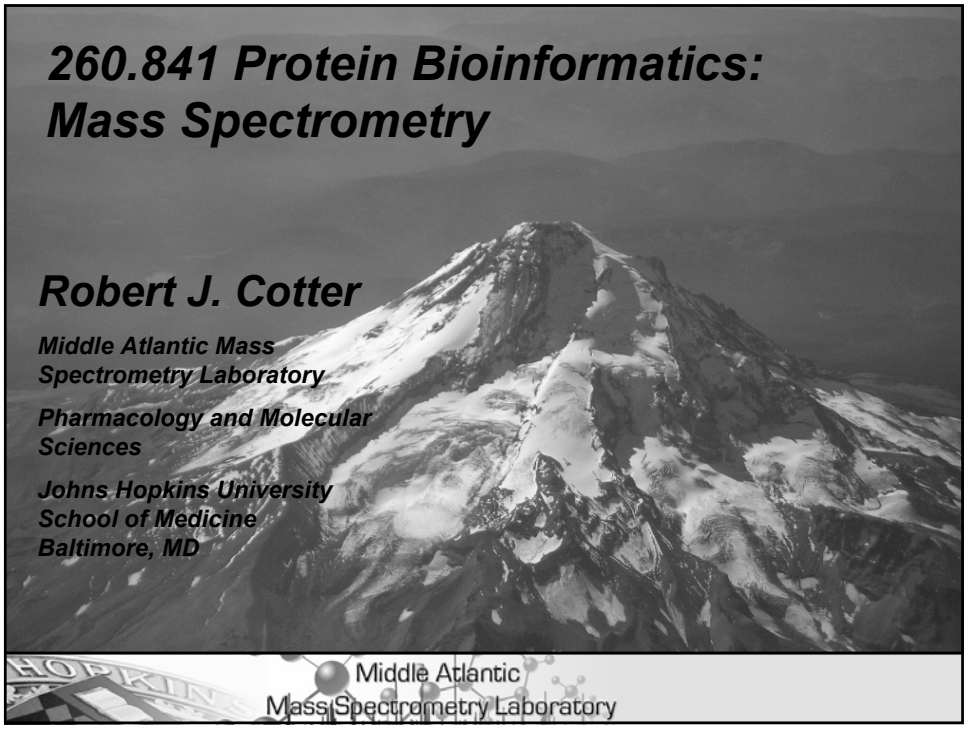
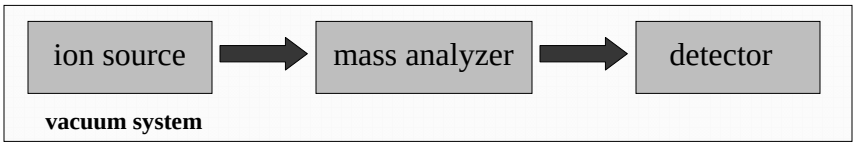


What is a mass spectrometer?



Chemical	TOF: time-of-flight	multiplier
EI: electron impact	ITMS: ion trap mass spectrometer	channelplate
CI: chemical ionization	FTMS: Fourier transform mass spectrometer	
Biological	…. and combinations of these (QTOF, TOF/TOF, IT/TOF)	

MALDI: matrix-assisted laser desorption/ionization

ESI: electrospray ionization

AP/MALDI: atmospheric pressure MALDI

a balance for weighing molecules

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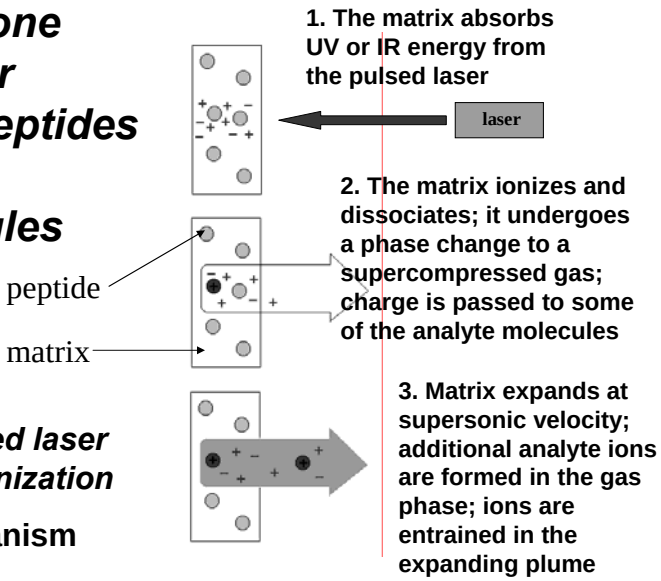
MALDI is one method for ionizing peptides and other biomolecules

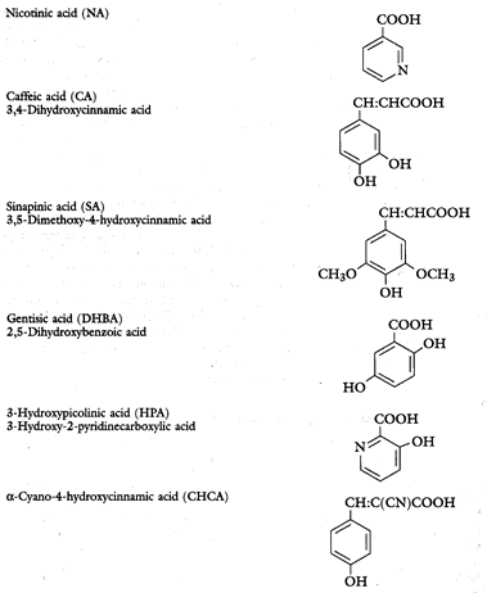


Matrix-assisted laser desorption/ionization

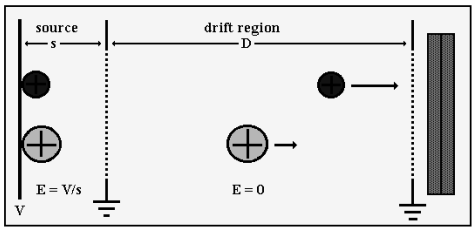
MALDI mechanism

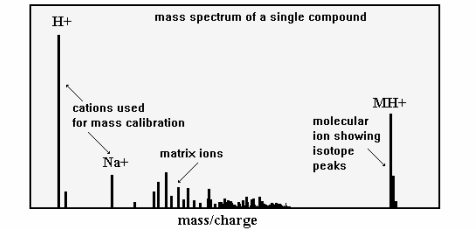
The MALDI "matrix":

In MALDI, samples are deposited in solutions of a UV-absorbing matrix, which absorbs the laser energy, ionizes and then protonates the sample molecules



MALDI is most often used with a time-of-flight (TOF) mass spectrometer:





Short source region (s) with a high field for extracting the ions

Longer field-free drift region (D)

Flight times follow a simple square root dependence on mass:

$$t = \left(\frac{m}{2eV} \right)^{1/2} D$$

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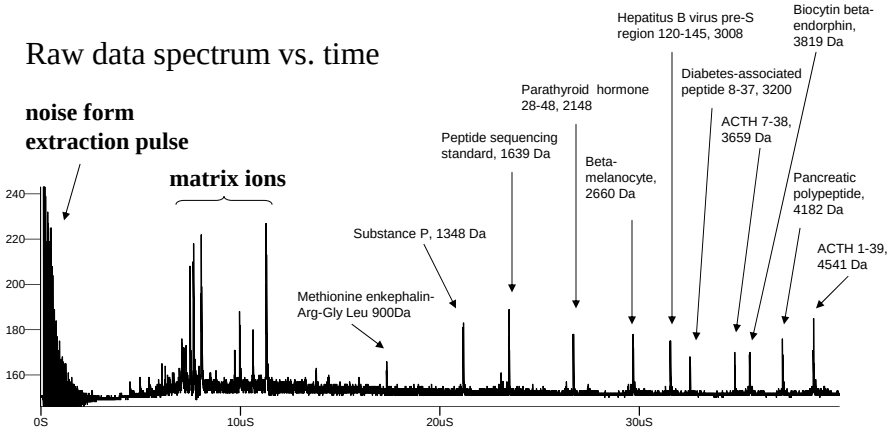
Time-of-flight mass spectrum.

Raw data spectrum vs. time



Mass spectrum of a mixture of 11 peptides obtained with pulsed (delayed) extraction on a linear TOF.

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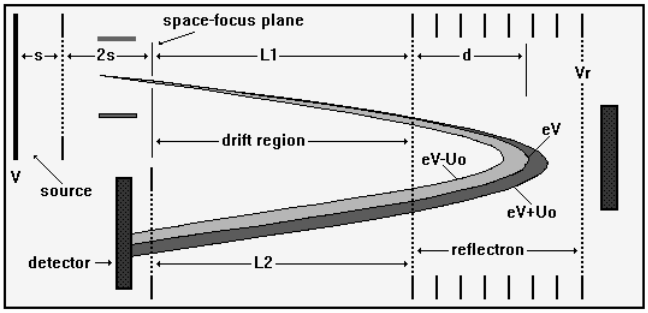
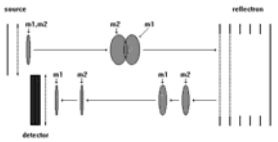
Mass resolution is improved using a "single-stage" reflectron mass analyzer



Flight times are still proportional to the square root of the mass:

$$t = \left(\frac{m}{2eV}\right)^{1/2} [L_1 + L_2 + 4d]$$



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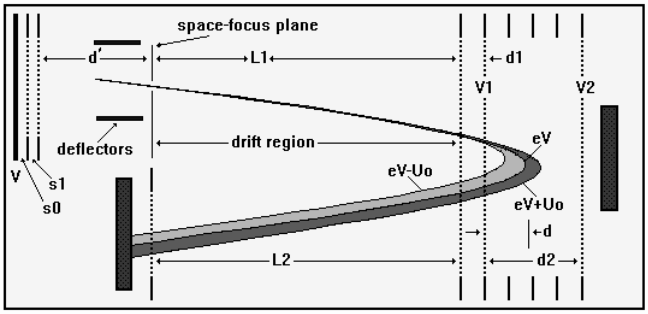
Reflectrons compensate for the initial kinetic energy spread of ions.



