Mass Spectrometry on the Cinco de Mayo

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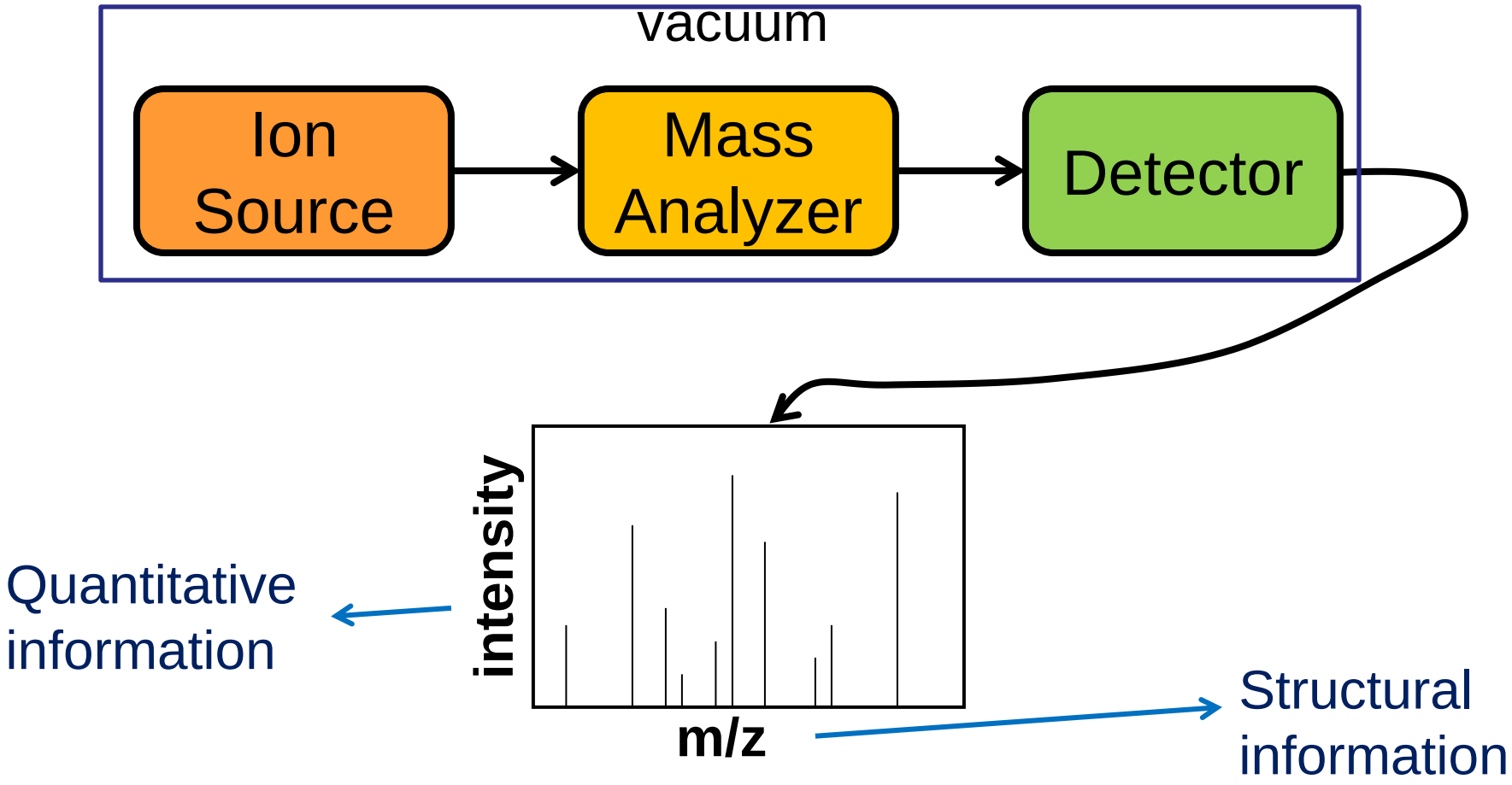
Images courtesy of David Fenyö, Nathan Yates, Wilhelm Haas, Michael McCoss, AB-SCIEX, Thermo

Outline of today's talk

- Overview - mass spectrometry in proteomics
- How mass spectrometers work
- Looking at spectra - mass and isotopes
- Quantitative proteomics

What does a mass spectrometer do?

- Measures mass/charge (m/z) of intact ions and fragments



Use of mass spectrometry in proteomics research

protein identification
protein processing:
    proteolytic
    post-translational modifications (100's)
quaternary structure
complex formation
steady-state levels
turnover rates
structural studies (H/D exchange)

comparison of samples (disease & controls)

Mass spectrometry is an enabling technology* in the field of proteomics

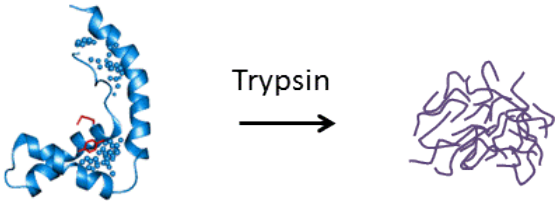
	Peptide Sequencing	
	Edman degradation	Mass spectrometry
Material required	~pmol (6×10^{11} molecules)	≤fmol 6×10^{8} molecules
Time (data collection)	~10 hours	10 msec

Wikipedia

*An **enabling technology** is an invention or innovation, that can be applied to drive radical change in the capabilities of a user or culture. Enabling technologies are characterized by rapid development of subsequent derivative technologies, often in diverse fields.

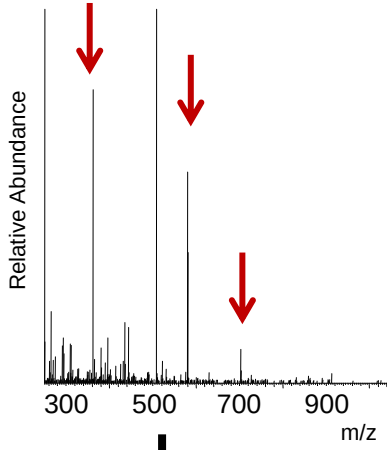
Equipment and/or methodology that, **alone or in combination** with associated technologies, provides the means to increase performance and capabilities of the user, product or process.

The mass of a single peptide provides sequence constraints but is not sufficient for identification



1. MS1 (MS) spectra measure intact peptide ions

MS



507.303

observed m/z, z=2

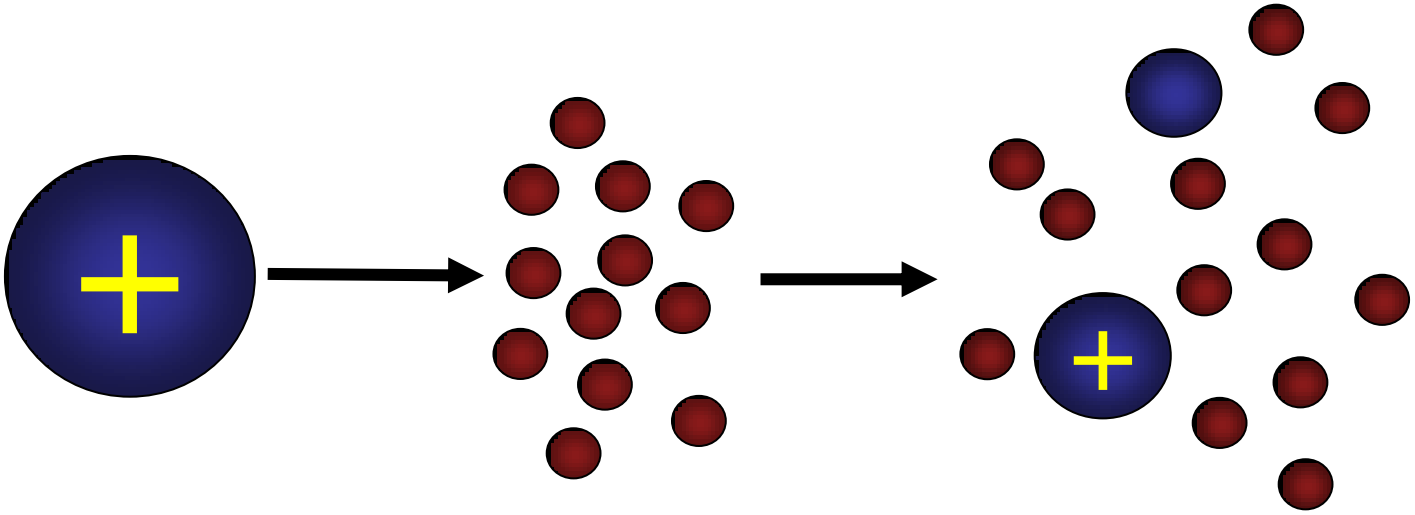
Predicted tryptic peptides in database

507.2962	K.RLNIVQDR.F
507.3031	K.ANELLINVK.Y
507.3031	K.IIAIDINNK.K
507.3031	R.VLNLPSVGSK.S
507.3088	R.LNVLSNVVR.K
507.3088	K.SPKSNKKPK.R
507.3213	K.AIILGAQSIK.C
...	

The precursor mass is not enough information for ID

Wilhelm Haas

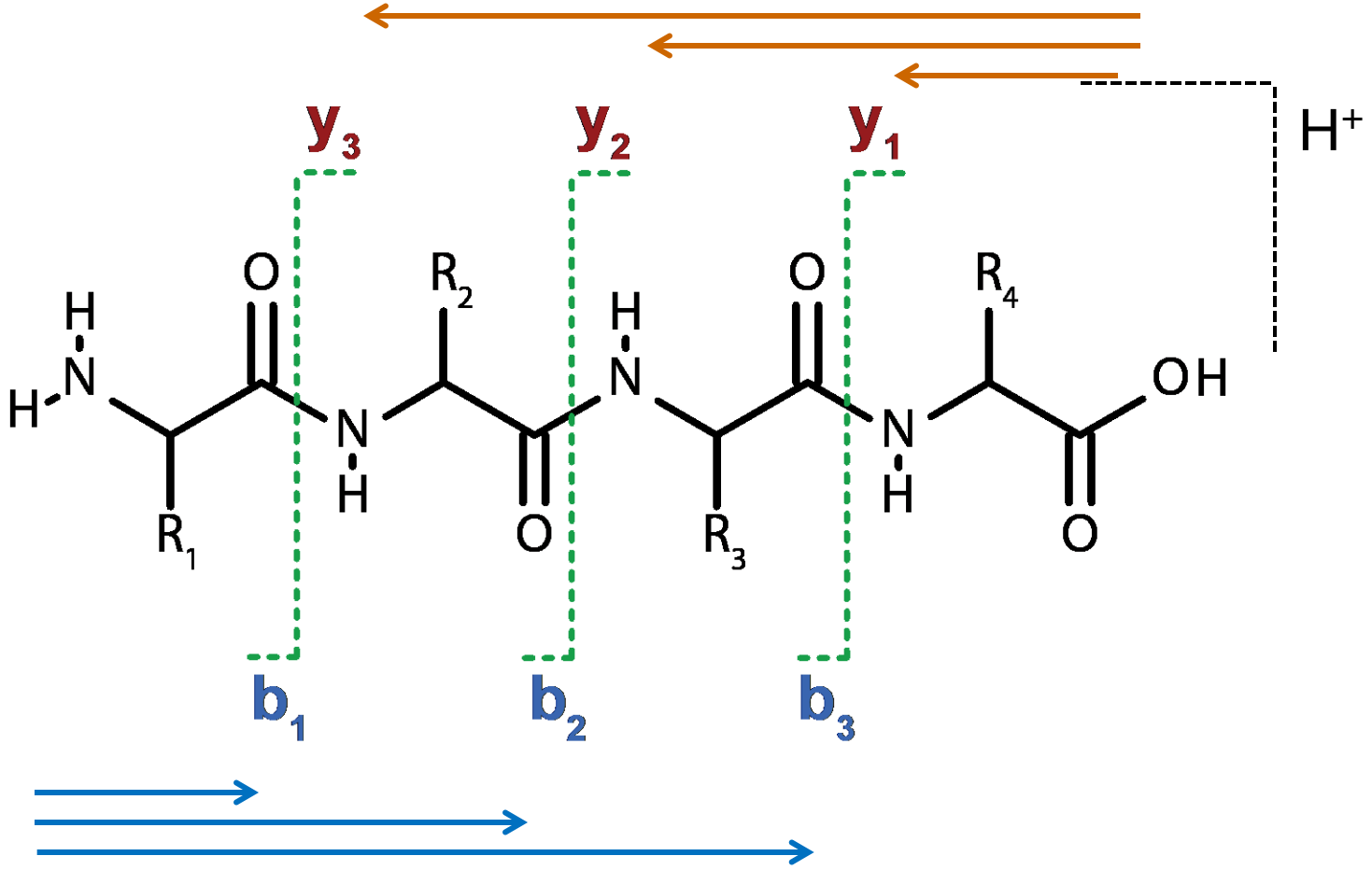
Collision-Induced Dissociation (CID)



- Kinetic energy of parent ions is increased
- Parent ions undergo energy converting collisions
- Parent ions fall apart into product ions and neutrals
- Also referred to collision-activated dissociation (CAD)

