



Skyline tutorial webinar #24

# SKYLINE FOR LIPIDOMICS

**Michele Wölk**

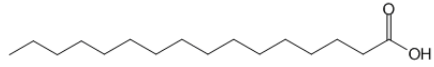
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03.12.2024

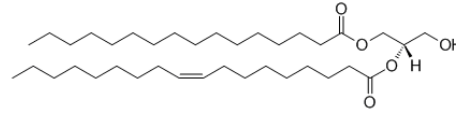


# Lipids and their roles

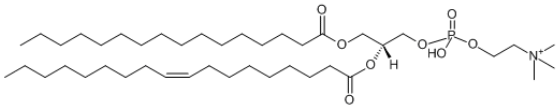
... are hydrophobic or amphipathic small molecules with large spectrum of biological functions



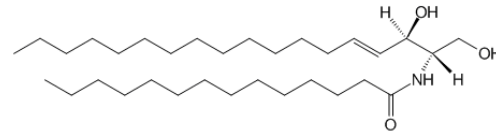
**Fatty Acyls:** hexadecanoic acid



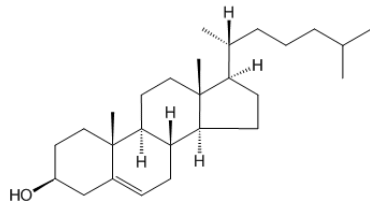
**Glycerolipids:** 1-hexadecanoyl-2-(9Z-octadecenyl)-sn-glycerol



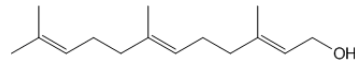
**Glycerophospholipids:** 1-hexadecanoyl-2-(9Z-octadecenyl)-sn-glycerol-3-phosphocholine



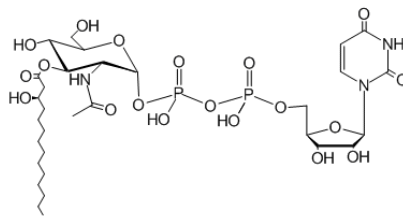
**Sphingolipids:** N-(tetradecanoyl)-sphing-4-enine



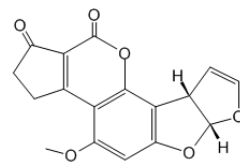
**Sterol Lipids:** cholest-5-en-3 $\beta$ -ol



**Prenol Lipids:** 2E,6E-farnesol



**Saccharolipids:** UDP-3-O-(3R-hydroxy-tetradecanoyl)- $\alpha$ D-N-acetylglucosamine

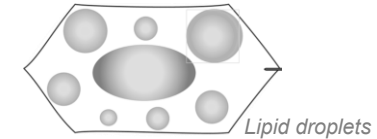


**Polyketides:** aflatoxin B1

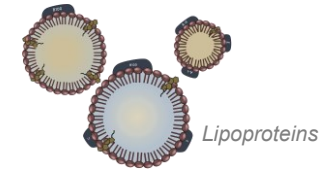
Building cellular and organelle membranes



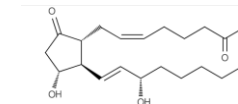
Energy storage



Transport



Cell signaling

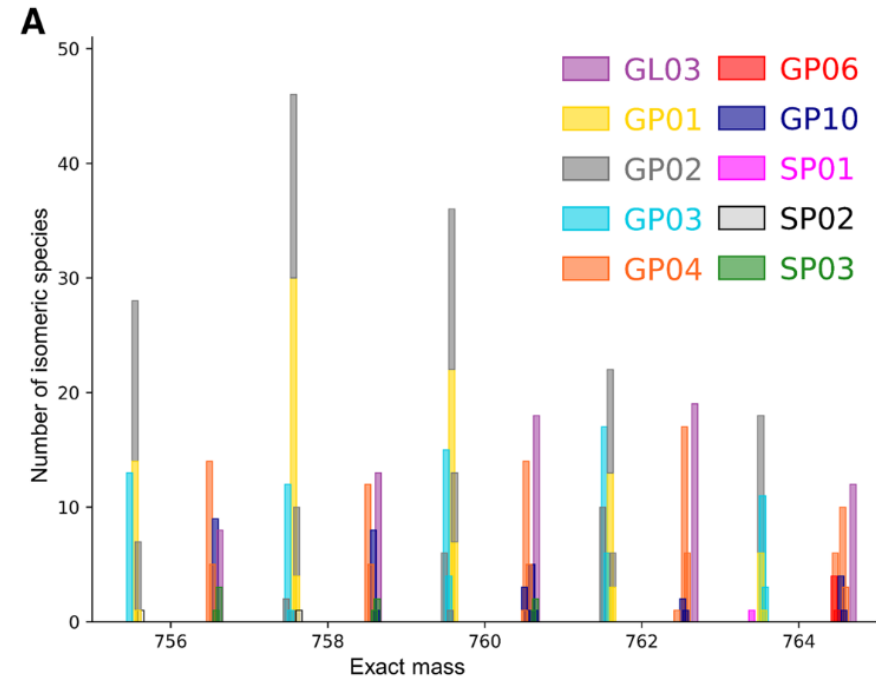


# MS-based lipidomics



Very high number of lipid molecular species occupy relatively narrow  $m/z$  space  
e.g., 40 000 species over  $m/z$  range from 250 till 1250

- **High diversity** of natural lipidomes
- High-number of **isomeric and isobaric** species
  - Unresolved isobars at chosen MS resolution
  - Adduct formation increases complexity further
- **High dynamic range** of lipid concentrations - ionization suppression
- Different chemical properties of lipids – competition for a charge during ionization



possible isomers and potentially overlapping isobars for phospholipids ( $m/z$  755–765) within 5 ppm mass accuracy

