Chapter 1 Exercises

Jeremy Wong

CH-419 – Protein Mass Spectrometry and Proteomics

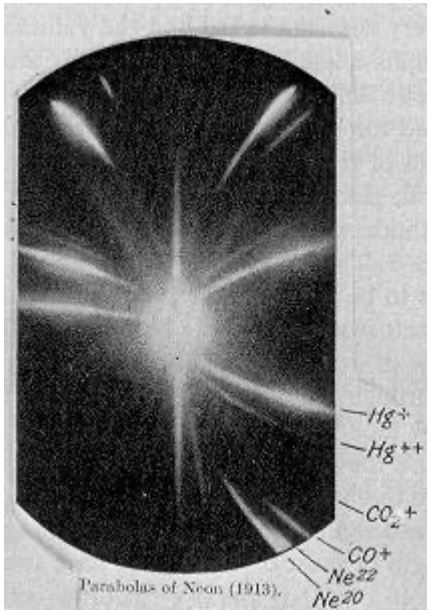
Mar 16, 2022

Question 1

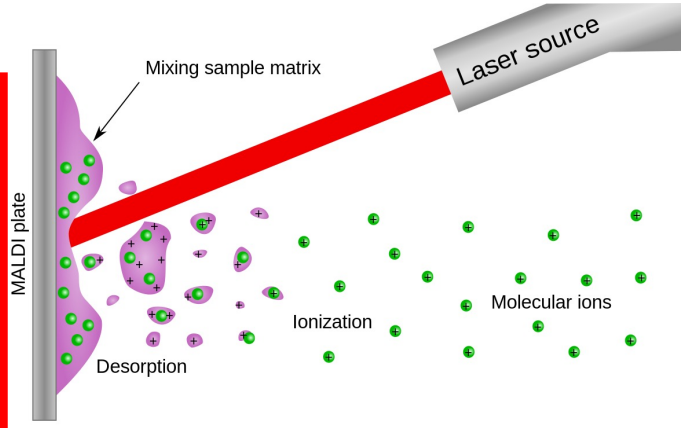
Who is considered as the “father” of mass spectrometry?

□ Thomson □ Tanaka □ Watson □ Makarov

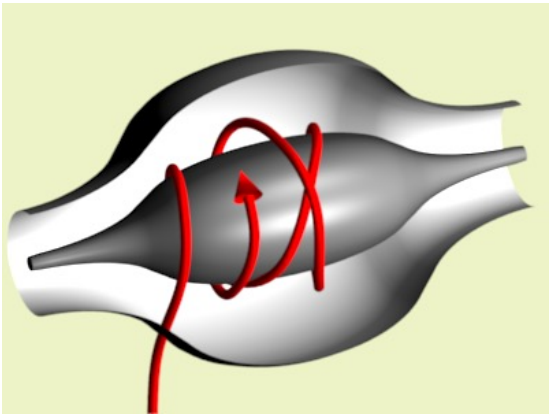
Question 1



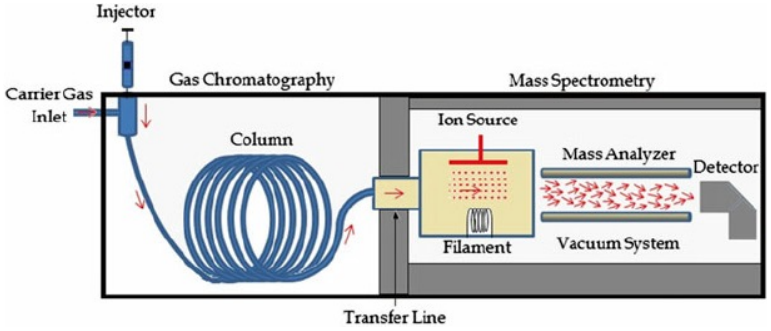
Thompson: separation of Ne isotopes, **first example of mass spectrometry**



Tanaka: soft laser desorption ionization for macromolecules



Markarov: Orbit Trap

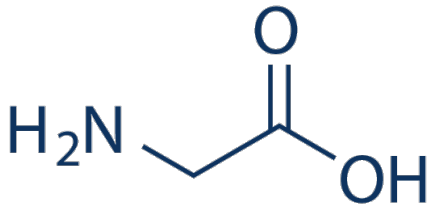


Watson: Gas chromatography coupled mass spectrometry (GC-MS)

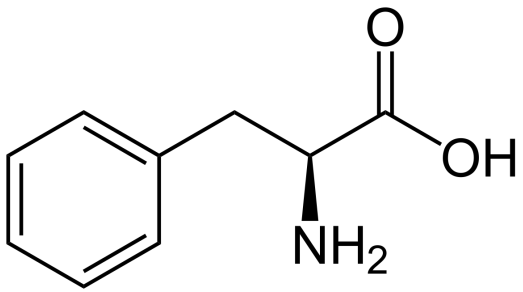
Question 2

What amino acid(s) could be phosphorylated?
□ Glycine □ Serine □ Threonine □ Tryptophan

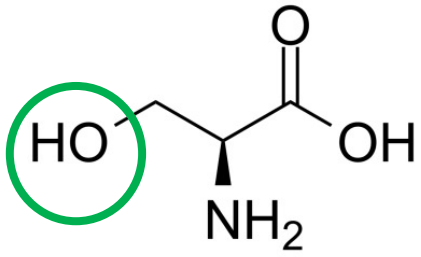
Question 2



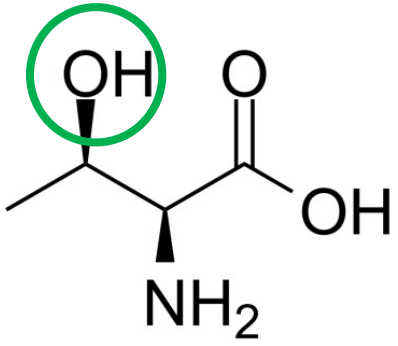
Gly, no OH sidechain



Phe, no OH sidechain



Ser, yes OH sidechain



Thr, yes OH sidechain

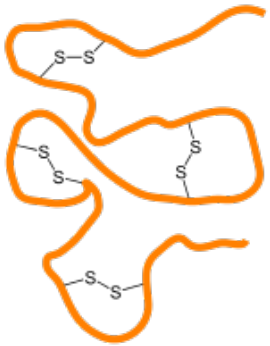
- Amino acids with side chain hydroxy groups can be phosphorylated

Question 3

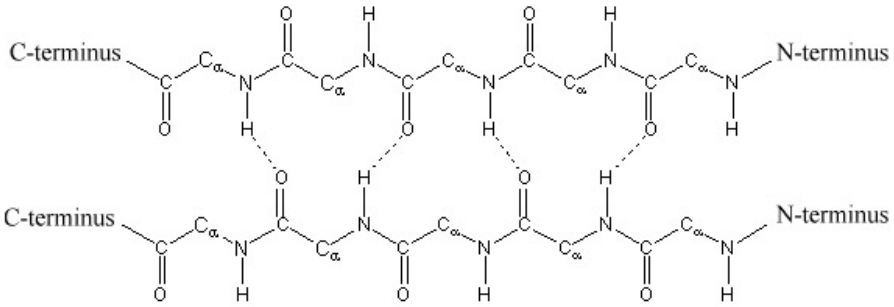
What is typical of protein secondary structure?

□ Disulfide bridges □ β-sheet □ α-helix □ Hydrophobic interactions

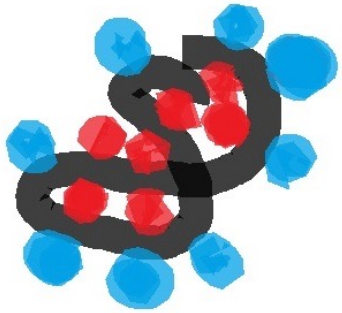
Question 3



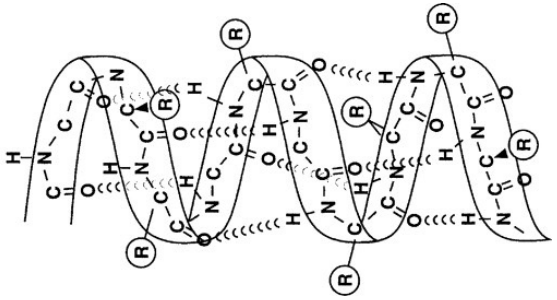
Disulfide: tertiary



Beta sheets: secondary



Hydrophobic interactions: tertiary



Alpha helix: secondary

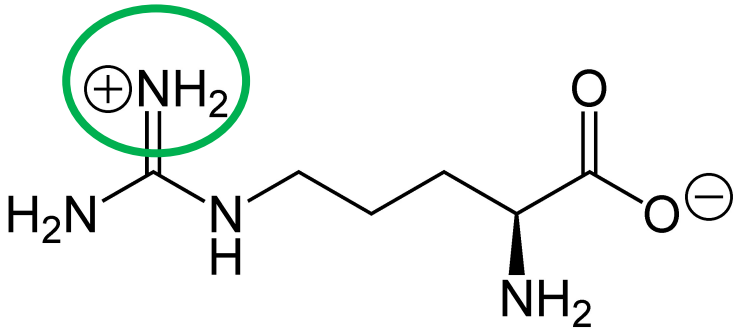
- Secondary structure refers to regular, recurring arrangements in space of adjacent amino acid residues in a polypeptide chain. **It is maintained by hydrogen bonds between amide hydrogens and carbonyl oxygens of the peptide backbone.**

Question 4

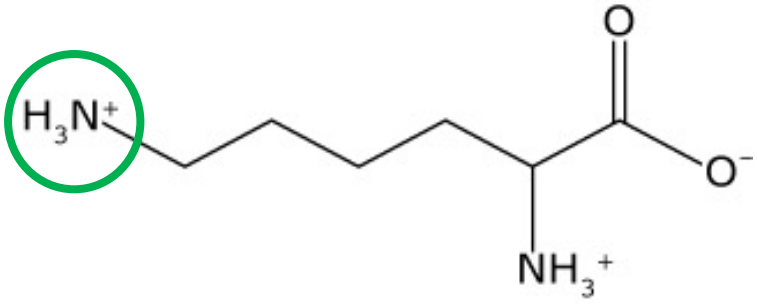
What amino acid(s) present a charged side chain?
□ Arginine □ Lysine □ Glycine □ Leucine

