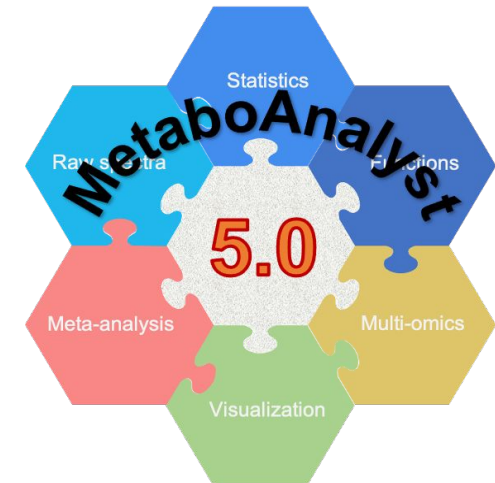


Spectra processing, **functional integration** and covariate adjustment of global metabolomics data using MetaboAnalyst 5.0

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




Schedule

Part I: 12:00 PM – 2:00 PM

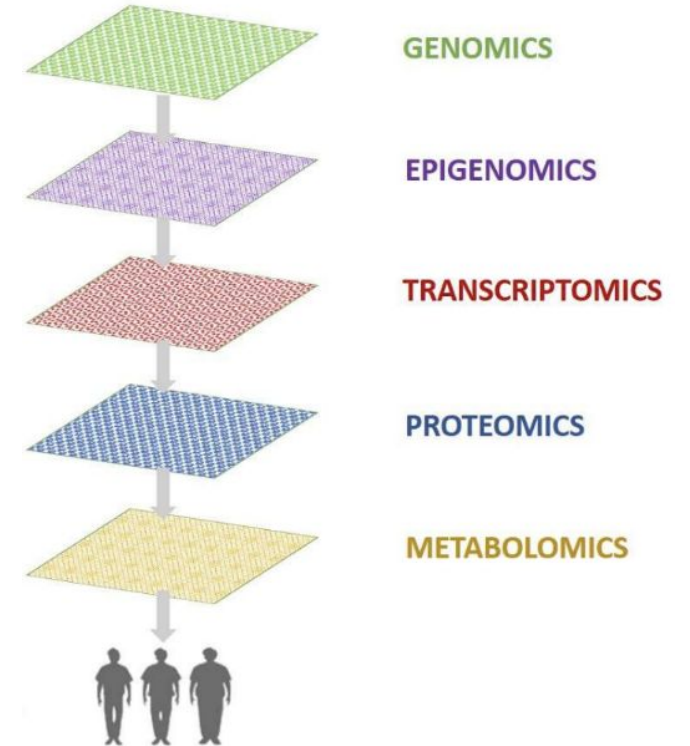
- 12:00 – 12:30: Opening lecture (Jeff)
- 12:30 – 12:45: Logistics
- 12:50 – 1:10: Section 1: LC-MS spectral processing and functional analysis (Qiang)
- 1:10 – 2:00: Interactive protocol exercise

Part II: 2:15PM – 4:15PM

- 
- 2:15 – 2:30: Section 2: multi-omics integration using pathways and networks (Yao)
 - 2:30 – 3:10: Interactive protocol exercise
 - 3:10 – 3:30: Section 3: Complex meta-data lecture (Jessica)
 - 3:30 – 4:00: Interactive protocol exercise
 - 4:00 – 4:15: Summary (Jeff)

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.