Nathan Yates

Typical CID fragmentation pattern of peptides

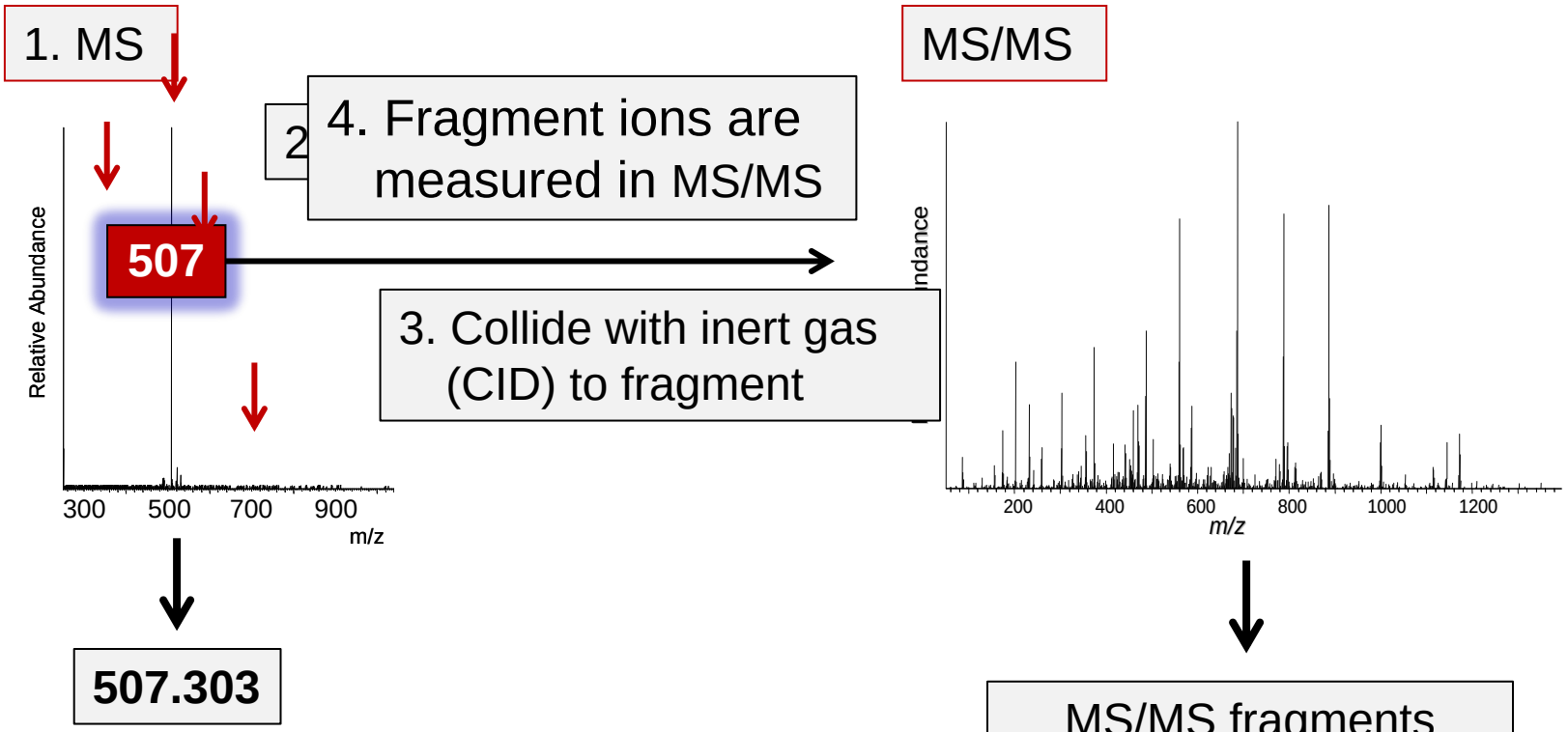


Combined residue mass for two amino acids

		Gly	Ala	Ser	Pro	Val	Thr	Cys	Lxx	Asn	Asp	Gln	Lys	Glu	Met	His	Phe	Arg	Cmc	Tyr	Trp
	AA	57	71	87	97	99	101	103	113	114	115	128	128	129	131	137	147	156	161	163	186
Gly	57	114																			
Ala	71	128	142																		
Ser	87	144	158	174																	
Pro	97	154	168	184	194																
Val	99	156	170	186	196	198															
Thr	101	158	172	188	198	200	202														
Cys	103	160	174	190	200	202	204	206													
Lxx	113	170	184	200	210	212	214	216	226												
Asn	114	171	185	201	211	213	215	217	227	228											
Asp	115	172	186	202	212	214	216	218	228	229	230										
Gln	128	185	199	215	225	227	229	231	241	242	243	256									
Lys	128	185	199	215	225	227	229	231	241	242	243	256	256								
Glu	129	186	200	216	226	228	230	232	242	243	244	257	257	258							
Met	131	188	202	218	228	230	232	234	244	245	246	259	259	260	262						
His	137	194	208	224	234	236	238	240	250	251	252	265	265	266	268	274					
Phe	147	204	218	234	244	246	248	250	260	261	262	275	275	276	278	284	294				
Arg	156	213	227	243	253	255	257	259	269	270	271	284	284	285	287	293	303	312			
Cmc	161	218	232	248	258	260	262	264	274	275	276	289	289	290	292	298	308	317	322		
Tyr	163	220	234	250	260	262	264	266	276	277	278	291	291	292	294	300	310	319	324	326	
Trp	186	243	257	273	283	285	287	289	299	300	301	314	314	315	317	323	333	342	347	349	372

Nathan Yates

Sequencing a peptide ion using tandem MS (MS/MS)



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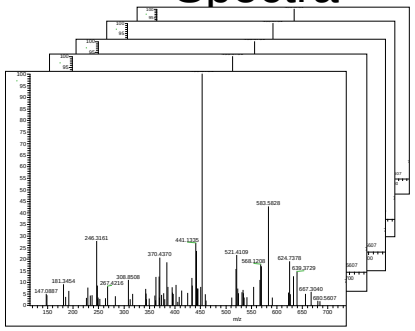
Sequencing a peptide ion using tandem MS (MS/MS)

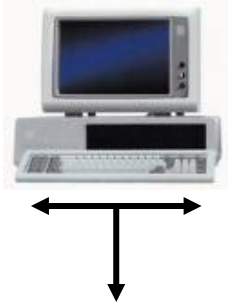
Mass Spec

Experimental Spectra



Precursor & fragment ions

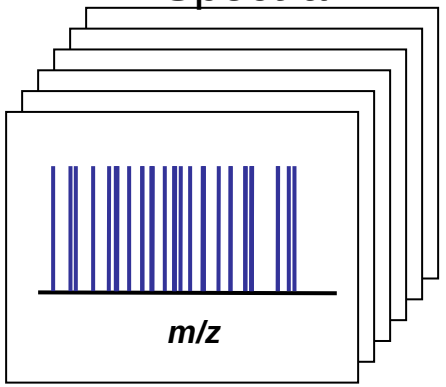
Search Engine



Peptide ID

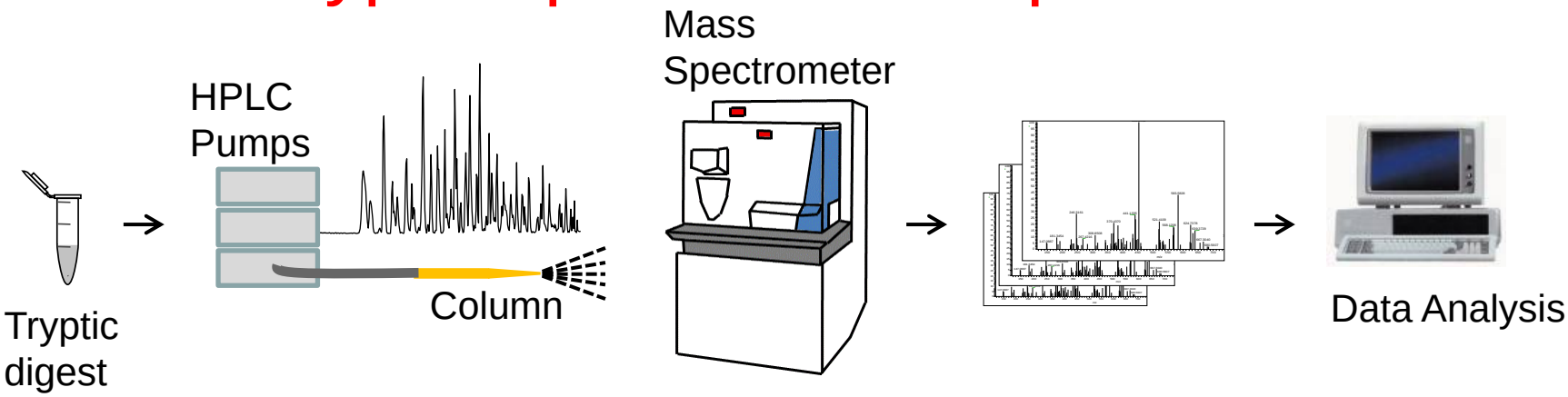
Database

Theoretical Spectra



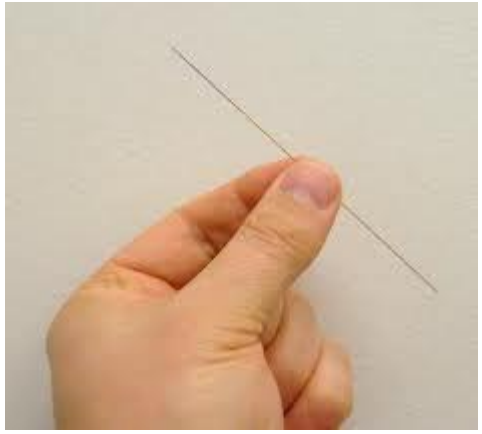
All rely on the database peptide sequences
(largely predicted from DNA and RNA sequences)

A typical proteomics experiment

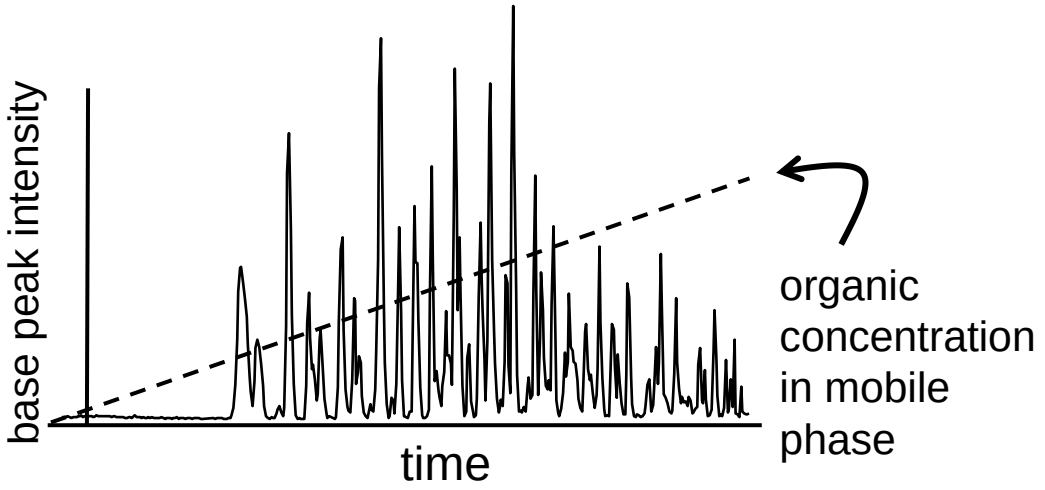


LC-MS/MS

Separate microgram quantities of peptides on a capillary C18 column



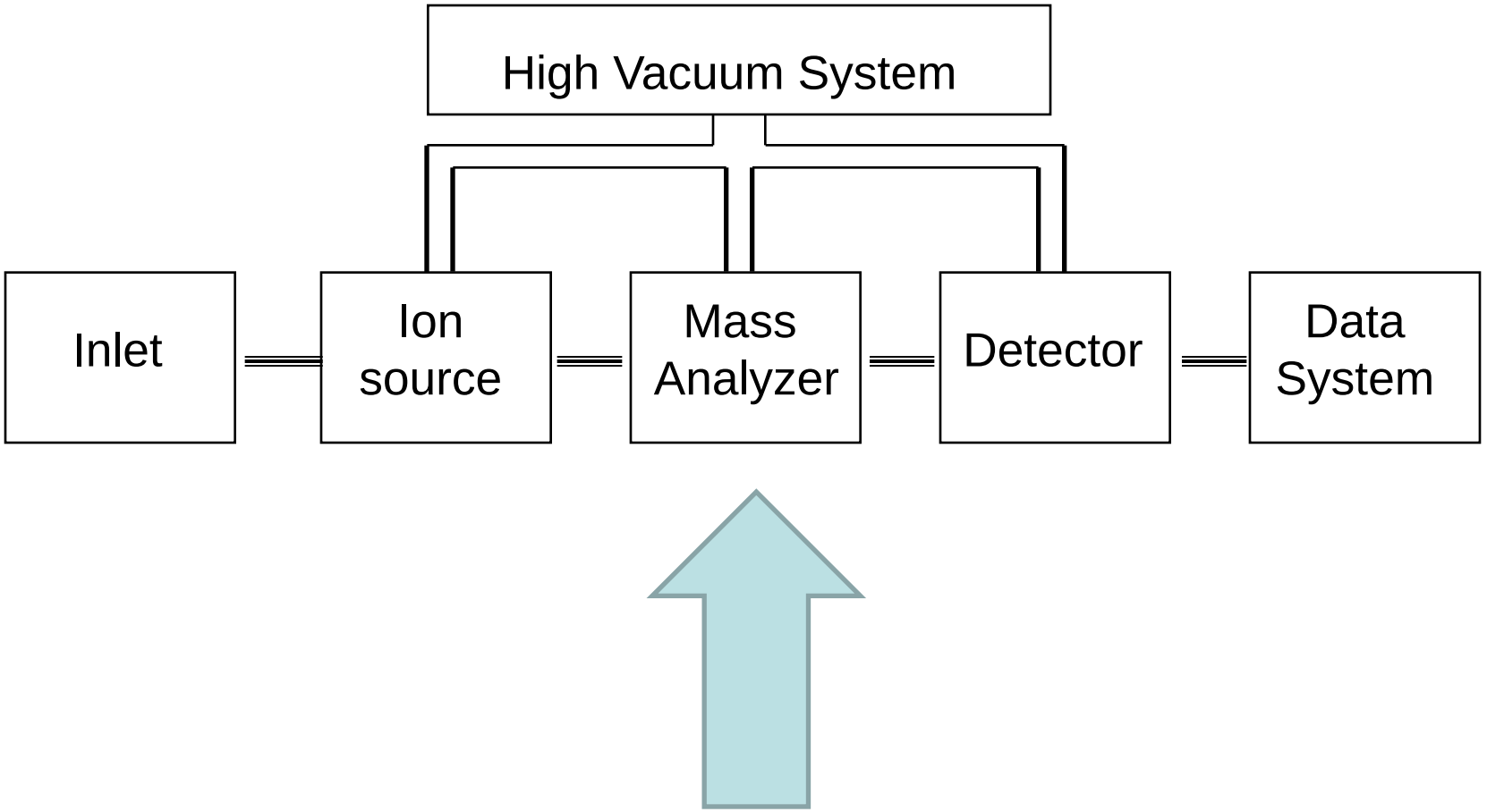
Column I.D. = 75 µm



We can identify 10,000 peptides in a 90 min run

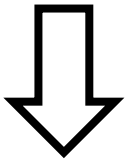
Wilhelm Haas

How do mass spectrometers work?



