LIPIDOMICS

ANALYSIS OF PHOSPHATIDYLCHOLINE SPECIES IN BIOLOGICAL SAMPLES

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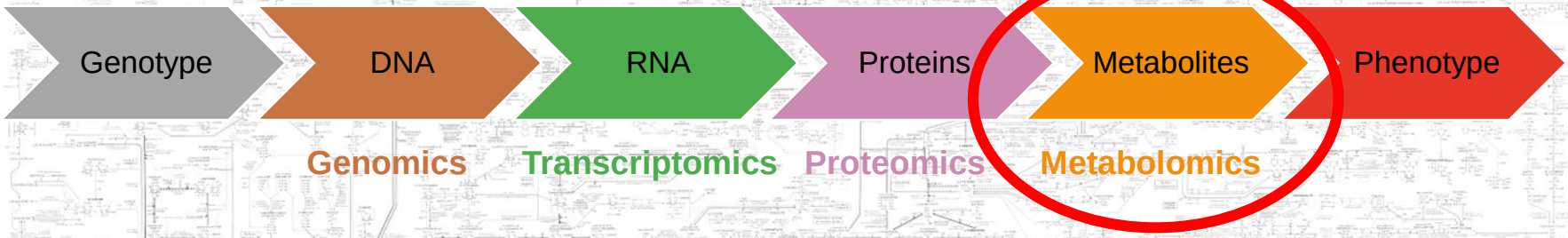
Lipidomics



Sub-discipline of Metabolomics

- Carbohydrates
- Amino acids
- Nucleotides
- Lipids → **Lipidomics**

Functions of lipids

- Main constituents of bio-membranes
- Energy source and storage
- Cell signalling

Application areas for lipidomics

- Correlation with several diseases
  - Cardiovascular disease
  - Inflammatory diseases
  - Neurological disorders
  - Cancer
  - Diabetes
  - Obesity
  - Eye diseases
  - Etc.

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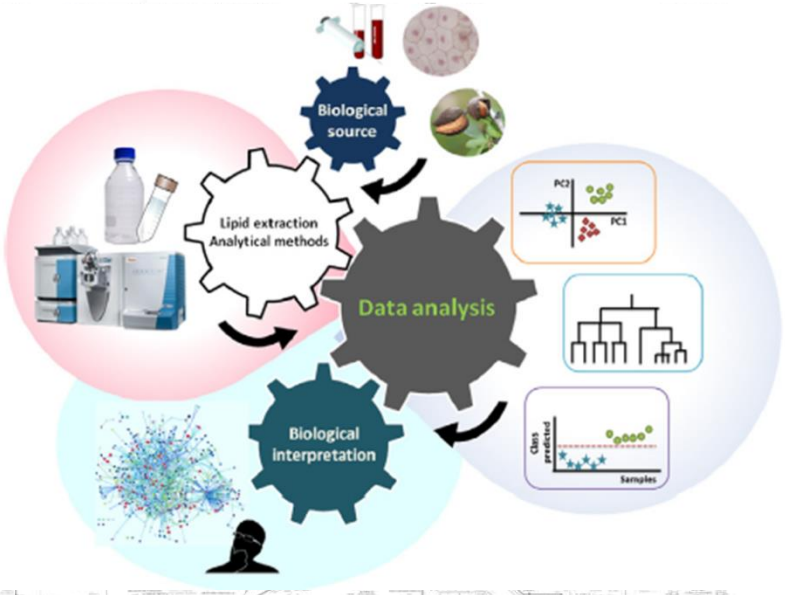
Lipidomics

