A Quantitative Positive/Negative Switching Method for Shotgun Lipidomics via High Resolution LC-MS/MS from any Biological Source

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Integrating Different –*Omics* Approaches can Reveal insight into Biological Systems of Disease

Global Phosphoproteomics: Triple SILAC, ~12,000 pSTY sites (20 mg protein, 15x10E7 cells) x 3

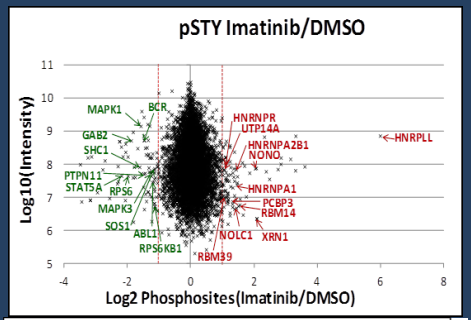
Targeted Metabolomics: 300 polar metabolites, >150 ^{13}C labeled metabolites (3x10E6 cells) x 3

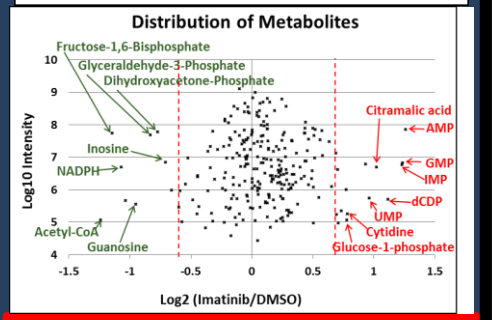
Untargeted Lipidomics: ~1,500 lipid molecules identified (1.5x10E7 cells) x 3

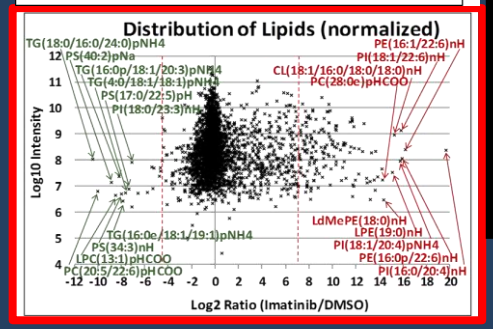
RP-C18, DDA, HCD

HILIC, SRM, +/- switch

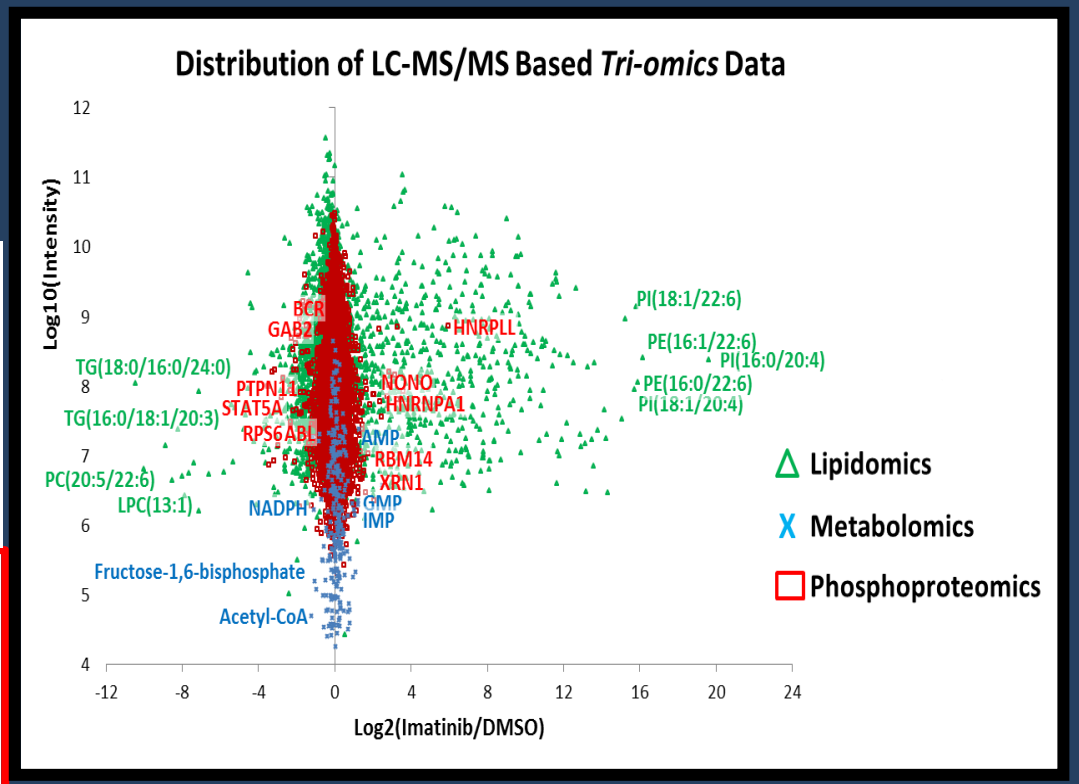
RP-C18, DDA, HCD, +/- switch







Imatinib treated H929 myeloma cells

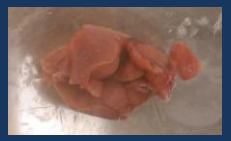


Breitkopf et al., *Anal Chem*, 2015

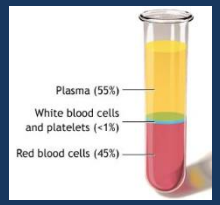
Platform for *Untargeted* Lipidomics

Cancer cells

Tumor tissue

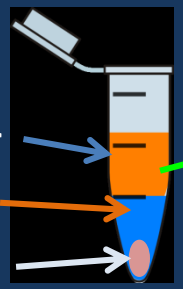


Blood plasma



flies

methyl-tert-butyl ether (MTBE)



Matyash et al, J. Lipid Res., 2008.

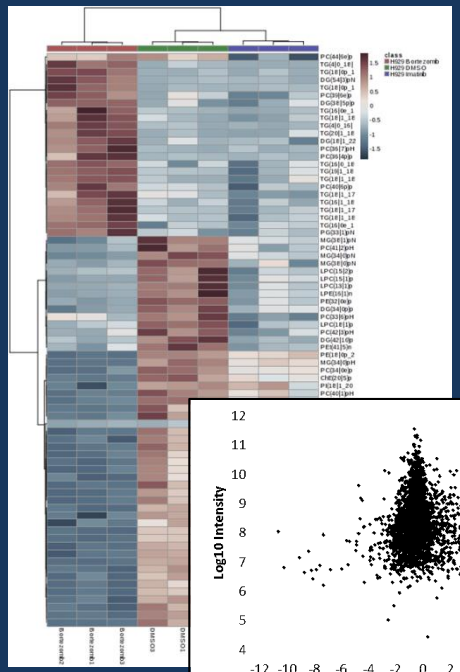
Thermo QExactive Plus/HF Agilent 1100/1200

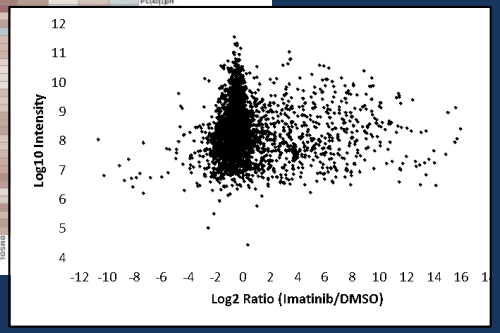
2.1 mm x 10 cm C_{18} (low pH, high organic)

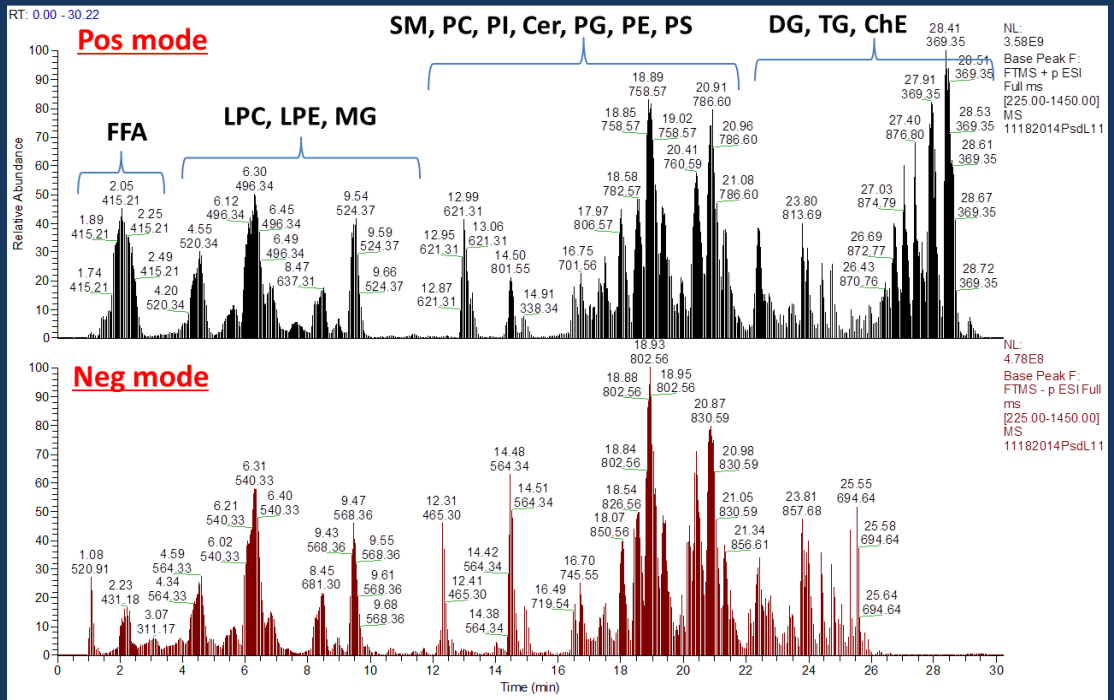
260 µL/min

Pos/Neg polarity switching (~10 points/peak)

HCD-DDA (Top 10) pos *and* neg mode







Fatty Acid Synthesis

Lipid Arrays

Glycolysis
↓
TCA cycle
↓
Acetyl-coA
↓ **ACC**
Malonyl-coA
↓ **FAS**

Palmitate (C16:0) —**SCD1 desaturation**→ Palmitoleate (C16:1) →

- increase insulin sensitivity (high conc. in liver)
- inhibit the destruction of insulin-secreting pancreatic beta cells

↓ **elongation**

Stearate (C18:0) —**SCD1 desaturation**→ Oleate (C18:1) →

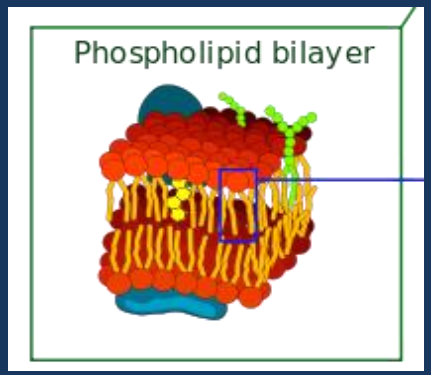
- Mostly present as triglycerides (olive oil, animal fat, etc.)

