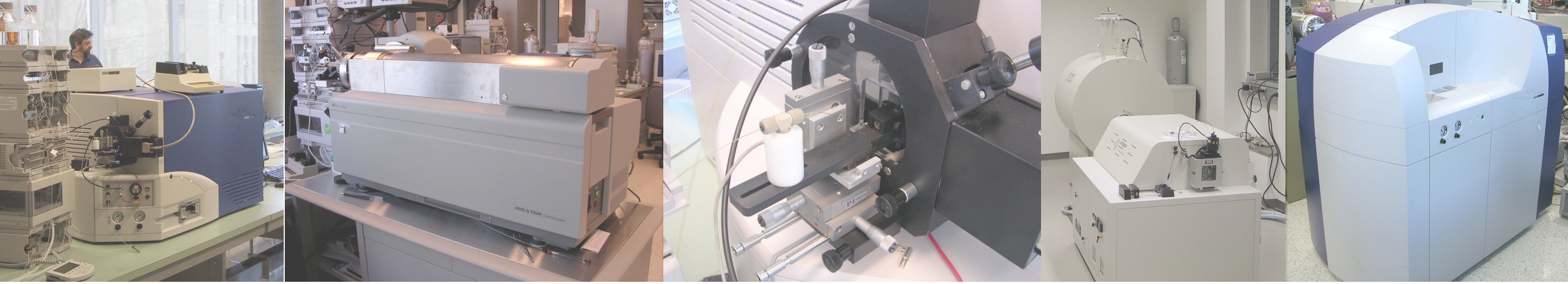

MASS SPECTROMETRY
in proteomics

Simon Letarte
May 2006

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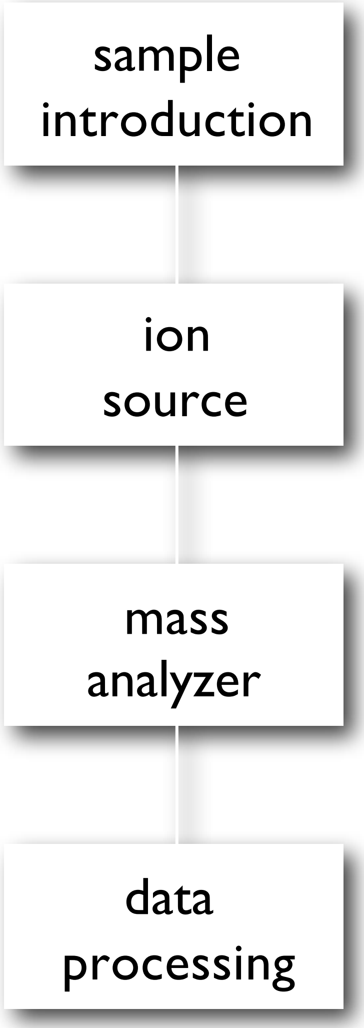
Outline

- fundamental concepts
- proteomics – a special case
- ion sources
- mass analyzers
- hybrid instruments
- quantification

Typical MS configuration



sample introduction

ion source

mass analyzer

data processing

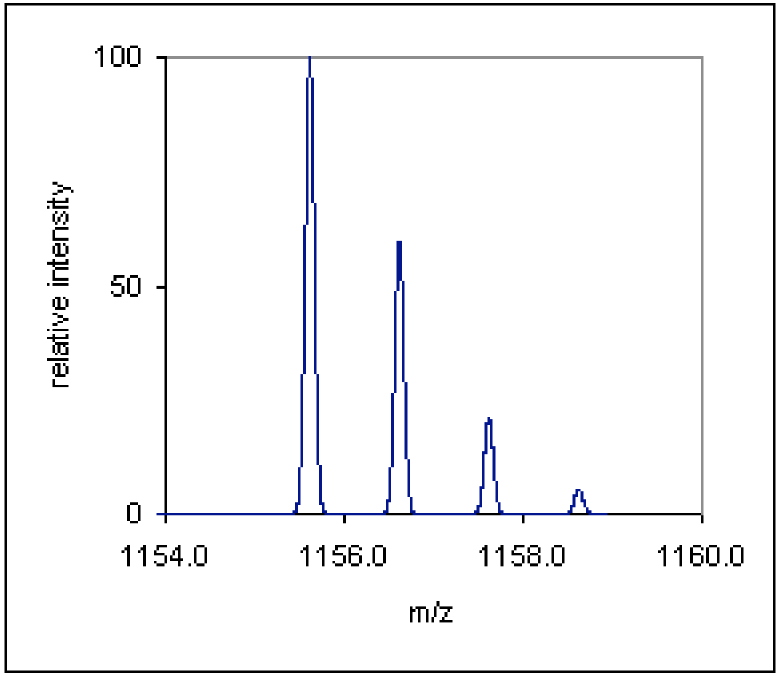
What is mass spectrometry ?

- ions are charged molecules
- measure the m/z of ions in the gas phase
- m/z unit is the Thomson (Th)
  - information about the molecular mass
  - information about the structure

Types of masses

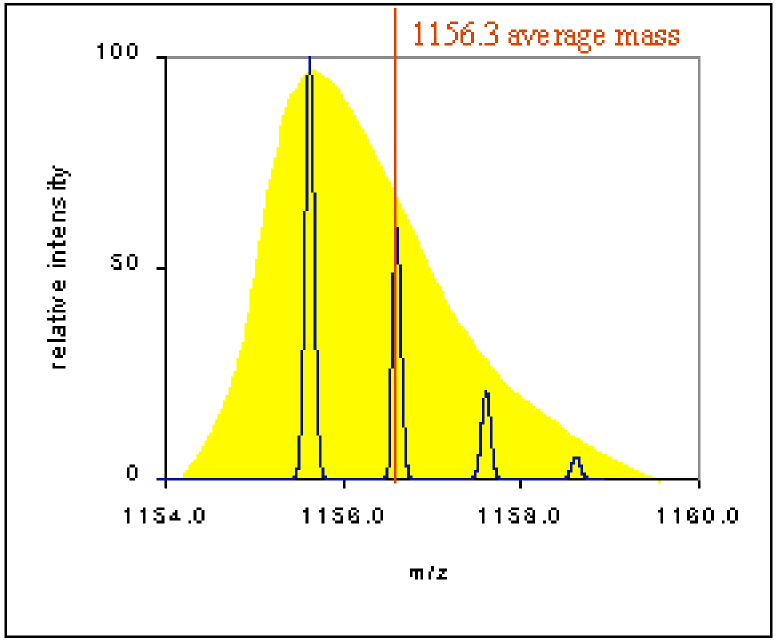
monoisotopic

1155.6



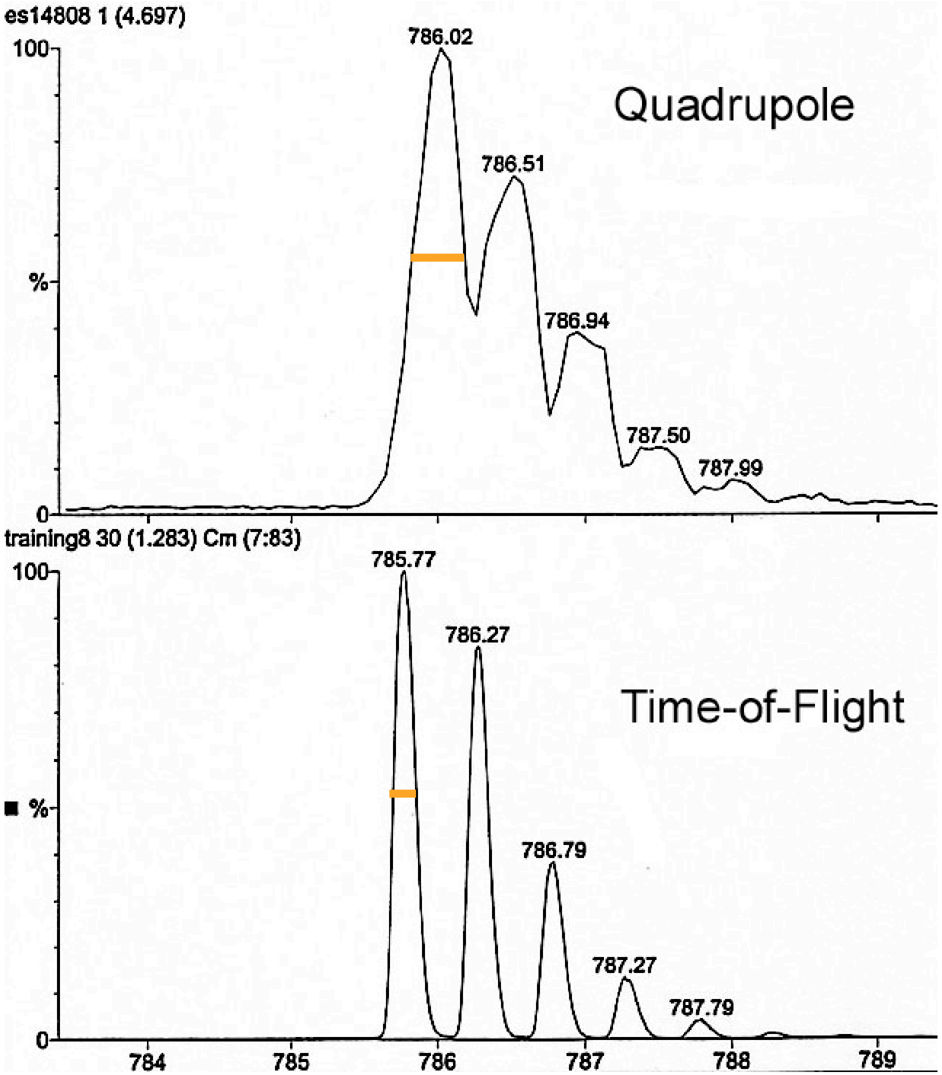
average

1156.3



Resolving power

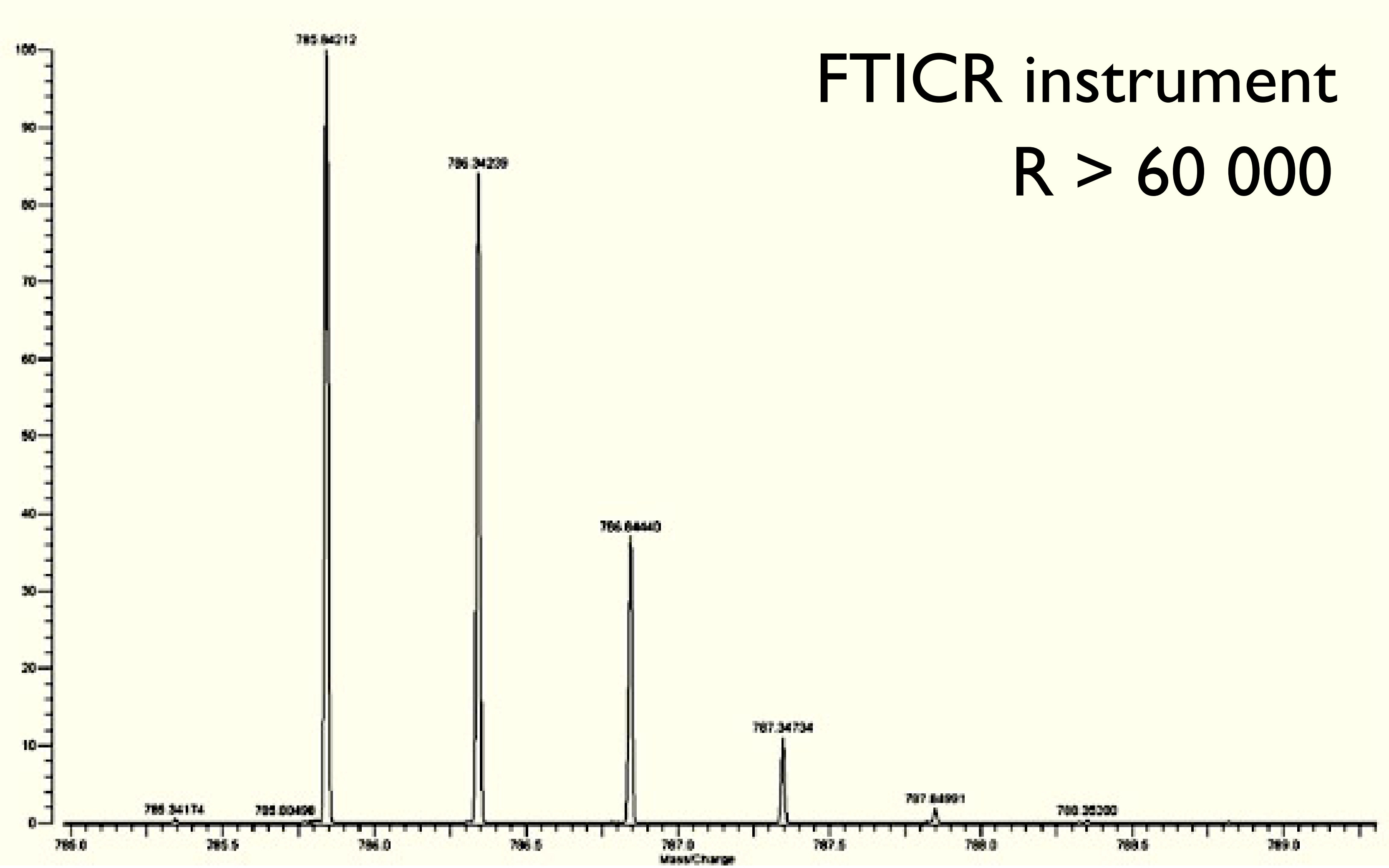
R=M/ΔM



786 / 0.5 = 1572

786 / 0.1 = 7860

High resolving power



FTICR instrument

R > 60 000

Mass accuracy

Difference between the experimental m/z and the calculated value of the chemical formula.

$$ppm = \frac{M_{meas} - M_{calc}}{M_{calc}} x10^6$$

1715.851	SSKMPTVGTEERPPGK
1715.859	QPAQSQNLSPLSGFSR
1715.849	KSFSQYAHLSQHQR
1715.888	AVEKGAVHPAMANHLR
1715.847	EGYLEEESLKVQHR

Proteomics

a special case

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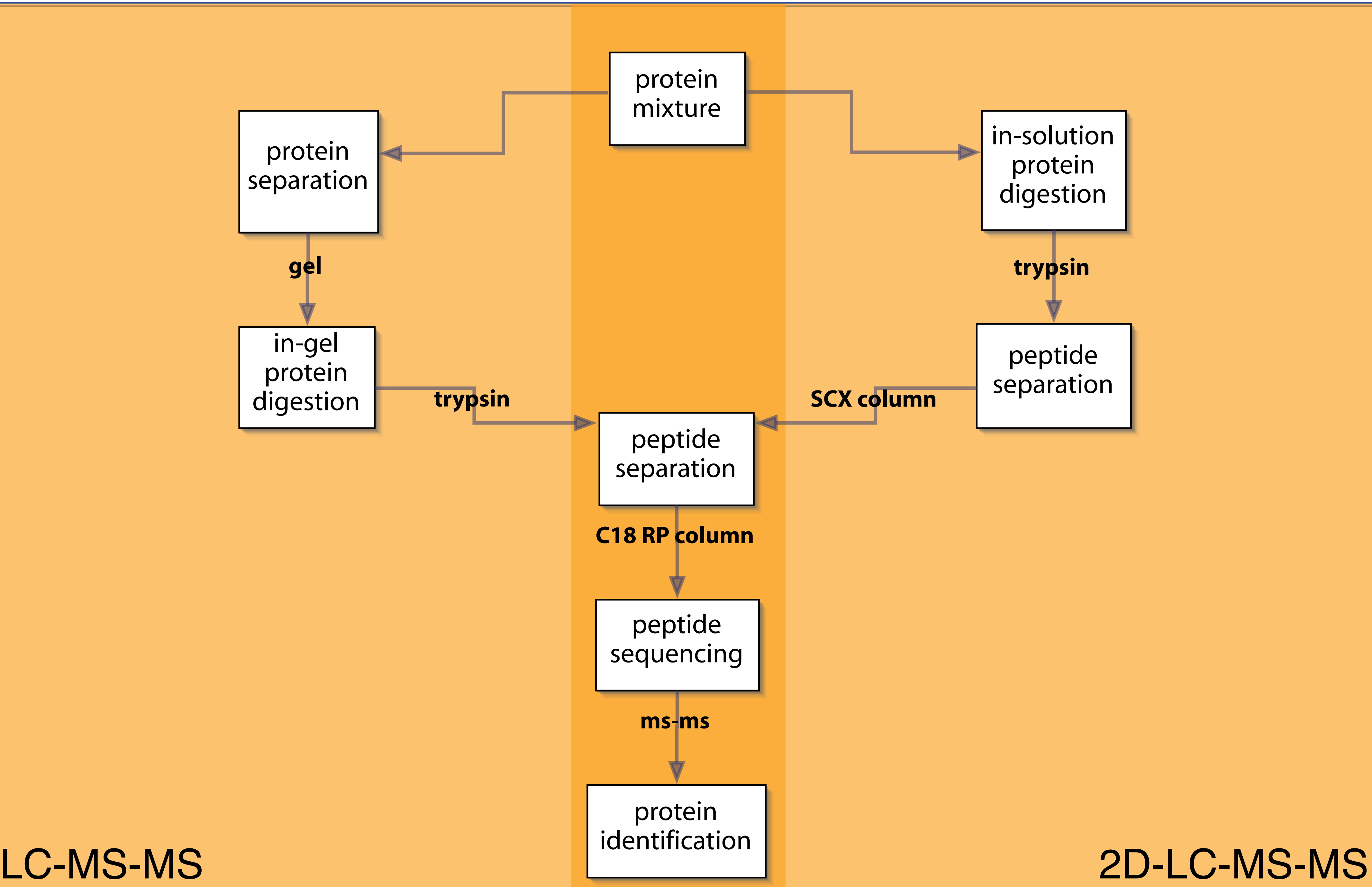
Simplifying the sample

- 1D or 2D gel + RP HPLC
- 2D HPLC
- capillary electrophoresis
- cystein-based enrichment
- antibody-based enrichment
- glycocapture

1D LC vs 2D LC


protein mixture
protein separation
gel
in-gel protein digestion
trypsin
in-solution protein digestion
trypsin
peptide separation
SCX column
peptide separation
C18 RP column
peptide sequencing
ms-ms
protein identification
LC-MS-MS
2D-LC-MS-MS

Ionization Sources

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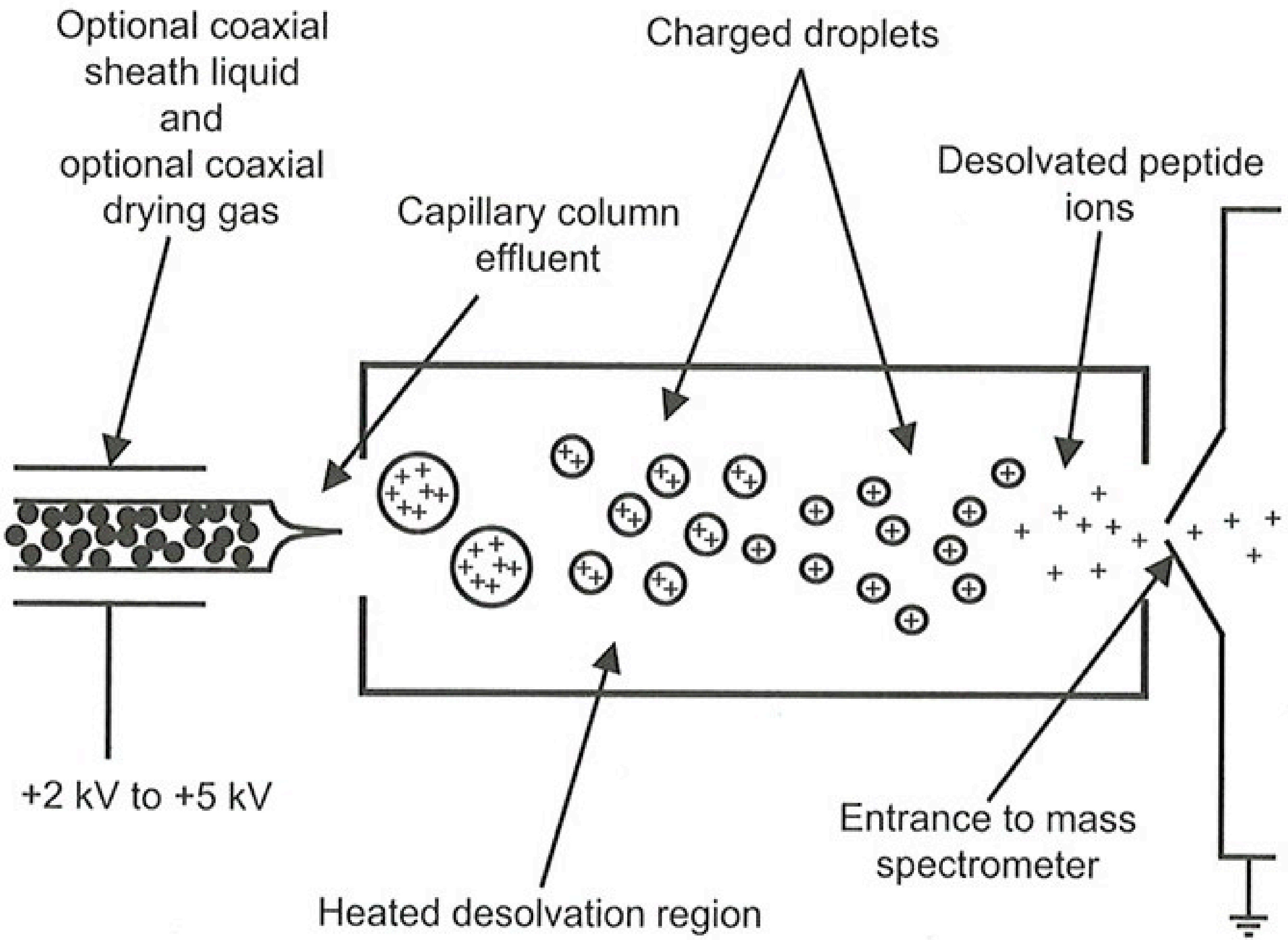
Electrospray

