

MASS SPECTROMETRY BASED METABOLOMICS AND LIPIDOMICS

Darshak Gadara

Supervisor - Dr. Zdenek Spacil

Targeted Proteomics and Metabolomics Group

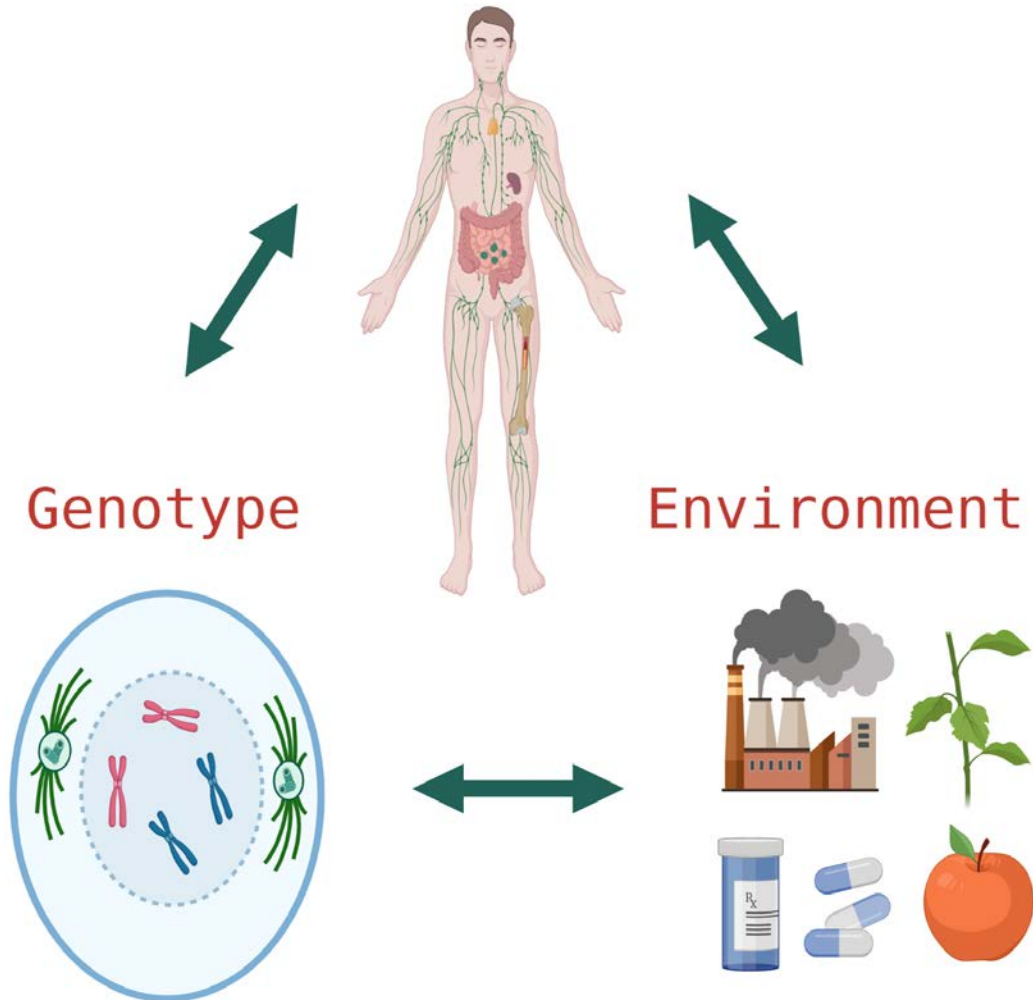
RECETOX, MUNI, Brno

Flow of Presentation

- Systematic Data Processing of Untargeted Metabolomics
- *Application* - Case/Control Fecal Sample of CVID
- High-Throughput Microbore LC-MS Lipidomics
- *Application* - APOE3 vs APOE 4 of iPSC Cerebral Organoid
- *Application* - 3D-Hepatospheroids to Study Hazardous Compounds

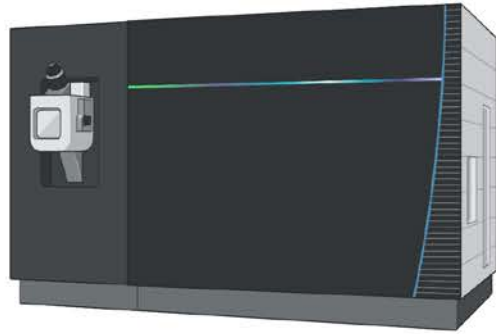
Why Metabolomics?

Phenotype (Metabolome)

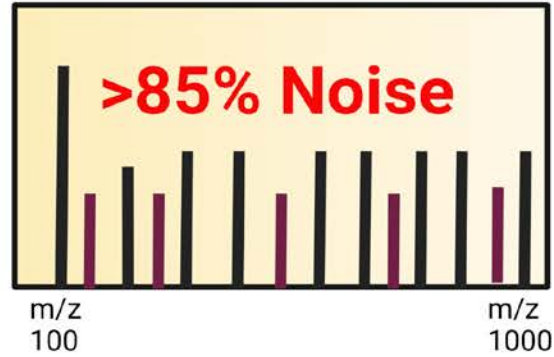


- Metabolites are the endpoint of biological reactions
- Constantly synthesized, degraded, and interacting within the system and with the environment
- Represents dynamic relation between genotype and environment
- Extremely important in clinical diagnosis

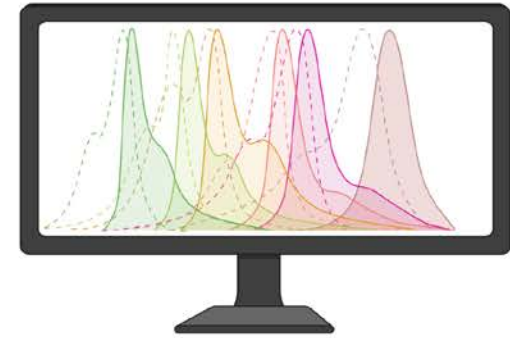
Challenge of Data Processing



LC-MS (high resolution)



