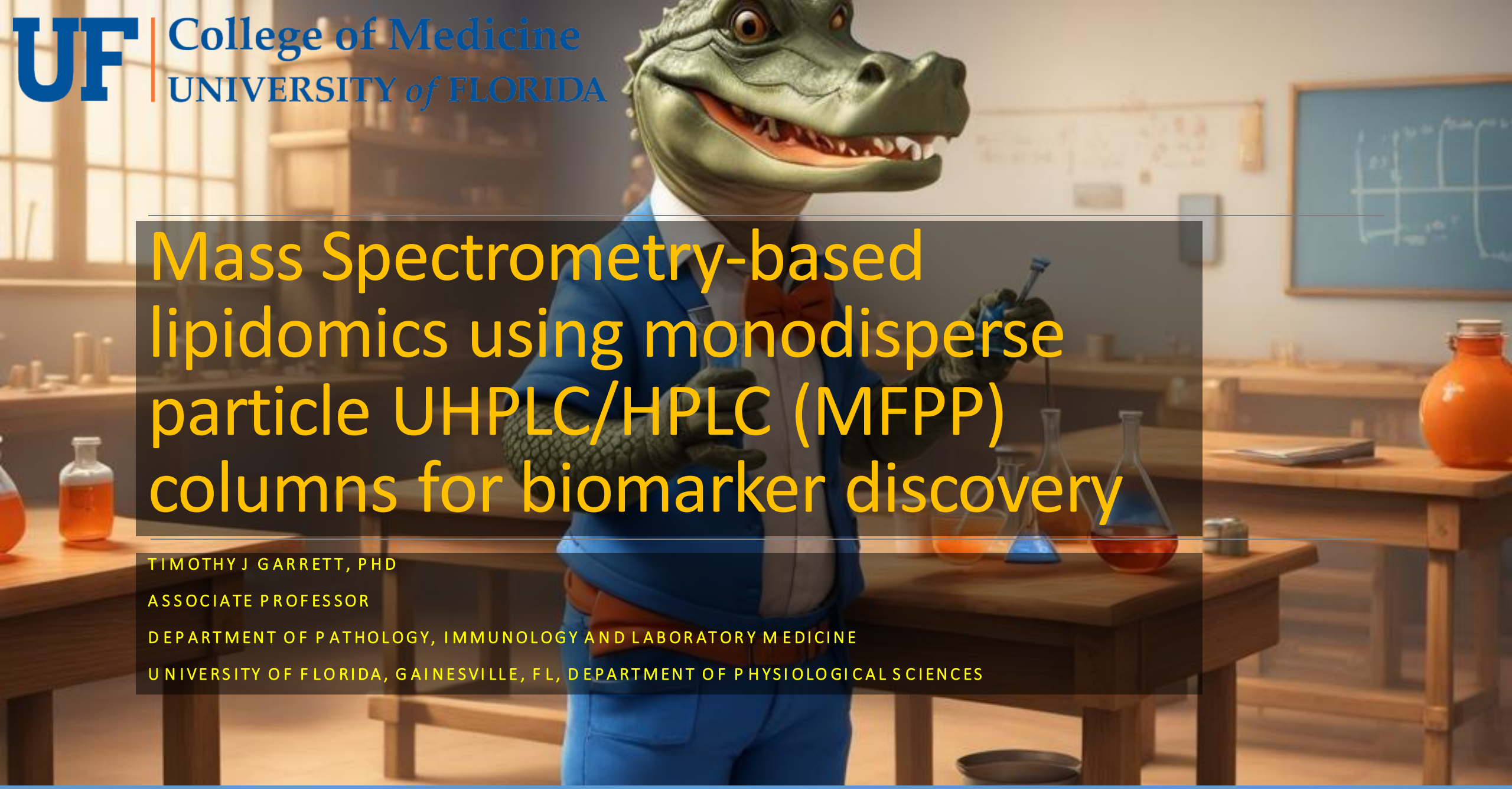

UF College of Medicine
UNIVERSITY of FLORIDA

Mass Spectrometry-based lipidomics using monodisperse particle UHPLC/HPLC (MFPP) columns for biomarker discovery

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Background photo credit to Don Chace


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WALL STREET JOURNAL

Outline

- The Southeast Center for Integrated Metabolomics
- The ‘Omics era
- Lipidomics
- Unknown rare disease example
- Monodisperse particle columns and lipids

Goals and Mission of Research and the Center

Provide metabolomic and lipidomic analyses to the broader scientific community

Focus on driving technology and high-throughput service analysis

Build open-source tools to aid in analysis and interpretation

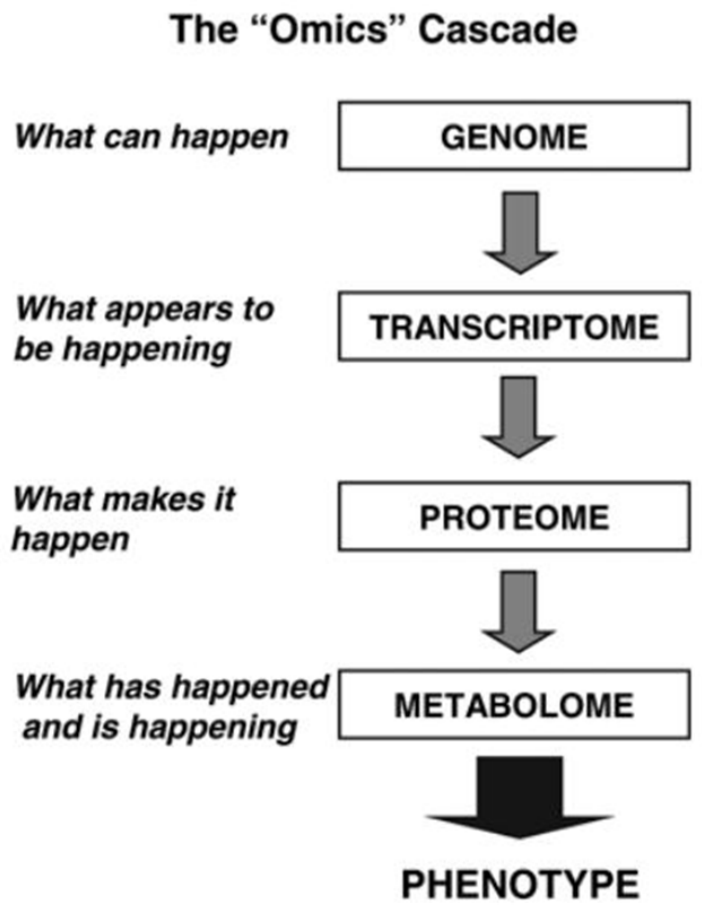
Provide grants and collaborative opportunities in metabolomics/lipidomics

Train scientists/postdocs/graduate/undergraduate students in metabolomic and lipidomic tools and technologies

Metabolomic analyses to the broader community

approximate locations

The era of Omics technology



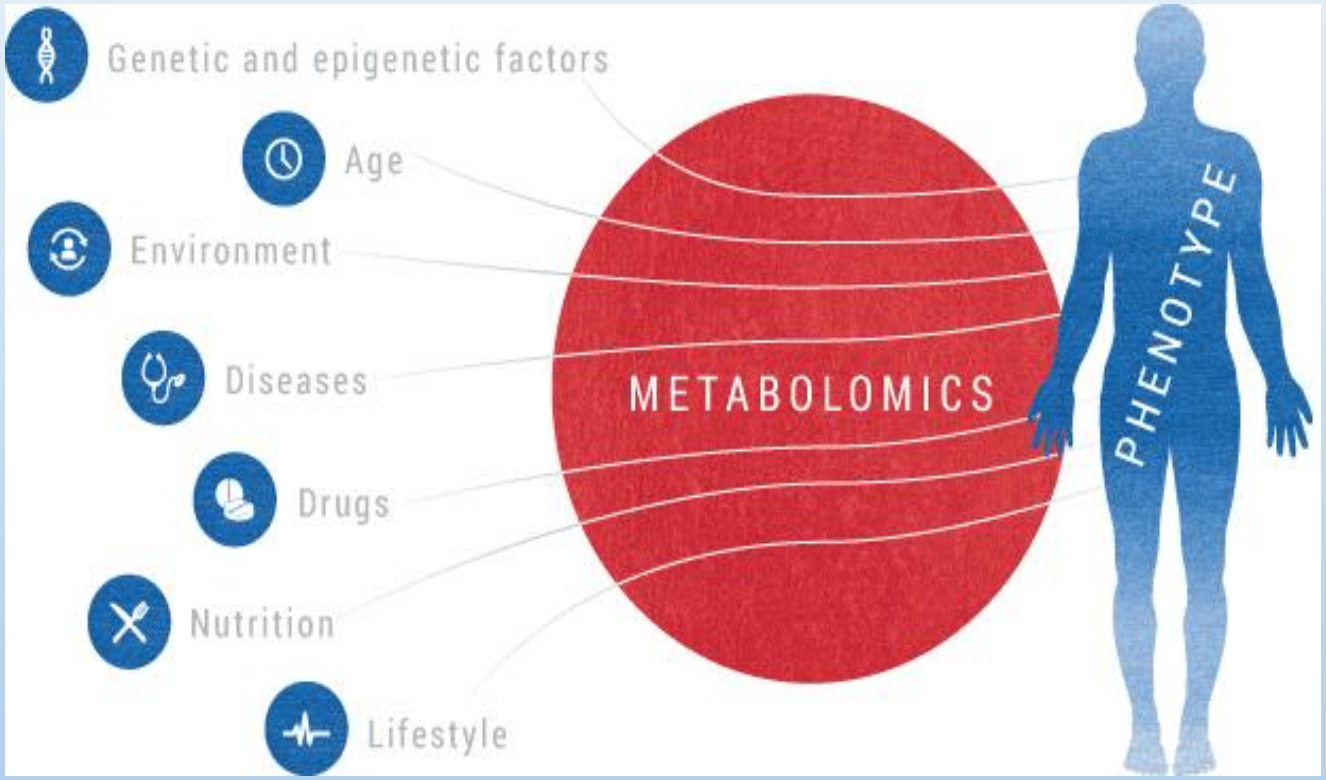
Metabolome is the collection of small molecules in cells, tissue, plasma, urine, tissue, etc.

Metabolomics is the measure of those metabolites

The comprehensive characterization of small molecules in a given system

Mass Spectrom Rev. 2007 Jan-Feb;26(1):51-78

Metabolomics



We are diverse and so our metabolome is reflective of that!

http://www.metanomics-health.com/en/why-metabolomics.html

Lipidomics

Lipids are involved in numerous diseases

Lipids have been implicated in virus replication for many years

Lipids are used for energy (storage and utilization), signaling, cellular formation and numerous other areas

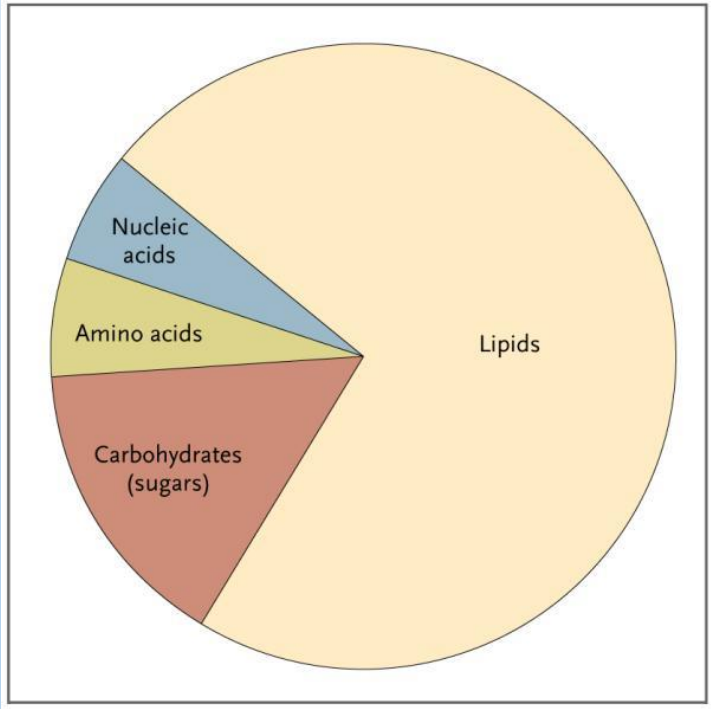
Lipids are ubiquitous across species, but can also be highly unique to species

- e.g. Lipid A in bacteria

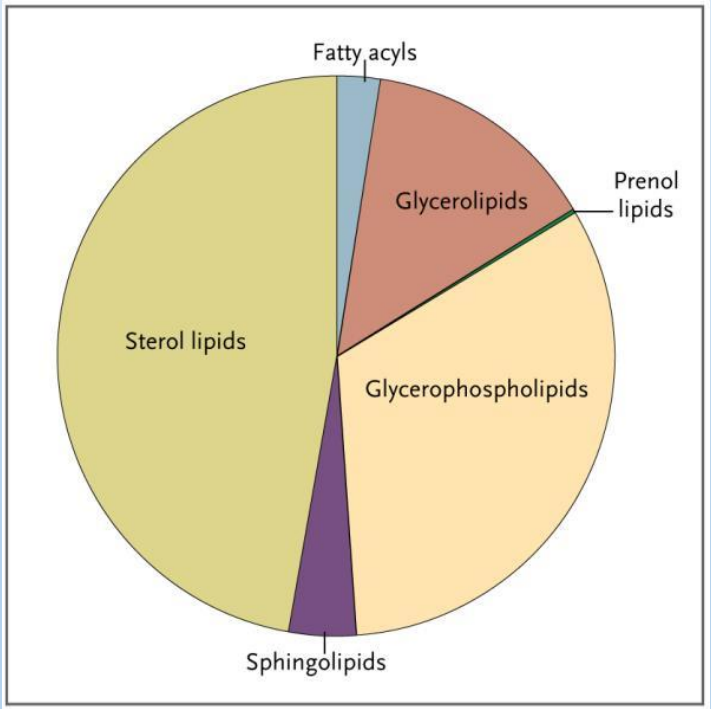
Measurement of lipids is important to understand biological processes in humans, plants, bacteria, etc

Lipid distribution in plasma

DISTRIBUTION BY WEIGHT

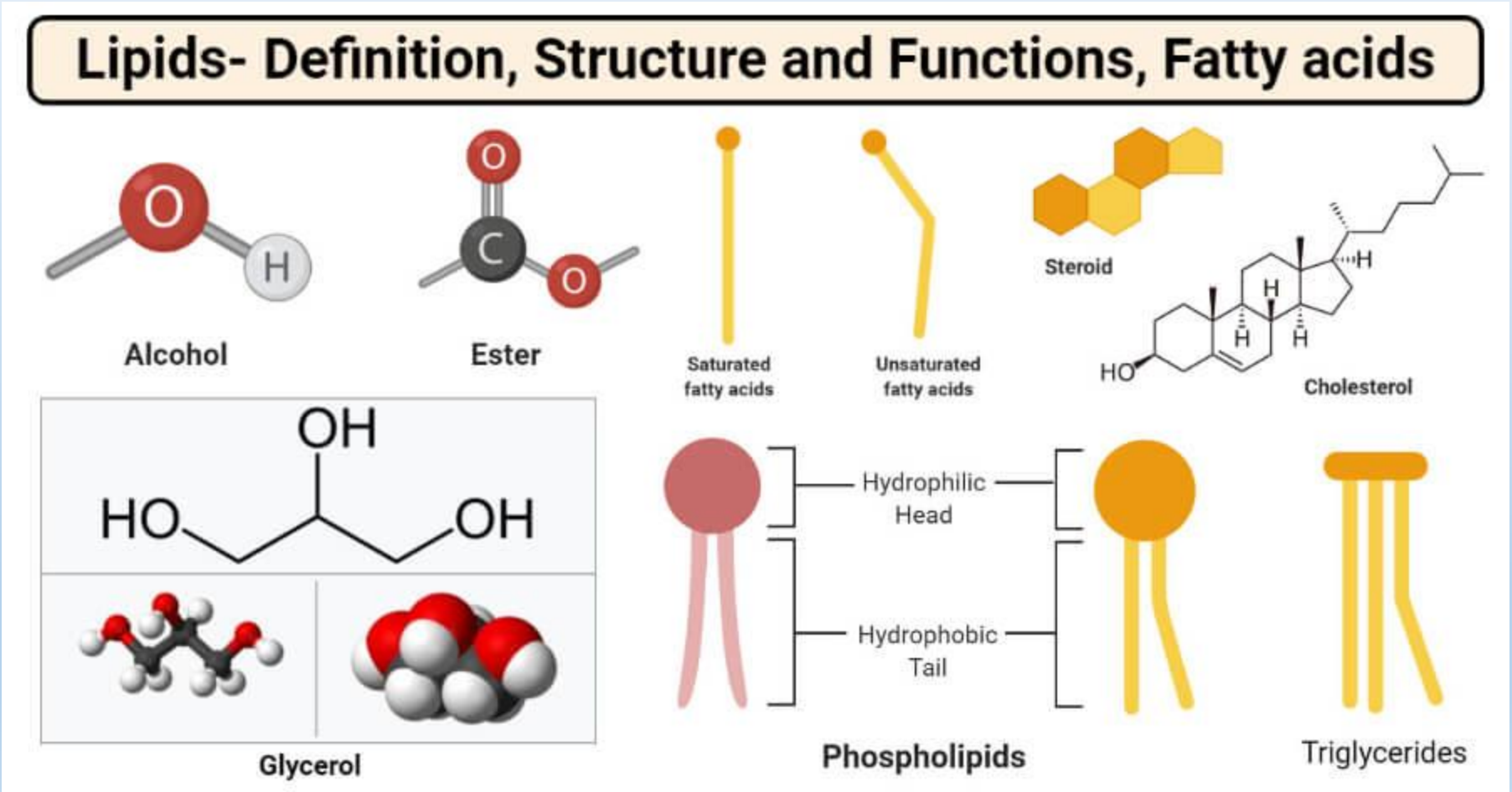


DISTRIBUTION OF LIPIDS BY MOLARITY



N Engl J Med. 2011 Nov 10; 365(19): 1812–1823.

Lipid Diversity



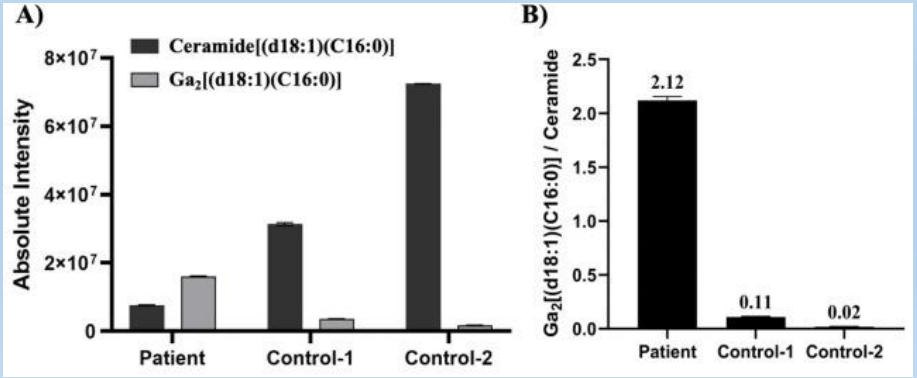
https://thebiologynotes.com/lipids/

LipidMatch

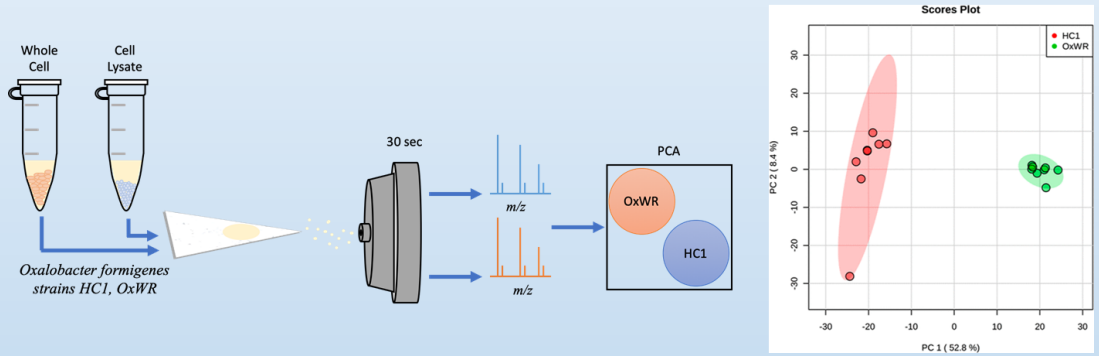
IE-Omics

Software/tools

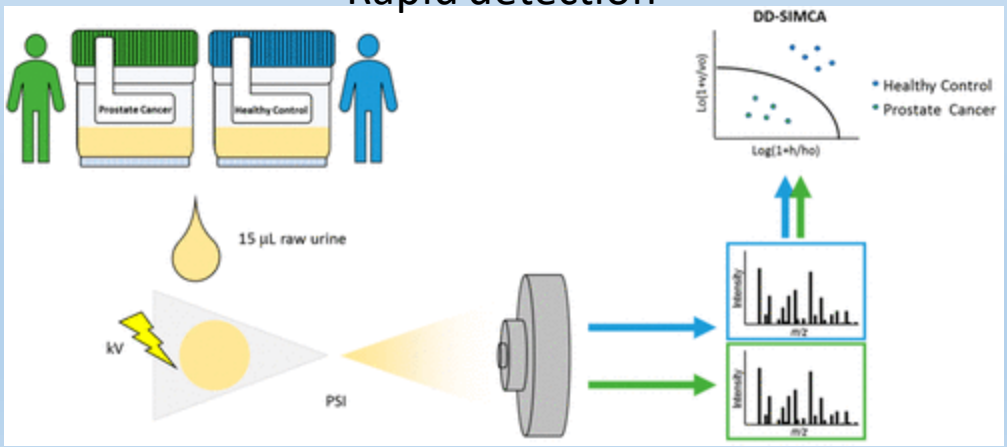
Garrett Lab



Personalized disease diagnostics



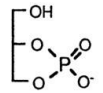
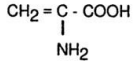
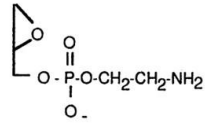
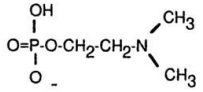
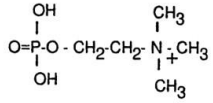
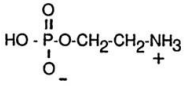
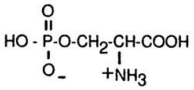
Rapid detection



Lipid Identification

Lipids follow well-defined fragmentation patterns with tandem mass spectrometry

1. Example for Phospholipid classes
2. Can identify based on head group fragmentation
3. Tails can be identified with enough signal intensity
4. That means we can develop software to identify common fragments and report the class level and fatty acyl chains
5. We can't necessarily identify where the double bond is or which carbon the fatty acyl chain is attached to

specificity	polarity	fragment structure	scan mode	fragment type	optimal collision offset
all $[M-H]^-$ ions of glycerophospho-lipids	neg.		precursor of 153	glycerol-phosphate $-H_2O$	+50 V
phosphatidyl-inositol	neg.		precursor of 241	head group $-H_2O$	+45 V
phosphatidyl-serine	neg.		neutral loss of 87	head group $-H_3PO_4$	+28 V
phosphatidyl-ethanolamine	neg.		precursor of 196	dilyso $-H_2O$	+50 V
sphingomyelin	neg.		sCID + precursor of 168	head group (demethyl-ated)	sCID +65 & +40 V
phosphatidyl-choline and sphingomyelin	pos.		precursor of 184	head group	-35 V
phosphatidyl-ethanolamine	pos.		neutral loss of 141	head group	-25 V
phosphatidyl-serine	pos.		neutral loss of 185	head group	-22 V

PNAS March 18, 1997 94 (6) 2339-2344; https://doi.org/10.1073/pnas.94.6.2339

Lipid Identification Software: MS/MS identification

	MS/MS confirmation	RT filter	Structural Resolution	Application	Libraries (Types)	Customizable MS^2 Libraries	MS/MS Visualization
LipidMatch Flow	rule (*m/z*) & rank (int)	No	Correct	DDA, DIA	71	In Excel	Yes (Table)
GREAZY	Baysian (*m/z*)	No	Over-annotates	DDA	24	In Code	No
LipidBlast	Modified Dot Prod (*m/z* & int)	No	Over-annotates	DDA	28	In Excel	Yes
MS-DIAL v2.84	Modified Dot Prod (*m/z* & int)	Yes	Over-annotates	DDA, DIA	42	In Excel	Yes
LipidSearch v4.2.10	rule (*m/z* & int)	No	Over-annotates	DDA, MS^3	83	No	Yes
SimLipid	rule (*m/z* & int)	Yes	Over-annotates	DDA, DIA	53+	User-Interface	Yes
Liquid	Probability (int quartiles)	No	Over-annotates	DDA	56	In Code	Yes
LipiDex	Modified Dot Prod (*m/z* & int)	Yes	Correct	DDA	36	User-Interface	No
LDA2 v2.6.3	rule (*m/z* & int)	Yes	Correct	DDA, MS^3	14	User-Interface	Yes
LipidFrag	in silico	No	Correct	DDA	LipidMaps*	No	Yes (MetFrag)
LipidHunter	rule (*m/z*) & rank (int)	No	Correct	DDA	8	In Excel	Yes
Lipid Annotator	Bayesian Probability (*m/z* & int)	No	Correct	DDA, DIA, IM-DIA	58	No	Yes

LipidMatch Open Source Software

LipidMatch

Inputs:

MS/MS In-silico Libraries:
>300,000 species across >60 lipid types

Feature Tables (Multiple Platforms):

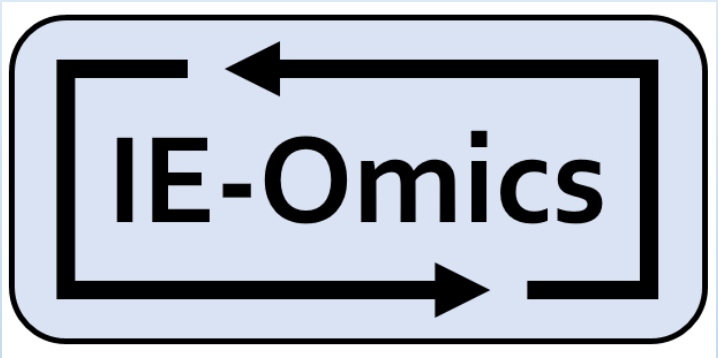
XCMS Online | MZmine 2 | MS-DIAL | Compound Discoverer

MS/MS Data (not vendor specific):
AIF, IE-ddMS^2, ddMS^2, Targeted

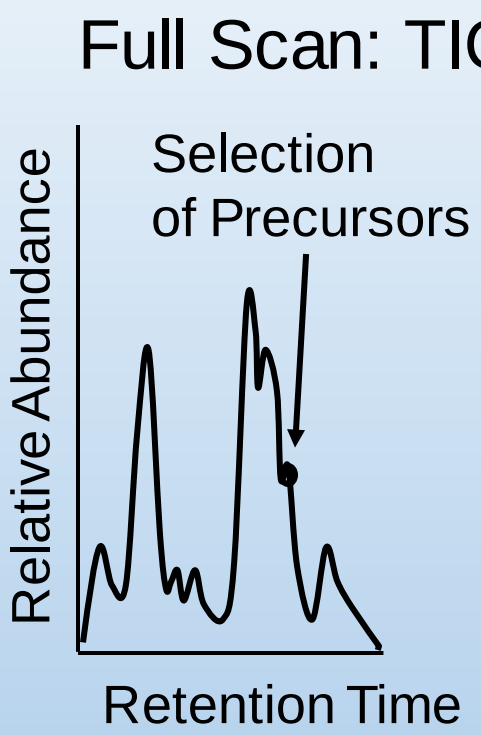
→ **Lipid identification (written in R)** → **Annotated lipids appended to feature table**

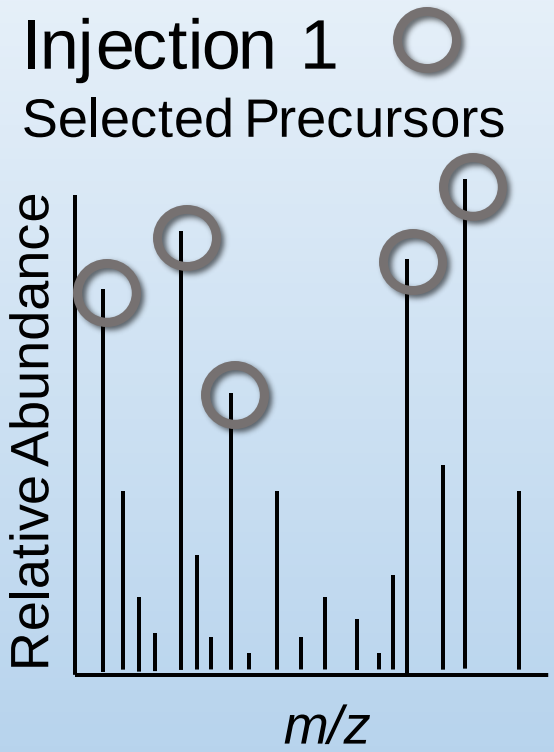
"LipidMatch: an automated workflow for rule-based lipid identification using untargeted high-resolution tandem mass spectrometry data", J.P. Koelmel, N.M. Kroeger, C.Z. Ulmer, J.A. Bowden, R.E. Patterson, J.A. Cochran, C.W.W. Beecher, T.J. Garrett, R.A. Yost, *BMC Bioinformatics*, 18(1), 331 (2017).

