TOWARDS THE USE OF LIPIDOMICS IN CLINICAL PRACTICE

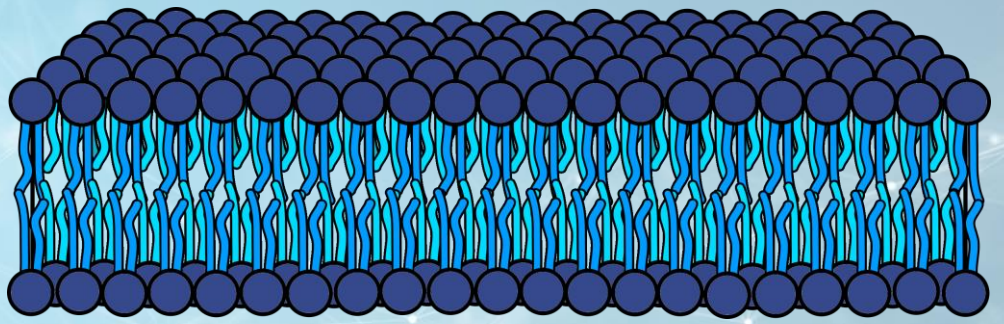
Identification and Quantification of Oxidized Lipids in LC-MS Lipidomics Data

Christoph A. Krettler

Background - Lipids

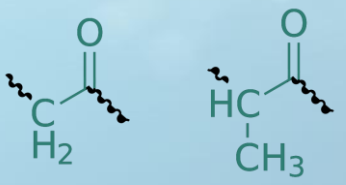
- Biomolecules soluble in nonpolar solvents

- Functions
  - Membrane structure
  - Energy and heat source
  - Signaling processes

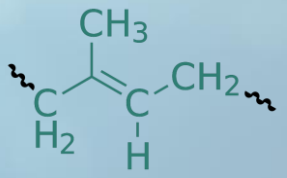


- Totality of lipids = lipidome
  - Tens of thousands to millions, depending on resolution

- Structural diversity
  - Classified in eight categories (LIPID MAPS)
  - Classes and subclasses

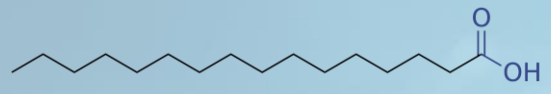


Isoprene
building blocks

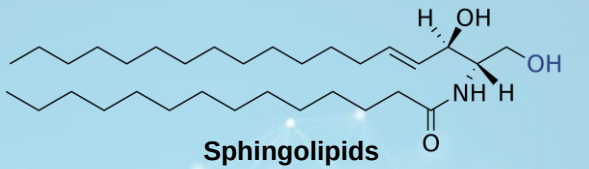


Ketoacyl
building blocks

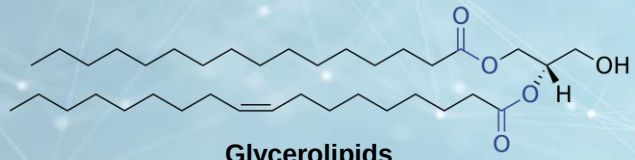
Background - Lipids



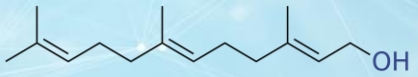
Fatty Acyls
(hexadecanoic acid)



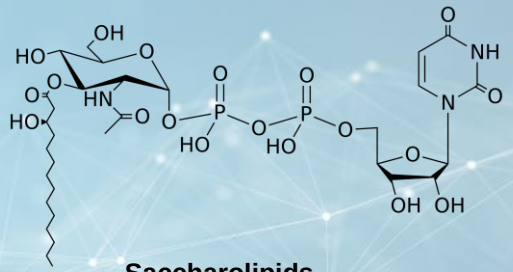
Sphingolipids
(N-(tetradecanoyl)-sphing-4-enine)



