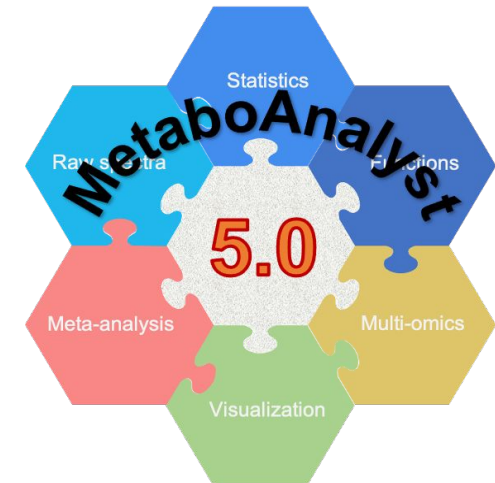


Spectra processing, **functional integration** and covariate adjustment of global metabolomics data using MetaboAnalyst 5.0

Section II: Functional analysis of
metabolomics data and integration with
other omics data

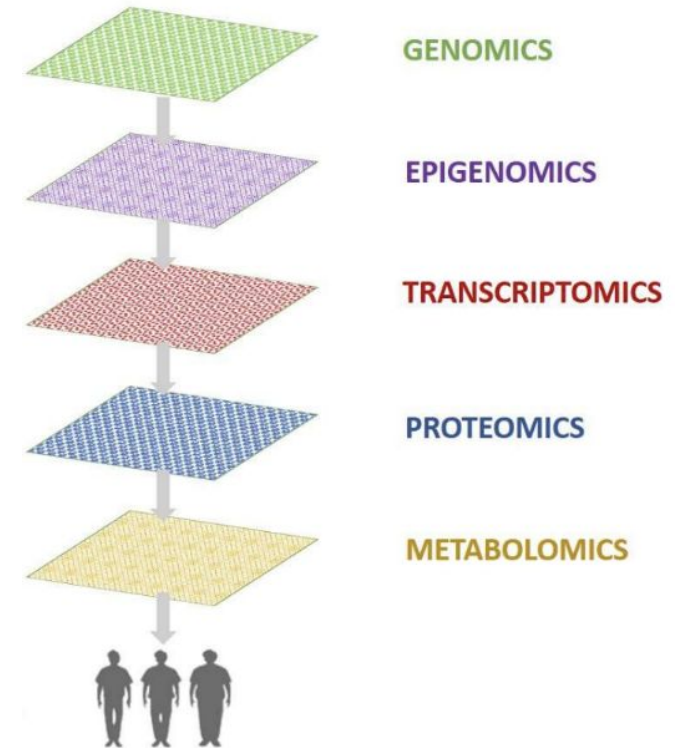
TA: Yao Lu
(yao.lu5@mail.mcgill.ca)

18th Annual Conference of the Metabolomics Society
METABOLOMICS 2022
Valencia, Spain | JUNE 19-23
Pre-Conference Workshops



Why multi-omics integration ?

- Biological processes are the complex results from multiple interactions among different omics layers.
- The integrative analysis combines the information from multi-omics data to provide an enhanced readout of cellular processes.
- Existing knowledge of other omics facilitates the annotation and interpretation of global metabolomics.