LC-MS Spectra



Data Processing



Full Scan Mode

Unbiased Measurement



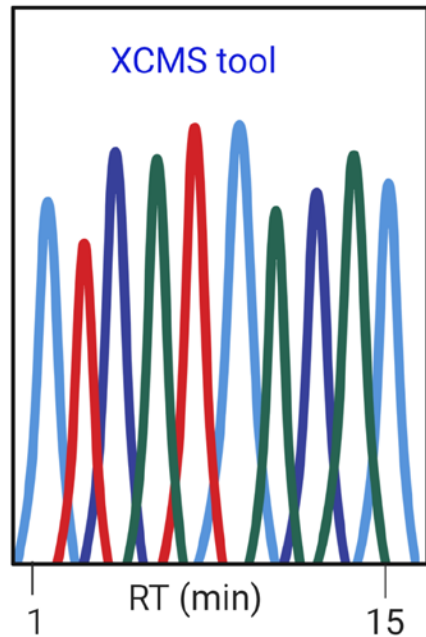
Contaminants
+
Background
+
Metabolites



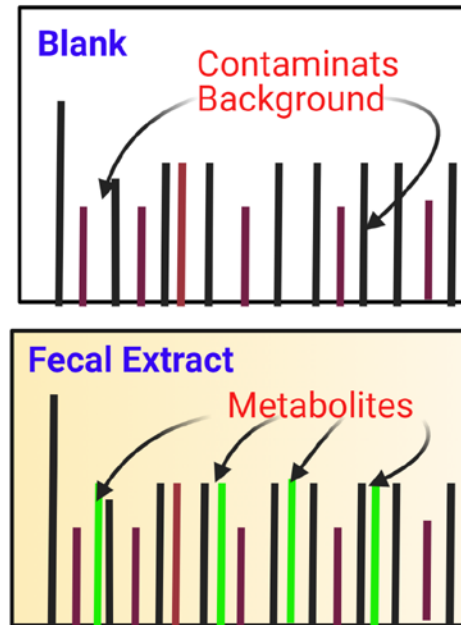
Major Challenge
Thousands of peaks
No Consensus

Metabolomics Data Processing Workflow

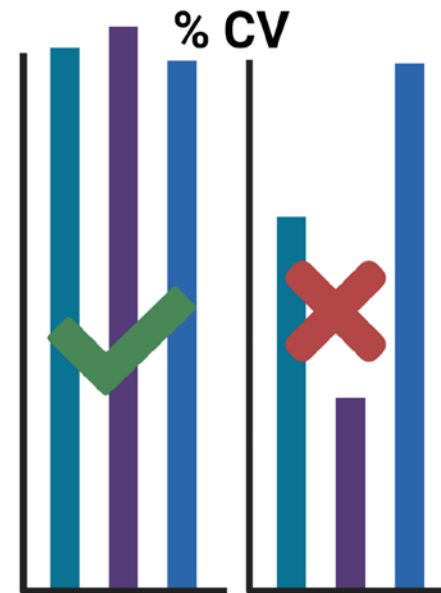
Peak Peaking



Noise Filtering



Quanti. Filtration



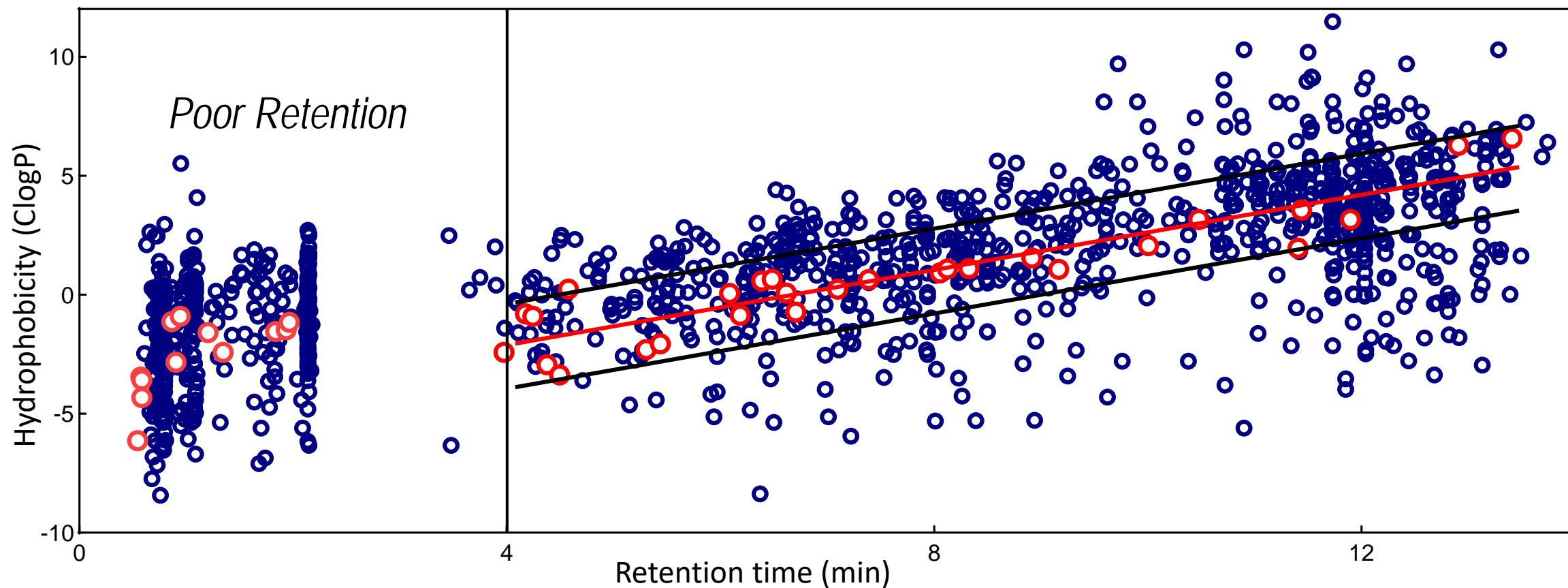
Database Annotation



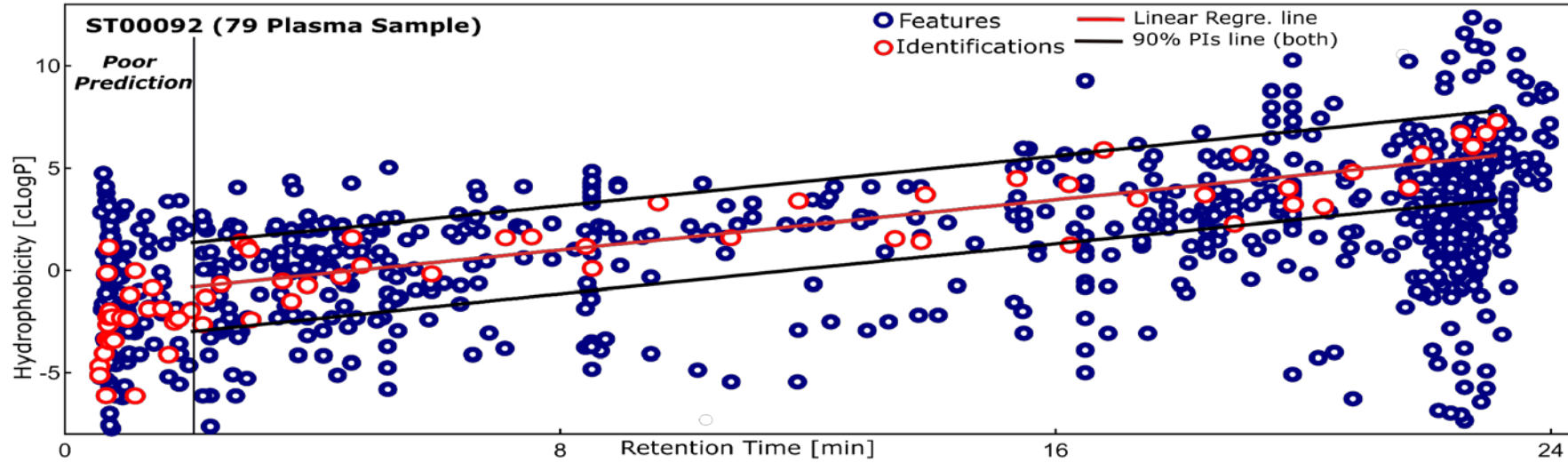
Gadara, D., Systematic Feature Filtering in Exploratory Metabolomics: Application toward Biomarker Discovery. *Anal. Chem.* **2021**, 93 (26), 9103–9110.
<https://doi.org/10.1021/ACS.ANALCHEM.1C00816>.

