

[www.lipidmaps.org](http://www.lipidmaps.org)

# LIPID MAPS Lipidomics Workshop

April 28, 2007

## Lipid Analysis by Mass Spectrometry

Introduction and Challenges

Neutral and Phospholipids

**Robert C. Murphy**

Department of Pharmacology

University of Colorado

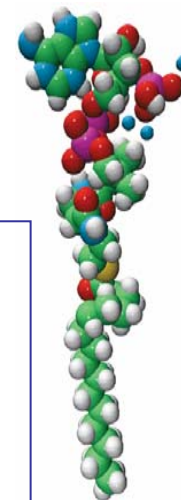
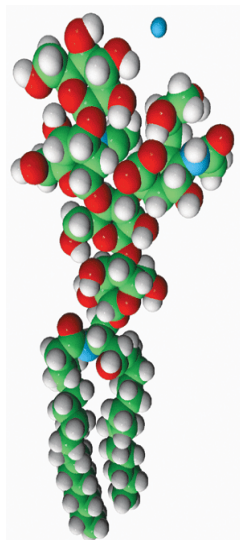
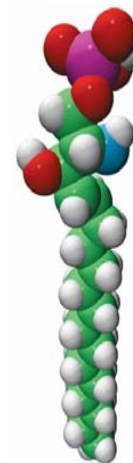
Health Sciences Center

Other LIPID MAPS Neutral Lipid Core members:

Robert Barkley

Miguel Gijon

Jessica Krank



## Outline:

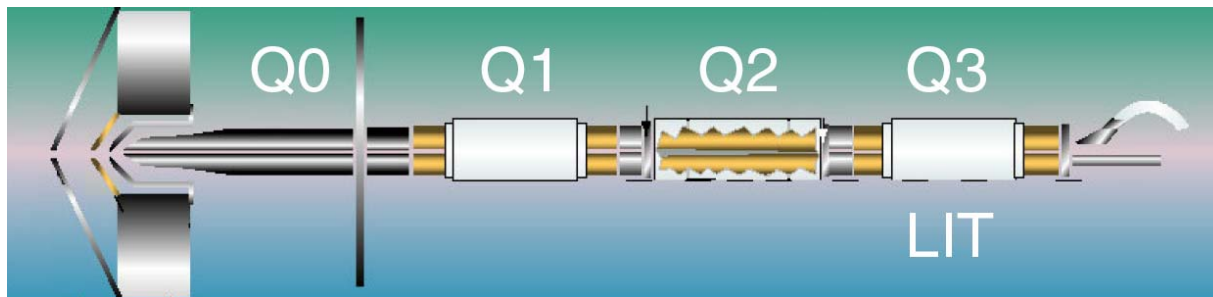
- A. Electrospray Ionization of Neutral Lipids
- B. NL- tandem mass spectrometry-TAGs /CE
- C. Compound identification: Qualitative  
Analysis/Challenges/artifacts
- D. Quantitation: Internal standards, etc.
- E. Data analysis/visualization: Lipid Profiler
- F. Phospholipids
- G. LC/MS/MS Quantitation
- H. Other strategies

# Neutral Lipids: Mass spectrometric Challenges

- Formation of gas phase ions
  - Desorption/Spray Ionization
    - Attachment of charging species
- Complex mixture - molecular species
  - Signal divided by total number of components
  - Hundreds of species

# Mass Spectrometry

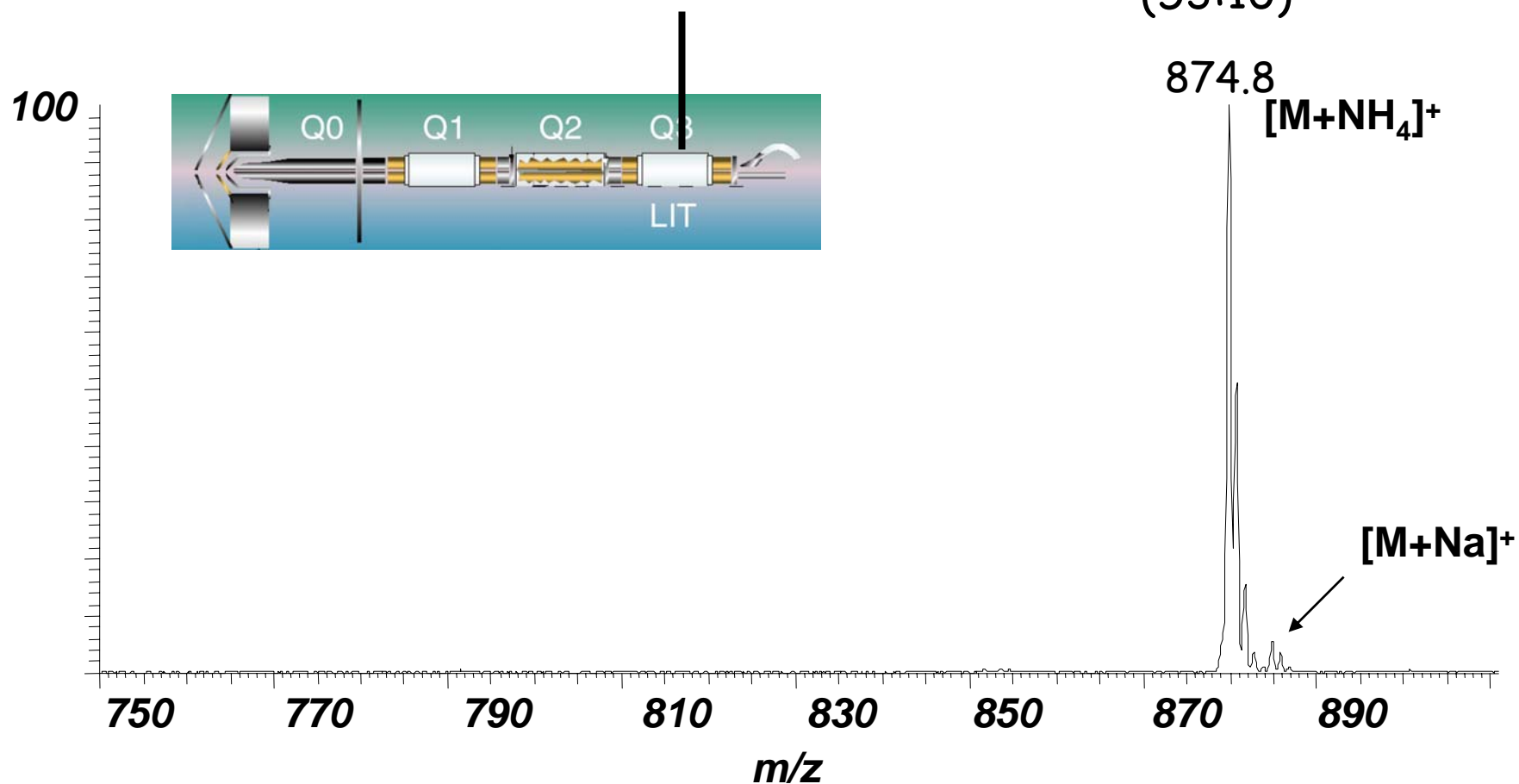
- Electrospray Ionization-Neutral Lipids
  - Tandem Mass spectrometry
    - Product ions
    - Precursor Ions
    - Neutral loss



# Challenge: Neutral Lipids

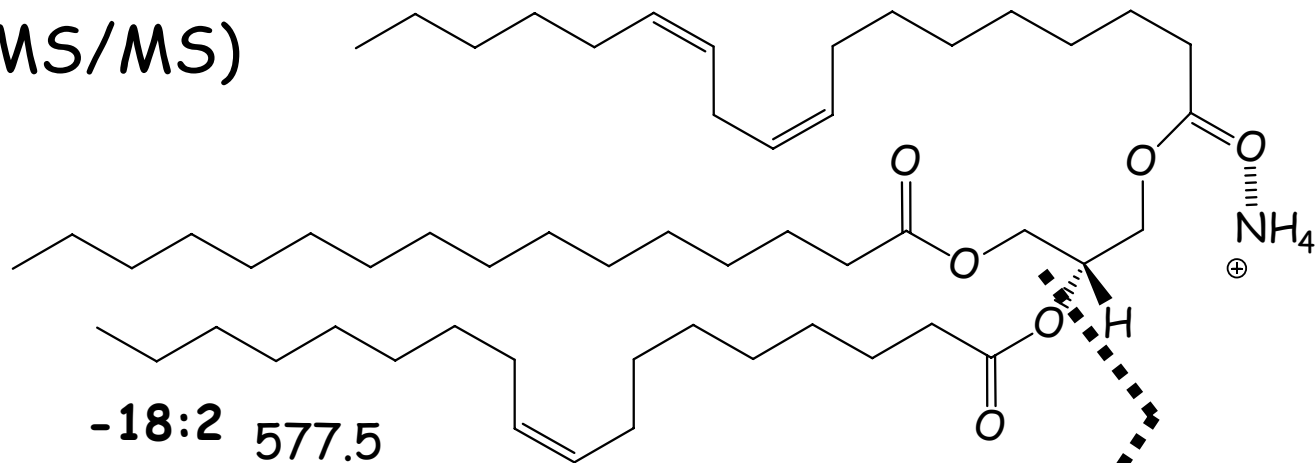
- ESI- no structural information

Scan  $m/z$  500 to 1000



# Product ions (MS/MS)

m/z 874.8  
16:0/18:1/18:2 TAG



100

-18:2 577.5

601.5

-16:0

601  
(-273)

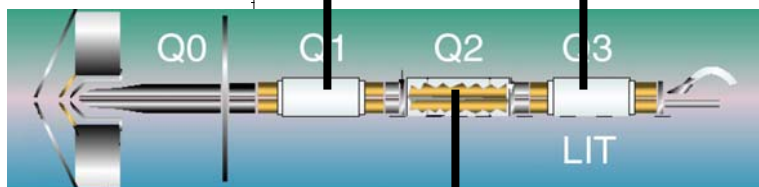
+H  
-NH<sub>3</sub>

-18:1  
575.5

Product ion Scan

m/z 874.8

Scan



Collisional Activation

[M+NH<sub>4</sub>]<sup>+</sup>  
874.8

[M+H]<sup>+</sup>  
857.8

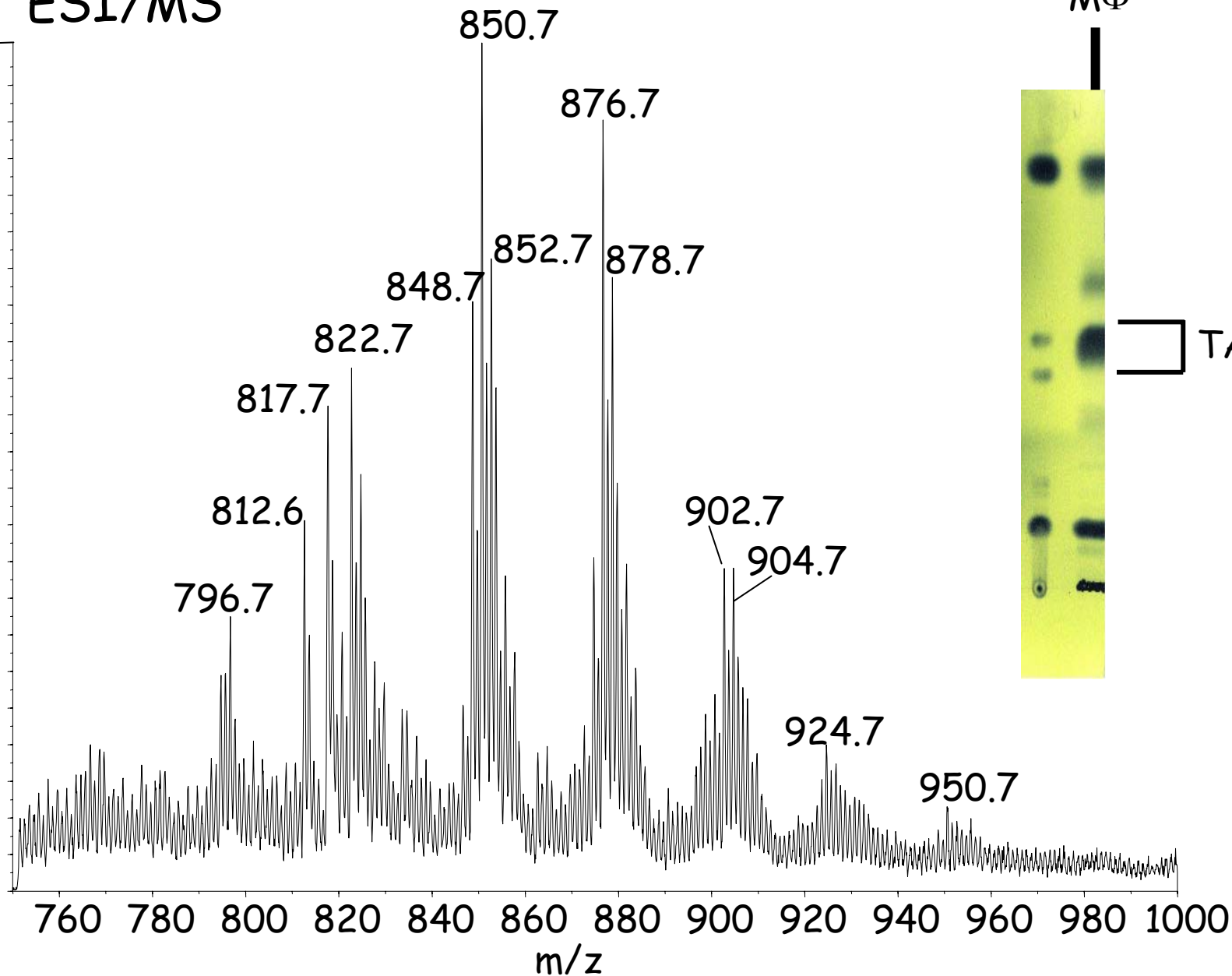
200 300 400 500 600 700 800 900

m/z

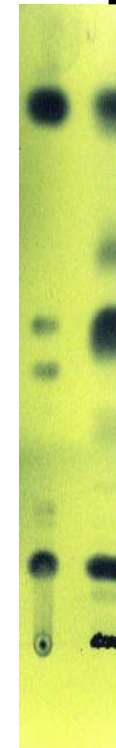
# ESI/MS

$2.3 \times 10^6$

Ion Abundance  
(counts/sec)

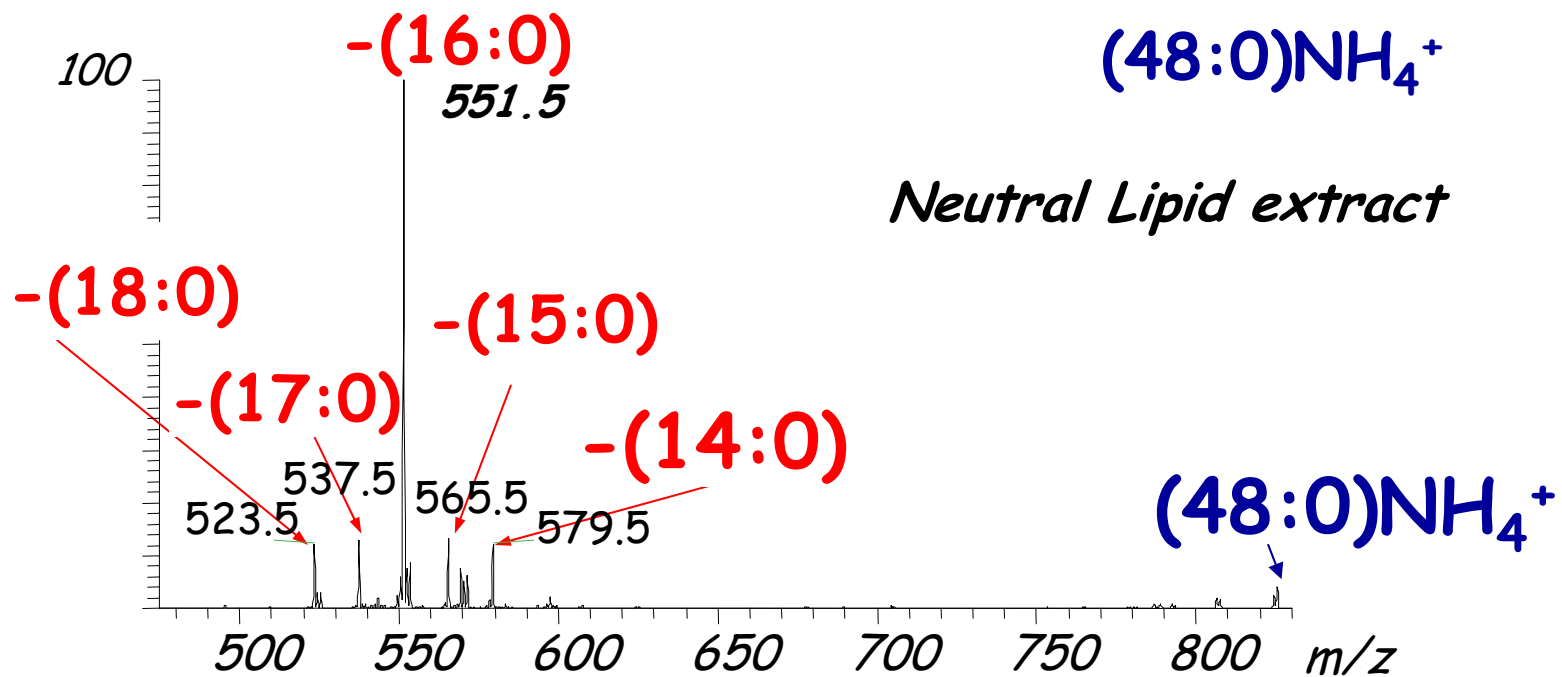


$M\Phi$



TAGs

# Product Ion Scan $m/z$ 824.7 TAGs from RAW 264.7 cells





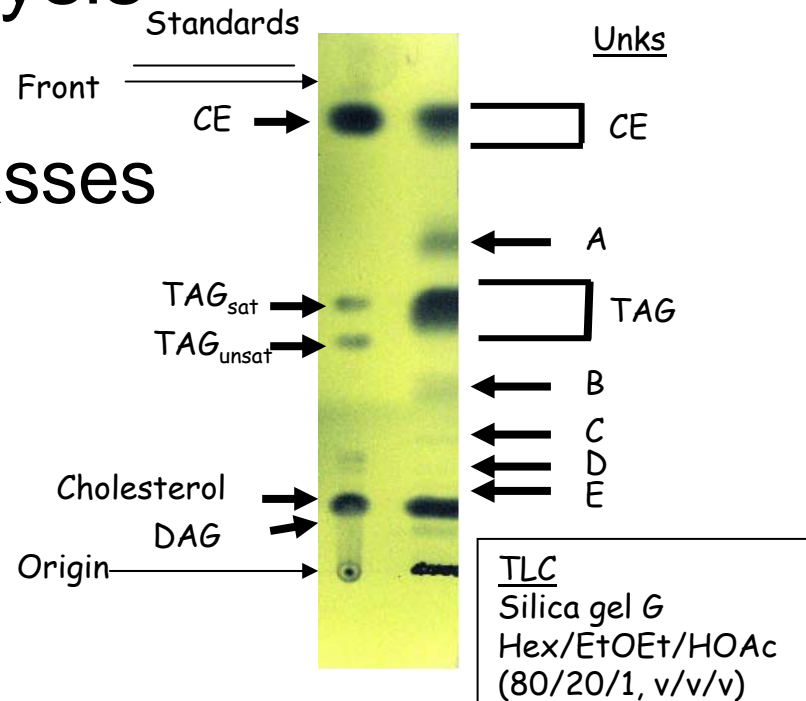
# List of MS<sup>3</sup> identified TAGs From 6 different m/z values

