Application of Lipidomics to identify new phospholipid disorders

Frédéric M. Vaz

Laboratory Genetic Metabolic
Academic Medical Center, Am
The Netherlands

ık van Lenthe
Femke Stet
ırtin Vervaart
Wim Kulik
nald Wanders

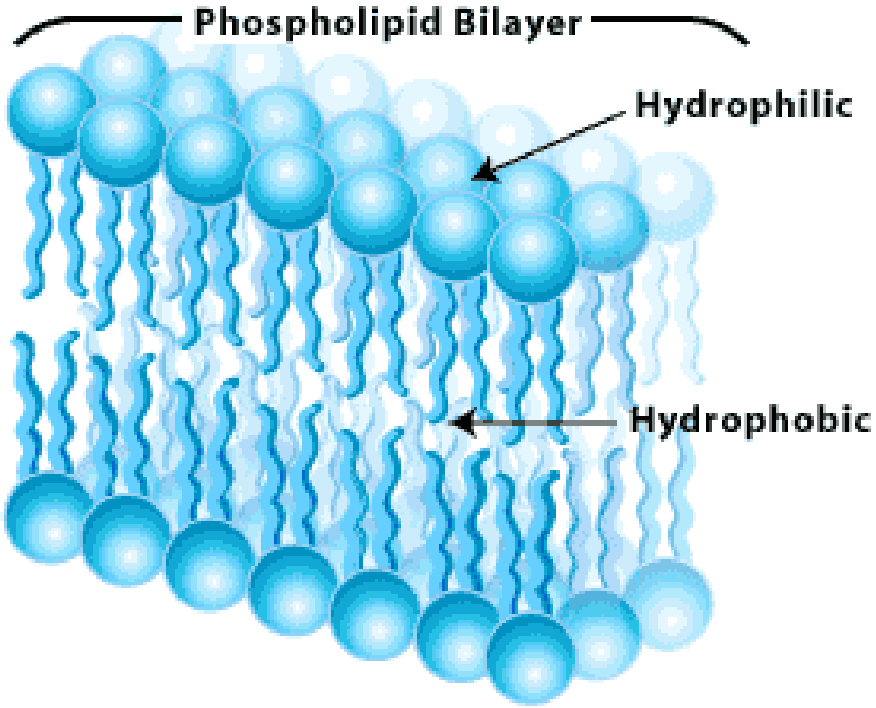
Bioinformatica
Pras - Raves
ristin Christin
he van Kampen

Phospholipids, lipidomics and applications

- Phospholipid structure and measurement with emphasis on cardiolipin and Barth syndrome (3-MGAuria type II)
- Recent developments: Lipidomics pipeline
- Another 3-MGAuria: MEGDEL syndrome, the functional defect elucidated using a Lipidomics approach

Phospholipids

- Phospholipids are important membrane components but also are involved in signal transduction/signaling



Two

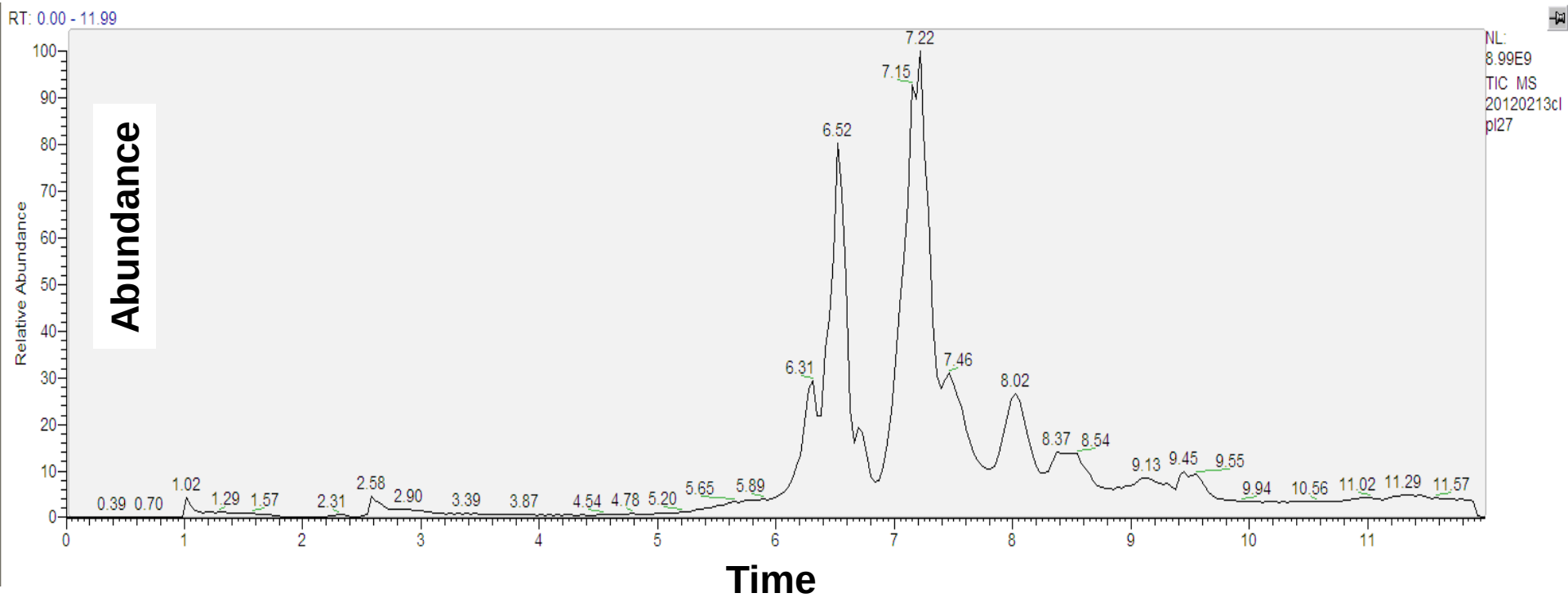
Side-chains

- Head groups:
  - Choline
  - Ethanolamine
  - Serine
  - Inositol
  - Glycerol
  - …

- Fatty acids:
  - Palmitic acid (16:0)
  - Stearic acid (18:0)
  - Palmitoleic acid (16:1)
  - Oleic acid (18:1)
  - Linoleic acid (18:2)
  - …

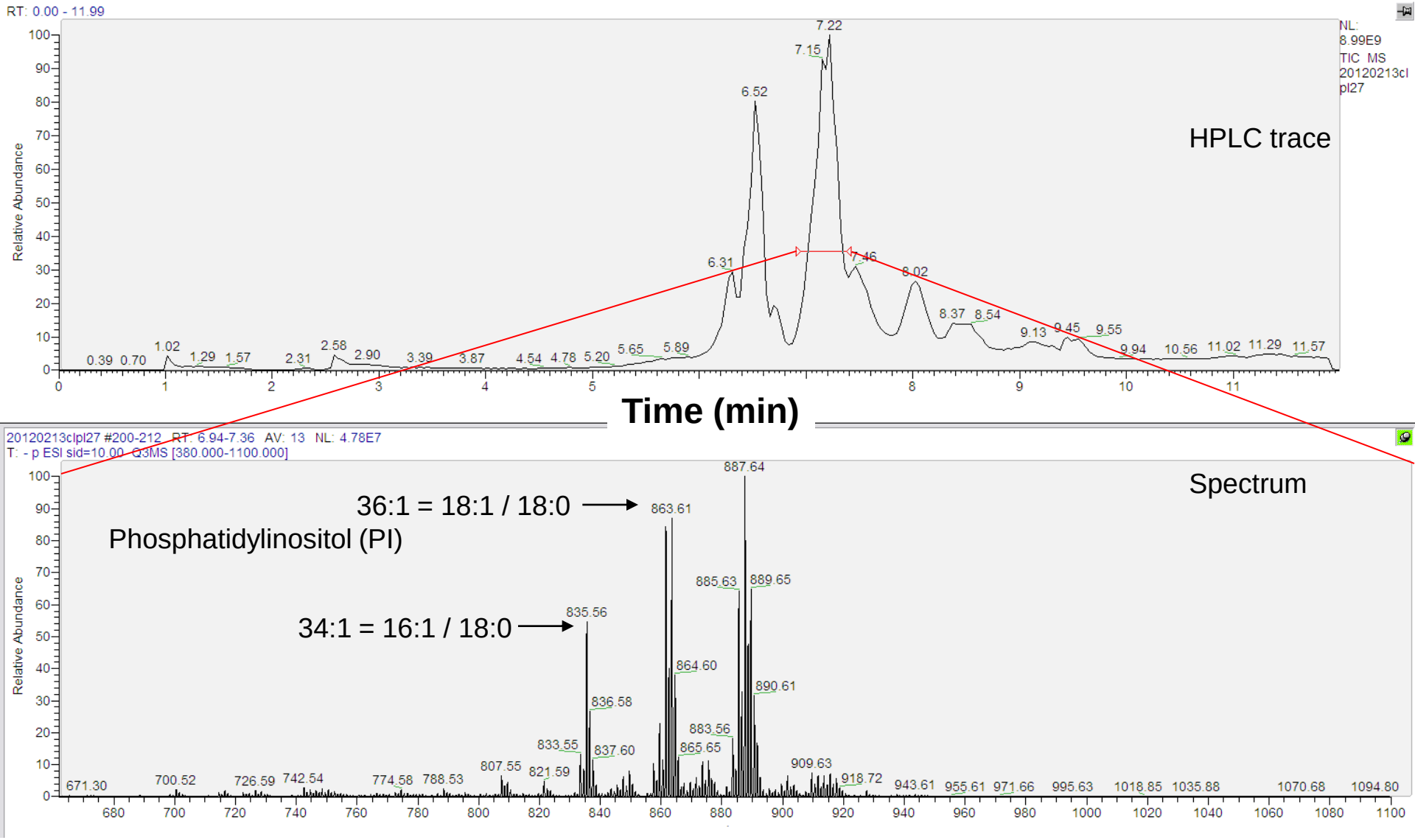
Technique

- Extraction of lipids followed by HPLC MS
- Two modes; negative and positive scan

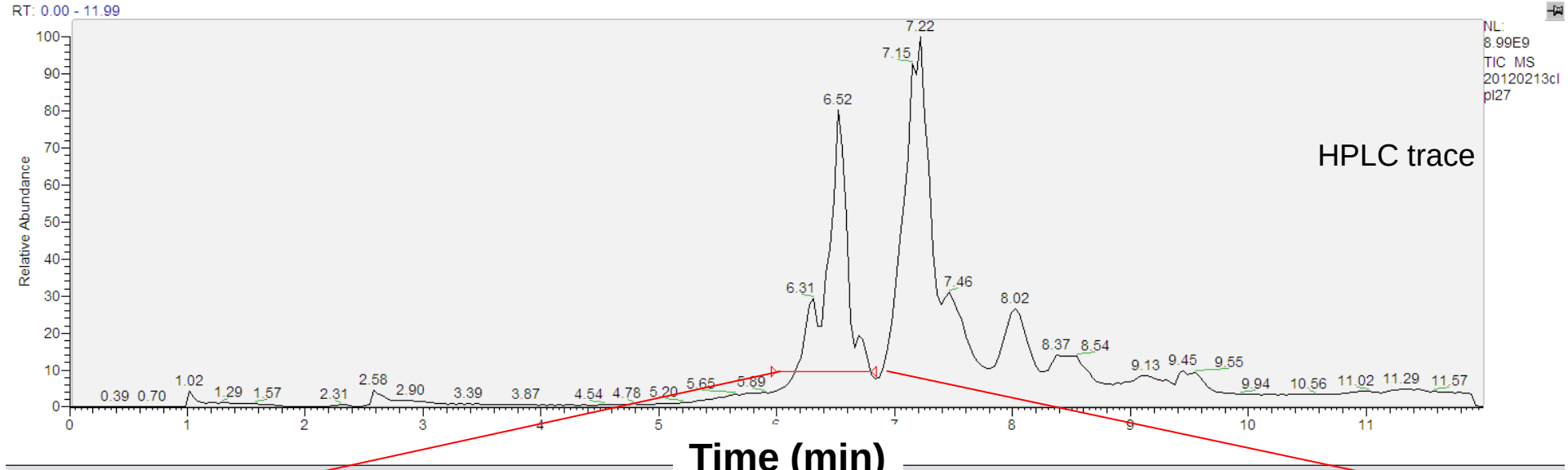


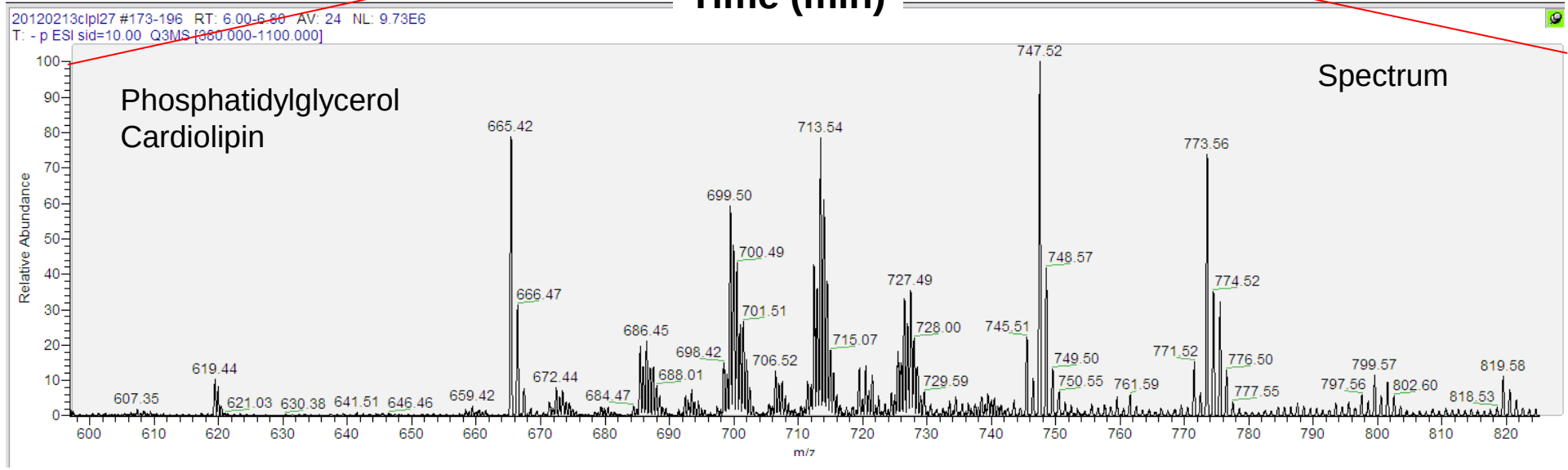
- HPLC profile of negative scan; “total ion current”

Technique; negative scan (MS)



Technique; negative scan (MS)