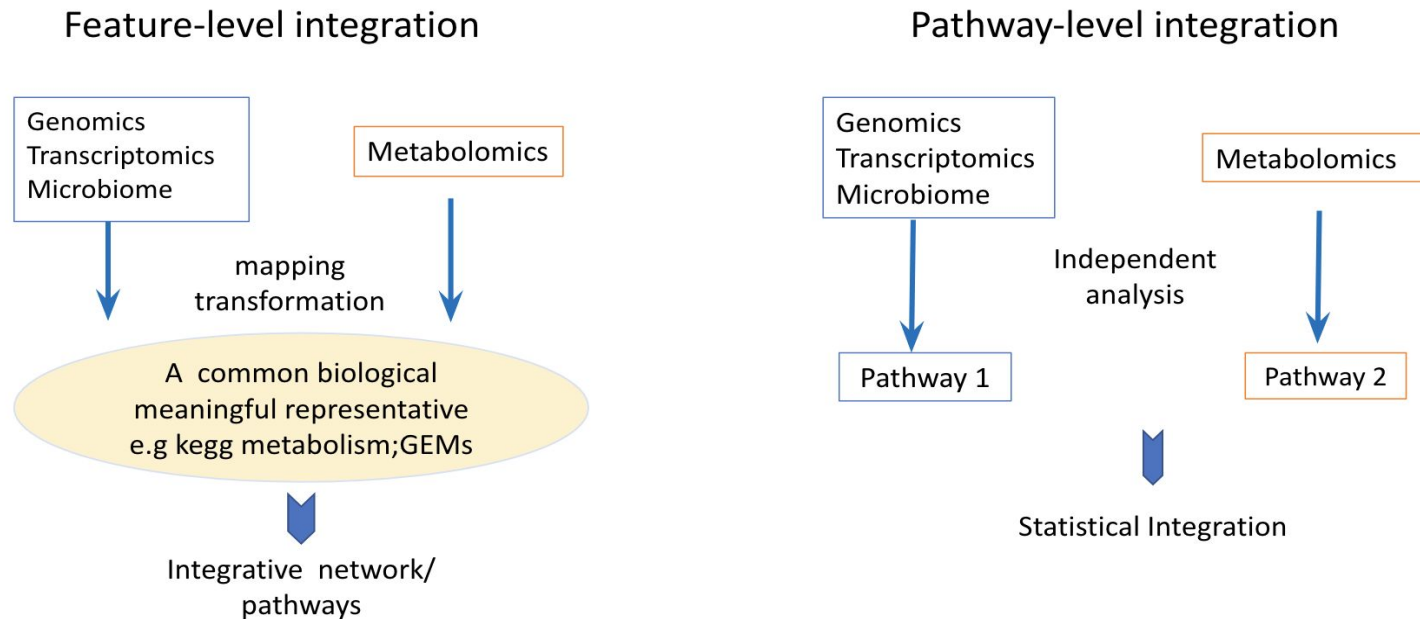


Approaches for omics data integration:

Mainly categories for integration:

- knowledge-driven: interaction metabolic network;
- statistics-driven: univariate or multivariate correlation between variables in different omics layers

Strategies:





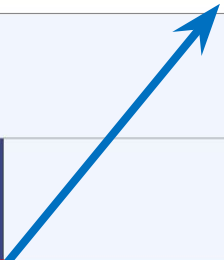
MetaboAnalyst 5.0 - user-friendly, streamlined metabolomics data analysis

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- [Tutorials](#)
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- [APIs](#)
- [Update History](#)
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- [COVID-19 Data](#)
- [About](#)

Module Overview

| Input Data Type | Available Modules (click on a module to proceed, or scroll down for more details) | | | | | |
|--|---|---------------------------------------|---------------------|---------------------------|------------------|-----------------|
| Raw Spectra (mzML, mzXML or mzData) | | | | LC-MS Spectra Processing | | |
| MS Peaks (peak list or intensity table) | | | Functional Analysis | Functional Meta-analysis | | |
| Annotated Features (compound list or table) | | Enrichment Analysis | Pathway Analysis | Joint-Pathway Analysis | Network Analysis | |
| Generic Format (.csv or .txt table files) | Statistical Analysis [one factor] | Statistical Analysis [metadata table] | Biomarker Analysis | Statistical Meta-analysis | Power Analysis | Other Utilities |

