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MASS SPECTROMETRY & STANFORD CHEMISTRY

Brief intro to Mass Spectrometers

Ionization: ESI & APCI

SUMS Instrumentation

MS – Organic, Organometallic &
Biomolecule

HPLC-MS





The Mass Spectrometer: Operation

Steps in generating a mass spectrum:

1. Produce ions
2. Separate or filter ions
3. Detect ions
4. Process the data



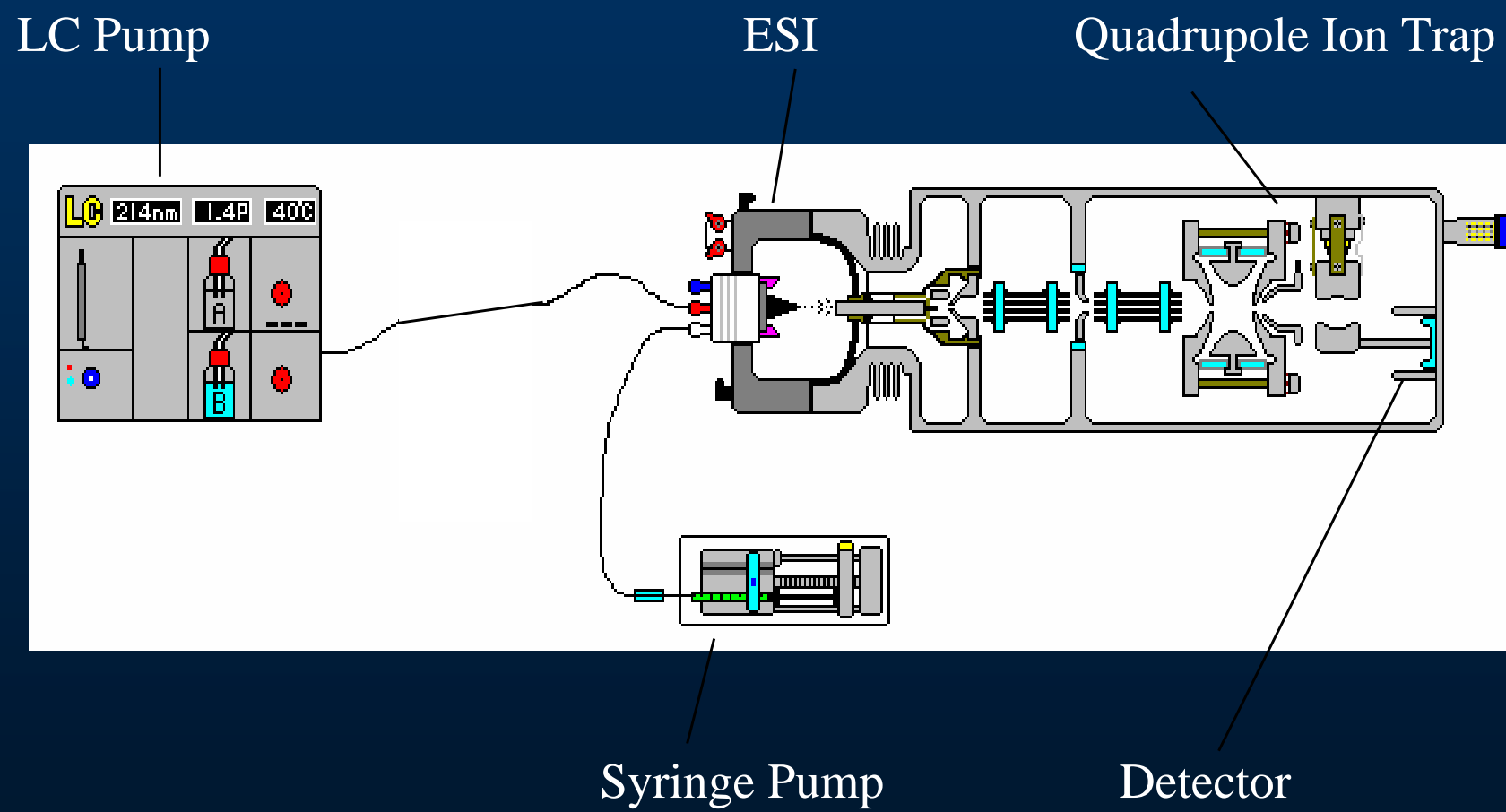
The Mass Spectrometer: Components

1. Ion source/interface
2. Mass analyzer, including:
 - a. Mass analyzer (quadrupole, ion trap, TOF, *etc.*)
 - b. Vacuum system
 - c. Some electronics
3. Detector
4. Data storage, (processing), and output device (usually a computer)





Quadrupole Ion Trap



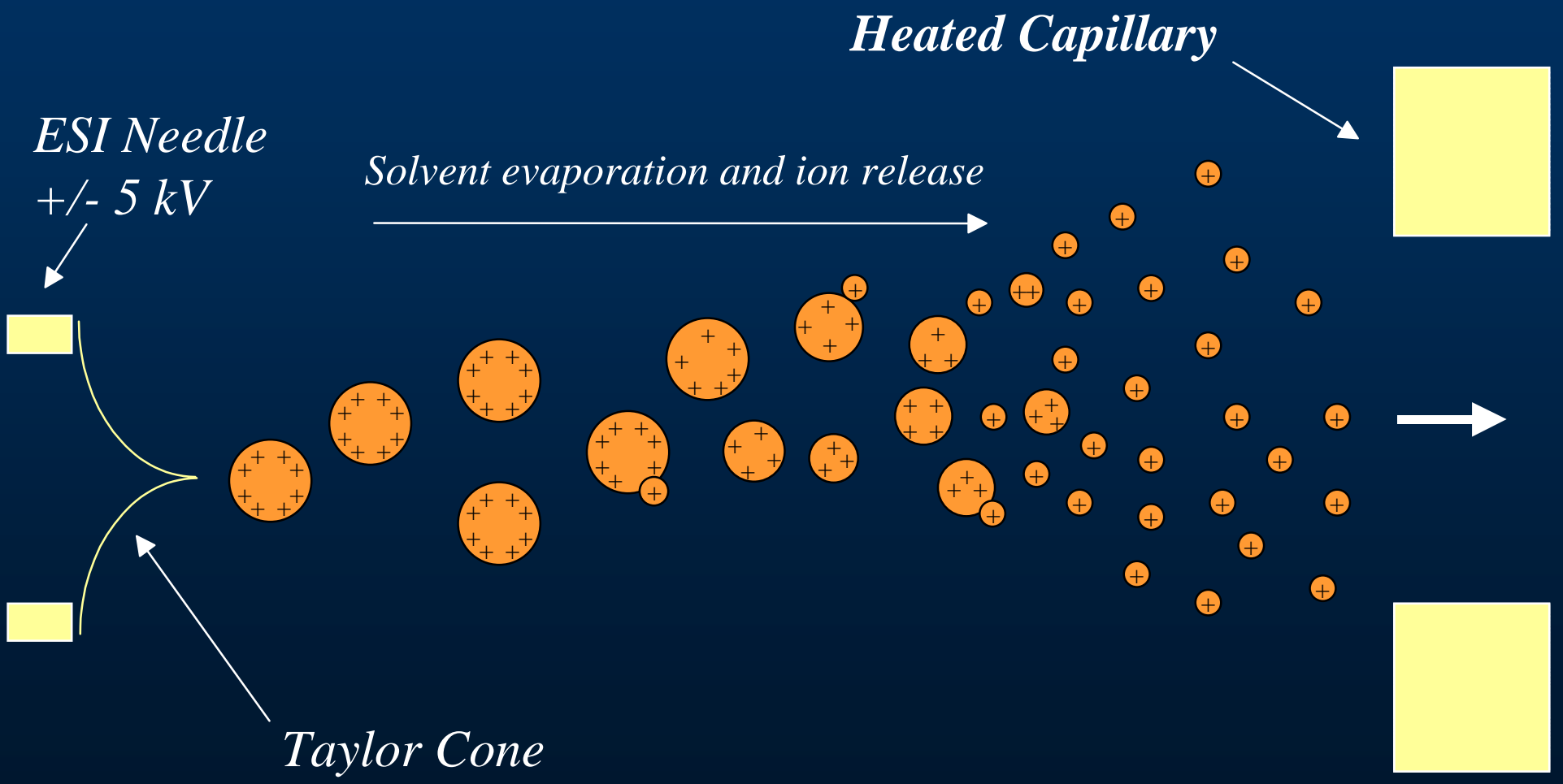


What is API?

- Atmospheric Pressure Ionization
 - ESI – Electrospray Ionization
 - Solution-phase process (for the most part)
 - APCI – Atmospheric Pressure Chemical Ionization
 - Gas-phase process
- An interface between HPLC and Mass Detection
 - Designed to separate and ionize analytes from HPLC solvents



Electrospray – Basic Layout





APCI: Atmospheric Pressure Chemical Ionization

Mechanism for positive ion formation

Primary ion formation:



Secondary ion formation:



Analyte ion formation:



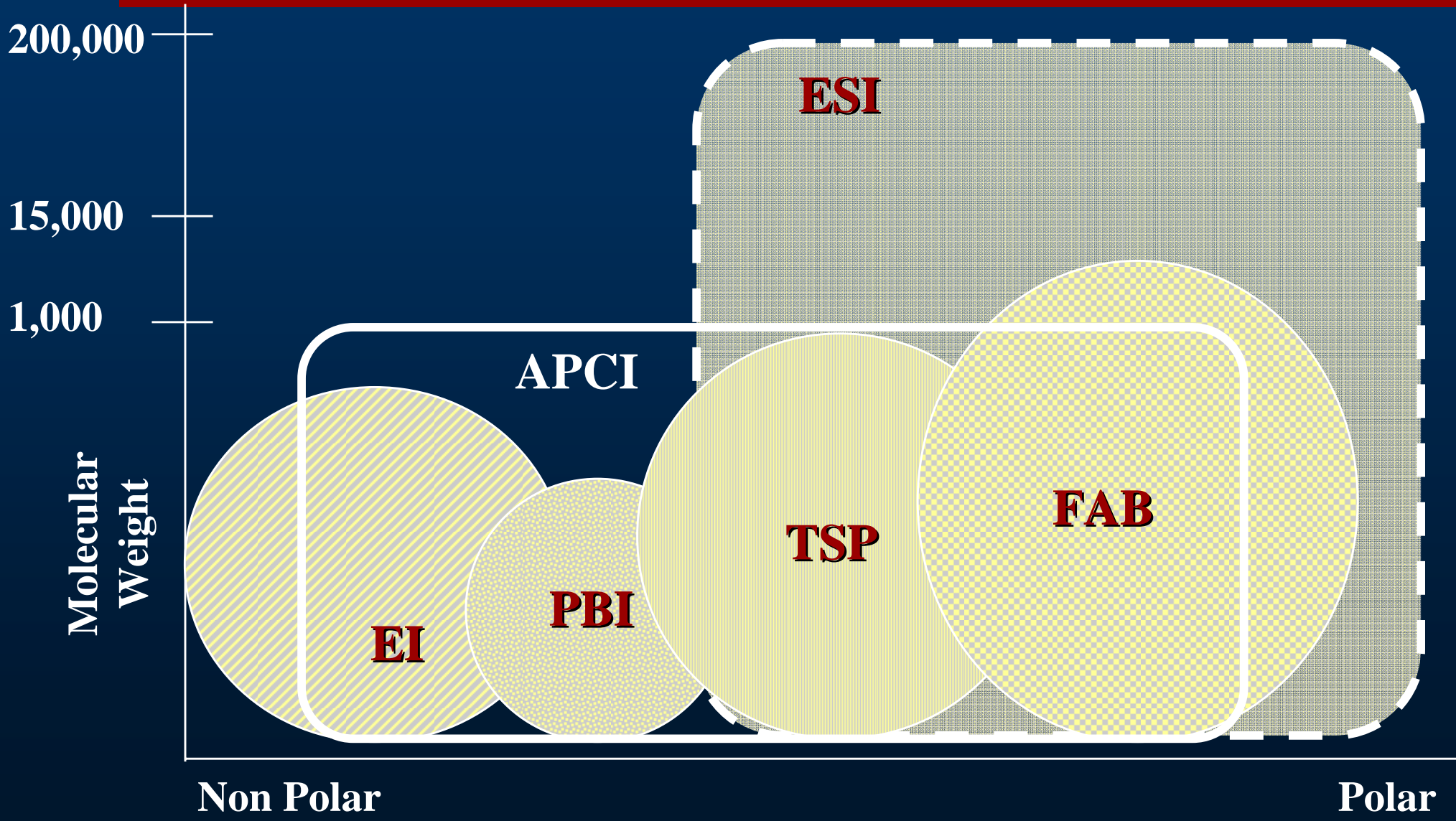


ESI or APCI ?

- Many compounds can be analyzed by both techniques with different sensitivities
- ESI is for highly polar compounds
- ESI is for molecular weights >1000 amu
- ESI is for thermally fragile compounds
- APCI generally gives more fragmentation



Analyte Compatibility



-
-
-
-
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-
-
-
-

SUMS Instrumentation





LCQ Classic MS

- Quadrupole Ion Trap LC-MS
- ThermoFinnigan Surveyor HPLC & LCQ "Classic" MS
 - MW determination
 - Analytical LC-MS
 - MSⁿ





LCQ Deca XP Plus MS

- LC Packings Capillary HPLC System & ThermoFinnigan LCQ Deca XP Plus MS

- Capillary LC-MS
- Protein identification & characterization
- Complex protein mixture analysis
- Assays, quantitation

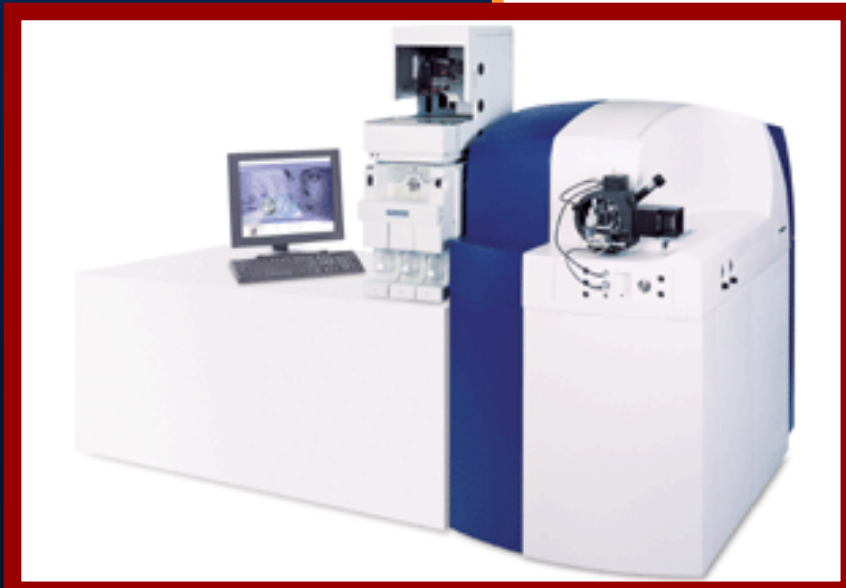
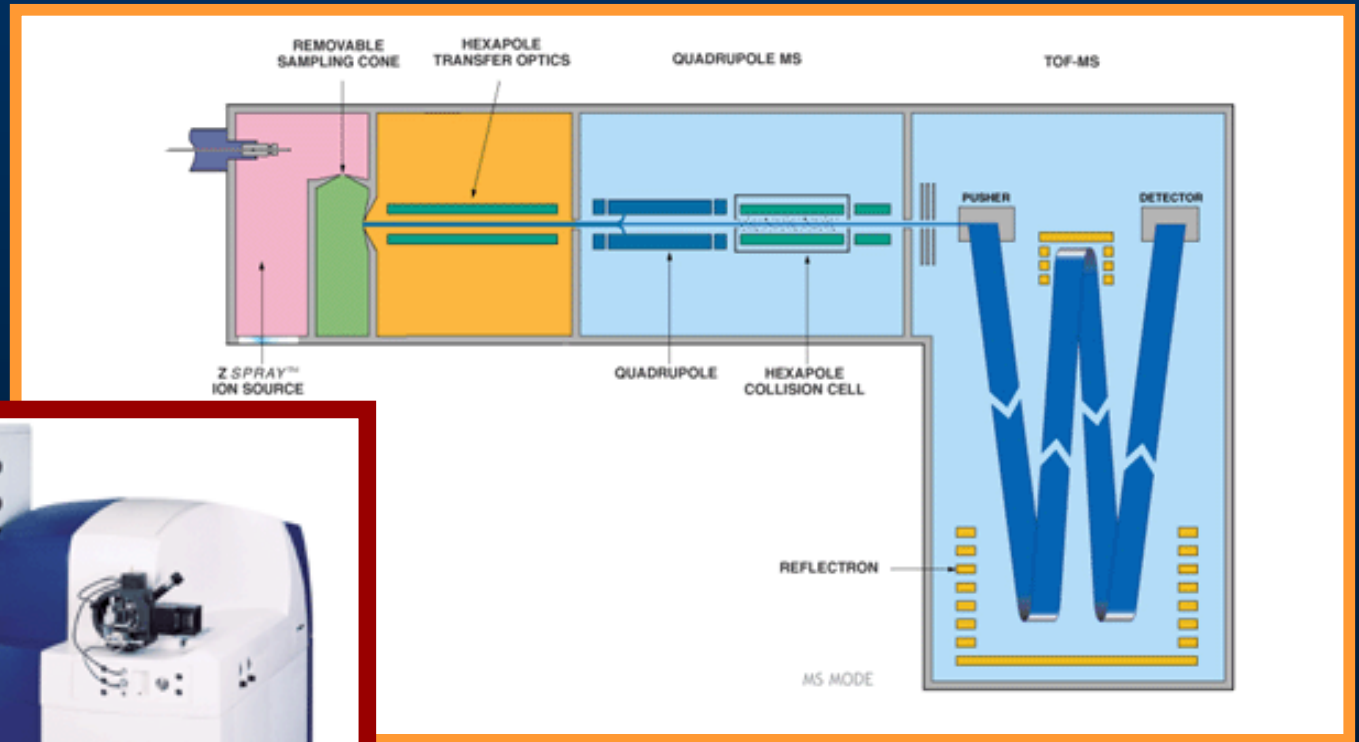




Q-ToF API

- Hybrid Tandem Quadrupole – Time of Flight MS

Micromass
Q-Tof



- High resolution MS
- Protein identification & characterization
- *De novo* peptide sequencing
- Post-translational modification ID



Mass Spec Examples



Organic Compounds

Organometallic Compounds

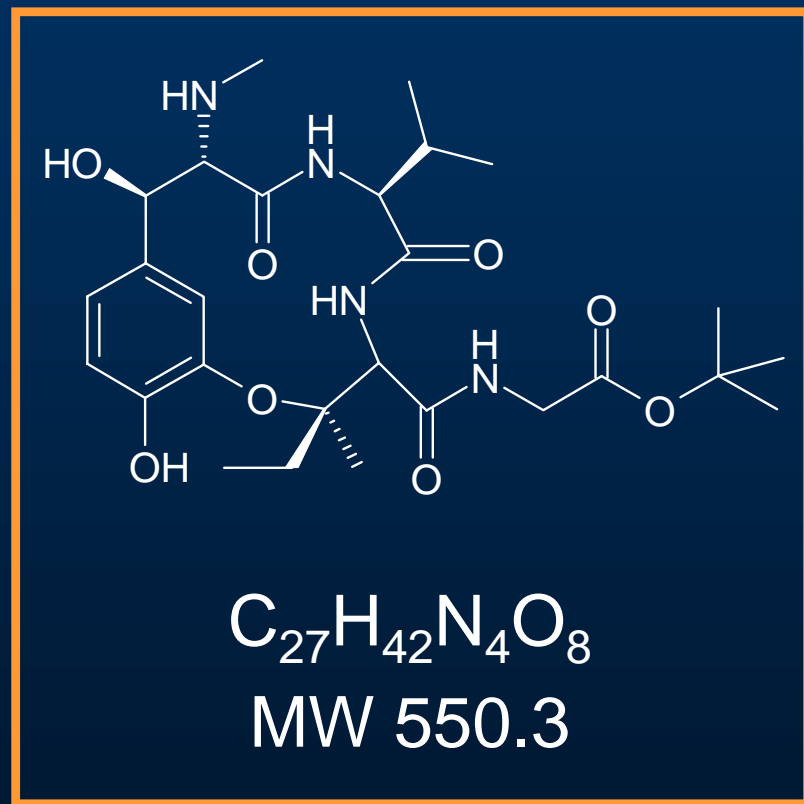
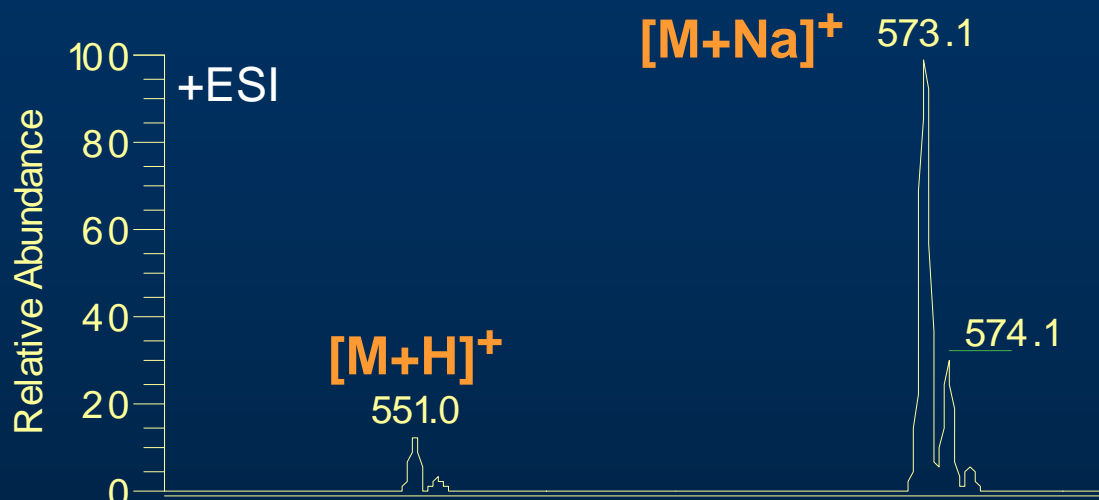
Biomolecules

