Integrating 4D peak picking of LC-TIMS-MS/MS data into GNPS feature based molecular networking for 4D Metabolomics and 4D Lipidomics analysis

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Introduction

As throughput of metabolomic and lipidomic analyses continuously expands, effective workflows for analyzing the resulting datasets are of increasing importance. Molecular networking in recent years has become a vital tool in the metabolomics community as it quickly allows the identification of compounds with similar fragmentation patterns which are often structurally related.

While this approach mainly focusses on the fragment spectra, important information can be deduced from the precursor spectra, i.e. intensity, accurate mass, isotopic pattern as well as Collision Cross Sections (CCS) of the analytes which provide crucial information about the analytes concentration and identity.

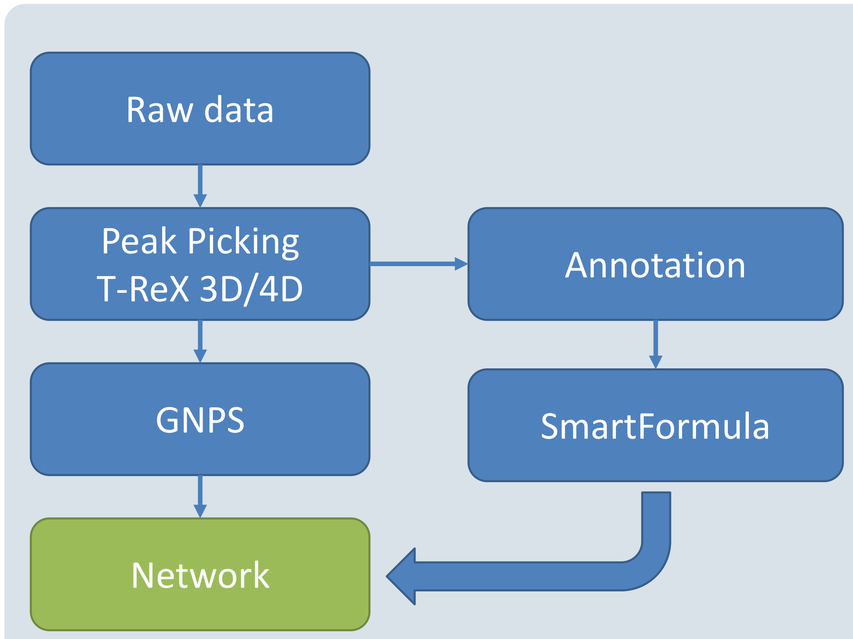

Fig. 1: Workflow of MetaboScape and GNPS integration. First, Peak picking is performed by T-ReX algorithms. Dedicated mgf export allows processing in GNPS while further annotations can be used to enrich the resulting network.

The nodes in the resulting molecular network are enriched by useful information about the precursor ions like the intensity in individual samples, molecular formula, annotation, CCS values, group mean intensity and maximum intensity. The information are important indicators to assess distribution of a specific analyte between sample groups (by group mean).

Additionally, the maximum intensity can help to determine if a purification of the analyte is feasible, i.e. to perform structure elucidation via NMR or assess its biological activity. Likewise, interpretation of the resulting molecular network is greatly simplified by displaying generated molecular formulas instead of precursor masses as node labels.

Summary

To the best of our knowledge, this workflow enables the integration of 4D Metabolomics/Lipidomics data into GNPS feature based molecular networking for the first time. Furthermore, the additional metadata greatly eases the interpretation of resulting networks.

This allows the following:

- Assess distribution of analytes from distinct compound classes between sample groups (by mean group intensity)
- Asses if purification of a compound from any sample of the batch is feasible, i.e. for structure elucidation by NMR (by maximum intensity).
- Ion mobility separation is taken into account, allowing for analysis of similar fragmenting isomers which would otherwise be handled as a single node.

[1] Wang, Mingxun, et al.; *Nat. Biotechnol.*, 34, 828 (**2016**)

[2] Nothias, L.-F., et al.; *Nat. Methods*, 17, 905 (2**020**)

