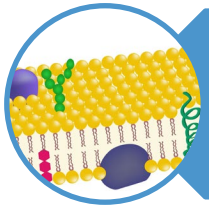
Development and Application of a Multidimensional Lipid Database Containing Liquid Chromatography, Ion Mobility Spectrometry, and Mass Spectrometry Separation Characteristics in Skyline

Amie Solosky

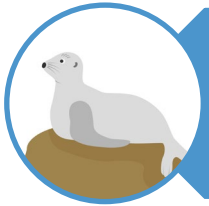
Skyline User Meeting
ASMS 2024

Outline



Lipid Overview

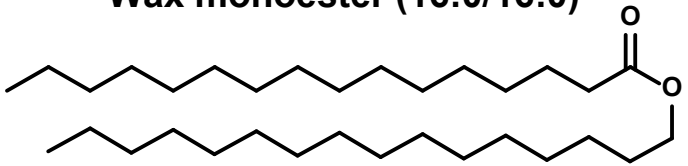
Database Creation



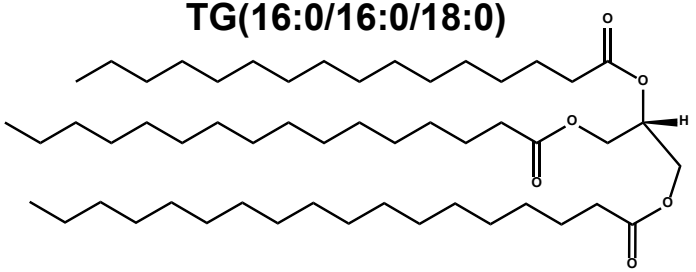
Sea Lion Application

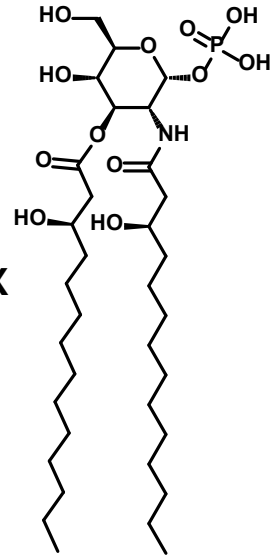
What is a lipid?

Wax monoester (16:0/16:0)

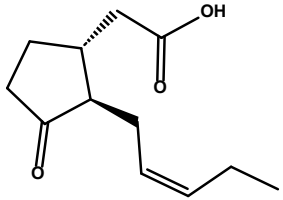


TG(16:0/16:0/18:0)





Lipid X



Jasmonic acid

Common denominator?

Lipids do not dissolve in water!

Lipidomics - why are lipids so important?

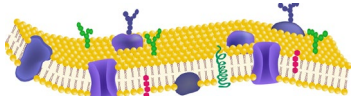
Cell signaling

Inflammation

Lipid Functions

Energy Storage

Lipid Biosynthesis

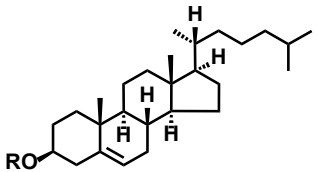


Cell Membrane

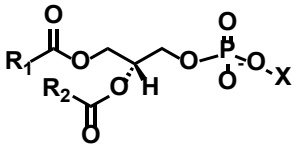
Fahy, E., et. al. *J Lipid Res* **2009**, 50, S9-14. DOI: 10.1194/jlr.R800095-JLR200

Lipid structure

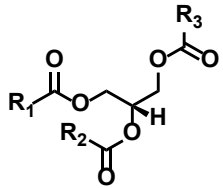
Sterol Lipids



Glycerophospholipids



Glycerolipids



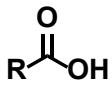
Lipid Speciation

Category

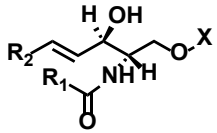
8

lipid categories

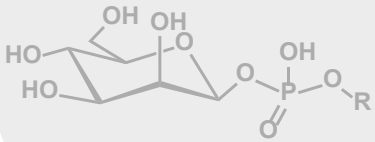
Fatty Acids



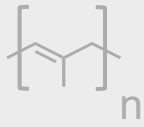
Sphingolipids



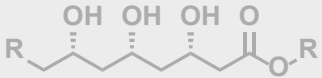
Saccharolipids



Prenol Lipids



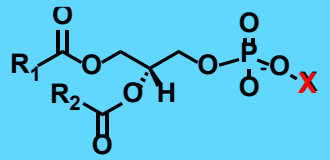
Polyketides



Fahy, E., et. al. *J Lipid Res* **2009**, 50, S9-14. DOI: 10.1194/jlr.R800095-JLR200

Lipid classes

Glycerophospholipids

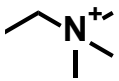


X =

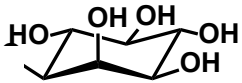
Lipid Speciation

Category
8
lipid categories

Class
Common lipids vary by head group

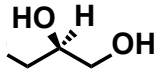


PC



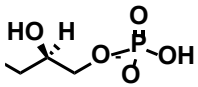
PI

PA

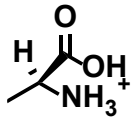


PG

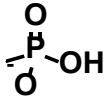
PE



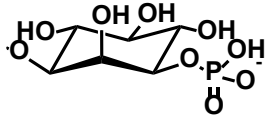
PGP



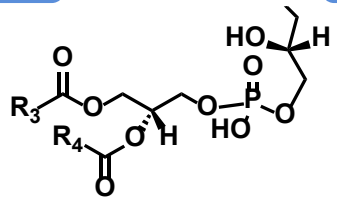
PS



PPA



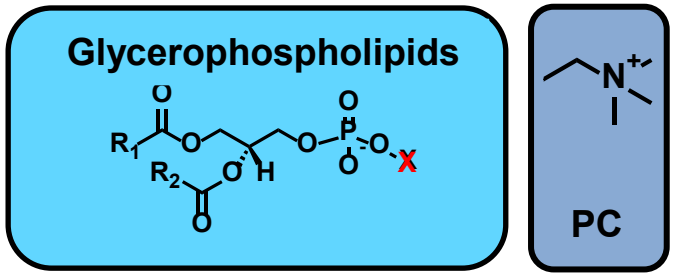
PIP



CL

Fahy, E., et. al. *J Lipid Res* **2009**, 50, S9-14. doi: 10.1194/jlr.R800095-JLR200

