

Laboratory Techniques and Medical Device Approvals

**Mass spectrometry for
biomolecules II**

Applications

Dr. Anne Zeck, anne.zeck@nmi.de
Natural and Medical Sciences Institute (NMI)
at the University of Tübingen

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

Why is it important?

Examples for identification of proteins and characterization of protein modifications

1. Natural rubber latex (NRL) has been increasingly used in a variety of medical devices. But: NRL can induce allergic reactions. → Identification and analysis of latex proteins that are allergenic is very important
2. Manufacturer of medical devices are required to control their manufacturing process in such a way that no biological contaminants remain in the final product. If e.g. enzymes are used to manufacture bone implants the removal of the enzyme needs to be proven. → analysis methods need to be developed
3. To increase “biocompatibility” and reduce immunogenicity new generation of medical devices may be coated with proteins → analysis for quality control is necessary
4. Stability of medical devices containing biological material needs to be proven. Is degradation / modification of the proteins occurring ?

Mass spectrometry of proteins

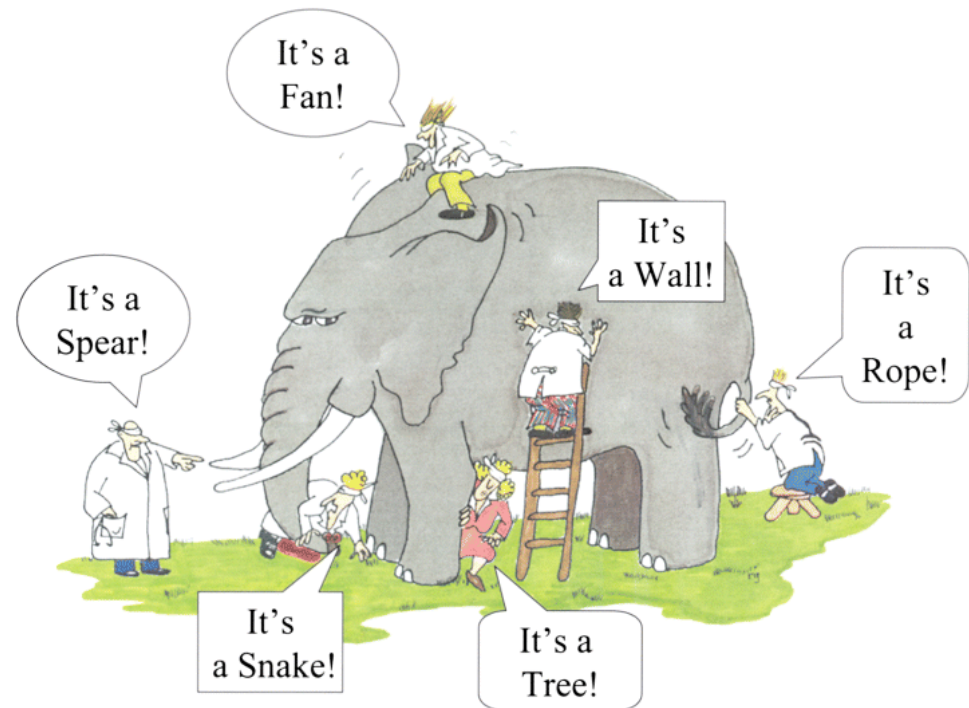
How do we identify and characterize proteins by MS ?

Three ways to identify proteins by mass spectrometry

1. Measurement of the intact protein mass
 - + Fast, sensitive method
 - + “whole” picture of the protein (integrity, modification,...)
 - Not possible for complex samples
2. Peptide mass fingerprint (PMF): enzymatic digestion of intact proteins and measurement of the peptide masses. Match of peptide masses theoretical protein digest
 - + analysis of complex samples possible
 - + more detailed information of modification
 - More complex sample preparation -> method artefacts possible
3. PMF with fragmentation of peptides in the mass spectrometer
 - highest degree of information but most complex dataset

Intact protein analysis *versus* peptide mass fingerprint

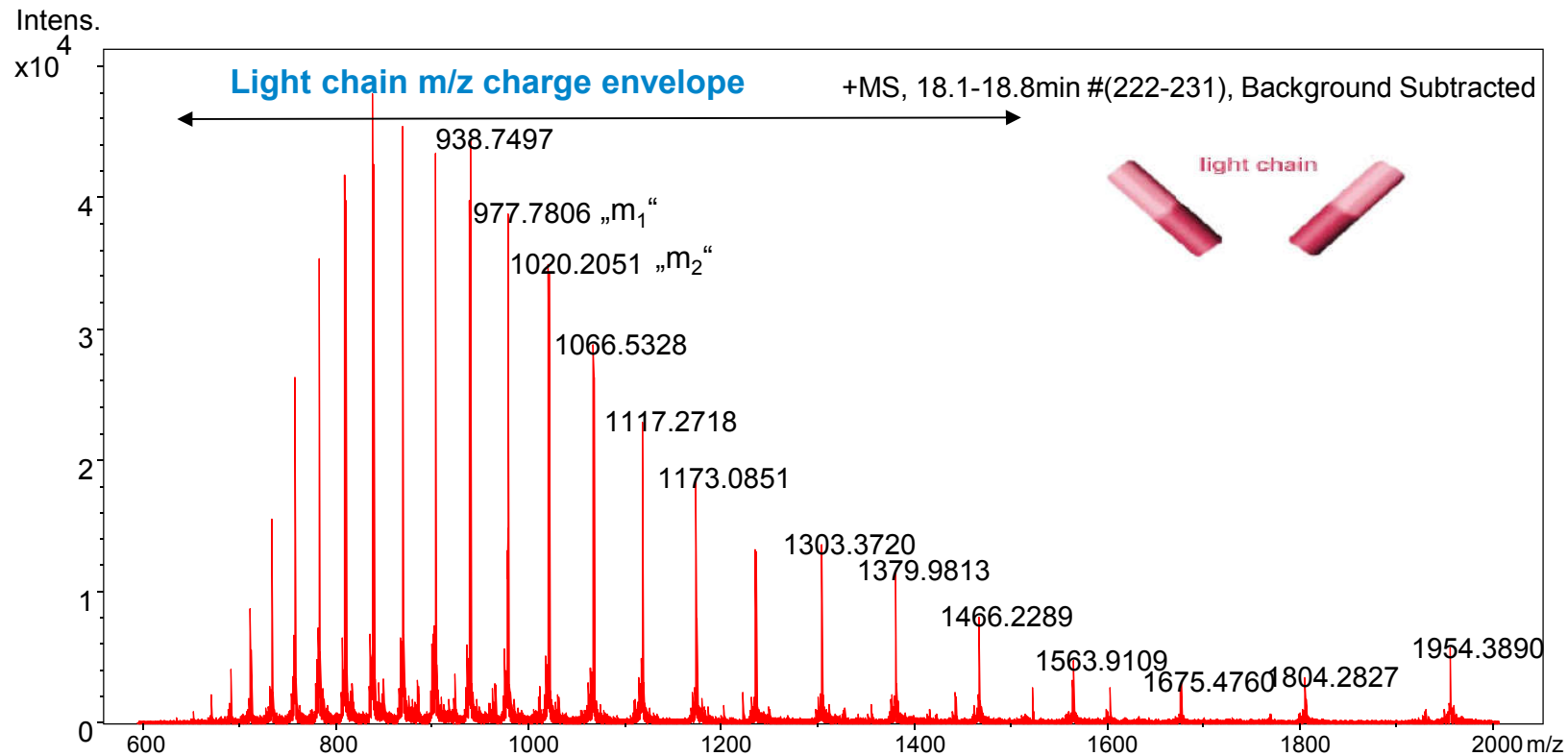
- Work with well characterized biomolecules in your bioassays
- Establish structure-function relationship of proteins
- Elucidate function of proteins



<http://logixicf.com/ecobuildtrends/perspectives-seeing-the-whole-elephant/>

Mass spectrometry of intact proteins

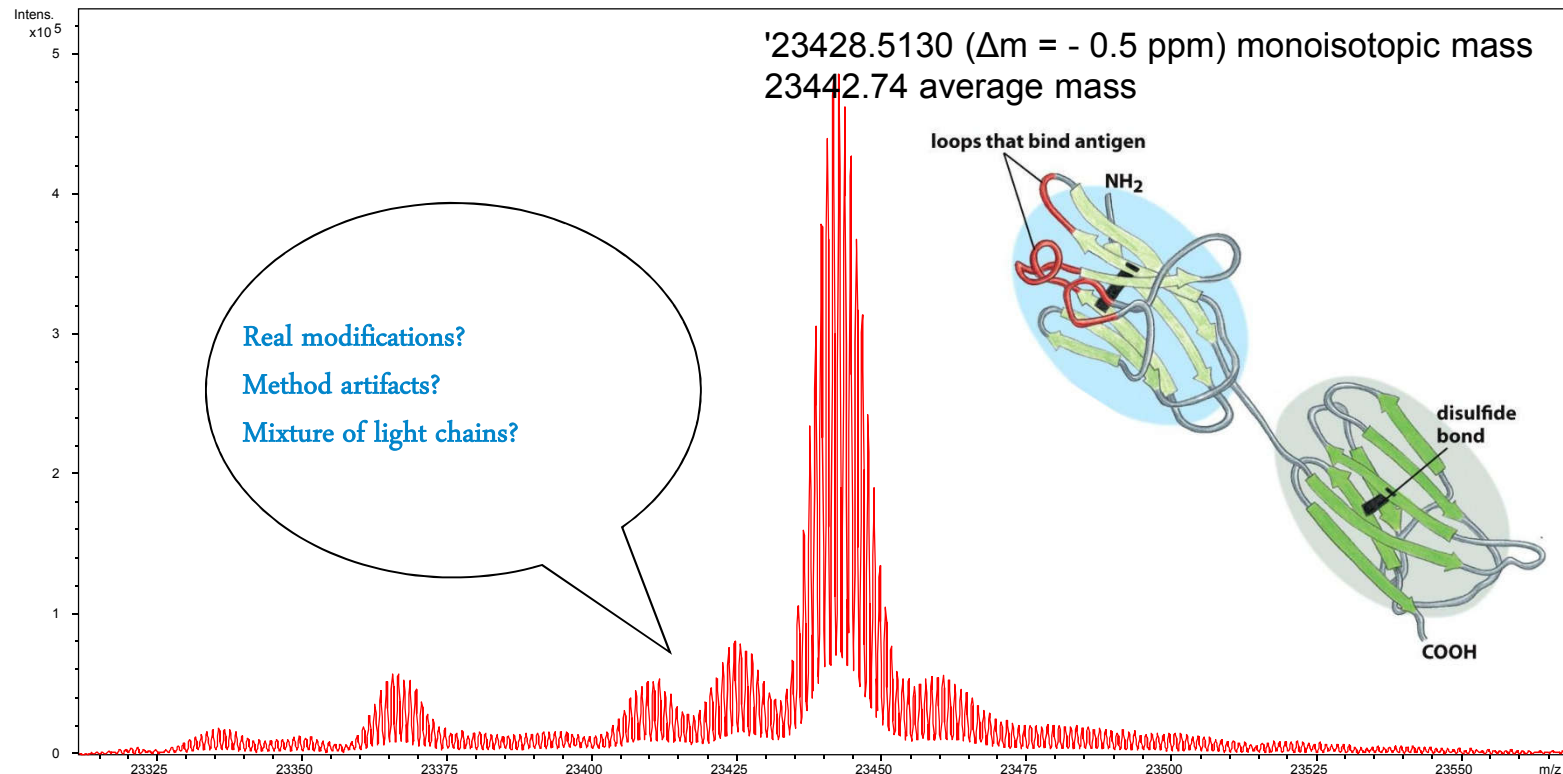
ESI spectrum of the light chain of an antibody (25 kDa)



