

Laboratory Techniques and Medical Device Approvals

Mass spectrometry for biomolecules II

Applications

Dr. Anne Zeck,
Natural and Medical Sciences Institute (NMI)
at the University of Tübingen
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Overview

- Mass spectrometry of proteins
 - Applications
- Mass spectrometry of peptides
 - Application
 - Fragmentation
- Mass spectrometry of small molecules
 - Glycans, amino acids, chemotherapeutics
- MS fingerprint of bacteria

Mass spectrometry of proteins

Determination of the intact protein molecular mass from multiple-charge electrospray mass spectra 1

M = molecular mass of the protein (**unknown**)

z = charge of the protein (**unknown**)

$M[H]$ is the rel. mass of a proton = 0.99862349 (~1)

$$\left(\frac{m}{z}\right) = \frac{M + z * M[H]}{z} \quad \rightarrow \quad M = z * \left(\left(\frac{m}{z}\right) - M[H]\right)$$

(m/z) = value measured by the mass spectrometer (**known**)

Two adjacent peaks in the spectrum of a protein have $(m/z)_1$ and $(m/z)_2$ where

$$z_1 = z_2 + 1$$

Mass spectrometry of proteins

Determination of the intact protein molecular mass from multiple-charge electrospray mass spectra 2

$$\left(\frac{m}{z}\right)_1 = \frac{M + z_1 * M[H]}{z_1}$$

$$\left(\frac{m}{z}\right)_2 = \frac{M + z_2 * M[H]}{z_2}$$

$z_1 = z_2 + 1$ for adjacent m/z peaks

$$M = \frac{\left(\left(\frac{m}{z}\right)_1 - M[H]\right) * \left(\left(\frac{m}{z}\right)_2 - M[H]\right)}{\left(\left(\frac{m}{z}\right)_2 - \left(\frac{m}{z}\right)_1\right)}$$

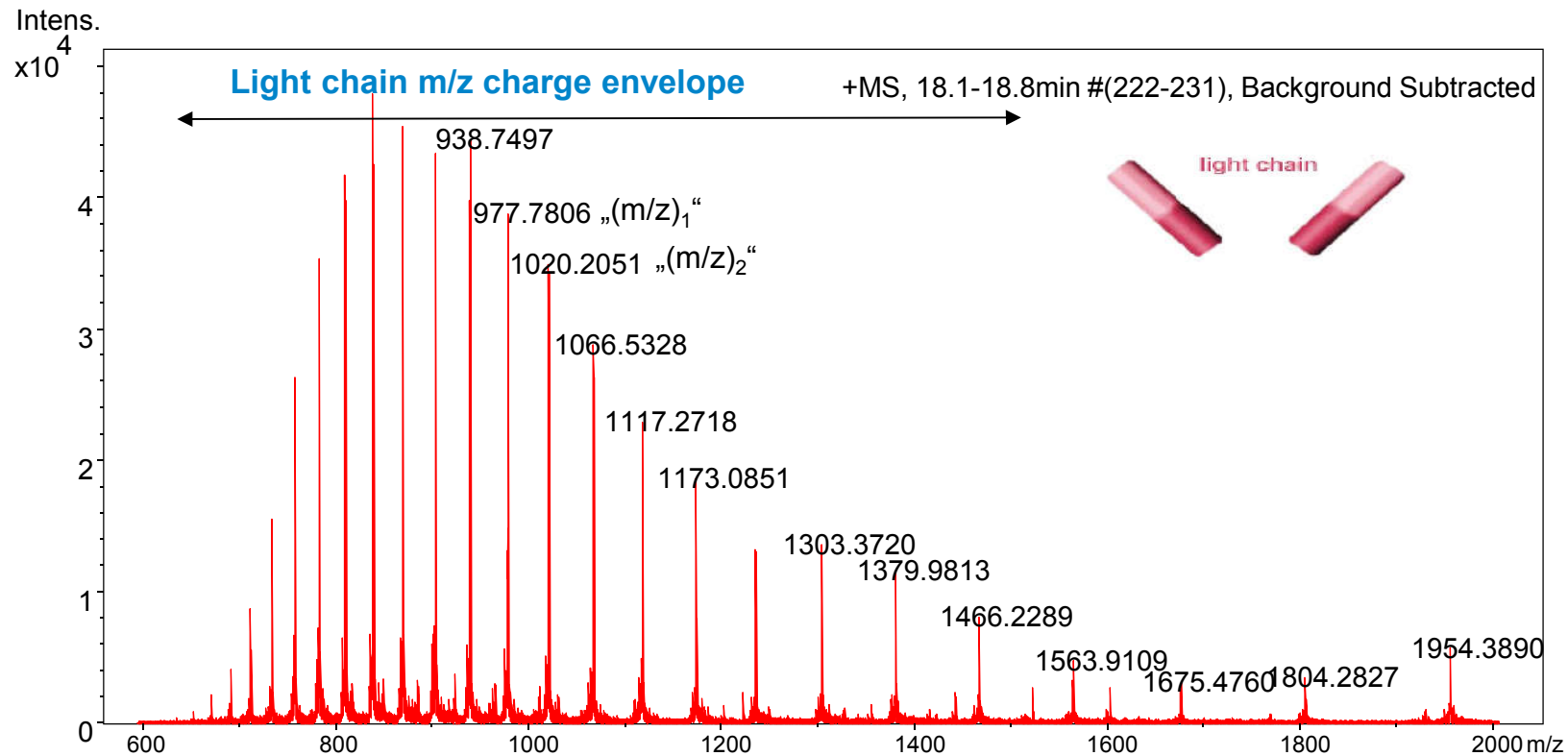
$$M \sim \frac{\left(\left(\frac{m}{z}\right)_1 - 1\right) * \left(\left(\frac{m}{z}\right)_2 - 1\right)}{\left(\left(\frac{m}{z}\right)_2 - \left(\frac{m}{z}\right)_1\right)}$$

<http://www.sepscience.com/Information/Archive/MS-Solutions/247-/MS-Solutions-16-Determination-of-Intact-Protein-Molecular-Mass--from-MultipleCharge-Electrospray-Mass-Spectra>

Mass spectrometry of proteins

ESI spectrum of the light chain of an antibody (25 kDa)

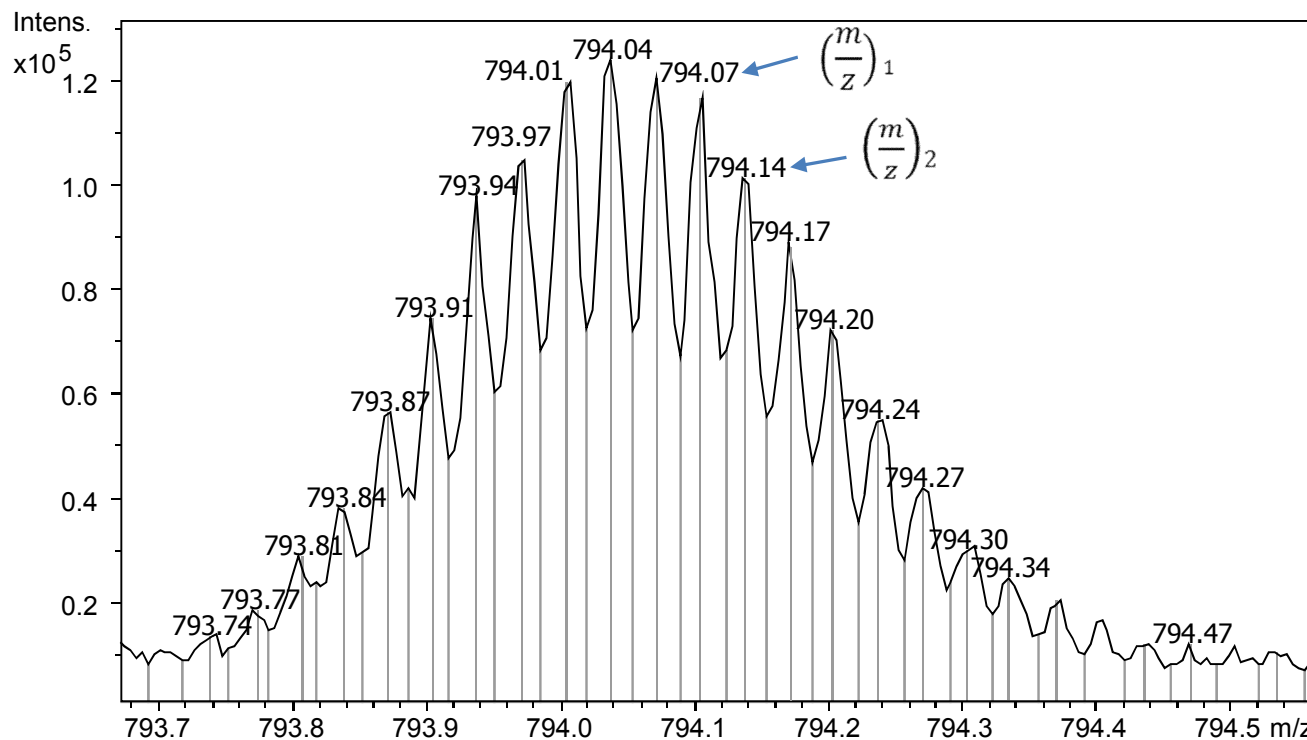
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charge deconvolution $z=...$

Mass spectrometry of proteins

Determination of the intact protein molecular mass from *isotopically resolved* mass spectra



