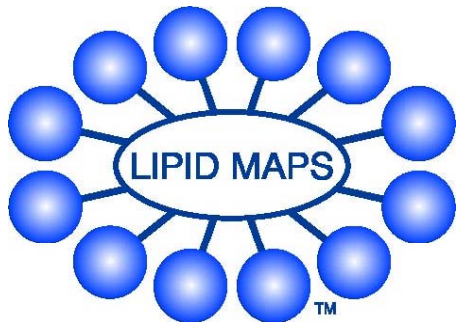




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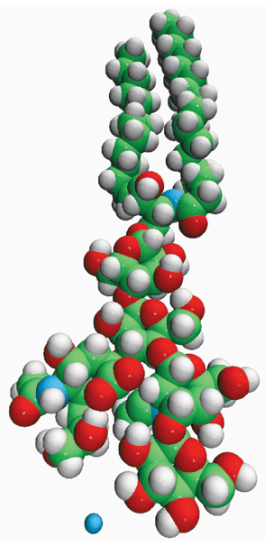
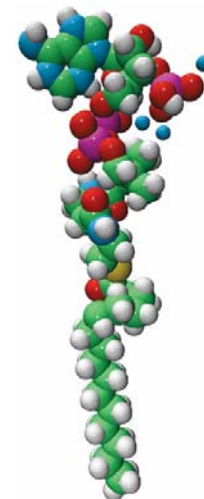
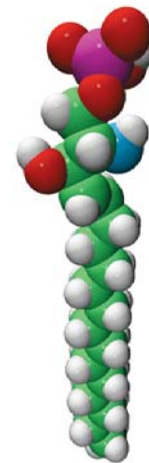
Novel Lipid Analysis

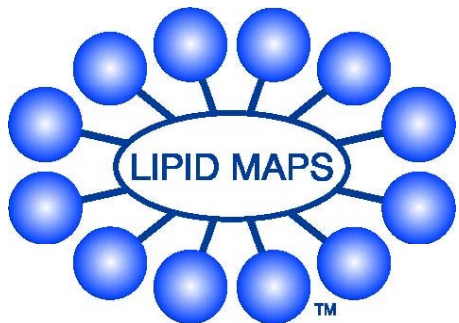
Teresa A. Garrett

Department of Biochemistry
Duke University School of Medicine
Durham, NC

Other LIPID MAPS Core K members:

Chris Raetz
Ziaqiang Guan
Reza Kordestani
Andrea Ryan





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Novel Lipid Analysis

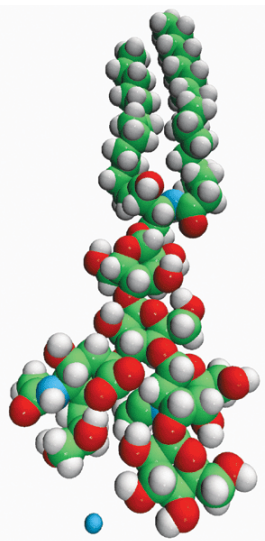
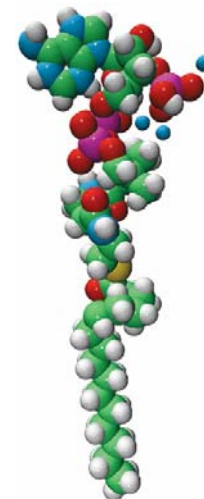
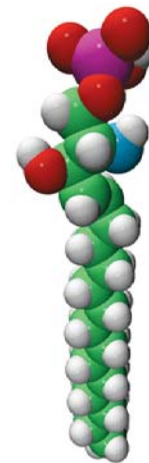
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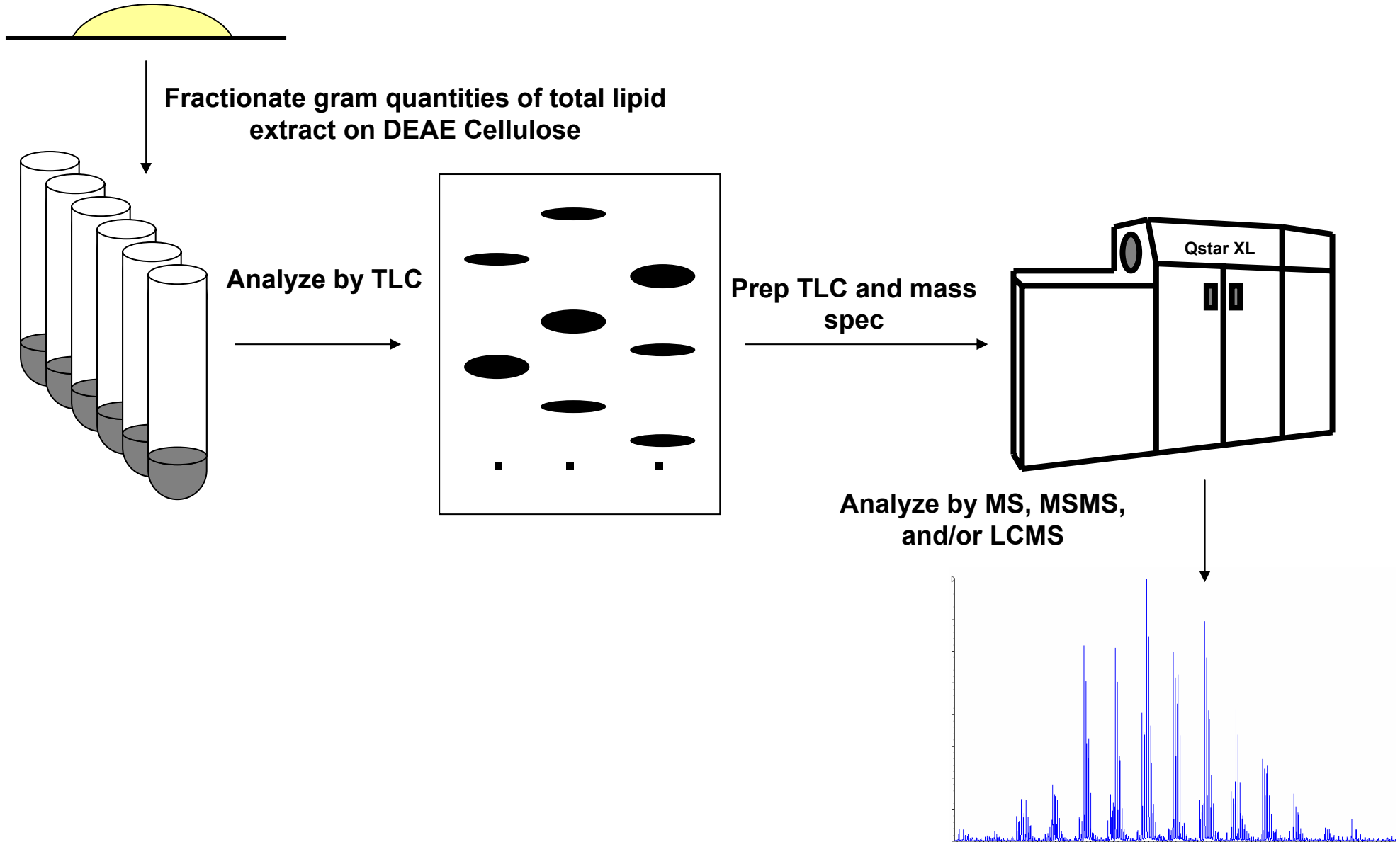
Other Core K Objectives

Quantification of cardiolipin, dolichols, and coenzyme Q

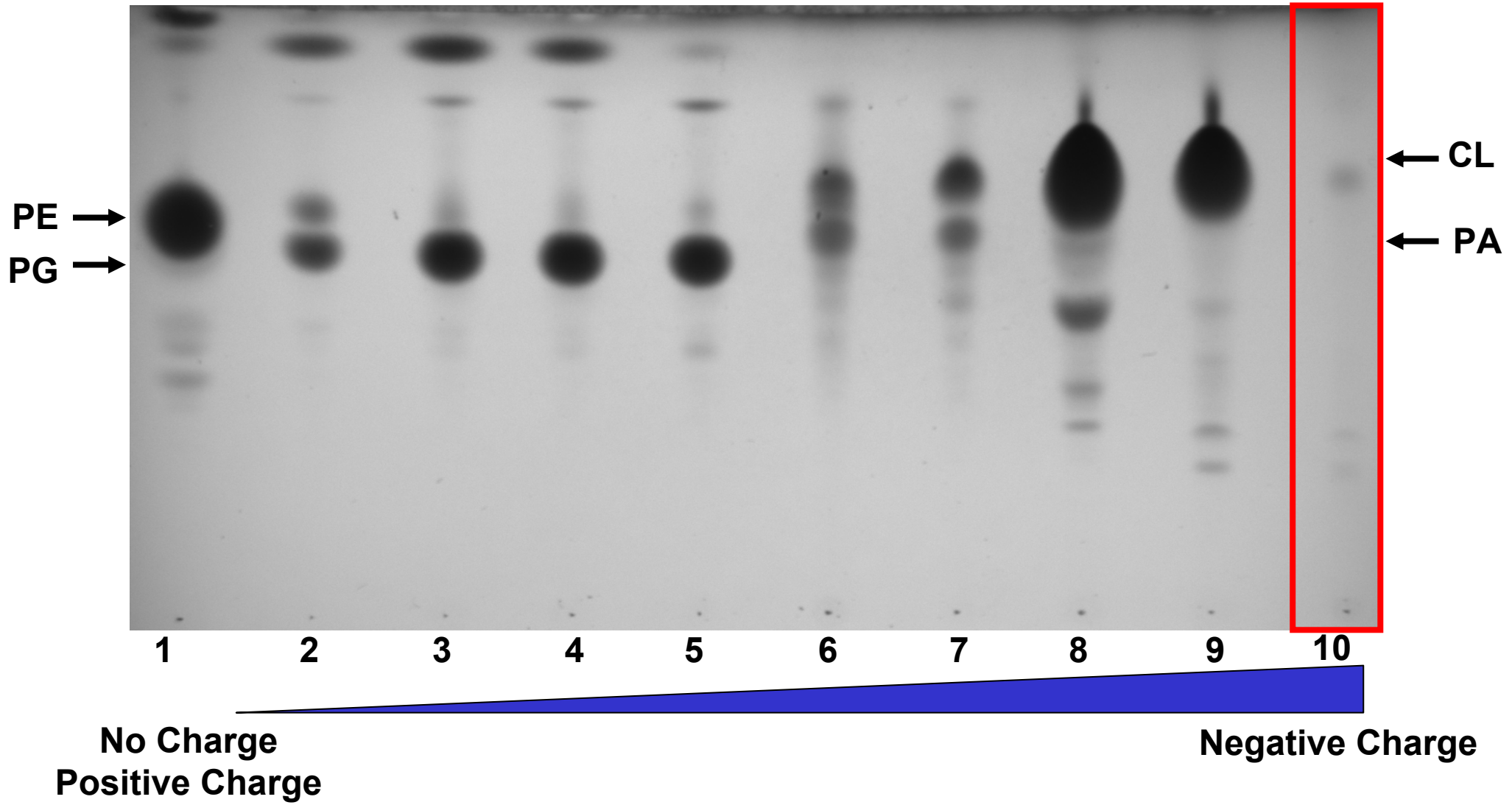
Methods in Enzymology. 2007. Volume 424.
"Lipidomics and Bioactive Lipids".
Edited by H. Alex Brown.



