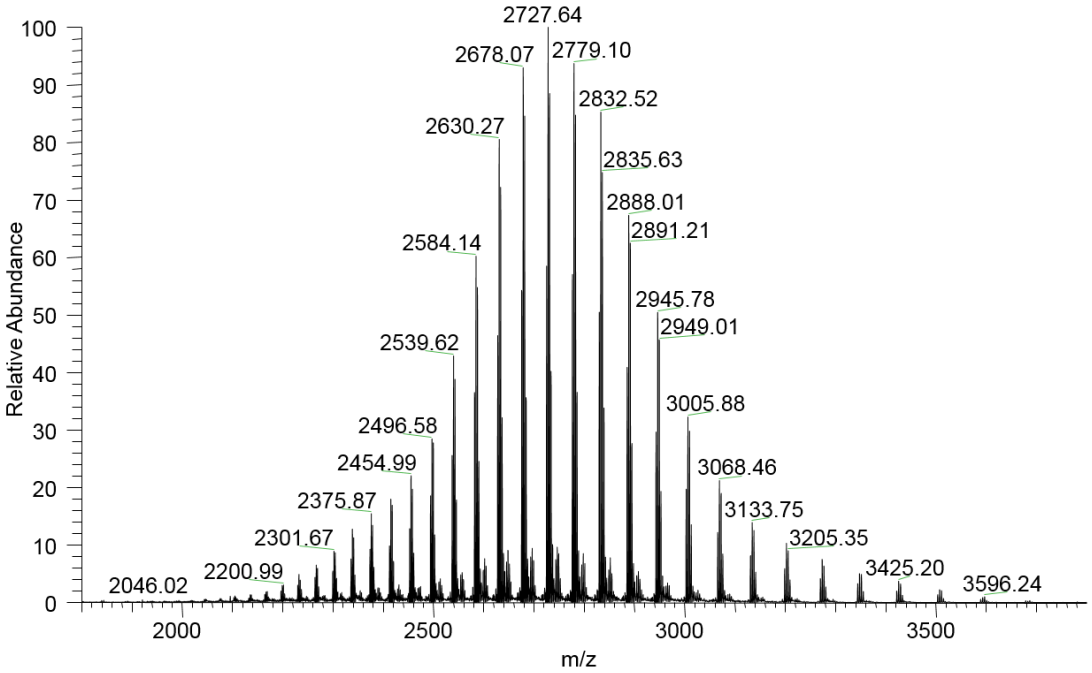
Protein analysis using mass spectrometry

Michael Stadlmeier



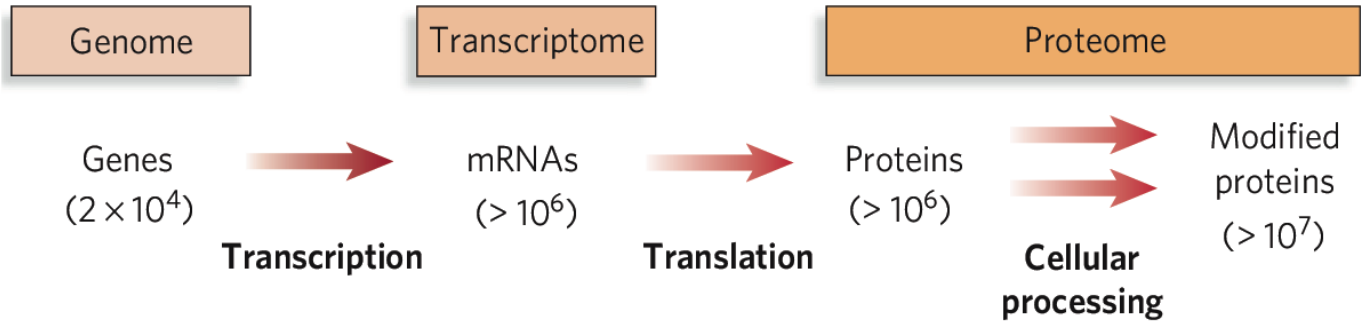
Literature

http://www.carellgroup.de/teaching/master

What is Proteomics?

The proteome is:

- the entire set of proteins in a given cell at a certain time
- highly dynamic
- very complex



→ Mass spectroscopy is ideally suited for proteomics!

Outline

1) **Mass spectrometry in general**
    1) What is MS?
    2) Why MS?
    3) Definitions

2) **Mass spectrometer**
    1) Principles
    2) Details

3) **Applications**
    1) Intact proteins
    2) Protein Identification
    3) Quantification

4) **Research in Progress**

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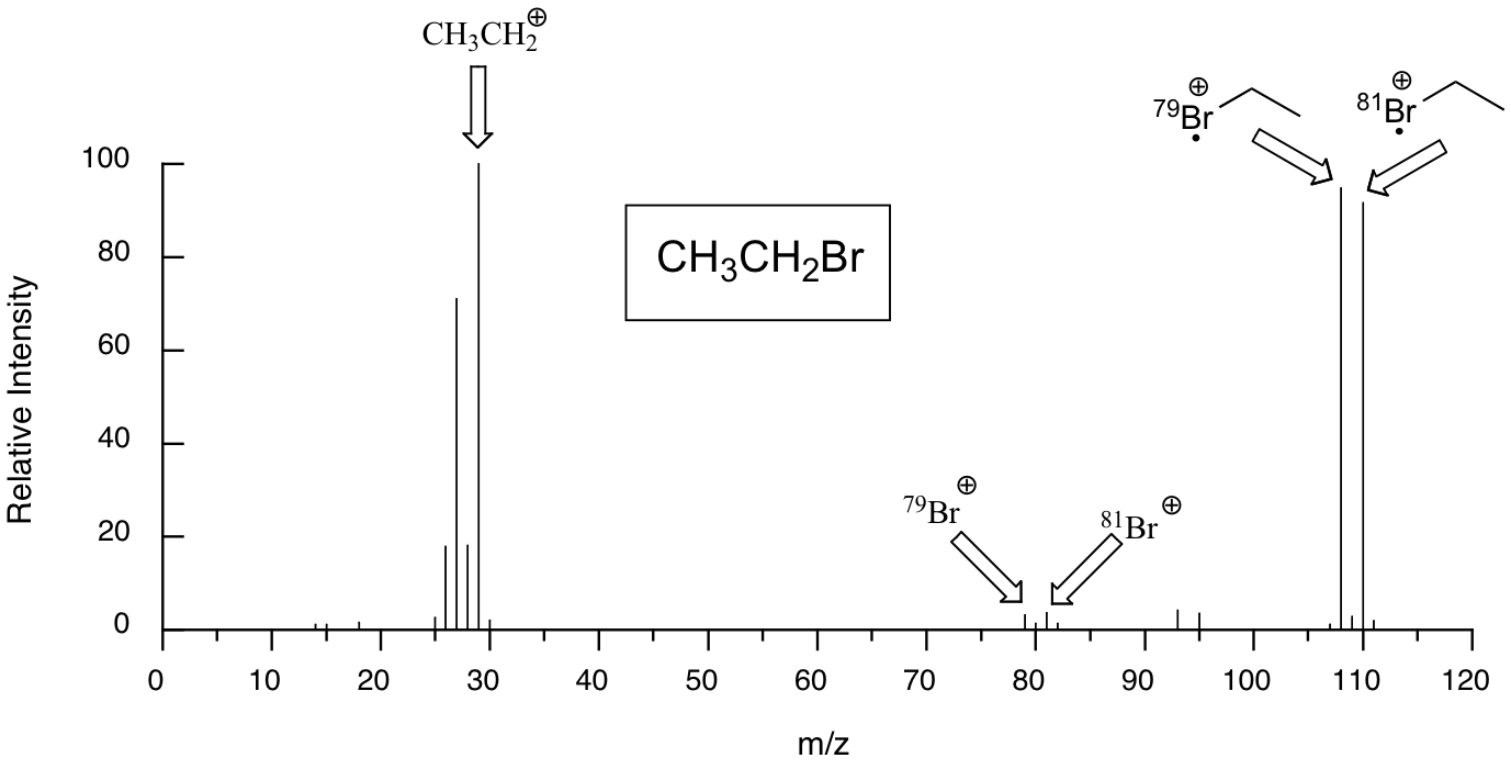
What‘s mass spectrometry

- A mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio

- identify type/amount of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions.



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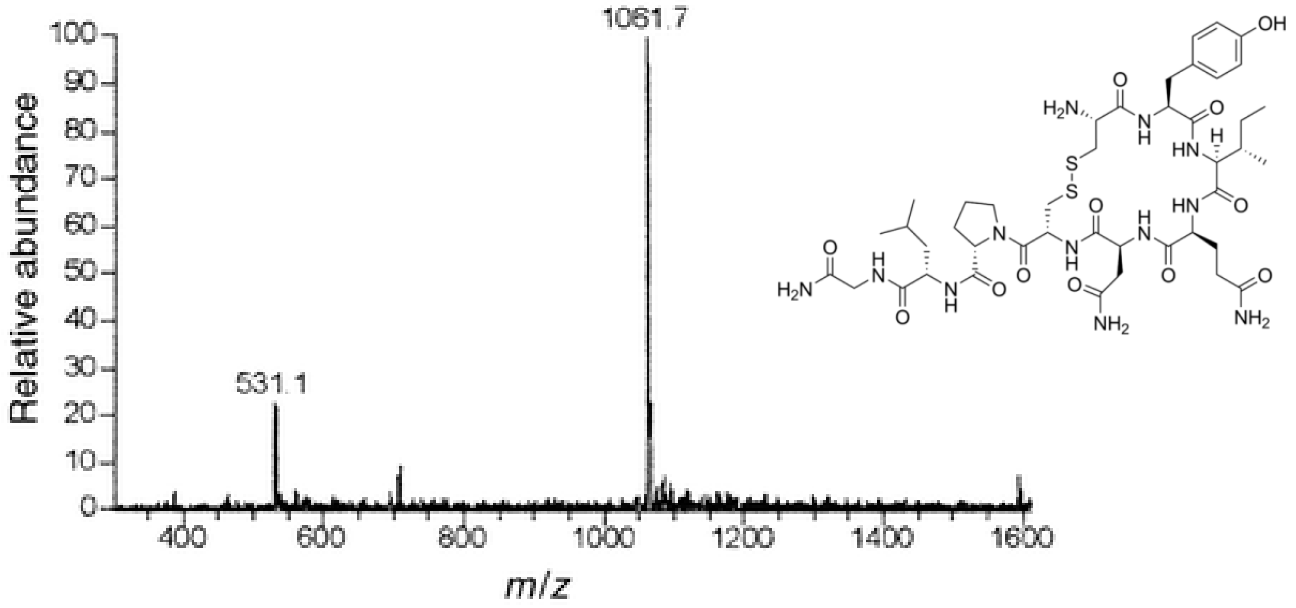

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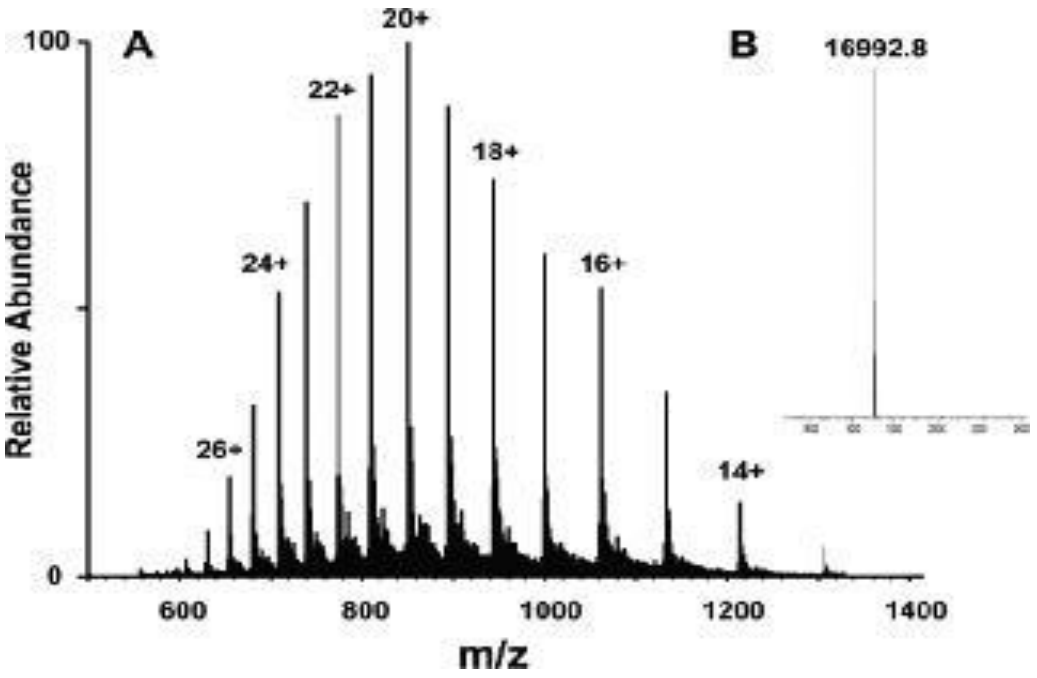
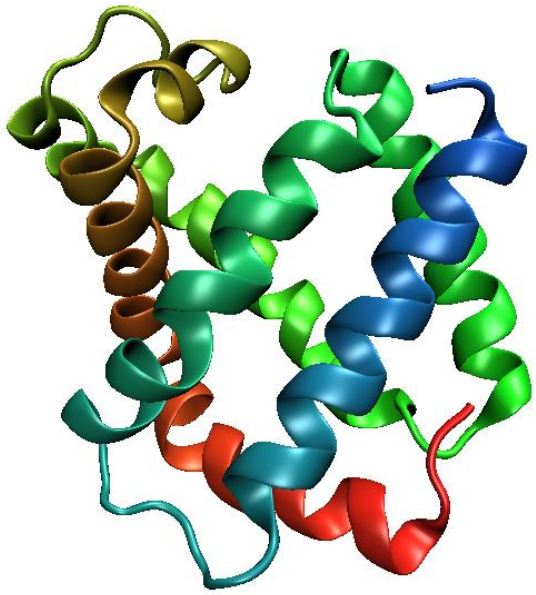




Why mass spectrometry?

- **Universal** detector: DNA, RNA, proteins, lipids, small molecules (compare to Edmann)
- Enables to **identify** and not only to detect (compare to immunoassays)
- Enables **quantification** if a standard/reference is introduced (compare to WB)
- Rather **easy** method (compare to crystallography)
- Fast easy data interpretation (compare to NMR)
- **Sensitive** down to attomoles (compare to SDS-PAGE)
- …

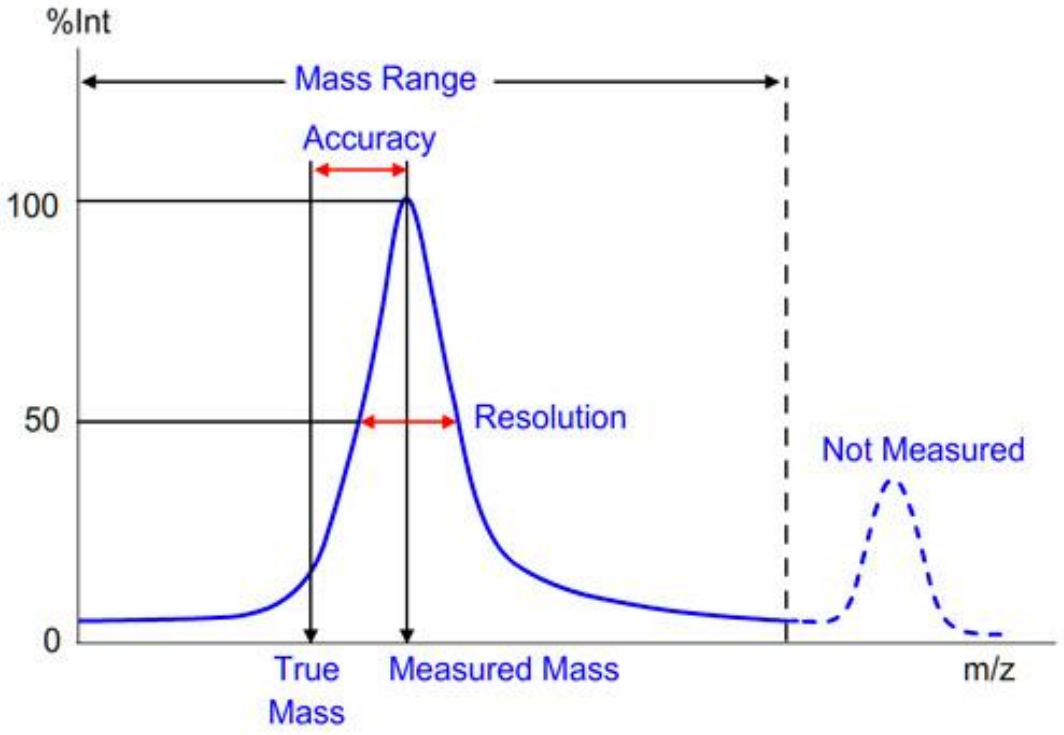
Important! Take a good look at it for the exam preparation ;)

Definitions: Accuracy, Mass Range, Speed

Mass Accuracy is the determined difference between the calculated (true) exact mass and the measured mass. [difference often in parts per million (ppm)]

The **Mass Range** is the m/z range in which ions are detected.

The **Speed** is defined by the number of spectra per unit time.



Quality of mass spectrometers

The quality of a mass spectrometer can be defined by:

- Resolution

- Mass accuracy

- Sensitivity

- Scan speed

- Mass Range

- Price and Reliability ☺

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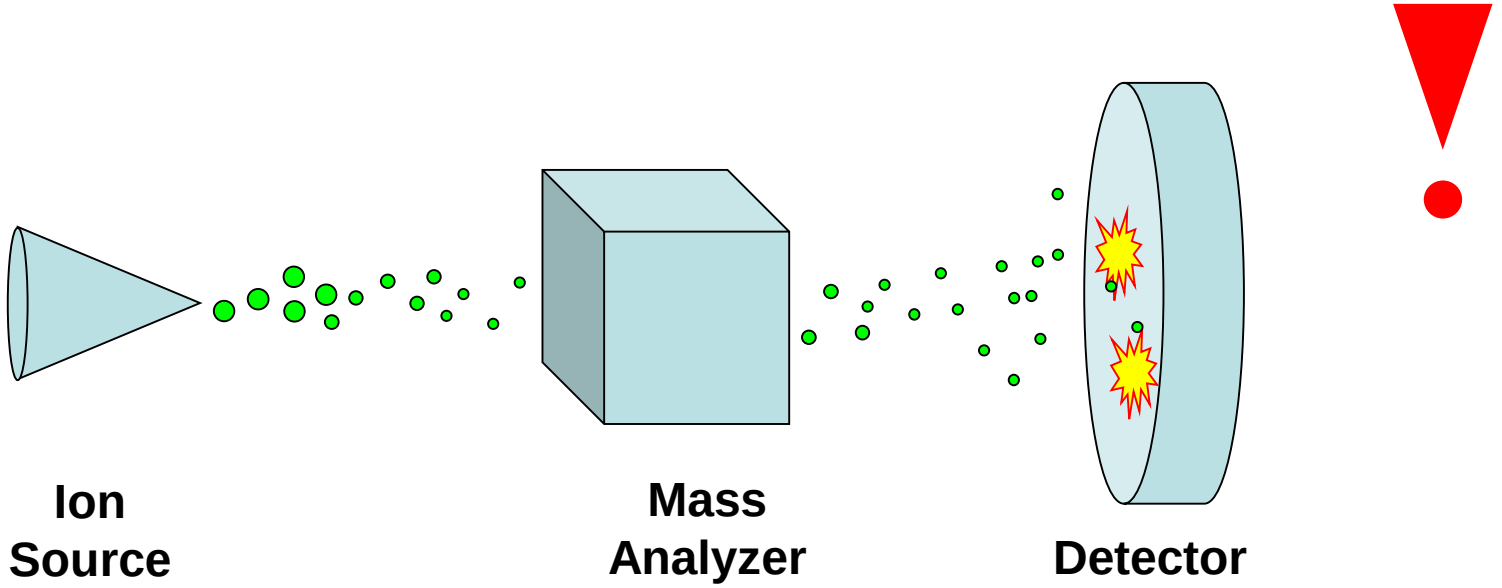
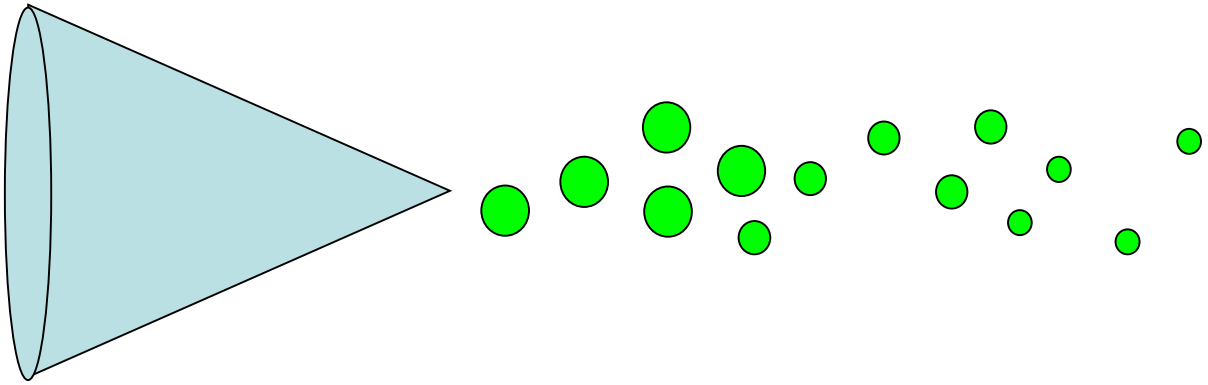
Components of a mass spectrometer



Inlet	Ionizer		
Target plate	MALDI	TOF	Electron Multiplier
HPLC / nLC	ESI	sector	Array Detector
GC		Quadrupole	Orbitrap
others		Ion Trap	FTMS

