ÚOCHB AV ČR
IOCB PRAGUE

Workflow and applications in lipidomics

RNDr. Vladimír Vrkoslav, Ph.D.

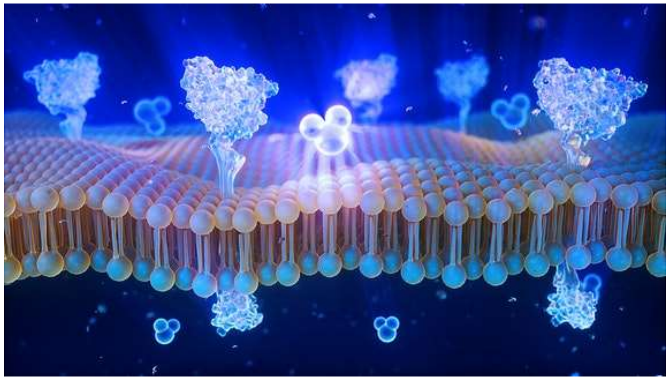
Lipids

Definition:

- hydrophobic or amphiphilic molecules, soluble in nonpolar solvents
- hydrophobic or amphipathic small molecules that originate entirely or in part by carbanion-based condensations of thioesters and/or by carbocation - based condensations of isoprene units. *J. Lipid Res.* 50: S9-S14, (2009)

Funcions:

- storing energy
- signaling processes
- structural components of cell membranes



→ crucial role in many biological processes

imbalance in homeostasis can lead to serious conditions (chronic inflammation, diabetes, neurodegenerative diseases,…)

https://www.lipidmaps.org

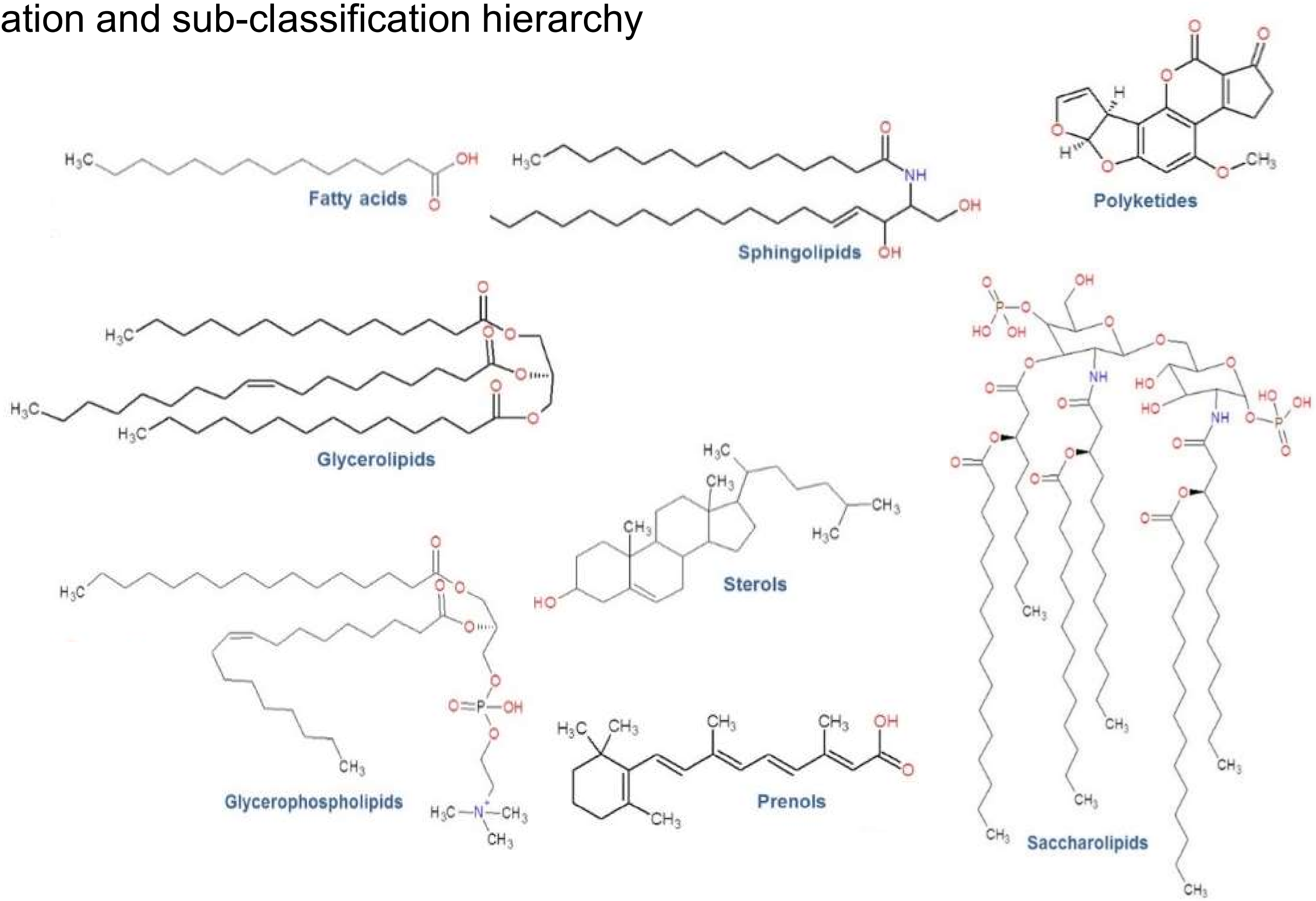
Lipid Classification System

https://www.lipidmaps.org/

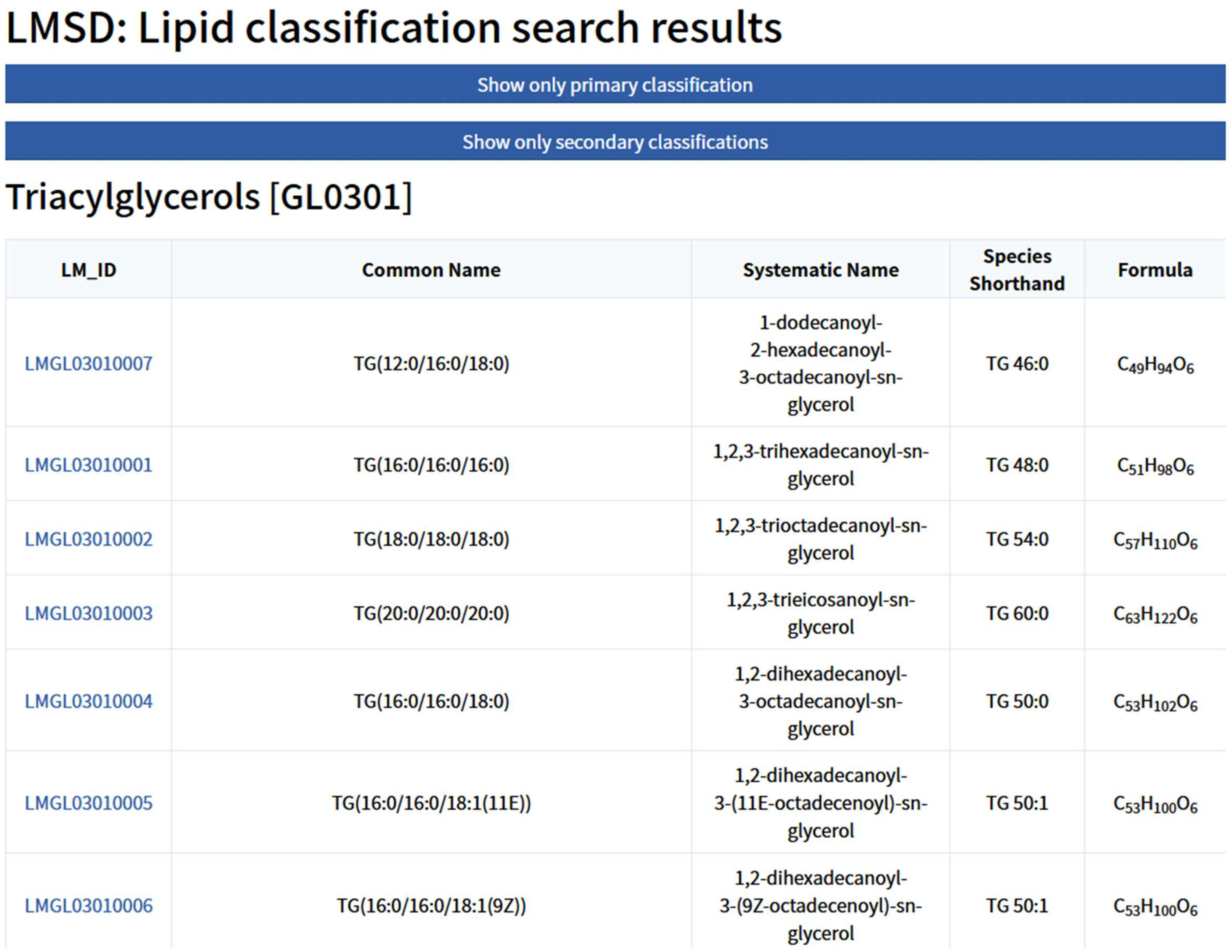
Eight lipid categories:

each with its own classification and sub-classification hierarchy

Category	Abbrev.
Fatty acids	FA
Glycerolipids	GL
Glycero-phospholipids	GP
Sphingolipids	SP
Sterol lipids	ST
Prenol lipids	PR
Saccharolipids	SL
Polyketides	PK



Mass Spectrometry Reviews, Volume: 40, Issue: 3, Pages: 162-176, DOI: (10.1002/mas.21627)



LMSD: Lipid classification search results

Triacylglycerols [GL0301]

LM_ID	Common Name	Systematic Name	Species Shorthand	Formula
LMGL03010007	TG(12:0/16:0/18:0)	1-dodecanoyl-2-hexadecanoyl-3-octadecanoyl-sn-glycerol	TG 46:0	$C_{49}H_{94}O_6$
LMGL03010001	TG(16:0/16:0/16:0)	1,2,3-trihexadecanoyl-sn-glycerol	TG 48:0	$C_{51}H_{98}O_6$
LMGL03010002	TG(18:0/18:0/18:0)	1,2,3-trioctadecanoyl-sn-glycerol	TG 54:0	$C_{57}H_{110}O_6$
LMGL03010003	TG(20:0/20:0/20:0)	1,2,3-trieicosanoyl-sn-glycerol	TG 60:0	$C_{63}H_{122}O_6$
LMGL03010004	TG(16:0/16:0/18:0)	1,2-dihexadecanoyl-3-octadecanoyl-sn-glycerol	TG 50:0	$C_{53}H_{102}O_6$
LMGL03010005	TG(16:0/16:0/18:1(11E))	1,2-dihexadecanoyl-3-(11E-octadecenoyl)-sn-glycerol	TG 50:1	$C_{53}H_{100}O_6$
LMGL03010006	TG(16:0/16:0/18:1(9Z))	1,2-dihexadecanoyl-3-(9Z-octadecenoyl)-sn-glycerol	TG 50:1	$C_{53}H_{100}O_6$

