Small Molecules in a Core Setting: Targeted Metabolic Flux to Untargeted Lipidomics

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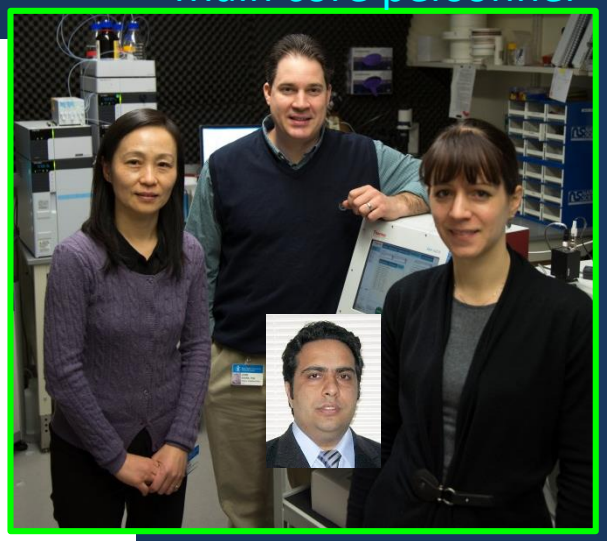
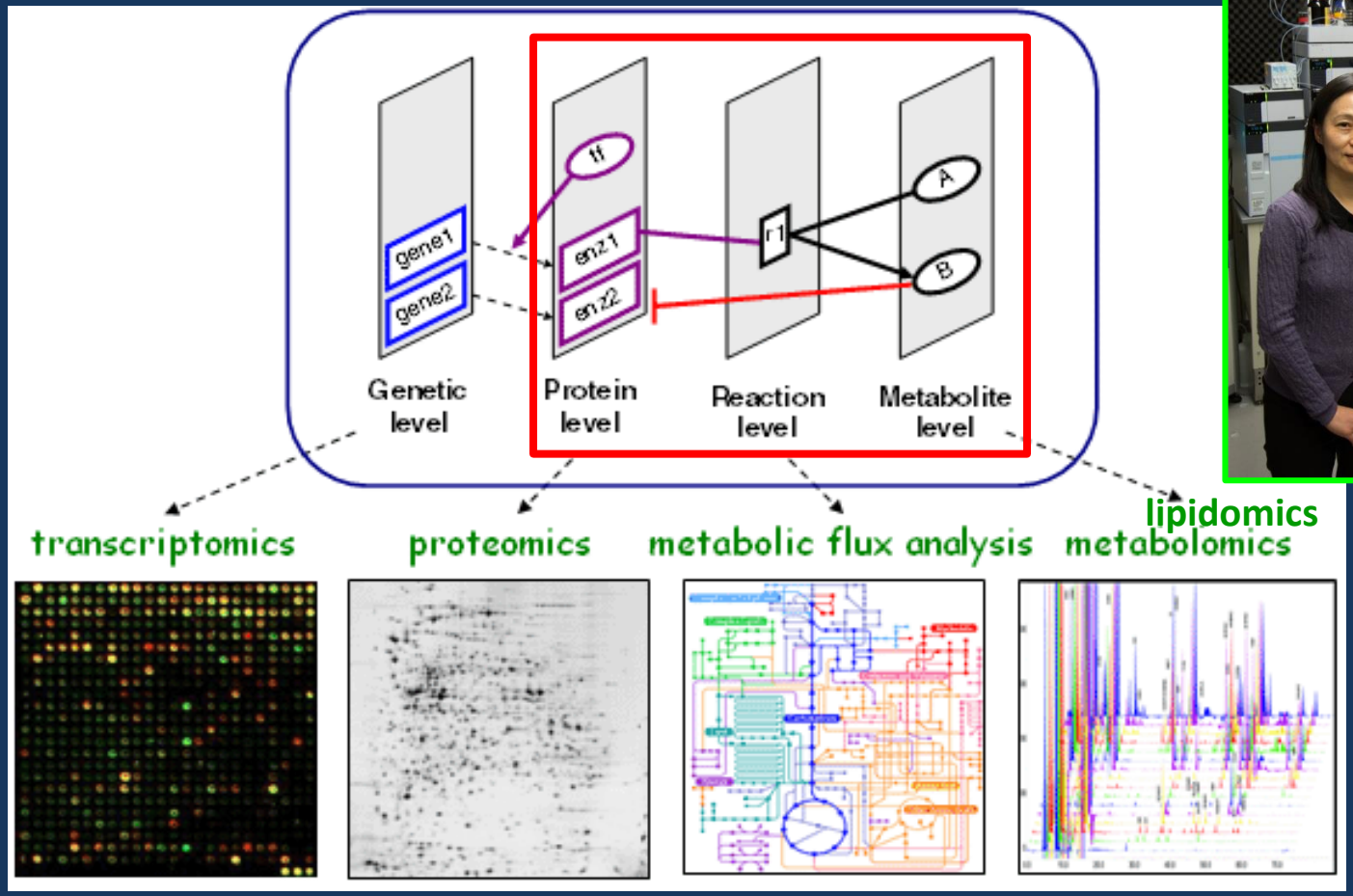
HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

BIDMC Mass Spectrometry Core
- Integrating Different –*Omics* Approaches

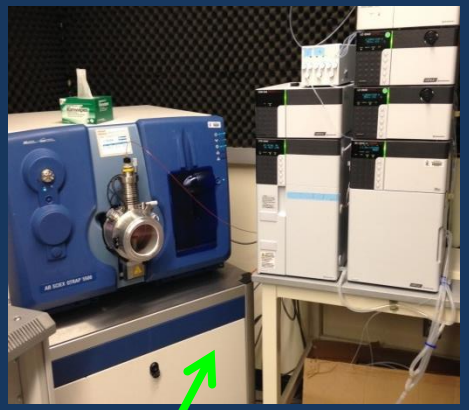
Main core personnel





BIDMC Mass Spec Equipment & Binning Process

AB/SCIEX 5500 QTRAP (***METABOLOMICS***)



-Targeted metabolomics
-13C/15N metabolic flux
-Targeted drug quant
-Extremely fast
-Pos/Neg switching

Thermo Orbitrap Elite (***PROTEOMICS***)

-SILAC/TMT/Label-free quantitation
-Protein Identification
-PTM mapping
-Global Phosphoproteomics
-High resolution

Thermo QExactive Plus Orbitrap (***LIPIDOMICS*** & Proteomics)

-Untargeted lipidomics
-Lipidomic 13C/15N flux (*soon*)
-Untargeted Metabolomics
-SILAC/TMT quantitation
-Post-Translation Mod mapping
-Global Phosphoproteomics
-Label-free protein quantitation
-High resolution and high-throughput

Everybody pays

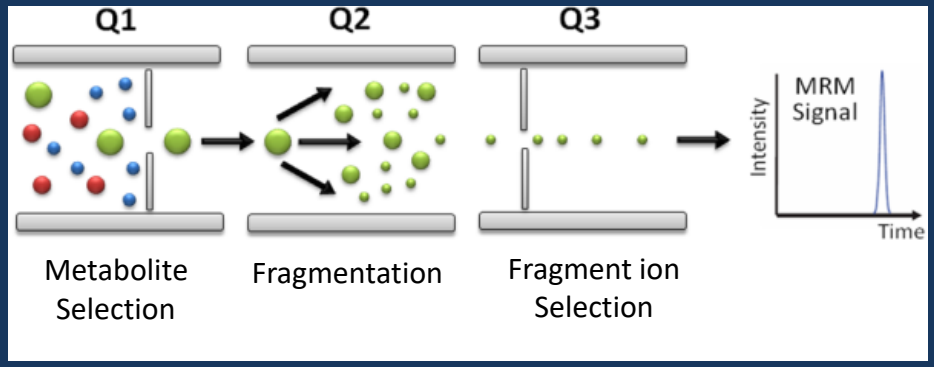
- Different rates (grants, affiliation, collaborators, etc.)
- All goes through core system

Targeted Polar Metabolite Profiling Platform

+/- switching

Amide XBridge
HILIC - 1 column
4.6mm x 15cm
pH=9.0, NH_4^+
400 µL/min

Selected Reaction Monitoring (SRM) ~300 transitions (270 unique metabolites -^{12}C & ^{13}C)

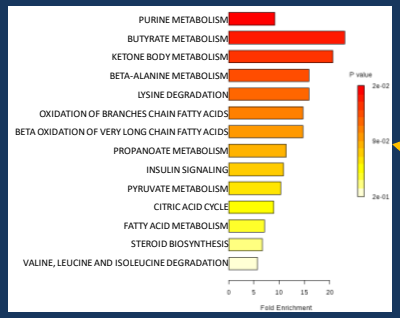


Extract metabolites with **80% methanol**
From cells, tumor tissue, fluids, etc.

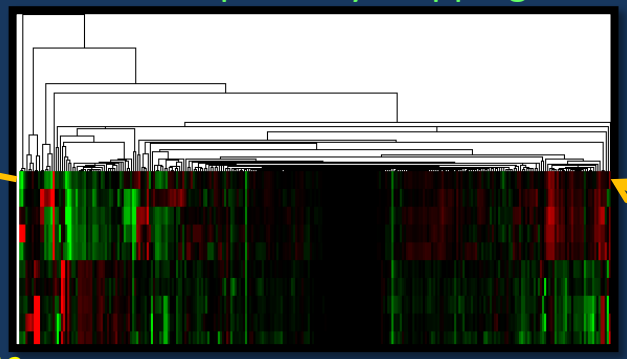
Cancer cells

Mean R^2 = 0.978
Mean CV= 0.12
FWHH = ~9 seconds
Cycle time = 1.67 sec
3-4 msec dwell
10-14 points per peaks

Bioinformatics
MarkerView
Metaboanalyst.ca



Clustering (MatLab, Metaboanalyst.ca)
KEGG pathway mapping



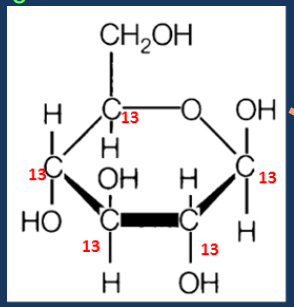
MultiQuant v2.0 Peak Area integration software

Sample Name	avg DMSO	avg BEZ	avg BKM	avg U0126
3-phosphoglycerate	1330385.962	871663.9395	1038599.88	943607.0569
3-phospho-serine	152487.2158	83097.6478	73986.5328	31000.0000
D.glyceraldehdye.3-phosphate	254477.5084	293208.1613	209570.2472	194280.8415
dihydroxy.acetone-phosphate	357217.2808	274197.6204	227350.7244	210859.1435
fructose.1,6-bisphosphate	1059370.361	808511.4636	1082381.874	682001.4833
fructose.6-phosphate	1471332.891	1019002.062	1046811.137	1054938.996
glucose.1-phosphate	761216.5713	605856.8664	815435.949	811274.948
glucose.6-phosphate	955670.737	704956.8497	635987.2986	741371.4211
hexose-phosphate	19302214.34	14375529.9	20558058.05	16548067.87
lactate	95442398.42	94957148.33	103044596.9	101662913.9
phosphoenolpyruvate	258107.2535	217613.2952	323347.9553	382350.6681

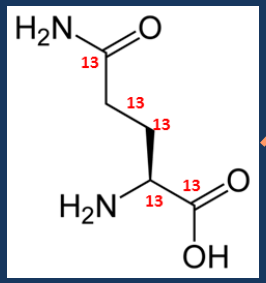
Yuan, et. al., ***Nature Protocols***, 2012.