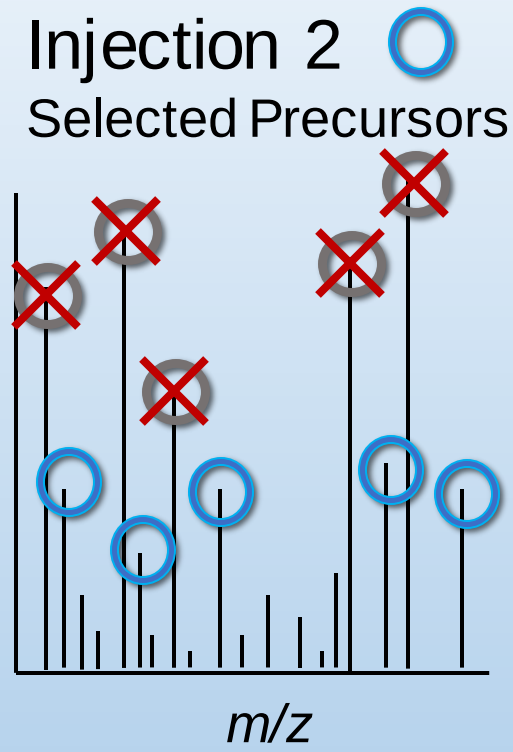
Expanding lipid coverage



Iterative Exclusion (IE) ddMS^2-topN



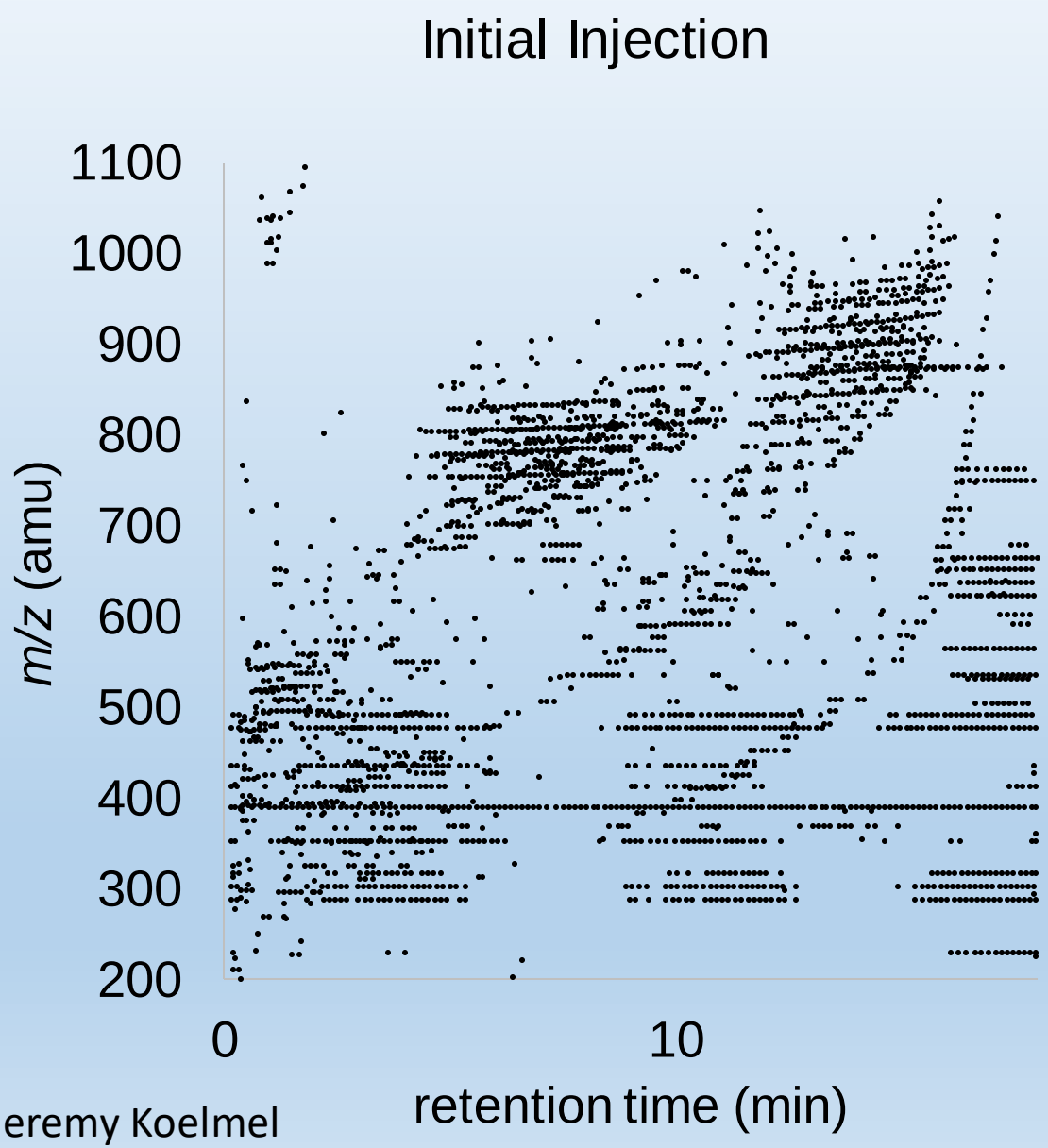


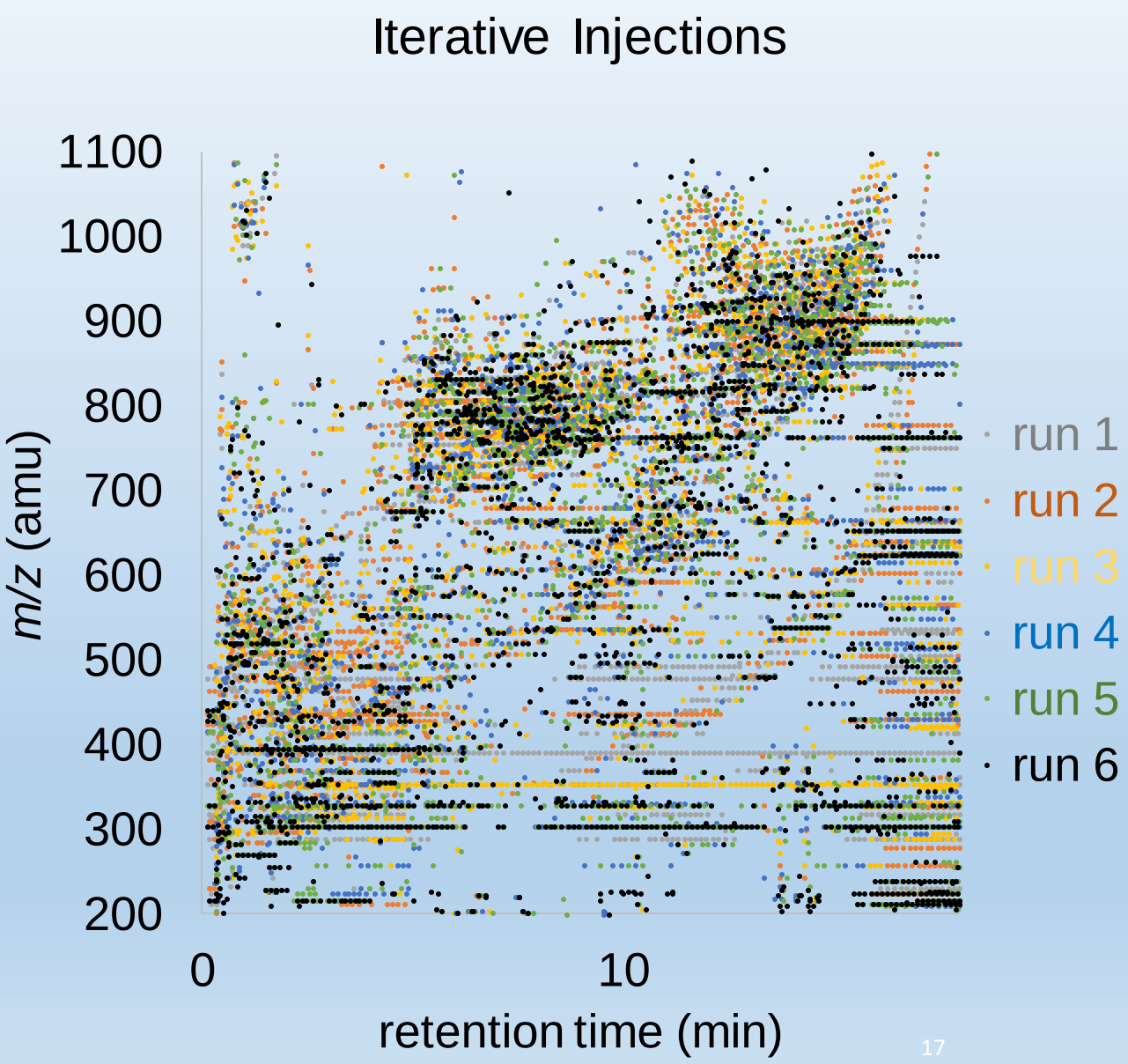


…Injection n

Jeremy P. Koelmel, Nicholas M. Kroeger, Emily L. Gill, Candice Z. Ulmer, John A. Bowden, Rainey E. Patterson, Richard A. Yost, Timothy J. Garrett: Expanding lipidome coverage using LC-MS/MS data-dependent acquisition with automated exclusion list generation. *Journal of the American Society for Mass Spectrometry*. (2017) 28: 908.

Increase Fragmentation Coverage with IE





Let's talk about Biomarkers

Biomarkers are…

Objective characteristics of biological process

Quantifiable (can be semi-quantitative)

Consistently expressed (reliable)

Accurate at defining disease

Defined from observational research and use observational tools

- Screening platforms (i.e. what we do)

Reality

Screening assays (i.e. metabolomics) have many technical issues

- Suffer from low ‘n’ with high features (e.g. 10 subjects vs 4000 variables)

How many biomarkers have been translated to clinical practice?

Less than 1% (Kern, SE. Cancer Res, 2012, 72, 6097-6101)

- An insurance paid test to diagnose a patient

Discovery is exciting…validation is not

- Validation is time-consuming and requires many more samples
- Validation is more expensive and can result in failure (not very exciting)

Statistical significance can be subject to bias especially if using techniques such as PLS-DA and not performing validation testing

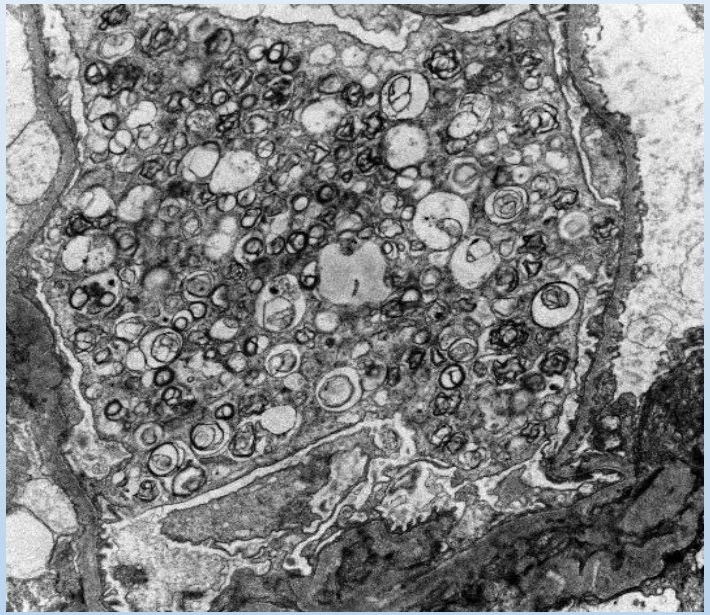
- AI represents a new challenge for translation

Omics Biomarker Characterization of Rare Disorders

- In the last few years, metabolomics/lipidomics has made it possible to better understand the metabolic processes associated with several rare disorders and proved to be a powerful tool for their clinical investigation
- Due to the rapid expansion of metabolomics/lipidomics analysis in the clinical laboratory, Omics analyses can begin to play a more prominent role in the characterization of rare disorders especially borderline or late onset (not identified at birth)
- Metabolomics/lipidomics can aid in the early diagnosis of rare disorders, or in helping to better understand different manifestations of rare disorders

Case Study in precision diagnostics

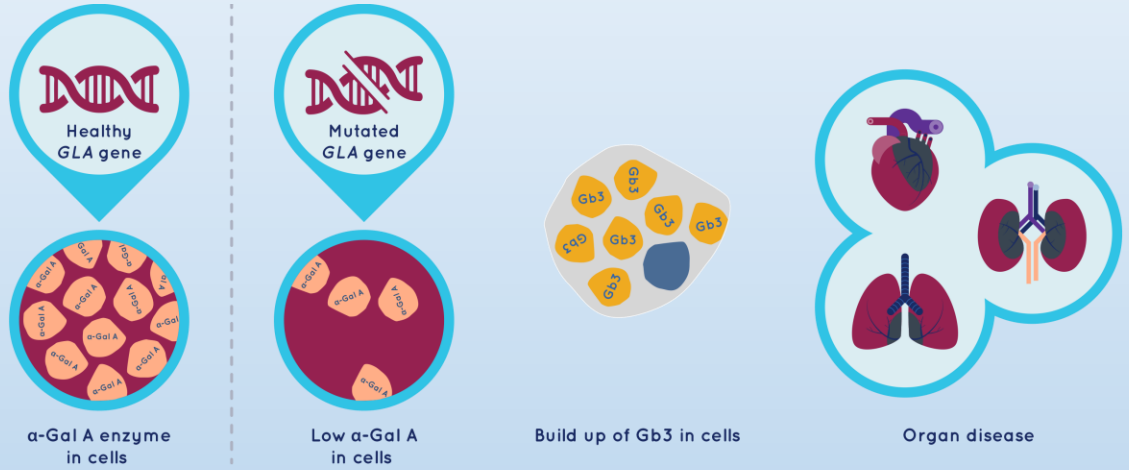
- Approached by William Clapp to try and help characterize the disease of a female patient
- The patient is a 57–year old female who presented with proteinuria
- She has a history of breast cancer and has a history of diabetes
- Proteinuria was believed secondary to diabetes; however, her proteinuria seemed more severe than that associated with her diabetes
- A renal biopsy was performed for clinical pathology, and electron microscopy revealed abundant lamellated lipid inclusions-no clear diagnosis for Fabry though



Electron micrograph showing numerous myeloid bodies within podocyte cytoplasm

Fabry Disease

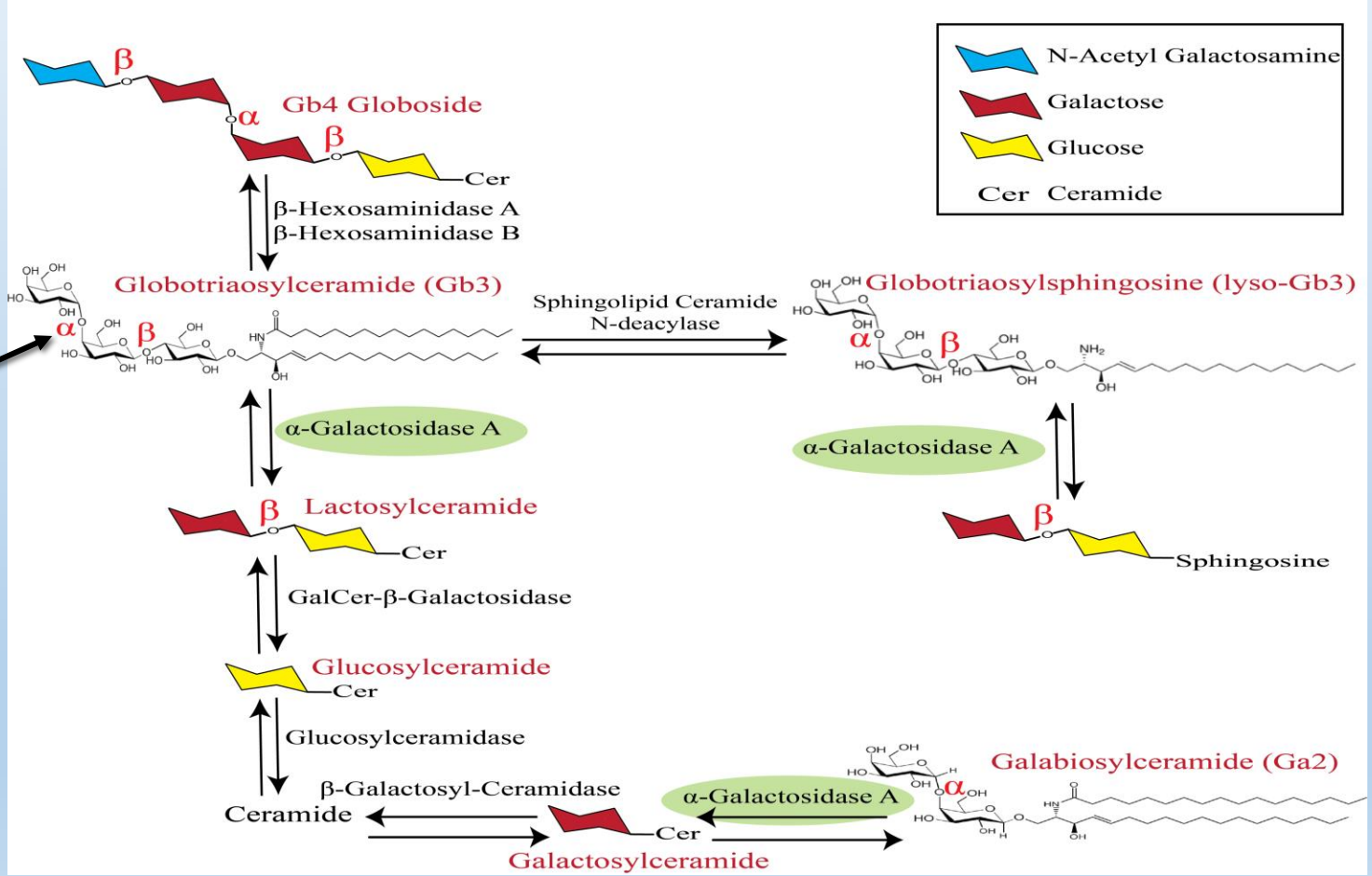
- Caused by alterations (mutations) in the α-galactosidase A (*GLA*) gene
- Absent or markedly deficient activity of the lysosomal enzyme, α-galactosidase A (α-Gal A)
- α-Gal A functions to break down complex sugar-lipid molecules called glycolipids
- The spectrum of disease in heterozygous female patients is broad and ranges from asymptomatic to mild and to severe disease



- Renal Failure
- Burning Pain
- Cardiovascular Disease
- Fatigue, Fever and Body Aches
- Febrile Episode

Boutin, M. & C. Auray-Blais, 2015. *J Am Soc Mass Spectrom,* 26**,** 499-510
Guffon, N. & A. Fouilhoux, **2004**, *J Inherit Metab Dis,* 27**,** 221-7.

Fabry lipid pathway



- α-Gal A is only able to break down α bonds in glyocosphingolipids
- Reduction in α-GAL A causes an increase in Gb3 and lysoGb3 in classical Fabry
- Traditional analysis for Fabry identification is measurement of Gb3 and lysoGb3

Glycolipid Biomarkers in Fabry Disease

1. Globotriaosylceramide (Gb_3)

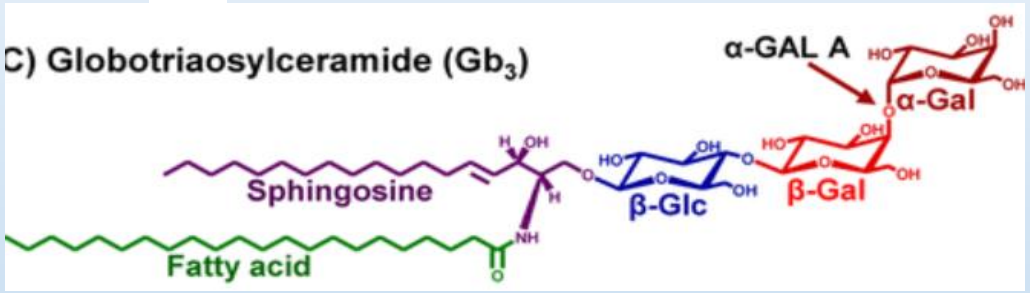
   Gb3(d18:1)(C16:0)

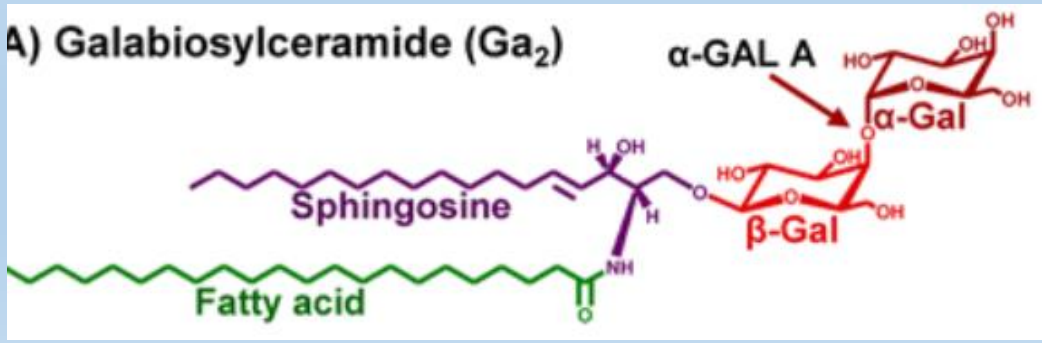
2. Globotriaosylsphingosine (lyso-Gb_3)

   Lyso-Gb3(d18:1)

3. Galabiaosylceramide (Ga2)

   Ga2(d18:1)(C16:0)





Guffon, N. & A. Fouilhoux, **2004**, J Inherit Metab Dis, 27, 221-7.
Auray-Blais, C., M. Boutin, R. Gagnon, F. O. Dupont, P. Lavoie & J. T. Clarke, **2012**, *Anal Chem,* 84**,** 2745-53.

Experimental Procedures:

- Received 3 renal biopsy samples
  - (2 Controls, 1 Patient)
- Homogenized samples and weight normalized
  - 1-3 mg of tissue
- Folch Extraction for lipidomics analysis
  - Chloroform:Methanol
- Untargeted lipidomics Study
  - Data Analysis by LC-HRMS/MS
  - LipidMatch for identification

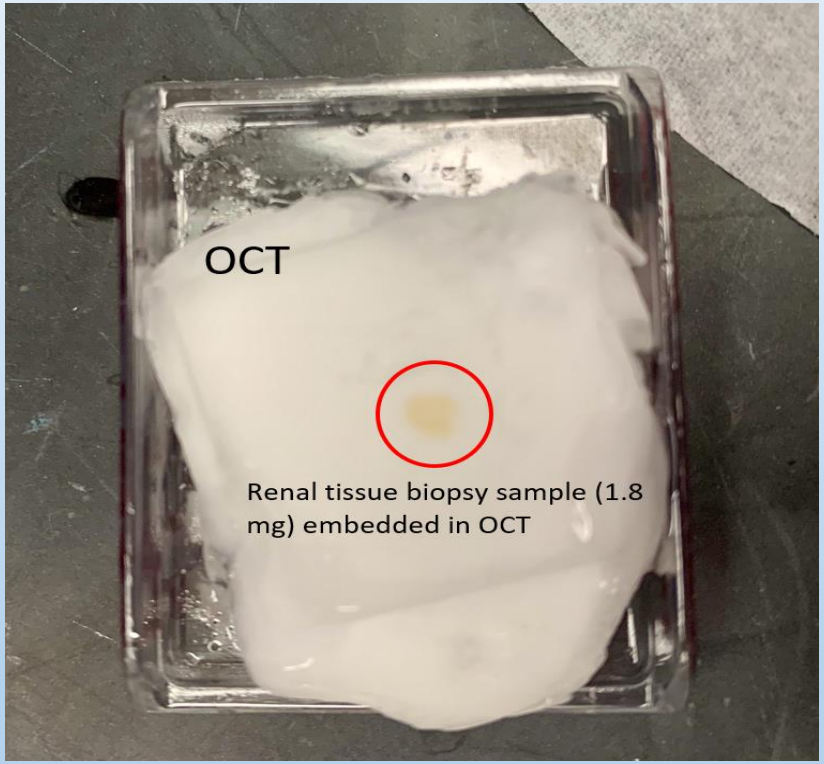


Photo of patient renal tissue biopsy sample embedded in OCT, showing the lipidomics analysis can be conducted on the leftover sample without disruption in normal patient care

Looking at Current Clinical Biomarkers

1) Globotriaosylceramide (Gb_3)

Gb_3 Related Biomarker	Expected Mass (m/z)	Patient	Control-1	Control-2
Gb_3[(d18:1)(C16:0)]	1024.6784	nd	nd	nd
Gb_3[(d18:2)(C16:0)]	1022.6701	nd	nd	nd
Gb_3[(d18:2)(C22:1)]	1104.7417	nd	nd	nd
Gb_3[(d18:2)(C18:0)]	1051.7092	nd	nd	nd
Gb_3[(d18:1)(C14:0)] + Gb_3[(d16:1)(C16:0)]	996.6636	nd	nd	nd

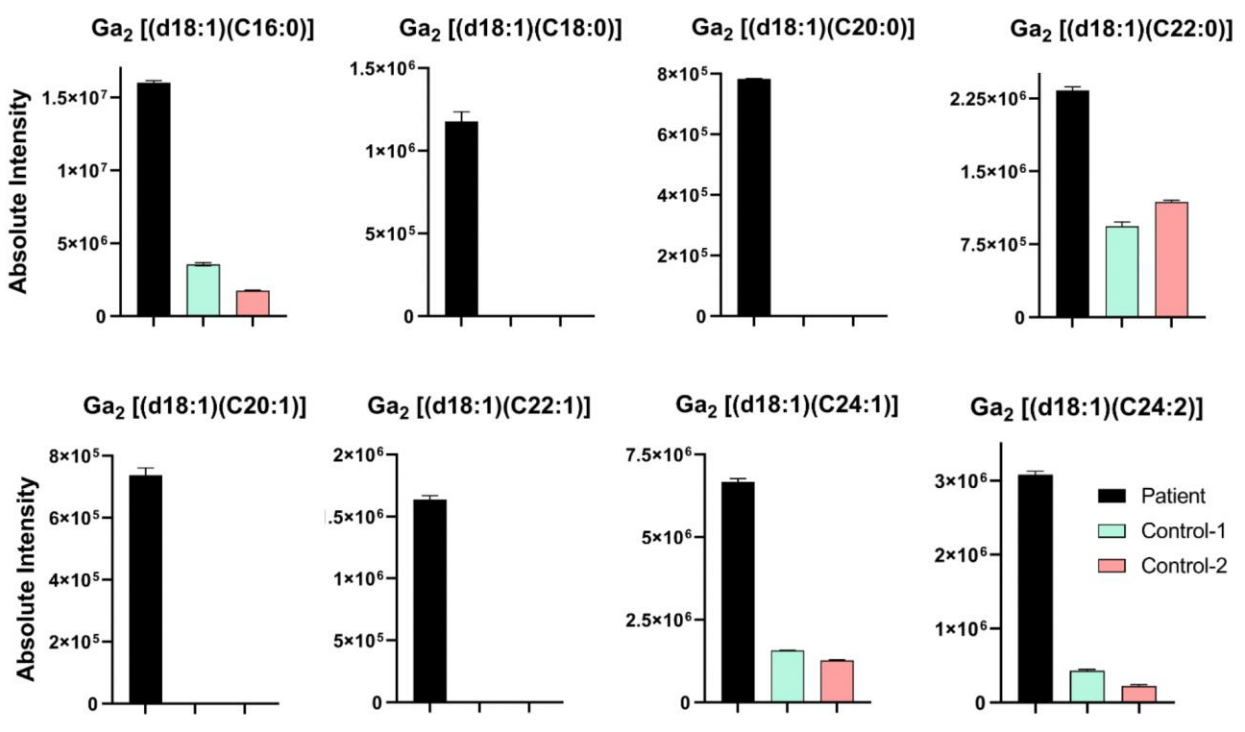
2) Globotriaosylsphingosine (lyso-Gb_3)

