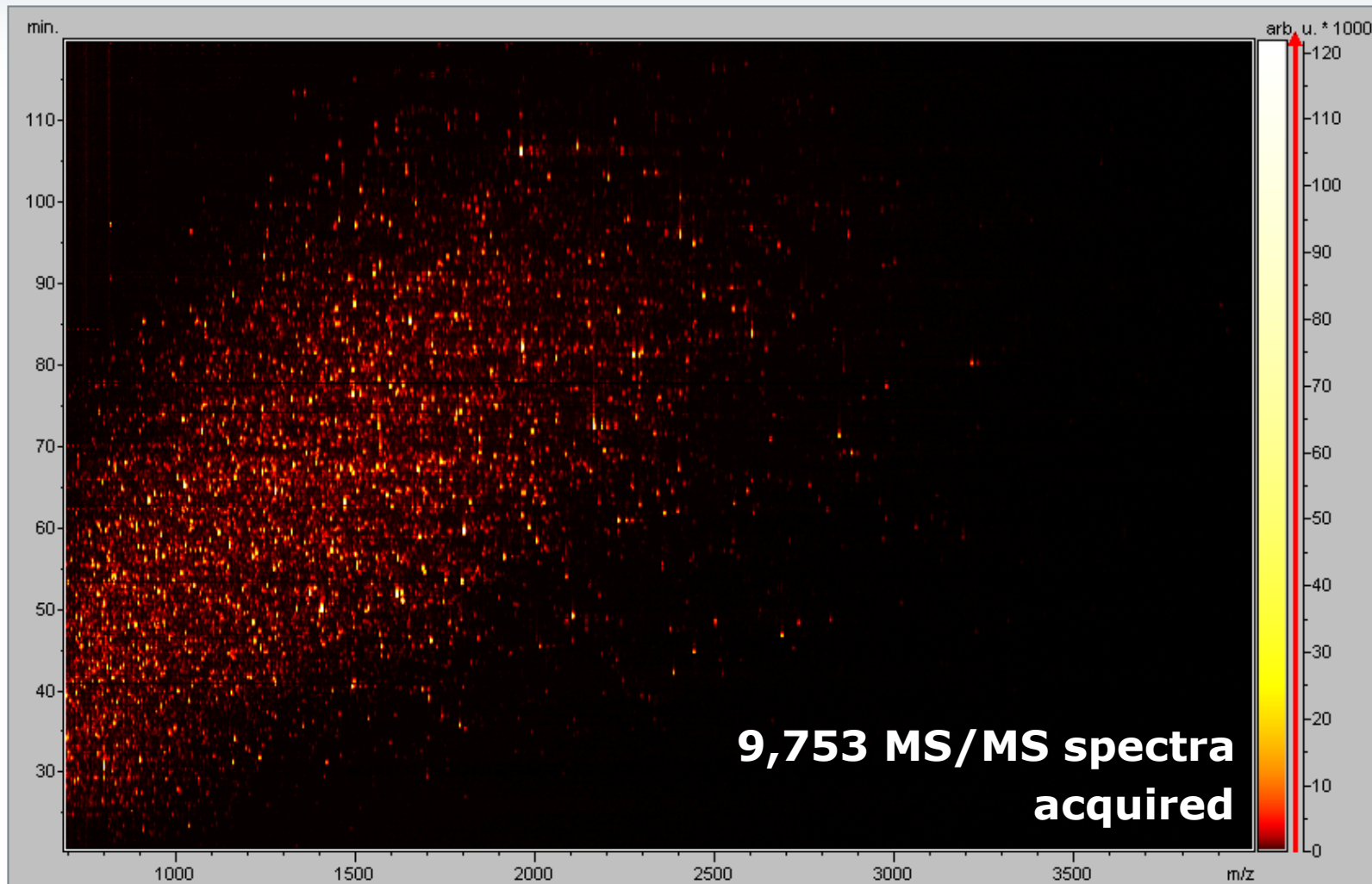
LC-MALDI analysis of 500ng E.coli digest (**gradient I**)



ultrafleXtreme:

## Analysis of complex proteomic samples

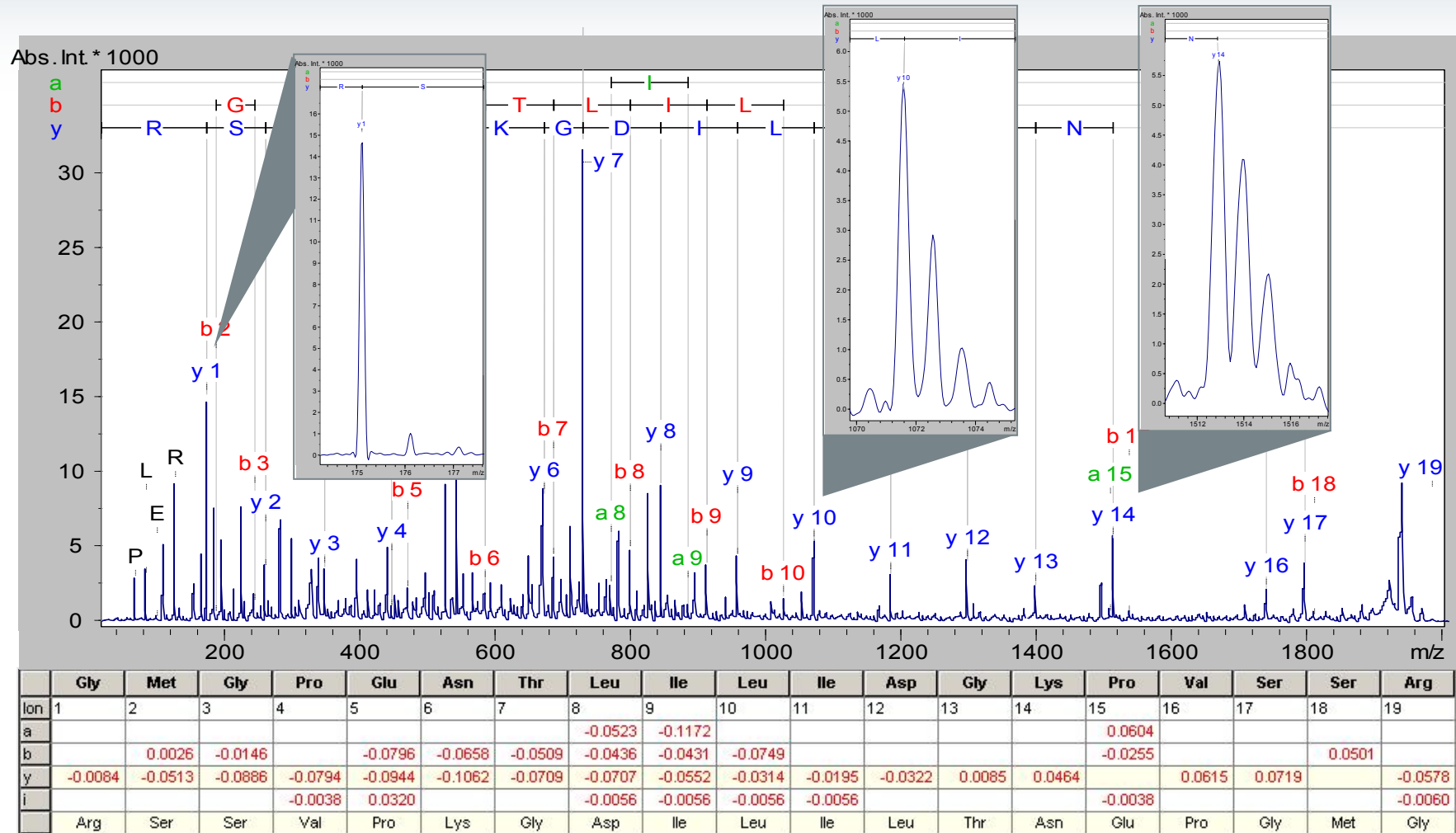
LC-MALDI analysis of 500ng E.coli digest (gradient I)



ultrafleXtreme:

# Analysis of complex proteomic samples

LC-MALDI analysis of 500ng E.coli digest (gradient I): MS/MS mass accuracy



Average fragment mass deviation: **0.045Da**



# ultrafleXtreme: Analysis of complex proteomic samples



LC-MALDI analysis of 500ng E.coli digest: Data organization & analysis in ProteinScope

ProteinScope - Demo - hbappprotein1

File Edit View Window Help

Project Navigator Spectrum

Cmpd\_5258 [MS\_0]

Cmpd\_5258 [MS/MS\_(1797.83)]

LC-MS Survey Gel

min. \*1000

200 150 100 50

1000 2000 3000 m/z

Main View

Info Proteins & Peptides

Protein	MW [kDa]	pI	Similar Proteins	Scores	Peptides	SC	RMS90 [p...]	Rank
Ferrienterobactin receptor precursor - ...	82.1	5.3	1	4369.6 (M:4369.6)	48	66.2	4.96	1
Formate acetyltransferase 1 - Escheri...	85.3	5.6	2	3408.6 (M:3408.6)	51	62.8	5.16	2
Elongation factor Tu 1 OS=Escherichia...	43.3	5.2	9	3265.9 (M:3265.9)	51	83.8	4.51	3
Chaperone protein dnaK - Escherichia ...	69.1	4.7	2	2933.4 (M:2933.4)	44	67.7	4.92	4
60 kDa chaperonin - Escherichia coli O...	57.3	4.7	3	2883.7 (M:2883.7)	41	58.2	4.75	5

m/z meas.	Δ m/z...	Rt [min]	Scores	P	Sequence	Modifications
2707.3343	-0.71	86.17	107.4 (M:107.4)	0	K.IEAGYVAVGQNAVGTDLQWDNVPK.A	
1646.8306	-4.75	57.33	104.6 (M:104.6)	0	R.IPEGLAGGTEGKFNK.A	
2000.0231	-12.50	74.17	102.8 (M:102.8)	0	R.GMGPELTLIDGKPVSSR.N	Oxidation: 2
2784.3618	-5.11	104.17	99.6 (M:99.6)	0	K.AEIFSLFAENMELTDSTIVTPGLR.F	Oxidation: 12
2949.4644	-3.29	83.83	98.3 (M:98.3)	0	R.NKIEAGYVAVGQNAVGTDLQWDNVPK.A	
2827.3309	-2.31	58.83	93.5 (M:93.5)	0	R.MKDLSSNTQALGTGNTGGAGIDGVSTTDR.S	Oxidation: 1
1307.6508	-1.79	99.33	93.2 (M:93.2)	0	R.DGWLAVGTWFR.N	

Sequence Table Processing View Protocol Navigator

10 20 30 40 50 60 70

MNKKIHSLAL LVMLGIYGVA Q&QEPDTPV SHDDTIVVTA AEQNLQAPGV STITADEIRK NPVARDVSKI

80 90 100 110 120 130 140

IRTMPGVNLT GNSTSGQRGN NRQIDIRGMG PENTLILIDG KPVSSRNSVR QGWRGERDTR GDTSWVPEM

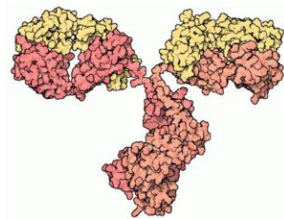
150 160 170 180 190 200 210

IERIEVLRGP AAARYGNAA GGVVNIITKK GSGEWHGSWD AYPNAPEHKE EGATKRTNFS LTGPLGDEFS

220 230 240 250 260 270 280

## ultrafleXtreme:

Unparalleled performance in **MALDI top-down** terminal sequencing of **intact proteins**