The "dual-stage" reflectron mass analyzer provides "second order" focusing.

Flight times are proportional to the square root of mass.

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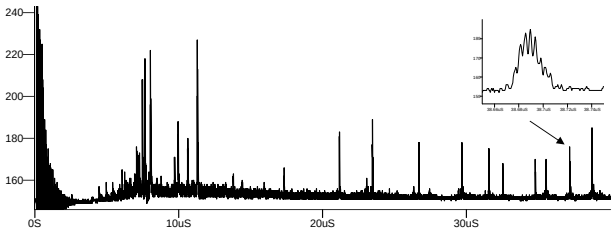
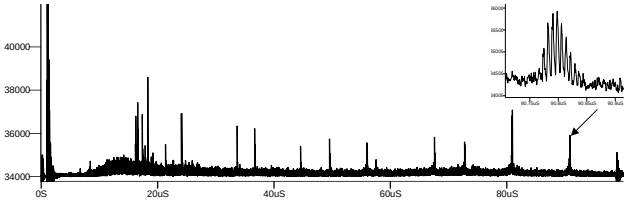
Comparison of linear and reflectron mass spectra

Mass spectrum of a mixture of 11 peptides obtained on a <u>linear</u> TOF



Mass spectrum of a mixture of 9 peptides obtained on a <u>reflectron</u> TOF



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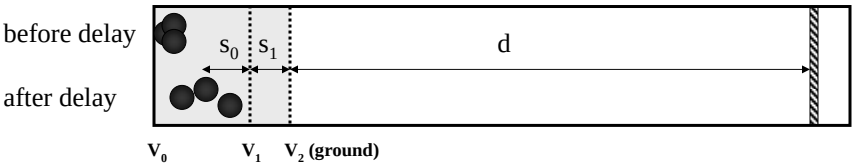
Another way to improve mass resolution is using "delayed extraction"":*

During the delay period, ions with different energies array themselves in different locations.



When the extraction pulse is applied the slower ions will be accelerated the most, and will catch up with the faster ions when they reach the detector

*also known as "pulsed extraction" or "time-lag focusing"

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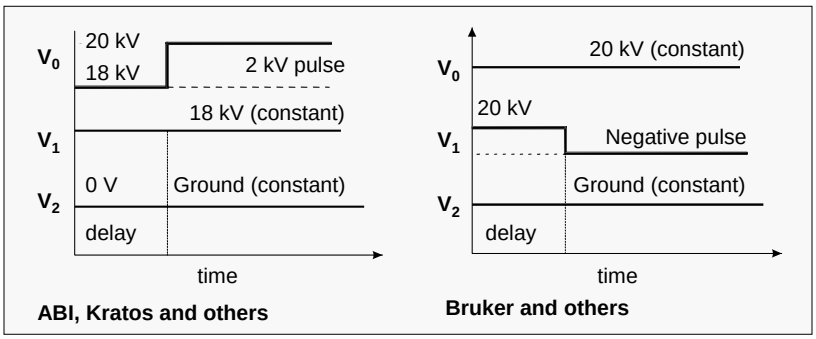
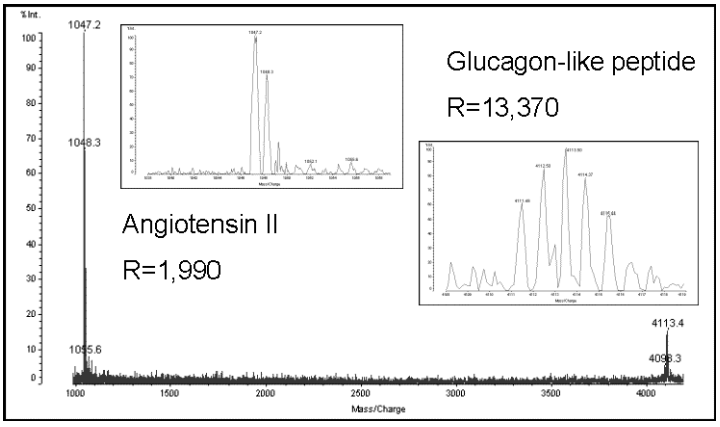
A number of different methods are used to pulse the field in the first extraction region:



Whittal, R.M.; Li, L., **Anal. Chem. 67** (1995) 1950-1954
Brown, R.S.; Lennon, J.J., **Anal Chem. 67** (1995) 1998-2003.
Vestal, M.L.; Juhasz, P.; Martin, S.A, **Rapid Commun. Mass Spectrom. 9** (1995) 1044-1050.

Maximum mass resolution results from a combination of a reflectron and delayed extraction



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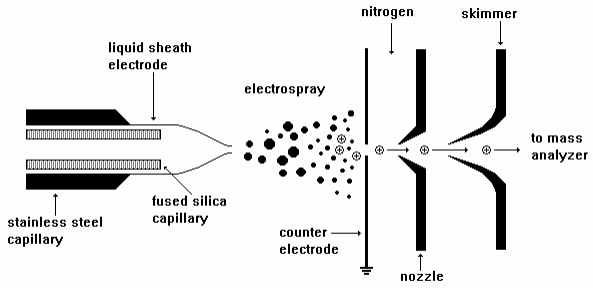
Electrospray ionization (ESI)

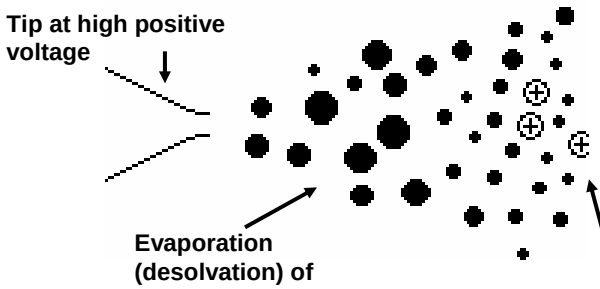


Introduced by John Fenn:

Yamashita, M.; Fenn, J.B., **J. Phys. Chem. 88** (1984) 4451.
Whitehouse, C.M.; Dreyer, R.N.; Yamashita, M.; Fenn, J.B., **Anal. Chem. 57** (1985) 675.
Fenn, J.B.; Mann, M.; Meng, C.K.; Wong, S.F.; Whitehouse, C.M., **Science 246** (1989) 64.

Based on an ion evaporation model:

Iribarne, J.V.; Thomson, B.A., **J. Chem. Phys. 64** (1976) 2287.
Thomson, B.A.; Iribarne, J.V., **J. Chem. Phys. 71** (1979) 4451.



Evaporation (desolvation) of positively-charged droplets until the *Rayleigh limit* is reached.

Ejection of positively charged ions from droplet.

Favors ejection of multiply-charged ions

ESI produces multiply-charged ions

Ions have the formula:

$(M + nH)^{+n}$

From which one can determine M.

Average *m/z* are around 1000, which is compatible with quadrupole mass spectrometers and ion traps.

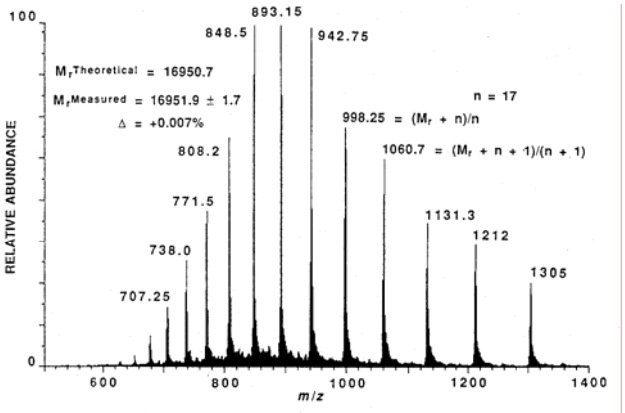
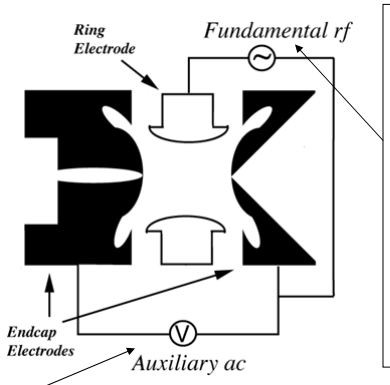


FIG. 3. ESI mass spectrum of equine myoglobin. Peaks *m*/*z* 707.25 through *m*/*z* 1305 are centroid values for these multiply protonated (24+ through 13+) ions. The simultaneous relation of one pair, *m*/*z* 998.25 and *m*/*z* 1060.7, is illustrated where the integer value of *n* is 17. Calculation of M_r as discussed in the text.

ESI is commonly used on quadrupole ion trap mass spectrometers (ITMS)



- **1.1 MHz**
- **Used to trap the ions**
- **used to scan the mass range in either *mass-selective instability* or *resonance ejection* modes**

- **supplementary RF for *resonance ejection* mode and high mass**
- **mass selection**
- **excitation in MS/MS mode**

Mathieu parameters:

$$q_z = \frac{8eV}{m(r_0^2 + 2z_0^2)\Omega_0^2}$$

$$a_z = \frac{-16eU}{m(r_0^2 + 2z_0^2)\Omega_0^2}$$

Mass selective instability mode: if dc voltage on the endcaps is zero, then scan along the a_z line (by varying the rf voltage); ion ejection occurs at the stability boundary when a_z = 0.908

The mass ejected is then given by:

$$\frac{m}{z} = \frac{8V}{(r_0^2 + 2z_0^2)\Omega_0^2 q_z}$$

Where *z* is the number of charges and Ω_0 is the angular drive frequency ($\Omega_0/2\pi$ = 1.1 MHz)

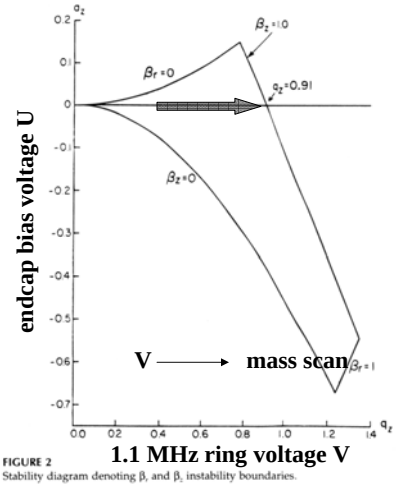


FIGURE 2
Stability diagram denoting β, and β, instability boundaries.

