

NetID User Guide

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1 Environment Setup

This section provides step-by-step instructions to set up the environment to run NetID algorithm in a local computer. A Windows system is recommended. Typical install time on a “normal” desktop computer is within a few hours.

1.1 Software installation

- Install R, Rstudio, Rtools40, ILOG CPLEX Optimization Studio (CPLEX), preferably at default location.

R(4.0.3): <https://www.r-project.org/>

RStudio: <https://rstudio.com/products/rstudio/download>

Rtools40: <https://cran.r-project.org/bin/windows/Rtools/ow>

CPLEX(12.10): <https://www.ibm.com/academic/technology/data-science>

- You need to add R and Rtools40 to Environmental Variables PATH, with instruction provided at the end.

1.2 Code download

1.2.1 Via Git (recommended)

1. Install **git** via <https://support.rstudio.com/hc/en-us/articles/200532077?version=1.3.1093&mode=desktop>
2. In Rstudio, go to File → New project → Version control → Git, enter <https://github.com/LiChenPU/NetID.git> for URL, select a subdirectory, and create project.
3. You should be able to see all files in place under your selected subdirectory. Use pull option to check for latest updates.

1.2.2 Via Github

1. Go to website <https://github.com/LiChenPU/NetID>, hit the green **code** button, select download zip, and unzip files.

1.3 Package dependency installation

Most of the dependent packages can be installed by running the R script `NetID_packages.R` in the `get started` folder. See **Troubleshooting** section for possible errors.

The package, **cplexAPI**, connecting R to CPLEX, requires additional installation steps.

1. Go to website: <https://cran.r-project.org/web/packages/cplexAPI/index.html>, look for **Package source**, and download `cplexAPI_1.4.0.tar.gz`. In the same page, look for **Materials**, and open the **INSTALL** link
2. Unzip the folder `cplexAPI` to the **desktop**, follow the installation guide to modify the file `Makevars.win`.
Note: Replace `\` in the `Makevars.win` file into `/` in order for R to recognize the path.

- For example, the `-I"${CplexStudioDir}\cplex\include"` should be replaced with path such as:
`-I"C:/Program Files/IBM/ILOG/CPLEX_Studio1210/cplex/include"`
 - The `-L"${CplexStudioLib}"` should be replaced with path such as:
`-L"C:/Program Files/IBM/ILOG/CPLEX_Studio1210/cplex/bin/x64_win64"`
3. In command line, run line below to build package, change `${Username}` to actual name.
`R CMD build --no-build-vignettes --no-manual --md5 "C:\Users\${Username}\Desktop\cplexAPI"`
 a new package `cplexAPI_1.4.0.tar.gz` will be built under the default path (for example, `C:\Users\${Username}`)
 4. In command line, run line below to install package.
`R CMD INSTALL --build --no-multiarch .\cplexAPI_1.4.0.tar.gz`
 If you see `DONE (cplexAPI)`, then the package installation is successful.
 - *Note:* if error occurs relating to `__declspec(dllimport deprecated)`, you need to go to `C:\Program Files\IBM\ILOG\CPLEX_Studio1210\cplex\include\ilcplex` (or other installation path), open the file `cpconst.h`, go to the line indicated in the error message or search for `__declspec(dllimport deprecated)`, add `_` to `__declspec(dllimport deprecated)`, making it to `__declspec(dllimport_deprecated)`. Save file and repeat *step 4*.
 5. To take a short venture using CPLEX in R, refer to **Package cplexAPI – Quick Start** in <https://cran.r-project.org/web/packages/cplexAPI/index.html>.

2 Using NetID

This section will use yeast negative-mode dataset and mouse liver negative-mode dataset as examples to walk through the NetID workflow.

- *Note 1:* If other *El-MAVEN* version was used, check the “`raw_data.csv`” for the column number where the first sample is located, and specify that in the `NetID_run_script.R` file. For example, In *El-MAVEN* (version 7.0), `first_sample_col_num` is set at 15 as default. If *El-MAVEN* (version 12.0) is used, `first_sample_col_num` should be set at 16.
- *Note 2:* for more advanced uses, scoring and other parameters can be edited in `NetID_function.R` and `NetID_run_script.R`. Read the manuscript method section for detailed explanation on parameters.

2.1 Yeast negative-mode dataset

In the `Sc_neg` folder, file `raw_data.csv` is the output from **Elmaven** recording MS information, and is the input file for **NetID**. MS2 is not collected for this dataset.