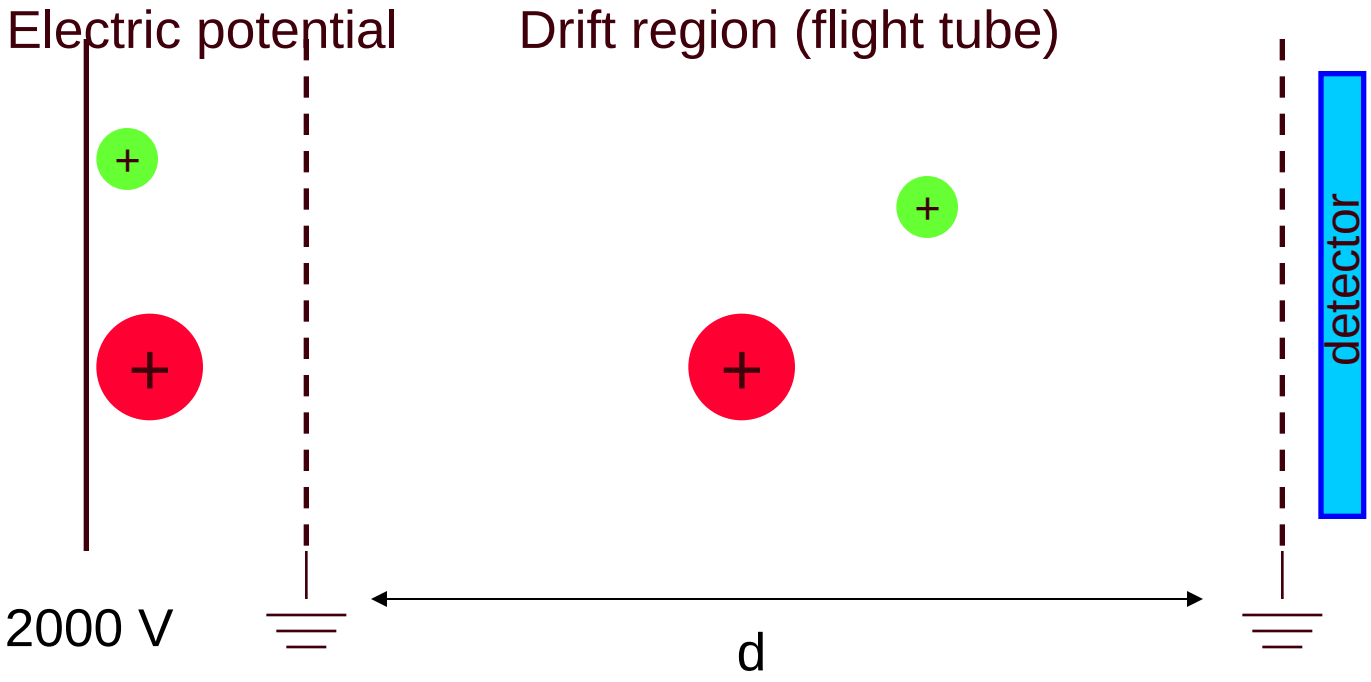
Mass analyzers use forces to manipulate ions

$$\bar{F} = m\bar{a} = m\frac{d\bar{v}}{dt} = z(\bar{E} + \bar{v} \times \bar{B})$$



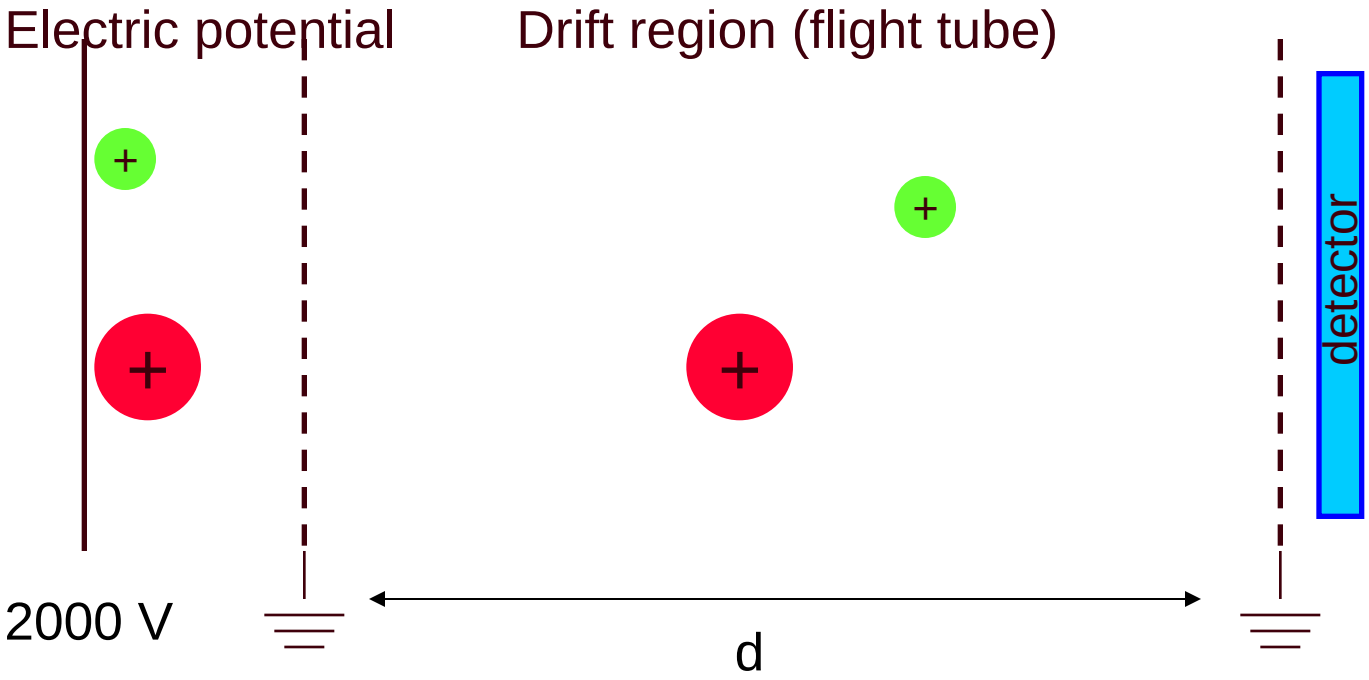
$$\frac{m}{z}\frac{d\bar{v}}{dt} = \bar{E} + \bar{v} \times \bar{B}$$

Time-of-flight (TOF) mass analyzer



- Ions are formed in pulses.
- The drift region is field free.
- Measures the time for ions to reach the detector.
- Small ions reach the detector before large ones.

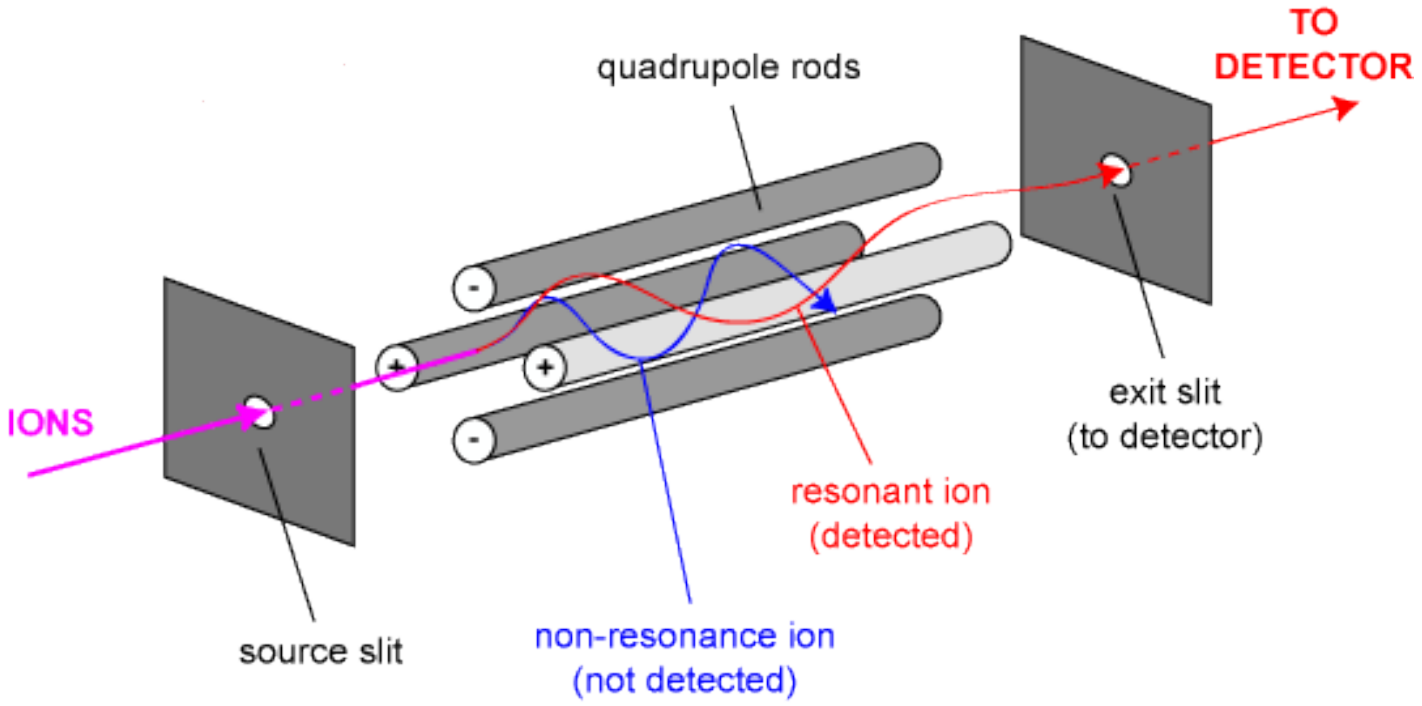
Time-of-flight (TOF) mass analyzer



- Energy uptake is $E_{el}=qV=ezV$ where e=charge/electron
- Conversion of potential energy to kinetic energy. $ezV = ½ mv^2$
- The drift region is field free. Known distance, d.
- Measures the time for ions to reach the detector. $v=d/t$
  - $m/z=2eVv^2=2eVd^2/t^2$

Quadrupole mass analyzer

Oscillating electric fields, operates as a mass filter.



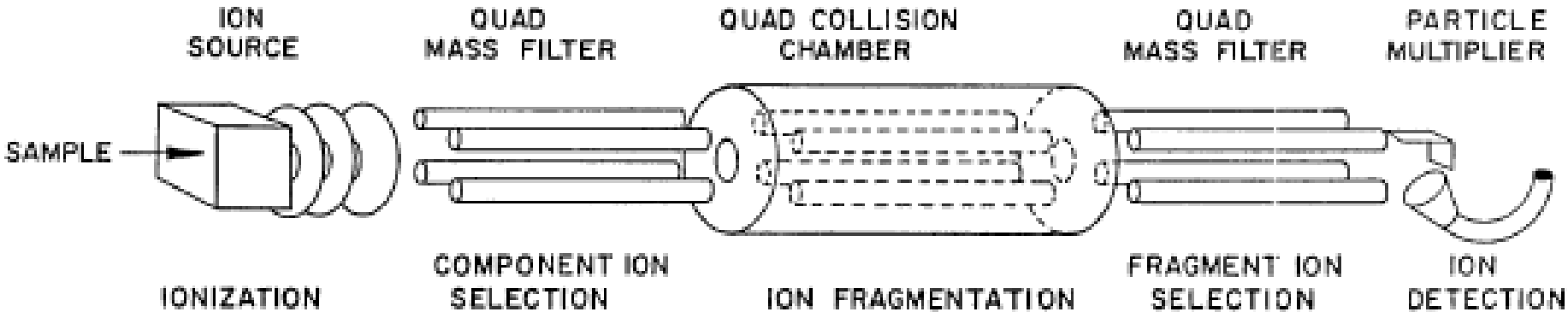
- Has four parallel metal rods.
- Lets one mass pass through at a time.
- Can scan through all masses or sit at one fixed mass.

Quadrupoles have variable ion transmission modes

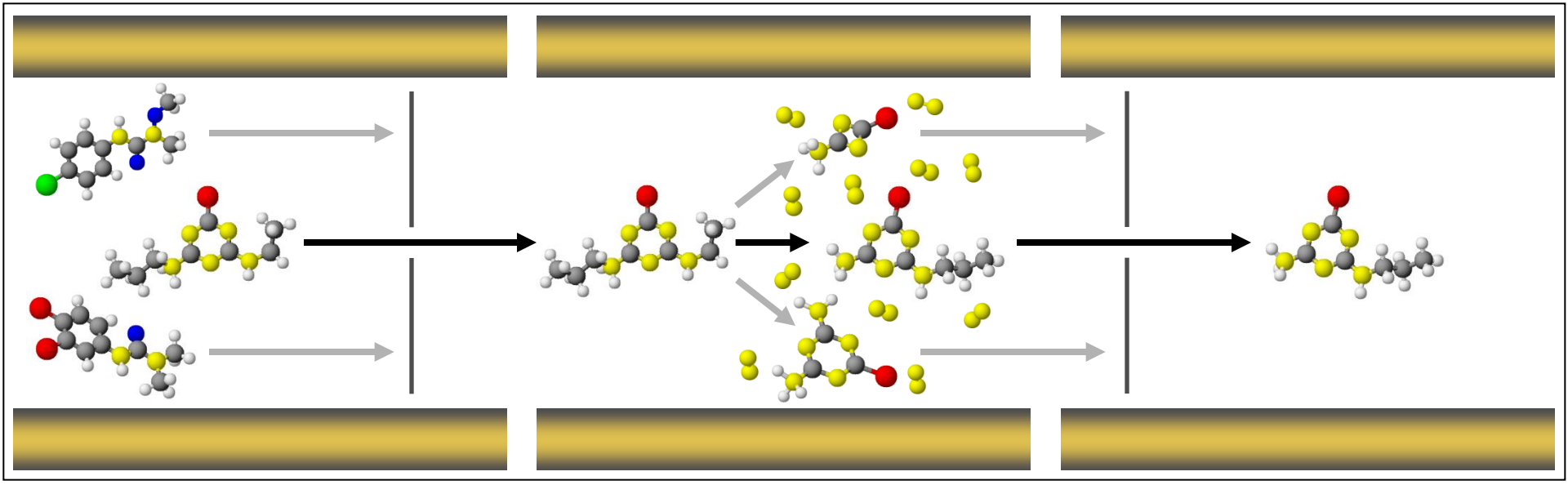
mass scanning mode
(let different m/z through as function of time, collect mass spectrum)

single mass transmission mode
let single m/z through, measure intensity

Triple quadrupole MS (tandem in space)