Lipid nomenclature



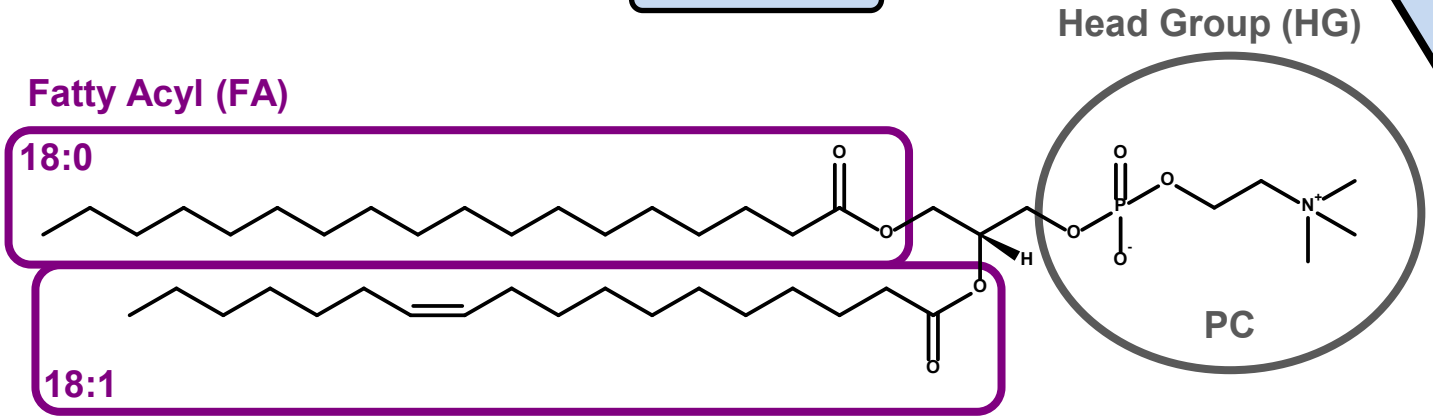
Lipid Speciation

Category
8
lipid categories

Class
Common lipids vary by head group

Lipid
FAs

PC(18:0_18:1) → PC(18:0/18:1)



Targeted vs. untargeted analysis

Targeted Analysis

- Pre-determined list of analytes
- Only detect known features
- Quantitative
- Rapid & sensitive
- Regulatory methods

Baker Lab Analysis

Data Collection → Untargeted

Data Analysis → Suspect Screening

Skyline

Non-targeted Analysis

- Feature finding
- Requires high-resolution mass spectrometry
- *Vex* multidimensional data
- consuming
- ative
- ratory

Unknown unknowns

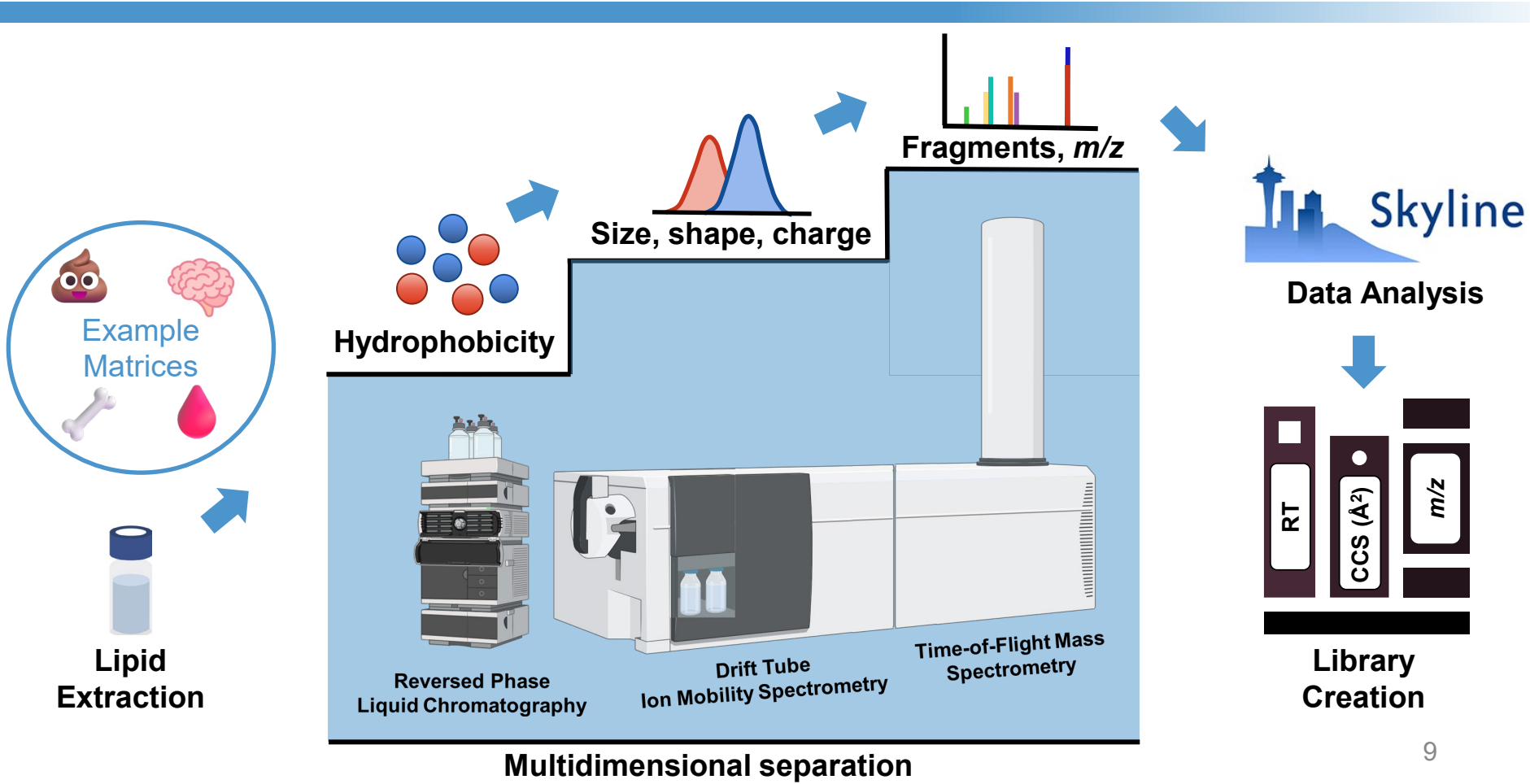
Discovery

Known unknowns

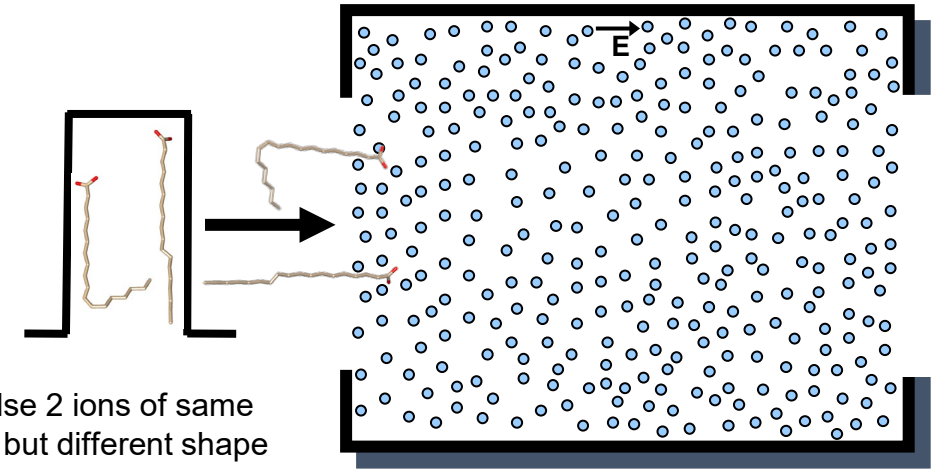
Suspect Screening

https://www.cnn.com/2020/01/23/politics/trump-epa-repeals-obama-water-regulation/index.html