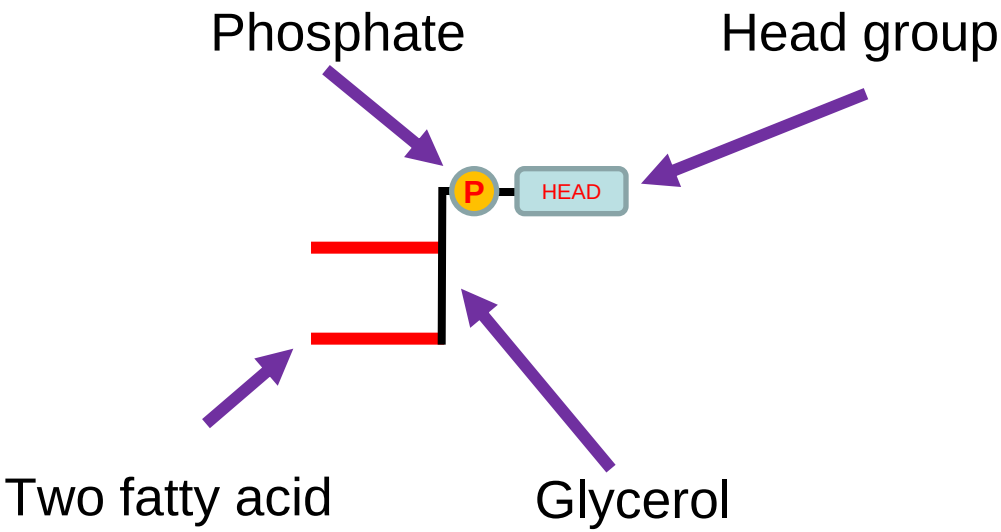




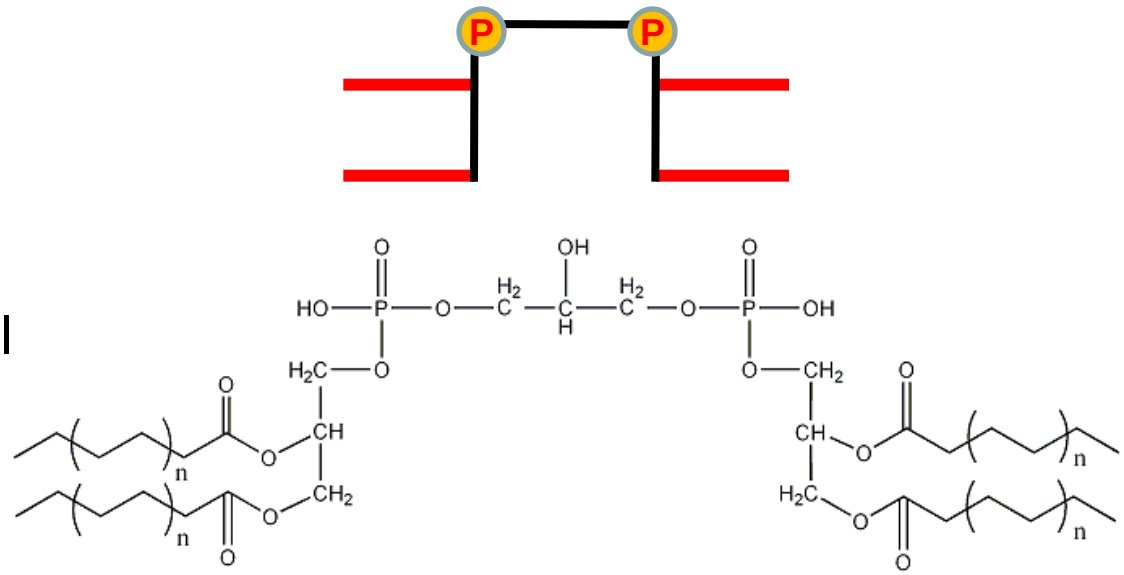
Cardiolipin (CL)

- Mitochondrial phospholipid with an unusual structure

"Regular" phospholipid structure



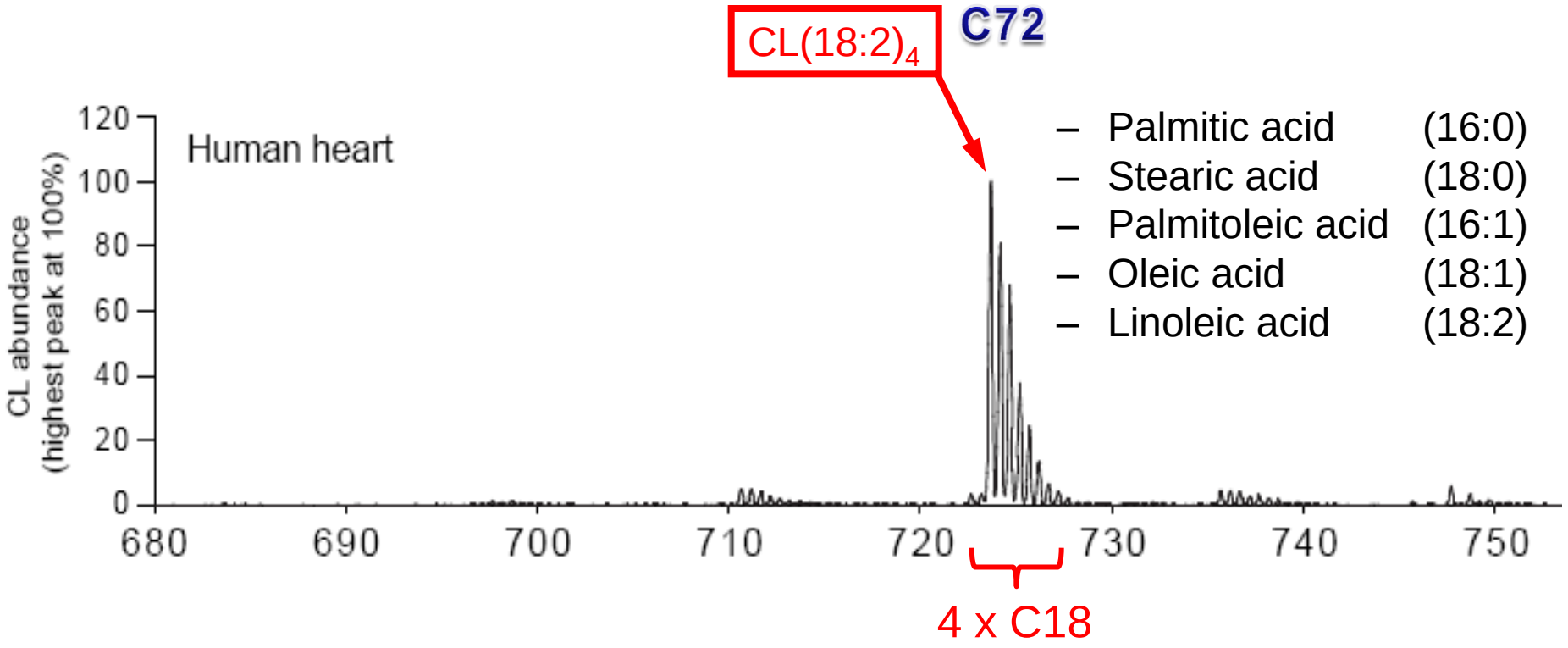
Cardiolipin



Functions of cardiolipin

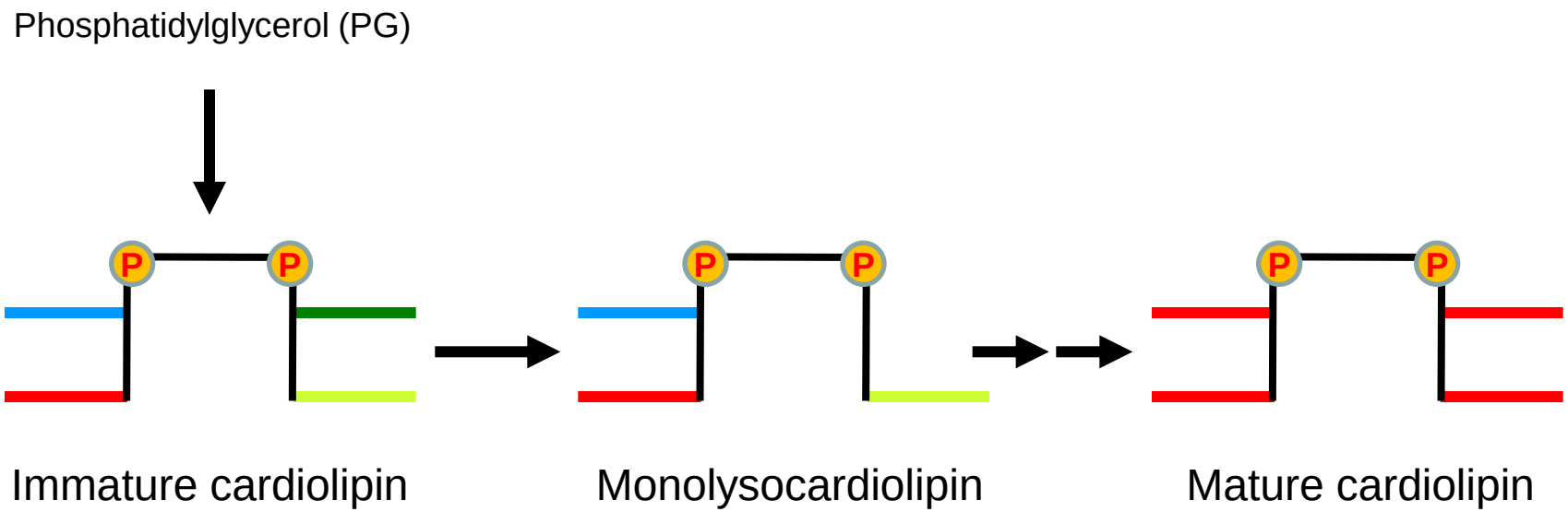
- Important constituent of mitochondrial membrane
- Mitochondrial energy metabolism
  - Essential for oxidative phosphorylation
- Mitochondrial protein import
- Type II (mitochondria-mediated) apoptosis

Cardiolipin in the human heart



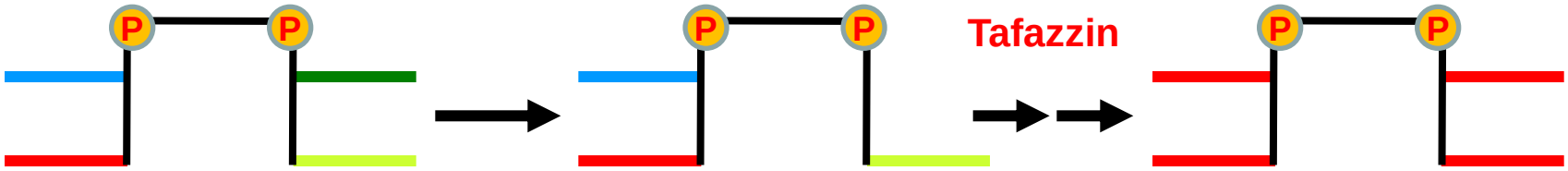
- Cardiolipin contains almost exclusively linoleic acid
- How to get the specific composition of cardiolipin?

Cardiolipin synthesis and remodeling



- Cardiolipin is actively remodeld to achieve the mature acylcomposition
- This remodeling is deficient in Barth syndrome

Cardiolipin remodeling

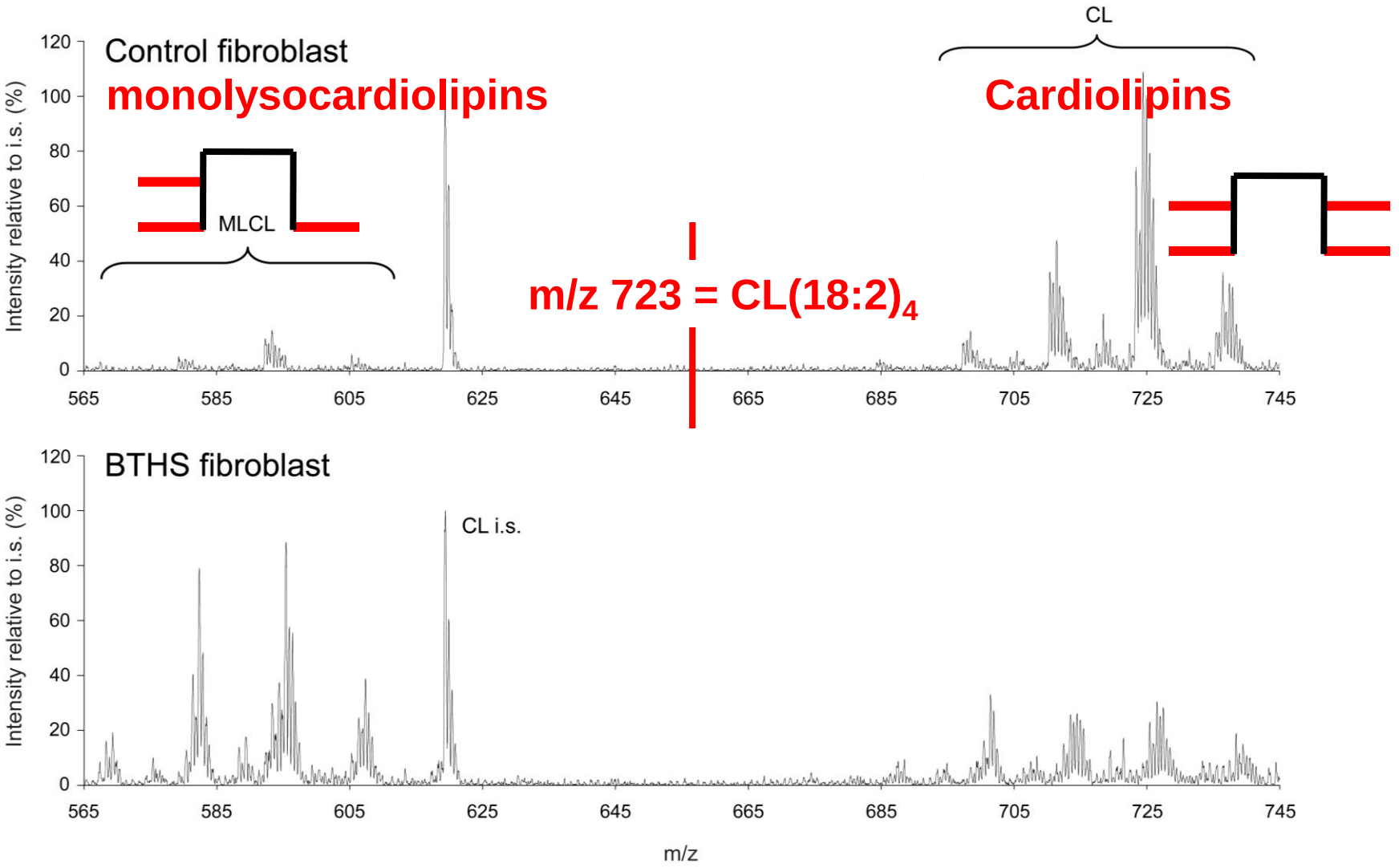


Immature cardiolipin Monolysocardiolipin Mature cardiolipin

Barth syndrome patients have:

- Lower CL levels
- Higher MLCL levels
- Altered CL and MLCL composition

Cardiolipins in Barth syndrome

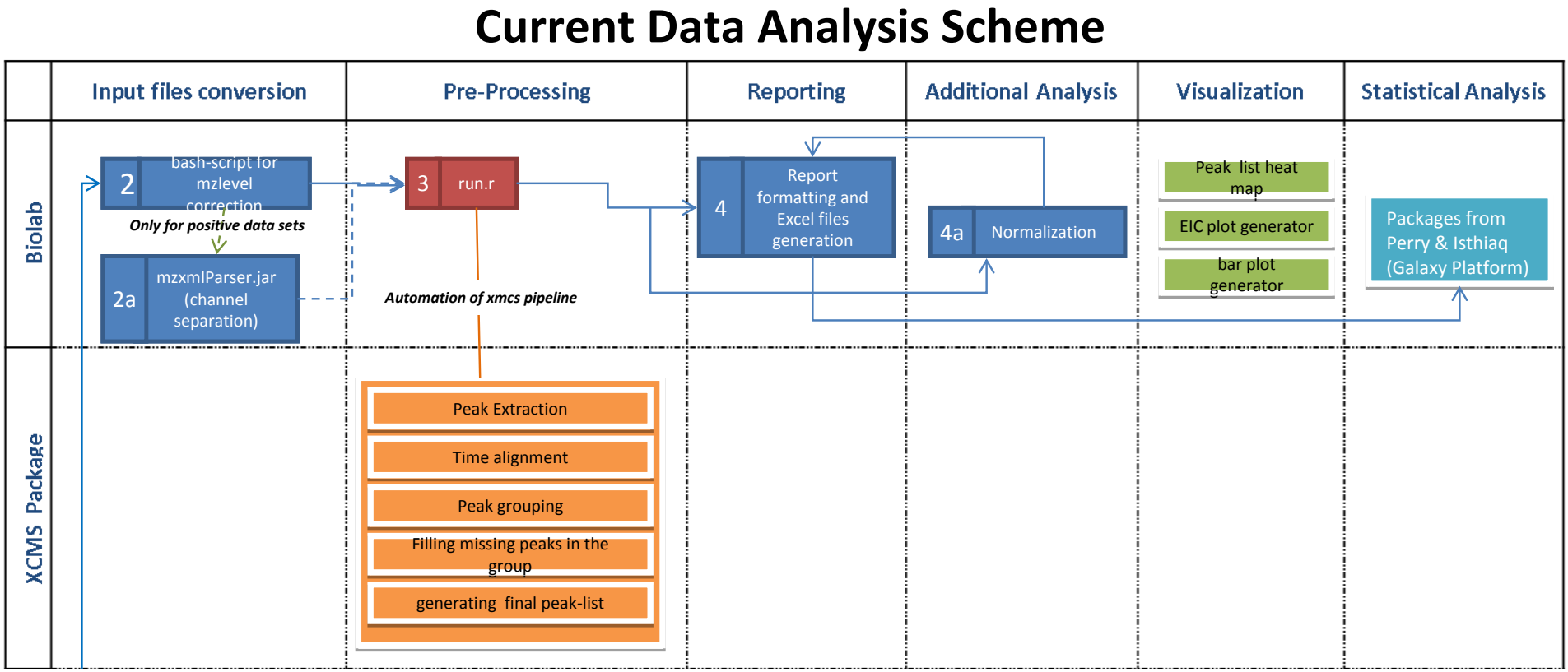


Indeed: lower CL, higher MLCL, altered composition!

Is there something wrong with phospholipids?

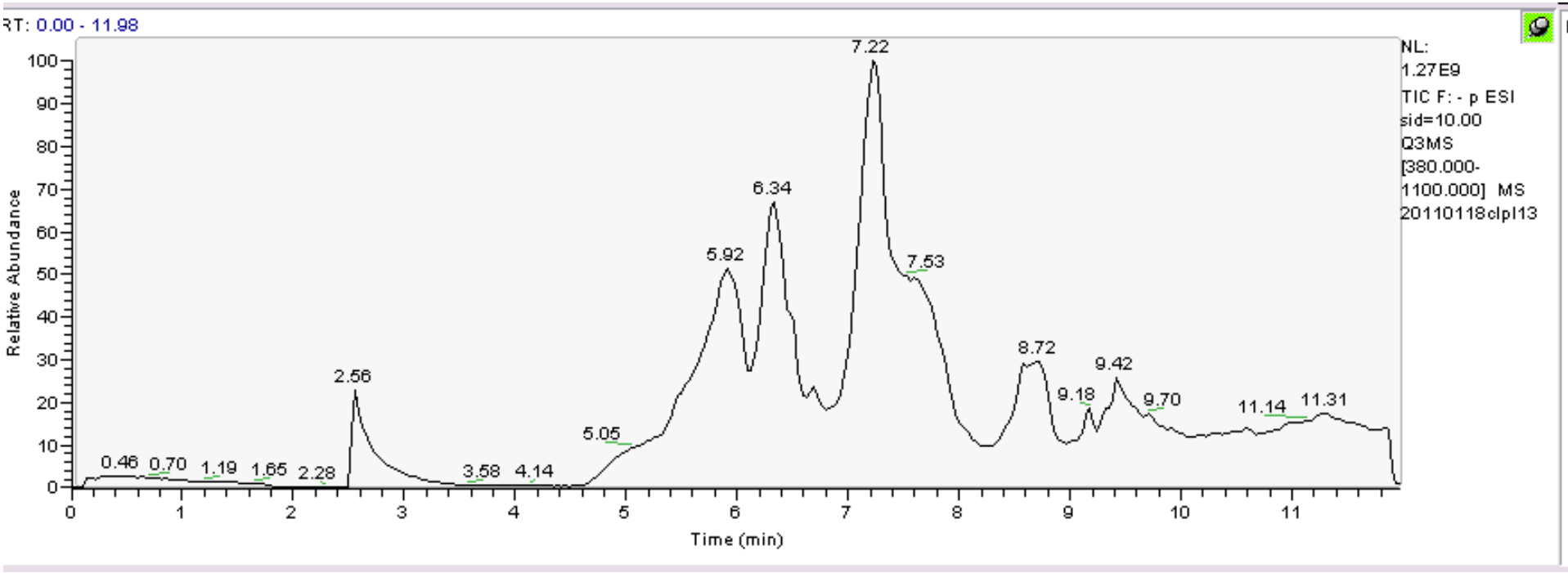
- Frequently asked question
- Analysis is relatively simple
- Data-analysis, however, is labor intensive and biased
- Development of a “pipeline” to analyze this data, fast and in an unbiased manner
- But what is a pipeline? How does it work?

The pipeline

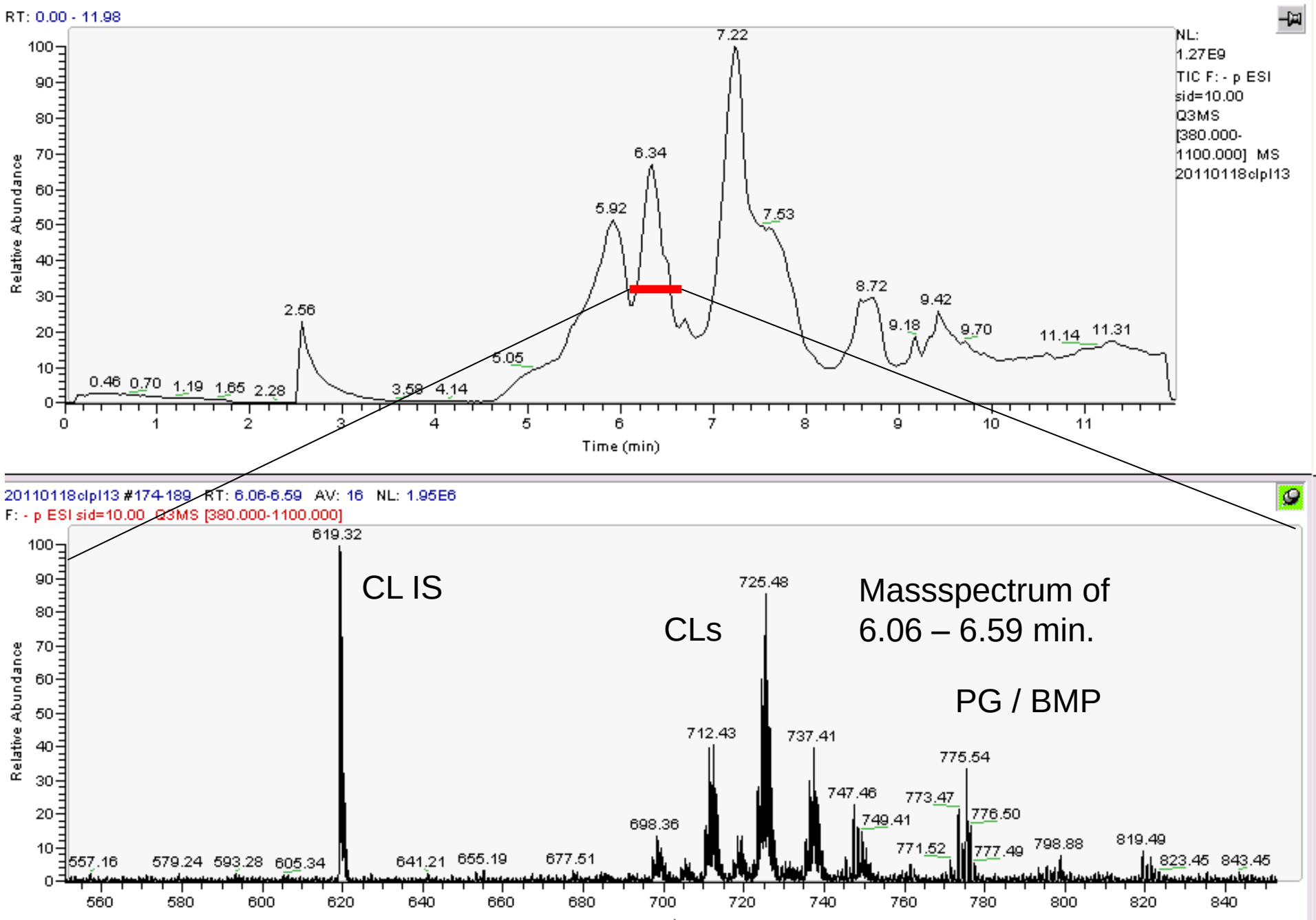


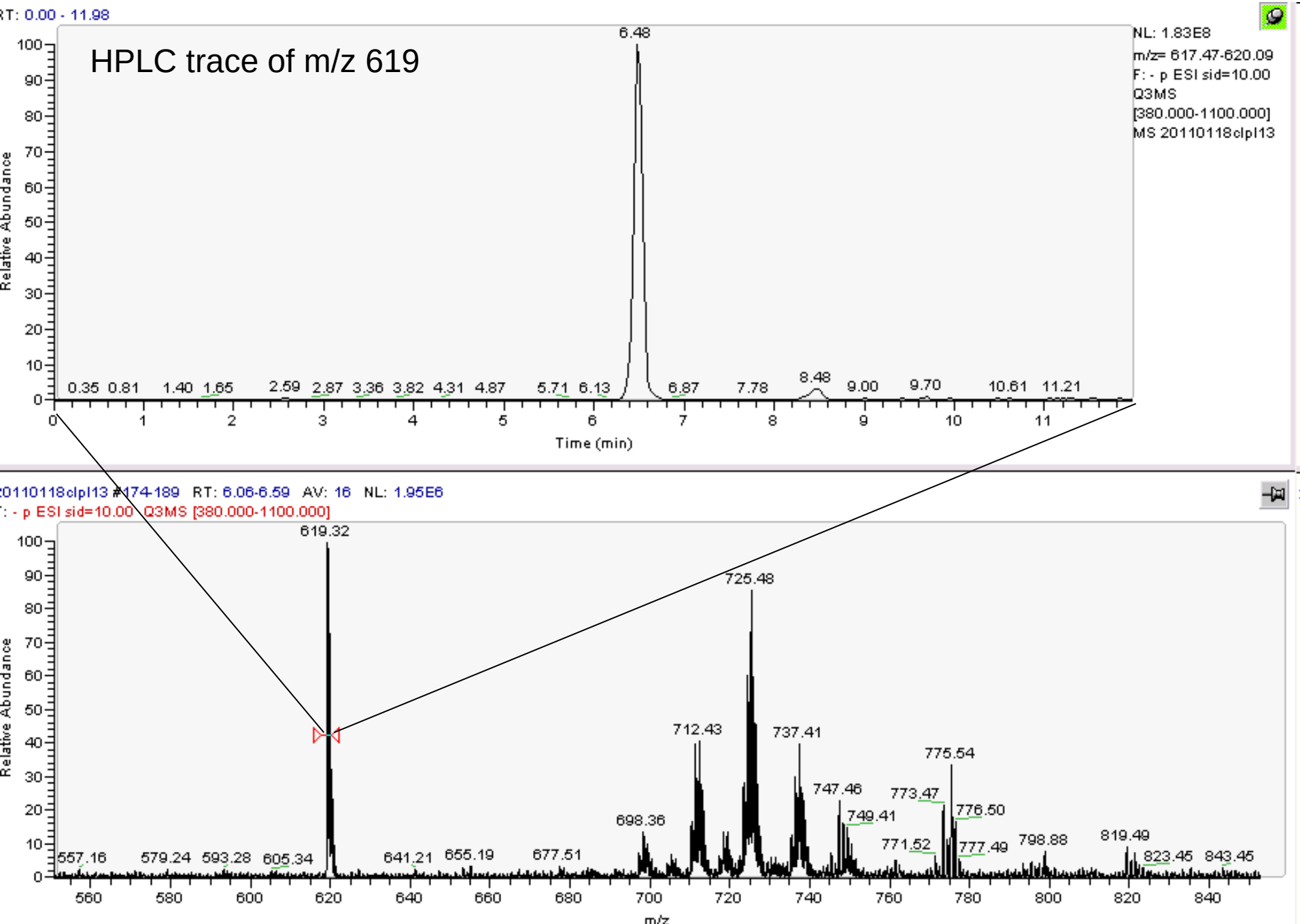
- But what does the pipeline really do?

