Functional Genomics and Proteomics
National Centre for Biomolecular Research
Faculty of Science · Masaryk University

CEITEC

Protein characterization by mass spectrometry

C7250

Part I

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Proteomics CF, CEITEC-MU
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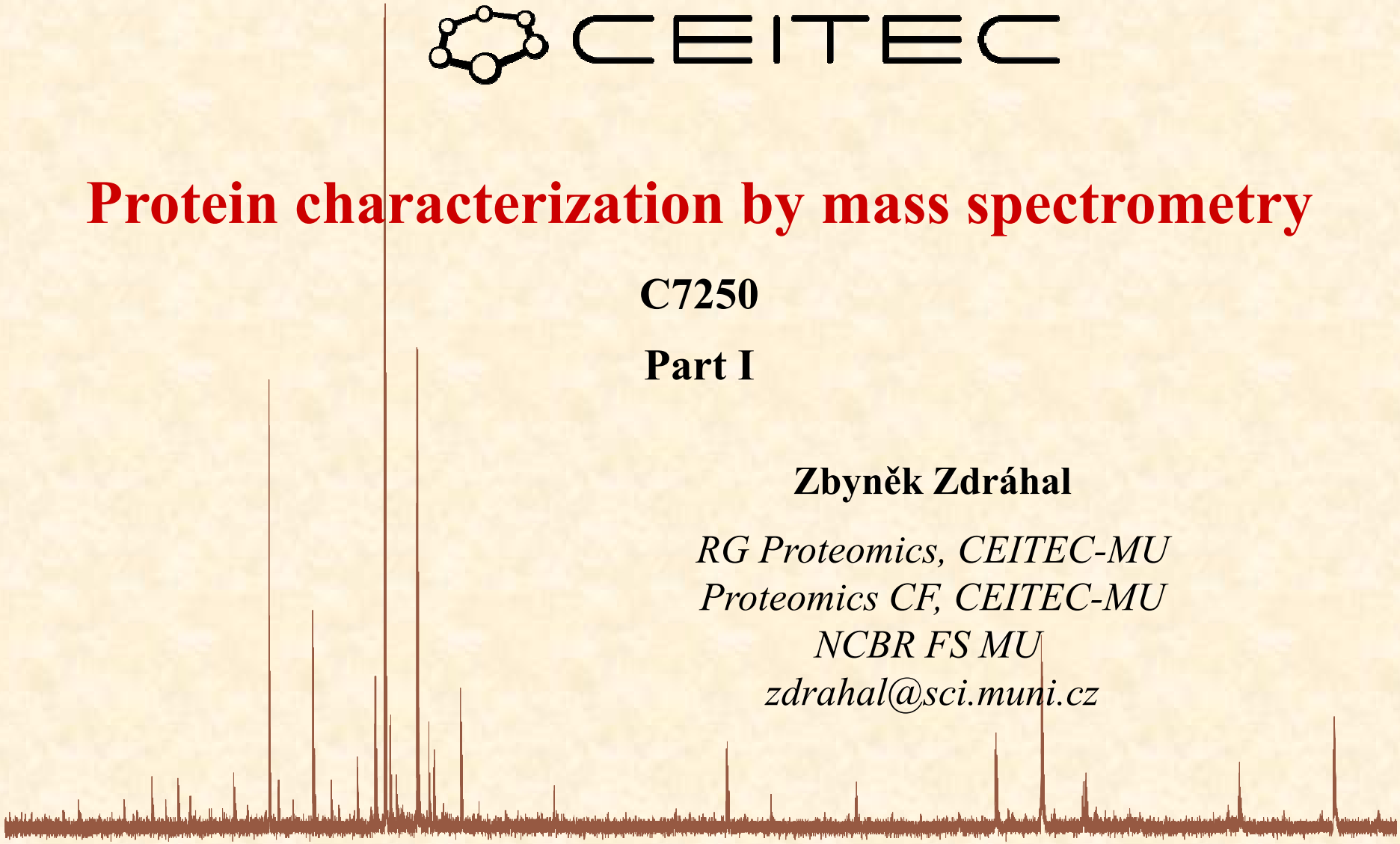

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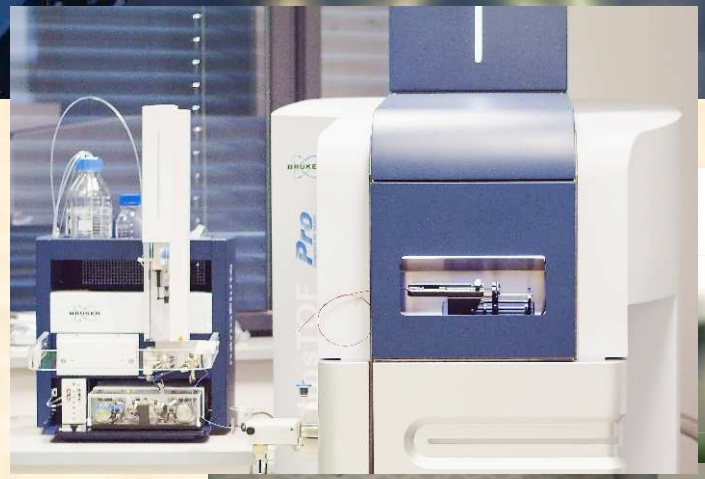
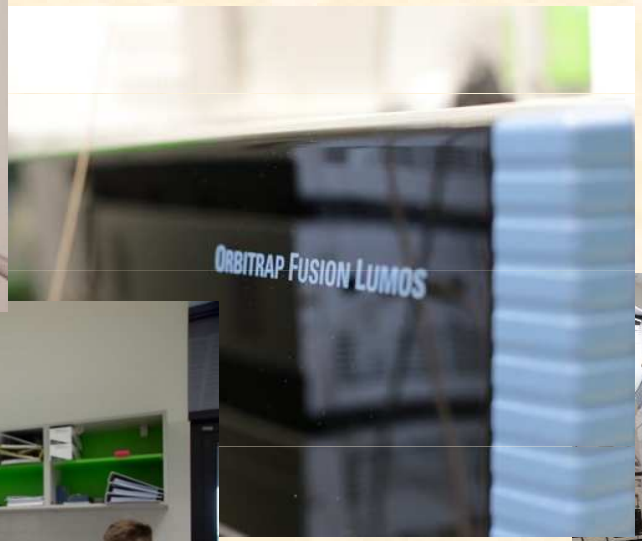
CF: www.ceitec.eu/proteomics-core-facility/cf95
RG: www.ceitec.cz/proteomika-zbynek-zdrahal/rg49

CIISB
Czech Infrastructure for Integrative Structural Biology





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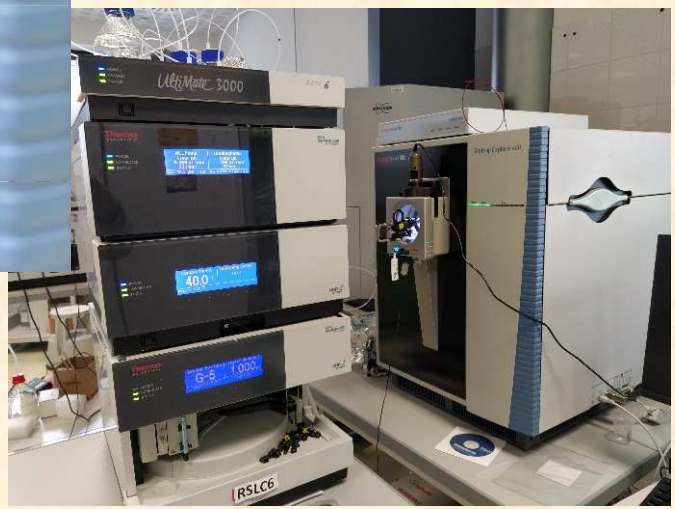


Aims of course:

- **applications/potential of mass spectrometry in proteomics**
- basic approaches of MS analysis
- „interpretation/validity“ of MS results

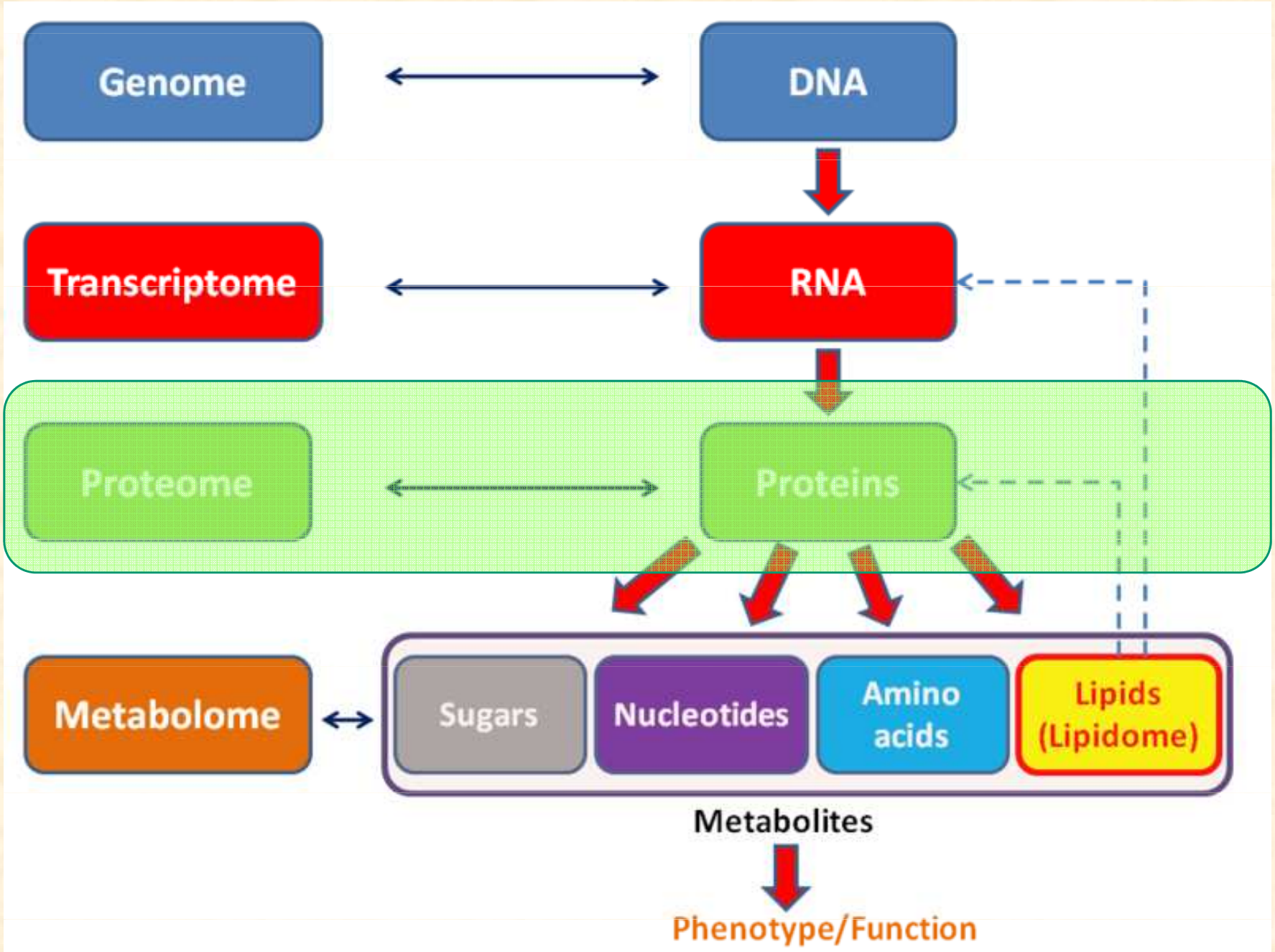
MS
CORE LAB

Proteins are responsible for both the structure and the functions of all living organisms.

Genes are simply the instructions for making proteins.

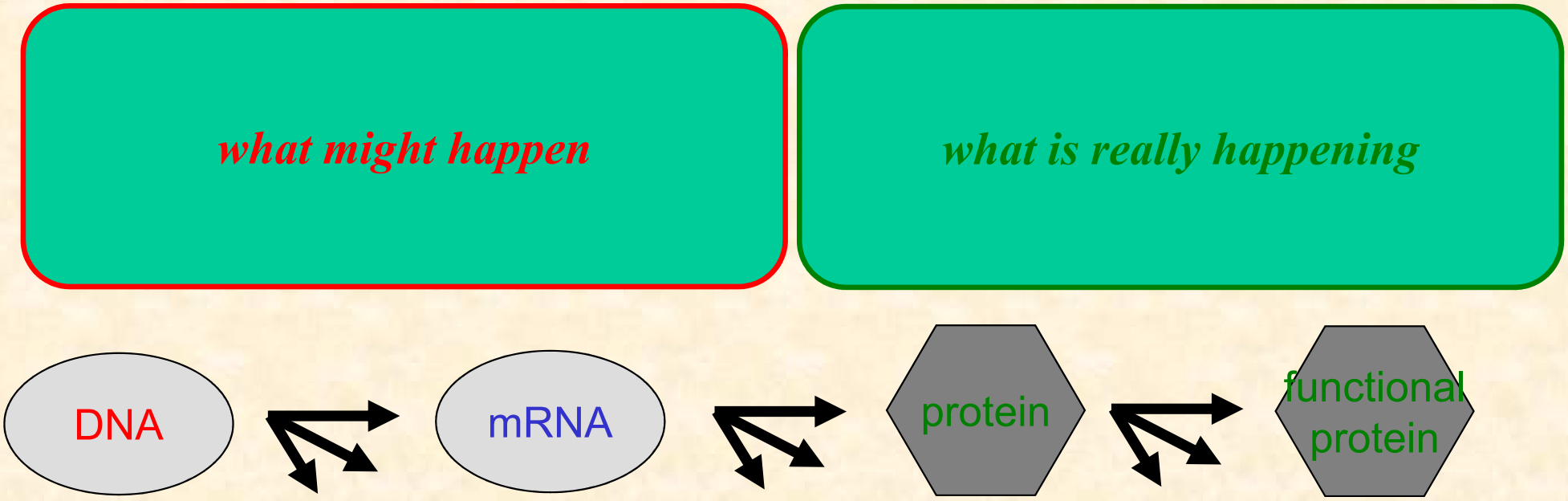
IT IS PROTEINS THAT MAKE LIFE.

http://www.biology-pages.info/P/Proteomics.html



http://en.wikipedia.org/wiki/File:Metabolomics_schema.png

Proteomics – discipline dealing with proteome analysis



transcription
posttranscriptional modifications
(alternative splicing etc.)

translation

posttranslational mods
Protein complexes

Proteomics - Why?

- several proteins/proteoforms might form from each gene, not possible to indicate them by DNA/RNA analysis
- there no direct correlation between mRNA content and final content of proteins
- functionality of protein depends frequently on its interaction with other proteins or DNA/RNA
- only at protein level epigenetics factors of gene expression regulation are detectable

The Desperate Man, Gustave Courbet

Proteome

the word “proteome” is derived from **PROTE**ins expressed by a gen**OME**, and it refers to all the proteins produced by an organism

Marc Wilkins in 1994

the complement of proteins expressed in a cell, tissue, or organism by a genome

the entire complement of proteins found in an organism over its entire life cycle, or in a particular cell type **at a particular time under defined environmental conditions.**

The entire set of proteins expressed by a genome, cell, tissue or organism at a certain time. More specifically, it is the set of expressed proteins in a given type of cell or organism, at a given time, under defined conditions.

proteotype

Genome vs Proteome

the same genome

Genome *versus* Proteome

relat
DNA

4 ba

effic
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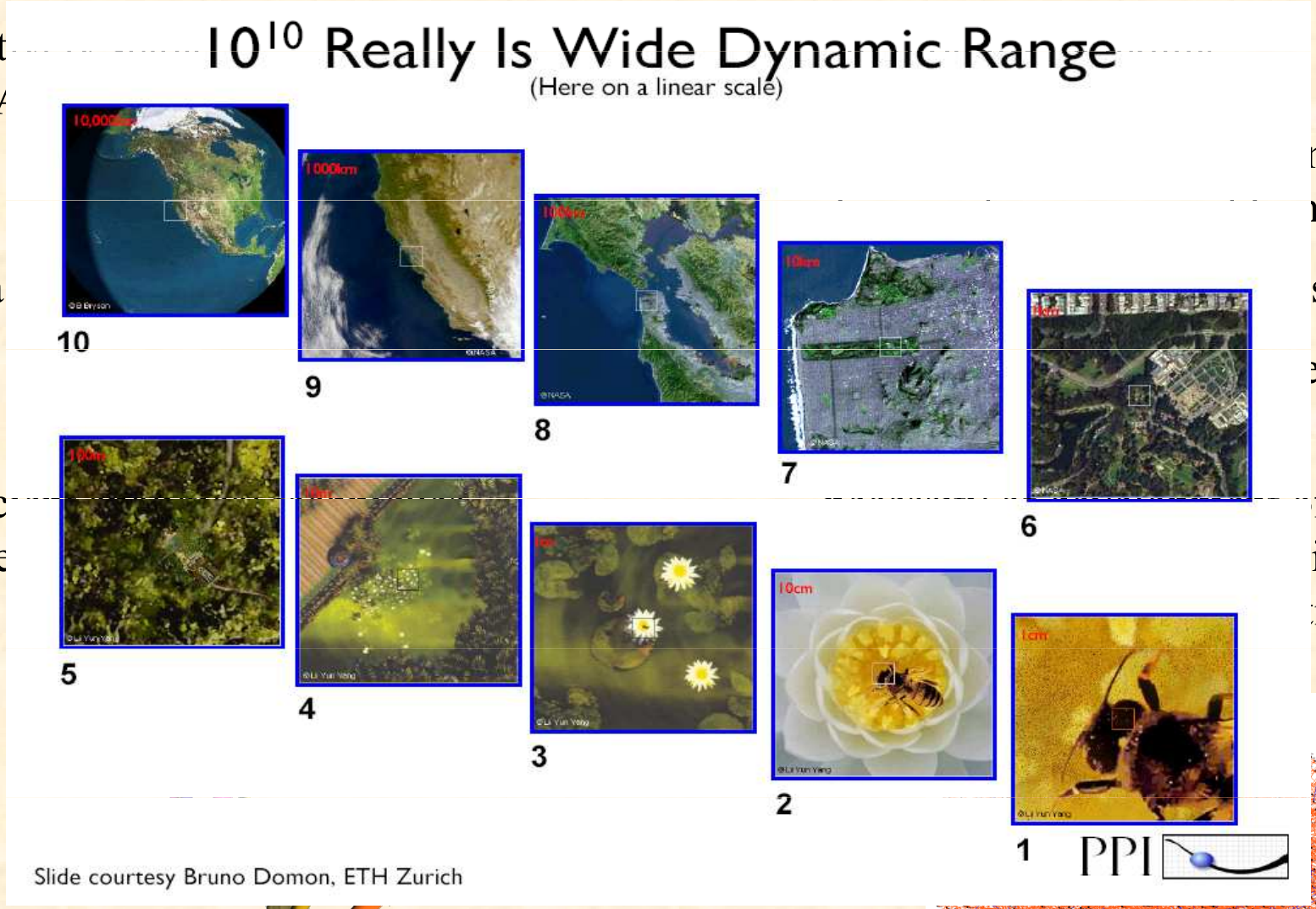

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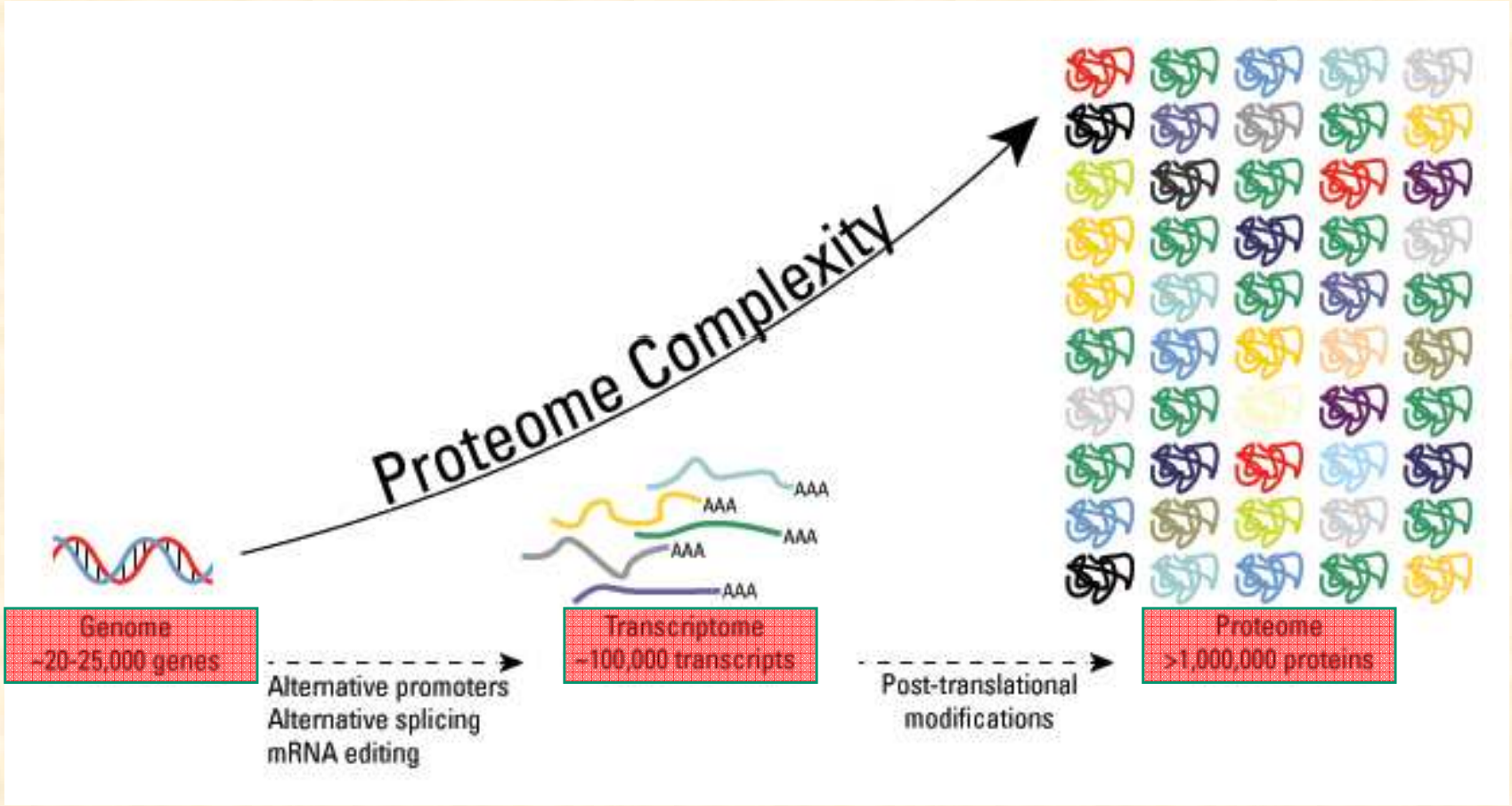
Genome *vs* Traskriptome *vs* Proteome



Estimates for human

http://www.piercenet.com/method/overview-post-translational-modification

Proteome complexity

Estimates from deep proteomics and transcript profiling suggest that about half the human genome is expressed in proteins at over 20 copies per cell in a given cell type (i.e., about 10,000 of the 20,000 human genes)[28]. Assuming this expression threshold of 10,000 genes and allowing for detection of ~100 proteoforms for each gene product, one then multiplies these two to arrive at a measurement target of 1,000,000 distinct proteoforms in a given cell type. A 2016 estimate based on trends in databases indicated that the number may be ~6 million proteoforms[29]. Better estimates of this proteoform diversity are needed, and are analogous to the extrapolations of the number of human genes using expressed sequence tags (ESTs) in the year 2000 (ref. 30).

Aebersold R. et al, Nat. Chem. Biol., 14, 206–214 (2018)

74 unique histone H4 proteoforms out of [illegible] **possible** (based on know[illegible] [illegible]ected in differentiating human [illegible]s.

Phanstiel D. et al, PNAS, 105, 4093–4098 (2008)

The nature behaves rationally or technologies are still not developed enough

Proteomics

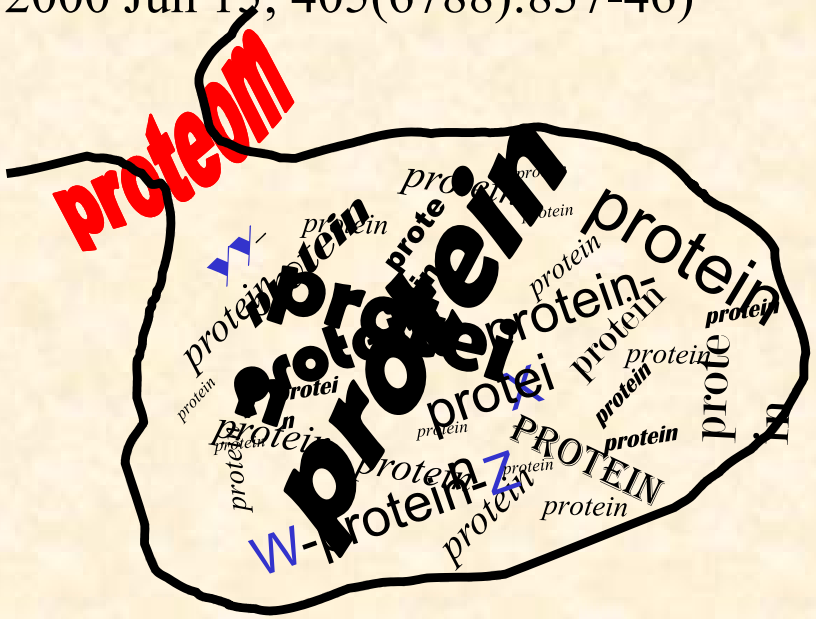
Proteomics is the **large-scale study of proteins**, particularly their structures and functions.

The first is the more classical definition, restricting **the large-scale analysis of gene products to studies involving only proteins**.

The second and more inclusive definition **combines protein studies with analyses that have a genetic readout such as mRNA analysis, genomics, and the yeast two-hybrid analysis** (Pandey A, Mann M Nature. 2000 Jun 15; 405(6788):837-46)

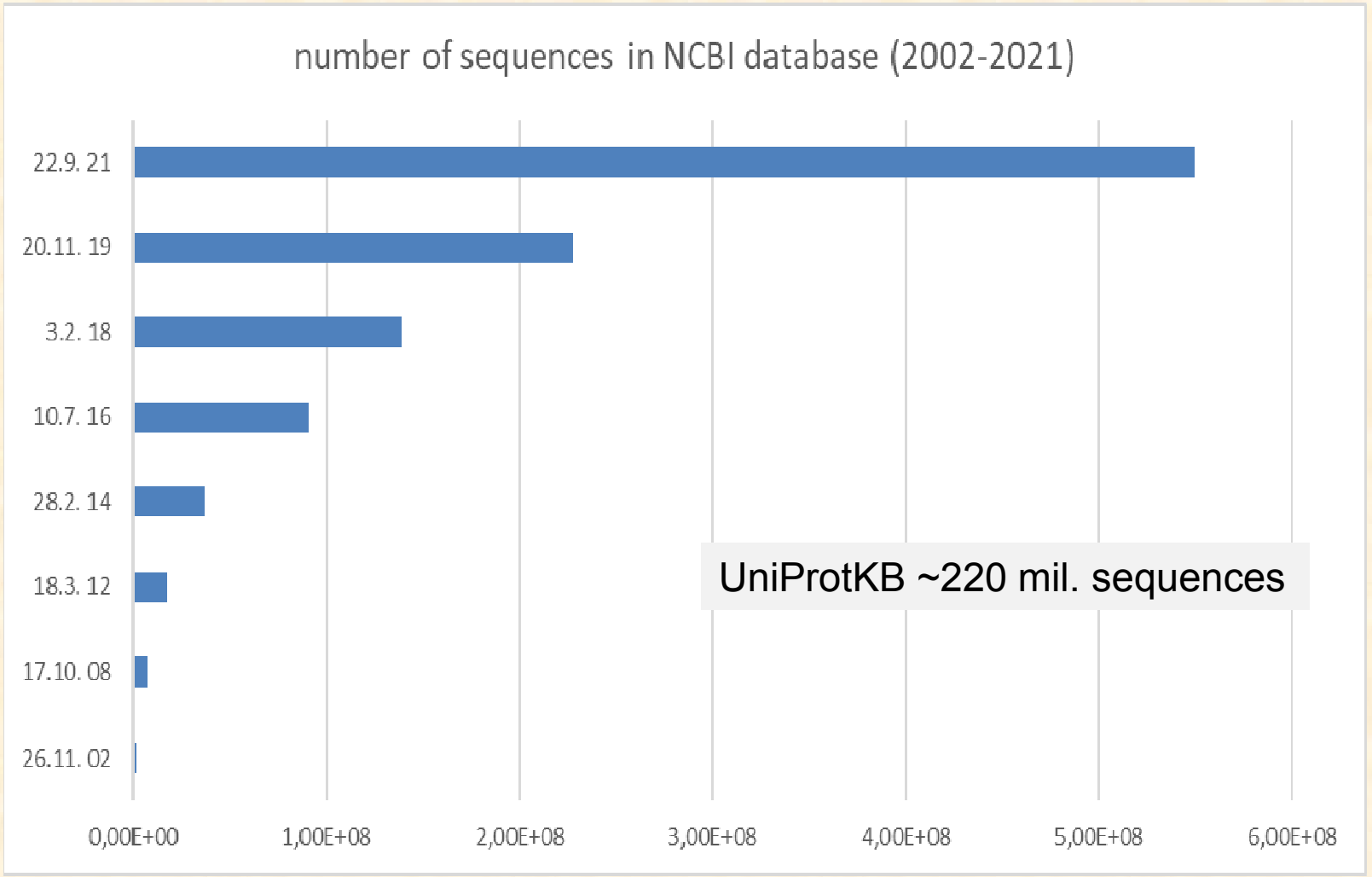
Proteomics has been enabled by the accumulation of :

- **DNA and protein sequence databases**
- improvements in **mass spectrometry**
- **computer algorithms for database searching.**



Increase in knowledge of genomes

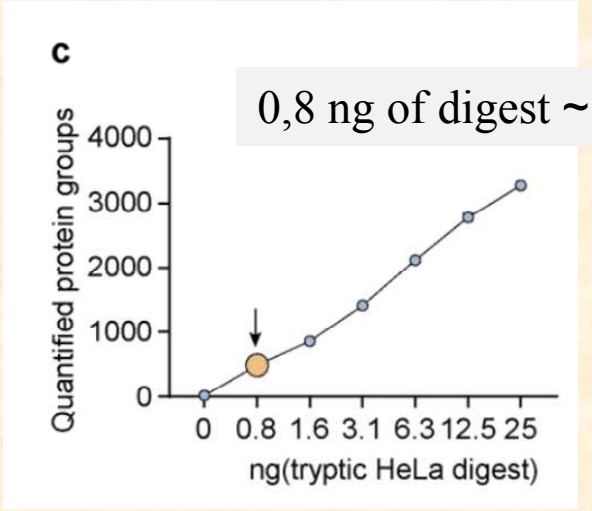
protein characterization by MS is in principle based on knowledge of primary sequence



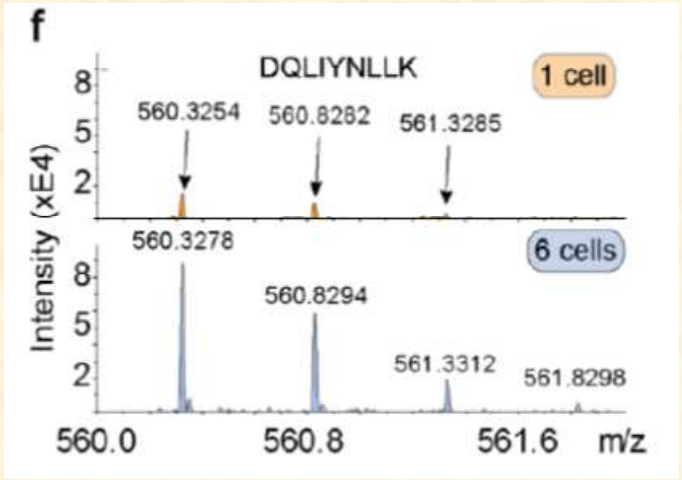
Single cell proteomics

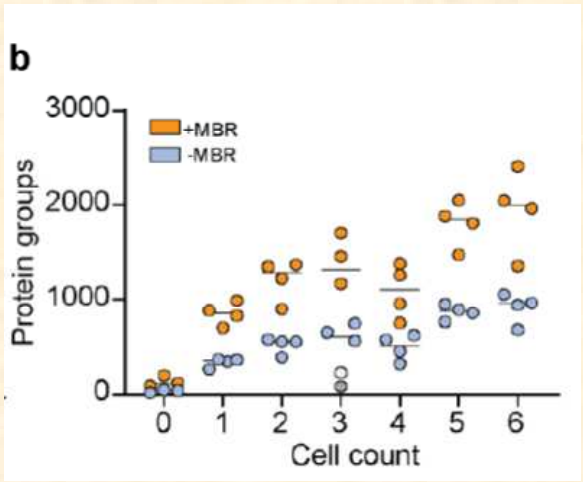
Mass spectrometry technology progress

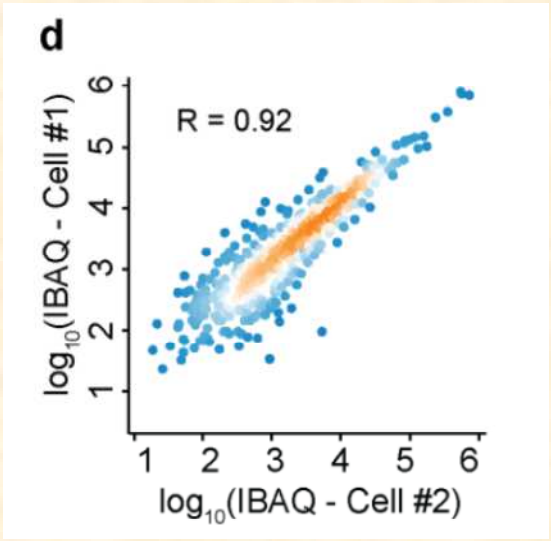
HeLa cells, FACS, LC-MS/MS (TimsTOF Pro)