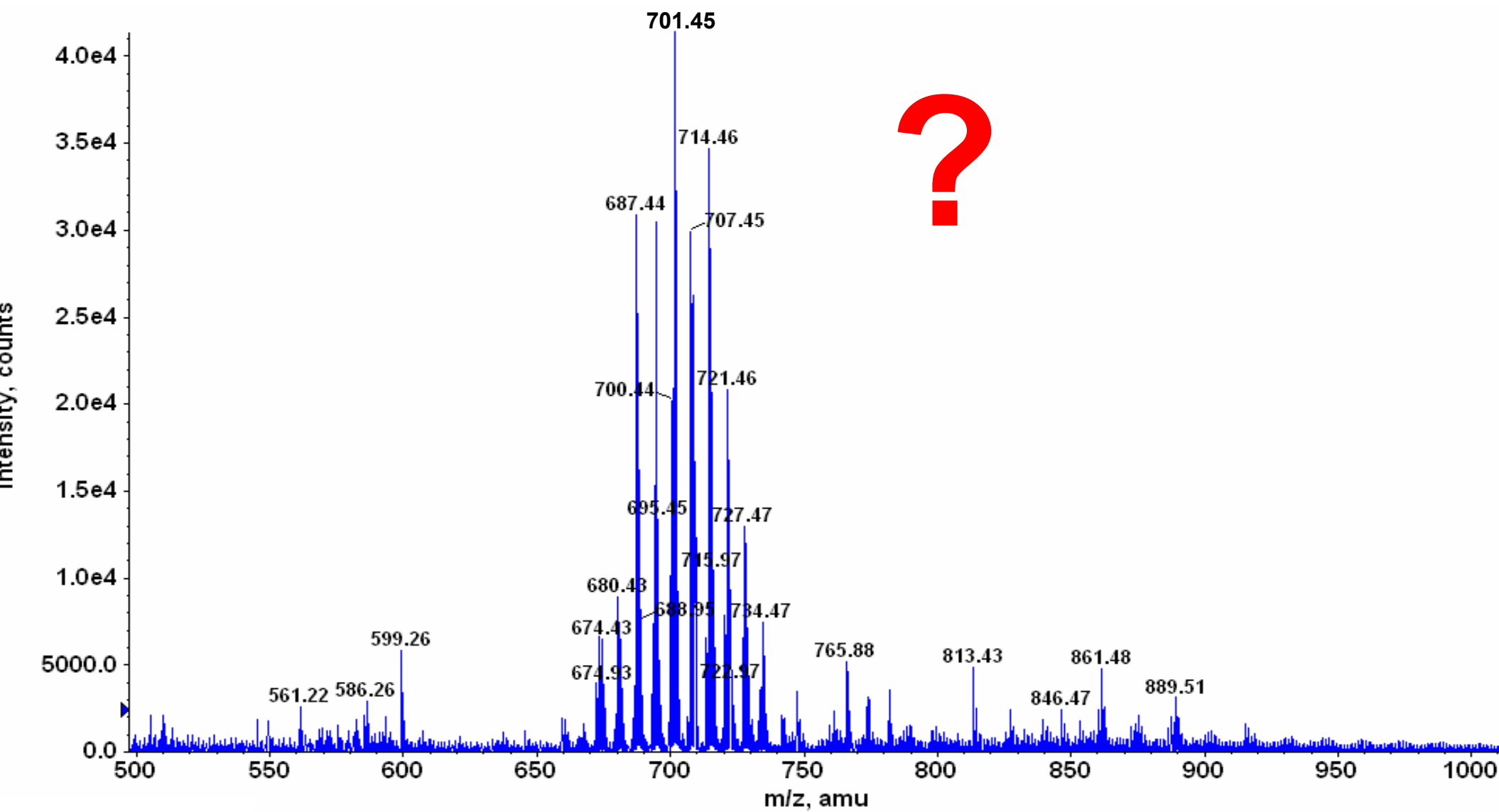
Identifying Novel Lipids



Anion Exchange Chromatography of *E. coli* Total Lipids



ESI-MS of Fraction #10



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Show Possible Structures

Find mass, number of carbons, number of double bonds, abbreviation, MS/MS product ions (neutral loss), formula, and ion based on input criteria, with links to structure and isotopic distribution.

[Cardiolipins](#) (Search by mass, mass tolerance (+/- m/z), radyl group abbreviation, and/or ion.)

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Mass Spectrometry

Show Possible Glycerophospholipid Structures

Enter a mass (m/z) value and corresponding ion type:

☐ Nominal Mass ☒ Exact Mass

Mass Tolerance (+/- amu)

Radyl group abbreviation (eg. 12:0)

Headgroup

Ion

Sort by

Records per page

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Mass Spectrometry

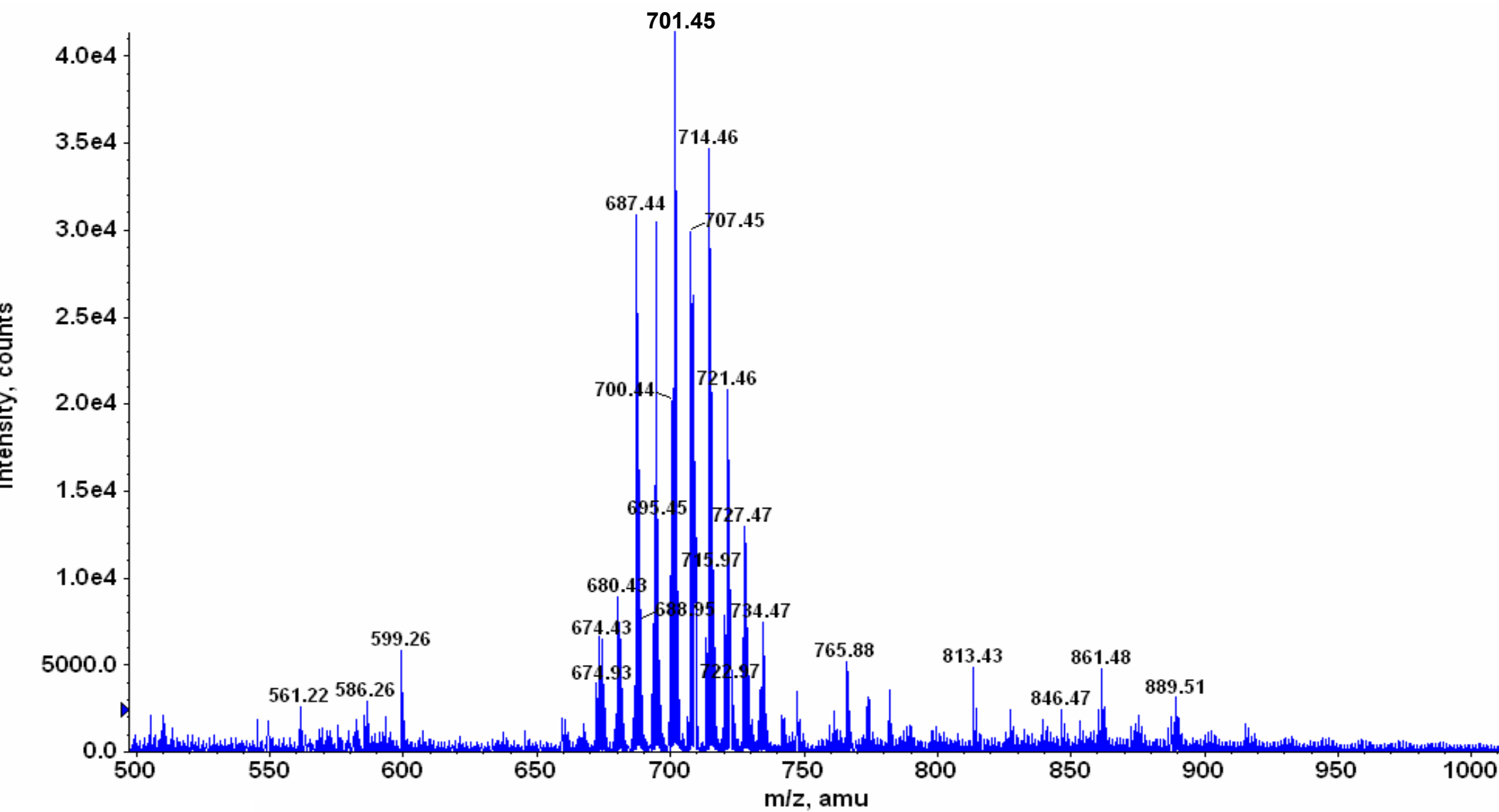
Possible Glycerophospholipid Structures

Exact Mass: Mass Tolerance: [New Search](#)