Multi-omics integration

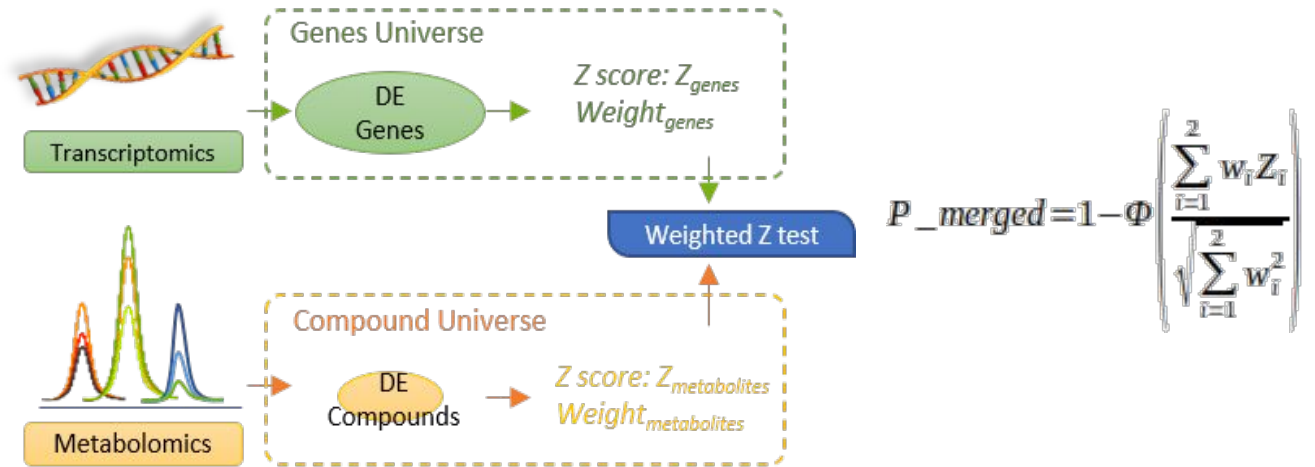


Integration of targeted metabolomics with other omics

❖ Join in feature level

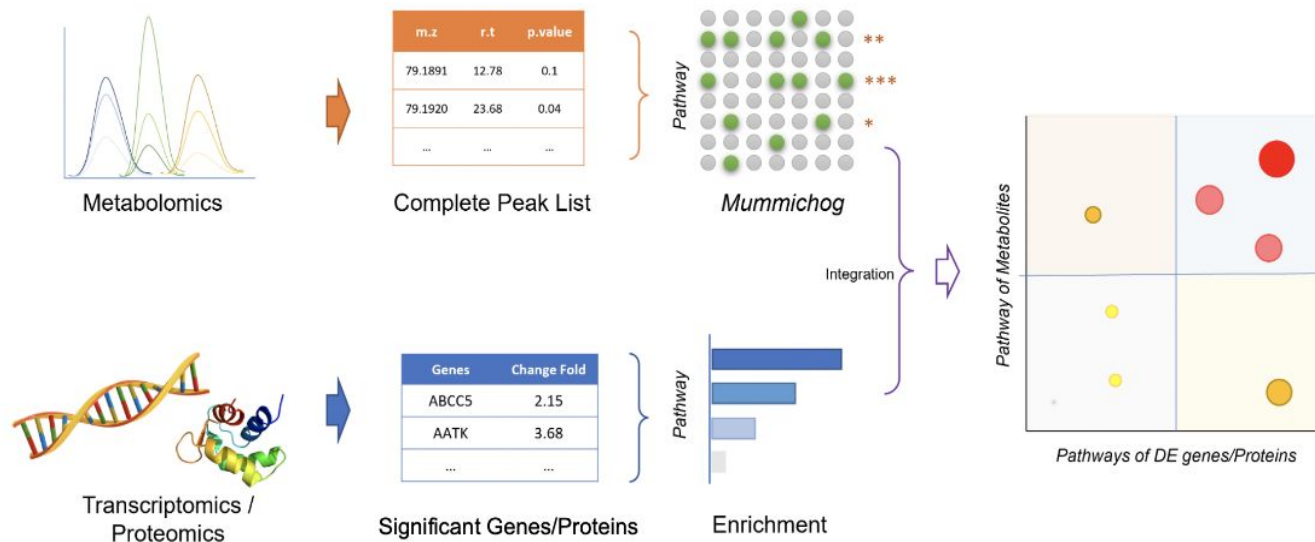
❖ Joint in pathway level:

- Combine **unweighted** p values:
i.e metabolite: 0.5, gene: 0.5;
- Combined based on the **overall** weights:
i.e metabolite: 0.2, gene: 0.8 for all pathways;
- Combined based on the **pathway-level** weights:
i.e. pathway A - metabolite 0.1, gene 0.9;
pathway B - metabolite 0.4, gene 0.6.



| | |
|---------------------|---|
| Enrichment analysis | <input checked="" type="radio"/> Hypergeometric Test <input type="radio"/> Fisher's Exact Test |
| Topology measure | <input checked="" type="radio"/> Degree Centrality <input type="radio"/> Betweenness Centrality <input type="radio"/> Closeness Centrality |
| Integration method | <input checked="" type="radio"/> Combine queries <input type="radio"/> Combine p values (unweighted) <input type="radio"/> Combine p values (overall) <input type="radio"/> Combine p values (pathway-level) |