RT vs ClogP Relationship

1224 Features $\xrightarrow[\text{Filtration}]{\text{RTvsClogP}}$ 839 Features



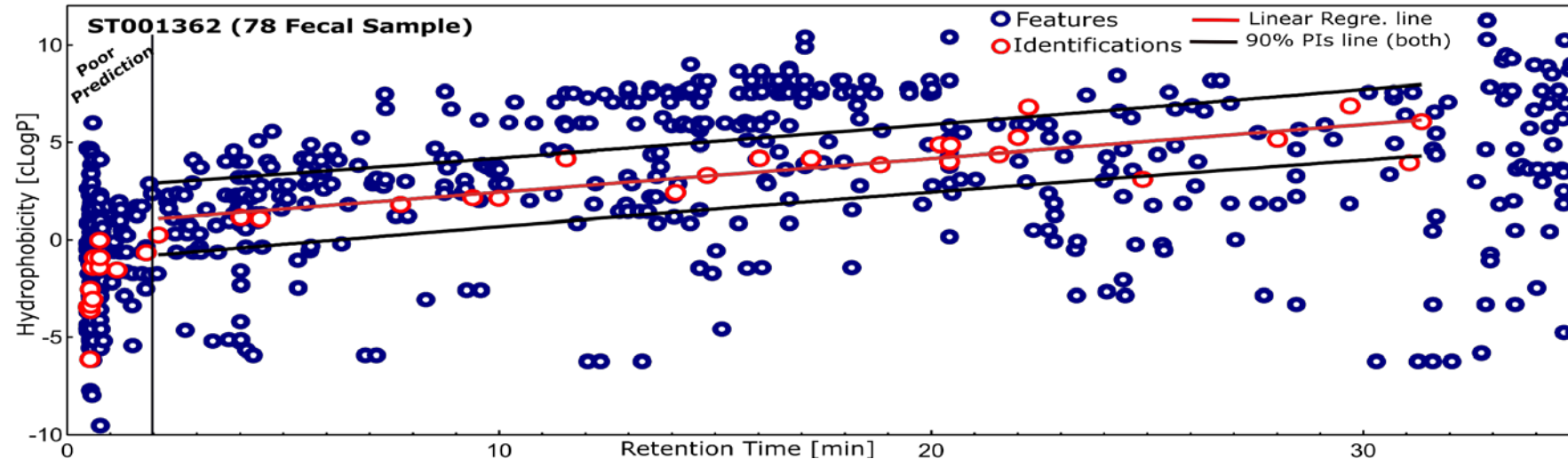
RTvsClogP Filtration on other Datasets



752 Features

RTvsClogP
↓
Filtration

447 Features



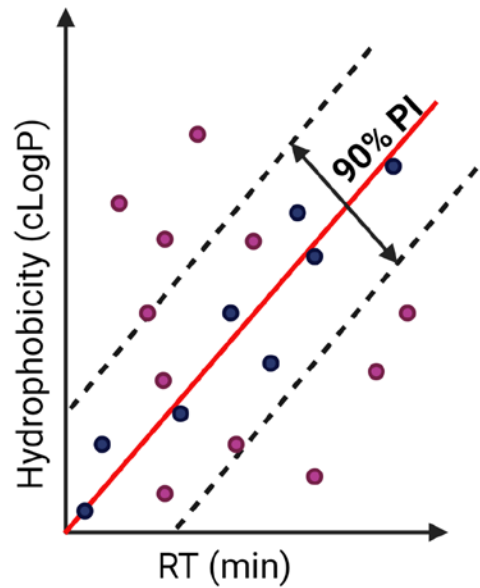
736 Features

RTvsClogP
↓
Filtration

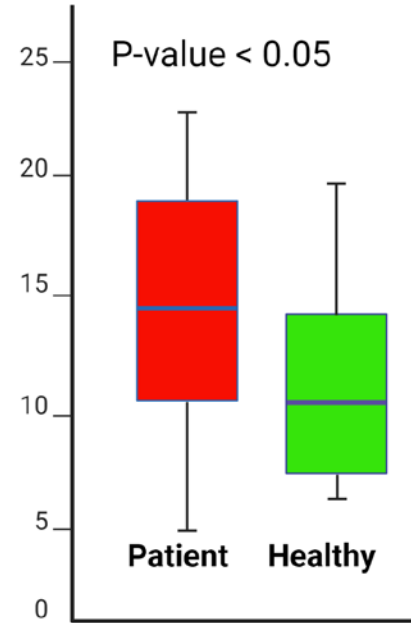
447 Features

Metabolomics Data Processing Workflow

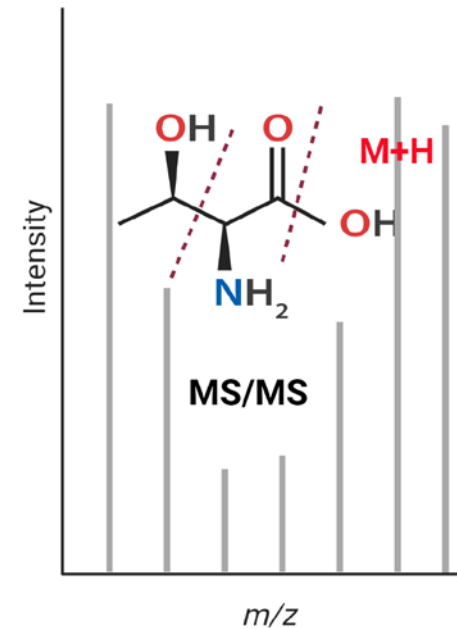
RT Filtering



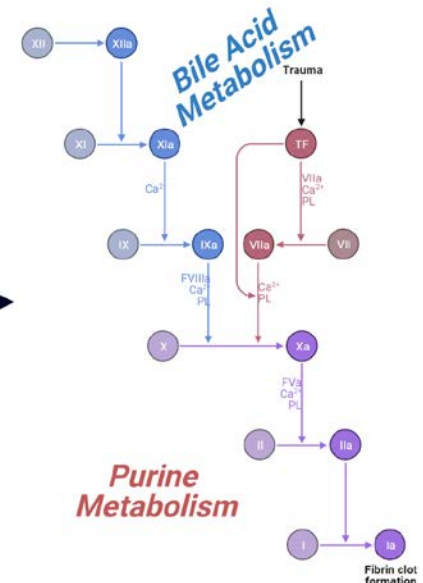
Statistical Analysis



Structure Elucida.



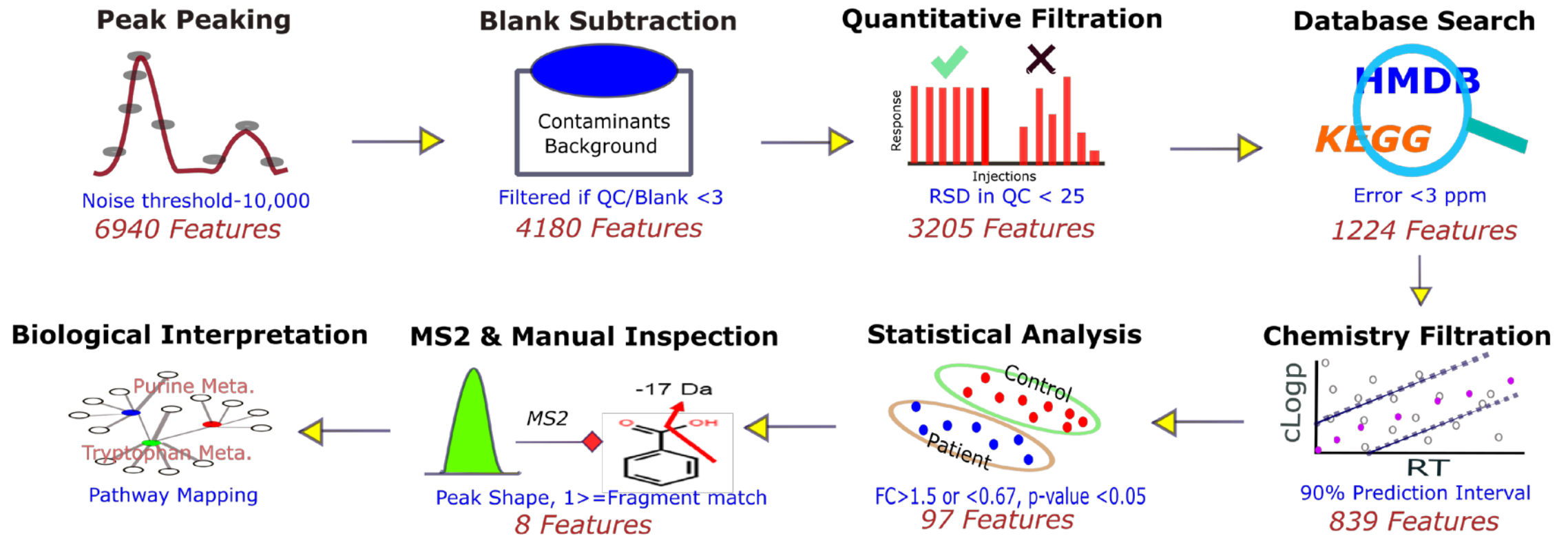
Interpretation



Application in Clinical Case/Control sample

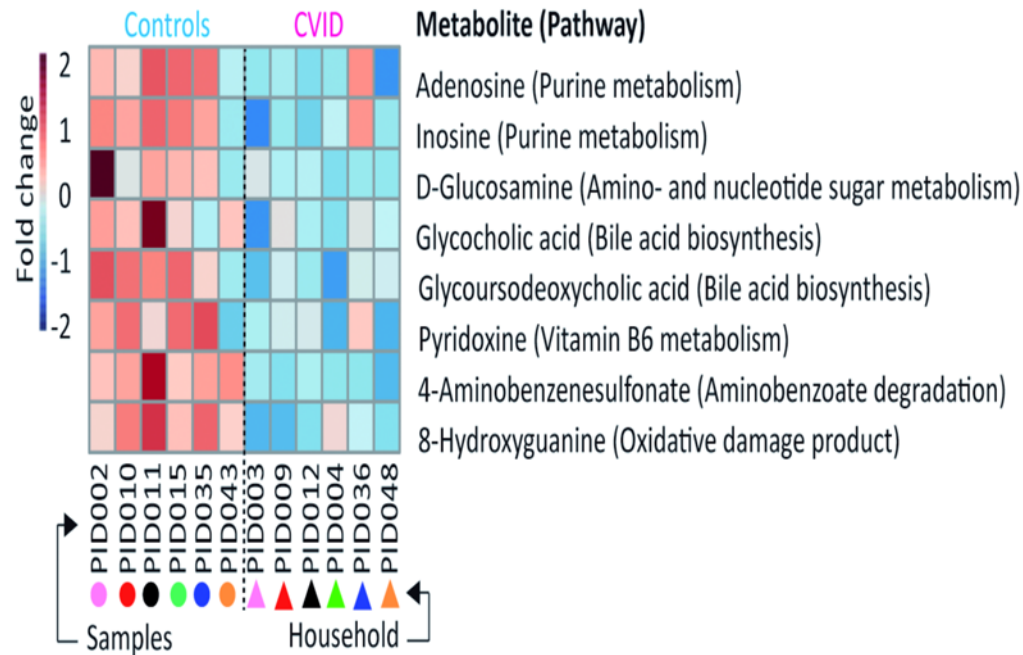
Common Variable Immunodeficiency Disease (CVID) - inadequate antibody responses and low levels of immunoglobulins

Case/Control Fecal sample (n=6) subjected to developed metabolomics data processing workflow



Interpretation of metabolomics data for CVID vs healthy subject

Eight metabolites differ significantly between CVID vs Healthy subject ($p < 0.05$)



In collaboration with Prof. David Smajs group, Faculty of medicine

- *Purine metabolites are important anti-inflammatory signal molecules modulating inflammatory response*

Welihinda AA et al. Cell Signal (2016) 28:552–60

He B et al. J Exp Med (2017) 214:107–23

- *A decreased level of “purine-like” molecules was previously detected in blood of CVID patients*

