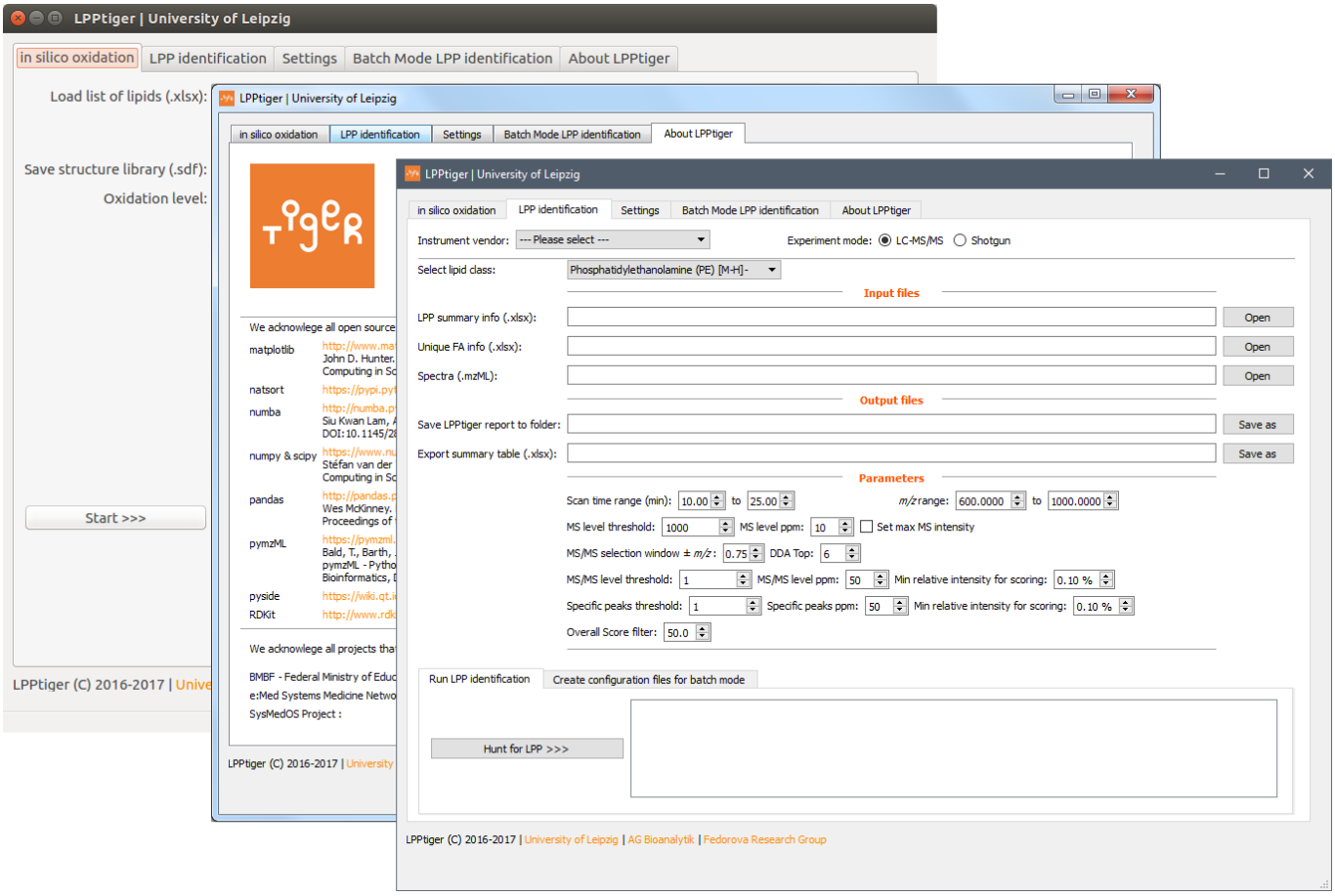
User Guide To LPPtiger



For LPPtiger Beta version 9th November 2017

User guide version 10th November 2017

UNIVERSITÄT LEIPZIG

Table of Contents

1. Introduction

LPPtiger is an open-source program aimed to predict and identify lipid peroxidation products (LPPs) from LC-MS and shotgun MS based lipidomics data.

Main features of LPPtiger are:

- Prediction of specific oxLipidome from input initial lipidome by *in silico* oxidation
- Five-criteria scoring algorithms
- Unique interactive HTML output with integrated annotated six-panel spectra plot
- Highly customizable for different modifications and instruments
- User-friendly graphic interface
- Native support for parallel processing
- Cross platform
- Free and open-source
- Rapid and transparent source code development managed by online public repository

LPPtiger can gradually reduce the processing time of LPP identification. The interactive HTML report provides an organized view of assigned spectra, which enhance the experience of high-throughput identification of LPPs.

2. License

LPPtiger is Dual-licensed

- For academic and non-commercial use: GPLv2 License
  https://www.gnu.org/licenses/old-licenses/gpl-2.0.en.html

- For commercial use:
  Please contact the SysMedOS team by email.
  https://home.uni-leipzig.de/fedorova/

- **Please cite our publication in an appropriate form.**

  LPPtiger software for lipidome-specific prediction and identification of oxidized phospholipids from LC-MS datasets

  Zhixu Ni, Georgia Angelidou, Ralf Hoffmann & Maria Fedorova

  Scientific Reports 7, Article number: 15138 (2017)

  doi:10.1038/s41598-017-15363-z

  https://www.nature.com/articles/s41598-017-15363-z

3. Download and install LPPtiger

There are two versions of LPPtiger. The Windows executable version and the source code version. General information and installation instructions can be found here:

https://bitbucket.org/SysMedOs/lpptiger

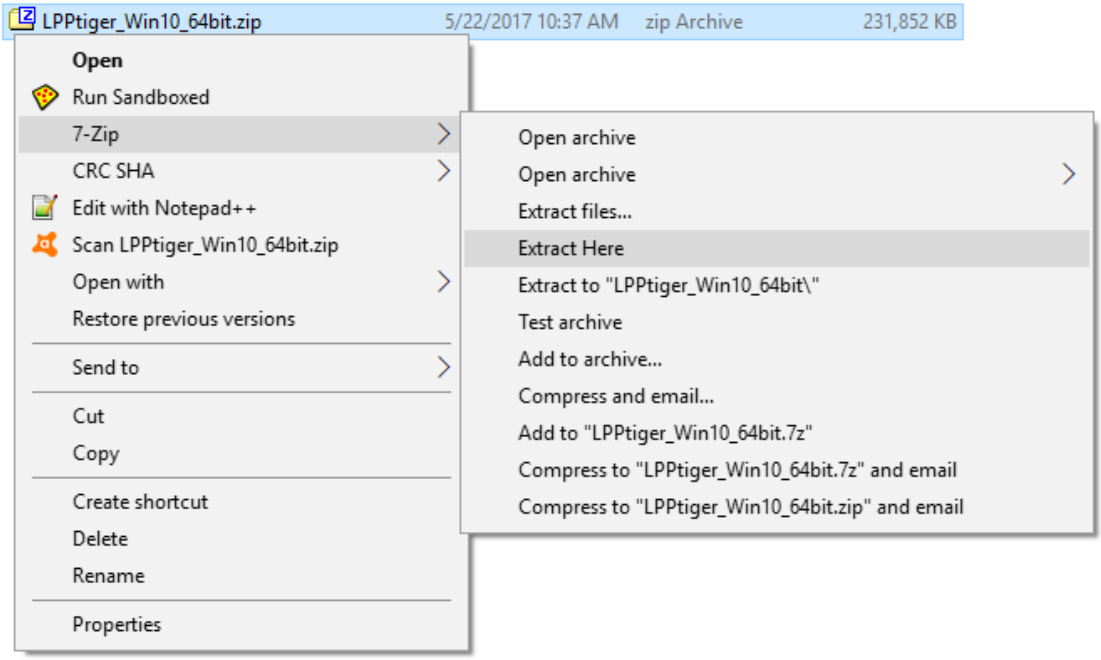
- Windows executable version
  - Executable versions are provided for Windows 7, 8 and 10 64bit system only.
  - Please read the instructions of LPPtiger Windows version: https://bitbucket.org/SysMedOs/lpptiger_exe
  - Download link: https://bitbucket.org/SysMedOs/lpptiger_exe/downloads/
- Source code version
  - For developers or other platform users (Linux, Windows server editions), LPPtiger source code is available.
  - Please read the instructions of LPPtiger source code version: https://bitbucket.org/SysMedOs/lpptiger
- Other files provided:
  - Test files to be used with this user guide:
    - The sample spectra in .mzML format
    - Templates and other sample outputs
    - Downloaded link: https://bitbucket.org/SysMedOs/lpptiger_exe/downloads/
  - Updates of LPPtiger user guide
    - Please check the following link for the latest version of LPPtiger user guide https://bitbucket.org/SysMedOs/lpptiger_exe/downloads/

- Other software required to cooperate with LPPtiger:
  - .mzML spectra converter and viewer:
    - ProteoWizzard MSconvert and SeeMS
    - http://proteowizard.sourceforge.net/
  - SDF structure library viewer:

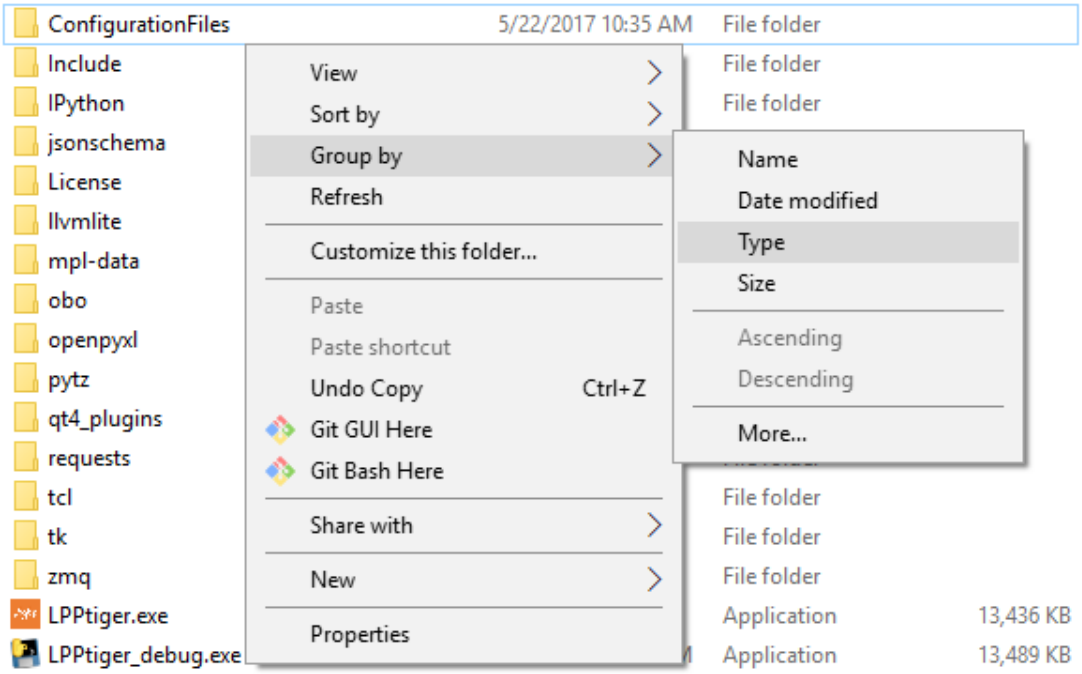
    Please install an appropriate SDF file viewer based on your interests.
    - ChemAxon Instant JChem (cross platform, academic license available) https://www.chemaxon.com/products/instant-jchem-suite/instant-jchem/
    - Progenesis SDF Studio (Windows only, free to use) http://www.nonlinear.com/progenesis/sdf-studio/

The installation of Windows version is provided here:

We recommend using the 7-zip program to unpack the LPPtiger zip package (7-Zip is a free and open source software to zip and unzip files. 7-Zip can be downloaded from http://www.7-zip.org).

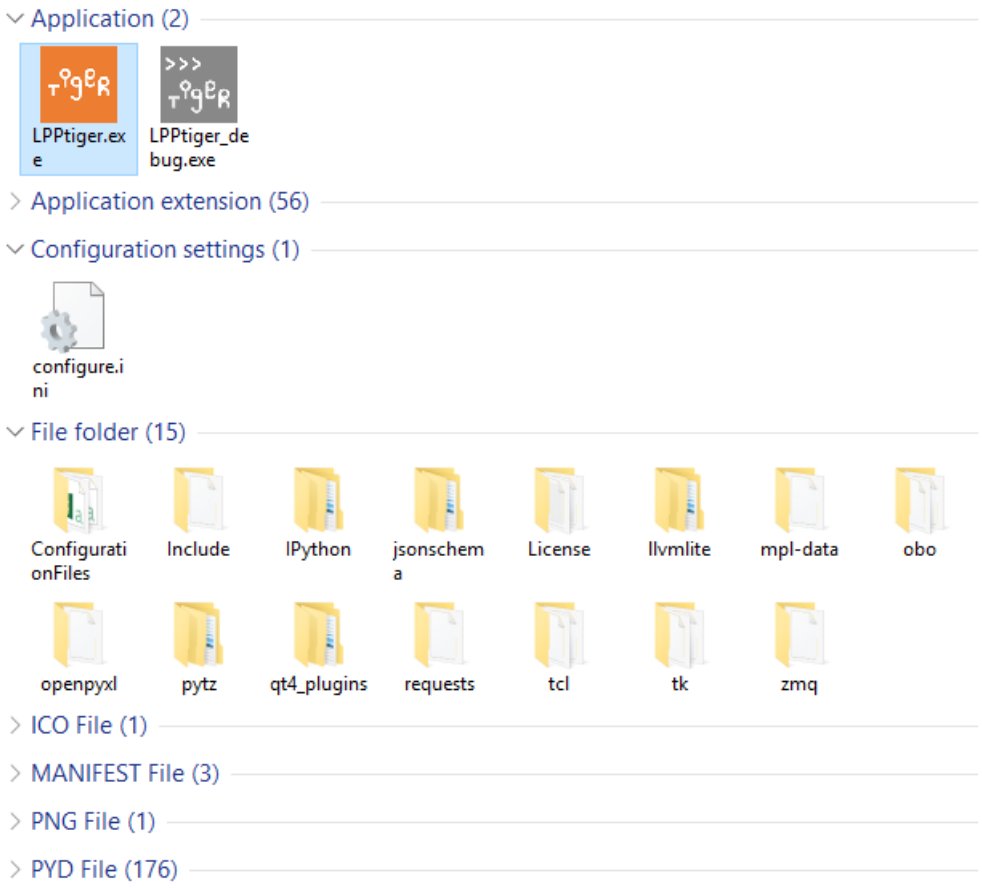


After the extraction, we recommend sorting the content of the folder using the “Group by” function.



There are two excitable files named “LPPtiger.exe” and “LPPtiger_debug.exe” which corresponding to the original LPPtiger program and a debug mode of LPPtiger respectively.

The debug mode will show the background information in a separated command line window that connected to the LPPtiger interface. If you meet any problem during the run, please launch the “LPPtiger_debug.exe”, apply the same settings, and make screenshots of the command line contents. Please do not be hesitated to report an issue to LPPtiger issue tracker: https://bitbucket.org/SysMedOs/lpptiger/issues



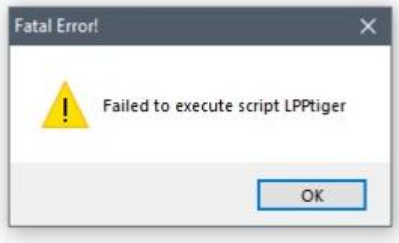
The default settings were saved in “configure.ini” file.

The default configuration files were provided in the “ConfigurationFiles” folder.

The Licenses of LPPtiger dependencies were saved in the “License” folder.

!!! General Notes

- LPPtiger might be blocked by anti-virus software, e.g., Avast, Kaspersky, etc., If you see some warning like blow, please try to start LPPtiger.exe again. It LPPtiger still got blocked, please give LPPtiger enough permission or add LPPtiger to the white list and try to launch LPPtiger again.



- We recommend creating a shortcut of “LPPtiger.exe” to your desktop.

How to uninstall LPPtiger?

LPPtiger is a green software, which means you can simply delete the LPPtiger program folder (and shortcuts if there is any) to uninstall it.

4. Data conversion to .mzML

LPPtiger is designed to work with .mzML files obtained from LC-MS/MS and shotgun data-dependent acquisition experiments. Original data should be converted to .mzML files using ProteoWizard MSconvert tool.

- Go to http://proteowizard.sourceforge.net/downloads.shtml
- Download the version suitable for your system
- Install ProteoWizard
- Open MSConvert from ProteoWizard folder
- For **SCIEX** .wiff files please try ProteoWizard Version 3.0.10158 from: http://teamcity.labkey.org:8080/repository/download/bt83/380712:id/pwiz-setup-3.0.10158-x86_64.msi

!!! General Notes

- The conversion of .mzML is the most critical step for LPPtiger workflow. The .mzML file provided in the LPPtiger test file were converted by ProteoWizard version 3.0.9134.
- The ProteoWizard version you download from the ProteoWizard website might be different.
- The .mzML file converted from specific instruments by different ProteoWizard version might be different, thus they might not compatible with LPPtiger. Please find the suitable ProteoWizard version to convert your raw files and keep using the same ProteoWizard version for further analysis if there are not critical updates of ProteoWizard.
- If you failed to convert or the .mzML cannot be processed by LPPtiger, please read our wiki to understand the LPPtiger specific requirement of the .mzML file. Please contact ProteoWizard team to get a proper version to convert your files.
- Please read LPPtiger wiki about essential data sections in the .mzML files at: https://bitbucket.org/SysMedOs/lpptiger/wiki/LPPtiger%20.mzML%20format%20requirements

4.1 Convert raw data to .mzML

To generate a **.mzML file** containing both MS survey scan and MS/MS information:

- Please create a new folder and save .mzML file to the new folder. (Save mzML file to the previous location might overwrite the previous converted files.)
- Input your data in .raw format
- Click “Add”
- Choose binary coding precision **“32 bit”** to minimize the file size
- Other parameters (e.g. ScanTime, mzWindow, Threshold) can be specified

Options	Required parameters
Binary coding precision	32-bit
Write index	True
Use zlib compression	True
TPP compatibility	True
Package in gzip	False

Options	Suggested parameters
Use numpress options	False to all
MS level	Do not apply filter here (Important for Waters .raw files)
Threshold peak filter	Absolute intensity, Most intense, e.g., 10 for Q-ToF; 100 for Orbitrap; 10 for Agilent and SCIEX instruments
Subset → Scan Time	Scan time range in seconds
Subset → *m/z* Window	Do not apply any filter here

4.2 Check converted .mzML file by ProteoWizard SeeMS tool

It is very important to check your converted mzML file before starting to work with LPPtiger. The SeeMS tool in the ProteoWizard program folder provides an excellent way to view .mzML files.