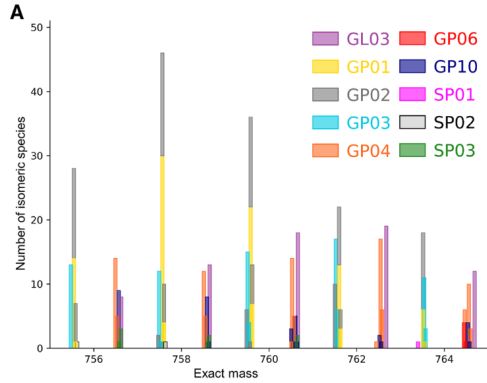
GP01 – PC

GP03 – PS

GP02 – PE

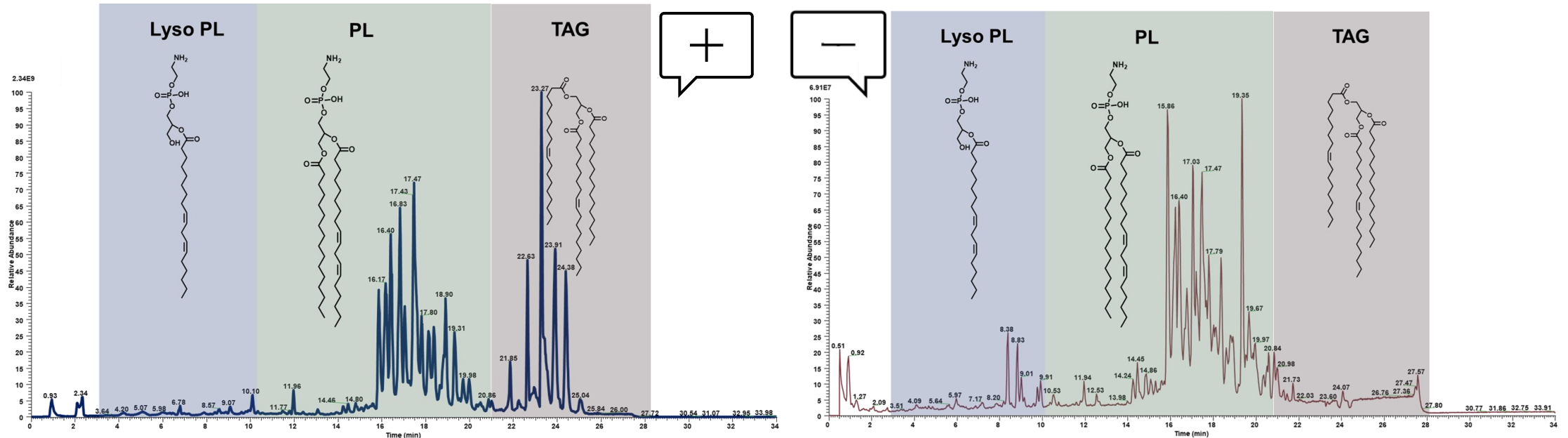
GP04 – PG

# LC- MS-based lipidomics



- Good separation of lipid species crucial for high confident identification and annotation
- Obtained level of structural information depends on lipid class and ionization mode/efficiency

## RPC-MS in positive and negative ionization modes

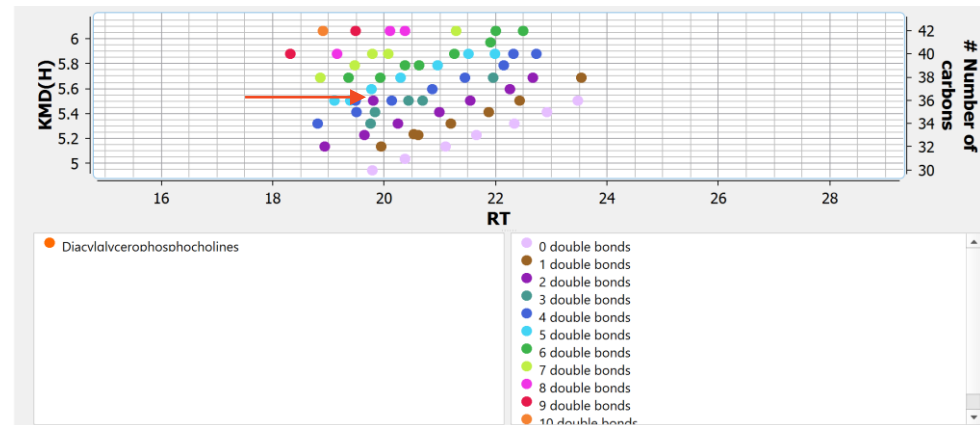
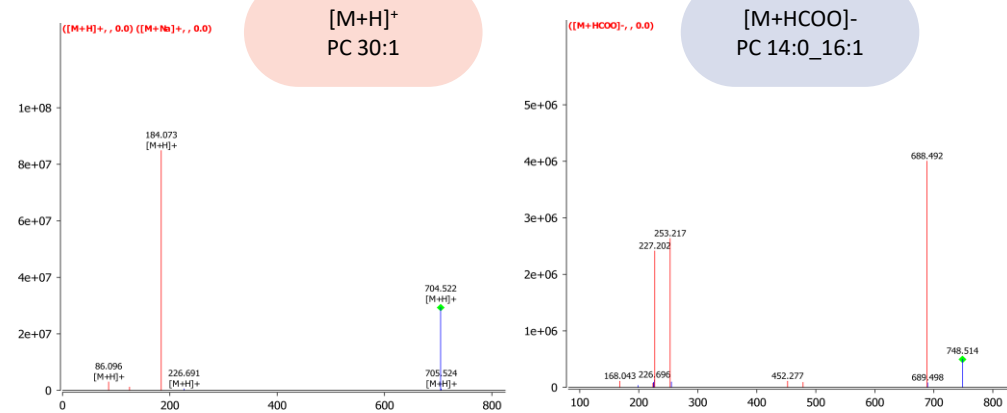
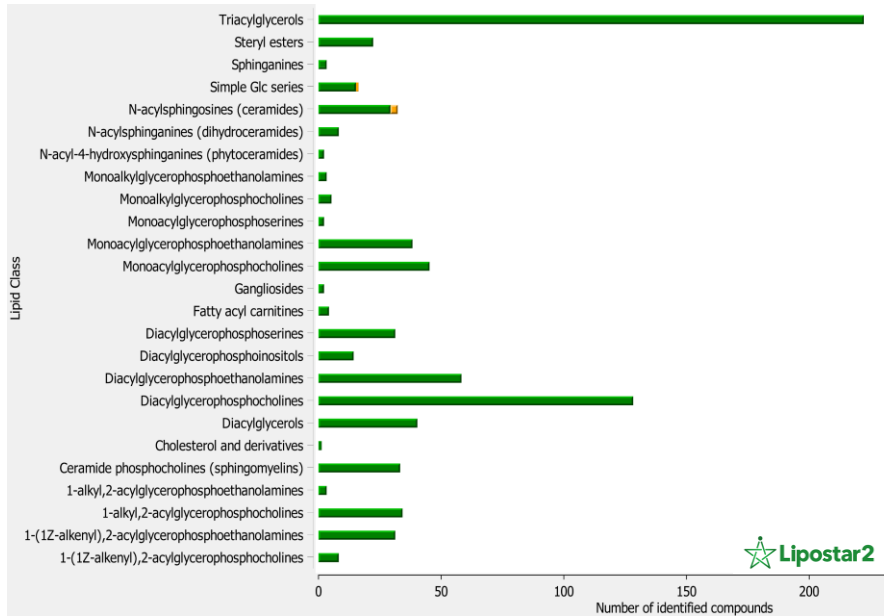