Multidimensional separation technique for lipids

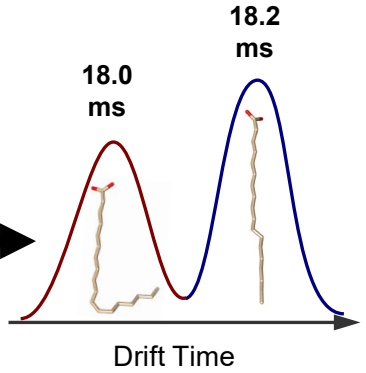


Drift Tube Ion Mobility Spectrometry (DTIMS)

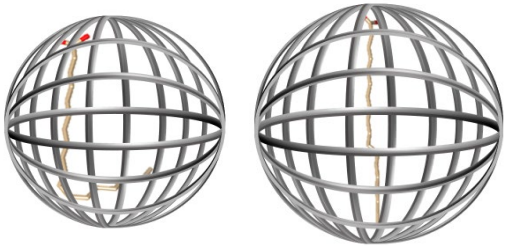


Pulse 2 ions of same *m/z* but different shape

Drift Cell



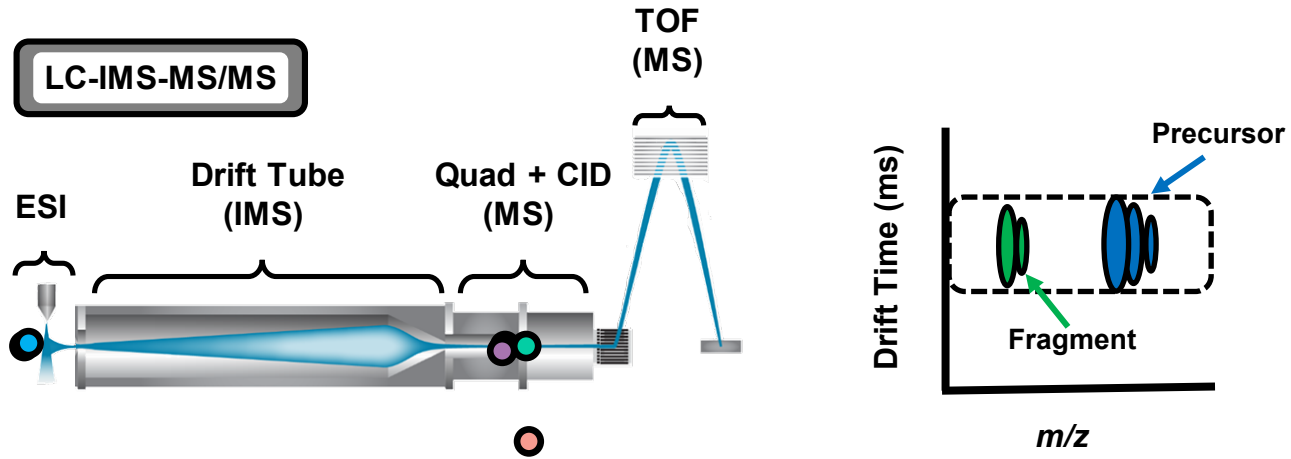
Isomers separate in time with peak heights representing amounts



Higher drift time
=
Larger Collision Cross Section (CCS, Å^2)

Fragmentation

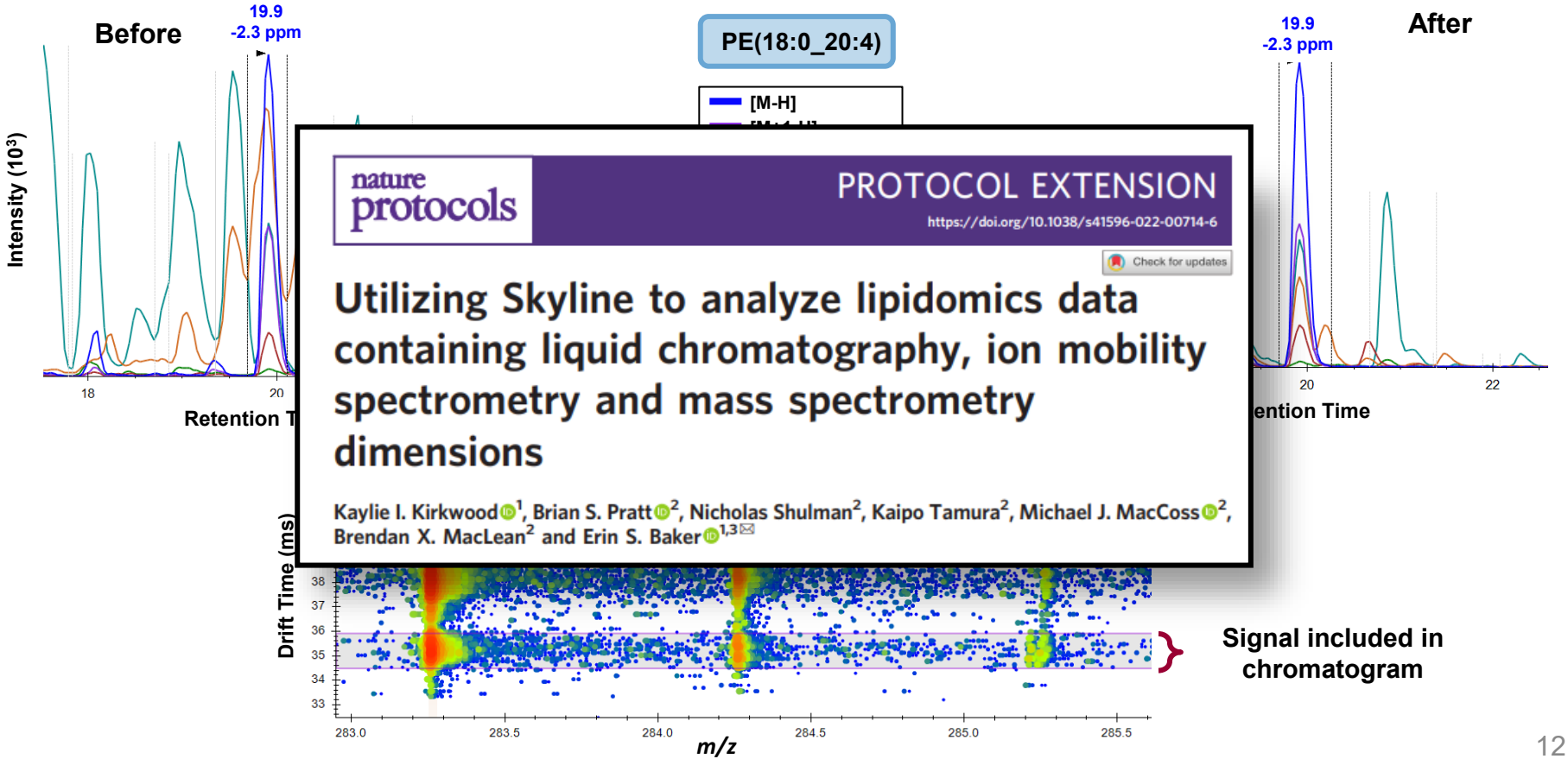
- **DIA All Ions** – alternating precursor and fragment scans without precursor isolation



