

# TurbOmics & TurboPutative

A web-server for multi-omics analysis, data handling and metabolite classification in untargeted metabolomics

This website is free to use and open-source to all users and there is no login requirement

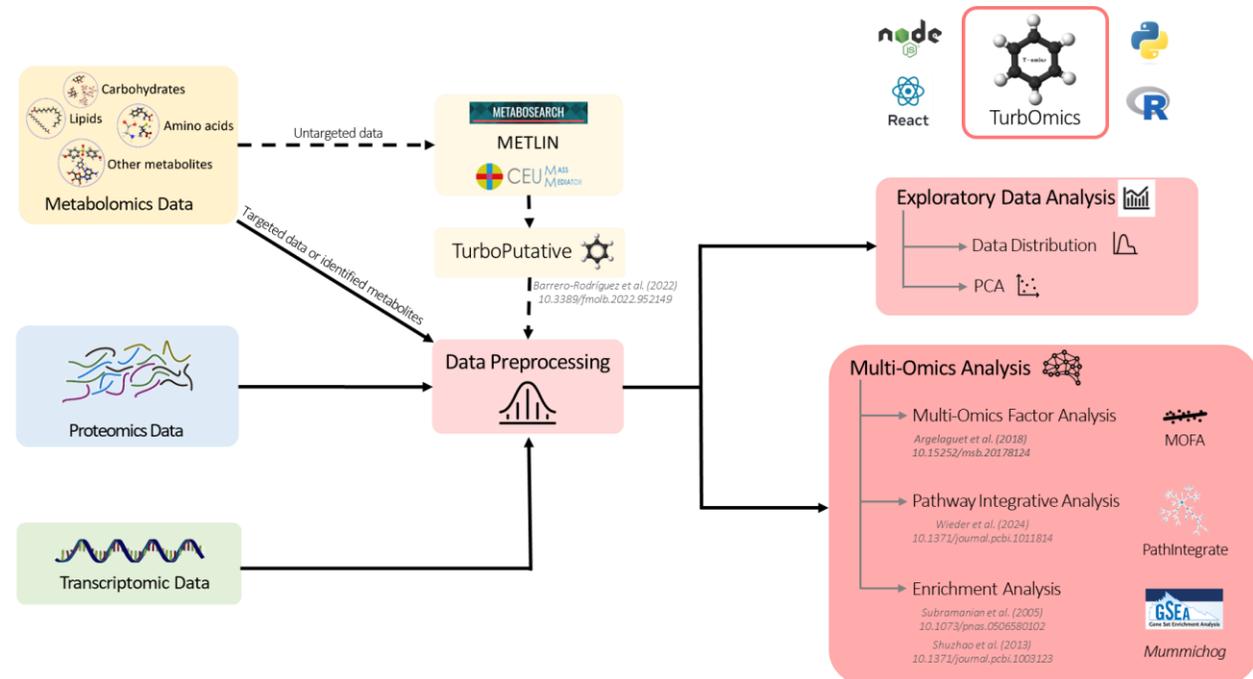
## A step-by-step Tutorial

# Overview

**TurbOmics** is an intuitive web-based platform designed to facilitate the multi-omics analysis of metabolomics data alongside proteomics and transcriptomics. It supports both **identified metabolites** from targeted-based experiments (LC/CE-MS) as well as those where the metabolite's identification is available (i.e. NMR or GC-MS). Additionally, TurbOmics offers tools to **putatively annotate** features from untargeted metabolomics based on their mass-to-charge ( $m/z$ ) ratio by sequentially and internally running CMM and TurboPutative.

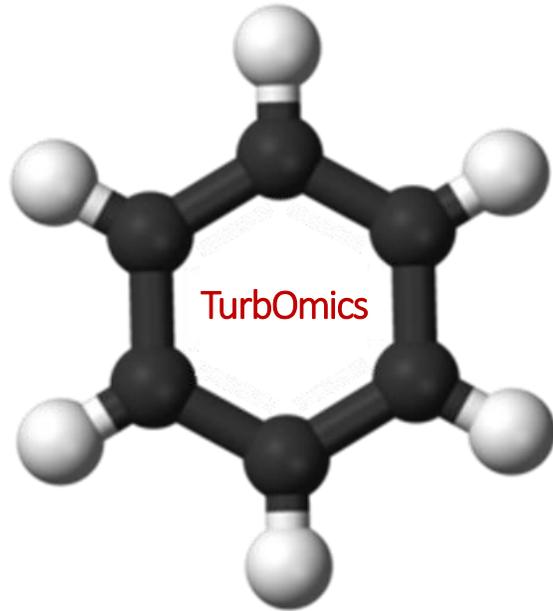
The platform provides a streamlined workflow for **exploratory data analysis** and **multi-omics integration**, through three main modules:

- **Multi-Omics Factor Analysis**
- **Pathway Integrative Analysis**
- **Enrichment Analysis**



# Content

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## □ INTRODUCTION

## □ EXAMPLES

### □ Case study (1): ATHEROSCLEROSIS

UNTARGETED LIPIDOMICS, PROTEOMICS and TRANSCRIPTOMICS from lorenzo *et al*, Nature 2021

- DATA PREPARATION
- EXPLORATORY ANALYSIS
- MULTI-OMICS FACTOR ANALYSIS
- ENRICHMENT ANALYSIS

### □ Case study (2): COVID-19

TARGETED METABOLOMICS and PROTEOMICS from Su *et al.*, Cell 2020

- DATA PREPARATION
- PATHWAY INTEGRATIVE ANALYSIS

## □ SUMMARY

# Introduction

## Background

Multi-omics integration provides a **comprehensive view of biological systems**, but current bioinformatics tools struggle to incorporate metabolomics data, especially for untargeted annotation and for offering alternatives to predefined knowledge bases. Moreover, advanced integration methods often require programming and statistical expertise, limiting their accessibility to non-specialist users.

## The web application

TurbOmics, is a user-friendly web-based platform that enables researchers with diverse backgrounds **to analyze transcriptomics, proteomics, and metabolomics/lipidomics data** using an **integrative workflow** that includes advanced algorithms for **multi-omics integration**, while addressing key challenges associated with untargeted metabolomics data.

## Data Formats

TurbOmics uses a structured data frame system composed of three types of tables that should be provided **in TSV format** :

- i. **Experimental metadata** (e.g. treatment, sex, time);
- ii. **Omics metadata** (e.g. ChEBI ID, gene names, annotations, RT, categories)
- iii. **Omics quantification** (abundancies or concentrations).

## Expected Results

TurbOmics enables users to **map biomolecules** from different omics layer, **facilitating biological interpretation of multiomics results**. Users can explore and export data tables and visualize plots as results of:

- i. Exploratory Analysis
- ii. MOFA
- iii. Pathway Integrative Analysis
- iv. Enrichment Analysis

# Try TurbOmics

GO to <https://proteomics.cnice.es/TurboPutative/home>

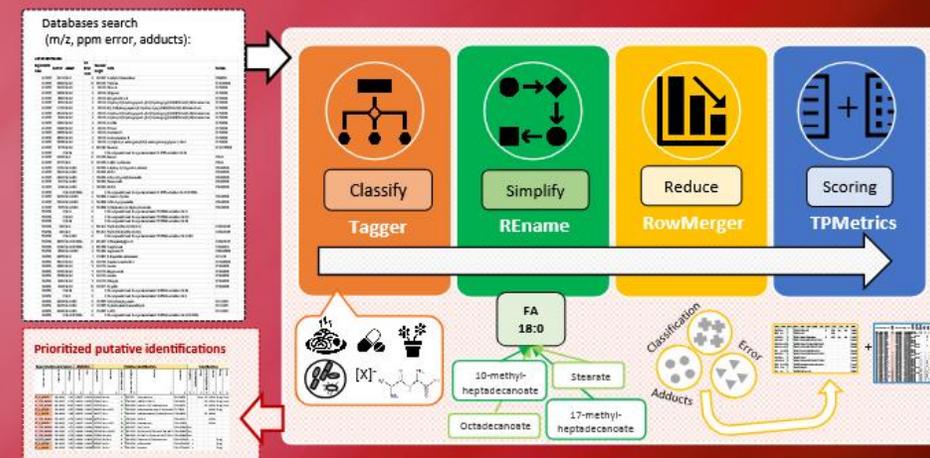
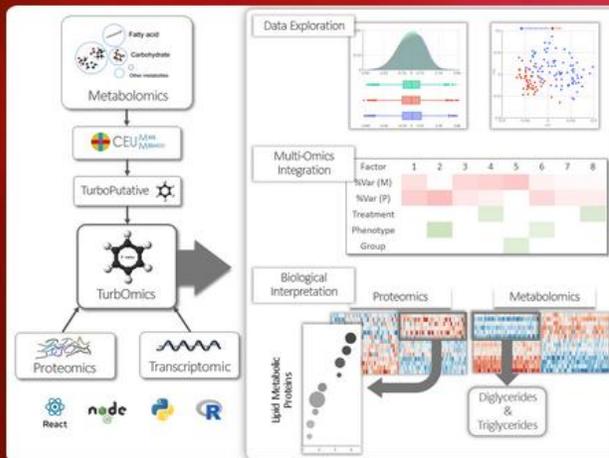
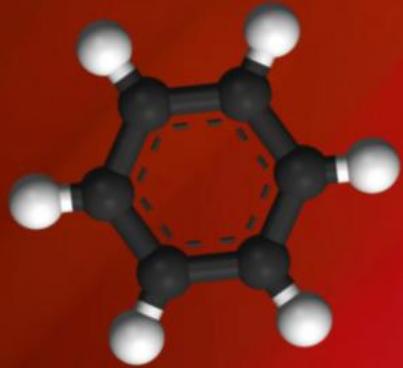
## TurbOmics & TurboPutative

A web-server for multi-omics analysis, data handling and metabolite classification in untargeted metabolomics

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 Try TurbOmics

 Try TurboPutative



# Application to multi-omics datasets including untargeted lipidomics data

nature

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Article | Published: 02 December 2020

## **ALDH4A1 is an atherosclerosis auto-antigen targeted by protective antibodies**

[Cristina Lorenzo](#), [Pilar Delgado](#), [Christian E. Busse](#), [Alejandro Sanz-Bravo](#), [Inmaculada Martos-Folgado](#), [Elena Bonzon-Kulichenko](#), [Alessia Ferrarini](#), [Ileana B. Gonzalez-Valdes](#), [Sonia M. Mur](#), [Raquel Roldán-Montero](#), [Diego Martinez-Lopez](#), [Jose L. Martin-Ventura](#), [Jesús Vázquez](#), [Hedda Wardemann](#) & [Almudena R. Ramiro](#) 

[Nature](#) **589**, 287–292 (2021) | [Cite this article](#)

**19k** Accesses | **124** Citations | **128** Altmetric | [Metrics](#)

- UNTARGETED LIPIDOMICS
- PROTEOMICS
- TRANSCRIPTOMICS

# i. New data upload

To get started, users can upload their experimental data directly from the **Main Page**. Previous analyses can be accessed using the **Find Job** field by entering the corresponding job ID. Once a dataset is loaded, the **Results** section provides access to a suite of tools for in-depth analysis and interpretation.

