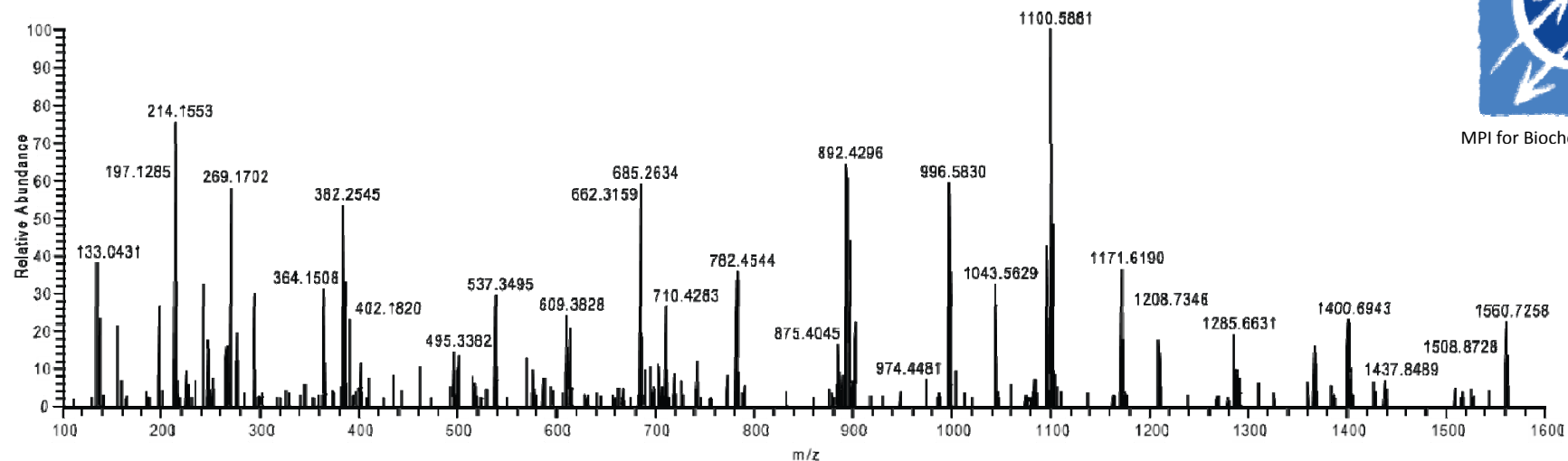


How to get everything out of an MS/MS spectrum

Tami Geiger
Max Planck Institute of Biochemistry



MPI for Biochemistry

How to get everything out of an MS/MS spectrum

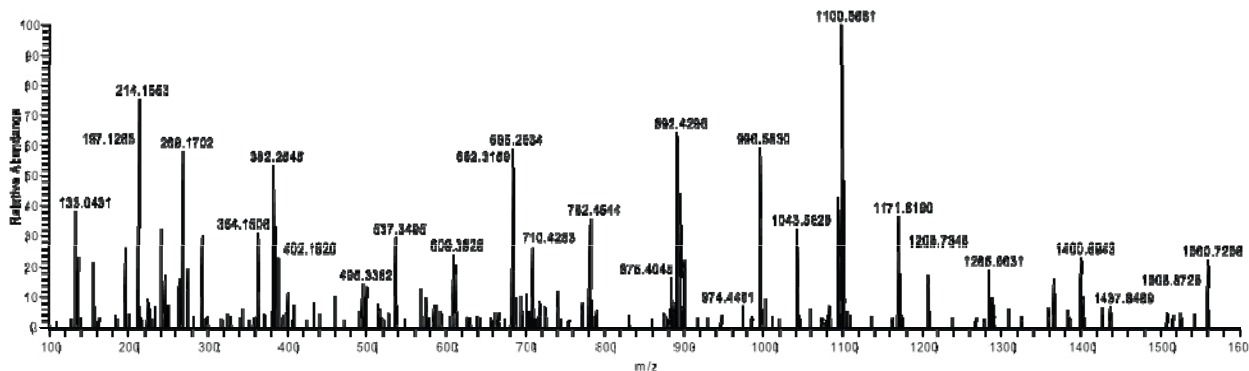
- Mobile proton model
- Fragmentation methods: CID (ion trap/ quadrupole), ECD, ETD
- Peptide fragments in the MS/MS spectra