Steady-State Metabolic Flux Analysis:
"SILAC" version for metabolomics

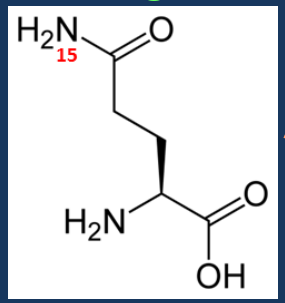
$^{13}C_6$-labeled glucose



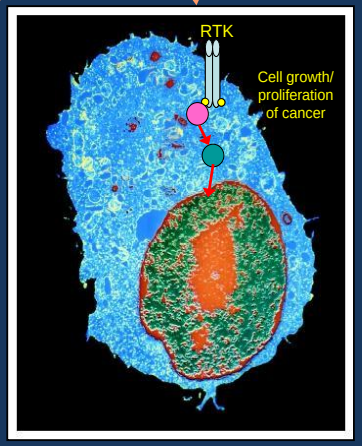
$^{13}C_5$-labeled glutamine



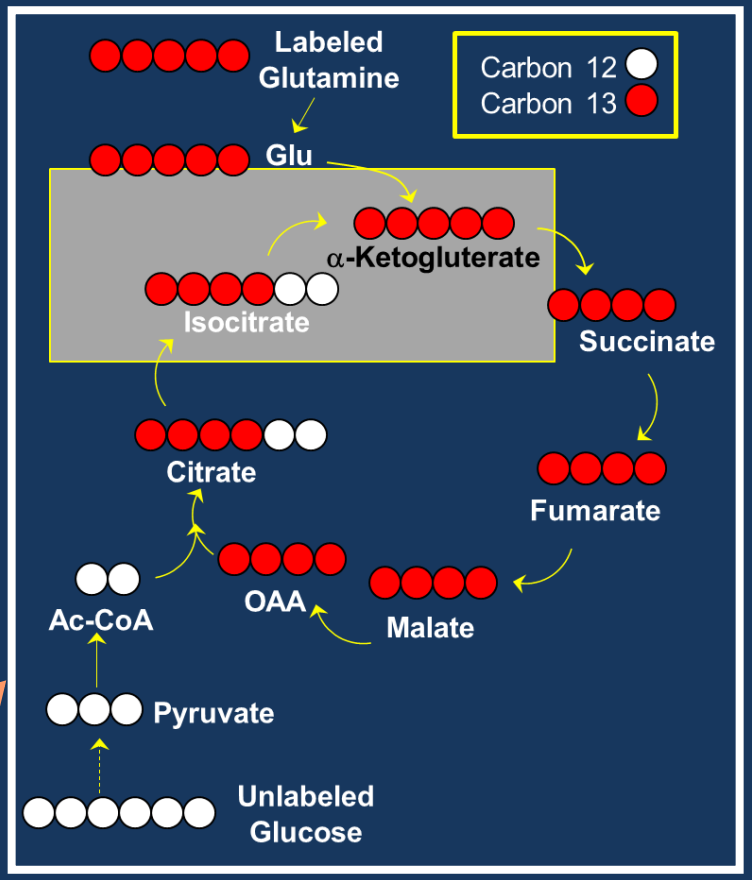
^{15}N-labeled glutamine



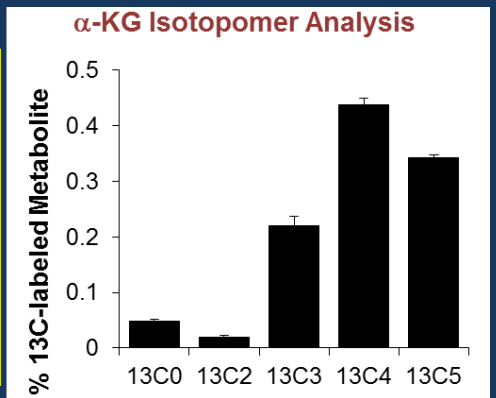
Cambridge Isotope Laboratories (CIL)



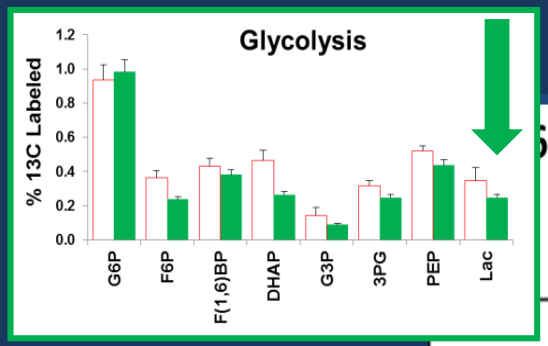
Cancer cell

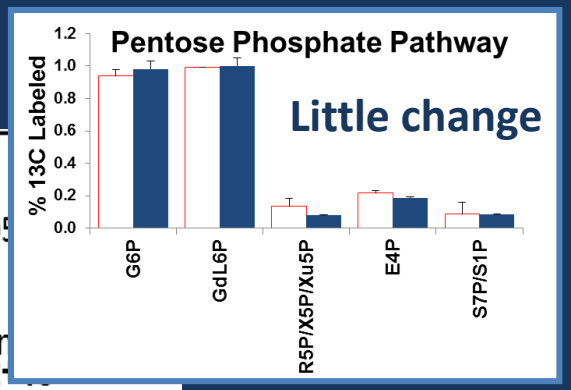


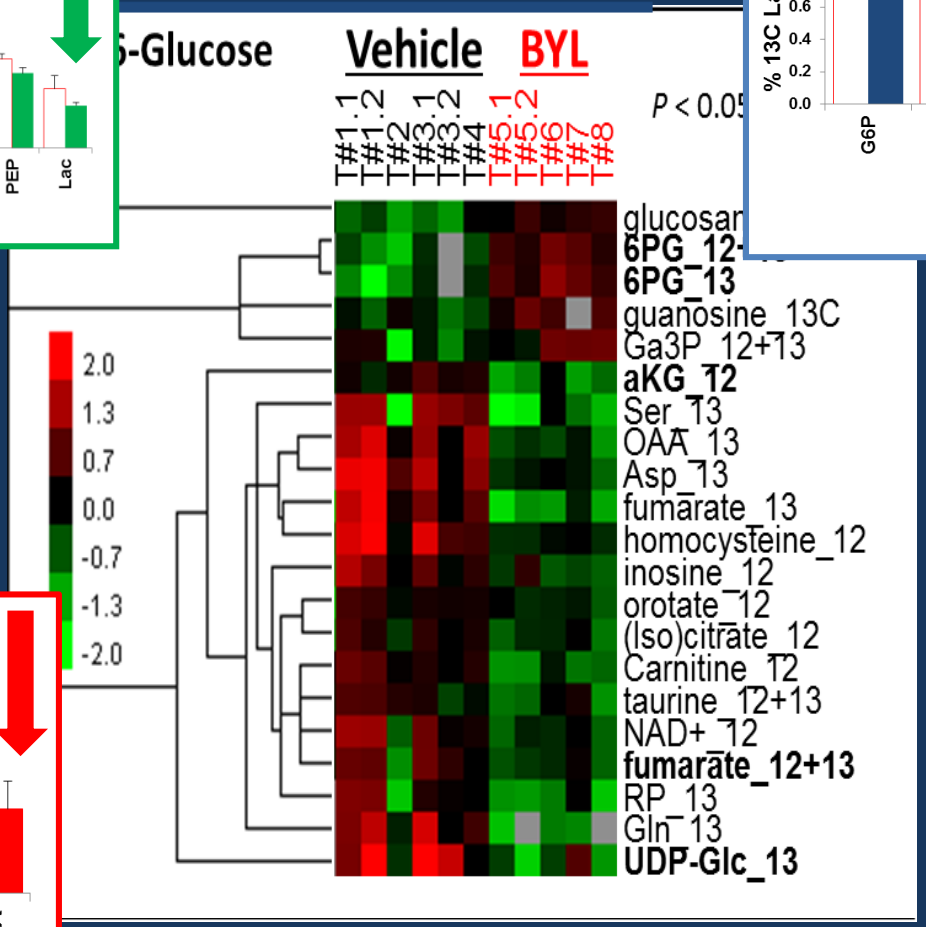
Q1	Q2	Dwell	Compound	CE
145	101	3	a-ketoglutarate	-12
146	101	3	a-ketoglutarate_13C1(2)	-12
146	102	3	a-ketoglutarate_13C1	-12
147	102	3	a-ketoglutarate_13C2(2)	-12
147	103	3	a-ketoglutarate_13C2	-12
148	103	3	a-ketoglutarate_13C3(2)	-12
148	104	3	a-ketoglutarate_13C3	-12
149	104	3	a-ketoglutarate_13C4(2)	-12
149	105	3	a-ketoglutarate_13C4	-12
150	105	3	a-ketoglutarate_13Q	-12



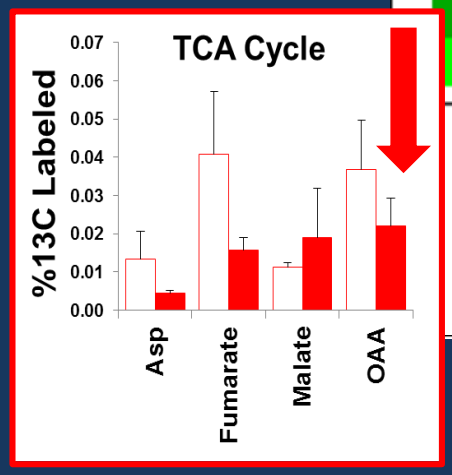
Significantly ($P < 0.05$) differentially regulated metabolites in *labeled* mouse *tumors* upon PI3K Inhibitor treatment







Vehicle
PI3K Inhibitor



^{13}C-glucose solution
(2g/kg; fasted O/N)

***In Vivo* Labeling of mice**
IP injection

Test blood glucose/sac mice

LC-MS/MS (SRM)

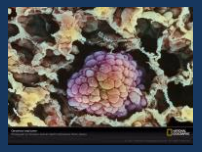
Ward & Thompson, *Cancer Cell*, 2012

Glycolysis and TCA Cycle Are Affected by PI3K inhibition

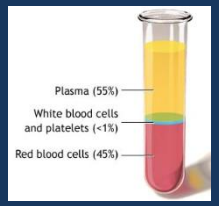
Ashish Juvekar, Costas Lyssiotis, Lewis Cantley, Gerburg Wulf

Platform for *Untargeted* Lipidomics

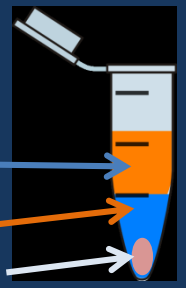
Cancer cells



Blood plasma



methyl-tert-butyl ether (MTBE) or Chl:MeOH 2:1)



