Paving the way towards analysis of *Caenorhabditis elegans* individuality— Development of single worm lipidomics based on nanoLC-TIMS-MS/MS

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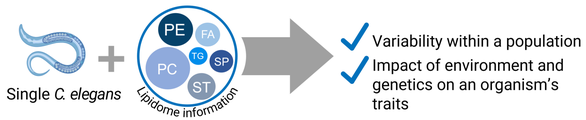
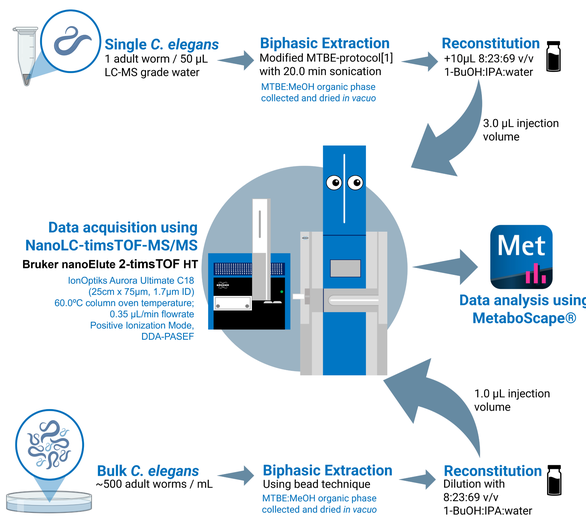
Overview



- Variability within a population
- Impact of environment and genetics on an organism's traits

Caenorhabditis elegans is extensively studied as a model organism for developmental, behavioral, and disease research. To the best of our knowledge, *C. elegans* lipidomics has been solely examined through bulk extraction methods. Here, we present a **single worm lipidomics method** leveraging the low sample requirements and higher sensitivity of **nanoLC-TIMS-MS/MS** in analysing and annotating lipids from individual *C. elegans*.

Methods



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4D Lipidomics

Single worm samples exhibit different lipid profiles compared to bulk extracts.

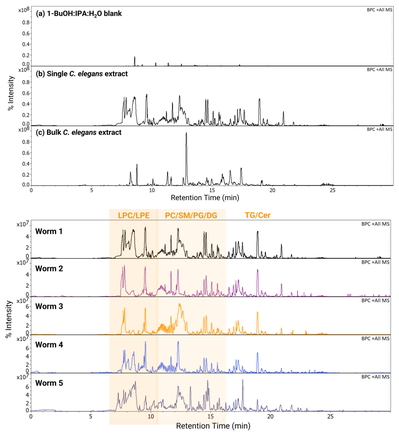

Figure 1. Normalized base peak chromatograms of blanks (a) and samples (b,c) show differences in nLC-MS positive mode profiles. Representative chromatograms of single *C. elegans* (**b**), and bulk worm sample (**c**) have distinct visual differences in the lipid profiles particularly in the LPC/LPE region.

Figure 2. Representative nLC-MS profiles of individual worms. Samples 1 to 5 vary considerably in terms of relative intensities in the LPC/LPE and PC/SM regions. Peak shapes are comparably similar in the TG/Cer portion, with some noticeable changes in intensities.

Lipid class coverage between individual and bulk *C. elegans* are comparable across classes, with thousands of features detected and putatively annotated with MetaboScape.

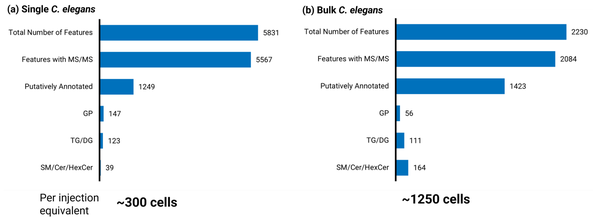

Figure 3. Positive ion mode datasets analyzed with MetaboScape show 1249 4D features detected and annotated from the individual worm (a) and 2084 in bulk (b) extracts. Same parameters were used for these datasets, with slight adjustments on the recursive feature extraction in MetaboScape. Lipid classes detected by nanoLC-TIMS-PASEF included PCs, SMs, and TGs which were previously reported to be present in *C. elegans* [2].

Chemometrics

PE and TG features contribute more to the variance between bulk and single *C. elegans* samples.