b and y ions

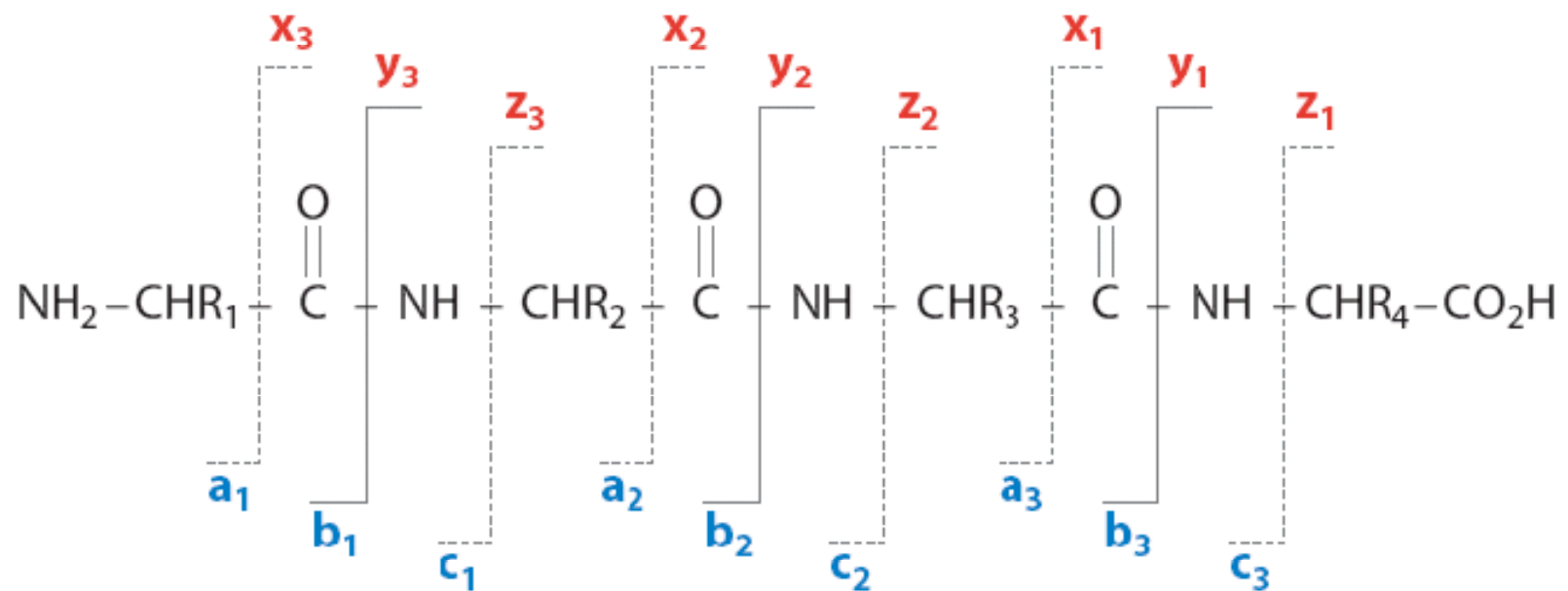
neutral losses

reporter ions

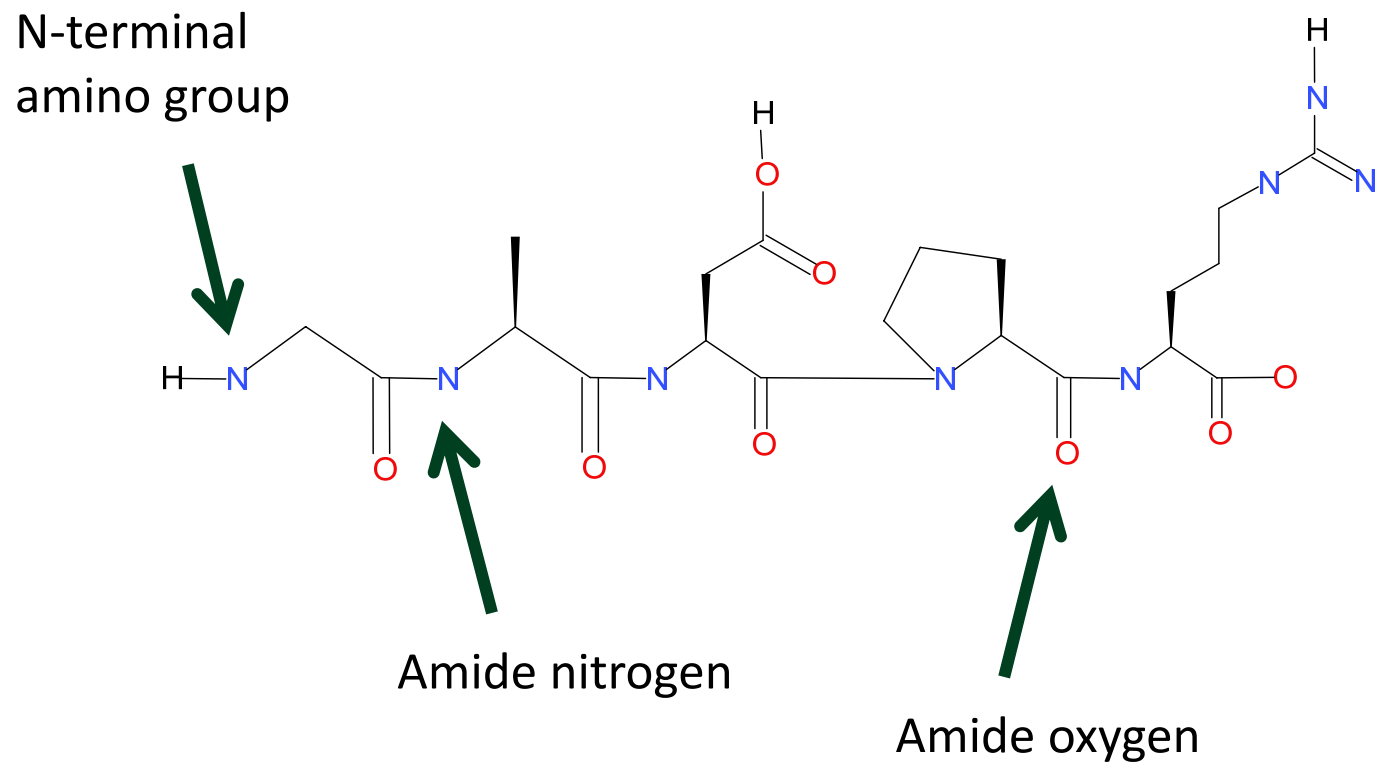
Sequence effects



Fragmentation is mainly on the peptide backbone

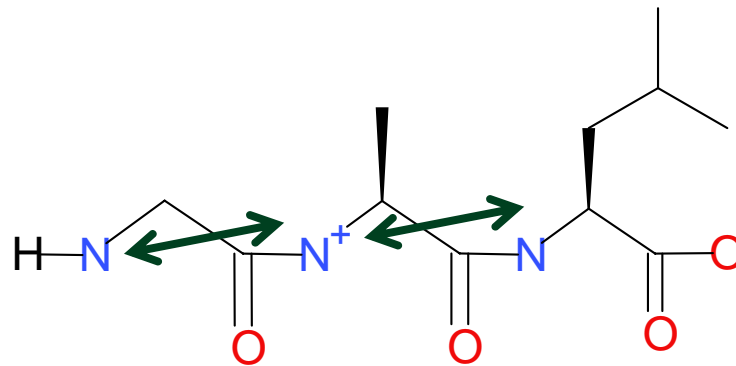


Mobile proton model > Protonation of peptide backbone at different positions



Mobile proton model > Peptide excitation induces mobility

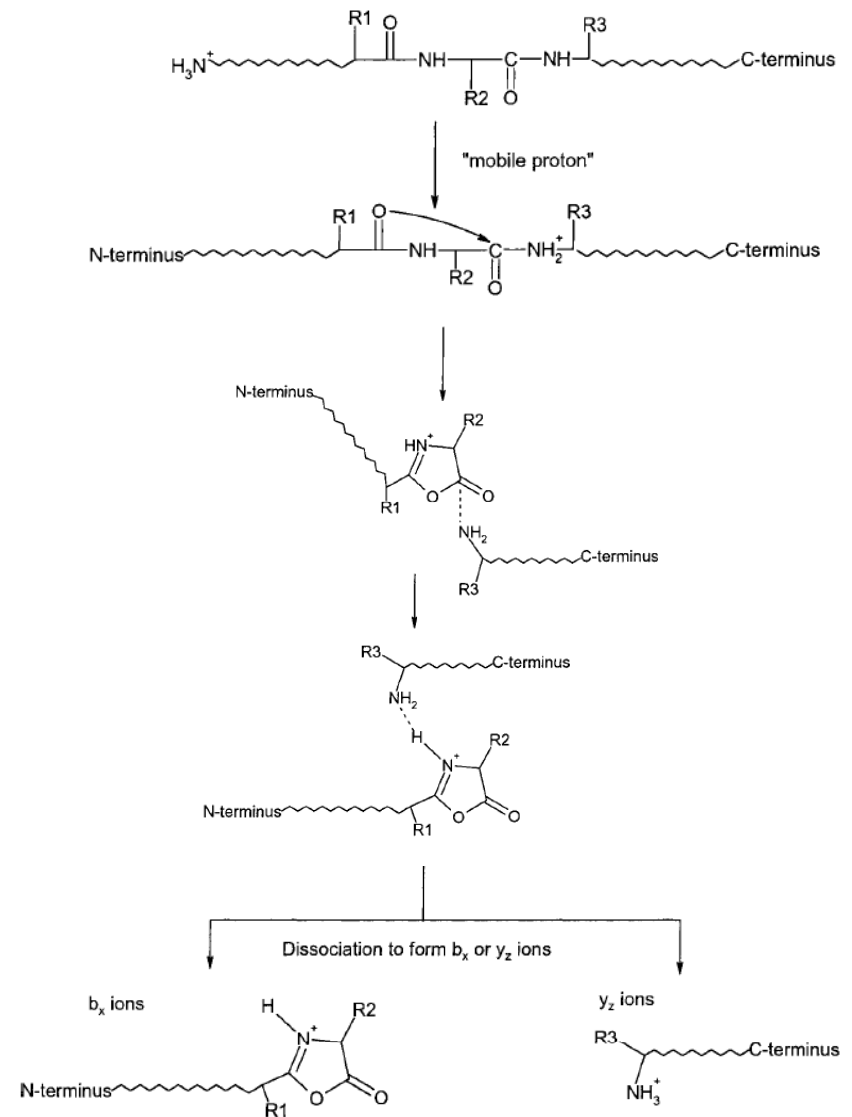
With excitation a proton located at the N-terminus starts moving along the peptide backbone:



By protonation, the carbon atom becomes a likely target of nucleophilic attack.

Suggested mechanism of formation of b- and y-ions

complementary b- and y-ions
if multiply charged!



Mobile proton model

Proton mobility classification (Kapp et al.):

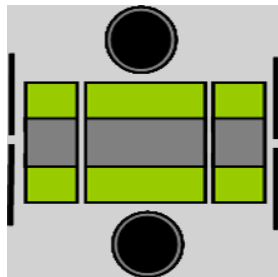
- nonmobile: # of ionizing protons \leq # of R
- partially mobile: # of R $<$ # of ionizing protons \leq combined # of R,K,H
- mobile: # of ionizing protons $>$ combined # of R,K,H

- **SGSpSQELDV****K****PpSASPQER**

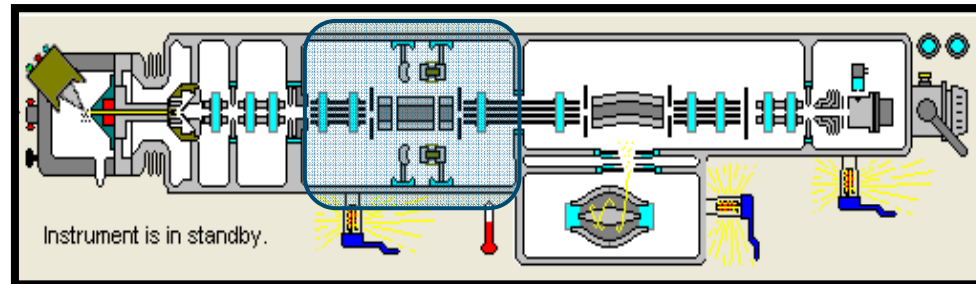
- 1+: nonmobile
- 2+: partially mobile
- 3+: mobile

CID (CAD) > Collision-induced (activated) dissociation (in ion traps)

Low energy CID in iontraps



Linear iontrap

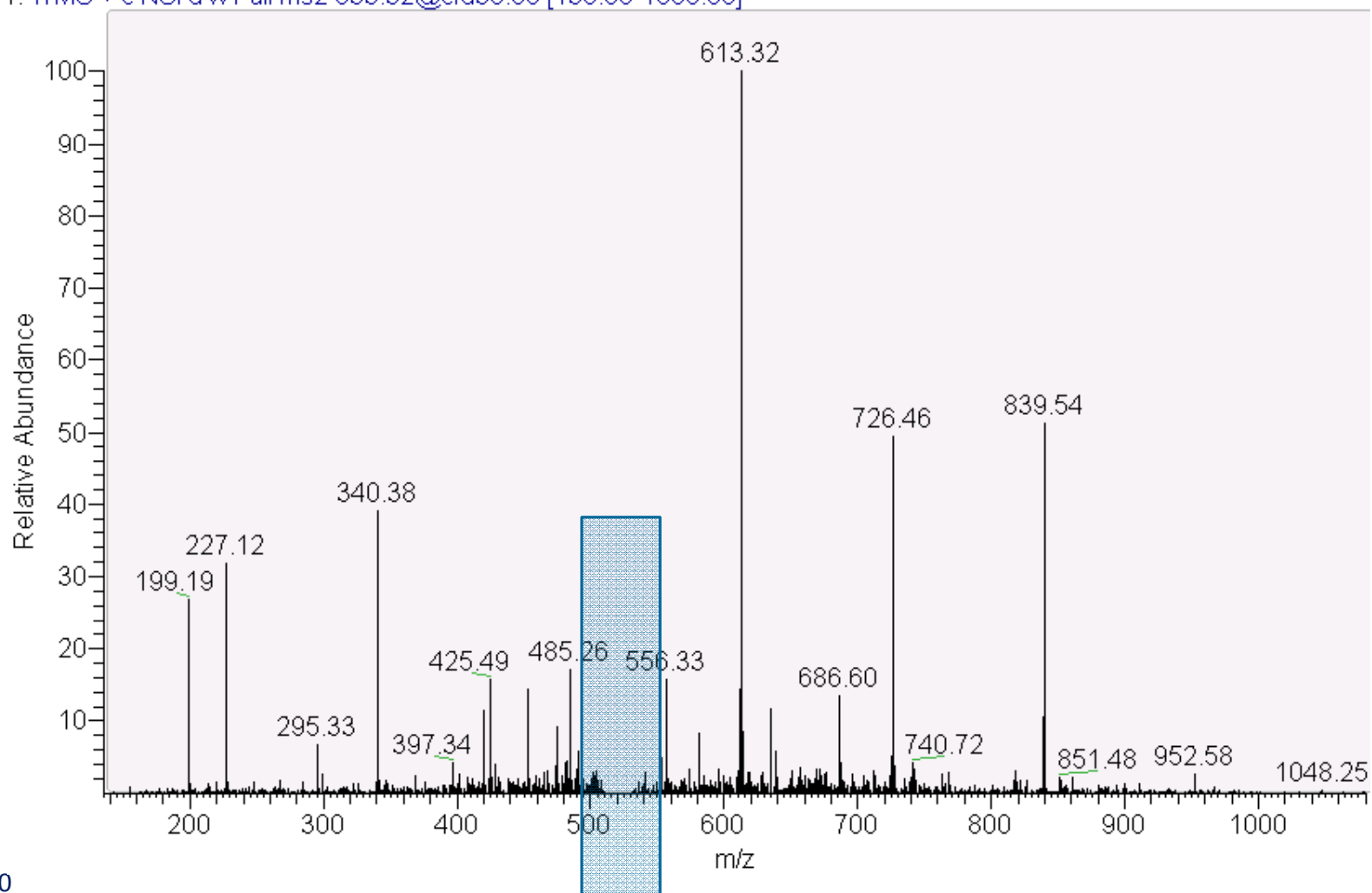


LTQ-Orbitrap

- Mainly b- and y-ions
- Collision with Helium
- (-) 1/3 cut-off
- (-) long activation time, loss of labile modifications, e.g. Phosphorylation

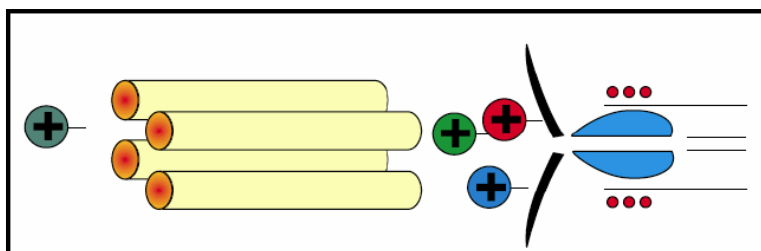
CID Spectrum

20090716_Velos5_GaSt_QC_L1_HCD_CID_ETD_ETD #10596 RT: 66.77 AV: 1 NL: 1.16E5
T: ITMS + c NSI d w Full ms2 533.32@cid35.00 [135.00-1080.00]