Question 4

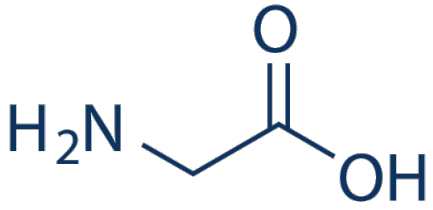
Lecture slide: 13



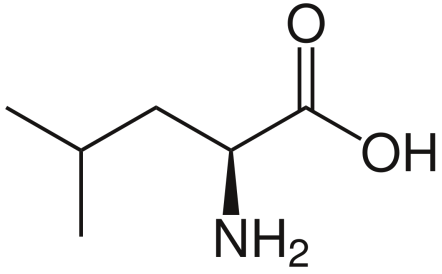
Arg, charged guanidinium group



Lys, charged amine



Gly, no charge on sidechain



Leu, no charge on sidechain

Question 5

What is single-letter code for glycine, arginine, and lysine?
□ G, R, L □ G, A, K □ G, R, K □ Y, A, N

Lecture slides: 13, 15

Question 5

Amino acids	Three-letter code	Single letter code	Amino acids	Three-letter code	Single letter code
Alanine	Ala	A	Leucine	Leu	L
Arginine	Arg	R	Lysine	Lys	K
Asparagine	Asn	N	Methionine	Met	M
Aspartic acid	Asp	D	Phenylalanine	Phe	F
Cysteine	Cys	C	Proline	Pro	P
Glutamine	Gln	Q	Serine	Ser	S
Glutamic acid	Glu	E	Threonine	Thr	T
Glycine	Gly	G	Tryptophan	Trp	W
Histidine	His	H	Tyrosine	Tyr	Y
Isoleucine	Ile	I	Valine	Val	V

Table 1. Notations of amino acids in three-letter and single letter codes.

- It is GRK

Question 6

What is the heavier amino acid?

□ Glycine □ Leucine □ Tryptophan □ isoleucine

Question 6

Lecture slide: 15

Amino acid	1-letter code	3-letter code	Chemical formula(-H_2O)	Monoisotopic mass (-H_2O)	Average mass (-H_2O)
Alanine	A	Ala	C_3H_5ON	71.03711	71.0788
Arginine	R	Arg	$C_6H_{12}ON_4$	156.10111	156.1875
Asparagine	N	Asn	$C_4H_6O_2N_2$	114.04293	114.1038
Aspartic Acid	D	Asp	$C_4H_5O_3N$	115.02694	115.0886
Cysteine	C	Cys	C_3H_5ONS	103.00919	103.1388
Glutamic Acid	E	Glu	$C_5H_7O_3N$	129.04259	129.1155
Glutamine	Q	Gln	$C_5H_8O_2N_2$	128.05858	128.1307
Glycine	G	Gly	C_2H_3ON	57.02146	57.0519
Histidine	H	His	$C_6H_7ON_3$	137.05891	137.1411
Isoleucine	I	Ile	$C_6H_{11}ON$	113.08406	113.1594
Leucine	L	Leu	$C_6H_{11}ON$	113.08406	113.1594
Lysine	K	Lys	$C_6H_{12}ON_2$	128.09496	128.1741
Methionine	M	Met	C_5H_9ONS	131.04049	131.1926
Phenylalanine	F	Phe	C_9H_9ON	147.06841	147.1766
Proline	P	Pro	C_5H_7ON	97.05276	97.1167
Serine	S	Ser	$C_3H_5O_2N$	87.03203	87.0782
Threonine	T	Thr	$C_4H_7O_2N$	101.04768	101.1051
Tryptophan	W	Trp	$C_{11}H_{10}ON_2$	186.07931	186.2132
Tyrosine	Y	Tyr	$C_9H_9O_2N$	163.06333	163.176
Valine	V	Val	C_5H_9ON	99.06841	99.1326

Trp is heaviest

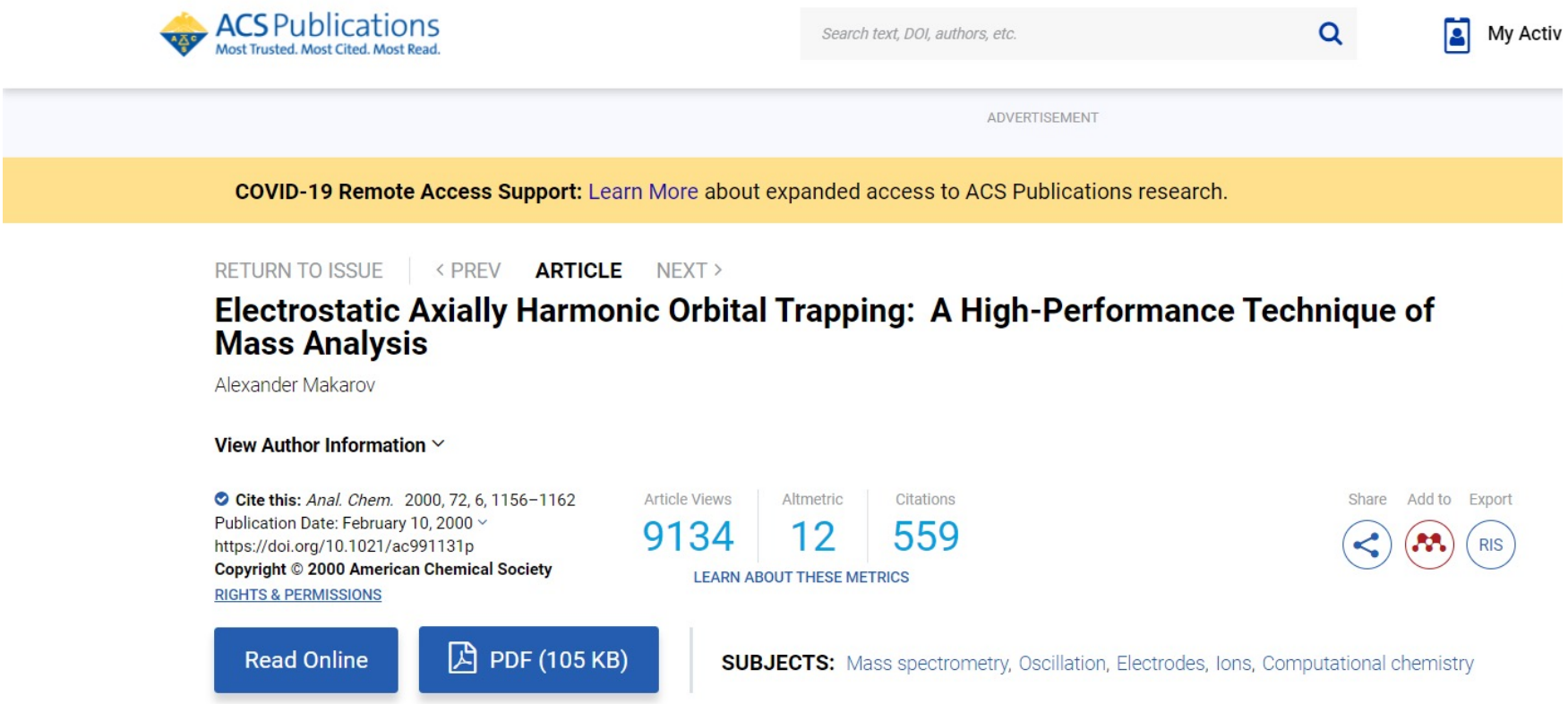
Question 7

When was the Orbitrap analyzer invented?

□ 1910 □ 1953 □ 2000 □ 2015

Question 7

Lecture slide: 17



- 2000, by Alexander Makarov

Question 8

Lecture slide: 18

What is the SI unit of mass?
□ Dalton [Da] □ Unified atomic mass [u] □ Thomson [Th] □ Dimensionless

A: unified atomic mass [u]

Question 9

What is the nominal mass of insulin ($C_{257}H_{383}N_{65}O_{77}S_6$)?

□ 5801 u □ 5797 u □ 5803.638 u □ 5899.621 u

Question 9

Lecture slide: 22

What is the nominal mass of insulin ($C_{257}H_{383}N_{65}O_{77}S_6$)?
□ 5801 u □ 5797 u □ 5803.638 u □ 5899.621 u

- Nominal masses are rounded to the nearest 1 (for each atom). So you can immediately filter out the last 2 choices
- Check the nominal mass with https://www.lfd.uci.edu/~gohlke/molmass/
- Nominal mass is 5801 u

Question 10

What is the charge state of a positive ion with its isotopic peaks separated by 0.25?

□ z = 1 □ z = 2 □ z = 3 □ z = 4

Question 10

What is the charge state of a positive ion with its isotopic peaks separated by 0.25?

□ z = 1 □ z = 2 □ z = 3 □ z = 4

- As discussed in the lecture, we expect the difference of mass between each isotopic peak to be 1 u
- This is because we do not have special cases such as Br or Cl in biological systems (doesn't jump by 2 u)
- We are looking at mass to charge, so m/z
- **Now we know m = 1, m/z = 0.25, solve for z**
- **1/z = 0.25, z = 4**

Question 11

11- A mass spectrometer achieves an accuracy of 5 ppm. What is the Δm in Da at m/z = 1000?

□ 0.001 Da □ 0.002 Da □ 0.005 Da □ 0.01 Da

- Given: error = 5 ppm, actual m/z = 1000 m/z
- We need to calculate the **Δm**
- **Error = (Δm/ (actual m/z))*10^6 , units = ppm**
- So: 5 ppm = (Δm/ (1000m/z))*10^6)

- **Δm = 5/0.001 = 0.005 Da**

Question 12

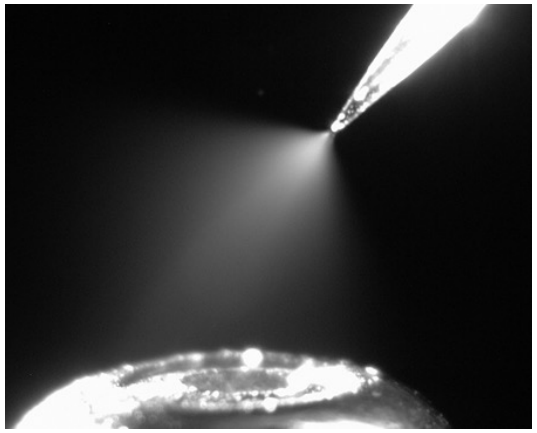
What is ESI?

