Peptide ion fragmentation in mass spectrometry

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6-4681

Where we are so far

- We've discussed the nature of the problem, how we might attack it and what we believe in
- Matt Renfrow has told you
  - how (remarkably) we get peptide and protein molecular ions into gas phase
  - the importance of isotopes in mass spectrometry
  - how we measure the *m/z* values of the ions
- We also talked about:
  - how to measure the molecular weight of a protein
  - How to fragment a protein into smaller pieces to get a peptide mass fingerprint and hence "identify" it

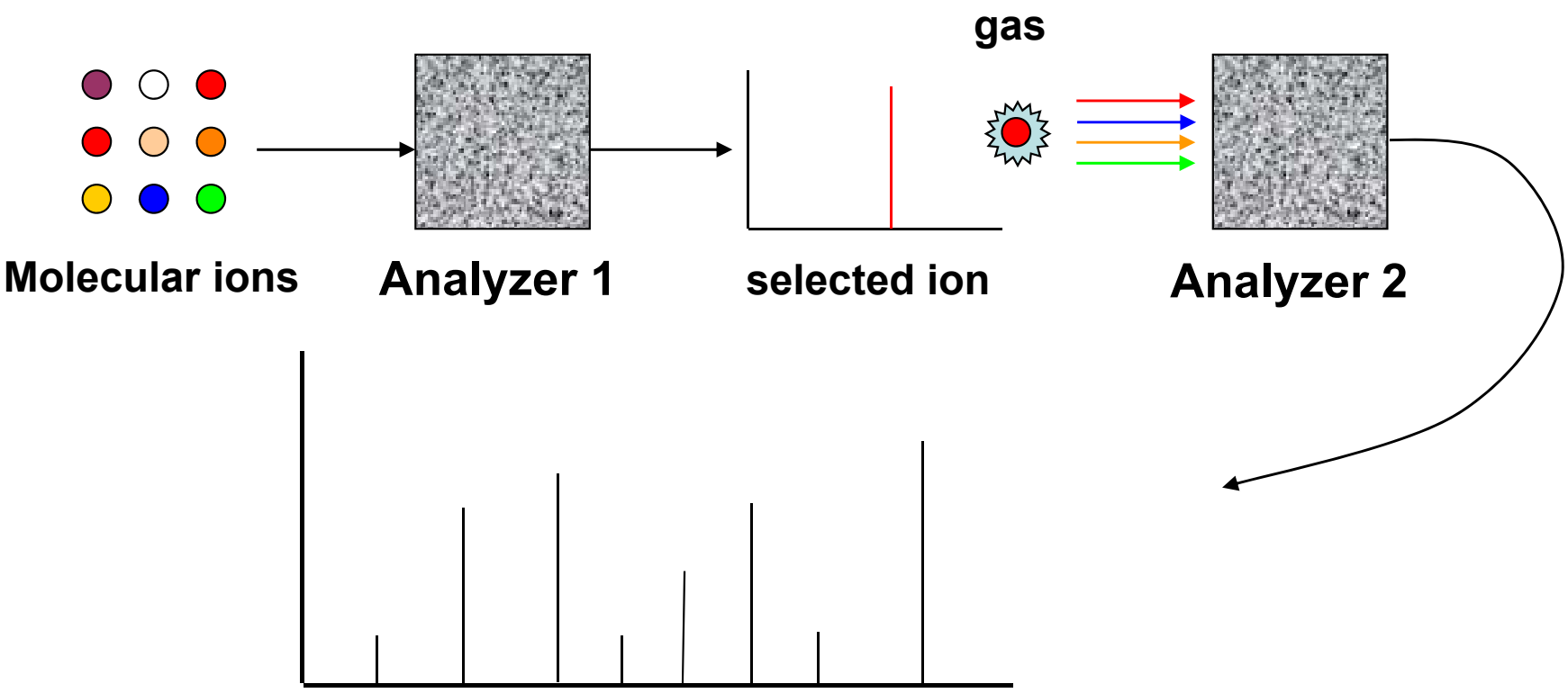
Lecture goals

- Value of fragmentation in determining structure
- How peptides fragment
  - Interpreting the tandem mass spectrum
- Automating identification of peptides from their fragment ions
  - pros and cons
- Controlling fragmentation
  - Choice of ionization and fragmentation methods

Why ion fragmentation provides useful information

- **Compounds can have the same empirical formula, i.e., the same molecular weight or *m/z*, but be different chemically.**
- **Breaking them into parts (fragmenting them) helps to identify what they are.**
- **Each of the following peptides gives rise to exactly the same *m/z* for the $[M+2H]^{2+}$ ion**
  - **NH_2VFAQHLK-COOH NH_2VAFQHLK-COOH**
  - **NH_2VFQHALK-COOH NH_2VHLAFQK-COOH**
- **In proteomics we want to distinguish these peptides**

What is MS-MS (tandem mass spectrometry)?



Collision-induced dissociation

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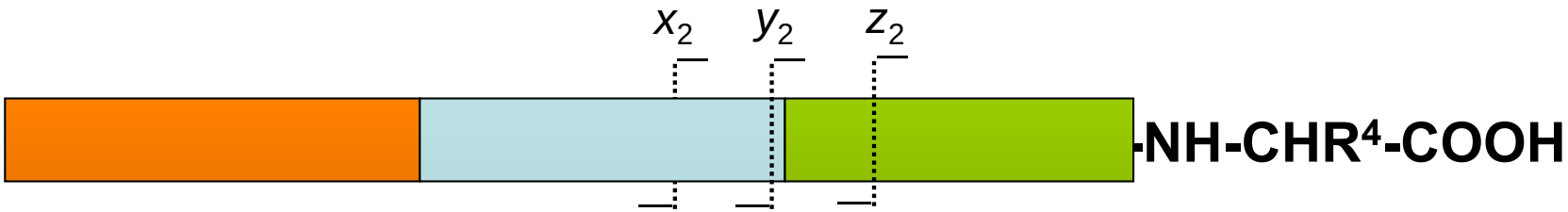
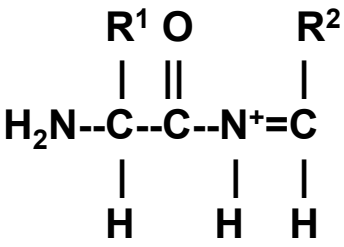
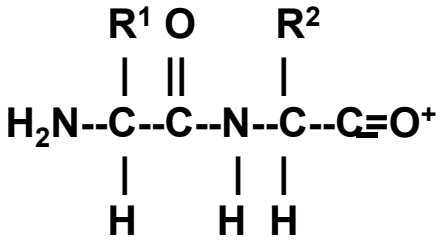
Fragmenting a peptide

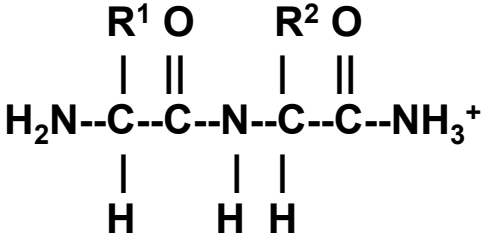




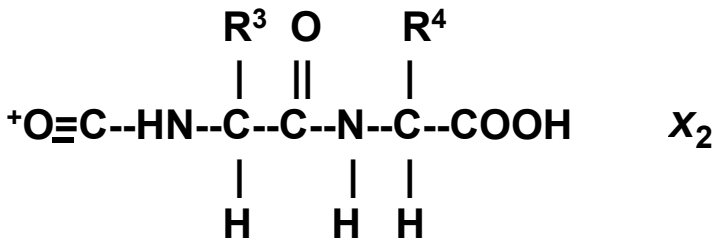
a_2



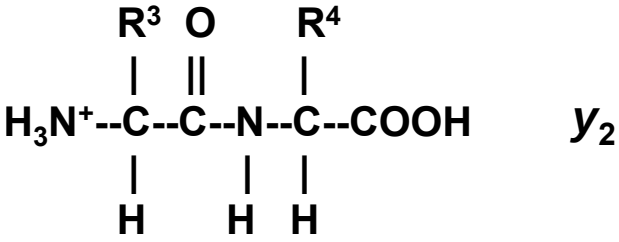
b_2



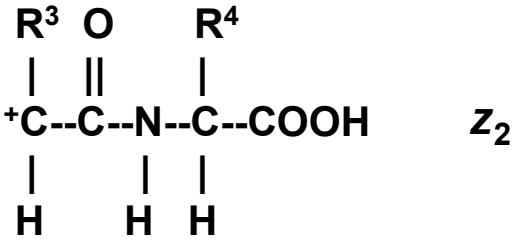
c_2



x_2



y_2



z_2

Adapted from http://www.matrixscience.com/help/fragmentation_help.html

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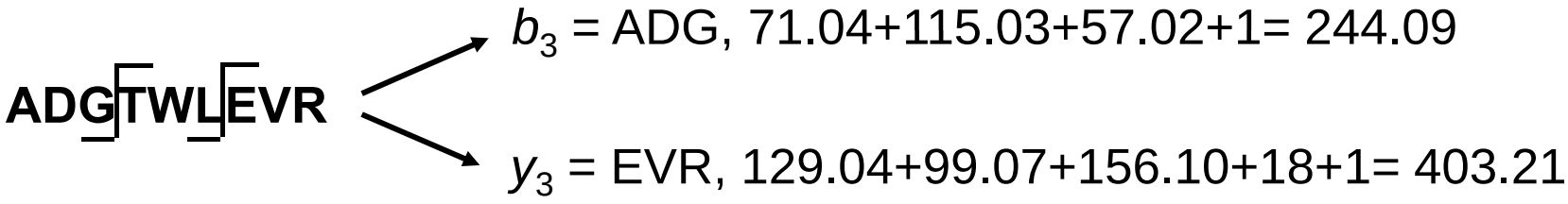
Calculating expected *b*- and *y*-ion fragments

Alanine	71.037	Leucine	113.084
Arginine	156.101	Lysine	128.094
Asparagine	114.043	Methionine	131.040
Aspartic acid	115.027	Phenylalanine	147.068
Cysteine	103.009	Proline	97.053
Glutamic acid	129.043	Serine	87.032
Glutamine	128.058	Threonine	101.048
Glycine	57.021	Tryptophan	186.079
Histidine	137.059	Tyrosine	163.063
Isoleucine	113.084	Valine	99.068

b_n = [residue masses + 1] - these come from the N-terminus
y_n = [residue masses + H_2O + 1] = these come from the C-terminus



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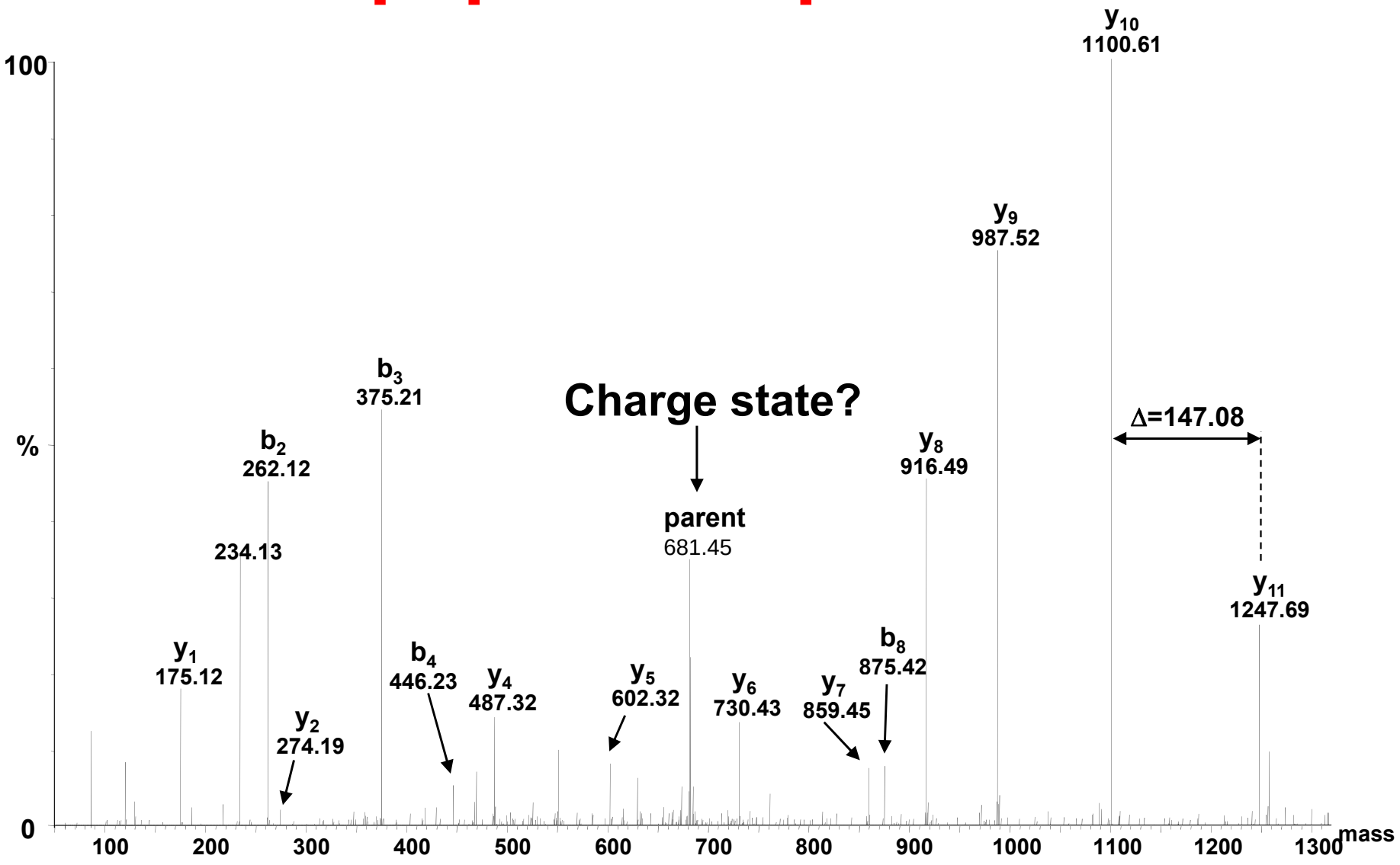
Identification of daughter ions and peptide sequence



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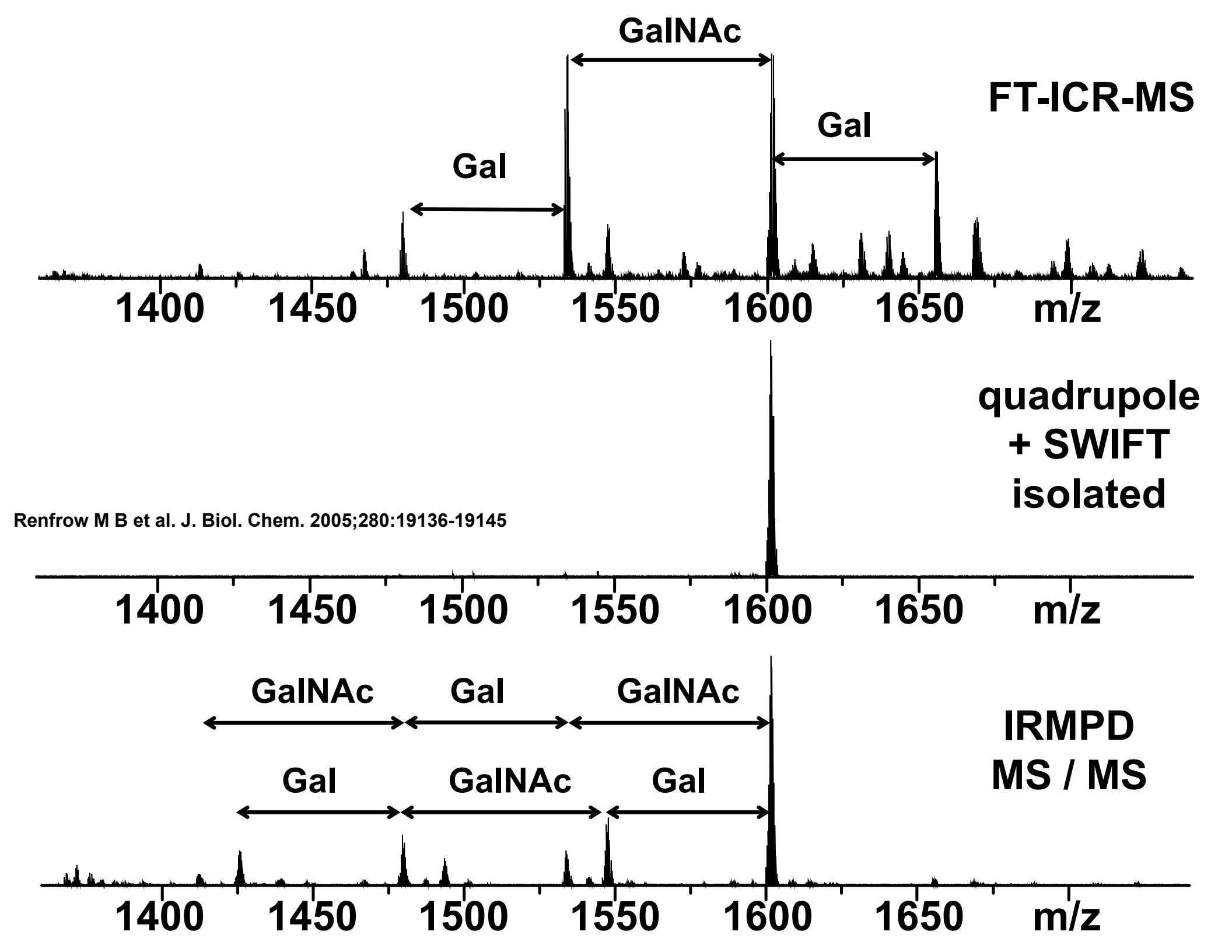
What's in a peptide MSMS spectrum?