$$\left(\frac{m}{z}\right)_1 = \frac{M + z \cdot M[H]}{z}$$

$$\left(\frac{m}{z}\right)_2 = \frac{M + \mathbf{1} + z \cdot M[H]}{z}$$



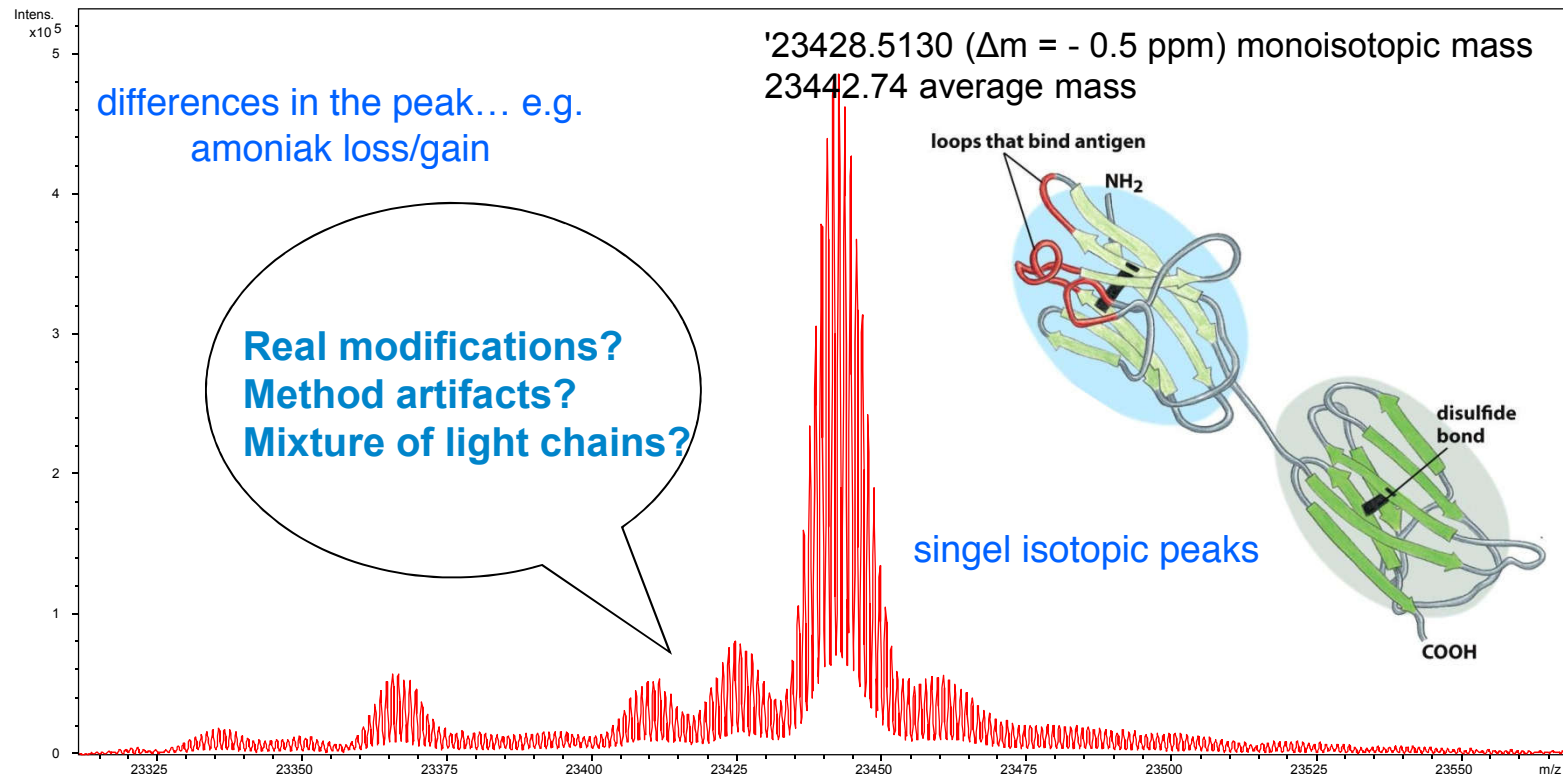
$$z = \frac{1}{\left(\left(\frac{m}{z}\right)_2 - \left(\frac{m}{z}\right)_1\right)}$$

$$M_{aver.} \sim \frac{\left(\frac{m}{z}\right)_{\text{max Intensity}}}{z}$$

All peaks have the same charge!
They differ in the amount of carbon atoms with mass 13.
 $\Delta M(\text{C}^{13} - \text{C}^{12}) = \mathbf{1}$

Mass spectrometry of proteins

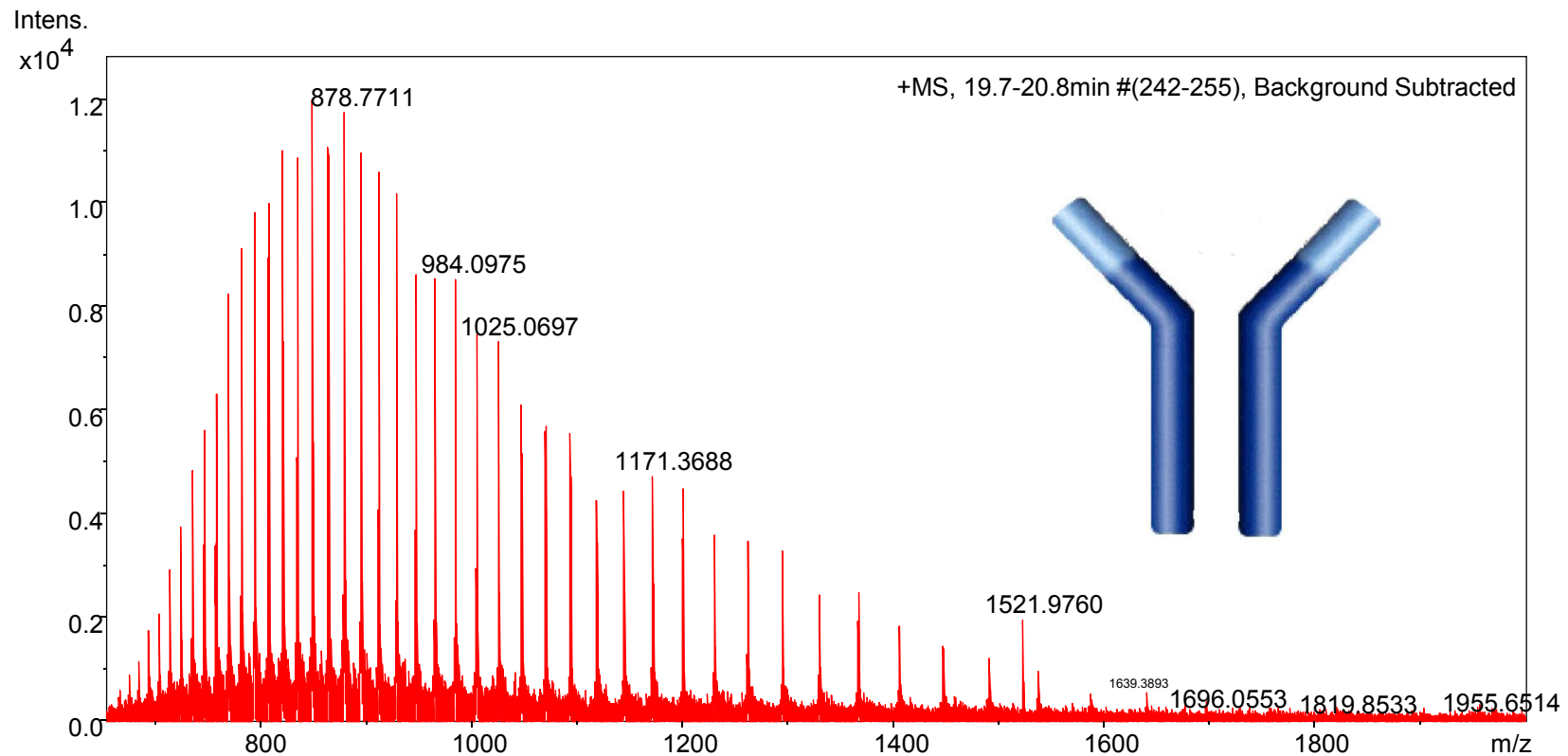
Exact mass; modifications: deconvoluted spectrum



- Isotopical resolution of Ab LC with FT-ICR, Orbitrap and modern Q-TOF instruments
- ➔ monoisotopic mass can be determined
- Deamidation ($\Delta m = 1$ Da) can be detected at the level of light chain !

Mass spectrometry of proteins:

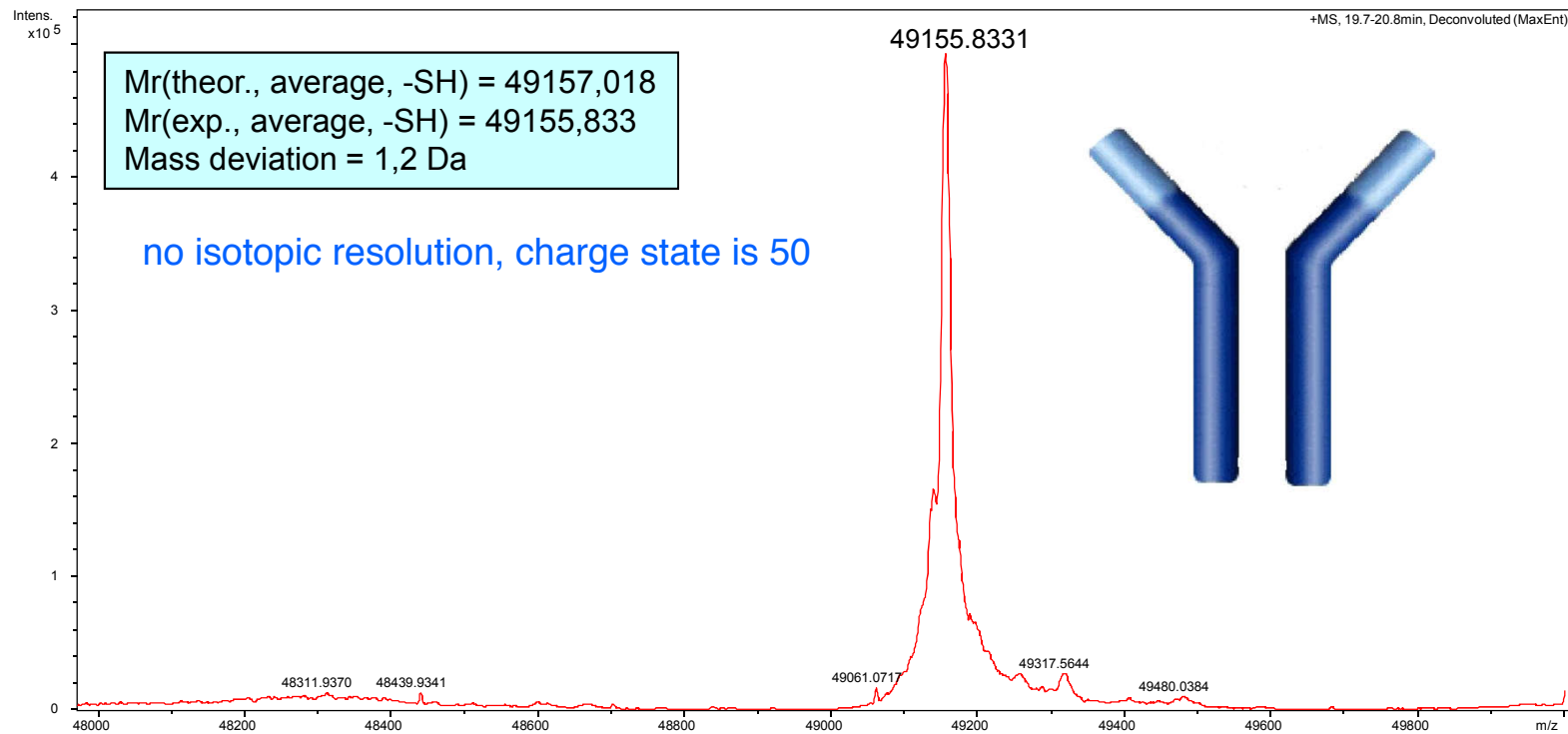
ESI spectrum heavy chain of an antibody (50 kDa)



