Serial-Omics: From Breast Tumors to Bodily Fluids to Dried Blood Spots
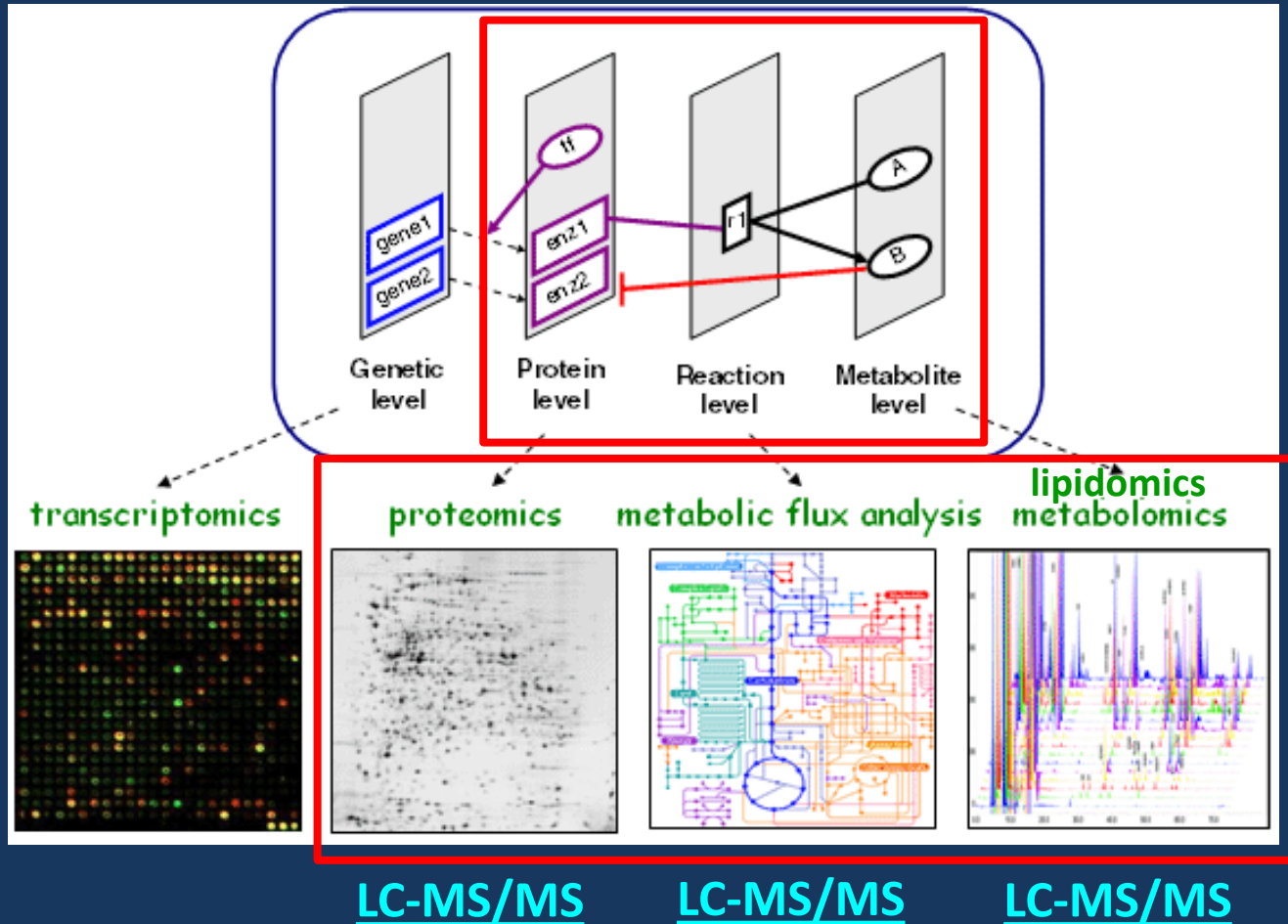
John M. Asara, Ph.D.
Beth Israel Deaconess Medical Center
Harvard Medical School
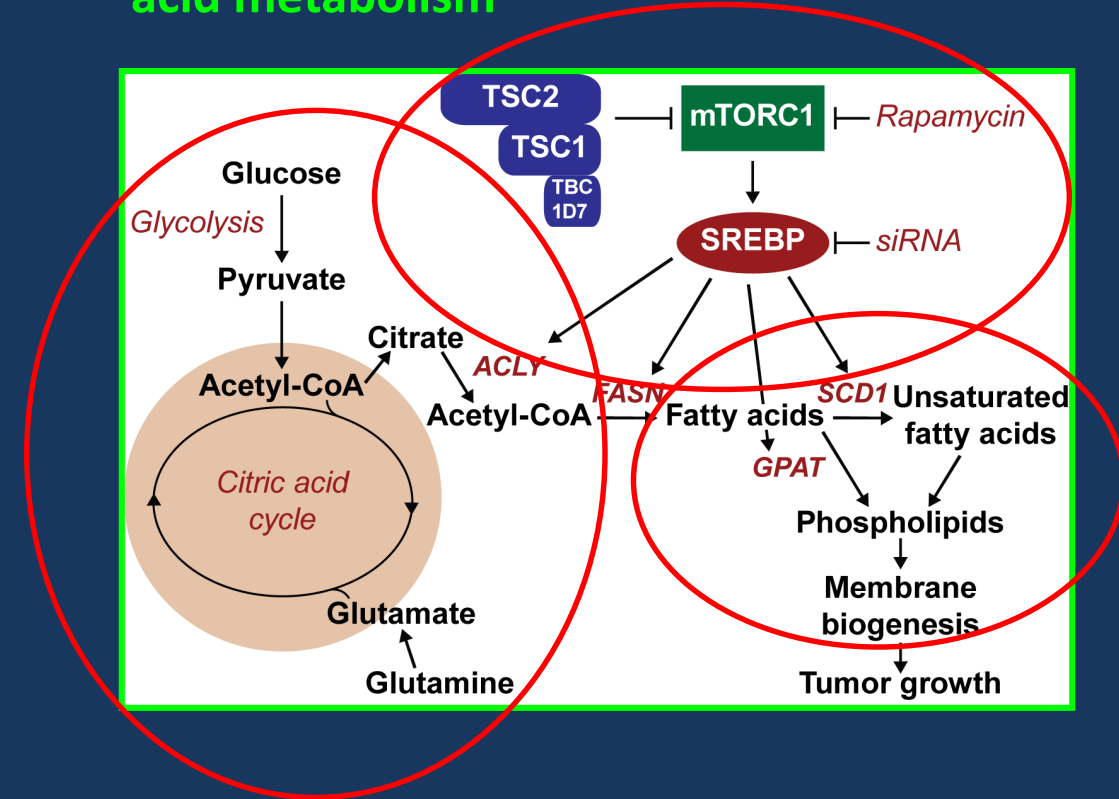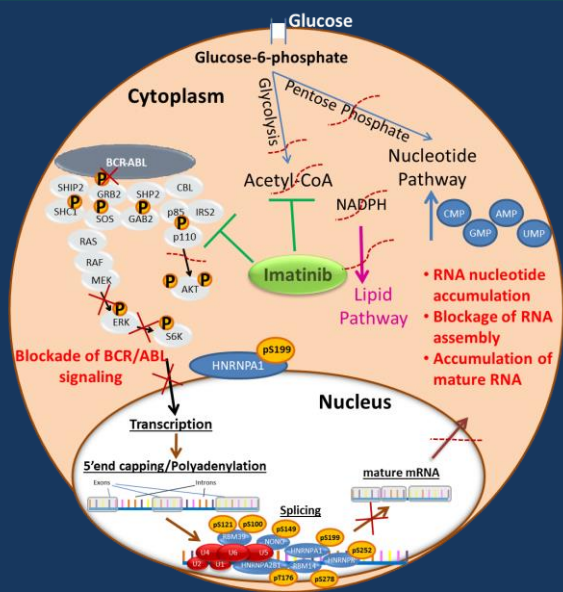
Biomarkers: Qualitative Analysis
06/07/2018

Cancer Center   Beth Israel Deaconess Medical Center   HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

- Integrating Different *–Omics* Approaches

transcriptomics   proteomics   metabolic flux analysis   lipidomics metabolomics

LC-MS/MS   LC-MS/MS   LC-MS/MS

Cell signaling to central carbon and fatty acid metabolism

Separate *Omics* experiments

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

H929 cells/multiple myeloma

△ Lipidomics
X Metabolomics
□ Phosphoproteomics
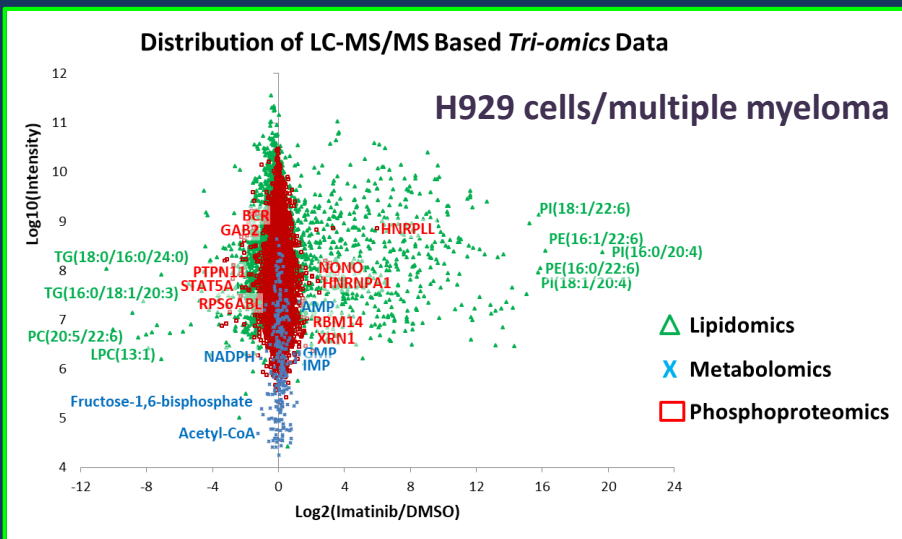
Breitkopf et. al., 2015, *Anal. Chem.*

Serial-*Omics* experiments

Our current approach

Matyash, 2008, J. Lipid Res.

methyl-tert-butyl ether (MTBE/MeOH)