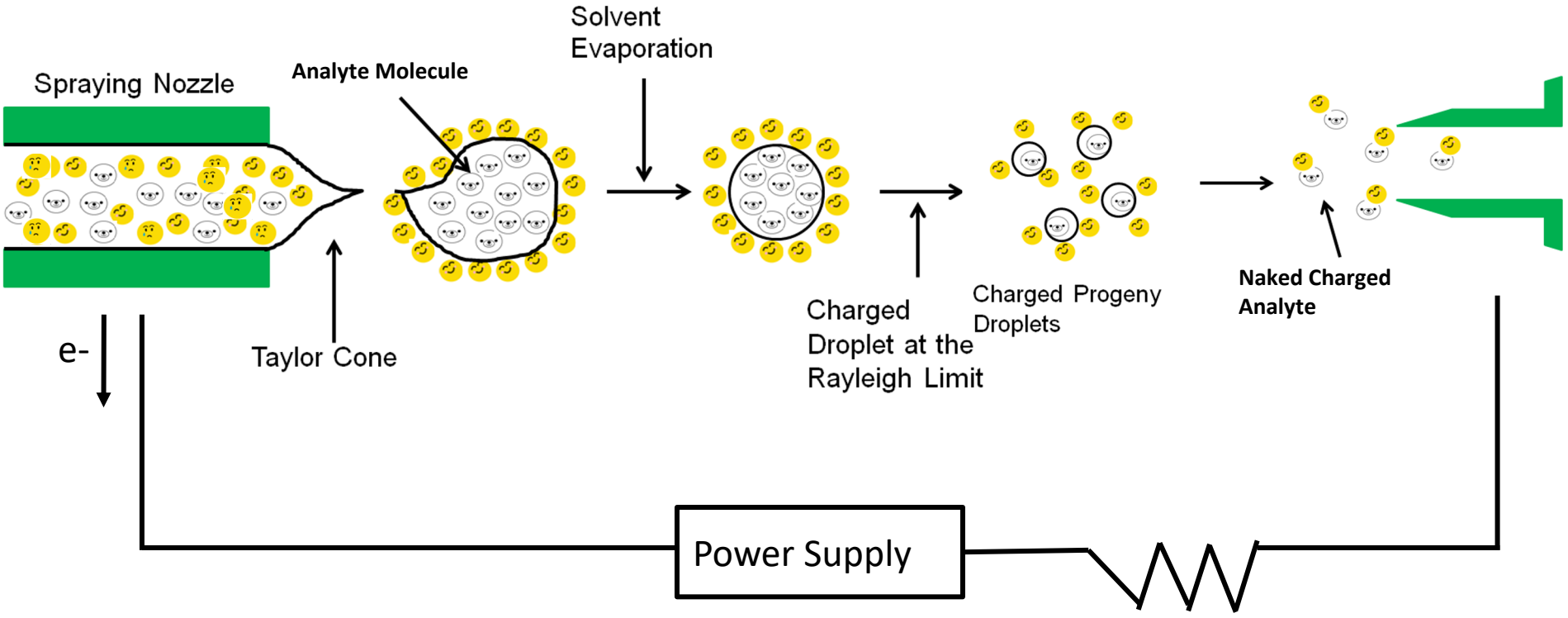
□ An analyzer □ A detector □ An ionization method

□ None of those

Electrospray Ionization (ESI)

- Ionization of protein without fragmentation
- Intolerant to salt, protein sample must be desalted prior to analysis.





- **An ionization method!**

Lecture slides: 37-38

Question 13

The ESI spectrum of a protein gives a charge state envelope. What is charge state of my peak m1 at m/z = 1199.08 knowing that the next peak of lower charge is m2 at m/z = 1332.20?

□ z1 = 8 □ z1 = 9 □ z1 = 10 □ z1 = 8

Question 13

- Given: peak m1 = 1199.08 m/z and m2 = 1332.20 m/z
- We need to calculate **the charge state of peak 1**

- The equation for m/z: $\frac{m}{z} = \frac{Mass\ Protein + zH}{z}$

- We know that m1 and m2 differ by a charge state of 1, higher m/z = lower charge state!
- So $(m/z)_{m1} = ((M_P) + zH)/z$
- Similarly, $(m/z)_{m2} = ((M_P) + (z-1)H)/(z-1)$
- Now rearrange both for M_P and set them equal to each other
- $M_P = (z(m/z)_{m1}) - zH = ((z-1)\ (m/z)_{m2}) - zH + M_H$
- $z(1199.08) = (z(1332.20) - 1332.20) + 1$
- $(1199.08 - 1332.20)z = -1331.20$
- **$z = (-1331.20)/ -133.12$**

- **z = 10. So, the charge state of peak one is 10**

Question 14

From question #13, what is the average molecular mass of this protein?

□ 11980.81 Da □ 12580.76 Da □ 14960.23 Da □ 10279.67 Da

Question 14

Lecture slide: 39

- The equation for m/z: $\frac{m}{z} = \frac{Mass\ Protein + zH}{z}$

- We know that m/z of peak 1 is 1199.08 m/z
- From our calculation, we also know z = 10^+
- This also means **10 H^+** is present in this charge state
- Mass of H^+ is 1

- So, plugging in these values into the formula after rearrangement:

- M_P = (1199.08)(10) – 10
- **M_P = 11980.80 Da**

Question 15

Lecture slide: 40

What flow rate is typical of the micro-spray mode?
□ 10 µL/min □ 300 nL/min □ 1 µL/min □ 1 nL/min

A: 300 nL/min

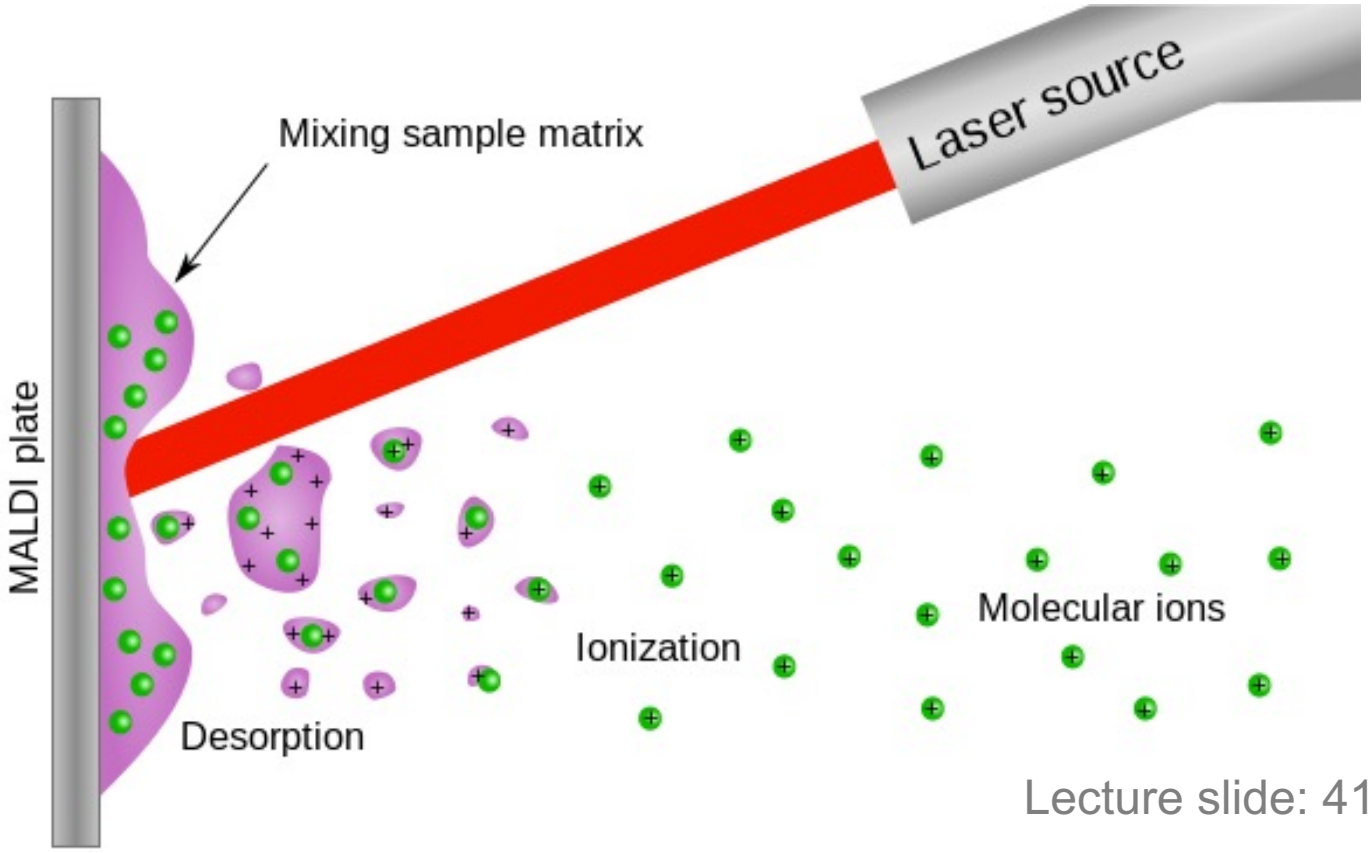
Question 16

What does MALDI stand for?

- □ Matrix-assisted laser desorption/ionization
- □ Metal-assisted laser desorption/ionization
- □ Metal-assisted light desorption/ionization
- □ Matrix-assisted light dissolution/ionization

Matrix-Assisted Laser Desorption/Ionization

- MALDI is a solid-to-gas phase, surface, vacuum ionization technique
- MALDI immediately became important because of its ability to softly ionize very large biomolecules



Lecture slide: 41

Question 17

What mass analyzer can offer the best resolution?