The screenshot shows the data upload interface with several panels and annotations:

- Navigation:** Home, TurboPutative, TurbOmics, Web Services, Help, Contact us. Search Job field.
- Buttons:** Load the Untarget Lipidomic sample data, Load the Target Metabolomic sample data, Upload from R, Create new job.
- Annotation (1):** Data files upload. Upload the experimental metadata (e.g., treatment, sex, time). First column must contain **sample IDs**.
- Annotation (2):** Choose appropriate organism. Organism: Mouse - Mus musculus.
- Table:**

	n
Meta-variables	18
Transcriptomic features	11999 / 11999
Metabolomic features	465 / 465
Proteomic features	4114 / 4114
- Upload Panels:**
  - Experimental Metadata (File name: experimental\_metadata.tsv)
  - Transcriptomic Metadata (File name: transcriptomic\_metadata.tsv)
  - Metabolomic/Lipidomic Metadata (File name: metabolomic\_metadata.tsv)
  - Proteomic Metadata (File name: proteomic\_metadata.tsv)
  - Transcriptomic Quantifications (File name: transcriptomic\_quantifications.tsv)
  - Metabolomic/Lipidomic Quantifications (File name: metabolomic\_quantifications.tsv)
  - Proteomic Quantifications (File name: proteomic\_quantifications.tsv)
- Annotation:** Upload the metadata from each omics dataset (e.g., ChEBI ID, RT, UniProt ID, gene name). Rows = **biomolecules**, Columns = **information**.
- Annotation:** Upload the quantifications from each omics dataset (e.g., abundancies, concentrations). Rows = **biomolecules**; Columns = **samples IDs**.

# ii. Load example dataset

To get started, users can upload their experimental data directly from the **Main Page**. Previous analyses can be accessed using the **Find Job** field by entering the corresponding job ID. Once a dataset is loaded, the **Results** section provides access to a suite of tools for in-depth analysis and interpretation.

**(1a)** Load directly the example containing the **metadata** and the **quantitative data** from all omics layers

**(1b)** Download the sample data files

**(1c)** Job ID: UntargetedSamplePaper

Organism: Mouse - Mus musculus

Choose appropriate organism

Experimental Metadata

Upload or drop a file right here TSV

File name: experimental\_metadata.tsv

n	18
Meta-variables	4
Transcriptomic features	11999 / 11999
Metabolomic features	465 / 465
Proteomic features	4114 / 4114

Transcriptomic Metadata

Upload or drop a file right here TSV

File name: transcriptomic\_metadata.tsv

Metabolomic/Lipidomic Metadata

Upload or drop a file right here TSV

File name: metabolomic\_metadata.tsv

Proteomic Metadata

Upload or drop a file right here TSV

File name: proteomic\_metadata.tsv

Transcriptomic Quantifications

Upload or drop a file right here TSV

File name: transcriptomic\_quantifications.tsv

Metabolomic/Lipidomic Quantifications

Upload or drop a file right here TSV

File name: metabolomic\_quantifications.tsv

Proteomic Quantifications

Upload or drop a file right here TSV

File name: proteomic\_quantifications.tsv

# Example of uploaded untargeted lipidomics dataset by TSV format

Home TurboPutative TurbOmics Web Services Help Contact us Search Job

Mouse_ID	Treatment	Batch
LD2169	A12	Batch1
LD2170	A12	Batch1
LD2171	A12	Batch1
LD2243	A12	Batch2
LD2244	A12	Batch2
LD2245	A12	Batch2
LD2162	B1-8	Batch1
LD2163	B1-8	Batch1

Organism  
 Mouse - Mus musculus

Create new job

fid	Apex m/z	RT [min]	Platform	Mode
P1	760.58465	19.341	C18	POS
P2	876.80088	23.952	C18	POS
P3	782.56882	18.116	C18	POS
P4	758.56897	18.518	C18	POS
P6	806.56868	17.913	C18	POS
P7	874.78481	23.42	C18	POS
P8	848.76963	23.348	C18	POS
P9	784.58393	19.02	C18	POS
P10	810.59934	19.6	C18	POS

n	18
Meta-variables	4
Transcriptomic features	11999 / 11999
Metabolomic features	2935 / 2935
Proteomic features	4114 / 4114

ID	LD2169	LD2170	LD2171	LD2243	LD2244	LD2245
P1	12.658	12.877	12.694	12.952	12.789	13.034
P2	11.453	11.128	10.891	10.861	11.403	10.520
P3	11.802	11.962	11.790	11.774	11.248	11.823
P4	11.660	11.889	11.820	11.959	11.741	11.795
P6	11.463	11.854	11.719	11.646	11.442	11.686
P7	10.601	9.946	10.344	10.225	10.761	9.830
P8	10.490	9.860	10.149	10.050	10.214	9.721
P9	11.146	11.270	11.375	11.359	11.406	11.126
P10	10.940	11.125	10.867	10.817	10.551	10.836

Experimental Metadata

Upload or drop a file right here TSV

File name: experimental\_metadata.tsv

Metabolomic/Lipidic Metadata

Upload or drop a file right here TSV

File name: metabolomic\_metadata.tsv

Proteomic Metadata

Upload or drop a file right here TSV

File name: proteomic\_metadata.tsv

Metabolomic/Lipidic Quantifications

Upload or drop a file right here TSV

File name: metabolomic\_quantifications.tsv

Proteomic Quantifications

Upload or drop a file right here TSV

File name: proteomic\_quantifications.tsv

# Data format for the three uploaded tables

The consistency and linkage between these tables rely on matching identifiers. Therefore, it is essential that:

- The sample identifiers in the first column of the experimental metadata table match the column headers (i.e., the first row) of the omic quantification tables.
- The biomolecule identifiers in the first column of both the omics metadata and omics quantification tables match exactly.

**Experimental Metadata**

Sample_ID	Condition	Treatment	Batch
S001	Control	None	Batch1
S002	Control	None	Batch1
S003	Control	None	Batch2
S004	Control	None	Batch2
S005	Control	Drug_A	Batch1
S006	Control	Drug_A	Batch1
S007	Control	Drug_A	Batch2
S008	Control	Drug_A	Batch2
S009	Control	Drug_B	Batch1
S010	Control	Drug_B	Batch1
S011	Control	Drug_B	Batch2
S012	Control	Drug_B	Batch2
S013	Disease	None	Batch1
S014	Disease	None	Batch1
S015	Disease	None	Batch2
S016	Disease	None	Batch2
S017	Disease	Drug_A	Batch1
S018	Disease	Drug_A	Batch1
S019	Disease	Drug_A	Batch2
S020	Disease	Drug_A	Batch2
S021	Disease	Drug_B	Batch1
S022	Disease	Drug_B	Batch1
S023	Disease	Drug_B	Batch2
S024	Disease	Drug_B	Batch2

**Metabolomics Metadata**

Metabolite_ID	Name	Biol_Function	Chemical_Class	ChEBI_ID	Pathway
HMDB0000122	Glucose	Energy metabolism	Carbohydrate	17234	Glycolysis
HMDB0000190	Lactate	Anaerobic metabolism	Organic acid	24996	Cori cycle
HMDB0000538	ATP	Cellular energy	Nucleotide	15422	Oxidative Phospho.
⋮	⋮			⋮	⋮

**Metabolomics Quantification**

Metabolite_ID	S001	S002	S003	...	S010	S011	S012
HMDB0000259	5.3	6.1	5.8	...	5.9	5.4	4.8
HMDB0000562	10.5	11.2	9.9	...	10.9	10.3	9.5
HMDB0000243	7.9	8.4	7.5	...	7.8	7.1	6.8
⋮	⋮	⋮	⋮	...	⋮	⋮	⋮
HMDB0000259	25.7	26.3	24.9	...	25.4	24.1	23.3
HMDB0000562	6.8	7.2	6.5	...	6.7	6.3	5.8
HMDB0000243	3.2	3.8	3.1	...	3.5	3.2	2.8

**Transcriptomics Metadata**

Transcript_ID	Gene_Name	Biol_Function	Chromosome	Pathway_Association
ENST00000269305	TP53	Tumor suppression	17	p53 signaling
ENST00000352993	BRCA1	DNA repair	17	Homologous recomb.
ENST00000229239	GAPDH	Glycolysis	12	Metabolism
⋮	⋮			⋮

**Transcriptomics Quantification**

Transcript_ID	S001	S002	S003	...	S010	S011	S012
ENST00000269305	12.3	14.2	11.8	...	12.8	11.9	10.7
ENST00000352993	8.7	9.1	8.5	...	8.4	7.9	7.2
ENST00000229239	20.4	21.2	19.8	...	19.5	18.6	17.3
⋮	⋮	⋮	⋮	...	⋮	⋮	⋮
ENST00000324450	10.5	11.2	9.9	...	10.3	9.5	8.7
ENST00000377970	7.9	8.4	7.5	...	7.1	6.8	6
ENST00000361624	25.7	26.3	24.9	...	24.1	23.3	22

# iii. Normalization, scaling and Missing values Imputation

- **Normalization techniques** are used to correct for systemic and technical variations that can obscure biological signals and lead to misleading conclusions.
- Different omics datasets often exhibit varying value ranges due to differences in technological platforms and the normalization methods applied during data generation. These disparities can be addressed **standardizing** omics data by setting the mean of each biomolecule to 0 and the standard deviation to 1.
- Once these parameters are set, the user can launch the analysis > create new job

(5) Create new job

**Transcriptomic Quantifications**

Upload or drop a file right here TSV

File name: transcriptomic\_quantifications.tsv

Apply:

Center & Scale

No missing value detected

**Metabolomic/Lipidomic Quantifications**

Upload or drop a file right here TSV

File name: metabolomic\_quantifications.tsv

Apply:

Center & Scale

**Proteomic Quantifications**

Upload or drop a file right here TSV

File name: proteomic\_quantifications.tsv

Apply:

Center & Scale

No missing value detected

**User can apply different type of normalization and scaling**

None (3)

Log2

Median & Log2

VSN

- TurbOmics handles **missing values** independently for each omic type. This design allows users to apply different filtering and imputation methods tailored to the technical and methodological characteristics of each omics dataset, increasing the flexibility and robustness of the workflow.

**(4a) Metabolomic Features vs MV Threshold**

Accepted Features

Missing Values Threshold

Selected MV Threshold: 0.2

Users can interactively adjust this threshold and observe in real time how many biomolecules are retained.

