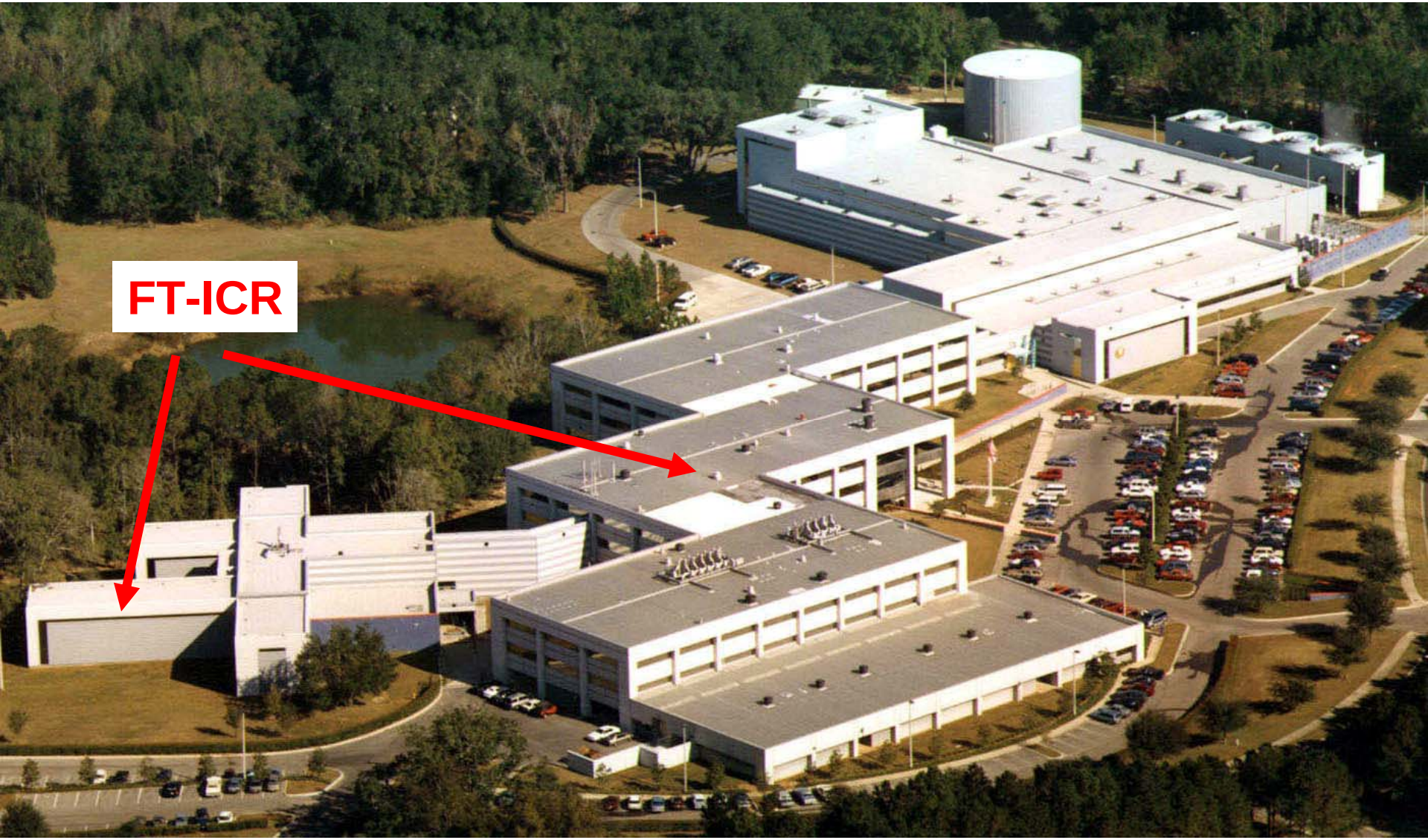
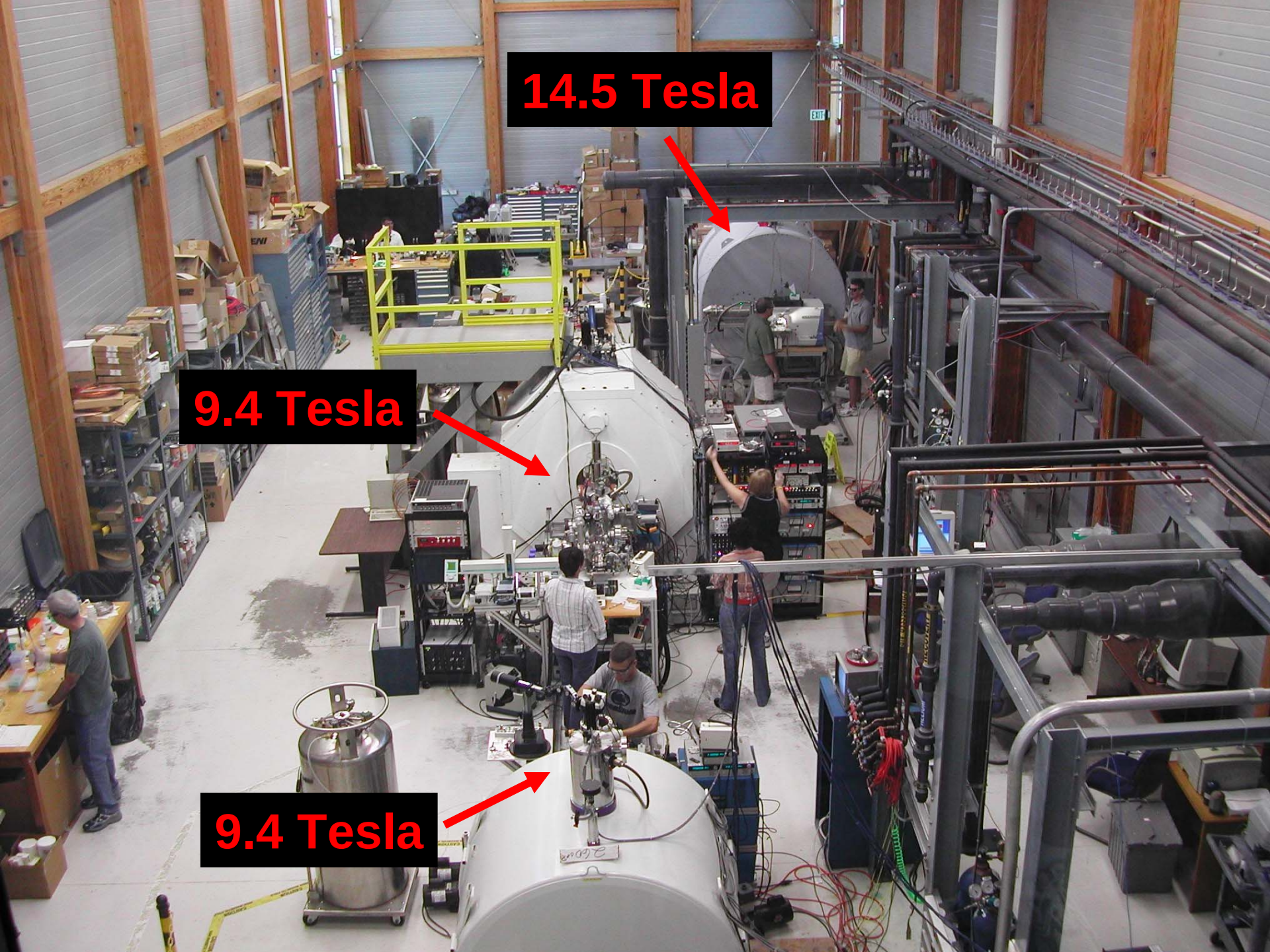
National High Magnetic Field Laboratory at Florida State University



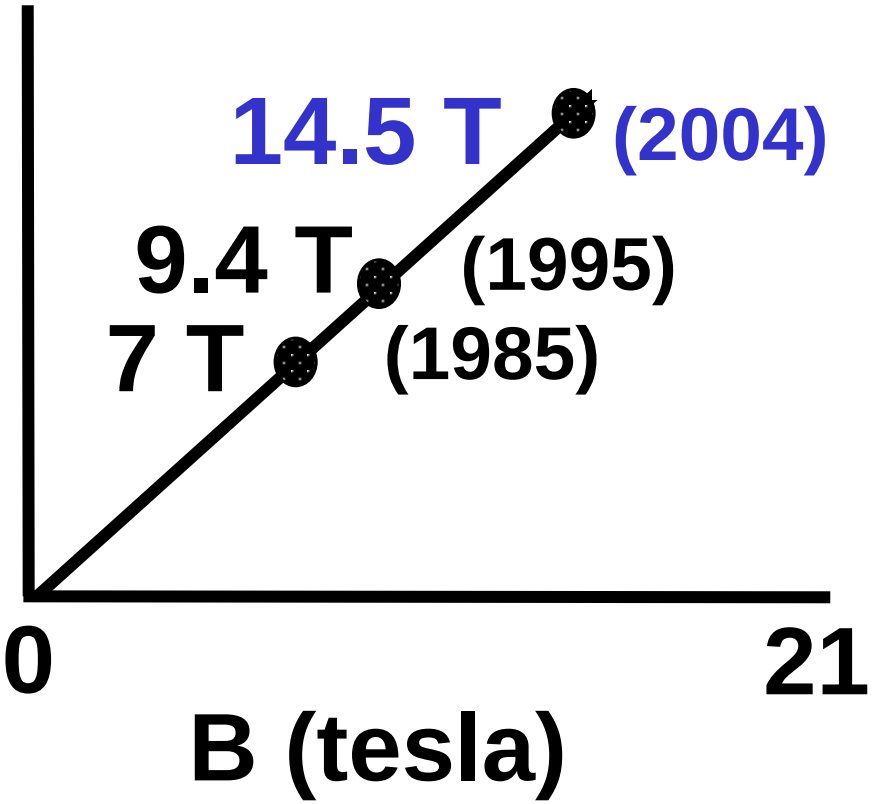

14.5 Tesla
9.4 Tesla
9.4 Tesla

Higher Magnetic Field

Highest Non-Coalesced Mass

Mass Resolving Power

Scan Speed (LC/MS)

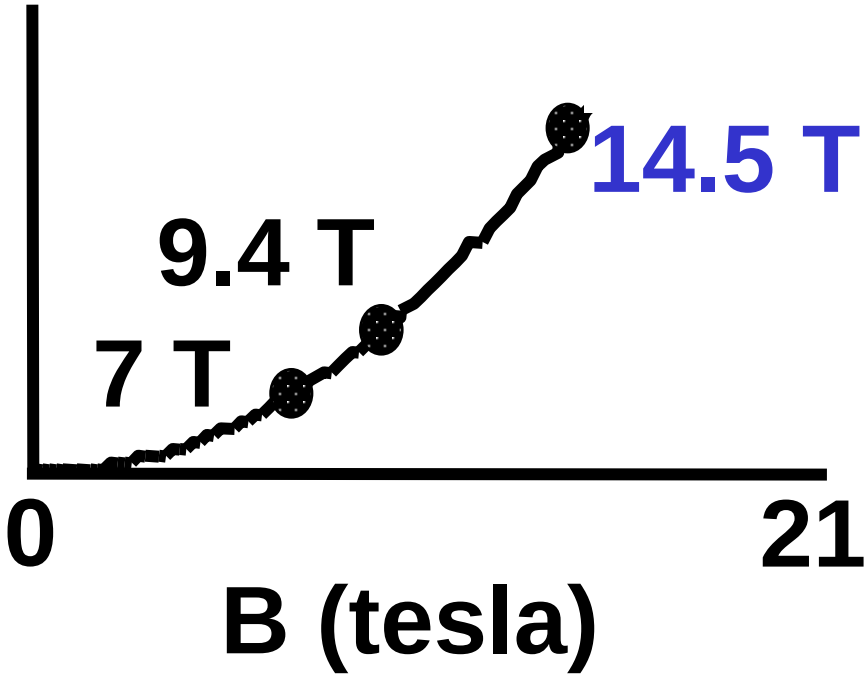


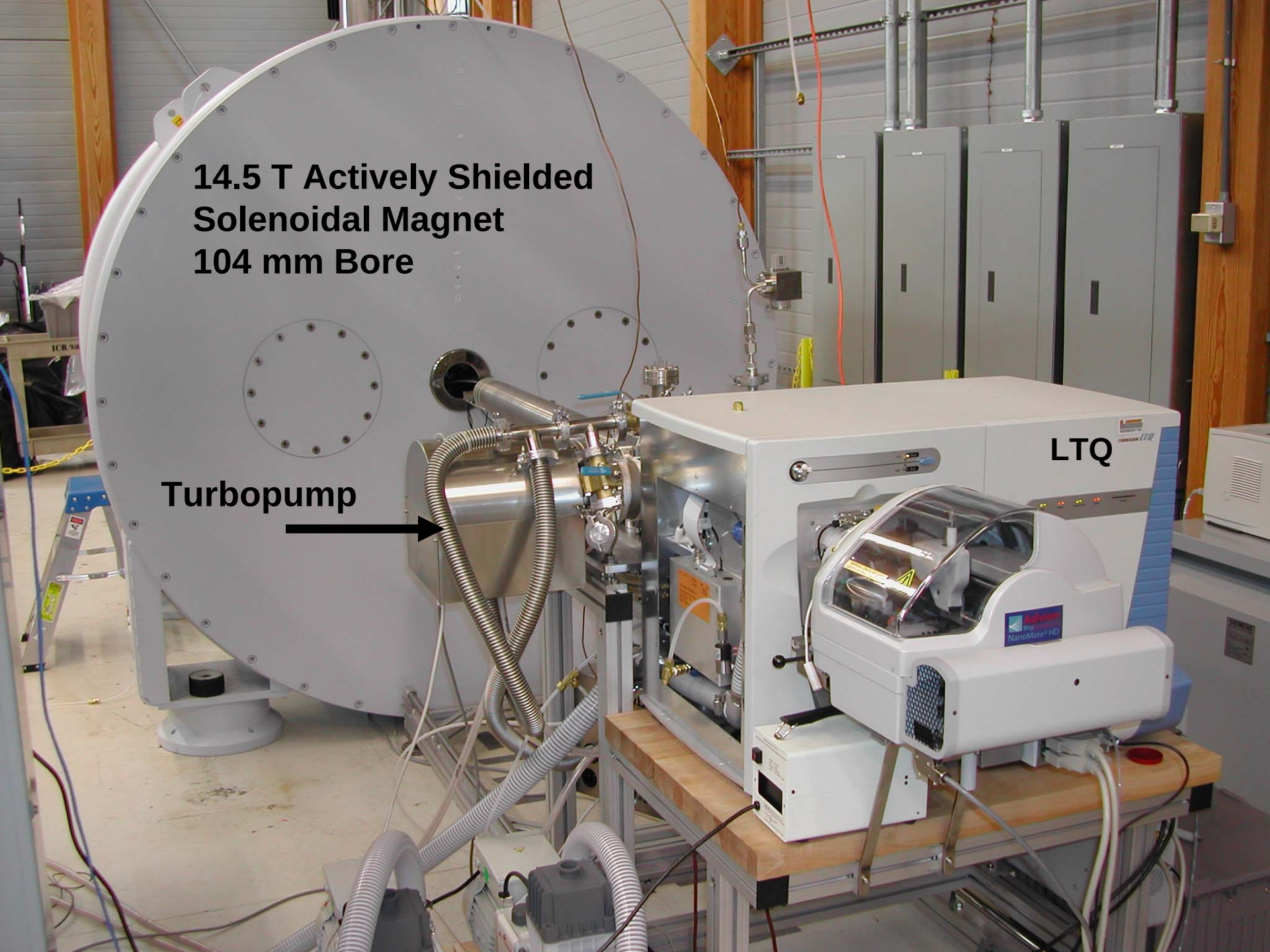
Ion Energy

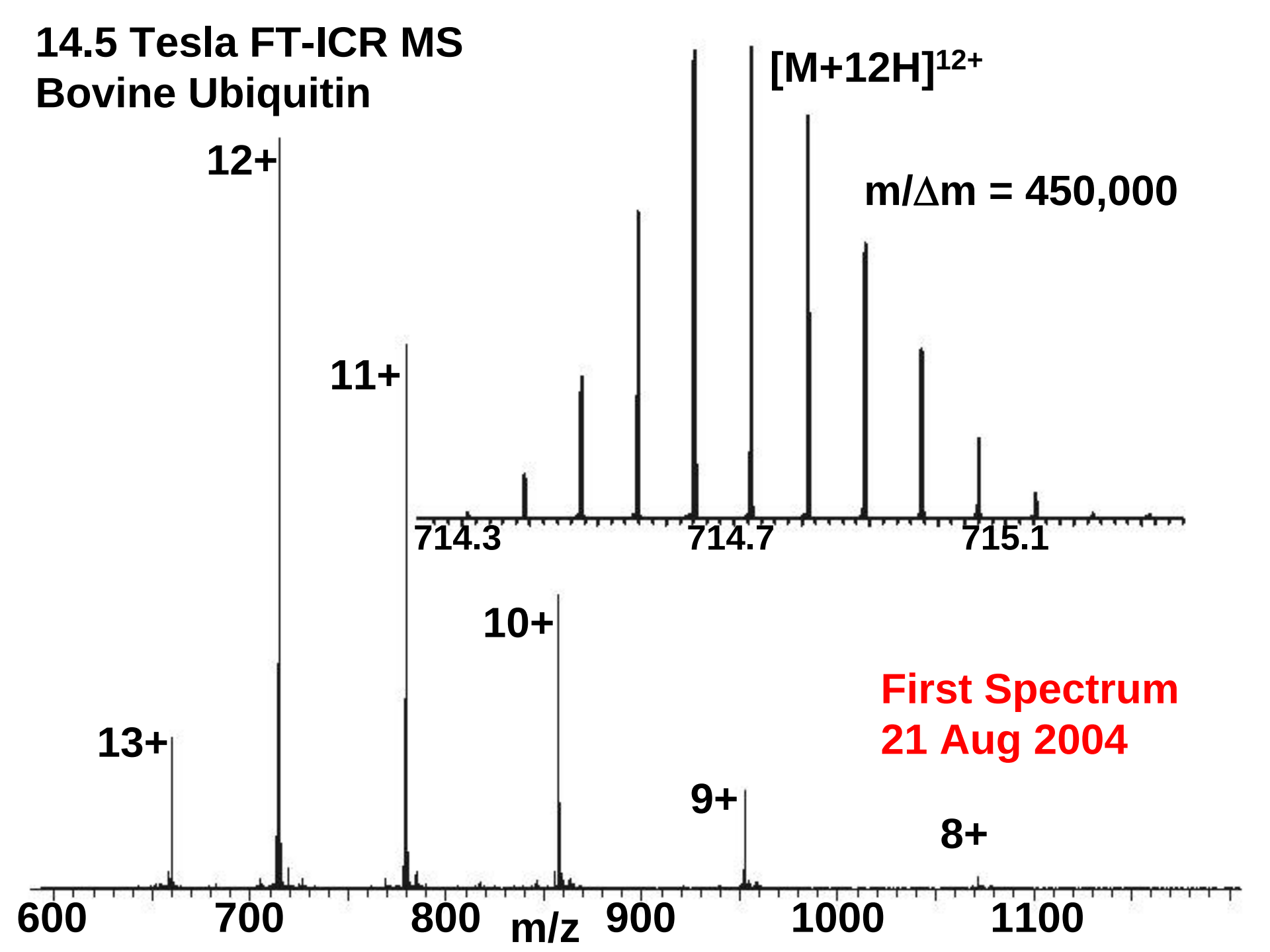
Number of Ions

Upper Mass Limit

Ion Trapping Period



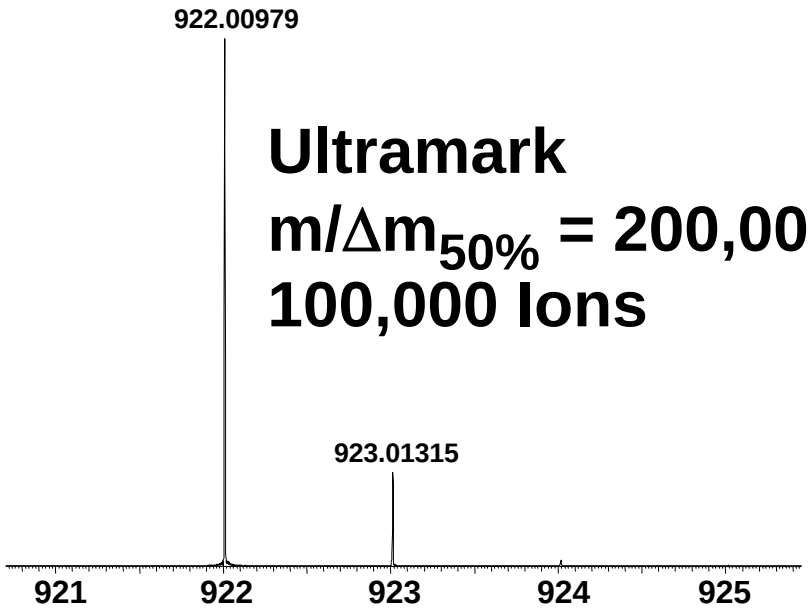

14.5 T Actively Shielded
Solenoidal Magnet
104 mm Bore
Turbopump
LTQ


14.5 Tesla FT-ICR MS
Bovine Ubiquitin
[M+12H]12+
m/Δm = 450,000
714.3
714.7
715.1
12+
11+
10+
13+
9+
8+
First Spectrum
21 Aug 2004
600
700
800
m/z
900
1000
1100

Single vs. Double Precision Centroid Determination

Glu-Fibrinogen Peptide Selected Ion Mode – Single Precision Mass Determination

Scan #	Measured Mass	Error (ppb)
1	785.84198	-51
2	785.84198	-51
3	785.84204	25
4	785.84198	-51
5	785.84204	25
6	785.84204	25
7	785.84204	25
8	785.84198	-51
9	785.84198	-51
10	785.84204	25