LC-MS

High Resolution MS

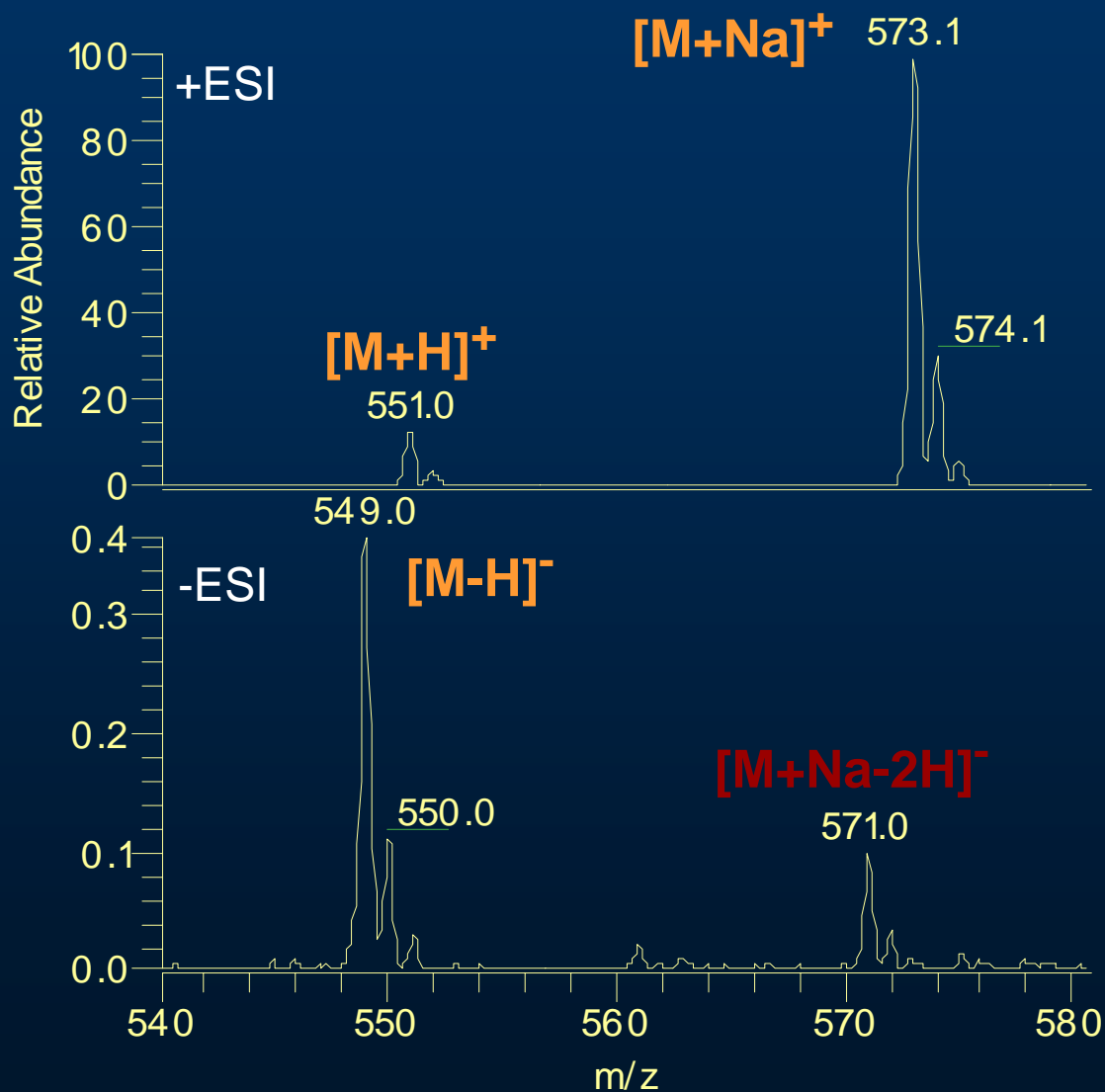


Commonly Observed Ions in ESI



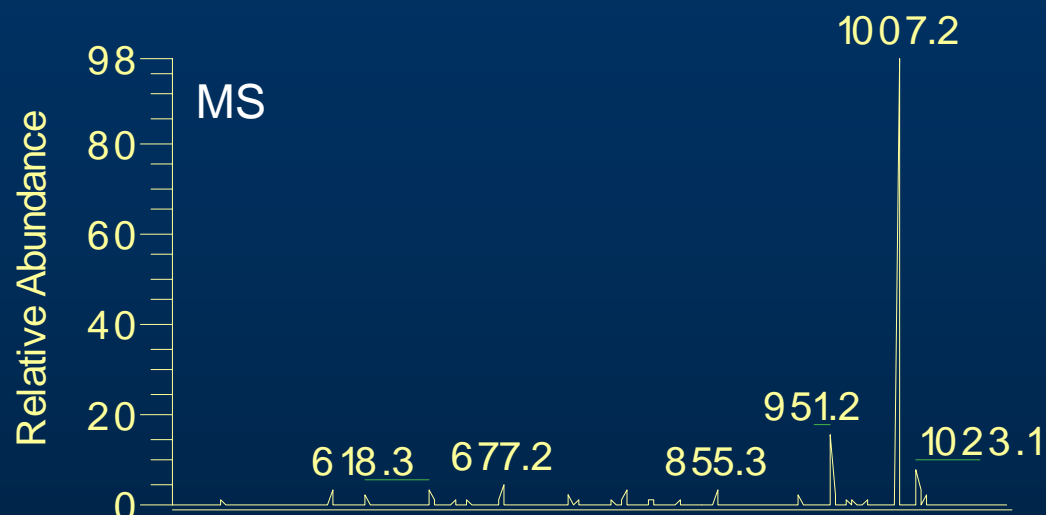


Commonly Observed Ions in ESI





Na⁺ Adduct

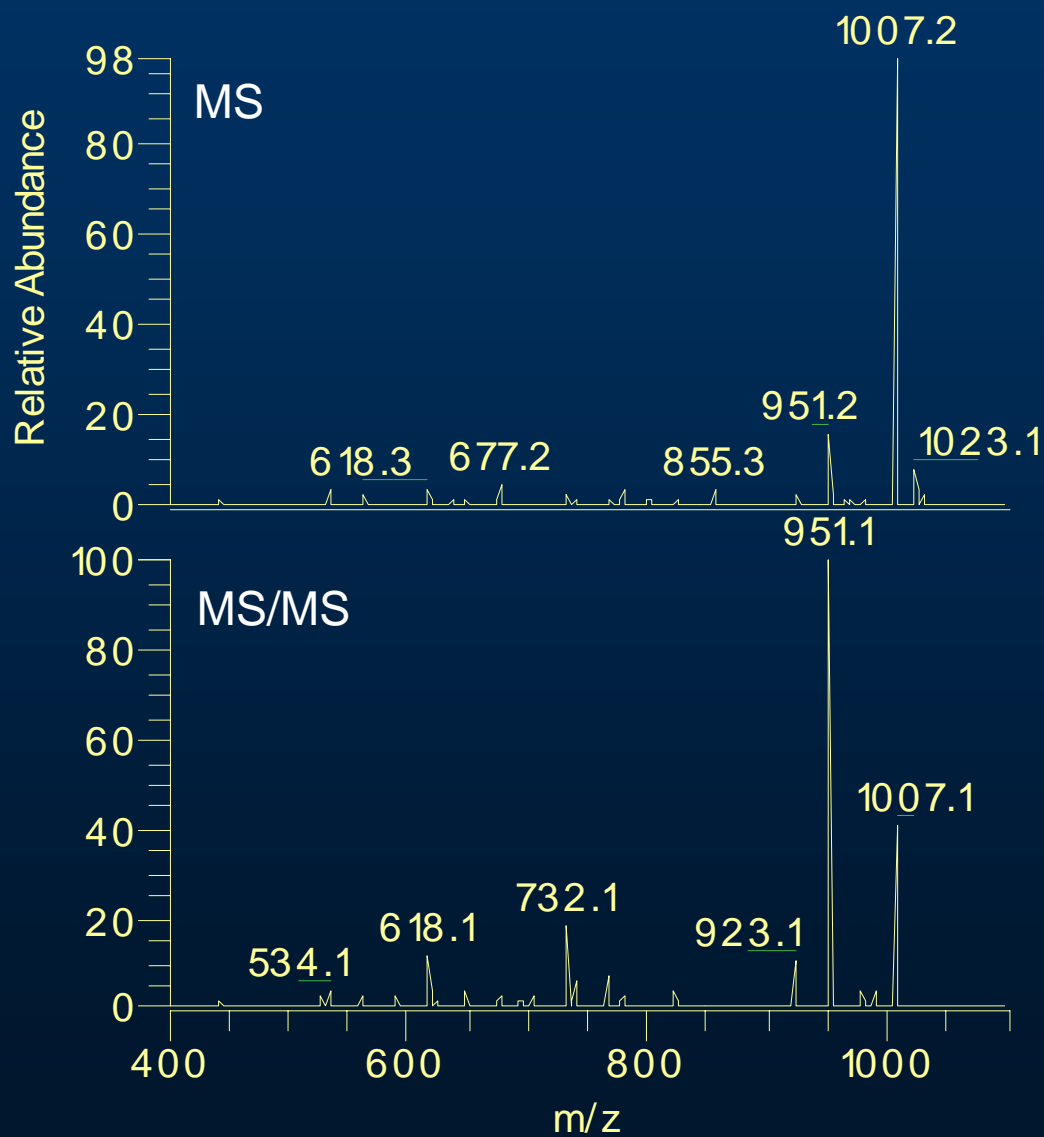


Hiroko Tanaka

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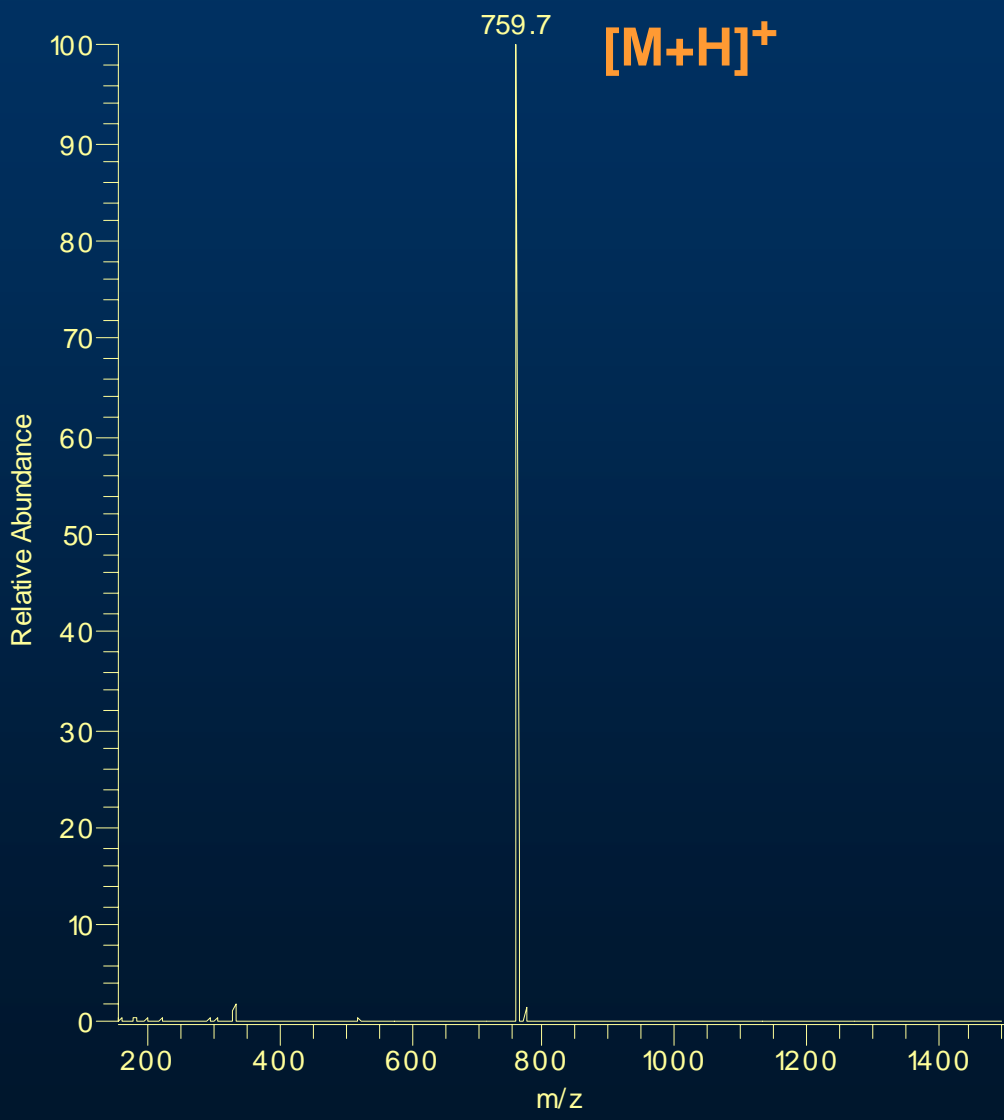
MS/MS Does Not Displace Na⁺