Non-polar Lipids
Thermo QExactive Plus

Targeted Polar Metabolites
AB/SCIEX 5500 QTRAP

Untargeted Polar Metabolites
Thermo QExactive HF

Phophophopeptides & Global Proteins
Thermo QExactive HF
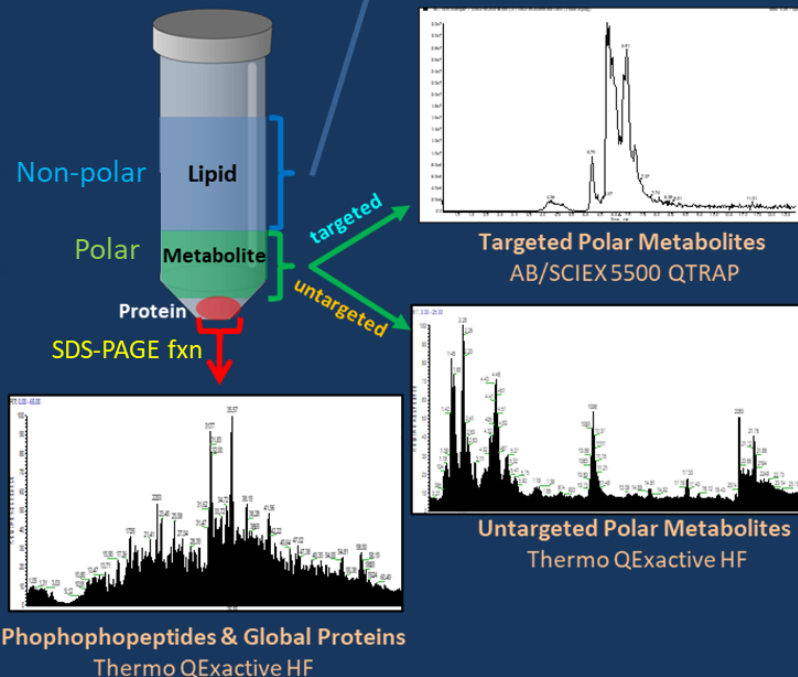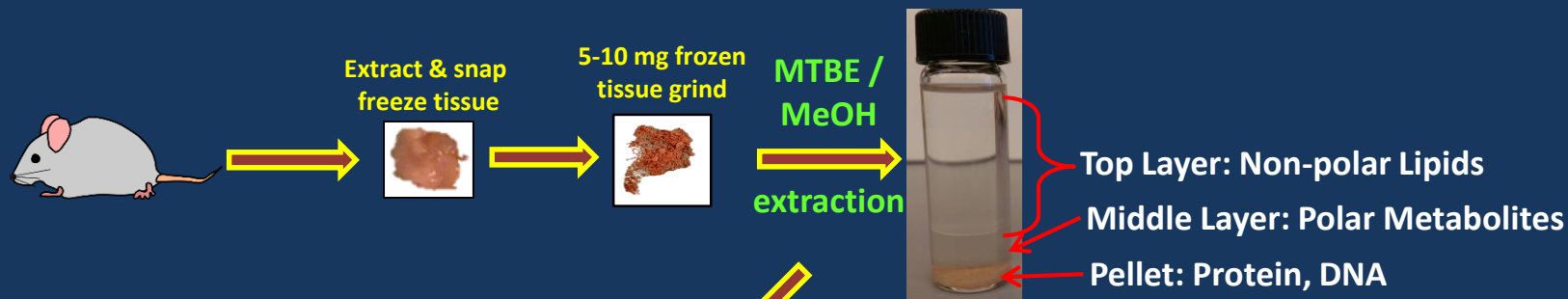
Breitkopf et. al, 2017, *Sci Reports*

Willmitzer lab, Max-Planck Institute, Germany -Plants

Ahrends lab, Univ. of Aberdeen, UK   -Stem cells

+ more

Serial-Omics Platform Workflow

Applications
- **Breast/Lung Tumors**
- **Dried Blood Spots**
- **Urine**
- **Hair**

Extract & snap freeze tissue

5-10 mg frozen tissue grind

MTBE / MeOH extraction

Top Layer: Non-polar Lipids
Middle Layer: Polar Metabolites
Pellet: Protein, DNA

Non-polar Lipids
Speed-Vac Dry
Resuspend in 50/50 IPA/MeOH

DDA, HCD, +/- switch, RP-C18
^{13}C lipid flux

QE+/HF 1100/1200

LipidSearch software
Elements software
MetaboAnalyst
FluxSearch

Polar Metabolites
Speed-Vac Dry
Resuspend in H_2O

SRM, **+/-** switch, 300 unlabeled targets
185 $^{13}C/^{15}N$ targets, Amide-HILIC

5500/6500

MultiQuant
MetaboAnalyst

DDA, HCD, +/- switch

1100
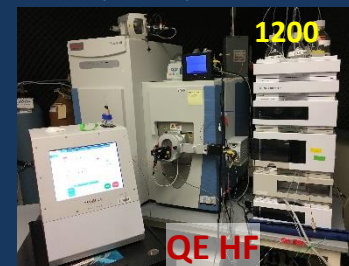
QE+

Elements, FluxSearch

Peptides/Phosphopeptides
Pellet dissolved in
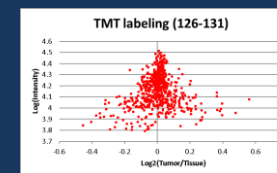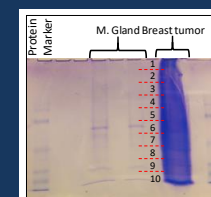lysis buffer/SDS-PAGE

Or Digest pellet with Trypsin
Fractionation
IMAC/TiO2
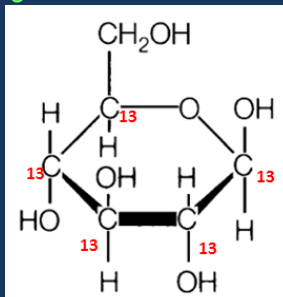TMT labeling (126-131)

DDA, HCD, RP-C18

SDS-PAGE Gel Fractionation

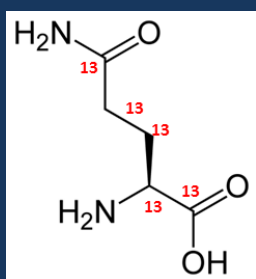TMT labeling (126-131)

1200

QE HF

Mascot / Scaffold
MaxQuant

Steady-State Metabolic Profiling and Flux Analysis:

$^{13}C_6$-labeled glucose

$^{13}C_5$-labeled glutamine

^{15}N-labeled glutamine

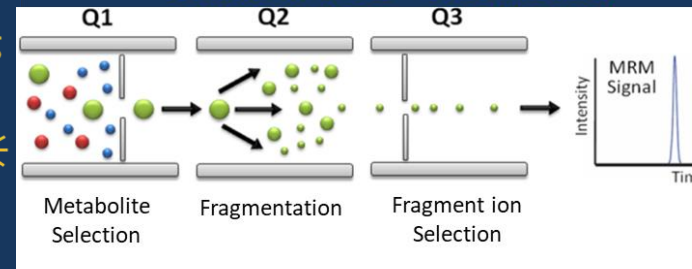Cancer cells

Shimadzu Prominence UFLC

5500/6500 QTRAP
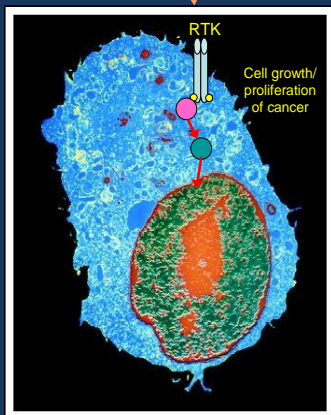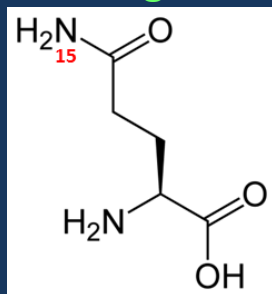
+/- switching

Amide HILIC
4.6 mm x 10cm
pH=9.0, NH$_4^+$
400 µL/min

Selected Reaction Monitoring (SRM) ~300 metabolite transitions (~150 ^{13}C metabolites)

Q1	Q2	Q3
Metabolite Selection	Fragmentation	Fragment ion Selection

MRM Signal

Yuan *et. al.*, 2012, ***Nat. Protoc.***

Labeled Glutamine

Carbon 12 (white)
Carbon 13 (red)

Glu

α-Ketogluterate

Isocitrate

Succinate

Citrate

Fumarate

Ac-CoA

OAA

Malate

Pyruvate

Unlabeled Glucose

Q1	Q3	Isotopologue	Fragment
90	45	M+3	F+2
89	45	M+2	F+2
89	44	M+2	F+1
88	44	M+1	F+1
88	43	M+1	F+0
87	43	M+0	F+0
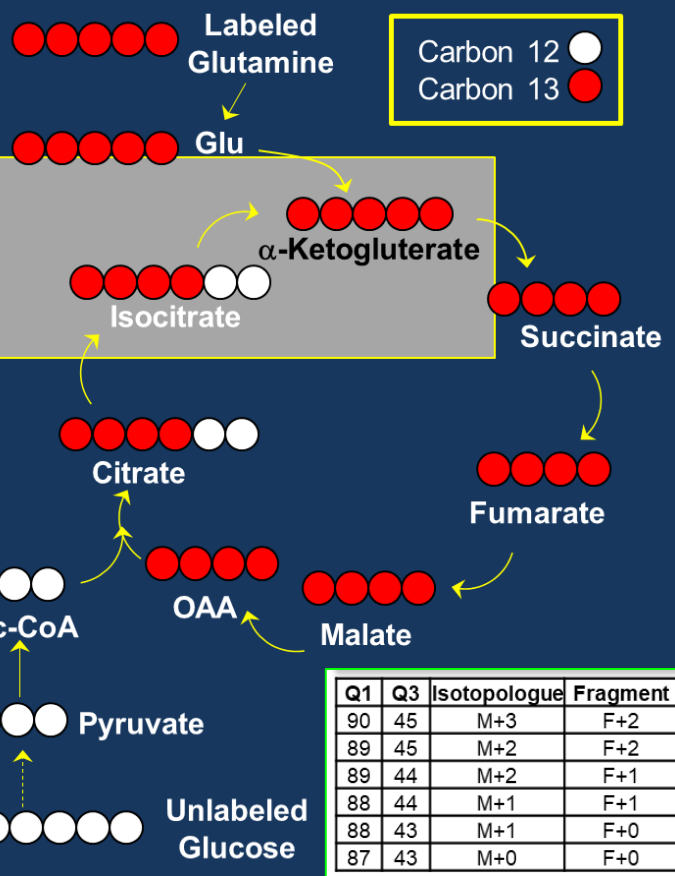
Yuan *et. al*, 2018, ***Nat. Protoc.***

In vivo labeling of mice

Monitor blood glucose (30-60 min)

Sacrifice, process samples

LC-MS/MS (SRM)

$^{13}C_6$-glucose solution (2g/kg; fasted O/N)

glycolysis:
glucose-1-phosphate
fructose-1,6-bisphosphate
fructose-6-phosphate
glyceraldehdye-3-phosphate
sn-glycerol-3-phosphate
3-phosphoglycerate
serine
phosphoenolpyruvate
lactate
alanine

PPP:
D-glucono-δ-lactone-6-phosphate
erythrose-4-phosphate
ribose-5-phosphate
sedoheptulose-7-phosphate
SBP

TCA:
a-ketoglutarate
oxaloacetate
succinate
fumarate
asparagine
aspartate
glutamate
glutamine

%^{13}C

RTK
Cell growth/ proliferation of cancer

Platform for *Untargeted* Lipidomics/Metabolomics/Proteomics
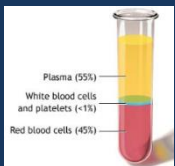
Blood plasma **Cancer cells** **Tumor tissue**

MTBE

Lipid layer
Aqueus layer
Solid pellet
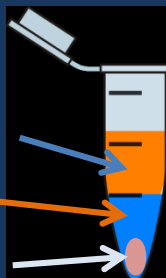
Thermo QExactive Plus/HF Agilent 1100/1200
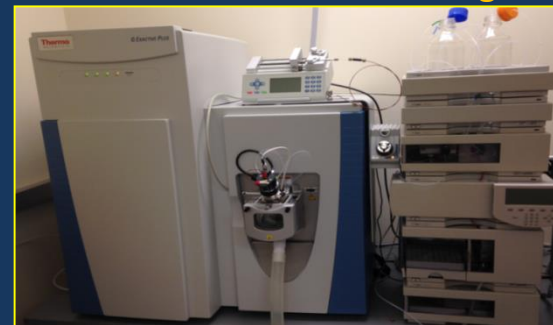
•identification based on *high mass accuracy MS and **MS2** data*