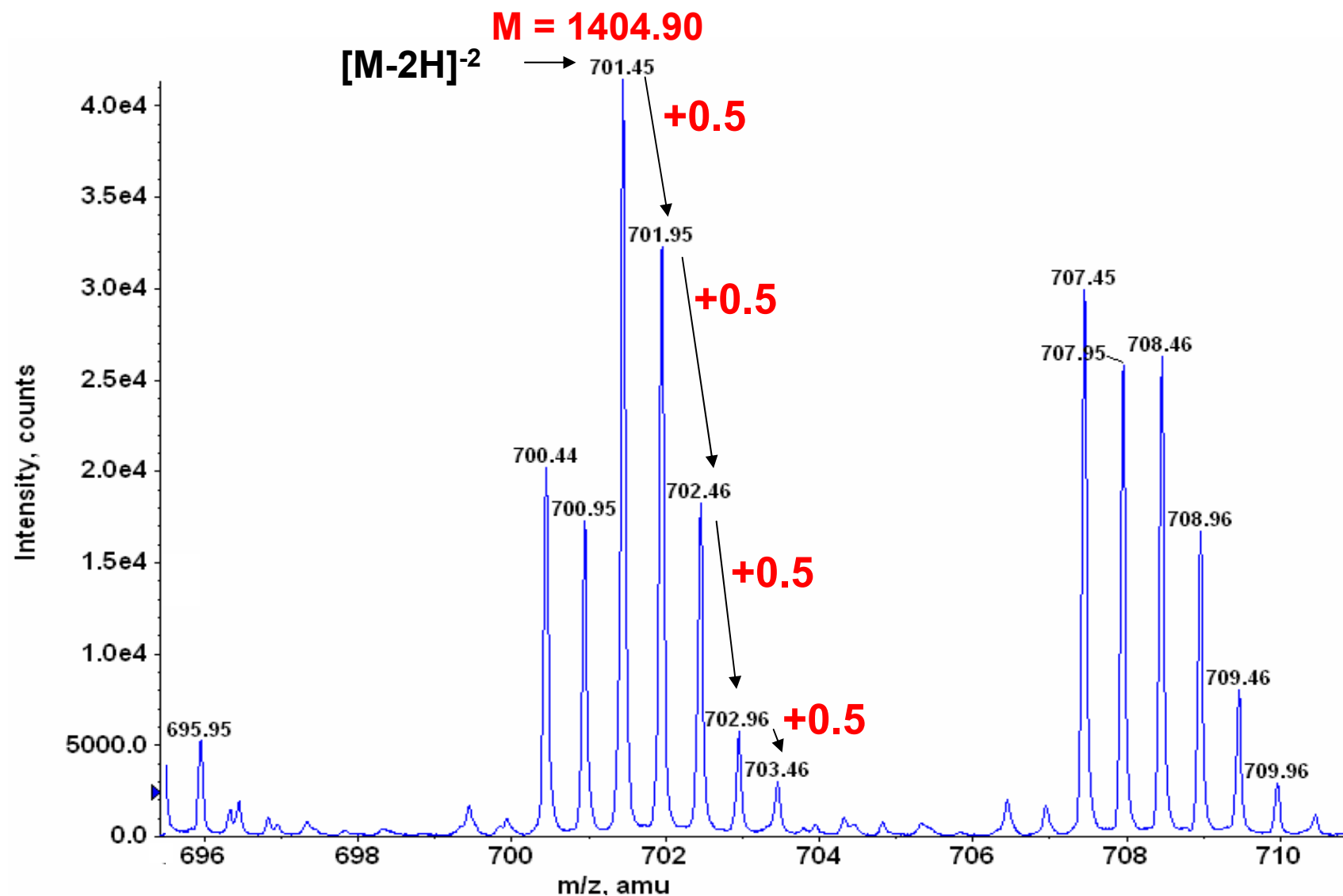
C=Number of Carbons; DB=Number of double bonds; sn1('1),sn2,...=MS/MS Product Ions (neutral loss)

Mass	C	DB	Abbrev.	M-sn1-H	M-sn1-H2O-H	M-sn2-H	M-sn2-H2O-H	sn1 acid(-)	sn2 acid(-)	HG	Formula
701.2704	20	3	20:3(82,112,142)/0:0	413.0251	395.0145	687.2911	669.2805	305.2481	30.982	GPIInSP	C29H52O15P2
702.4346	30	2	10:0/20:2(112,142)	548.2988	530.2882	412.1736	394.163	171.1385	307.2637	GPser	C36H66NO10P
702.4346	30	2	12:0/18:2(92,122)	520.2675	502.2569	440.2049	422.1943	199.1698	279.2324	GPser	C36H66NO10P
702.4346	30	2	13:0/17:2(92,122)	506.2519	488.2413	454.2206	436.21	213.1855	265.2168	GPser	C36H66NO10P
702.4346	30	2	17:2(92,122)/13:0	454.2206	436.21	506.2519	488.2413	265.2168	213.1855	GPser	C36H66NO10P
702.4346	30	2	14:1(92)/16:1(92)	494.2519	476.2413	466.2206	448.21	225.1855	253.2168	GPser	C36H66NO10P
702.4346	30	2	16:1(92)/14:1(92)	466.2206	448.21	494.2519	476.2413	253.2168	225.1855	GPser	C36H66NO10P
702.4346	30	2	18:2(92,122)/12:0	440.2049	422.1943	520.2675	502.2569	279.2324	199.1698	GPser	C36H66NO10P
702.4346	30	2	20:2(112,142)/10:0	412.1736	394.163	548.2988	530.2882	307.2637	171.1385	GPser	C36H66NO10P
701.4394	31	3	13:0/18:3(92,122,152)	505.2567	487.2461	441.2254	423.2148	213.1855	277.2168	GPgro	C37H67O10P
701.4394	31	3	18:3(92,122,152)/13:0	441.2254	423.2148	505.2567	487.2461	277.2168	213.1855	GPgro	C37H67O10P
701.5122	36	1	14:0/22:1(132)	491.3138	473.3032	381.2043	363.1937	227.2011	337.3107	GPA	C39H75O8P
701.5122	36	1	19:0/17:1(92)	421.2356	403.225	451.2825	433.2719	297.2794	267.2324	GPA	C39H75O8P
701.5122	36	1	17:1(92)/19:0	451.2825	433.2719	421.2356	403.225	267.2324	297.2794	GPA	C39H75O8P
701.5122	36	1	14:1(92)/22:0	493.3295	475.3189	379.1886	361.178	225.1855	339.3263	GPA	C39H75O8P

ESI-MS of Fraction #10



Major Ions of Fraction #10



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Enter a mass (m/z) value and corresponding ion type:

☐ Nominal Mass ☒ Exact Mass

Mass Tolerance (+/- m/z)

Radyl group abbreviation (eg. 12:0)

Ion

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Mass Spectrometry

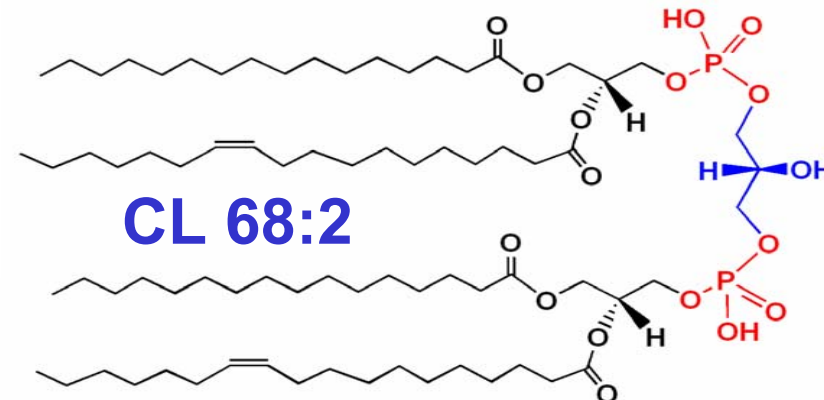
Possible Cardiolipin Structures

Exact Mass: Mass Tolerance: [New Search](#)

C=Number of Carbons; DB=Number of double bonds; sn1('1'),sn2...=MS/MS Product Ions (neutral loss)

Mass	C	DB	Abbreviation	sn1('1')	sn2('1')	sn1('2')	sn2('2')	Formula	Ion
701.4939	68	2	10:0/18:0/18:2(9Z,12Z)/22:0	615.4208	559.3582	561.3738	531.3269	C77H146O17P2	M-2H
701.4939	68	2	10:0/18:0/22:0/18:2(9Z,12Z)	615.4208	559.3582	531.3269	561.3738	C77H146O17P2	M-2H
701.4939	68	2	10:0/18:1(9Z)/18:1(9Z)/22:0	615.4208	560.366	560.366	531.3269	C77H146O17P2	M-2H
701.4939	68	2	10:0/18:1(9Z)/22:0/18:1(9Z)	615.4208	560.366	531.3269	560.366	C77H146O17P2	M-2H
701.4939	68	2	10:0/18:2(9Z,12Z)/18:0/22:0	615.4208	561.3738	559.3582	531.3269	C77H146O17P2	M-2H
701.4939	68	2	10:0/18:2(9Z,12Z)/20:0/20:0	615.4208	561.3738	545.3425	545.3425	C77H146O17P2	M-2H
701.4939	68	2	10:0/18:2(9Z,12Z)/22:0/18:0	615.4208	561.3738	531.3269	559.3582	C77H146O17P2	M-2H
701.4939	68	2	10:0/20:0/18:2(9Z,12Z)/20:0	615.4208	545.3425	561.3738	545.3425	C77H146O17P2	M-2H
701.4939	68	2	10:0/20:0/20:0/18:2(9Z,12Z)	615.4208	545.3425	545.3425	561.3738	C77H146O17P2	M-2H
701.4939	68	2	10:0/22:0/18:0/18:2(9Z,12Z)	615.4208	531.3269	559.3582	561.3738	C77H146O17P2	M-2H

