Τίτλος διαλέξεων:

Φασματομετρία Μάζας για Βιοχημικούς και Βιολόγους

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Τμήμα Χημείας
Πανεπιστήμιο Κρήτης

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Γραφείο: Α206

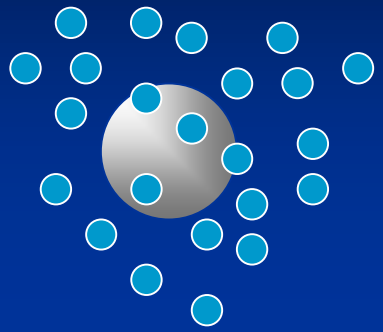
Εφαρμογές Τεχνικών Φασματομετρίας Μάζας

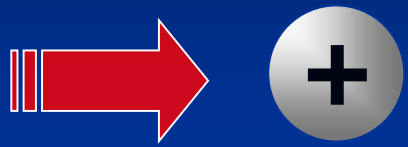
- Χημικοί
  - Προσδιορισμός σχετικής μοριακής μάζας
  - Προσδιορισμός στοιχειακής σύστασης
  - Συμπληρωματικά στοιχεία για τον χαρακτηρισμό ενώσεων
  - Ταυτοποίηση ενώσεων
  - Ποσοτικοποίηση ιχνοποσοτήτων αναλύτη σε περιβαλλοντικά ή βιολογικά δείγματα

Εφαρμογές Τεχνικών Φασματομετρίας Μάζας

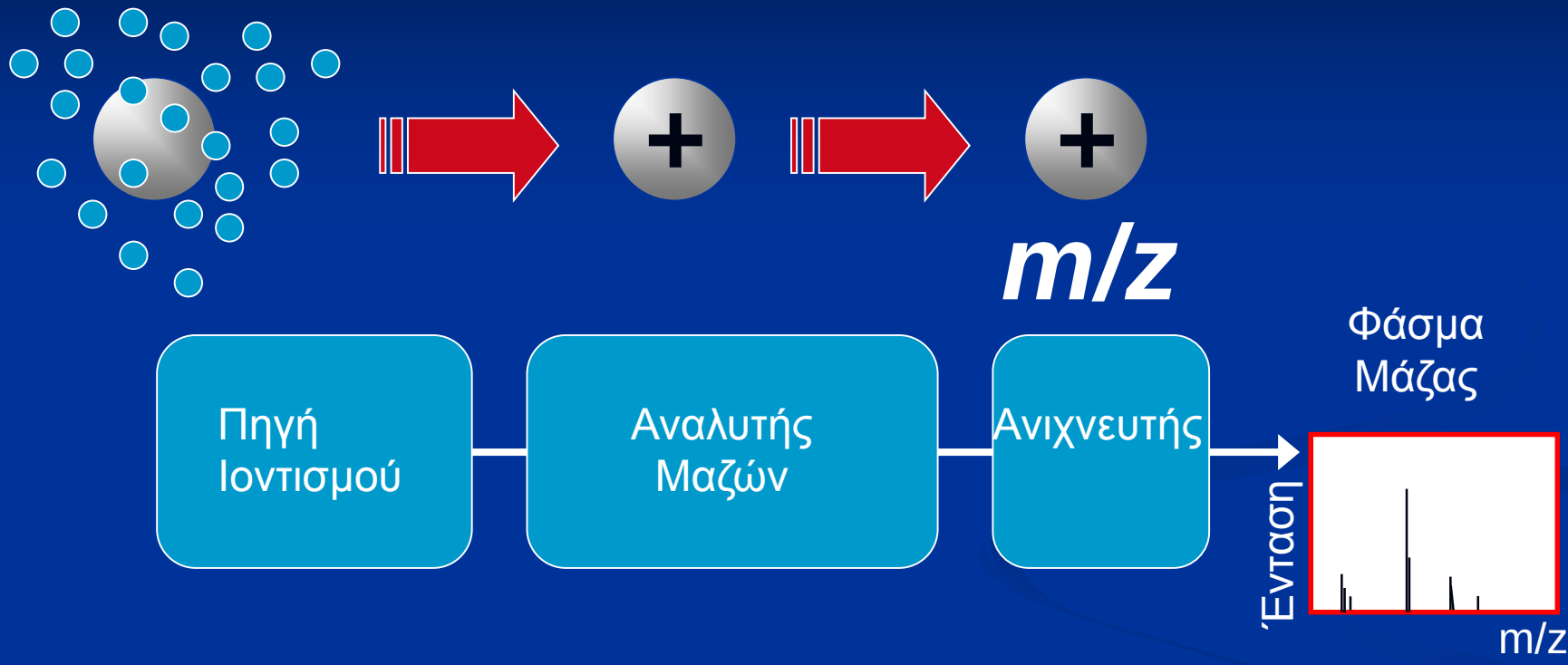
- Βιοχημικοί και Μοριακοί Βιολόγοι
  - Προσδιορισμός σχετικής μοριακής μάζας πρωτεϊνών και άλλων βιομορίων
  - Προσδιορισμός αλληλουχίας αμινοξέων σε πεπτίδια (proteomics)
  - Χαρακτηρισμός μεταβολιτών (metabolomics)
  - Μελέτη μη-ομοιοπολικών αλληλεπιδράσεων μεταξύ βιομορίων

Βασικές Διεργασίες MS

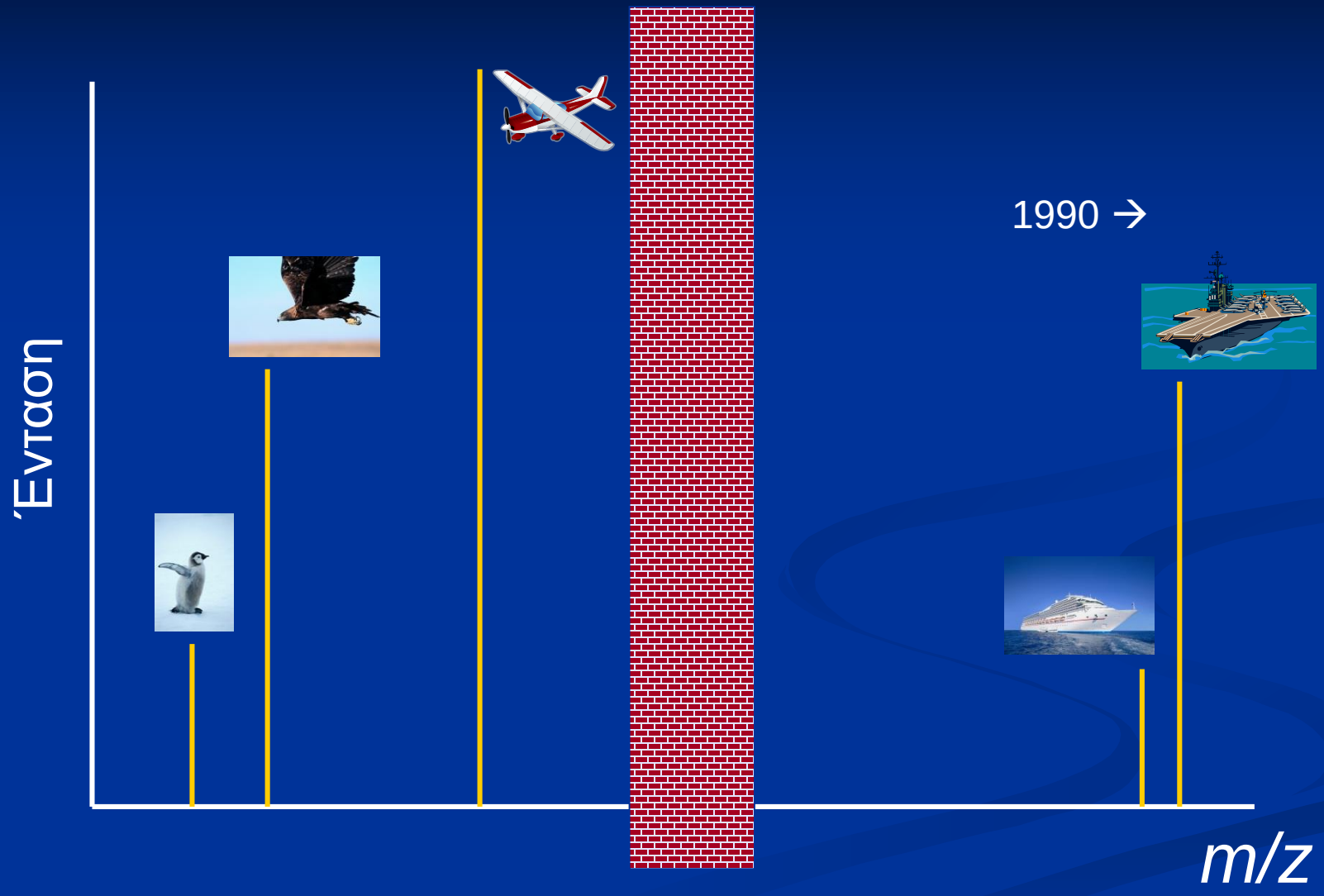




m/z


m/z
Πηγή Ιοντισμού
Αναλυτής Μαζών
Ανιχνευτής
Φάσμα Μάζας
Ένταση
m/z

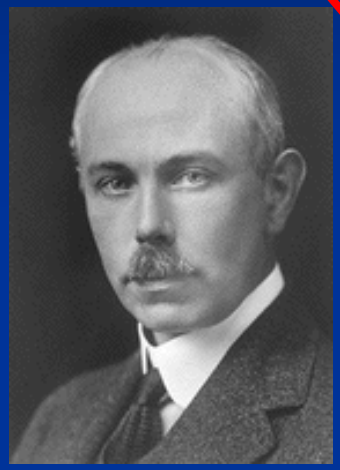
Φάσμα μάζας



Ιστορική Αναδρομή

J.J. Thomson

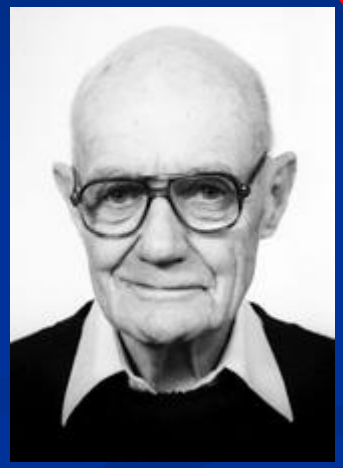
1906 Nobel Φυσικής



Francis W. Aston

1922 Nobel Χημείας

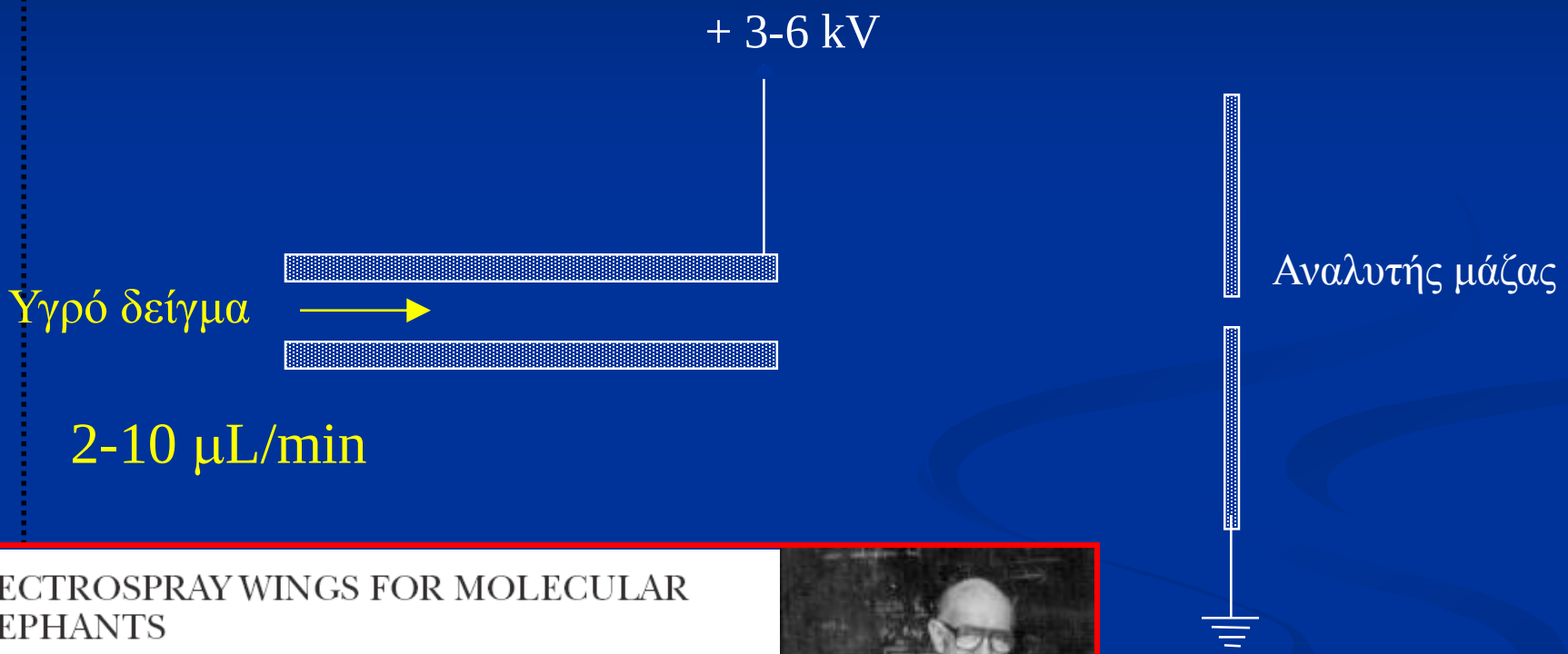
Koichi Tanaka



John B. Fenn

2002 Nobel Χημείας

ΦΑΣΜΑΤΟΜΕΤΡΙΑ ΜΑΖΩΝ ΗΛΕΚΤΡΟΨΕΚΑΣΜΟΥ
Electrospray Mass Spectrometry



2-10 μL/min

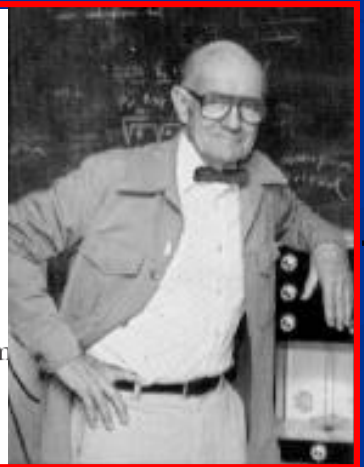
ELECTROSPRAY WINGS FOR MOLECULAR ELEPHANTS

Nobel Lecture, December 8, 2002

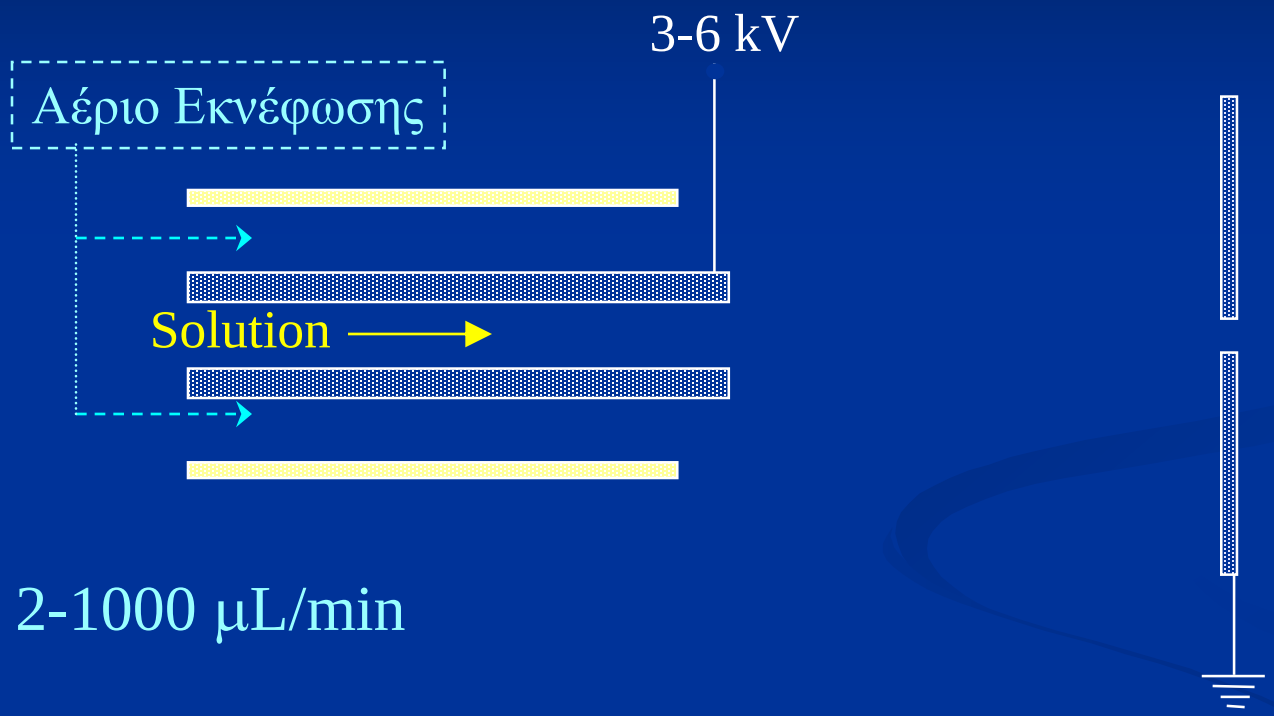
by

JOHN B. FENN

Department of Chemistry, Virginia Commonwealth University, Richm
Virginia 2384-2006, USA.

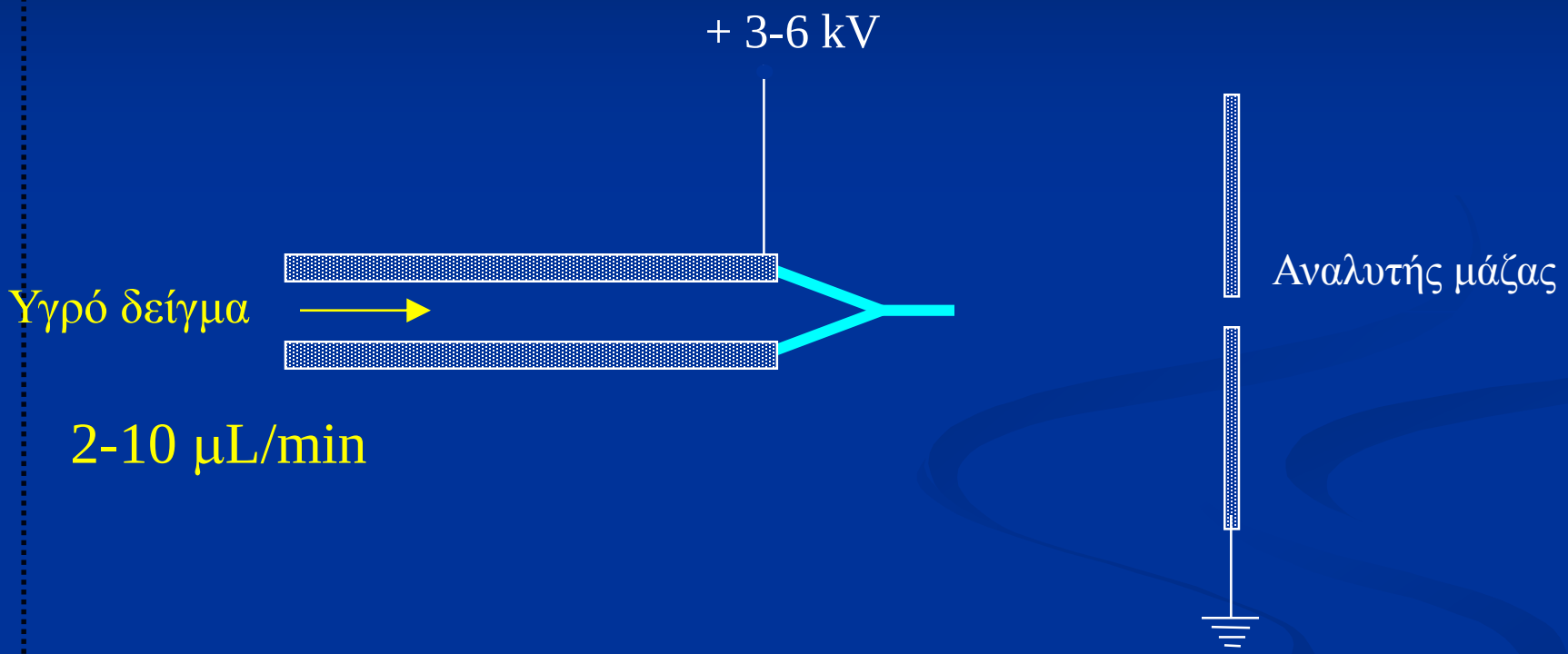


ΦΜ Ηλεκτροψεκασμού



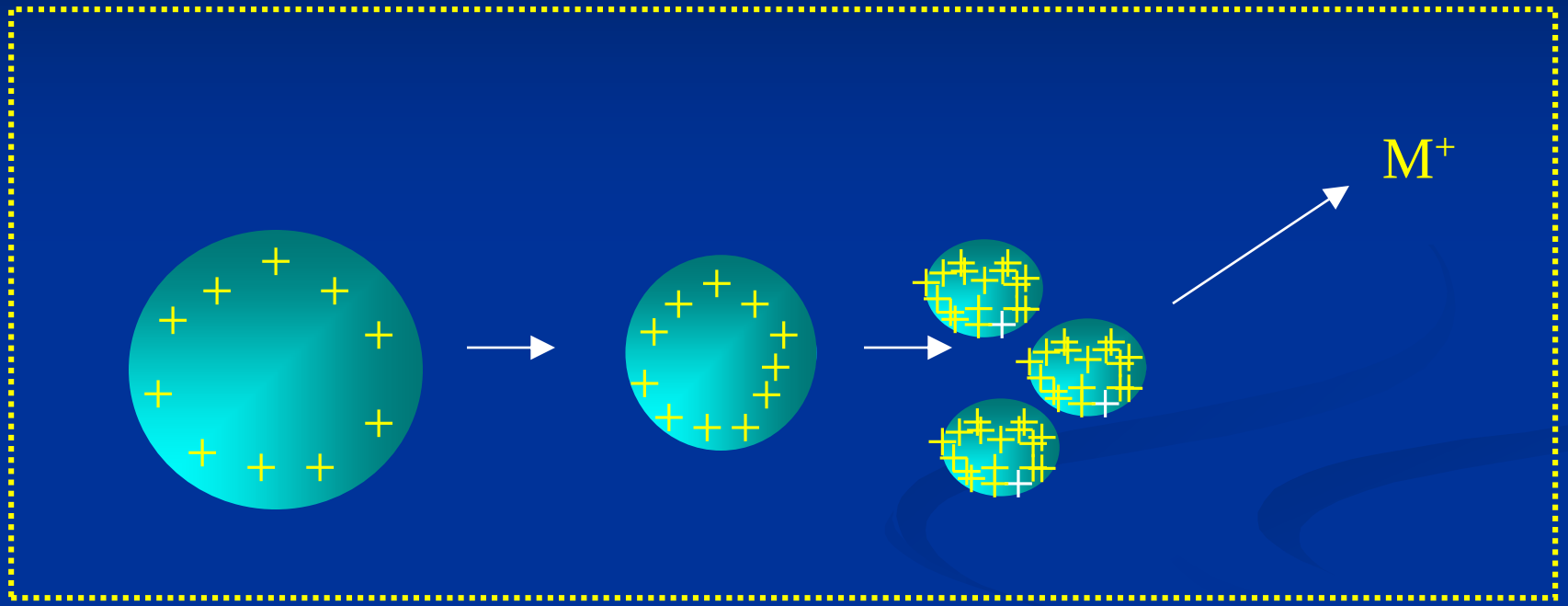
Ηλεκτροψεκασμός με βοήθεια αερίου εκνέφωσης