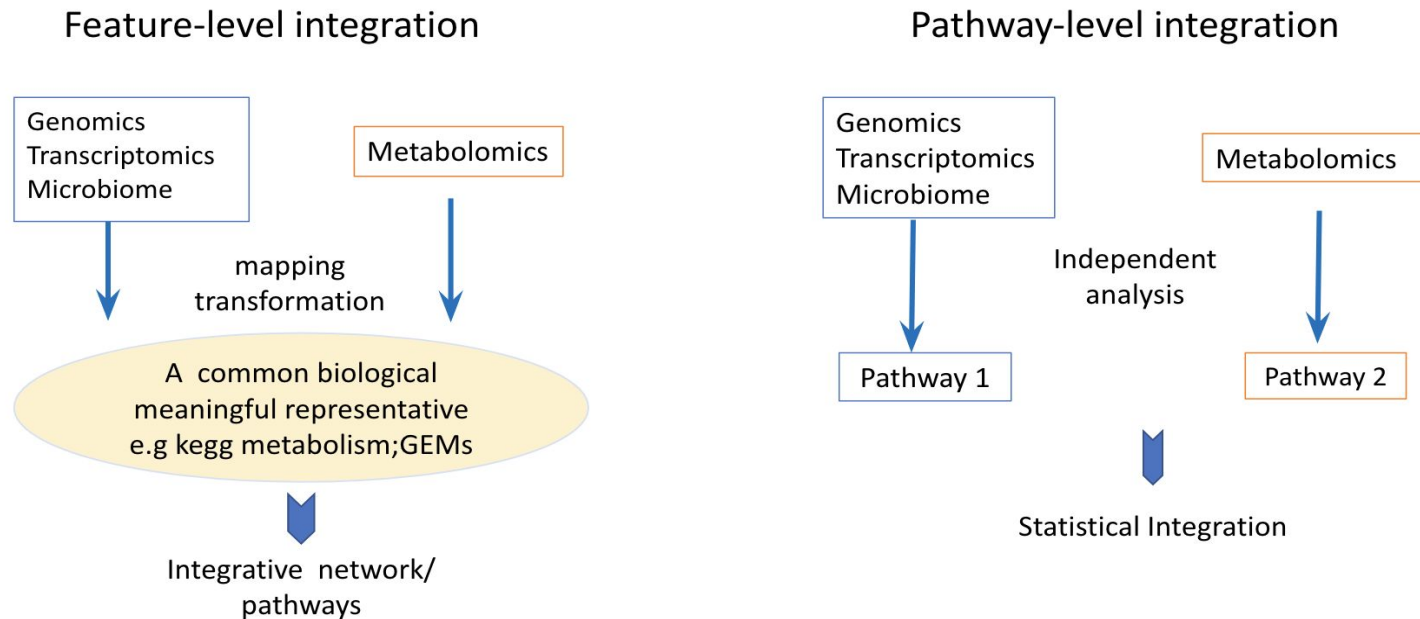


Approaches for omics data integration:

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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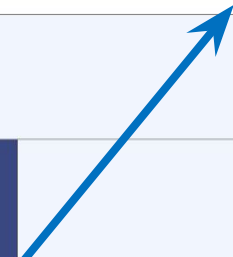
[COVID-19 Data](#)