HPLC profile (TIC)



Spectrum of selected timeframe




HPLC trace of m/z 619
RT: 0.00 - 11.98
NL: 1.83E8
m/z= 617.47-620.09
F: - p ESI sid=10.00
Q3MS
[380.000-1100.000]
MS 20110118clpl13
Relative Abundance
Time (min)
6.48
0.35 0.81 1.40 1.65 2.59 2.87 3.36 3.82 4.31 4.87 5.71 6.13 6.87 7.78 8.48 9.00 9.70 10.61 11.21
20110118clpl13 #174-189 RT: 6.06-6.59 AV: 16 NL: 1.95E6
T: - p ESI sid=10.00 Q3MS [380.000-1100.000]
619.32
725.48
712.43
737.41
775.54
747.46
773.47
776.50
749.41
698.36
771.52
777.49
798.88
819.49
557.16 579.24 593.28 605.34 641.21 655.19 677.51 823.45 843.45
m/z

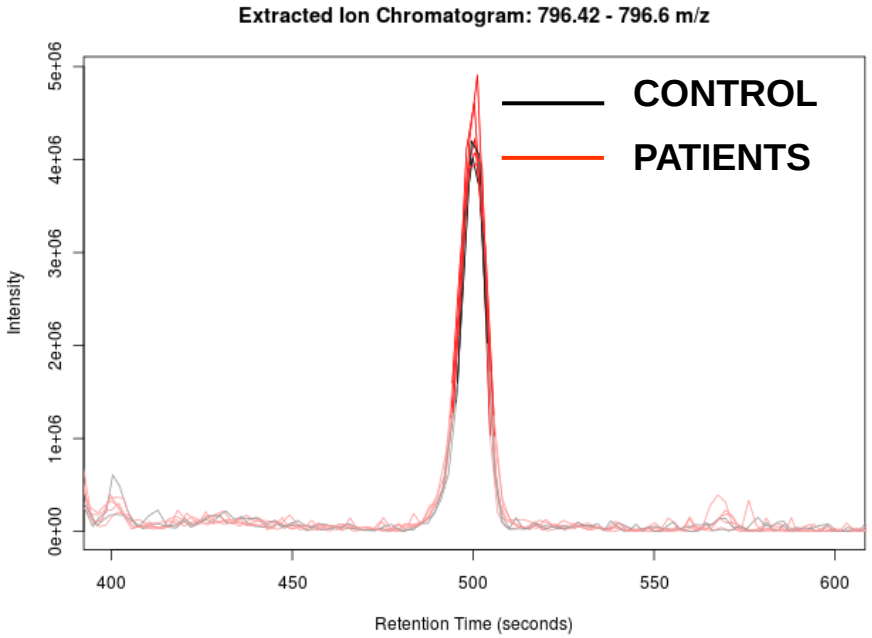
The pipeline

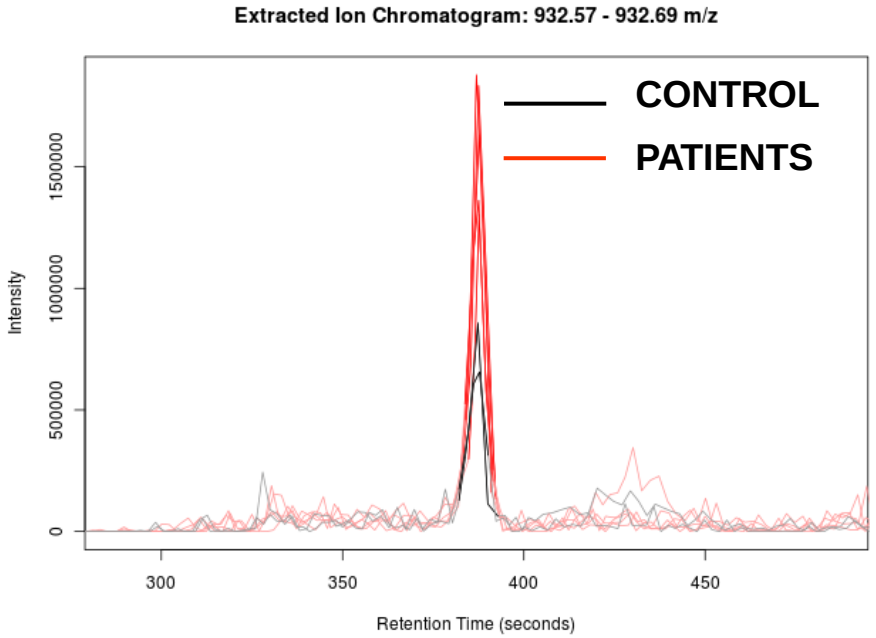
- In essence, the pipeline does this for every m/z value.
- Then it integrates the area of the chromatographic peak
- Generates a “peaklist” listing retentiontime, m/z value and area

The peaklist

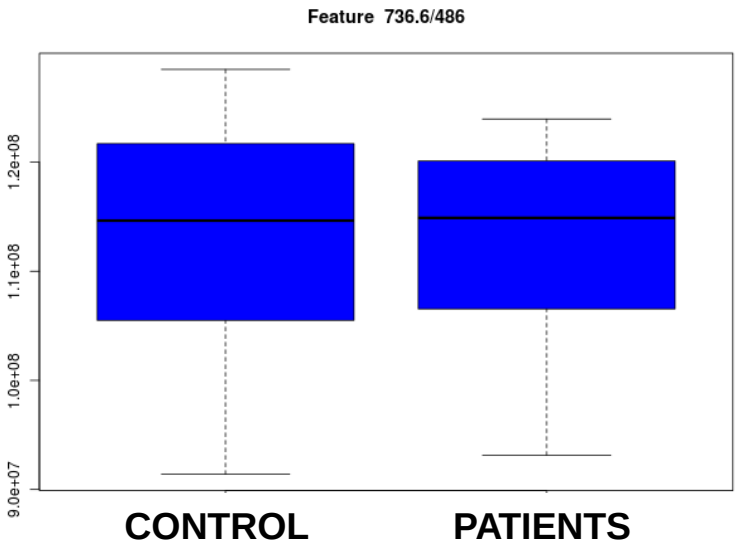
	tstat	pvalue	mzmed	mzmin	mzmax	rtmed	rtmin
1	-18.787266	1.681569e-09	777.5184	777.4782	777.5807	378.7836	361.3063
2	-16.43899	3.201833e-08	776.5000	776.4219	776.567	378.7889	358.6286
3	-17.286205	4.279428e-08	775.5062	775.4645	775.55	378.1312	357.4936
4	-9.862654	1.595528e-07	1084.782	1084.7112	1084.8334	313.9773	303.9761
5	6.404811	1.392417e-05	699.4335	699.3374	699.4512	377.5719	376.308
6	6.671633	2.341280e-05	686.3802	686.3544	686.4755	379.603	377.1957
7	-5.987861	2.937940e-05	820.4871	820.4531	820.5205	354.0506	353.0109
8	6.193218	3.215256e-05	699.9173	699.8677	699.9654	377.4913	376.308
9	6.379906	3.845940e-05	428.5979	428.564	428.6564	373.5914	365.5613
10	-6.012029	3.944758e-05	819.4759	819.4543	819.5162	354.0375	353.0109
11	-5.43208	7.405838e-05	564.9256	564.8171	564.9375	635.3374	628.0686
12	-5.628626	8.196840e-05	417.8037	417.7612	417.889	638.6079	628.0686
13	5.746326	8.947048e-05	672.8597	672.778	672.9229	379.7681	379.2079
14	-5.587583	9.379562e-05	817.4597	817.4356	817.5126	353.7516	351.0847
15	-5.23837	1.379943e-04	822.4882	822.4386	822.5757	354.6527	353.2115
16	-5.021188	1.606485e-04	821.4956	821.4555	821.5389	354.0506	353.0109
17	-5.216035	1.664028e-04	805.4557	805.4182	805.4894	355.4843	354.0506
					1086.844		

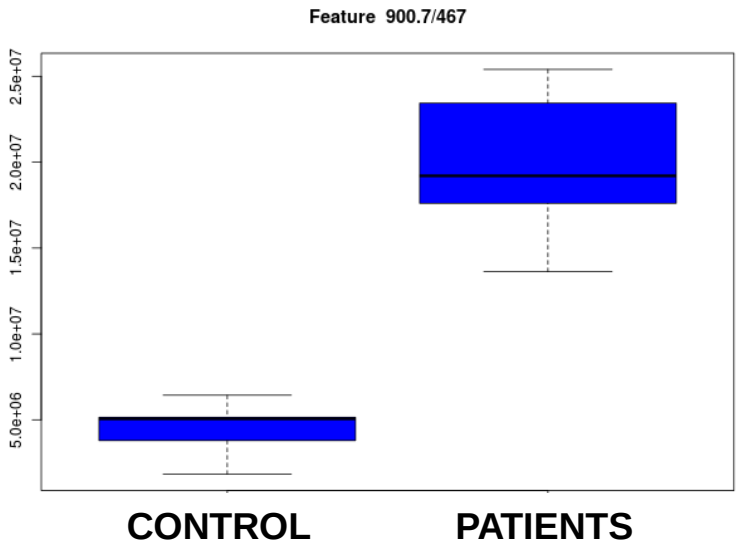
Visualization in extracted ion-chromatographic overlays of data sets per *m/z* value and R_t





Visualization by Box & Whiskers plots of data sets of peaks per *m/z* value and R_t





Phospholipid analysis/pipeline

- Analysis of major phospholipid classes:
  - PE, PC, PI, PG, CL, SM etc.
  - Determination of molecular composition of the species (fatty acid side-chains)
- The pipeline allows fast and unbiased quantification of all ions in the analysis.
- Example of the power of the analysis/pipeline: **MEGDEL syndrome**

MEGDEL syndrome

GMZ

Femke Stet

Henk van Lenthe

Martin Vervaart

Wim Kulik

Frederic Vaz

Radboud University Nijmegen

Saskia Wortmann

Arjan de Brouwer

Eva Morava

Ron Wevers

Bioinformatica

Christin Christin

Antoine van Kampen

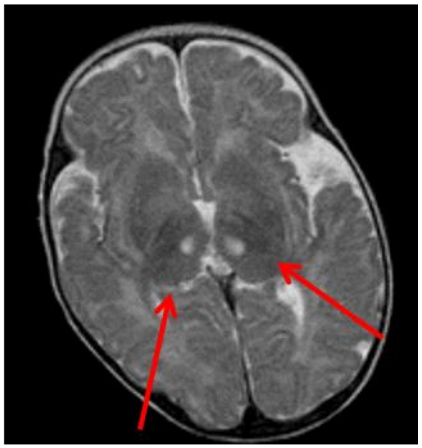
3-Methylglutaconic aciduria syndromes

- Type II: **Barth syndrome** (X-linked, DCM, cyclic neutropenia, hypotonia, normal cognitive function, *TAZ*)
- Type IV: “unclassified” “mitochondrial dysfunction” (*TMEM70, POLG, RYR1, SUCLA2…..*)

New subtype of type IV

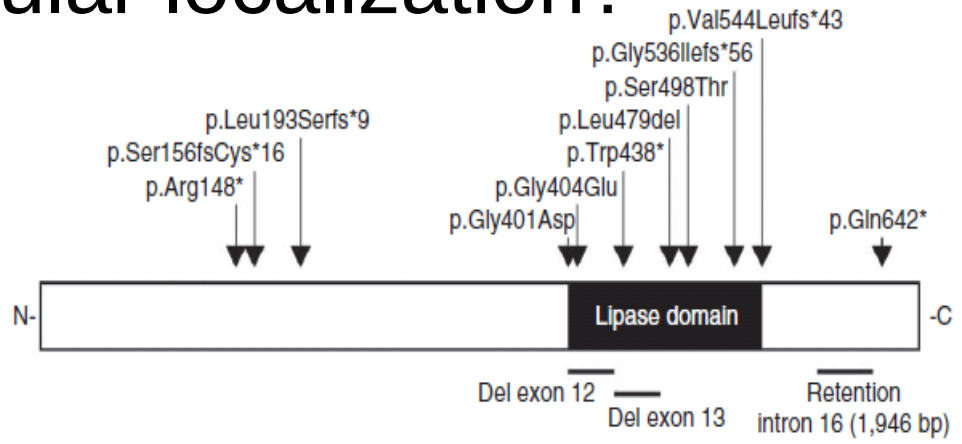
- Termed MEGDEL syndrome:
  - 3-MEthylGlutaconic aciduria
    (30-300 umol/mmol creatinine, n<20)
  - Hypotonia/spasticity/dystonia
  - Deafness
  - Encephalopathy, psychomotor retardation
  - Leigh like syndrome/disease
  - OXPHOS dysfunction
    (mild decrease in ATP production and complex I)
  - Lactic acidemia (predominantly neonatal)
  - Liver dysfunction (predominantly neonatal)

MEGDEL syndrome



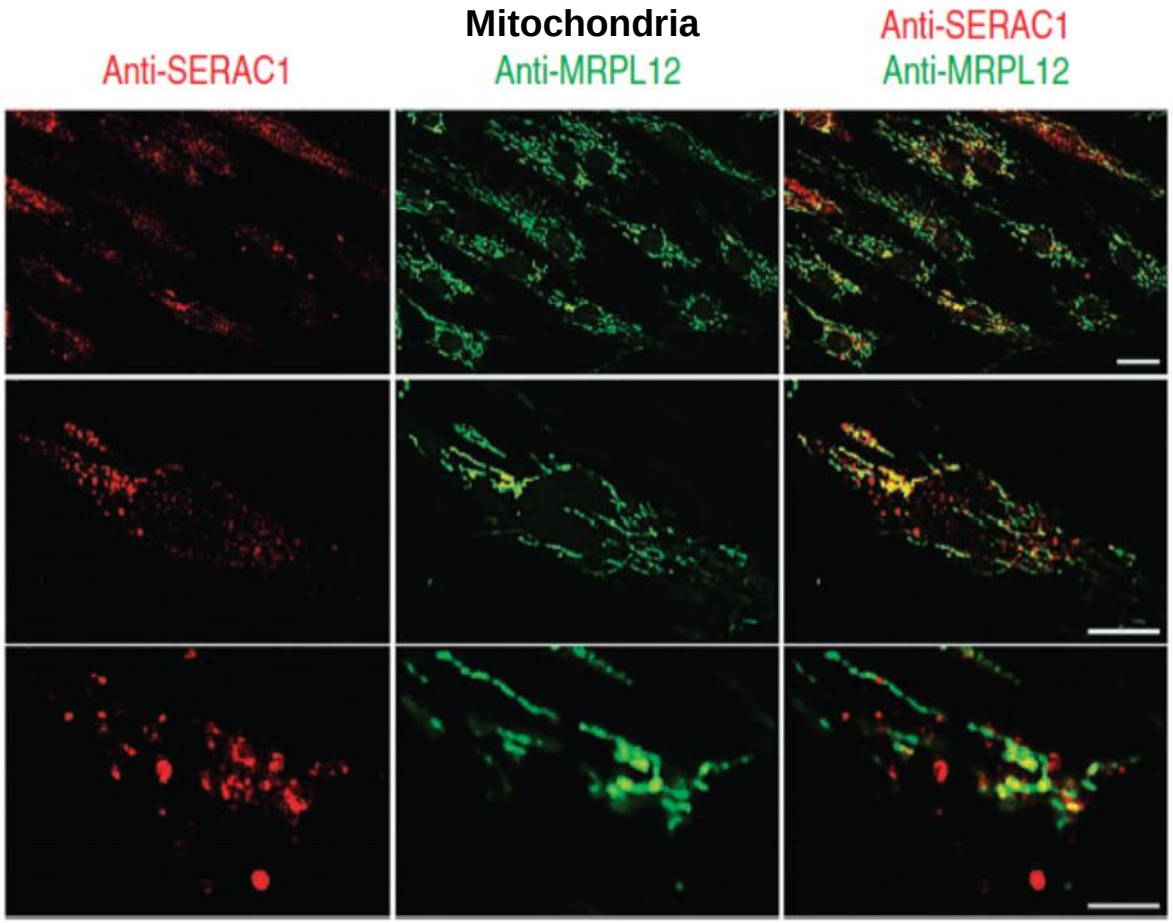
Exome sequencing (Nijmegen)

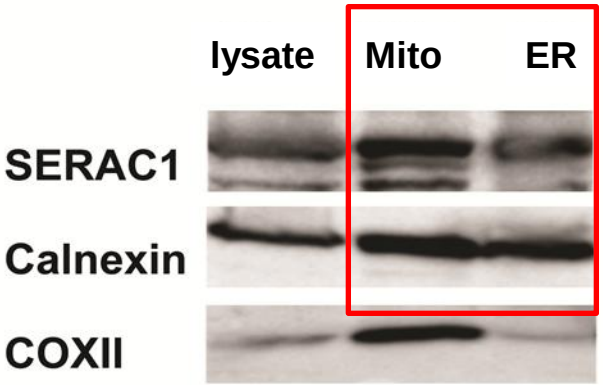
- 2 patients exome sequencing → both mutation in *SERAC1*
- All other patients (n=16) also found to have mutation in *SERAC1*
- Function unknown
- *SERAC1* subcellular localization?