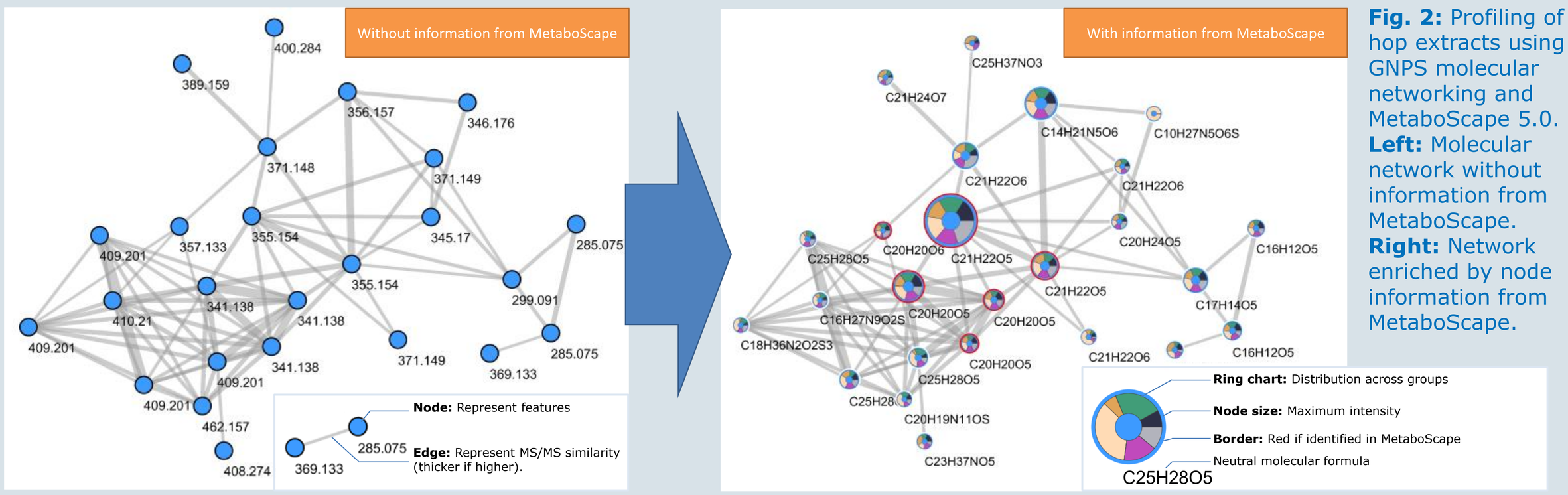

Fig. 2: Profiling of hop extracts using GNPS molecular networking and MetaboScape 5.0. **Left:** Molecular network without information from MetaboScape. **Right:** Network enriched by node information from MetaboScape.

Herein, we present a workflow to integrate analyte information for untargeted profiling from the software MetaboScape into GNPS feature based molecular networking.

Results

The unique workflow of this study (Fig. 1) allows the integration of three- and four-dimensional Time aligned Region complete eXtraction (T-ReX) peak picking results as well as annotation workflows of MetaboScape with GNPS feature based molecular networking.

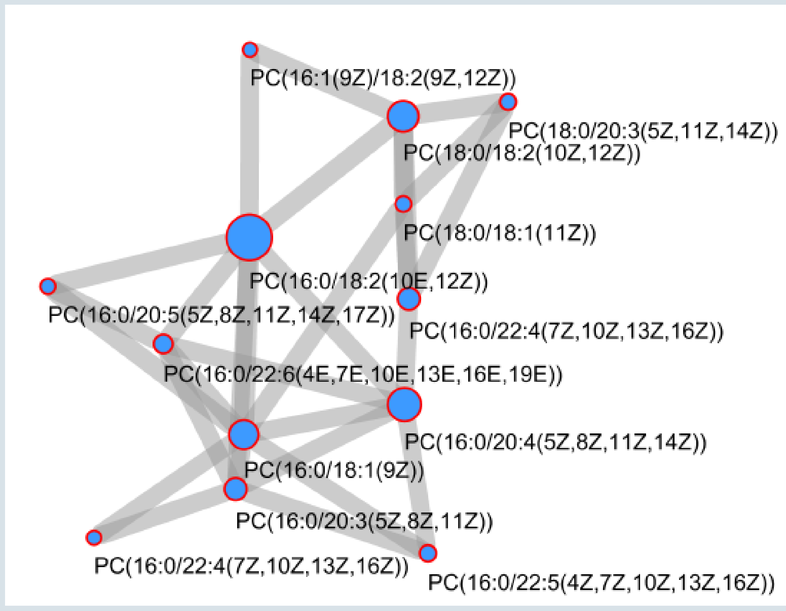

Fig. 3: Subset of a PC cluster from a 4D Lipidomics experiment. Peak picking by T-ReX 4D enables processing of 4D data in GNPS feature based networking.

Conclusions

- The presented workflows leverages the peak picking performance of T-ReX algorithms for use in GNPS feature based molecular networking.
- Allows integration of 4D data into GNPS for 4D Metabolomics and Lipidomics.
- Integrates reliable molecular formula generation based on HRAM and isotopic pattern.

4D Metabolomics

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