Selected Reaction Monitoring (SRM) on a triple quadrupole mass spectrometer

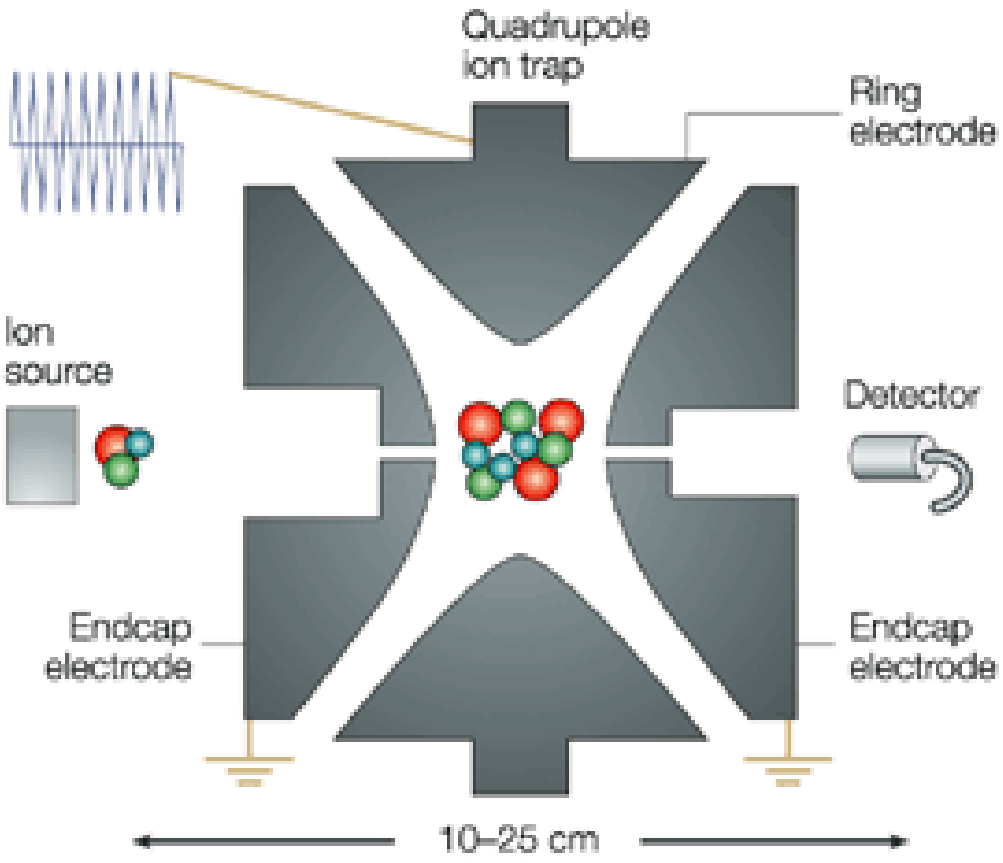


Select
precursor ion in Q1

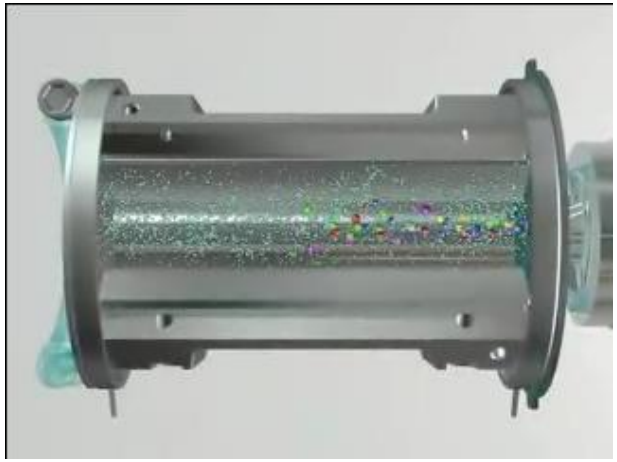
Fragment
precursor ion in Q2
(Collision cell containing inert gas)

Select
product ion in Q3

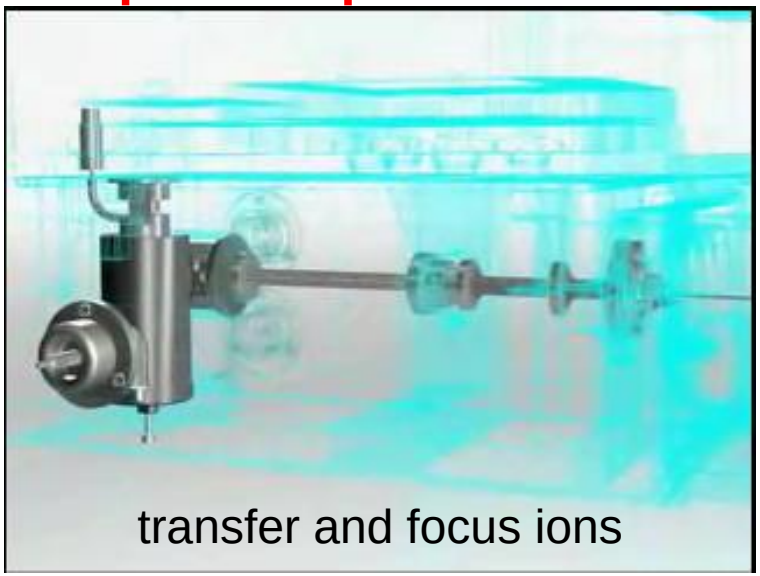
Ion Trap Mass Spectrometer



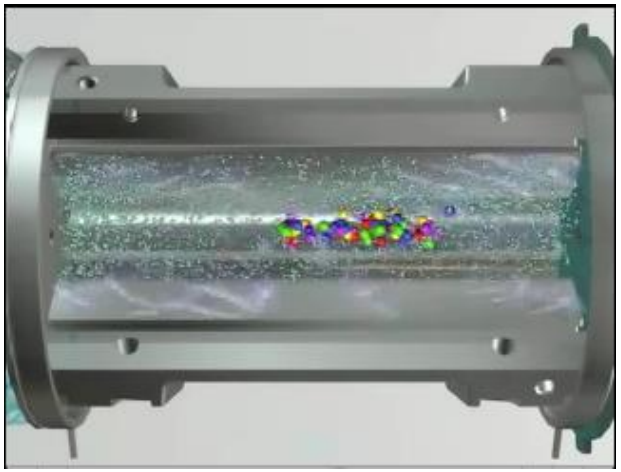
Ion Trap MS process



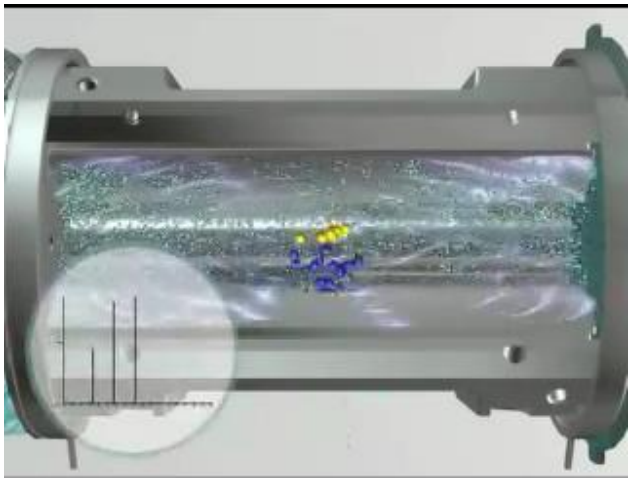
accumulate ions



transfer and focus ions

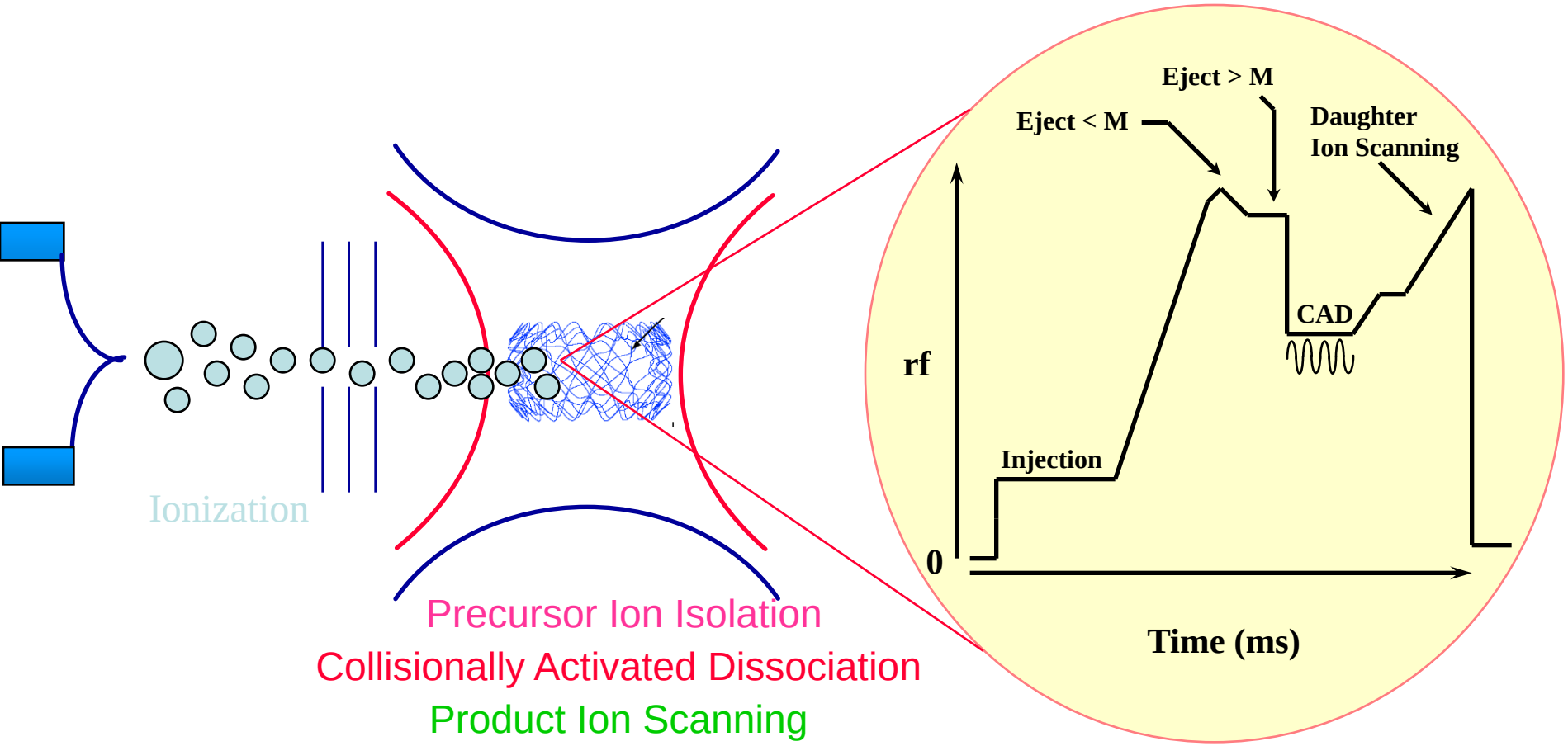


compress ion cloud in center



apply resonance voltage
to eject ions selectively

MS/MS on an ion trap mass spectrometer
Tandem-in-Time (allows MSn)