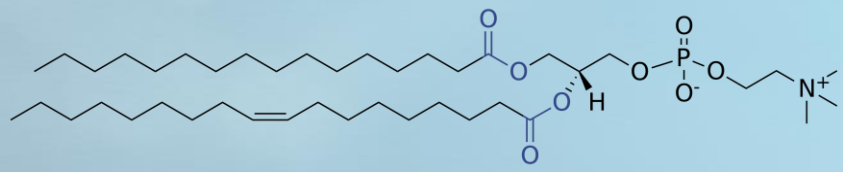
Glycerolipids
(1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycerol)



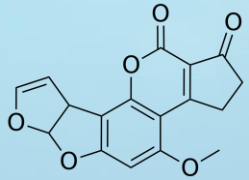
Prenol lipids
(2E,6E-farnesol)



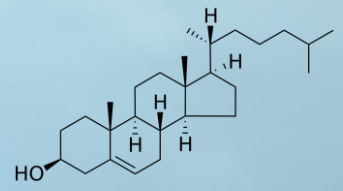
Saccharolipids
(UDP-3-O-(3R-hydroxy-tetradecanoyl)-αD-N-acetylglucosamine)



Glycerophospholipids
(1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine)



Polyketides
(aflatoxin B1)



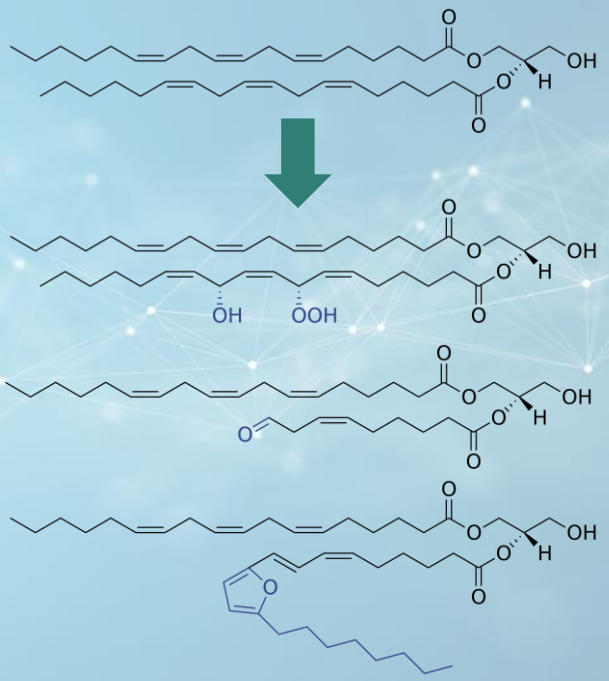
Sterol lipids
(choles-5-en-3β-ol)

Background - Oxidized Lipids

- Lipid peroxidation
  - Initiated by free radicals or enzymes
  - (Unsaturated) fatty acids are major targets
  - Degree of unsaturation ~ oxidation rate

- Long-chain products
- Short-chain products
- Cyclization

- Pathological roles
  - Age-related and chronic diseases
  - Atherosclerosis and immune response
  - Mutagenic and carcinogenic properties
  - Change in membrane integrity → apoptotic events



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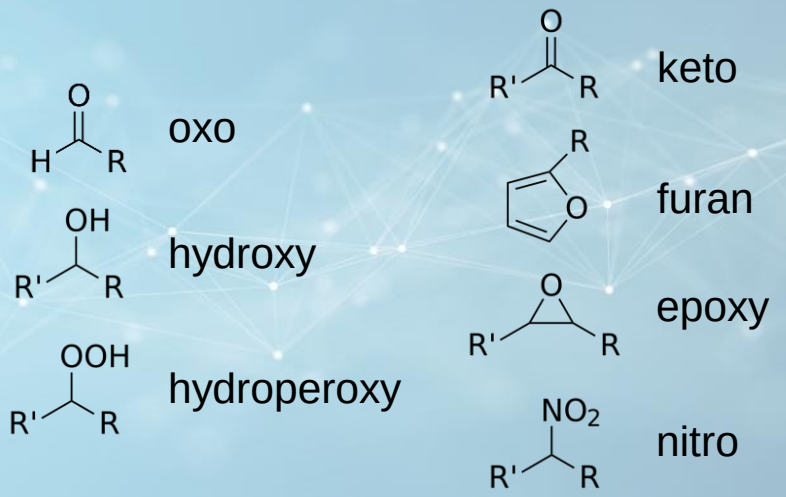