↓ **elongation**

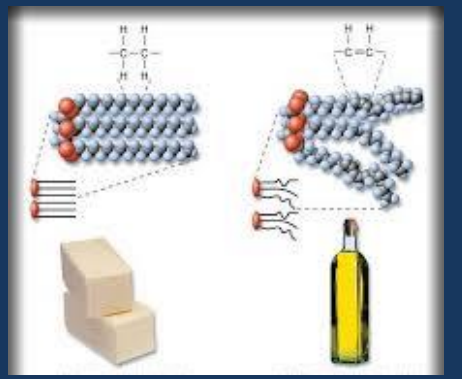
Arachidate (C20:0) —**desaturation**→ Eicosenoate (20:1) →

↓

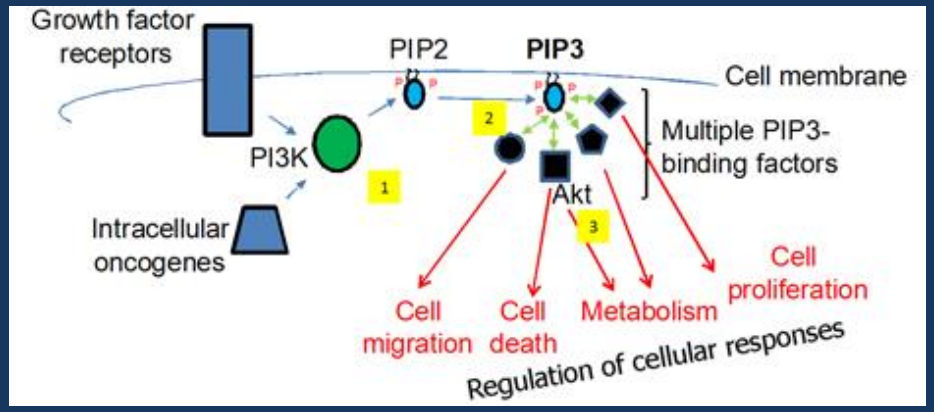
Membrane structure



Fats

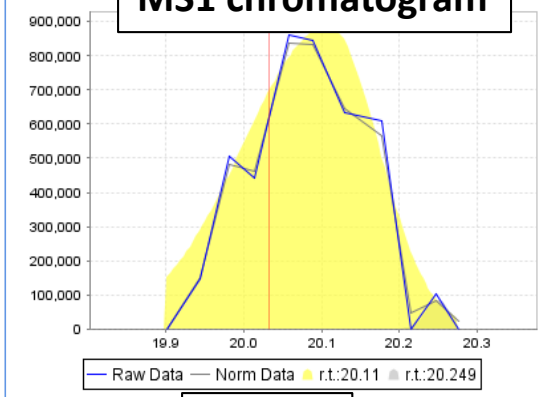


Signaling Lipids

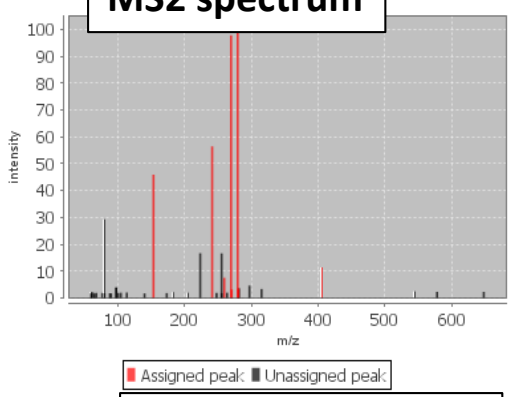


Thermo LipidSearch MS2 Based Identification Process

MS1 chromatogram



MS2 spectrum



Scoring

Match Lipid

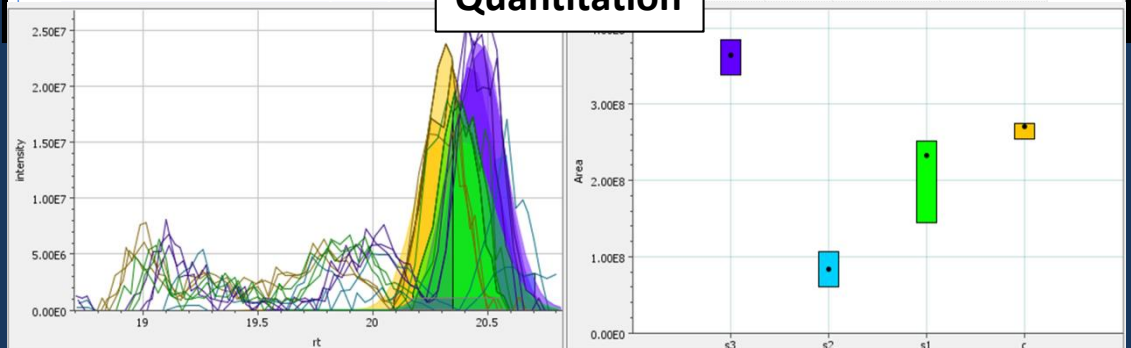
LipidIon	M-Sc.	T-Sc.	Sc.	St.
PI(17:0/18:2)-H	43.3	0.4	72.2	
PI(18:2/17:0)-H	43.3	0.4	72.2	
PI(16:0/19:2)-H	11.2	0.4	28	
PI(19:2/16:0)-H	11.2	0.4	28	
PI(17:1/18:1)-H	10.1	0.4	25.2	
PI(18:1/17:1)-H	10.1	0.4	25.2	
PI(11:0/24:2)-H	7.3	0.4	24.4	
PI(13:0/22:2)-H	7.3	0.4	24.4	
PI(15:0/20:2)-H	7.3	0.4	24.4	
PI(15:1/20:1)-H	7.3	0.4	24.4	
PI(16:1/19:1)-H	7.3	0.4		

HCD fragmentation

Match Detail

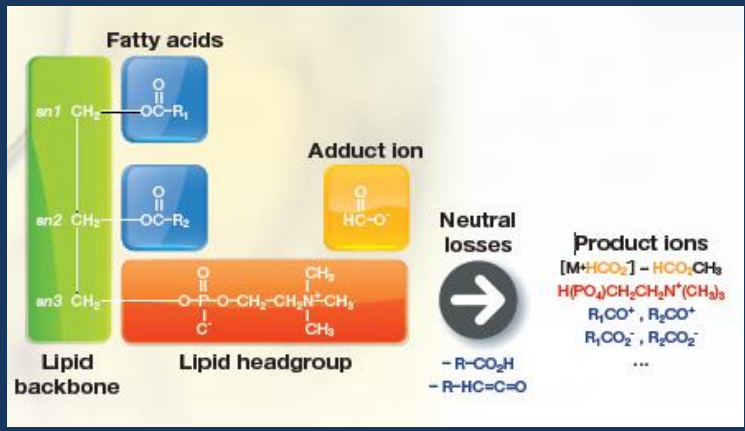
ObsMz	Type	It.(%)	Frag.	Delta(Da)
152.9949	MS2	45.906	GP-H3O	-0.0009
172.7457	MS2	1.882	-	-
182.1563	MS2	2.169	-	-
204.3326	MS2	2.012	-	-
223.0009	MS2	16.663	-	-
241.0119	MS2	56.445	PH(inositol)-H	0
247.5671	MS2	1.936	-	-
255.1215	MS2	1.828	-	-
255.2328	MS2	16.623	-	-
259.0225	MS2	7.551	IP	0.0001
263.2816	MS2	2.008	-	-
269.249	MS2	97.74	FA(17:0)-H	0.0004
270.2529	MS2	3.21	FA(17:0)-H [is	1.0043
	MS2	100	FA(18:2)-H	0.0003

Quantitation



- Untargeted identification based on *FRAGMENTATION and high mass accuracy MS and MS2 data*