Callery EL et al, Sci Rep (2019) 9:e7239

- *Dysregulation of bile acid metabolism related to bacterial dysbiosis has been reported for several conditions*

Zheng X et al. BMC Biol. 2017 Dec 14; 15(1):120

Wang Y et al. mSystems. 2019 Dec 17; 4(6)

- *Decreased plasma levels of pyridoxine (B6) observed CVID patients*

Bierwirth J et al. Eur J Clin Nutr (2008) 62:332–5

Summary of Metabolomics

- We developed a systematic metabolomics data processing workflow for clinical biomarker discovery, explaining individual feature filtering stages
- Identified eight metabolites in stool samples which clearly distinguished between CVID patients and controls

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**analytical
chemistry**

Systematic Feature Filtering in Exploratory Metabolomics: Application toward Biomarker Discovery

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





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ORIGINAL RESEARCH article
Front. Immunol., 14 May 2021 | <https://doi.org/10.3389/fimmu.2021.671239>



Patients With Common Variable Immunodeficiency (CVID) Show Higher Gut Bacterial Diversity and Levels of Low-Abundance Genes Than the Healthy Housemates

 Juraj Bosák¹,  Matej Lexa²,  Kristýna Fiedorová^{3,4},  Darshak C. Gadara⁵,  Lenka Mícenková⁵,  Zdeněk Spacil⁵,  Jiří Litzman^{4,6},  Tomáš Freiberger^{3,4} and  David Šmajš^{1*}

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⁴Department of Clinical Immunology and Allergology, Faculty of Medicine, Masaryk University, Brno, Czechia
⁵RECETOX Center, Faculty of Science, Masaryk University, Brno, Czechia

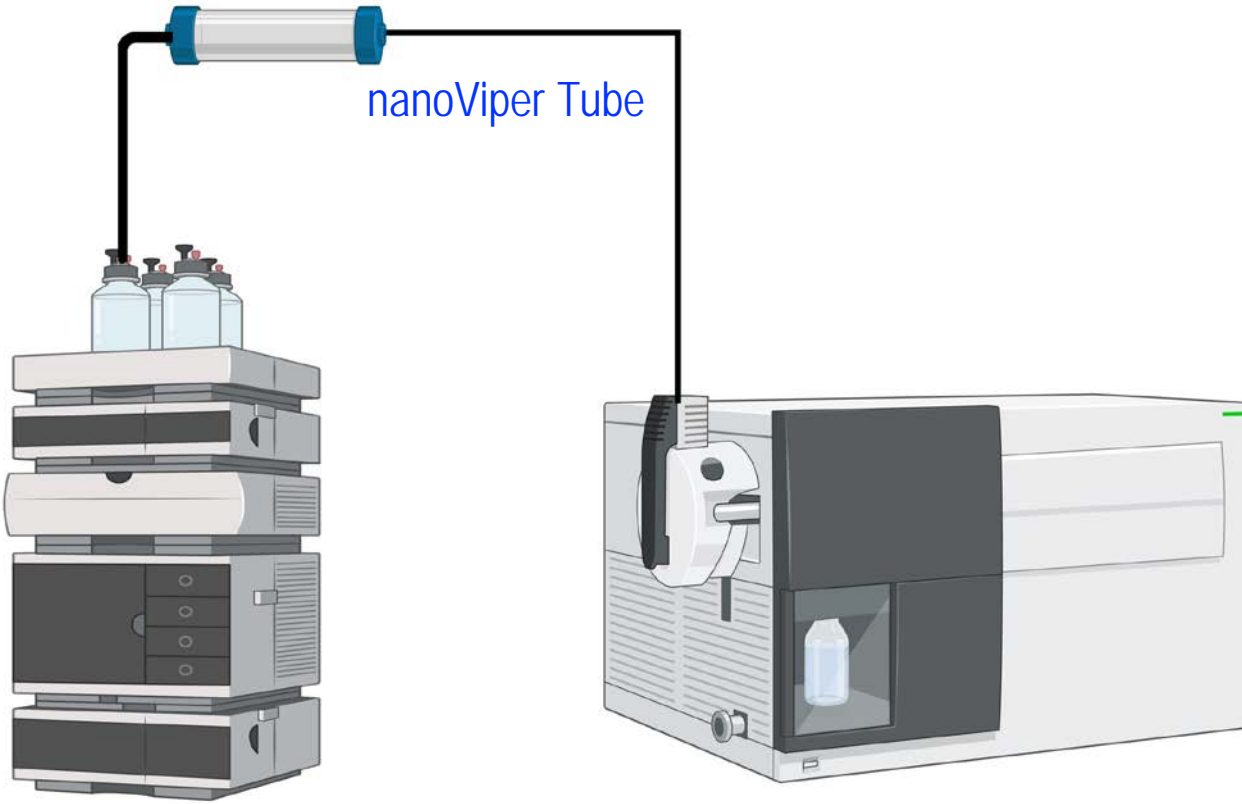
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LC Gradient and Flow Optimization

Microbore Column
1 mm I.D.

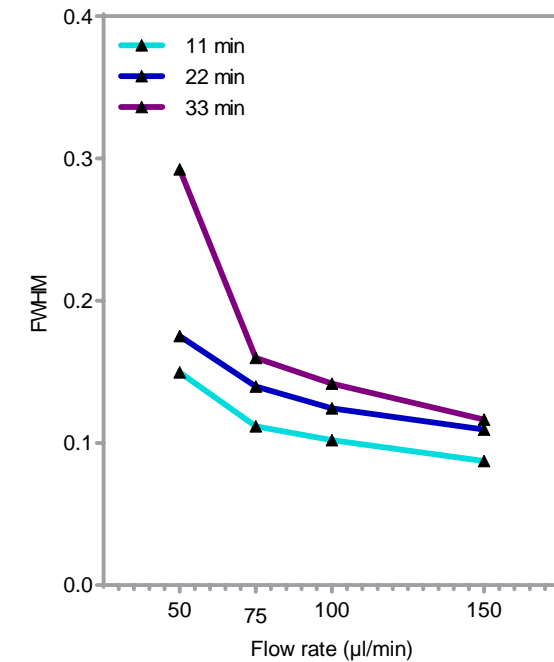
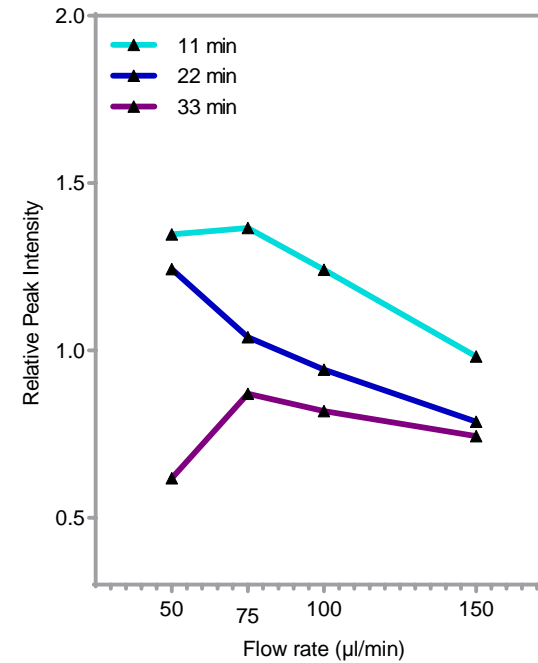
nanoViper Tube