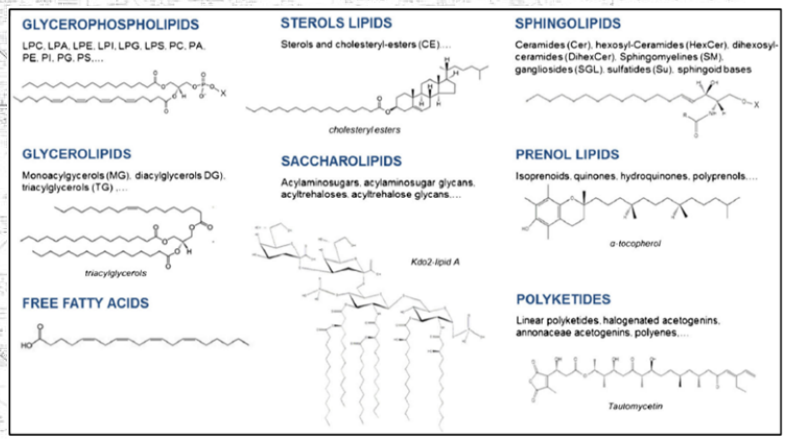


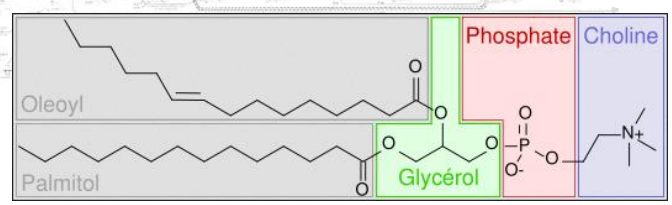
Analytical approaches

- Several extraction procedures
- Several chromatographic techniques (or no separation)
  - (RP, NP, HILIC, SFC)
- Mass spectrometry: high- vs. low-resolution

Challenges in Lipidomics:

- Highly diverse class of biomolecules
- > 40.000 species (ref. LipidMAPS)
  - 8 categories
  - Classes and subclasses
- Several isobaric and isomeric species
- Automated data analysis
  - Commercial solutions
  - Open access solutions





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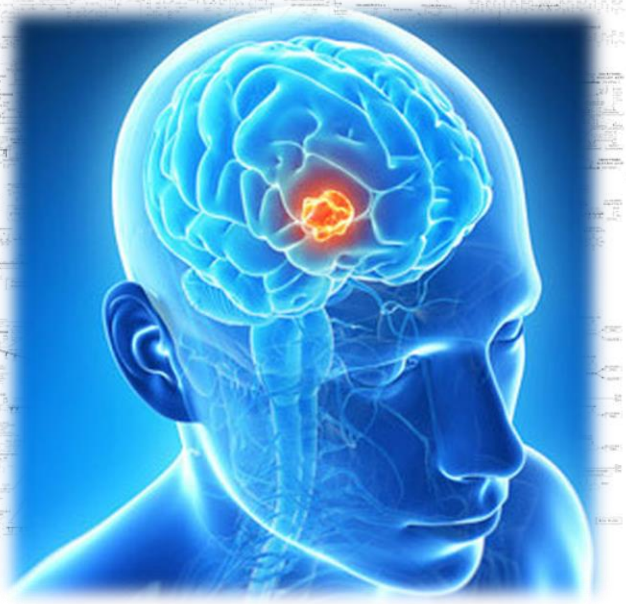
Outline of current project

General aspects

- Collaboration with University Vienna (Prof. Gerner) and General Hospital Vienna
- Investigate molecular patterns or mechanisms in progression and therapy of different kinds of brain tumors
- Cerebrospinal fluid (CSF) and serum samples
- Different sampling timepoints allowing for trending of compound patterns
- Analysis of different substance classes (proteomics, different lipid classes)

Our contribution

- Analysis of (lyso-) phosphatidylcholines
- Specific extraction method
- HPLC-MS (Orbitrap)



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Experimental

Extraction

- Several methods published: Folch (1957), Bligh & Dyer (1959), MTBE, BUME,...
- Our approach: acidified Bligh & Dyer
  - Liquid liquid extraction method ($CHCl_3$, MeOH, 10 mM HCl)
  - 3 extraction steps
  - $CHCl_3$ phases are collected and brought to dryness with N2 stream
  - Redissolution in HPLC eluent
- Recovery (tested adding 5 Standards) > 80%