**(4b)**

Select

Please select imputation type

**KNN** Choose from several imputation techniques to handle missing values in the remaining biomolecules

Minimum

Mean

Median

# Putative Annotation

TurbOmics provides two alternative approaches for handling putative annotations of metabolite:

**a) User-provided annotations:** Users can upload a **metabolomics metadata table** that includes identifications or putative annotations previously obtained using tools such as [MetaboSearch](#) or Ceu Mass Mediator ([CMM](#)), optionally simplified with TurboPutative (TPMetrics table). Option recommended for larger datasets).

**b) Automated annotation generation:** TurbOmics can automatically generate putative annotations for all detected features in a metabolomics experiment by internally executing [CMM](#) and [TurboPutative](#). Option recommended for dataset with less that 500 features.

Search Job

Job ID: 45329iGzy4

[Back to results](#) [Create new job](#)

	n
Meta-variables	4
Transcriptomic features	11999 / 11999
Metabolomic features	465 / 465
Proteomic features	4114 / 4114

Transcriptomic Metadata

Upload or drop a file right here TSV

File name: transcriptomic\_metadata.tsv

Obtain Putative Annotations from CMM & TurboPutative?

Metabolomic features will be annotated using [Ceu Mass Mediator](#) and simplified using [TurboPutative](#).

NO  YES

Proteomic Metadata

Upload or drop a file right here TSV

File name: proteomic\_metadata.tsv

Transcriptomic Quantifications

Upload or drop a file right here TSV

File name: transcriptomic\_quantifications.tsv

Metabolomic/Lipidomic Quantifications

Upload or drop a file right here TSV

File name: metabolomic\_quantifications.tsv

Proteomic Quantifications

Upload or drop a file right here TSV

File name: proteomic\_quantifications.tsv

Apply:

Normalization: *None*

Apply:

Normalization: *None*

Apply:

Normalization: *None*

# Putative Annotation

To enable automated annotation, users must upload a metadata table containing the *m/z*, retention time (RT), and ionization mode (positive or negative) for each feature.

CMM compares the *m/z* values of detected features against in-house libraries and external databases such as [HMDB](#), [LipidMaps](#), [METLIN](#), and [KEGG](#).

The resulting candidate annotations are then processed by the four modules of TurboPutative: **Tagger**, **Rename**, **RowMerger**, and **TPMetrics**. The final output, **TPFilter**, provides the most likely annotation for each feature and is integrated with the user-supplied metabolomics/lipidomics metadata table.

## i. Upload the metabolomics/lipidomics metadata table

Metabolomic Putative Annotations

(1)

Set Columns

m/z Column  
Apex m/z

Retention Time Column  
RT [min]

Ionisation Mode Column  
Mode

Positive Ion Value  
POS

Negative Ion Value  
NEG

User data matrix

fid	Apex m/z	RT [min]	Platform	Mode
P1	760.58465	19.341	C18	POS
P1002	577.51885	18.018	C18	POS
P1004	784.58414	23.702	C18	POS
P1006	853.68138	21.396	C18	POS
P101	812.65201	21.395	C18	POS
P1012	850.57197	21.487	C18	POS

Metabolomic/Lipidomic Metadata

Annotate Features >>

Choose the columns label in your data that contain *m/z*, retention time (RT), and ionization mode (positive or negative) for each feature

PUTATIVE ANNOTATIONS PARAMETERS (CMM)

(2)

Tolerance (ppm)

Number  
5

Specify the parts-per-million (ppm) tolerances for *m/z*-based searches in CMM

13

# Putative Annotation

ii. Select potential adducts for both positive and negative ionization modes

(3) Click on the buttons to choose the potential adducts that can be formed in the analytical set-up (.i.e. mobile phases) used by the researcher

Positive Adducts

Select All

Grid of buttons for Positive Adducts:

M+H	M+2H	M+Na	M+K	M+NH <sub>4</sub>	M+H-H <sub>2</sub> O	M+H-NH <sub>4</sub>	2M+H	2M+Na	M+H-HCOONa	2M+H-H <sub>2</sub> O	M+3H	M+2H+Na
M+H+2K	M+H+2Na	M+3Na	M+H+Na	M+H+K	M+ACN+2H	M+2Na	M+2ACN+2H	M+3ACN+2H	M+CH <sub>3</sub> OH+H	M+ACN+H		
M+2Na-H	M+IsoProp+H	M+ACN+Na	M+2K-H	M+DMSO+H	M+2ACN+H	M+IsoProp+Na+H	2M+NH <sub>4</sub>	2M+K	2M+ACN+H			
2M+ACN+Na	3M+H	3M+Na	M+H-2H <sub>2</sub> O	M+NH <sub>4</sub> -H <sub>2</sub> O	M+Li	2M+2H+3H <sub>2</sub> O	M+H+CH <sub>3</sub> COOH	M+H+CH <sub>3</sub> COONa	M+F+H			

Negative Adducts

Select all

Grid of buttons for Negative Adducts:

M-H	M+Cl	M+HCOOH-H	M-H-H <sub>2</sub> O	M-H+HCOONa					
M-H+CH <sub>3</sub> COONa	2M-H	M-3H	M-2H	M+Na-2H					
M+K-2H	M+CH <sub>3</sub> COOH-H	M+Br	M+TFA-H						
2M+HCOOH-H	2M+CH <sub>3</sub> COOH-H	3M-H	M+F	M+F+H					

# Putative Annotations

## iii. Select TPMetric parameters (Turboputative)

(6)

Annotate Features >>

RT is used by the TPMetrics module to prioritize annotations based on correlation analyses between features.

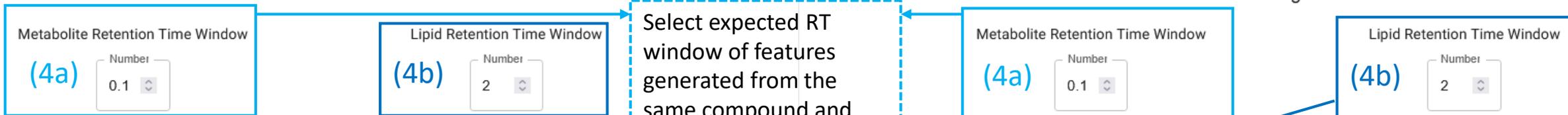
These criteria are based on the concept that signals coming from the same molecular entity and those sharing the same biochemical class show a common elution behavior in the LC-MS and a similar correlation pattern across the samples.

➤ Once these parameters are set, the user can launch the analysis > annotate features

### TURBOPUTATIVE PARAMETERS (TPMetrics)

Mode Positive

Mode Negative



Specify the most likely adducts formed in MS for each compound class by entering the **adduct type** in the corresponding field.

#### Lipid Class and Possible Adducts (5)

	Lipid	Adduct
1	PC	M+H
2	PC	M+Na
3	PC	M+K
4	PE	M+H
5	PE	M+Na
6	PG	M+NH4
7	PI	M+NH4
8	PA	M+NH4
9	LPC	M+H
10	LPC	M+Na
11	LPC	M+H-H2O
12	LPC	M+K
13	LPE	M+H
14	LPE	M+H-H2O
15	LPE	M+Na
16	SM	M+H

(5)

Select the expected RT window of features belonging to the **same compound class** (e.g. LPC, CAR)

This option is only available for lipids since they can easily be classified using the abbreviations extracted from the Lipid Maps Structure Database (LMSD) (Sud et al., 2007) and the Goslin package (Kopczynski et al., 2020) incorporated into TurboPutative.

#### Lipid Class and Possible Adducts (5)

	Lipid	Adduct
1	PC	M+HCOOH-H
2	PC	M+Cl
3	PE	M-H
4	PE	M-H+HCOONa
5	PS	M-H
6	PG	M-H
7	PG	M-H+HCOONa
8	PI	M-H
9	PI	M-H+HCOONa
10	PA	M-H
11	PA	M-H+HCOONa
12	LPC	M+HCOOH-H
13	LPC	M+Cl
14	LPE	M-H
15	LPE	M-H+HCOONa
16	LPS	M-H

(5)

# Putative Annotation

Home TurboPutative TurbOmics Web Services Help Contact us Search Job Job ID: 0lnJ00bl3B

Main page

PUTATIVE ANNOTATION

DATA DISTRIBUTION

PCA

MULTIOMICS FACTOR ANALYSIS

ENRICHMENT ANALYSIS

PATHWAY INTEGRATIVE ANALYSIS

Putative annotation process is running...

CMM	TurboPutative	Results
Running CMM...	Running TurboPutative...	
Positive Mode 742 / 742 batches <span>Done</span>	TurboPutative <span>Processing</span>	—
Negative Mode 229 / 237 batches <span>Processing</span>	TurboPutative <span>Waiting</span>	—

Home TurboPutative TurbOmics Web Services Help Contact us Search Job Job ID: 4eYOz8s8nD

Main page

PUTATIVE ANNOTATION

DATA DISTRIBUTION

PCA

MULTIOMICS FACTOR ANALYSIS

ENRICHMENT ANALYSIS

PATHWAY INTEGRATIVE ANALYSIS

The final results from **TPFilter** provide the most likely annotation for each feature. This is later integrated with the metabolomics/lipidomics metadata table that was previously supplied by the user (slide 7)

CMM	TurboPutative	Results
Waiting...	Idle	
Positive Mode 742 / 742 batches <span>Done</span>	TurboPutative <span>Done</span>	<a href="#">View POS Results</a>
Negative Mode 237 / 237 batches <span>Done</span>	TurboPutative <span>Done</span>	<a href="#">View NEG Results</a>

Click "View POS Results" or "View NEG Results" to download the putative annotation results for features detected in positive and negative polarity mode

# Exploratory data analysis: Data Distribution

The Exploratory Data Analysis module provides researchers with an **initial overview and visualization of the data uploaded to the platform.**

In TurbOmics, exploratory analysis is conducted independently for each omics dataset.

To support this process, the platform includes two dedicated modules: Data Distribution and Principal Component Analysis (PCA).

The screenshot shows the TurbOmics web interface. At the top, there is a navigation bar with 'Home', 'TurboPutative', 'TurbOmics', 'Web Services', 'Help', and 'Contact us'. A search bar is on the right. Below the navigation bar, there is a 'Main page' button and a sidebar with various analysis modules: 'PUTATIVE ANNOTATION', 'DATA DISTRIBUTION', 'PCA', 'MULTIOMICS FACTOR ANALYSIS', 'ENRICHMENT ANALYSIS', and 'PATHWAY INTEGRATIVE ANALYSIS'. The main content area is titled 'Data Distribution' and has tabs for 'Transcriptomics', 'Metabolomics', and 'Proteomics'. The 'Metabolomics' tab is active. A 'Group by' dropdown menu is set to 'Treatment'. Below this, there is a 'Download Normalized Data' button and a 'Data Distribution' button. The main visualization consists of a density plot and a box plot. The density plot shows three overlapping distributions (red, green, blue) with a legend below it: A12 (blue), B1-8 (red), and PBS (green). The box plot shows three box plots corresponding to these groups. To the right of the plot is a table with columns 'TP\_Name' and 'TG'. The table contains 17 rows of data. Annotations are present: (1) A red dashed box around the 'Group by' dropdown with the text 'Select a metadata category to explore the distribution of a particular group of samples'. (2) A green dashed box around the 'TP\_Name' dropdown with the text 'Select the column to be displayed from the Biomolecule Table lists (e.g. putative annotation, ChEBI IDs, gene name, etc.)'. (3) A blue dashed box around the 'TG' input field with the text 'Users can filter a set of biomolecules based on a specific column from the Omics Metadata Table to displayed them in the density plot'.