Lyso-Gb_3 Related Biomarker	Expected Mass (m/z)	Patient	Control-1	Control-2
lyso-Gb_3	786.4487	nd	nd	nd
lyso-Gb_3 (– C_2H_4)	758.4174	nd	nd	nd
lyso-Gb_3 (– C_2H_4 + O)	774.4123	nd	nd	nd
lyso-Gb_3 (– H_2)	784.4331	nd	nd	nd
yso-Gb_3 (– H_2 + O)	800.4280	nd	nd	nd
lyso-Gb_3 (+ O)	802.4436	nd	nd	nd
lyso-Gb_3 (H_2O_2)	820.4542	nd	nd	nd
lyso-Gb_3 (H_2O_3)	836.4491	nd	nd	nd

- These are the current targeted lipids for Fabry disease identification
- Accumulation would be present in Males with Fabry
- Accumulation for Females could occur, but to a lesser extent depending on enzyme activity
- These tests would suggest that the patient doesn't have Fabry since no accumulation is observed

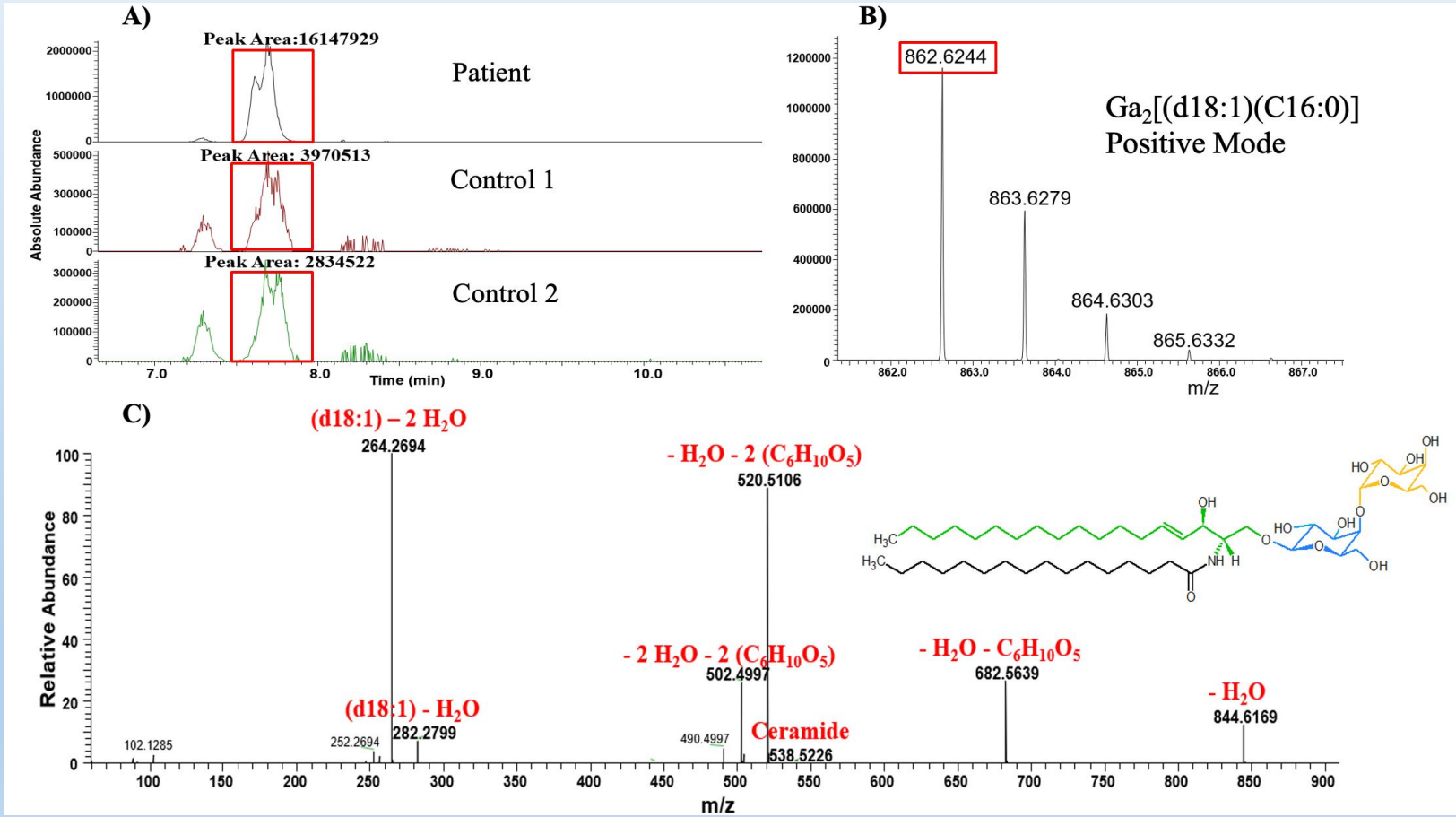
Additional biomarker analysis

3) Galabiaosylceramide (Ga2)



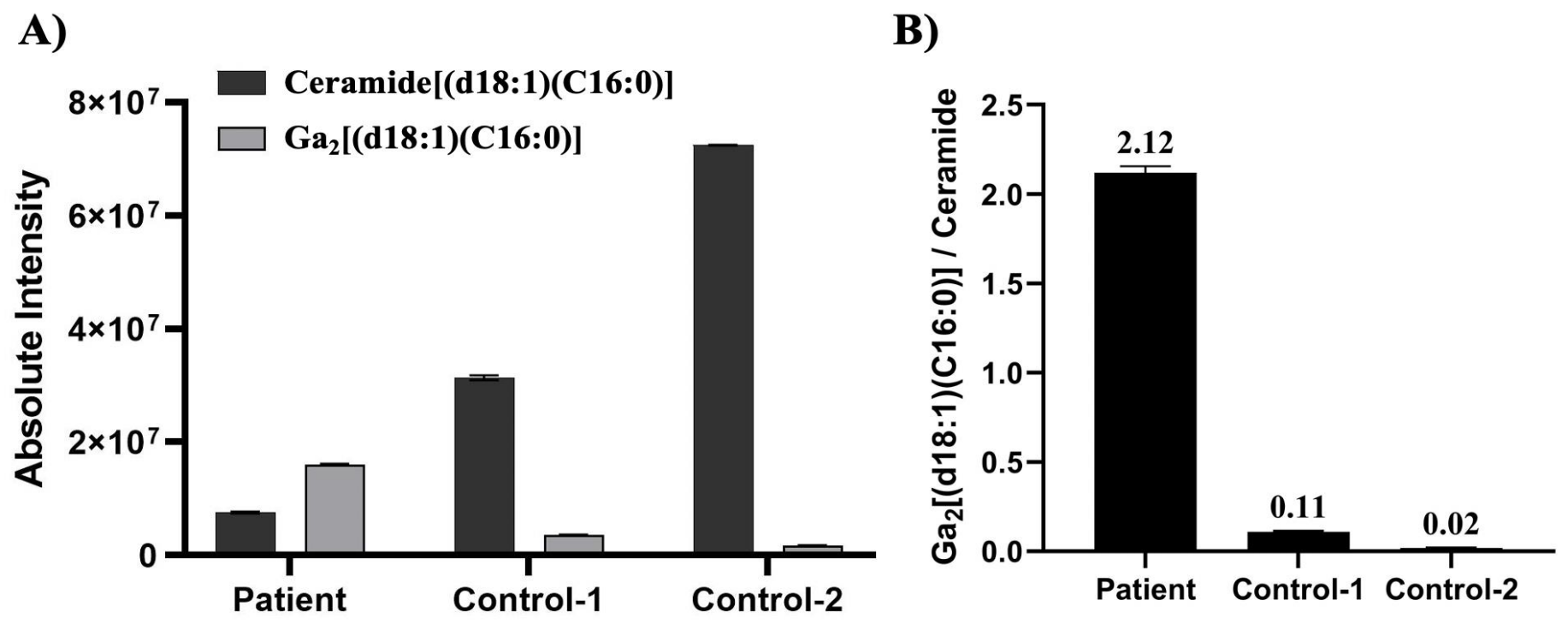
- Analyzing the sample by LC-HRMS enable a deeper dive into other lipids
- The Ga2-related lipid biomarker level was *substantially* higher in the patient's renal tissue biopsy than in the two control samples
- These results confirm that this patient has some residual α-Gal A enzyme activity that has broken down Gb3 and lyso-Gb3 lipids but is not sufficient to degrade all Ga2 lipids, Fabry disease now almost confirmed

Confirmation of signal in raw data

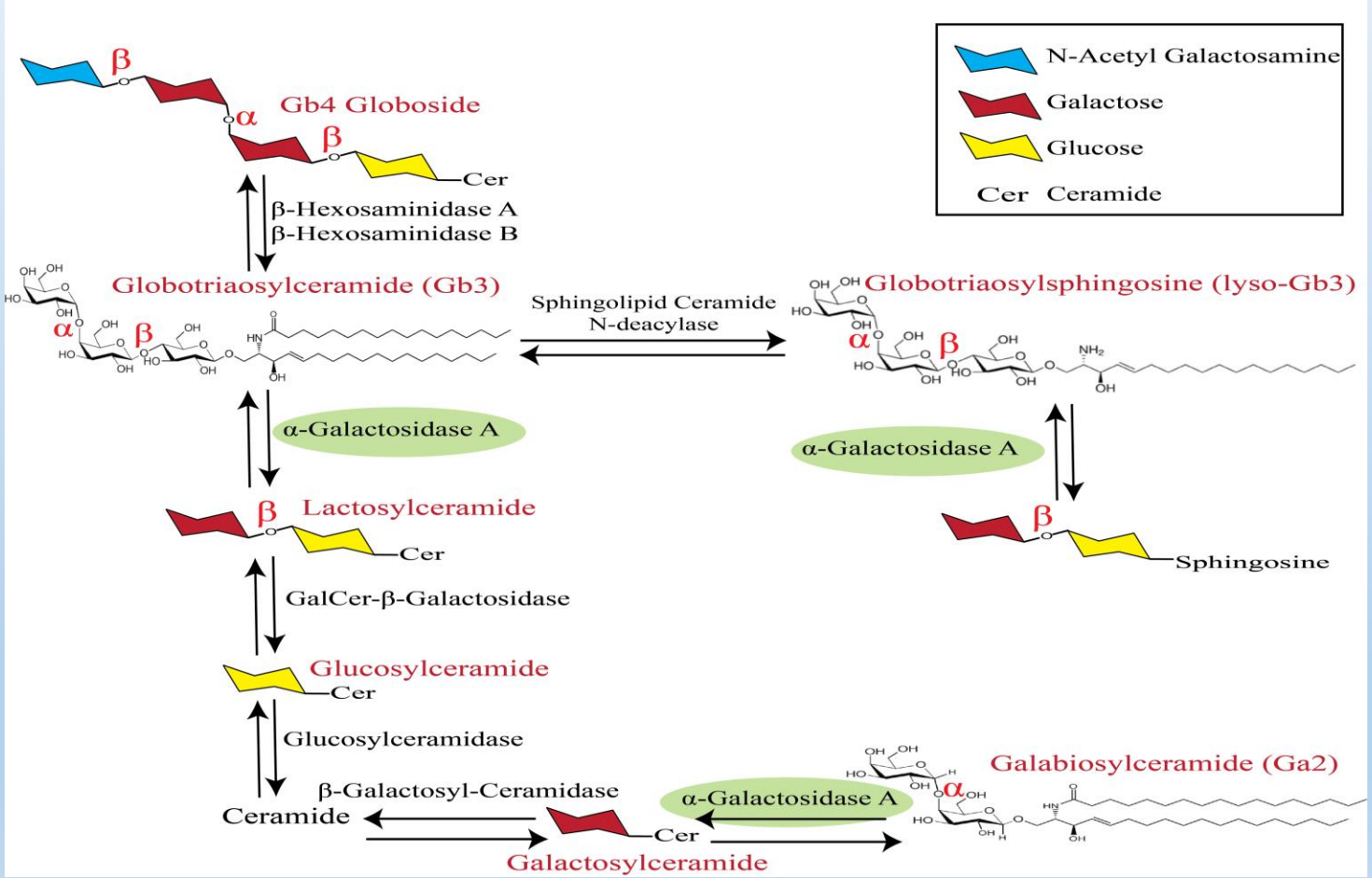


Ratiometric Metabolomics

- Diagnosing a N of 1 or rare disease requires additional confidence
- Ratiometric approaches are used in newborn screening as a metric of enzyme activity
- In the case of Fabry disease, we evaluated the ratio of Ga2[(d18:1)(C16:0)] to its corresponding ceramide (ceramide[(d18:1)(C16:0)]) to better demonstrate the increased level of Ga2[(d18:1)(C16:0)] in the patient
- The Ga2[(d18:1)(C16:0)]/Ceramide[(d18:1)(C16:0)] ratio increased more than 20 times in the patient sample compared to the two control samples



Revisiting the metabolism



Sufficient activity to metabolize Gb3

Insufficient activity to metabolize Ga2

α-galactosidase

2 isoforms exist

- α-Gal A encoded by GLA
- α-Gal B encoded by NAGA
- α-Gal A accounts for most of the activity in normal tissues while α-Gal B often accounts for residual activity
- Both act on the alpha linkage of galactose in Gb3 and Ga2

WGS has not been conducted yet, but will be conducted to evaluate activity of difference genes

We have not measured the activity of either A or B isoforms

- There could be differences in enzyme activity and thus would be useful to measure

There are over 1000 mutations in the GLA gene known and thus additional characterization is needed

- MS can offer a method to analyze for products related to genes and enzyme activity and add phenotypic information related to mutations

B. Winchester and E. Young. Chapter 18 Biochemical and genetic diagnosis of Fabry disease in Fabry disease: Perspectives from 5 years of FOS, Eds A. Mehta, M. Beck, G. Sunder-Plassmann, Oxford, 2006.

Summary

LC-HRMS in the identification of a single Female Fabry patient was explored

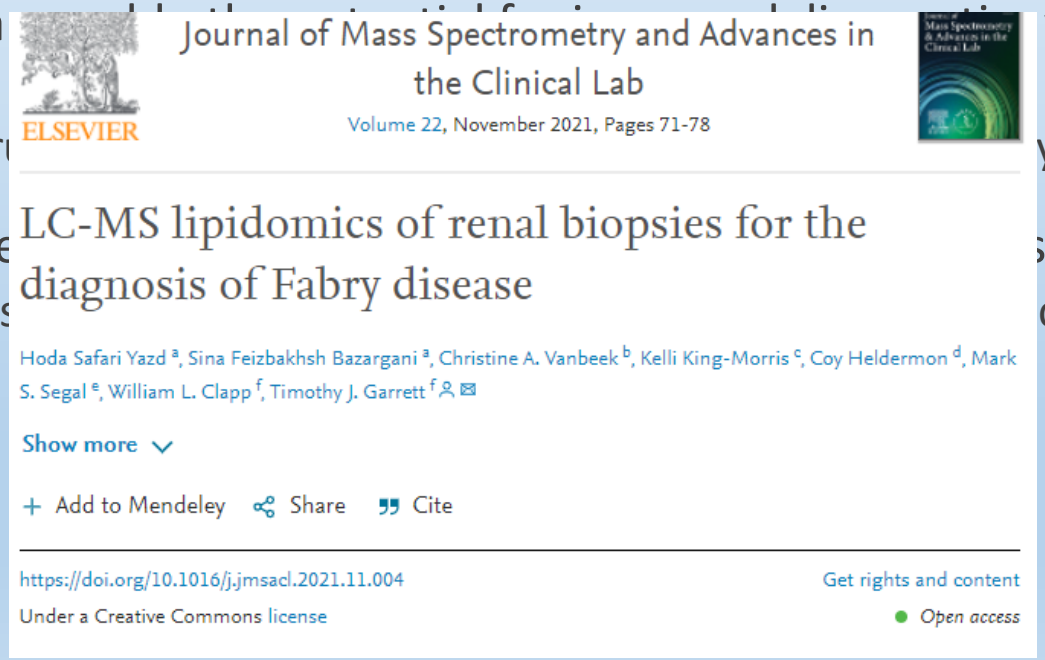
The ratio of Ga2[(d18:1)(C16:0)]/Ceramide[(d18:1)(C16:0)] was over 20 times higher in the Fabry patient

HRMS approaches ca… when working with known enzymatic processes

- Overall, the cost of r… y available

The process worked e…

- We can work with tis… cterization of mutations



ELSEVIER

Journal of Mass Spectrometry and Advances in the Clinical Lab

Volume 22, November 2021, Pages 71-78

LC-MS lipidomics of renal biopsies for the diagnosis of Fabry disease

Hoda Safari Yazd [a], Sina Feizbakhsh Bazargani [a], Christine A. Vanbeek [b], Kelli King-Morris [c], Coy Heldermon [d], Mark S. Segal [e], William L. Clapp [f], Timothy J. Garrett [f]

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Towards improving lipid analysis for biomarker identification

The common lipid separation is with a C18 column

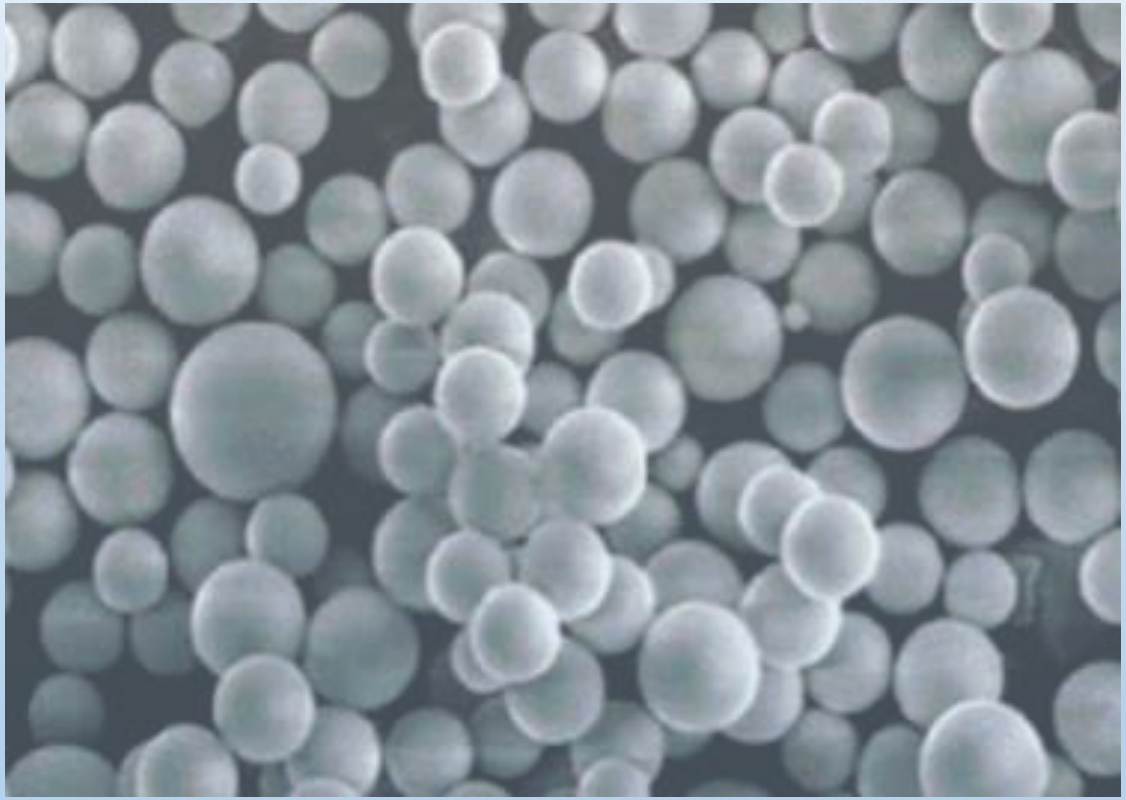
Better separation often leads to improved biomarker identification

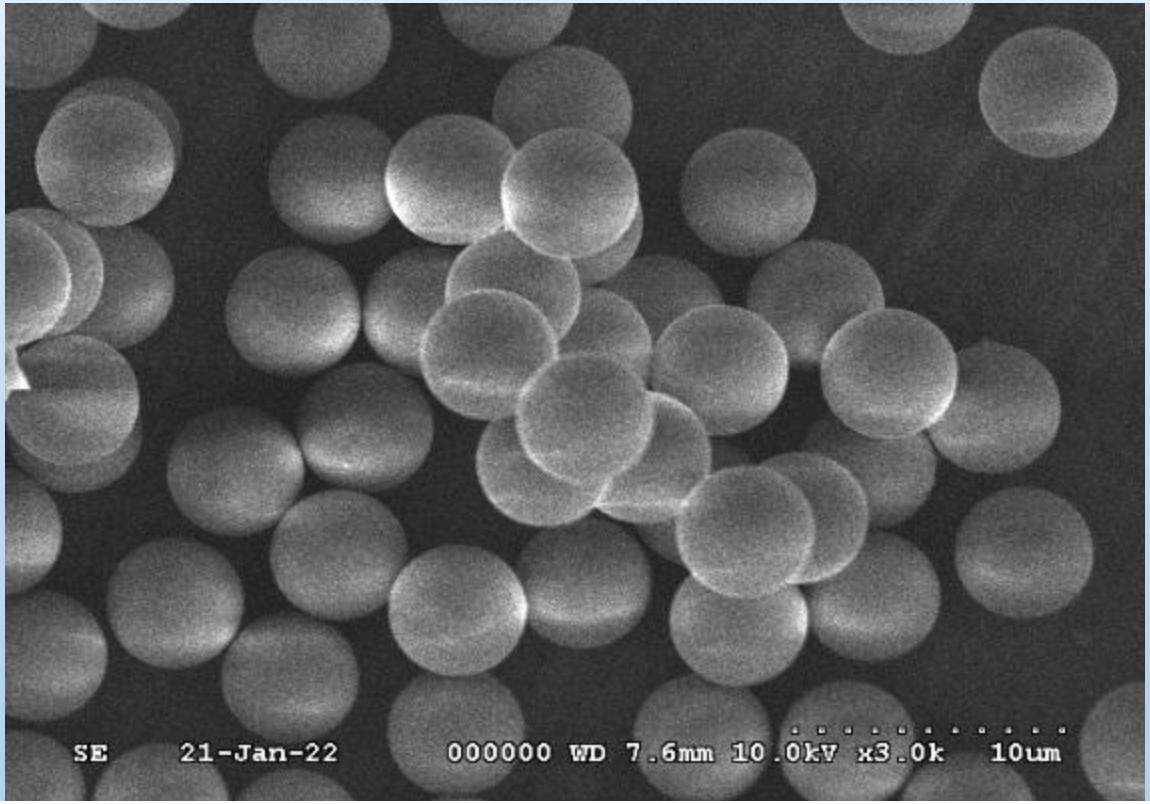
Some think that a C30 would be better for lipids and several methods have been developed

We tested 5 columns to evaluate overall lipid separation in potential biomarker studies

- ThermoFisher Accucore C30 50 x 2.1mm, 2.6 µm
- Fortis Evosphere C12 50 x 2.1mm, 3 µm (monodisperse)
- Fortis Evosphere C12 30 x 2.1mm, 1.7 µm (monodisperse)
- Waters BEH C18 50 x 2.1 mm, 1.7 µm
- Ace C8 50 x 2.1mm, 3 µm

Polydisperse vs Monodisperse

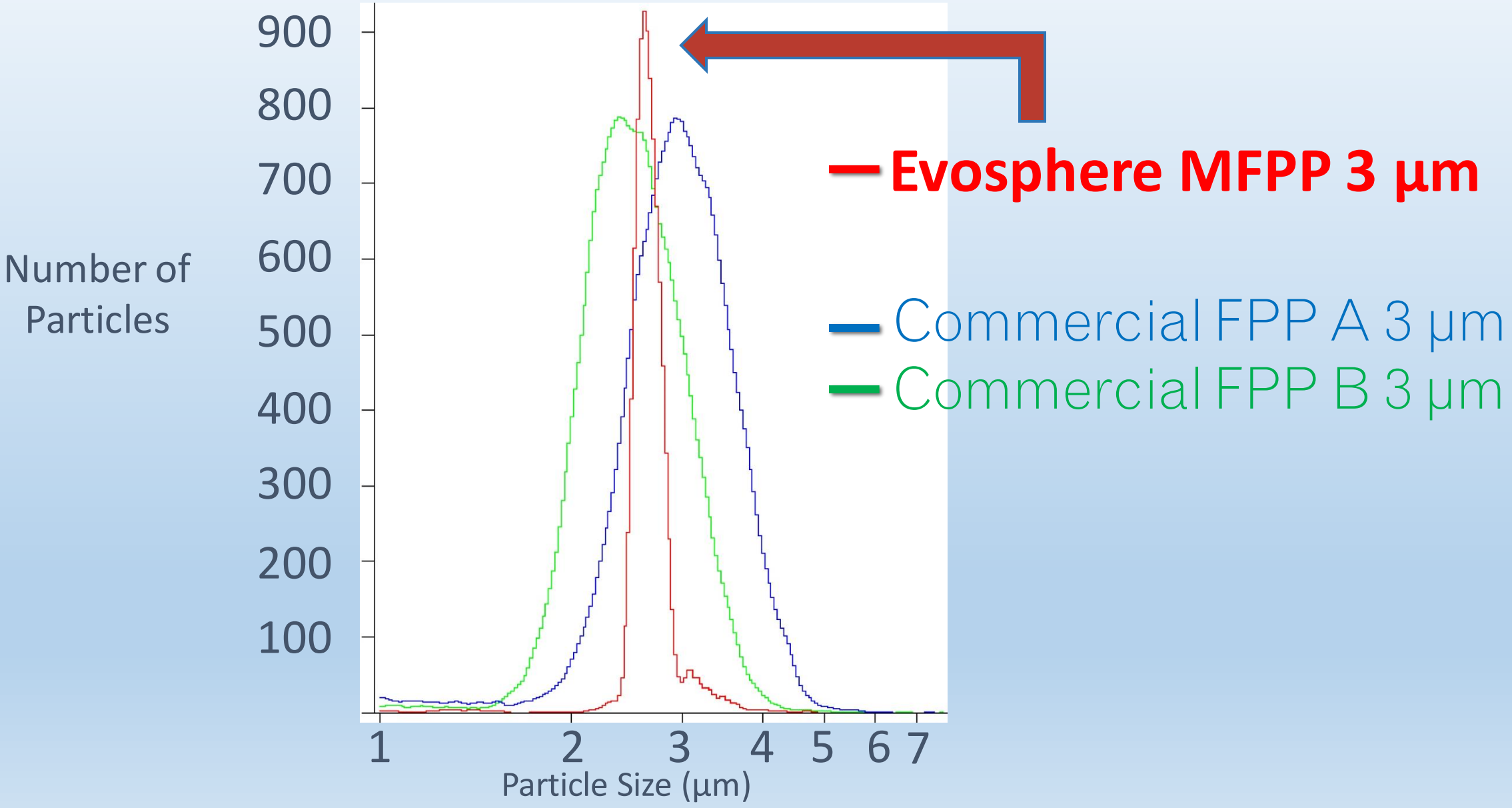




Polydisperse
Fully Porous Particle

Monodisperse
Fully Porous Particle

Particle Size Distribution Comparison



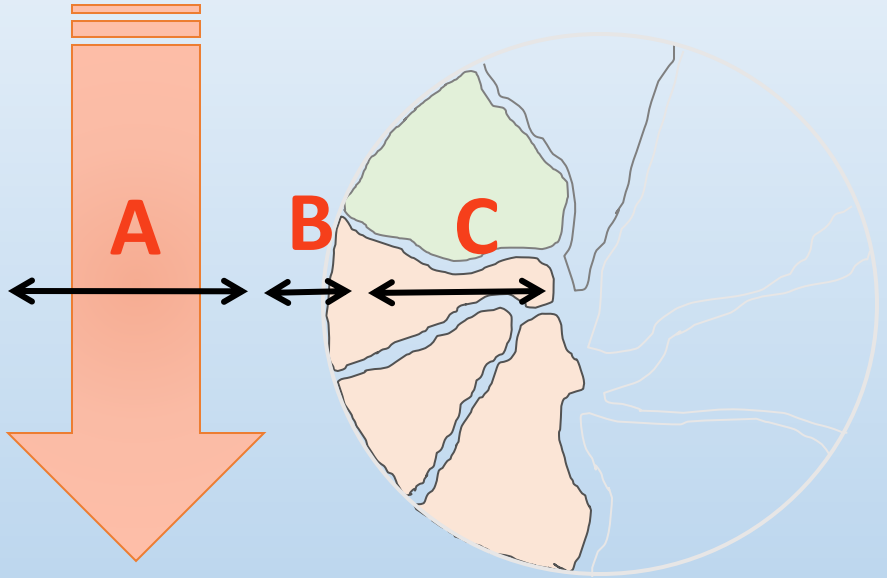
How does MFPP impact band broadening?