2.1 mm x 10 cm C_{18} (lipidomics); 75 μm x 10 cm C_{18} (proteomics, pos. only)

4.6 mm x 10 cm amide HILIC (metabolomics)

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

HCD-DDA (Top 8) pos *and* neg mode

LipidSearch

18 main lipid classes and >66 subclasses

Elements/Scaffold (Mascot)

>550 identified metabolites (NIST & HMDB)
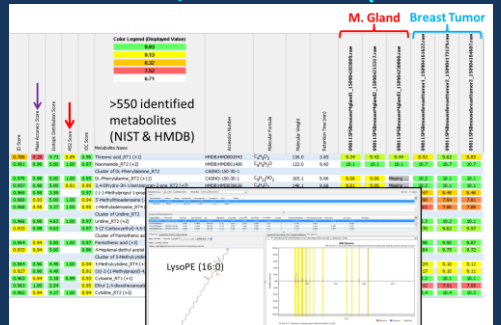
NIST 2017: 652,475 MS/MS spectra; Protein DBs

Ceramide Ganglioside

PC, LPA

DG, LPC

Triglycerides

Pos mode FFA LPC, LPE, MG SM, PC, PI, Cer, PG, PE, PS DG, TG, ChE

Neg mode
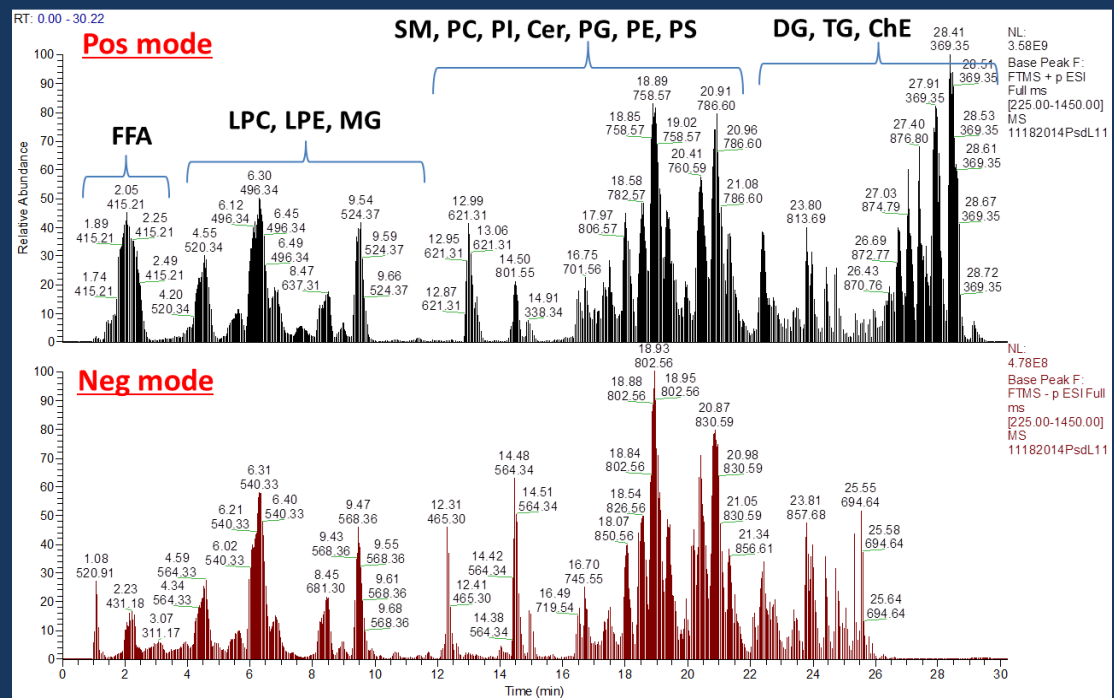
FluxSearch: Untargeted $^{13}C/^{15}N$ Flux-Omics

Poster 427, Mon, June 4, He Huang *et. al.*
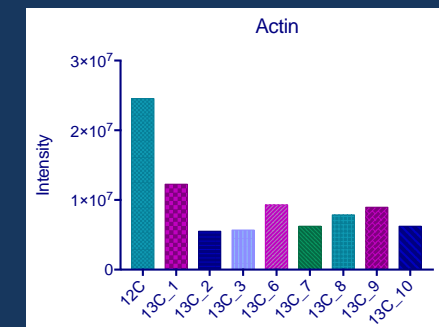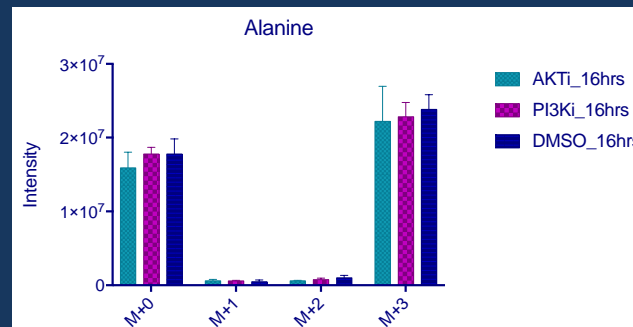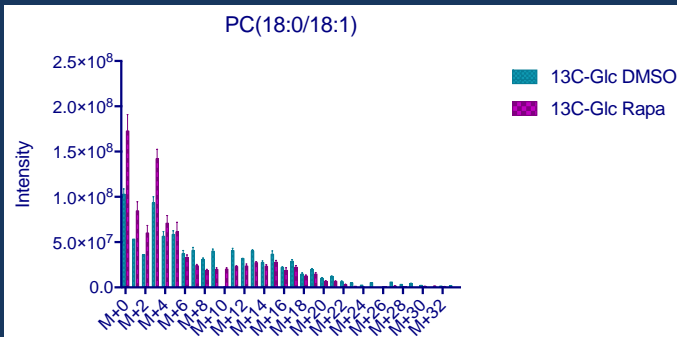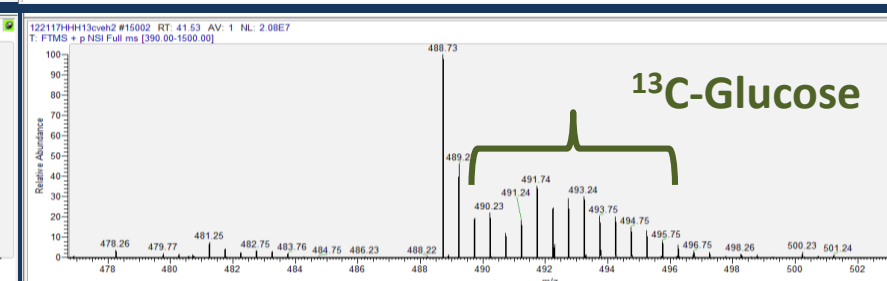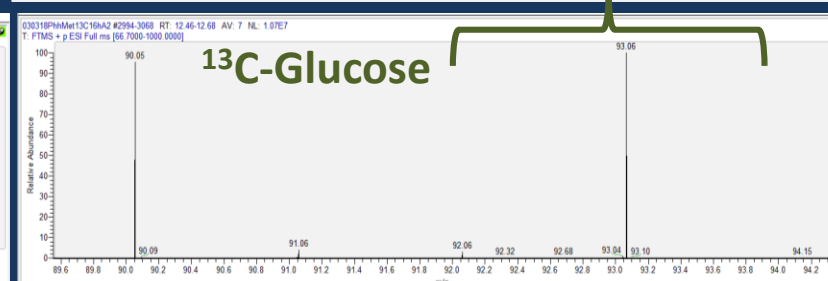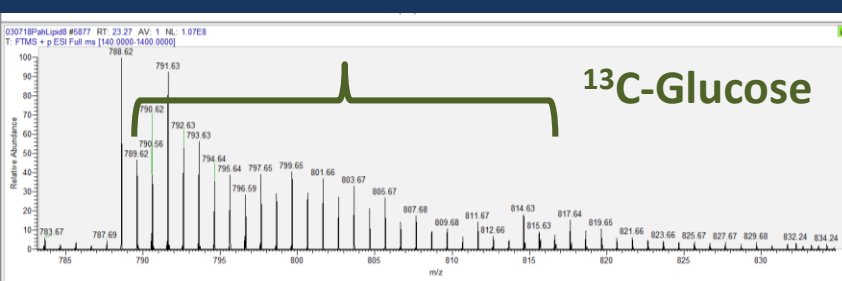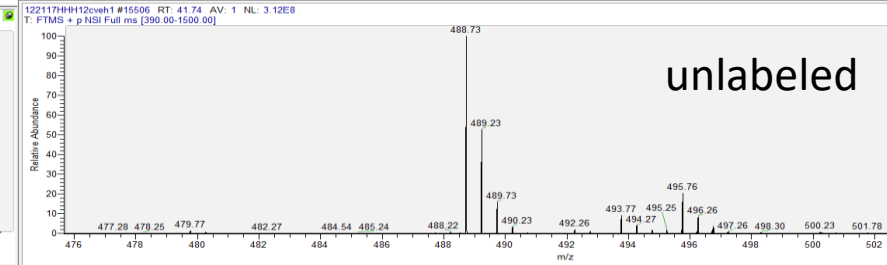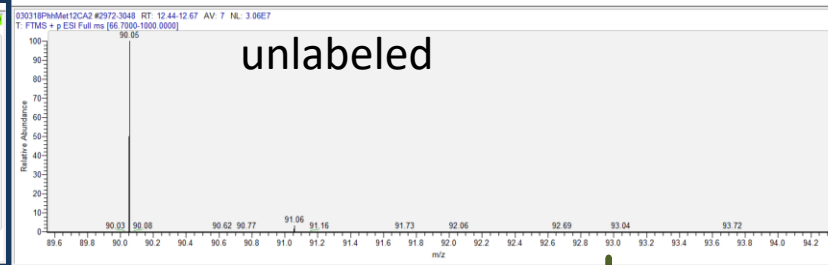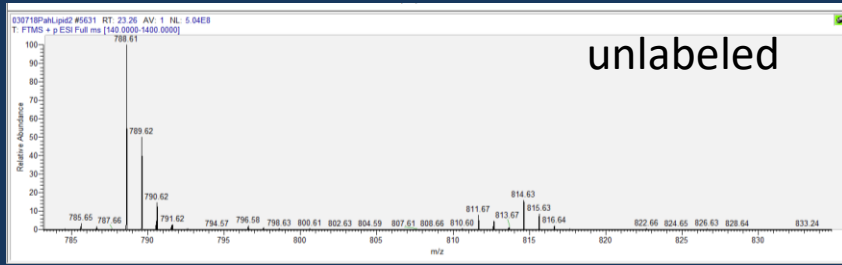
Serial-Omics of Mouse Breast Tumor vs. Mammary Gland
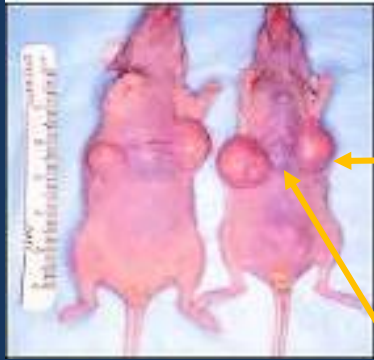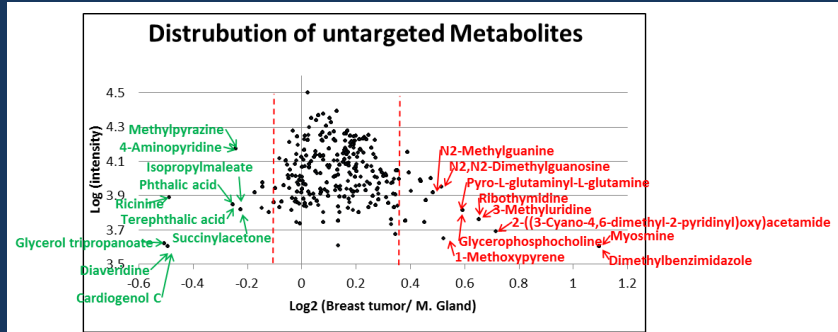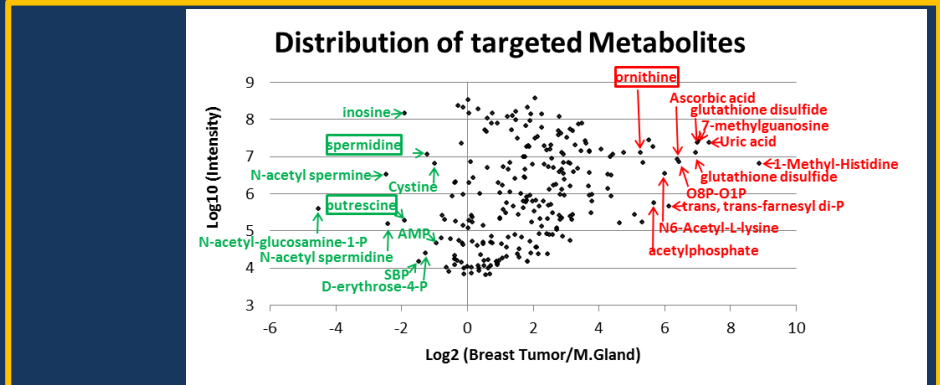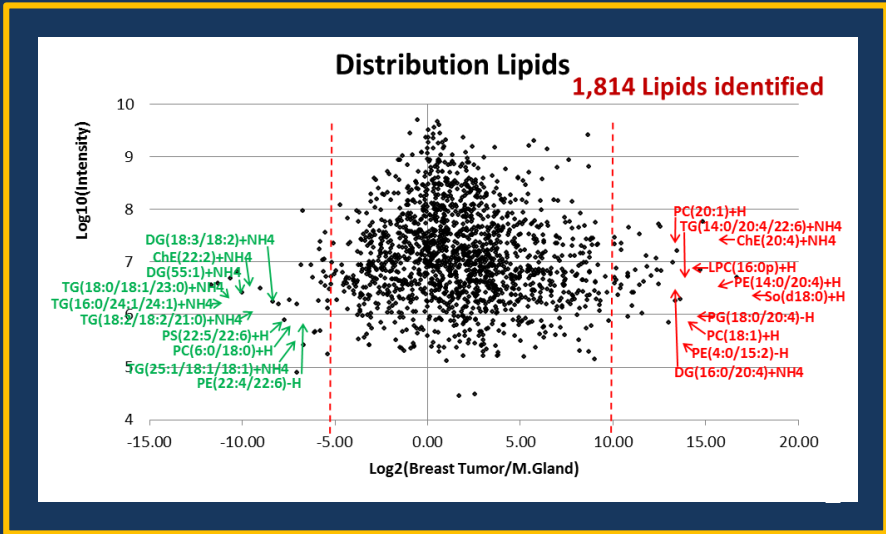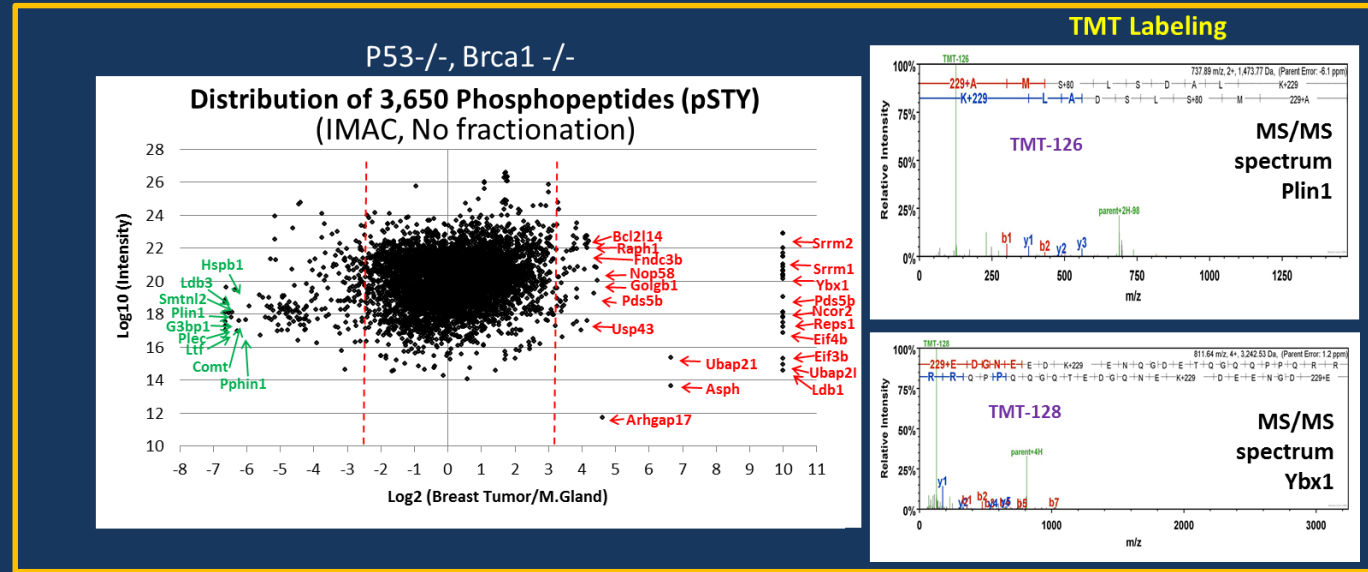
Merging the Phosphoproteome-Metabolome-Lipidome.....