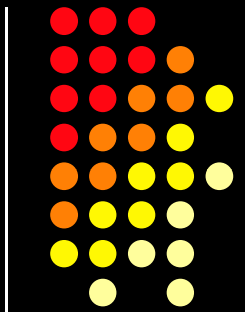
- In most cases, some, but rarely all, of the theoretic *b*- and *y*-ions are observed
- Besides *b*- and *y*-ions, other types of fragmentation can occur to form a_n and x_n ions, as well as also losing CO, NH_3 and H_2O groups
- Internal cleavage reactions can occur at acidic (Asp - Glu) residue sites

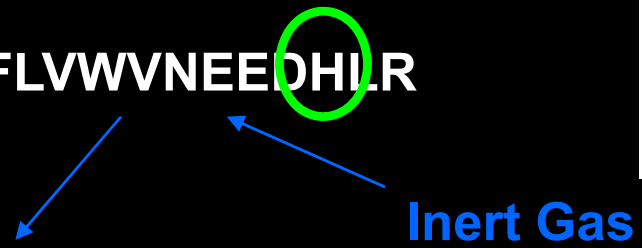

GalNAc
Gal
Gal
FT-ICR-MS
1400
1450
1500
1550
1600
1650
m/z
quadrupole
+ SWIFT
isolated
Renfrow M B et al. J. Biol. Chem. 2005;280:19136-19145
1400
1450
1500
1550
1600
1650
m/z
GalNAc
Gal
GalNAc
Gal
GalNAc
Gal
IRMPD
MS / MS
1400
1450
1500
1550
1600
1650
m/z

Identifying a peptide by de novo sequencing

- **Take the partial sequence that can be identified manually and submit it to PROWL (http://prowl.rockefeller.edu/) - click on PROTEINFO and enter sequence - select all species**
- **Use suggested sequences to fill in the gaps and then check all theoretical ions using MS-Product at http://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msproduct**

CID Peptide Fragmentation





N-terminal b ions m/z			C-terminal y ions m/z
102	T	FLVWVNEEDHLR	1556
249	TF	LVWVNEEDHLR	1409
362	TFL	VWVNEEDHLR	1296
461	TFLV	WVNEEDHLR	1197
647	TFLVW	VNEEDHLR	1911
746	TFLVWV	NEEDHLR	912
860	TFLVWVN	EEDHLR	798
989	TFLVWVNE	EDHLR	669
118	TFLVWVNEE	DHLR	540
1233	TFLVWVNEED	HLR	425
1370	TFLVWVNEEDH	LR	288
1483	TFLVWVNEEDHL	R	175

The ion's mass which would be affected by a modified **H?**

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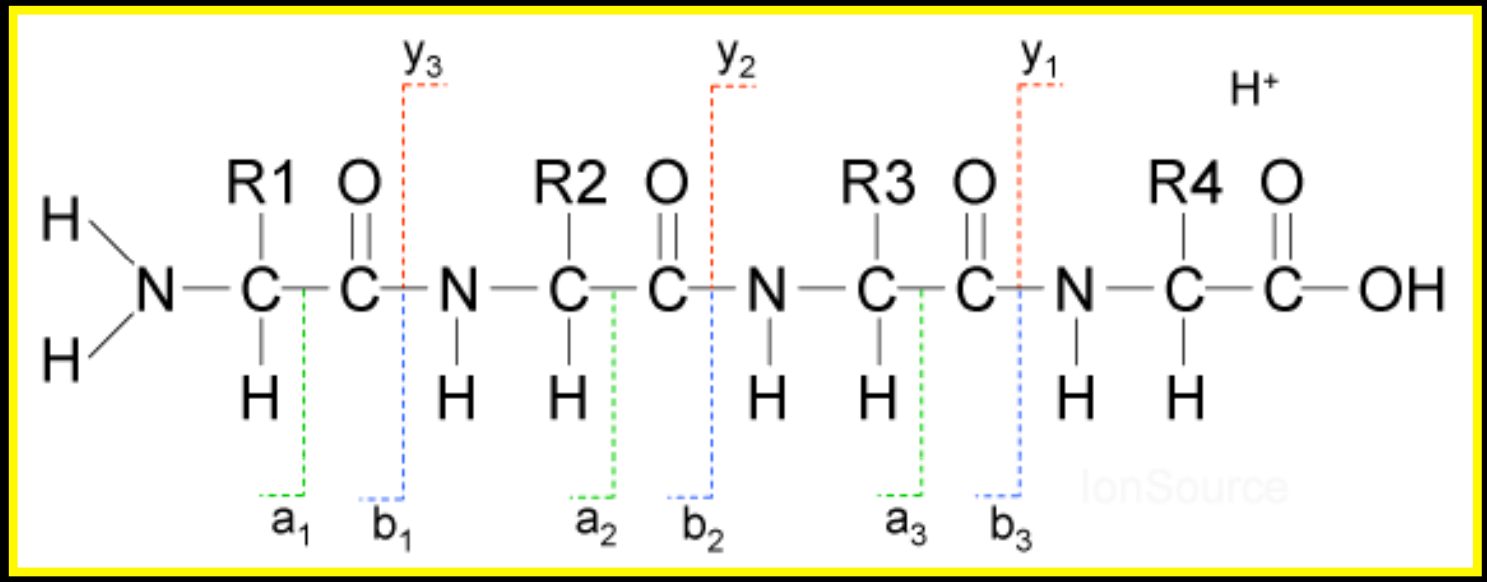
Fragmentation by CID

(Collision Induced Dissociation)



http://www.ionsource.com/tutorial/DeNovo/nomenclature.htm

The most common fragments observed with ion trap, triple quadrupole, and QTOF mass spectrometers

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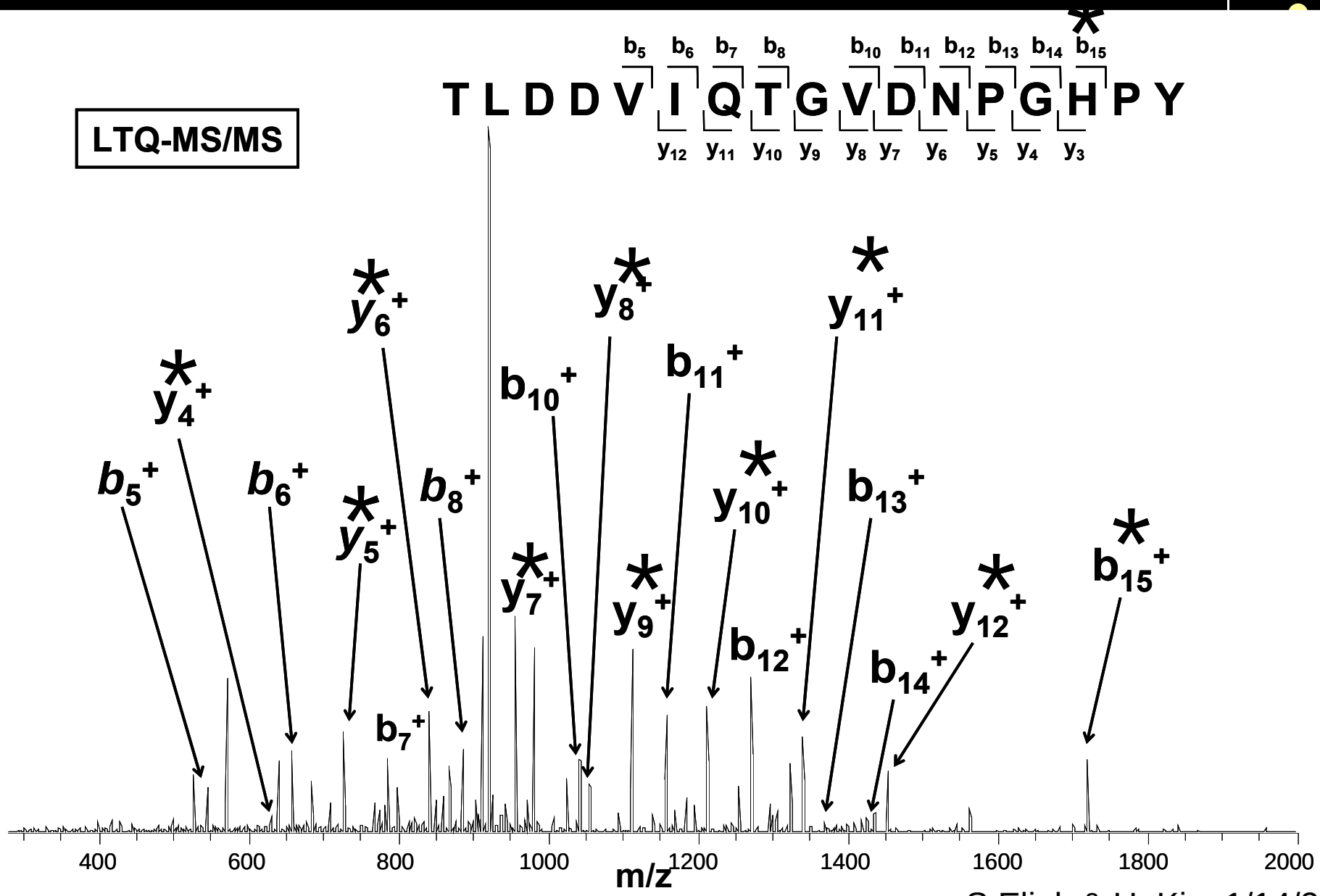
4HNE Michael Adduction of H^{66}



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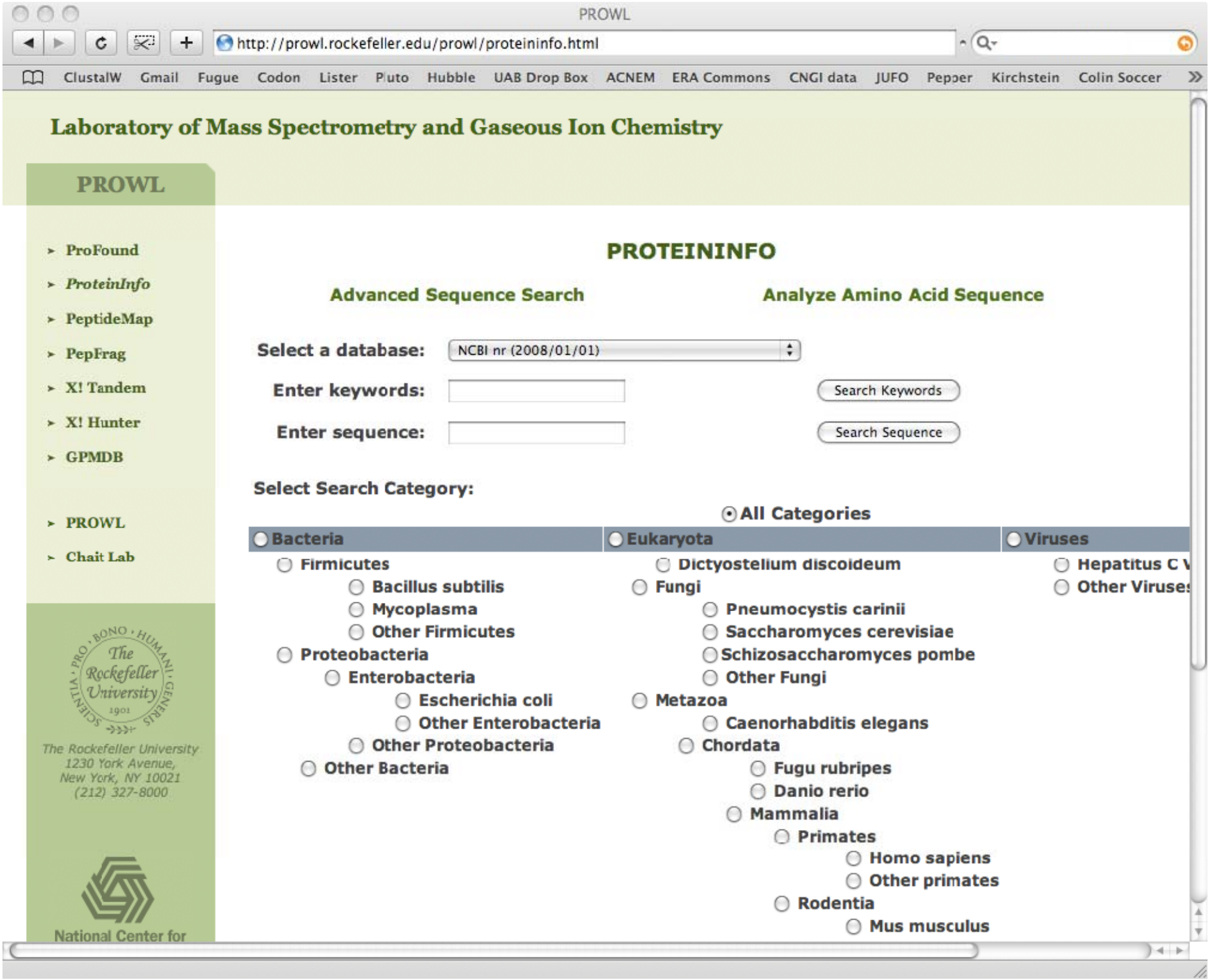
PROWL
http://prowl.rockefeller.edu/prowl/proteininfo.html
ClustalW Gmail Fugue Codon Lister Pluto Hubble UAB Drop Box ACNEM ERA Commons CNGI data JUFO Pepper Kirchstein Colin Soccer
Laboratory of Mass Spectrometry and Gaseous Ion Chemistry
PROWL
ProFound
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PeptideMap
PepFrag
X! Tandem
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Chait Lab
The Rockefeller University
1230 York Avenue,
New York, NY 10021
(212) 327-8000
National Center for
PROTEININFO
Advanced Sequence Search
Analyze Amino Acid Sequence
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Enter keywords:
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Enter sequence:
Search Sequence
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Firmicutes
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Fungi
Pneumocystis carinii
Saccharomyces cerevisiae
Schizosaccharomyces pombe
Other Fungi
Metazoa
Caenorhabditis elegans
Chordata
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Rodentia
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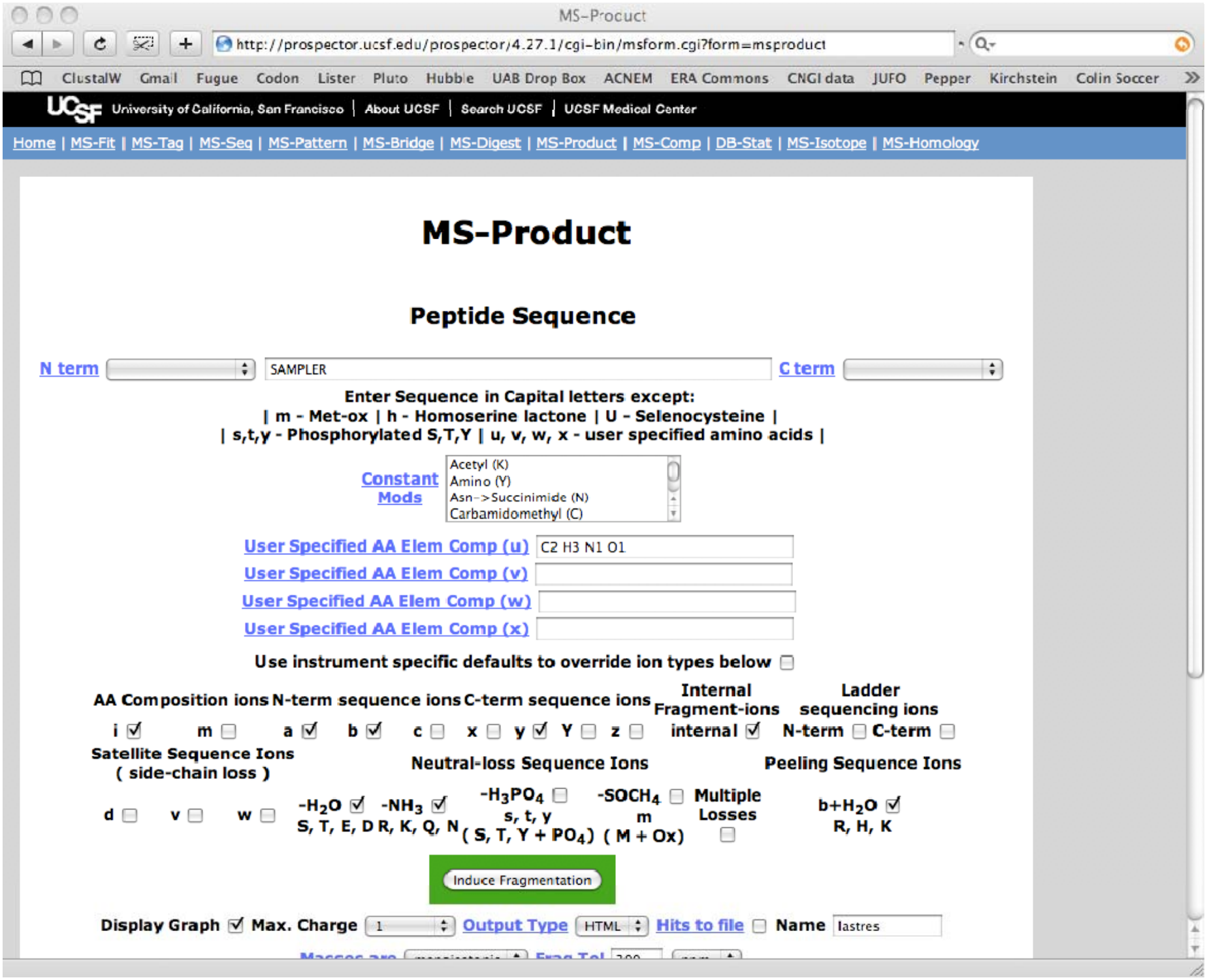
Hepatitus C V
Other Viruses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Home | MS-Fit | MS-Tag | MS-Seq | MS-Pattern | MS-Bridge | MS-Digest | MS-Product | MS-Comp | DB-Stat | MS-Isotope | MS-Homology
MS-Product
Peptide Sequence
N term
SAMPLER
C term
Enter Sequence in Capital letters except:
| m - Met-ox | h - Homoserine lactone | U - Selenocysteine |
| s,t,y - Phosphorylated S,T,Y | u, v, w, x - user specified amino acids |
Constant Mods
Acetyl (K)
Amino (Y)
Asn->Succinimide (N)
Carbamidomethyl (C)
User Specified AA Elem Comp (u)
C2 H3 N1 O1
User Specified AA Elem Comp (v)
User Specified AA Elem Comp (w)
User Specified AA Elem Comp (x)
Use instrument specific defaults to override ion types below
AA Composition ions
i m
N-term sequence ions
a b c
C-term sequence ions
x y Y z
Internal Fragment-ions
internal
Ladder sequencing ions
N-term C-term
Satellite Sequence Ions (side-chain loss)
d v w
Neutral-loss Sequence Ions
-H2O S, T, E, D
-NH3 R, K, Q, N
-H3PO4 s, t, y (S, T, Y + PO4)
-SOCH4 m (M + Ox)
Multiple Losses
Peeling Sequence Ions
b+H2O R, H, K
Induce Fragmentation
Display Graph
Max. Charge
1
Output Type
HTML
Hits to file
Name
lastres

Other ions observed in CID peptide fragmentation

Immonium and Related Ions												
	87.06	120.08	86.10	---	---	102.05	84.08 101.11 129.10	88.04	87.06	72.08	72.08	70.07 87.09 100.09 112.09
N-terminal ions												
a-NH_3 ions	---	217.10	330.18	401.22	458.24	587.28	715.38	830.40	944.45	1043.52	1142.58	---
a ions	---	234.12	347.21	418.24	475.27	604.31	732.40	847.43	961.47	1060.54	1159.61	---
b-NH_3 ions	---	245.09	358.18	429.21	486.23	615.28	743.37	858.40	972.44	1071.51	1170.58	---
b-H_2O ions	---	---	---	---	---	614.29	742.39	857.42	971.46	1070.53	1169.59	---
b ions	---	262.12	375.20	446.24	503.26	632.30	760.40	875.43	989.47	1088.54	1187.61	---
	1	2	3	4	5	6	7	8	9	10	11	12
H -	N	F	L	A	G	E	K	D	N	V	V	R
y ions	---	1247.67	1100.61	987.52	916.48	859.46	730.42	602.33	487.30	373.26	274.19	175.12
y-NH_3 ions	---	1230.65	1083.58	970.50	899.46	842.44	713.39	585.30	470.27	356.23	257.16	158.09
y-H_2O ions	---	1229.66	1082.60	969.51	898.47	841.45	712.41	584.32-	---	---	--	---

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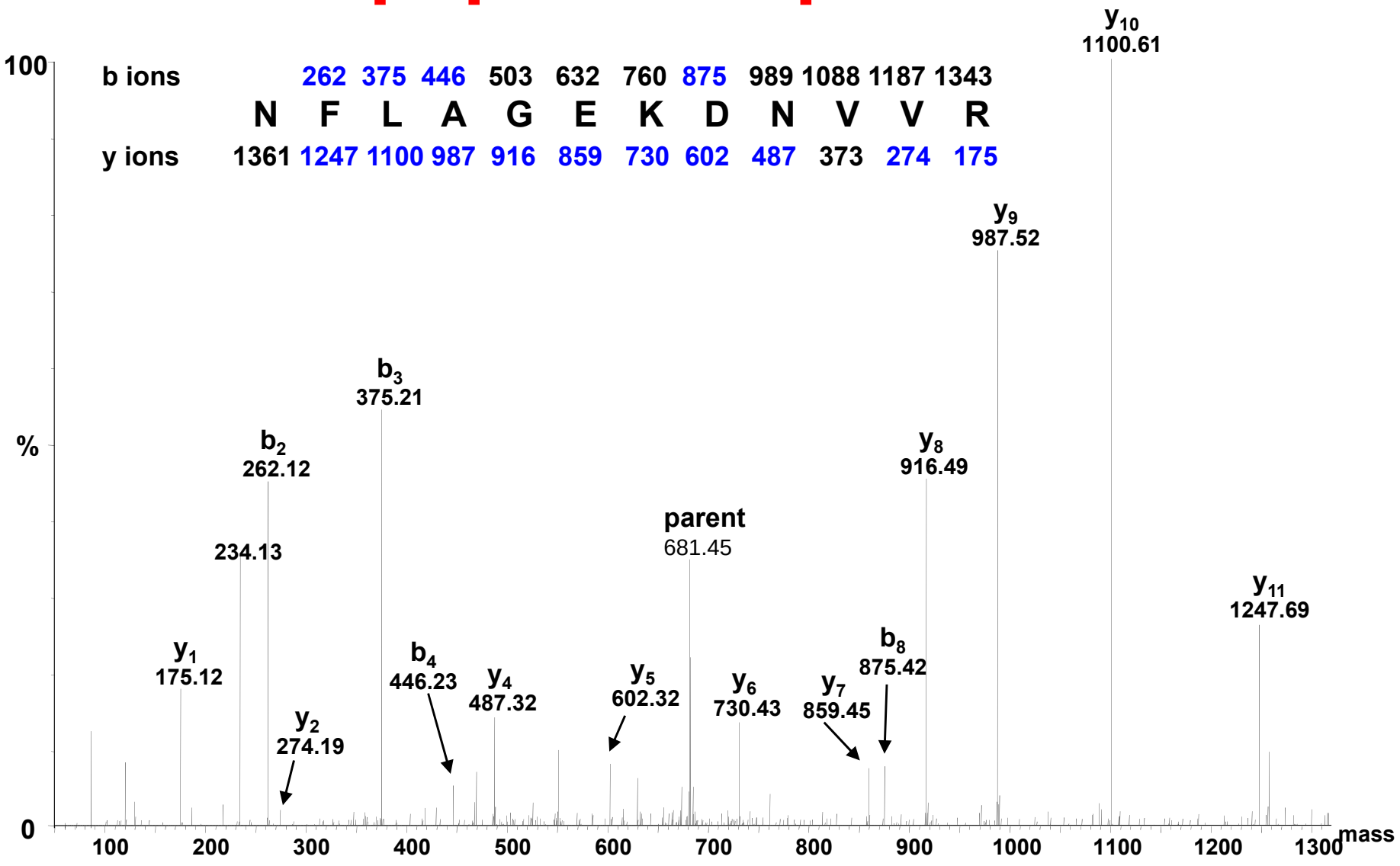
Identification of daughter ions and peptide sequence