UHPLC System

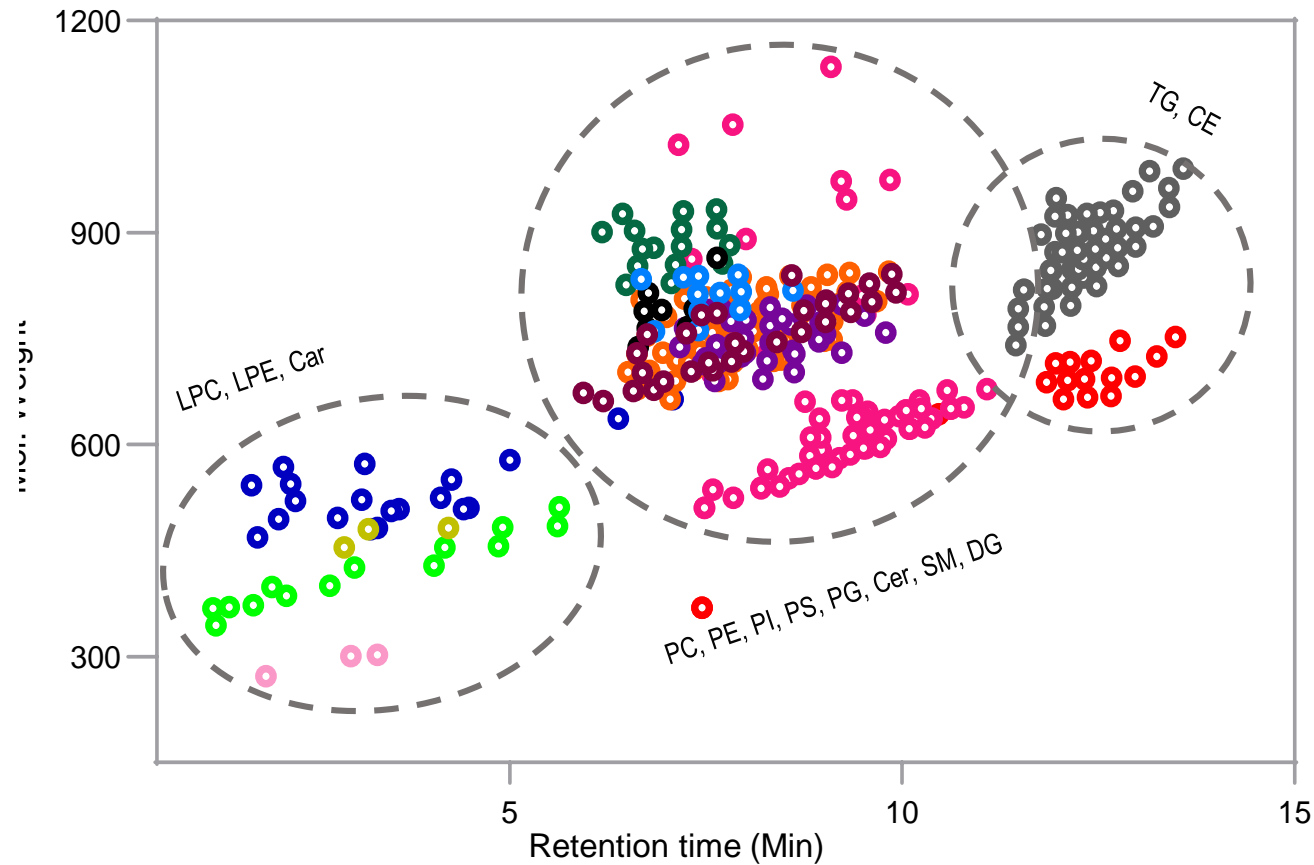
Four flow rate – 50, 75, 100, 120 $\mu\text{L}/\text{min}$