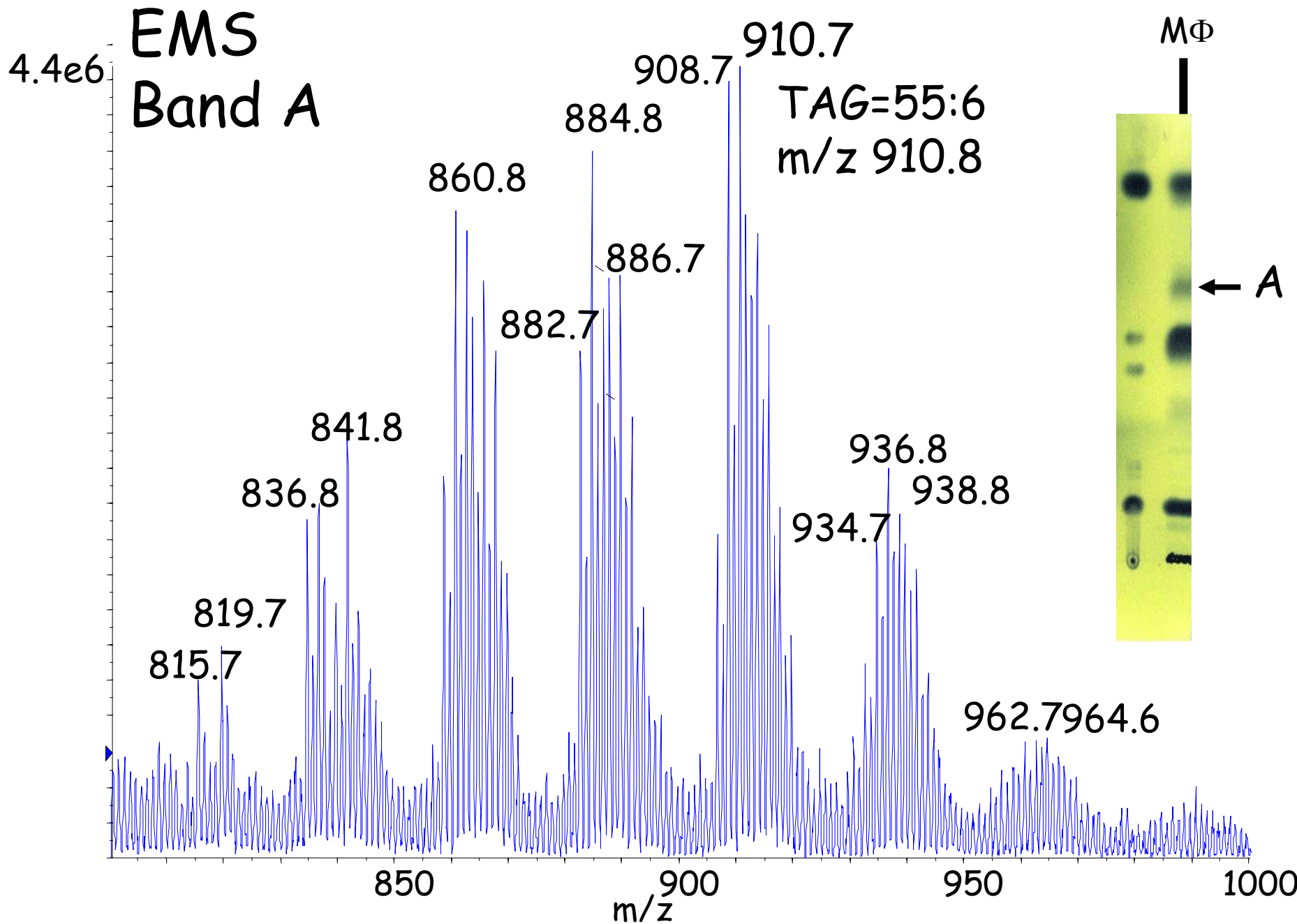
	<i>m/z</i> 824	<i>m/z</i> 822	<i>m/z</i> 820	<i>m/z</i> 838	<i>m/z</i> 836	<i>m/z</i> 834
	TG(48:0)	TG(48:1)	TG(48:2)	TG(49:0)	TG(49:1)	TG(49:2)
<b>Major</b>	16:0/16:0/16:0	16:0/16:0/16:1	16:0/16:1/16:1	16:0/16:0/17:0	15:0/16:0/18:1 16:0/16:1/17:0 16:0/16:0/17:1	15:0/16:1/18:1 16:0/16:1/17:1 16:1/16:1/17:0
<b>Minor</b>	14:0/16:0/18:0 15:0/16:0/17:0	14:0/16:0/18:1 14:0/16:1/18:0 15:0/16:1/17:0 15:0/16:0/17:1 15:0/15:0/18:1	14:0/16:1/18:1 14:0/16:0/18:2 15:0/16:1/17:1	15:0/16:0/18:0	14:0/17:0/18:1 15:0/16:1/18:0	15:1/16:0/18:1
<b>Trace</b>	15:0/15:0/18:0 12:0/18:0/18:0 14:0/17:0/17:0	14:0/17:0/17:1 14:1/17:0/17:0 15:1/16:0/17:0	12:0/18:1/18:1 14:0/17:1/17:1 14:1/16:0/18:1 15:0/15:0/18:2 15:0/15:1/18:1 15:1/16:0/17:1 14:1/17:0/17:1	12:0/17:0/18:0 13:0/18:0/18:0 14:0/16:0/19:0 15:0/15:0/19:0	13:0/16:0/20:1 14:0/16:0/19:1 14:0/17:1/18:0 14:1/17:0/18:0 15:0/15:0/19:1 15:1/16:0/18:0	13:0/16:0/20:2 14:0/15:0/20:2 14:0/17:1/18:1 14:0/17:0/18:2 14:1/17:0/18:1 15:0/16:0/18:2 15:0/17:1/17:1 15:1/17:0/17:1

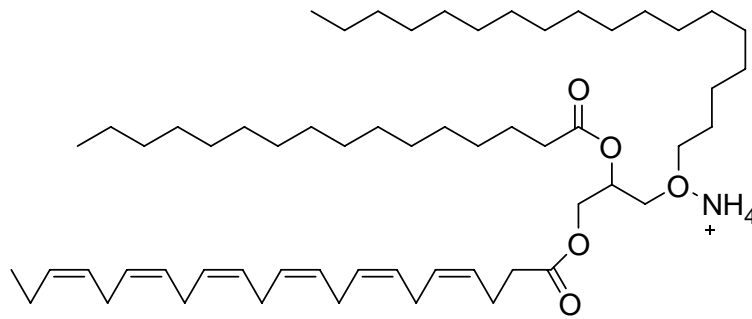
MS<sup>3</sup> data allowed for the unequivocal identification of 55 TAGs

# Crude Mixture or Purification of Lipids by Class

- Shotgun analysis
  - Use the power of MS to separate species
  - Unique product ions after collisional activation
- Separation prior to analysis
  - LC/MS and LC/MS/MS
  - Off line separation of classes
    - TLC (NP)
    - HPLC (NP and/or RP)
    - SPE





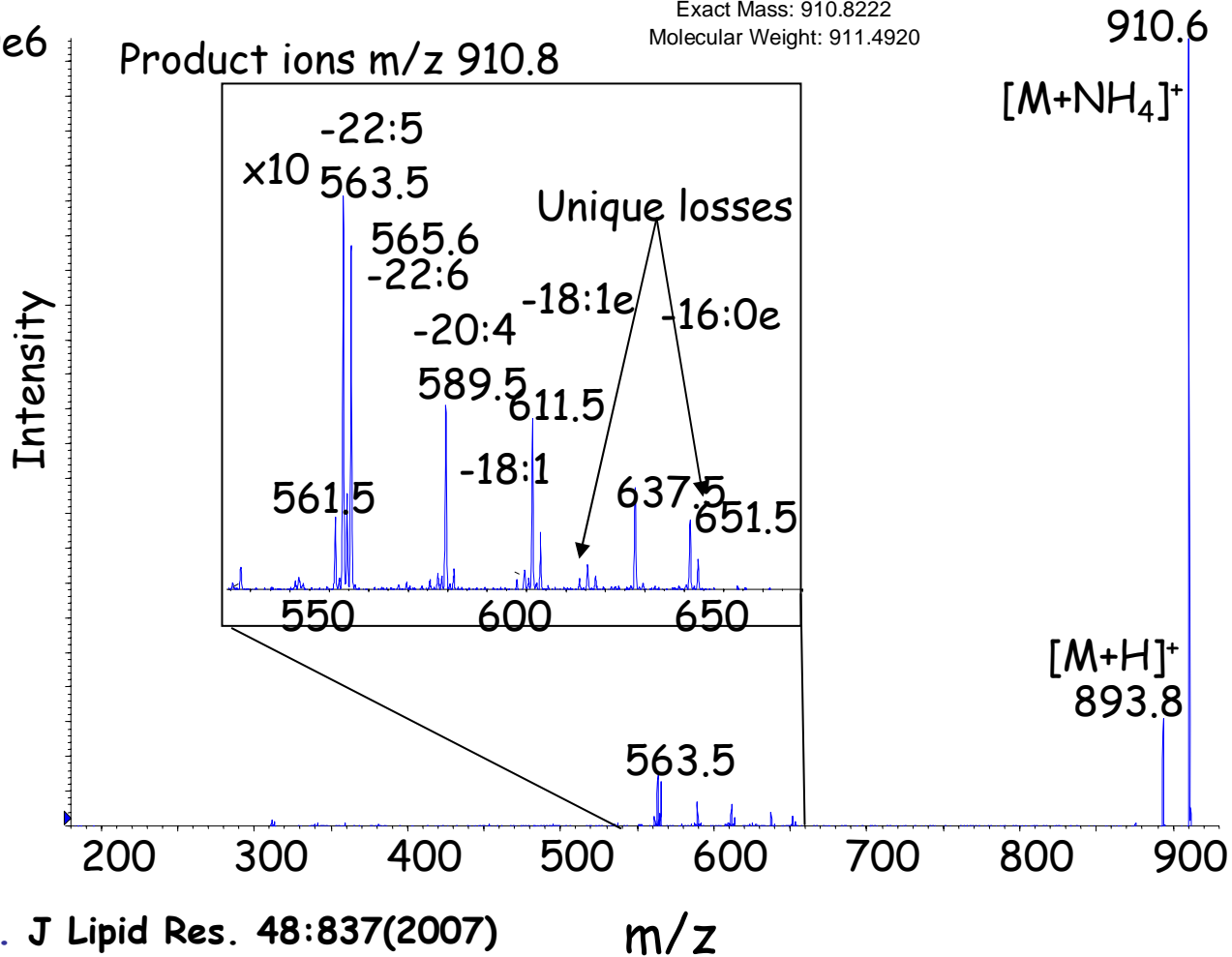


Chemical Formula:  $C_{59}H_{108}NO_5^+$

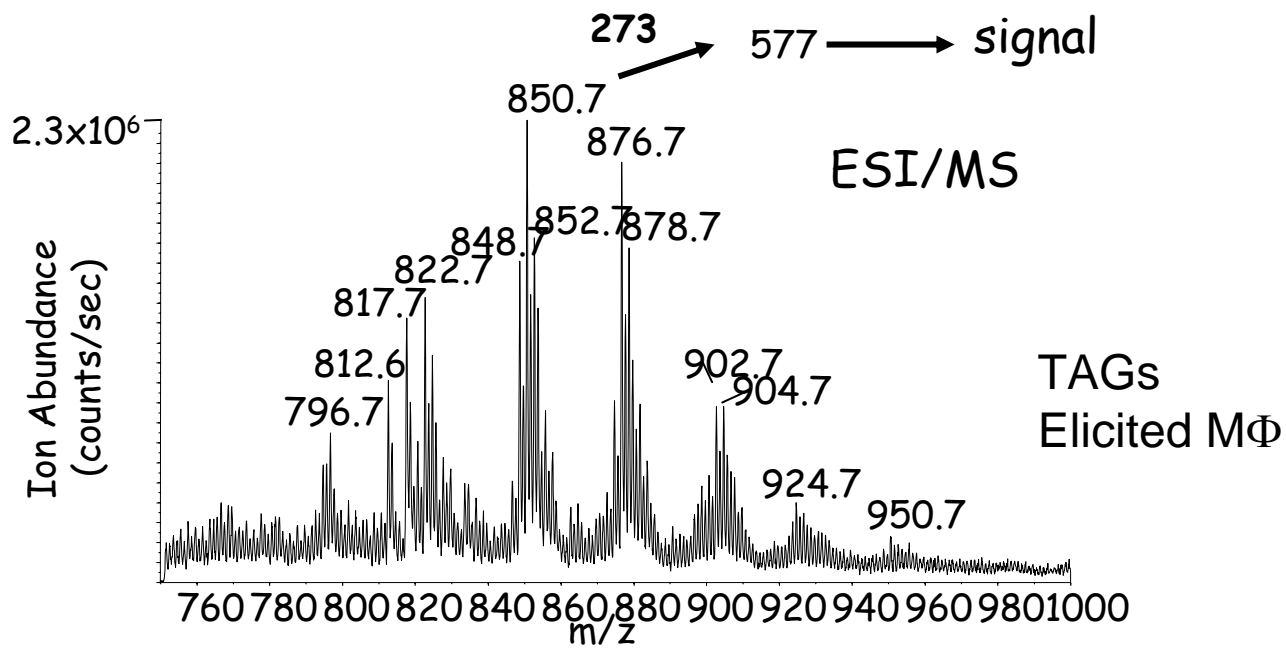
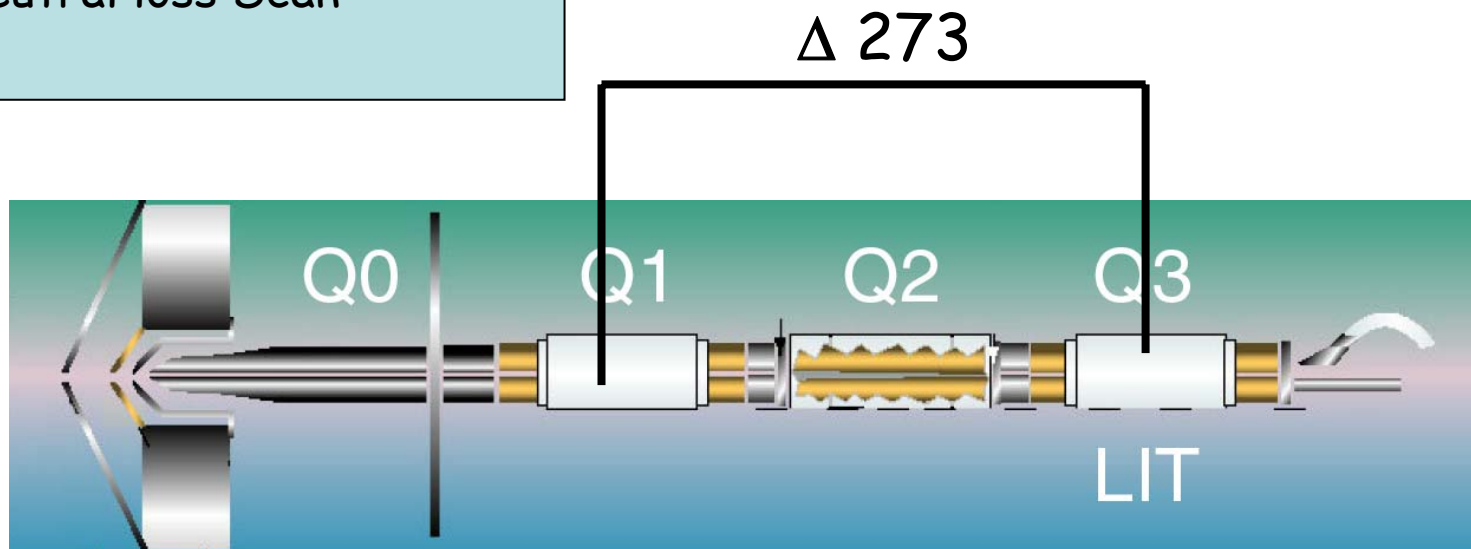
Exact Mass: 910.8222