0,8 ng of digest ~ 3 HeLa cells





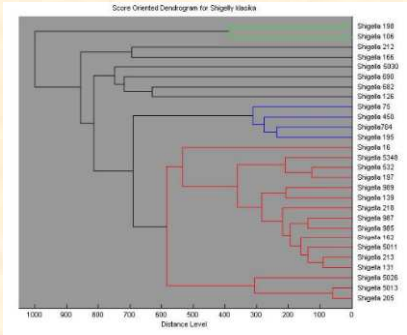


Brunner A.-D. et al. bioRxiv (2021), https://doi.org/10.1101/2020.12.22.423933

mass spectrometry

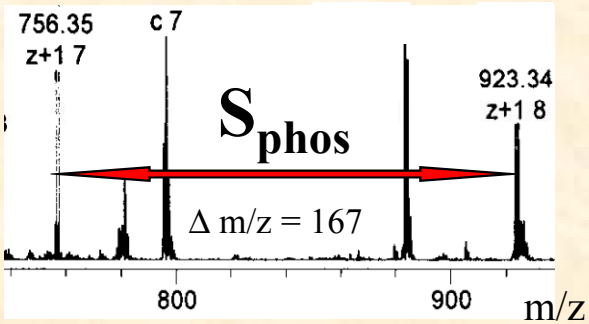
enables simultaneous qualitative and quantitative characterization of thousands of proteins
Analysis of „proteome“

Mass spectrometry in proteomics



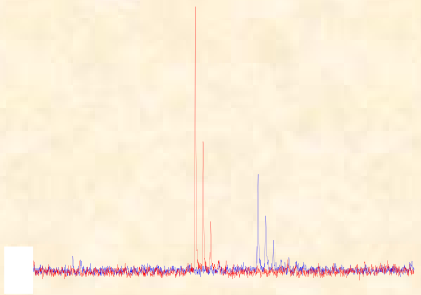
- ***Intact mass analysis***
  (MW, MALDI-MS profiling)

- ***Protein identification***
  (incl. protein complexes, de novo sequencing)



- ***Characterization of protein modifications***

- ***Protein quantification***
  (relative and absolute quantification)



- MALDI-MS imaging,
- 3D structure

Proteomic approaches

- Differential (expression) proteomics
- Functional proteomics
- Structural proteomics

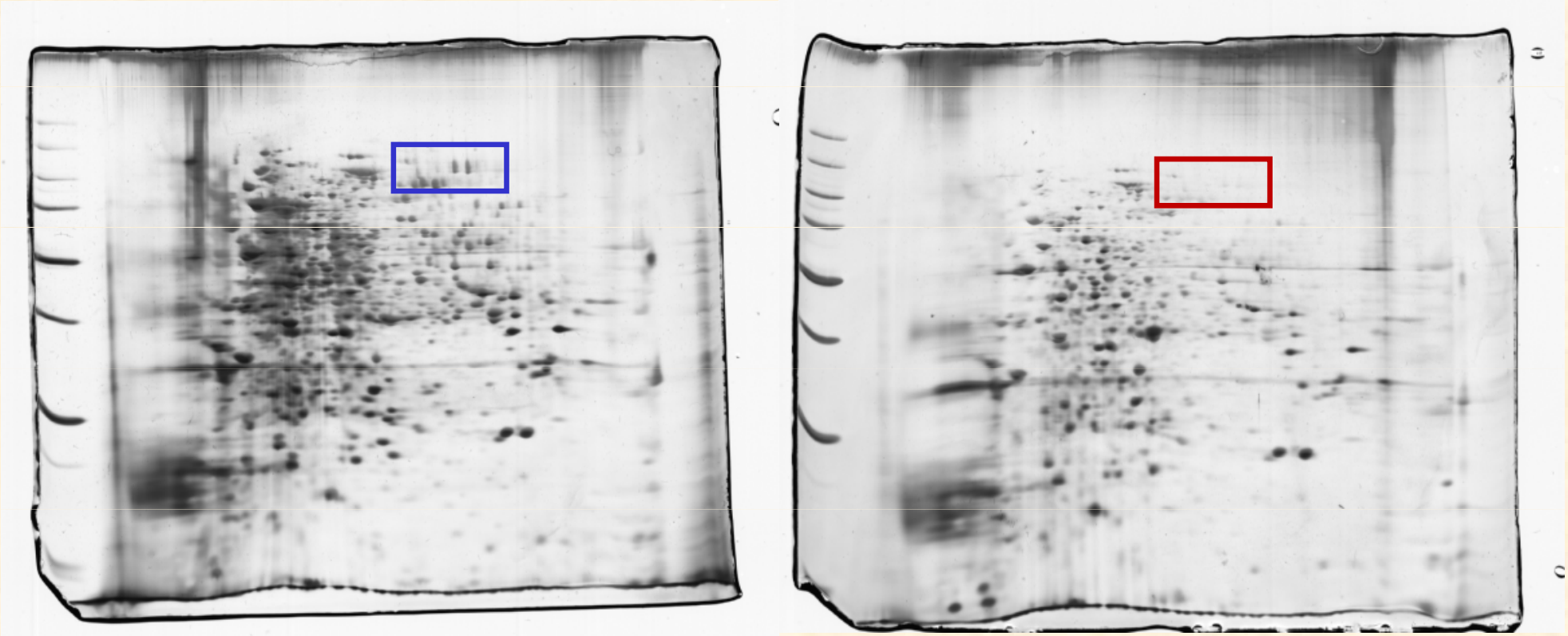
Differential (expression) proteomics

Qualitative and quantitative **comparison of proteomes**

aim – determination of changes at protein (and their forms (e.g. PTMs)) levels
which were induced by internal or extermal stimuli.

control

stress



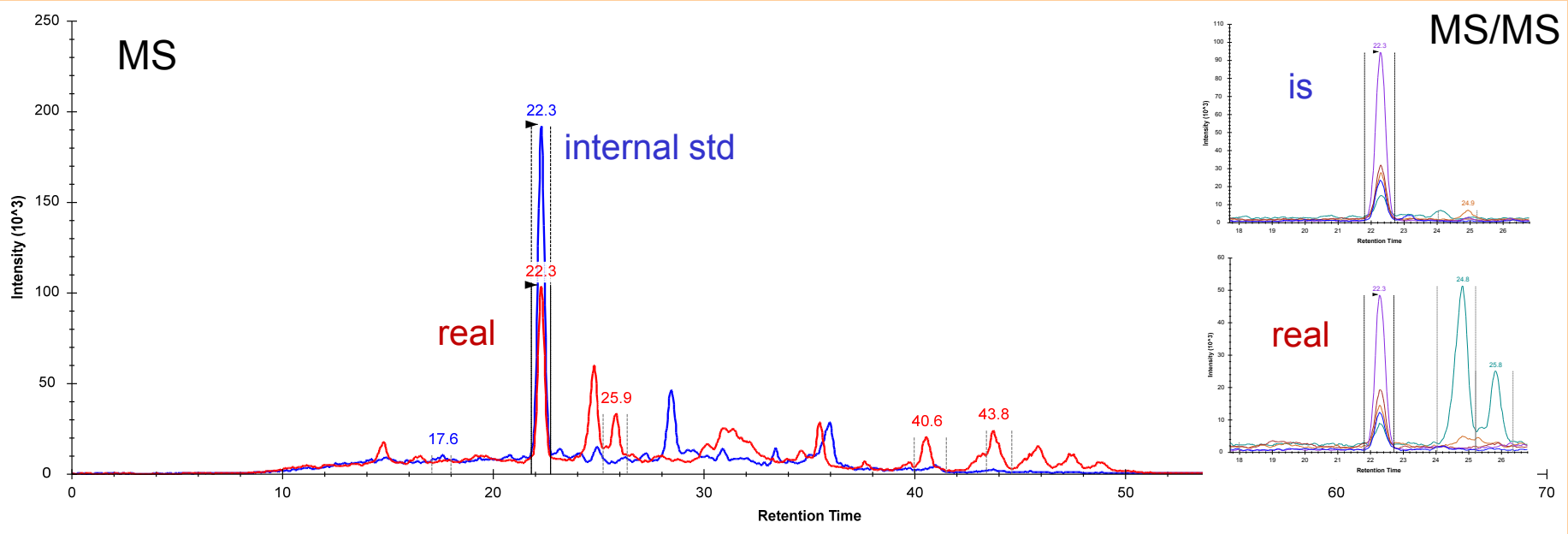
Rhodotorula glutinis

Cooperation with prof. Márová, FCH BUT Brno

Differential proteomics
Targeted approach

Monitoring of quantitative changes of **selected proteins** (e.g. biomarkers) in sample sets.

Determination of enterotoxin *(S. aureus)*

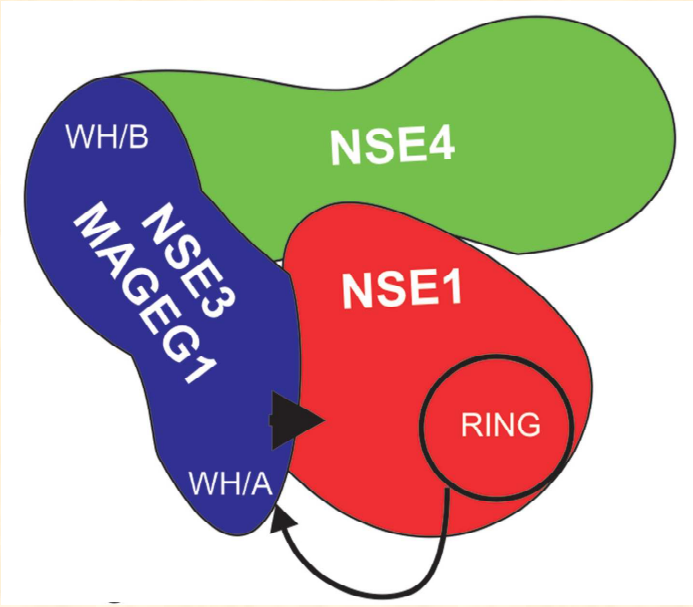


LC-MS/MS (MRM, PRM)

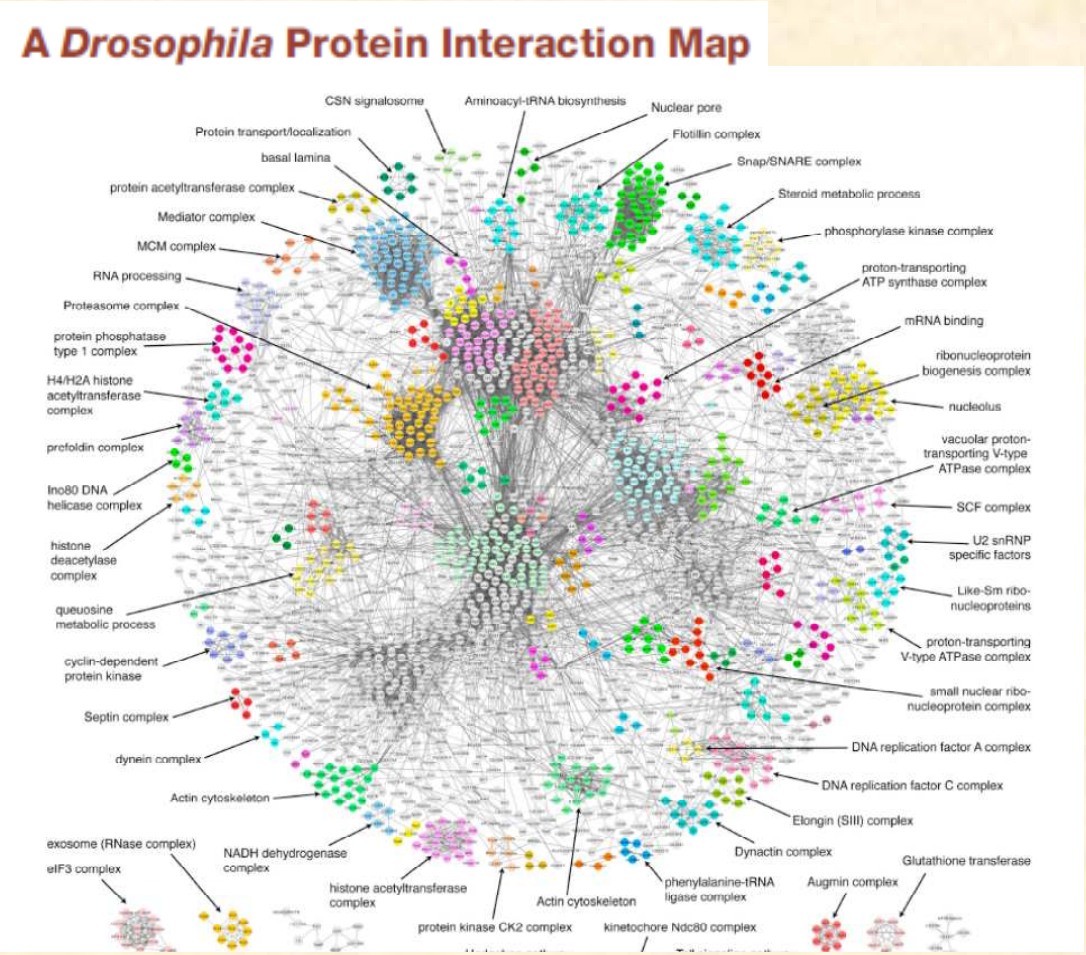
Functional proteomics

Study **of interactions of proteins** and their functional context

- protein-protein interactions
- architecture of protein complexes
- protein interactions with other types of molecules (RNA, DNA metabolites ...)



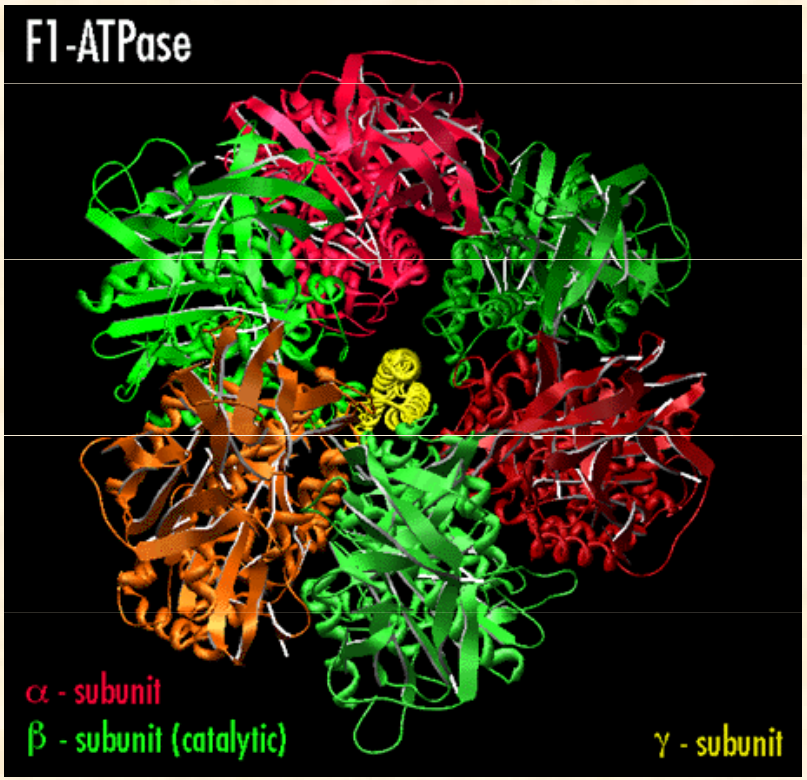
L. Kozakova et al.,
Cell Cycle, 14 (6), 920-930 (2015)



K.G. Guruharsha et al., Cell, 147, 690–703 (2011)

Structural proteomics

Study of higher levels of protein structure (tertiary, quaternary) and relation of a structure to protein function.



- X-ray crystalography
- NMR
- cryoEM
- MS (in minority)

Structure is formed by different types of bonds – ion interactions, hydrogen bridges, van der Waals forces or disulfidic bridges.

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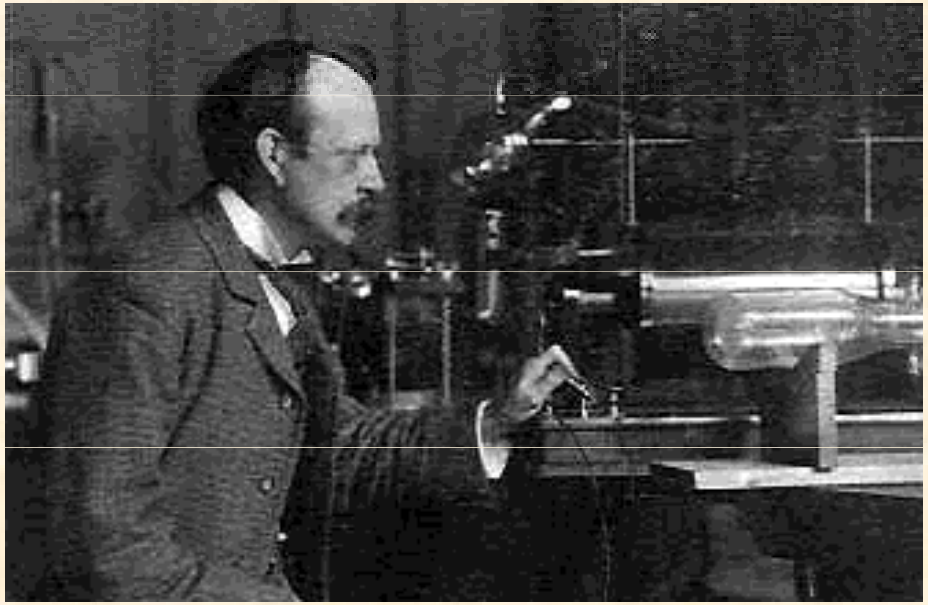
Faculty of Science Masaryk University

MS instrumentation and proteins

History of mass spectrometry



J.J. Thomson working with his cathode ray tube

Thomson J.J. (1856 – 1940)
On the Masses of the Ions in Gases at Low Pressures
Philosophical Magazine, **1899**, 48:295, p.547-567

1906 – Nobel prize for physics
for theoretical and experimental investigations on the conduction of electricity by gases

Eight of his students and his son also became Nobel Prize winners

Thomson's investigations into the action of electrostatic and magnetic fields on the nature of so called "anode rays" or "canal rays" would eventually result in the invention of the **mass spectrometer** (then called a *parabola spectrograph*) by **Francis Aston** (**Nobel Prize for Chemistry 1922**), **a tool which allows the determination of the mass-to-charge ratio of ions and which has since become an ubiquitous research tool in Chemistry**.

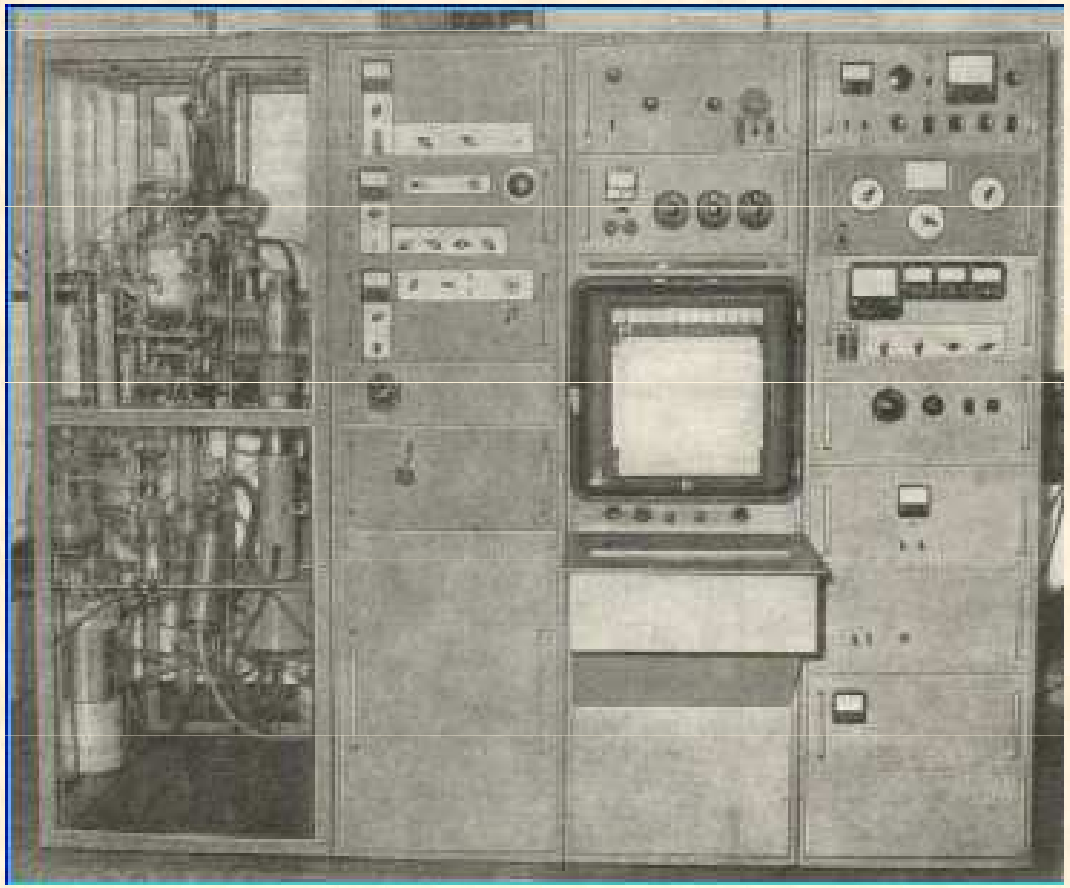
„ ... By this means there is attained what is known as a mass spectrogram, that is to say a series of lines in which each line corresponds to a certain atomic weight.“

Dr. H.G. Soderhaum - 1922

History of mass spectrometry

The first Czech mass spectrometer - 1953

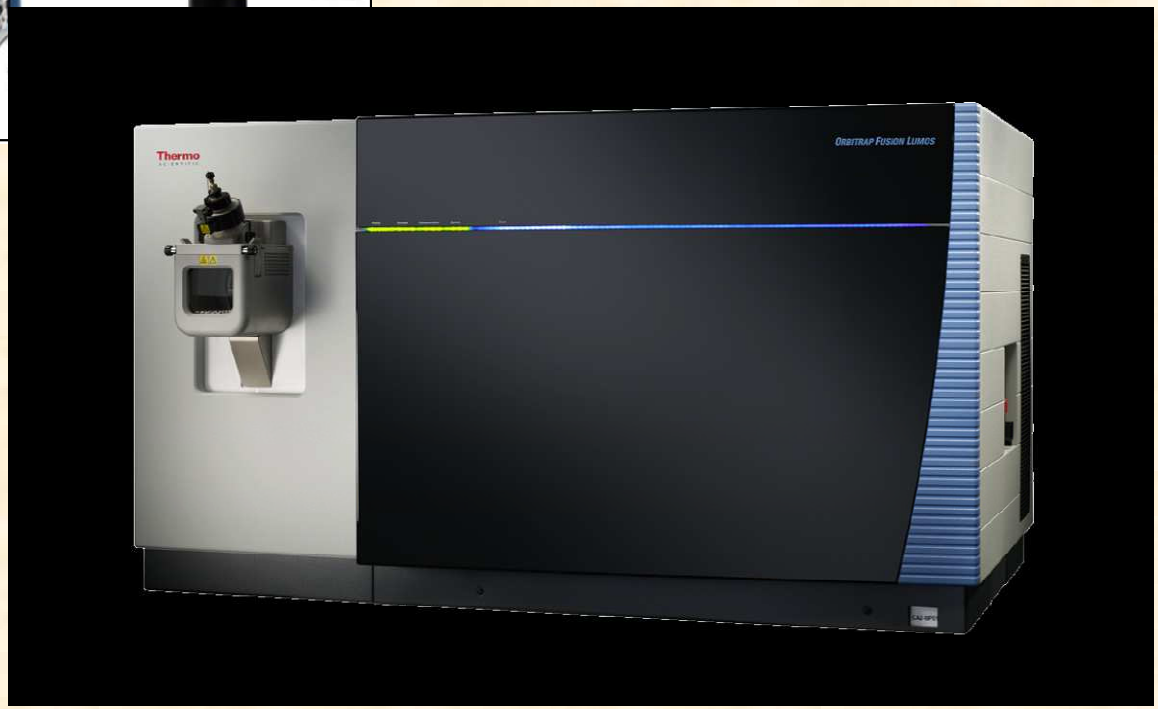
V. Čermák, V. Hanuš, Č. Jech, J. Cabicar



by courtesy of dr. M. Polášek

Mass spectrometry- today

Benchtop LC-MS/MS system
ion trap LTQ Velos (Thermo)

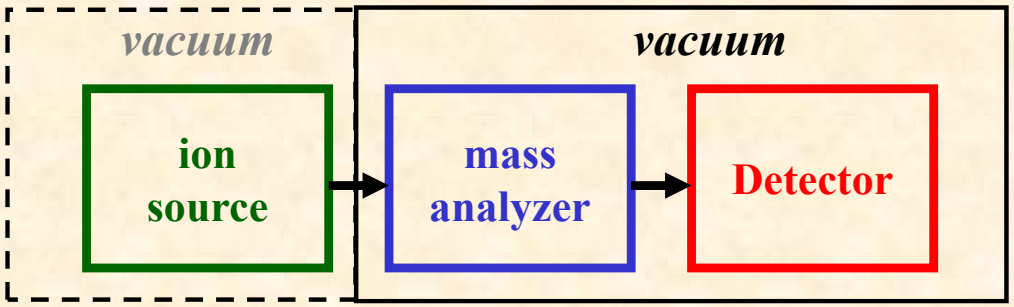


High resolution hybrid mass spectrometer Orbitrap Fusion™ Lumos™ Tribrid™ (Thermo)

Mass spectrometry

principle:

measurement of ratio of relative molecule mass and charge number (*m/z*) of ions of analyzed compounds



m – ion mass
z – number of charges

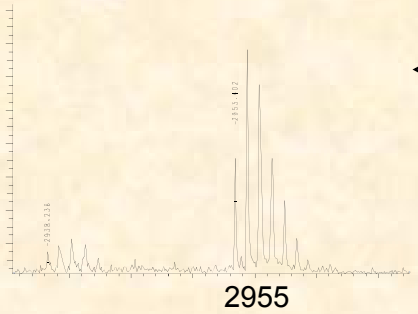
basic steps:

- **ionization of molecules of analyzed compounds**
- **ion separation according to their *m/z***
- **ion detection**

result:

- mass spectrum – dependence of ion intensity *vs* its *m/z*

a.i.



m/z

termination of ion mass
f a molecular ion
molecular mass

Da

Why vacuum in MS

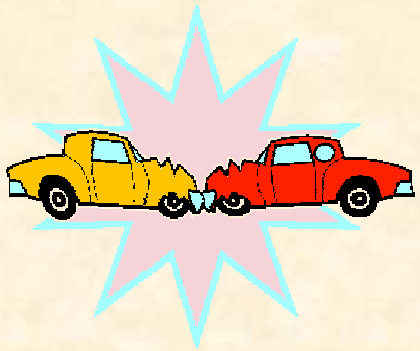
to prevent ions from unwanted collisions during their way from ion source to detector

The mean free path of a molecule

the average distance the molecule travels between two consecutive collisions with other moving particles

example: N_2 molecules

pressure (Torr)	free mean path (m)
760	$5{,}86 \times 10^{-8}$
1	$4{,}45 \times 10^{-5}$
10^{-3}	$4{,}45 \times 10^{-2}$
10^{-5}	$4{,}45 \times 10^{0}$
10^{-6}	$4{,}45 \times 10^{1}$
10^{-10}	$4{,}45 \times 10^{5}$



adopted from presentation of dr. M. Polášek

Ionization techniques

New „soft“ ionization techniques - ESI and MALDI

basic prerequisite for wide use of MS in biomolecule analysis

(Nobel prize 2002)

MALDI

matrix-assisted laser desorption/ionization

most often in combination with time-of-flight mass analyzer - **TOF**

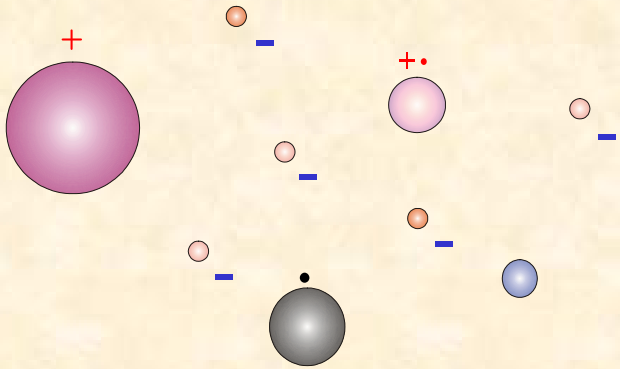
(MALDI-MS, MS/MS)

ESI

electrospray ionization

usually in combination with ion trap and hybrid mass spectrometers

(IT, QQQ, QTOF, IT-Orbitrap, IT-ICR etc.)