Ionisation



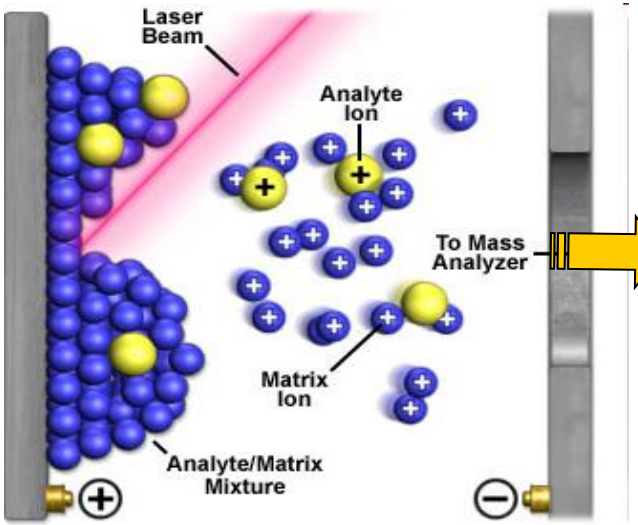
Ionizer

MALDI

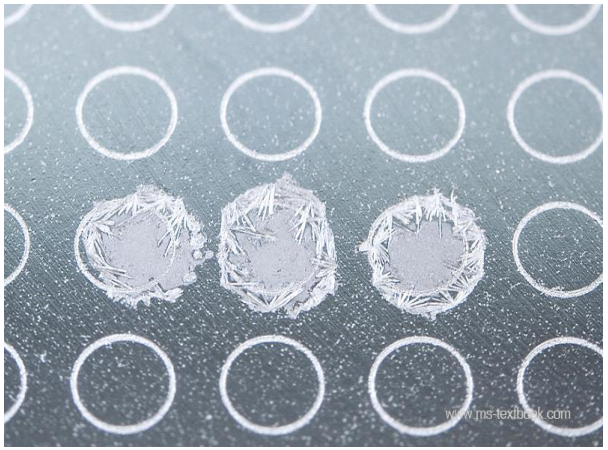
ESI

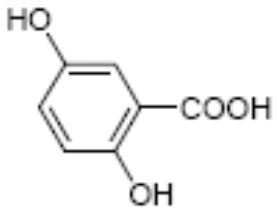
MALDI
(Matrix assisted laser desorbtion/ionization – time of flight)

- Sample ionization by a laser beam
- Fast/easy/robust (e.g. “Biotyper” in clinics)
- Matrix absorbs the laser energy and desorbs the samples
- Perfectly suited for biopolymers

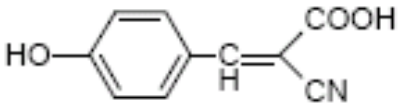


Matrices:





2,5 dihydroxy benzoic acid

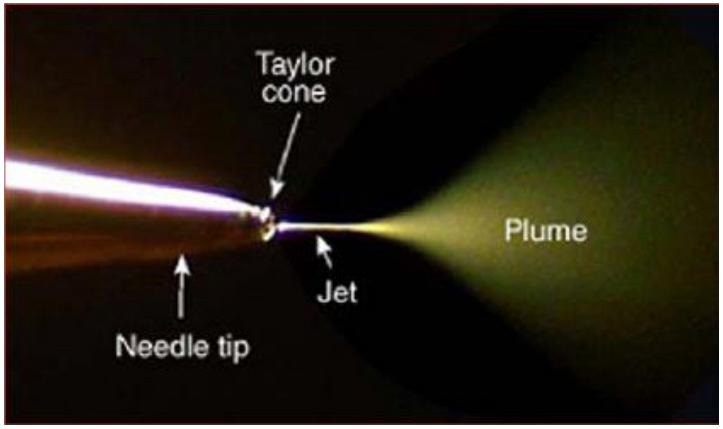


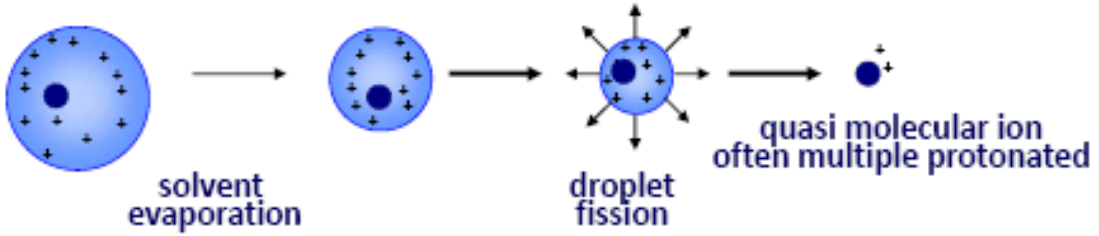
α-cyano-4-hydroxy cinnamic acid

Compound	Other Names	Applications
2,5-dihydroxy benzoic acid	DHB, Gentisic acid	peptides, nucleotides , oligonucleotides, oligosaccharides
3,5-dimethoxy-4-hydroxycinnamic acid	sinapic acid; sinapinic acid; SA	peptides, proteins, lipids
4-hydroxy-3-methoxycinnamic acid	ferulic acid	proteins
α-Cyano-4-hydroxycinnamic acid	CHCA	peptides, lipids, nucleotides
Picolinic acid	PA	oligonucleotides
3-hydroxy picolinic acid	HPA	oligonucleotides

ESI
(Electrospray Ionization)

- at needle mm-sized droplets are formed
- *via* drying gas and/or a heated capillary the droplets evaporate until nm-sized droplets remain
- through further solvent evaporation or ion emission quasi molecular ions are formed that are often multiple charged (protonated)





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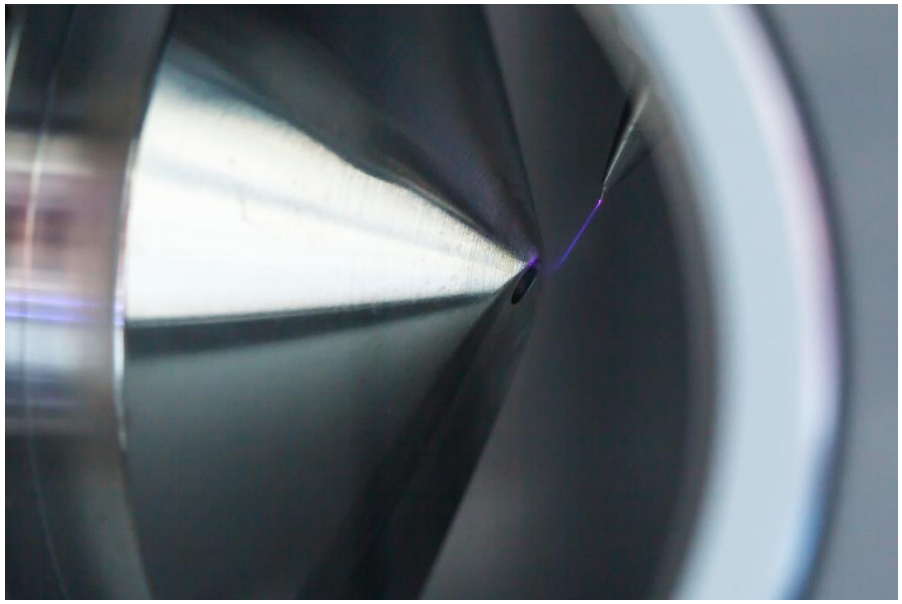

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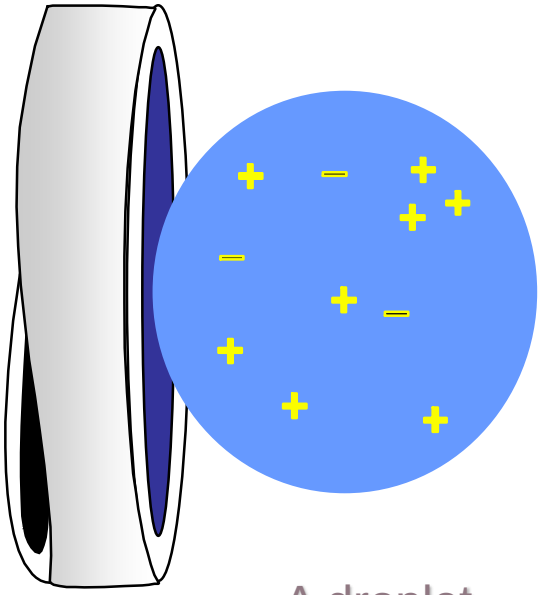
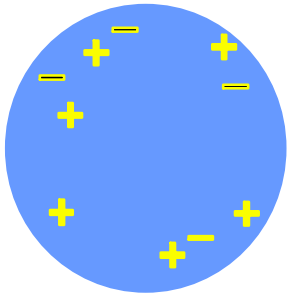
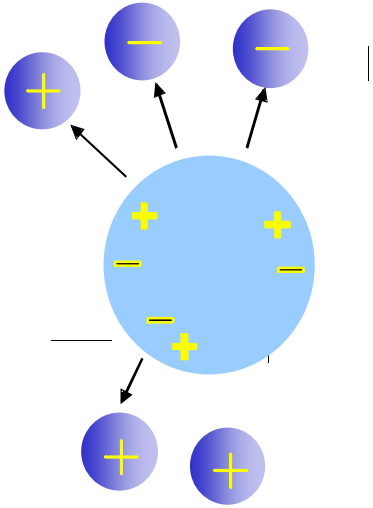


Capillary
+4 kV

A droplet containing (mostly positive) ions is formed.

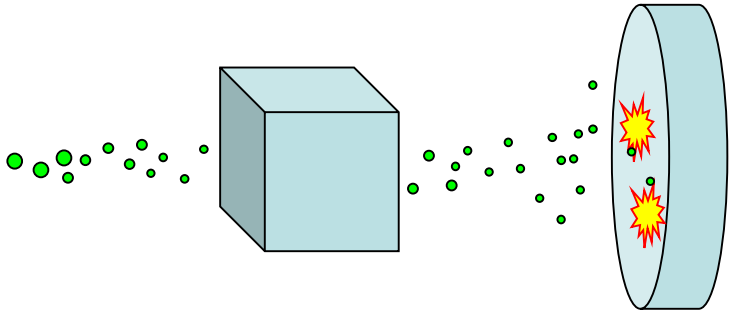


As the solvent evaporates, the field increases and the ions move to the surface.



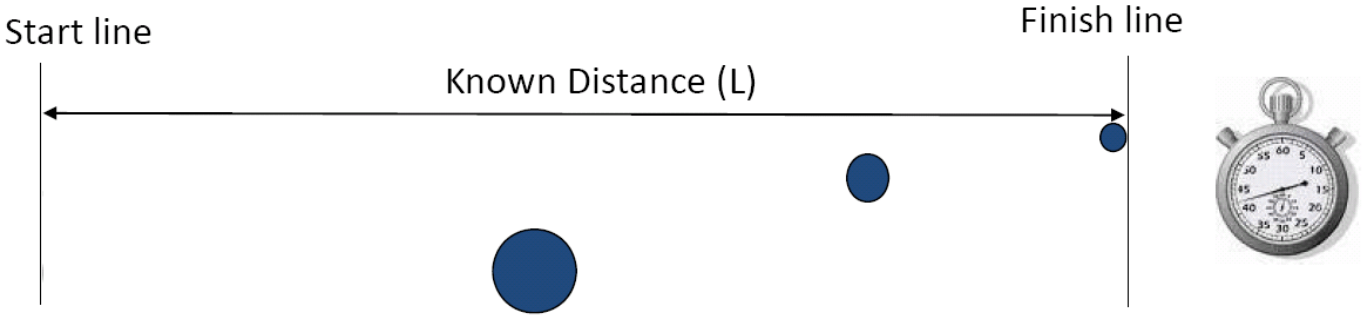
At the Rayleigh limit the droplet becomes unstable and releases smaller ion droplets.

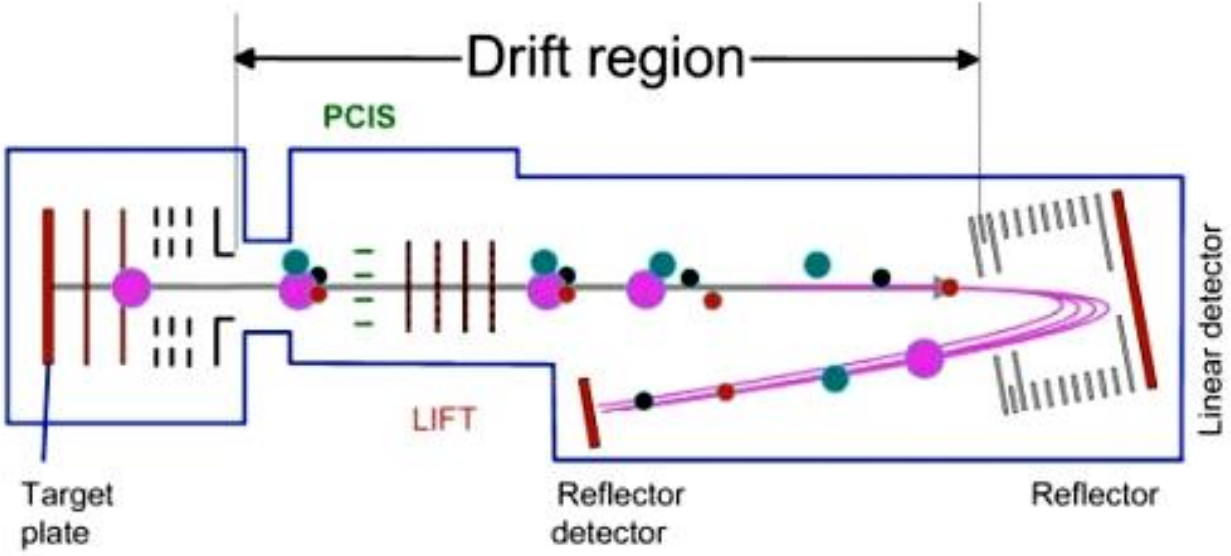
Mass analyzer/detector



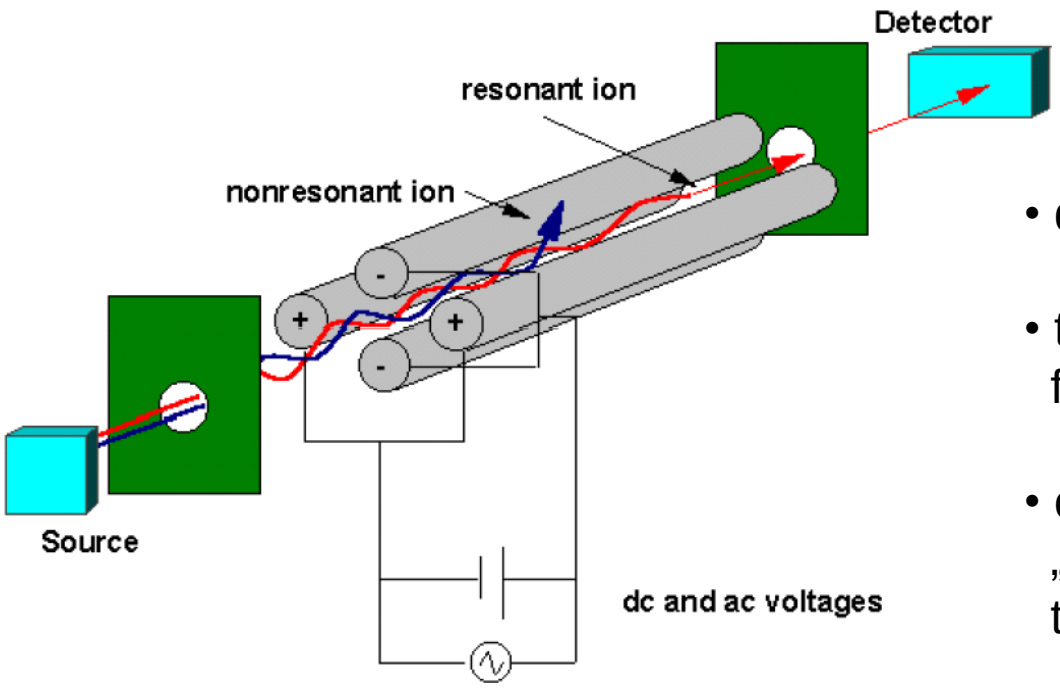
Mass Analyzer	Detector
TOF	Electron Multiplier
sector	Orbitrap
Quadrupole	FTMS
Ion Trap	

TOF
(Time of Flight)





Quadrupole



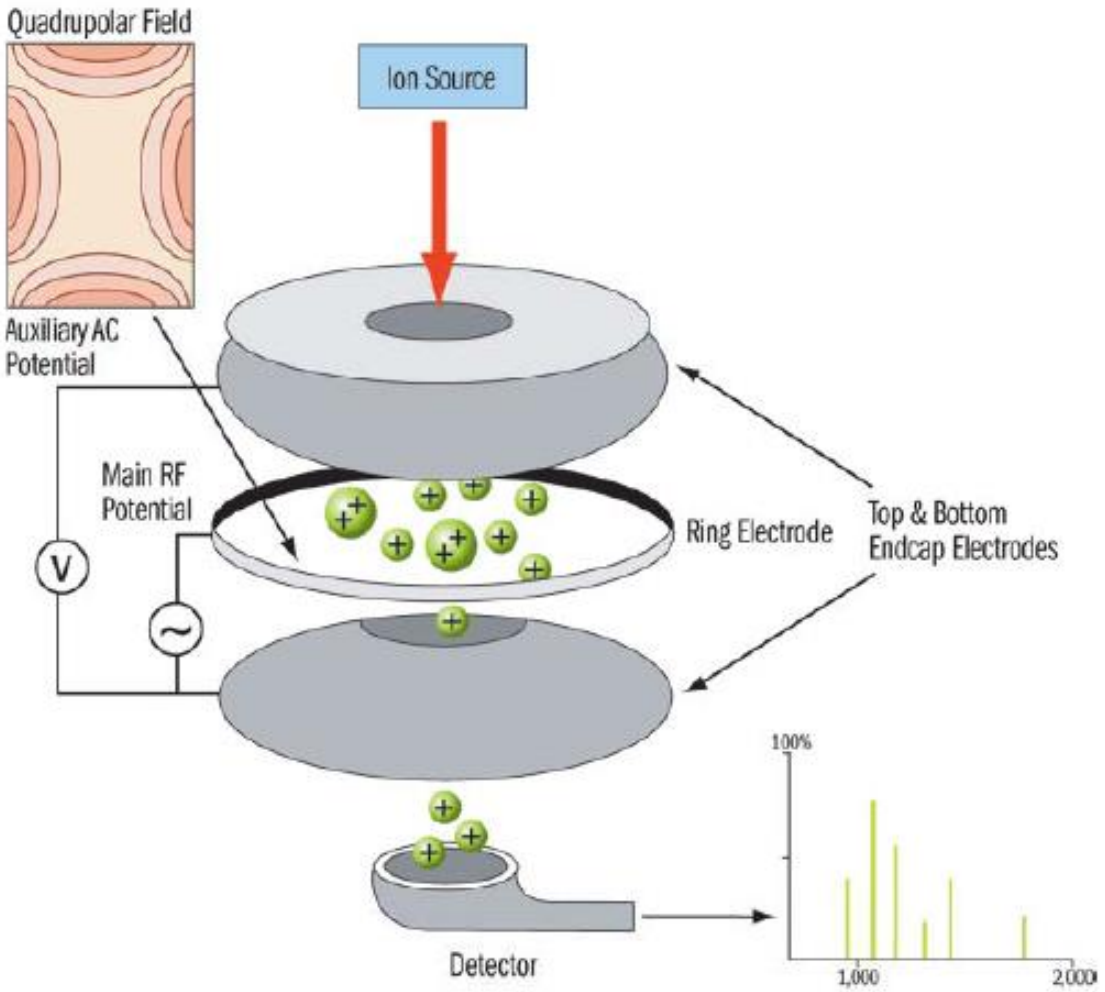
- quadrupole consists of 4 metal rods
- to opposing rods, a certain radio frequency (RF) voltage is applied
- only ions with a certain m/z-ratio are „resonant ions“ and have stable trajectories through the quadrupole
- to generate a mass spectrum, the voltage is changed and the whole mass range gets scanned and detected

Ion Trap

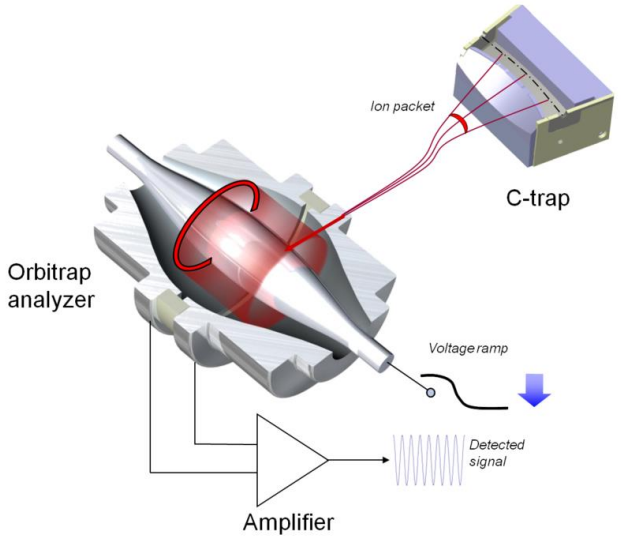
Ion traps are ion trapping devices that make use of a three-dimensional quadrupole field to trap and mass-analyze ions

invented by Wolfgang Paul (Nobel Prize1989)

Offer good mass resolving power, and MS^n capability.