2.1.1 Running the code

1. Open `code` folder → `NetID_run_script.R`
2. In the `# Setting path ####` section, set `work_dir` as `"../Sc_neg/"`.

```
# Setting path ####
{
```

```
setwd(dirname(rstudioapi::getSourceEditorContext()$path))
source("NetID_function.R")

work_dir = "../Sc_neg/"
setwd(work_dir)
printtime = Sys.time()
timestamp = paste(unlist(regmatches(printtime, gregexpr("[[:digit:]]+", printtime))), collapse = '')
}
```

3. In the `# Read data and files ####` section, set filename as `"raw_data.csv"`, set `MS2_folder` as `" "`.
set `ion_mode` as `-1` if negative ionization data is loaded, and `1` if positive ionization data loaded.

```
# Read data and files ####
{
  Mset = list()
  # Read in files
  Mset = read_files(filename = "raw_data.csv",
                    LC_method = "Hilic_25min_QE",
                    # "Hilic_Rutgers_QEPlus" "Hilic_25min_QE", lipids is empty
                    ion_mode = -1 # 1 for pos mode and -1 for neg mode
                    )
  Mset = read_MS2data(Mset,
                     MS2_folder = " ") # MS2
}
```

4. Keep all other parameters as default, and run all lines.

2.1.2 Expected outputs

1. In the console, error message should not occur. If optimization step is successful, you will see messages in the following format.

```
"Optimization ended successfull - integer optimal, tolerance - OBJ_value =
2963.71 (bestobjective - bestinteger) / (1e-10 + |bestinteger|) = 0.000048268"
95.74 sec elapsed
```

2. Three files will be generated in the `Sc_neg` folder. Expected run time on a "normal" desktop computer should be within an hour.
 - `NetID_output.csv` contains the annotation information for each peak.
 - `NetID_output.RData` contains node, edge and network information. The file will be used for network visualization in Shiny R app.
 - `.RData` records the environmental information after running codes. The file is mainly used for development and debugging.

2.2 Your own dataset

2.2.1 MS1 dataset preparation

1. File conversion. Use software ProteoWizard40 (version 3.0.11392) to convert LC-MS raw data files (.raw) into mzXML format. A command line script specifies the conversion parameter. Assuming the raw data are in D:/MS data/test. Type in the scripts below.

```
D:
cd D:/MS data/test
"C:\Program Files\ProteoWizard\ProteoWizard 3.0.11392\msconvert.exe"
*.raw --filter "peakPicking true 1-" --simAsSpectra --srmAsSpectra --mzXML
```

If **ProteoWizard** is installed in location other than C:\Program Files\ProteoWizard\ProteoWizard 3.0.11392\msconvert.exe, specify your path to where you can find the msconvert.exe file.

Expected outputs will be .mzXML files from .raw data.

2. **EI-MAVEN (version 7.0)** is used to generate a peak table containing m/z, retention time, intensity for peaks. Detailed guides for peak picking can be found in <https://elucidatainc.github.io/EIMaven/faq/>. After peak picking and a peak table tab has shown up, click export to CSV. Choose export all groups. In the pop-up saved window, choose format Groups Summary Matrix Format Comma Delimited. Save to the desired path.
3. Under the NetID folder, create a new folder NetID_test, copy the csv file from step 2 into the folder, and change the filename into raw_data.csv.

2.2.2 MS2 dataset preparation

NetID currently utilizes targeted MS2 data for better MS2 quality, and will incorporate data-dependent MS2 data in the future.