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Main page

PUTATIVE ANNOTATION

DATA DISTRIBUTION

PCA

MULTIOMICS FACTOR ANALYSIS

ENRICHMENT ANALYSIS

PATHWAY INTEGRATIVE ANALYSIS

Transcriptomics **Metabolomics** Proteomics

Group by Treatment

Download Normalized Data

Data Distribution

0.49  
0.3  
0.15  
0

-3.43 -2.05 -0.68 0 0.71 2.09 3.46

A12 B1-8 PBS

TP\_Name TG

P2 TG 52:2 // TG 52:2; LMSD{ TG 52:2 }

P7 TG 52:3 // TG 52:3; LMSD{ TG 52:3 }

P8 TG 50:2 // TG 50:2; LMSD{ TG 50:2 }

P11 TG 54:3 // TG 54:3; LMSD{ TG 54:3 }

P15 TG 50:3 // TG 50:3; LMSD{ TG 50:3 }

P16 TG 50:1; LMSD{ TG 50:1 } // TG 50:1

P19 TG 52:4 // TG 52:4; LMSD{ TG 52:4 }

P20 TG 54:4 // TG 54:4; LMSD{ TG 54:4 }

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(1) Select a metadata category to explore the distribution of a particular group of samples

(2) Select the column to be displayed from the Biomolecule Table lists (e.g. putative annotation, ChEBI IDs, gene name, etc.)

(3) Users can filter a set of biomolecules based on a specific column from the Omics Metadata Table to displayed them in the density plot

This module allows users to examine the **distribution of quantitative data** using **density plots** and **box plots**.

# Exploratory data analysis: Data Distribution

The Exploratory Data Analysis module provides researchers with an **initial overview and visualization of the data uploaded to the platform.**

In TurbOmics, exploratory analysis is conducted independently for each omics dataset.

To support this process, the platform includes two dedicated modules: Data Distribution and Principal Component Analysis (PCA).

The screenshot shows the TurbOmics web interface. The top navigation bar includes 'Home', 'TurboPutative', 'TurbOmics', 'Web Services', 'Help', and 'Contact us'. A search bar is on the right. The left sidebar lists analysis modules: PUTATIVE ANNOTATION, DATA DISTRIBUTION (highlighted), PCA, MULTIOMICS FACTOR ANALYSIS, ENRICHMENT ANALYSIS, and PATHWAY INTEGRATIVE ANALYSIS. The main content area has tabs for 'Transcriptomics', 'Metabolomics' (selected), and 'Proteomics'. A 'Group by' dropdown is set to 'Treatment'. Below this, there are dropdowns for 'TP\_Name' and 'TG'. The central visualization consists of a density plot with three overlapping curves (blue, red, green) and three corresponding box plots below it, all for the same x-axis range (-3.43 to 3.46). Annotations include: a pink dashed box pointing to a question mark icon with the text 'Click “?” for further information about the analysis'; a yellow dashed box pointing to a 'Download Normalized Data' button with the text 'Click “Download Normalized Data” to download the normalized omics layer matrix'; and a teal dashed box pointing to the 'Data Distribution' label with the text 'Click “Data Distribution” to download the density plot'. A legend at the bottom identifies the series as A12 (blue), B1-8 (red), and PBS (green).

Home TurboPutative TurbOmics Web Services Help Contact us Search Job Job ID: 4eYOz8s8nD

Main page

PUTATIVE ANNOTATION  
DATA DISTRIBUTION  
PCA  
MULTIOMICS FACTOR ANALYSIS  
ENRICHMENT ANALYSIS  
PATHWAY INTEGRATIVE ANALYSIS

Transcriptomics Metabolomics Proteomics

Group by Treatment

TP\_Name TG

Download Normalized Data

Data Distribution

Click “?” for further information about the analysis

Click “Download Normalized Data” to download the normalized omics layer matrix

Click “Data Distribution” to download the density plot

0.49  
0.3  
0.15  
0

-3.43 -2.05 -0.68 0 0.71 2.09 3.46

-3.43 -2.05 -0.68 0 0.71 2.09 3.46

A12 B1-8 PBS

TP_Name
P2 TG 52:2 // TG 52:2; LMSD{ TG 52:2 }
P7 TG 52:3 // TG 52:3; LMSD{ TG 52:3 }
P8 TG 50:2 // TG 50:2; LMSD{ TG 50:2 }
P11 TG 54:3 // TG 54:3; LMSD{ TG 54:3 }
P15 TG 50:3 // TG 50:3; LMSD{ TG 50:3 }
P16 TG 50:1; LMSD{ TG 50:1 } // TG 50:1
P19 TG 52:4 // TG 52:4; LMSD{ TG 52:4 }
P20 TG 54:4 // TG 54:4; LMSD{ TG 54:4 }

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This module allows users to examine the **distribution of quantitative data** using **density plots** and **box plots**.

# Exploratory data analysis: Principal Component Analysis (PCA)

Transcriptomics
Metabolomics
Proteomics

PCA / Cond.	1	2	3	4	5	6	7	8	9
%Variance	22.4	16.5	11.9	9.0	5.5	5.4	4.2	3.8	3.3
p-value [ Treatment ]	0.924	0.027	0.276	0.550	5.760e-4	0.194	0.652	0.599	0.801
p-value [ Ig_treated ]	0.689	0.777	0.462	0.635	9.945e-5	0.421	0.595	0.619	0.843
p-value [ A12_treated ]	0.879	0.014	0.399	0.537	0.197	0.066	0.349	0.603	0.509

(1) Select a **metadata category** to color samples accordingly

(3) Users can visualize and filter a specific sets of **biomolecules**

Color by: Treatment

(2) TP\_Name Filter text

ID	TP_Name	Loadings PCA1	Loadings PCA2
P1	PC 34:1; LMSD{ PC 34:1 } // PC 34:1	0.0105	0.763
P2	TG 52:2 // TG 52:2; LMSD{ TG 52:2 }	-0.0179	0.448
P3	PC 36:4; LMSD{ PC 36:4 } & PC 36:4	0.00254	0.137
P4	PC 34:2; LMSD{ PC 34:2 } // PC 34:2	0.01345	0.688
P6	PC 38:6; LMSD{ PC 38:6 } // PC 38:6	0.01692	0.993
P7	TG 52:3 // TG 52:3; LMSD{ TG 52:3 }	-0.01761	0.221

Select an **identifiers** from the **Biomolecule Table** lists. It helps users track which biomolecules are currently being analyzed.

TurbOmics computes the first ten principal components (PCs) and calculates correlations between the component scores and metadata variables. The percentage of variance explained by each component, along with the p-values from the correlations, is shown in a color-coded table, allowing users to quickly identify associations between quantitative data and metadata.

# Exploratory data analysis: Principal Component Analysis (PCA)

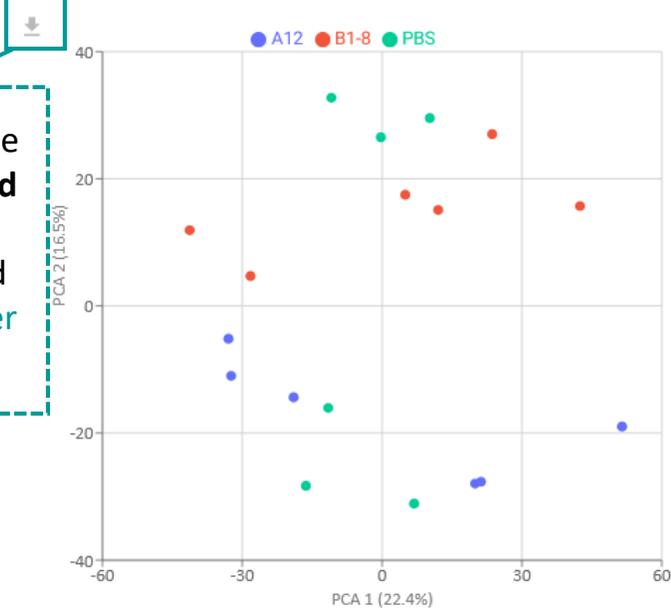
Click **"Table icon"** to download the PCA table in CSV format

PCA / Cond.	1	2	3	4	5	6	7	8	9
%Variance	22.4	16.5	11.9	9.0	5.5	5.4	4.2	3.8	3.3
p-value [ Treatment ]	0.924	0.027	0.276	0.550	5.760e-4	0.194	0.652	0.599	0.801
p-value [ Ig_treated ]	0.689	0.777	0.462	0.635	9.945e-5	0.421	0.595	0.619	0.843
p-value [ A12_treated ]	0.879	0.014	0.399	0.537	0.197	0.066	0.349	0.603	0.509



1D 2D

X axis: PCA 1  
Y axis: PCA 2  
Color by: Treatment



Click on the **"download icon"** to download PCA scatter plot

Click on the **"download icon"** to download PCA loading table

ID	TP_Name	Loadings PCA1	Loadings PCA2
P1	PC 34:1; LMSD{ PC 34:1 } // PC 34:1	0.0105	-0.03763
P2	TG 52:2 // TG 52:2; LMSD{ TG 52:2 }	-0.0179	0.00448
P3	PC 36:4; LMSD{ PC 36:4 } & PC 36:4	0.00254	0.02137
P4	PC 34:2; LMSD{ PC 34:2 } // PC 34:2	0.01345	0.00688
P6	PC 38:6; LMSD{ PC 38:6 } // PC 38:6	0.01692	0.00993
P7	TG 52:3 // TG 52:3; LMSD{ TG 52:3 }	-0.01761	0.02221

# Multi-Omics Factor Analysis

The **Multi-Omics Factor Analysis** module enables the unbiased and integrative decomposition of multi-omics quantitative data into latent factors. This module is based on the [MOFA](#) framework, which identifies latent factors that capture the main sources of variation across the analyzed datasets. TurbOmics supports the visualization of sample subgroups and shared patterns across different omics layers.