Ultramark

$m/\Delta m_{50\%}$ = 200,000

100,000 Ions

Single Precision Number = 32 bits

8 Bits for Exponent

$1/2^{24} = 6 \times 10^{-8}$

Double Precision Number = 64 bits

11 Bits for Exponent

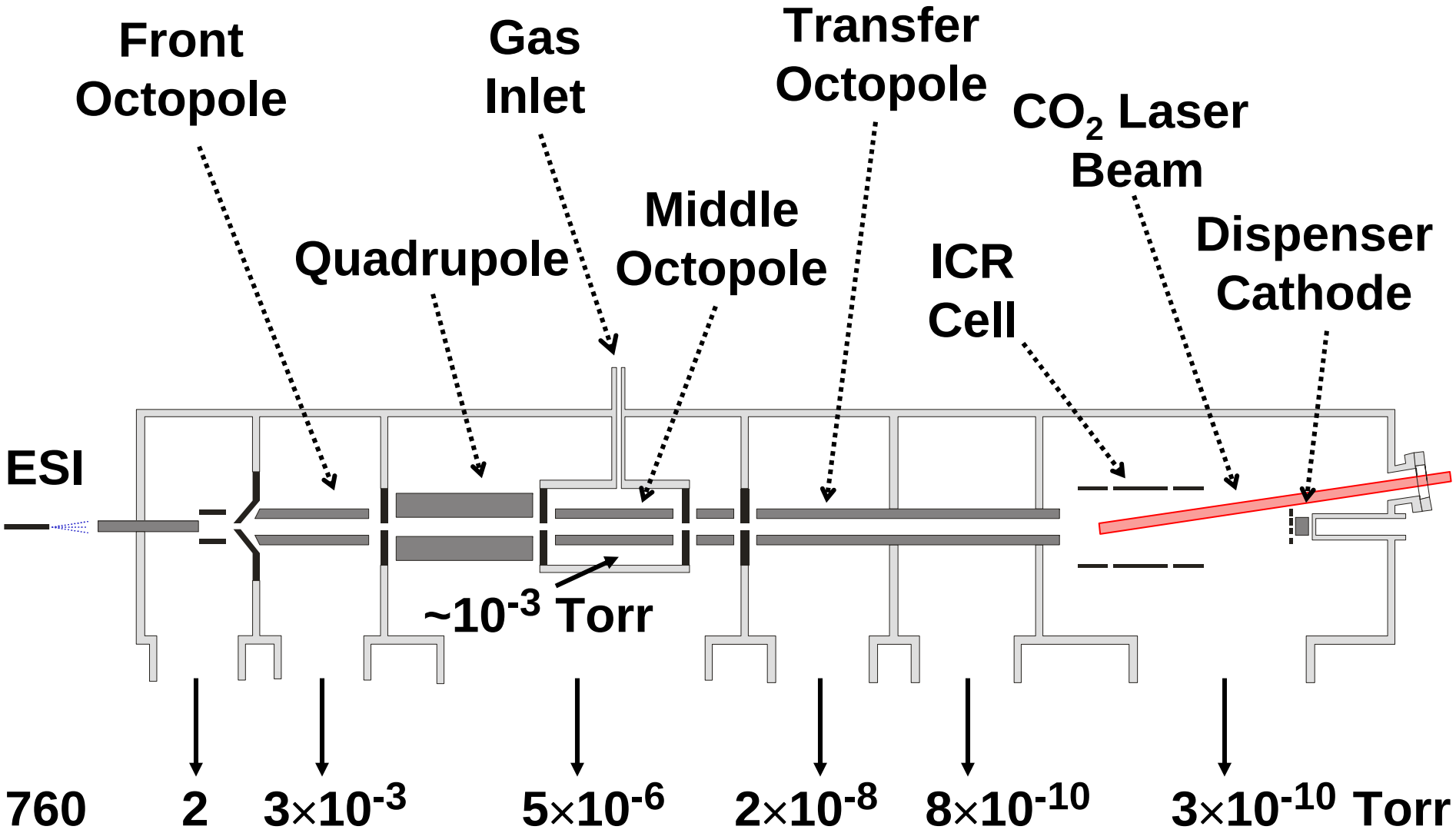
$1/2^{53} = 10^{-16}$

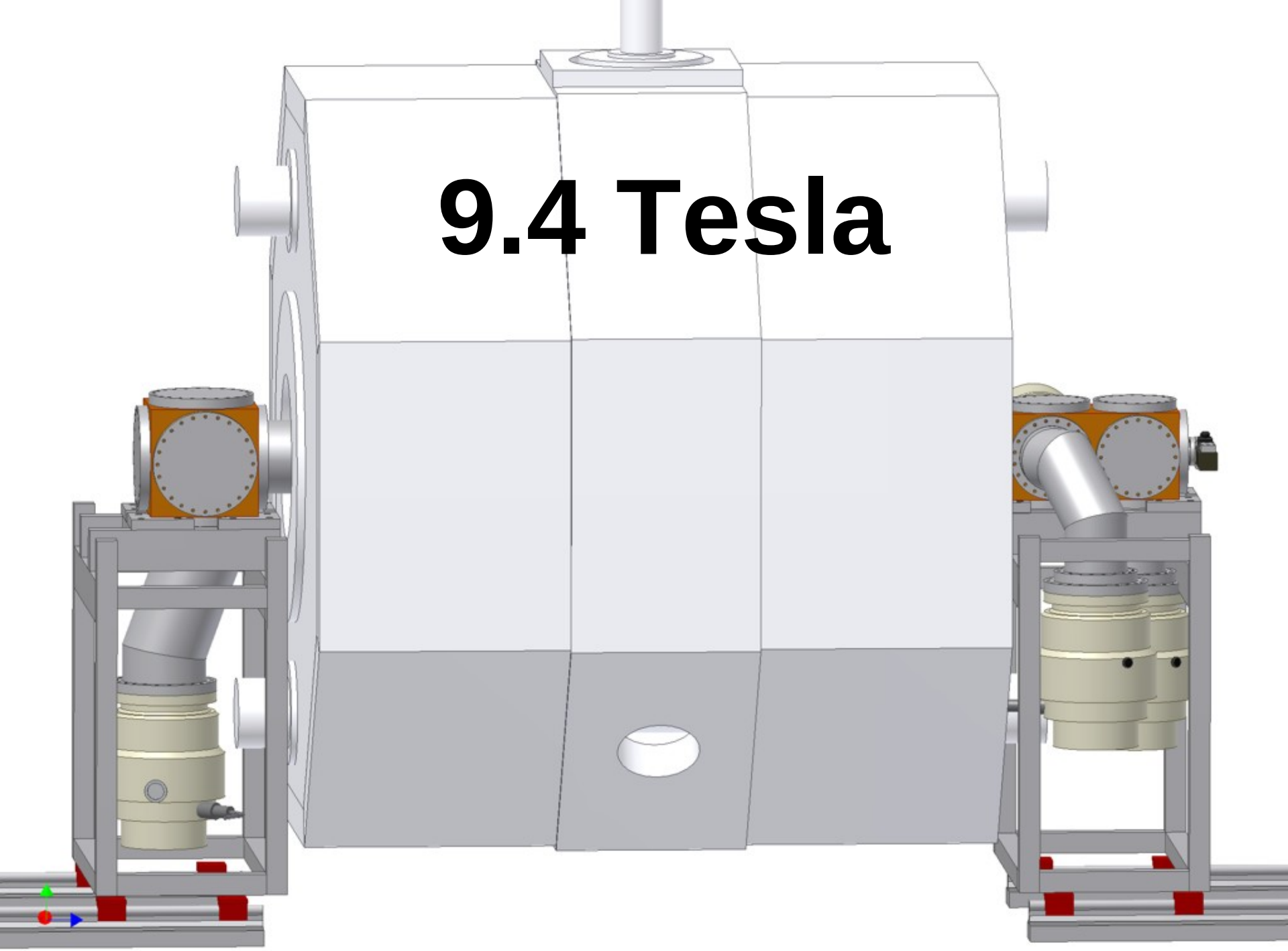
Ultramark Selected Ion Mode – Double Precision Mass Determination

Scan #	Measured Mass	Error (ppb)
1	922.00979	0
2	922.00978	-11
3	922.00979	0
4	922.00979	0
5	922.00978	-11
6	922.00979	0

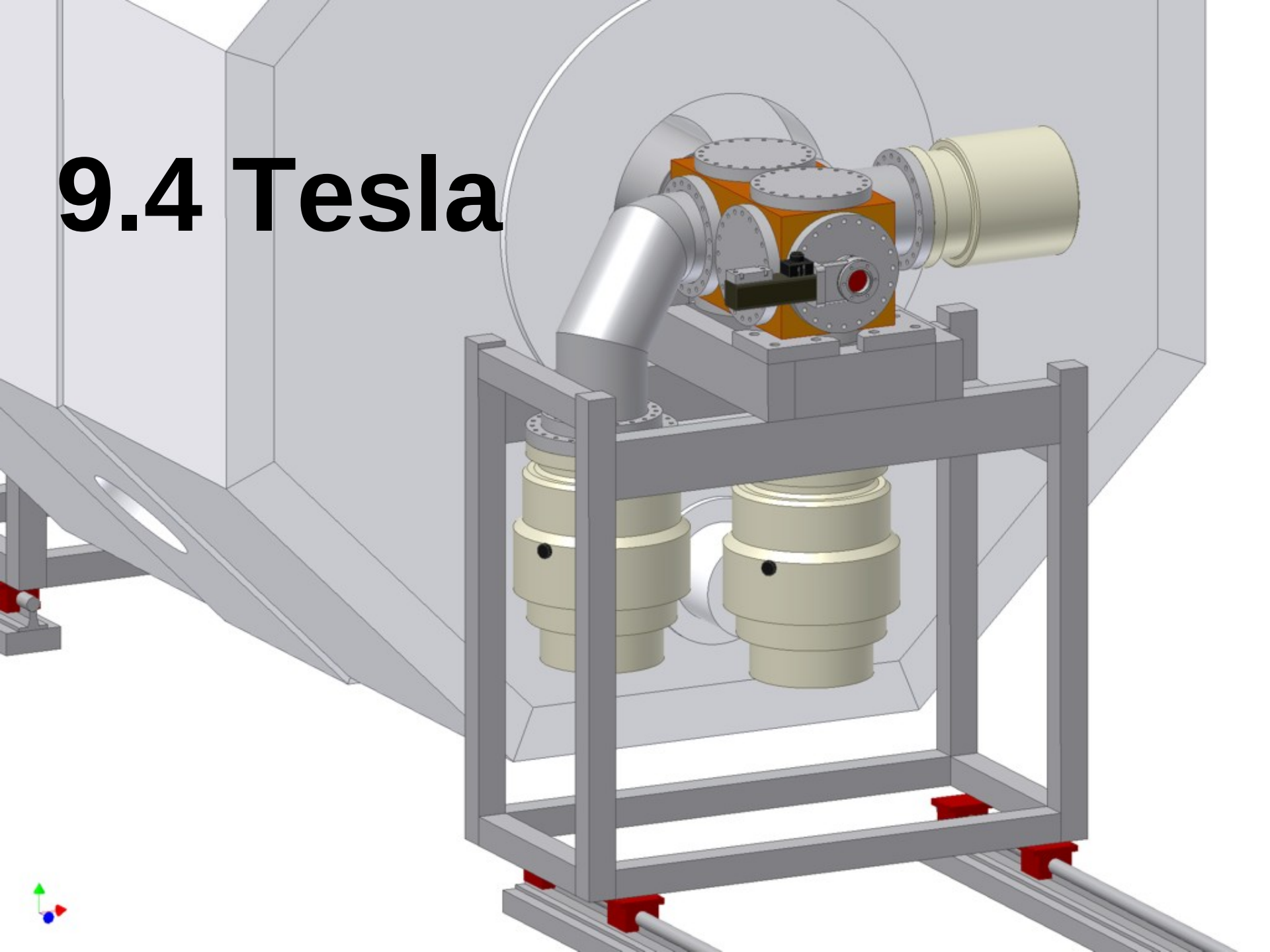
More Digits Needed from Software

It's not just the Magnet




9.4 Tesla

9.4 Tesla



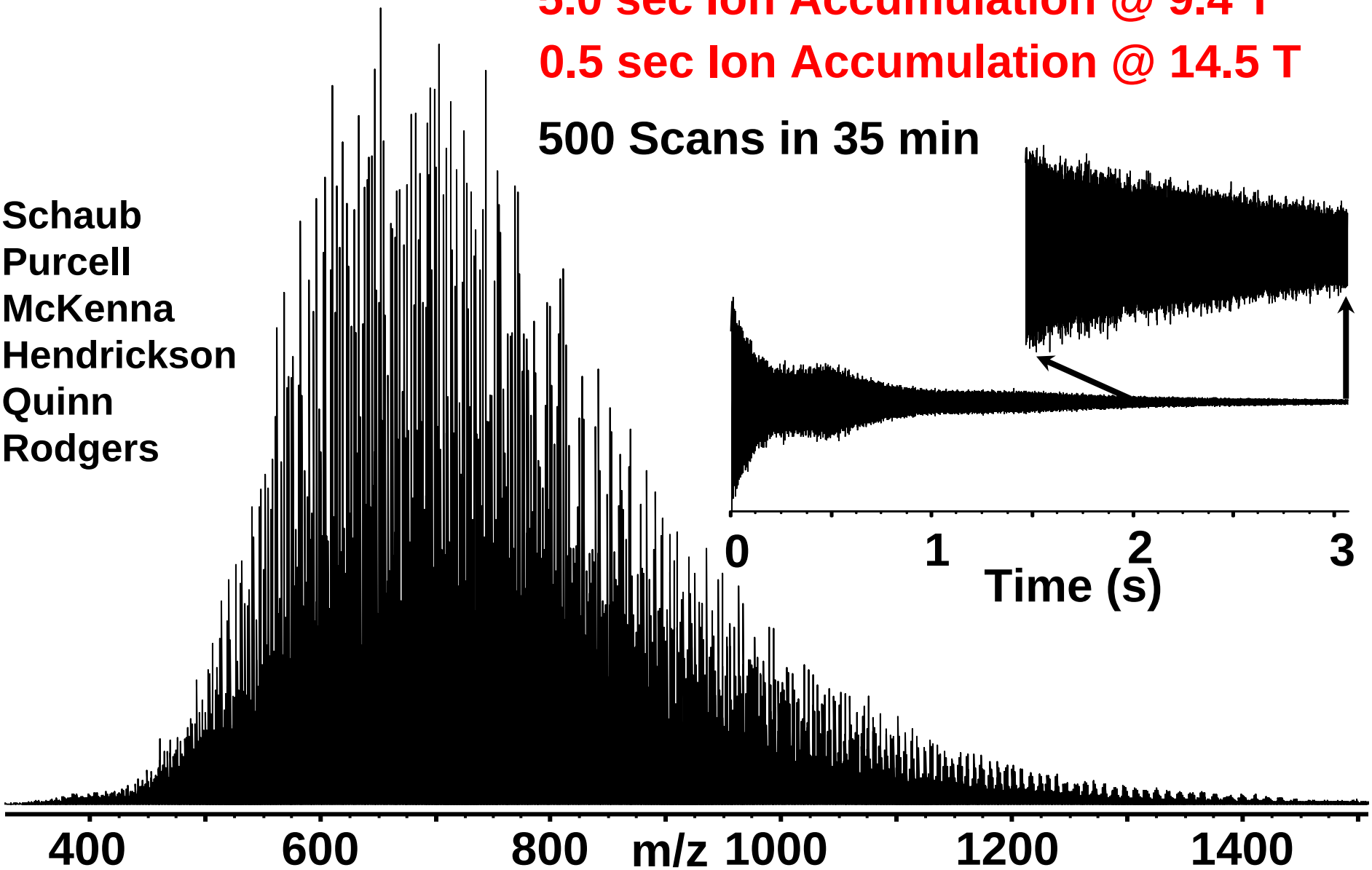
Arabian Light Crude Oil
Positive-Ion APPI 14.5 T FT-ICR MS