H: Height Equivalent to a Theoretical Plate

A: Eddy Diffusion

B: Longitudinal Diffusion

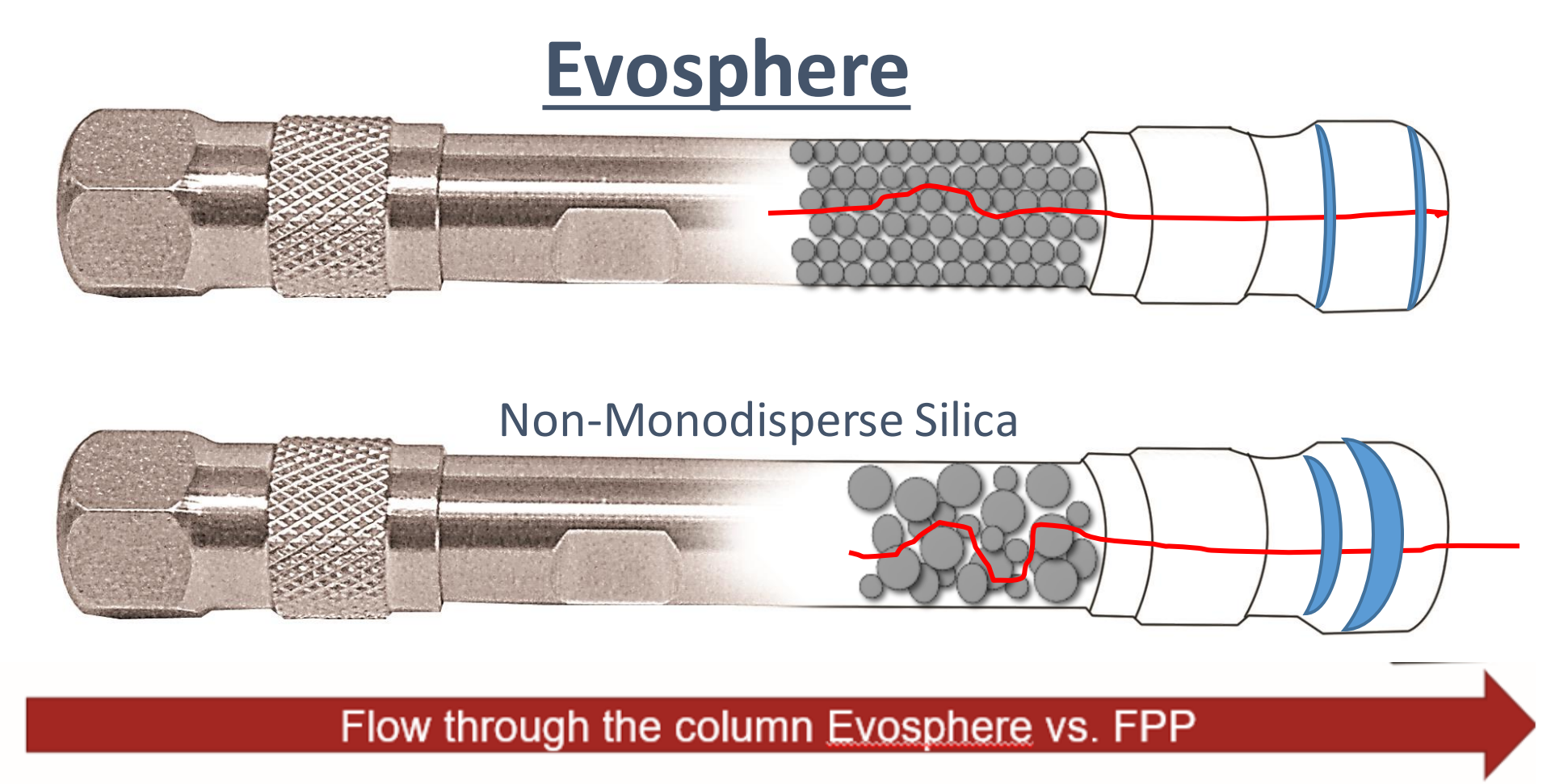
C: Resistance to Mass Transfer



Van Deemter Equation

$$H = A + \frac{B}{u} + Cu$$

What does this look like visually through the column?



Analysis details

INSTRUMENT

Thermo Orbitrap Exploris 120, Vanquish UHPLC

30,000 resolution in FS and 17,500 resolution for ddMS2

Positive ionization

- 3500 V
- 40 sheath gas
- 10 auxiliary gas
- 1.0 sweep gas
- 275C ion transfer tube
- 300C H-ESI probe

SEPARATION

A= 60/40 ACN/water with 10 mM AmFormate, 0.1% formic acid

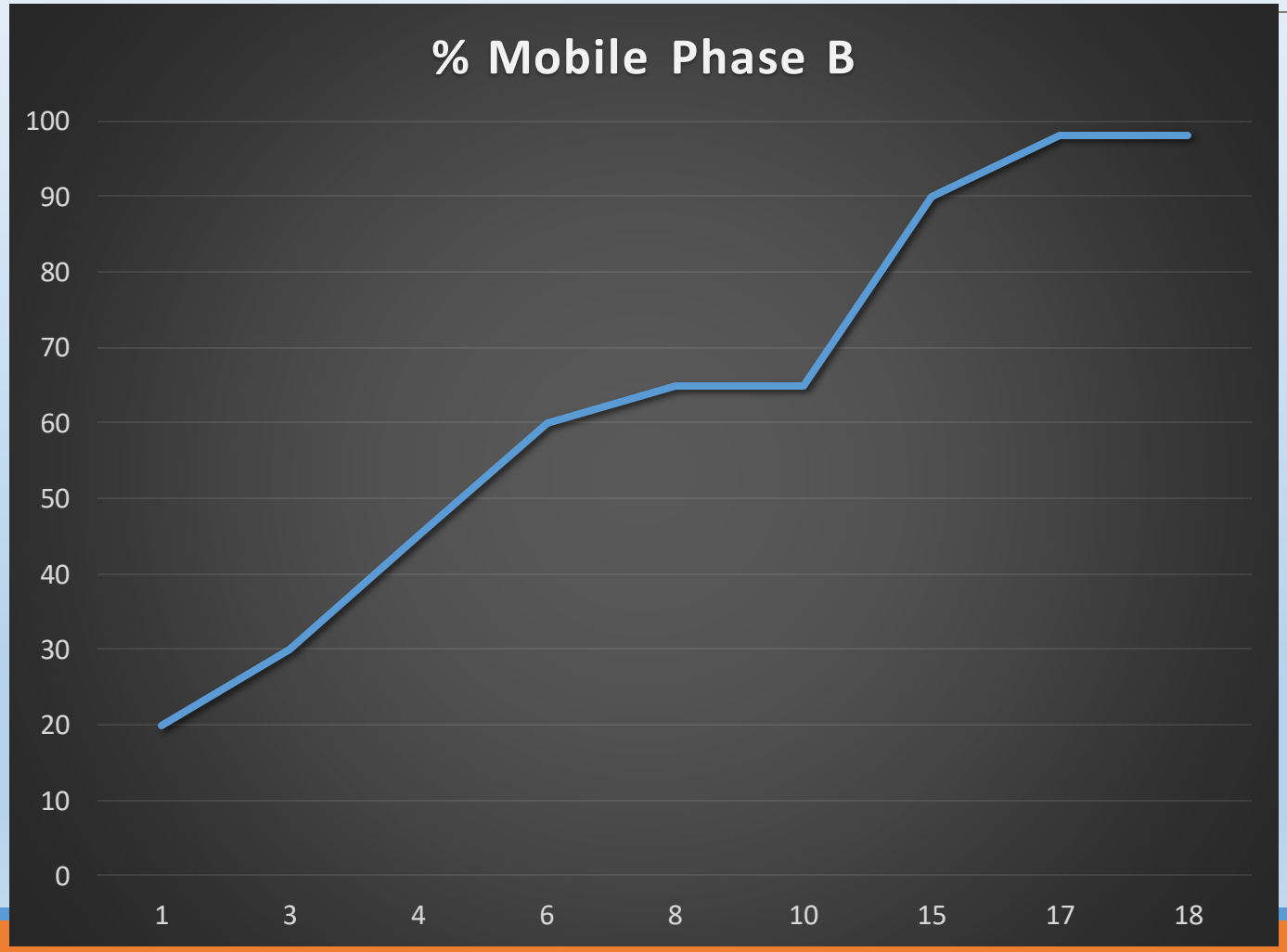
B=90/2/2 Isopropanol/ACN/Water with 10mM AmFormate, 0.1% formic acid

500 µL/min, 50C column T

Simple gradient elution from 80/20 to 2/98, 22 min injection to injection

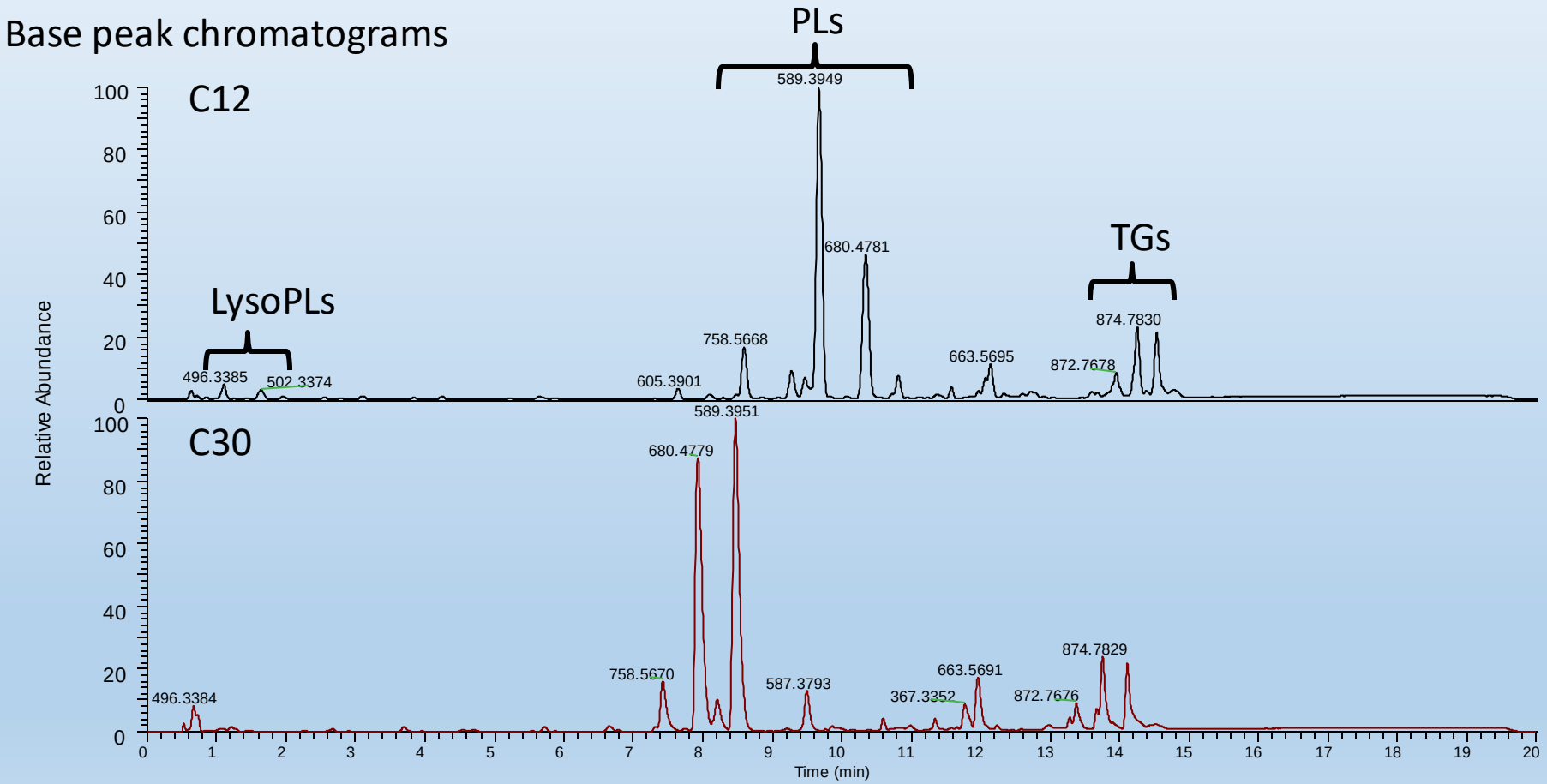
2 µL injection

Gradient Chart



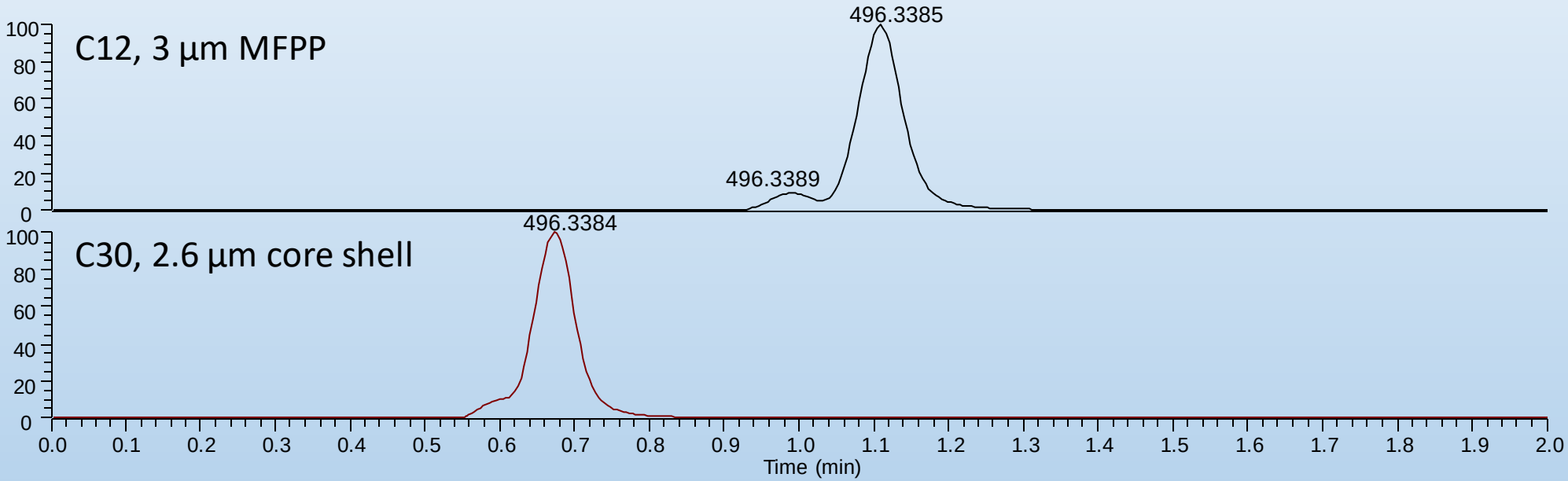
Time	%B
1 min	20
3 min	30
4 min	45
6 min	60
8 min	65
10 min	65
15 min	90
17 min	98
18 min	98

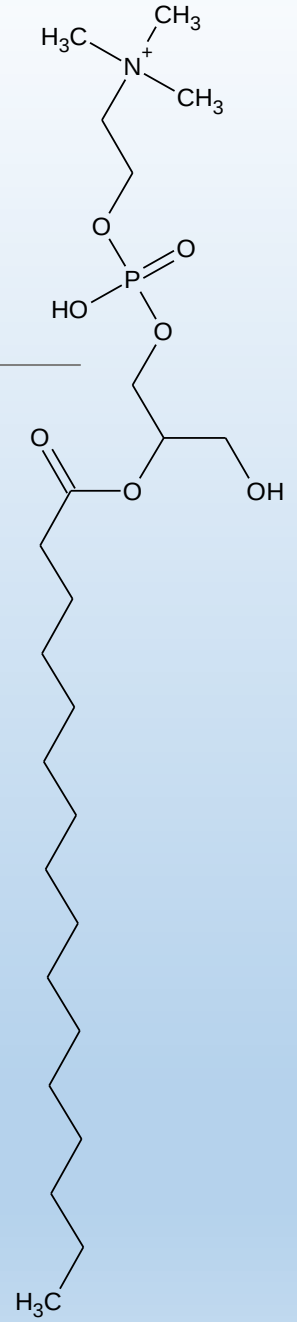
Dried plasma spot, Folch Extraction



Overall the C30 shows less retention for lipids than the C12, but lets look at some examples

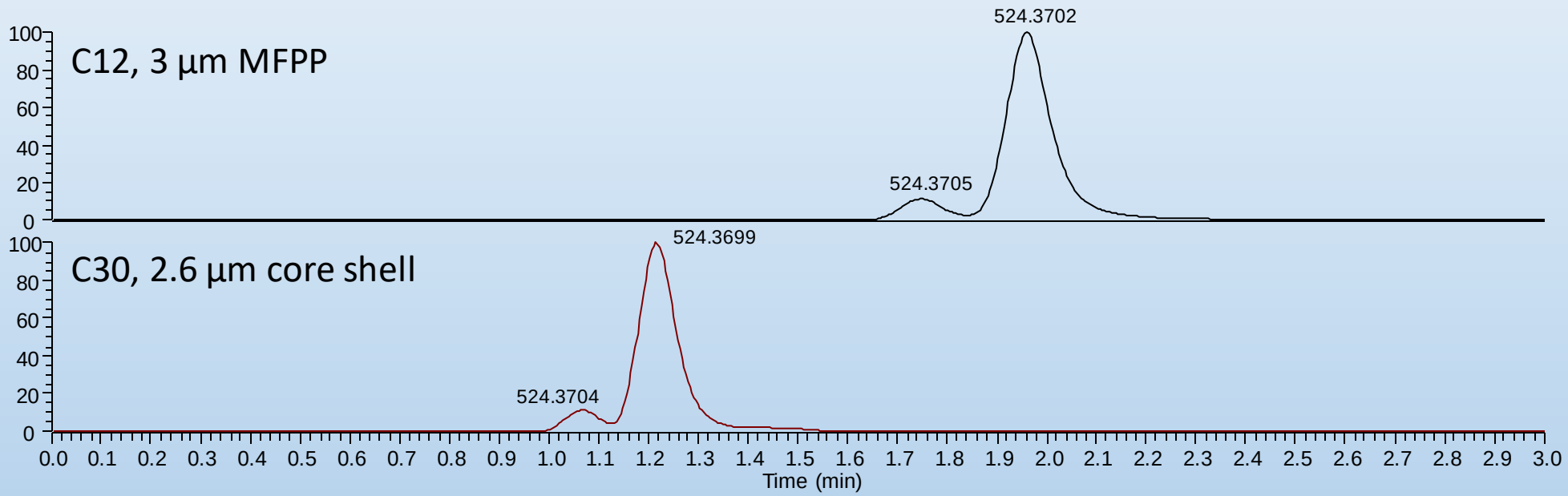
LysoPC 16:0, XIC for 496.3387





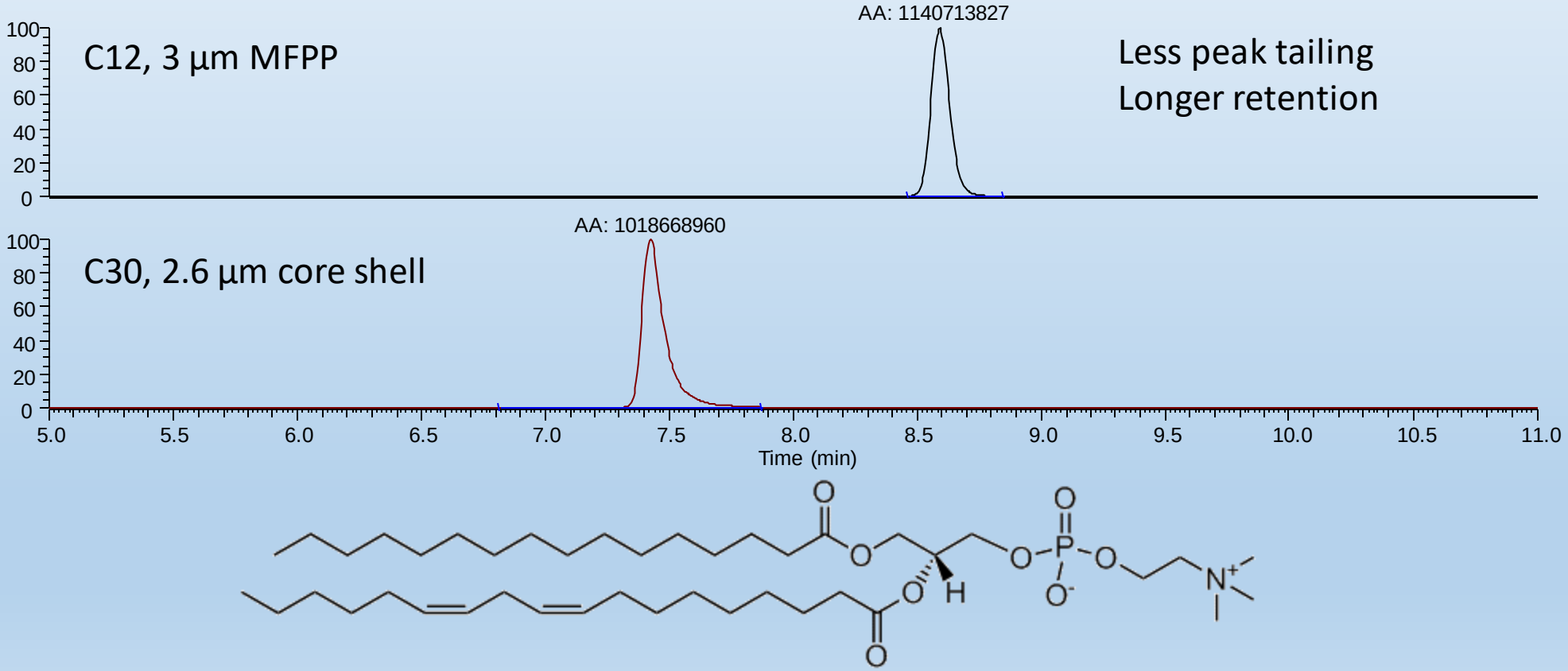
- LysoPC has 2 isomers (sn-1 and sn-2) and thus 2 peaks are expected
- Note the C12 3 µm MFPP column has better separation of the sn-1 and sn-2 isomers under the same conditions