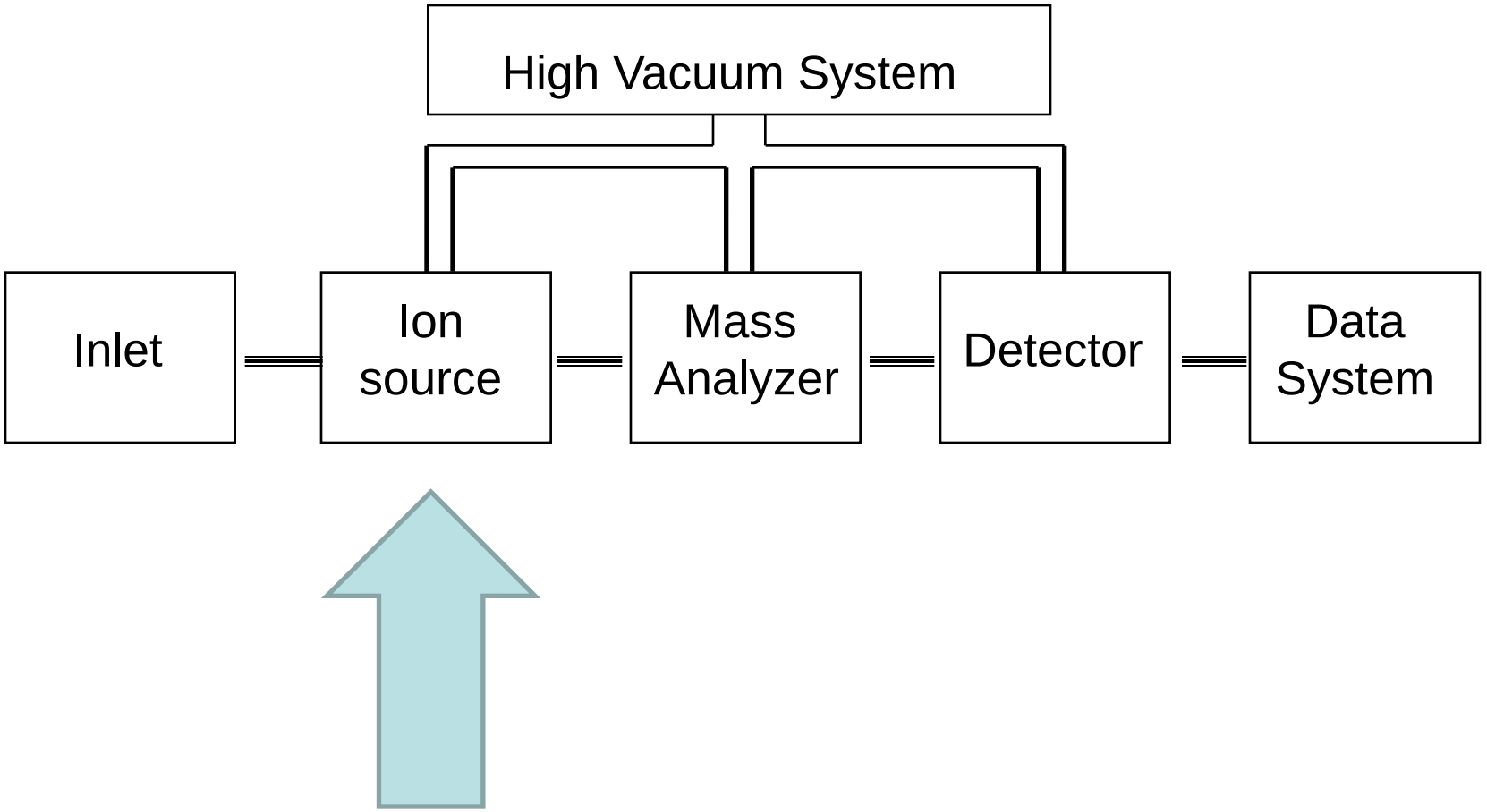


Precursor Ion Isolation

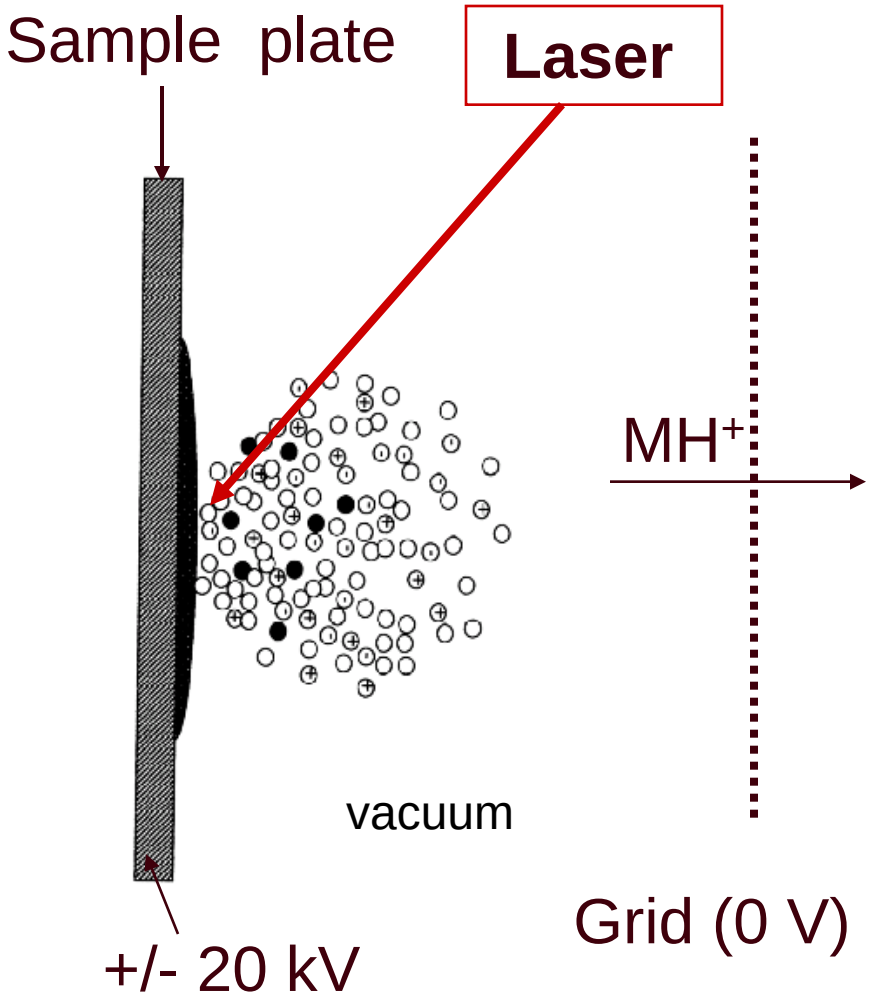
Collisionally Activated Dissociation

Product Ion Scanning

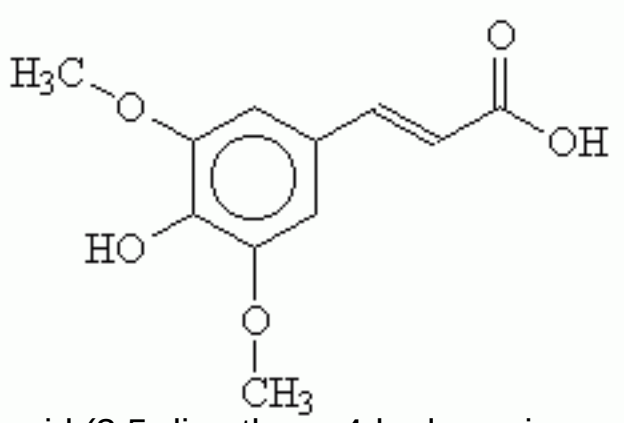
How do mass spectrometers work?



Matrix Assisted Laser Desorption/Ionization (MALDI)



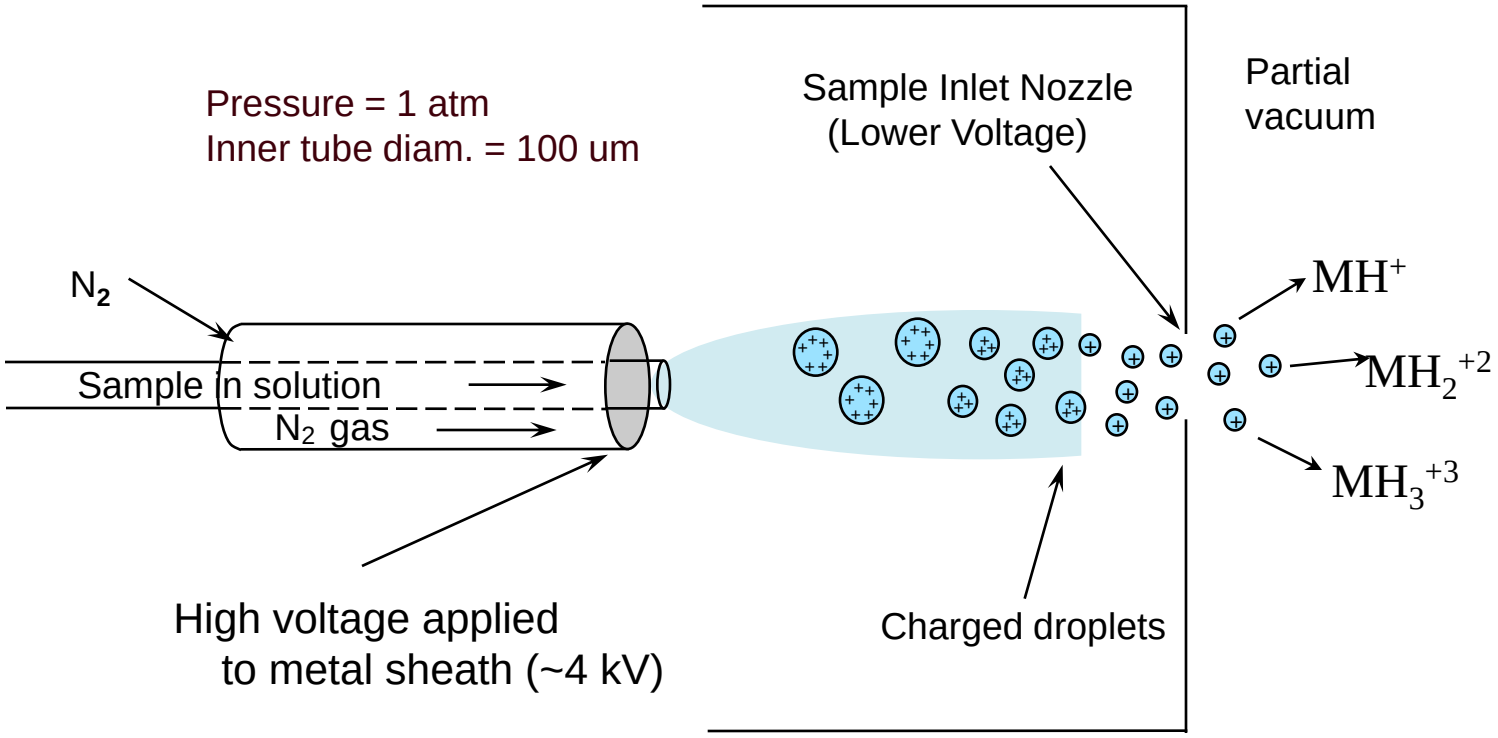
1. Sample is mixed with matrix (X) and dried on plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules (M) are ionized by proton transfer:
   $XH^+ + M \rightarrow MH^+ + X$



Sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid)
MH+ 225, 207
Frequently used MALDI Matrix
for proteins, large peptides

Electrospray Ionization (ESI)

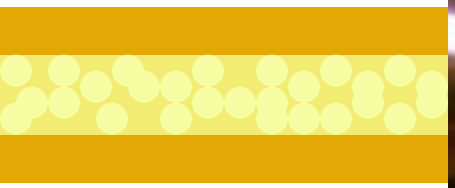
Frequently used with liquid chromatography (LC-MS)



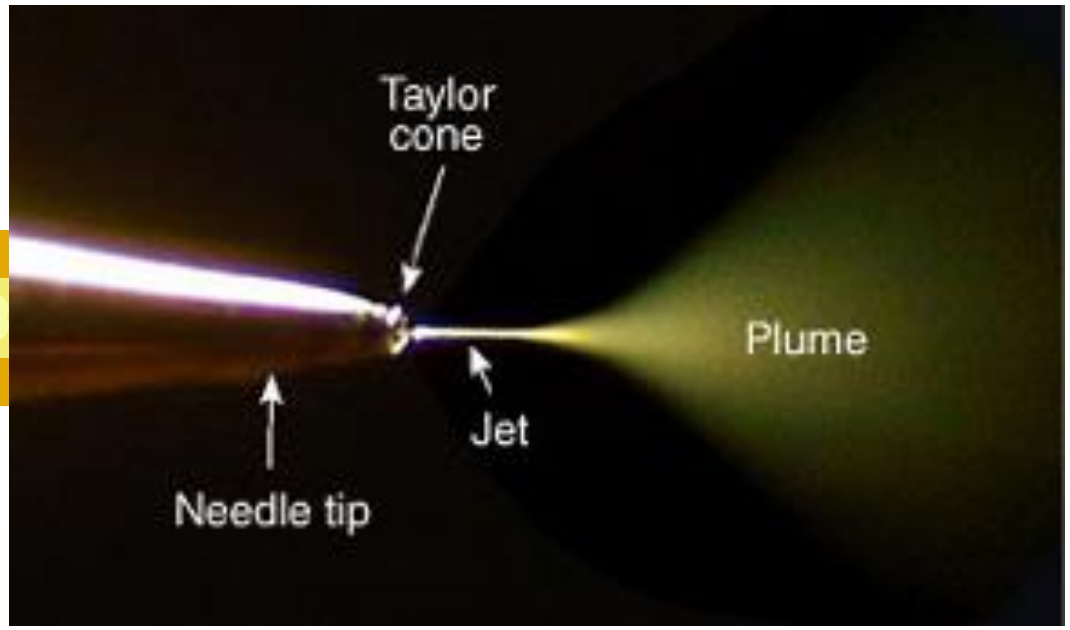
Electrospray Ionization (ESI)

Frequently used with liquid chromatography (LC-MS)

From µLC

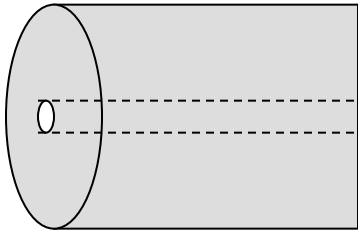


Electrospray Ionization



Protonated Molecules

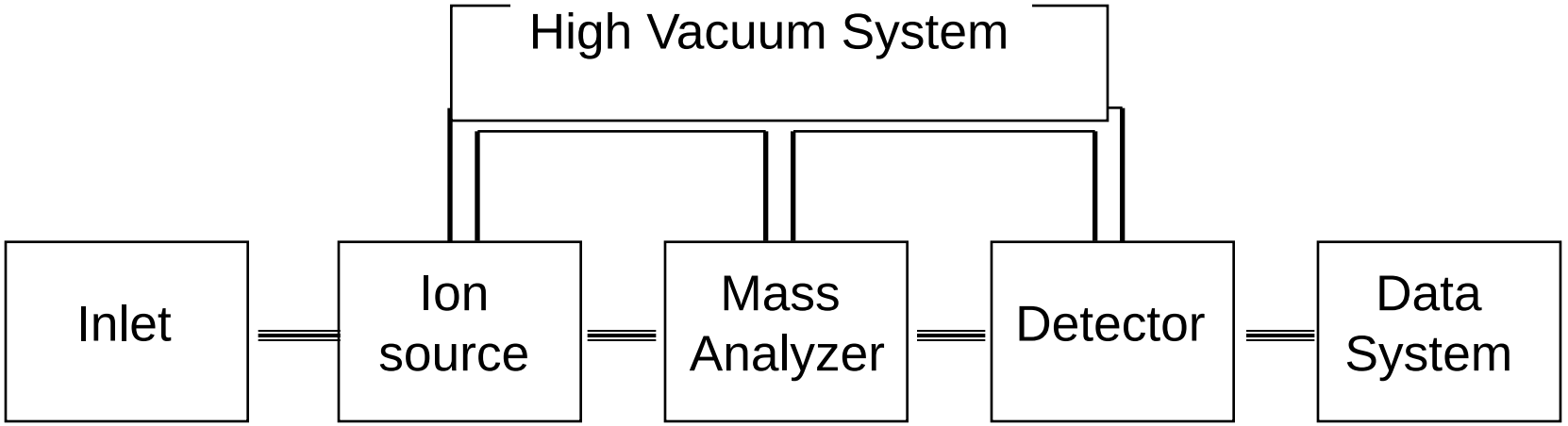
$(M+H)^+$



To MS

Nanospray (typically ~0.2 µL/min) do not use sheath gas.

Mass spectrometers are frequently named based on their ion source & mass analyzer



MALDI
Electrospray

TOF
Quadrupole (Q)
Ion trap
Orbitrap (orbi)
Hybrid: TOF-TOF
QQQ
Q-TOF
Q-Orbi
Ion trap-Orbi

Mass Spectra

- Mass units
- Isotopes
  - the good, the bad & the ugly

How is mass defined?

- Numerical value to the intrinsic property of “mass” is based in reference to the most abundant isotope of carbon, ^{12}C (6 protons & 6 neutrons).
- One unit of mass is defined as a Dalton (Da).
- A Da is defined as 1/12 the mass of one ^{12}C atom.
- Thus, one ^{12}C atom has a mass of 12.0000… Da.

