

June 5 - 9, 2016

Short Courses
June 4 - 5

San Antonio,
Texas

**64th Conference on
Mass Spectrometry
and Allied Topics**

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

John M. Asara, Ph.D.

Cancer Center



Beth Israel Deaconess
Medical Center



HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

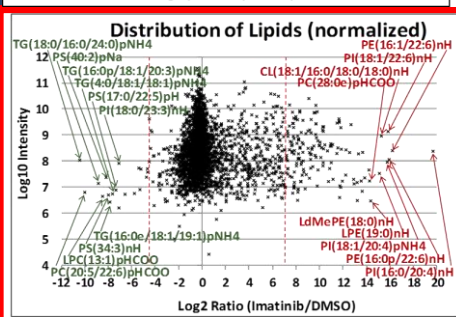
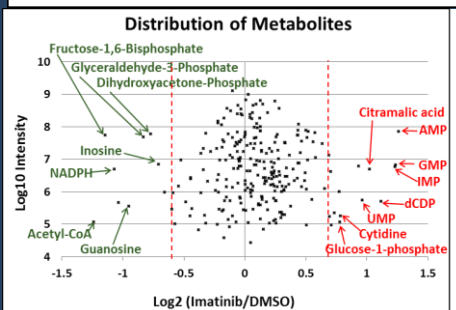
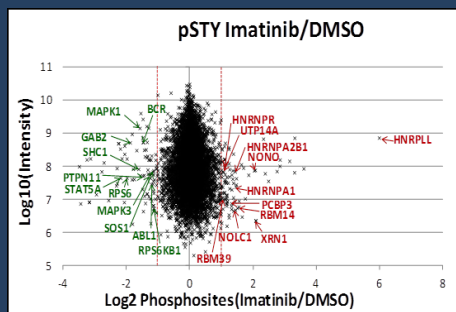
Integrating Different –Omics Approaches can Reveal insight into Biological Systems of Disease

Global Phosphoproteomics: Triple SILAC, ~12,000 pSTY sites (20 mg protein, 15x10⁷ cells) x 3

Targeted Metabolomics: 300 polar metabolites, >150 ¹³C labeled metabolites (3x10⁶ cells) x 3

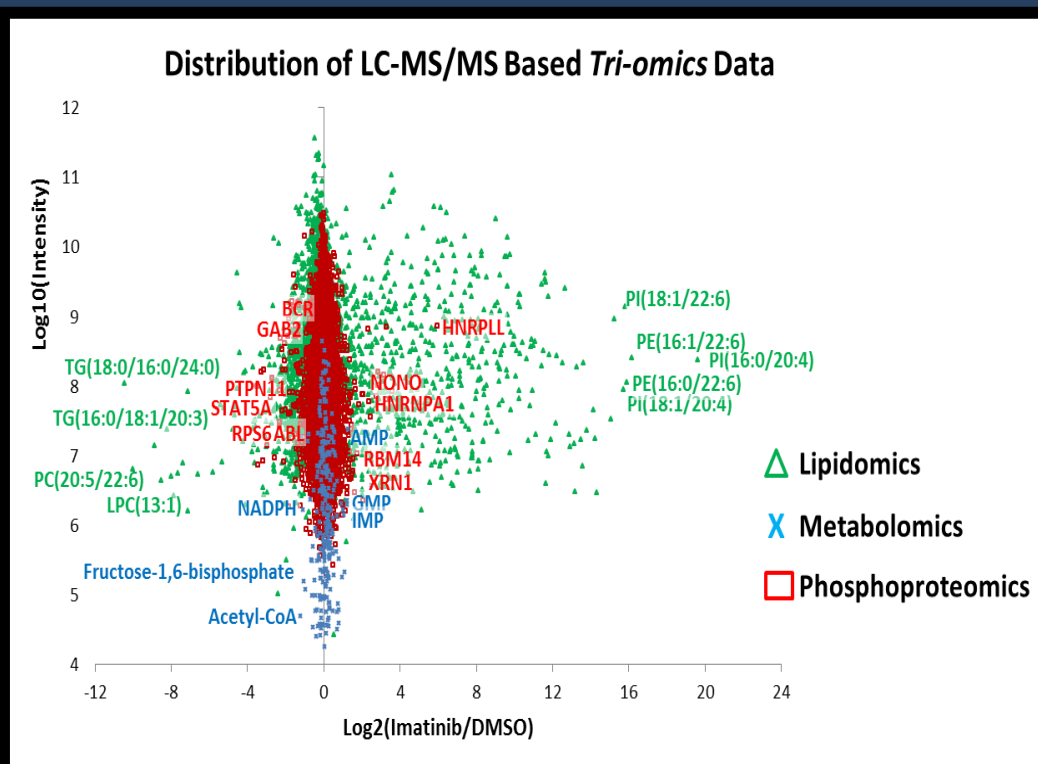
Untargeted Lipidomics: ~1,500 lipid molecules identified (1.5x10⁷ cells) x 3

RP-C18, DDA, HCD



Imatinib treated H929 myeloma cells

Distribution of LC-MS/MS Based *Tri-omics* Data



▲ Lipidomics
× Metabolomics
■ Phosphoproteomics

HILIC, SRM, +/- switch

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

Platform for *Untargeted* Lipidomics

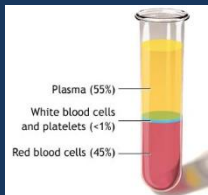
Tumor tissue

Cancer cells



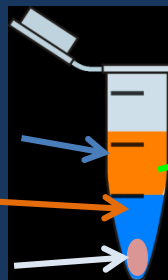
Blood plasma

flies



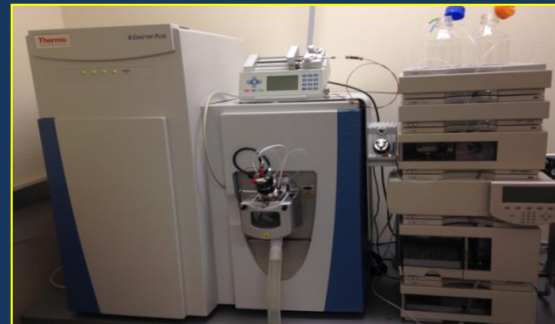
methyl-tert-butyl ether (MTBE)

Lipid layer
Aqueous layer
Solid pellet



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

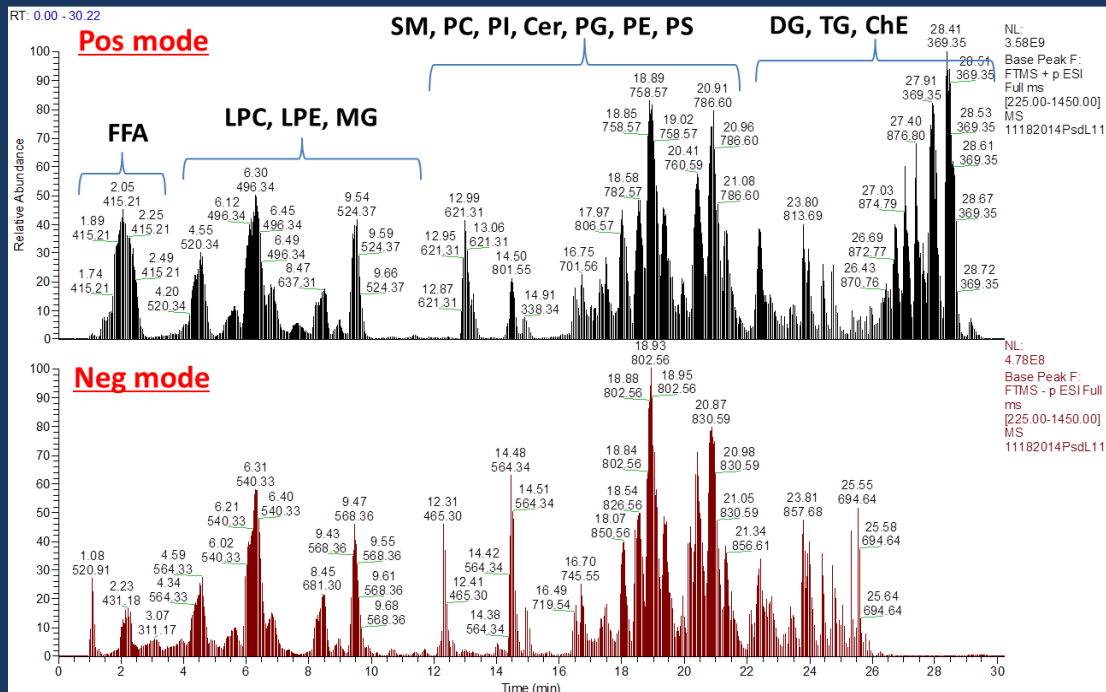
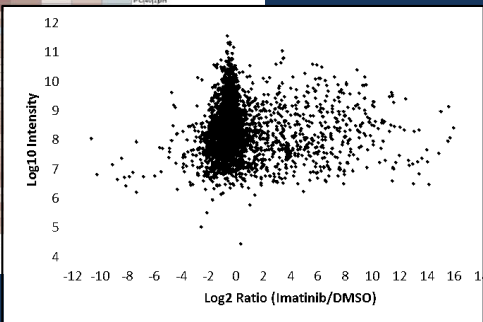
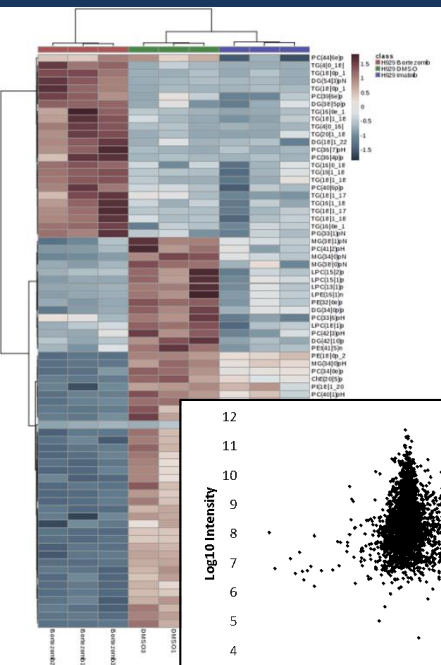
Thermo QExactive Plus/HF Agilent 1100/1200