# Lipidomics workflow



## ① Deep profiling and accurate annotation of lipidome of interest

- ✓ Generate a representative pooled sample
- ✓ Select optimal lipid extraction protocol
- ✓ Perform deep LC-MS/MS analysis using orthogonal LC and MS methods
- ✓ Perform accurate lipid annotation based on MS/MS fragmentation patterns and RT mapping

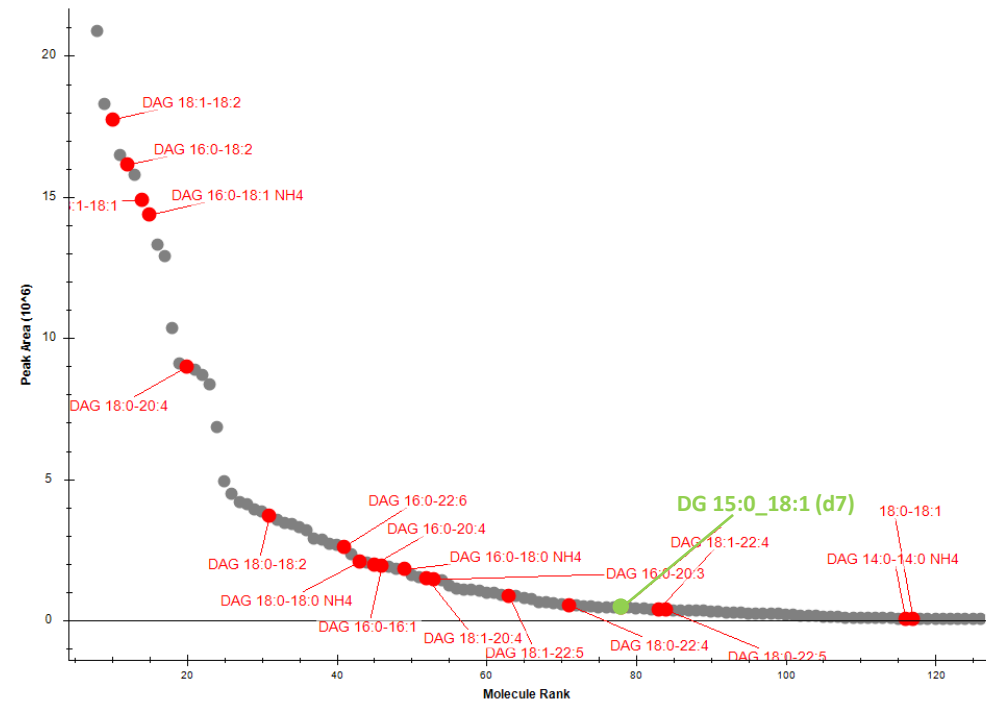
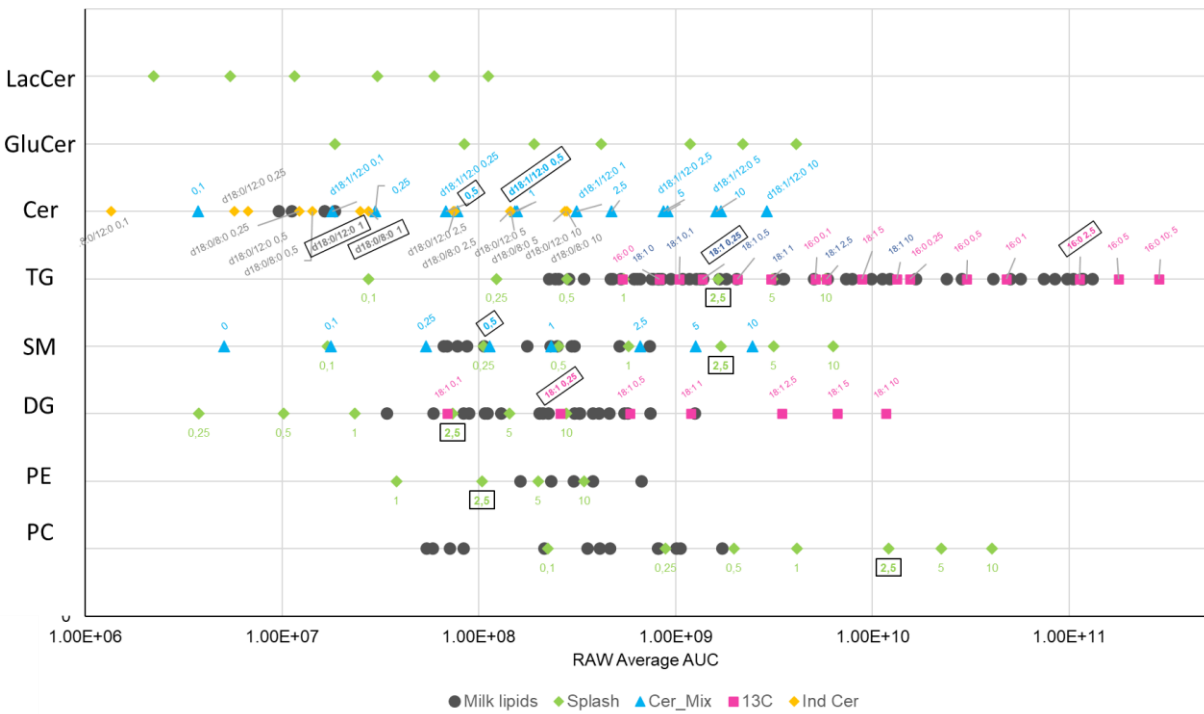


# Lipidomics workflow



**② Design of lipidome-specific ISTD mixture**

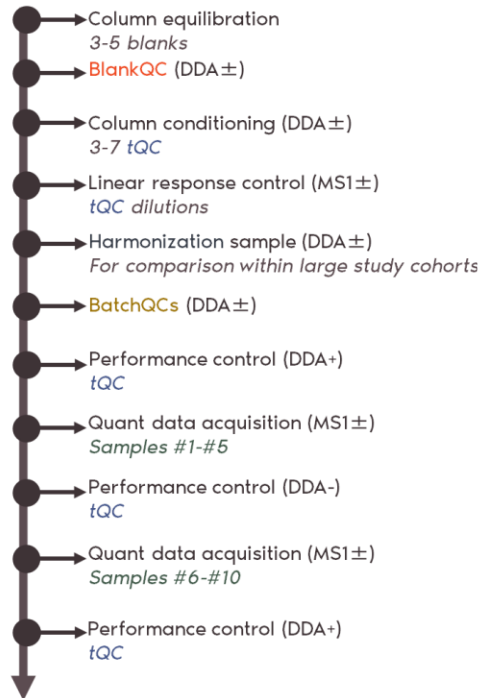
- ✓ Select lipidome-specific set of ISTDs
- ✓ Determine assay LDR, LOD and LOQ in sample matrix
- ✓ Define optimal ISTD amounts for a studied lipidome
- ✓ Design a final ISTD mixture for 1-point calibration