Integration of untargeted metabolomics with other omics



Enrichment method for

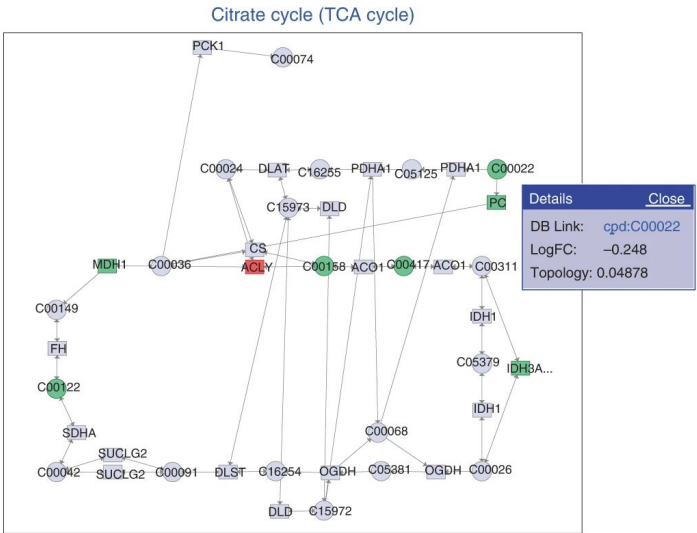
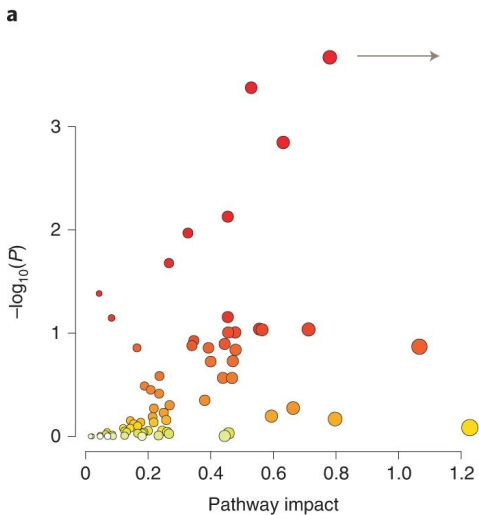
- untargeted metabolomics: Mummichog
- other omics: Hypergeometric test

Pathway p-value integration:

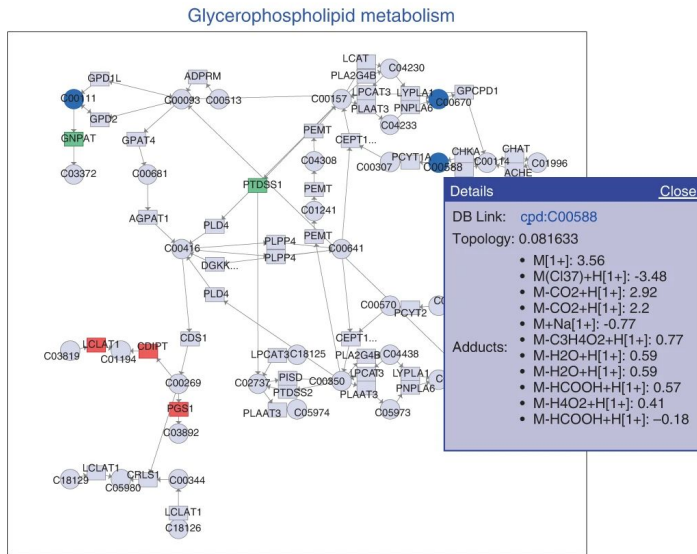
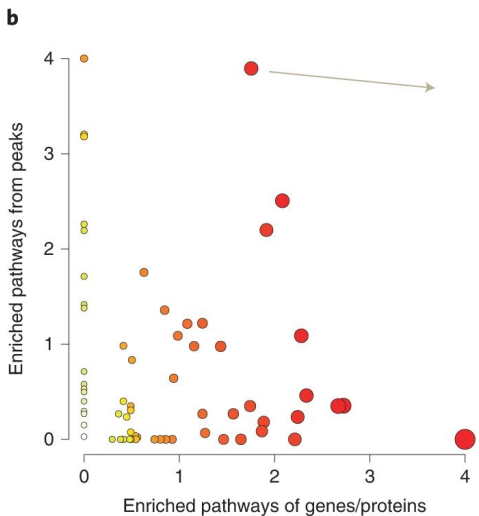
- Fisher's method
- Stouffer's methods