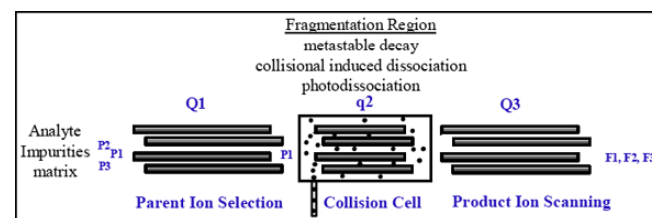


CID in quadrupole

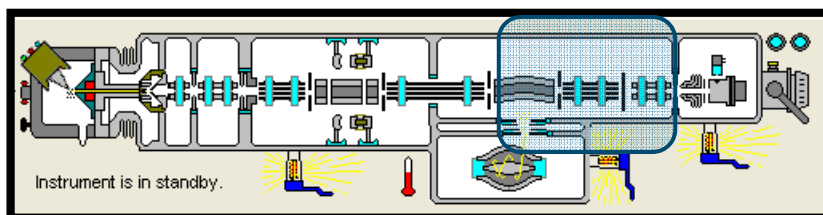
HCD – Higher-energy C-trap (induced) dissociation



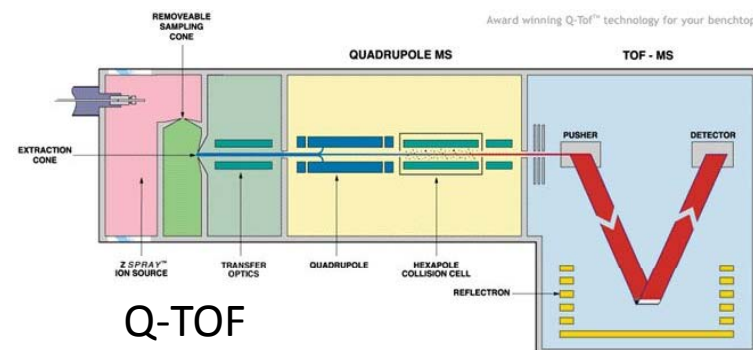
Quadrupole



Triple-quadrupole



LTQ-Orbitrap

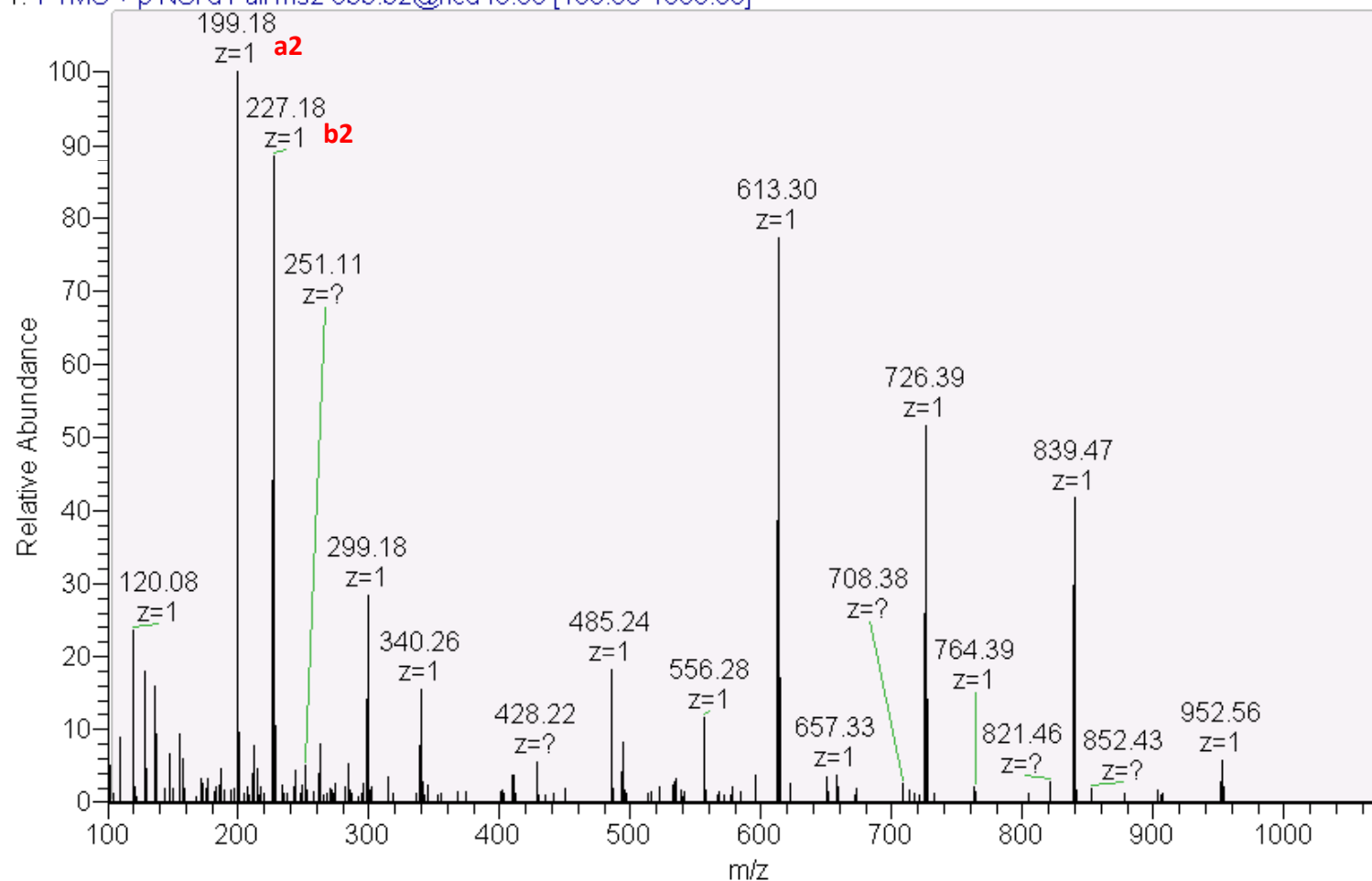


Q-TOF

- Common ions: a₂, b₂ ions; mainly γ -ions (also γ_1 and γ_2)

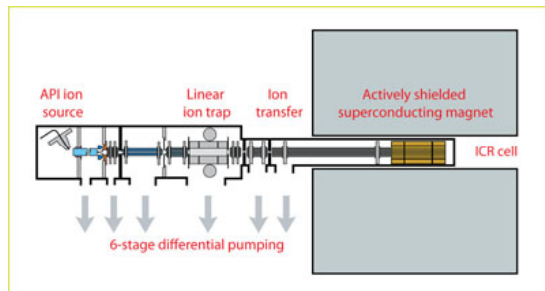
HCD - Spectrum

20090716_Velos5_GaSt_QC_L1_HCD_CID_ETD_ETD#10595 RT: 66.77 AV: 1 NL: 1.75E5
T: FTMS + p NSI d Full ms2 533.32@hcd40.00 [100.00-1080.00]

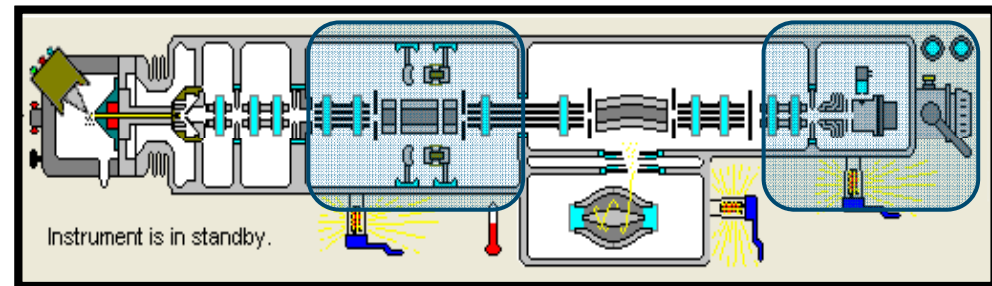


ECD- Electron capture dissociation

ETD-Electrontransfer dissociation



ECD > FT ICR



LTQ-Orbitrap-ETD

ECD

- Performed in superconducting magnet
- Reaction with a beam of electrons

- Generation of charge-reduced, radical peptides ions: $\text{MHn}(n-1)^+$
- Fragmentation between backbone amide and $\text{C}\alpha \rightarrow \text{c- and z'-ions}$
- Mainly for large, highly charged peptides.
- Ideal for peptides with labile bonds

ETD

- Reaction with one-electron donor (Fluoranthene radical anion)

Identification of peptides from MS/MS spectra

Database search

Common search engines: SEQUEST, Mascot, X!Tandem etc.

Step1: The search engine assigns MS/MS spectra to individual peptides in a protein database

Generates a list of all possible peptides in a database.

Compares the MS/MS spectrum to all theoretically possible peptides.

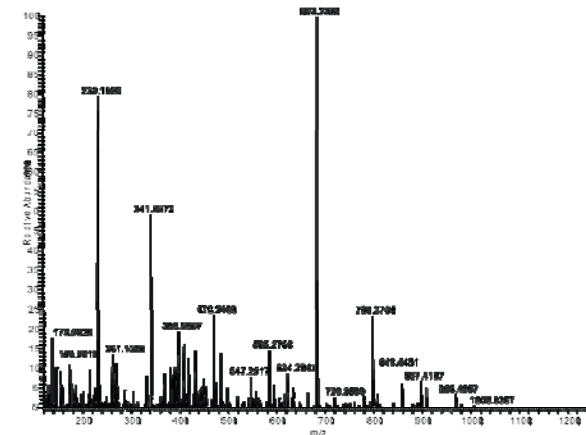
(uses enzyme information, modifications and the specified mass-range)

De-novo sequencing

Assign the masses of the peaks to obtain a full coverage of peptide amino acid sequence

Interpretation of MS/MS spectra

1. Evaluate spectrum quality- intensity of the signal:
2. Evaluate spectrum quality- unexplained peaks
3. Recognize the matched b and y ions
4. Recognize neutral loss fragments
5. Recognize low-mass reporter ions
6. Recognize sequence effects