Hiroko Tanaka

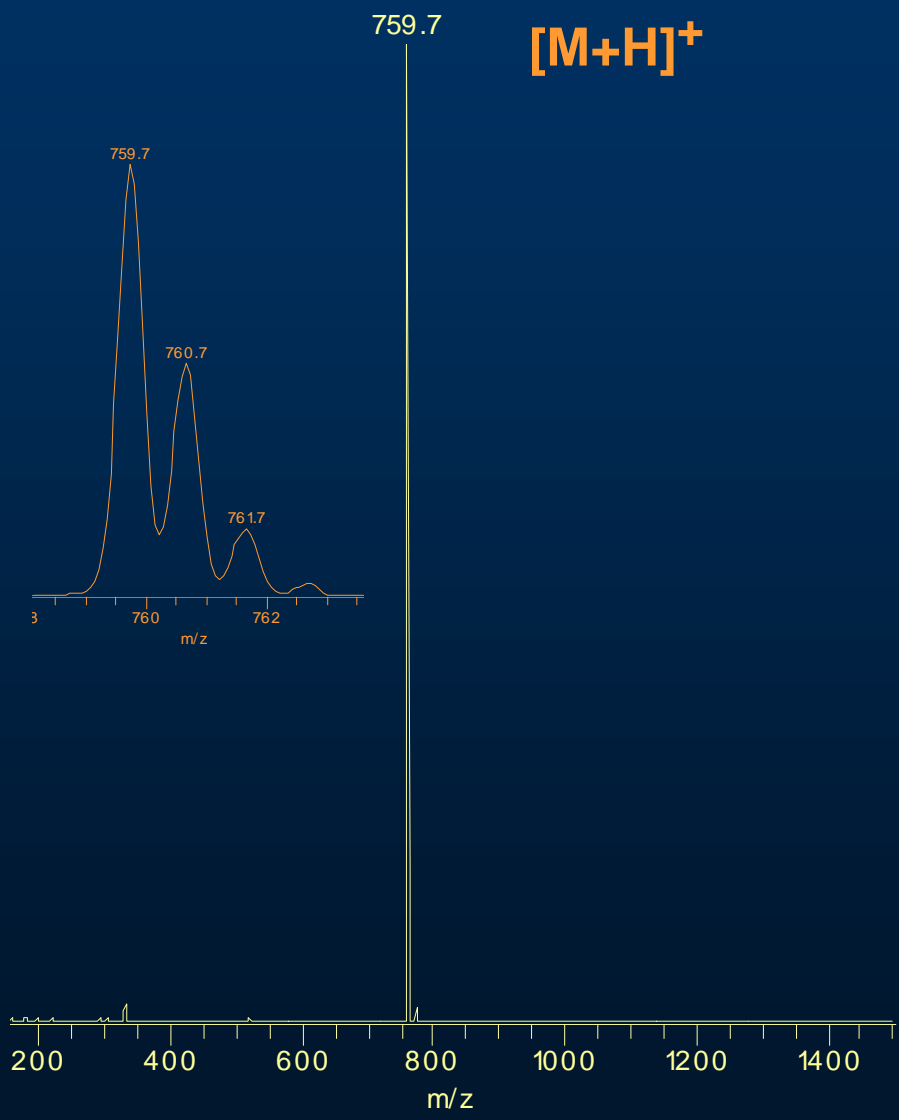


APCI of a Non-polar Compound



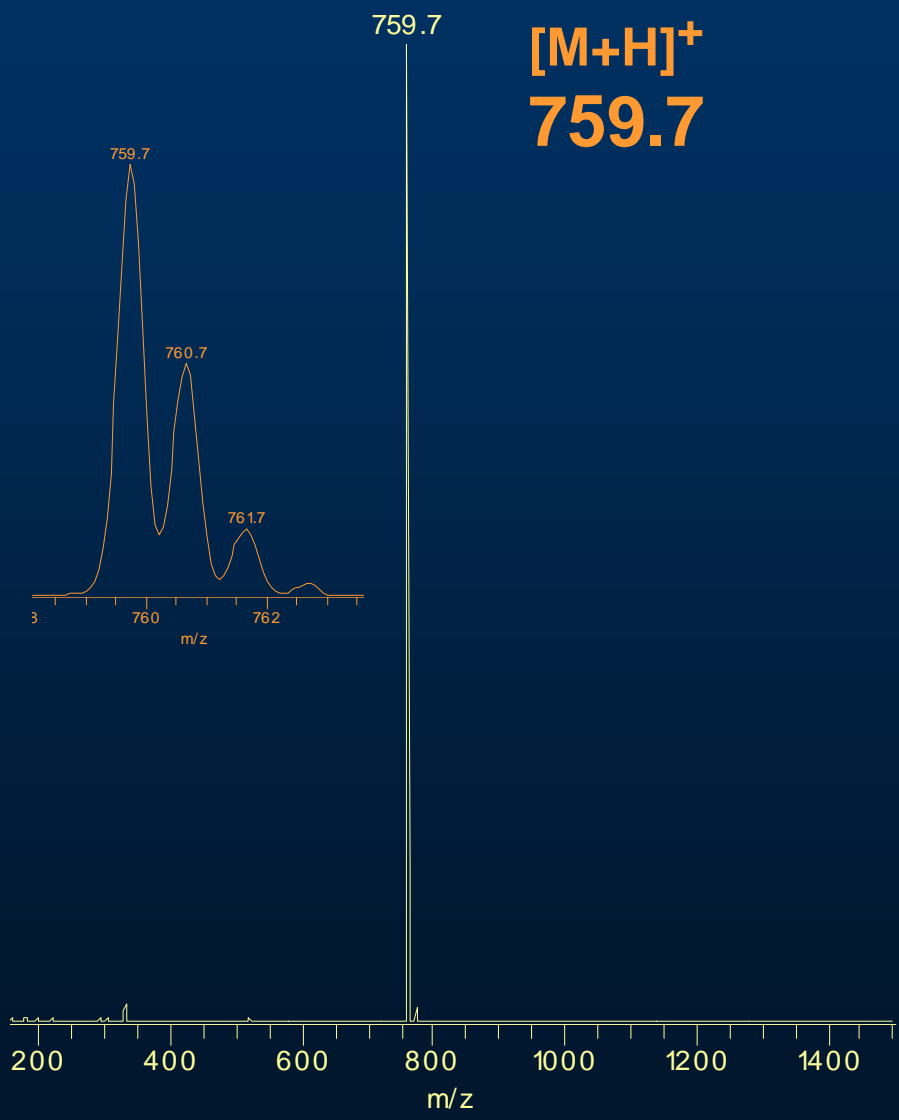


APCI of a Non-polar Compound

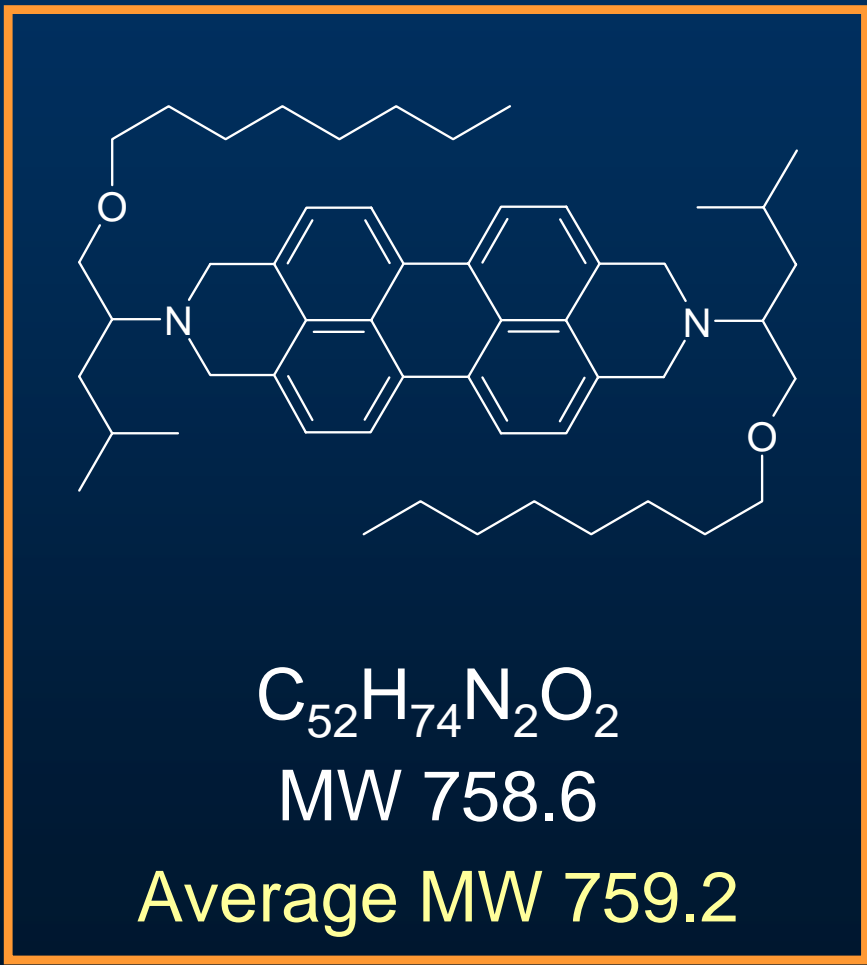




APCI of a Non-polar Compound

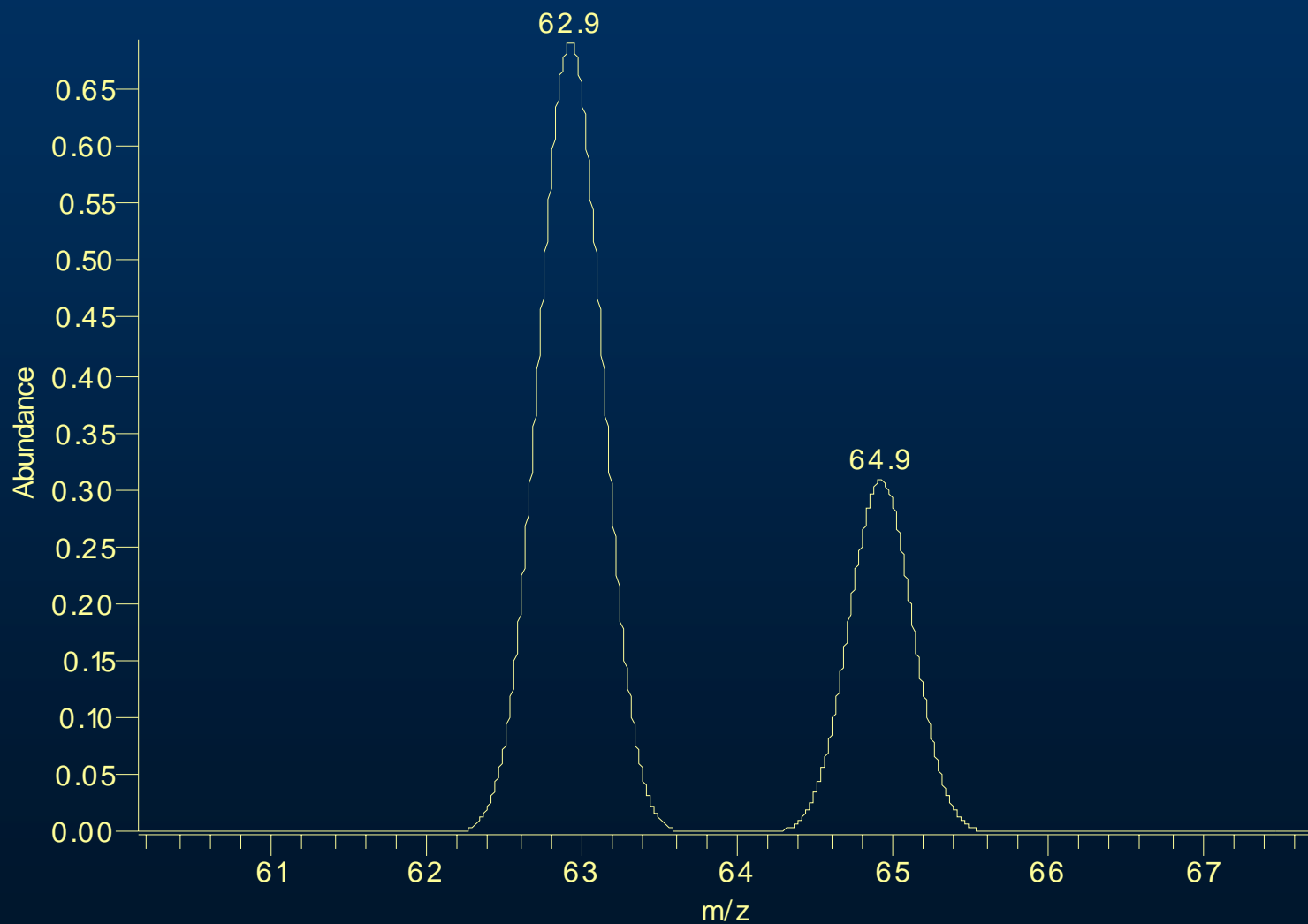


[M+H]⁺
759.7





Cu Isotope Pattern

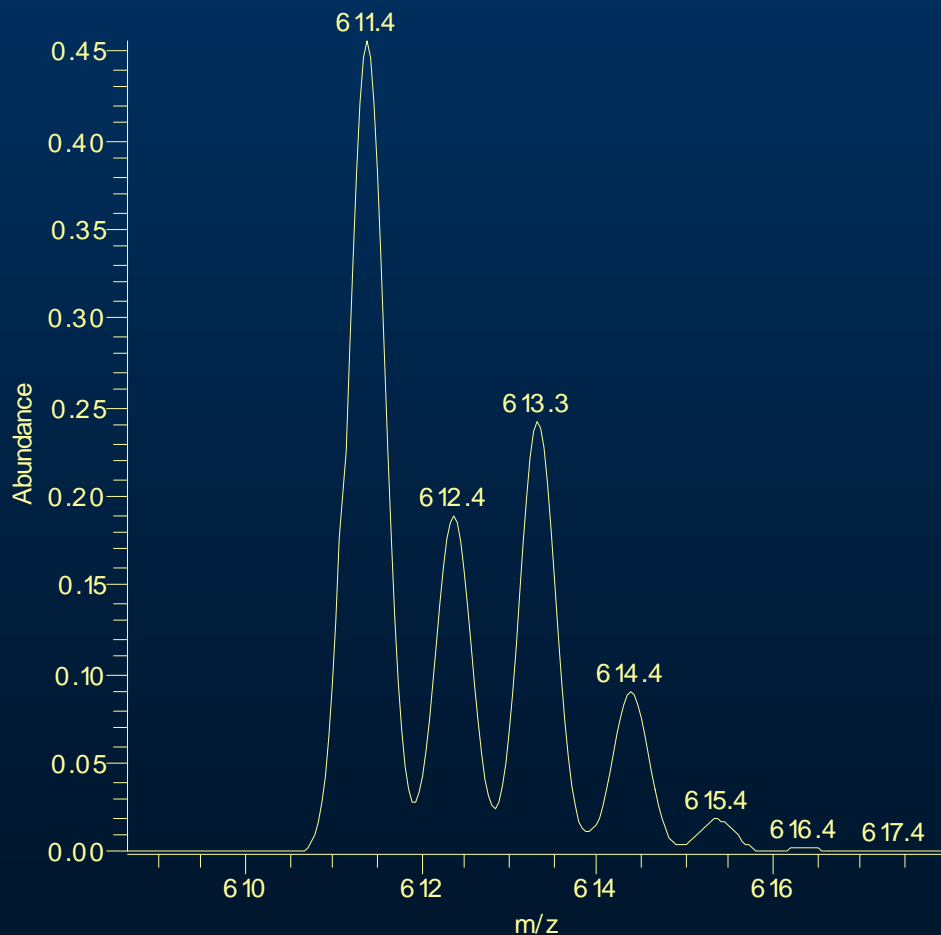


Simulation
Cu
Profile
Resolution:
Daltons 1
at 5%height
Charges 1
Chrg dist 0
Ions 2
Min Ion Ab. 1e-020
Min Ions 5000
Max Ions. 20000

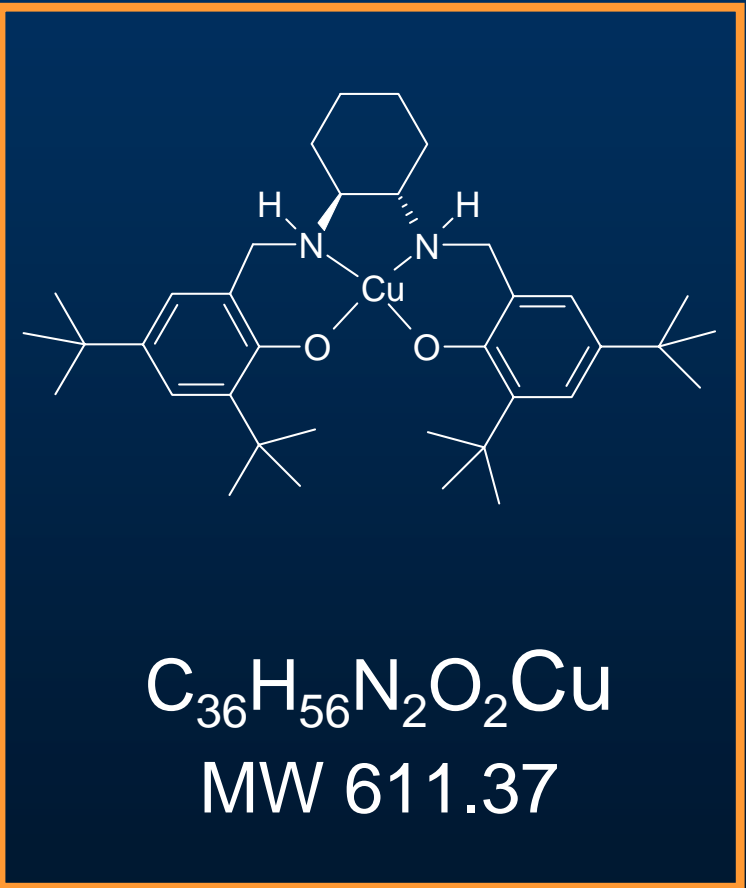


Cu Compound: Theoretical MS

C36 H56 N2 O2 Cu1

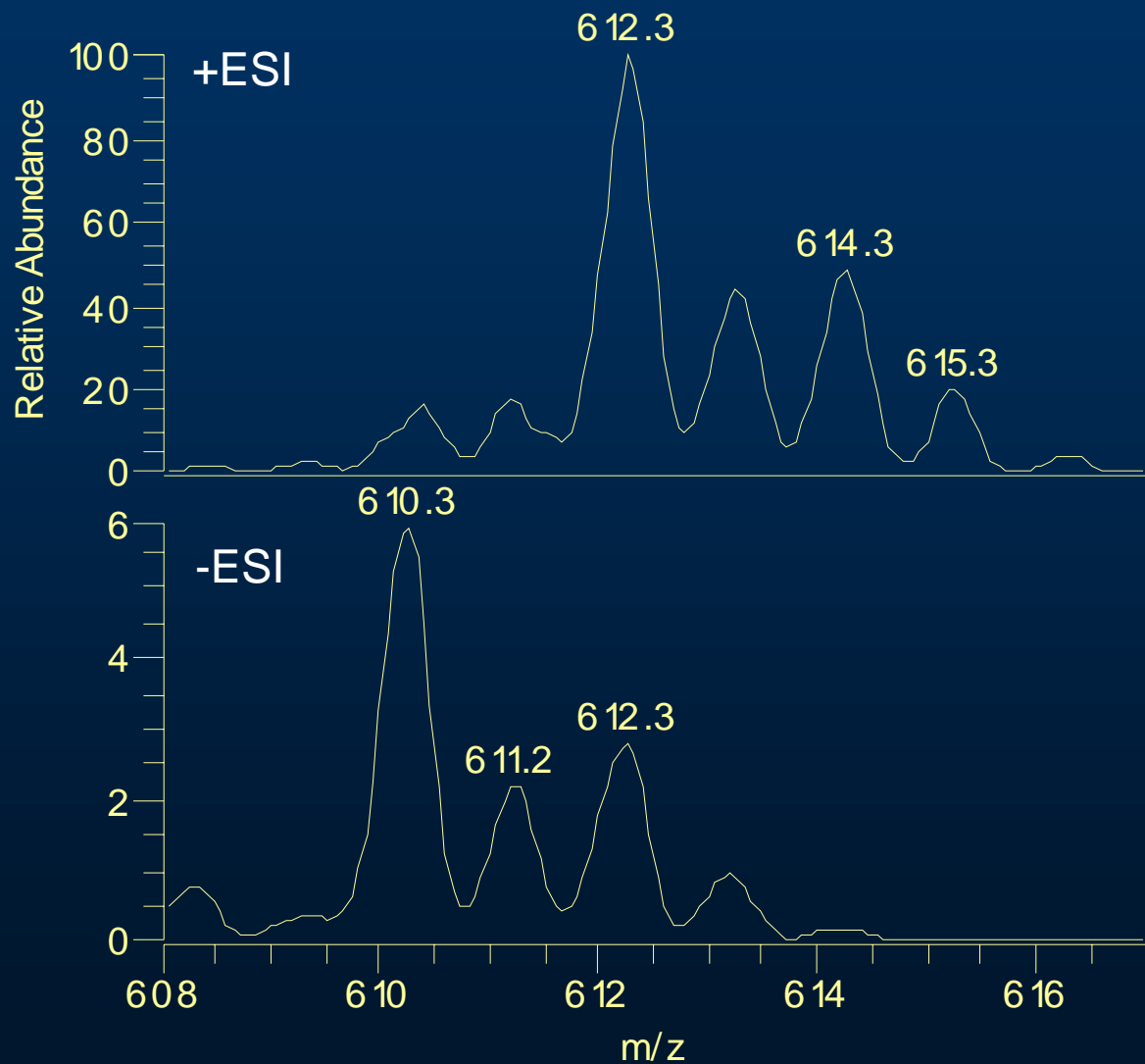


Simulation
C36H56N2O2Cu
Profile
Resolution:
Daltons 1
at 5%height
Charges 1
Chrg dist 0
Ions 3328
Min Ion Ab. 1e-020
Min Ions 5000
Max Ions. 20000



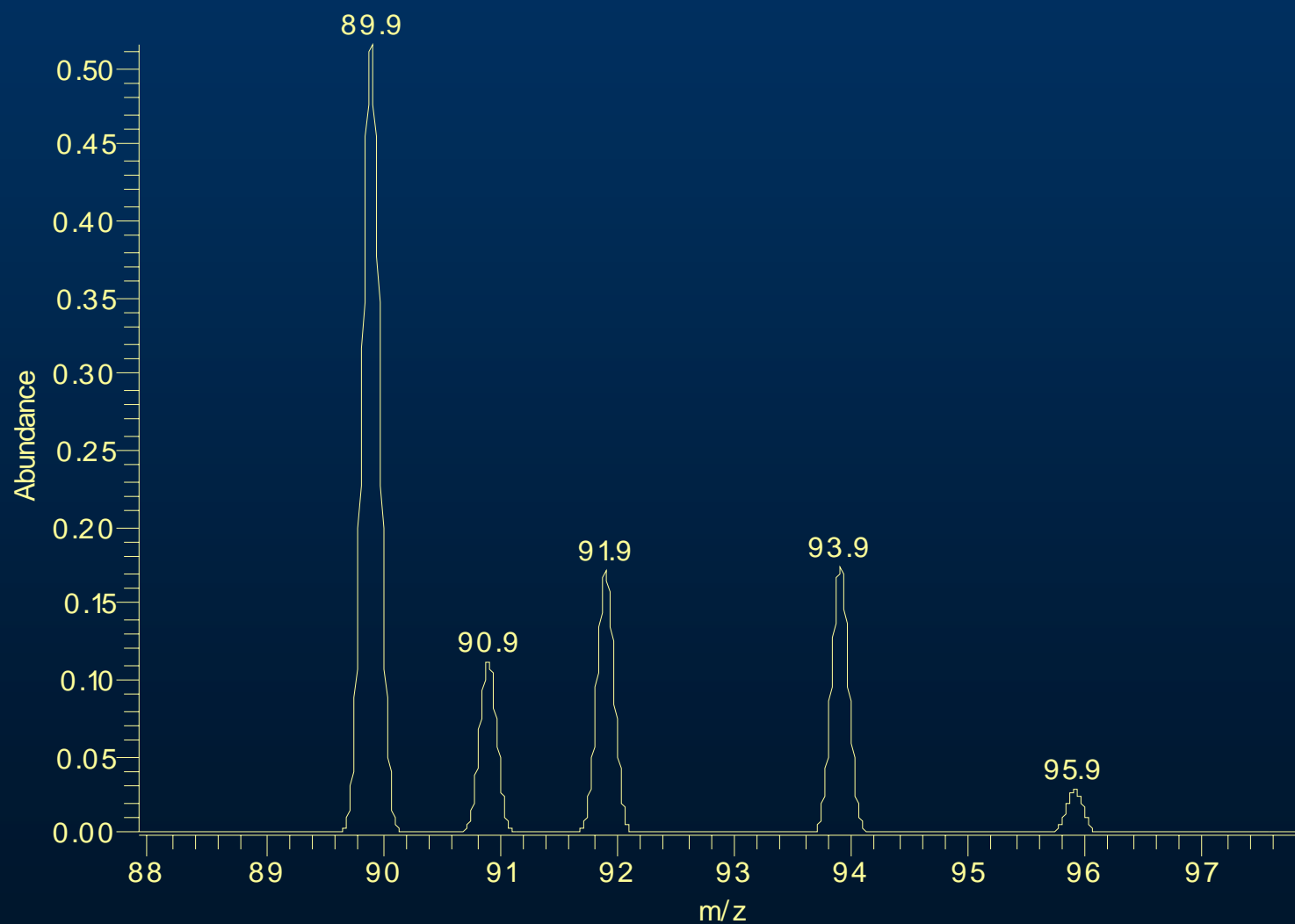


Cu Compound: +ESI and -ESI





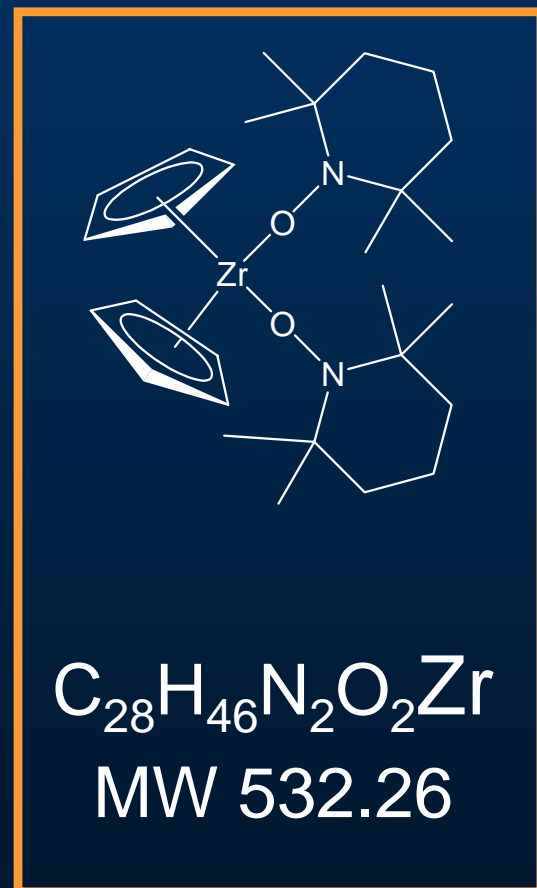
Zr Isotope Pattern



Simulation
Zr
Profile
Resolution:
Daltons 0.35
at 5%height
Charges 1
Chrg dist 0
Ions 5
Min Ion Ab. 1e-020
Min Ions 5000
Max Ions. 20000

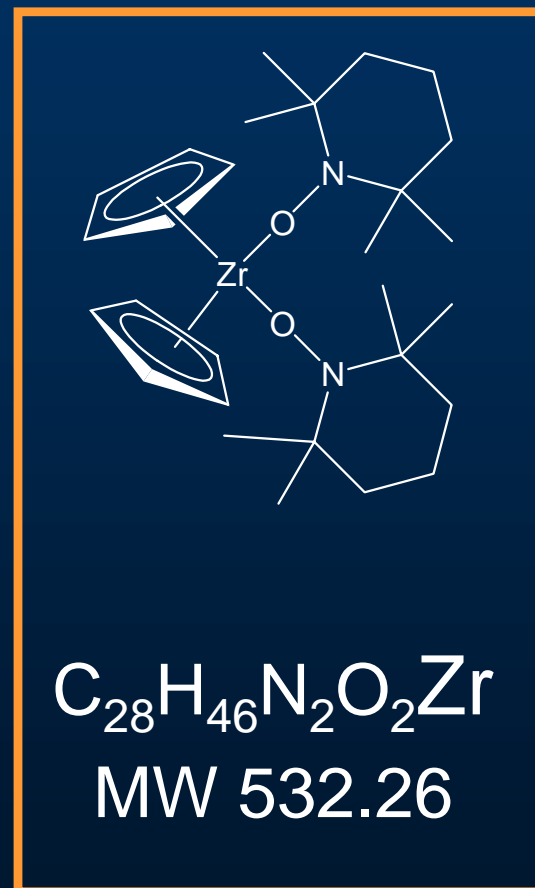
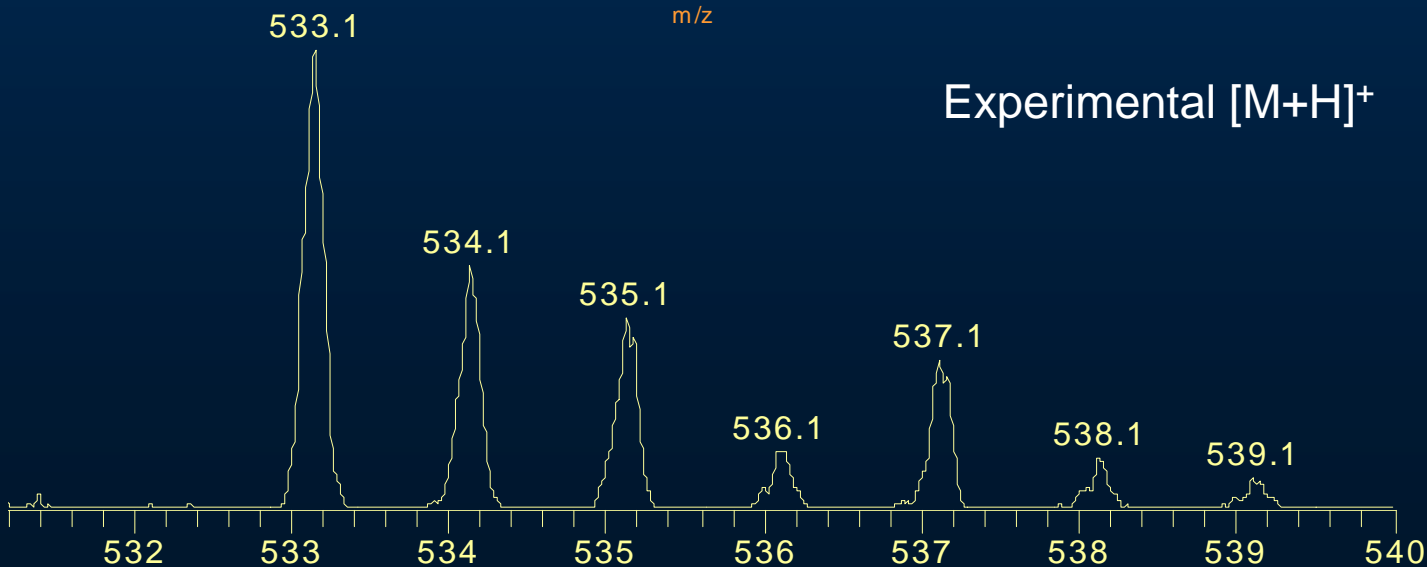
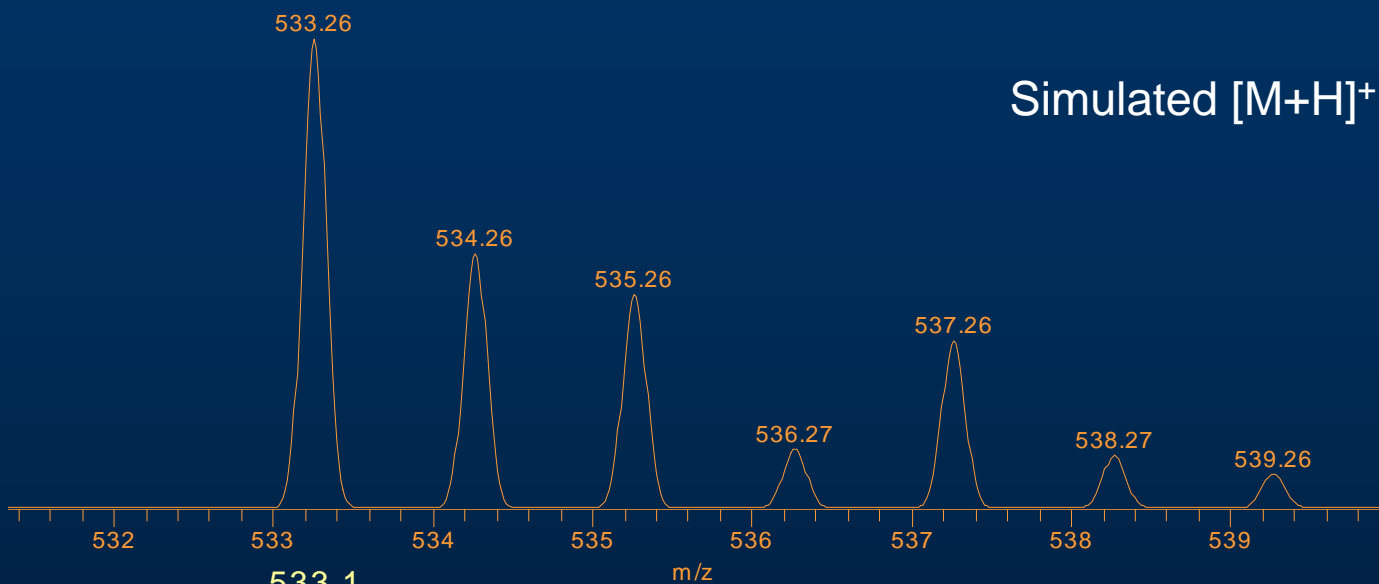