2.1 mm x 10 cm C₁₈ (low pH, high organic)
260 μ L/min

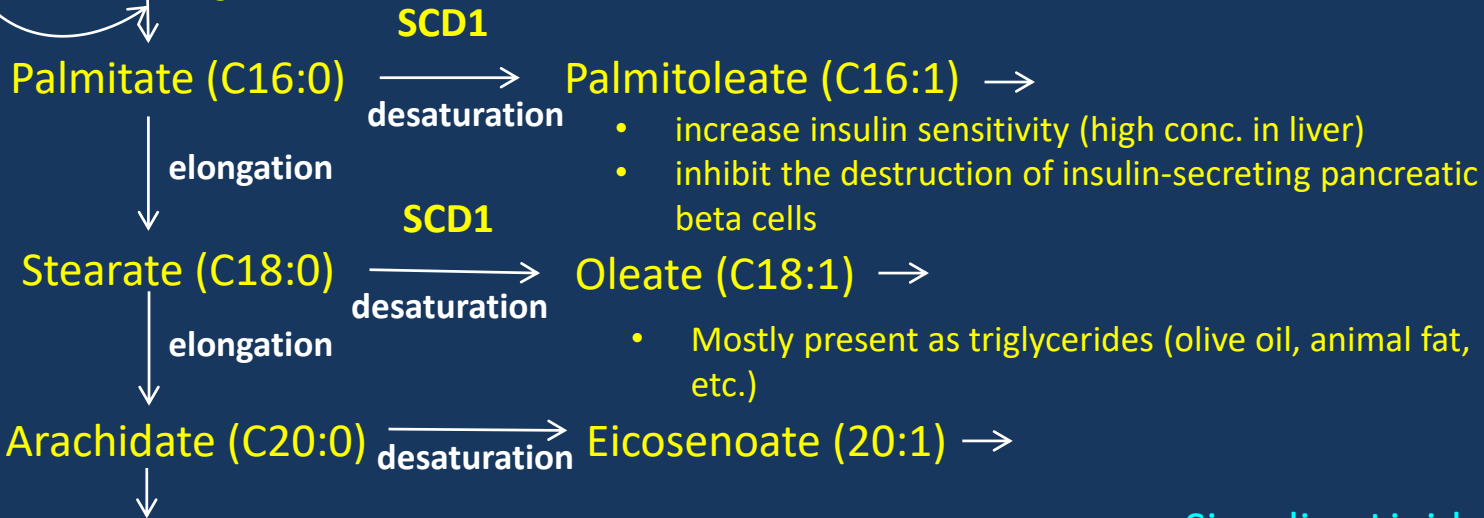
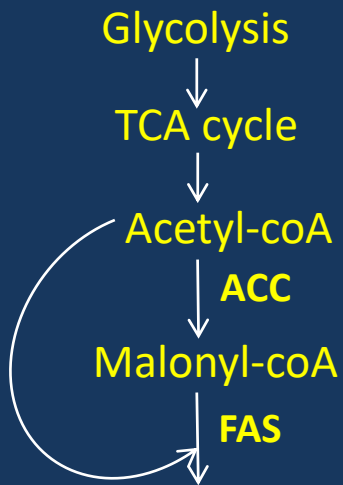
Pos/Neg polarity switching (~10 points/peak)

HCD-DDA (Top 10) pos and neg mode

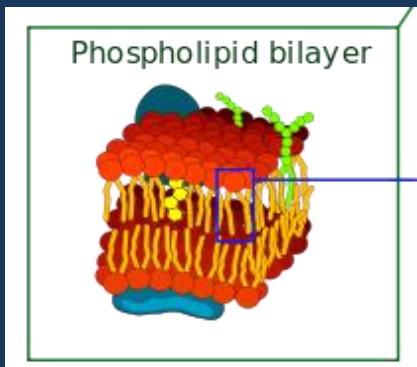


Fatty Acid Synthesis

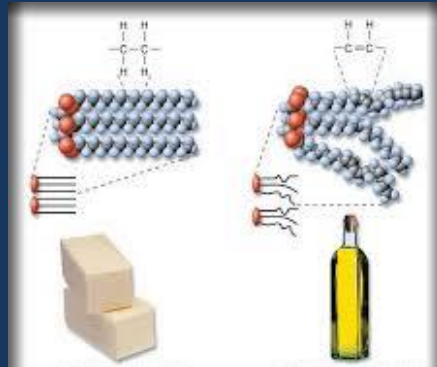
Lipid Arrays



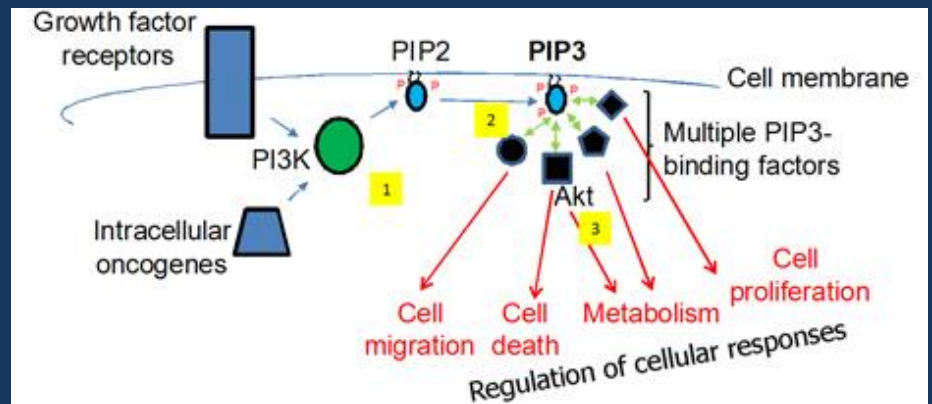
Membrane structure



Fats

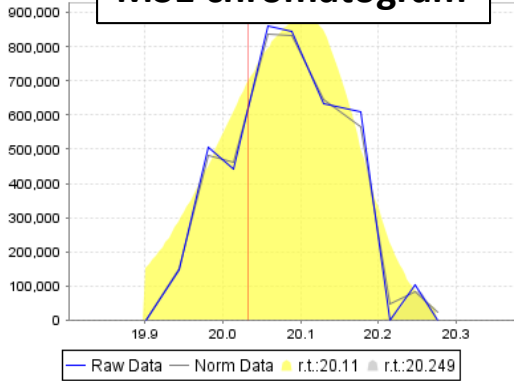


Signaling Lipids

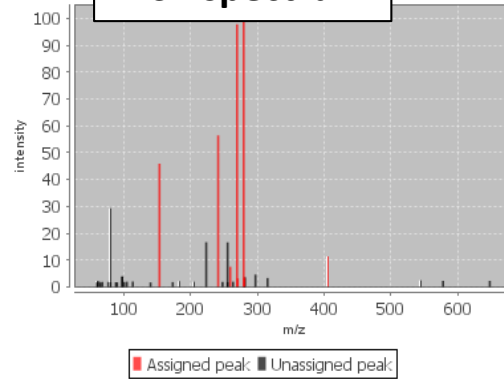


Thermo LipidSearch MS2 Based Identification Process

MS1 chromatogram



MS2 spectrum



Scoring

Match Lipid

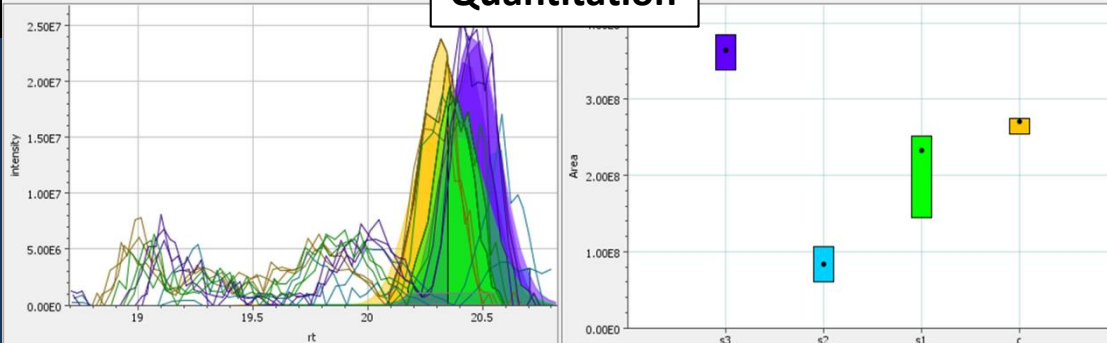
LipidIon	M-Sc.	T-Sc.	Acc.	St.
PI(17:0/18:2)-H	43.3	0.4	72.2	☑
PI(18:2/17:0)-H	43.3	0.4	72.2	☑
PI(16:0/19:2)-H	11.2	0.4	28	☑
PI(19:2/16:0)-H	11.2	0.4	28	☑
PI(17:1/18:1)-H	10.1	0.4	25.2	☑
PI(18:1/17:1)-H	10.1	0.4	25.2	☑
PI(11:0/24:2)-H	7.3	0.4	24.4	☑
PI(13:0/22:2)-H	7.3	0.4	24.4	☑
PI(15:0/20:2)-H	7.3	0.4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