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

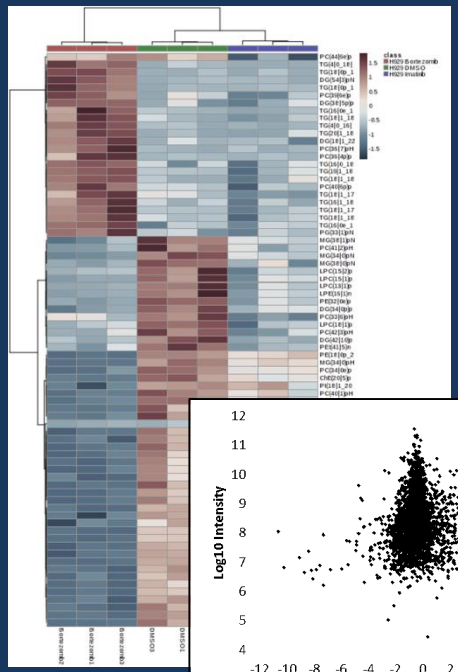
Thermo QExactive Plus **Agilent 1100**

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

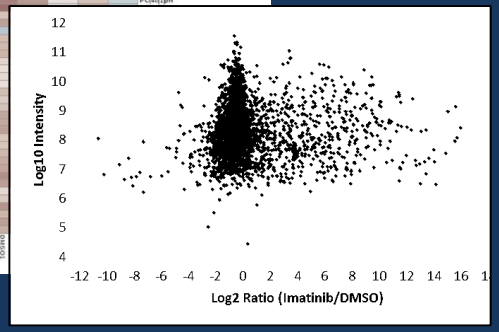
260 µL/min

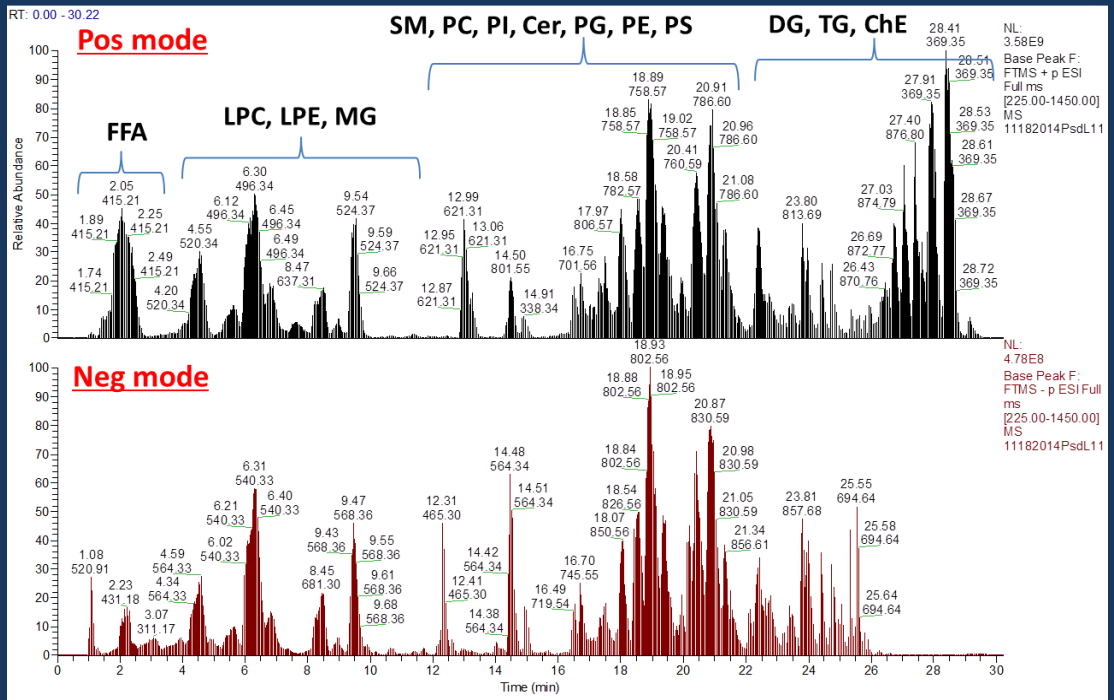
Pos/-Neg polarity switching (~10 points/peak)

DDA (Top 10) pos *and* neg mode (m/z 200-1500)



~ 10 points/peak





Fatty Acid Synthesis

TCA cycle → Acetyl-coA

Glycolysis → Acetyl-coA

Acetyl-coA —**ACC**→ Malonyl-coA

Malonyl-coA —**FAS**→ Palmitate (C16:0)

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

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

Palmitate (C16:0) —**elongation**→ Stearate (C18:0)

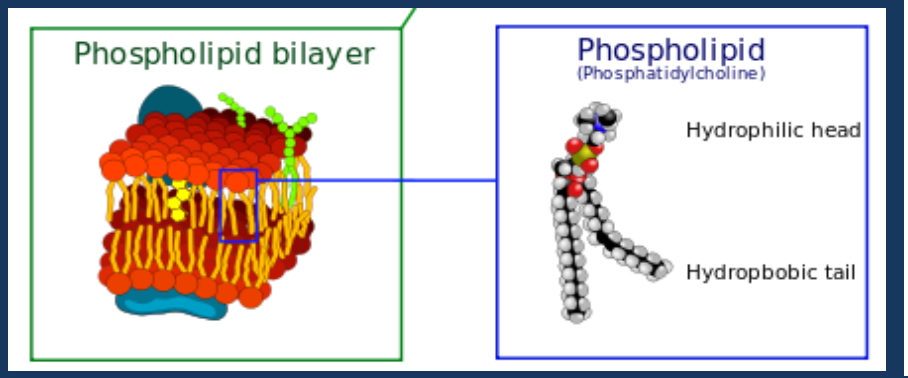
Stearate (C18:0) —**SCD1 desaturation**→ Oleate (C18:1)

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

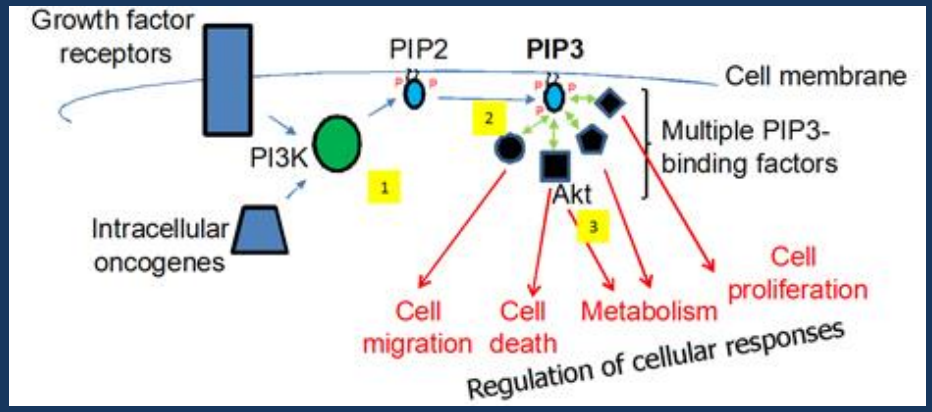
Stearate (C18:0) —**elongation**→ Arachidate (C20:0)

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

Membrane structure

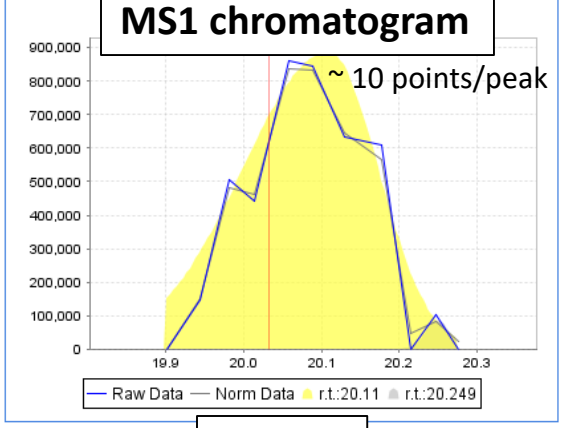


Signaling Lipids

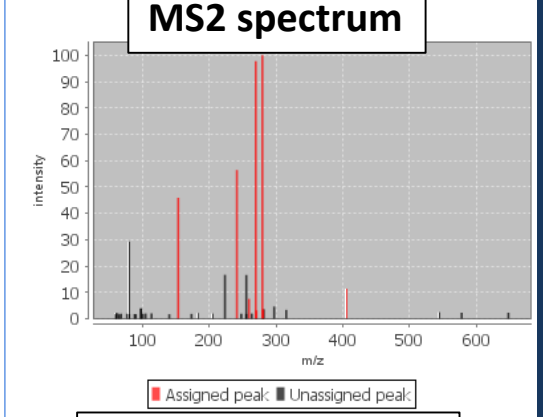


Thermo LipidSearch Identification/Quantitation Process

MS1 chromatogram

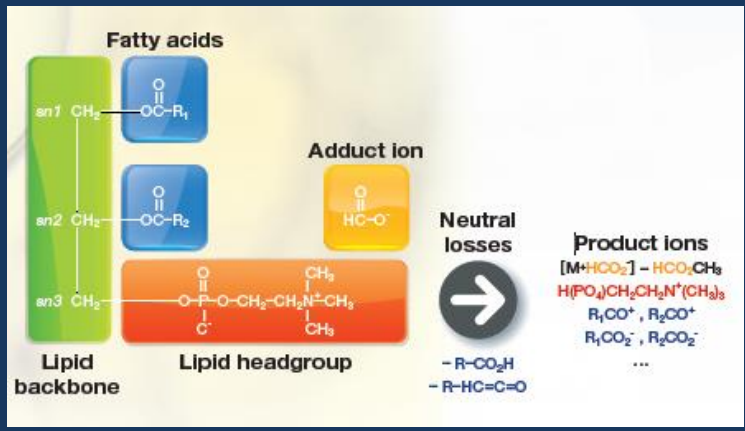


MS2 spectrum



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

Phospholipid



Scoring

Match Lipid

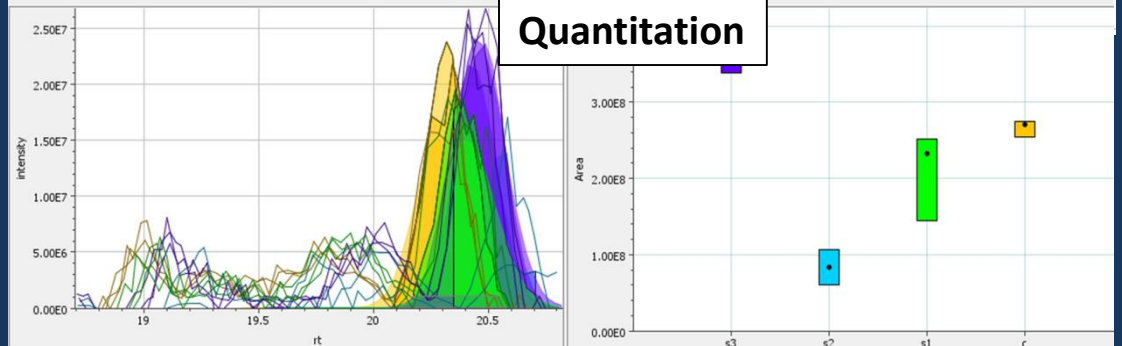
LipidIon	M-Sc.	T-Sc.	Acc.	St.
PI(17:0/18:2)-H	43.3	0.4	72.2	
PI(18:2/17:0)-H	43.3	0.4	72.2	
PI(16:0/19:2)-H	11.2	0.4	28	
PI(19:2/16:0)-H	11.2	0.4	28	
PI(17:1/18:1)-H	10.1	0.4	25.2	
PI(18:1/17:1)-H	10.1	0.4	25.2	
PI(11:0/24:2)-H	7.3	0.4	24.4	
PI(13:0/22:2)-H	7.3	0.4	24.4	
PI(15:0/20:2)-H	7.3	0.4	24.4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

HCD fragmentation

Match

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
279.2333	MS2	100	FA(18:2)-H	0.0003

Quantitation



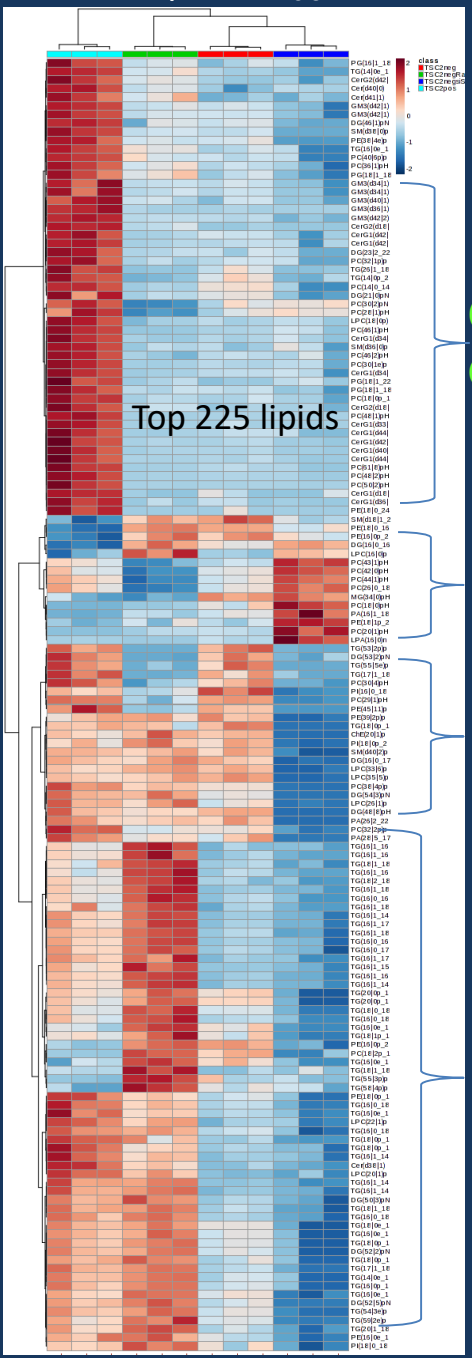
-Capable of identifying >1000 lipid ions in 30 min. with pos/neg switching