Please check following parameters of converted .mzML files.

- The MS level range.
    - **For Waters files**, the MS-Level 1 corresponding to MS survey scan, MS-Level 2 corresponding to DDA rank 1, MS-Level 3 corresponding to DDA rank 2, thus DDA rank N experiment should have MS-Level N+1 converted.
- The MS2 spectra *m/z* range
    - Please make sure that the MS2 spectra contain *m/z* range of specific fragments and neutral losses for identification
- The spectra intensity
    - Please check the intensity of structure representative peaks. Please adjust the "Absolute intensity" filter accordingly and convert again. Absolute intensity filter above 100000 for MS2 is NOT recommended.
    - Please start your own data with a relatively low threshold to finish the workflow of LPPtiger. You can adjust these conversion settings carefully based on the spectra quality and from preliminary LPPtiger results.

5 LPPtiger workflow overview

LPPtiger workflow is subdivided into five main steps.

I. ***In silico oxidation*:** extracts MS1 Data (m/z values and intensities) from .mzML files of different experiments and merges this data together into one file

II. **Identification of LPPs:** The previously generated *in silico* oxidation information was used for the identification of LPPs from selected spectrum. Results are exported as .xlsx tables and HTML files containing six-panel graphical images allowing to evaluate and verify the identification performance.

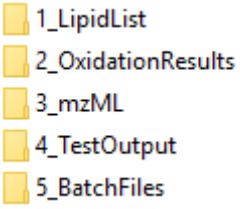
III. **Batch mode multiprocessing:** After the optimization of parameters for LPP identification, corresponding configuration files can be created for all spectra planned for LPP identification. These configuration files can be submitted to the batch mode tab of LPPtiger for multiprocessing of a list of files.

!!! General Notes

- Several .xlsx and .csv tables will be generated and used by LPPtiger. To avoid "comma vs dot" derived errors, please make sure that language on the computer is set to "US English" while working with LPPtiger.
- Note that during computational steps LPPtiger will display *"Not Responding"* message on the window title. The *in silico oxidation* process (level 2 and level 3) and identification steps might take very long time to run, during this time, LPPtiger GUI might freeze and do not respond to user actions, please be patient and wait until processing finishes. LPPtiger source code version users can monitor detailed information during the run. Windows executable version users can run the debug mode to monitor the background processes through the command line window.

In this user guide, we recommend you to use the provided sample data package that available from https://bitbucket.org/SysMedOs/lpptiger_exe/downloads/

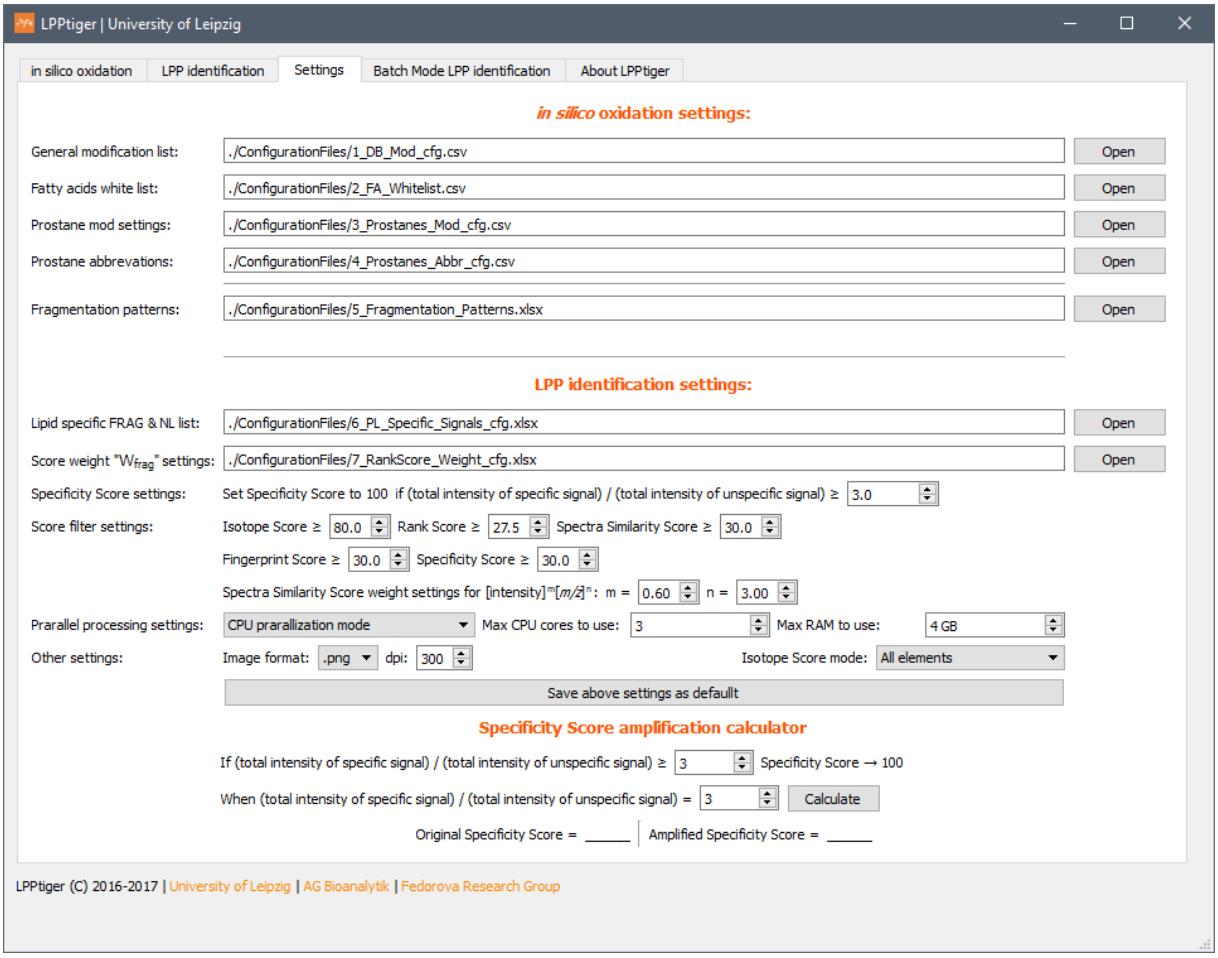
The template file and corresponding output can be found in corresponding subfolders.
We recommend constructing the same structure of sub-folders for your own projects.



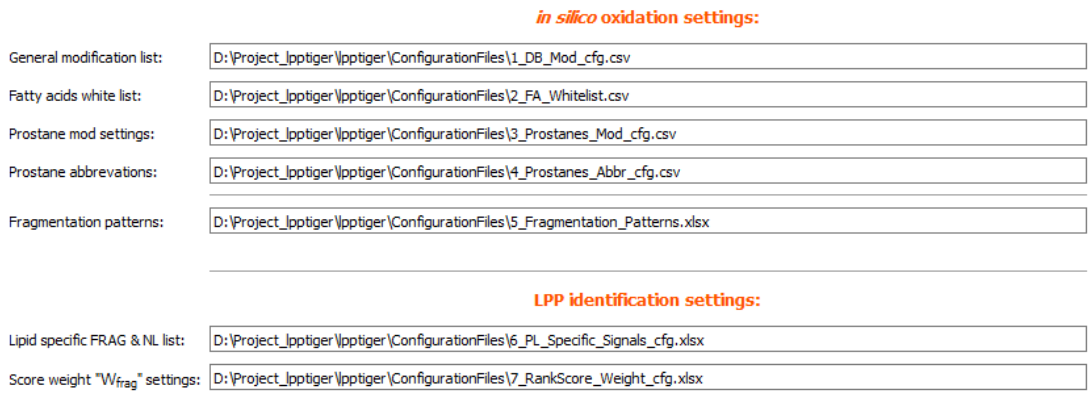
5.1 Before you start

Configuration files containing a fatty acid white list, PL specific product/neutral loss signals, and weight factor tables should be upload to the **Settings** tab. Instructions how to modify/create user specific configuration files are provided in Chapter 7.

The default configuration files are provided in the “ConfigurationFiles” folder under LPPtiger root folder. Please select the files according to the following instructions and press “save above settings as default” button to save the settings.



Please restart LPPtiger, and check the **Settings** tab to see if the configure files are loaded correctly before you start to use LPPtiger. An example is provided below.



Please go to Chapter 7 for more detailed description of each configuration file.

Parameters:

- **Specificity score settings:**
  Set the intensity fold, which defines a filter ratio of specific signal intensity against unspecific signals. LPPs with this ratio equal or more than the threshold will be assigned as 100 specificity score. This ratio is used to calculate the amplification factor of specificity score. The typical value we recommend is 5 (for Orbitrap data) or 3 (for Q-ToF data).
- **Score filter settings:**
  Score filter for individual scores: please read our publication for more information.
  Spectra similarity score weight settings: The parameter for similarity score calculation. Please read our publication for more details.
- **Parallel processing settings:**
  Parallel processing mode: The mode for parallel processing Level 3. LPPtiger is set to use CPU only for vectorized functions in parallel processing Level 3. An experimental GPU mode can be applied to Windows systems with CUDA compatible graphic cards. We recommend using default CPU only mode in the current version.
  Number of CPU cores: maximum number of subprocesses that can be used to process one file. This parameter is usually suggested to be the number of logical processors minus one, e.g., if you have a dual core CPU with hyper-threads, you get four logical processors and this parameter can be set to 3. We recommend using maximum five cores for a single file.
  Max RAM LPPtiger can use: we recommend at least 4 GB RAM and maximum 10 GB for 20 min LC-MS/MS .mzML file. Please reserve at least 2 GB RAM for the Windows systems, e.g., if you have 8 GB of RAM installed, this parameter can be set to 6 GB.
  # Note: For Windows users, the number of CPU cores, number of logical processors, and installed RAM can be read out from the task manager.
- **Other settings:**
  Image Format: available formats are ".png" and ".svg".
  dpi: we recommend to use 300 dpi or lower for preliminary research and 600 dpi for publication quality results.
  Isotope score mode: Set to "all elements" to consider isotopes from all elements, while "fast mode" considers carbon only.