Match Detail

HCD fragmentation

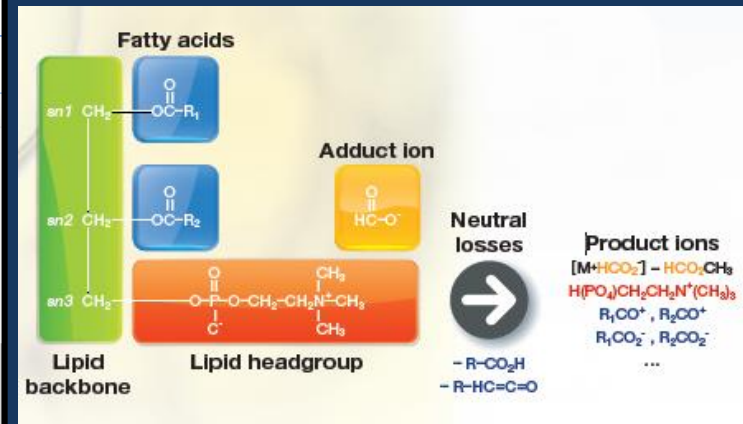
ObsMz	Type	lt.(%)	Frag.	Delta(Da)
152.9949	MS2	45.906	GP-H3O	-0.0009
172.7457	MS2	1.882	-	-
182.1563	MS2	2.169	-	-
204.3326	MS2	2.012	-	-
223.0009	MS2	16.663	-	-
241.0119	MS2	56.445	PH(inositol)-H 0	
247.5671	MS2	1.936	-	-
255.1215	MS2	1.828	-	-
255.2328	MS2	16.623	-	-
259.0225	MS2	7.551	IP	0.0001
263.2816	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



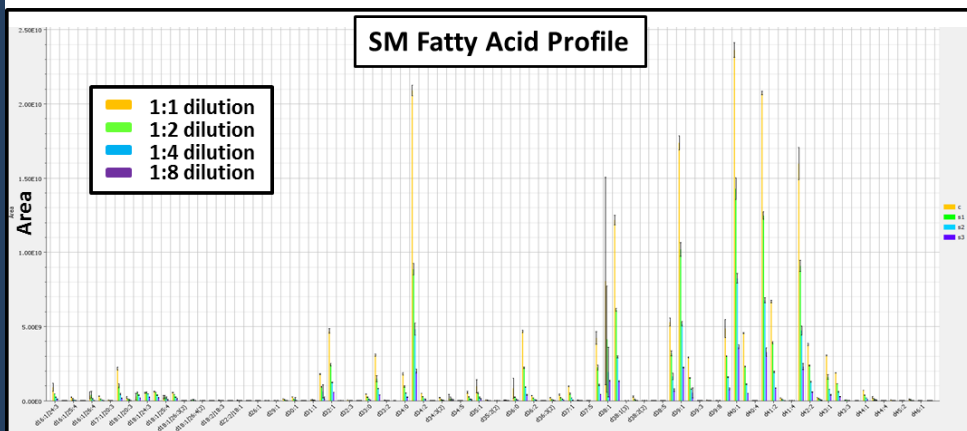
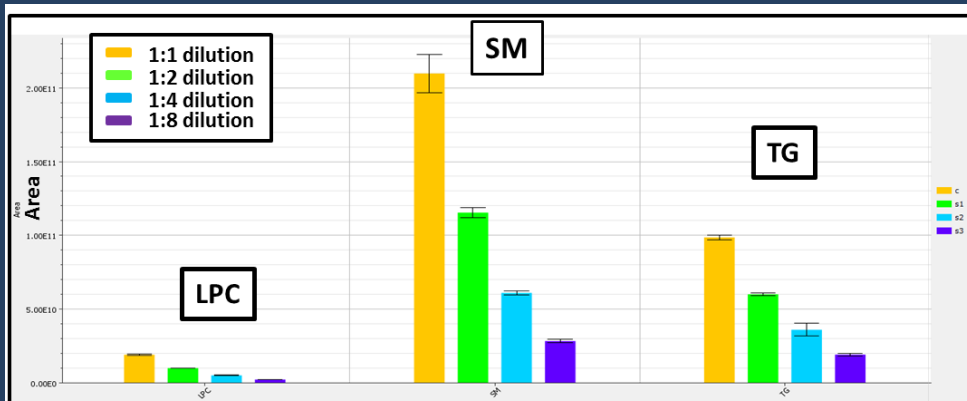
- Capable of identifying >1500 lipid ions in 30 min. with pos/neg switching from 66 subclasses of lipids
- Database assisted de novo interpretation

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	Sphingosine	So
	Sphingosine phosphate	SoP
Glycosphingolipids	Ceramides	Cer
	Ceramides phosphate	CerP
	Gangliosides	GM3
	Gangliosides	GM2
	Gangliosides	GM1
	Gangliosides	GD1a
	Gangliosides	GD1b
	Gangliosides	GD2

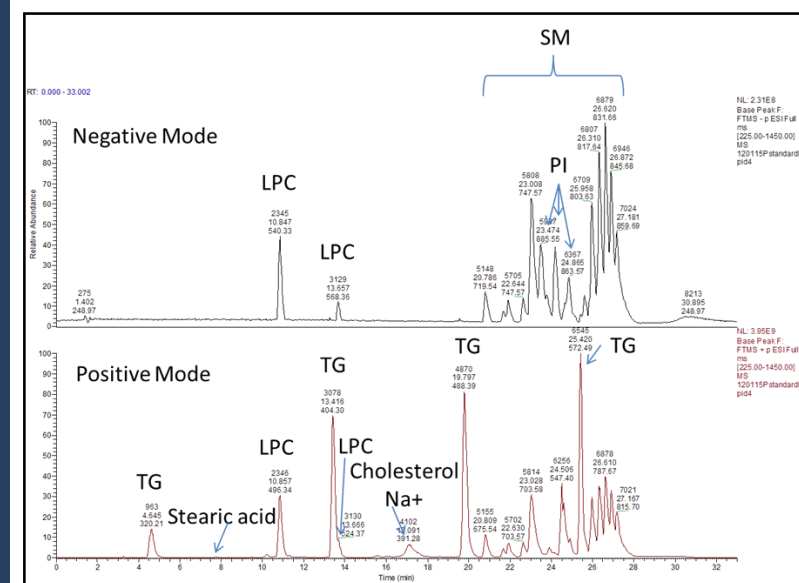
	Gangliosides	GD3
	Gangliosides	GT1a
	Gangliosides	GT1b
	Gangliosides	GT1c
	Gangliosides	GT2
	Gangliosides	GT3
	Gangliosides	GQ1c
	Gangliosides	GQ1b
Neutral Glycosphingolipids	Simple Glc series	CerG1
	Simple Glc series	CerG2
	Simple Glc series	CerG3
	Simple Glc series	CerG2GNAc1
	Simple Glc series	CerG3GNAc1
Steroid	Simple Glc series	CerG3GNAc2
	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

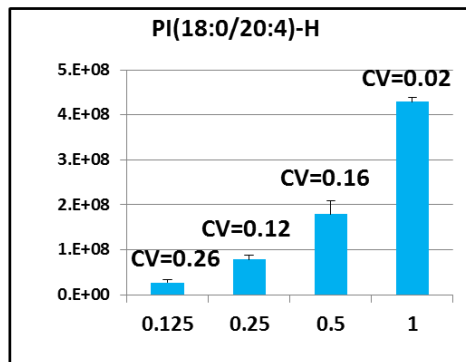
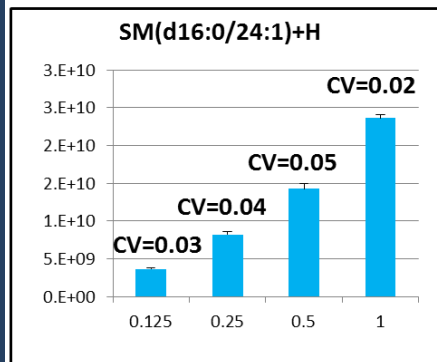
Lipid Standards Demonstrate Quantitative Reproducibility and Accuracy



Lipid standards



D)