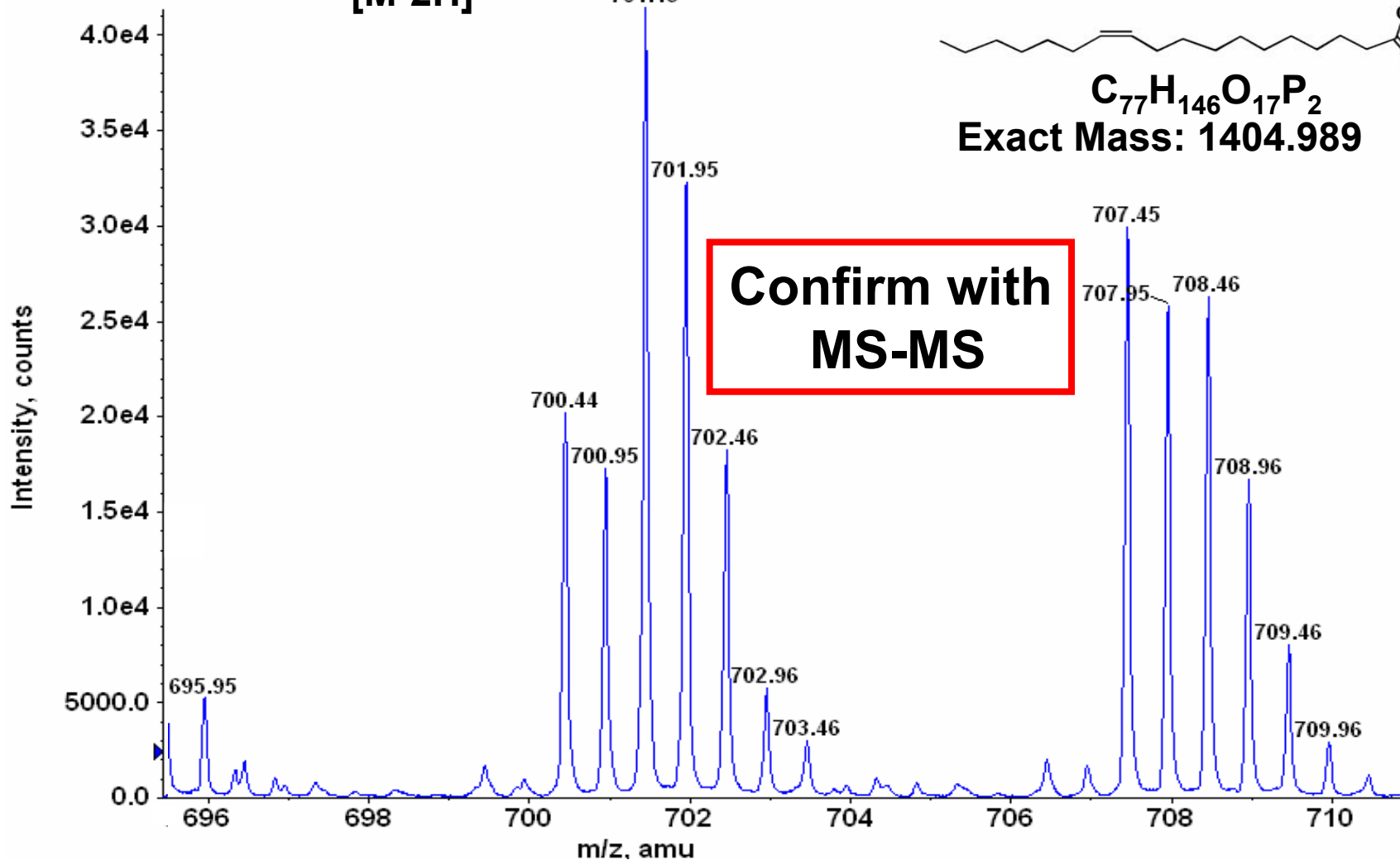
Cardiolipin Ions



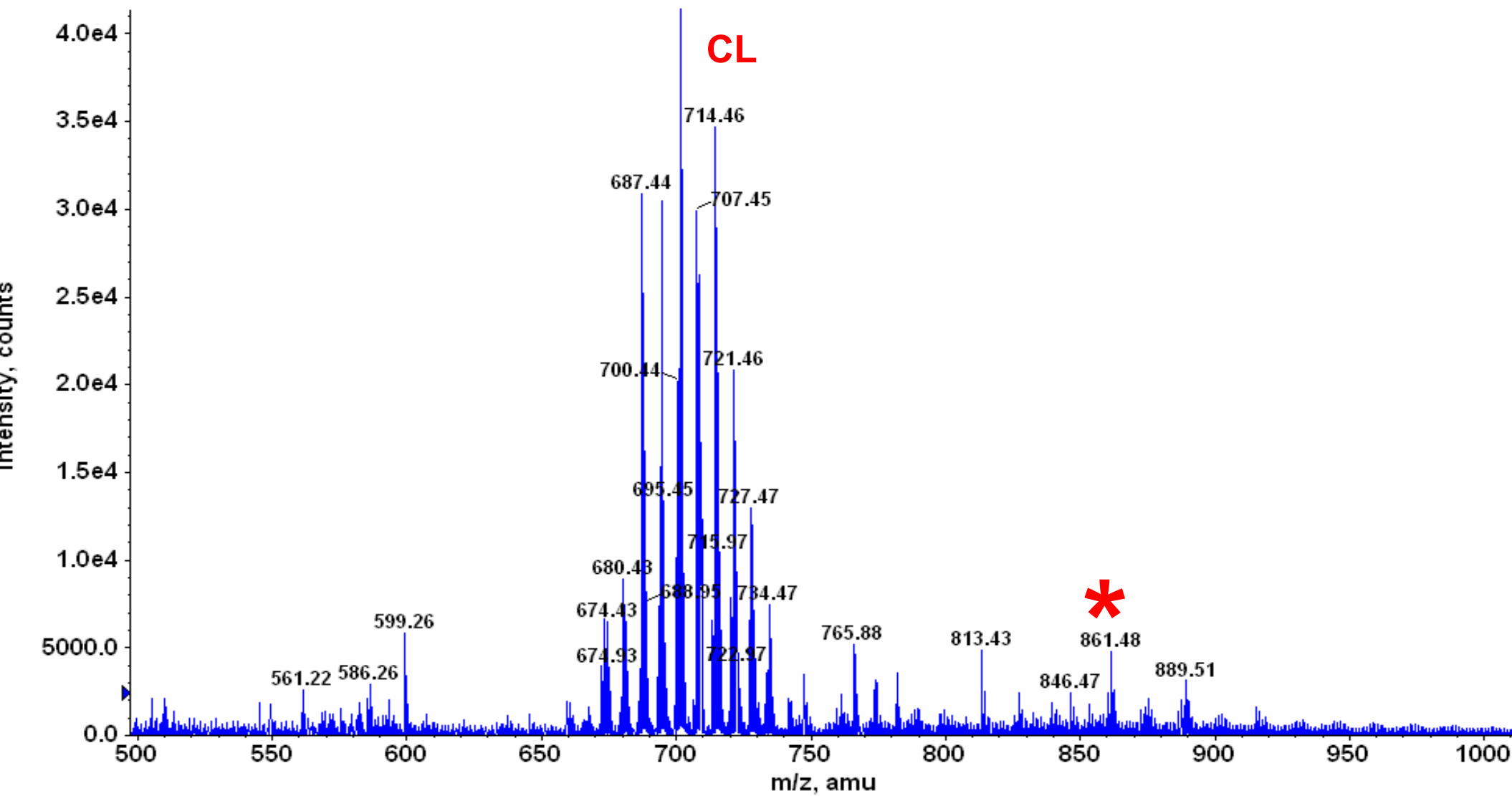
$C_{77}H_{146}O_{17}P_2$
Exact Mass: 1404.989