Signal density shows a highly charged protein.
~ 50 charges per molecule

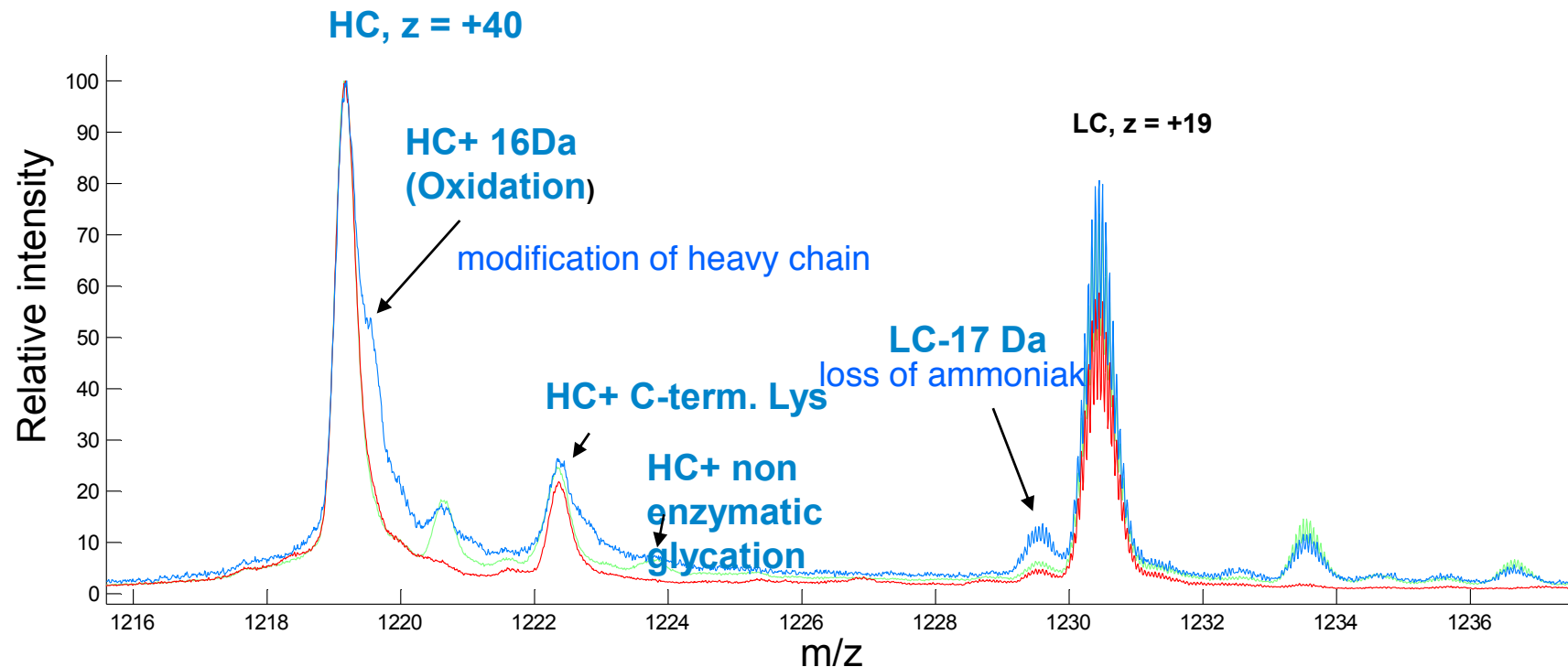
Mass spectrometry of proteins

Charge deconvoluted heavy chain of an antibody



Mass spectrometry of proteins

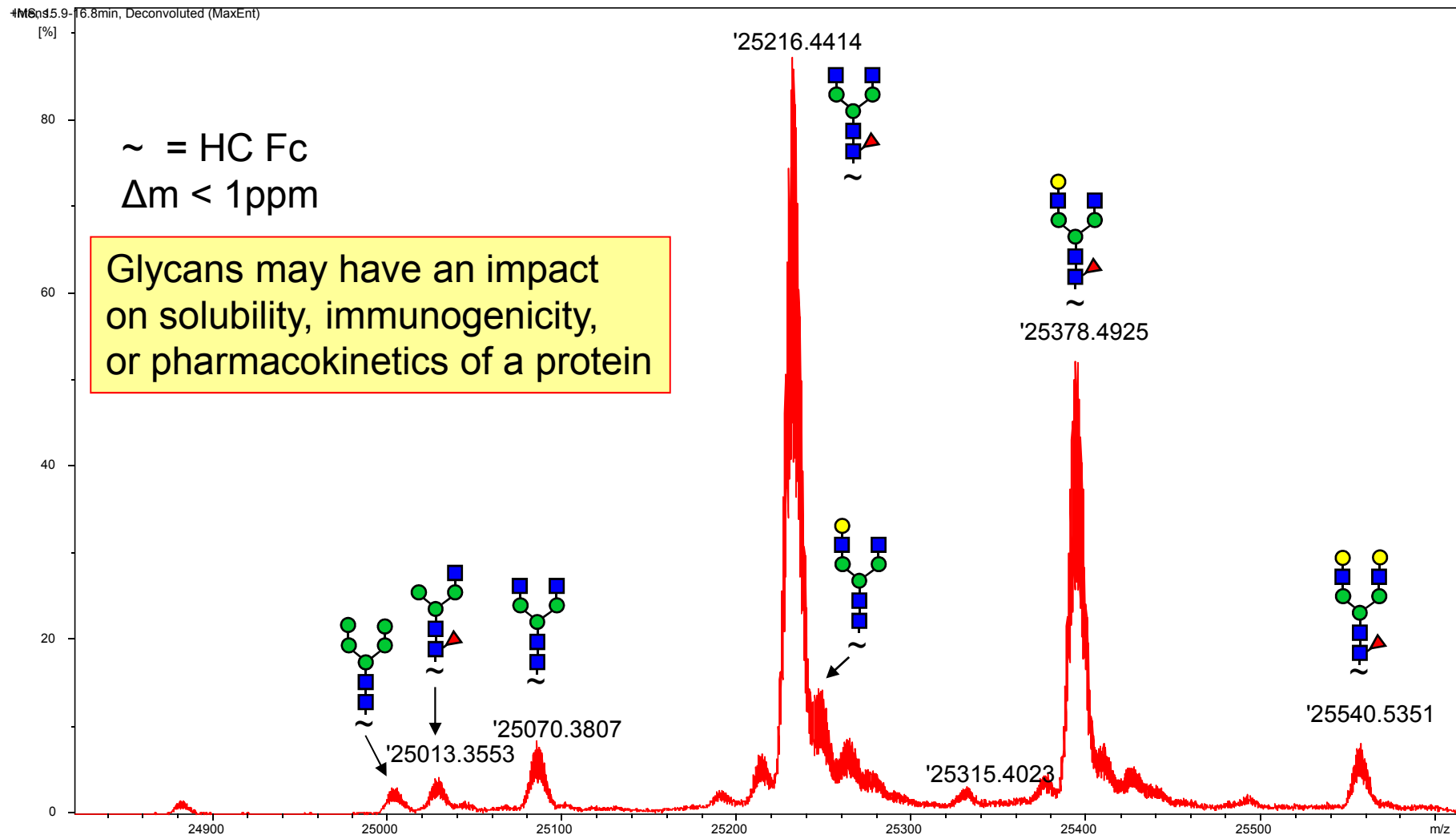
Detection of protein degradation



- ~ 30% oxidation of the heavy chain & and a slight increase in LC-17 Da for the light chain after storage for 6 weeks at 40 ° C in histidine formulation

Mass spectrometry of proteins

Determination of glycosylation

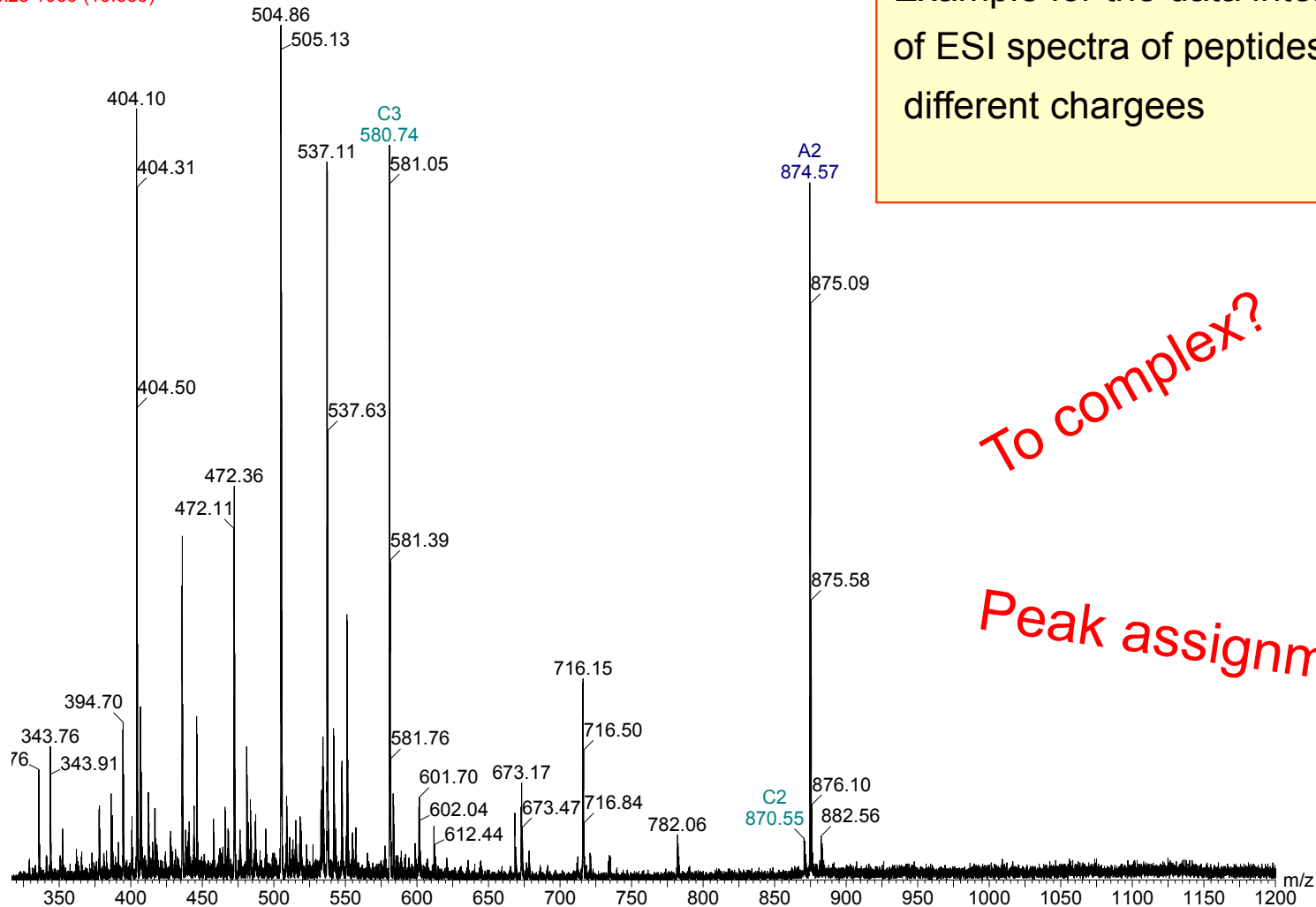


ESI Mass spectrometry of peptide mixtures

Direct infusion into mass spec

gag supermatrix25 1069 (19.659)