[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

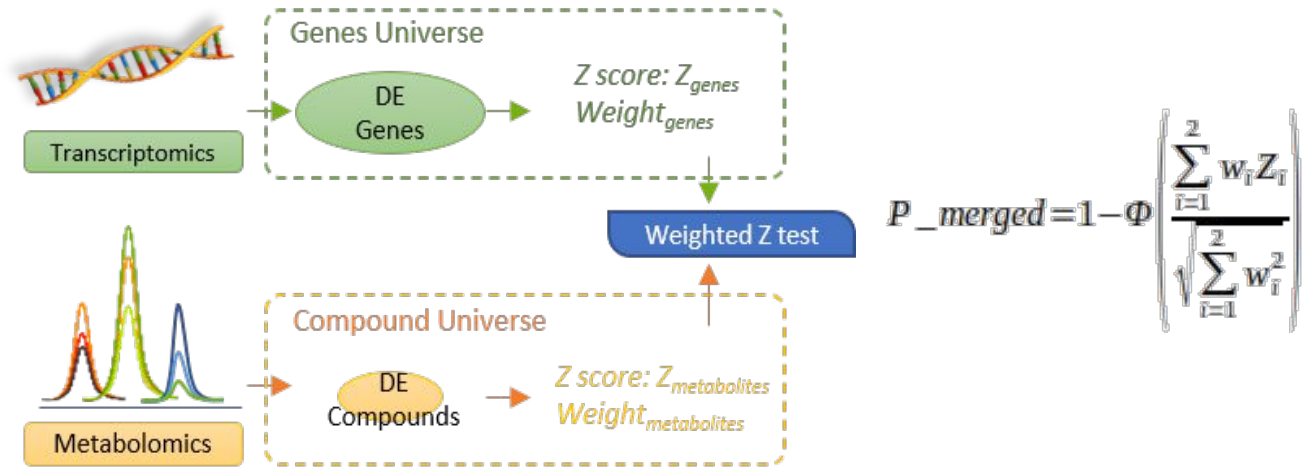


Integration of targeted metabolomics with other omics

❖ Join in feature level

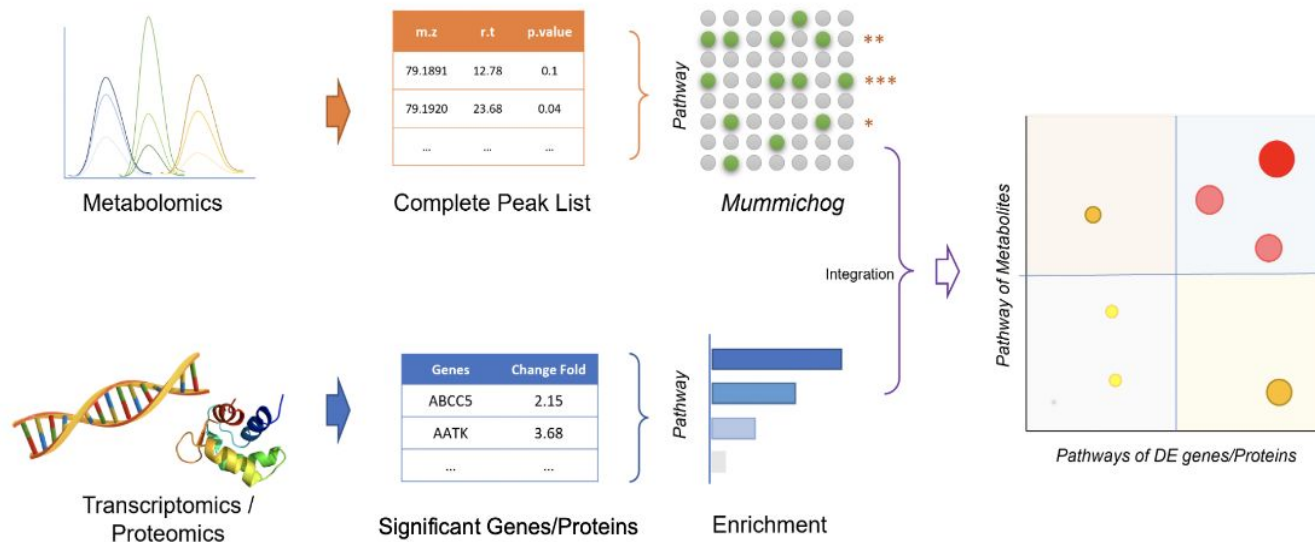
❖ Joint in pathway level:

- 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

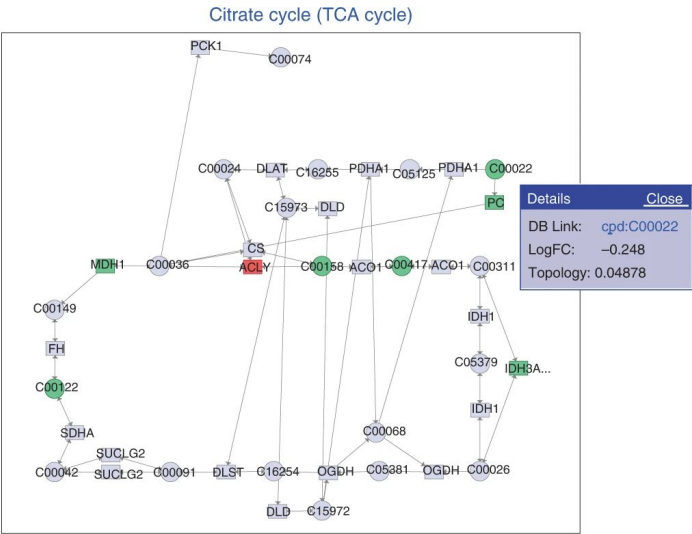
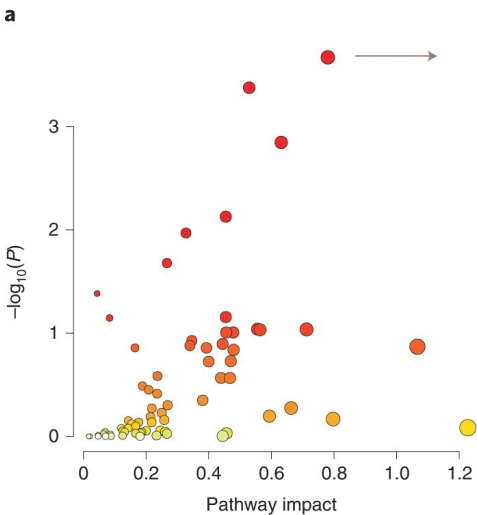
- untargeted metabolomics: Mummichog
- other omics: Hypergeometric test

Pathway p-value integration:

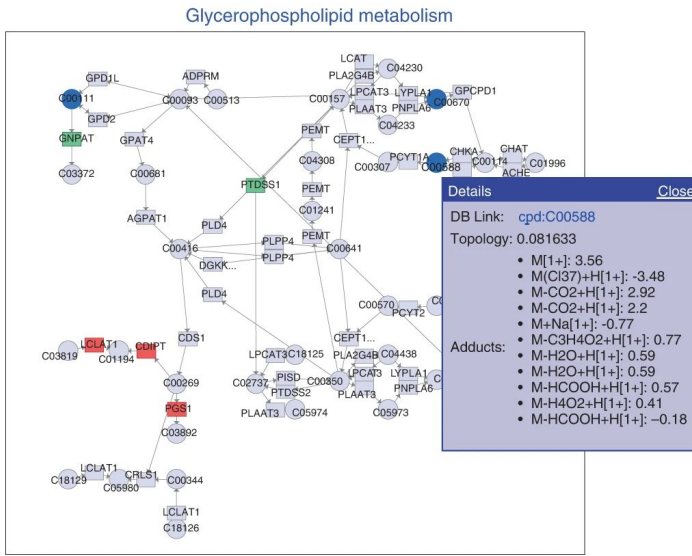
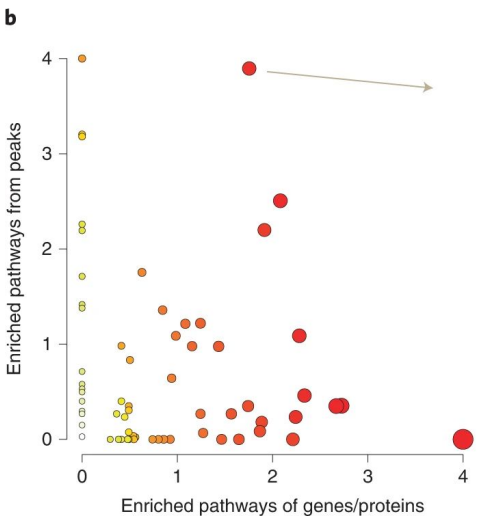
- Fisher's method
- Stouffer's methods