5.0 sec Ion Accumulation @ 9.4 T

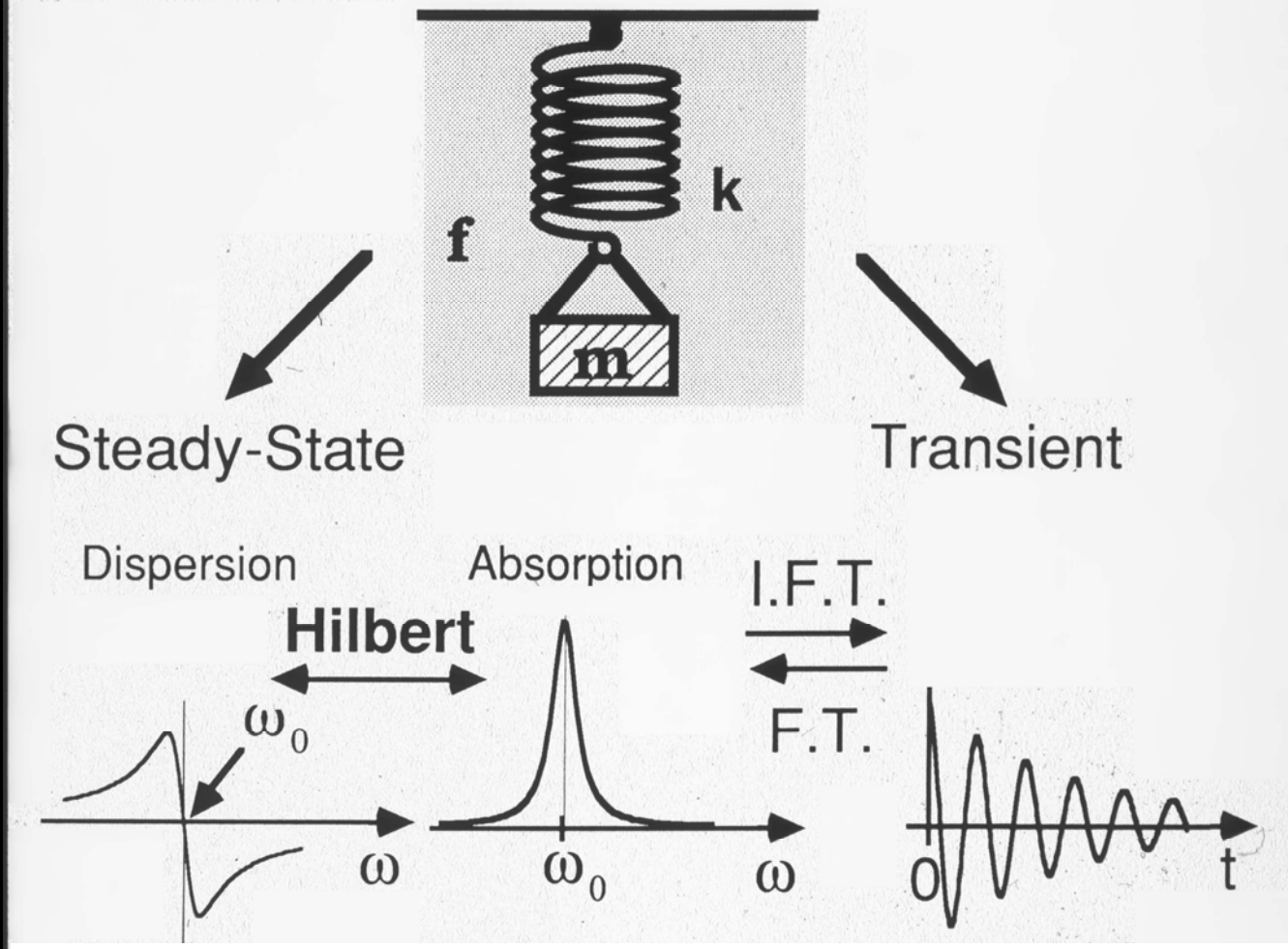
0.5 sec Ion Accumulation @ 14.5 T

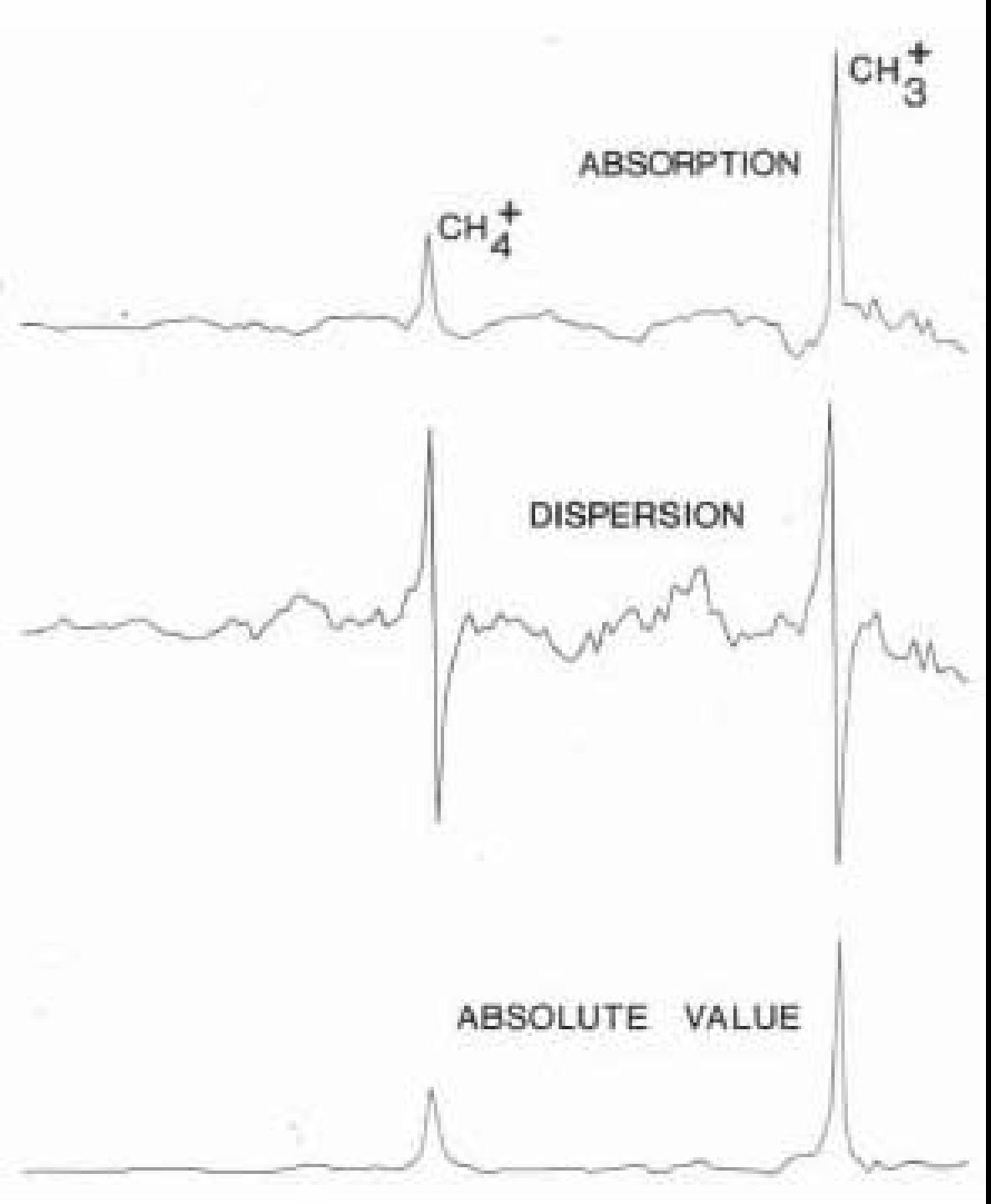
500 Scans in 35 min

Schaub
Purcell
McKenna
Hendrickson
Quinn
Rodgers

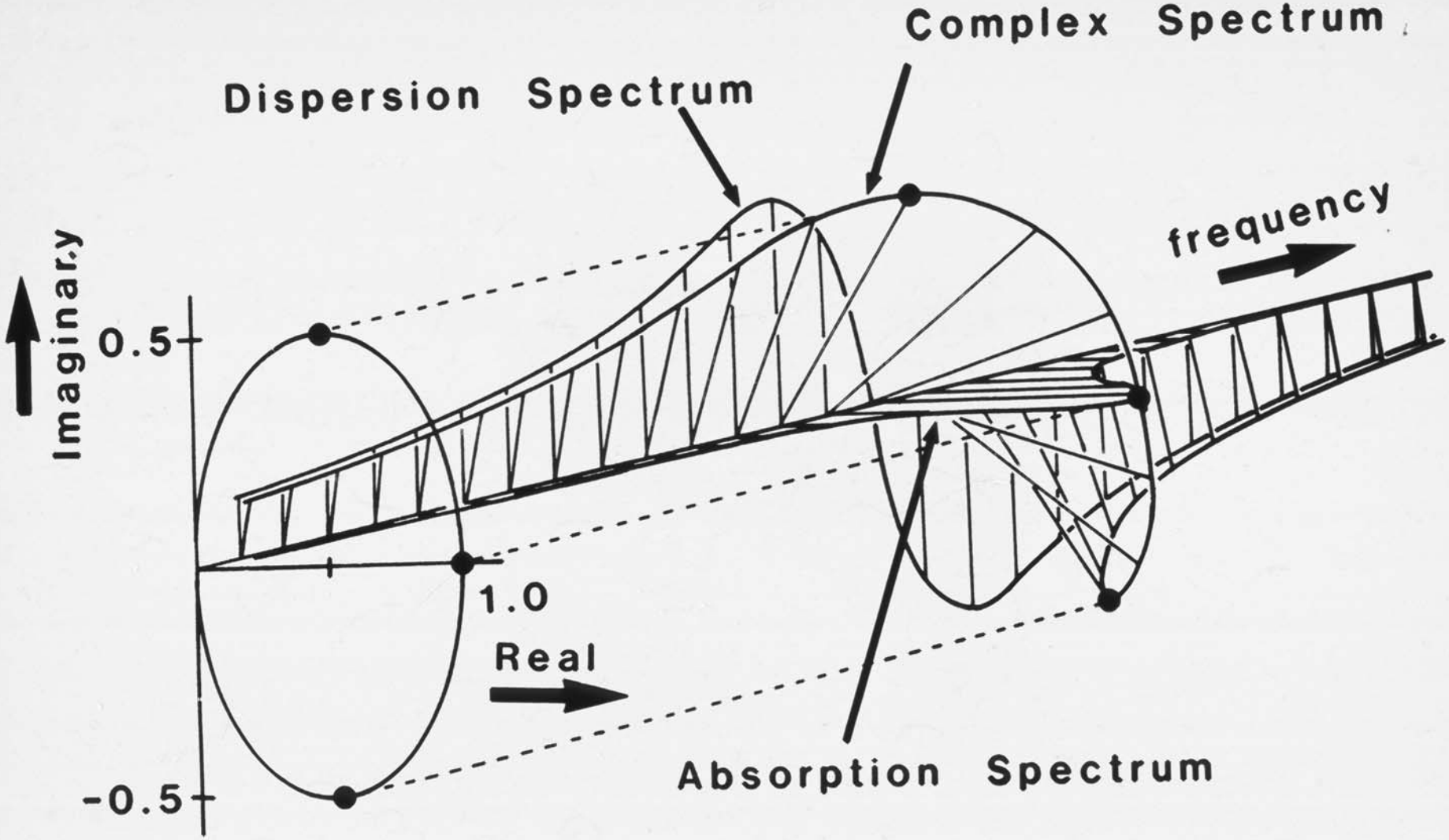


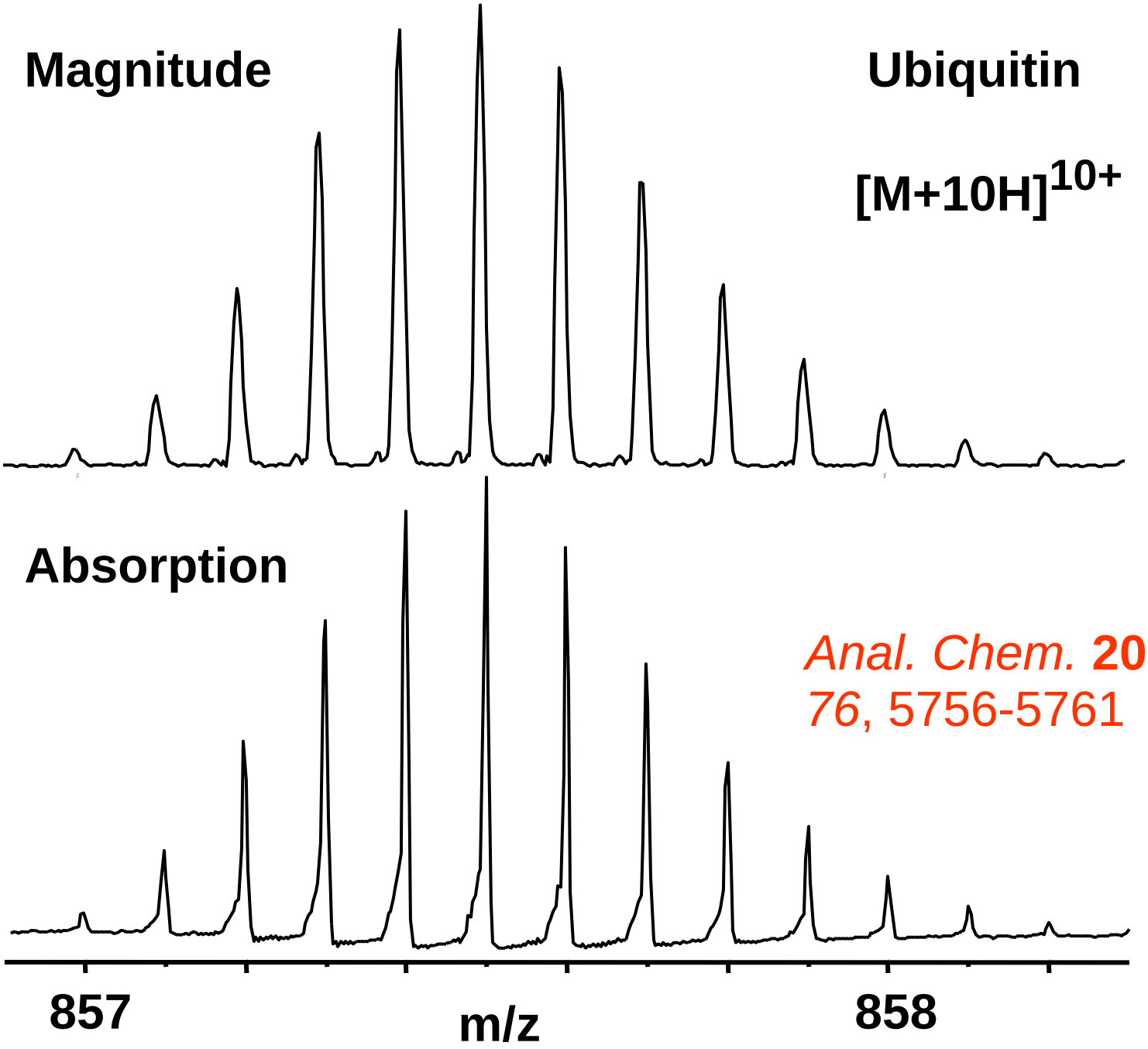
Phase Correction


k
f
m
Steady-State
Transient
Dispersion
Absorption
Hilbert
I.F.T.
F.T.
ω0
ω
ω0
ω
0
t



Comisarow & Marshall, "Selective-Phase Ion Cyclotron Resonance Spectroscopy," *Can. J. Chem.* **1974**, *52,* 1997-1999


Complex Spectrum
Dispersion Spectrum
frequency
Imaginary
0.5
1.0
Real
-0.5
Absorption Spectrum

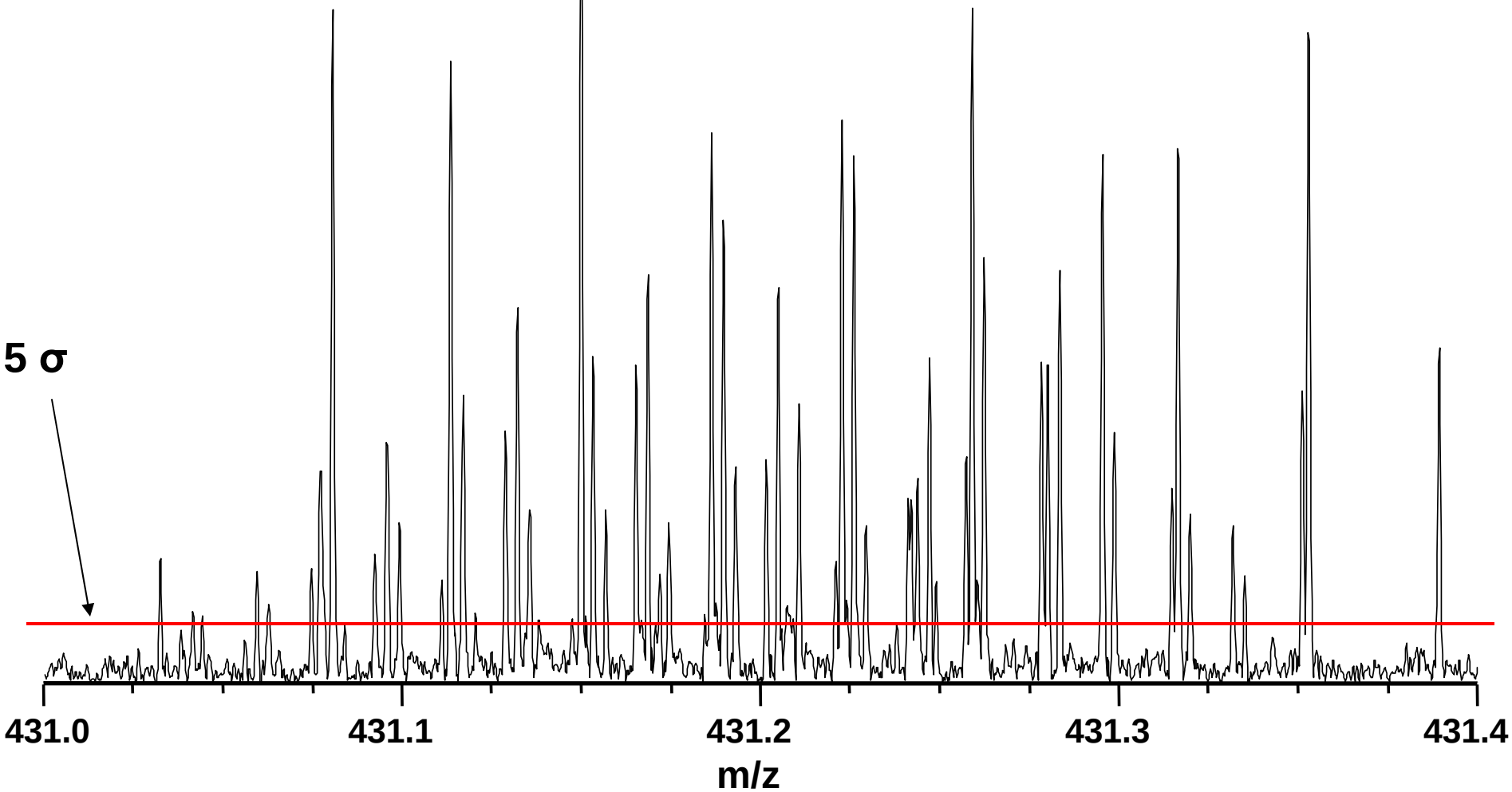


Anal. Chem. **2004**, *76*, 5756-5761

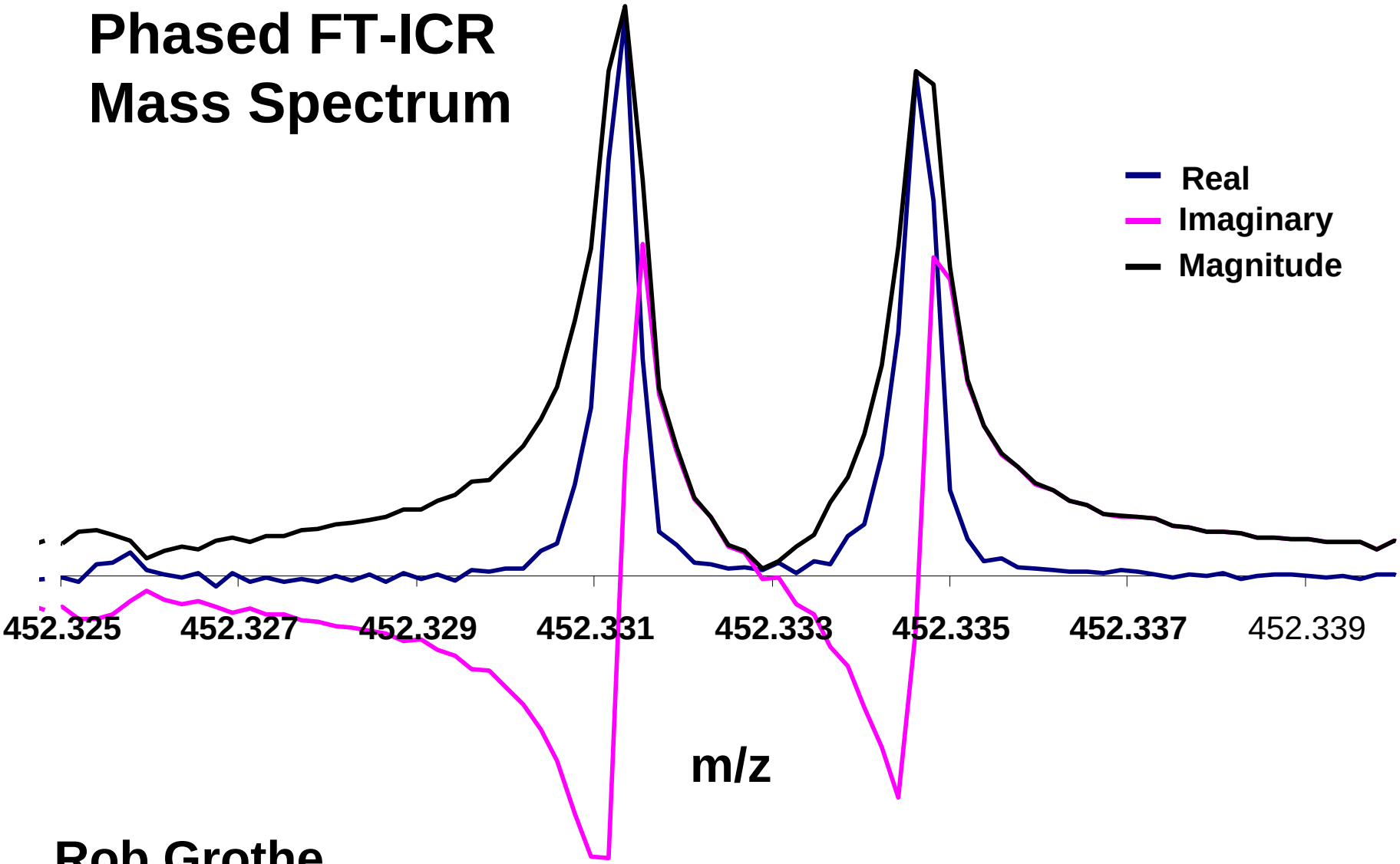
South American Crude Oil
Negative-Ion APPI 9.4 T FT-ICR MS

Anal. Chem. **2006**, *78*, 5906-5912

63 Spectral Peaks >5 σ Baseline Noise
62 Assigned Chemical Formulas

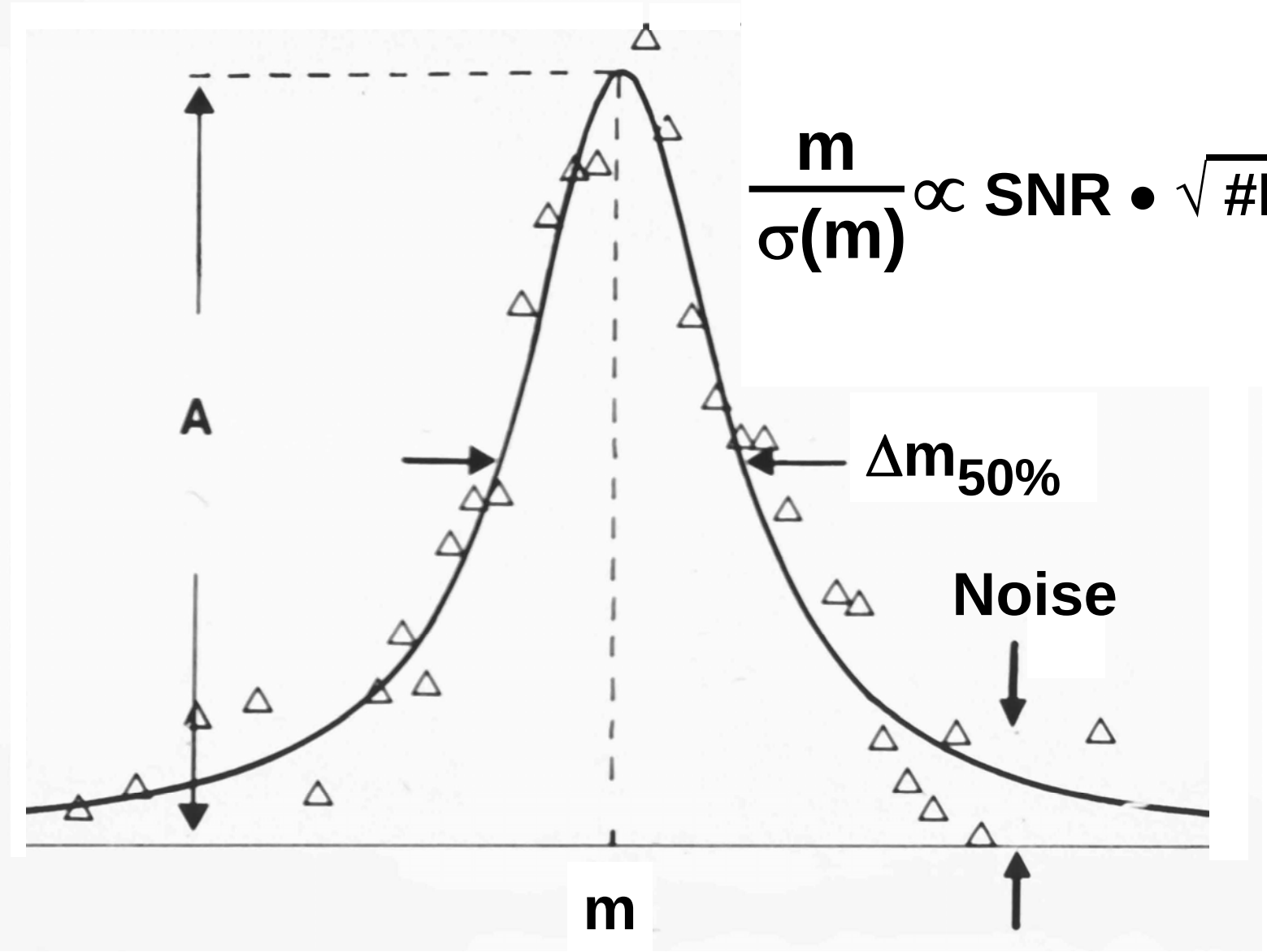


Phased FT-ICR Mass Spectrum



Rob Grothe
CedarsSinai/Thermo

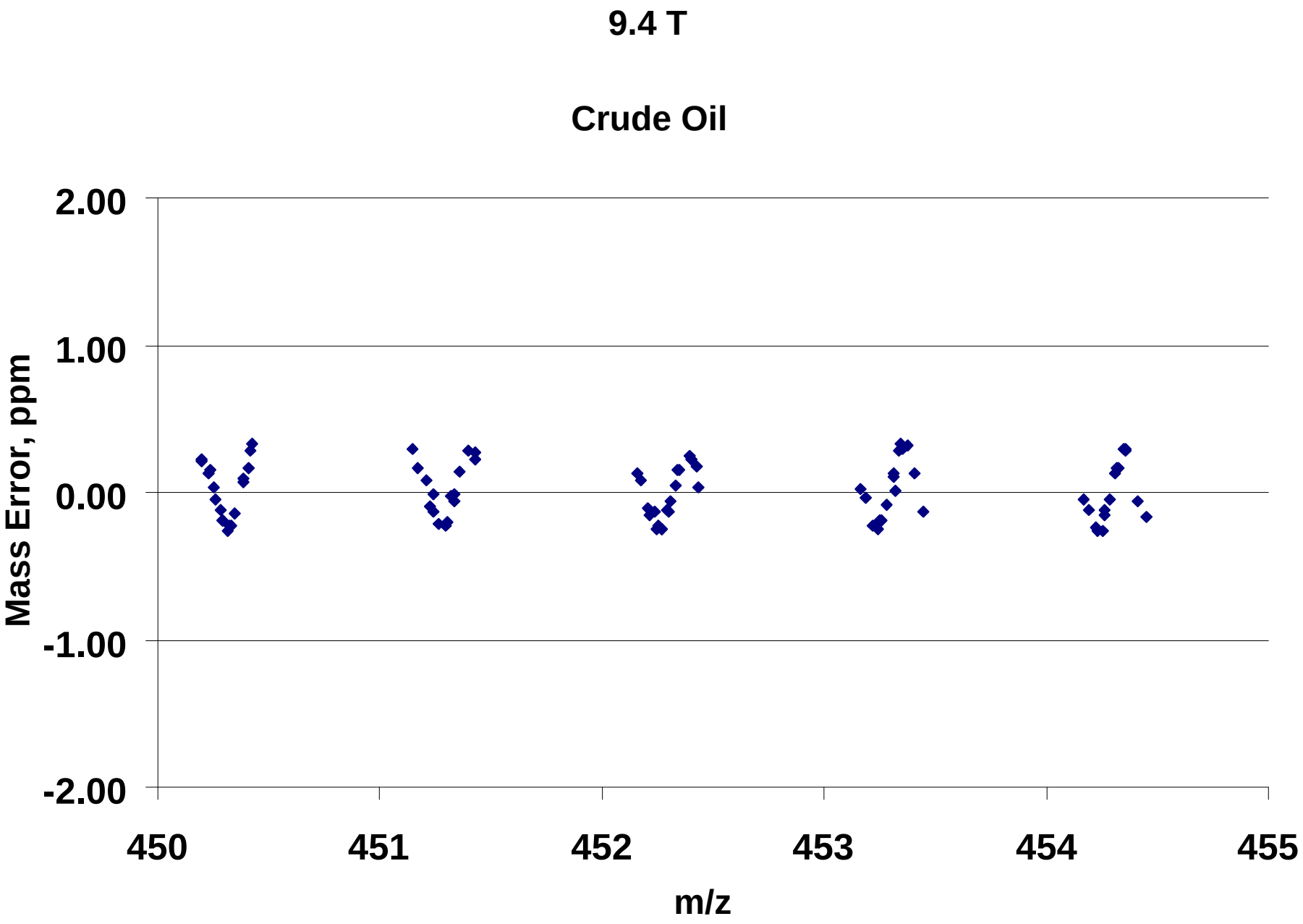
Mass Measurement Precision



$$\frac{m}{\sigma(m)} \propto \text{SNR} \bullet \sqrt{\#\text{Pts/Width}}$$

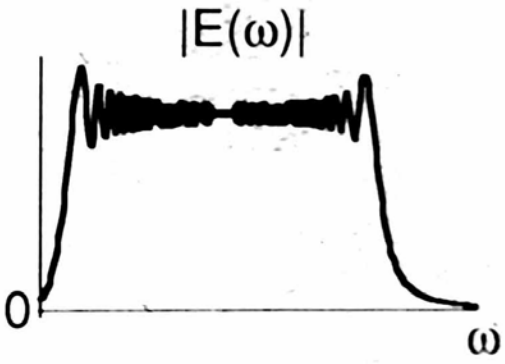
Chen, L.; Cottrell, C. E.; Marshall, A. G. "Effect of Signal-to-Noise Ratio and Number of Data Points upon Precision in Measurement of Peak Amplitude, Position, and Width in Fourier Transform Spectrometry", *Chemometrics and Intelligent Laboratory Systems* **1986,** *1*, 51-58