□ Quadrupole □ TOF □ FTICR □ Orbitrap

	TOF	Quad	Ion Trap	Orbitrap	FT-ICR
Resolving Power	very good	fair	fair	very good	excellent
Dynamic Range	very good	excellent	fair	fair	fair
Sensitivity	excellent	excellent	excellent	excellent	excellent
Speed	excellent	good	excellent	good	fair
Cost	150-300K	100K	100K	500K	1M
Maintenance	ave	ave	ave	ave	very high

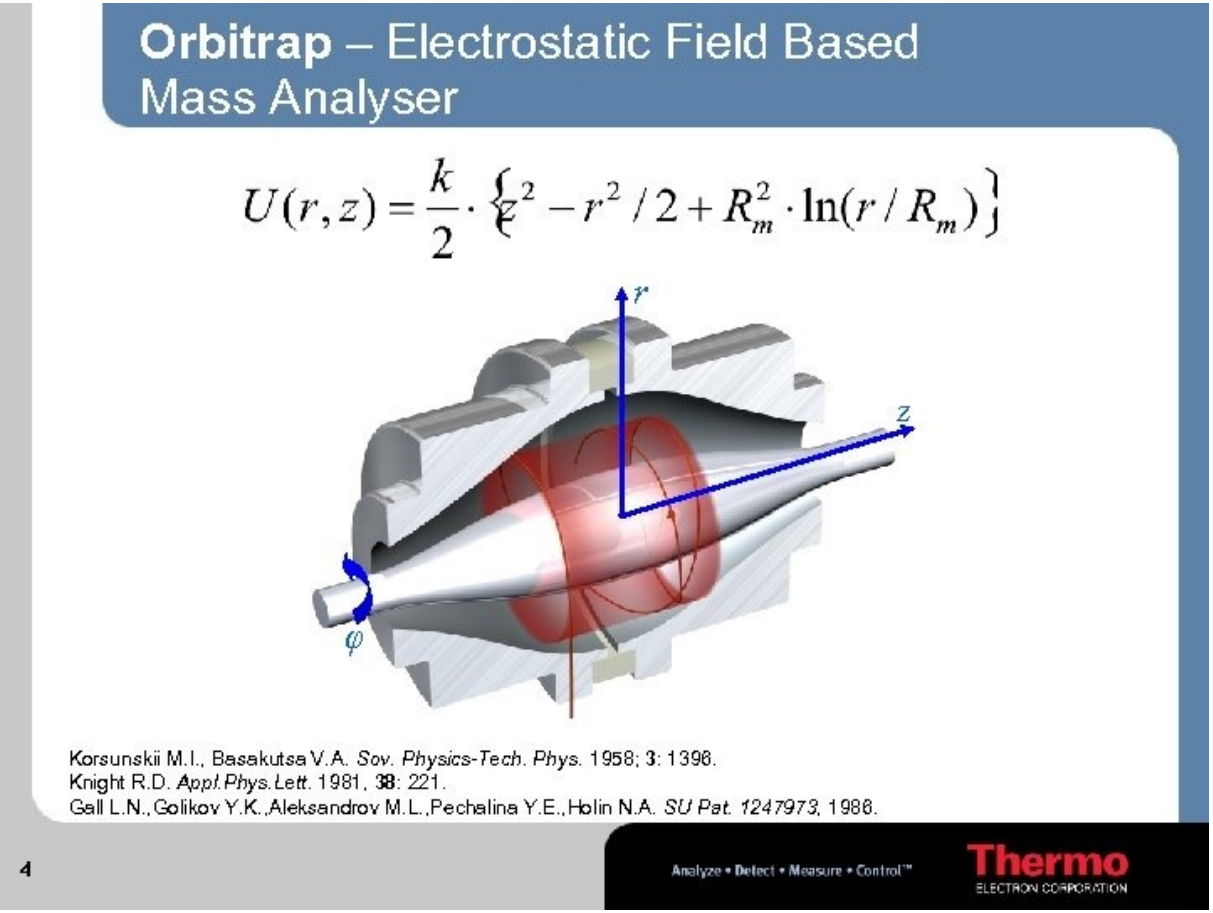
Question 18

Lecture slides: 49, 50

In an Orbitrap, the ions are trapped in an …?

□ Magnetic field □ Electrostatic field □ Ions are not trapped

□ Ions fly



Question 19

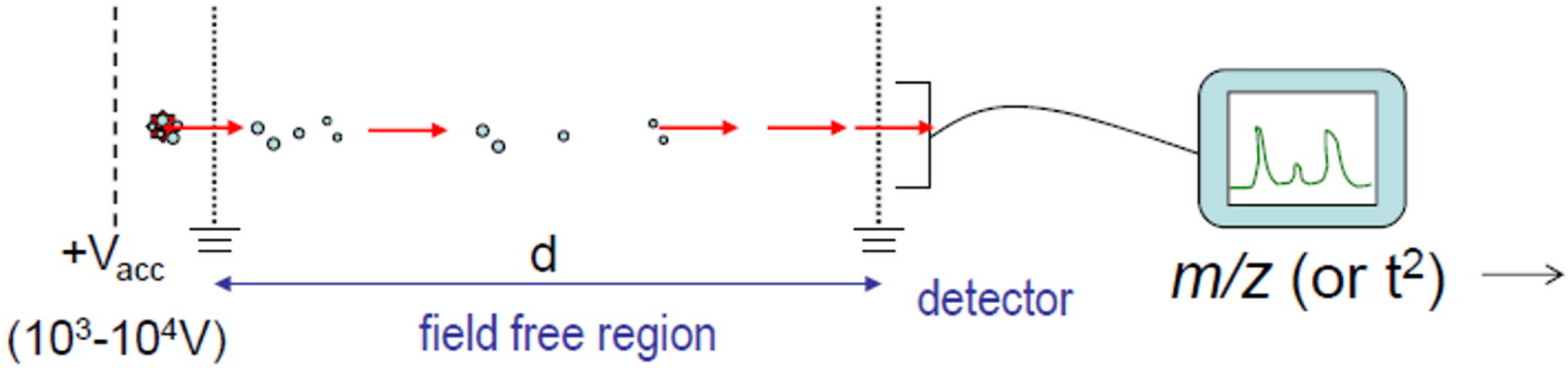
In a TOF analyzer, m/z is proportional to?

□ t^2 □ 1/t □ d □ None of these

Question 19

Lecture slides: 51, 52

- In the TOF extractor at the beginning of the flight region, ions encounter an electric field that accelerates all of them to a similar kinetic energy E.



- Potential energy in field region: $E_p = qU$
- All potential energy is converted into kinetic energy: $E_K = (1/2)mv^2$
- If $E_p = E_K$, then $qU = (1/2)mv^2$, or $qU = (1/2)m(d/t)^2$ (velocity is distance over time)
- Solving for t, we get:

$$t = \frac{d}{\sqrt{2U}}\sqrt{\frac{m}{q}} = k\sqrt{\frac{m}{q}}$$

- Then, the smaller the mass, the higher the velocity, smaller ions will first reach the detector
- **The m/z is directly proportional to the time it takes for the ion to make it to the detector, squared.**

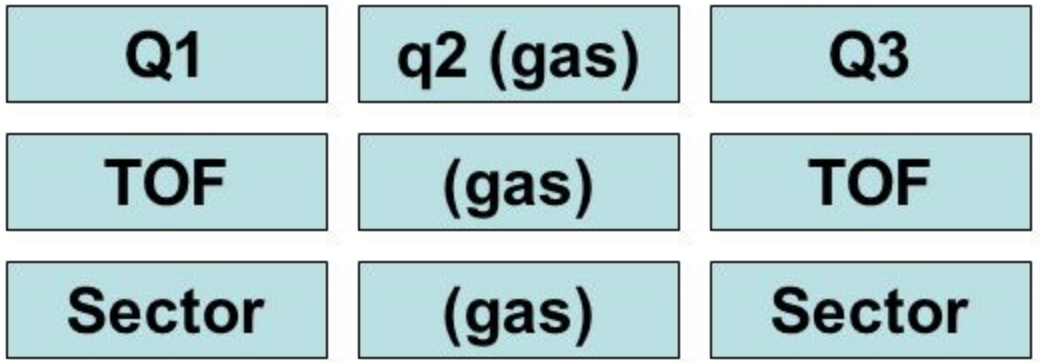
Question 20

In what type(s) of mass spectrometer can be performed MS/MS in time?

□ Q-TOF □ QqQ □ Ion trap □ FTICR

Tandem in Space vs. Tandem in Time MS/MS

- Space (Analyzers cannot trap, must be linked)
  - Triple Quad
  - TOF
  - Sector



- Many combinations of linked analyzers possible

- Time (Trapping analyzers)
  - Quadrupole Ion trap
  - ICR

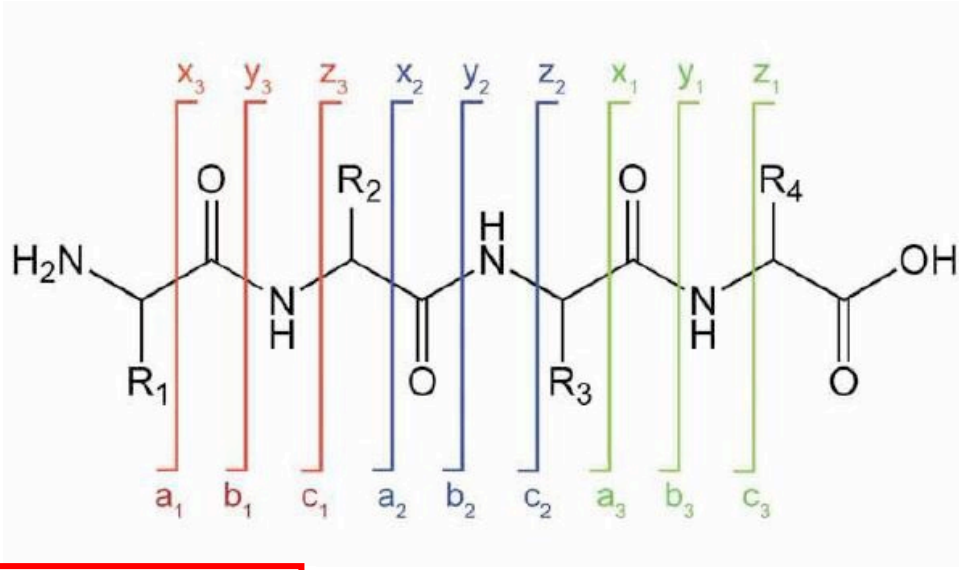
- Most activation by CID (CAD)

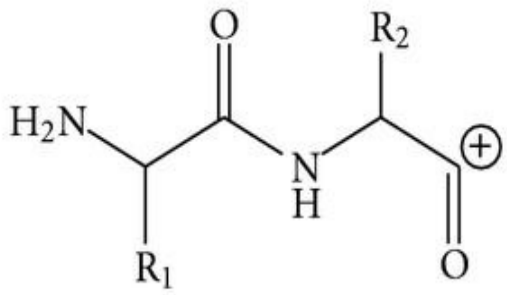
Lecture slide: 59

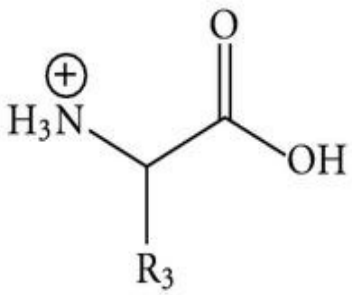
Question 21

What type(s) of ions are generated with collision-induced dissociation?