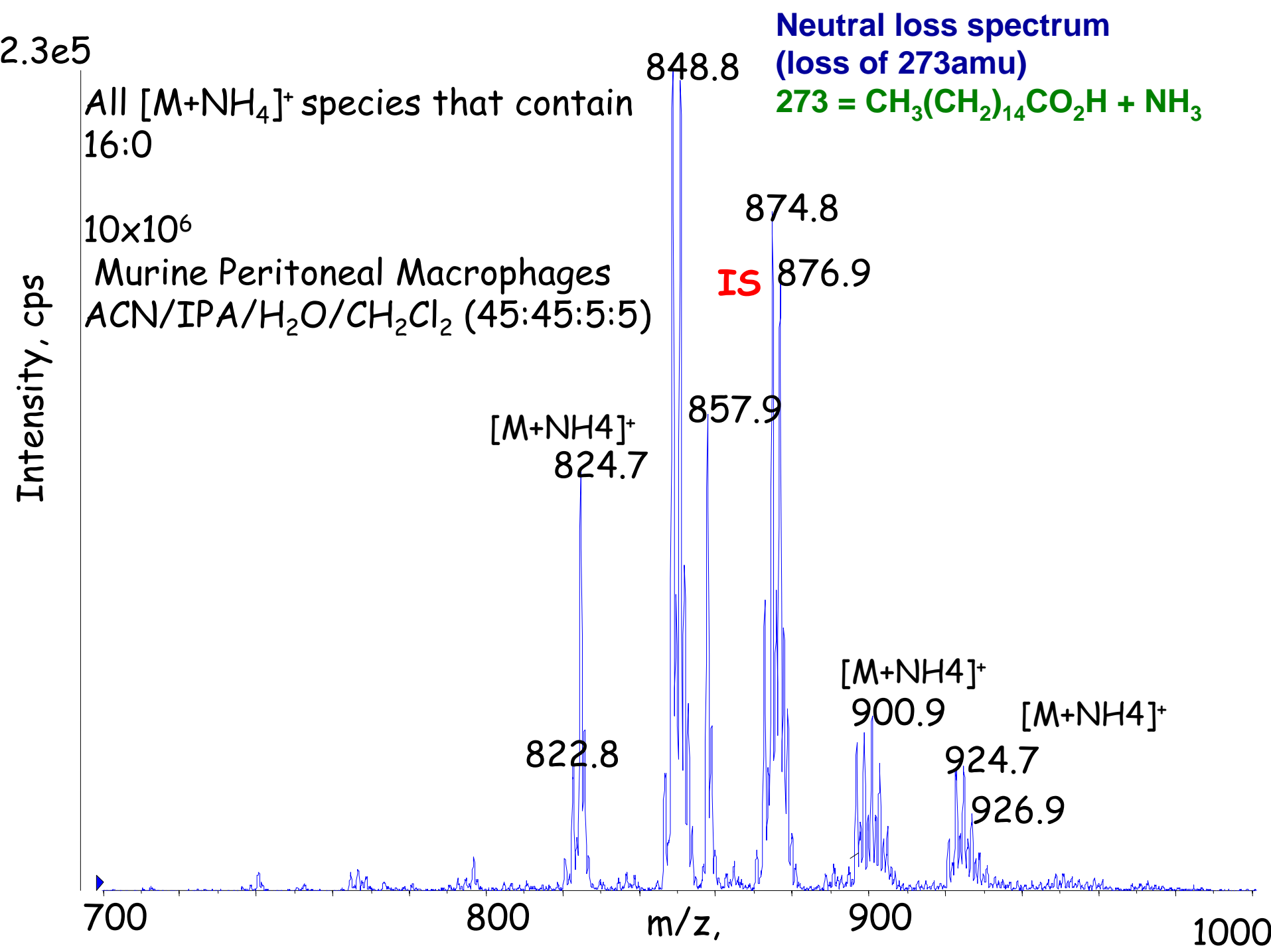
Molecular Weight: 911.4920

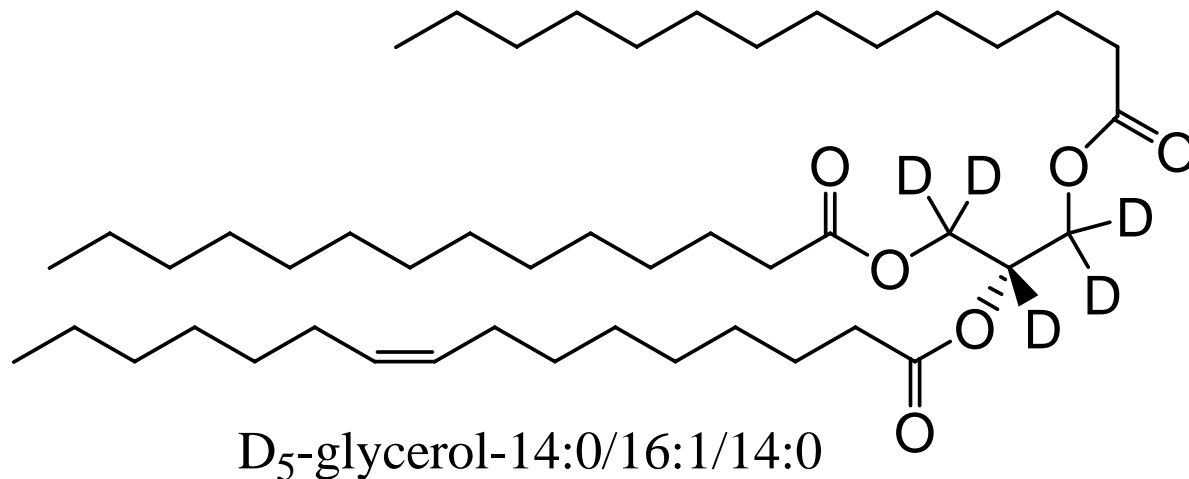
1.10e6



Neutral loss Scan







D<sub>5</sub>-glycerol-14:0/16:1/14:0

TAGs (9)

Avanti  
Polar  
Lipids

### DAGs (7)

sn-1	sn-2	sn-3	M+NH <sub>4</sub> <sup>+</sup>
19:0	OH	19:0	675.6
14:0	OH	14:0	535.5
15:0	OH	15:0	563.5
16:0	OH	16:0	591.5
17:0	OH	17:0	619.6
20:4	OH	20:4	687.5
20:0	OH	20:0	703.6

### 18- Fatty acyl groups

sn-1	sn-2	sn-3	M+NH <sub>4</sub> <sup>+</sup>
19:0	12:0	19:0	857.8
14:0	16:1	14:0	771.7
15:0	18:1	15:0	827.8
16:0	18:0	16:0	857.8
17:0	17:1	17:0	869.8
20:4	18:2	20:4	949.7
20:0	20:1	20:0	995.9
20:2	18:3	20:2	955.8
20:5	22:6	20:5	993.7



# Lipid Profiler

**Lipid Results** File View Help

File Name: 18Sep06 Spike Test b

Show Results  
 All  Confirmed  Confirmed and Tentative

Sample 9 Sample 10 Sample 11 Sample 12  
 Sample 1 Sample 2 Sample 3 Sample 4 Sample 5 Sample 6 Sample 7 Sample 8

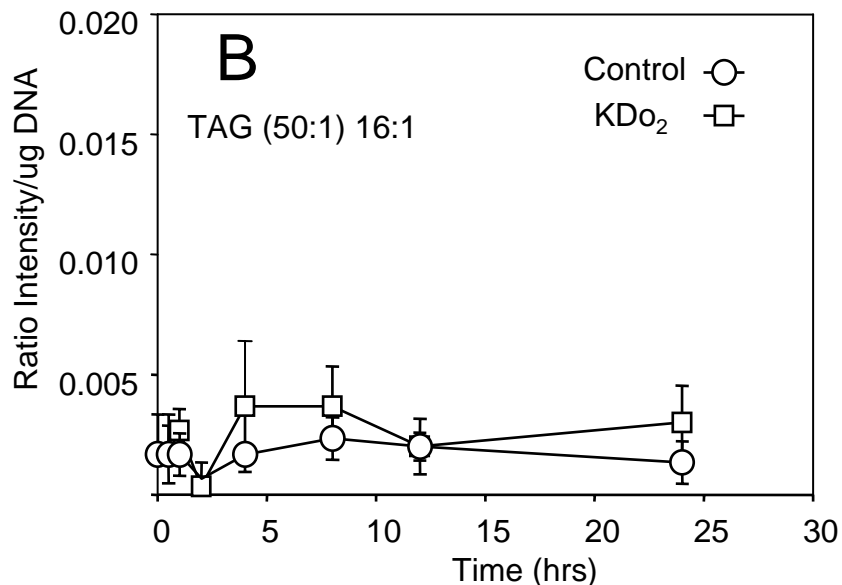
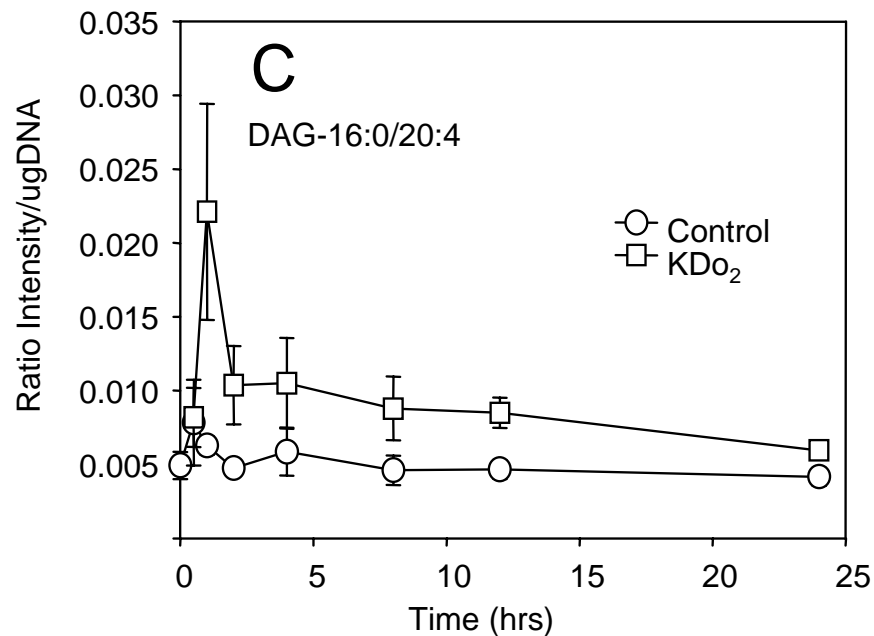
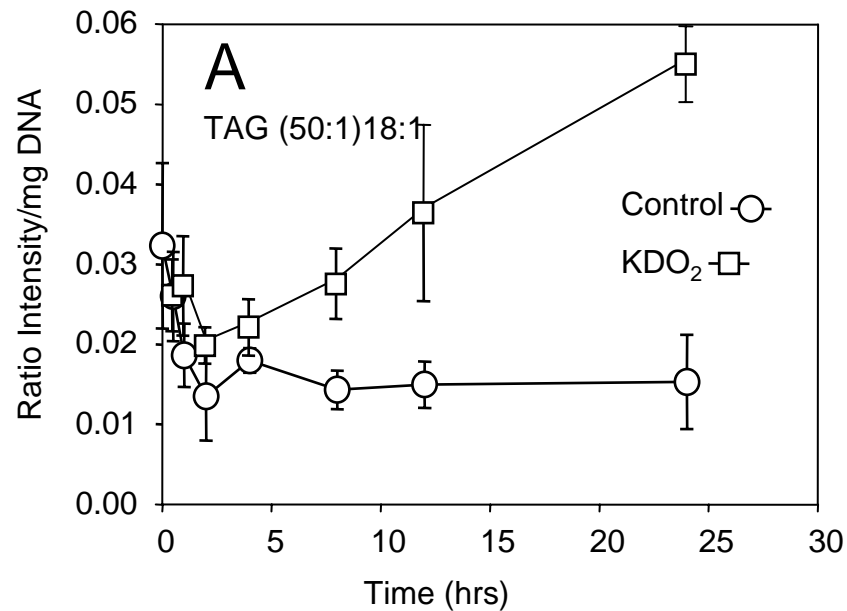
Sample Name: 0 ng 16:0/18:1/18:2 (C:\Documents and Settings\Administrator\Desktop\sept spike\18Sep06 Spike Test b.wiff)

PIS Scan	PIS m/z	m/z	Peak Intensity	Peak Area	TAG Name	IS Name
FA 16:0 (NH4)		273.20	591.6	1854.8		D5-DAG 16:0/16:0
FA 16:0 (NH4)		273.20	686.5	1011.3	TAG 39:6+NH4	
FA 16:0 (NH4)		273.20	824.9	403.5	TAG 48:0+NH4	
FA 16:0 (NH4)		273.20	848.8	413.3	TAG 50:2+NH4	
FA 16:0 (NH4)		273.20	856.8	909.2	TAG 51:5+NH4	
FA 16:0 (NH4)		273.20	857.8	17663.4		D5-TAG 16:0/18:0/16:0
FA 16:0 (NH4)		273.20	874.8	457.0	TAG 52:3+NH4	
FA 16:0 (NH4)		273.20	876.8	476.5	TAG 52:2+NH4	
FA 16:0 (NH4)		273.20	936.7	442.4	TAG 56:0+NH4	
FA 16:0 (NH4)		273.20	958.5	476.5	TAG 58:3+NH4	
FA 18:1 (NH4)		299.20	827.8	6556.1		D5-TAG 15:0/18:1/15:0
FA 18:1 (NH4)		299.20	874.8	634.5	TAG 52:3+NH4	
FA 18:1 (NH4)		299.20	876.9	537.2	TAG 52:2+NH4	
FA 18:1 (NH4)		299.20	898.8	418.1	TAG 54:5+NH4	
FA 18:1 (NH4)		299.20	900.8	719.5	TAG 54:4+NH4	
FA 18:1 (NH4)		299.20	902.8	427.8	TAG 54:3+NH4	
FA 18:1 (NH4)		299.20	906.6	498.3	TAG 54:1+NH4	
FA 18:1 (NH4)		299.20	962.7	865.4	TAG 58:1+NH4	
FA 18:1 (NH4)		299.20	984.6	1409.9	TAG 60:4+NH4	
FA 18:2 (NH4)		297.20	680.5	1745.4	TAG 38:2+NH4	
FA 18:2 (NH4)		297.20	872.8	697.7	TAG 52:4+NH4	
FA 18:2 (NH4)		297.20	874.8	476.5	TAG 52:3+NH4	
FA 18:2 (NH4)		297.20	898.8	697.7	TAG 54:5+NH4	
FA 18:2 (NH4)		297.20	900.8	401.1	TAG 54:4+NH4	
FA 18:2 (NH4)		297.20	960.5	430.3	TAG 58:2+NH4	
FA 18:2 (NH4)		297.20	982.7	588.3	TAG 60:5+NH4	

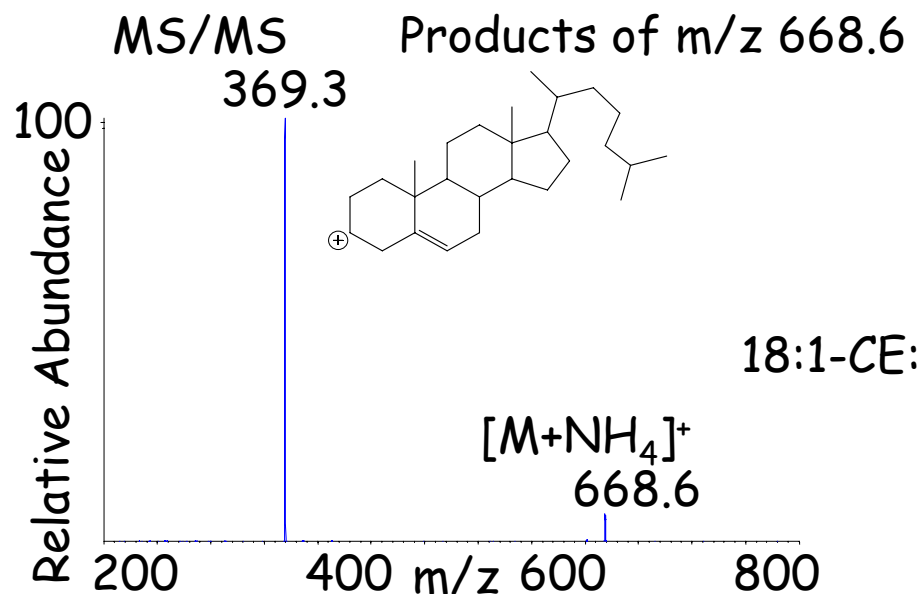
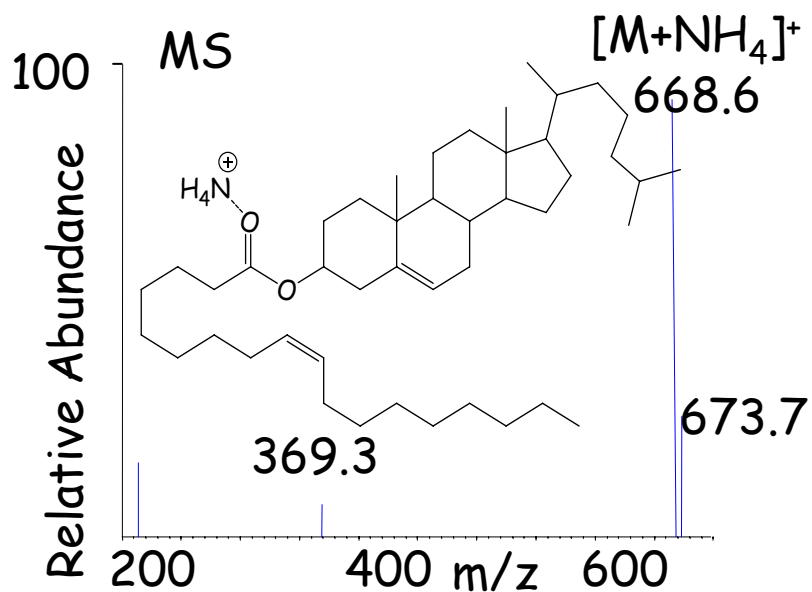
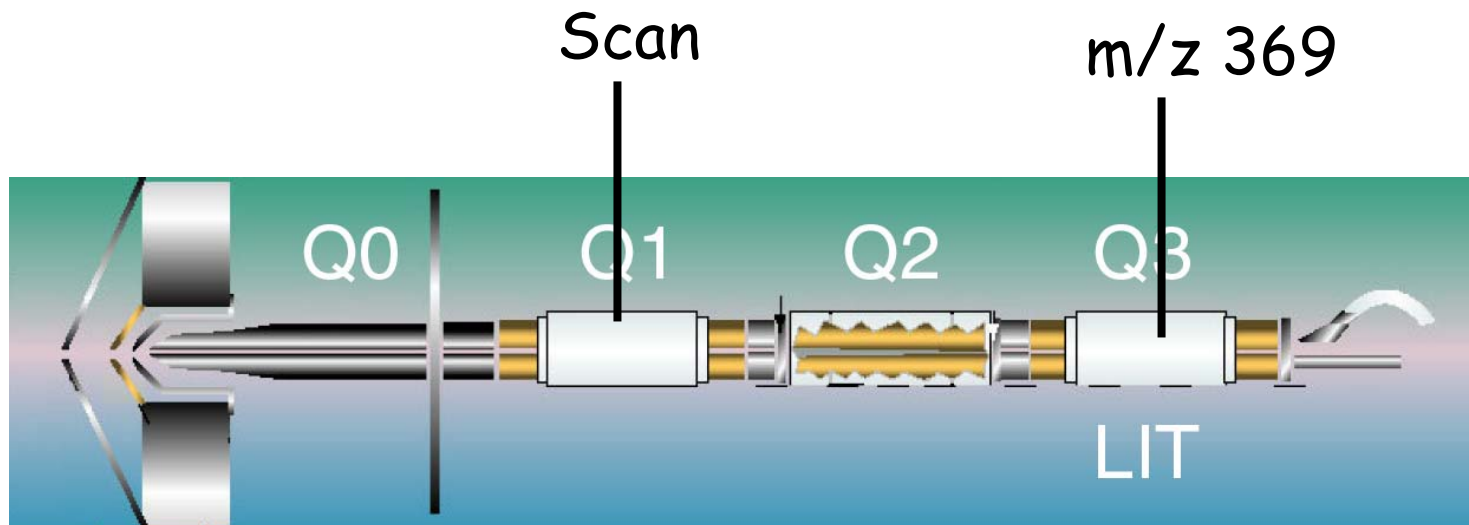