Nature Genetics, Wortmann et al, epub 10 june 2012

SERAC1 is localized to MAM's



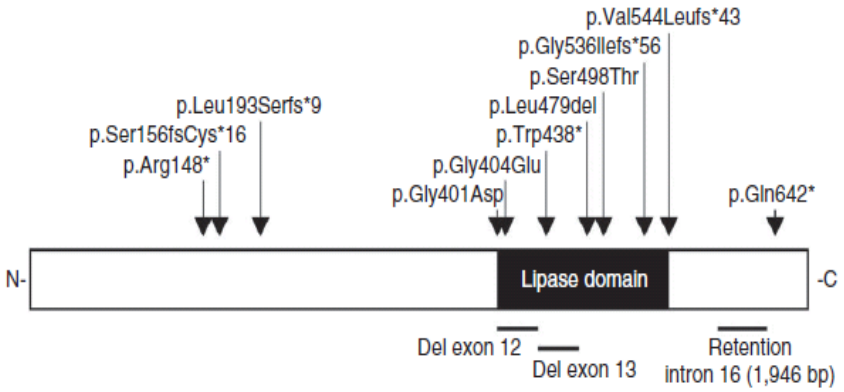


- SERAC1 is localized to mitochondria associated membranes (protK studies and IF).

Nature Genetics, Wortmann et al, epub 10 june 2012

SERAC1 characterization

- *SERAC1* protein has an acyltransferase/lipase domain
- MEGDEL patients have:
  - Methylglutaconic aciduria
  - Mitochondrial disease
- Phospholipids?



Nature Genetics, Wortmann et al, epub 10 june 2012

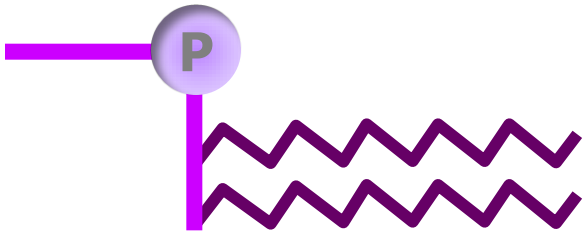
MEGDEL lipidomics experiment

- 5 MEGDEL fibroblast lines
- 10 control fibroblast lines

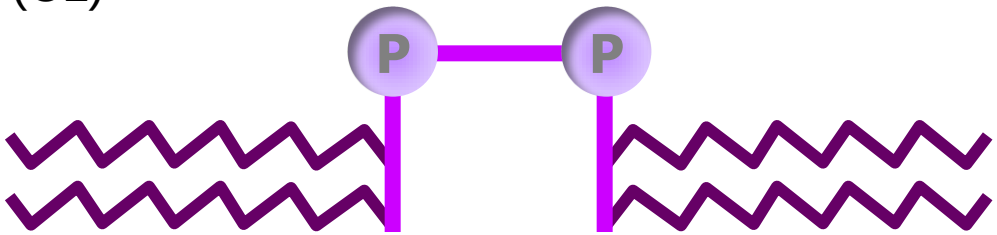
  [all cultured simultaneously, same medium, FCS etc…]
- Phospholipid analysis + bioinformatics pipeline

Structures of “the players”

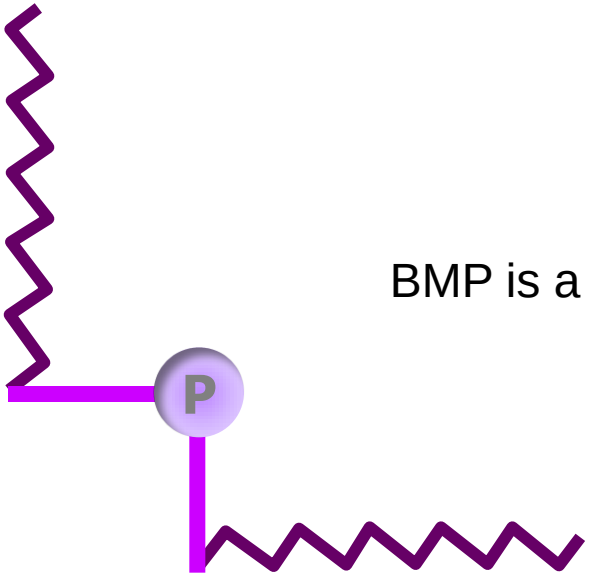
Phosphatidylglycerol (PG)

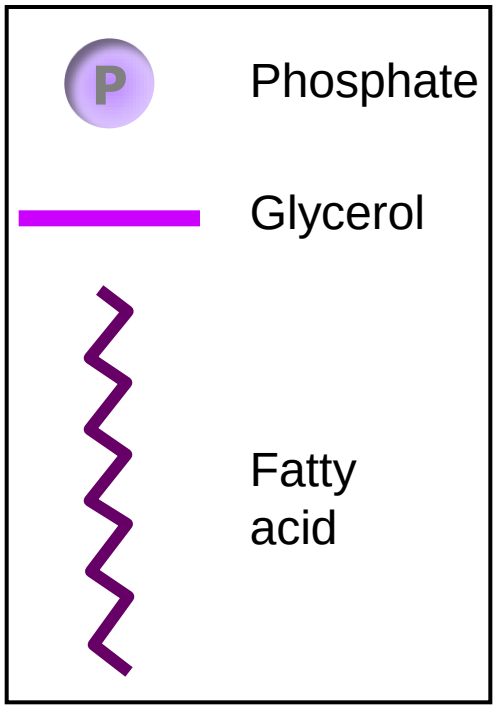


Cardiolipin (CL)



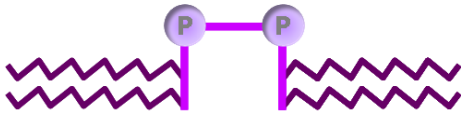
Bismonoacylglycerolphosphate (BMP)

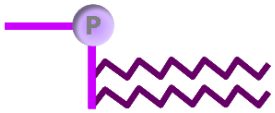




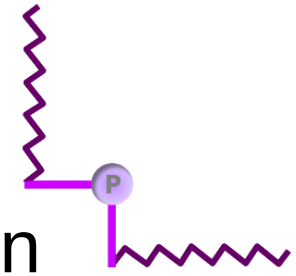
BMP is a structural isomer of PG

Functions



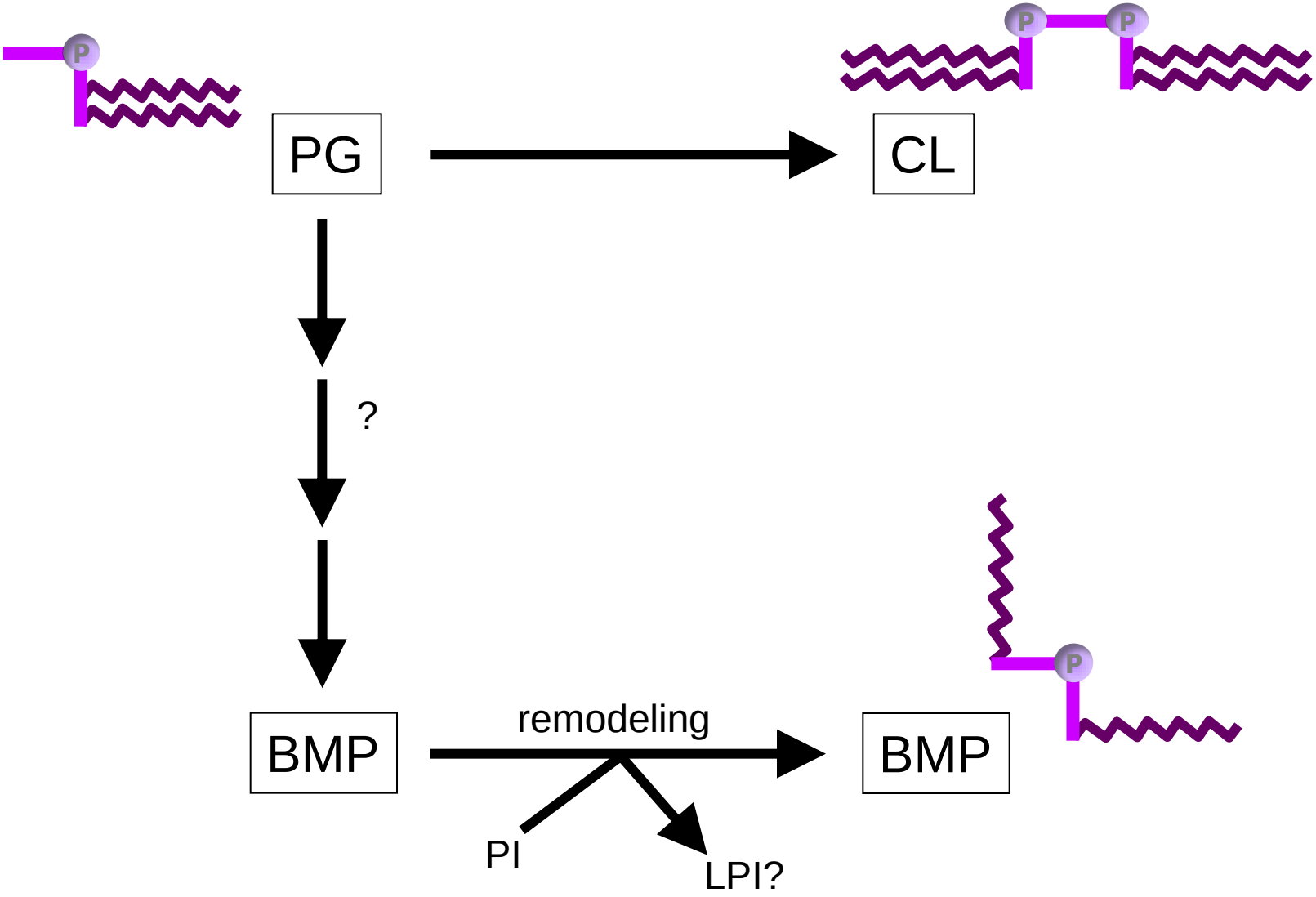


- *Cardiolipin (CL)* and *phosphatidylglycerol (PG)* are mitochondrial phospholipids that are important for mitochondrial function

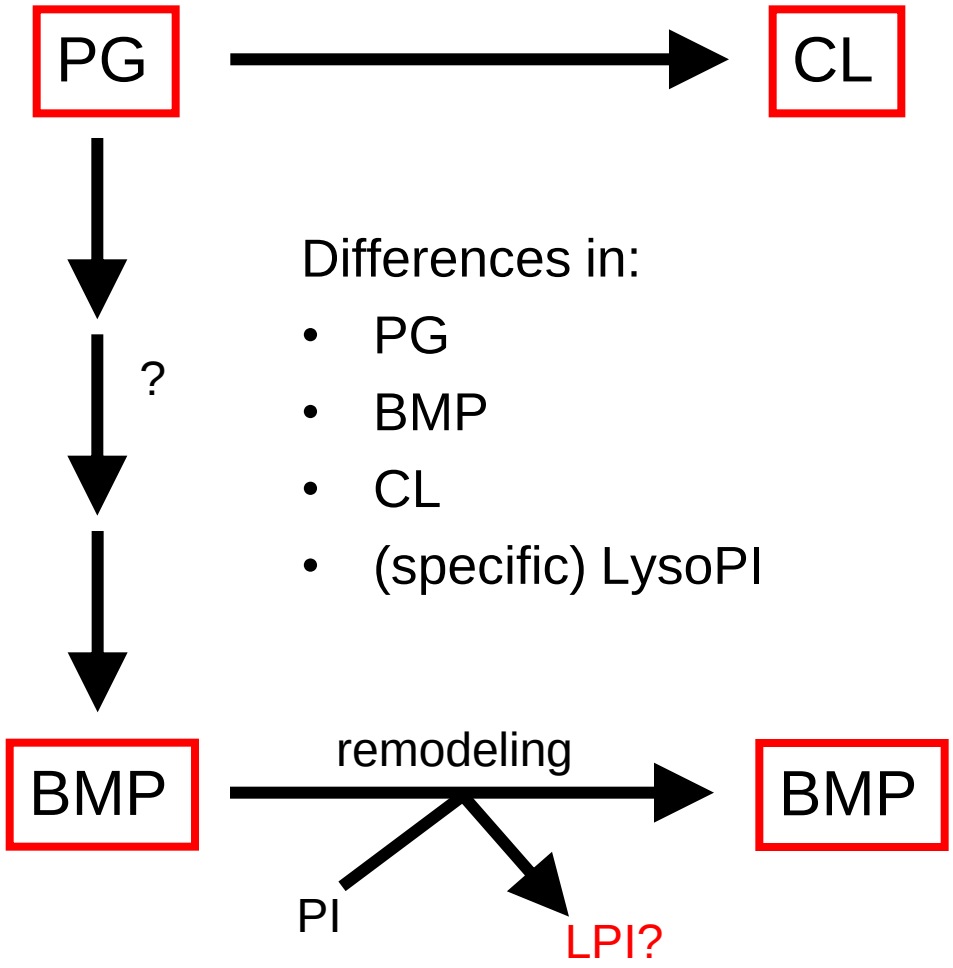


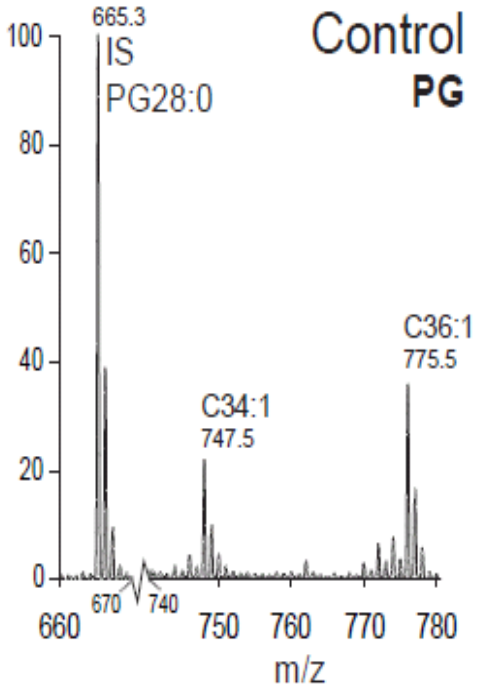
- *Bismonoacylglycerolphosphate (BMP)* is an endosomal phospholipid involved in the transport and breakdown of lipids and cholesterol.