LysoPC 18:0, XIC for 524.3702



Longer retained lysoPC shows similar results, improved separation with C12 MFPP

PC 34:2, XIC for 758.5700

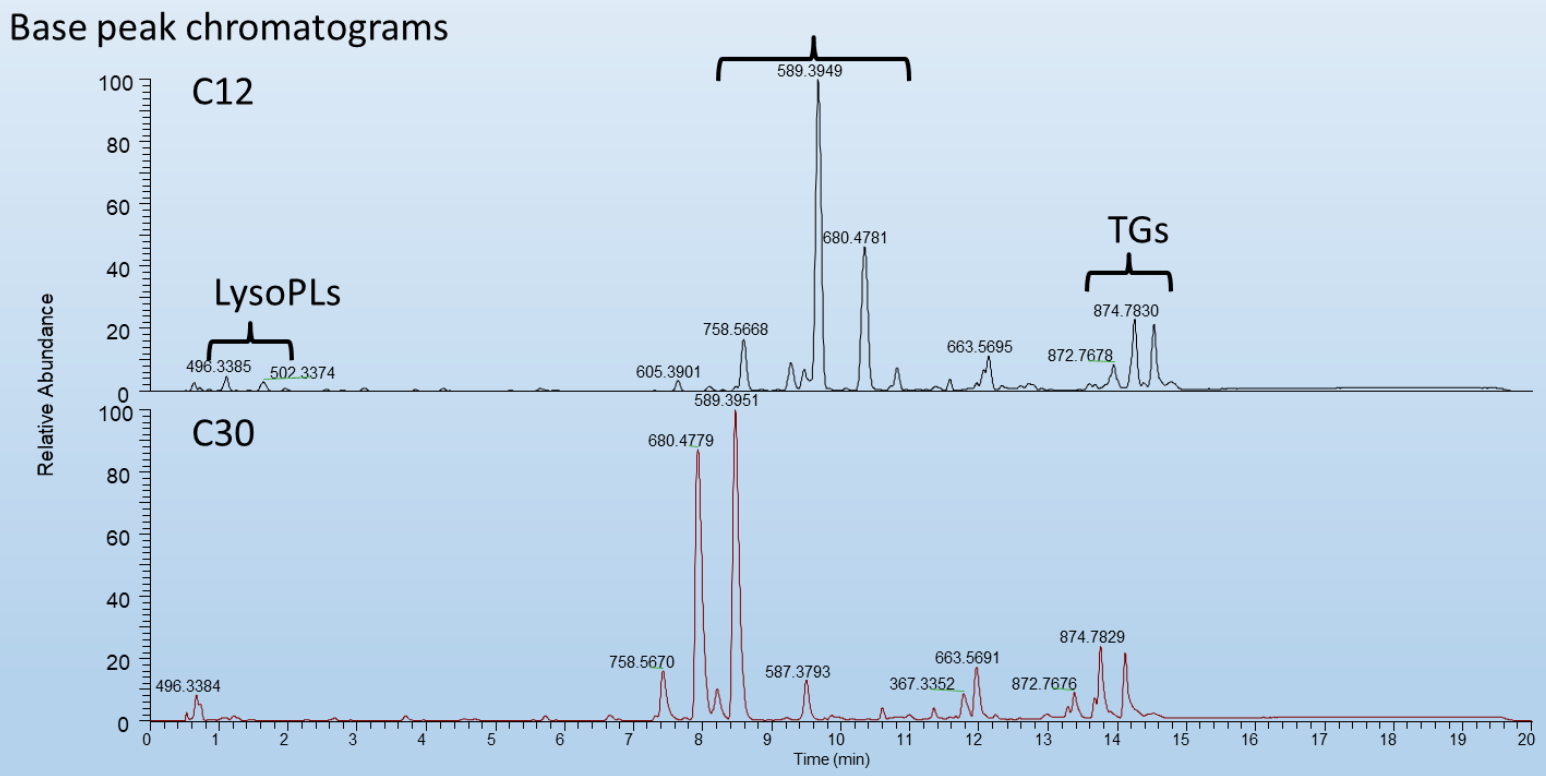


C12 vs C30 lipid separation

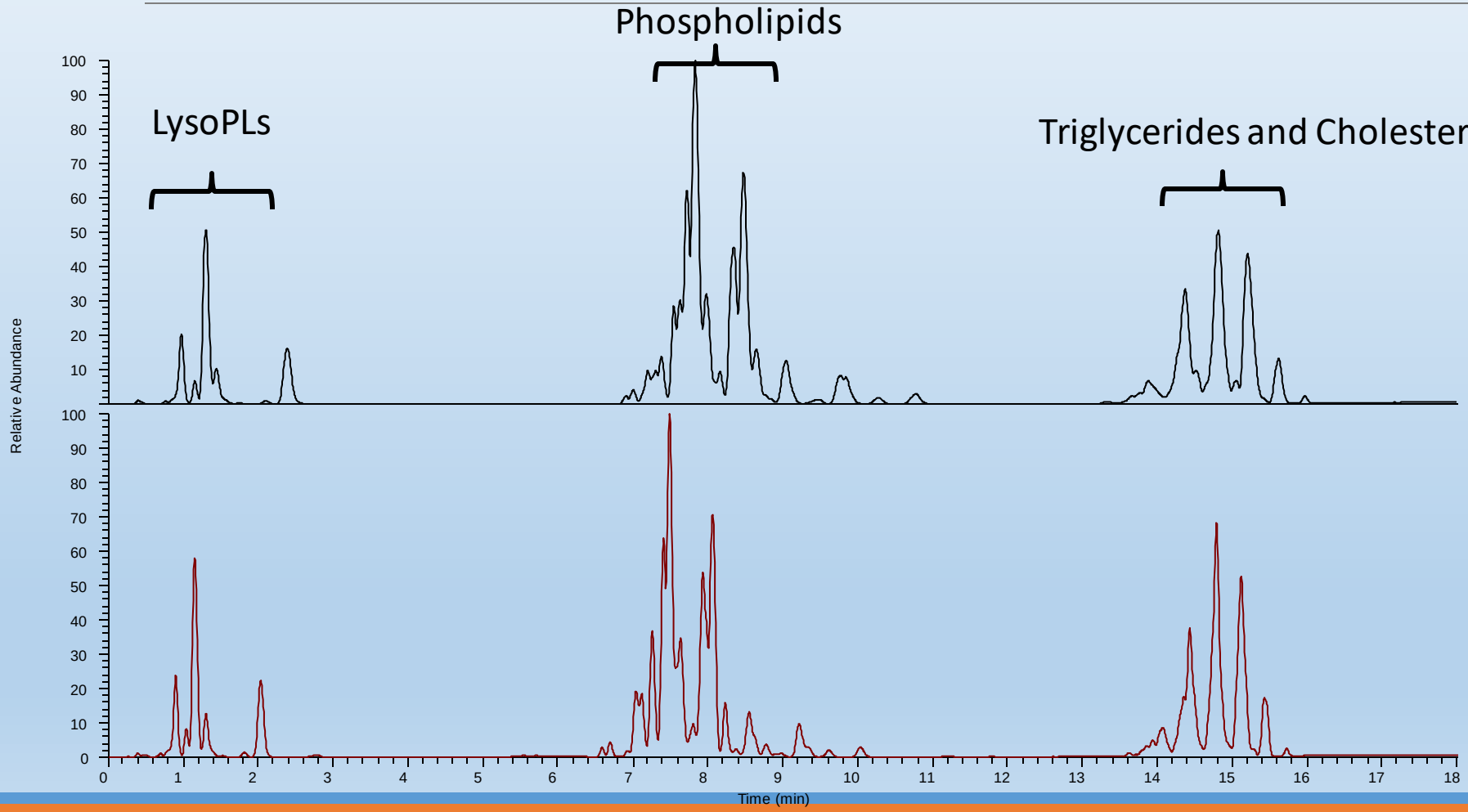
Longer retention was observed using a C12 MFPP 3 um vs a C30 core shell 2.6 um

Reduced peak tailing

Let's look at some other columns



Plasma, Folch extraction

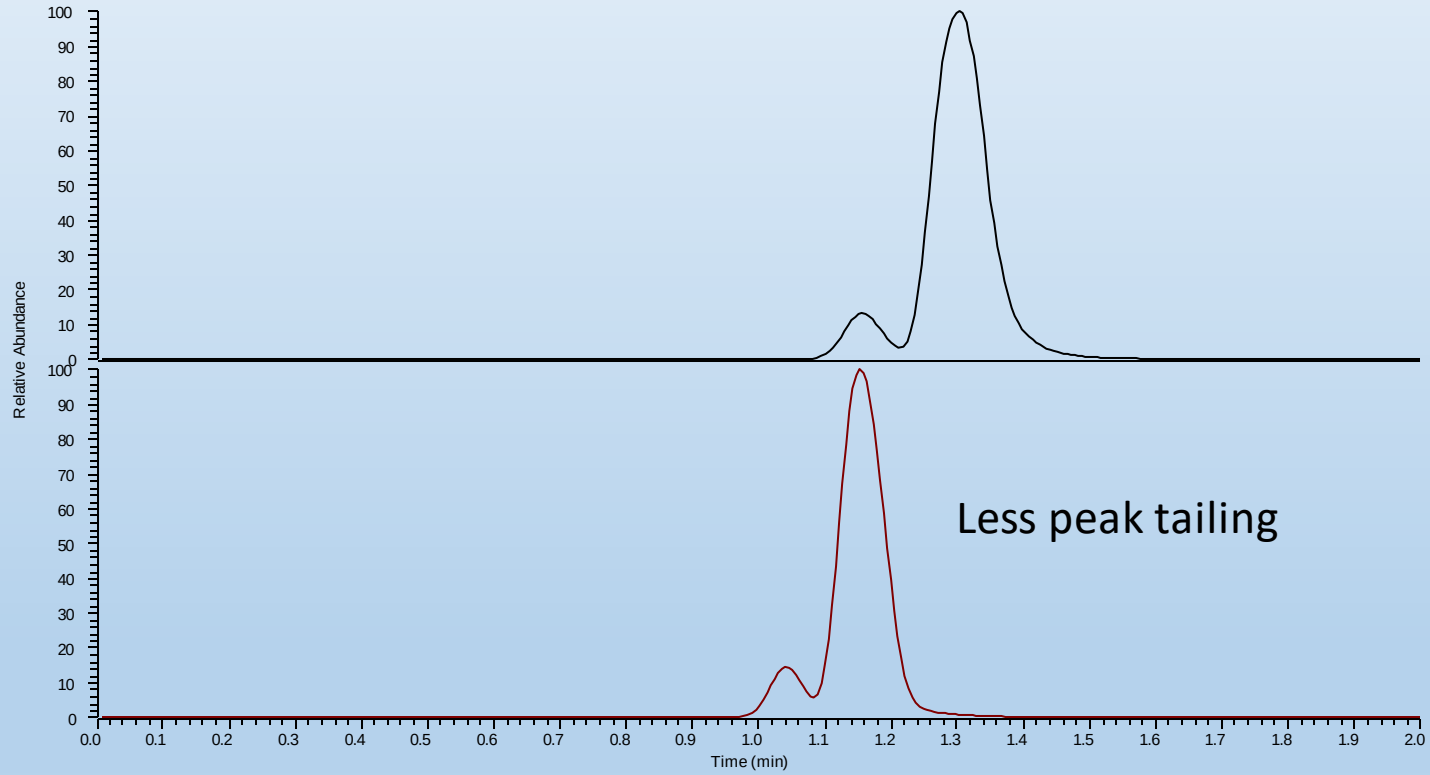


C18 50 x 2.1mm, 1.7 µm

C12 MFPP 50 x 2.1mm, 3.0 µm

C12 and C18 show very similar retention

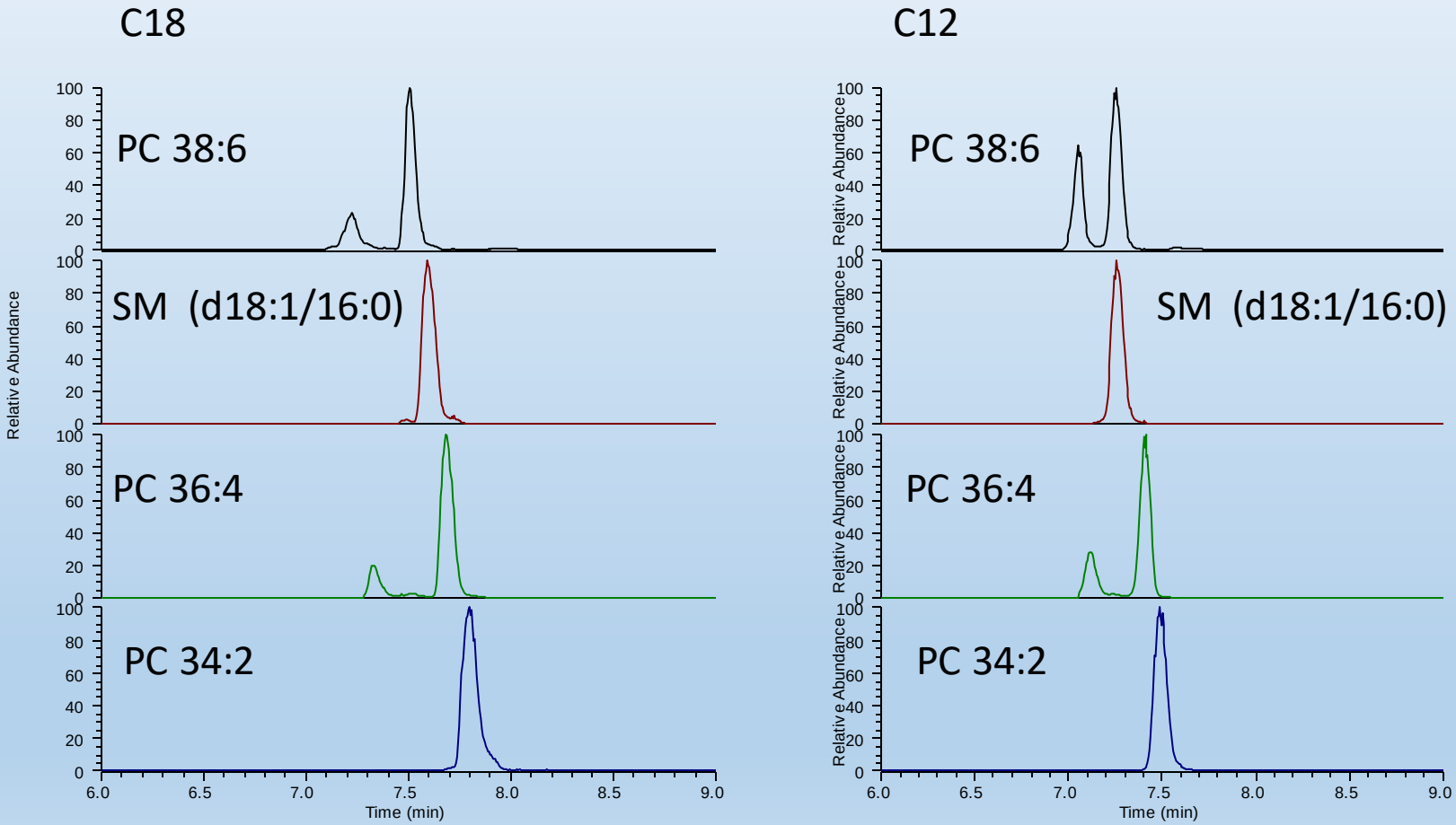
LysoPC 16:0, XIC for 496.3387



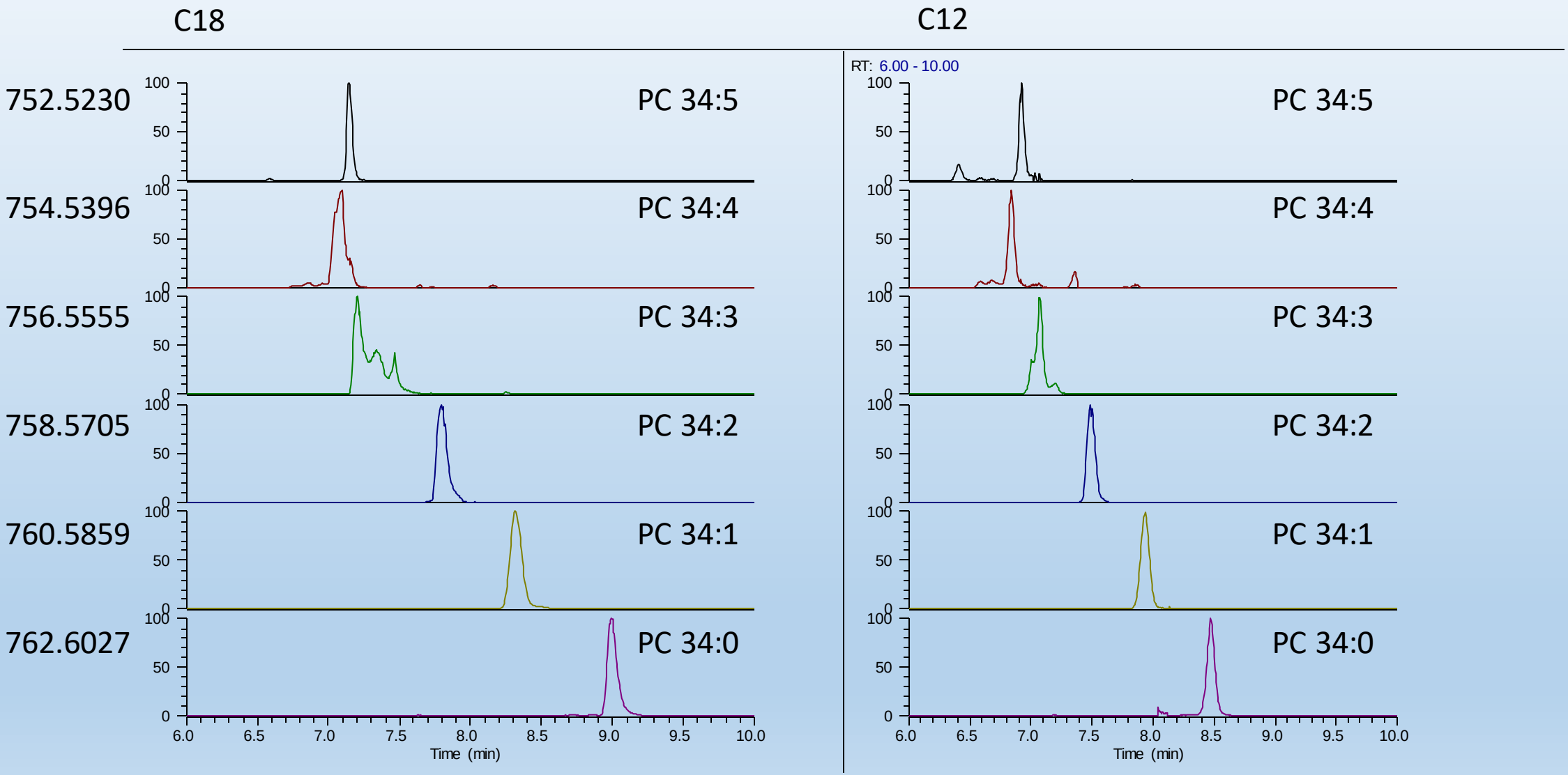
C18 50 x 2.1mm, 1.7 µm

C12 MFPP 50 x 2.1mm, 3.0 µm

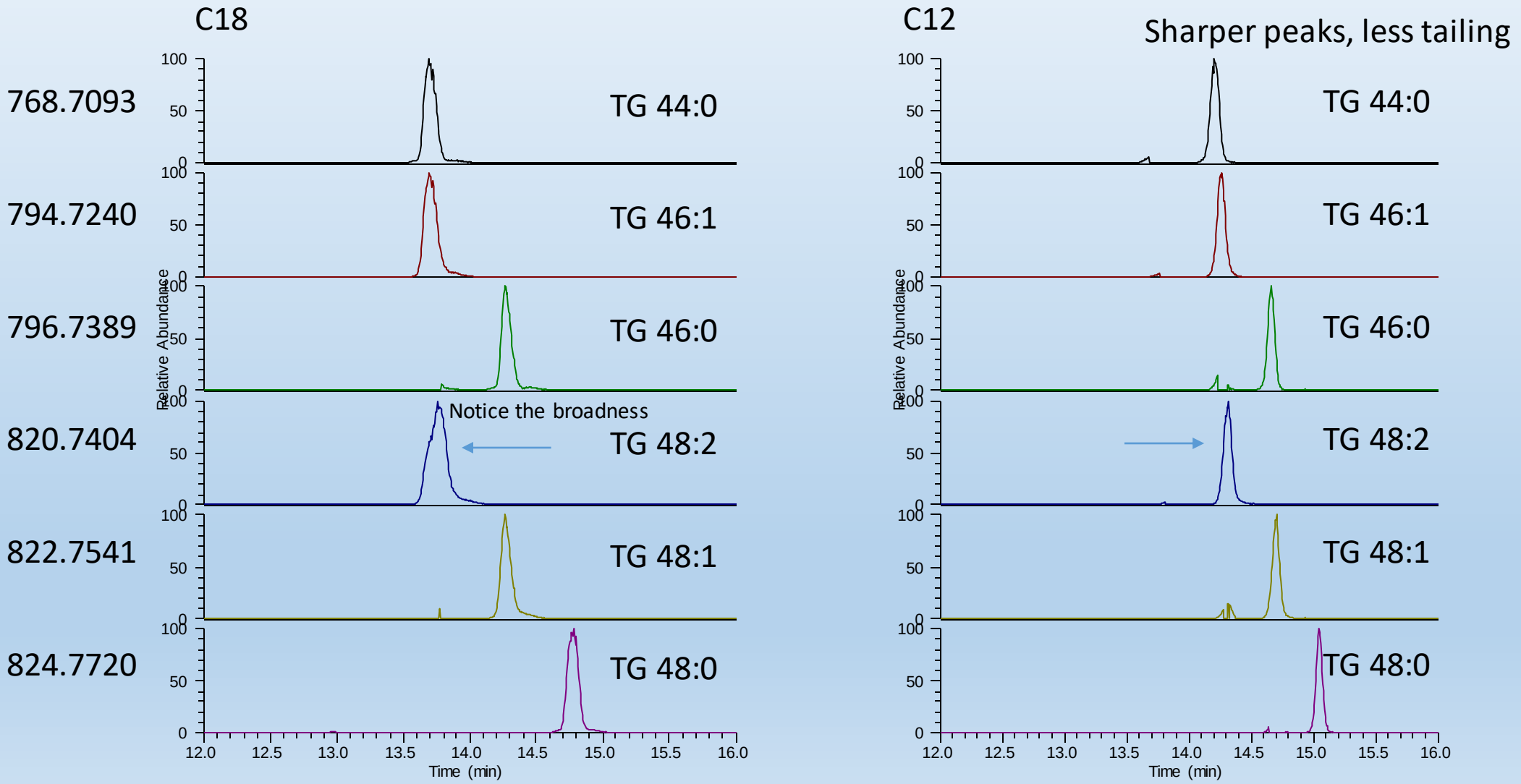
Phospholipids



Phosphatidylcholines (PC), different degrees of unsaturation



Triglycerides 44-48 total carbons



One challenge in biomarker discovery

Validation of a biomarker takes many samples

One of the failures of profiling techniques is the lack of large diverse sample sets to build reliable AI approaches

Long methods limit the ability to run large sample batches

500 samples at 23 min injection to injection is 8 days of instrument time (x2 because of positive and negative ionization=**16** total days)

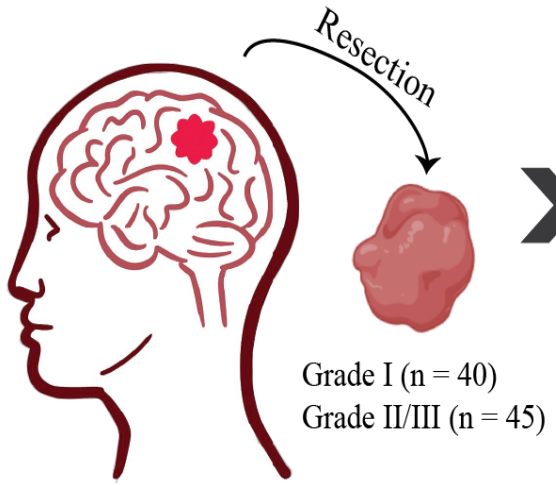
500 samples at 5 min is less than 2 days (**4** total days)

Could we use monodisperse columns to develop a rapid lipid method without sacrificing separation?

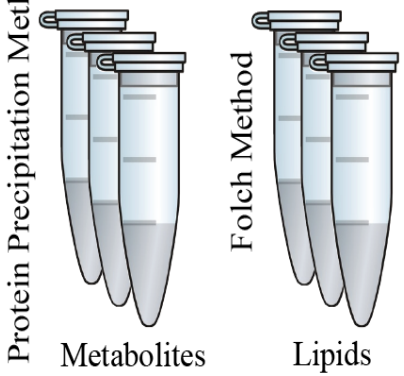
Example of a larger Study Design and Workflow

1. Experimental and Laboratory Analysis

Meningioma Tissue Collection

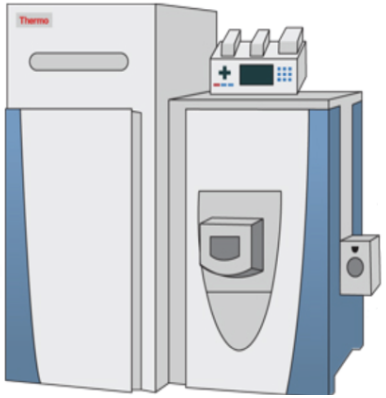


Sample Preparation

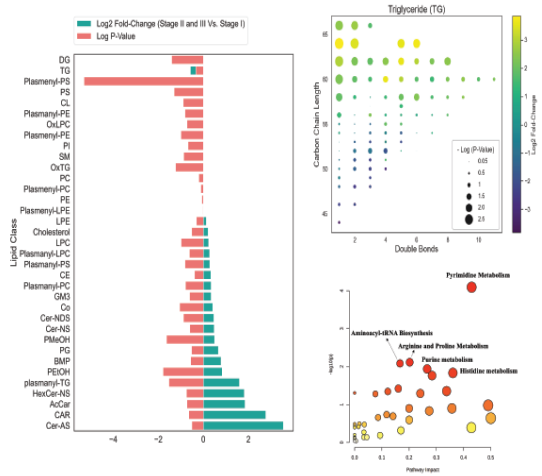


UHPLC-HRMS Analysis

Untargeted Metabolomics
Untargeted Lipidomics



Statistical and Pathway Analysis

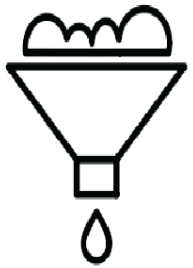


2. Artificial Intelligence and Statistical Analysis

Data Pre-Processing & Cleaning

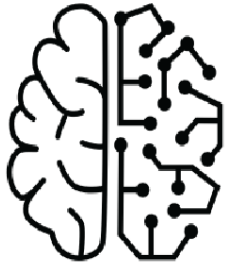
- Normalizing
- Transforming
- Scaling

Feature Selection & Dimension Reduction



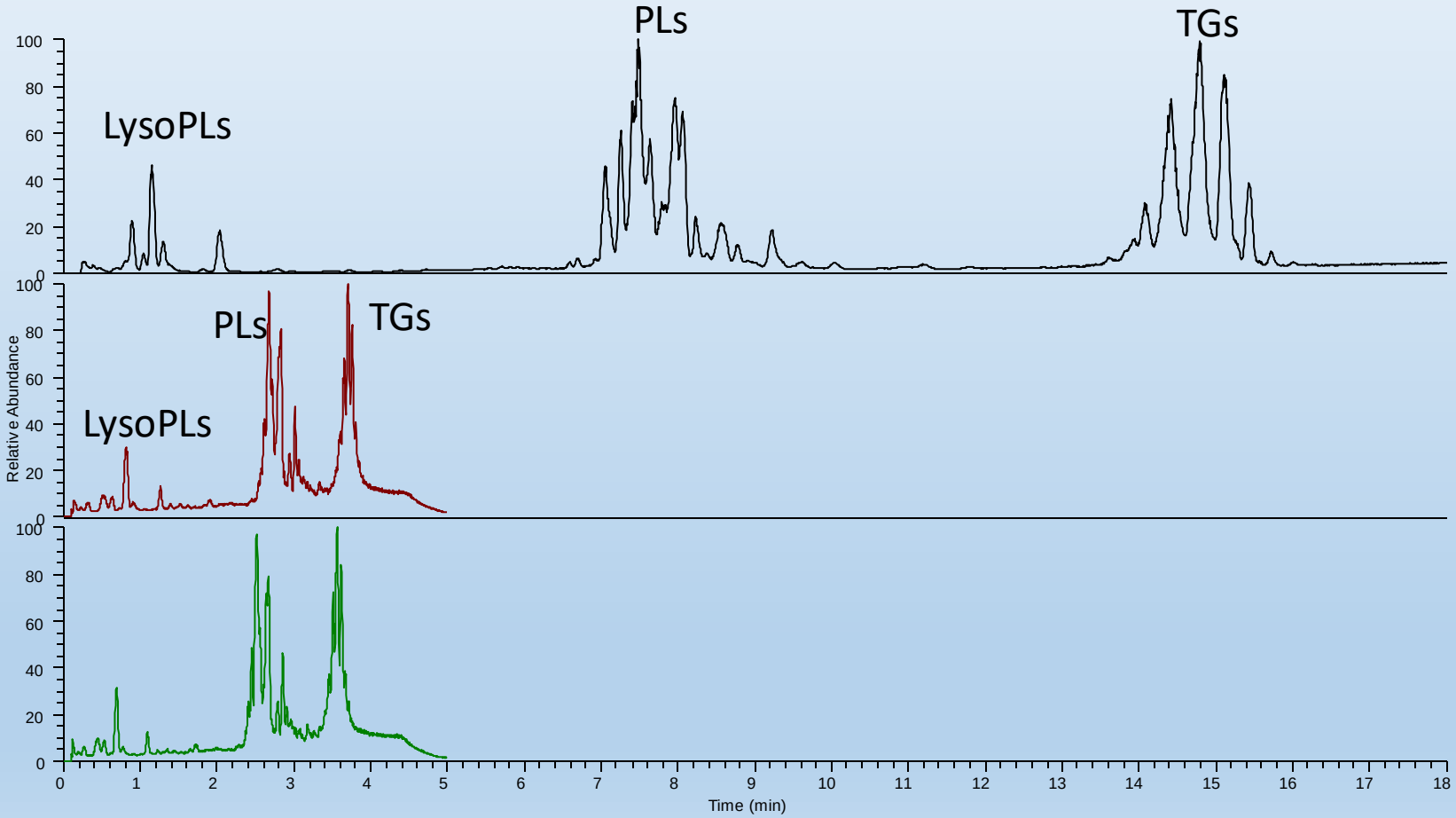
- Python's Scikit-Learn
- ExtraTreesClassifier
- Top 50 Features

Machine Learning & Model Training



- Distinguishing Important Metabolites
- Identifying Potential Biomarkers
- Meningioma Grade Classifying