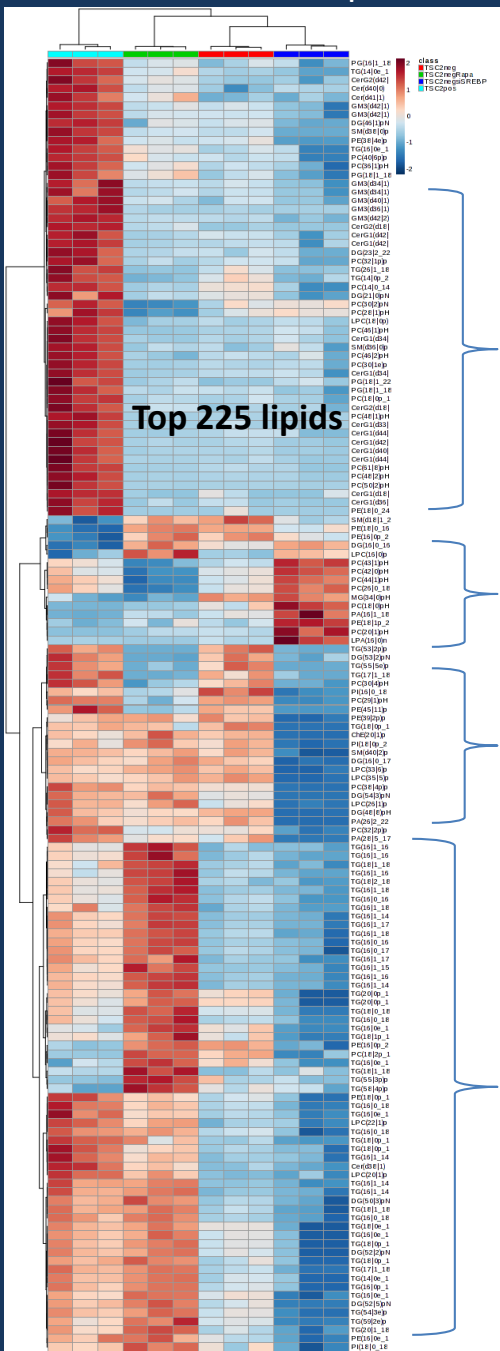


Clustering of TSC2 Lipidomics Data using *MetaboAnalyst*

TSC2+/+
TSC2-/-
siSREBP
TSC2-/-
Rapa

Biological replicates

$R^2 > 0.95$



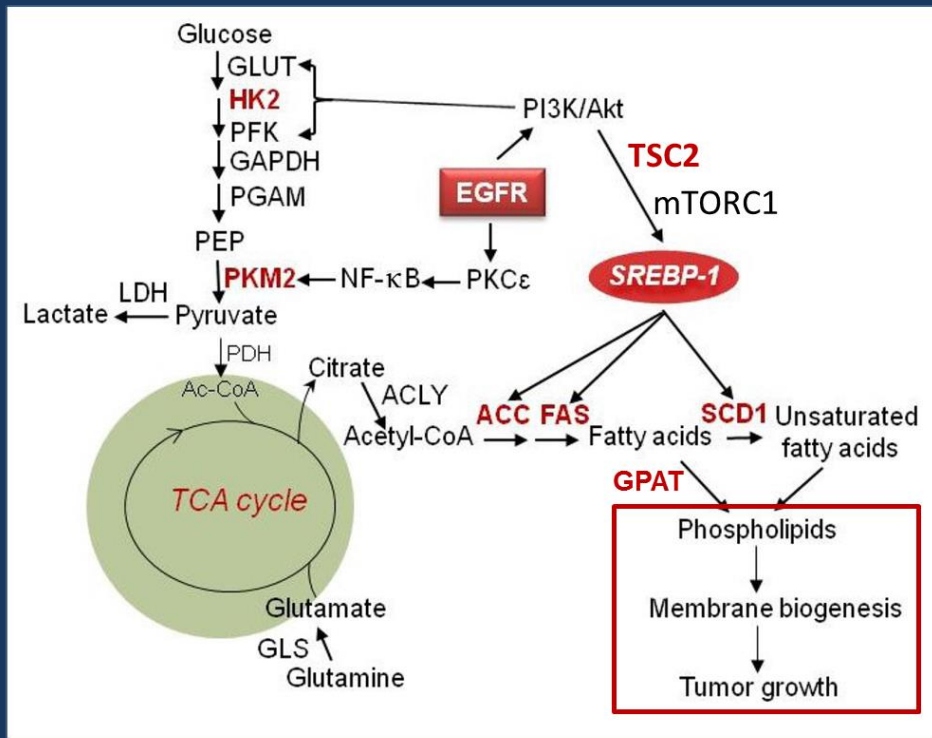
Ceramide
Ganglioside

PC, LPA

DG, LPC

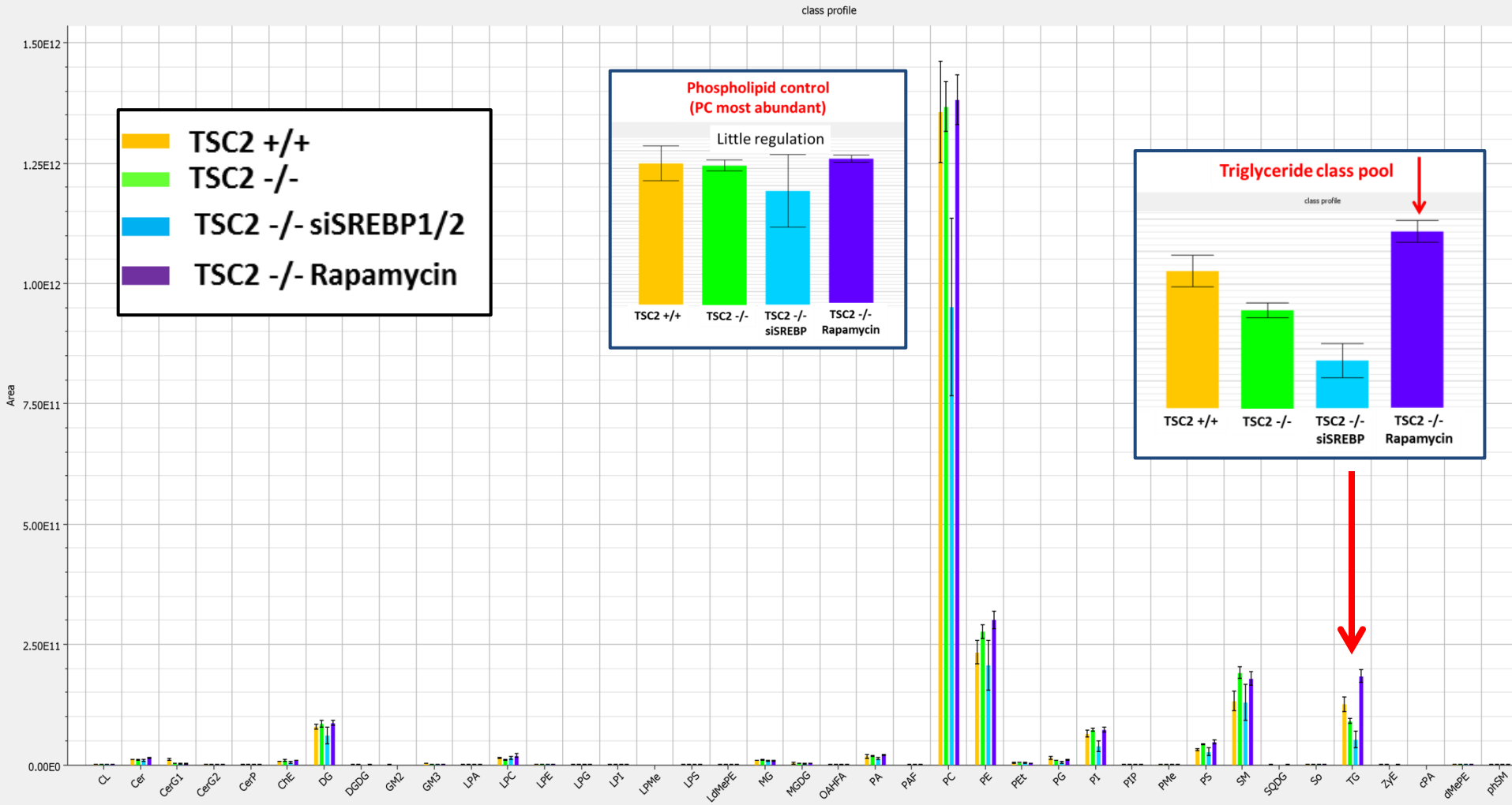
Triglycerides

De Novo Lipid Synthesis Pathway



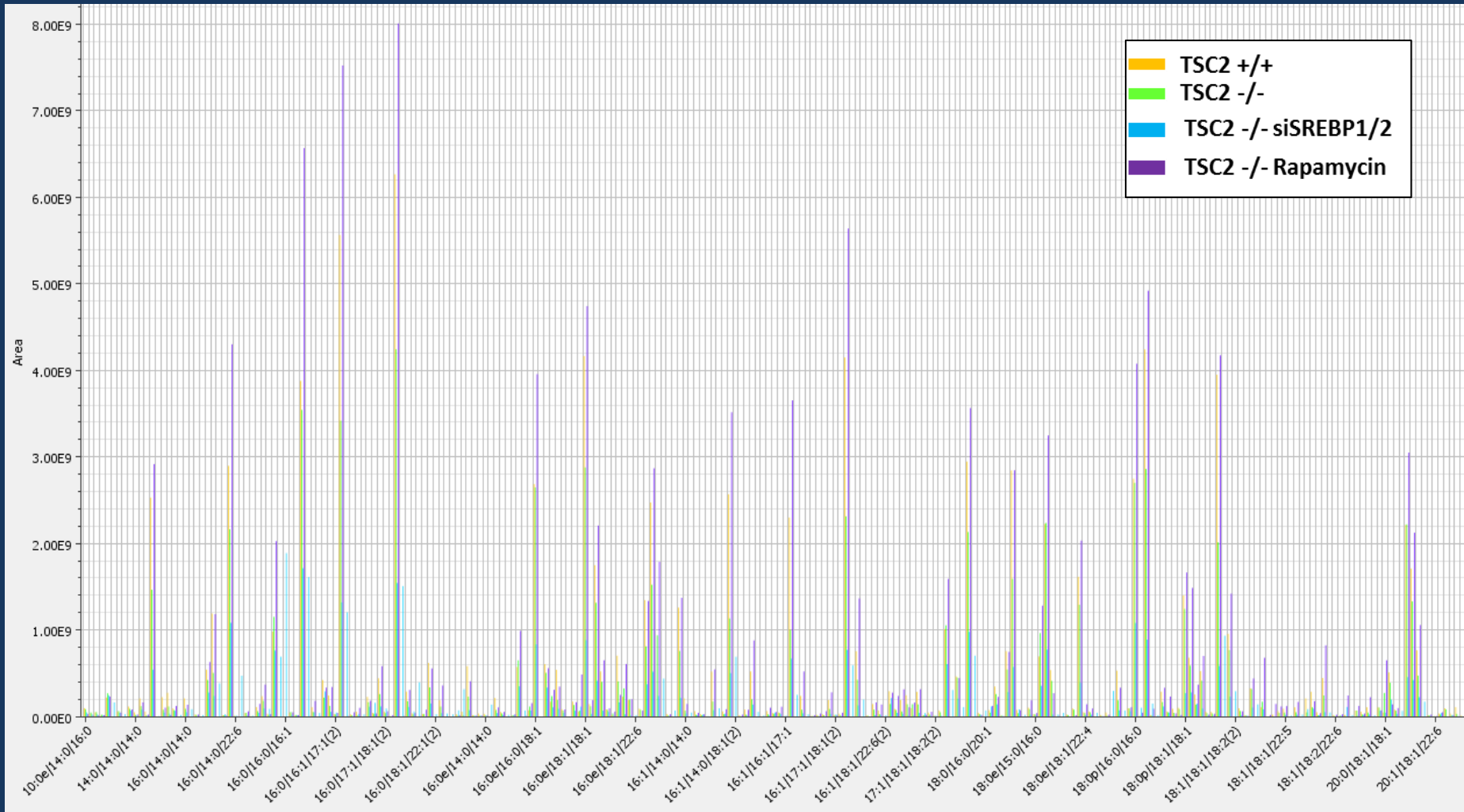
Ru et al, *Cancers* 2013, 5(4), 1469-1484