Drift time of fragments = drift time of precursor

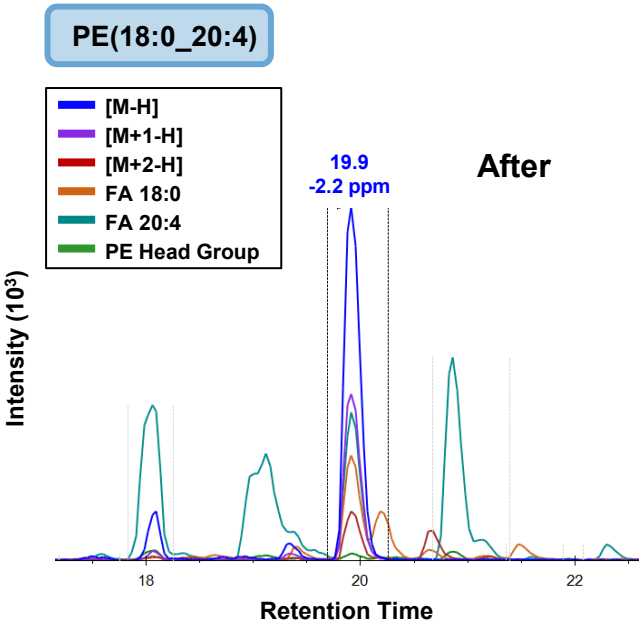
Baker et al. *J. Am. Soc. Mass Spectrom.* **2008**, 19(3), 411-419.
Figure created by Jack P. Ryan

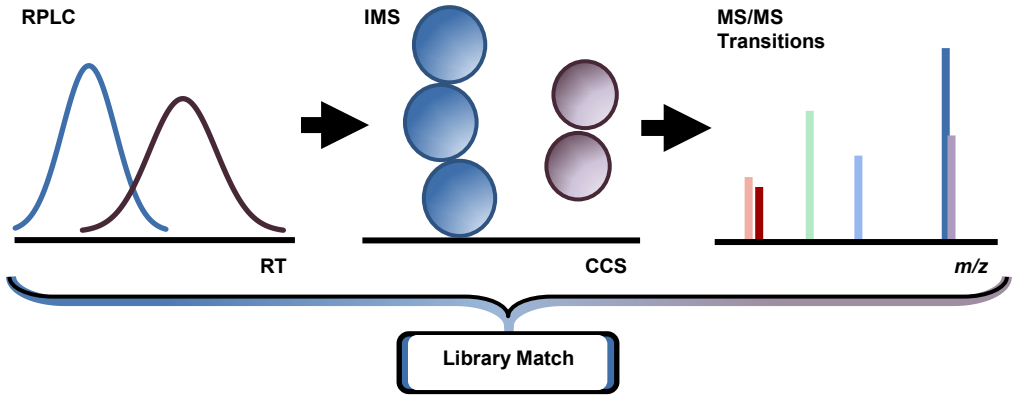
Drift time filtering to increase confidence

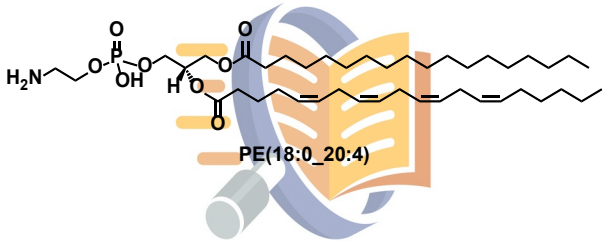


MacLean, B.X., et. al. *J Am Soc Mass Spectrom* **2018**, 29(11): 2182-2188.

Library match







MacLean, B.X., et. al. *J Am Soc Mass Spectrom* **2018**, 29(11): 2182-2188.