Lipidomics

- complete set of lipids produced in a given cell or organism = **lipidome**
- lipidomics – the study of the structure and function of the „complete“ set of lipids
  - **analysis of lipidome**; study of pathways and networks in biological systems

- **mass spectrometry** is the widely used analytical technique for lipidomics
  - We have developed an untargeted LC-MS method and offer cooperation

Untargeted lipidomics (profiling)

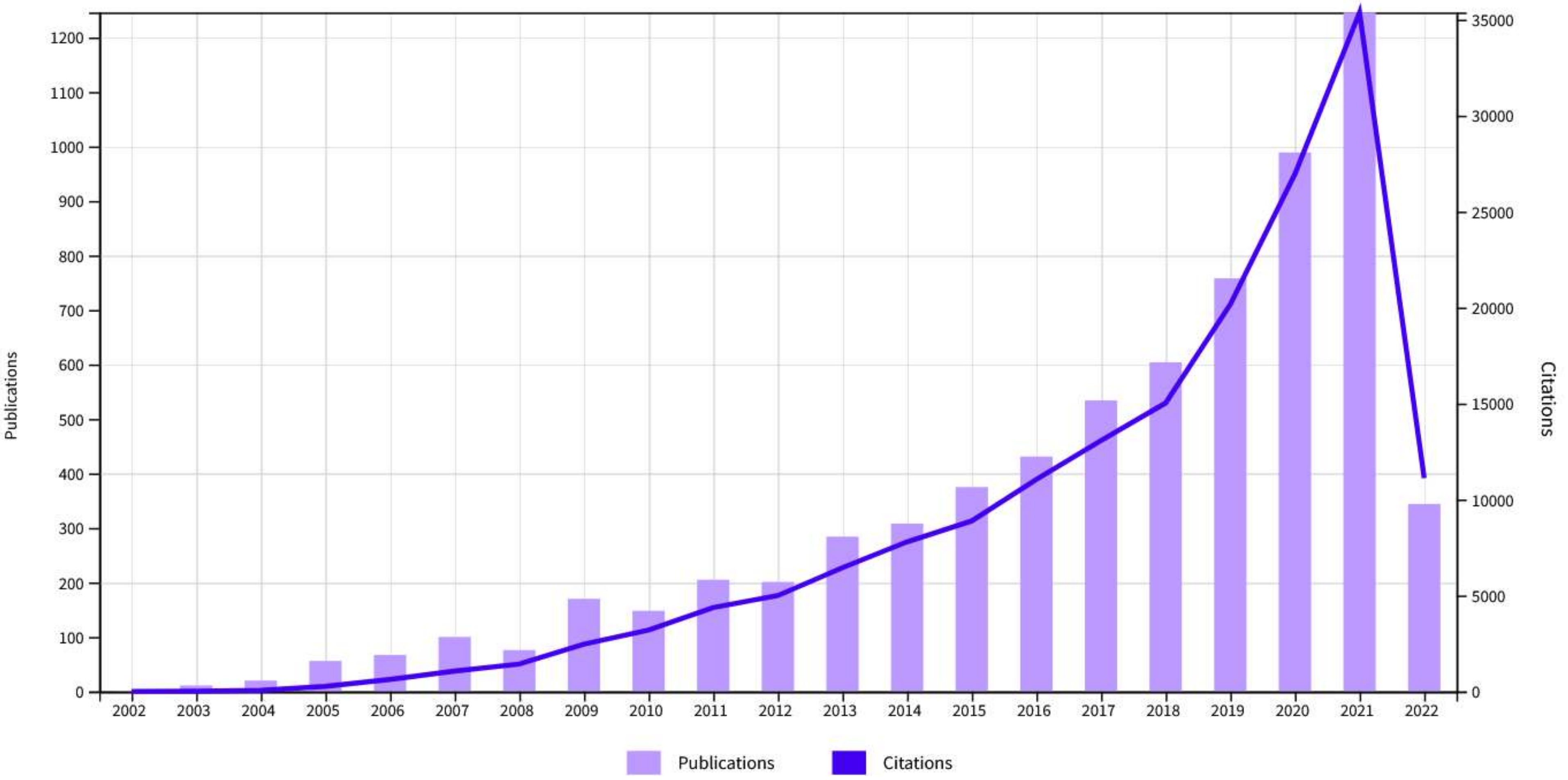
Targeted lipidomics (quantification)
- cooperation with Karel Čížek

LC-MS lipidomics

Shotgun (infusion) lipidomics

Lipidomics on WOS

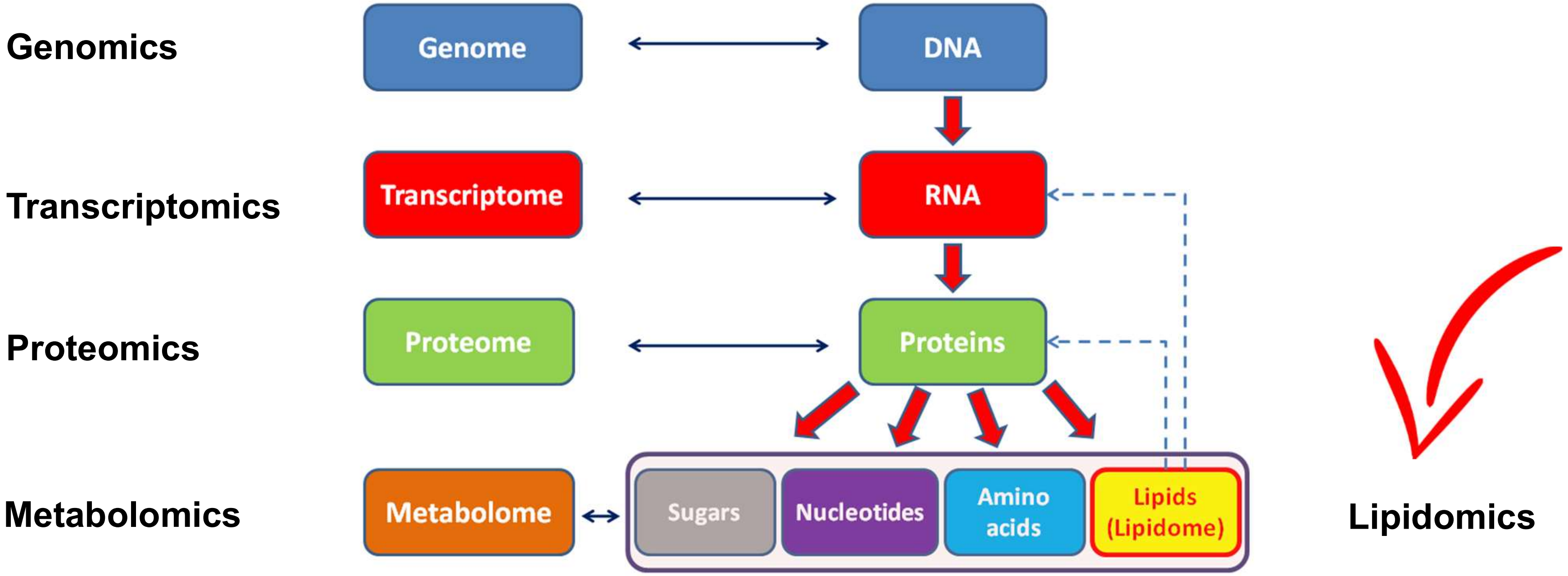
„Lipidomics“ 5/31/2022



https://www.lipidmaps.org

Omics technologies

- identification, characterization, and quantification of pools of biological molecules



Genomics

Genome

DNA

Transcriptomics

Transcriptome

RNA

Proteomics

Proteome

Proteins

Metabolomics

Metabolome

Sugars

Nucleotides

Amino acids

Lipids (Lipidome)

Lipidomics

Lipidome - subset of the metabolome

Untargeted lipidomic workflow

Sample preparation

biofluids
tissues
cells

→

extraction:
MTBE
BUME

→

Data acquisition

HPLC: RP C18

+

HRAM MS: Orbitrap Fusion Lumos

→

Data analysis

Identification of lipid species

↓

Basic statistic

HPLC method

Thermo Scientific™ Dionex™ UltiMate™ 3000

Mobile phase:

A 60:40 (v:v) acetonitrile / water

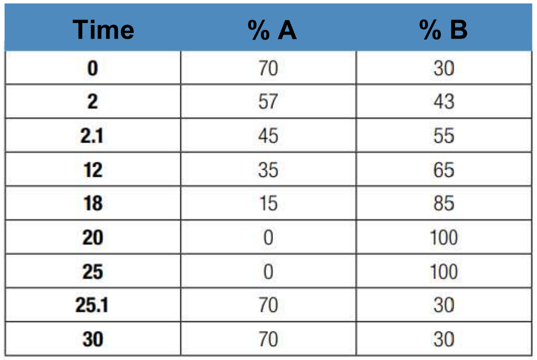
B 90:10 (v:v) IPA / acetonitrile

- 10 mM ammonium formate, 0.1% formic acid

HPLC method

Thermo Scientific™ Dionex™ UltiMate™ 3000

HPLC gradient



Time	% A	% B
0	70	30
2	57	43
2.1	45	55
12	35	65
18	15	85
20	0	100
25	0	100
25.1	70	30
30	70	30

Mobile phase:

A 60:40 (v:v) acetonitrile / water

B 90:10 (v:v) IPA / acetonitrile

- 10 mM ammonium formate, 0.1% formic acid

Column:

Waters ACQUITY UPLC BEH **C18** (2.1 x 100 mm, 1.7 µm)

operated at 45 °C

- flow rate of 180 µL/min

Mass spectrometry

Thermo Scientific™ Orbitrap Fusion™ Lumos™

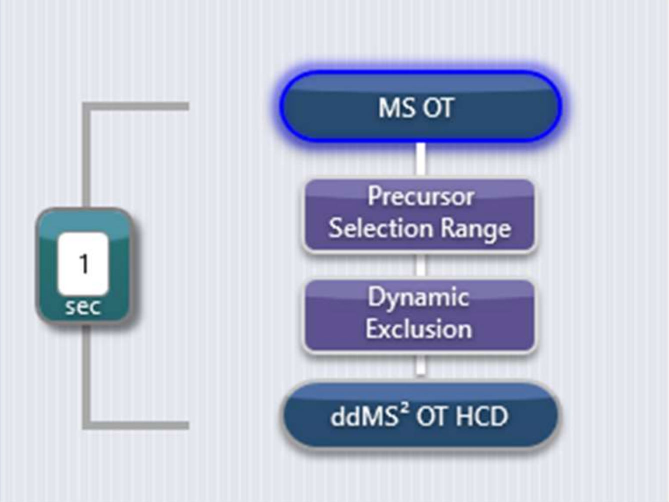
- HRAM mass spectrometer (high resolution, accurate mass)
- ionization H-ESI (heated electrospray)
- data-dependent acquisition (DDA) mode
- Positiv and negative mode
  - **separately** (in two chromatographic run) – more identified lipids (60 min per sample)
  - polarity switching (30 min per sample)