# RAW 264.7 Cells Stimulated with KDO<sub>2</sub>-Lipid A

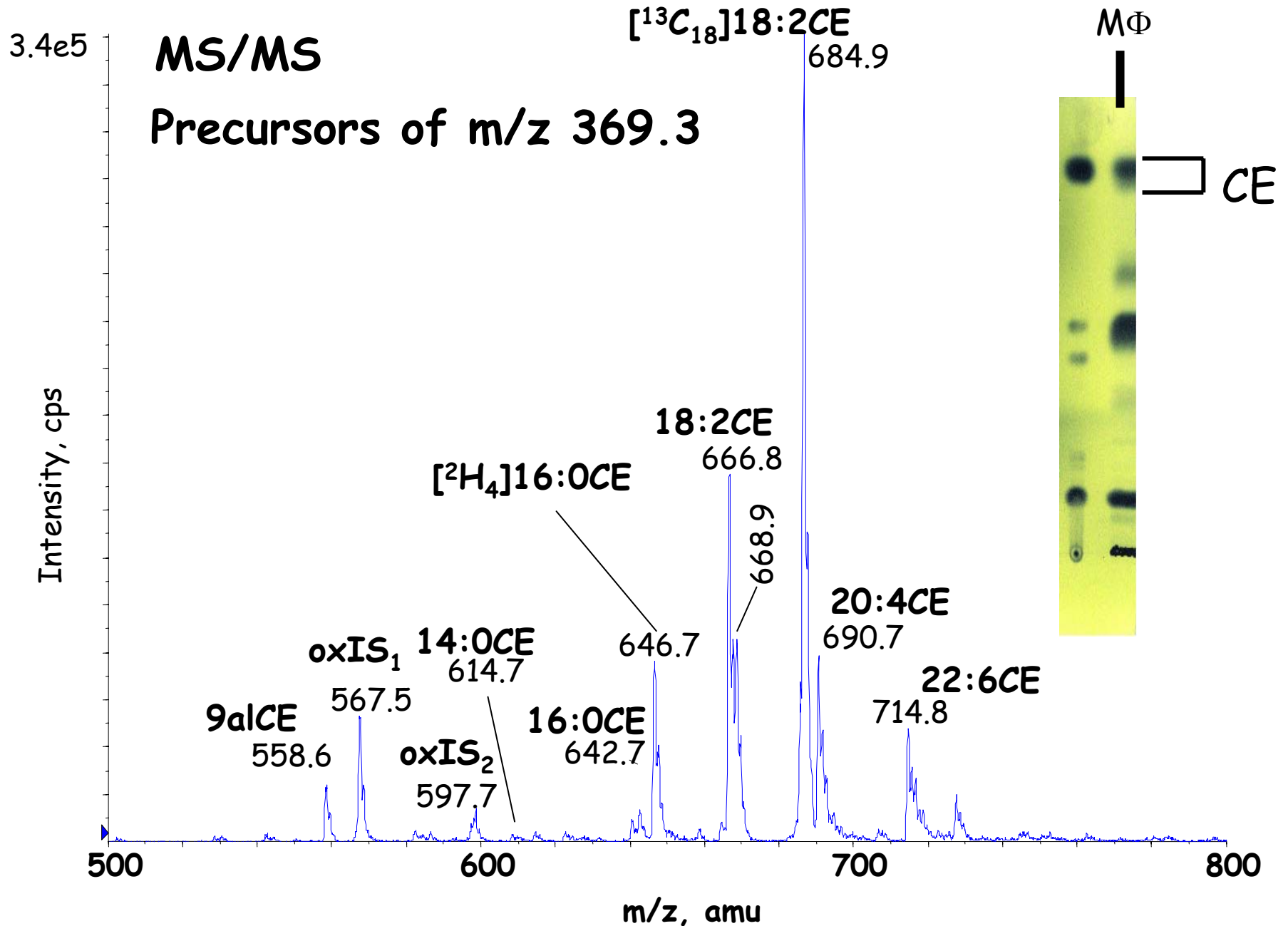
m/z 850



# Precursor ion scanning

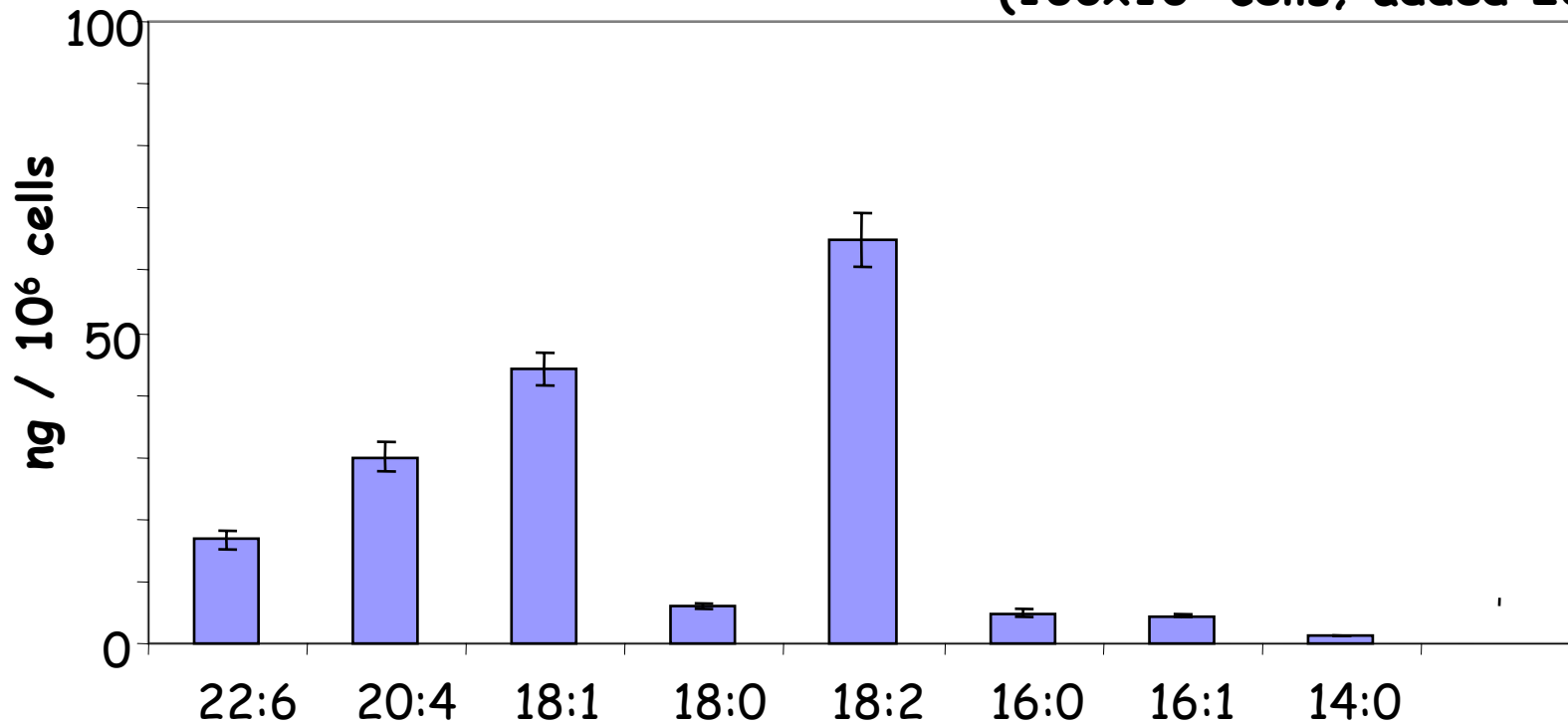


# Thioglycolate elicited Peritoneal Macrophage (C57B6)



# Peritoneal Macrophages (thioglycolate elicited)

( $100 \times 10^6$  cells; added 20ug IS)



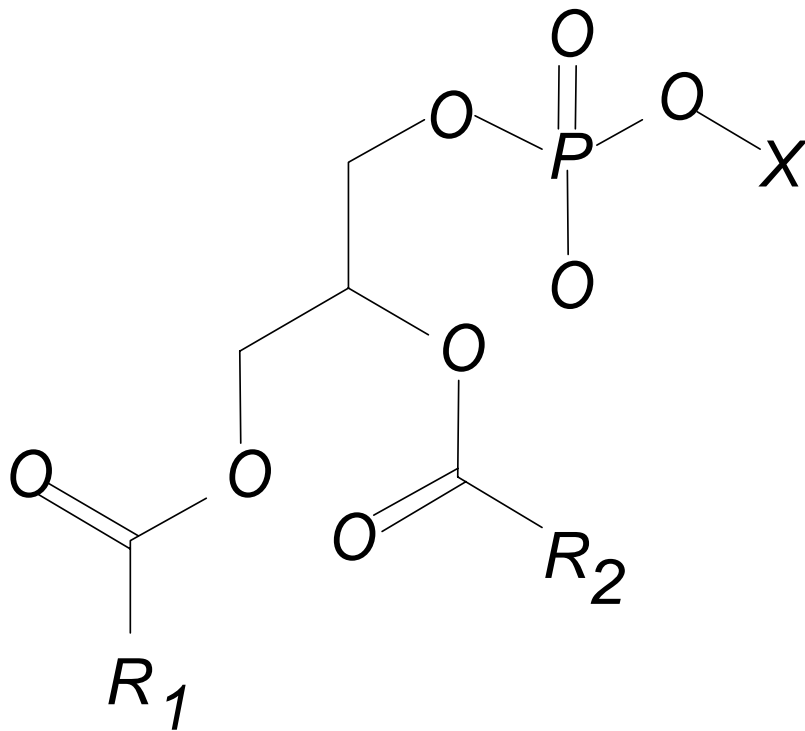
**Fatty Acid Esterified to Cholesterol**

# Phospholipids

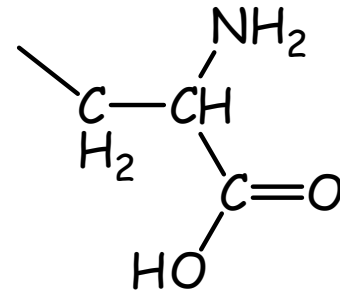
- Classes
- Quantitative and Qualitative analysis
  - Positive/negative ions
  - Isotope corrections
  - Quantity indicating ions and standard curves
- Chromatographic separation
  - Normal phase (LC)
  - Reverse phase(LC)
- Artifacts/challenges

# Glycerophospholipids

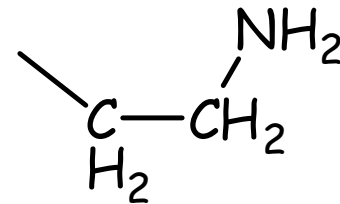
*X (Polar Head Group)*



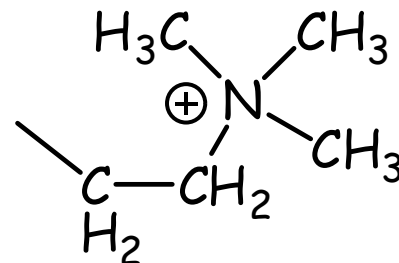
*1,2-diacyl*



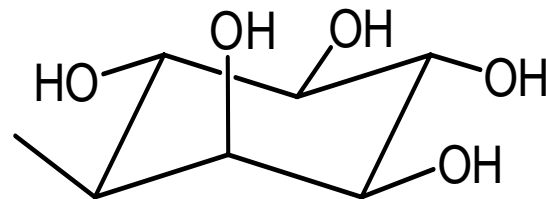
Serine (GPCer)



Ethanolamine (GPEtn)



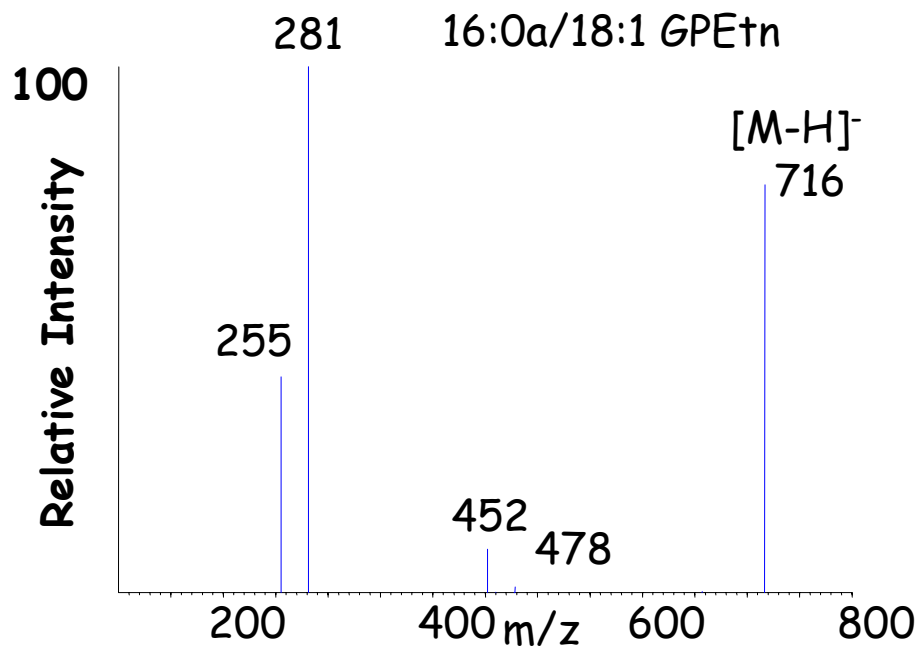
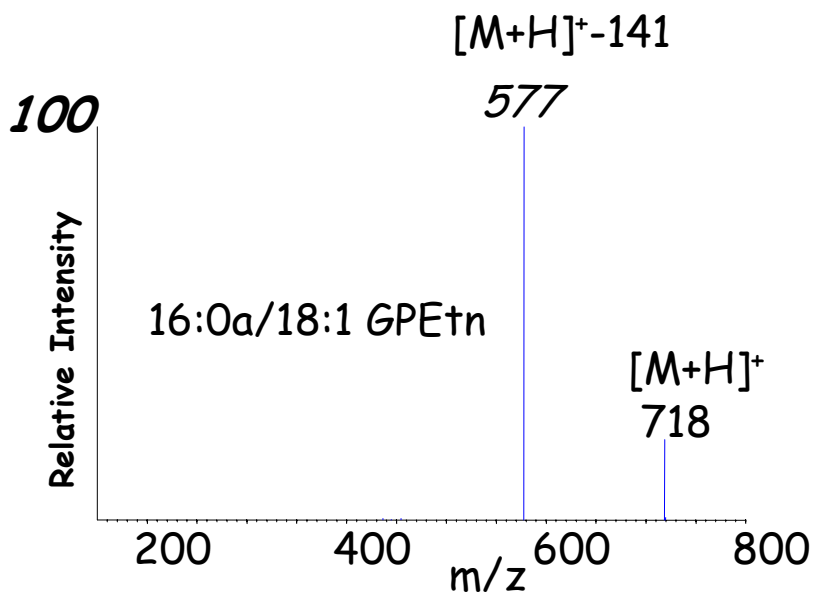
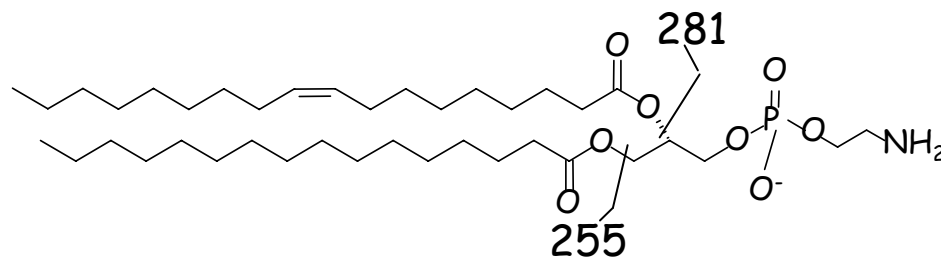
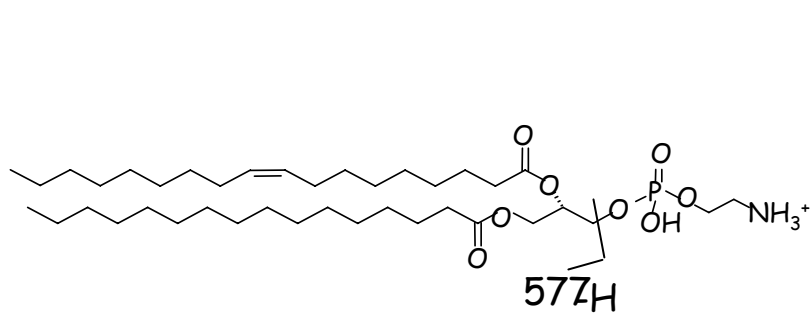
Choline (GPCho)