!!! General Notes

- **Spectra similarity score weight settings** and **Parallel processing mode** need to be saved to default configuration file and restart LPPtiger to make changes effective.
- **ALL other parameters can be changed temporarily.** You can change these parameters instantly without saving the parameters to test different combinations of parameters. However, we recommend you to save the optimized parameters to default configuration file.
- **The default configuration file** is saved as "configure.ini" in the LPPtiger folder. We recommend to back up the optimized parameters to prevent unexpected changes.

Please be sure that you have read the information listed in the **About** tab and agree with our license.

We recommend you to check LPPtiger project page regularly for the latest updates.

Source code users and developers can find additional information about the python libraries that used by LPPtiger.

5.2 Step I *in silico* oxidation of selected phospholipids

To start the *in silico* oxidation of phospholipids (PLs), a table of the selected PLs need to be prepared in a template as follows and save as ".xlsx" format.

In this example, we use two PLs, PC(16:0/18:1) and PC(16:0/18:2) and save as "PC_for_oxidation.xlsx"

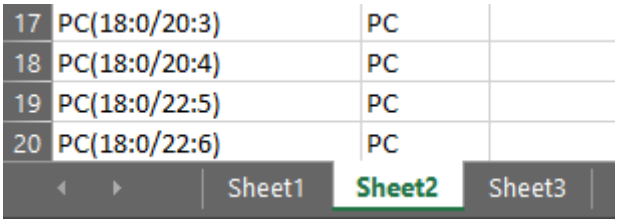
	A	B
1	phospholipids	class
2	PC(16:0/18:1)	PC
3	PC(16:0/18:2)	PC

!!! General Notes

- If you want to specify the Omega series of the FA, you can write as follows. Please check the configuration file to ensure that you have the corresponding FA in the FA list.

	A	B
1	phospholipids	class
2	PC(16:0/18:3)	PC
3	PC(16:0/18:3n-3)	PC
4	PC(16:0/18:2n-6)	PC

- FA without specified Omega series will take the first configuration in the FA list configuration file.
- Please put only one PL class in one table sheet.
    - LPPtiger can load .xlsx files containing multiple sheets, so you can put different classes of PLs into different sheets.
    - Please do not change the default names of the sheets, and keep them as ascending order.
    - Please avoid sheet names with brackets e.g., "Sheet3 (2)". Rename it as "Sheet4".

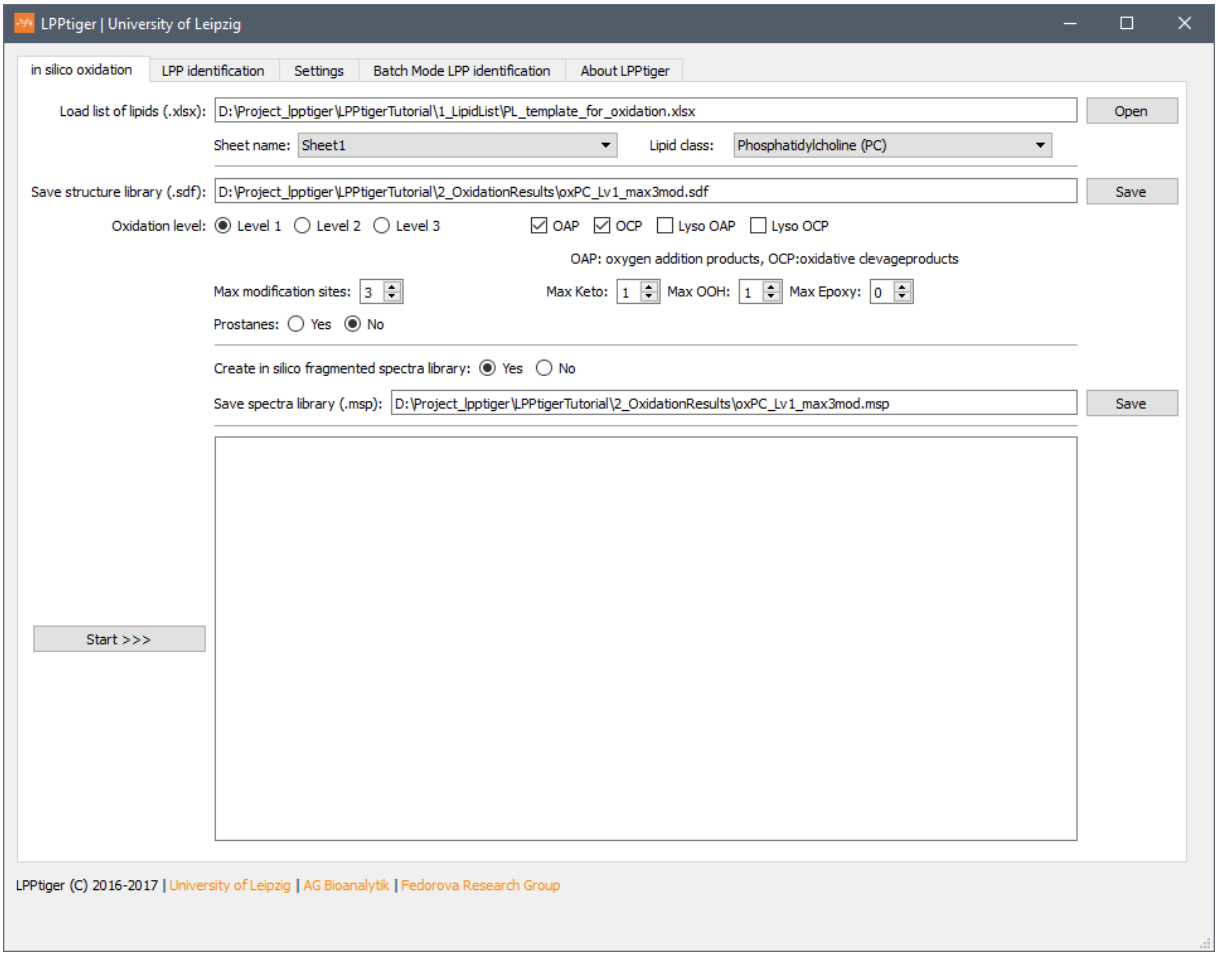


- Please check all FA residues in the file are present in the FA list configuration file.

Select the "TheoLPP" generator tab, click on "Open" button to load the PLs table, e.g., "PL_template_for_oxidation.xlsx".

Once the table is loaded, an "Excel Sheet name" selection list will appear below the path of the input PLs table. Please select the name of the sheet and the PL class accordingly.

To save the .sdf output, click on the save button and choose the folder and file names for the output.



Parameters in this step:

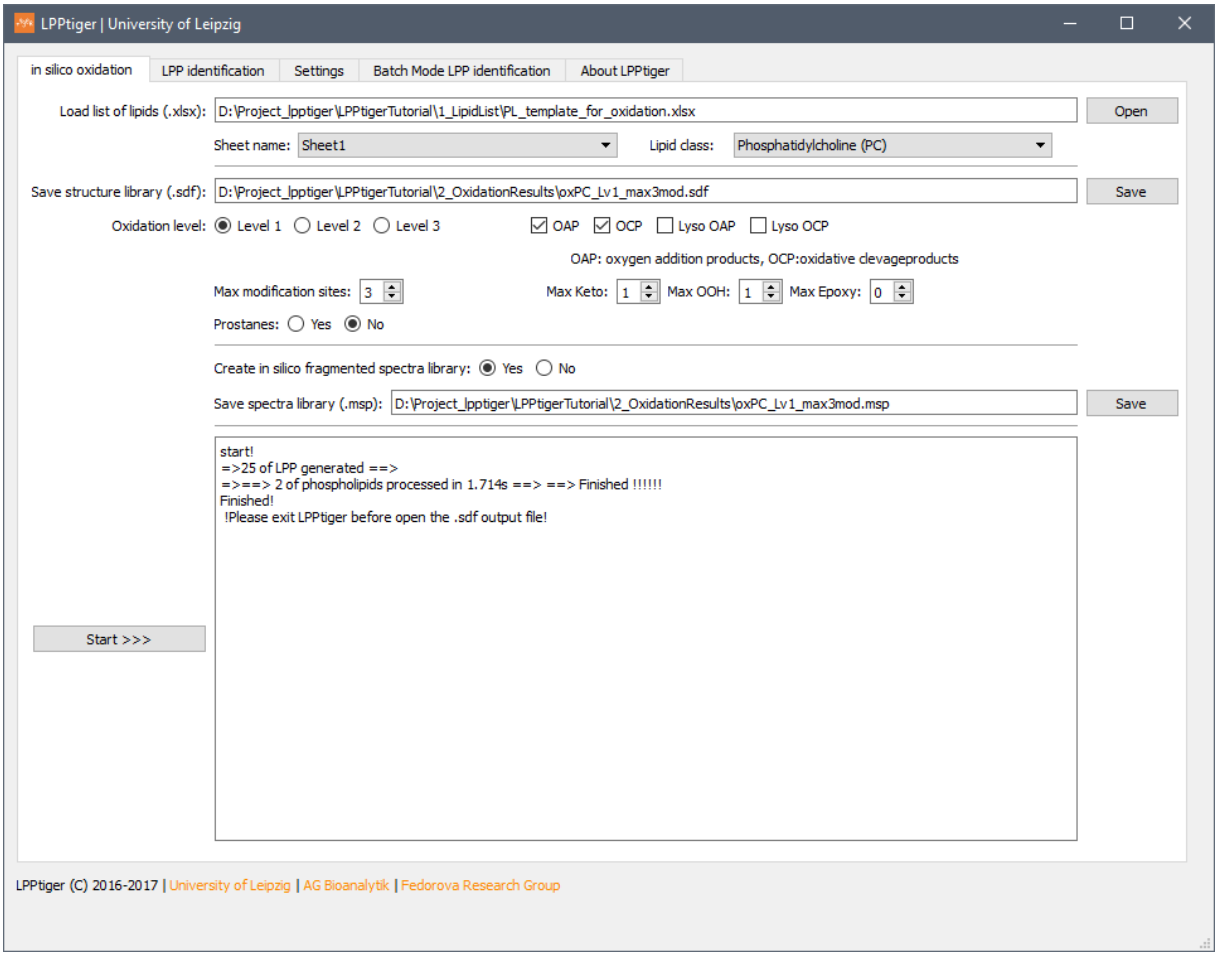
- **Oxidation level:**
  The level of oxidation according to predefined settings. See Chapter 7 for more information.
- **Type of the LPPs:**
  Multiple choices of which types of LPPs can be generated.
- **Max number of modification sites:**
  The maximum number of modification sites.
  The default value is set to 3. If the FA residue has less than 3 double bonds will have the max modification sites according to the number of double bonds, whereas the FA with more than 3 double bonds will have max 3 modifications.
- **Max number of individual modification type:**