Phospholipid

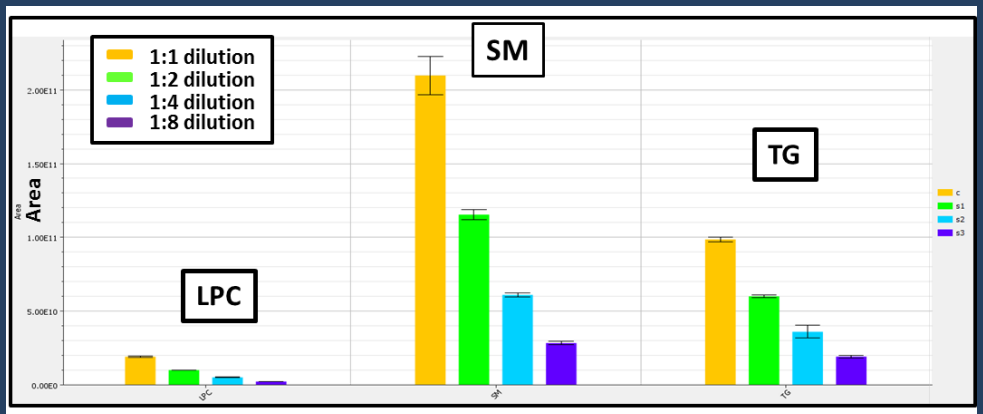


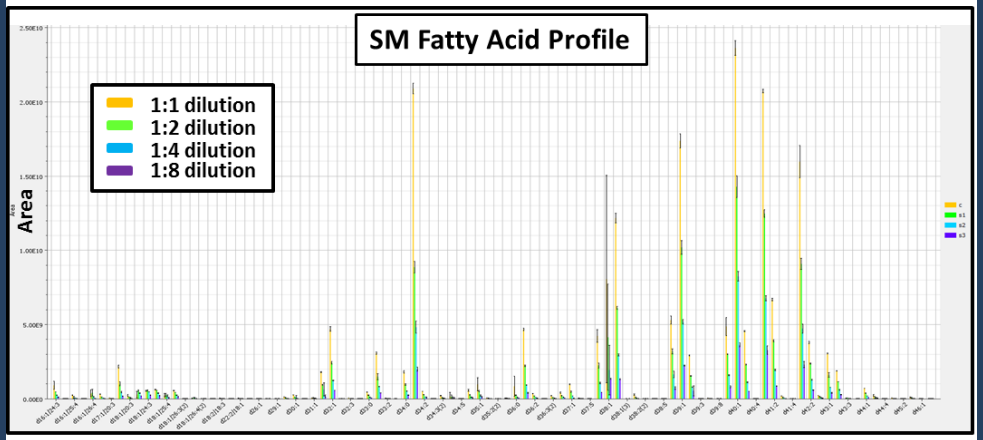
- Capable of identifying >1500 lipid ions in 30 min. with pos/neg switching from 66 subclasses of lipids
- Database assisted de novo interpretation

Lipid Family	Lipid Class	Abr.
P-Choline	lysophosphatidylcholine	LPC
	platelet-activating factor	PAF
	phosphatidylcholine	PC
P-Ethanol Amine	lysophosphatidylethanolamine	LPE
	lysodimethylphosphatidylethanolamine	LdMePE
	phosphatidylethanolamine	PE
	dimethylphosphatidylethanolamine	dMePE
P-Serine	lysophosphatidylserine	LPS
	phosphatidylserine	PS
P-Glycerol	lysophosphatidylglycerol	LPG
	phosphatidylglycerol	PG
P-Inositol	lysophosphatidylinositol	LPI
	phosphatidylinositol	PI
	phosphatidylinositol	PIP
	phosphatidylinositol	PIP2
	phosphatidylinositol	PIP3
P-Ethanol	lysophosphatidylethanol	LPEt
	phosphatidylethanol	PEt
P-Acid	lysophosphatidic acid	LPA
	phosphatidic acid	PA
	cyclic phosphatidic acid	cPA
P-Methanol	lysophosphatidylmethanol	LPMe
	phosphatidylmethanol	PMe
Sphingolipids	sphingomyelin	SM
	sphingomyelin(phytosphingosine)	phSM
Neutral glycerolipid	monoglyceride	MG
	diglyceride	DG
	triglyceride	TG
Fatty Acid	fatty acid	FA
	(O-acyl)-1-hydroxy fatty acid	OAHFA
Cardiolipin	Cardiolipin	CL
Sphingoid base	Sphingoshine	So
	Sphingoshine phosphate	SoP
Glycosphingolipids	Ceramides	Cer
	Ceramides phosphate	CerP
	Gangliosides	GM3
	Gangliosides	GM2
	Gangliosides	GM1
	Gangliosides	GD1a
	Gangliosides	GD1b
	Gangliosides	GD2
	Gangliosides	GD3
	Gangliosides	GT1a
	Gangliosides	GT1b
	Gangliosides	GT1c
	Gangliosides	GT2
	Gangliosides	GT3
	Gangliosides	GQ1c
	Gangliosides	GQ1b
Neutral Glycosphingolipids	Simple Glc series	CerG1
	Simple Glc series	CerG2
	Simple Glc series	CerG3
	Simple Glc series	CerG2GNAc1
	Simple Glc series	CerG3GNAc1
	Simple Glc series	CerG3GNAc2
Steroid	Cholesteryl Ester	ChE
	zymosteryl	ZyE
	Stigmasteryl ester	StE
	Sitosteryl ester	SiE
Coenzyme	Coenzyme	Co
Glycoglycerolipid	Monogalactosylmonoacylglycerol	MGMG
	Monogalactosyldiacylglycerol	MGDG
	Digalactosylmonoacylglycerol	DGMG
	Digalactosyldiacylglycerol	DGDG
	Sulfoquinovosylmonoacylglycerol	SQMG
	Sulfoquinovosyldiacylglycerol	SQDG

- **18 lipid classes and 66 subclasses can be identified via LC-MS/MS and LipidSearch**

Lipid Standards Demonstrate Quantitative Reproducibility and Accuracy





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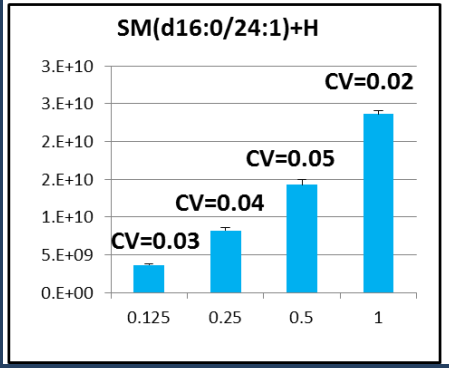
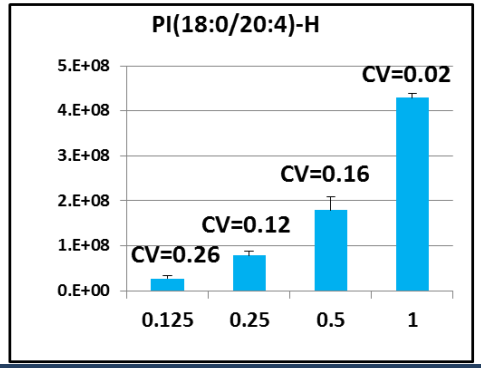
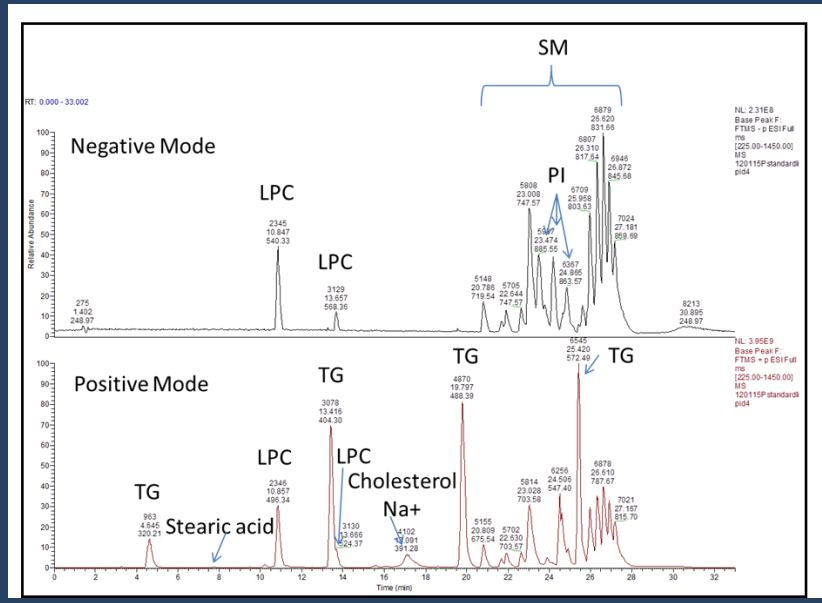
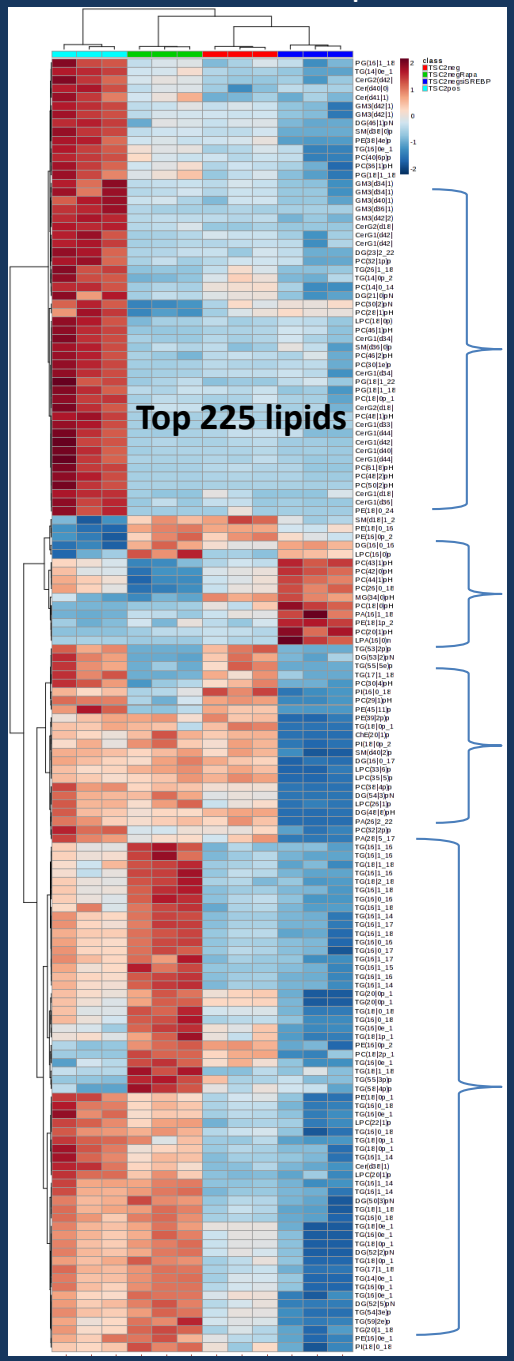