Faster methods



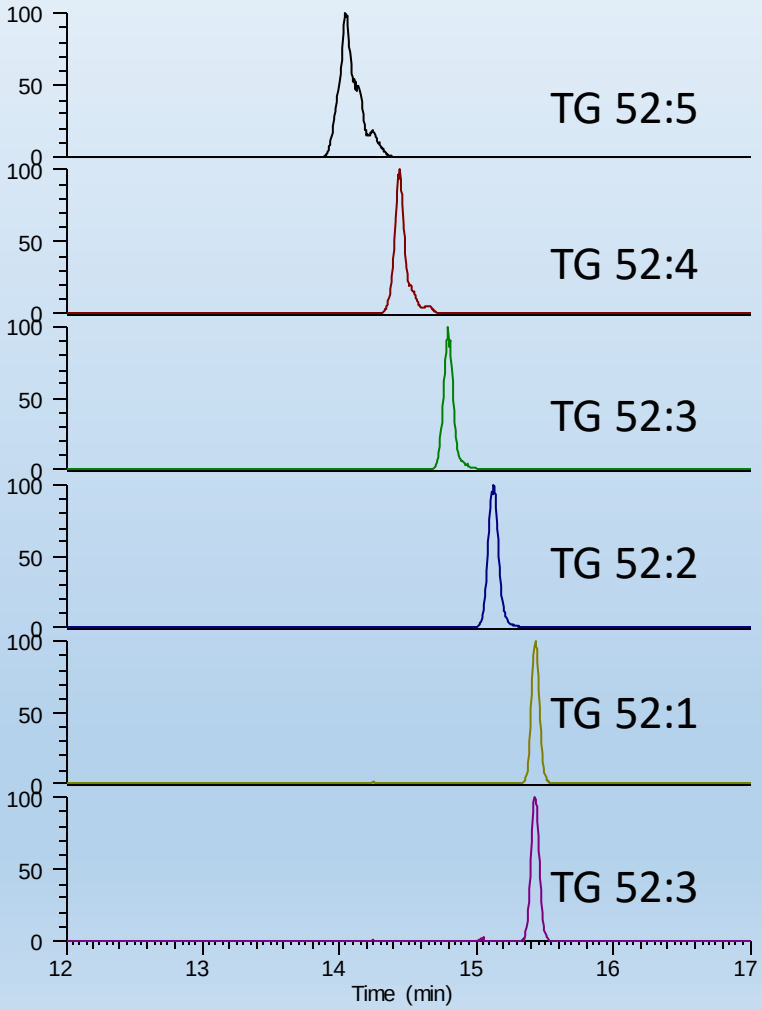
C12 50 mm, 3 um
22 min method
500 uL/min

C12 30 mm, 1.7 um
5 min method
500 uL/min

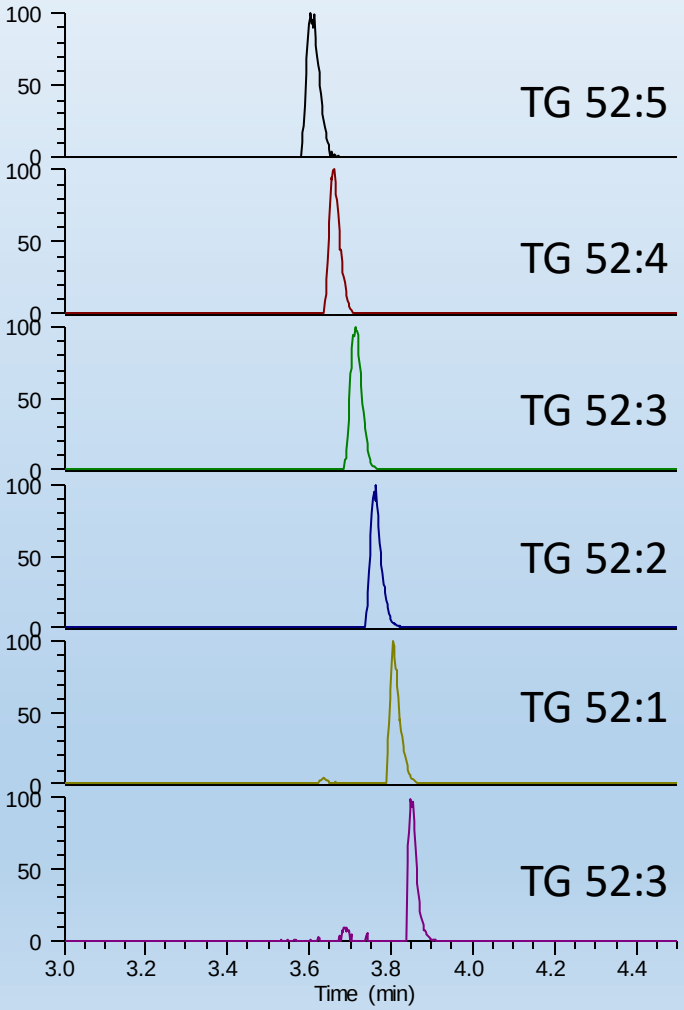
C12 30 mm, 1.7 um
5 min method
600 uL/min

TG with 52 total carbons

Evosphere C12-50mm, 22min

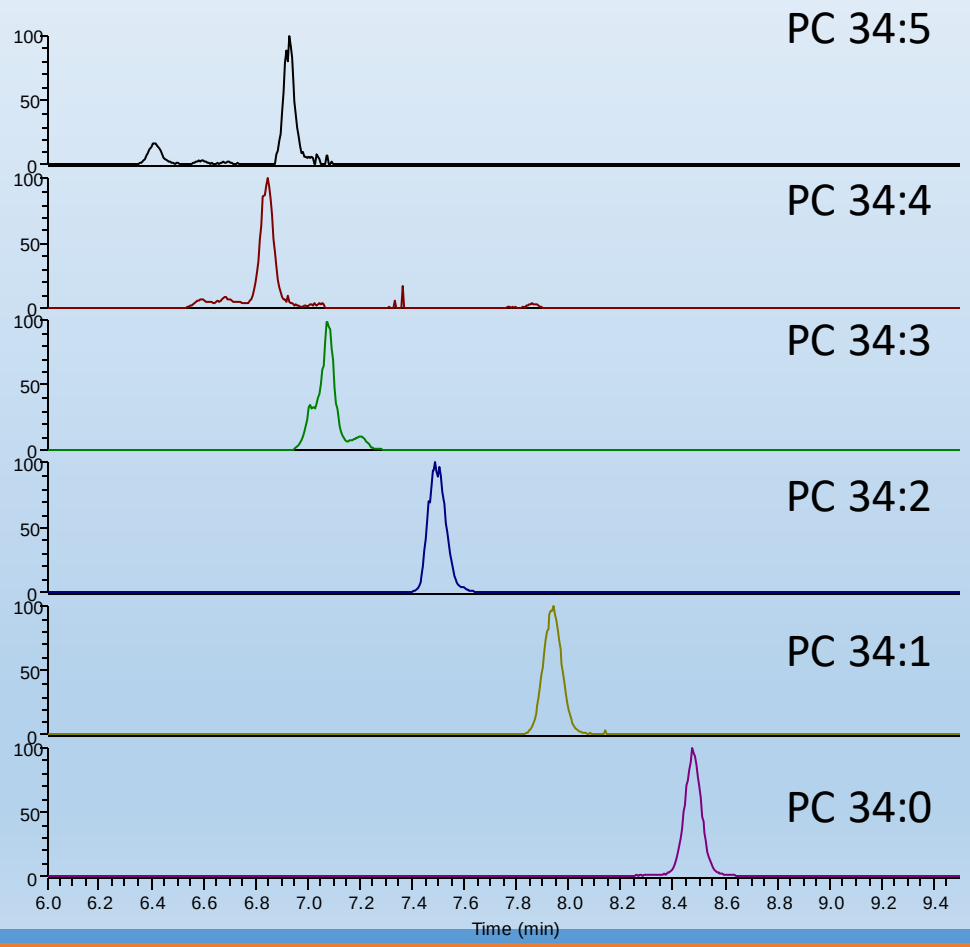


Evosphere C12-30mm, 5 min

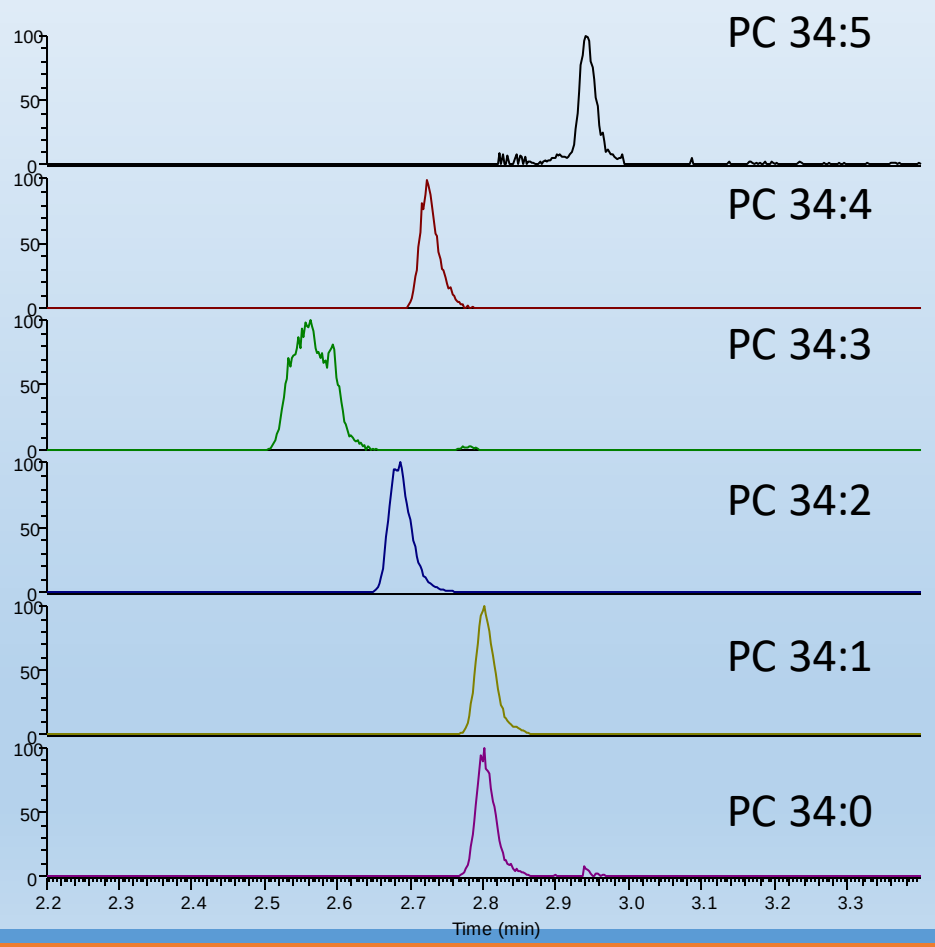


Phosphatidylcholines with different degrees of unsaturation

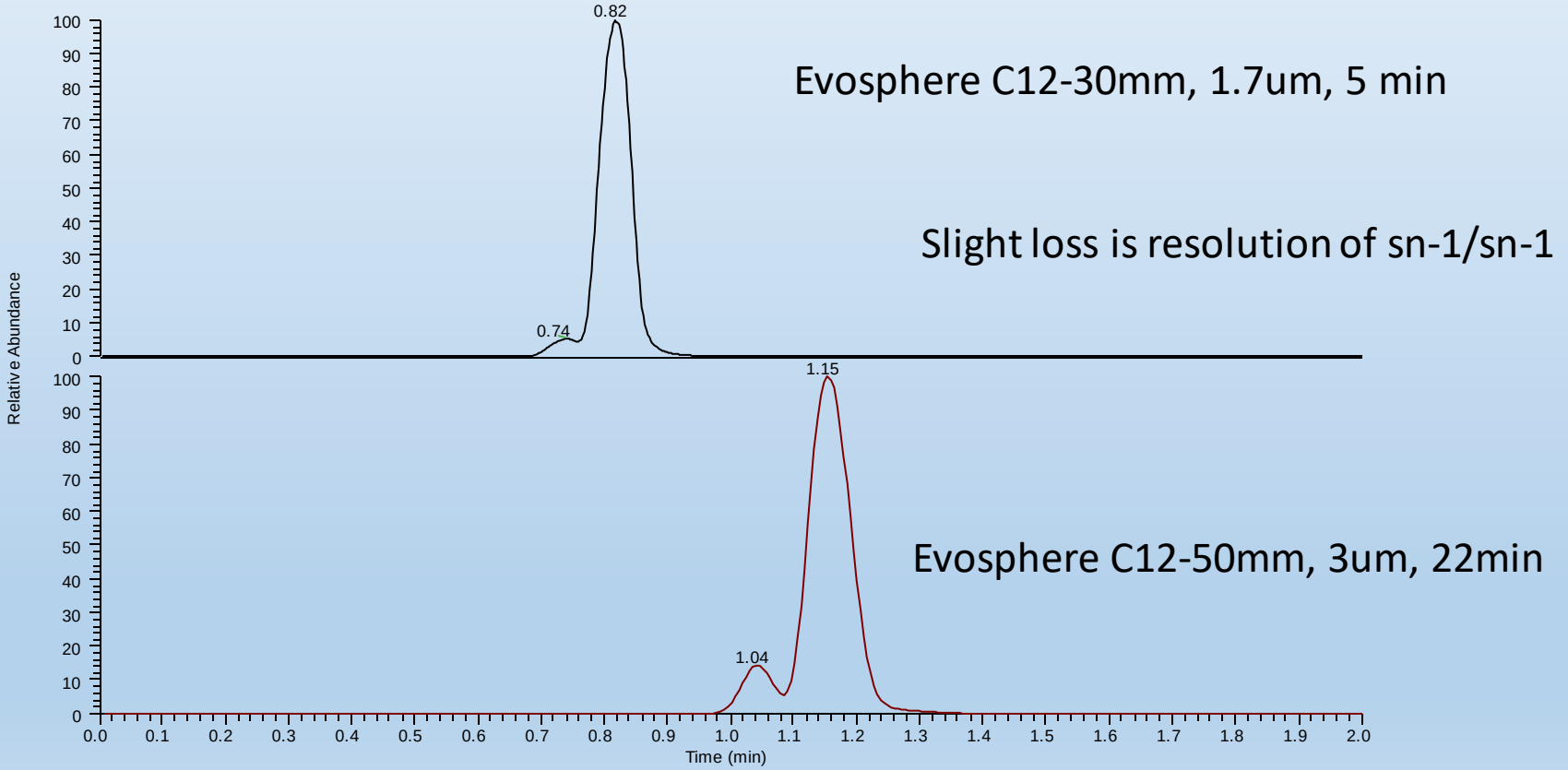
Evosphere C12-50mm, 22min



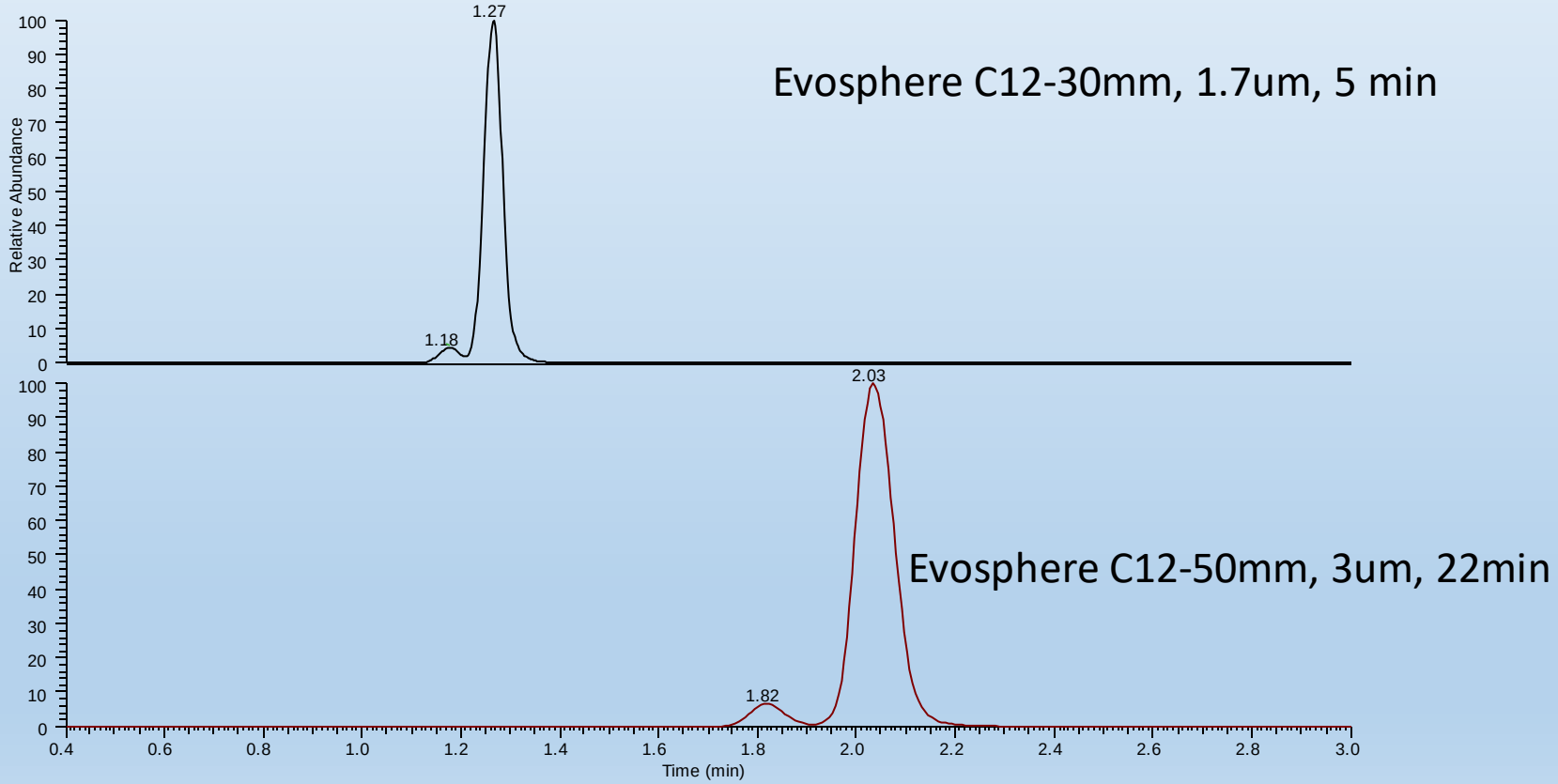
Evosphere C12-30mm, 5 min



LysoPC 16:0


0.82
0.74
1.15
1.04
Relative Abundance
Time (min)
Evosphere C12-30mm, 1.7um, 5 min
Slight loss is resolution of sn-1/sn-1
Evosphere C12-50mm, 3um, 22min

LysoPC 18:0


1.27
1.18
Evosphere C12-30mm, 1.7um, 5 min
2.03
1.82
Evosphere C12-50mm, 3um, 22min
Relative Abundance
Time (min)
100
90
80
70
60
50
40
30
20
10
0
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0

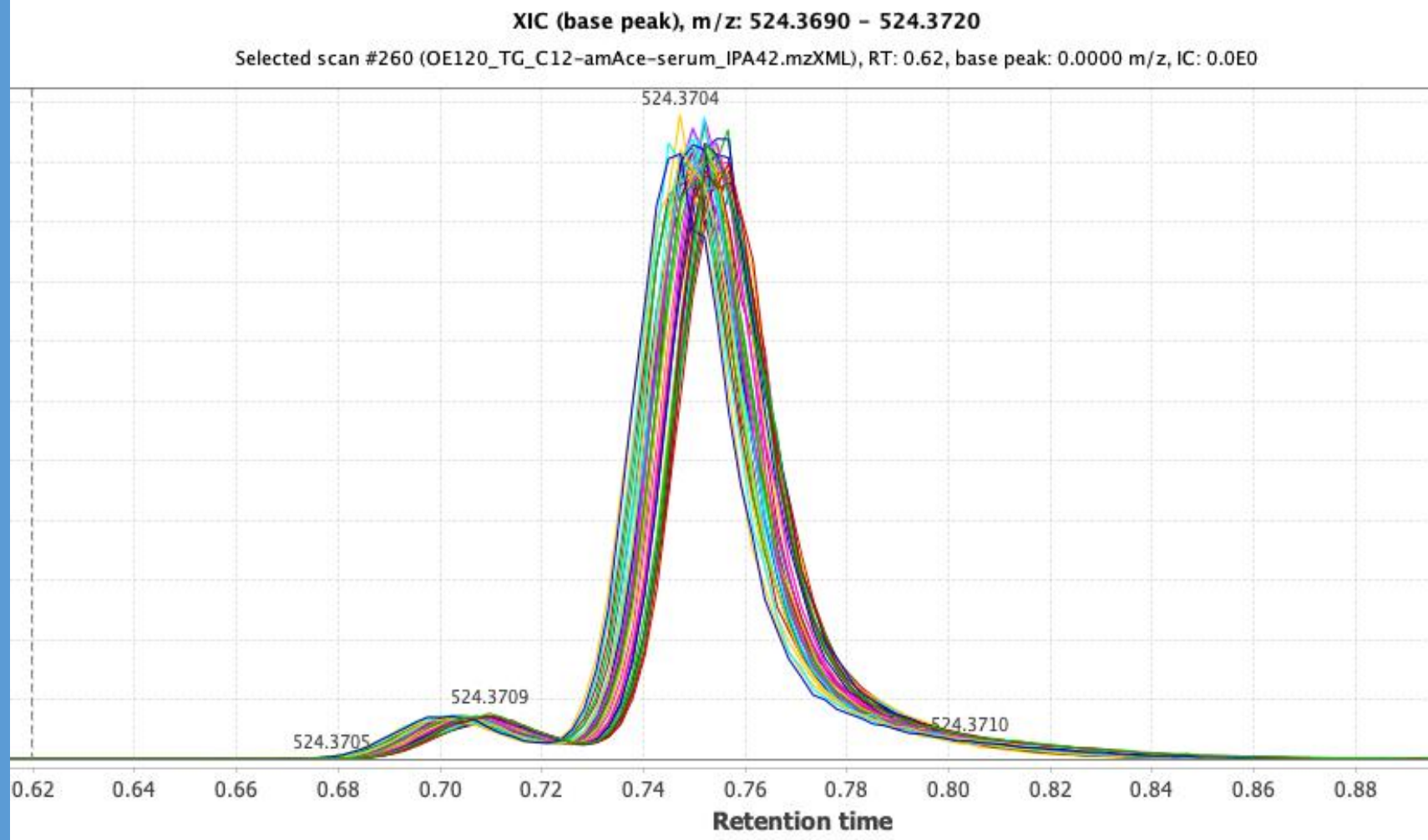
LysoPC 18:0

40 injections

5 min injection to injection

30 x 2.1mm, 1.7 um

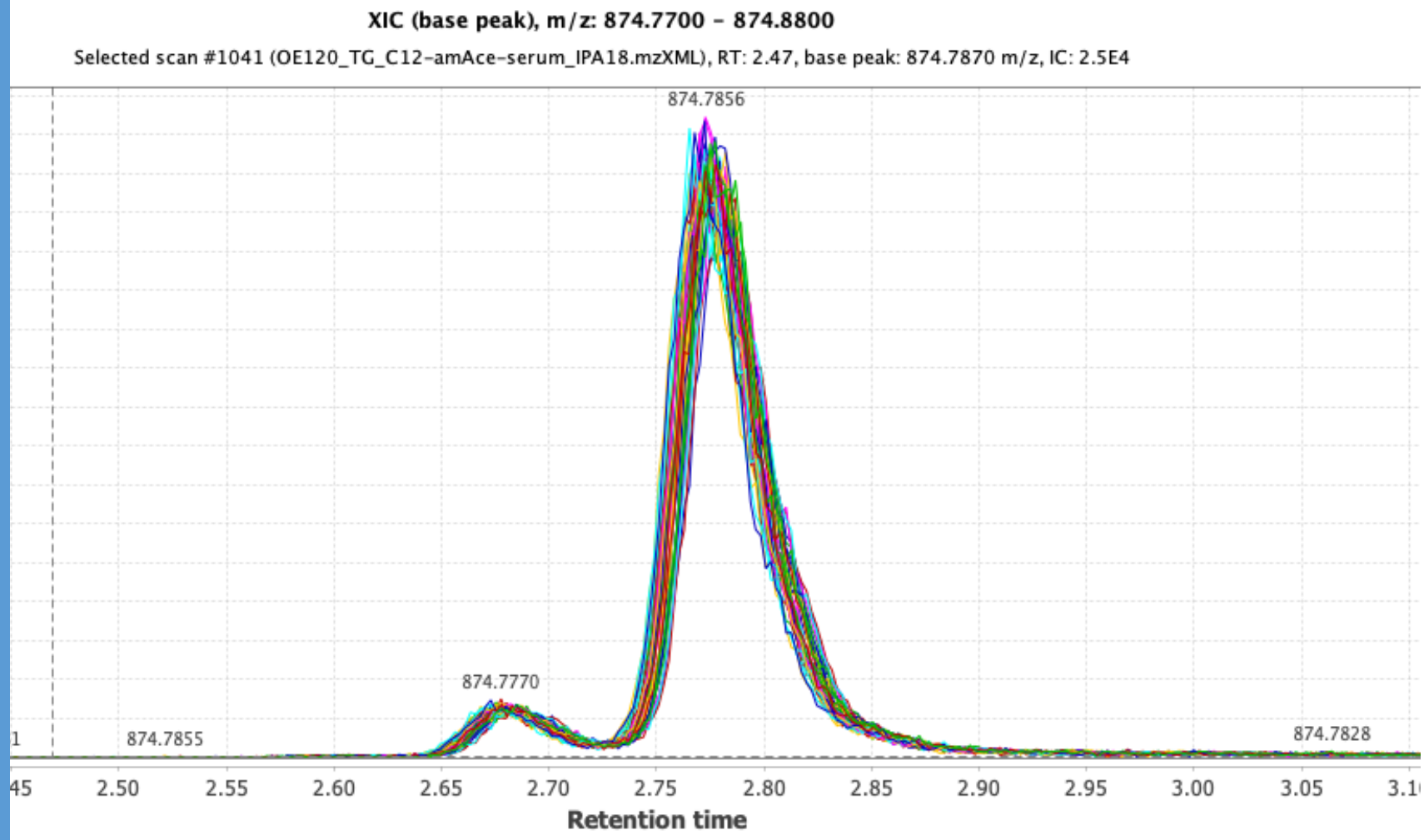
Excellent reproduciblity



TG 52:3

Retention time reproducibility is excellent at early and late retention times

Simple IPA extraction



Summary

Biomarker discovery is important for precision healthcare/diagnostics

Improvement in speed and performance of separations will lead to methods that are reproducible, reliable and efficient

Large-scale studies are necessary to build AI methods that are predictive

5 min methods for profiling are possible

Acknowledgements

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Jae Hwan Lee
Abdulkarim Alfaez
Michael Christopher
Geoff Faden
Ed Faden

NIH National Institutes of Health

UF PathLabs UNIVERSITY of FLORIDA

macmod SMARTER CHROMATOGRAPHY

TRAORDINARY JOY!

https://garrettlab.pathology.ufl.edu/

Thank you for your attention

Example of integrated ‘Omics

MENINGIOMA STUDY

METABOLOMICS, LIPIDOMICS AND AI

Hoda Safari Yazd, Sina Bazargani, Garrett Fitzpatrick, Richard A Yost, Jesse Kresak, Timothy J Garrett: Mass Spectrometry-Based Metabolomic and Lipidomic Characterization of Meningioma Grades using Machine Learning. *In preparation*

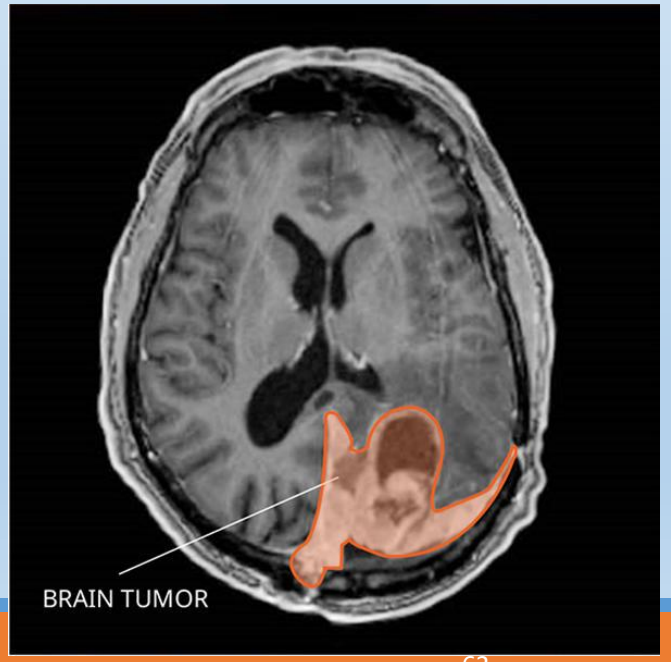
Meningioma Background

A usually noncancerous tumor that arises from the membranes surrounding the brain and spinal cord.