  The max number of each modification type. The max number of hydroxyl groups is equal to the maximum number of modification sites.

- **Generation of prostanes and other structures:**

  Enable or disable the function to generate prostanes and other specific structures.

- **Generation of spectra:**
  Enable or disable the function to generate predicted spectra library by *in silico* fragmentation algorithms and patterns.
  The control buttons to save the .msp spectra library is available when “Generation of spectra” option set to “Yes”. The prediction of spectra is only available when proper fragmentation pattern is defined. See Chapter 7 for more information.



!!! General Notes

- LPPtiger merged all positional isomers to reduce complexity, so the number of predicted structures on the GUI may larger than the number og final structures in the output files below.
- Please exit LPPtiger and wait few minutes until the system finish the generation of .sdf file

The program will generate three to four output files, e.g.,

- oxPC_Lv1_ max3mod.sdf
- oxPC_Lv1_max3mod.xlsx
- oxPC_Lv1_max3mod_FA_SUM.xlsx
- (optional) oxPC_Lv1_ max3mod.msp

The .sdf file is a structure library of all generated structures and annotations. All 17 predicted properties are stored accordingly in corresponding sections.

Please use ChemAxon InstantJ (view, search and modify) or Progenesis SDF studio (view only) to have a graphical view of the contents. The SDF files generated by LPPtiger can be imported by other software e.g., Progenesis QI for metabolomics to be used as structure library for LPP identification.

Developers can use any text editor or any programming language to extract, edit, and process all data entries inside the .sdf file.

The two .xlsx files stores essential parameters for predicted LPPs and all unique FA residues. These two files are used as input files for the identification steps. Users can edit the file to add and/or remove certain entries if necessary.

The optional .msp file is generated only when "create *in silico* spectra" is selected. The .msp file contains the predicted spectra generated by *in silico* fragmentation with annotation of each fragment ion. The .msp file is compatible with NIST MS search and MS peptide search programs, and are expected to be compatible with other programs which use spectra libraries in .msp format for identification.

!!! General Notes

Users can customize these two .xlsx files to shorten or enlarge the list of LPPs and corresponding FA lists.

Here are some remarks:

- Please back up these files before editing them.
- For the LPP summary .xlsx tables:

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Class	Abbreviation	MULA_NEUT	EXACT_MASS	-H]-_FORMU	[M-H]-_MZ	COO]-_FORM	M+HCOO]-_M	MSP_JSON	FINGERPRINT	LPP_SMILES	SN1_SMILES	SN2_SMILES
2	PC	PC(16:0/9:0<	C33H64NO9F	649.431869258			C34H65NO11	694.429523	{"[M-CH3]-":	[168.046, 171	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
3	PC	PC(16:0/9:0<	C33H64NO10	665.4267838	C33H63NO10	664.418959			{"[M-C3H9N-	[125.097, 143	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
4	PC	PC(16:0/12:1	C36H68NO9F	689.463169386			C37H69NO11	734.460824	{"[M-CH3]-":	[168.046, 206	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
5	PC	PC(16:0/12:1	C36H66NO10	703.442433942			C37H67NO12	748.440088	{"[M-CH3]-":	[168.046, 206	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
6	PC	PC(16:0/12:1	C36H68NO10	705.458084006			C37H69NO12	750.455738	{"[M-CH3]-":	[168.046, 206	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
7	PC	PC(16:0/12:1	C36H68NO10	705.4580840(	C36H67NO10	704.450259			{"[M-C3H9N-	[165.128, 168	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
8	PC	PC(16:0/12:1	C36H66NO11	719.4373485(	C36H65NO11	718.429523			{"[M-CH3]-":	[168.046, 197	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
9	PC	PC(16:0/12:1	C36H68NO11	721.452998626			C37H69NO13	766.450653	{"[M-CH3]-":	[168.046, 206	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
10	PC	PC(16:0/12:1	C36H68NO11	721.4529986(	C36H67NO11	720.445174			{"[M-CH3]-":	[168.046, 195	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
11	PC	PC(16:0/12:1	C36H68NO12	737.4479132(	C36H67NO12	736.440088			{"[M-CH3]-":	[168.046, 206	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
12	PC	PC(16:0/18:2	C42H80NO8F	757.56215515			C43H81NO10	802.559809	{"[M-CH3]-":	[168.046, 206	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
13	PC	PC(16:0/18:1	C42H82NO8F	759.577805214			C43H83NO10	804.575459	{"[M-CH3]-":	[168.046, 206	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
14	PC	PC(16:0/18:2	C42H78NO9F	771.541419706			C43H79NO11	816.539074	{"[M-CH3]-":	[168.046, 206	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC
15	PC	PC(16:0/18:2	C42H80NO9F	773.55706977			C43H81NO11	818.554724	{"[M-CH3]-":	[168.046, 206	[O-]P(OCC[N	OC(CCCCCC	OC(CCCCCC

  - The data type and abbreviations are critical for the program, please format the styles of the new entries according to existed fields.
  - The "FINGERPRINT_JSON" and "MSP_JSON" file should be entered properly as existed entries.
  - If you are not sure how to generate the data fields, please take a similar structure as a reference.
  - If any error appeared, please use the backup of the original file and try again.

- For the unique FA list:

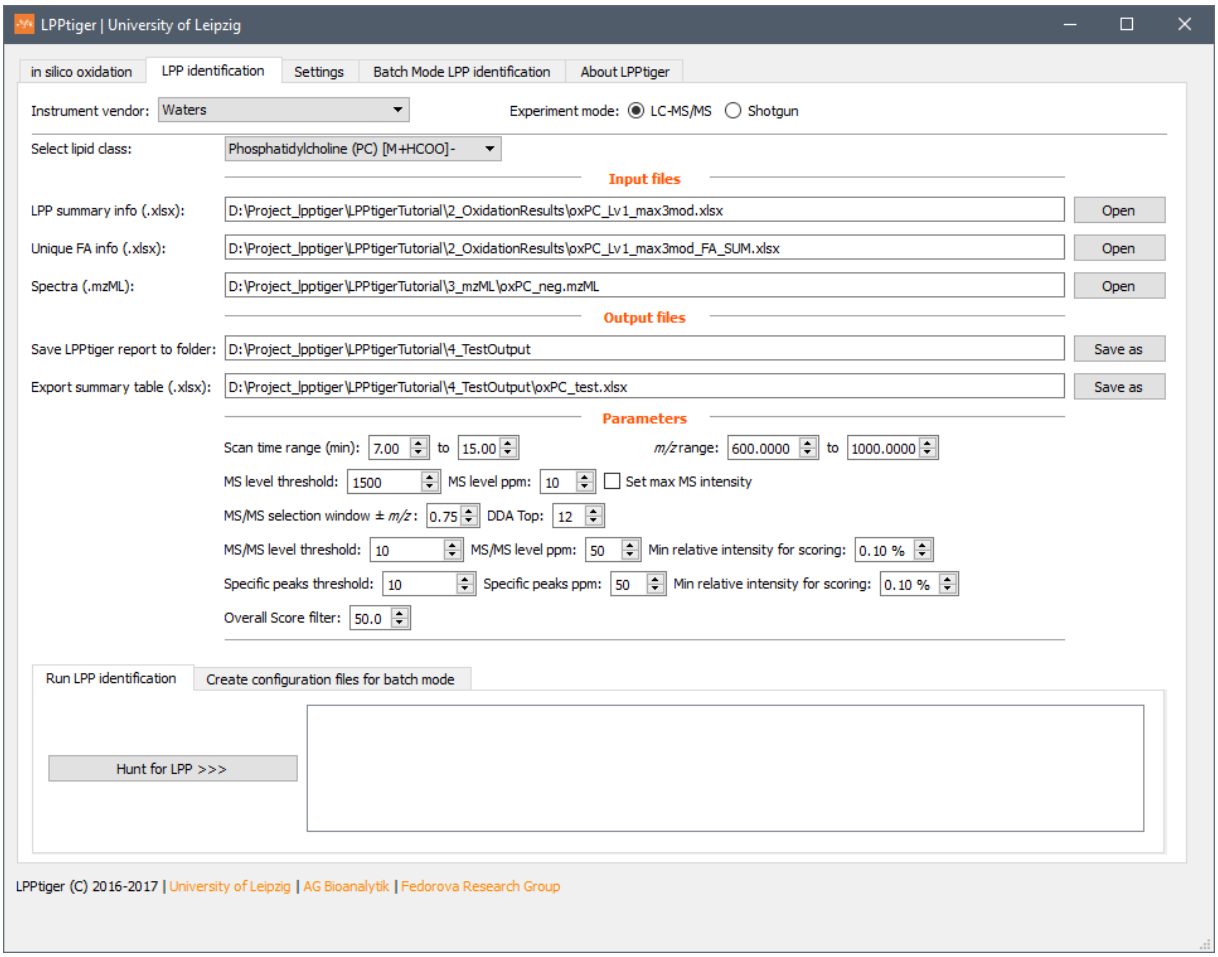
	A	B	C	D	E	F	G	H	I
1	FA	Link	C	DB	elem	mass	[M-H]-	[M-H2O-H]-	NL-H2O
2	9:0<CHO@C9	A	9	0	C9H16O3	172.109944	171.102119	154.099379	154.099379
3	9:0<COOH@(	A	9	0	C9H16O4	188.104859	187.097034	170.094294	170.094294
4	12:1[1xDB]<(	A	12	1	C12H20O3	212.141245	211.133419	194.13068	194.13068
5	12:1[1xDB,1x	A	12	1	C12H18O4	226.120509	225.112684	208.109944	208.109944
6	12:1[1xDB]<(	A	12	1	C12H20O4	228.136159	227.128334	210.125594	210.125594
7	12:1[1xDB,1x	A	12	1	C12H20O4	228.136159	227.128334	210.125594	210.125594
8	12:1[1xDB,1x	A	12	1	C12H18O5	242.115424	241.107599	224.104859	224.104859
9	12:1[1xDB,1x	A	12	1	C12H20O5	244.131074	243.123249	226.120509	226.120509
10	12:1[1xDB,1x	A	12	1	C12H20O5	244.131074	243.123249	226.120509	226.120509
11	16:0	A	16	0	C16H32O2	256.24023	255.232405	238.229665	238.229665
12	12:1[1xDB,1x	A	12	1	C12H20O6	260.125988	259.118163	242.115423	242.115423
13	18:2	A	18	2	C18H32O2	280.24023	279.232405	262.229665	262.229665
14	18:1	A	18	1	C18H34O2	282.25588	281.248055	264.245315	264.245315
15	18:2[2xDB,1x	A	18	2	C18H30O3	294.219495	293.21167	276.20893	276.20893

  - Please ensure that all new LPPs added to the LPP summary tables have their FA residues in this table.
  - The FA list should have all FA in the LPP summary table and can have more FA when it is necessary.
  - The data type and abbreviations are critical for the program, please format the styles of the new entries according to existed data.

- Add or remove any entry in the FA list may influence the specificity score due to the possible changes of total intensities of the unspecific peaks.
- If any error appeared, please use the backup original file and try again.

5.3 Step II Hunt for LPPs

The parameters are extremely important for the identification accuracy. Please use the following parameters for the test file, and find optimized values for your dataset.



Input files and mode selection:

- **Instrument vendor**
  Currently, LPPtiger supports .mzML files generated from .raw/.RAW files from **Waters** and **Thermo Fisher Scientific** instruments; preliminary support for mzML files from **Agilent** and **SCIEX** data were provided as early access feature. Working with mzML from other vendors might require adjustment of file format. (SCIEX users please check page 5 for more details)
- **Experiment Mode**
  experimental mode (LC-MS/MS or shotgun).
- **LPP summary info (.xlsx)**
  The summary table of all LPPs generated in *in silico* oxidation step
- **Unique FA info (.xlsx)**
  The summary table of unique FA residues generated in *in silico* oxidation step
- **Spectra (.mzML)**
  The spectra file in .mzML format to be searched for identification. Please make sure that the .mzML file selected matched to the vendor and experiment mode selections.
- **Save LPPtiger report to folder**

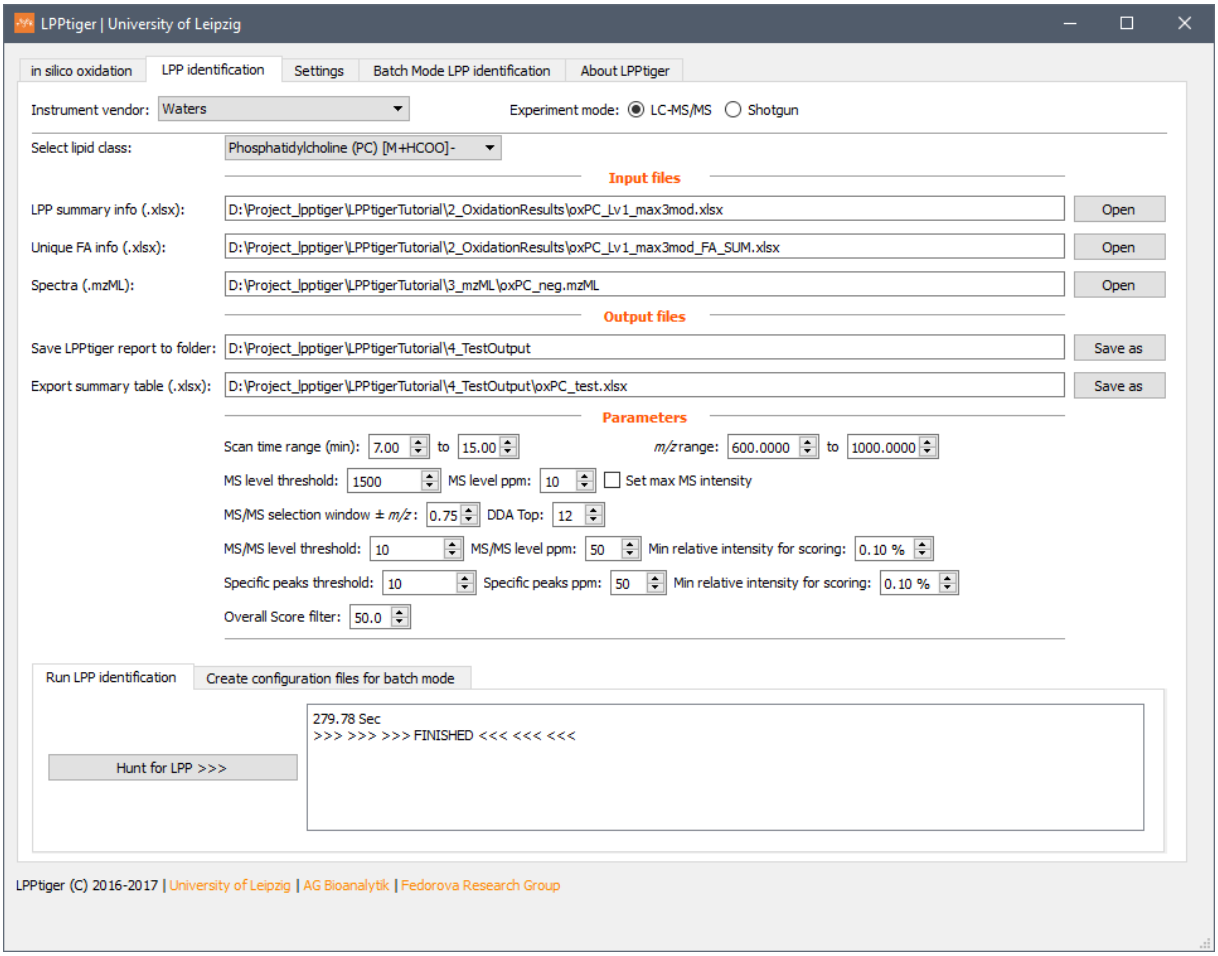
Choose the path for the HTML report file and corresponding image folder.

- **Export summary table (.xlsx)**
  Choose the path for the summary output table as a .xlsx file.

Parameters:

- **Ranges:** Scan time range and *m/z* range
- **Threshold:** MS level threshold, MS/MS level threshold, and specific peaks threshold. (optional: a max intensity filter can be defined to focus on LPPs in certain intensity range)
- **ppm:** MS level, MS/MS level, and specific peaks
- **Min relative intensity for scoring:** MS/MS level and specific peaks
- **Overall score filter:** Only proposed LPPs with a score higher than the filter will be considered for output.
- **Other parameters:** DDA top number and MS/MS precursor selection window

Click "***Hunt for LPP***" to start!
LPPtiger creates a log file named "LPPtiger_Params-Log_YYYY-MM-DD_HH-MM-SS.txt" and an HTML file with associated folder of image and "LPPtiger_Results_YYYY-MM-DD_HH-MM-SS.html".
You can open the HTML report in your web browser and refresh from time to time to check the latest results. It may take few minutes until the first image result generated. We recommend using Mozilla Firefox, Chrome or Chromium to review the HTML report.