- Charge deconvolution: $z = (m_2 + m_H) / (m_2 - m_1)$

Mass spectrometry of proteins

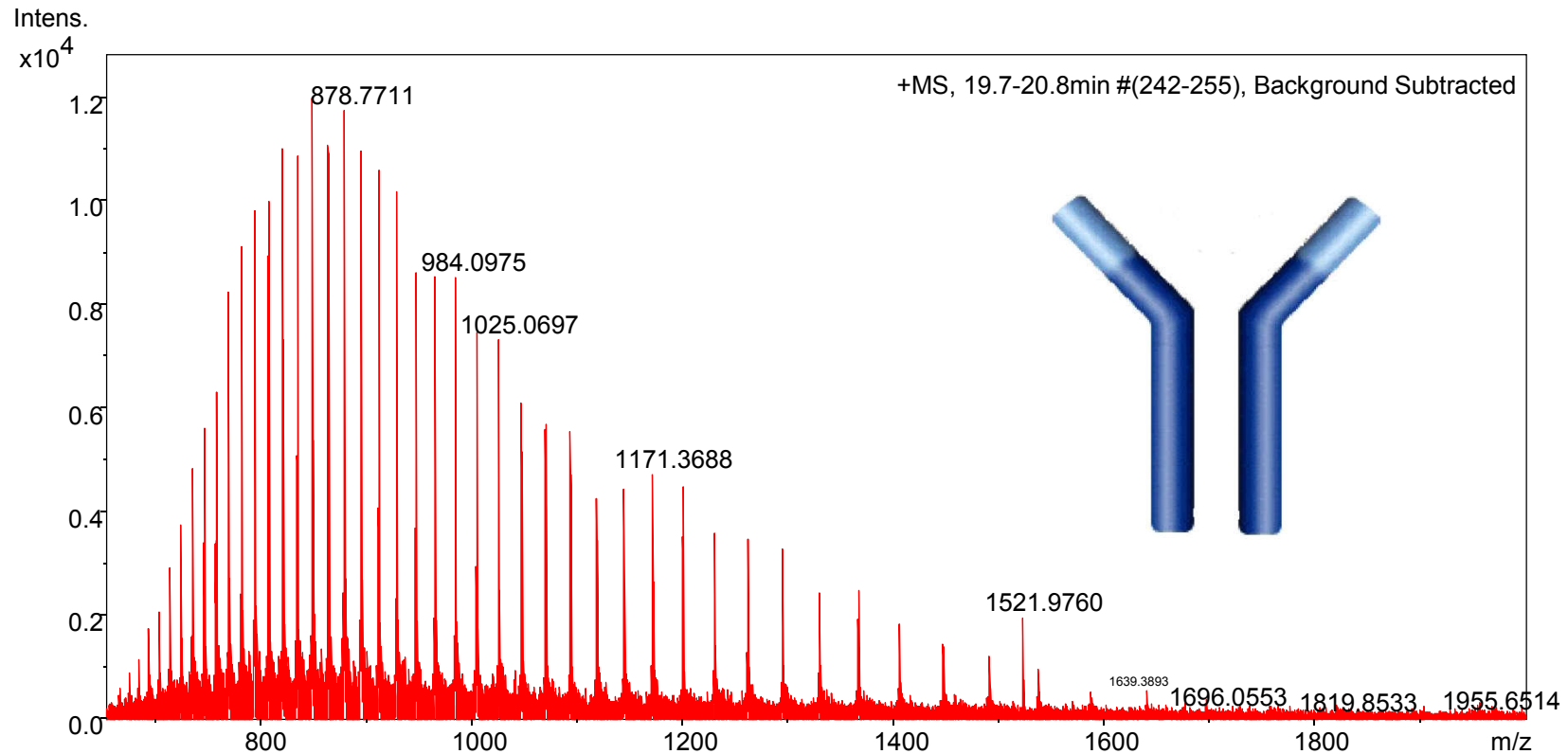
Exact mass; modifications: deconvoluted spectrum



- 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:

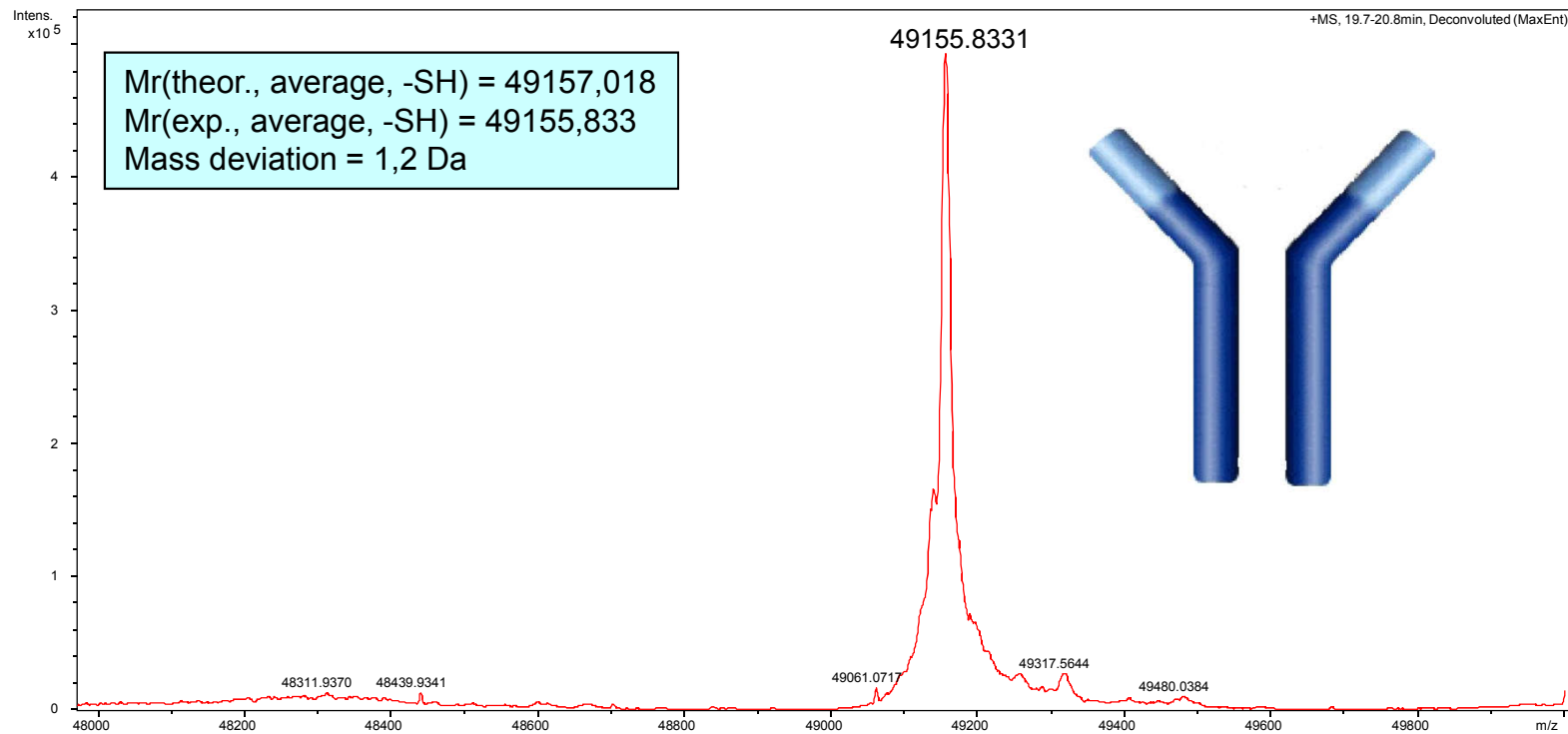
ESI spectrum heavy chain of an antibody (50 kDa)