Williams, J.D.; Cox, K.A.; Schwartz, J.C.; Cooks, R.G., in *Practical Aspects of Ion Trap Mass Spectrometry*, Volume II, Cairns, T., Ed., CRC Press, Boca Raton (1995), pp. 3-50

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Resonance ejection mode:

A supplementary rf voltage is applied to the endcaps

The fundamental rf voltage on the ring electrode is scanned

Ions are ejected "through a hole in the stability region"

Extension of mass range through axial modulation

Supplementary rf = 69.9 kHz
q_{eject} = 0.182
m/z = (0.91/0.182) x 650 = 5 x 650 = 3,250

Supplementary rf = 35.2 kHz
q_{eject} = 0.091
m/z = (0.91/0.091) x 650 = 10 x 650 = 6,500

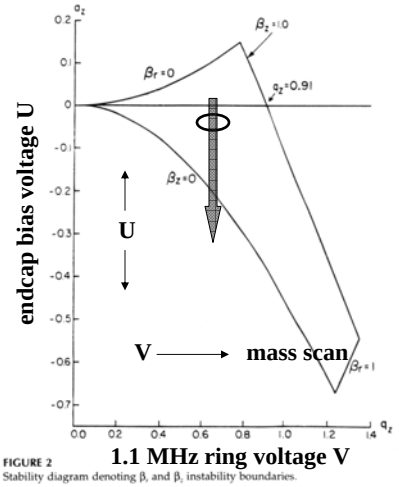

FIGURE 2
Stability diagram denoting β, and β, instability boundaries.

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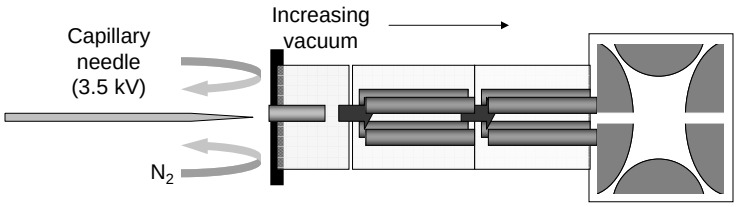
The "LCQ" ion trap mass spectrometry uses a quadrupole or octapole inlet system to interface to the atmosphere



Electrospray source at atmospheric pressure

RF-only quadrupole or octapole filters that collimate ions from high pressure ESI source.

Ion trap mass spectrometer

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Using mass spectrometry (MS) for peptide mapping and sequencing

Molecular weights obtained following the use of endo and exopeptidases

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Amino acid "residue" masses table

Amino acid	Three letter code	Single letter code	Nominal mass	Monoisotopic mass	Average mass
Alanine	Ala	A	71	71.037	71.079
Arginine	Arg	R	156	156.101	156.188
Asparagine	Asn	N	114	114.043	114.104
Aspartic acid	Asp	D	115	115.027	115.089
Cysteine	Cys	C	103	103.009	103.143
Glutamic acid	Glu	E	129	129.043	129.116
Glutamine	Gln	Q	128	128.059	128.131
Glycine	Gly	G	57	57.021	57.052
Histidine	His	H	137	137.059	137.141
Isoleucine	Ile	I	113	113.084	113.160
Leucine	Leu	L	113	113.084	113.160
Lysine	Lys	K	128	128.095	128.175
Methionine	Met	M	131	131.040	131.197
Phenylalanine	Phe	F	147	147.068	147.177
Proline	Pro	P	97	97.053	97.117
Serine	Ser	S	87	87.032	87.078
Threonine	Thr	T	101	101.048	101.105
Tryptophan	Trp	W	186	186.079	186.214
Tyrosine	Tyr	Y	163	163.063	163.176
Valine	Val	V	99	99.068	99.133

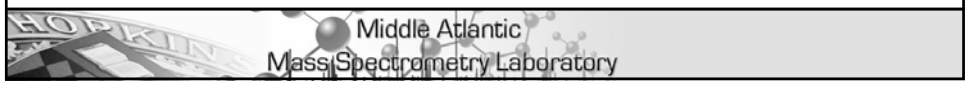
Chemical reagents and proteolytic enzymes for "peptide mapping"

Chemical reagents	Cleavage sites	Comments
Cyanogen bromide	after M	Homoserine –30.1 Da Homoserine lactone –48.1 Da
BNPS-skatole or DMSO + HCl	after W	
Acid hydrolysis	D/P then random	
Endopeptidases	Cleavage sites	
Trypsin	after K/R	
Endoproteinase Lys-C	after K	
Endoproteinase Asp-N	before D	
Endoproteinase Arg-C	after R	
Chymotrypsin	after F/W/Y/L	
Pepsin	after F/W/Y/L	
Thermolysin	before L/I/M/F/W	

Proteolytic enzymes for "peptide sequencing"

Exopeptidases	*Selectivity*
Carboxypeptidase A	stops at R/PX, sometimes G/S/D/E
Carboxypeptidase B	cleaves at R/K
Carboxypeptidase P	cleaves PX/D/E, sometimes stops at S/G
Carboxypeptidase Y	cleaves at PX/E, sometimes stops at K/R/S/G
Aminopeptidase M	non-specific
Leucine aminopeptidase	stops at or near K/R/P

Mass balancing: the sum of the molecular weights of n enzymatic fragments, minus n-1 water molecules, add up to the molecular weight of the intact protein.



Example. β-amyloid peptide (βA_{1-40}):

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMGGVV

tryptic digest:

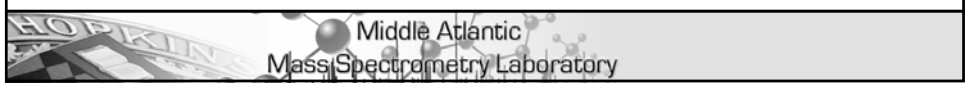
DAEFR	MW = 636.7	βA_{1-5}	MH^+ observed = 637.8
HDSGYEVHHQK	MW = 1336.5	βA_{6-16}	MH^+ observed = 1337.1
LVFFAEDVGSNK	MW = 1325.7	βA_{17-28}	MH^+ observed = 1326.7
GAIIGLMVGGVV	MW = 1085.5	βA_{29-40}	MH^+ observed = 1086.1

cyanogen bromide:

VGGVV	MW = 429.6	βA_{36-40}	MH^+ observed = 431.1
VGGVVIA	MW = 613.8	βA_{36-42}	MH^+ observed = 614.2
GAIIGLM	MW = 673.9	βA_{29-35}	MH^+ observed = 626.0
homoserine	MW = 643.8		
homoserine lactone	MW = 625.8		

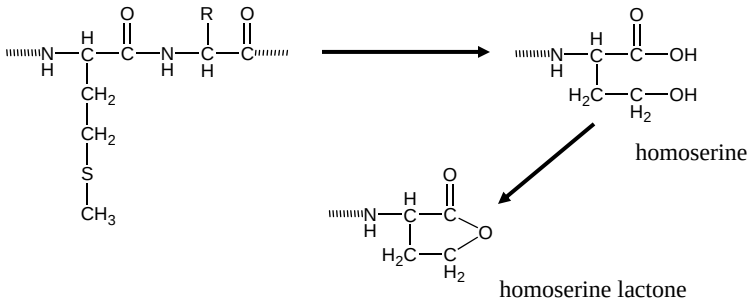
636.7 + 1336.5 + 1325.7 + 1085.5 – 3(18) = 4,329.9

Note that cyanogen bromide digestion revealed a longer amyloid peptide!



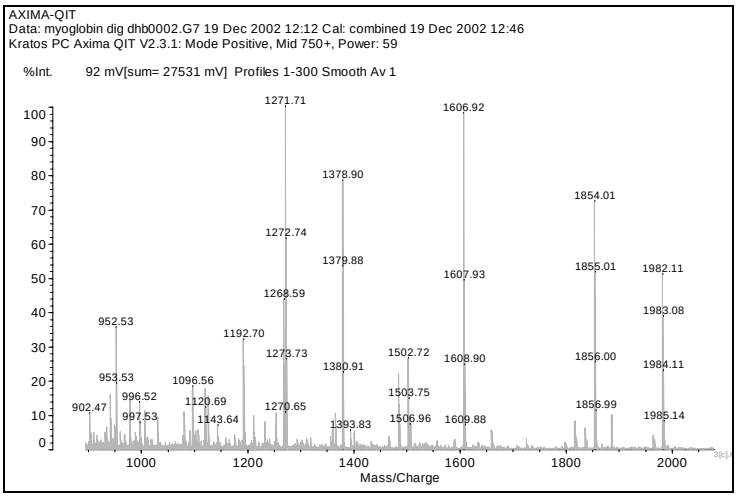
Chemical cleavage:

Cyanogen bromide (CNBr) cleaves the amide bond on the C-terminal side of a methionine residue:



forming a terminal homoserine (Δm = -30) or homoserine lactone (Δm = -48) residue