1. Prepare MS2 inclusion list
For targeted MS2 analysis, from the peak list generated in step 1, select the peaks (m/z, RT) that you want to perform MS2, and arrange them into multiple csv files that will serve as the inclusion lists to set up the PRM method on Thermo QExactive instrument. Instruction can be found in https://proteomicsresource.washington.edu/docs/protocols05/PRM_QExactive.pdf.
Note: Arrange the parent ions so as to avoid to perform many PRMs at same time. An example is shown below with the start and End time set as RT-1.5 and RT+1.5 (min) to have good chromatogram coverage.

```
library(readr)
read_csv("example.csv")
##
## -- Column specification -----
## cols(
##   Mass = col_double(),
##   Formula = col_logical(),
##   Formula_type = col_logical(),
##   Species = col_logical(),
##   CS = col_logical(),
##   Polarity = col_character(),
```

```
## Start = col_double(),
## End = col_double(),
## CE = col_double(),
## CE_type = col_character(),
## MSXID = col_logical(),
## Comment = col_character()
## )
## # A tibble: 16 x 12
##   Mass Formula Formula_type Species CS Polarity Start End CE CE_type
##   <dbl> <lgl> <lgl> <lgl> <lgl> <chr> <dbl> <dbl> <dbl> <chr>
## 1 499. NA NA NA NA Negative 0.456 3.46 30 NCE
## 2 722. NA NA NA NA Negative 0.733 3.73 30 NCE
## 3 403. NA NA NA NA Negative 1.06 4.06 30 NCE
## 4 211. NA NA NA NA Negative 1.20 4.20 30 NCE
## 5 328. NA NA NA NA Negative 1.40 4.40 30 NCE
## 6 149. NA NA NA NA Negative 1.59 4.59 30 NCE
## 7 151. NA NA NA NA Negative 2.69 5.69 30 NCE
## 8 335. NA NA NA NA Negative 2.70 5.70 30 NCE
## 9 143. NA NA NA NA Negative 4.07 7.07 30 NCE
## 10 89.0 NA NA NA NA Negative 5.67 8.67 30 NCE
## 11 283. NA NA NA NA Negative 6.92 9.92 30 NCE
## 12 202. NA NA NA NA Negative 8.79 11.8 30 NCE
## 13 160. NA NA NA NA Negative 10.3 13.3 30 NCE
## 14 216. NA NA NA NA Negative 11.4 14.4 30 NCE
## 15 125. NA NA NA NA Negative 12.0 15.0 30 NCE
## 16 230. NA NA NA NA Negative 12.9 15.9 30 NCE
## # ... with 2 more variables: MSXID <lgl>, Comment <chr>
```

2. Instrument setup

Set up the QExactive instrument so that it contains both “Full MS” and “PRM” scan events. For PRM setup, use the above file as inclusion list to perform targeted MS2 analysis. We typically use the following setting for MS2 analysis: resolution 17500, AGC target 1e6, Maximum IT 500 ms, isolation window 1.5 m/z. For a total of 1500 parent ions and 15 parent ions for each method, it requires a total of 100 runs, or ~42 hours using a 25-min LC method.

3. MS2 file conversion.

RawConverter (version 1.2.0.1, <http://fields.scripps.edu/rawconv/>) is used to convert the .raw file into .mzXML file that contains MS2 information. Keep the default parameters except setting **Environment Type** as **Data Independent**, and **Output Formats** as **mzXML**.

4. MS2 reading and cleaning.

A matlab code is used for MS2 reading and cleaning, which can be found in **CodeOcean** as a published capsule (<https://codeocean.com/capsule/1048398/tree/v1>). The csv files from 1 paired with the MS2 data files in mzXML format from 3 are the required input data. Refer to capsule description and **readme.md** file for more details of how the code works. In Brief,

- Prepare filename. Filenames for both csv and mzXML files should be named as **prefixNNN**, where prefix is the given file name and NNN is the 3 digits number in continuous order (e.g. M001.csv, M002.csv,... and M001.mzXML, M002.mzXML,... in the /data folder).

- Duplicate the capsule to your own account so you can edit and use the capsule. Upload your own files and remove the previous files in `/data` folder.
 - Specify the prefix and the range of numbers at the beginning section of the main code `Main_example.m`.
 - Set the main code as file to run in Code Ocean using the dropdown menu next to main code.
 - Click `reproducible run` to perform the batch processing.
 - The resulting output files in `.xlsx` format with the same filenames will appear in the timeline. Each `xlsx` file contains multiple tabs of cleaned MS2 spectra. The names of the tabs correspond to the row numbers of the `csv` file specifying the individual parent peak information.
5. Save files to folders.
Back to the `NetID_test` folder, create a new folder `MS2`, download all `xlsx` files from 4 into the folder.

2.2.3 Running the code

1. Open code folder → `NetID_run_script.R`.
2. In the `# Setting path ####` section, set `work_dir` as `"../NetID_test/"`.
3. In the `# Read data and files ####` section, set `filename` as `raw_data.csv`, set `MS2_folder` as `MS2`.
set `LC_method` to specify column to read for the retention time of known standards. (In folder `NetID` → `dependent` → `known_library.csv`, update the retention time info as needed.)
set `ion_mode` as `-1` if negative ionization data is loaded, and `1` if positive ionization data loaded.
4. Keep all other parameters as default, and run all lines.