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

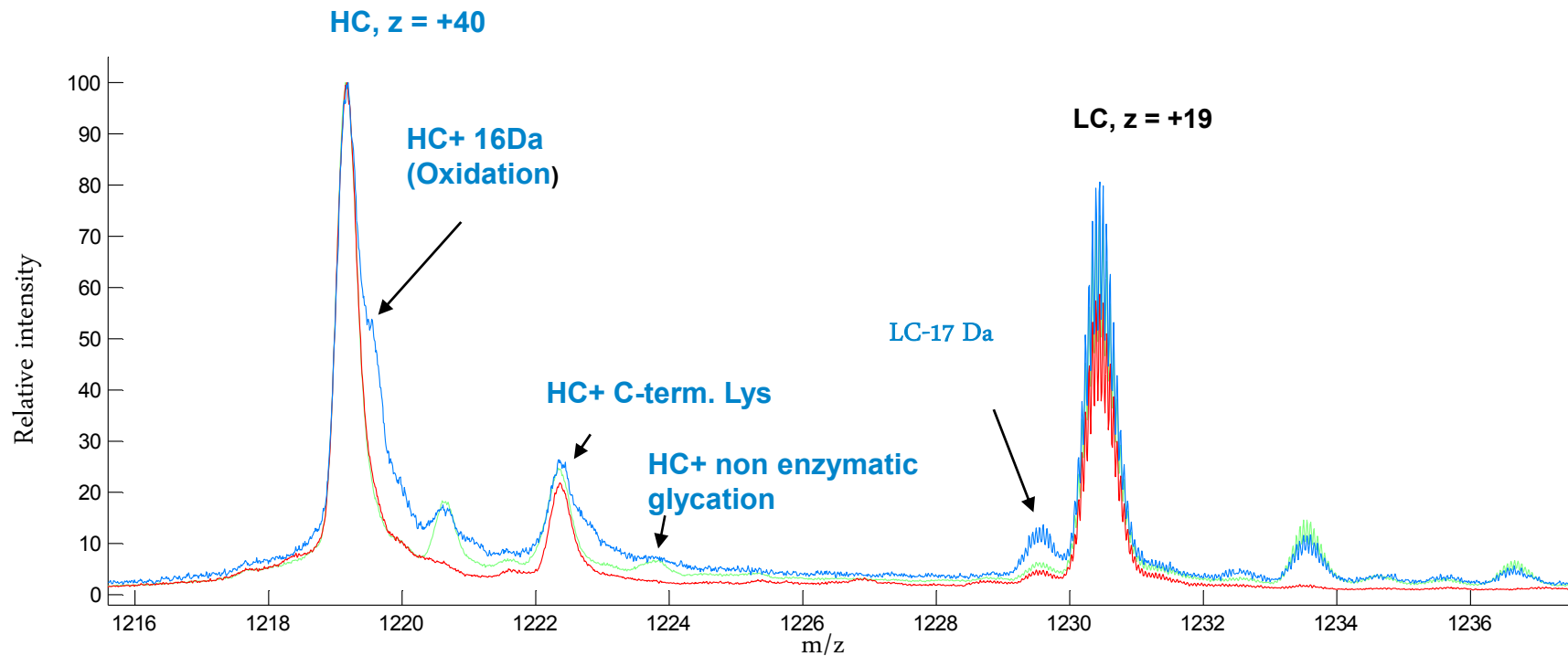
Mass spectrometry of proteins

Charge deconvoluted heavy chain of an antibody



Mass spectrometry of proteins

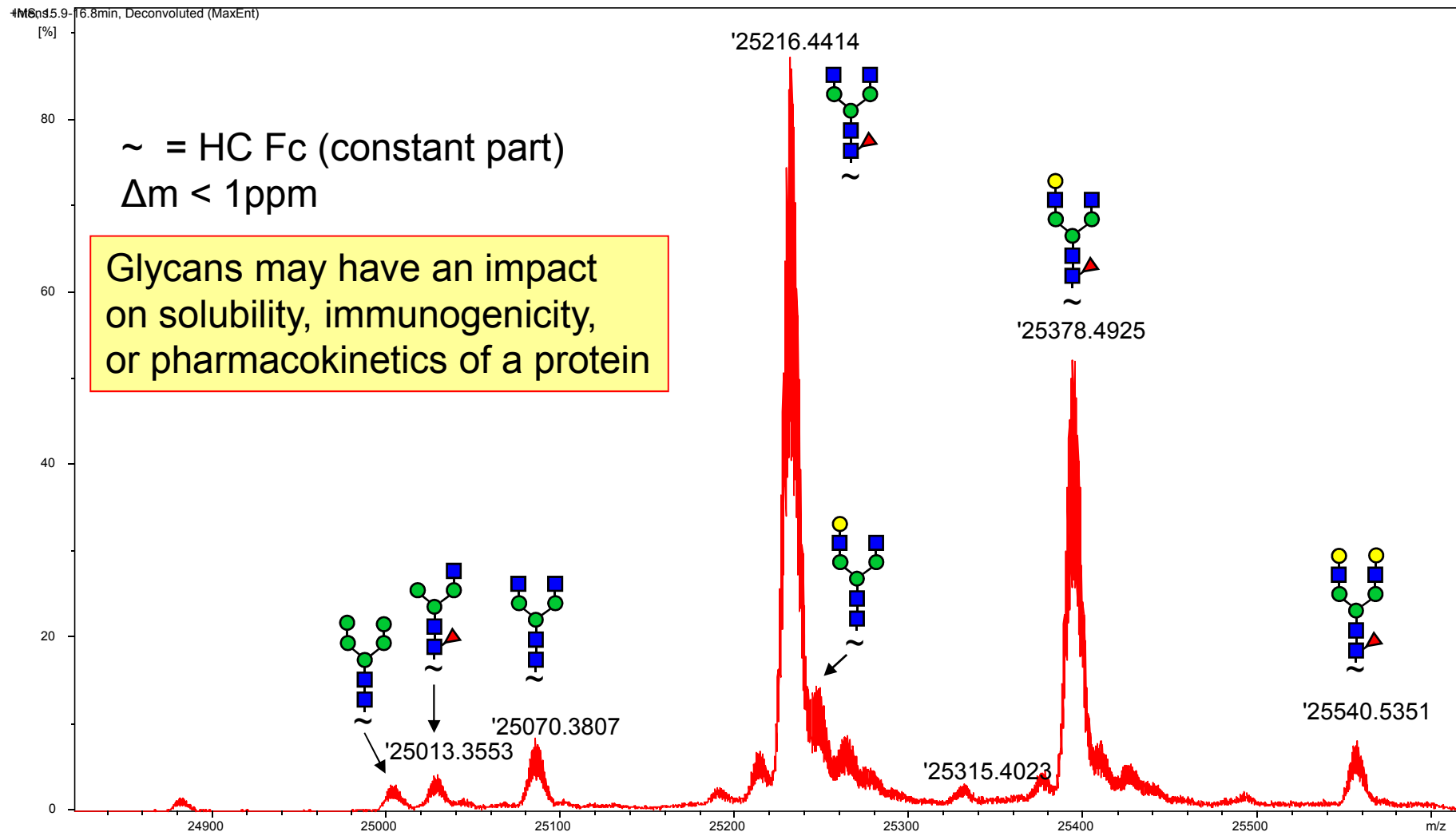
Detection of protein degradation



~ 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

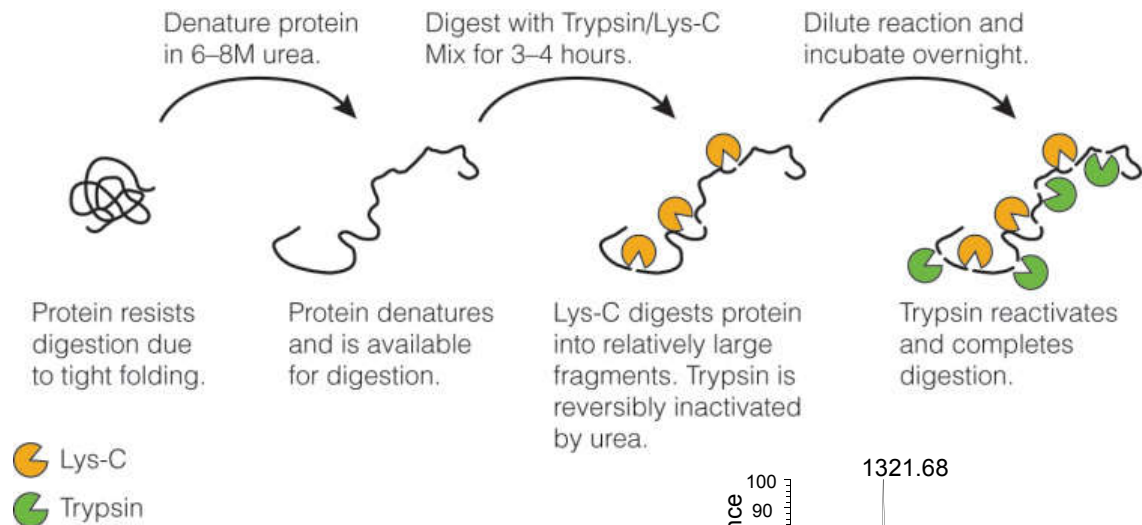
Mass spectrometry of proteins

Determination of glycosylation

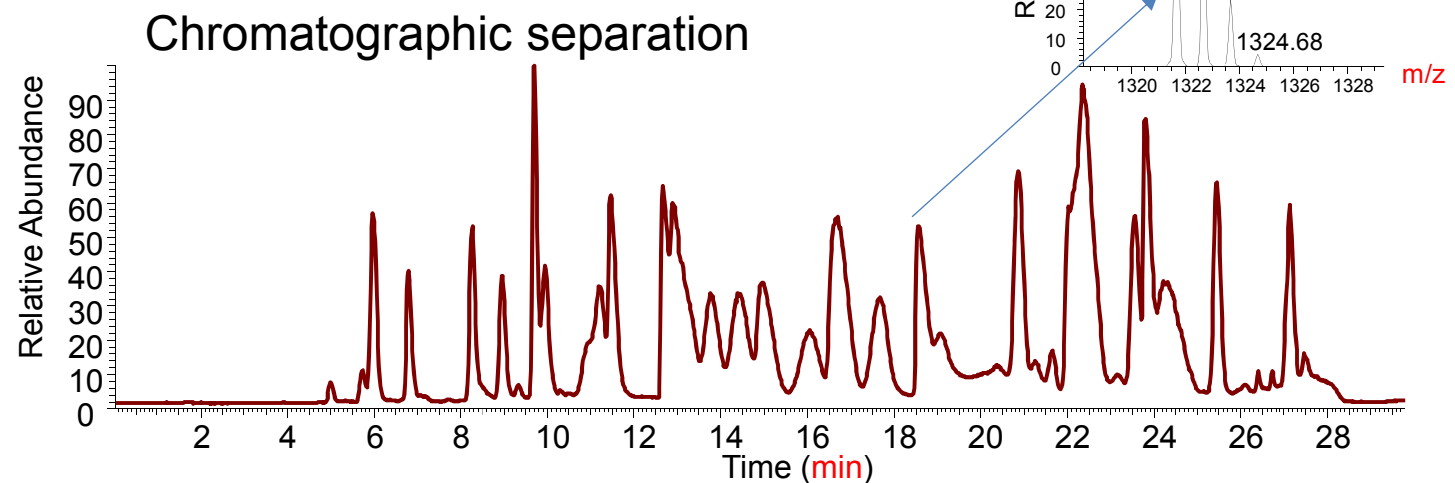


Peptide mass fingerprinting

Step 1: Enzymatic digestion of the protein

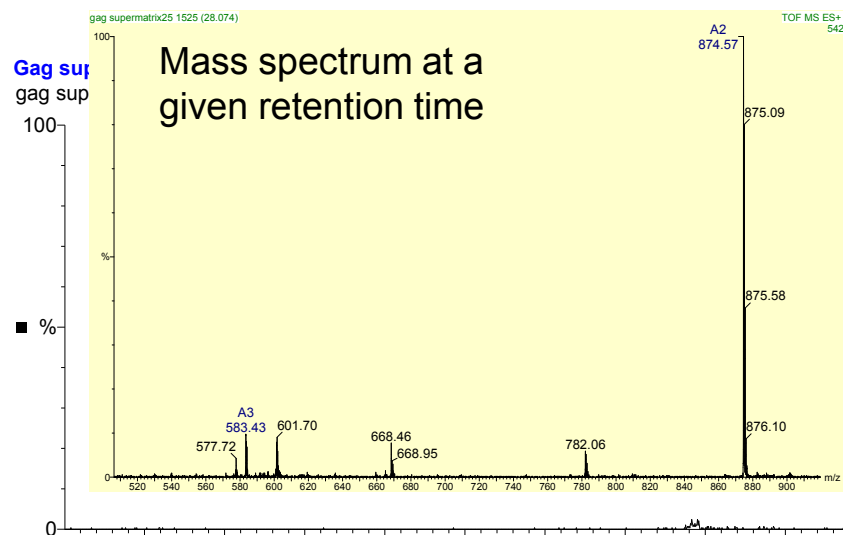


Step 2: Chromatographic separation of the peptides and mass measurement by MS



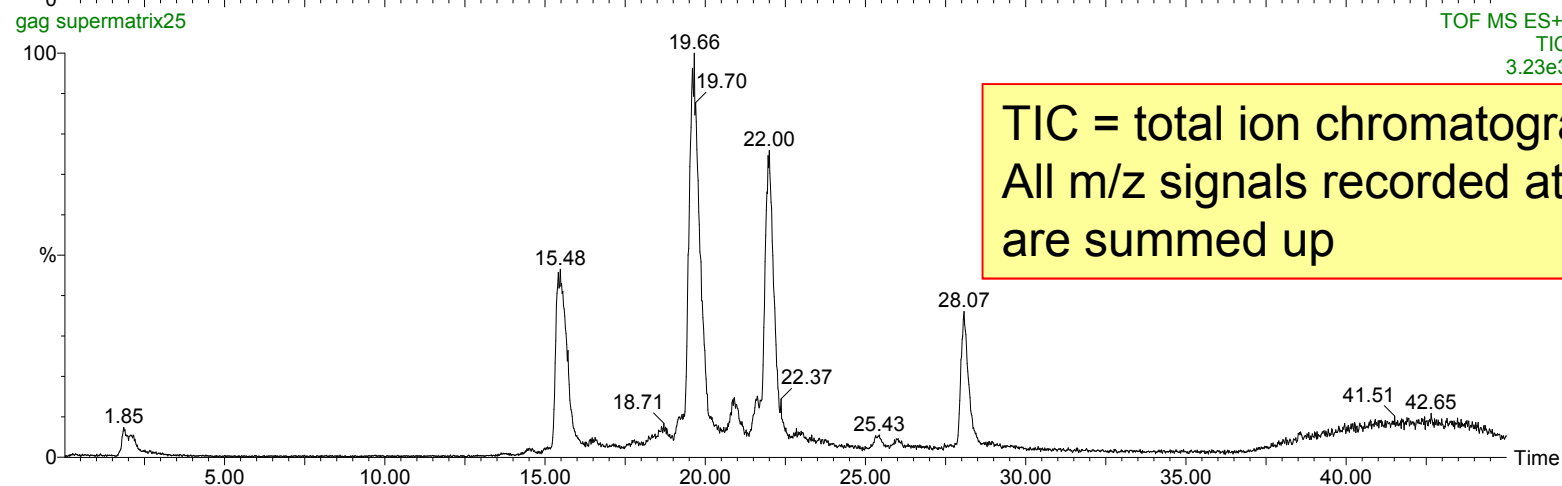
ESI mass spectrometry of peptide mixtures

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

Peptide mass fingerprinting

Step 3: Identification of the protein in a database by matching masses of a subset of the tryptic peptides against calculated values.