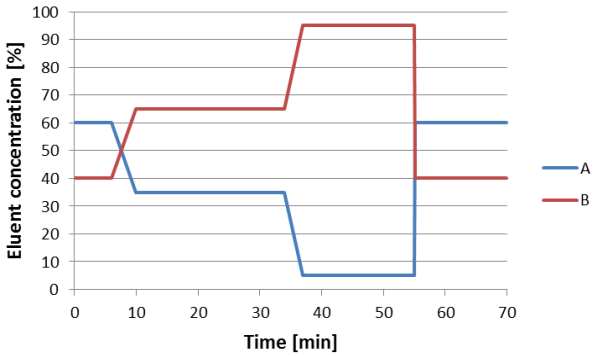
Analytical instrumentation (HPLC-MS)

- Agilent 1260 series HPLC
- LTQ Orbitrap XL mass spectrometer

Experimental

Chromatographic separation

- Phenomenex Kinetex C18 column (150 x 3 mm, 2,6 µm)
- Separation protocol based on Uhl et al 2011:
  - Eluent A (60/40 H2O/MeOH + 10 mM NH4-Ac + 1 mM HAc)
  - Eluent B (90/10 IPA/MeOH + 10 mM NH4-Ac + 1 mM HAc)
- Flowrate: 0,25 ml/min Injection volume: 10 µL



Mass spectrometry

- ESI positive mode
- Data dependent MS^2
  - 41 target analytes (8 LPC + 33 PC species)
  - Full scan in Orbitrap
  - MS^2 fragments scan in linear ion trap

→ exact mass, characteristic fragment (phosphocholine headgroup with m/z = 184,1 for PC´s or LPC´s) and RT allows for species characterization

→ e.g. PC aa C36:2 a

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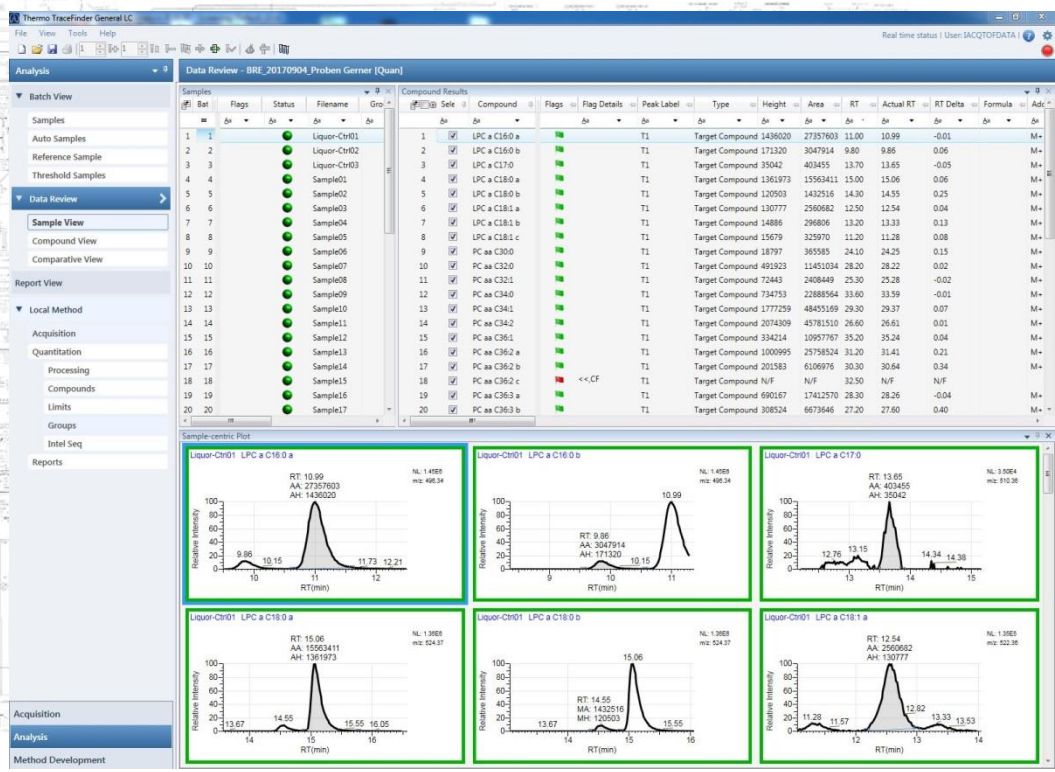
Experimental