10



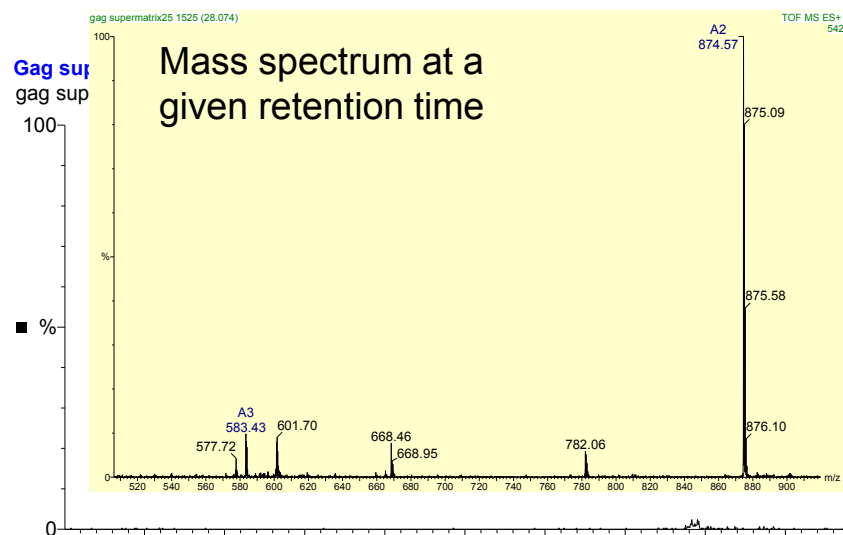
Example for the data interpretation of ESI spectra of peptides with different charges

To complex?

Peak assignment?

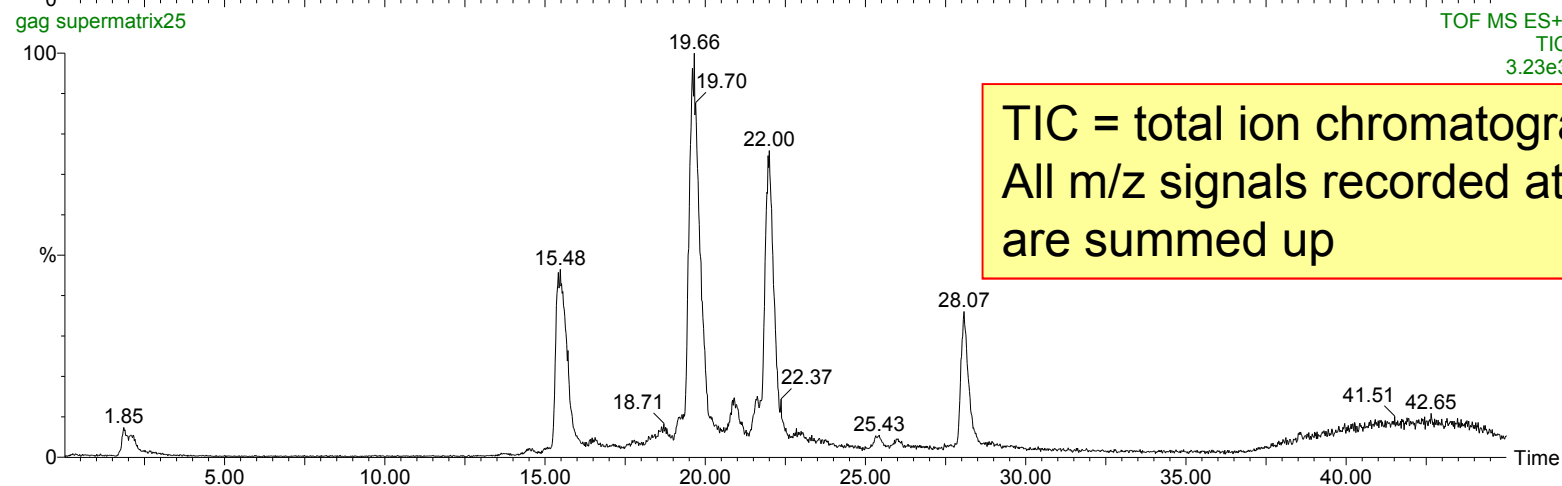
ESI mass spectrometry of peptide mixtures

HPLC separation prior to mass spec detection



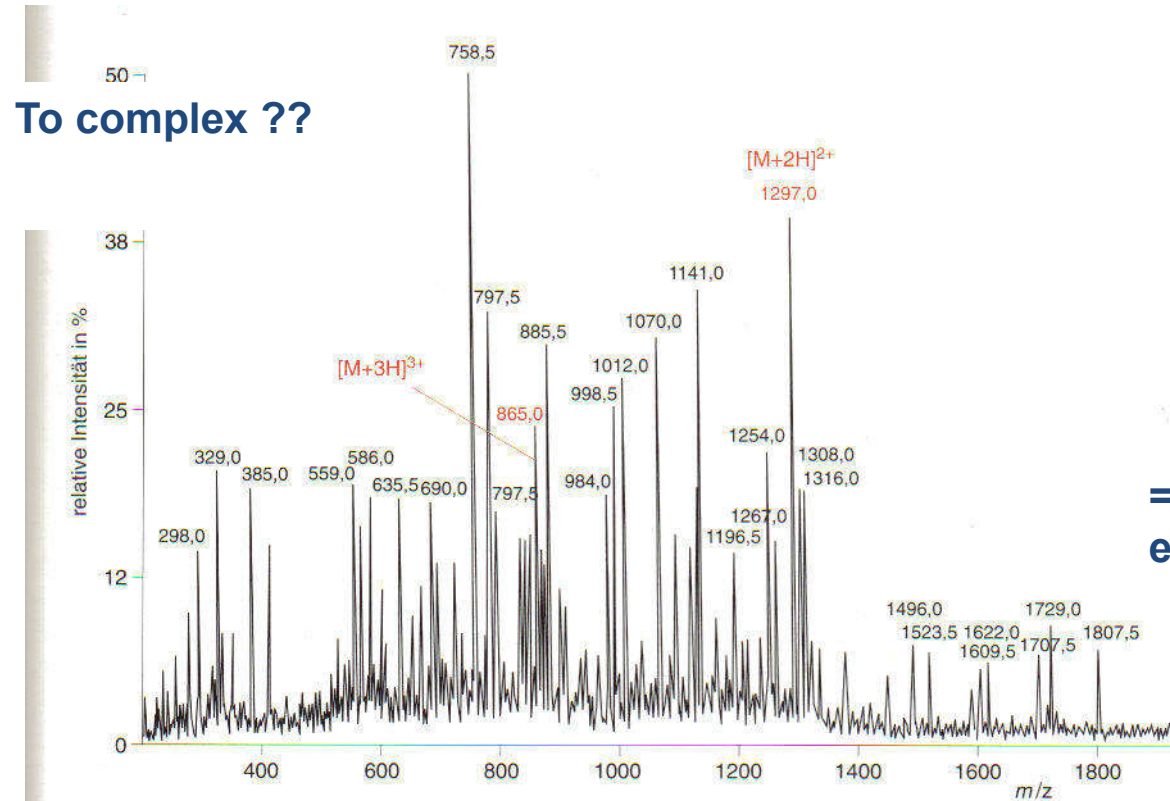
EIC = Extracted ion chromatogram
Only one m/z is displayed

HPLC-ESI-MS analysis enables the
identification of peptides by
retention time and molecular mass



TIC = total ion chromatogram
All m/z signals recorded at a given time
are summed up

ESI mass spectrometry of peptide mixtures



=> MS/MS based structure elucidation

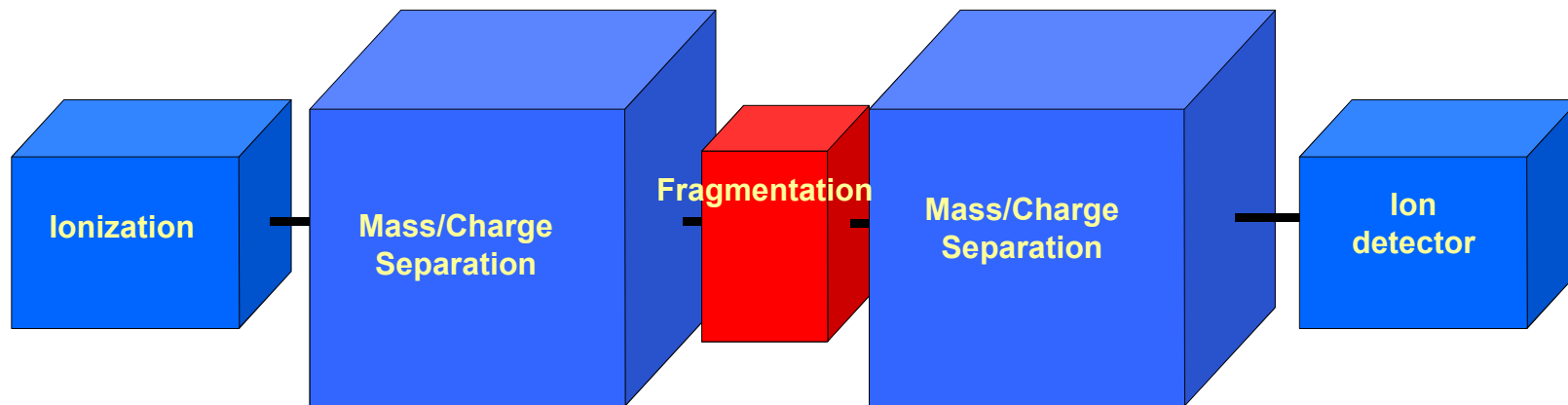
18.10 Elektrospray-Massenspektrum eines hydrophoben Transmembransegmentes (24-mer) ohne basische Aminosäuren. Das Spektrum weist einen $[M+2H]^{2+}$ -Peak bei 1297 amu und einen $[M+3H]^{3+}$ -Peak bei 865 amu auf. Desweiteren deutet die Auswertung der Fragmentierung darauf hin, dass das Hauptprodukt der gewünschten Sequenz entspricht.