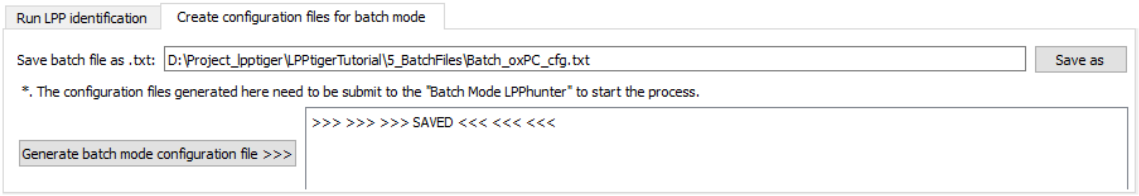
!!! General Notes

- LPPtiger will not respond during the run. The process may take very long, please be patient.

5.4 Step III batch mode

Instead of running individual files separately with manual input of all parameters. Users can save all parameters to a configuration text file which can be submitted to the integrated batch mode.

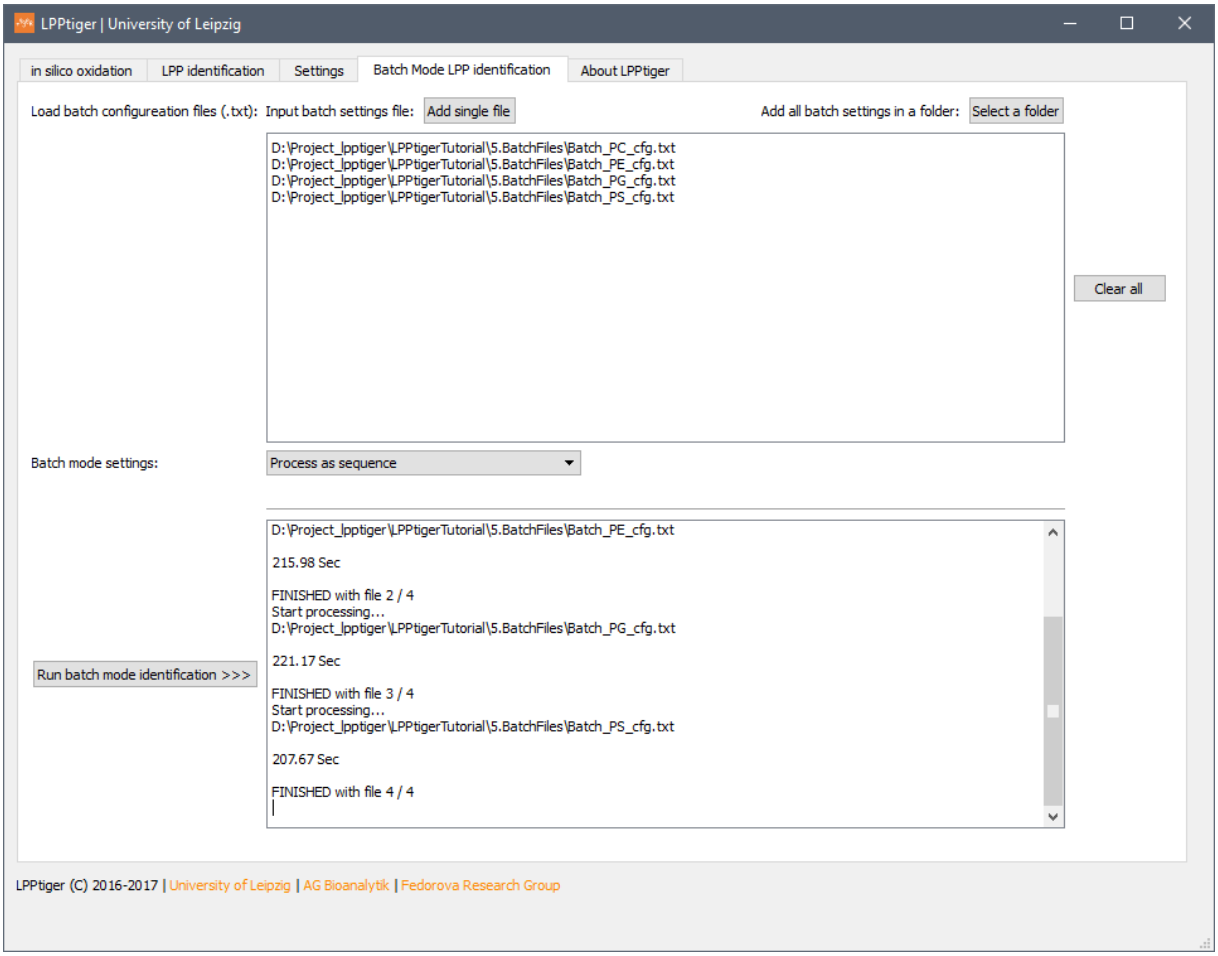
The configuration file can be generated from the “Create configuration files for batch mode” tab next to the “Run LPP identification” tab.



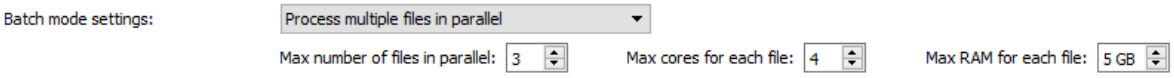
The saved configuration files can be edited by any text editor.

After generation of all configuration files, please click on the “Batch Mode LPP identification” tab, add individual files by “Add single file” or load all .txt configuration files by “select a folder”, then click on “Run Batch Mode” to run.

The default mode is set to process all configurations files one by one as a sequence.



However, for high configuration servers, optional mode to process several files in parallel is supported.



!!! General Notes

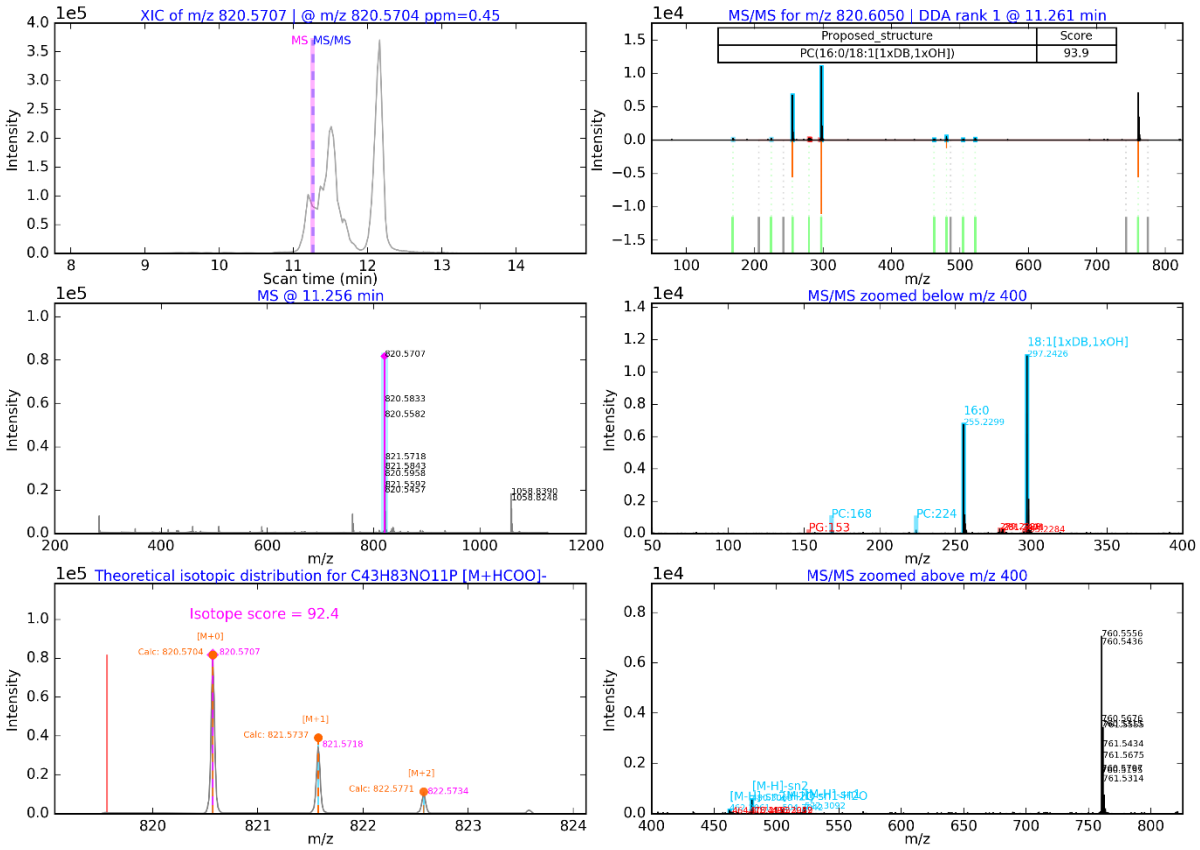
- The max core number and RAM size will overwrite the parameters in the configuration files.

6 LPPtiger output

For each submitted dataset LPPtiger provides the **output .xlsx table** which summarize lipid identities (bulk identification, proposed discrete structure, elemental composition, theoretical and observed *m/z* values, mass accuracy, retention time), identification metrics (LPPtiger and isotope scores, relative intensities of matched fragments, PL specific and unspecific signals) and data specific details (DDA rank, scan number).