□ b □ a □ z □ y







b and y ions

ESI or MALDI?

- It is relatively easy to distinguish whether a protein mass spectrometry is performed by **ESI** or **MALDI**.

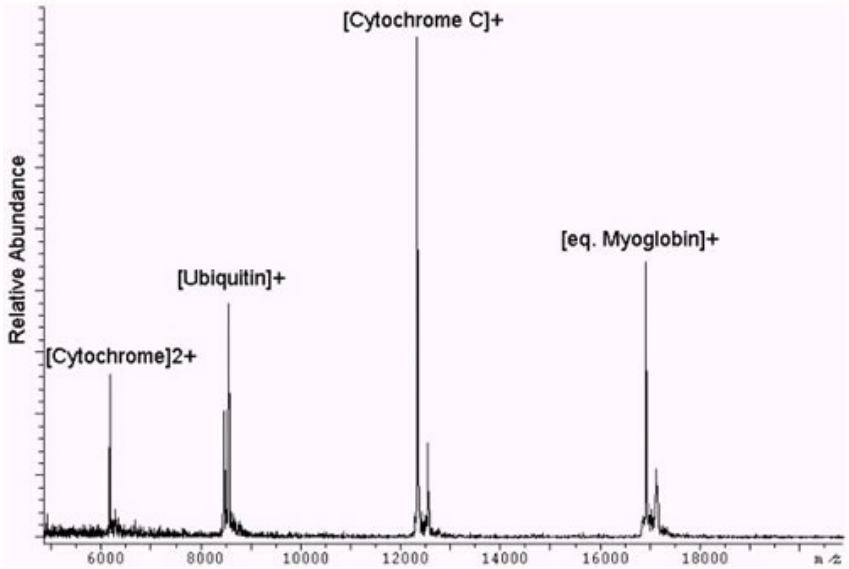
- Simply look at the **charge states** of the ions detected

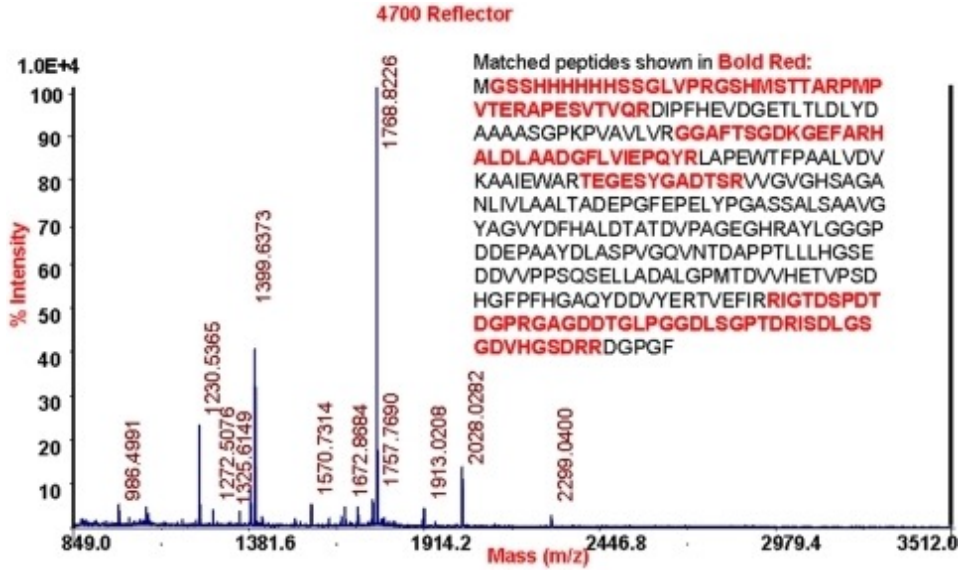
- Often cases in the analysis of big bio-macromolecules like proteins, **ESI generates ions of higher charge states, and also many different charge states**

- Often cases in the analysis of big bio-macromolecules like proteins, **MALDI generates ions of low charge states, most of the time just 1+ and 2+**

- **Therefore by simply looking at the number of charge state peaks and the charge states can help distinguish a spectra acquired by MALDI or ESI**

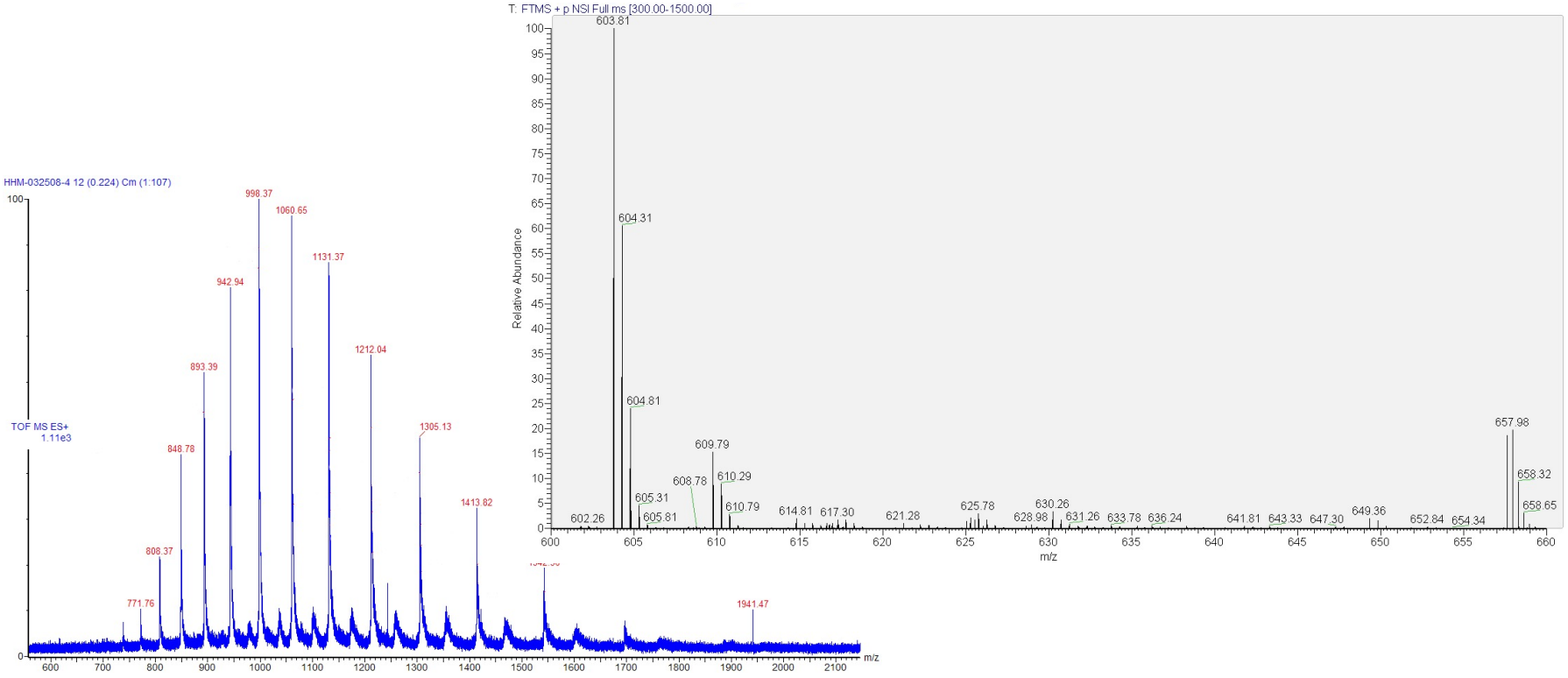
ESI or MALDI?





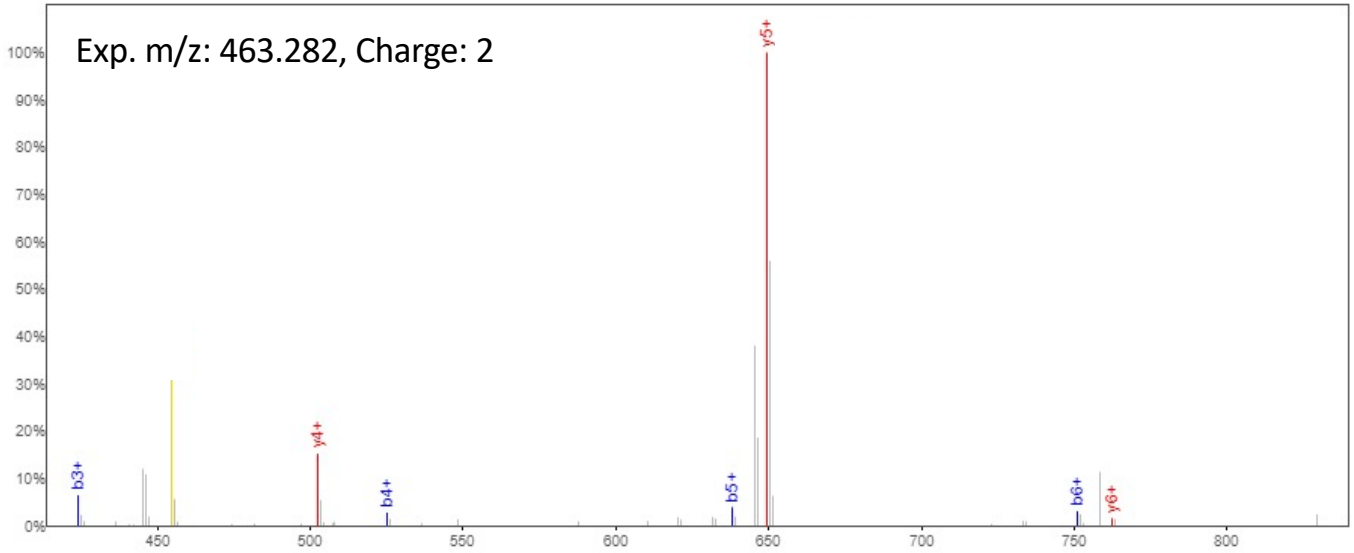
- Look at the MS1 spectra, we have peaks of multiple proteins
- All these proteins are of charge states of 1+ or 2+
- No higher charge states, not many different charge states
- **This is MALDI**

ESI or MALDI?