Orbitrap analyzers/detectors



- ion trap with no magnetic or RF-fields → trapping around cylindrical central electrode (@ 5kV)
- voltage ramp while the ions are injected leads to stable spiral trajectories → principle of electrodynamic squeezing
- ions move along the central electrode with a certain frequency: electrostatic attraction VS centrifugal force; they “orbit” the central electrode in the form of ion rings
- the moving ions create an image current in the outer electrodes, which is amplified and measured

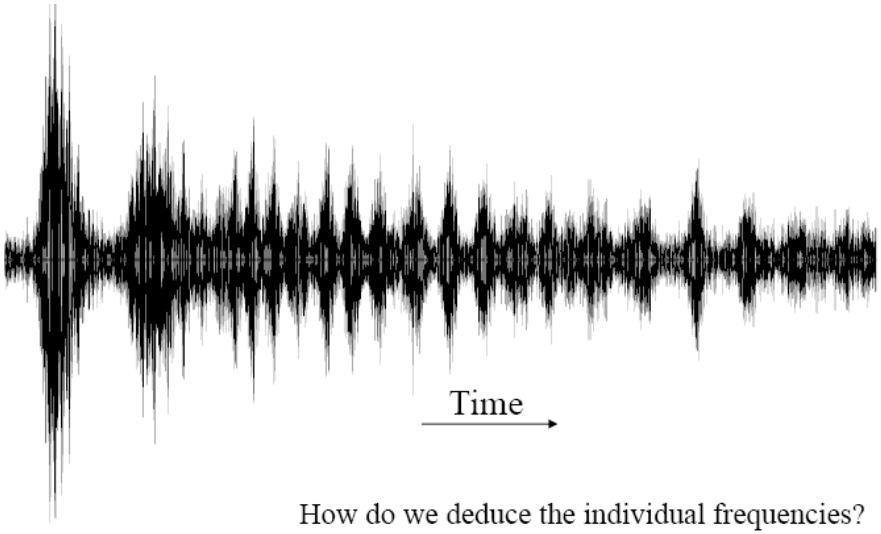
Fourier Transformation

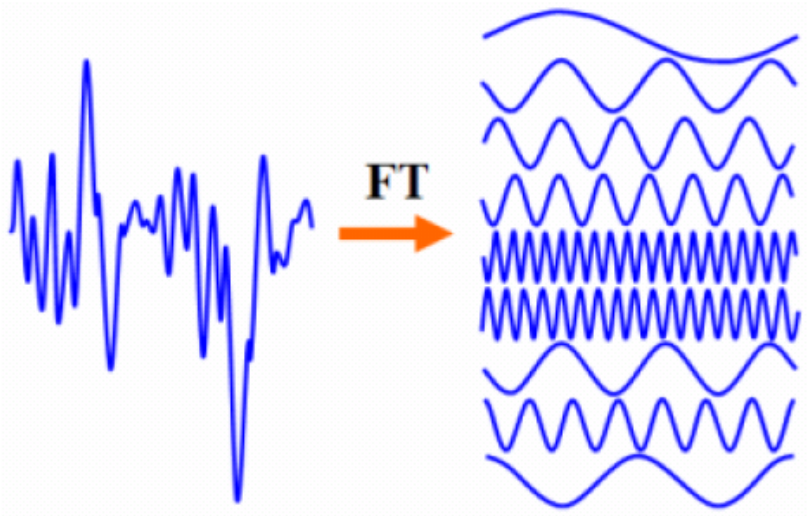
Samples are typically not just 1 ion but several ions and on top there will be ions with several different m/z values.

Luckily waves do not affect each other and so within the ‘messy’ image current, the waves are present intact.

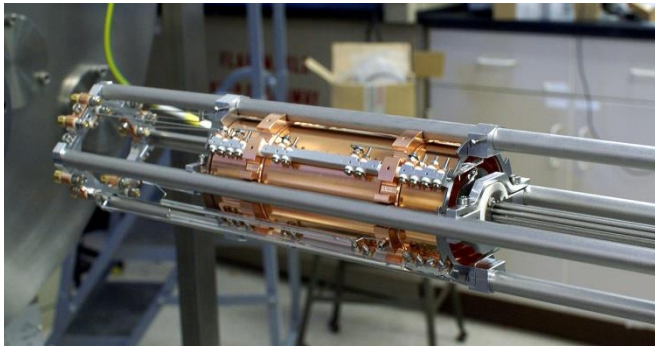
Fourier transform is a mathmatical technique which can deduce the frequencies present.

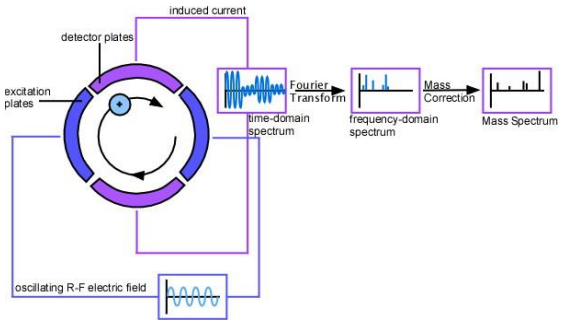
The measured frequencies are proportional to the m/z-ratio: $\omega = \sqrt{\frac{k}{m/z}}$





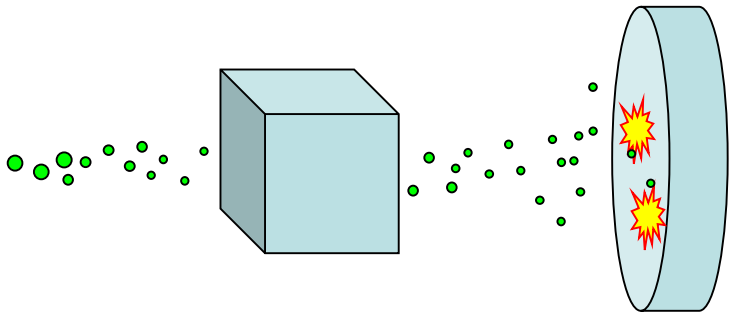
FT-Ion cyclotron resonance (FT-ICR)





- principle of operation is similar to the Orbitrap
- instead of electrical fields, a strong magnetic field is employed → He-cooling!
- the ions circle after excitation at their cyclotron frequencies (m/z-dependent)
- image current in the confining electrodes is detected and again, a mass spectrum is created by Fourier transformation

Mass analyzer/detector



Mass Analyzer | **Detector**

TOF
sector
Quadrupole
Ion Trap

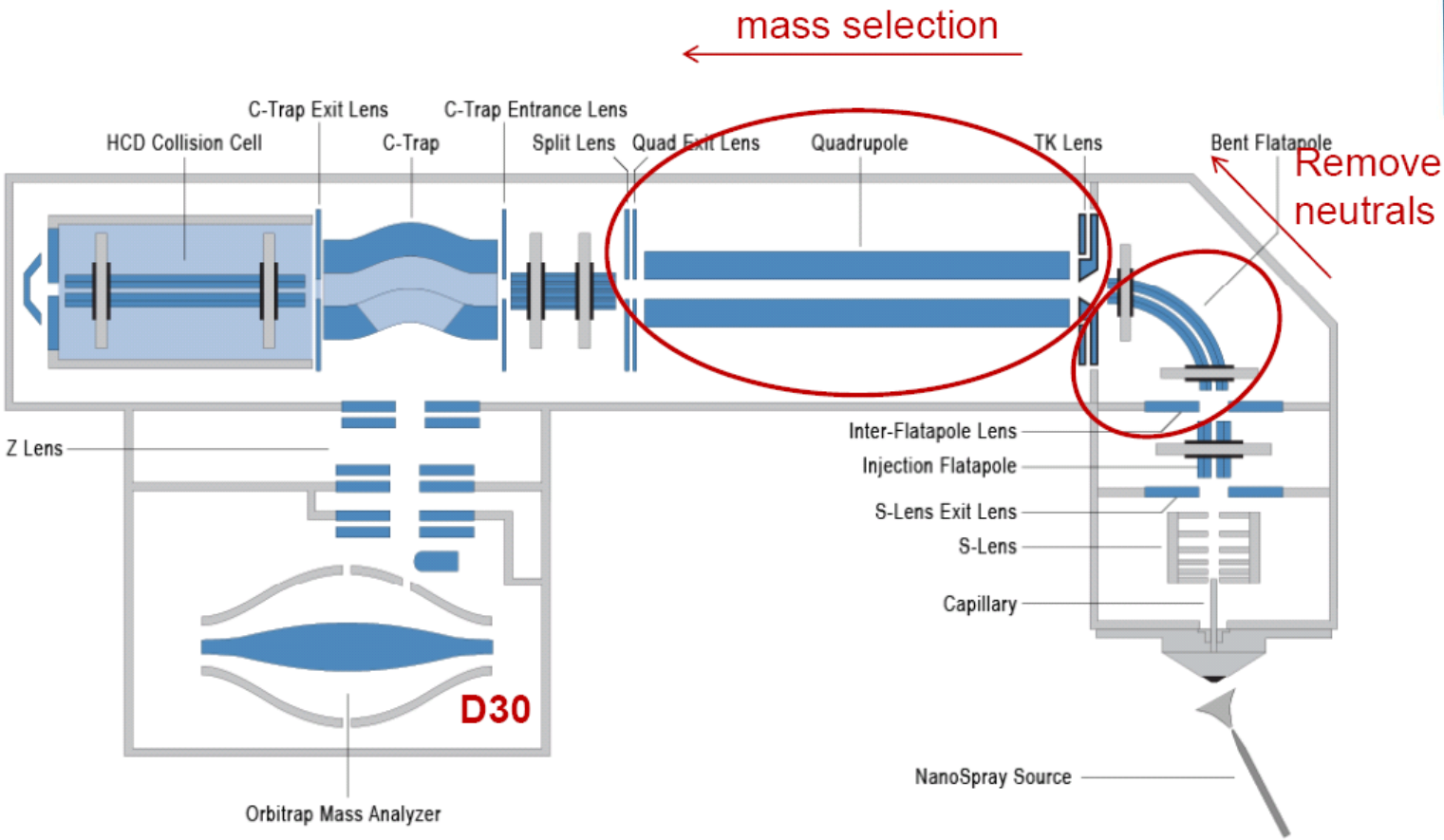
Electron Multiplier

Orbitrap
FTMS

- Time-of-Flight Analyzer (TOF)
  – Good resolution, exact mass, fast, no upper m/z limit, costly
- Quadrupole Analyzer (Q)
  – Low resolution, fast, cheap
- Ion Trap Mass Analyzer (QIT)
  – Fair resolution, all-in-one mass analyzer
- Orbitrap (FTMS)
  – High resolution, exact mass, costly
- Ion Cyclotron Resonance (FT-ICR, FTMS)
  – Highest resolution, exact mass, very costly (liquid helium cooling)

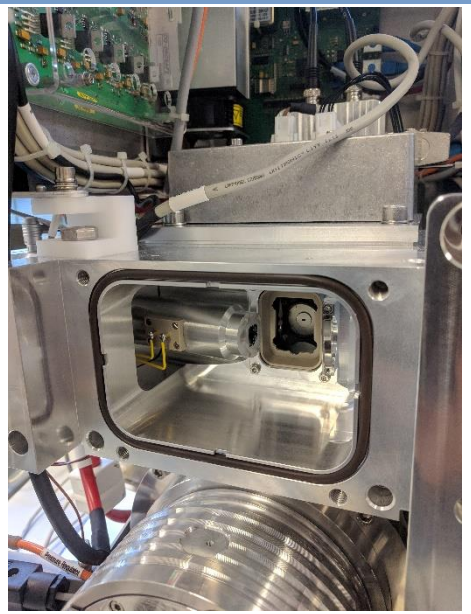
→ **most mass spectrometers combine different analyzers and detectors to give hybrid instruments and enable fragmentation experiments**

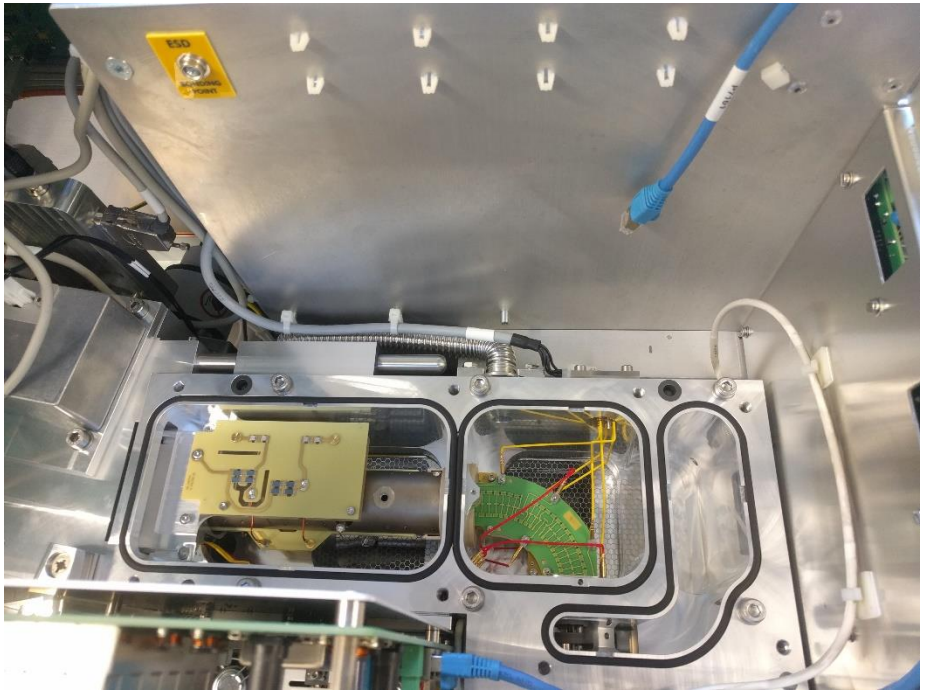
QExactive Operation Principle

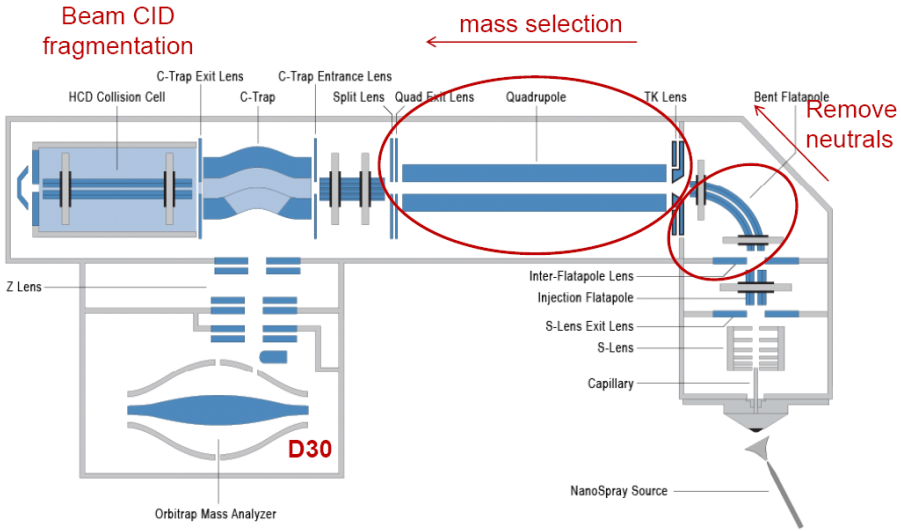


high resolution MS and MS/MS

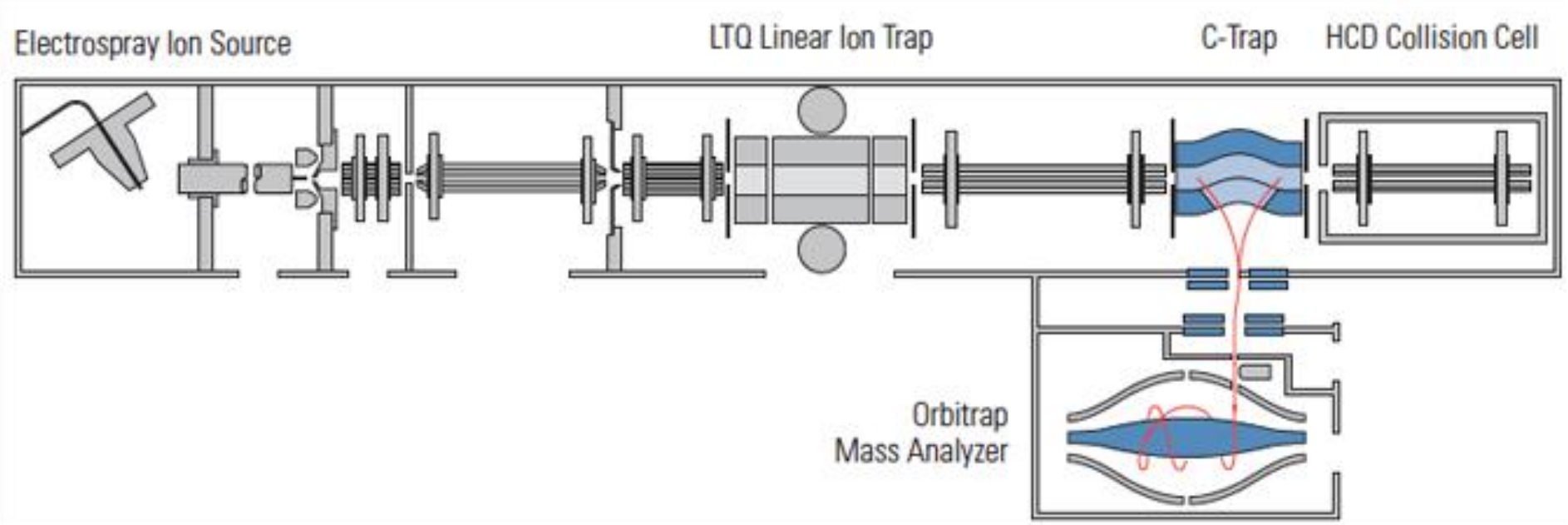
QExactive Operation Principle







LTQ Orbitrap Operation Principle



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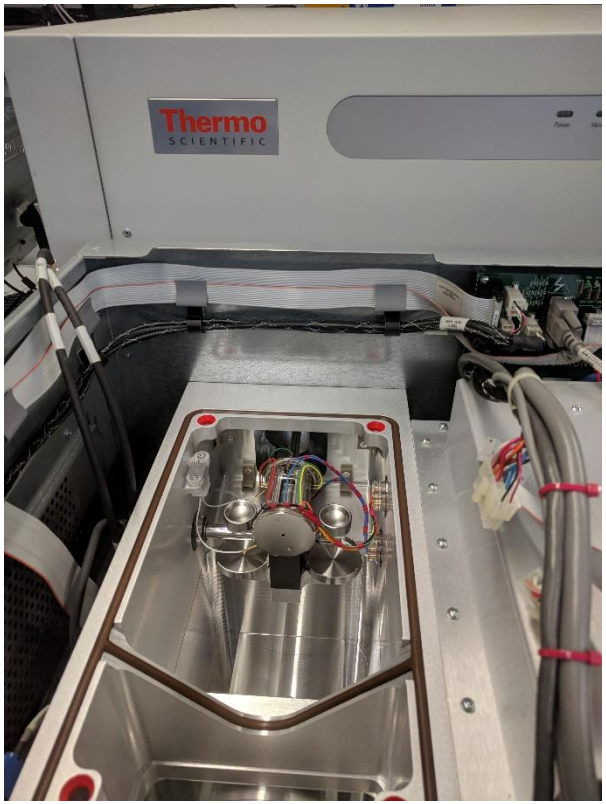
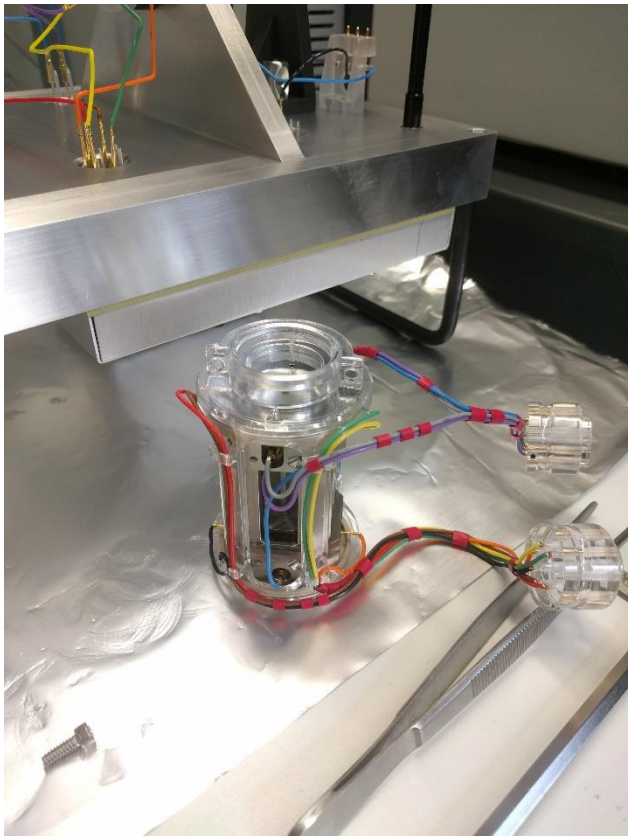
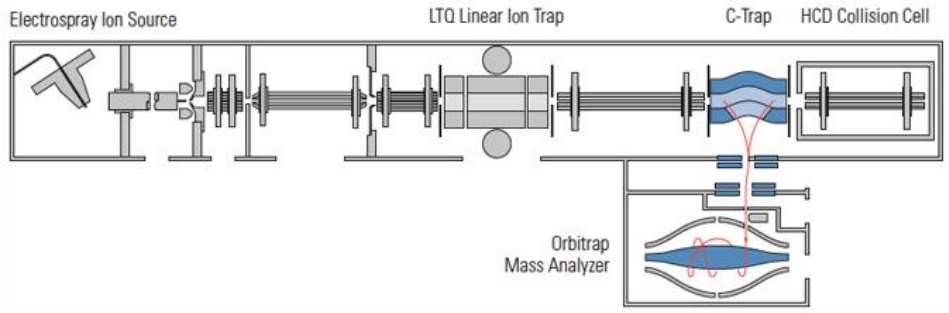