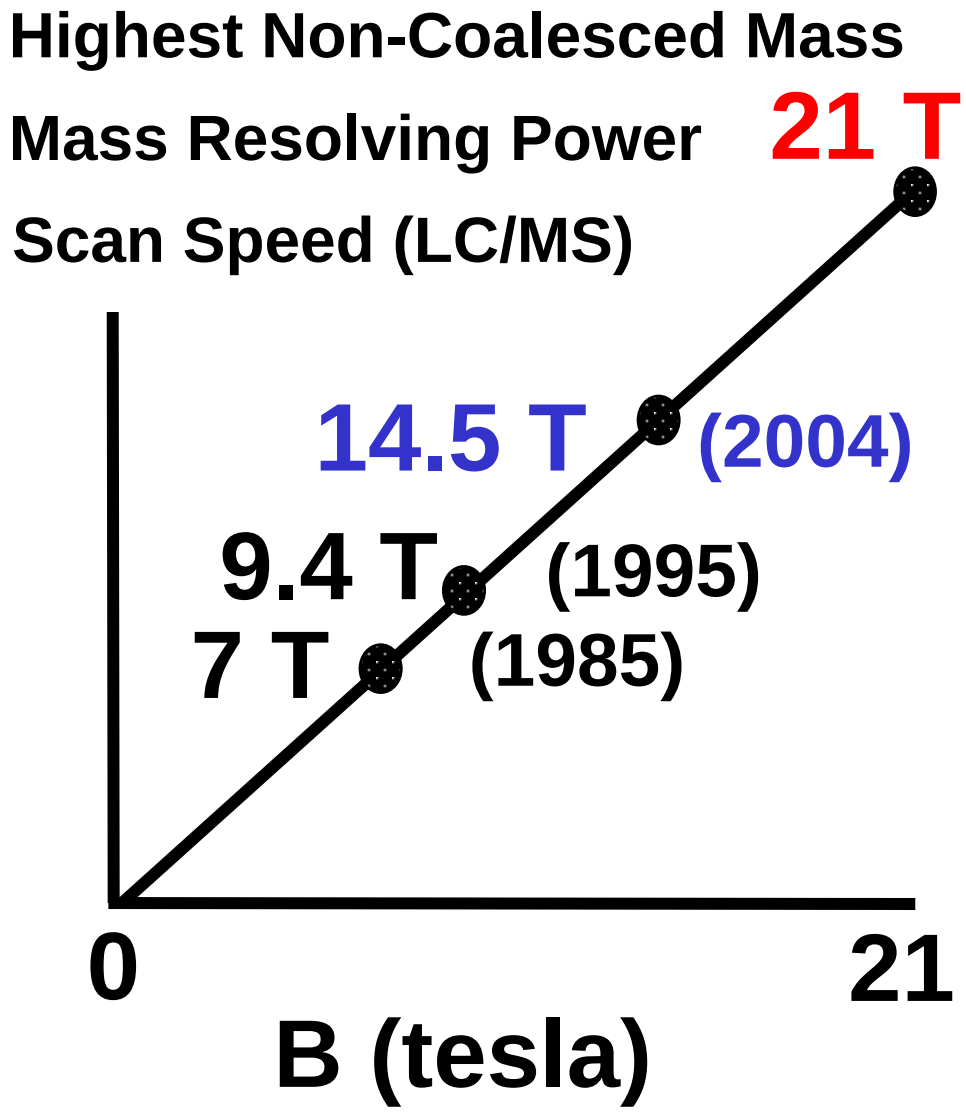
Systematic Mass Errors

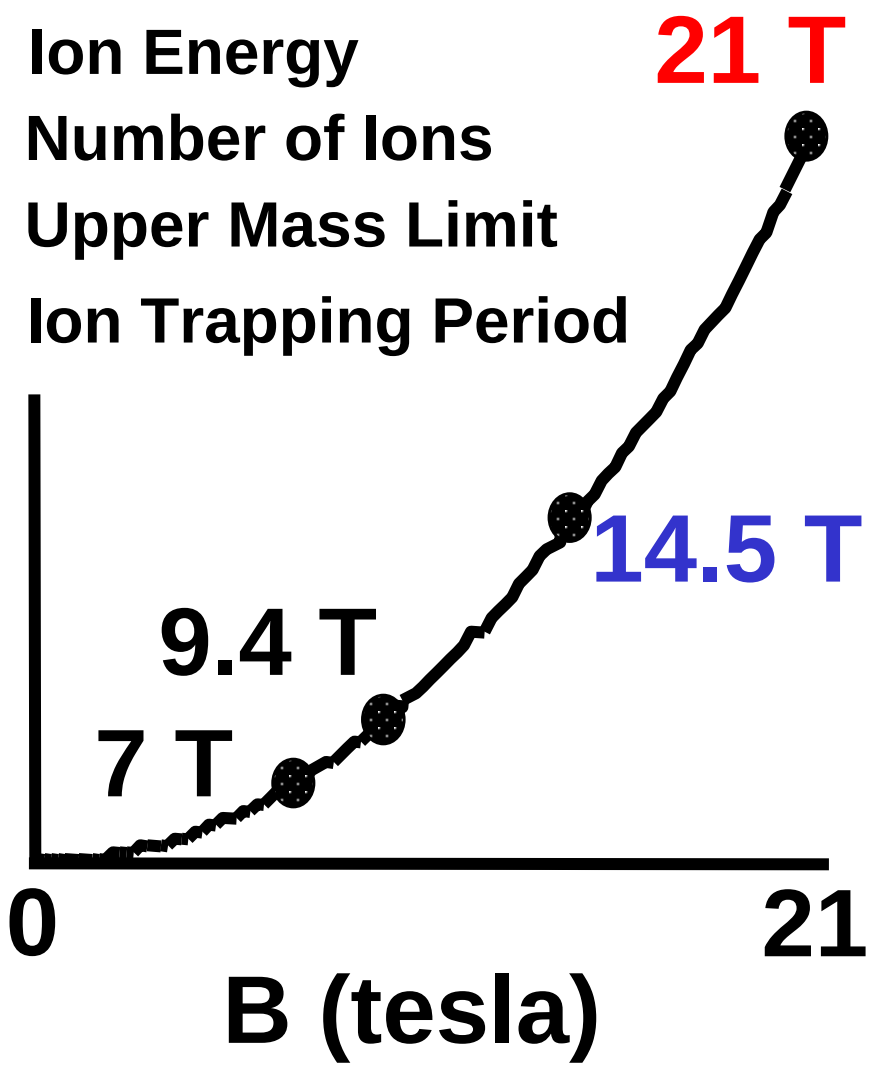

9.4 T
Crude Oil
2.00
1.00
0.00
-1.00
-2.00
Mass Error, ppm
450
451
452
453
454
455
m/z

e(t) = Frequency sweep excitation

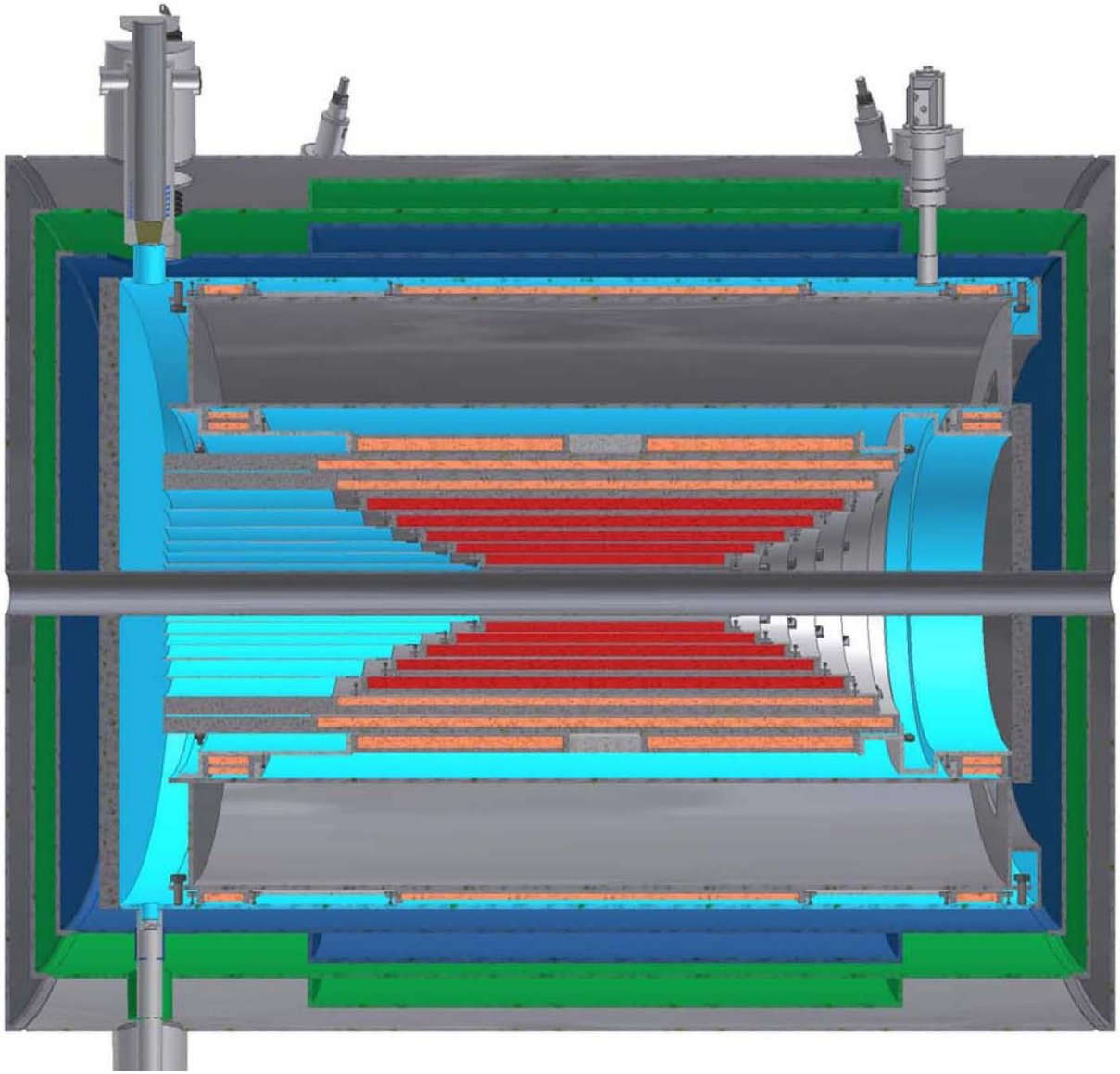
F.T.




Highest Non-Coalesced Mass
Mass Resolving Power
Scan Speed (LC/MS)
21 T
14.5 T
(2004)
9.4 T
(1995)
7 T
(1985)
0
21
B (tesla)


Ion Energy
Number of Ions
Upper Mass Limit
Ion Trapping Period
21 T
14.5 T
9.4 T
7 T
0
21
B (tesla)

21 T FT-ICR Magnet



Horizontal Bore

Cryocooler(s) for Extended Hold

2.2 K at Magnet

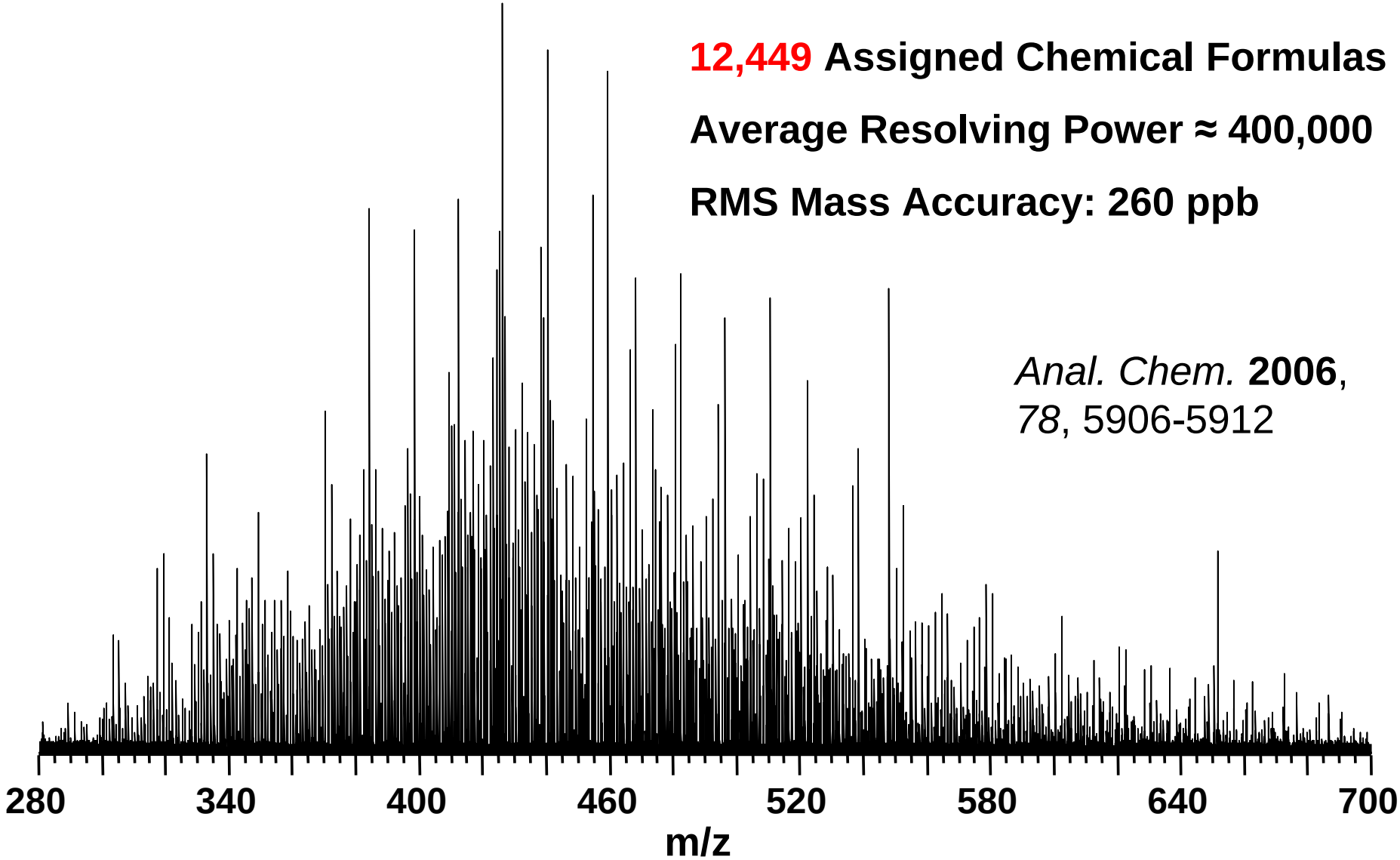
Active Shielding for Close Approach

Incremental becomes Transformative

Next-Highest Resolution
Mass Analyzer

High-Field FT-ICR MS

South American Heavy Crude Oil Negative-Ion Atmospheric Pressure Photoionization 9.4 T FT-ICR MS



12,449 Assigned Chemical Formulas

Average Resolving Power ≈ 400,000

RMS Mass Accuracy: 260 ppb

Anal. Chem. **2006**, *78*, 5906-5912

Arabian Light Crude Oil
Positive-Ion APPI 14.5 T FT-ICR MS

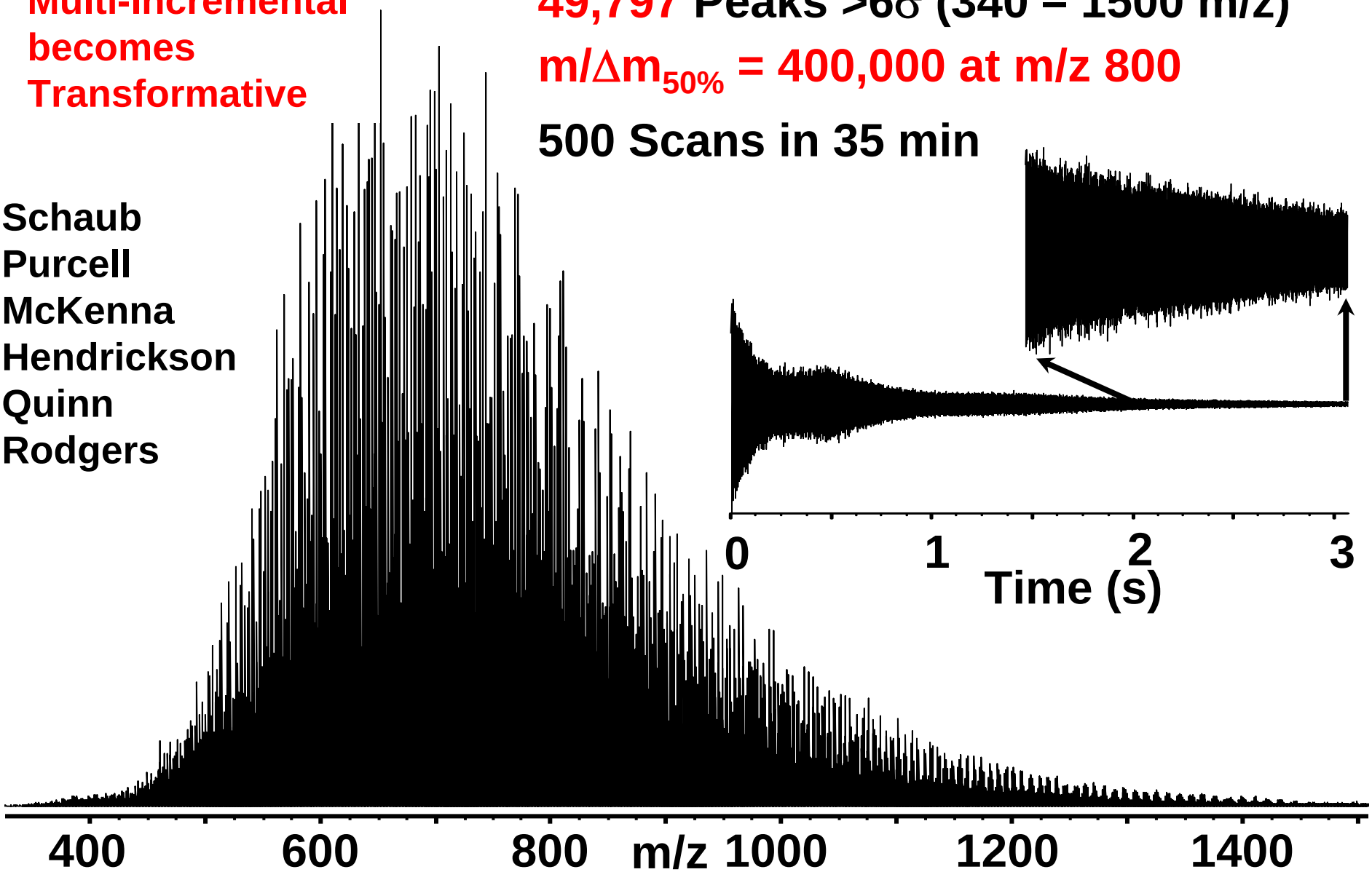
Multi-Incremental becomes Transformative

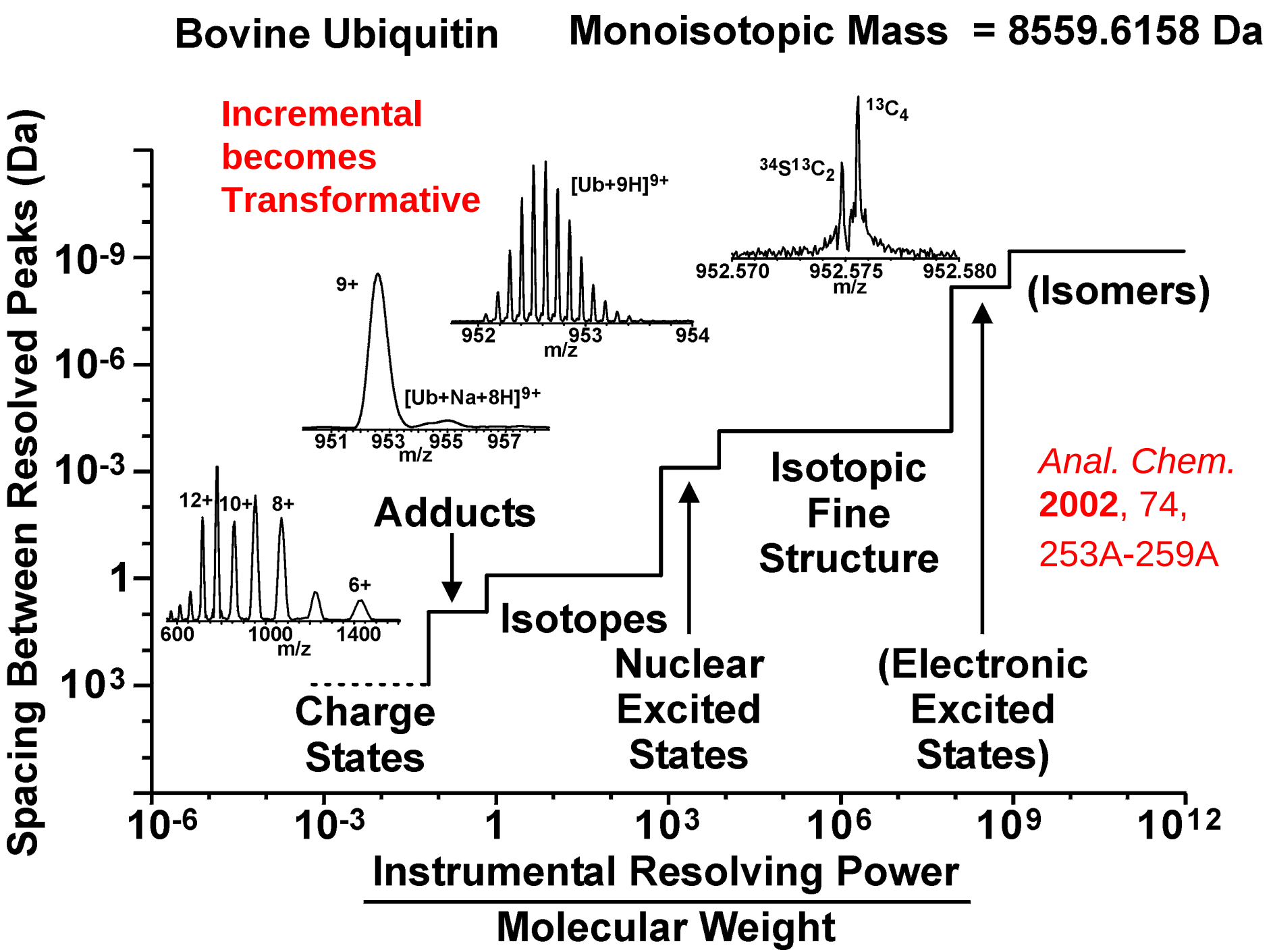
49,797 Peaks >6σ (340 – 1500 m/z)

$m/\Delta m_{50\%}$ = 400,000 at m/z 800

500 Scans in 35 min

Schaub
Purcell
McKenna
Hendrickson
Quinn
Rodgers

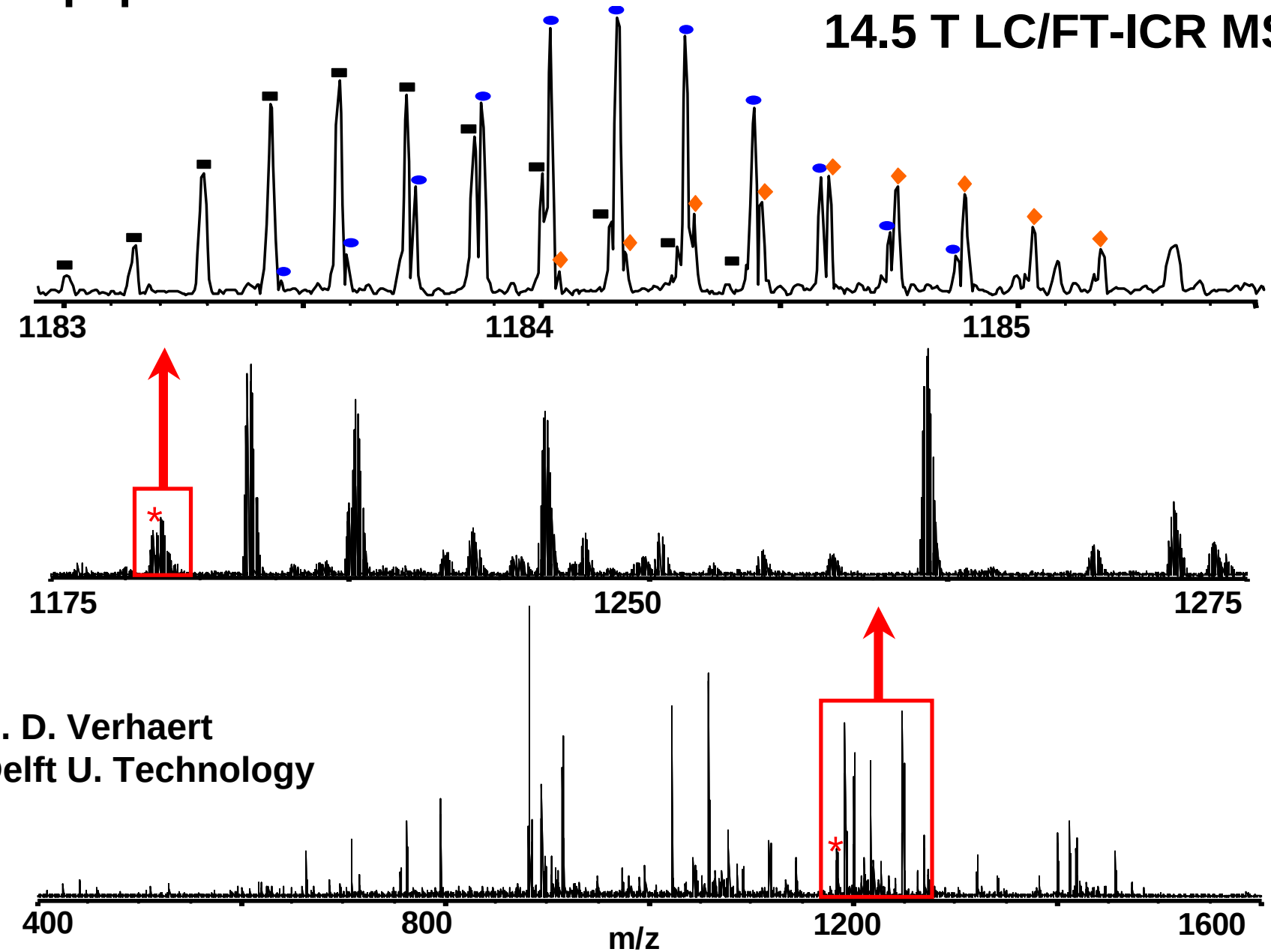



Bovine Ubiquitin
Monoisotopic Mass = 8559.6158 Da
Incremental becomes Transformative
Spacing Between Resolved Peaks (Da)
10-9
10-6
10-3
1
10 3
[Ub+9H]9+
952
953
954
m/z
9+
[Ub+Na+8H]9+
951
953
955
957
m/z
13C4
34S13C2
952.570
952.575
952.580
m/z
(Isomers)
12+
10+
8+
6+
600
1000
1400
m/z
Adducts
Isotopes
Charge States
Nuclear Excited States
Isotopic Fine Structure
(Electronic Excited States)
Anal. Chem. 2002, 74, 253A-259A
10-6
10-3
1
10 3
10 6
10 9
10 12
Instrumental Resolving Power
Molecular Weight