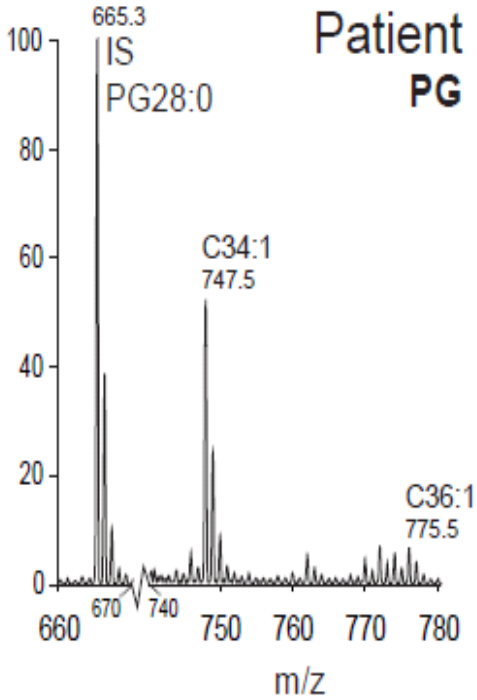
PG is a precursor of CL and BMP



Differences found by the pipeline

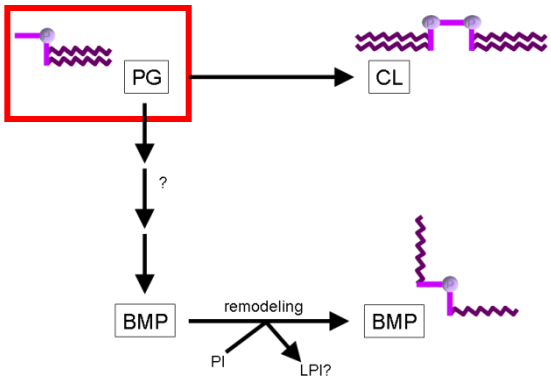


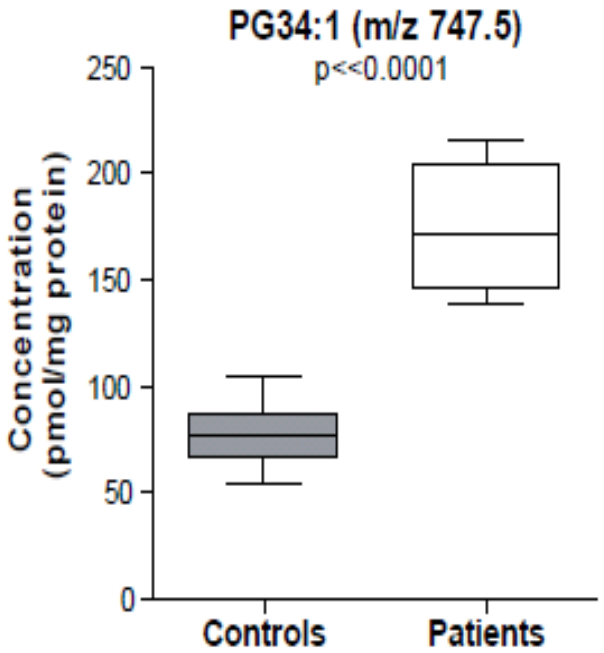


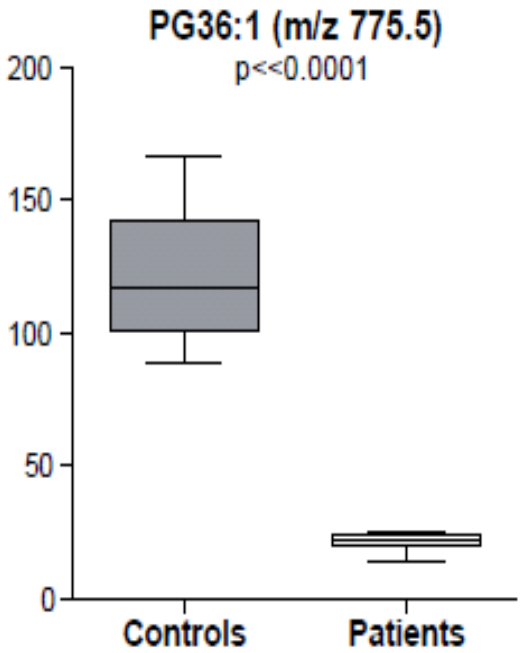


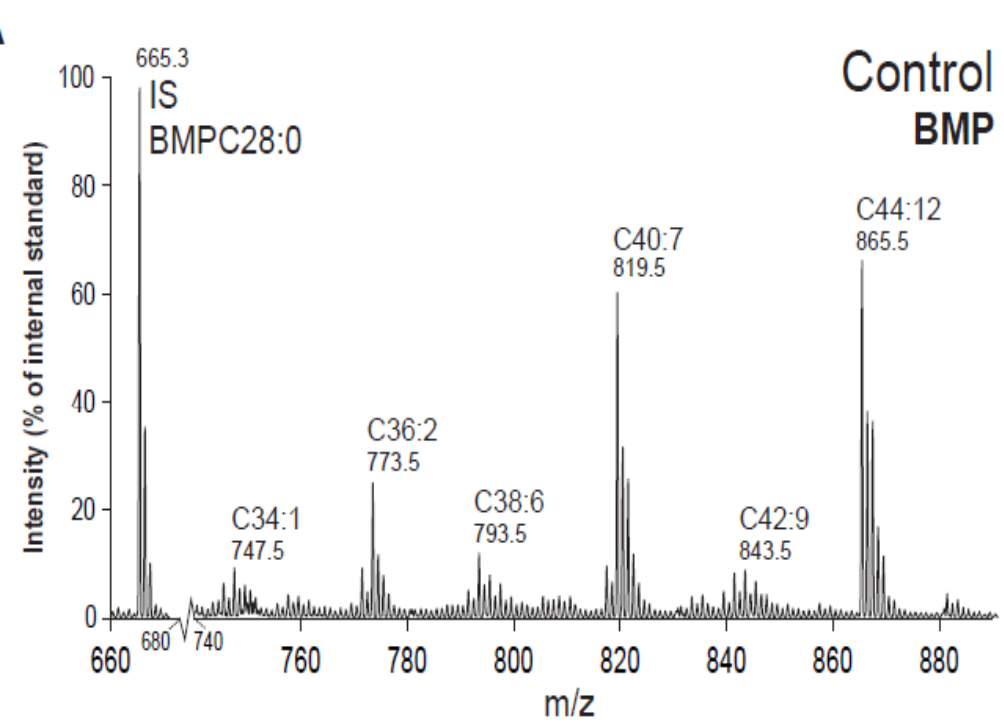
PG

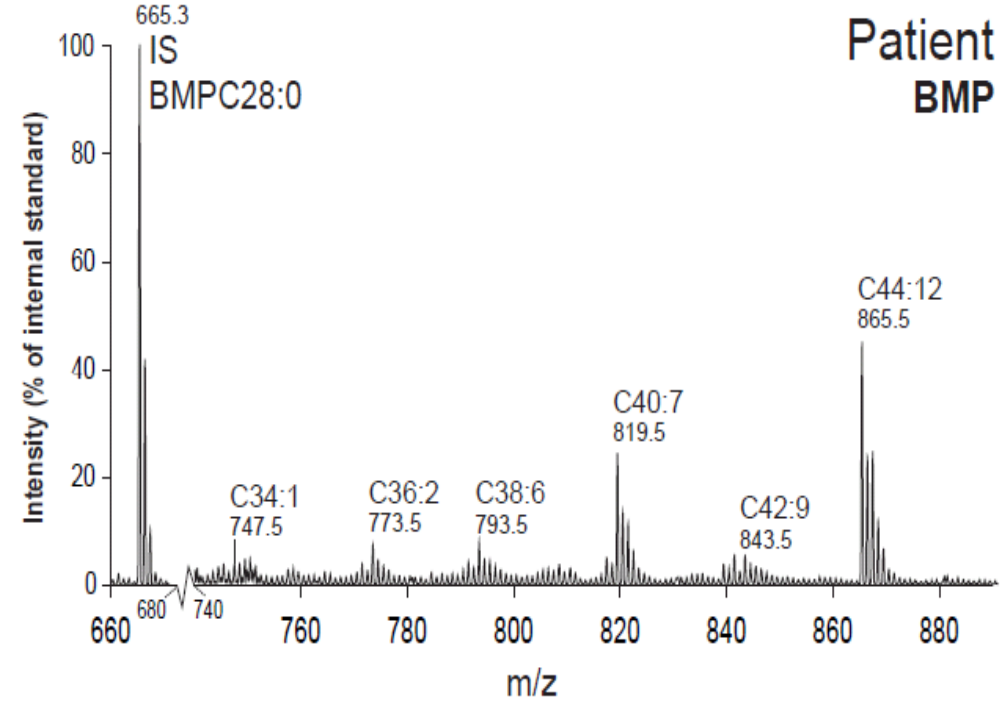
- Accumulation of PG34:1
- Deficiency of PG36:1





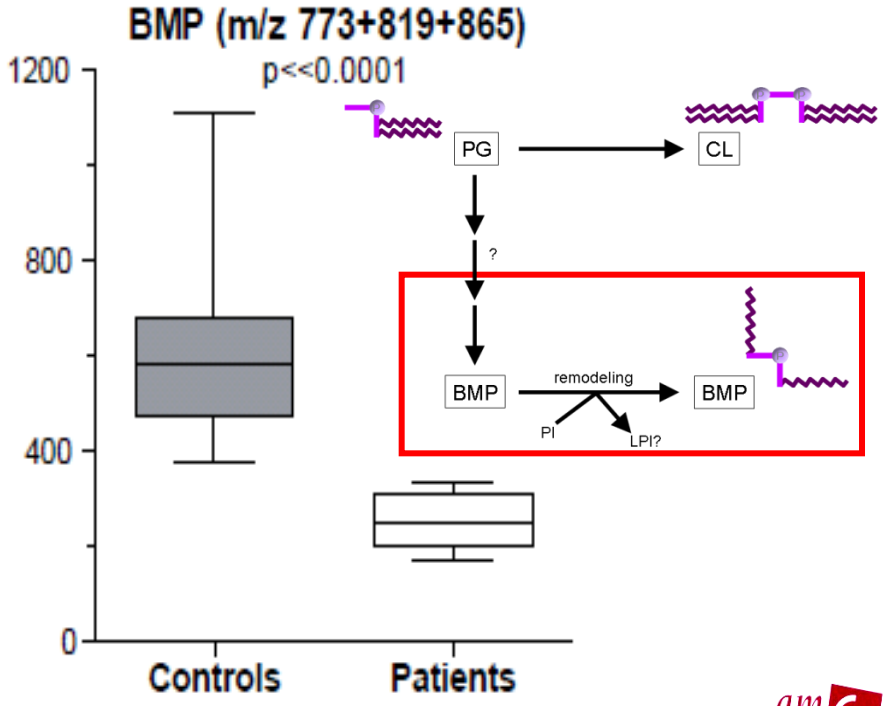




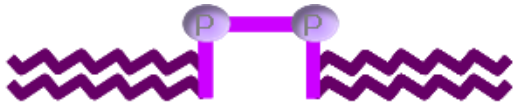


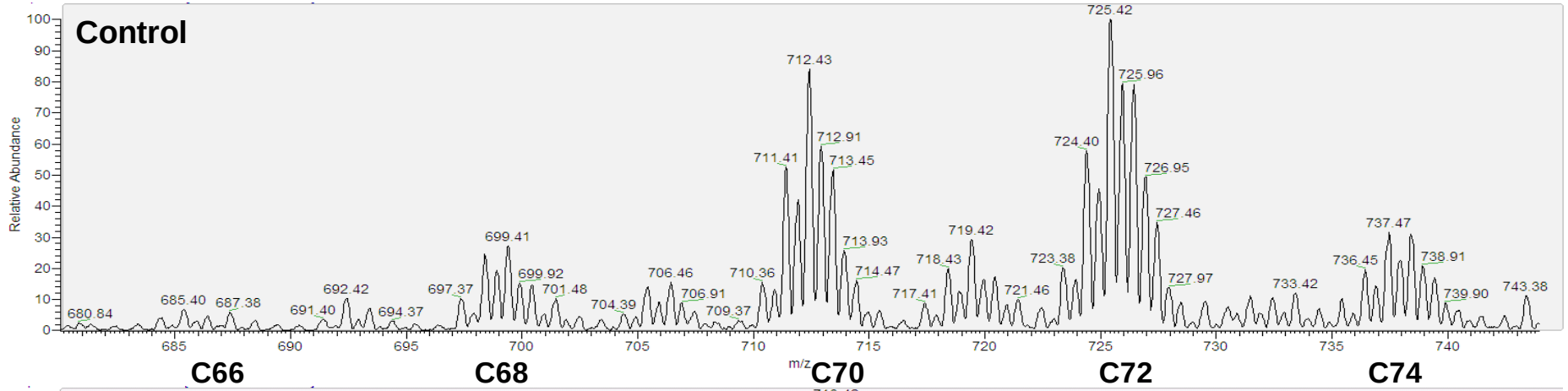
BMP

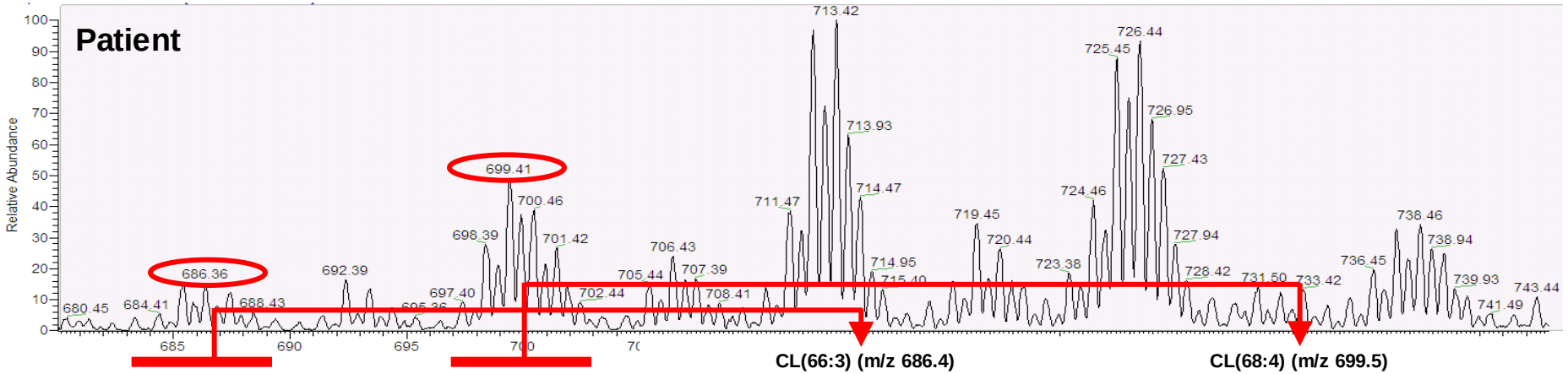
- Lower BMP levels
- Normal/same molecular composition of BMP