### Showcases:

- rapid quality control of **monoclonal antibodies**
- characterization of by-products in a **recombinant TAU protein**



ultrafleXtreme:

**MALDI top-down sequencing (MALDI-TDS) of intact proteins**



N T E R M S E Q U E N C E C T E R M





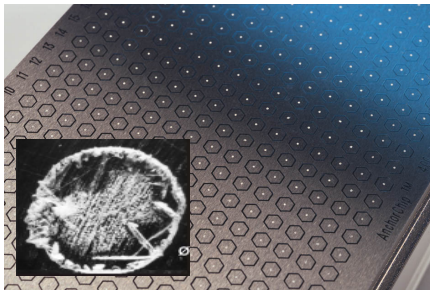
ultrafleXtreme:

**MALDI top-down sequencing (MALDI-TDS) of intact proteins**



N T E R M S E Q U E N C E C T E R M

+ MALDI matrix

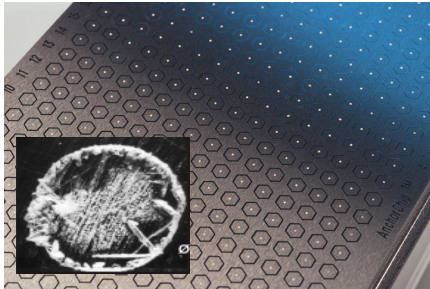


ultrafleXtreme:

**MALDI top-down sequencing (MALDI-TDS) of intact proteins**



+ MALDI matrix



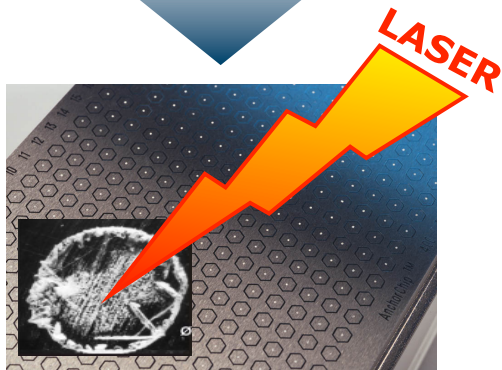
ultrafleXtreme TOF/TOF

ultrafleXtreme:

**MALDI top-down sequencing (MALDI-TDS) of intact proteins**



+ MALDI matrix



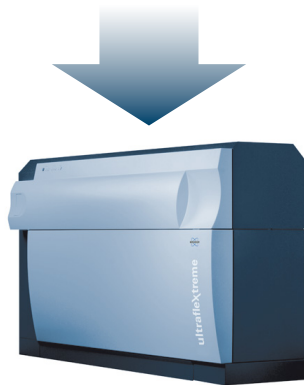
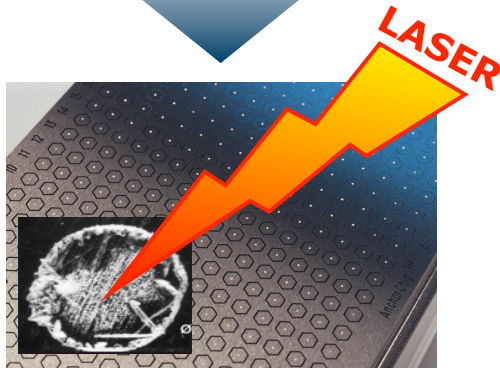
ultrafleXtreme TOF/TOF

ultrafleXtreme:

MALDI top-down sequencing (MALDI-TDS) of intact proteins

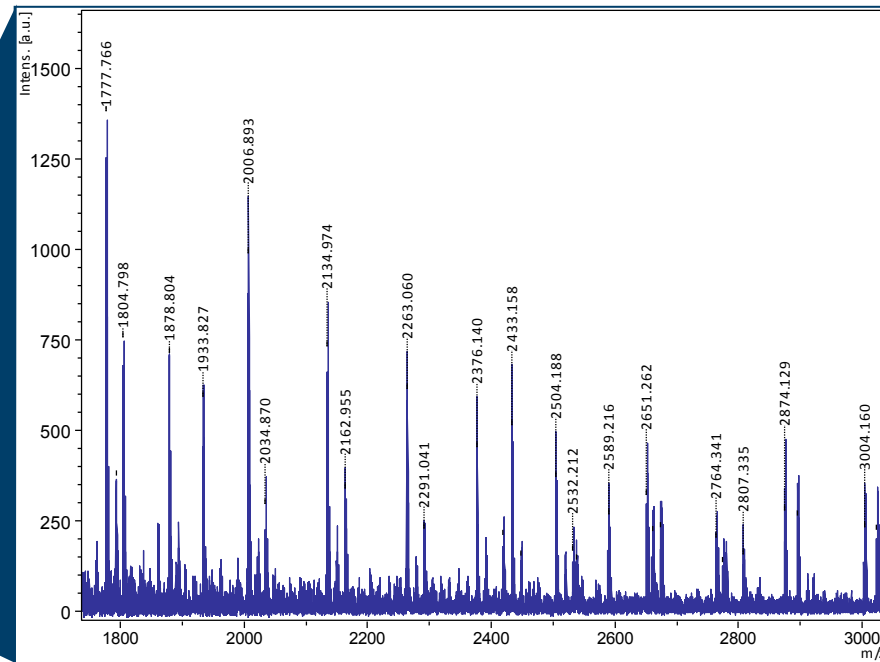


+ MALDI matrix



ultrafleXtreme TOF/TOF

In-source decay (ISD) upon MALDI process:



ultrafleXtreme:

**MALDI top-down sequencing (MALDI-TDS) of intact proteins**

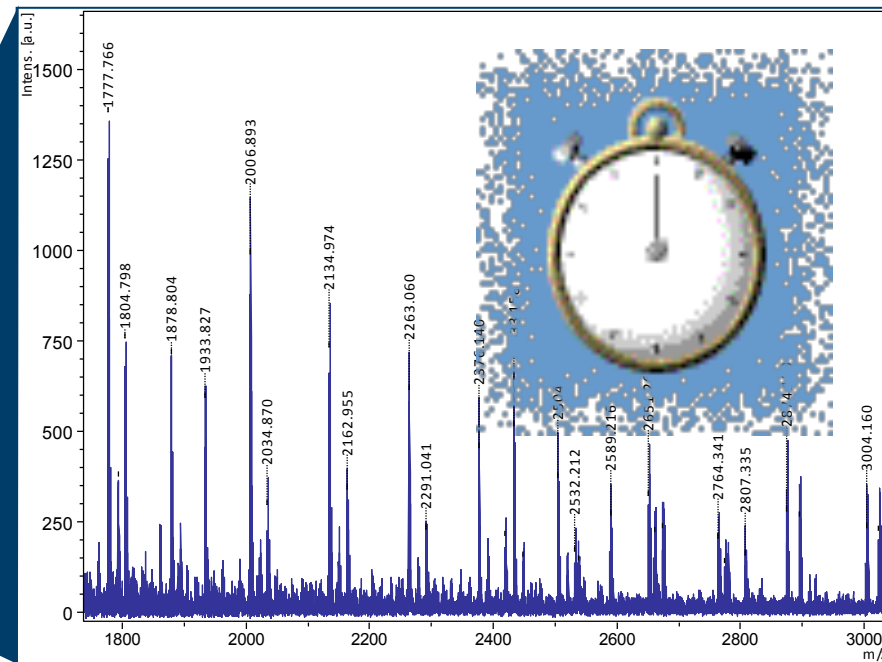


+ MALDI matrix



ultrafleXtreme TOF/TOF

**In-source decay (ISD) upon MALDI process:**



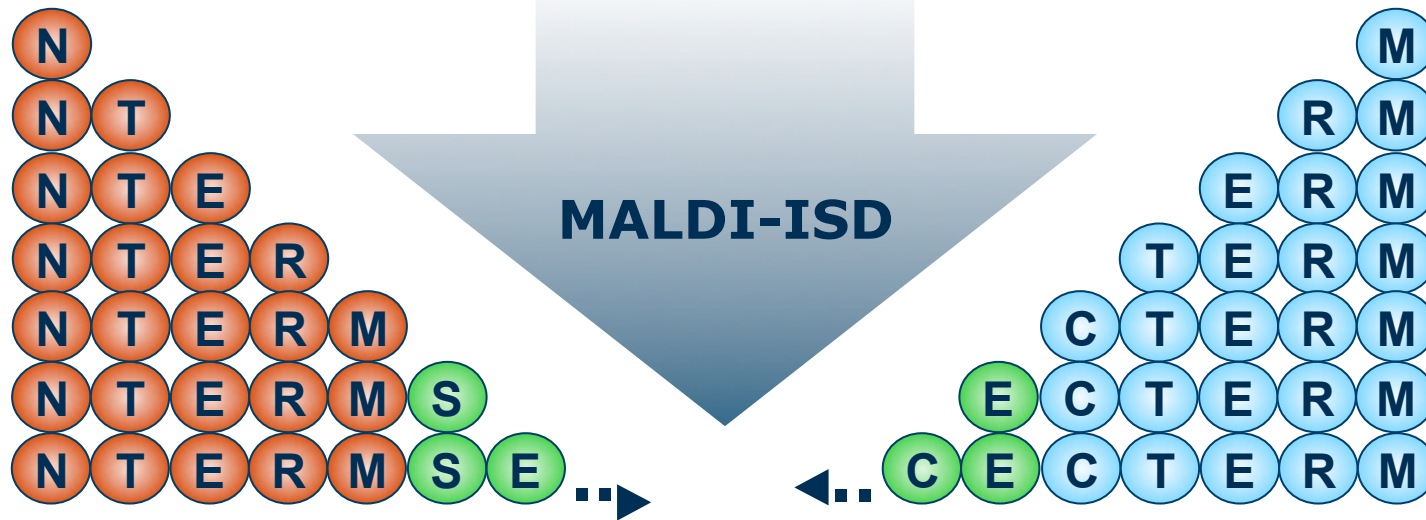
**Sample prep + data acquisition in minutes!**

ultrafleXtreme:

MALDI top-down sequencing of intact proteins

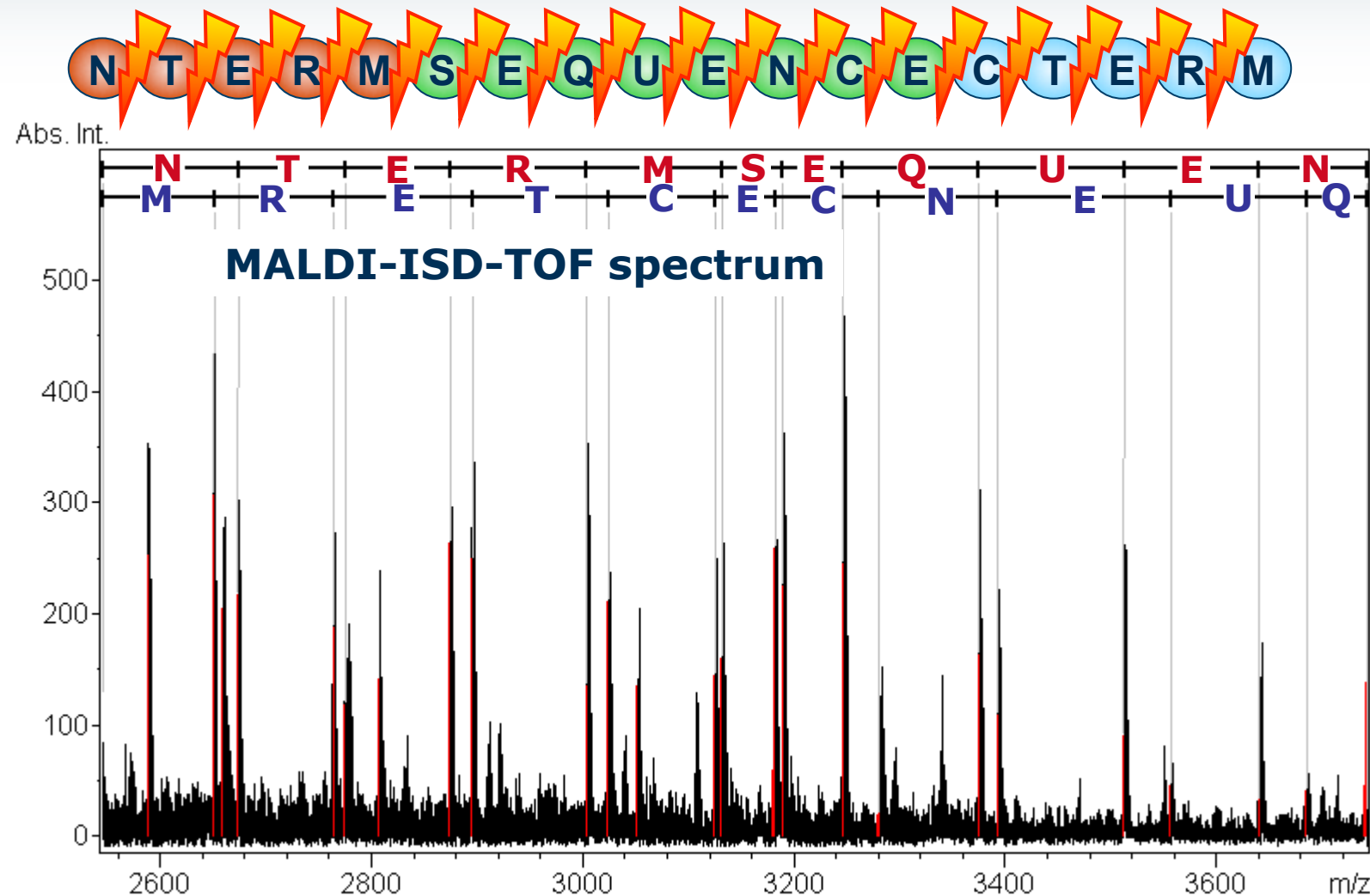


N T E R M S E Q U E N C E C T E R M



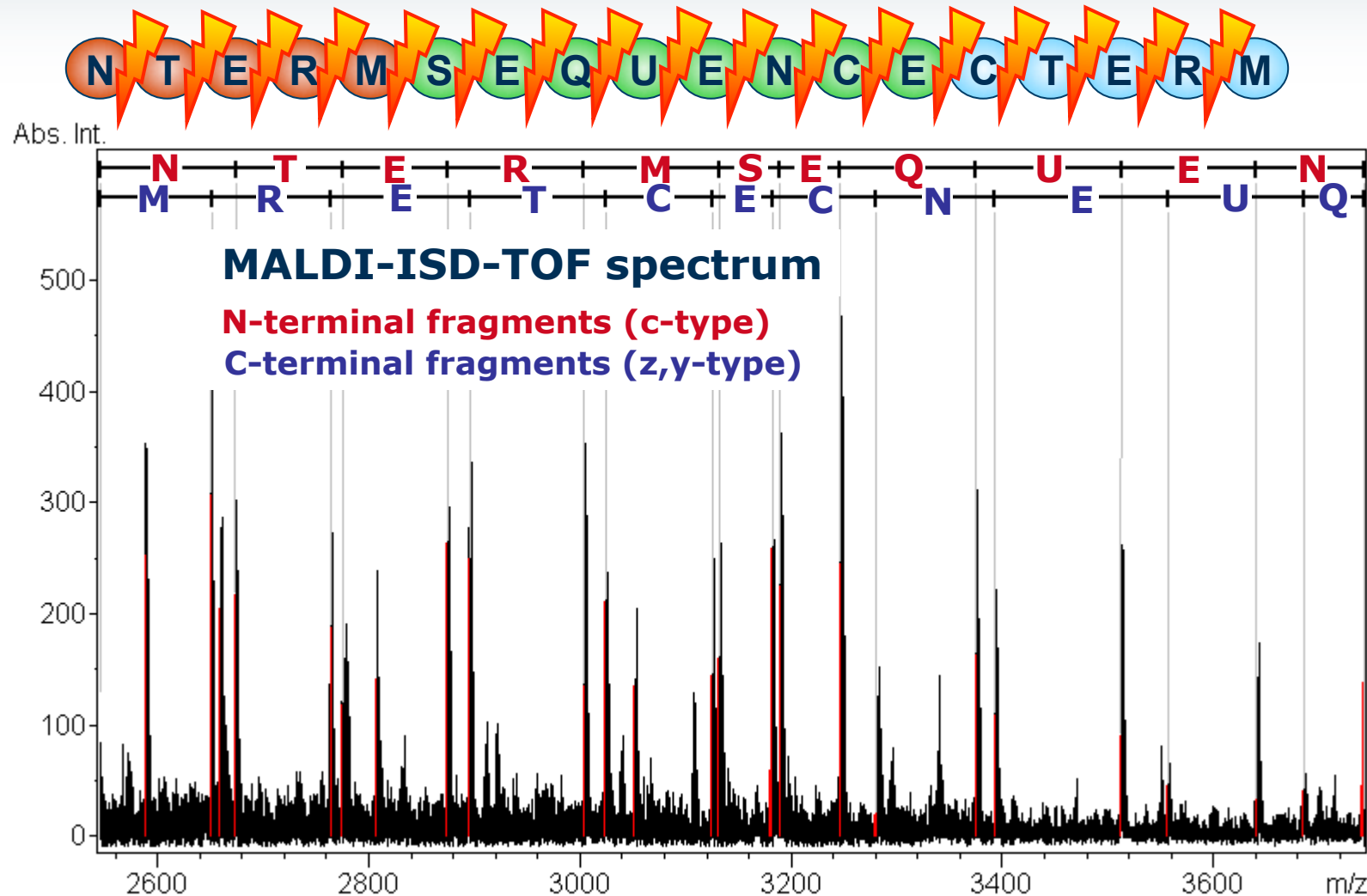
ultrafleXtreme:

MALDI top-down sequencing of intact proteins



ultrafleXtreme:

MALDI top-down sequencing of intact proteins





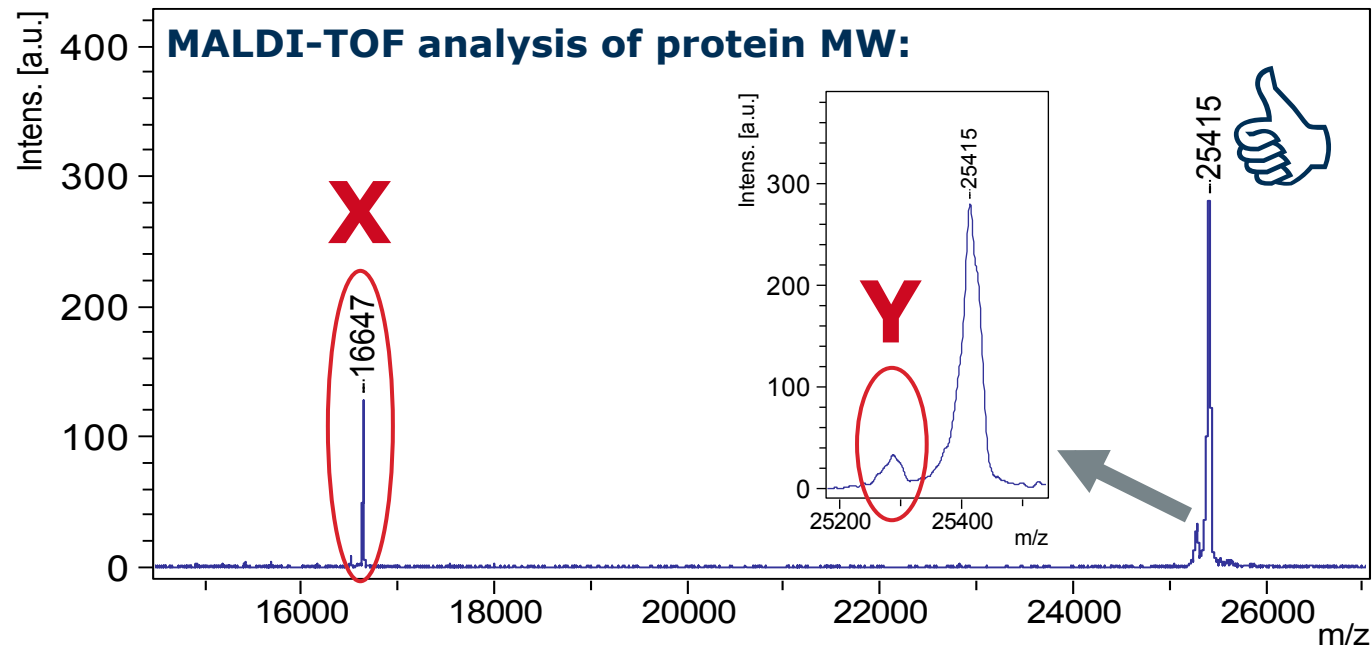
# ultrafleXtreme: Top-down sequencing of intact proteins



Characterization of by-products in a recombinant TAU protein  
(expected MW=25414Da)

(sample provided by Prof. M. Novak, Dr. B. Kovacech, Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava)

10	20	30	40	50	60	70							
MIATP	RGAAP	PGQKG	QANAT	RIPAK	TPPAP	KTPPS	SGEPP	KSGDR	SGYSS	PGSPG	TPGSR	SRTPS	LPTPP
80	90	100	110	120	130	140							
TREPK	KUAUU	RTPPK	SPSSA	KSRLQ	TAPUP	MPDLK	NUKSK	IGSTE	NLKHQ	PGGGK	UQIIN	KKLDL	SNUQS
150	160	170	180	190	200	210							
KCGSK	DNIKH	UPGGG	SUQIU	YKPVD	LSKVT	SKCGS	LGNIH	HKPGG	GQVEU	KSEKL	DFKDR	UQSKI	GSLDN
220	230	240											
ITHUP	GGGNK	KIETH	KLIFR	ENAKA	KTDHG	AE							



## MALDI top-down sequencing:

Characterization of minor components **X** and **Y**, resp.