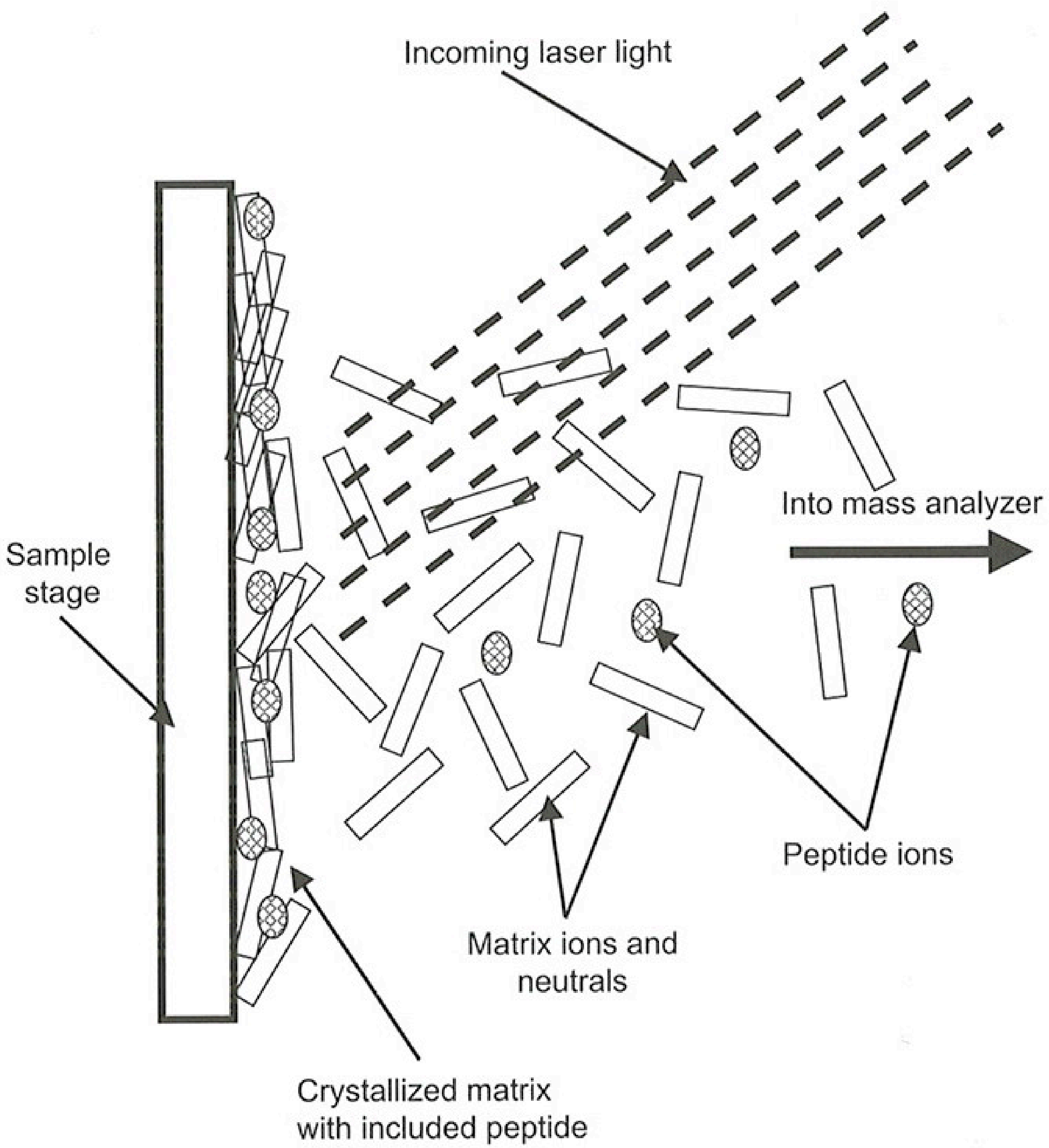


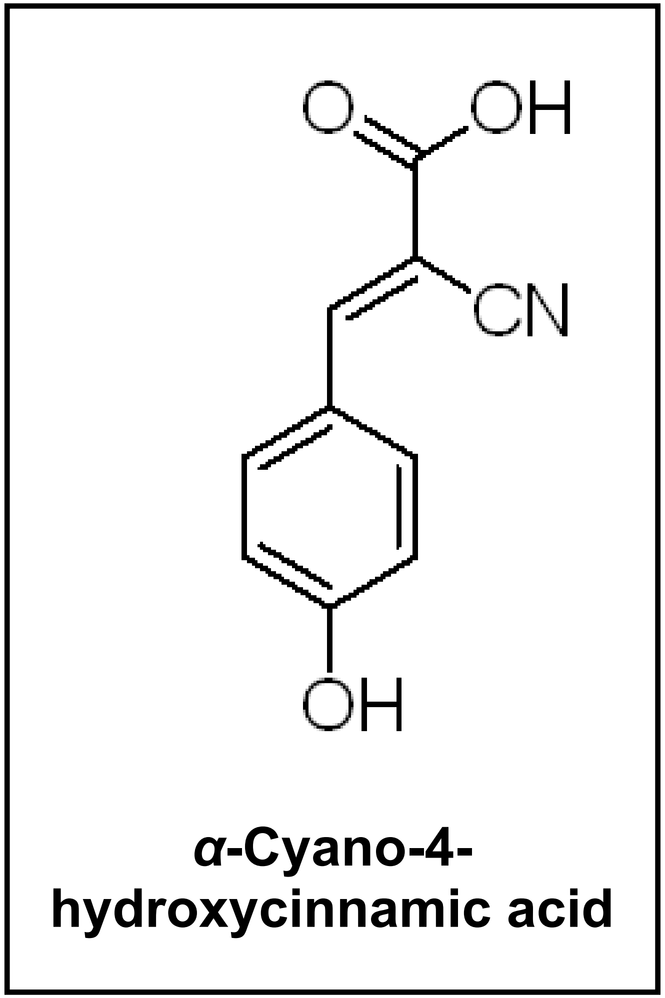
Kinter, M., Sherman, N.E., *Protein Sequencing and identification using tandem Mass Spectrometry*

Electrospray

- doubly and triply charged peptides
- high ionization efficiency
- good sensitivity
- peptides and polar compourneds
- perfect march for LC
- sensitive to salt, detergents

MALDI





α-Cyano-4-hydroxycinnamic acid

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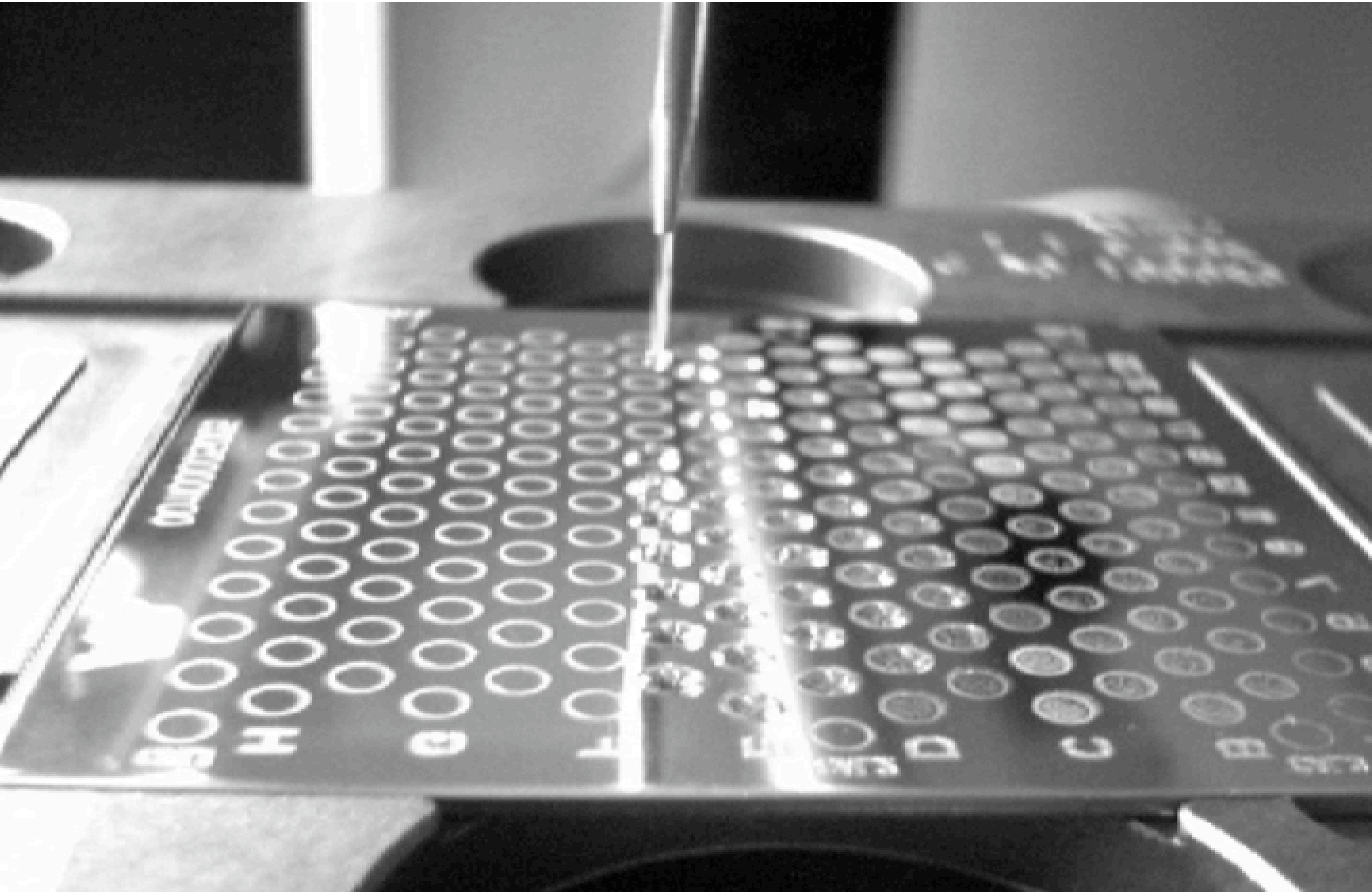
Offline LC-MALDI

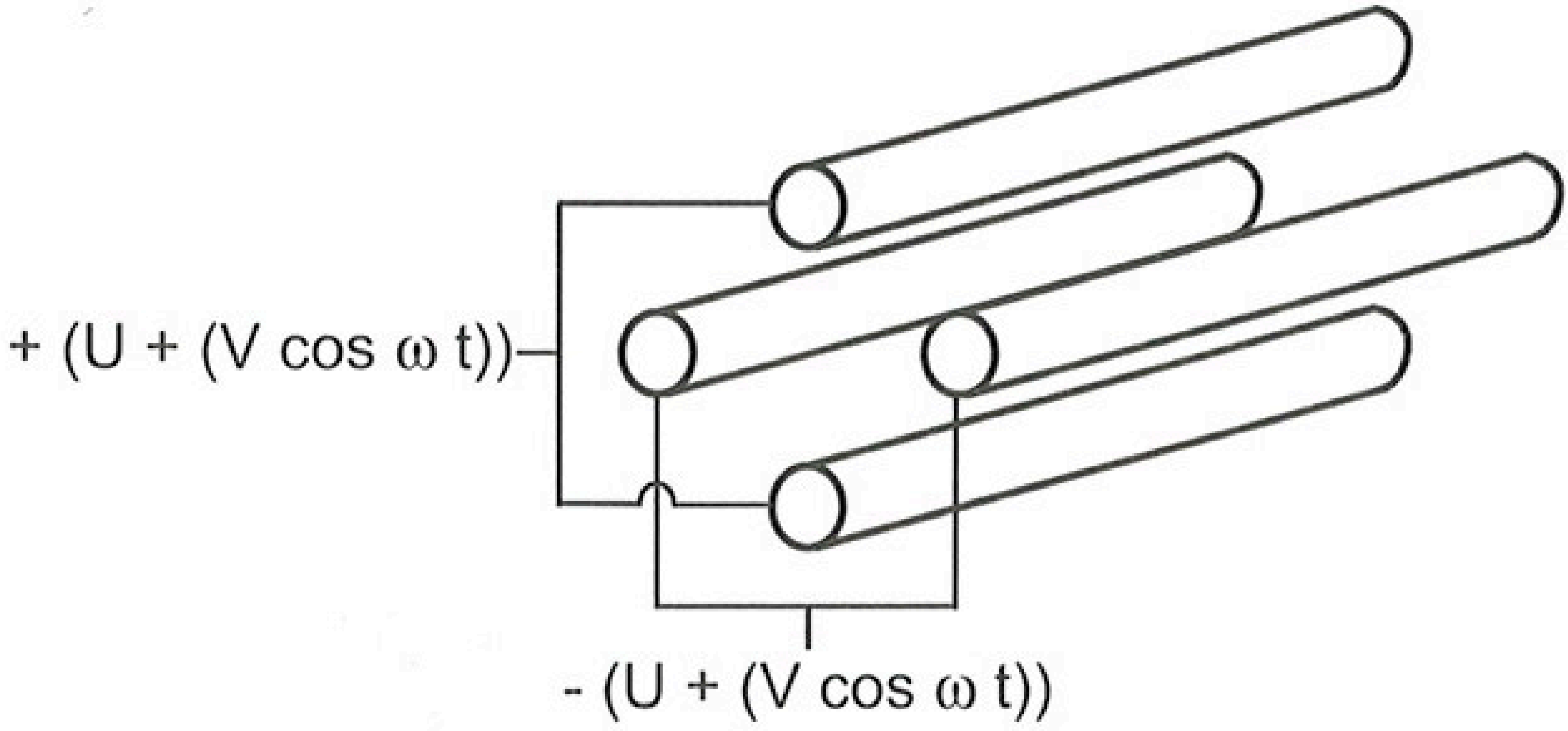


MALDI

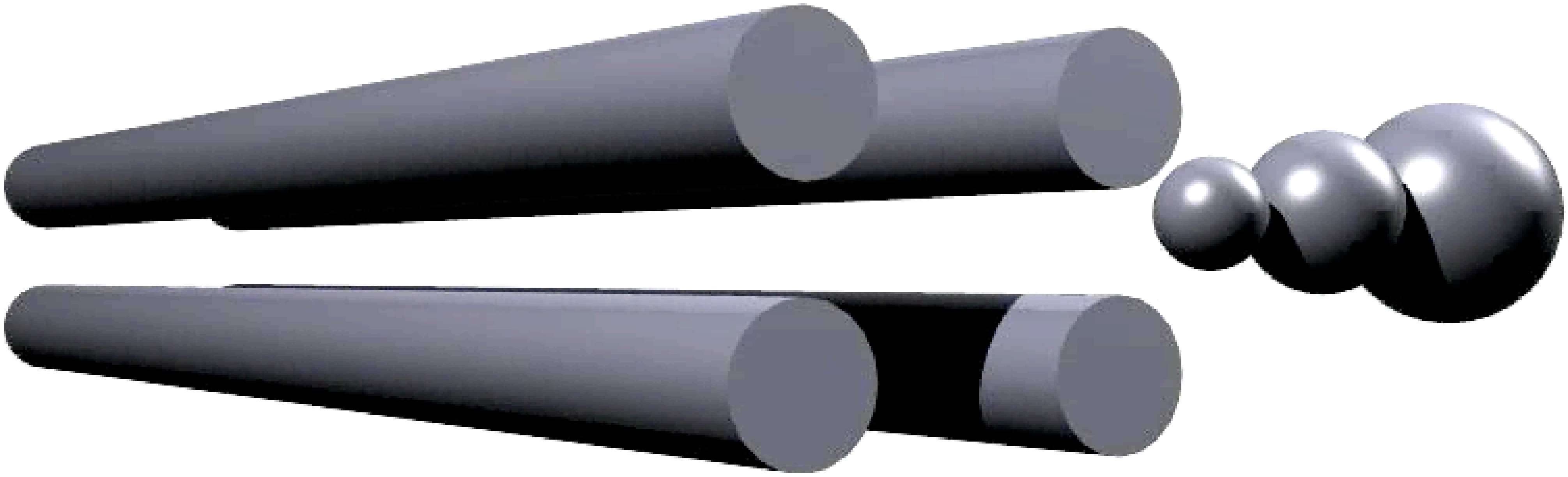
- solid phase samples
- tolerant to contamination
- pulsed ion source
- mostly singly charged ions
- mechanism will favor different ions than ESI