Peptide mapping: tryptic digest of myoglobin



MS obtained on a Kratos AXIMA Qit/TOF

PMF: Peptide Mass Fingerprinting

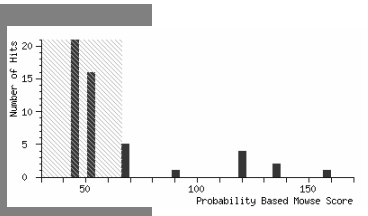
Taxonomy : Mammalia (mammals) (246067 sequences)
Timestamp : 19 Dec 2002 at 12:18:46 GMT
Top Score : 158 for gi|2506462, Myoglobin

Probability Based Mowse Score

Protein Summary Report

Switch to Concise Protein Summary Report

To create a bookmark for this report, right click this link: Protein Summary Report (../data/20021219/FtTtonYe.dat)



Index

	Accession	Mass	Score	Description
1.	gi|2506462	16941	158	Myoglobin
2.	gi|70561	16940	138	myoglobin [validated] - horse
3.	gi|2554649	16942	137	Myoglobin (Horse Heart) Mutant With Leu 104 Replaced By Asn (L104n)
4.	gi|494711	16967	118	Myoglobin (Horse Heart) Mutant With His 64 Replaced By Tyr (H64y)
5.	gi|2914321	16905	118	H64t Variant Of Myoglobin (Horse Heart) Recombinant Wild-Type Complexed With Azide
6.	gi|999870	16967	117	Myoglobin Mutant With His 93 Replaced By Tyr (H93y)
7.	gi|1942750	16969	117	Myoglobin (Horse Heart) Mutant With Ser 92 Replaced By Asp (S92d)
8.	gi|25029635	33033	88	similar to pORF2 [Mus musculus domesticus]
9.	gi|127664	17226	66	Myoglobin
10.	gi|127671	17034	65	MYOGLOBIN

Results List

1. gi|2506462 **Mass:** 16941 **Score:** 158
Myoglobin

Observed	Mr(expt)	Mr(calc)	Delta	Start	End	Miss	Peptide
941.48	940.47	940.47	0.01	146 -	153	1	YKELGFQG
1271.67	1270.66	1270.66	0.01	32 -	42	0	LFTGHPETLEK
1378.83	1377.82	1377.83	-0.01	64 -	77	0	HGTVVLTALGGILK
1502.68	1501.67	1501.66	0.01	119 -	133	0	HPGDFGADAQGAMTK
1506.94	1505.93	1505.93	0.00	64 -	78	1	HGTVVLTALGGILKK
1606.85	1605.84	1605.85	-0.01	17 -	31	0	VEADIAGHGQEVLIR
1815.88	1814.87	1814.90	-0.02	1 -	16	0	GLSDGEWQQVLNVWGK
1853.94	1852.93	1852.95	-0.02	80 -	96	0	GHHEAELKPLAQSHATK
1885.00	1883.99	1884.01	-0.02	103 -	118	0	YLEFISDAIIHVLHSK
1982.02	1981.01	1981.05	-0.04	79 -	96	1	KGHHEAELKPLAQSHATK

No match to: 931.53, 934.49, 949.50, 951.50, 952.52, 968.46, 978.46, 988.49, 1006.54

The masses of the tryptic peptides from the mass spectrum can be used to identify the parent protein

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Post-translational modifications will produce changes in the masses of tryptic fragments

Name	*Site*	*Modification*	*Δm*
N-terminal acetylation	terminal NH_2-	replaced by CH_3CONH-	+42
N-terminal formylation	terminal NH_2-	replaced by HCONH-	+28
N-terminal myristylation	terminal NH_2-	replaced by $CH_3(CH_2)_{12}CONH$-	+210
N-terminal palmitoylation	terminal NH_2-	replaced by $CH_3(CH_2)_{14}CONH$-	+238
C-terminal amidation	terminal –COOH	replaced by $–CONH_2$	-1
disulfide bonds	2 Cys –SH	replaced by –S-S-	-2
glycosylation (N-linked)	N-X-S/T	addition of sugar	
glycosylation (O-linked)	S/T	addition of sugar	
sulfation	-OH of Y	replaced by $–OSO_3H$	+80
phosphorylation	-OH of Y/S/T	replaced by $–OPO_3H_2$	+80
N-methylation	$-NH_2$ of K/R/H/Q	relaced by $–NHCH_3$	+14
O-methylesterification	-COOH of E/D	replaced by $–COOCH_3$	+14
carboxylation	$-NH_2$ of E/D	replaced by $–NHOCH_3$	+30
Hydroxylation	$-NH_2$ of P/K/D	replaced by –NHOH	+16

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Protein phosphorylation by kinases

Enzyme	*Consensus sequence*
protein kinase C	$(R/K_{1-3},X_{2-0})$-<u>S/T</u>-$(X_{2-0}, R/K_{1-3})$
cAMP-dependent PK	R-R-X-<u>S/T</u>
cGMP-dependent PK	R/K_{2-3}-X-<u>S/T</u>
casein kinase I	S[P]-X_{1-3}-<u>S/T</u>
casein kinase II	<u>S/T</u>-$(D/E/S[P])_{1-3},X_{2-0}$

X = unspecified peptide; S[P] = phosphorylated serine

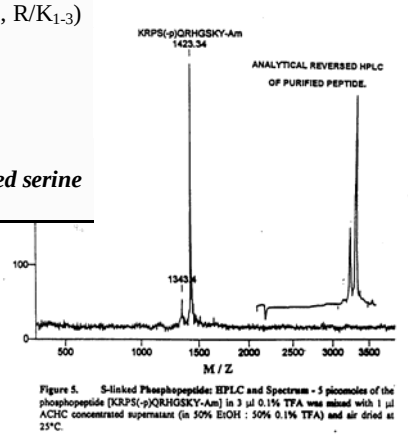


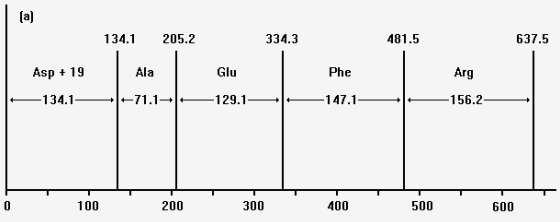
Figure 5. S-linked Phosphopeptide: HPLC and Spectrum - 5 picomoles of the phosphopeptide [KRPS(-p)QRHGSKY-Am] in 3 µl 0.1% TFA was mixed with 1 µl ACHC concentrated supernatant (in 50% EtOH : 50% 0.1% TFA) and air dried at 25°C.

Carboxypeptidase ladder sequencing

Digestion of the tryptic peptide DAEFR from βAmyloid with carboxypeptidase followed by mass spectral analysis of the ladder mixture

All masses are molecular masses

1	115.1	71.1	129.1	147.2	156.2	17	1		
H -	Asp -	Ala -	Glu -	Phe -	Arg -	OH -	H^+		637.7
1	115.1	71.1	129.1	147.2	17	1			
H -	Asp -	Ala -	Glu -	Phe -	OH -	H^+			481.5
1	115.1	71.1	129.1	17	1				
H -	Asp -	Ala -	Glu -	OH -	H^+				334.3
1	115.1	71.1	17	1					
H -	Asp -	Ala -	OH -	H^+					205.2
1	115.1	17	1						
H -	Asp -	OH -	H^+						134.1

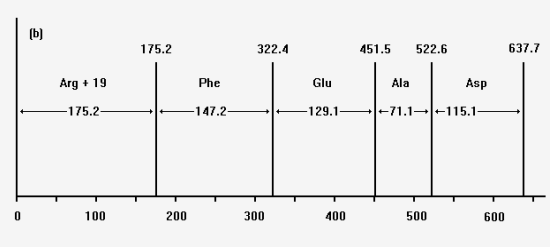


Aminopeptidase ladder sequencing

Digestion of the tryptic peptide DAEFR from βAmyloid with aminopeptidase followed by mass spectral analysis of the ladder mixture

All masses are molecular masses

1	115.1	71.1	129.1	147.2	156.2	17	1	
H -	Asp -	Ala -	Glu -	Phe -	Arg -	OH -	H^+	637.7
	1	71.1	129.1	147.2	156.2	17	1	
	H -	Ala -	Glu -	Phe -	Arg -	OH -	H^+	522.6
		1	129.1	147.2	156.2	17	1	
		H -	Glu -	Phe -	Arg -	OH -	H^+	451.5
			1	147.2	156.2	17	1	
			H -	Phe -	Arg -	OH -	H^+	322.4
				1	156.2	17	1	
				H -	Arg -	OH -	H^+	175.2



Example of "timed" carboxypeptidase ladder sequencing

In situ digestion: several aliquots of the intact peptide are place on different locations on the sample probe or slide. Enzyme (in excess) is added to each spot and quenched after a predetermined time by addition of the matrix solution.

Timed-course digests provide a means to maximize amino acid sequence information.

Patterson, D.H.; Tarr, G/E.; Regnier, F.E., Martin, S.A.; **Anal. Chem. 67** (1995) 3971-3978.

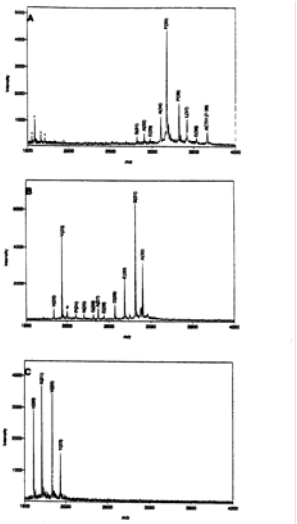


FIGURE 10.6 MALDI-TOF mass spectra of the (a) 1-min, (b) 5-min, and (c) 25-min aliquots from a time-dependent CPY digestion of ACTH 7–28 fragment (FRWGKPVGKKRRPVKVYPNGAEDE-SAEAPLE). Reprinted with permission from reference 12.