Background - Lipid Analysis

- Analyzing the entire complement of lipids
  - Biological samples
- Sample preparation
  - Major impact on quality and throughput
- Mass spectrometry (MS)
  - High throughput
  - Qualitative
  - Quantitative

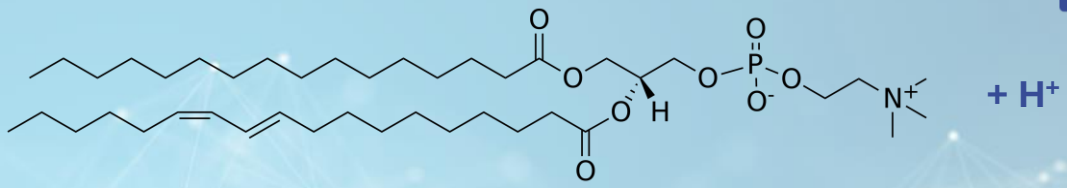
Background - Lipid Analysis

- Adduct formation during MS analysis

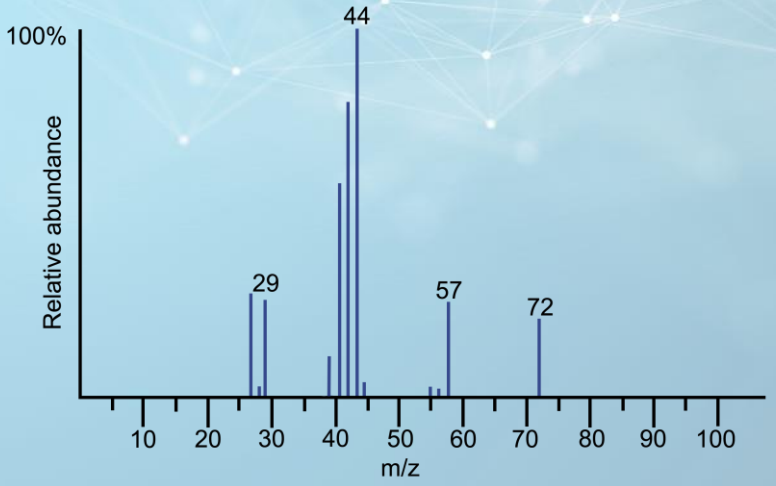
- Charged molecules
  - [M ± molecule]^{charge}
  - or
  - Lipid notation ± molecule^{charge}

- Output = Spectrum
  - Ion abundance vs. m/z ratio

- Tandem MS (MS^2)
  - Second step, fragmentation
  - → Structural information



PC(16:0_18:2) + H+



Background - Lipid Data Analyzer (LDA)[1]

- Developed at TU Graz
- 3D algorithm

- MS^1
  - Predefined target masses (masslist)

- MS^2
  - Decision rulesets
  - Fatty acid (FA) masslist

- Identification levels
  - Precursor mass (MS^1, no structural information)
  - Headgroup (MS^2)
  - Fatty acyl constituents (MS^2)
  - Fatty acyl *sn*-position (MS^2)

1 Hartler J, Triebl A, Ziegl A, Trötzmüller M, Rechberger GN, Zeleznik AO, et al. **Deciphering lipid structures based on platform-independent decision rules**. *Nature Methods* 2017;14(23), 1171-80

Background - Clinical Practice

- LDA - US patent application 2,013,012,6725
  - *[For...] monitoring quantitative changes of analytes and for monitoring progress or treatment of a disease…*
- New frontiers of health prevention and disease treatment
- Reliable analytical determination + biological role
  - → guide clinicians' decisional process
- Oxidized Lipids
  - *The majority of the oxidative stress tests available on the market use imprecise or non-optimized methodologies*[1]

1 Ferreri C, Chatgilialoglu C**. Membrane Lipidomics for Personalized Health**. Hoboken, NJ, USA: John Wiley & Sons Ltd, 2015.