MS method: negative mode

HPLC-MS run time

0 min

30 min

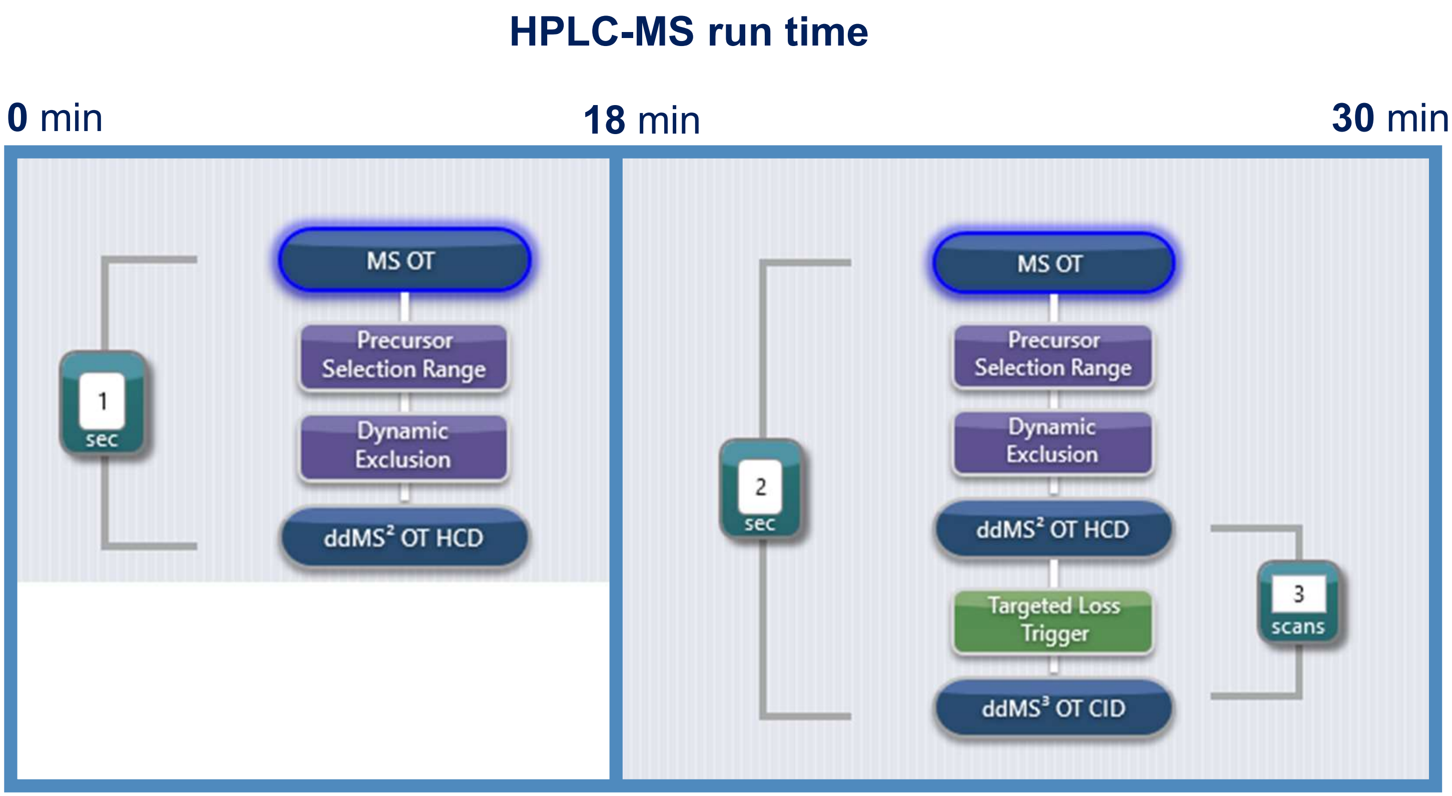


Full MS 120 000

250 – 1 200 m/z

MS^2 15 000

MS method: positive mode



Full MS 120 000

250 – 1 200 m/z

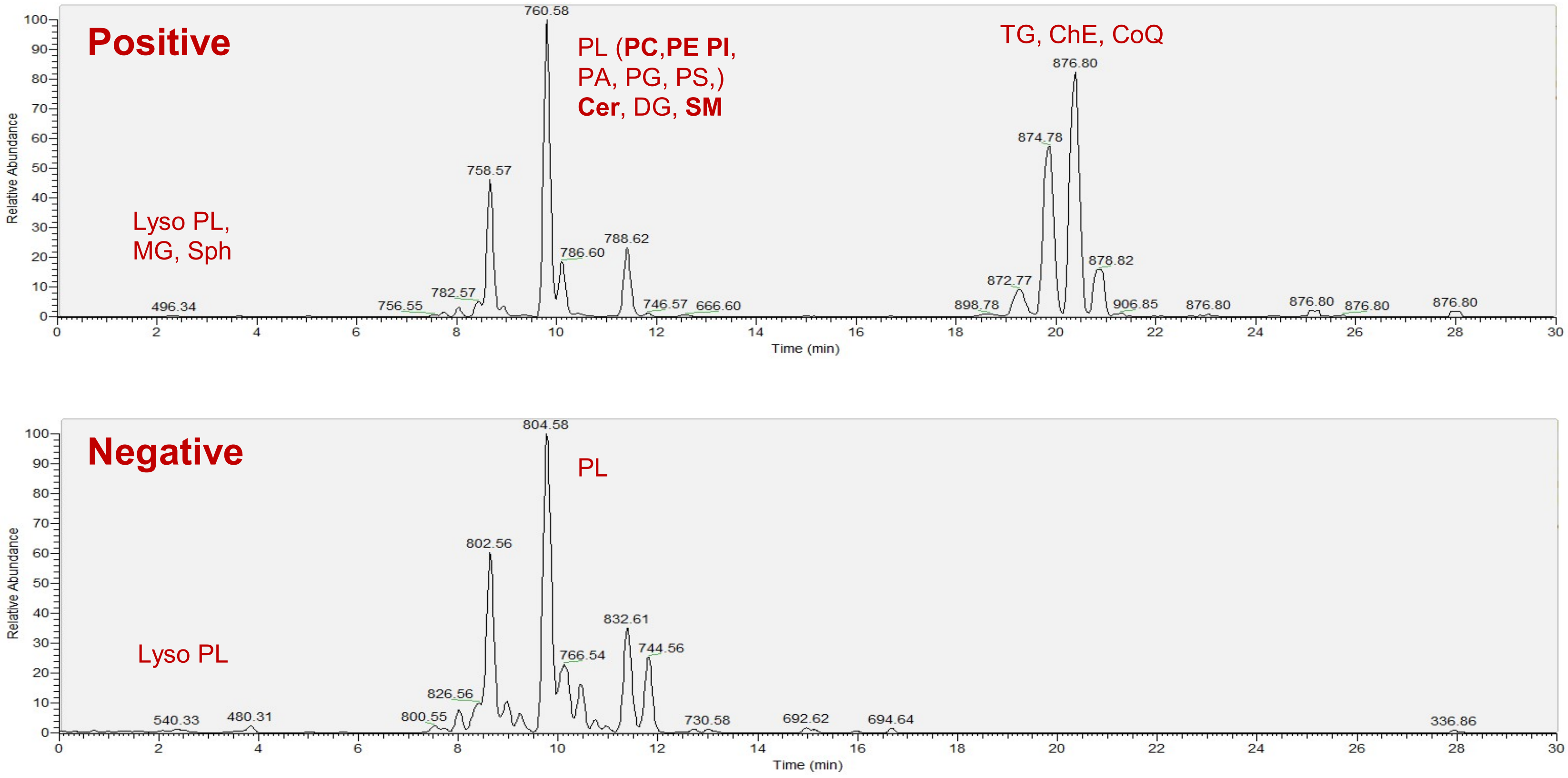
MS^2 15 000

FA neutral loss

MS^3 (top 3 intense product ions)

HPLC-MS

Base peak chromatograms of positive and negative ion modes of egg lipid extracts



Data analysis

Identification based on:

- retention time
- exact mass of molecular ions (molecular adduct) = elemental composition
- product ion spectra (MS/MS spectra) + exact mass of product ion = elemental composition of product ion

Raw files →

4.2 version

LipidSearch

(5.0 version is tested)

lipids annotation
alignment of RTs
integration of peaks

→ excel

normalization

→ MetaboAnalyst 5.0

statistical analysis

→ **final excel file:**

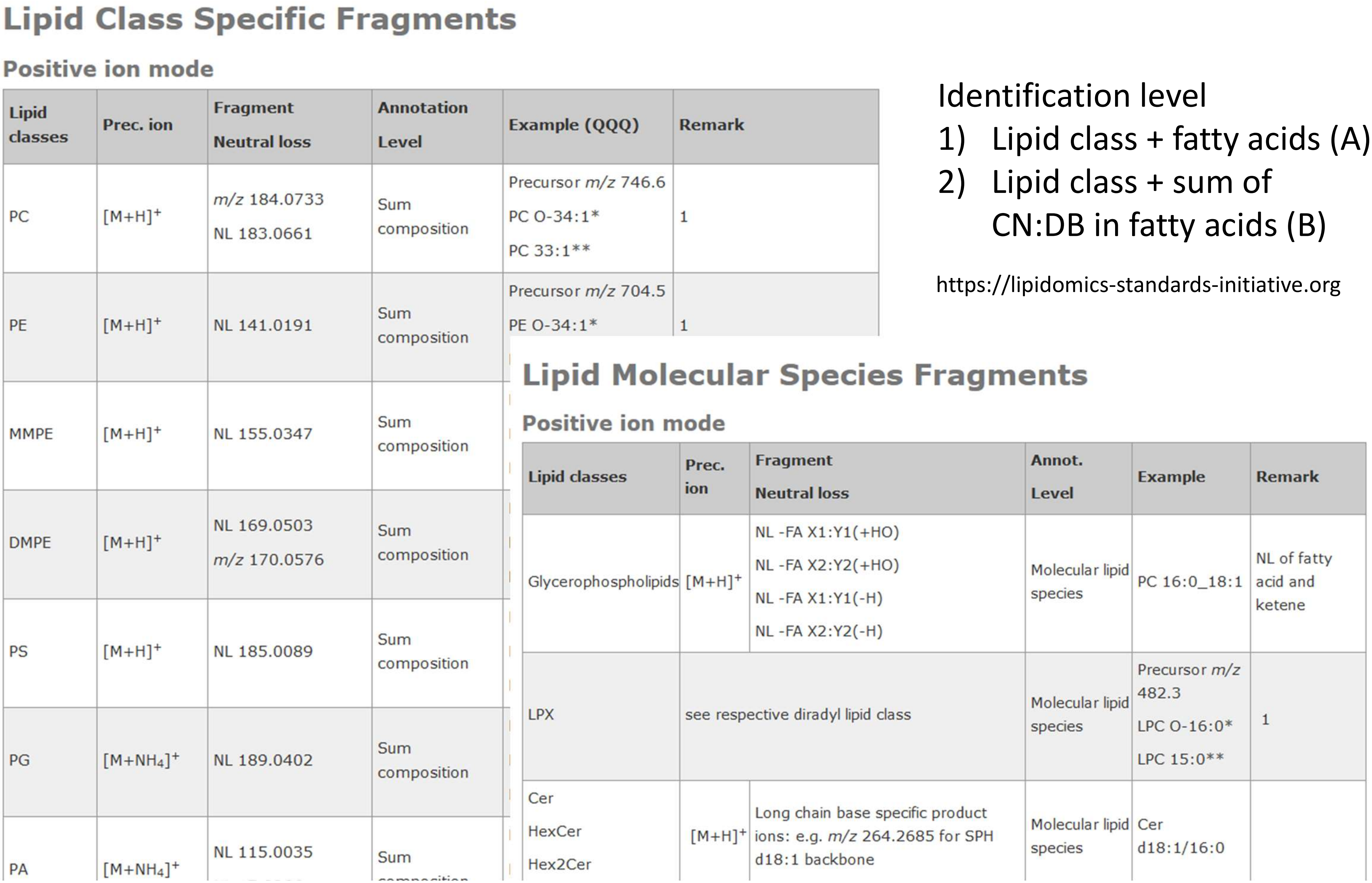
results data table
interactive dashboard
statistical analysis