ΦΑΣΜΑΤΟΜΕΤΡΙΑ ΜΑΖΩΝ ΗΛΕΚΤΡΟΨΕΚΑΣΜΟΥ
Electrospray Mass Spectrometry

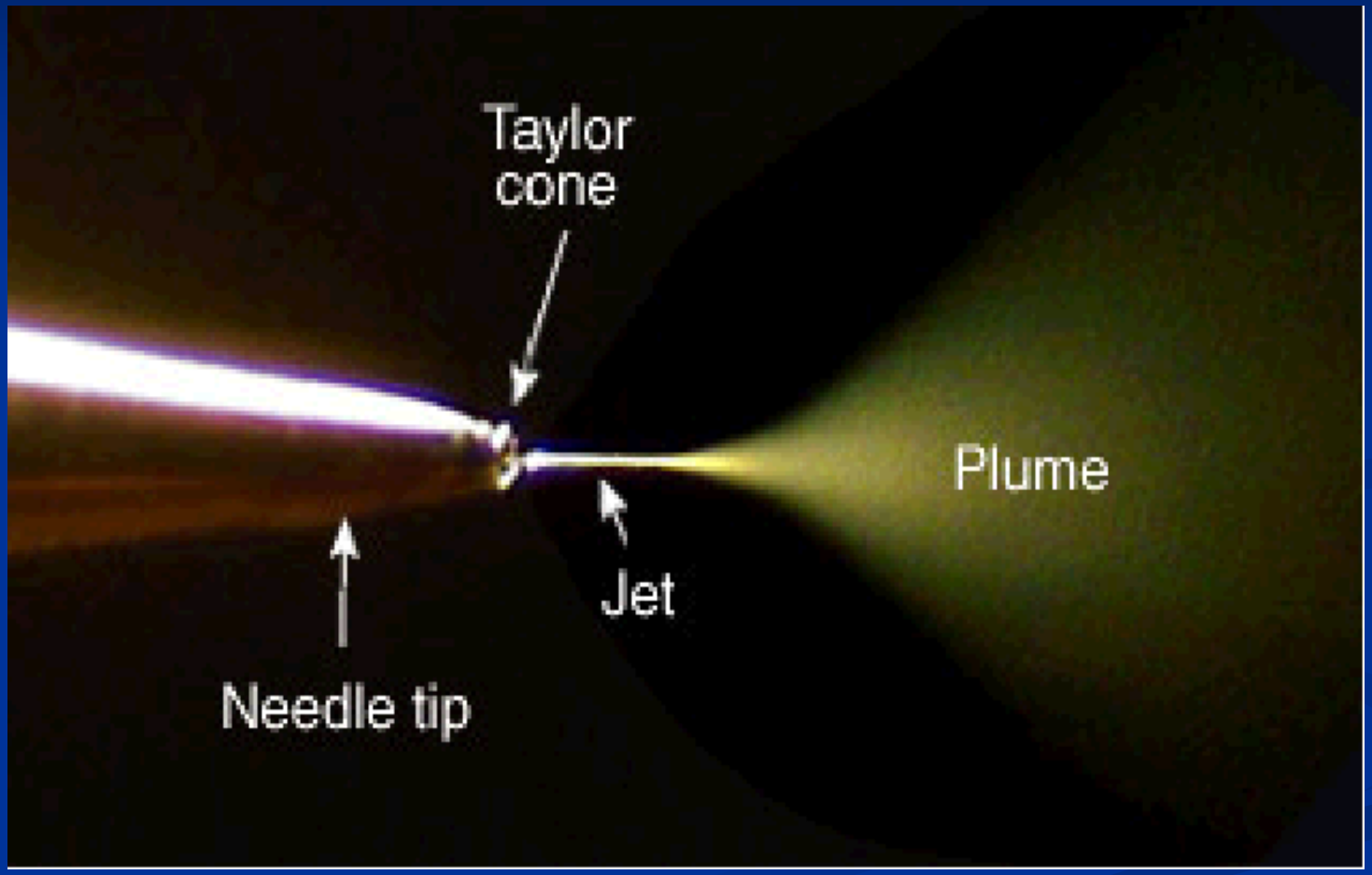


2-10 μL/min

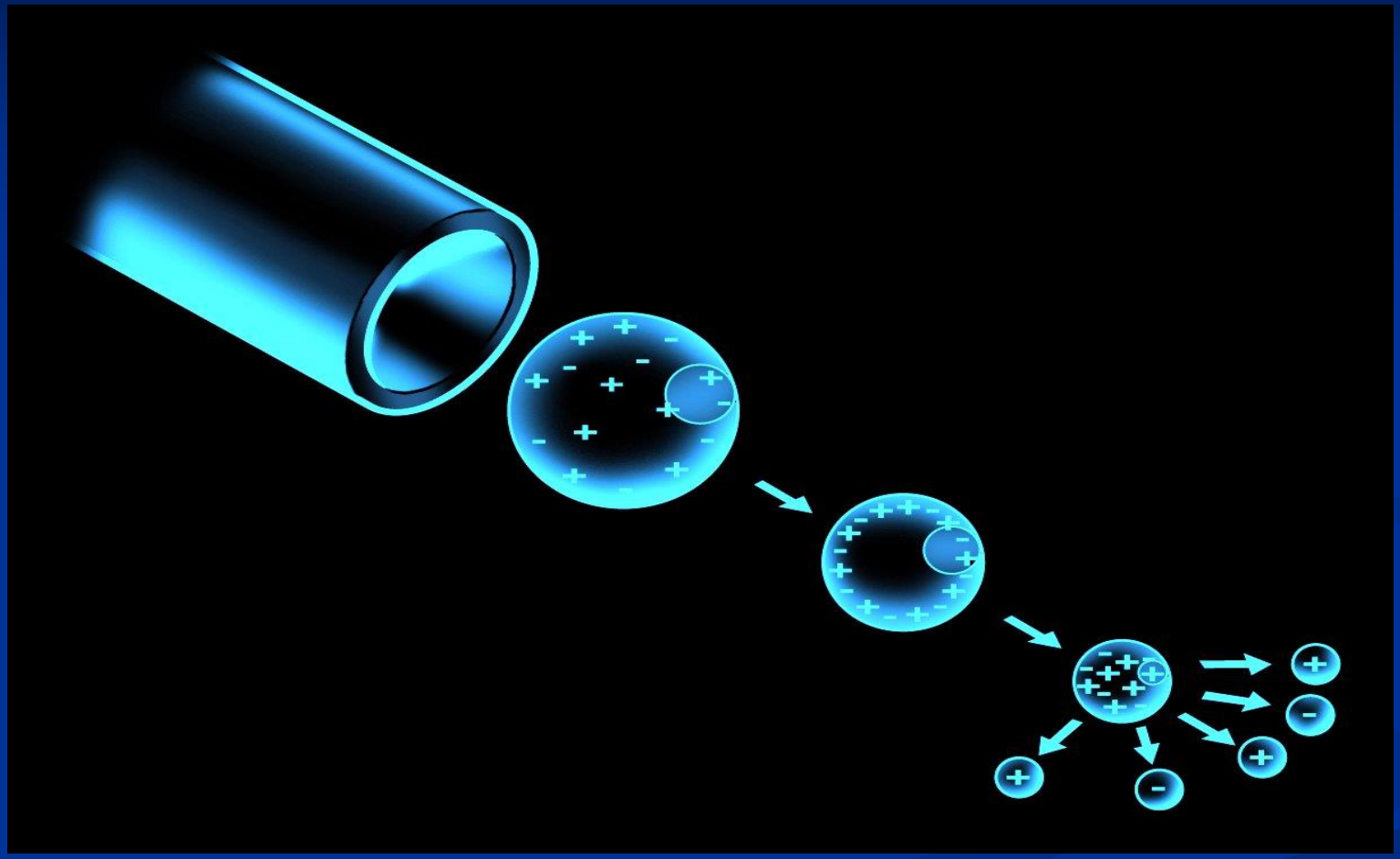
Εξαγωγή ιόντων στην αέρια φάση



The Perfect Image of Nanospray



Principles of Electrospray

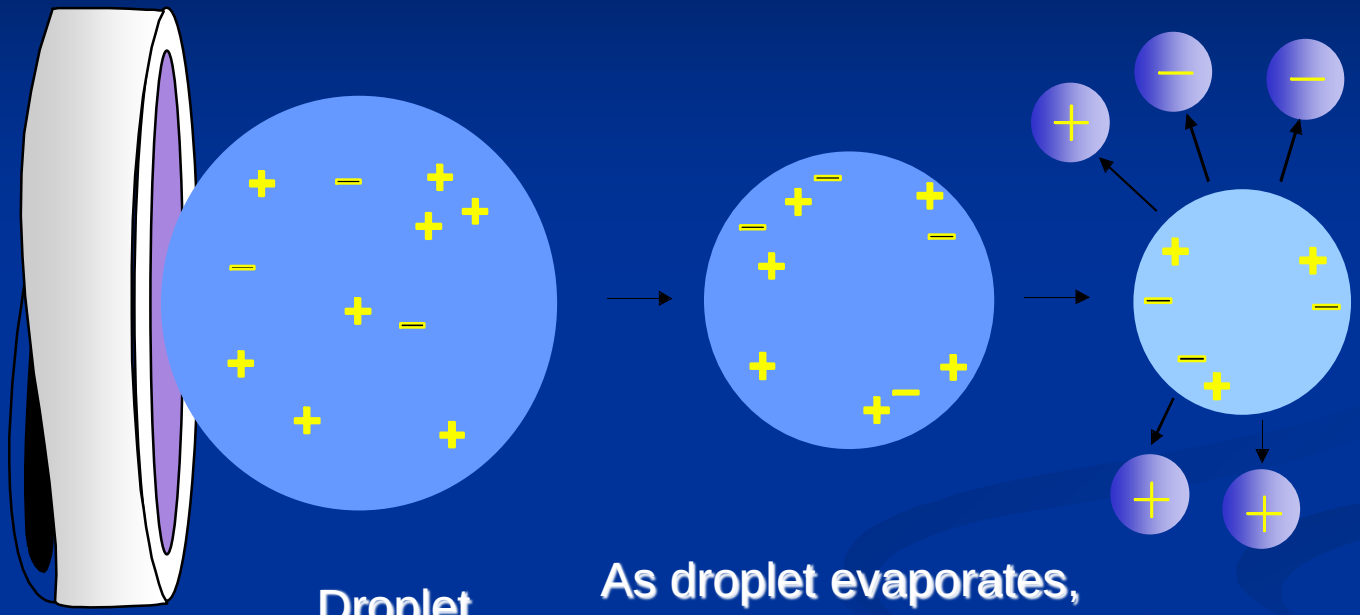


Chemistry Considerations

Electrospray Ionisation

- Ions are pre-formed in solution
- Technique works well with Polar analytes
- Good for Thermally labile analytes
- Good for Large Molecules (Proteins / Peptides)

Ion Evaporation Theory



Capillary
+4 kV

Droplet containing ions

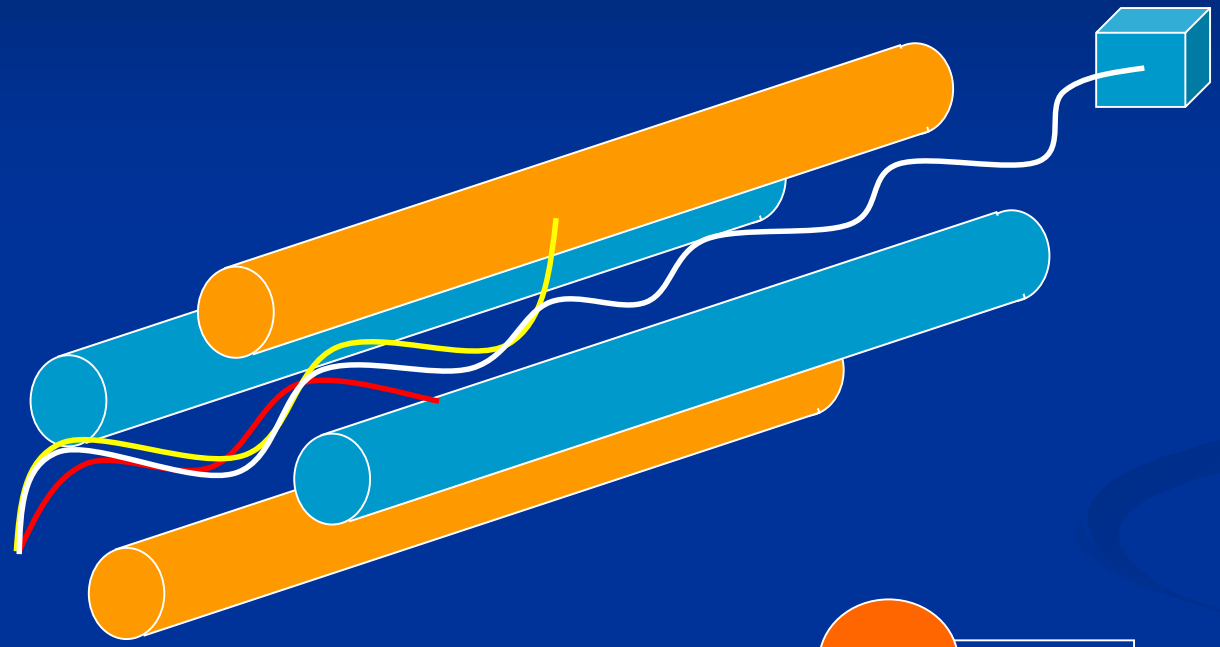
As droplet evaporates, field increases and ions move to surface

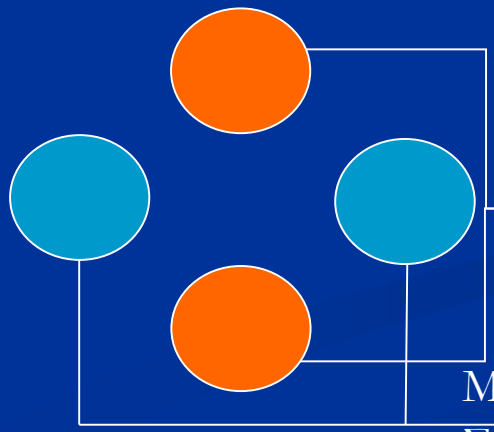
Raleigh Limit – Droplet Instability - releases smaller droplets -ions

Dole, Fenn, Kebarle

Αναλυτής Μαζών Τετραπόλου

L = 250 mm



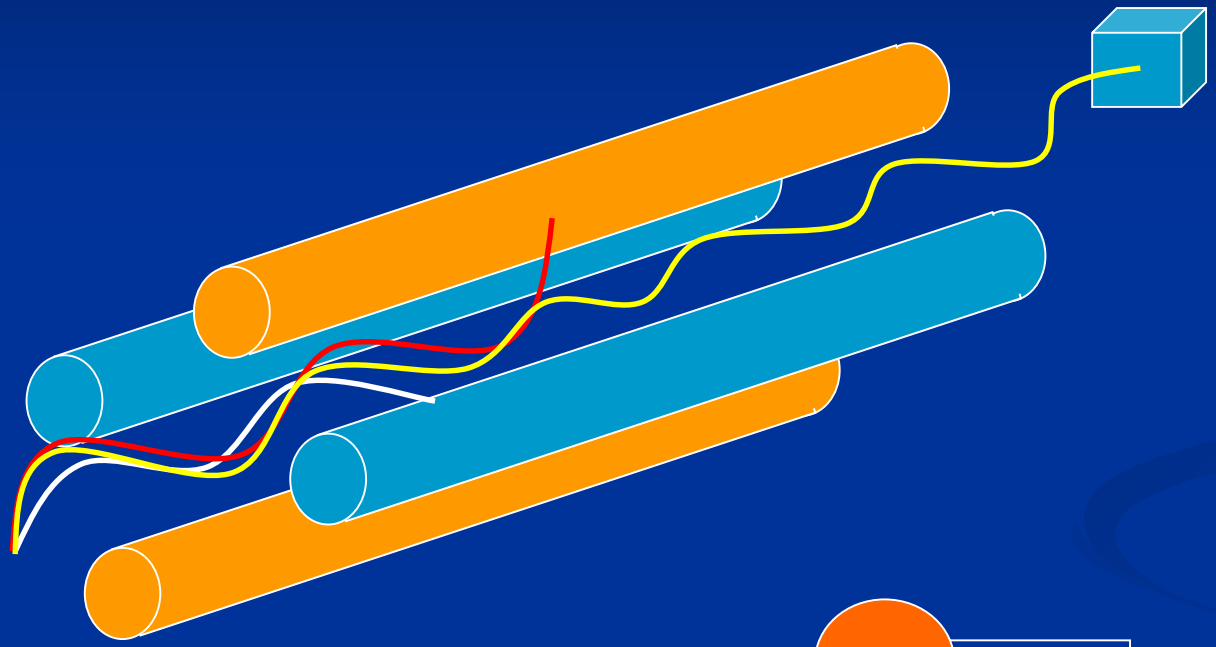


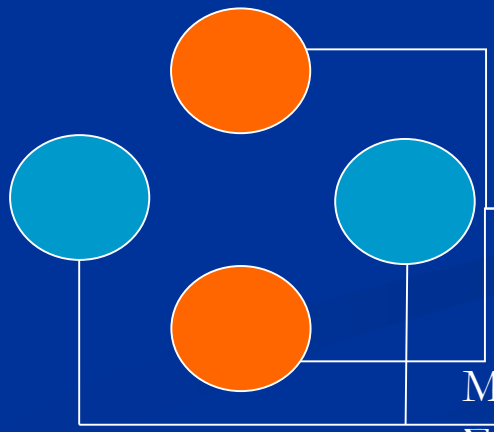
Μεταβλητό δυναμικό RF
Συνεχές δυναμικό + DC

Μεταβλητό δυναμικό RF (180ο δ.φάσεως)
Συνεχές δυναμικό - DC

Αναλυτής Μαζών Τετραπόλου

L = 250 mm



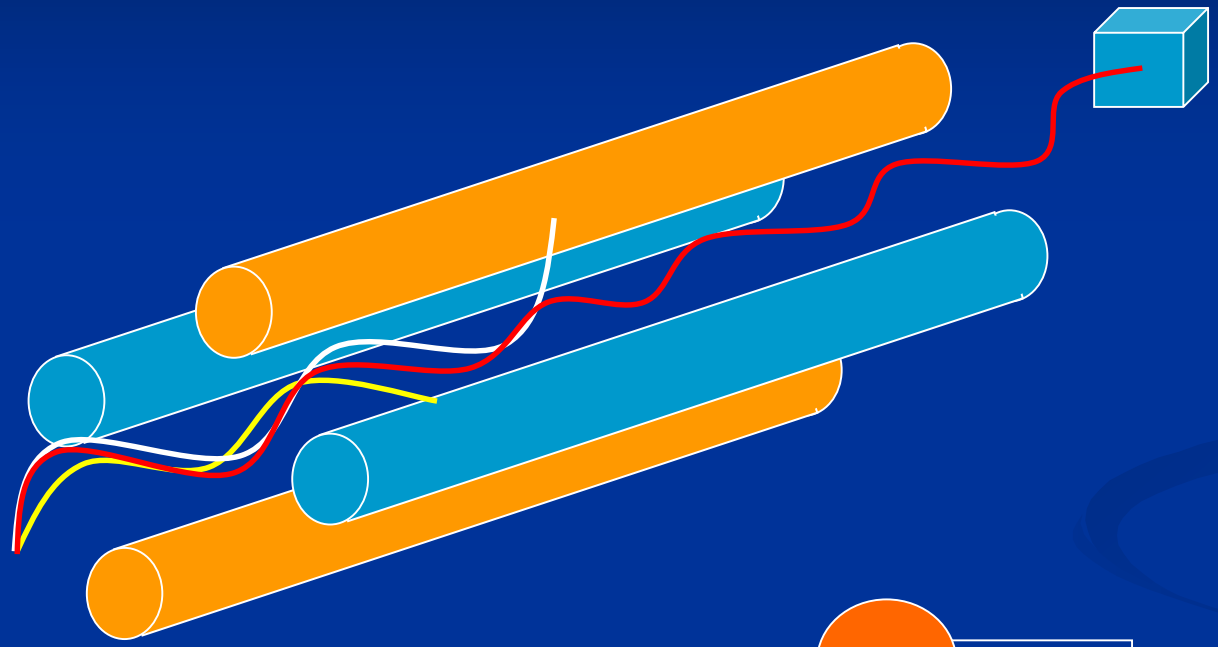


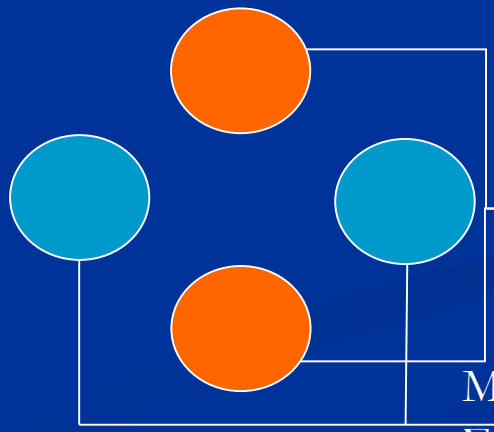
Μεταβλητό δυναμικό RF
Συνεχές δυναμικό + DC