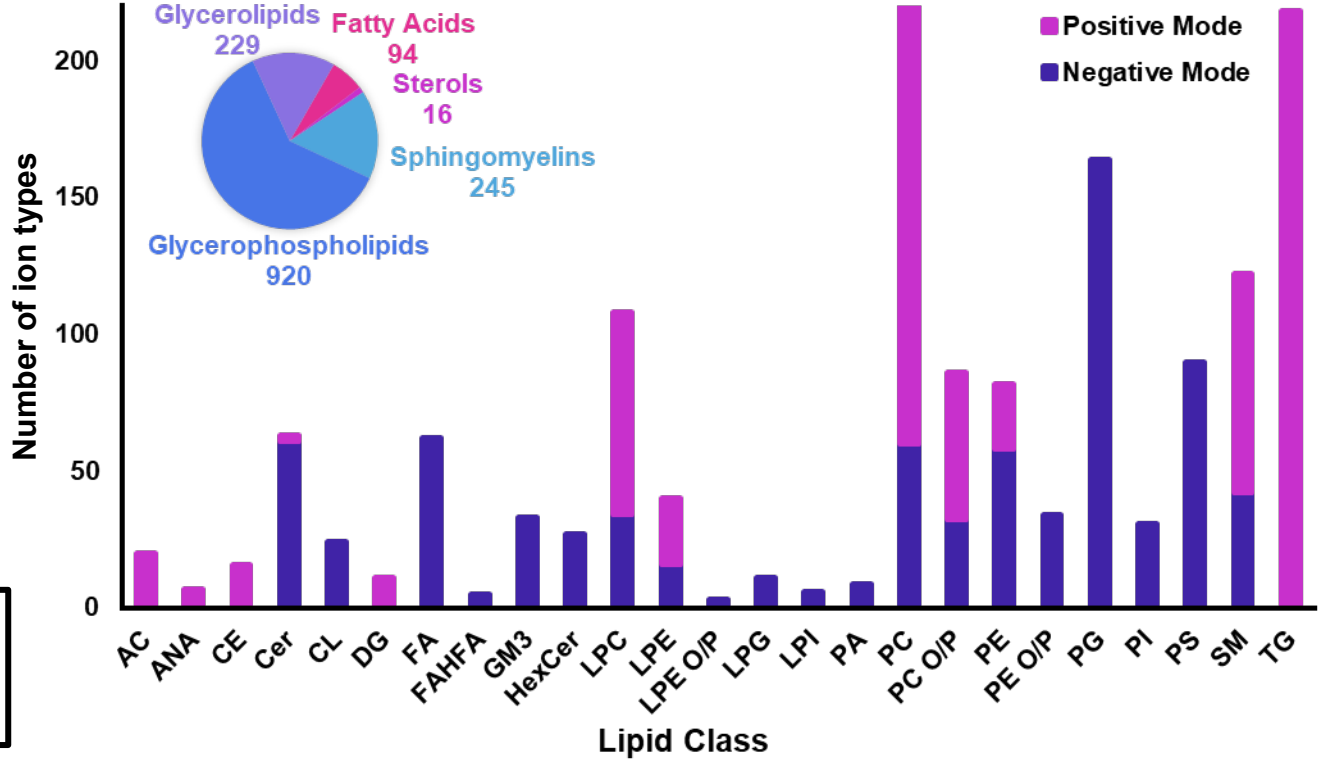
Baker Lab lipid library composition

Lipid Library Magicians

Dr. Kirkwood-Donelson

Dr. Odenkirk

1504 precursor ion types:
- 877 *unique* lipids
- 23 stable isotope labeled



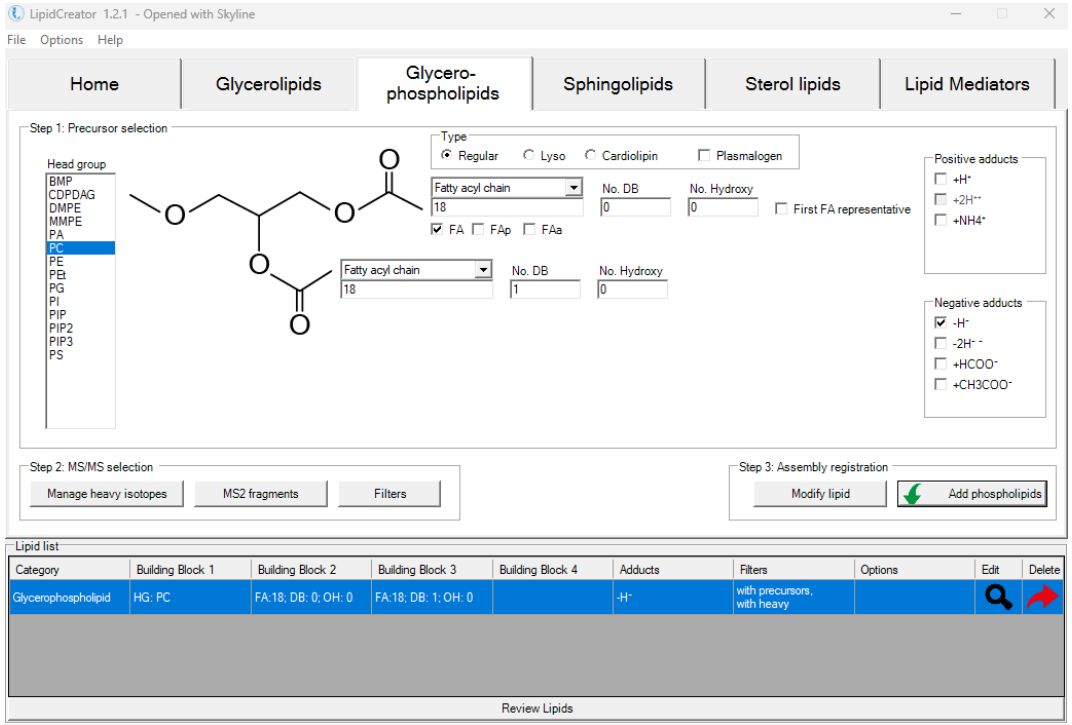
Kirkwood, K. I. et al., *JPR*. **2022**, *21*(1), 232-242. DOI: 10.1021/acs.jproteome.1c00820.
Kirkwood, K. I. et al. *Nature Protocols*. **2022**, *17*, 2415-2430. DOI: 10.1038/s41596-022-00714-6
Solosky, A.M. et al. *ABC*. **2024**. DOI: 10.1007/s00216-024-05351-4

You can do it too!

LipidCreator (in silico)

Transition lists can come from any source: feature finding, online databases, literature, Panorama

PanoramaWeb



Peng et al., *Nat Comm.* **2020**, 11. DOI: 10.1038/s41467-020-15960-z

Baker lab database

Home / Databases

Databases

Links to Baker Databases

Multidimensional RPLC-IMS-CID-MS Lipid Database

Baker-Lab-Lipid-Database_03_28_2024 Download

3/28/2024 Release Notes:

- This database contains information for 877 unique lipids from 1504 precursor [M+H]+, [M+NH4]+, [M+Na]+, [M-H]-, [M-2H]2-, [M+HCOO]- and [M+CH3COO]- ion types and their associated CID fragments (DOI: coming soon).
- This is an updated version of the Skyline library developed by Kirkwood et al., 2022 (DOI: 10.1021/acs.jproteome.1c00820).
- This database has 361 additional lipids, including heavy labeled internal standards by Avanti Polar Lipids
- A new matrix was added: California Sea Lion plasma

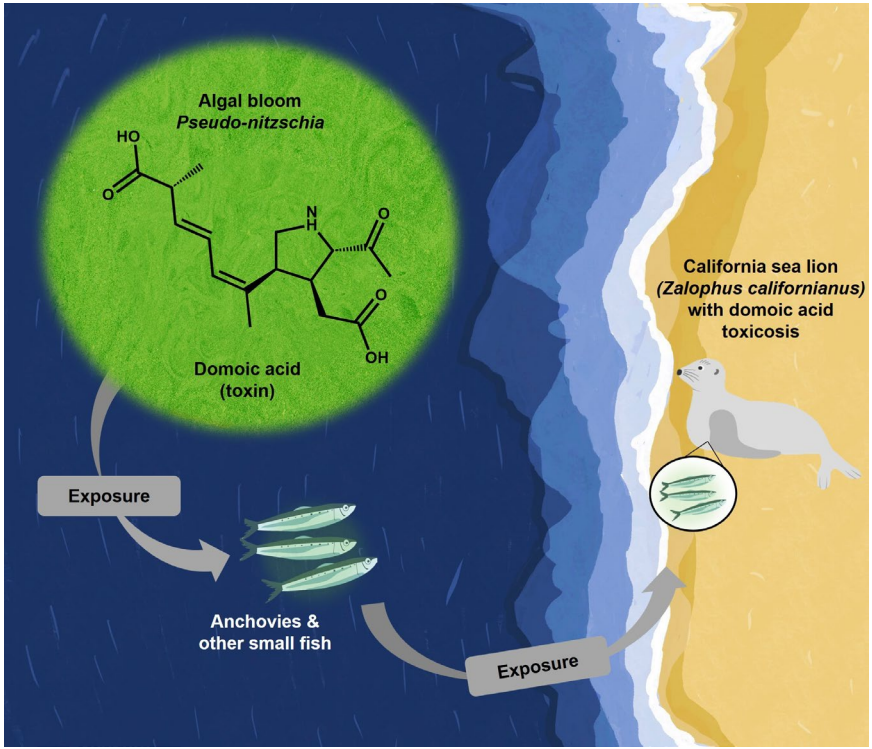
https://tarheels.live/bakerlab/databases/