Overview of Lipid Class regulation in TSC2^{-/-} MEFs



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

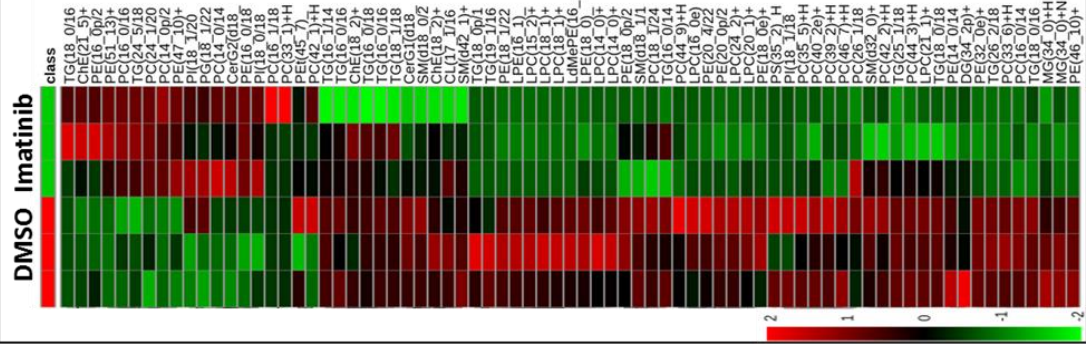
Triglyceride fatty acid composition in TSC2 MEFs using LipidSearch



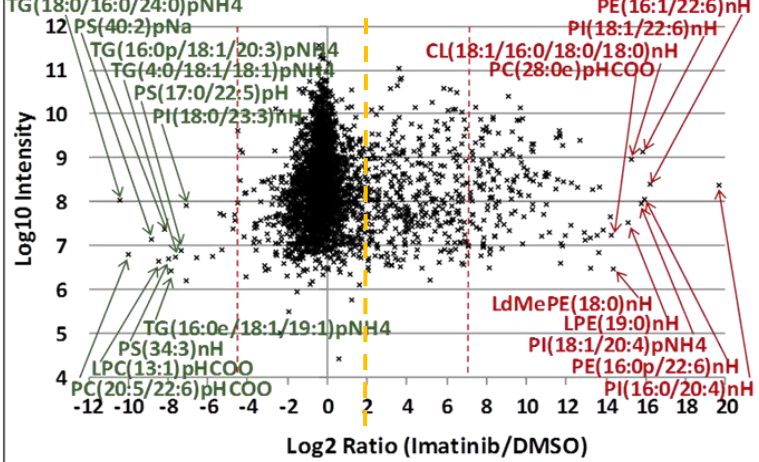
- Rapamycin rescues TG levels in TSC2^{-/-} MEFs similar to TSC2^{+/+} levels
- Many major TG fatty acids are basic building blocks (palmitate, oleate, etc.)

H929 Myeloma cancer cells

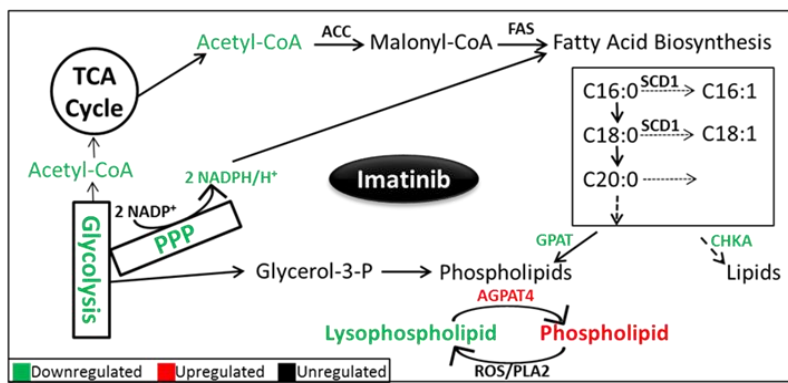
Heat Map Top 75 Lipid Features



Distribution of Lipids (normalized)

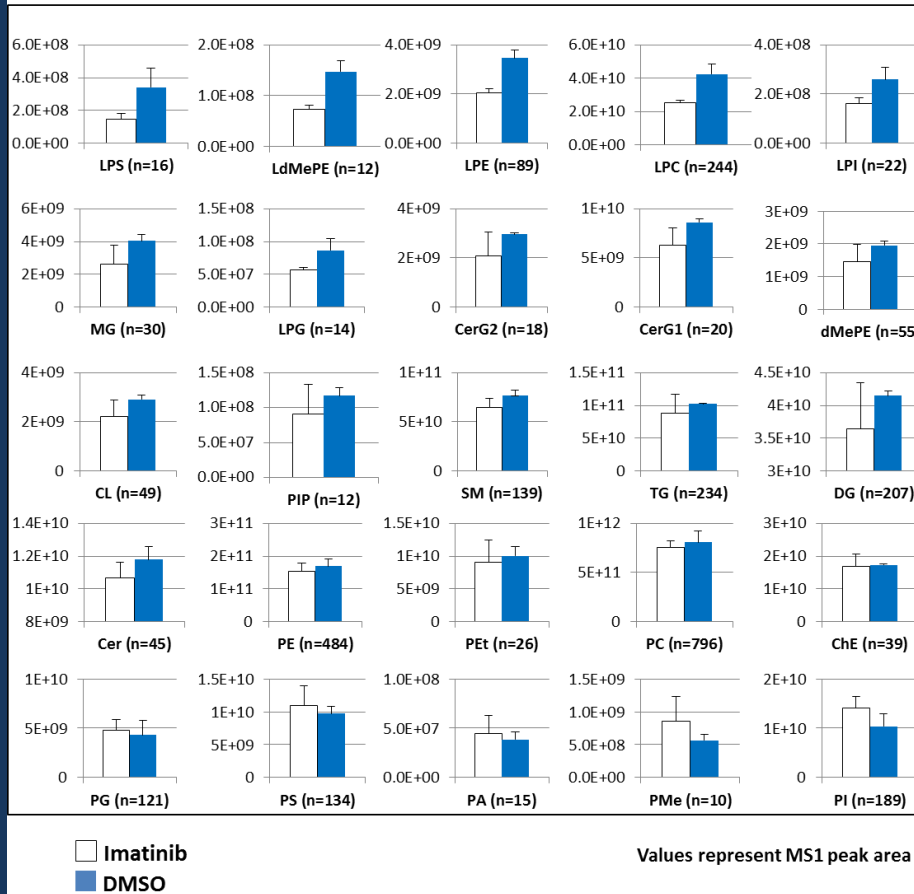


Lipidomics from Imatinib treated BCR/ABL cells for 1hr



H929 Myeloma cancer cells

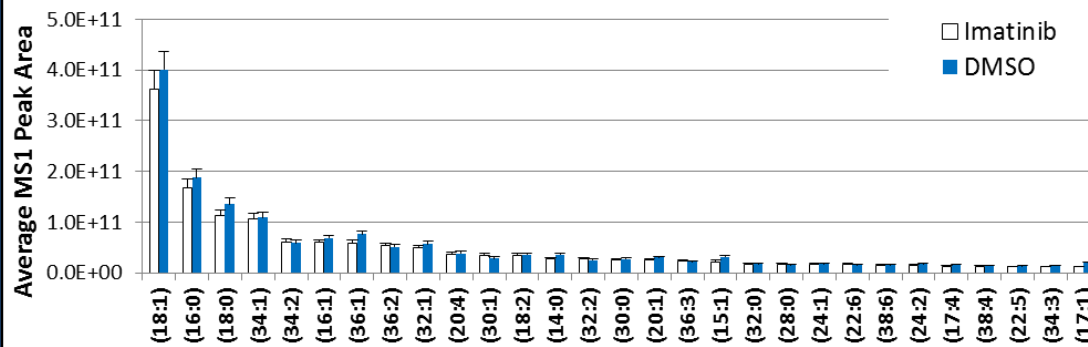
Lipid Class Quantification



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

More detail needed

Intensity of Fatty Acid Chains



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)

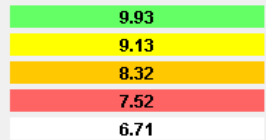
LipidSearch MS/MS spectral database not needed for MS2 identification

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

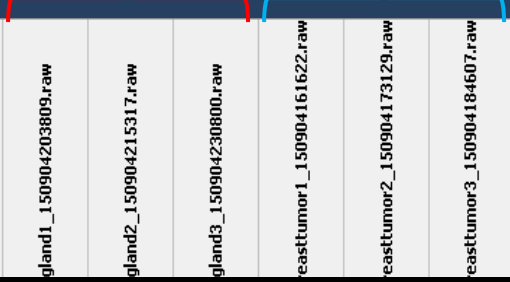
M. Gland Breast Tumor

Log10 Intensity

Color Legend (Displayed Value)



>550 identified



ID Score
0.786
0.981
0.979
0.957
0.969
0.968
0.968
0.966
0.933
0.964
0.933
0.964
0.927
0.963
0.963
0.962

Metabolite Group: LysoPE(16:0)0:0 Metabolites: 0.993 LysoPE(16:0)0:0

Theoretical m/z	Adducts	Charge	Average m/z	Δ Average m/z ...	Average RT	σ RT	Polarity
454.293	[M+H] ⁺	1	454.294	2.745	11.21	0.056	+
452.278	[M+] ⁻	-1	452.279	0.817	11.22	0.050	-

Features Matching Selected Ion

Sample Name	Record ID	ID Score	Raw Intensity	m/z	Aligned RT	Δ m/z AMU	Δ m/z PPM	m/z FWHM	RT FWHM	Charge	Isotopic Distribution ...	Mass Accuracy Score	MS2 Score	XIC Score	Attributes
10301SPS8KungTi...	NISTNO:1151364	0.93	4.29E9	454.294	11.14	0.001	2.894	0.008	0.29	1	0.762	0.904	0.994	0.720	
10301SPS8KungTi...	NISTNO:1151364	0.856	4.38E9	454.294	11.28	0.001	2.535	0.008	0.16	1	0.796	0.916	0.930	0.530	
10301SPS8KungTi...	NISTNO:1151364	0.939	4.24E9	454.294	11.25	0.001	2.456	0.008	0.20	1	0.815	0.918	0.988	0.337	
10301SPS8KungTu...	NISTNO:1151364	0.935	1.92E9	454.294	11.14	0.001	3.073	0.009	0.32	1	0.794	0.898	0.995	0.760	