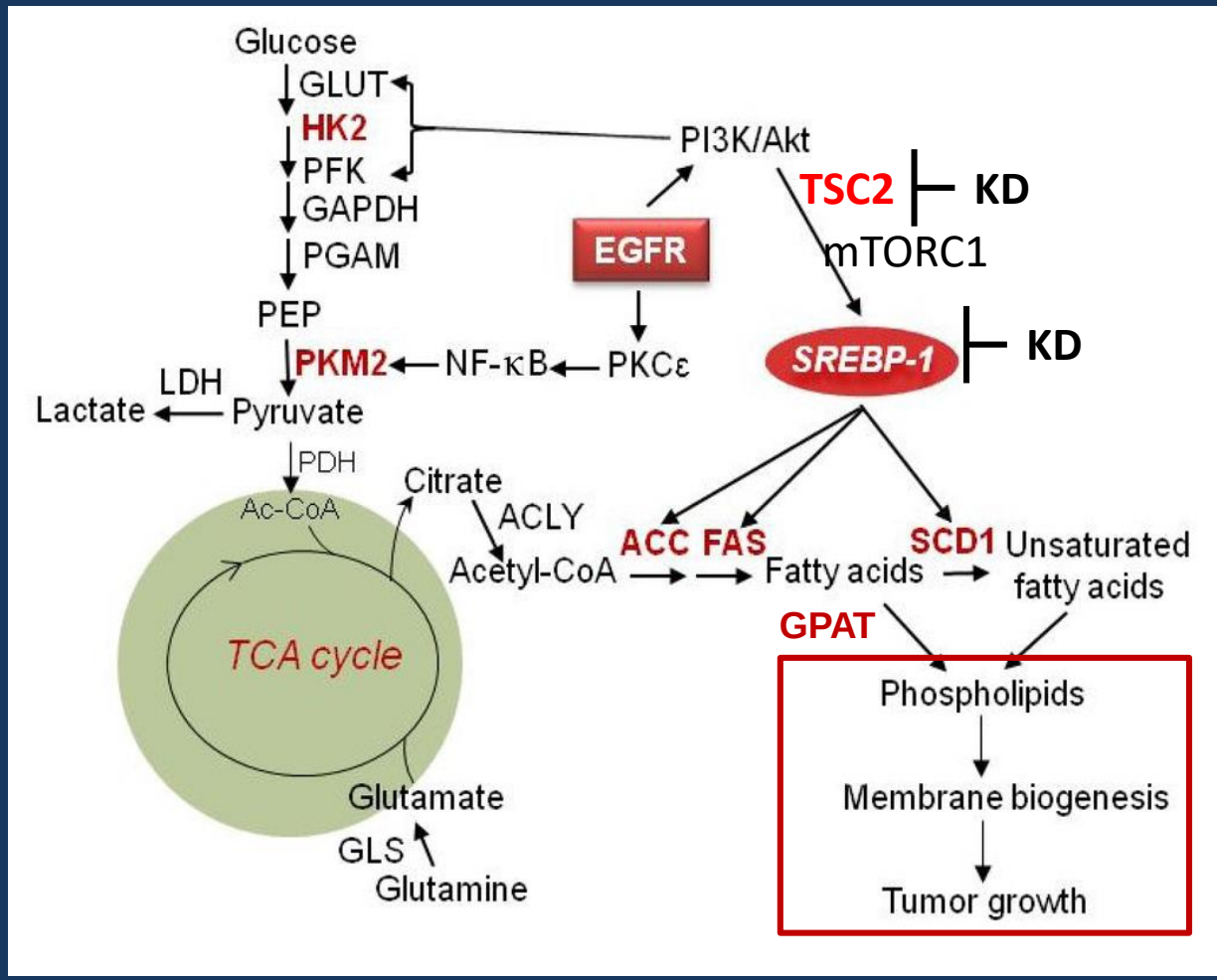
Susanne Breitkopf, Min Yuan, David Peake

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

- **39 lipid classes and 66 sub classes can be identified via LC-MS/MS and LipidSearch**

Investigation of TSC2 null MEFs on *De Novo* Lipid Synthesis using Untargeted Lipidomics

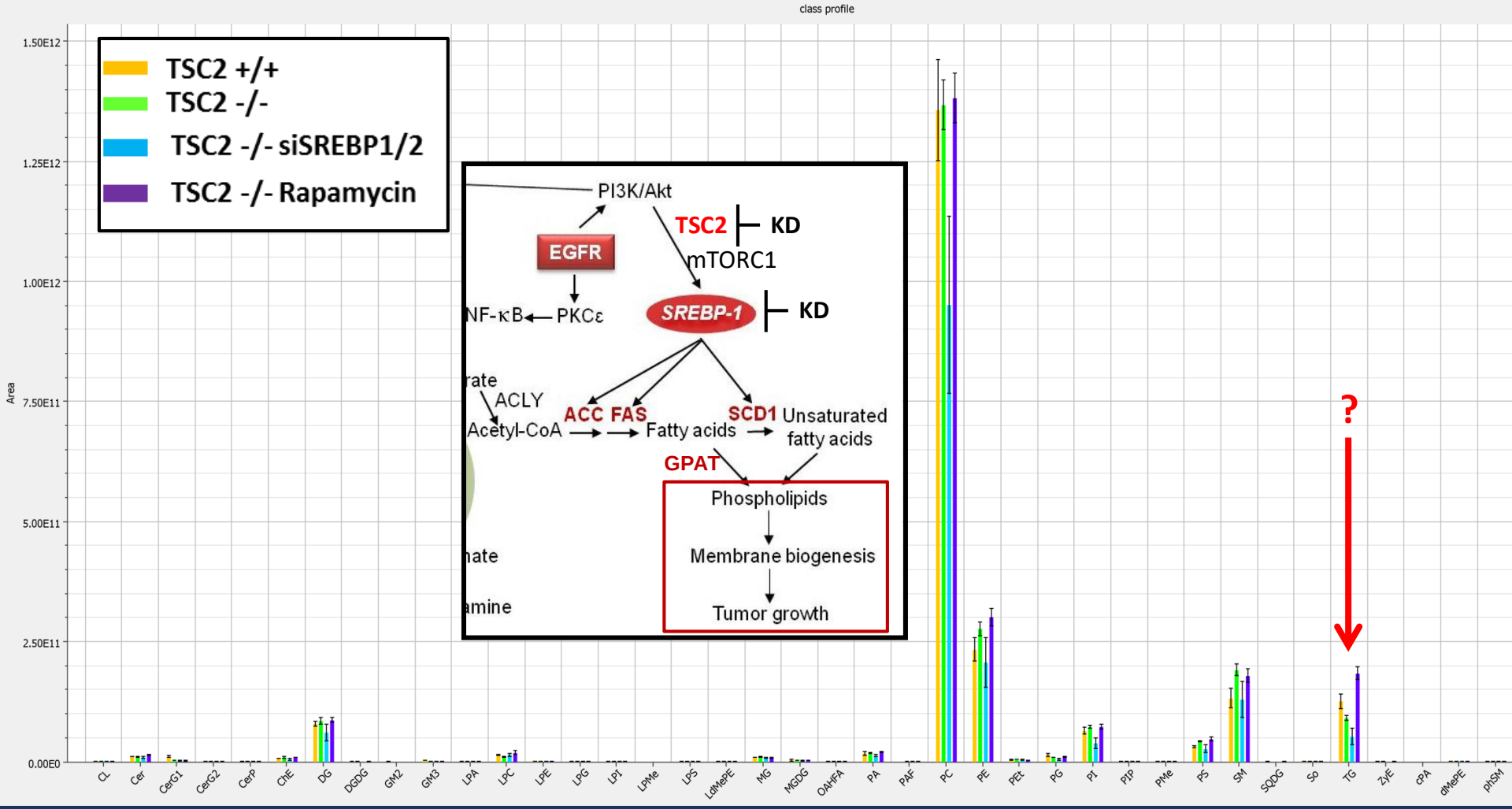




Ru et al, Cancers 2013, 5(4), 1469-1484

Stéphane Ricoult, Brendan Manning

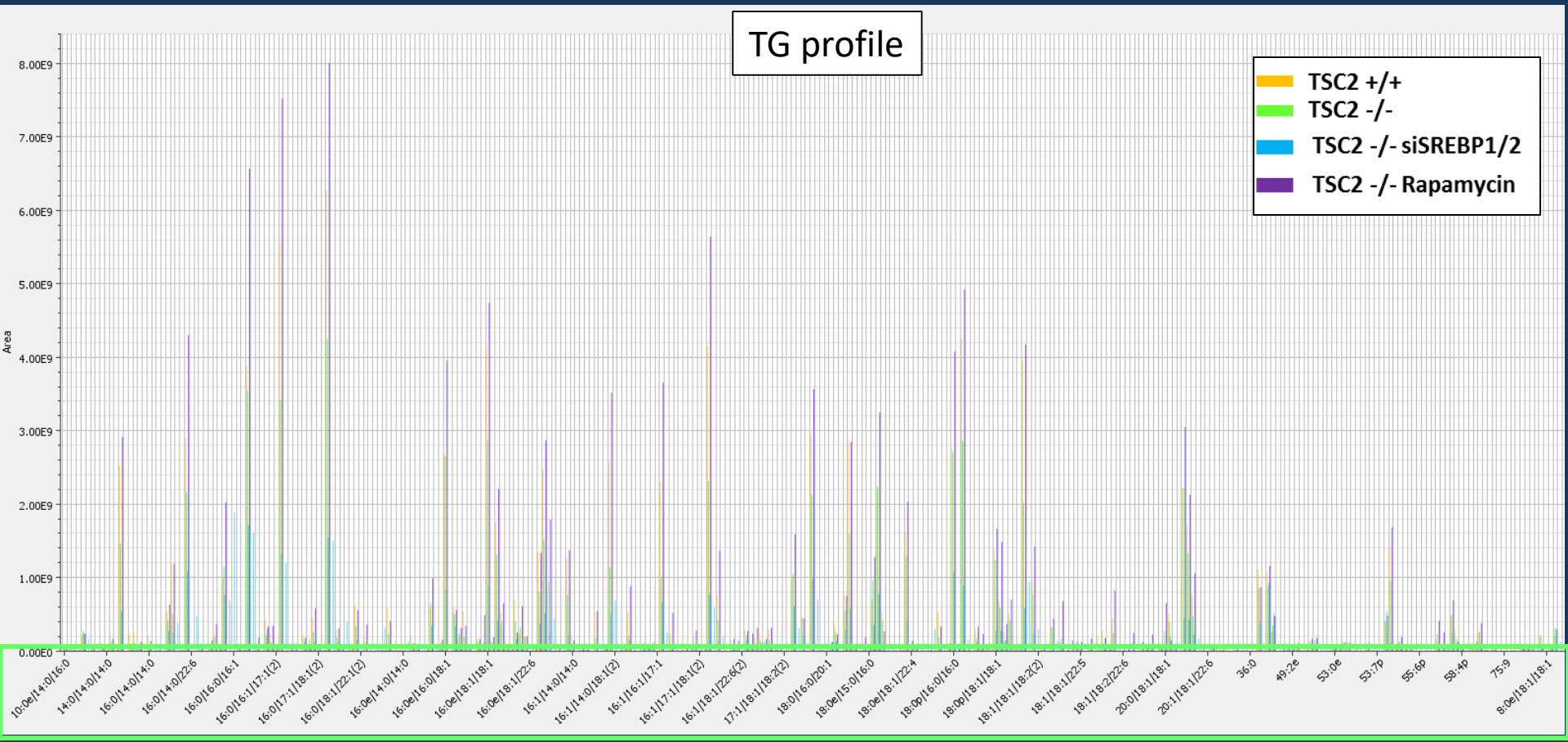
Overview of Lipid Class regulation in TSC2-/- MEFs