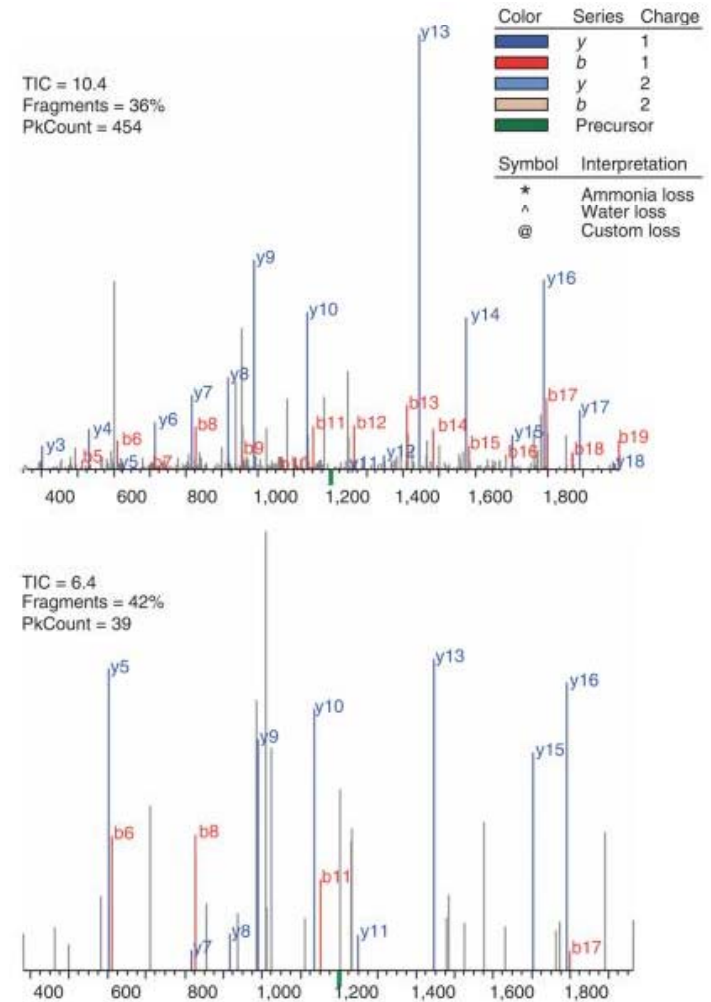
?

F I A T N P D T H G R

Evaluation of spectrum quality based on signal intensity

1. Evaluate spectrum quality- intensity of the signal:

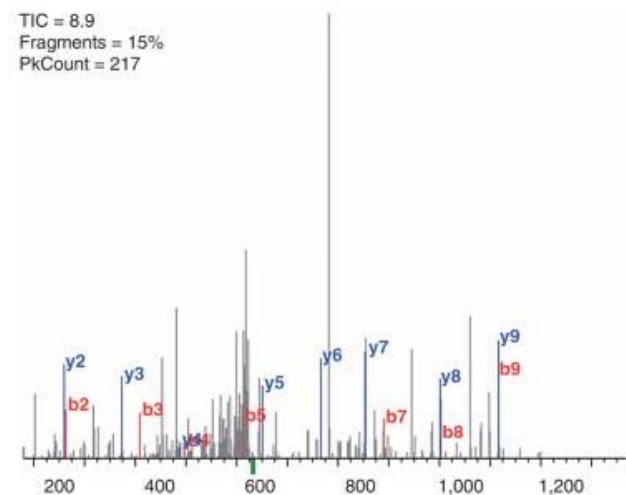
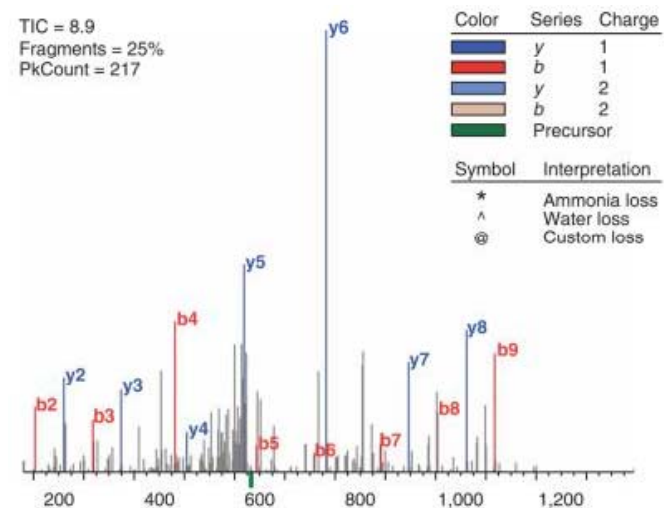
- Total ion current for the MS/MS spectrum
- Number of reported ions
- Difference in peak intensities
- Precursor total ion current







Evaluate spectrum quality > unexplained peaks

Are there multiple intense unexplained peaks?
What proportion of the intensity is explained by this sequence

Unexplained peaks can come from “contaminating” peptides.

