4D-Lipidomics investigation of in *C. elegans daf-2* mutants related to ageing and longevity

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Introduction

The small nematode *Caenorhabdtis elegans* is one of the premier biomedical model organisms and employed in different aspects of basic and applied science such as ageing and longevity research. The *C. elegans daf-2* mutant investigated in this study encodes for the insulin-like growth factor 1 (IGF-1) receptor. DAF-2 is part of the first metabolic pathway discovered to regulate the rate of aging. The mutant worms show extreme changes in animal phenotype, including increased lifespan. Furthermore, changes in the lipid content were reported. Comprehensive coverage of detected lipids with a corresponding MS/MS spectrum is required for confident lipidome characterization. With the timsTOF Pro system this is realized by the unique PASEF (Parallel Accumulation Serial Fragmentation) acquisition mode. The PASEF scan mode offers the possibility to generate MS/MS spectra with uncompromised high data quality at high acquisition speeds for lipid profiling compared to traditional DDA analyses. PASEF can generate clean MS/MS spectra by separation of isobaric lipid species co-eluting from the LC. Additionally, Trapped Ion Mobility Separation (TIMS) provides reproducible true CCS values for increased confidence in lipid identification. In this study we showcase a fully integrated workflow for evaluating 4D-Lipidomics™ data in one software solution: MetaboScape®. Comparing lipid extracts from *C. elegans* wild type and *daf-2* mutants this enabled the pinpointing of characteristic lipids and their confident identification, including matching of measured and predicted CCS values.

References

(1) Matyash V., et al. J Lipid Research, 2008, 49(5):1137-46
(2) http://fiehnlab.ucdavis.edu/projects/LipidBlast
(3) Kind T., et al. Nature Methods, 2013, 10:755-758
(4) Tsugawa H., et al. Nature Methods, 2015, 12:523-526
(5) Zhou Z., et al. Analytical chemistry, 2017, 89(17):9559-9566.
(6) Picache J., et al Chemical Science, 2019, 10:983-993

Methods

Lipids from *C. elegans* were extracted based on a protocol from Matyash *et al. [1].* For ESI positive and negative mode measurements 2 µl and 10 µl were injected, respectively.
The 20 minute reversed phase based LC separation was performed using an Elute UHPLC system (Bruker Daltonics) and a Bruker intensity C18 column (100 x 2.1 mm, 1.9 µm).
Column temperature was set to 55°C.
LC Buffer A: Acetonitrile / water (60:40, 10 mM NH4Formate, 0.1% FA)
LC Buffer A B: Isopropanol / acetonitrile (90:10, 10 mM NH4Formate, 0.1% FA)
The MS data was acquired in positive and negative ESI mode using a timsTOF Pro instrument (Bruker Daltonics) in PASEF mode.
Data was evaluated using MetaboScape 5.0.

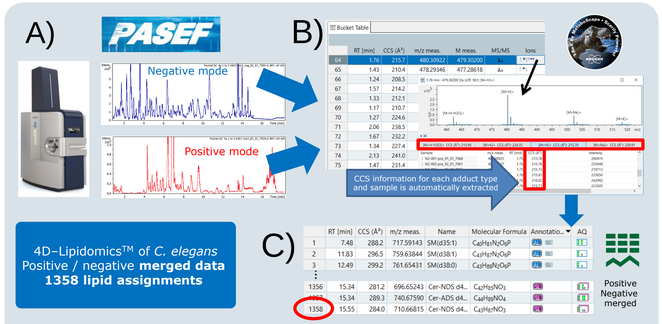

Fig. 1 4D-Lipidomics – PASEF and MetaboScape T-ReX® 4D:
A) Base peak chromatograms of selected pooled Quality Control sample from *C. elegans* lipid extracts measured in positive mode (bottom, red) and negative mode (top, blue) by LC-PASEF using a timsTOF Pro instrument.
B) MetaboScape: T-ReX stands for Time aligned Region complete eXtraction. T-ReX 4D automatically extracts five complementary criteria which can be used for high confident lipid identification: Retention time, accurate precursor mass (including adduct information), isotopic pattern, MS/MS spectra, CCS values
C) Automatic lipid assignment of T-ReX 4D extracted lipids by LipidBlast [2-4] and custom Analyte list: 1358 lipid assignments in positive / negative mode merged data.