The screenshot displays the TurbOmics web interface. On the left is a navigation menu with categories: PUTATIVE ANNOTATION, DATA DISTRIBUTION, PCA, MULTIOMICS FACTOR ANALYSIS (highlighted in blue), ENRICHMENT ANALYSIS, and PATHWAY INTEGRATIVE ANALYSIS. The main content area is divided into two sections. The top section, labeled (1), shows a color-coded table summarizing factors computed by MOFA. The bottom section, labeled (2), shows a 2D projection plot of Factor4 versus Treatment, with a control panel above it. A third annotation, labeled (3), points to the 'Group by' dropdown in the control panel. A fourth annotation, labeled (4), points to a table icon in the top left of the table area.

(1) Color-coded table summarizes the factors computed by MOFA

Factor / Cond.	1	2	3	4	5	6	7
%Var (Tran.)	46.57	14.05	0.19	8.10	2.19	1.13	3.22
%Var (Meta.)	3.75	9.46	20.07	13.93	3.78	3.31	1.30
%Var (Prot.)	1.40	15.39	8.11	4.23	18.07	11.22	2.76
p-value [ Treatment ]	0.630	0.384	0.610	0.076	0.945	0.006	0.367
p-value [ Ig_treated ]	0.564	0.581	0.944	0.422	0.759	0.002	0.612
p-value [ A12_treated ]	0.717	0.433	0.370	0.023	0.772	0.043	0.394

(2) Users can select and visualize a specific MOFA factor

(3) Select a metadata category to classify samples

(4) Click "Table icon" to download the MOFA table in CSV format

Projections

1D 2D

Factor: Factor4

Group by: Treatment

Show Labels

Factor4

Treatment: A12, B1-8, PBS

# Multi-Omics Factor Analysis: Feature Loading Analysis

(4) Users can examine the distribution of factor loadings for each omics dataset and select the top-n biomolecules with the highest positive or negative loadings for visualization in a heatmap.

Home Main page

PUTATIVE ANNOTATION

DATA DISTRIBUTION

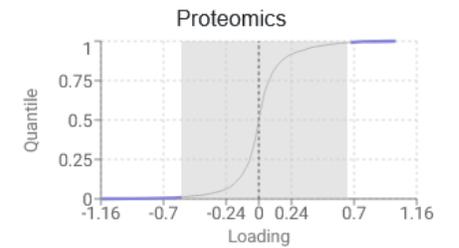
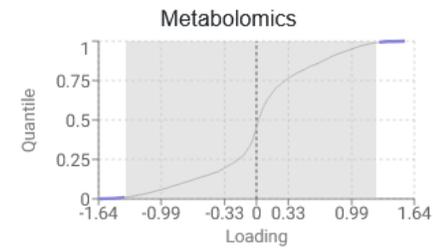
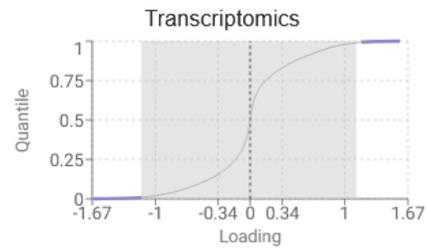
PCA

MULTIOMICS FACTOR ANALYSIS

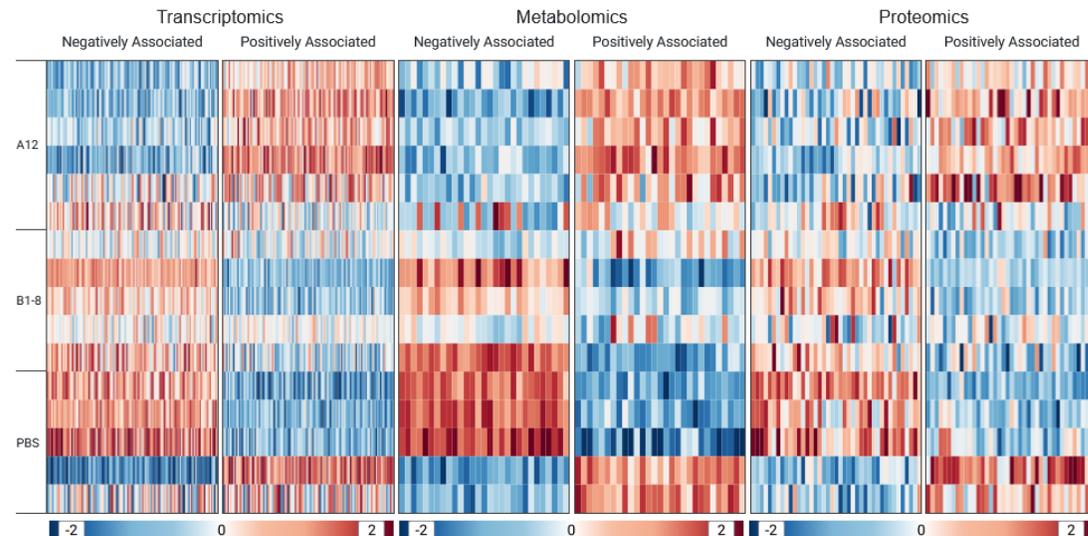
ENRICHMENT ANALYSIS

PATHWAY INTEGRATIVE ANALYSIS

Feature Loading Analysis: Factor4



Help Download



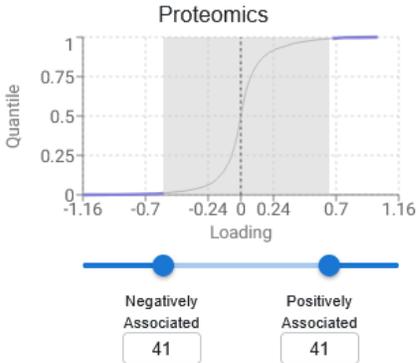
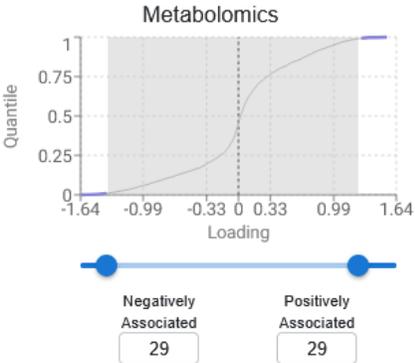
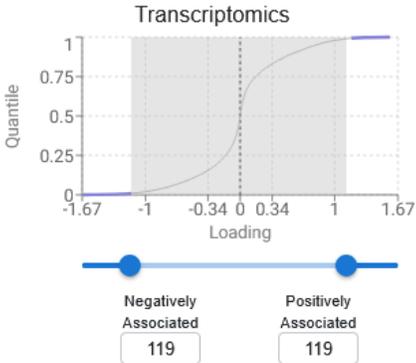
EXPLORE FEATURES

# Multi-Omics Factor Analysis: Feature Loading Analysis

Home Main page

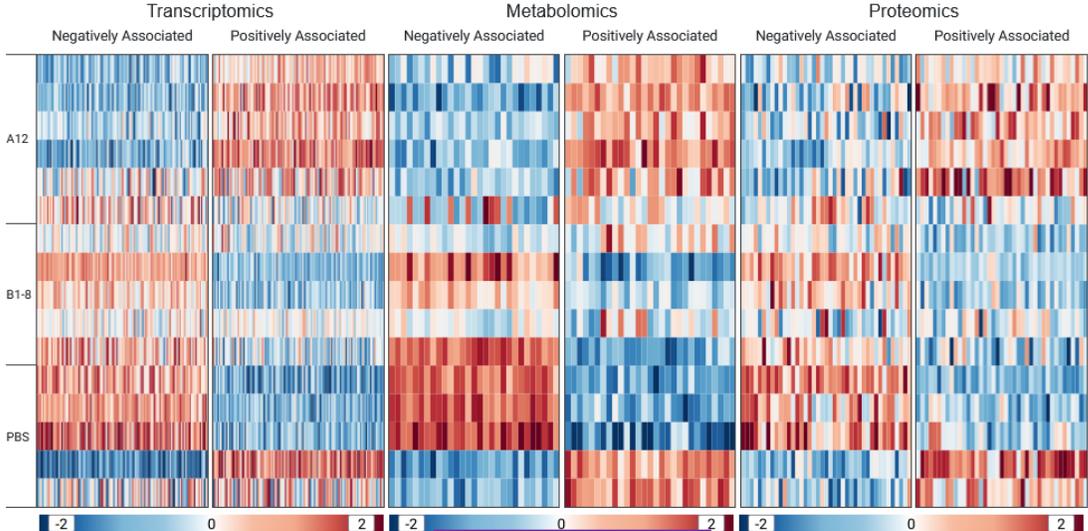
- PUTATIVE ANNOTATION
- DATA DISTRIBUTION
- PCA
- MULTIOMICS FACTOR ANALYSIS
- ENRICHMENT ANALYSIS
- PATHWAY INTEGRATIVE ANALYSIS

Feature Loading Analysis: Factor4



Click "?" for further information about the analysis

Click on the "download icon" to download heat maps



Select "EXPLORE FEATURE" to explore the top positively and negatively associated biomolecules for the selected factor within each omics.

(5)

EXPLORE FEATURES ↗

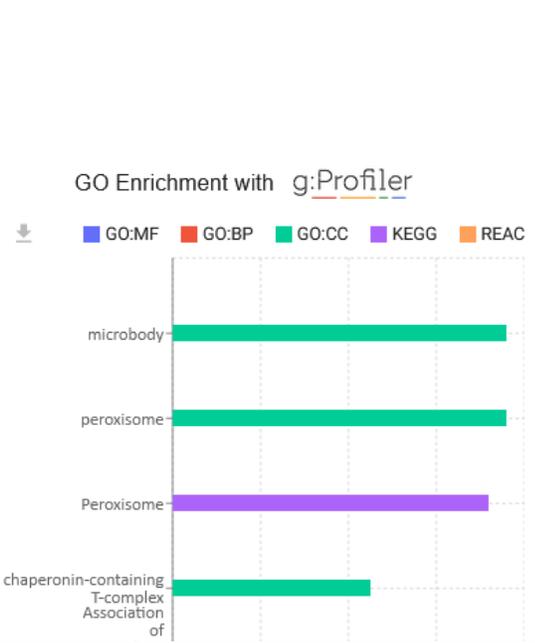


# Multi-Omics Factor Analysis

➤ Example of ORA (over-representation analysis) of the proteomics data within Factor 4 in MOFA

Explore Features: Factor4 vs Treatment

Transcriptomics      Metabolomics      **Proteomics**



To aid in the biological interpretation of MOFA results, **ORA** is performed using databases such as KEGG, REACTOME, and GO. This analysis helps identify significantly enriched biological pathways, processes, or functions among the selected biomolecules.

EXPORT ALL DATA

ID	GN	Description
A2AKK5	Acnat1	acyl-coenzyme A amino acid N-acyltransferase 1
Q922Q1	Mtarc2	mitochondrial amidoxime reducing component 2
A2AKD6	Pxmp4	peroxisomal membrane protein 4
Q9JJW0	Pxmp4	peroxisomal membrane protein 4
Q99LC9	Pex6	peroxisomal biogenesis factor 6
Q8VCI5	Pex19	peroxisomal biogenesis factor 19
Q9DC50	Crot	camitine O-octanoyltransferase

By clicking on a category, you can view all biomolecules associated it, with the ones selected for the factor highlighted in blue.