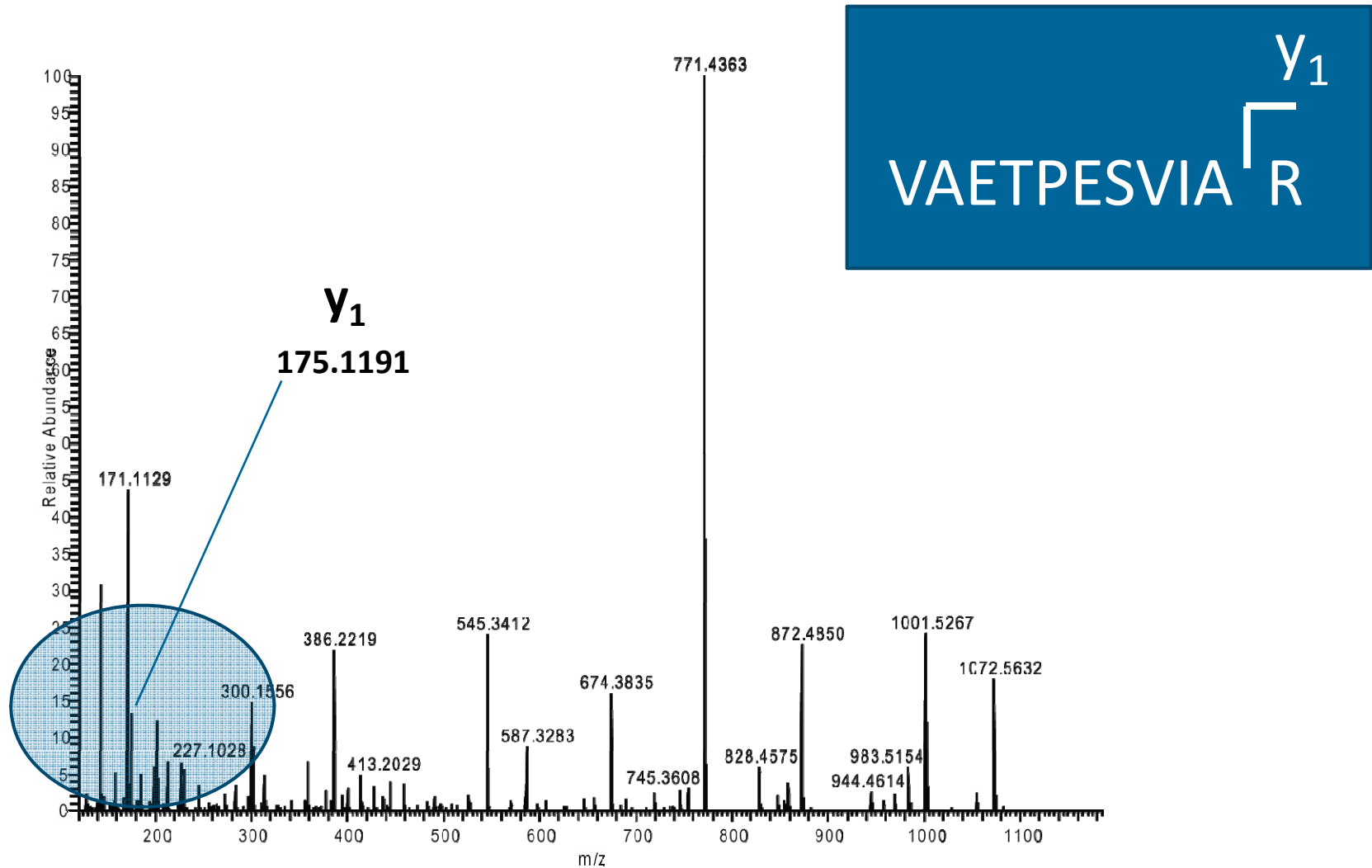


Recognizing b and y ions > identifying the amino acid mass differences

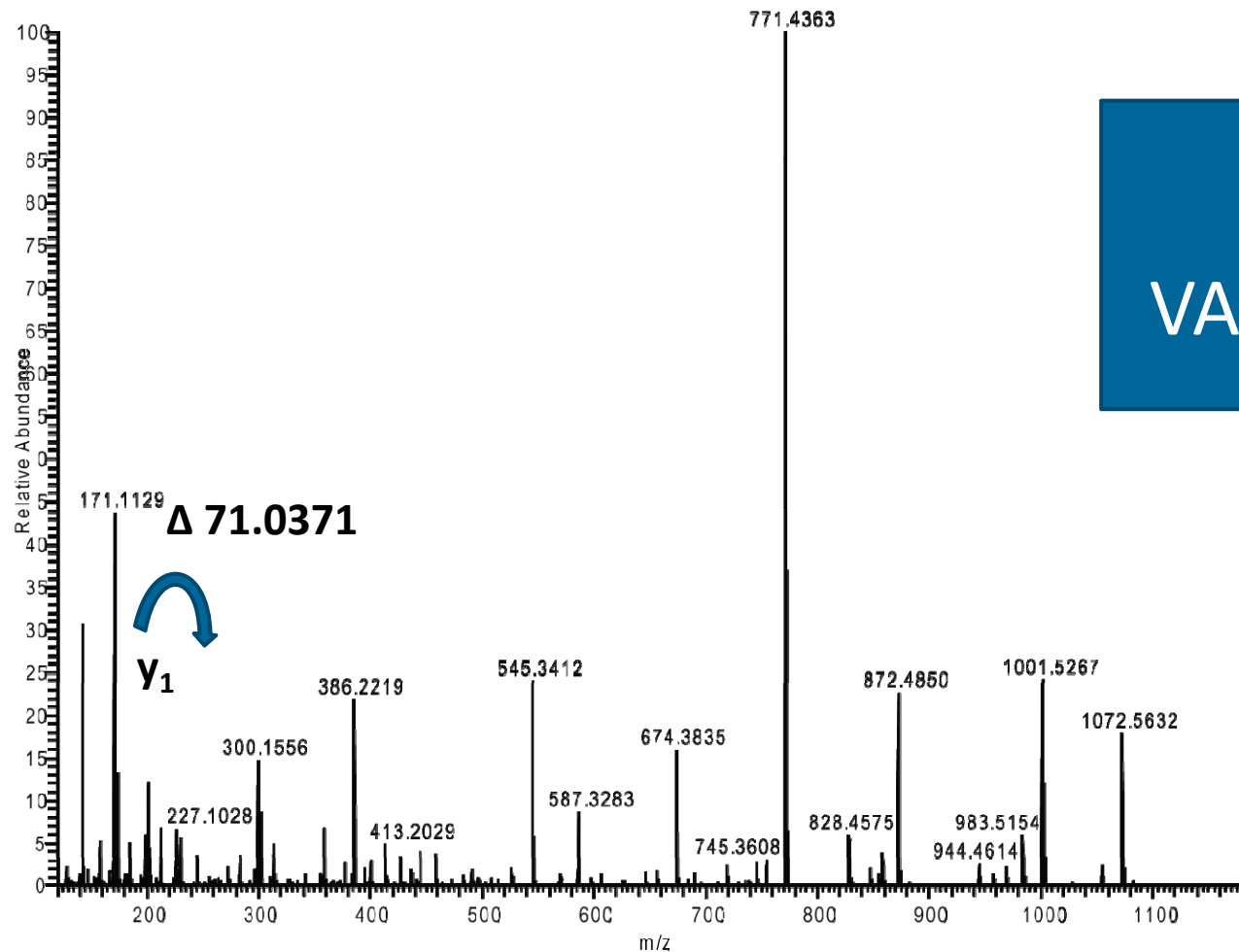
symbol	composition	structure	monoisotopic	average
Ala A	Alanine C ₃ H ₅ NO	$\begin{array}{c} \text{CH}_3 \\ \\ \text{-NH-CH-CO-} \end{array}$	71.03711	71.0788
Arg R	Arginine C ₆ H ₁₂ N ₄ O	$\begin{array}{c} \text{CH}_2\text{-(CH}_2\text{)}_2\text{-NH-C-NH}_2 \\ \\ \text{-NH-CH-CO-} \end{array}$	156.10111	156.1876
Asn N	Asparagine C ₄ H ₆ N ₂ O ₂	$\begin{array}{c} \text{CH}_2\text{-CONH}_2 \\ \\ \text{-NH-CH-CO-} \end{array}$	114.04293	114.1039
Asp D	Aspartic Acid C ₄ H ₅ NO ₃	$\begin{array}{c} \text{CH}_2\text{-COOH} \\ \\ \text{-NH-CH-CO-} \end{array}$	115.02694	115.0886
Cys C	Cysteine C ₃ H ₅ NOS	$\begin{array}{c} \text{CH}_2\text{-SH} \\ \\ \text{-NH-CH-CO-} \end{array}$	103.00919	103.1448
Gln Q	Glutamine C ₅ H ₈ N ₂ O ₂	$\begin{array}{c} \text{CH}_2\text{-CH}_2\text{-CONH}_2 \\ \\ \text{-NH-CH-CO-} \end{array}$	128.05858	128.1308
Glu E	Glutamic Acid C ₅ H ₇ NO ₃	$\begin{array}{c} \text{CH}_2\text{-CH}_2\text{-COOH} \\ \\ \text{-NH-CH-CO-} \end{array}$	129.04259	129.1155
Gly G	Glycine C ₂ H ₃ NO	$\text{-NH-CH}_2\text{-CO-}$	57.02146	57.0520
His H	Histidine C ₆ H ₇ N ₃ O		137.05891	137.1412
Ile I	Isoleucine C ₆ H ₁₁ NO	$\begin{array}{c} \text{CH(CH}_3\text{)-CH}_2\text{-CH}_3 \\ \\ \text{-NH-CH-CO-} \end{array}$	113.08406	113.1595
Leu L	Leucine C ₆ H ₁₁ NO	$\begin{array}{c} \text{CH}_2\text{-CH(CH}_3\text{)}_2 \\ \\ \text{-NH-CH-CO-} \end{array}$	113.08406	113.1595
Lys K	Lysine C ₆ H ₁₂ N ₂ O	$\begin{array}{c} \text{CH}_2\text{-(CH}_2\text{)}_3\text{-NH}_2 \\ \\ \text{-NH-CH-CO-} \end{array}$	128.09496	128.1742
Met M	Methionine C ₃ H ₉ NOS	$\begin{array}{c} \text{CH}_2\text{-CH}_2\text{-S-CH}_3 \\ \\ \text{-NH-CH-CO-} \end{array}$	131.04049	131.1986
Phe F	Phenylalanine C ₉ H ₉ NO	$\begin{array}{c} \text{CH}_2\text{-Ph} \\ \\ \text{-NH-CH-CO-} \end{array}$	147.06841	147.1766
Pro P	Proline C ₅ H ₇ NO		97.05276	97.1167
Ser S	Serine C ₃ H ₅ NO ₂	$\begin{array}{c} \text{CH}_2\text{-OH} \\ \\ \text{-NH-CH-CO-} \end{array}$	87.03203	87.0782
Thr T	Threonine C ₄ H ₇ NO ₂	$\begin{array}{c} \text{CH(OH)-CH}_3 \\ \\ \text{-NH-CH-CO-} \end{array}$	101.04768	101.1051
Trp W	Tryptophan C ₁₁ H ₁₀ N ₂ O		186.07931	186.2133
Tyr Y	Tyrosine C ₉ H ₉ NO ₂		163.06333	163.1760
Val V	Valine C ₅ H ₉ NO	$\begin{array}{c} \text{CH(CH}_3\text{)}_2 \\ \\ \text{-NH-CH-CO-} \end{array}$	99.06841	99.1326

To calculate peptide mass- add the mass of the terminal atoms.

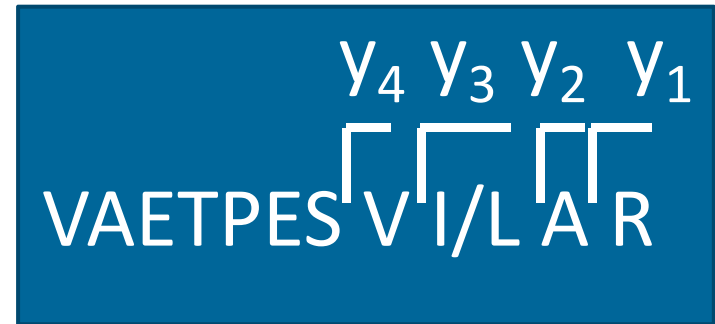
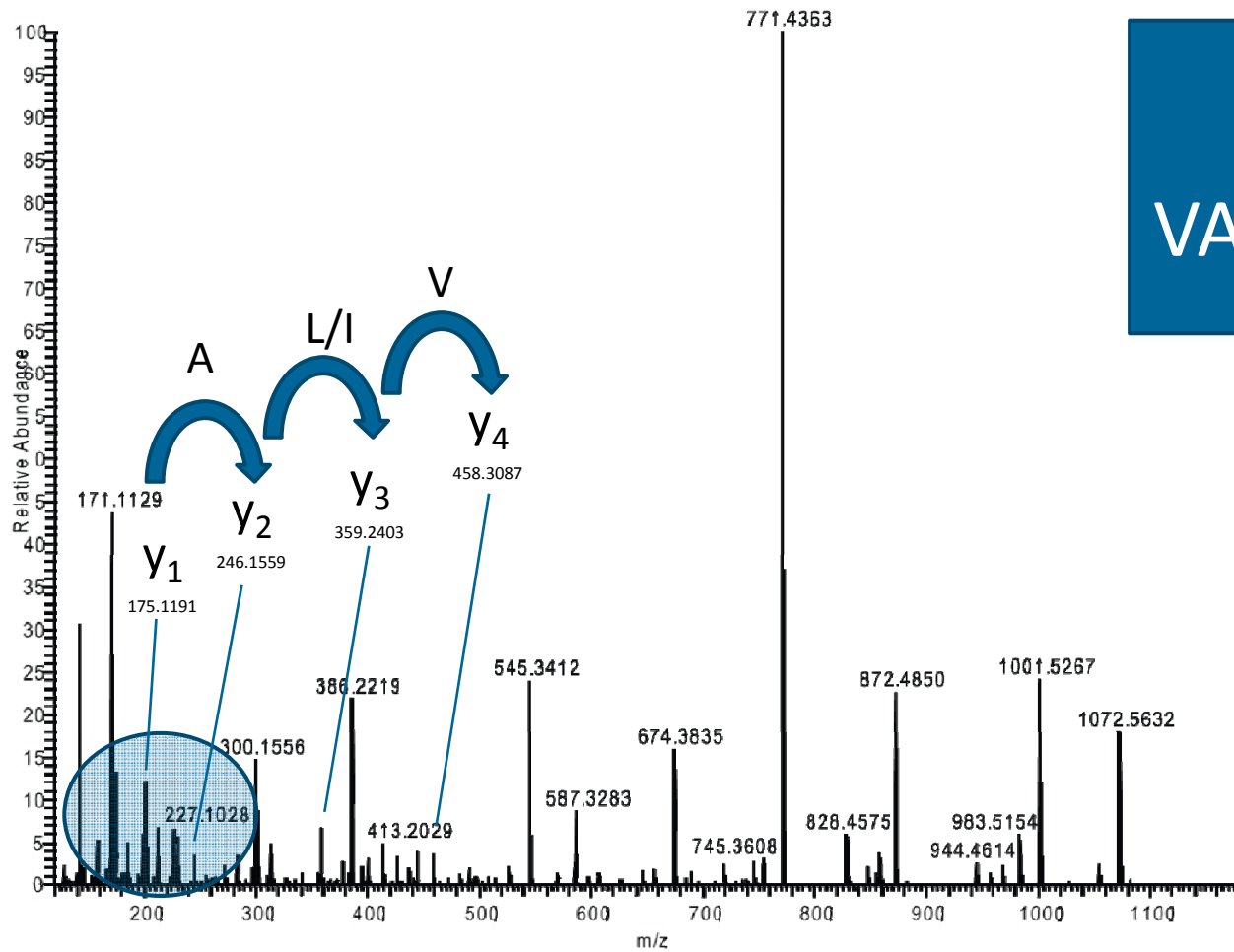
Recognizing b and y ions >y₁ reporter ion