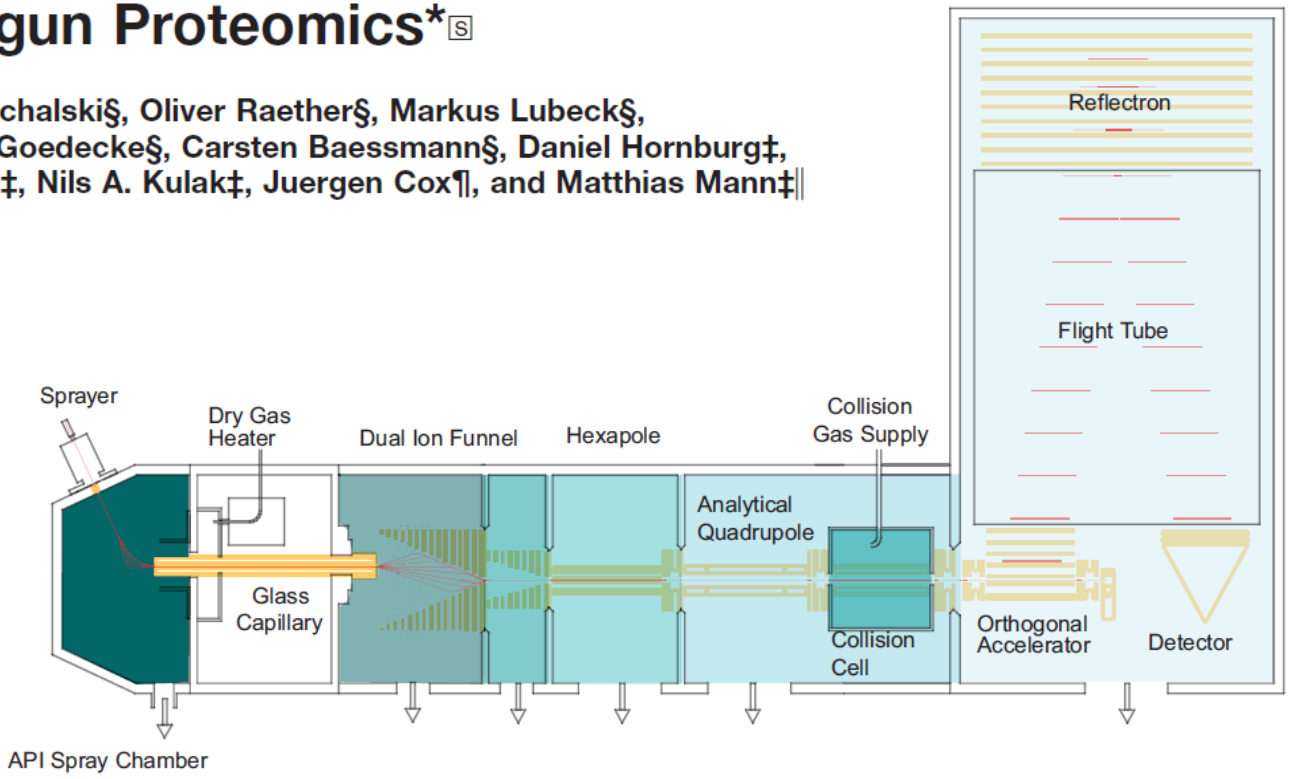




QTOF Operation Principle

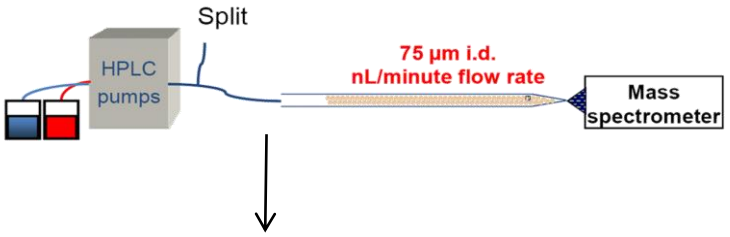
The Impact II, a Very High-Resolution Quadrupole Time-of-Flight Instrument (QTOF) for Deep Shotgun Proteomics*

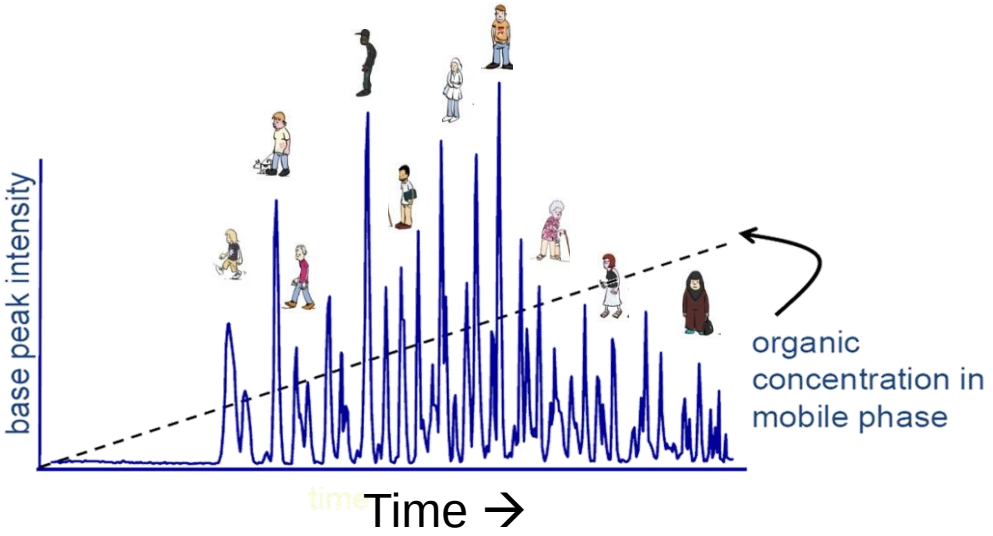
Scarlet Beck‡, Annette Michalski§, Oliver Raether§, Markus Lubeck§, Stephanie Kaspar§, Niels Goedecke§, Carsten Baessmann§, Daniel Hornburg‡, Florian Meier‡, Igor Paron‡, Nils A. Kulak‡, Juergen Cox¶, and Matthias Mann‡‖



Liquid Chromatography coupled to MS – Why?

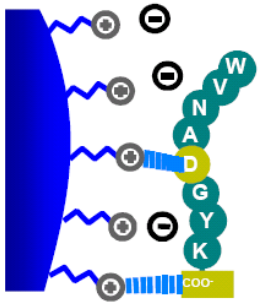
Because it makes life easier



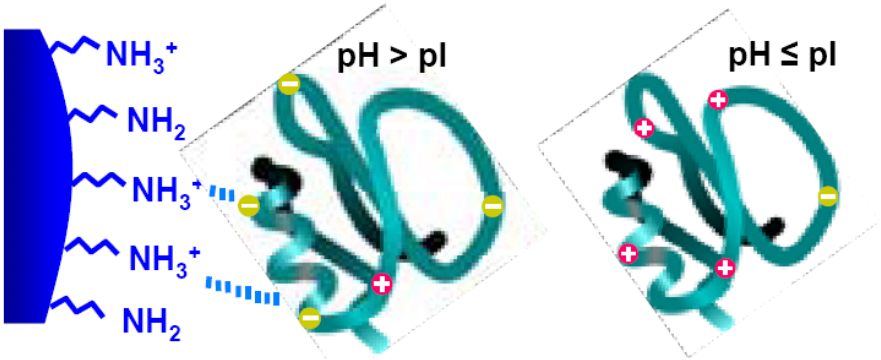


Liquid Chromatography

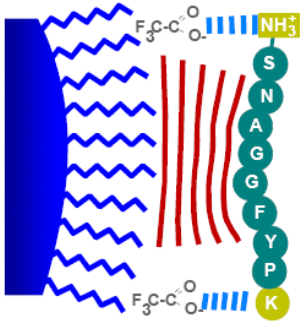
Chromatographic modes for proteins & peptides



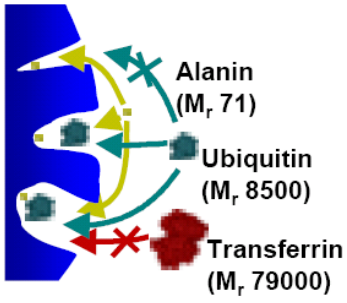
Ion exchange-HPLC:
salt gradient



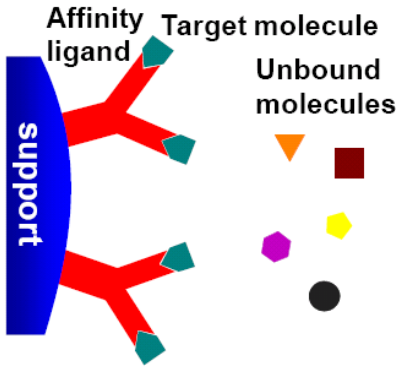
Chromatofocusing:
pH gradient



(Ion-pair)-reversed-phase HPLC:
acetonitrile gradient (in trifluoroacetic acid)



Size exclusion-HPLC



Affinity chromatography:
pH- or salt gradient

Applications of Orbitrap spectrometers

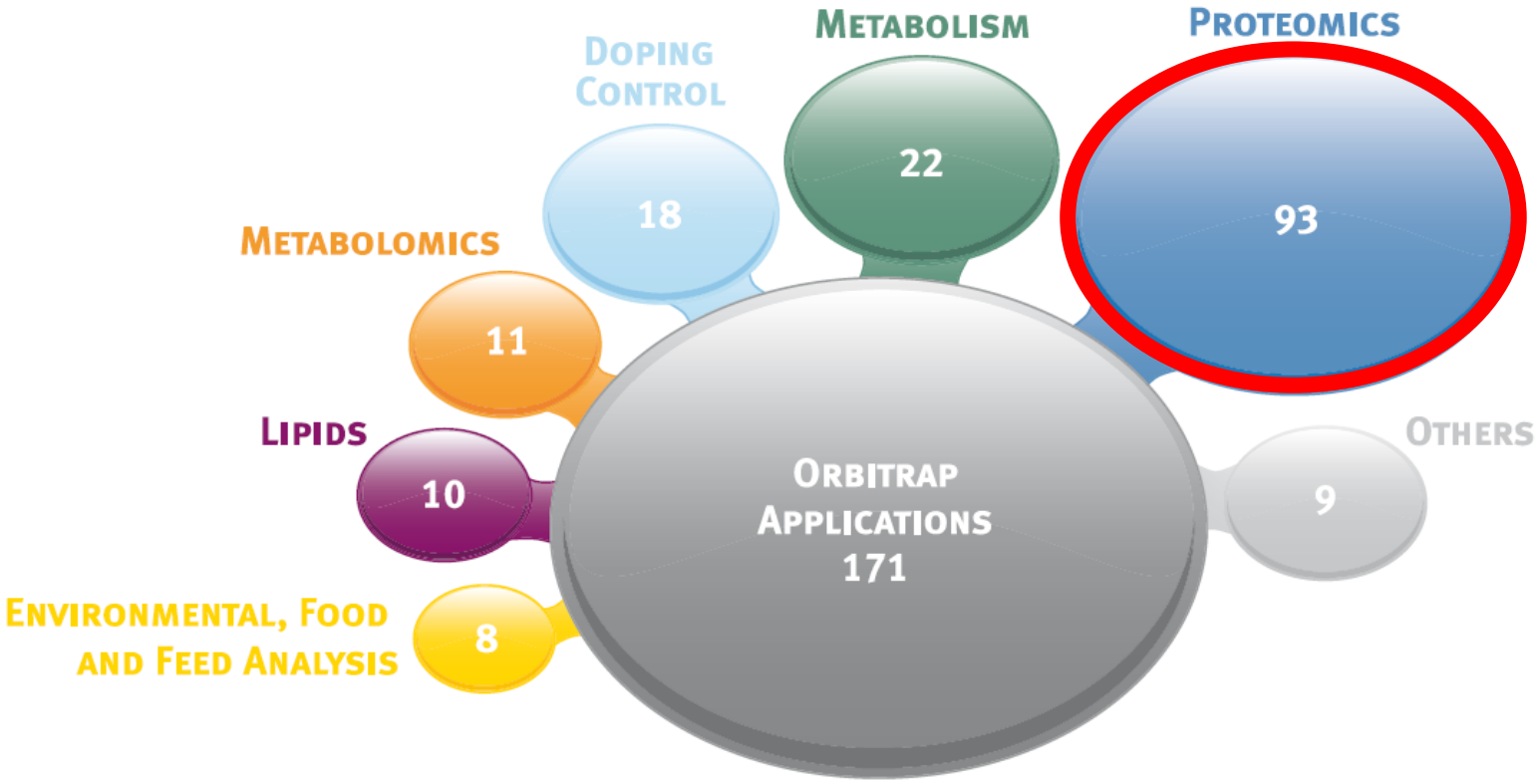


Figure 1. Representation of specific application areas using the Orbitrap mass analyzer in published peer-reviewed journals with the number of publications stated.

Lit: Thermo Scientific LTQ Orbitrap - An Overview of the Scientific Literature (2010)

Outline

1) Mass spectrometry in general
    1) What is MS?
    2) Why MS?
    3) Definitions

2) Mass spectrometer
    1) Principles
    2) Details

3) Applications
    1) Intact proteins
    2) Protein Identification
    3) Quantification

4) Research in Progress

Protein Analysis

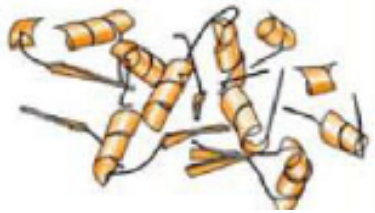
Identification (of Proteins)

→ Based on MASS-TO-CHARGE-RATIO of ions (m/z)

Intact protein analysis (top-down approach)
→ Based on full length protein mass

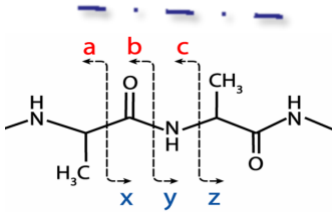
digest

Peptide mass fingerprint (PMF)
→ Based on petide masses



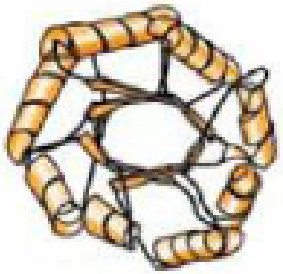
MS/MS

de novo sequencing / database search (bottom-up approach)
→ Based on amino acid sequence

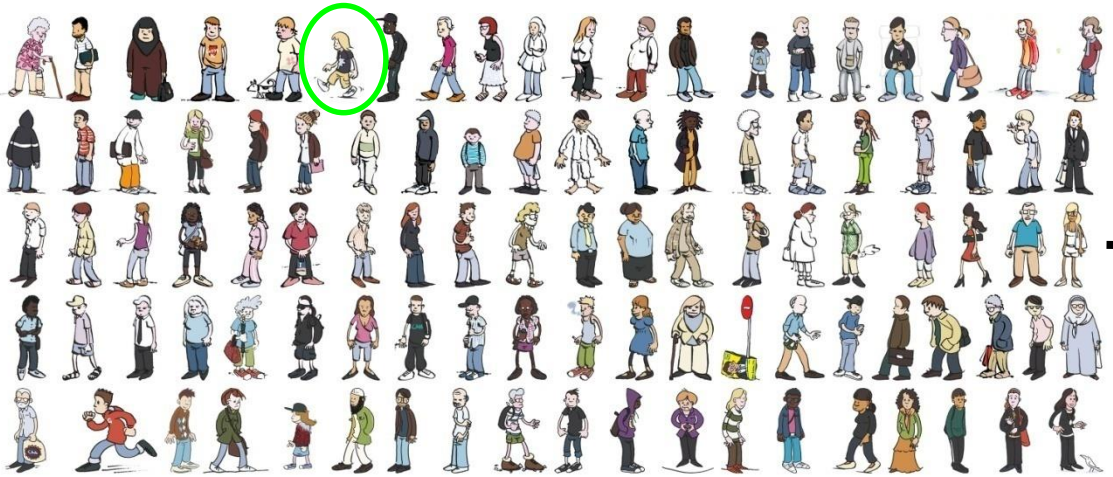


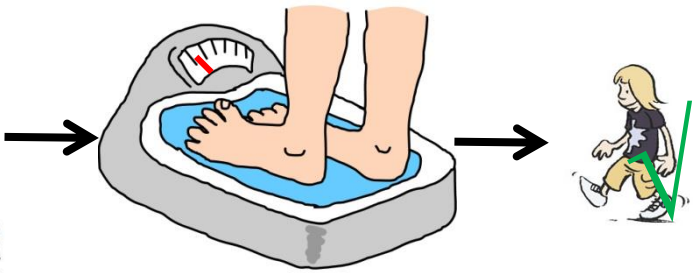
Intact protein analysis - Top-down approach

Identification (of proteins)



Intact protein analysis (Top-Down)
→ Based on full length protein mass

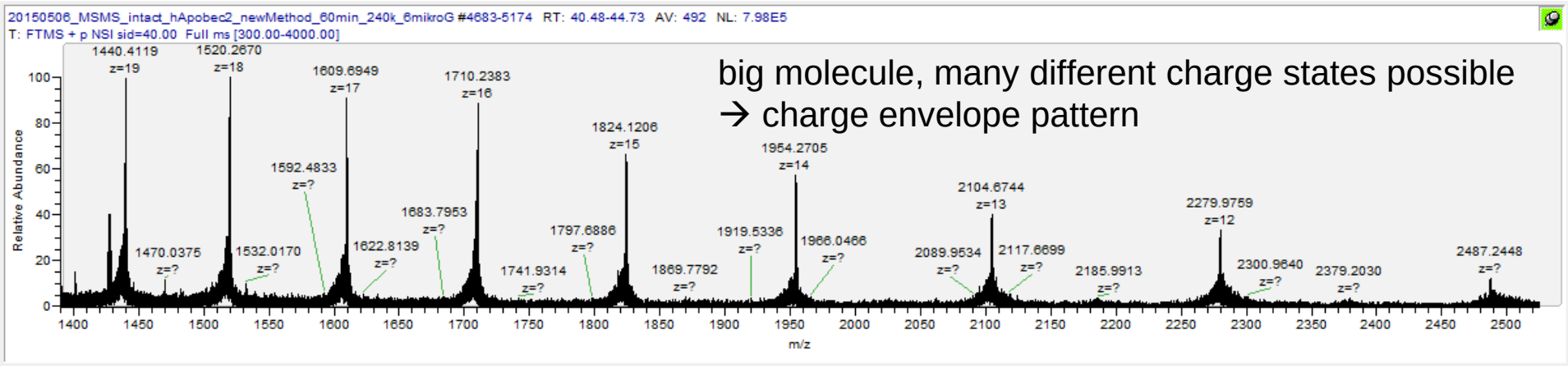


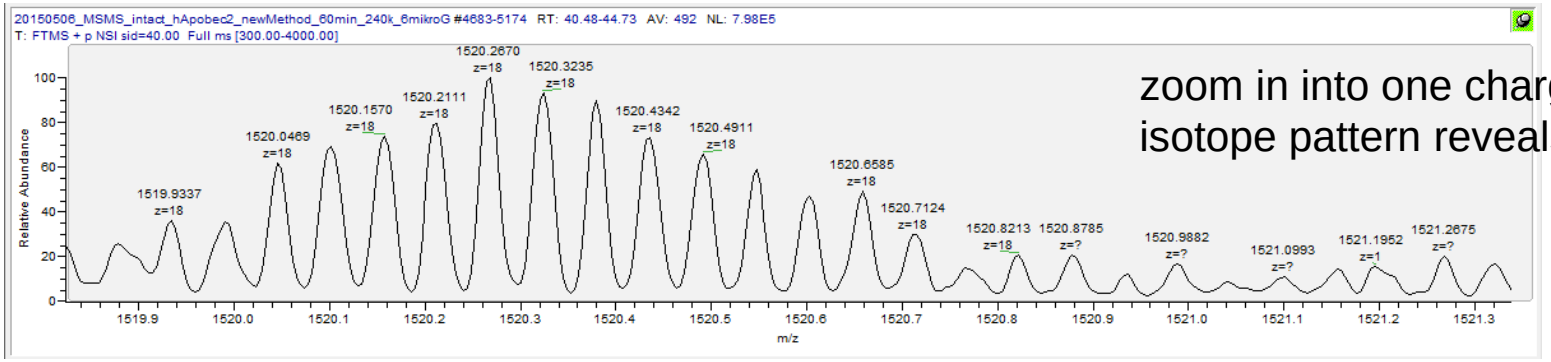


Intact protein analysis

Top-Down approach

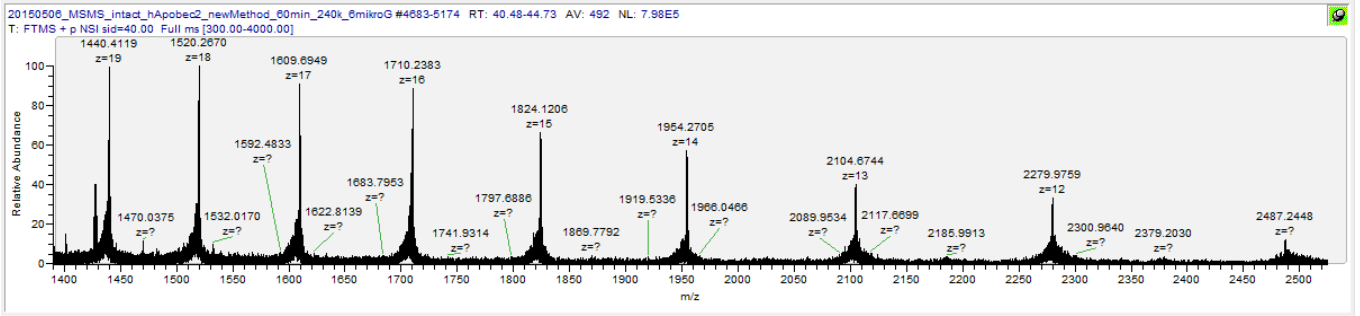
- measurement of the intact protein mass
- very clean sample required
- check for protein purity, truncated version, …