Meningiomas account for 37% of primary brain tumors in the US.

Meningiomas are classified into WHO Grades 1, 2, and 3.

	Grade I	Grade II	Grade III
Frequency	75%	20-35%	1-3%
Treatment	Gross total resection	Gross total resection +/- Radiotherapy	Gross total resection +/- Radiotherapy
Survival	Same as age-matched controls	modest decrease	18-40 months
Recurrence	5 year - 5%	5 year - 40%	Frequent



Purpose

Investigate the metabolomic profile of meningiomas

- Grade classification with 2 and 3 is currently difficult
- Compare low-grade and high-grade meningiomas
- Find new biomarkers capable of differentiating different stages of meningioma's
- Identify potential metabolites which may correlate with disease free and overall survival

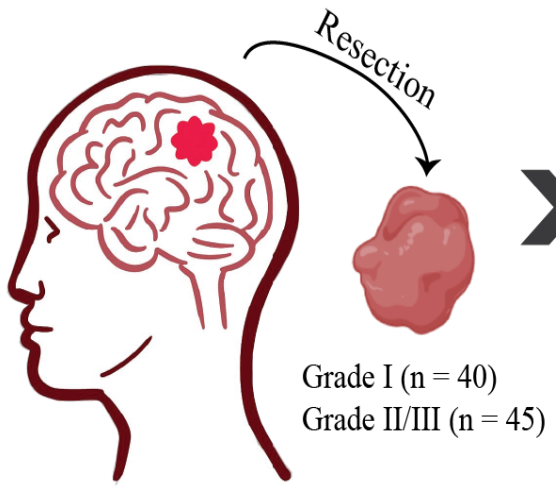
Overarching goal to use metabolomic data to identify biomarkers for disease diagnostics

Collaboration with anatomical pathology (Jesse Kresak, MD)

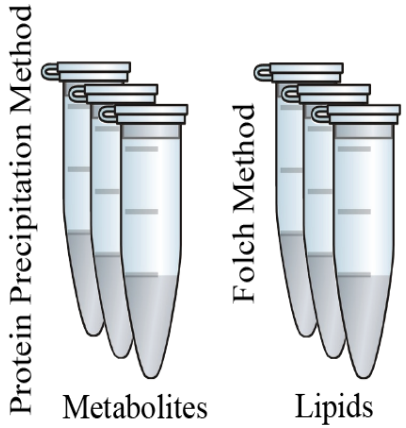
Study Design and Workflow

1. Experimental and Laboratory Analysis

Meningioma Tissue Collection

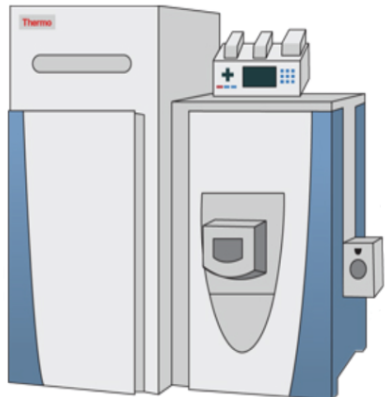


Sample Preparation

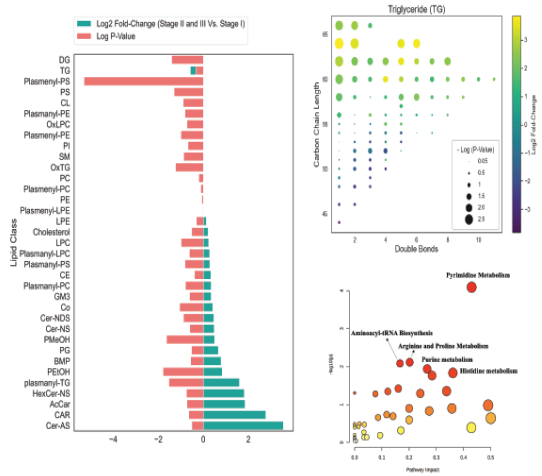


UHPLC-HRMS Analysis

Untargeted Metabolomics
Untargeted Lipidomics



Statistical and Pathway Analysis



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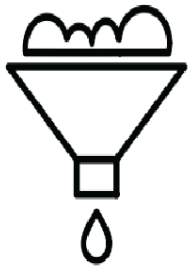
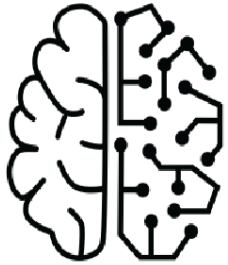
- Transforming
- Scaling

Feature Selection & Dimension Reduction



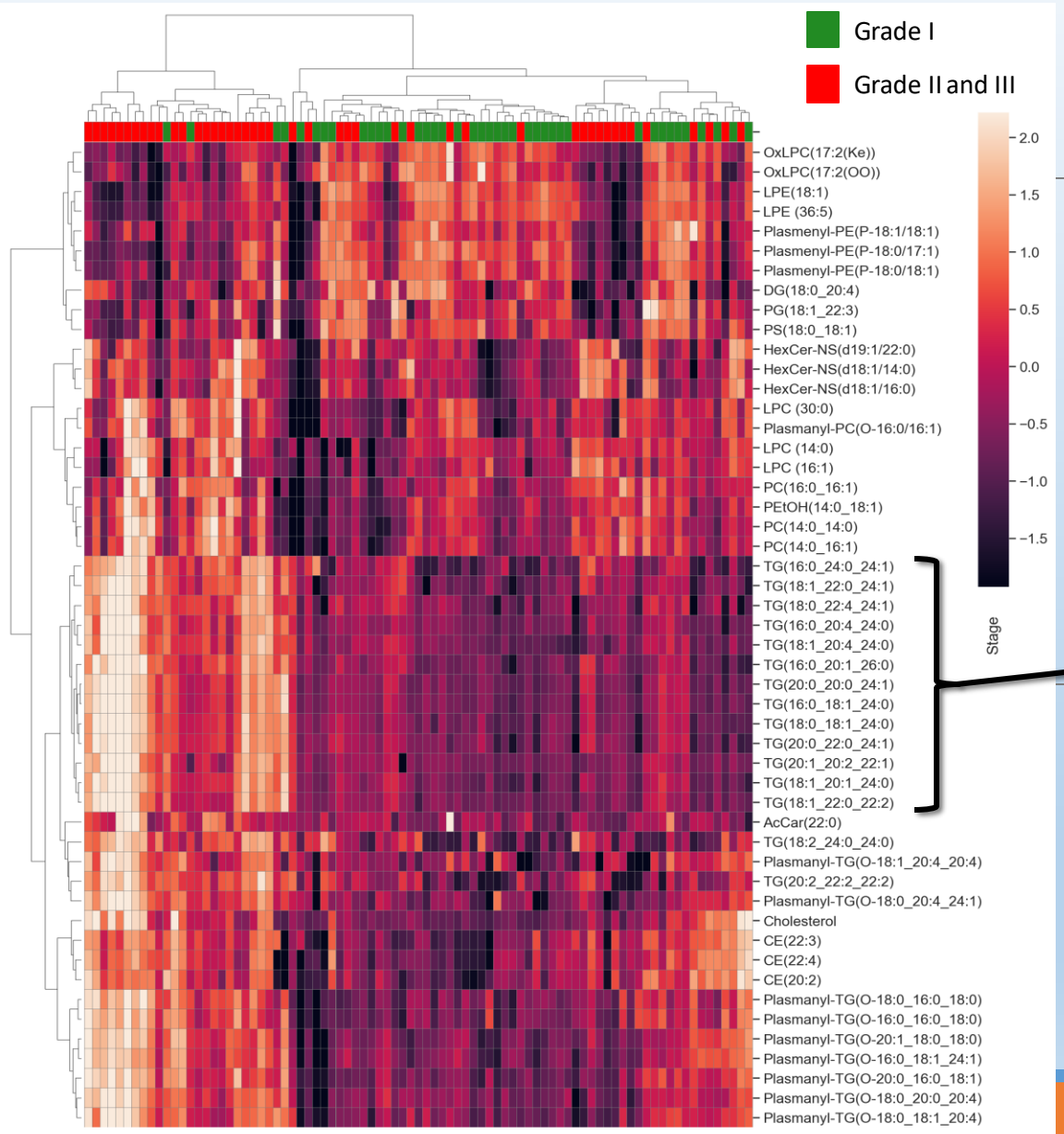
- Python's Scikit-Learn
- ExtraTreesClassifier
- Top 50 Features

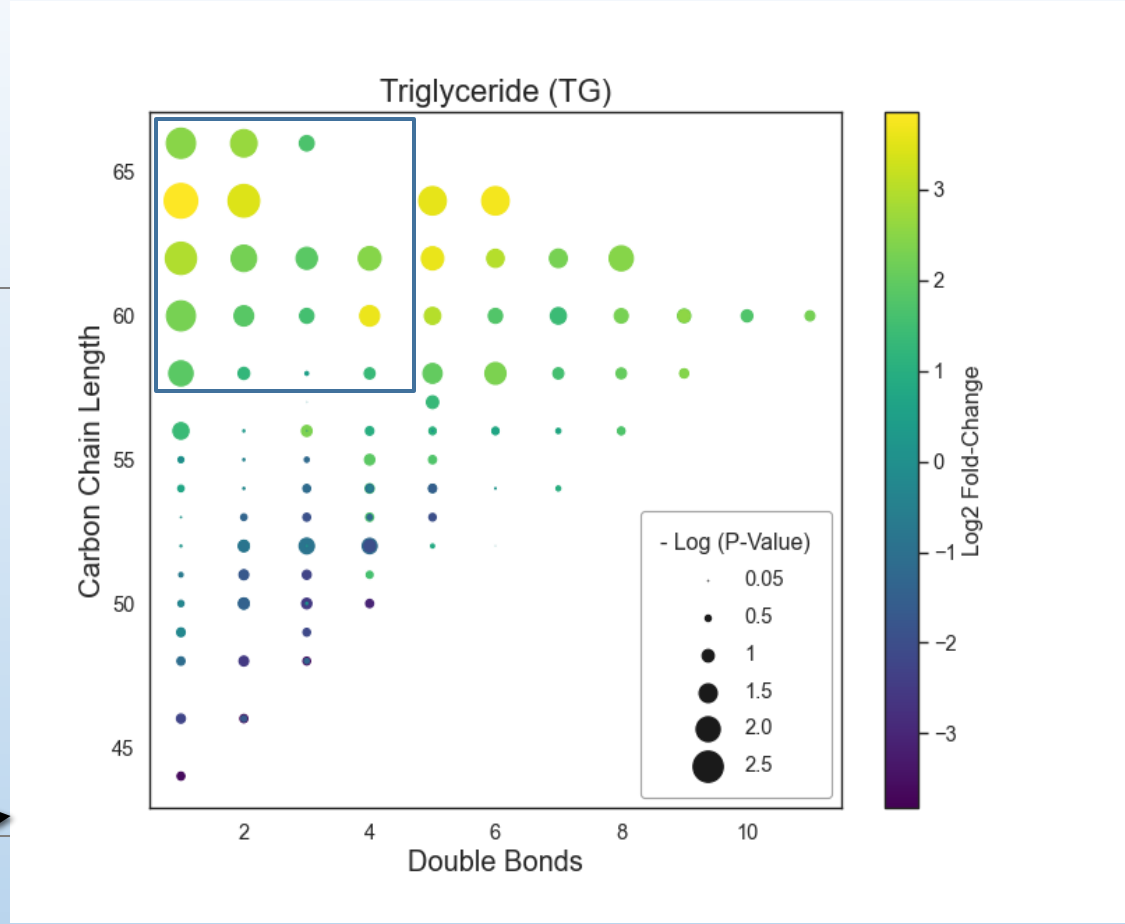
Machine Learning & Model Training



- Distinguishing Important Metabolites
- Identifying Potential Biomarkers
- Meningioma Grade Classifying

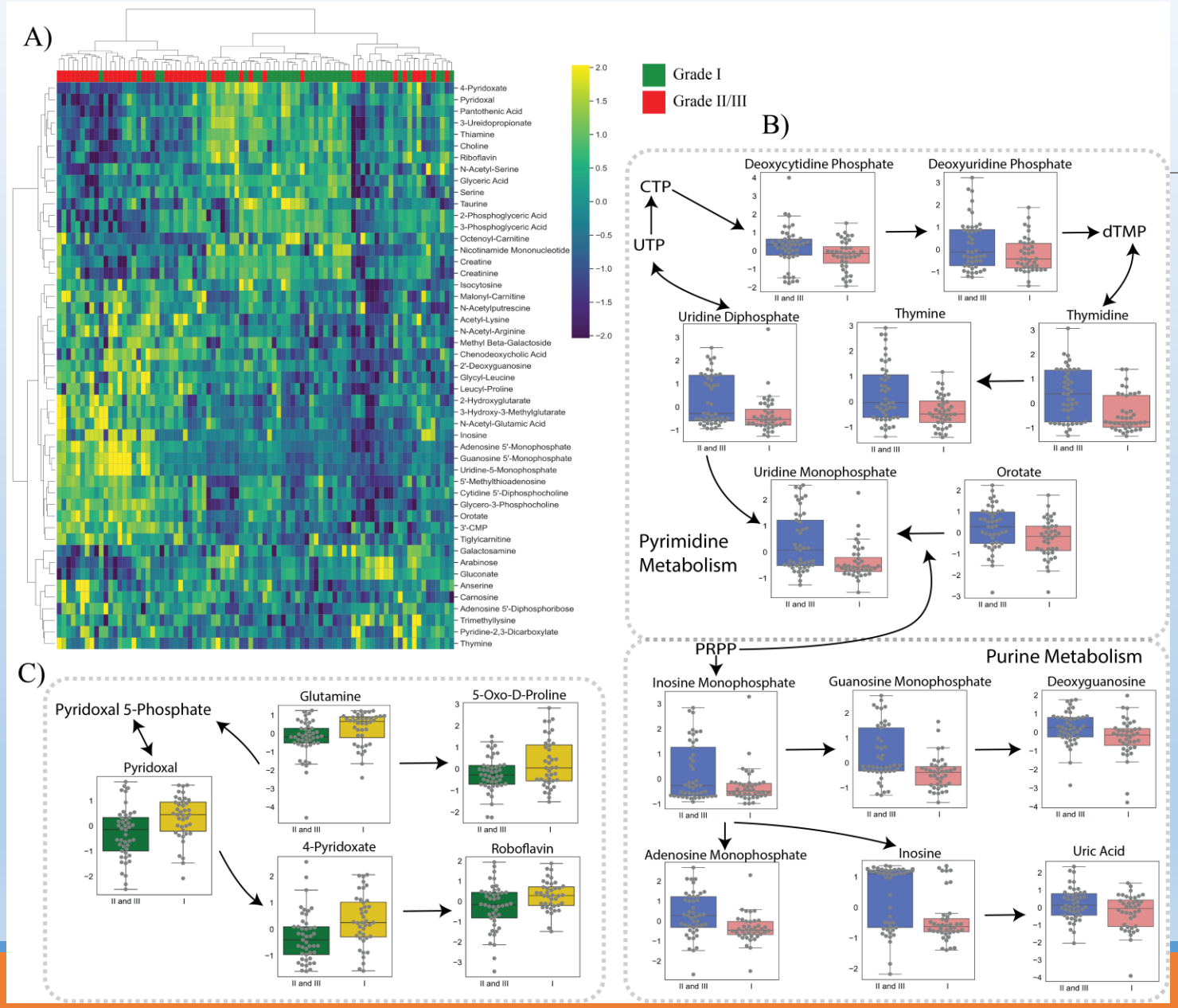
Lipidomics





- Key Results:
  - TG Levels **higher** in grade 2 and 3
  - **Higher** levels of long chain TGs in 2/3
- Cancer cells require lipids for growth
  - Obtain fatty acids from lipogenesis

Metabolomics



Pathways implicated

Key Results:

- Pyrimidine and Purine metabolism is <u>upregulated</u> in grade II/III
- Vitamin B6 metabolism is <u>downregulated</u> in grade II/III

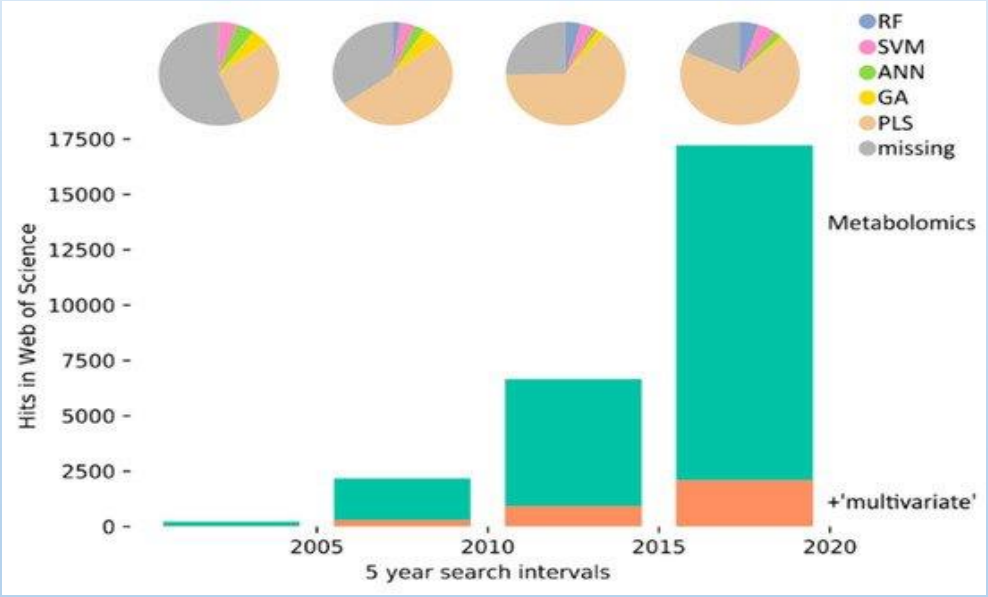
Multi-Omics Data Analysis

MS Metabolomics data analysis is complicated:

- Large data environment
- Nonlinear data
- Heterogeneous data

Machine learning methods applied to MS-based multi-omics ease data analysis and can support clinical decisions, guide metabolic engineering, and stimulate fundamental biological discoveries

The integration of multiple omics levels will enhance our understanding of the interactions among the different biological layers



History of machine learning (ML) in metabolomics

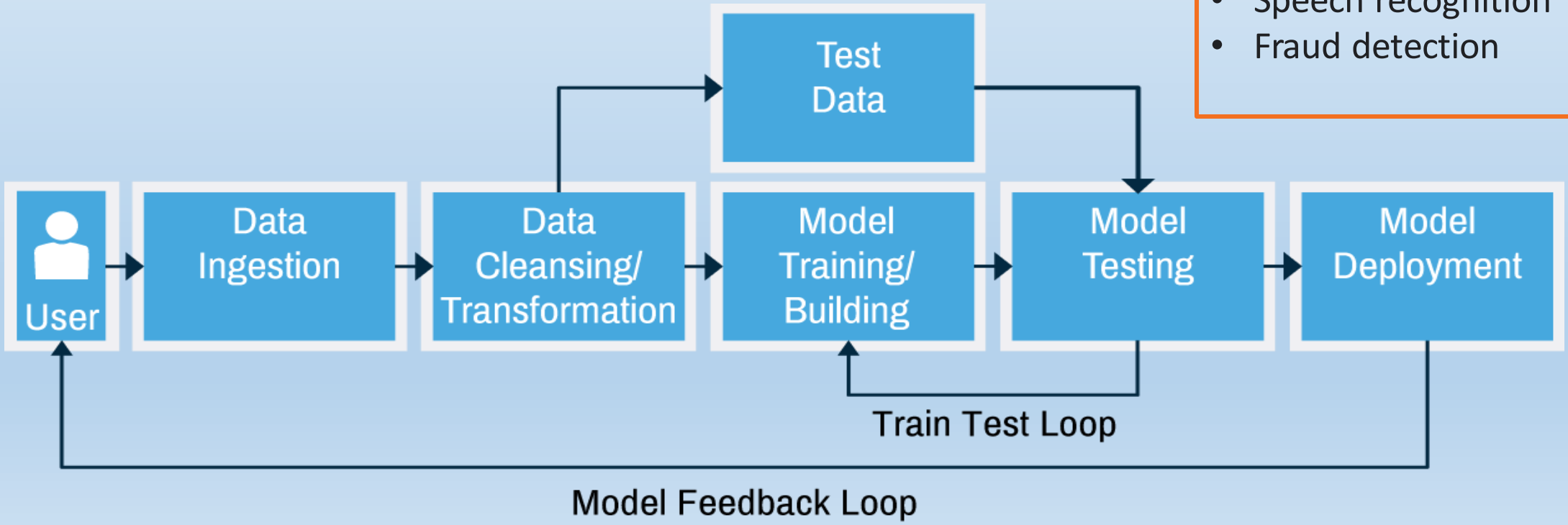
Liebal, U. W.; Phan, A. N. T.; Sudhakar, M.; Raman, K.; Blank, L. M., Machine Learning Applications for Mass Spectrometry-Based Metabolomics. *Metabolites* **2020,** *10* (6).

Machine Learning

Machine learning is the process of teaching a computer system how to make accurate predictions when fed data.

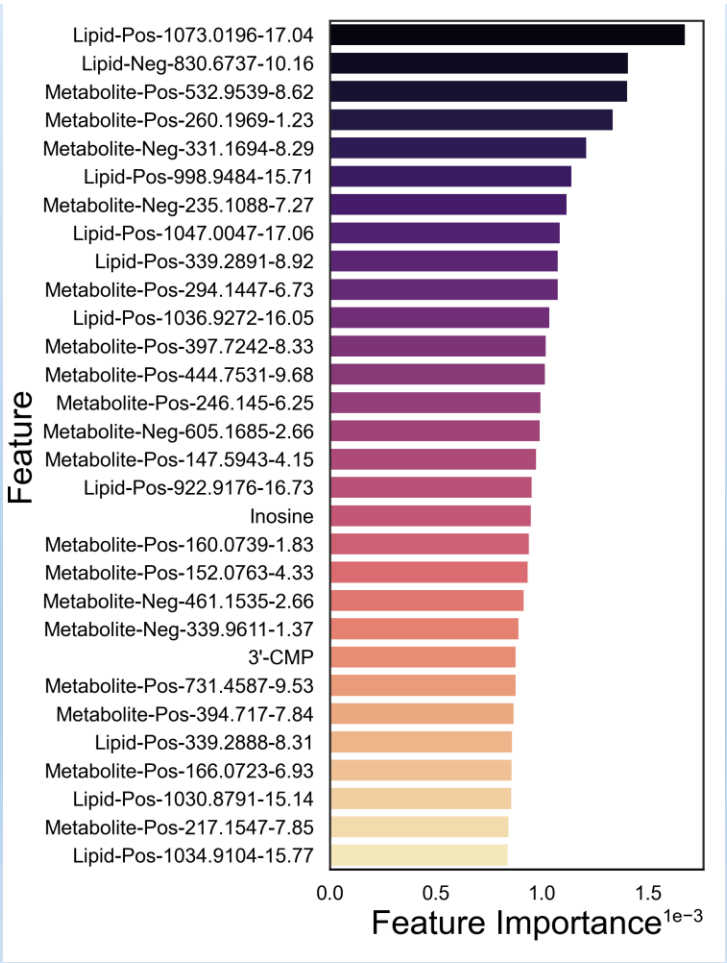
Machine Learning Applications:

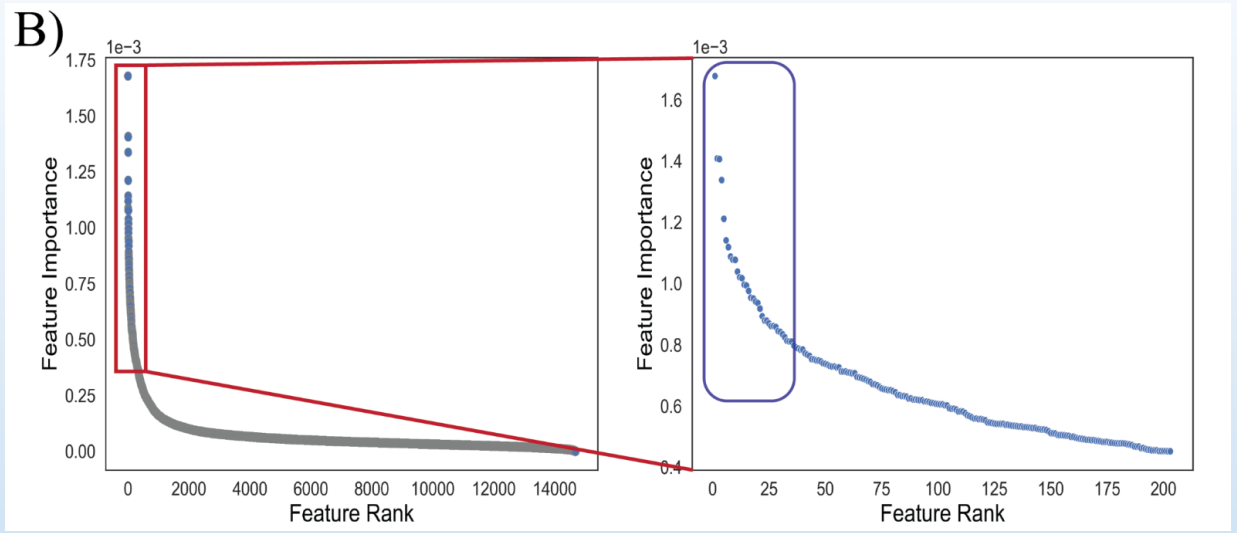
- Face detection
- Handwriting recognition
- Computer vision
- **Healthcare**
- Voice interfaces
- Speech recognition
- Fraud detection

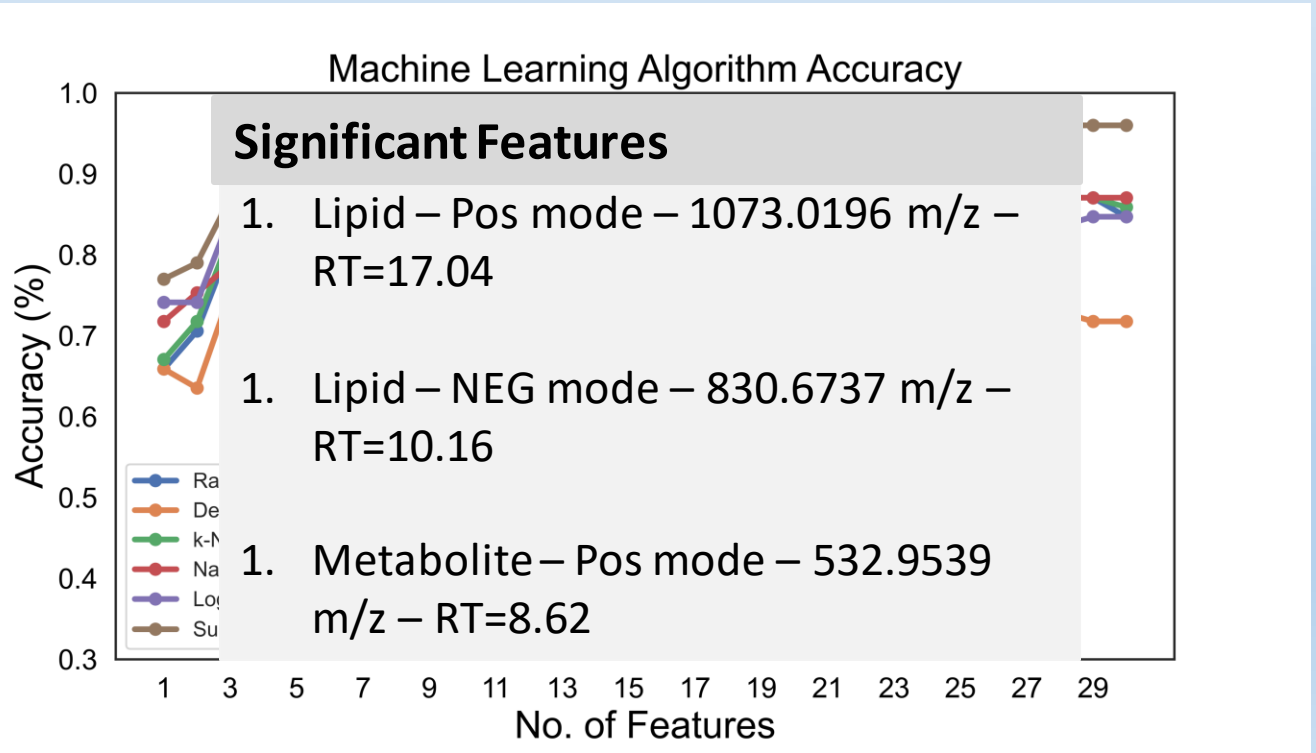


Feature Selection

- **> 17,000 Features detected**
- **Python Scikit-Learn Package**
- **ExtraTrees Package**
  - Classification and regression based on an ensemble of decision trees.