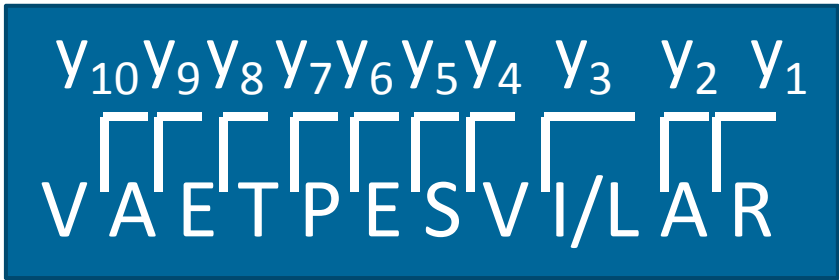
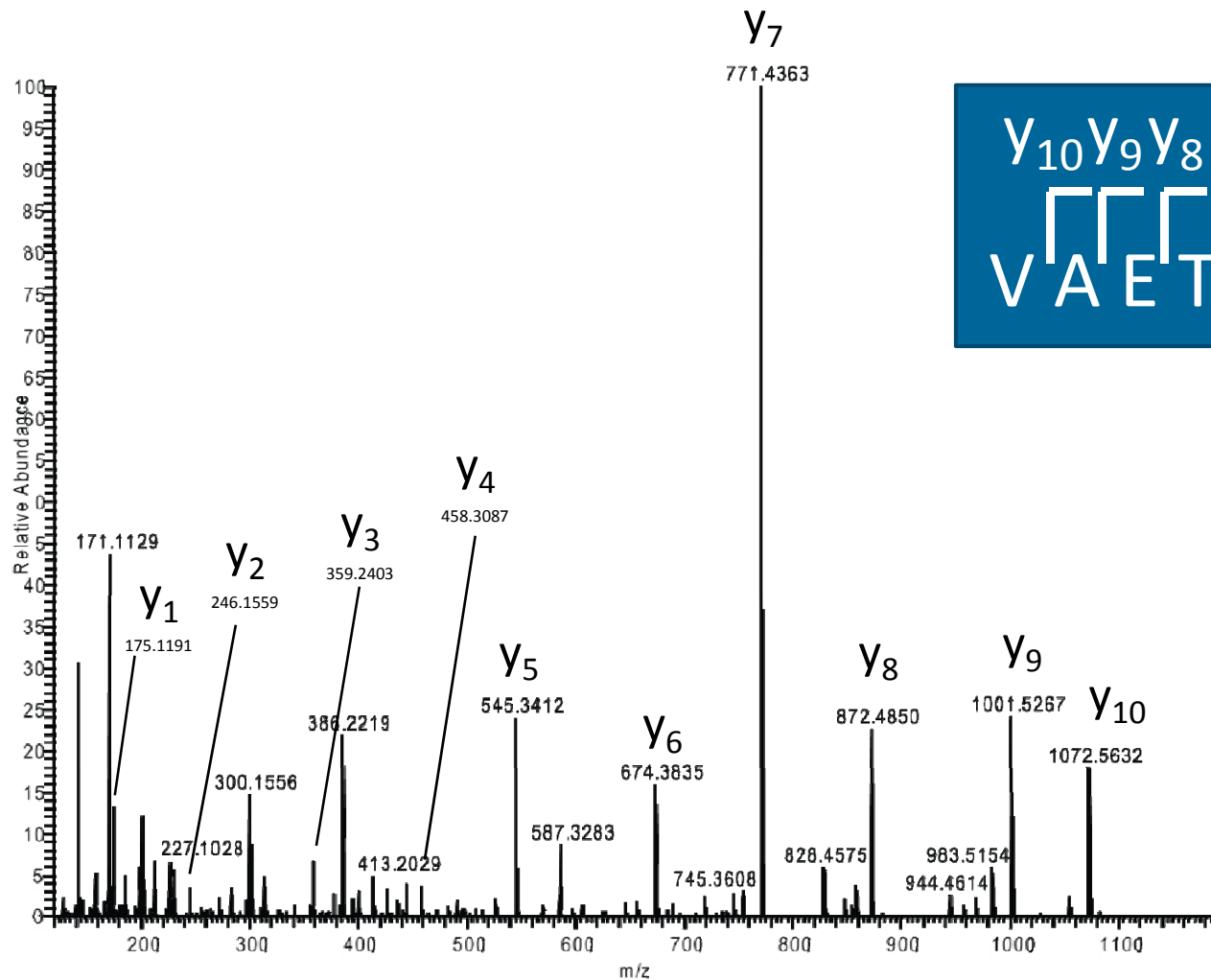
Recognizing b and y ions $>y_1, y_2$



Recognizing b and y ions > y_1, y_2, y_3, y_4

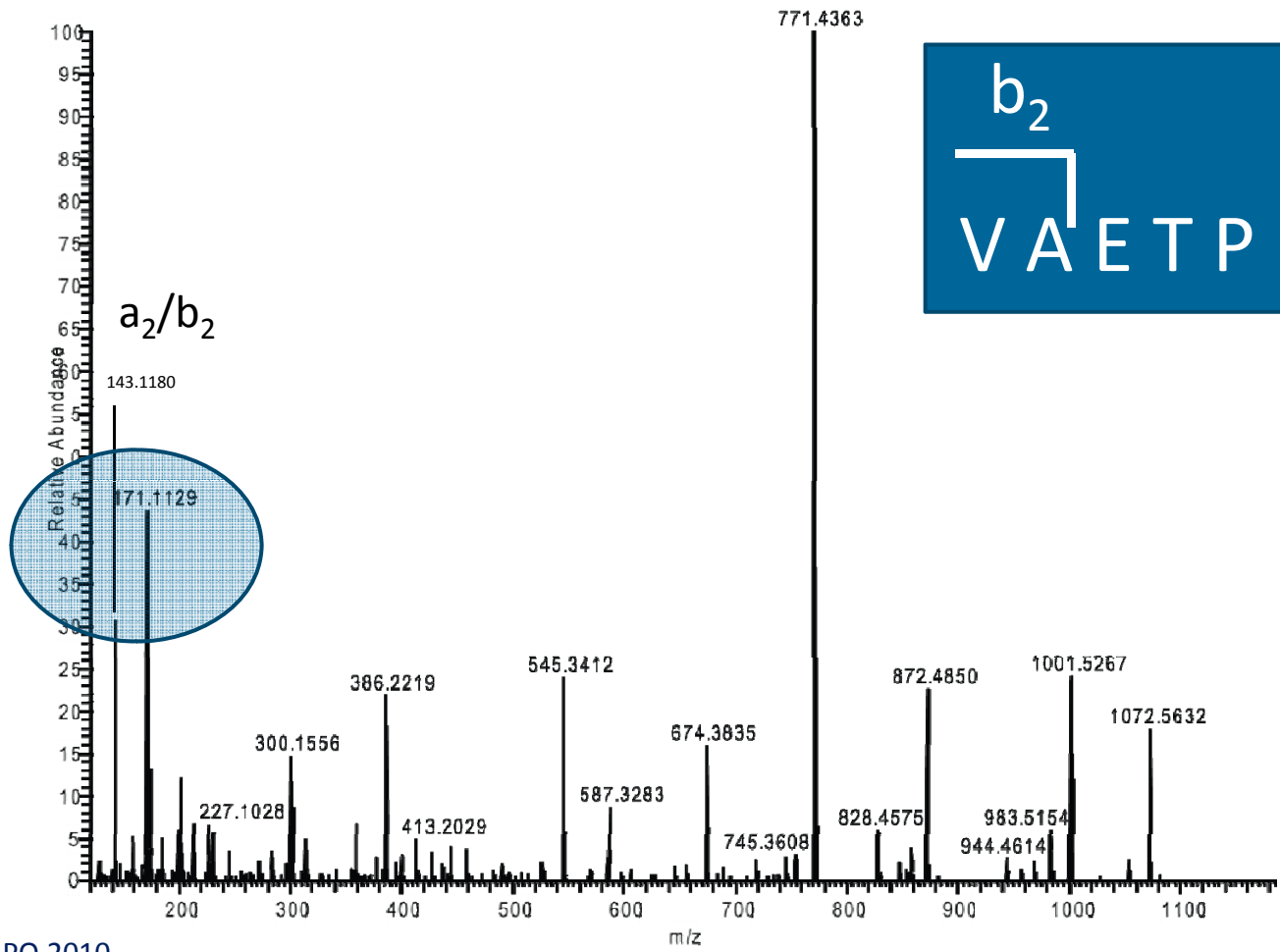


Recognizing b and y ions >The complete y series



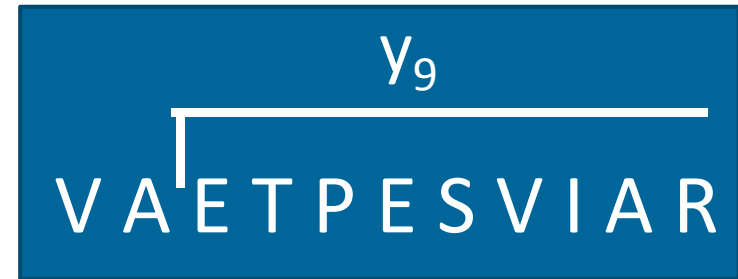
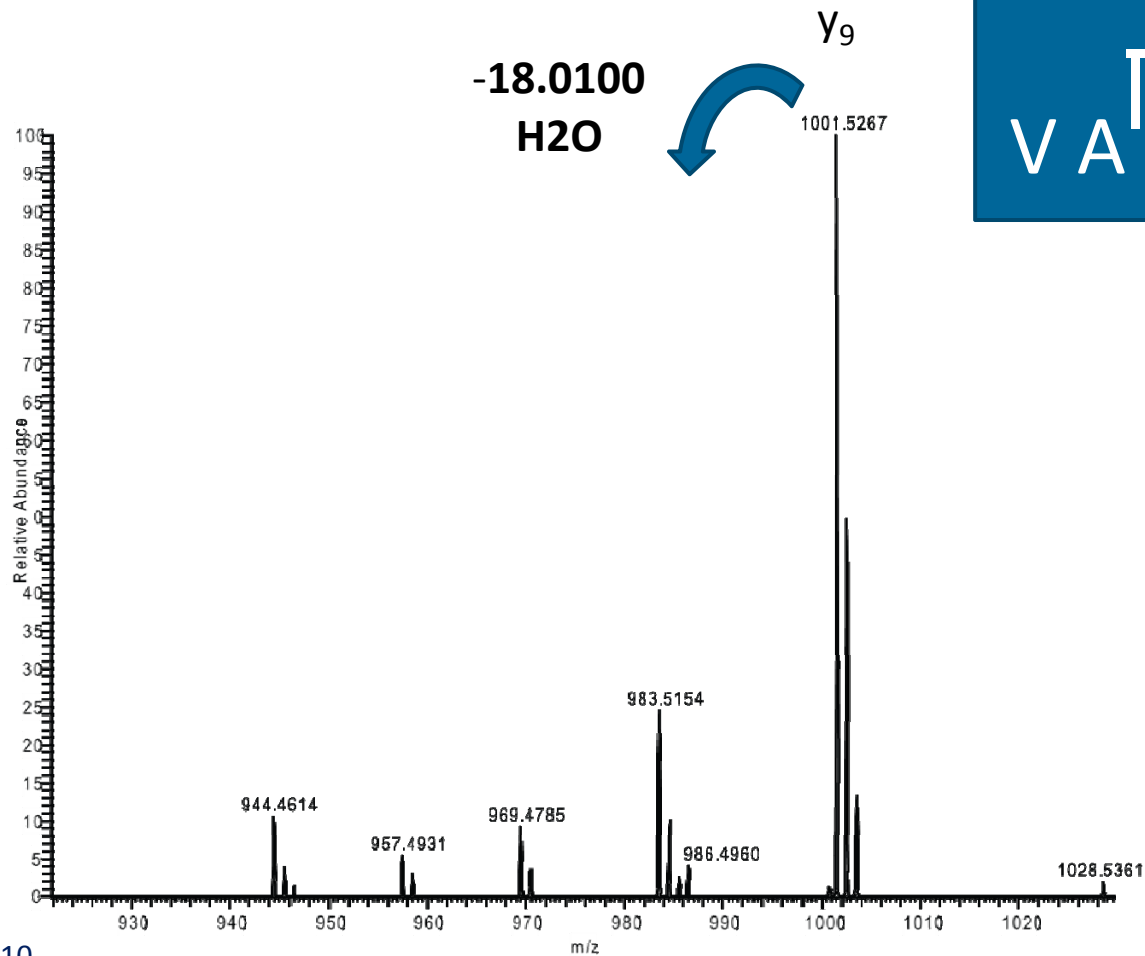
Recognizing b and y ions >a₂/b₂ pair

a₂ ion results from a neutral loss of carbon monoxide from b₂ ion > loss of 28 Da