$[M-2H]^{-2} \rightarrow 701.45$ **M = 1404.90**

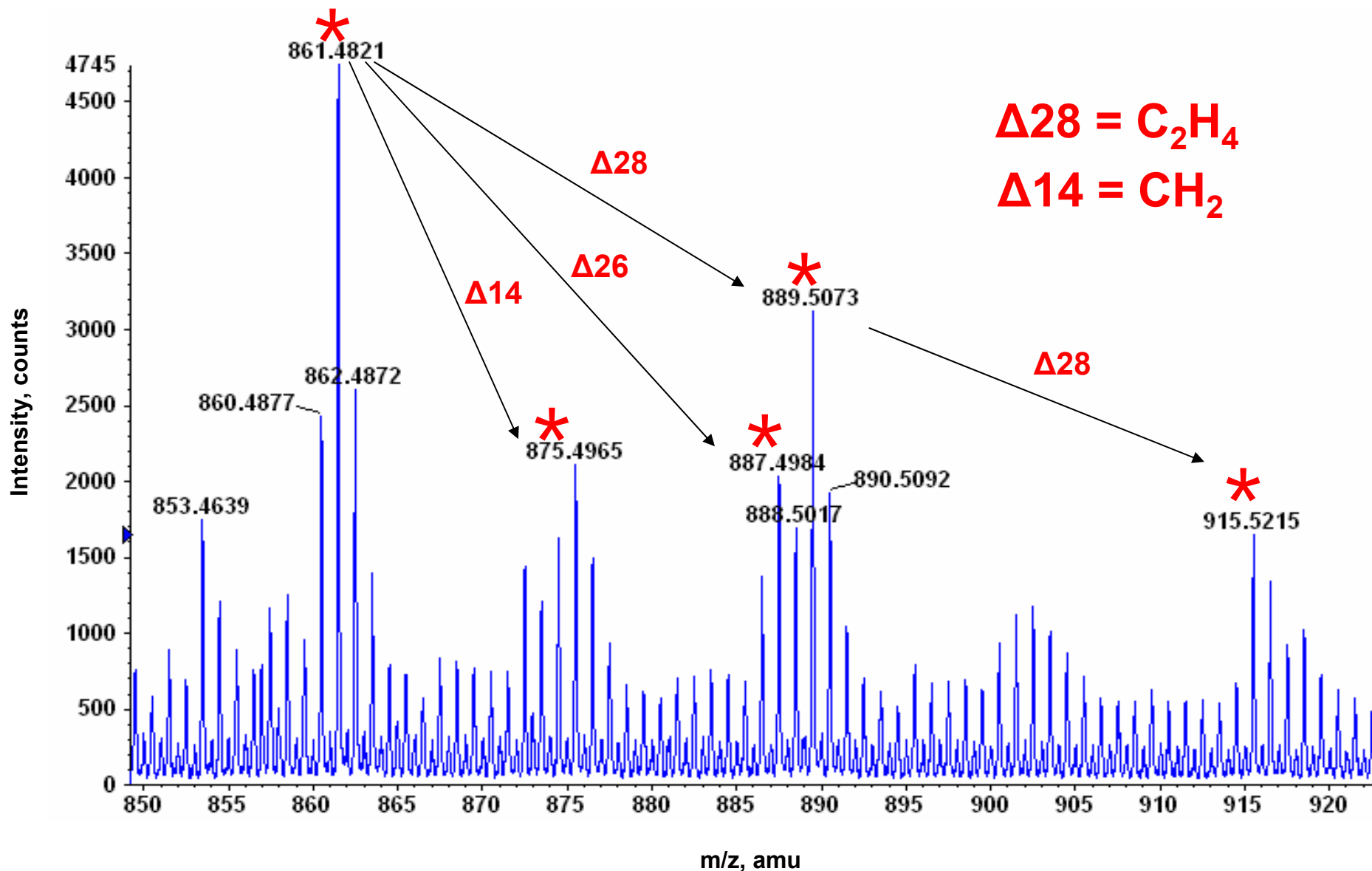
Confirm with
MS-MS



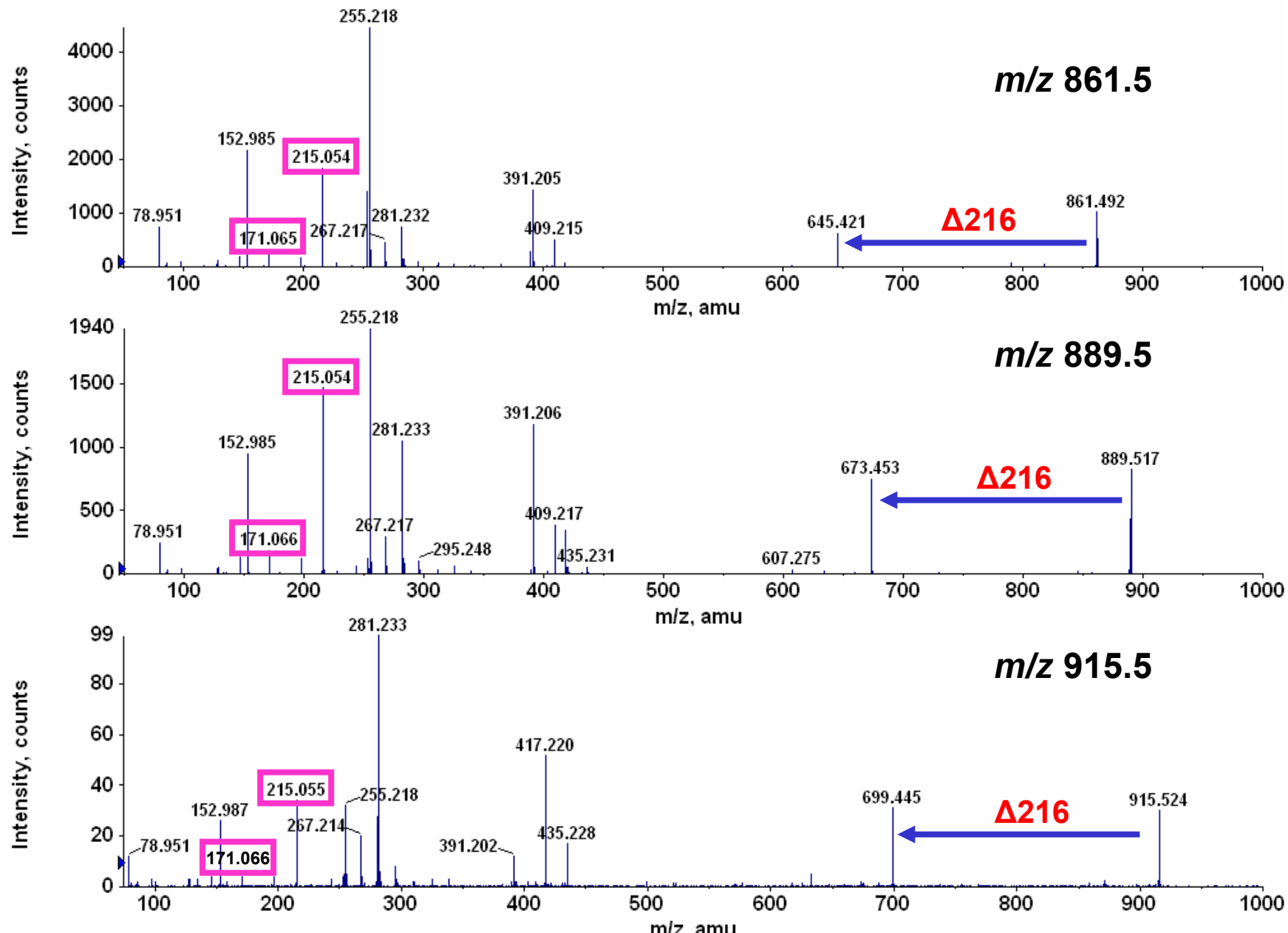
ESI-MS of Fraction #10



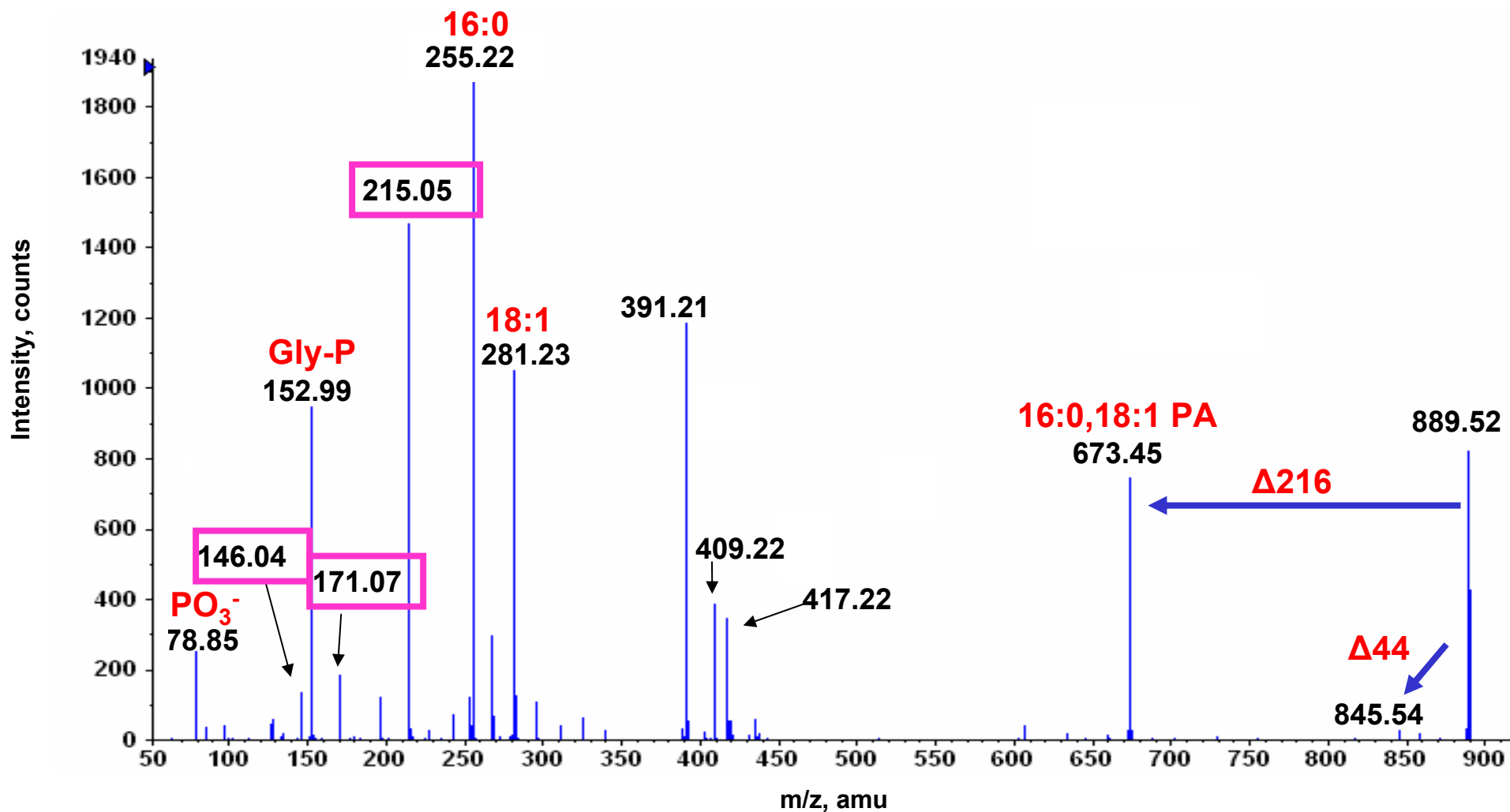
ESI-MS of Unknown Ions



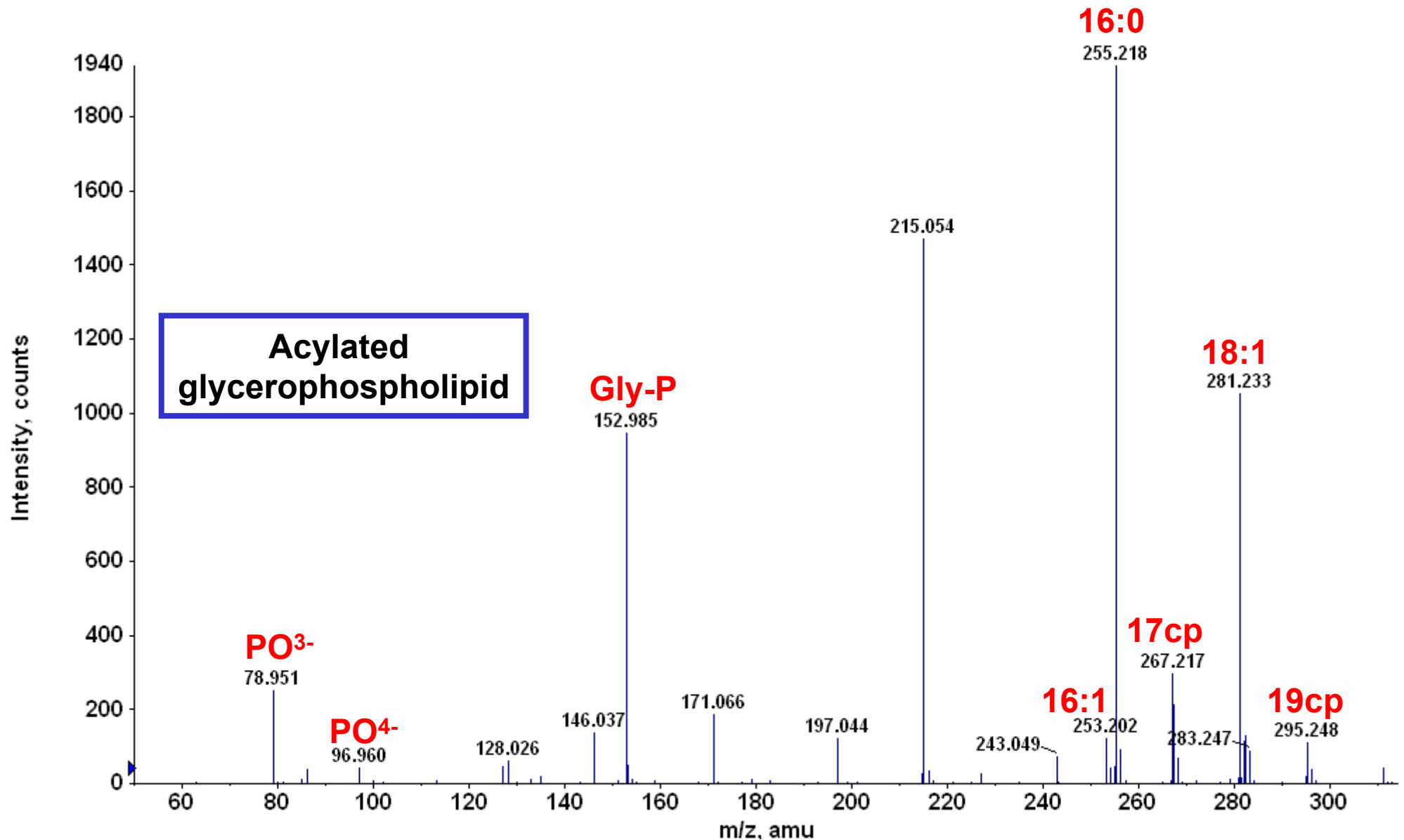
ESI-MSMS of Unknown Ions



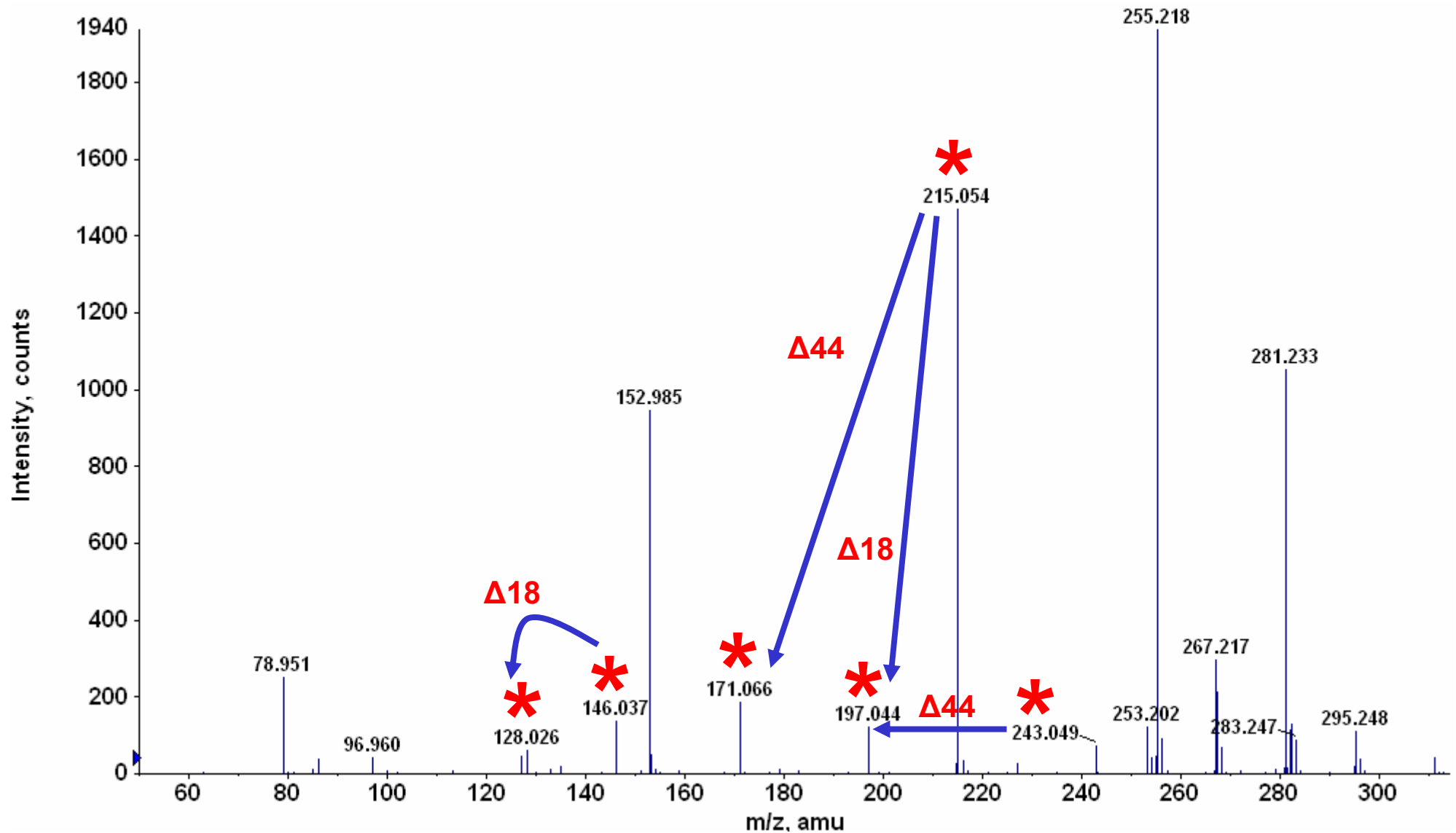