2.2.4 Expected outputs

Similar to the `demo` file, the console will print out message indicating optimization step is successful, and three files `NetID_output.csv`, `NetID_output.RData` and `.RData` will be generated in the `NetID_test` folder

2.3 Advanced Setting

2.3.1 Using other libraries

2.3.1.1 Using provided libraries NetID provides 4 libraries for the user to choose: **HMDB**, **YMDB**, **PubChem**, **PubChem Bio-pathway only**.

To select the desired database, change `HMDB_library_file` = `"../dependent/hmdb_library.csv"` to `../dependent/ymdb_library.csv`, `../dependent/pbcm_library.csv` or `../dependent/pbcm_library-bio.csv`.

```
Mset = read_files(filename = "raw_data.csv",
                  LC_method = "Hilic_25min_QE", # "Hilic_Rutgers_QEPlus" "Hilic_25min_QE", lipids is empty
                  ion_mode = -1, # 1 for pos mode and -1 for neg mode
                  HMDB_library_file = "../dependent/hmdb_library.csv"
                  )
```

2.3.1.2 Design your own library

1. A workable library requires following columns.

```
read_csv("../dependent/hmdb_library.csv")
##
## -- Column specification -----
## cols(
##   accession = col_character(),
##   iupac_name = col_character(),
##   name = col_character(),
##   SMILES = col_character(),
##   status = col_character(),
##   formula = col_character(),
##   mass = col_double(),
##   rdbe = col_double(),
##   category = col_character()
## )
## # A tibble: 114,014 x 9
##   accession iupac_name      name SMILES      status formula  mass  rdbe category
##   <chr>      <chr>        <chr> <chr>      <chr> <chr>  <dbl> <dbl> <chr>
## 1 HMDB000000~ (2S)-2-amino~ 1-Me~ CN1C=NC(~ quant~ C7H11N~ 169.    4 Metabol~
## 2 HMDB000000~ propane-1,3-d~ 1,3~ NCCCN      quant~ C3H10N2 74.1    0 Metabol~
## 3 HMDB000000~ 2-oxobutanoic~ 2-Ke~ CCC(=O)C~ quant~ C4H6O3 102.    2 Metabol~
## 4 HMDB000000~ 2-hydroxybuta~ 2-Hy~ CCC(O)C(~ quant~ C4H8O3 104.    1 Metabol~
## 5 HMDB000000~ (1S,10R,11S,1~ 2-Me~ [H][C@@]~ quant~ C19H24~ 300.    8 Metabol~
## 6 HMDB000000~ (3R)-3-hydrox~ (R)~ C[C@@H]~ quant~ C4H8O3 104.    1 Metabol~
## 7 HMDB000000~ 1-[(2R,4S,5R)~ Deox~ OC[C@H]1~ quant~ C9H12N~ 228.    5 Metabol~
## 8 HMDB000000~ 4-amino-1-[(2~ Deox~ NC1=NC(~ quant~ C9H13N~ 227.    5 Metabol~
## 9 HMDB000000~ (1S,2R,10R,11~ Cort~ [H][C@@]~ quant~ C21H30~ 346.    7 Metabol~
## 10 HMDB000000~ (1S,2R,10S,11~ Deox~ [H][C@@]~ quant~ C21H30~ 330.    7 Metabol~
## # ... with 114,004 more rows
```

2. To build your own library, make a csv file in the same format as the one shown above, and set HMDB_library_file = "../dependent/hmdb_library.csv" to your desired directory in NetID_run_script.R.