Example: Identification of **Papain** from *Carica papaya* in a bovine sponge sample for bone implants

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: 21%

Protein amino acid sequence with matched peptides shown in Bold Red

1 MAMIPSISKL LFVAICLFVY MGLSFGDFSI VGYSQNDLTS TERLIQLFES
51 WMLKHNNKIYK NIDEKIYRFE IFKDNLKYID ETNKKNNNSYW LGLNVFADMS
101 NDEFKEKYTG SIAGNYTTTE LSYEEVLNDG DVNIPEYVDW RQK**GAVTPVK**
151 NQGSCGSCWA FSAVVTIEGI IKIR**TGNLNE YSEQELDCD** RRSYGCNGGY
201 PWSALQLVAQ YGIHYRNTYP YEGVQRYCRS REKGPYAAKT DGVRQVQPYN
251 EGALLYSIAN QPVSVVLEAA GK**DFQLYRGG IFVGPCGNK**V DHAVAAVGYG
301 PNYILIK**NSW GTGWGENGYI** RIKRGTGNSY GVCGLYTSSF YPVKN

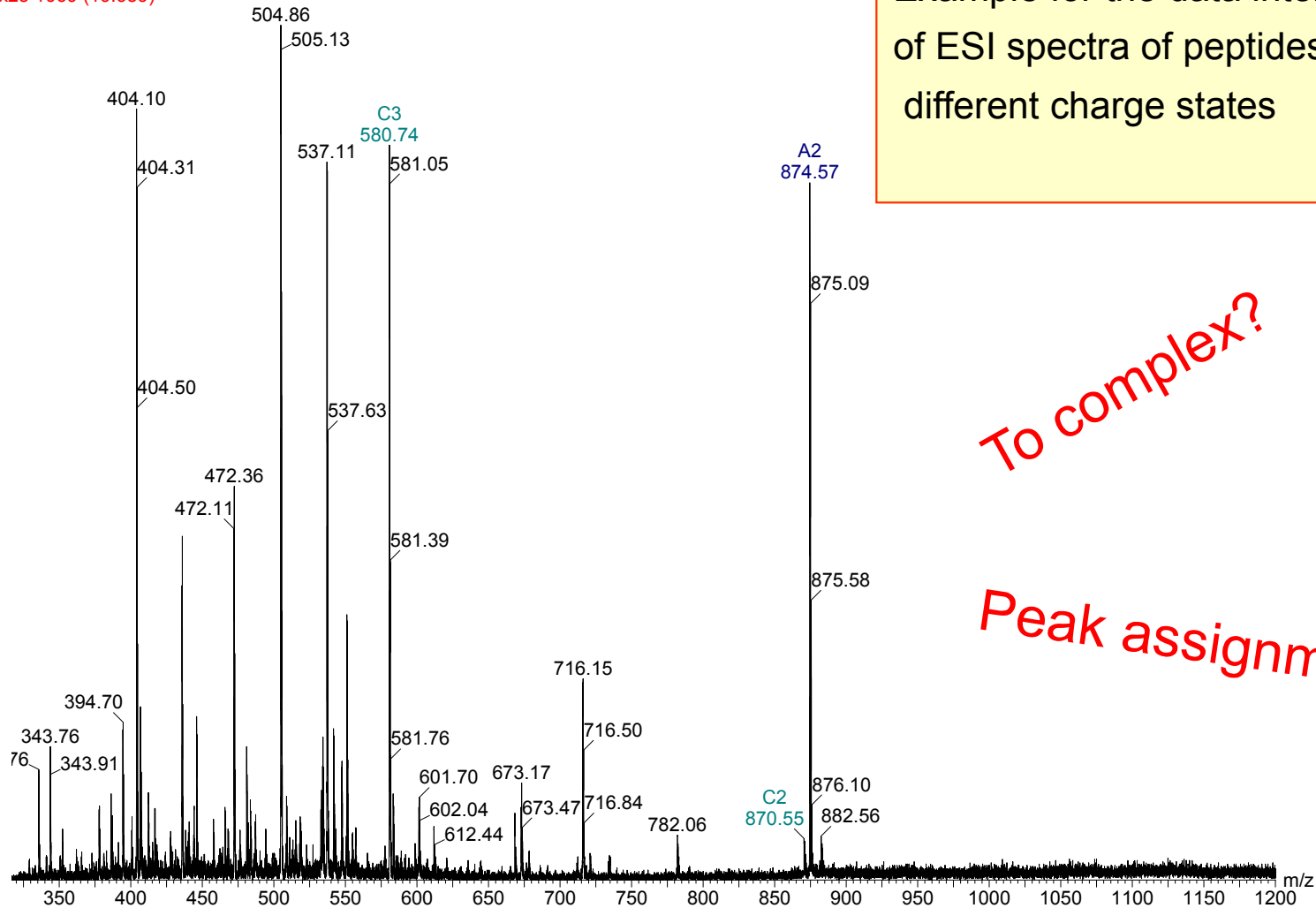
Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
144 - 150	336.2074	670.4002	670.4014	-0.0012	0	K. GAVTPVK .N (Ions score 36)
217 - 226	613.7936	1225.5726	1225.5727	-0.0001	0	R. NTYPYEGVQR .Y (Ions score 27)