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

Stéphane Ricoult, Brendan Manning

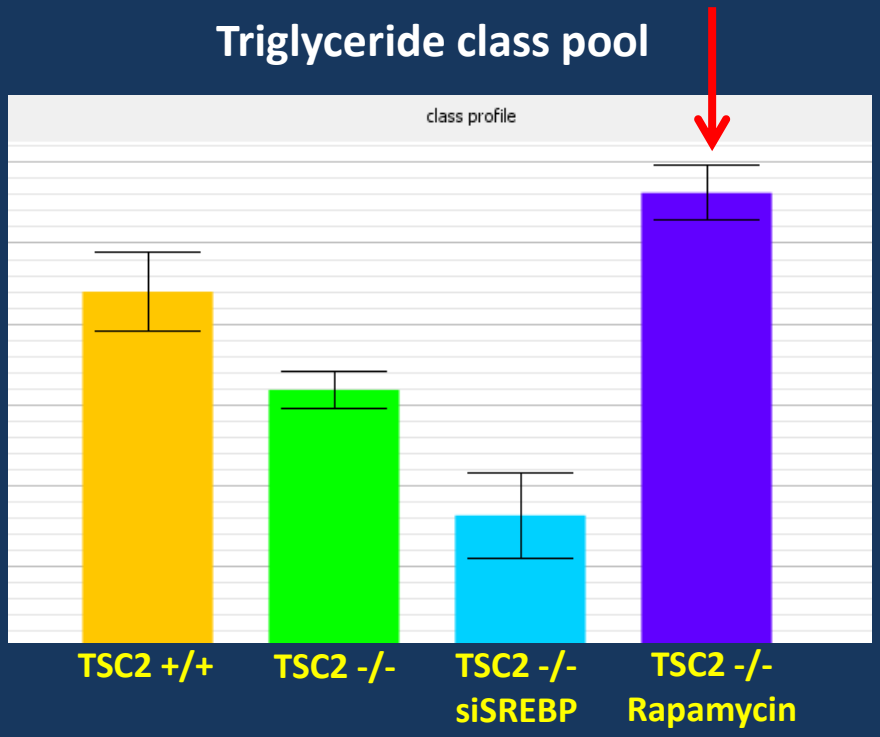
Triglyceride (TG) fatty acid composition in TSC2 MEFs



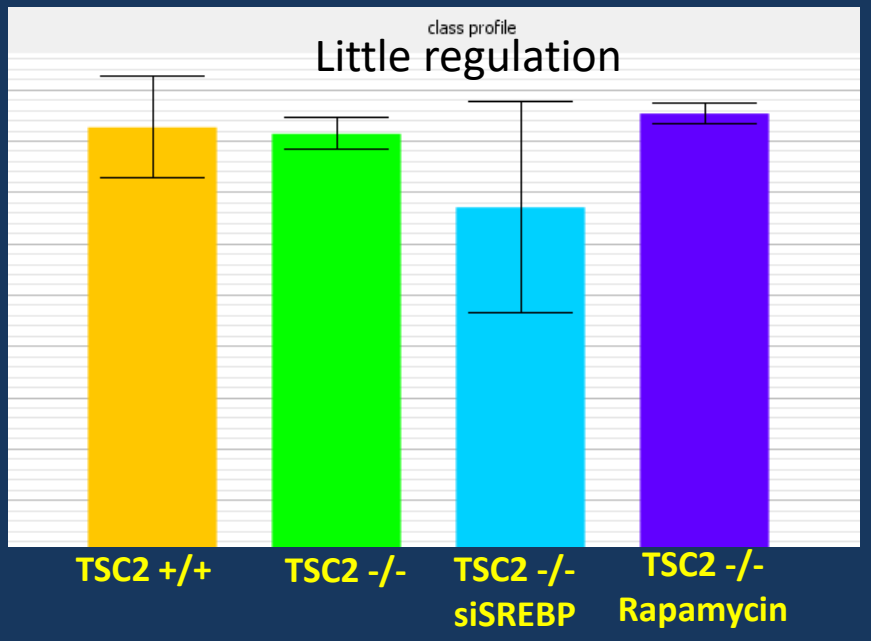
- Rapamycin rescues TG levels in TSC2-/- MEFs similar to TSC2+/+ levels
- Many major TG fatty acids are basic building blocks (palmitate, oleate, etc.)

Stéphane Ricoult, Brendan Manning

Triglyceride class pool



Phospholipid control (PC most abundant)

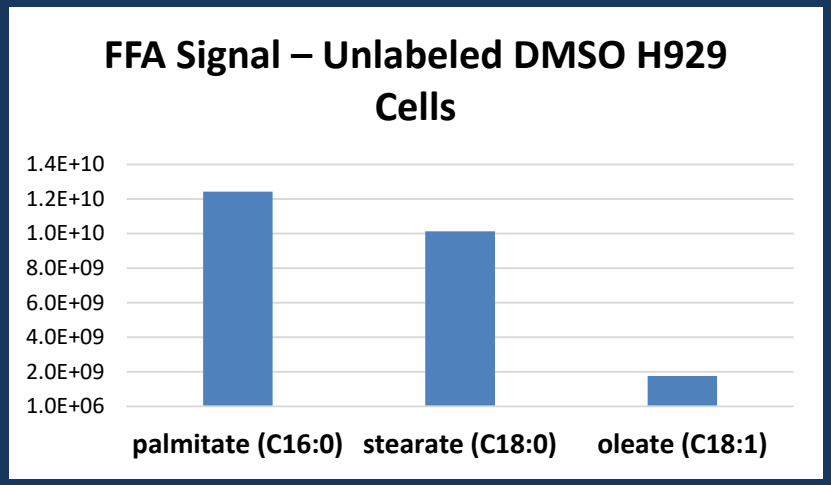


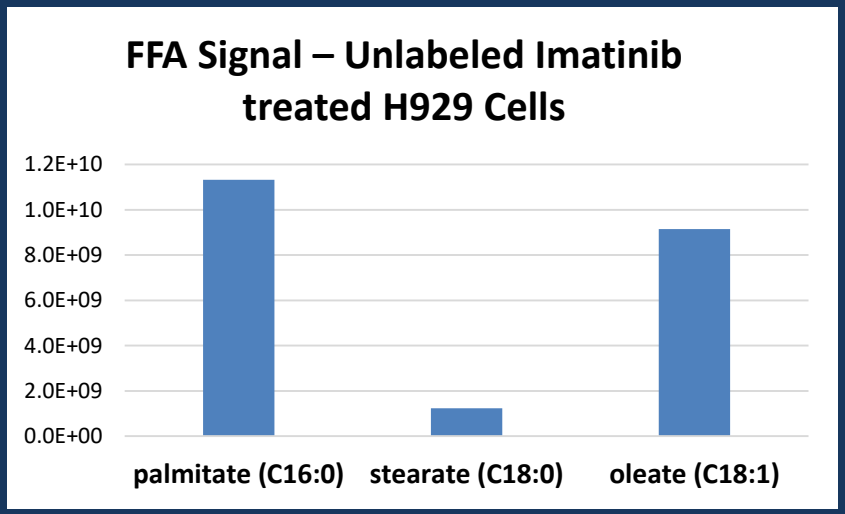
Patient Triglyceride Levels Rise in Response to Rapamycin

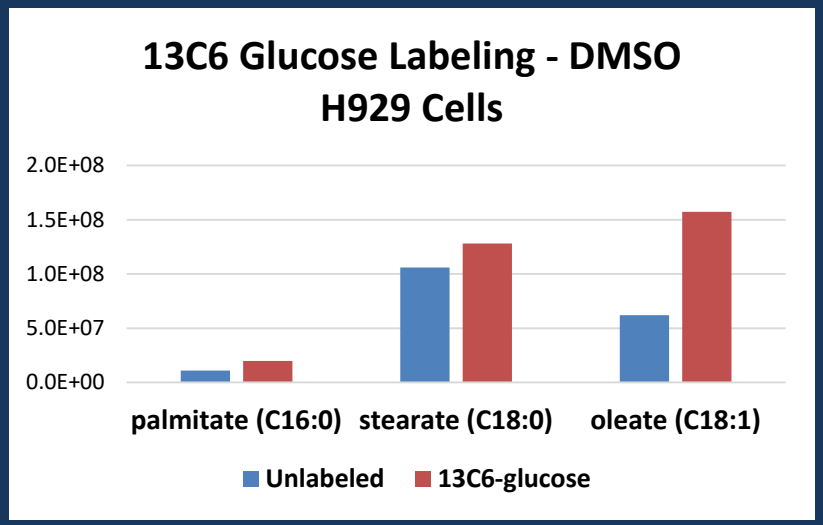
Triglyceride Level Before/ After Sirolimus					Hyperlipidemia	
Day −1[b]	Day 14 Stop Drug	Day 28	Day 56	Change	Before	After
mg/dl				%		
169	465[a]	ND	ND	175	II a	II b
123	294	211	163	139	II a	II b
739	1709	652	ND	131	II b	II b
248	628	313	155	153	II b	II b
156	340	162	137	118	N	II b
315	392	475	298	24	II b	II b
				123		
				0.04		

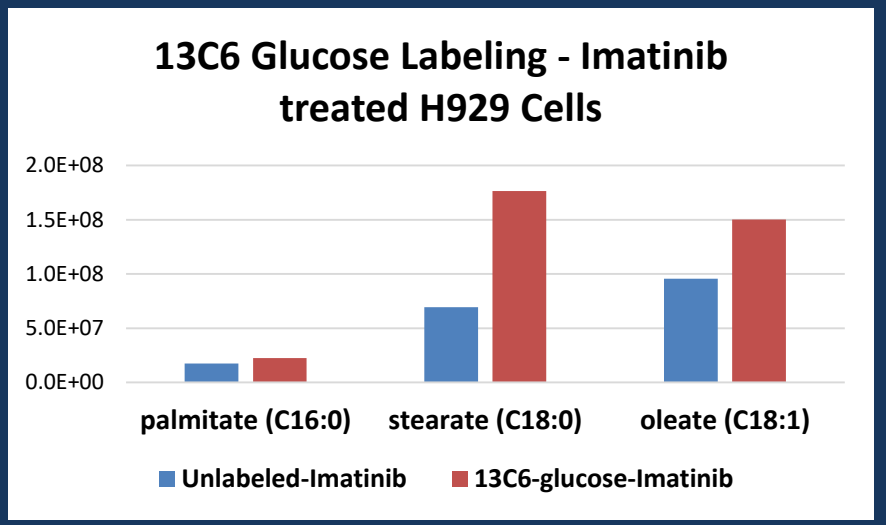
Morrisett et. al., *J. Lipid Research*, 2002