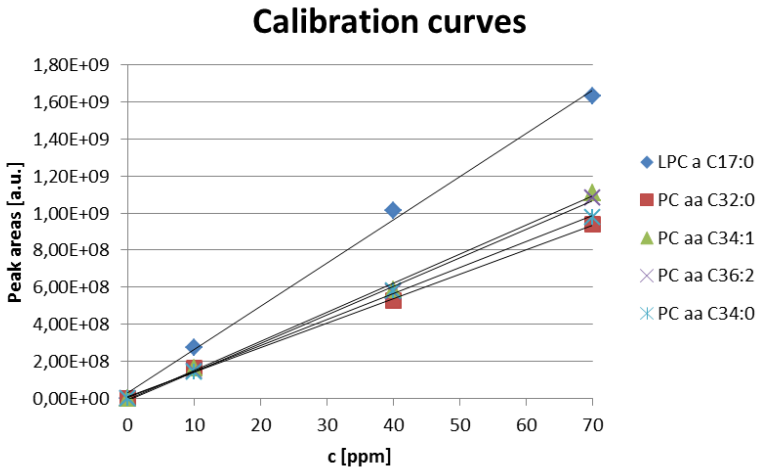
Data analysis

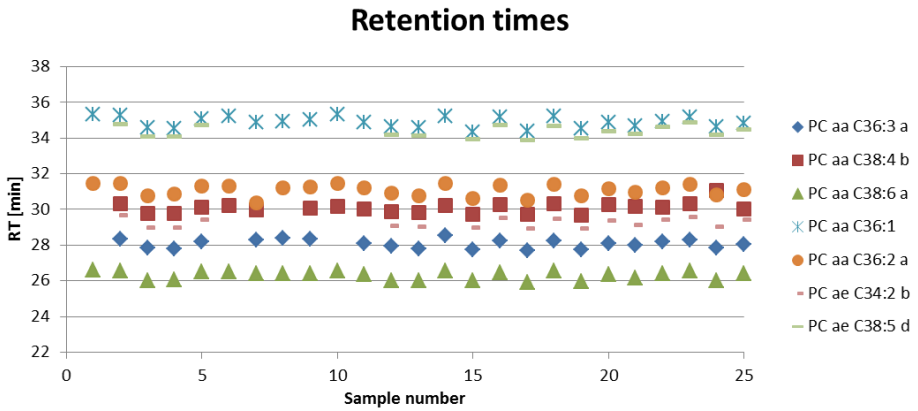
- Software: commercial vs. open access
- TraceFinder Software (Thermo)
- Automatic peak integration using Orbitrap full scan data
  - 41 target species
  - Mass range window: 10 ppm
  - RT window: 120 sec
- Structure confirmation of each feature
  - MS^2 data (fragment m/z 184,1)