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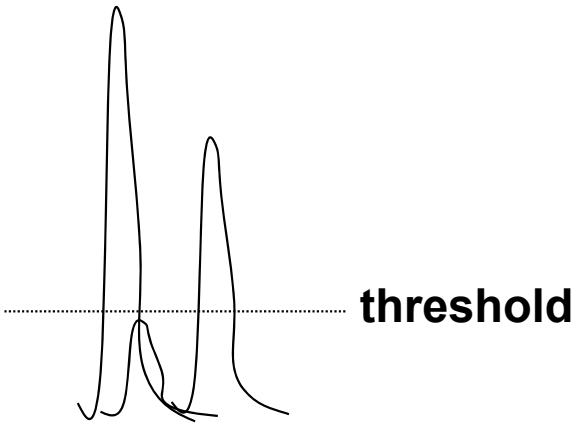
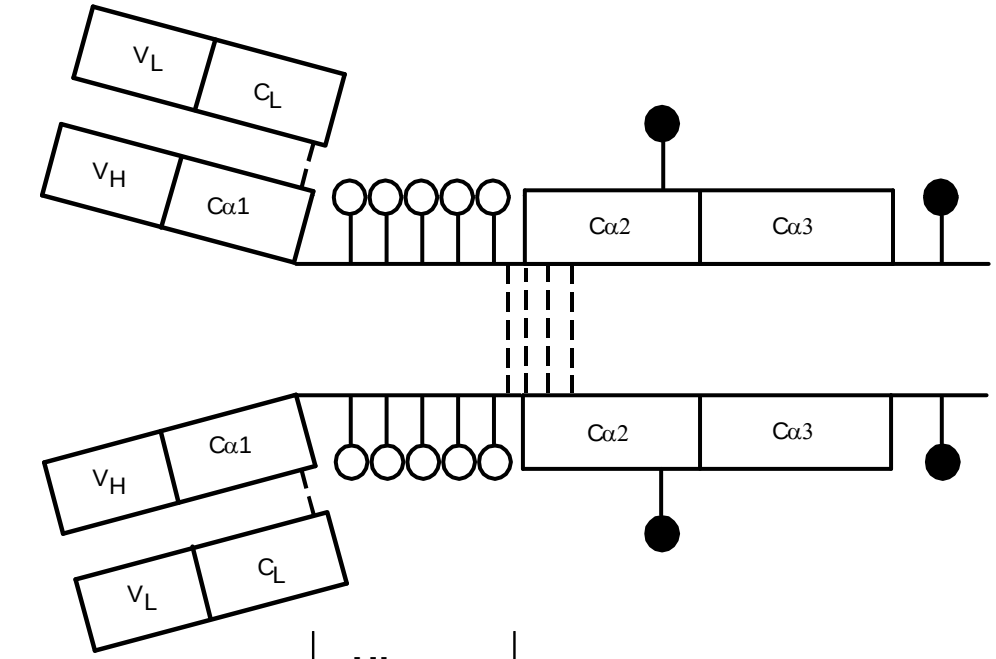
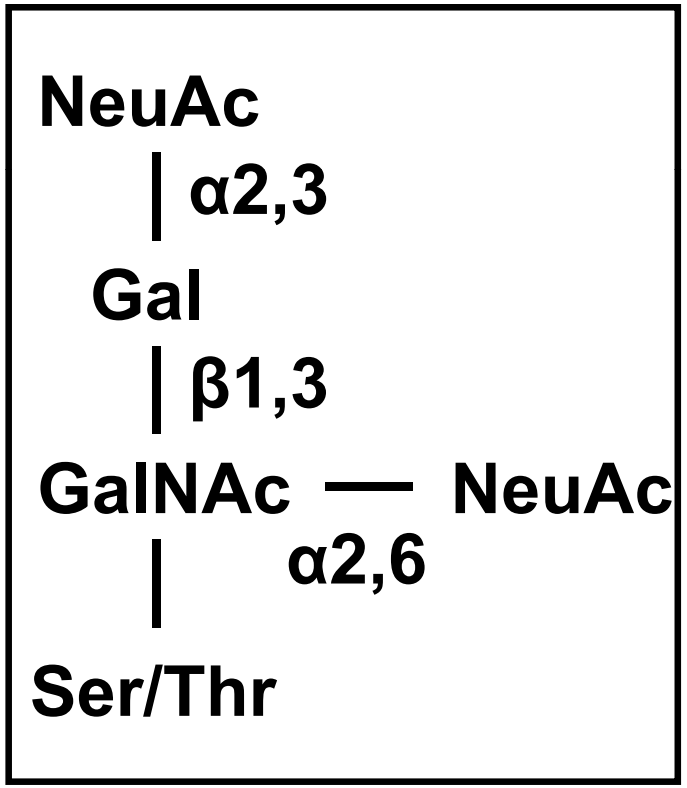
Issues in MS-MS experiment

- At any one moment, several peptides may be co-eluting
- Data-dependent operation:
  - The most intense peptide molecular ion is selected first (must exceed an initial threshold value)
  - A 2-3 Da window is used (to maximize the signal)
  - The ion must be in 2^+ or 3^+ state
  - Since the ion trap scan of the fragment ions takes ~ 1 sec, only the most intense ions will be measured
  - However, can use an exclusion list on a subsequent run to study minor ions







Trypsin

Pepsin

CHVK **HYTNPSQDVTVPCPVPSTPPTPSPSTPPTPSPSCCHPRL** SLHR

CH1

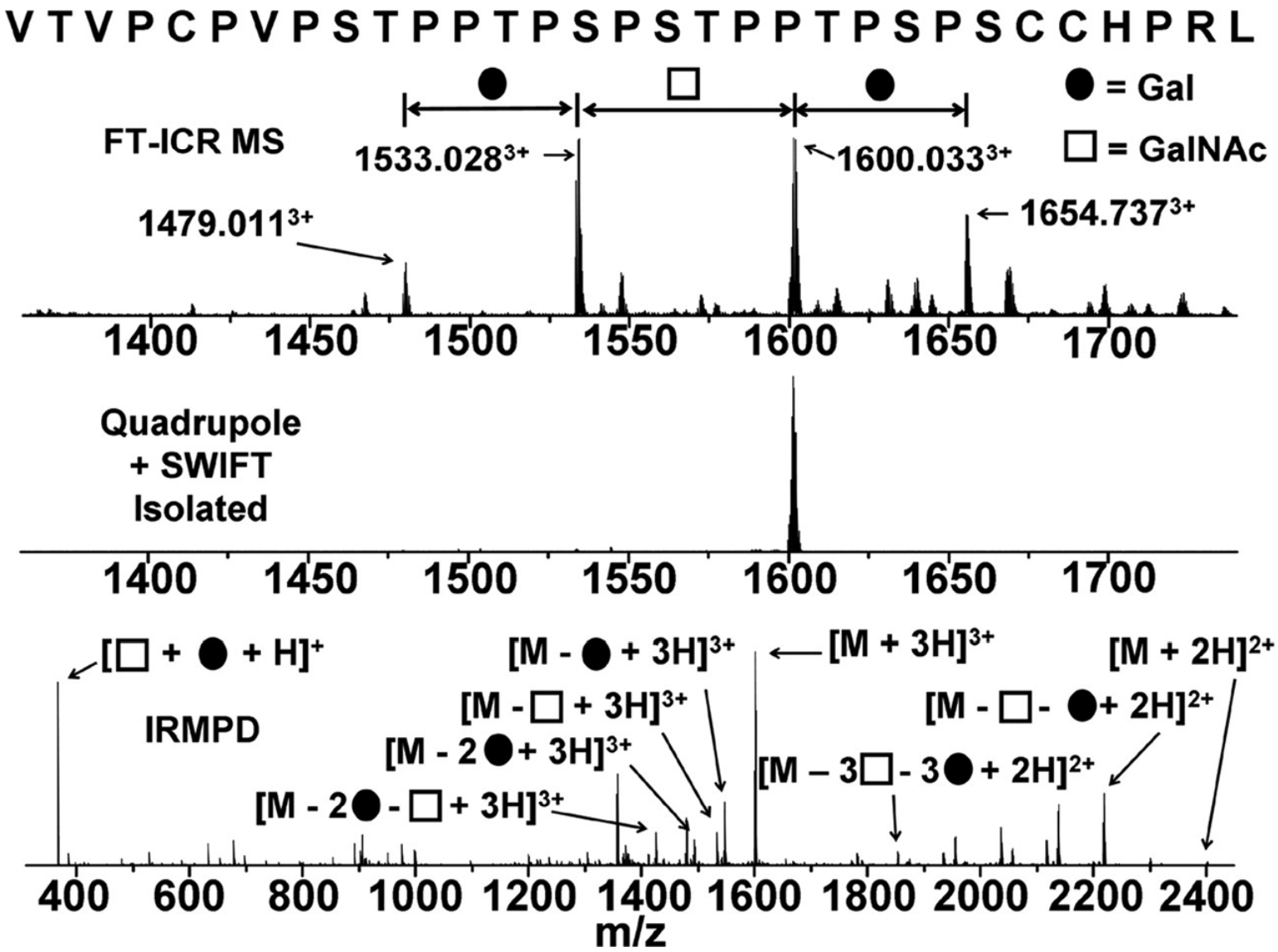
(CHO) CHO CHO CHO (CHO)

CH2

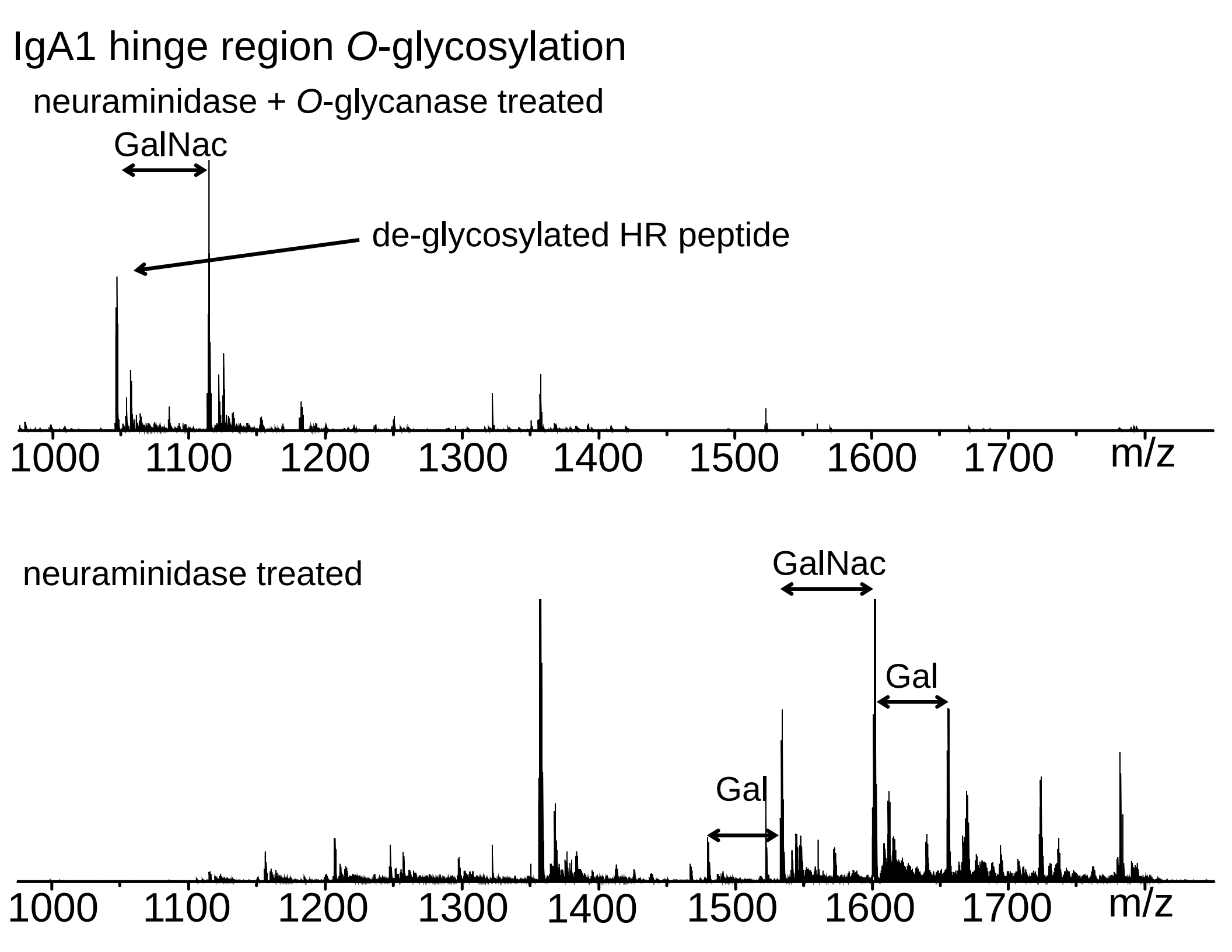
-Pro-Val-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser-Cys-

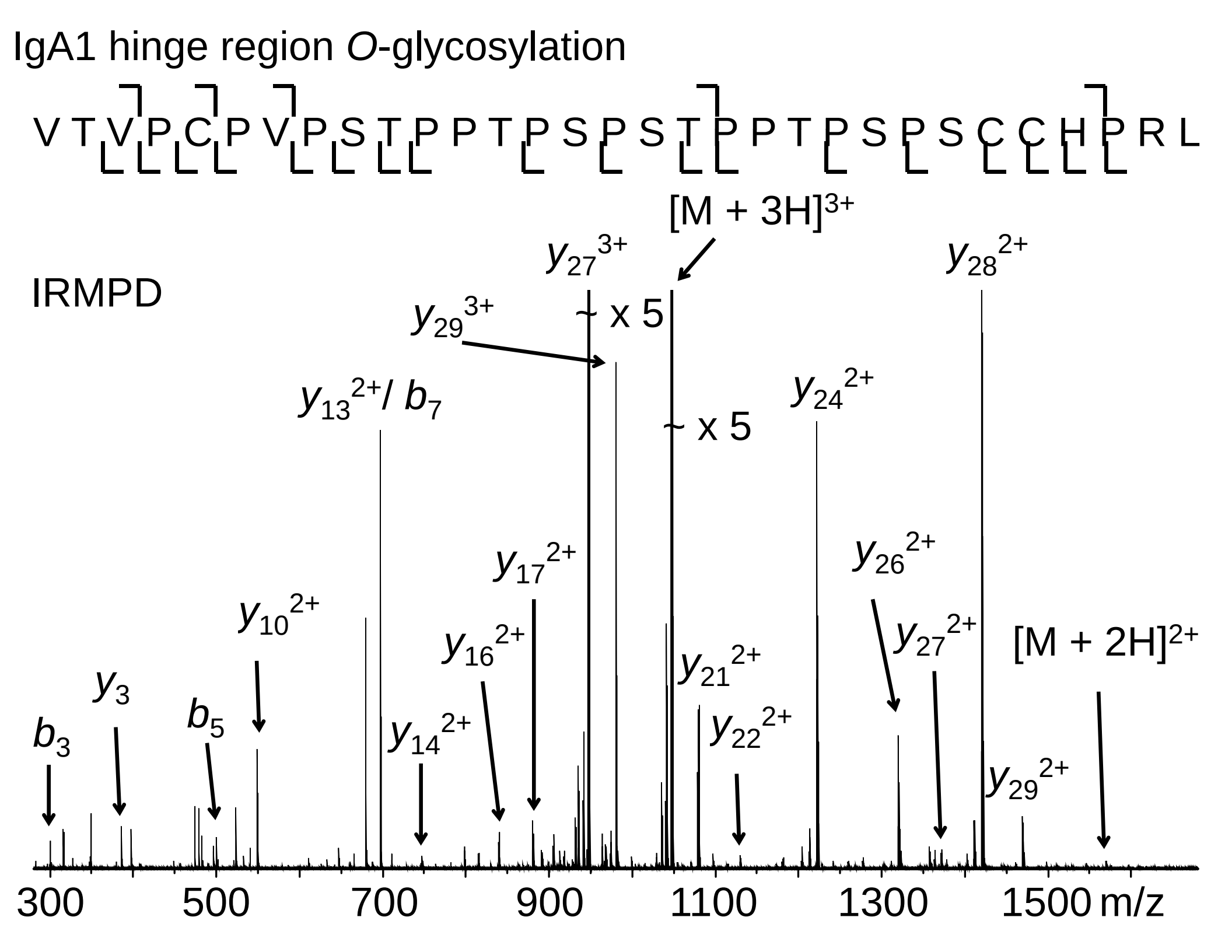
225 228 230 232 236

ESI FT-ICR positive ion mass spectra of isolated IgA1 HR peptide.