Most regulated -omics in mouse breast tumors and mammary gland

C — Most regulated Metabolites in Breast Tumor

A — Most regulated pSTY sites normalized by protein level

B — Most regulated Lipids in Breast Tumor

Lipid Classes
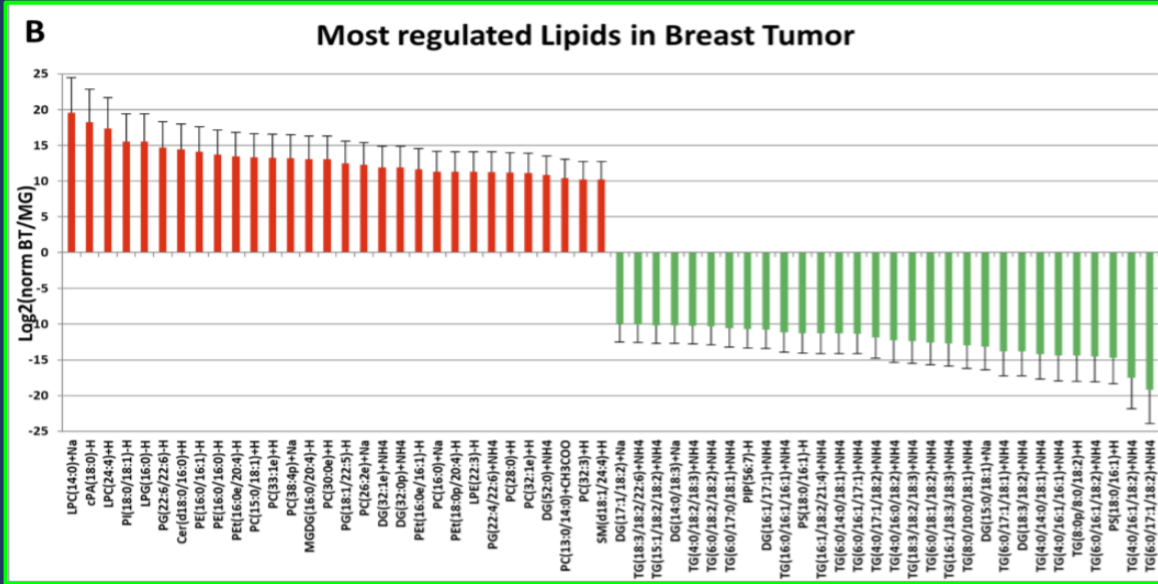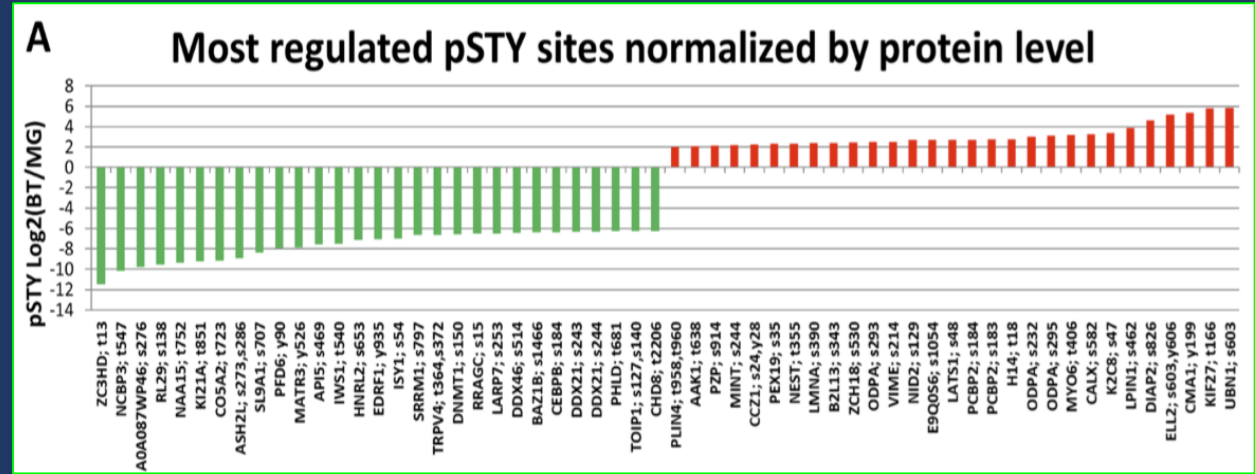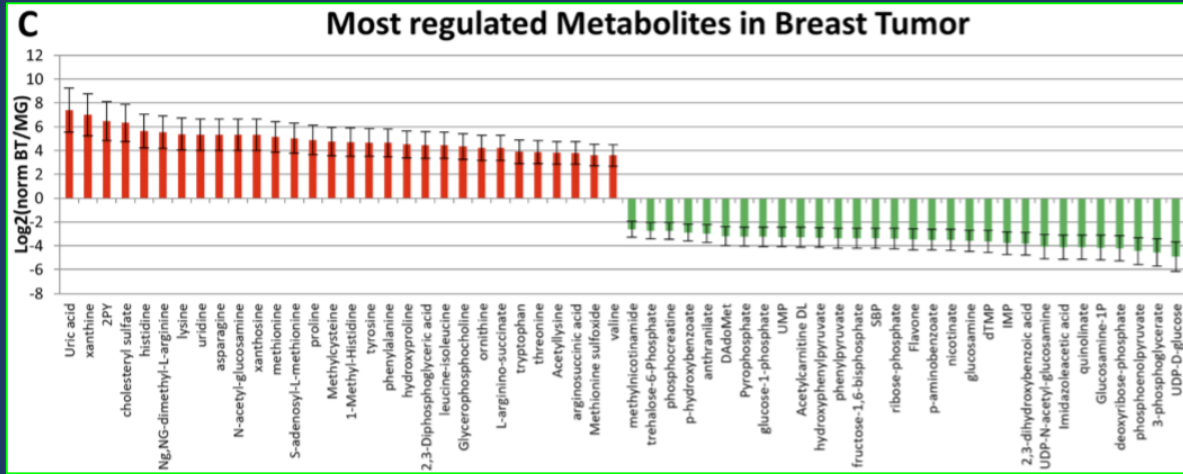
Lipids: TG and DG load much higher in MG, Phospholipids much higher in tumor