Lipid standards



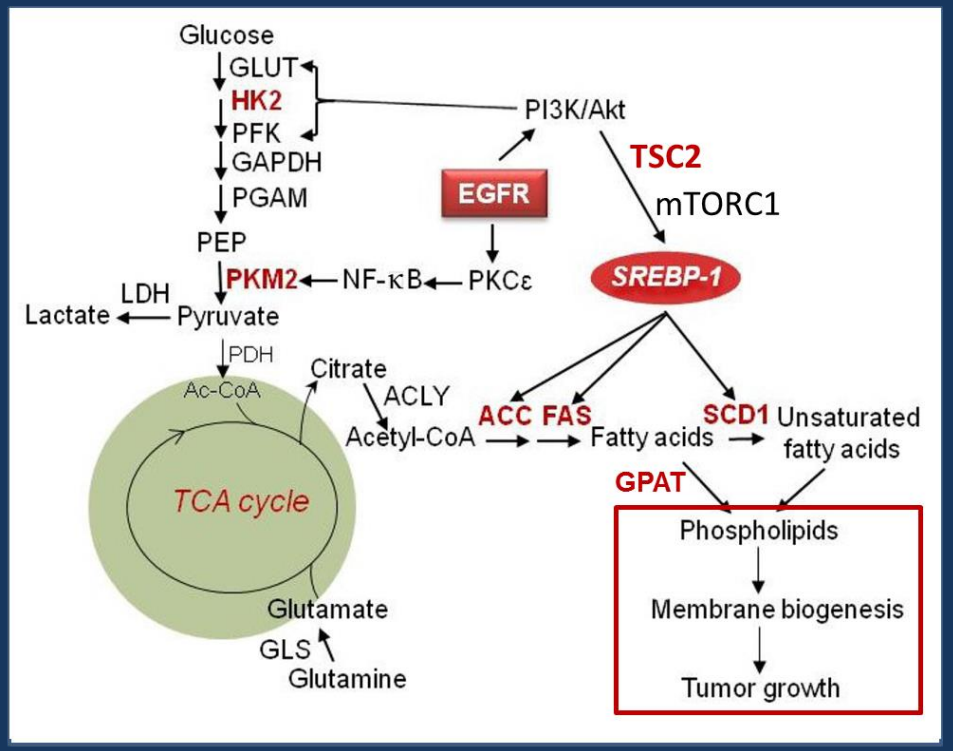
Clustering of TSC2 Lipidomics Data using *MetaboAnalyst*



Biological replicates

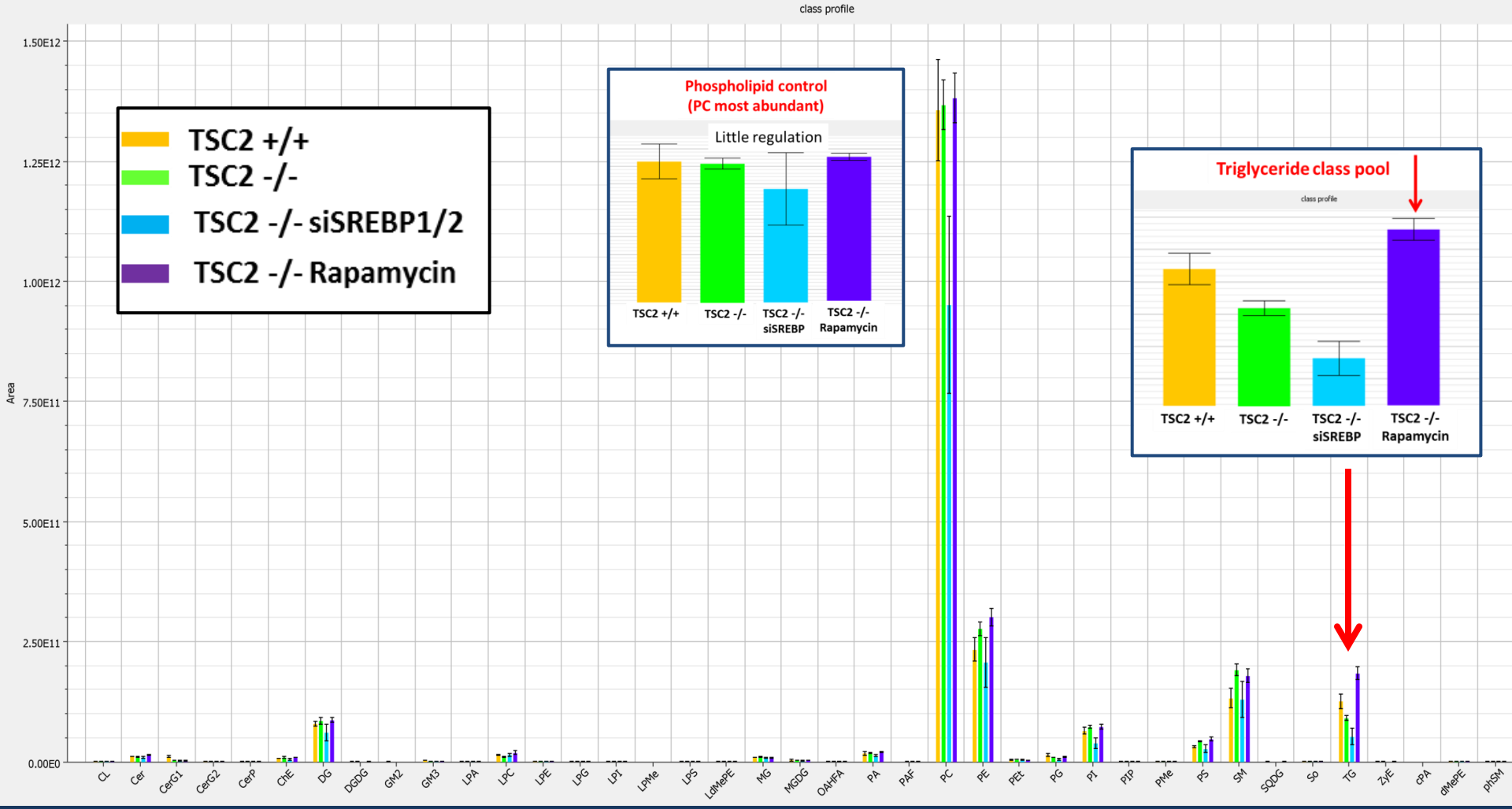
$R^2 > 0.95$

De Novo Lipid Synthesis Pathway



Ru et al, Cancers 2013, 5(4), 1469-1484

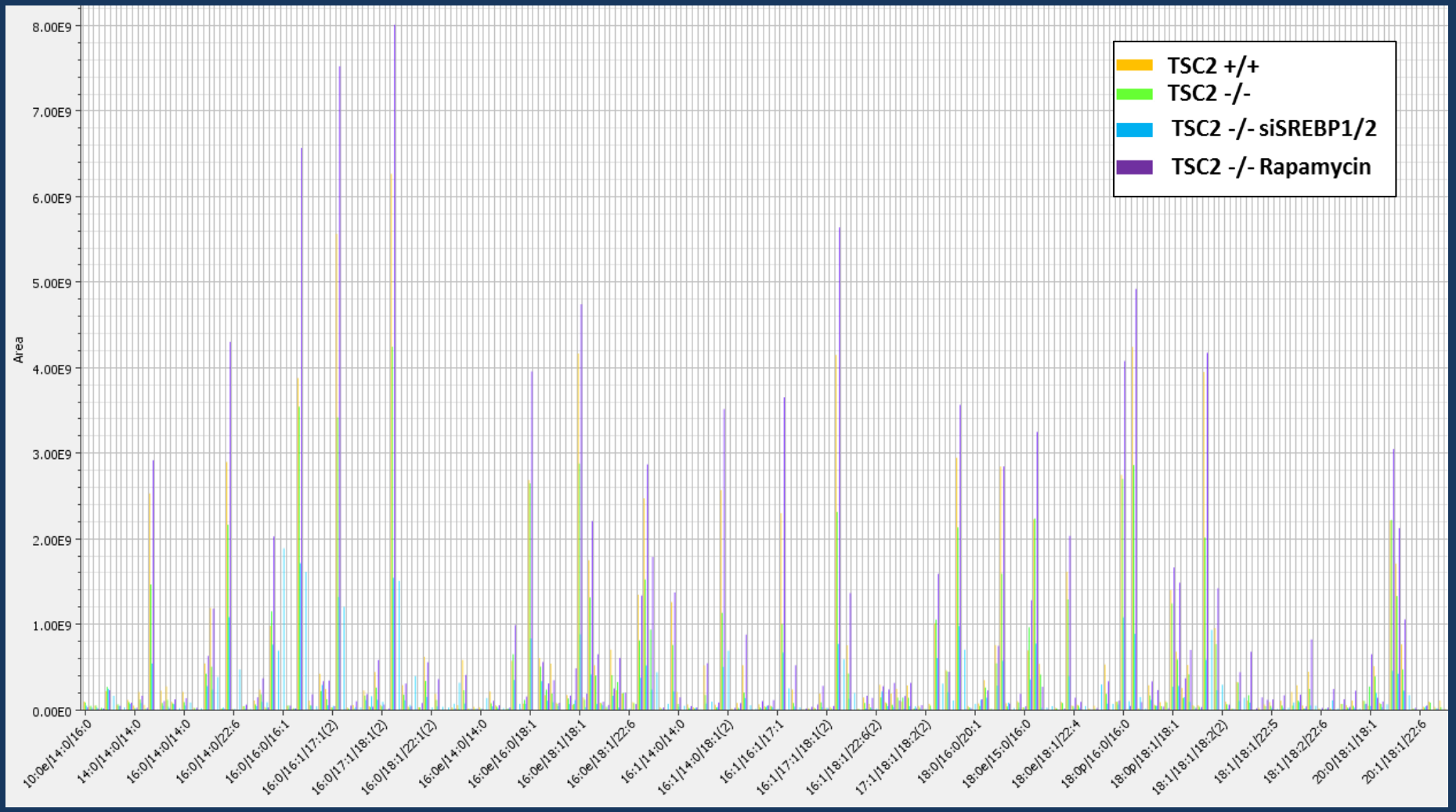
Overview of Lipid Class regulation in TSC2-/- MEFs