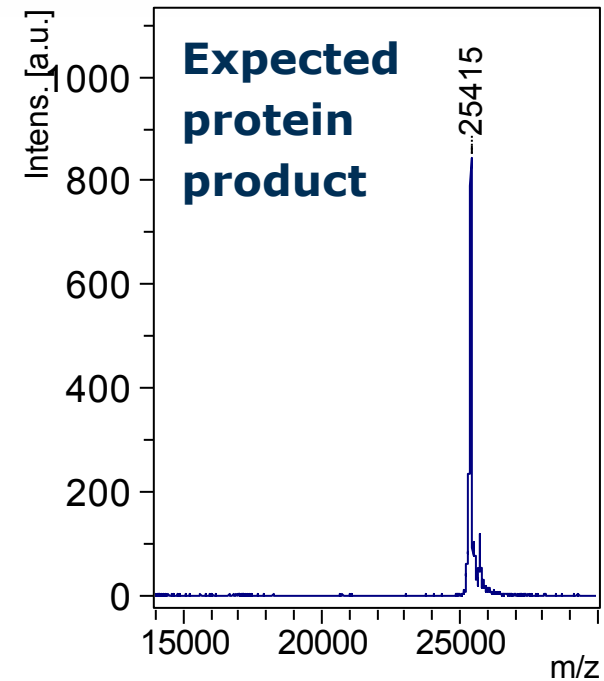
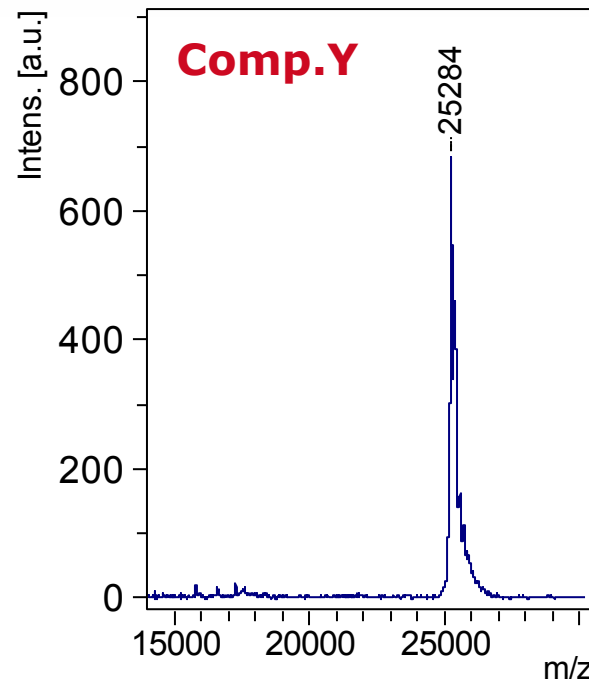
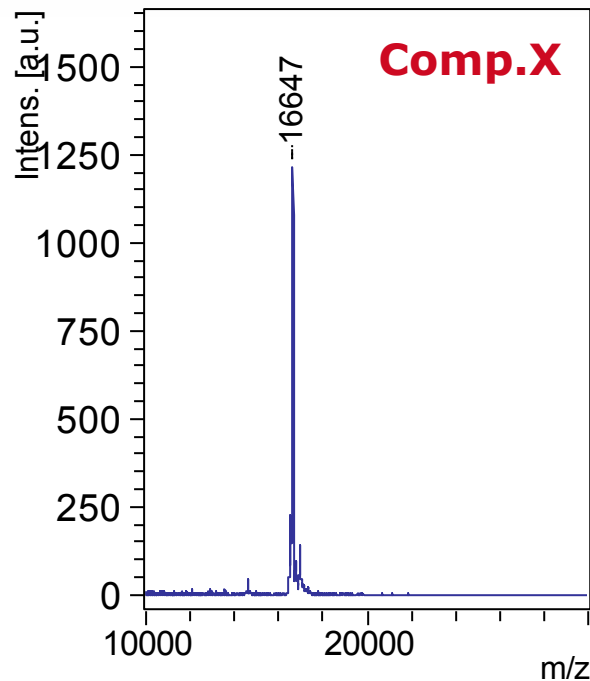
# ultrafleXtreme: Top-down sequencing of intact proteins



Characterization of by-products in a recombinant TAU protein  
(expected MW=25414Da)

(sample provided by Prof. M. Novak, Dr. B. Kovachech, Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava)

## Pre-fractionation by HPLC:



## MALDI top-down sequencing

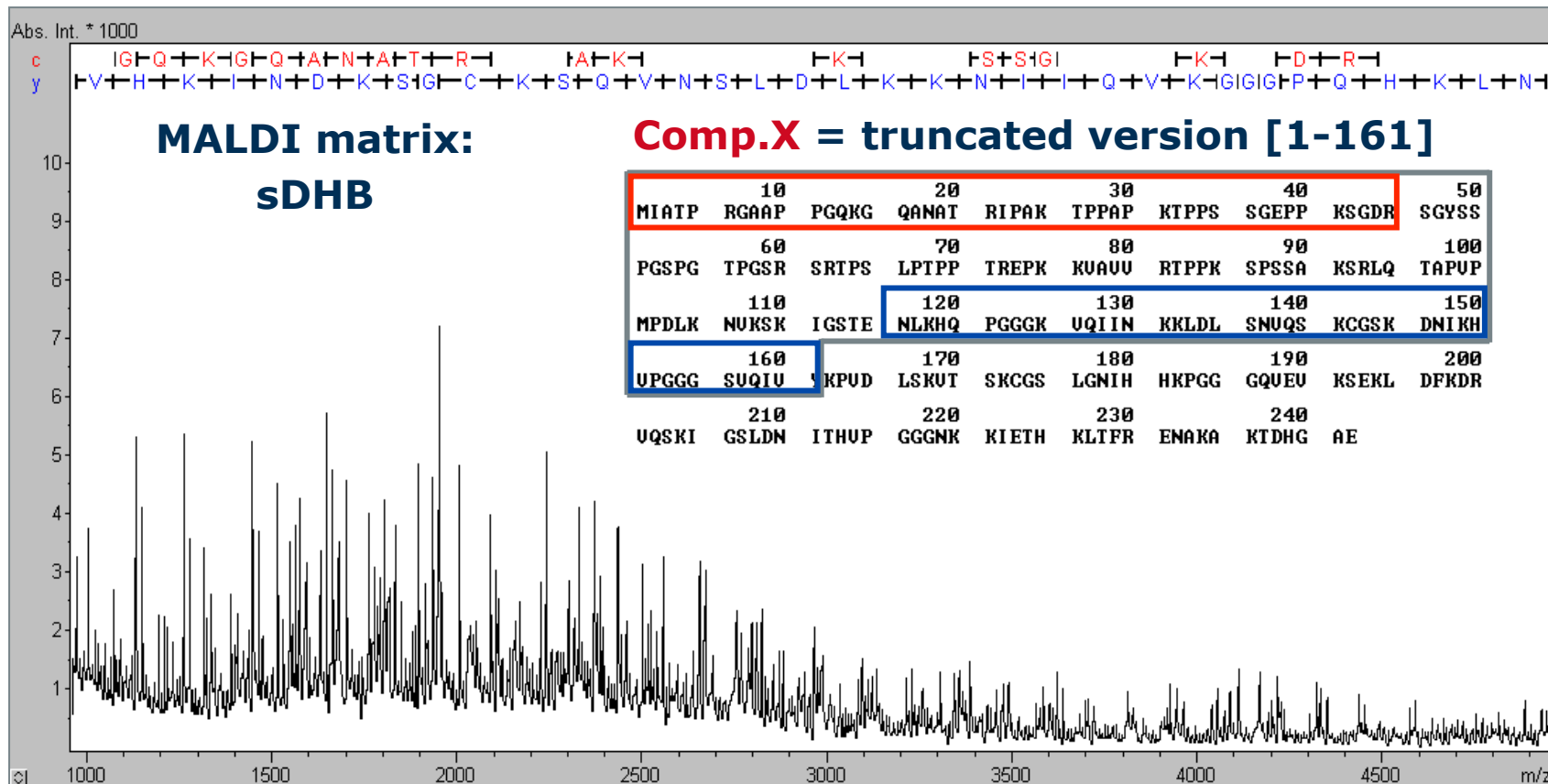
# ultrafleXtreme: Top-down sequencing of intact proteins



Characterization of by-products in a recombinant TAU protein  
(expected MW=25414Da)

(sample provided by Prof. M. Novak, Dr. B. Kovachech, Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava)

## MALDI-ISD-TOF spectrum of compound X (16647Da)



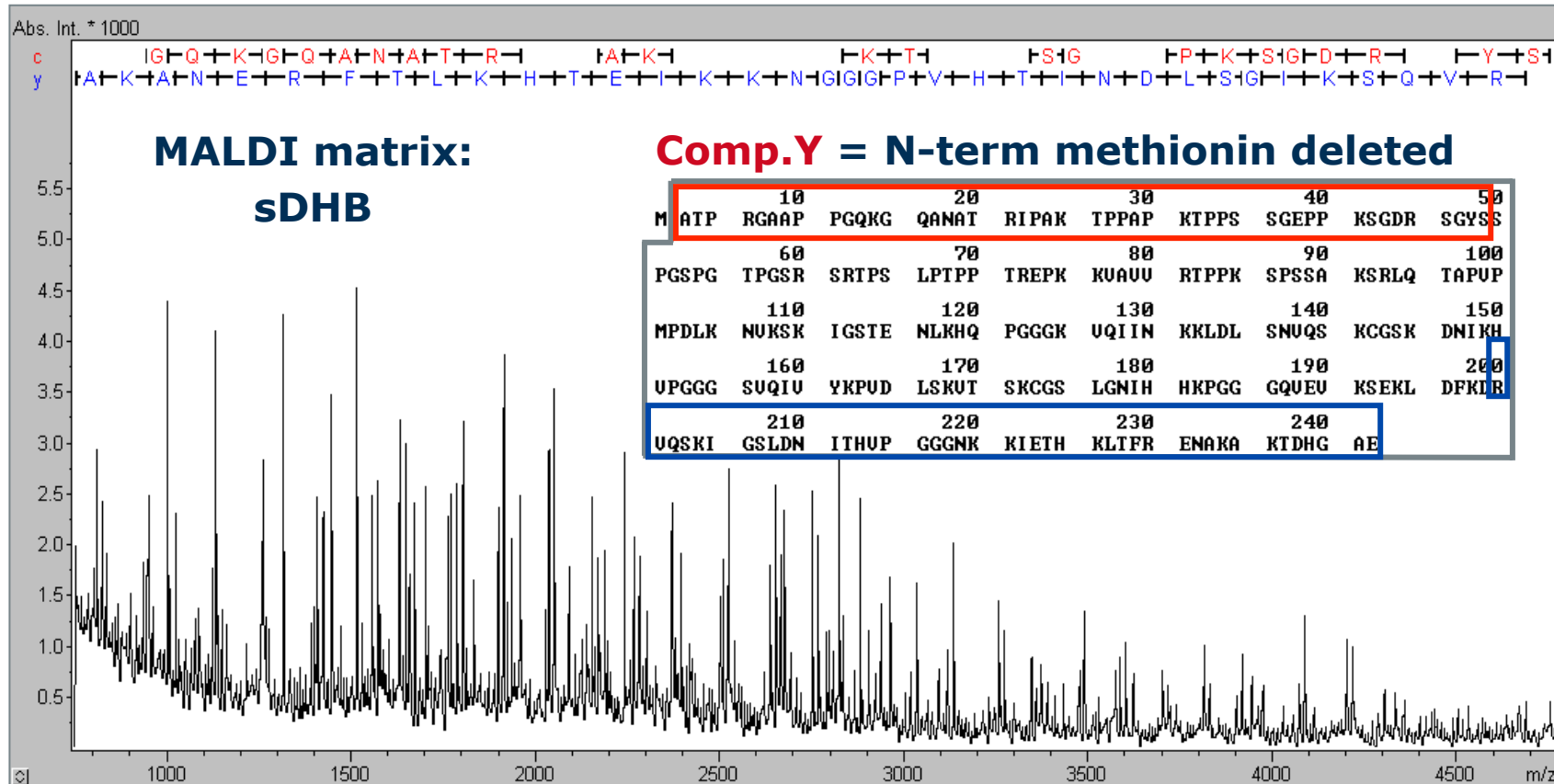
# ultrafleXtreme: Top-down sequencing of intact proteins