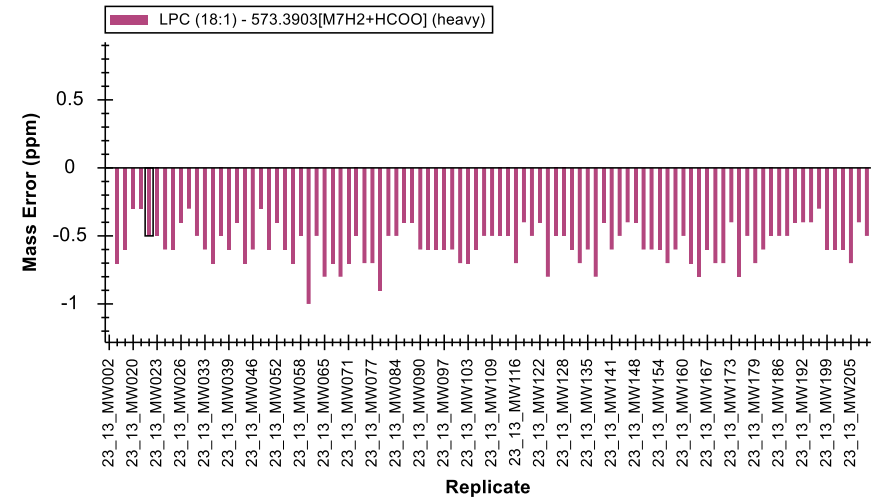
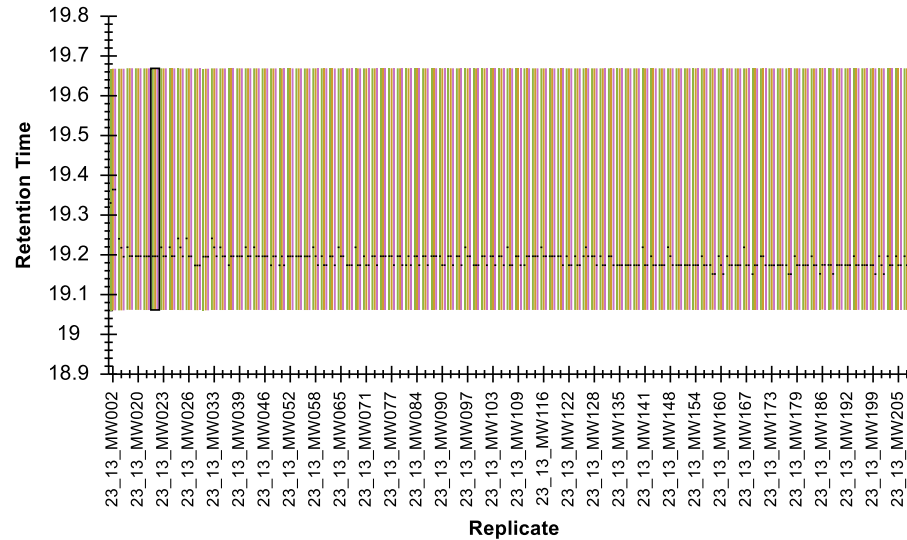


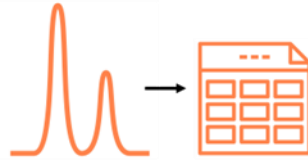
### 3 Batch extraction and data acquisition

- ✓ Randomize cohort samples, divide into batches and create QCs
- ✓ Spike with ISTD mixture and extract lipid
- ✓ Condition LC-MS system and run all necessary QCs
- ✓ Run individual samples, include tQC after every 10<sup>th</sup> injection



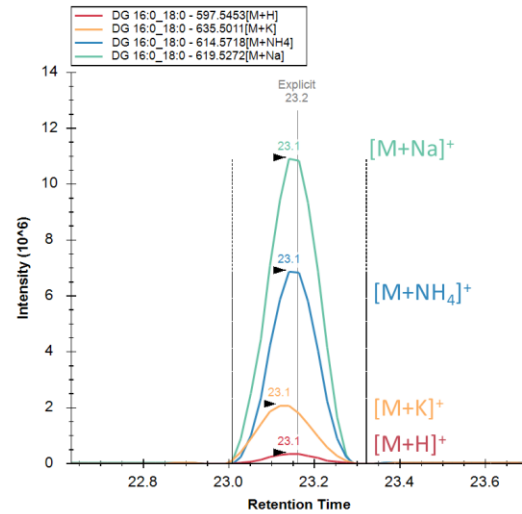
Monitor retention time stability & mass error over time