Zr Compound: Theoretical MS



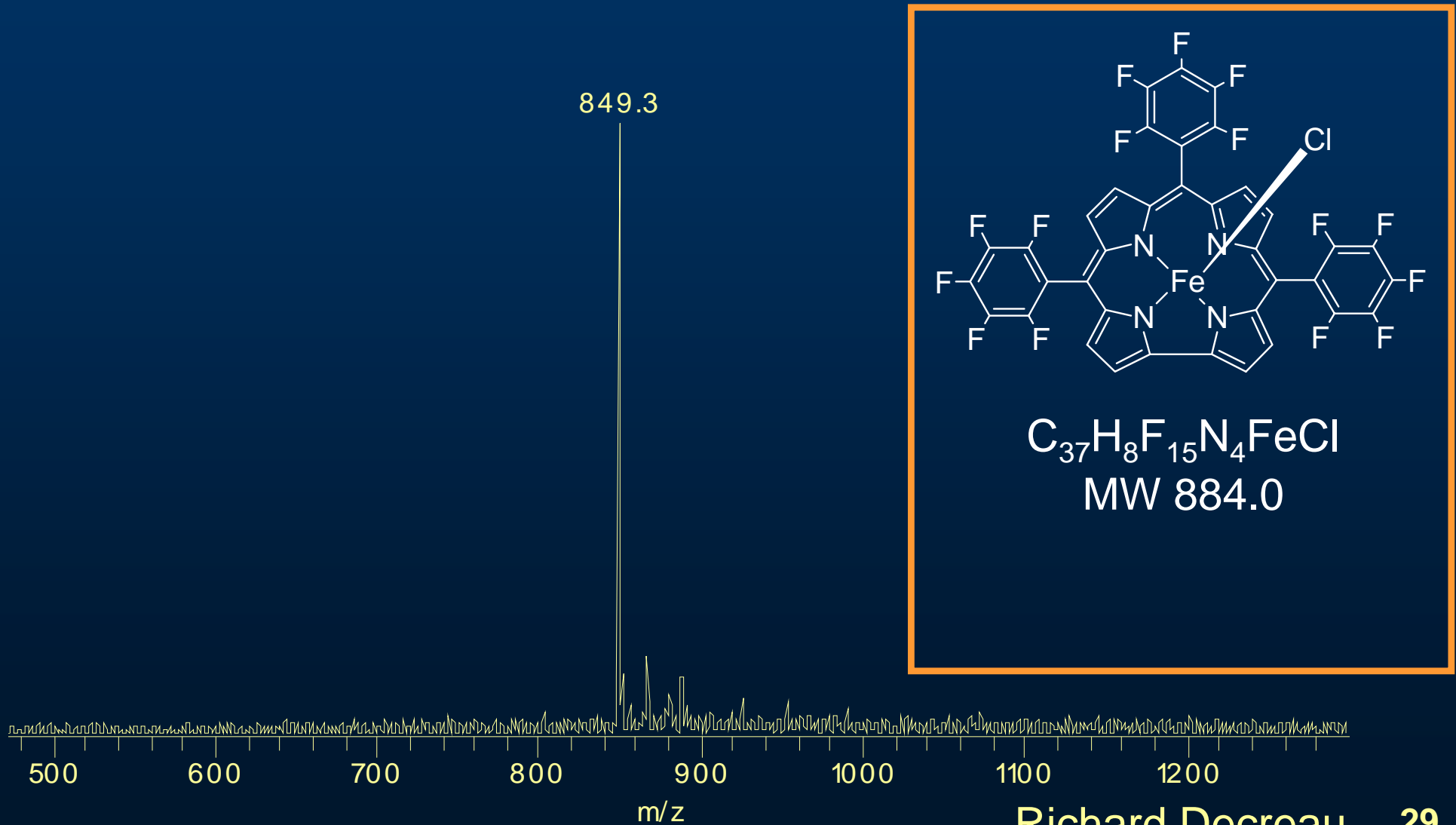


Theoretical vs. Experimental



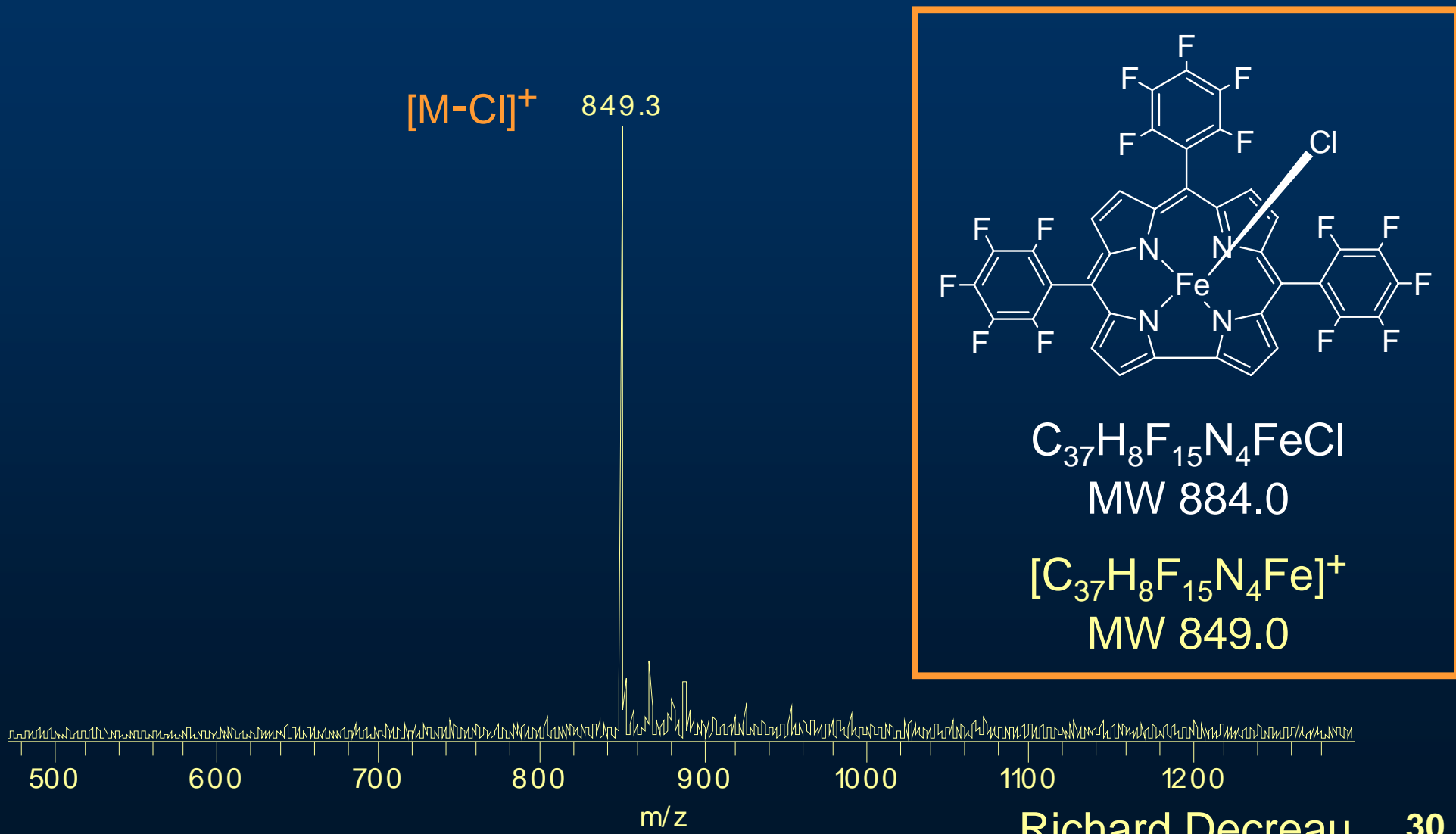


Loss of Counterion



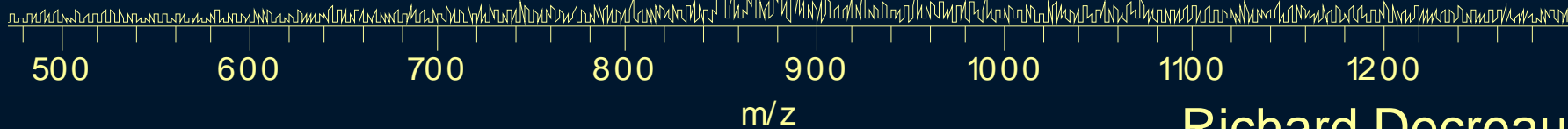
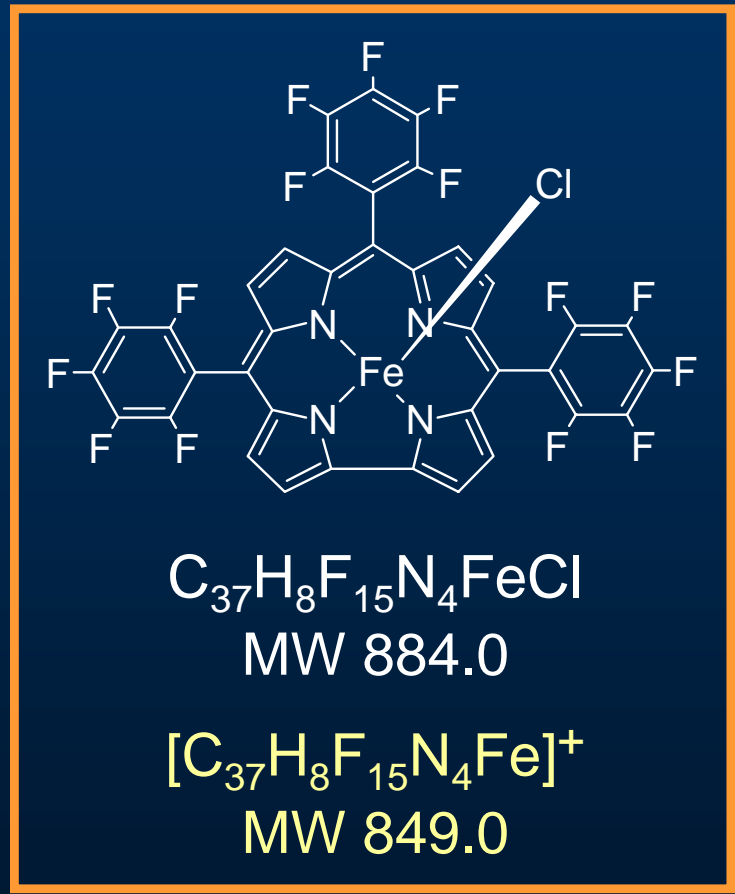
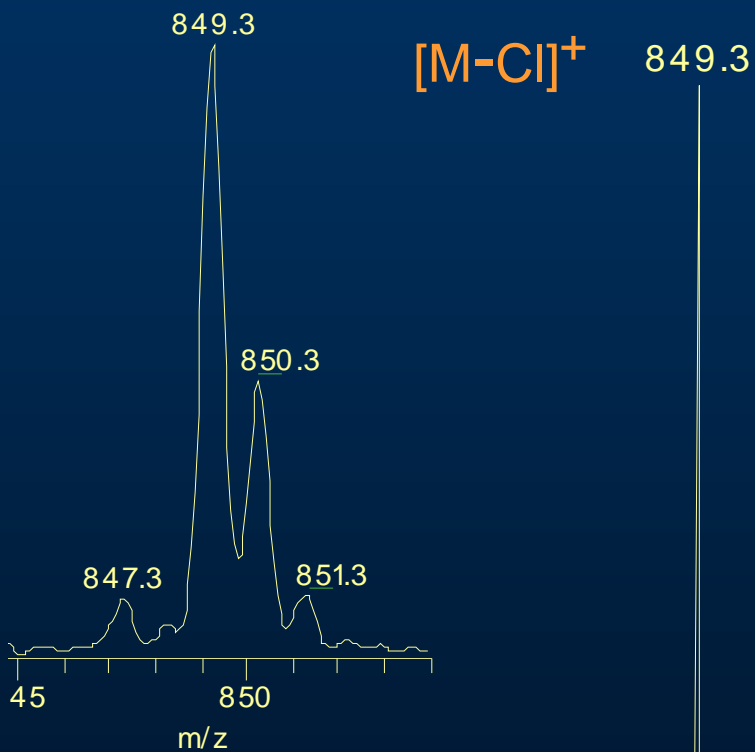