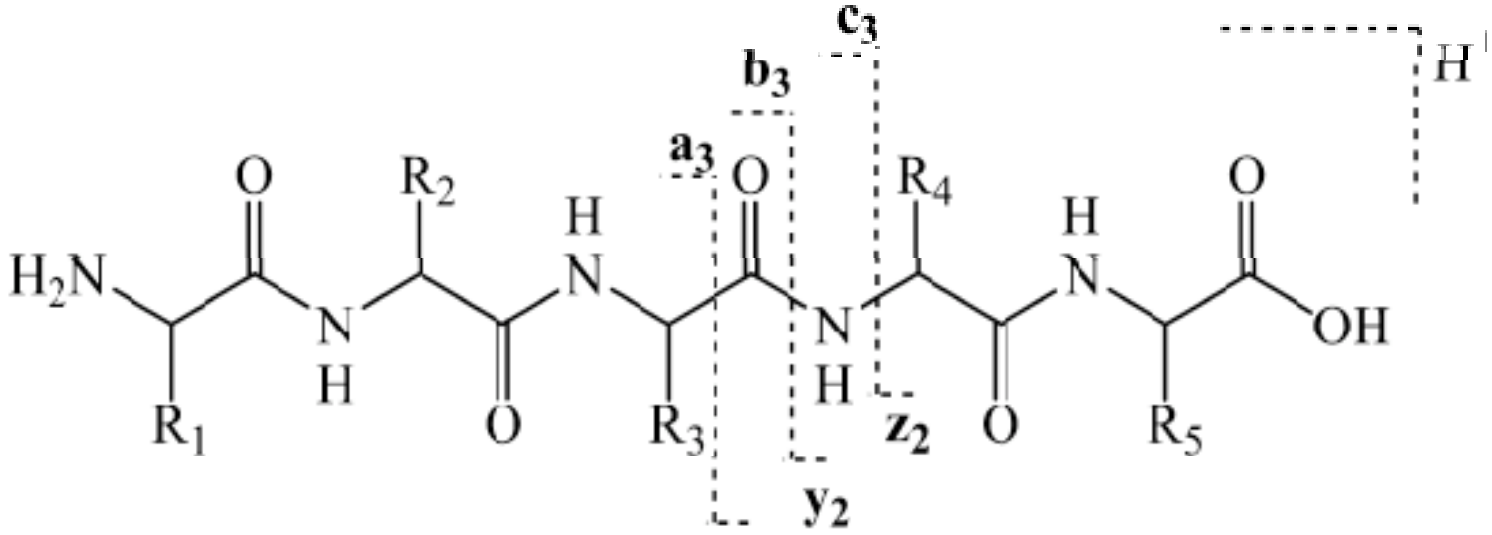


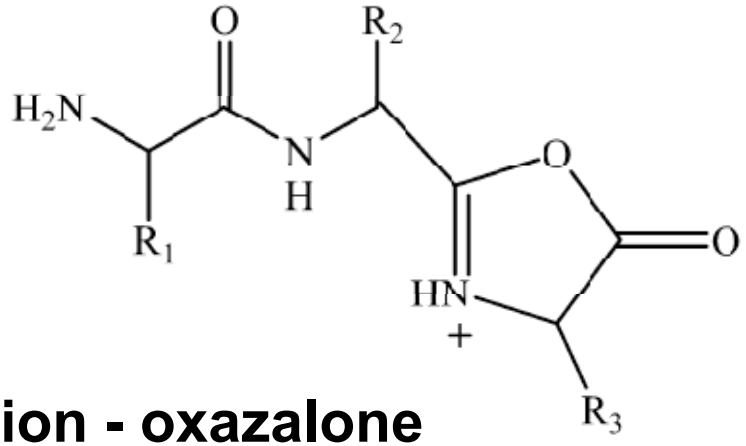
Renfrow M B et al. J. Biol. Chem. 2005;280:19136-19145


IgA1 hinge region O-glycosylation
neuraminidase + O-glycanase treated
GalNac
de-glycosylated HR peptide
1000
1100
1200
1300
1400
1500
1600
1700
m/z
neuraminidase treated
GalNac
Gal
Gal
1000
1100
1200
1300
1400
1500
1600
1700
m/z


IgA1 hinge region O-glycosylation
V T V P C P V P S T P P T P S P S T P P T P S P S C C H P R L
IRMPD
[M + 3H]3+
y27 3+
y28 2+
y29 3+
~ x 5
~ x 5
y13 2+/ b7
y24 2+
y17 2+
y26 2+
y10 2+
y16 2+
y27 2+
[M + 2H]2+
y21 2+
y3
b5
b3
y14 2+
y22 2+
y29 2+
300
500
700
900
1100
1300
1500
m/z

Let's take a closer look at fragmentation

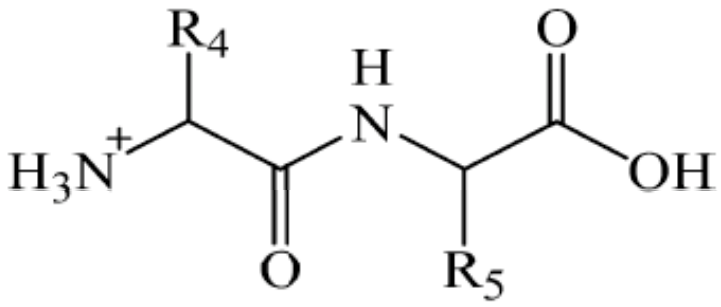




b_3 ion - oxazalone

no b_1 ion

y_2

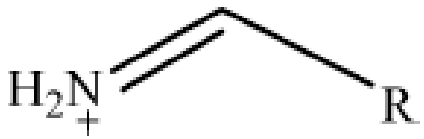


Wysocki et al. 2005

Other amino acid fragment ions

m/z values of common immonium ions

Immonium ion (*m/z*)	Amino acid residue	Major (M) or minor (m) peak
60.04	S	M
70.07	R or P	M
72.08	V	M
73.00	R	m
74.06	T	M
84.08	K or Q	M
86.1	I or L	M
87.09	N or R	M
88.04	D	M
100.09	R	m
101.11	K or Q	M
102.06	E	M
104.05	M	M
110.07	H	M
112.09	R	M
120.08	F	M
126.06	P	M
129.1	K or Q	m
136.08	Y	M
138.07	H	m
159.09	W	M



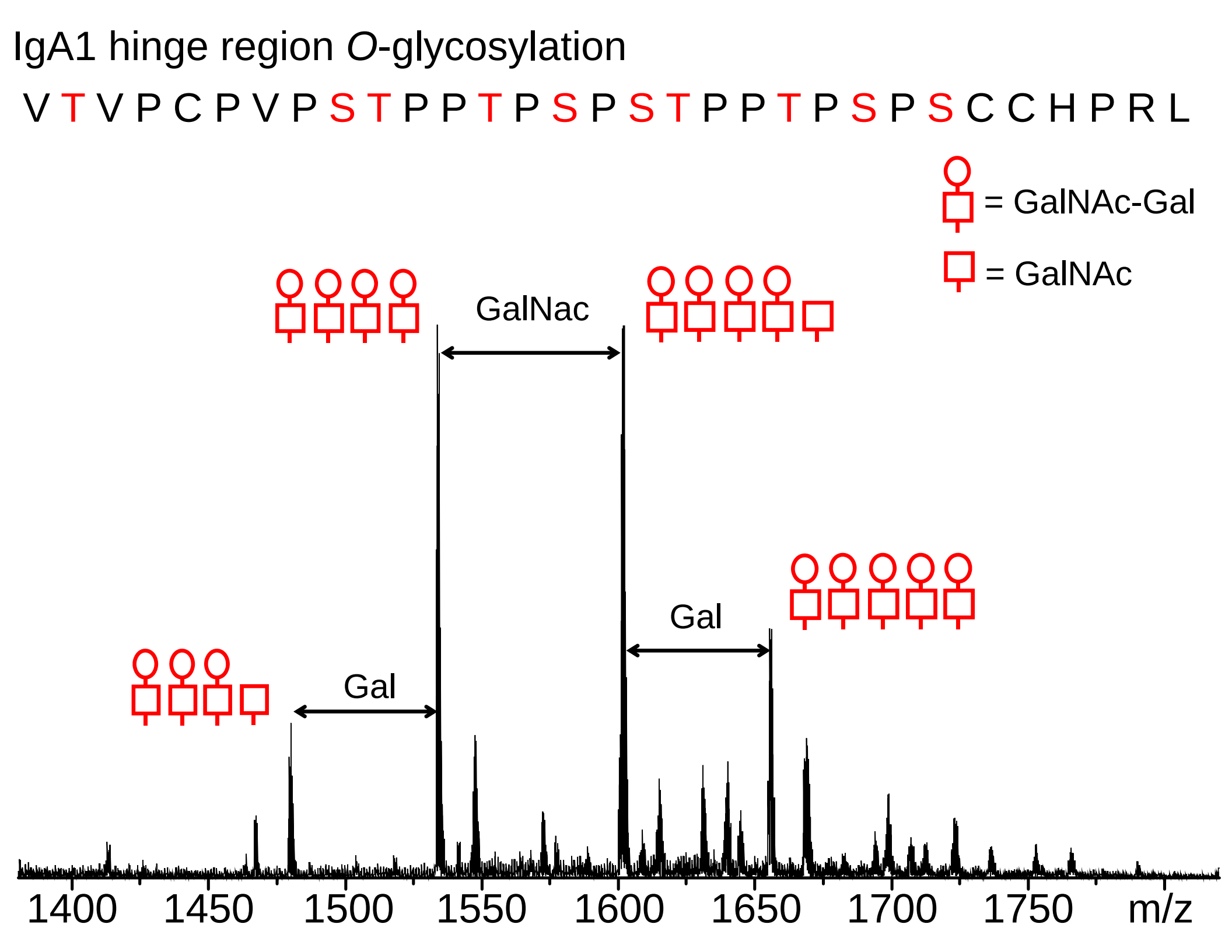
Wysocki et al. 2005

Detecting posttranslational modifications (PTMs) by MS

- A key issue is that the energy of ionization or the collisional process should not exceed the dissociational energy of the PTM

- MALDI-TOF MS with a N_2 laser causes fragmentation of a nitrated tyrosine residue
  - Use ESI to make the molecular ion
  - Go to another laser wavelength (YAG laser at 355 nm or IR)

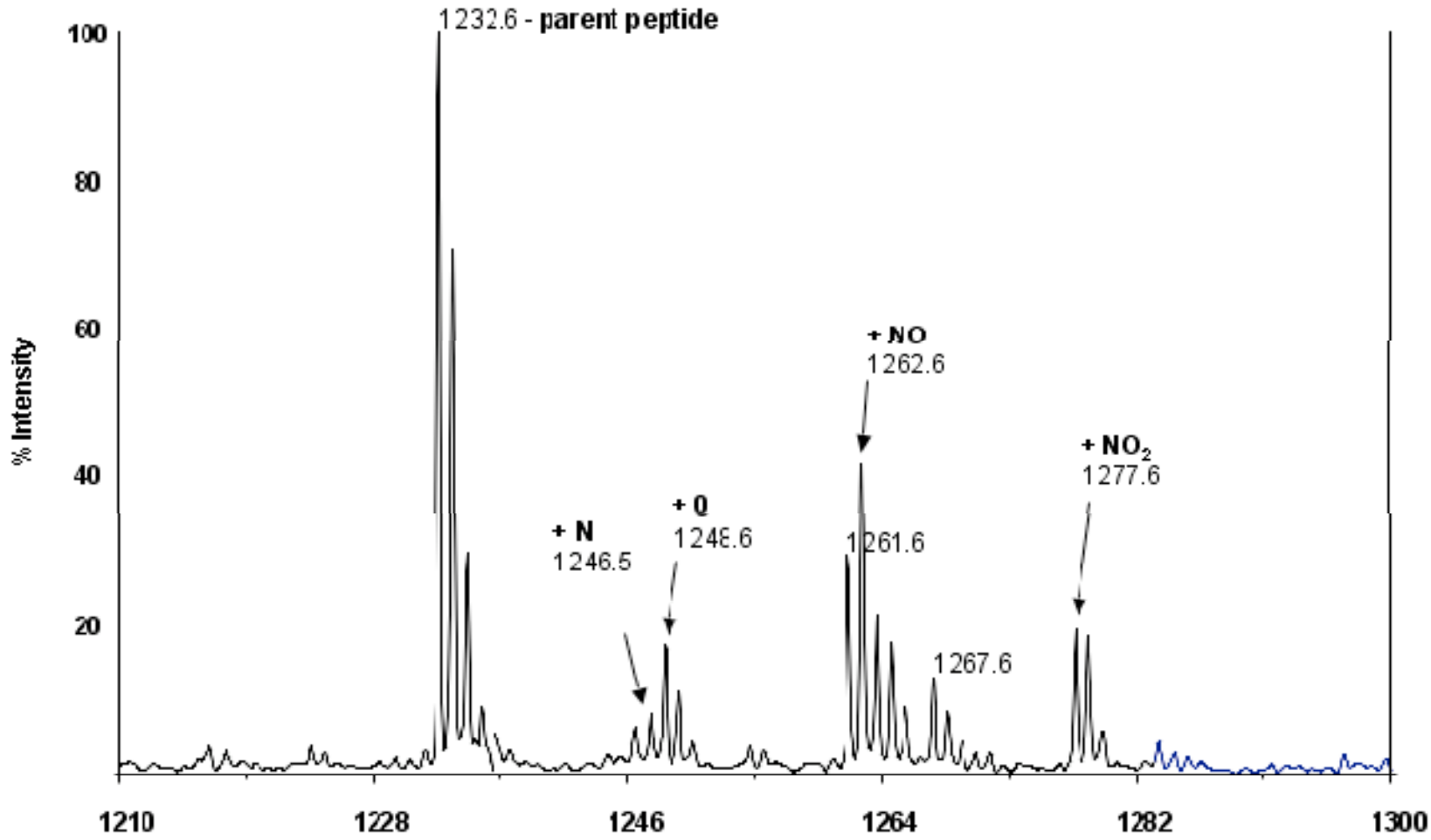
- O-glucosyl and phospho groups fragment more easily than the peptide to which they are attached
  - Use electron capture dissociation


IgA1 hinge region O-glycosylation
V T V P C P V P S T P P T P S P S T P P T P S P S C C H P R L
= GalNAc-Gal
= GalNAc
GalNac
Gal
Gal
1400
1450
1500
1550
1600
1650
1700
1750
m/z

Types of fragmentation (1)

- **Collision-induced dissociation (CID)**
  - **Also called CAD (collision-activated dissociation)**
  - **Multiply charged peptide ions are isolated by an *m/z* based filter**
  - **Selected ions are accelerated into a field of inert gas (He, N_2, Ar, Xe) at moderate pressure**
  - **The energy gained in collision events increases vibrational and stretching modes of the peptide backbone (and anything attached to it!)**
  - **The increased motion of the energized peptide causes breaks that occur typically at the peptide bond**
    - **Side chain groups can also be broken, some times more easily than the peptide chain**

Fragmentation of nitrated peptides in MALDI-TOF experiment



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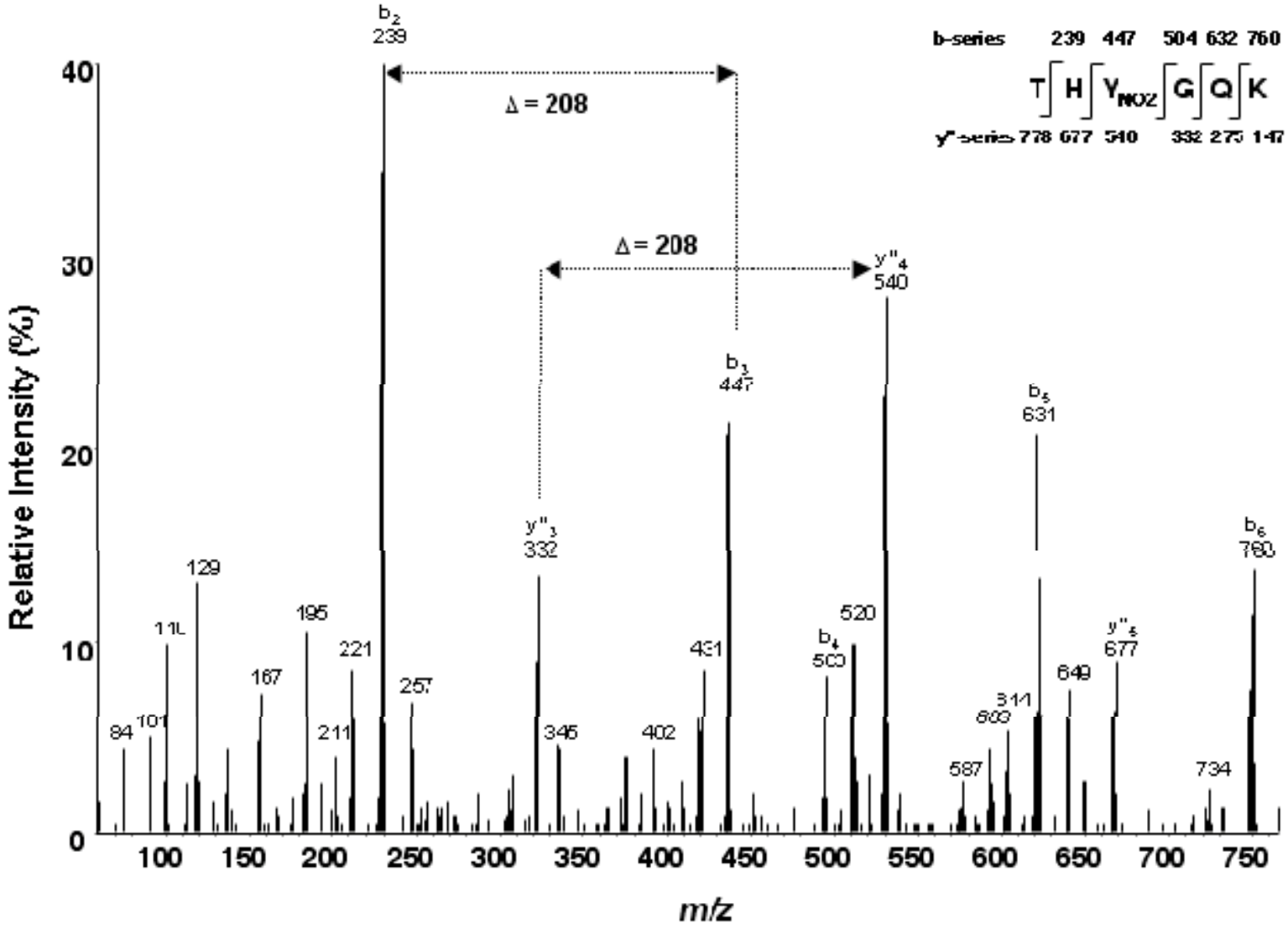
ESI-tandem MS of a nitrated peptide



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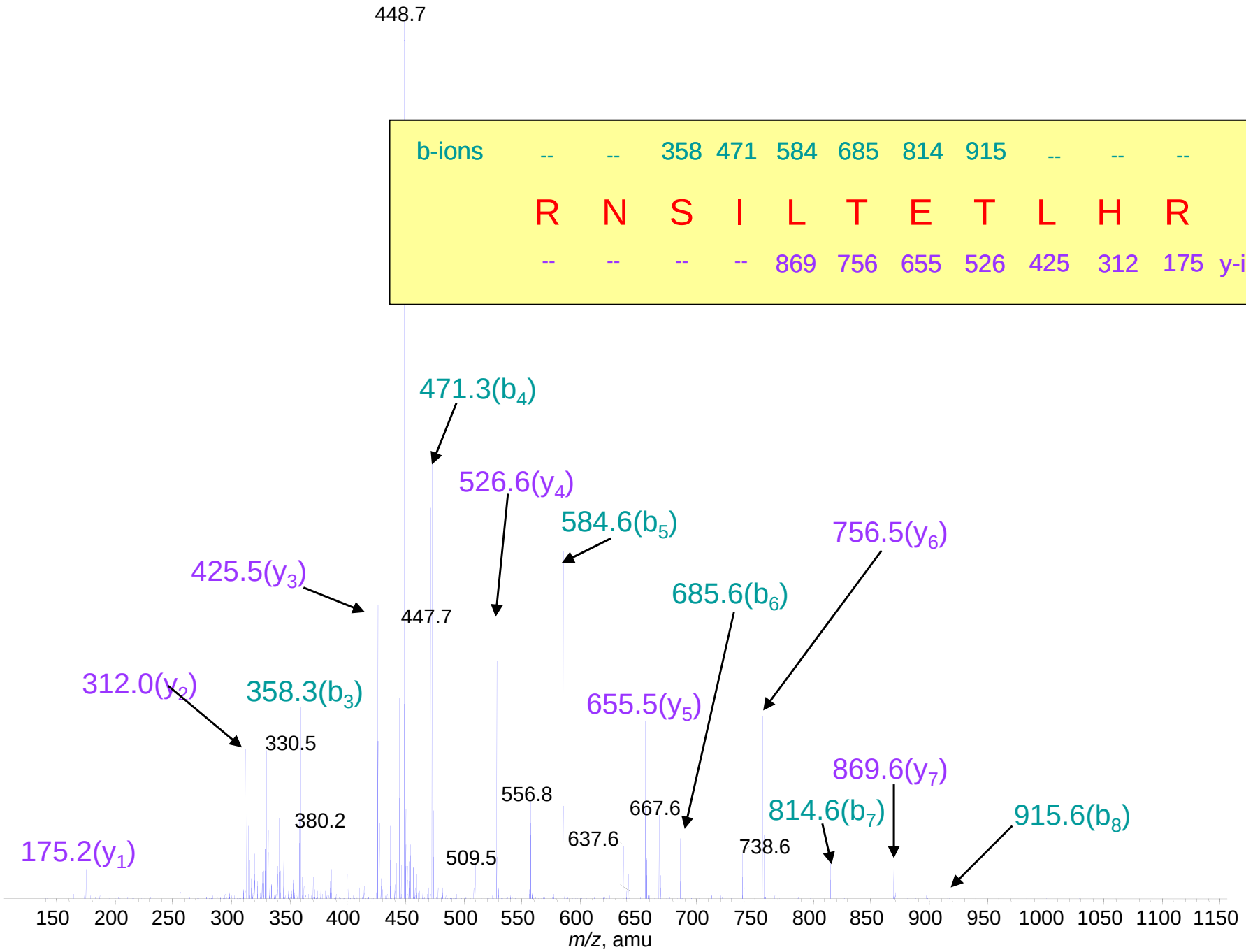
aa658-668 RNSILTETLHR Unmodified *m/z* 447.37(3^+) 1339.09 Da