Mass Analyzers

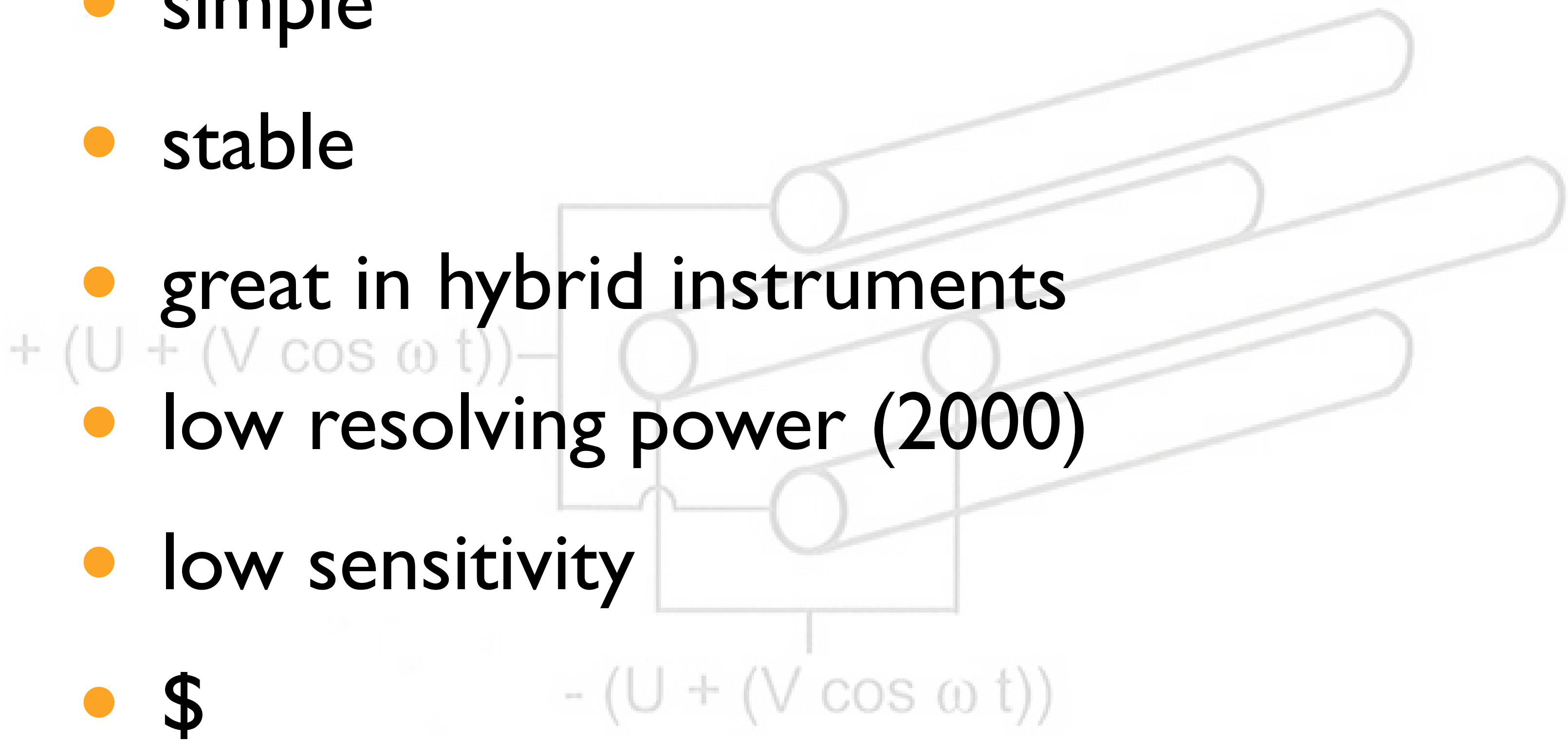
Quadrupole



Quadrupole

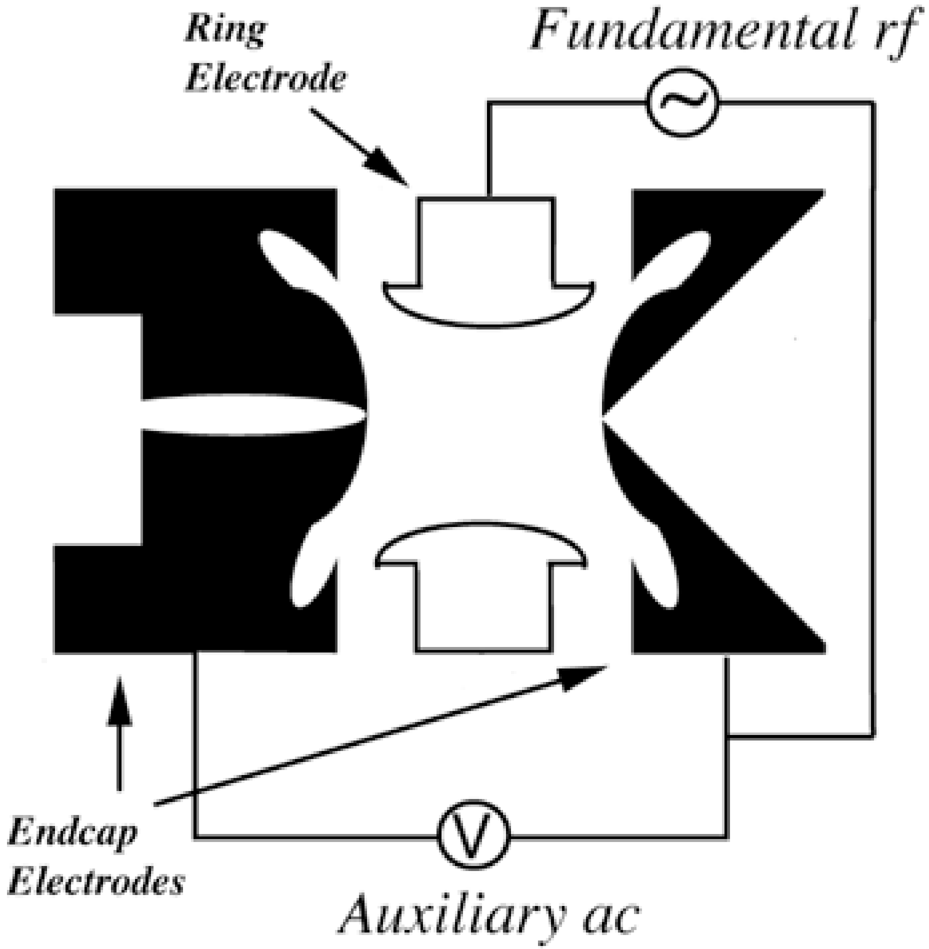


Quadrupole

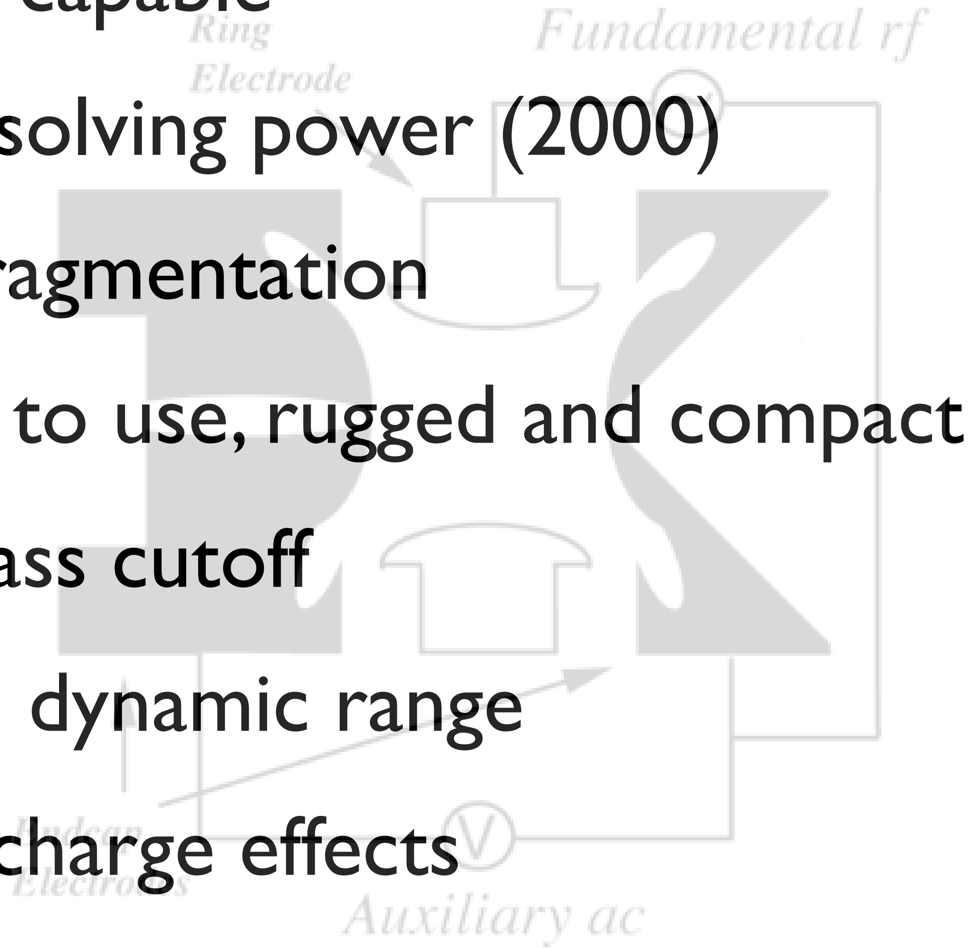


- simple
- stable
- great in hybrid instruments
- low resolving power (2000)
- low sensitivity
- $

Ion trap

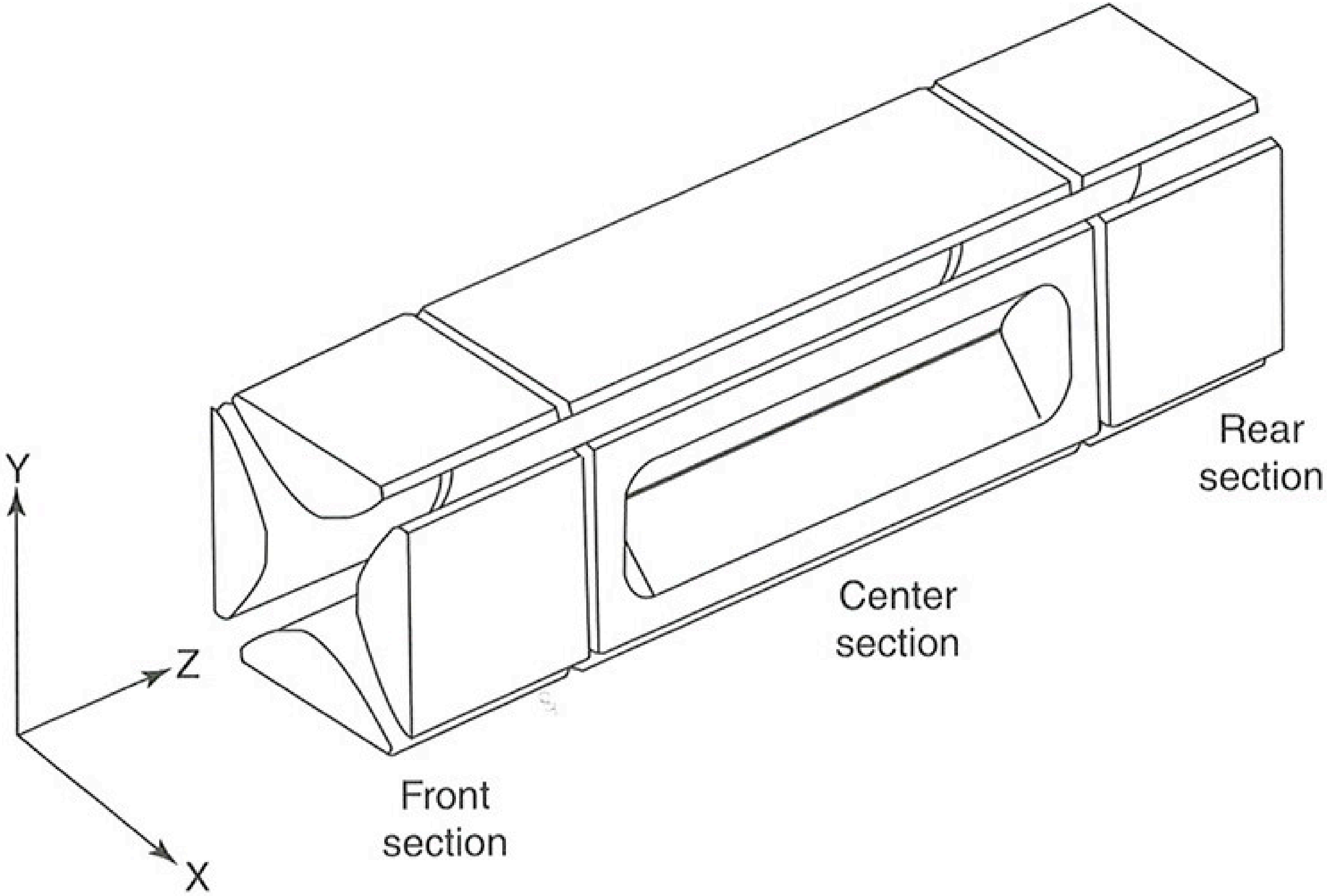

Ring Electrode
Fundamental rf
~
V
Endcap Electrodes
Auxiliary ac

Ion trap



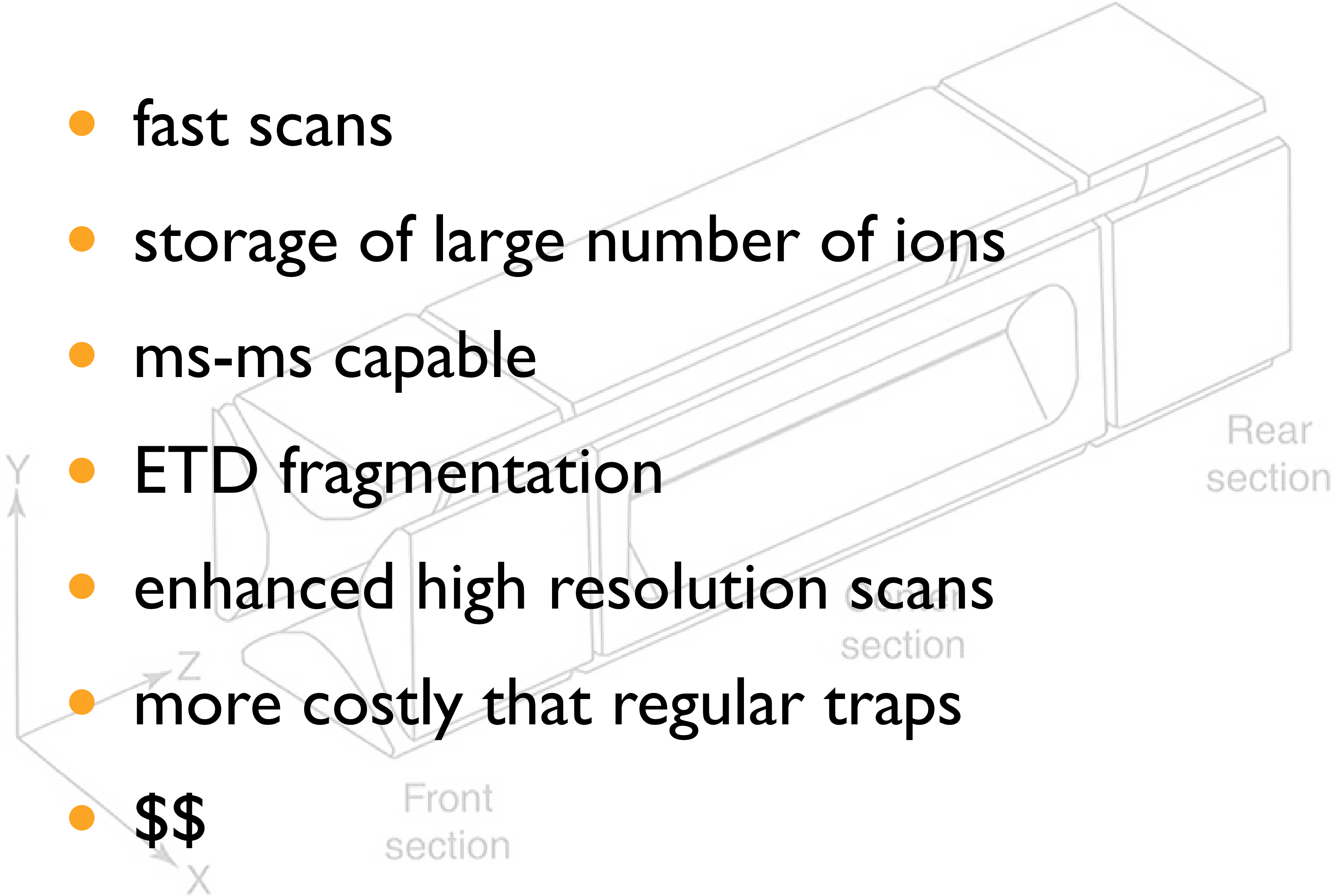
- ms-ms capable
- low resolving power (2000)
- ETD fragmentation
- simple to use, rugged and compact
- low mass cutoff
- limited dynamic range
- space-charge effects
- $

Linear ion trap



March, R.E., Todd, J.F.J., *Quadrupole Ion Trap Mass Spectrometry*

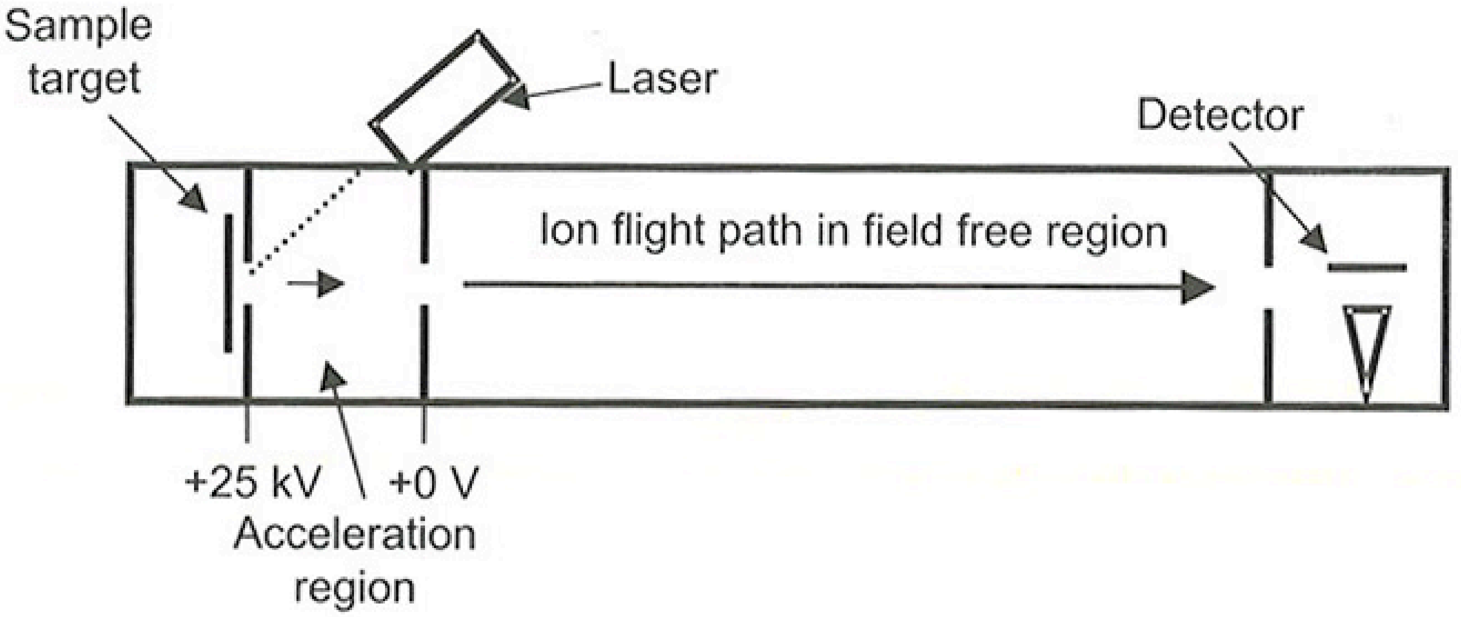
Linear ion trap



- fast scans
- storage of large number of ions
- ms-ms capable
- ETD fragmentation
- enhanced high resolution scans
- more costly that regular traps
- $$

Time-of-flight

A. Linear time-of-flight mass spectrometer



$$ZeE = \frac{mv^2}{2} \qquad v = \sqrt{\frac{2ZeE}{m}} \qquad t = \frac{d}{v} \qquad v = \sqrt{\frac{m}{z}} \times const.$$

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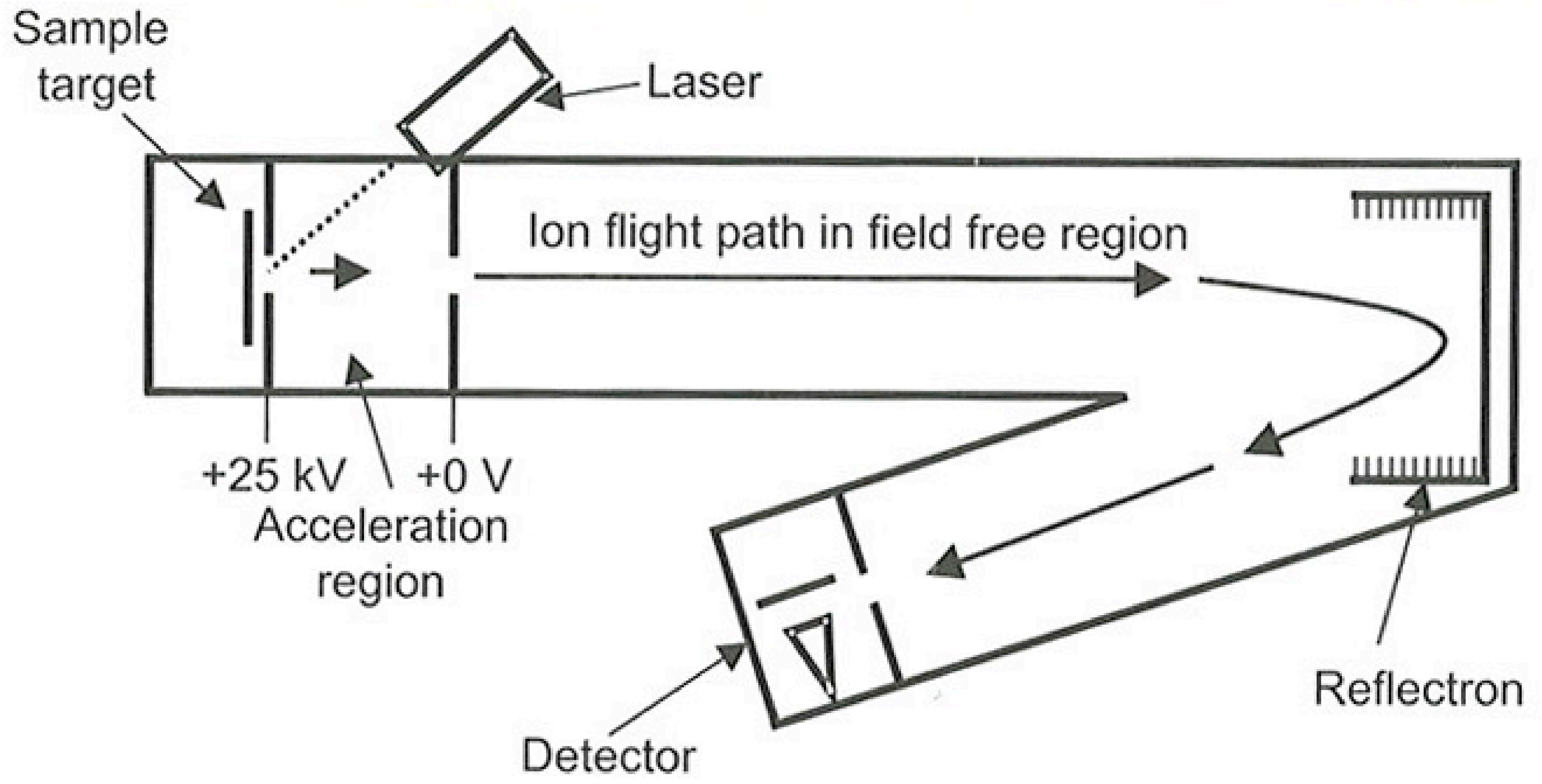
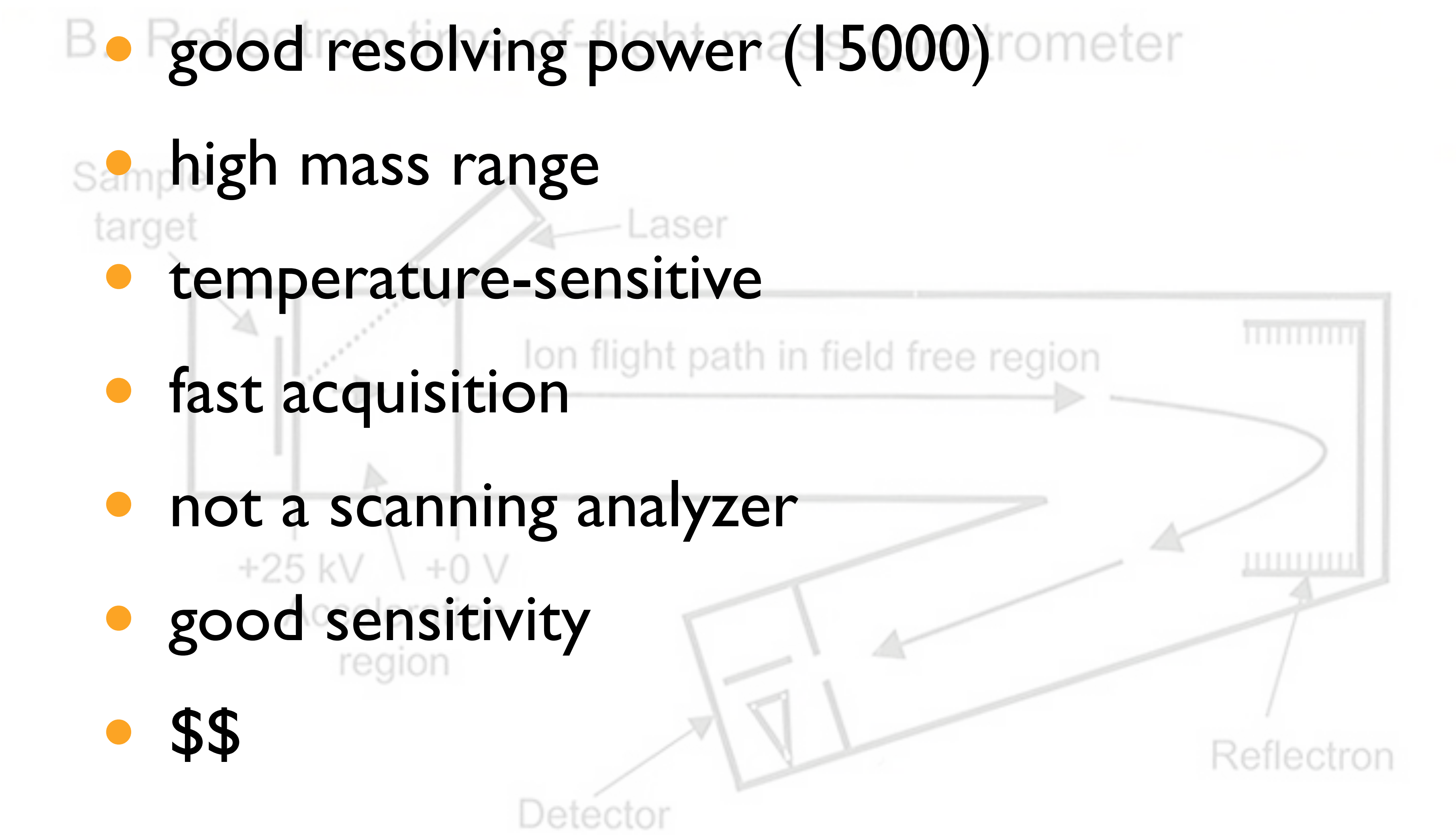
Re-Tof