Metabolites: urea cycle, TCA cycle high, parts of glycolysis, PPP low in tumor
  -Not glucose driven, glutamine driver

Phosphoproteomics/proteomics: Transcription and splicing, signaling proteins high

Total Protein — Mammary Gland / Breast Tumor — Total Phosphosites

2728 | 1915 | 149   ↑ Kinase activity in tumor   938 | 29 | 105

Serial-Omics Integrated Data and Pathway Model (Lipidomics, Metabolomics and Proteomics)

Mouse Breast Tumor

Breitkopf et al, 2017, *Sci. Reports*
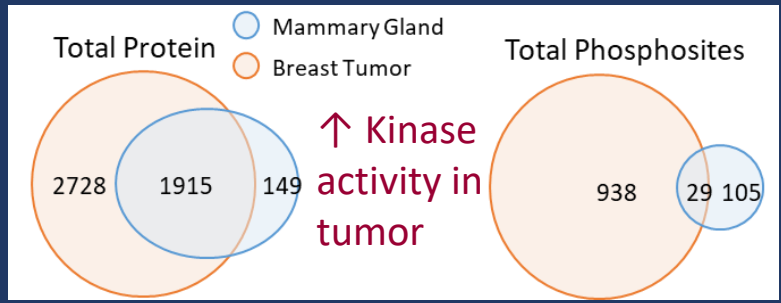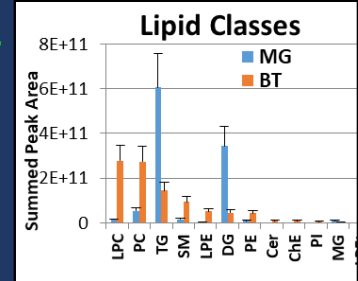
Serial-Omics of Lung Tumor vs Normal Mouse Lung Tissue

EGFR T790M model
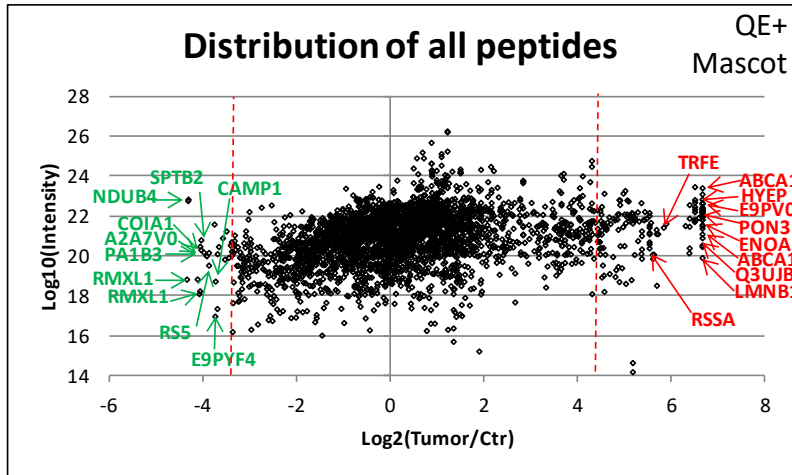
RMXL1, part of Ribonucleo complex, is down-regulated
ENOA, enolase in glycolysis, growth control, Tumor-associated antigen is up-regulated
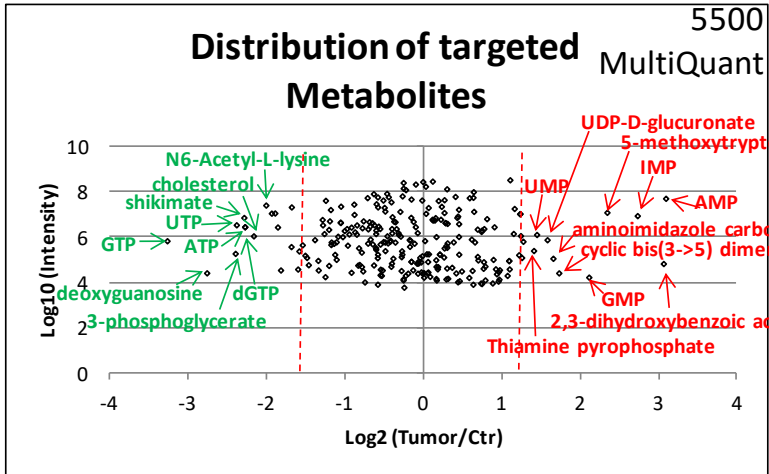
Glycolysis and TCA Cycle are down-regulated
PPP, Purine and RNA metabolism are up-regulated

-Omics assembly under construction
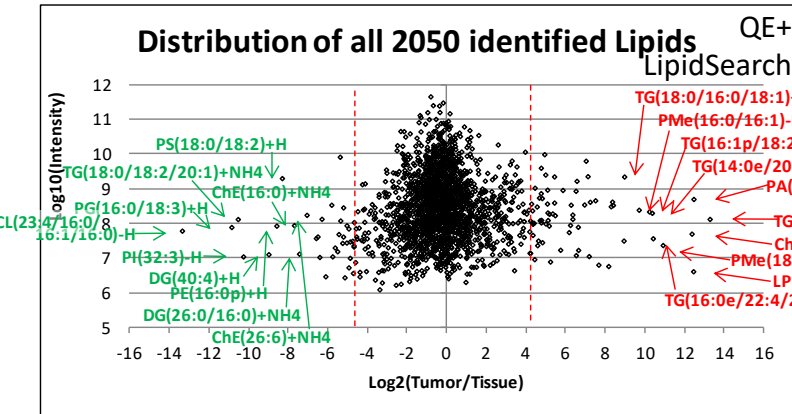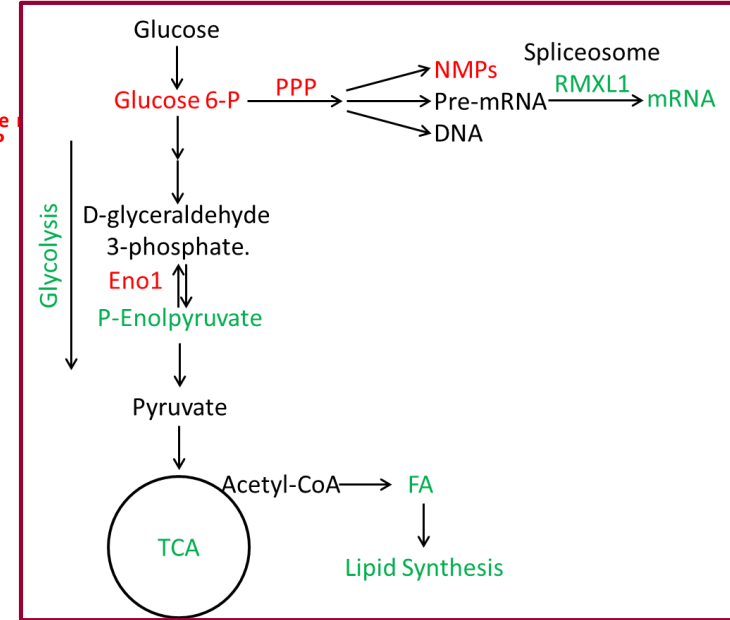
PC and SM are downregulated, ChE and TG are up-regulated
Fatty Acid Biosynthesis is down-regulated

Dan Tenen, BIDMC
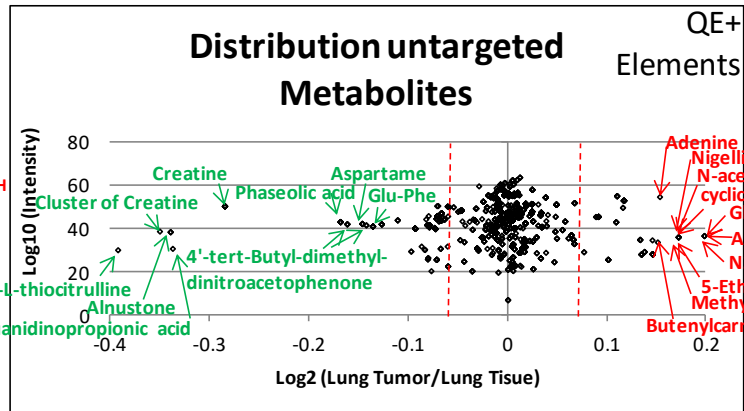
Serial-Omics of *Dried Blood Spots*

Blood spot collection

DBS (stored 2-3 years at RT)

MTBE extraction

DBS

- Non-polar lipidomics
- Polar metabolomics/peptidomics
- proteomics/phosphoproteomics