Loss of Counterion



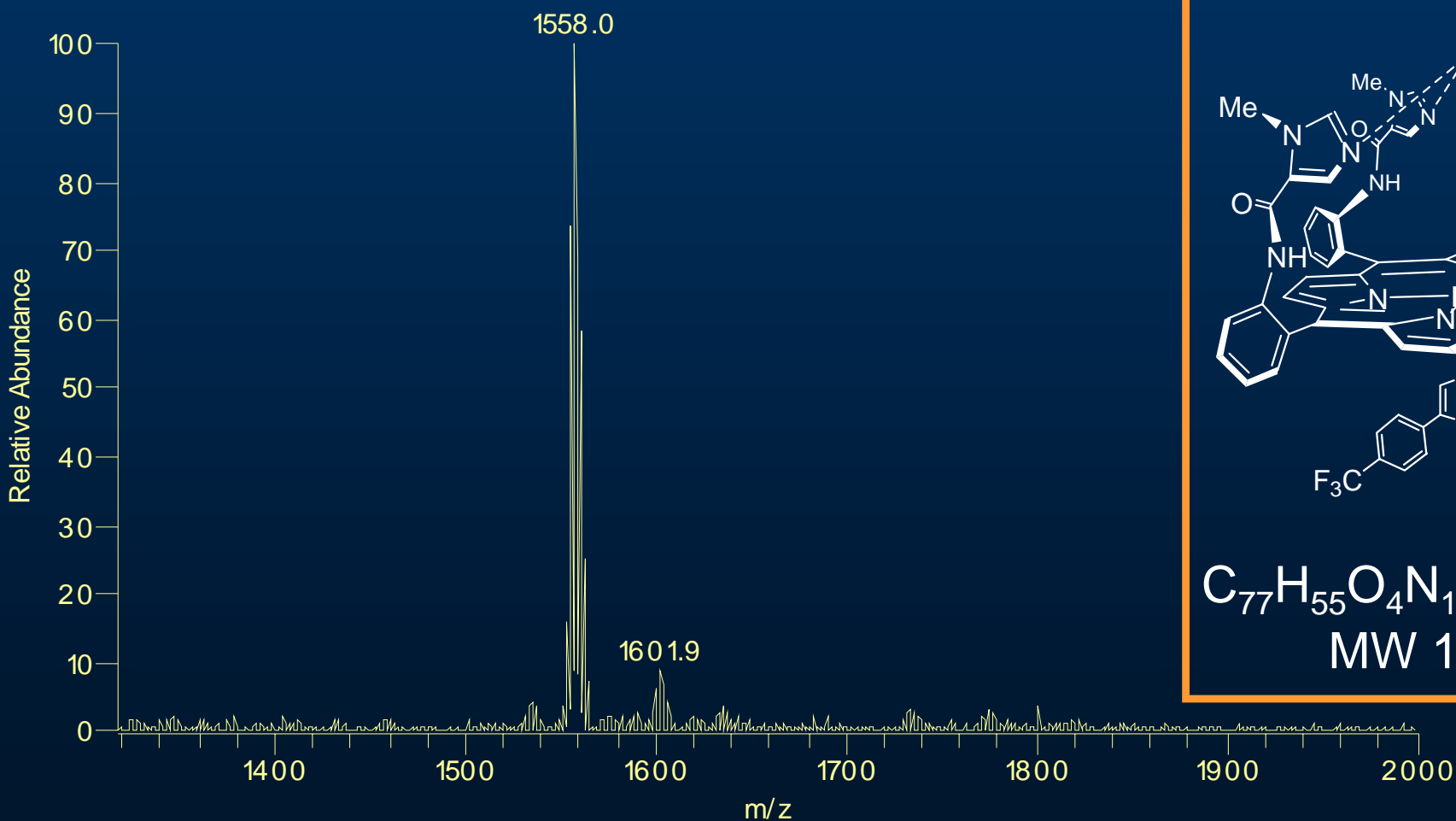


Loss of Counterion



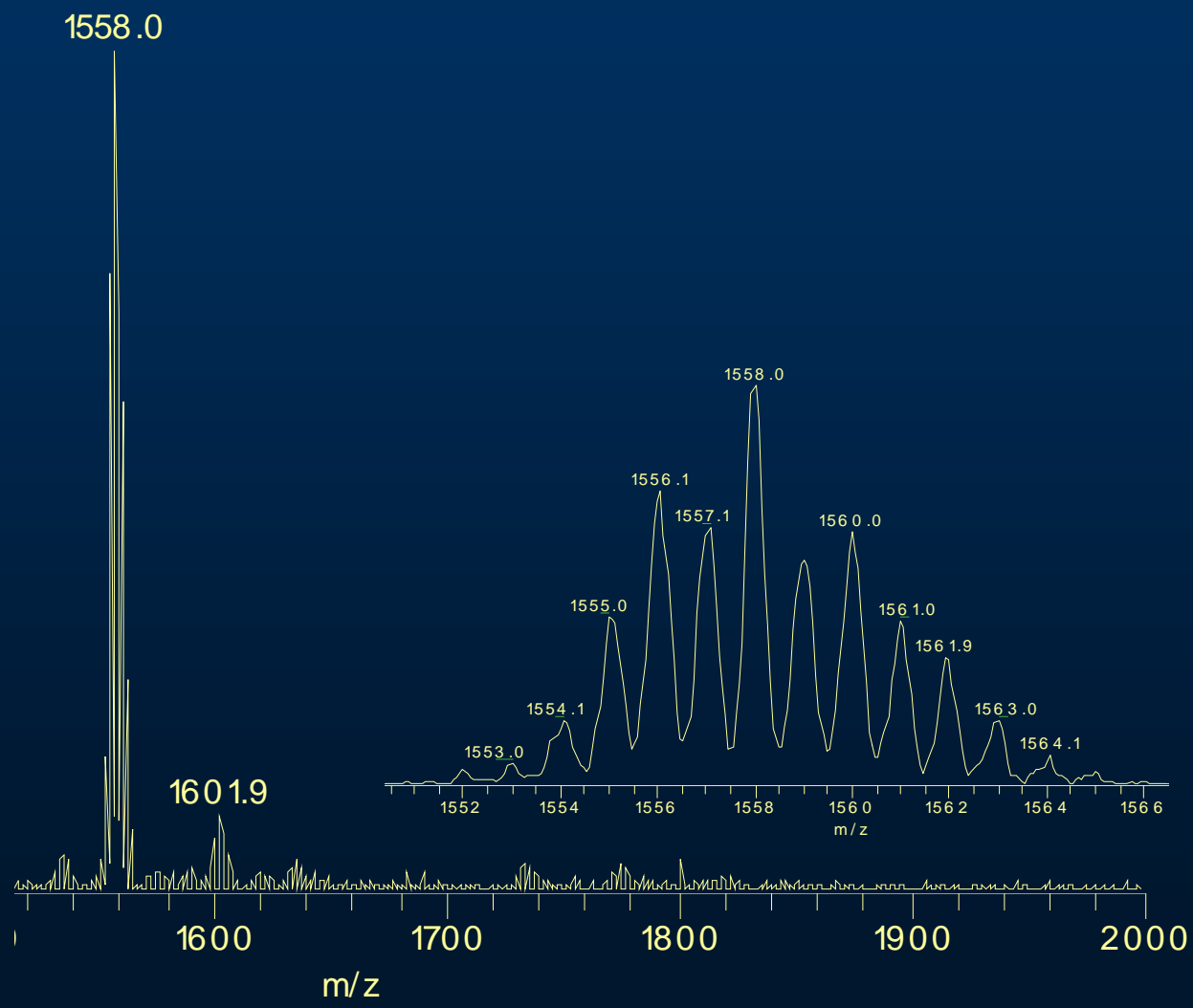


FePd-Porphyrin



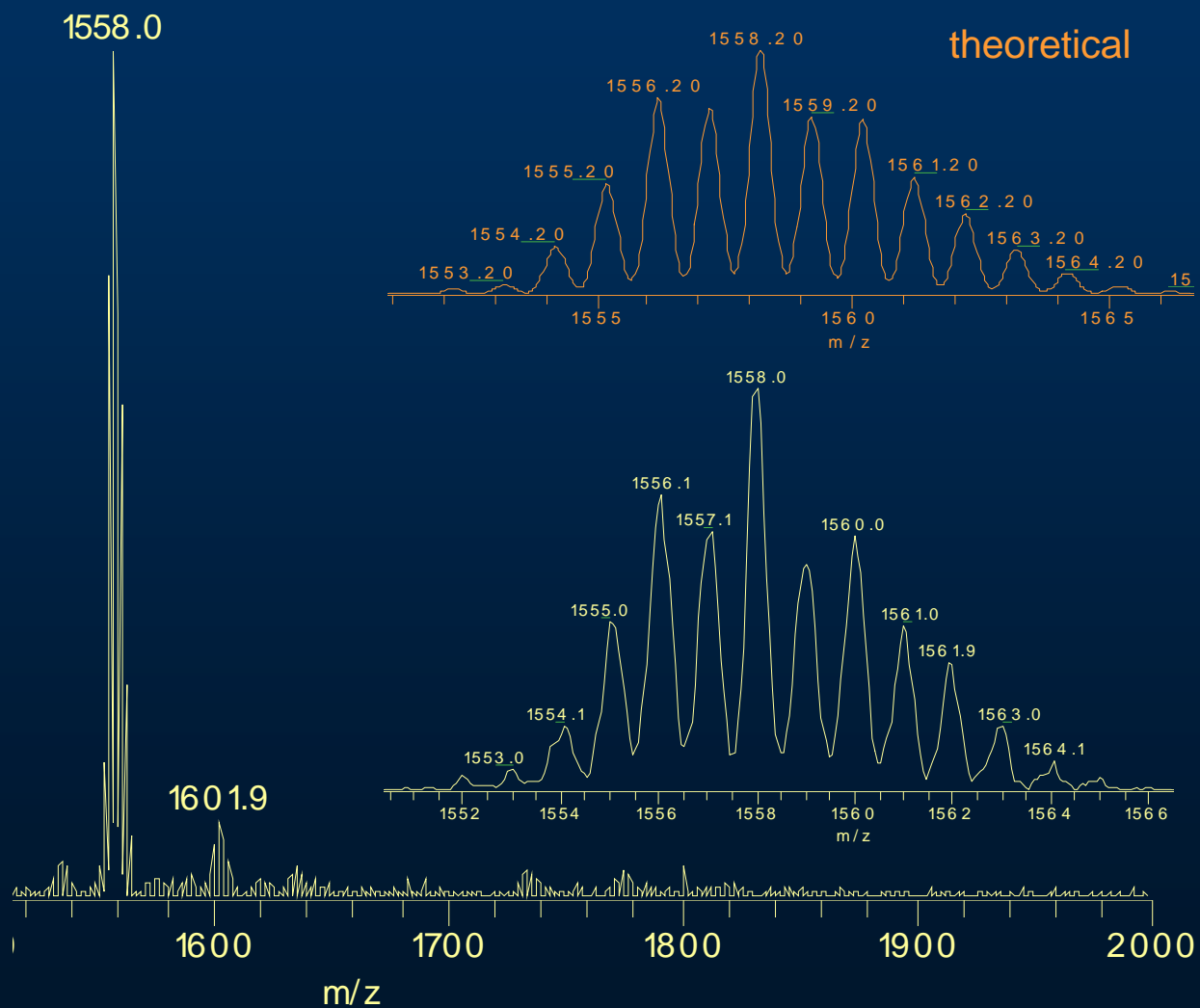


$[M]^+$



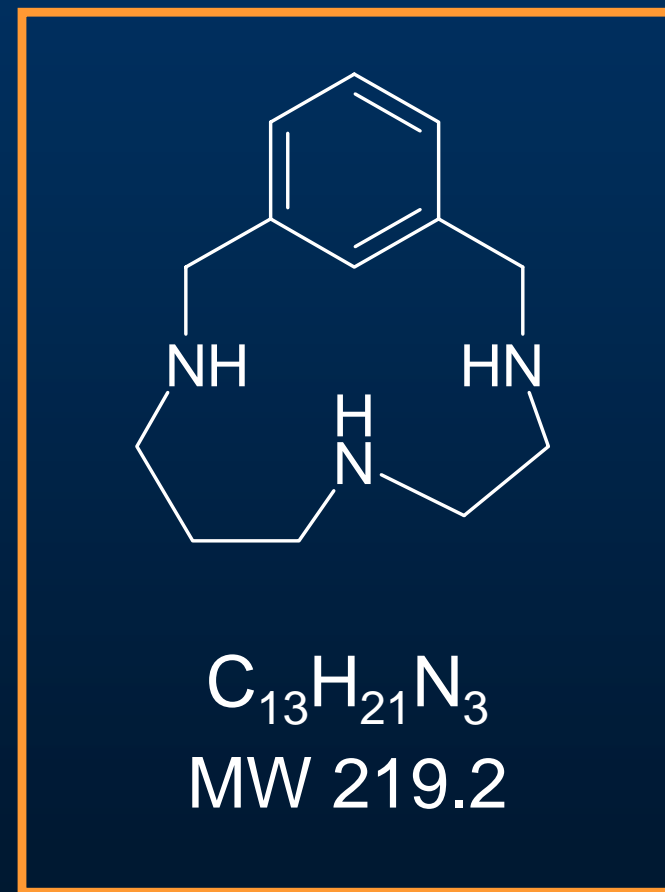
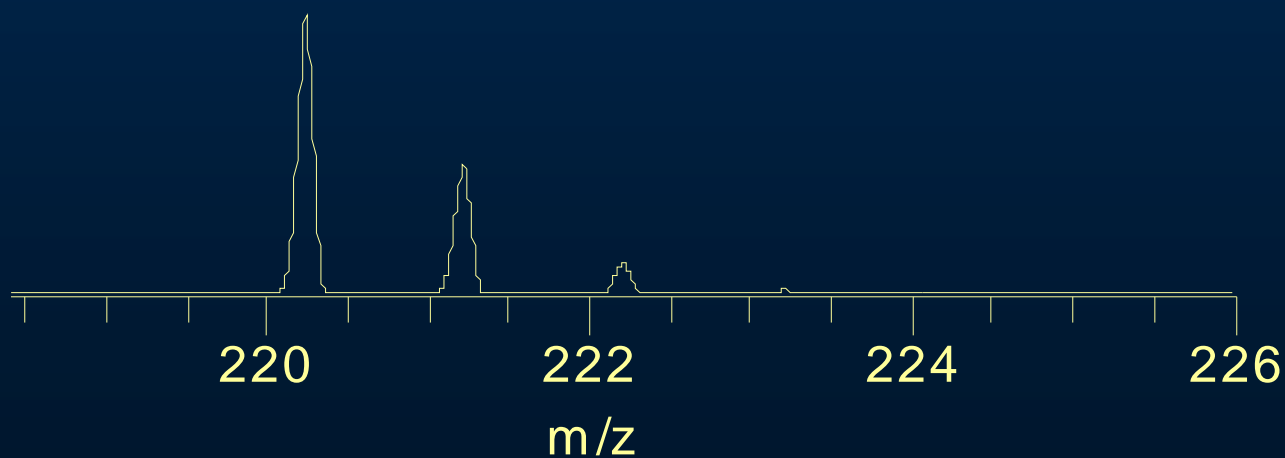


Fe(II) Oxidized to Fe(III)



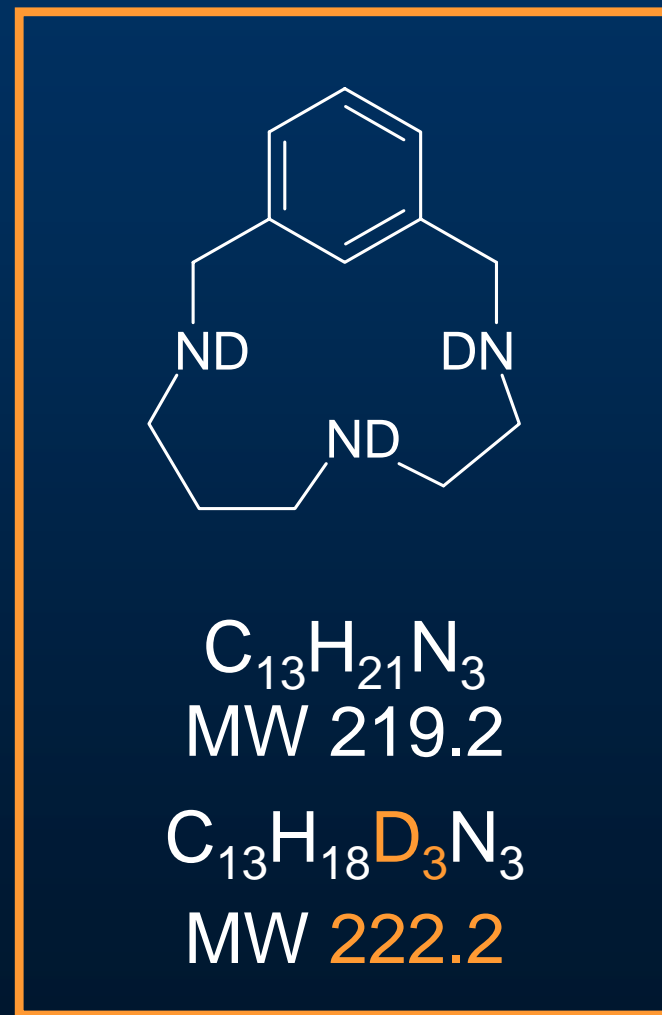


Exchangeable Hydrogens



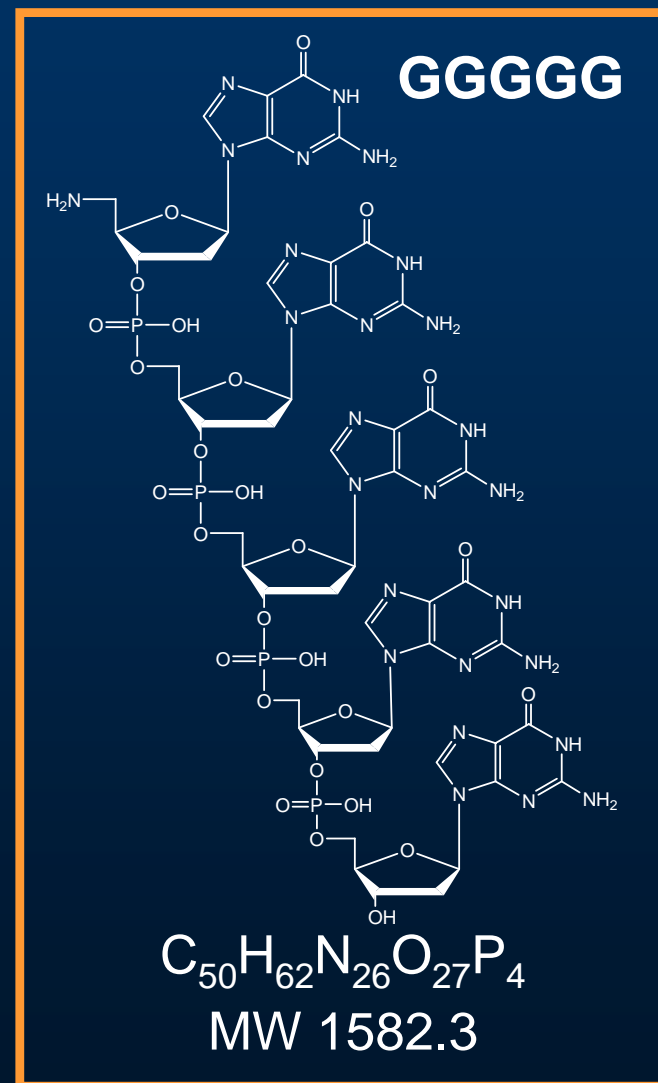
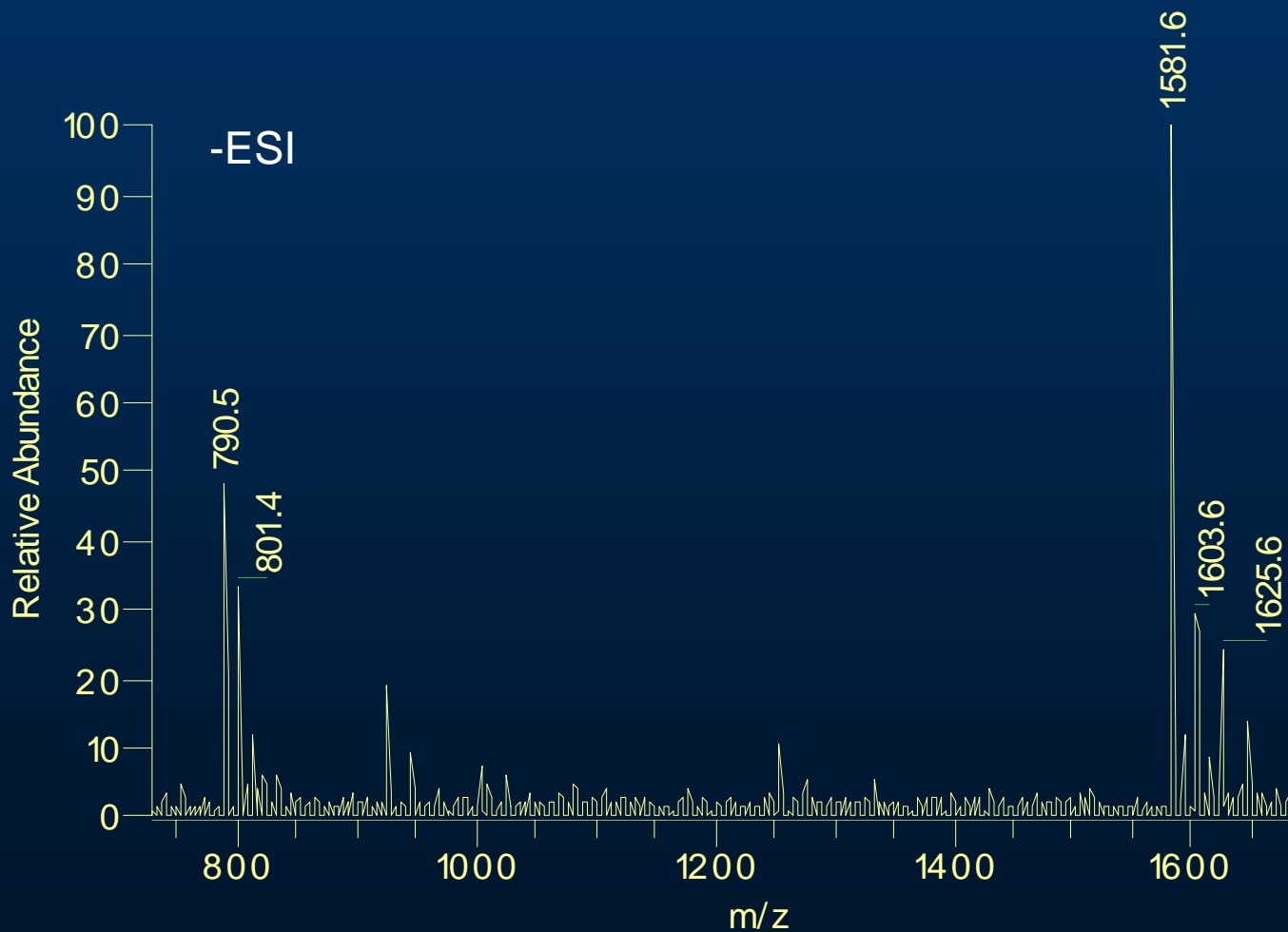


H/D Exchange Over Time



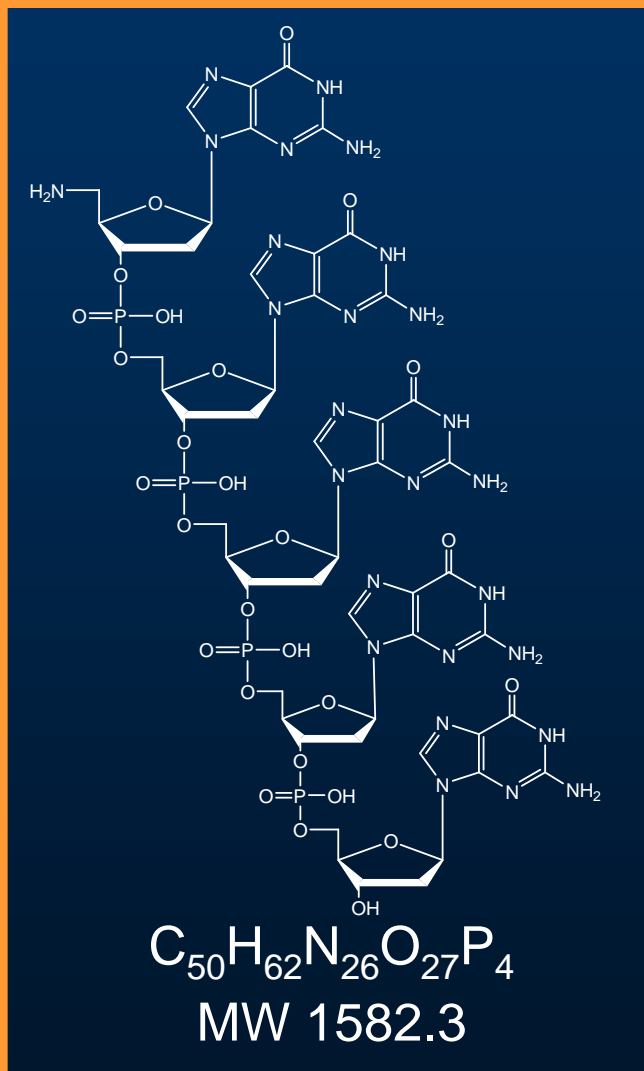
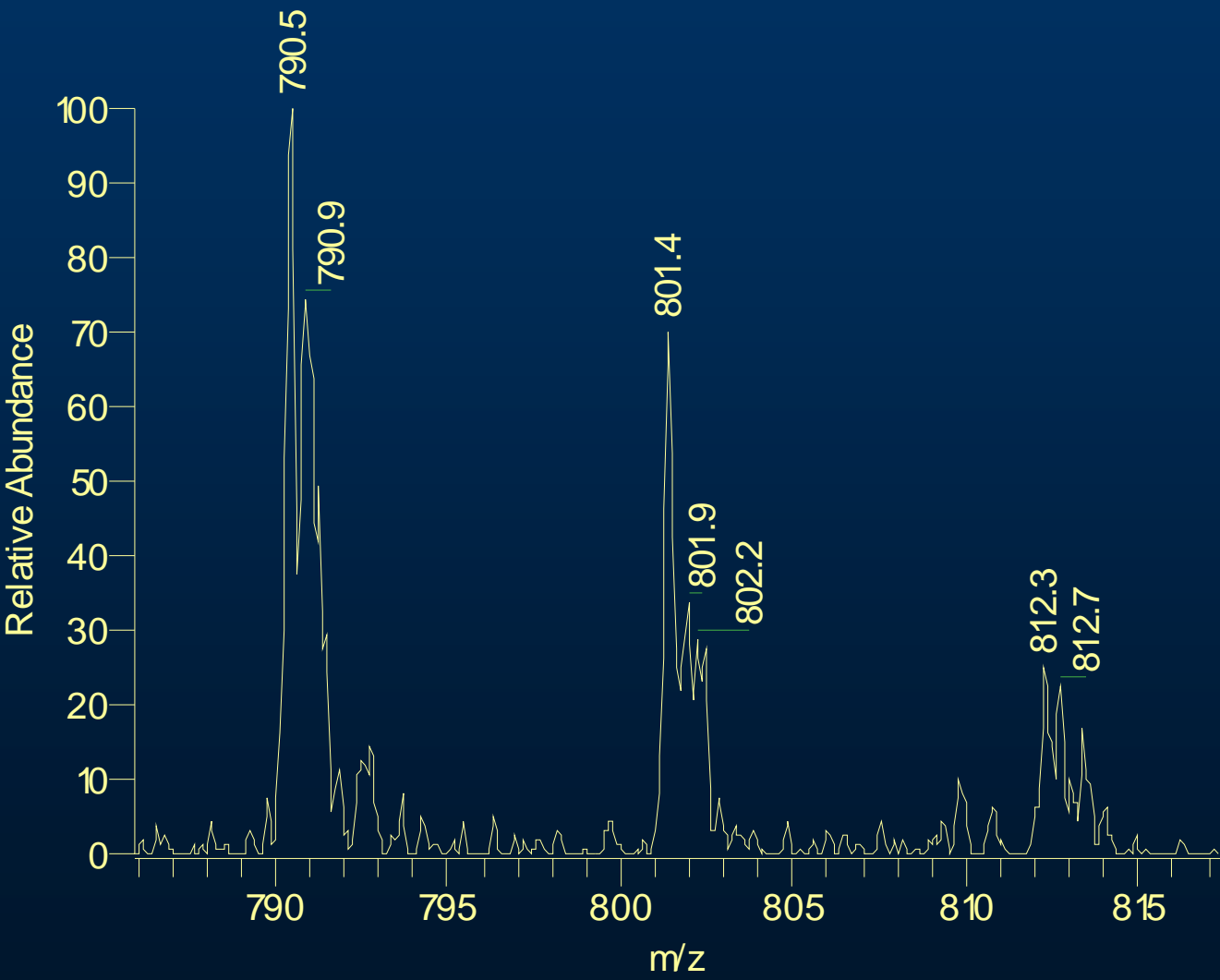