Μεταβλητό δυναμικό RF (180ο δ.φάσεως)
Συνεχές δυναμικό - DC

Αναλυτής Μαζών Τετραπόλου

L = 250 mm

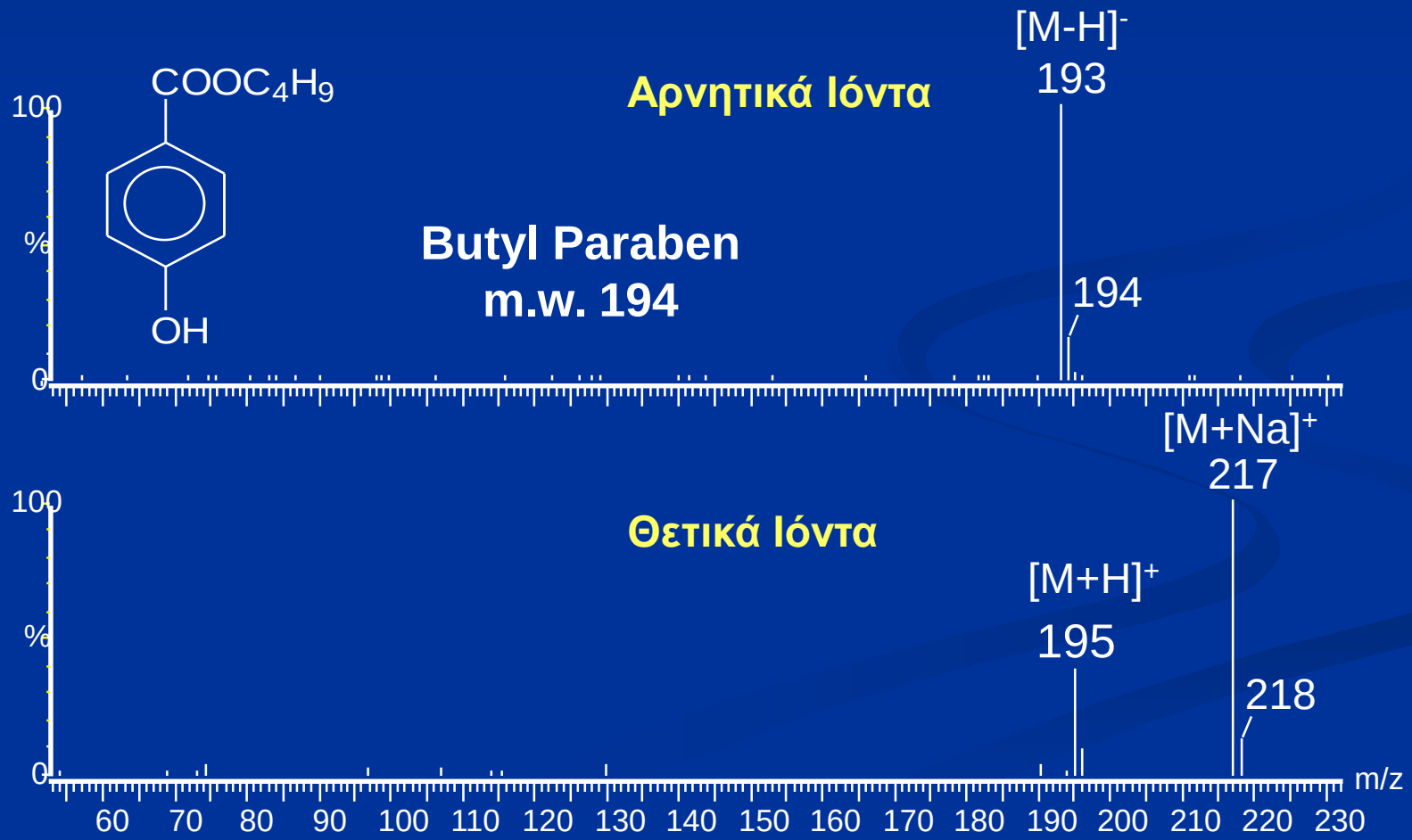


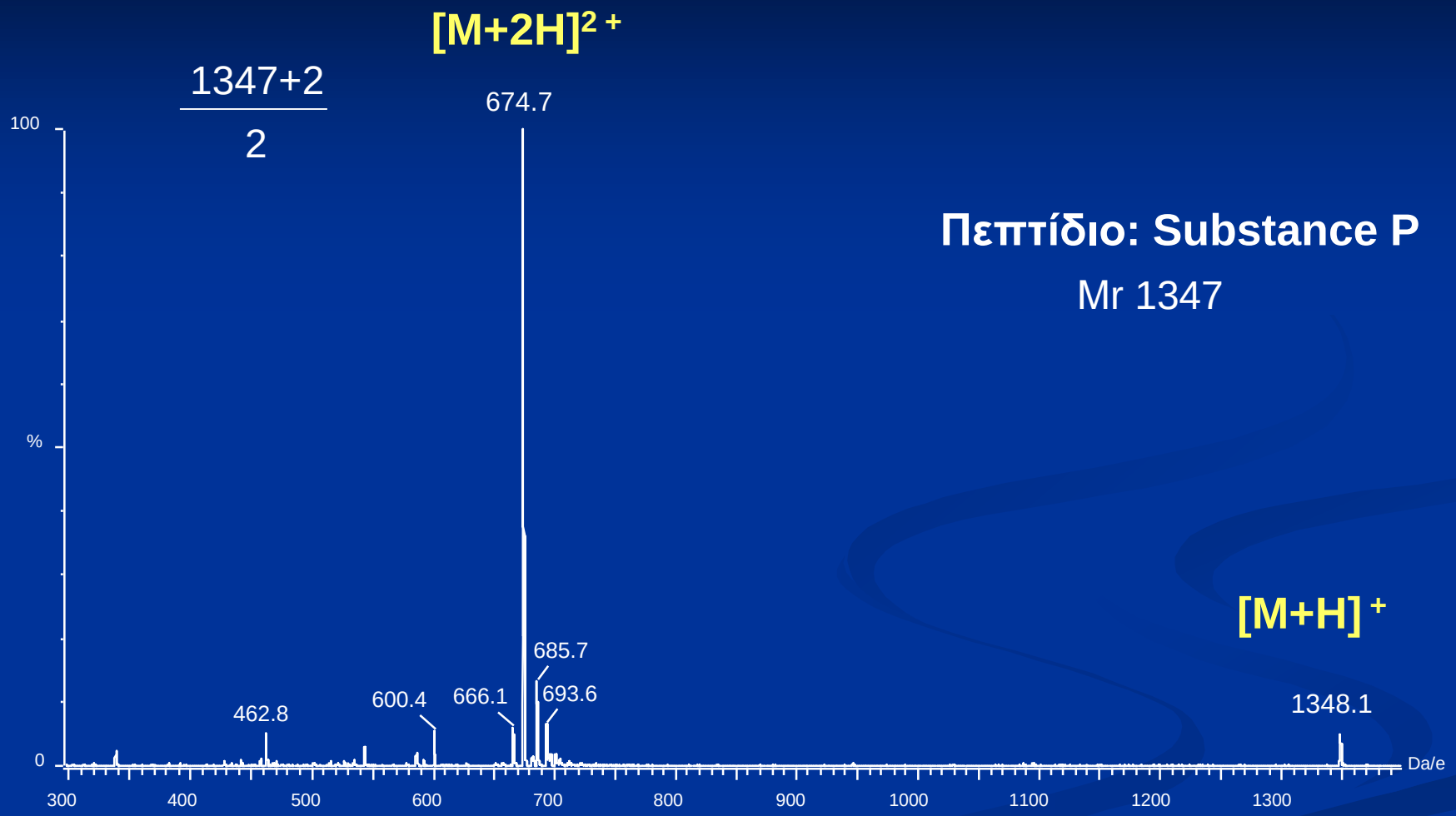


Μεταβλητό δυναμικό RF
Συνεχές δυναμικό + DC

Μεταβλητό δυναμικό RF (180ο δ.φάσεως)
Συνεχές δυναμικό - DC

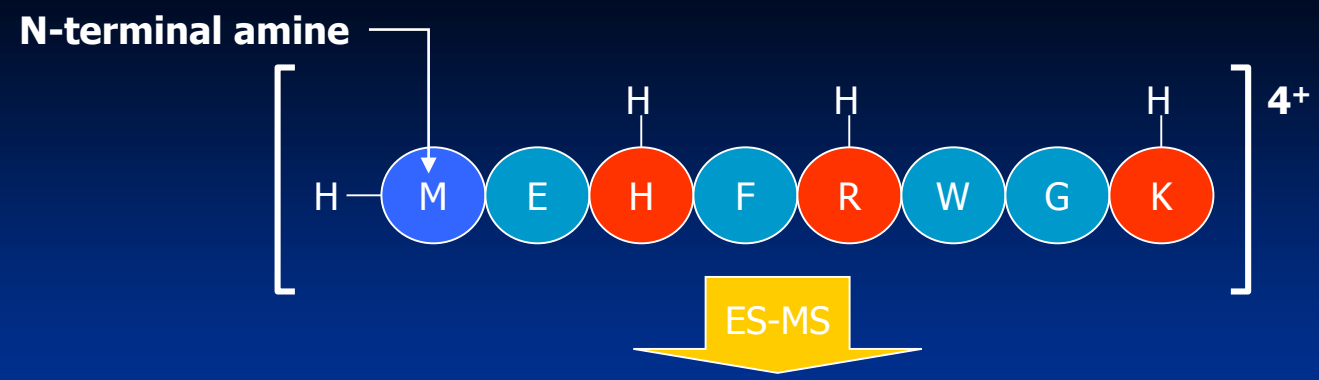
- Βασικές Ομάδες ($-NH_2$) → $[M+H]^+$
- Όξινες Ομάδες (-COOH, -OH) → $[M-H]^-$



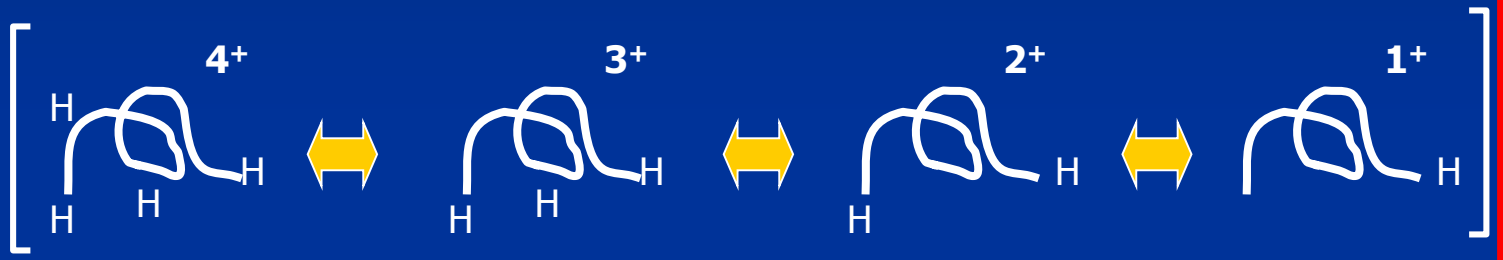


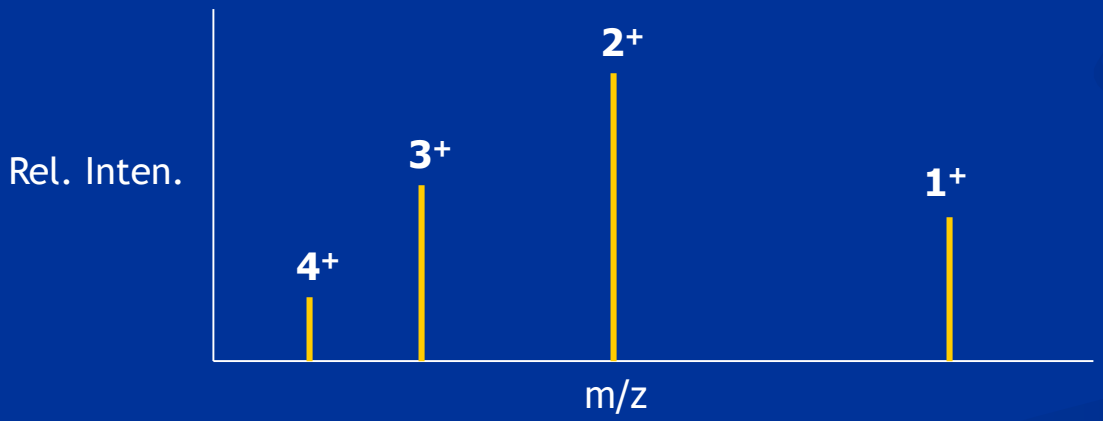
Πεπτίδιο: Substance P

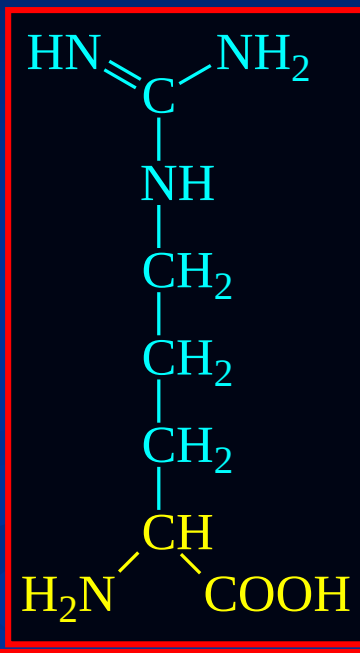
Mr 1347

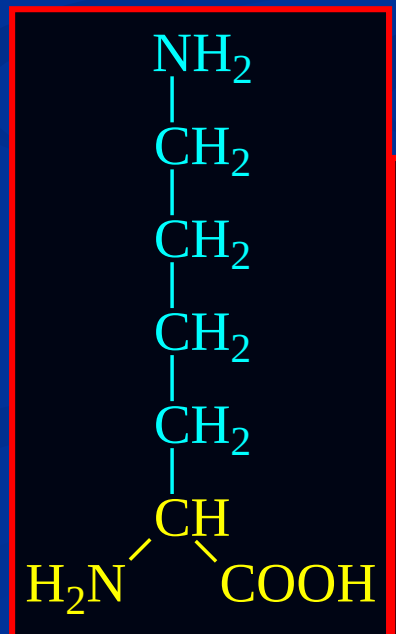

N-terminal amine
H
M
E
H
F
R
W
G
K
4+

ES-MS

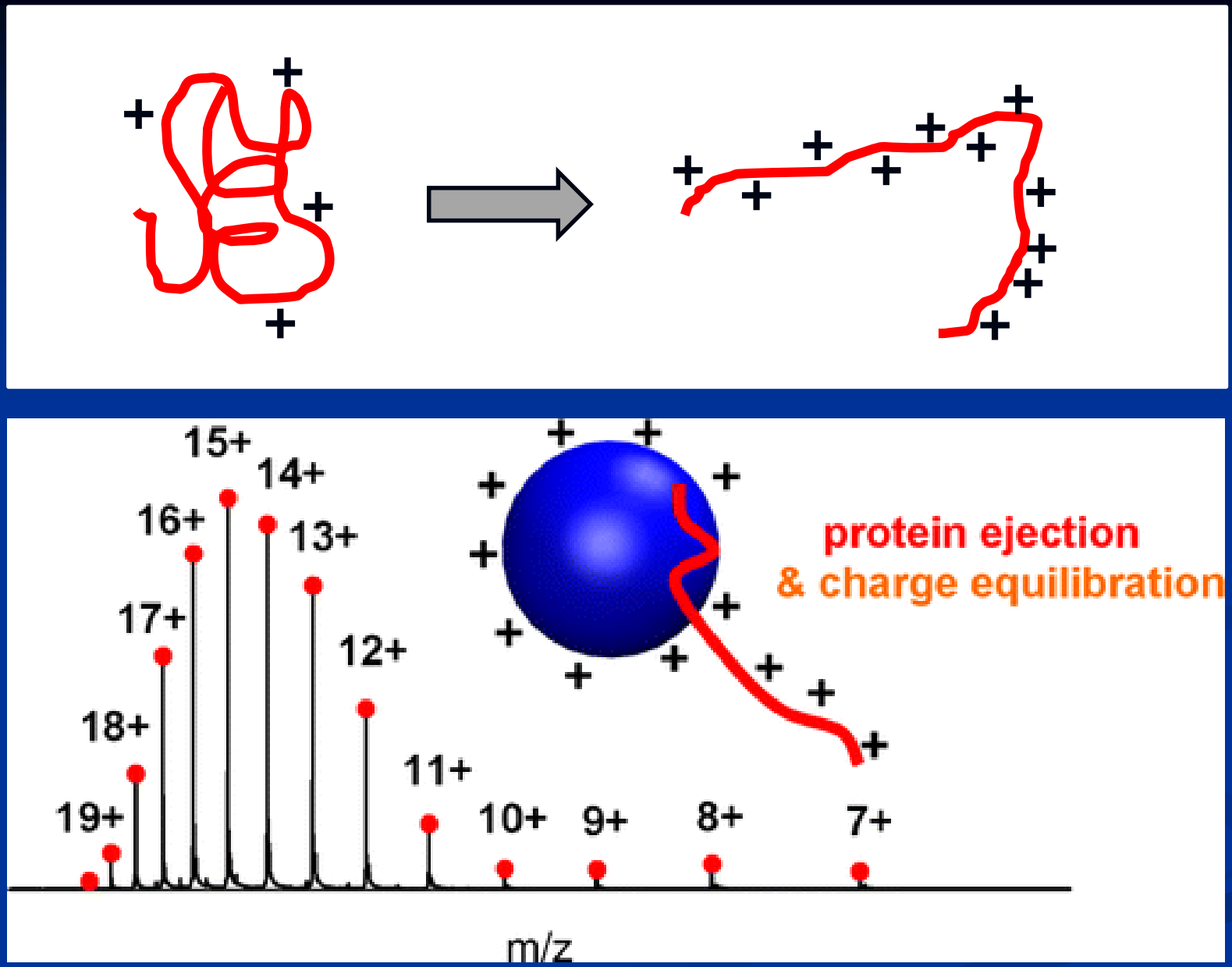

4+
3+
2+
1+


Rel. Inten.
4+
3+
2+
1+
m/z


HN
NH2
C
NH
CH2
CH2
CH2
CH
H2N
COOH


NH2
CH2
CH2
CH2
CH2
CH
H2N
COOH


N
NH
CH2
CH
H2N
COOH


15+
14+
16+
13+
17+
12+
18+
11+
19+
10+
9+
8+
7+
m/z
protein ejection
& charge equilibration

$$[M+(z+1)H]^{(z+1)+}$$

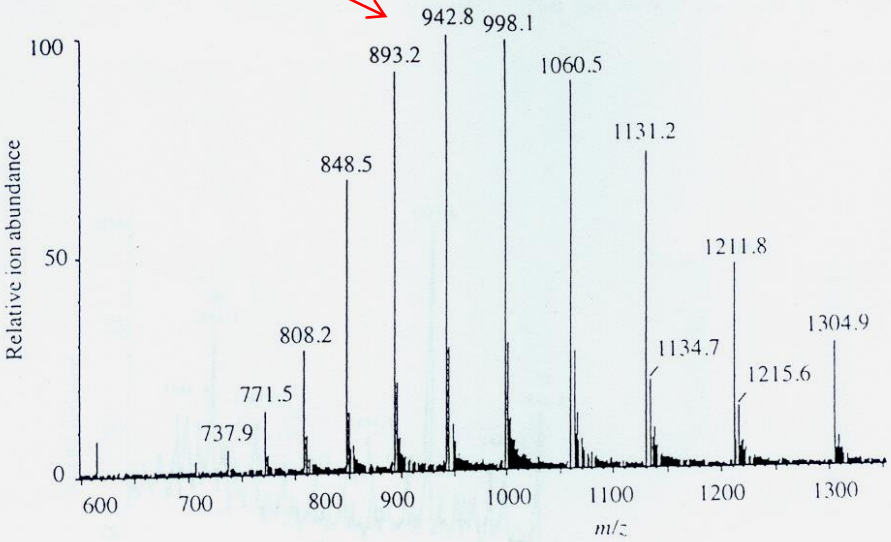
$$[M+zH]^{+}$$

$$m_2 = \frac{m}{z} = \frac{M_r + (z+1)\,m_p}{z+1}$$

$$m_1 = \frac{m}{z} = \frac{M_r + z\,m_p}{z}$$

$$m_1 = \frac{M_r + z}{z}$$

$$m_2 = \frac{M_r + z + 1}{z+1}$$

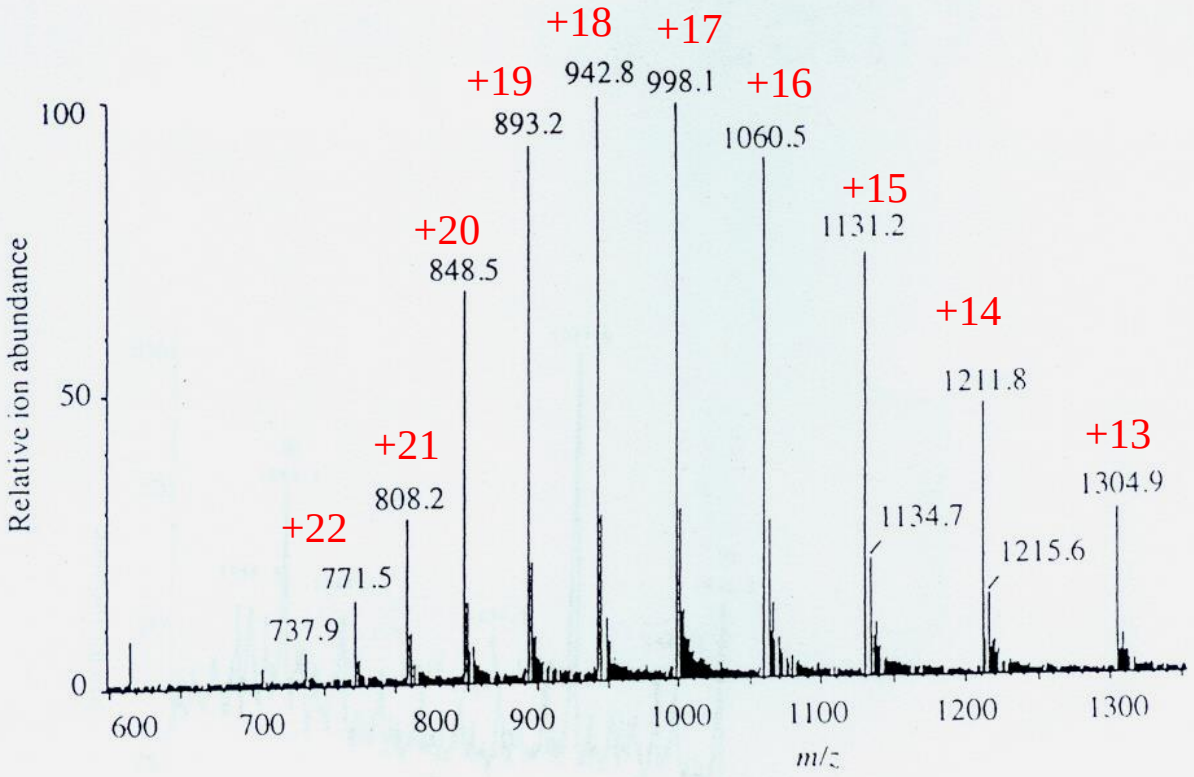


$$z = \frac{m_2 - 1}{m_1 - m_2}$$

$$M_r = z(m_1 - 1)$$

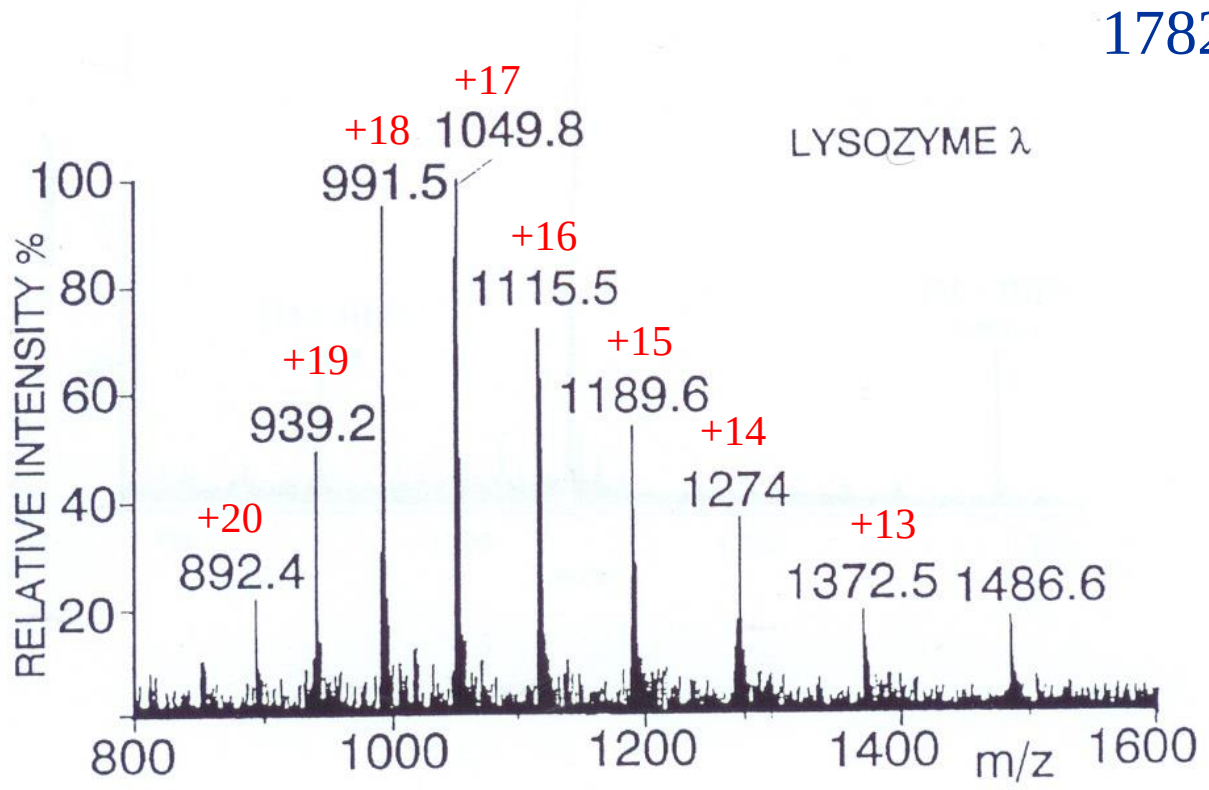
Προσδιορισμός μοριακών μαζών Πρωτεϊνών με Φασματομετρία μάζας Ηλεκτροψεκασμού