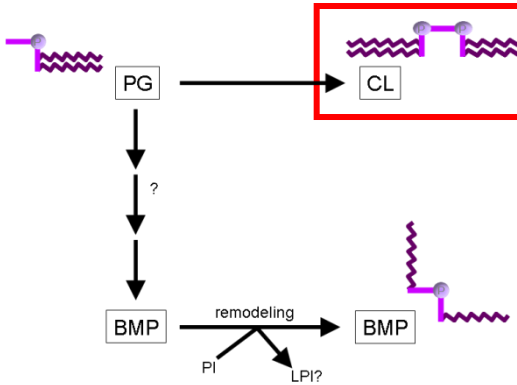


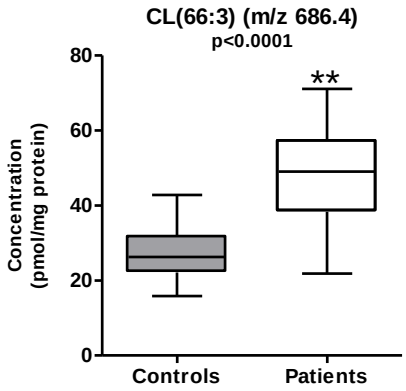
Cardiolipin

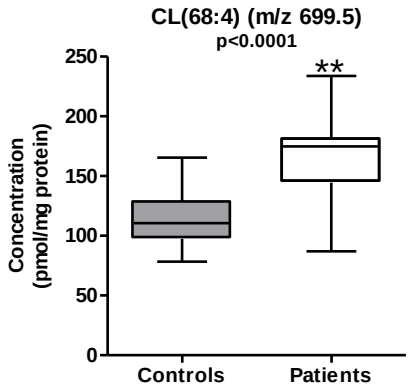




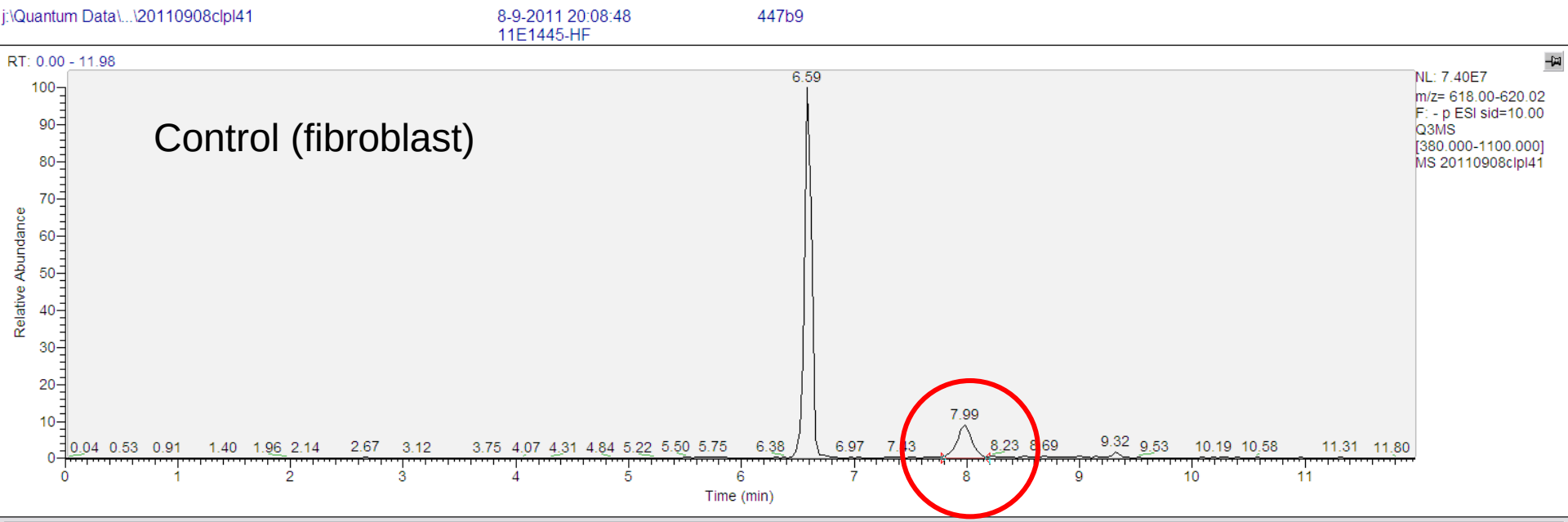


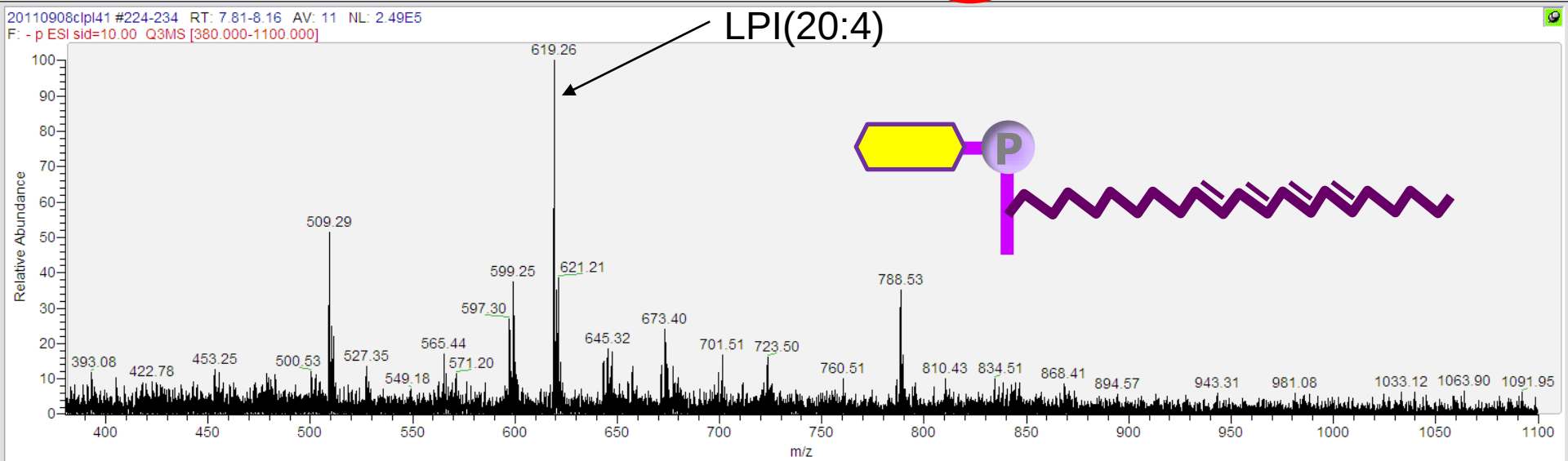




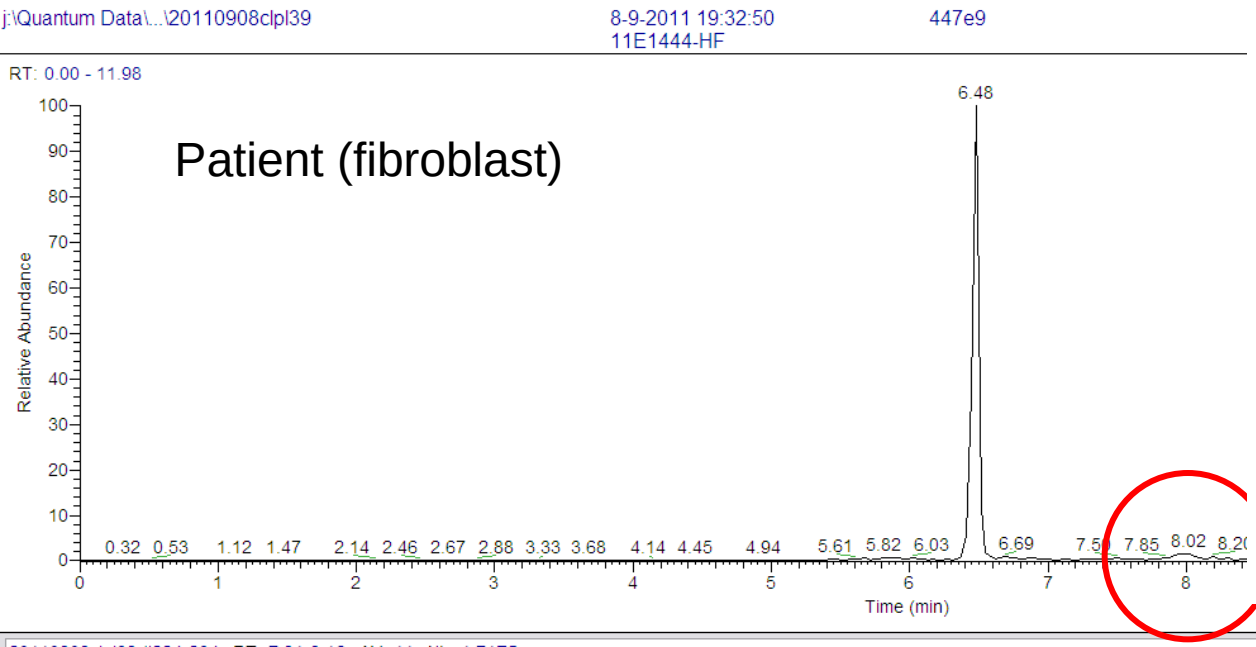


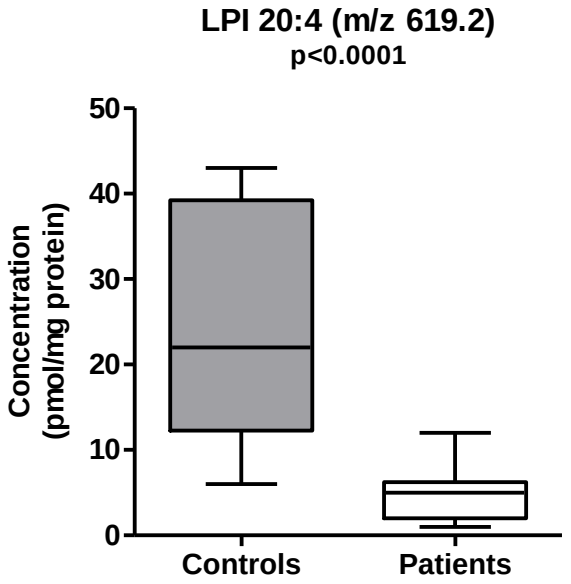
The surprise of the pipeline

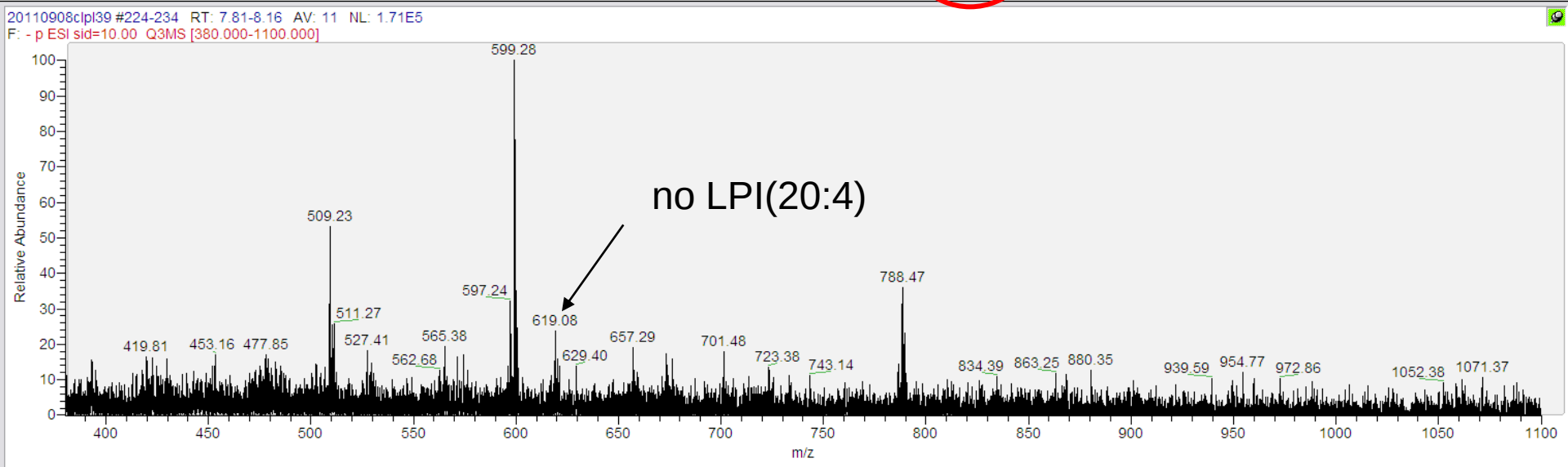




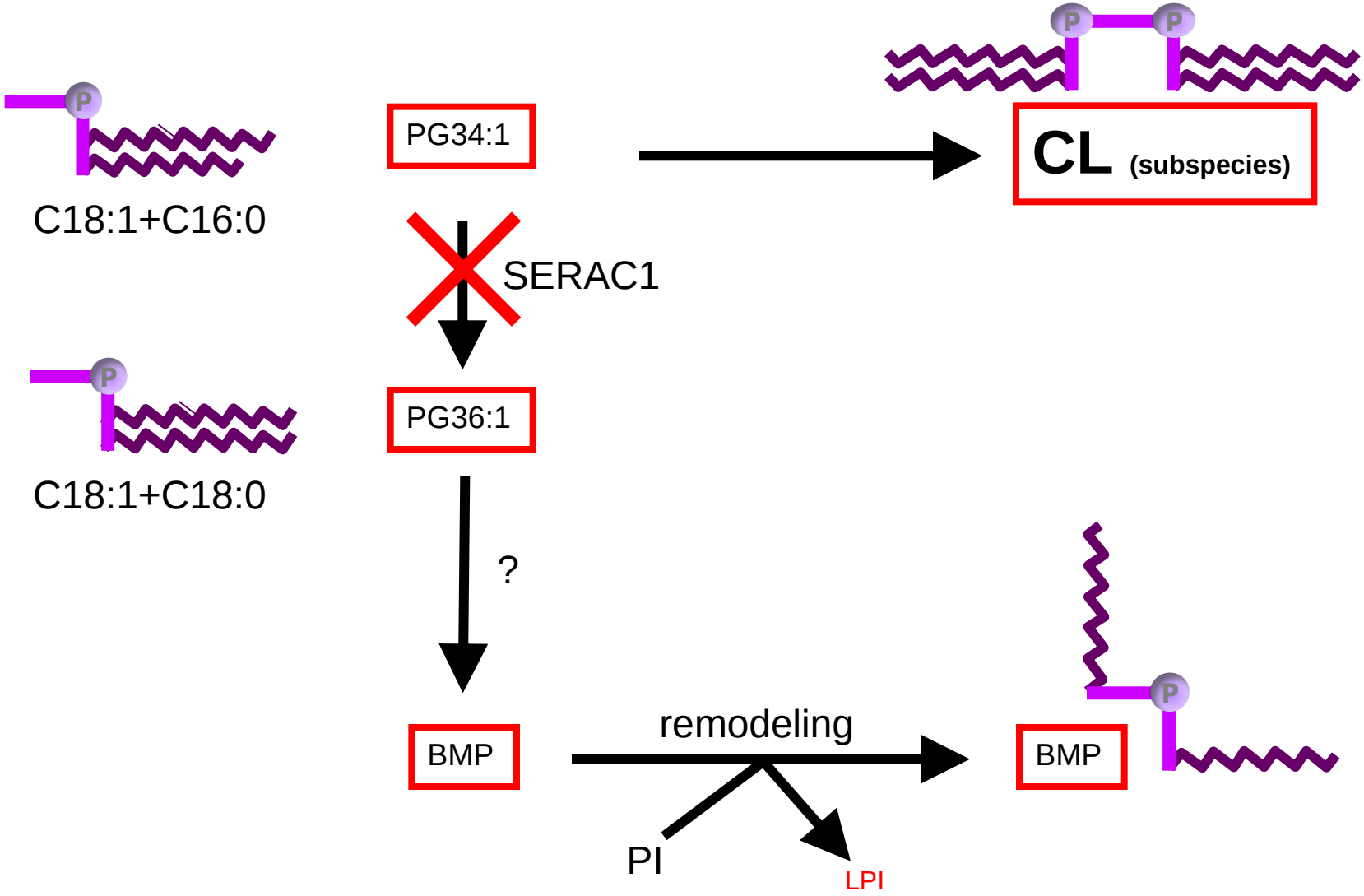
The surprise of the pipeline





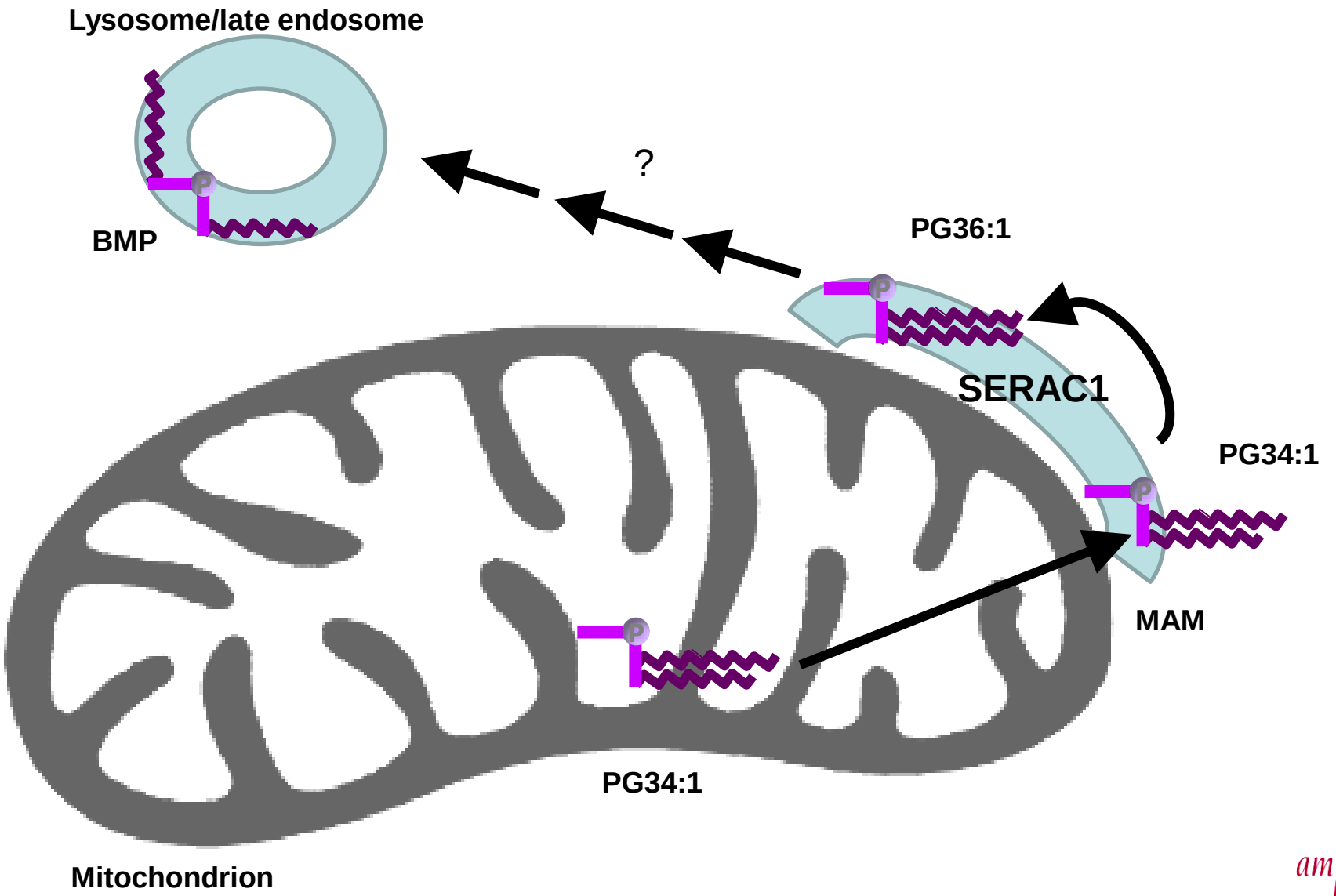


Hypothesized function of SERAC1



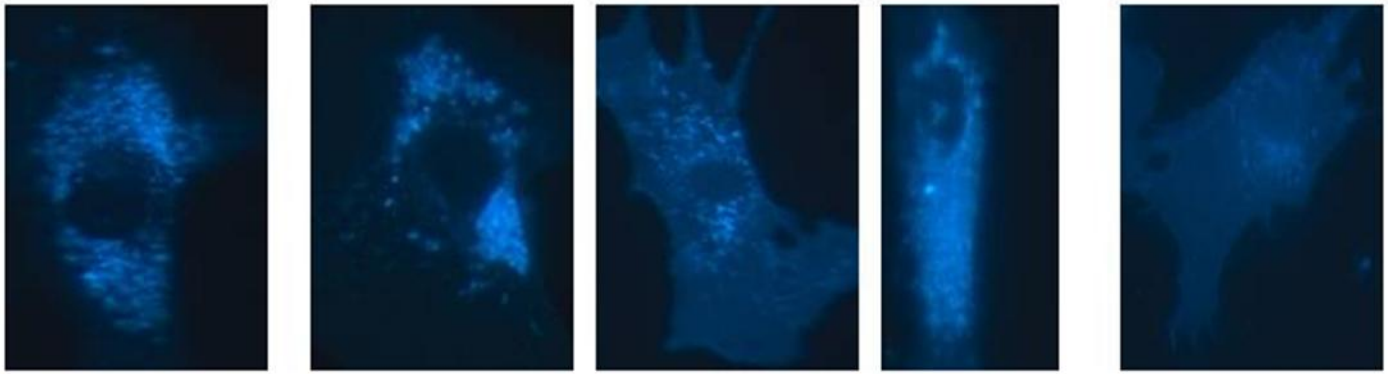
SERAC1 is a PG transacylase involved in the formation of PG36:1, which is a precursor of BMP.

SERAC1 function in cellular context



BMP function

- BMP is needed to export cholesterol from lysosomes