B. Reflectron time-of-flight mass spectrometer



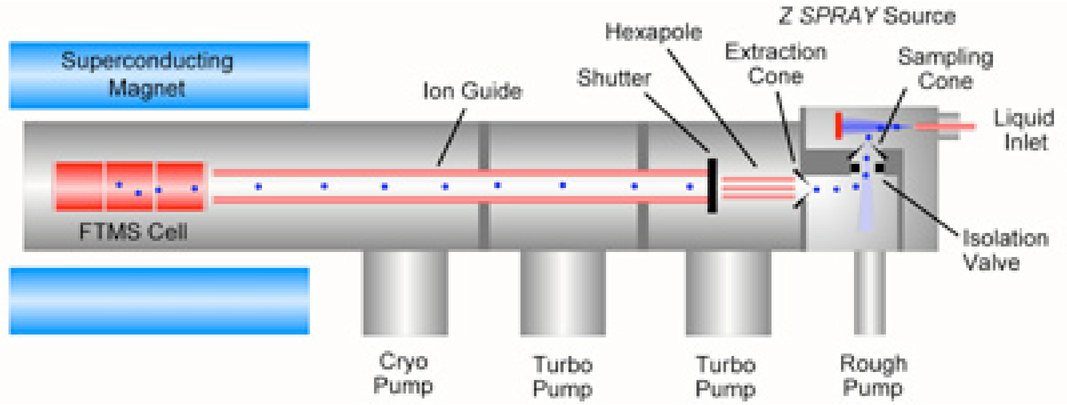
Kinter, M., Sherman, N.E., *Protein Sequencing and identification using tandem Mass Spectrometry*

Time-of-flight

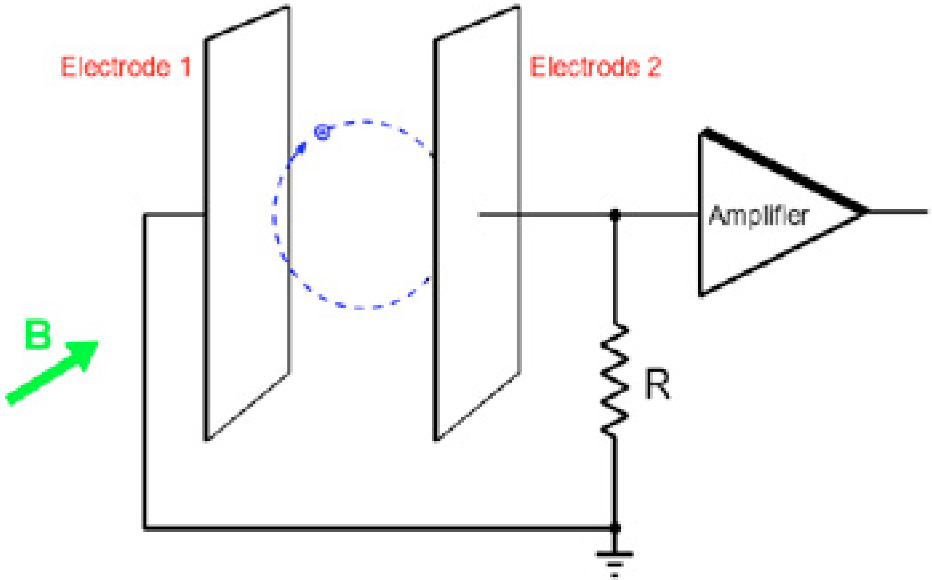


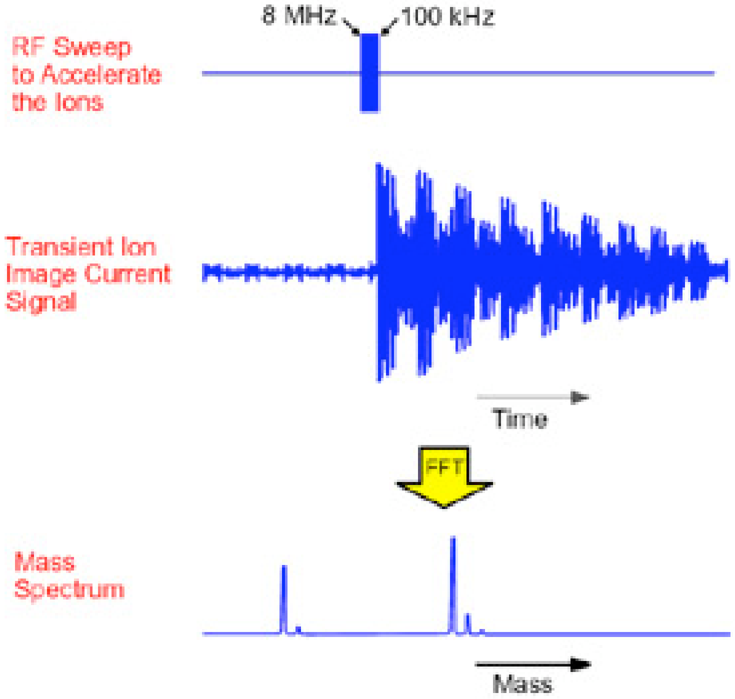
- good resolving power (15000)
- high mass range
- temperature-sensitive
- fast acquisition
- not a scanning analyzer
- good sensitivity
- $$

FTICR



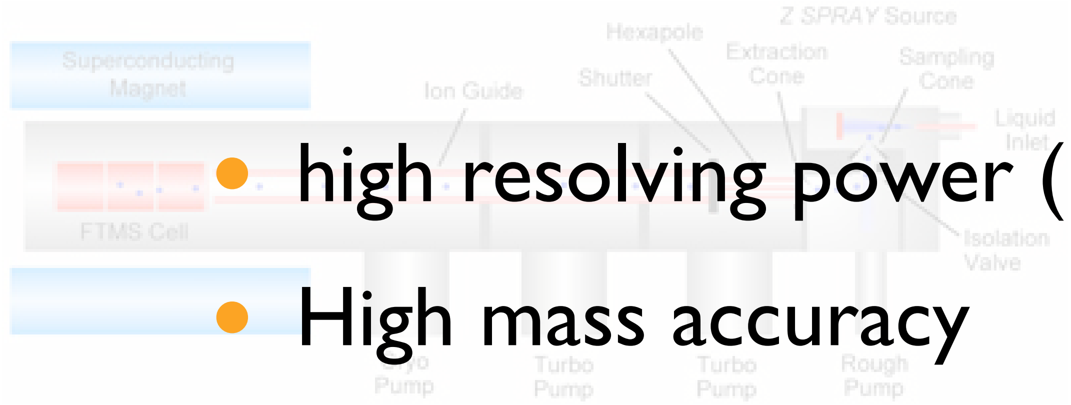
$$\omega = \frac{zB}{m}$$





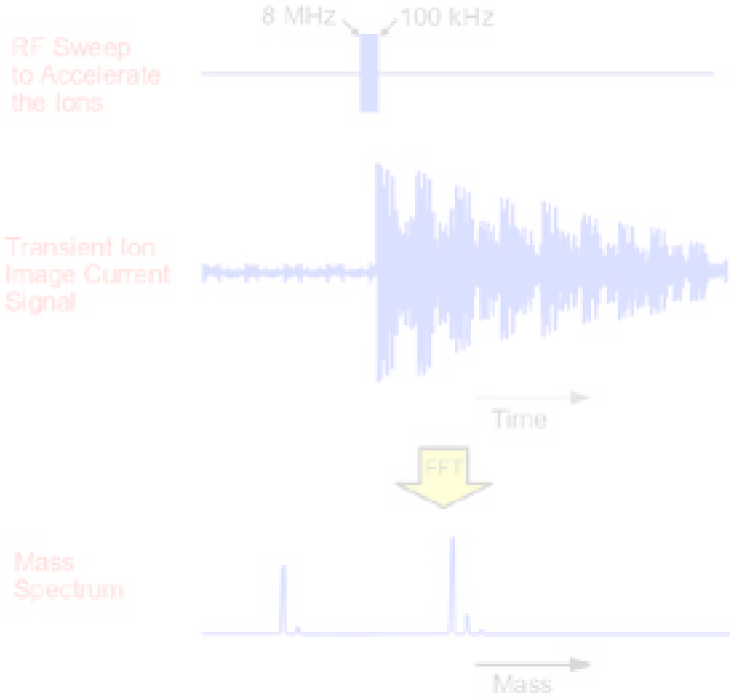
www.ionspec.com

FTICR



$$\omega = \frac{zB}{m}$$

- high resolving power (100 000)
- High mass accuracy
- ECD and IRMPD fragmentation
- high maintenance
- huge files
- charge-space effects
- $$$$



Hybrid Instruments

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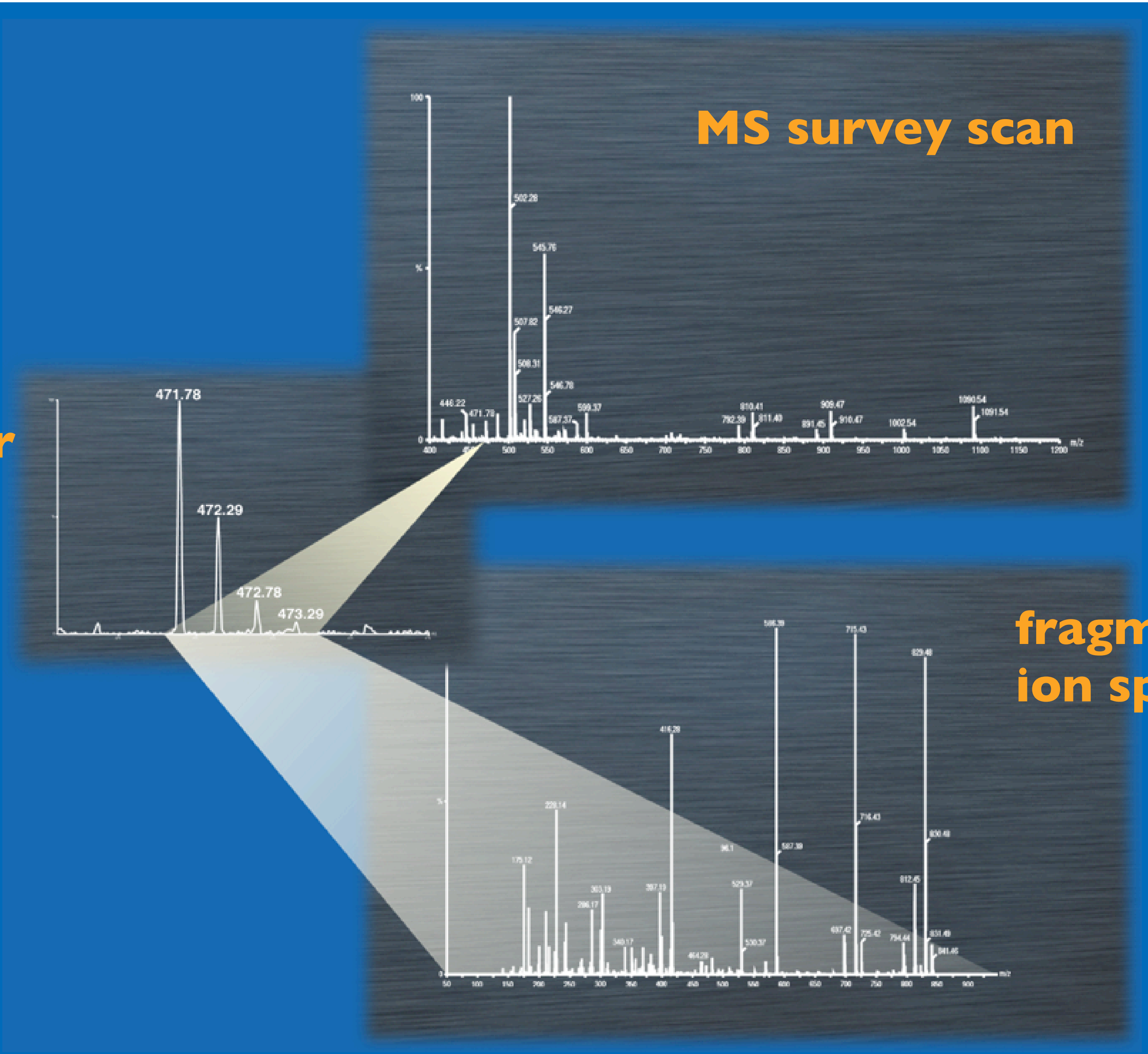
Tandem mass spectrometry

- mass spectrum acquisition
- precursor selection
- precursor fragmentation
- fragment analysis
- precursor analysis
- neutral loss analysis
- multiple reaction monitoring

Automatic precursor selection

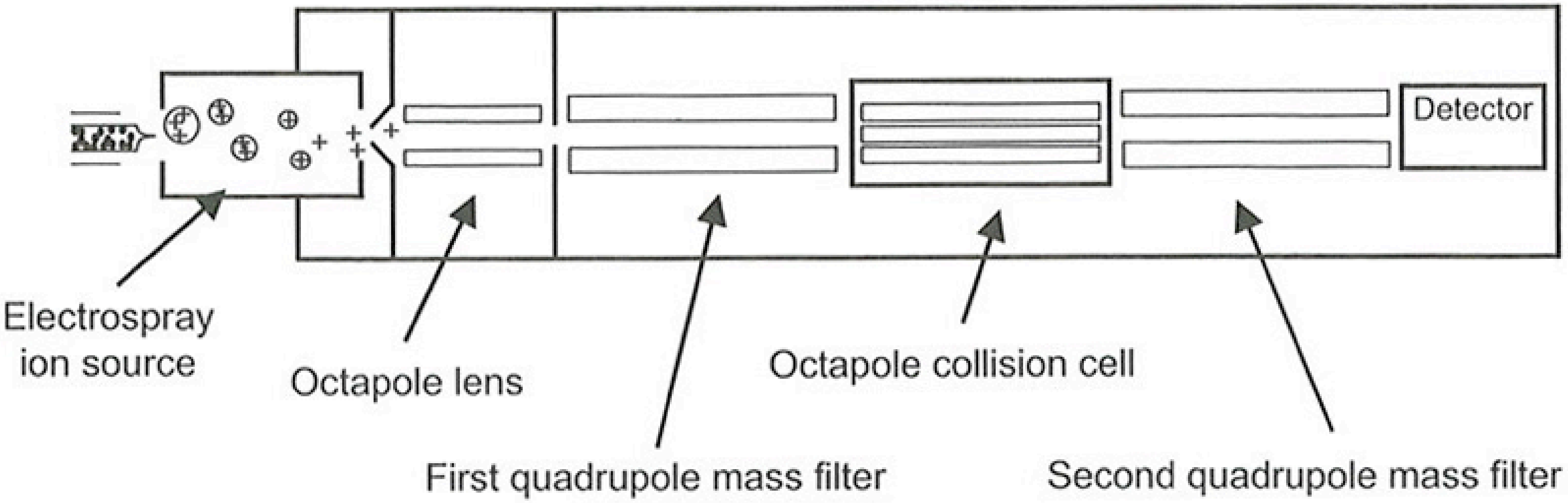


MS survey scan

precursor selection

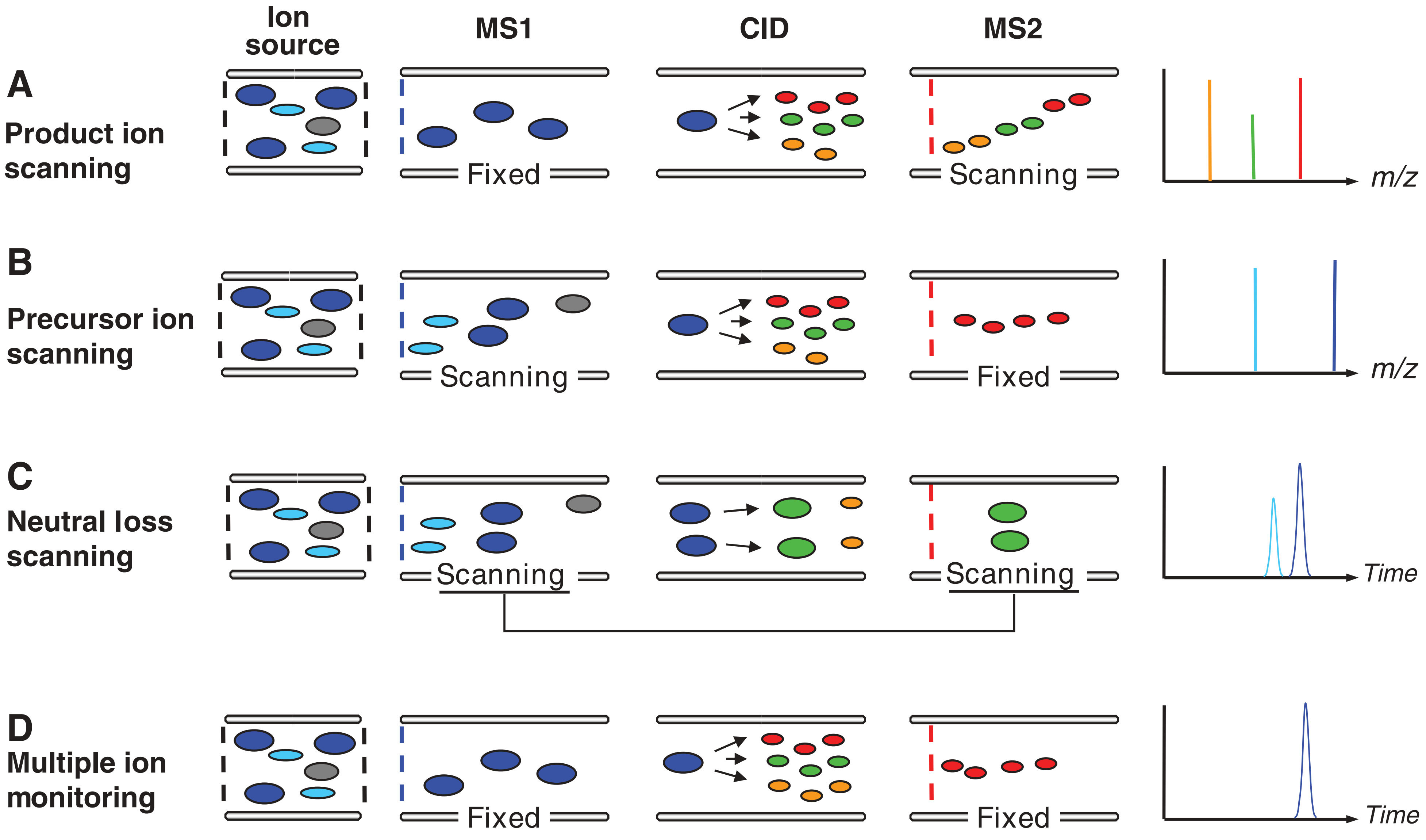
fragment ion spectrum

Triple-quad / Q-trap



Kinter, M., Sherman, N.E., *Protein Sequencing and identification using tandem Mass Spectrometry*