Identification – specific fragments

Identification level

1) Lipid class + fatty acids (A)
2) Lipid class + sum of CN:DB in fatty acids (B)

https://lipidomics-standards-initiative.org



Lipid Class Specific Fragments

Positive ion mode

Lipid classes	Prec. ion	Fragment Neutral loss	Annotation Level	Example (QQQ)	Remark
PC	$[M+H]^+$	*m/z* 184.0733 NL 183.0661	Sum composition	Precursor *m/z* 746.6 PC O-34:1* PC 33:1**	1
PE	$[M+H]^+$	NL 141.0191	Sum composition	Precursor *m/z* 704.5 PE O-34:1*	1
MMPE	$[M+H]^+$	NL 155.0347	Sum composition		
DMPE	$[M+H]^+$	NL 169.0503 *m/z* 170.0576	Sum composition		
PS	$[M+H]^+$	NL 185.0089	Sum composition		
PG	$[M+NH_4]^+$	NL 189.0402	Sum composition		
PA	$[M+NH_4]^+$	NL 115.0035	Sum		

Lipid Molecular Species Fragments

Positive ion mode

Lipid classes	Prec. ion	Fragment Neutral loss	Annot. Level	Example	Remark
Glycerophospholipids	$[M+H]^+$	NL -FA X1:Y1(+HO) NL -FA X2:Y2(+HO) NL -FA X1:Y1(-H) NL -FA X2:Y2(-H)	Molecular lipid species	PC 16:0_18:1	NL of fatty acid and ketene
LPX	see respective diradyl lipid class		Molecular lipid species	Precursor *m/z* 482.3 LPC O-16:0* LPC 15:0**	1
Cer HexCer Hex2Cer	$[M+H]^+$	Long chain base specific product ions: e.g. *m/z* 264.2685 for SPH d18:1 backbone	Molecular lipid species	Cer d18:1/16:0	

Example results - data table

- cell samples

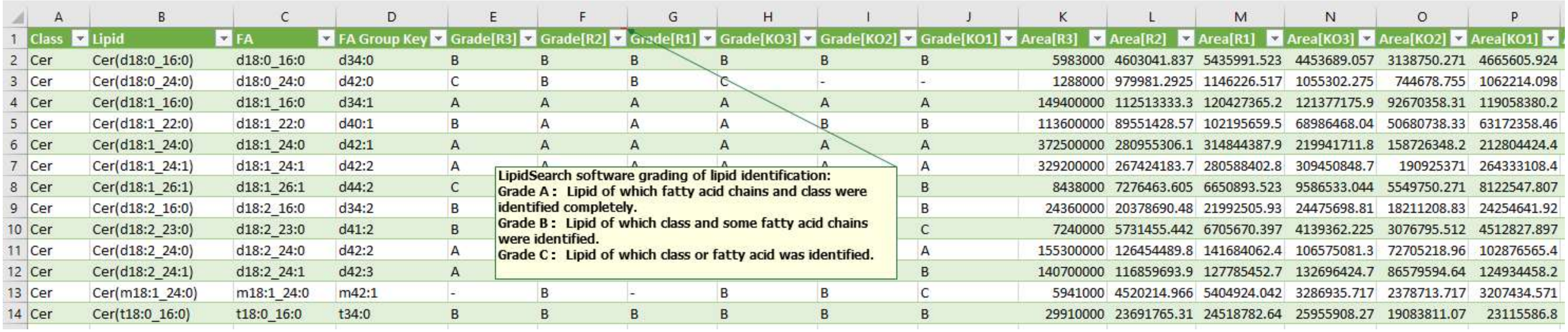
  KO cells (gene deletion)

  rescue cells (gene re-expression)

(group of Kvido Stříšovský)

Lipid identification

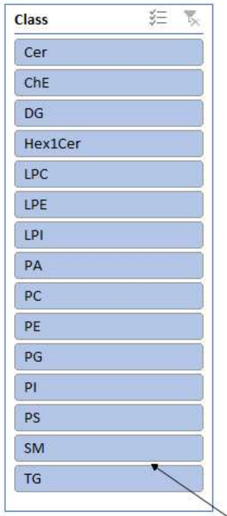
Peak area (for relative quantification)



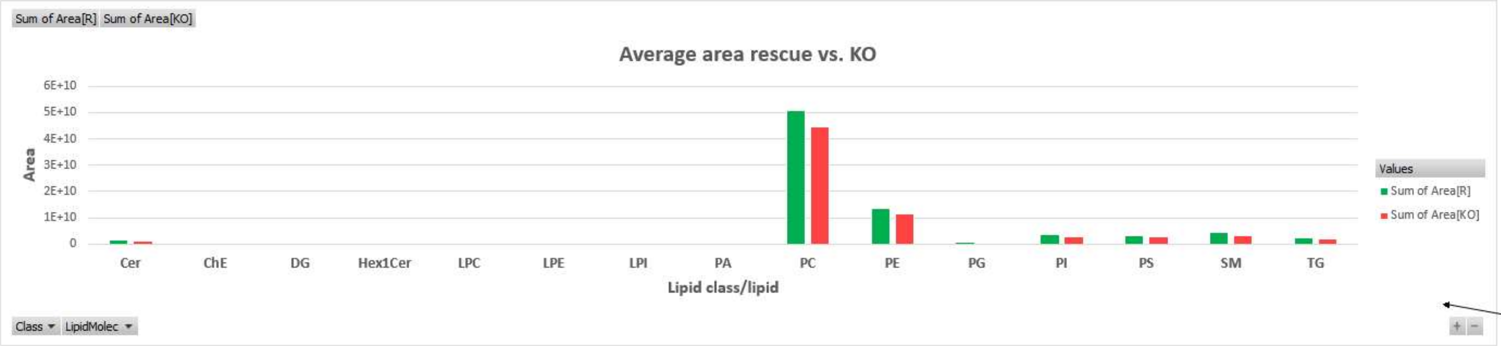
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Class	Lipid	FA	FA Group Key	Grade[R3]	Grade[R2]	Grade[R1]	Grade[KO3]	Grade[KO2]	Grade[KO1]	Area[R3]	Area[R2]	Area[R1]	Area[KO3]	Area[KO2]	Area[KO1]
2	Cer	Cer(d18:0_16:0)	d18:0_16:0	d34:0	B	B	B	B	B	B	5983000	4603041.837	5435991.523	4453689.057	3138750.271	4665605.924
3	Cer	Cer(d18:0_24:0)	d18:0_24:0	d42:0	C	B	B	C	-	-	1288000	979981.2925	1146226.517	1055302.275	744678.755	1062214.098
4	Cer	Cer(d18:1_16:0)	d18:1_16:0	d34:1	A	A	A	A	A	A	149400000	112513333.3	120427365.2	121377175.9	92670358.31	119058380.2
5	Cer	Cer(d18:1_22:0)	d18:1_22:0	d40:1	B	A	A	A	B	B	113600000	89551428.57	102195659.5	68986468.04	50680738.33	63172358.46
6	Cer	Cer(d18:1_24:0)	d18:1_24:0	d42:1	A	A	A	A	A	A	372500000	280955306.1	314844387.9	219941711.8	158726348.2	212804424.4
7	Cer	Cer(d18:1_24:1)	d18:1_24:1	d42:2	A	A	A	A	A	A	329200000	267424183.7	280588402.8	309450848.7	190925371	264333108.4
8	Cer	Cer(d18:1_26:1)	d18:1_26:1	d44:2	C					B	8438000	7276463.605	6650893.523	9586533.044	5549750.271	8122547.807
9	Cer	Cer(d18:2_16:0)	d18:2_16:0	d34:2	B					B	24360000	20378690.48	21992505.93	24475698.81	18211208.83	24254641.92
10	Cer	Cer(d18:2_23:0)	d18:2_23:0	d41:2	B					C	7240000	5731455.442	6705670.397	4139362.225	3076795.512	4512827.897
11	Cer	Cer(d18:2_24:0)	d18:2_24:0	d42:2	A					A	155300000	126454489.8	141684062.4	106575081.3	72705218.96	102876565.4
12	Cer	Cer(d18:2_24:1)	d18:2_24:1	d42:3	A					B	140700000	116859693.9	127785452.7	132696424.7	86579594.64	124934458.2
13	Cer	Cer(m18:1_24:0)	m18:1_24:0	m42:1	-	B	-	B	B	C	5941000	4520214.966	5404924.042	3286935.717	2378713.717	3207434.571
14	Cer	Cer(t18:0_16:0)	t18:0_16:0	t34:0	B	B	B	B	B	B	29910000	23691765.31	24518782.64	25955908.27	19083811.07	23115586.8

LipidSearch software grading of lipid identification:
Grade A : Lipid of which fatty acid chains and class were identified completely.
Grade B : Lipid of which class and some fatty acid chains were identified.
Grade C : Lipid of which class or fatty acid was identified.