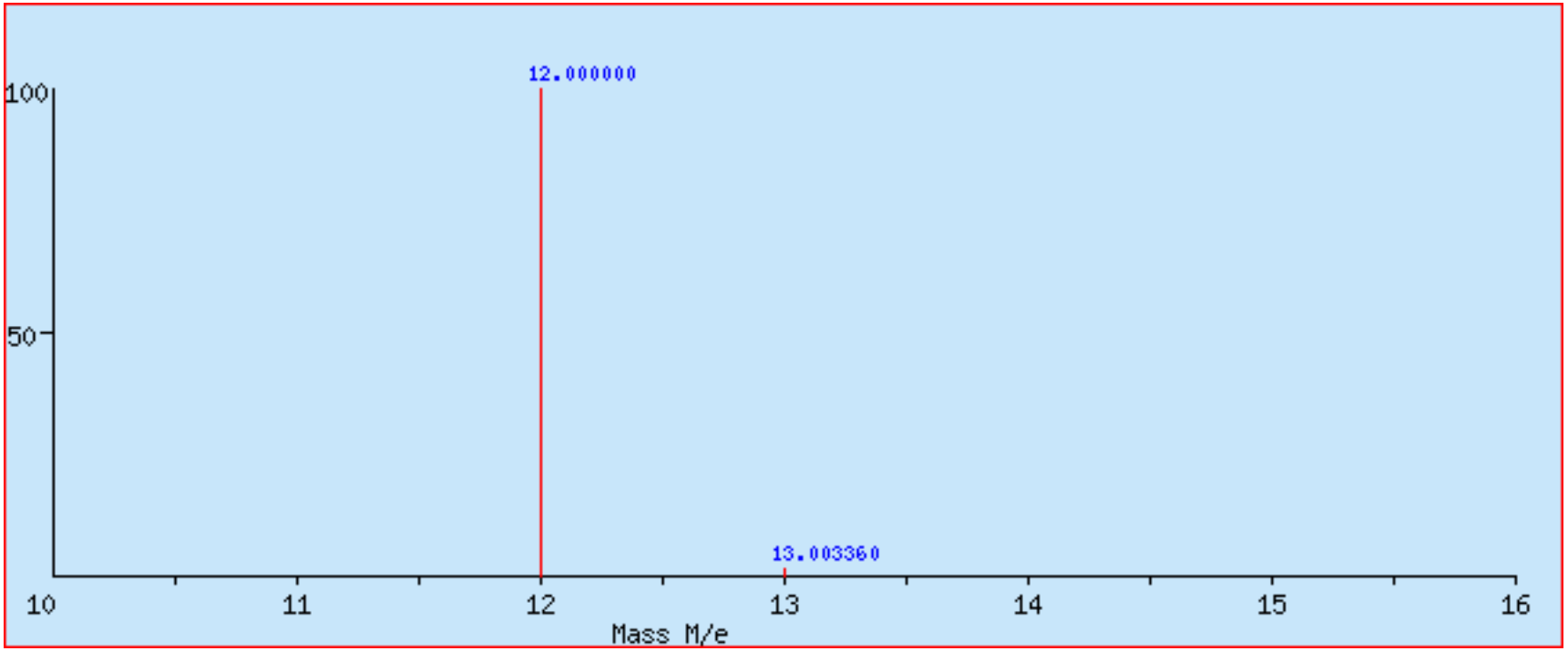
Most elements have >1 stable isotope

For example, most carbon atoms have a mass of 12 Da, but in nature, 1.11% of C atoms have an extra neutron, making their mass 13 Da.

Isotope composition of molecules depends on molecular formula and isotope distribution of component atoms (binomial distribution).

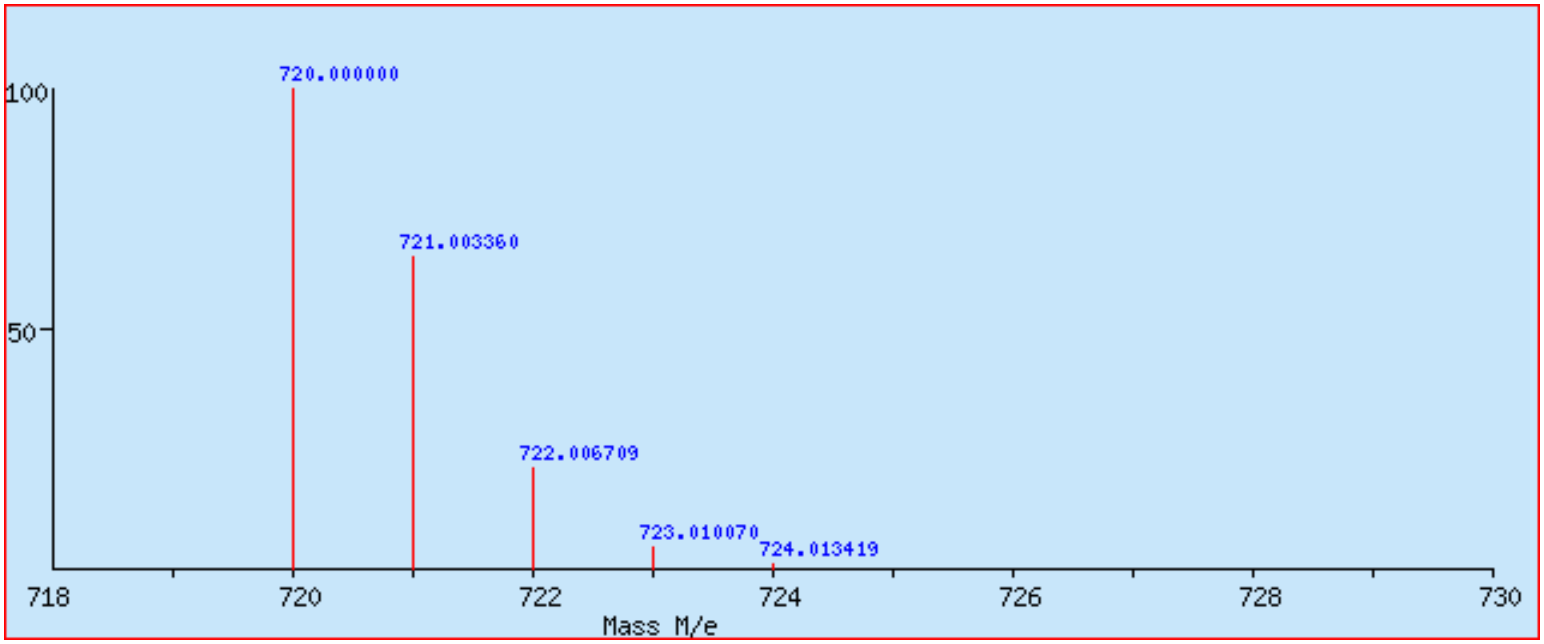
Element	**Isotope**	**Abundance**
Hydrogen	1H	99.985
	2H	0.015
Carbon	12C	98.890
	13C	1.110
Nitrogen	14N	99.630
	15N	0.370
Oxygen	16O	99.759
	17O	0.037
	18O	0.204

Isotope pattern $(C_1)^{+1}$



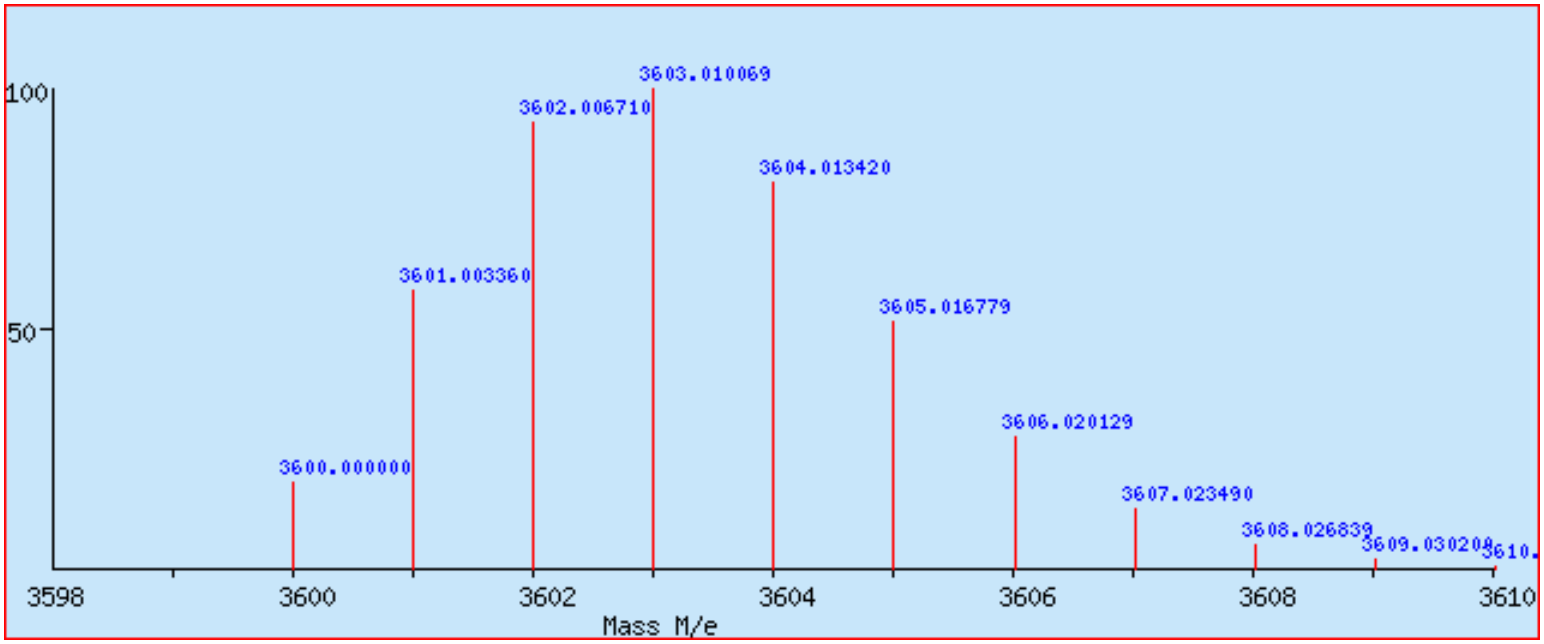
Monoisotopic peak, $^{12}C_1$~ 99% of total $(0.989)^1$

Isotope Pattern $(C_{60})^{+1}$



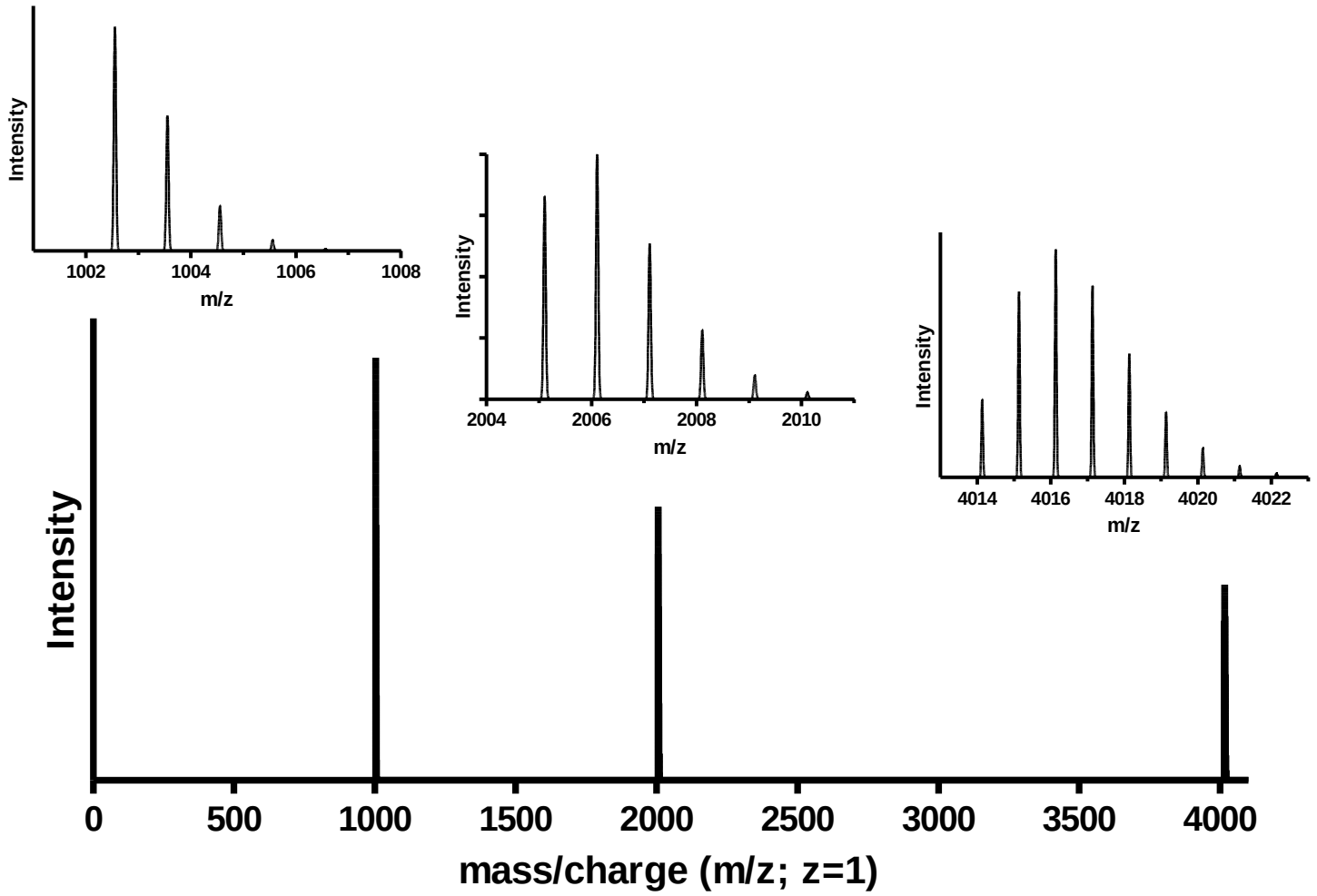
Monoisotopic peak, $^{12}C_{60}$~ 51% of total $(0.989)^{60}$