Ladder sequencing of a phospho-peptide:

For phosphopeptides, amino and carboxypeptidase ladder sequencing is carried out in the same manner, but the sequence includes a phosphoserine which has a residue mass of:

87 + 80 = 167

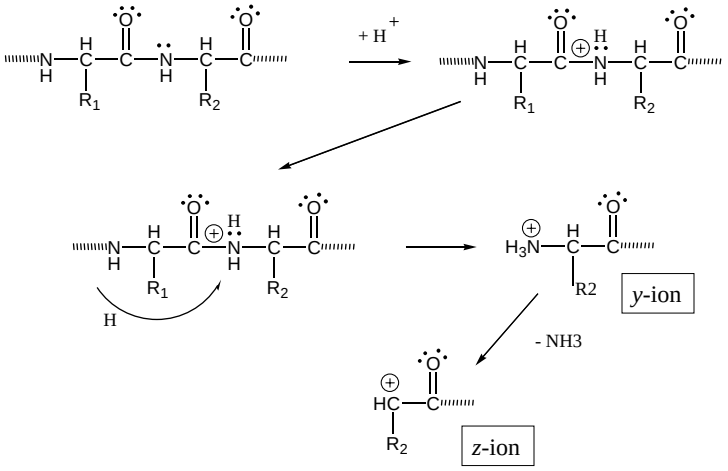
```
 1    87.1  156.2 167.1 57.1  71.1  113.2 128.2  17   1
H –  Ser – Arg – Ser – Gly – Ala – Leu – Lys –  OH – H+   MW = 799.0
                  |
                 PO3H

      1    156.2 167.1 57.1  71.1  113.2 128.2  17   1
     H –  Arg – Ser – Gly – Ala – Leu – Lys – OH – H+    MW = 711.9
                  |
                 PO3H

           1    167.1 57.1  71.1  113.2 128.2  17   1
          H –  Ser – Gly – Ala – Leu – Lys – OH – H+    MW = 555.7
                  |
                 PO3H

                 1    57.1  71.1  113.2 128.2  17   1
                H –  Gly – Ala – Leu – Lys – OH – H+    MW = 388.6

                      1    71.1  113.2 128.2  17   1
                     H –  Ala – Leu – Lys – OH – H+    MW = 331.5

                           1    113.2 128.2  17   1
                          H –  Leu – Lys – OH – H+    MW = 260.4

                                1    128.2  17   1
                               H –  Lys – OH – H+    MW = 47.2
```


Sequencing by fragmentation: tandem and hybrid mass spectrometers

The tandem mass spectrometer:

- two or more mass analyzers (or mass analysis steps: “tandem in time”)
- the ability to select a ions of a particular mass observed in a mass spectrum and to observe its fragments in a subsequent spectrum
- a means for activating the ions to induce fragmentation

C-terminal ions: the “y” ions



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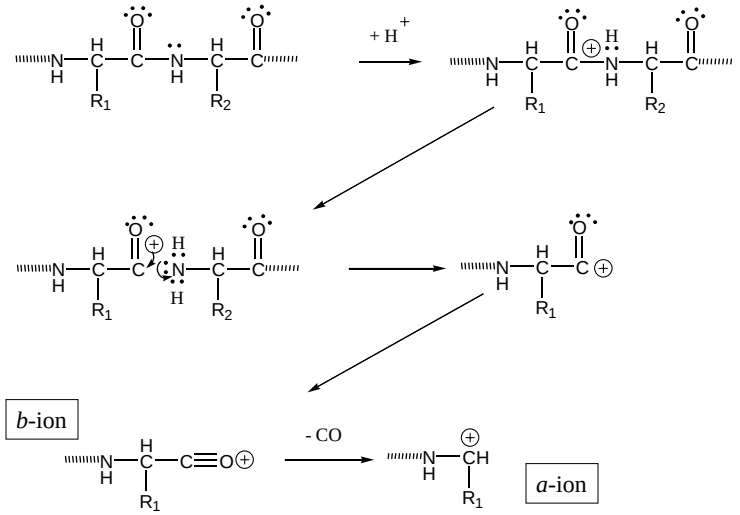
N-terminal ions: the “a” and “b” ions



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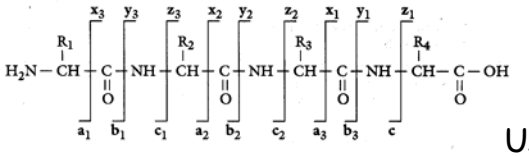
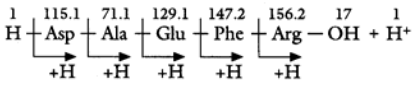
Sequence fragment nomenclature

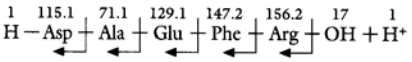


Use these schemes to verify the masses in the next figure

y-ion masses can be determined for a known sequence:



b-ion masses can also be determined:

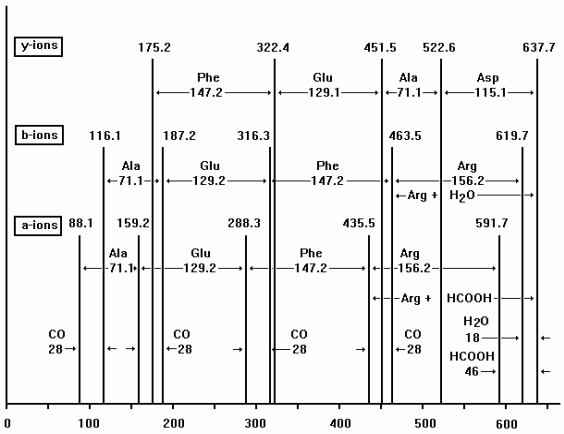


a-ion masses are determined by subtracting 28 from the masses of the *b*-ions

Amino acid sequencing by fragmentation.

De novo sequencing of an unknown is complicated by the fact that one does not know *a priori* which type of fragmentation will occur.

Approaches include the location of peaks 28 mass units apart (*a* and *b* series) and noting that the molecular ion is a *y* ion).



Tandem Mass Spectrometry

Mass filters vs. mass analyzers
Mass selection
Collision-induced dissociation (CID)

In a normal configuration:

Mass filter: passes a single mass

Collision gas: He or Xe

Collision-induced dissociation

High energy (1-20 Kev) single collisions, or

Low energy (10-50 ev multiple collisions.

Mass analyzer: records a mass spectrum

Normal mode = product ion scan

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Some examples of tandem (and hybrid) instruments

***Tandem in time*:**
Ion trap mass spectrometer (ITMS)
Fourier transform mass spectrometer (FTMS)
Linear ion trap/FTMS (LTQ-FT)

***Tandem in space*:**
Triple quadrupoles
Quadrupole/time-of-flight (QTOF)
Time-of-flight/time-of-flight (TOF/TOF)
Ion trap/time-of-flight (trapTOF, Qit/TOF)

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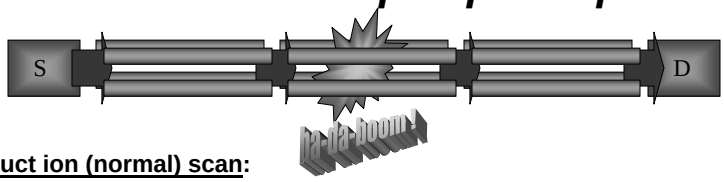
Scan modes of the triple quadrupole:



Product ion (normal) scan:

mass filter:	**RF-only mode:**	**mass analyzer:**
single precursor mass		Scan product masses for selected precursor

Precursor ion scan:

mass analyzer:	**RF-only mode:**	**mass filter:**
Scan precursor masses for selected product		single product mass

Constant neutral loss (CNL) scan (*reaction ion monitoring*):

mass analyzer:	**RF-only mode:**	**mass analyzer:**
scan precursor masses		scan product masses differing by constant mass difference

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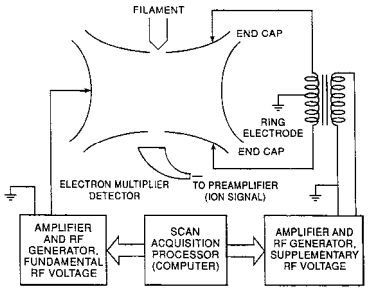
The ion trap mass spectrometer is a "tandem" mass spectrometer



Fundamental rf applied to ring electrode (1.1 MHz)

dc and excitation voltages applied to the end caps

Trapping cycle: fixed amplitude of the fundamental rf voltage

Ion ejection cycle: remove unwanted ions by symmetric or asymetric pulses on endcaps; *stored waveform inverse Fourier transform* (SWIFT) or *filtered noise field* techniques

Mass analysis cycle:mass selective instability mode: scan fundamental rf voltage; mass range approx. 650 resonance ejection mode: set supplemental rf voltage on endcaps and then scan fundamental rf voltage

MS/MS mode: low amplitude supplemental rf voltage applied to endcaps

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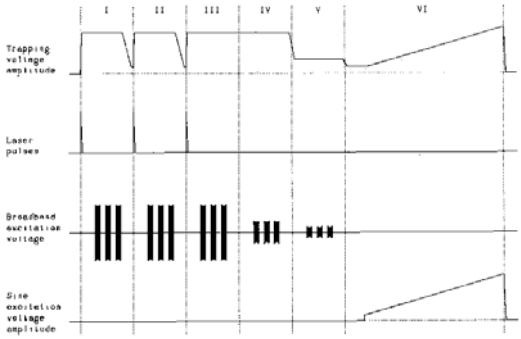
Tandem-in-time

I, II and III: trapping cycle: fundamental (1.1 MHz) RF voltage on ring electrode

IV: mass isolation cycle (MS1): resonant ejection of all but selected ion, using high amplitude supplementary RF on ring electrode

V: excitation cycle (low energy CID): low amplitude supplementary RF voltage on endcaps

VII: mass analysis cycle (MS2): resonance ejection mode, high amplitude supplementary RF voltage on endcaps while scanning the amplitude of the fundamental RF voltage on the ring electrode