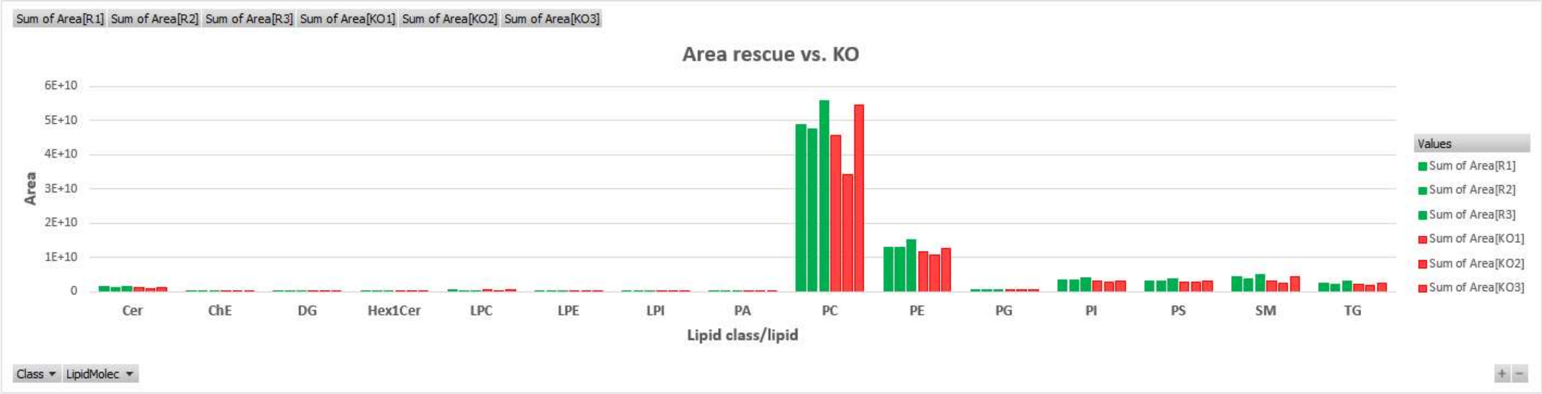
Example results - interactive dashboard



Click and select lipid class.
Ctrl + click select multiple classes.
Clear filter icon: top right

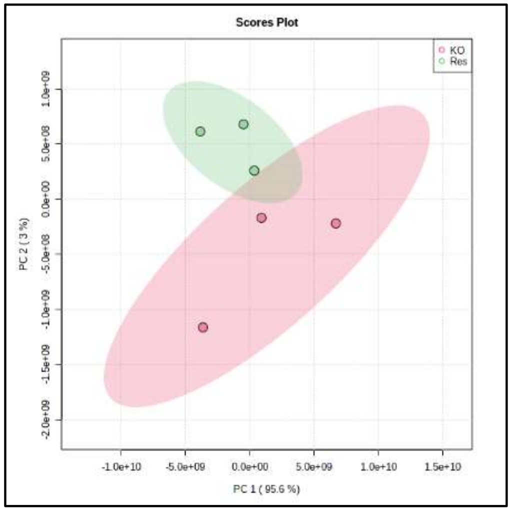


Click + for zooming
Click - for unzooming

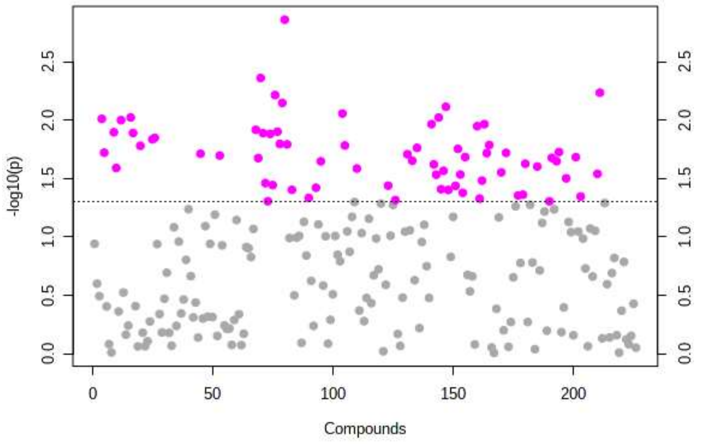


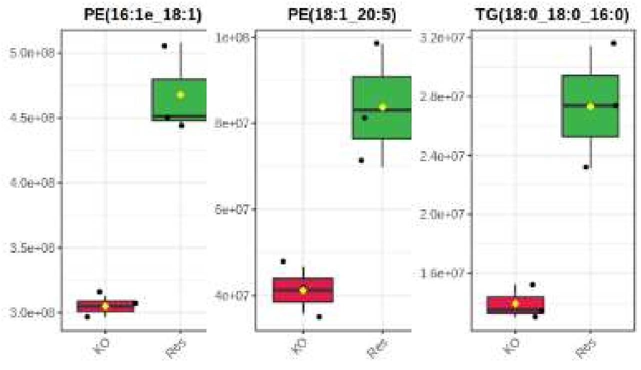
Example results - statistical analysis

PCA

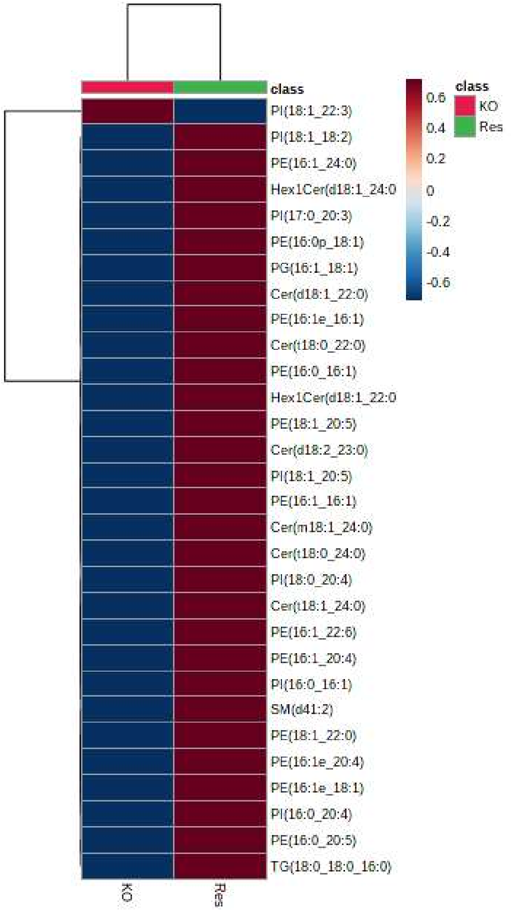


T-test





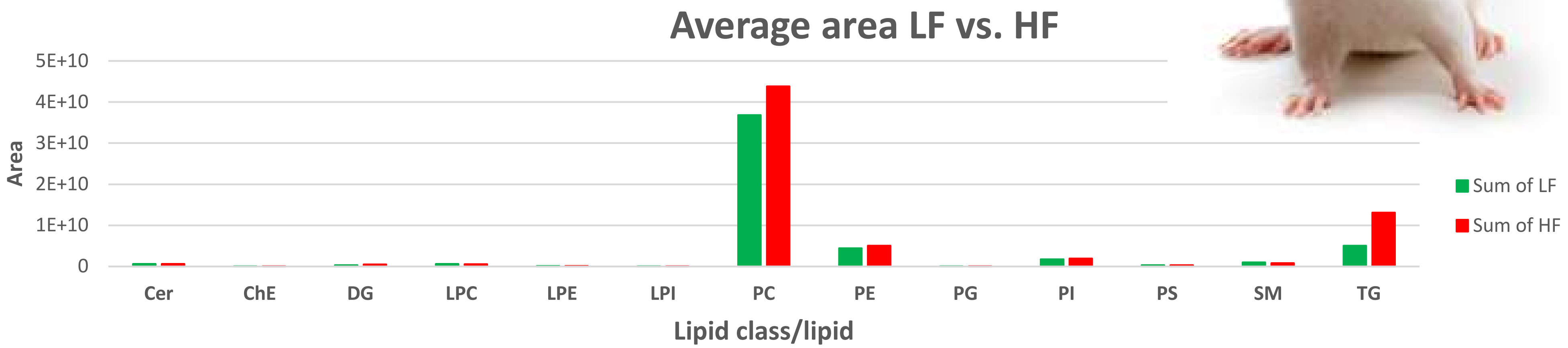
Hierarchical clustering heatmaps

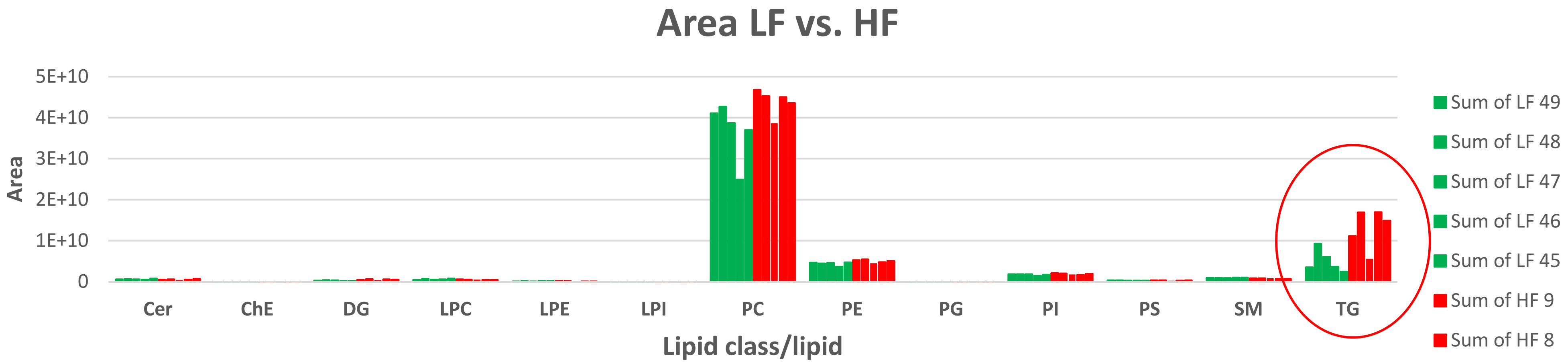


IOCB Prague

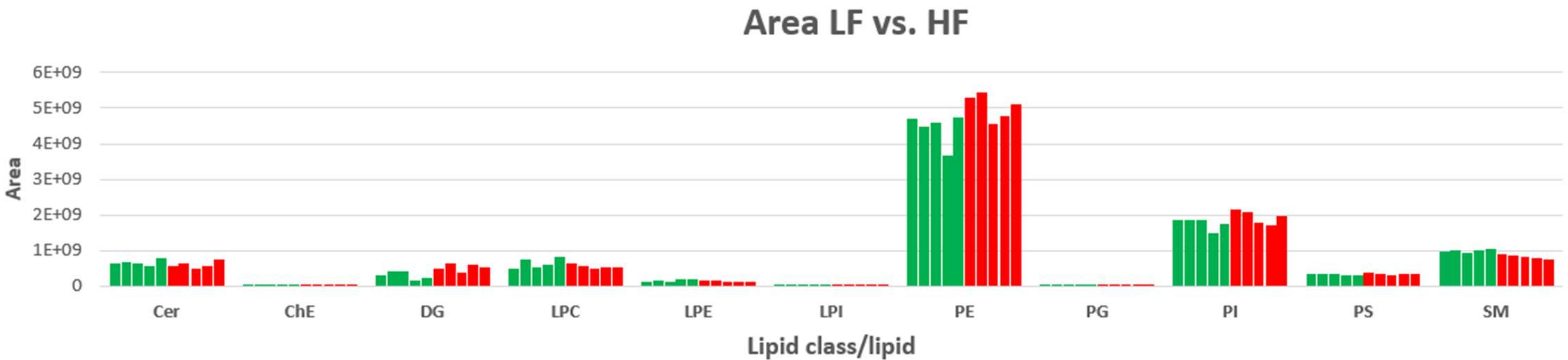
Project WKY rats

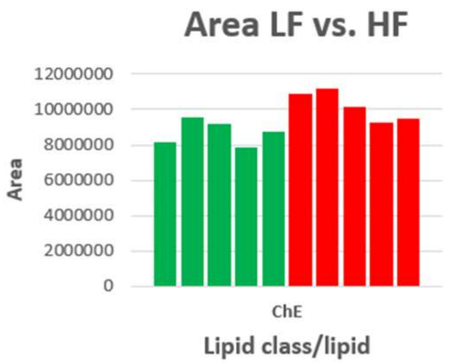
Wistar Kyoto rats; high-fat diet; (group of Lenka Maletínská)