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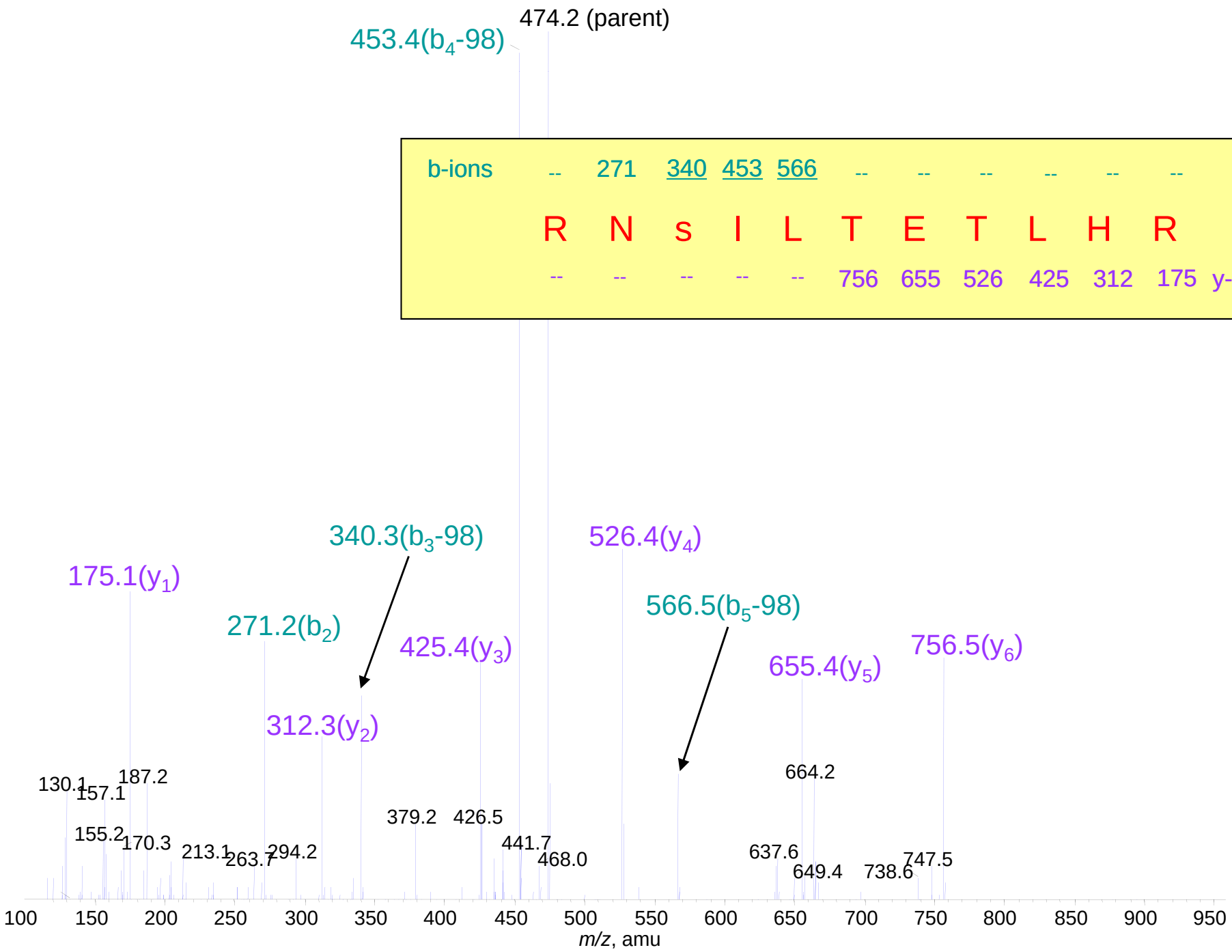
aa658-668 RNsILTETLHR Phosphorylated *m/z* 474.00(3^+) 1419.00 Daltons



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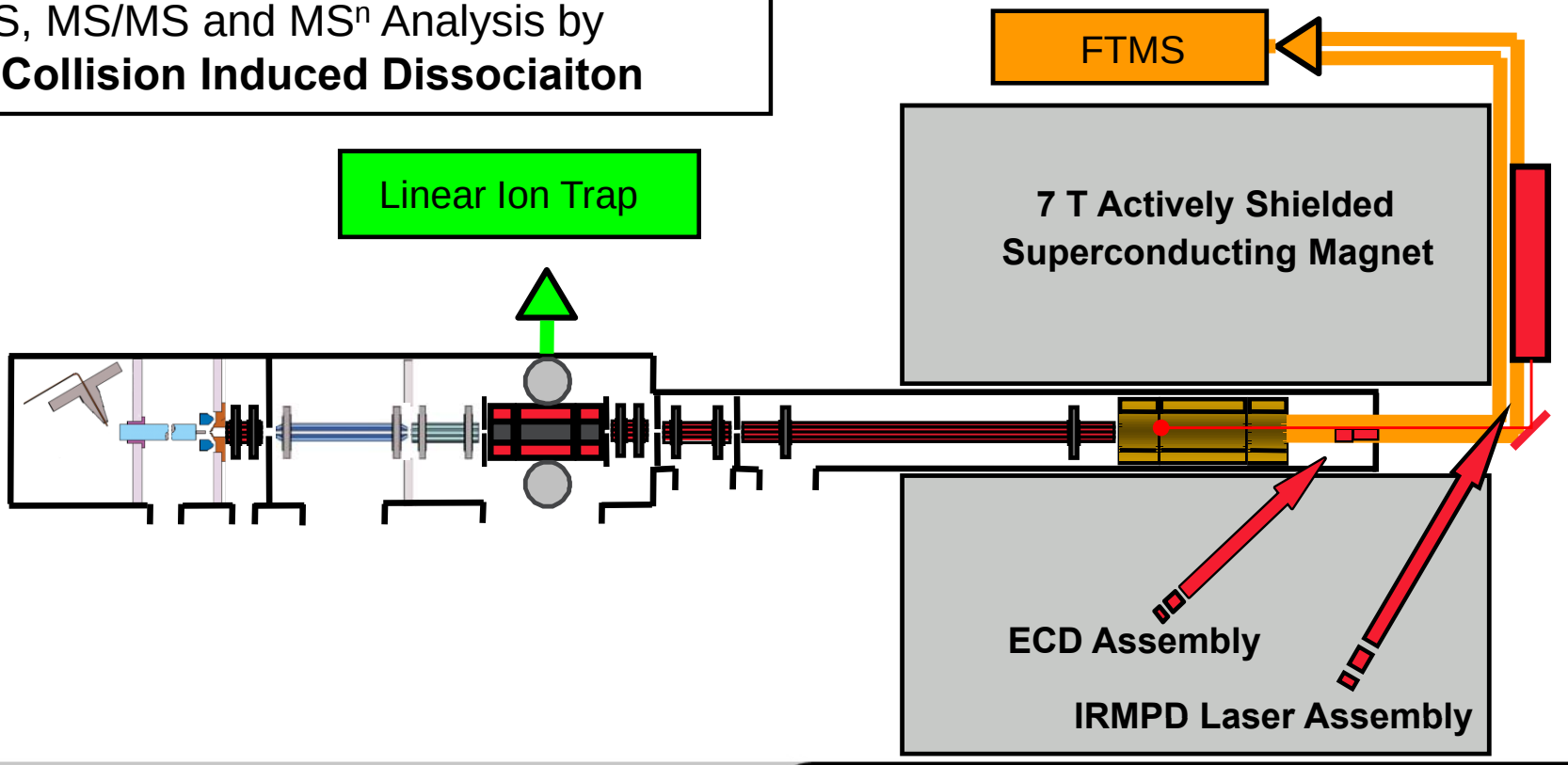
Types of fragmentation (2) IRMPD

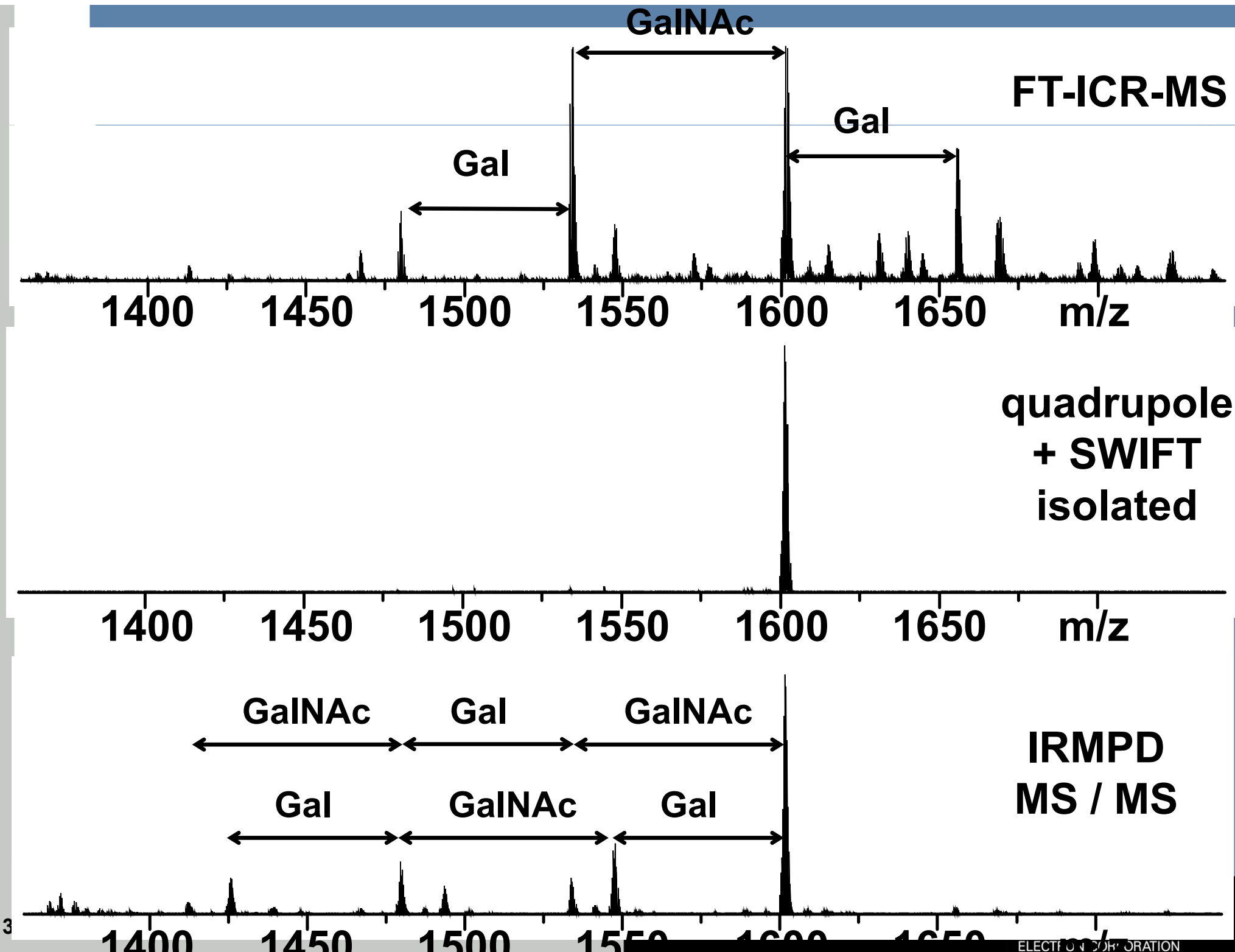
- **InfraRed Multi-Photon Dissociation**
  - **Used in FT-ICR instruments where a vacuum better than 1 x 10^{-10} torr is necessary for the analysis of peptide ions**
  - **The infra-red radiation is delivered by an IR laser operating at 10.6 microns**
  - **No gas is involved**
  - **In this case, the fragmentation is induced in the ICR cell**
  - **Effects are essentially equivalent to CID**

ECD and IRMPD with the Finnigan LTQ FT

FTICR MS
- **Electron Capture Dissociation (ECD)**
- **Infra-Red Multiphoton Dissociation (IRMPD)**

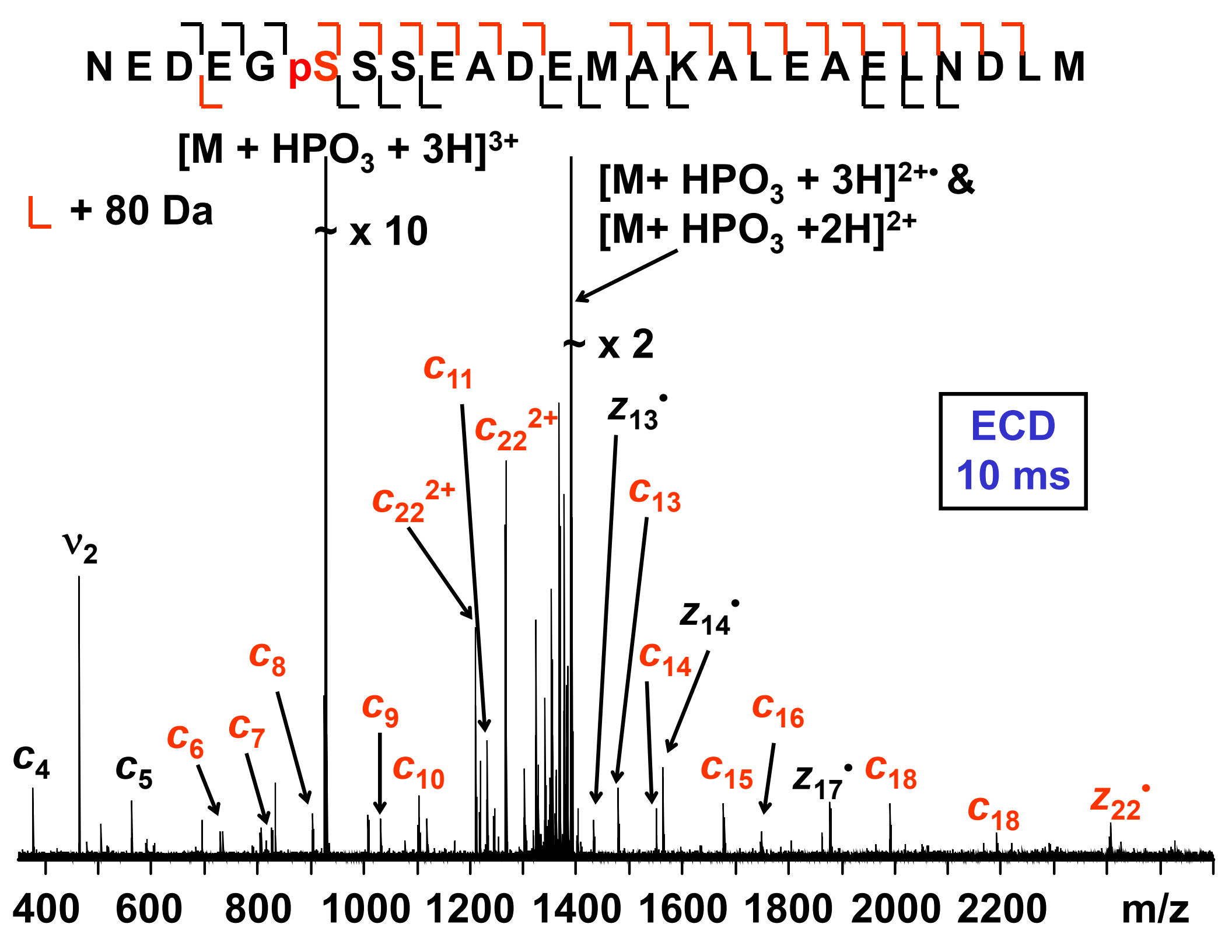
Linear Ion Trap MS
- MS, MS/MS and MS^n Analysis by **Collision Induced Dissociaiton**




GalNAc
Gal
Gal
FT-ICR-MS
1400
1450
1500
1550
1600
1650
m/z
quadrupole
+ SWIFT
isolated
1400
1450
1500
1550
1600
1650
m/z
GalNAc
Gal
GalNAc
Gal
GalNAc
Gal
IRMPD
MS / MS
1400
1450
1500

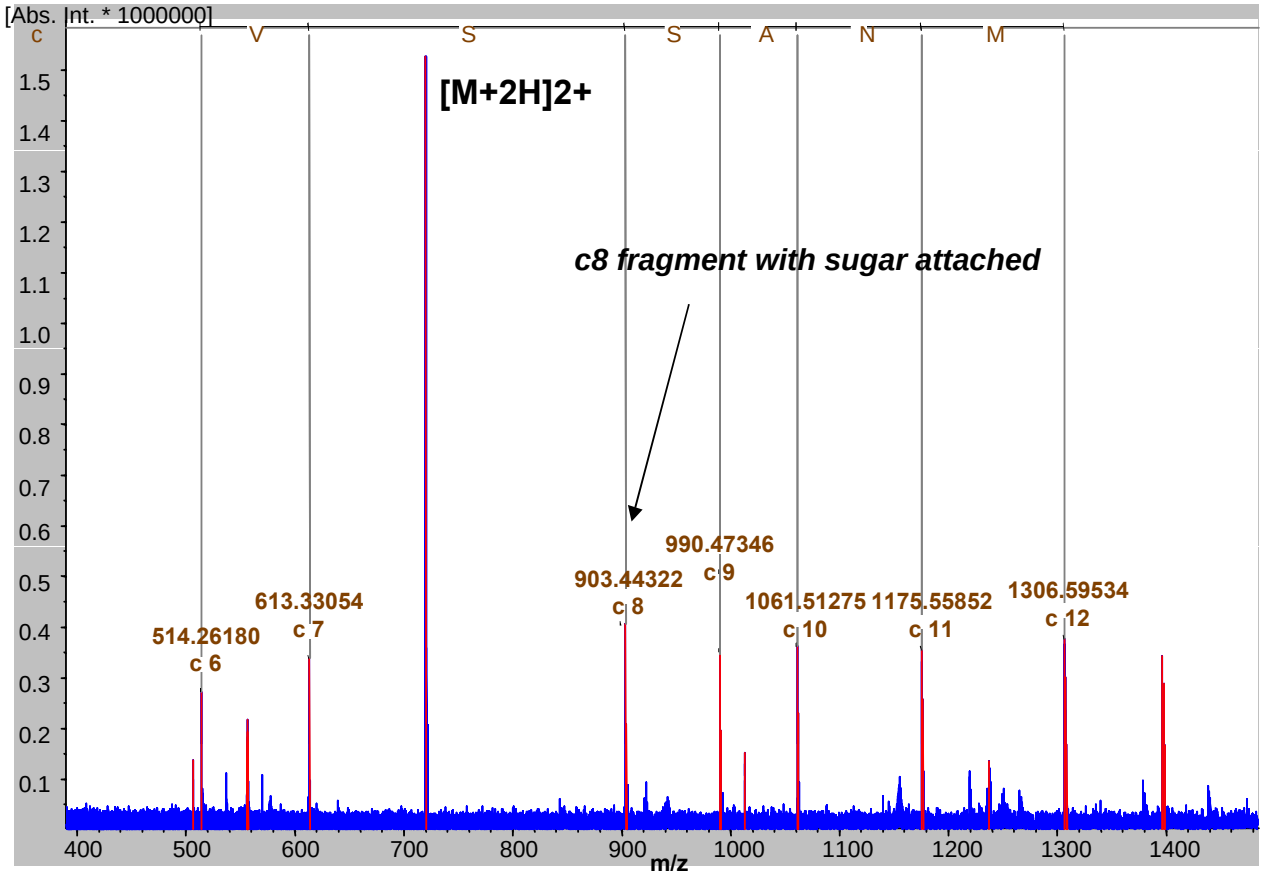
Types of fragmentation (3) ECD

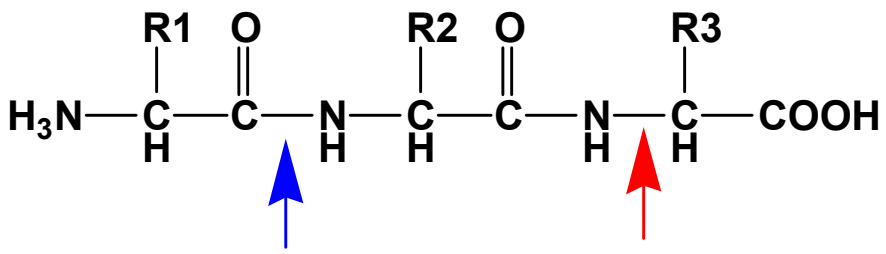
- **Electron Capture Dissociation**
  - **Used in an ICR cell of an FT-MS instrument**
  - **Low energy electrons interact with the multiply charged peptide and are absorbed**
  - **They disturb bonding of the peptide backbone and cleave it without altering the side chain**
  - **Yields *c*- and *z*-ions**
  - **MS-MS spectra often very clean, but low sensitivity**
  - **In conjunction with an IR laser, ECD can fragment whole proteins (top-down)**