Result exploration

Integration of
targeted
metabolomics



Integration of
untargeted
metabolomics



Network viewer

View style: **KEGG style** Background: **Black** Pathway name: **Hide** Compound name: **Show** Gene name: **Show** Download: **--Please Select--** Highlight: **Yellow**

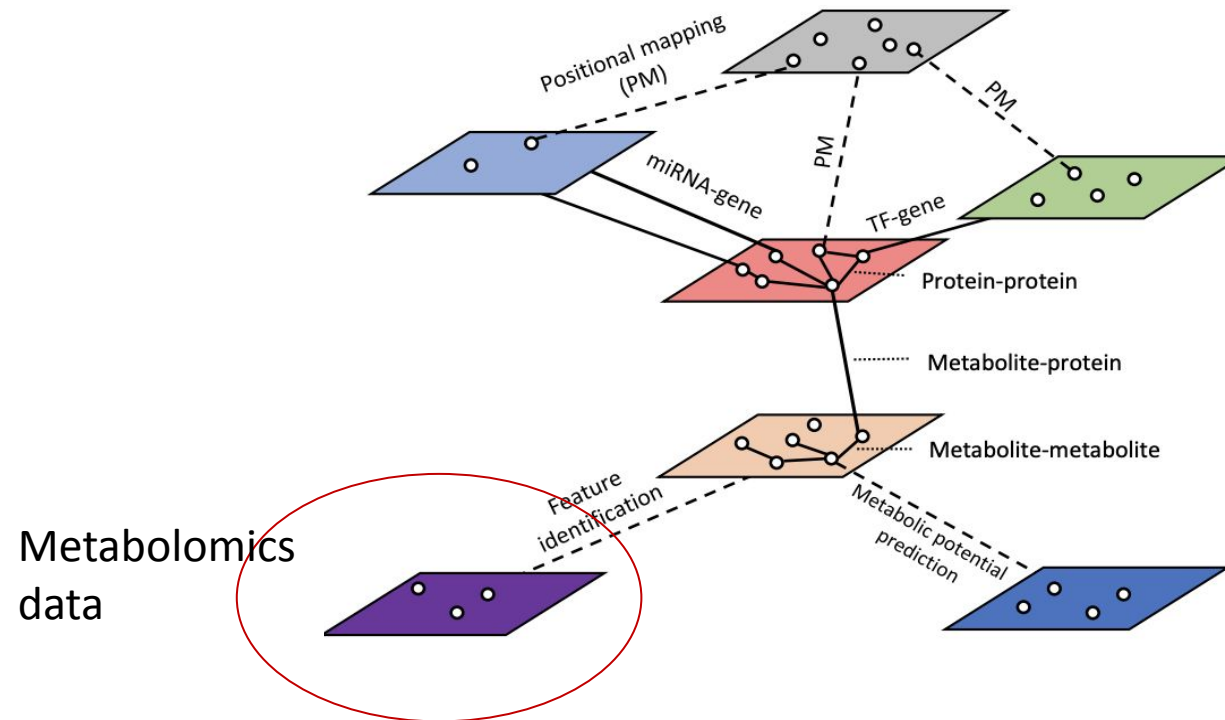
| <input type="checkbox"/> | Name | Hits | P-value | Color |
|-------------------------------------|---|------|------------|--------|
| <input type="checkbox"/> | Ether lipid metabolism | 7 | 0.00000897 | |
| <input type="checkbox"/> | Glycerophospholipid metabolism | 11 | 0.000011 | |
| <input type="checkbox"/> | Drug metabolism - other enzyme | 8 | 0.000101 | |
| <input type="checkbox"/> | Inositol phosphate metabolism | 10 | 0.000207 | |
| <input type="checkbox"/> | Valine, leucine and isoleucine | 10 | 0.000512 | |
| <input checked="" type="checkbox"/> | Fatty acid metabolism | 8 | 0.000614 | Yellow |
| <input type="checkbox"/> | Citrate cycle (TCA cycle) | 8 | 0.00115 | |
| <input checked="" type="checkbox"/> | Biosynthesis of unsaturated fatty acids | 6 | 0.00291 | Yellow |
| <input type="checkbox"/> | Pyrimidine metabolism | 10 | 0.00431 | |
| <input type="checkbox"/> | One carbon pool by folate | 5 | 0.00848 | |
| <input type="checkbox"/> | Carbon fixation in photosynthesis | 4 | 0.0157 | |
| <input type="checkbox"/> | Purine metabolism | 14 | 0.0163 | |
| <input type="checkbox"/> | Glutathione metabolism | 6 | 0.0167 | |
| <input type="checkbox"/> | Alanine, aspartate and glutamate | 7 | 0.0167 | |
| <input type="checkbox"/> | Biosynthesis of secondary metabolites | 51 | 0.0224 | |