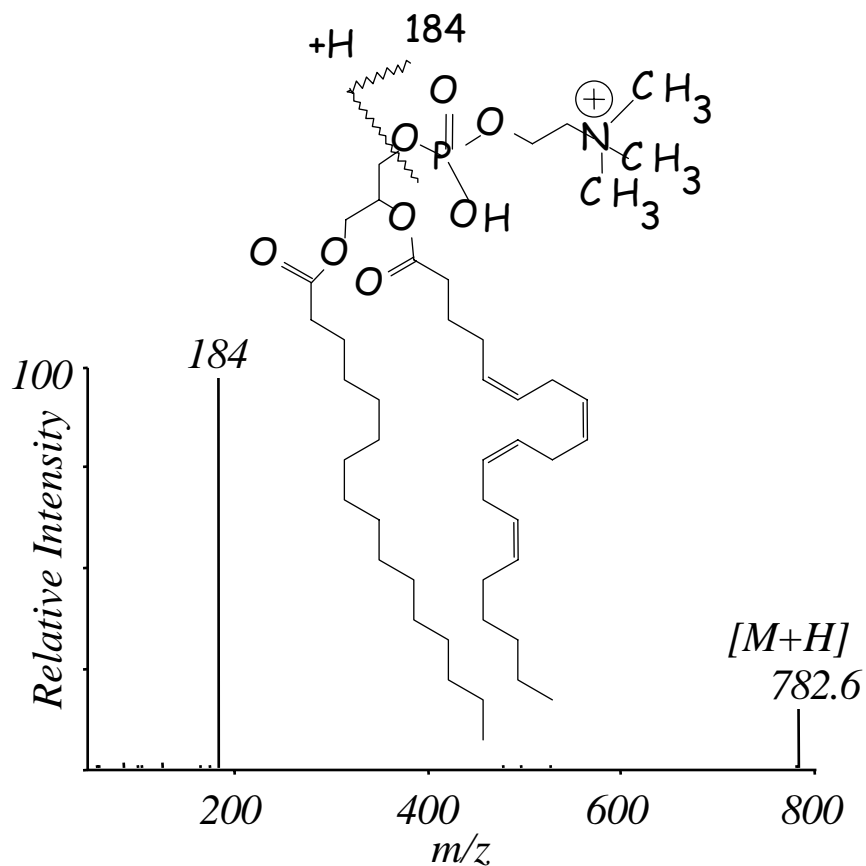
Inositol (GPIIns)

# CID 16:0a/18:1-GPEtn

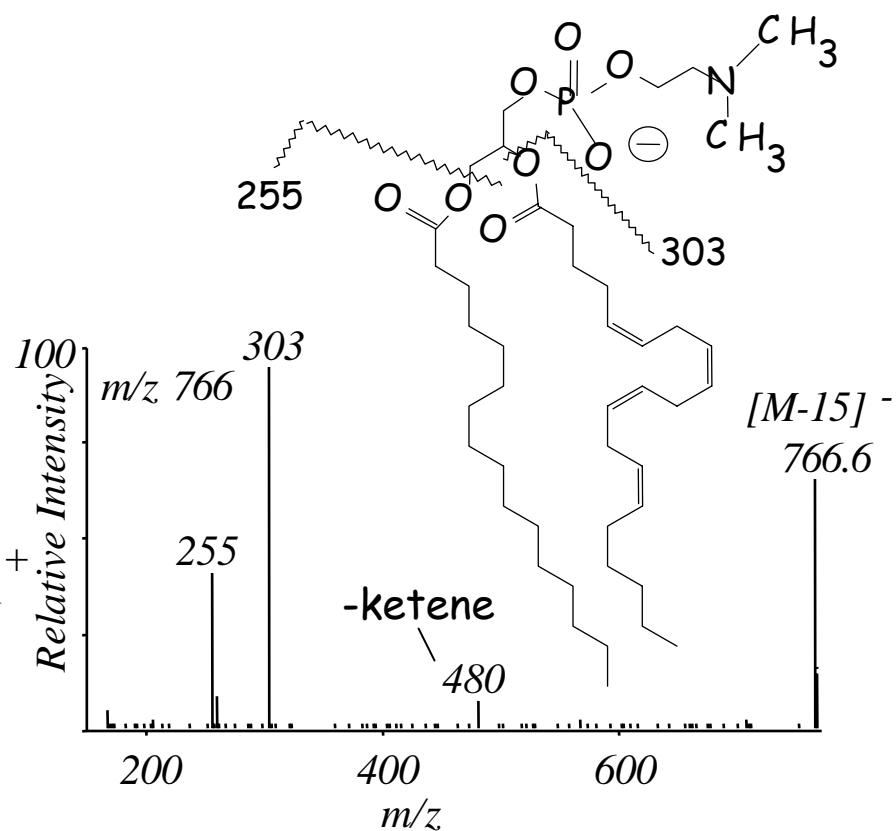
## Positive and negative product ions



# ESI-MS/MS (tandem quadrupole)



Positive Ions

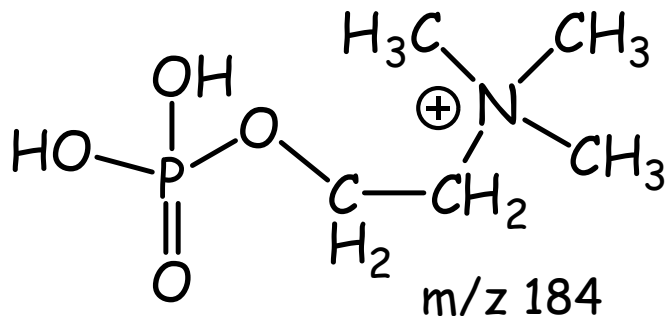


Negative Ions

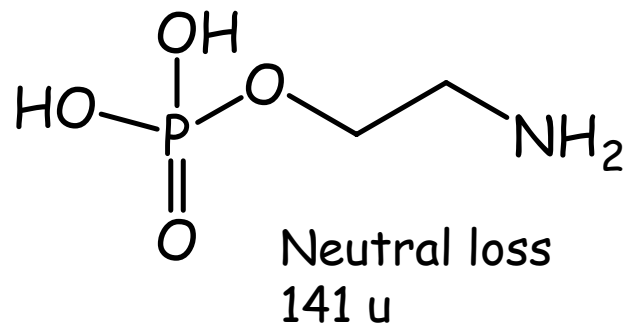


# Diagnostic Product Ions from Positive $[M+H]^+$ Glycerophospholipids (CID)

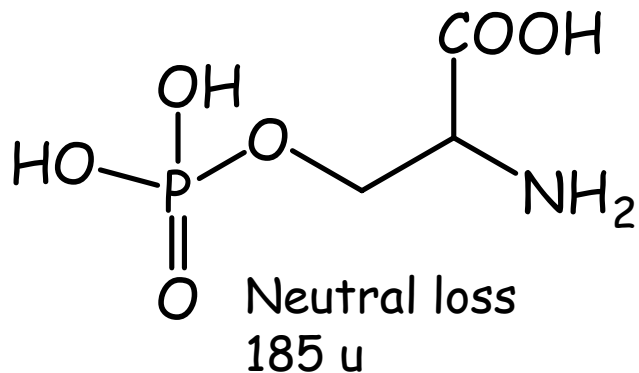
## *GPCho*



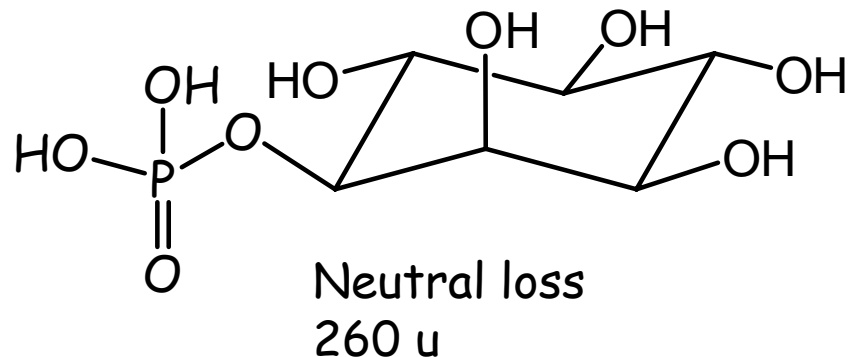
## *GPEtn*



## *GPSer*



## *GPIIns*



# Common Negative Ions of Glycerophospholipids (CID)

*GPCho*

*GPSer*

*GPEtn*

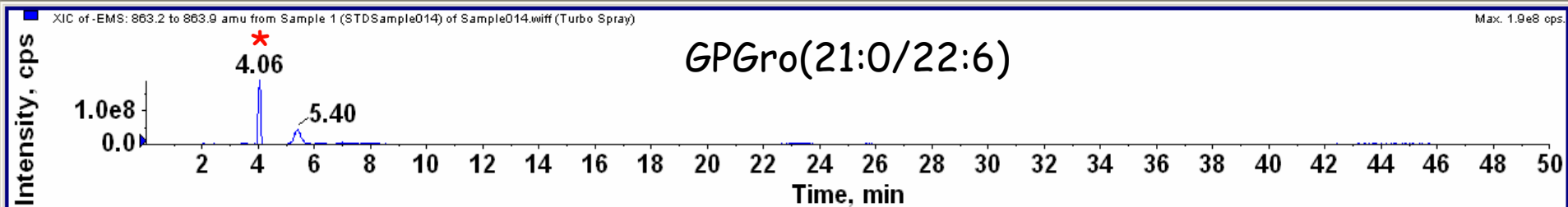
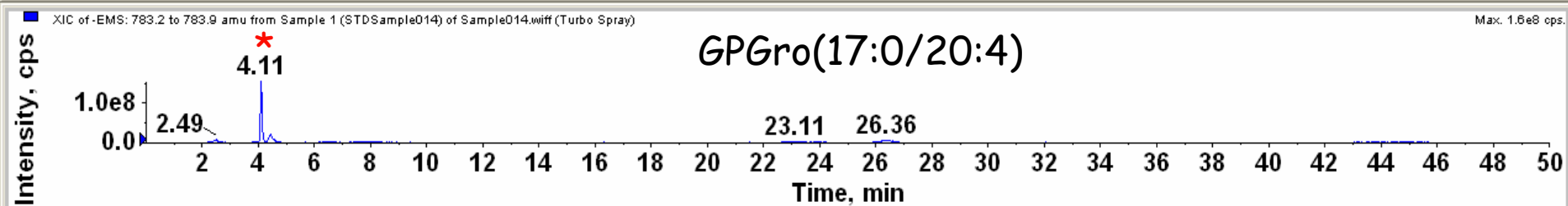
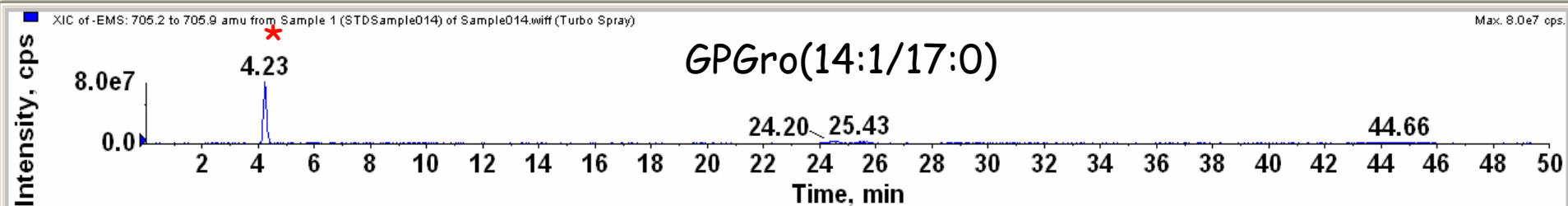
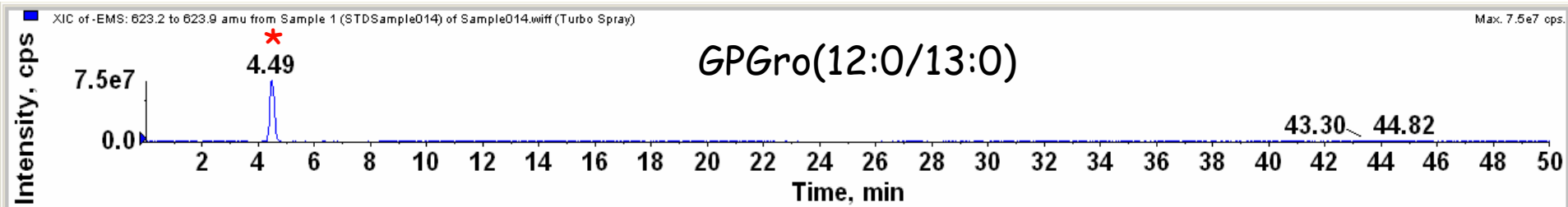
*GPIns*

[M-15]<sup>-</sup>

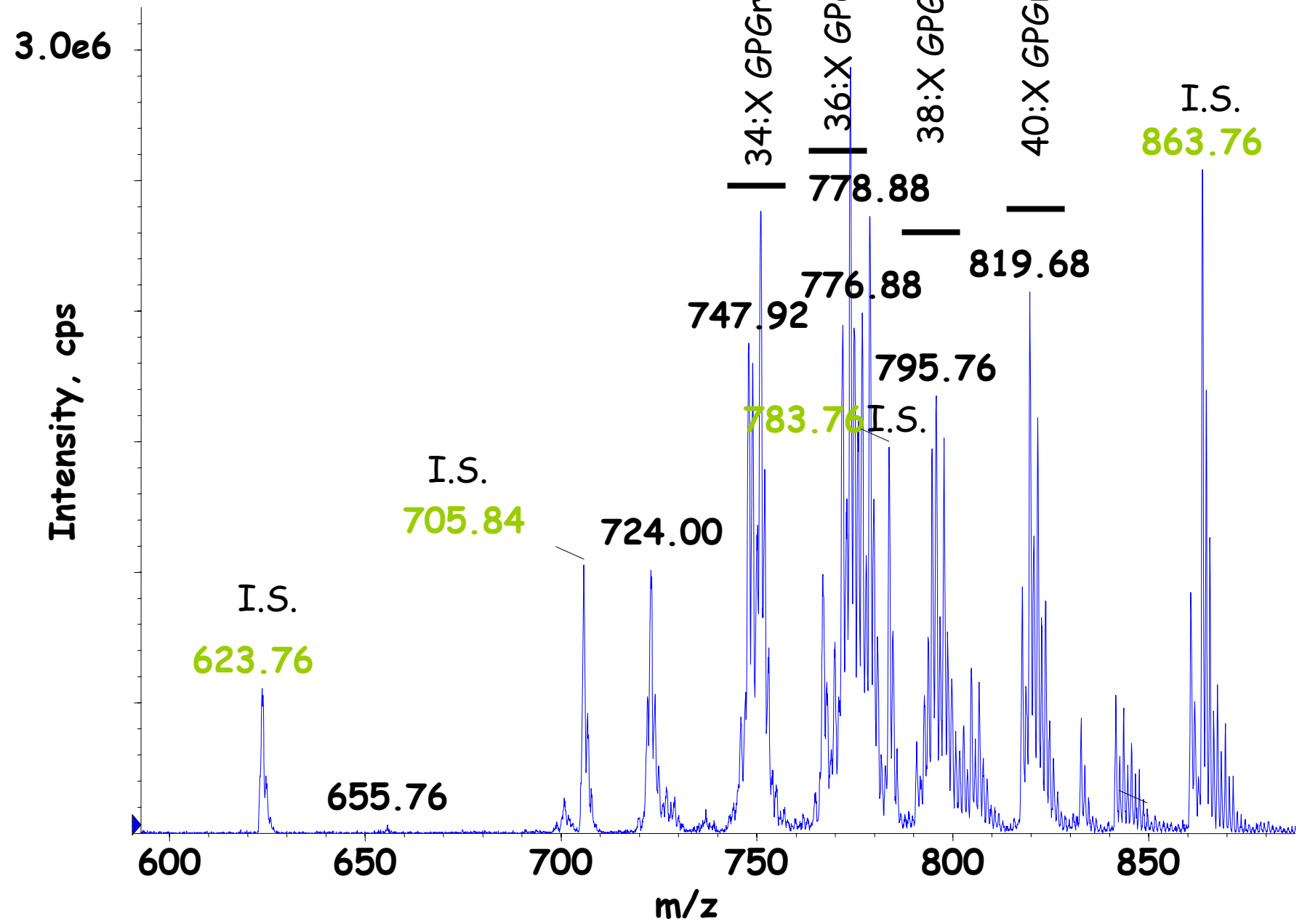
---

$R_1COO^-$	$R_1COO^-$	$R_1COO^-$	$R_1COO^-$
$R_2COO^-$	$R_2COO^-$	$R_2COO^-$	$R_2COO^-$
$[M-H]^- - R_2COOH$	$[M-H]^- - 87$	$[M-H]^- - R_2COOH$	$[M-H]^- - R_2COOH$
$[M-H]^- - R_2C=C=O$		$[M-H]^- - R_2C=C=O$	$[M-H]^- - R_2C=C=O$
	$[M-H]^- - R_2COOH - 87$		m/z 241
	m/z 153	m/z 153	m/z 153