Isotope Pattern $(C_{300})^{+1}$

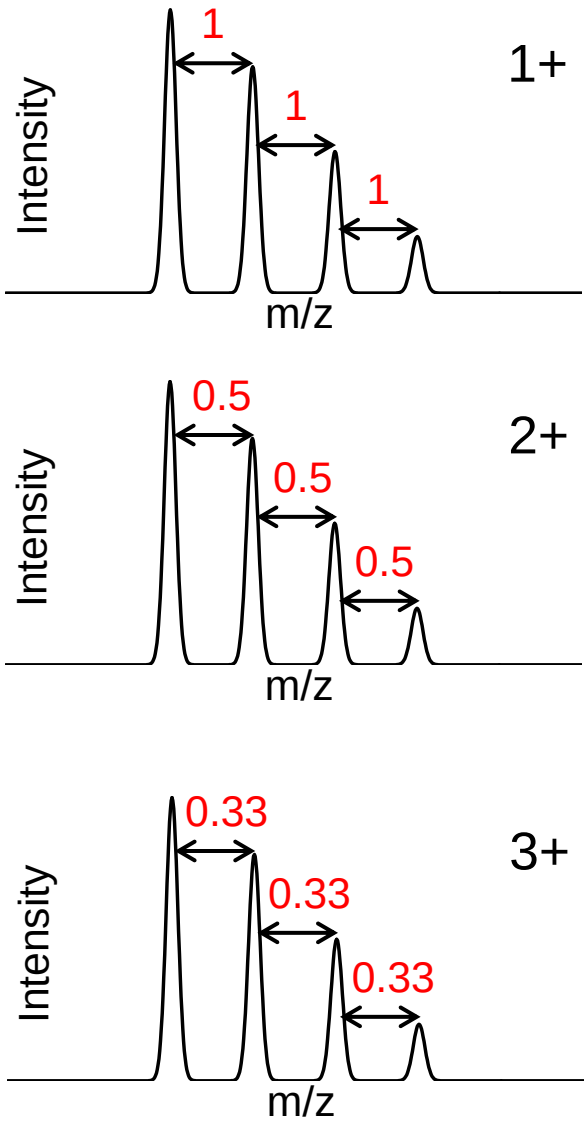


Monoisotopic peak, $^{12}C_{300}$~ 4% of total $(0.989)^{300}$

Effect of isotope abundance on mass measurements



Isotope clusters allow charge state determination



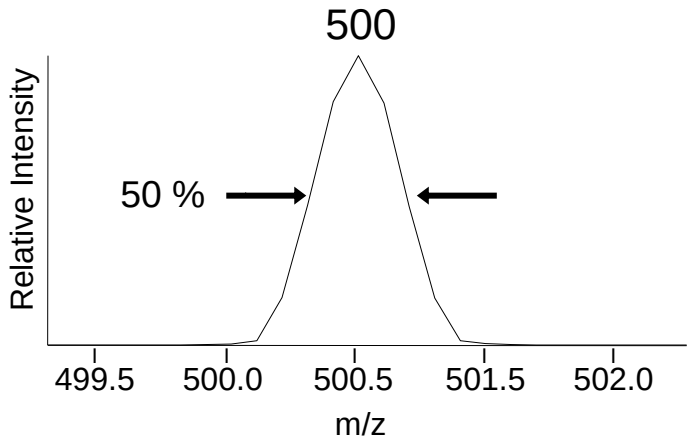
David Fenyö

Resolution

$$R = \frac{M}{\Delta M} = \text{resolving power}$$

Resolution = minimum peak separation, ΔM,
which allows to distinguish two ion species

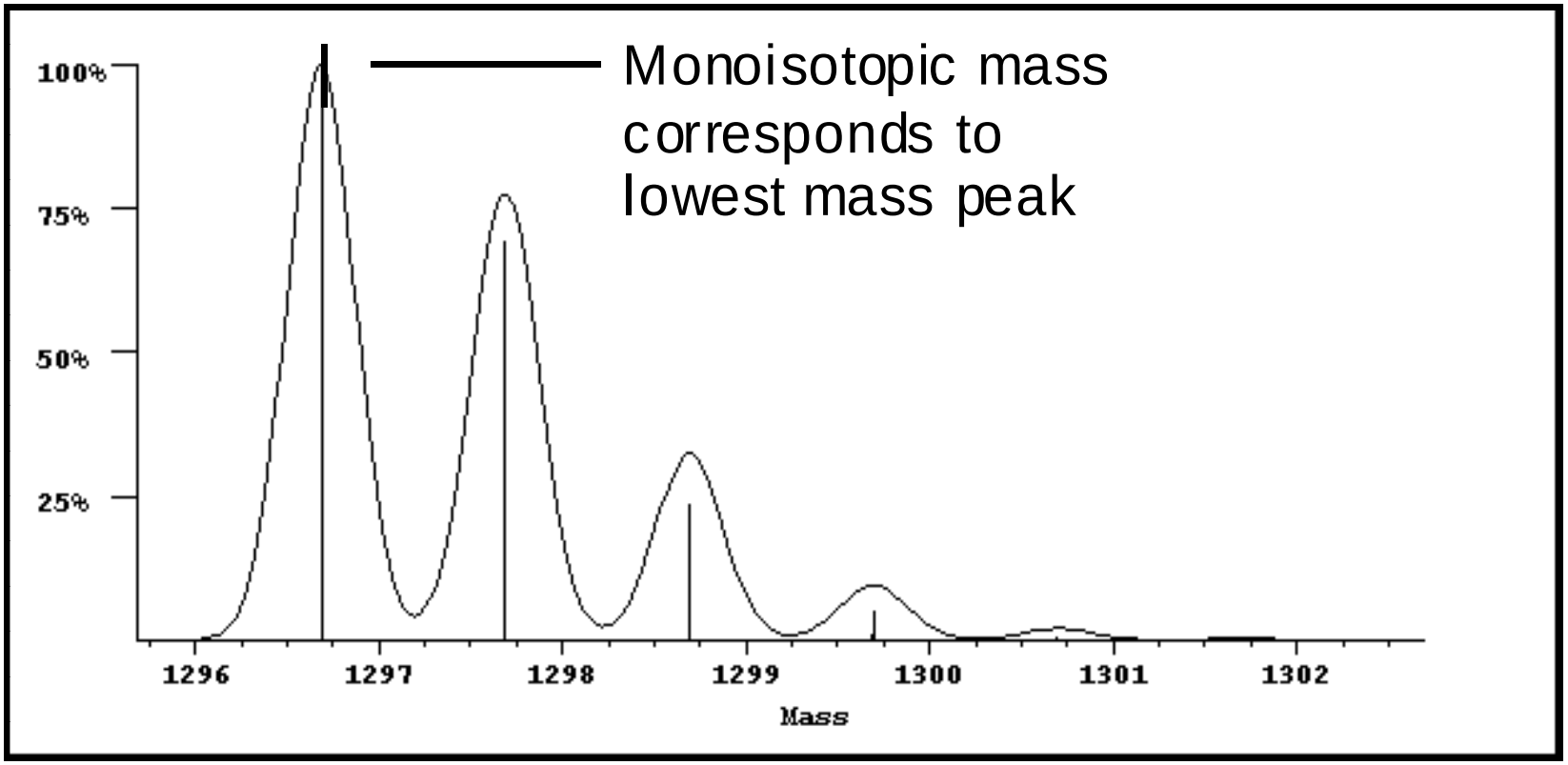
ΔM = full width at half maximum (FWHM)



Resolution = $M/\Delta M = 500/0.5 = 1000$

David Fenyö

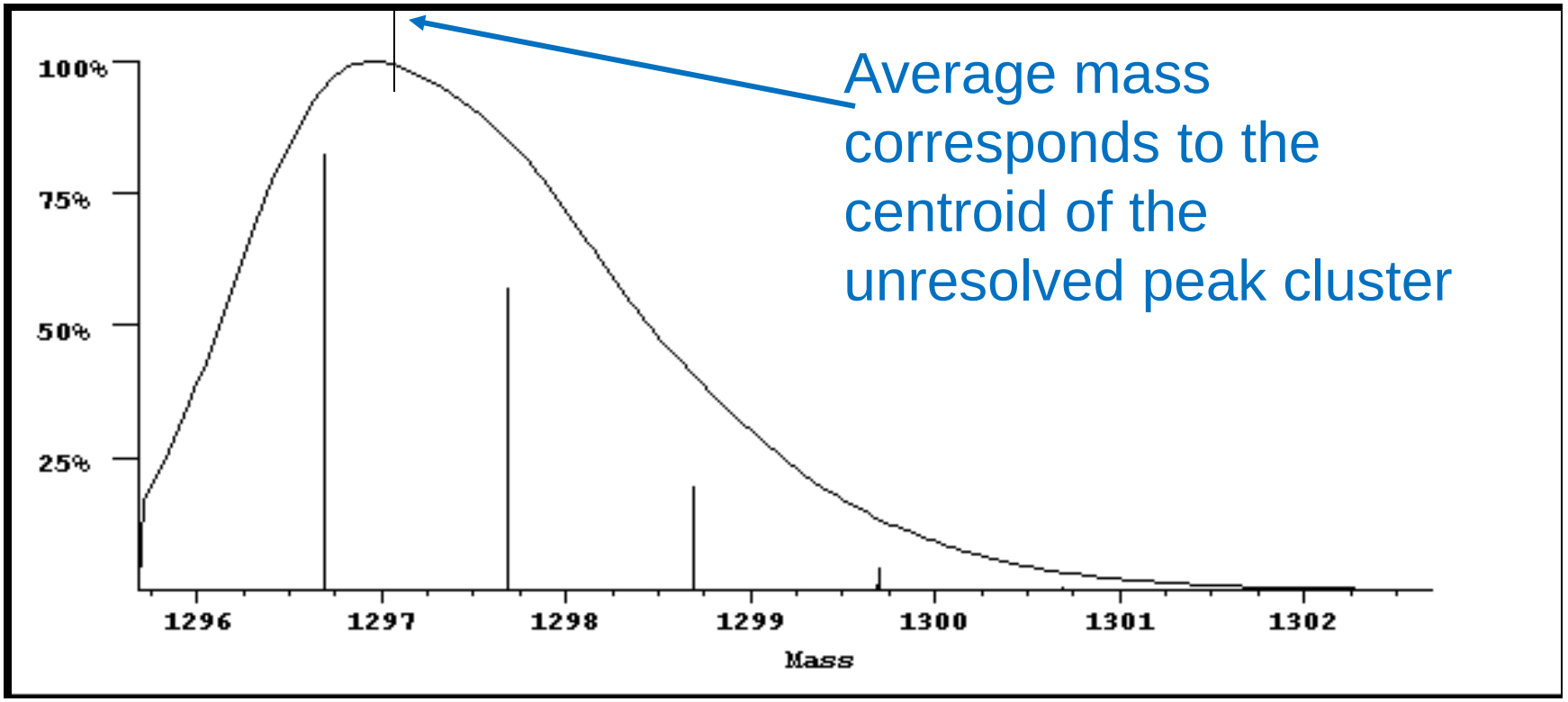
Monoisotopic mass



When the isotopes are clearly resolved the **monoisotopic mass** is used as it is the most accurate measurement.

Spacing of adjacent isotope peaks ($\Delta m/z$, measured) gives z as know Δm)

Average mass



When the isotopes are not resolved, the centroid of the envelope corresponds to the weighted average of all the isotope peaks in the cluster, which is the same as the average or chemical mass.

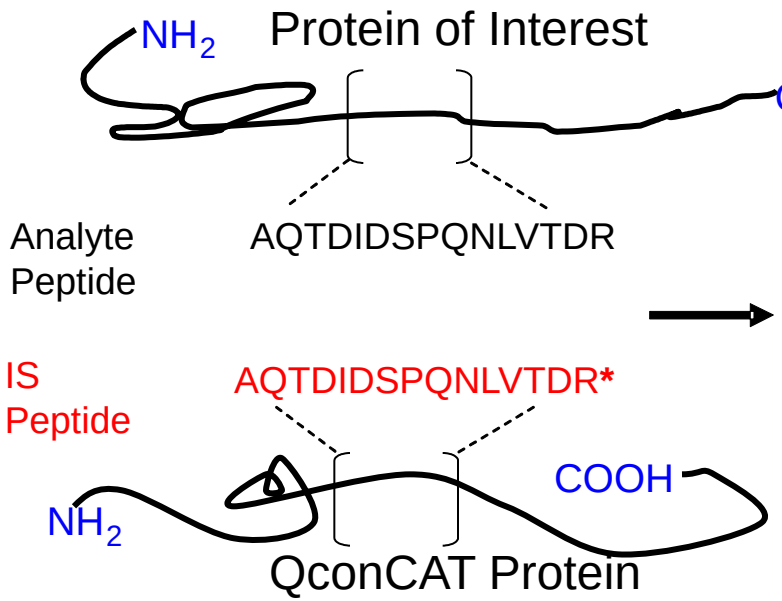
Why we typically analyze peptides instead of intact proteins

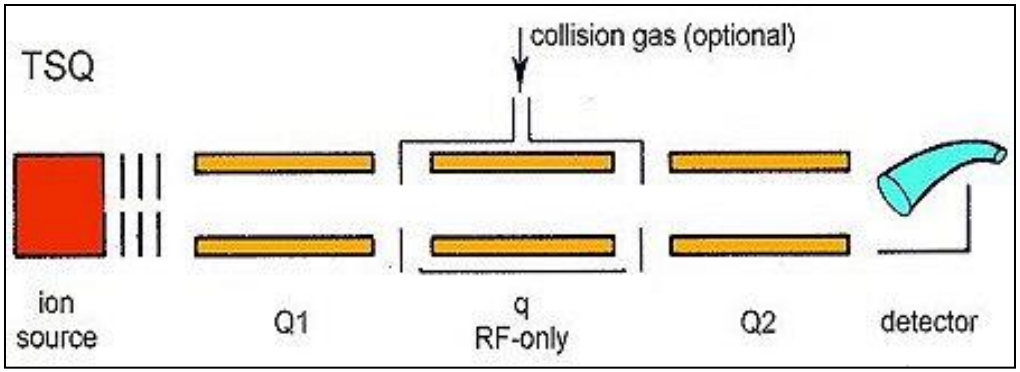
- Protein heterogeneity:
  - proteolytic processing
  - post translational modifications
- Stability in gas phase
- Natural isotope abundance
- Better resolution and accuracy at lower masses

Quantitative proteomics

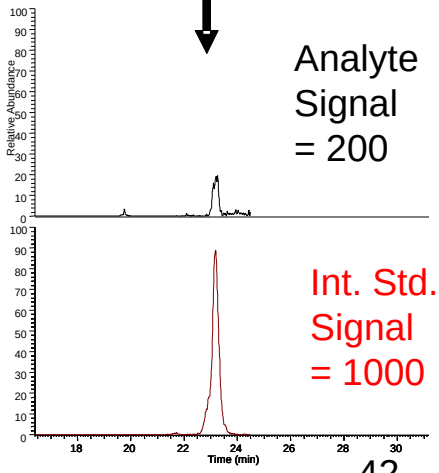
- Label-free
- Stable isotope labeled (^{13}C,^{15}N, and/or ^{18}O)
  - Metabolic labeling
  - Synthetic peptides and proteins
  - Chemical modification