Hits (Biosynthesis of unsaturated fatty acids)

| <input checked="" type="checkbox"/> | ID | Name | Expr. |
|-------------------------------------|------------------------|--|-----------|
| <input checked="" type="checkbox"/> | C01530 | Stearic acid | -0.602 |
| <input checked="" type="checkbox"/> | C00249 | Palmitic acid | -0.535 |
| <input checked="" type="checkbox"/> | K10203 | Elongation of very long chain fatty acid | 0.693 |
| <input checked="" type="checkbox"/> | K08764 | Sterol carrier protein 2 | 0.3859999 |
| <input checked="" type="checkbox"/> | K07513 | Acetyl-CoA acyltransferase | -0.522 |
| <input checked="" type="checkbox"/> | K10250 | Elongation of very long chain fatty acid | 1.003 |

Multi-omics integrative analysis in OmicsNet

- ❖ OmicsNet is dedicated to the Integration of different omics data in the current molecular interaction **knowledge framework** combined with enhanced visualization using 3D and 2D **network**.



Integration within knowledge-based multi-omics network

Targeted metabolomics:

Metabolite list

Untargeted metabolomics:

list of metabolite
annotated and putative
metabolites

Microbial taxa

Gene/Protein list



metabolite-protein
metabolite-metabolite

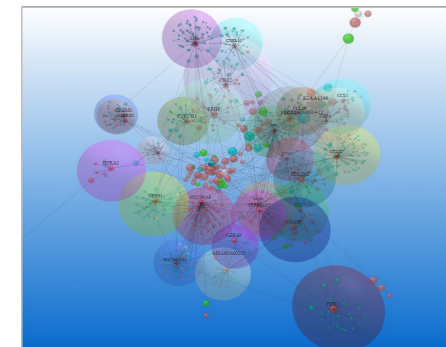
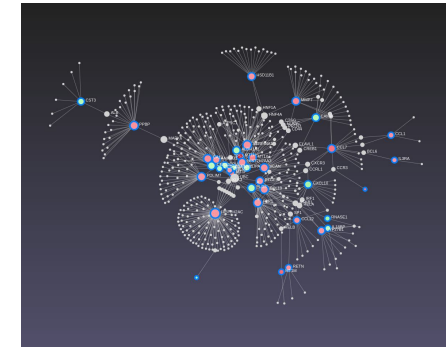
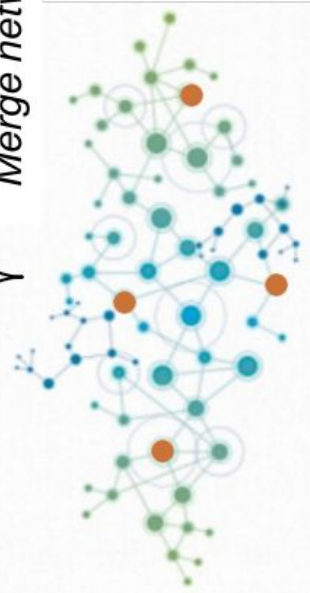


microbe-metabolite

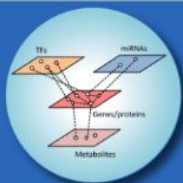


Gene-protein-metabolite

Merge networks



Further network exploration...