Oligonucleotides: -ESI



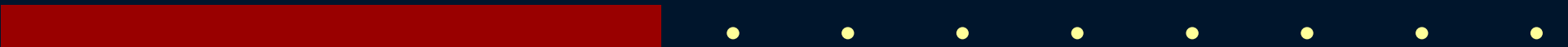
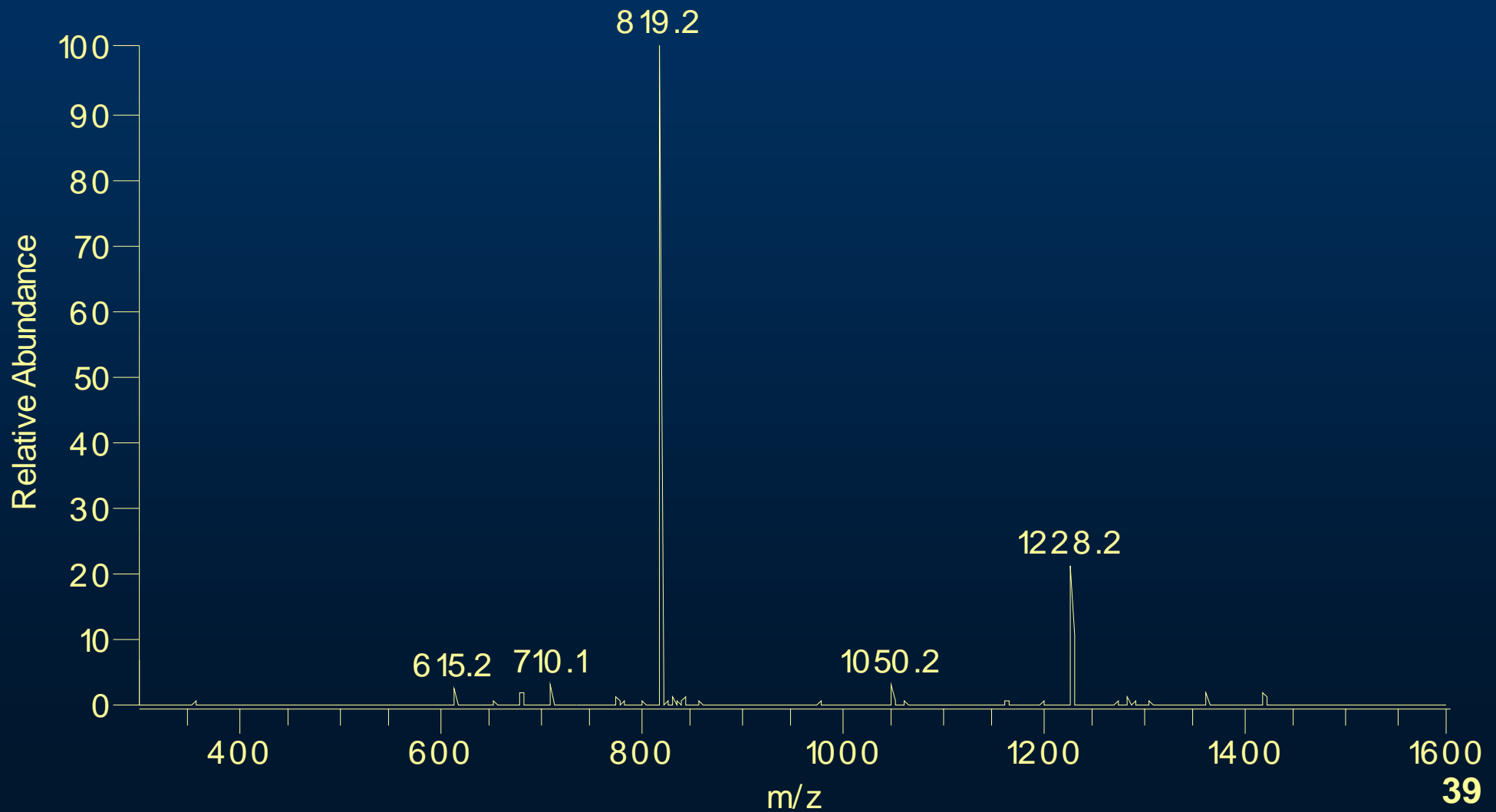


Doubly Charged Ion $[M-2H]^{2-}$



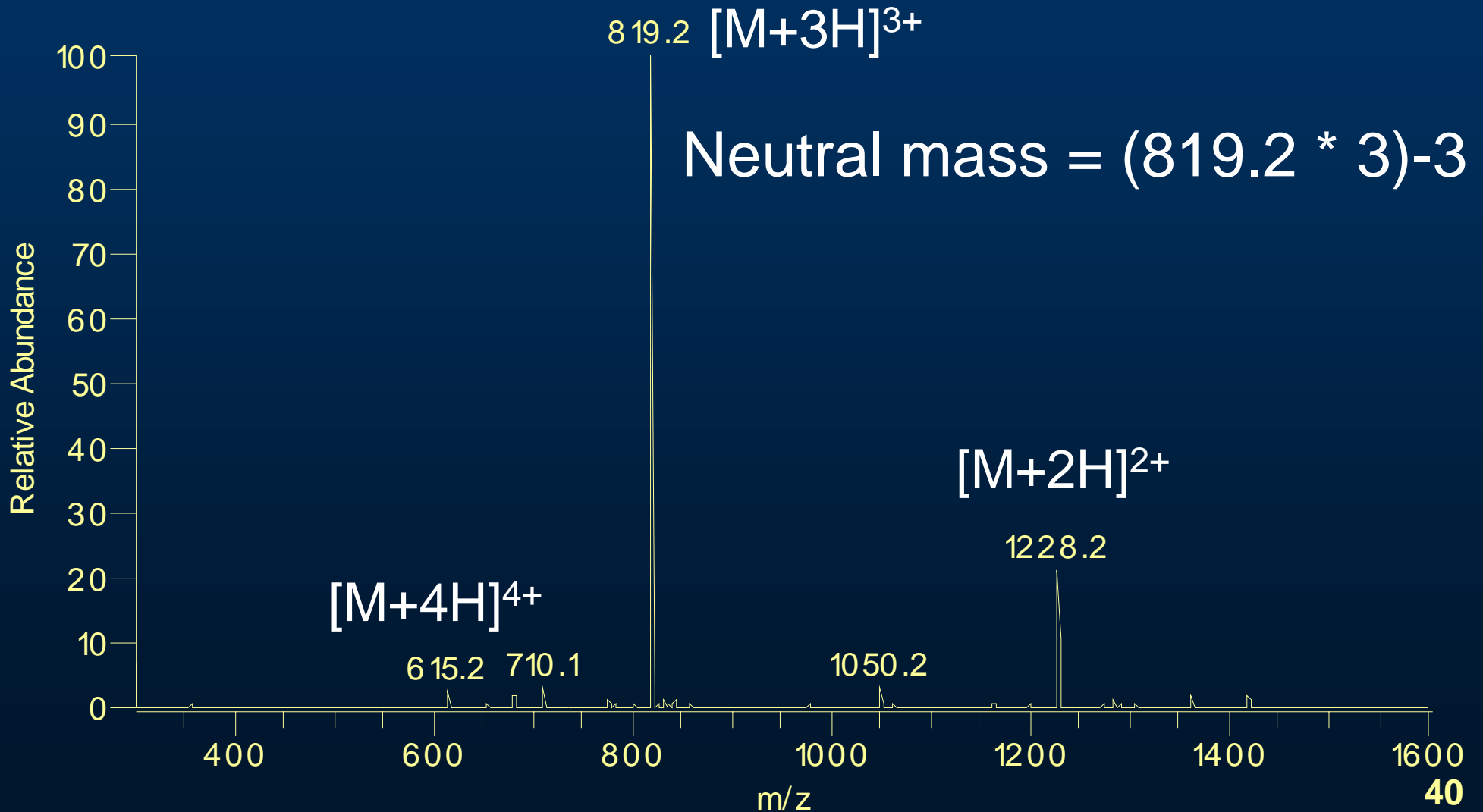


Peptide Charge States





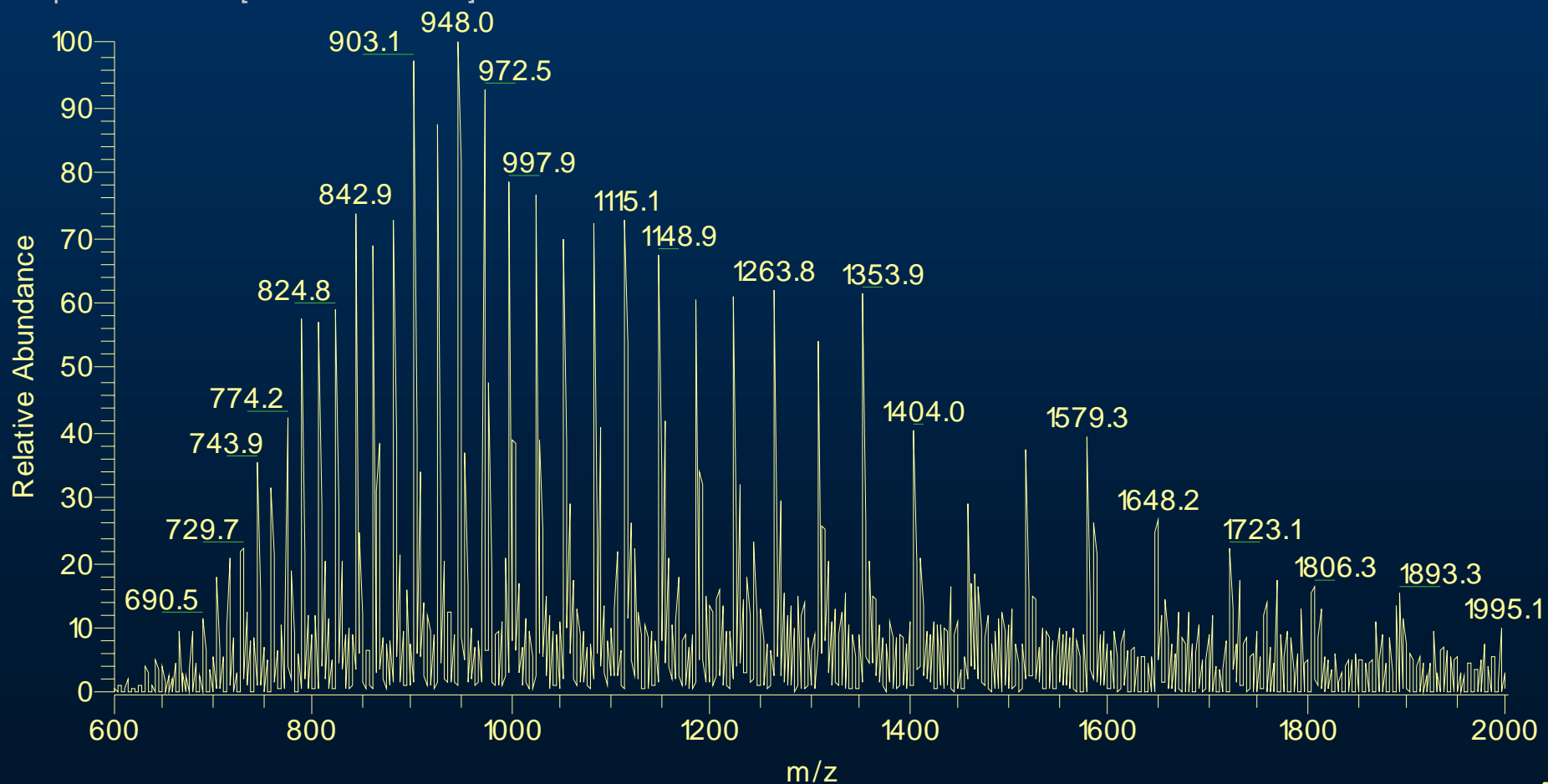
Peptide MW = 2455 Da





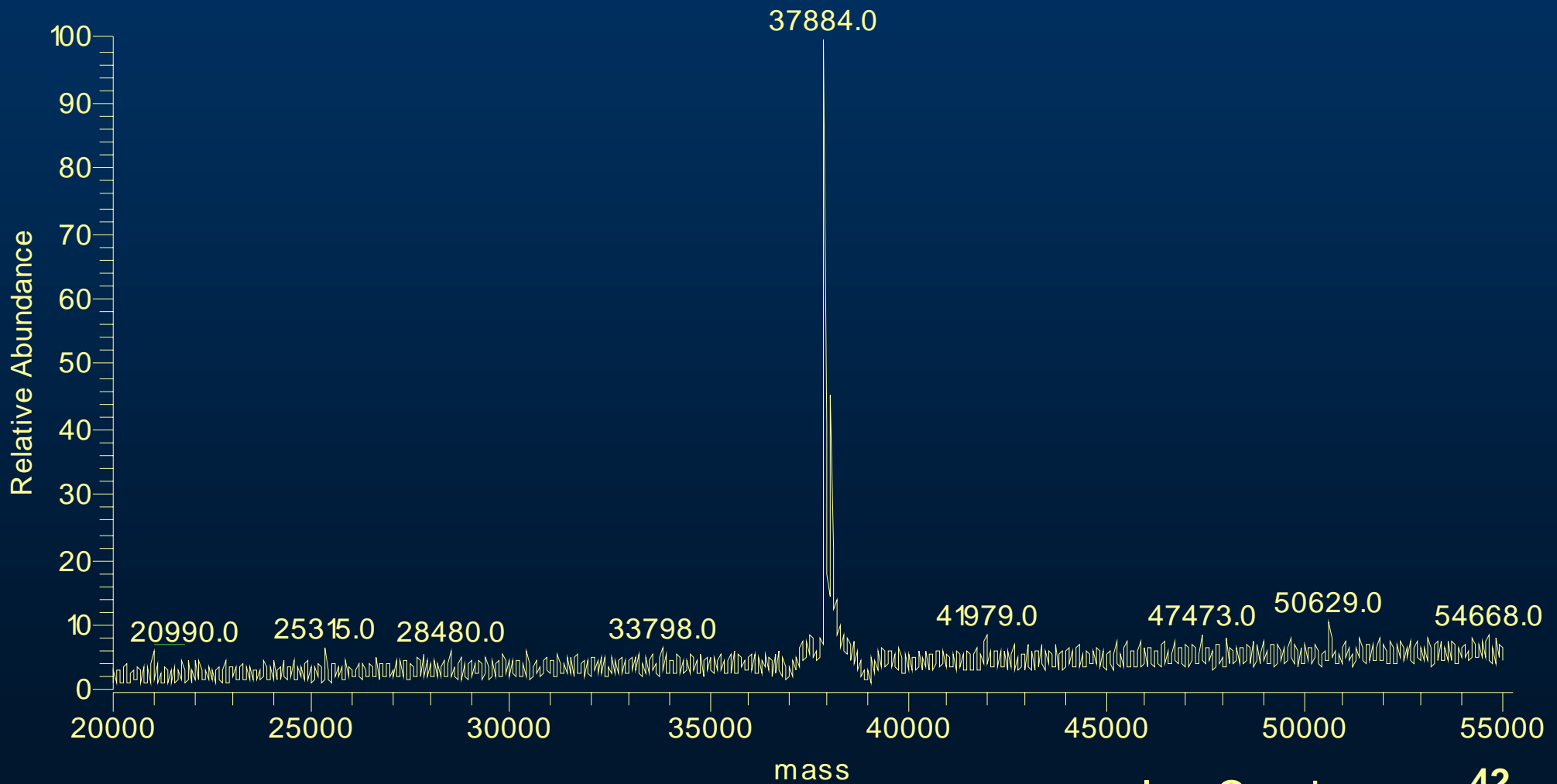
Proteins: Multiple charge states

031009_12216' # 1042-1147 RT: 27.33-30.13 AV: 106 NL: 2.73E7
T: +p ESI Full ms [400.00-2000.00]