$^{13}C_6$ – Glucose Tracing of Free Fatty Acids in H929 myeloma cells







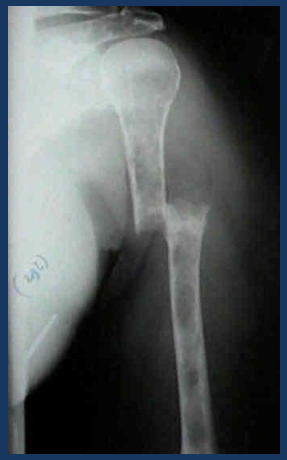


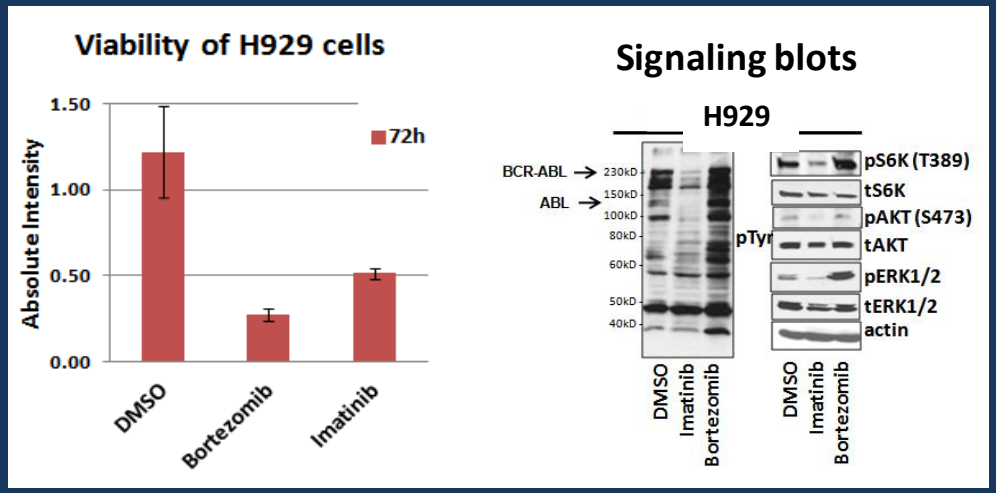
- LipidSearch software *needs* to account for stable isotope labeling……..
- Setting up SRM scans to some labeled species…..

Susanne Breitkopf

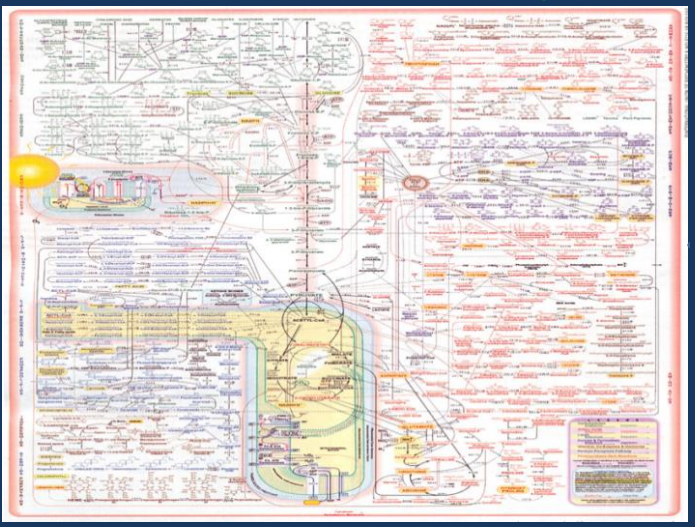
Cross-Omics Study in BCR-ABL H929 Myeloma Cancer Cells with the Kinase Inhibitor Imatinib

Multiple Myeloma: a cancer of the plasma cells in bone marrow





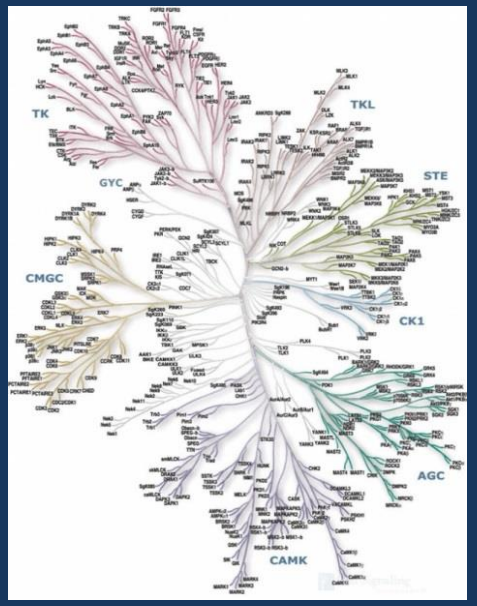
Human metabolome/lipidome



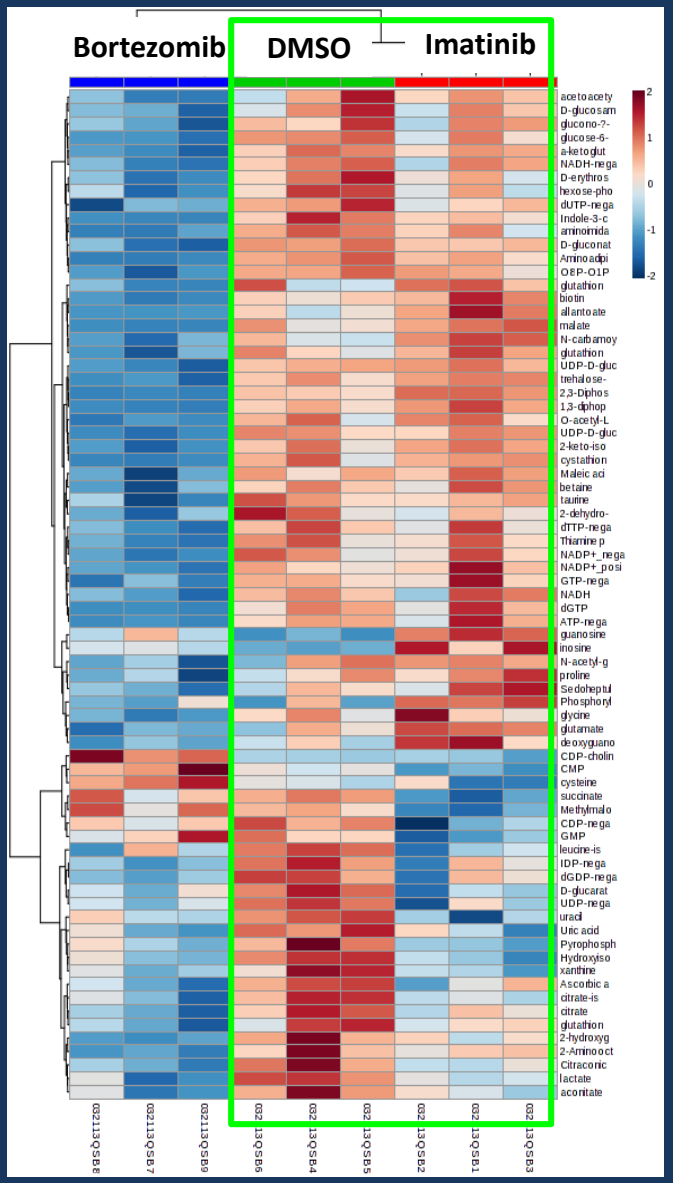
Metabolomics/ Lipidomics ***Phosphoproteomics***

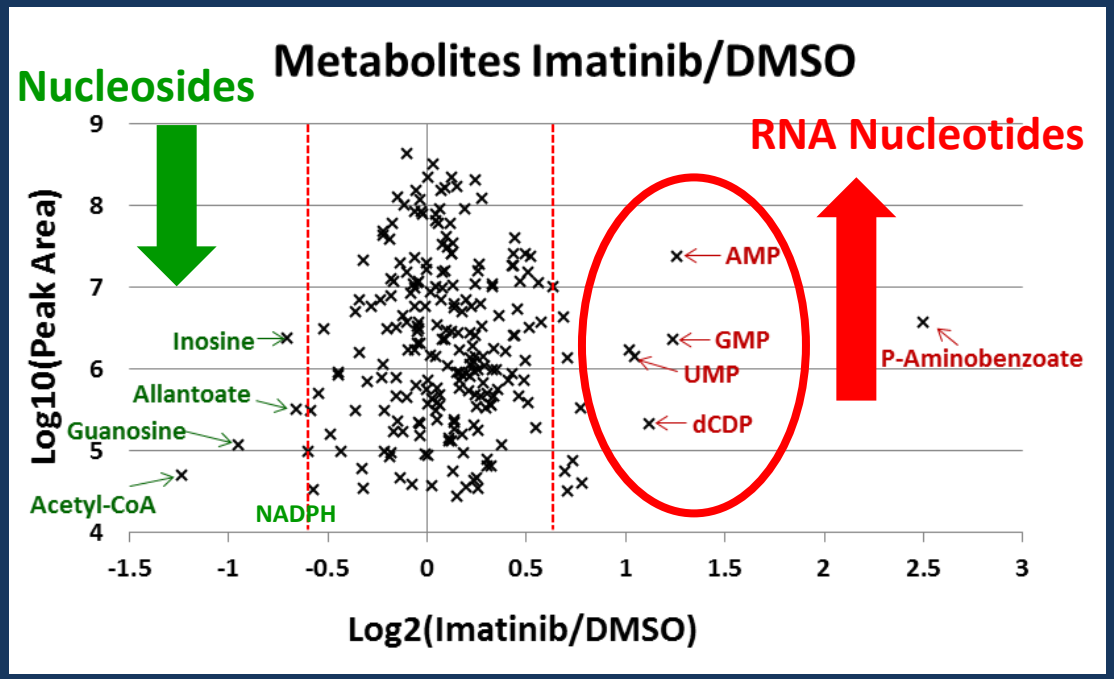
???