Recognizing neutral loss fragments > water loss

H₂O loss @ Glu, Asp, Ser, Thr

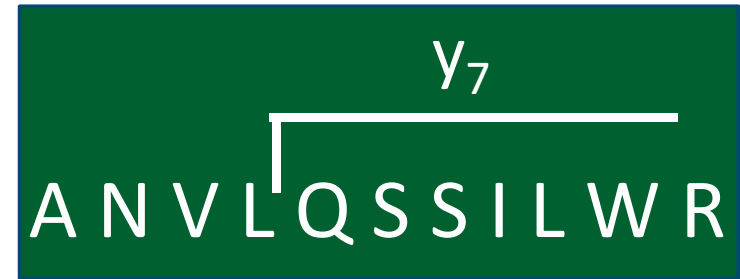
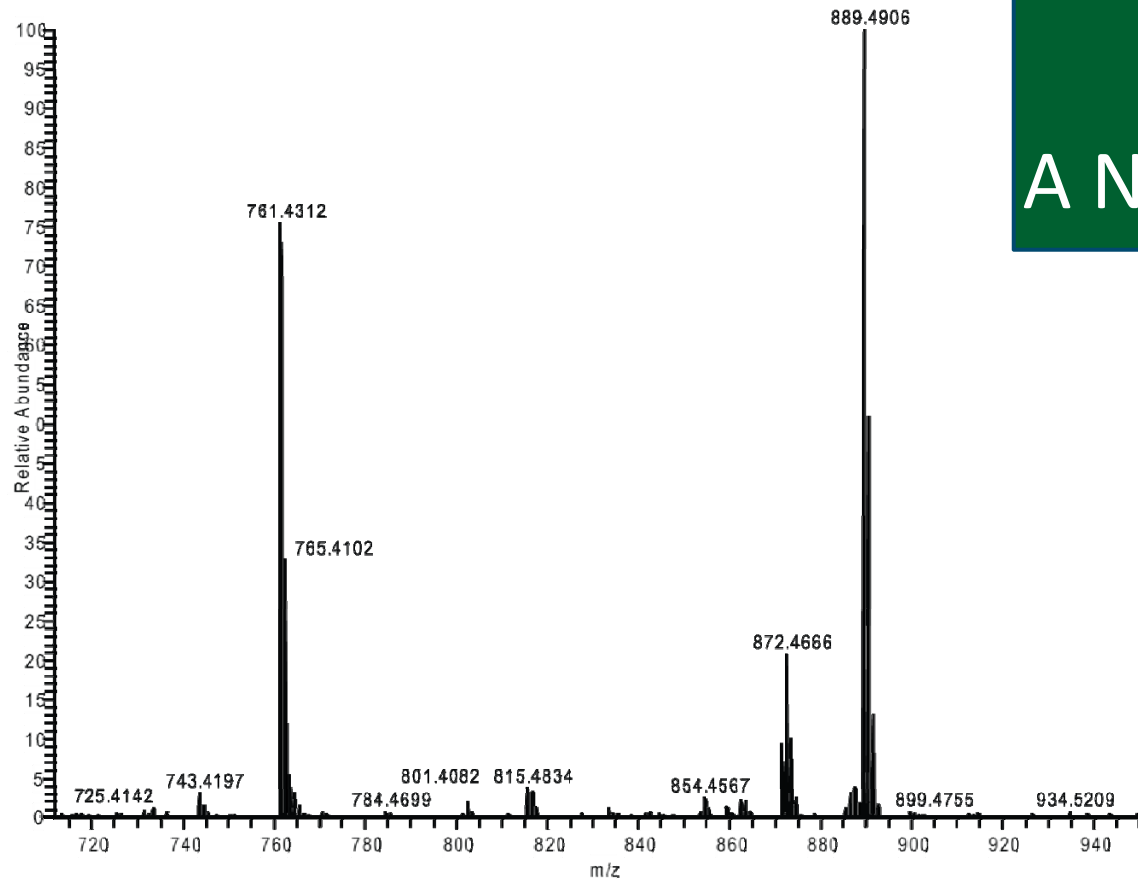


Recognizing neutral loss fragments > ammonia loss

NH₃ loss @ Asn, Gln

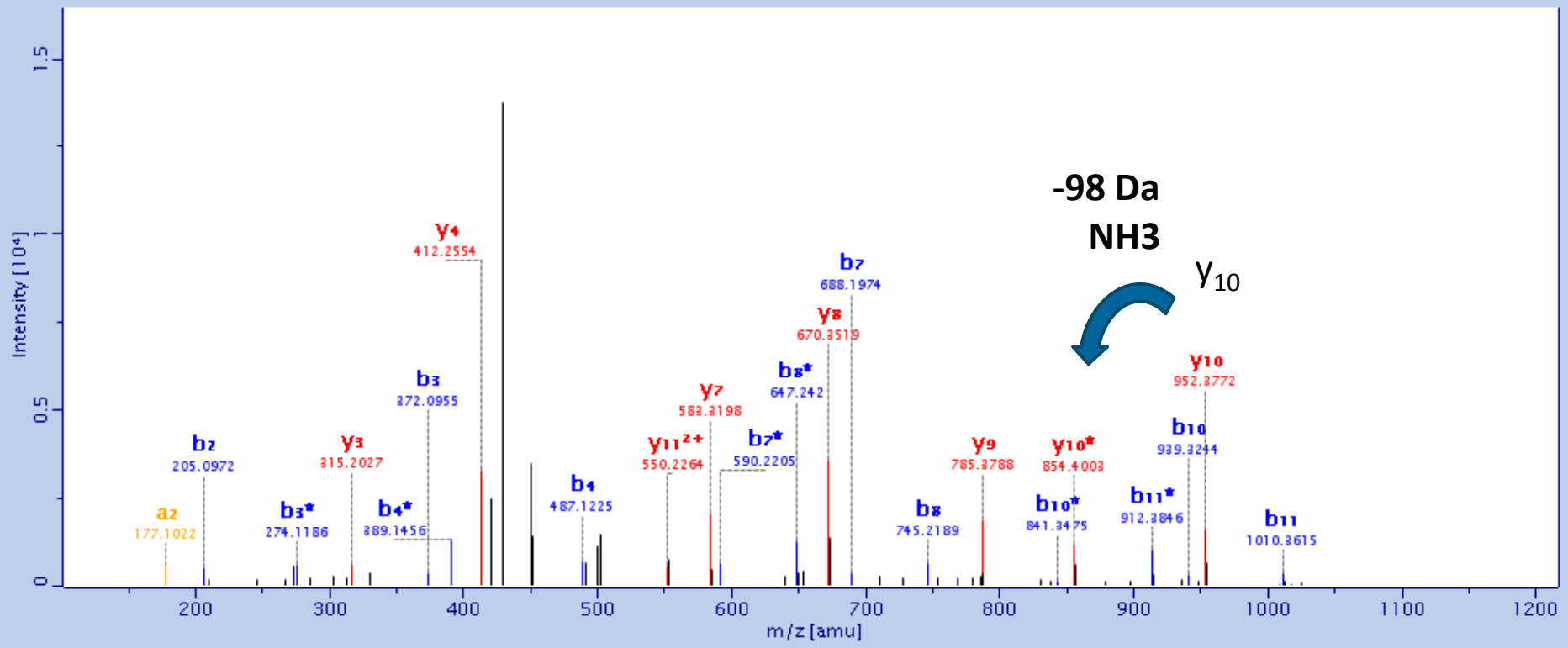
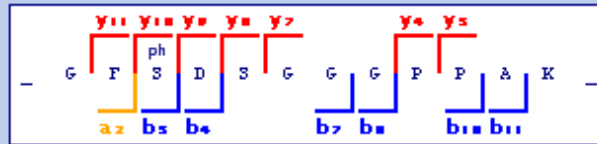
-17.0260
NH₃ 