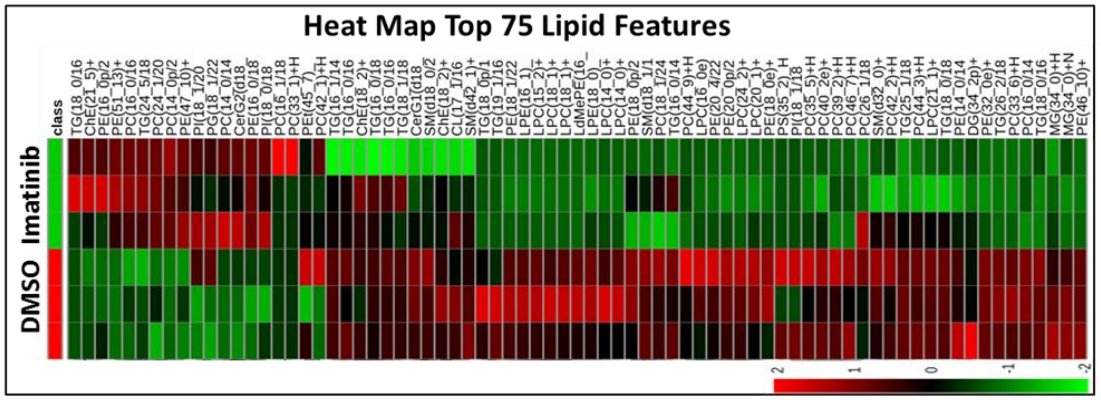
Triglyceride (TG) levels significantly increase in Rapamycin treated cells -hyperlipidemia in patients

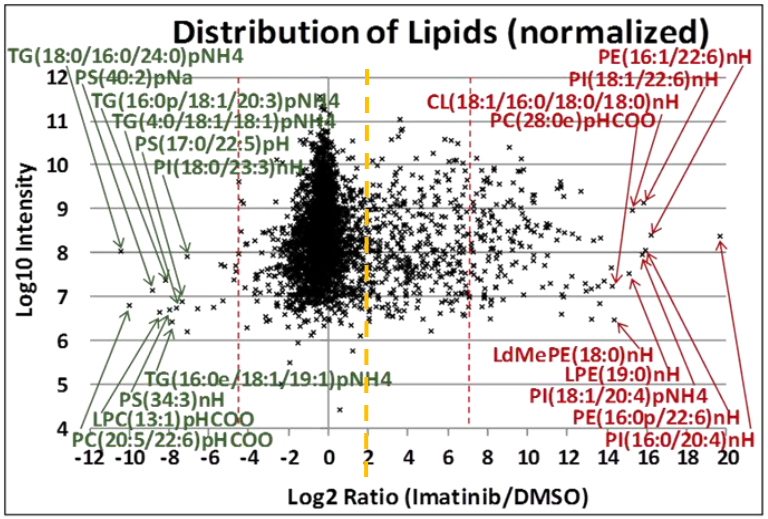
Morrisett et. al., *J. Lipid Research*, 2002

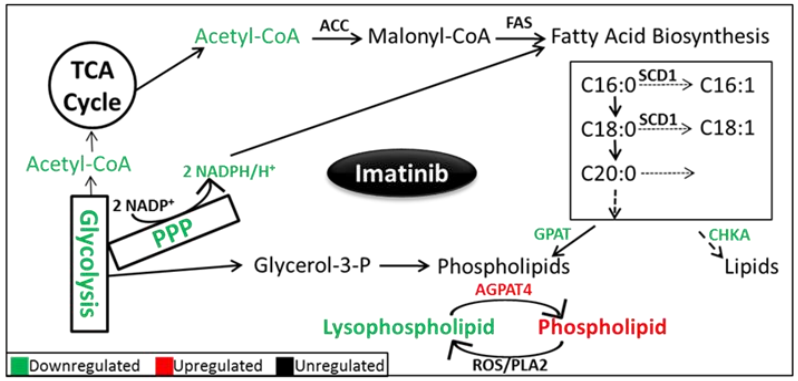
Triglyceride fatty acid composition in TSC2 MEFs using LipidSearch



- Rapamycin rescues TG levels in TSC2-/- MEFs similar to TSC2+/+ levels
- Many major TG fatty acids are basic building blocks (palmitate, oleate, etc.)

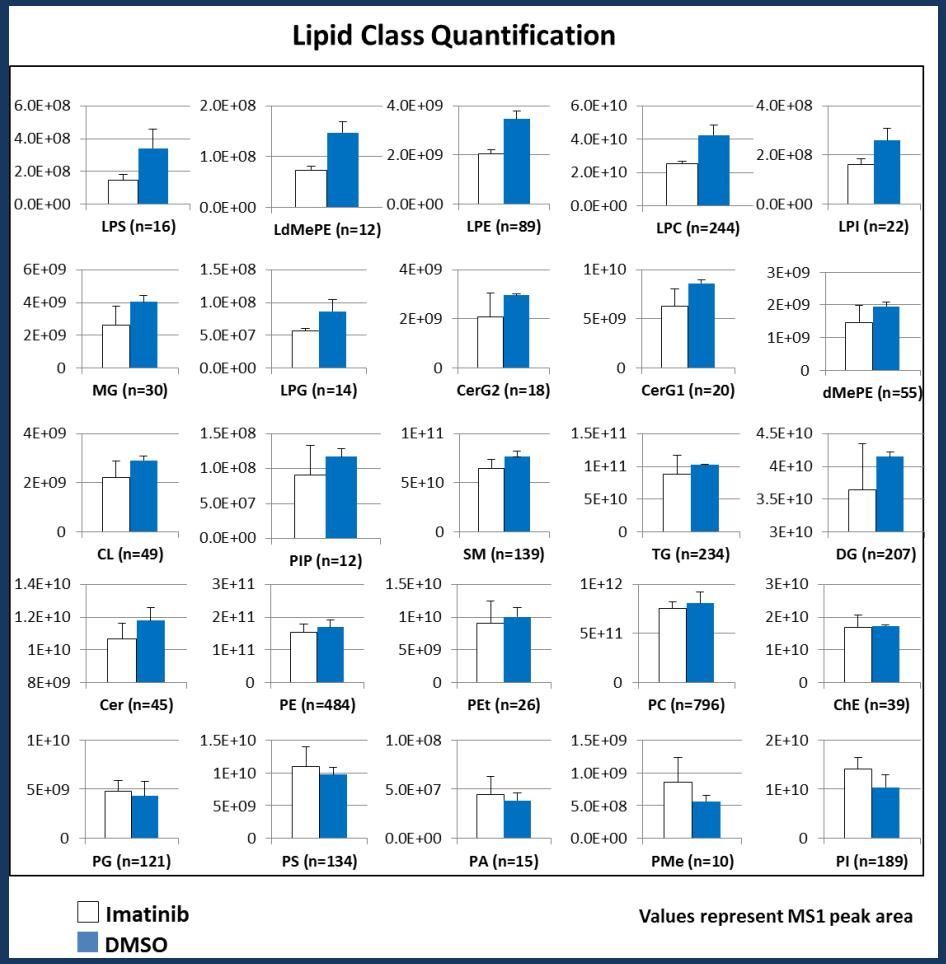






H929 Myeloma cancer cells

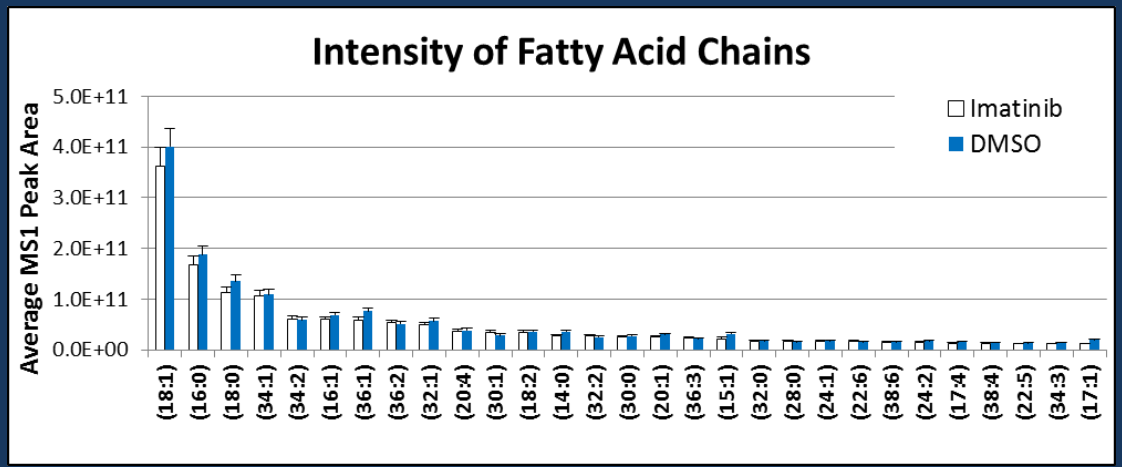
Lipidomics from Imatinib treated BCR/ABL cells for 1hr