Human kinome

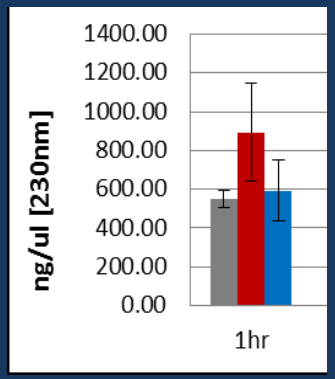


Distribution of Imatinib Regulated Polar Metabolites by Targeted LC-MS/MS

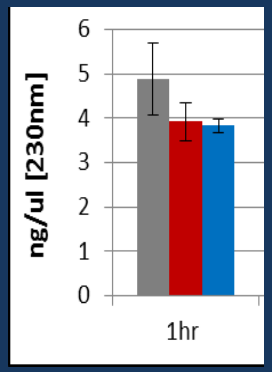




Cytoplasmic RNA



Nascent RNA

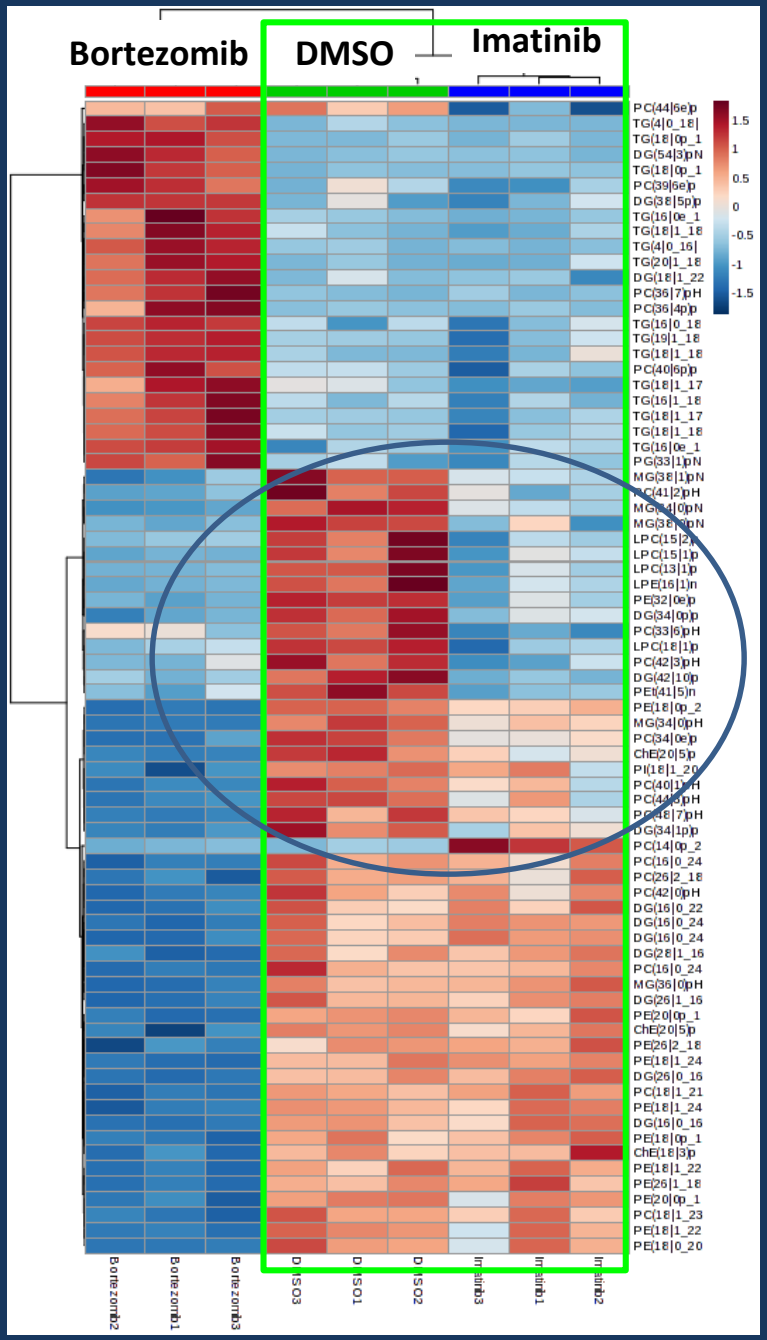


RNA degradation inhibited

RNA synthesis inhibited

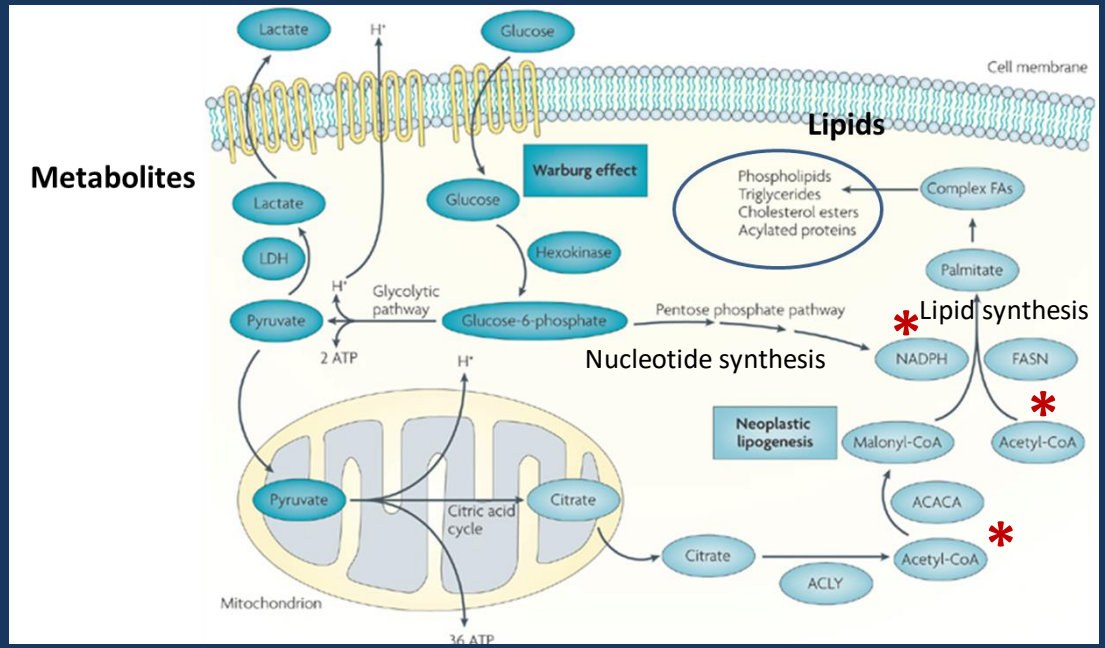
Susanne Breitkopf, Min Yuan, Katja Helenius, Costas Lyssiotis

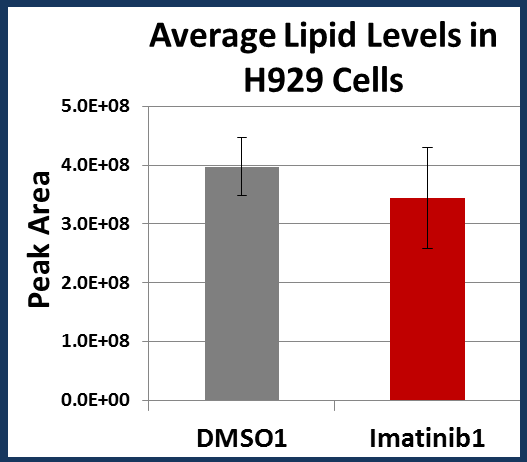
Distribution of Imatinib Regulated Lipids by Targeted LC-MS/MS



H929 Lipidomics Heat Map

Top 100 features





Lipid synthesis inhibited

Susanne Breitkopf, Min Yuan

Triple SILAC Phosphoproteomics Platform


Lys0, Arg0


Lys4, Arg6


Lys8, Arg10

Imatinib 1 µM Bortezomib **200 nM** **DMSO control**

Lysis Lysis Lysis

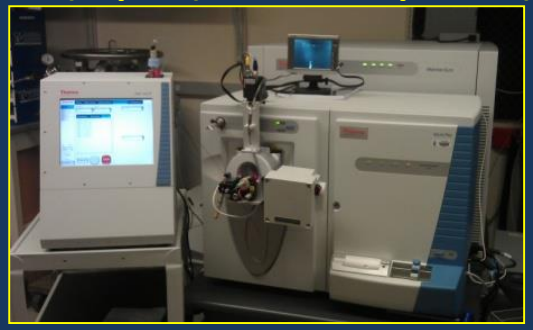
Mix 1:1:1

Trypsin digestion

Offline SCX chromatography

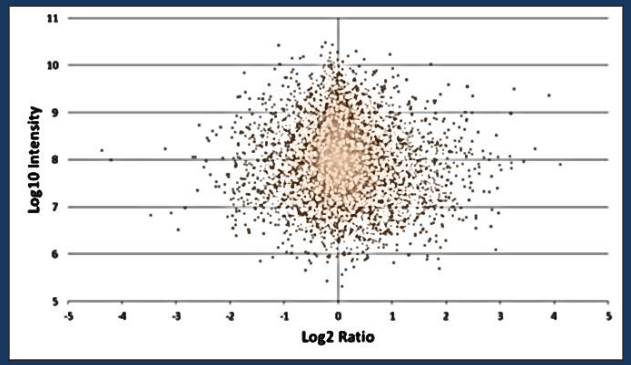
IMAC/Fe^{3+}

2hr LC/MS/MS via HCD (Top 12) (2X) / CID (Top 20) on Orbitrap Elite (1X)