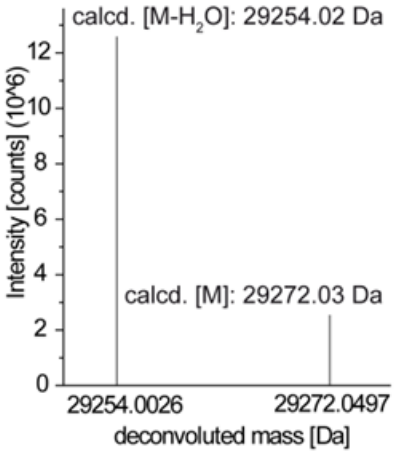




Top-Down approach

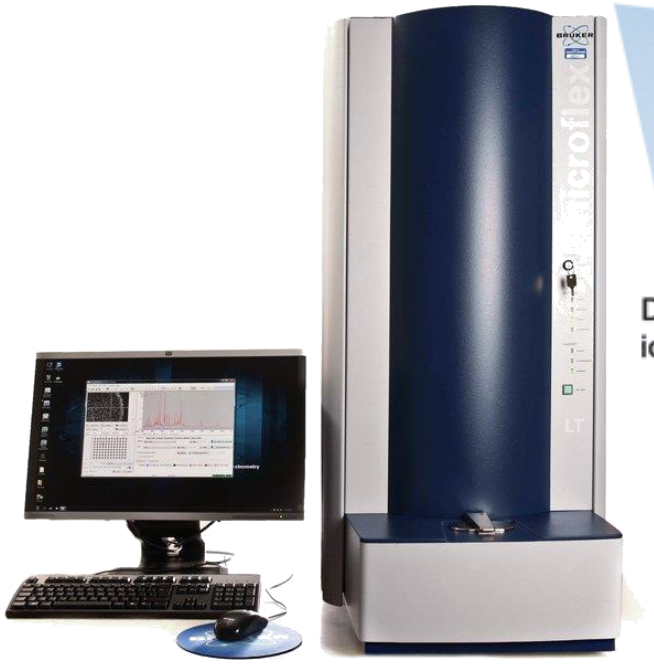
- spectra need to be deconvoluted to give intact protein mass (M or $M+H^+$)



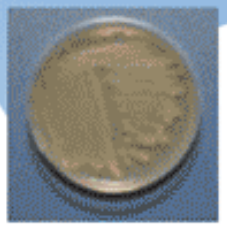


Intact Protein Analysis - Biotyper

Several proteins can be used to identify organisms

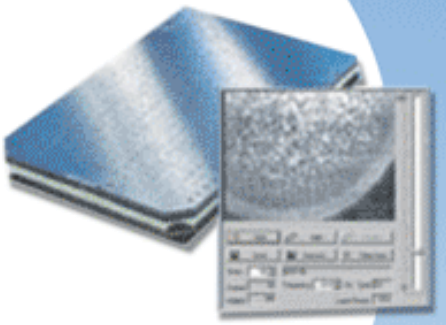


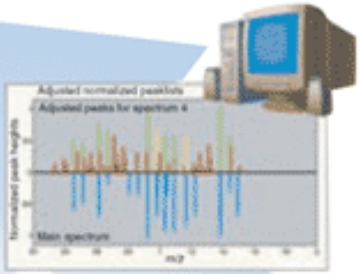
Unknown
'single colony'



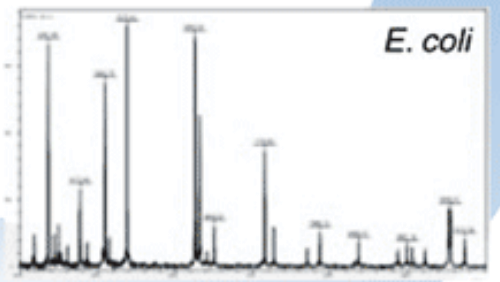
Sample preparation:
- direct thin layer without any treatment
- direct measurement of cell extracts

Add HCCA matrix solution





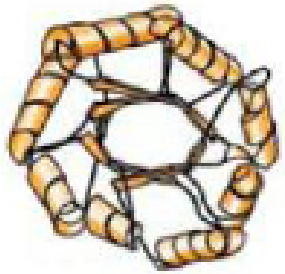
Data evaluation
identification and classification



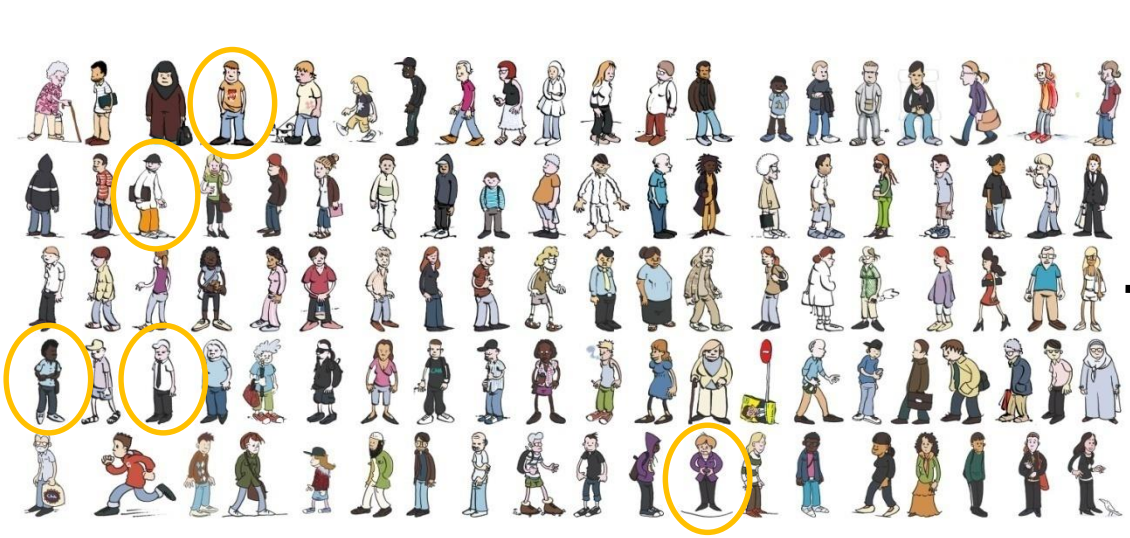
Acquisition of MALDI-TOF MS spectra
Detection limit:
- 10^5 cells (ground steel target)
→ approx. 0.5 µg biological material
- 5 x 10^3 cells (400) µm AnchorChip™ target)
→ ~25 ng biological material

Intact Protein Analysis – Top-down approach

Identification (of proteins)

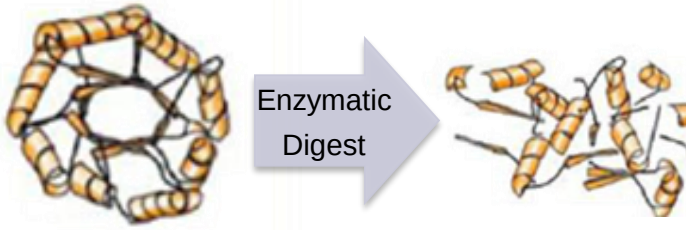


Problem: Similar proteins or modifications



Protein Analysis – peptide mass fingerprinting

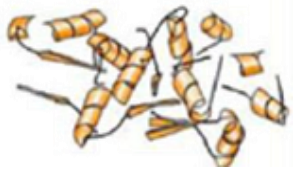
Identification (of Proteins)

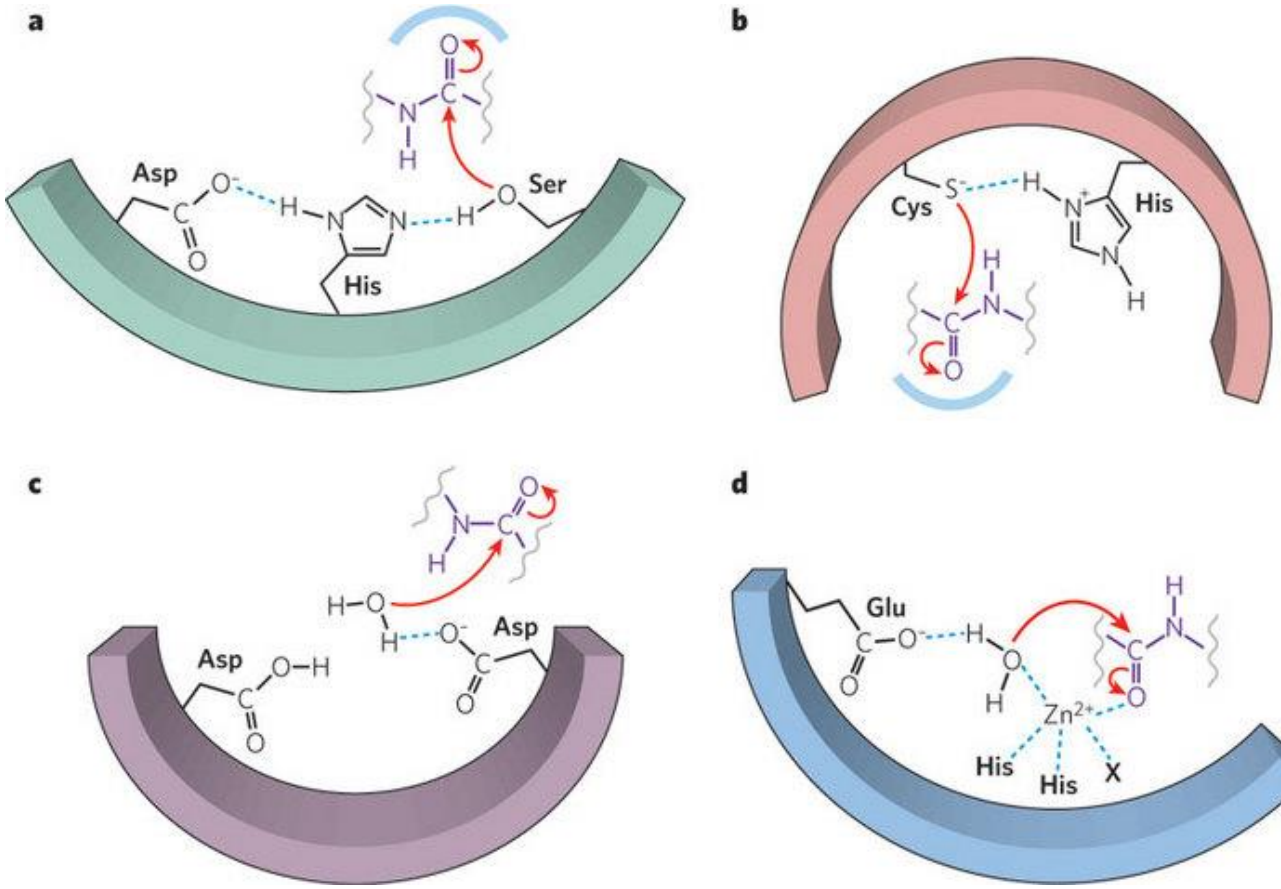


Peptide mass fingerprint (PMF)
→ Based on petide masses

Protein Analysis - Proteases

Enzymatic
Digest

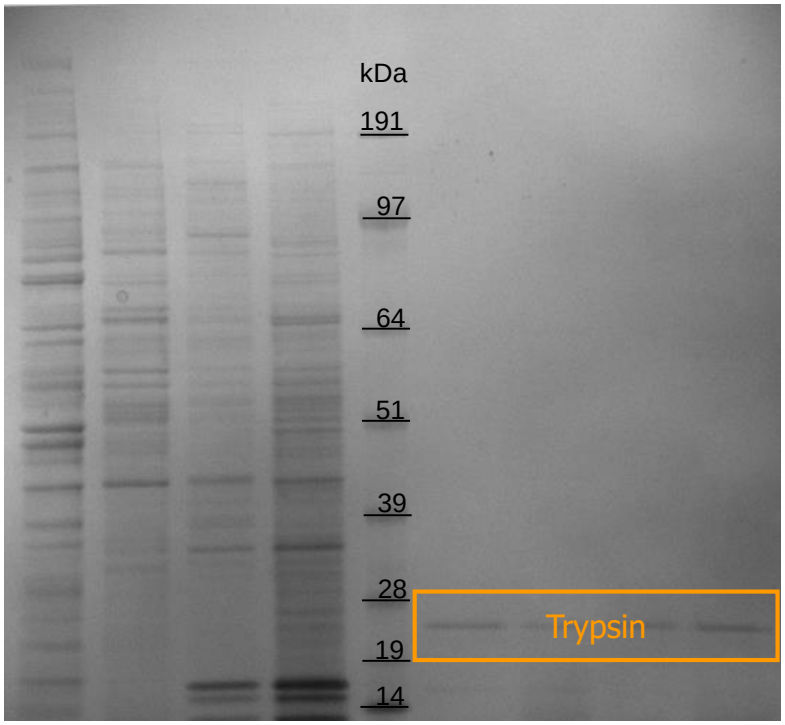




e.g.

a) Serinprotease
b) Cysteinprotease
c) Aspartatprotease
d) Metalloprotease

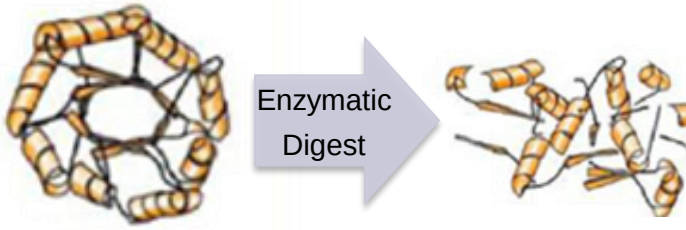
Protein Analysis - Trypsin



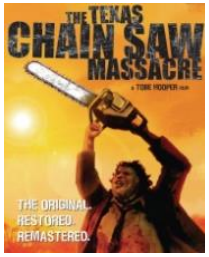
- Trypsin cleaves after the basic amino acids arginin and lysine
- often modified by chemical treatment or mutation to be more resistant to autolysis
- reversible inhibition in acidic pH; irreversible inhibition with PMSF (phenylmethyl-sulfonylfluoride)
- yields often good peptides for proteomics (good length, charge state minimum 2+: Lys/Arg + N-Terminus)

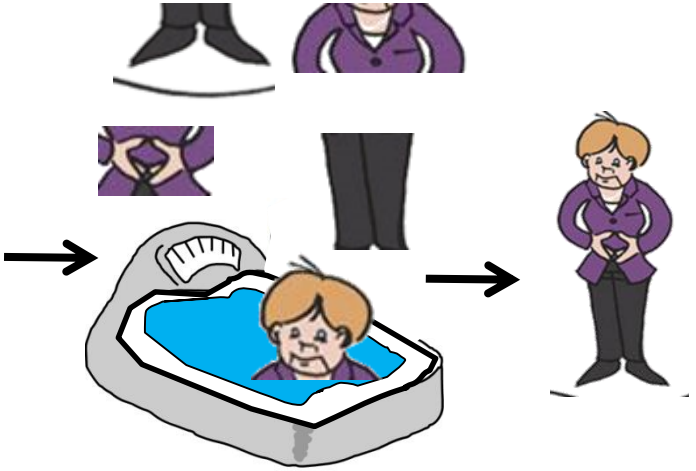
Protein Analysis – peptide mass fingerprinting

Identification (of Proteins)



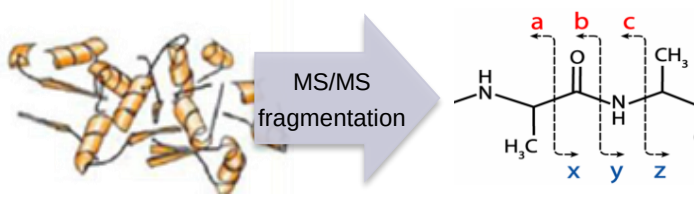
Problem: in complex mixtures, peptide masses can be very similar!

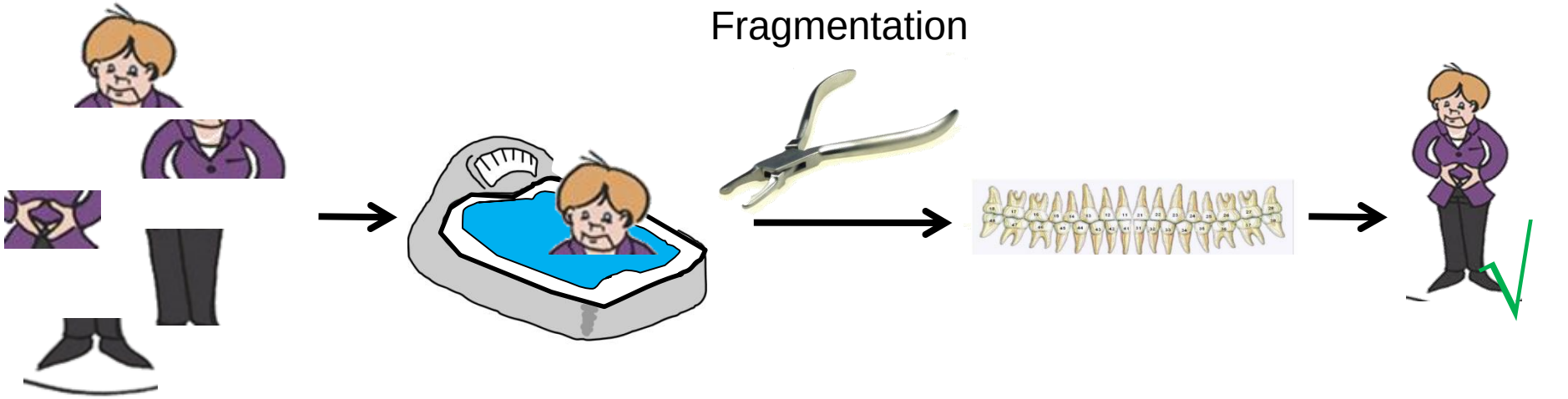




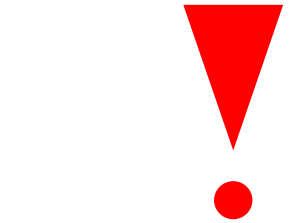
Identification (of Proteins)

de novo sequencing / database search
→ Based on amino acid sequence





Bottom-up-approach

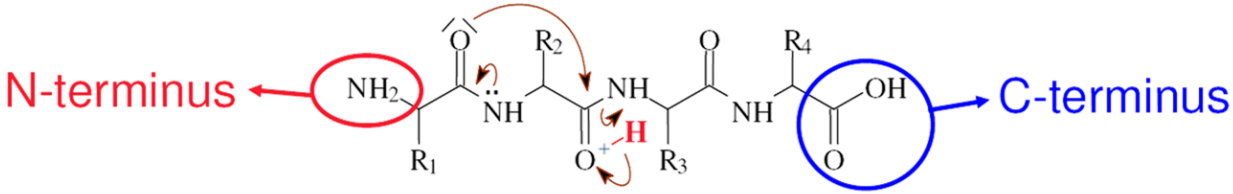


de novo sequencing / database search
→ Based on amino acid sequence

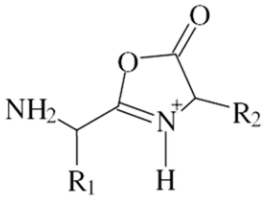
- **identification** of proteins in complex mixtures is often the central point in proteomics experiments
- bottom-up-approach: digest complex protein mixture and analyse peptides by peptide mass AND product ion masses after fragmentation
- **fragmentation** of peptides in the mass spectrometer (in the gas phase) is possible by **MS²** (AKA MS/MS, Tandem-MS)
- bottom-up-approach enables the use of **database searching** → automatable
- also gives: identification of **modification(s)** (with site of modification(s)), **quantification** methods

Fragmentation (MS/MS or MS^2)