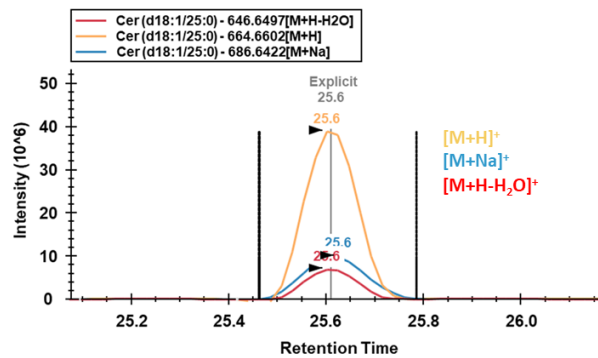


## ④ Data processing for semi-absolute quantification

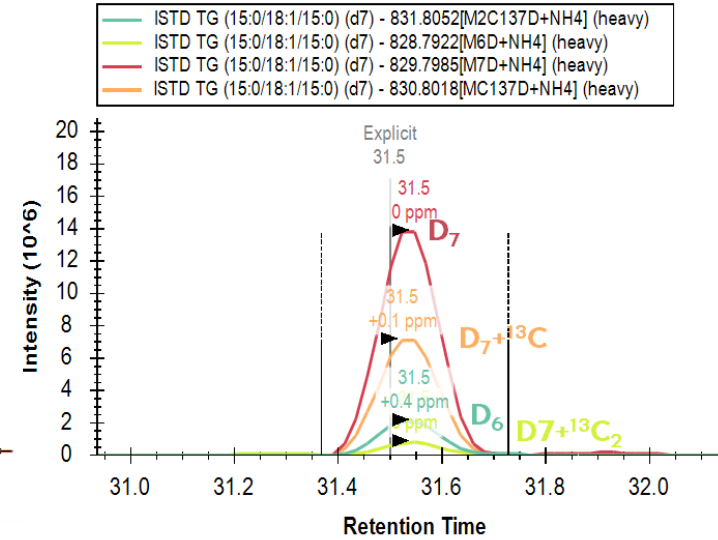
- ✓ Perform peak area integration considering all necessary ionization adducts and ISF ions
- ✓ Perform isotopic corrections and correction for incomplete ISTD labelling
- ✓ Quantify endogenous lipids based on the added ISTDs
- ✓ Prepared reporting summary and upload data to the public repository



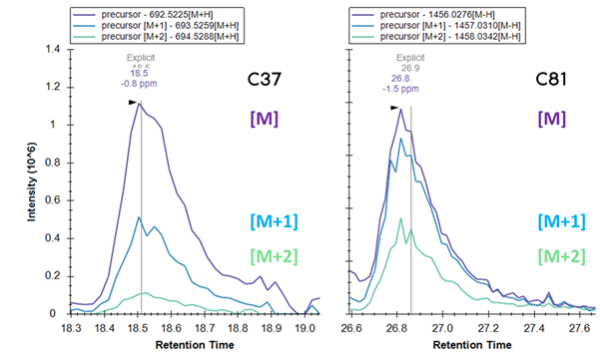
DG 16:0\_18:0  
Identification based on MS2 of [M+NH<sub>4</sub>]<sup>+</sup>  
Consider all adducts for accurate quan



In-source fragmentation



Incomplete labelling of deuterated Standards:  
consider all isotopologues for accurate quan



Isotope abundance



# The tutorial



I. Brief introduction to data set



II. Set up Skyline for small molecules



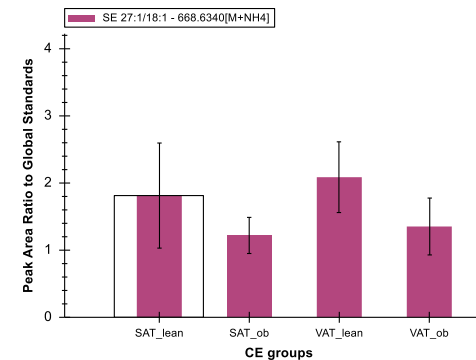
III. Create a transition list using Lipid Creator



IV. Relative quantification: normalize to deuterated internal standard



V. Performing a basic group comparison



# Lipidomics tool guide



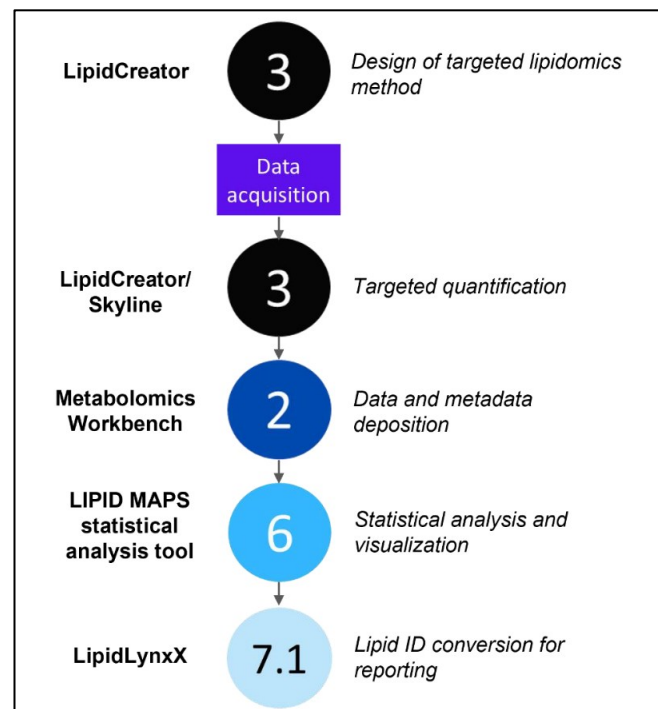
Perspective | Published: 21 December 2022

## Guiding the choice of informatics software and tools for lipidomics research applications

Zhixu Ni, Michele Wölk, Geoff Jukes, Karla Mendivelso Espinosa, Robert Ahrends, Lucila Aimo, Jorge Alvarez-Jarreta, Simon Andrews, Robert Andrews, Alan Bridge, Jeremy C. Clair, Matthew J. Conroy, Eoin Fahy, Caroline Gaud, Laura Goracci, Jürgen Hartler, Nils Hoffmann, Dominik Kopczyk, Ansgar Korf, Andrea F. Lopez-Clavijo, Adnan Malik, Jacobo Miranda Ackerman, Martijn R. Molenaar, Claire O'Donovan, ... Maria Fedorova [+ Show authors](#)

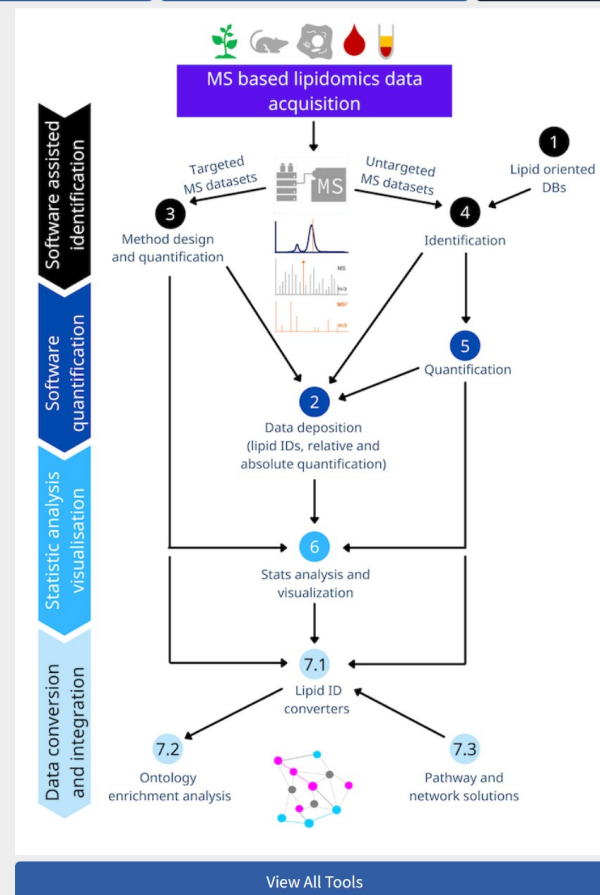
*Nature Methods* 20, 193–204 (2023) | [Cite this article](#)