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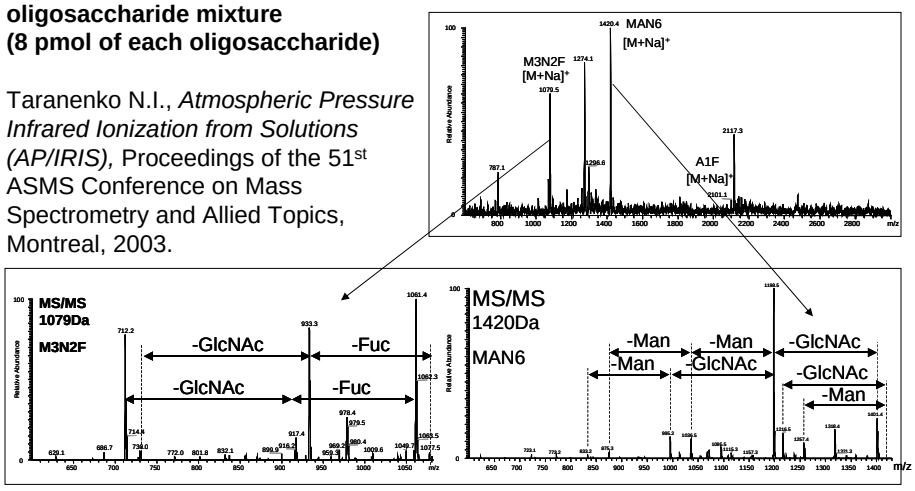
IR AP MALDI from 0.1 % TFA solution

AP/IRIS MS spectrum of 3-oligosaccharide mixture (8 pmol of each oligosaccharide)

Taranenko N.I., *Atmospheric Pressure Infrared Ionization from Solutions (AP/IRIS),* Proceedings of the 51st ASMS Conference on Mass Spectrometry and Allied Topics, Montreal, 2003.



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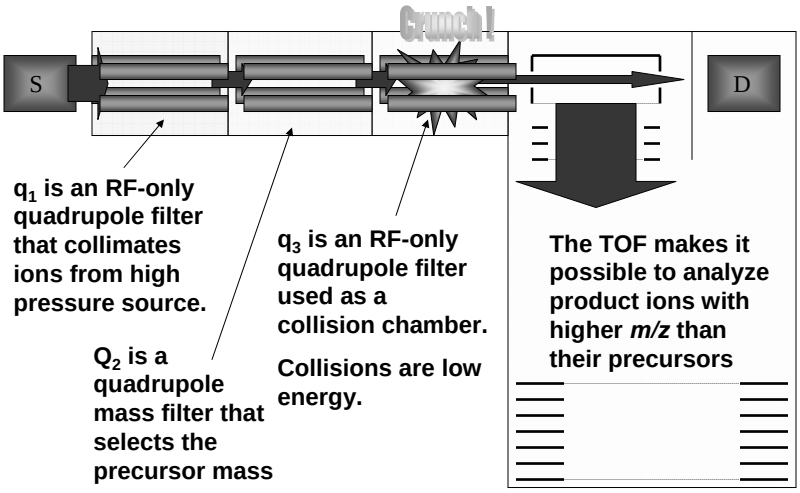
Combined quadrupole and time-of-flight mass spectrometers (QTOF)



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MS/MS spectrum of doubly charged ion on a QTOF mass spectrometer

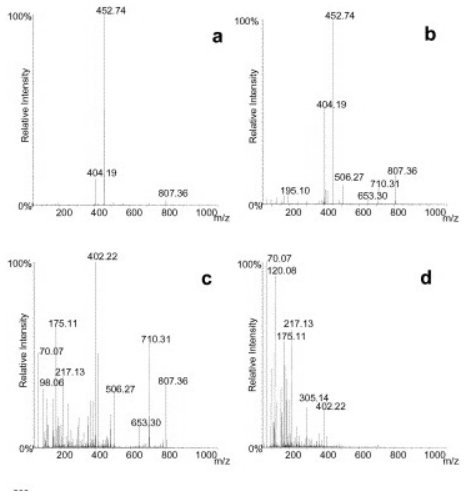

Figure 2. Fragment ion spectra of doubly-charged Bradykinin 2–9 (*m/z* 452.74) obtained on a hybrid quadrupole-time of flight instrument at (a) 20 V, (b) 25 V, (c) 35 V, and (d) 45 V potential difference.

Rogalski, J.C.; Lin, M.S.; Sniatynski, M.J.; Taylor, R.J.; Youhnovski, N.; Przybylski, M.; Kast, J. *Statistical evaluation of electrospray tandem mass spectra for optimized peptide fragmentation,* **J. Am. Soc. Mass Spectrom. 16** (2005) 505-514.

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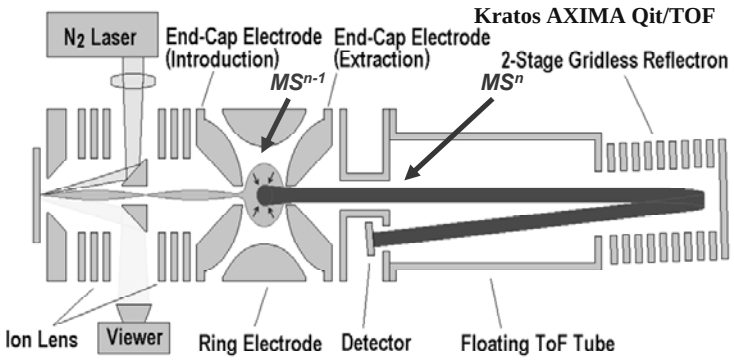
Combined ion trap and time-of-flight mass spectrometer (trapTOF)



Compared with QTOF: can carry out MS^{n-1} steps in the ion trap

Compared with ion trap alone: obtain high resolution MS^n spectrum

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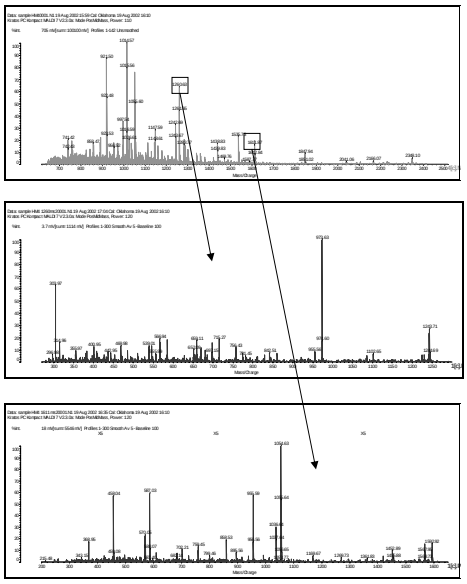
MALDI MS and MS/MS spectra obtained on a trapTOF

- **Mass selection from an ion trap provides high accuracy, narrow mass range**
- **Full kinetic energy of product ions provides high mass resolution in MS^n modes**



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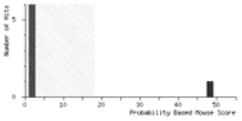
Both MS/MS spectra give the same ID

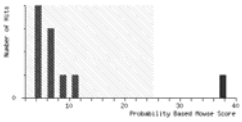


MS/MS of 1611

Mascot Search Results

```
Search title     : digest
MS data file     : C:\Program Files\Kompact\data\Customers\Oklahoma\mass lists\HM4 1611 ms2.txt
Database         : NCBInr 20020814 (1030915 sequences; 326041867 residues)
Taxonomy         : Drosophila (fruit flies) (28122 sequences)
Timestamp        : 19 Aug 2002 at 16:03:04 GMT
Significant hits: gi|5921205          ATP synthase alpha chain, mitochondrial precursor (Protein bellwether)

1. gi|5921205           Mass: 59384   Total score: 48  Peptides matched: 1
1 1611.15 1610.14 1609.87 0.27 0 48 1 TGAIVDVPVGDELLGR
```



MS/MS of 1260

Mascot Search Results

```
Search title     :  digest
MS data file     : C:\Program Files\Kompact\data\Customers\Oklahoma\mass lists\HM4 1260 ms2.txt
Database         : NCBInr 20020814 (1030915 sequences; 326041867 residues)
Taxonomy         : Drosophila (fruit flies) (28122 sequences)
Timestamp        : 19 Aug 2002 at 16:17:32 GMT
Significant hits: gi|5921205          ATP synthase alpha chain, mitochondrial precursor (Protein bellwether)
1. gi|5921205           Mass: 59384   Total score: 38  Peptides matched: 1
1 1260.70 1259.69 1259.64 0.06 0 38 1 SAEISNILEER
```

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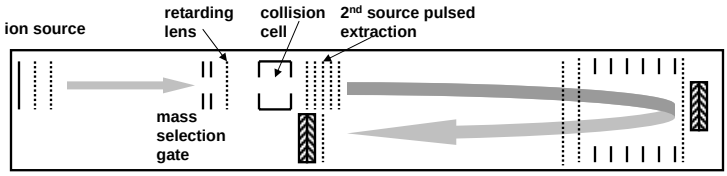
Tandem time-of-flight (TOF/TOF) mass spectrometers



Applied BioSystems

- 20 keV ions decelerated to 1-2 keV
- product ions are reaccelerated by 18 keV
- does not record metastable products formed in MS1
- can be operated with (CID) or without (PSD) gas

CID = collision-induced disssociation
PSD = post-source decay (metastable decomposition)

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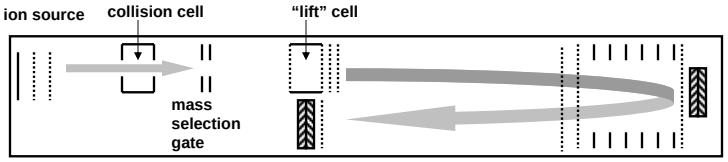
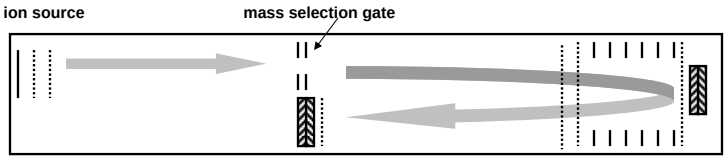
Bruker Daltonics