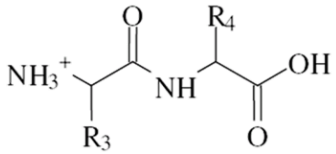
- collision with **gas** (CID, HCD) or reaction with chemical reagent (ETD) leads to **cleavage of a peptide in the MS**
- fragmentation by:
  - **CID**: collisional induced dissociation
  - **HCD**: higher-energy collisional dissociation
  - **ETD**: electron transfer dissociation
  - …
- **peptide-bond** (CO-NH) is usually the weakest
- fragmentation is **directed by protonation**



protonation at carboxyl oxygen
→ leads to formation of fragment ions



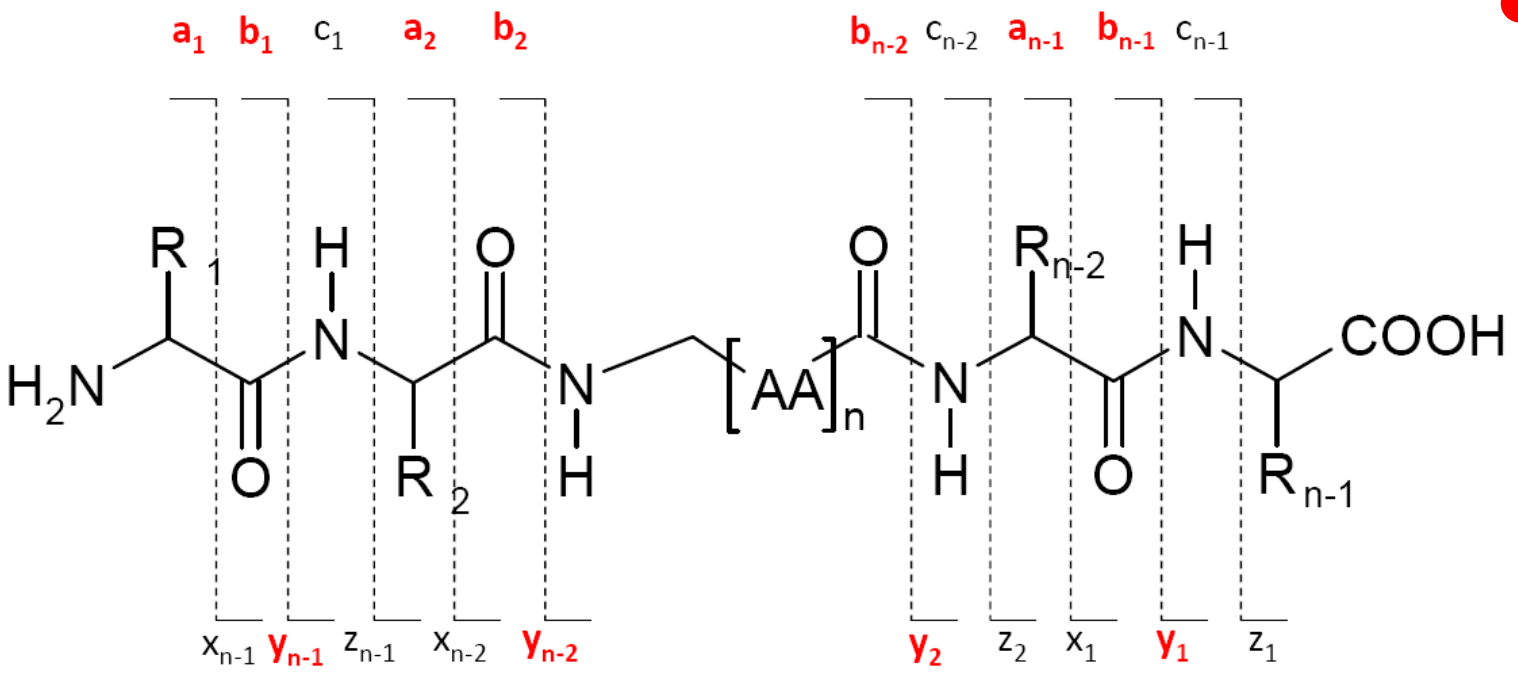
b-ion type



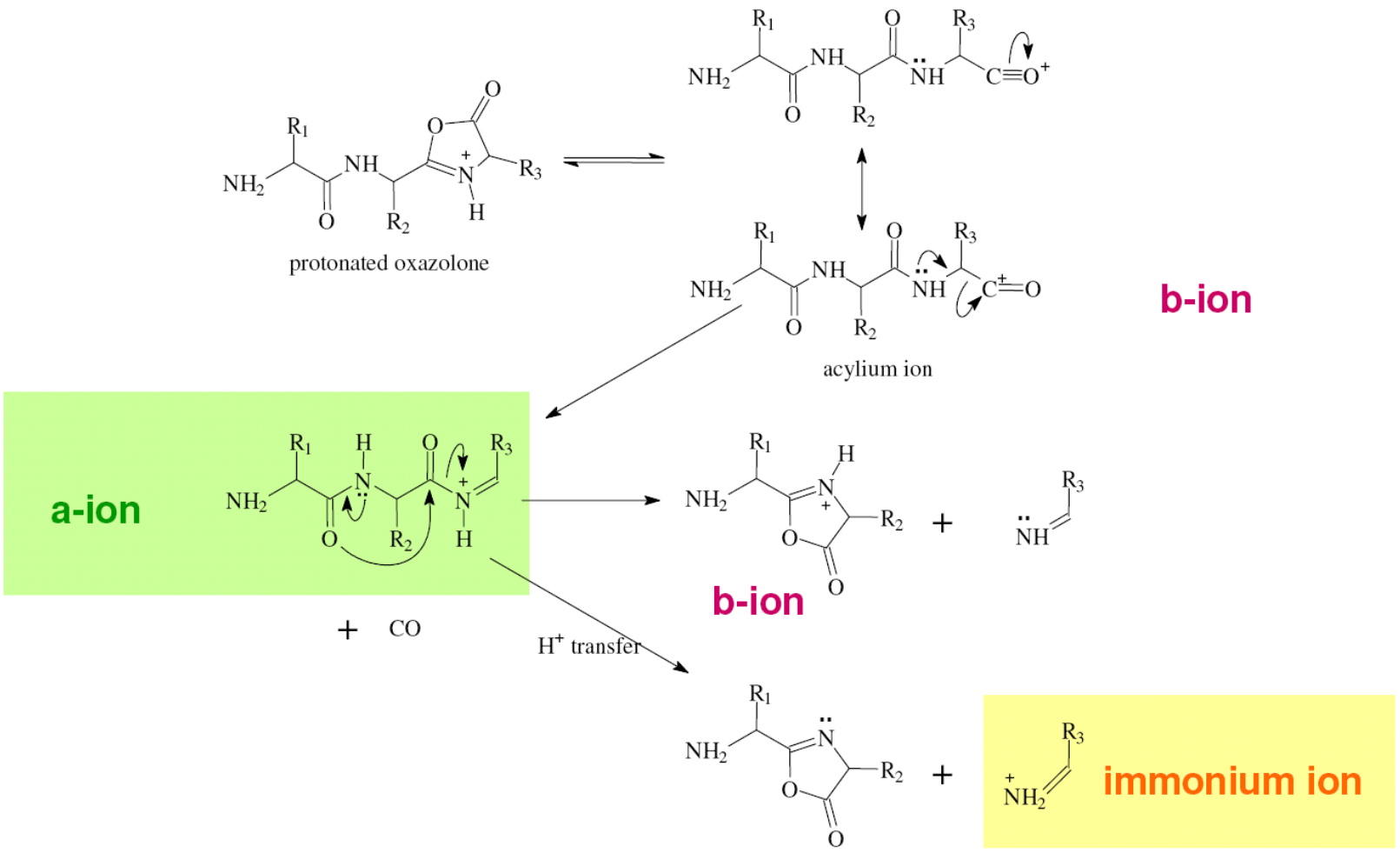
y-ion type

Fragmentation (MS/MS or MS^2)

Only fragments that are charged are detected!



Fragmentation (MS/MS or MS^2)



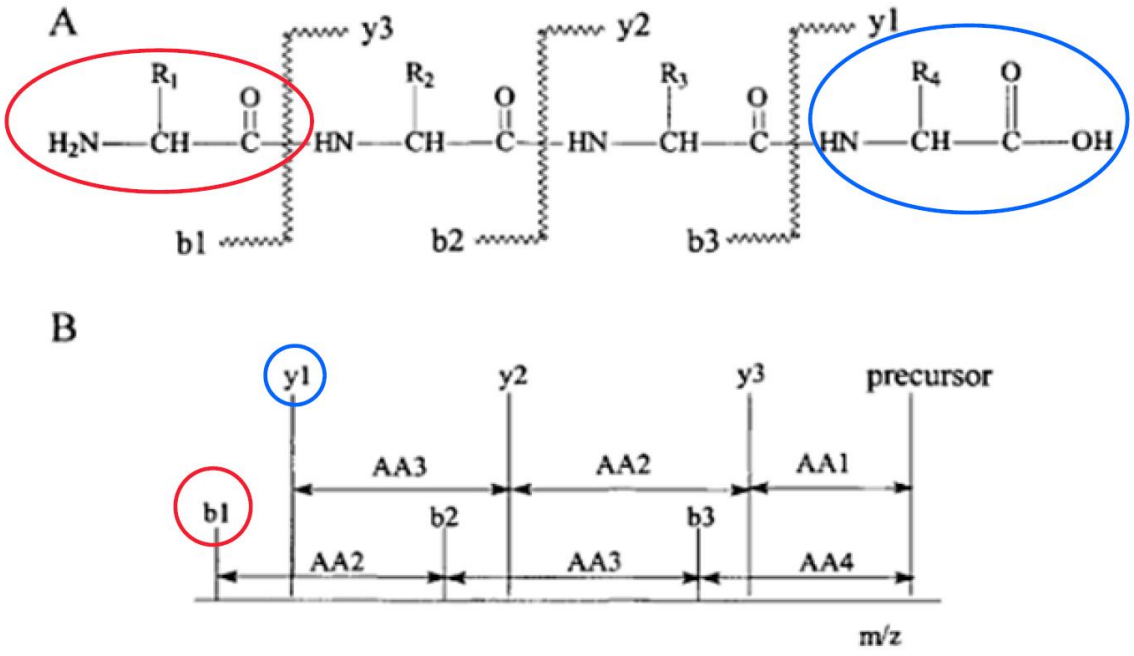
Fragmentation (MS/MS or MS^2)

Any of the peptide bonds *might* break, hard to predict which ones *will* break

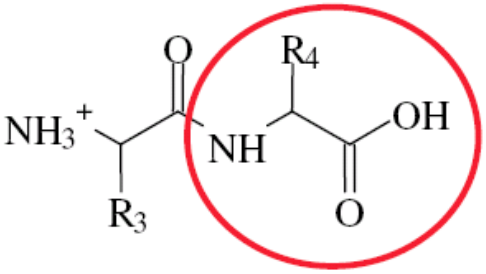
Peptide: S-G-F-L-E-E-D-E-L-K

MW	ion			ion	MW
88	b_1	S	GFLEEDELK	y_9	1080
145	b_2	SG	FLEEDELK	y_8	1022
292	b_3	SGF	LEEDELK	y_7	875
405	b_4	SGFL	EEDELK	y_6	762
534	b_5	SGFLE	EDELK	y_5	633
663	b_6	SGFLEE	DELK	y_4	504
778	b_7	SGFLEED	ELK	y_3	389
907	b_8	SGFLEEDE	LK	y_2	260
1020	b_9	SGFLEEDEL	K	y_1	147

How to start sequencing:

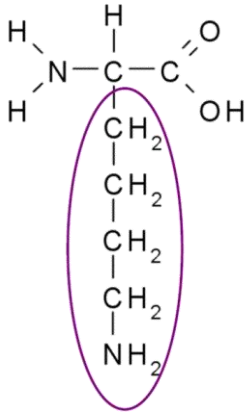


Rules for Fragmentation



y-ion type

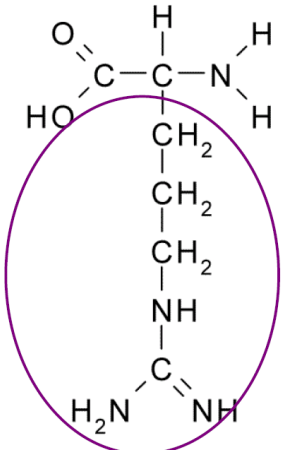
Last amino acid after tryptic digest: R or K



Lysine (K)

128.09496

147.11281



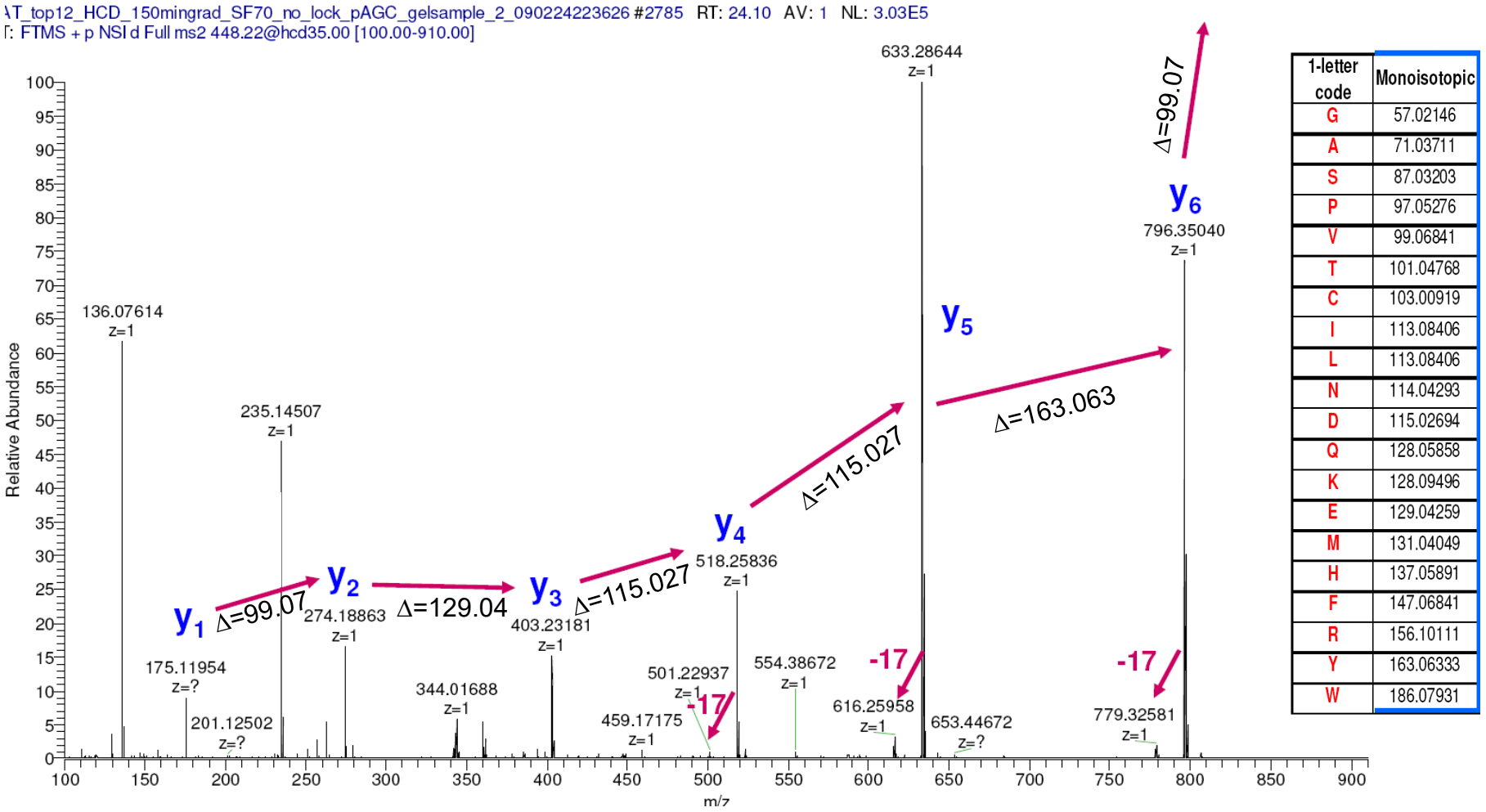
Arginine (R)

156.10111

175.11896

One Example

HCD spectrum; m/z 448.22 $(M+2H)^{2+}$ MH^{+} 895.42223

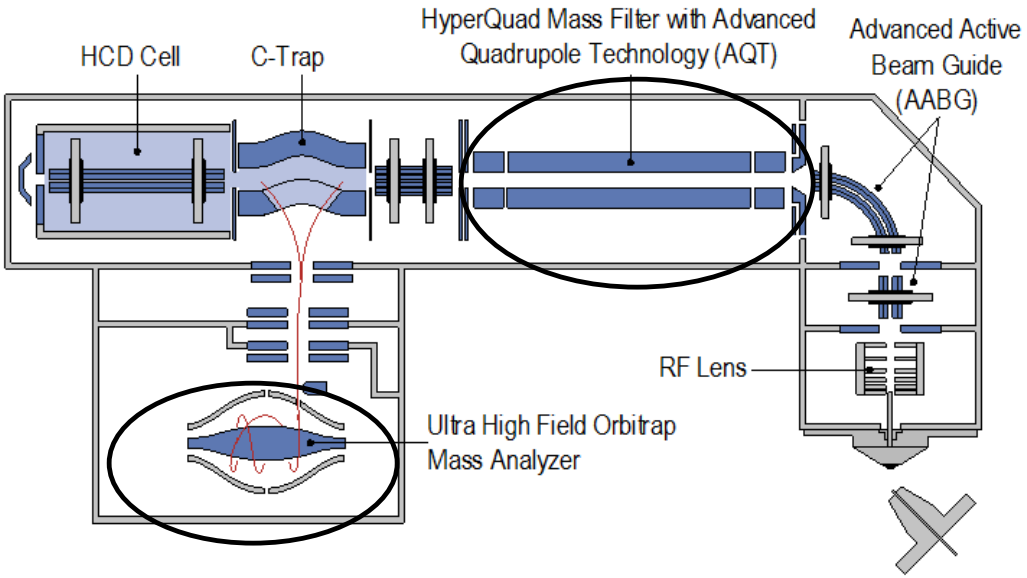


1-letter code	Monoisotopic
G	57.02146
A	71.03711
S	87.03203
P	97.05276
V	99.06841
T	101.04768
C	103.00919
I	113.08406
L	113.08406
N	114.04293
D	115.02694
Q	128.05858
K	128.09496
E	129.04259
M	131.04049
H	137.05891
F	147.06841
R	156.10111
Y	163.06333
W	186.07931

Bottom-up-approach – technical realization

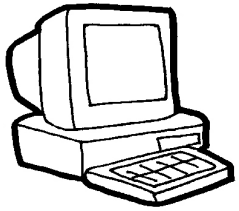
The QExactive HF

isolation



measurement of high-resolution Full scan spectra (MS^1)
and of high-resolution fragmentation (MS^2)-spectra

Interpretation of Bottom-Up data



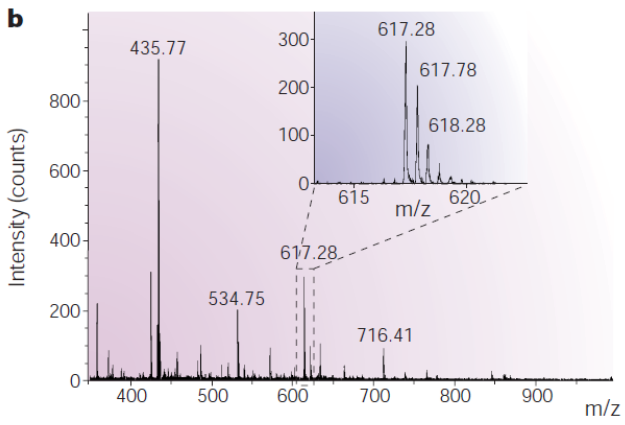
- proteomics bottom-up data is **very complex**
- certain **algorithms** enable the identification of peptides and therefore proteins
- **popular algorithms**:
    - Sequest
    - Mascot (Matrix Science)
    - Andromeda (implemented in the MaxQuant software, MPI for Biochemistry)
- spectra are searched against **sequence databases** (FASTA-format)
- **scoring** of identified peptides/proteins by different statistical methods

→ bottom-up data can be **analyzed automatically**!

Interpretation of Bottom-Up data

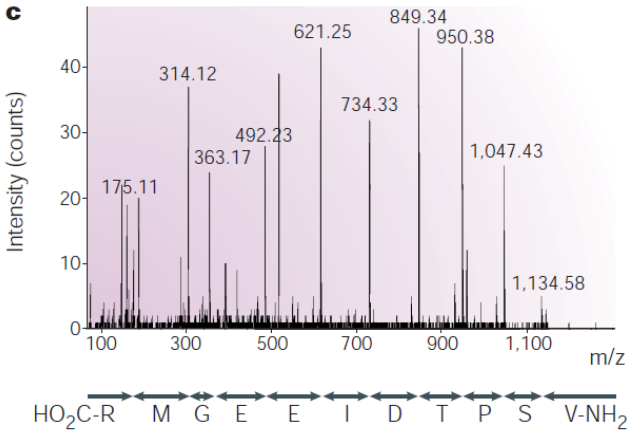
Bottom-Up approach

MS



isolation & fragmentation

MS/MS

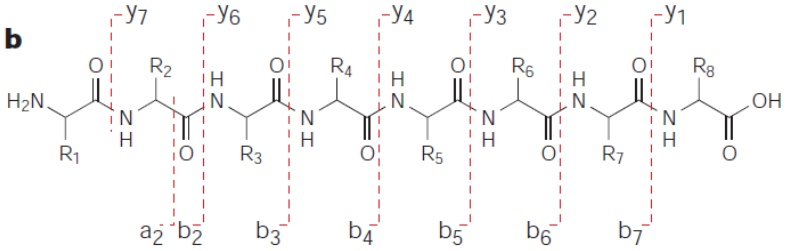


Full scan

determine intact peptide mass

MS²-scan

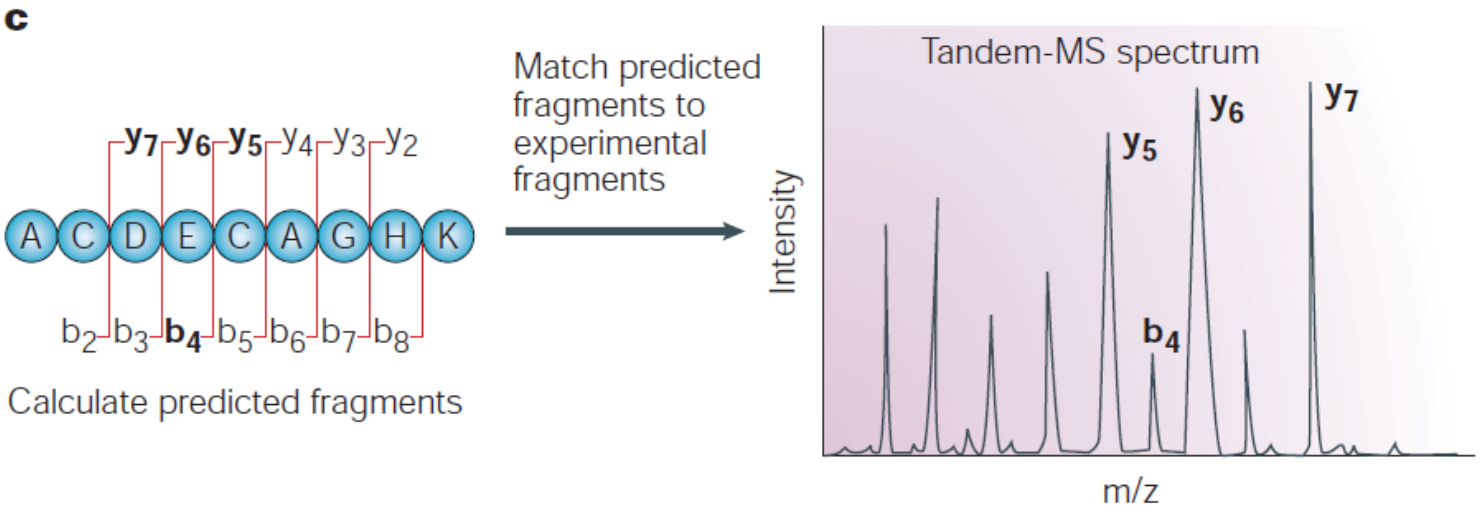
fragment peptide and get sequence information