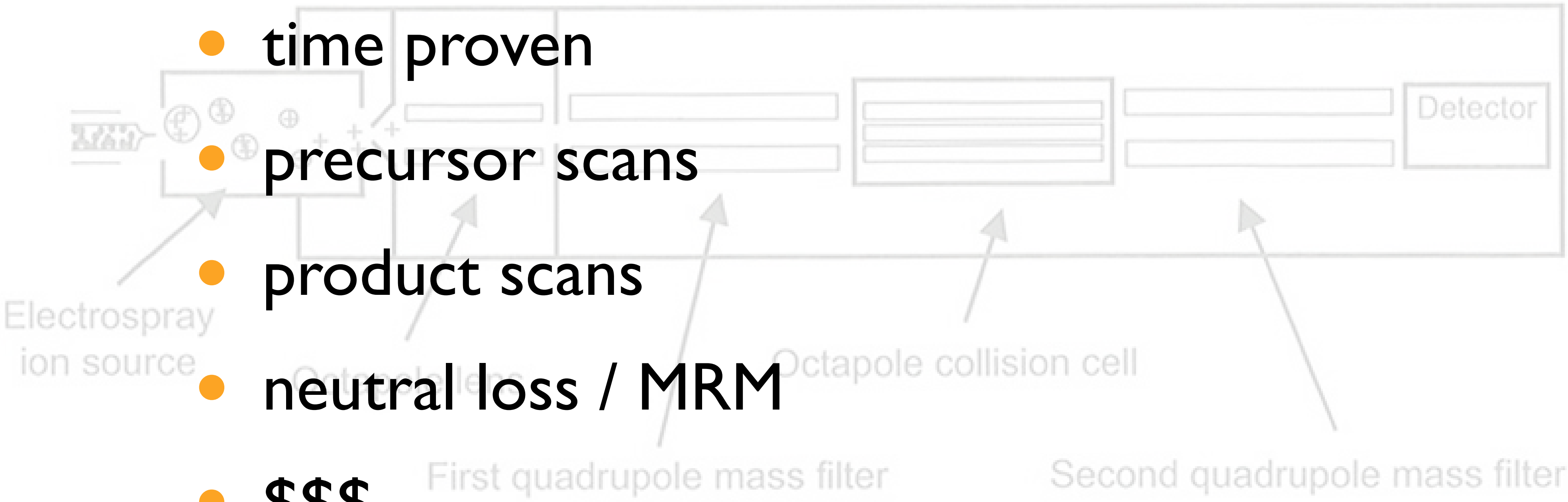
Types of scans



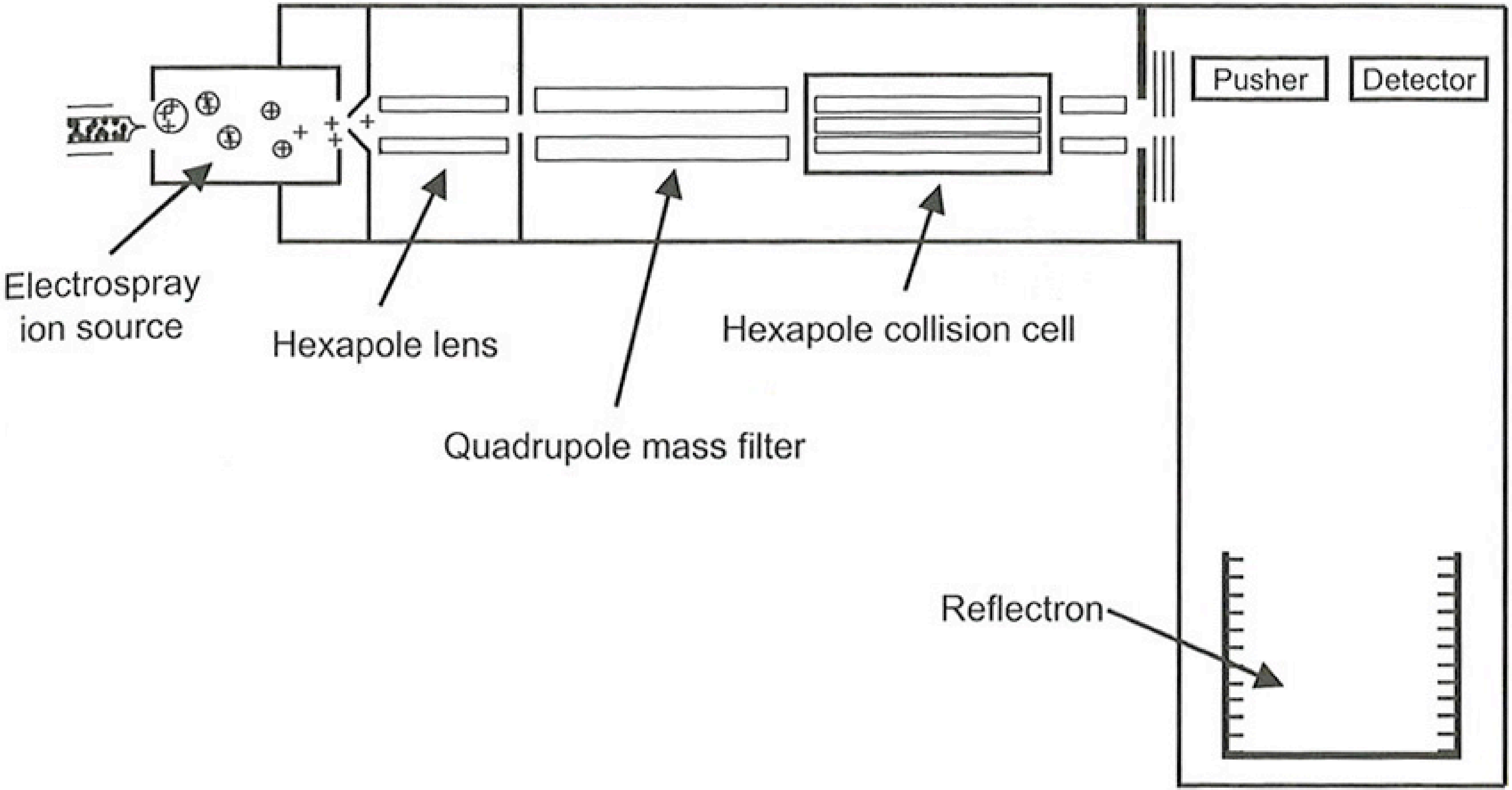
Domon, B., Aebersold., R., *Mass Spectrometry and Protein Analysis*, Science 312, 2006.

Triple-quad / Q-trap

- rugged hybrid instrument
- time proven
- precursor scans
- product scans
- neutral loss / MRM
- $$$

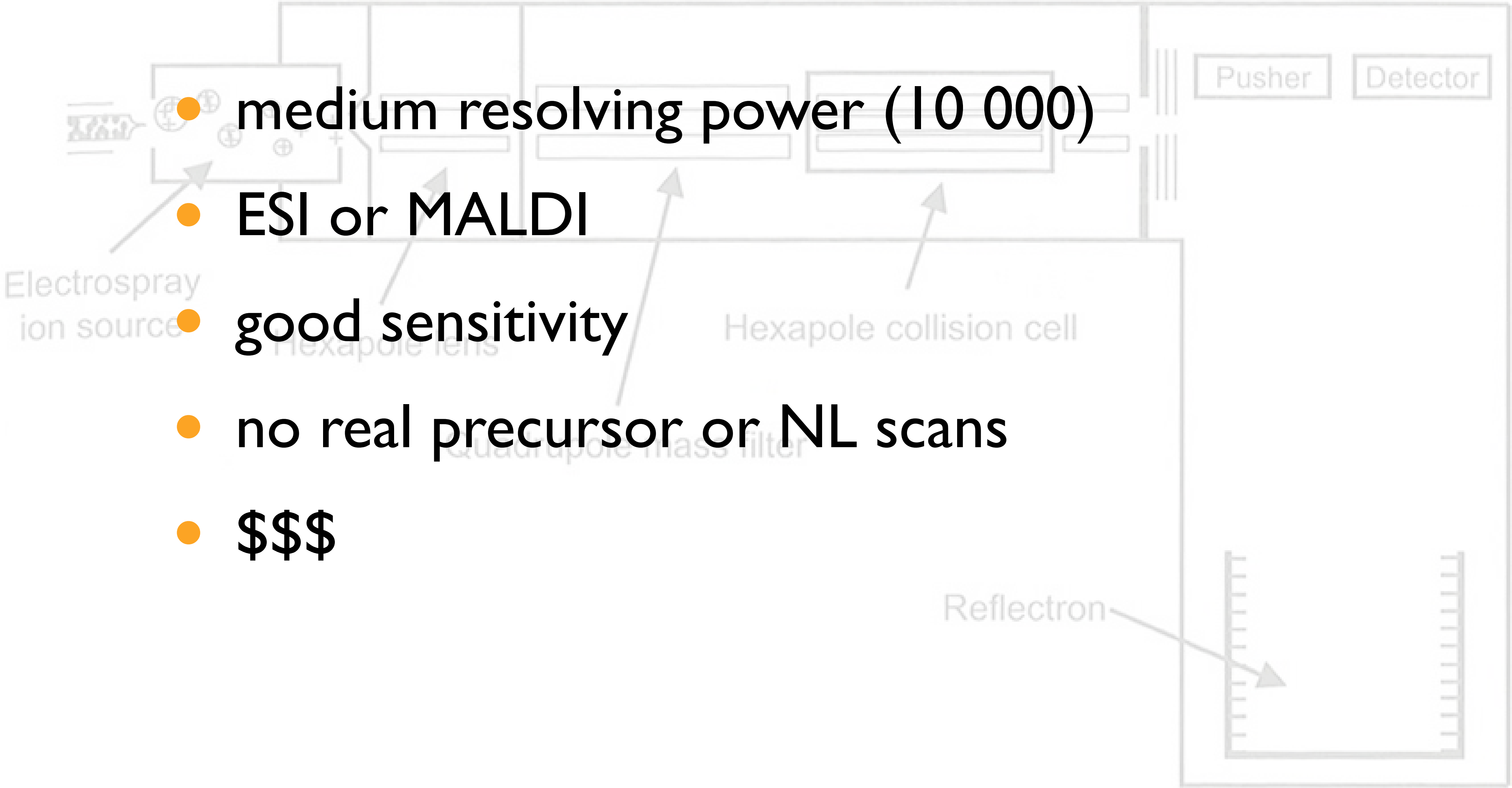


Q-Tof



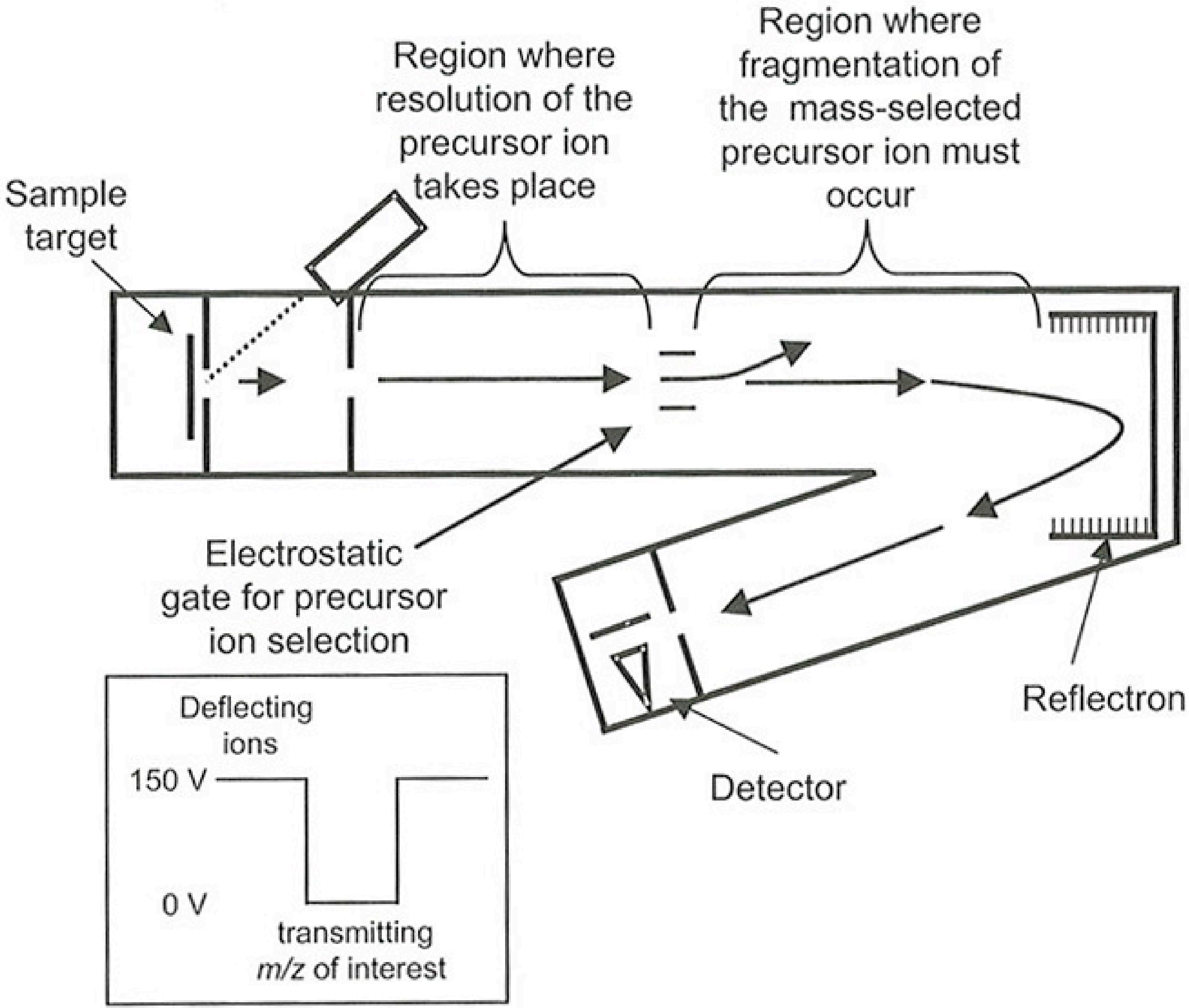
Kinter, M., Sherman, N.E., *Protein Sequencing and identification using tandem Mass Spectrometry*

Q-Tof



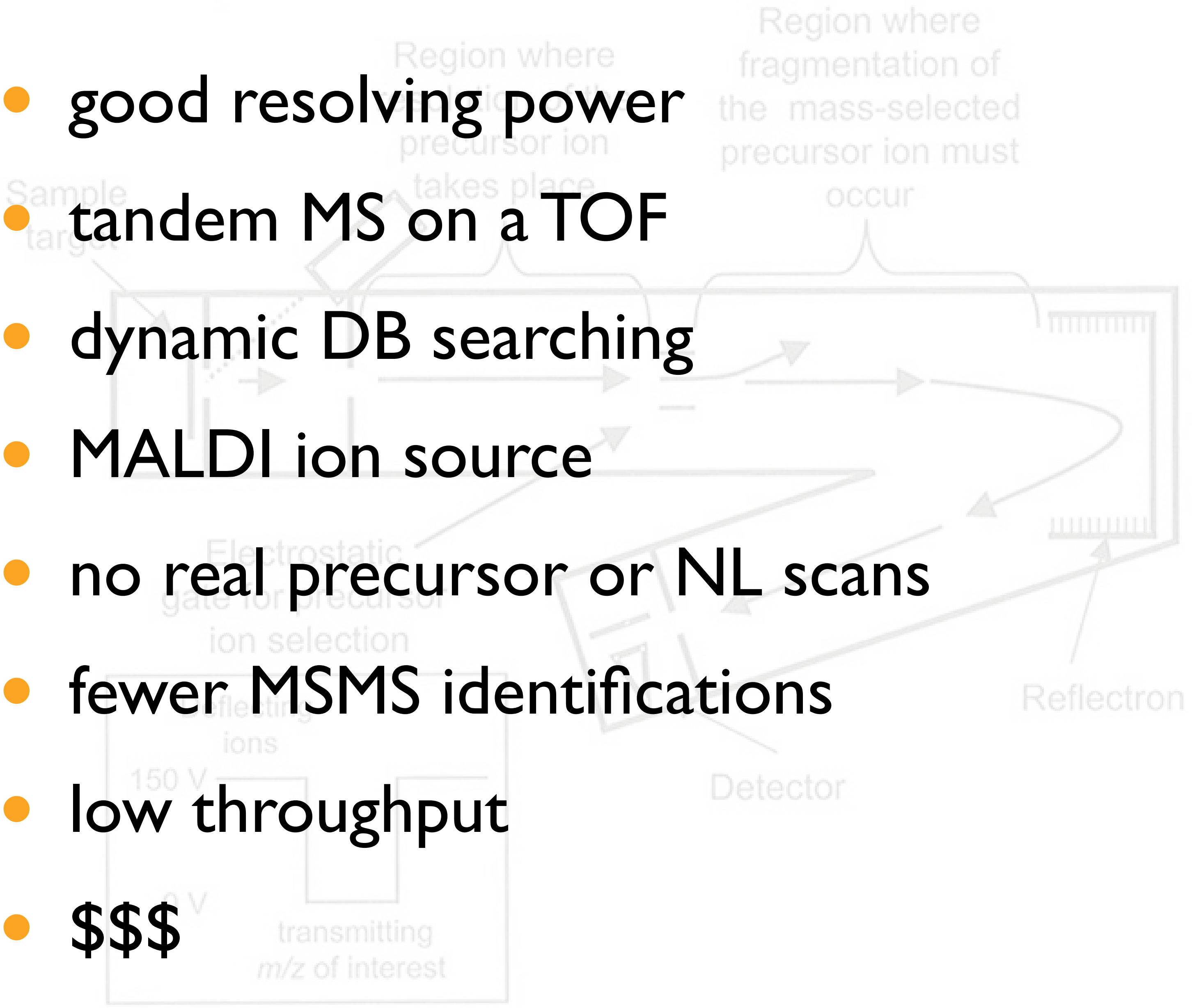
- medium resolving power (10 000)
- ESI or MALDI
- good sensitivity
- no real precursor or NL scans
- $$$

Tof-Tof



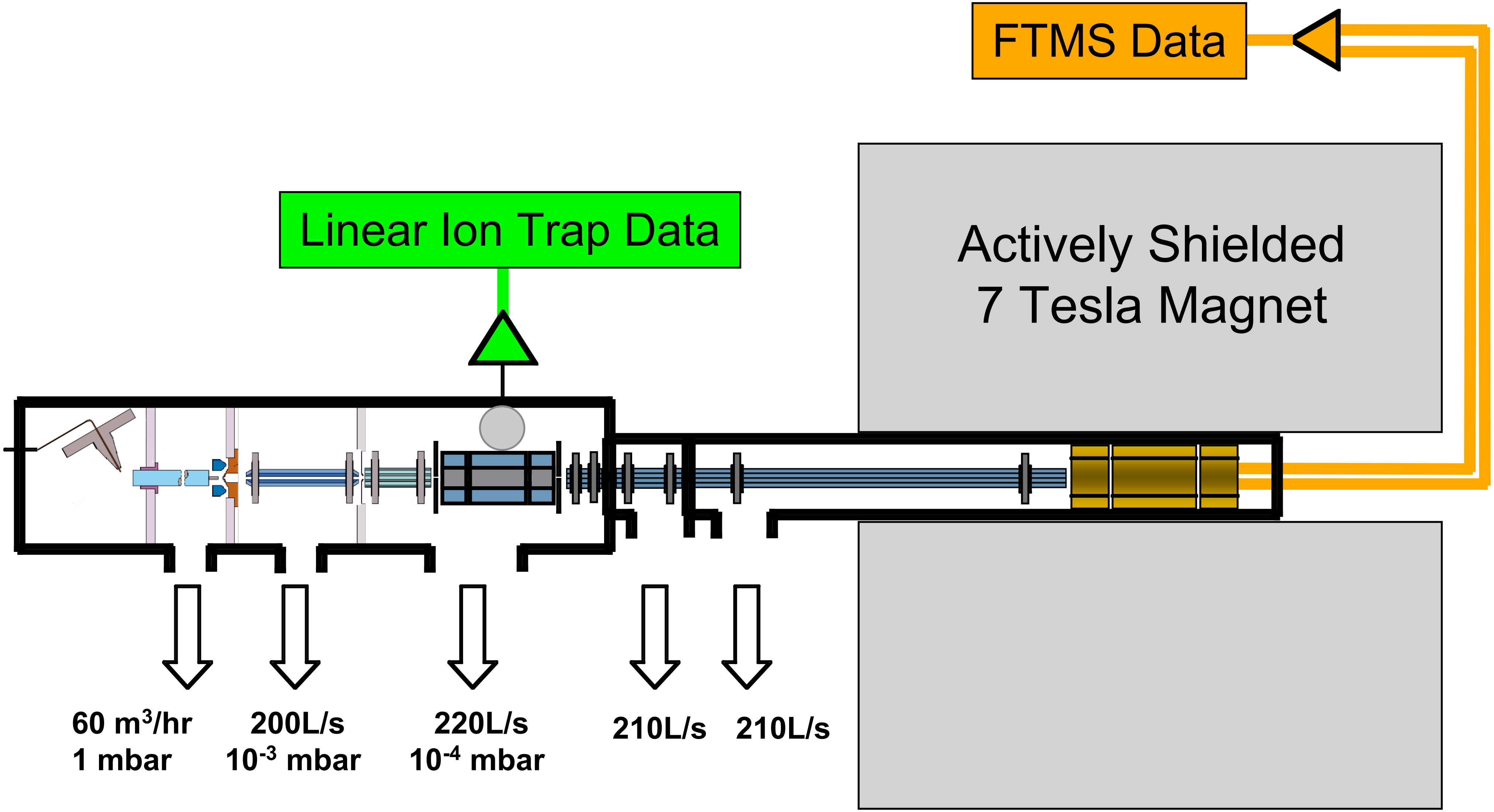
Kinter, M., Sherman, N.E., *Protein Sequencing and identification using tandem Mass Spectrometry*