Kirkwood, K. I. et al., *JPR*. **2022**, *21*(1), 232-242. DOI: 10.1021/acs.jproteome.1c00820.
Kirkwood, K. I. et al. *Nature Protocols*. **2022**, *17*, 2415-2430. DOI: 10.1038/s41596-022-00714-6
Solosky, A.M. et al. *ABC*. **2024**. DOI: 10.1007/s00216-024-05351-4

California sea lions (Zalophus californianus)

https://www.pier39.com/sealions/

DAT & Sea lions (*Zalophus Californianus*)



Symptoms:

- Seizures
- Heart issues
- Fatal

Challenges:
No diagnostic tools
Half-life of domoic acid is < 48 hours

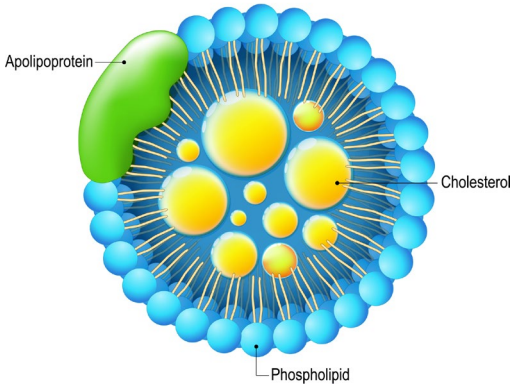
McClain, A. M et al. *Front. Vet. Sci.* **2023**, *10*. DOI: 10.3389/fvets.2023.1245864.

Previous proteomic study

Dr. Ben Neely, NIST

APOLIPOPROTEINS

(proteins that bind lipids to form lipoproteins)



PLoS One. 2015 Apr 28;10(4):e0123295. doi: 10.1371/journal.pone.0123295. eCollection 2014.

Proteomic Analysis of Plasma from California Sea Lions (Zalophus californianus) Reveals Apolipoprotein E as a Candidate Biomarker of Chronic Domoic Acid Toxicosis

Benjamin A Neely [1], Jason A Ferrante [2], J Mauro Chaves [1], Jennifer L Soper [3], Jonas S Almeida [4], John M Arthur [5], Frances M D Gulland [3], Michael G Janech [6]

Hypothesis: Apolipoprotein dysregulation indicates that lipids are informative of DAT

Experimental design (lipidomics)

Rescued Sea Lion Samples

14 DAT*
17 non-DAT
13 males, 18 females
All ages

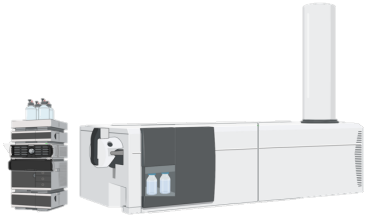
*Diagnosed based on histological exam of brain tissues & clinical signs

Lipidomic Workflow

Experimental | **Data Analysis**

sea lion plasma

- **Modified-Folch lipid extraction**



37 min LC-Drift Tube IMS-CID-MS
- **m/z 50 to 1700**

Skyline

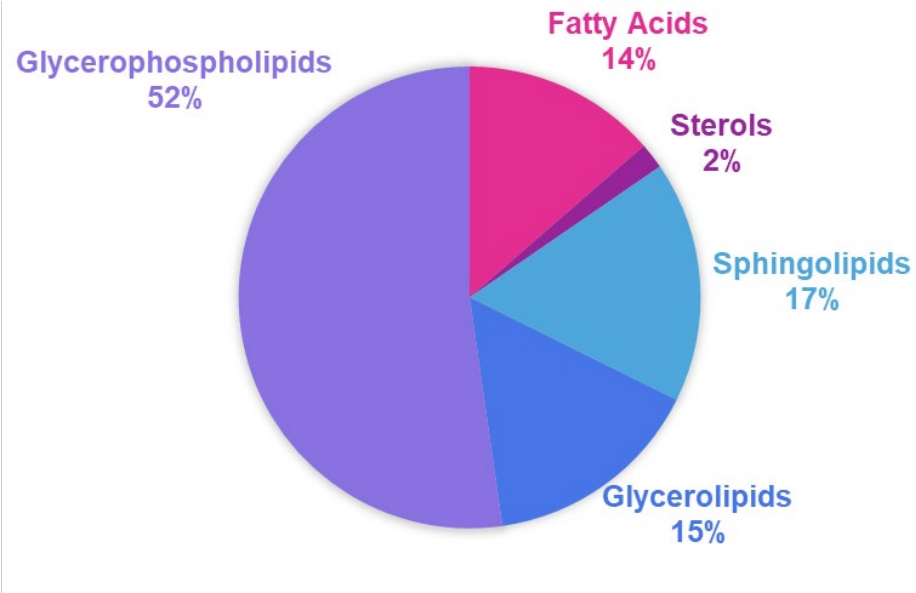
RStudio MetaboAnalyst 5.0

- **Suspect Screening**
- **Statistics**
- **Data visualization**

Kirkwood, K. I. et al., *Nat Protoc.*, **2022**, *17* (11), 2415-2430.
Pang, Zhiqiang et al. *Nucleic Acids Res.* **2021**, *49*(W1), W388-W396.
Created with BioRender.com