20k Accesses | 30 Citations | 67 Altmetric | [Metrics](#)



## Lipidomics Tools Guide

MS Analysis	Structure Drawing Tools	Statistical Analysis Tools
Nomenclature Tools	Lipid Analysis Software	Lipidomics Tools Guide



### Reference

Guiding the choice of informatics software and tools for lipidomics research applications, *Nature Methods*, 2022, DOI: 10.1038/s41592-022-01710-0

# The tutorial data set: AdipoAtlas



## Cell Reports Medicine

Volume 2, Issue 10, 19 October 2021, 100407

Article

### AdipoAtlas: A reference lipidome for human white adipose tissue

Mike Lange<sup>1,2,7</sup>, Georgia Angelidou<sup>1,2</sup>, Zhixu Ni<sup>1,2,8</sup>, Angela Criscuolo<sup>1,2,3</sup>, Jürgen Schiller<sup>4</sup>, Matthias Blüher<sup>5,6</sup>, Maria Fedorova<sup>1,2,8,9</sup>

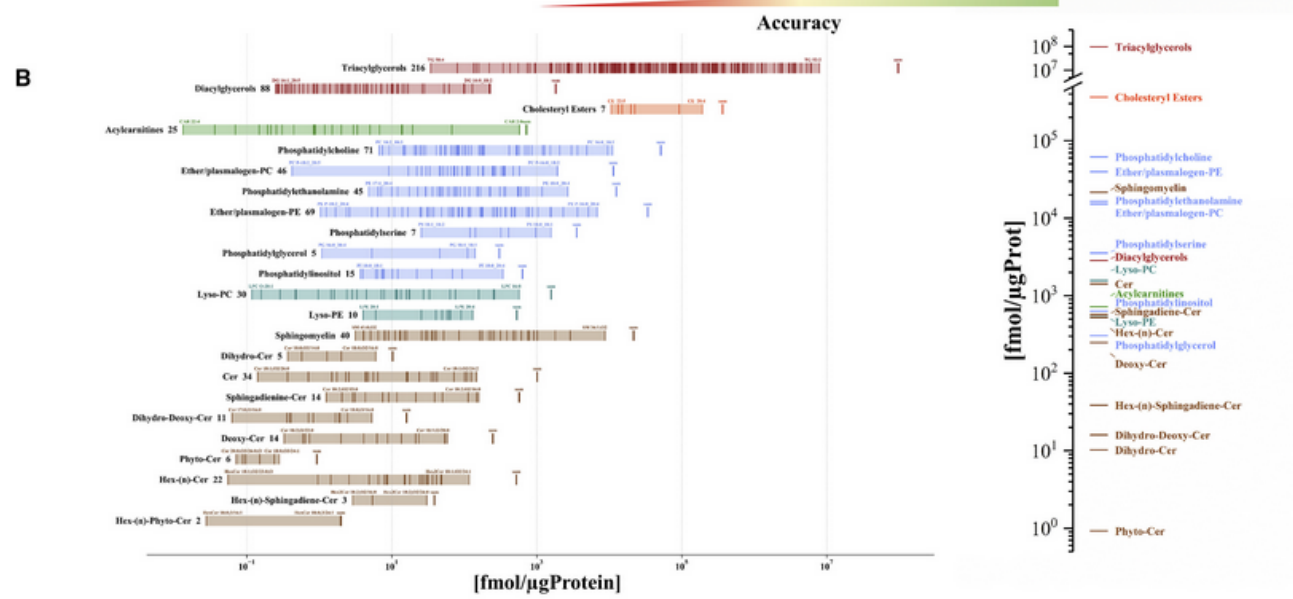
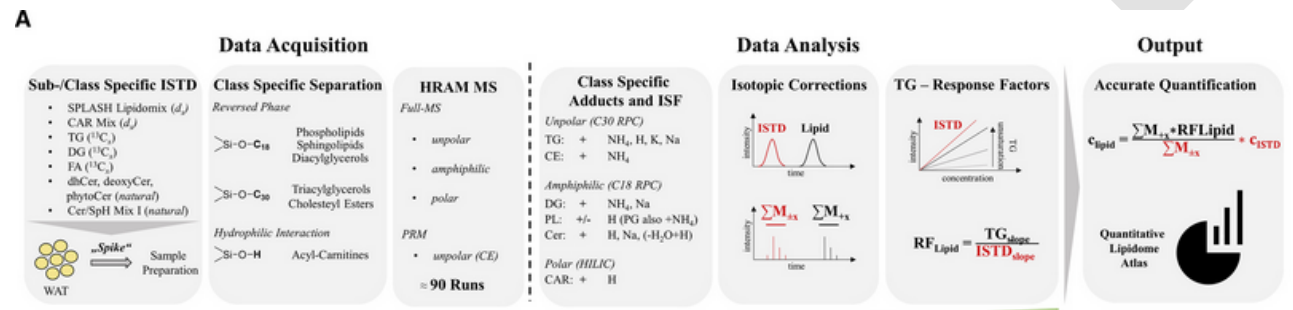
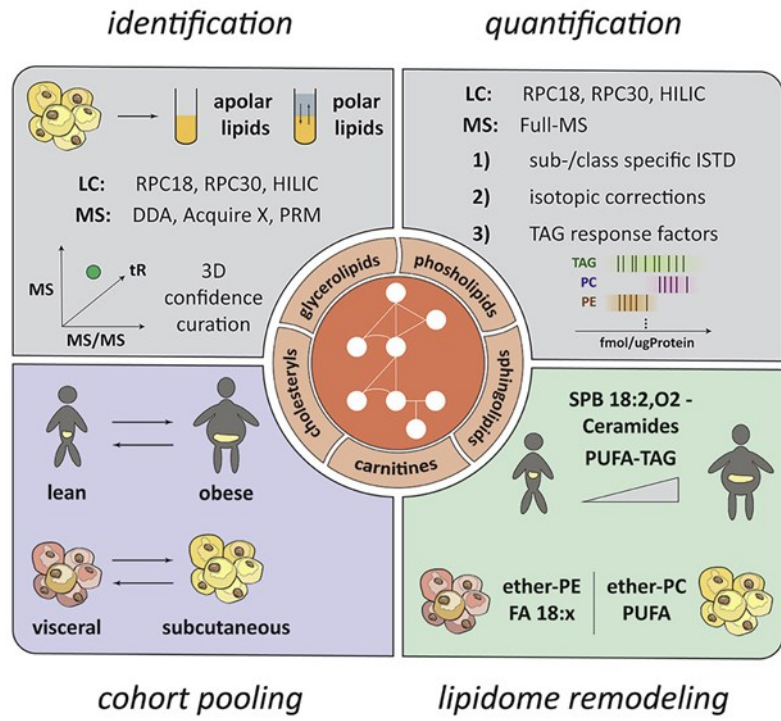
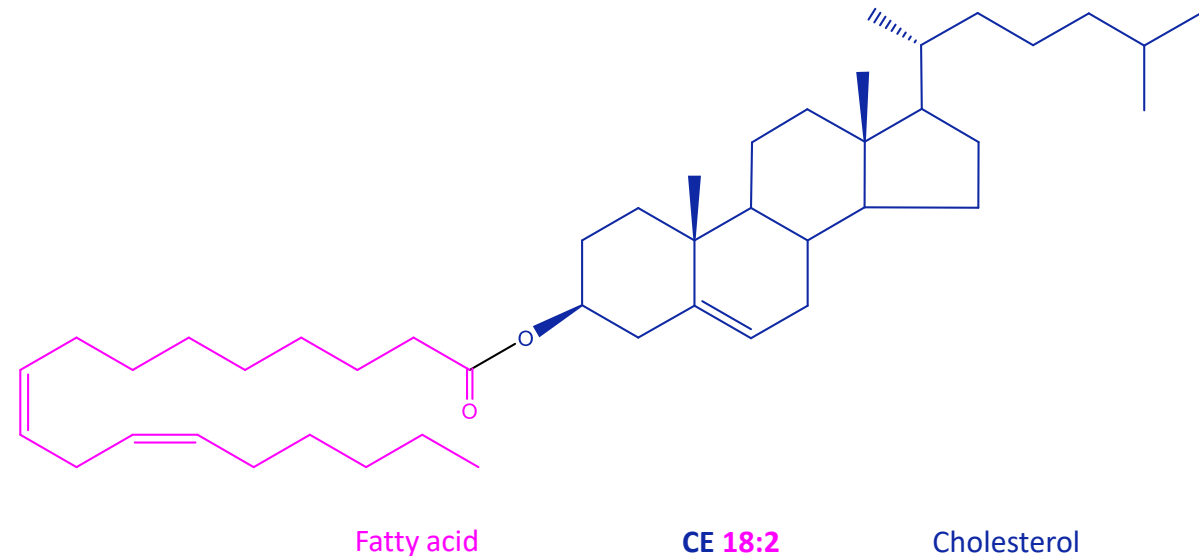