Three different gradient length – 11, 22, 33 min

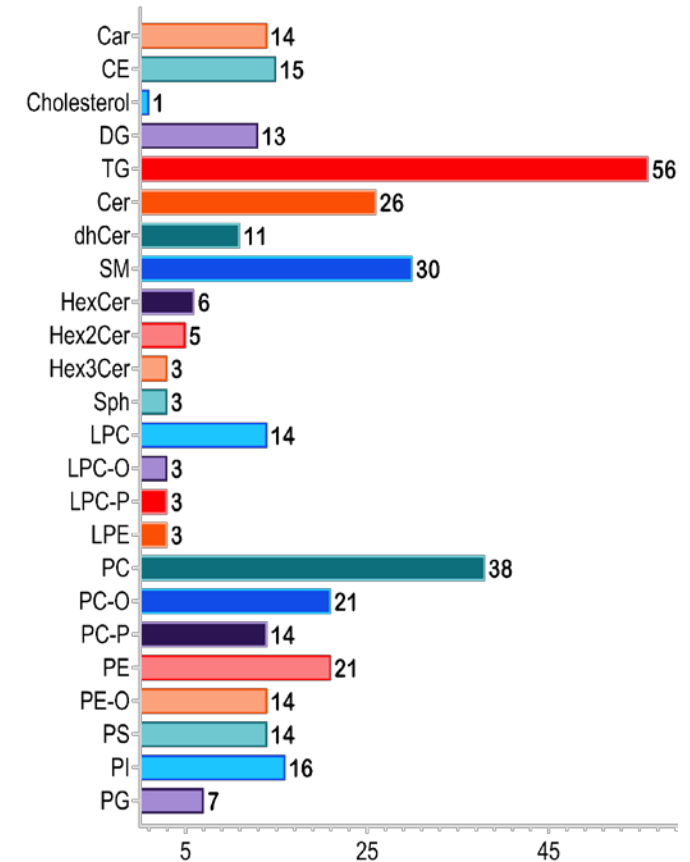


Single Cerebral Organoid Lipidomics

Reverse Phase Separation

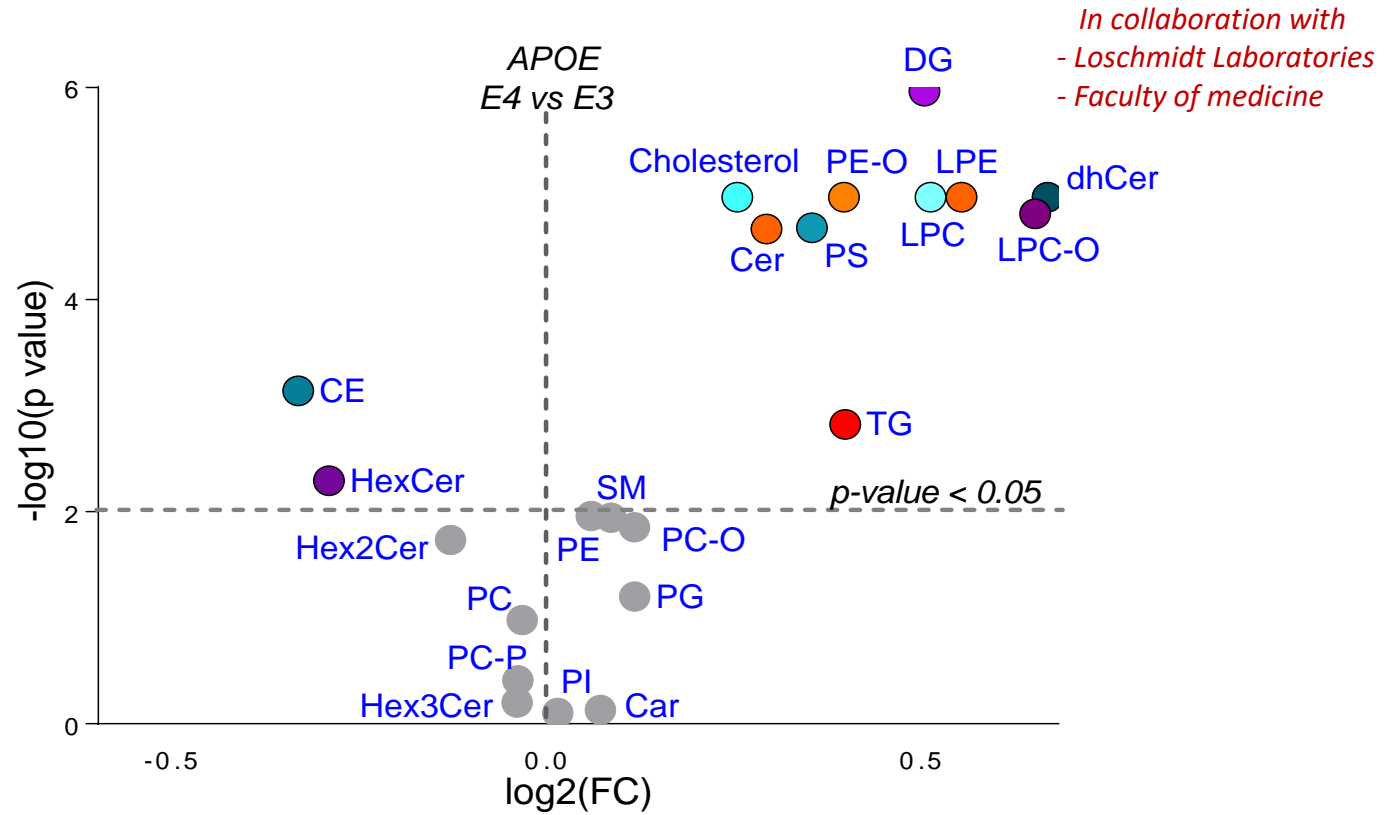
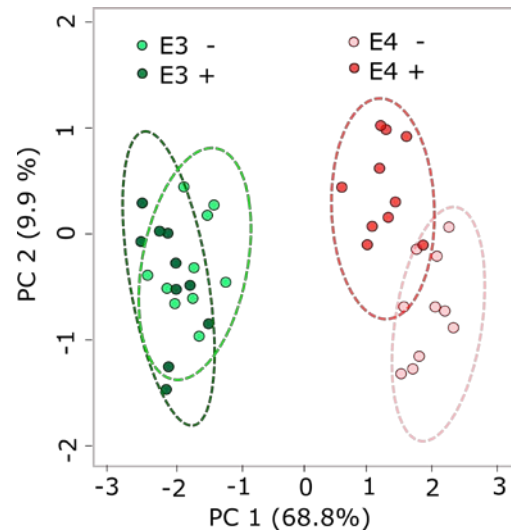
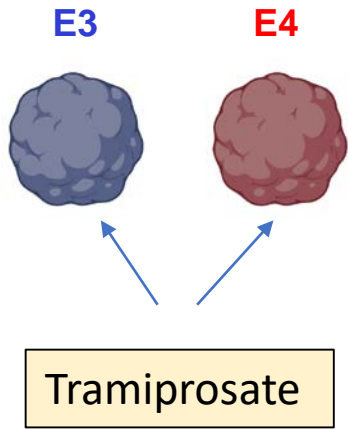


~ 350 lipid species from single cerebral organoid



APOE3 vs APOE4 Lipidomics

Cerebral Organoids
APOE Phenotypes (n=10)