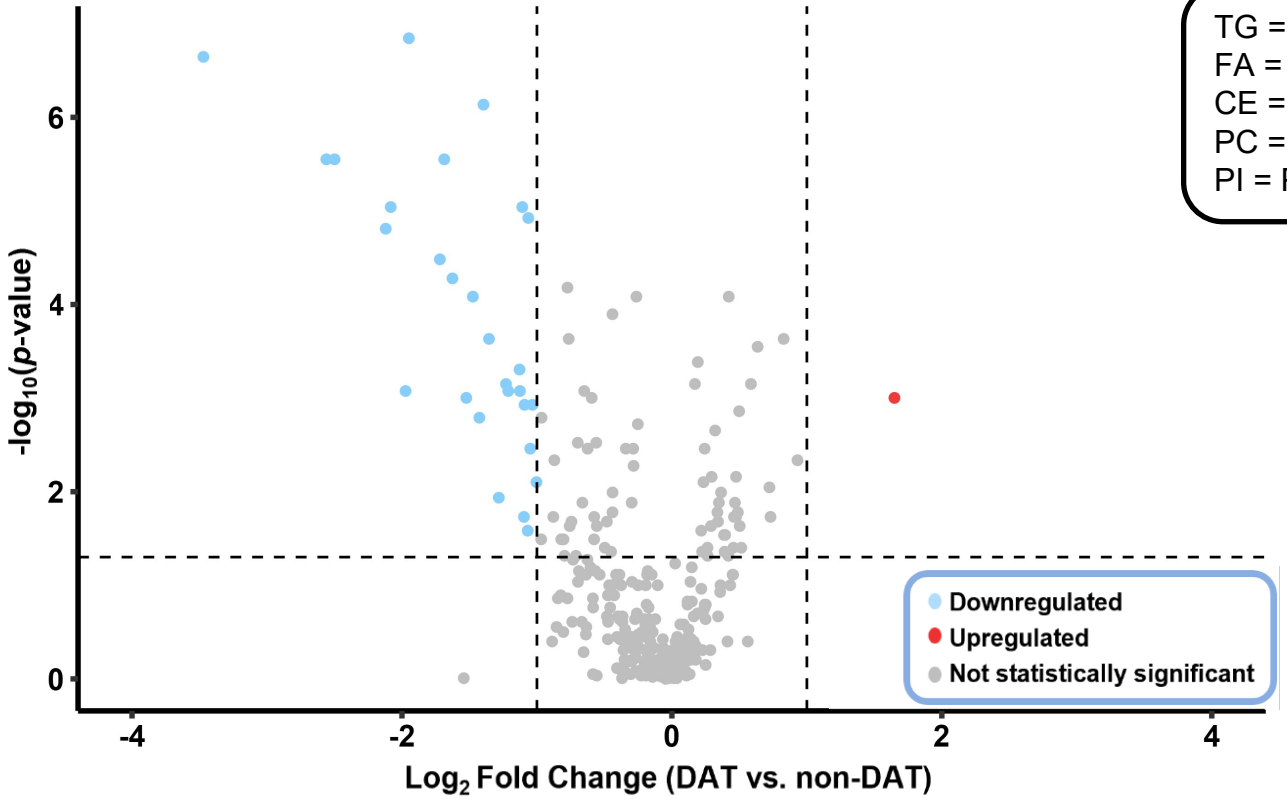
Lipid category trends

Detected Lipids: 331



Solosky, A.M. et al. *bioRxiv*, **2024**.

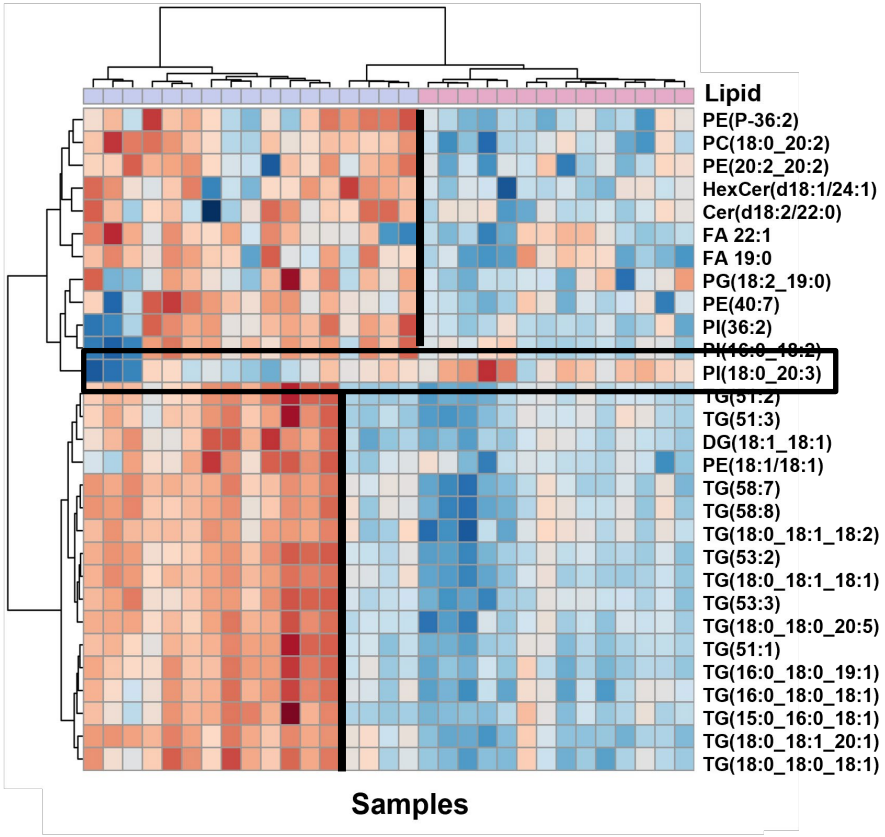
Significance testing

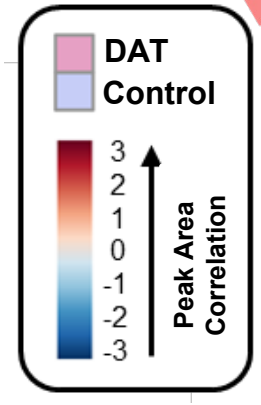


TG = Triglyceride
FA = Fatty acid
CE = Cholesteryl ester
PC = Phosphatidylcholine
PI = Phosphatidylinositol

Solosky, A.M. et al. *bioRxiv*, **2024**.