Tof-Tof



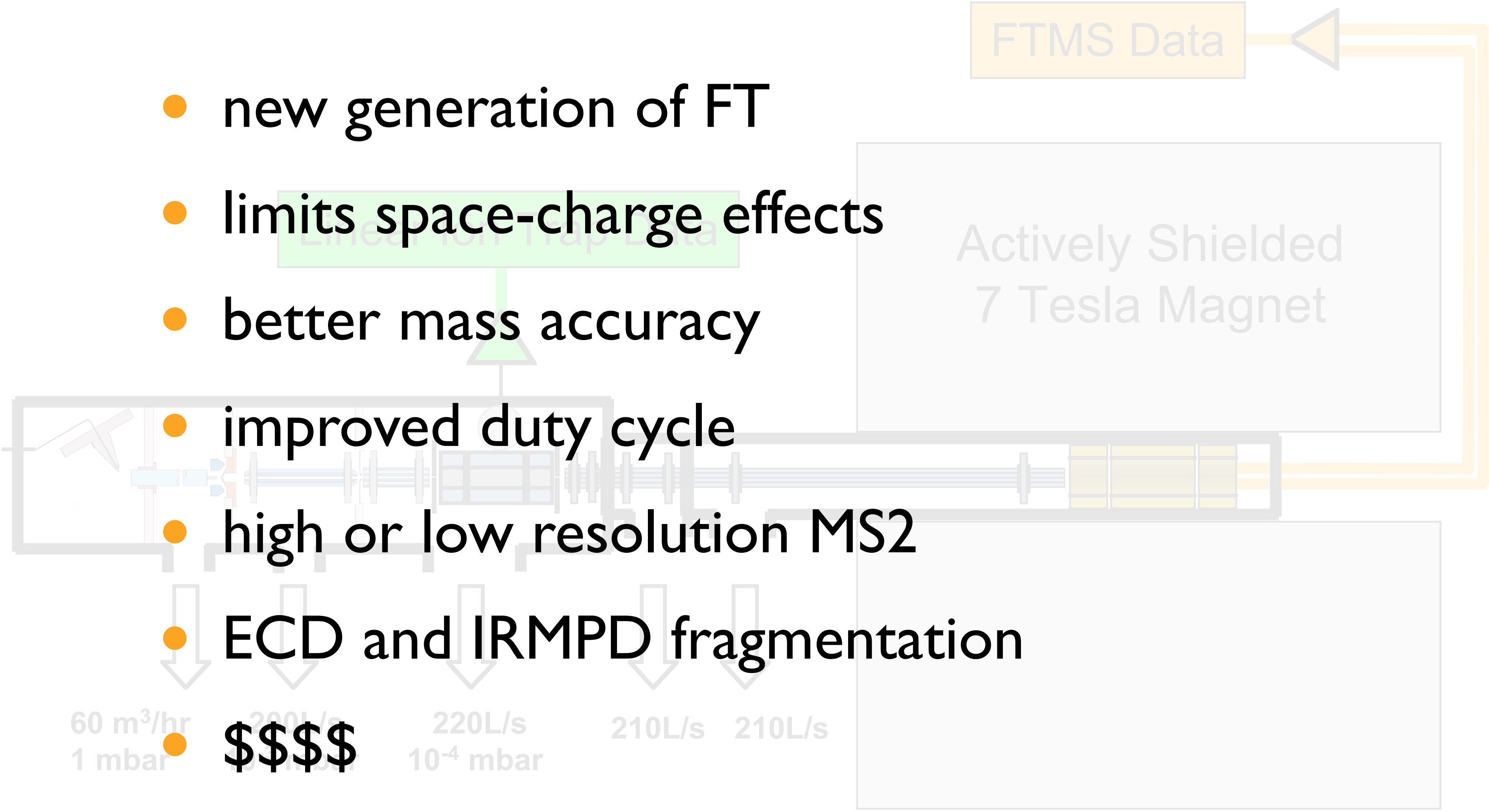
- good resolving power
- tandem MS on a TOF
- dynamic DB searching
- MALDI ion source
- no real precursor or NL scans
- fewer MSMS identifications
- low throughput
- $$$

quad/trap FTICR

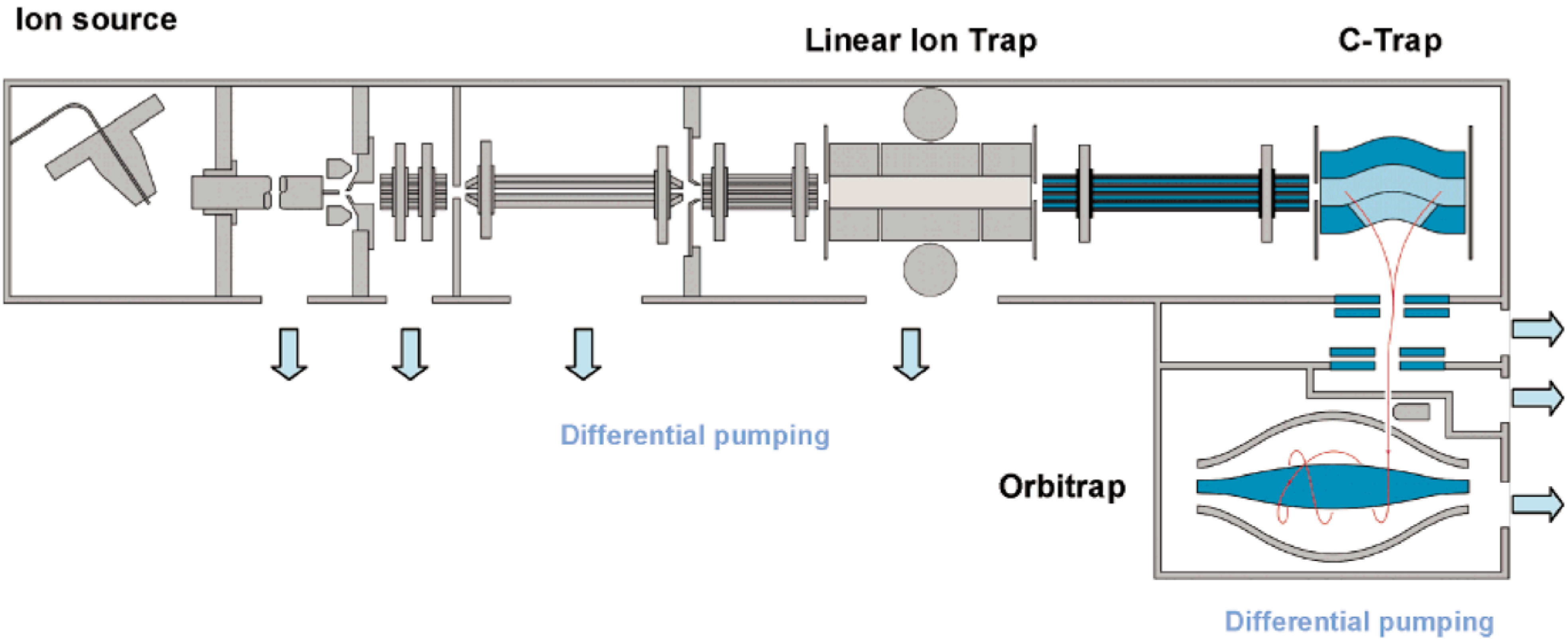


quad/trap FTICR

- new generation of FT
- limits space-charge effects
- better mass accuracy
- improved duty cycle
- high or low resolution MS2
- ECD and IRMPD fragmentation
- $$$$

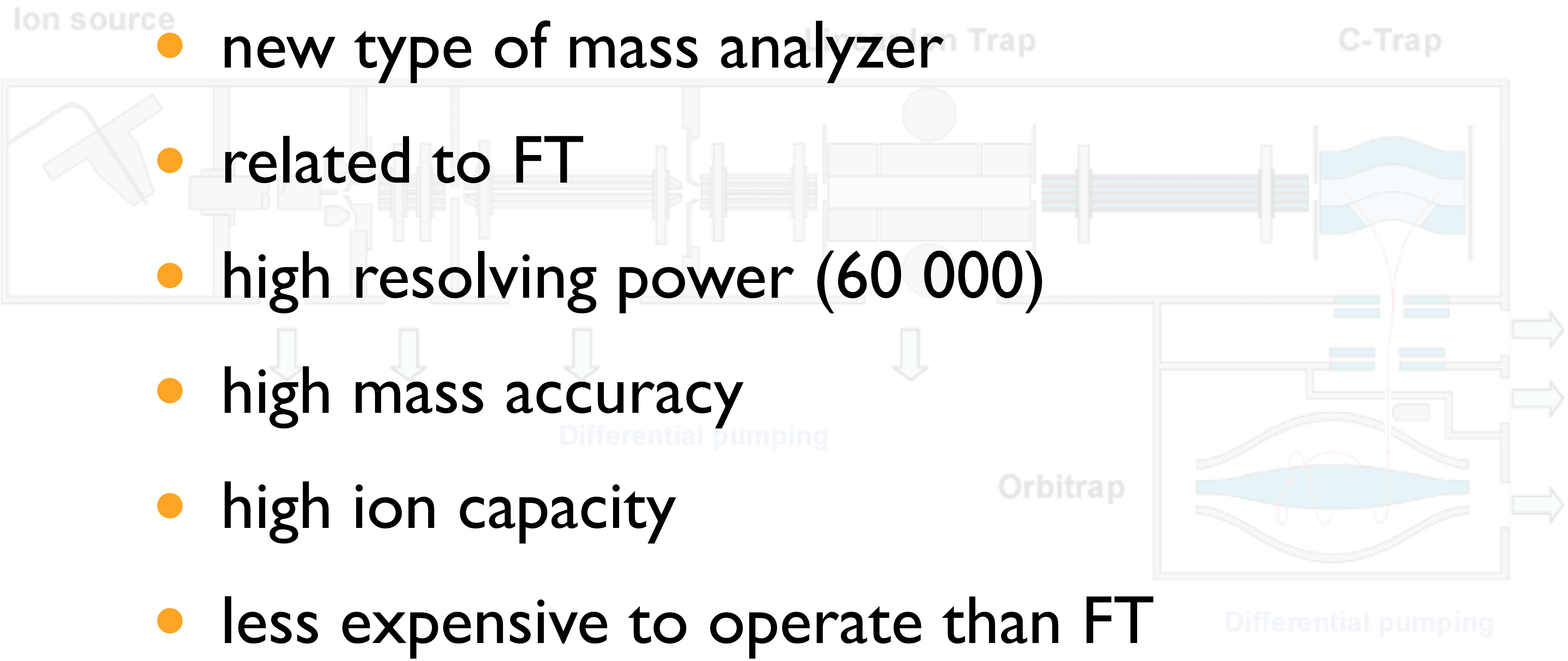


LTQ-orbitrap



Yates, J. R.; Cociorva, D.; Liao, L.; Zabrouskov, V. Anal. Chem. 2006, 78, 493-500.

LTQ-orbitrap



- new type of mass analyzer
- related to FT
- high resolving power (60 000)
- high mass accuracy
- high ion capacity
- less expensive to operate than FT

Instrumentation summary

Table 1. Characteristics and performances of commonly used types of mass spectrometers. Check marks indicate available, check marks in parentheses indicate optional. +, ++, and +++ indicate possible or moderate, good or high, and excellent or very high, respectively. Seq., sequential.

	IT-LIT	Q-Q-ToF	ToF-ToF	FT-ICR	Q-Q-Q	QQ-LIT
Mass accuracy	Low	Good	Good	Excellent	Medium	Medium
Resolving power	Low	Good	High	Very high	Low	Low
Sensitivity (LOD)	Good		High	Medium	High	High
Dynamic range	Low	Medium	Medium	Medium	High	High
ESI	✓	✓		✓	✓	✓
MALDI	(✓)	(✓)	✓			
MS/MS capabilities	✓	✓	✓	✓	✓	✓
Additional capabilities	Seq. MS/MS				Precursor, Neutral loss, MRM	
Identification	++	++	++	+++	+	+
Quantification	+	+++	++	++	+++	+++
Throughput	+++	++	+++	++	++	++
Detection of modifications	+	+	+	+		+++

Domon, B., Aebersold., R., *Mass Spectrometry and Protein Analysis*, Science 312, 2006.

Quantification

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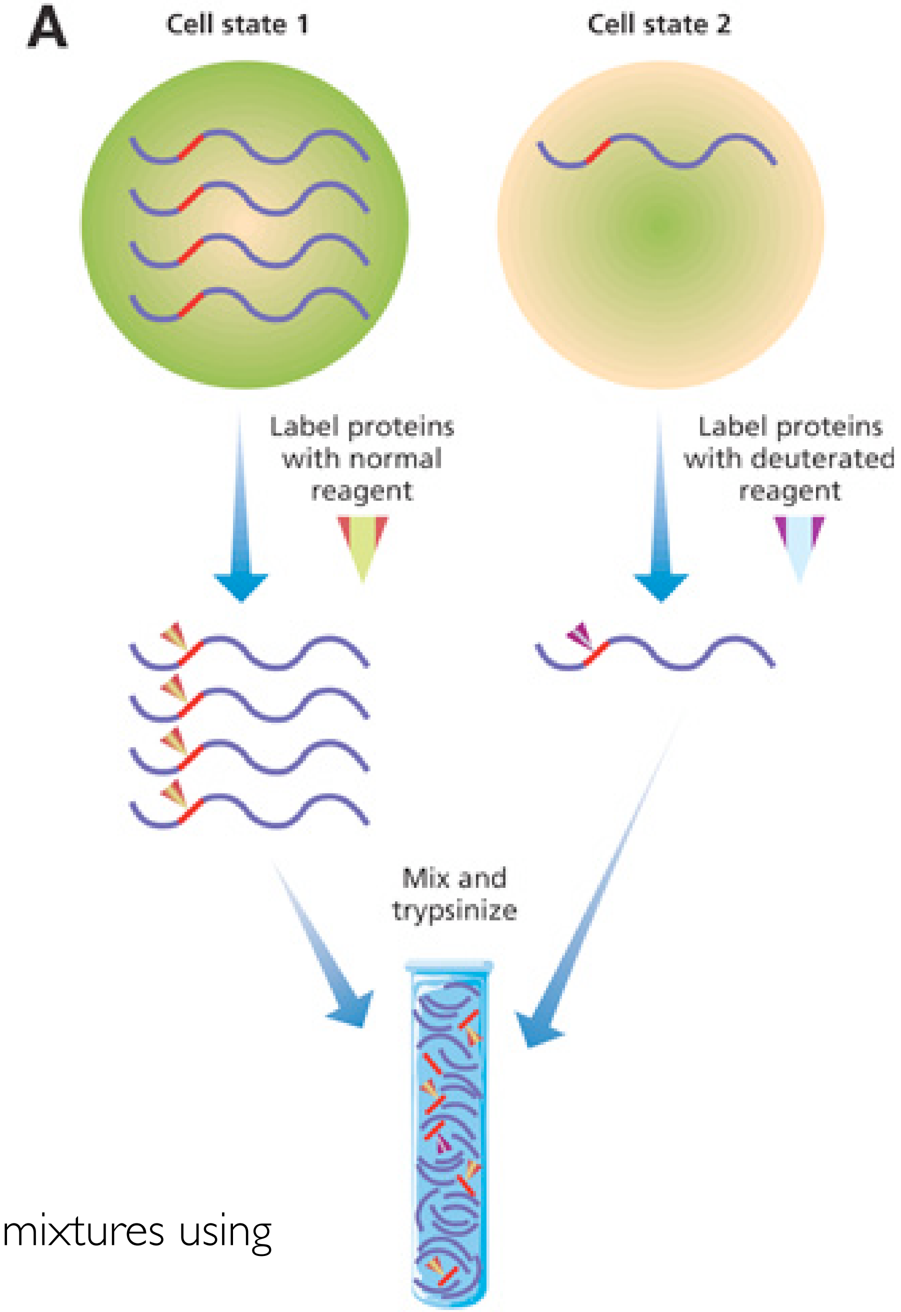
ICAT

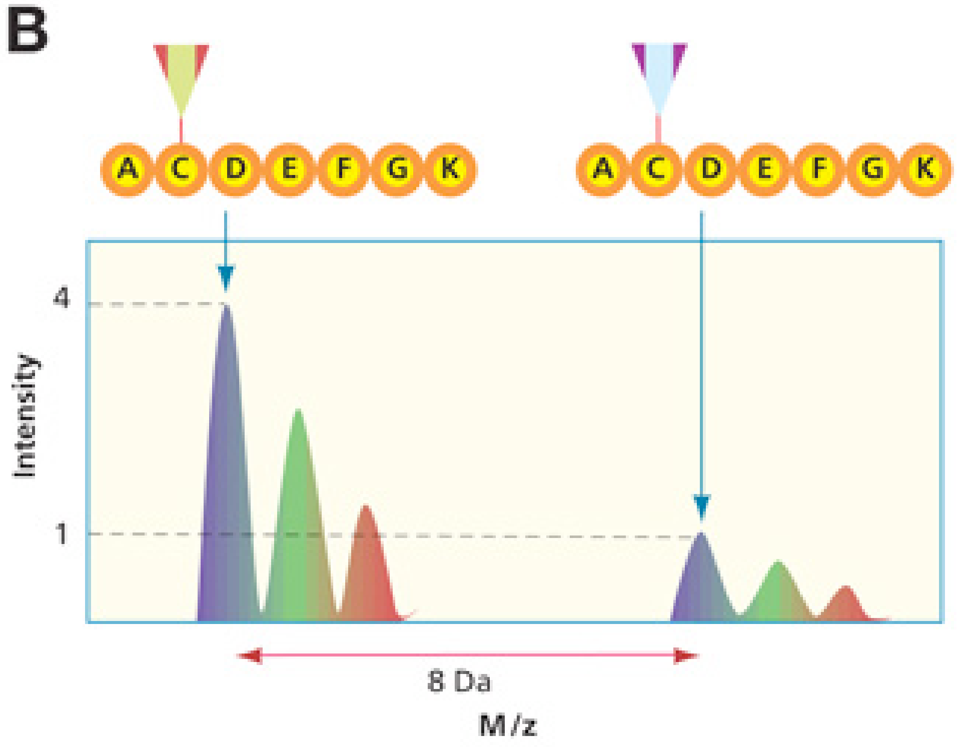
- 2 cell states
- Label with light and heavy reagent
- Combine and digest



Gygi, S. P., et al. (1999). "Quantitative analysis of complex protein mixtures using isotope-coded affinity tags." Nature biotechnology 17: 994-999.