Multi-omics Integration via Biological Networks

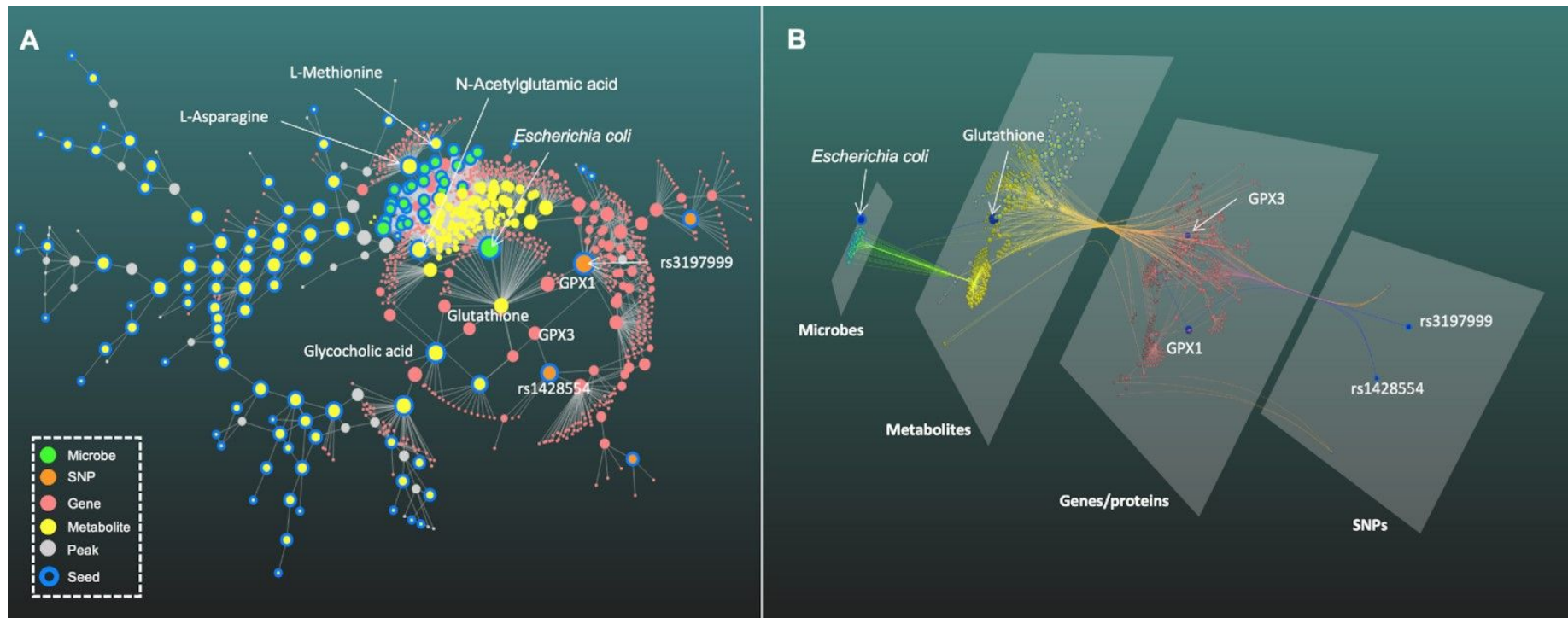
| Objective | Click on a panel below to start | | | | |
|--|---------------------------------|----------|-----------------------|-------------|-------------|
| Explore your networks in 2D or 3D space | | | A Graph File | | |
| Annotate SNPs, taxa, or LC-MS peaks for network analysis | | SNPs | Microbial Taxa | LC-MS Peaks | |
| Network analysis of one or more list(s) of molecules | Genes | Proteins | Transcription Factors | miRNAs | Metabolites |

▶ Proceed

↺ Reset

A comprehensive case study

- ❖ Potential mechanical investigation and hypothesis generation



*IBD case study using OmicsNet (<https://www.omicsnet.ca/OmicsNet/docs/Tutorial.xhtml>)

Questions for exploration:

- ❑ Which integration strategy is more powerful for your testing dataset and why ?
- ❑ What are the advantages and weaknesses of joint-pathway analysis compared to interaction networks?

Tutorials

- Publication: <https://www.nature.com/articles/s41596-022-00710-w>
- Or our manuscript:
<https://www.dropbox.com/s/7184c4dheeiiz2p/NP-MetaboAnalyst-2022.pdf?dl=0>
→ **Stage 3: pathway-level integration of multiple datasets**
- [OmicsNet.ca](https://www.omicsnet.ca)

Got questions?

- <https://www.omicsforum.ca>
- If your question is not covered, please create a new topic – we will try to answer them in the coming days