- 8 keV collisions with products accelerated by lift cell
- records metastables (PSD) and collision (CID) products



Kratos AXIMA

- PSD with curved-field reflectron



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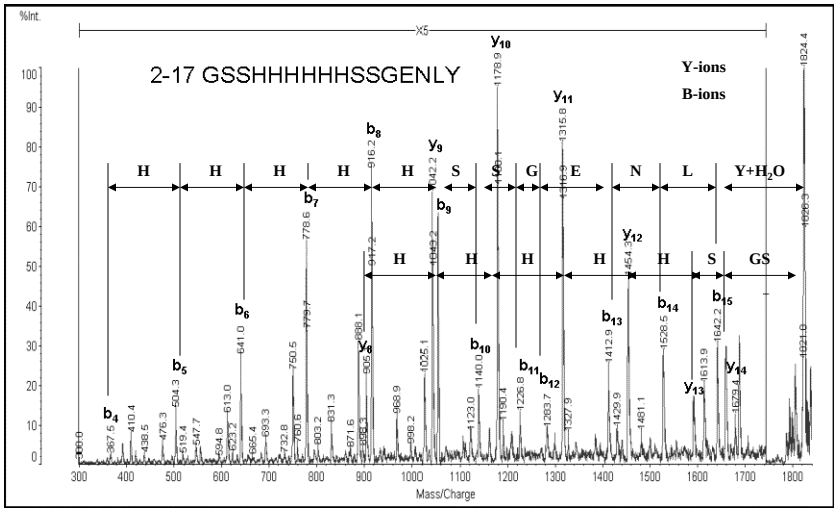
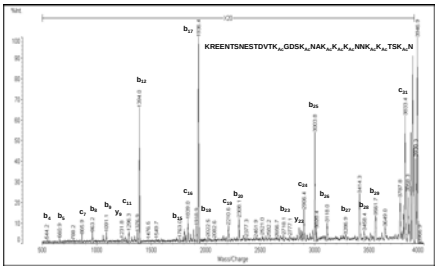
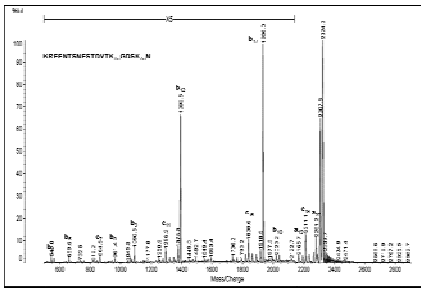
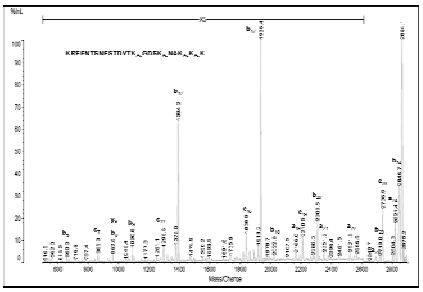
MS/MS or sequence spectrum of 2-17 from HAT



Middle Atlantic Mass Spectrometry Laboratory







MS/MS analysis of acetylated peptides from histone acetyl transferase

Thompson, P.; Wang, D.; Wang, L.; Fulco, M.; Pediconi, N.; Zhang, D.; An W.; Ge Q.; Roeder, R.G.; Wong, J.; Levrero, M.; Sartorelli, V.; Cotter, R.J. and Cole, P.A., *Regulation of the p300 HAT Domain via a Novel Activation Loop.* **Nature: Struct. Mol. Biol. 11** (2004) 308-15.

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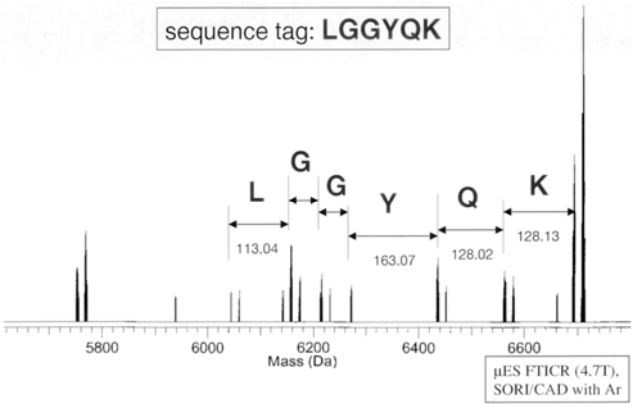
Sequence tags

For identification we need only a partial sequence …



FTMS/MS of a bacterial agent biomarker with a mass of 6710.5

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… which can be combined with a molecular weight to obtain an identification with very high specificity

Here two peptides are found with the same sequence segment, but only one has the correct molecular weight

BLAST sequence tag query:

only 2 sequences returned (out of > 550 000 in Swiss PROT)

P06554: Small acid-soluble spore protein - SASP-2 - from ***B.cereus***
64 AA, MW: **6710.5** Da, tag at the C-terminus

SRSTNKLAVP GAESALDQMK YEIAQEFGVQ LGADATARAN GSVGG
EITKR LVSLAEQQ**LG GYQK**

P45157: Exodeoxyribonuclease V, B chain - EX5B_HAEIN- from ***H.influenzae***
1211 AA, MW: 139 857 Da, tag between 232 - 237 AA

... LLKADLGKDL QVEIENKQAL SVPIQIFLPQ Y**LGGYQK**ALN ...

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High energy and low energy collisions

High energy collisions

- precursor ion kinetic energy: E_1 = 1-20 keV
- single collision conditions
- product ion energy: $$E_2 = \frac{m_2}{m_1} E_1$$
- used in instruments in which mass measurement depends upon kinetic energy, i.e. TOF/TOF mass spectrometers
- collision energy (Erel) in the center-of-mass frame

$$E_{rel} = \frac{m_n}{m_n + m_M} E_M$$

where *M* is the molecular ion and *n* is the collision gas

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High energy and low energy collisions

Protein	*MW*	*1 keV*	*8 keV*	*20 keV*
Substance P	1,348	2.97 ev	23.7 ev	59.4 ev
Ubiquitin	8,566	0.47 ev	3.7 ev	9.3 ev
Cytochrome C	12,328	0.32 ev	2.6 ev	2.5 ev
C fragment of tetanus toxin	51,819	0.08 ev	0.6 ev	1.5 ev
Bovine serum albumin	66,430	0.06 ev	0.5 ev	1.2 ev

Low energy collisions

- precursor ion kinetic energy = 10-50 ev
- activation through multiple collisions
- used in instruments in which the ion residence time is long

quadrupole ion traps (ITMS)
linear ion traps (LTQ)
Fourier transform mass spectrometers (FTMS)

High energy and low energy collisions

Some observations:

- both high energy and low energy CID are less effective for high mass because of the relativistic effect
- CID generally produces more internal fragment ions and single residue ions for amino acid composition
- low energy CID raises the internal energy slowly and has a tendency to break the weakest bonds: at proline and glutamic acid residues, losses of phosphate and other charged PTMs
- high energy CID produces more “remote site” fragmentation

What is needed…..

- Ability to fragment higher masses
- Ability to fragment without losing PTMs

Use something other than collisions

New methods of ion activation

On an FTMS:

- **IRMPD**
- **ECD**

on an ion trap:

- **ETD**

Method	*Characteristics*
Metastable fragmentation Post-source decay	- Primarily b, y ions
Low energy collision induced dissociation (Low energy CID)	- Multiple low energy (20 – 200 ev) collisions - Used primarily for instruments with long ion residence times, i.e. quadrupole, ion trap or FTMS
High energy collision induced dissociation (High energy CID)	- Single high energy (1 keV to 20 keV) collisions - Primarily b, y ions (amide bond cleavage) - Some side chain losses for distinguishing residues of the same mass - Additional acyl and immonium internal ions
Infrared multiphoton dissociation (IRMPI)	- Similar to CID, but activation energy does not depend upon relative kinetic energy - Better for high mass - Requires high ion residence times for good cross section
Electron capture dissociation (ECD)	- Fewer neutral losses than CID, such as phosphate and sugar - More c and z ions (C-N bond cleavage) - Activation does not depend upon relative kinetic energy - Better for high mass - Used for "top down" proteomics - Requires high ion residence times for good cross section

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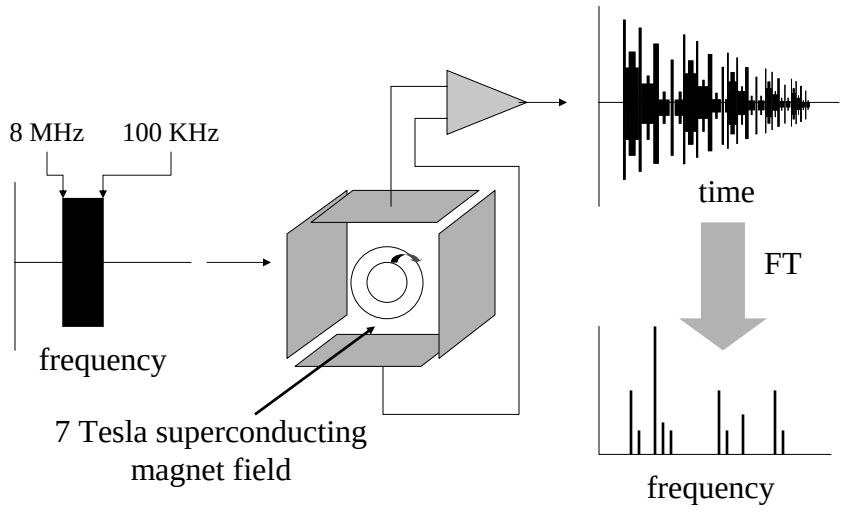
Fourier transform mass spectrometer



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Fourier transform mass spectrometer

What are the advantages?