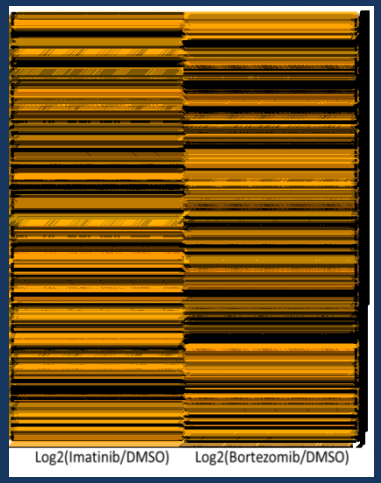


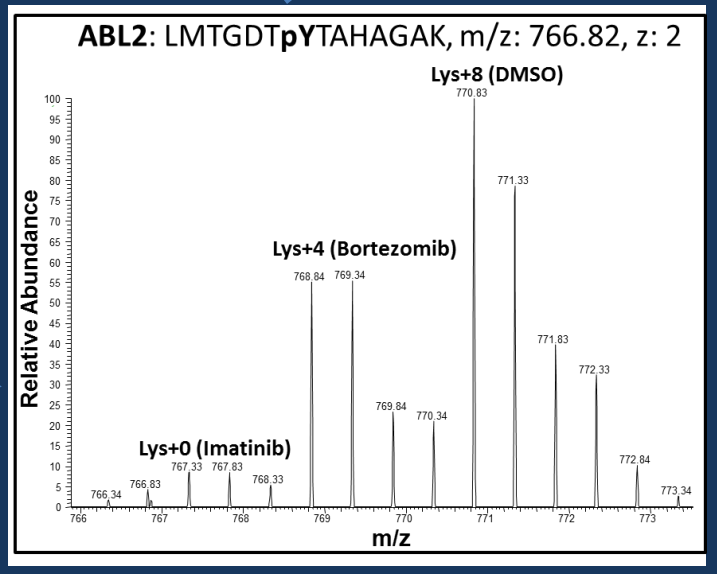
MaxQuant identification/quantification

Peak distribution



Heat Map - Ratios



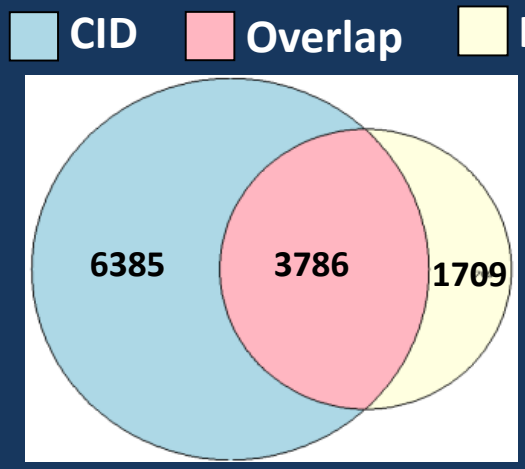


Triple SILAC

Susanne Breitkopf, Min Yuan

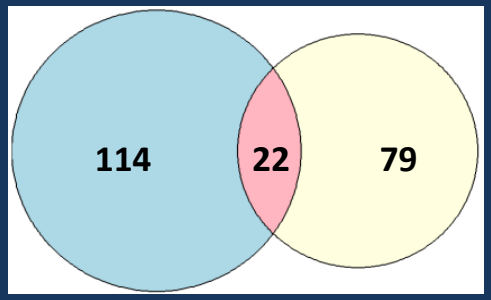
11,880 total unique phospho-STY sites

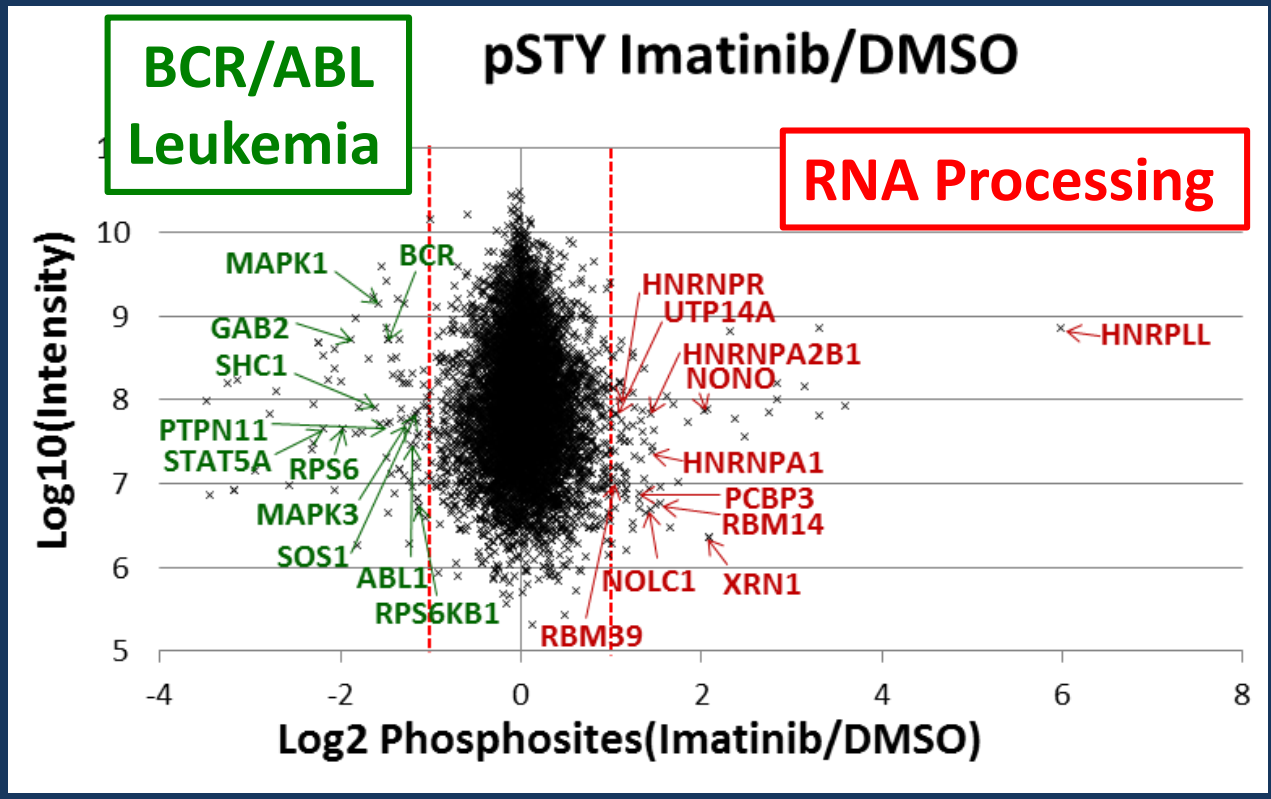
3,121 Total phosphoproteins



Down-regulated pSTY Imatinib

Up-regulated pSTY Imatinib



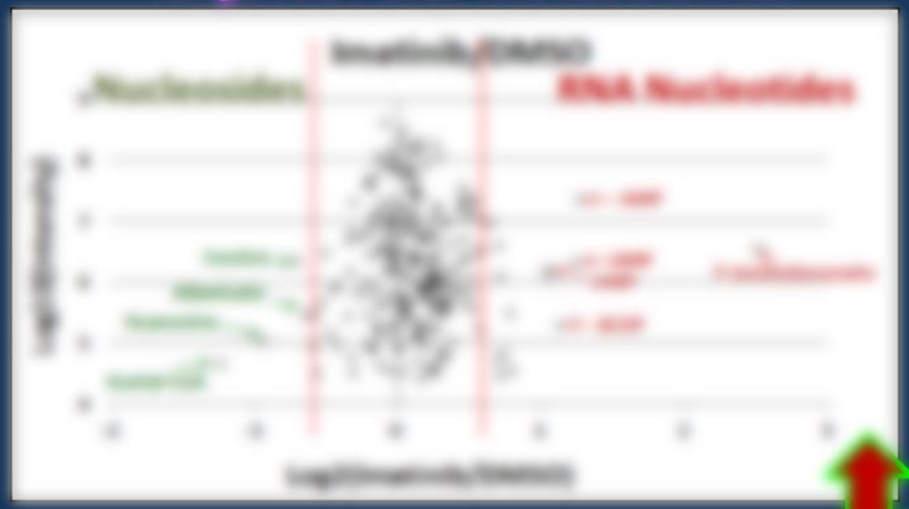


BCR-ABL in H929 known from our previous work……

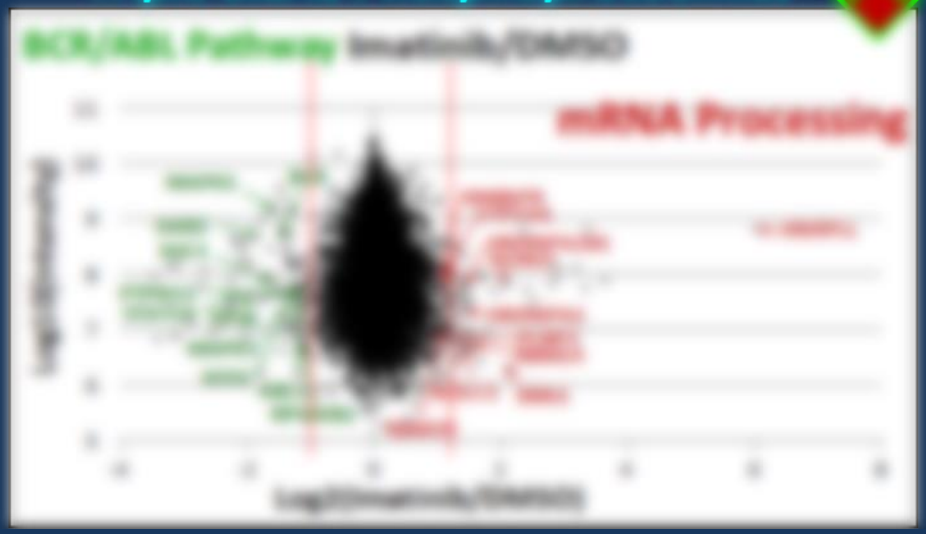
Breitkpf et. al., 2012, PNAS

Merging the Phosphoproteome-Metabolome-Lipidome.....

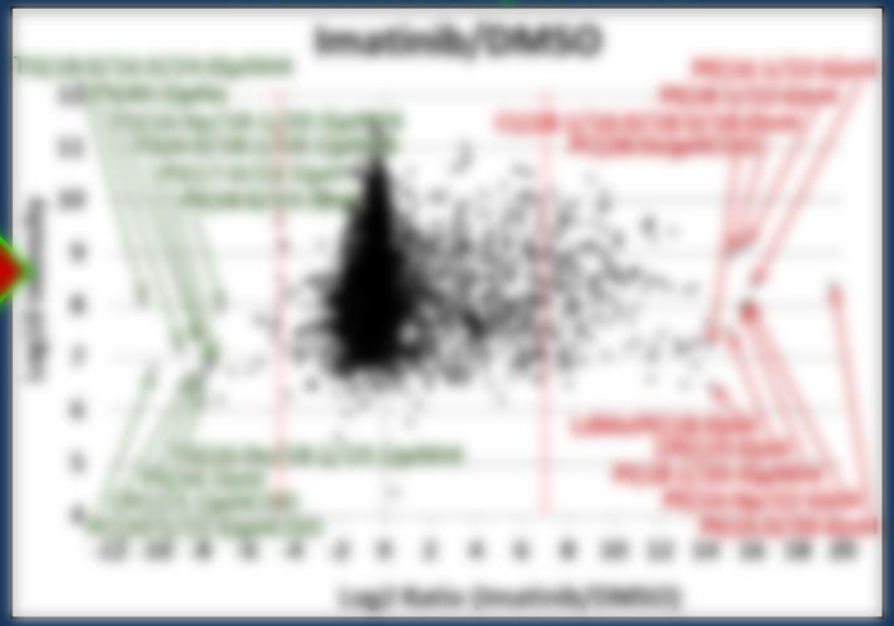
Targeted Polar Metabolomics



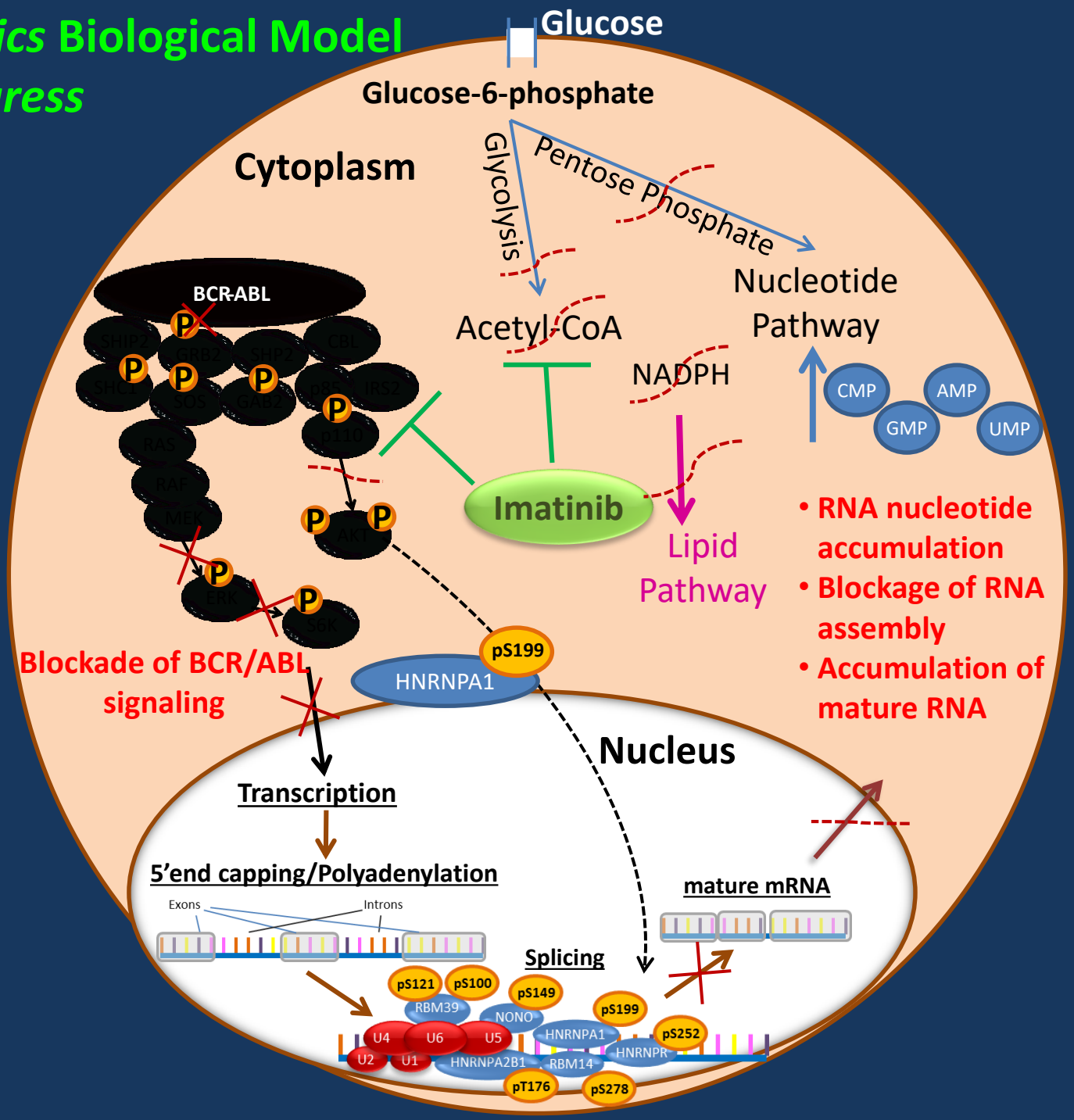
Triple SILAC Phosphoproteomics



Non-Polar Lipidomics



Cross-Omics Biological Model
- In progress



Acknowledgements

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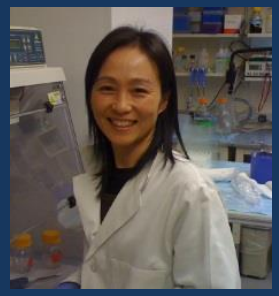
Susanne Breitkopf



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