Aims of the Project

- Identify LDA implementation gaps
- Implementation gap → oxidized lipids
- Implement novel features
- Generate LDA appropriate masslists
- Extend the LDA decision ruleset
- Analyze datasets with the extended LDA version
  - + Benchmarking

Results - Novel Features

- Extended FA masslist
  - New column: oxidation-state
  - Modifications separated by ;
- Extended masslist
  - New column: oxidation-state
  - Modifications separated by ;
- Example
  - oxMGDG(36:6)
  - oxMGDG(36:6[OH])
  - oxMGDG(36:6[2OH])
  - oxMGDG(36:6[3OH])
  - oxMGDG(36:6[4OH])

New FA masslist; excerpt

Name		dbs	C	H	O	mass	oxidation-state
2	:	0	2	4	2	60.0211205	;OH;2OH;3OH;4OH
3	:	0	3	6	2	74.0367706	;OH;2OH;3OH;4OH
4	:	0	4	8	2	88.0524206	;OH;2OH;3OH;4OH
5	:	0	5	10	2	102.068071	;OH;2OH;3OH;4OH
6	:	0	6	12	2	116.083721	;OH;2OH;3OH;4OH
7	:	0	7	14	2	130.099371	;OH;2OH;3OH;4OH
8	:	0	8	16	2	144.115021	;OH;2OH;3OH;4OH
9	:	0	9	18	2	158.130671	;OH;2OH;3OH;4OH
10	:	0	10	20	2	172.146321	;OH;2OH;3OH;4OH
11	:	0	11	22	2	186.161971	;OH;2OH;3OH;4OH

New MGDG masslist; excerpt

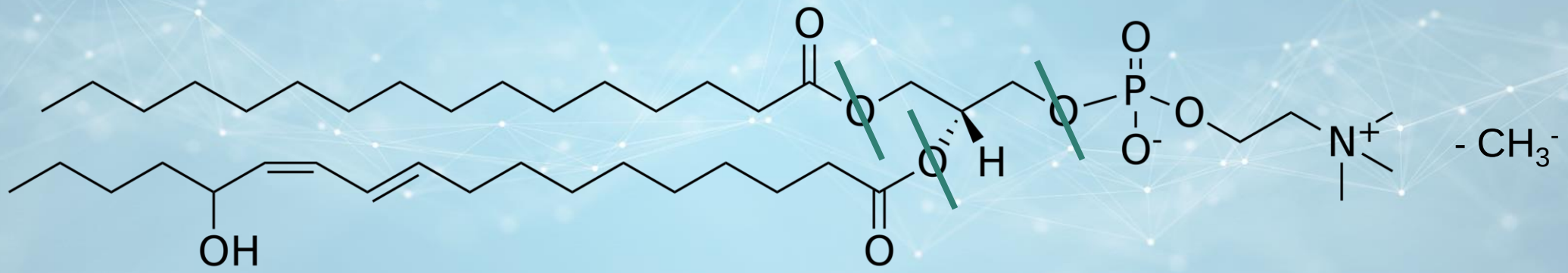
Name		dbs	C	H	O	mass(form[+NH4] name[NH4])	mass(form[+Na name[Na])	mass(form[+H] name[H])	oxidation-state
20	:	0	29	54	10	580.4055235	585.360919	563.3789744	;OH;2OH;3OH;4OH
20	:	1	29	52	10	578.3898734	583.345269	561.3633243	;OH;2OH;3OH;4OH
20	:	2	29	50	10	576.3742234	581.3296189	559.3476743	;OH;2OH;3OH;4OH
20	:	3	29	48	10	574.3585733	579.3139688	557.3320242	;OH;2OH;3OH;4OH
20	:	4	29	46	10	572.3429232	577.2983188	555.3163741	;OH;2OH;3OH;4OH
21	:	0	30	56	10	594.4211736	599.3765691	577.3946245	;OH;2OH;3OH;4OH
21	:	1	30	54	10	592.4055235	597.360919	575.3789744	;OH;2OH;3OH;4OH
21	:	2	30	52	10	590.3898734	595.345269	573.3633243	;OH;2OH;3OH;4OH
21	:	3	30	50	10	588.3742234	593.3296189	571.3476743	;OH;2OH;3OH;4OH
21	:	4	30	48	10	586.3585733	591.3139688	569.3320242	;OH;2OH;3OH;4OH