# LC/MS Analysis (A. Brown)



Foam Cell GPGro Region of LC/MS Experiment



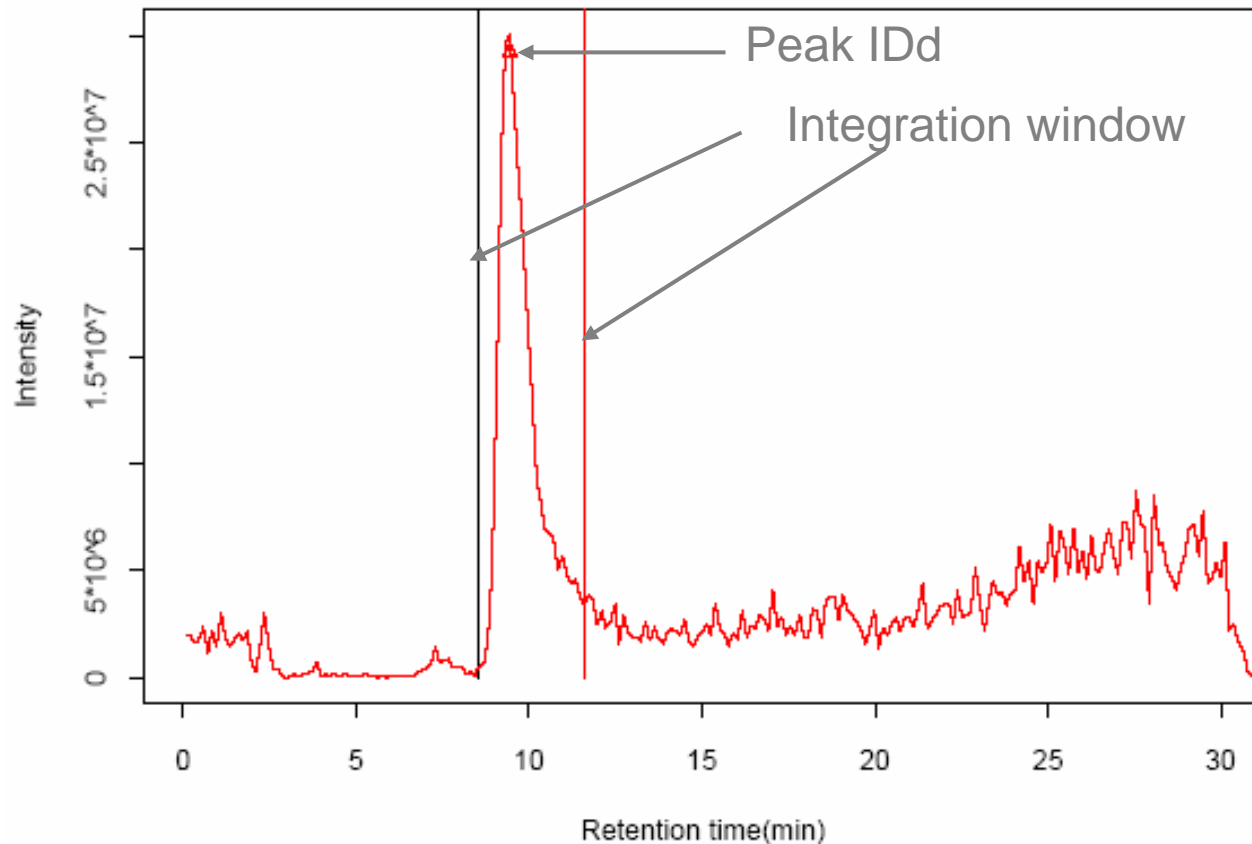
# Avanti Lipid Maps Standards

25:0 GPA	31:1 GPA	37:4 GPA	43:6 GPA
25:0 GPCho	31:1 GPCho	37:4 GPCho	43:6 GPCho
25:0 GPEtn	31:1 GPEtn	37:4 GPEtn	43:6 GPEtn
25:0 GPGro	31:1 GPGro	37:4 GPGro	43:6 GPGro
25:0 GPIIns	31:1 GPIIns	37:4 GPIIns	43:6 GPIIns
25:0 GPSer	31:1 GPSer	37:4 GPSer	43:6 GPSer
13:0 Lyso GPA	17:1 Lyso GPA		
13:0 Lyso GPCho	17:1 Lyso GPCho		
37:4 GPIIns(3)P	37:4 GPIIns(4)P	37:4 GPIIns(5)P	
37:4 GPIIns(3,4)P2	37:4 GPIIns(3,5)P2	37:4 GPIInsI(4,5)P2	
37:4 GPIIns(3,4,5)P3			

# LC-MS Computational Analysis

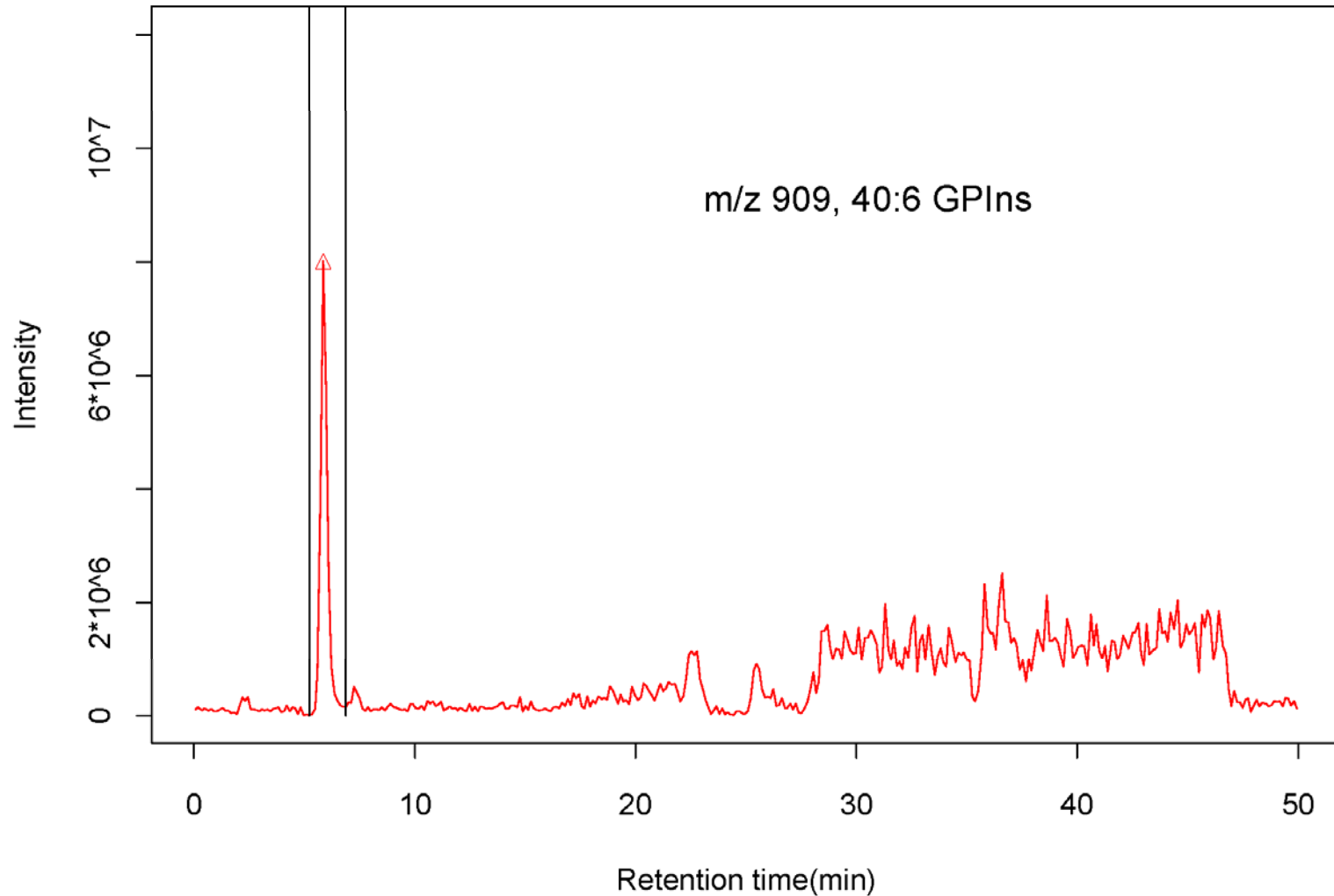
- XIC ASCII data is automatically generated from fullscan data for all  $m/z$  values, peaks found, integrated, and aligned across files (samples).
- Visual checks and error checking per  $m/z$  are used to confirm automated results.

XIC from  $m/z$  631.06 to 631.96 (Peak fragmentation indicates 31:1 GPA standard)



# LC-MS Analysis (cont'd)

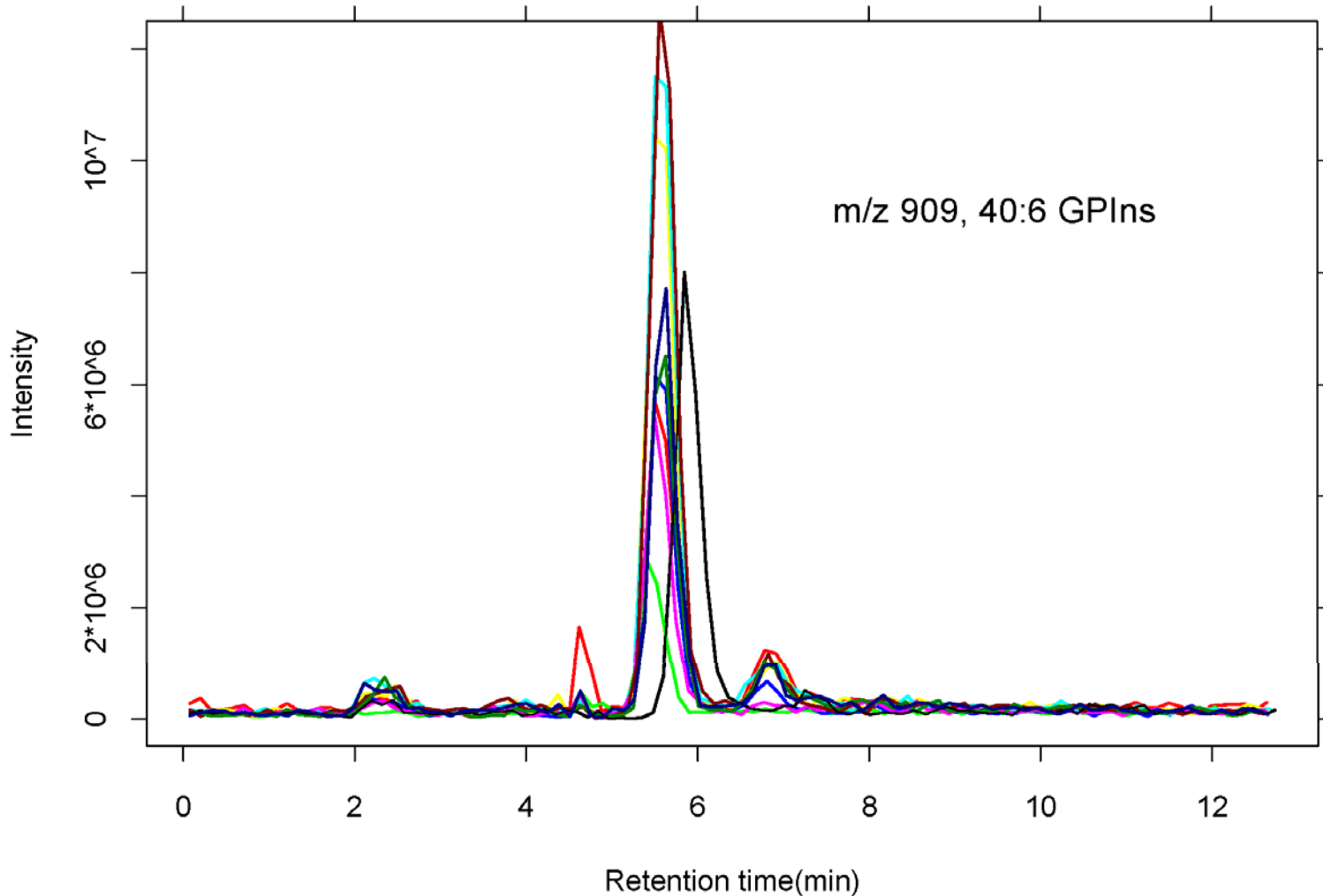
XIC from  $m/z$  909.06 to 909.96 (LMAPS Raw cell)



- Peaks areas are normalized to fixed odd-carbon internal standards (when available)
- Even carbon titrations are used for estimating quantities in the extract.

## Multiple XICs (10 spectra) from $m/z$ 909.06 to 909.96 (LMAPS RAW cell)

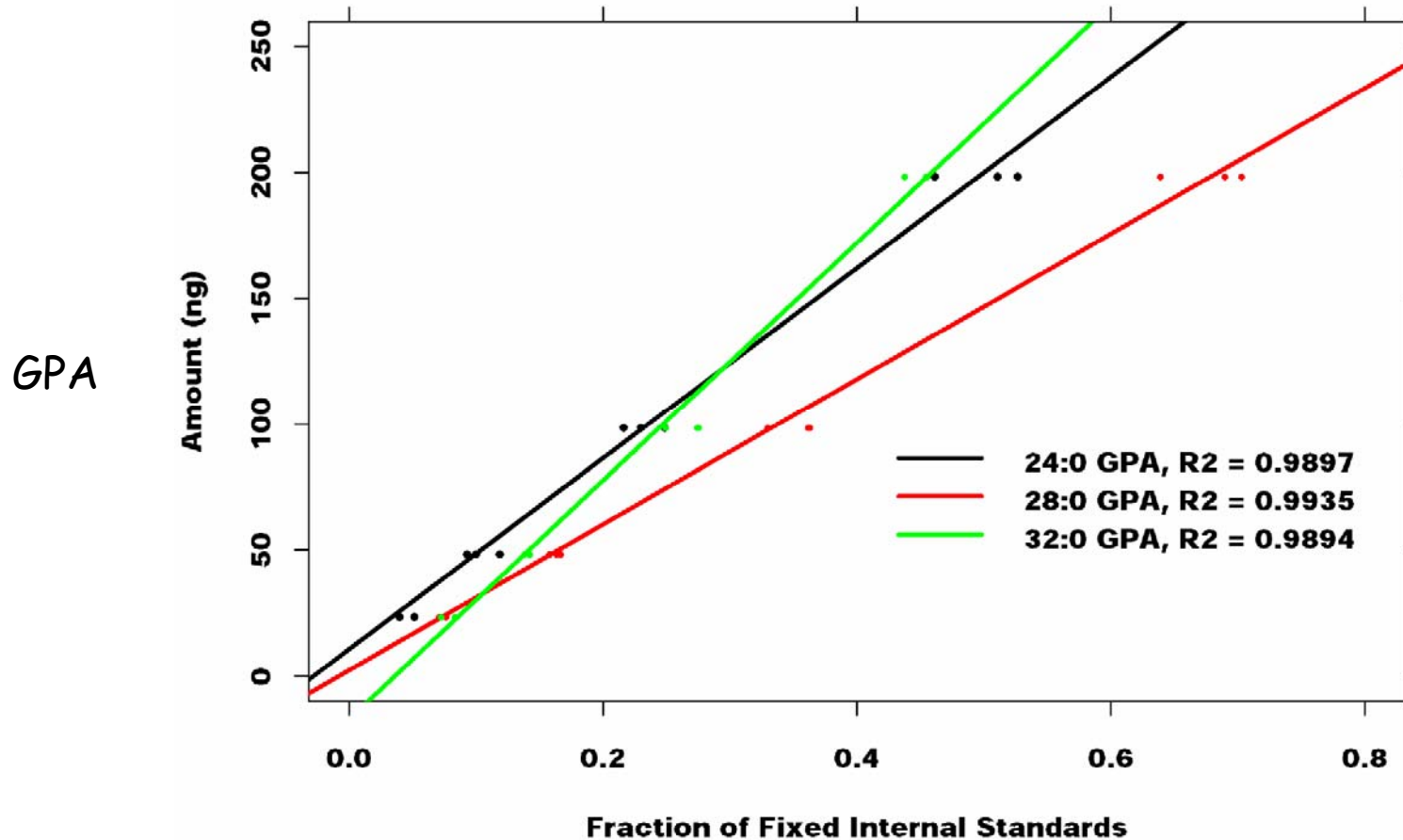
- Determine peak areas using automated integration algorithms.
- Align integrated values across samples (confirm alignment graphically).
- Normalize areas to fixed internal standard areas.
- Currently do this for ~130 glycerophospholipid analytes.