References Quantitative Charts Feature 3D Plot

Mass: 453.286 Formula: C₂₁H₄₁NO₂P
NAME: LysoPE(16:0)0:0
SMILES: [H]C(O)O(COC(=O)CCCCCCCCCCCCCCCCO)P(=O)(O)CCN

LysoPE (16:0)

MS2 Spectra
454.29 m/z, 1+, 453.29 Da, Reference Instrument: Thermo Finnigan Elite Orbitrap, Collision Energy: 31.0 eV

16 out of 17 reference masses were matched within 0.5 Da

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

#	Visible	Star	ID Score	Mass Accuracy Score	Isotope Distribution Score	MS ₂ Score	XIC Score	Metabolite Name	Accession Number	Molecular Formula	Molecular Weight	Retention Time (min)	lipid1.raw	lipid2.raw	lipid3.raw	raw	raw	raw	
1	✓		0.999	1.00	1.00		0.84	ent-17-Hydroxy-16beta-kauran-19-ol_RT2 (+15)	HMDB:HMDB36721	C ₃₀ H ₅₂ O ₂	304.2	11.74	6.99	10.7	10.8	Missing Value	9.31	9.29	
2	✓		0.998	1.00	1.00		0.74	Cluster of Neogrolin_RT2	HMDB:HMDB30053				6.98	10.8	10.8	Missing Value	9.40	9.38	
2.1	✓		0.998	1.00	1.00		0.74	Neogrolin_RT2 (+5)	HMDB:HMDB30053	C ₂₇ H ₅₀ O ₂	328.2	11.23	6.98	10.8	10.8	Missing Value	9.40	9.38	
2.2	✓		0.998	1.00	1.00		0.74	ent-17-Acetoxy-16beta-kauran-19-ol_RT2 (+5)	HMDB:HMDB36722	C ₃₂ H ₅₀ O ₃	346.3	11.20	6.98	10.8	10.8	Missing Value	9.40	9.38	
3	✓		0.997	1.00	1.00		0.90	LysopC(15:0)_RT5 (+3)	HMDB:HMDB10381	C ₂₇ H ₄₈ NO ₂ P	481.3	12.36	10.9	11.0	11.1	9.05	9.41	9.42	
4	✓		0.996	0.99	1.00		0.87	LysopE(18:2(9Z,12Z)/0:0)_RT1 (+1)	HMDB:HMDB11507	C ₂₇ H ₄₈ NO ₂ P	477.3	6.31	10.6	10.5	10.6	9.33	9.47	9.42	
5	✓		0.994	0.98	1.00		0.82	Cluster of Tridecyl chlorate_RT2	HMDB:HMDB35477				12.20	Missing Value	9.93	10.0	Missing Value	8.45	Missing Value
5.1	✓		0.996	1.00	0.99		0.58	Tridecyl chlorate_RT2 (+11)	HMDB:HMDB35477				12.22	Missing Value	10.0	10.1	Missing Value	8.45	Missing Value
5.2	✓		0.996	1.00	0.99		0.58	O-Arachidonyl Ethanolamine_RT3 (+1)	HMDB:HMDB08043	C ₂₆ H ₄₈ NO ₂	384.5	29.17	10.9	Missing Value	10.9	10.7	10.8	Missing Value	10.7
6	✓		0.995	1.00	0.99		0.96	TG(14:0)_6:0(20:1(11Z)) (+32)	HMDB:HMDB08049	C ₃₆ H ₇₀ O ₂	542.6	22.10	10.3	Missing Value	10.3	10.4	Missing Value	Missing Value	Missing Value
7	✓		0.994	0.98	1.00		0.82	PC(18:4)_2:2(12Z,15Z)/20:0)_RT2	HMDB:HMDB08273	C ₃₈ H ₇₄ NO ₂ P	609.6	12.22	10.6	10.7	10.8	Missing Value	Missing Value	Missing Value	Missing Value
8.1	✓		0.994	0.99	1.00		0.85	PC(20:0)/18:4(6Z,9Z,12Z,15Z))_RT2	HMDB:HMDB08368	C ₃₈ H ₇₄ NO ₂ P	609.6	12.22	10.3	10.5	10.6	Missing Value	Missing Value	Missing Value	Missing Value
8.2	✓		0.981	0.95	1.00		0.83	PC(20:3(5Z,8Z,11Z)/18:1(9Z))_RT2	HMDB:HMDB08431	C ₃₈ H ₇₄ NO ₂ P	609.6	12.22	Missing Value	Missing Value	Missing Value	Missing Value	Missing Value	Missing Value	
9	✓		0.994	1.00	0.99		0.80	PC(20:4(5Z,8Z,11Z,14Z)/18:0)_RT2	HMDB:HMDB07988	C ₃₈ H ₇₄ NO ₂ P	609.6	12.22	10.5	10.5	10.1	8.87	9.00	9.01	
10	✓		0.994	1.00	0.99		0.87	PC(16:0)/22:4(7Z,10Z,13Z,16Z))_RT2	HMDB:HMDB08049	C ₃₈ H ₇₄ NO ₂ P	609.6	12.22	9.80	9.86	9.89	9.52	9.68	9.72	
11	✓		0.994	1.00	0.99		0.87	PC(18:0)/20:4(8Z,11Z,14Z,17Z))_RT2	HMDB:HMDB08079	C ₃₈ H ₇₄ NO ₂ P	609.6	12.22	10.2	9.20	9.24	10.4	10.4	10.4	
12	✓		0.993	0.99	0.99		0.74	PC(18:1(9Z)/20:3(5Z,8Z,11Z))_RT2	HMDB:HMDB08113	C ₃₈ H ₇₄ NO ₂ P	609.6	12.22	Missing Value	Missing Value	Missing Value	Missing Value	Missing Value	Missing Value	
13	✓		0.993	1.00	0.99		0.82	PC(18:3(6Z,9Z,12Z)/20:1(11Z))_RT2	HMDB:HMDB08176	C ₃₈ H ₇₄ NO ₂ P	609.6	12.22	10.5	10.5	10.1	8.87	9.00	9.01	
14	✓		0.992	1.00	0.99		0.98	PC(20:1(11Z)/18:3(6Z,9Z,12Z))_RT2	HMDB:HMDB08304	C ₃₈ H ₇₄ NO ₂ P	609.6	12.22	10.2	9.20	9.24	10.4	10.4	10.4	
15.1	✓		0.992	0.98	1.00		0.97	PC(20:3(5Z,8Z,11Z)/18:1(11Z))_RT2	HMDB:HMDB08367	C ₃₈ H ₇₄ NO ₂ P	609.6	12.22	10.6	10.7	10.8	Missing Value	Missing Value	Missing Value	
15.2	✓		0.861	0.60	0.99		0.97	PC(20:3(5Z,8Z,11Z)/18:1(11Z))_RT2	HMDB:HMDB08367	C ₃₈ H ₇₄ NO ₂ P	609.6	12.22	10.6	10.7	10.8	Missing Value	Missing Value	Missing Value	
16	✓		0.992	0.99	0.99		0.82	Scladonol (18:4)	HMDB:HMDB08049	C ₃₈ H ₇₄ NO ₂ P	609.6	12.22	10.6	10.7	10.8	Missing Value	Missing Value	Missing Value	

>25 possibilities

M. Gland Breast Tumor

Elements Search with NIST (MS2) yields 322

Features Matching Selected Ion

Sample Name	Record ID	ID Score	Raw Abundance	m/z	Aligned RT	Δ m/z AMU	Δ m/z PPM	m/z PHEH	RT PHEH	Charge	Isotopic Distribution
042816P@BreastTumor10mg@q42.raw	NIST01-1213950	0.977	2.23E10	548.372	9.12	0.001	1.900	0.02	8.24	1	0.959
042816P@BreastTumor10mg@q42.raw	NIST01-1213950	0.972	2.08E10	548.372	9.16	0.001	2.107	0.02	8.33	1	0.944

References

Quantitative Charts

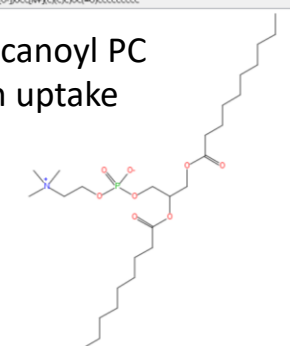
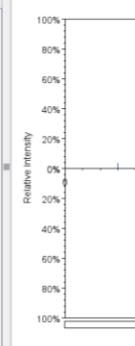
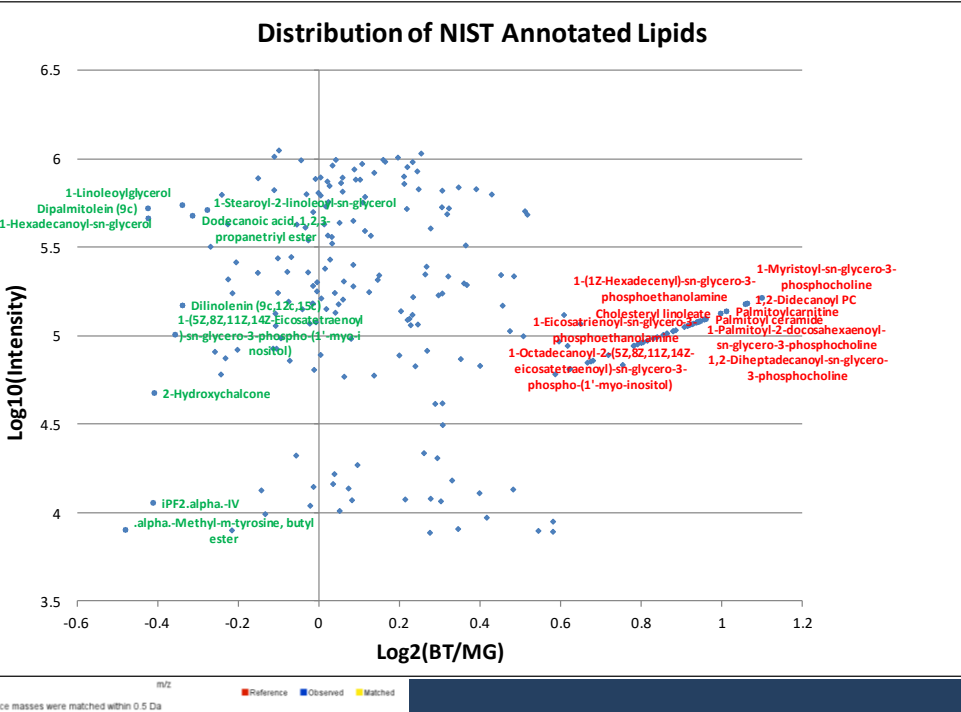
Feature 3D Plot