ESI Mass spectrometry of peptide mixtures

Direct infusion into mass spec

gag supermatrix25 1069 (19.659)

10

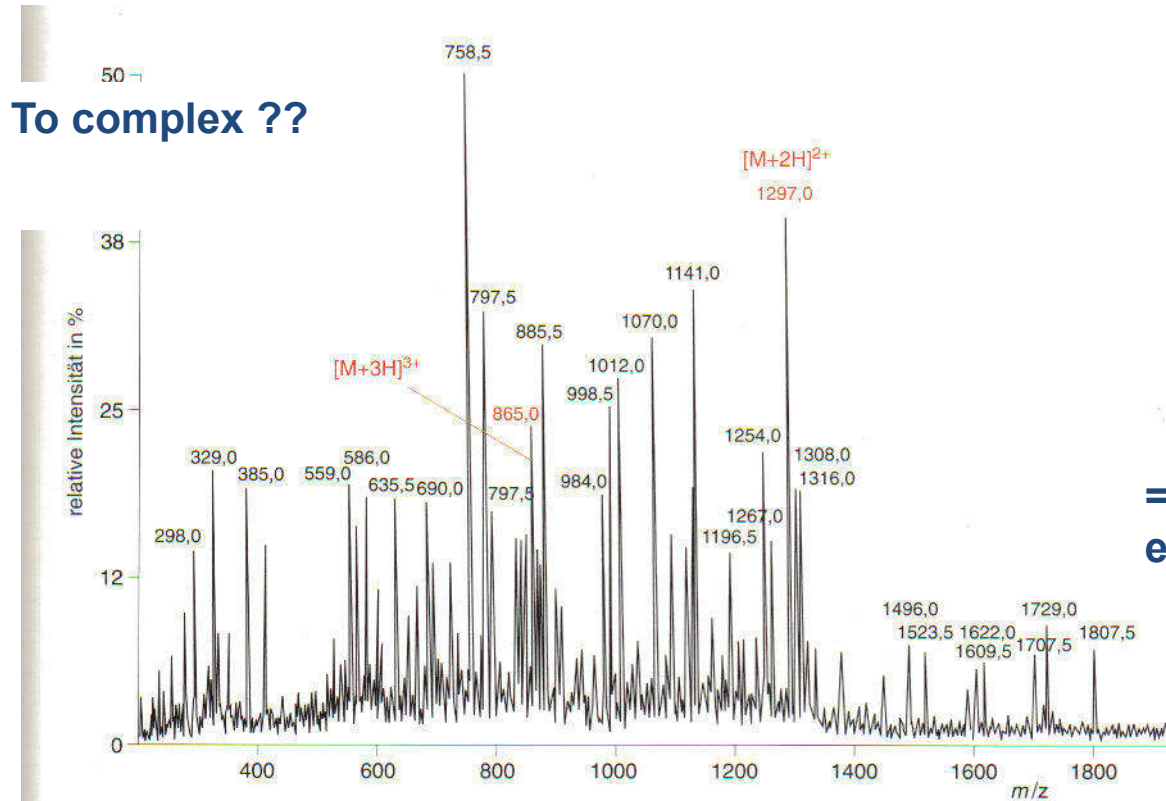


Example for the data interpretation of ESI spectra of peptides with different charge states

To complex?

Peak assignment?

ESI mass spectrometry of peptide mixtures



=> MS/MS based structure elucidation

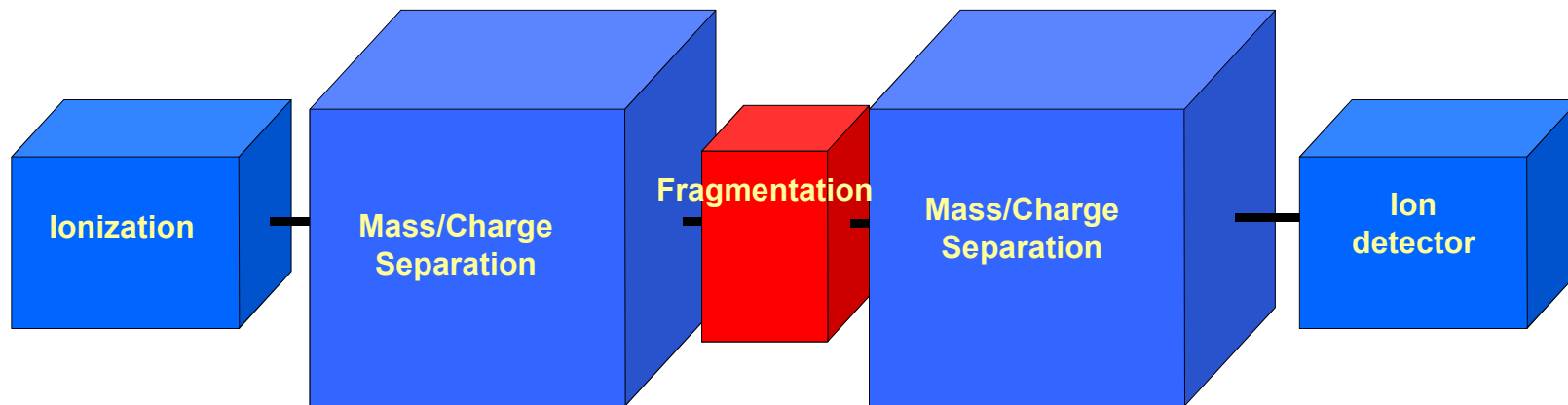
18.10 Elektrospray-Massenspektrum eines hydrophoben Transmembransegmentes (24-mer) ohne basische Aminosäuren. Das Spektrum weist einen $[M+2H]^{2+}$ -Peak bei 1297 amu und einen $[M+3H]^{3+}$ -Peak bei 865 amu auf. Desweiteren deutet die Auswertung der Fragmentierung darauf hin, dass das Hauptprodukt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

Tandem mass spectrometer

Fragmentation of molecular ion

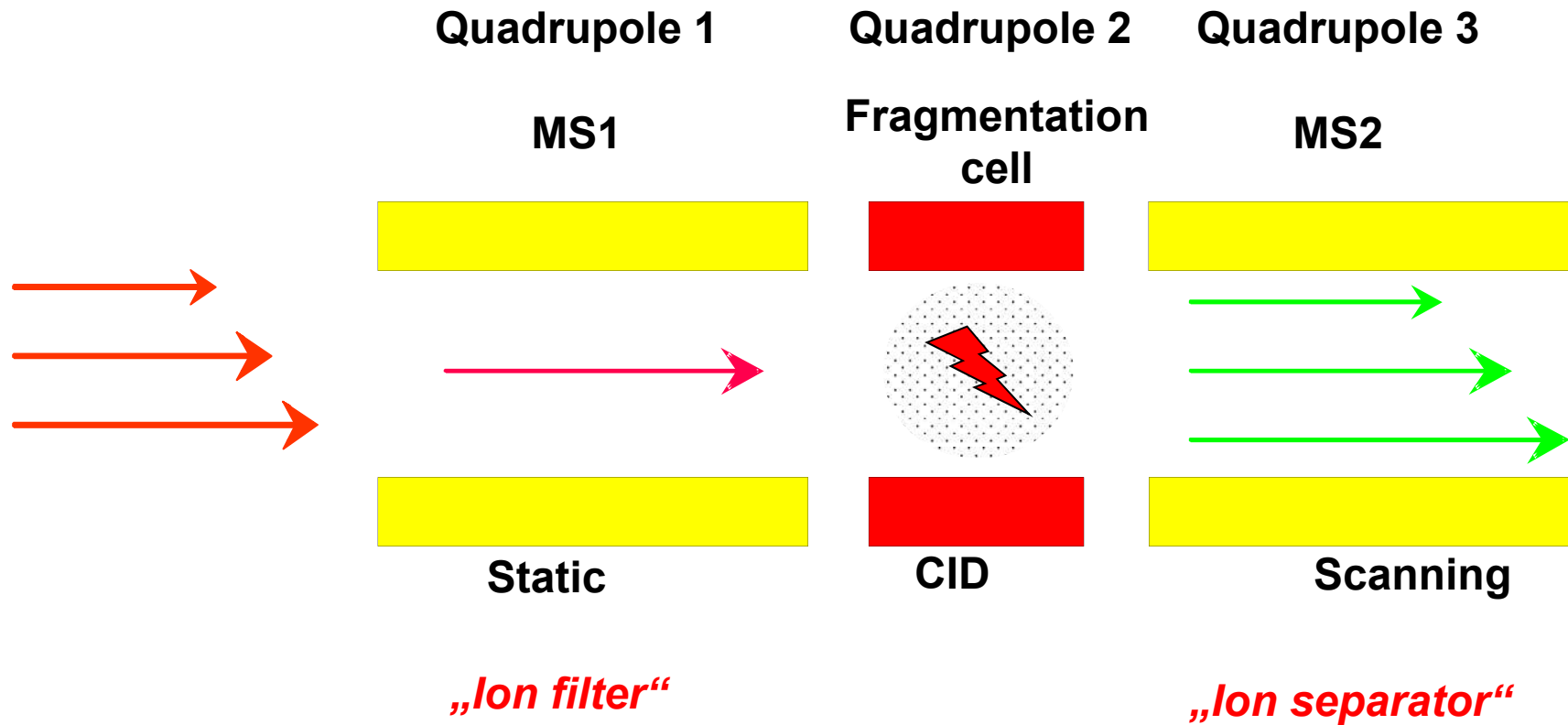
=> Structure elucidation by mass spectrometry