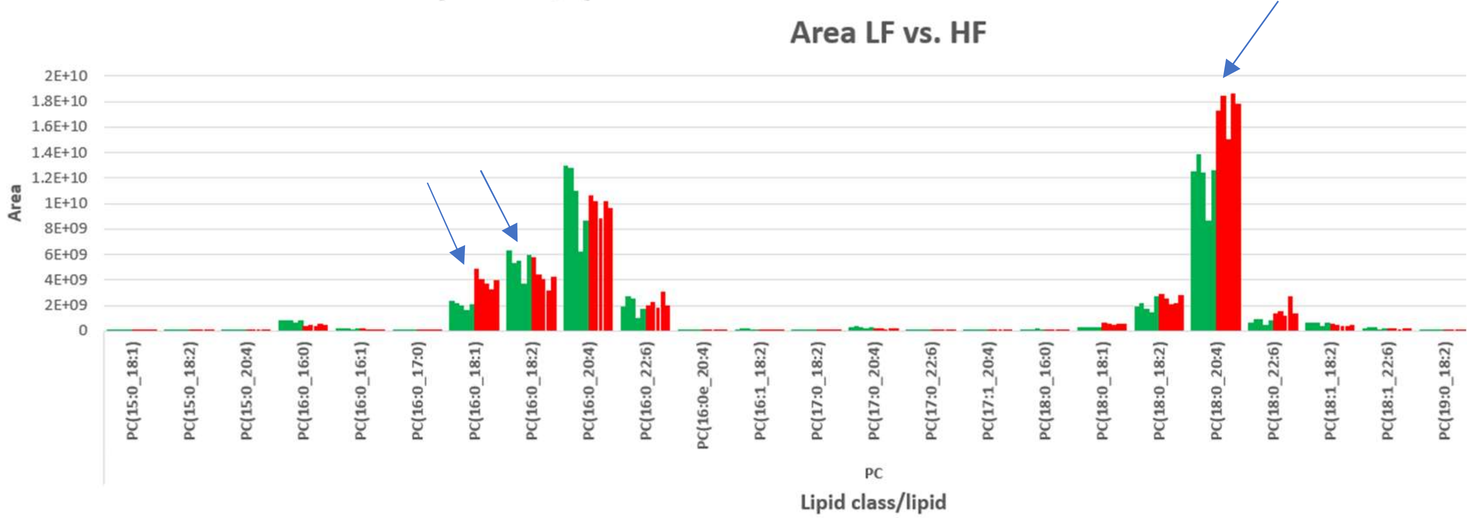




WKY rats

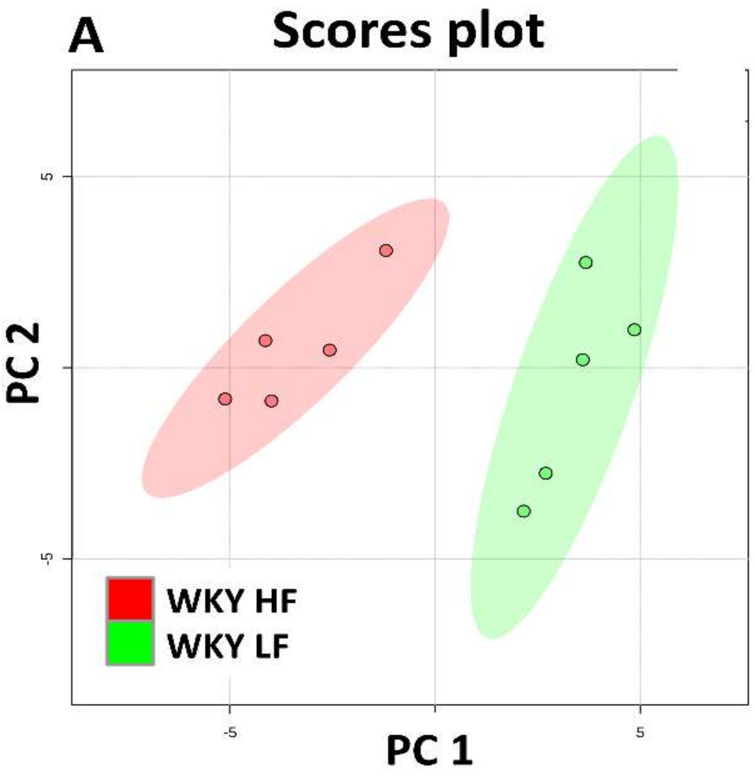

Area LF vs. HF
6E+09
5E+09
4E+09
3E+09
2E+09
1E+09
0
Area
Cer
ChE
DG
LPC
LPE
LPI
PE
PG
PI
PS
SM
Lipid class/lipid


Area LF vs. HF
12000000
10000000
8000000
6000000
4000000
2000000
0
Area
ChE
Lipid class/lipid

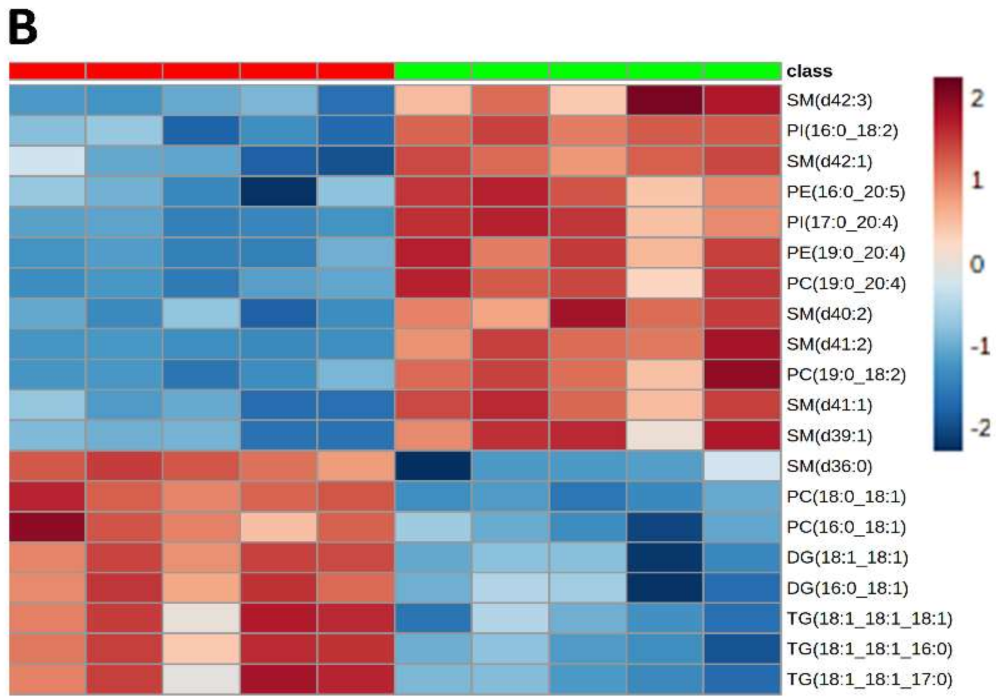

Area LF vs. HF
2E+10
1.8E+10
1.6E+10
1.4E+10
1.2E+10
1E+10
8E+09
6E+09
4E+09
2E+09
0
Area
PC(15:0_18:1)
PC(15:0_18:2)
PC(15:0_20:4)
PC(16:0_16:0)
PC(16:0_16:1)
PC(16:0_17:0)
PC(16:0_18:1)
PC(16:0_18:2)
PC(16:0_20:4)
PC(16:0_22:6)
PC(16:0e_20:4)
PC(16:1_18:2)
PC(17:0_18:2)
PC(17:0_20:4)
PC(17:0_22:6)
PC(17:1_20:4)
PC(18:0_16:0)
PC(18:0_18:1)
PC(18:0_18:2)
PC(18:0_20:4)
PC(18:0_22:6)
PC(18:1_18:2)
PC(18:1_22:6)
PC(19:0_18:2)
PC
Lipid class/lipid

