

Confident annotation of GPNAE and HexGPNAE in *Caenorhabditis elegans* aided by homologous series extension in LC-MS/MS data

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Introduction

Lipids can be systematically described by their head groups, side chains, double bonds, and modifications. They form grids in LC-MS/MS data based on this systematic nature. We present a workflow that can detect and extend these grids by detecting and extrapolating homologous series for molecular formulas, retention times, and collision cross sections, revealing potential yet unannotated lipid candidates or validating RT and CCS values of annotated lipid candidates relative to their peers.

This new functionality allowed us to semi-automatically derive and visualize homologous series of lipid-like glycerophospho-N-acylethanolamides (GPNAE) and hexosylglycerophospho-N-acylethanolamides (HexGPNAE) molecules in *Caenorhabditis elegans* and revealed prospective positions (exact molecular formula and m/z , extrapolated RT and CCS) of additional members.

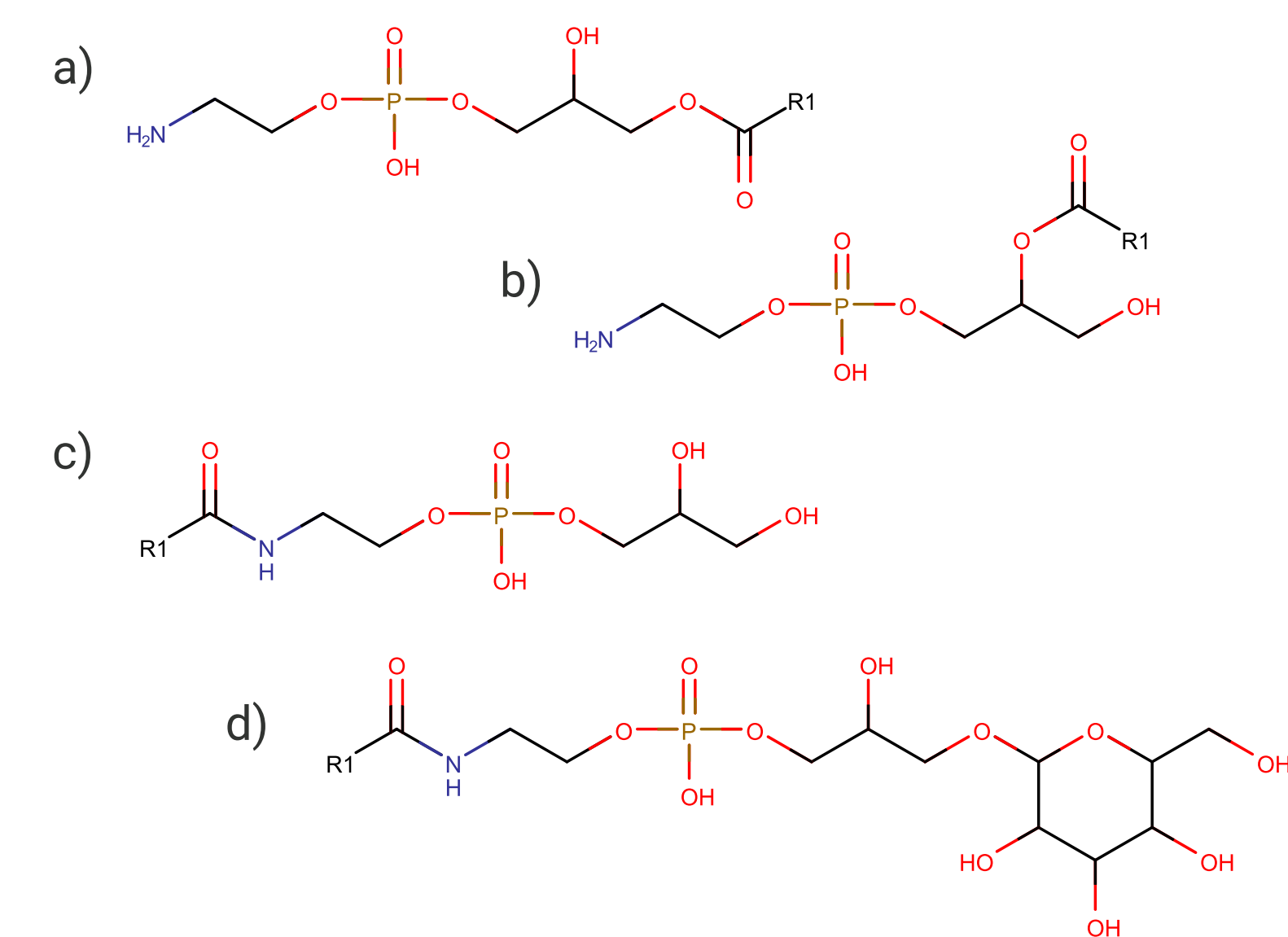


Fig. 1 Example structures for a) sn1- and b) sn2-lysophosphatidyl-ethanolamines (LPEs), c) glycerophospho-N-acylethanolamides (GPNAE) and d) hexosylglycerophospho-N-acylethanolamides (HexGPNAE)