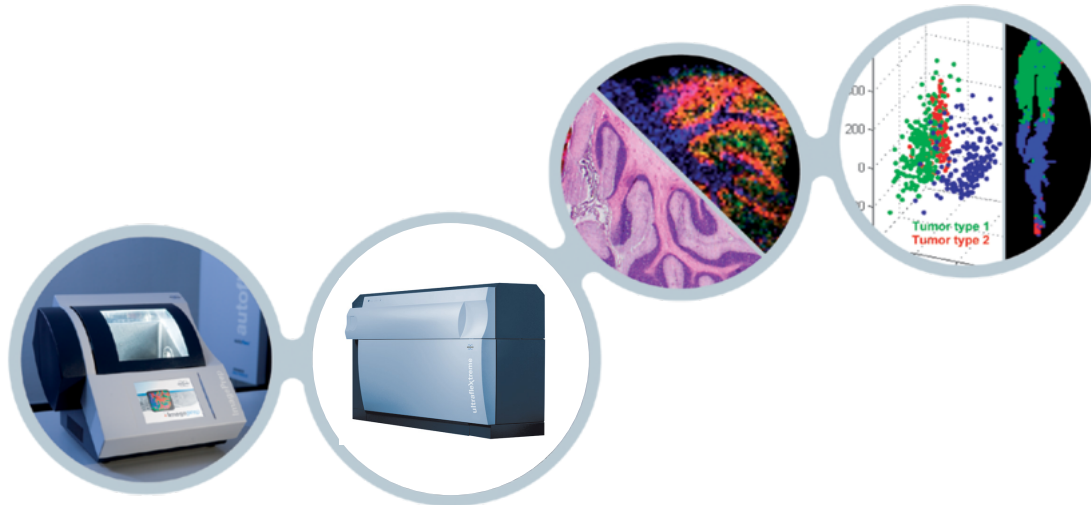
Characterization of by-products in a recombinant TAU protein  
(expected MW=25414Da)

(sample provided by Prof. M. Novak, Dr. B. Kovacech, Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava)

## MALDI-MS/MS spectrum of compound Y (25284Da)



ultrafleXtreme:  
Integrated part of Bruker's complete **MALDI Imaging** solution



**Ultimate performance in MALDI Imaging:**

- **20 $\mu$ m** spatial resolution (smartbeam-II)
  - True **kHz acquisition** speed
- Laser-based ion source **self-cleaning within 15min**

# Principle of MALDI Imaging



34

*M. Stoeckli et al. / Analytical Biochemistry 311 (2002) 33–39*

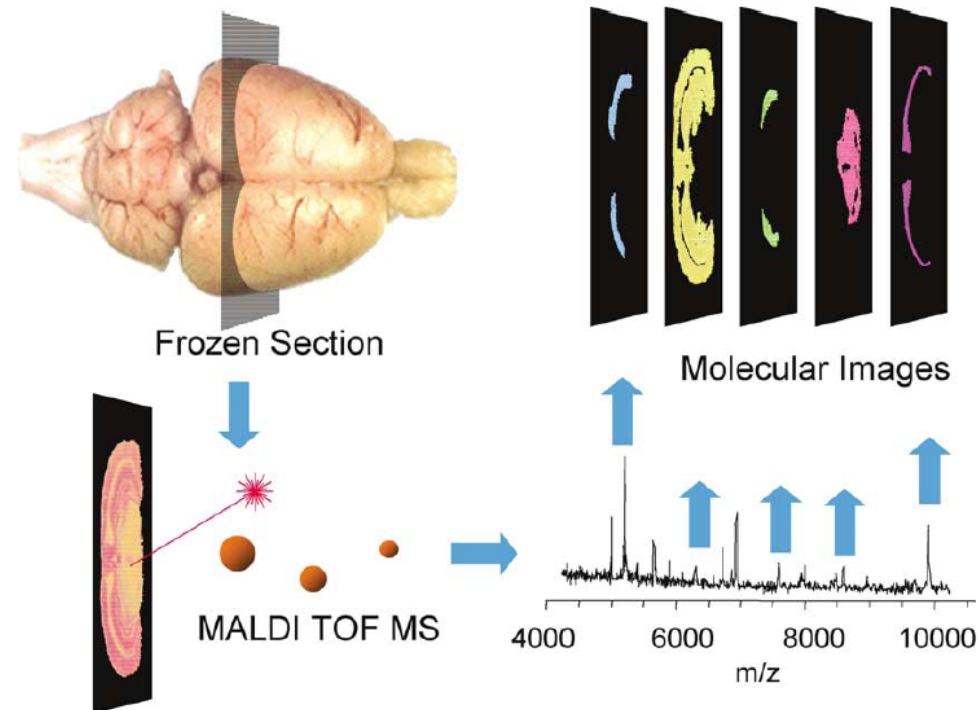
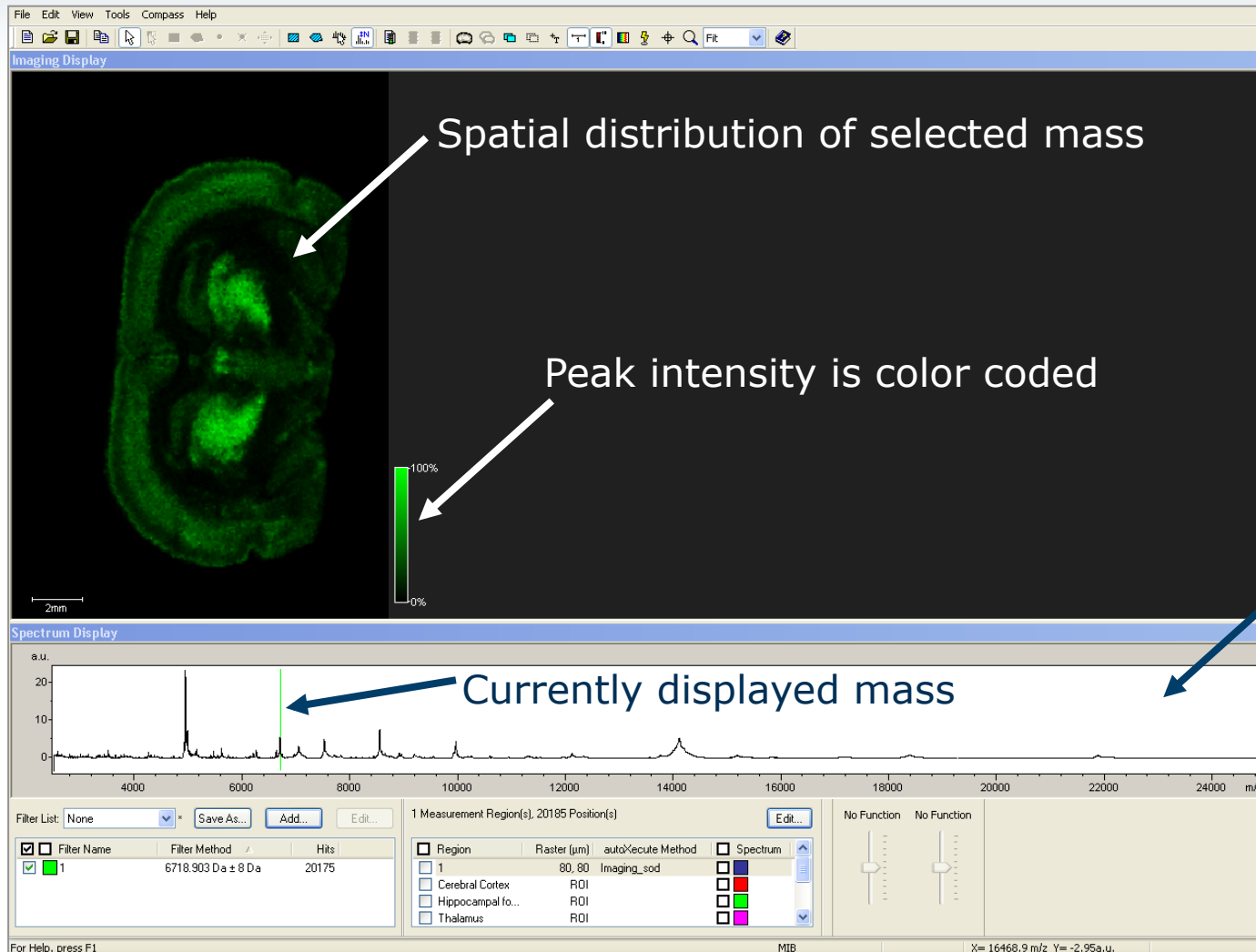


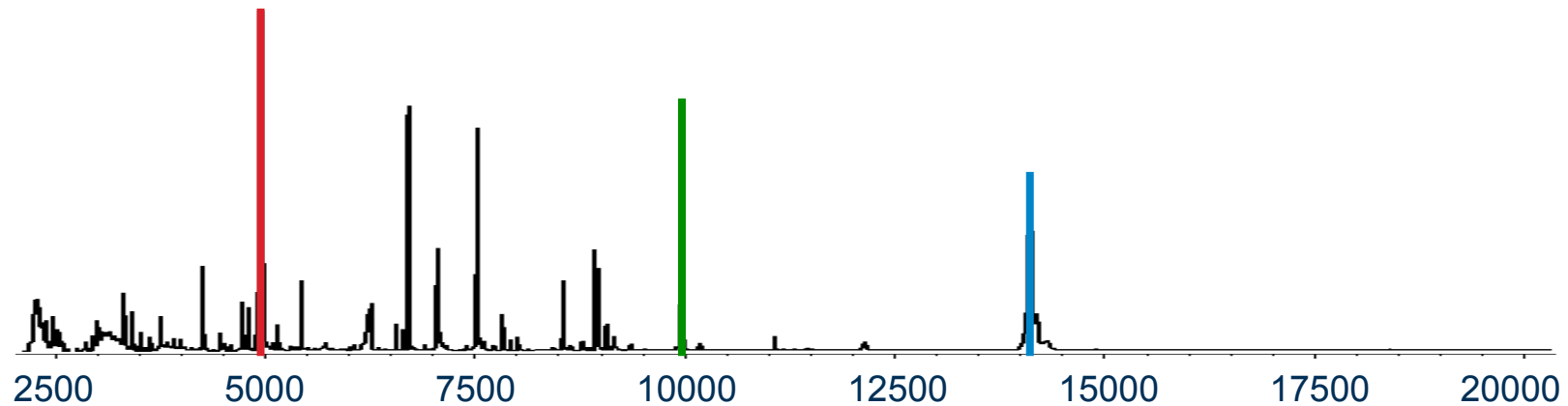
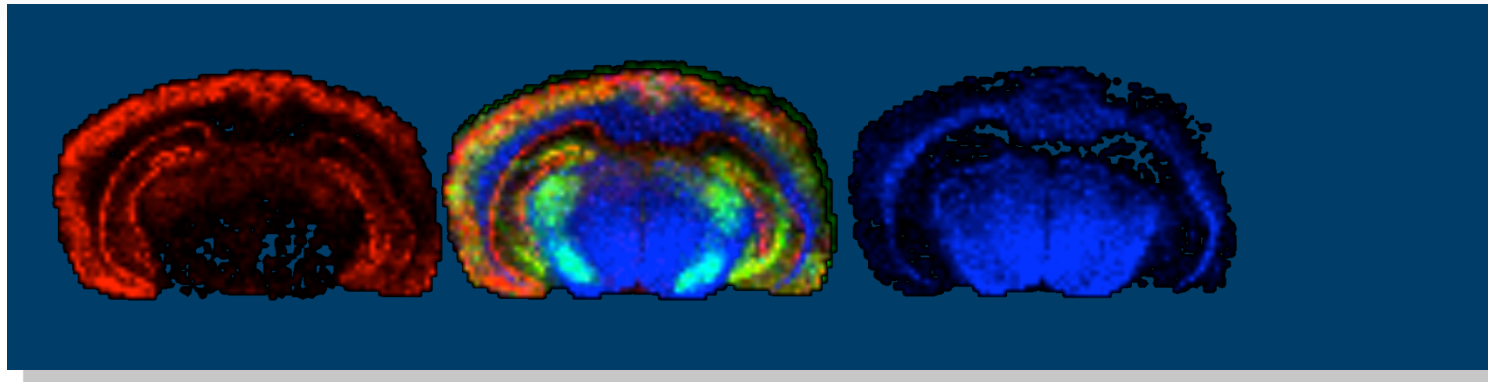
Fig. 1. MALDI MS imaging process: Cryostatic sections are fixed and coated with matrix, before they are analyzed in a TOF MS. Specific software is used for image acquisition and calculation of image data.