N E D E G pS S S E A D E M A K A L E A E L N D L M
[M + HPO3 + 3H]3+
+ 80 Da
~ x 10
[M+ HPO3 + 3H]2+• &
[M+ HPO3 +2H]2+
~ x 2
c11
c22 2+
z13•
c22 2+
c13
ECD
10 ms
ν2
z14•
c8
c14
c9
c16
c6
c7
c4
c5
c10
c15
z17•
c18
c18
z22•
400 600 800 1000 1200 1400 1600 1800 2000 2200 m/z

Sequencing O-GlcNAc peptides by ECD FT-ICR-MS

Casein kinase II - AGGSTPVSSANMMSG





b ion cleavage **c ion cleavage**

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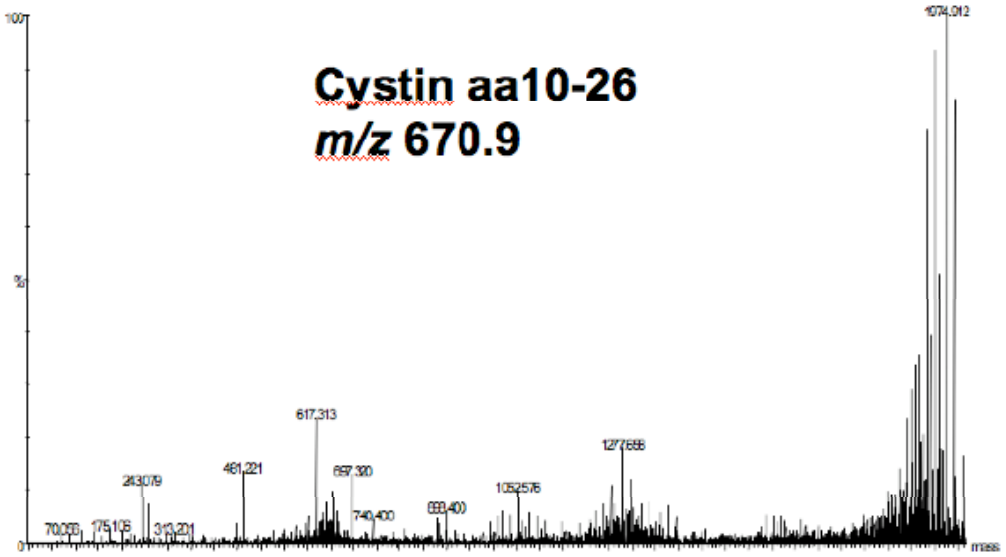
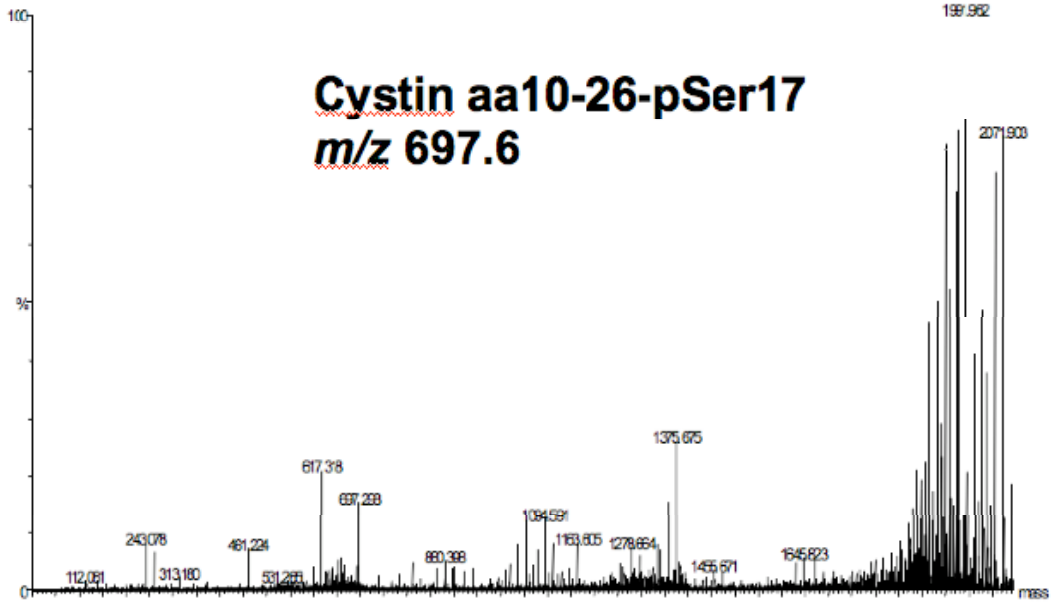
CID spectra of Arg-rich peptide



Furry spectra due to -NH_3 losses from Arg residues



Spectra are uninterpretable

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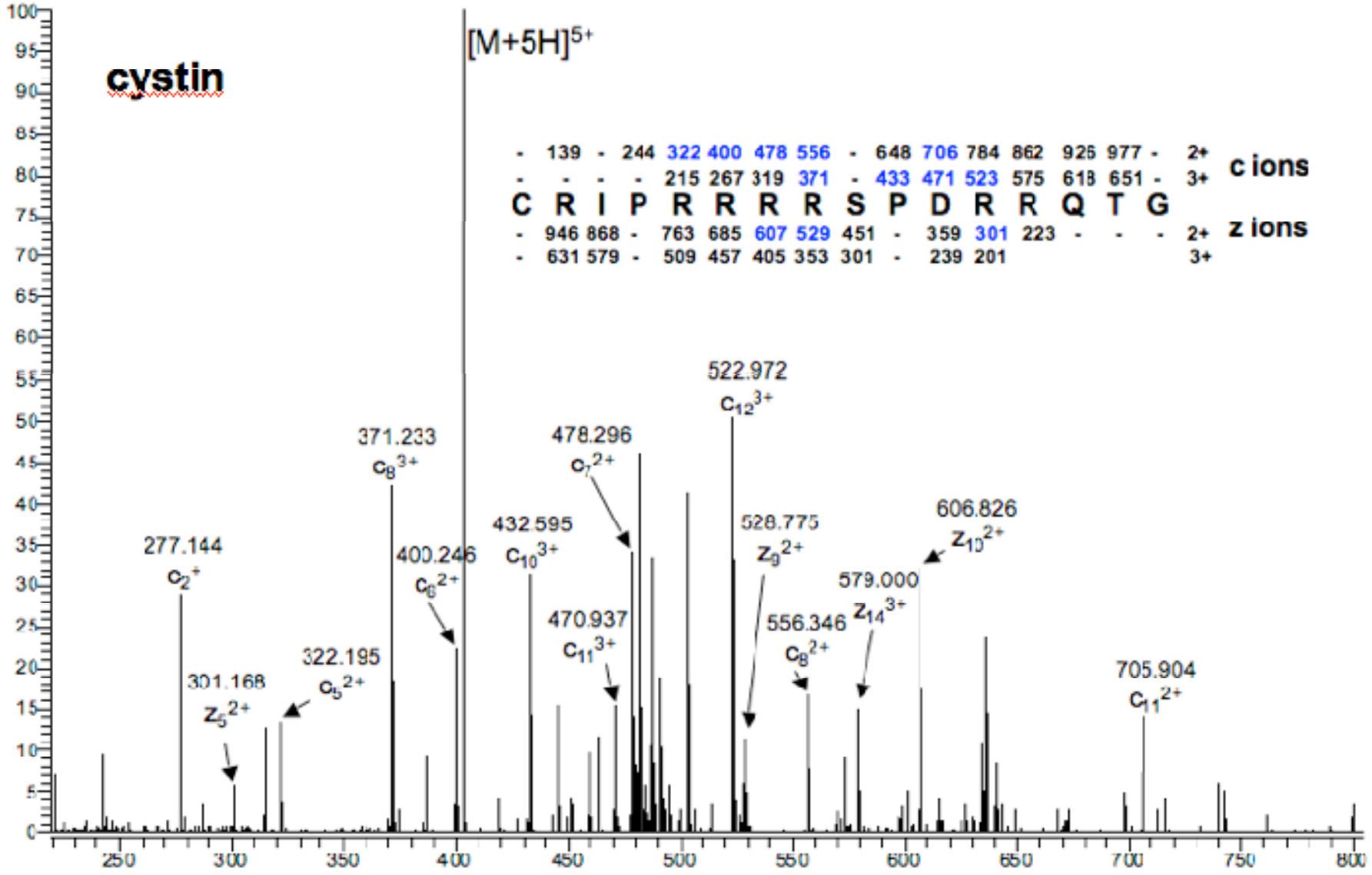
ECD spectra of cystin peptide



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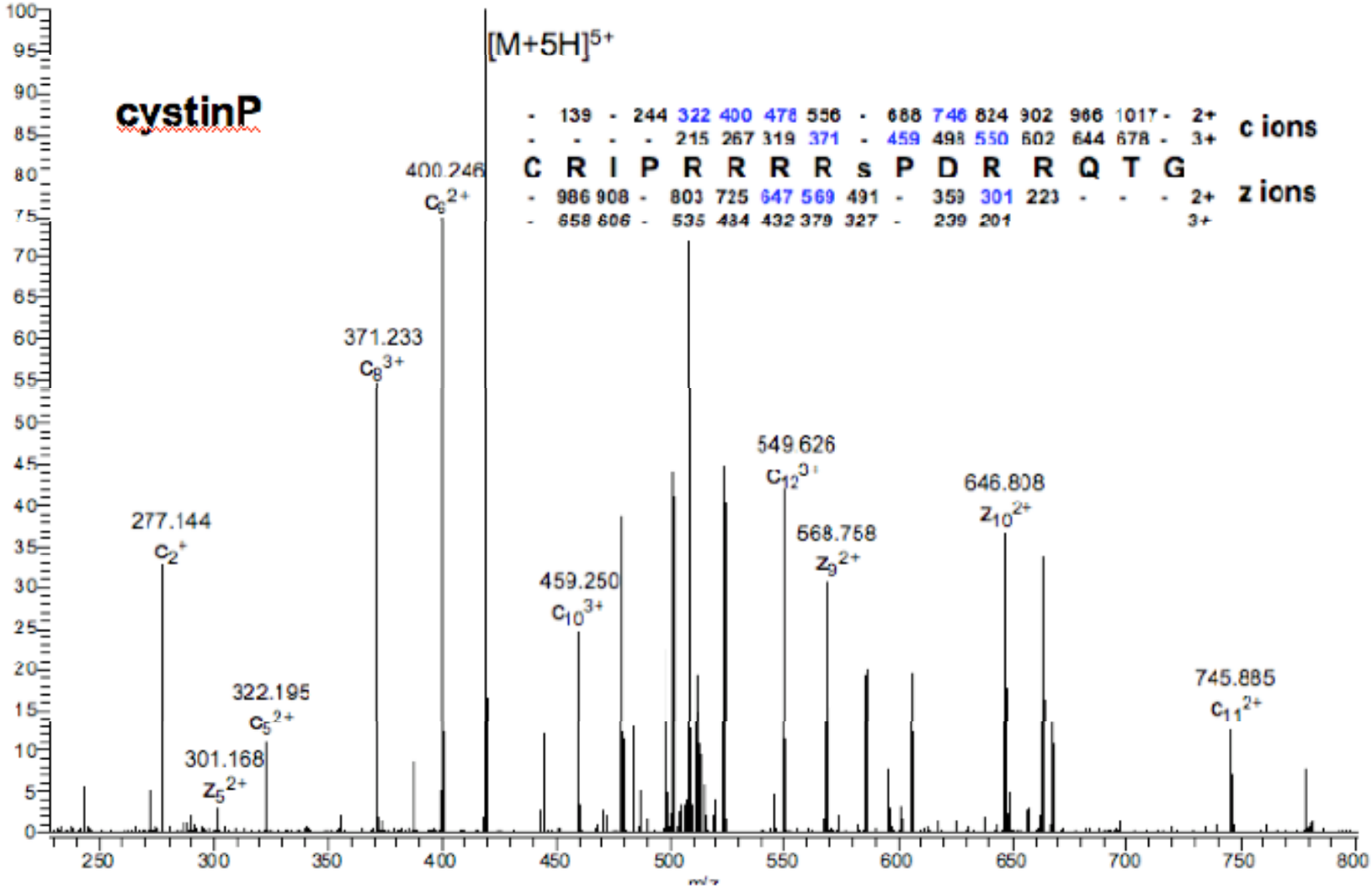
ECD spectra of phosphorylated cystin peptide



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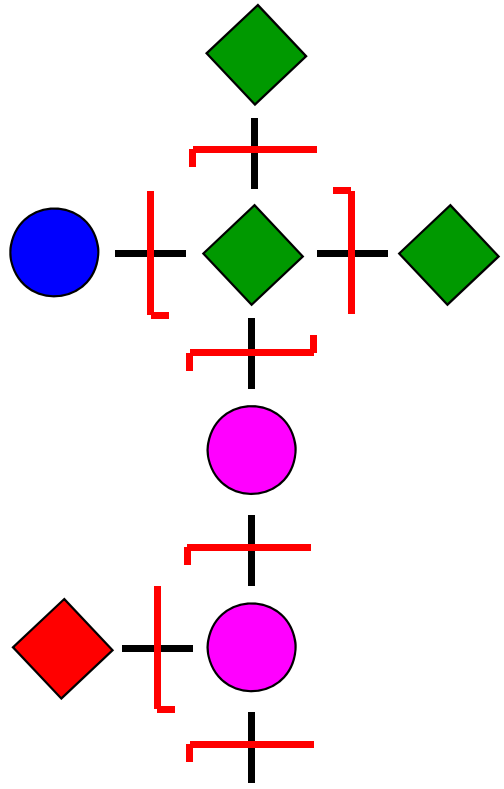
IRMPD Fragmentation pattern

● = GlcNAc

◆ = Fuc

◆ = Man

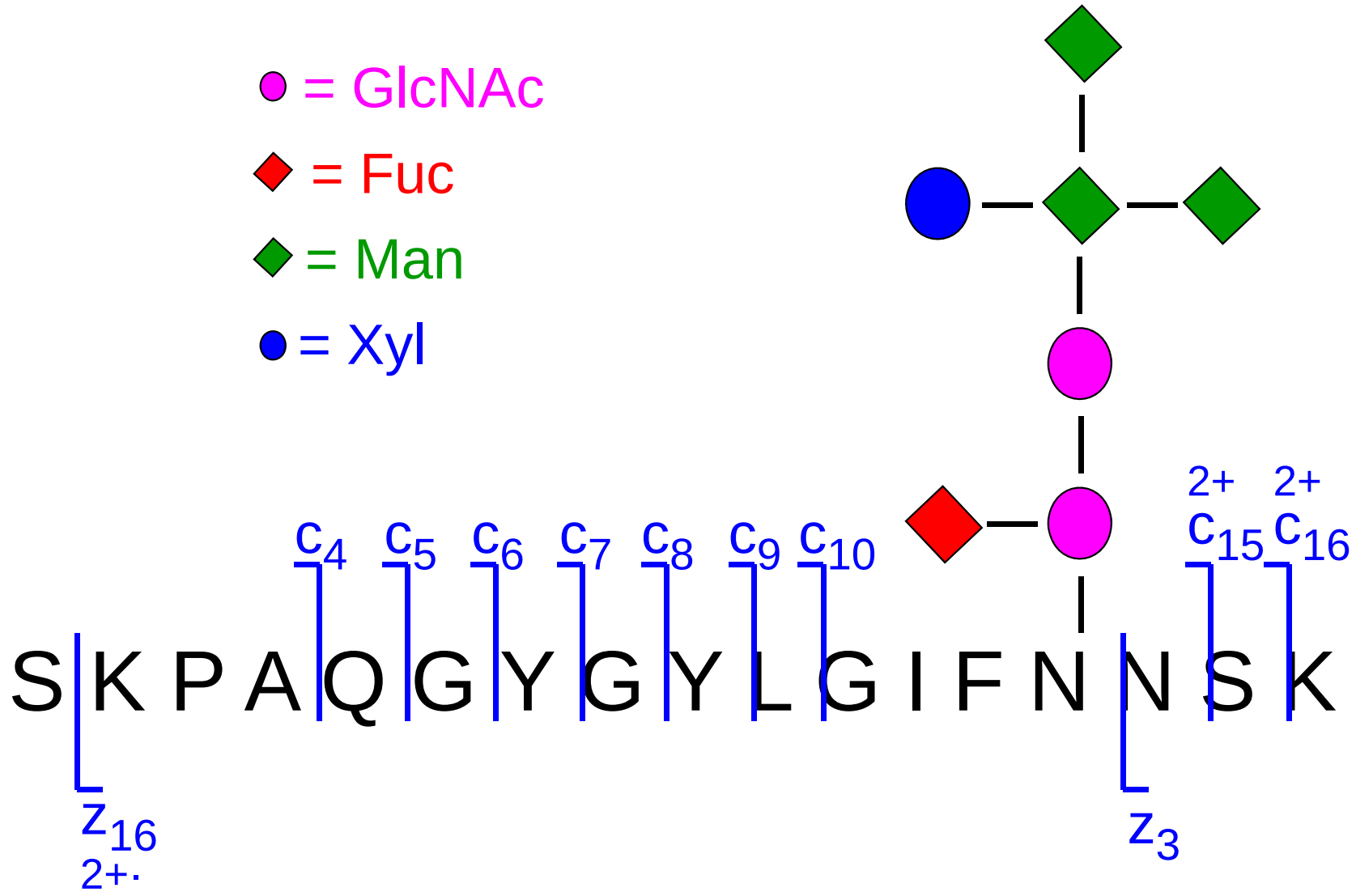
● = Xyl



S K P A Q G Y G Y L G I F N N S K

K. Håkansson, H. J. Cooper, M. R. Emmett, C. E. Costello, A. G. Marshall, C. L. Nilsson, *Anal. Chem.* **73**, 4530 (2001).