Example results - statistical analysis

PCA

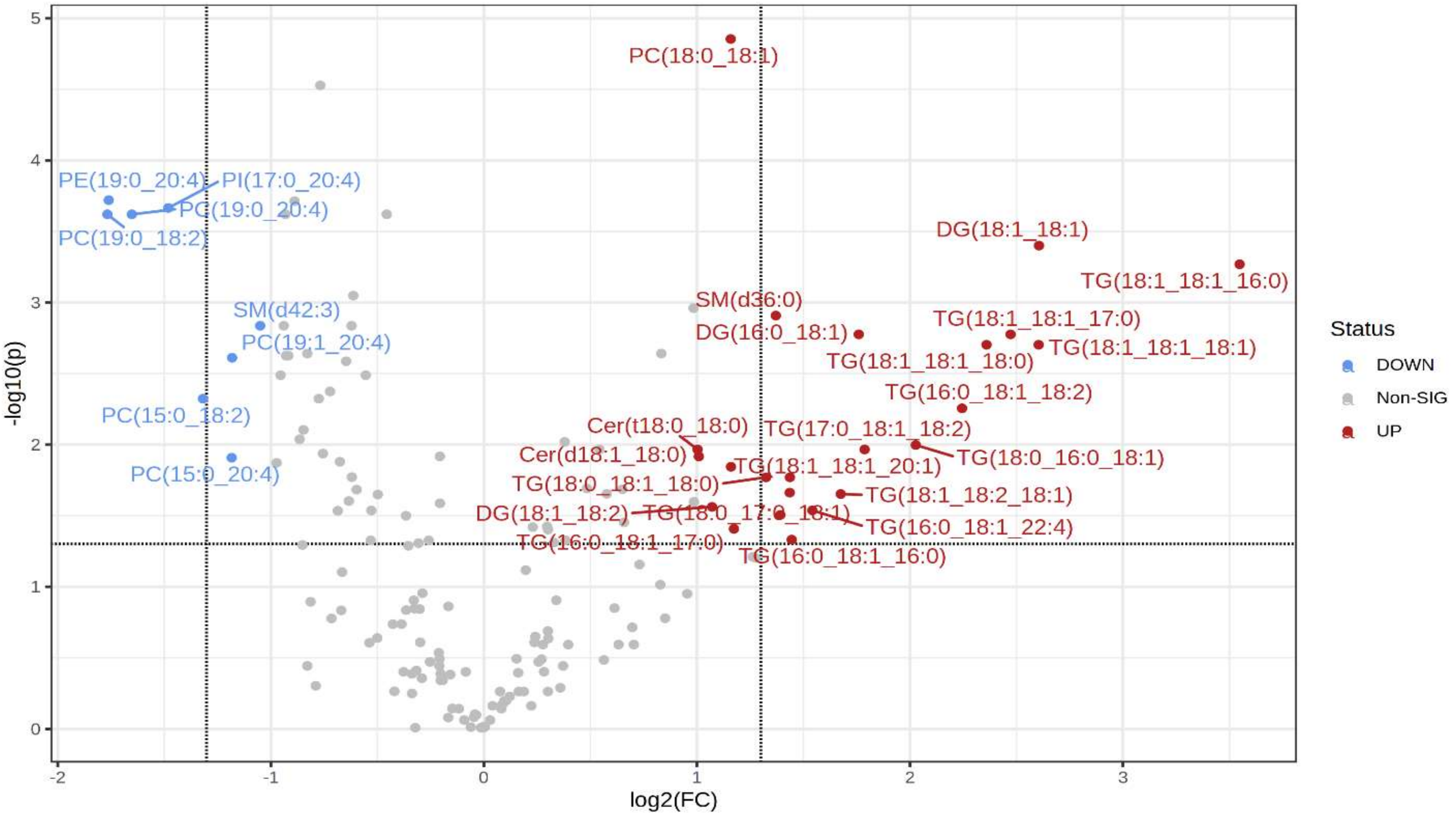


Hierarchical clustering heatmaps



Example results - statistical analysis

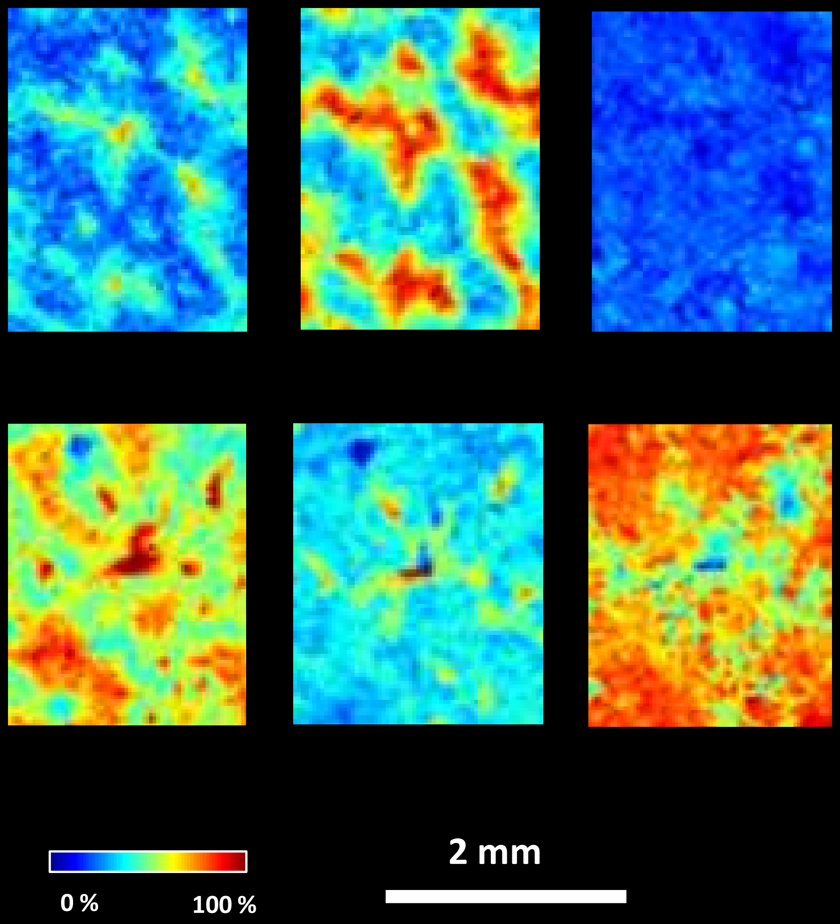
Vulcano plot

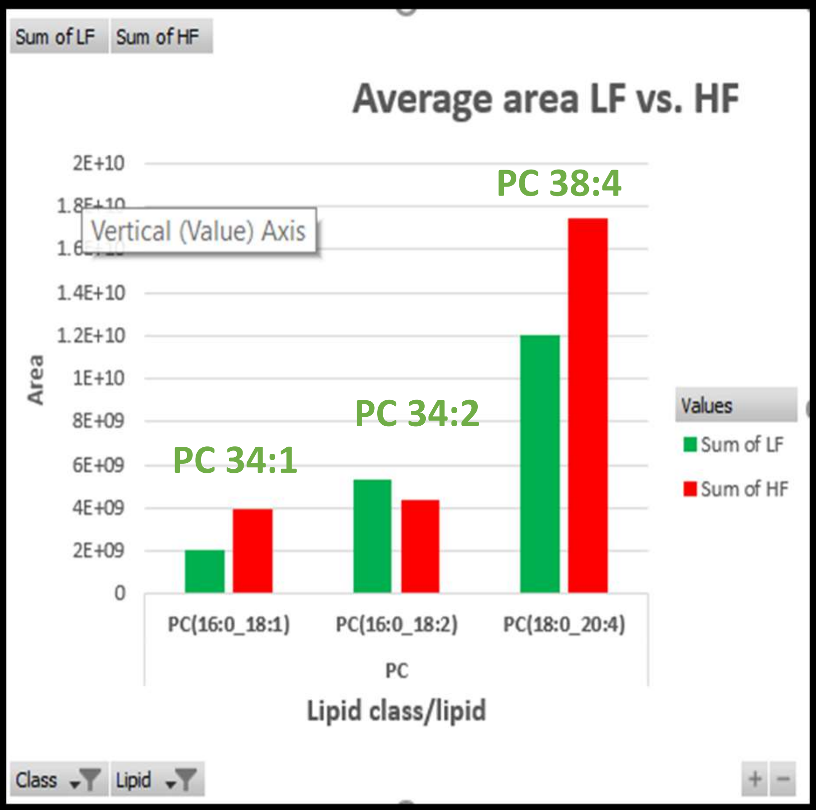


WKY rats

control model PC 34:1 PC 34:2 PC 38:4

HF model

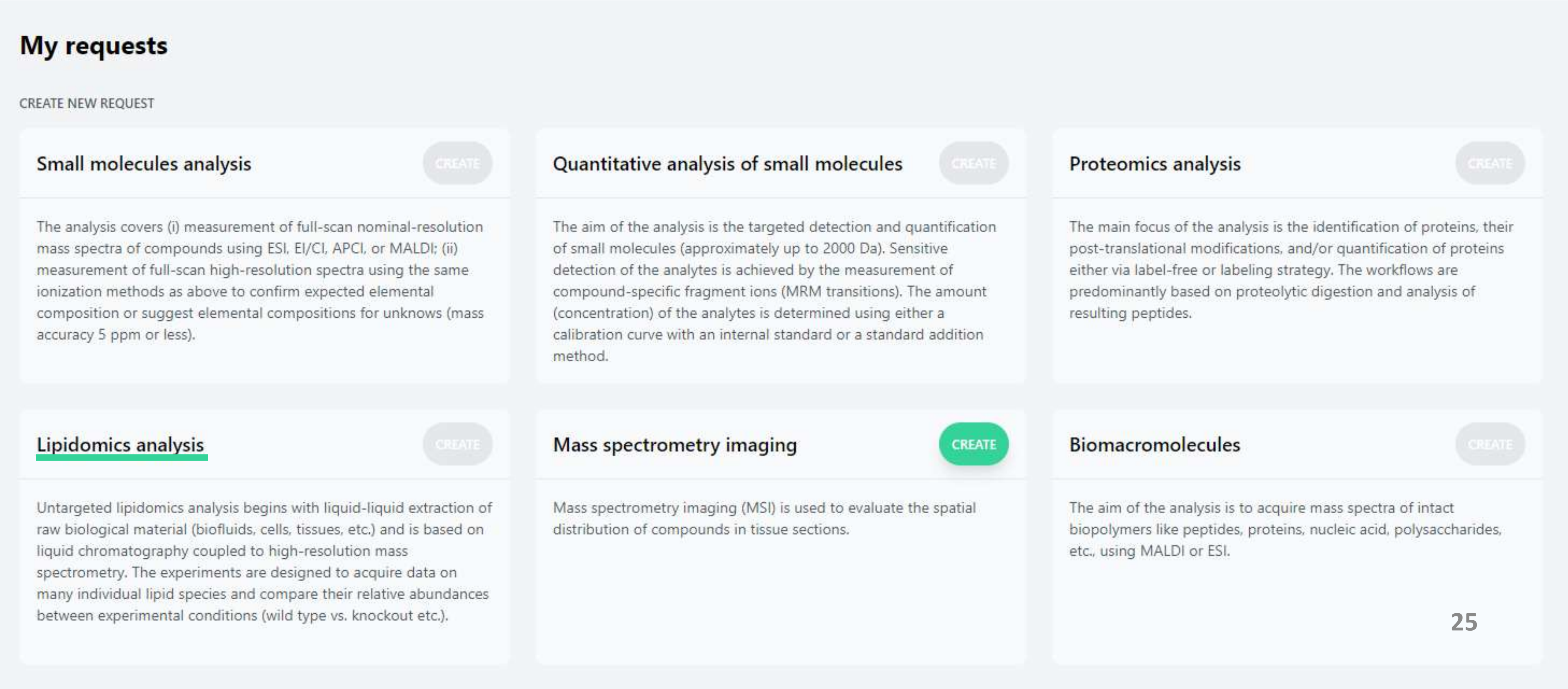




New REQUEST

How can apply for lipidomic analysis

- Contact Vladimír Vrkoslav, Štěpán Strnad and discuss your project



My requests

CREATE NEW REQUEST

Small molecules analysis

The analysis covers (i) measurement of full-scan nominal-resolution mass spectra of compounds using ESI, EI/CI, APCI, or MALDI; (ii) measurement of full-scan high-resolution spectra using the same ionization methods as above to confirm expected elemental composition or suggest elemental compositions for unknowns (mass accuracy 5 ppm or less).

Quantitative analysis of small molecules

The aim of the analysis is the targeted detection and quantification of small molecules (approximately up to 2000 Da). Sensitive detection of the analytes is achieved by the measurement of compound-specific fragment ions (MRM transitions). The amount (concentration) of the analytes is determined using either a calibration curve with an internal standard or a standard addition method.

Proteomics analysis

The main focus of the analysis is the identification of proteins, their post-translational modifications, and/or quantification of proteins either via label-free or labeling strategy. The workflows are predominantly based on proteolytic digestion and analysis of resulting peptides.

Lipidomics analysis

Untargeted lipidomics analysis begins with liquid-liquid extraction of raw biological material (biofluids, cells, tissues, etc.) and is based on liquid chromatography coupled to high-resolution mass spectrometry. The experiments are designed to acquire data on many individual lipid species and compare their relative abundances between experimental conditions (wild type vs. knockout etc.).