Tandem mass spectrometer

Fragmentation of molecular ion

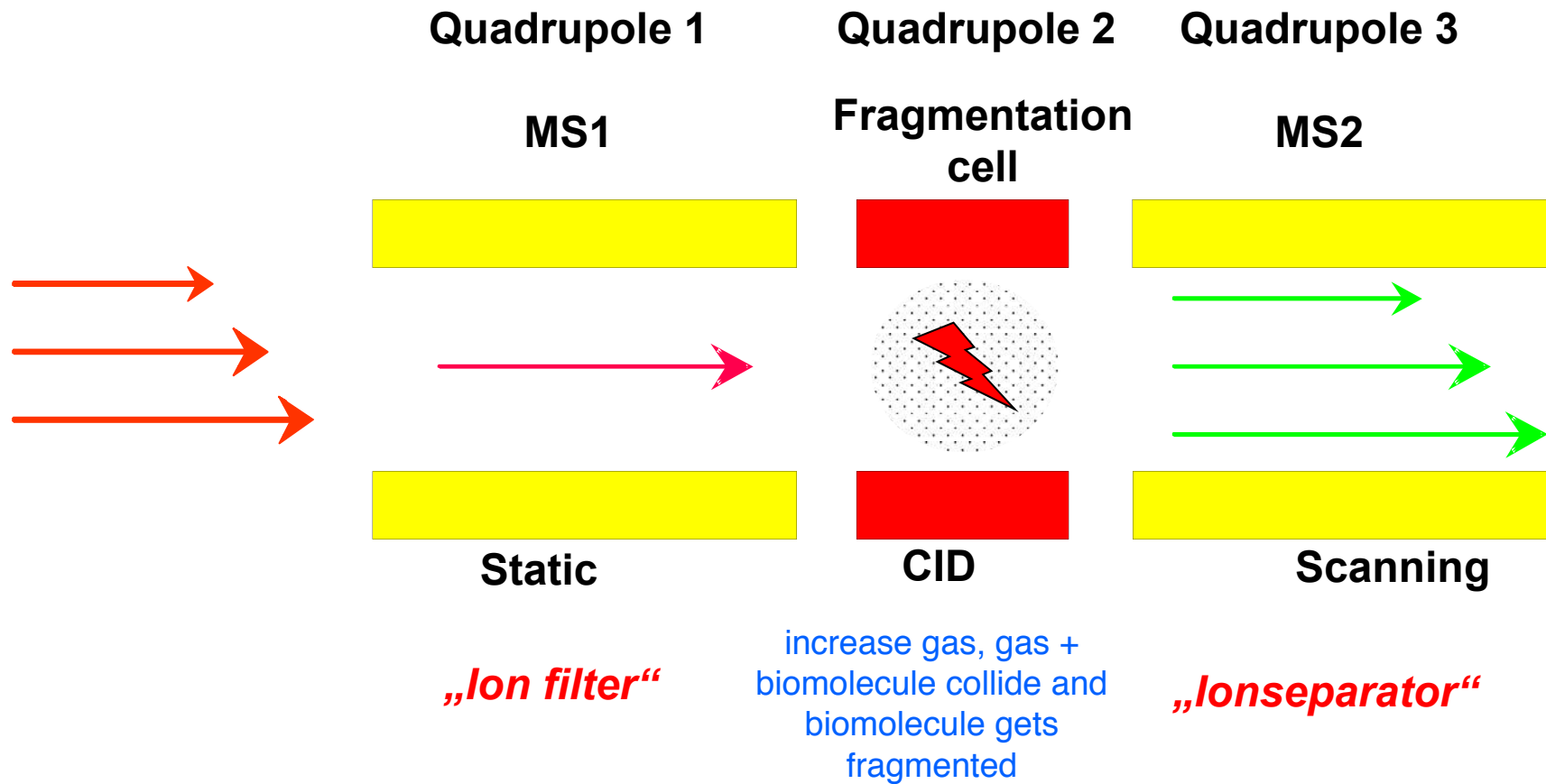
=> Structure elucidation by mass spectrometry

Instrument with MS-MS capability



Fragmentation – Principle

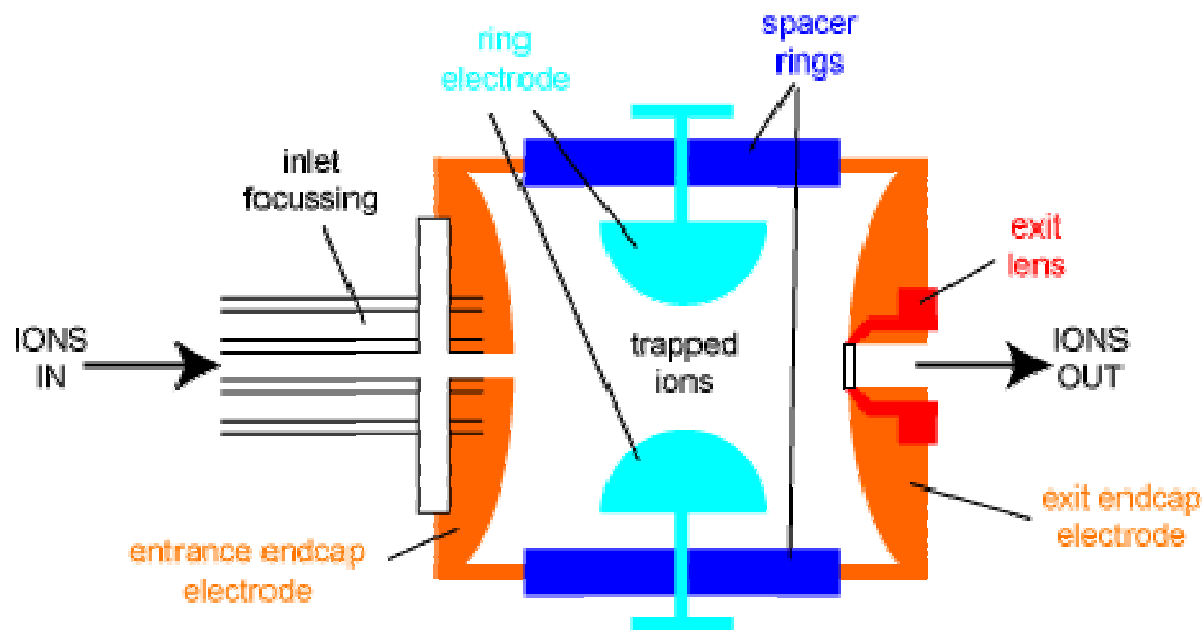
Triple quadrupole instruments



CID: collision induced dissociation

Fragmentation – Principle

Linear ion trap – fragmentation MS^n

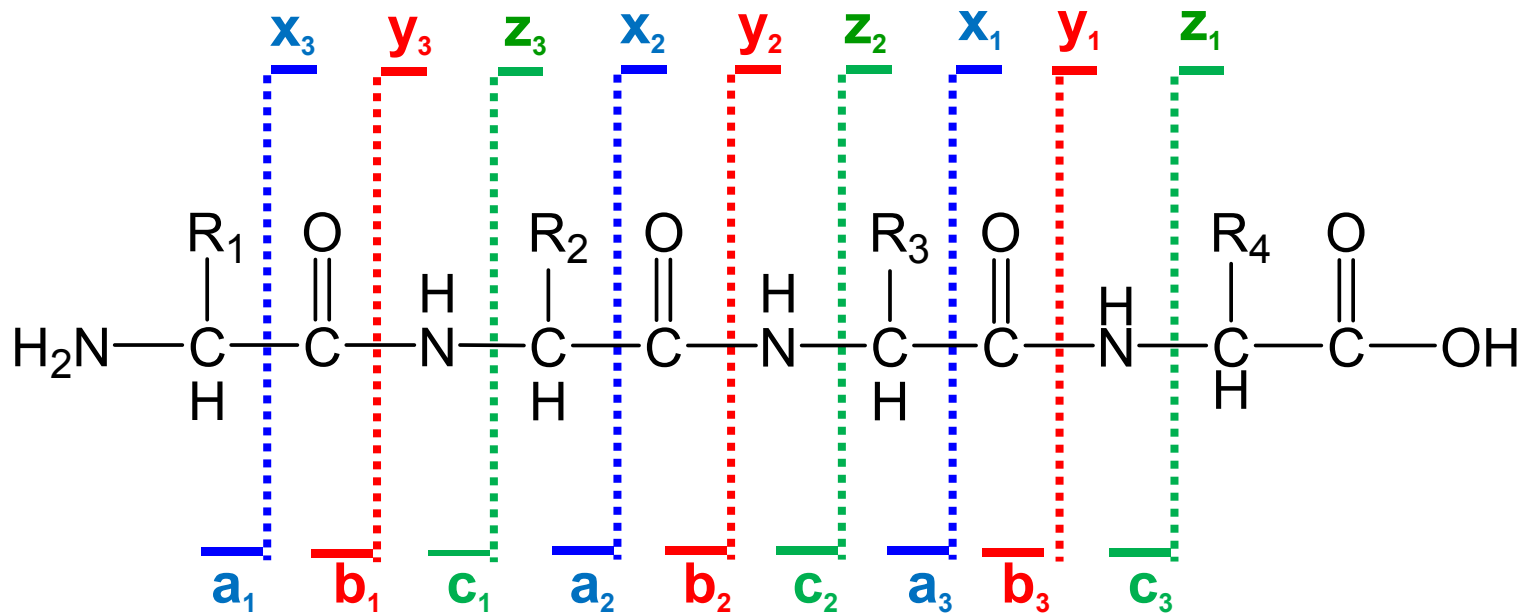


CID: collision induced dissociation
Fragmentation gas, e.g. nitrogen, helium,

Fragmentation – Principle

Fragmentation of peptides by CID

Nomenklatur Roepstorff, P., Fohlmann, J., 1984, *Biomed. Mass Spectrom.* **11** (11): 601.



N-terminal

$\text{a}_i, \text{b}_i, \text{c}_i$

i: number amino acids in fragment

Remind: a = „amino terminus“
z = „C terminus“

C-terminal

$\text{x}_i, \text{y}_i, \text{z}_i$

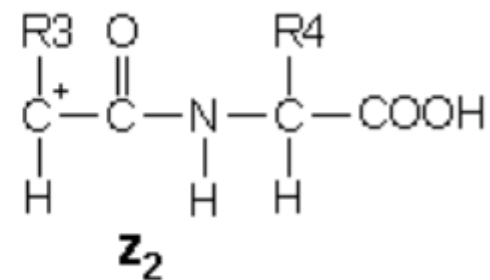
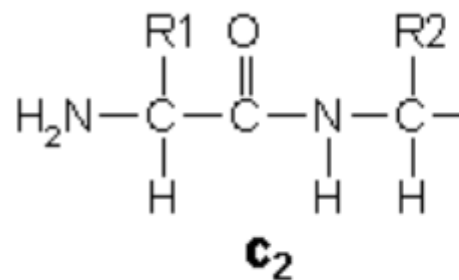
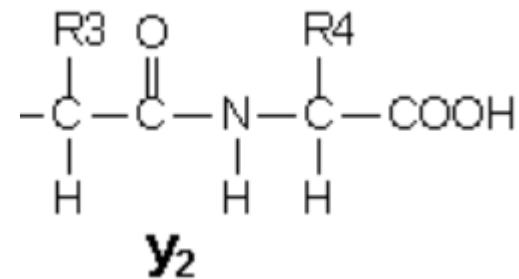
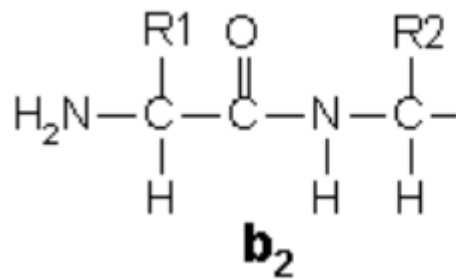
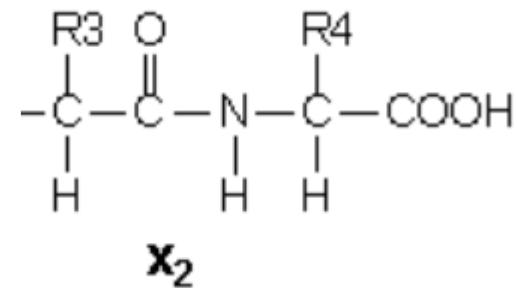
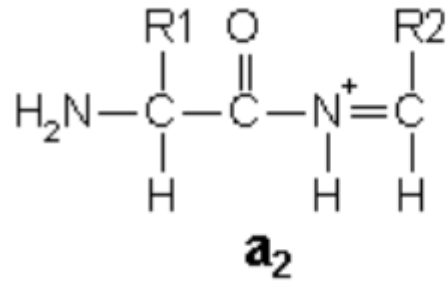
i: number amino acids in fragment

homogeneous
fraction, probabilities are equal



Fragmentation – Principle

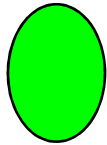
Peptide fragments (low energy MSMS)