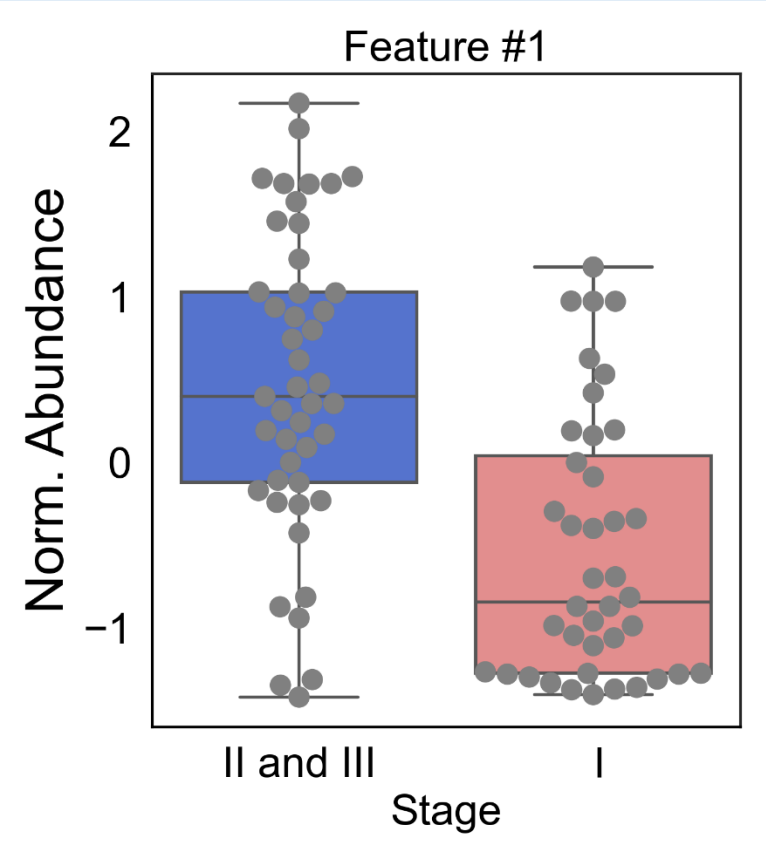
Significant Features

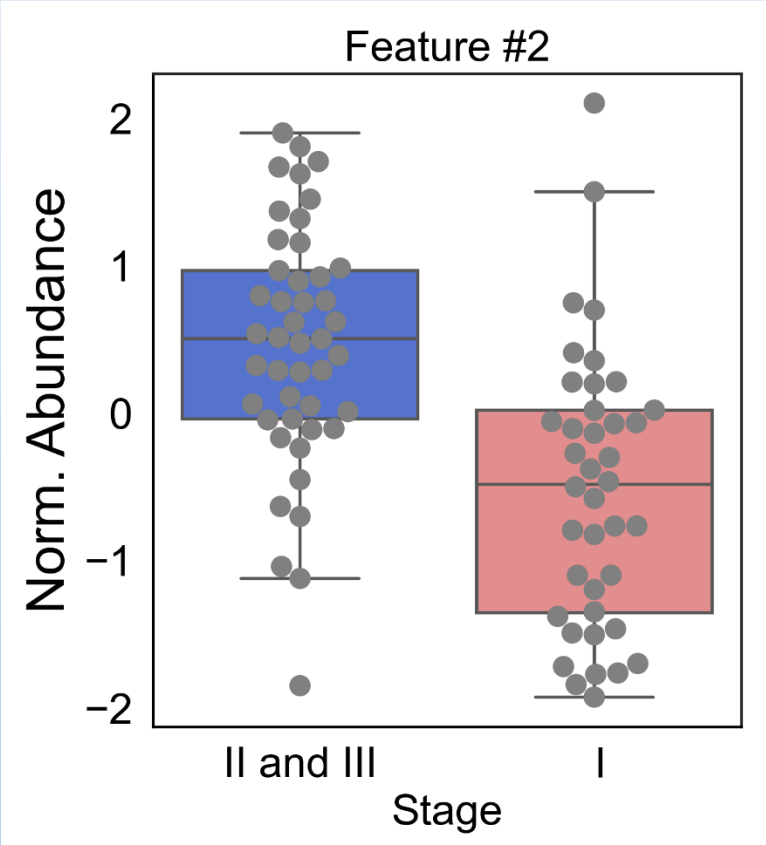
1. Lipid – Pos mode – 1073.0196 m/z – RT=17.04

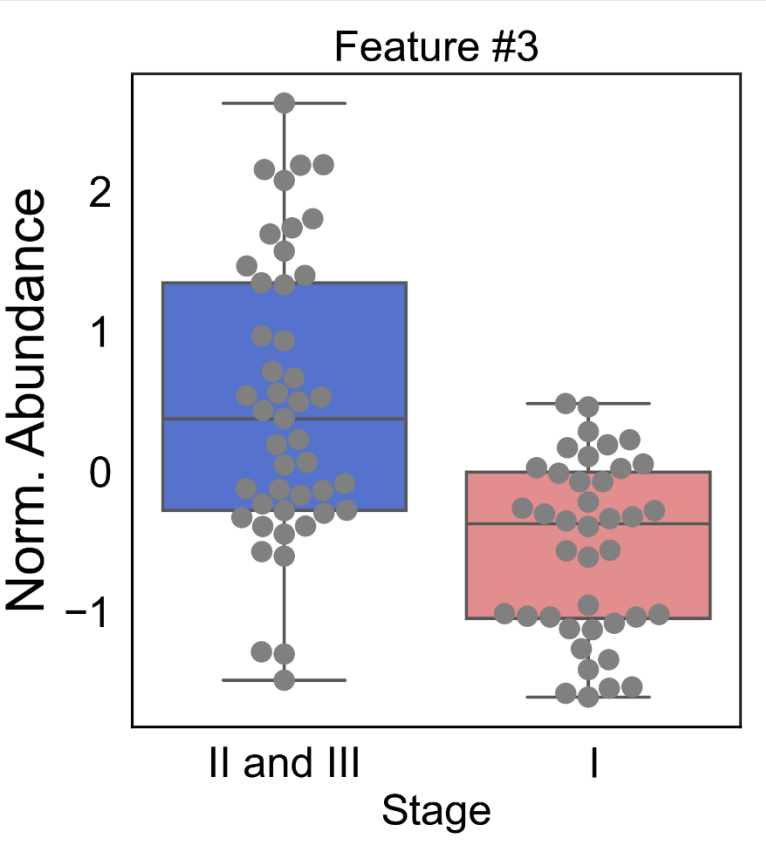
1. Lipid – NEG mode – 830.6737 m/z – RT=10.16

1. Metabolite – Pos mode – 532.9539 m/z – RT=8.62

Response for 3 of the features

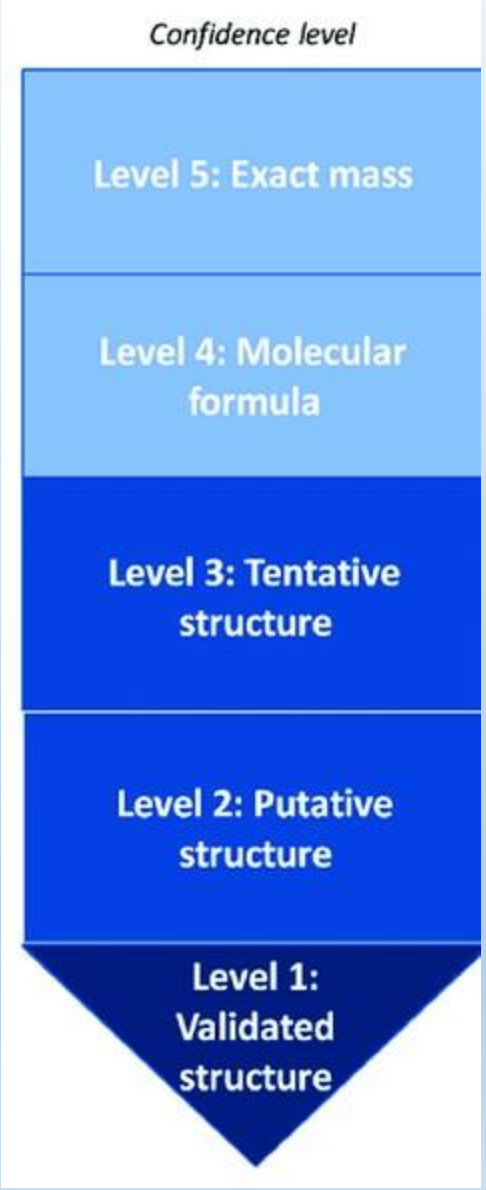






All are elevated in Stage 2/3

Unknown Identification



- Metabolomics/chemical database search based on the exact mass
- Fragmentation spectrum
- Standard check (if available)

Significant Features

1. Lipid – Pos mode – 1073.0196 m/z – RT=17.04
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1. TG(18:2)(24:0)(24:0)
2. GalCer(d18:0/22:0)
3. Peptide with 14 Amino-Acid

Summary of Meningioma

Combine machine learning using lipids and metabolites identified 5 unknowns to classify Grade 1 vs Grade 2/3

- MS/MS interpretation was used to identify 2 of the 5 unknowns as lipids
- 1 of the unknowns is a small peptide from the metabolite analysis
- 2 are still unknown, these will require NMR for fraction collection

Cannot classify Grade 2 vs Grade 3 because of small sample sizes

- Only had 15 grade 3 and it is still difficult by current histopathology to classify grade 2 and 3

Biological interpretation is on going

- Lipids are harnessed by cancer cells
- The increased TG content could be localized to lipid droplet accumulation in tumors
- The longer chain, but not significantly increased PUFA content could be significant
- 24:0 and 24:1 were associated with TG

Medulloblastoma

A primary central nervous system tumor

Cause is currently unknown

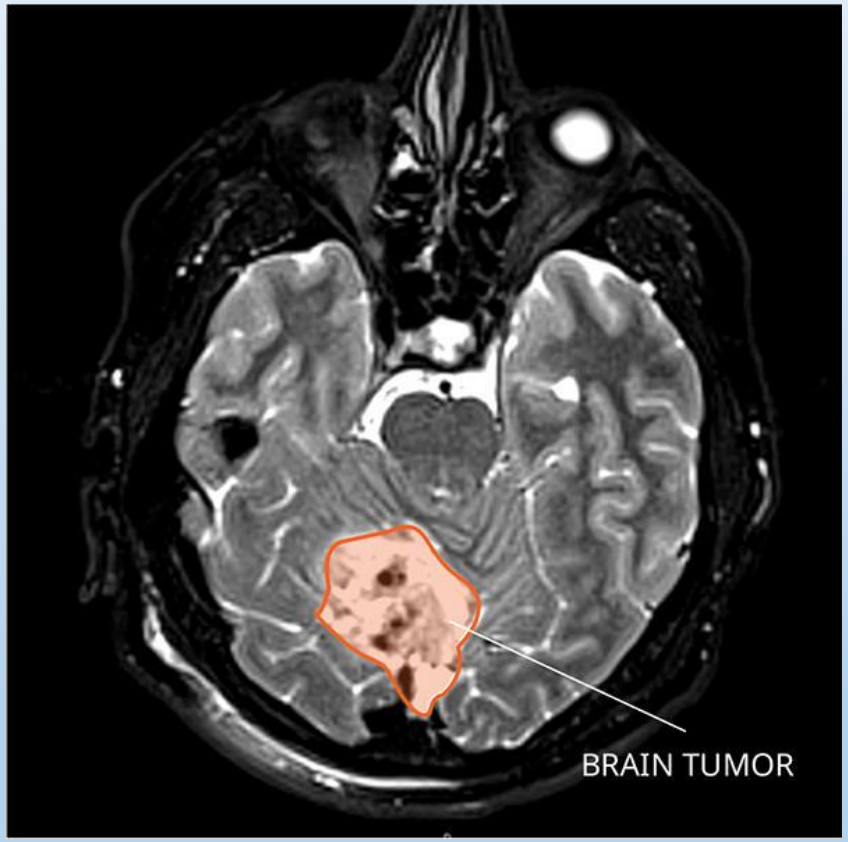
It is the most common malignant tumor of the cerebellum in children accounting for 10-15% of CNS tumors

It can invade and disseminate into the CSF

Current diagnosis

- Clinical assessment
- Imaging
- Histopathology of biopsies

No current biomarker test using CSF exists



https://www.cancer.gov/rare-brain-spine-tumor/tumors/medulloblastoma

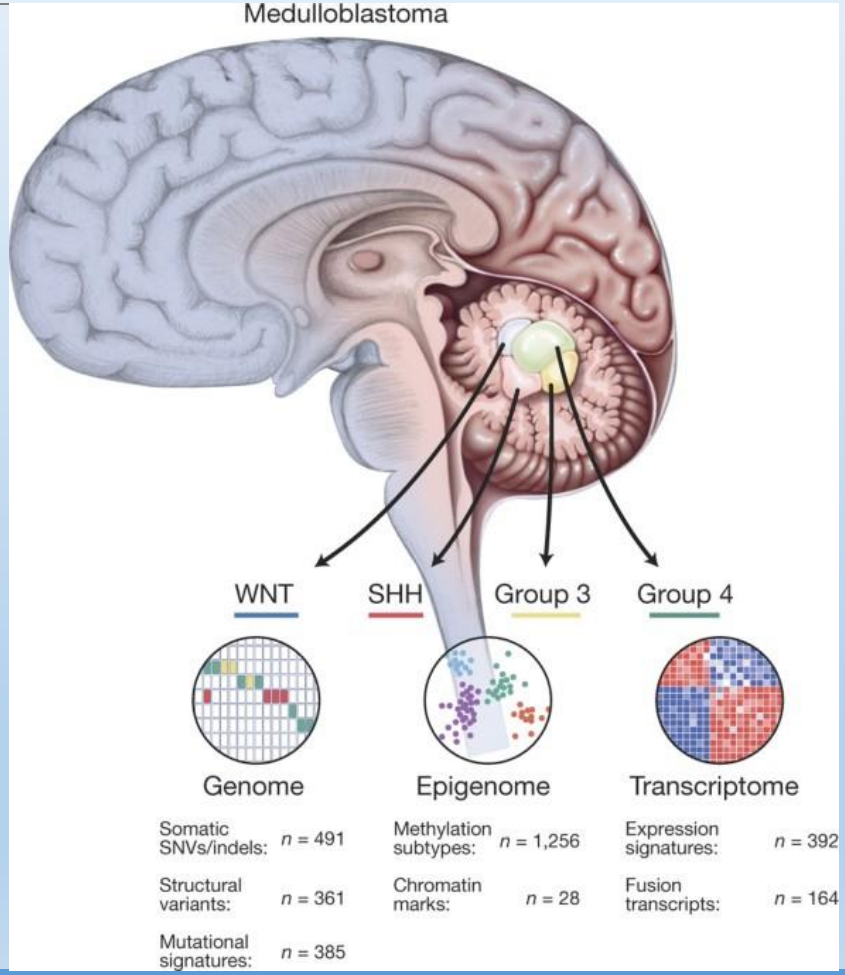
Subtypes of Medulloblastoma

- WNT (wingless) activated
- SHH (sonic hedgehog) activated
- Group 3
- Group 4

Can we use CSF for biomarker ID and mechanistic analysis?

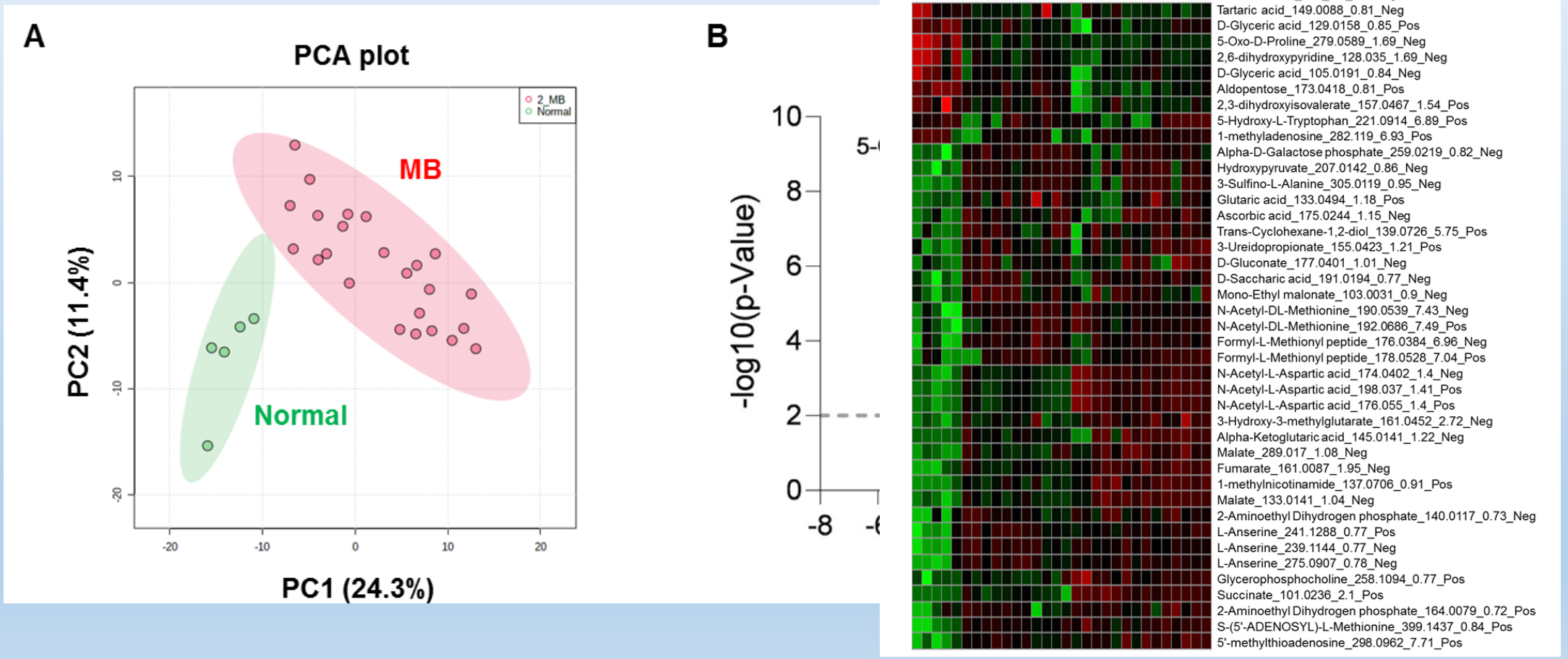
Liquid biopsies represent an alternative to invasive tissue biopsies, thus exploration of metabolites/lipids in CSF could help to better understand medulloblastoma

Collaboration with Dr. Ranjan Perera



Northcott, P., Buchhalter, I., Morrissy, A. et al. Nature 547, 311–317 (2017). https://doi.org/10.1038/nature22973

Global metabolomics clearly delineates MB from normal



Global lipidomics in MB vs normal

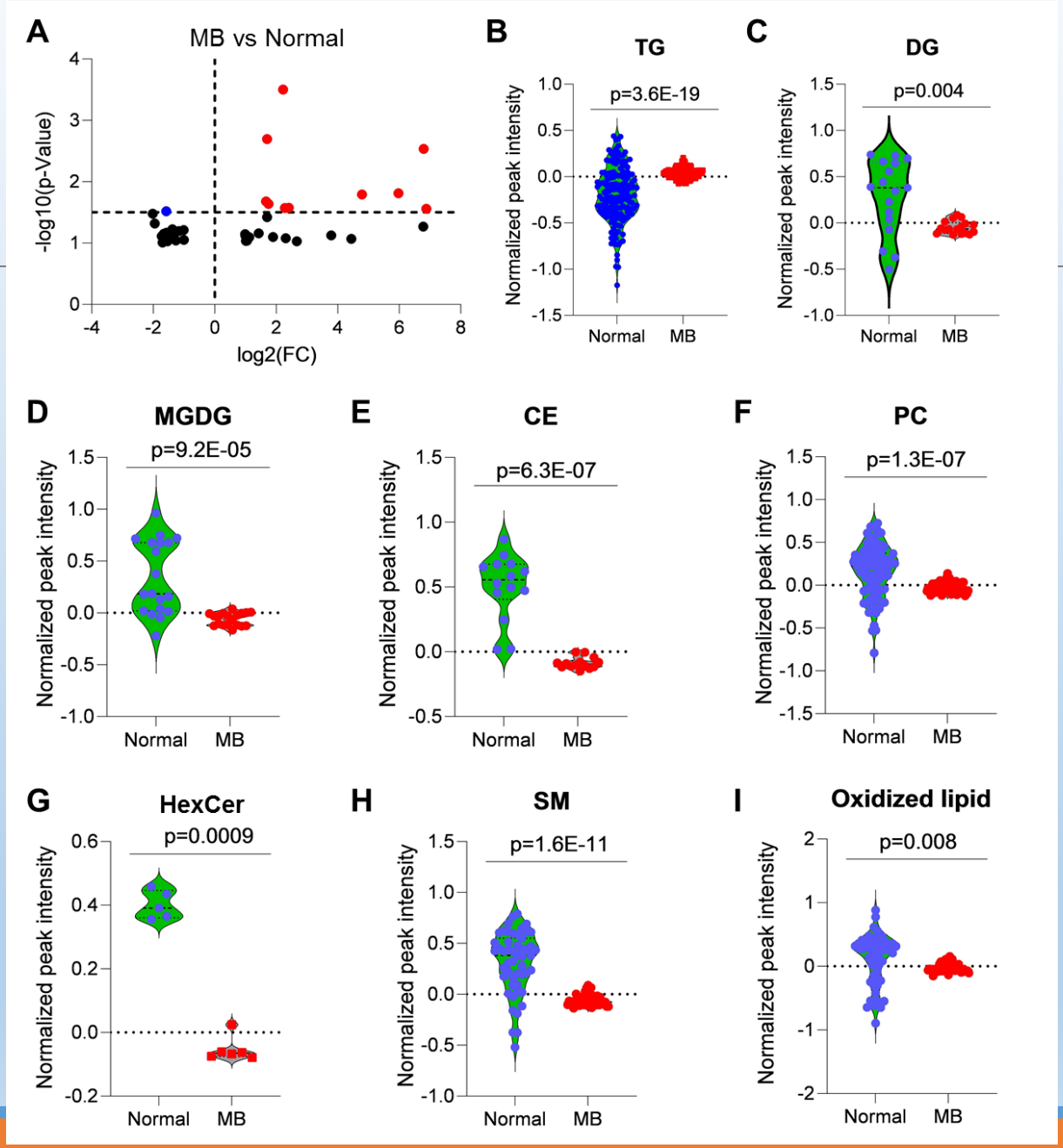
We identified over 700 lipids in the CSF

Class-based analysis identified several lipid classes as significantly regulated lower in MB

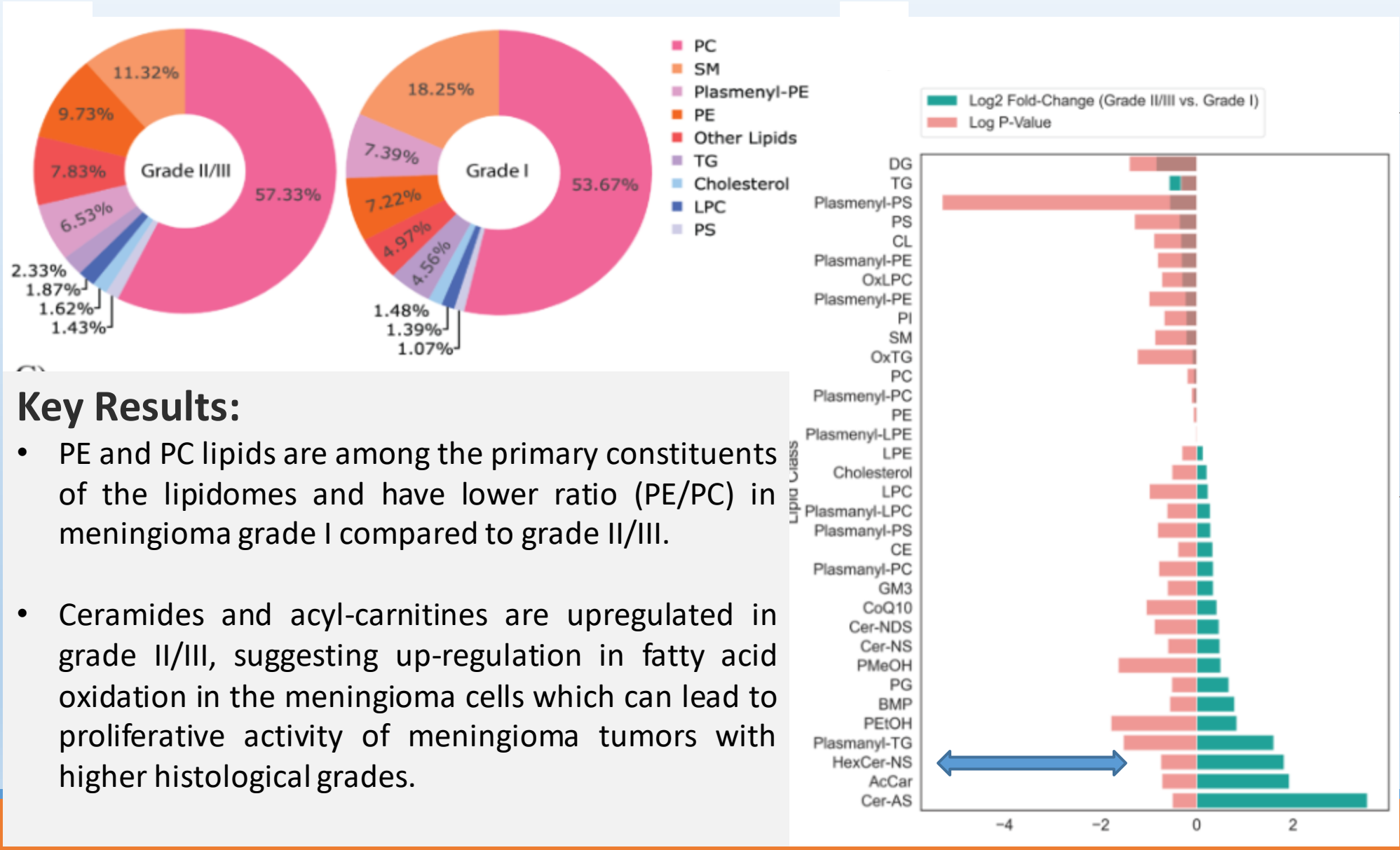
TGs were elevated overall (possible link to hypoxia or pH control)

Hexosylceramides (HexCer) were the most consistently down regulated lipid class

- HexCer are abundant in the brain
- Decrease in CSF levels could translate to utilization for tumor growth in the brain



Lipidomics in Meningioma tissues



Key Results:

- PE and PC lipids are among the primary constituents of the lipidomes and have lower ratio (PE/PC) in meningioma grade I compared to grade II/III.

- Ceramides and acyl-carnitines are upregulated in grade II/III, suggesting up-regulation in fatty acid oxidation in the meningioma cells which can lead to proliferative activity of meningioma tumors with higher histological grades.