Figure 3: Quantitative representation of human WAT lipidome and description of analytical strategy used. (A) Schematic depiction of the quantitative lipidomics workflow. (B) Quantitative distribution of lipid class and corresponding lipid molecular species within subclasses of human WAT. Total lipid class concentration is represented by bold lines (SUM), and each single lipid molecular species is represented by thin lines.

# The tutorial data set: PRM data for Cholesteryl esters



- Esters of cholesterol with ester bond formed between the carboxylate group of a fatty acid and the hydroxyl group of cholesterol
- Found in lipid droplets & lipoproteins
- Transport and storage of cholesterol
- Nomenclature: **CE X:Y**

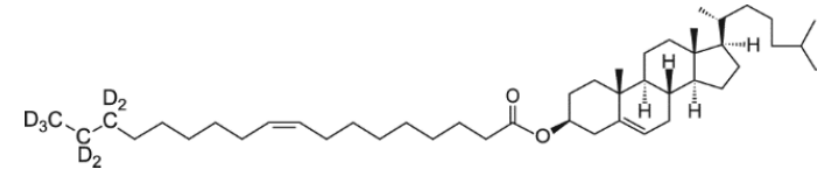
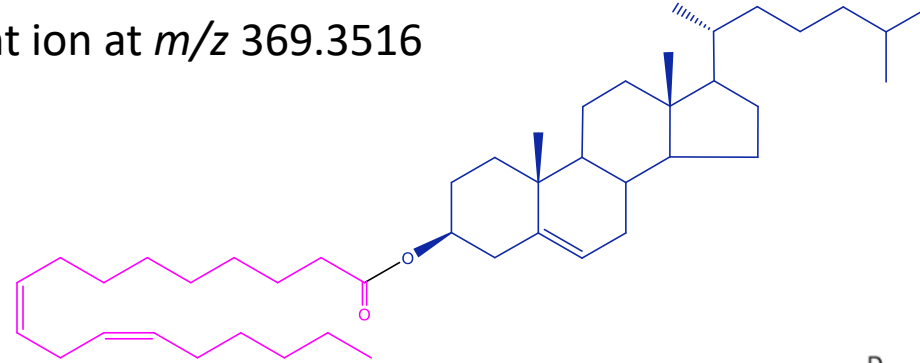


# The tutorial data set: PRM data for Cholesteryl esters

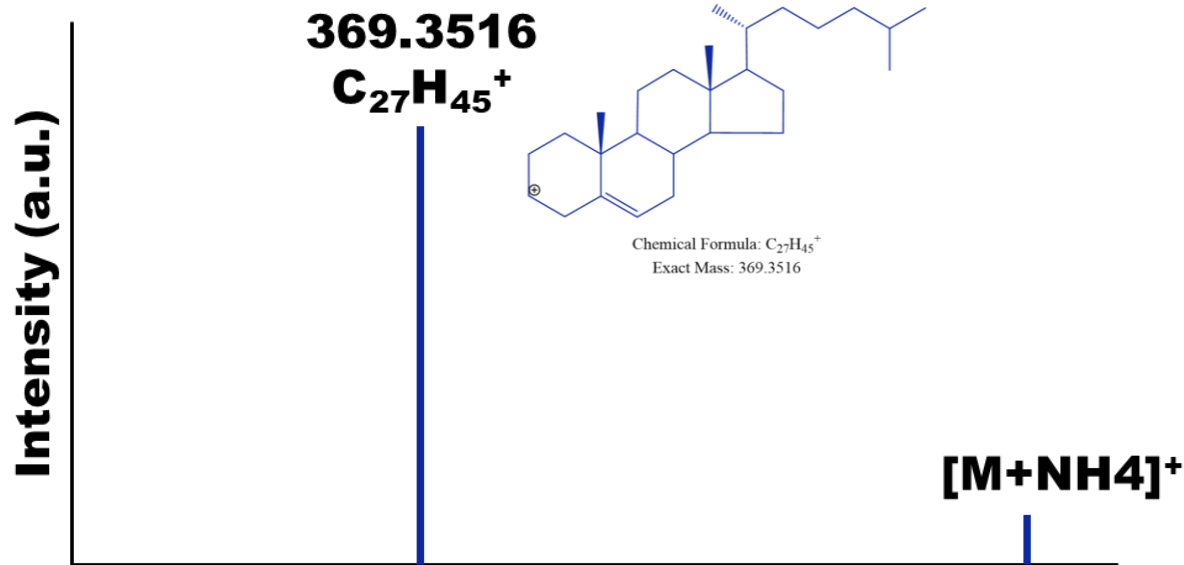


For our transition list:

CE X:Y results in fragment ion at  $m/z$  369.3516



Internal standard (d7 @ acyl chain)



CE	Retention time (min)
CE 18:1 (d7)	24.99
CE 18:1	24.99
CE 18:2	23.00
CE 20:3	23.29
CE 20:4	21.68
CE 22:4	23.71
CE 22:5	22.04
CE 22:6	20.96

# LipidCreator within Skyline for generation of transition lists



Article | [Open Access](#) | [Published: 28 April 2020](#)

## LipidCreator workbench to probe the lipidomic landscape

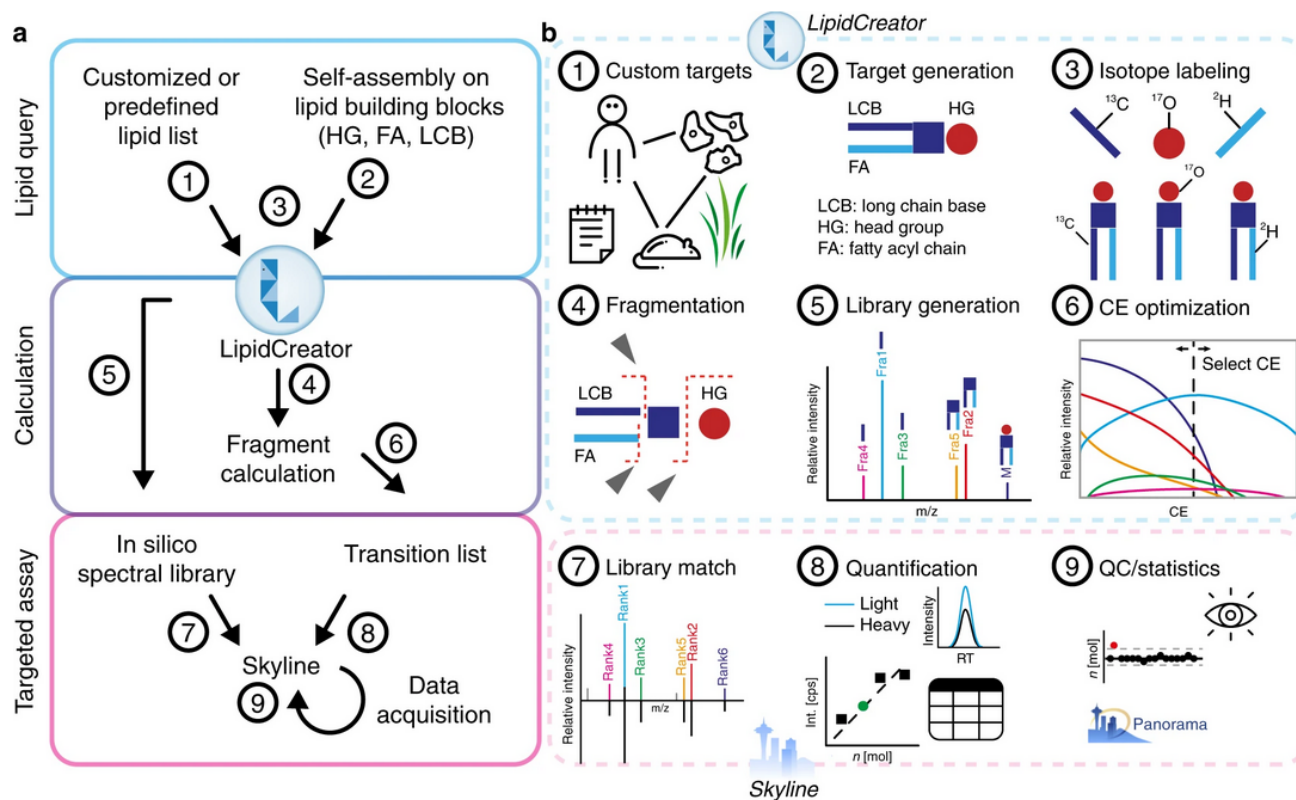
[Bing Peng](#), [Dominik Kopczynski](#), [Brian S. Pratt](#), [Christer S. Ejsing](#), [Bo Burla](#), [Martin Hermansson](#), [Peter Imre Benke](#), [Sock Hwee Tan](#), [Mark Y. Chan](#), [Federico Torta](#), [Dominik Schwudke](#), [Sven W. Meckelmann](#), [Cristina Coman](#), [Oliver J. Schmitz](#), [Brendan MacLean](#), [Mailin-Christin Manke](#), [Oliver Borst](#), [Markus R. Wenk](#), [Nils Hoffmann](#) & [Robert Ahrends](#) ✉

[Nature Communications](#) **11**, Article number: 2057 (2020) | [Cite this article](#)

8294 Accesses | 39 Citations | 144 Altmetric | [Metrics](#)

**Fig. 1: The LipidCreator workbench and its integration into Skyline.**

From: [LipidCreator workbench to probe the lipidomic landscape](#)







### LipidHunter 2

- Identification of unmodified lipids

[github.com/SysMedOs/lipidhunter](https://github.com/SysMedOs/lipidhunter)

### LipidLynxX

- Convert, equalize & link Lipid abbreviations

[github.com/SysMedOs/LipidLynxX](https://github.com/SysMedOs/LipidLynxX)

### LPptiger 2

- Prediction & identification of **oxidized** Lipids

[github.com/LMAI-TUD/lpptiger2](https://github.com/LMAI-TUD/lpptiger2)



LIPID MAPS  
Tools Guide



LPptiger2  
Project Page

For more information about our research & lipidomics applications visit  
[fedorovalab.net](https://fedorovalab.net)