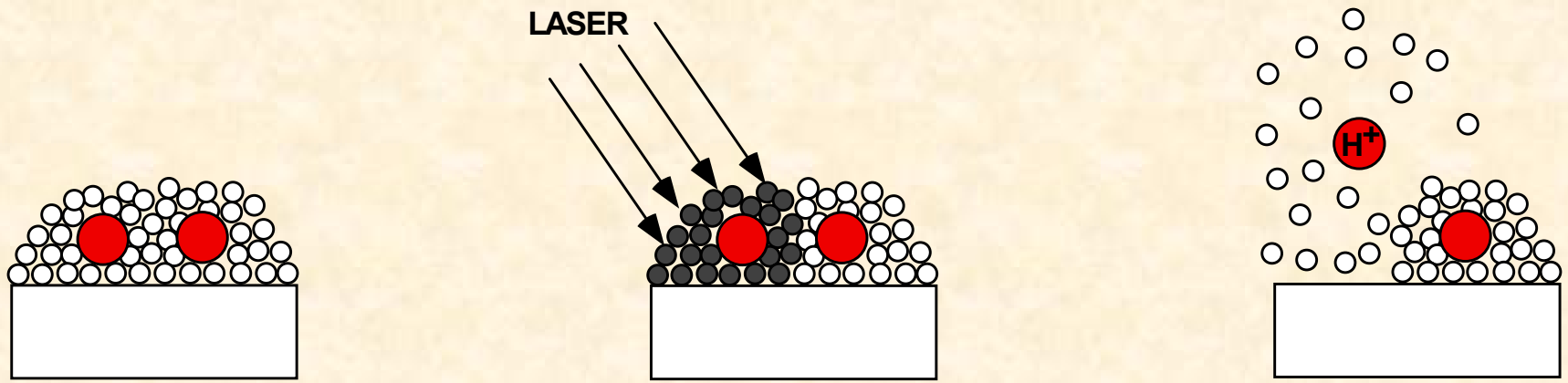
MALDI

Desorption-ionization process

Sample embedded in light-absorbing matrix

LASER-excitation of matrix molecules

Sample desorption and protonation



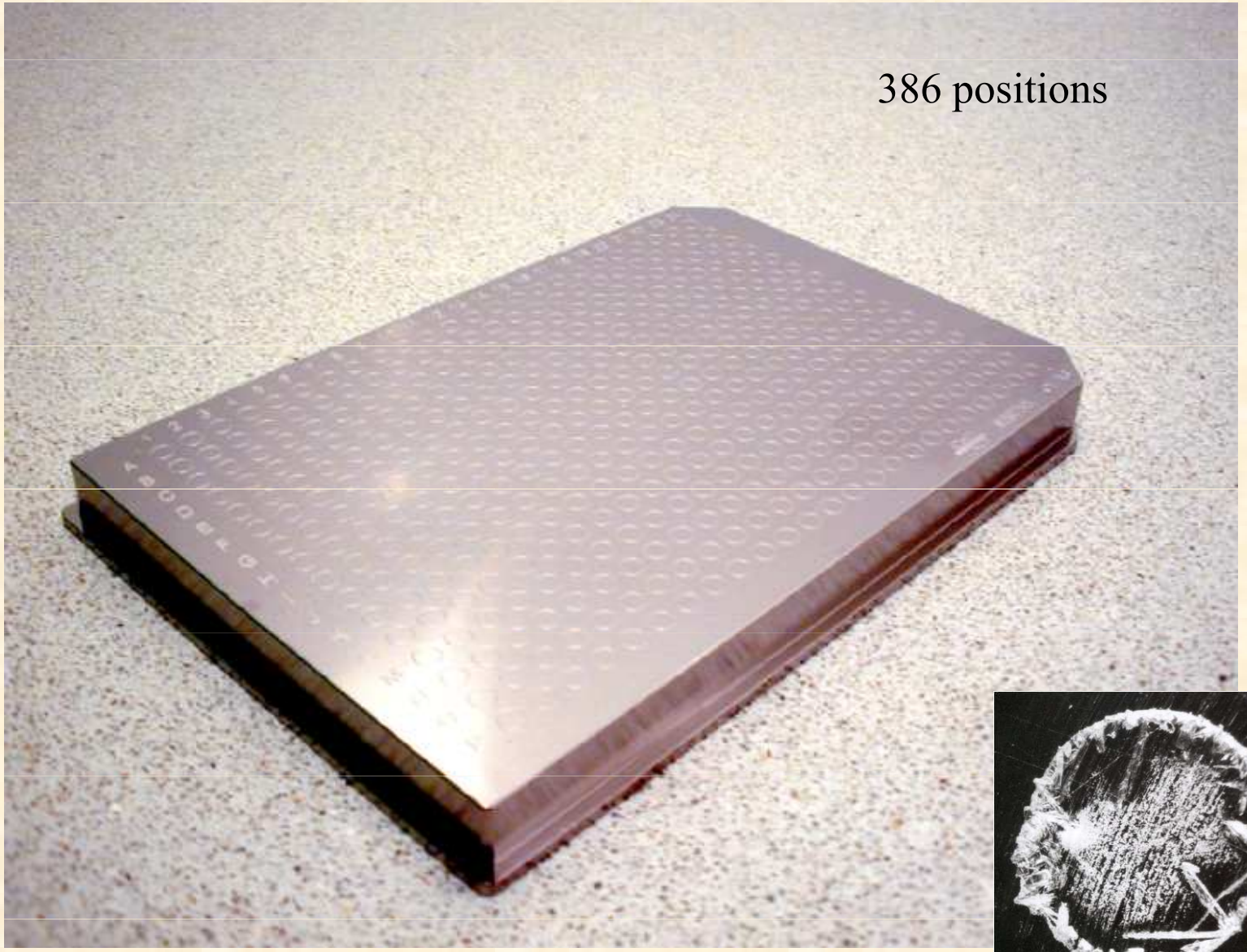
matrix is low mass compound capable to absorb laser radiation

e.g. Dihydroxybenzoic acid (for UV laser)

- Soft ionization without fragmentation
- Simple spectra
- Sample storage on sample target

pictures by courtesy of Dr. Sauerland (Bruker)

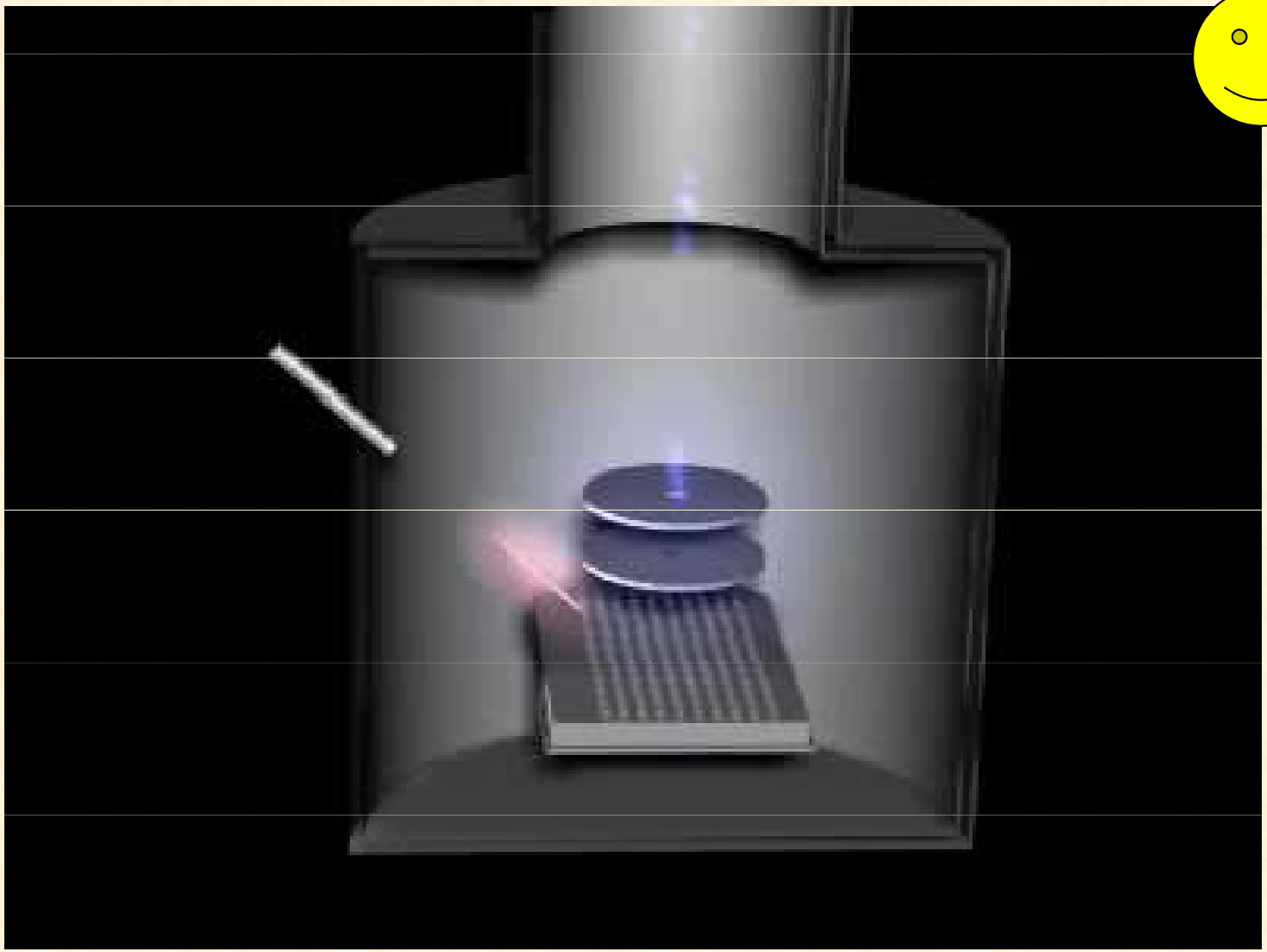
Sample target for MALDI-MS Ultraflextreme (Bruker)



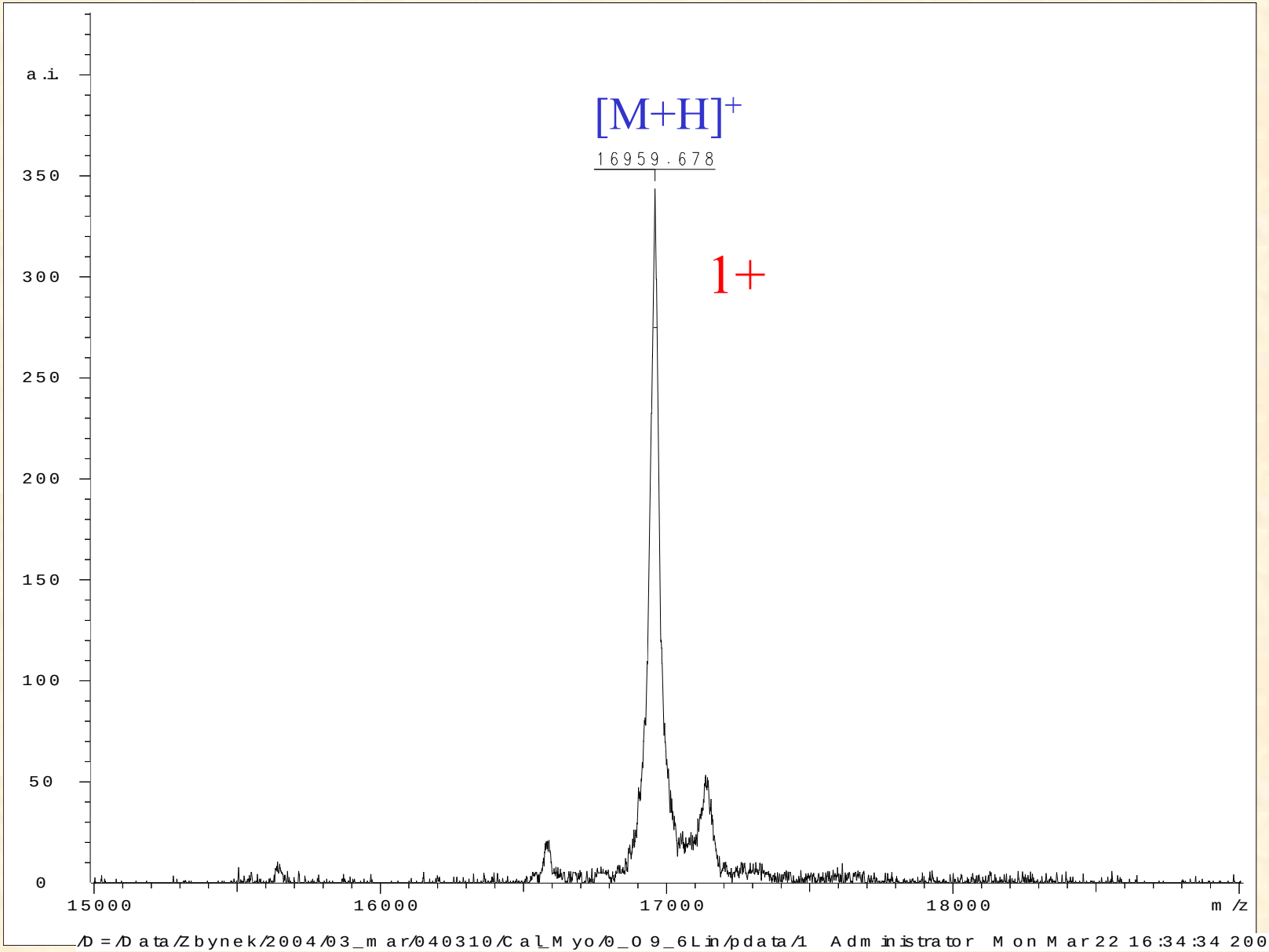
Sample spot with DHB matrix

MALDI – ionization

First part, cca 30s

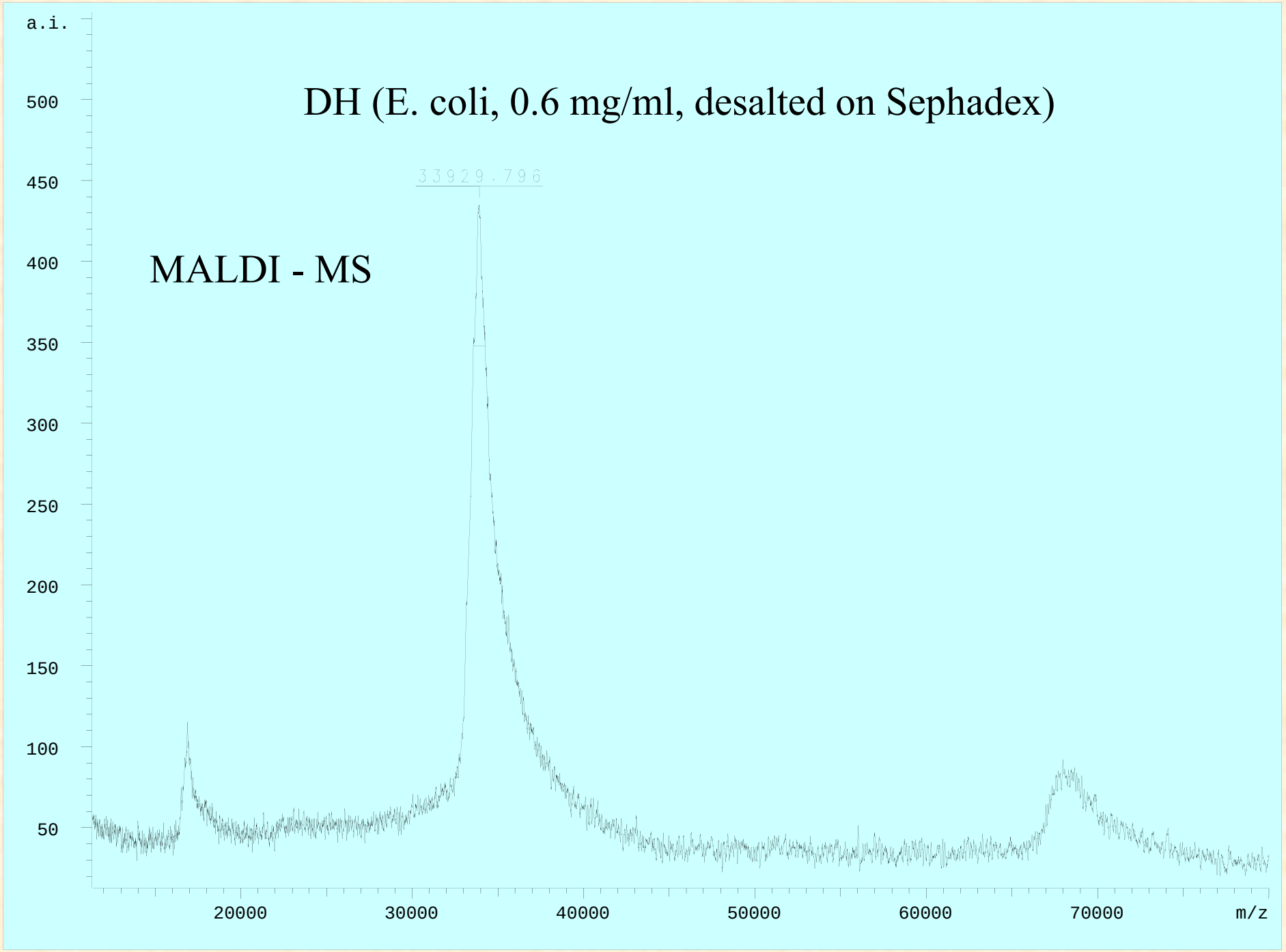


MALDI-TOF spectrum of myoglobin (16 951 Da)

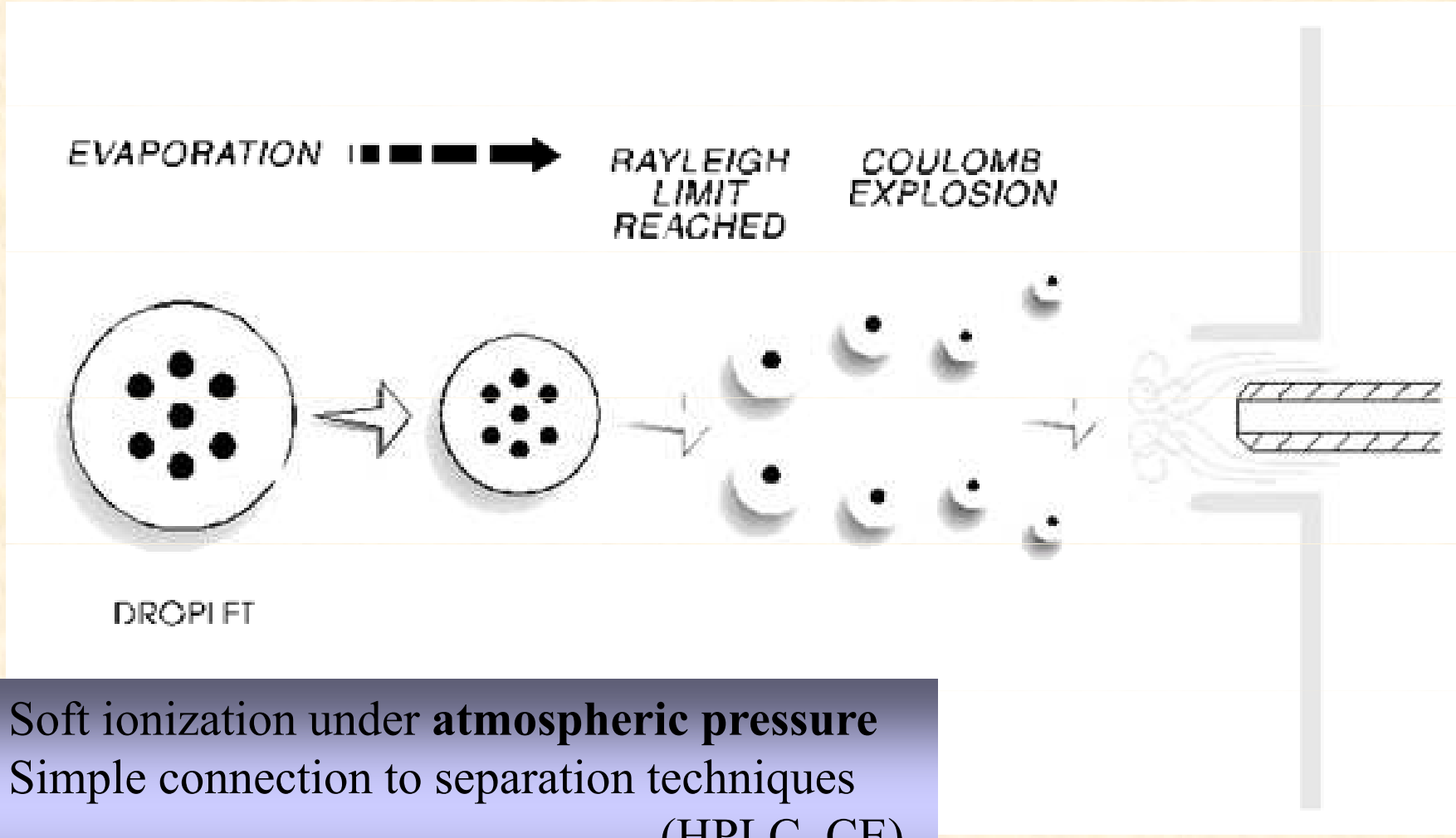


Salt content influence on MALDI ionization

C7250



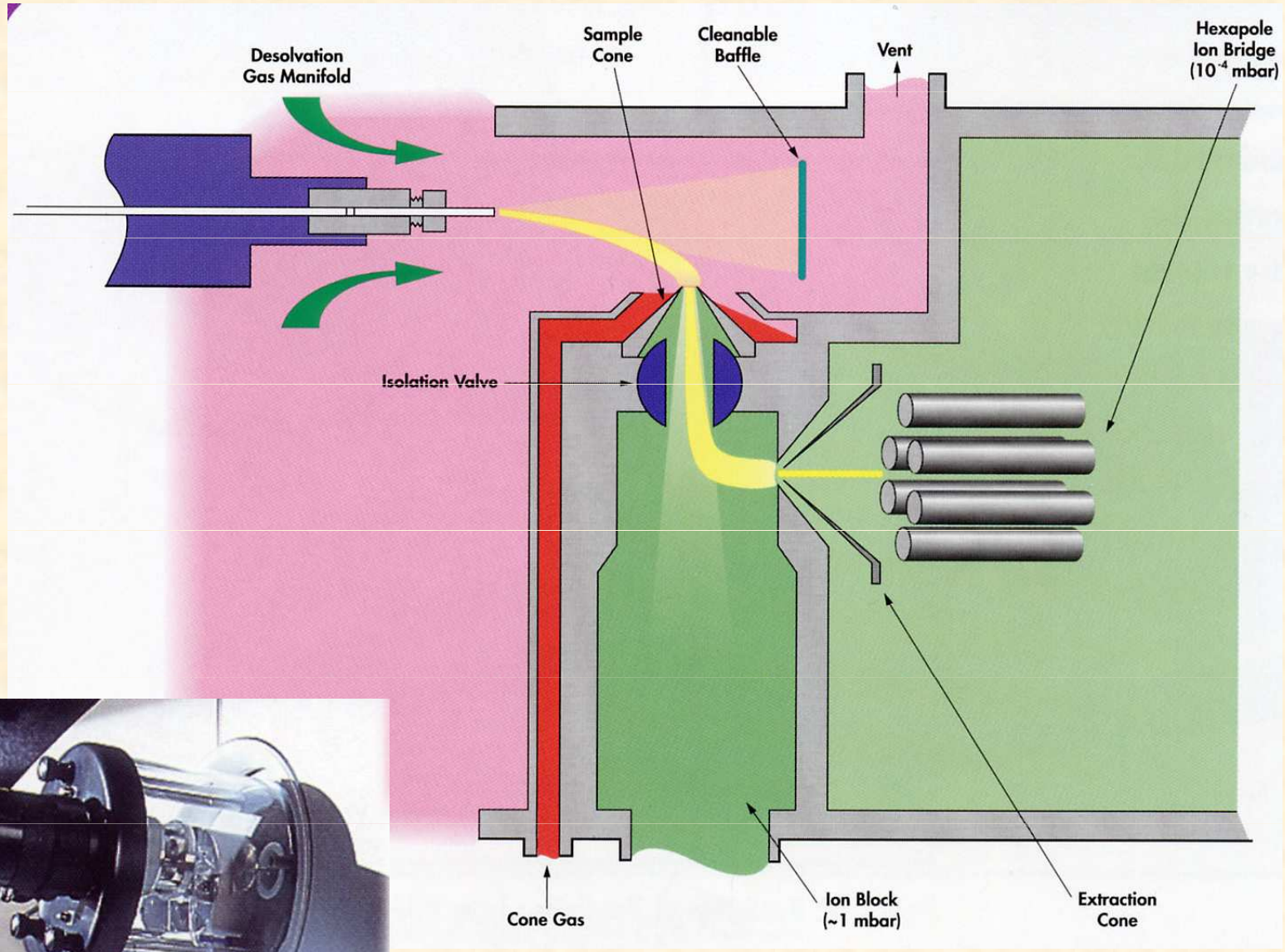
Electrospray Ionization



- Soft ionization under **atmospheric pressure**
- Simple connection to separation techniques (HPLC, CE)
- Multiply-charged ions

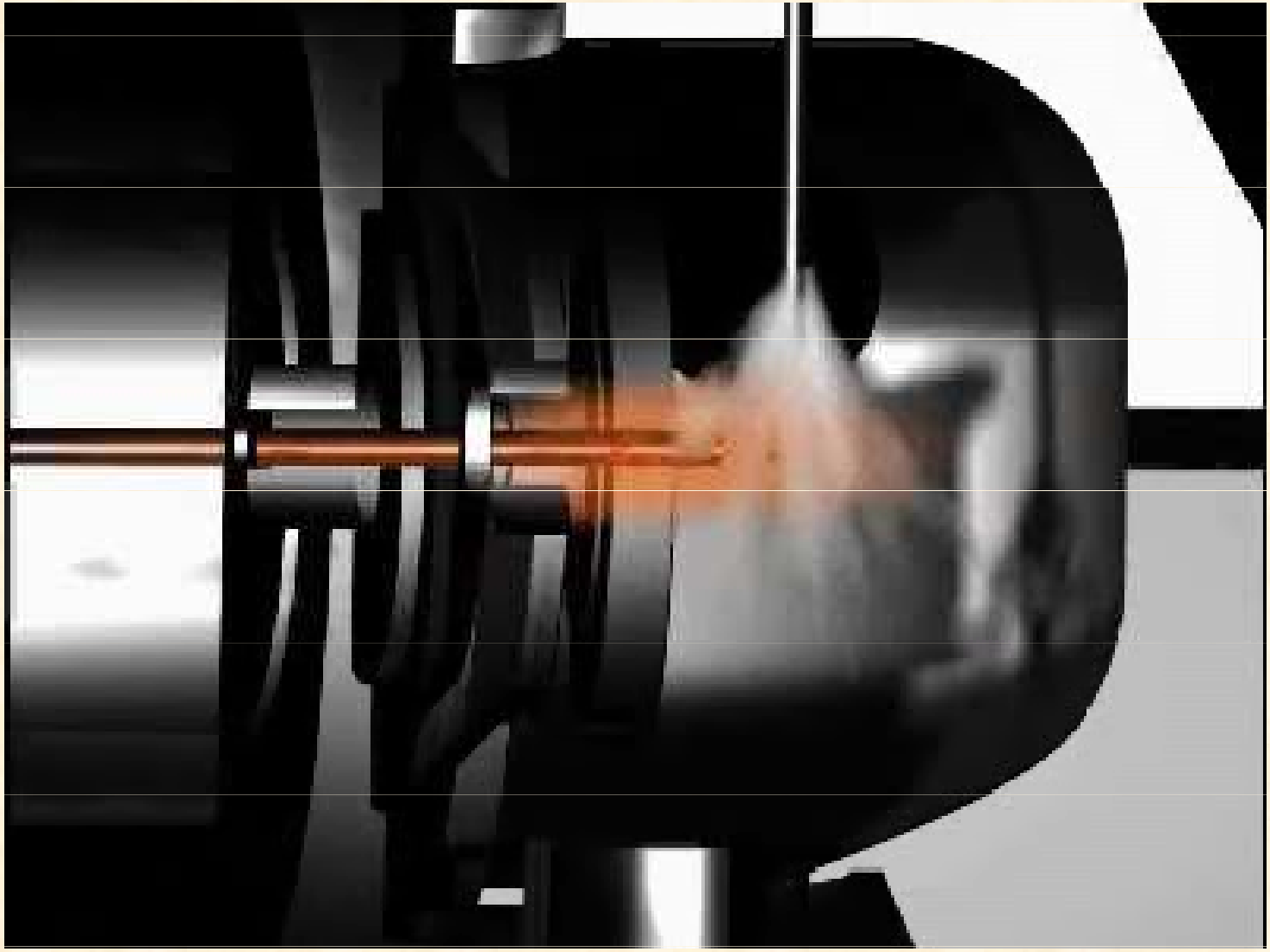
M. Wilm, Principles of Electrospray Ionization, MCP, 2011; 10 (7)

ESI – z spray

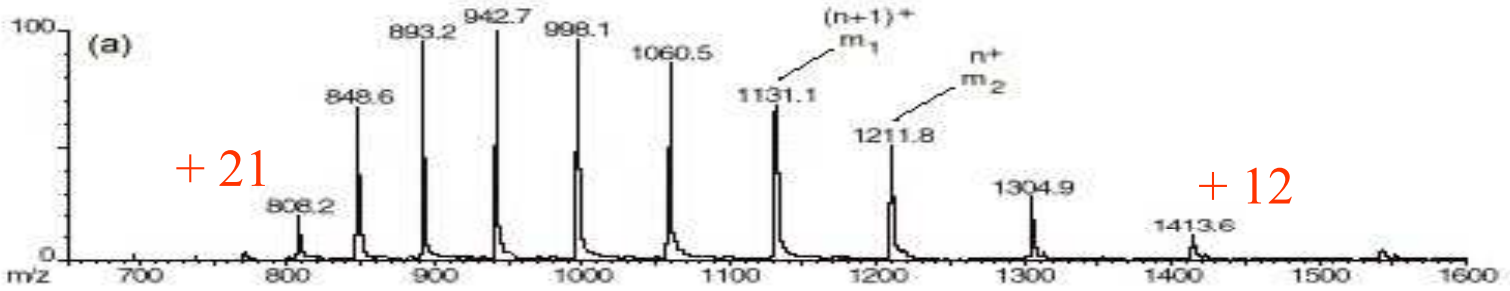

Desolvation
Gas Manifold
Sample
Cone
Cleanable
Baffle
Vent
Hexapole
Ion Bridge
(10⁻⁴ mbar)
Isolation Valve
Cone Gas
Ion Block
(~1 mbar)
Extraction
Cone

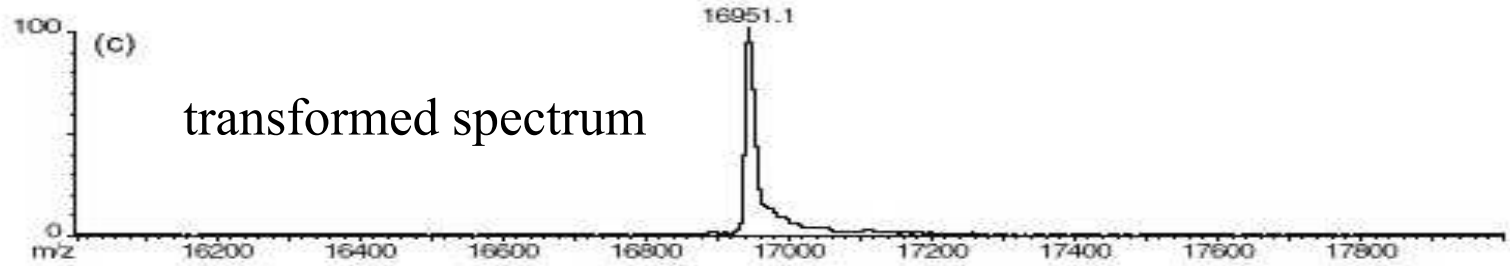
ESI – ionization

(orthogonal geometry)



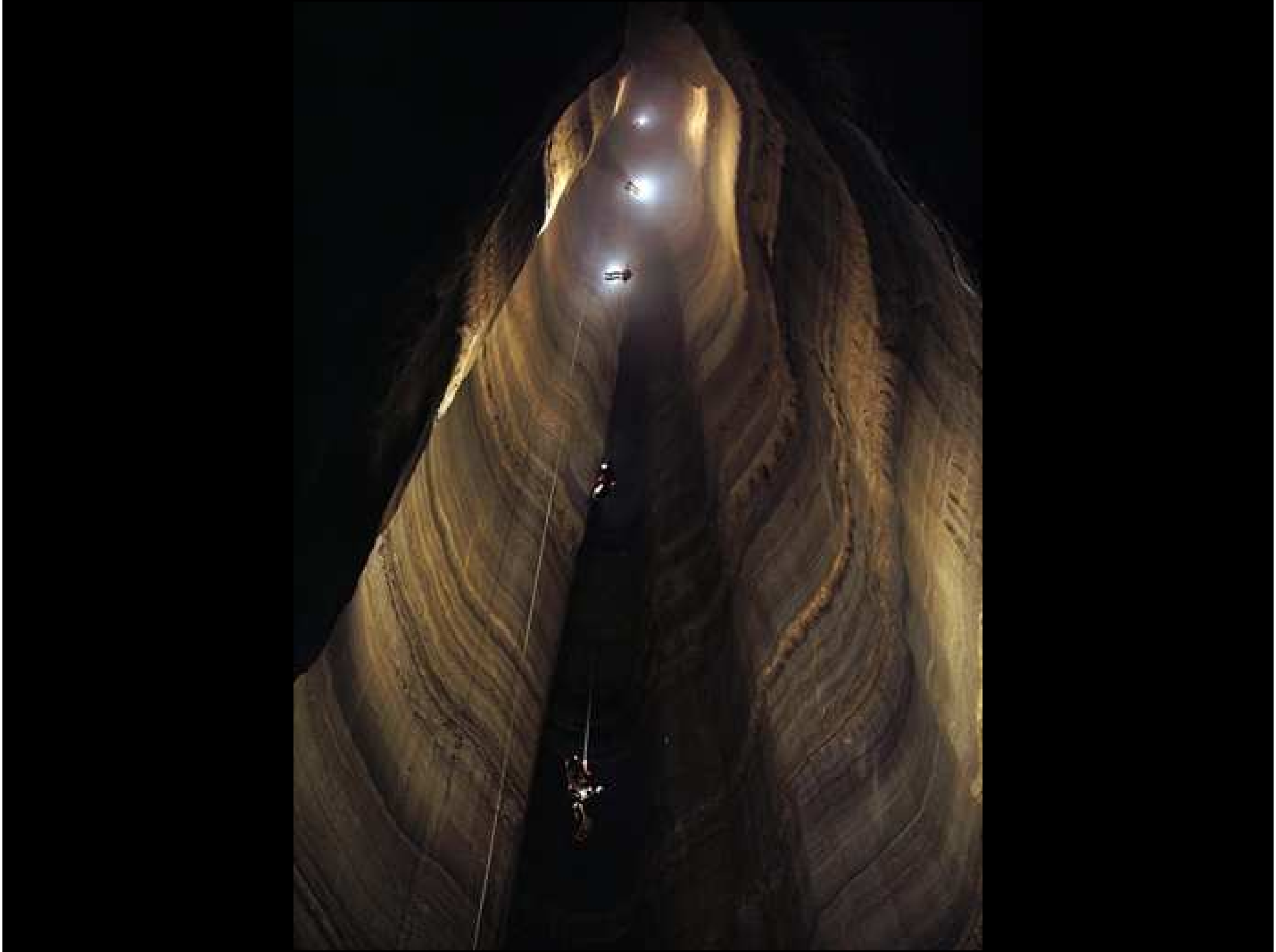
ESI spectrum of myoglobin (16 951 Da)





(b)

Mass to Charge Ratio (m/z)	No. of charges (n)	Molecular Mass (RMM)
1542.04	11	16951.40
1413.59	12	16950.95
1304.93	13	16950.94
1211.80	14	16951.11
1131.12	15	16951.62
1060.46	16	16951.26
998.11	17	16950.67
942.75	18	16951.30
893.15	19	16950.71
848.57	20	16951.25
808.21	21	16951.14
771.49	22	16950.72
	Mean	16951.09
	S.D.	±0.30