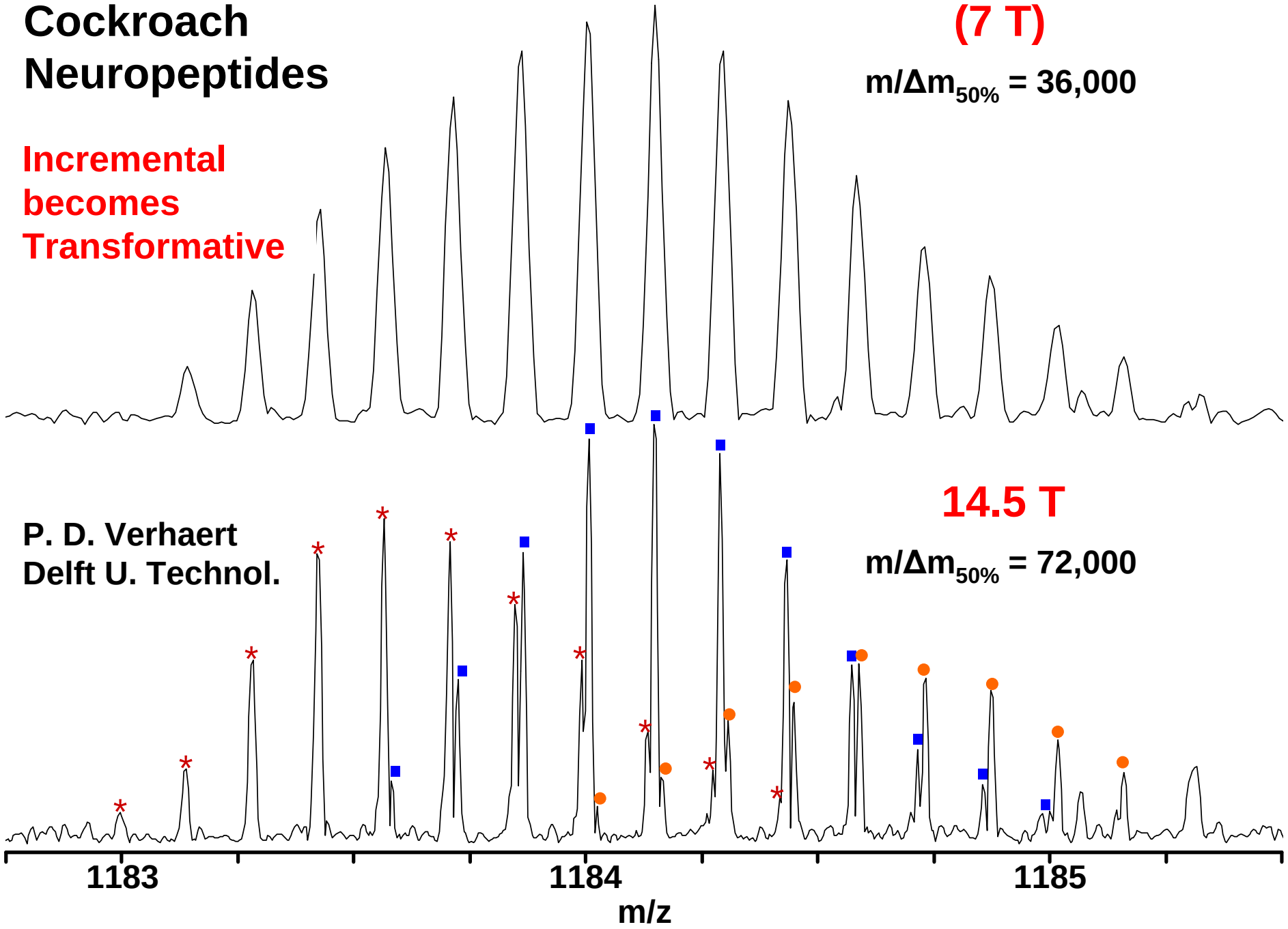
Cockroach Neuropeptides

$m/\Delta m_{50\%}$ = 200,000 at m/z 400 @ 1 Scan/sec

14.5 T LC/FT-ICR MS



P. D. Verhaert
Delft U. Technology


Cockroach
Neuropeptides
Incremental
becomes
Transformative
(7 T)
m/Δm50% = 36,000
14.5 T
m/Δm50% = 72,000
P. D. Verhaert
Delft U. Technol.
1183
1184
1185
m/z

APPI

$$M \xrightarrow{-\,e^-} M^{+\bullet} \qquad {}^{13}C$$

$$RH \xrightarrow{-\,e^-} RH^{+\bullet} \xrightarrow{M} MH^+ \qquad {}^{12}CH$$

****

Nitrogen Rule

McLafferty, *Interpretation of Mass Spectra*, 1993

	Nominal Mass	
	Odd	**Even**
$N_0, N_2, N_4, \ldots$	MH^+	$M^{+\bullet}$
$N_1, N_3, N_5, \ldots$	$M^{+\bullet}$	MH^+

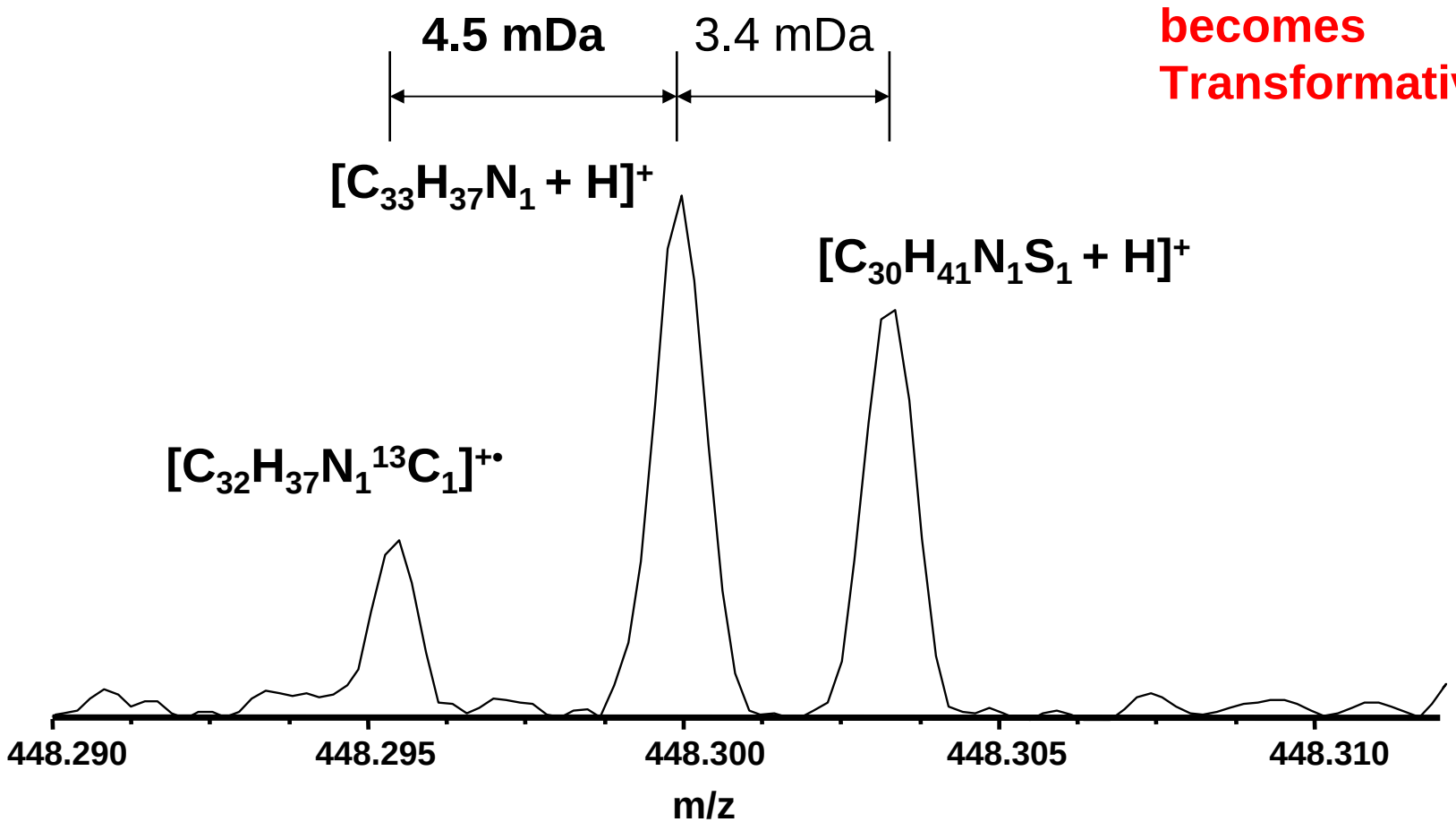
Ultrahigh Mass Resolving Power

3.4 mDa → C_3 versus SH_4

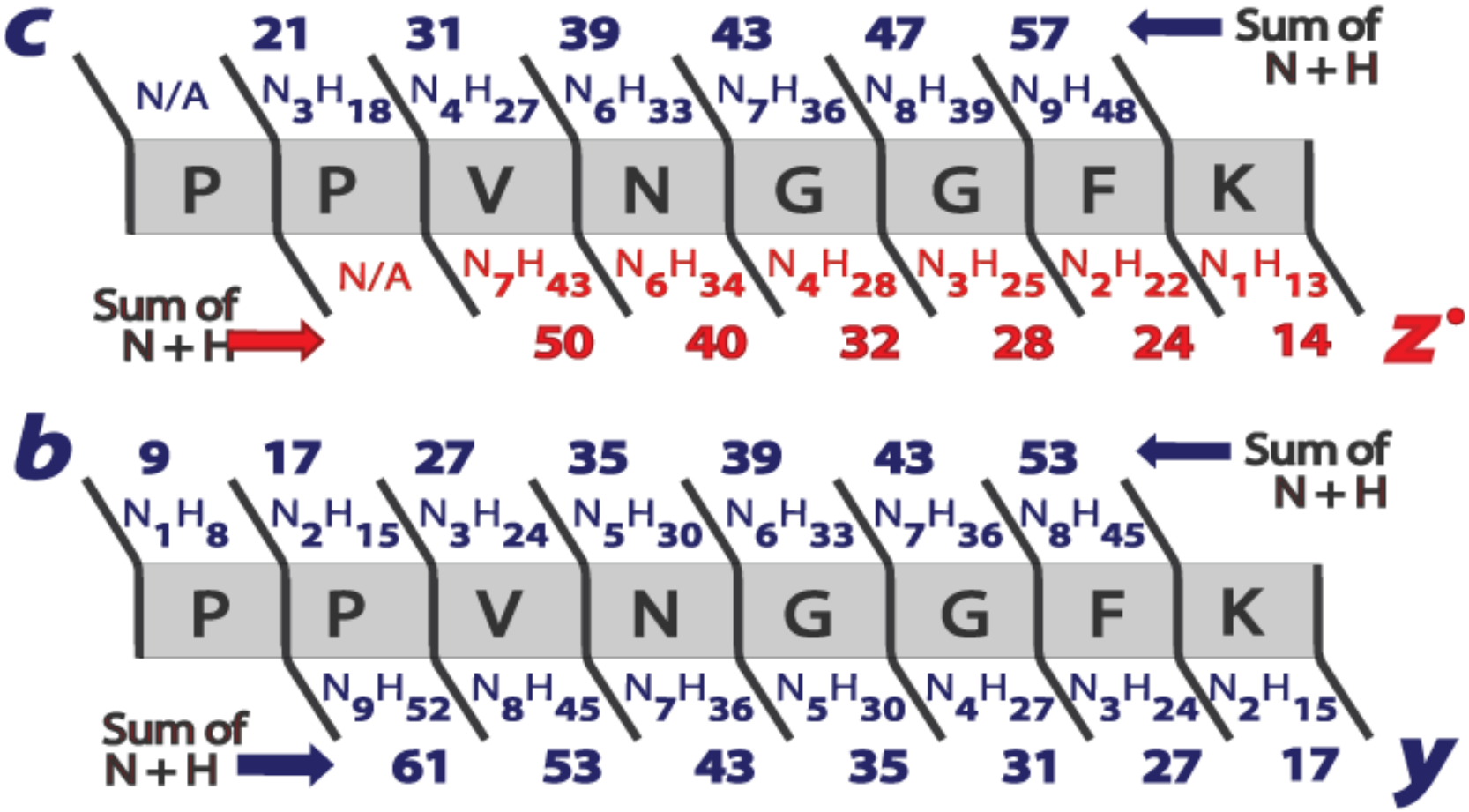
4.5 mDa → ^{13}C versus CH

South American Crude Oil

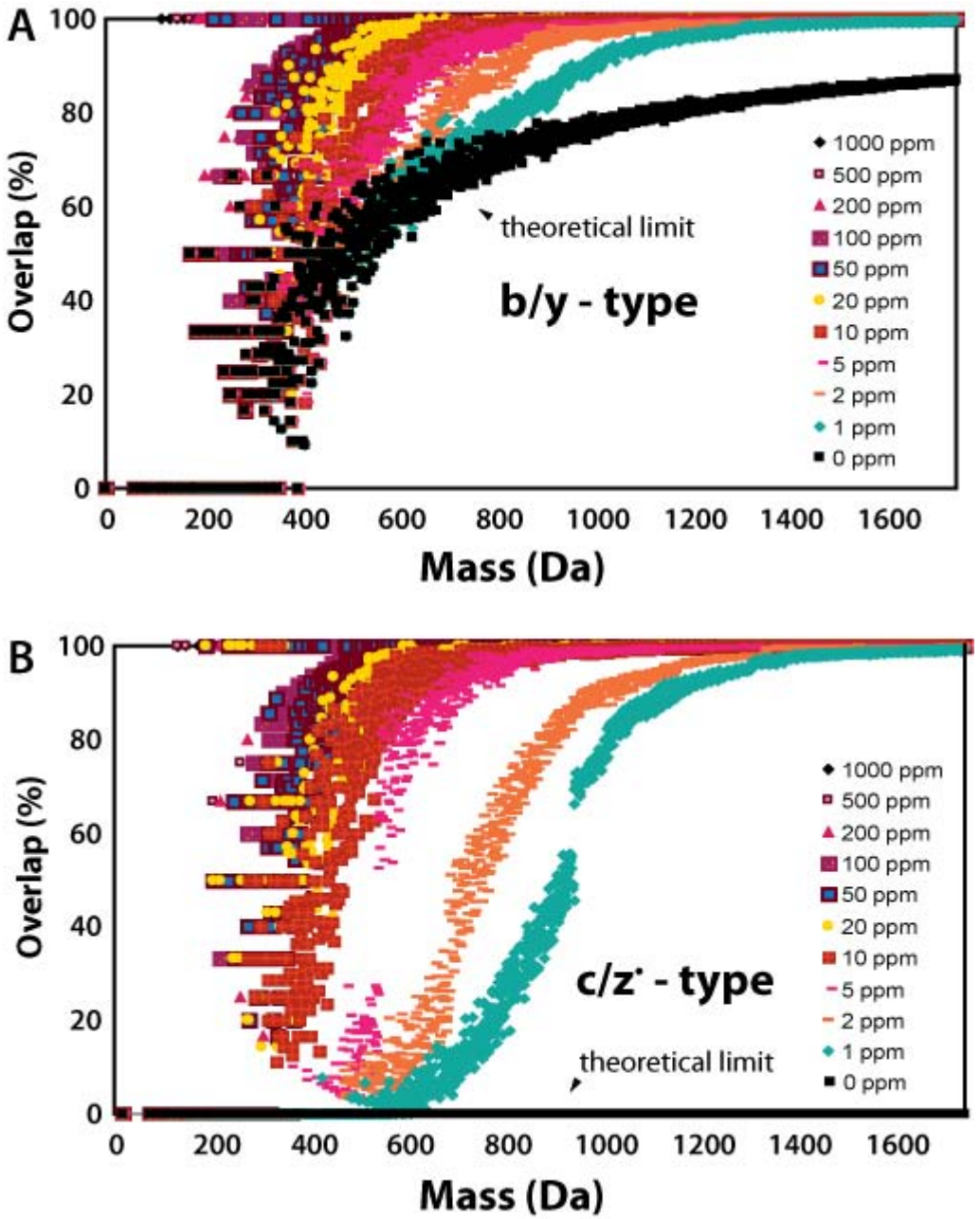
Incremental becomes Transformative



(N + H) Rule

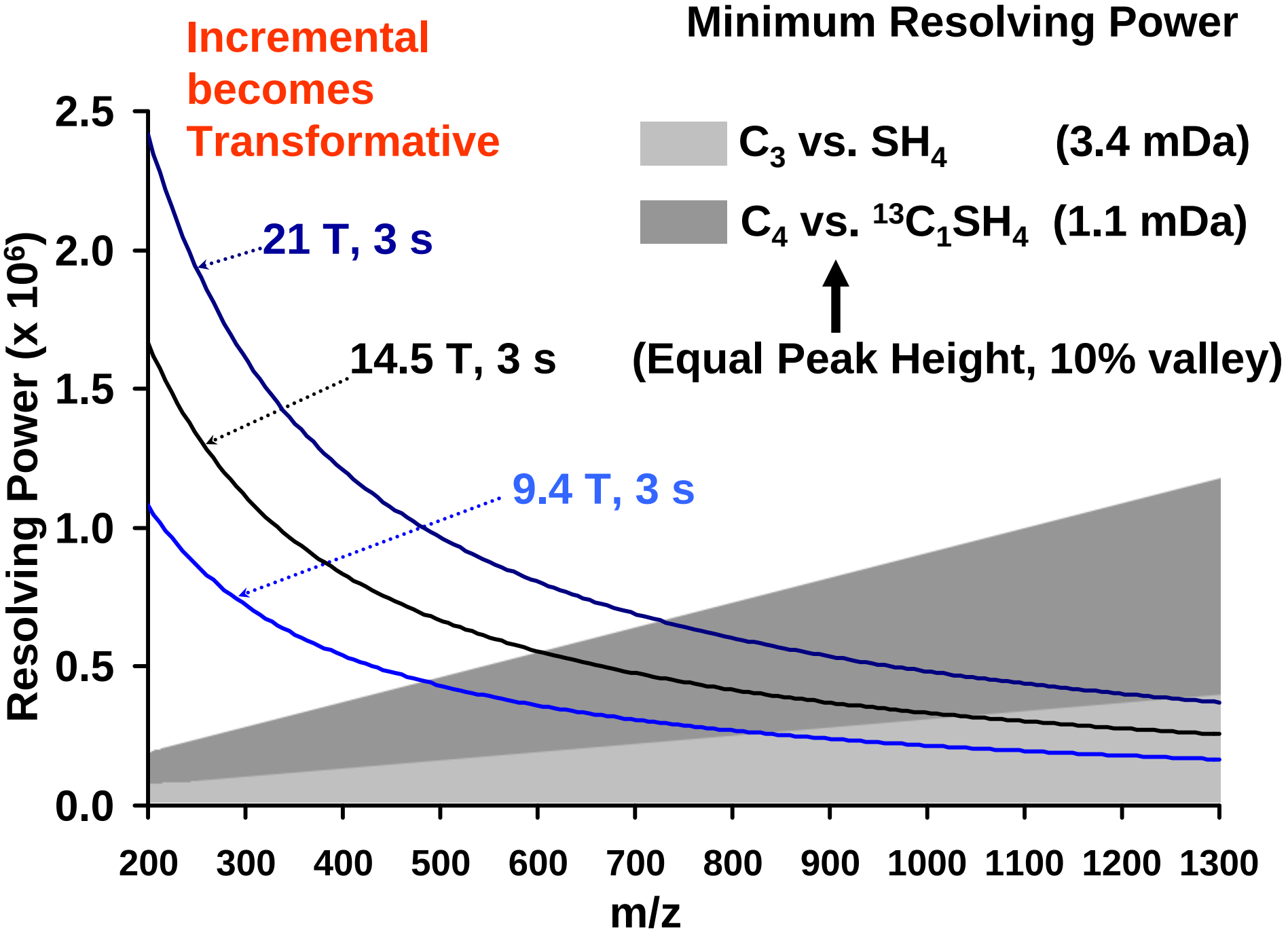


S. L. Hubler, A. Jue, J. Keith, B. C. McAlister, G. Carciun, J. J. Coon *JACS* **2008** (in press)

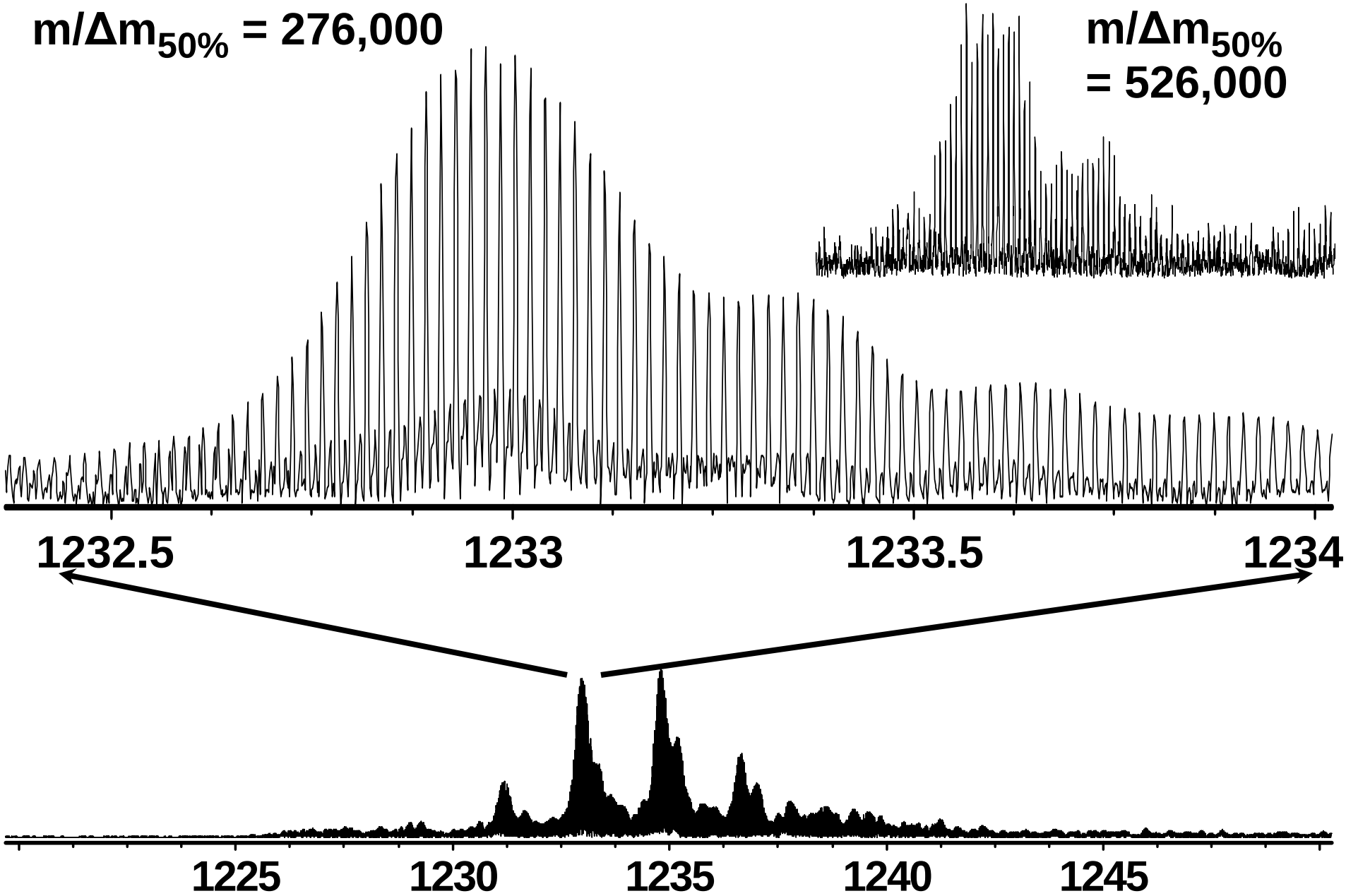


Incremental becomes Transformative

S. L. Hubler, A. Jue, J. Keith, B. C. McAlister, G. Carciun, J. J. Coon *JACS* **2008** (in press)


Minimum Resolving Power
Incremental becomes Transformative
C_3 vs. SH_4 (3.4 mDa)
C_4 vs. $^{13}C_1SH_4$ (1.1 mDa)
(Equal Peak Height, 10% valley)
21 T, 3 s
14.5 T, 3 s
9.4 T, 3 s
Resolving Power (x 10^6)
2.5
2.0
1.5
1.0
0.5
0.0
200
300
400
500
600
700
800
900
1000
1100
1200
1300
m/z

BSA, Unpurified, Selected Charge State, 100 Transients



The best reason for higher magnetic field:

Experiments that can be performed only with heroic difficulty at low field become routine at high field.