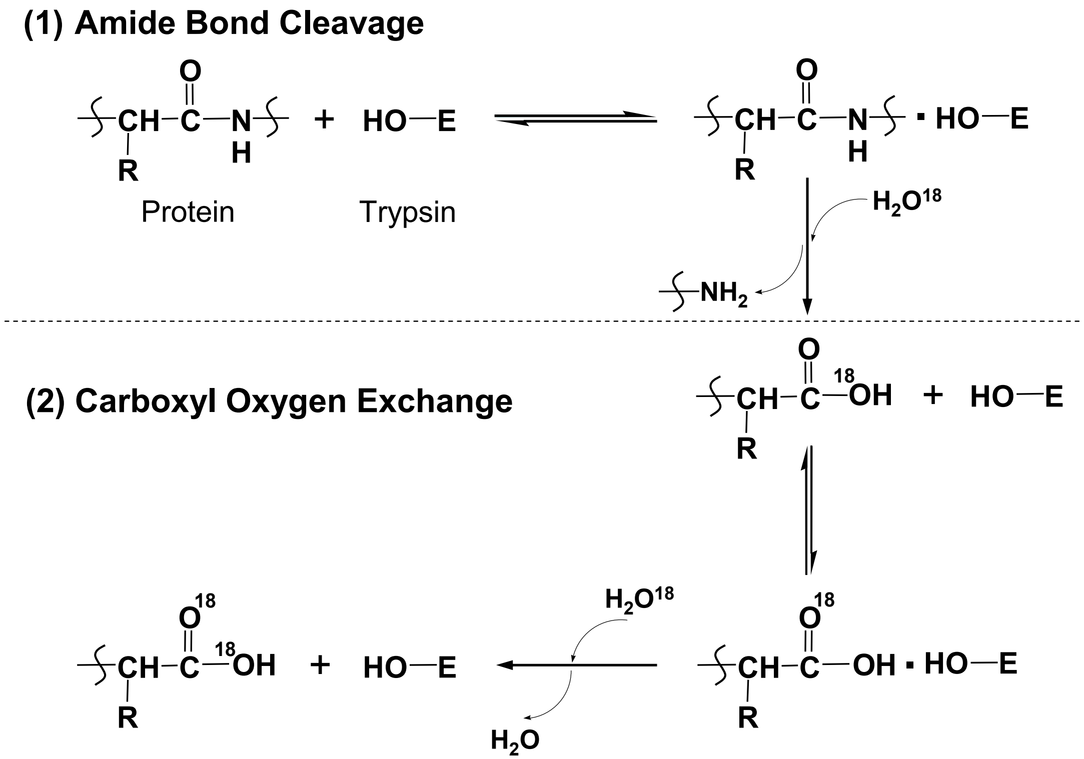
ICAT

- Retrieve cystein-containing peptide
- Analyze by MS
- Find pairs separated by 8 Da



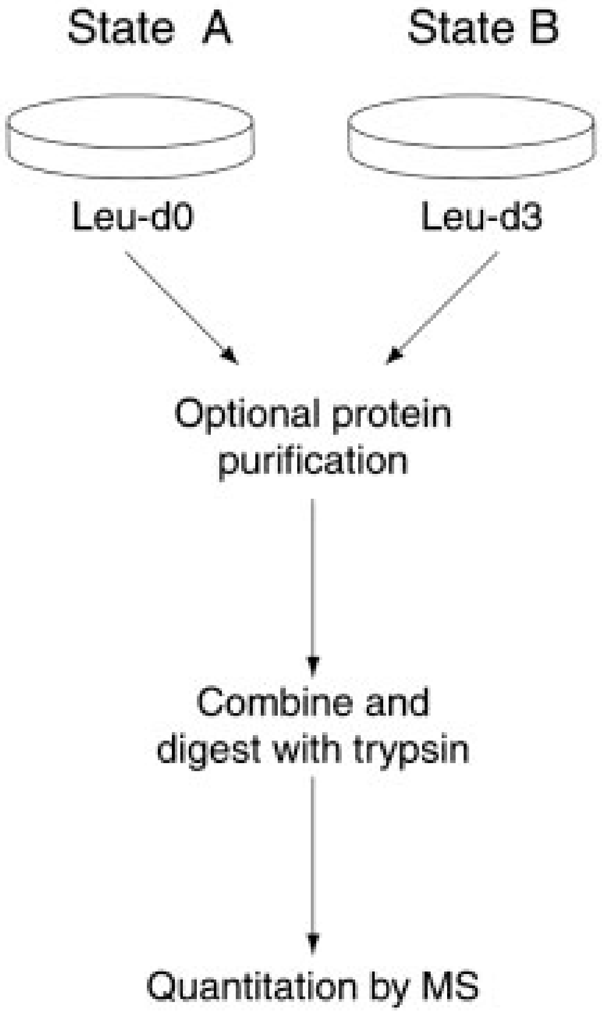
18O labeling

- metabolic process
- 4 dalton separation
- trypsin digestion performed in labeled water



SILAC

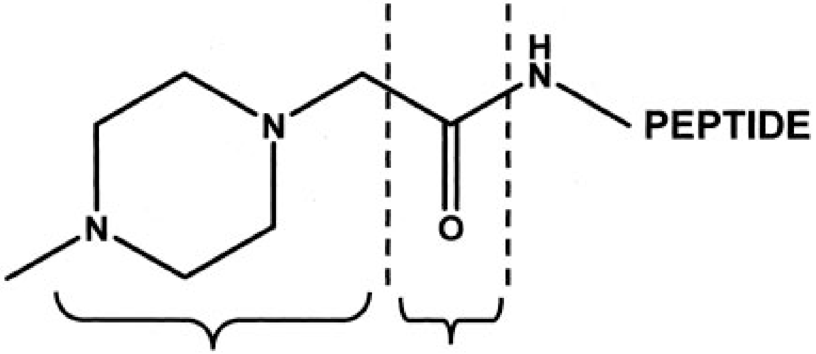
- Culture medium contains labeled amino acid
- Let the cells incorporate the labels
- Difficult for mammalian cells
- Multiplicity of labels



Ong, S.-E. et al., *Stable Isotope Labeling by Amino Acids in Cell Culture, SILAC, as a Simple and Accurate Approach to Expression Proteomics*, Mol. Cell. Proteomics 2002, 1.

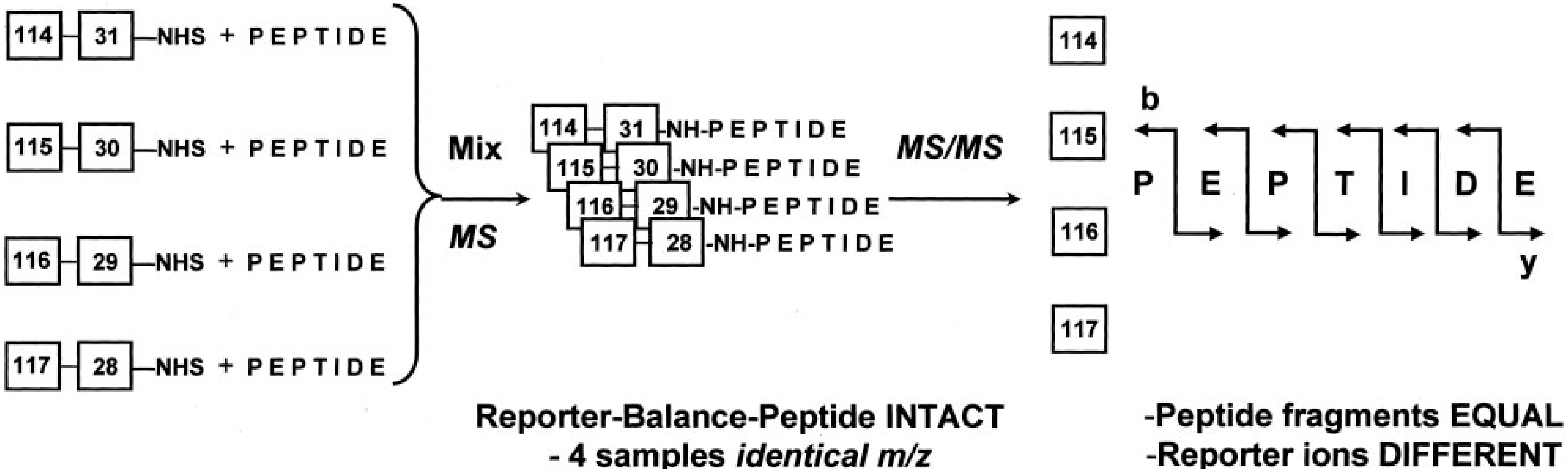
iTRAQ

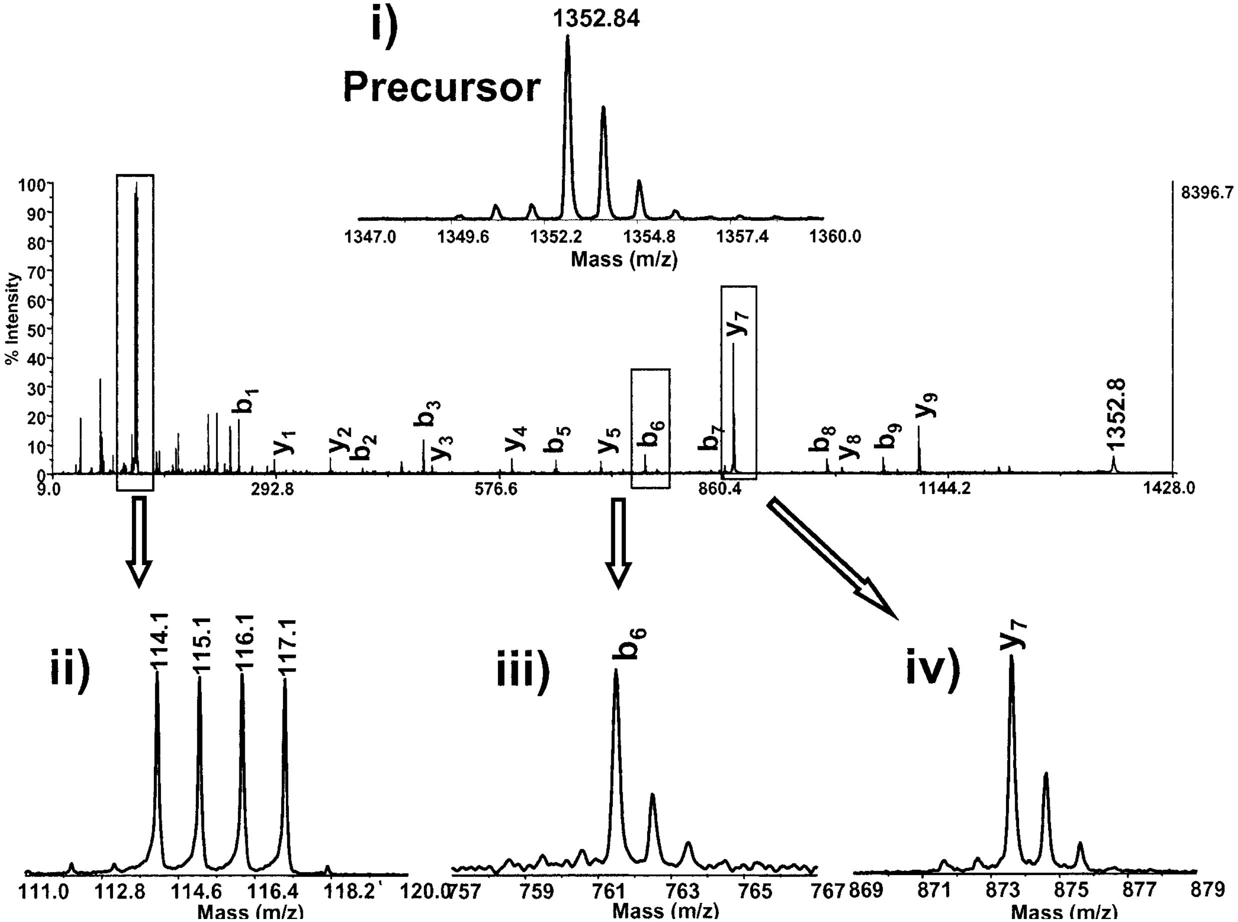
- Reagent attached to the N-terminal
- All cell states have the same precursor masse
- Quantitation is performed in MS-MS



m/z 114	(+1)	^{13}C	^{13}C ^{18}O	(+3)
m/z 115	(+2)	$^{13}C_2$	^{18}O	(+2)
m/z 116	(+3)	$^{13}C_2$ ^{15}N	^{13}C	(+1)
m/z 117	(+4)	$^{13}C_3$ ^{15}N		(+0)

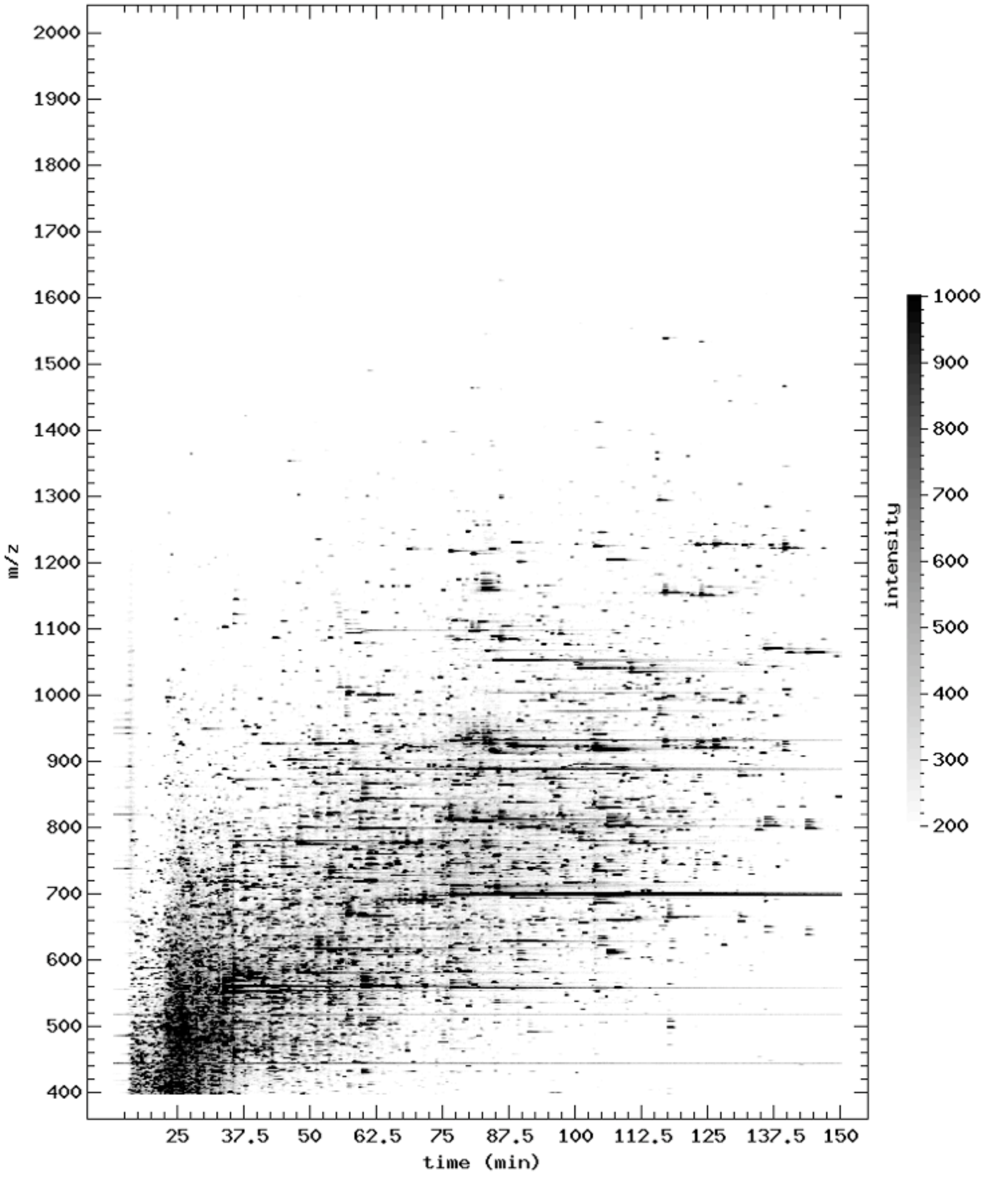
Ross, P. L et al. (2004), *Multiplexed Protein Quantitation in Saccharomyces cerevisiae Using Amine-reactive Isobaric Tagging Reagents*, Mol. Cell. Proteomics 2004, 2, 1154-1169.


114 31 NHS + PEPTIDE
115 30 NHS + PEPTIDE
116 29 NHS + PEPTIDE
117 28 NHS + PEPTIDE
Mix
MS
114 31 -NH-PEPTIDE
115 30 -NH-PEPTIDE
116 29 -NH-PEPTIDE
117 28 -NH-PEPTIDE
MS/MS
114
115
116
117
b
P E P T I D E
y
Reporter-Balance-Peptide INTACT
- 4 samples identical m/z
-Peptide fragments EQUAL
-Reporter ions DIFFERENT

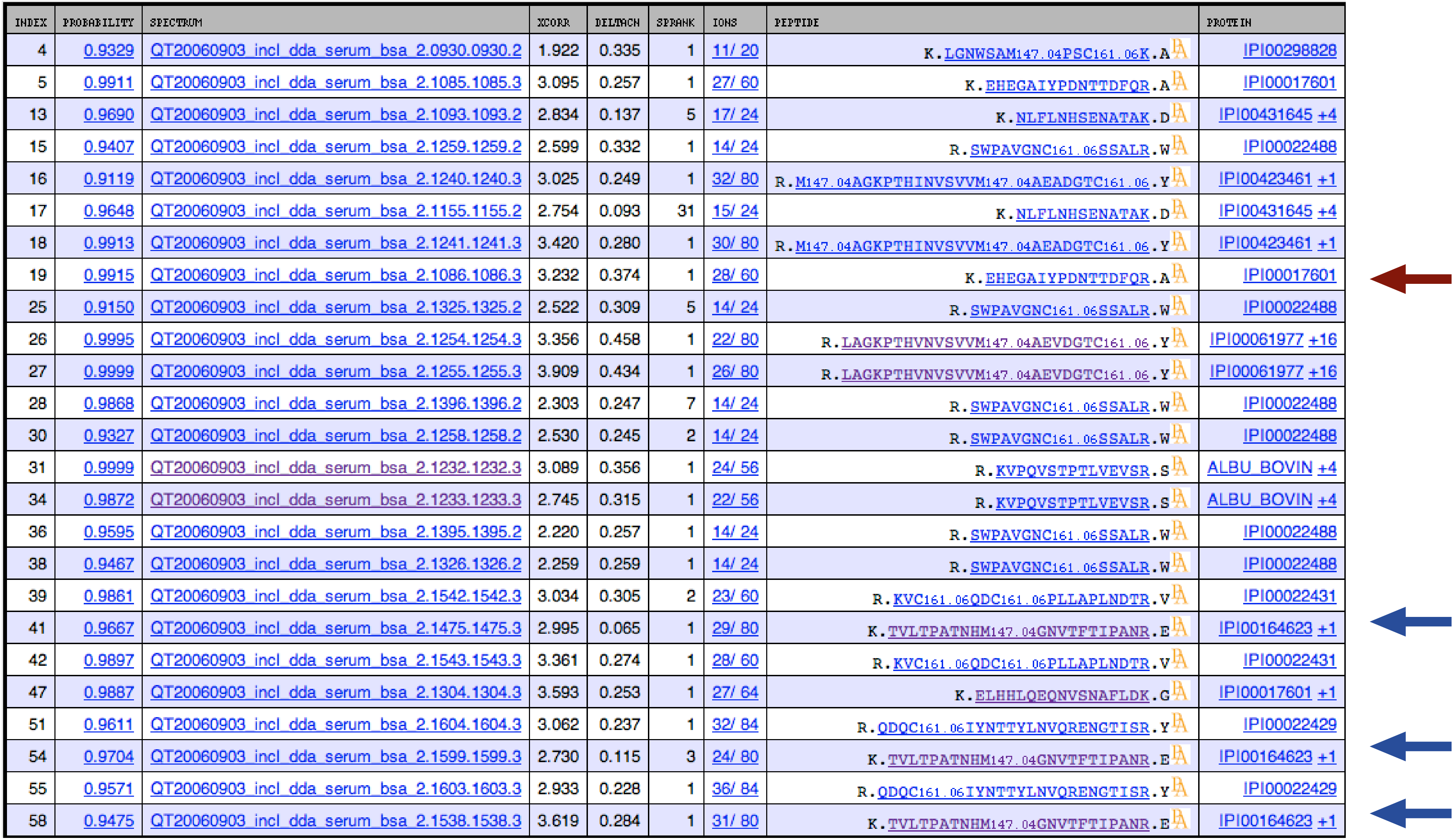

i)
Precursor
1352.84
1347.0
1349.6
1352.2
1354.8
1357.4
1360.0
Mass (m/z)
8396.7
% Intensity
100
90
80
70
60
50
40
30
20
10
0
b1 y1 y2 b2 b3 y3 y4 b5 y5 b6 b7 y7 b8 y8 b9 y9
1352.8
9.0
292.8
576.6
860.4
1144.2
1428.0
ii)
114.1
115.1
116.1
117.1
111.0
112.8
114.6
116.4
118.2
120.0
Mass (m/z)
iii)
b6
757
759
761
763
765
767
Mass (m/z)
iv)
y7
869
871
873
875
877
879
Mass (m/z)

MS1 quantitation (Label-free)

- Compare same peptide in different runs
- Need to align runs
- Computationally intensive
- Internal standards helps