Mass: 565.374 Formula: C₃₈H₇₄NO₂P

NAME: 1,2-Didecanoyl PC

SMILES: CCCCCCCCCC=OCCCC(COP(=O)(O)C(=O)CCCCCCCC)C(=O)CCCCCCCC

1,2-didecanoyl PC
Insulin uptake

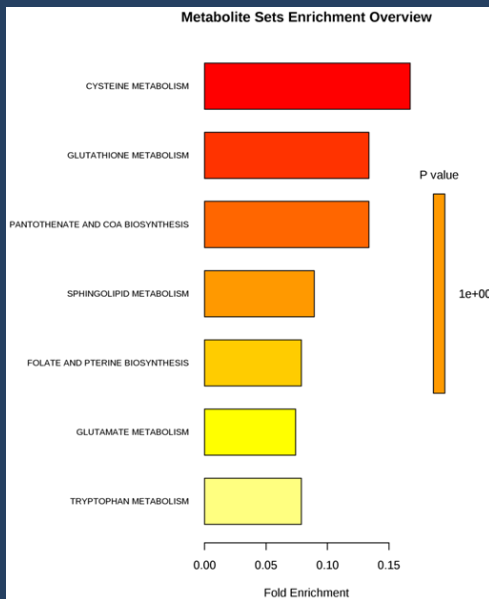
How Do You Perform Informatics of Lipid Data?

NIST MS2 IDs from Elements software

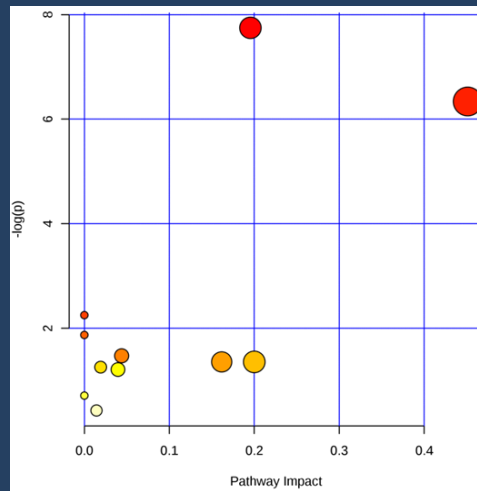
Convert to KEGG/HMDB using Fiehn lab tool

MetaboAnalyst Pathway Enrichment/Mapping

Pathway Enrichment



Pathway Mapping



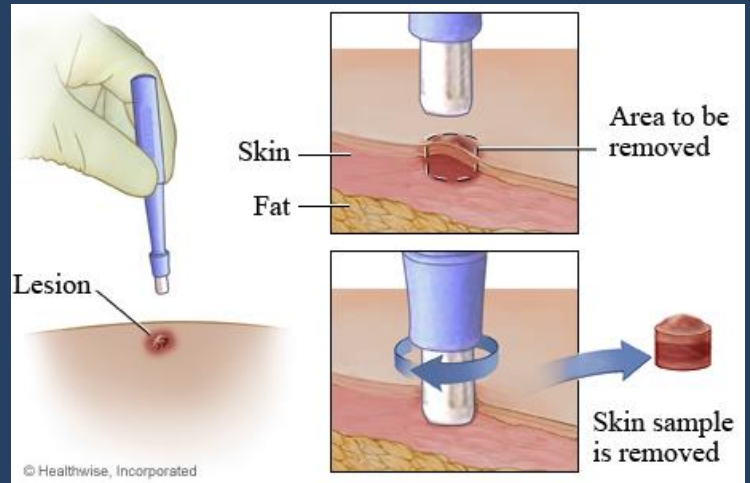
Identified Pathways

Pathway Name	Total	Hits	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	0.0292	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	0.2358	1.2181	1.0	1.0	0.0
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	14	1	0.2104	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

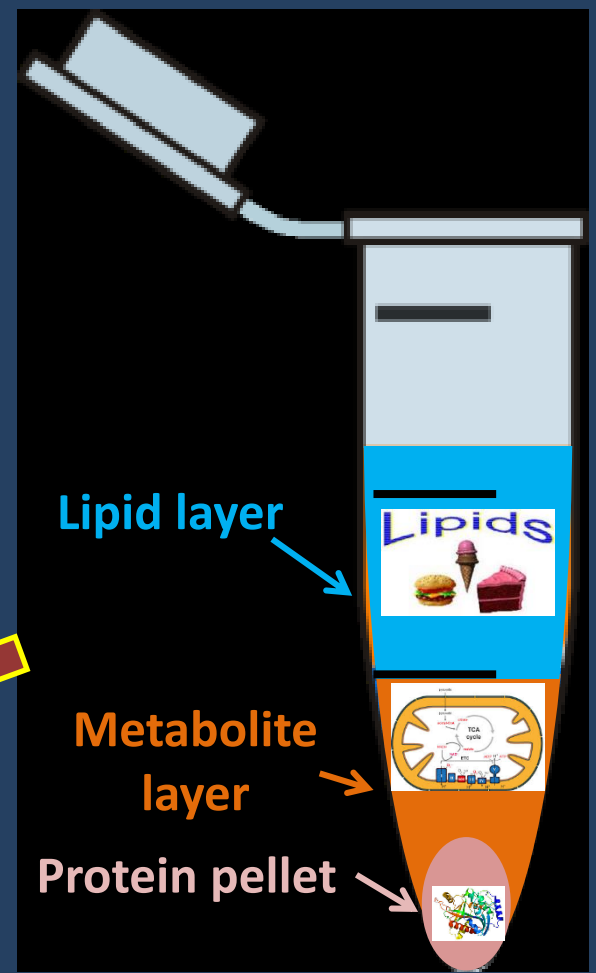


As little as 5-10 mg starting material

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

Methyl tert-butyl ether (MTBE)

Mainly used for lipid extractions

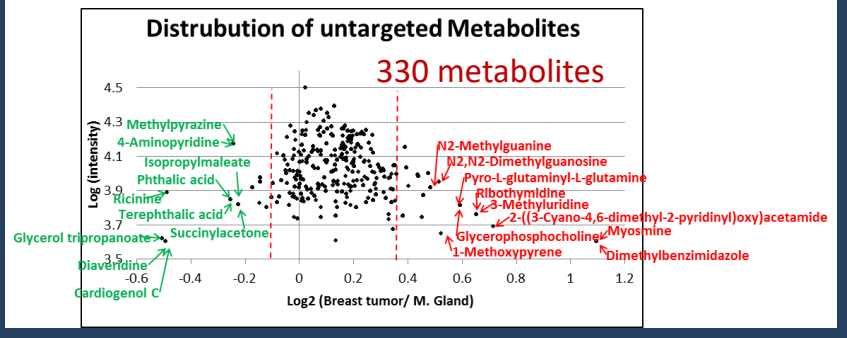
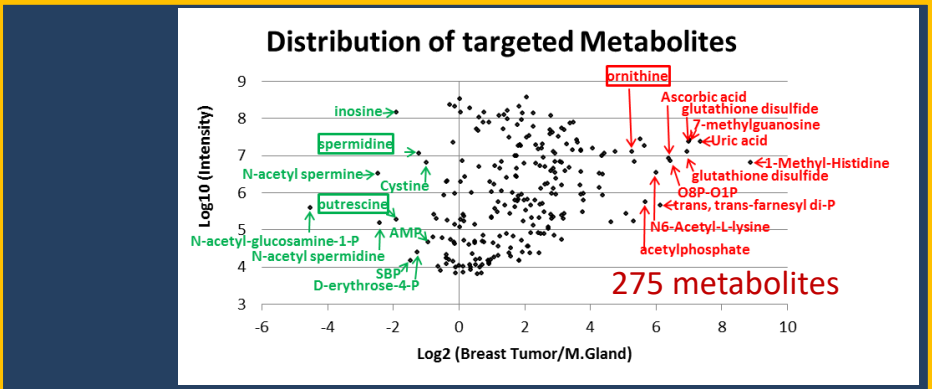
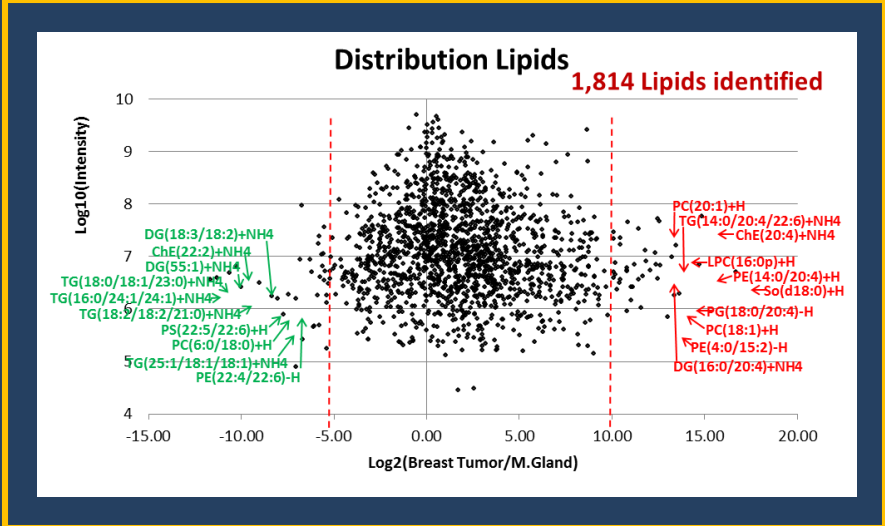
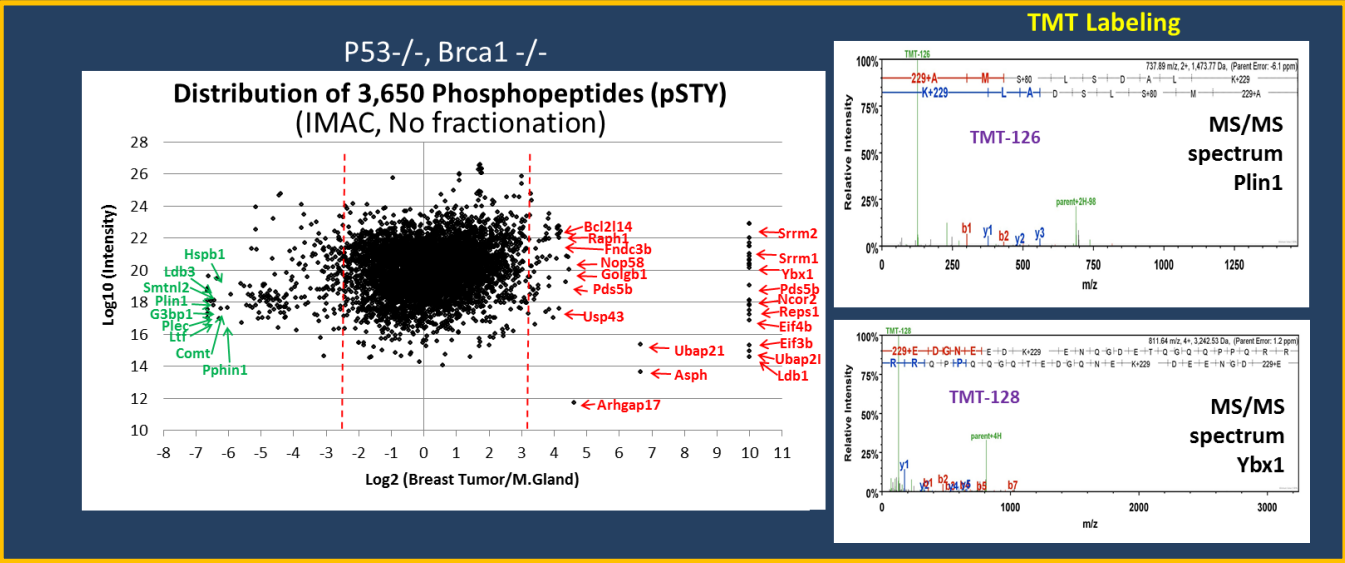