## Monitoring of Biomarker Tissue Distribution



Overall average spectrum





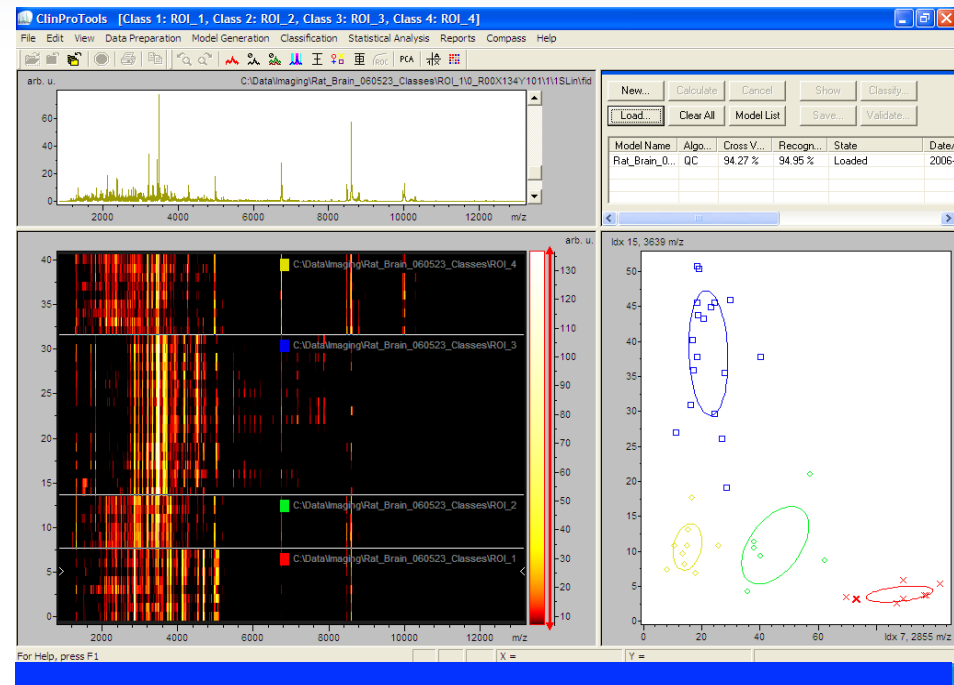
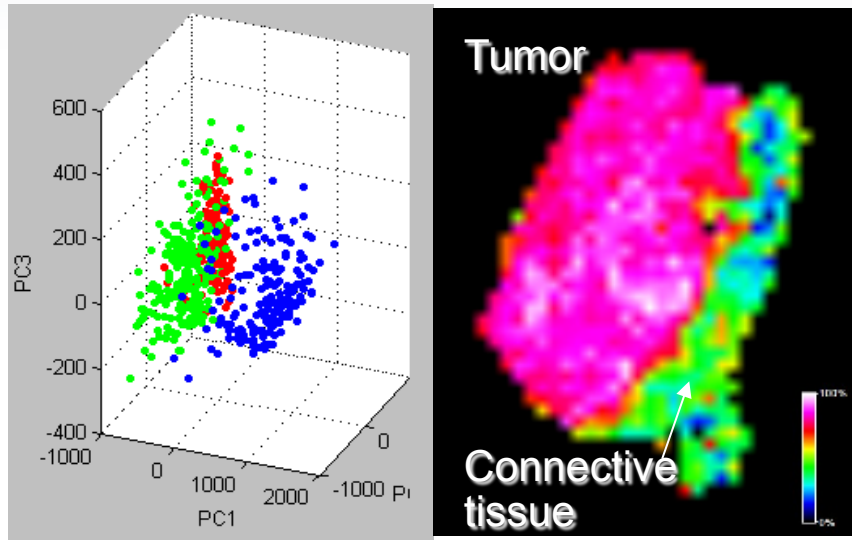
MALDI Imaging is a **multiplexing** technique!



ultrafleXtreme:

ClinproTools software:

Toolbox for statistical analysis of MALDI Imaging data



ClinproTools provides algorithms required for both **unsupervised** and **supervised** multivariate data analysis.

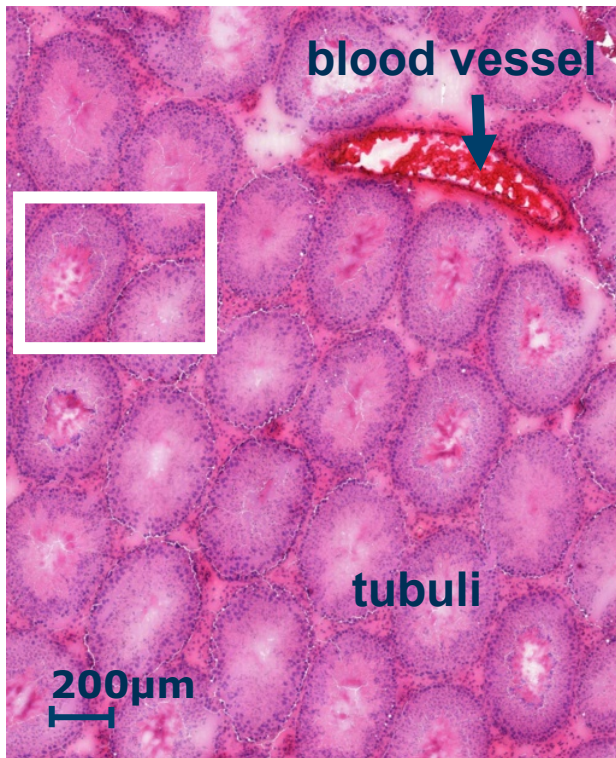
# ultrafleXtreme:

Ultimate performance in MALDI Imaging:

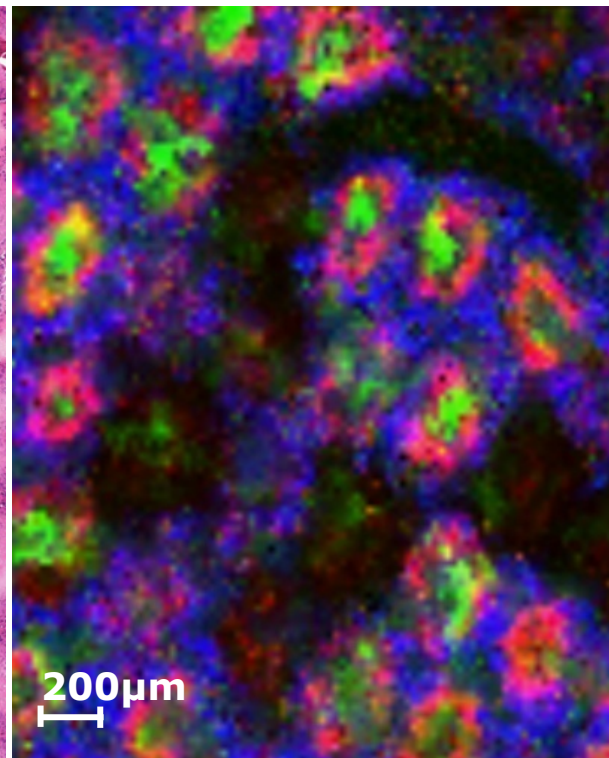
High resolution (**20 $\mu$ m**) imaging of rat testis performed @ **khz speed**