ECD Fragmentation Pattern

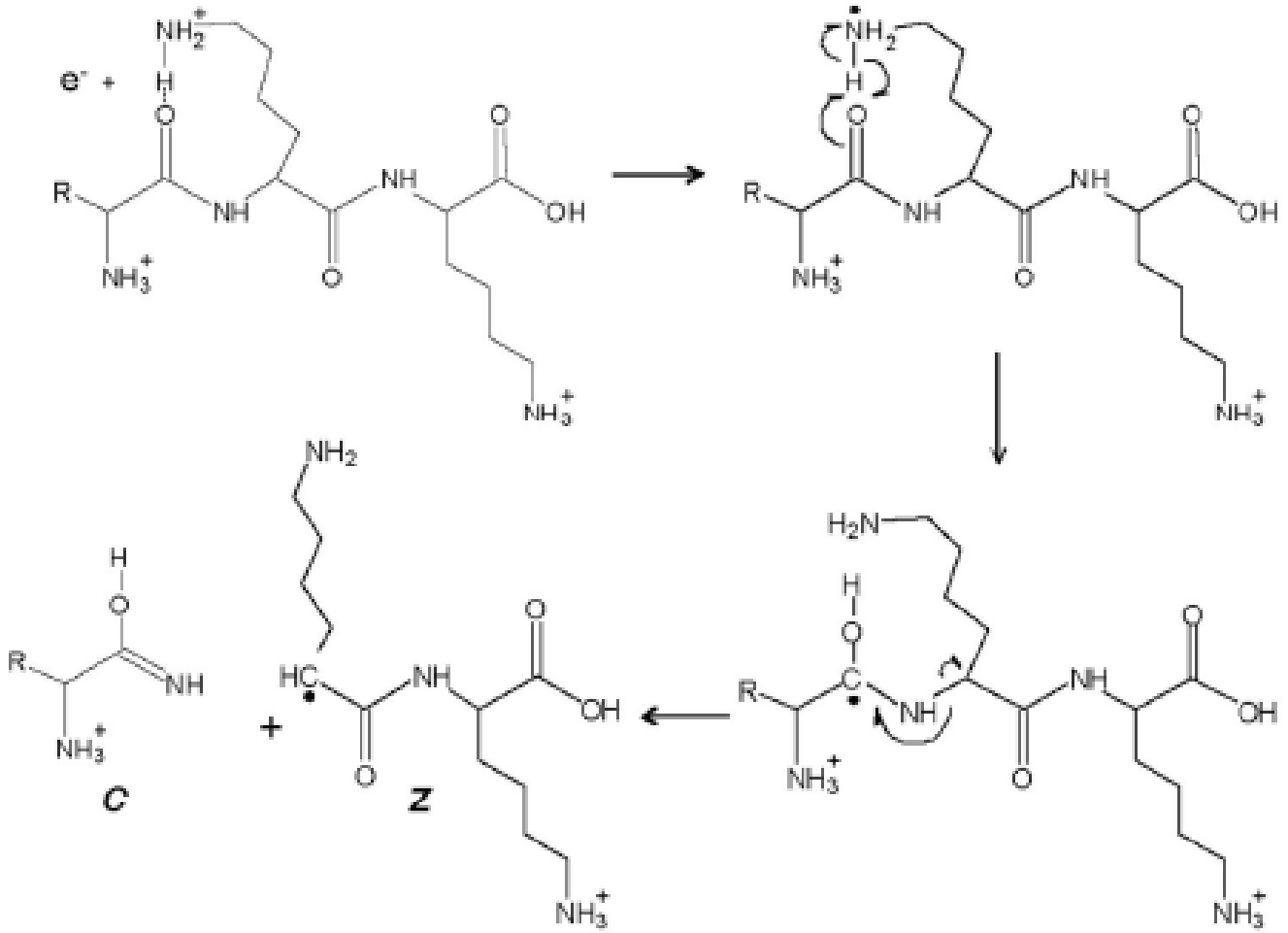


K. Håkansson, H. J. Cooper, M. R. Emmett, C. E. Costello, A. G. Marshall, C. L. Nilsson, *Anal. Chem.* **73**, 4530 (2001).

Types of fragmentation (4) ETD

- **Electron Transfer Dissociation**
  - **The electron is provided by an electron donating chemical species, a radical anion (azobenzene, fluoranthene) directly infused as a reagent gas, or from their precursors introduced by ESI - 9-anthracenecarboxylic acid, 2-fluoro-5-iodobenzoic acid, and 2-(fluoranthene-8-carbonyl)benzoic acid)**

Electron transfer dissociation



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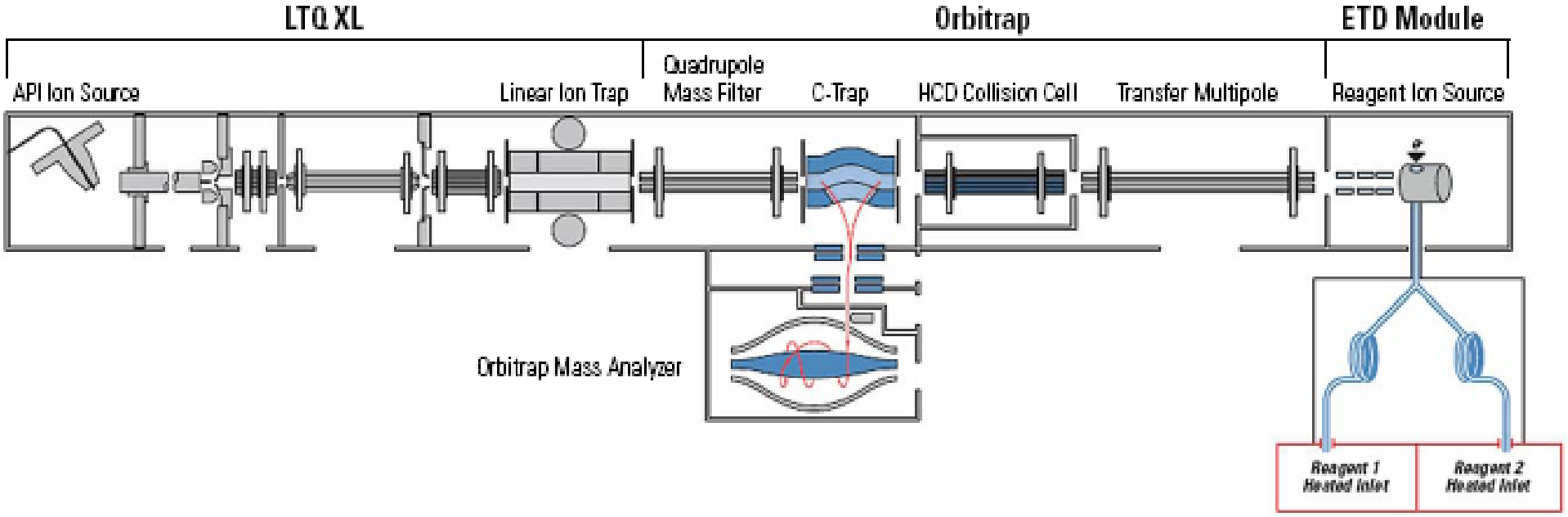

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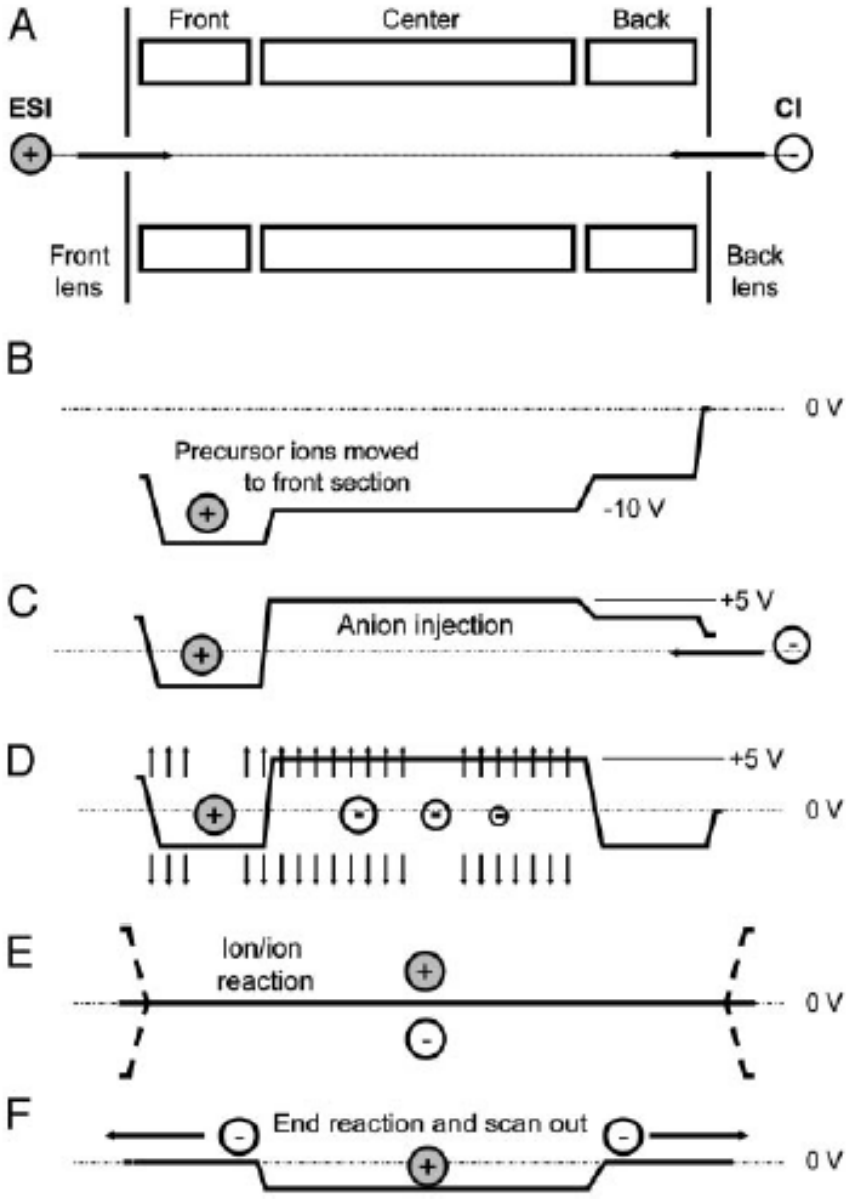
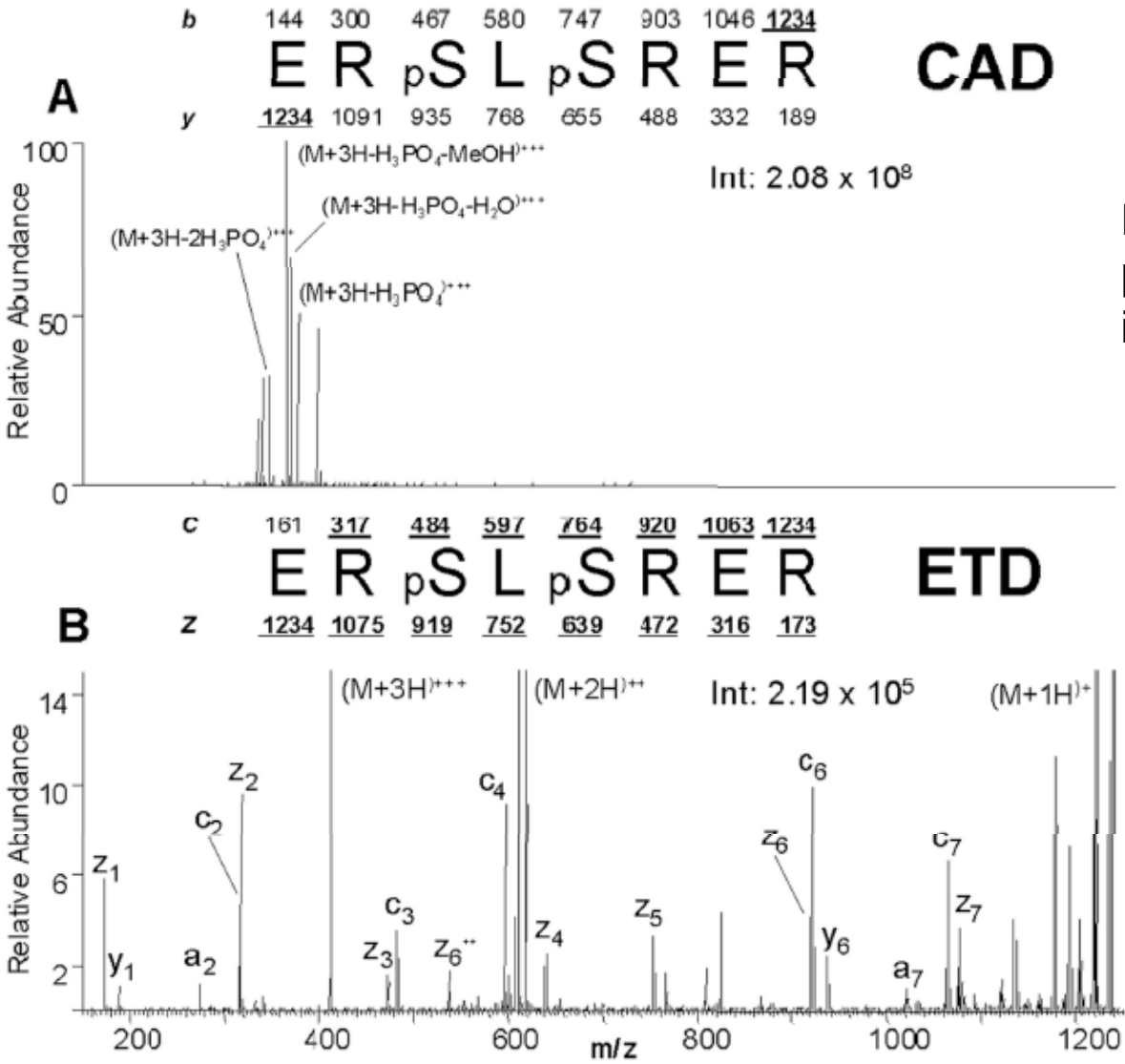


Fig. 4. Schematic of steps involved in the operation of the LTQ mass spectrometer for peptide sequence analysis by ETD. (*A*) Injection of multiply protonated peptide molecules (precursor ions) generated by ESI. (*B*) Application of a dc offset to move the precursor ions to the front section of the linear 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 po-

Syka, *et al.*, *PNAS* 2004

CAD versus ETD for phosphopeptide



Phosphopeptide loses phosphate, but no sequence information is obtained

Rich series of *c* and *z* ions

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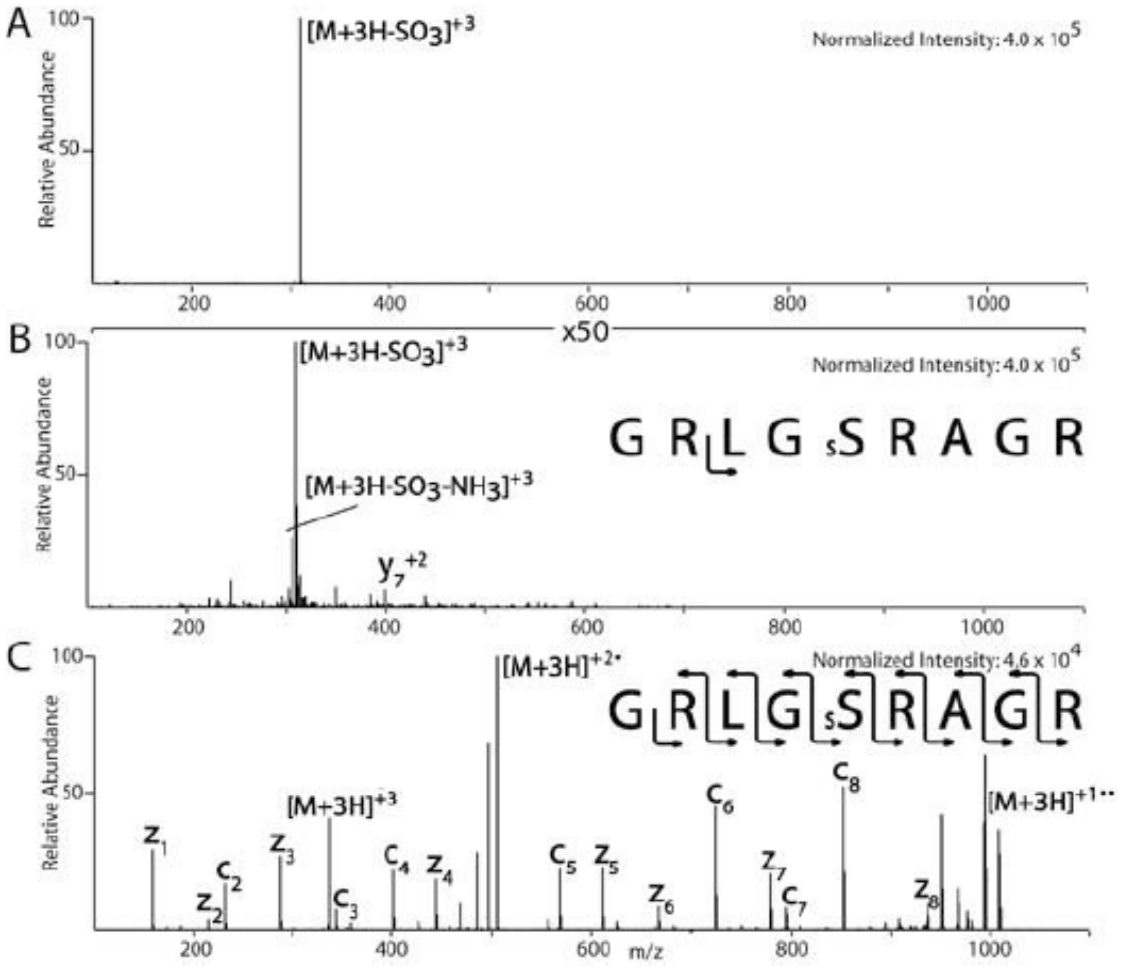
ETD better for sulfonated peptides