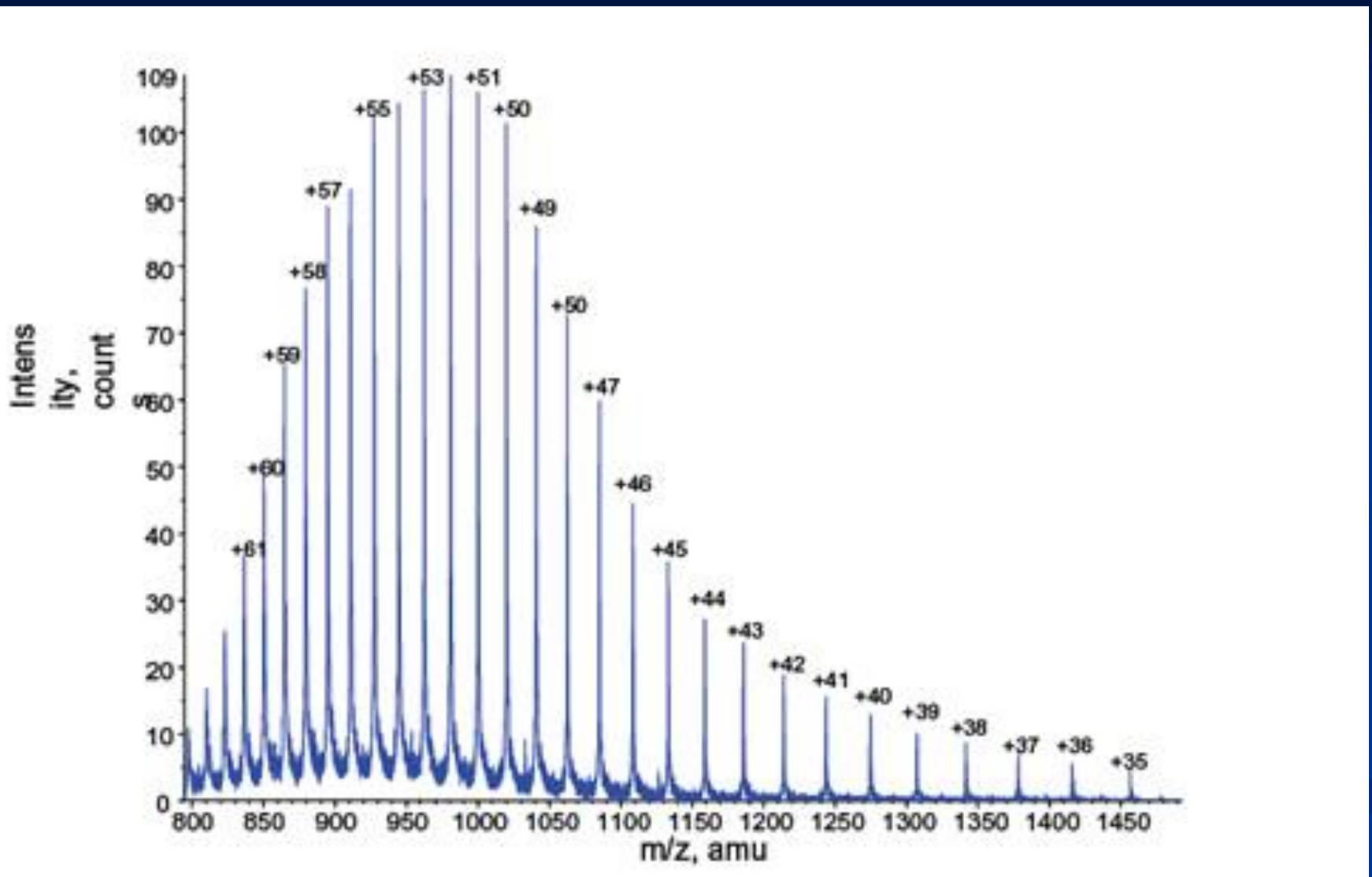
16951,5



Ακρίβεια προσδιορισμού μάζας 0,01 %

Προσδιορισμός μοριακών μαζών Πρωτεϊνών με ΦΜ Ηλεκτροψεκασμού




Intens
ity,
count
109
100
90
80
70
60
50
40
30
20
10
0
+61
+60
+59
+58
+57
+55
+53
+51
+50
+49
+50
+47
+46
+45
+44
+43
+42
+41
+40
+39
+38
+37
+36
+35
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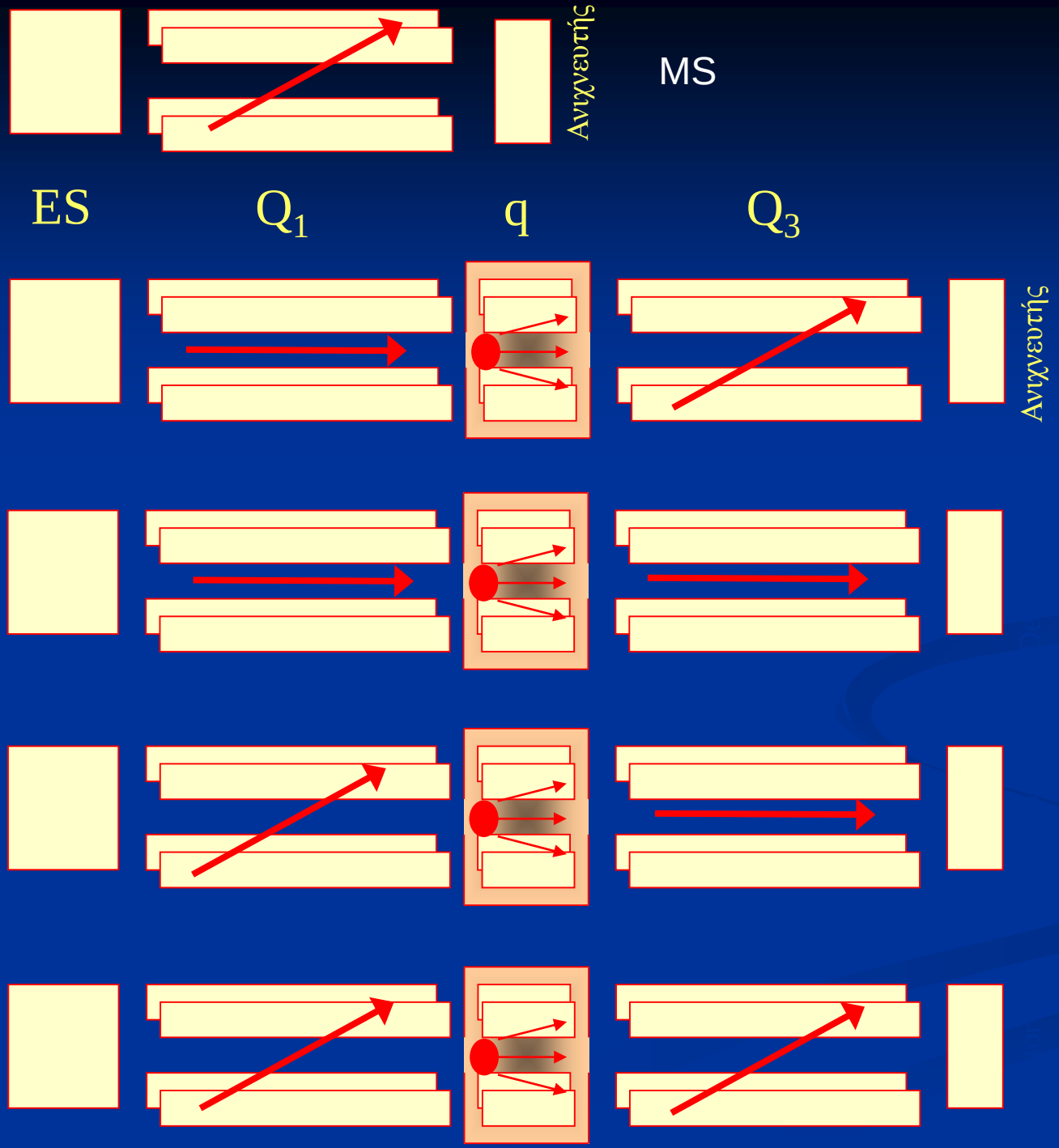
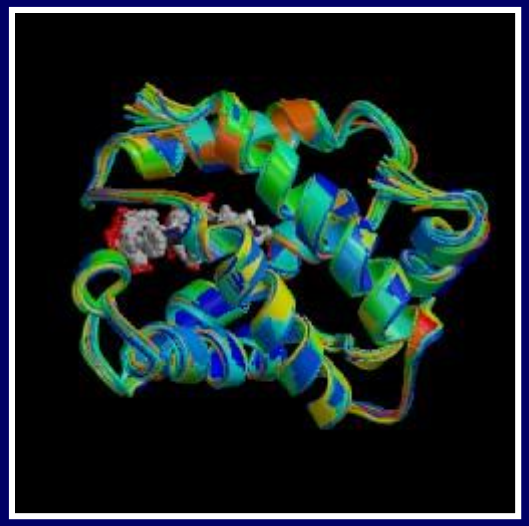
1100
1150
1200
1250
1300
1350
1400
1450
m/z, amu


Ανιχνευτής
MS
ES
Q_1
q
Q_3
Ανιχνευτής
MS/MS
Παρακολούθηση Επιλεγμένων αντιδράσεων
Σάρωση πρόδρομων ιόντων
Σάρωση απώλειας ουδετέρων

Mass Spectrometry for Protein Analysis: Protein Sequencing

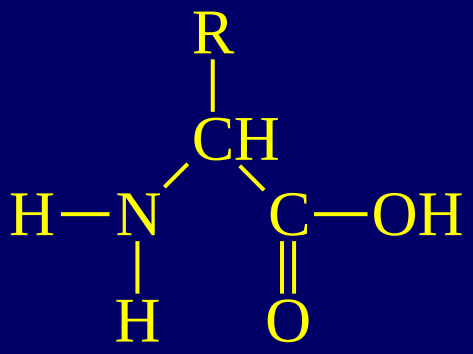


ΔΙΑΛΕΞΗ 2η
05 Νοεμβρίου 2014

Lecture by Dr Spiros A. Pergantis

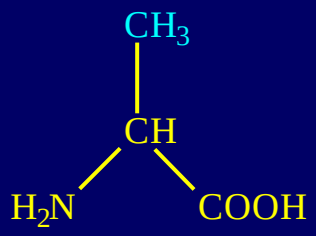
Introduction: What are Proteins?

• Proteins are polyamides, their monomeric units are comprised of 20 different α-amino acids

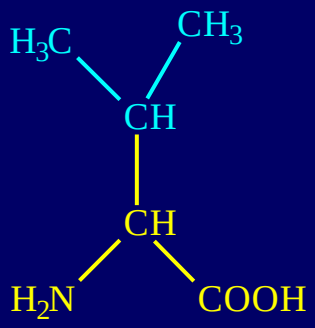


• Enzymatic proteins (catalysts for such functions as digestion)
• Structural proteins e.g. collagen and elastin (connective tissue)
• Contractile proteins e.g. myosin (muscle movement)
• Transport proteins e.g. hemoglobin molecules (oxygen transport)
• Storage proteins e.g. casein (major source of aa for baby mammals)
• Hormonal proteins e.g. insulin (regulates sugar conc. in the blood)
• Receptor proteins (build into the membrane of nerve cells)
• Defensive proteins (antibodies to protect against diseases)

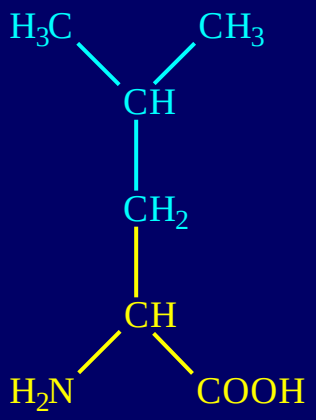
Neutral and hydrophobic aminoacids



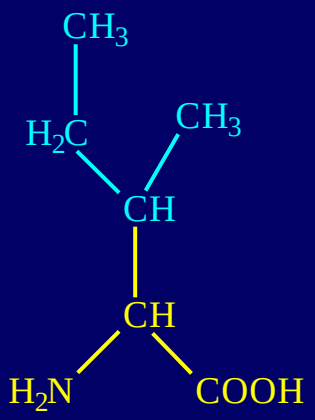
Alanine



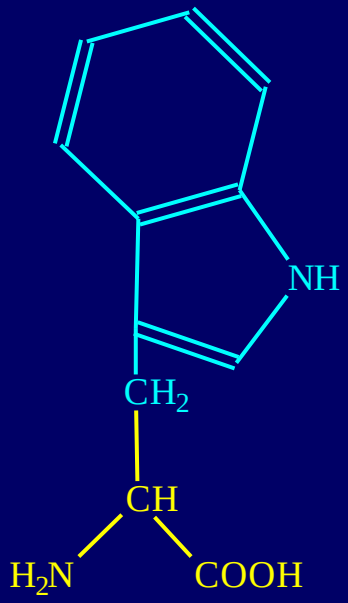
Valine



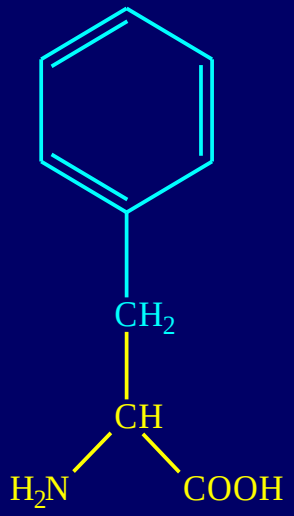
Leucine



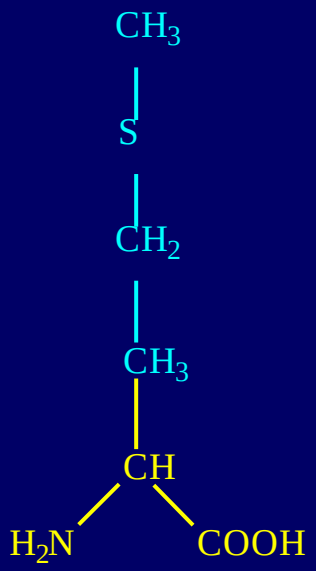
Isoleucine



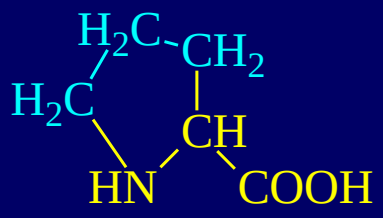
Tryptophan



Phenylalanine

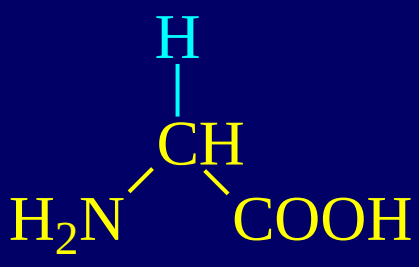


Methionine

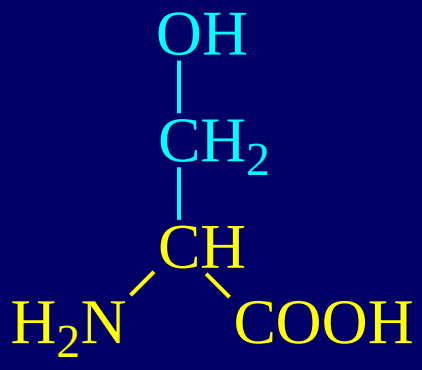


Proline

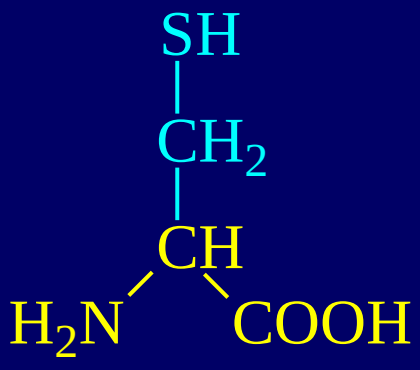
Neutral polar aminoacids



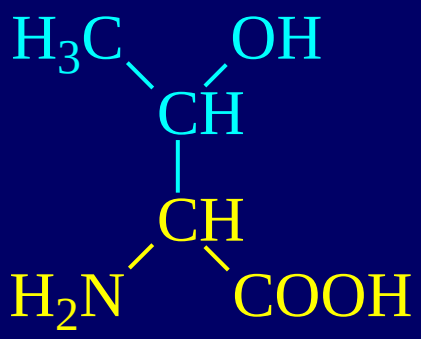
Glycine



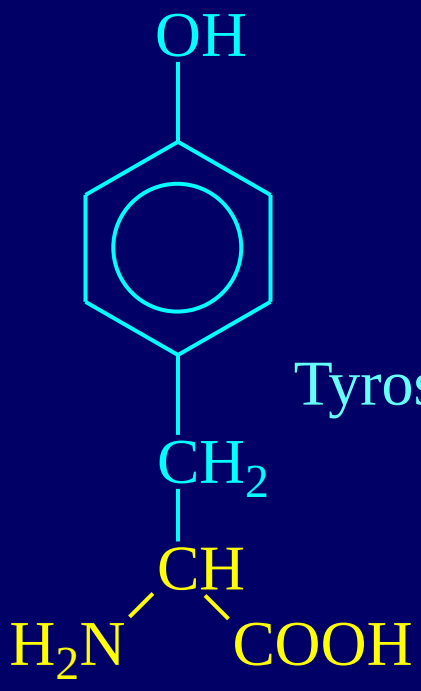
Serine



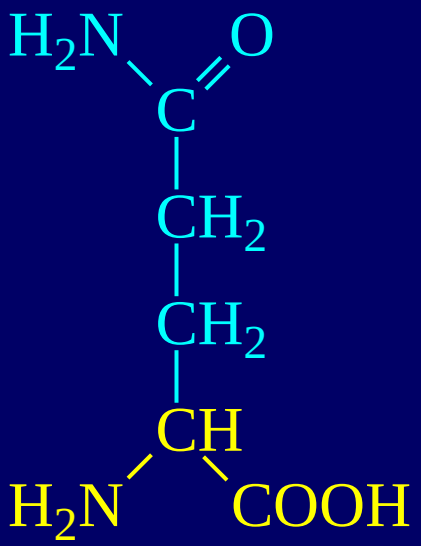
Cysteine



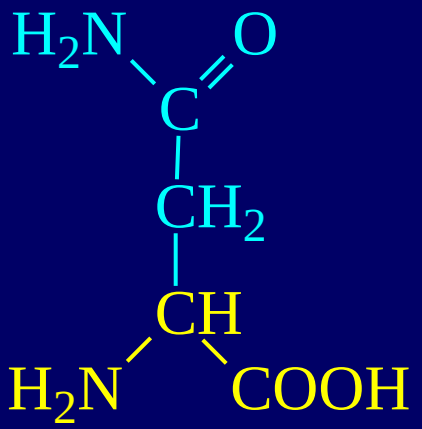
Threonine



Tyrosine

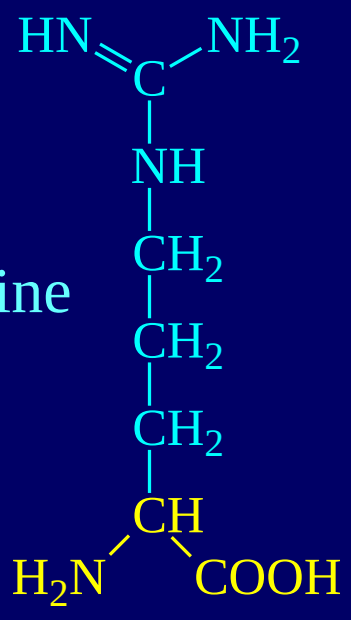


Glutamine



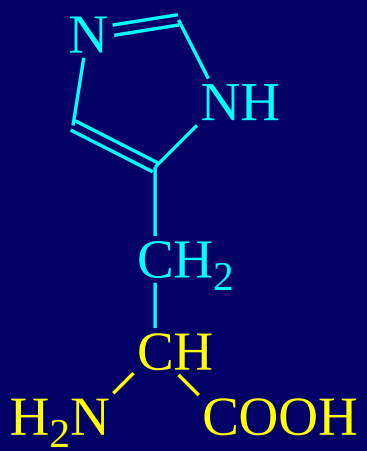
Asparagine

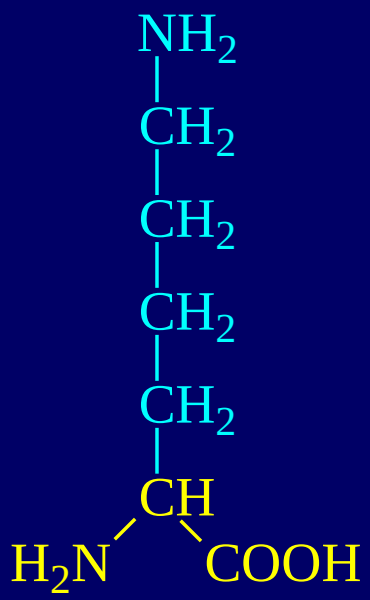
Basic aminoacids



Arginine

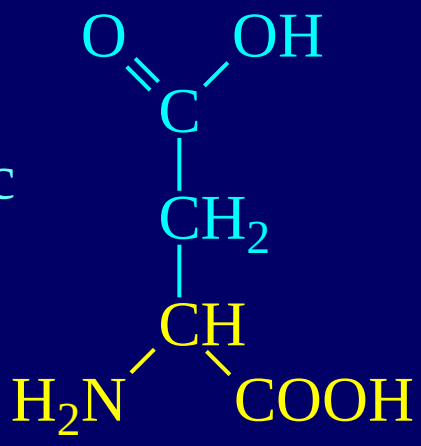
Histidine



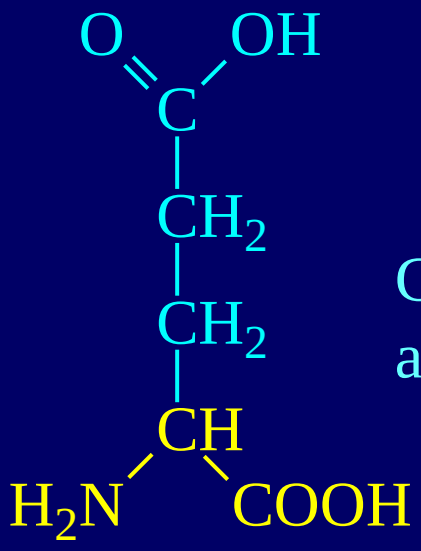


Lysine

Acidic aminoacids

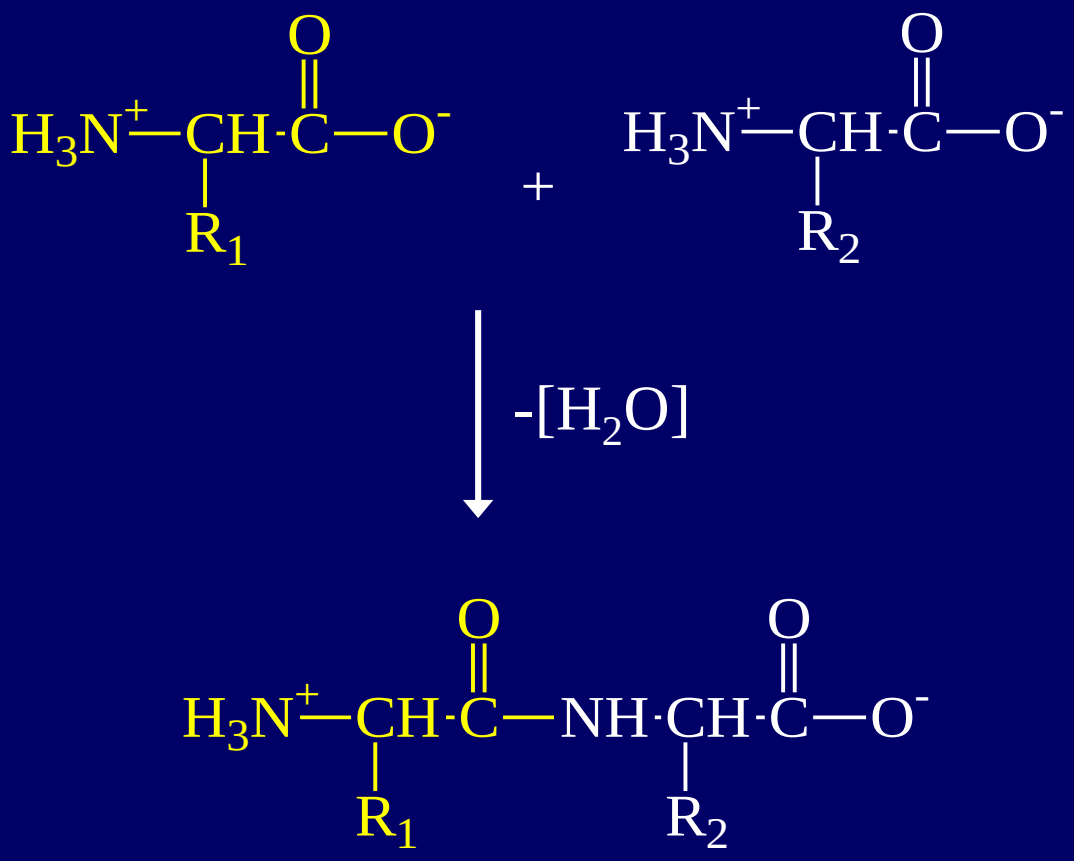


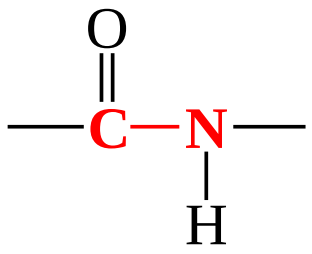
Aspartic acid



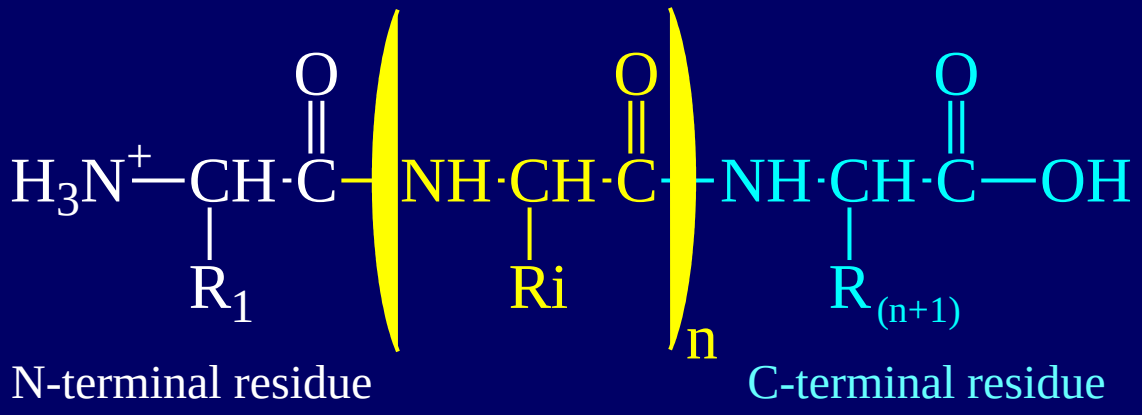
Glutamic acid

Primary structure of a protein is the exact sequence of the different α-amino acids along the protein chain (also refered to as the covalent structure of the protein).





Peptide bond
or
peptide linkage



By convention, protein and peptide sequences are written with the N-terminal amino acid on the left and the C-terminal residue on the right.

Polymers containing:

2 amino acid residues are called **dipeptides**

3 -//- **tripeptides**

3-10 -//- **oligopeptides**

Many -//- **polypeptides**

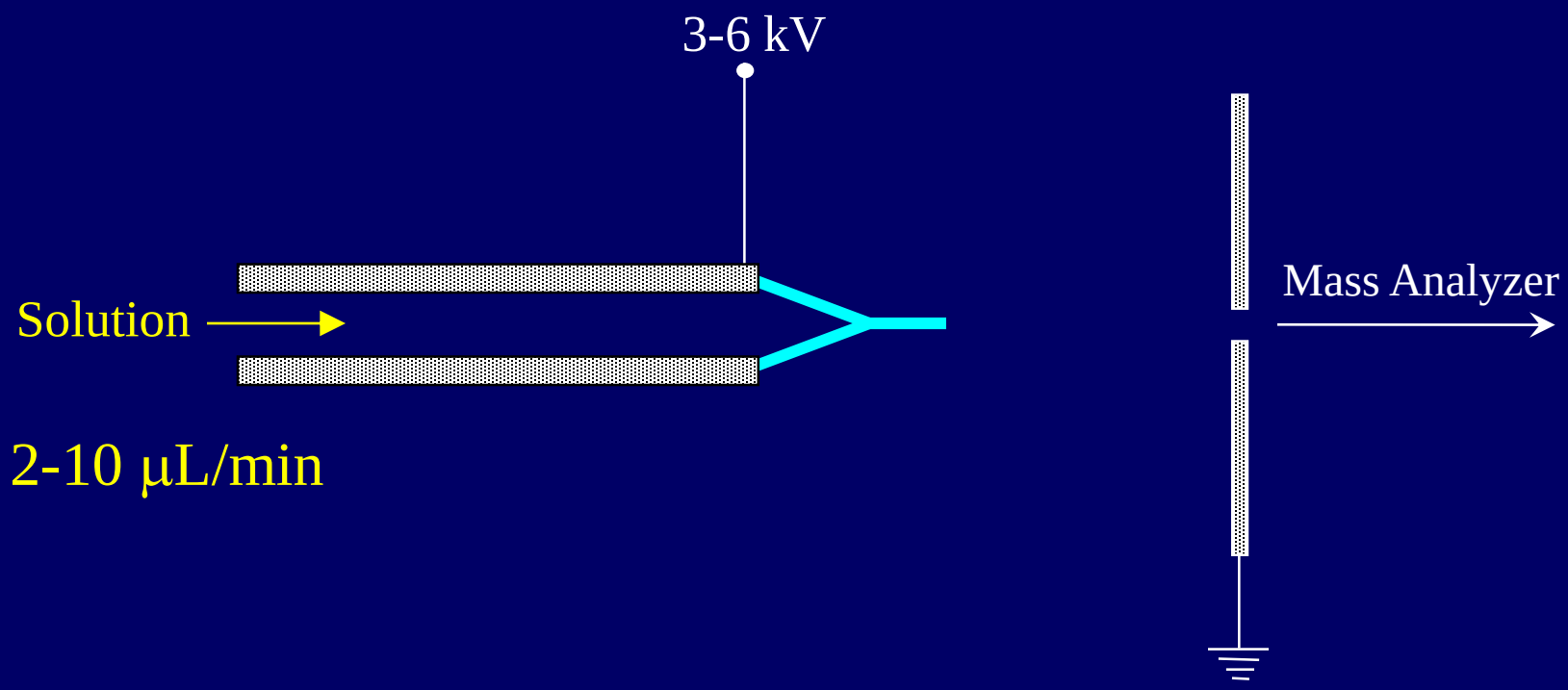
Proteins are molecules that contain one or more polypeptide chains

Mass Spectrometric Analysis of Proteins

Steps in procedure:

1. M_r determined by electropsray (ESP) or MALDI.
2. Samples of protein are digested with proteases like trypsin and chymotrypsin.
3. Each of the digests is fractionated by HPLC or CE.
4. The M_r of each peptide is determined by ESP or MALDI, subsequently collision induced dissociation is used to gather information on the sequence.
5. The sequence of as many of the oligopeptides as possible are deduced from the data.
6. By looking for identical overlapping sequences, individual peptide sequences are assembled into the full sequence of the protein.