- Accumulation of cholesterol in MEGDEL syndrome?



- BMP deficiency results in cellular cholesterol accumulation.

Conclusions (1)

- MEGDEL syndrome is new subtype of 3-methylglutaconic aciduria
- MEGDEL syndrome is, like Barth syndrome, a defect in phospholipid metabolism
- A lot of “loose ends”

Conclusions (2)

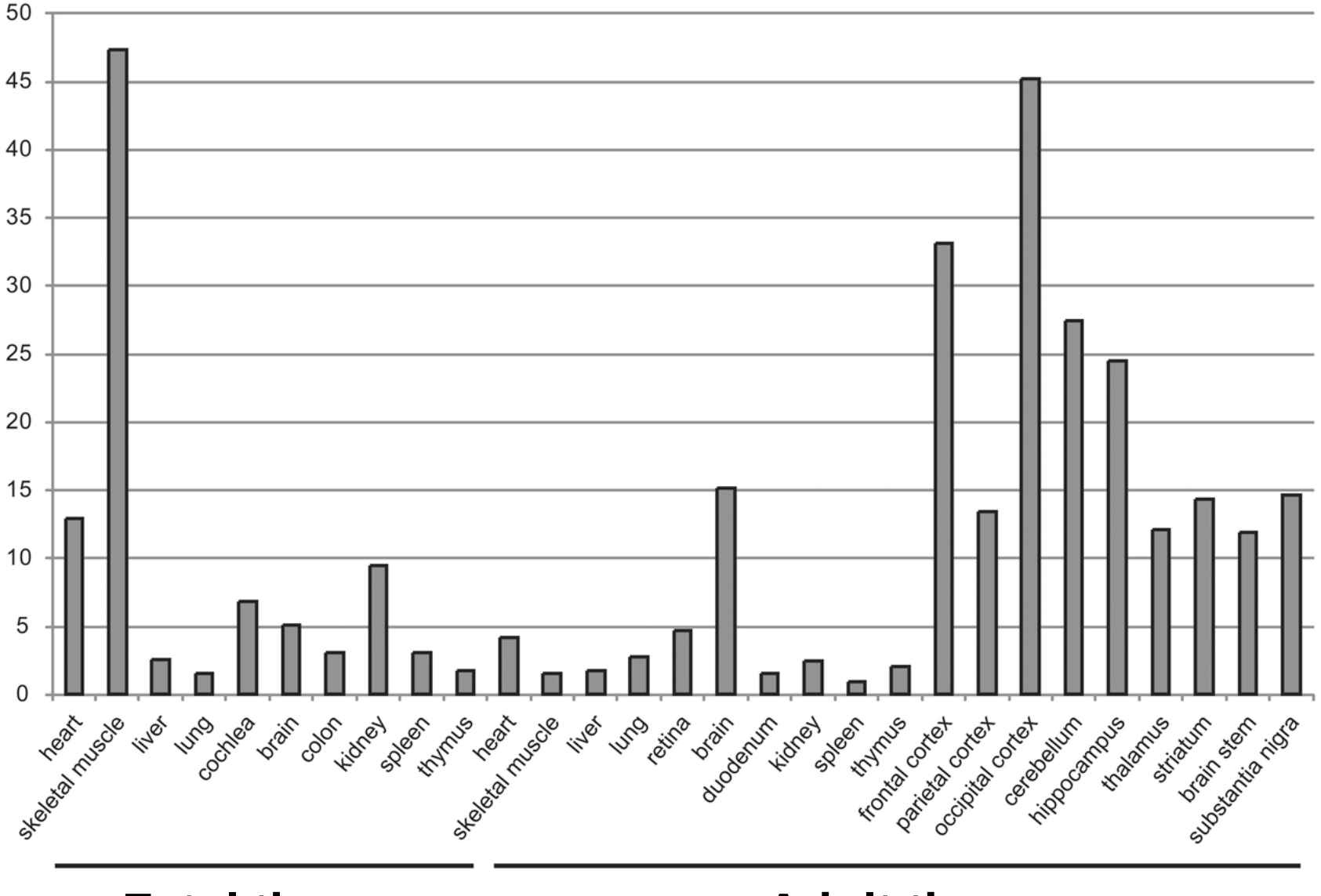
- Phospholipid analysis in combination with automated analysis of the raw data is a powerfull research/diagnostic tool

- This combined approach elucidated the functional defect in MEGDEL syndrome

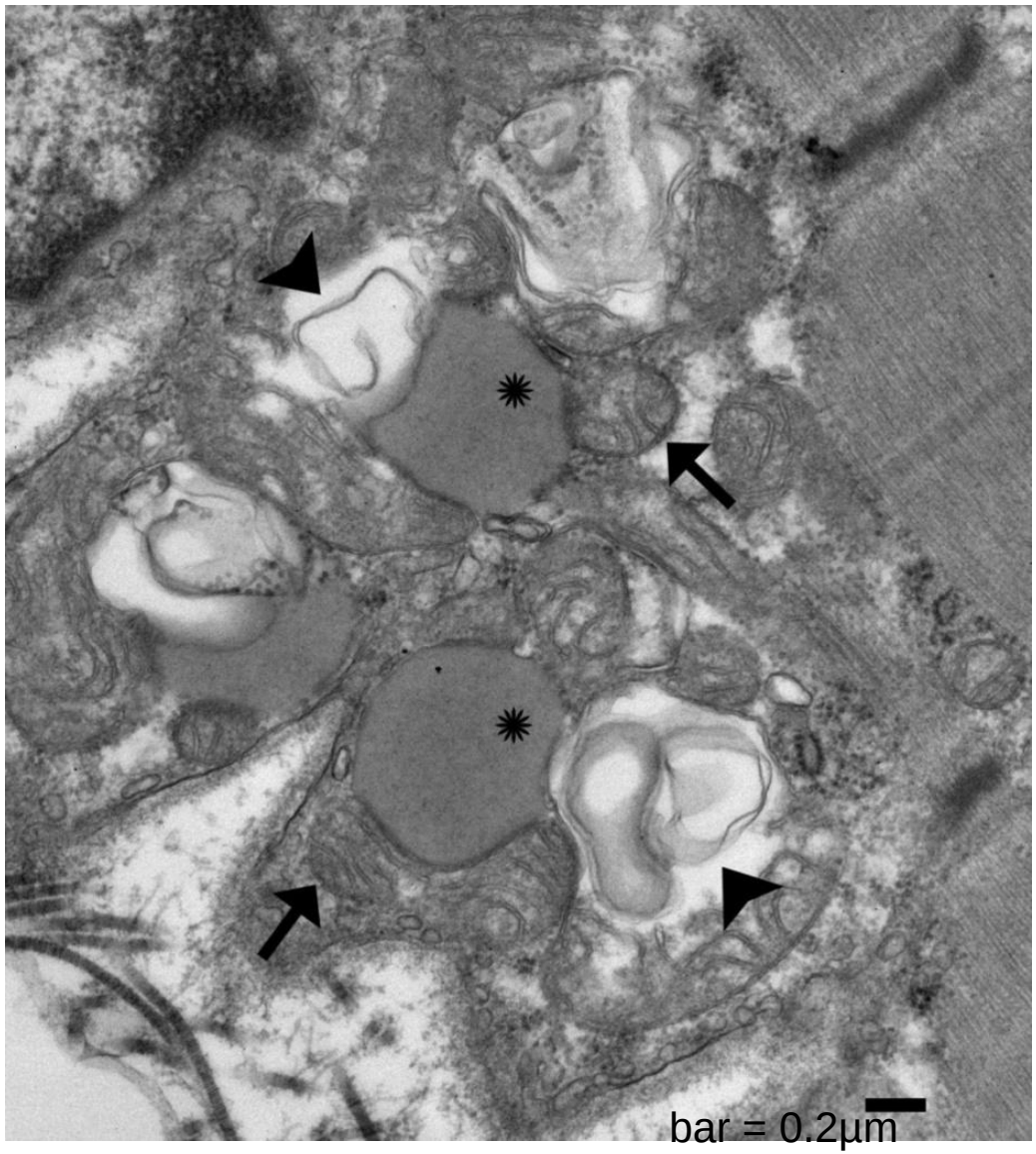
Thank you for your attention!

Questions?

SERAC1 expression profile



Perspective/Discussion



Mitochondria (arrows)
lysosomes with fat droplets (asterisks)
membranous remnants (arrowheads)