- **High mass accuracy:**
  - **2 ppm (external calibration)**
  - **1 ppm (internal calibration)**
- **Therefore, better protein identifications from mass fingerprinting**
- **Very high mass resolution**
- **Ability to carry out IRMPD and ECD, as well as CID**
- **Therefore, able to do "top-down" proteomics**

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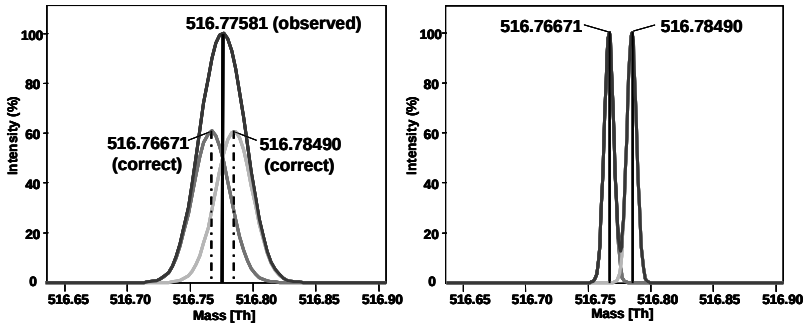
Fourier transform mass spectrometer



Monoisotopic MH_2^{+2} peaks for the peptides DRVYVHPF (*m/z* 516.76671) and KRPPGFSPF (*m/z* 516.78490) at resolving powers of 15,000 and 56,700 , where the difference in mass between these two peptides is 18.2 mmu or 35 ppm.

Courtesy: ThermoFinnegan

Salmonella GroEl (GMQFDRGYL) *m/z* 543.75575
Mouse hsp (GMKFDRGYI) *m/z* 543.77390; 33 ppm,

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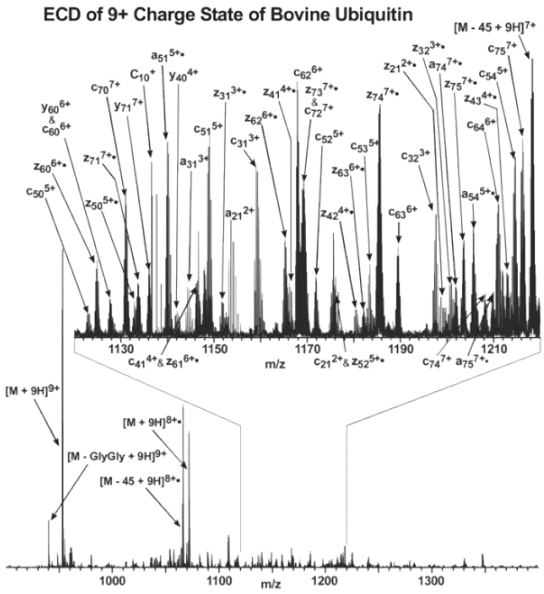
ECD on a Fourier transform mass spectrometer

Håkansson, Chalmers, M.J.; Quinn, J.P.; McFarland, M.A.; Hendrickson, C.L.; Marshall, A.G., *Combined Electron Capture and Infrared Multiphoton Dissociation for Multistage MS/MS in a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer,* **Anal. Chem. 75** (2003) 3256-3262.



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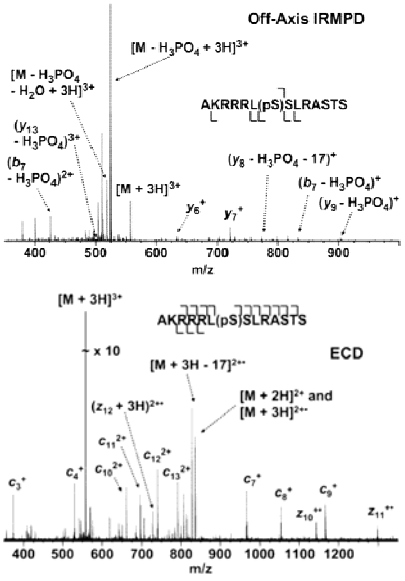
Comparison of IRMPD and ECD on a Fourier transform mass spectrometer

IRMPD fragmentation is similar to CID

Håkansson, Chalmers, M.J.; Quinn, J.P.; McFarland, M.A.; Hendrickson, C.L.; Marshall, A.G., *Combined Electron Capture and Infrared Multiphoton Dissociation for Multistage MS/MS in a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer,* **Anal. Chem. 75** (2003) 3256-3262.



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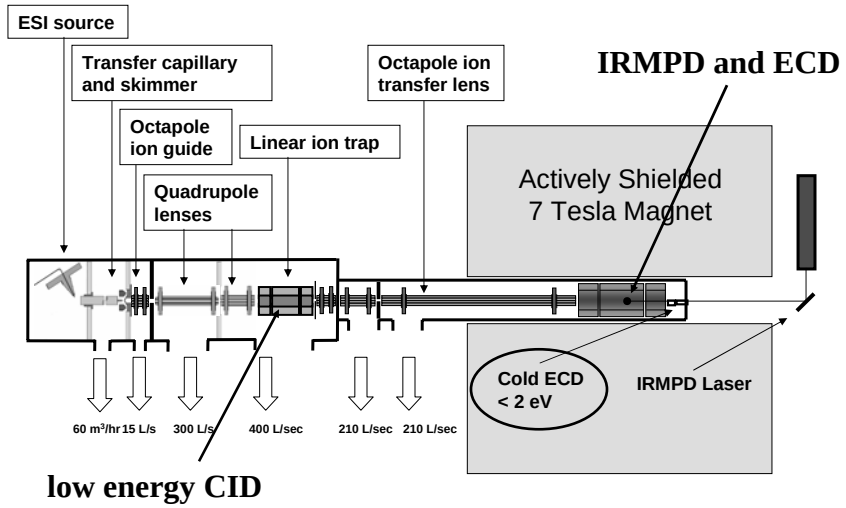
Linear ion trap/FTMS



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Electron transfer dissociation (ETD) on a linear ion trap (LTQ)

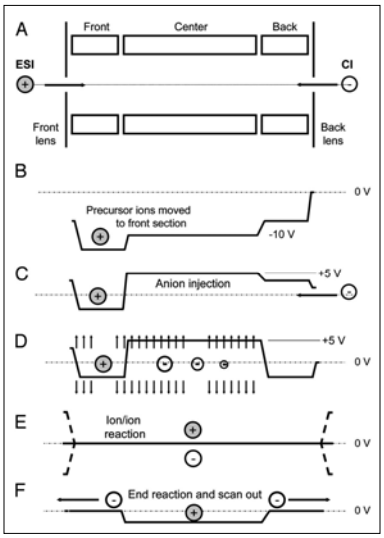

Fig. 4. Schematic of steps involved in the operation of the LTQ mass spectrometer for peptide sequence analysis by ETD. (*A*) Injection of multiply protonated peptide molecules (precursor ions) generated by ESI. (*B*) Application of a dc offset to move the precursor ions to the front section of the linear trap. (*C*) Injection of negatively charged reagent ions from the CI source into the center section of the linear trap. (*D*) Application of a supplementary dipolar broadband ac field to eject all ions except those within 3 mass-unit windows centered around the positively charged precursor ions and the negatively charged electron-donor reagent ions. (*E*) Removal of the dc potential well and application of a secondary RF voltage (100 V zero to peak, 600 kHz) to the end lens plates of the linear trap to allow positive and negative ion populations to mix and react. (F) Termination of ion/ion reactions by axial ejection of negatively charged reagent ions while retaining positive ions in the center section of the trap. This is followed by mass-selective, radial ejection of positively charged fragment ions to record the resulting MS/MS spectrum.

Syka, John E. P. et al. (2004) Proc. Natl. Acad. Sci. USA 101, 9528-9533

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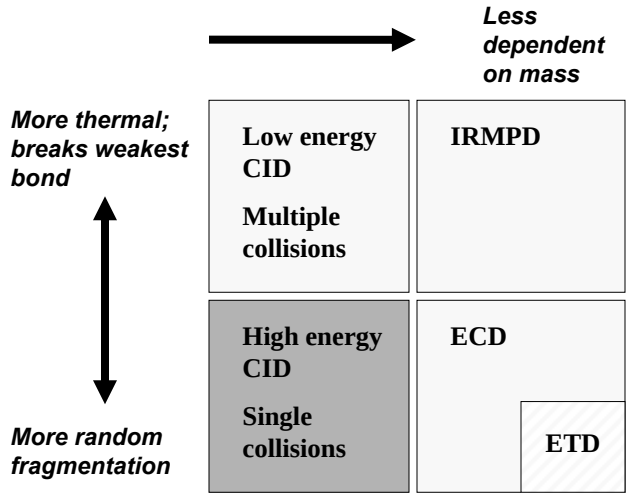
Summary of activation methods

- ☐ Requires long residence times
- ☐ LTQ
- ■ TOF/TOF only



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References

McCloskey, J.A., ed.; **Methods in Enzymology 193** (1990).

Packer, N.H.; Harrison, M.J., *Glycobiology and Proteomics: Is Mass Spectrometry the Holy Grail?* **Electrophoresis 19** (1998) 1872-1882.

Vestling, M.M.; Fenselau, C., *Surfaces for Interfacing Protein Gel Electrophoresis Directly with Mass Spectrometry*, **Mass Spectrom. Rev. 14** (1995) 169-178.

Siuzdak, G., *Mass Spectrometry for Biotechnology*, Academic Press, NY (1996)

Willoughby, R.; Sheehan, E.; Mitrovich, S., *A Global View of LC/MS*, Global View Publishing, Pittsburgh (1998).

Cotter, R.J., *Time-of-Flight Mass Spectrometry: Instrumentation and Applications in Biological Research*, American Chemical Society, Washington DC (1997).

Handout available electronically on the Middle Atlantic Mass Spectrometry Laboratory website: http://www.hopkinsmedicine.org/mams

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