Instrument with MS-MS capability



Fragmentation – Principle

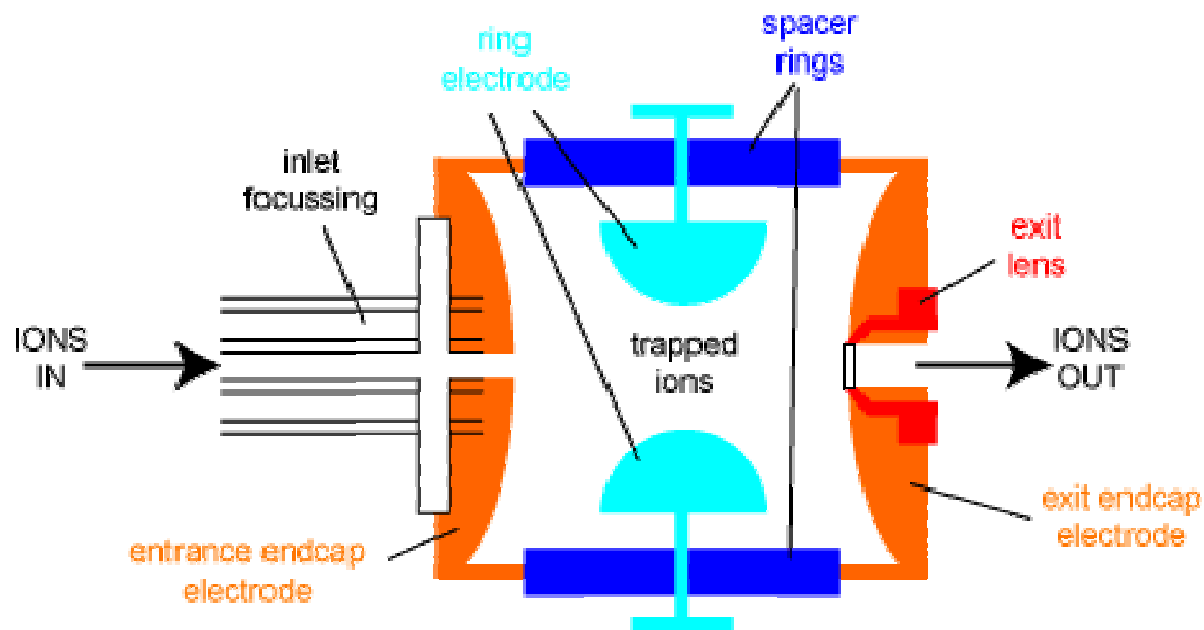
Triple quadrupole instruments



CID: collision induced dissociation

Fragmentation – Principle

Linear ion trap – fragmentation MS^n

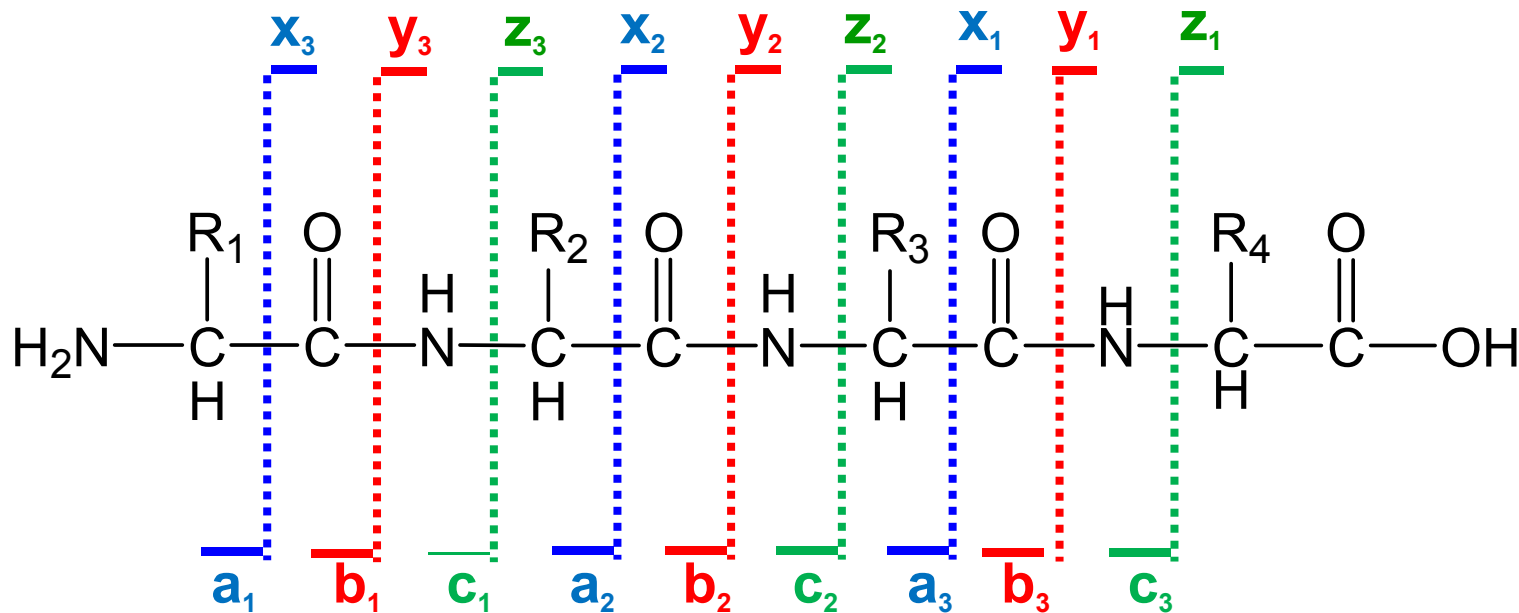


CID: collision induced dissociation
Fragmentation gas, e.g. nitrogen, helium,

Fragmentation – Principle

Fragmentation of peptides by CID

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



N-terminal

a_i, b_i, c_i

i: Zahl AS im Fragment

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

C-terminal

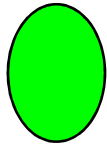
x_i, y_i, z_i

i: Zahl AS im Fragment

Fragmentation – Principle

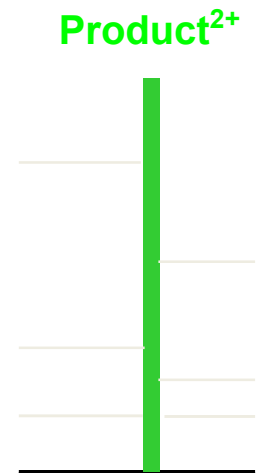
Peptide fragments (low energy MSMS)

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

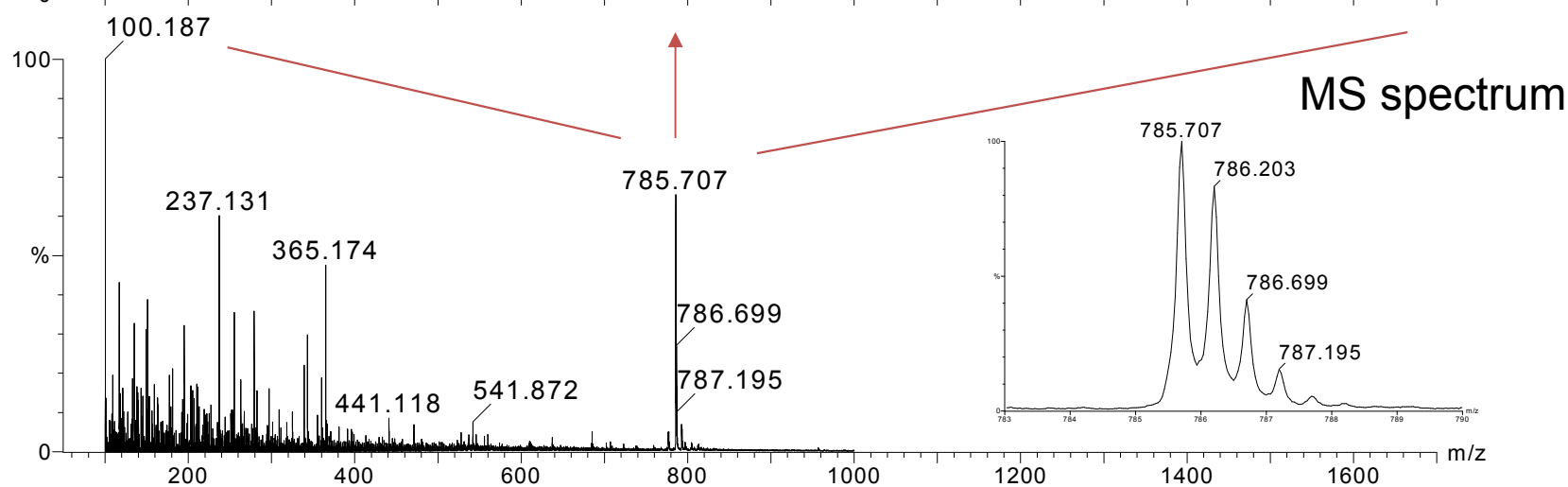
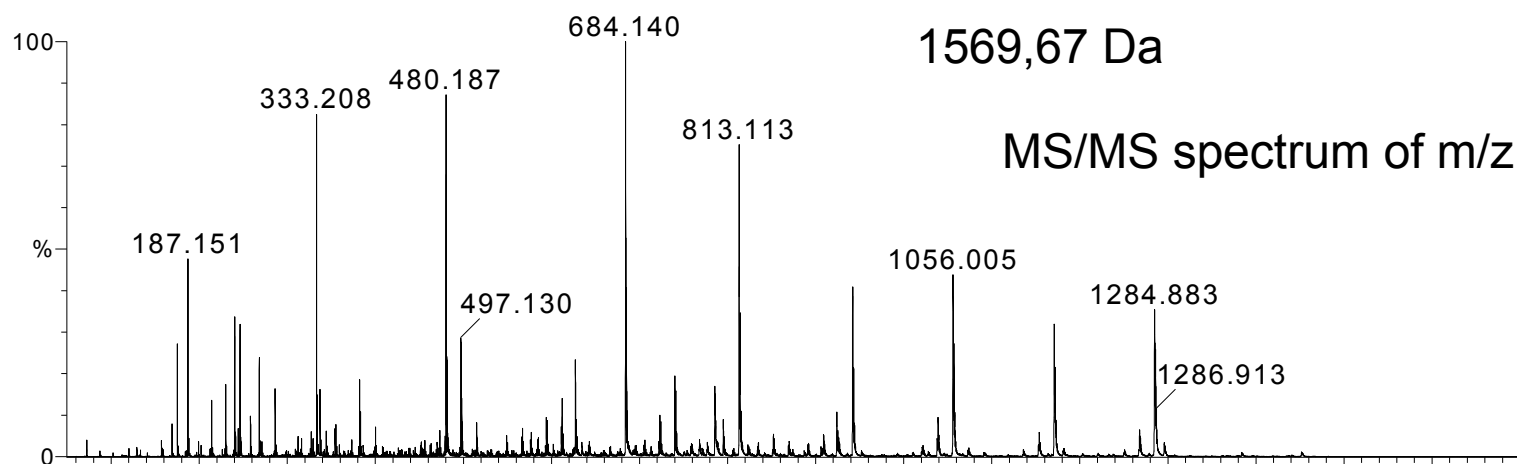
Fragmentation – Example