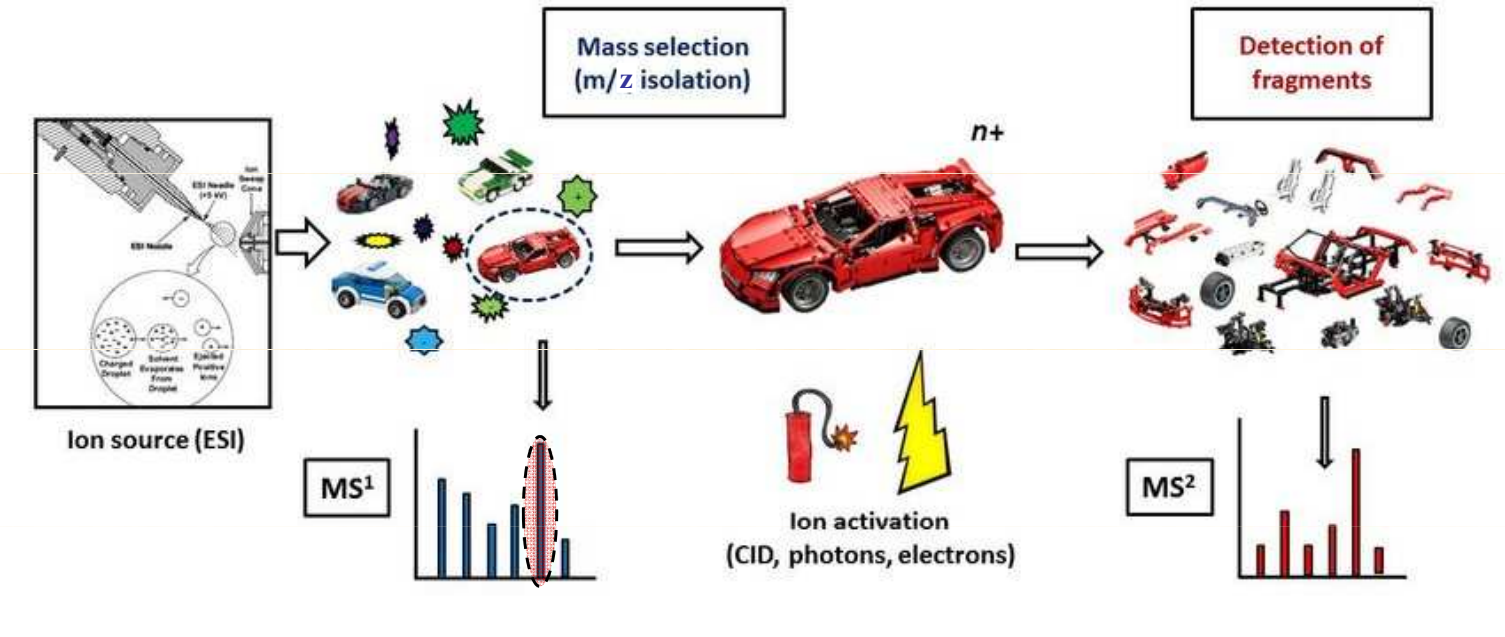


Mass analyzers

MS and MS/MS

MS

MS/MS
tandem mass spectrometry
MS^2

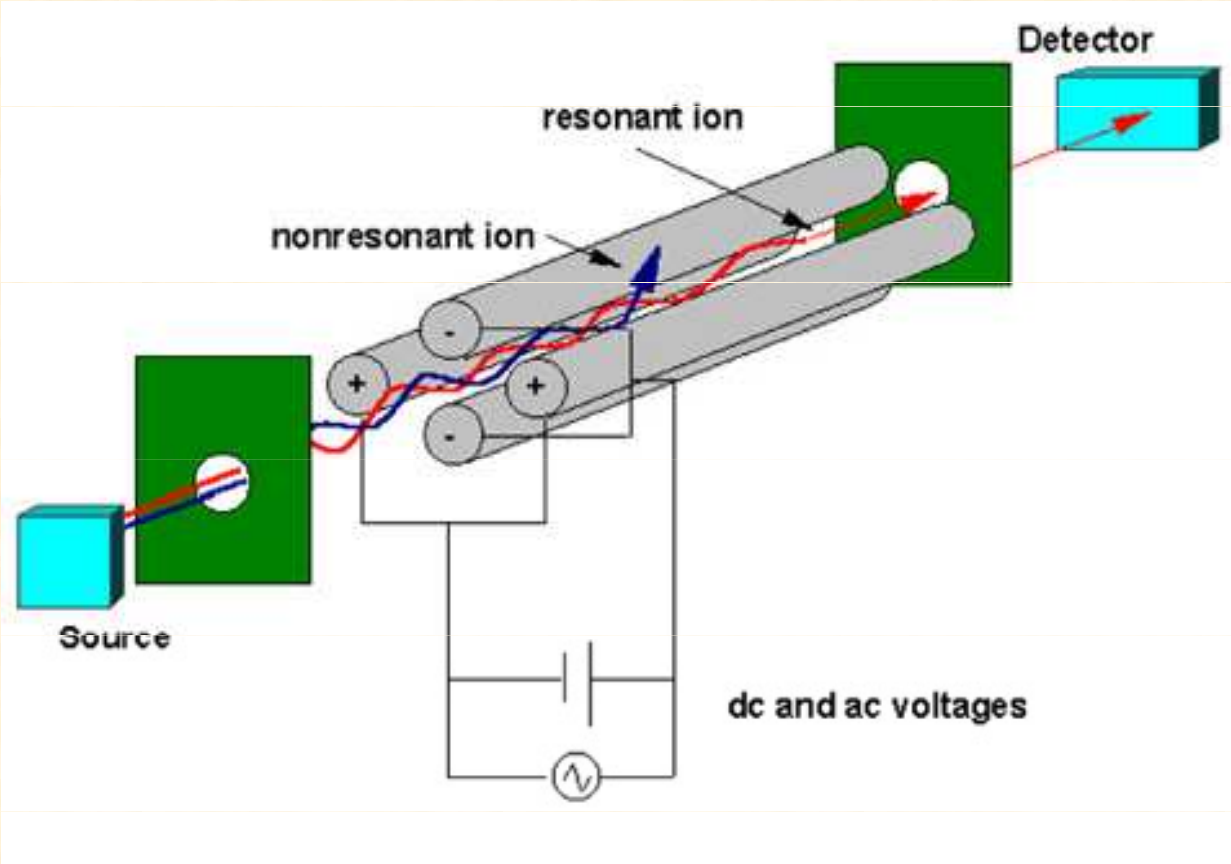


m/z of the whole ion

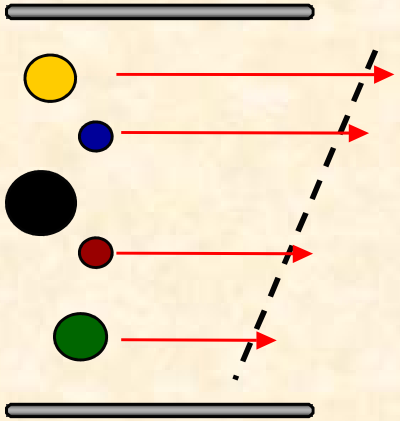
m/z of the fragments derived from the ion

Quadrupole analyzer (Q)

- mass filter
- limited mass range ($m/z < 4\,000$)
- low resolution
- discrimination of high mass ions
- MS/MS not possible

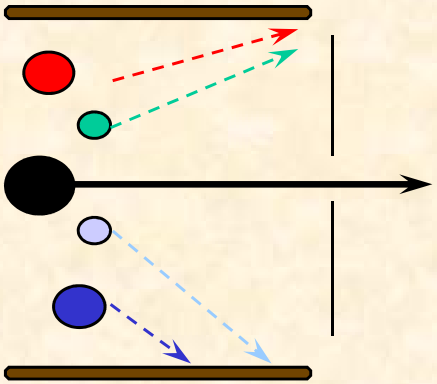


Full Scan



Q scan

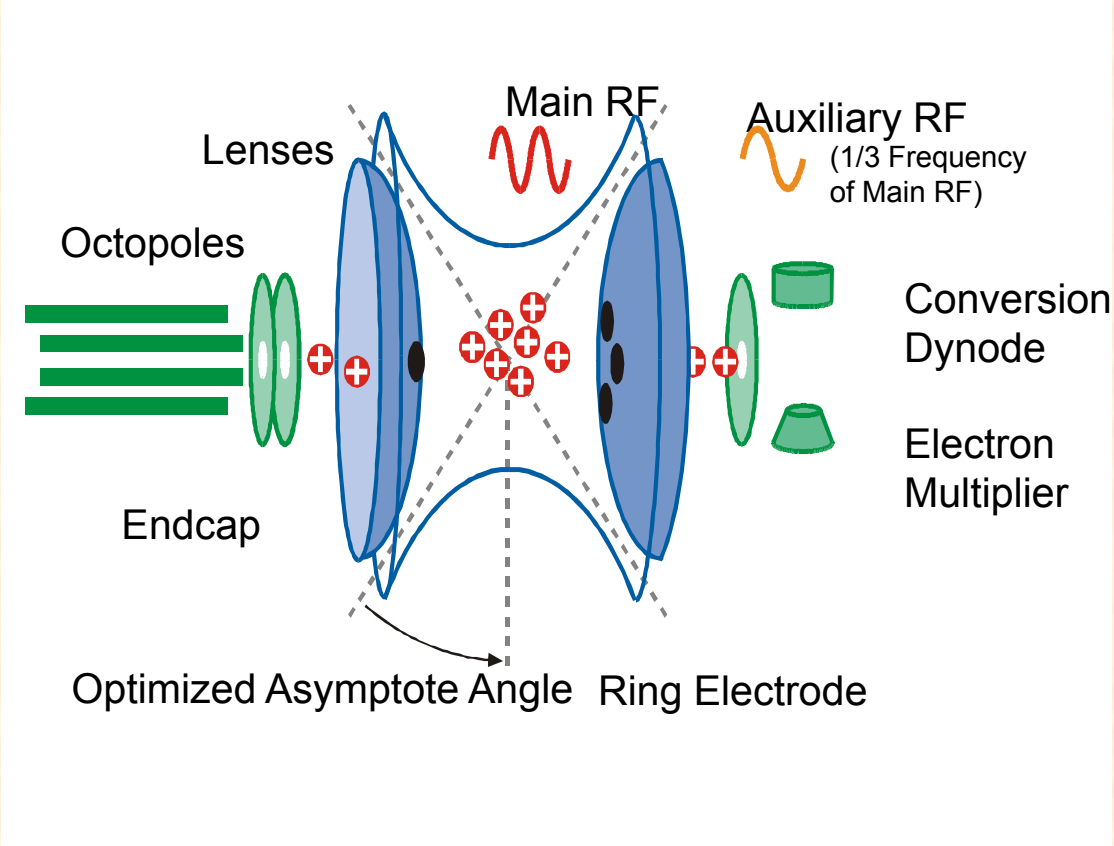
Selected ion monitoring



Q fix

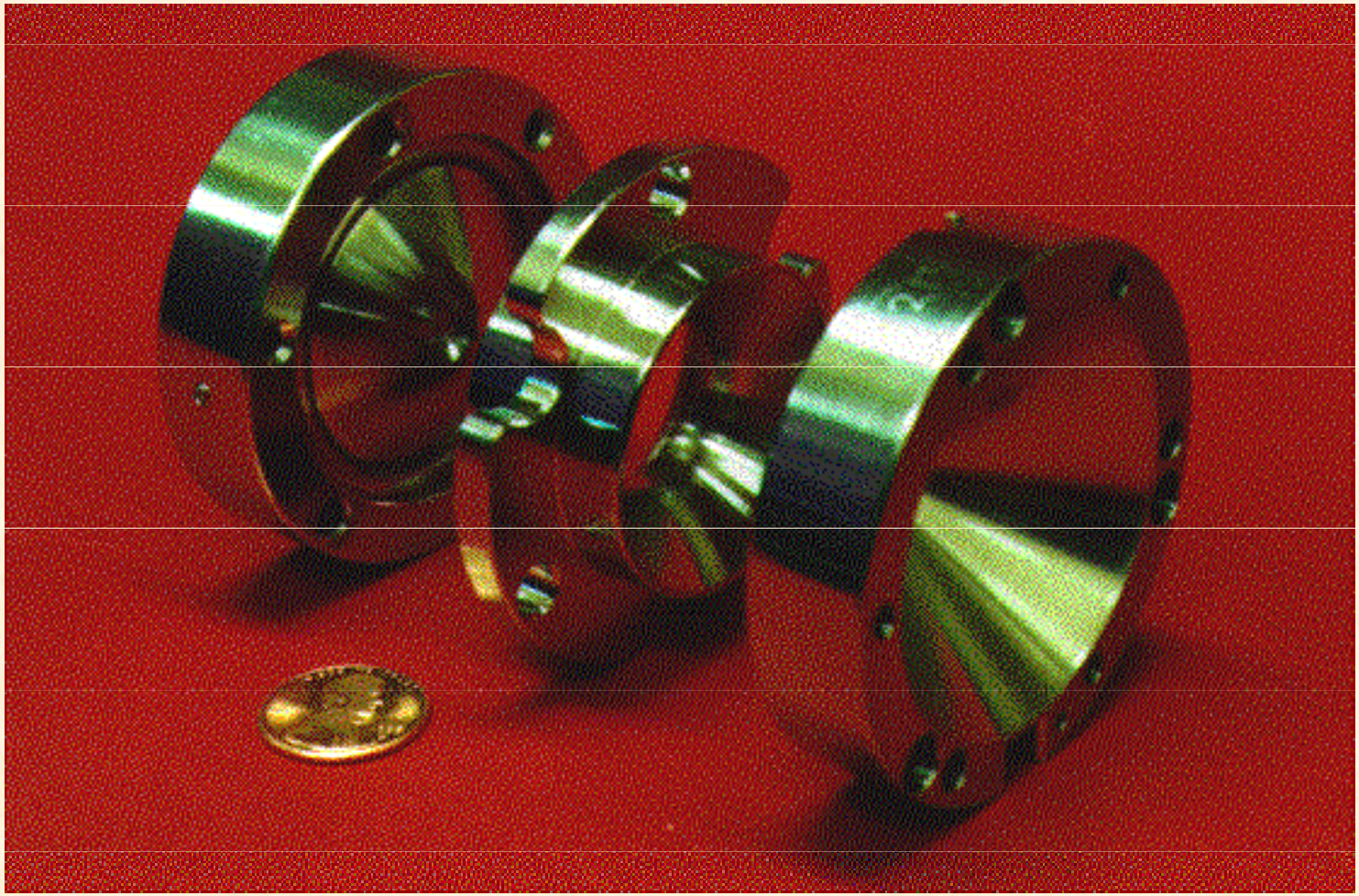
Ion trap (IT)

- limited mass range (m/z < 6 000, 20 000)
- low resolution
- enables MS/MS (MS^n up to 10)



pictures by courtesy of Dr. Sauerland (Bruker)

C7250



Ion trap

IT-MS

MS scan

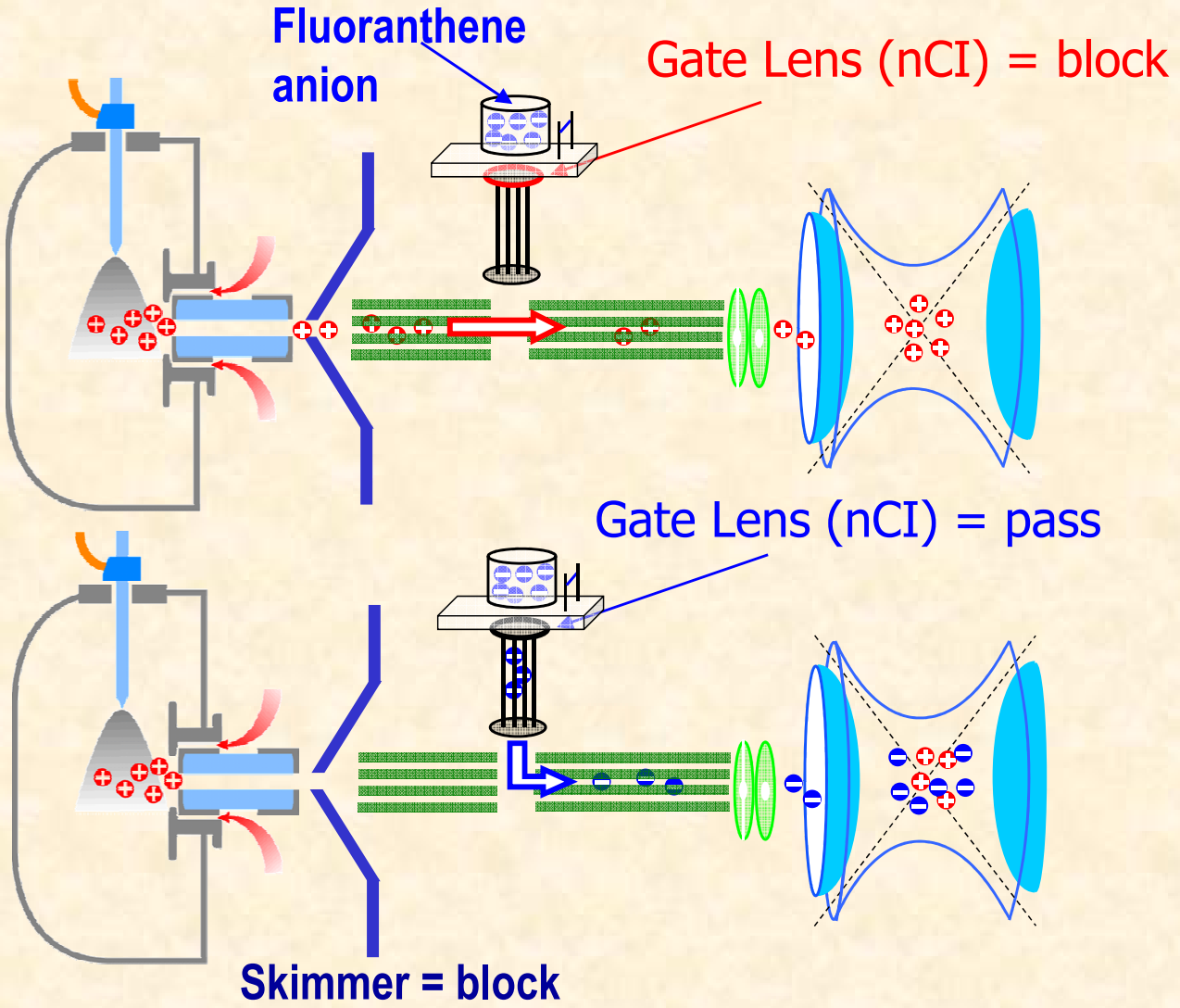
- ion capture
- sequential ion ejecting out from the trap according to *m/z*
- ion detection

MS/MS scan

- ion capture
- isolation of ions with selected *m/z* (precursors)
- excitation and fragmentation of isolated ions
- fragment detection (product ions)

ETD in the HCT$_{ultra}$

1. **Electrospray ion accumulation**
2. **Precursor ion isolation**
3. **Reactant anion accumulation (nCI source)**
4. **ETD fragmentation**
5. **Scan**



by courtesy of Dr. Arnd Ingendoh (Bruker)

Reactant Anion Production

BRUKER

Presentation 17th-Oct-2005

negative Chemical Ionization (nCI) Source

1st Step:

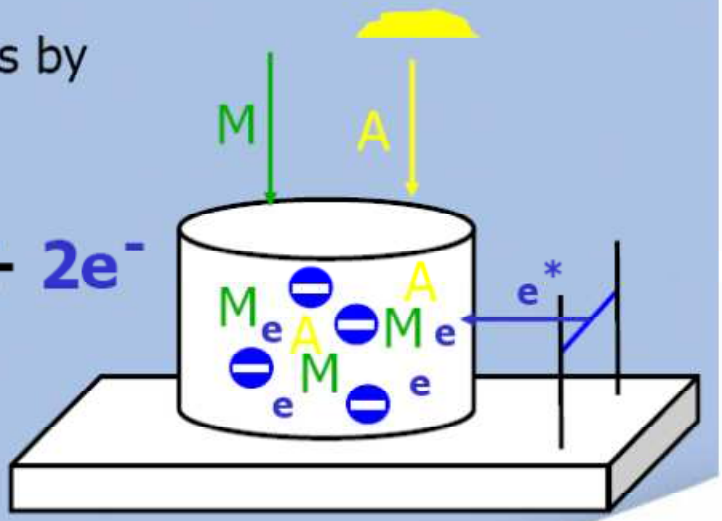
generation of low energy electrons by EI of CH_4 (Mediator)

$$M + e^{-*} \rightarrow M^{+\bullet} + 2e^-$$

2nd Step:

electron attachment to **flouranthene**

$$A + e^- \rightarrow A^{-\bullet} (=\ominus)$$



BRUKER DALTONICS

Enabling Life Science Tools Based on Mass Spectrometry™

by courtesy of Dr. Arnd Ingendoh (Bruker)

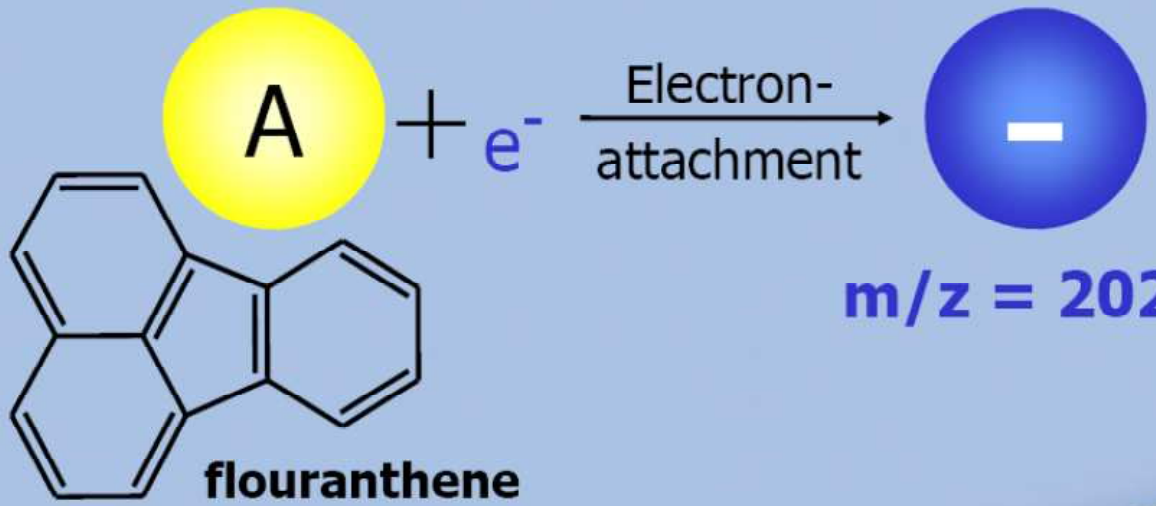
Theory

BRUKER

Presentation 17th-Oct-2005

Reactant anions generation

Electron Carrier → Reactant Anion $^{\cdot -}$



BRUKER DALTONICS

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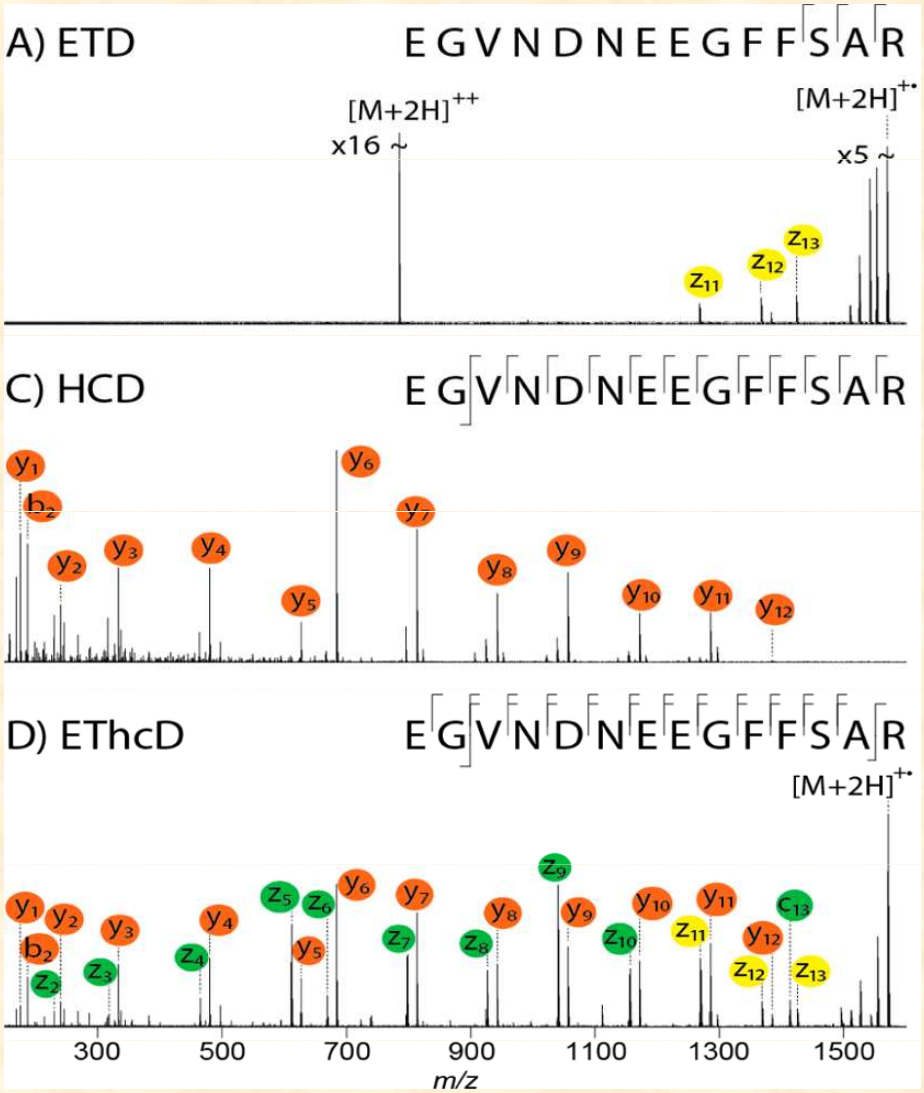
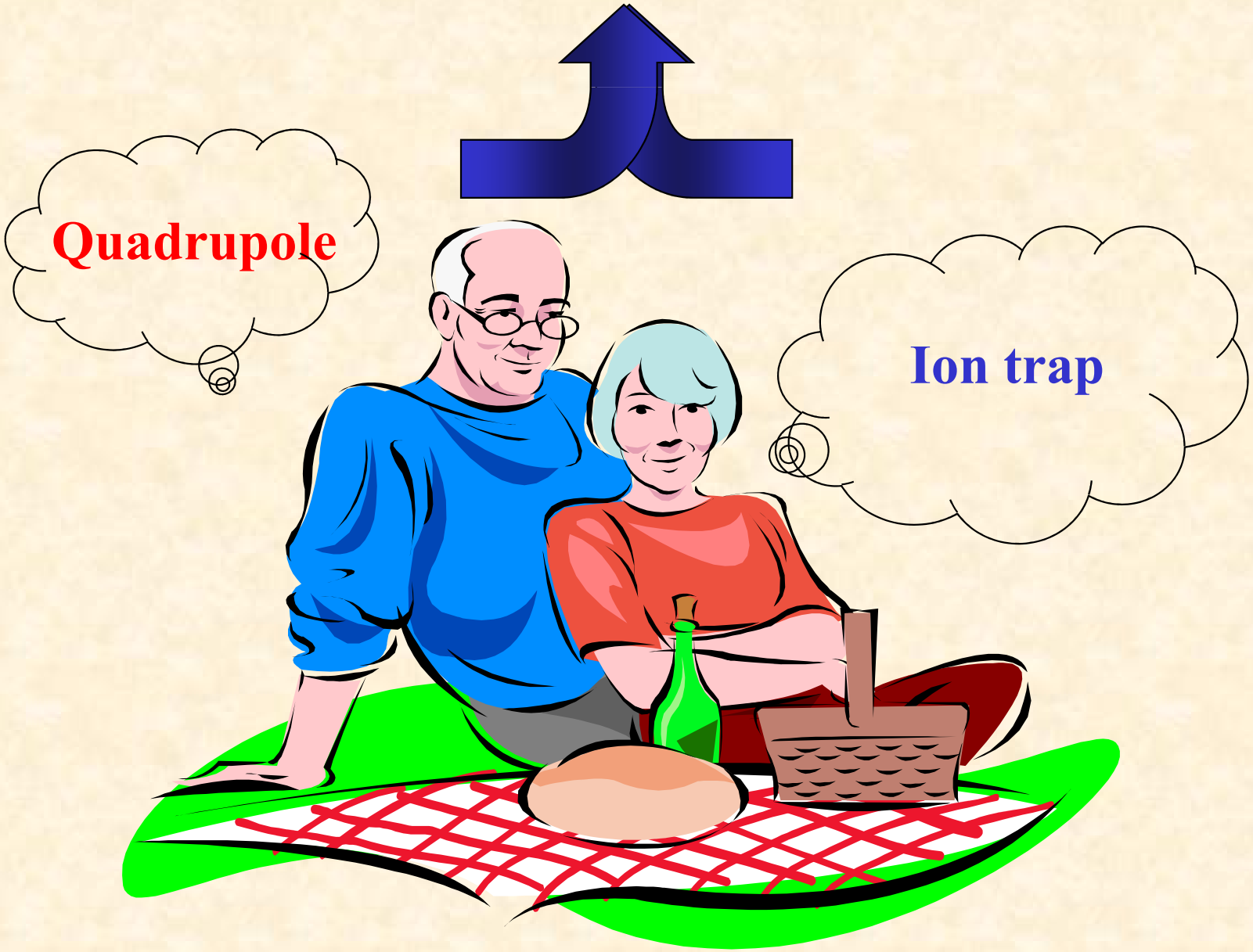
by courtesy of Dr. Arnd Ingendoh (Bruker)

Combining Electron-Transfer and Higher-Energy Collision EThcD



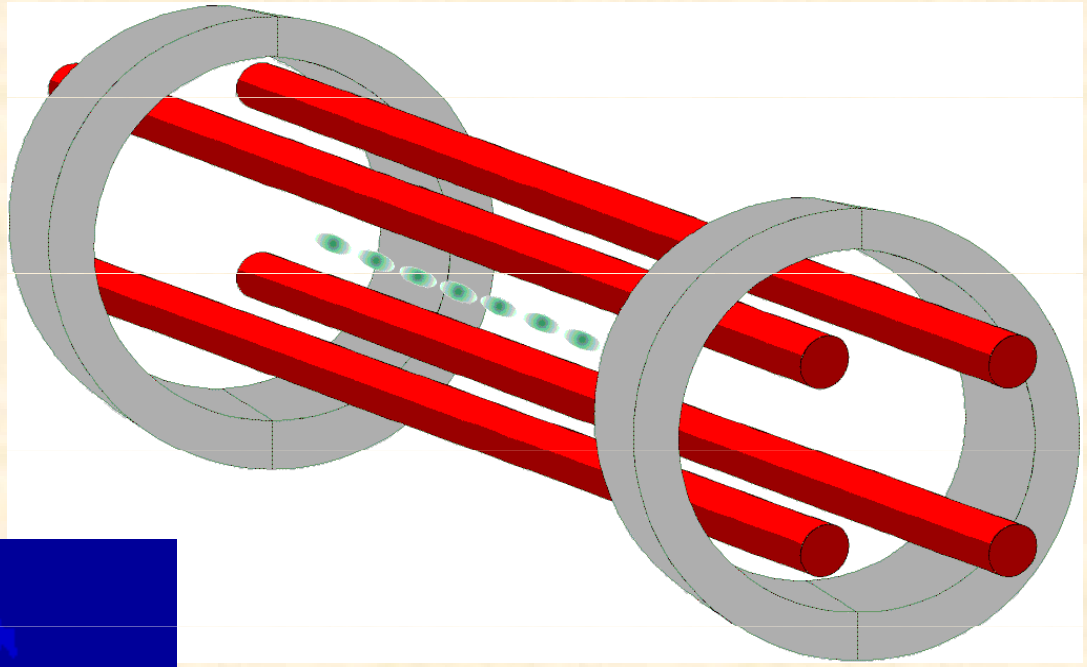
Frese et al., Anal. Chem. 2012, 84, 9668−9673

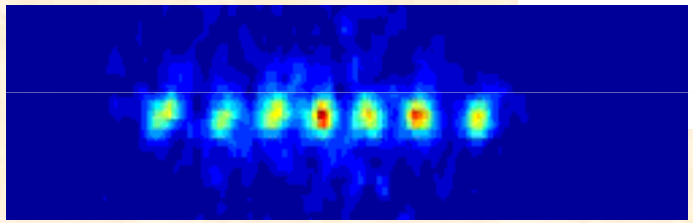
Linear ion trap



Linear ion trap

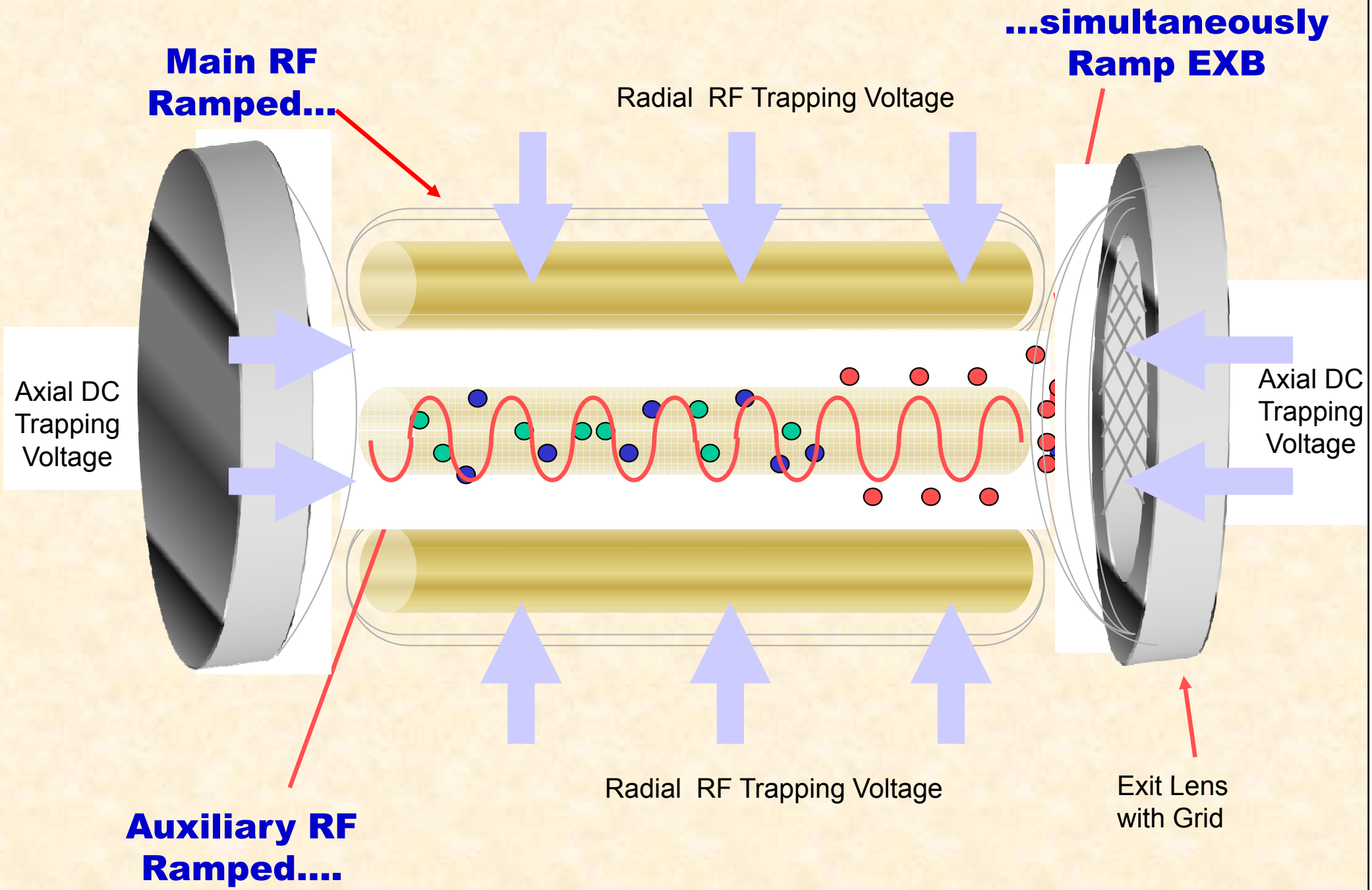
- displays advantages of quadrupole and ion trap
- limited mass range (m/z < 6 000)
- increased ion capacity by order ➡ sensitivity increase
- enables MS/MS **(MS^n)**