Electrospray Ionization Mass Spectrometry



Sample preparation requirements

Because of the need for protons protein samples are dissolved in protic volatile solvent systems.

Approximately 1 mM of salt

However, up to 100 mM NH_4Ac is often used

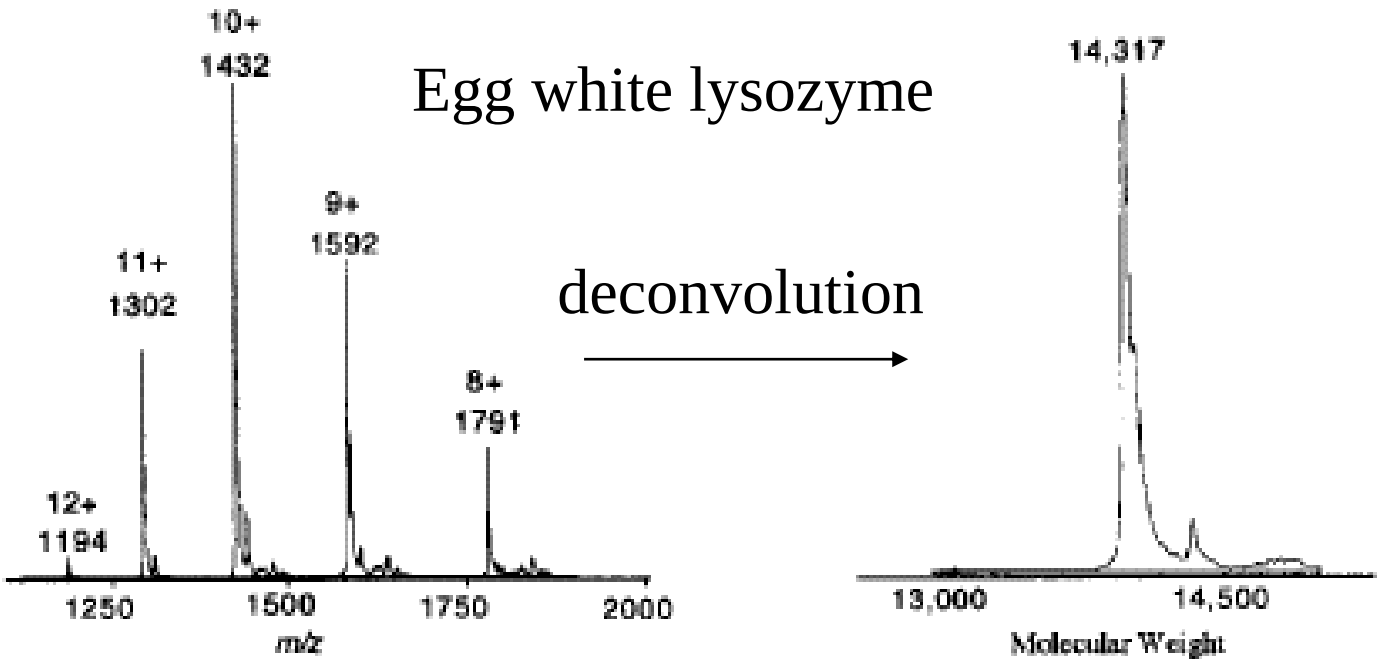
Alkali and alkaline salts are detrimental to signal

Phosphate buffers are detrimental to signal

Requires approx. 1 pmol

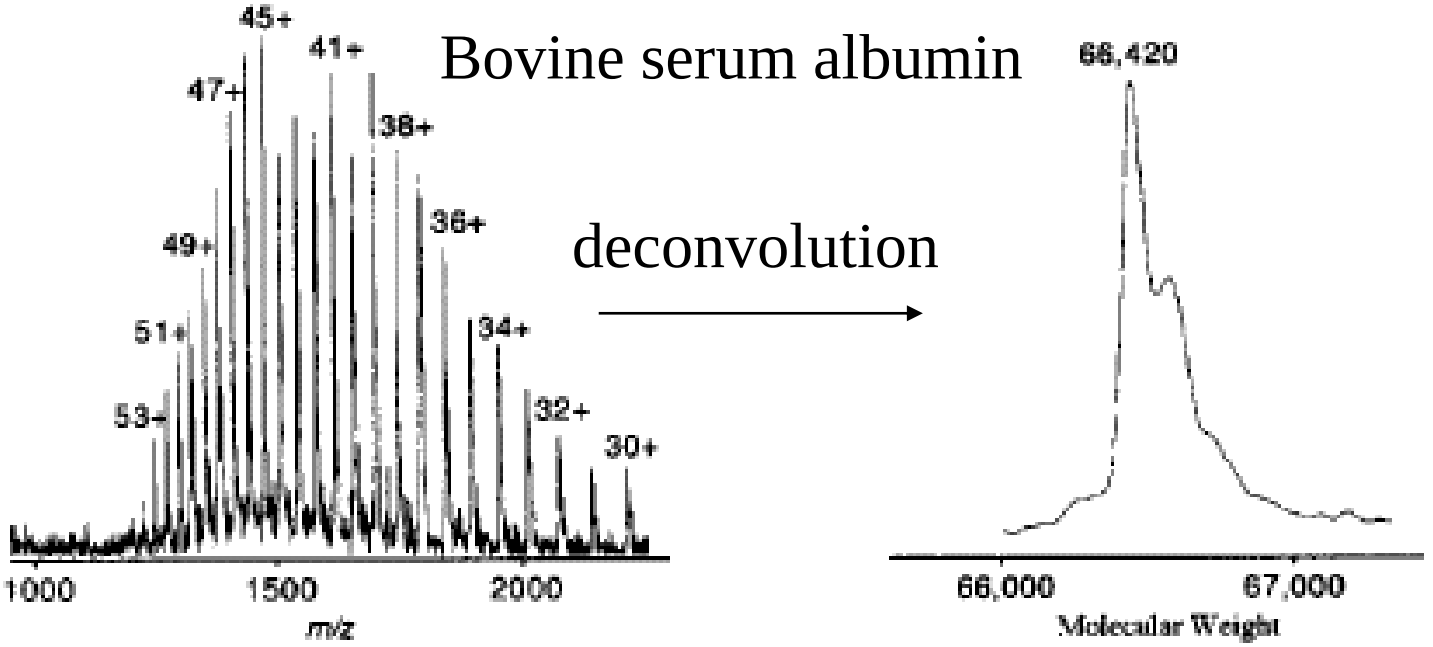
(less sample required when using nanospray)

Determination of protein Mr using ESP-MS



Mass accuracy of 0.01 % can be expected

Determination of protein Mr using ESP-MS



Mass Spectrometric Analysis of Proteins

Steps in procedure:

1. M_r determined by electropsray (ESP) or MALDI.
2. Samples of protein are digested with proteases like trypsin and chymotrypsin.

Protein digestion

First, useable CID data usually can only be obtained from peptides less than 2-3 kDa, and trypsin generally produces peptides of this size. It seems that the easiest spectra to interpret are those obtained from doubly-charged precursors, where the resulting fragment ions are mostly singly-charged with only a few doubly-charged fragments. Doubly-charged precursors also fragment such that most of the peptide bonds break with comparable frequency, such that one is more likely to derive a complete sequence.

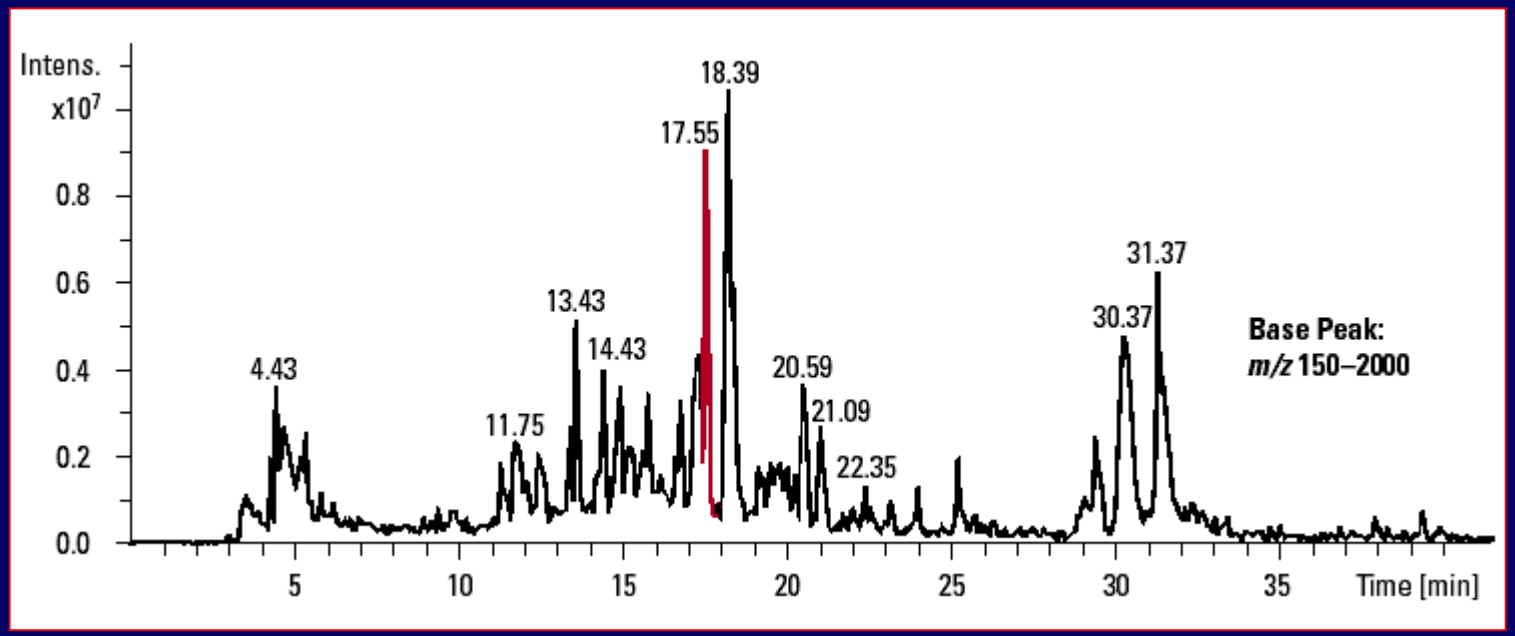
The second reason for using trypsin proteolysis has to do with the desirability of placing basic residues, notably arginine, at the C-terminus of a peptide. It is a general observation in low energy CID that the presence of arginine in the middle of a peptide will often result in the absence of fragmentations at several contiguous peptide bonds adjacent to the arginine. Trypsin cleaves on the C-terminal side of arginine and lysine. By putting the basic residues at the C-terminus, peptides fragment in a more predictable manner throughout the length of the peptide.

Mass Spectrometric Analysis of Proteins

Steps in procedure:

1. M_r determined by electropsray (ESP) or MALDI.
2. Samples of protein are digested with proteases like trypsin and chymotrypsin.
3. Each of the digests is fractionated by HPLC or CE.

HPLC ESP-MS/MS of peptide digests



Chromatogram generated from 1pmol of material injected onto HPLC.

Mass Spectrometric Analysis of Proteins

Steps in procedure:

1. M_r determined by electropsray (ESP) or MALDI.
2. Samples of protein are digested with proteases like trypsin and chymotrypsin.
3. Each of the digests is fractionated by HPLC or CE.
4. The M_r of each peptide is determined by ESP or MALDI, subsequently collision induced dissociation is used to gather information on the sequence.

Amino acid sequencing in an unknown peptide using ESP-MS/MS

The peptide we are about to sequence was obtained from methionyl human growth hormone.

Initially the protein was digested using trypsin.

The resulting peptide mixture was separated and detected using HPLC – ESP – MS/MS.

An ESP mass spectrum was recorded for each peptide.

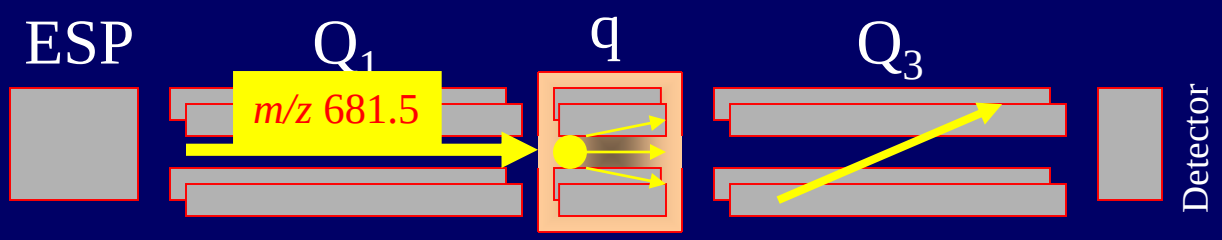
The mass spectrum of the peptide under investigation contained a base peak at m/z 681.5

What is the peptide's M*r*?

M*r* 1361

Amino acid sequencing in an unknown peptide using ESP-MS/MS

The sequence of the peptide can be investigated by carrying out collision-induced fragmentation (CID) of the $[M+2H]^{2+}$ ions occurring at *m/z* 681.5

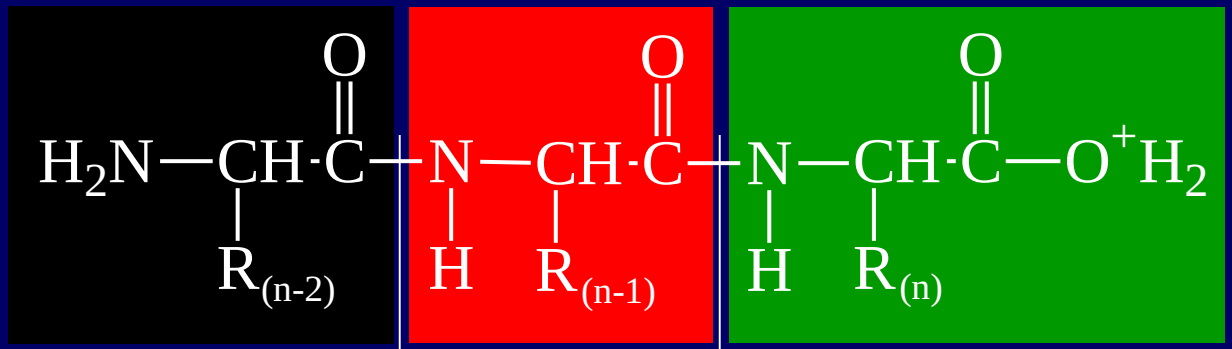


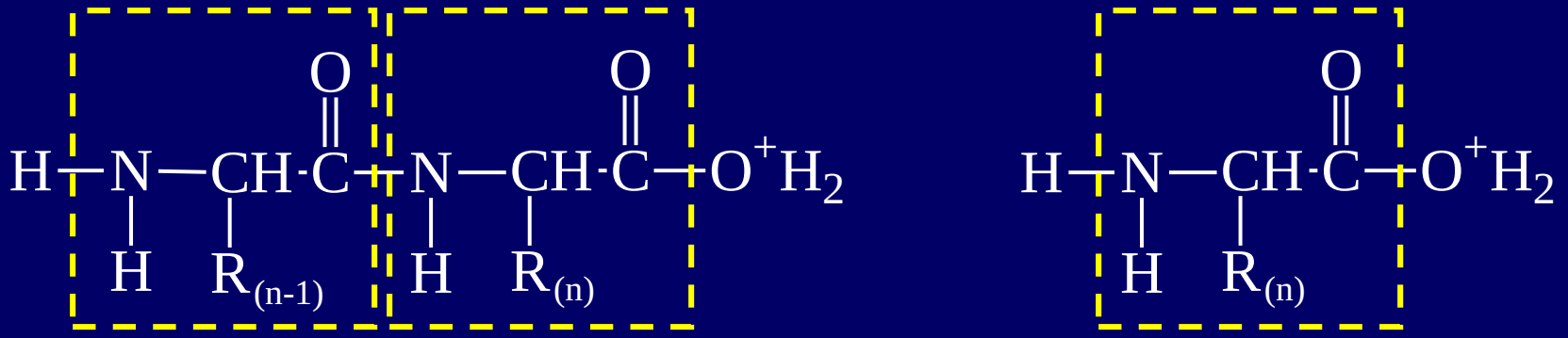
Q1 is setup to only allow ions with *m/z* 681.5 through.

CID is occurs in the collision cell (q)

Q3 is used to separate the resulting product ions, and thus allow for Recording of the product mass spectrum.

Amino acid sequencing in an unknown peptide using ESP-MS/MS





Amino acid sequencing in an unknown peptide using ESP-MS/MS

Product ions along with their approximate RA following CID of diprotonated unknown peptide.

m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue mass
Glycine	Gly	G	57
Alanine	Ala	A	71
Serine	Ser	S	87
Proline	Pro	P	97
Valine	Val	V	99
Threonine	Thr	T	101
Cysteine	Cys	C	103
Isoleucine	Ile	I	113
Leucine	Leu	L	113
Asparagine	Asn	N	114
Aspartic acid	Asp	D	115
Glutamine	Gln	Q	128
Lysine	Lys	K	128
Glutamic acid	Glu	E	129
Methionine	Met	M	131
Histidine	His	H	137
Phenylalanine	Phe	F	147
Arginine	Arg	R	156
Tyrosine	Tyr	Y	163
Tryptophan	Trp	W	186

y-type ions

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol		Residue mass
Glycine	Gly	G		57
Alanine	Ala	A		71
Serine	Ser	S		87
Proline	Pro	P		97
Valine	Val	V		99
Threonine	Thr	T		101
Cysteine	Cys	C		103
Isoleucine	Ile	I		113
Leucine	Leu	L		113
Asparagine	Asn	N		114
Aspartic acid	Asp	D		115
Glutamine	Gln	Q		128
Lysine	Lys	K	128+19=147	128
Glutamic acid	Glu	E		129
Methionine	Met	M		131
Histidine	His	H		137
Phenylalanine	Phe	F		147
Arginine	Arg	R	156+19=175	156
Tyrosine	Tyr	Y		163
Tryptophan	Trp	W		186

m/z	RA (%)	m/z	RA (%)
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Trypsin hydrolyses proteins at the C-terminus of all lysine and arginine residues

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

-Arg

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue ma	+175
Glycine	Gly	G	57	232
Alanine	Ala	A	71	246
Serine	Ser	S	87	262
Proline	Pro	P	97	272
Valine	Val	V	99	274
Threonine	Thr	T	101	276
Cysteine	Cys	C	103	278
Isoleucine	Ile	I	113	288
Leucine	Leu	L	113	288
Asparagine	Asn	N	114	289
Aspartic acid	Asp	D	115	290
Glutamine	Gln	Q	128	303
Lysine	Lys	K	128	303
Glutamic acid	Glu	E	129	304
Methionine	Met	M	131	306
Histidine	His	H	137	312
Phenylalanine	Phe	F	147	322
Arginine	Arg	R	156	331
Tyrosine	Tyr	Y	163	338
Tryptophan	Trp	W	186	361

m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

-Gly-Arg

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue ma	+232
Glycine	Gly	G	57	289
Alanine	Ala	A	71	303
Serine	Ser	S	87	319
Proline	Pro	P	97	329
Valine	Val	V	99	331
Threonine	Thr	T	101	333
Cysteine	Cys	C	103	335
Isoleucine	Ile	I	113	345
Leucine	Leu	L	113	345
Asparagine	Asn	N	114	346
Aspartic acid	Asp	D	115	347
Glutamine	Gln	Q	128	360
Lysine	Lys	K	128	360
Glutamic acid	Glu	E	129	361
Methionine	Met	M	131	363
Histidine	His	H	137	369
Phenylalanine	Phe	F	147	379
Arginine	Arg	R	156	388
Tyrosine	Tyr	Y	163	395
Tryptophan	Trp	W	186	418

m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

-Met-Gly-Arg

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue ma	+363
Glycine	Gly	G	57	420
Alanine	Ala	A	71	434
Serine	Ser	S	87	450
Proline	Pro	P	97	460
Valine	Val	V	99	462
Threonine	Thr	T	101	464
Cysteine	Cys	C	103	466
Isoleucine	Ile	I	113	476
Leucine	Leu	L	113	476
Asparagine	Asn	N	114	477
Aspartic acid	Asp	D	115	478
Glutamine	Gln	Q	128	491
Lysine	Lys	K	128	491
Glutamic acid	Glu	E	129	492
Methionine	Met	M	131	494
Histidine	His	H	137	500
Phenylalanine	Phe	F	147	510
Arginine	Arg	R	156	519
Tyrosine	Tyr	Y	163	526
Tryptophan	Trp	W	186	549

m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

-Leu/Ile-Met-Gly-Arg

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue ma	+476
Glycine	Gly	G	57	533
Alanine	Ala	A	71	547
Serine	Ser	S	87	563
Proline	Pro	P	97	573
Valine	Val	V	99	575
Threonine	Thr	T	101	577
Cysteine	Cys	C	103	579
Isoleucine	Ile	I	113	589
Leucine	Leu	L	113	589
Asparagine	Asn	N	114	590
Aspartic acid	Asp	D	115	591
Glutamine	Gln	Q	128	604
Lysine	Lys	K	128	604
Glutamic acid	Glu	E	129	605
Methionine	Met	M	131	607
Histidine	His	H	137	613
Phenylalanine	Phe	F	147	623
Arginine	Arg	R	156	632
Tyrosine	Tyr	Y	163	639
Tryptophan	Trp	W	186	662

m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

-Thr-Leu/Ile-Met-Gly-Arg

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue ma	+577
Glycine	Gly	G	57	634
Alanine	Ala	A	71	648
Serine	Ser	S	87	664
Proline	Pro	P	97	674
Valine	Val	V	99	676
Threonine	Thr	T	101	678
Cysteine	Cys	C	103	680
Isoleucine	Ile	I	113	690
Leucine	Leu	L	113	690
Asparagine	Asn	N	114	691
Aspartic acid	Asp	D	115	692
Glutamine	Gln	Q	128	705
Lysine	Lys	K	128	705
Glutamic acid	Glu	E	129	706
Methionine	Met	M	131	708
Histidine	His	H	137	714
Phenylalanine	Phe	F	147	724
Arginine	Arg	R	156	733
Tyrosine	Tyr	Y	163	740
Tryptophan	Trp	W	186	763

m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

-Gln/Lys-Thr-Leu/Ile-Met-Gly-Arg

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue ma	+705
Glycine	Gly	G	57	762
Alanine	Ala	A	71	776
Serine	Ser	S	87	792
Proline	Pro	P	97	802
Valine	Val	V	99	804
Threonine	Thr	T	101	806
Cysteine	Cys	C	103	808
Isoleucine	Ile	I	113	818
Leucine	Leu	L	113	818
Asparagine	Asn	N	114	819
Aspartic acid	Asp	D	115	820
Glutamine	Gln	Q	128	833
Lysine	Lys	K	128	833
Glutamic acid	Glu	E	129	834
Methionine	Met	M	131	836
Histidine	His	H	137	842
Phenylalanine	Phe	F	147	852
Arginine	Arg	R	156	861
Tyrosine	Tyr	Y	163	868
Tryptophan	Trp	W	186	891

m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

-Leu/Ile-Gln/Lys-Thr-Leu/Ile-Met-Gly-Arg

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue ma	+818
Glycine	Gly	G	57	875
Alanine	Ala	A	71	889
Serine	Ser	S	87	905
Proline	Pro	P	97	915
Valine	Val	V	99	917
Threonine	Thr	T	101	919
Cysteine	Cys	C	103	921
Isoleucine	Ile	I	113	931
Leucine	Leu	L	113	931
Asparagine	Asn	N	114	932
Aspartic acid	Asp	D	115	933
Glutamine	Gln	Q	128	946
Lysine	Lys	K	128	946
Glutamic acid	Glu	E	129	947
Methionine	Met	M	131	949
Histidine	His	H	137	955
Phenylalanine	Phe	F	147	965
Arginine	Arg	R	156	974
Tyrosine	Tyr	Y	163	981
Tryptophan	Trp	W	186	1004

