

Integrating MetaboScape and Polly™ for the analysis of LC-TIMS-MS and LC-MS based fluxomics

Heino M. Heyman¹, Heiko Neuweger², Pawel Konrad Lorkiewicz³, Bradford G. Hill³, Kyle Fulghum³, Shefali Lathwal⁴, Avijit Zutshi⁴, Brian Dranka⁵, Swetabh Pathak⁴, Abhishek Jha⁵
¹Bruker Scientific LLC, Billerica, MA; ²Bruker Daltonics GmbH, Bremen, Bremen; ³University of Louisville, Louisville, KY; ⁴Elucidata, New Delhi, Delhi; ⁵Elucidata, Cambridge, MA

INTRODUCTION

LC-MS based fluxomics studies provide researchers the ability to detect and quantify the incorporation of isotopically labeled atoms into an organism's metabolites. Ion mobility separation adds a further dimension for the detection and annotation of metabolites in the labeled and unlabeled samples need streamlined workflows and quality controls. Here we present a combination of MetaboScape and Polly™, a cloud platform for analyzing and interpreting omics data, for the analysis of LC-TIMS-MS and LC-MS measurements to assess differences in incorporation of ¹³C into downstream metabolites *in-vivo*.

METHODS

We performed a time-course feeding experiment using male, C57Bl/6J mice (age 12-15 wks). Mice were first acclimated to non-labeled glucose-containing liquid diet for 48h. Following acclimation time, mice fasted for 6h then were provided a ¹³C₆-glucose-containing liquid diet for either 18h (n=3; c8553, c8556, c8557) or 36h (n=2; c8554, c8555). After feeding, mice were sacrificed and the hearts freeze-clamped. Heart tissues were pulverized and extracted with 60% acetonitrile. Extracted metabolites were separated on a reversed-phase column (UPLC BEH C18 1.7μm, 100Å, 2.1 x 150mm), and data were acquired on a timsTOF Pro (Bruker) with (timsON) and without (timsOFF) ion mobility separation. LC-MS/MS (timsON and timsOFF) data were processed using MetaboScape, EI-MAVEN and Polly™.

MetaboScape

Putative annotations of metabolites in unlabeled samples with and without ion mobility separation was done using MetaboScape and the compounds present in the Bruker HMDB Metabolite Library exploiting accurate mass match (< 2ppm) and isotopic pattern fit (< 20 mSigma). Annotations including retention times were then transferred to EI-MAVEN and further pushed to PollyPhi for in depth flux analysis of labeled samples and projections on metabolic pathways of the central carbon metabolism.

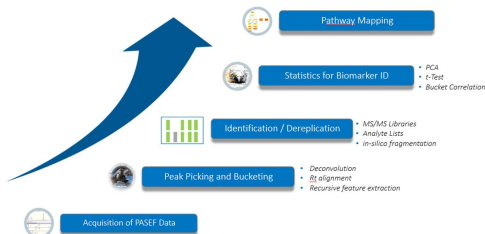


Figure 1. MetaboScape Workflow. For analysis of unlabeled discovery metabolomics data.

EI-MAVEN PollyPhi Workflow

The EI-MAVEN PollyPhi Workflow enables automated peak picking and subsequent visualization of data after natural abundance correction as follows:

- The .d files were converted to .mzML using MSConvert, an open-source mass-spec file conversion tool and uploaded on EI-MAVEN.
- A compound database with metabolite names, formula and retention time was also uploaded to EI-MAVEN.
- Peaks of ¹³C-labeled form of each metabolite was curated automatically using the information provided in the compound database.
- These curated peaks were then pushed to PollyPhi using the built-in EI-MAVEN PollyPhi Integration.
- Natural abundance correction was performed and the results were visualized in PollyPhi.



Figure 2. EI-MAVEN PollyPhi Workflow. For analysis of labeled data.

RESULTS

MetaboScape

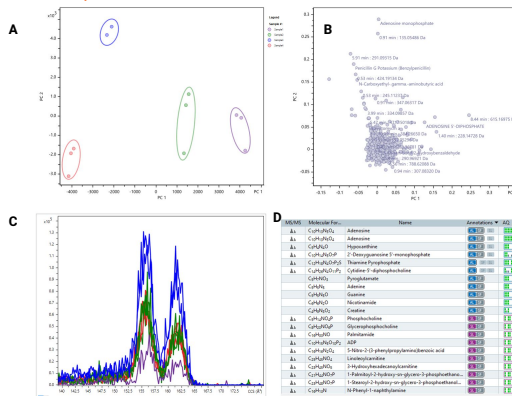


Figure 3. Unlabeled cohort analysis with MetaboScape. A) PCA scores plot showing samples clustering based on their loadings shown in B. C) An Extracted Mobilogram (EIM) for Adenosine. D) Bucket table showing annotations made with the use of an Analyte list (containing CCS values and MSMS spectra) and Spectral libraries.

Fractional Enrichment and Pool Total Plots generated from PollyPhi validate the experimental design

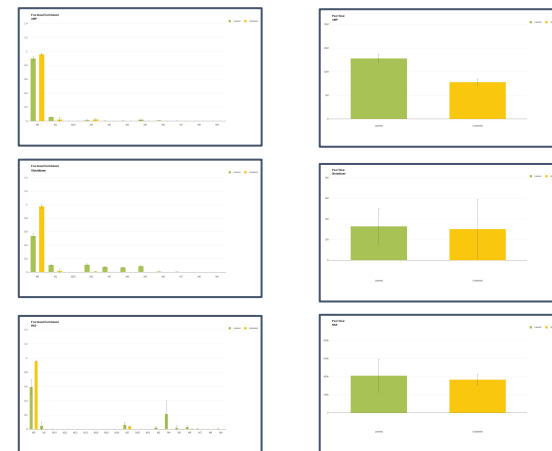


Figure 4. Fractional Enrichment and Pool Total Plot. Consistent with the experimental design, samples in the Unlabeled cohort exhibit maximum enrichment for M0 whereas samples in the Labeled cohort exhibit enrichment for multiple labels.

CONCLUSION

- Putative annotations of a diverse range of metabolites in unlabeled samples with and without ion mobility separation was done using MetaboScape.
- EI-MAVEN's unique machine learning algorithm combined with its rapid processing capabilities allowed this data to be curated in less than 5 minutes.
- Using PollyPhi, we observed a consistent increase in the occurrence of higher order isotopologues for AMP, ATP, NAD, Glutathione etc. thus validating the experimental design.
- Successful integration of two software solutions for the annotation and analysis of both LC-TIMS-MS and LC-MS fluxomics data.

REFERENCES

- MetaboScape; <https://www.bruker.com/products/mass-spectrometry-and-separations/ms-software/metaboscape.html>
- EI-MAVEN v0.10.0; <https://github.com/ElucidataInc/EI-Maven>
- PollyPhi; <https://docs.elucidata.io/Apps/Metabolomic%20Data/EI-MAVEN%20Phi%20Relative%20C-MS.html>