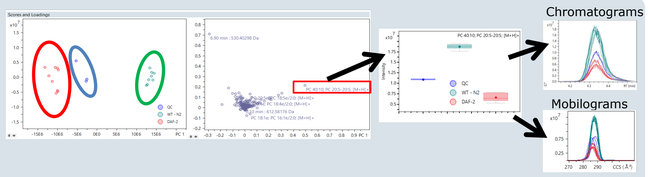

Fig. 2 4D-Lipidomics - Statistics:
Principal Component Analysis (PCA) of 4D-Lipidomics data. The scores and loadings plot shows clear separation of wild type and *daf-2* mutant lipid extracts (3 biological and 3 technical replicates). Pooled QC samples (3 samples) cluster in the middle between wild type and *daf-2* mutants. One Loading contributing to wild type and mutant sample separation is highlighted. Interlinked displays and access to raw data in MetaboScape® enables quick visual validation of statistical changes observed in PCA. The Box plot, chromatogram and mobilogram view for the lipid assigned as PC 40:10 are shown.

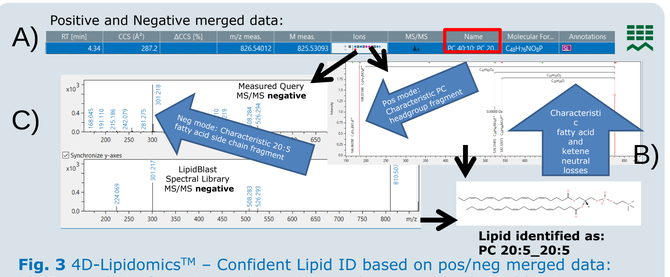

Fig. 3 4D-Lipidomics™ – Confident Lipid ID based on pos/neg merged data:
A) Screenshot of positive and negative mode merged bucket tables in MetaboScape highlighting the extracted lipid PC 40:10.
B) Screenshot of MS/MS spectrum of $[M+H]^+$ automatically annotated with molecular formula for fragment ions and neutral losses in SmartFormula3D. The characteristic headgroup fragment 184 m/z validates the automatic ID as phosphocholine. Neutral losses and fragment ions indicate side chains to be 20:5 fatty acids.
C) Measured MS/MS spectrum of $[M+HCOOH-H]^-$ (top) and LipidBlast MS/MS library spectrum (bottom). Characteristic 301 m/z side fragment validates lipid ID as PC 20:5_20:5.

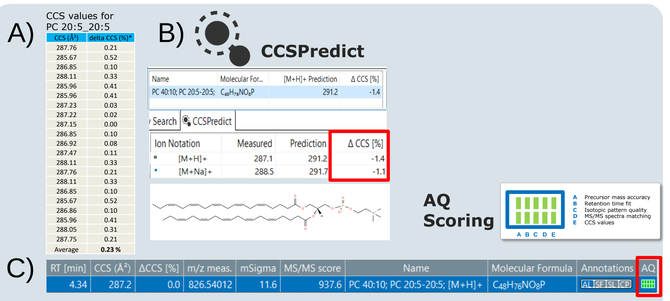

Fig. 4 4D-Lipidomics™ – Increased confidence in Lipid ID based on CCSPredict:
A) Highly reproducible CCS values automatically extracted by T-ReX4D: For PC 20:5_20:5 the average delta CCS across 21 analysis is 0.23%.
B) CCSPredict** machine learning based prediction of CCS value in MetaboScape increases confidence in lipid ID: low deviation of measures vs. predicted CCS value for both $[M+H]^+$ and $[M+Na]^+$.
C) Analyte List based annotation enables quick de-replication and Annotation Quality scoring (AQ Scoring). Five complementary measures in AQ scoring provide users with a quick graphical feedback on confidence in identification.

*|(CCS measured - mean CCS) / mean CCS x 100|
**is based on LipidCCS from [5]

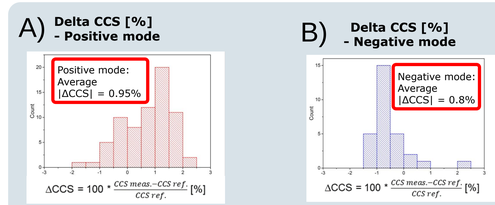

Fig. 5 Deviation of CCS values measured on a timsTOF Pro versus CCS values from the public CCS Compendium repository [6].
A) CCS values of 70 measured lipids in *C. elegans* vs. CCS compendium.
B) CCS values of 30 measured lipids in *C. elegans* vs. CCS compendium.

Summary

The 4D-Lipidomics™ workflow was presented as a powerful tool for the deep profiling of the *C. elegans* lipidome. This is the basis for an investigation of characteristic changes induced by the *daf-2* mutation and a first step for a better understanding of how this mutation relates to an increased lifespan.

Conclusions

- The presented 4D-Lipidomics™ workflow enables deep profiling of the *C. elegans* lipidome
- MetaboScape automatically assigned IDs to > 1300 lipid features in a positive / negative mode merge data set
- MetaboScape could pinpoint relevant lipids and enables their confident identification
- Machine learning based CCS prediction provided further confidence in lipid ID
- CCS values generated on a timsTOF Pro system can be matched to public CCS repositories with high accuracy

4D-Lipidomics™ timsTOF Pro