m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

-Gly or Trp -Leu/Ile-Gln/Lys-Thr-Leu/Ile-Met-Gly-Arg

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue ma	+875
Glycine	Gly	G	57	932
Alanine	Ala	A	71	946
Serine	Ser	S	87	962
Proline	Pro	P	97	972
Valine	Val	V	99	974
Threonine	Thr	T	101	976
Cysteine	Cys	C	103	978
Isoleucine	Ile	I	113	988
Leucine	Leu	L	113	988
Asparagine	Asn	N	114	989
Aspartic acid	Asp	D	115	990
Glutamine	Gln	Q	128	1003
Lysine	Lys	K	128	1003
Glutamic acid	Glu	E	129	1004
Methionine	Met	M	131	1006
Histidine	His	H	137	1012
Phenylalanine	Phe	F	147	1022
Arginine	Arg	R	156	1031
Tyrosine	Tyr	Y	163	1038
Tryptophan	Trp	W	186	1061

m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

-(Glu-Gly or Trp)-Leu/Ile-Gln/Lys-Thr-Leu/Ile-Met-Gly-Arg

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue ma	+1004
Glycine	Gly	G	57	1061
Alanine	Ala	A	71	1075
Serine	Ser	S	87	1091
Proline	Pro	P	97	1101
Valine	Val	V	99	1103
Threonine	Thr	T	101	1105
Cysteine	Cys	C	103	1107
Isoleucine	Ile	I	113	1117
Leucine	Leu	L	113	1117
Asparagine	Asn	N	114	1118
Aspartic acid	Asp	D	115	1119
Glutamine	Gln	Q	128	1132
Lysine	Lys	K	128	1132
Glutamic acid	Glu	E	129	1133
Methionine	Met	M	131	1135
Histidine	His	H	137	1141
Phenylalanine	Phe	F	147	1151
Arginine	Arg	R	156	1160
Tyrosine	Tyr	Y	163	1167
Tryptophan	Trp	W	186	1190

m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

-Glu-(Glu-Gly or Trp)-Leu/Ile-Gln/Lys-Thr-Leu/Ile-Met-Gly-Arg

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue ma	+1133
Glycine	Gly	G	57	1190
Alanine	Ala	A	71	1204
Serine	Ser	S	87	1220
Proline	Pro	P	97	1230
Valine	Val	V	99	1232
Threonine	Thr	T	101	1234
Cysteine	Cys	C	103	1236
Isoleucine	Ile	I	113	1246
Leucine	Leu	L	113	1246
Asparagine	Asn	N	114	1247
Aspartic acid	Asp	D	115	1248
Glutamine	Gln	Q	128	1261
Lysine	Lys	K	128	1261
Glutamic acid	Glu	E	129	1262
Methionine	Met	M	131	1264
Histidine	His	H	137	1270
Phenylalanine	Phe	F	147	1280
Arginine	Arg	R	156	1289
Tyrosine	Tyr	Y	163	1296
Tryptophan	Trp	W	186	1319

m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

-Leu/Ile-Glu-(Glu-Gly or Trp)-Leu/Ile-Gln/Lys-Thr-Leu/Ile-Met-Gly-Arg

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue ma	+1246
Glycine	Gly	G	57	1303
Alanine	Ala	A	71	1317
Serine	Ser	S	87	1333
Proline	Pro	P	97	1343
Valine	Val	V	99	1345
Threonine	Thr	T	101	1347
Cysteine	Cys	C	103	1349
Isoleucine	Ile	I	113	1359
Leucine	Leu	L	113	1359
Asparagine	Asn	N	114	1360
Aspartic acid	Asp	D	115	1361
Glutamine	Gln	Q	128	1374
Lysine	Lys	K	128	1374
Glutamic acid	Glu	E	129	1375
Methionine	Met	M	131	1377
Histidine	His	H	137	1383
Phenylalanine	Phe	F	147	1393
Arginine	Arg	R	156	1402
Tyrosine	Tyr	Y	163	1409
Tryptophan	Trp	W	186	1432

Mr 1361

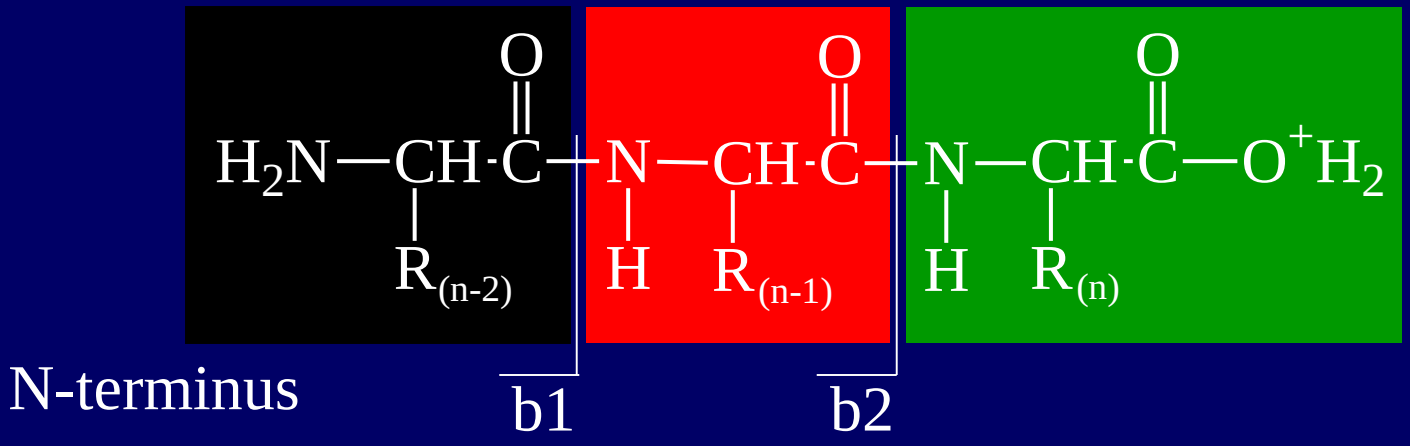
m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4

Step #4

Amino acid sequencing in an unknown peptide using ESP-MS/MS

Asp-Leu/Ile-Glu-(Glu-Gly or Trp)-Leu/Ile-Gln/Lys-Thr-Leu/Ile-Met-Gly-Arg

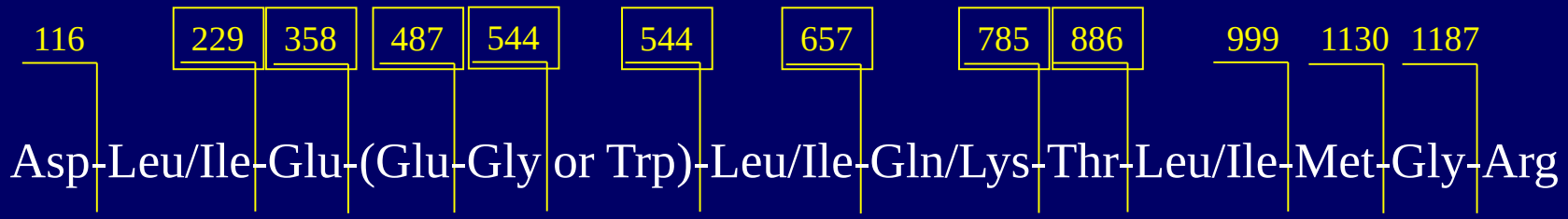
Amino acid sequencing in an unknown peptide using ESP-MS/MS



amino acid residues

Amino acid sequencing in an unknown peptide using ESP-MS/MS

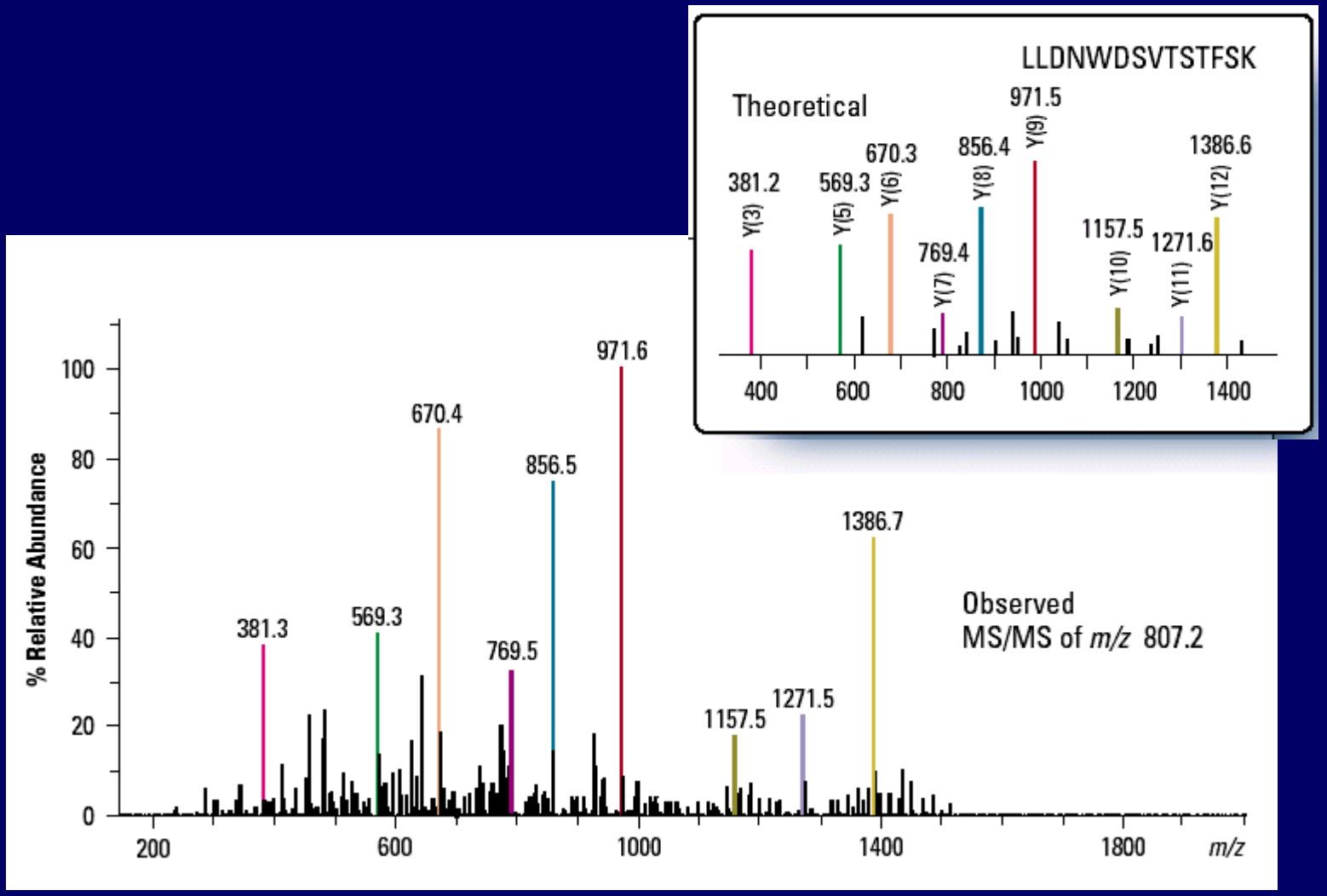
b-type ions



Masses of amino acid residues (-NHCHRCO-)

Name	Symbol	Symbol	Residue mass
Glycine	Gly	G	57
Alanine	Ala	A	71
Serine	Ser	S	87
Proline	Pro	P	97
Valine	Val	V	99
Threonine	Thr	T	101
Cysteine	Cys	C	103
Isoleucine	Ile	I	113
Leucine	Leu	L	113
Asparagine	Asn	N	114
Aspartic acid	Asp	D	115
Glutamine	Gln	Q	128
Lysine	Lys	K	128
Glutamic acid	Glu	E	129
Methionine	Met	M	131
Histidine	His	H	137
Phenylalanine	Phe	F	147
Arginine	Arg	R	156
Tyrosine	Tyr	Y	163
Tryptophan	Trp	W	186

m/z	*RA (%)*	*m/z*	*RA (%)*
85	12	544	25
102	13	566	13
142	6	577	48
175	7	629	8
201	100	657	14
229	95	705	92
232	28	785	4
259	20	818	29
298	7	858	5
316	20	875	96
358	46	886	4
363	29	987	10
383	10	1004	80
400	14	1116	10
476	27	1133	46
487	16	1246	4



Excellent match between the observed MS/MS spectrum from the most abundant ion (*m/z* 807.2) in the chromatographic peak at 17.55 minutes and the theoretical y-ion series predicted for a tryptic peptide from human apolipoprotein, one of the proteins in the sample mixture.

Mass Spectrometric Analysis of Proteins

Steps in procedure:

1. M_r determined by electropsray (ESP) or MALDI.
2. Samples of protein are digested with proteases like trypsin and chymotrypsin.
3. Each of the digests is fractionated by HPLC or CE.
4. The M_r of each peptide is determined by ESP or MALDI, subsequently collision induced dissociation is used to gather information on the sequence.
5. The sequence of as many of the oligopeptides as possible are deduced from the data.

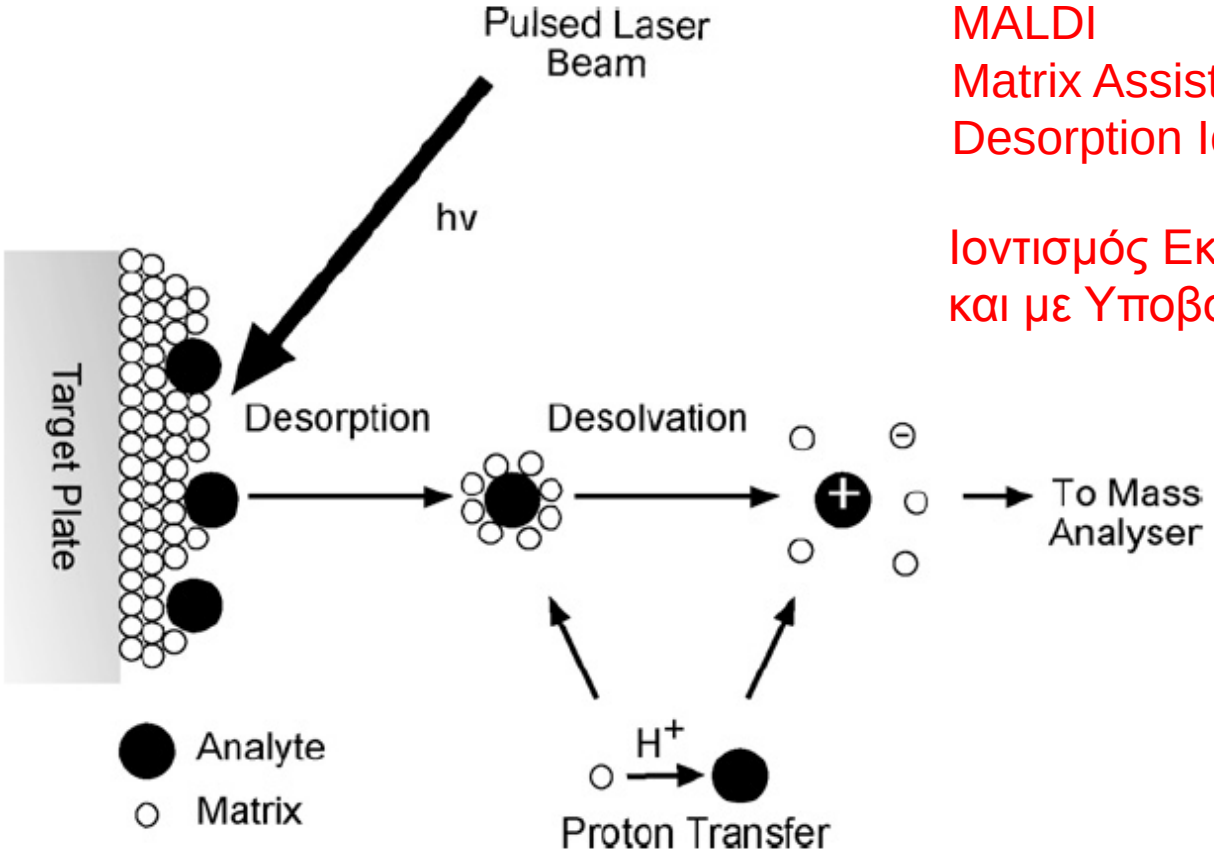
Mass Spectrometric Analysis of Proteins

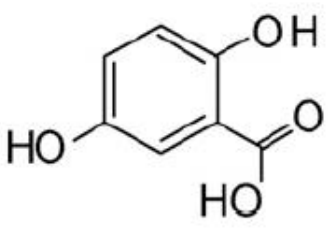
Steps in procedure:

1. M_r determined by electropsray (ESP) or MALDI.
2. Samples of protein are digested with proteases like trypsin and chymotrypsin.
3. Each of the digests is fractionated by HPLC or CE.
4. The M_r of each peptide is determined by ESP or MALDI, subsequently collision induced dissociation is used to gather information on the sequence.
5. The sequence of as many of the oligopeptides as possible are deduced from the data.
6. By looking for identical overlapping sequences, individual peptide sequences are assembled into the full sequence of the protein.

MALDI
Matrix Assisted Laser
Desorption Ionization

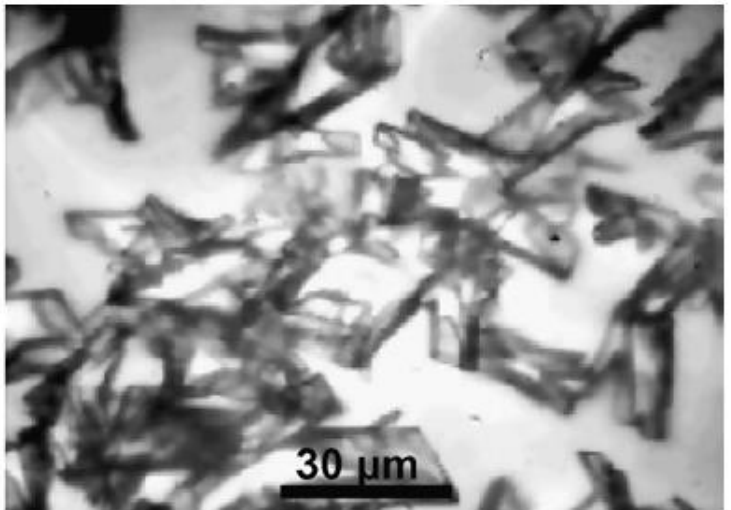
Ιοντισμός Εκρόφησης με Λέιζερ
και με Υποβοήθηση Μήτρας