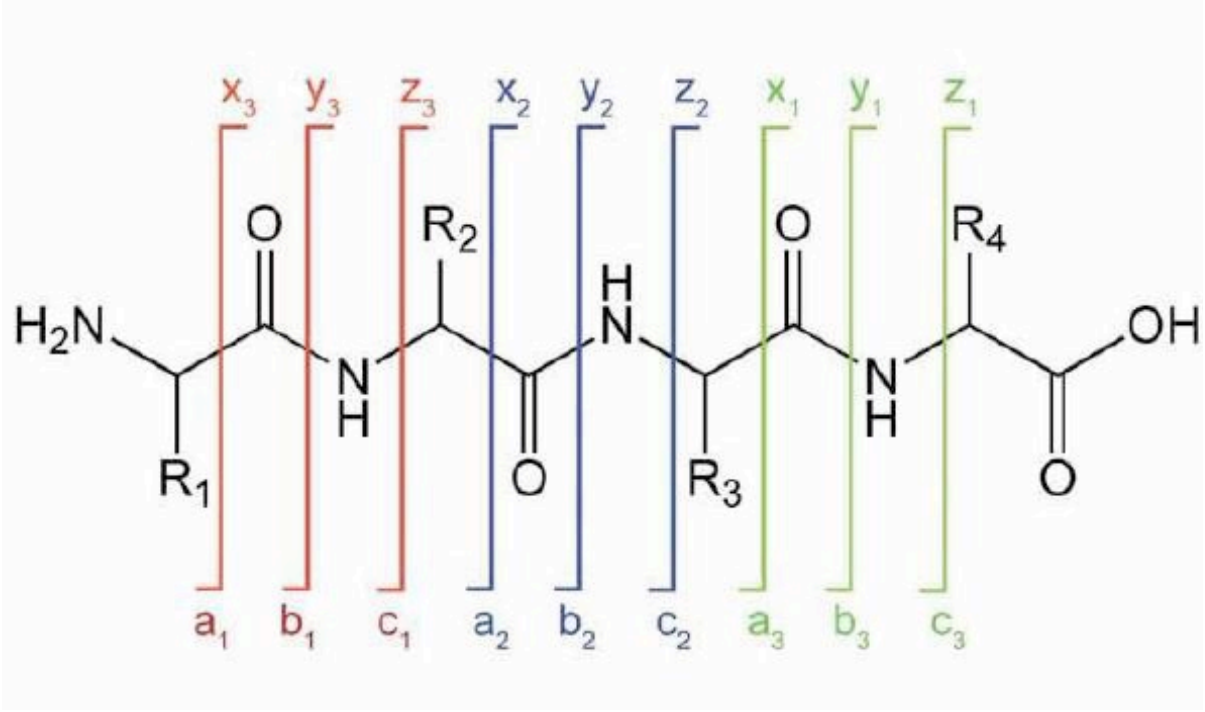
- Look at the MS1 spectra, there is a distribution of multiple charge states
- Many different charge states, most likely higher than 1+ and 2+
- **This is ESI**

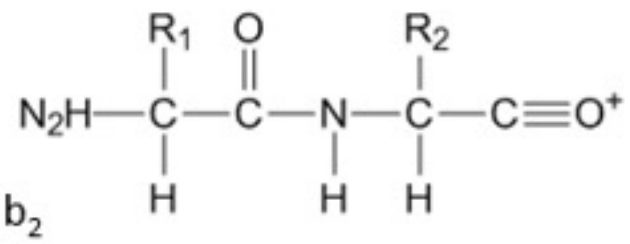
Determine the sequence of the fully tryptic peptide given the following experimental tandem mass spectrum

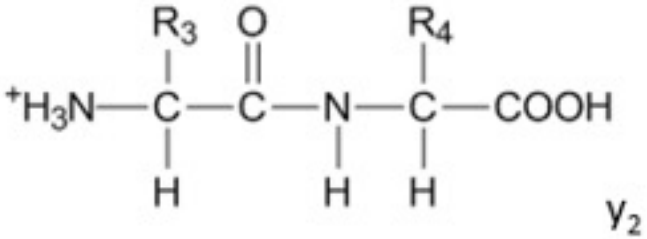


b+	#	Seq	#	y+
	1		7	
	2		6	762.4872
424.2231	3		5	649.4032
525.2708	4		4	502.3348
638.3548	5		3	
751.4389	6		2	
	7		1	

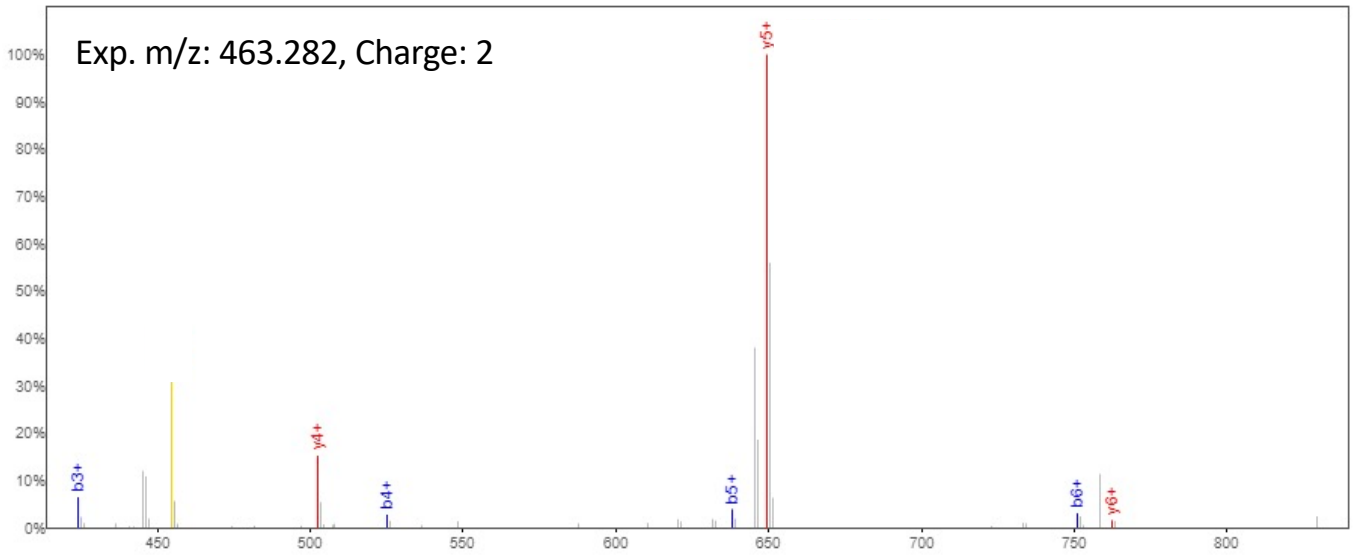
Fragmentation by CID generates b and y ions







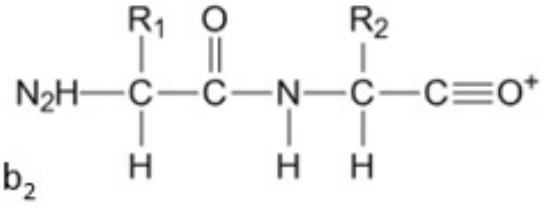
Use what we are given…



b+	#	Seq	#	y+
	1		7	
	2		6	762.4872
424.2231	3		5	649.4032
525.2708	4		4	502.3348
638.3548	5		3	
751.4389	6		2	
	7		1	

- MS1 data: parent ion peptide m/z = 463.282, z = 2
- **So, the mass of this parent peptide is (463.282)*2 - 2 = 924.564 Da**
- Note: in the slides to follow, I provided a chart of amino acid residual masses. You can feel free to also use the one provided in the lecture!