H929 Myeloma cancer cells

Decrease in Lipid Classes and Fatty Acids with 1 hr imatinib treatment

More detail needed



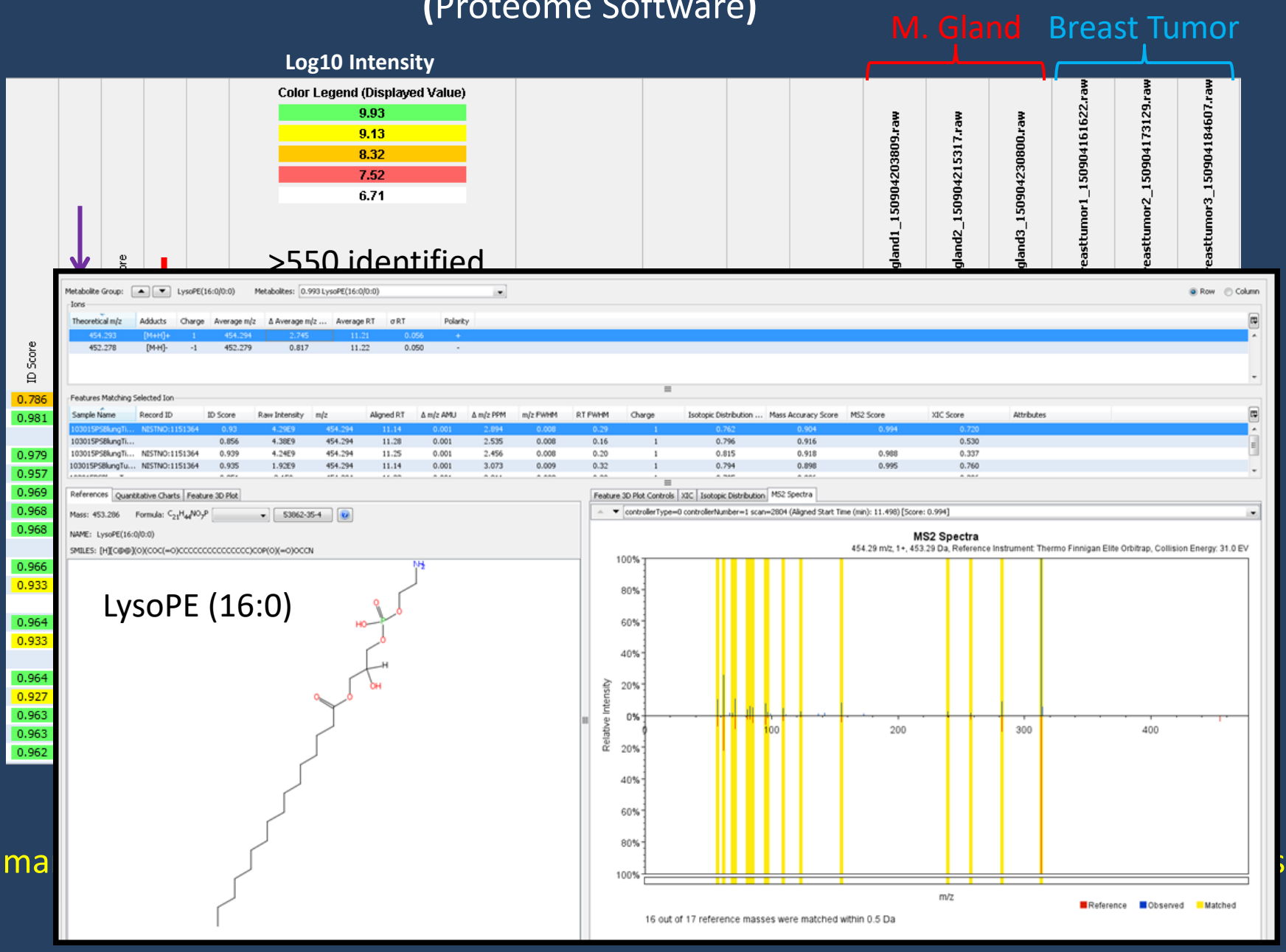
Spectral library matching using NIST and Elements software

NIST: 234,284 MS/MS spectra (open access)

mzCloud 146, 686 filtered MS/MS spectra (Thermo)
METLIN 72,268 MS/MS spectra (Agilent)
LipidSearch MS/MS spectral database not needed for MS2 identification

Untargeted Metabolomics/Lipidomics (unknowns) using *Elements* Software

(Proteome Software)

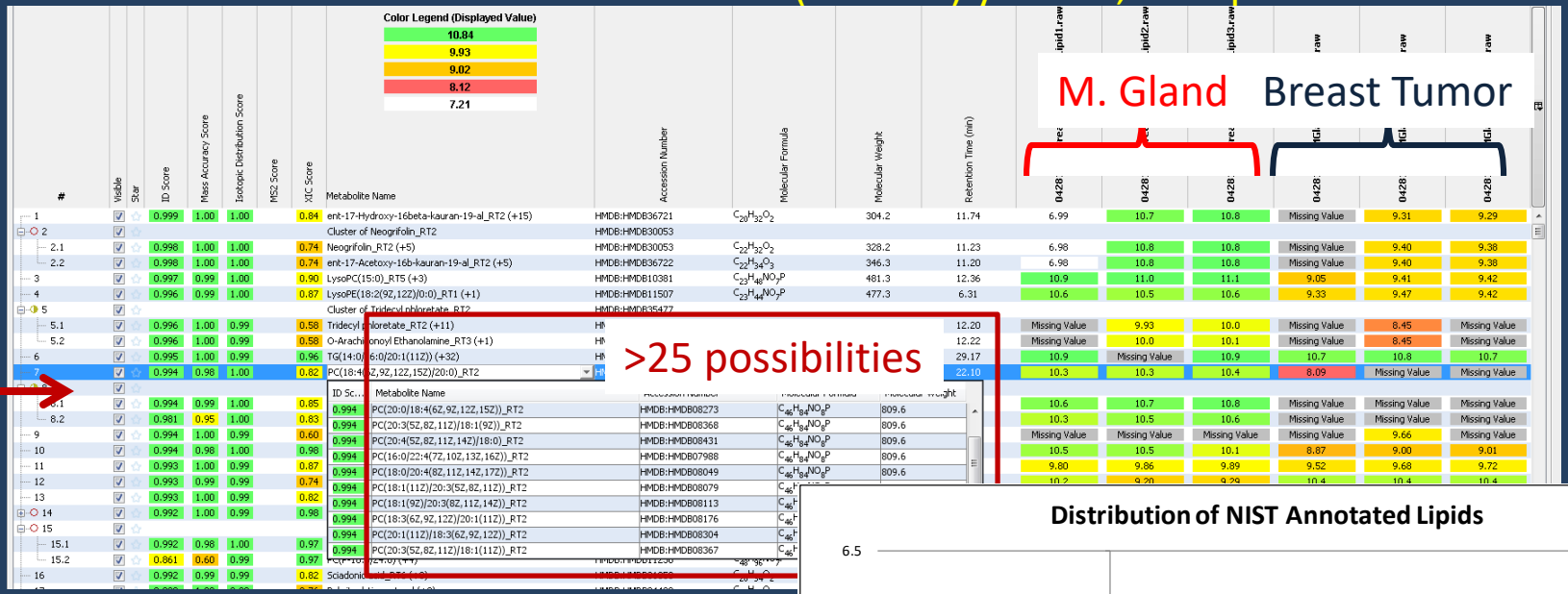


The ma

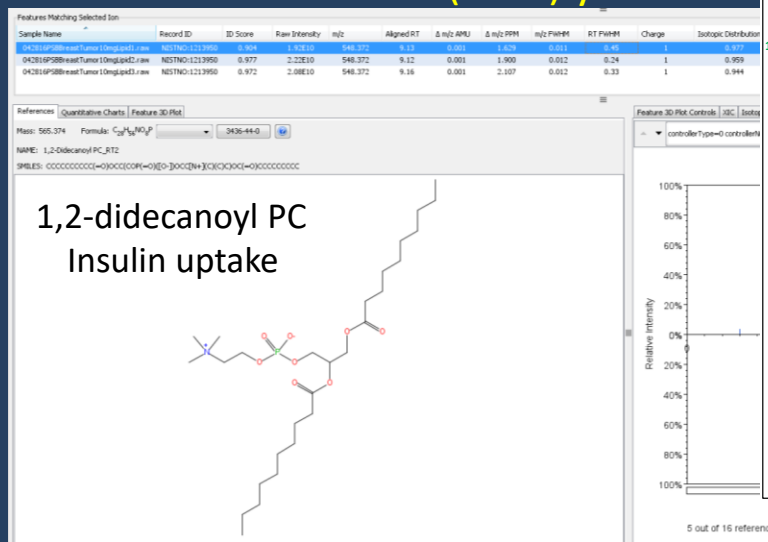
I need MS2 fragmentation data in order to trust an identification

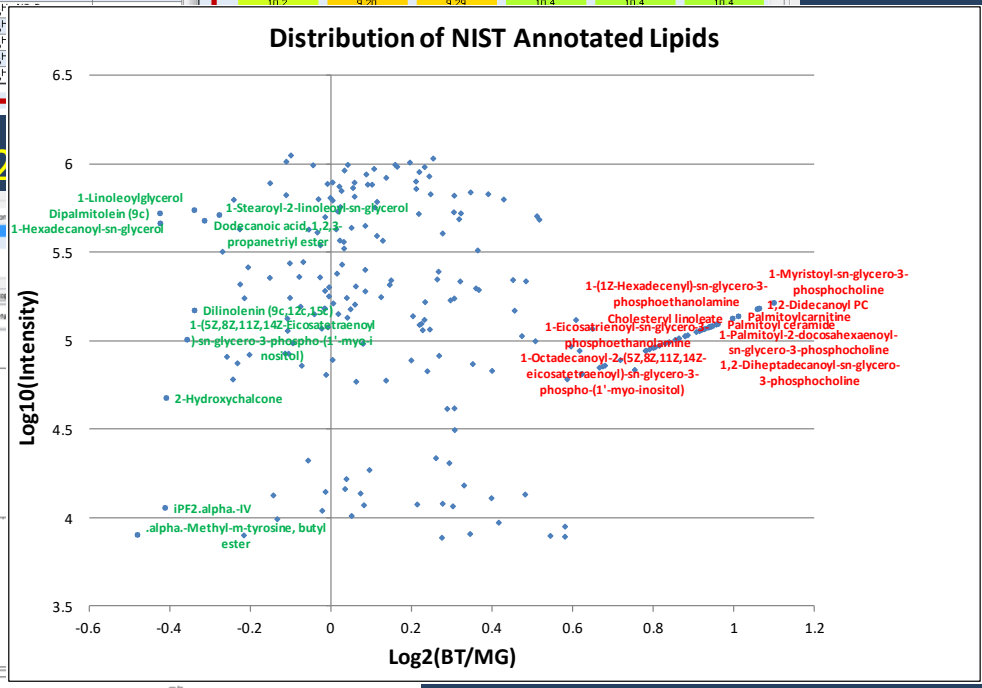
- Even sub 1-2 ppm DB searches can be ambiguous in structure without MS2

Elements Search with HMDB (no MS2) yields 1,313 lipid IDs



Elements Search with NIST (MS2) yields 322





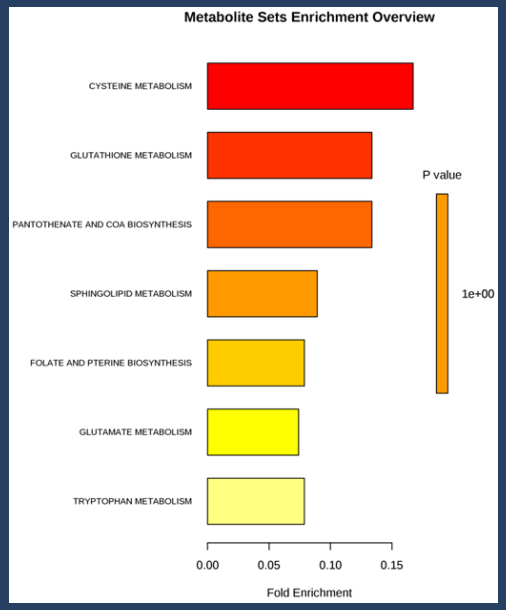
How Do You Perform Informatics of Lipid Data?