Example: Petroleomics

Breakout Group Discussion Topics

Magnet

Horizontal vs. Vertical

Bore Diameter vs. Field Strength

Drift Rate

Std. Conductor vs. Hybrid

Shielding

Cryo-Cooling

Breakout Group Discussion Topics

Instrumentation

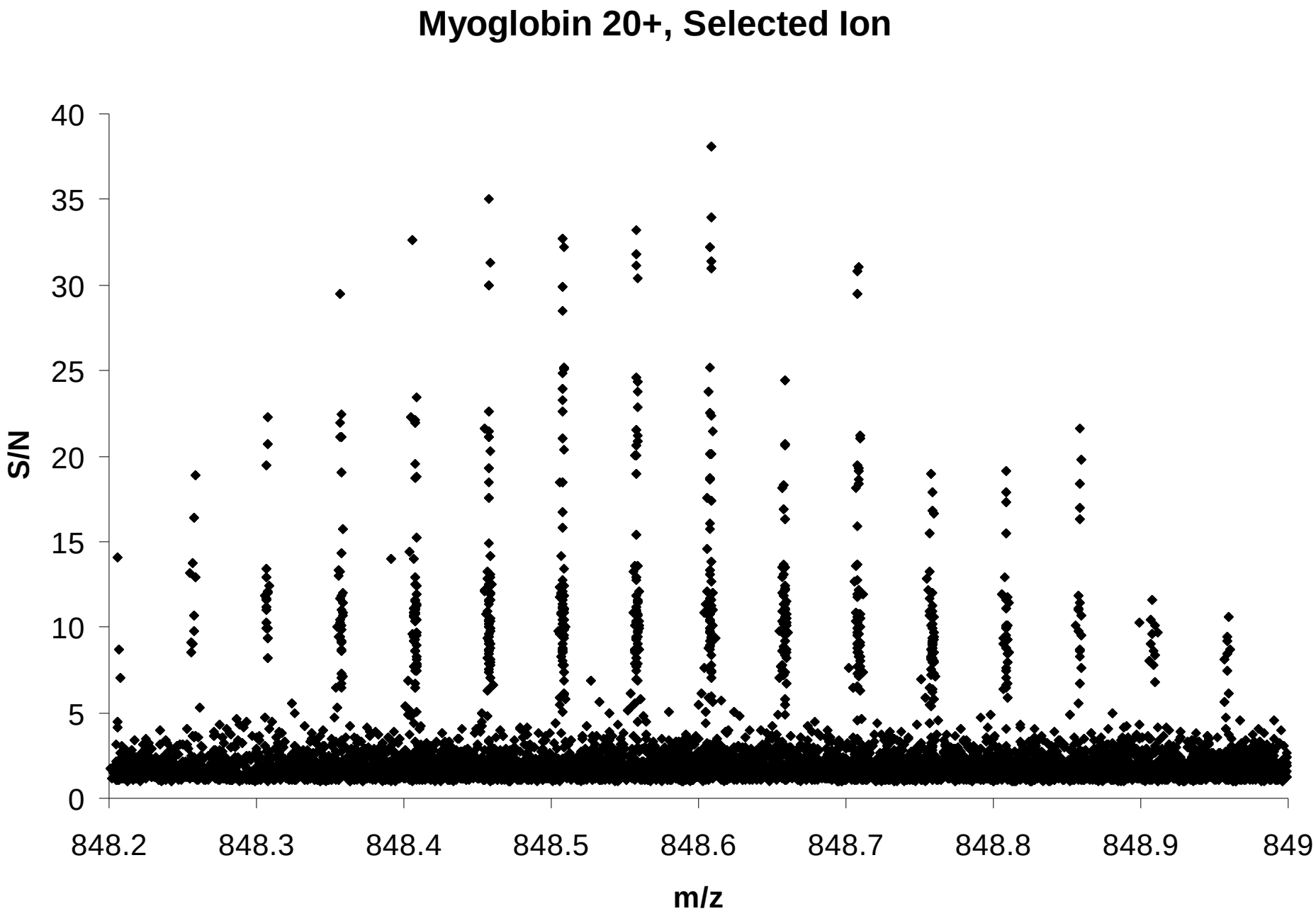
Ion Sources: ESI/FD/APPI/ASAP/Chip…
External Ion Accumulation
Ion Funnel (PNNL)
Ext'l Ion Ejection/Transmission/Trapping
ICR Excitation/Detection (Cold?)
Data Manipulation (Phasing, Calibration, Mass Errors, Data Searching)
Automation
Ion Simulations to Guide Expt'l Design

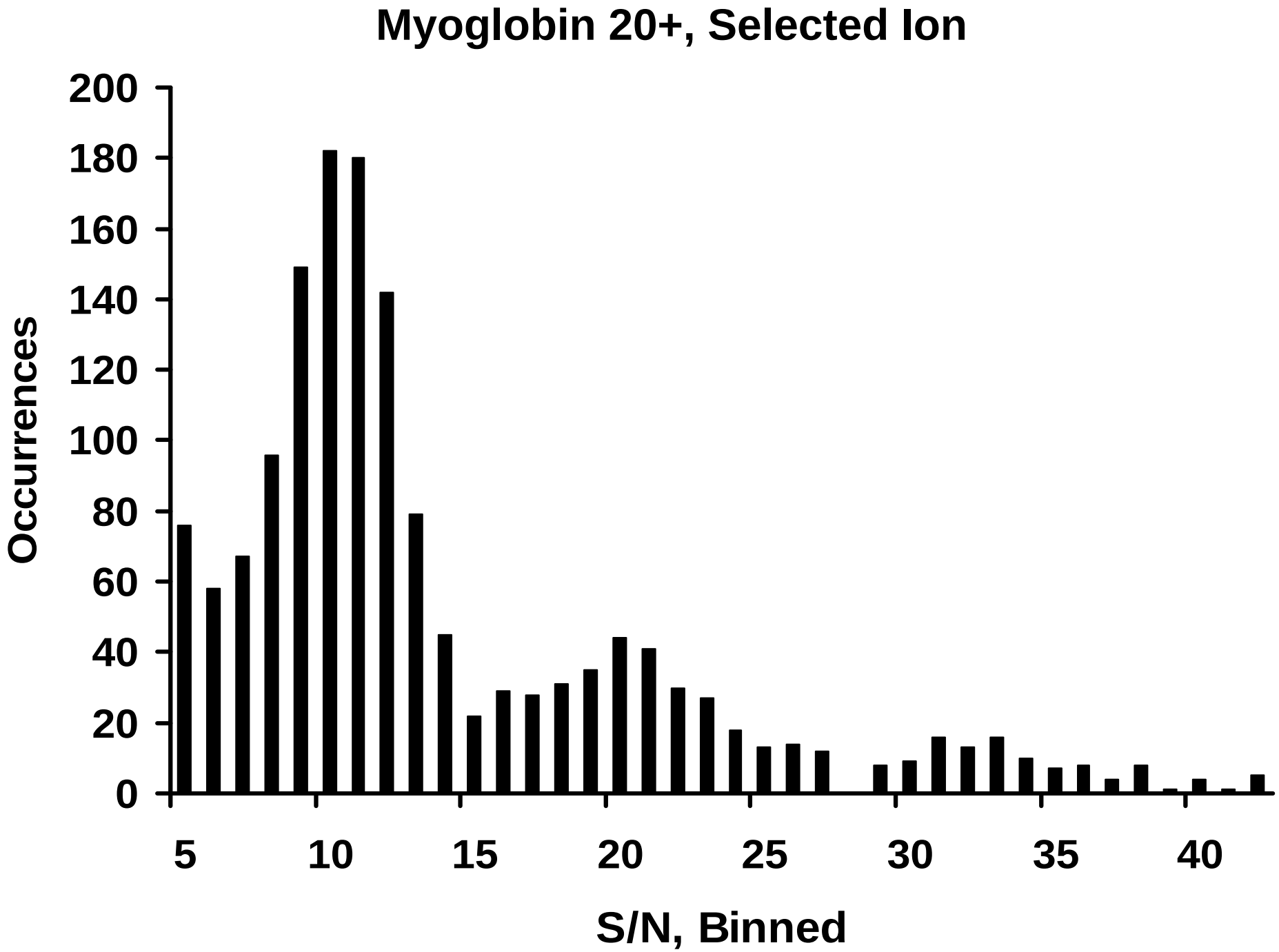
Breakout Group Discussion Topics

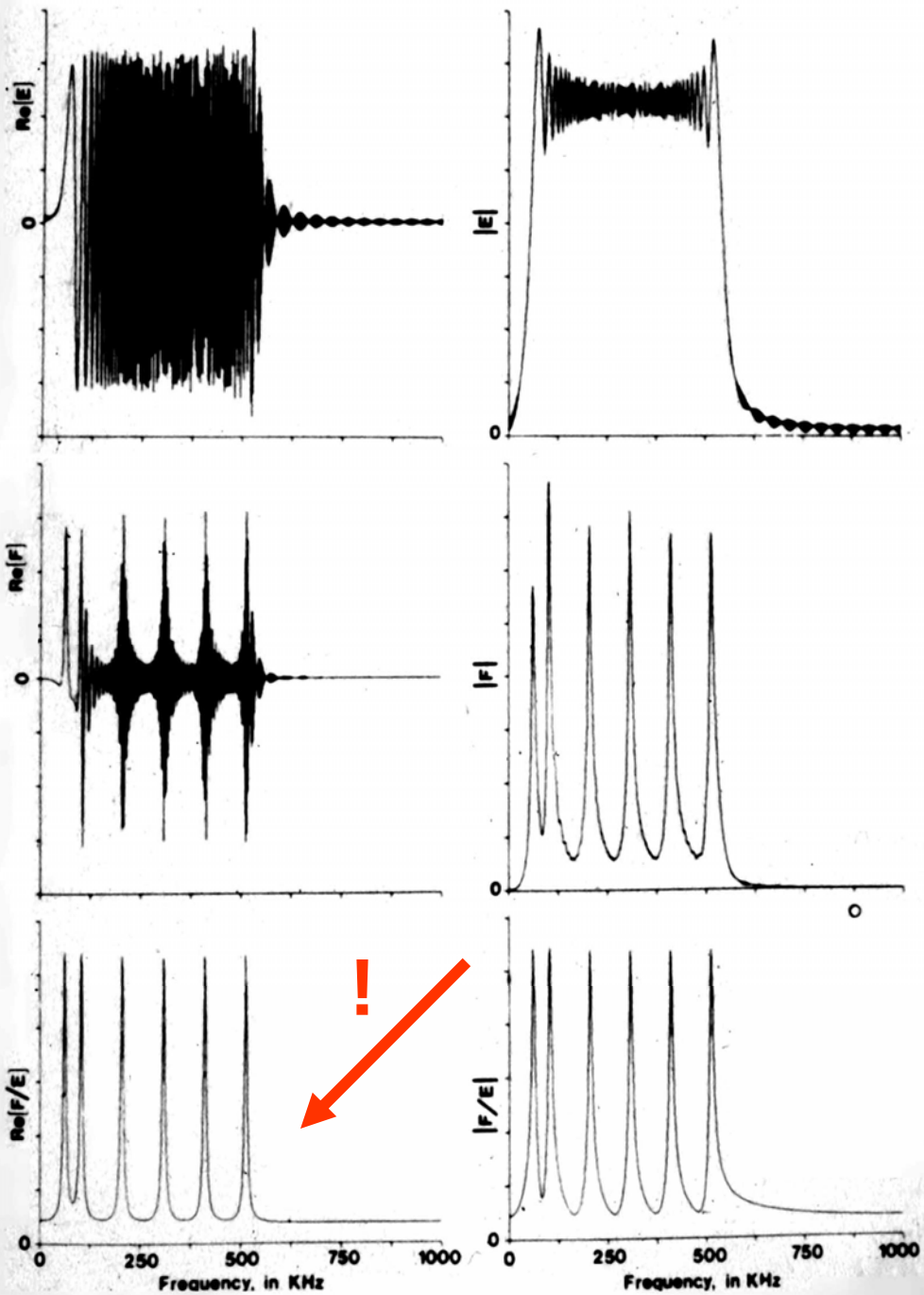
Applications

Proteomics, Lipidomics, Glycomics, etc.
Petroleomics/Environmental
Protein Post-Translational Modifications
Top-Down vs. Bottom-Up Sequencing
MS:MS (ECD, IRMPD, CAD, ETD, BIRD, etc.)
Data Mining, Pattern Recognition
H/D Exchange

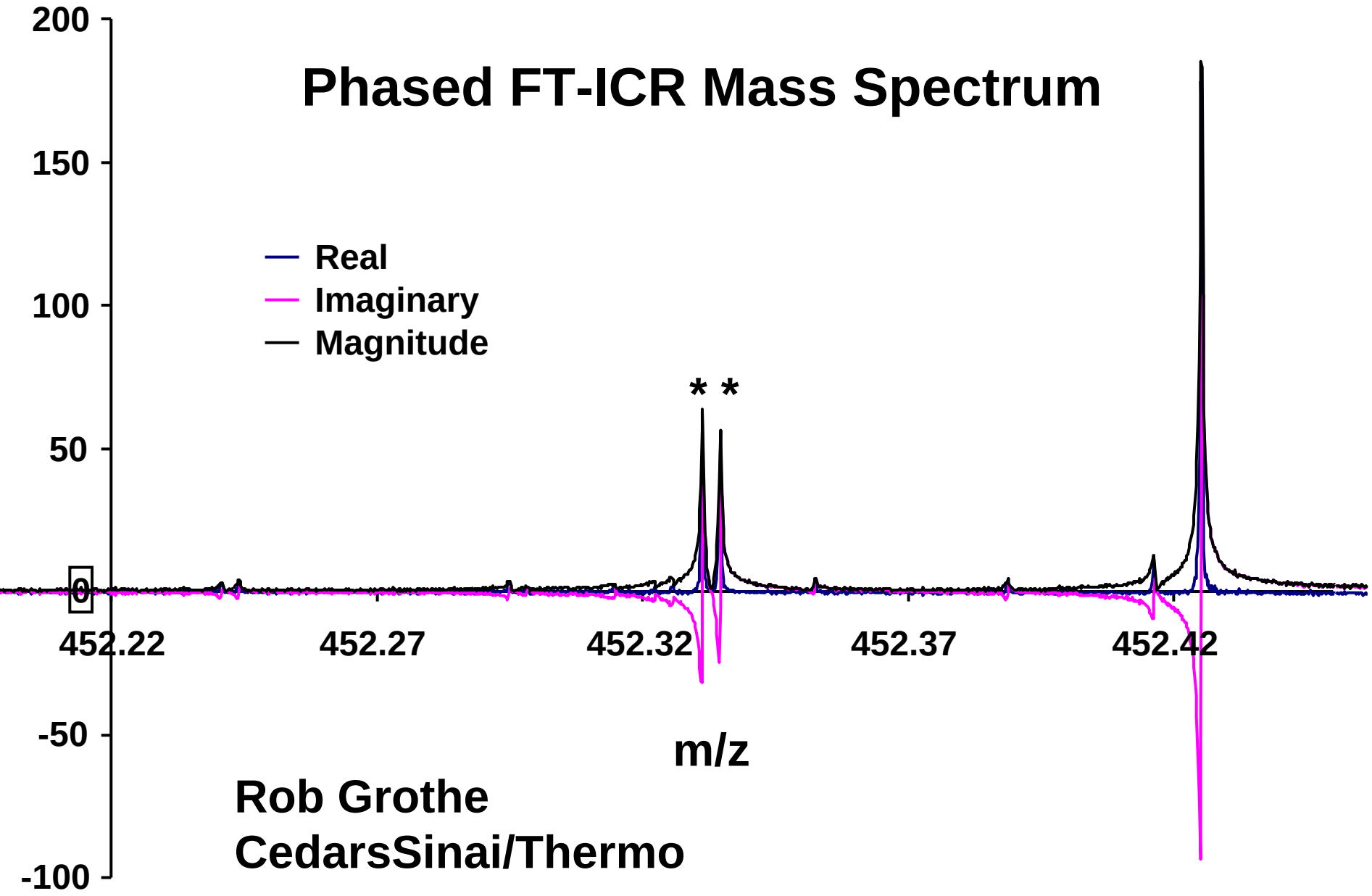
End of AGM Talk (Remaining Slides for CLH, if needed)


Myoglobin 20+, Selected Ion
S/N
40
35
30
25
20
15
10
5
0
848.2
848.3
848.4
848.5
848.6
848.7
848.8
848.9
849
m/z

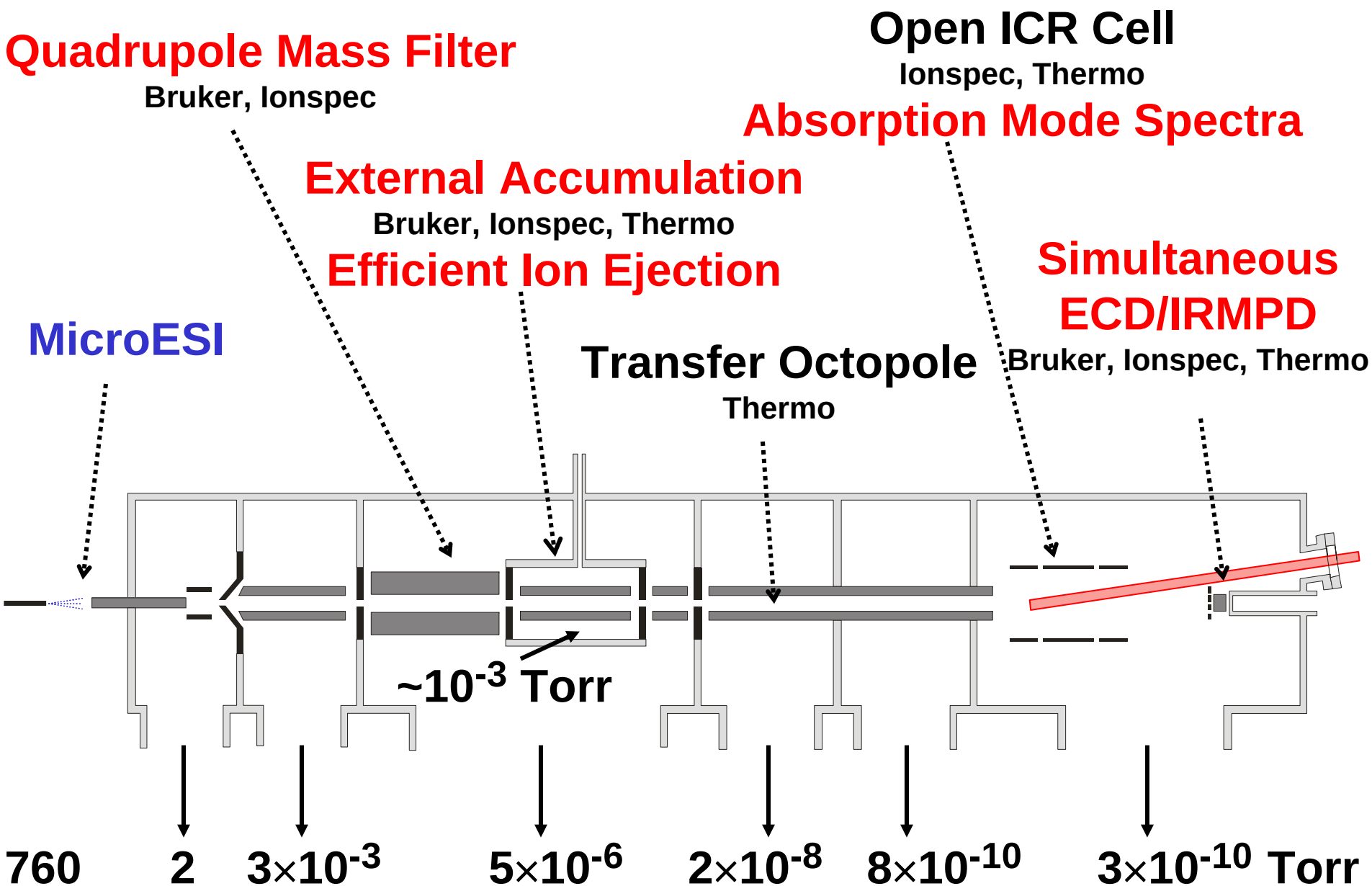

Myoglobin 20+, Selected Ion
Occurrences
200
180
160
140
120
100
80
60
40
20
0
5
10
15
20
25
30
35
40
S/N, Binned



Marshall, A. G. "Convolution Fourier Transform Ion Cyclotron Resonance Spectroscopy", *Chem. Phys. Lett.* **1979**, *63*, 515-518


Phased FT-ICR Mass Spectrum
Real
Imaginary
Magnitude
* *
200
150
100
50
0
-50
-100
452.22
452.27
452.32
452.37
452.42
m/z
Rob Grothe
CedarsSinai/Thermo