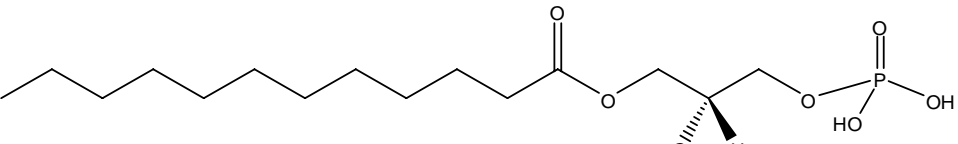
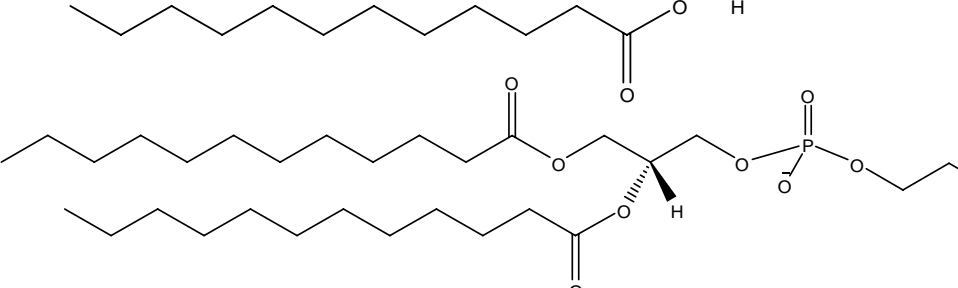
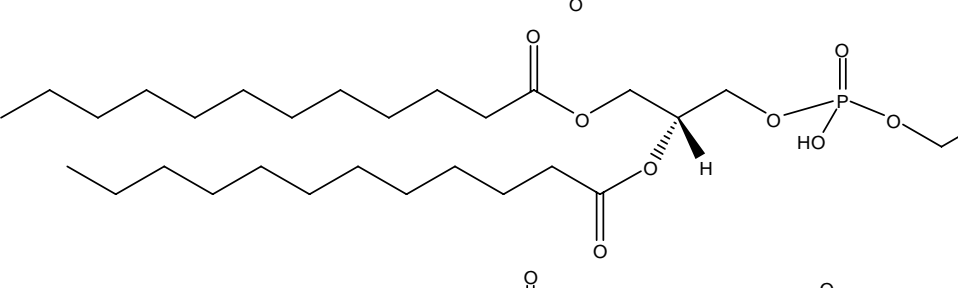
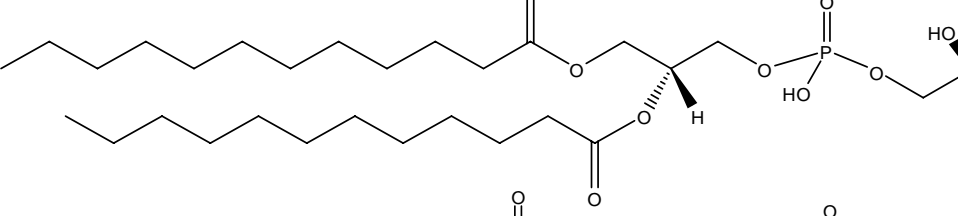
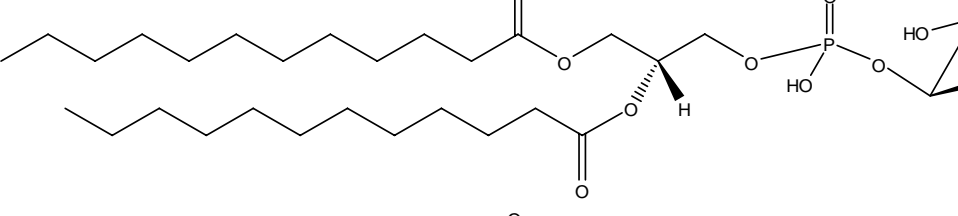
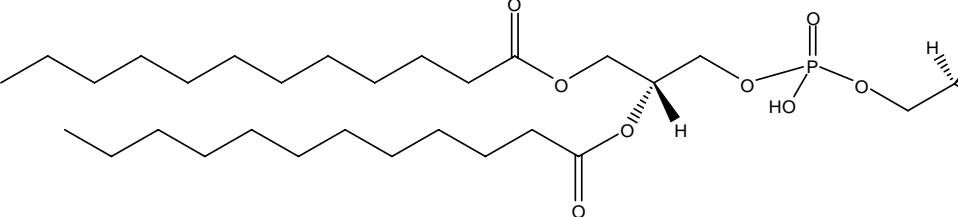
# Standard curves from integrated peak XIC data

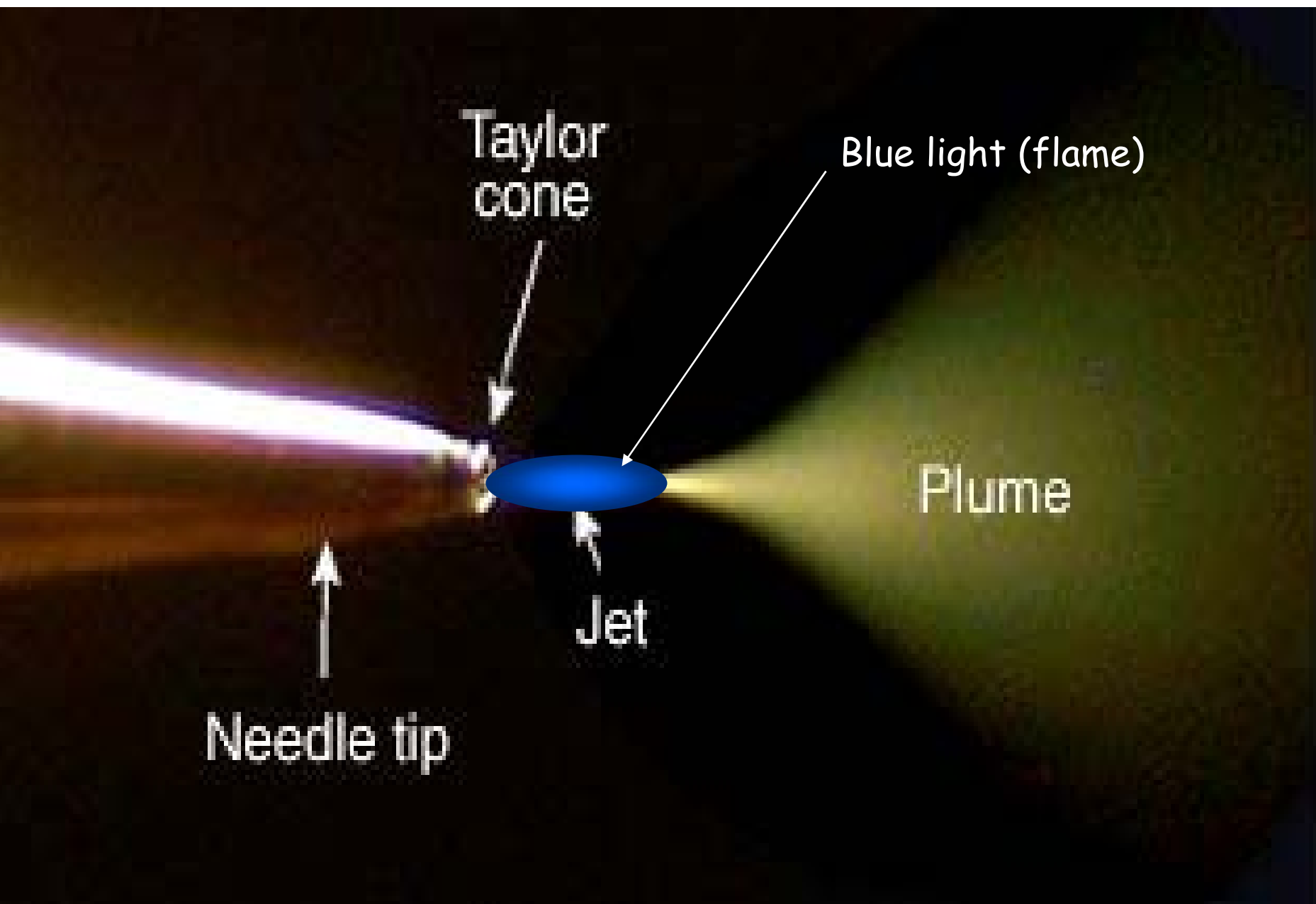
$$\text{Amount}(\text{ng}) = \text{slope} * (\text{fraction of odd carbon std}) + \text{intercept}$$



- Peak areas normalized to mean of 4 fixed odd carbon internal standard peak areas within the class (as in KDO2 time-course data set).
- 11 even carbon GPA standard curves generated.

# Glycerophospholipid Parts List (RAW 264.7 cells)

	<u>Lipid Class</u>	<u>Lipids Identified</u>
	GPA	101
	GPCho	106
	GPEtn	136
	GPGro	105
	GPIIns	84
	GPSer	68



Taylor  
cone

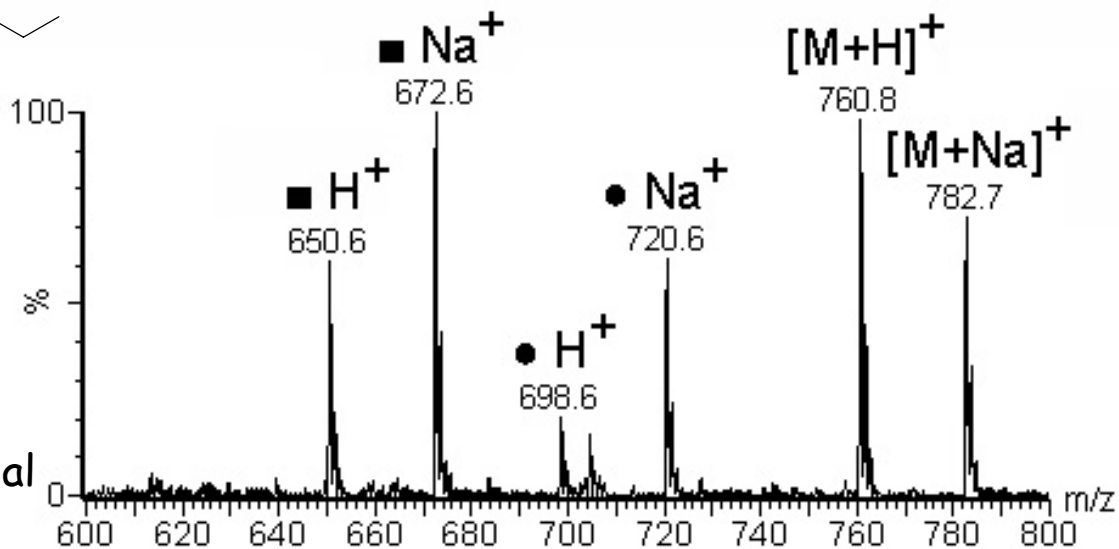
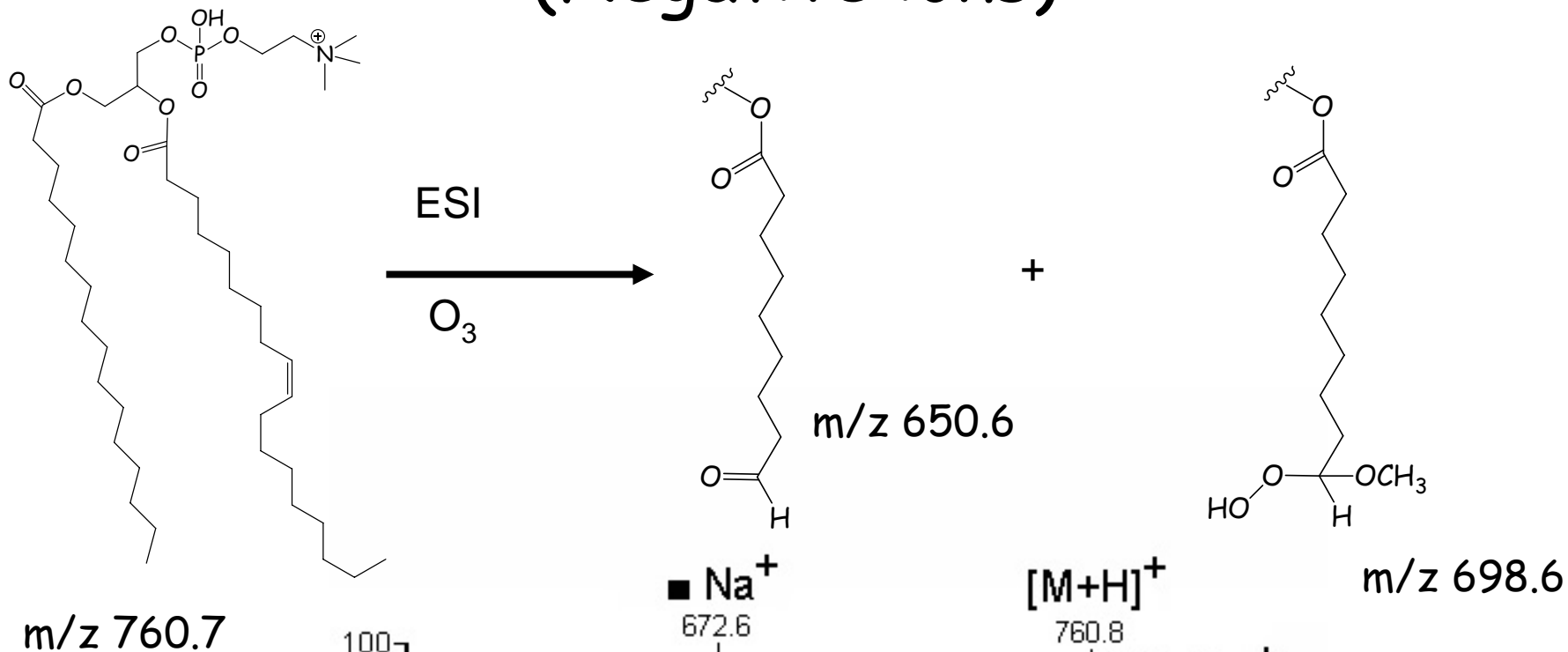
Blue light (flame)

Plume

Jet

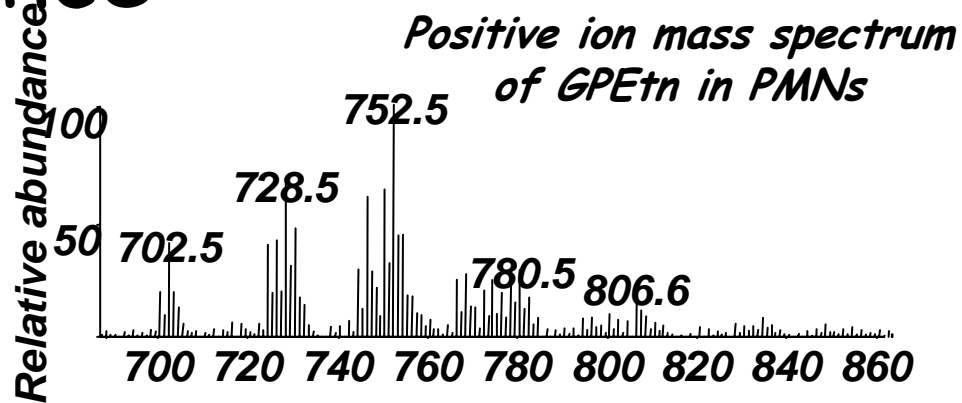
Needle tip

# Ozonolysis during ESI (Negative ions)



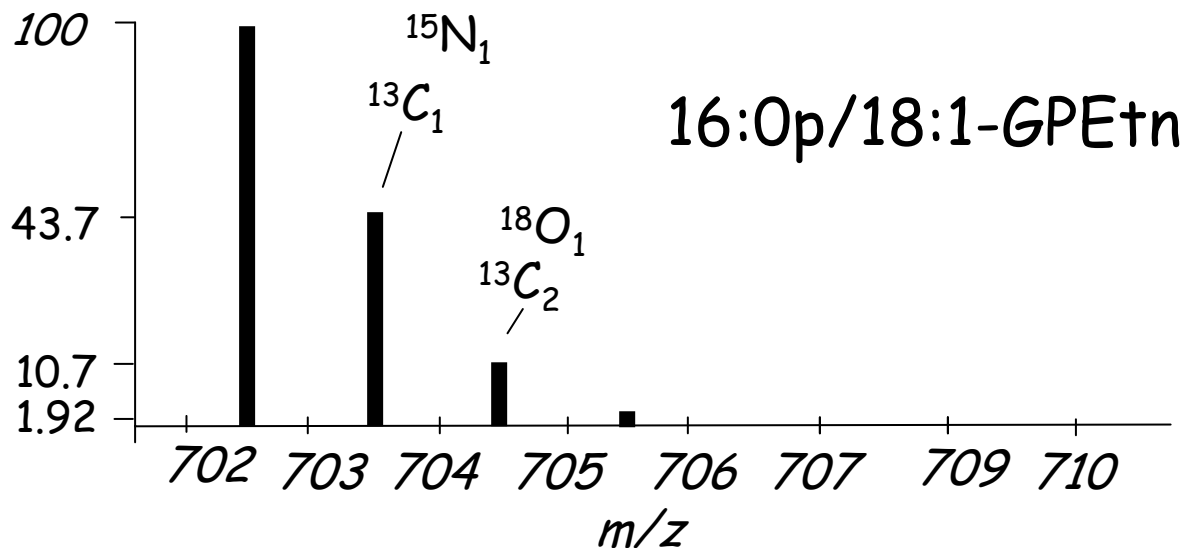
# Isotopic Abundance

Isotope	% Natural Abundance
$^1\text{H}$	99.98
$^2\text{H}$	0.015
$^{12}\text{C}$	98.9
$^{13}\text{C}$	1.1
$^{14}\text{N}$	99.64
$^{15}\text{N}$	0.36
$^{16}\text{O}$	99.8
$^{18}\text{O}$	0.2
$^{19}\text{F}$	100
$^{28}\text{Si}$	92.2
$^{29}\text{Si}$	4.7
$^{30}\text{Si}$	3.1