Let's start with b-ions



b_2

b+	#	Seq	#	y+
	1		7	
	2		6	762.4872
424.2231	3		5	649.4032
525.2708	4		4	502.3348
638.3548	5		3	
751.4389	6		2	
	7		1	

Amino acid	1-letter code	3-letter code	Chemical formula(-H_2O)	Monoisotopic mass (-H_2O)	Average mass (-H_2O)
Alanine	A	Ala	C_3H_5ON	71.03711	71.0788
Arginine	R	Arg	$C_6H_{12}ON_4$	156.10111	156.1875
Asparagine	N	Asn	$C_4H_6O_2N_2$	114.04293	114.1038
Aspartic Acid	D	Asp	$C_4H_5O_3N$	115.02694	115.0886
Cysteine	C	Cys	C_3H_5ONS	103.00919	103.1388
Glutamic Acid	E	Glu	$C_5H_7O_3N$	129.04259	129.1155
Glutamine	Q	Gln	$C_5H_8O_2N_2$	128.05858	128.1307
Glycine	G	Gly	C_2H_3ON	57.02146	57.0519
Histidine	H	His	$C_6H_7ON_3$	137.05891	137.1411
Isoleucine	I	Ile	$C_6H_{11}ON$	113.08406	113.1594
Leucine	L	Leu	$C_6H_{11}ON$	113.08406	113.1594
Lysine	K	Lys	$C_6H_{12}ON_2$	128.09496	128.1741
Methionine	M	Met	C_5H_9ONS	131.04049	131.1926
Phenylalanine	F	Phe	C_9H_9ON	147.06841	147.1766
Proline	P	Pro	C_5H_7ON	97.05276	97.1167
Serine	S	Ser	$C_3H_5O_2N$	87.03203	87.0782
Threonine	T	Thr	$C_4H_7O_2N$	101.04768	101.1051
Tryptophan	W	Trp	$C_{11}H_{10}ON_2$	186.07931	186.2132
Tyrosine	Y	Tyr	$C_9H_9O_2N$	163.06333	163.176
Valine	V	Val	C_5H_9ON	99.06841	99.1326

- Take a look at residual masses (amino acid – H_2O) and the structure of b ions
- **B ions miss an OH at C terminal (compared to peptide)****
- We know the parent peptide is 924.564 Da, technically we also know what the b7 ion look like.
- Therefore, b7 ion is simply 924.564 – mass OH = 924.564 - 17.00274 = 907.5613

Find the m/z difference from ion to ion

b+	#	Seq	#	y+
	1		7	
	2		6	762.4872
424.2231	3		5	649.4032
525.2708	4		4	502.3348
638.3548	5		3	
751.4389	6		2	
907.5613	7		1	

- 101.0 (-T)

- 113.1 (-I or L)

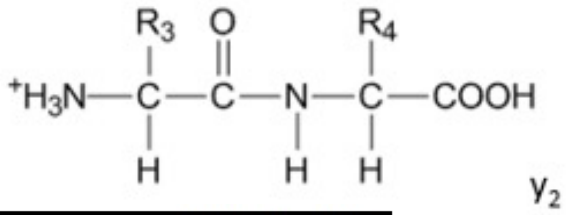
- 113.1 (-I or L)

- 156.1 (-R)

Amino acid	1-letter code	3-letter code	Chemical formula(-H_2O)	Monoisotopic mass (-H_2O)	Average mass (-H_2O)
Alanine	A	Ala	C_3H_5ON	71.03711	71.0788
Arginine	R	Arg	$C_6H_{12}ON_4$	156.10111	156.1875
Asparagine	N	Asn	$C_4H_6O_2N_2$	114.04293	114.1038
Aspartic Acid	D	Asp	$C_4H_5O_3N$	115.02694	115.0886
Cysteine	C	Cys	C_3H_5ONS	103.00919	103.1388
Glutamic Acid	E	Glu	$C_5H_7O_3N$	129.04259	129.1155
Glutamine	Q	Gln	$C_5H_8O_2N_2$	128.05858	128.1307
Glycine	G	Gly	C_2H_3ON	57.02146	57.0519
Histidine	H	His	$C_6H_7ON_3$	137.05891	137.1411
Isoleucine	I	Ile	$C_6H_{11}ON$	113.08406	113.1594
Leucine	L	Leu	$C_6H_{11}ON$	113.08406	113.1594
Lysine	K	Lys	$C_6H_{12}ON_2$	128.09496	128.1741
Methionine	M	Met	C_5H_9ONS	131.04049	131.1926
Phenylalanine	F	Phe	C_9H_9ON	147.06841	147.1766
Proline	P	Pro	C_5H_7ON	97.05276	97.1167
Serine	S	Ser	$C_3H_5O_2N$	87.03203	87.0782
Threonine	T	Thr	$C_4H_7O_2N$	101.04768	101.1051
Tryptophan	W	Trp	$C_{11}H_{10}ON_2$	186.07931	186.2132
Tyrosine	Y	Tyr	$C_9H_9O_2N$	163.06333	163.176
Valine	V	Val	C_5H_9ON	99.06841	99.1326

Now y-ions

b+	#	Seq	#	y+
	1		7	
	2		6	762.4872
424.2231	3		5	649.4032
525.2708	4		4	502.3348
638.3548	5		3	
751.4389	6		2	
	7		1	



y_2

Amino acid	1-letter code	3-letter code	Chemical formula(-H_2O)	Monoisotopic mass (-H_2O)	Average mass (-H_2O)
Alanine	A	Ala	C_3H_5ON	71.03711	71.0788
Arginine	R	Arg	$C_6H_{12}ON_4$	156.10111	156.1875
Asparagine	N	Asn	$C_4H_6O_2N_2$	114.04293	114.1038
Aspartic Acid	D	Asp	$C_4H_5O_3N$	115.02694	115.0886
Cysteine	C	Cys	C_3H_5ONS	103.00919	103.1388
Glutamic Acid	E	Glu	$C_5H_7O_3N$	129.04259	129.1155
Glutamine	Q	Gln	$C_5H_8O_2N_2$	128.05858	128.1307
Glycine	G	Gly	C_2H_3ON	57.02146	57.0519
Histidine	H	His	$C_6H_7ON_3$	137.05891	137.1411
Isoleucine	I	Ile	$C_6H_{11}ON$	113.08406	113.1594
Leucine	L	Leu	$C_6H_{11}ON$	113.08406	113.1594
Lysine	K	Lys	$C_6H_{12}ON_2$	128.09496	128.1741
Methionine	M	Met	C_5H_9ONS	131.04049	131.1926
Phenylalanine	F	Phe	C_9H_9ON	147.06841	147.1766
Proline	P	Pro	C_5H_7ON	97.05276	97.1167
Serine	S	Ser	$C_3H_5O_2N$	87.03203	87.0782
Threonine	T	Thr	$C_4H_7O_2N$	101.04768	101.1051
Tryptophan	W	Trp	$C_{11}H_{10}ON_2$	186.07931	186.2132
Tyrosine	Y	Tyr	$C_9H_9O_2N$	163.06333	163.176
Valine	V	Val	C_5H_9ON	99.06841	99.1326

- Take a look at residual masses (amino acid – H_2O) and the structure of y ions
- **y ions have 1 extra H at the N terminal (compared to peptide)****
- We know the parent peptide is 924.564 Da, technically we also know what y7 ion look like.
- Therefore, y7 ion is simply 924.564 + mass H = 924.564 + 1.007825 = 925.5718

Find the m/z difference from ion to ion

b+	#	Seq	#	y+
	1		7	925.5718
	2		6	762.4872
424.2231	3		5	649.4032
525.2708	4		4	502.3348
638.3548	5		3	
751.4389	6		2	
907.5613	7		1	

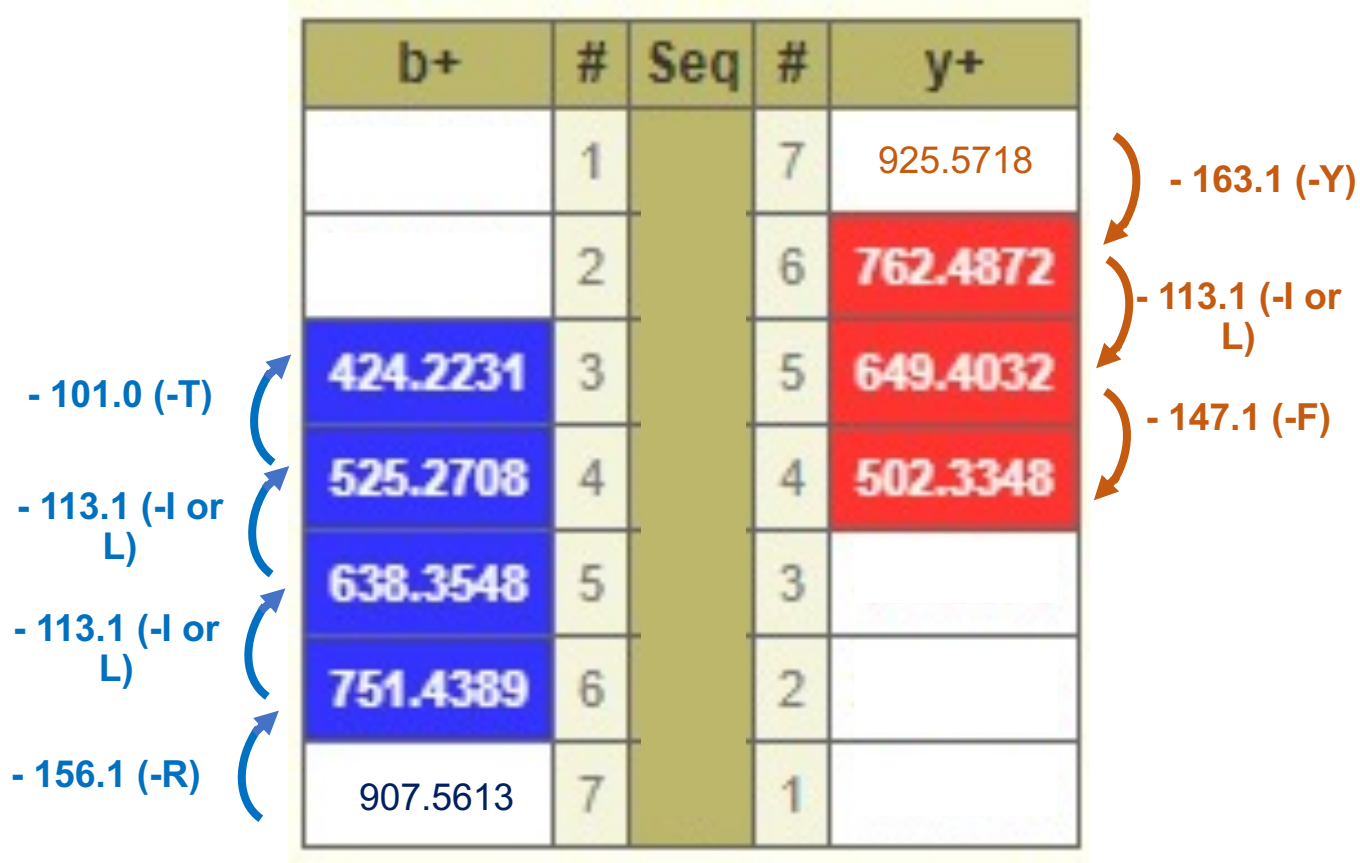
- 163.1 (-Y)