MSMS of Unknown Ion 889.52



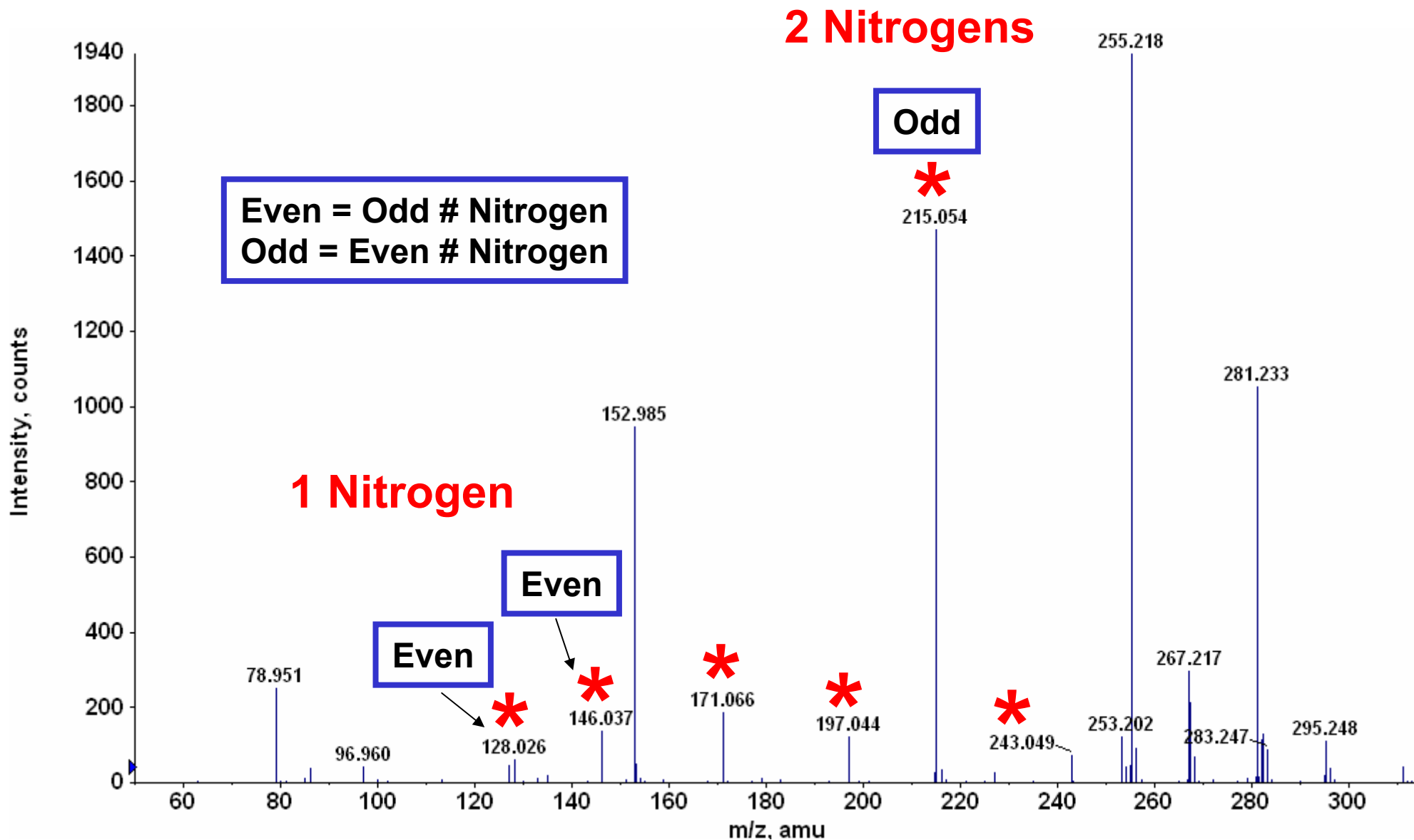
MSMS of Unknown Ion 889.52



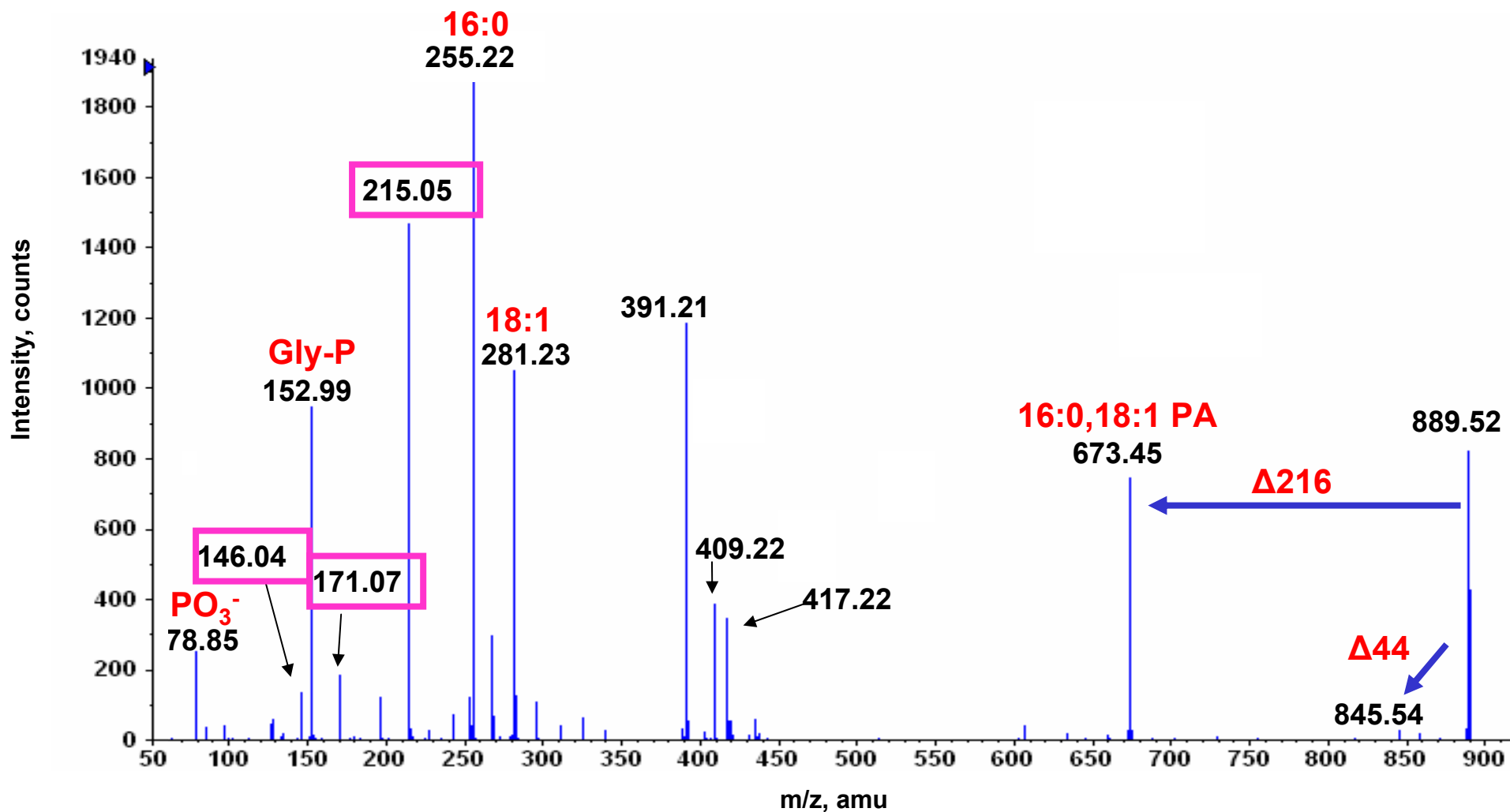
MSMS of Unknown Ion 889.52



MSMS of Unknown Ion 889.52



MSMS of Unknown Ion 889.52



Elemental composition Calculator

Calculators

Elemental Composition | Hypermass | Elemental Targeting | Mass Property | Isotopic Distribution