Hierarchical clustering results

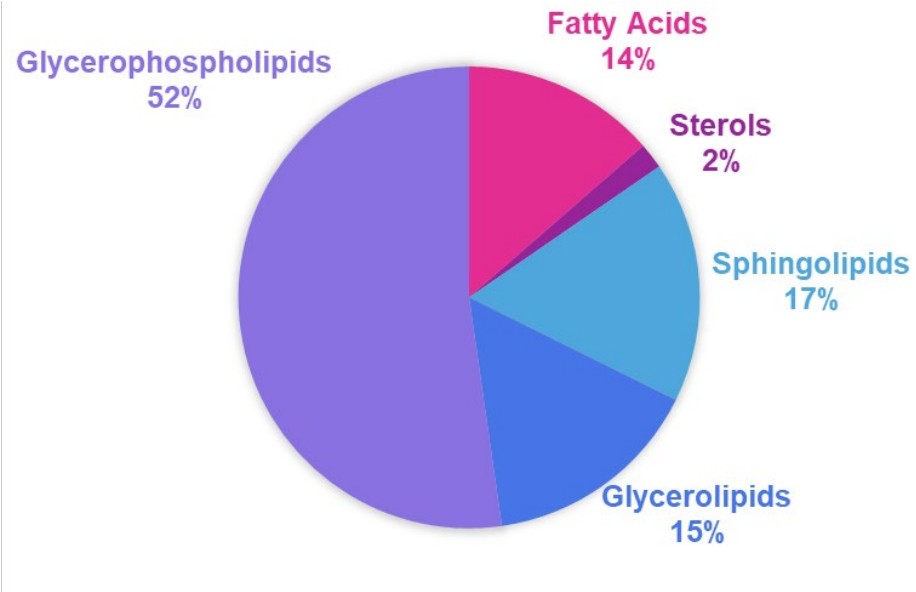




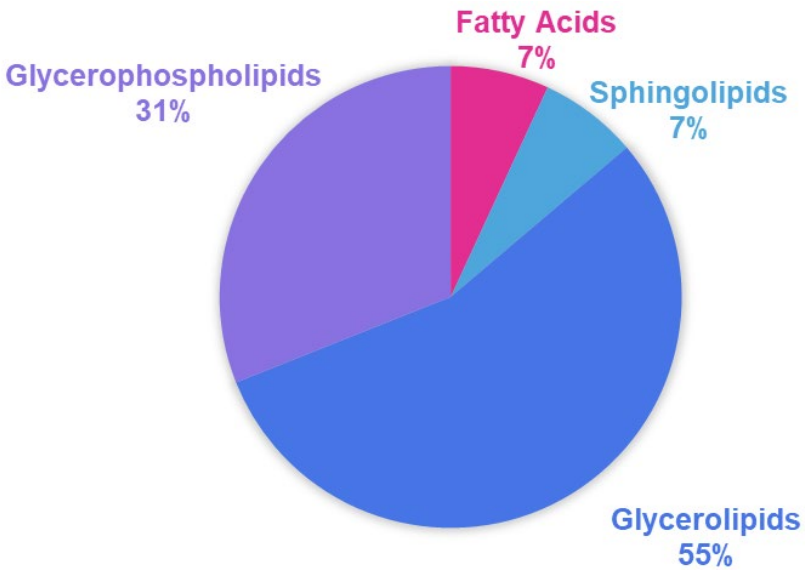
Pang, Zhiqiang et al. *Nucleic Acids Res*. **2021**, *49* (W1), W388-W396
Solosky, A.M. et al. *bioRxiv,* **2024**.

Lipid category trends

Detected Lipids: 331



Significant Lipids: 29



Solosky, A.M. et al. *bioRxiv*, **2024**.

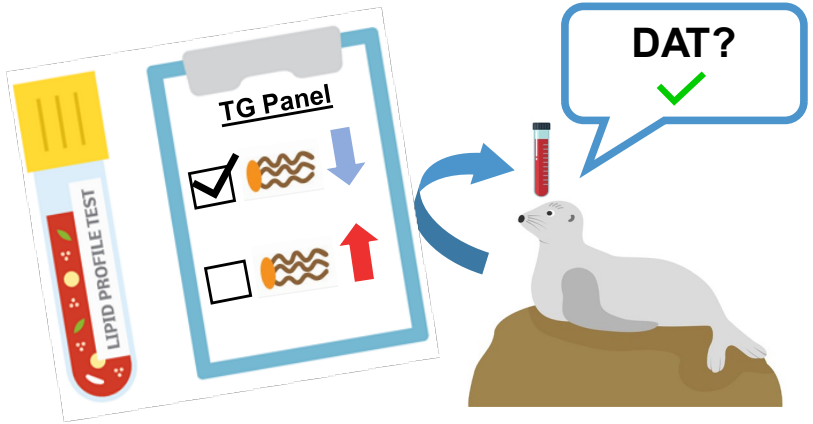
Summary

Conclusions:

- 29 significant lipids & 50% are TGs
- **Triglycerides are dysregulated in DAT sea lions**
- This is a trend **unique to DAT** sea lions!
- Skyline was *essential* to these findings

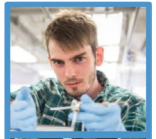
Future Directions:

- Develop a triglyceride blood panel to aid in diagnosis of DAT
- Test more samples to validate results



Solosky, A.M. et al. *bioRxiv*, **2024**.

Check out the Baker Lab at ASMS!



James

WP 475 - Cross Platform Assessment of Feature Detection as a Function of Measured Resolving Power in IMS

Guozhi

TP 366 - Lipids as Indicators of Successful Fecal Microbiota Transplantations

Jessie

TP 250 - Non-Targeted Analysis Feature Screening: Sample Classification and Feature Identification

Jack

WP - Utilizing High Resolution IMS-MS for Opioid Profiling

Sunday – Agilent Users Meeting – Oligonucleotide Sequencing using IMS-MS/MS

Anna

ThOD - Comparing Target Screening, Suspect Screening, and Unknown Discovery Workflows in Non-Targeted Analyses of PFAS

Ashlee

TP 101 - An Assessment of Serum Lipidomic Perturbations due to PFAS Exposures

Amie

TP 348 - Building a Multidimensional LC-IMS-MS Oxylipin Library

Sunday - Skyline Users Meeting – Developing a Multidimensional Lipid Database

Greg

TP 103 - Evaluating Environmental Factors in Systemic Autoimmune Rheumatic Diseases

Emily

MP 196 - Assessing Antidepressant Pharmaceuticals in the Environment

Sarah

TP 055 - Evaluating Bone Marrow Lipidomic Changes and Their Potential Association with Alzheimer's Disease

Kara

TP 079 - Extraction Optimization and Non-Targeted Analyses of (PFAS) in Mammalian Milk

Allison

TP 116 - A Lipidomic Exploration of the Impact of PFOA Exposure on SARS-CoV-2 Infection

Haley

TP 115 - Evaluating Lipidomic Changes in Mice Exposed to Wildfire-Relevant Smoke from Different Fuel and Burn Conditions

Skyline used in the project

Acknowledgements

Baker Lab Members

Principal Investigator
- Dr. Erin S. Baker

Research Assistant Professor
- Prof. James Dodds

Postdoctoral Researchers
- Dr. Guozhi Zhang
- Dr. Jessie Chappel

Graduate Students
- Jack Ryan
- Anna Boatman
- Ashlee Falls
- Greg Kudzin
- Allison Fry
- Emily Crawford
- Haley Jostes
- Kara Joseph
- Sarah Clark

Past Graduates with Significant Contributions
- Dr. Karen Butler
- Dr. Melanie Odenkirk
- Dr. Kaylie Kirkwood Donelson
- Dr. Nancy Lee Alexander

Contributing Undergraduate Students
- Iliana Claudio (alum)
- Quentin DuVal

Sources of Funding
- NIEHS P42 ES027704 & P42 ES031009
- NIGMS RM1 GM145416 & R01 GM141277
- US EPA

@BakerLabMS
@AmieSolosky

Collaborators

U Washington
Mike MacCoss
Brendan MacLean

NIST
Ben Neely

College of Charleston
Alison Bland
Michael Janech

University of California Davis
Frances M.D. Gulland

The Marine Mammal Center®

Skyline