Results

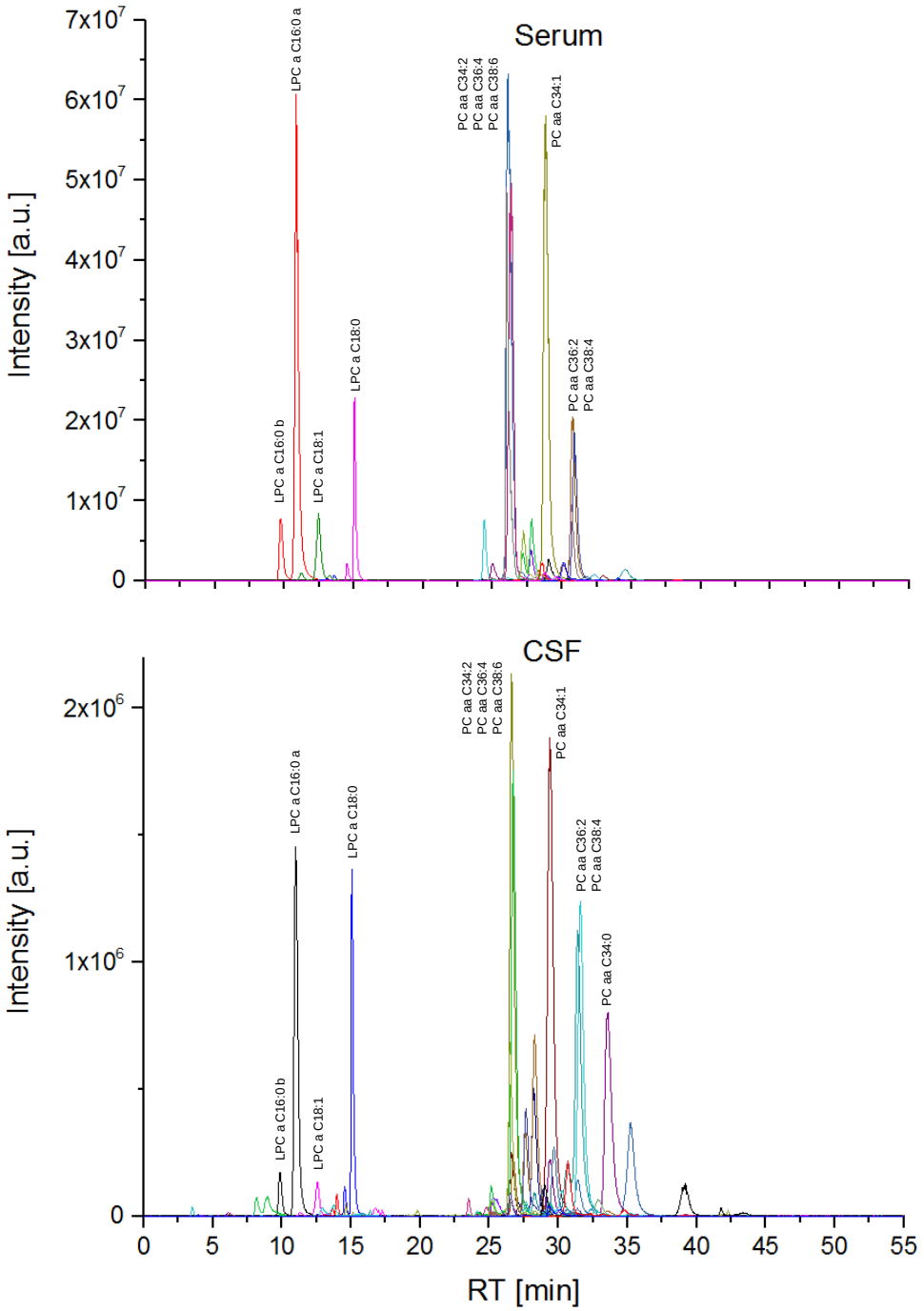
- Sequence with 25 samples + standards + controls
- Linearity between 10 ppb and 70 ppm (5 Standards)
  - $R^2 > 0,99$
- LOQ: 10 ppb
- RT´s: low variance over whole sequence
  - RSD´s ≤ 1%
  - Max. deviation: average + 3% (PC aa C38:4 b)
  - Column is thoroughly flushed after ~10 injections
- Mass accuracy: < 3,6 ppm





Chromatograms

- Overlay of EIC´s for all targets
- Serum vs. CSF (pooled control samples)
- Clear separation of PCs and LPCs
- Partially coelution of species



Conclusion and outlook

- Method for analysis of PC´s and LPC´s established
- First batch of samples analyzed
- Interpretation of results still open
- More samples to be analyzed
- But still potential to improve method
  - Alternative extraction methods: improve recovery, easier handling, predilution of serum?
  - Improve separation for PC species
  - Try to improve automation in data analysis

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Thank you for your attention

This work was conducted under the frame of „Programm Interreg ATCZ52 Österreich – Tschechische Republik: Infrastruktur für Metabolomik-Forschung und Klinische Chemie“.

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