- 113.1 (-I or L)

- 147.1 (-F)

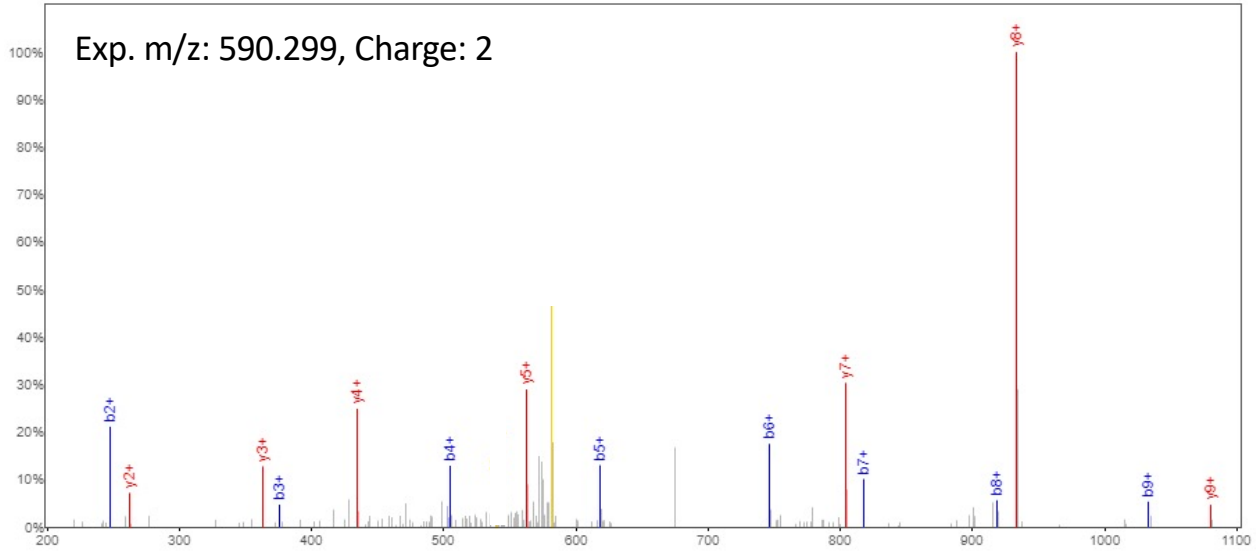
Amino acid	1-letter code	3-letter code	Chemical formula(-H_2O)	Monoisotopic mass (-H_2O)	Average mass (-H_2O)
Alanine	A	Ala	C_3H_5ON	71.03711	71.0788
Arginine	R	Arg	$C_6H_{12}ON_4$	156.10111	156.1875
Asparagine	N	Asn	$C_4H_6O_2N_2$	114.04293	114.1038
Aspartic Acid	D	Asp	$C_4H_5O_3N$	115.02694	115.0886
Cysteine	C	Cys	C_3H_5ONS	103.00919	103.1388
Glutamic Acid	E	Glu	$C_5H_7O_3N$	129.04259	129.1155
Glutamine	Q	Gln	$C_5H_8O_2N_2$	128.05858	128.1307
Glycine	G	Gly	C_2H_3ON	57.02146	57.0519
Histidine	H	His	$C_6H_7ON_3$	137.05891	137.1411
Isoleucine	I	Ile	$C_6H_{11}ON$	113.08406	113.1594
Leucine	L	Leu	$C_6H_{11}ON$	113.08406	113.1594
Lysine	K	Lys	$C_6H_{12}ON_2$	128.09496	128.1741
Methionine	M	Met	C_5H_9ONS	131.04049	131.1926
Phenylalanine	F	Phe	C_9H_9ON	147.06841	147.1766
Proline	P	Pro	C_5H_7ON	97.05276	97.1167
Serine	S	Ser	$C_3H_5O_2N$	87.03203	87.0782
Threonine	T	Thr	$C_4H_7O_2N$	101.04768	101.1051
Tryptophan	W	Trp	$C_{11}H_{10}ON_2$	186.07931	186.2132
Tyrosine	Y	Tyr	$C_9H_9O_2N$	163.06333	163.176
Valine	V	Val	C_5H_9ON	99.06841	99.1326

Combine findings from b and y ions



- You count b ions from N-term to C term, vice versa for y ions.
- So the peptide sequence here is: N term to C term
- **Y-(L/I)-(F)-T-(L/I)-(L/I)-R**

Determine the sequence of the fully tryptic peptide given the following experimental tandem mass spectrum



b+	#	Seq	#	y+
	1		10	
247.1441	2		9	1080.5208
376.1867	3		8	933.4524
505.2293	4		7	804.4098
618.3134	5		6	
746.3719	6		5	562.2831
817.4090	7		4	434.2245
918.4567	8		3	363.1874
1033.4837	9		2	262.1397
	10		1	

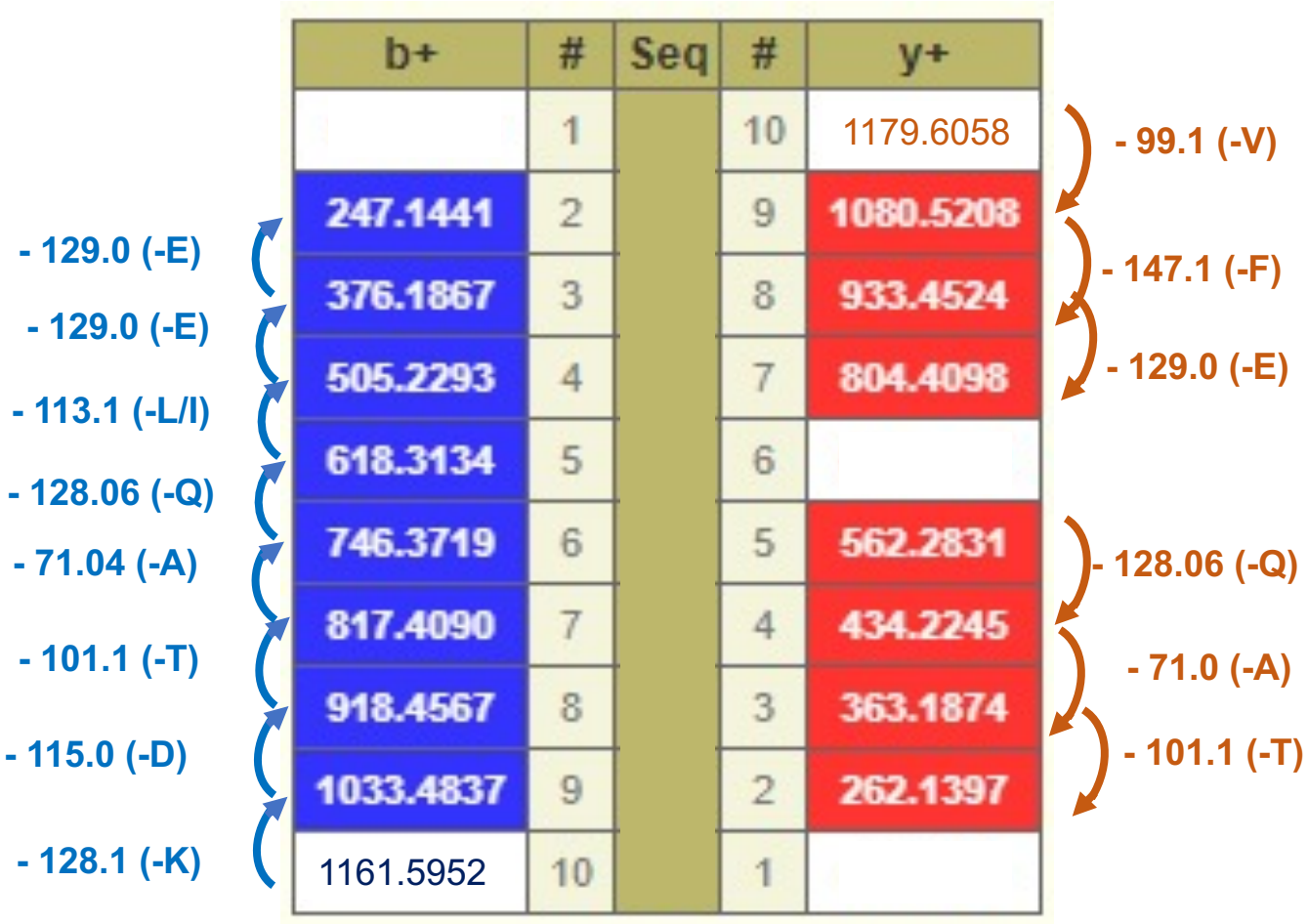
- Parent peptide: m/z = 590.299, Charge = 2
- **Mass peptide = (590.299*2)-2 = 1178.598**

Combine findings from b and y ions

b+	#	Seq	#	y+
	1		10	1179.6058
247.1441	2		9	1080.5208
376.1867	3		8	933.4524
505.2293	4		7	804.4098
618.3134	5		6	
746.3719	6		5	562.2831
817.4090	7		4	434.2245
918.4567	8		3	363.1874
1033.4837	9		2	262.1397
1161.5952	10		1	

- B ion lacks OH, so b10 ion = 1178.598 – 17.00274 = 1161.5952
- Y ion has an extra H, so y10 ion = 1178.598 + 1.007825 = 1179.6058

Combine findings from b and y ions



Amino acid	Symbol	Residue Mass, amu (monoisotopic)
Glycine	G	57.02
Alanine	A	71.04
Serine	S	87.03
Proline	P	97.05
Valine	V	99.07
Threonine	T	101.05
Cysteine	C	103.01
Isoleucine	I	113.08
Leucine	L	113.08
Asparagine	N	114.04
Aspartic Acid	D	115.03
Glutamine	Q	128.06
Lysine	K	128.09
Glutamic Acid	E	129.04
Methionine	M	131.04
Histidine	H	137.06
Phenylalanine	F	147.07
Arginine	R	156.10
Tyrosine	Y	163.06
Tryptophan	W	186.08

- Sequence from N term to C term:
- **V-F-E-E-(L/I)-Q-A-T-D-K**