Input parameters

Target m/z: 215.054 amu

Tolerance: 300 mDa

Result type: Elemental

Max num of results: 100

Min DBE: -0.5 Max DBE: 50

Electron state: OddAndEven

Num of charges: -1

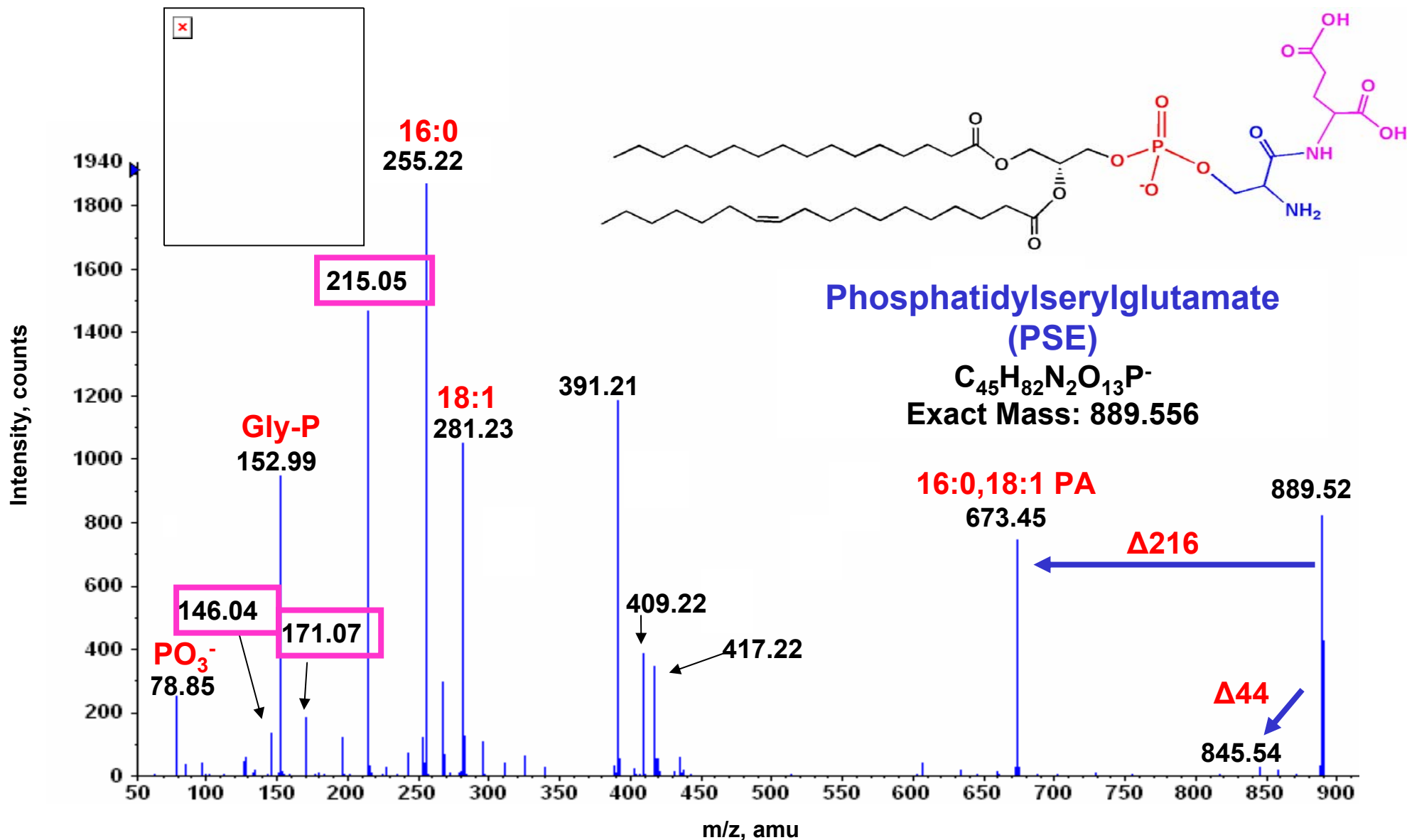
☐ Add water ☐ Add proton

Set elements and limits

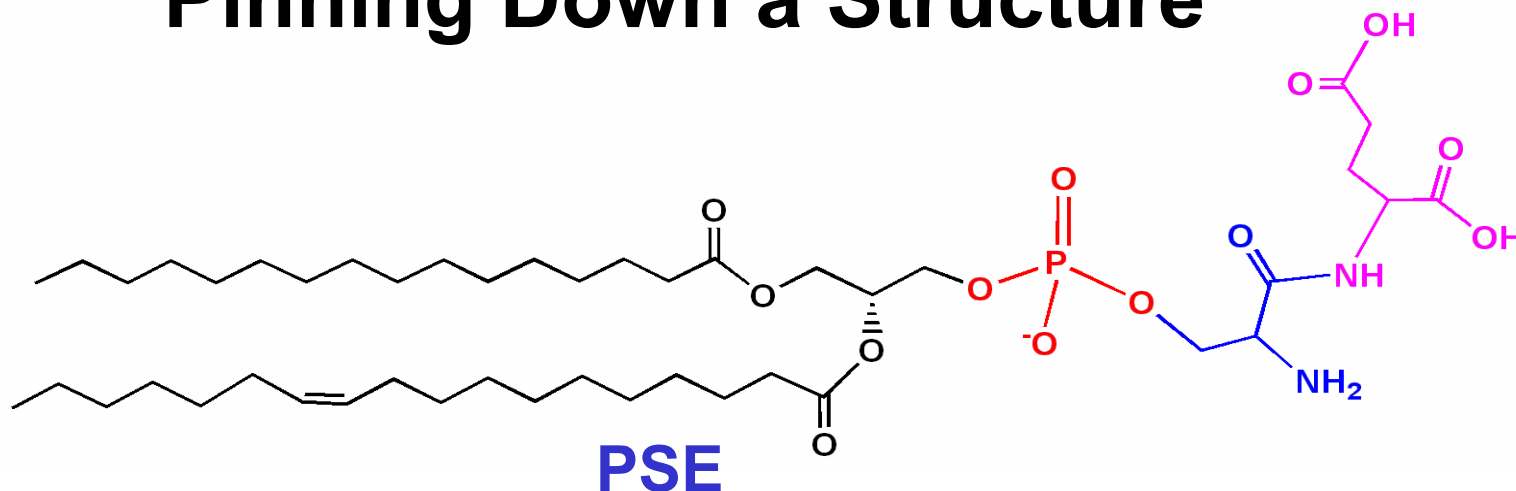
Calculate Show isotopic Export to file Help