# of Identifications

Stored for several years at RT, 25°C

~5 μL blood

170

597

316

☐ Proteins ☐ Lipids ☐ Metabolites

Collaboration with *CT Analytical, Bethany CT*
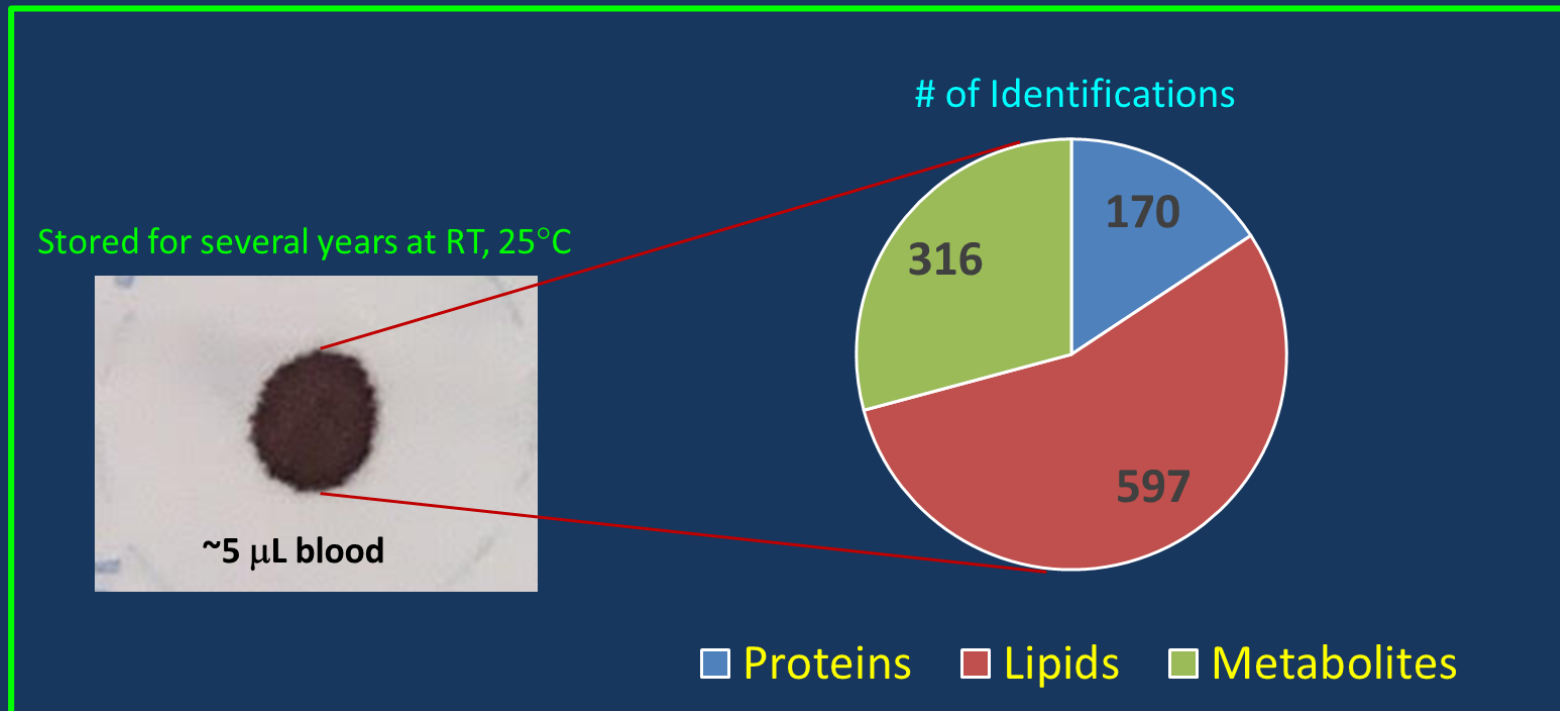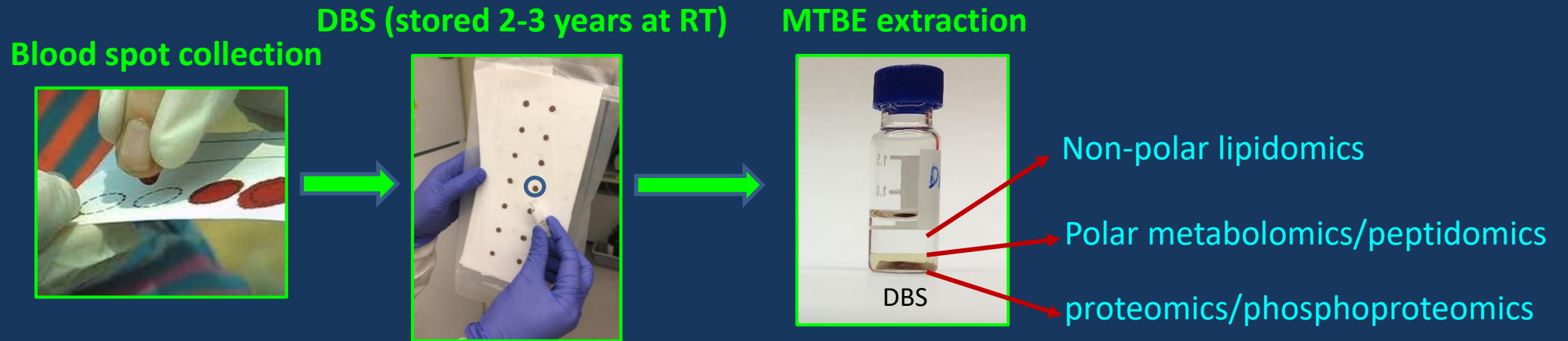
Analysis of the MTBE protein precipitate and Dried Blood Spot paper

DBS, post tryptic digestion

DBS   Control paper

C_{18} Zip tip

TiO2 tip

LC-MS/MS

DBS   Control paper
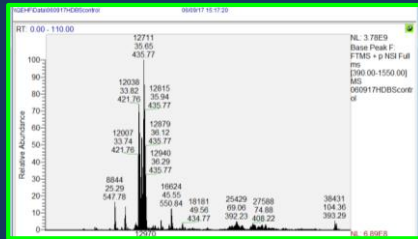
170 DBS proteins identified:

- Hemoglobin (α,β,δ) 52% & Albumin 10% of total signal

- No significant PO_4 info

Proteins identified from 5μL of 2-3 year old dried blood spot

~170 proteins

Pie chart labels include:
- Hemoglobin subunit beta, 643
- Hemoglobin subunit alpha, 574
- Serum albumin, 294
- Hemoglobin subunit delta, 322
- Complement C3, 75
- Carbonic anhydrase 1, 56
- Alpha-2-macroglobulin, 54
- Spectrin alpha chain, erythrocytic 1, 58
- Peroxiredoxin-2, 56
- Spectrin beta chain, erythrocytic, 50
- Apolipoprotein B-100, 44
- Alpha-1-antitrypsin, 37
- Titin, 9
- Band 3 anion transport protein, 31
- Complement C4-A, 27
- Apolipoprotein A-I, 25
- Fibrinogen beta chain, 21
- Carbonic anhydrase 2, 24
- Ankyrin-1, 21
- Catalase, 20
- Ig gamma-1 chain C region, 22
- Ig alpha-1 chain C region, 15
- Fibrinogen gamma chain, 18
- Ig kappa chain C region, 18
- Fibrinogen alpha chain, 18
- Ceruloplasmin, 15
- Haptoglobin, 15
- Immunoglobulin lambda constant 2, 13
- Glyceraldehyde-3-phosphate dehydrogenase, 12
- protein 4.2, 11
- protein band 3, 10
- Keratin, cytoplasmic, 10
- Actin, cytoplasmic 1, 10
- Alpha-1-...
- Lysine-specific demethylase PHF2, 8
- Hemoglobin subunit gamma-2, 112
- Phosphoglycerate kinase 1, 8
- Alpha-1-acid glycoprotein 1, 7
- Ig gamma-2 chain C region, 16
- Selenium...
- Hemopexin, 7
- Peroxiredoxin...
- Protein 4...
- Paraoxonase...
- ...late kinase...
- ...glutamine...
- ...mitochondrial 2...
- cytoplasmic 2...
- Erythrocyte... phosphate
- Ubiquitin-like modifier-activating enzyme NEDD8...

Dried Blood Spot Lipidomics & Metabolomics by LC-MS/MS

class profile

~597 lipid ions

Lipid Classes identified:

Cer, CerG2, CerG3GNac1, DG, LPC, LPE, LPI, LdMePE, MG, OAHFA, PA, PC, PE, Pet, PI, PS, SM, TG, cPA, dMePE, etc.
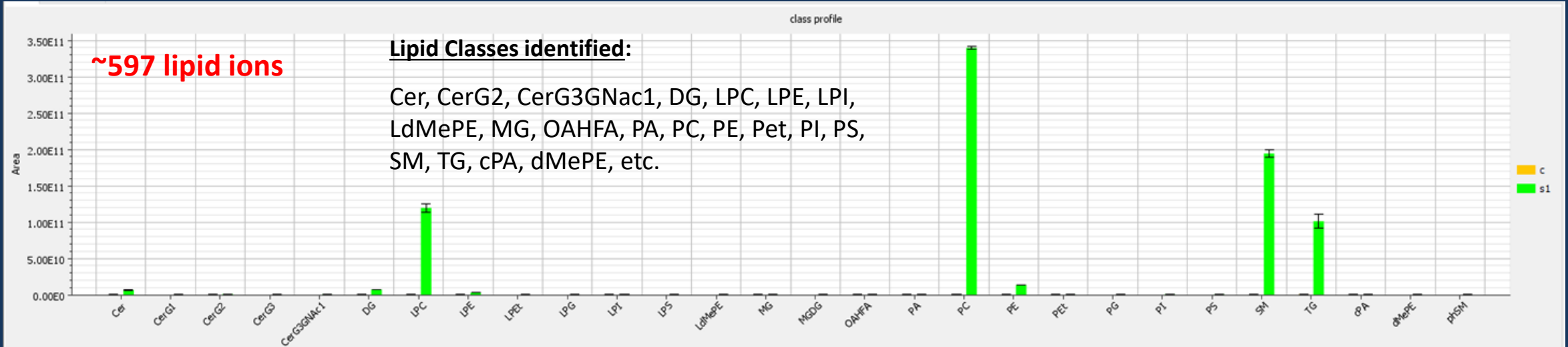
Elements metabolite ID

ID Score	MS2 Score	Metabolite Name	Molecular Formula	Dried Blood Spot (Log2 Peak Area)
0.716	0.88	Creatinine	C 4H 7N 3O	10.5
0.915	0.94	Hypoxanthine	C 5H 4N 4O	10.3
0.81	0.68	Edetic Acid	C 10H 16N 2O 8	10.3
0.978	0.98	Niacinamide	C 6H 6N 2O	10.2
0.972	0.98	Phosphoric acid	H 3O 4P	10.2
0.919	0.93	Alpha-D-Glucose 1,6-bisphosphate	C 6H 14O 12P 2	10.2
0.655	0.86	Cytosine	C 4H 5N 3O	10.2
0.941	0.91	2,3-Diphosphoglyceric acid	C 3H 8O 10P 2	10.1
0.973	1	L-Isoleucine	C 6H 13NO 2	10
0.885	0.85	Acetic acid, (aminooxy)-	C 2H 5NO 3	9.98
0.948	1	3-Phosphoglyceric acid	C 3H 7O 7P	9.86
0.971	0.99	L-Carnitine	C 7H 15NO 3	9.77
0.987	1	DL-Phenylalanine	C 9H 11NO 2	9.74
0.955	0.97	N-Methyl-a-aminoisobutyric acid	C 5H 11NO 2	9.72
0.929	0.98	Phosphoenolpyruvic acid	C 3H 5O 6P	9.7
0.67	0.95	Diethanolamine	C 4H 11NO 2	9.6
0.971	0.99	2-Imidazolecarboxaldehyde	C 4H 4N 2O	9.54
0.98	1	Creatine	C 4H 9N 3O 2	9.49
0.679	0.87	Senecioic acid	C 5H 8O 2	9.49
0.956	0.93	4-Pyridoxic acid	C 8H 9NO 4	9.46
0.986	0.99	Hexanoylcarnitine	C 13H 25NO 4	9.42
0.908	0.97	Deoxygalactonojirimycin	C 6H 13NO 4	9.42
0.954	0.91	Cadaverine	C 5H 14N 2	9.42
0.976	0.98	Citric acid	C 6H 8O 7	9.4
0.633	0.29	Glucaric acid	C 6H 10O 8	9.32
0.958	1	L-Glutamic acid	C 5H 9NO 4	9.3
0.899	0.87	3-Hydroxydodecanoic acid	C 12H 24O 3	9.25
0.699	0.79	Myristoleic acid	C 14H 26O 2	9.25
0.661	0.61	Gamma-Aminobutyric acid	C 4H 9NO 2	9.25
0.938	0.99	Succinic acid	C 4H 6O 4	9.24
0.991	1	L-Tyrosine	C 9H 11NO 3	9.21
0.941	1	Adenosine monophosphate	C 10H 14N 5O 7P	9.19
0.722	1	L-Pipecolic acid	C 6H 11NO 2	9.18
0.763	0.94	Adenine	C 5H 5N 5	9.18
0.889	0.87	Serricornin	C 11H 22O 2	9.16

Top 35 untargeted metabolites by abundance

316 metabolites (untargeted + targeted)