Methods



Mixed stage *C. elegans* reference samples were extracted with 50% H₂O/50% MeOH and separation of lipid-like molecules was achieved on a Kinetex C18 column (100 mm x 2.1 mm, 1.7 μm particle size) and a linear gradient from 100% H₂O + 0.1% formic acid to 100% ACN + 0.1% formic acid. Mass spectrometric detection was performed using a Bruker timsMetabo™ TIMS-TOF-MS/MS in positive and negative ionization mode.

Data was analyzed in Bruker MetaboScape® 2025b with T-ReX® 4D processing. Detected Features and their corresponding MS/MS spectra were subjected to dedicated MassQL queries and manual inspection, to create an initial Target List. Example MassQL query to find GPNAE and HexGPNAE are shown below (Damiani *et al.*, 2025). The annotated features were then selected to detect and extrapolate homologous series, and to identify further features, which are associated to these series.



GPNAE & HexGPNAE:

```
QUERY scaninfo(MS2DATA) WHERE
MS2PROD=96.969619:TOLERANCEMZ=0.005 AND
MS2PROD=78.959054:TOLERANCEMZ=0.005 AND
MS2PROD=152.995834:TOLERANCEMZ=0.005 AND
MS2PROD=171.006399:TOLERANCEMZ=0.005
```

HexGPNAE:

```
QUERY scaninfo(MS2DATA) WHERE
MS2PROD=96.969619:TOLERANCEMZ=0.005 AND
MS2PROD=78.959054:TOLERANCEMZ=0.005 AND
MS2PROD=152.995834:TOLERANCEMZ=0.005 AND
MS2PROD=171.006399:TOLERANCEMZ=0.005 AND
MS2PROD=333.059222:TOLERANCEMZ=0.005
```