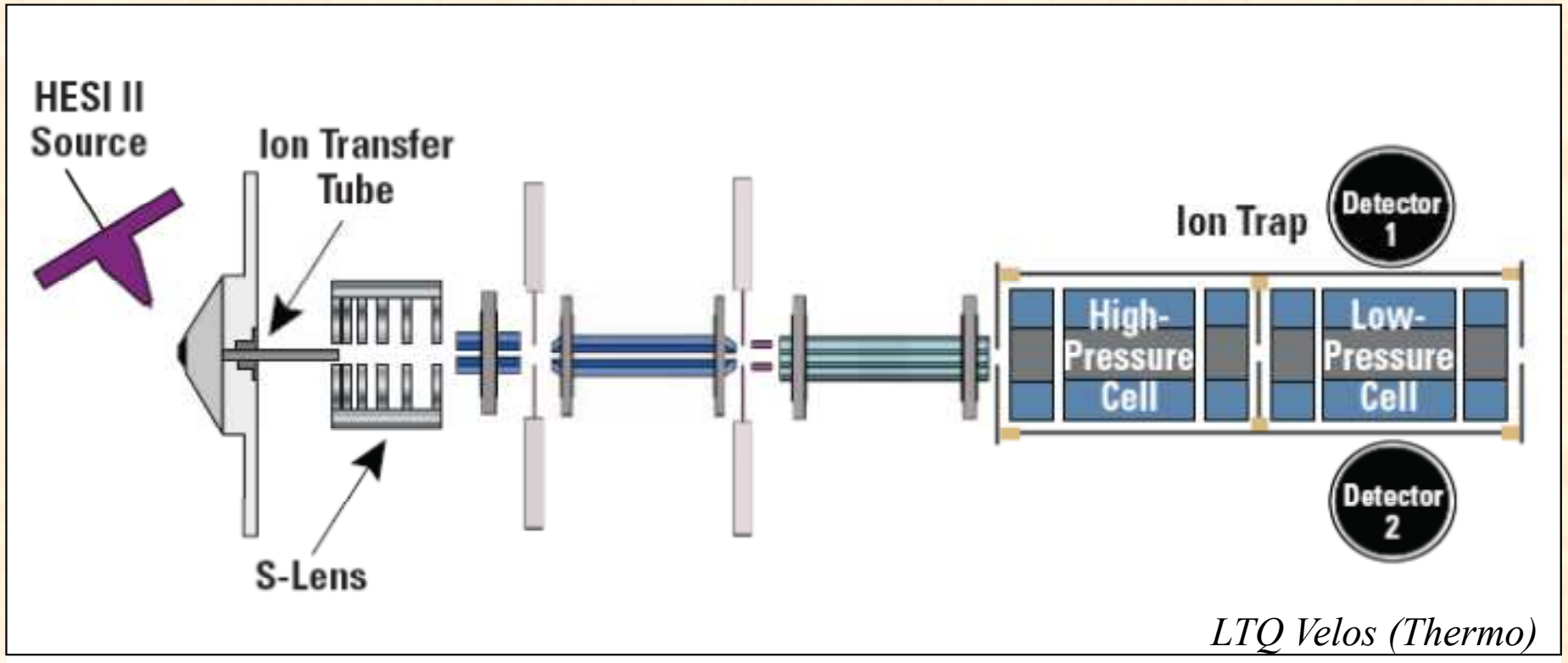


Trapping Forces in a Linear Ion Trap

C7250



Dual pressure ion trap



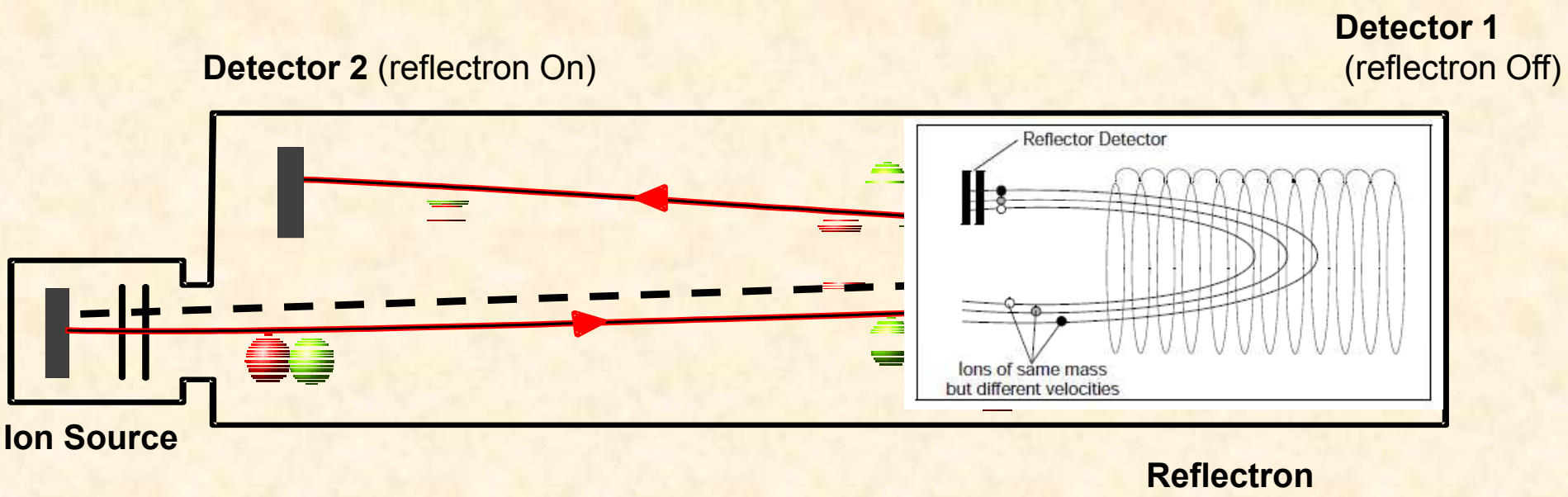
LTQ Velos (Thermo)

- HP chamber – increase of efficiency of ion traping and their fragmentation
- LP chamber – improves resolution and scan speed

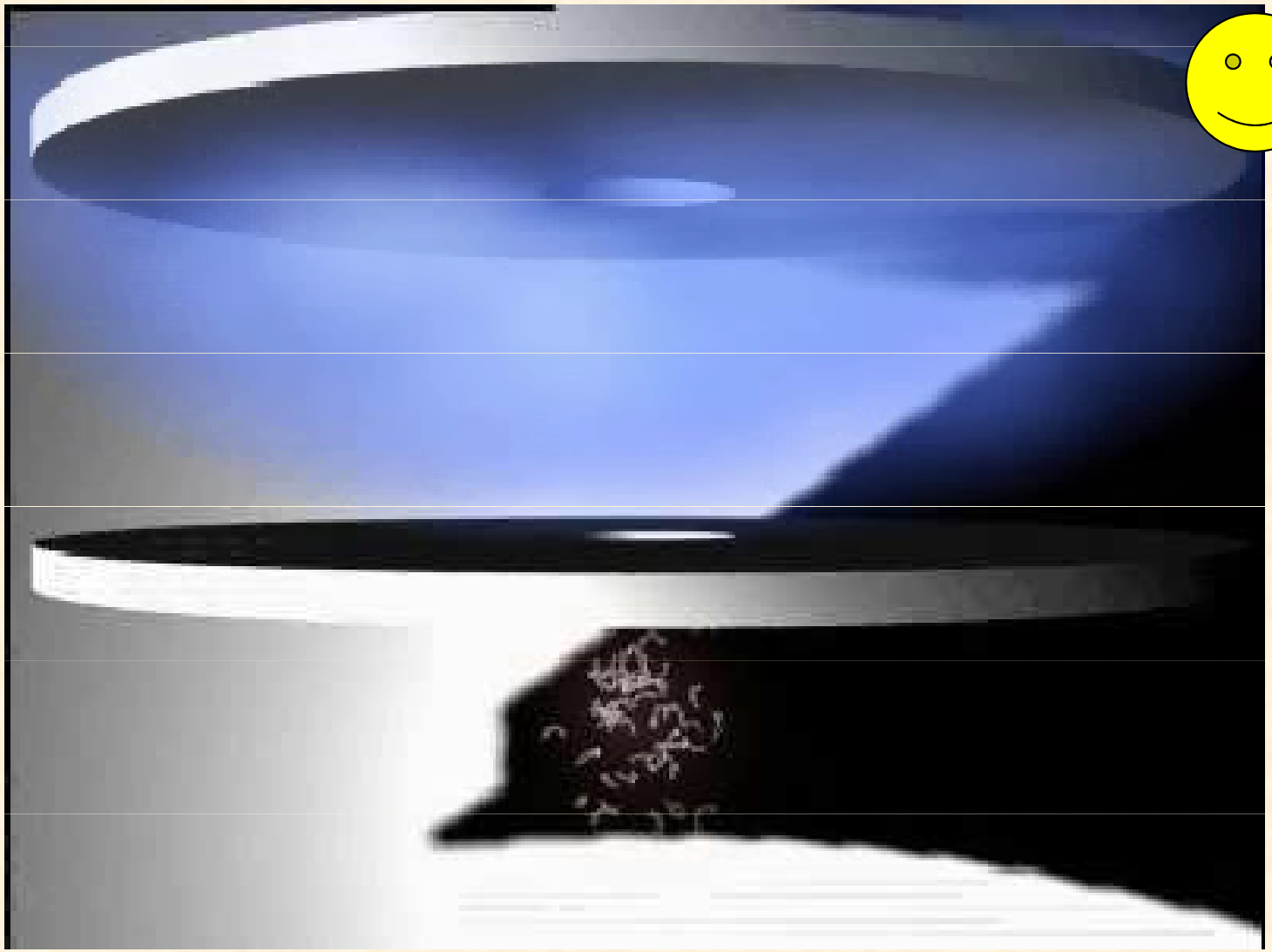
Time-of-Flight analyzer (TOF)

$$E = 1/2mv^2$$

- „unlimited“ mass range (m/z < 1 000 000)
- fast scanning
- high resolution (R až 60 000)
- enables MS/MS by PSD (post source decay) – not used today
- MALDI



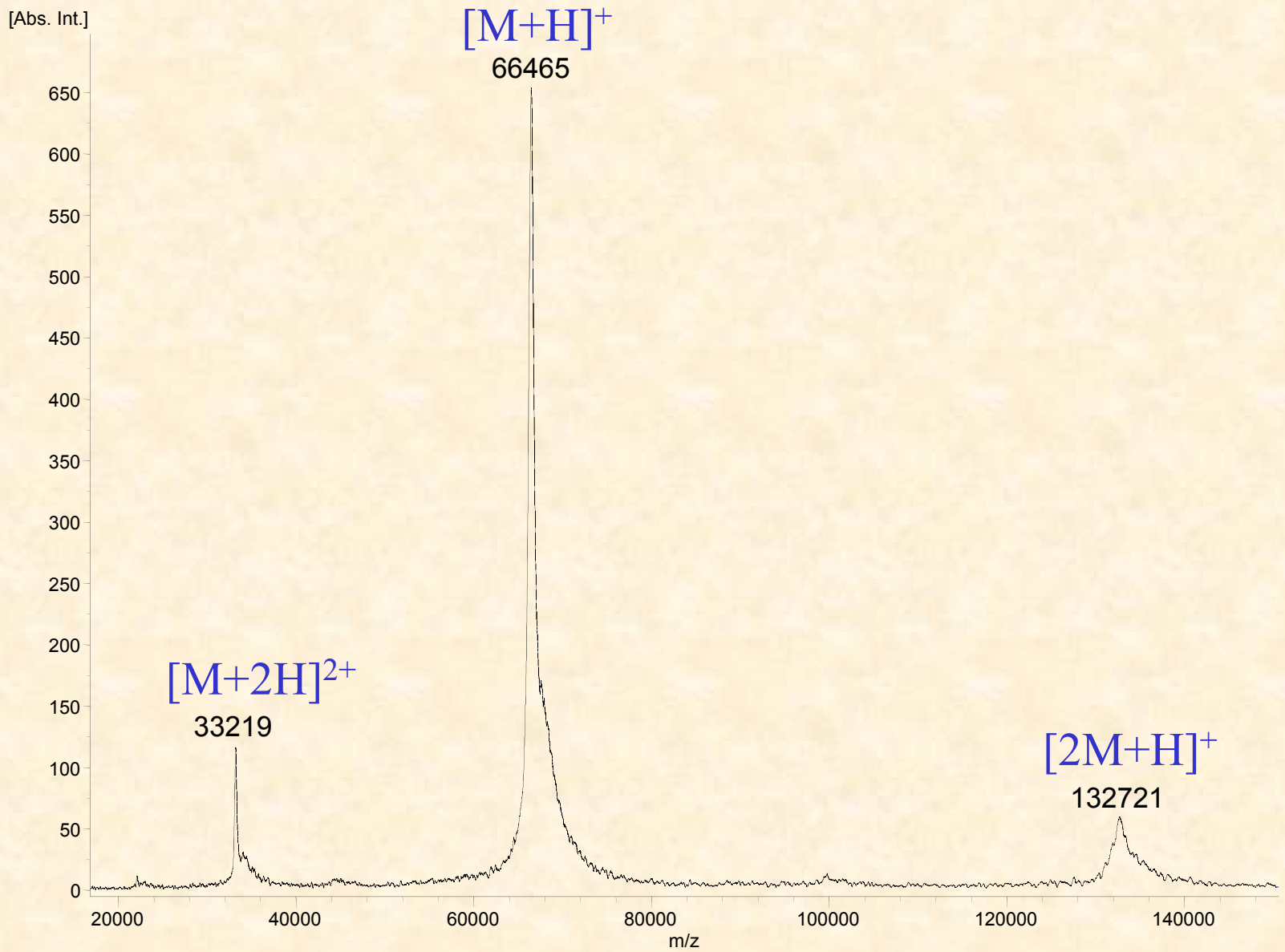
TOF – ion separation



Second part

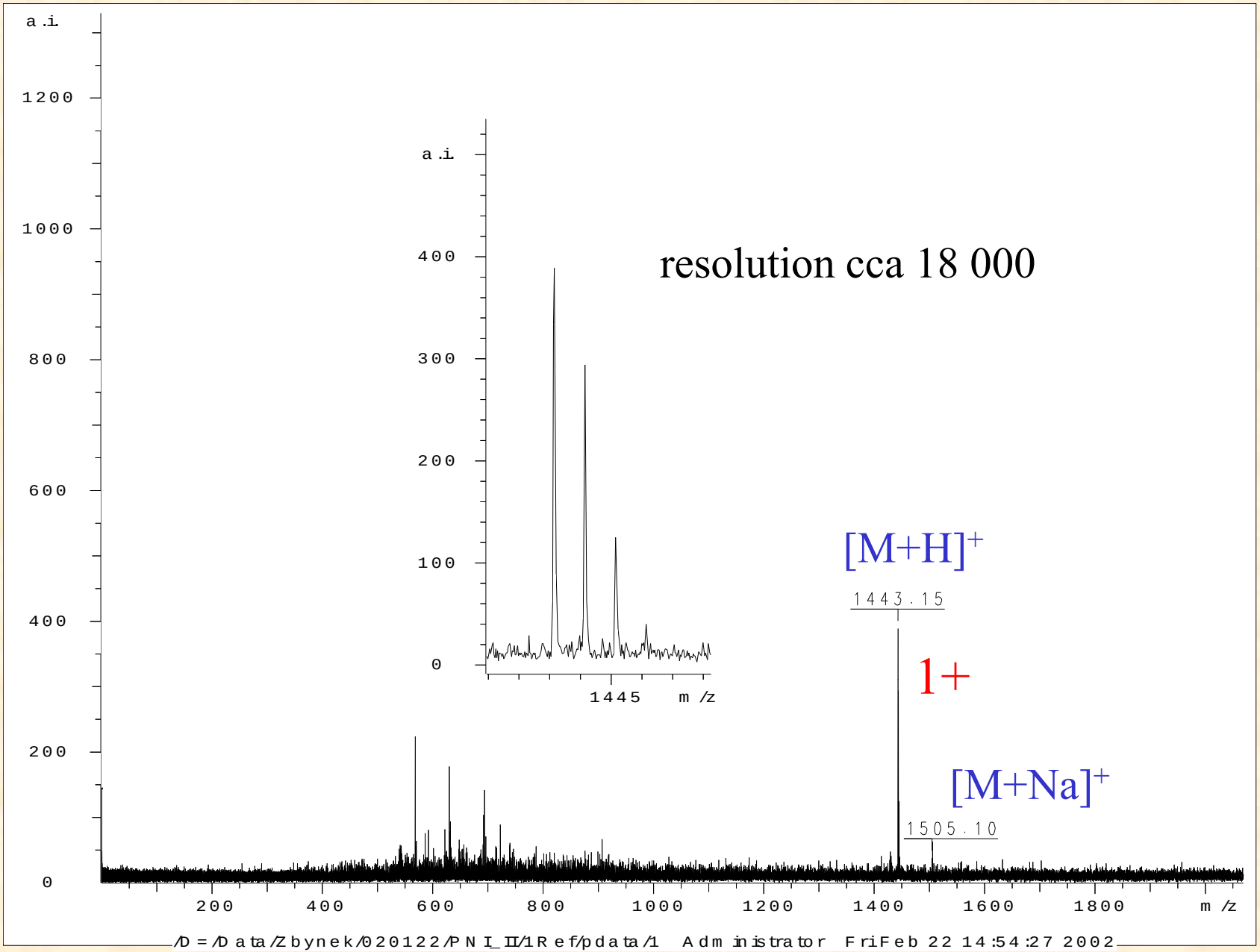
C7250

Protein (BSA, 66.4 kDa, ≈15 pmol on spot)

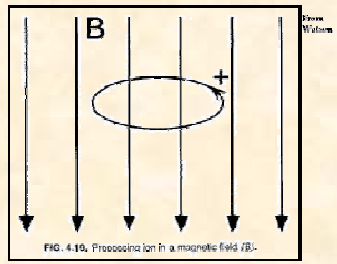


C7250

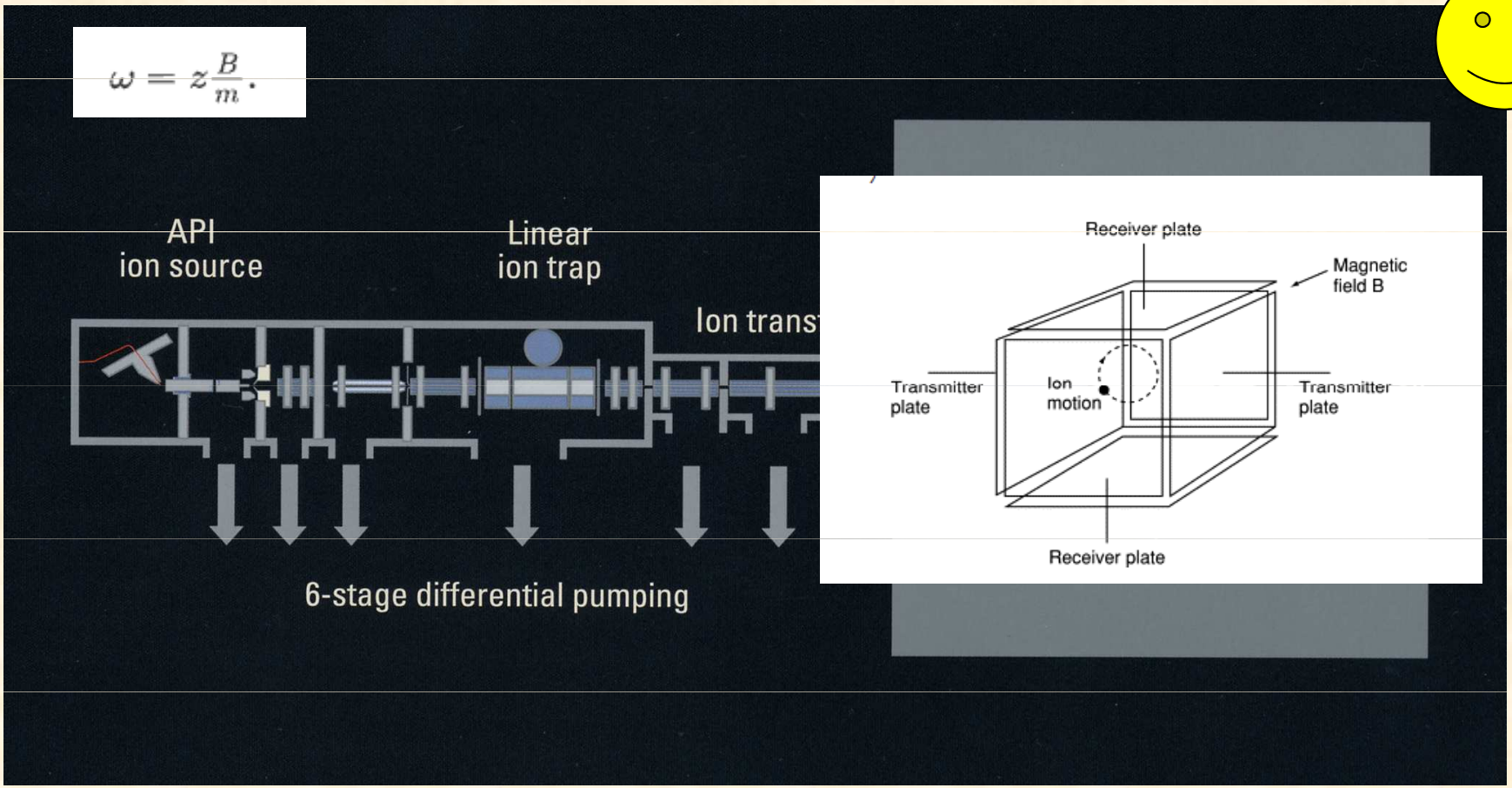
Peptide (≈ 50 fmol on spot)



Ion cyclotron resonance with Fourier transformation
FT- ICR MS



- ultimate resolution (R >10 000 000)
  - ➡ determination of elemental composition
- enables MS^n, top-down approaches
- disadvantage (magnet up to 15T, liquid He), high operational costs



Significance of high resolution

Mass of elements is not whole numbers

H	1,0079
C	12,0107
N	14,0067
O	15,9994

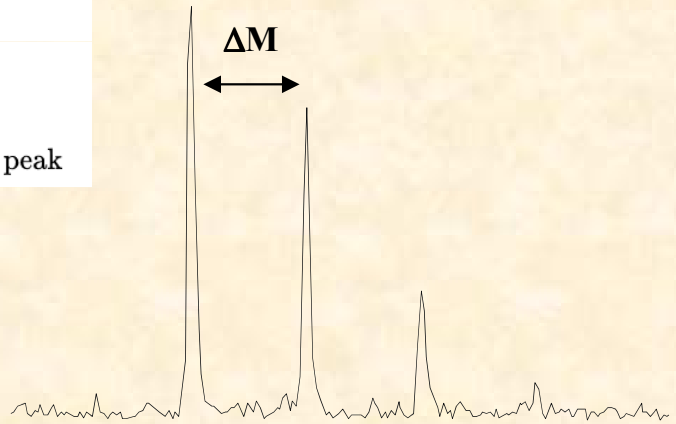
$$R = \frac{M}{\Delta M} = \text{resolution}$$

ΔM = resolving power

M = mass of the (second) peak

C_2H_3O	**43,0184**
C_3H_7	**43,0547**

In an simplified way, resolution value indicates lowest distinguishable mass difference



R	ΔM	at mass 43
100	0,43	
1000	0,043	
10000	**0,0043**	

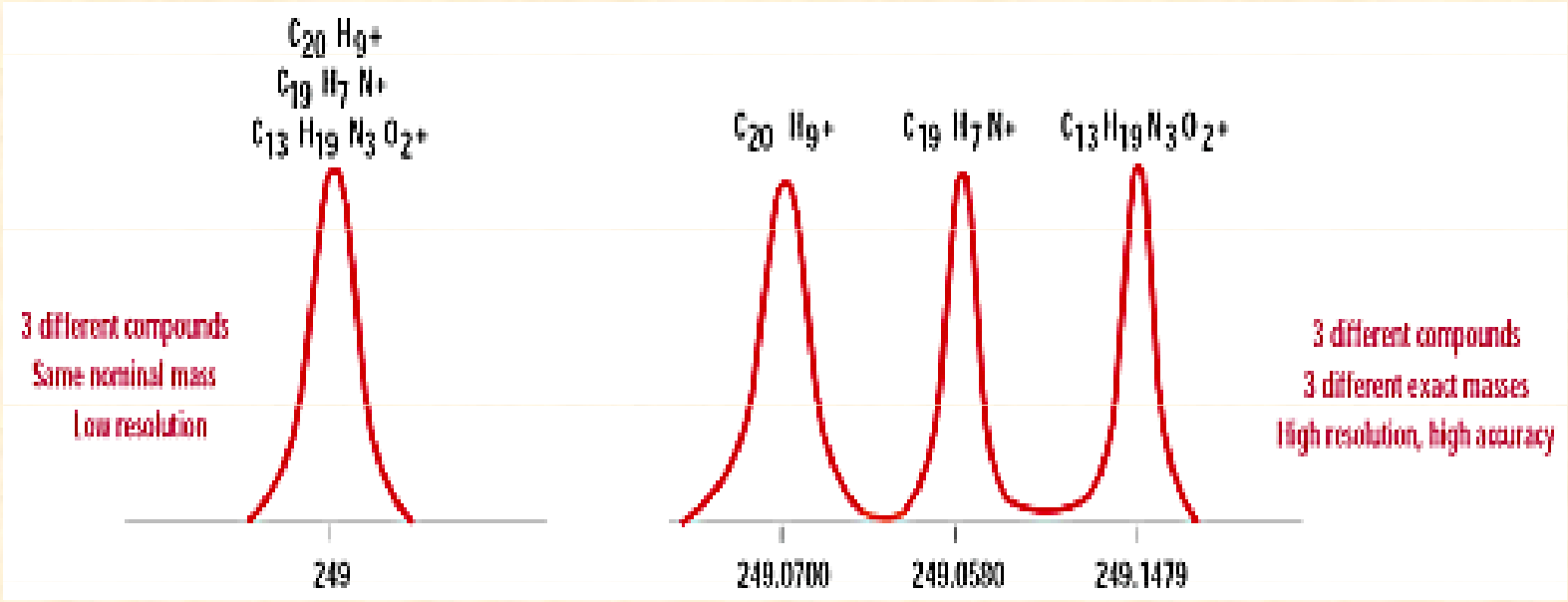
M5

Sufficient resolution results in possibility to deduce elemental composition

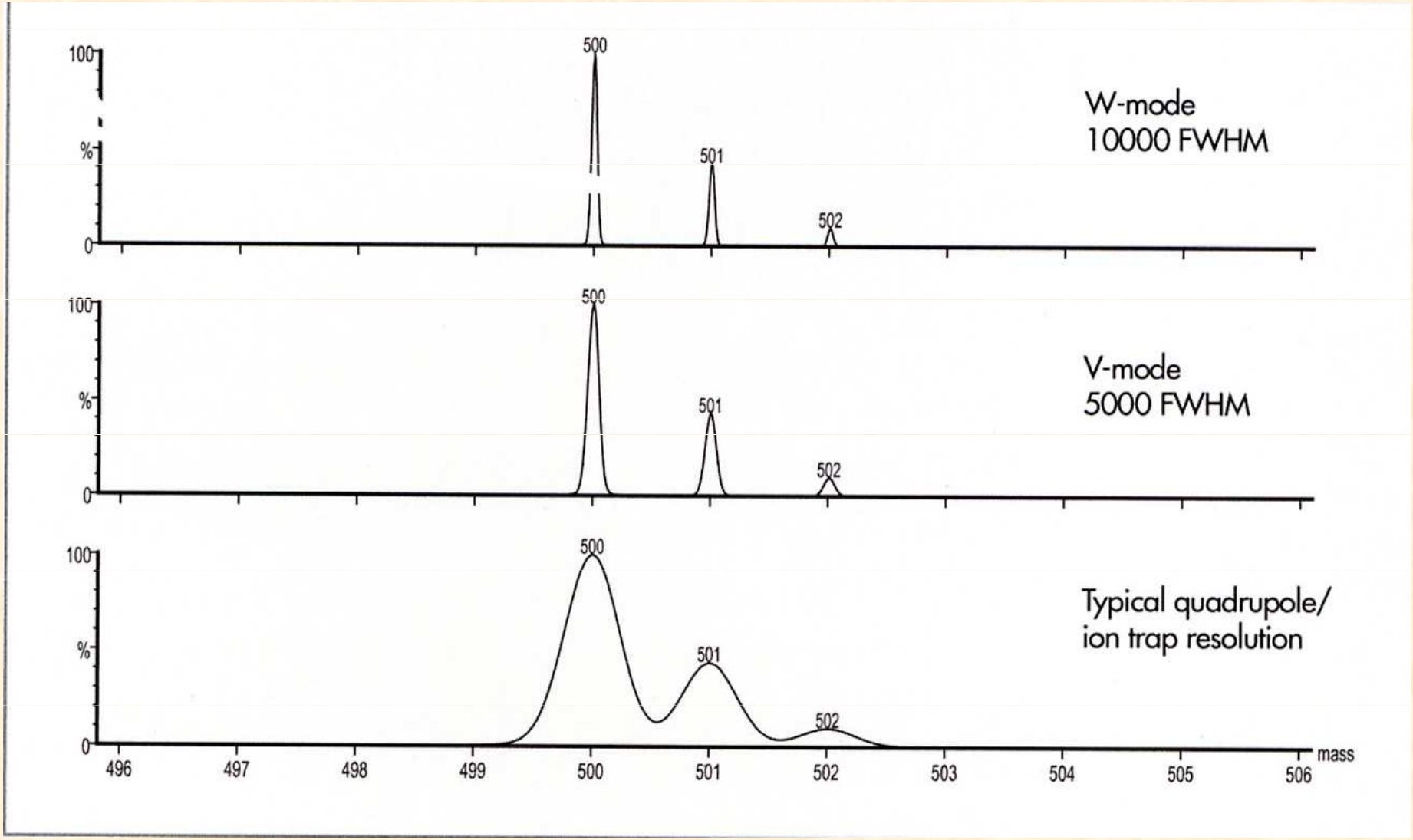
In case of compounds above we need resolution of 1185

M5 Smallest mass difference Δm between two equal magnitude peaks such that the valley between them is a specified fraction of the peak height
MU, 10/27/2009