MetaboAnalyst Pathway Enrichment

METABOLITES AFFECTED BY GENDER
CREATINE DEFICIENCY, GUANIDINOACETATE...
RHABDOMYOLYSIS
HEART FAILURE
CRITICAL ILLNESS (MAJOR TRAUMA, SEVERE...
MYOCARDIAL ISCHEMIA
METHYLMALONIC ACIDURIA (MMA)
DIABETES MELLITUS (MODY),...
2,4-DIENOYL-COA REDUCTASE DEFICIENCY
3-HYDROXYISOBUTYRIC ACIDURIA
CARNITINE DEFICIENCY, MYOPATHIC
FEEDING: VEGANIAN [DD]
GLUTARIC ACIDURIA
HAWKINSINURIA
PANCREATIC CANCER
PREMENSTRUAL DYSPHORIC DISORDER
ISOVALERIC ACIDEMIA
HYPERBARIC OXYGEN EXPOSURE
3-METHYL-CROTONYL-GLYCINURIA
3-METHYLGLUTACONIC ACIDURIA (TYPE I),...
CARNITINE TRANSPORTER DEFECT, PRIMARY...
CARNITINE-ACYLCARNITINE TRANSLOCASE...
HEPATIC ENCEPHALOPATHY
HYPERORNITHINEMIA WITH GYRATE ATROPHY (HOGA)
LYSINURIC PROTEIN INTOLERANCE (LPI)
MYOCARDIAL INFARCTION
MEDIUM CHAIN ACYL-COA DEHYDROGENASE...
REFRACTORY LOCALIZATION-RELATED EPILEPSY
ARGININEMIA, HYPERARGININEMIA, ARGINASE...
CARNITINE PALMITOYL TRANSFERASE...
LESCH-NYHAN SYNDROME
LONG CHAIN ACYL-COA DEHYDROGENASE...
TYROSINEMIA I
VALPROATE THERAPY: ANTICONVULSANT...
METABOLITES AFFECTED BY EXERCISE
DIFFERENT SEIZURE DISORDERS
NEONATAL INTRAHEPATIC CHOLESTASIS
AGING-RELATED METABOLITES
ACUTE SEIZURES
EARLY MARKERS OF MYOCARDIAL INJURY
ASPHYXIA [DD]
PHENYLKETONURIA
PYRUVATE DEHYDROGENASE DEFICIENCY (E3)
CONTINUOUS AMBULATORY PERITONEAL DIALYSIS...
ANOXIA
MALNUTRITION
PROPIONIC ACIDEMIA
MAPLE SYRUP URINE DISEASE
LONG-CHAIN-3-HYDROXYACYL-COA...
SCHIZOPHRENIA

P value

5e-02

5e-01

9e-01

Fold Enrichment

Serial-Omics of Horse Urine and Mane Hair

Horse mare

JF Sierra Flame

Horse gelding

Phoenix

333 µL Urine specimen

Mane Hair specimen
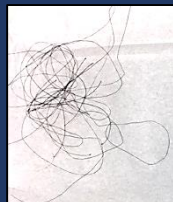
MTBE/MeOH extraction

Non-polar phase (Lipid)

Polar phase (Metabolite)

Precipitate (Protein)

Tryptic digest TiO2

Lipidomics

- MS
+ MS
- MS/MS
+ MS/MS

Metabolomics

- MS
+ MS
- MS/MS
+ MS/MS
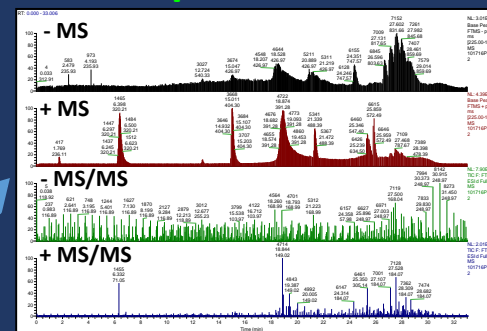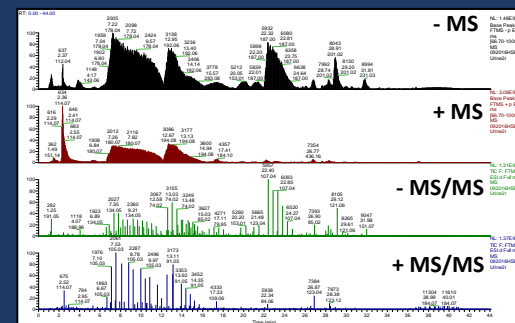
Proteomics

+ MS
+ MS/MS

Serial-Omics of Horse Urine

Horse mare urinary system

333 µL specimen

Serial-omics Molecule Distribution

46 Proteins

474 Metabolites

10 Lipids

- Omics catalog
- Biomarkers
- Tracers of drugs, feed, plants, supplements, etc.
- Health status (disease presence or progression)

Yuan et. al, *PloS ONE*, 2017

Horse Urine Serial-omics Pathway Analysis

Proteomics

~50 proteins, ≥3 spectra, Horse DB

Pie chart of proteins including: Uromodulin, 376; Calcium-activated chloride channel regulator 1, 101; Keratin, type I cytoskeletal 10, 66; Protein AMBP, 56; Immunoglobulin lambda light chain, 47; Keratin, type II cytoskeletal 1, 44; Kininogen-1, 42; Keratin, type II cytoskeletal 5, 33; Keratin, type I cytoskeletal 1, 26; Fibulin-like extracellular matrix protein 1, 25; EGF-containing fibulin-like extracellular matrix protein 2h 1, 24; Cytokeratin-2e, 24; growth factor, 23; Keratin, type I cytoskeletal 6A, 21; Keratin, type I cytoskeletal 16, 20; Protein disulfide; Pantetheinase, 18; Beta-globin, 17; Antithrombin-III, 16; Trypsinogen, 16; Epithelial cell adhesion molecule; Cadherin-6, 14; Keratin, type I cytoskeletal 16; Cadherin-5; Corticosteroid-binding globulin, 13; Hemopexin A-2; Far upstream...

PANTHER GO-Slim Biological Process
Total # Genes: 45   Total # process hits: 101

Click to get gene list for a category:
- biological adhesion (GO:0022610)
- biological regulation (GO:0065007)
- cellular component organization or biogenesis (GO:0071840)
- cellular process (GO:0009987)
- developmental process (GO:0032502)
- immune system process (GO:0002376)
- localization (GO:0051179)
- metabolic process (GO:0008152)
- multicellular organismal process (GO:0032501)
- reproduction (GO:0000003)
- response to stimulus (GO:0050896)

Metabolomics

Metabolite Sets Enrichment Overview (Fold Enrichment vs pathway, colored by P value):
- CITRIC ACID CYCLE
- UREA CYCLE
- ALANINE METABOLISM
- TAURINE AND HYPOTAURINE METABOLISM
- MALATE-ASPARTATE SHUTTLE
- BETAINE METABOLISM
- HISTIDINE METABOLISM
- ASPARTATE METABOLISM
- INSULIN SIGNALLING
- PYRUVATE METABOLISM
- METHIONINE METABOLISM
- GLYCINE, SERINE AND THREONINE METABOLISM
- ARGININE AND PROLINE METABOLISM
- GLUCONEOGENESIS
- BILE ACID BIOSYNTHESIS

Top 60 metabolites

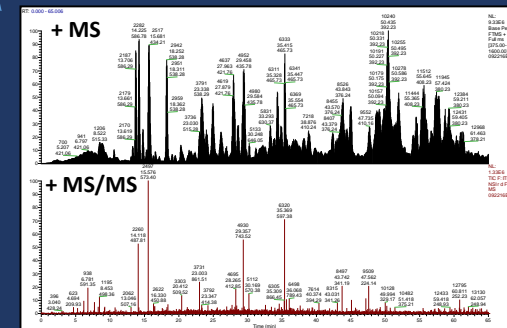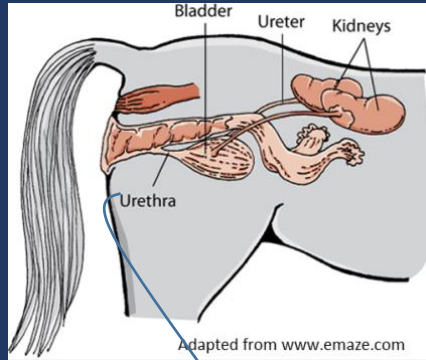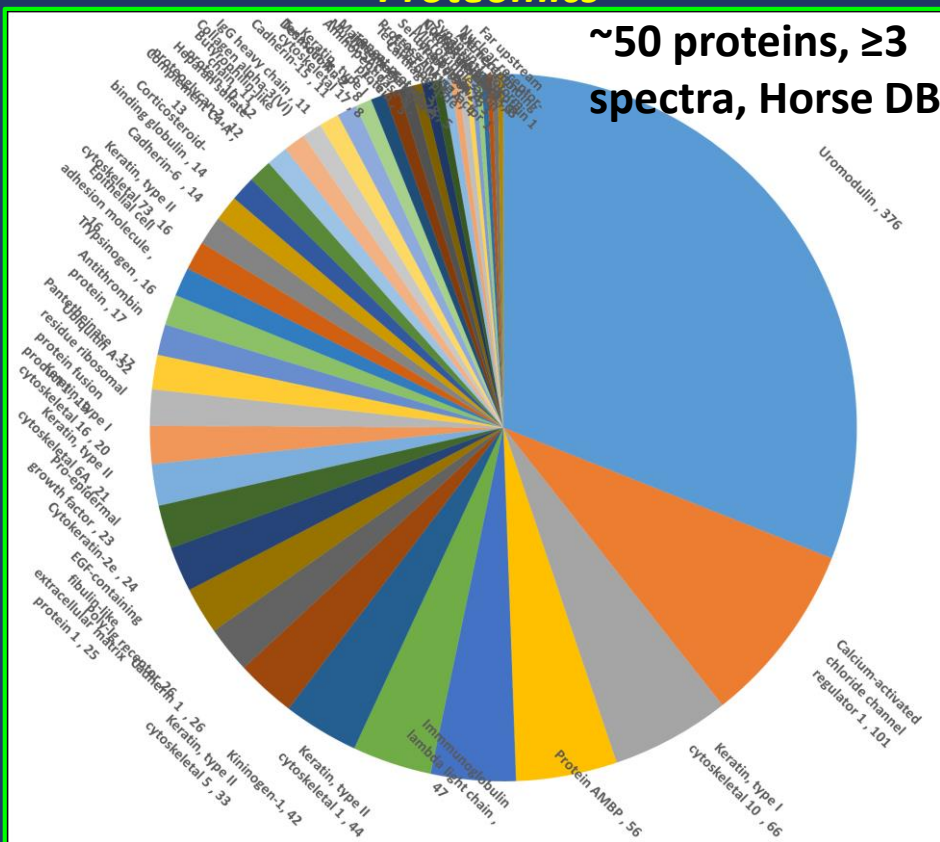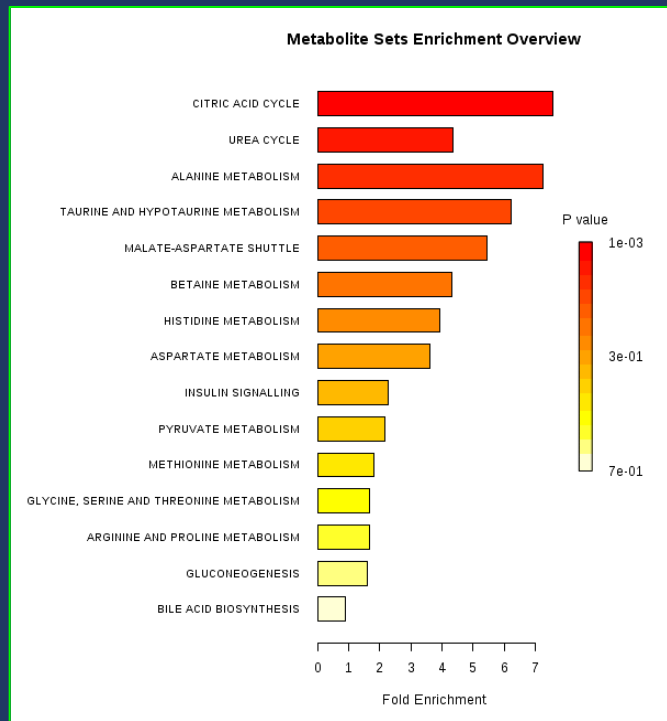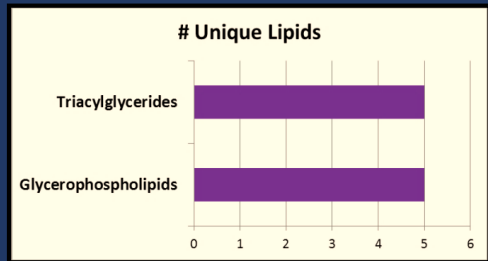Table II. Top 60 identified metabolites by intensity from 474 unique equine urine metabolites