ETD

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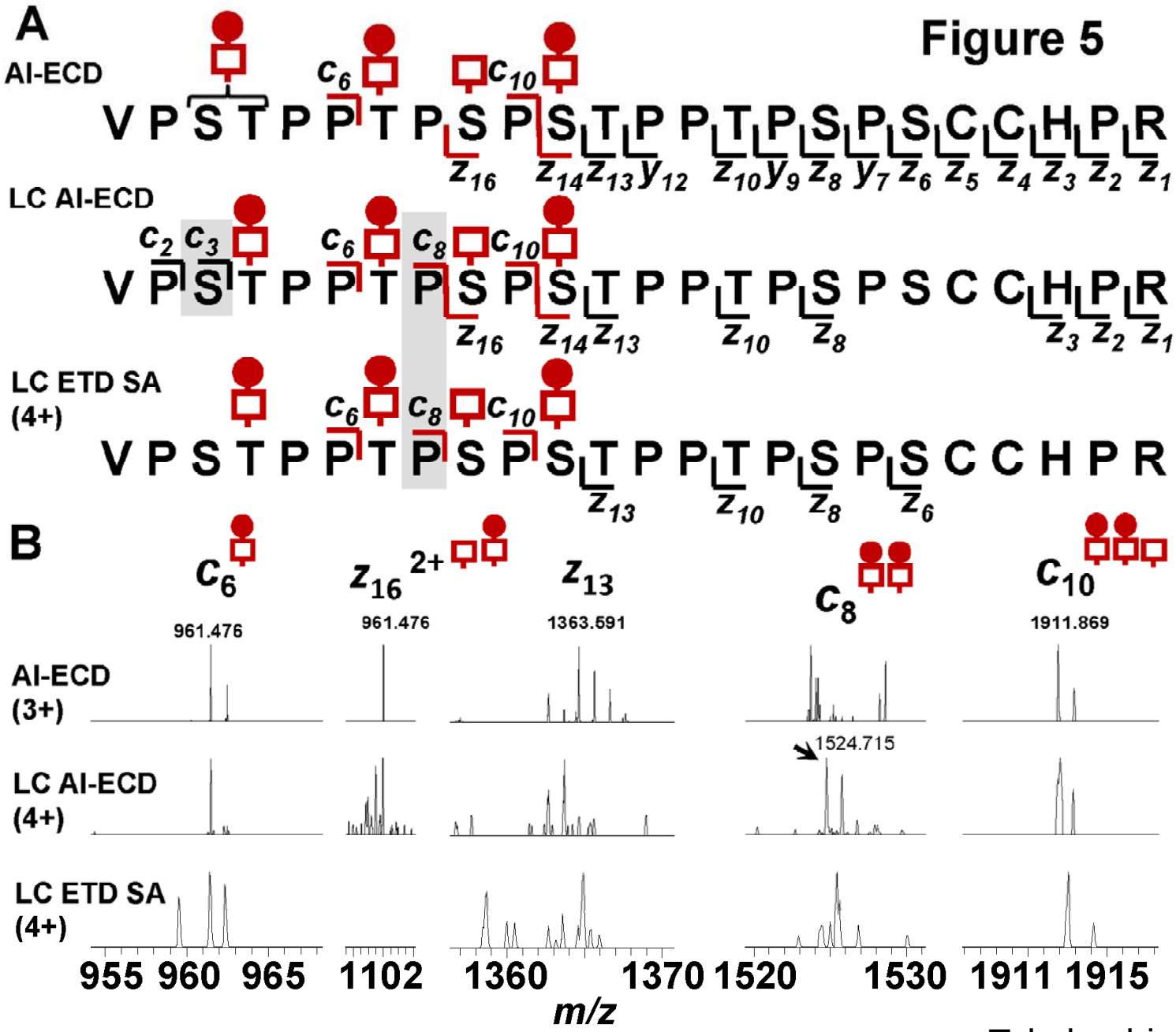
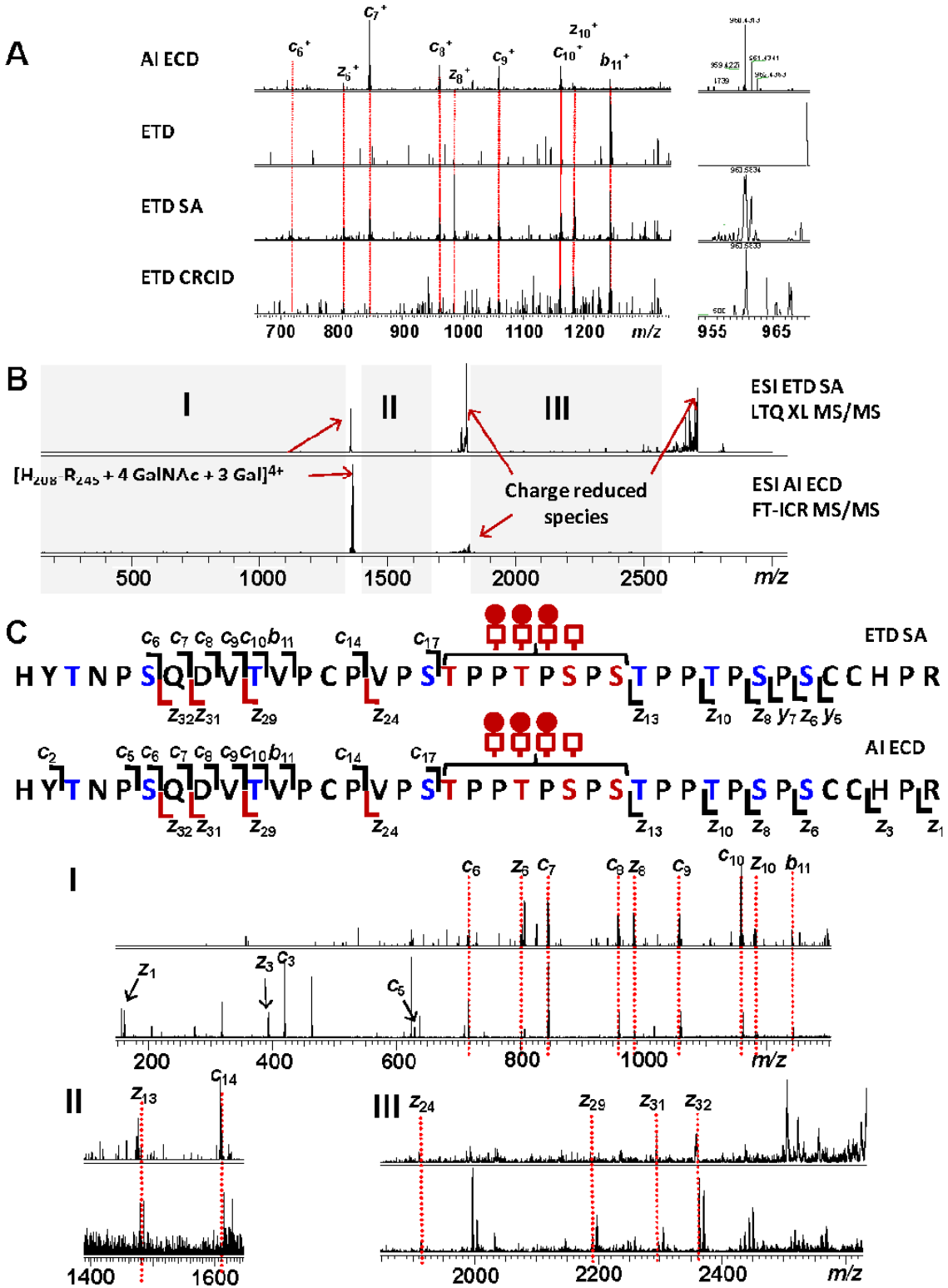


Figure 5

Takahashi *et al., MCP*, 2010



Takahashi *et al., MCP*, 2010

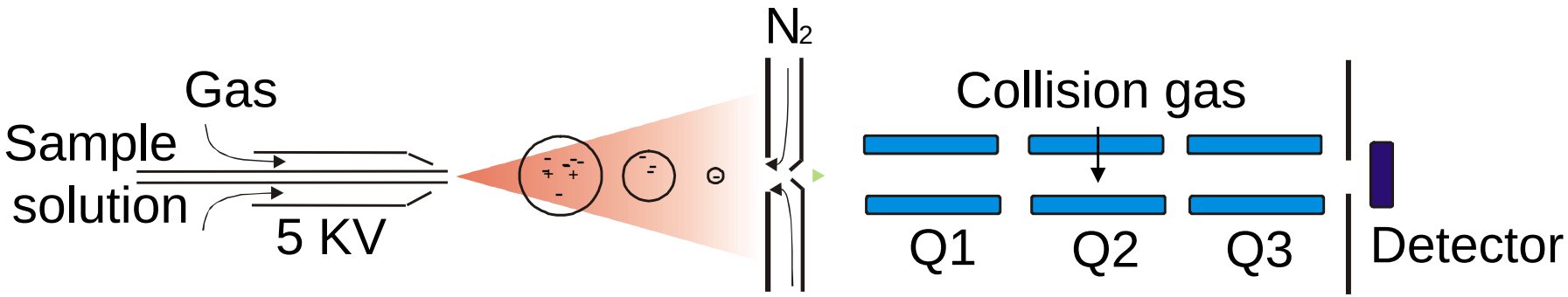
What affects fragmentation?

- Chemical composition
- Peptides
  - Amino Acid Sequence
  - Adjacent Amino Acids
  - Charge State
  - Location of Charge
  - Size
  - Mechanism of Fragmentation
  - Double Bonds
  - Electrophiles
  - Proton Mobility
  - Gas phase chemistry

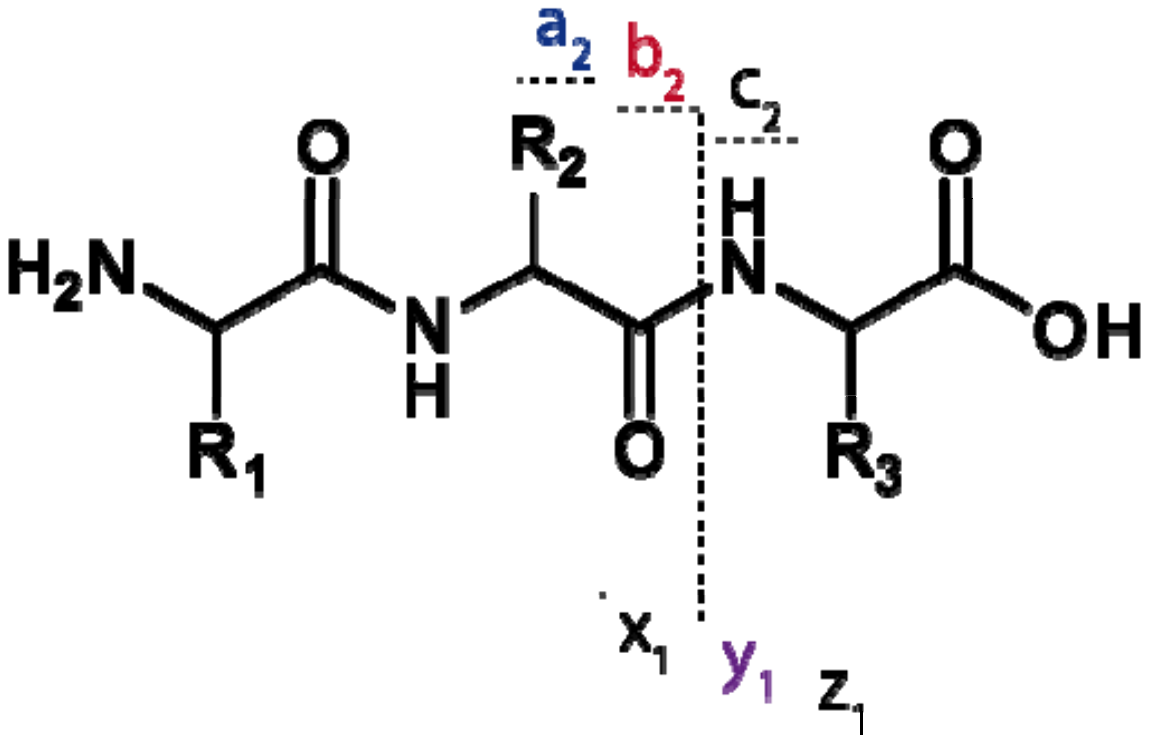
What can you do with a fragment besides identify?

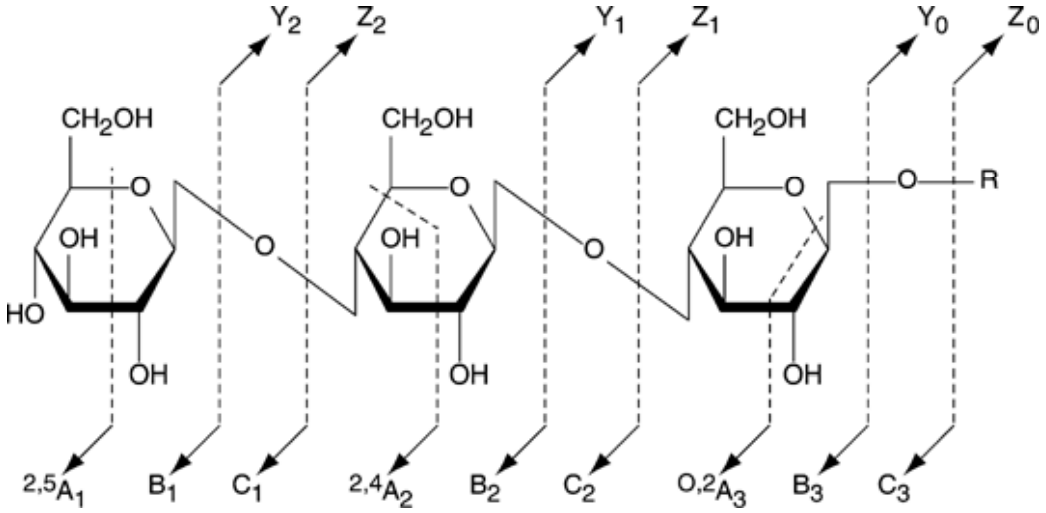
- Localize PTMs
- Use it to establish signatures for specific ion species
    - Biomarker
- Use it to quantify
- Use it to convict a felon
- Add a fragment to create an isobaric tag for quantitative comparison.
- Use it to filter out the information (spectra) you want

Tandem mass spectrometry on a triple quadrupole instrument

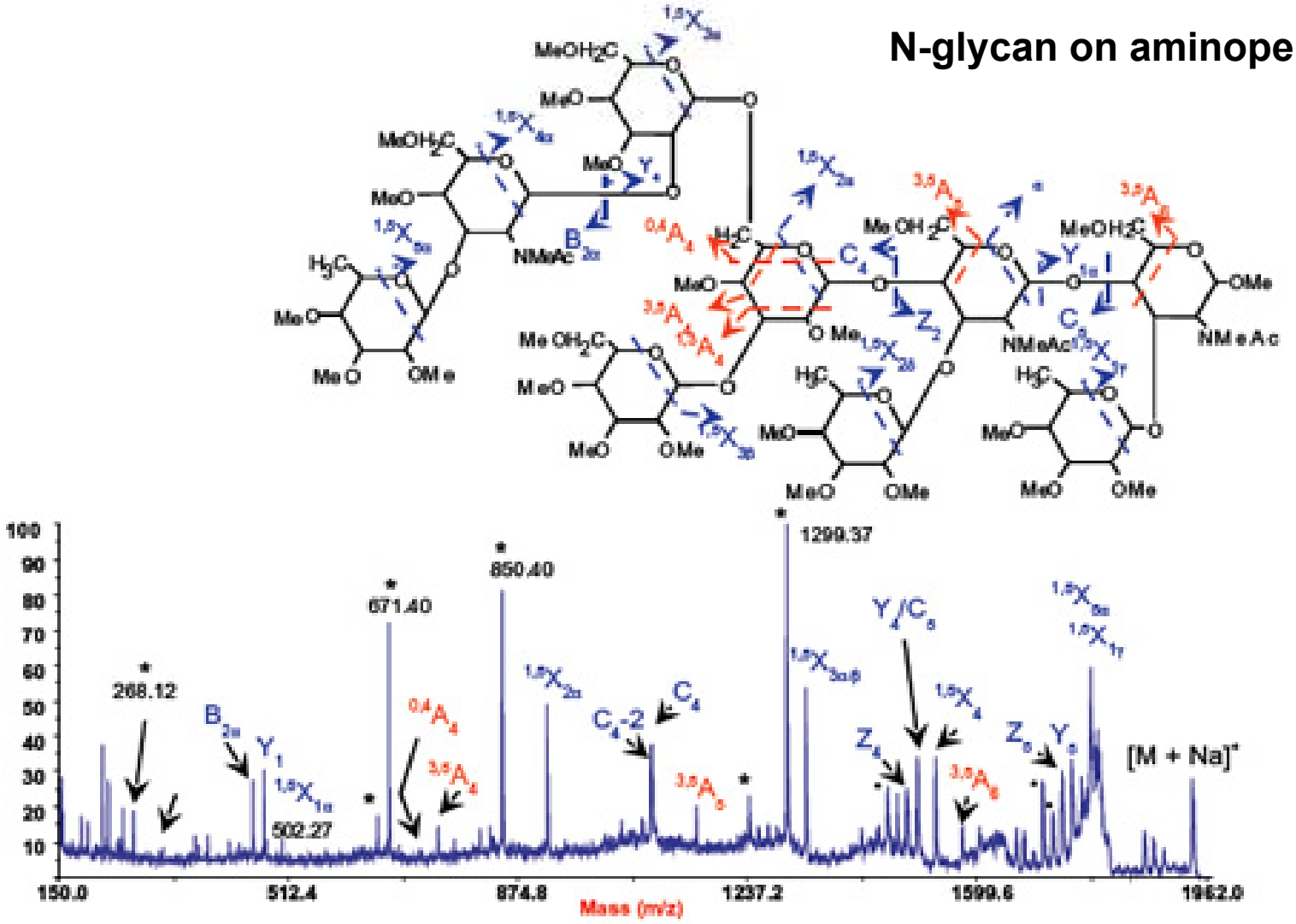


- **Daughter ion spectra**
- **Parent ion spectra**
- **Multiple reaction ion scanning**


a2
b2
c2
O
R2
H
N
O
H2N
N
OH
H
R1
O
R3
x1
y1
z1

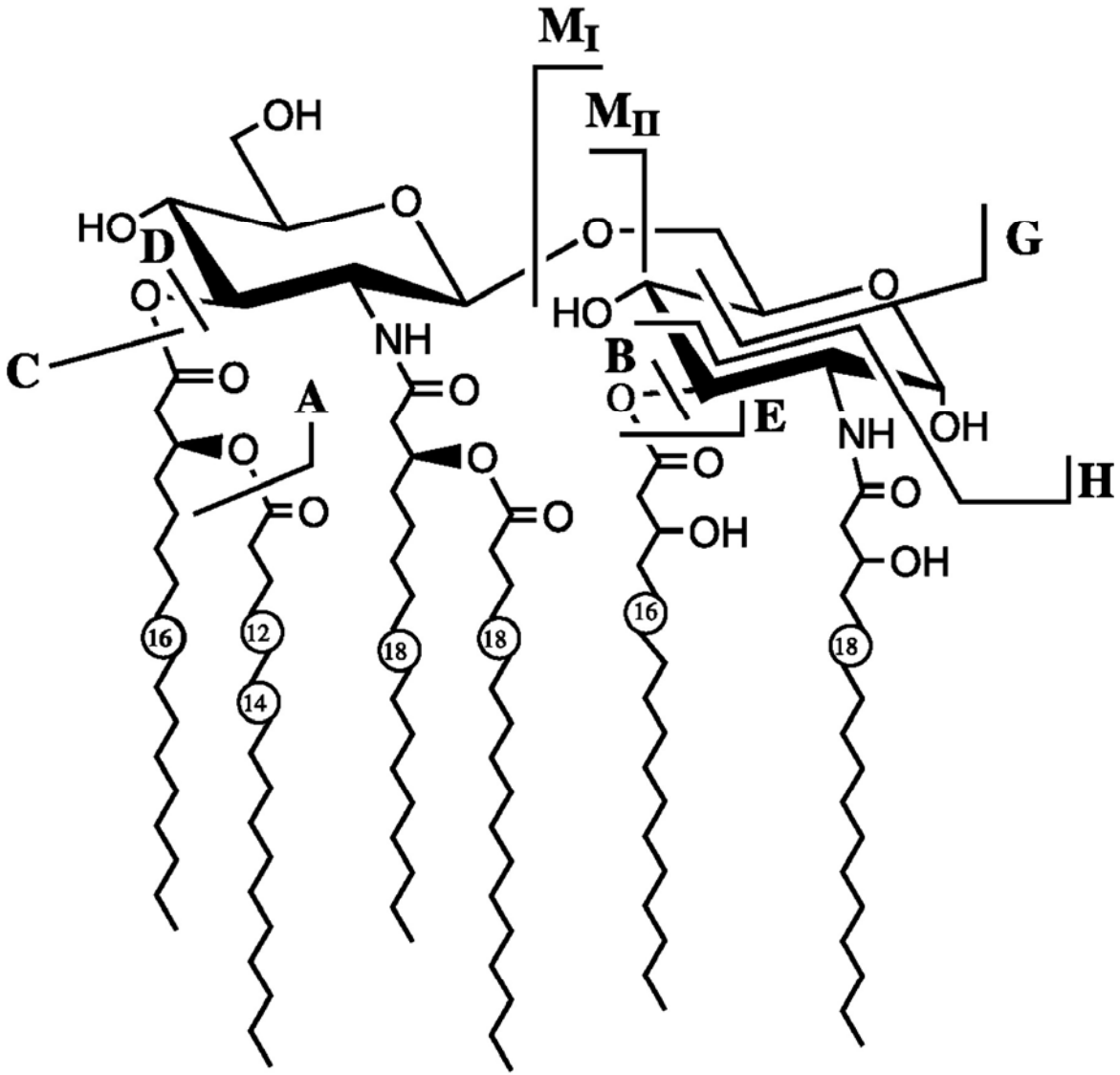

Y2
Z2
Y1
Z1
Y0
Z0
CH2OH
CH2OH
CH2OH
O
O
O
O
R
OH
OH
OH
HO
O
O
OH
OH
OH
2,5A1
B1
C1
2,4A2
B2
C2
0,2A3
B3
C3

N-glycan on aminopeptidase 1



Stephens *et al., Eur J. Biochem*, 2004

fragmentation pattern of dephosphorylated lipid A derived from LPS1 (LA1

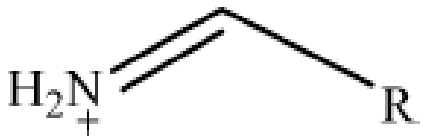


Moran et al., *JBC* 2002

Other amino acid fragment ions

m/z values of common immonium ions

Immonium ion (*m/z*)	Amino acid residue	Major (M) or minor (m) peak
60.04	S	M
70.07	R or P	M
72.08	V	M
73.00	R	m
74.06	T	M
84.08	K or Q	M
86.1	I or L	M
87.09	N or R	M
88.04	D	M
100.09	R	m
101.11	K or Q	M
102.06	E	M
104.05	M	M
110.07	H	M
112.09	R	M
120.08	F	M
126.06	P	M
129.1	K or Q	m
136.08	Y	M
138.07	H	m
159.09	W	M



Wysocki et al. 2005