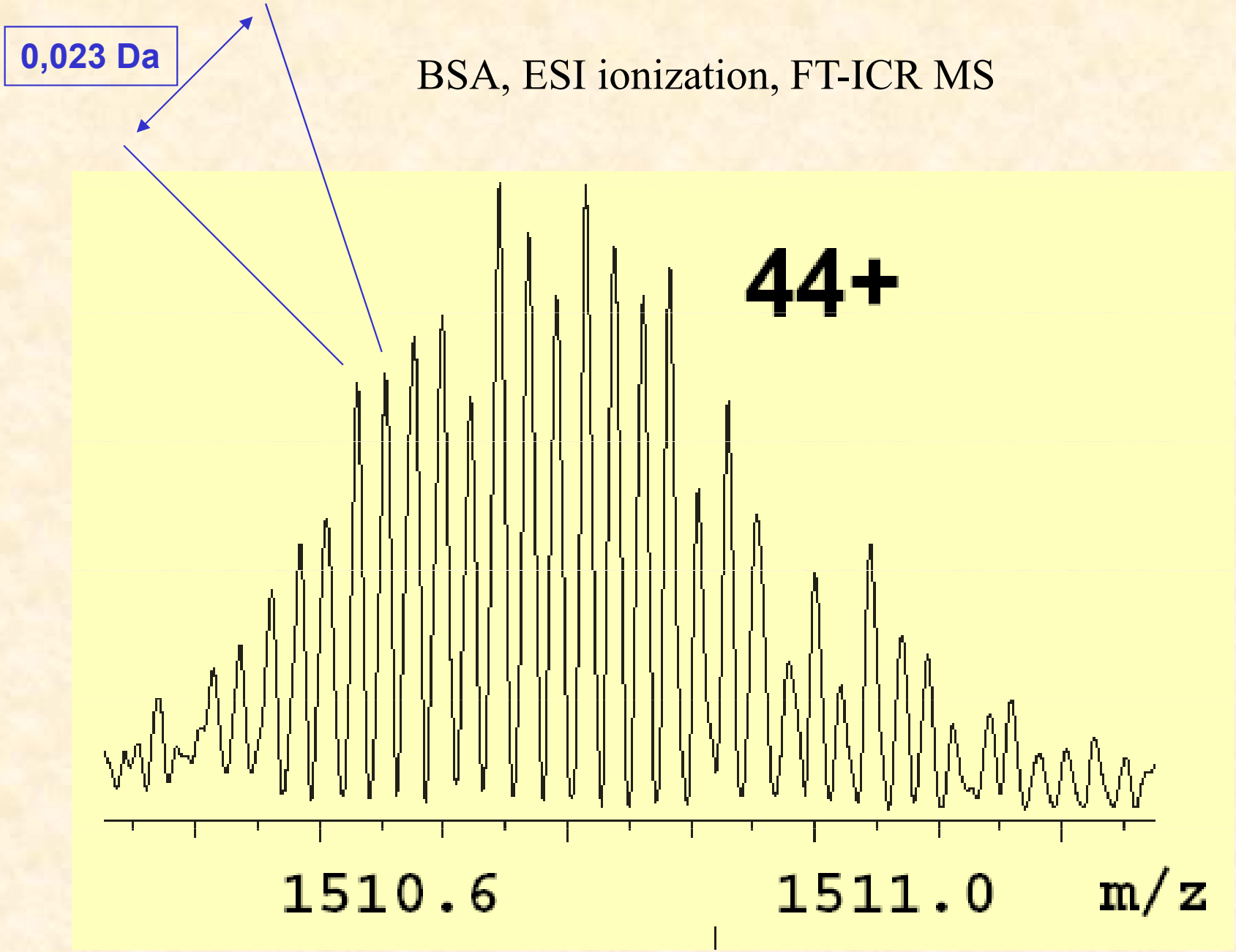
Significance of high resolution



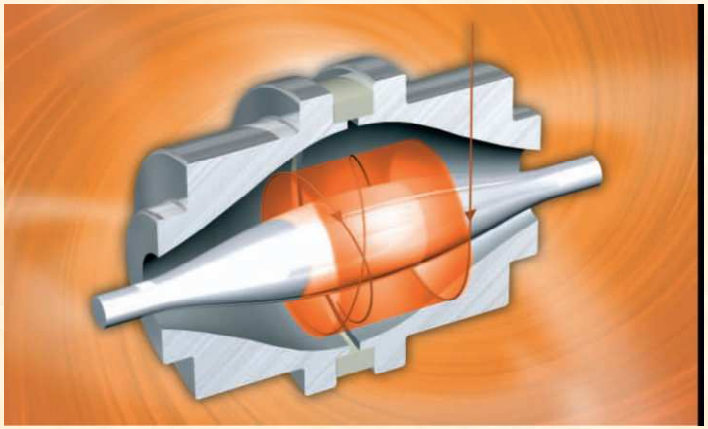
Significance of high resolution



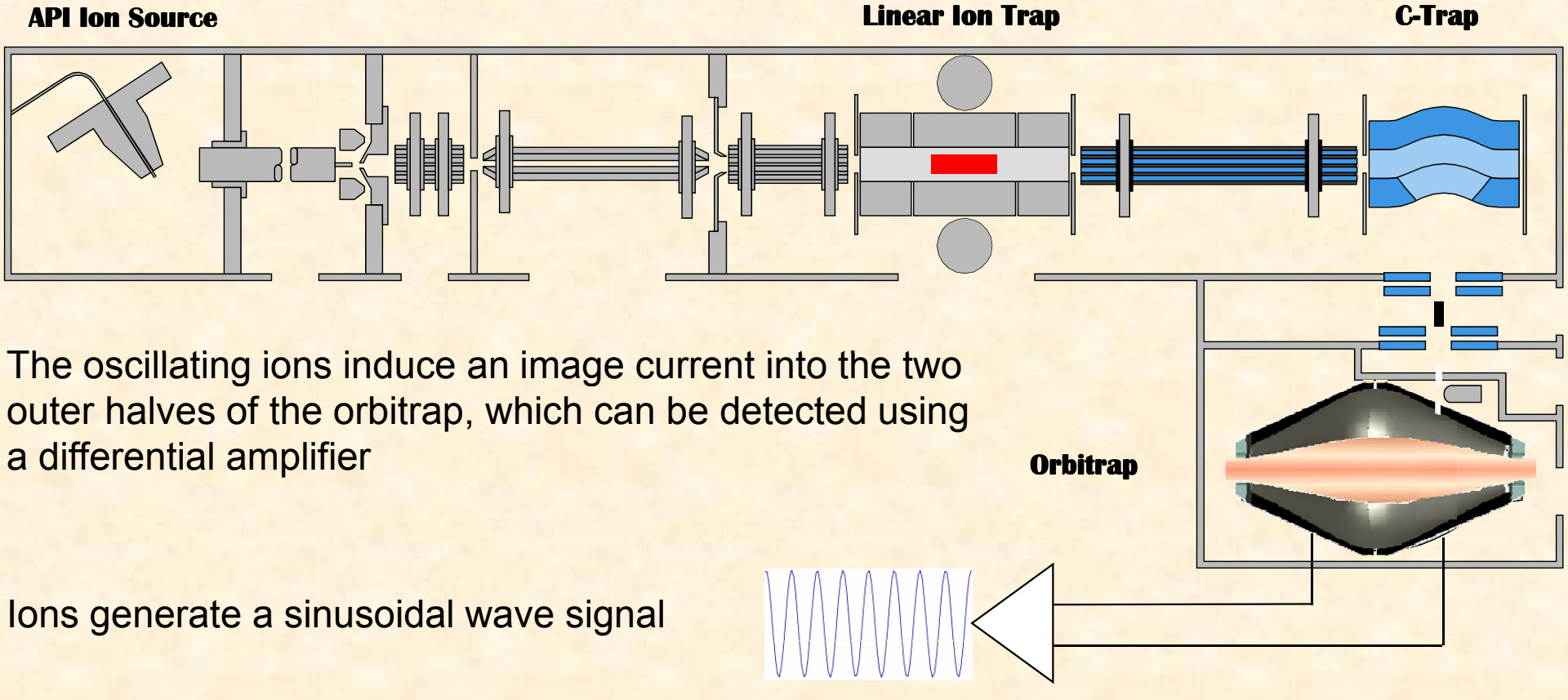
BSA, ESI ionization, FT-ICR MS



Orbitrap



- high resolution in MS and MS/MS
  (up to 1 000 000, but w/o magnet)
- limited mass range $m/z < 4\,000$
- ESI



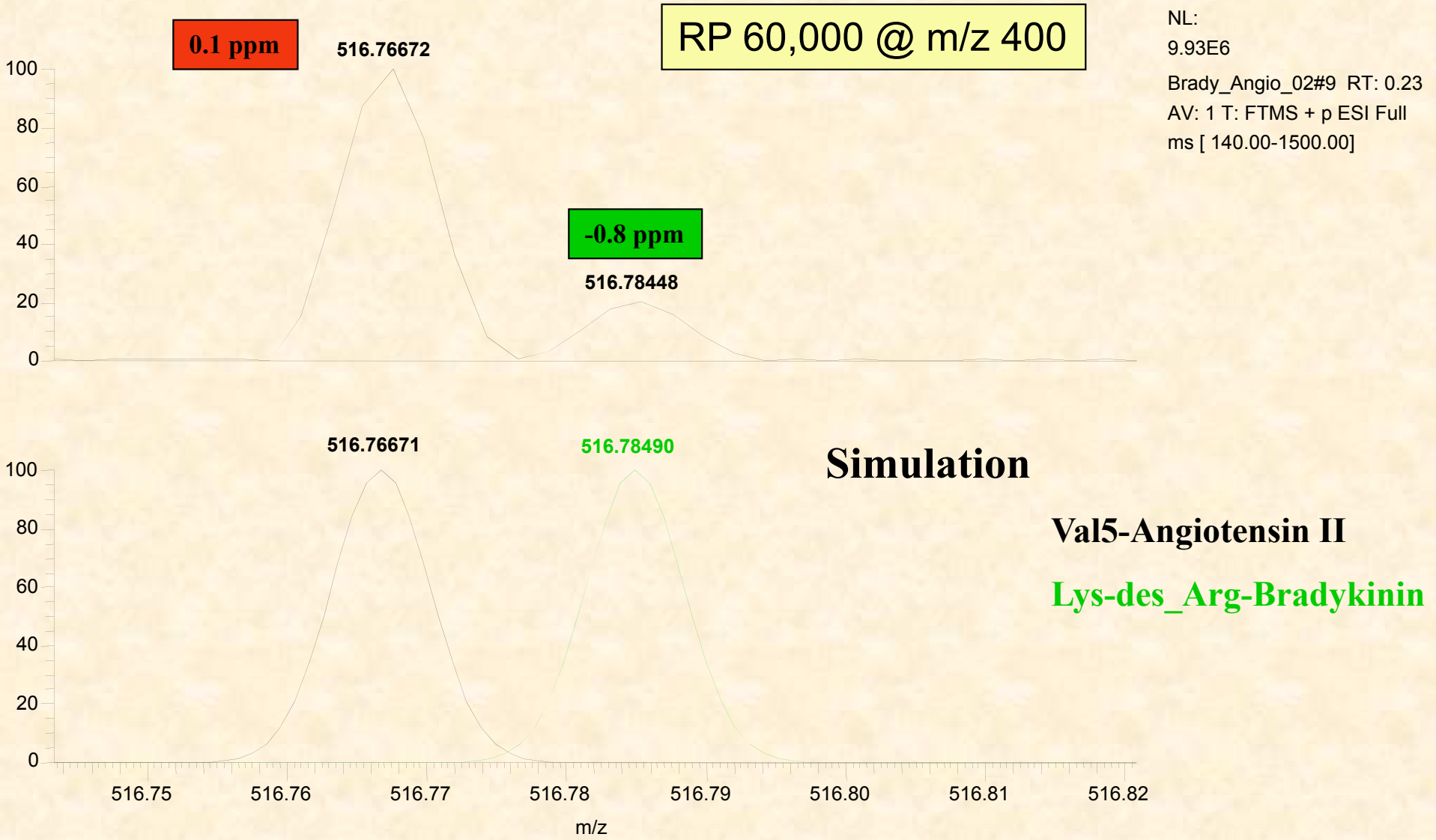
The oscillating ions induce an image current into the two outer halves of the orbitrap, which can be detected using a differential amplifier

Ions generate a sinusoidal wave signal

with permission Ing. Petr Verner (Thermo)

C7250

Full Scan – High Resolution, Zoom-in



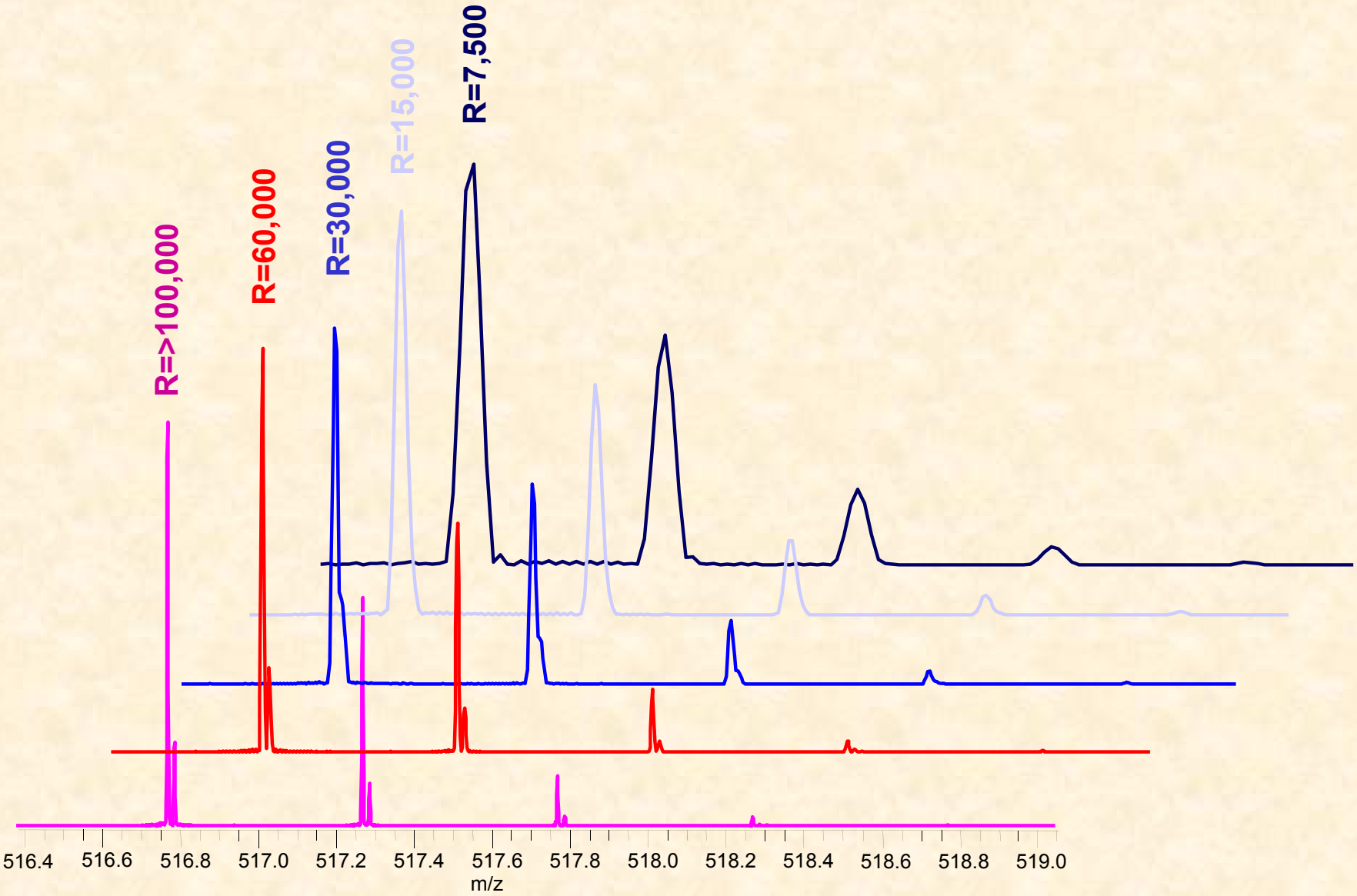
Simulation

Val5-Angiotensin II

Lys-des_Arg-Bradykinin

with permission Ing. Petr Verner (Thermo)

Simulations at different resolution settings



with permission Ing. Petr Verner (Thermo)

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Basic approaches of data acquisition in MS

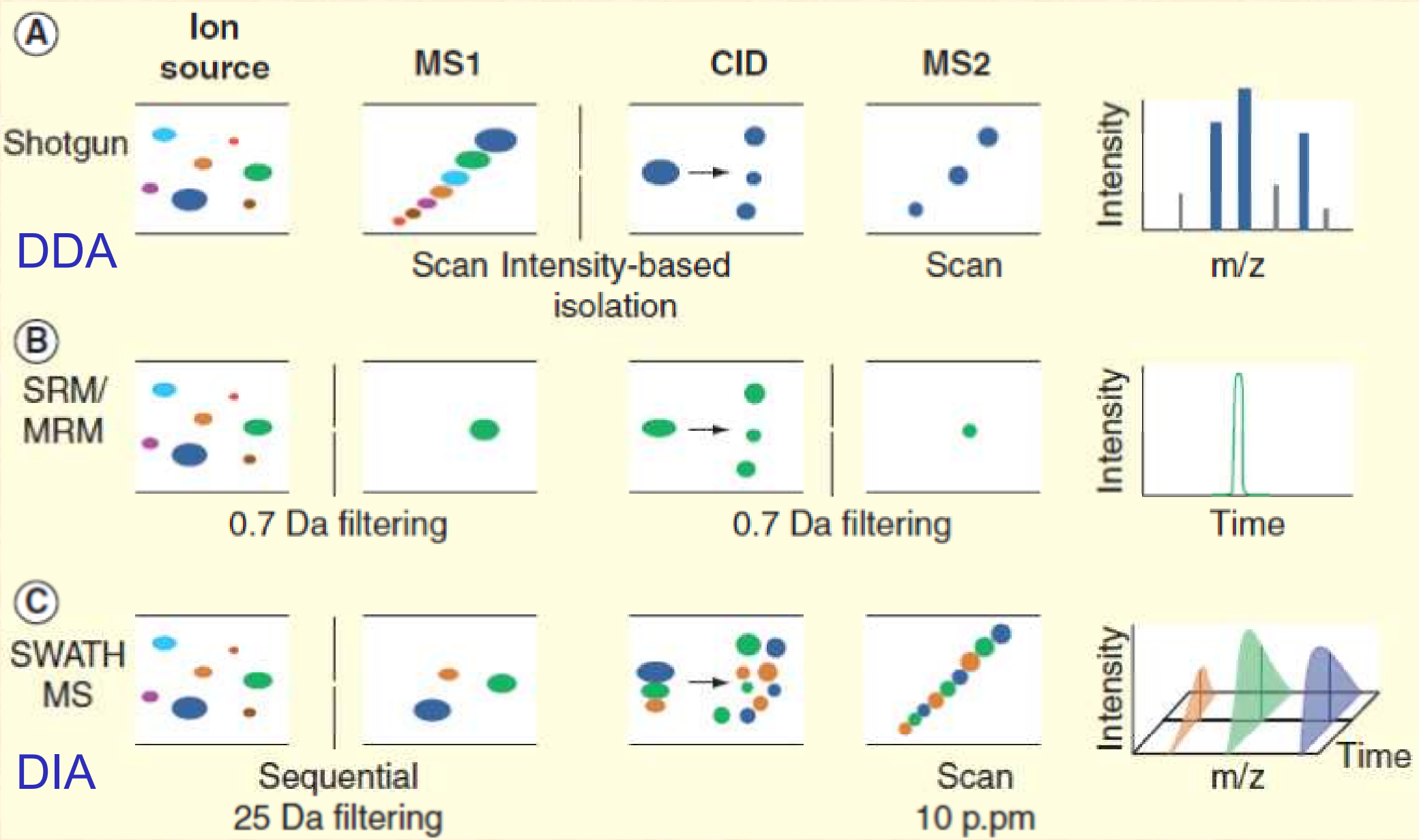
DDA – Data Dependent Acquisition

One precursor selected for MS/MS at a time

DIA – Data Independent Acquisition

Set of precursors is fragmented simultaneously

Acquisition modes

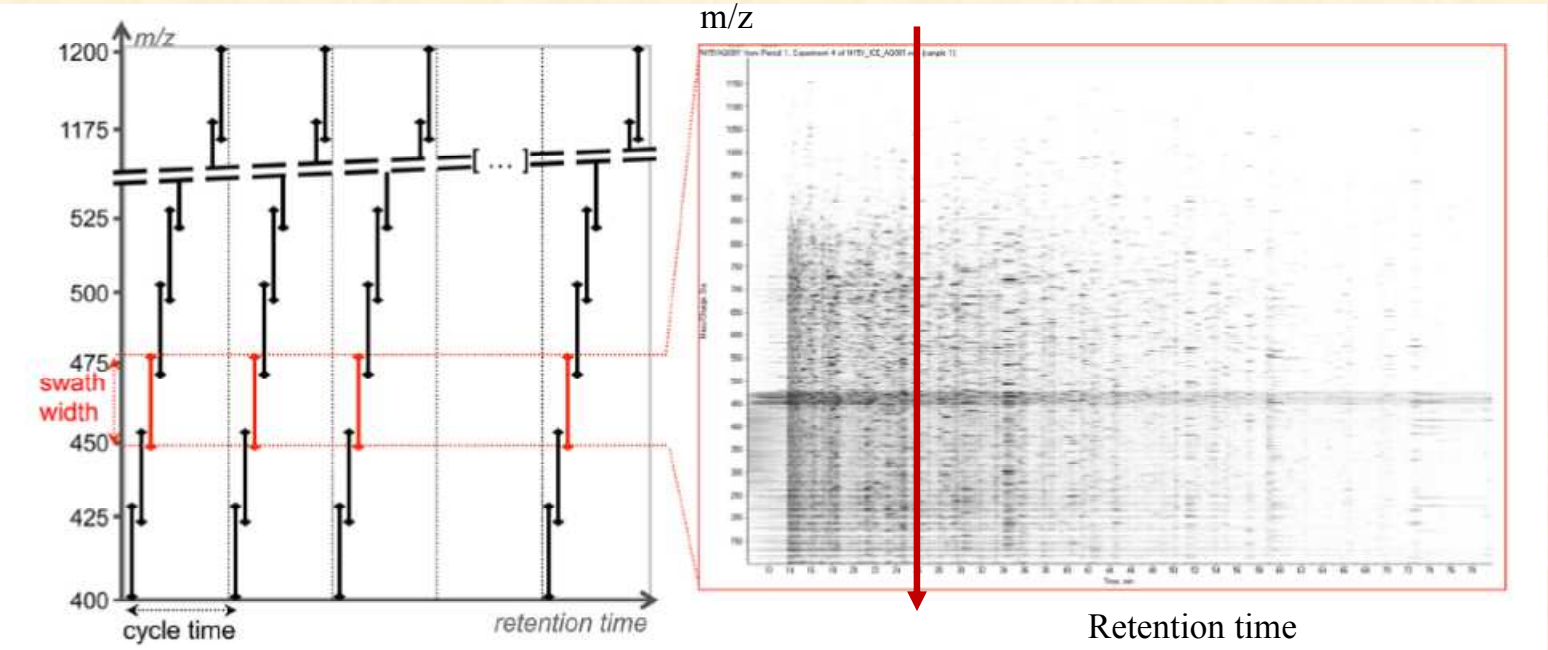


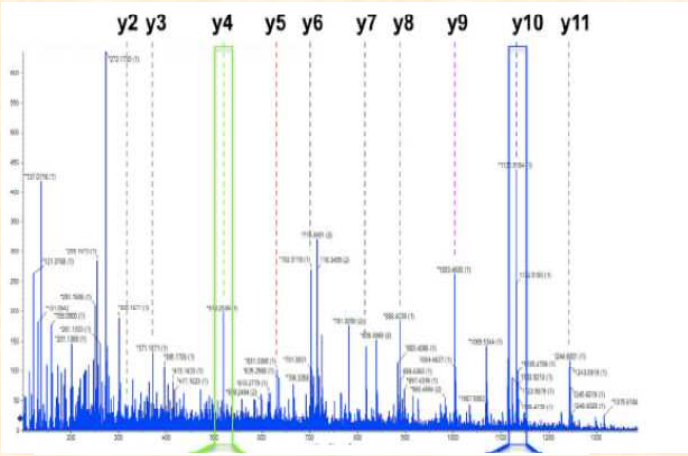
Liu et al, Expert Rev. Mol. Diagn., 13(8), 811 (2013)

SWATH MS

C7250

Q-TOF, MS/MS < 10 ppm





full MS/MS spectrum

Gillet et al, MCP, 11, 1-17 (2012)

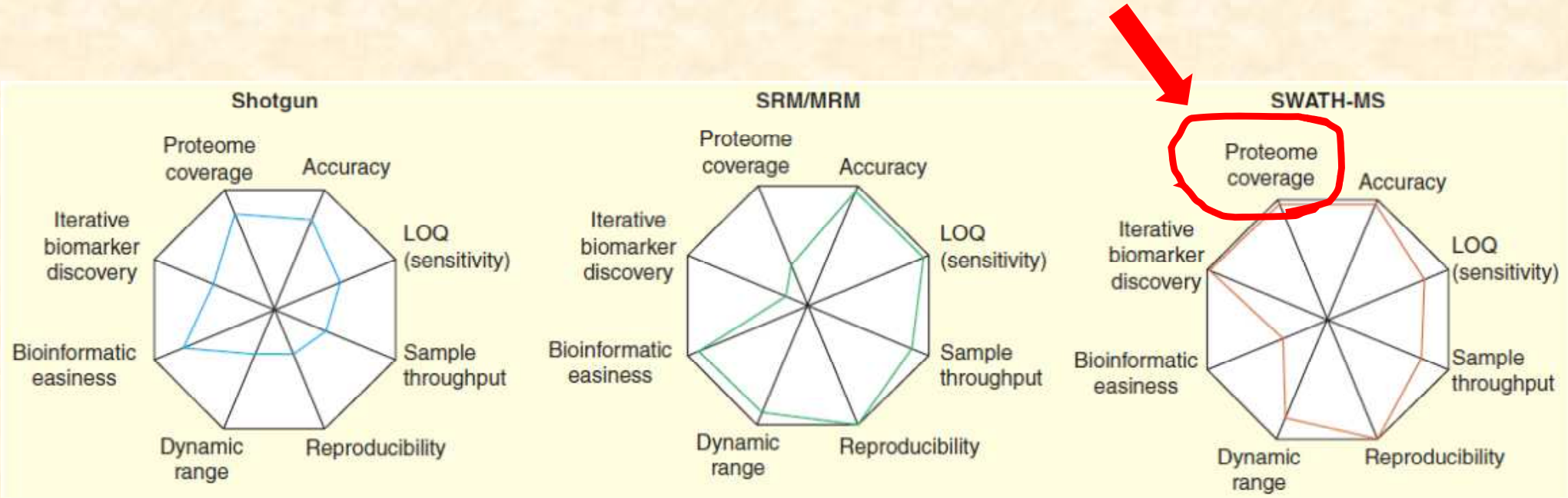
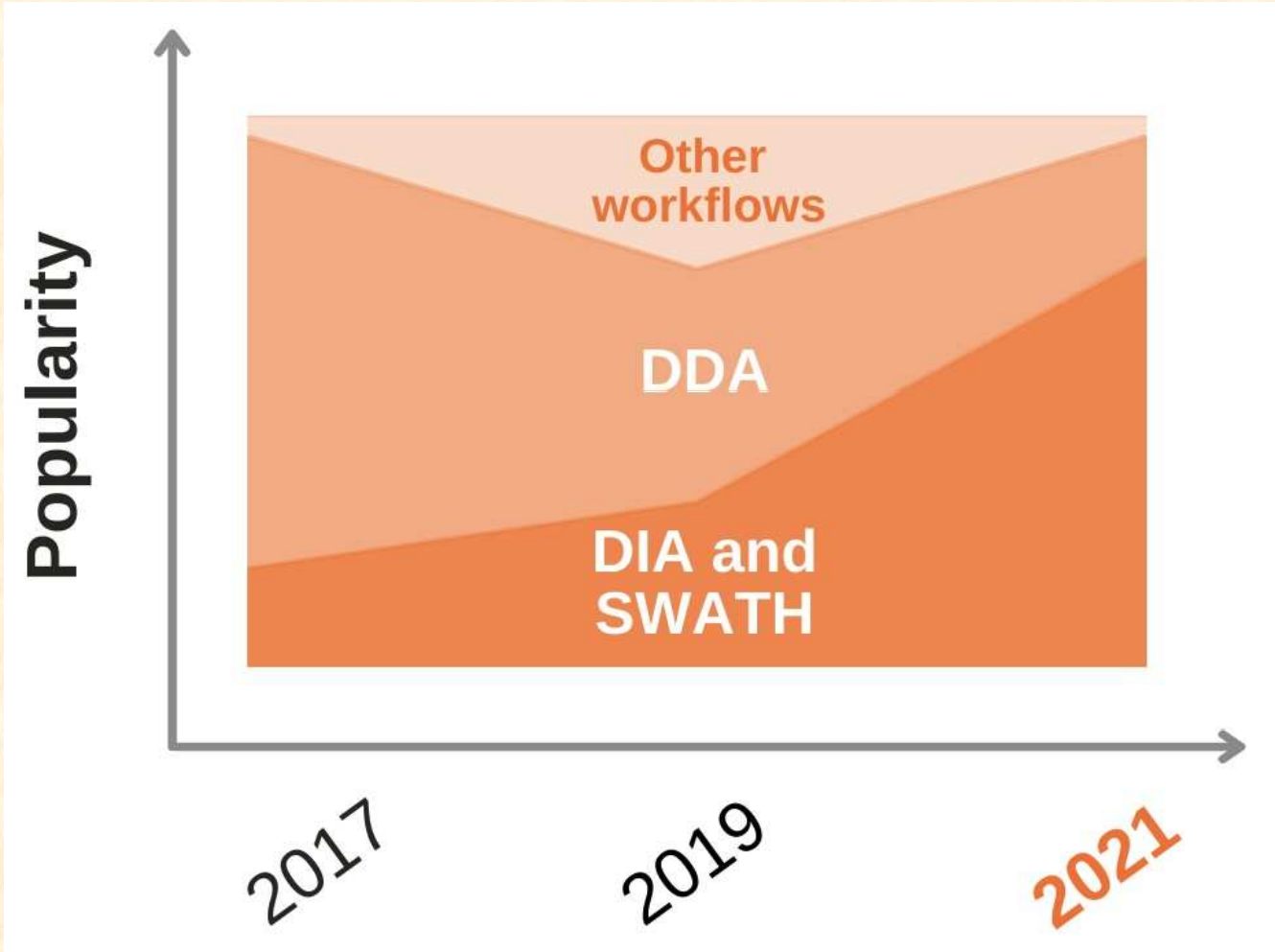


Figure 3. Performance profiles comparing technical advantages and disadvantages of shotgun proteomics, SRM and SWATH MS. In the radar chart, analytical variables are presented on axes staring from the same point and each variable is represented by a spoke. The length of a spoke indicates the magnitude of the variables. Note that SWATH-MS combines the strengths of shotgun and SRM technologies; however, requires more powerful bioinformatic tools for data analysis.
LOQ: Limit of quantification; MRM: Multiple reaction monitoring; MS: Mass spectrometry; SRM: Selected reaction monitoring.

Liu et al, Expert Rev. Mol. Diagn., 13(8), 811 (2013)



Unleash the power DIA combined with short gradients (evosep.com)

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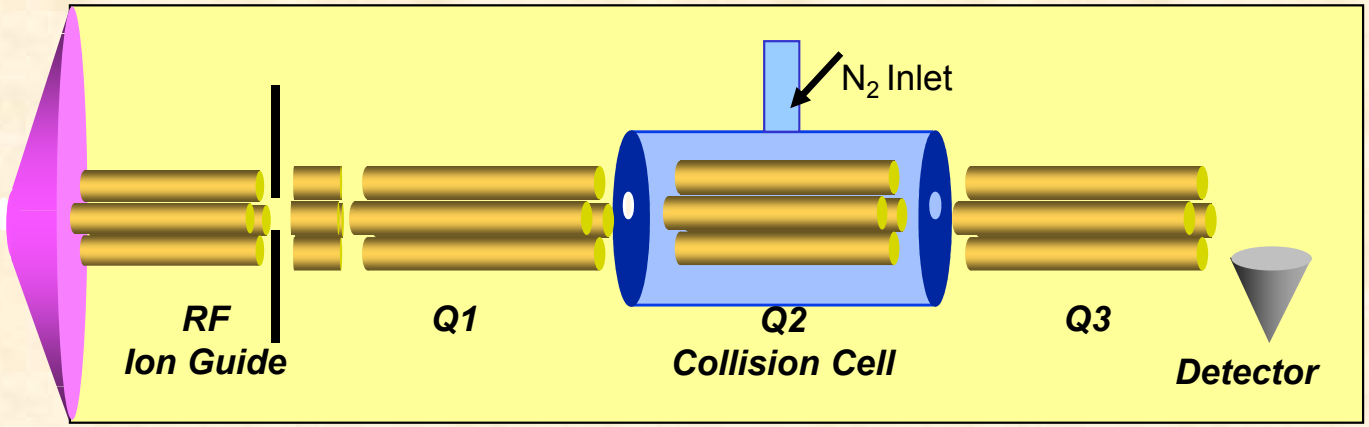
Hybrid systems

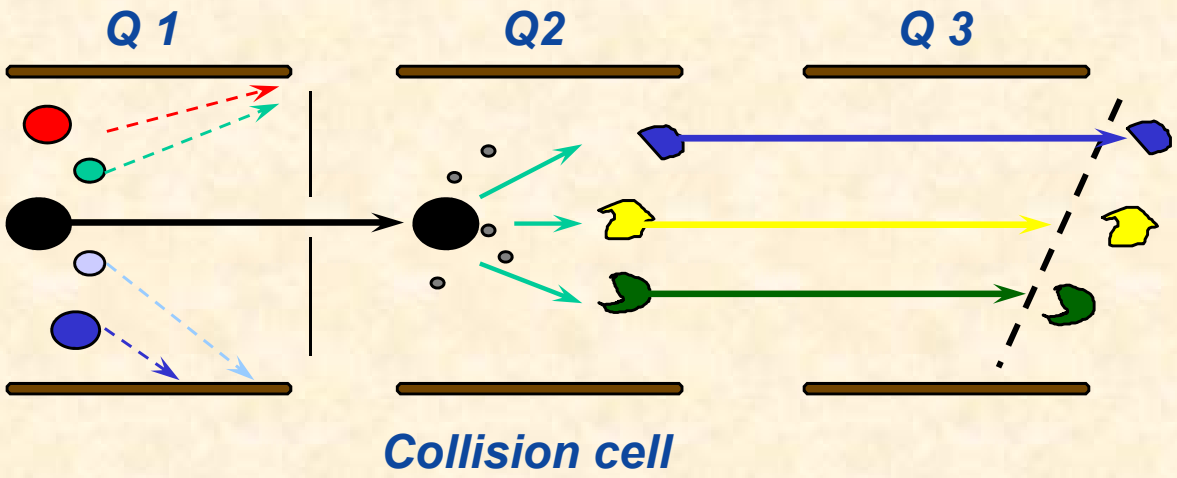
Triple quadrupole, 3-Q

(original design, not hybrid)

- robust
- quantification
- limited mass range ($m/z < 4\,000$)
- enables MS/MS (**MS^2**)
- variety of scan modes
- low resolution
- ESI



Product Ion Scan



Q1 fix
Q3 scan

- quadrupole **Q1** transmits into collisional cell **only ions with selected *m/z***
- quadrupole **Q3 analyzes all fragments** formed in collisional cell by CID (originated from selected ions (precursors) transmitted by Q1)

M1

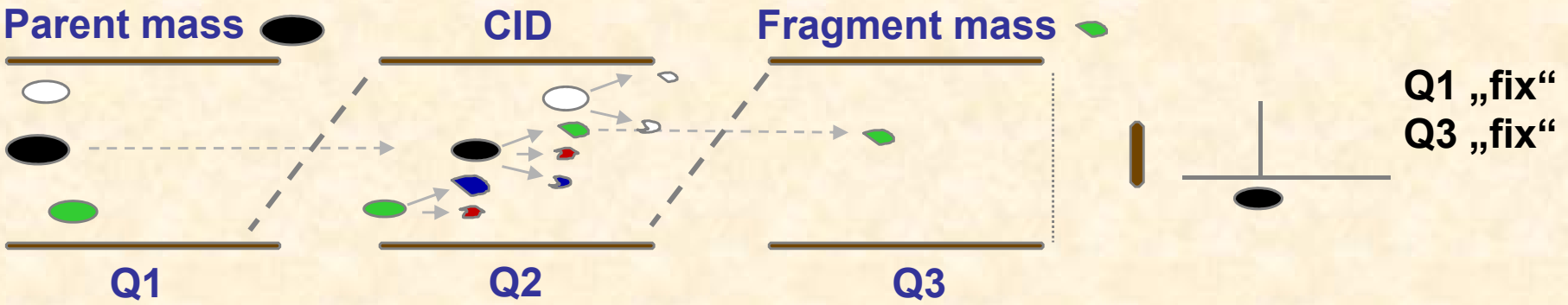
ABI documents

Snímek 81

M1 Product ion scan consists of selecting a precursor ion of a chosen mass-to-charge ratio and determining all of the product ions resulting from collision-induced-dissociation (CID)

MU, 10/27/2009

Selected reaction monitoring, SRM
Multiple reaction monitoring, MRM



- quadrupole **Q1** and **Q3** are fixed to selected values of *m/z* (Q1-precursor and Q3- selected fragment), **only precursors displaying production of selected fragment** during fragmentation in collisional cell **are recorded**
- enables to follow tens of reactions (transitions) during analytical run (MRM)

improved sensitivity
detection of low abundant components (e.g. biomarkers in complex samples)

M4

ABI documents

Snímek 82

M4 Selected reaction monitoring consists of selecting a fragmentation reaction. For this scan Q1 and Q3 are focused on selected masses There is no scan. The method is analogous to selected ion monitoring in MS but here the ions selected by Q1 are only detected if they produced a given fragment. The absence of scanning allows one to focus on the prcursor and fragment ions over longer times, increasing the sensitivity as for SIM but this sensitivity is now associated with a high increase in selectivity.