2.3.2 Modifying emperical_rules.csv

emperical_rules.csv can also be created or modified to support specific biotransformation. A workable emperical_rules requires following columns. * name and note is not necessary. * category includes: Biotransform, Natural_abundance, Adduct, Fragment and Radical * rdbe is calculated using the formula_rdbe function of the package lc8 * direction states the possible direction of transformation: 1 means from larger mass to smaller mass; 0 means the opposite; -1 means both direction are possible.


```

read_csv("../dependent/empirical_rules.csv")
##
## -- Column specification -----
## cols(
##   category = col_character(),
##   name = col_character(),
##   formula = col_character(),
##   mass = col_double(),
##   direction = col_double(),
##   rdbe = col_double(),
##   note = col_character()
## )
## # A tibble: 84 x 7
##   category      name formula      mass direction  rdbe note
##   <chr>         <chr> <chr>      <dbl>      <dbl> <dbl> <chr>
## 1 Biotransform O-HN  O1N-1H-1  0.984        0      0 Deamination
## 2 Biotransform NH3-O N1H3O-1    1.03        0     -1 Transamination
## 3 Biotransform H2    H2         2.02        0     -1 Hydrogenation
## 4 Biotransform CH2   C1H2       14.0        0      0 Methylation
## 5 Biotransform NH    N1H1       15.0        0      0 Amination
## 6 Biotransform O     O1         16.0        0      0 Hydroxylation
## 7 Biotransform N1H3  N1H3       17.0        0     -1 Amination (+NH3)
## 8 Biotransform H2O   H2O1       18.0        0     -1 Hydration
## 9 Biotransform C0    C1O1       28.0        0      1 Formylation (+C0)
## 10 Biotransform C2H4 C2H4       28.0        0      0 Beta oxidation
## # ... with 74 more rows

```

2.3.3 Score Setting

To be updated.

3 NetID Visualization

This section provides instruction to visualize and explore **NetID** output results in either **Cytoscape** software or interactive **Shiny R app**. After running **NetID** algorithm, it will export one **.R** and two **.csv** files (**cyto_node.csv** and **cyto_edges.csv**), storing the nodes and edges of the output network.

3.1 Cytoscape

1. install **Cytoscape** Download **Cytoscape** (<https://cytoscape.org/download.html>) and follow installation instruction to install onto your computer.
2. Load the example **NetID** output into **Cytoscape**
 - Run **Cytoscape**, click **import network from file system**, and load **cyto_edges.csv**, set **edge_id** column as the key, set **node1** as source node, set **node2** column as target node, and the rest columns as edge attribute.
 - Click **import table from file**, load **cyto_node.csv**, set **node_id** column as the key, and the rest columns as node attribute.

- Select `subnetwork`, set `styles`, and explore the network with various functionalities inside **Cytoscape**.
- 3. Explore in Cytoscape
- 4. Export

The network as well as the curated subnetworks can be exported for future analysis or sharing with others. An example network file `example.cys` is included along with the two `.csv` files, which is created using **Cytoscape** version 3.8.2

3.2 Shiny App

This part provides instruction to visualize and explore **NetID** output results in the interactive **Shiny R app**. A 21-inch or larger screen is recommended for best visualization.

3.2.1 Runing Shiny App

1. Open `code` folder → `R_shiny_App.R`.
2. In the `# Read in files ####` section, set `datapath` as `../Sc_neg/`
3. Keep all other parameters as default, and run all lines.
4. A Shiny app will pop up.

3.2.2 Searching peaks of interest

1. On the left panel, you can enter a `m/z` or a formula to search your peak of interest. For example, 180.0631 or C6H12O6 will automatically update the data table on the right. Enter 0 to restore full list for the data table.
2. Change ionization and ppm window to adjust calculated `m/z`.
3. On the right, you can explore the peak list in an interactive data table, including global text search on top right, specifying ranges for numeric column or searching text within character columns, ranking each column etc.

The screenshot shows the Shiny App interface with three main sections labeled a, b, and c. Section a is a text input field labeled 'Enter a m/z or formula of interest' containing the value 'C6H12O6'. Section b is a dropdown menu labeled 'Select ionization' with 'M' selected, and a text input field labeled 'ppm' with the value '3'. Section c is a data table with columns: peak_id, medMz, medRt, log10_inten, class, formula, and ppm_error. The table shows one entry with peak_id 5587, medMz 180.0631, medRt 13.61, log10_inten 5.3, class Metabolite, formula C6H12O6, and ppm_error 1.6. Above the table is a search bar and a 'Show 5 entries' dropdown. Below the table are filters for each column, all set to 'All', and a 'Showing 1 to 1 of 1 entries' message with 'Previous' and 'Next' buttons.

peak_id	medMz	medRt	log10_inten	class	formula	ppm_error
5587	180.0631	13.61	5.3	Metabolite	C6H12O6	1.6

3.2.3 Network Visualization

1. Peak ID, formula and class determines the center node for the network graph. Peak ID will be automatically updated by the first line in the data table if a `m/z` or formula is given. Alternatively, you can manually enter Peak ID.
2. The degree parameter controls how far the network expands from the center node. Degree 1 means only nodes directly connected to the center node will be shown and degree 2 means nodes connected to degree 1 will be shown, etc.