Peptide fragments (low energy MSMS)

H₂N-EGVNDNEEGFFSAR-OH

1569,67 Da

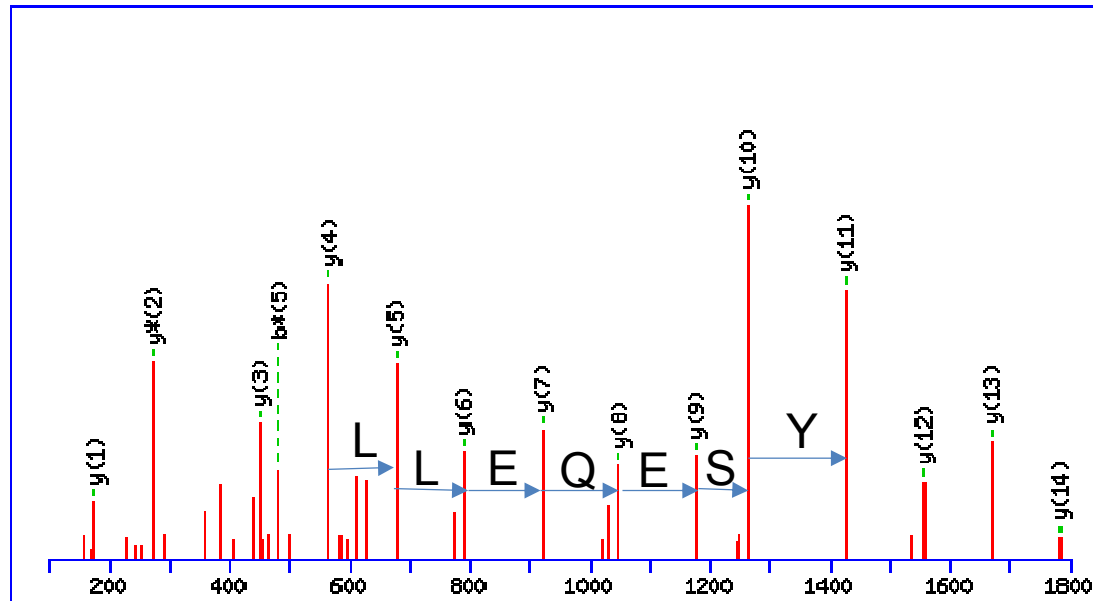
MS/MS spectrum of m/z 785.7



Fragmentation – Identification of a peptide

Difference of fragment masses is characteristic for amino acids

Parent ion 1028.4522 Da, $[M+2H]^{2+}$



#	b ion theor. mass	Seq.	y ion theoretical mass	#
1	102.0550	T		17
2	159.0764	G	1954.8447	16
3	273.1193	N	1897.8232	15
4	386.2034	L	1783.7803	14
5	500.2463	N	1670.6963	13
6	629.2889	E	1556.6533	12
7	792.3523	Y	1427.6107	11
8	879.3843	S	1264.5474	10
9	1008.4269	E	1177.5154	9
10	1136.4855	Q	1048.4728	8
11	1265.5281	E	920.4142	7
12	1378.6121	L	791.3716	6
13	1491.6962	L	678.2876	5
14	1606.7231	D	565.2035	4
15	1766.7538	C	450.1765	3
16	1881.7807	D	290.1459	2
17		R	175.1190	1

BUT: terminal amino acid information is usually missing!

Identification by MS/MS – peptide sequence tag

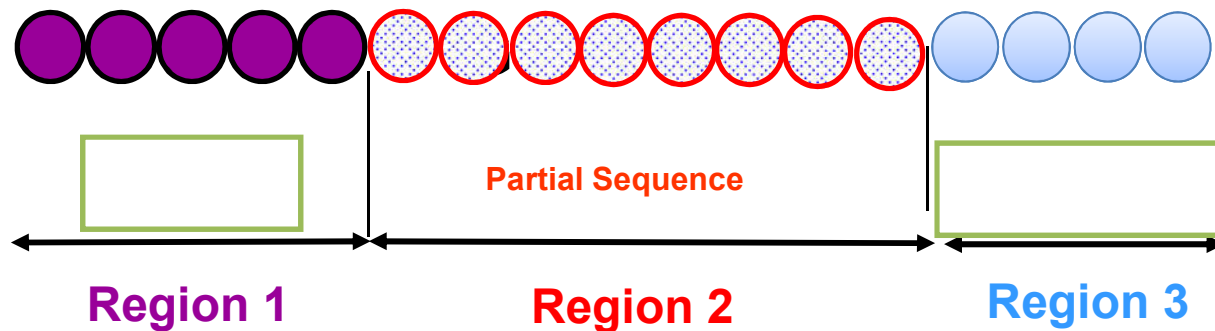
Sequence Tag = 4 informations for the protein identification using databases

- 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)

Peptide measured molecular wt = 1569.7



Identification of a protein using a sequence tag

Find a Protein

Search by: Pattern & MW

1345.70

Find

Mass Accuracy [%]: .005

1.00

< MW [kDa] <

1000.00

Peptides required for match:

Max numb. missed cleavages:

Sequence Pattern:

(734.39)SVS(IL)(1120.60)

Sort

Remove

Clear...

Highlight

Add

Protein Info

Sort

Acce. Number:

swiss|P00921

CAH2_BOVIN

CARBONIC

ANHYDRASE I

Seq. Type

☒ AA Seq

☐ DNA Seq

970.441

972.549

978.477

1000.519

1011.535

1017.488

1345.691

1580.810

2097.870

2197.211

2252.148

2353.099

pl: 8.95

28508.3 Da

25 peptides

SHHWGYGKHGPHWHKDF

PIANGERQSPVNIDTKA

VVQDPALKPLALVGEA

TSRRMVNNGHSFNVEYD

DSQDKAVLKDGPLTGT

RLVQFHFWGSSQGSEH

TVDRKKYAAELHLVHWN

TKYGDFTAAQQPDGLA

VVGVLKVGDNALPQK

VLDALDSIKTKGKSTDF

PNFDPGSLLPNVLDYWT

YPSLTTPPLLESVTWI

VLKEPISVSSQQMLKFR

TLNFNAEGEPPELLMLAN

WRPAQPLKNRQVRGFPK

//

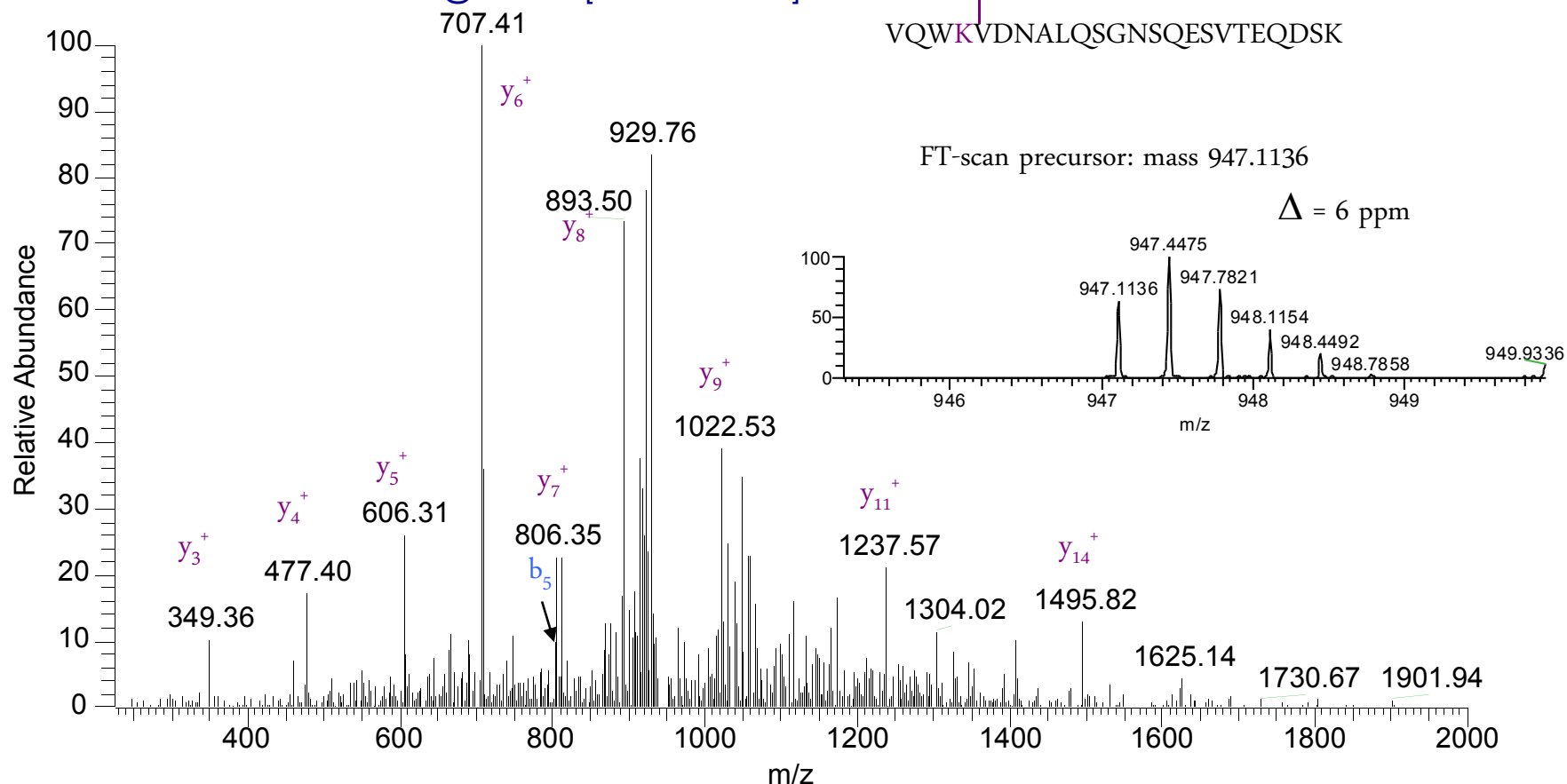
nrdb.index(10Jan99)

	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

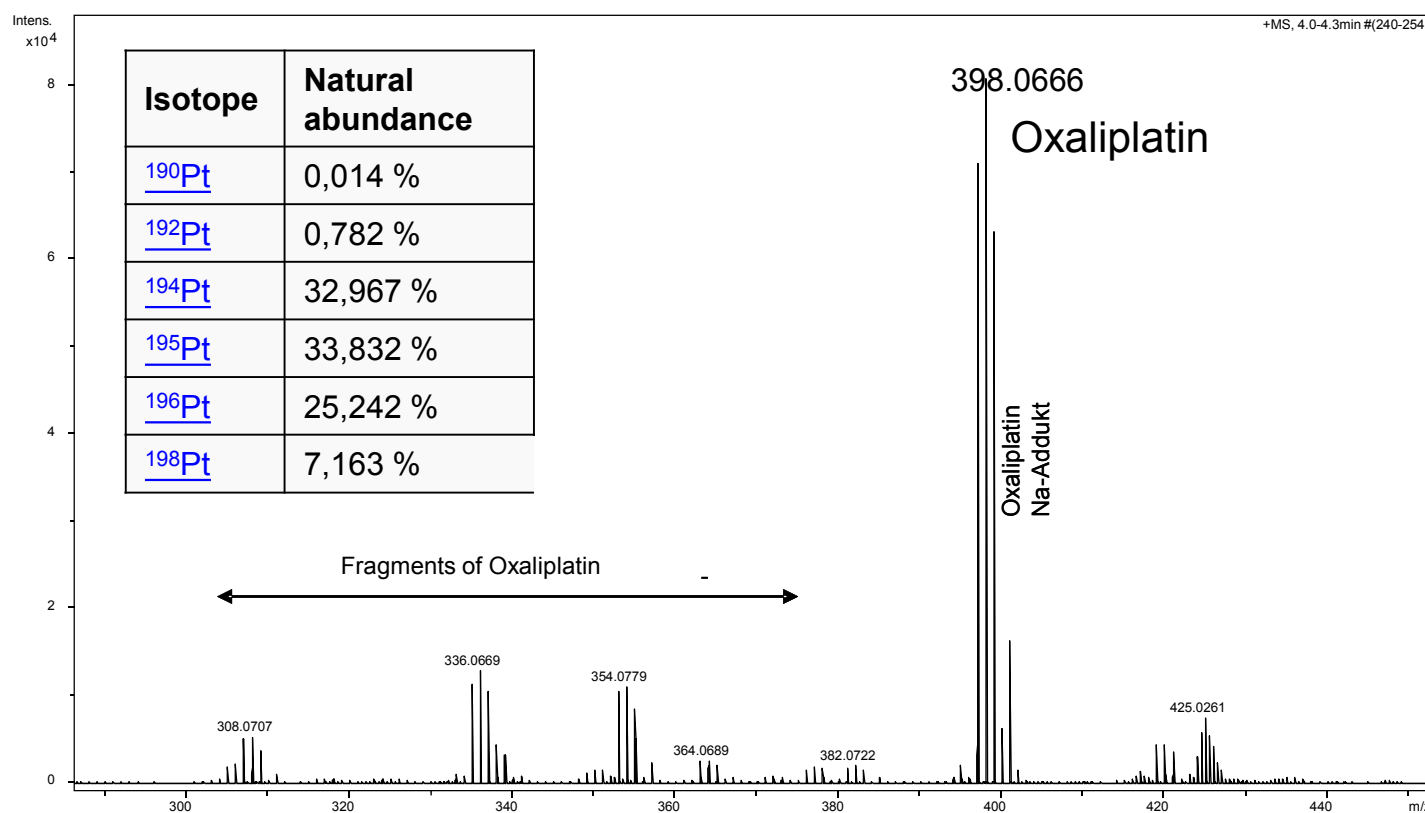
LC-MS examples: site specific ID of non enzymatic glycation

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]

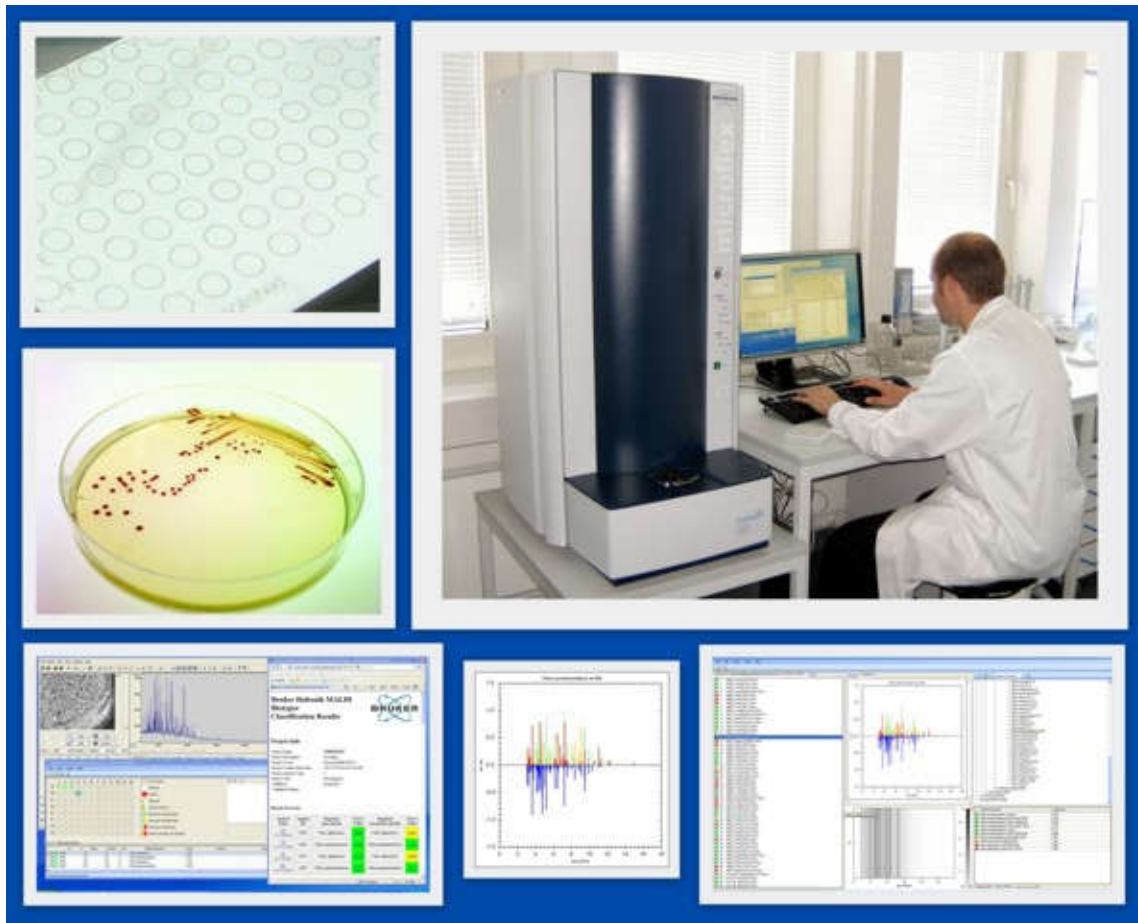


Mass spectrometry of chemotherapeutics

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.

NMI
schafft Ergebnisse



➔Faster than PCR or metabolic pathway Analysis

- Generic experimental setup
- robust method, minimal sample preparation



- 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
- → can be classified as „Class 1 Medical Device“ intended for use in the diagnosis of disease

Hot topics (applications):

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