Horse mare polar metabolites (Untargeted run)	Accession	Molecular Formula	Avg MS1 Log10 Peak Area
Hippuric acid	HMD800714	C 9H 9NO 3	11.8
Phenylacetylglycine	HMD800821	C 10H 11NO 3	11.6
2-Hydroxy-4-trifluoromethyl benzoic acid	HMD860715	C 8H 5F 3O 3	11.6
Creatinine	HMD800562	C 4H 7N 3O	11.4
p-Cresol glucuronide	HMD11686	C 13H 16O 7	11.3
Buntansin A	HMD835086	C 11H 8O 5	10.9
Acetohydroxamic Acid	HMD814691	C 2H 5NO 2	10.8
Mesoridazine	HMD815068	C 21H 26N 2OS 2	10.6
Mammeigin	HMD830785	C 25H 24O 5	10.6
Monomethyl phenylphosphonate	HMD831868	C 7H 9O 3P	10.6
Neoisoliquiritin	HMD837317	C 21H 22O 9	10.5
Geranylgeranylcysteine	HMD811678	C 23H 37NO 3S	10.4
4-Hydroxy-8-methoxy-2H-furo[2,3-h]-1-benzopyran-2-one	HMD832659	C 12H 8O 5	10.4
N'-Hydroxysaxitoxin	HMD833664	C 10H 17N 7O 5	10.4
4-Hydroxyphenyl-2-propionic acid	HMD841683	C 9H 10O 3	10.4
cis-Mulberroside A	HMD831726	C 26H 32O 14	10.3
N-Methylphthalimide	CASNO:550-44-7	C 9H 7NO 2	10.3
Phlorizin	HMD836634	C 21H 24O 10	10.3
L-leucyl-L-proline	HMD11175	C 11H 20N 2O 3	10.2
Benzocaine	HMD804992	C 9H 11NO 2	10.2
Vanilloloside	HMD832013	C 14H 20O 8	10.2
apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)]	HMD859607	C 7H 15N 3O 2	10.1
Trimethylamine N-oxide	HMD800925	C 3H 9NO	10.1
Acetaminophen	HMD801859	C 8H 9NO 2	10.1
D-(-)-Isoascorbic acid	CASNO:89-65-6	C 6H 8O 6	10.1
Butyrylcarnitine	HMD802013	C 11H 21NO 4	10.1
DOPA sulfate	HMD802028	C 9H 11NO 7S	10.0
Pilocarpine	HMD815217	C 11H 16N 2O 2	10.0
Pyrogallol-2-O-glucuronide	HMD860017	C 12H 14O 9	10.0

Horse mare polar metabolites (Targeted run)	Accession	Molecular Formula	Avg Q3 Log10 Peak Area
2-Hydroxy-2-methylbutanedioic acid	C02612	C5H8O5	7.7
citrate	C00158	C6H8O7	7.3
2-Isopropylmalic acid	C02504	C7H12O5	7.3
1-Methyl-Histidine	C01152	C7H11N3O2	7.3
betaine	C00719	C5H11NO2	7.2
aconitate	C00417	C6H6O6	7.1
oxaloacetate	C00036	C4H4O5	7.1
Acetylcarnitine DL	C02571	C9H18NO4	7.1
allantoin	C01551	C4H6N4O3	7.1
Urea	C00086	C4H2O	6.9
Acetyllysine	C02727	C8H16N2O3	6.8
N6-Acetyl-L-lysine	C02727	C8H16N2O3	6.7
2-hydroxygluterate	C02630	C5H8O5	6.7
D-sedoheptulose-1-7-phosphate	C05382	C7H15O10P	6.6
Phenylpropiolic acid	HMD800563	C9H6O2	6.6
DL-Pipecolic acid	C00408	C6H11NO2	6.5
Citraconic acid	C02226	C5H6O4	6.5
Ascorbic acid	C00072	C6H8O6	6.5
3-hydroxybuteric acid	C01089	C4H8O3	6.5
Ng,NG-dimethyl-L-arginine	C03626	C8H18N4O2	6.3
Kynurenic acid	C01717	C10H7NO3	6.3
p-hydroxybenzoate	C00156	C7H6O3	6.3
taurine	C00245	C2H7NO3S	6.2
succinate	C00042	C4H6O4	6.2
Aminoadipic acid	C00956	C6H11NO4	6.2
Atrolactic acid	HMD800475	C9H10O3	6.2
pantothenate	C00864	C9H17NO5	6.1
2-dehydro-D-gluconate	C00629	C6H10O7	6.1
orotate	C00295	C5H4N2O4	6.1
glutamine	C00064	C5H10N2O3	6.1

Lipidomics (negligible)

Unique Lipids
- Triacylglycerides
- Glycerophospholipids

Diet Specific Metabolism Hay/Grain

- Neoisoliquirtin
- 4-hydroxy-2-propionic acid
- Cis-Mulberroside A
- Phlorizin
- Buntansin A
- Mammeigin

} Plant derived

Serial-Omics of Horse Mane Hair

Pulled mane hair

Phoenix

14 hair strands
7 follicles

MTBE extraction

Lipidomics
Metabolomics
Proteomics

312 Metabolites

492 Lipids

391 Proteins

MW & Peak Intensity Distribution of Horse Mane Hair-Omics

- Proteins
- Metabolites
- Lipids

Works on human hair as well!

Identified Proteins and Bioinformatics Analysis of Horse Mane Hair

Top 50 Proteins (391 total) by Intensity

391 protein IDs
≥3 spectra
Horse DB

- **A wide variety of proteins can be detected beyond just keratins, hair is complex!**

Metabolomics Pathway Enrichment and Lipid Profile in Horse Mane Hair

Mane Hair Lipid Profile

492 lipid IDs

class profile

- **Hair resembles fatty material....**

Labels on chart: DG, TG, PC, PE, Cer, ChE

Mane Hair Metabolite Pathways

Amino acids

Pathways listed (top to bottom): Arginine and Proline Metabolism, Phenylalanine and Tyrosine Metabolism, Alanine Metabolism, Urea Cycle, Warburg Effect, Glycine and Serine Metabolism, Butyrate Metabolism, Ammonia Recycling, Phenylacetate Metabolism, Malate-Aspartate Shuttle, Aspartate Metabolism, Nicotinate and Nicotinamide Metabolism, Cysteine Metabolism, Oxidation of Branched Chain Fatty Acids, Purine Metabolism, Mitochondrial Beta-Oxidation of Short..., Propanoate Metabolism, Histidine Metabolism, Methionine Metabolism, Mitochondrial Beta-Oxidation of Long..., Methylhistidine Metabolism, Pyruvate Metabolism, Citric Acid Cycle, Glutamate Metabolism, Ethanol Degradation, Gluconeogenesis, Nucleotide Sugars Metabolism, Riboflavin Metabolism, Betaine Metabolism, Glutathione Metabolism, Pantothenate and CoA Biosynthesis, Carnitine Synthesis, Transfer of Acetyl Groups into Mitochondria, Biotin Metabolism, Fatty acid Metabolism, Phytanic Acid Peroxisomal Oxidation, Thiamine Metabolism, Mitochondrial Beta-Oxidation of Medium..., Selenoamino Acid Metabolism, Pentose Phosphate Pathway, Cardiolipin Biosynthesis, Lysine Degradation, Starch and Sucrose Metabolism, Phosphatidylethanolamine Biosynthesis, Taurine and Hypotaurine Metabolism, Amino Sugar Metabolism, Inositol Metabolism, Glucose-Alanine Cycle, Ketone Body Metabolism, Beta-Alanine Metabolism

P value legend: 9e-03, 2e-01, 5e-01

Fold Enrichment (x-axis: 0, 1, 2, 3, 4, 5)

312 metabolite IDs

TEM

Keratin-lipid cross-links

Table I. Chemical composition of integral hair lipid

	Hair shaft (%)	IRS (%)	Whole hair follicle (%)
Fatty acid	29.60	49.90	49.10
Phytosphingosine	37.20	23.30	26.30
Ceramide	26.90	23.20	20.20
Cholesterol	5.10	2.50	3.70
Cholesterol sulfate	1.10	0.20	0.30
Cholesterol oleate	0.20	0.80	0.30

IRS, inner root sheath.

Lee et. al., 2005, *J. Investig. Dermatol*

Summary: Single Liquid-Liquid Extractions can be used for Multi-Omics from Tumors, DBS, Urine, Hair, etc.

Needle Biopsy

< 5-10 mg

Dried Blood Strips

~ 5 μL

Methyl tert-butyl ether (MTBE)

Lipid layer

Metabolite layer

Protein pellet

LC-MS/MS

Untargeted lipidomics	Targeted lipidomics

Untargeted Metabolomics/peptidomics	Targeted metabolomics

Untargeted Proteomics	TMT labeling (126-131)	TiO2 phosphoproteomics

RECYCLE!

Assay Development

SRM, PRM, etc.

- Merge –omics data
- Save money/reagents
- Reduce chemical waste
- Improve reproducibility (one prep)
- *Biomarker discovery*
- *Robust assay development*

Acknowledgements

Beth Israel Deaconess Medical Center / Harvard Medical School
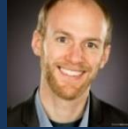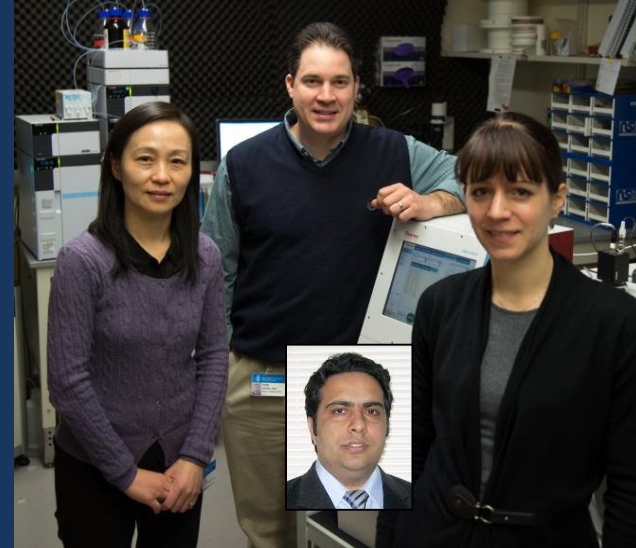
Susanne Breitkopf
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He Huang
Dan Tenen
Brendan Manning

Sierra and Phoenix

Thermo Fisher Scientific

Proteome Software

Applications in Horse Racing and Olympic Eventing

Hip 70
$2,000,000

Fasig-Tipton/Photos by Z

Dried Blood/Urine Spot Collection

MTBE extraction

metabolomics proteomics lipidomics

Assay development

Muscle damage
Inflammation
ROS
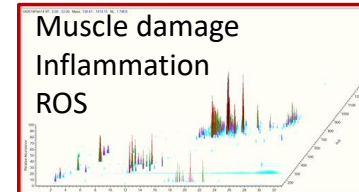
Purchase decisions, healthcare, training decisions