Fragmentation – Principle

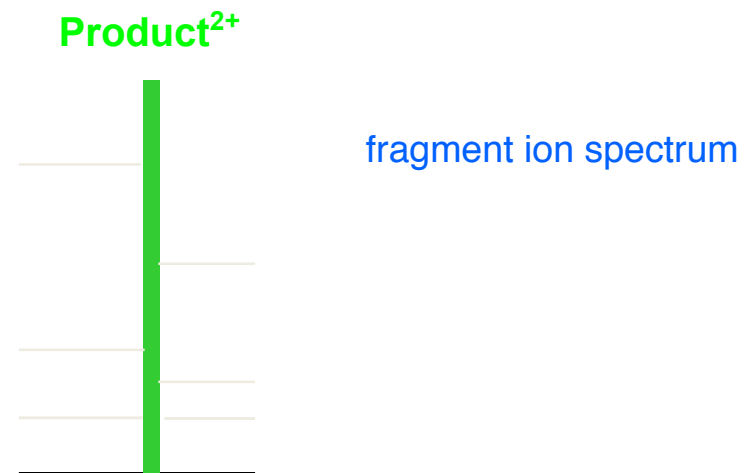
Peptide fragments (low energy MSMS)

Peptide $[M+2H]^{2+}$



Product Ion Scan

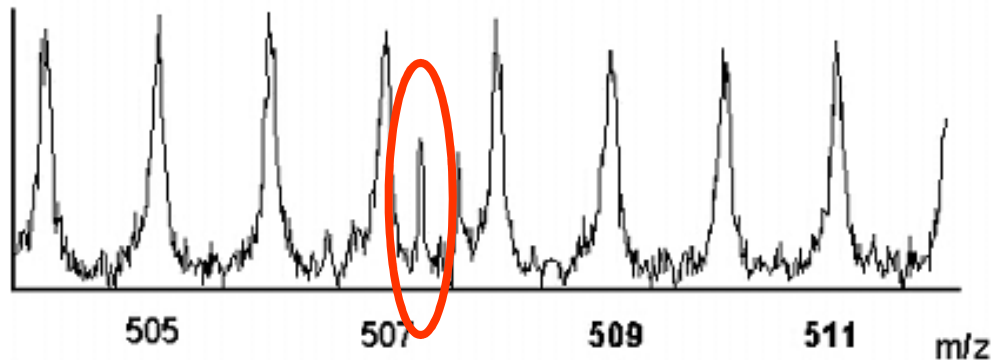
Product Ion Spectrum of a Particular Peptide



Attention: The fragmentation of multiply charged ions produces fragments with higher m/z ratio than the precursor ion showed

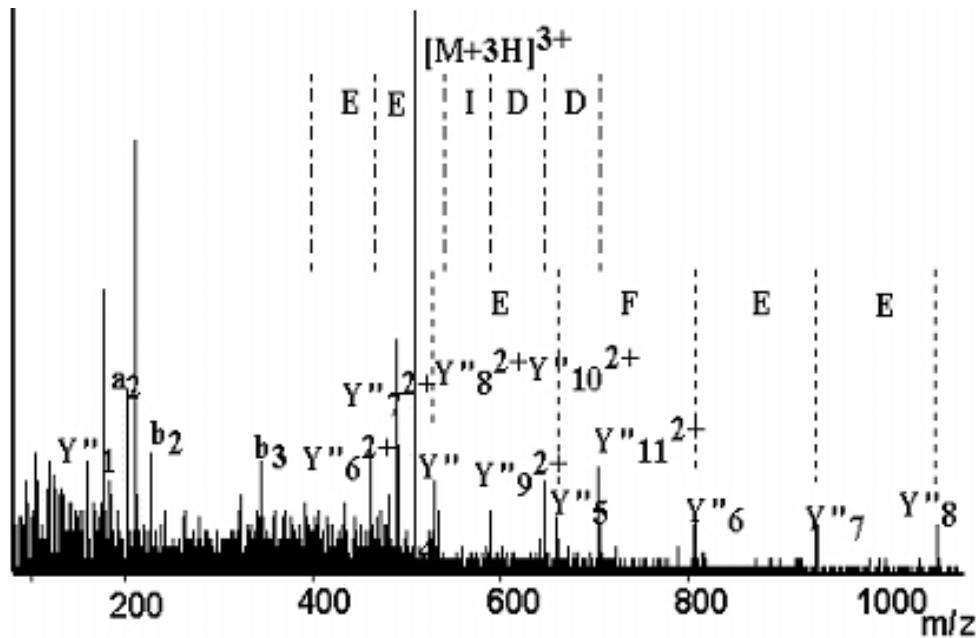
Fragmentation – Example

Peptide fragments (low energy MSMS)



MS spectrum

singly charged
chemical background
triply charged peptide ion



MS/MS spectrum

High mass range only
contains fragment ions
originating from peptide

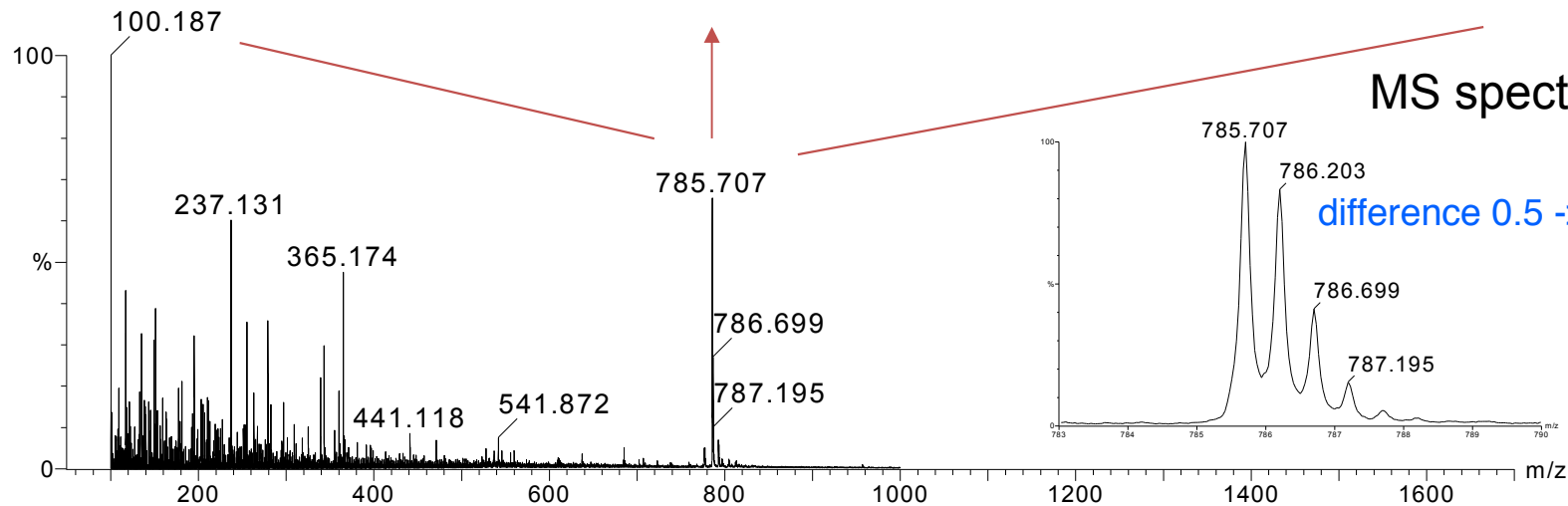
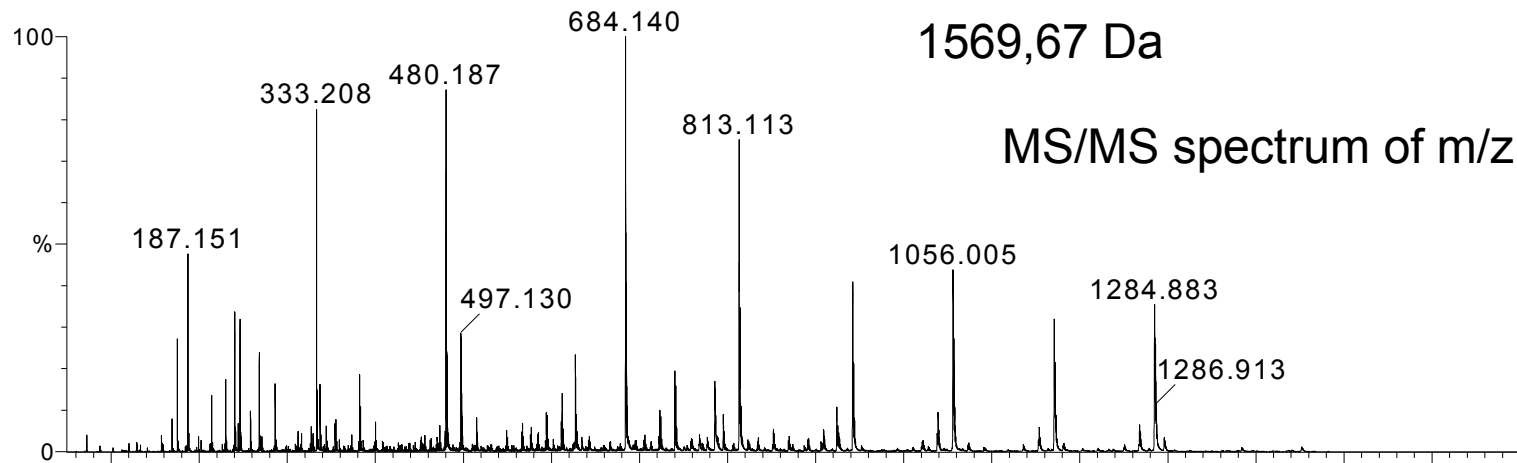
Exam: know charge + mass of spectrum (below)
difference between mono average isotopic peaks(?)
two kinds of ionization