EXPORT ALL DATA

Select	GO	Filter by GO	FDR
<input type="radio"/>	GO:0042579	GO:CC	microbody 2.57e-8
<input checked="" type="radio"/>	GO:0005777	GO:CC	peroxisome 2.57e-8
<input type="radio"/>	KEGG:04146	KEGG	Peroxisome 6.35e-8
<input type="radio"/>	GO:0005832	GO:CC	chaperonin-containing T-complex 3.25e-5
<input type="radio"/>	REAC:R-MMU-390471	REAC	Association of TriC/CCT with target proteins during biosynthesis 4.17e-5

# Multi-Omics Factor Analysis

➤ Example of ORA (over-representation analysis) of the proteomics data within Factor 4 in MOFA

Explore Features: Factor4 vs Treatment

Transcriptomics | Metabolomics | Proteomics

Select Column Containing ID: Field: fid  
Select ID Format: Field: UNIPROT\_GN\_ACC

GO Enrichment with g:Profiler

Legend: GO:MF (blue), GO:BP (red), GO:CC (green), KEGG (purple), REAC (orange)

microbody  
peroxisome  
Peroxisome  
chaperonin-containing T-complex Association of

Click on the "download icon" to download the enrichment bar plot

Click on the "download icon" to download the GO enrichment table

EXPORT ALL DATA

Click on the "download icon" to download category table

ID	GN	Description
A2AKK5	Acnat1	acyl-coenzyme A amino acid N-acyltransferase 1
Q922Q1	Mtarc2	mitochondrial amidoxime reducing component 2
A2AKD6	Pxmp4	peroxisomal membrane protein 4
Q9JJW0	Pxmp4	peroxisomal membrane protein 4
Q99LC9	Pex6	peroxisomal biogenesis factor 6
Q8VCI5	Pex19	peroxisomal biogenesis factor 19
Q9DC50	Crot	camitine O-octanoyltransferase

EXPORT ALL DATA

Select	GO	Type	Name	FDR
<input type="radio"/>	GO:0042579	GO:CC	microbody	2.57e-8
<input checked="" type="radio"/>	GO:0005777	GO:CC	peroxisome	2.57e-8
<input type="radio"/>	KEGG:04146	KEGG	Peroxisome	6.35e-8
<input type="radio"/>	GO:0005832	GO:CC	chaperonin-containing T-complex	3.2e-5
<input type="radio"/>	REAC:R-MMU-390471	REAC	Association of TriC/CCT with target proteins during biosynthesis	4.17e-5

# Enrichment Analysis

This module allows the user to perform Enrichment Analysis using the **Gene Set Enrichment Analysis (GSEA)** algorithm on each omics dataset independently. Users can switch between omics using the navigator bar at the top of the page to explore results for different data types.

If you are working with **untargeted metabolomics/lipidomics data**, you can choose to perform enrichment analysis using the **Mummichog algorithm**.

The screenshot shows the 'Untargeted Lipidomics Data' section of a web application. The interface includes a top navigation bar with links like 'Home', 'TurboPutative', 'TurbOmics', 'Web Services', 'Help', and 'Contact us'. A search bar is located in the top right corner. The main content area is divided into three tabs: 'Transcriptomics', 'Metabolomics' (selected), and 'Proteomics'. The 'Metabolomics' tab is active, showing the 'Mummichog' configuration panel. This panel includes dropdown menus for 'Apex m/z Column' (set to 'Apex m/z'), 'Retention Time Column (min)' (set to 'RT [min]'), and 'Ion Mode Column' (set to 'Mode'). There are also dropdowns for 'Positive Ion Value' and 'Negative Ion Value', both set to 'POS'. Below this, there are two 'Select Enrichment Ranking Metric' dropdowns, one set to 'GSEA Ranking Metric' and another to 'MOFA'. To the right, there is a 'Select MOFA Factor' dropdown set to 'Factor2'. A 'Complement the analysis with your Pathways (Optional)' section contains an 'UPLOAD NEW PATHWAY' button, a text input showing 'Uploaded: BA\_mouse.gmt', and a 'Select Pathways' dropdown with 'BA\_human, BA\_mouse' selected. A 'RUN QEA' button is at the bottom center. Three callouts provide additional information: (1) 'Users need to provide the following additional columns from the untargeted metabolomics/lipidomics metadata table: Apex m/z, Retention Time (min), Ion Mode' (orange dashed box); (2) 'Users must specify the metric used for the enrichment analysis. Different options are available' (blue dashed box); (3) 'Users can upload custom pathways in GMT format, enabling the integration of personalized metabolite-based pathway definitions' (green dashed box). The word 'Optional' is written in green text next to the pathway section.

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ENRICHMENT ANALYSIS

PATHWAY INTEGRATIVE ANALYSIS

## Untargeted Lipidomics Data

Transcriptomics **Metabolomics** Proteomics

(1) Users need to provide the following additional columns from the untargeted metabolomics/lipidomics metadata table: **Apex m/z, Retention Time (min), Ion Mode**

Mummichog

Select Apex m/z Column  
Apex m/z  
Apex m/z

Select Retention Time Column (min)  
RT column (min)  
RT [min]

Select Ion Mode Column  
Ion Mode Column  
Mode

Positive Ion Value  
Positive Ion  
POS

Negative Ion Value  
Negative Ion  
POS

Select Enrichment Ranking Metric  
GSEA Ranking Metric

Select Enrichment Ranking Metric  
GSEA Ranking Metric  
MOFA

Select MOFA Factor  
MOFA Factor  
Factor2

Complement the analysis with your Pathways (Optional)

Optional

Users can upload **custom pathways in GMT format**, enabling the integration of personalized metabolite-based pathway definitions

Users must specify the **metric used for the enrichment analysis**. Different options are available

(2)

(3) RUN QEA >

# Enrichment Analysis: example using untargeted lipidomics data

Results of the Enrichment Analysis can be explored, providing insights into the biological pathways, processes, or functions that are significantly associated with the conditions or experimental groups in the study.

Click "EXPORT ALL DATA" to download the enrichment table

Enrichment Analysis: MOFA | Factor6

Custom KEGG REACTOME Mummichog (+) **Mummichog (-)**

↓ EXPORT ALL DATA

Search

<input type="checkbox"/>	Pathway	pval	Overlap Size	Pathway Size	Features
<input type="checkbox"/>	Arachidonic acid metabolism	6.72e-4	3	5	N238,N889
<input type="checkbox"/>	Porphyrin metabolism	9.24e-3	1	1	N320
<input type="checkbox"/>	Glycerophospholipid metabolism	1.50e-2	2	7	N238,N889
<input type="checkbox"/>	Vitamin A (retinol) metabolism	1.61e-2	1	2	N889
<input type="checkbox"/>	Omega-6 fatty acid metabolism	1.61e-2	1	2	N238,N889
<input type="checkbox"/>	Leukotriene metabolism	2.36e-2	1	3	N238,N889
<input type="checkbox"/>	Prostaglandin formation from arachidonate	3.04e-2	1	4	N238,N889
<input type="checkbox"/>	De novo fatty acid biosynthesis	4.54e-2	1	12	N238,N889

# Enrichment Analysis: example using proteomics data

- Main page
- PUTATIVE ANNOTATION
- DATA DISTRIBUTION
- PCA
- MULTIOMICS FACTOR ANALYSIS
- ENRICHMENT ANALYSIS
- PATHWAY INTEGRATIVE ANALYSIS

Transcriptomics Metabolomics **Proteomics**

(1) Select column containing Proteomics ID  
Proteomics ID column  
UniProt\_ID

Users need to specify the column in their proteomic metadata table that contains the biomolecule identifiers.

(2) Select Enrichment Ranking Metric  
GSEA Ranking Metric  
Mean difference  
t-test  
PCA  
MOFA  
Custom

Users must specify the metric used for the enrichment analysis.

Select GSEA Ranking Metric  
GSEA Ranking Metric  
MOFA

Select MOFA Factor  
MOFA Factor  
Factor4

(3) RUN QEA >

Click "EXPORT ALL DATA" to download the enrichment table

Enrichment Analysis: MOFA | Factor4

Custom HALLMARK GO:MF GO:CC GO:BP **KEGG** REACTOME

EXPORT ALL DATA

Pathway	pval	padj	ES	NES	N. Leading Edge	Size
Lysosome	2.39e-7	1.00e-4	-0.6794	-2.1791	22	57
Peroxisome	6.83e-7	1.00e-4	0.6794	2.1046	27	62
Propanoate metabolism	4.00e-4	3.75e-2	0.6996	1.8407	9	29
Tight junction	6.00e-4	4.18e-2	-0.5444	-1.7862	29	63
Proteasome	1.30e-3	7.15e-2	0.622	1.7728	18	40
Protein processing in endoplasmic reticulum	1.40e-3	7.15e-2	0.501	1.6364	19	90
Bacterial invasion of epithelial cells	1.90e-3	7.95e-2	-0.56	-1.6941	18	43
Salmonella infection	2.10e-3	7.95e-2	-0.4155	-1.5004	25	120

✓ For Pathway Integrative Analysis see slides 36-40 of the present tutorial

# Application to multi-omics datasets in a COVID-19 study, including targeted metabolomics data

Volume 183, Issue 6, 10 December 2020, Pages 1479-1495.e20

Article

## Multi-Omics Resolves a Sharp Disease-State Shift between Mild and Moderate COVID-19

Yapeng Su<sup>1</sup>, Daniel Chen<sup>1</sup>, Dan Yuan<sup>1,2</sup>, Christopher Lausted<sup>1</sup>, Jongchan Choi<sup>1</sup>, Chengzhen L. Dai<sup>1</sup>, Valentin Voillet<sup>3,4</sup>, Venkata R. Duvvuri<sup>1</sup>, Kelsey Scherler<sup>1</sup>, Pamela Troisch<sup>1</sup>, Priyanka Baloni<sup>1</sup>, Guangrong Qin<sup>1</sup>, Brett Smith<sup>1</sup>, Sergey A. Kornilov<sup>1</sup>, Clifford Rostomily<sup>1</sup>, Alex Xu<sup>1</sup>, Jing Li<sup>5</sup>, Shen Dong<sup>6</sup>, Alissa Rothchild<sup>7</sup>, Jing Zhou<sup>8</sup>...James R. Heath<sup>1,2,21</sup>  

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- TARGETED METABOLOMICS
- PROTEOMICS
- TRANSCRIPTOMICS

# Load example dataset

To get started, users can upload their experimental data directly from the **Main Page**. Previous analyses can be accessed using the **Find Job** field by entering the corresponding job ID. Once a dataset is loaded, the **Results** section provides access to a suite of tools for in-depth analysis and interpretation.