Results - Masslists

- Calculated programmatically
- Defined in XML file

Modification	Fatty Acyl (FA) Mass Formula
oxo, keto, epoxy, furan	FA + O - H_2
hydroxy	FA + O
hydroperoxy	FA + O_2
bromo	FA + Br - H
chloro	FA + Cl - H
fluoro	FA + Fl - H
nitro	FA + NO_2- H

Results - Rulesets



oxPC(16:0_18:2[OH]) - CH_3^-

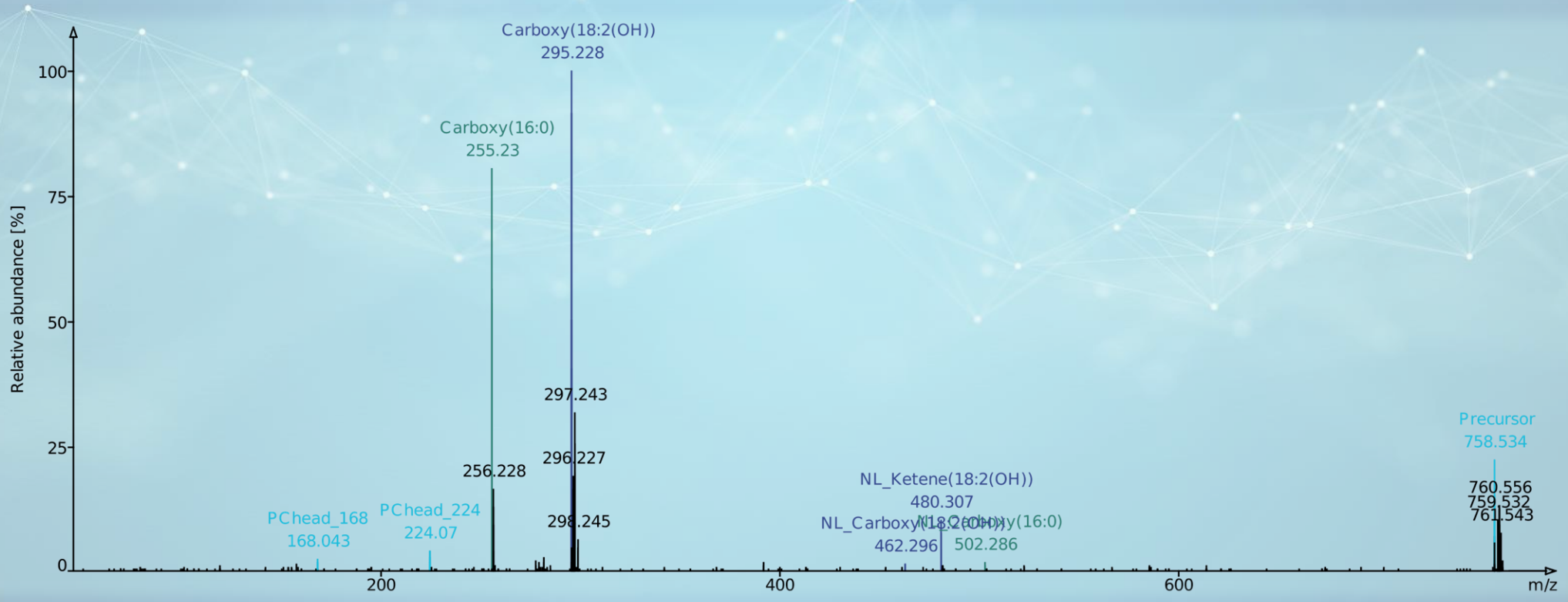
Results - Rulesets

PC_-CH3.frag.txt

```
[GENERAL]
AmountOfChains=2
BasePeakCutoff=0.01%
RetentionTimePostprocessing=true


[HEAD]
!FRAGMENTS
Name=NL_PChead_60     Formula=$PRECURSOR-C2O2H4  Charge=1   MSLevel=2   mandatory=false
Name=PChead_168       Formula=C4H11NO4P          Charge=1   MSLevel=2   mandatory=false


[CHAINS]
!FRAGMENTS
Name=Carboxy          Formula=$CHAIN-H           Charge=1   MSLevel=2   mandatory=false

[POSITION]
!INTENSITIES
Equation=Carboxy[2]*0.5>Carboxy[1]                                       mandatory=false
```