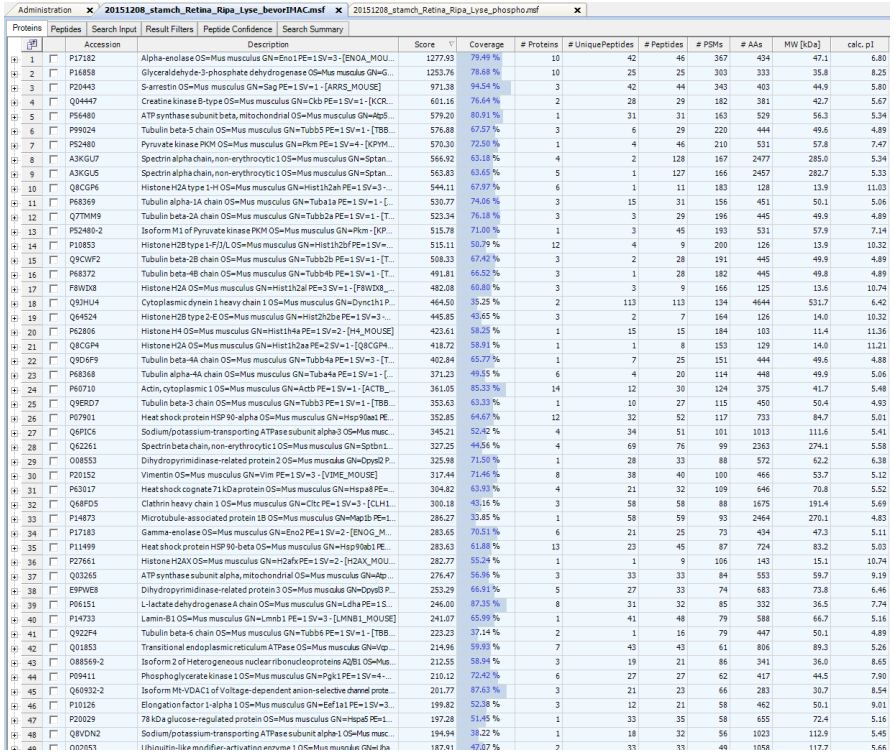
Interpretation of Bottom-Up data

Simplified action of algorithm

- ***in silico* digest** of proteins (input: protease used & species origin of sample)
- calculation of expected fragment ion masses (input: mass and modified AAs of expected modifications)
- matching of calculated to experimental MS^2-spectrum
- scoring

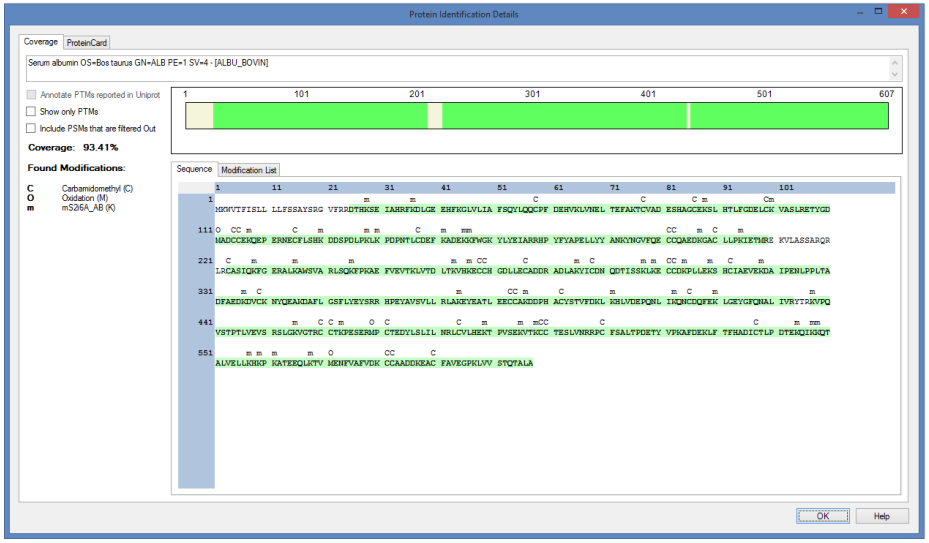


Example of data



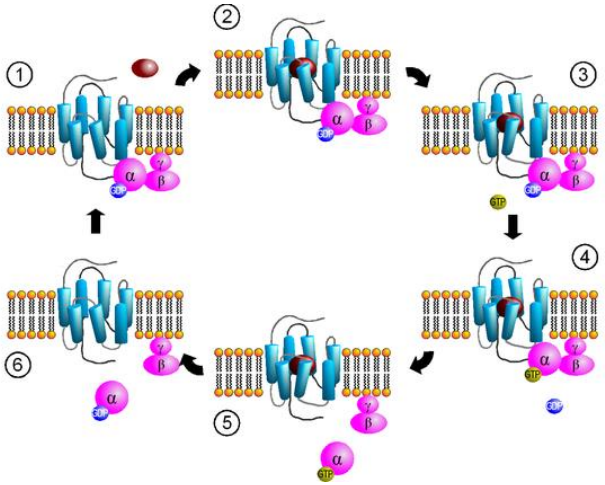
	Accession	Description	Score	Coverage	# Proteins	# UniquePeptides	# Peptides	# PSMs	# AAs	MW [kDa]	calc. pI
1	P17182	Alpha-enolase OS=Mus musculus GN=Eno1 PE=1 SV=3 - [ENOA_MOU...	1277.93	79.49 %	10	42	46	367	434	47.1	6.80
2	P16858	Glyceraldehyde-3-phosphate dehydrogenase OS=Mus musculus GN=G...	1253.76	78.68 %	10	25	25	303	333	35.8	8.25
3	P20443	S-arrestin OS=Mus musculus GN=Sag PE=1 SV=1 - [ARRS_MOUSE]	971.38	94.54 %	3	42	44	343	403	44.9	5.80
4	Q04447	Creatine kinase B-type OS=Mus musculus GN=Ckb PE=1 SV=1 - [KCR...	601.16	76.64 %	2	28	29	182	381	42.7	5.67
5	P56480	ATP synthase subunit beta, mitochondrial OS=Mus musculus GN=Atp5...	579.20	80.91 %	1	31	31	163	529	56.3	5.34
6	P99024	Tubulin beta-5 chain OS=Mus musculus GN=Tubb5 PE=1 SV=1 - [TBB...	576.88	67.57 %	3	6	29	220	444	49.6	4.89
7	P52480	Pyruvate kinase PKM OS=Mus musculus GN=Pkm PE=1 SV=4 - [KPYM...	570.30	72.50 %	1	4	46	210	531	57.8	7.47
8	A3KGU7	Spectrin alpha chain, non-erythrocytic 1 OS=Mus musculus GN=Sptan...	566.92	63.18 %	4	2	128	167	2477	285.0	5.34
9	A3KGU5	Spectrin alpha chain, non-erythrocytic 1 OS=Mus musculus GN=Sptan...	563.83	63.65 %	5	1	127	166	2457	282.7	5.33
10	Q8CGP6	Histone H2A type 1-H OS=Mus musculus GN=Hist1h2ah PE=1 SV=3 -...	544.11	67.97 %	6	1	11	183	128	13.9	11.03
11	P68369	Tubulin alpha-1A chain OS=Mus musculus GN=Tuba1a PE=1 SV=1 - [...	530.77	74.06 %	3	15	31	156	451	50.1	5.06
12	Q7TMM9	Tubulin beta-2A chain OS=Mus musculus GN=Tubb2a PE=1 SV=1 - [T...	523.34	76.18 %	3	3	29	196	445	49.9	4.89
13	P52480-2	Isoform M1 of Pyruvate kinase PKM OS=Mus musculus GN=Pkm - [KP...	515.78	71.00 %	1	3	45	193	531	57.9	7.14
14	P10853	Histone H2B type 1-F/J/L OS=Mus musculus GN=Hist1h2bf PE=1 SV=...	515.11	50.79 %	12	4	9	200	126	13.9	10.32
15	Q9CWF2	Tubulin beta-2B chain OS=Mus musculus GN=Tubb2b PE=1 SV=1 - [T...	508.33	67.42 %	3	2	28	191	445	49.9	4.89
16	P68372	Tubulin beta-4B chain OS=Mus musculus GN=Tubb4b PE=1 SV=1 - [T...	491.81	66.52 %	3	1	28	182	445	49.8	4.89
17	F8WIX8	Histone H2A OS=Mus musculus GN=Hist1h2al PE=3 SV=1 - [F8WIX8_...	482.08	60.80 %	3	3	9	166	125	13.6	10.74
18	Q9JHU4	Cytoplasmic dynein 1 heavy chain 1 OS=Mus musculus GN=Dync1h1 P...	464.50	35.25 %	2	113	113	134	4644	531.7	6.42
19	Q64524	Histone H2B type 2-E OS=Mus musculus GN=Hist2h2be PE=1 SV=3 -...	445.85	43.65 %	3	2	7	164	126	14.0	10.32
20	P62806	Histone H4 OS=Mus musculus GN=Hist1h4a PE=1 SV=2 - [H4_MOUSE]	423.61	58.28 %	1	15	15	184	103	11.4	11.36
21	Q8CGP4	Histone H2A OS=Mus musculus GN=Hist1h2aa PE=2 SV=1 - [Q8CGP4...	418.72	58.91 %	1	1	8	153	129	14.0	11.21
22	Q9D6F9	Tubulin beta-4A chain OS=Mus musculus GN=Tubb4a PE=1 SV=3 - [T...	402.84	65.77 %	1	7	25	151	444	49.6	4.88
23	P68368	Tubulin alpha-4A chain OS=Mus musculus GN=Tuba4a PE=1 SV=1 - [...	371.23	49.55 %	6	4	20	114	448	49.9	5.06
24	P60710	Actin, cytoplasmic 1 OS=Mus musculus GN=Actb PE=1 SV=1 - [ACTB_...	361.05	85.33 %	14	12	30	124	375	41.7	5.48
25	Q9ERD7	Tubulin beta-3 chain OS=Mus musculus GN=Tubb3 PE=1 SV=1 - [TBB...	353.63	63.33 %	1	10	27	115	450	50.4	4.93
26	P07901	Heat shock protein HSP 90-alpha OS=Mus musculus GN=Hsp90aa1 PE...	352.85	64.67 %	12	32	52	117	733	84.7	5.01
27	Q6PIC6	Sodium/potassium-transporting ATPase subunit alpha-3 OS=Mus musc...	345.21	52.42 %	4	34	51	101	1013	111.6	5.41
28	Q62261	Spectrin beta chain, non-erythrocytic 1 OS=Mus musculus GN=Sptbn1...	327.25	44.56 %	4	69	76	99	2363	274.1	5.58
29	O08553	Dihydropyrimidinase-related protein 2 OS=Mus musculus GN=Dpysl2 P...	325.98	71.50 %	1	28	33	88	572	62.2	6.38
30	P20152	Vimentin OS=Mus musculus GN=Vim PE=1 SV=3 - [VIME_MOUSE]	317.44	71.46 %	8	38	40	100	466	53.7	5.12
31	P63017	Heat shock cognate 71 kDa protein OS=Mus musculus GN=Hspa8 PE=...	304.82	63.93 %	4	21	32	109	646	70.8	5.52
32	Q68FD5	Clathrin heavy chain 1 OS=Mus musculus GN=Cltc PE=1 SV=3 - [CLH1...	300.18	43.16 %	3	58	58	88	1675	191.4	5.69
33	P14873	Microtubule-associated protein 1B OS=Mus musculus GN=Map1b PE=1...	286.27	33.85 %	1	58	59	93	2464	270.1	4.83
34	P17183	Gamma-enolase OS=Mus musculus GN=Eno2 PE=1 SV=2 - [ENOG_M...	283.65	70.51 %	6	21	25	73	434	47.3	5.11
35	P11499	Heat shock protein HSP 90-beta OS=Mus musculus GN=Hsp90ab1 PE...	283.63	61.88 %	13	23	45	87	724	83.2	5.03
36	P27661	Histone H2AX OS=Mus musculus GN=H2afx PE=1 SV=2 - [H2AX_MOU...	282.77	55.24 %	1	1	9	106	143	15.1	10.74
37	Q03265	ATP synthase subunit alpha, mitochondrial OS=Mus musculus GN=Atp...	276.47	56.96 %	3	33	33	84	553	59.7	9.19
38	E9PWE8	Dihydropyrimidinase-related protein 3 OS=Mus musculus GN=Dpysl3 P...	253.29	66.91 %	5	27	33	74	683	73.8	6.46
39	P06151	L-lactate dehydrogenase A chain OS=Mus musculus GN=Ldha PE=1 S...	246.00	87.35 %	8	31	32	85	332	36.5	7.74
40	P14733	Lamin-B1 OS=Mus musculus GN=Lmnb1 PE=1 SV=3 - [LMNB1_MOUSE]	241.07	65.99 %	1	41	48	79	588	66.7	5.16
41	Q922F4	Tubulin beta-6 chain OS=Mus musculus GN=Tubb6 PE=1 SV=1 - [TBB...	223.23	37.14 %	2	1	16	79	447	50.1	4.89
42	Q01853	Transitional endoplasmic reticulum ATPase OS=Mus musculus GN=Vcp...	214.96	59.93 %	7	43	43	61	806	89.3	5.26
43	O88569-2	Isoform 2 of Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Mus...	212.55	58.94 %	3	19	21	86	341	36.0	8.65
44	P09411	Phosphoglycerate kinase 1 OS=Mus musculus GN=Pgk1 PE=1 SV=4 -...	210.12	72.42 %	6	27	27	62	417	44.5	7.90
45	Q60932-2	Isoform Mt-VDAC1 of Voltage-dependent anion-selective channel prote...	201.77	87.63 %	3	21	23	66	283	30.7	8.54
46	P10126	Elongation factor 1-alpha 1 OS=Mus musculus GN=Eef1a1 PE=1 SV=3...	199.82	52.38 %	3	12	21	58	462	50.1	9.01
47	P20029	78 kDa glucose-regulated protein OS=Mus musculus GN=Hspa5 PE=1...	197.28	51.45 %	1	33	35	58	655	72.4	5.16
48	Q8VDN2	Sodium/potassium-transporting ATPase subunit alpha-1 OS=Mus musc...	194.94	38.22 %	1	18	32	56	1023	112.9	5.45
49	Q02053	Ubiquitin-like modifier-activating enzyme 1 OS=Mus musculus GN=Uba...	187.91	47.07 %	2	33	33	49	1058	117.7	5.66

- list of all identified proteins
- scores
- sequence coverage
- list of identified peptides
- type and site of modification



Application example: Phosporylation sites

- Phosphorylations of serine-, tyrosine- or threonine-residues
- play important role in regulatory processes
- phosphogroups are attached to proteins by kinases; phosphatases remove phosphogroups
- phosphopeptides are not very abundant and need to be enriched



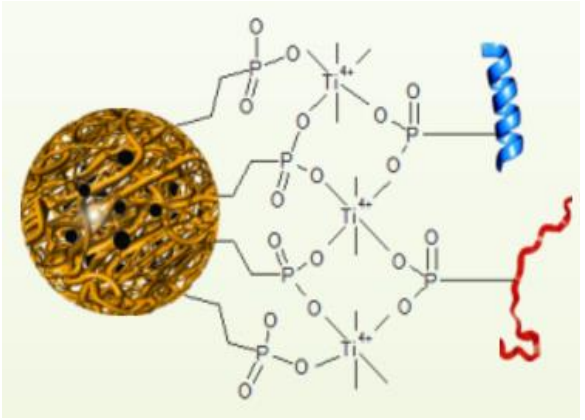
G-protein coupled receptors are downregulated by phosphorylation

High-throughput phosphoproteomics reveals *in vivo* insulin signaling dynamics

Sean J Humphrey, S Babak Azimifar & Matthias Mann

Application example: Phosporylation sites

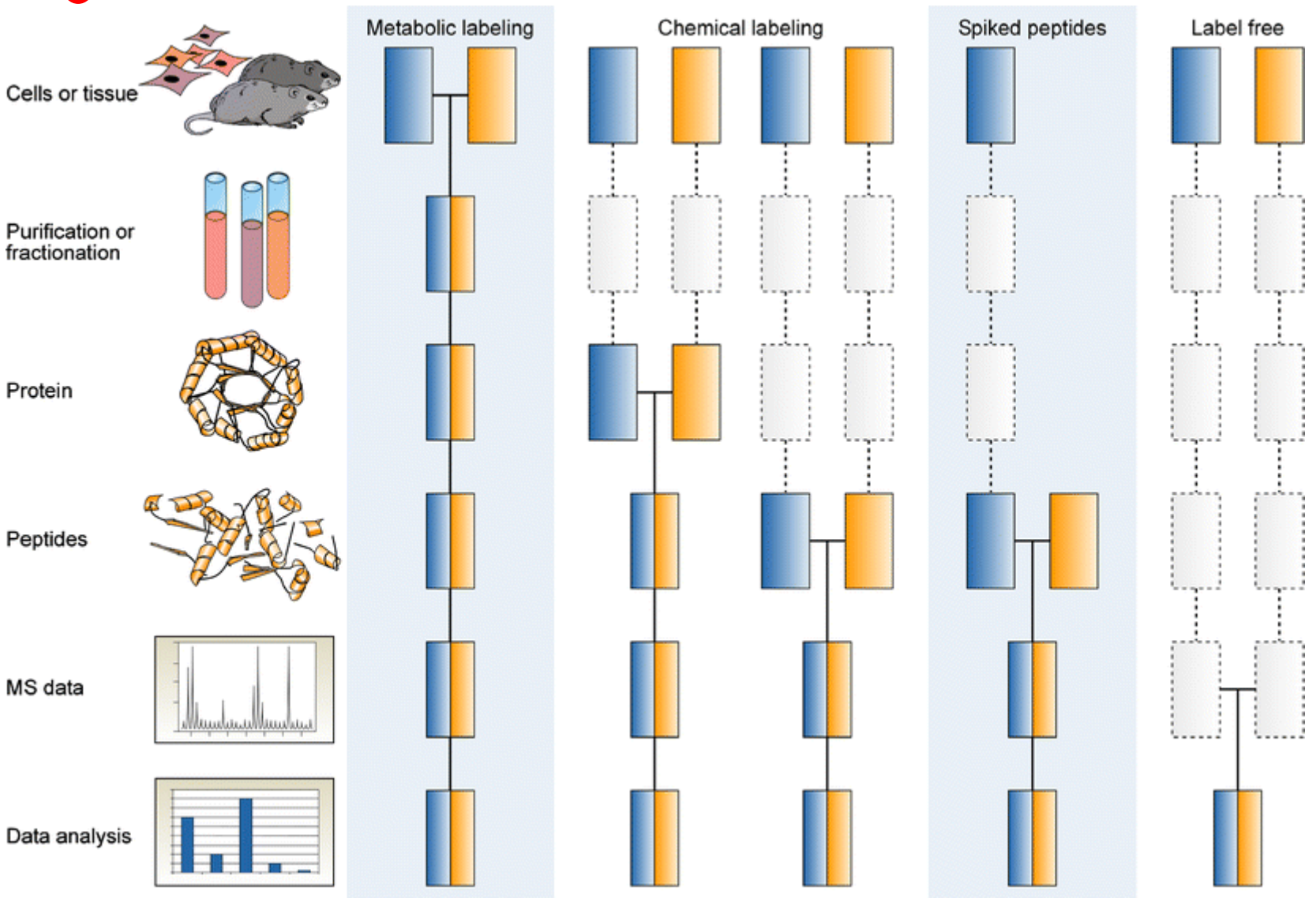
-phosphopeptides need to be enriched
→**IMAC: Immobilized metal ion affinity chromatography**
- most popular with Fe, Ti
- for example magnetic beads, covered with Ti^{4+}-ions
→enrichment of phophopeptides and elution with phosphate-containing buffer



	A2	Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group Accessions	Modifications	ΔCn	phosphoRS Site Probabilities	q-Value	PEP	XCorr	Charge	MH+ [Da]	ΔM [ppm]	RT [min]	# Missed Cleavages
1		VNHEPEPASGASPGATPKsP...	8	2	1	Q6P1B9	S20(Phospho)	0.0000	S(9): 0.0; S(12): 0.0; T(16)...	0	2.41e-20	8.10	4	2607.25649	0.04	44.26	1
2		GNKsPsPPPDGSPAATPEIR	2	3	1	Q6P1B9	S4(Phospho); S6(Phospho)	0.0000	S(4): 100.0; S(6): 100.0; S(...	0	1.56e-08	4.74	3	2134.92093	0.10	39.99	1
3		AQPSDNAPEKGNKsPsPPPD...	13	3	1	Q6P1B9	S14(Phospho); S16(Phosph...	0.0000	S(4): 0.0; S(14): 100.0; S(1...	0	4.55e-16	4.66	3	3172.40439	1.84	38.80	2
4		VNHEPEPASGAsPGATPK	4	3	1	Q6P1B9	S12(Phospho)	0.0000	S(9): 0.0; S(12): 100.0; T(1...	0	1.77e-11	4.57	3	1938.89499	-0.41	37.58	0
5		AQPSDNAPEKGNKSPsPPPD...	10	3	1	Q6P1B9	S16(Phospho)	0.0000	S(4): 0.0; S(14): 1.1; S(16)...	0	1.77e-07	4.16	4	3092.43349	0.41	37.58	2
6		AQPSDNAPEKGNKsPsPPPD...	3	3	1	Q6P1B9	S14(Phospho); S16(Phosph...	0.0000	S(4): 98.2; S(14): 98.2; S(1...	0	5.58e-05	3.56	4	3252.36855	1.13	38.60	2
7		GNKSPsPPPDGSPAATPEIR	4	3	1	Q6P1B9	S6(Phospho)	0.0000	S(4): 0.6; S(6): 99.4; S(12)...	0	9.8e-07	2.52	3	2054.95237	-0.98	38.44	1
8		VNHEPEPASGAsPGATPKsP...	4	2	1	Q6P1B9	S12(Phospho); S20(Phosph...	0.0000	S(9): 1.4; S(12): 97.3; T(16...	0	0.000529	2.05	3	2687.22562	1.08	46.43	1

Quantitative Proteomics

Quantificaton methods



MS-quantification methods

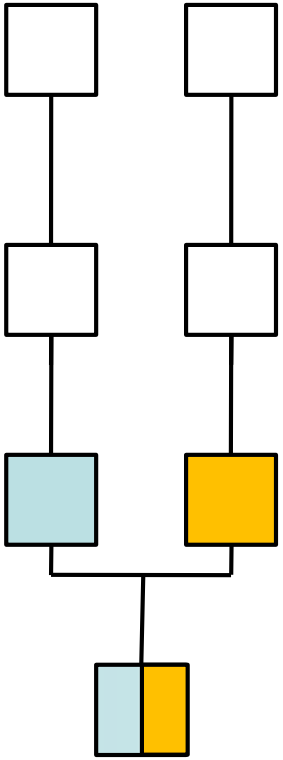
Isobaric tagging of peptides

cells/tissue

proteins

peptides

MS-analysis



heavy label
light label
no label

Methods:

- iTRAQ (isobaric Tag for Relative and Absolute Quantification)
- TMT (tandem mass tags)

Pros:

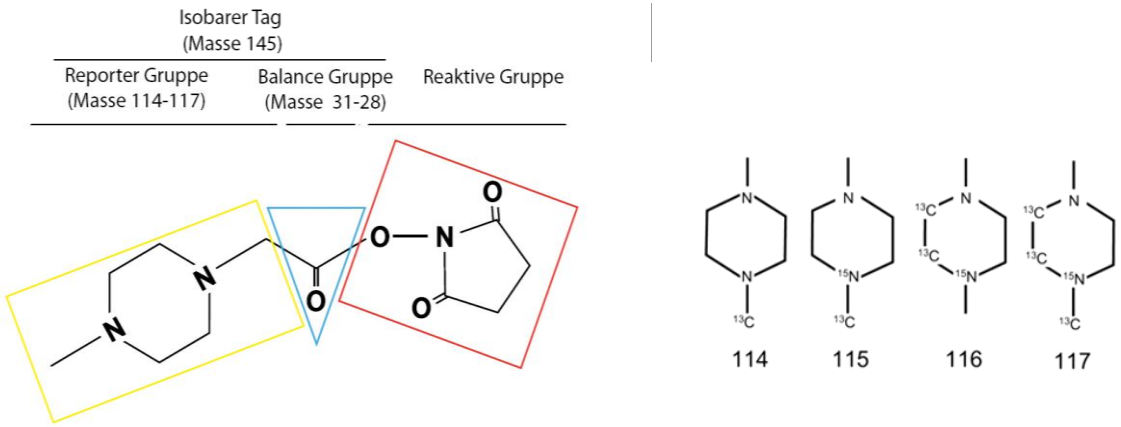
- flexibility
- relative or absolute
- easy
- high dynamic range
- enables multiplexing (up to 11 samples in one measurement!)