Quantification of proteins by targeted MS/MS using internal standards





QQQ



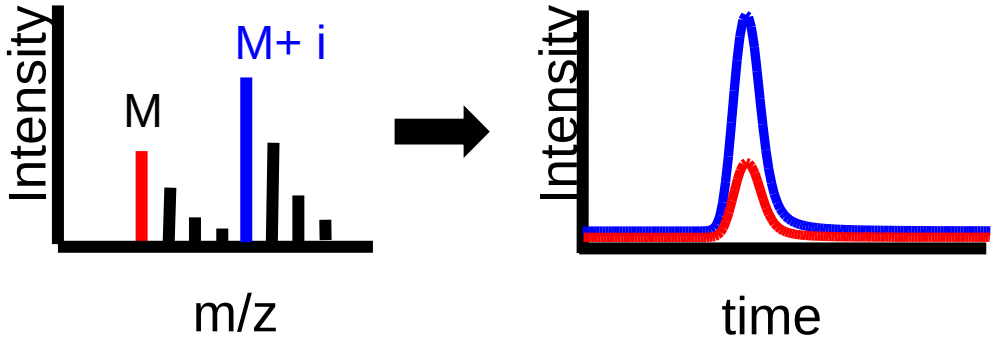
LC-SRM

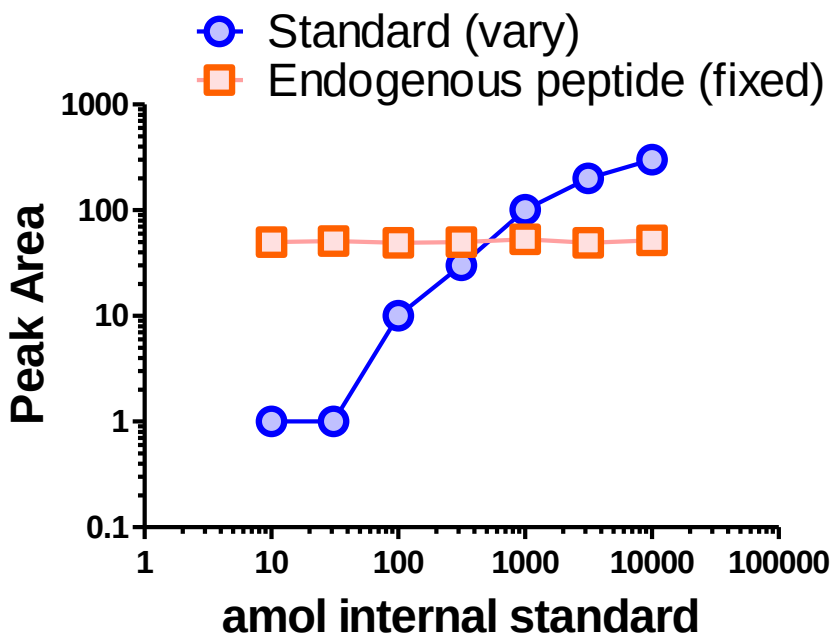
QQQ Detection: Selected Reaction Monitoring provides the high sensitivity and amino acid selectivity needed to detect peptides in complex mixtures

Quantification: Peptide concentrations are obtained by multiplying the internal standard concentration by the signal ratio of the analyte / internal standard

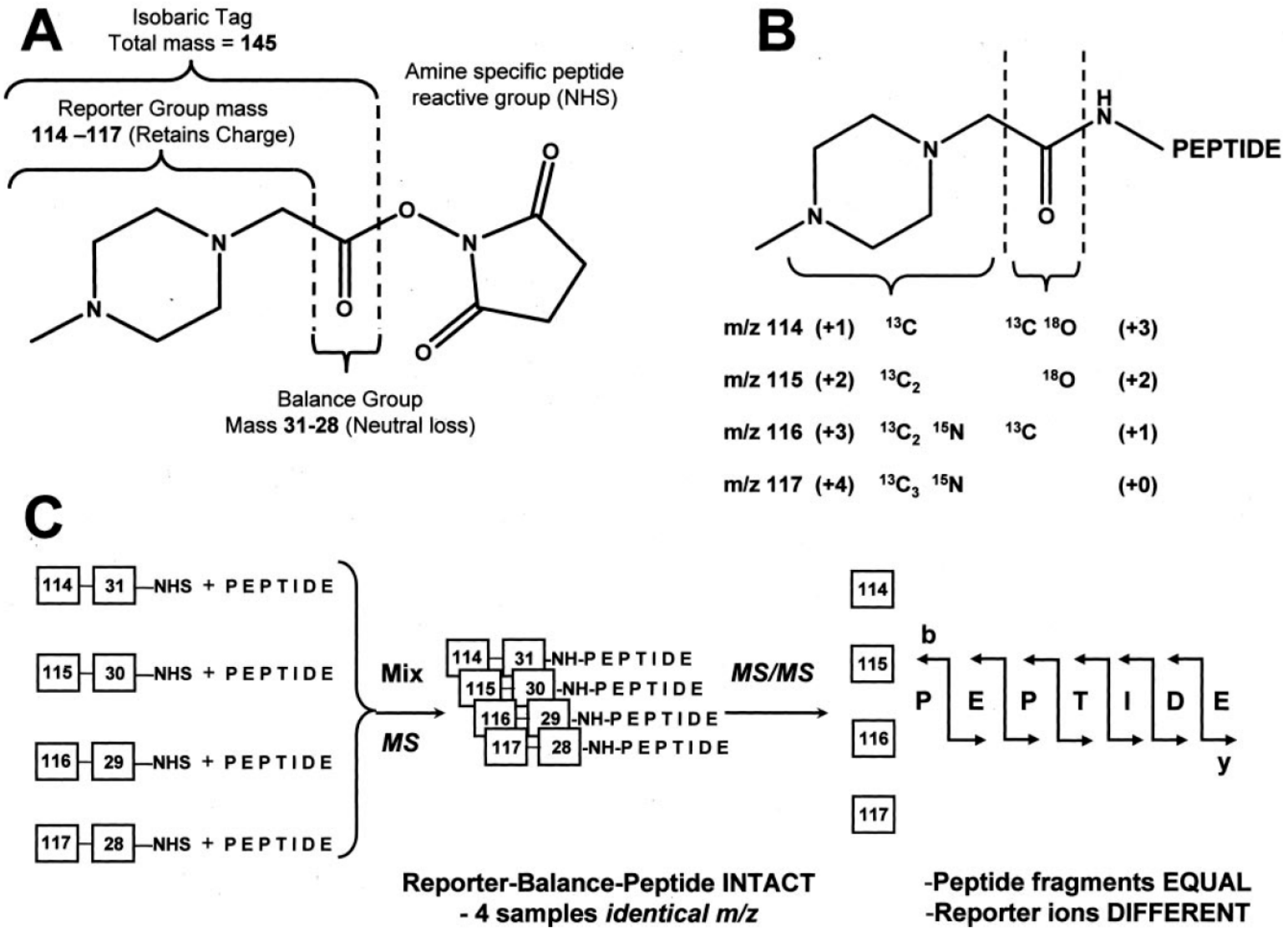
Quantitative analysis using an internal standard

Sample (m/z = M) Spiked with
Internal Standard (m/z = M+i)

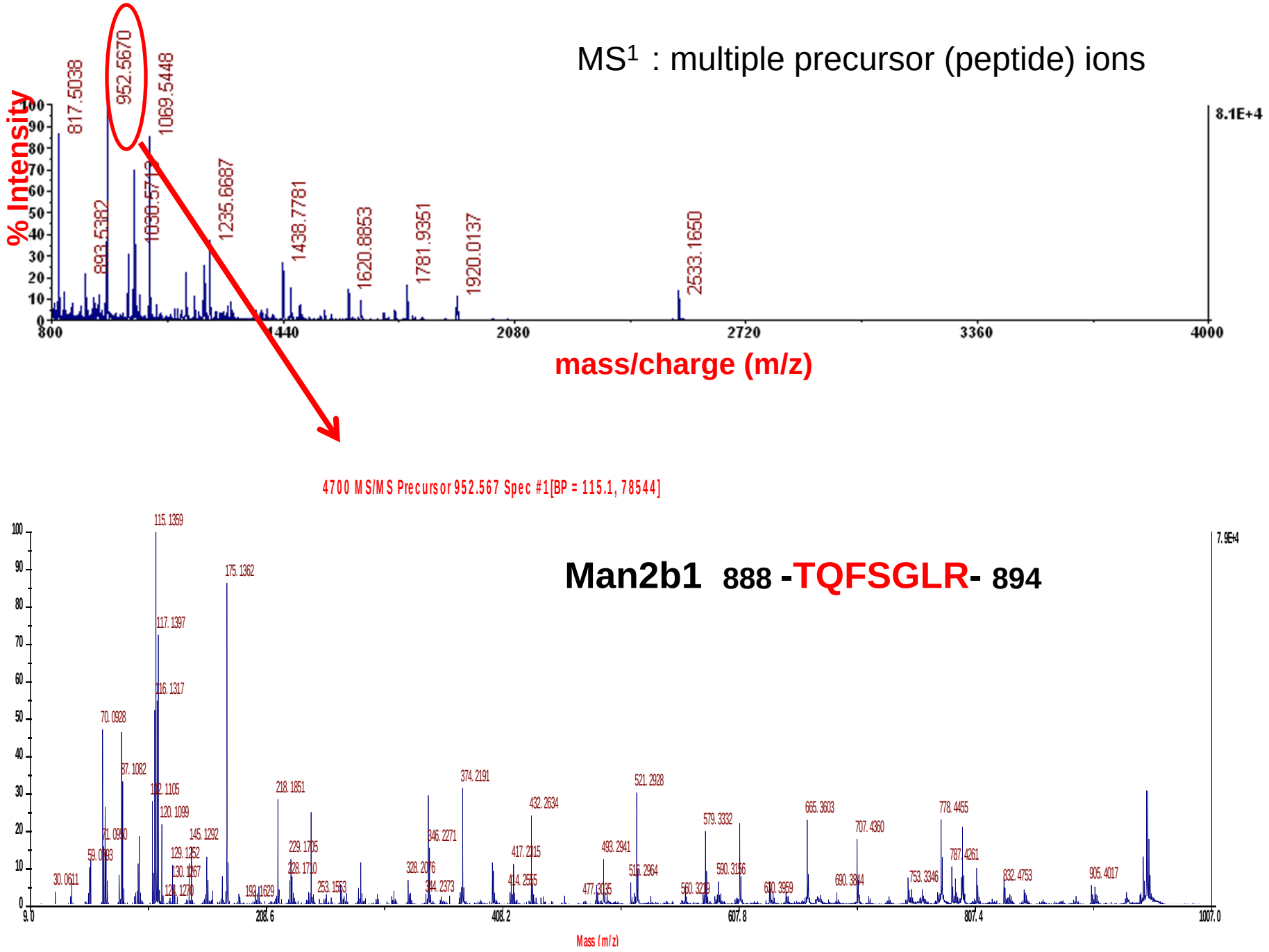


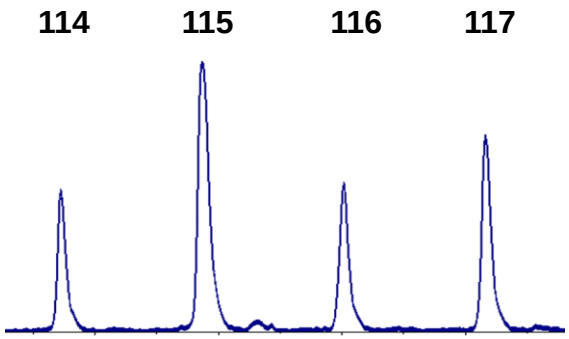


Quantitiatve Proteomics: isobaric labeling reagents

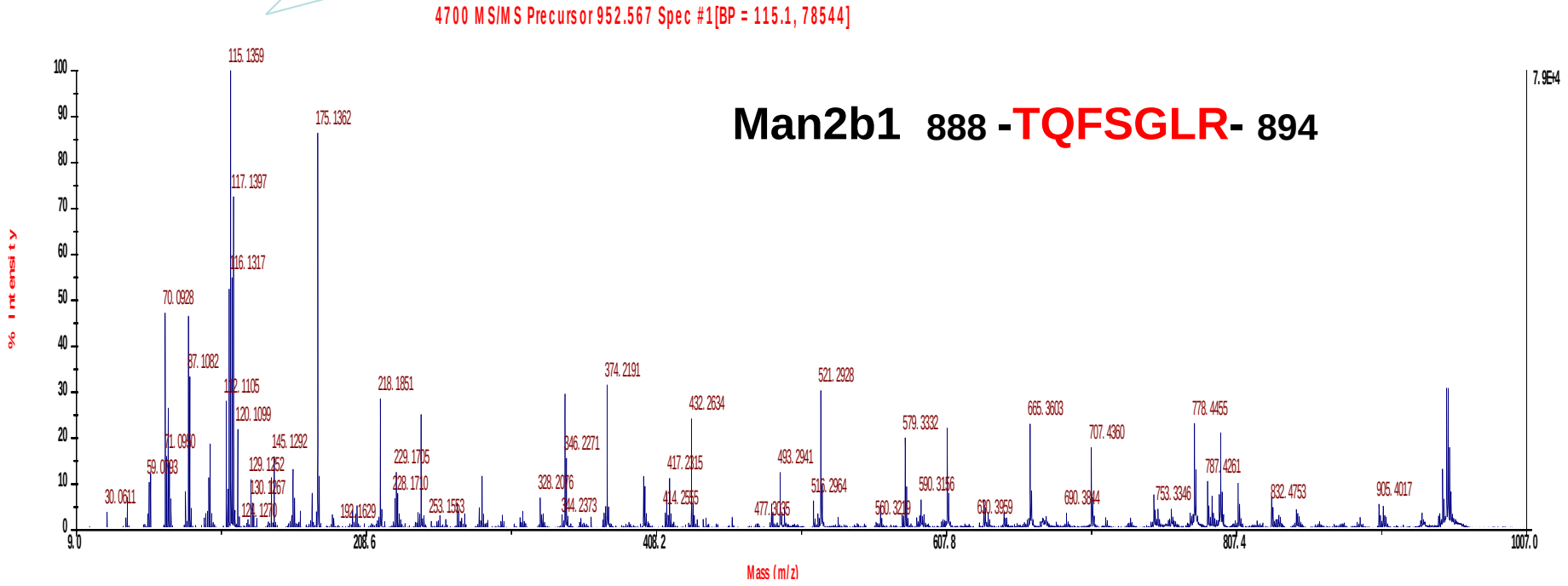


Mol. Cell. Proteomics 3, 1154-1169(2004)


MS1 : multiple precursor (peptide) ions
% Intensity
817.5038
952.5670
1069.5448
893.5382
1030.5712
1235.6687
1438.7781
1620.8853
1781.9351
1920.0137
2533.1650
8.1E+4
800
1440
2080
2720
3360
4000
mass/charge (m/z)
4700 MS/MS Precursor 952.567 Spec #1[BP = 115.1, 78544]
Man2b1 888 -TQFSGLR- 894
7.9E+4
Mass (m/z)



Expanded scale Reporter ion region



Parting message

Progress in science depends on

new techniques,

new discoveries, and

new ideas,

probably in that order

Sydney Brenner, 20 March 1980

Biology in the 1980's, talk at the

Friedrich Miescher Institute, Basel, Switzerland