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**

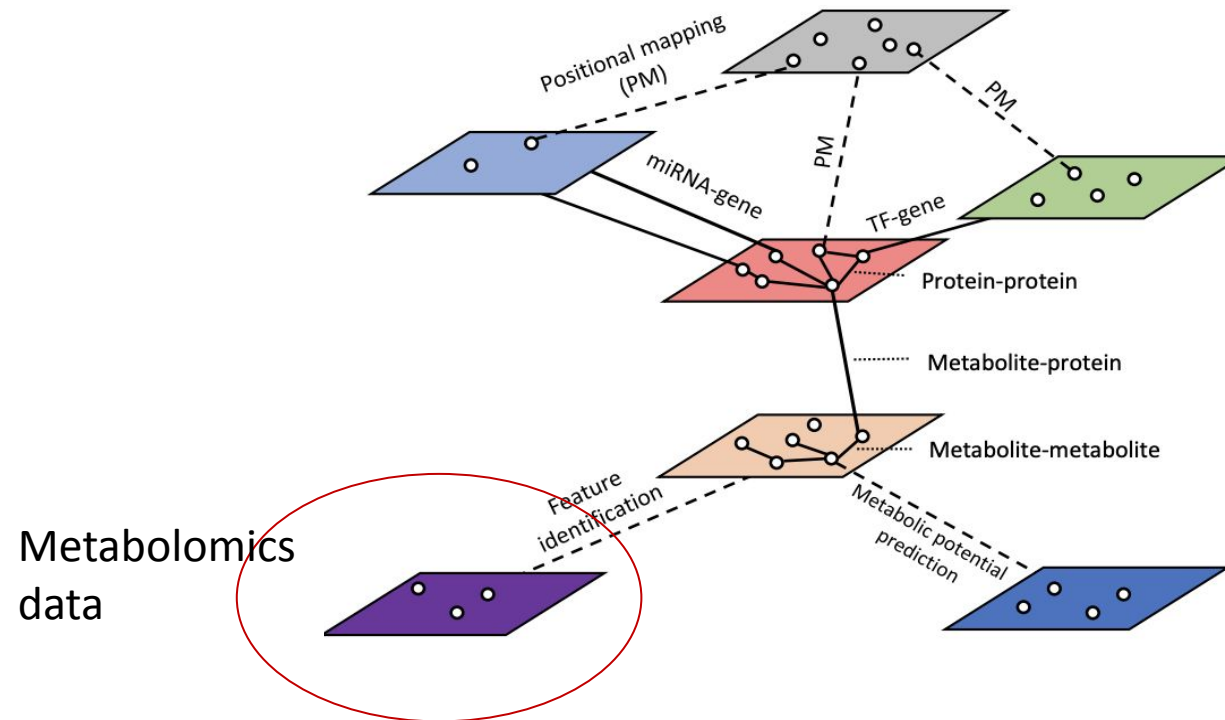
<input type="checkbox"/>	Name	Hits	P-value	Color
<input type="checkbox"/>	Ether lipid metabolism	7	0.00000897	
<input type="checkbox"/>	Glycerophospholipid metabolism	11	0.000011	
<input type="checkbox"/>	Drug metabolism - other enzyme	8	0.000101	
<input type="checkbox"/>	Inositol phosphate metabolism	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

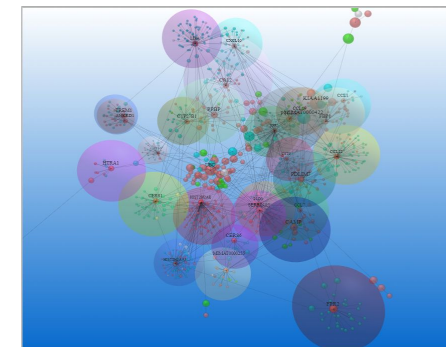
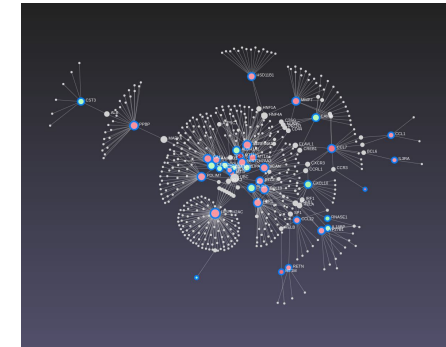
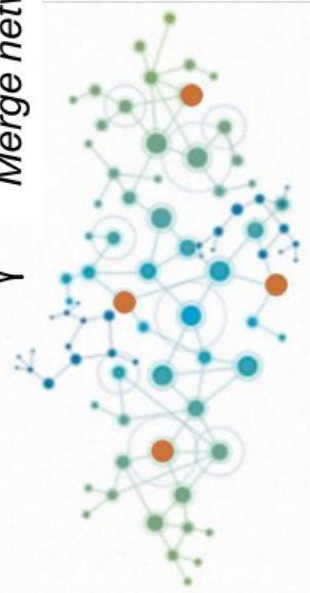


microbe-metabolite

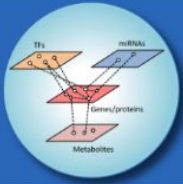


Gene-protein-metabolite

Merge networks



Further network exploration...