NIST MS2 IDs from Elements software

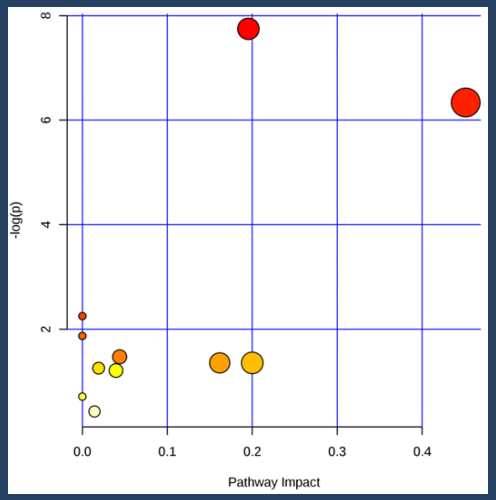
Convert to KEGG/HMDB using Fiehn lab tool

MetaboAnalyst Pathway Enrichment/Mapping

Pathway Enrichment



Pathway Mapping



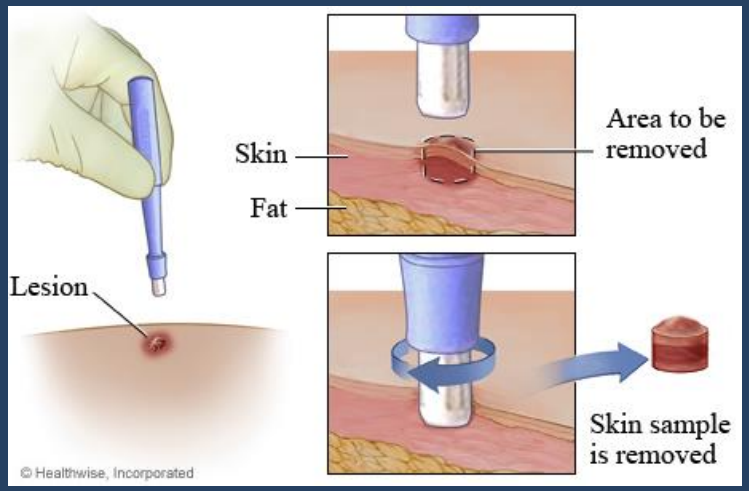
Identified Pathways

Pathway Name	Total	Hit	p	-log(p)	Holm p	FDR	Impact
Sphingolipid metabolism	21	4	0.0069634	4.9671	0.571	0.571	0.04261
Glycerophospholipid metabolism	30	3	0.10292	2.2738	1.0	1.0	0.25525
Linoleic acid metabolism	6	1	0.20828	1.5689	1.0	1.0	0.0
alpha-Linolenic acid metabolism	9	1	0.2958	1.2181	1.0	1.0	0.0
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	14	1	0.42104	0.86504	1.0	1.0	0.0439
Pantothenate and CoA biosynthesis	15	1	0.44332	0.81347	1.0	1.0	0.32653
Tryptophan metabolism	40	2	0.45603	0.7852	1.0	1.0	0.11562
Folate biosynthesis	16	1	0.46476	0.76623	1.0	1.0	0.0
Pentose and glucuronate interconversions	16	1	0.46476	0.76623	1.0	1.0	0.2
Starch and sucrose metabolism	19	1	0.52434	0.64562	1.0	1.0	0.03958
Glutathione metabolism	26	1	0.63919	0.44756	1.0	1.0	0.07824
Arachidonic acid metabolism	36	1	0.75748	0.27776	1.0	1.0	0.0
Drug metabolism - cytochrome P450	56	1	0.8914	0.11497	1.0	1.0	0.01429

LipidSearch results, while MS2 based, have no searchable annotation……

Our Goal is To Perform *Tri-Omics* from Single Tumor Biopsies

Typical Needle Biopsy



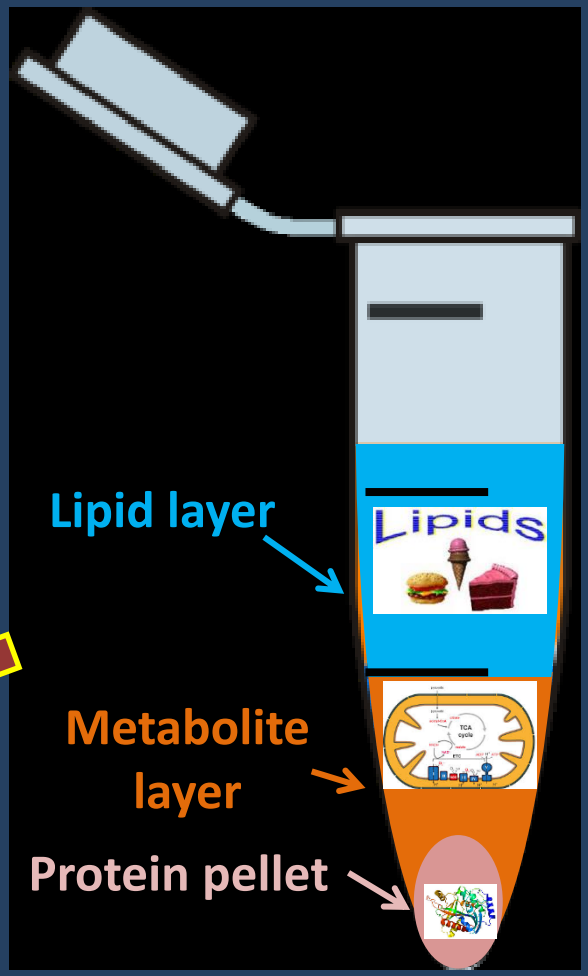
As little as 5-10 mg starting material

Labeling ($^{13}C/^{15}N$)

TMT

Methyl tert-butyl ether (MTBE)

Mainly used for lipid extractions



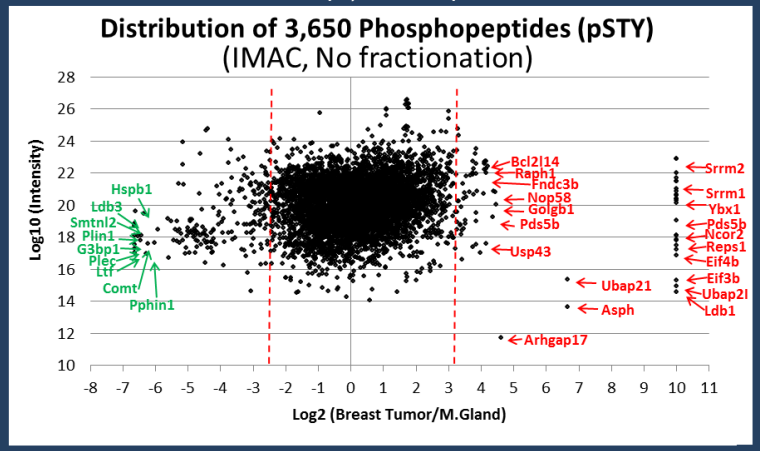
Serial-Omics "Super-Omics"

LC-MS/MS

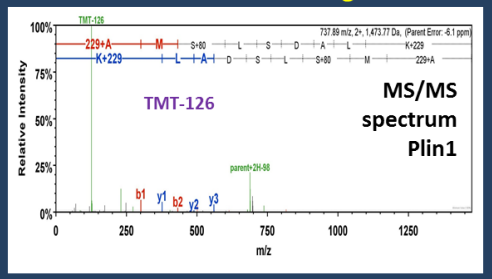
Metabolomics, Lipidomics and Phosphoproteomics)

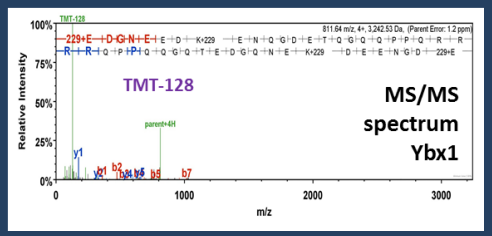
Serial-Omics on Mouse Breast Tumor vs Mammary Gland Tissue