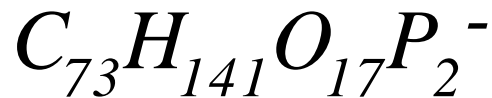
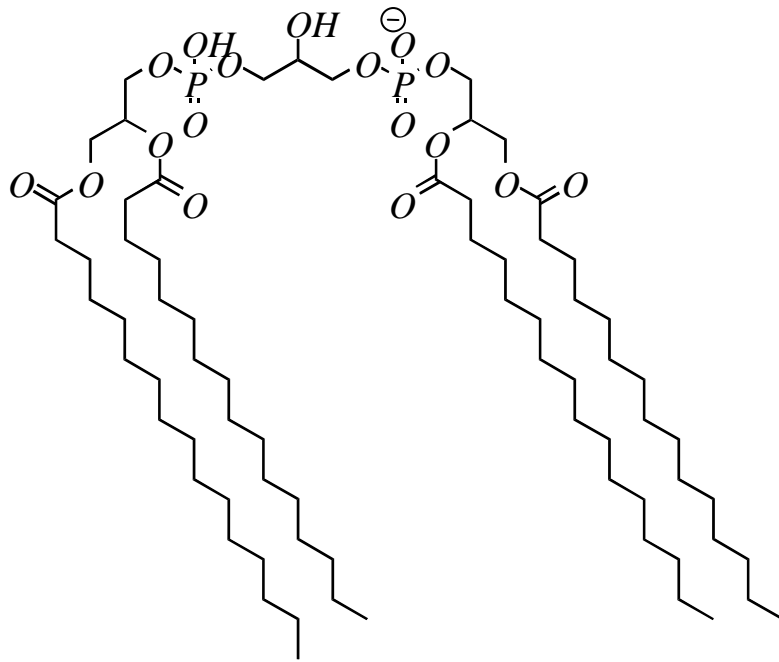


64.0%  
Total  
Ion current  
 $^{12}\text{C}_{38}$

$[\text{M}+\text{H}]^+$   
 $m/z$  702.5437  
 $\text{C}_{38}\text{H}_{77}\text{O}_7\text{NP}$

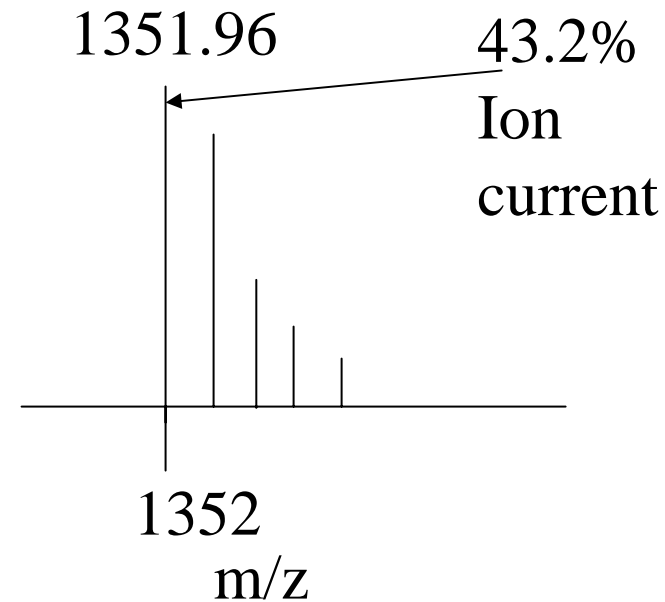


# Exact mass and Isotopes

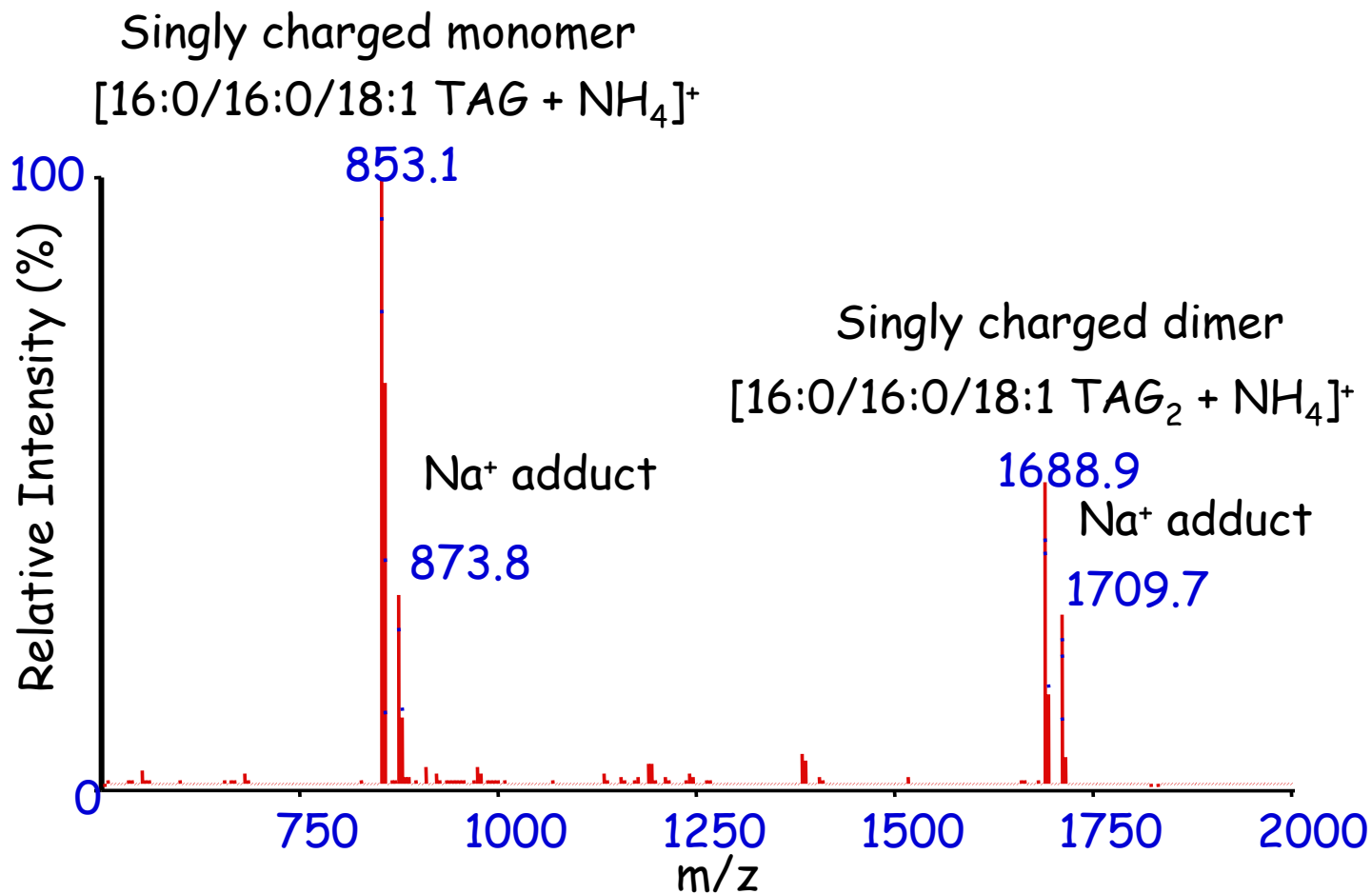


*Exact Mass: 1351.96*

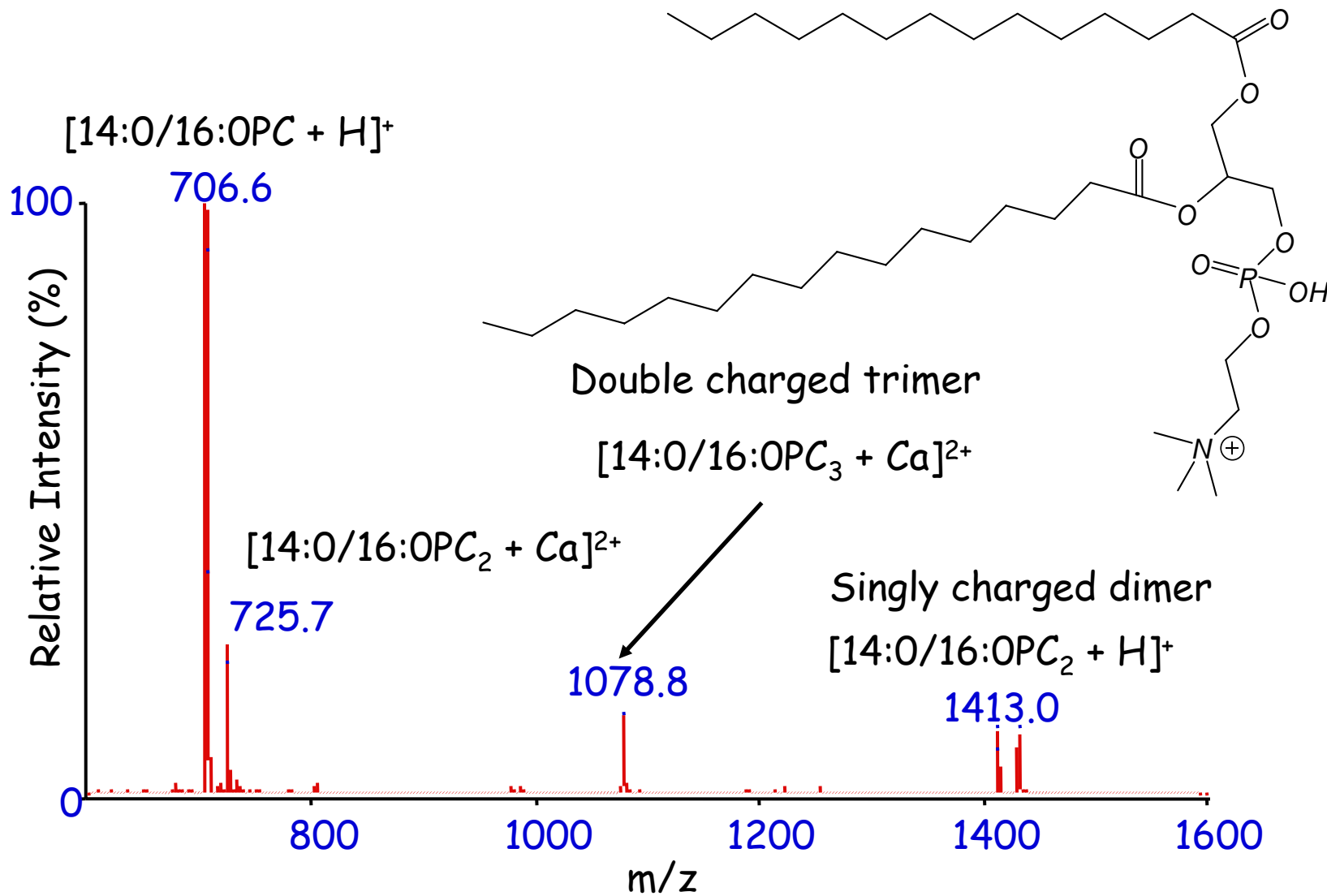
Cardiolipin  
Mitochondrial lipid



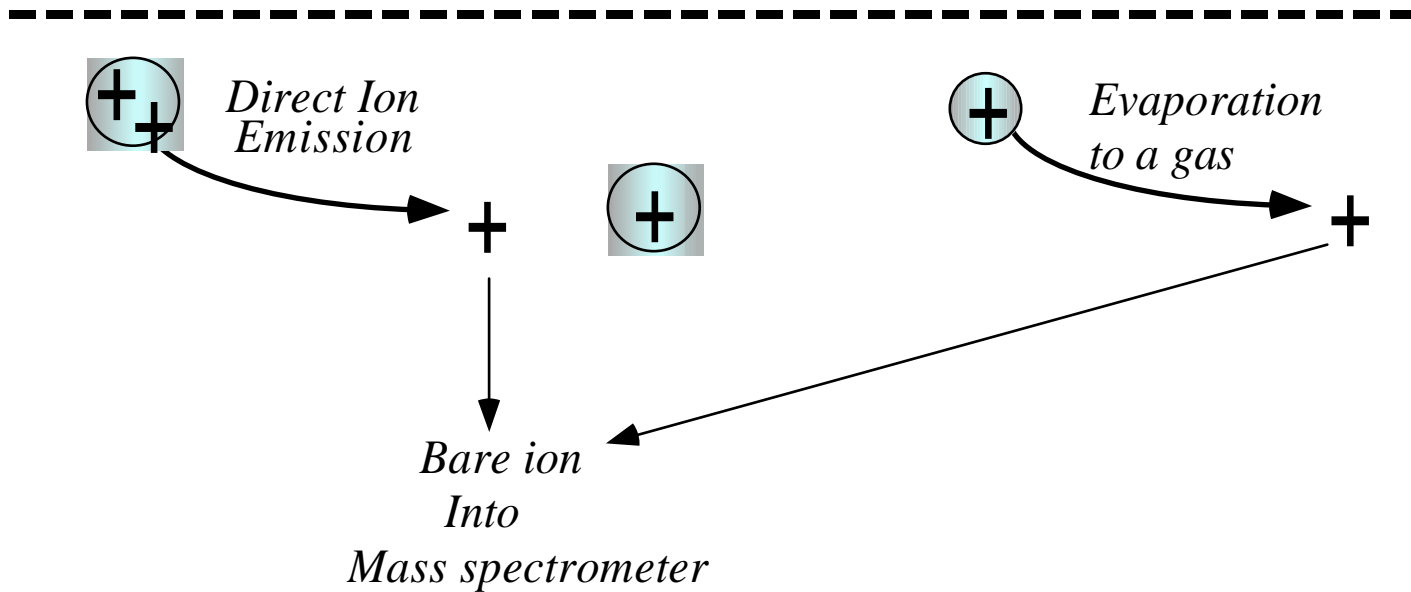
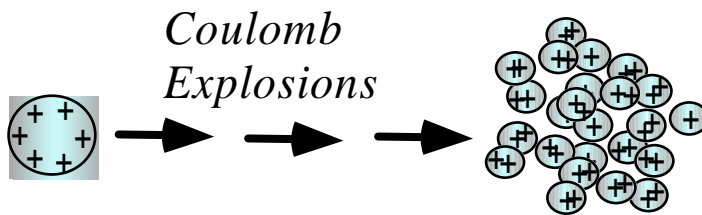
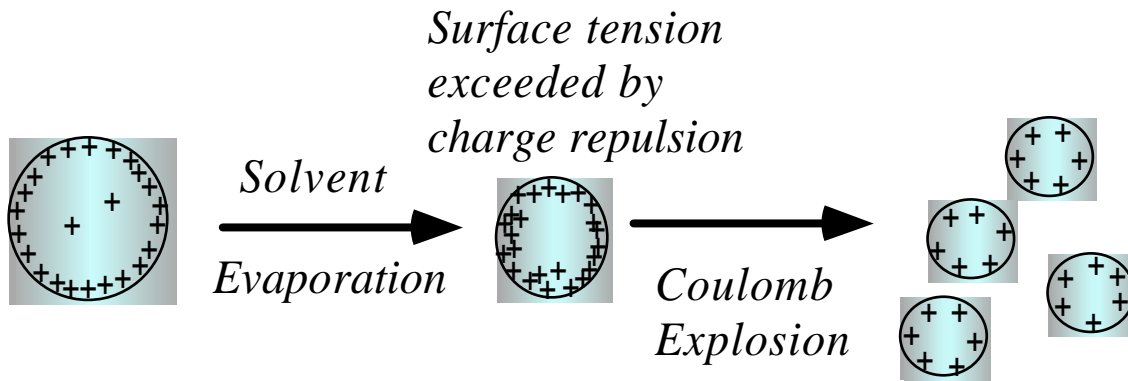
# 16:0/16:0/18:1-TAG ESI-MS



# 14:0a/16:0 GPCho ESI-MS







# References

- Triglyceride analysis
  - Qualitative MS/MS and MS<sup>3</sup>
    - McAnoy et al. *J Amer Soc Mass Spectrom.* 16:1498 (2005)
  - Quantitation by neutral loss scanning
    - Murphy et al. *Anal. Biochem.* In press 2007
- Phospholipid analysis
  - Qualitative analysis by ESI Mass Spectrometry
    - Pulfer and Murphy, *Mass Spectrom. Rev.* 22:332 (2003)
    - RCMurphy Mass Spectrometry of Phospholipids (2002).
  - Quantitation
  - Rouzer, R.A., Ivanova, P.T., Byrne, M.O., Milne, S.B., Marnett, L.J., and Brown, H.A., (2006) Lipid profiling reveals arachidonate deficiency in RAW264.7 cells: Structural and functional implications., *Biochemistry* 45: 14795-14808.
  - Milne, S.B., Ivanova, P.T., Forrester, J.S., and Brown, H.A., (2006) Lipidomics: Analysis of cellular lipids by ESI-MS. *Methods* 39: 92-103. Edited by V. Bankaitis. Elsevier Press.
  - Callender, H.L., Forrester, J.S., Ivanova, P., Preininger, A., Milne, S., and Brown, H.A., (2007) Quantification of diacylglycerol species from biological extracts by electrospray ionization mass spectrometry. *Analytical Chemistry* 79: 263-272.
  - 4. Ivanova, P.T., Milne, S.B., Byrne, M.O., Xiang, Y., and Brown, H.A. (2007) Glycerophospholipid Identification and Quantitation by Electrospray Mass Spectrometry. *Methods in Enzymology: Lipidomics and Bioactive Lipids*. Volume 1. Edited by H. Alex Brown, Elsevier Press. In press.
- Web sites for tools:
  - [www.lipidmaps.org](http://www.lipidmaps.org)
  - [www2.uchsc.edu/pharmacology/RCMweb1](http://www2.uchsc.edu/pharmacology/RCMweb1)—use Microsoft Internet Explorer

# Acknowledgements

- Analysis of neutral lipids
  - Jessica Krank
  - Miguel Gijon
  - Patrick Hutchins
- Analysis of Phospholipids
  - Alex Brown (Vanderbilt University)
- Lipid Profiler
  - Eva Duchoslav (Applied Biosystems, Canada)
- Support: National Institutes of Health  
LipidMaps GM069338