Y₇



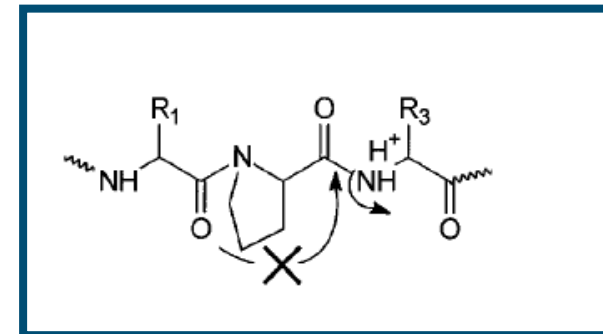
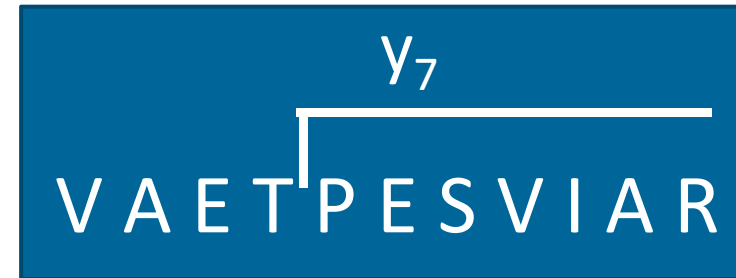
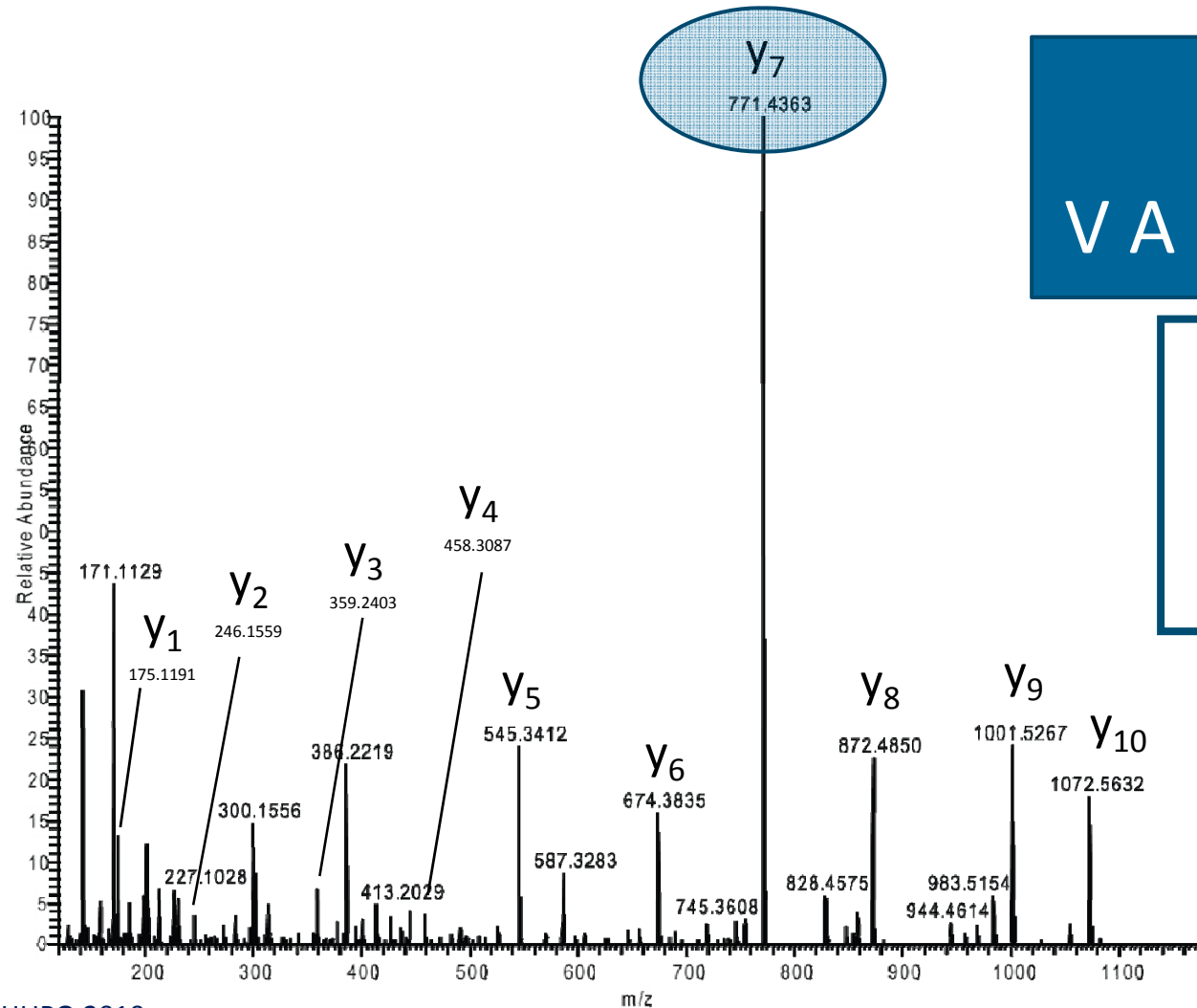
Recognizing neutral loss fragments > phosphate

H_3PO_4 @ pSer and pThr (98 Da) or HPO_3 @ pTyr and pThr (80 Da)



Sequence effects > the proline effect

Proline effect- intense fragment ions N-terminal of Pro

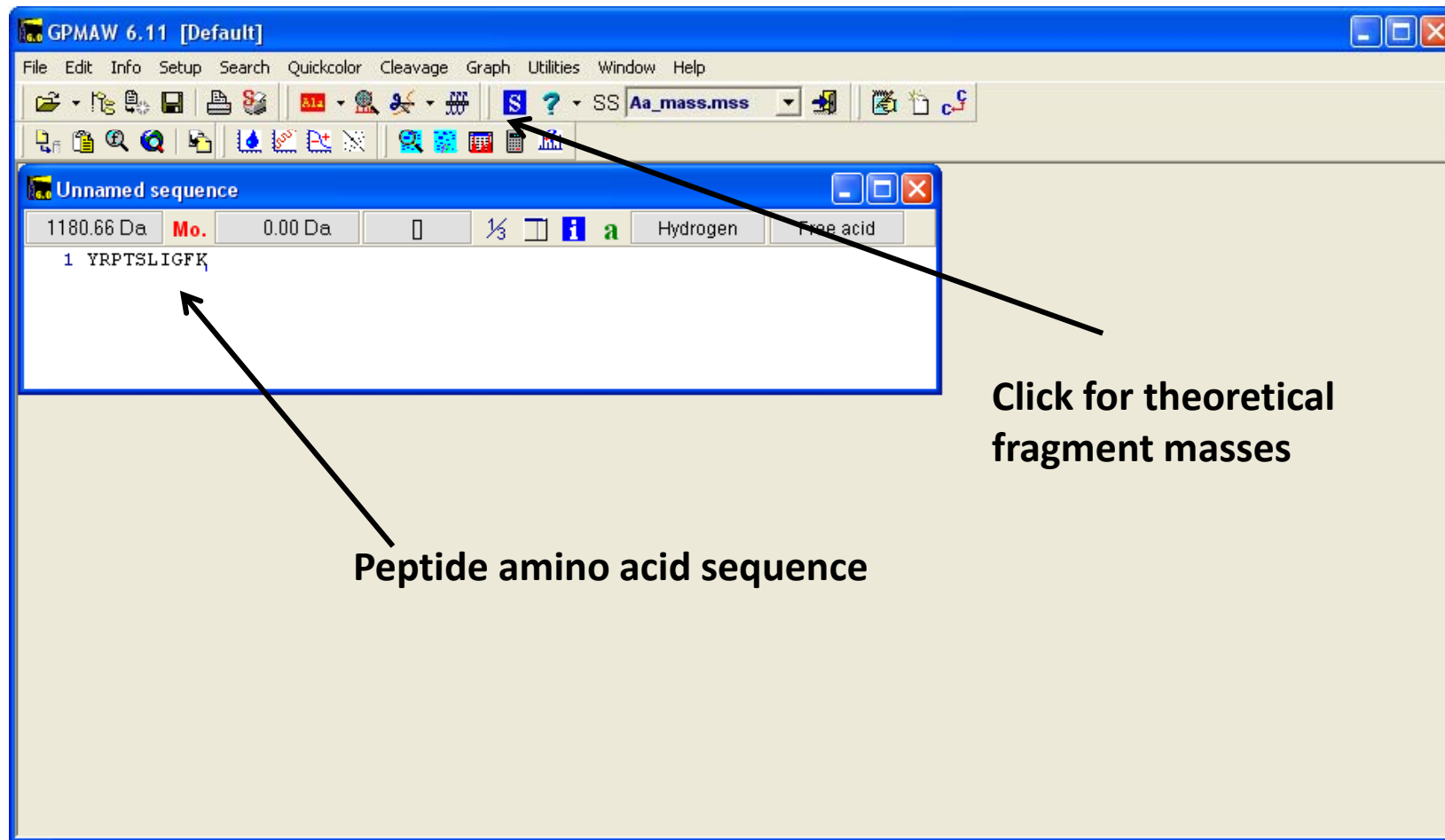


Sequence effects > fragmentation preferences

- **Proline** effect- intense fragment ions N-terminal of **Pro**.
- Side chain of **Asp** can cleave the peptide bond to its C-terminal side.
- Branched hydrophobic side chains **Val, Ile, Leu** favor fragmentation on their C-terminal side.
- **Gly** and **Ser** favor fragmentation to their N-terminal side.
- Peptide bond **Asn-Gly** is very labile.
- **His** side chain can attack its own C-terminal side to have the b-ion.

Increased number of modifications does not always improve peptide identification. Fragment identification should be related to the likelihood of the reaction and intensity of the fragments.

Tools for validation of MS/MS spectra > GPMMAW



Tools for validation of MS/MS spectra > GPMMAW

GPMAW 6.11 [Default]

File Edit Setup Search Utilities Window Help

SS Aa_mass.mss

Unnamed sequence

MS/MS fragmentation - C56 H88 N14 O14

YRPTSLIGFK MH+ 1181.6677 Mo S MS 2 N: Hydrogen - C: Free acid

Backbone fragments			Fragment losses			Internal fragments			
a	b	c''	x	y''	z				
136.076	164.071	183.112	1 Tyr 10	-	-				
292.177	320.172	339.213	2 Arg 9	1042.570	1018.605			999.564	
389.230	417.225	436.266	3 Pro 8	886.469	862.504			843.463	
490.278	518.273	537.314	4 Thr 7	789.416	765.451			746.410	
577.310	605.305	624.346	5 Ser 6	688.368	664.403			645.362	
690.394	718.389	737.430	6 Leu 5	601.336	577.371			558.330	
803.478	831.473	850.514	7 Ile 4	488.252	464.287			445.246	
860.499	888.494	907.535	8 Gly 3	375.168	351.203			332.162	
1007.568	1035.563	1054.604	9 Phe 2	318.146	294.182			275.141	
-	-	-	10 Lys 1	171.078	147.113			128.072	
- Ion charge state: 2									
68.542	82.539	92.060	1 Tyr 10	-	-			-	
146.592	160.590	170.110	2 Arg 9	521.788	509.806			500.286	
195.119	209.116	218.637	3 Pro 8	443.738	431.756			422.235	
245.643	259.640	269.161	4 Thr 7	395.212	383.229			373.709	
289.159	303.156	312.677	5 Ser 6	344.688	332.705			323.185	
345.701	359.698	369.219	6 Leu 5	301.172	289.189			279.669	
402.243	416.240	425.761	7 Ile 4	244.630	232.647			223.127	
430.753	444.751	454.271	8 Gly 3	188.088	176.105			166.585	
504.288	518.285	527.806	9 Phe 2	159.577	147.595			138.074	
-	-	-	10 Lys 1	86.043	74.060			64.540	
- Related immonium ions:									
74.06[T]	60.04[S]	70.07[P]	30.03[G]	86.10[I]	86.10[L]	136.08[Y]	120.08[F]	101.11[K]	129.11[R]

Immonium ions for HCD

Singly charged

Doubly charged

Summary>interpretation of MS/MS spectra

- Evaluate spectrum quality.
- Identify low Mw reporter ions.
- Recognize b and y ions.
- Identify neutral losses.
- Recognize sequence effects.
- Ion trap data- y-ions have double the intensity of b-ions; low mass cutoff.
- Quadrupole data- under-representation of b-ions
- Peptide bonds in the middle of the peptide cleave more readily than nearer to the terminus

Acknowledgments

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Annette Michalski

Gabi Stoehr

NagarjunaNagaraj



MPI for Biochemistry

THANK YOU FOR YOUR ATTENTION