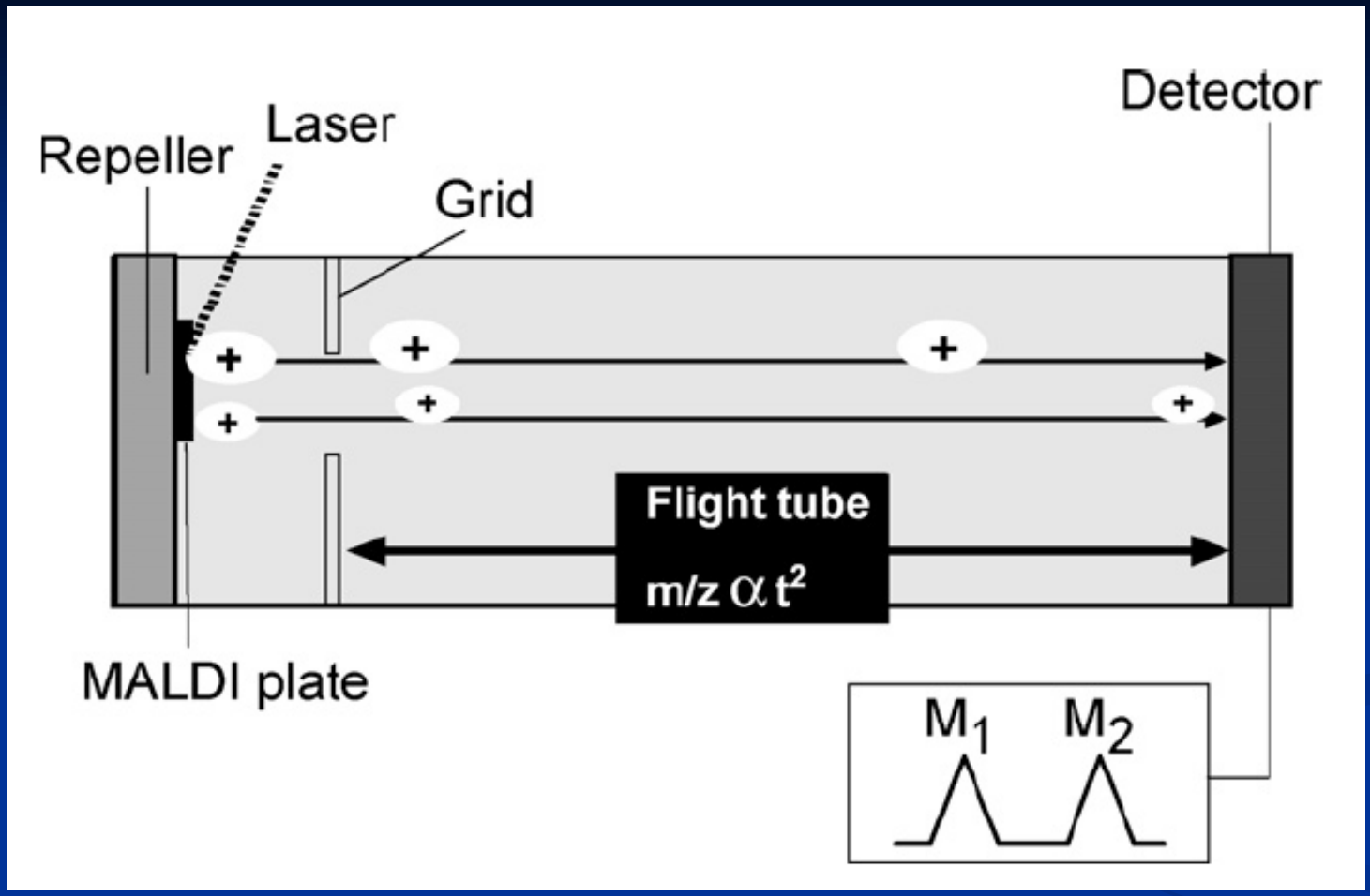




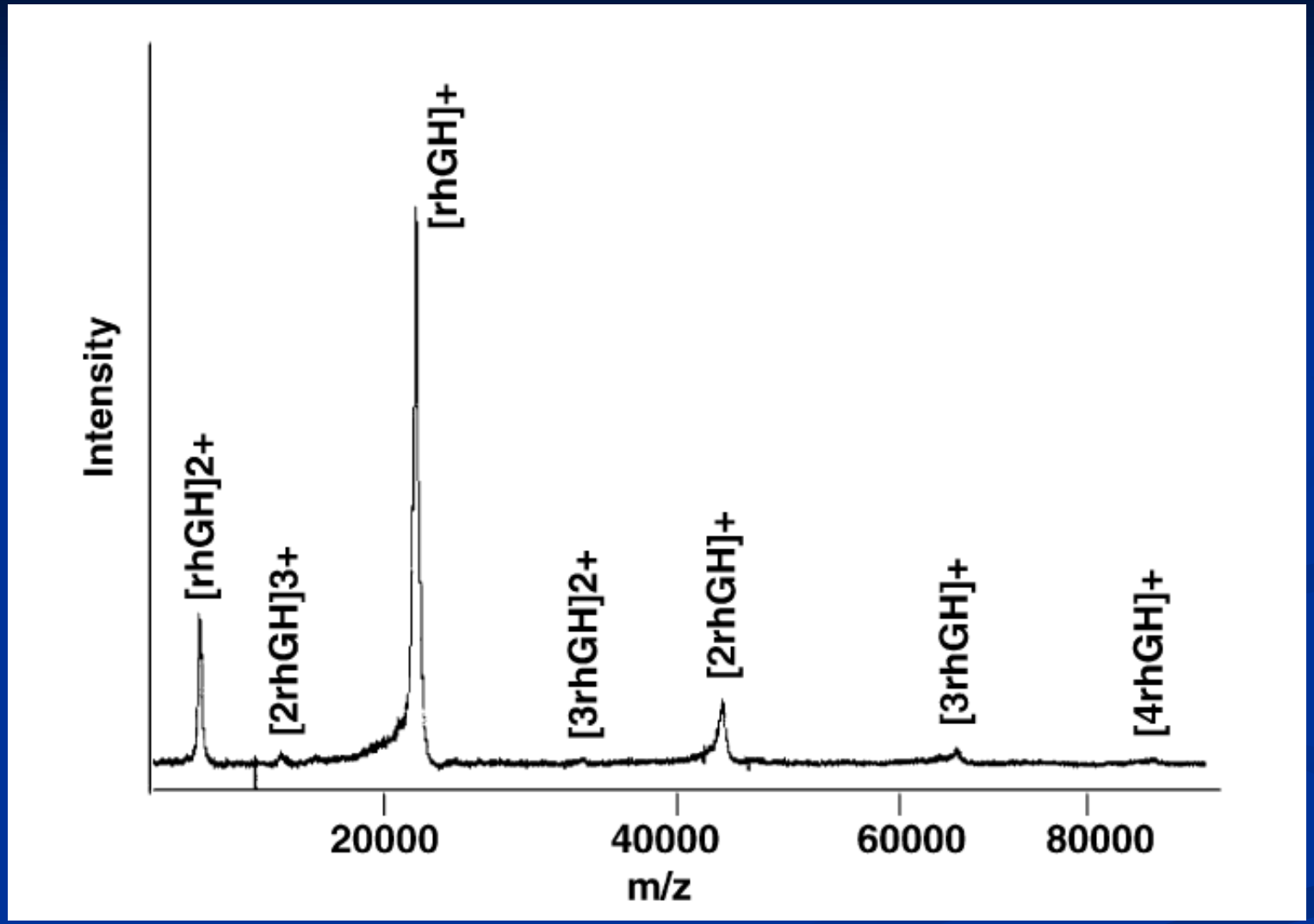
DHB

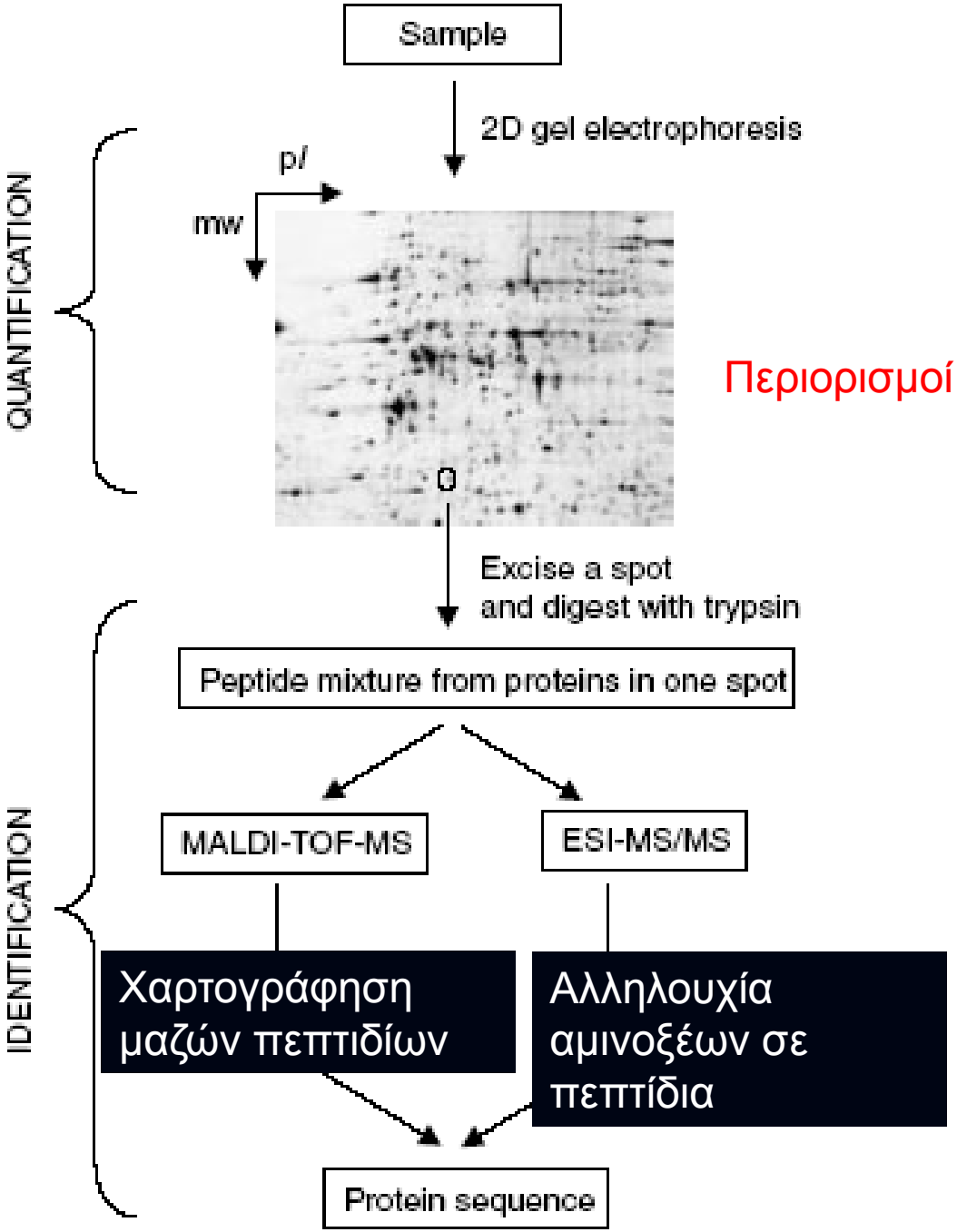
Protein
+
Excess Matrix
Evaporate Solvent



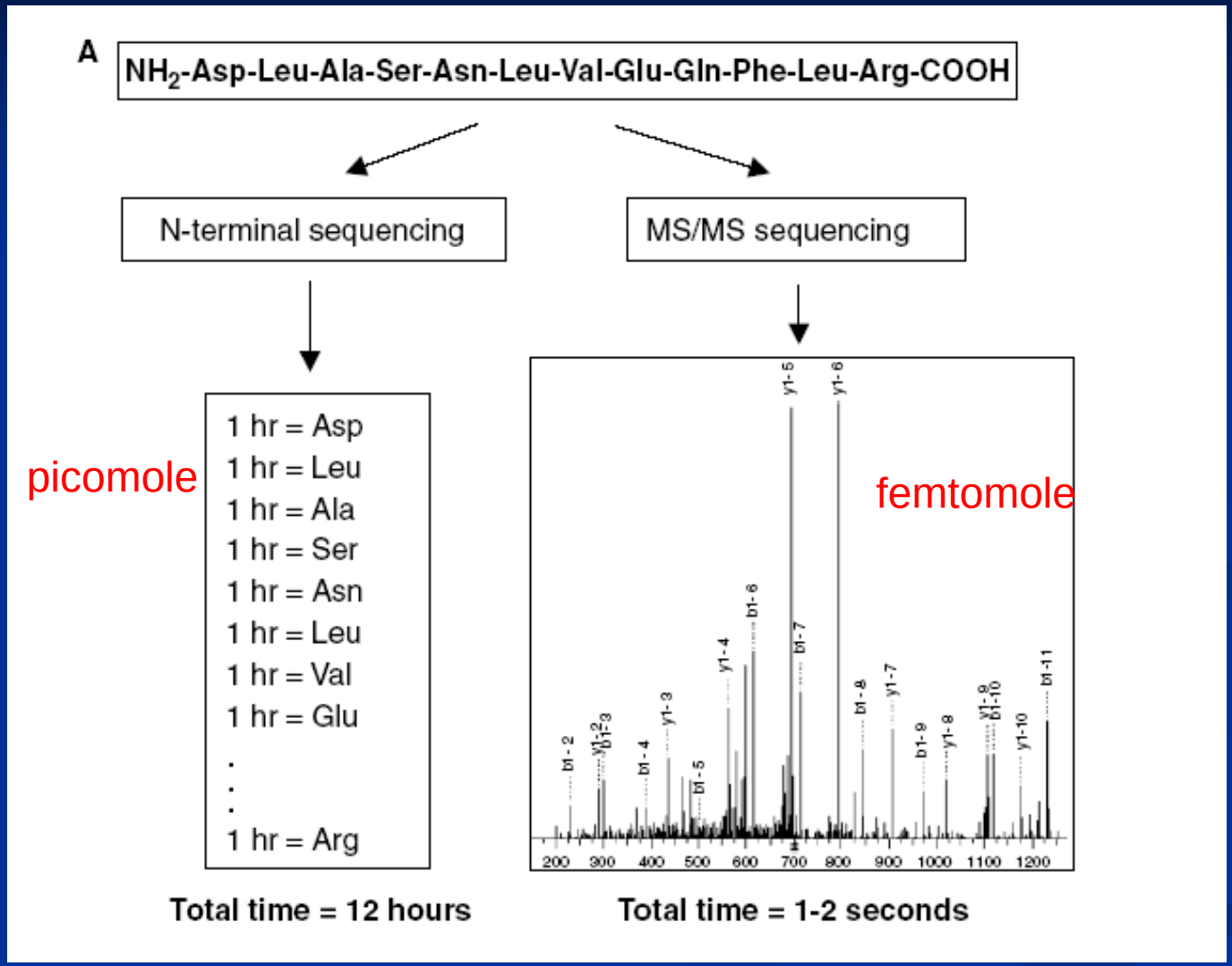


Ιοντισμός Εκρόφησης με Λέιζερ και με Υποβοήθηση Μήτρας

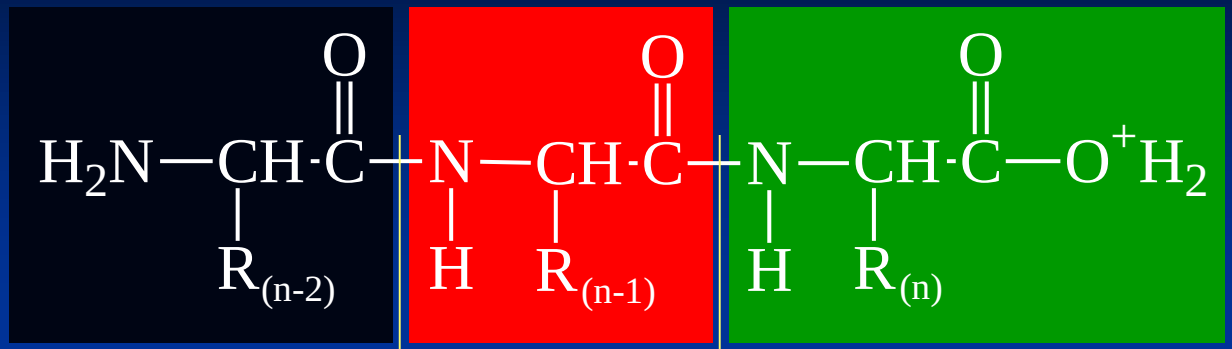

Intensity
[rhGH]2+
[2rhGH]3+
[rhGH]+
[3rhGH]2+
[2rhGH]+
[3rhGH]+
[4rhGH]+
20000
40000
60000
80000
m/z



Περιορισμοί


A
NH2-Asp-Leu-Ala-Ser-Asn-Leu-Val-Glu-Gln-Phe-Leu-Arg-COOH
N-terminal sequencing
MS/MS sequencing
picomole
1 hr = Asp
1 hr = Leu
1 hr = Ala
1 hr = Ser
1 hr = Asn
1 hr = Leu
1 hr = Val
1 hr = Glu
1 hr = Arg
femtomole
Total time = 12 hours
Total time = 1-2 seconds

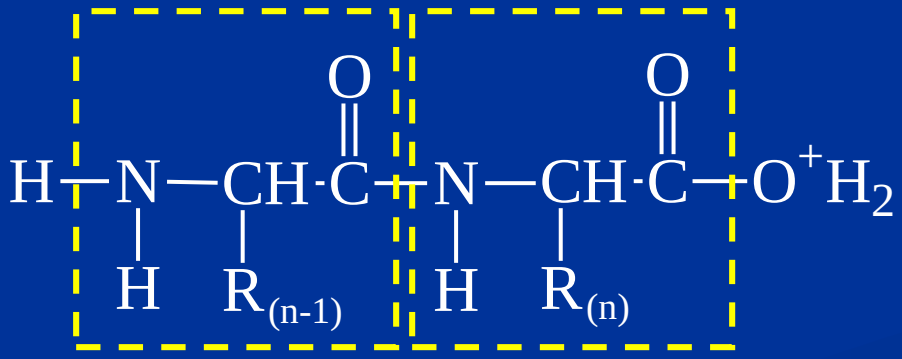
Προσδιορισμός Αλληλουχίας αμινοξέων σε πεπτίδιο με ES-MS/MS

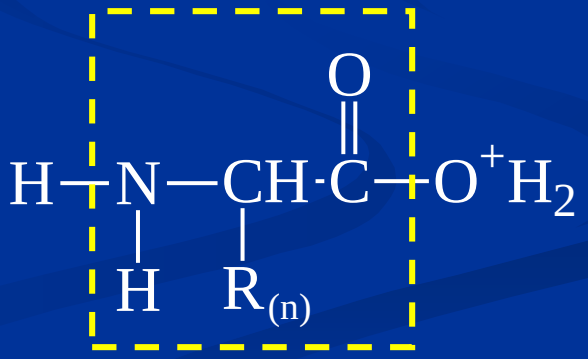


C-terminus

y2

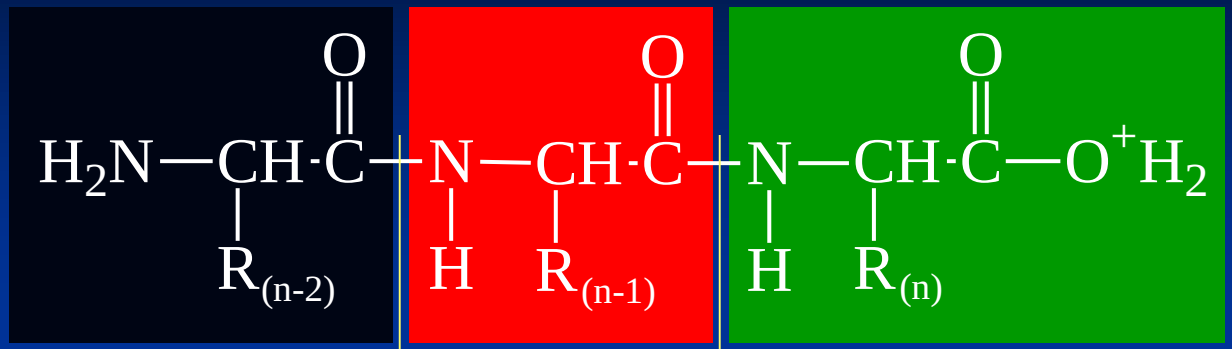
y1





amino acid residues

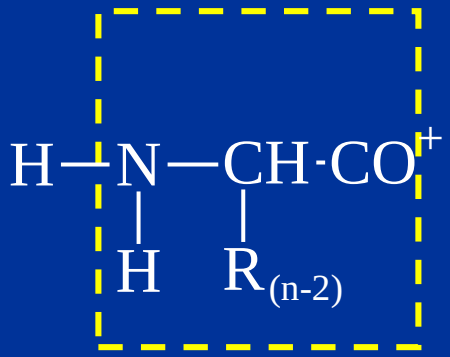
Προσδιορισμός Αλληλουχίας αμινοξέων σε πεπτίδιο με ES-MS/MS

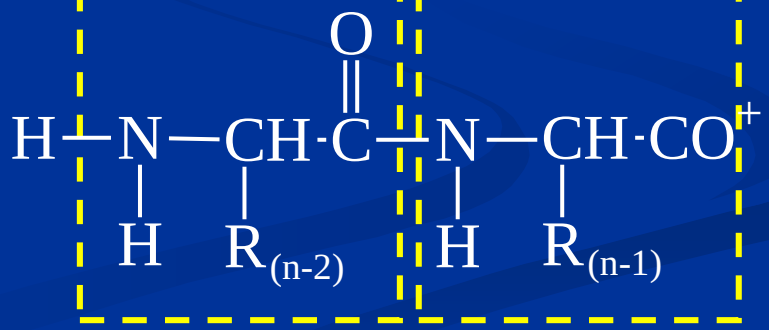


N-terminus

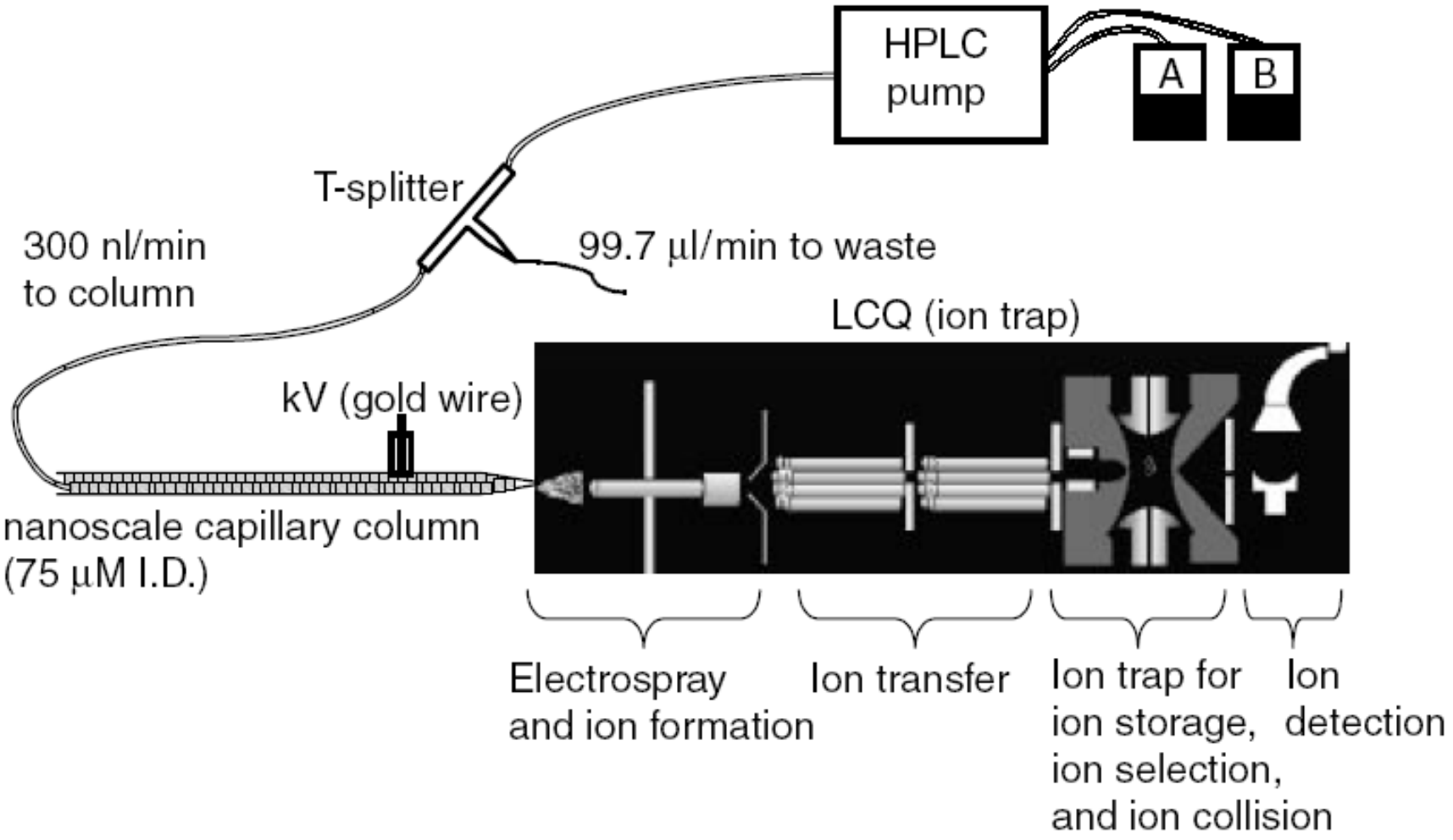
b1

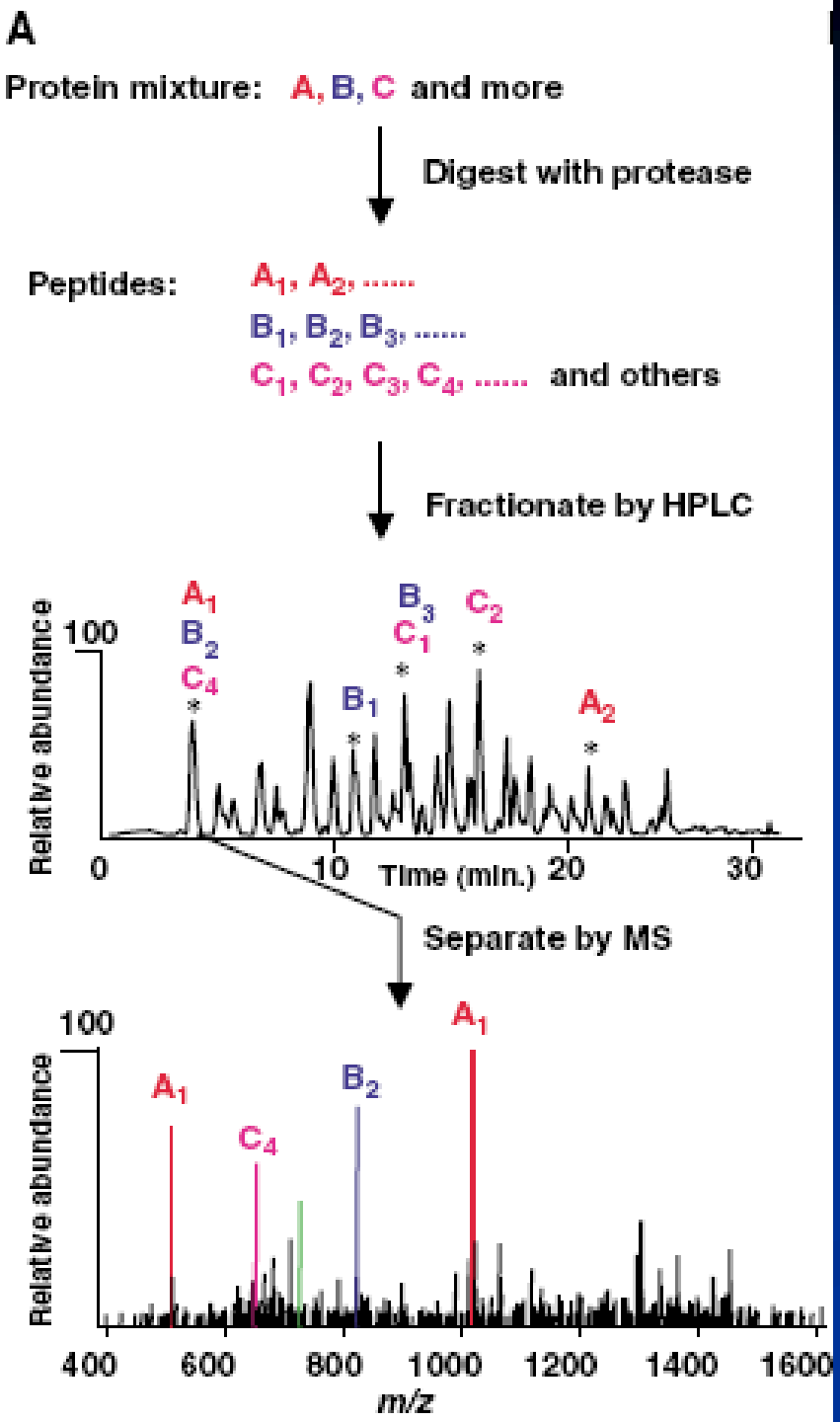
b2





amino acid residues


HPLC
pump
A
B
T-splitter
300 nl/min
to column
99.7 µl/min to waste
LCQ (ion trap)
kV (gold wire)
nanoscale capillary column
(75 µM I.D.)
Electrospray
and ion formation
Ion transfer
Ion trap for
ion storage,
ion selection,
and ion collision
Ion
detection



B

Events during 12 seconds of LC-MS/MS:

.....

4'0"-1": MS of peptides eluted

4'1"-3": MS/MS of m/z 1018, A_1

4'3"-5": MS/MS of m/z 820, B_2

4'5"-7": MS/MS of m/z 509, A_1

4'7"-9": MS/MS of m/z 650, C_4

4'9"-11": MS/MS of m/z 724

4'11"-12": MS again

.....

5 sequencing attempts in 11 seconds

800 sequencing attempts in 30 minutes

B

Events during 12 seconds of LC-MS/MS:

.....

4'0"-1": MS of peptides eluted

4'1"-3": MS/MS of m/z 1018, A_1

4'3"-5": MS/MS of m/z 820, B_2

4'5"-7": MS/MS of m/z 509, A_1

4'7"-9": MS/MS of m/z 650, C_4

4'9"-11": MS/MS of m/z 724

4'11"-12": MS again

.....

5 sequencing attempts in 11 seconds

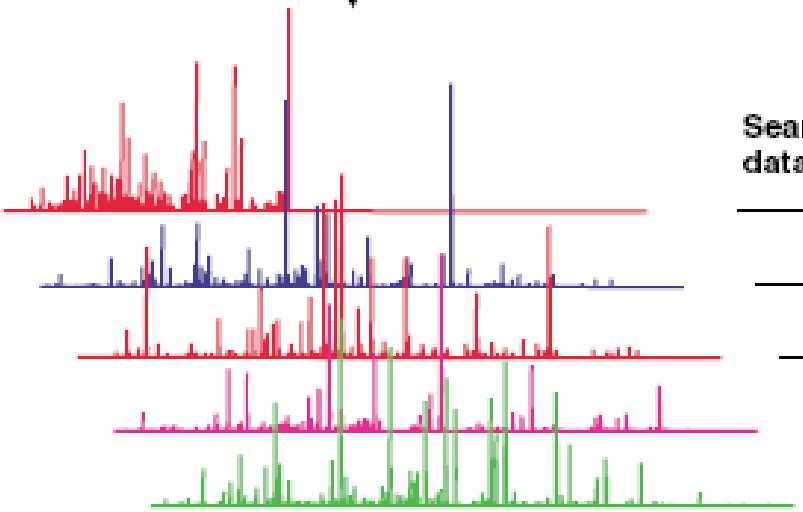
800 sequencing attempts in 30 minutes

Identification of proteins:
A, B, C and .....