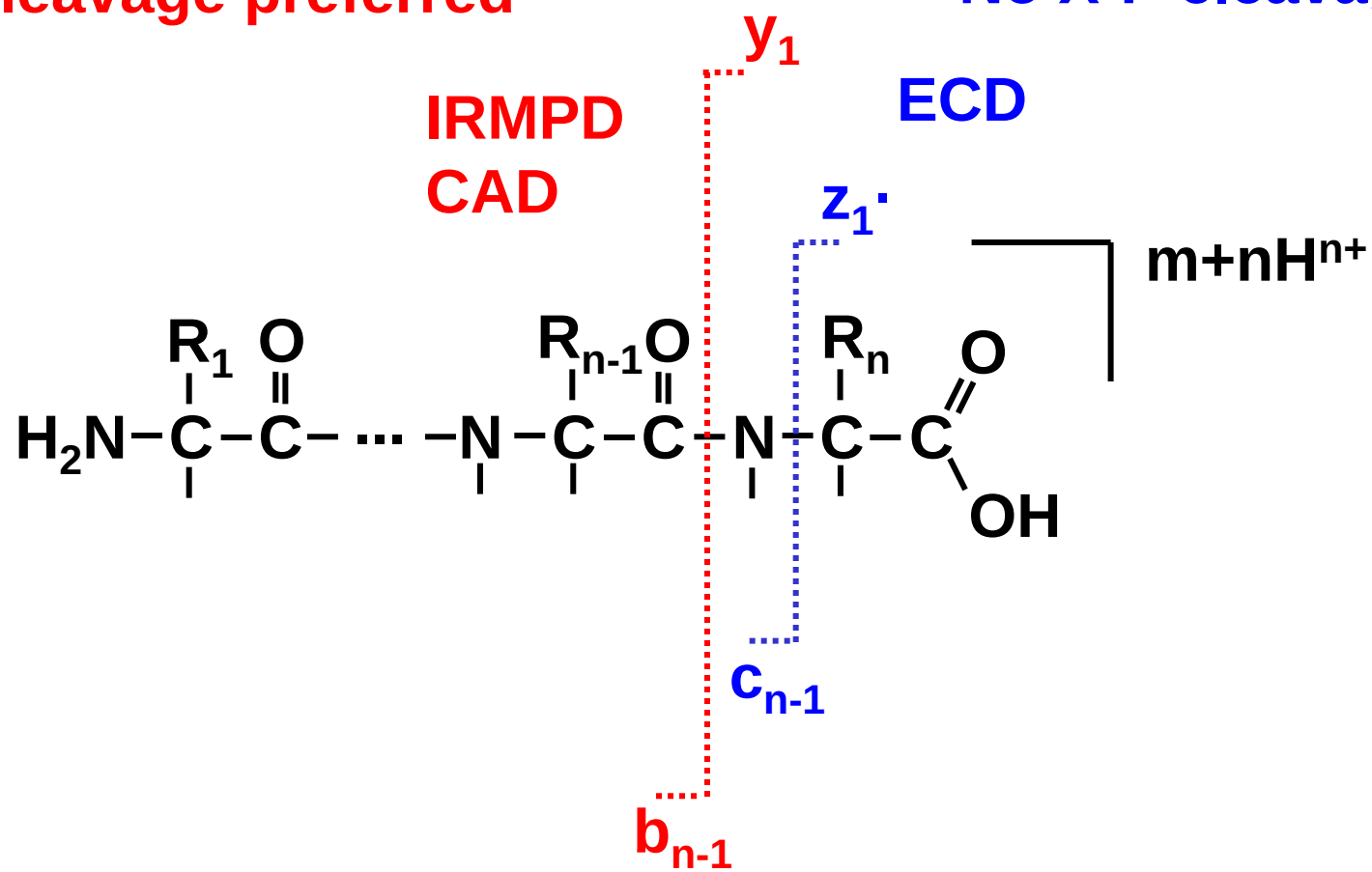
NHMFL Instrumentation Development



Peptide Ion Fragmentation

Labile bonds cleaved;
X-P cleavage preferred

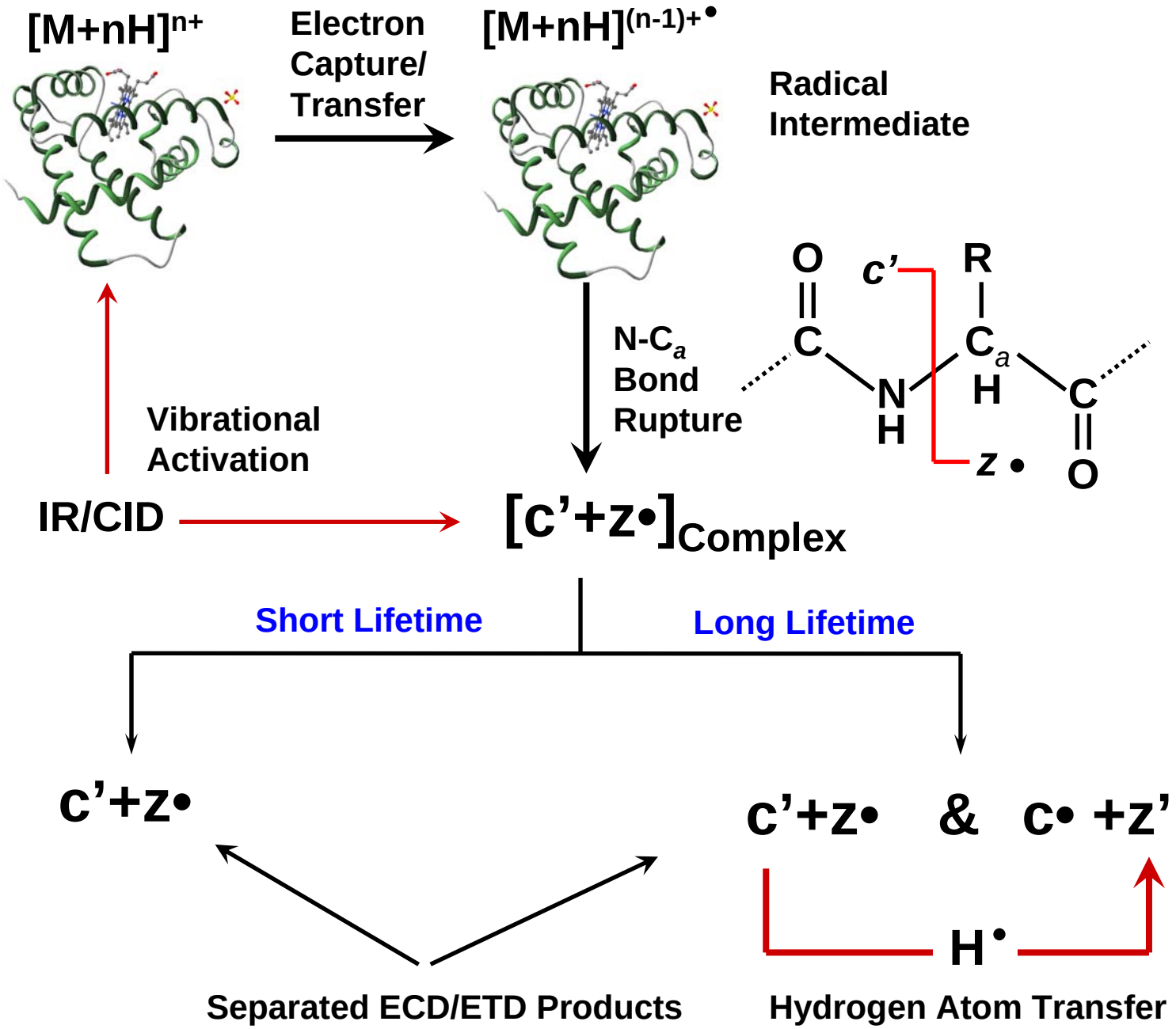
Labile bonds retained;
No X-P cleavage

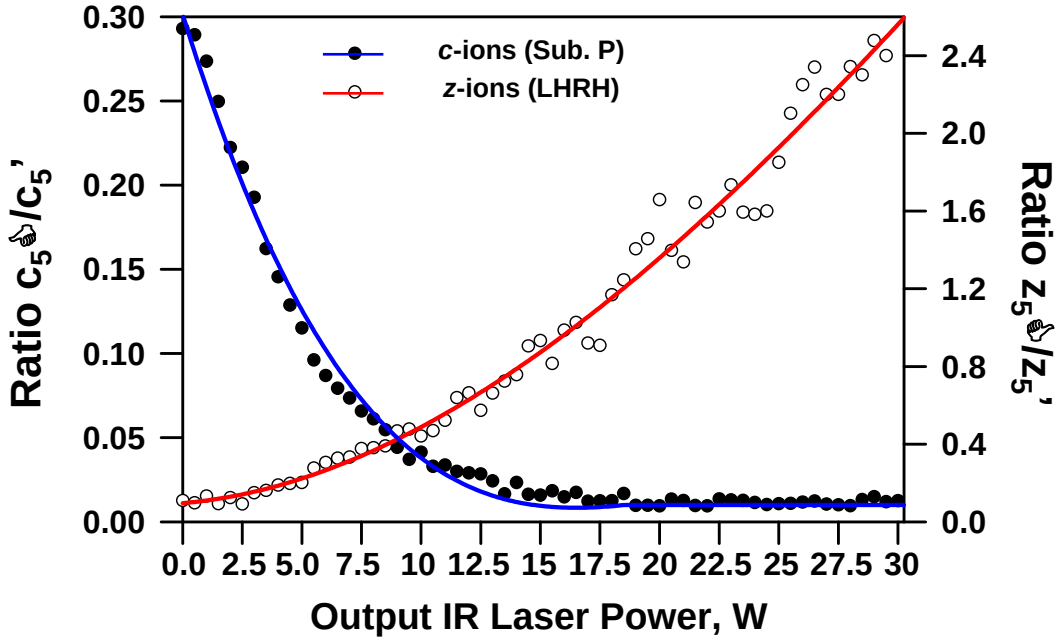


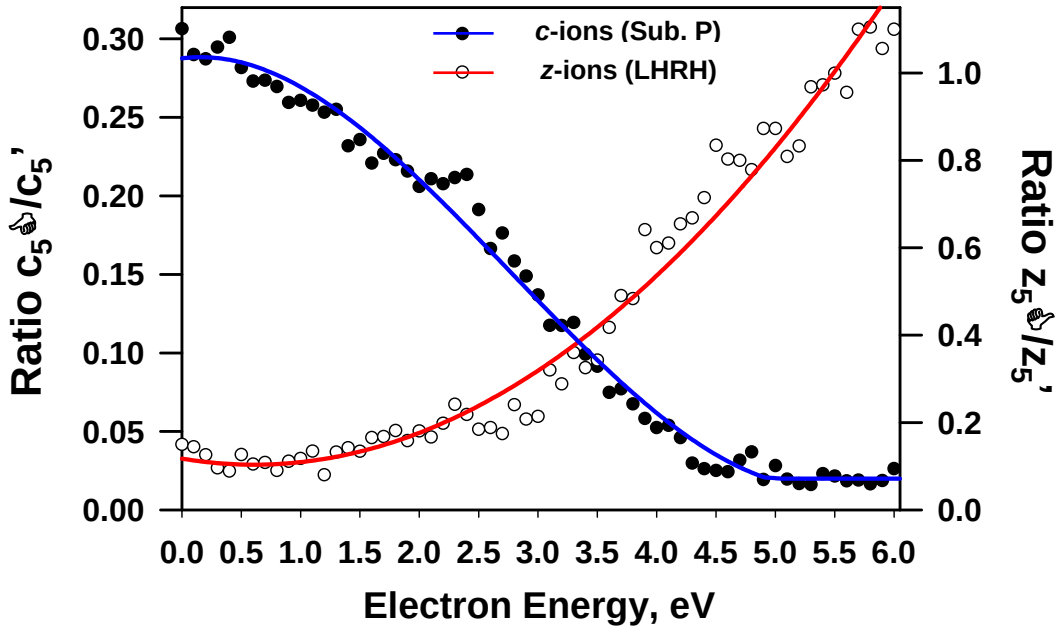
Zubarev, R. A.; Horn, D. M.; Fridriksson, E. K.; Kelleher, N. L.; Kruger, N. A.

Lewis, M. A.; Carpenter, B. K.; McLafferty, F. W. "Electron Capture Dissociation for Structural Characterization of Multiply Charged Protein Cations", *Anal. Chem.* **2000,** *72*, 563-573

MS/MS: Distinguishing N-Terminal vs. C-Terminal Products

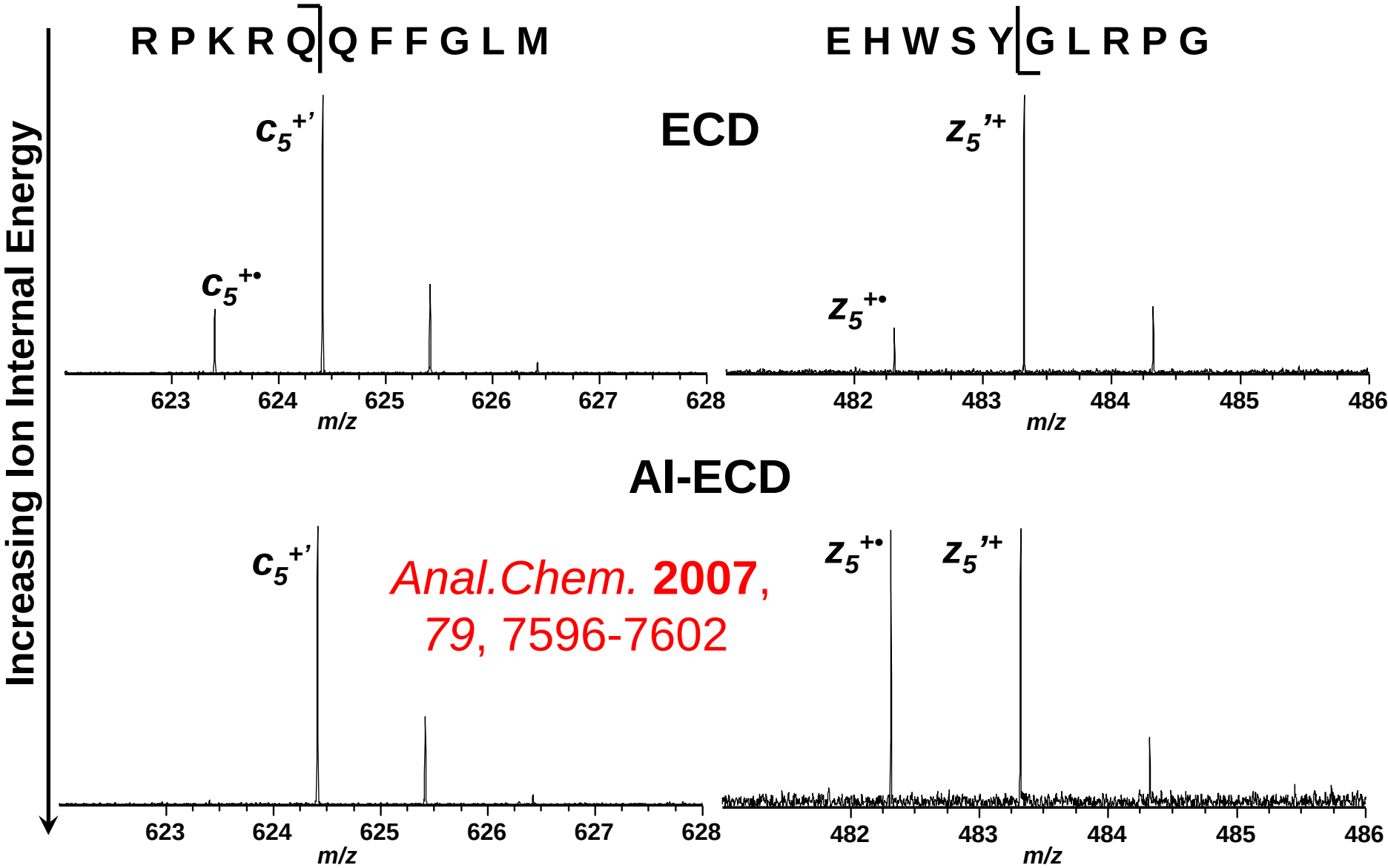

[M+nH]n+
Electron Capture/ Transfer
[M+nH](n-1)+•
Radical Intermediate
N-Ca Bond Rupture
O
c'
R
C
Ca
H
N
H
C
O
z •
Vibrational Activation
IR/CID
[c'+z•]Complex
Short Lifetime
Long Lifetime
c'+z•
c'+z• & c• +z'
H•
Separated ECD/ETD Products
Hydrogen Atom Transfer





Anal.Chem. **2007**, *79*, 7596-7602

Hydrogen Atom Rearrangement between ECD Products



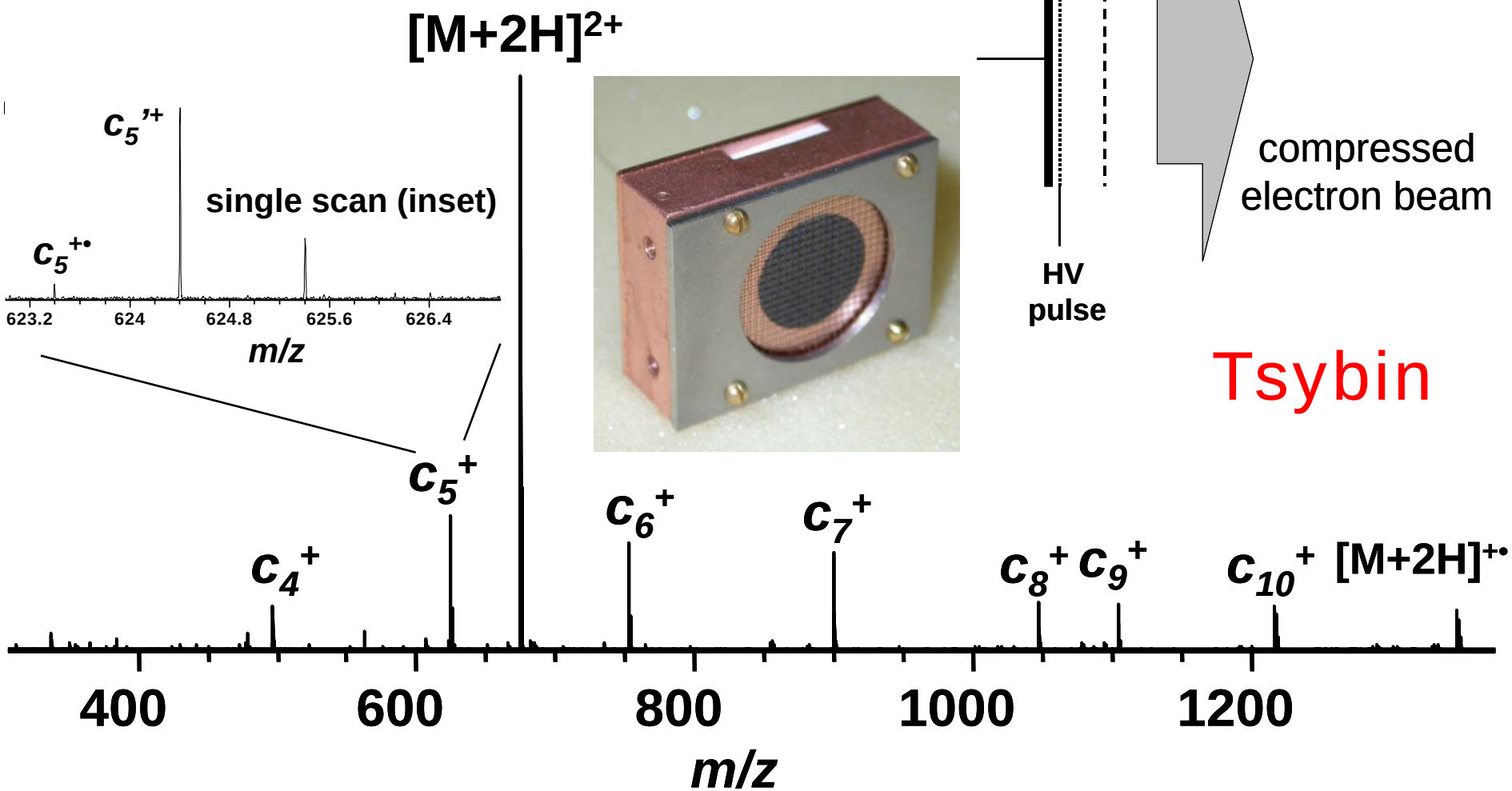
$$[c+H]^+ - H^\bullet = c^{+\bullet} \xrightarrow{H^\bullet} z^{+\bullet} + H^\bullet = [z+H]^+$$

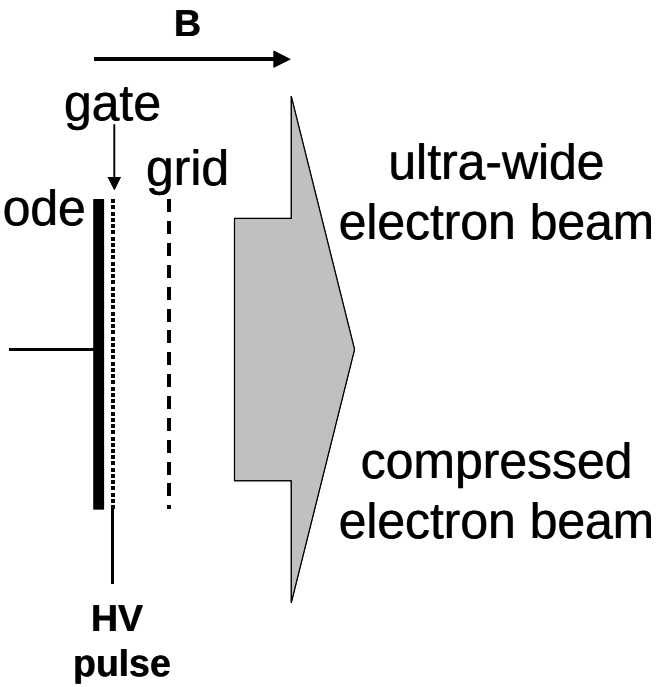
Cold Cathode

Cold Electron Injection

H-RPKPQQFFGLM-NH_2

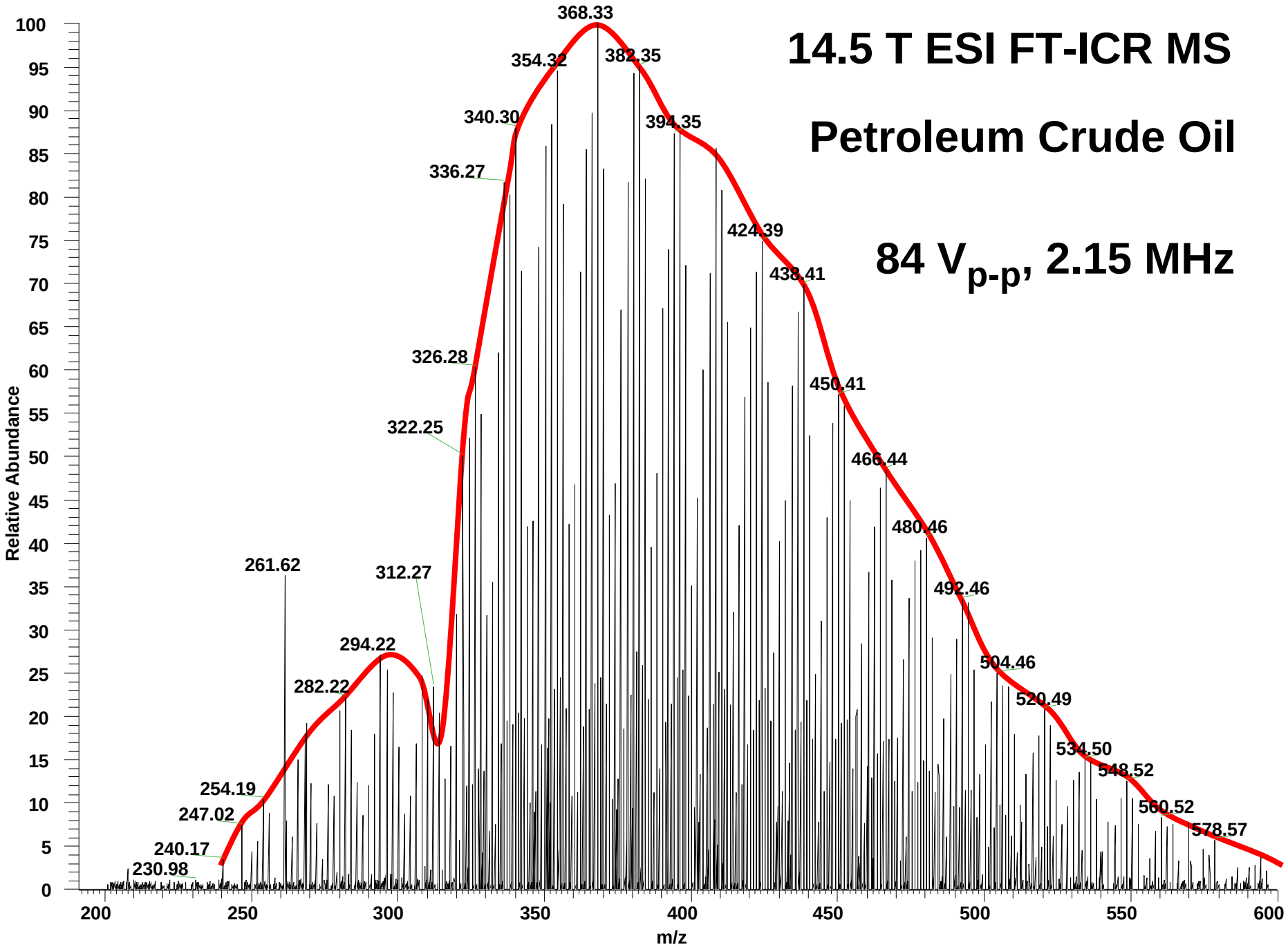
- **Ultra-Large Emitter**
- **No Thermal Activation**
- **Fast Switch On/Off**
- **High Electron Flux**



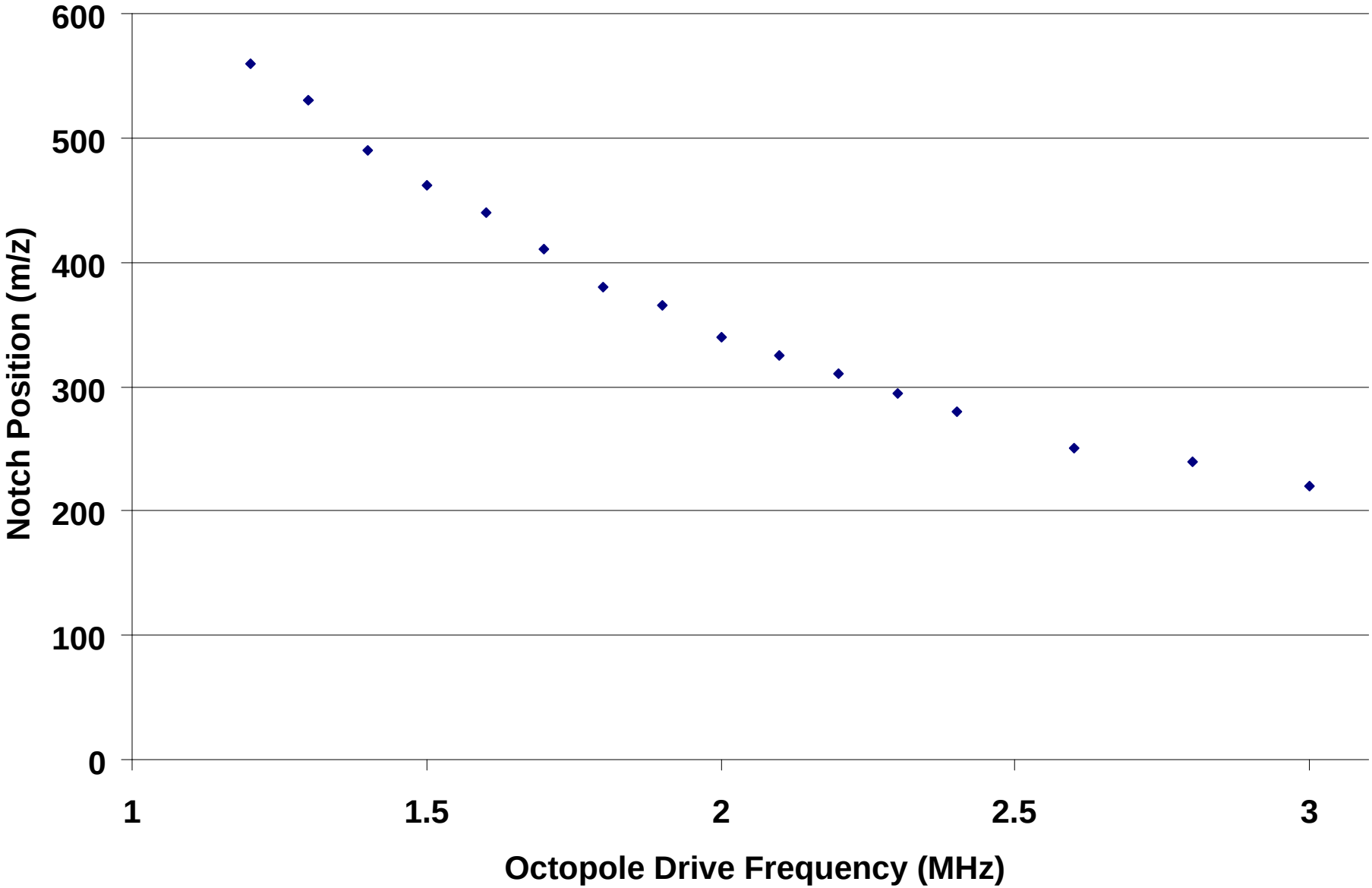


Tsybin

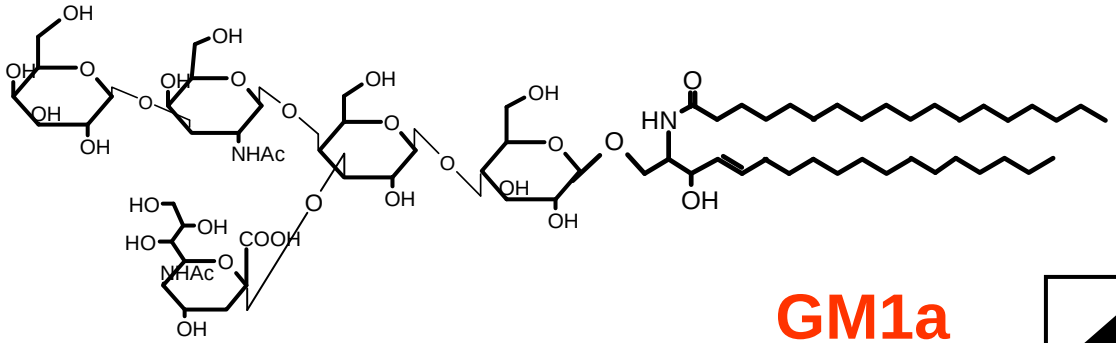
Ion Transmission


14.5 T ESI FT-ICR MS
Petroleum Crude Oil
84 Vp-p, 2.15 MHz
Relative Abundance
m/z
368.33
354.32
382.35
340.30
394.35
336.27
424.39
438.41
326.28
450.41
322.25
466.44
480.46
261.62
312.27
492.46
294.22
504.46
282.22
520.49
534.50
548.52
254.19
560.52
247.02
578.57
240.17
230.98