Home TurboPutative TurbOmics Web Services Help Contact us

Search Job

Create new job

(1a) Load directly the example containing the **metadata** and the **quantitative data** from all omics layers

(1b) Download the sample data files

(2) Choose appropriate organism

Organism: Mouse - Mus musculus

Experimental Metadata

Upload or drop a file right here TSV

File name: experimental\_metadata.tsv

	n
Meta-variables	18
Transcriptomic features	4
Metabolomic features	11999 / 11999
Proteomic features	465 / 465
	4114 / 4114

Transcriptomic Metadata

Upload or drop a file right here TSV

File name: transcriptomic\_metadata.tsv

Metabolomic/Lipidomic Metadata

Upload or drop a file right here TSV

File name: metabolomic\_metadata.tsv

Proteomic Metadata

Upload or drop a file right here TSV

File name: proteomic\_metadata.tsv

Transcriptomic Quantifications

Upload or drop a file right here TSV

File name: transcriptomic\_quantifications.tsv

Metabolomic/Lipidomic Quantifications

Upload or drop a file right here TSV

File name: metabolomic\_quantifications.tsv

Proteomic Quantifications

Upload or drop a file right here TSV

File name: proteomic\_quantifications.tsv

- ✓ For Normalization, Scaling and Missing Values Imputation see slide 11 of the present tutorial

# Putative Annotation

Home TurboPutative TurbOmics Web Services Help Contact us

Search Job

Job ID: 72f42rH8UT

Back to results Create new job

Organism: Human - Homo sapiens

Load the Untarget Lipidomic sample data

Load the Target Metabolomic sample data

Upload from R

Experimental Metadata

Transcriptomic Metadata

Transcriptomic Quantifications

Metabolomic/Lipidomic Metadata

Metabolomic/Lipidomic Quantifications

Proteomic Metadata

Proteomic Quantifications

	n
Meta-variables	6
Metabolomic features	750 / 1050
Proteomic features	454 / 464

Obtain Putative Annotations from CMM & TurboPutative?  
Metabolomic features will be annotated using [CeU Mass Mediator](#) and simplified using [TurboPutative](#).

NO YES

Apply: Normalization: None Center & Scale

Apply: Normalization: None Center & Scale

In this example, metabolites identification are already provided

- ✓ For Exploratory analysis see pp.17-20 of the present tutorial
- ✓ For Multiomics Factor Analysis (MOFA) see pp. 21-26 of the present tutorial
- ✓ For Enrichment Analysis see pp. 27-29 of the present tutorial

# Pathway Integrative Analysis

This section leverages the **PathIntegrate** library to identify pathways that are most strongly associated with a condition of interest. By using single-sample pathway analysis (ssPA) approaches, PathIntegrate transforms molecular-level abundance data into pathway-level matrices, enabling a more comprehensive understanding of how different pathways contribute to the observed biological outcomes. In this implementation, Principal Component Analysis (PCA) is used for summarizing the data at the pathway level.

## ➤ Single-View Framework

The screenshot displays the PathIntegrate web application interface. The top navigation bar includes links for Home, TurboPutative, TurbOmics, Web Services, Help, and Contact us, along with a search bar and a job ID (72f42rH8UT). The left sidebar contains a navigation menu with options: Main page, DATA DISTRIBUTION, PCA, MULTIOMICS FACTOR ANALYSIS, ENRICHMENT ANALYSIS, and PATHWAY INTEGRATIVE ANALYSIS. The main content area is titled "Single-View" and "Multi-View". It features three numbered steps: (1) "1. Select the Metadata" with a "Group" dropdown and "Group 1" and "Group 2" dropdowns; (2) "2. Select the Omics Mapping" with "Metabolomics ID Column" and "Proteomics ID Column" dropdowns, and "ChEBI ID Type" and "Uniprot Protein ID Type" dropdowns; and (3) "RUN ANALYSIS >". An "Optional" section titled "Complement the analysis with your Pathways (Optional)" includes an "UPLOAD NEW PATHWAY" button, an "Uploaded: BA\_mouse.gmt" label, and a "Select Pathways" dropdown menu showing "BA\_human, BA\_mouse" and "BA\_mouse". Annotations with dashed boxes and arrows point to these elements: an orange box around step 1 with the text "Specify the comparison groups to be considered in the pathways analysis"; a blue box around step 2 with the text "Select the Identifiers for each omics layer to use in the pathways analysis"; and a green box around the optional section with the text "Users can upload custom pathways in GMT format, enabling the integration of personalized metabolite-based pathway definitions".

**Single-View approach** computes more comprehensive pathway scores, using Partial Least Squares Discriminant Analysis (PLS-DA) on a single pathway-level matrix that combines information from all omics

Users can upload **custom pathways in GMT format**, enabling the integration of personalized metabolite-based pathway definitions

# Pathway Integrative Analysis

**R-squared (R<sup>2</sup>)** value representing the proportion of variance in the response variable explained by the model. An **empirical p-value** is, calculated by performing ten permutations on the response vector (y) and comparing the simulated R<sup>2</sup> values with the actual one. The minimum p-value that can be obtained is 0.1, reflecting the reliability of the model.

## ➤ Single-View Framework results



# Pathway Integrative Analysis

➤ Single-View Framework results

DATA DISTRIBUTION

PCA

MULTIOMICS FACTOR ANALYSIS

ENRICHMENT ANALYSIS

PATHWAY INTEGRATIVE ANALYSIS

a)

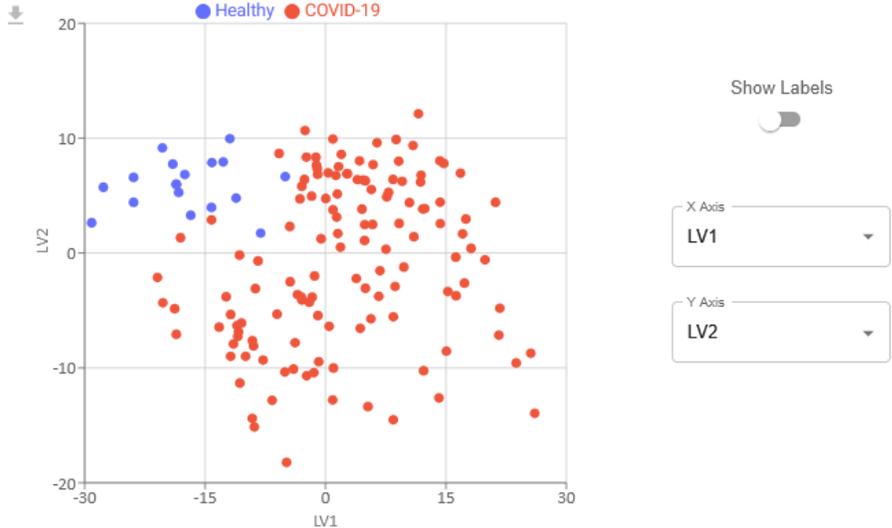
EXPORT TABLE

Summary statistics

Model R2 = 0.8287

p-value ≤ 0.1

Latent Variable	R2	p-value
1	0.2998	4.97e-13
2	0.0973	0.00011
3	0.1609	3.97e-7
4	0.1911	2.48e-8
5	0.0796	0.00049



Click on the “EXPORT TABLE” to download the results tables:

a) Pathways analysis model with statistical summary

b) Relevant Pathway displaying the pathways most strongly associated with the outcome;

c) Pathways exploration, with biomolecules associated with a specific pathway

b)

EXPORT TABLE

Select	Path_ID	Name	N.	VIP
<input type="radio"/>	R-HSA-216083	Integrin cell surface interactions	4	3.1342
<input checked="" type="radio"/>	R-HSA-156580	Phase II - Conjugation of compounds	9	2.4516
<input type="radio"/>	R-HSA-448424	Interleukin-17 signaling	5	2.3862
<input type="radio"/>	R-HSA-5687128	MAPK6/MAPK4 signaling	4	2.2312
<input type="radio"/>	R-HSA-83936	Transport of nucleosides and free purine and pyrimidine bases across the plasma membrane	4	2.1508
<input type="radio"/>	R-HSA-74217	Purine salvage	4	2.0680

c)

EXPORT TABLE

Phase II - Conjugation of compounds

PLOT HEATMAP

#	Omic	ID	Name	Loading
Q145	Proteomics	P50225	SULT1A1   sulfotransferase family 1A member 1	0.601
Q304	Proteomics	P21964	COMT   catechol-O-methyltransferase	0.5437
Q378	Proteomics	O60760	HPGDS   hematopoietic prostaglandin D synthase	0.4682
M227	Metabolomics	25858	1,7-dimethylxanthine	0.3108
Q306	Proteomics	P23526	AHCY   adenosylhomocysteinase	0.0862
M391	Metabolomics	27905	phenyl hydrogen sulfate	0.0823

# Pathway Integrative Analysis

## ➤ Multi-View Framework

The screenshot shows the web application interface for Pathway Integrative Analysis. The top navigation bar includes links for Home, TurboPutative, TurbOmics, Web Services, Help, and Contact us, along with a search bar and a job ID (72f42rH8UT). The left sidebar contains a navigation menu with options: Main page, DATA DISTRIBUTION, PCA, MULTIOMICS FACTOR ANALYSIS, ENRICHMENT ANALYSIS, and PATHWAY INTEGRATIVE ANALYSIS (highlighted in blue). The main content area is divided into three numbered steps:

- (1) 1. Select the Metadata:** This step includes a dropdown for 'Metadata Column', a 'Group' dropdown, and two sub-drops for 'Group 1' (containing 'He...') and 'Group 2' (containing 'C ...'). An orange dashed box highlights this step with the text: "Specify the **comparison groups** to be considered in the pathways analysis".
- (2) 2. Select the Omics Mapping:** This step includes dropdowns for 'Metabolomics ID Column' (ChEBI\_ID) and 'Proteomics ID Column' (UniProt\_ID), and corresponding 'ID Type' dropdowns (ChEBI and Uniprot Protein ID). A blue dashed box highlights this step with the text: "Select the **Identifiers** for each omic layer to use in the pathways analysis".
- (3) RUN ANALYSIS >** A blue button with a right-pointing arrow, highlighted with a purple box.

On the right side, there is an 'Optional' section titled "Complement the analysis with your Pathways (Optional)". It features an "UPLOAD NEW PATHWAY" button, a status "Uploaded: BA\_mouse.gmt", and a "Select Pathways" dropdown menu with the selection "BA\_human, BA\_mouse". A green dashed box highlights this section with the text: "Users can upload **custom pathways in GMT format**, enabling the integration of personalized metabolite-based pathway definitions".

**Multi-View approach** employs a multi-block partial least squares (MB-PLS) latent variable model. It preserves the block structure of each omics view, enabling you to see the contribution of each omics dataset to the prediction of the outcome variable.