3. Biochemical graph shows biochemical connections. Abiotic graph shows abiotic connections. Node labels and Edge labels determines if the graph show node or edge labels. Optimized only determines whether to show only the optimal annotations or all possible annotations in the network.
4. When setting parameters, hit plot to see the network graph.

a

Peak ID

5587

Formula

C6H12O6

Class

Metabolite

b

Degree

1

d

Plot

c

☒ Biochemical graph

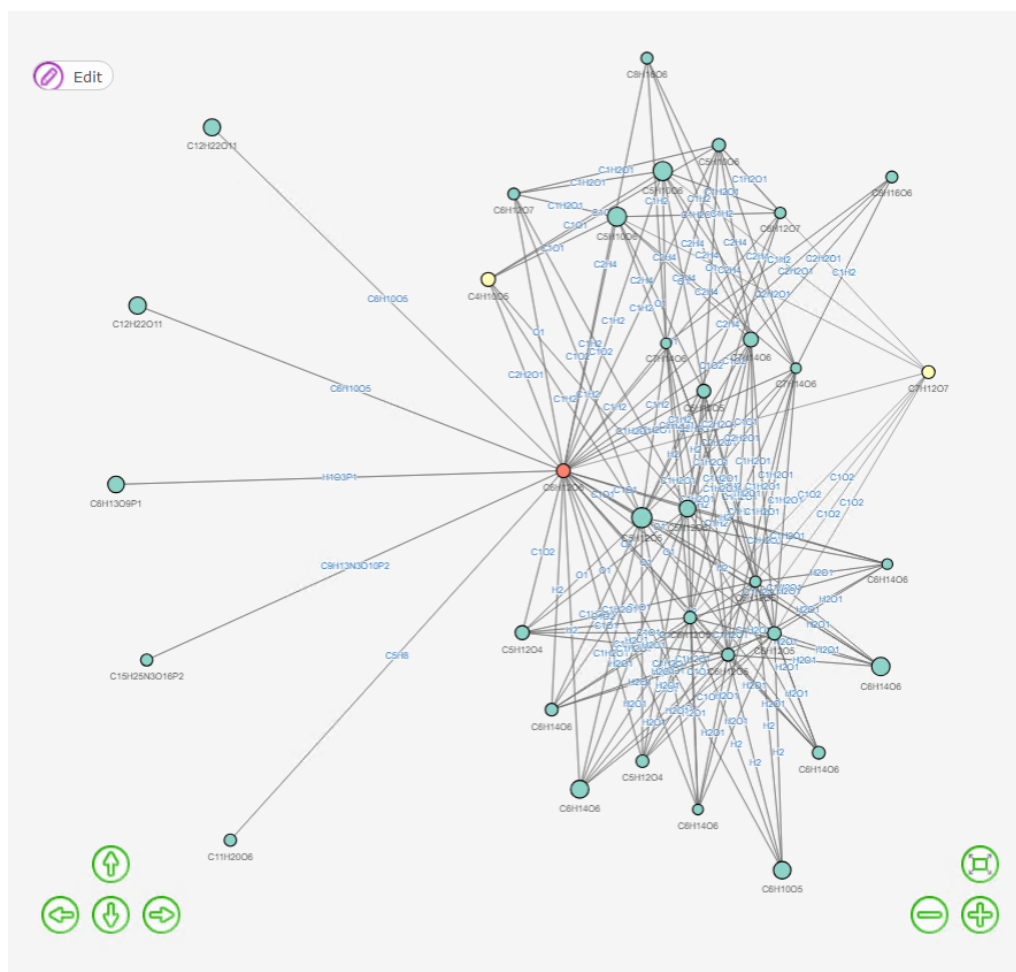
☐ Abiotic graph

☒ Node labels

☒ Edge labels

☒ Optimized only

5. A sample network graph is shown below (a different center node may give less complicated graph). You may edit the nodes or edges (top left), move figures with the arrow buttons (bottom left), and zoom in/out or center figure (bottom right).