Cons:

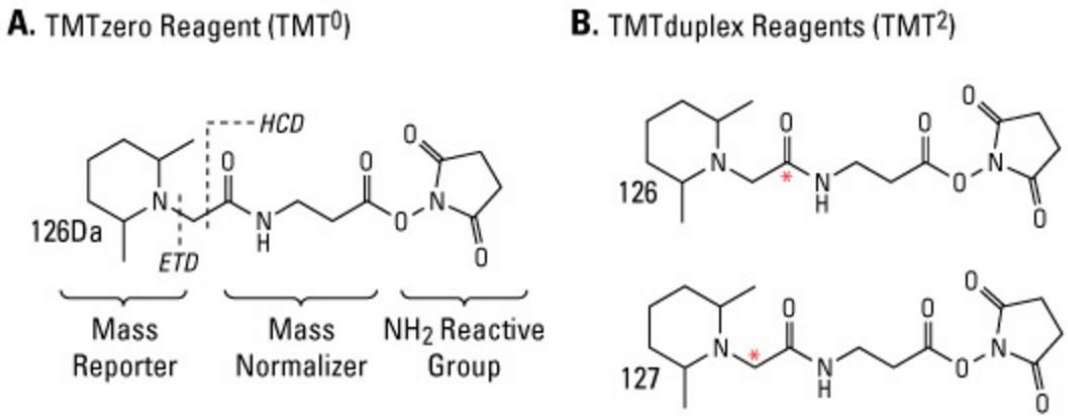
- late introduction
- quality control is necessary

Isobaric tagging reagents

iTRAQ

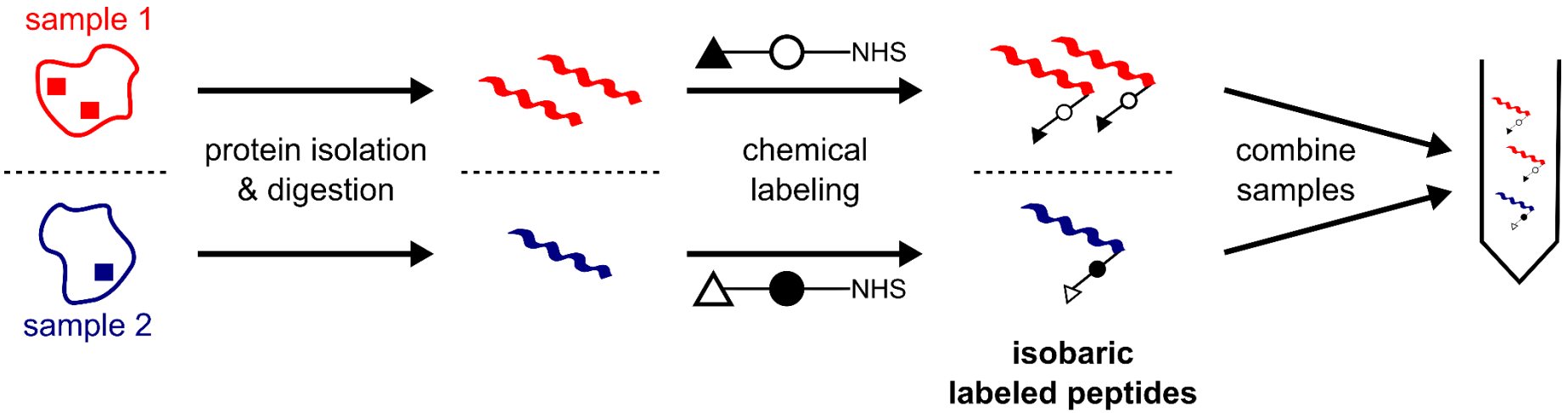


TMT



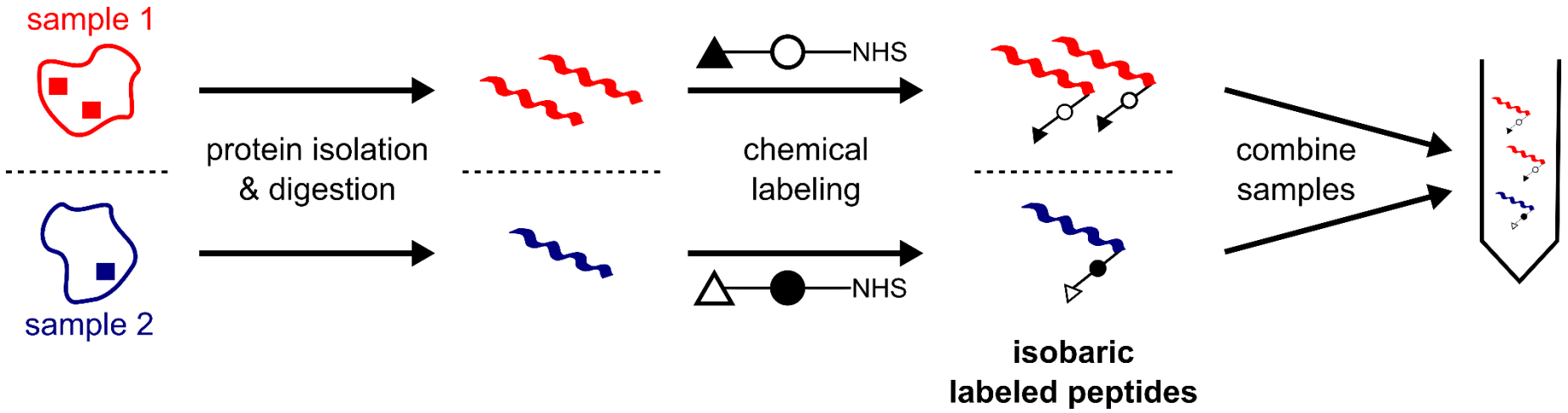
MS-quantification methods

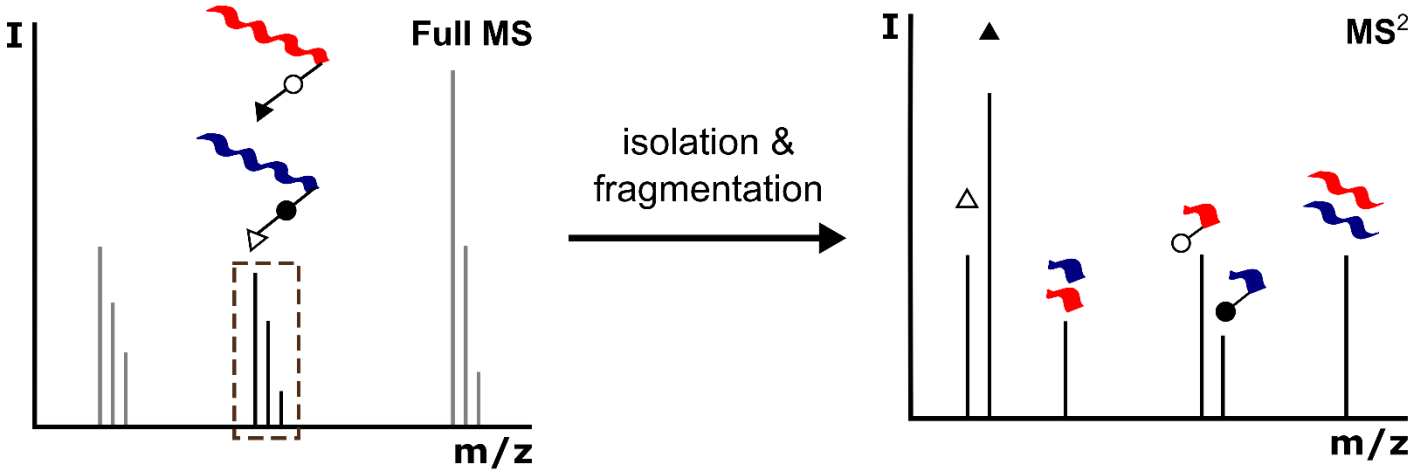
Isobaric tagging of peptides

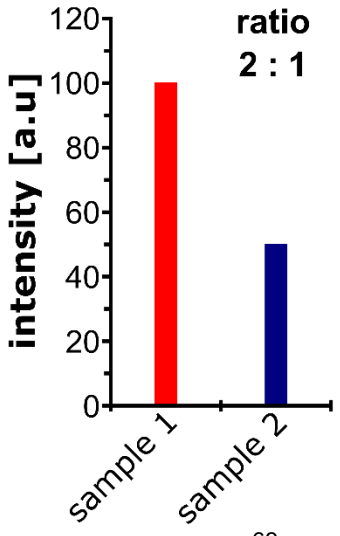


MS-quantification methods

Isobaric tagging of peptides







MS-quantification methods

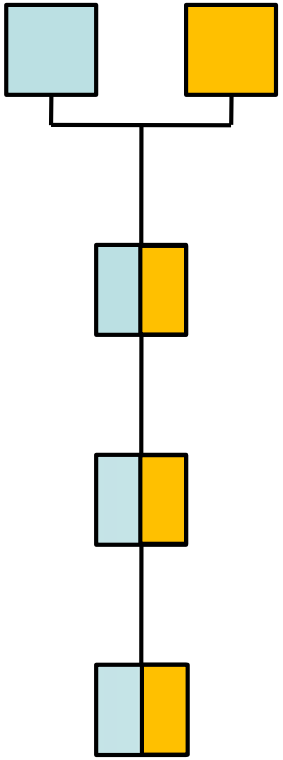
metabolic modifications

cells/tissue

proteins

peptides

MS-analysis



heavy label
light label
no label

methods:

- SILAC (stable isotope labeling by amino acids in cell culture)

Pros:

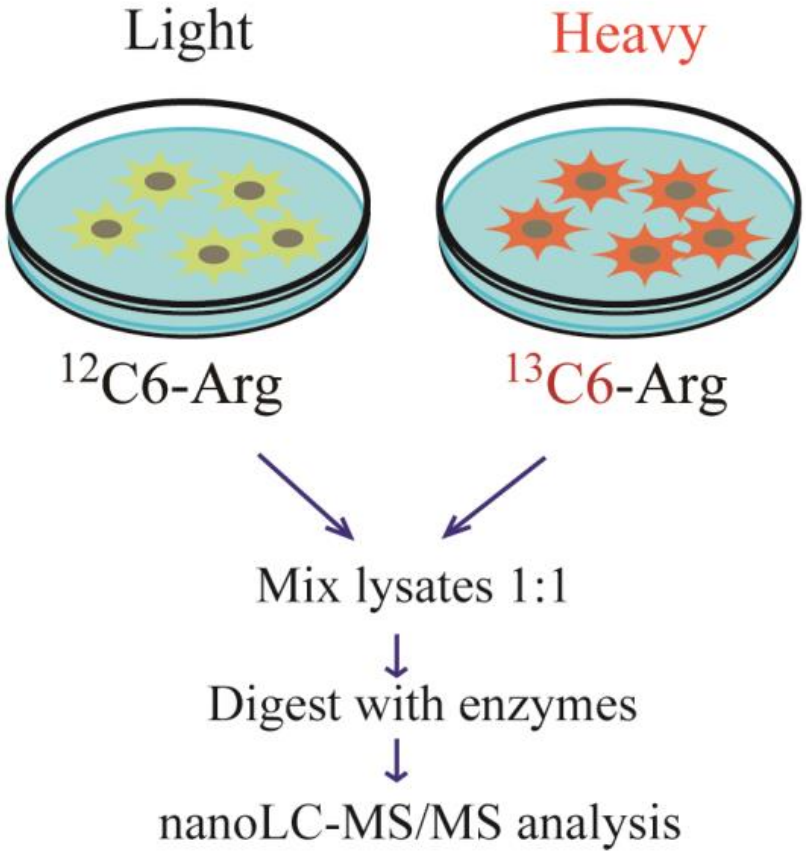
- very early labeling
- possible in live cells
- measurement of multiple samples combined

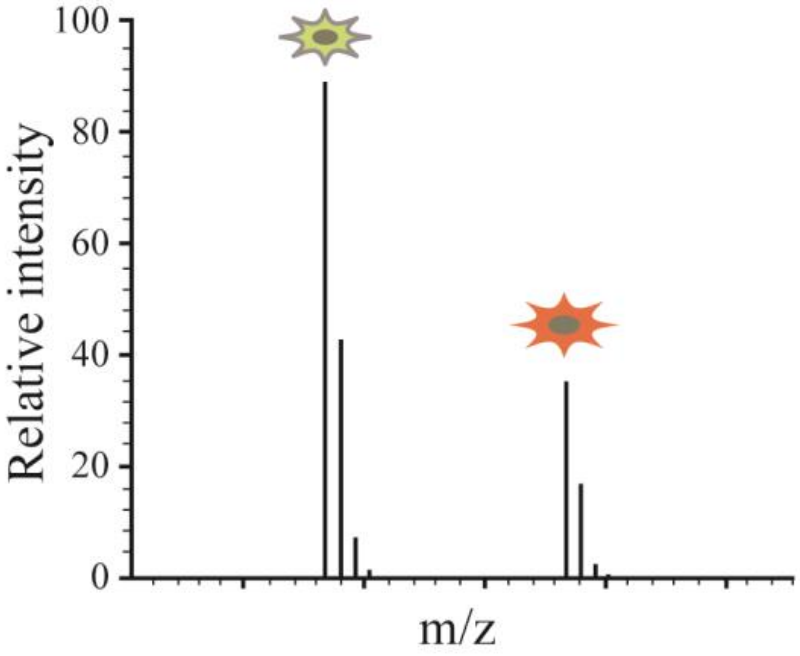
Cons:

- expensive
- time consuming
- only feasable for cell culture samples

MS-quantification methods

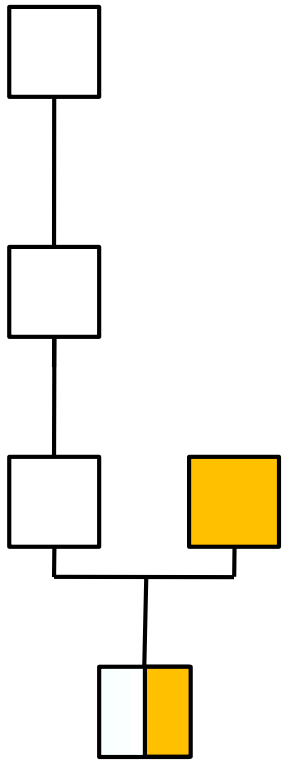
metabolic modifications





MS-quantification methods

isotopically labelled standard peptides



heavy label
light label
no label

Methods:

- AQUA (absolute quantification)

Pros:

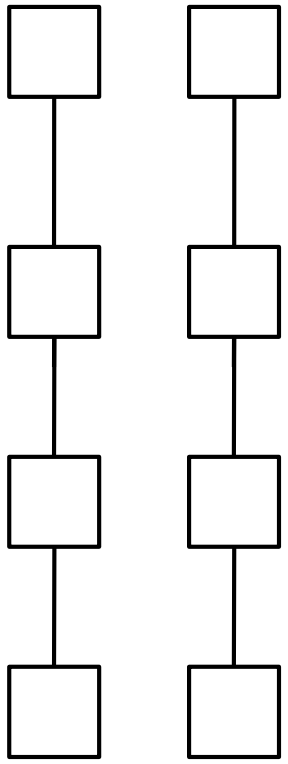
- simple
- absolute quantification possible

Cons:

- very late introduction
- very expensive
- small dynamic range
- labelled peptides necessary

MS-quantification methods

Label-free quantification



heavy label
light label
no label

modified after M.Mann et al.

Methods:

- precursor signal intensity
- spectral counting

Pros:

- cheap
- no sample preparation necessary
- different samples can be compared after measurement

Cons:

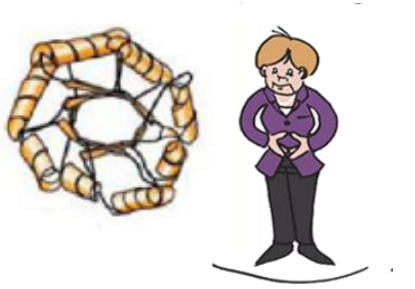
- high variance
- small difference in sample preparation can lead to big differences in quantification
- not very robust
- need to measure every sample in triplicates→ long measurement times

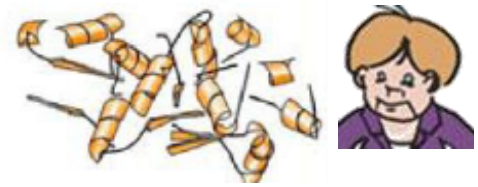
Summary

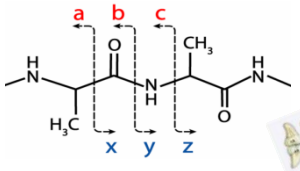
- Mass spectromety is a powerful tool
- Universal detector
- Enables Identification and quantification
- Various types/methods for each application

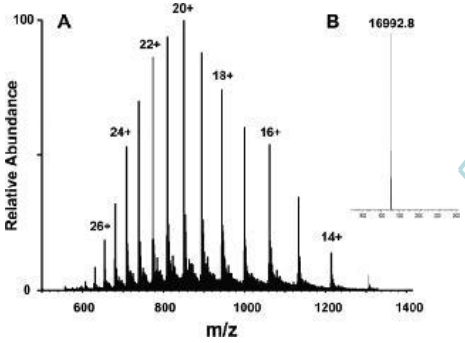
Protein Analysis – different methods to ID proteins

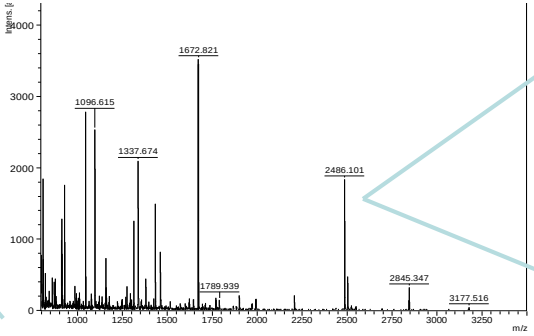
Identification (of Proteins)

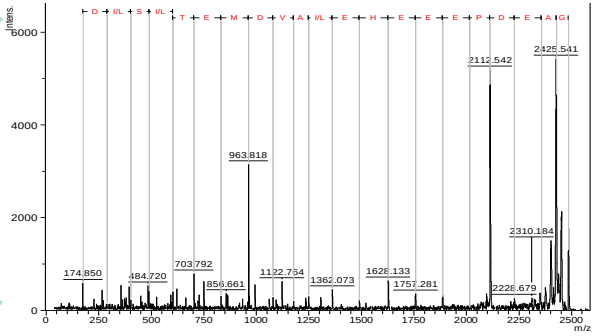












Top-Down → intact protein
→ modified? pure? truncated?
→ quantification of isoforms

PMF → intact peptides
→ which proteins in the mix?
→ modified sequences?

Bottom-Up → MS/MS
→ sequence?
→ identification?
→ quantities?
→ ...

Outline

1) Mass spectrometry in general
    1) What is MS?
    2) Why MS?
    3) Definitions

2) Mass spectrometer
    1) Principles
    2) Details

3) Applications
    1) Intact proteins
    2) Protein Identification
    3) Quantification

4) Research in Progress

Thank you for your interest!

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