# Pathway Integrative Analysis

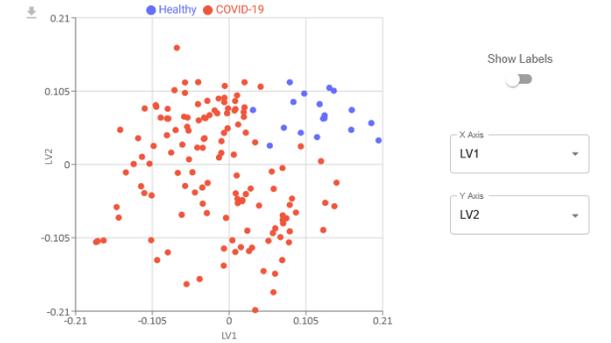
a)

EXPORT TABLE

Summary statistics

Model R2 = 0.7864  
p-value ≤ 0.1

Latent Variable	Metabolomics (%)	Proteomics (%)	R2	p-value
1	16.44	83.56	0.3007	4.54e-13
2	55.46	44.54	0.1025	6.88e-5
3	53.56	46.44	0.142	2.17e-6
4	85.37	14.63	0.1777	8.60e-8
5	88.31	11.69	0.0635	0.00194



## Multi-View Framework results

Click on the "EXPORT TABLE" to download results :

- a) Pathways analysis model with statistical summary;
- b) Relevant Pathway displaying the pathways most strongly associated with the outcome;
- c) Pathways exploration with biomolecules associated with a specific pathway

EXPORT TABLE

b1) Metabolomics

Select	Path_ID	Name	N.	VIP
<input type="radio"/>	R-HSA-373076	Class A/1 (Rhodopsin-like receptors)	6	2.5551
<input checked="" type="radio"/>	R-HSA-156580	Phase II - Conjugation of compounds	5	2.5417
<input type="radio"/>	R-HSA-556833	Metabolism of lipids	11	2.5026
<input type="radio"/>	R-HSA-416476	G alpha (q) signalling events	4	2.4952
<input type="radio"/>	R-HSA-1483206	Glycerophospholipid biosynthesis	4	2.4916
<input type="radio"/>	R-HSA-425393	Transport of inorganic cations/anions and amino acids/oligopeptides	5	2.4465
<input type="radio"/>	R-HSA-5619102	SLC transporter disorders	11	2.4452

EXPORT TABLE

b2) Proteomics

Select	Path_ID	Name	N.	VIP
<input type="radio"/>	R-HSA-3007120	MAPK9/MAPK9 signaling	4	1.5404
<input type="radio"/>	R-HSA-5669034	TNFs bind their physiological receptors	7	1.5163
<input checked="" type="radio"/>	R-HSA-156580	Phase II - Conjugation of compounds	4	1.5101
<input type="radio"/>	R-HSA-8957322	Metabolism of steroids	4	1.5058
<input type="radio"/>	R-HSA-8979227	Triglyceride metabolism	4	1.4659
<input type="radio"/>	R-HSA-163560	Triglyceride catabolism	4	1.4659
<input type="radio"/>	R-HSA-168255	Influenza Infection	4	1.4357
<input type="radio"/>	R-HSA-5218859	Regulated Necrosis	8	1.4325

EXPORT TABLE

c1) Phase II - Conjugation of compounds

PLOT HEATMAP

#	ID	Name	Loading
M227	25858	1,7-dimethylxanthine	0.7268
M391	27905	phenyl hydrogen sulfate	0.3355
M710	37946	dopamine 3-O-sulfate	-0.065
M82	9008	salicylic acid	-0.0822
M435	17864	taurothiocholic acid sulfate	-0.5902

EXPORT TABLE

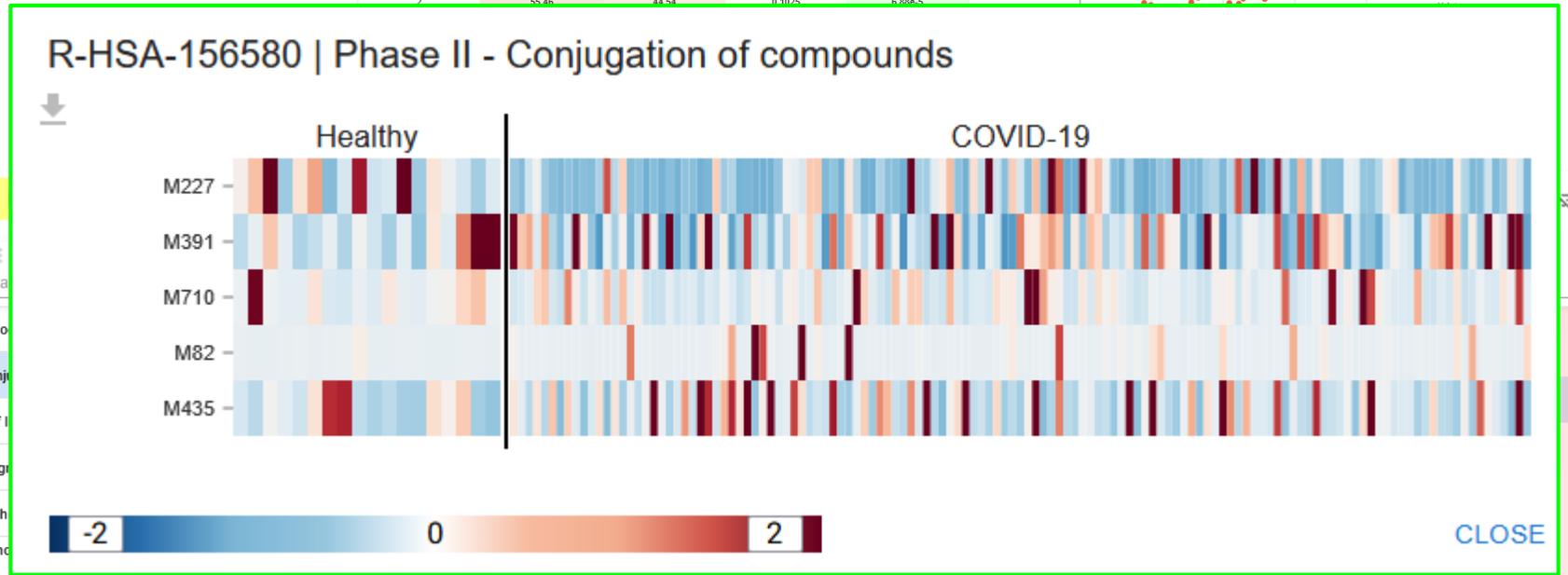
c2) Phase II - Conjugation of compounds

PLOT HEATMAP

#	ID	Name	Loading
Q145	P50225	SULT1A1   sulfotransferase family 1A member 1	0.6379
Q304	P21964	COMT   catechol-O-methyltransferase	0.6269
Q378	O60760	HPGDS   hematopoietic prostaglandin D synthase	0.3758
Q306	P23526	AHCY   adenosylhomocysteinase	0.2426

# Pathway Integrative Analysis

## ➤ Multi-View Framework results



EXPORT TABLE

Select Path\_ID Name

Filter by Path\_ID Filter by Name

<input type="radio"/>	R-HSA-373076	Class A/1 (Rho
<input checked="" type="radio"/>	R-HSA-156580	Phase II - Conji
<input type="radio"/>	R-HSA-556833	Metabolism of I
<input type="radio"/>	R-HSA-416476	G alpha (q) sig
<input type="radio"/>	R-HSA-1483206	Glycerophosph
<input type="radio"/>	R-HSA-425393	Transport of inc
<input type="radio"/>	R-HSA-5619102	SLC transporter disorders

Click on "PLOT HEATMAPS" to show heatmap visualization of biomolecule abundance or quantification values associated with the selected pathway

Phase II - Conjugation of compounds

PLOT HEATMAP

ID	Name	Loading
6858	1,7-dimethylxanthine	0.7268
7905	phenyl hydrogen sulfate	0.3355
M710	dopamine 3-O-sulfate	-0.065
M82	salicylic acid	-0.0822
M435	taurothiocholic acid sulfate	-0.5902

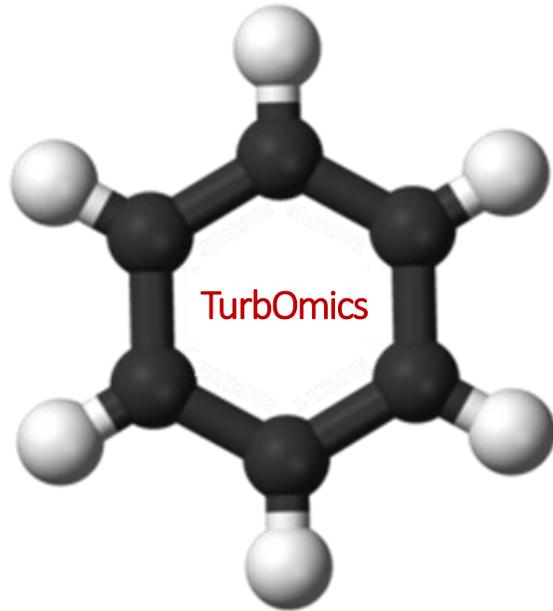
EXPORT TABLE

Phase II - Conjugation of compounds

PLOT HEATMAP

#	ID	Name	Loading
Q145	P50225	SULT1A1   sulfotransferase family 1A member 1	0.6379
Q304	P21964	COMT   catechol-O-methyltransferase	0.6269
Q378	O60760	HPGDS   hematopoietic prostaglandin D synthase	0.3758
Q306	P23526	AHCY   adenosylhomocysteinase	0.2426

# Summary



For additional details and explanation, please visit:  
<https://proteomics.cnice.es/TurboPutative/turbomicshelp>

If you have any questions, please contact us:  
[metabolomics.cnice@gmail.com](mailto:metabolomics.cnice@gmail.com)

- TurbOmics is an intuitive web-based platform designed to facilitate the **multi-omics analysis of metabolomic/ lipidomics data alongside proteomics and transcriptomics**.
- It supports both **targeted** experiments and those where metabolite **identification** is available (e.g., LC/CE-MS/MS, NMR or GC-MS data).
- Additionally, TurbOmics offers tools to **putatively annotate features** from untargeted metabolomics based on their mass-to-charge (m/z) ratio.
- The platform provides a streamlined workflow for **exploratory data analysis** and **multi-omics integration** through i) **Multi-Omics Factor Analysis**, ii) **Pathway Integrative Analysis** and iii) **Enrichment Analysis**.
- Users can upload their experimental data directly from the **Main Page**. Previous analyses can be accessed using the **Find Job** field by entering the corresponding job ID.
- Once a dataset is loaded, the **Results** section provides access to a suite of tools for in-depth analysis and interpretation where users can explore and export data tables and visualize plots of the results.