Sequences of all peptides:
A_1, A_2,
B_1, B_2, B_3,
C_1, C_2, C_4, and .....

Analyze by MS/MS
(Top 5 ions, see panel B)

Output all data



Search database

5 peptide sequences:

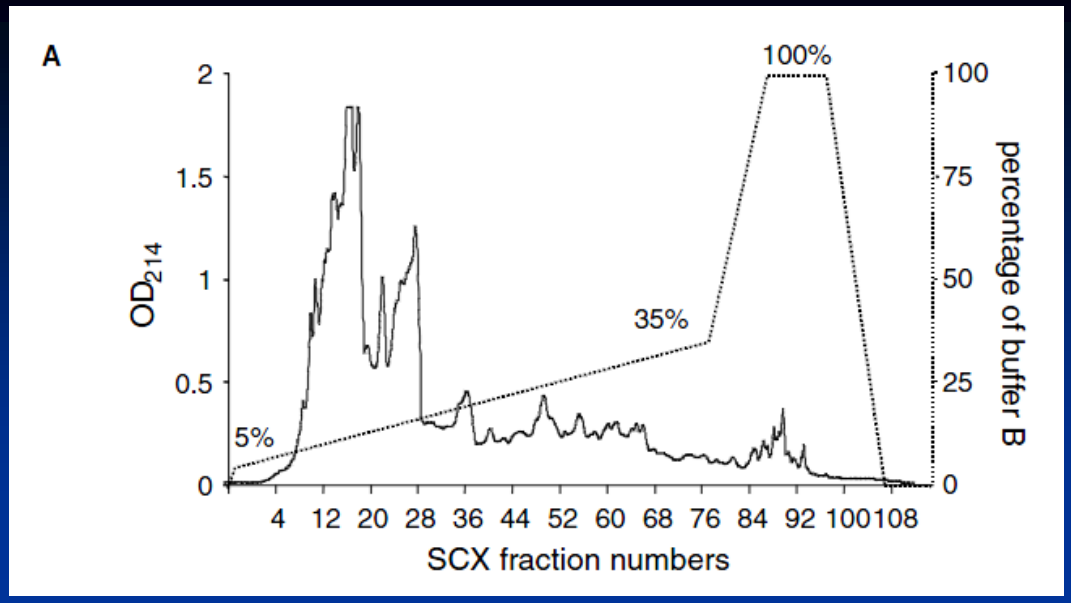
LLTTIADAAK

EFNDPSNAGLQNGFK

LLTTIADAAK

SAGGNYVVFGEAK

EDDVEEAVQAADR


A
100%
35%
5%
OD_{214}
percentage of buffer B
SCX fraction numbers
0
0.5
1
1.5
2
0
25
50
75
100
4 12 20 28 36 44 52 60 68 76 84 92 100108

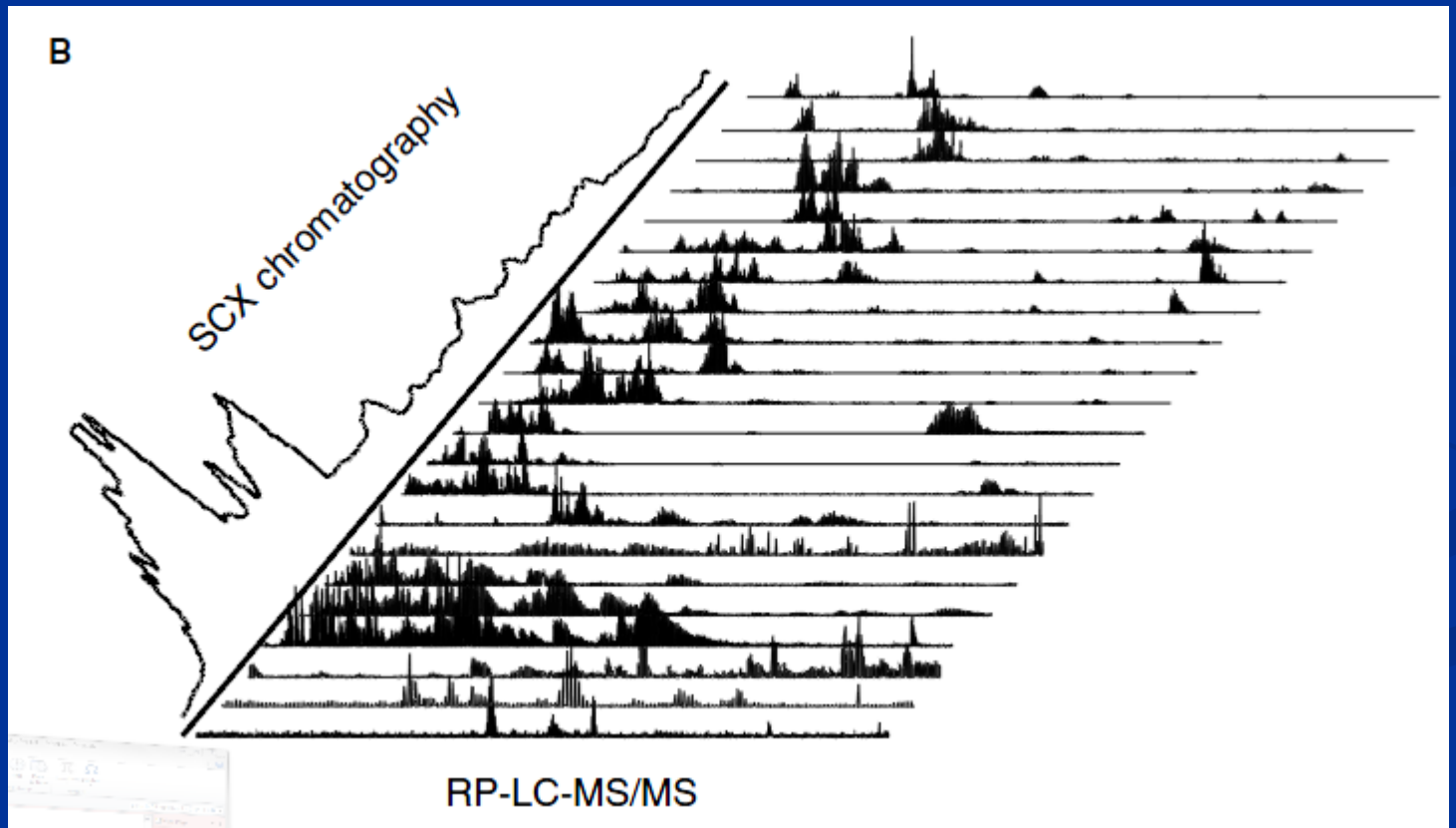
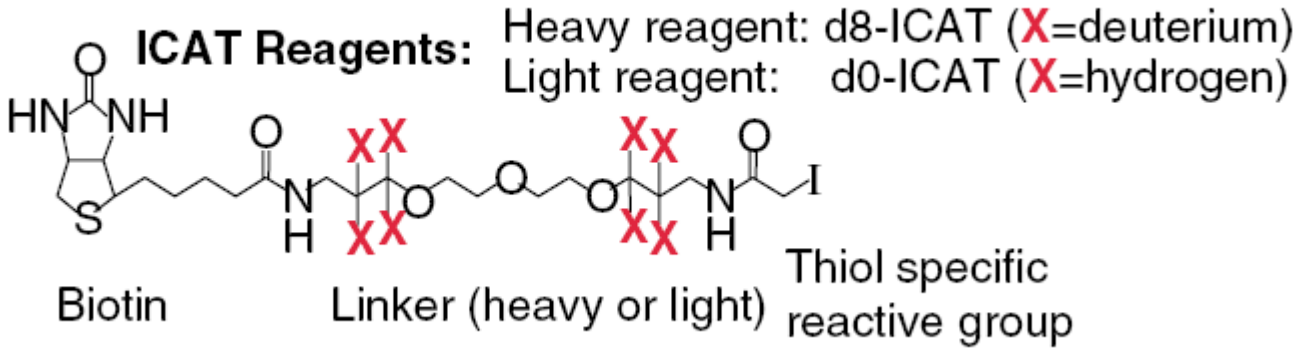
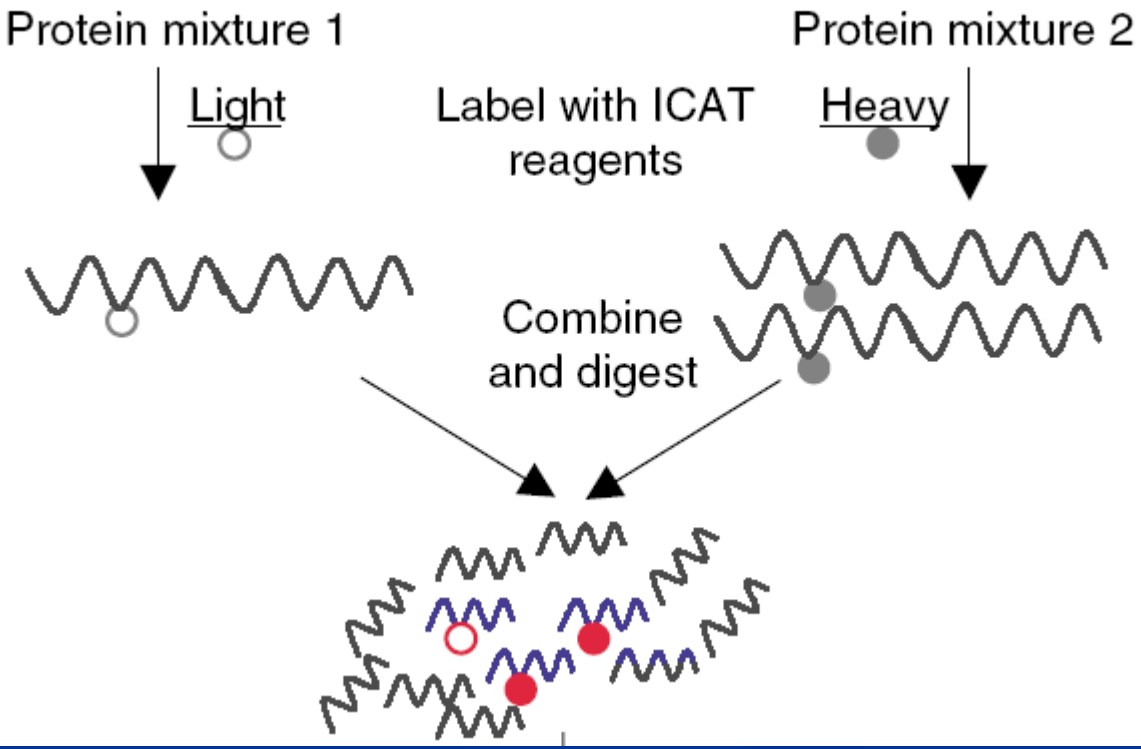

B
SCX chromatography
RP-LC-MS/MS

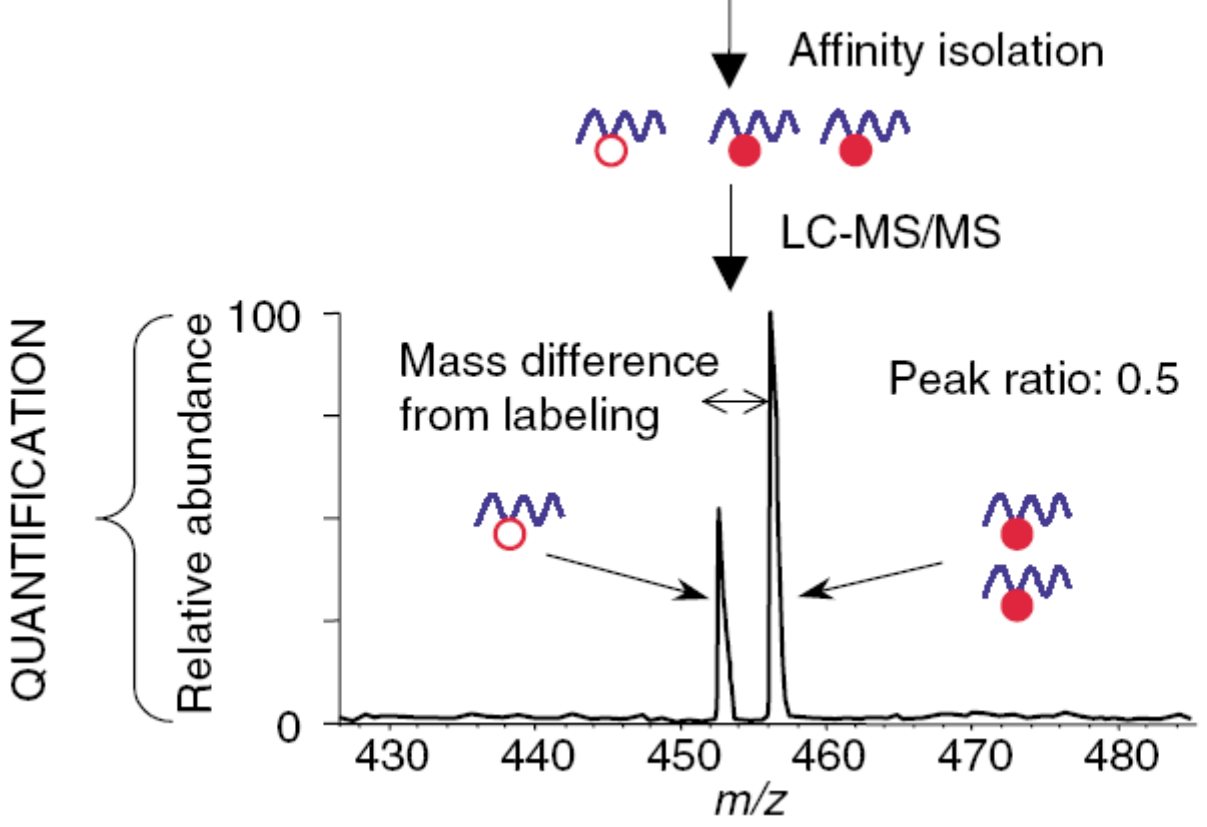
Figure 7. Multi-dimensional peptide chromatography permits the analysis of thousands of proteins from a single sample. In the example shown, 1 mg of whole cell yeast extract was proteolyzed with trypsin under reducing and denaturing conditions. (A) The highly complex peptide solution was separated in the first dimension by strong cation-exchange (SCX) chromatography with UV detection and fraction collection every minute (solvent A, 5 mM phosphate buffer, 25% acetonitrile, pH 3.0; solvent B, the same as A with 350 mM KCl). (B) The collected fractions were then analyzed individually by the nanoscale microcapillary LC/MS/MS techniques described in Fig. 6. As a result, more than 12 000 unique peptides were sequenced and more than 1600 unique proteins were identified from 80 fractions using previously established criteria for database matching.[9]

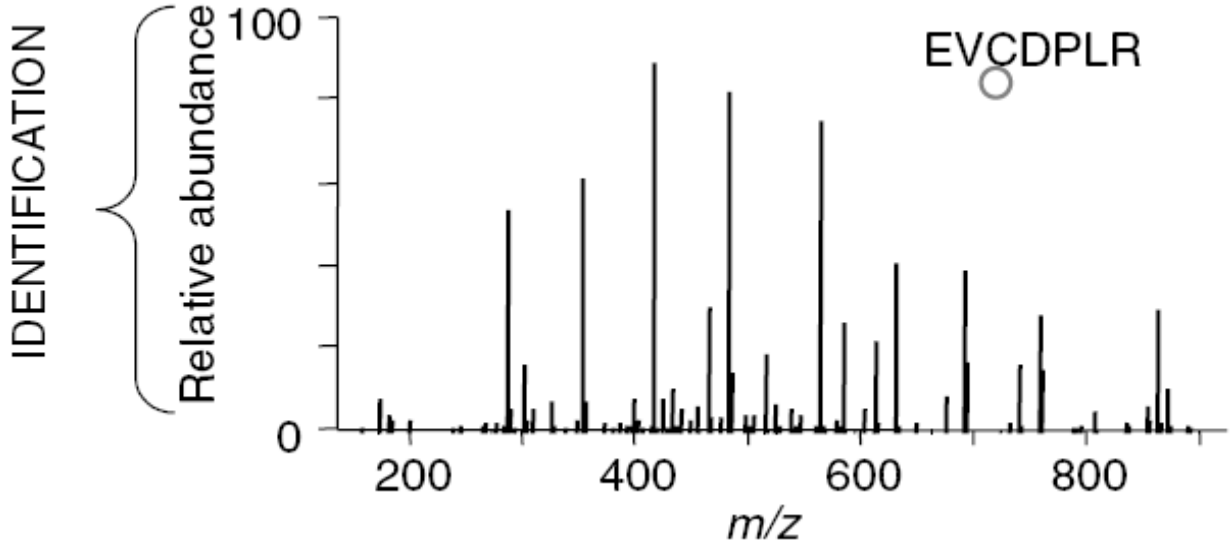
A

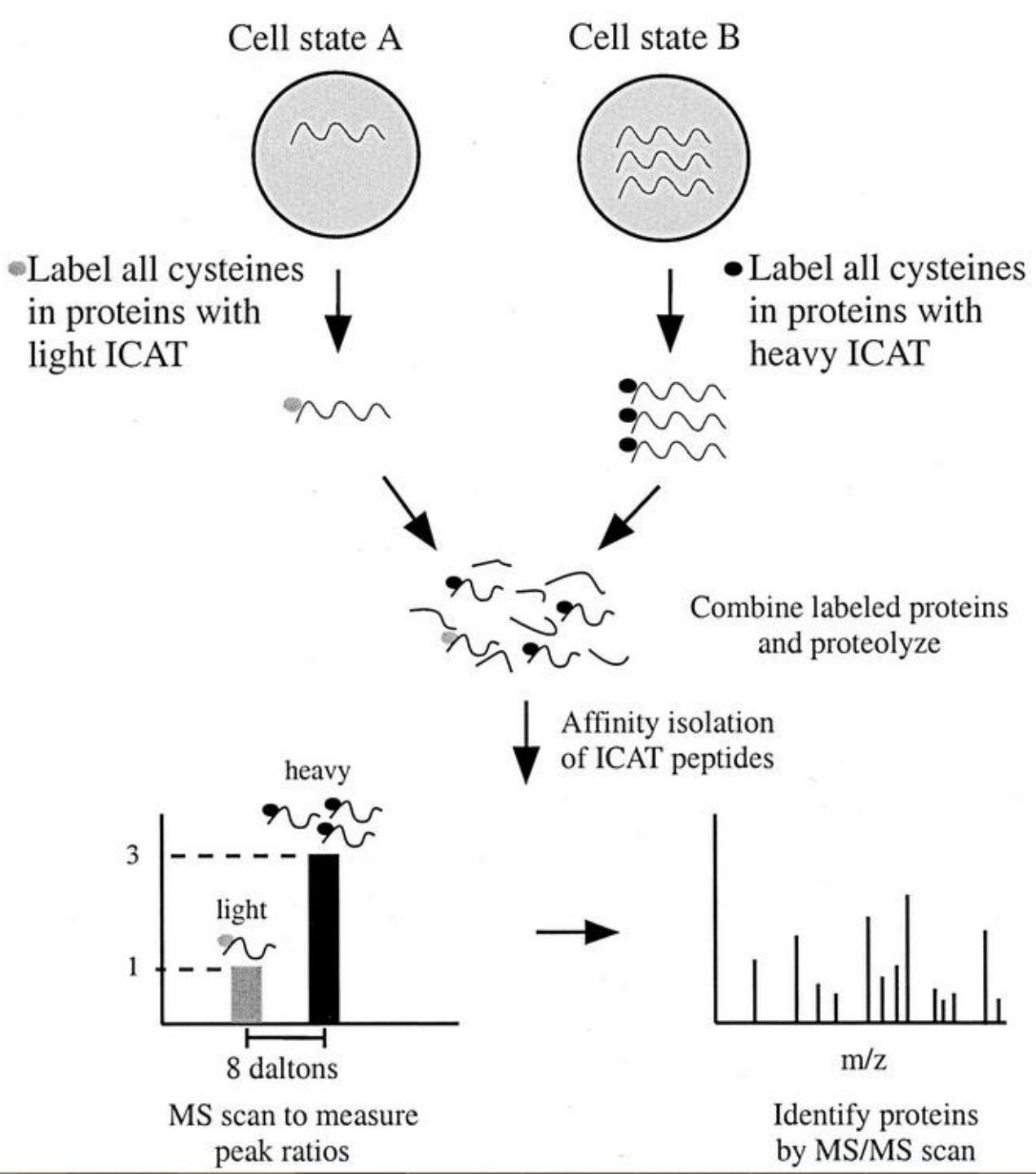

ICAT Reagents:
Heavy reagent: d8-ICAT (X=deuterium)
Light reagent: d0-ICAT (X=hydrogen)
Biotin
Linker (heavy or light)
Thiol specific reactive group

B


Protein mixture 1
Light
Label with ICAT reagents
Protein mixture 2
Heavy
Combine and digest


Affinity isolation
LC-MS/MS
QUANTIFICATION
Relative abundance
100
0
Mass difference
from labeling
Peak ratio: 0.5
430
440
450
460
470
480
m/z


IDENTIFICATION
Relative abundance
100
0
EVCDPLR
200
400
600
800
m/z


Cell state A
Cell state B
Label all cysteines in proteins with light ICAT
Label all cysteines in proteins with heavy ICAT
Combine labeled proteins and proteolyze
Affinity isolation of ICAT peptides
heavy
light
3
1
8 daltons
MS scan to measure peak ratios
m/z
Identify proteins by MS/MS scan

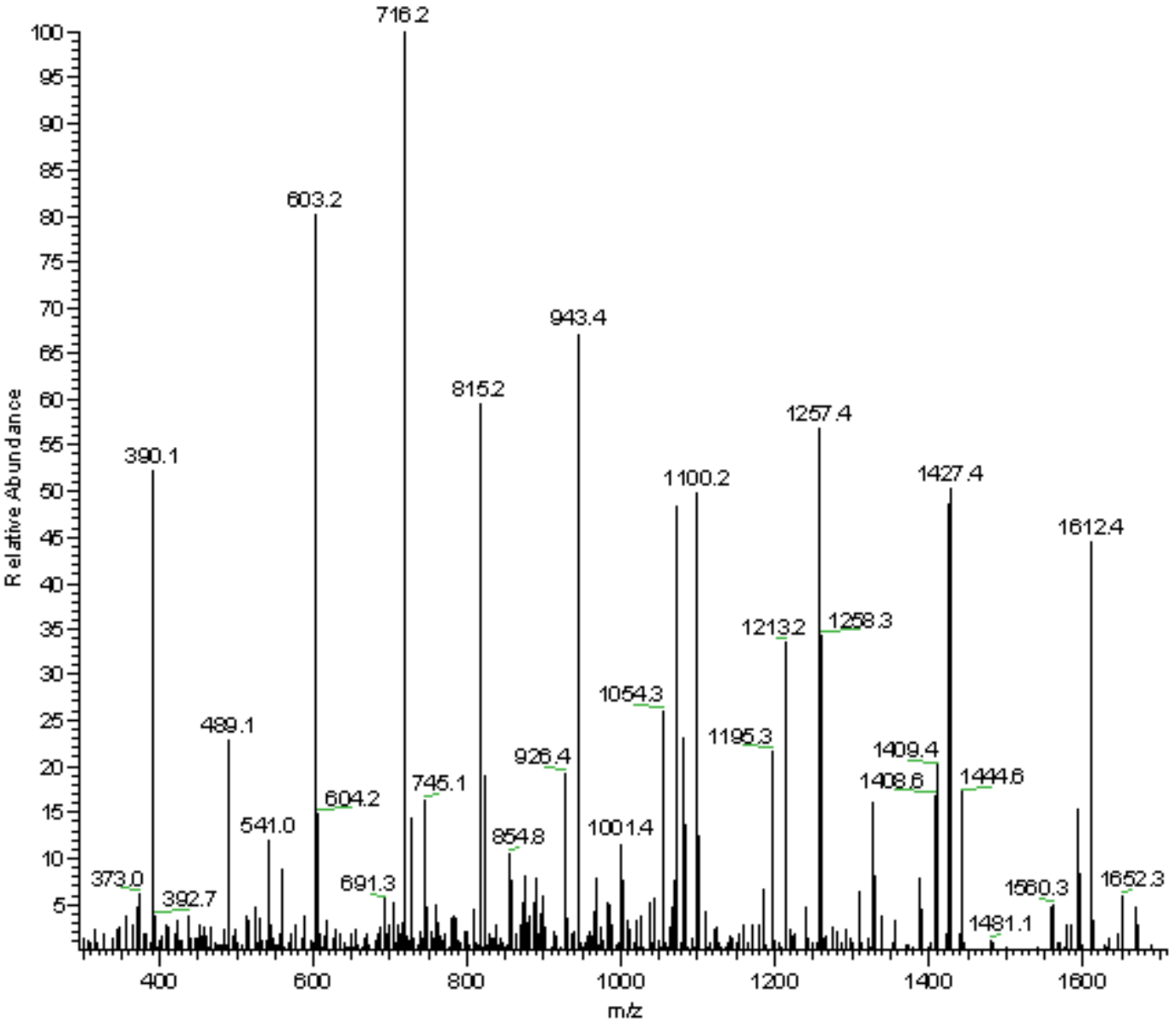


Figure 1. This is an MS/MS spectrum of the tryptic peptide GLSDGEWQQVLNVWGK. This data was collected on an ion trap mass spectrometer.