Deconvoluted Protein Mass

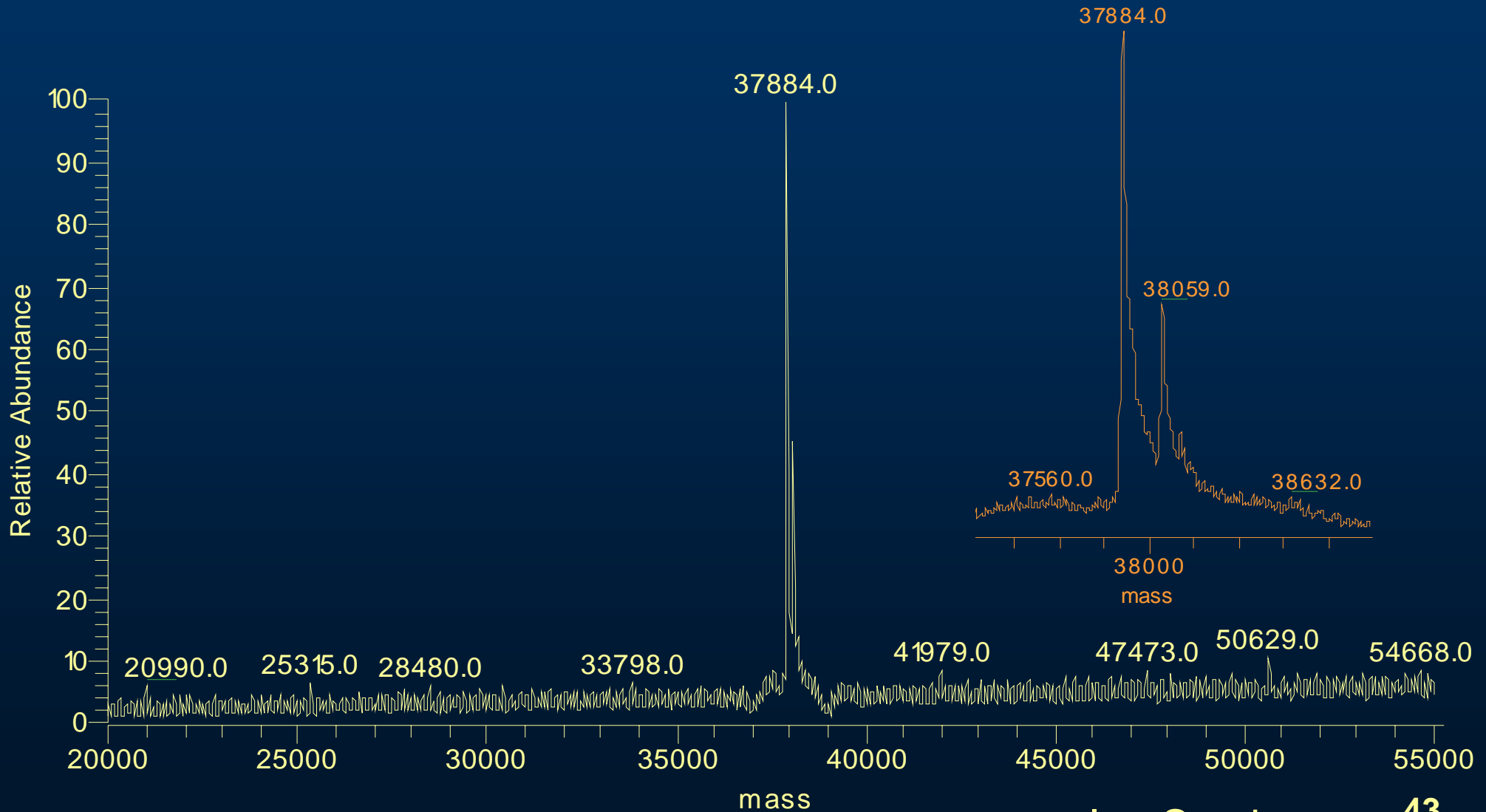


Ian Suydam

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Deconvoluted Protein Mass

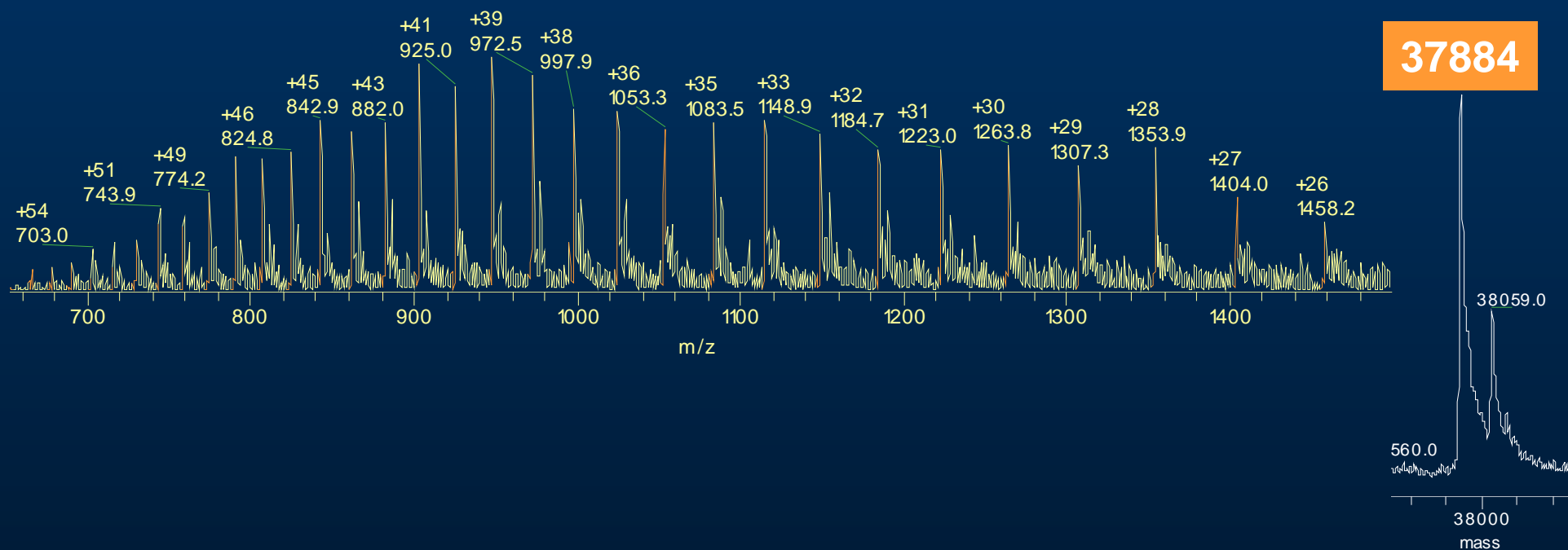


Ian Suydam

43

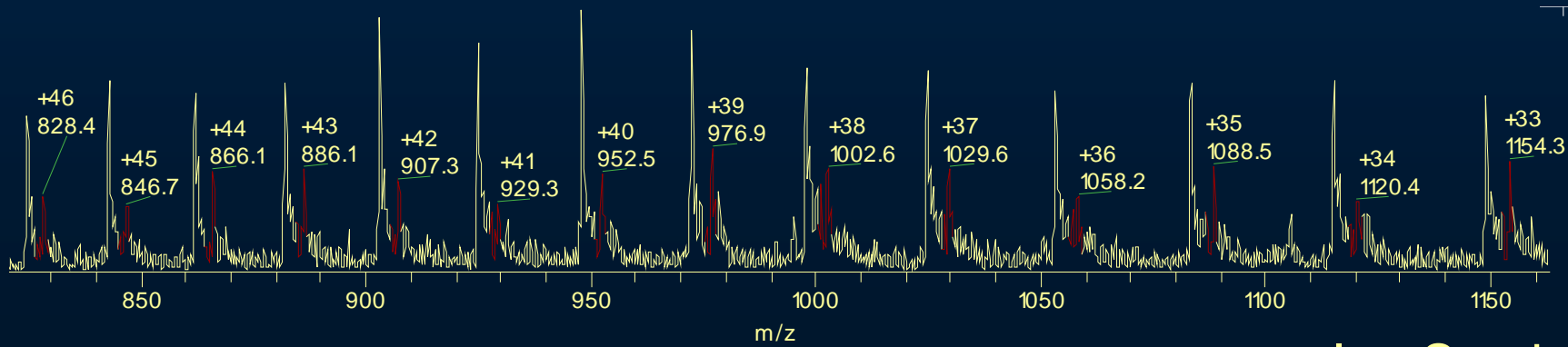
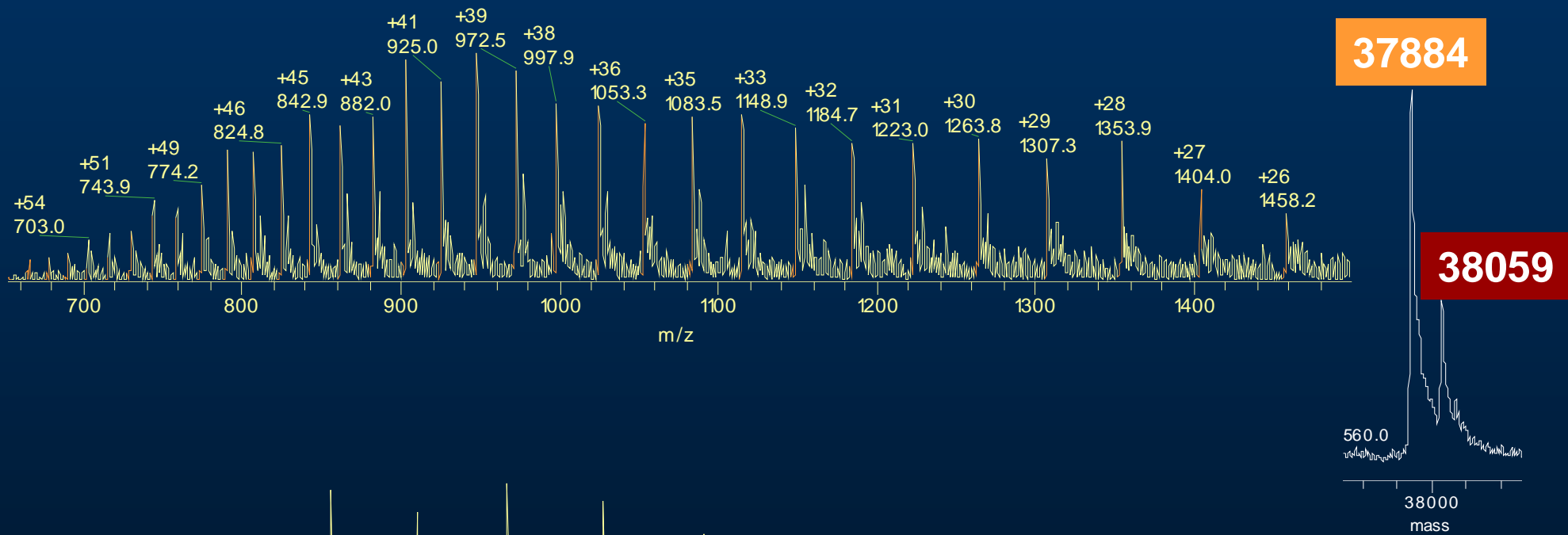


Charge State Series





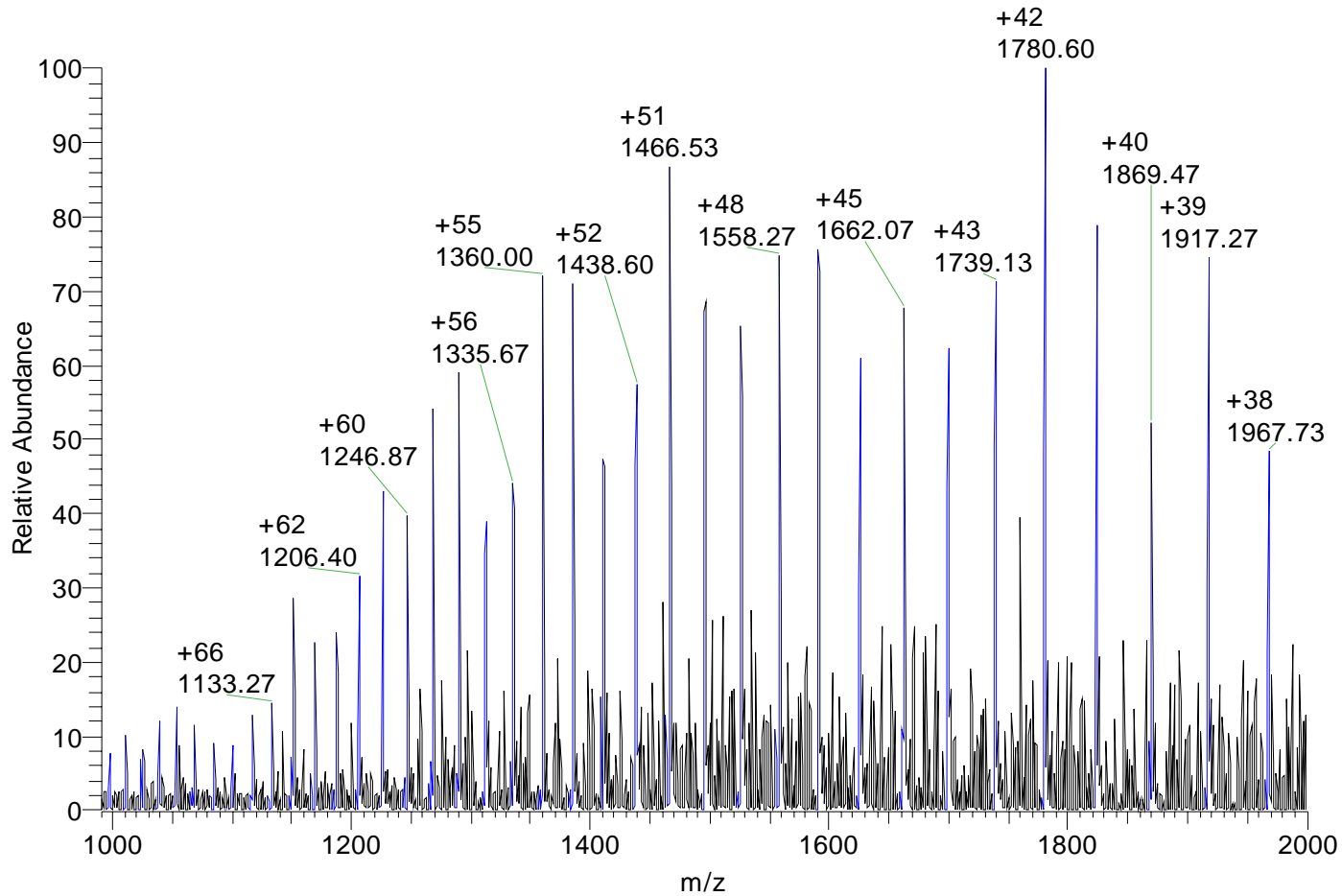
Charge State Series





75 kDa Protein Charge States

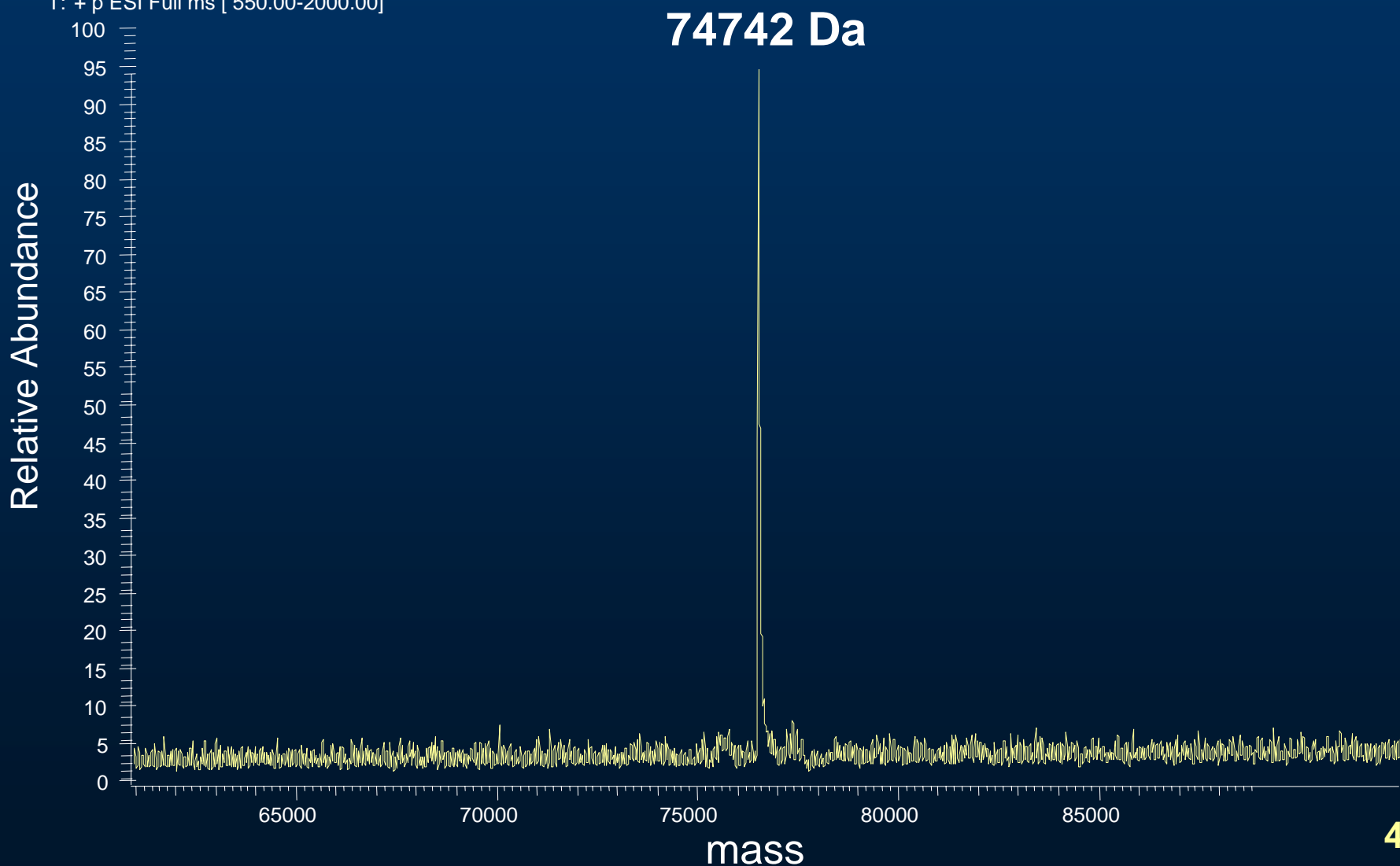
011105_4139 #281-317 RT: 10.11-11.44 AV: 37 NL: 1.76E6
T: + p ESI Full ms [550.00-2000.00]





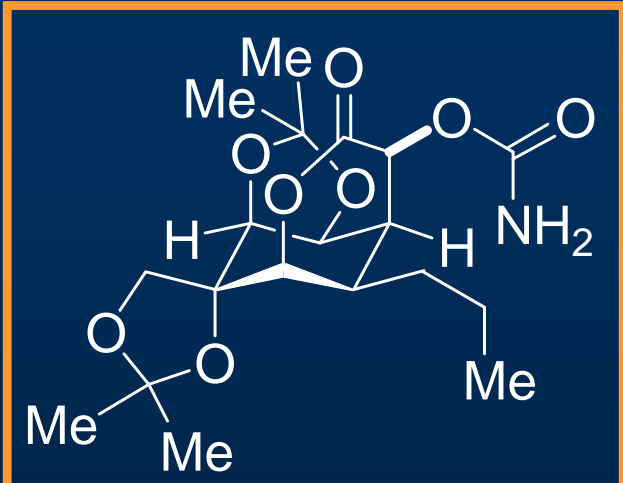
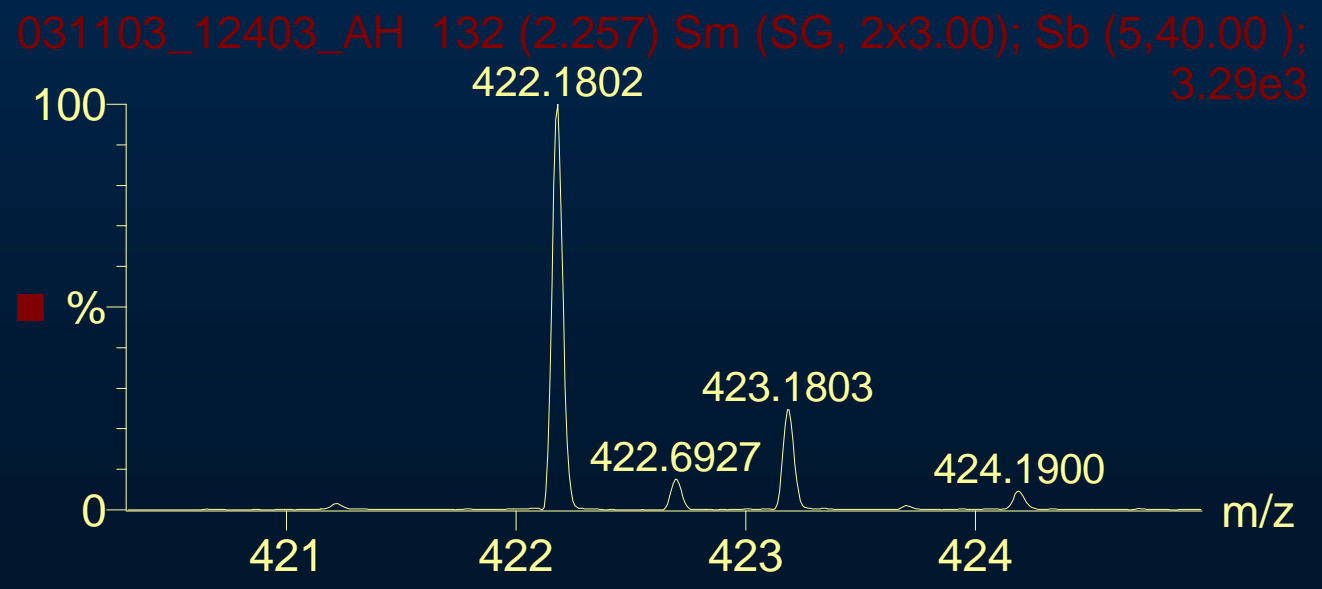
75 kD Protein Deconvoluted

1 RT: 0.00 P: + NL: 2.82E7
T: + p ESI Full ms [550.00-2000.00]





High-Resolution MS - Q-ToF



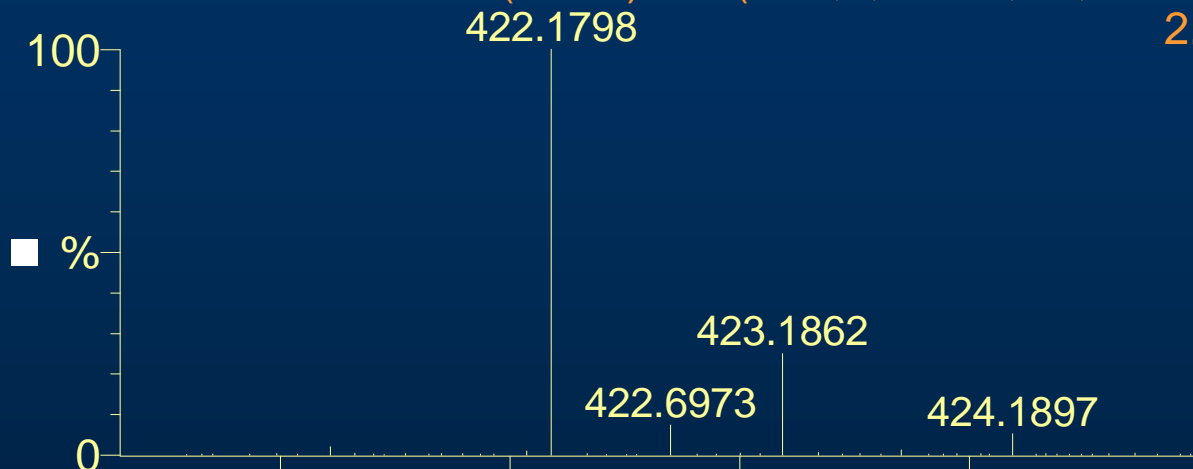
M (neutral)
 $C_{19}H_{29}NO_8$
MW 399.1893

$[M+Na]^+$
 $C_{19}H_{29}NO_8Na$
MW 422.1791

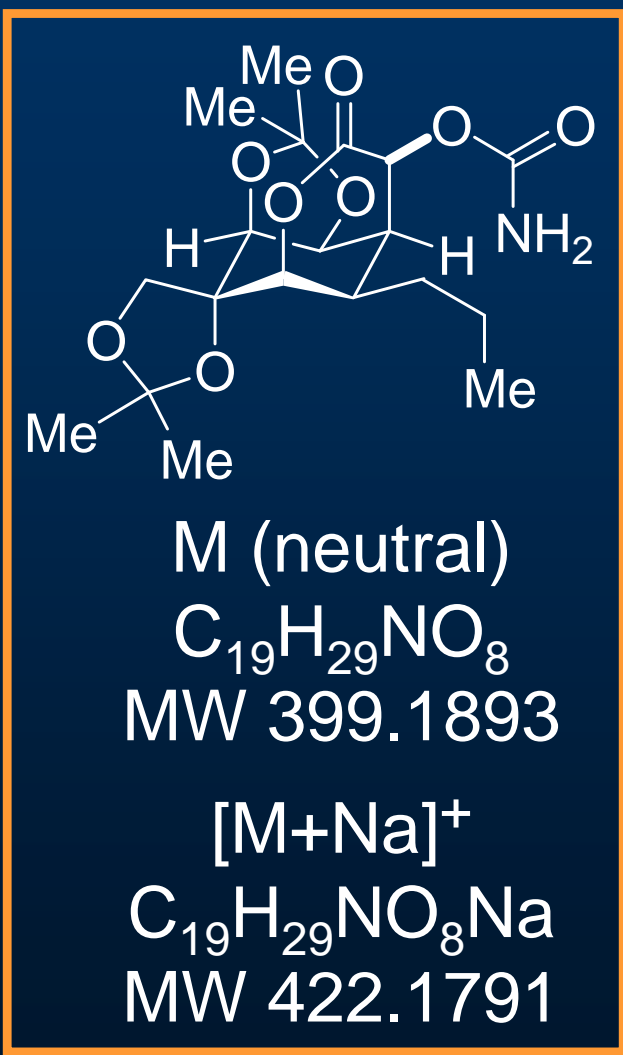


Centroided Spectrum

031103_12403_AH 132 (2.257) AM (Cen,4, 80.00, Ar,5000.0,0.0) 2.26e4



031103_12403_AH 132 (2.257) Sm (SG, 2x3.00); Sb (5,40.00); 3.29e3





Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

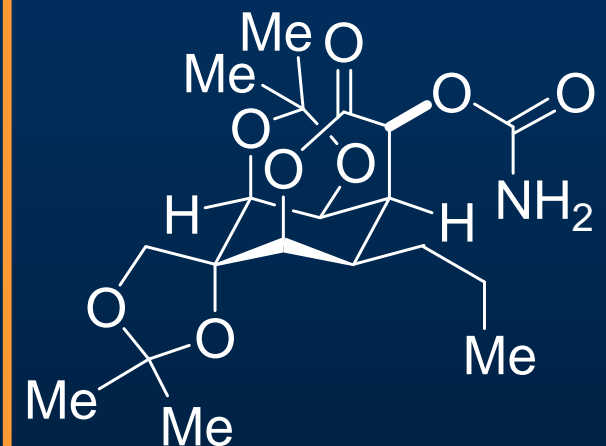
Monoisotopic Mass, Odd and Even Electron Ions

810 formula(e) evaluated with 8 results within limits (up to 50 closest results for each)

Mass	Calc. Mass	mDa	PPM	DBE	Formula	Score	C	H	N	O	Na
422.1798	422.1801	-0.3	-0.8	9.0	C19 H26 N4 O7	4	19	26	4	7	
422.1804	422.1804	-0.6	-1.5	10.5	C20 H25 N5 O4 Na	3	20	25	5	4	1
422.1791	422.1791	0.7	1.7	5.5	C19 H29 N O8 Na	5	19	29	1	8	1
422.1788	422.1788	1.0	2.3	4.0	C18 H30 O11	6	18	30		11	
422.1783	422.1783	1.5	3.6	22.0	C31 H22 N2	8	31	22	2		
422.1815	422.1815	-1.7	-4.0	8.5	C21 H28 N O8	1	21	28	1	8	
422.1818	422.1818	-2.0	-4.7	10.0	C22 H27 N2 O5 Na	2	22	27	2	5	1
422.1777	422.1777	2.1	4.9	6.0	C17 H27 N4 O7 Na	7	17	27	4	7	1



AH XII-20
031103_12403_AH 132 (2.257) AM (Cen,4, 80.00, Ar,5000.0,0.00,1.00); Sm (SG, 2x3.00); Sb (5,40.00
2.26e4



[M+Na]⁺

C₁₉H₂₉NO₈Na

MW 422.1791



Elemental Composition Report

422.1791 amu, 1.7 ppm, C₁₉H₂₉NO₈Na

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

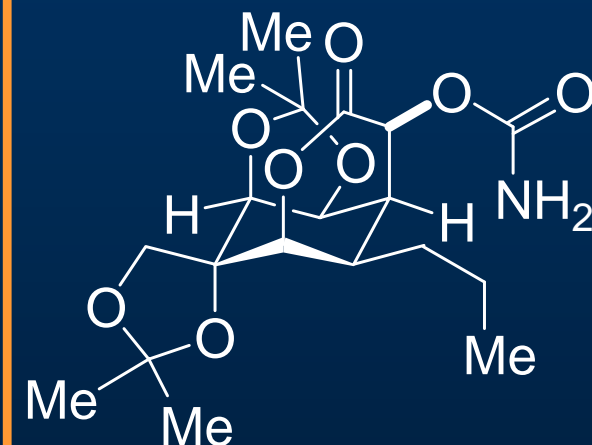
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