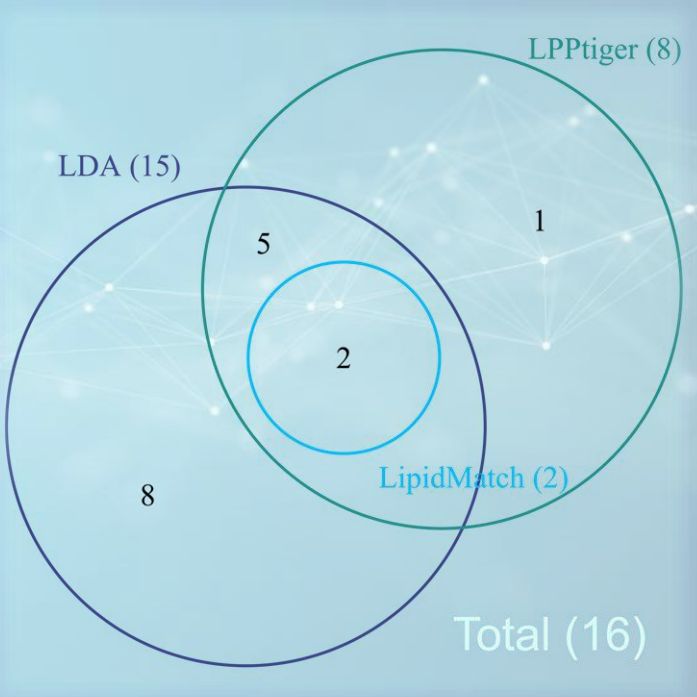
Results - Rulesets

oxPC(34:2[OH]) - CH_3^- → oxPC(16:0_18:2[OH]) - CH_3^-



Results - Benchmarking

- LDA vs. LPPtiger vs. LM (dataset 1) [1]
  - Comparison proves reliability of LDA implementations
  - LDA covers $[M\text{-}CH_3]^-$ adduct → 9 identifications
  - $[M+HCO_2]^-$ adduct → LDA (10), LPPtiger (8), LM (3)
  - LDA - four false positives (4 out of 19)



Venn diagram (without false positives)

1 Ni Z, Angelidou G, Hoffmann R, Fedorova M. **LPPtiger software for lipidome-specific prediction and identification of oxidized phospholipids from LC-MS datasets**. *Scientific Reports* 2017;7(11), 15138.

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- LDA vs. manual curation (dataset 2) [2]
  - Similar results for most classes
  - MGDG(~85%), DGDG (~93%), …

Comparison for oxTGs

Modification	LDA	Manual Curation
0 OH	68	71
1 OH	41	58
2 OH	14	23
3 OH	8	12
4 OH	2	9

1 Ni Z, Angelidou G, Hoffmann R, Fedorova M. **LPPtiger software for lipidome-specific prediction and identification of oxidized phospholipids from LC-MS datasets**. *Scientific Reports* 2017;7(11), 15138.

2 Riewe D, Wiebach J, Altmann T. **Structure Annotation and Quantification of Wheat Seed Oxidized Lipids by High-Resolution LC-MS/MS**. *Plant Physiology* 2017;175(8), 600-18.

Summary

- Challenges
  - No universal nomenclature
  - Ambiguities – isomers, isobars

- LDA (https://genome.tugraz.at/lda) → **Identification of oxidized lipids**
  - Comparisons prove reliability
  - LDA better overall performance (F_1 0.86)
  - LPPtiger (F_1 0.67), LM (F_1 0.22) comparison for dataset 3 only
  - False positives and missed identifications → non-optimized settings
    → **Use in clinical practice and for personalized strategies**

- Future
  - Disambiguation of oxidized isomers, isobars
  - Advanced statistical features
  - In-silico modelling of fragmentation