Multi-omics Integration via Biological Networks

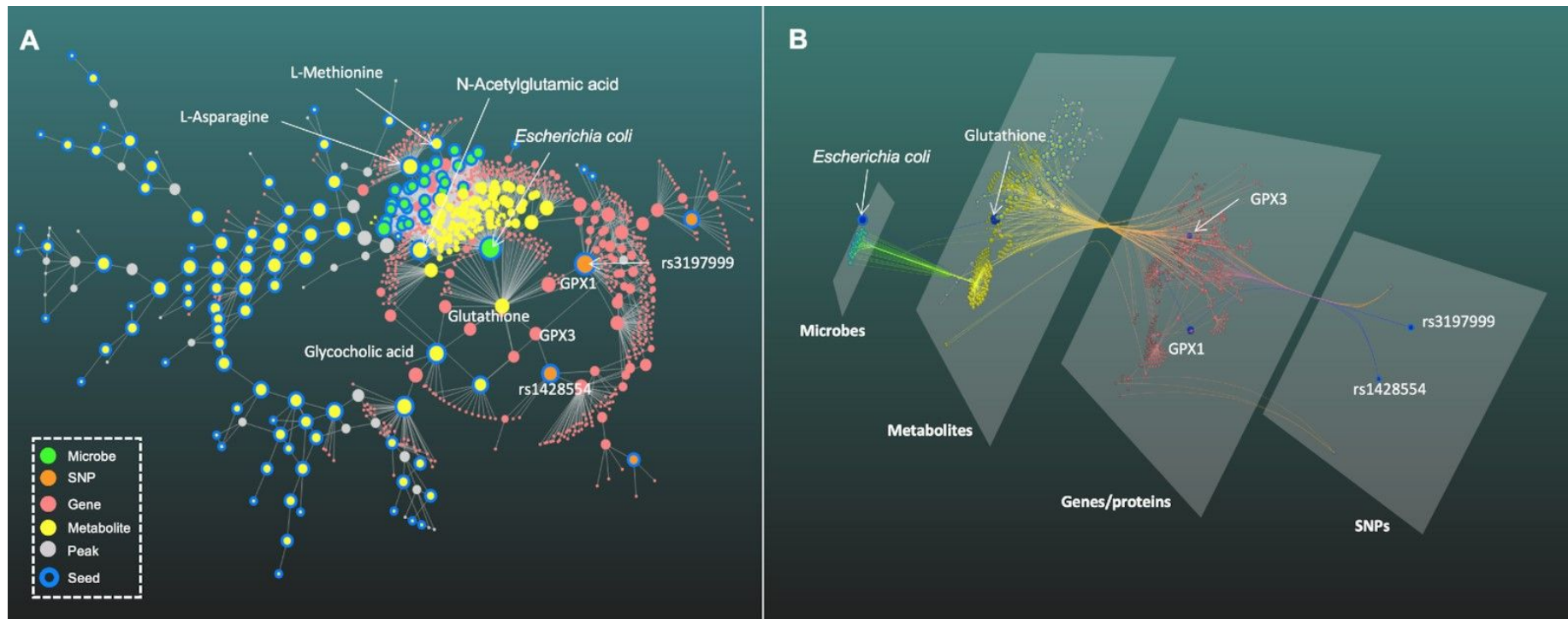
Objective	Click on a panel below to start				
Explore your networks in 2D or 3D space			A Graph File		
Annotate SNPs, taxa, or LC-MS peaks for network analysis		SNPs	Microbial Taxa	LC-MS Peaks	
Network analysis of one or more list(s) of molecules	Genes	Proteins	Transcription Factors	miRNAs	Metabolites

▶ 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