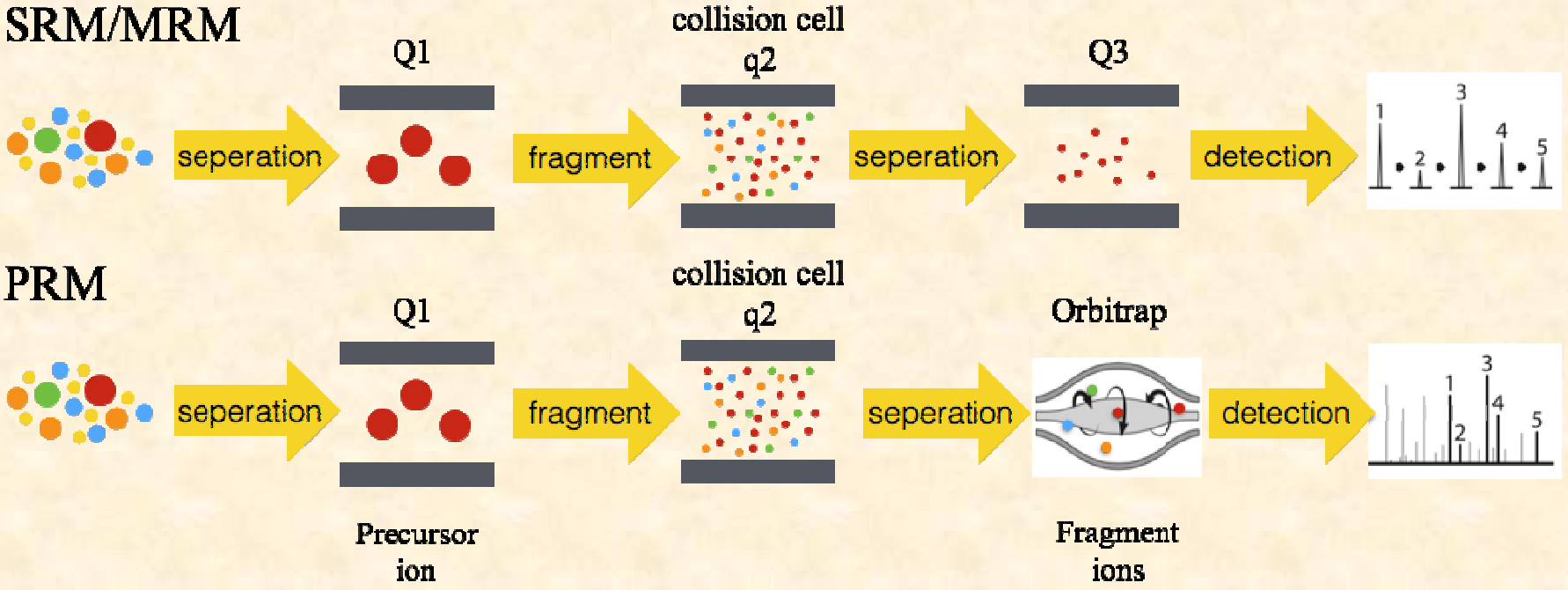
MU, 10/27/2009

Z1

Parallel reaction monitoring (PRM) Z2

C7250

- Q-Orbitrap MS
- similar to SRM/MRM
- all fragments of selected precursor are deected
  with high resolution/accuracy for determination of *m/z*



- Simplier method adjustment
- improved precursor identification and quantification securing high sensitivity

https://www.creative-proteomics.com/services/parallel-reaction-monitoring-prm.htm

Snímek 83

Z1 PRM is based on Q-Orbitrap as the representative quadrupole-high resolution mass spectrum platform. Unlike the SRM, which performs one transition at a time, the PRM performs a full scan of each transition by a precursor ion, that is, parallel monitoring of all fragments from the precursor ion. First, the PRM uses the quadrupole (Q1) to select the precursor ion, and the selection window is usually m/z≤2; then, the precursor ion is fragmented in the collision cell (Q2); finally, Orbitrap replaces Q3, scans all product ions with high resolution and high accuracy. Therefore, PRM technology not only has the SRM/MRM target quantitative analysis capabilities, but also have the qualitative ability. (1) The mass accuracy can reach to ppm level, which can eliminate the background interference and false positive better than SRM / MRM, and improve the detection limit and sensitivity in complex background effectively; (2) Ful scan of product ions, without the need to select the ion pair and optimize the fragmentation energy, easier to establish the assay; (3) a wider linear range: increased to 5-6 orders of magnitude

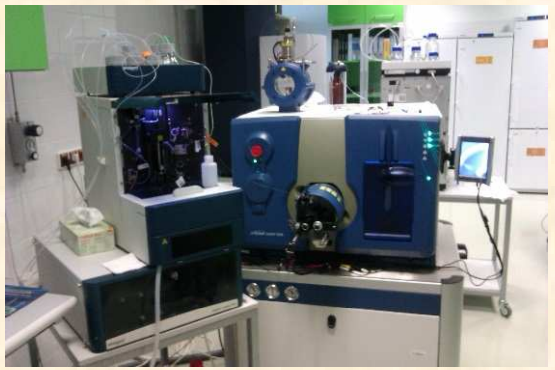
Zbynek, 2/21/2019

Z2 Parallel reaction monitoring (PRM) is an ion monitoring technique based on
high-resolution and high-precision mass spectrometry. The principle of this technique is comparable to SRM/MRM, but it is more convenient in assay development for absolute quantification of proteins
and peptides. It is most suitable for quantification of multiple proteins in complex sample with an attomole-level detection.

Zbynek, 2/21/2019

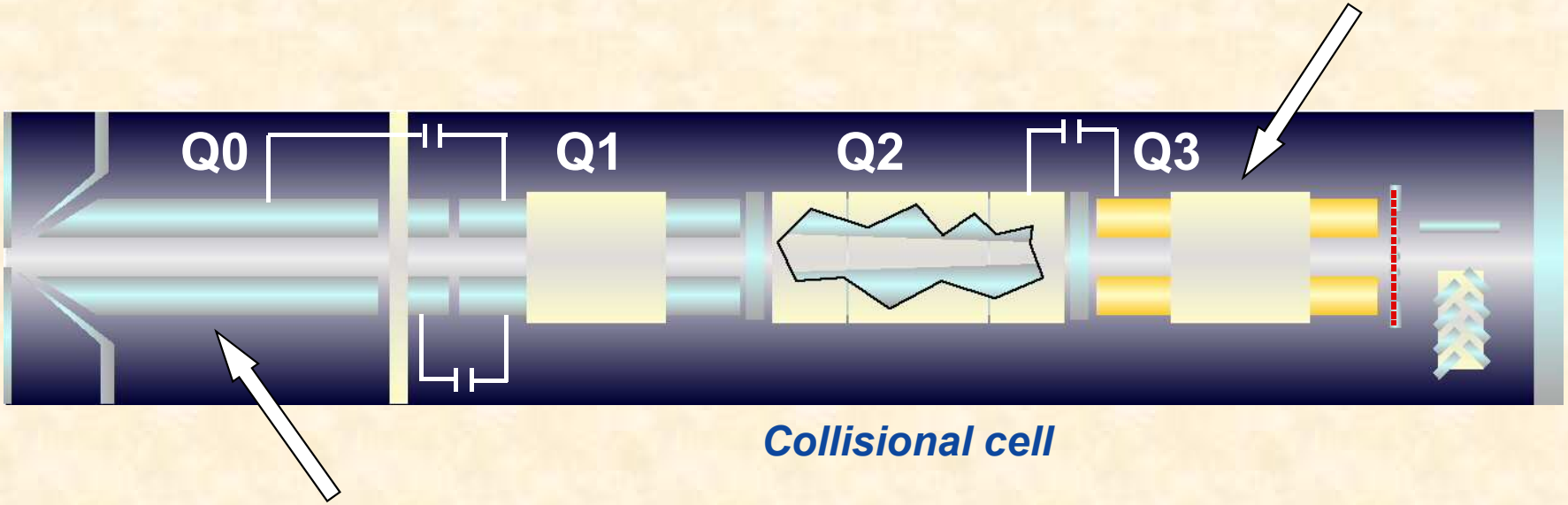
3-Q

(LIT instead of Q3)



Linear ion trap

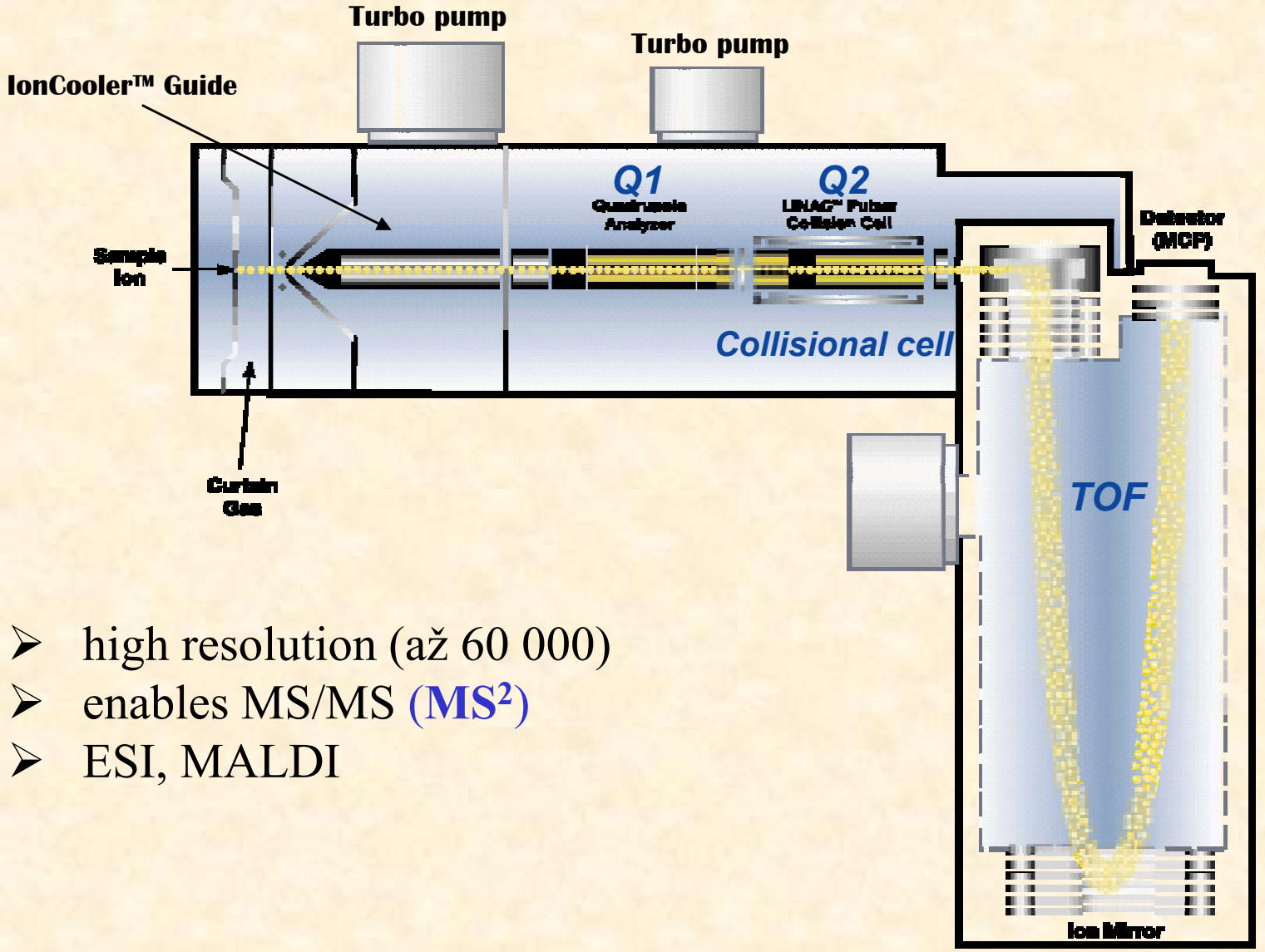
- increased sensitivity (enhanced scans)
- enables MS/MS (MS^n)
- low resolution
- ESI



accumulation of ions during LIT scan (reduced loss of ions)

Q-TOF

(TOF instead of Q3)



- high resolution (až 60 000)
- enables MS/MS (**MS²**)
- ESI, MALDI

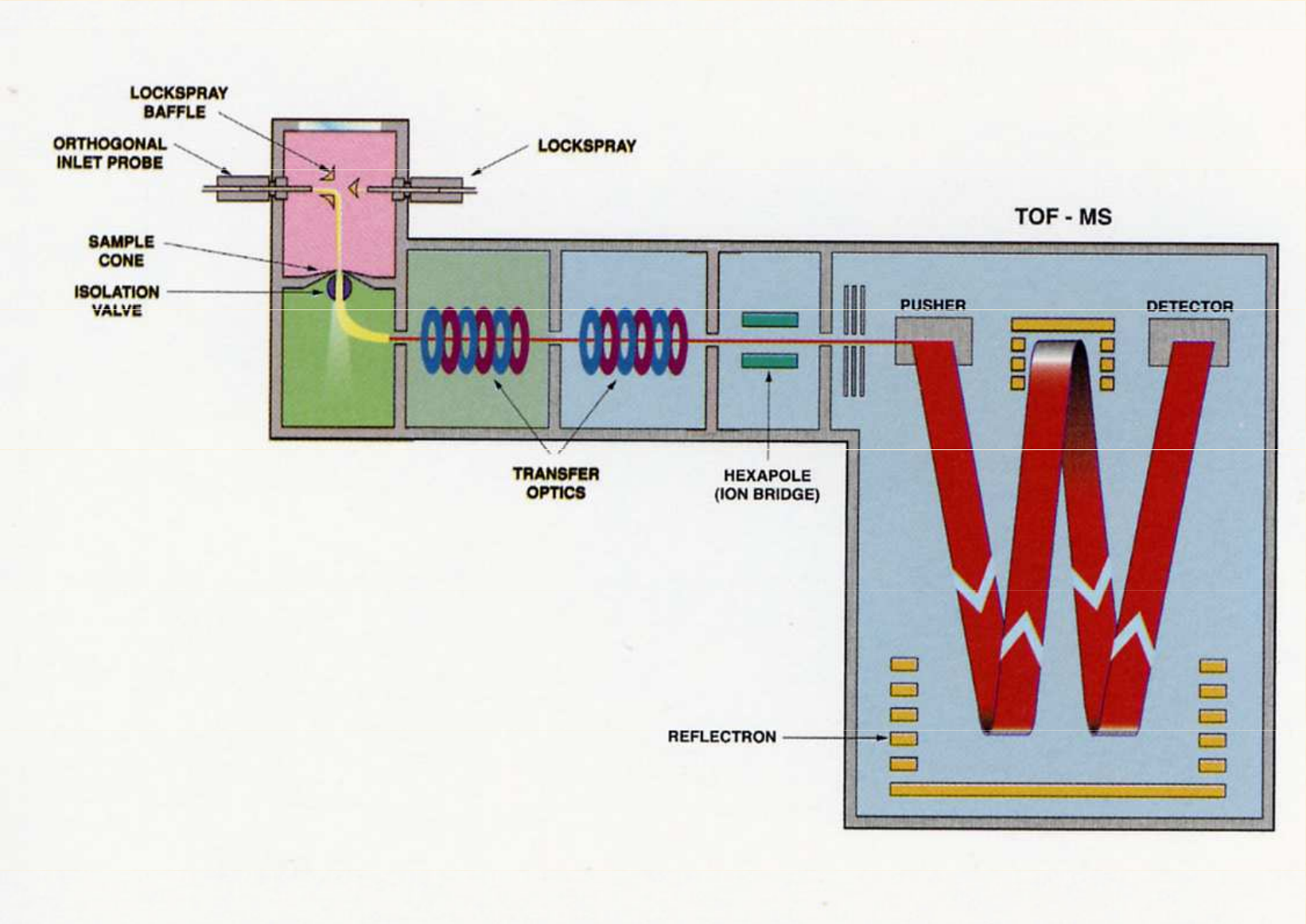
C7250

Q-TOF

Bruker

Q-TOF

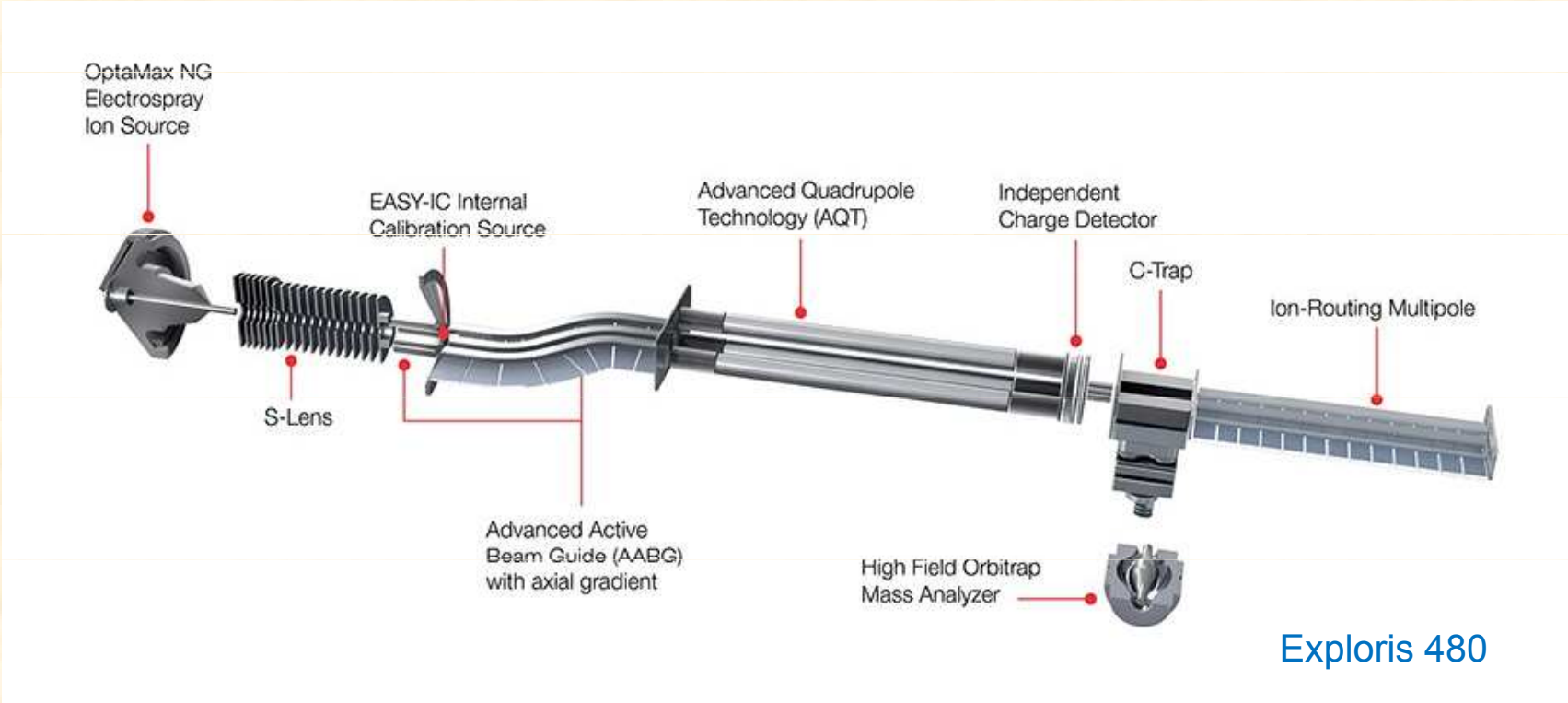
(TOF - W design)



Q-Orbitrap

(Orbitrap instead of Q3)

- ✓ high resolution up to 480 000
- ✓ *m/z* range < 6000
- ✓ HCD, ETD

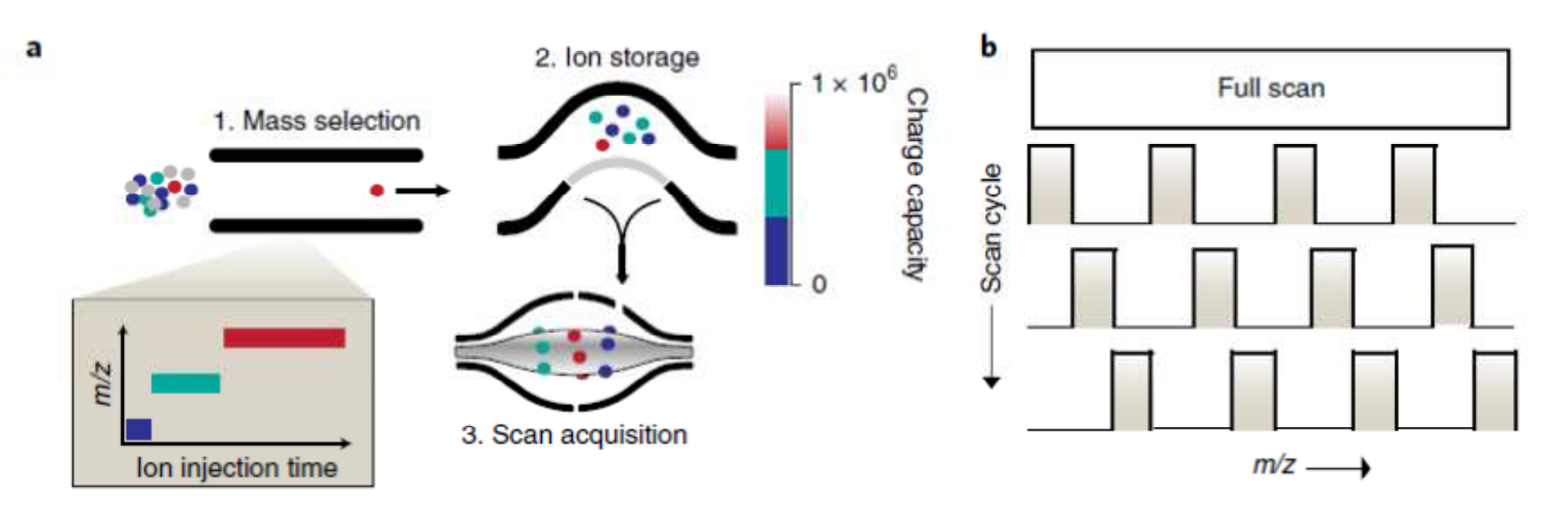


Thermo

Q-Orbitrap

BoxCar acquisition method (extended dynamic range)

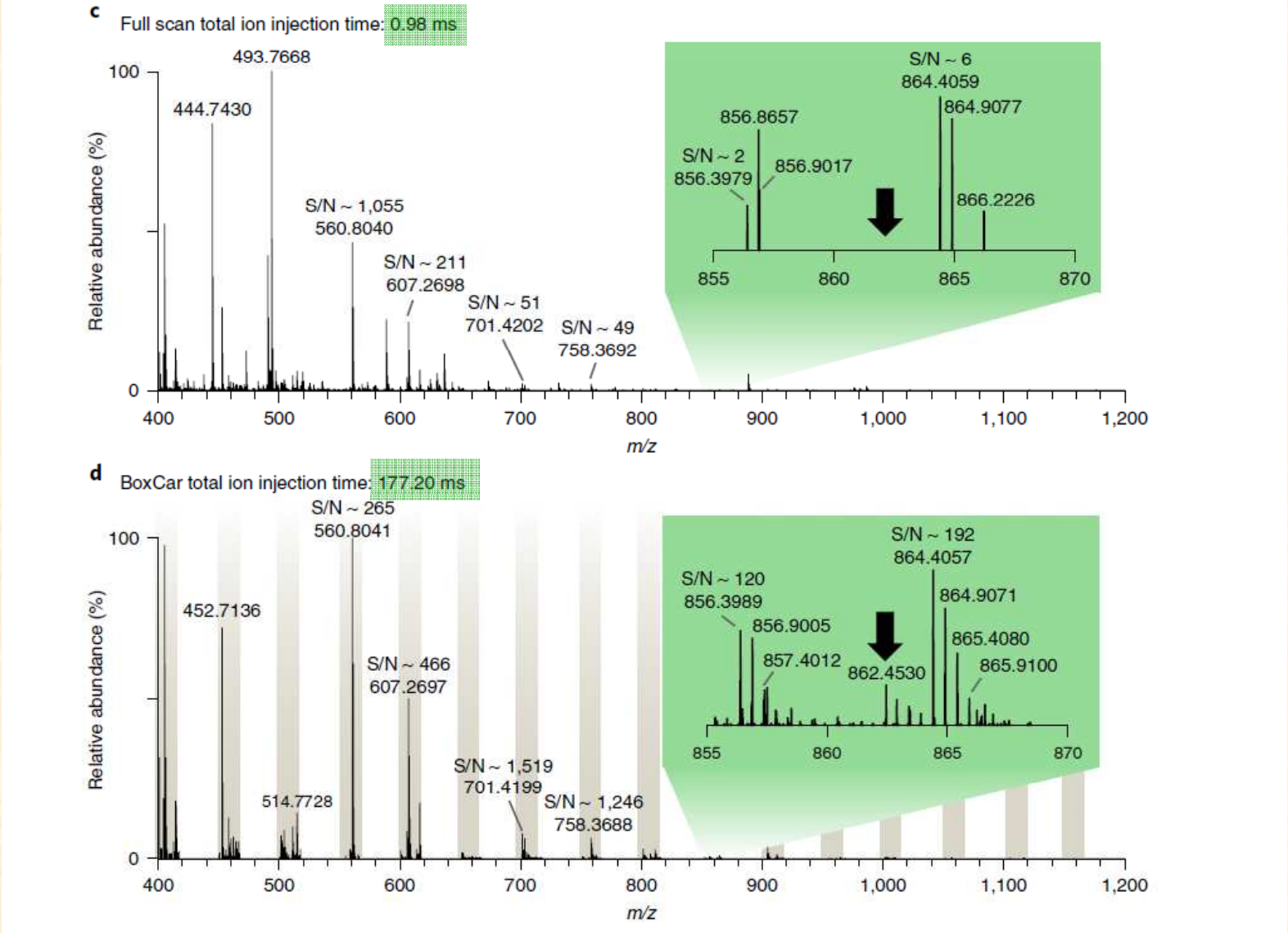
- limitation – C-trap capacity (1 000 000 charges)
- capacity is often saturated by high abundant ions
- BoxCar collects ions in C-trap in narrow m/z segments allowing to accumulate low abundant ions increasing number of identified peptides and extending dynamic range
- MS/MS scans are restricted
- creation of peptide library is required for identification (mostly done at MS level)



Meier F. et al., Nature Methods, 15, 440-448 (2018)

Q-Orbitrap

BoxCar acquisition method

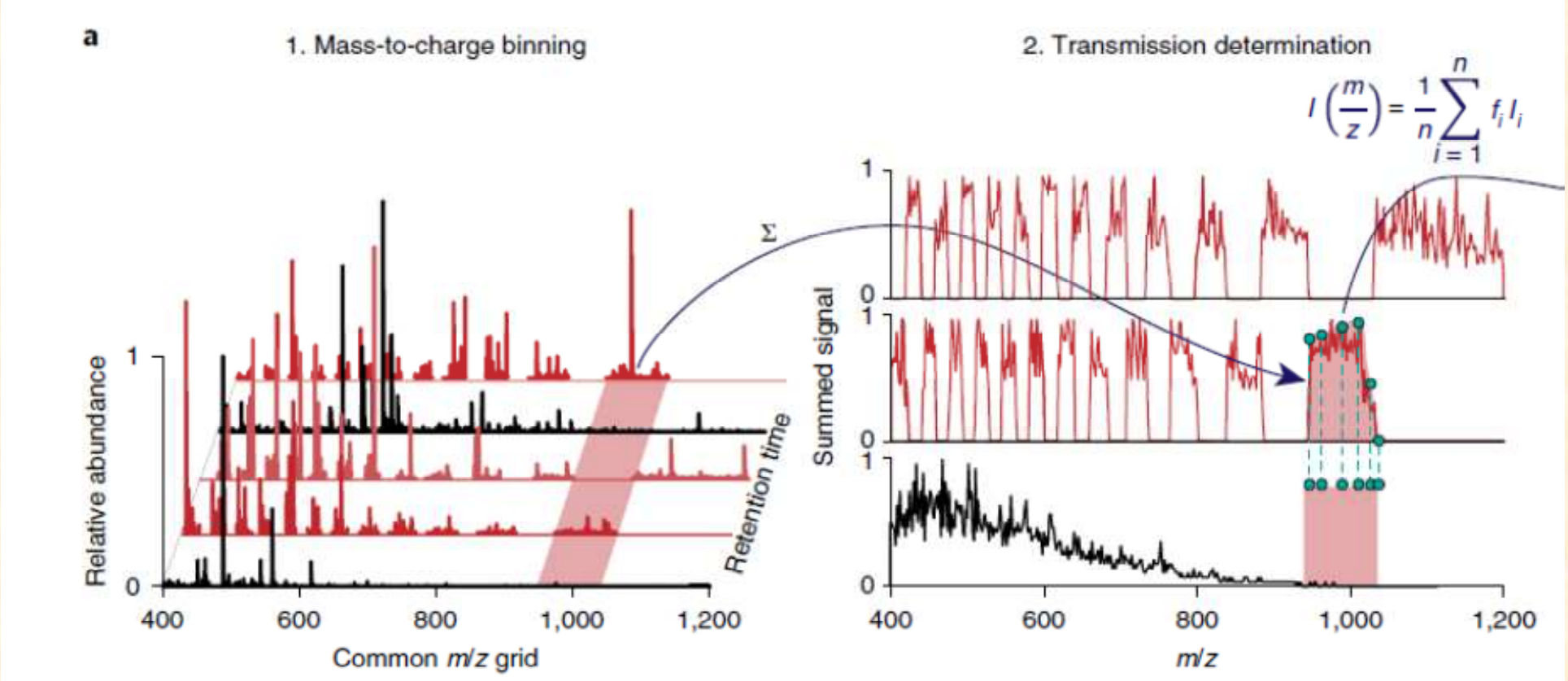


Meier F. et al., Nature Methods, 15, 440-448 (2018)

Q-Orbitrap

C7250

BoxCar acquisition method



MaxQuant assembles the full scan (black traces) and BoxCar scans (red traces) of an acquisition cycle to a single high-dynamic-range scan. First, all spectra are transformed to a common high-resolution *m*/*z* grid, and the signals from each scan (here **one full scan and two BoxCar scans**) are integrated over the entire LC elution time (step 1). From the integrated signals, the shape of the quadrupole transmission function for each BoxCar scan is globally determined by a pointwise comparison to the full scan (step 2). The resulting relative transmission factors for each *m*/*z* bin in each BoxCar scan are used as weights for calculating the average signal intensity from the full scan and the BoxCar scans. These hybrid spectra are taken as a replacement for standard full scans in all subsequent processing steps without further adjustments (step 3).