Spectral counting



INDEX	PROBABILITY	SPECTRUM	XCORR	DELTACN	SPRANK	IONS	PEPTIDE	PROTEIN
4	0.9329	QT20060903_incl_dda_serum_bsa_2.0930.0930.2	1.922	0.335	1	11/ 20	K.LGNWSAM147.04PSC161.06K.A	IPI00298828
5	0.9911	QT20060903_incl_dda_serum_bsa_2.1085.1085.3	3.095	0.257	1	27/ 60	K.EHEGAIYPDNTTDFQR.A	IPI00017601
13	0.9690	QT20060903_incl_dda_serum_bsa_2.1093.1093.2	2.834	0.137	5	17/ 24	K.NLFLNHSENATAK.D	IPI00431645 +4
15	0.9407	QT20060903_incl_dda_serum_bsa_2.1259.1259.2	2.599	0.332	1	14/ 24	R.SWPAVGNC161.06SSALR.W	IPI00022488
16	0.9119	QT20060903_incl_dda_serum_bsa_2.1240.1240.3	3.025	0.249	1	32/ 80	R.M147.04AGKPTHINVSVVM147.04AEADGTC161.06.Y	IPI00423461 +1
17	0.9648	QT20060903_incl_dda_serum_bsa_2.1155.1155.2	2.754	0.093	31	15/ 24	K.NLFLNHSENATAK.D	IPI00431645 +4
18	0.9913	QT20060903_incl_dda_serum_bsa_2.1241.1241.3	3.420	0.280	1	30/ 80	R.M147.04AGKPTHINVSVVM147.04AEADGTC161.06.Y	IPI00423461 +1
19	0.9915	QT20060903_incl_dda_serum_bsa_2.1086.1086.3	3.232	0.374	1	28/ 60	K.EHEGAIYPDNTTDFQR.A	IPI00017601
25	0.9150	QT20060903_incl_dda_serum_bsa_2.1325.1325.2	2.522	0.309	5	14/ 24	R.SWPAVGNC161.06SSALR.W	IPI00022488
26	0.9995	QT20060903_incl_dda_serum_bsa_2.1254.1254.3	3.356	0.458	1	22/ 80	R.LAGKPTHVNVSVVM147.04AEVDGTC161.06.Y	IPI00061977 +16
27	0.9999	QT20060903_incl_dda_serum_bsa_2.1255.1255.3	3.909	0.434	1	26/ 80	R.LAGKPTHVNVSVVM147.04AEVDGTC161.06.Y	IPI00061977 +16
28	0.9868	QT20060903_incl_dda_serum_bsa_2.1396.1396.2	2.303	0.247	7	14/ 24	R.SWPAVGNC161.06SSALR.W	IPI00022488
30	0.9327	QT20060903_incl_dda_serum_bsa_2.1258.1258.2	2.530	0.245	2	14/ 24	R.SWPAVGNC161.06SSALR.W	IPI00022488
31	0.9999	QT20060903_incl_dda_serum_bsa_2.1232.1232.3	3.089	0.356	1	24/ 56	R.KVPQVSTPTLVEVSR.S	ALBU_BOVIN +4
34	0.9872	QT20060903_incl_dda_serum_bsa_2.1233.1233.3	2.745	0.315	1	22/ 56	R.KVPQVSTPTLVEVSR.S	ALBU_BOVIN +4
36	0.9595	QT20060903_incl_dda_serum_bsa_2.1395.1395.2	2.220	0.257	1	14/ 24	R.SWPAVGNC161.06SSALR.W	IPI00022488
38	0.9467	QT20060903_incl_dda_serum_bsa_2.1326.1326.2	2.259	0.259	1	14/ 24	R.SWPAVGNC161.06SSALR.W	IPI00022488
39	0.9861	QT20060903_incl_dda_serum_bsa_2.1542.1542.3	3.034	0.305	2	23/ 60	R.KVC161.06QDC161.06PLLAPLNDTR.V	IPI00022431
41	0.9667	QT20060903_incl_dda_serum_bsa_2.1475.1475.3	2.995	0.065	1	29/ 80	K.TVLTPATNHM147.04GNVTFTIPANR.E	IPI00164623 +1
42	0.9897	QT20060903_incl_dda_serum_bsa_2.1543.1543.3	3.361	0.274	1	28/ 60	R.KVC161.06QDC161.06PLLAPLNDTR.V	IPI00022431
47	0.9887	QT20060903_incl_dda_serum_bsa_2.1304.1304.3	3.593	0.253	1	27/ 64	K.ELHHLQEQNVSNAFLDK.G	IPI00017601 +1
51	0.9611	QT20060903_incl_dda_serum_bsa_2.1604.1604.3	3.062	0.237	1	32/ 84	R.QDQC161.06IYNTTYLNVQRENGTISR.Y	IPI00022429
54	0.9704	QT20060903_incl_dda_serum_bsa_2.1599.1599.3	2.730	0.115	3	24/ 80	K.TVLTPATNHM147.04GNVTFTIPANR.E	IPI00164623 +1
55	0.9571	QT20060903_incl_dda_serum_bsa_2.1603.1603.3	2.933	0.228	1	36/ 84	R.QDQC161.06IYNTTYLNVQRENGTISR.Y	IPI00022429
58	0.9475	QT20060903_incl_dda_serum_bsa_2.1538.1538.3	3.619	0.284	1	31/ 80	K.TVLTPATNHM147.04GNVTFTIPANR.E	IPI00164623 +1

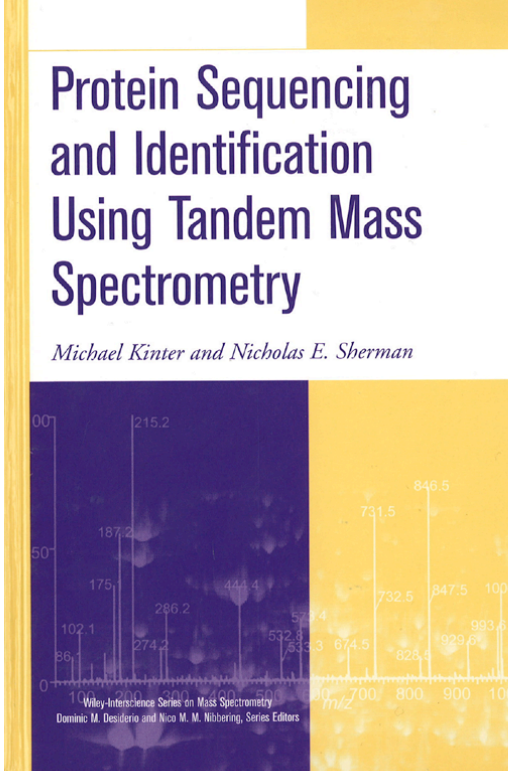
Spectral counting

- MSMS-based method
- Data is searched with Sequest or Mascot
- Count the number of hits per peptide
- Indirect assessment of abundance
- Robust for high abundance peptides
- Quantify the big trends in the data
- 2 orders of magnitude dynamic range
- Simple to do perform

Quantification summary

technique	software	+	-
ICAT	ASAP ratio Xpress	sample enrichment	only cystein peptides
18O	-	cheap	small mass difference
SILAC	ASAP ratio	choice in aa target	need to grow cells
iTRAQ	LIBRA	no interference	most intense peaks
Label-free	Corra	wide dynamic range	sensitive to exp. conditions
Spectral Counting	Sequest Mascot	cheap, easy, works on legacy data	limited dynamic range

References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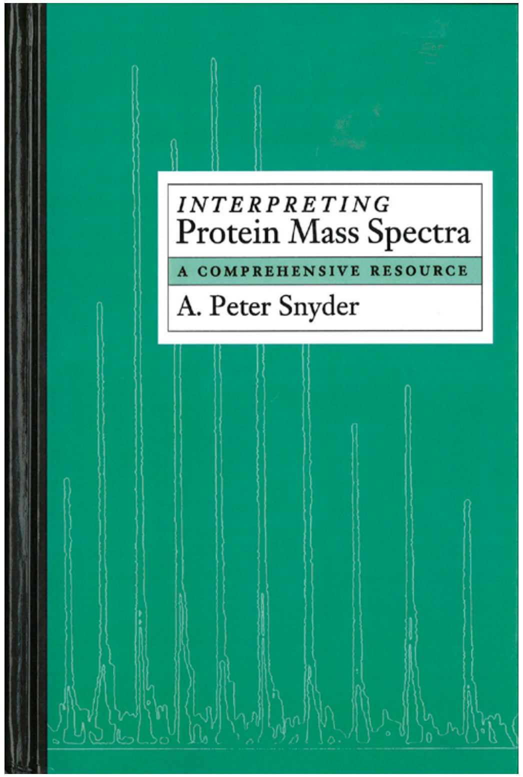
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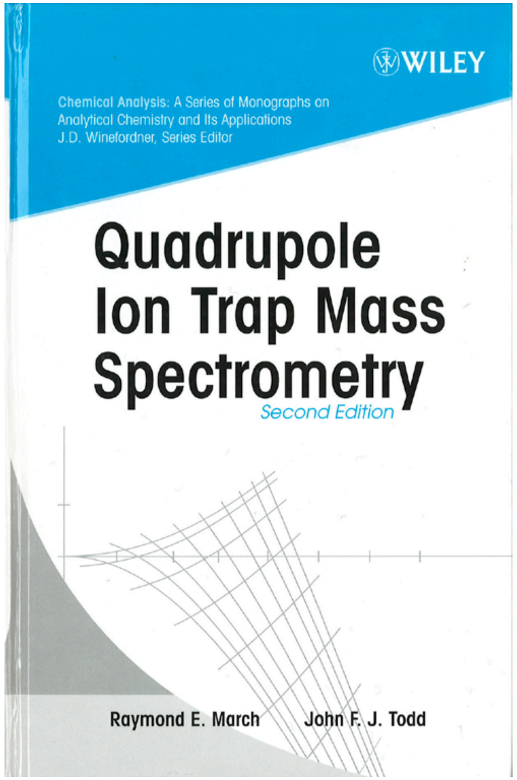
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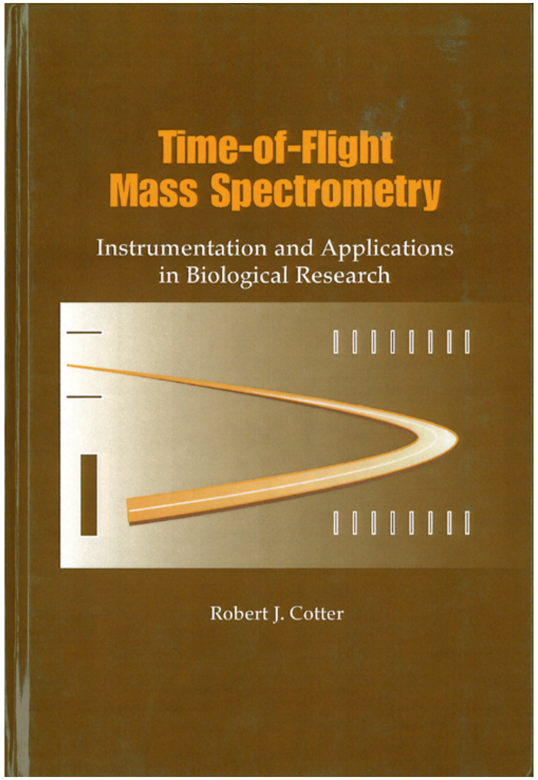
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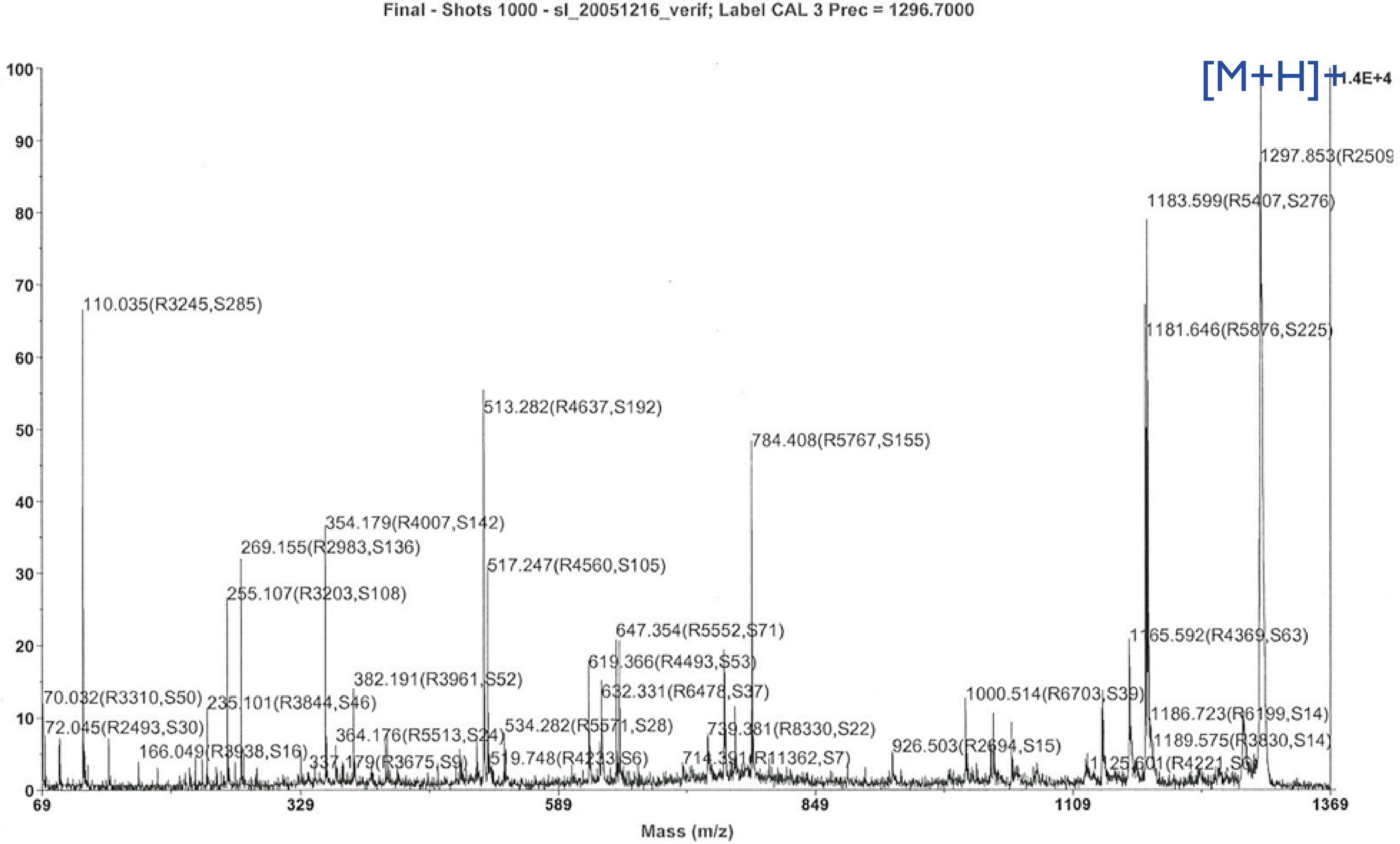
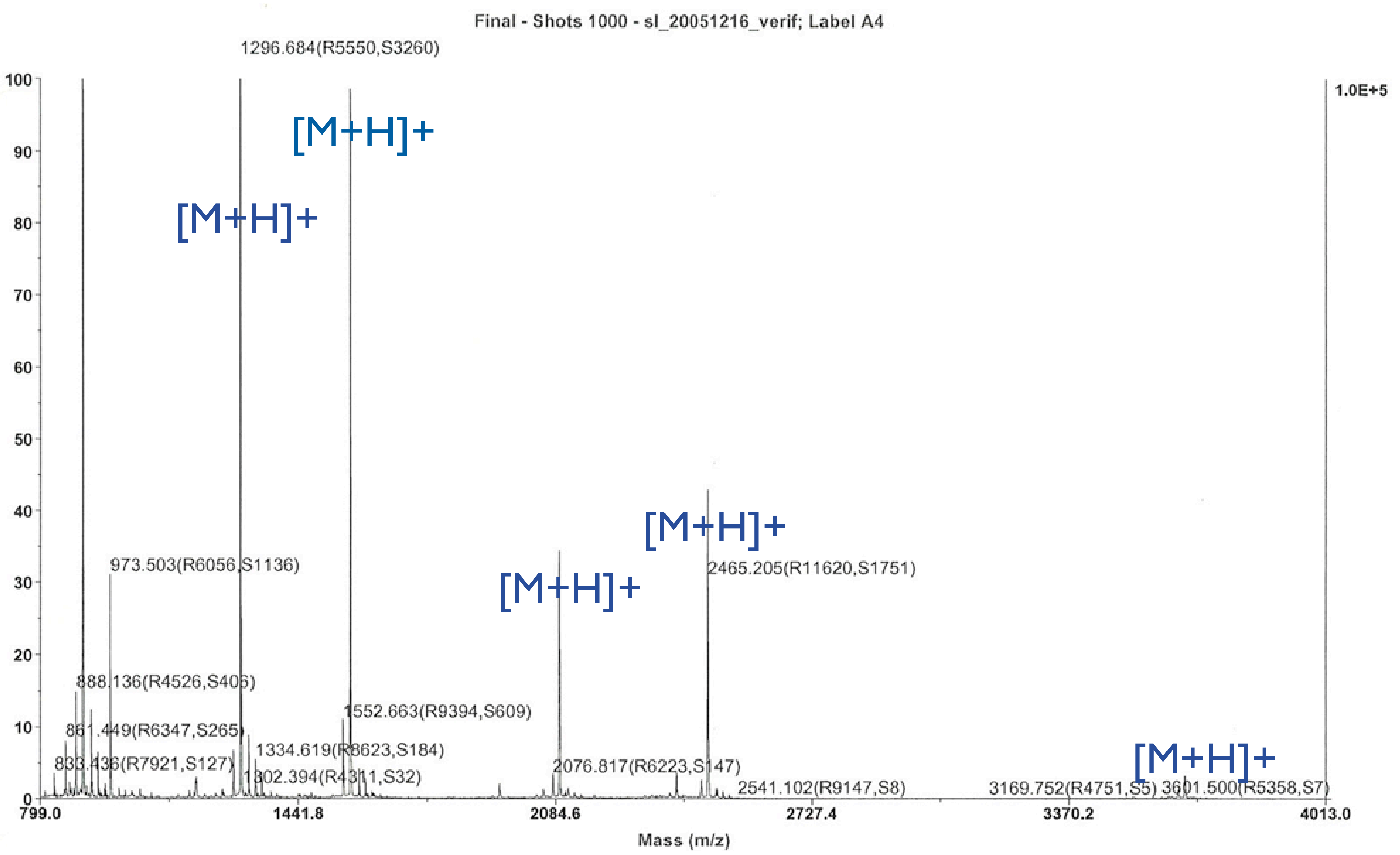
Fragment ions

|<----------------------fragments ------------------------>|

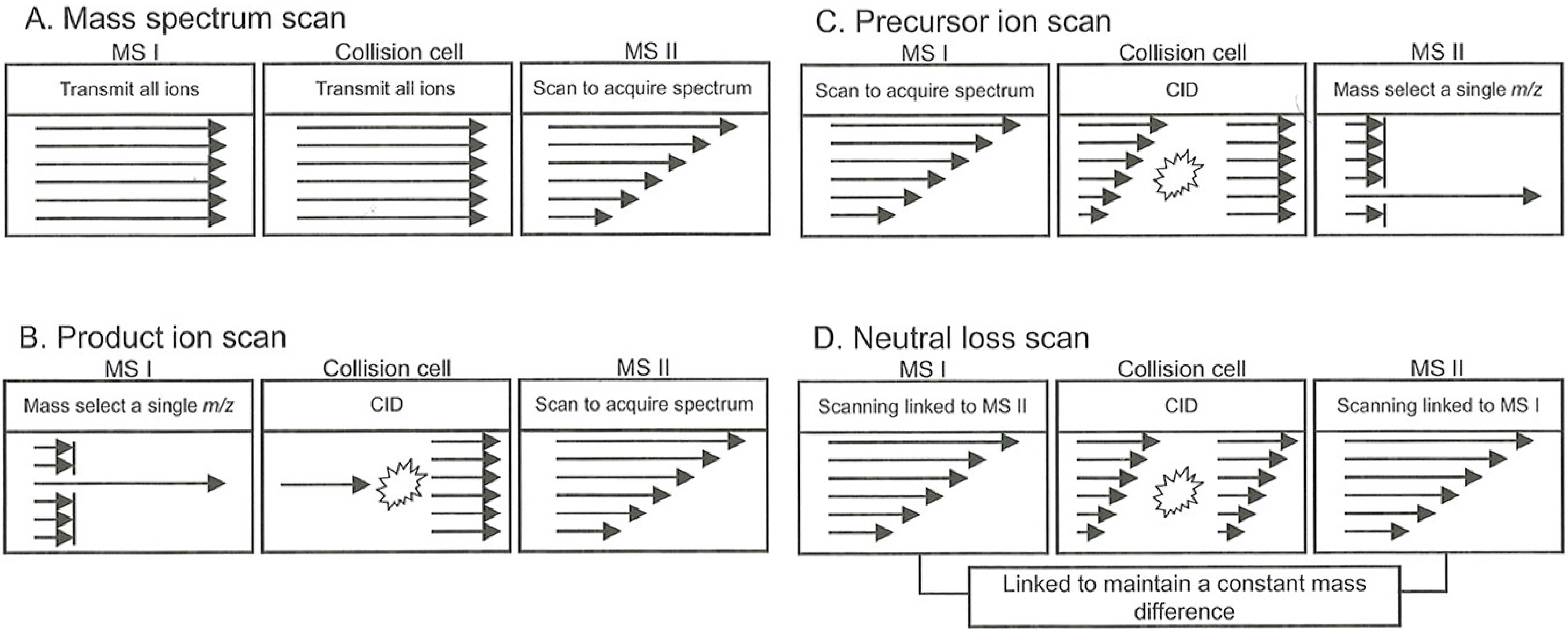


[M+H]+

(pseudo)Molecular ions



Types of scans



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