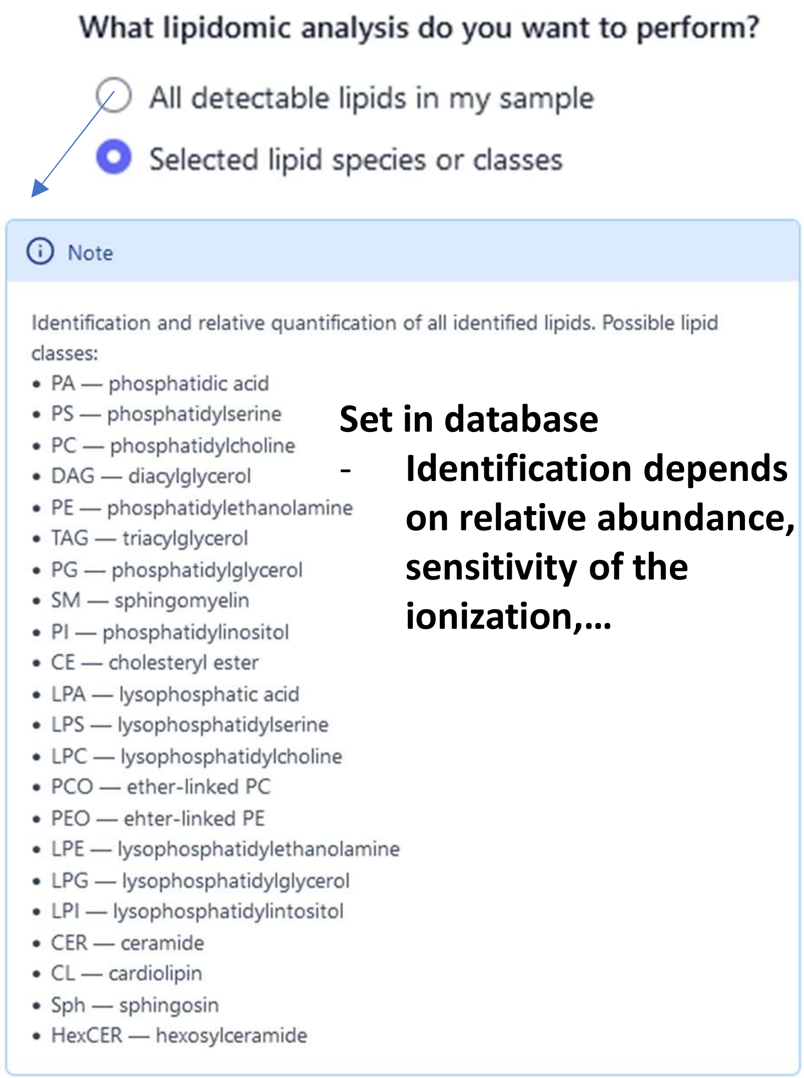
Mass spectrometry imaging

Mass spectrometry imaging (MSI) is used to evaluate the spatial distribution of compounds in tissue sections.

Biomacromolecules

The aim of the analysis is to acquire mass spectra of intact biopolymers like peptides, proteins, nucleic acid, polysaccharides, etc., using MALDI or ESI.

Lipidomic project in reQest 2



What lipidomic analysis do you want to perform?

○ All detectable lipids in my sample

● Selected lipid species or classes

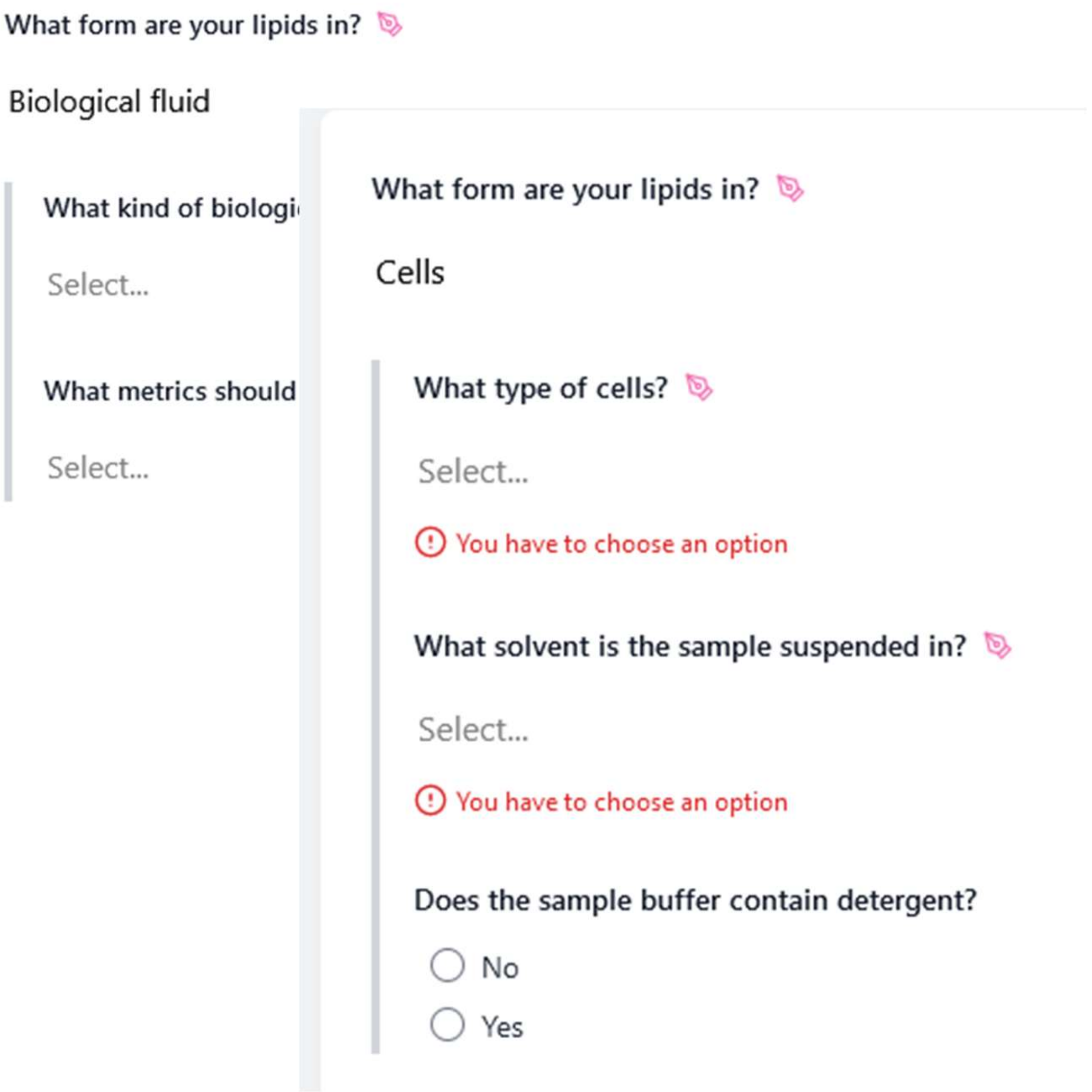
Note

Identification and relative quantification of all identified lipids. Possible lipid classes:

- PA — phosphatidic acid
- PS — phosphatidylserine
- PC — phosphatidylcholine
- DAG — diacylglycerol
- PE — phosphatidylethanolamine
- TAG — triacylglycerol
- PG — phosphatidylglycerol
- SM — sphingomyelin
- PI — phosphatidylinositol
- CE — cholesteryl ester
- LPA — lysophosphatic acid
- LPS — lysophosphatidylserine
- LPC — lysophosphatidylcholine
- PCO — ether-linked PC
- PEO — ehter-linked PE
- LPE — lysophosphatidylethanolamine
- LPG — lysophosphatidylglycerol
- LPI — lysophosphatidylintositol
- CER — ceramide
- CL — cardiolipin
- Sph — sphingosin
- HexCER — hexosylceramide

Set in database

- **Identification depends on relative abundance, sensitivity of the ionization,…**



What form are your lipids in?

Biological fluid

What kind of biologi

Select...

What metrics should

Select...

What form are your lipids in?

Cells

What type of cells?

Select...

ⓘ You have to choose an option

What solvent is the sample suspended in?

Select...

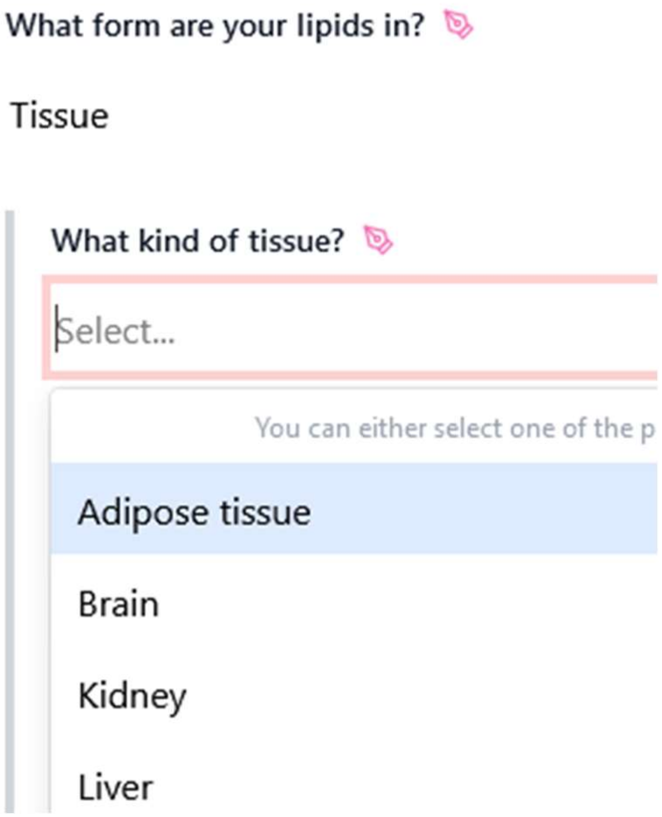
ⓘ You have to choose an option

Does the sample buffer contain detergent?

○ No

○ Yes

Lipidomic project in reQest 2



What form are your lipids in?

Tissue

What kind of tissue?

Select...

You can either select one of the p

Adipose tissue

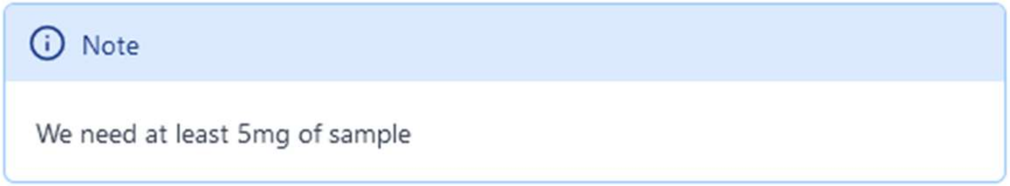
Brain

Kidney

Liver

How many individual samples do you provide?

Up to 10 samples, all samples we measure usualy two times



Note

We need at least 5mg of sample

Normalization !

- Cells
  - number of cells
  - total protein concentration
- Tissues
  - mass
  - protein concentration

Acknowledgment

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RNDr. Vladimír Vrkoslav, Ph.D.; Ing. Štěpán Strnad, Ph.D.

IOCB Prague
Flemingovo nám. 542/2
166 10 Praha 6
Czech Republic

Thank you for your attention.