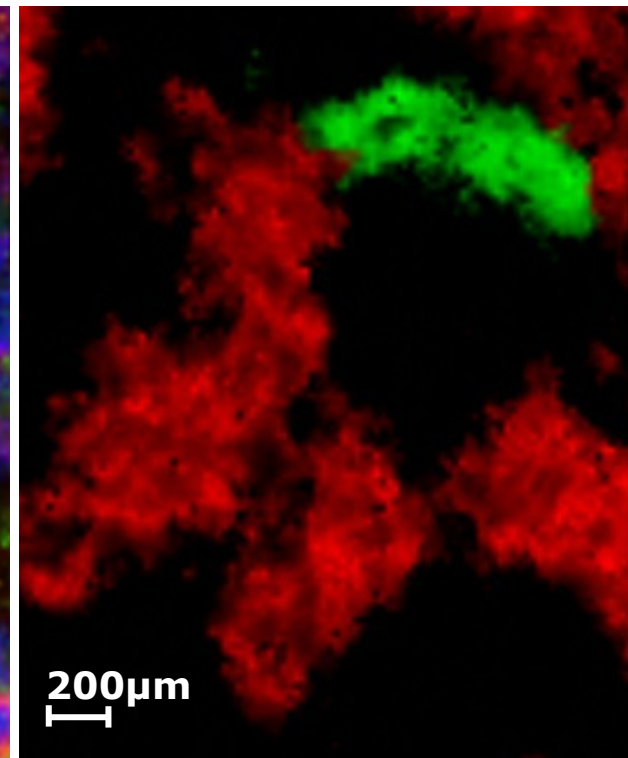
## Histological image



## MALDI images



**3457 Da** **5455 Da** **10261 Da**



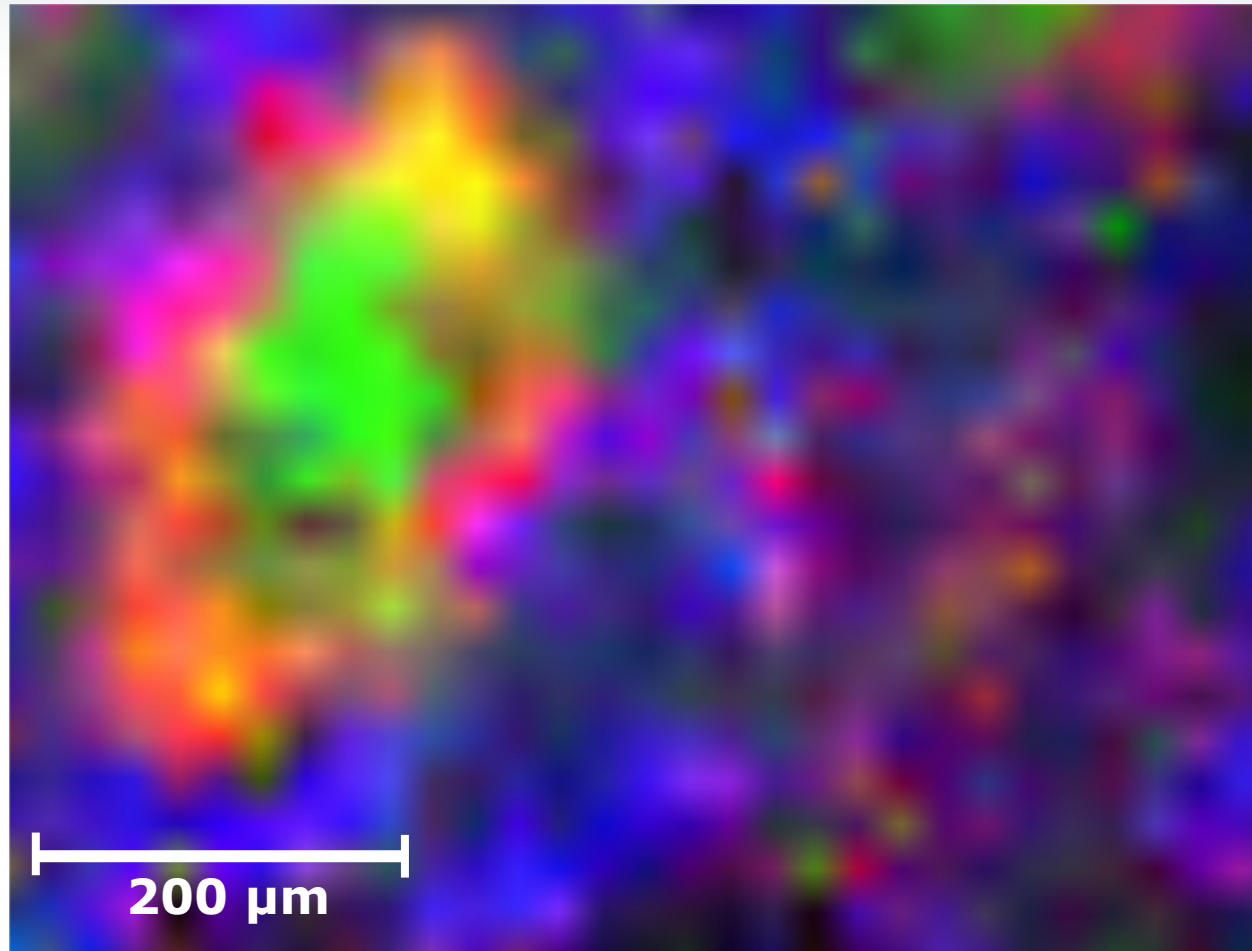
**6263 Da** **2396 Da**

Sample by courtesy of Charles Pineau,  
Rennes, France

# ultrafleXtreme:

Ultimate performance in MALDI Imaging:

High resolution (**20 $\mu$ m**) imaging of rat testis performed @ **khz speed**



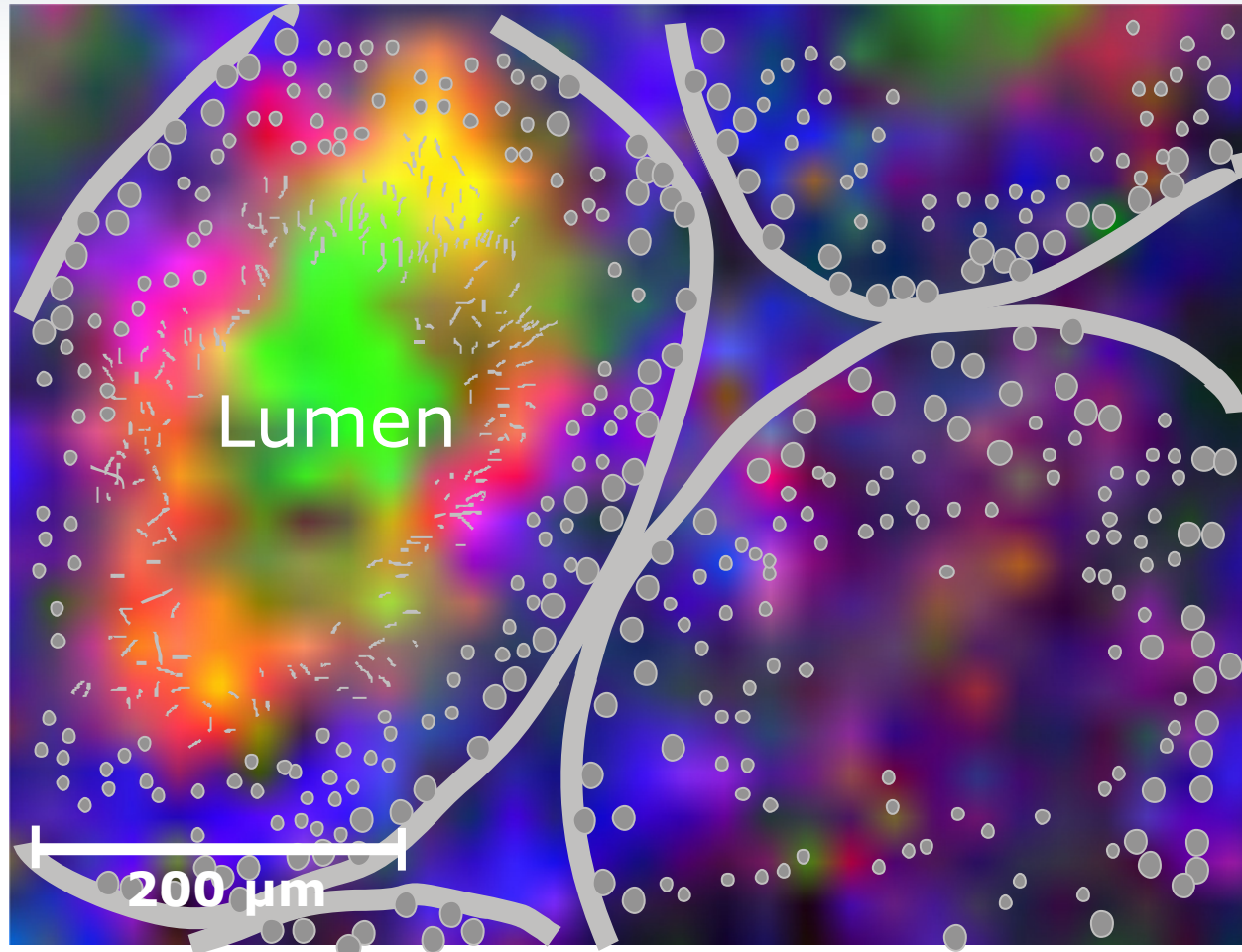
3457 Da 5455 Da 10261 Da



# ultrafleXtreme:

Ultimate performance in MALDI Imaging:

High resolution (**20 $\mu$ m**) imaging of rat testis performed @ **khz speed**



3457 Da 5455 Da 10261 Da



ultrafleXtreme:

**TLC/HPTLC-MALDI** coupling

for compound detection

&

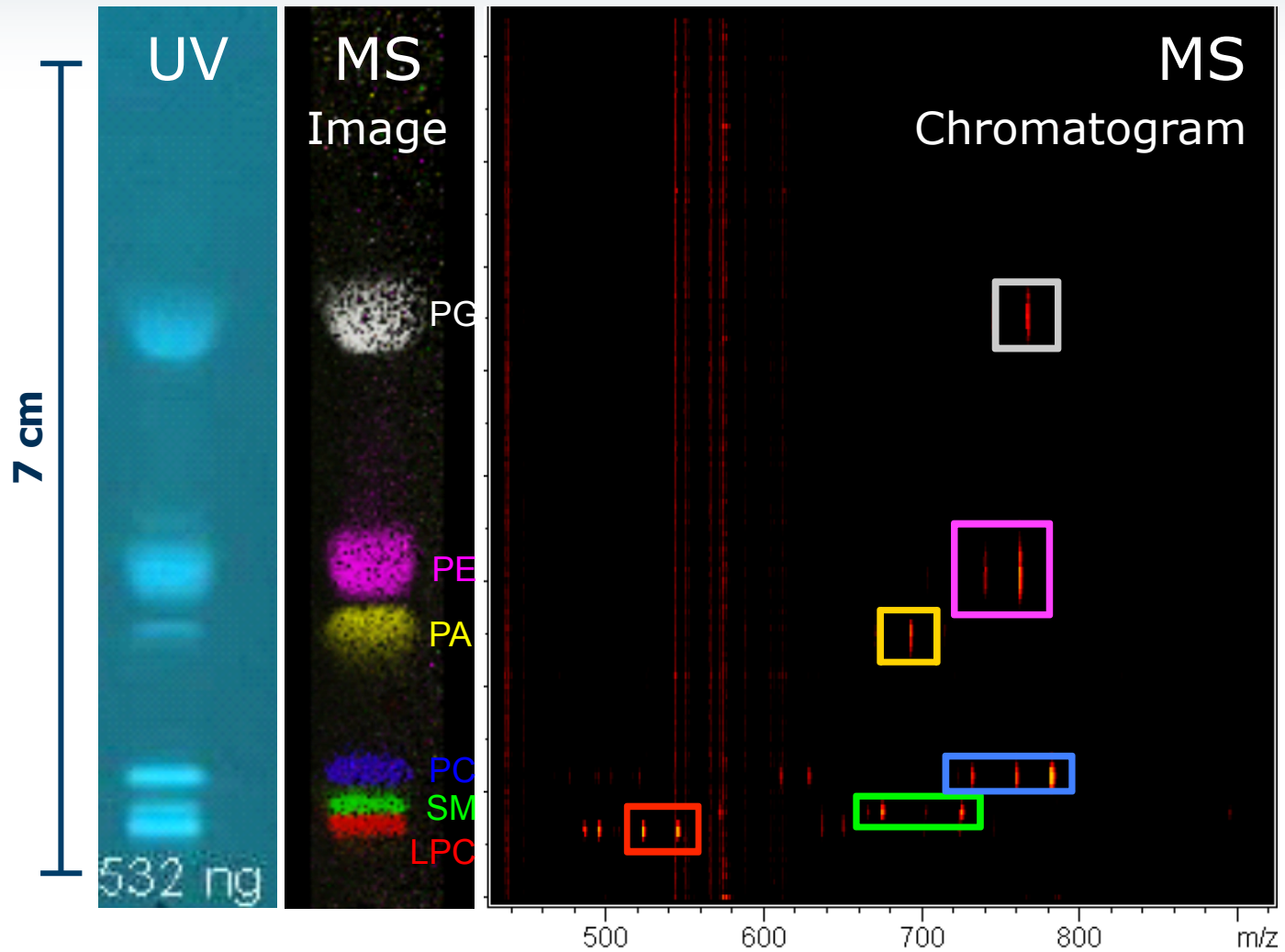
structural analysis

(e.g. of lipids)