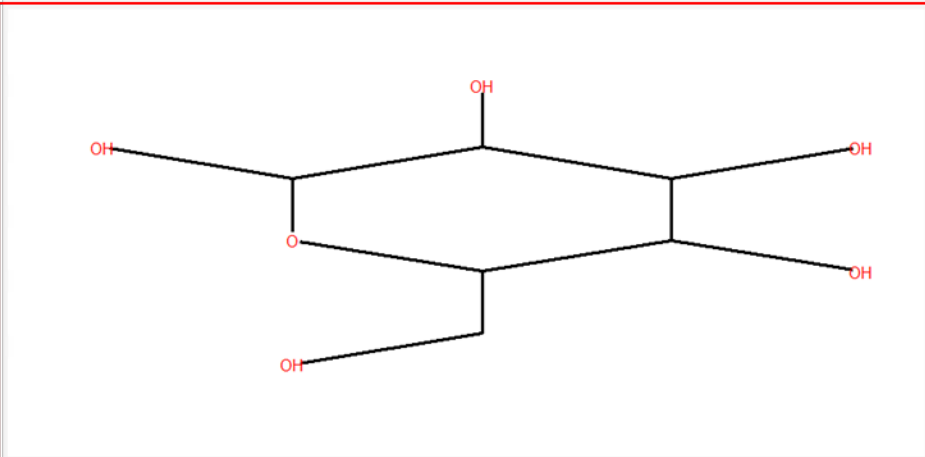
- You can use the “Download plot” button to download a html webpage to visualize the network graph independent of the Shiny app, and the “Download csv” button to download the information of the nodes in the network. The download buttons will appear after hitting the plot button. Note: edits within the Shiny app will not go into the html file.

3.2.4 Possible structures exploration

A figure + data table is provided to explore structures of the selected node in the network graph.

- The figure shows the chemical structure of the annotated metabolites. If the node is annotated as a putative metabolite, only the known parts of the putative metabolite will be shown.
Scroll left or right, or select the entry number, to visualize different annotations. Right click and select to save image.
- In the data table, class has 3 possible entries: Metabolite if it is documented in database such as HMDB library; Putative metabolite if it is transformed from a metabolite through a biotransformation edge; and Artifact if it is transformed by an abiotic edge. Use the download button to download the data table

a



D-Glucose C₆H₁₂O₆

<- 1 -> Download csv

b

Show 10 entries Search:

	class	annotation	origin	note
1	Metabolite	D-Glucose C ₆ H ₁₂ O ₆	HMDB_library	HMDB0000122
2	Metabolite	D-Galactose C ₆ H ₁₂ O ₆	HMDB_library	HMDB0000143
3	Metabolite	D-Mannose C ₆ H ₁₂ O ₆	HMDB_library	HMDB0000169
4	Metabolite	myo-Inositol C ₆ H ₁₂ O ₆	HMDB_library	HMDB0000211
5	Metabolite	3-Deoxyarabinohexonic acid C ₆ H ₁₂ O ₆	HMDB_library	HMDB0000346

4 Troubleshooting

4.1 Failing to install package `lc8`

Reinstall the packages `devtools` and `digest`.

4.2 Cannot find `cpLexAPI` even if the installation seems successful

Check **R** version used in **RStudio** to see if `cpLexAPI` is installed under the same R version library. Which R library `cpLexAPI` goes to depends on the R path specified in `Environment Variables`.

4.3 Add **R** to `PATH`

1. Go to `Environment Variables`:
search `PATH` in windows → open edit `Environment Variables` → `Environment Variables` or
`control panel` → `system and security` → `System` → `Advanced system Settings` (on your left) → `Advanced` → `Environment Variables`
2. In the lower Panel select the `Path Variable` and select `Edit`, add the R path (`C:\Program Files\R\R-4.0.3\bin\x64`, if installed at default location) to the `Path Variable`.
3. You may need to restart computer for the R path to take effect.

4.4 Add **Rtools40** to `PATH`

1. Add the path `C:\Rtools\bin` to the `Path Variable` in `Environment Variables`
2. Run the line in **R**:

```
writeLines('PATH="{RT00LS40_HOME}\\usr\\bin;{PATH}"', con = "~/Renvi  
ron")
```


Use the line below in R console to check for successfully adding Rtools40

```
Sys.which("make")
```


Expected output: `## "C:\\rtools40\\usr\\bin\\make.exe"`