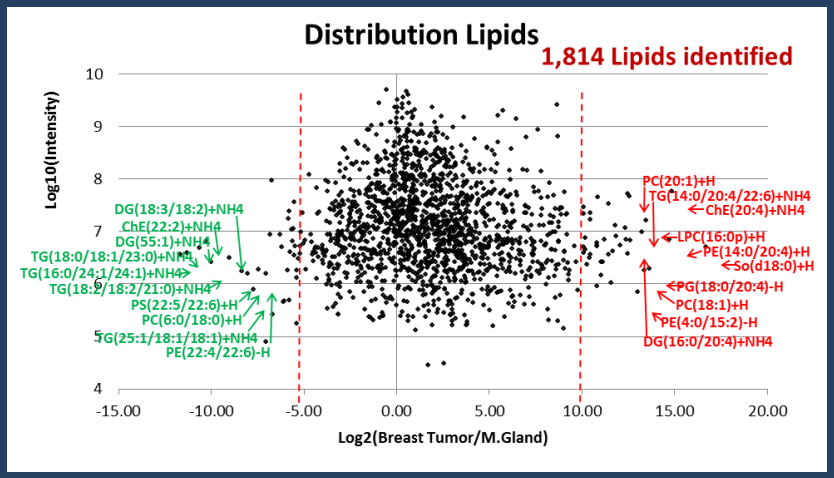
P53-/-, Brca1 -/-

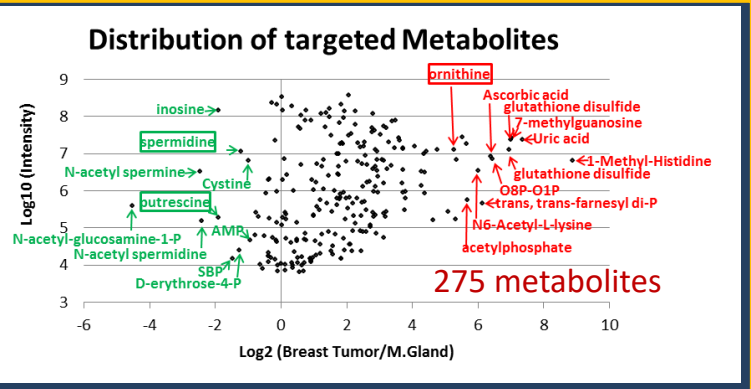


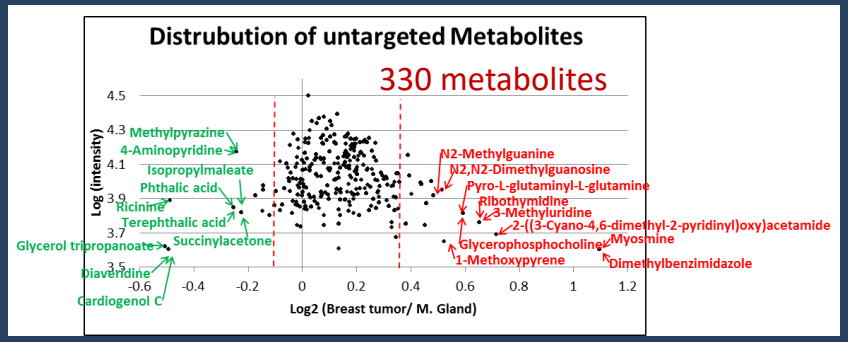
TMT Labeling



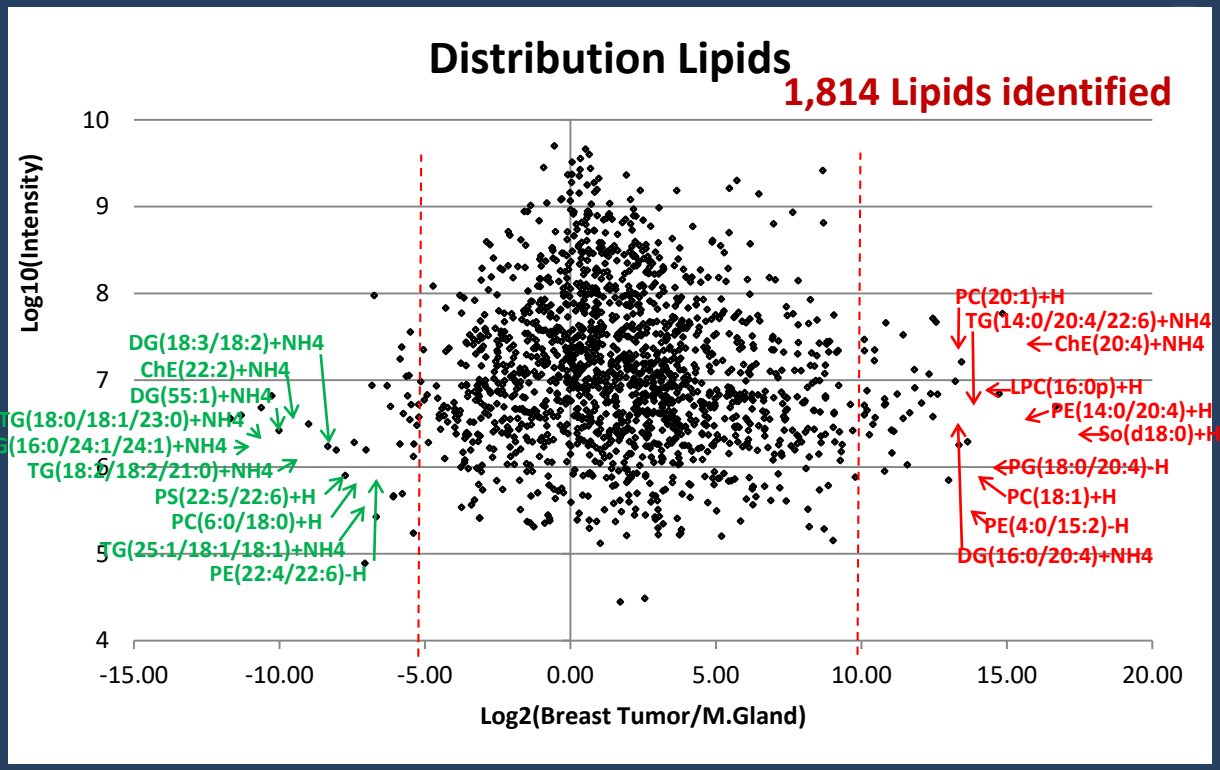


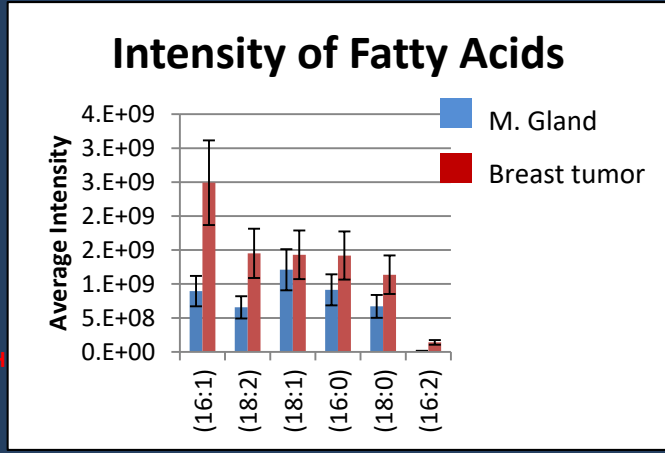


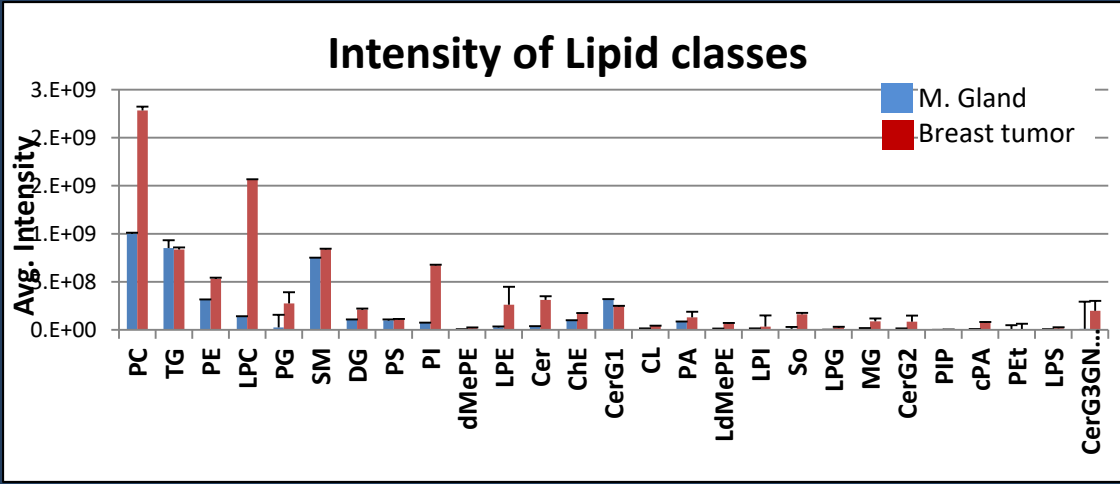




Serial-Omics: Lipidomics from *Upper Phase*-Breast Tumor vs. Mam Gland







Phospholipid Synthesis

↑

Fatty Acid Synthesis

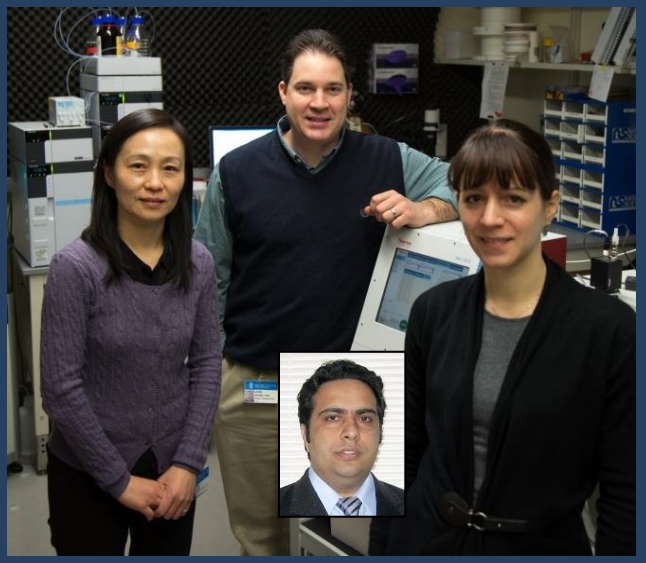
- **Phospholipids are most up-regulated especially PC, PE, LPC, PI, etc.**

Acknowledgements

Beth Israel Deaconess Medical Center / Harvard Medical School




Susanne Breitkopf
Stephane Ricoult
Ying Xu
Min Yuan
Hui Lui
Gerburg Wulf
Elena Levantini
Brendan Manning



Thermo Fisher Scientific

David Peake

Funding

NIH grants 5P01CA120964, R35CA197459
NIH DF/HCC Support 5P30CA006516
NIH 1S10OD010612