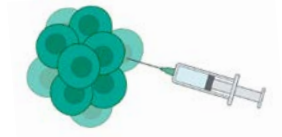
Single 3D-Hepatospheroid Lipidomics



200+ lipid species
Characterized

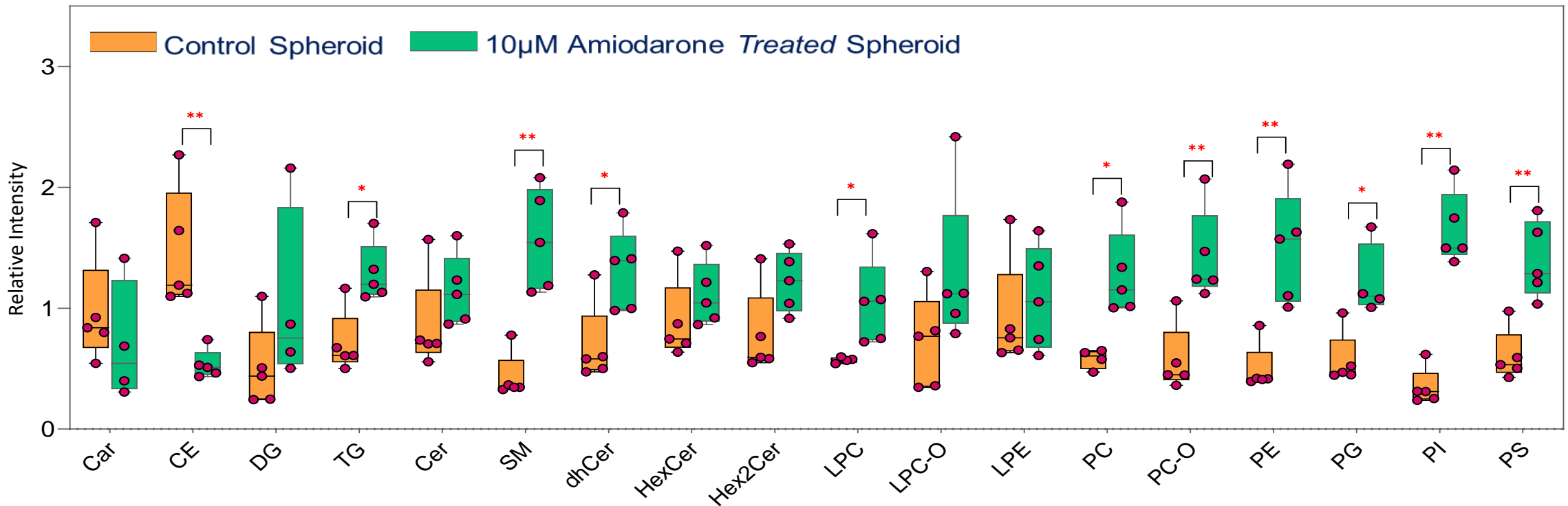


Control
Spheroid



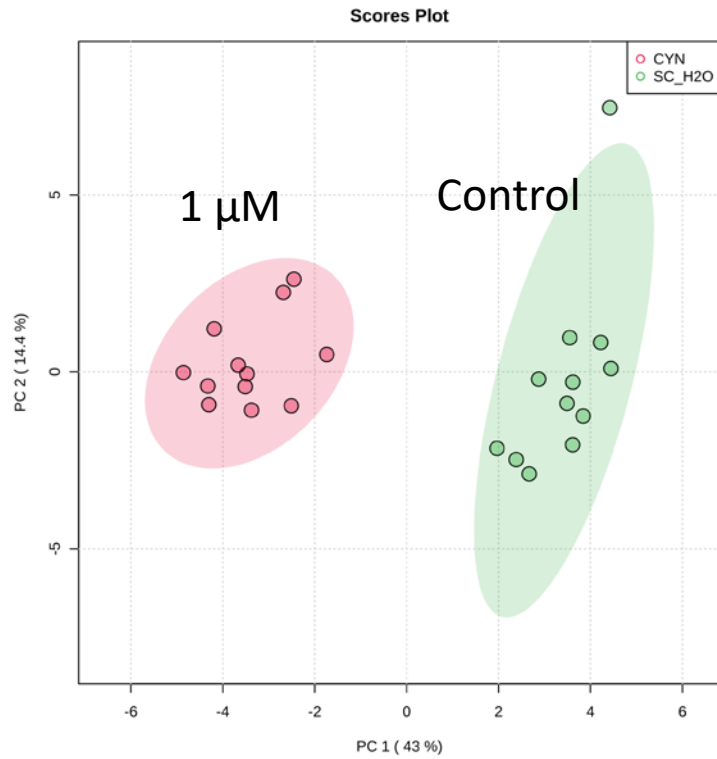
Spheroid
+ 10µM Amiodarone

*In collaboration with
- Marina Grossi
Pavel Babica's group*



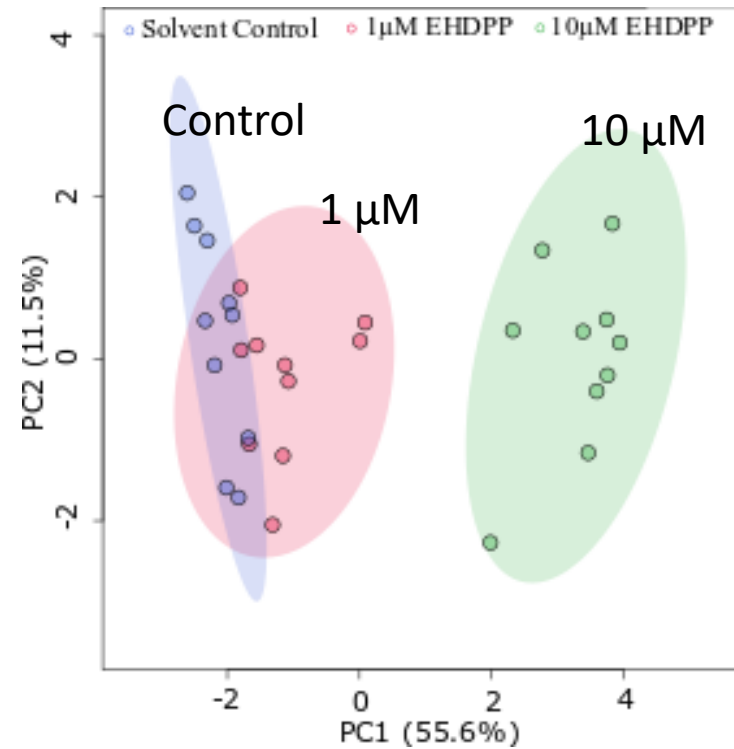
Other Hazardous Chemicals?

Cynotoxins _CYN



- Riju Roy Chowdhury
Dr. Pavel Babica's group

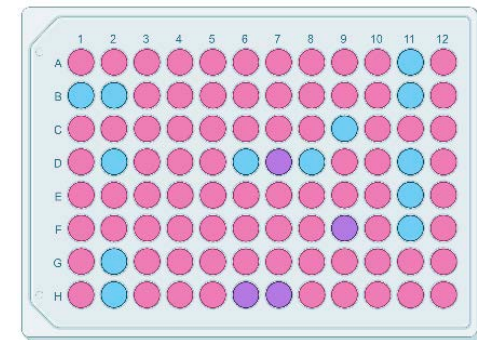
Flam Retardant - EHDPP



- Chander Negi
Prof. Ludek Blaha's group

Several chemicals

TM3 & TM4 Cell lines (2D)



Data Visualization is Ongoing

- Eliska Sychrova
Dr. Iva Sovadinova's group

Summary of Lipidomics

- μ LC-MS/MS lipidomics workflow allows sensitive, high-throughput and robust measurement of lipidome from small volume in vitro samples such as single hepatospheroid or single cerebral organoid.
- Application demonstrated on 5 different projects (1 first author + several co-authors manuscript under preparation)
- This work paves the way for a more routine application of μ LC-MS/MS lipidomics in high-throughput in vitro toxicity screening.

ACKNOWLEDGEMENT

- Sincerely Thankful to
- Supervisor- Dr.Zdenek Spacil
- Lab-members
- Collaborators

Thank you all for listening

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