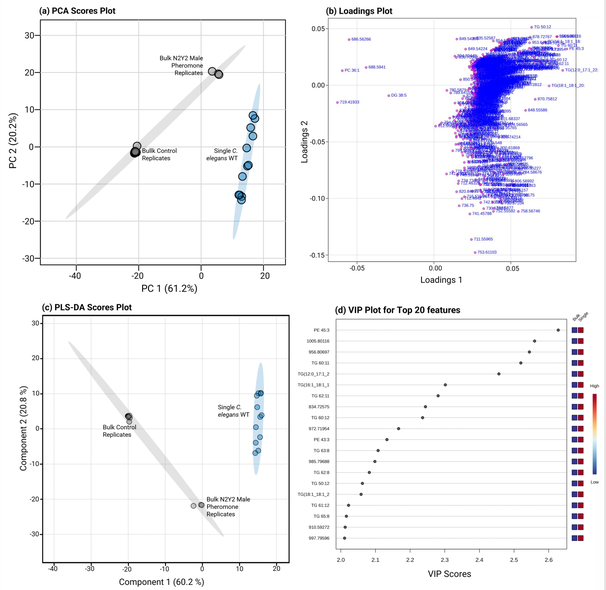

Figure 4. Comparison between bulk and single *C. elegans*. Multivariate analysis via MetaboAnalyst 6.0 show distinct separation between the bulk versus single *C. elegans* samples. In the PCA scores plot (**a**), majority of the variance is in PC 1, with TG and PE features contributing to the clustering of the samples according to the loadings plot (**b**). Moreover, more abundant PC and DG contribute further to the separation of mutant worms from the control in the bulk sample. Supervised analysis using PLS-DA (**c**) substantiated the observed clustering trend in the PCA. Overall, most PE and TG lipids comprise the top 20 contributors (**d**) to the variance between bulk and single worm samples—all of which were more abundant in single worms.

Annotations

CCS values allow verification of annotated features along with MS/MS spectra and library matches.

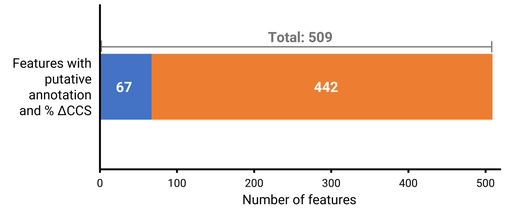

Figure 5. Collision cross section (CCS) values obtained from TIMS-MS for lipids, annotated using rule-based methods in MetaboScape 2025, were automatically compared to class-specific CCS models. The %ΔCCS values resulting from class-specific comparison were used to identify which putative annotations will be prioritized for verification. This was done by comparing measured CCS to reference values obtained from standards and their corresponding adducts (CCS Compendium, LipidMaps). CCS prediction is also possible using LipidCCS[3], in case both repositories do not have CCS information available. While DDA lipidomics can improve lipid annotation using MS/MS-based fragmentation [4], it is highly dependent on the quality of fragmentation and reference libraries. CCS values, which are innate structural properties of molecules [5], provide a secondary layer of verification of annotated MS/MS spectra, improving lipid identification.

Highlights and outlook

- Analyzing **individual *C. elegans*** is **possible** with nanoLC-TIMS-MS/MS.
- Lipid classes (PC, PE, SM, TG) previously reported in *C. elegans* have been **detected and annotated in single worm** samples, including several Cer and HexCer species.
- Lipid species (SM, TG, PI, Cer) with lower abundances **benefit from PASEF**, as it allows the selection and fragmentation of multiple precursors.
- The number of worms that can be analyzed individually is limited, considering that currently, **harvesting *C. elegans* remain labor-intensive**.
- Sample **preparation and extraction of single *C. elegans*** will be optimized for nLC-MS applications.
- Optimizing **annotation and statistics parameters with IMS-MS** information will enhance insights into biological variations between individual worms.

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Conflict of Interest Disclosure

Authors N. Balasubramanian, L. Abel, V. Jeck, A. Barsch and S. Meyer, are employees of Bruker Corporation or one of its subsidiaries ("Bruker"). Bruker manufactures and sells analytical instruments including mass spectrometers and software. Bruker mass spectrometers and software were used in this study.

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