Interactive detection, extrapolation and extension of homologous series

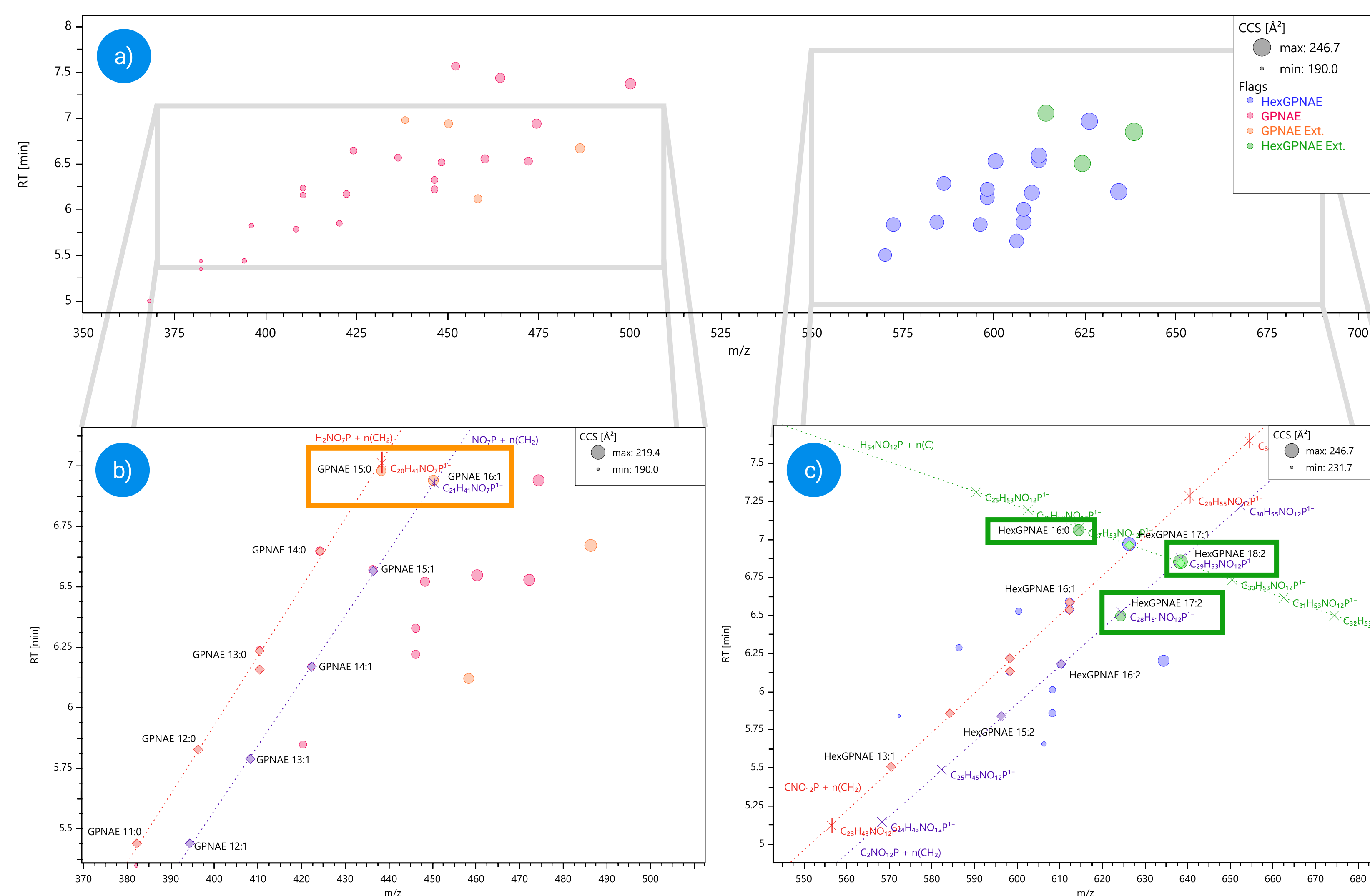


Fig. 2 a) displays features that were annotated as GPNAE (red) or HexGPNAE (blue) using a combination of a manually curated Target List, and class-specific MassQL queries in MetaboScape. Further lipids for both classes were later annotated, based on their positioning along respective homologous series (GPNAE in orange, HexGPNAE in green.)

Fig. 2 b) and c) show, how the selection of annotated features in MetaboScape 2025b can automatically detect, display and extrapolate respective homologous series, suggesting the positions of further potential members in m/z , RT and mobility. GPNAE and HexGPNAE which were revealed due to analysis of homologous series, are marked in orange and green boxes, respectively.

In Fig. 2b) the lipids GPNAE 15:0 and 16:1 were not annotated via Target List or MassQL, but could be confirmed by their positions in two homologous series 11:0, 12:0, 13:0 ... (red dotted line) and 12:1, 13:1, 14:1 ... (purple dotted line).

In Fig. 2c) the lipids HexGPNAE 17:2 and 18:2 were not annotated via Target List or MassQL, but could be confirmed by its position in two homologous series 15:2, 16:2, ... (purple dotted line). HexGPNAE 16:0 was revealed due to its position along the new homologous series 16:0, 17:1, 18:2, ... (green dotted line.)

References

Damiani *et al.*, Nat Methods. 2025 May 12.

Charbonnet *et al.*, Environ Sci Technol Lett. 2022 Jun 14, 9(6):473-481.

Competing interest statement: Authors Nikolas Kessler, Aiko Barsch and Matthew R. Lewis 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.

Homologous series support annotations

The annotation confidence framework introduced by Charbonnet *et al.* for PFAS, particularly levels 2c and 3c, emphasizes the value of homologous series in supporting compound identification. This concept translates seamlessly to 4D-Lipidomics, where lipids often exist as structurally related series differing by defined chemical increments. In this context, the detection of multiple members within a homologous lipid series—each exhibiting consistent trends across retention time, ion mobility, and fragmentation behaviour—can significantly bolster the confidence of annotations.

For instance, even if a lipid species lacks one or more characteristic MS/MS fragments, its alignment with a well-characterized series can justify a level 2c or 3c classification, enhancing interpretability and reducing false positives. This approach leverages the multidimensional data of 4D-Lipidomics to provide a robust, systematic framework for lipid identification, mirroring the rigor applied in PFAS analysis.

Conclusion

- MassQL queries helped to lay out a starting matrix of MSMS-validated lipid species.
- MetaboScape 2025b allows to interactively select lipid species, to detect and extrapolate homologous series in m/z , RT and CCS.
- Lipid species which were not annotated automatically, can be confirmed by their respective homologous series.
- New lipid species in *C. elegans* were detected and annotated based on respective homologous series.

MetaboScape 2025b