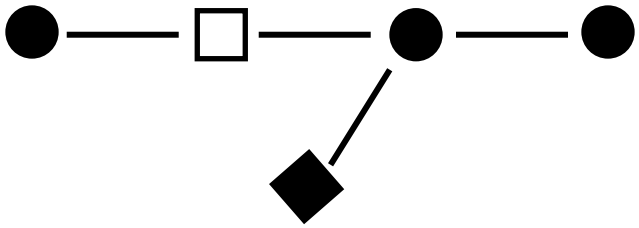
14.5 T FT-ICR MS



Gangliosides



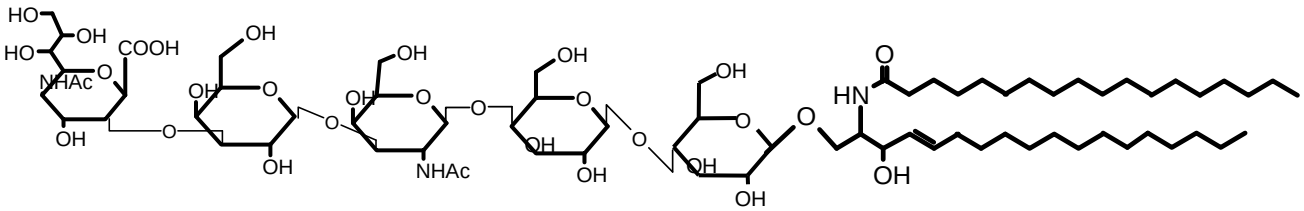
GM1a

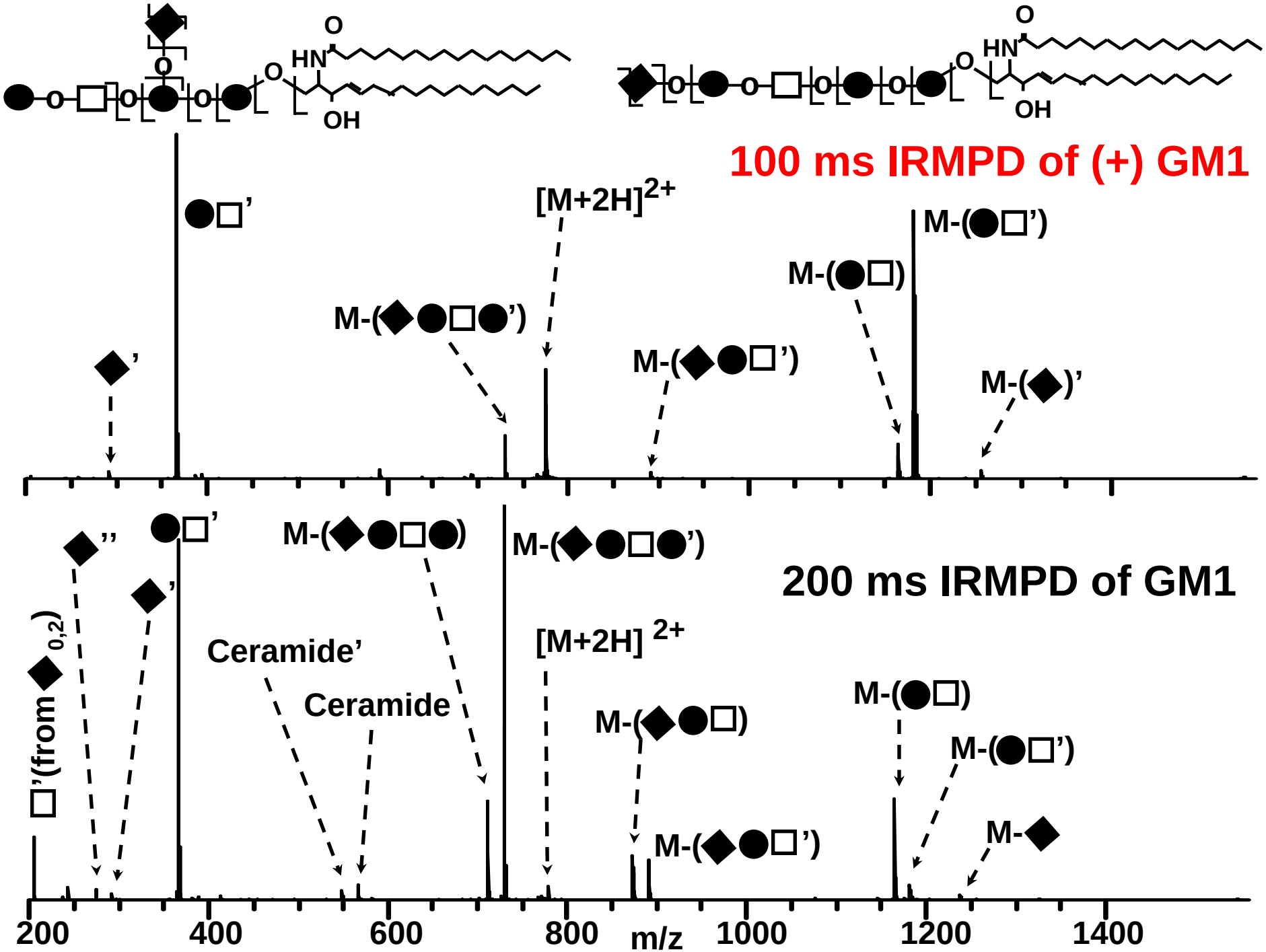


- ◆ **NeuAc = N-Acetylneuraminic Acid (Sialic Acid)**
- □ **HexNAc = N-Acetylhexosamine**
- ● **Hex = Hexose**

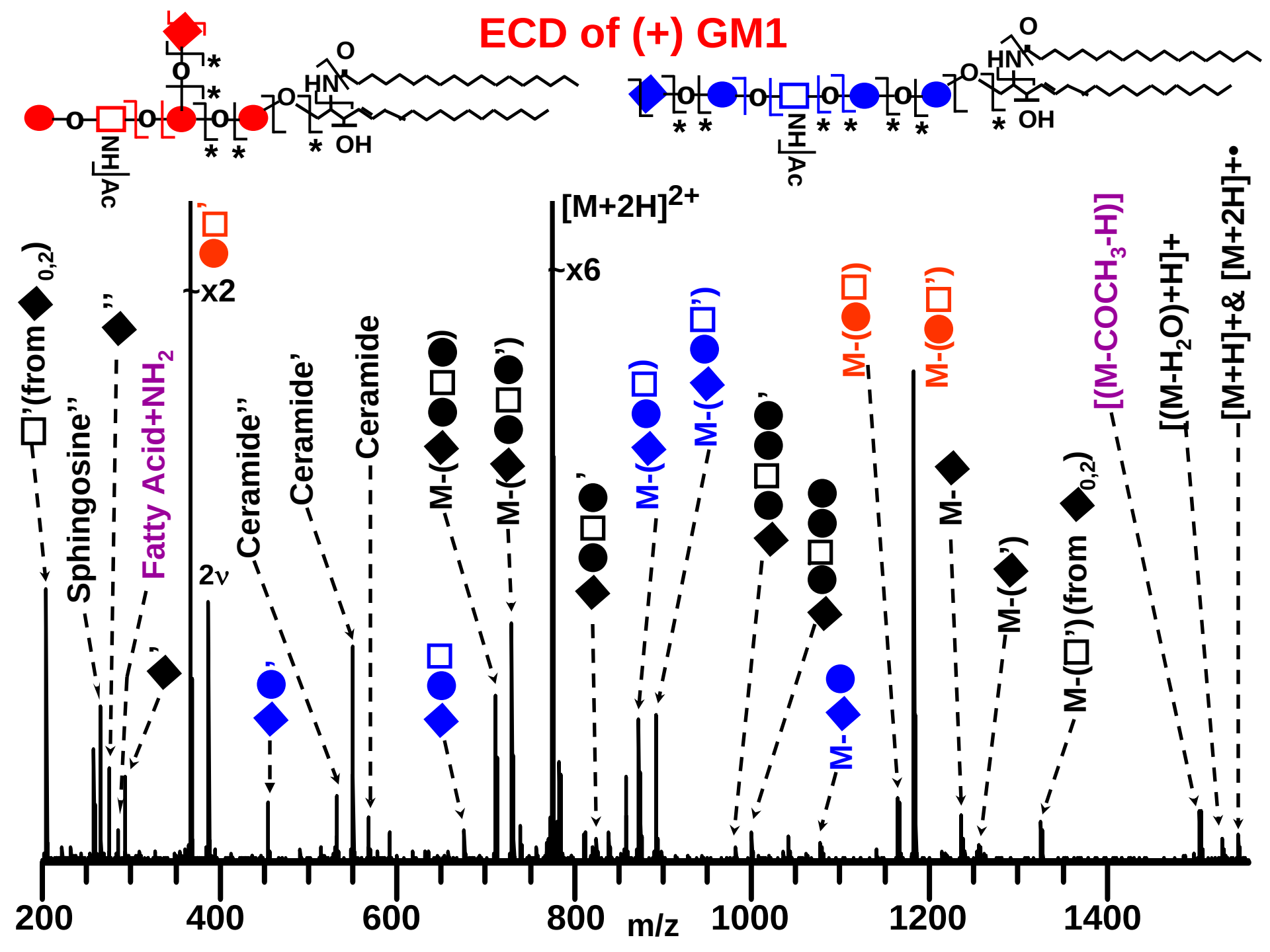
GM1b

JASMS **2005**, *16*, 752-762




100 ms IRMPD of (+) GM1
200 ms IRMPD of GM1
[M+2H]2+
M-(●□')
M-(●□)
M-(◆●□●')
M-(◆●□')
M-(◆)'
◆'
●□'
◆''
M-(◆●□●)
Ceramide'
Ceramide
M-(◆●□)
M-(●□)
M-(●□')
M-◆
M-(◆●□')
□'(from ◆0,2)
200
400
600
800
m/z
1000
1200
1400

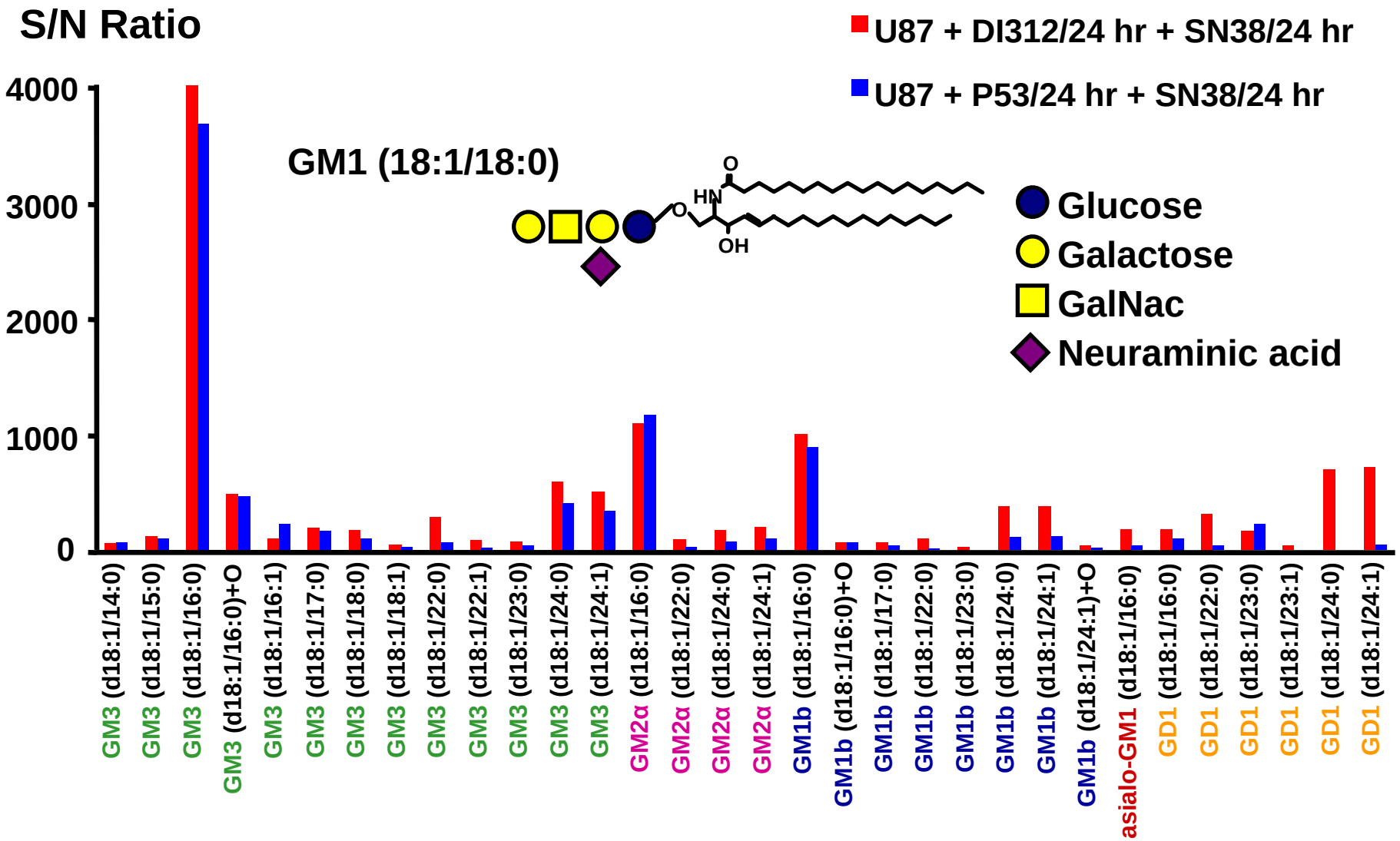
ECD of (+) GM1


[M+2H]2+
~x2
~x6
2ν
Sphingosine''
Fatty Acid+NH2
Ceramide''
Ceramide'
Ceramide
M-(●□●)
M-(●□●')
M-(●□●●)
M-(●□●●●)
M-(●□●●●●)
M-(●□●●●●●)
M-(●□●●●●●●)
M-(●□●●●●●●●)
M-(●□●●●●●●●●)
M-(●□●●●●●●●●●)
M-(●□●●●●●●●●●●)
M-(●□●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●)
M-(●□●●●)
M-(●□●●●●●●●●●●●●●●●●●●●

MS/MS:
FT-ICR + Ext'l CAD

Gangliosides

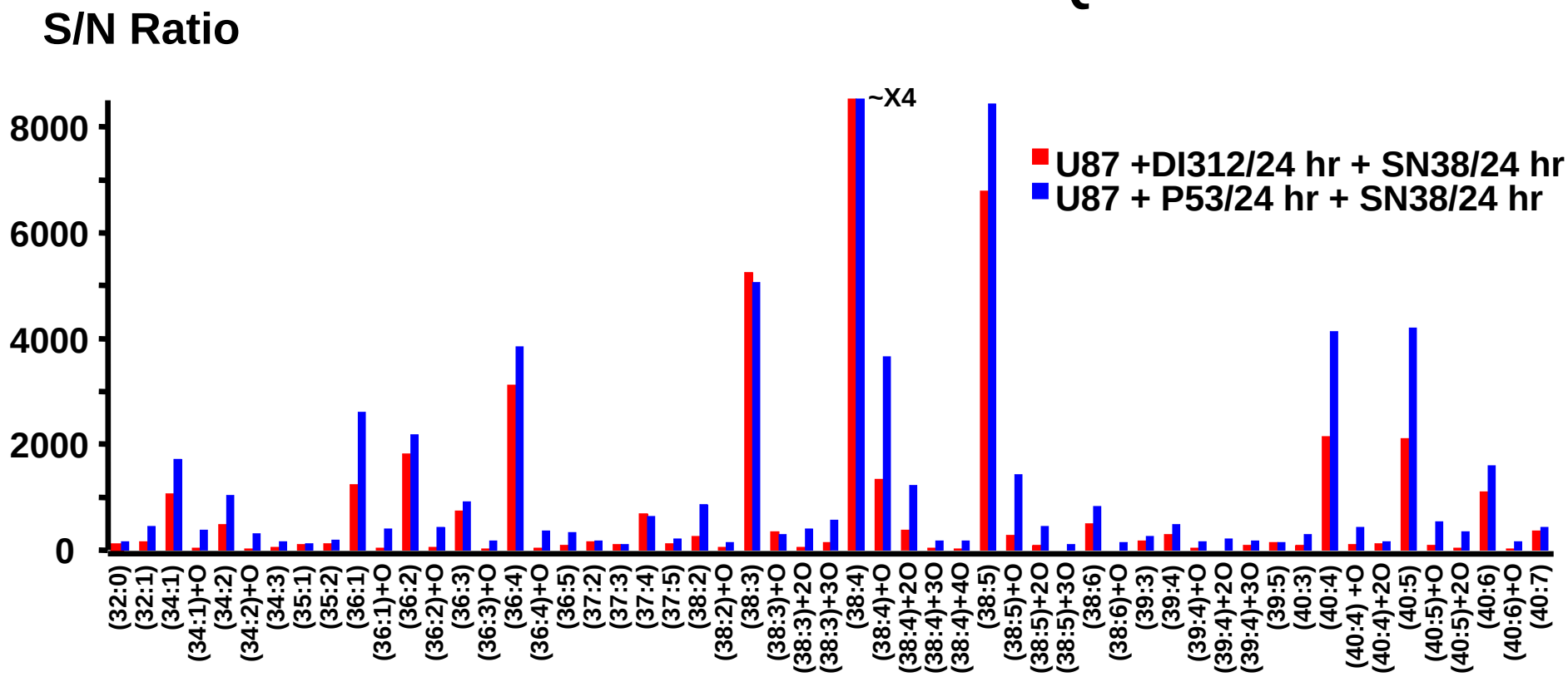
LTQ 14.5 T FT-ICR MS

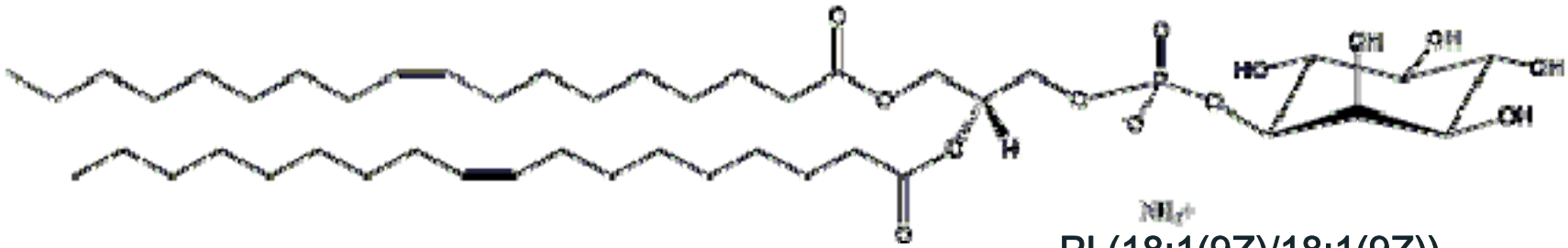


Anal.Chem. **2007**, *79*, 8423-8430

Phosphatidylinositols

LTQ 14.5 T FT-ICR MS



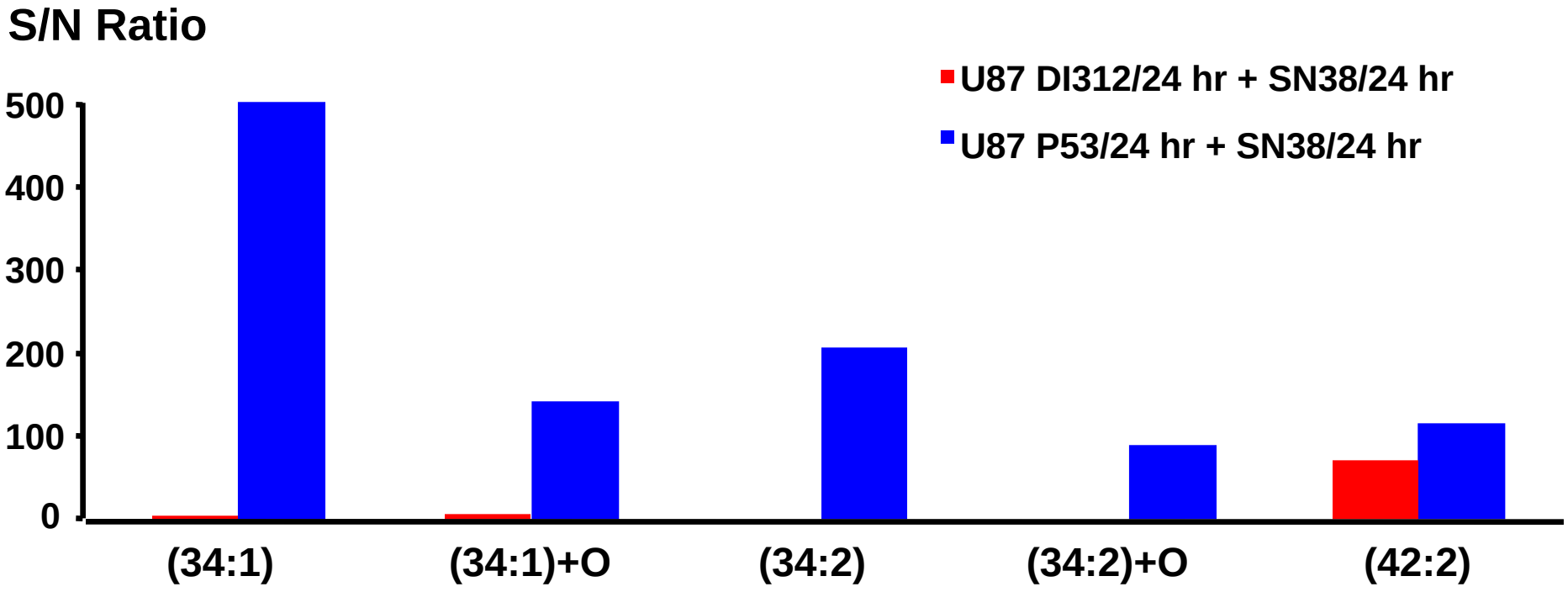


PI (18:1(9Z)/18:1(9Z))

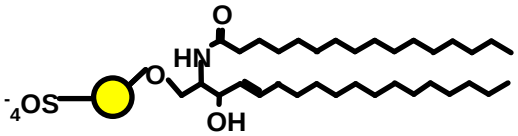
Anal.Chem. **2007**, *79*, 8423-8430

Sulfatides

LTQ 14.5 T FT-ICR MS



Sulfatide (34:1)



Galactose

Anal.Chem. **2007**, *79*, 8423-8430