Fragmentation – Example

Peptide fragments (low energy MSMS)

H₂N-EGVNDNEEGFFSAR-OH
1569,67 Da

MS/MS spectrum of m/z 785.7

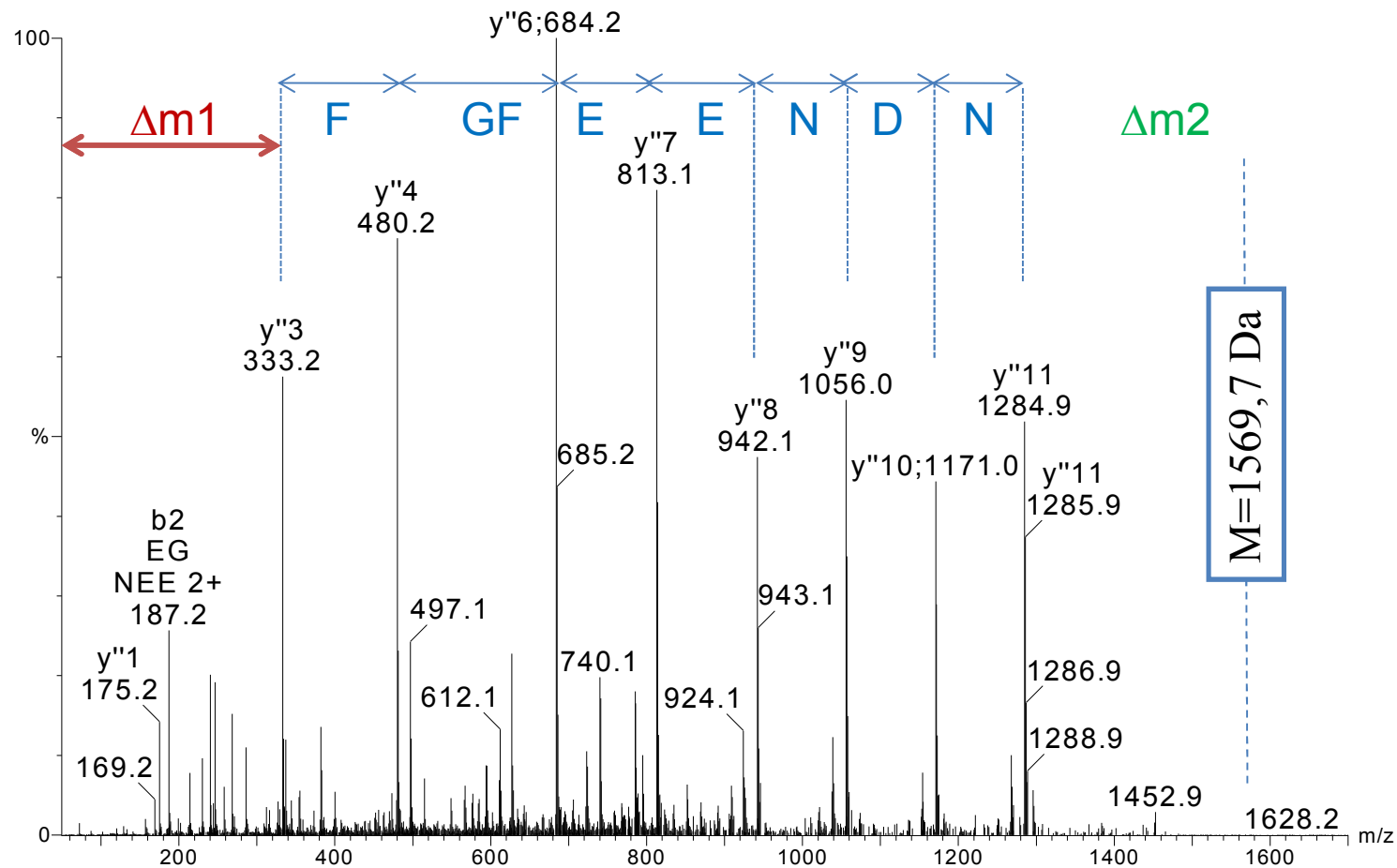


Fragmentation – Example

Identification by MS/MS – peptide sequence tag

Elternion 785,7 Da, $[M+2 H]^{2+}$

H₂N- EGVNDNEEGFFSAR-OH



Fragmentation – Identification

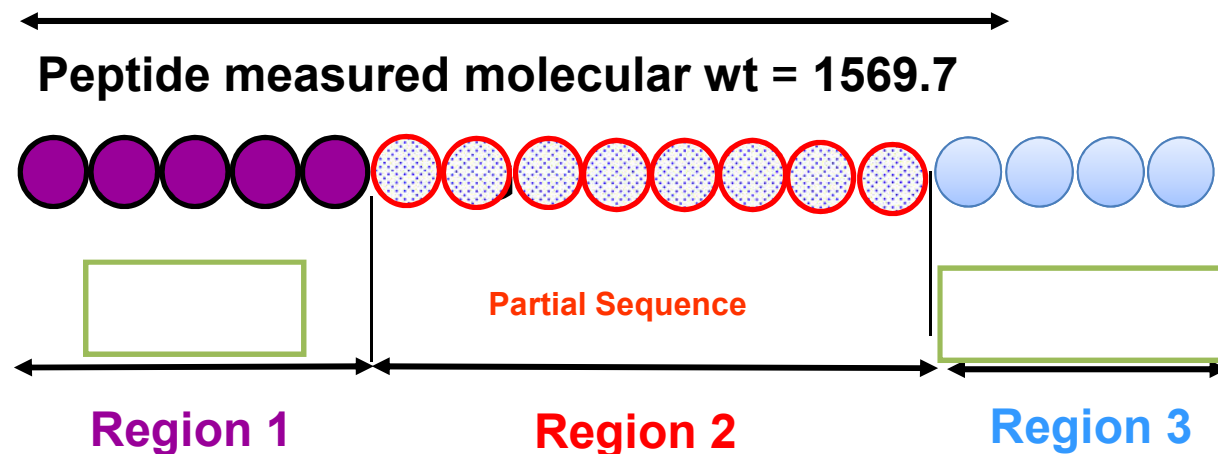
Identification by MS/MS – peptide sequence tag

Sequence Tag = 4 informations for the protein identification using databases

- Molecular weight of the peptide (monoisotopic masse) **(MW)**
- Part of the sequence (Sequence Tag) **(region 2)**
- Mass fragment of the N-terminus **(region 1)**
- Mass fragment of the C-Terminus **(region 3)**

Region 1

Sequence Tag (333.2) E G F E E N D N (1284.9)



Identification of a protein using a sequence tag

identified
protein in
sample

Find a Protein

Search by: Pattern & MW

Mass Accuracy [%]: ☐ .005

< MW [kDa] <

Peptides required for match:

Max numb. missed cleavages:

Sequence Pattern:

Protein Info

Acce. Number:

CAH2_BOVIN
CARBONIC
ANHYDRASE I

Seq. Type

☒ AA Seq
☐ DNA Seq

SHHWGYGKHGPHWHKDF
PIANGERQSPVNIDTKA
VVQDPALKPLALVYGEA
TSRRMVNNGHSFNVEYD
DSQDKAVLKDGPLTGT
RLVQFHFHWGSSQGSEH
TVDRKKYAAELHLVHWN
TKYGDFTAAQQPDGLA
VVGVLKVGDNALPALQK
VLDALDSIKTKGKSTDF
PNFDPGSLLPNVLDYWT
YPSLTTPPLLESVTWI
VLKEPISVSSQQMLKFR
TLNFNAEGEPELLMLAN
WRPAQPLKNRQVRGFPK
//

970.441
972.549
978.477
1000.519
1011.535
1017.488
1345.691
1580.810
2097.870
2197.211
2252.148
2353.099

pI: 8.95
28508.3 Da
25 peptides

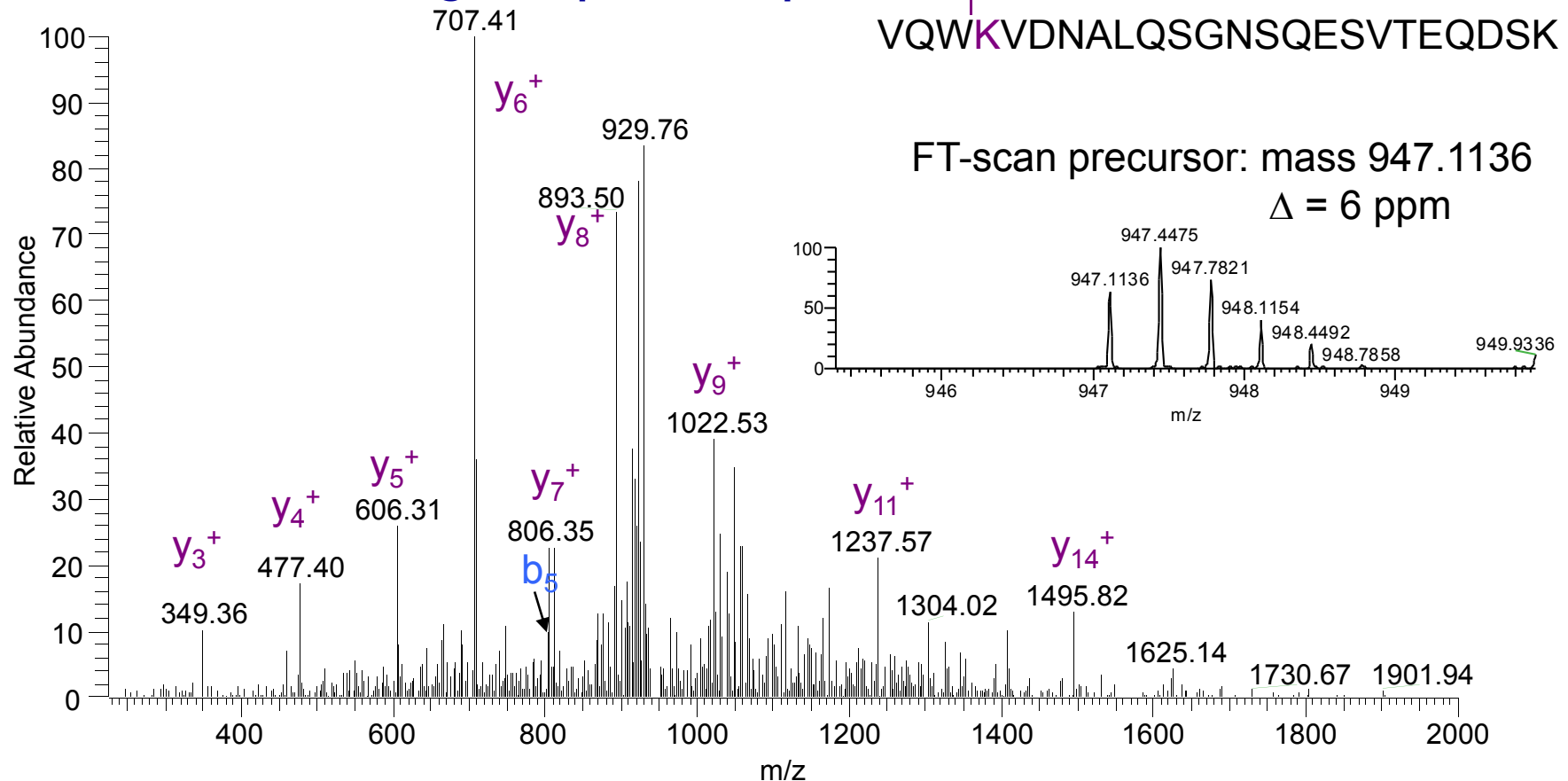
nrdb.index(10Jan99)