Serial-Omics "Super-Omics"

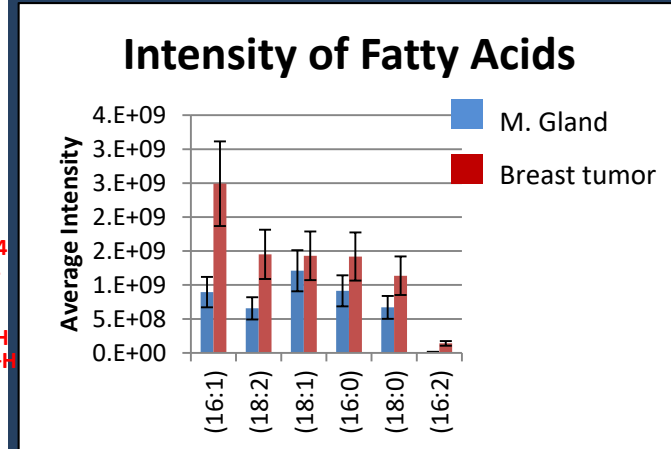
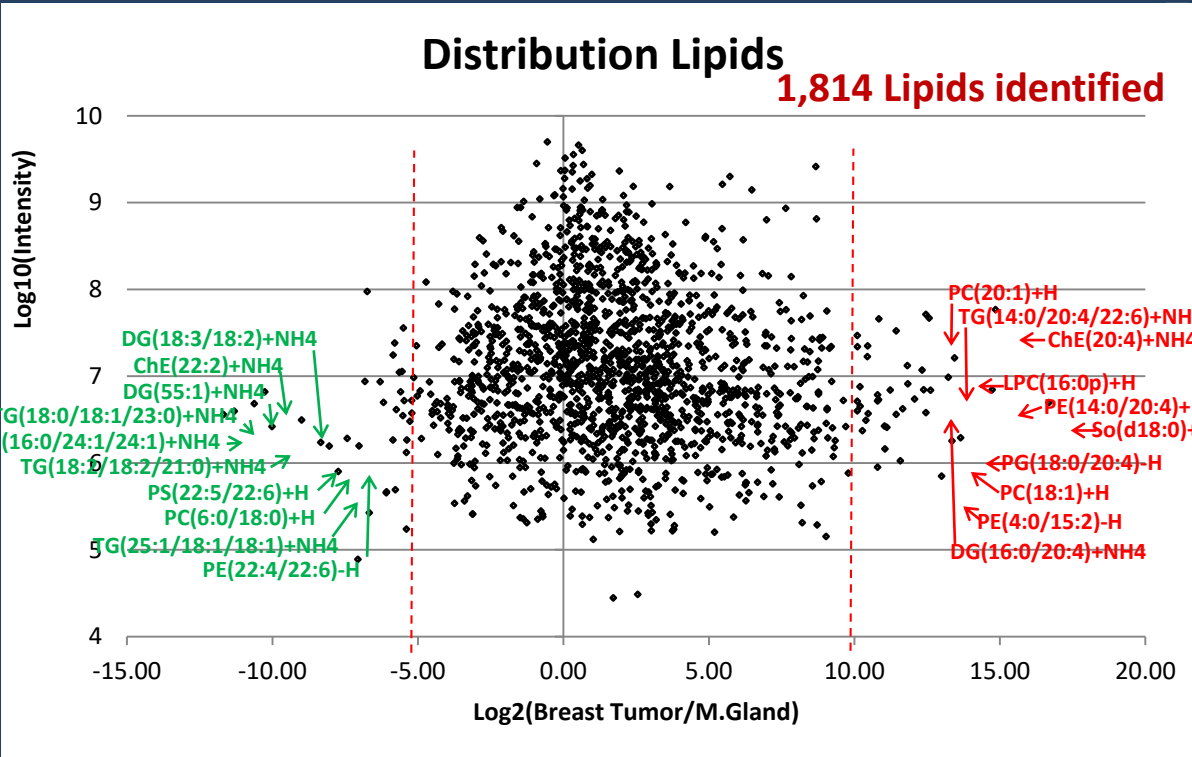
LC-MS/MS

Metabolomics, Lipidomics and Phosphoproteomics)

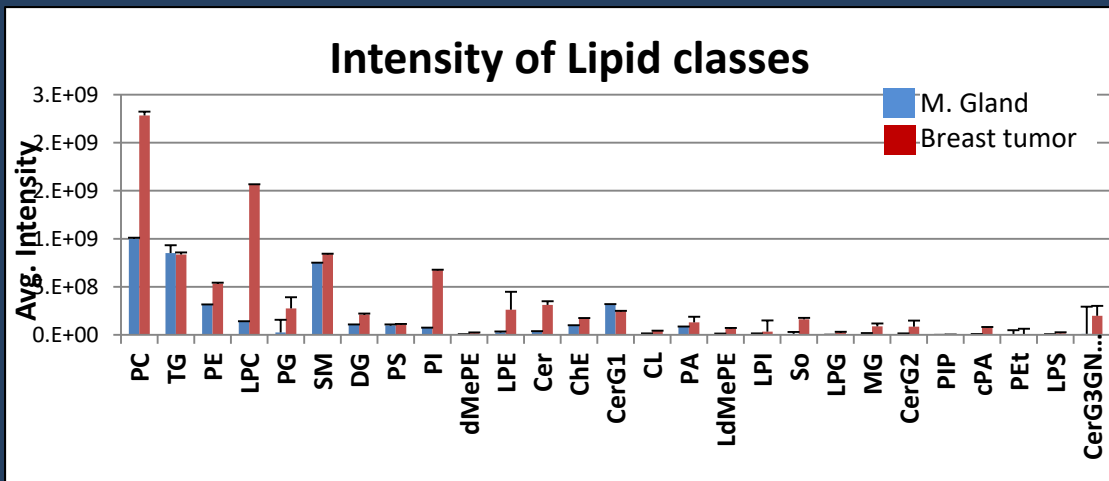
Serial-Omics on Mouse Breast Tumor vs Mammary Gland Tissue



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.

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Ying Xu



Min Yuan

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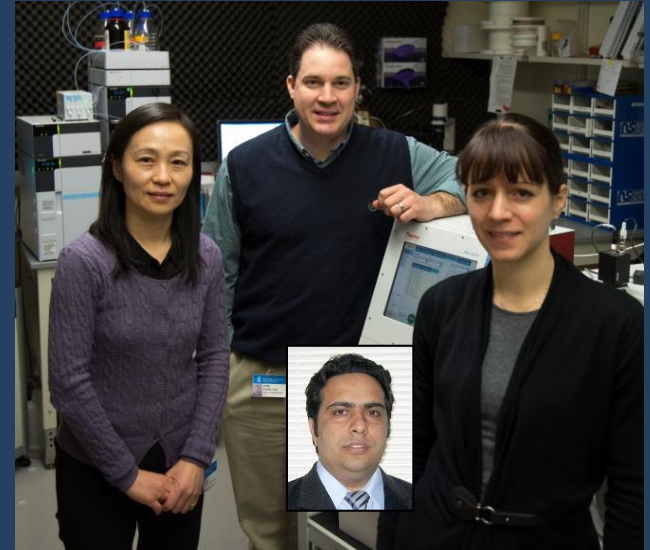


Gerburg Wulf

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Brendan Manning



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