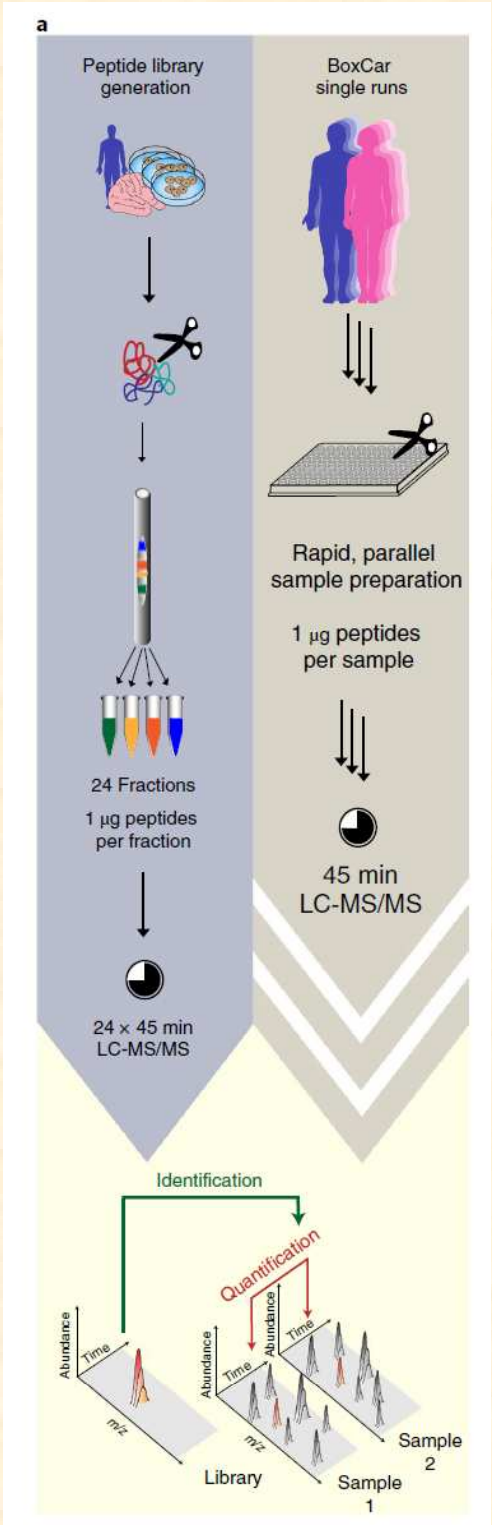
Meier F. et al., Nature Methods, 15, 440-448 (2018)

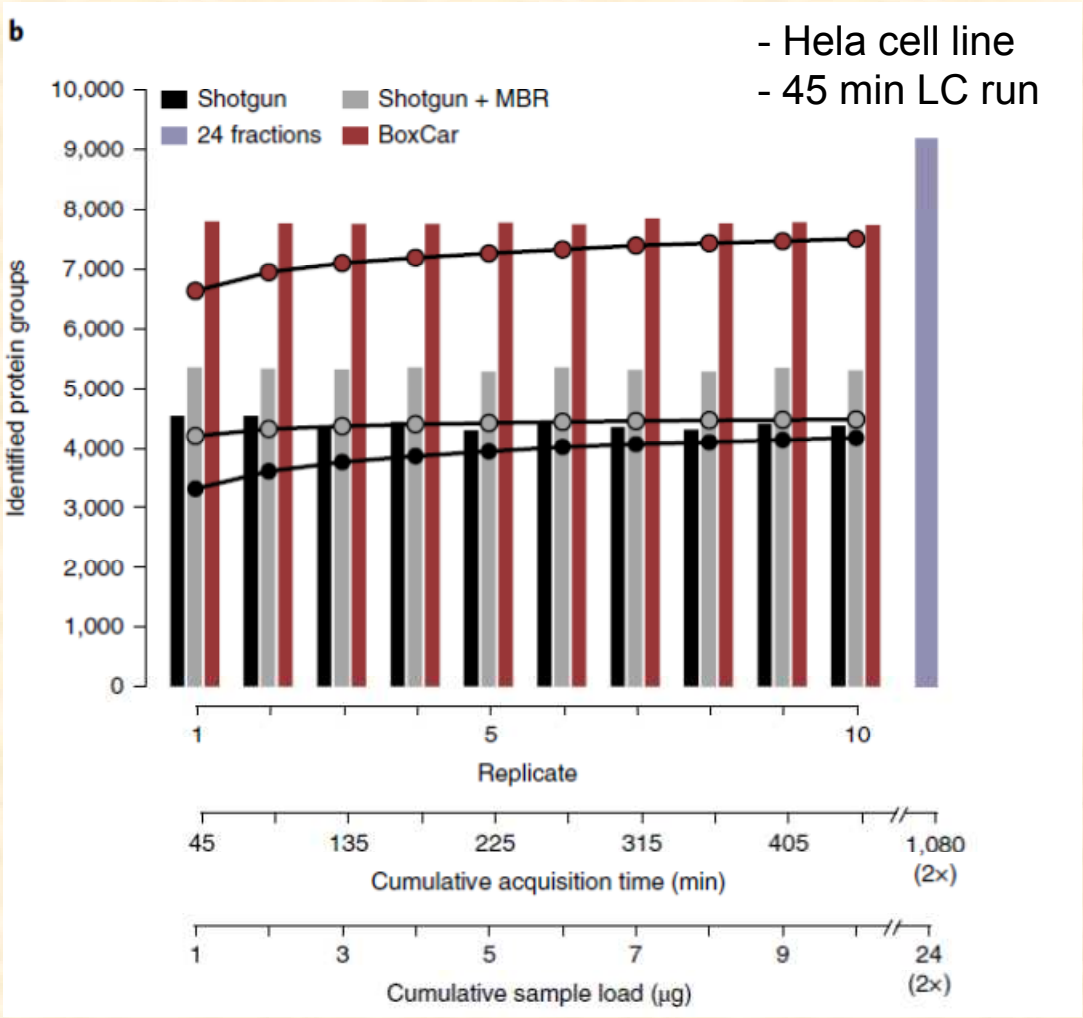
Q-Orbitrap

C7250

BoxCar acquisition method

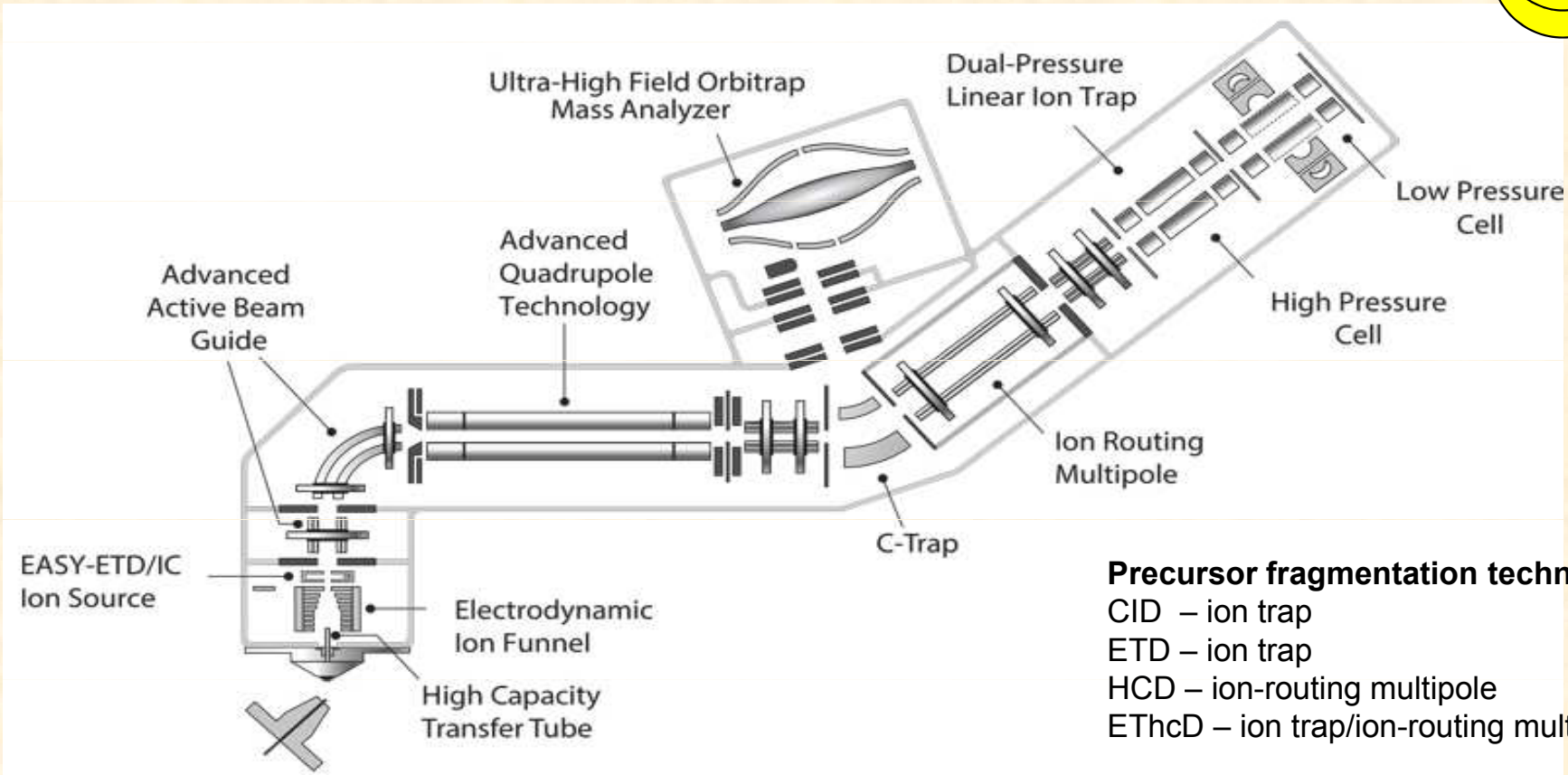
- generation of a peptide library
- analysis of individual samples





Meier F. et al., Nature Methods, 15, 440-448 (2018)

Orbitrap Fusion™ Lumos Tribrid



Precursor fragmentation techniques:
CID – ion trap
ETD – ion trap
HCD – ion-routing multipole
EThcD – ion trap/ion-routing multi pole

Ion separation/detection:
Ion trap – low resolution
Orbitrap – high resolution

ETD HD – high dynamic range ETD providing significantly increased fragment ion coverage

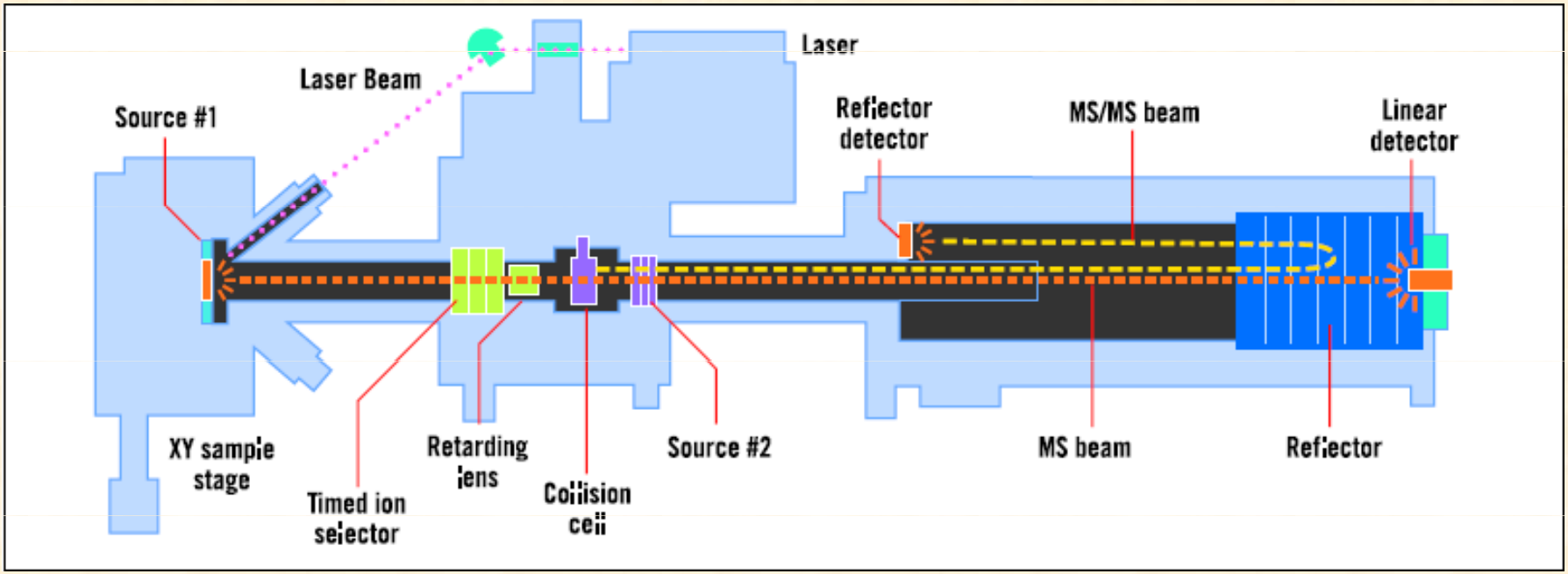
Resolution Orbitrap
15 000–1 000 000 (FWHM) at *m/z* 200

TOF-TOF

- relatively high resolution (< 30 000)
- enables MS/MS (MS^2)
- MALDI
- enables off-line connection with LC (LC-MALDI approach)

Bruker

AB Sciex



ion mobility + mass spectrometry

C7250

Trapped Ion Mobility Spectrometry

timsTOF™

- **coupling ion mobility separation with mass spectrometry**
- **ion mobility brings additional separation dimension**
- **ion mobility allows separation of structural isomers**
  (in general, compounds with the same or close m/z differing in ccs)

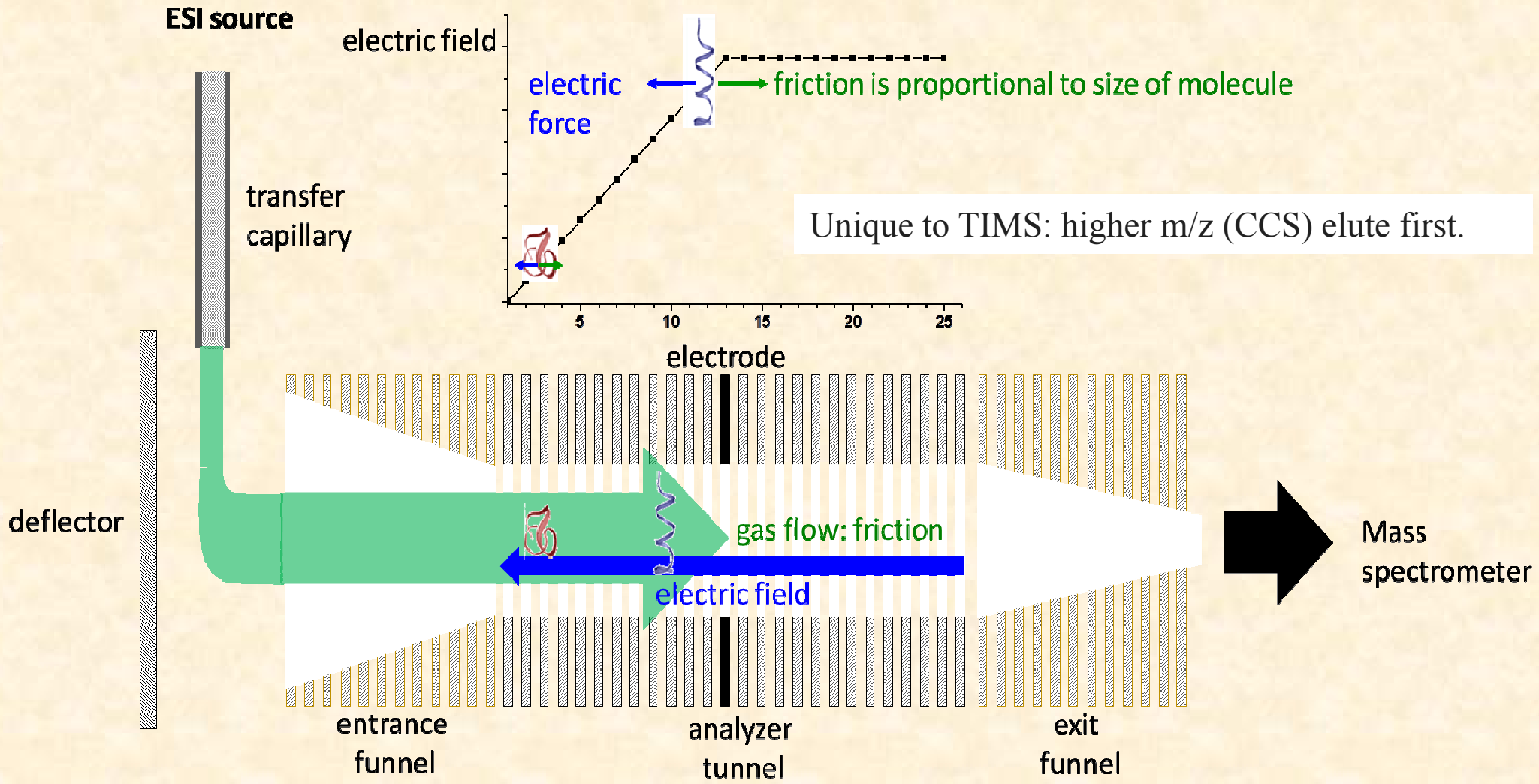
The **TIMS analyzer** is a segmented rf ion guide wherein ions are mobility-analyzed using an electric field that holds ions stationary against a moving gas, unlike conventional drift tube ion mobility spectrometry where the gas is stationary. Ions are initially trapped, and subsequently eluted from the TIMS analyzer over time according to their mobility (K).

TIMS theory – Michelmann K. et al.: J. Am. Soc. Mass Spectrom. 26,14-24 (2015)

Trapped Ion Mobility Spectrometry

timsTOF™

Ion mobility **separates** compounds based on their **collisional cross section (CCS)**, which is primarily **a function of three-dimensional shape**.

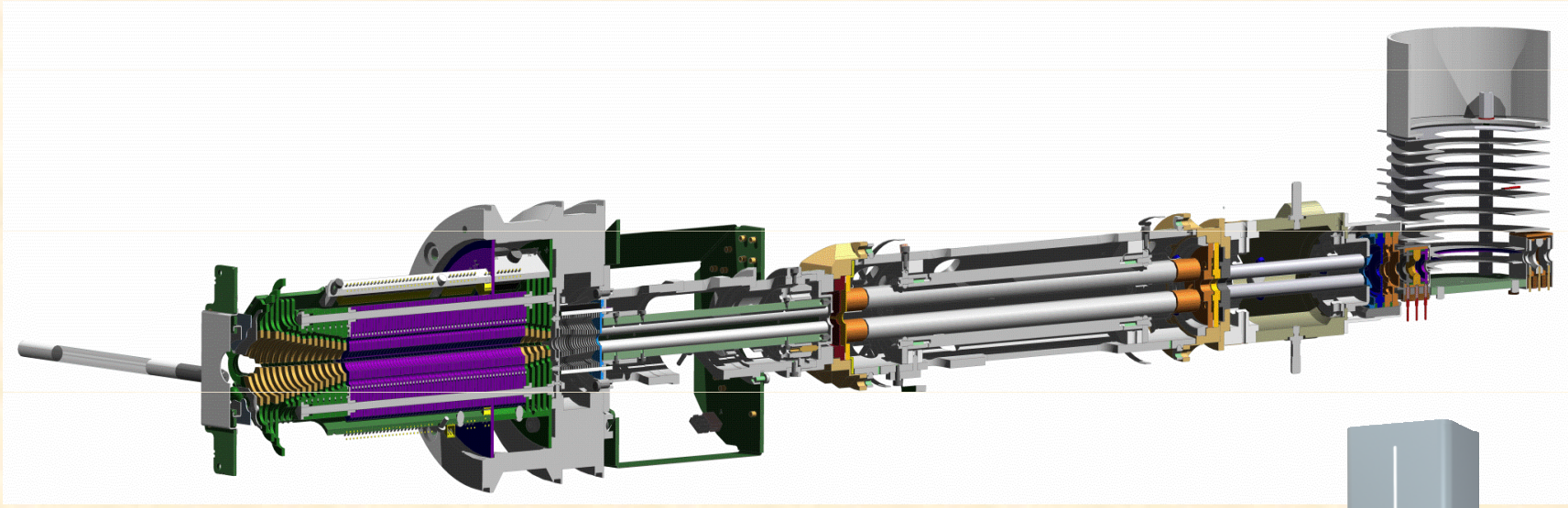


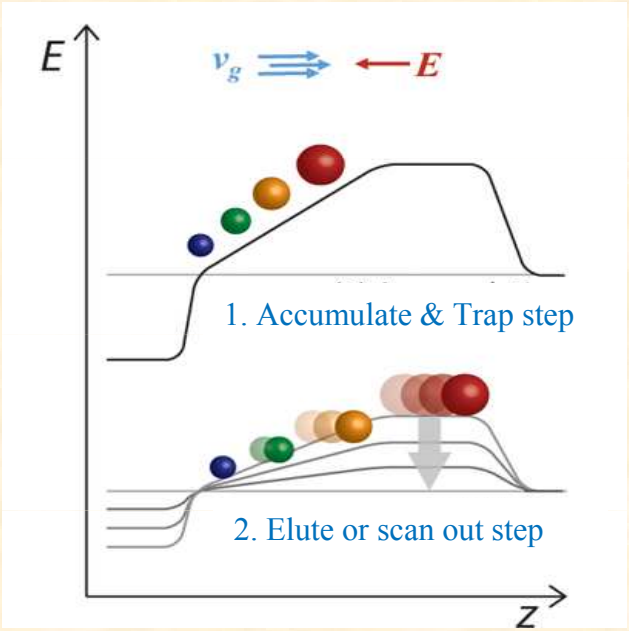
Unique to TIMS: higher m/z (CCS) elute first.

with courtesy of M. Boháč (Bruker)

Trapped Ion Mobility Spectrometry

timsTOF™





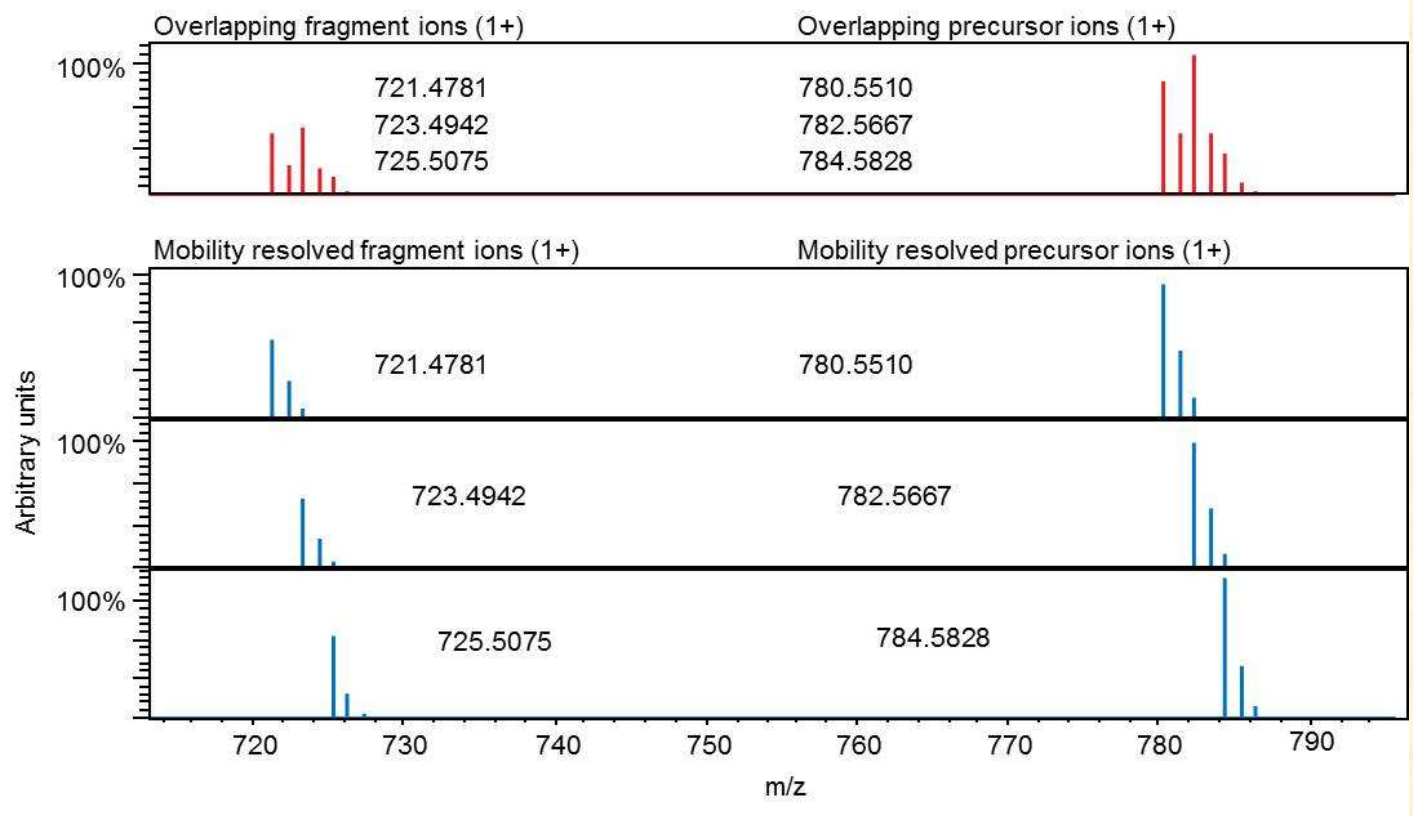
~10 x higher sensitivity

with courtesy of M. Boháč (Bruker)

Trapped Ion Mobility Spectrometry

timsTOF™

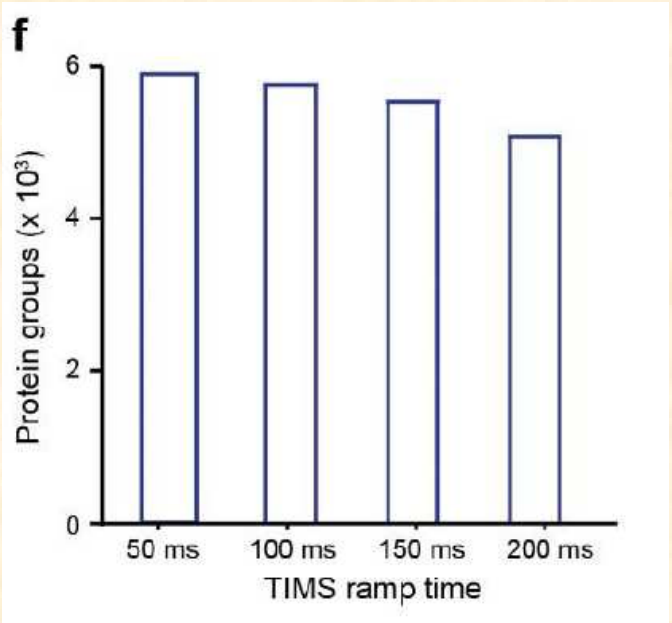
resolution of coeluting compounds with overlapping isotopic patterns



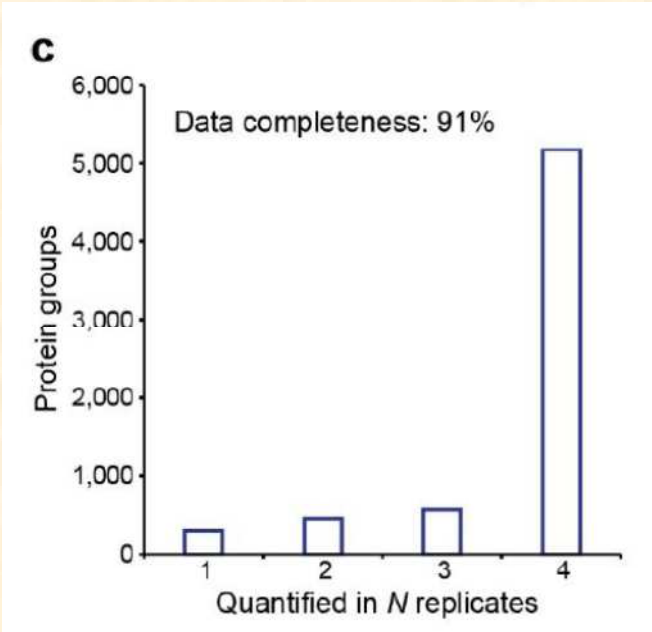
Trapped Ion Mobility Spectrometry

timsTOF™

- analysis of HeLa digest (200 ng)
- 120 min LC run



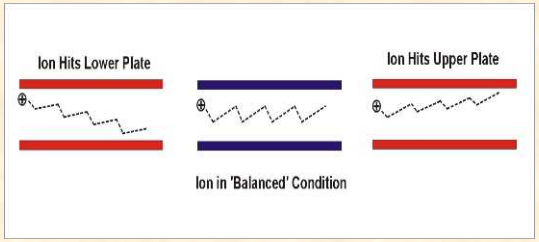
Average number of protein group identifications in a single run (N=4) with different TIMS settings



Number of proteins quantified in *N* out of four replicates.

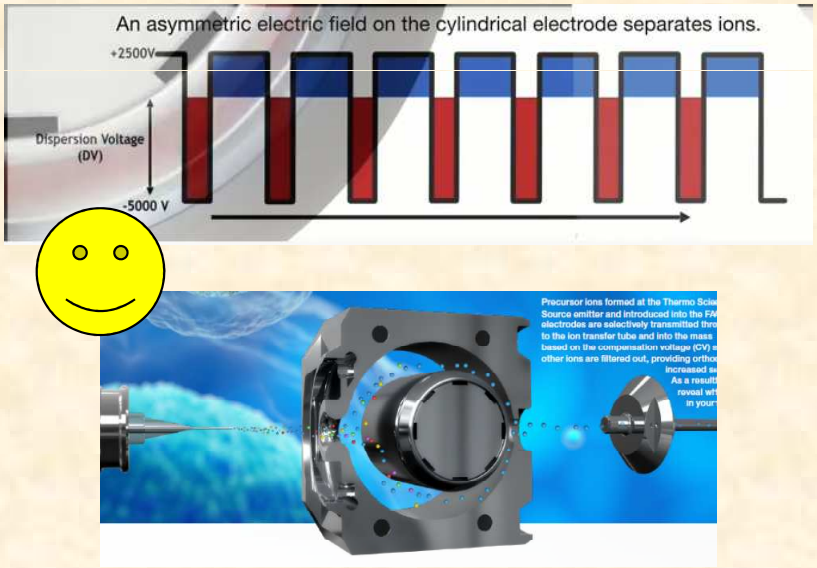
BioRxiv, https://doi.org/10.1101/336743

Field Asymmetric Ion Mobility Spectrometry
FAIMS

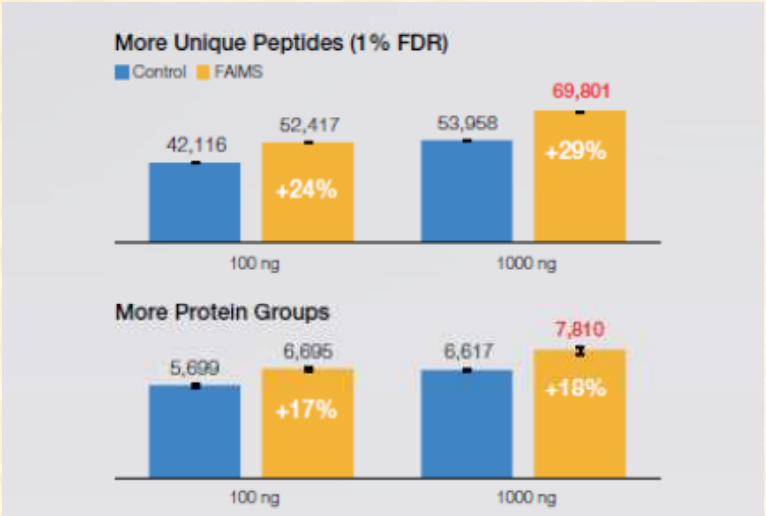


If a mixture of ions of different sizes and types is introduced between two metal plates, the application of high voltage in an appropriate waveform to the plates will create a condition where some types of ions drift and hit the metal plates while other types of ions remain between the plates.

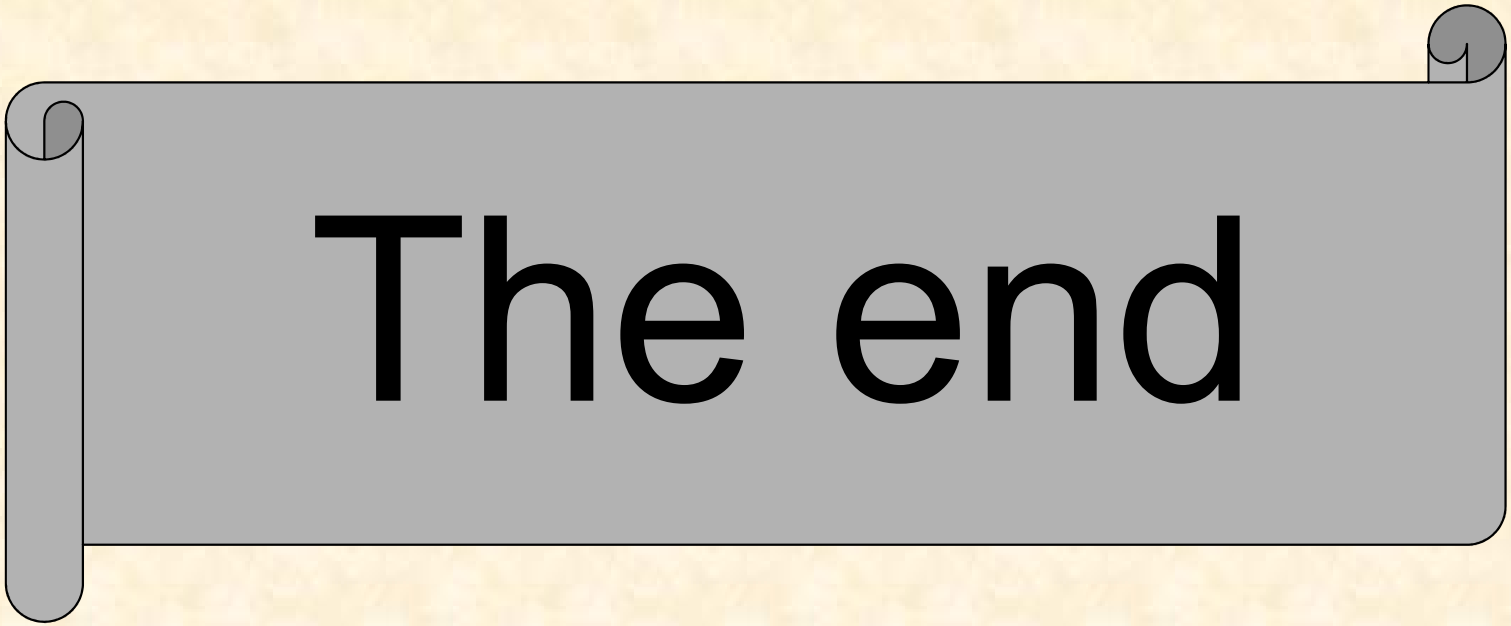
basic principle: *http://www.faims.com/howpart1.htm*



Analysis of tryptic digest of HeLa cell lysate



FAIMS – Thermo Fisher


The end