	Index	Peptide starts at	Acc. Num.	MW [Da]	Protein Name
1	18791	208	swiss P00921	28508.30	CAH2_BOVIN CARBONIC ANHYDRASE I

Fragmentation – identification of modifications

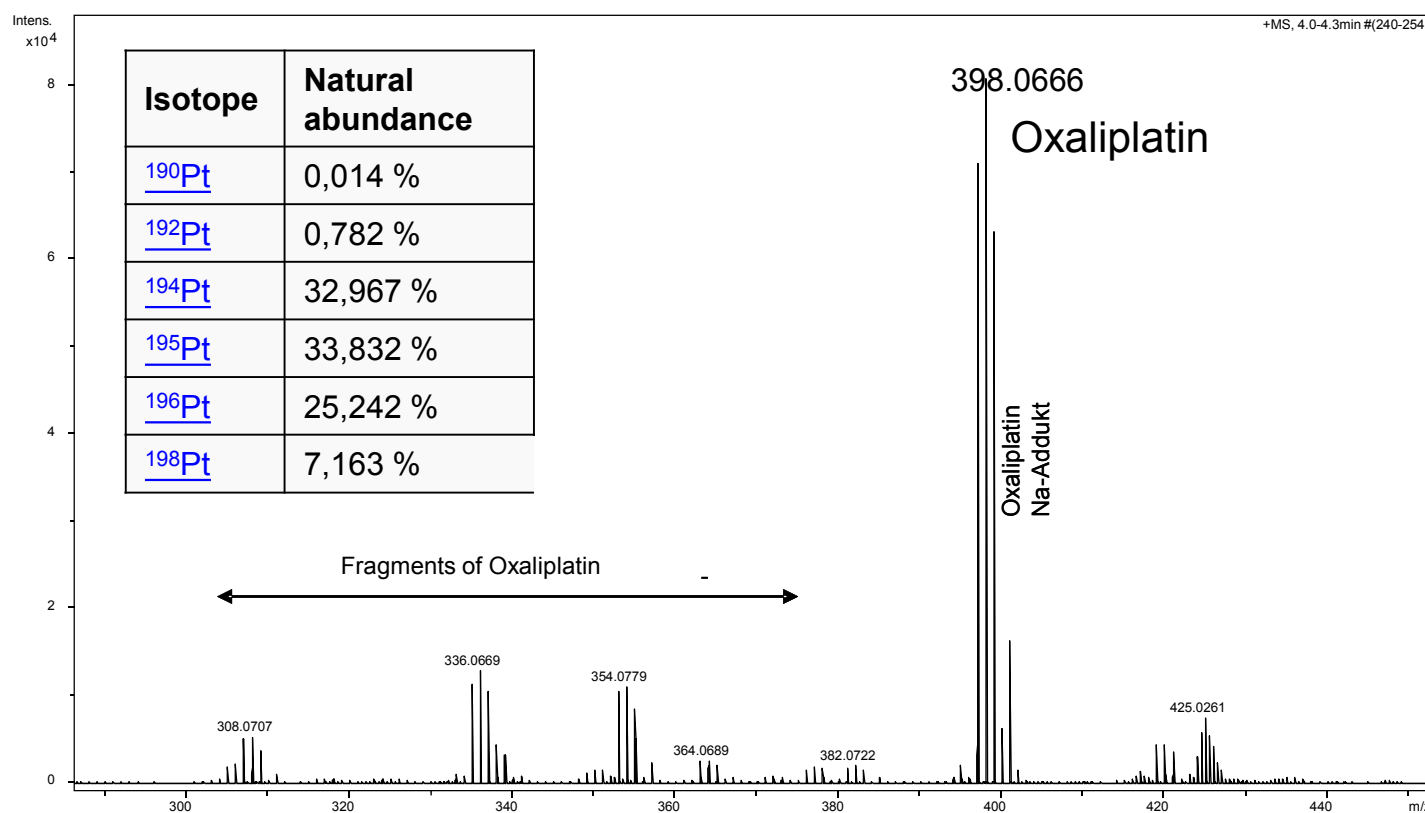
LC-MS examples: site specific ID of non enzymatic glycation

AZ070116 #3560 RT: 41.46 AV: 1 NL: 2.68E3
T: ITMS + c NSI d Full ms2 947.45@cid35.00 [225.00-2000.00]

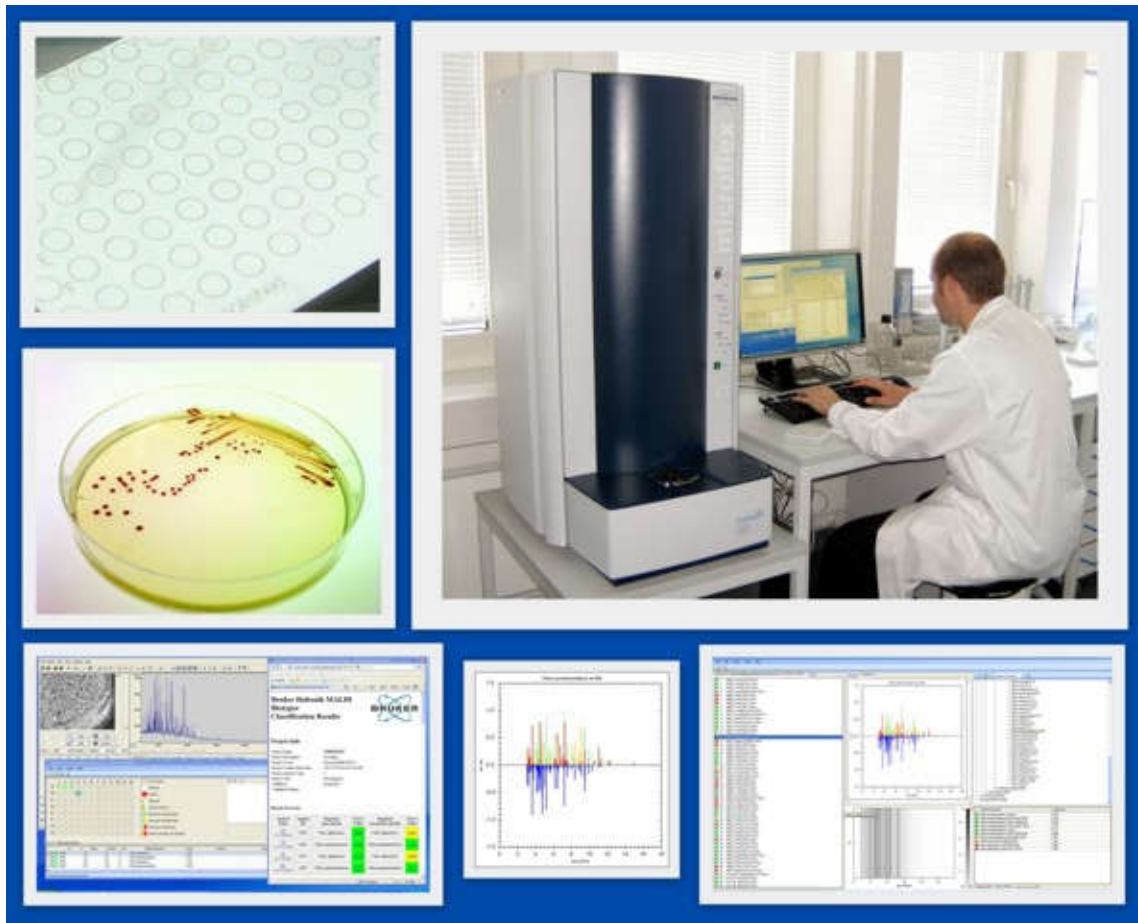


Mass spectrometry of chemotherapeutics

Application: off-line monitoring of Oxaliplatin during chemotherapeutic treatment



Mass spectrometry based identification of microorganisms using their molecular fingerprint (MALDI Biotyper)



Workflow:

1. Applying a small amount of biological material directly On a MALDI target plate in a thin film
2. The thin microbial film is overlaid with 2 μ l of a saturated acid matrix Solution
3. Mass spectra are acquired between 2000 and 20 000 Da in positive mode
4. Identification of bacteria, yeast, fungi by using the proteomics fingerprint and peak-matching / multivariate analysis.

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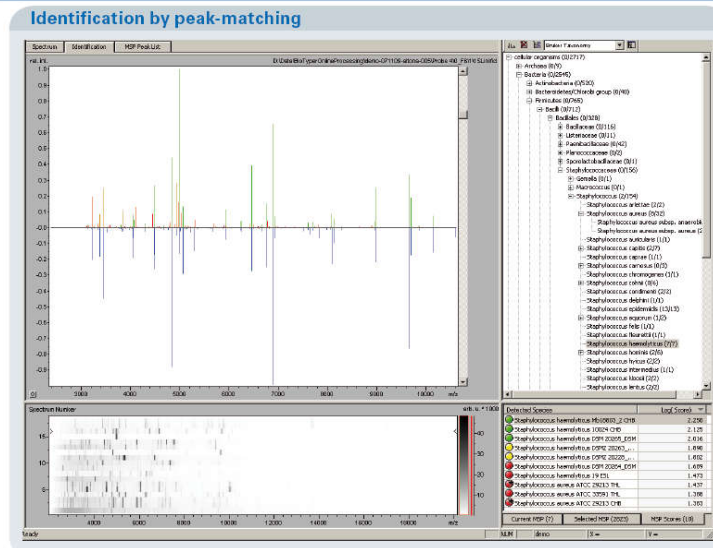


➔Faster than PCR or metabolic pathway Analysis

- Generic experimental setup
- robust method, minimal sample preparation

Applications:

- clinical routine monitoring,
- environmental & taxonomical research,
- food processing quality control



Summary: Mass Spectrometry in the Clinics

Mass Spectrometry:

- can measure multiple analytes with high sensitivity
- often faster and cheaper than other methods

Hot topics (applications):

- Endocrines & Biomarkers:
 - vitamin D, steroids, testosterone
- Therapeutic drug monitoring
 - Immunosuppressants, pain panel drugs,...
- Inborn errors of metabolism
 - newborn blood spot analysis: acyl carnitines, amino acids,...
- Toxicology research:
 - Drugs of abuse, opiates, barbiturates, amphetamines.....