Development of Fatty Acid Lipid Standards for Membrane Bilayer Imaging

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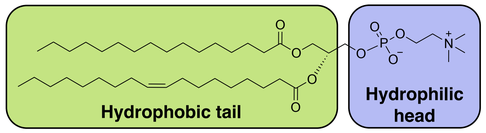
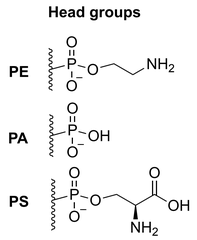
Background

- Lipids are essential class of biomolecules found in all forms of life and in the membranes of cells. They play vital roles in cell signaling, and analyzing and imaging lipids can further our understanding of biological systems.
- Lipids are organized with a polar head group and a non-polar fatty acid tail.





Hydrophilic head groups: Phosphatidylethanolamine (PE), Phosphatic acid (PA), Phosphatidylserine (PS)

- Lipids can be difficult to analyze because conventional methods damage their structure. Common methods that work for analyzing lipids are:

Fluorescence Microscopy
- Fluorescent signal is found in all membranes of organelles.

Liquid Chromatography-Mass Spectrometry (LC-MS)
- Liquid chromatography separates the compounds by polarity, and mass spectrometry ionizes compounds to determine molecular weight for the precise identification of lipid species.

High-Pressure Liquid Chromatography (HPLC)
- Compounds are separated using high-pressure liquid chromatography to enhance performance.
- Paired with a fluorescence detector, retention times of lipids can be observed to identify specific lipids when compared to appropriate standards.

Project Introduction

This project aims to develop standards for click-tag labeling by attaching a fluorophore to fatty acids and lipids providing insight into which species are labeled during lipid imaging.

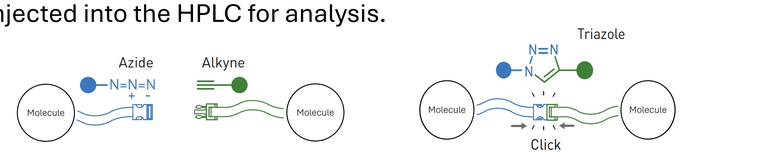
Aim 1: Prepare and fluorescently label free fatty acid standards and determine their retention time on the HPLC.

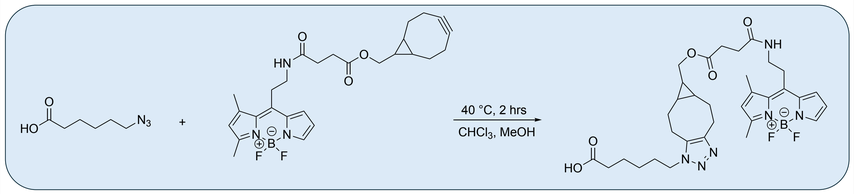
Aim 2: Prepare four separate fluorescently labeled phospholipids with a clickable analog and determine their retention times on the HPLC.

Aim 3: Determine an HPLC method that separates fluorescently labeled free fatty acids from fluorescently labeled phospholipids.

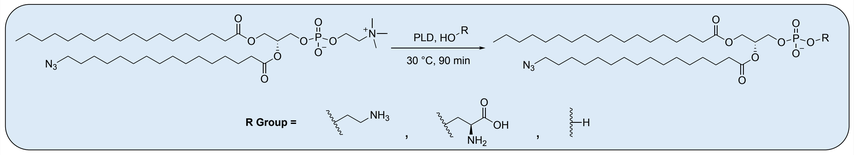
Methodology

- Fatty acids and lipids were clicked to a BODIPY fluorophore and injected into the HPLC for analysis.

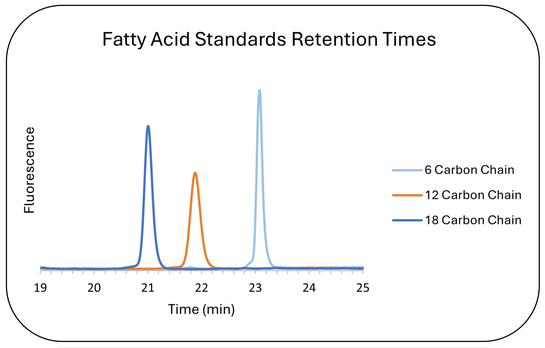


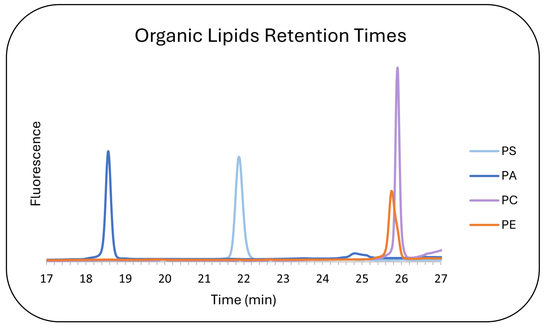


- Phospholipase D (PLD) catalyzes transphosphatidylation, replacing the head group with functionalized alcohols to produce clickable lipid standards.



Results





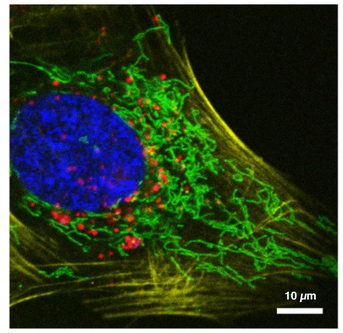
- Fatty acids with longer carbon chains are more hydrophobic and experience less retention on the column. The fatty acid traces are sufficiently separated.

Conclusions

- After running multiple fatty acids of various lengths through HPLC, the result is that longer fatty acid chains, which are less polar, have shorter retention times. From this, a broad conclusion can be drawn that less polar molecules have shorter retention times.
- Through developing accurate standards for common fatty-acids, this project stands as a keystone for future characterization of more complex lipids.

Future Work

- Next steps are to determine an HPLC method that will separate fluorescently labeled free fatty acid from all fluorescently labeled phospholipid standards.
- Characterization of lipids labeled with clickable fatty acids will enable live-cell and super resolution imaging strategies that can be used to understand lipid and membrane function during health and disease.



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