# ultrafleXtreme:

HPTLC-MALDI coupling for lipid analysis: 532ng/band of a standard lipid mixture

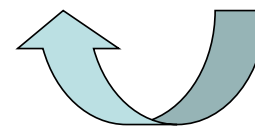
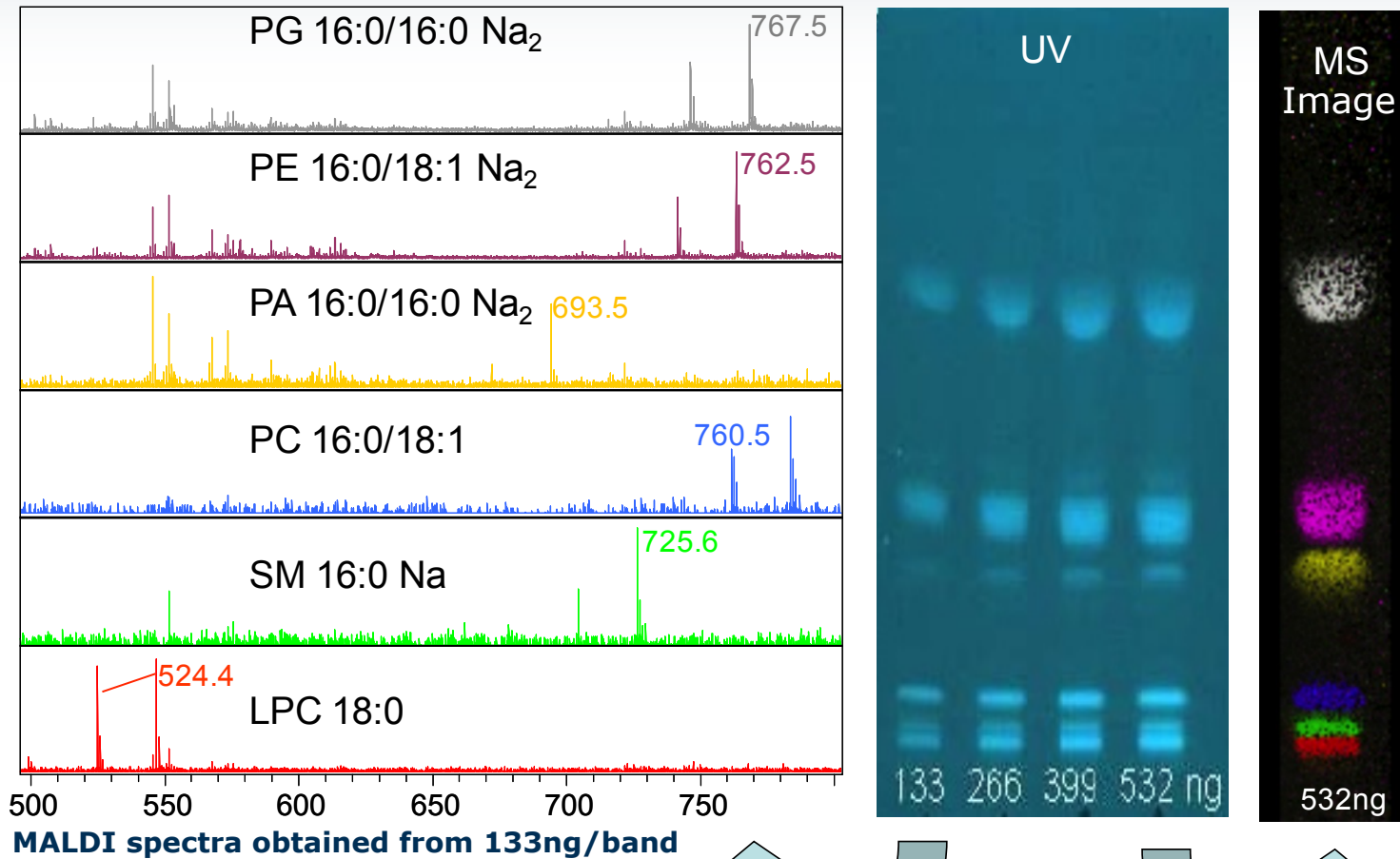


ultrafleXtreme:

HPTLC-MALDI coupling for lipid analysis:

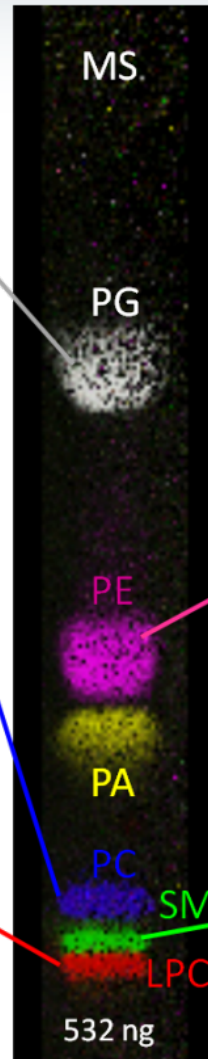
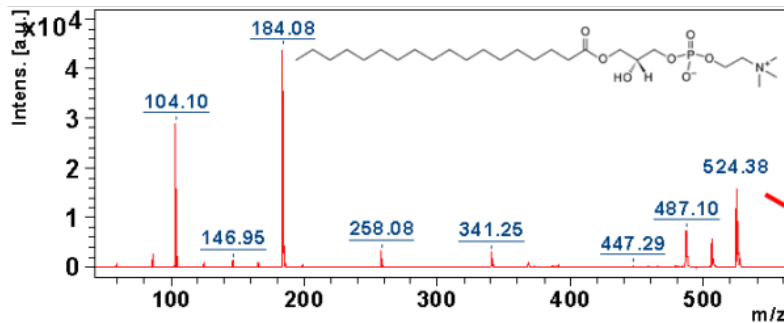
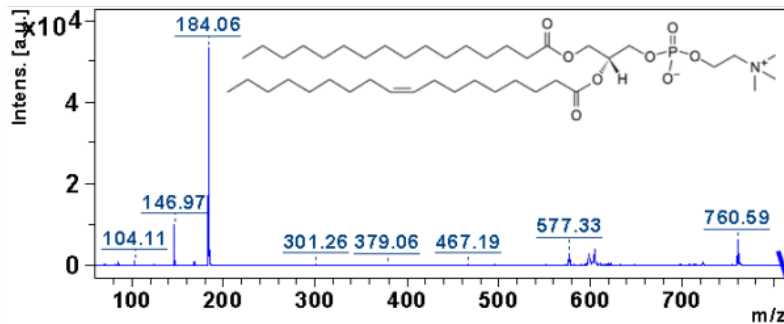
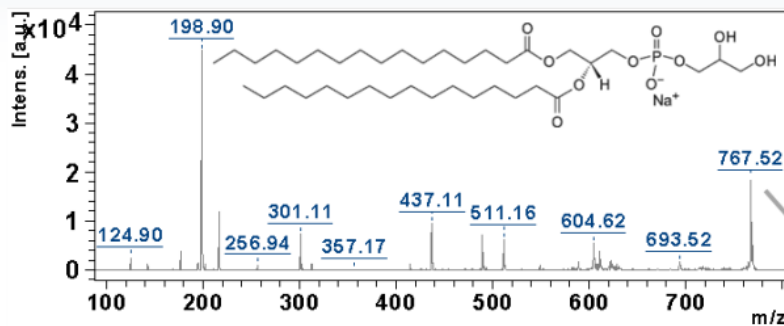


Sensitivity shown for 133 - 532ng/band of a standard lipid mixture

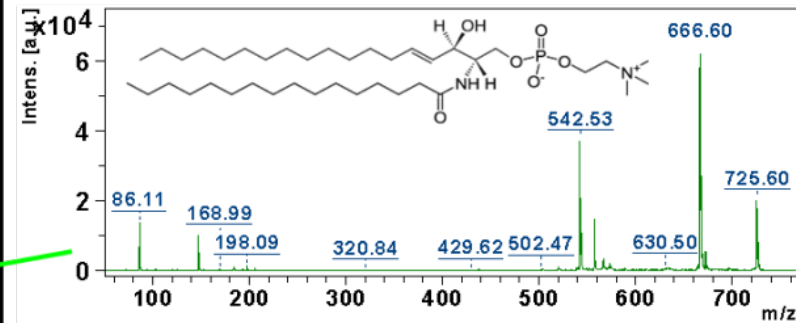
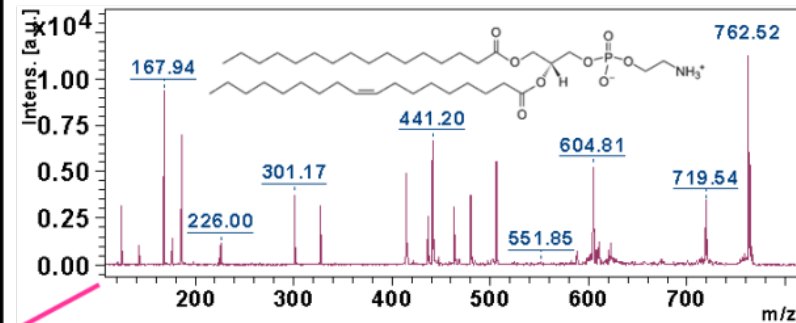


# ultrafleXtreme:

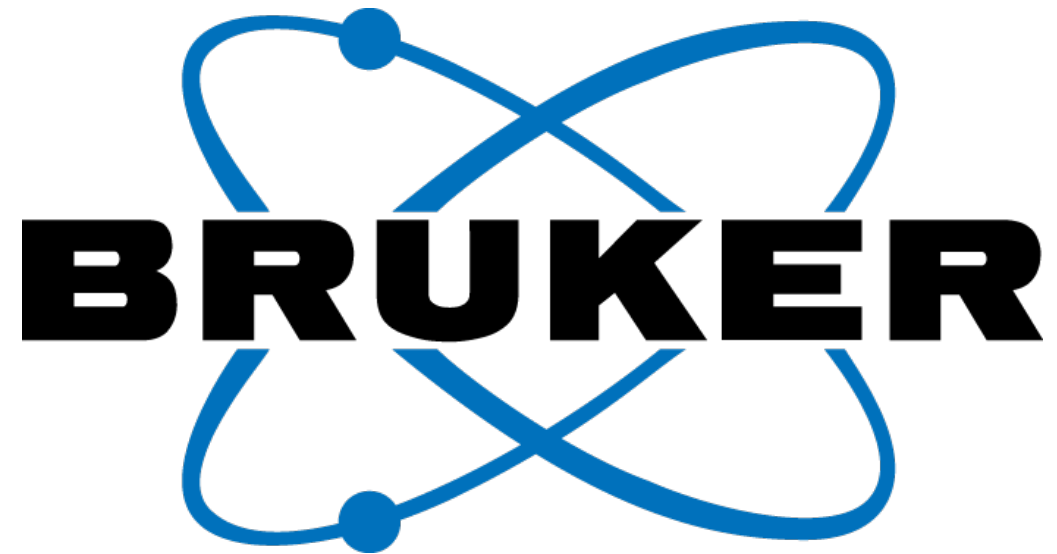
## HPTLC-MALDI coupling for lipid analysis: Structural elucidation by MS/MS



MS/MS Analyses right off  
the HPTLC plate







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