810 formula(e) evaluated with 8 results within limits (up to 50 closest results for each)

Mass	Calc. Mass	mDa	PPM	DBE	Formula	Score	C	H	N	O	Na
422.1798	422.1801	-0.3	-0.8	9.0	C19 H26 N4 O7	4	19	26	4	7	
422.1804	422.1804	-0.6	-1.5	10.5	C20 H25 N5 O4 Na	3	20	25	5	4	1
422.1791	422.1791	0.7	1.7	5.5	C19 H29 N O8 Na	5	19	29	1	8	1
422.1788	422.1788	1.0	2.3	4.0	C18 H30 O11	6	18	30		11	
422.1783	422.1783	1.5	3.6	22.0	C31 H22 N2	8	31	22	2		
422.1815	422.1815	-1.7	-4.0	8.5	C21 H28 N O8	1	21	28	1	8	
422.1818	422.1818	-2.0	-4.7	10.0	C22 H27 N2 O5 Na	2	22	27	2	5	1
422.1777	422.1777	2.1	4.9	6.0	C17 H27 N4 O7 Na	7	17	27	4	7	1

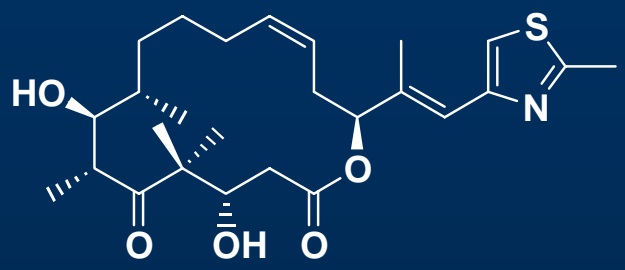
AH XII-20
031103_12403_AH 132 (2.257) AM (Cen,4, 80.00, Ar,5000.0,0.00,1.00); Sm (SG, 2x3.00); Sb (5,40.00



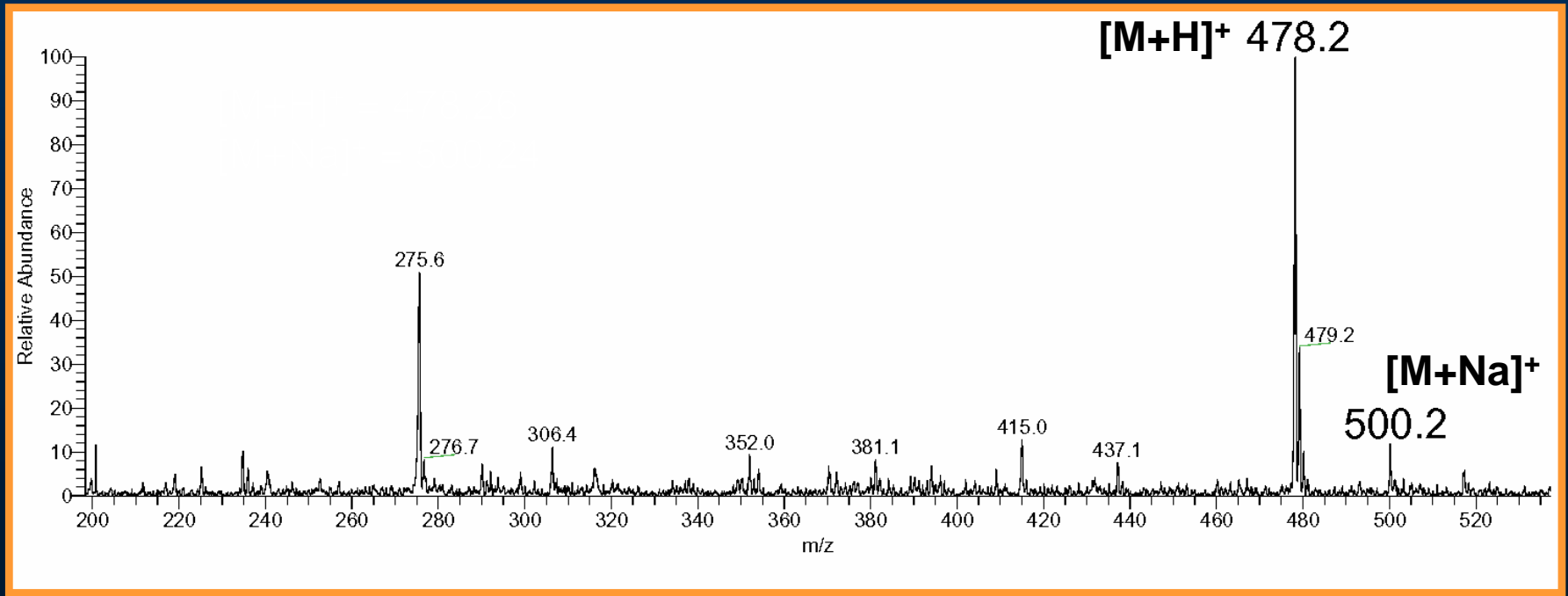
[M+Na]⁺
C₁₉H₂₉NO₈Na
MW 422.1791



ESI-MS of Epothilone C



$C_{28}H_{39}NO_5S$
MW: 477.25

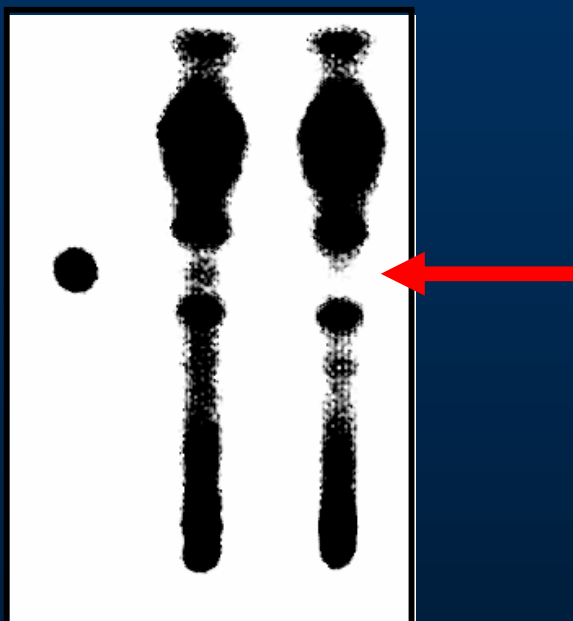


Epothilone C from *E. coli* culture broth



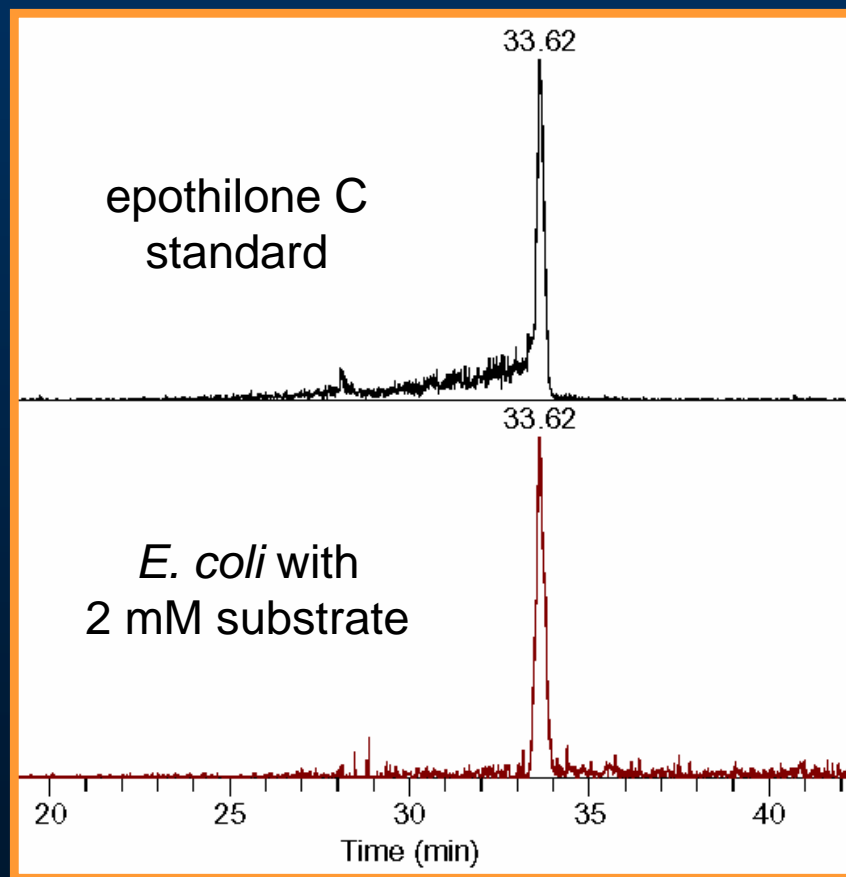
Epothilone C Biosynthesis is Reconstituted in *E. coli*

¹⁴C Radio-TLC assay



- 1. EpoC standard
- 2. 2 mM substrate
- 3. Negative control

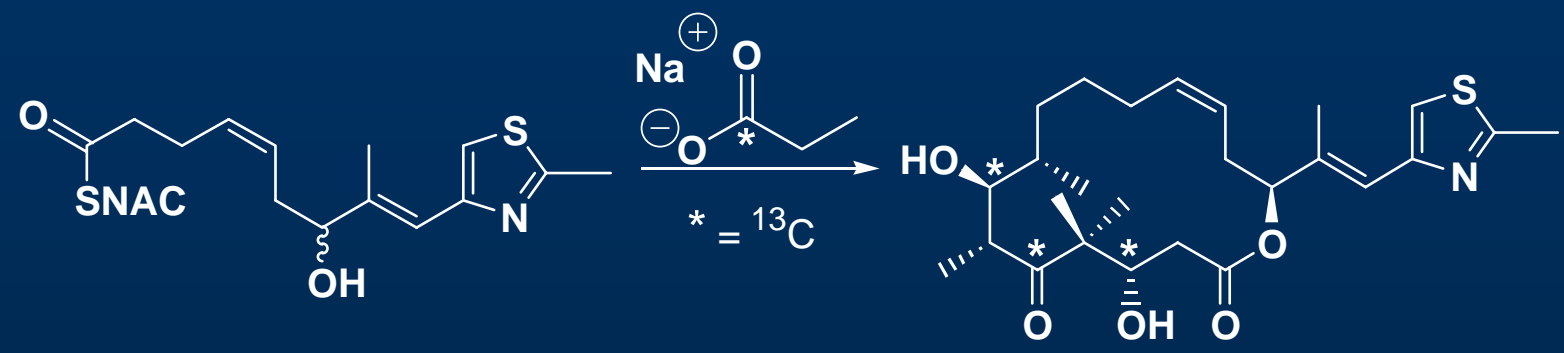
LC/MS analysis
Extracted Ion Chromatogram of [M+H]⁺



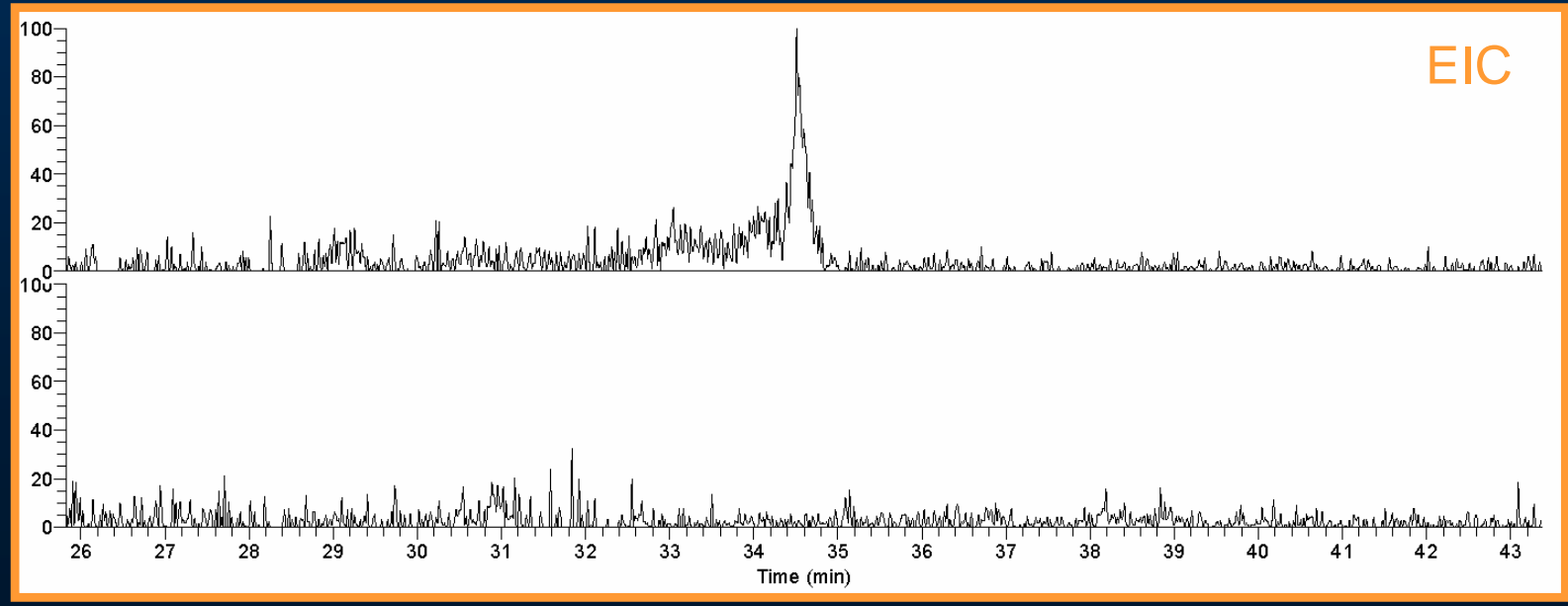
Epothilone C is observed in metabolically engineered *E. coli* cultures



Characterization by Isotopic Derivatization



¹²C propionate feeding

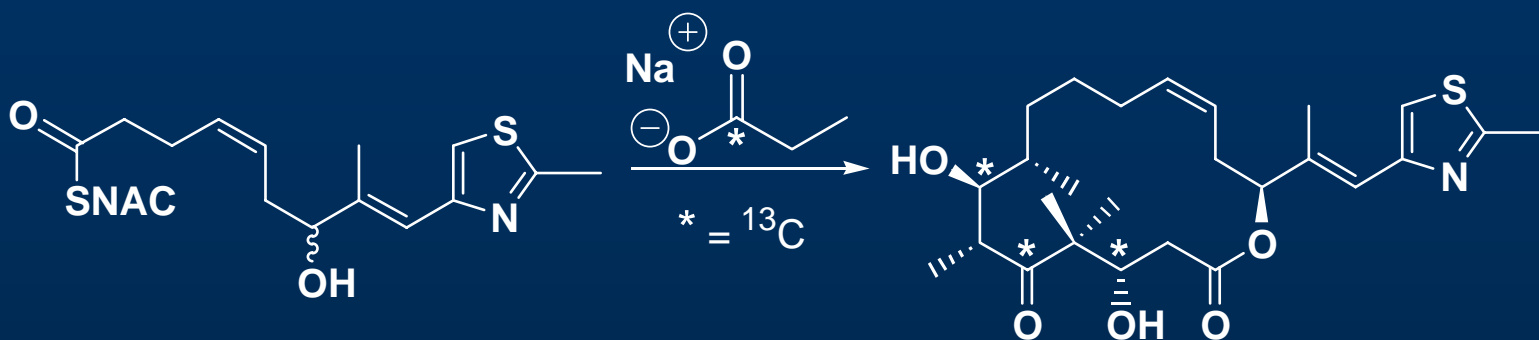


¹²C
m/z = 478.3

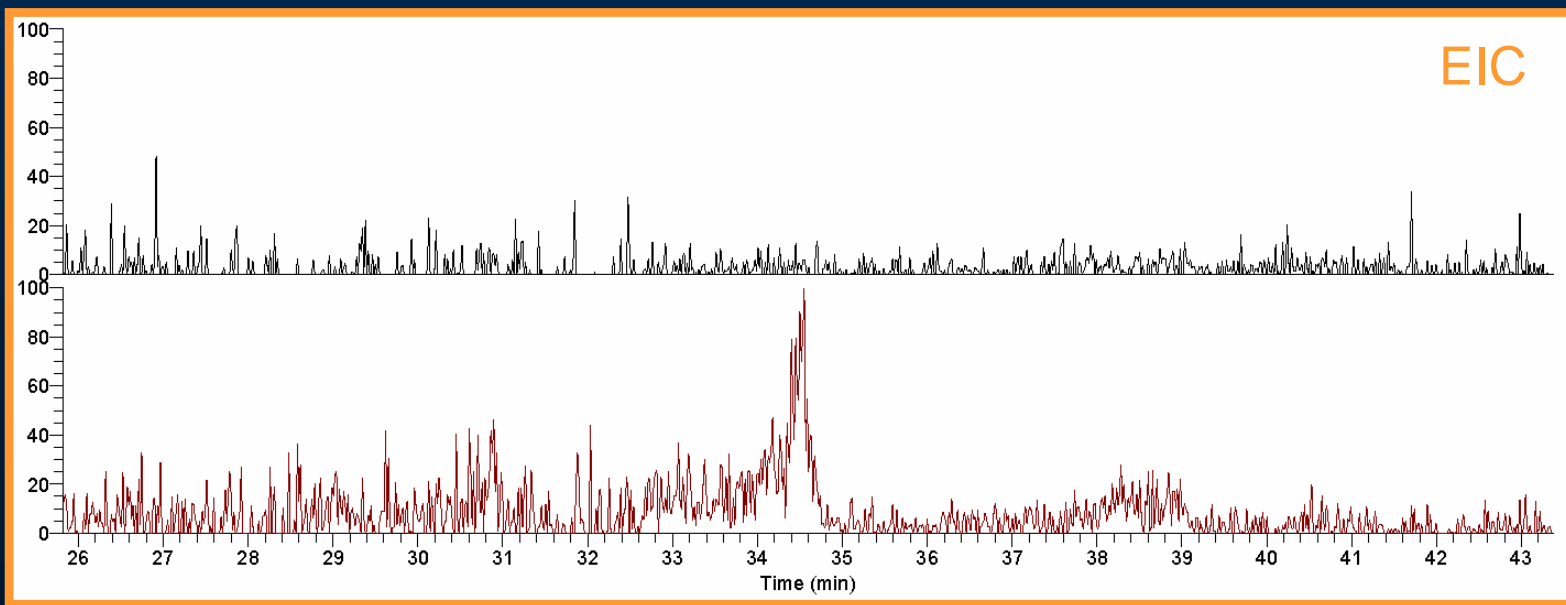
¹³C
m/z = 481.3



^{13}C Propionate increases mass by 3 Da



^{13}C propionate feeding



^{12}C
 $m/z = 478.3$

^{13}C
 $m/z = 481.3$



Conclusion



Resources

Acknowledgements





http://mass-spec.stanford.edu

The screenshot shows the website for the Vincent Coates Foundation Mass Spectrometry Laboratory at Stanford University. The page features a navigation menu on the left with buttons for SUMS HOME, INSTRUMENTS, SERVICES, SAMPLE PREP, RATES, FORMS, FAQ, PUBLICATIONS, TOOLS & LINKS, and CONTACT INFO. The main content area includes a welcome message, a description of the laboratory's facilities and services, and contact information. A footer contains a link to the webmaster and the date of the last modification.

 **Stanford University**
MASS SPECTROMETRY

[SUMS HOME](#)

Welcome to the web home of the **Vincent Coates Foundation Mass Spectrometry Laboratory**. The laboratory is named in honor of a generous gift from Vincent and Stella Coates, given for the purpose of supporting the mass spectrometry facility as a core resource for researchers throughout the University and elsewhere. The laboratory is also a [Bio-X core facility](#), supported by James H. Clark and the Bio-X initiative in the spirit of interdisciplinary communication and collaboration.

At this time, we have in operation two quadrupole ion trap mass spectrometers and one hybrid quadrupole-time of flight MS which are equipped with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources.

Routine services include molecular weight determination, MS_n, LC-MS, and protein identification by proteolytic digest, LC-MS/MS and database search. Custom analyses are available; please [contact SUMS](#) to discuss.

Please check back regularly, as the website is constantly being developed and updated in response to user feedback. It is our hope that these pages will be a valuable resource to you.

SUMS
Stanford University
Keck Science Building
Room 328
380 Roth Way
Stanford, CA
94305-5080

[Stanford Home](#)

Comments & questions to the [Webmaster](#)

Last Modified 9.9.03

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<http://mass-spec.stanford.edu>

 **Stanford University**
MASS SPECTROMETRY

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Online Tools & Links

- SUMS HOME
- INSTRUMENTS
- SERVICES
- SAMPLE PREP
- RATES
- FORMS
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- PUBLICATIONS
- TOOLS & LINKS
- CONTACT INFO

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Stanford University
Keck Science Building
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Stanford, CA
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Useful web tools, links, and resources

STANFORD ORGANIZATIONS

- [Bio-X](#)
- [Protein & Nucleic Acid Facility](#)
- [NMR Facility](#)
- [Stanford Libraries](#)

ONLINE TOOLS & CALCULATORS

- [Mascot](#) - online database searching for protein ID
- [ExPASy](#) - proteomics tools
- [BLAST](#) - NCBI Basic Local Alignment Search Tool
- [Entrez](#) - NCBI database search and retrieval system
- [ChemCalc](#) - molecular mass calculator
- [MS Tools](#) - from SIS; includes graphical periodic table
- [IonSource](#) - collection of tools and tutorials

REFERENCE DATA

- [Delta Mass](#) - database of protein post-translational modifications
- [List of Elements](#) - exact masses & isotopic abundances

TUTORIALS & INFORMATION

- How [electrospray](#) works
- How [ion trap](#) mass spectrometers work
- How to [sequence peptides](#) from MS/MS data
- [Tutorials](#) at [Base Peak](#)

ORGANIZATIONS

- [ABRE](#) - Association of Biomolecular Resource Facilities
- [ASMS](#) - American Society for Mass Spectrometry
- [BAMS](#) - Bay Area Mass Spectrometry
- [CASSS](#) - California Separation Science Society
- [Proteome Society](#)



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- DuBois Lab
Andrew Hinman
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- Chaitan Khosla
- Al Smith
- Tom Wandless

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