	Formula ...	Calculated	mDa Error	ppm Error	D
1	C4 H11 N2 O8	215.052	1.9108	8.8854	0.5
2	C8 H12 N2 O3 P	215.0591	-5.1043	-23.7354	4.5
3	C11 H7 N2 O3	215.0462	7.7842	36.1966	9.5
4	C4 H12 N2 O6 P	215.0438	10.1516	47.2053	0.5
5	C8 H11 N2 O5	215.0673	-13.3452	-62.0554	4.5
6	C11 H8 N2 O P	215.0379	16.025	74.5165	9.5
7	C7 H7 N2 O6	215.0309	23.0402	107.1374	5.5
8	C5 H16 N2 O5 P	215.0802	-26.2338	-121.9875	-0.5
9	C12 H11 N2 O2	215.0826	-28.6013	-132.9963	8.5
10	C7 H8 N2 O4 P	215.0227	31.2811	145.4574	5.5
11	C5 H15 N2 O7	215.0884	-34.4746	-160.3074	-0.5
12	C3 H7 N2 O9	215.0157	38.2963	178.0783	1.5
13	C9 H16 N2 O2 P	215.0954	-41.4899	-192.9283	3.5
14	C3 H8 N2 O7 P	215.0074	46.5372	216.3983	1.5
15	C9 H15 N2 O4	215.1037	-49.7307	-231.2483	3.5
16	C13 H15 N2 O	215.1189	-64.9868	-302.1892	7.5
17	C10 H20 N2 O P	215.1318	-77.8754	-362.1213	2.5

MSMS of Unknown Ion 889.52



Pinning Down a Structure



Isotopic labeling

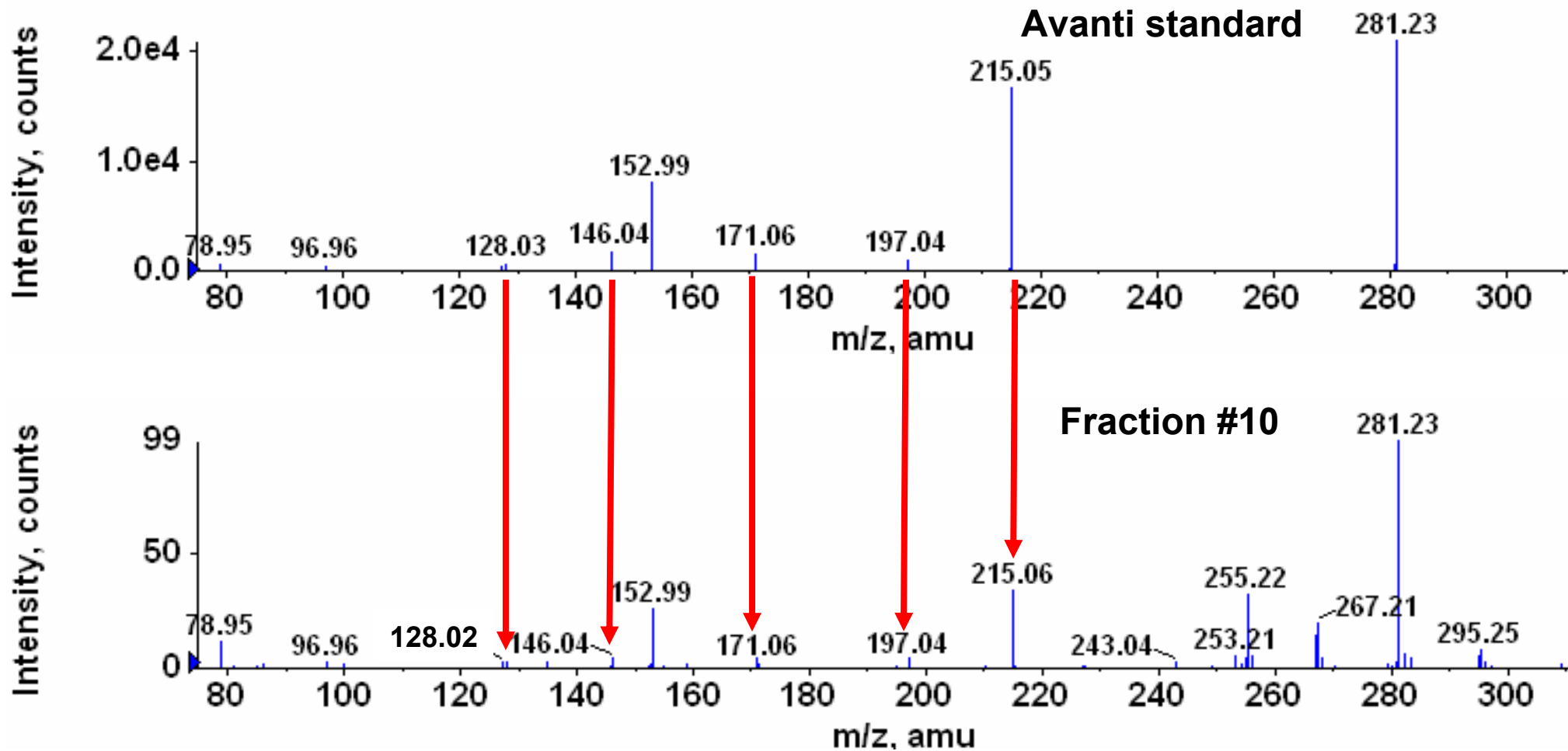
Purify and characterize by NMR and GC-MS

Compare MS, MSMS and LC-MS with synthetic standard

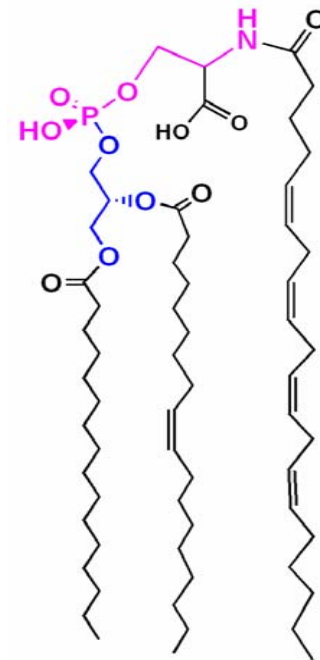
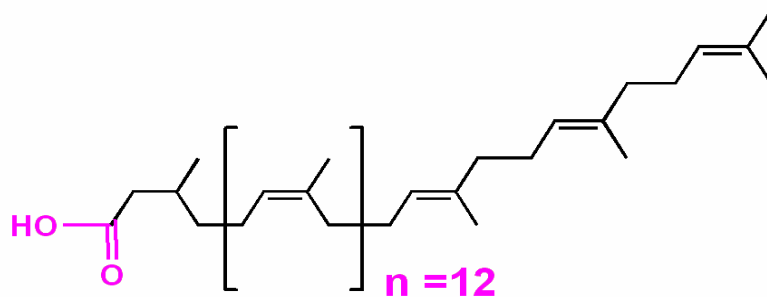
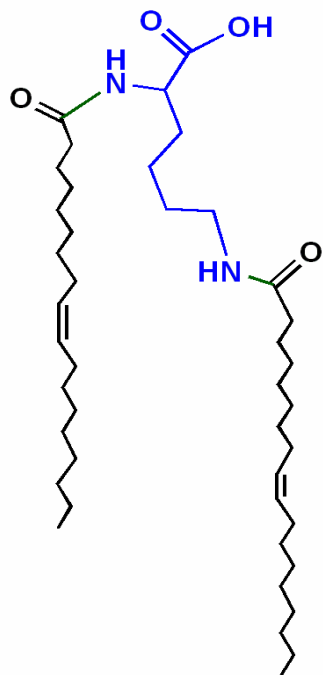
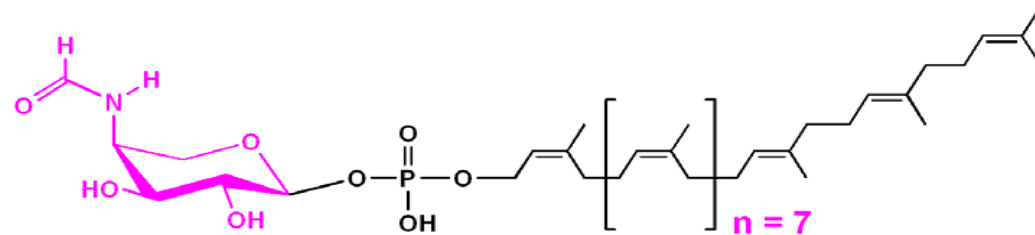
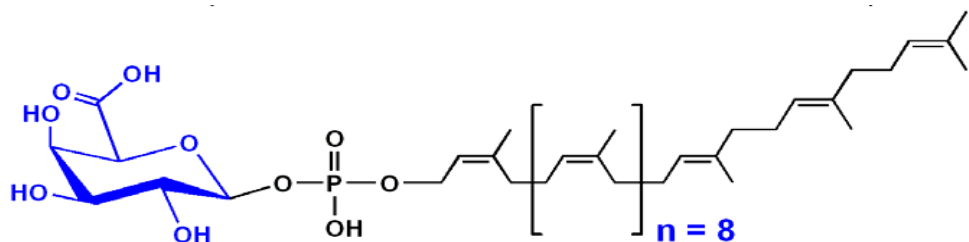
Develop an *in vitro* biosynthetic assay

MSMS of PSE

Standard v. Natural Product



New Structures Identified Using ESI-MS



Metabolite Identification



New pathways



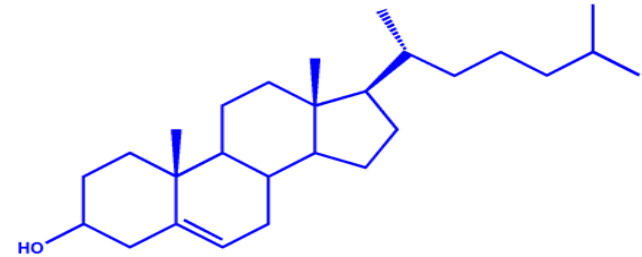
Enzyme identification



Drug Targets



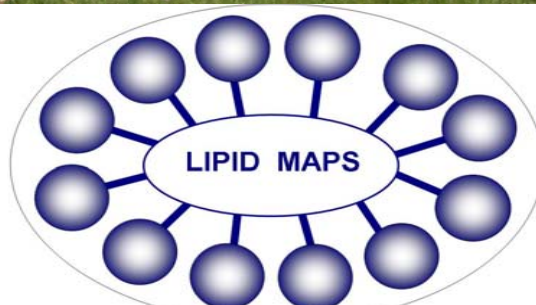
Genome annotation



>40 enzymes



Bob Murphy – UCHSC
Walt Shaw – Avanti Polar Lipids



NIH
1U54GM069338
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