PL class specific (marked here green) and unspecific (red) fragment ions are not considered for LPPtiger score calculations but can be used to provide additional confidence criteria:

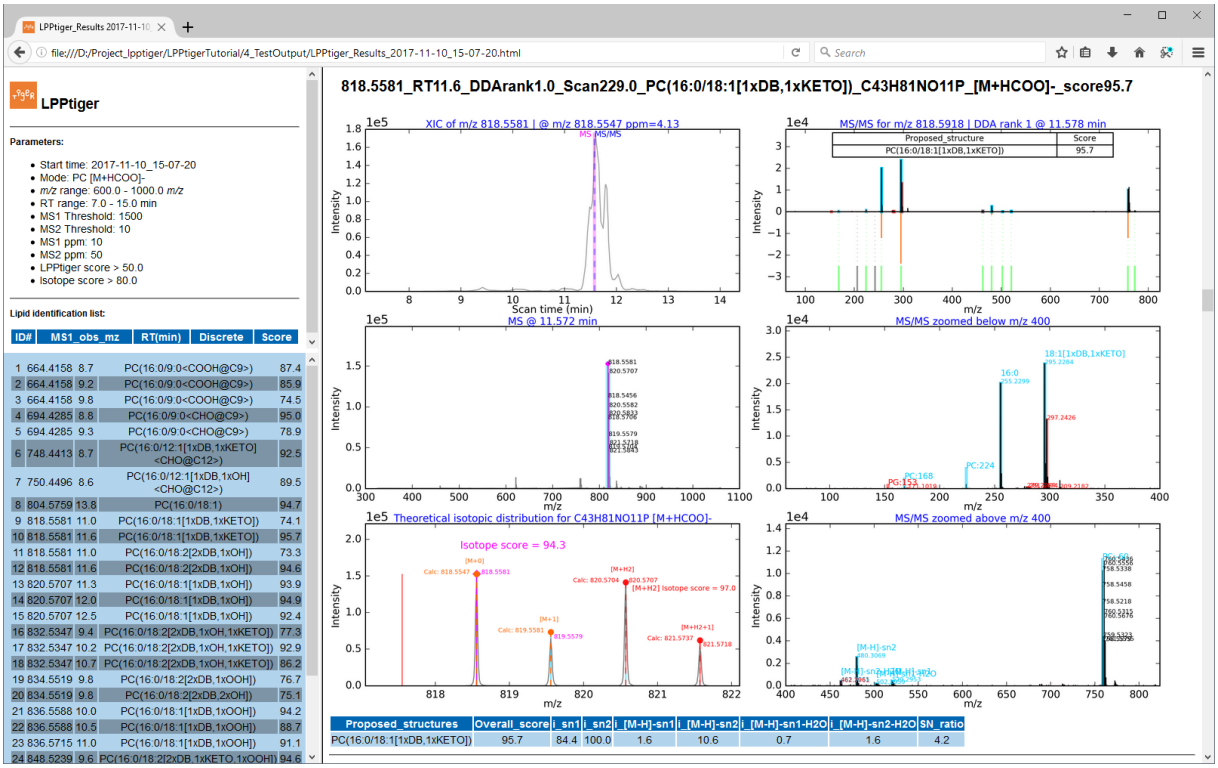
Furthermore, LPPtiger generates a separate **six-panel image for each identified lipid**:



Example of the six-panel image from LPPtiger .html report for precursor at m/z 846.5789 identified as PC(18:0/18:1[1xDB,1xKETO]).

A. extracted ion chromatogram (XIC)
B. corresponding MS scan
C. zoomed MS scan with isotope pattern and Isotope Score
D. MS/MS with identification information
E. MS/MS zoomed at the region of fatty acid fragments
F. MS/MS zoomed at the region of neutral loss signals

All output images are integrated and indexed in an informative **HTML report file** for manual reviewing. Availability of the graphical data representation and organized report files allows fast evaluation of identification results. The report HTML file with the built-in identification table and a log of the corresponding LPPtiger parameters provides a simple solution for data tracking and storage.



ID#	MS1_obs_mz	RT(min)	Discrete	Score
1	664.4158	8.7	PC(16:0/9:0<COOH@C9>)	87.4
2	664.4158	9.2	PC(16:0/9:0<COOH@C9>)	85.9
3	664.4158	9.8	PC(16:0/9:0<COOH@C9>)	74.5
4	694.4285	8.8	PC(16:0/9:0<CHO@C9>)	95.0
5	694.4285	9.3	PC(16:0/9:0<CHO@C9>)	78.9
6	748.4413	8.7	PC(16:0/12:1[1xDB,1xKETO]<CHO@C12>)	92.5
7	750.4496	8.6	PC(16:0/12:1[1xDB,1xOH]<CHO@C12>)	89.5
8	804.5759	13.8	PC(16:0/18:1)	94.7
9	818.5581	11.0	PC(16:0/18:1[1xDB,1xKETO])	74.1
10	818.5581	11.6	PC(16:0/18:1[1xDB,1xKETO])	95.7
11	818.5581	11.0	PC(16:0/18:2[2xDB,1xOH])	73.3
12	818.5581	11.6	PC(16:0/18:2[2xDB,1xOH])	94.6
13	820.5707	11.3	PC(16:0/18:1[1xDB,1xOH])	93.9
14	820.5707	12.0	PC(16:0/18:1[1xDB,1xOH])	94.9
15	820.5707	12.5	PC(16:0/18:1[1xDB,1xOH])	92.4
16	832.5347	9.4	PC(16:0/18:2[2xDB,1xOH,1xKETO])	77.3
17	832.5347	10.2	PC(16:0/18:2[2xDB,1xOH,1xKETO])	92.9
18	832.5347	10.7	PC(16:0/18:2[2xDB,1xOH,1xKETO])	86.2
19	834.5519	9.8	PC(16:0/18:2[2xDB,1xOOH])	76.7
20	834.5519	9.8	PC(16:0/18:2[2xDB,2xOH])	75.1
21	836.5568	10.0	PC(16:0/18:1[1xDB,1xOOH])	94.2
22	836.5568	10.5	PC(16:0/18:1[1xDB,1xOOH])	88.7
23	836.5715	11.0	PC(16:0/18:1[1xDB,1xOOH])	91.1
24	848.5239	9.6	PC(16:0/18:2[2xDB,1xKETO,1xOOH])	94.6

Proposed_structures	Overall_score	i_sn1	i_sn2	i_[M-H]-sn1	i_[M-H]-sn2	i_[M-H]-sn1-H2O	i_[M-H]-sn2-H2O	SN_ratio
PC(16:0/18:1[1xDB,1xKETO])	95.7	84.4	100.0	1.6	10.6	0.7	1.6	4.2

Screenshot of LPPtiger HTML output .html report file opened in Mozilla Firefox browser. The LPPtiger report file provides a simple and organized solution to review, store and exchange the assigned outputs. The rich information .html report file from LPPtiger has four major parts: (A) the main parameters used for identification, (B) the overall identification table, (C) the output images and (D) a relative intensity table for each identified lipid. Users can navigate between output images by clicking on the corresponding entry in the identification table. The original image can be accessed by clicking on the six-panel image (B).

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Proposed_structures	Formula_neutral	Formula_ion	Charge	Lib_mz	ppm	SN_ratio	Overall_score	Rank_score	Cosine_score	Fingerprint	SNR_score	Isotope_score
2	PC(16:0/9:0<COOH@C9>)	C33H64NO10P	C33H63NO10P	[M-H]-	664.419	-4.8	738.5	87.4	72.5	98.1	76.1	100	90.4
3	PC(16:0/9:0<COOH@C9>)	C33H64NO10P	C33H63NO10P	[M-H]-	664.419	-4.8	31.0	85.9	60	99.9	72.5	100	97.1
4	PC(16:0/9:0<COOH@C9>)	C33H64NO10P	C33H63NO10P	[M-H]-	664.419	-4.8	2.7	74.5	42.5	99.6	51.3	90.4	88.9
5	PC(16:0/9:0<CHO@C9>)	C33H64NO9P	C34H65NO11P	[M+HCOO]-	694.4295	-1.52	12.4	95	97	92.4	86.6	100	99.1
6	PC(16:0/9:0<CHO@C9>)	C33H64NO9P	C34H65NO11P	[M+HCOO]-	694.4295	-1.52	7.5	78.9	62.5	91.6	50	100	90.6
7	PC(16:0/12:1[1xDB,1xKETO]<CHO@C12>)	C36H66NO10P	C37H67NO12P	[M+HCOO]-	748.4401	1.6	17.8	92.5	97.5	72.1	95.7	100	97.3
8	PC(16:0/12:1[1xDB,1xOH]<CHO@C12>)	C36H68NO10P	C37H69NO12P	[M+HCOO]-	750.4557	-8.12	15.8	89.5	75	96.3	85.6	100	90.6
9	PC(16:0/18:1)	C42H82NO8P	C43H83NO10P	[M+HCOO]-	804.5755	0.58	3069.0	94.7	95	99.6	81.6	100	97.3
10	PC(16:0/18:1[1xDB,1xKETO])	C42H80NO9P	C43H81NO11P	[M+HCOO]-	818.5547	4.13	1.4	74.1	66.5	91.1	81.6	33.6	97.7
11	PC(16:0/18:2[2xDB,1xOH])	C42H80NO9P	C43H81NO11P	[M+HCOO]-	818.5547	4.13	1.4	73.3	66.5	91.1	77.5	33.6	97.7
12	PC(16:0/18:1[1xDB,1xKETO])	C42H80NO9P	C43H81NO11P	[M+HCOO]-	818.5547	4.13	4.2	95.7	95.5	97.5	91.3	100	94.3
13	PC(16:0/18:2[2xDB,1xOH])	C42H80NO9P	C43H81NO11P	[M+HCOO]-	818.5547	4.13	4.2	94.6	95.5	97.6	85.6	100	94.3
14	PC(16:0/18:1[1xDB,1xOH])	C42H82NO9P	C43H83NO11P	[M+HCOO]-	820.5704	0.45	28.8	93.9	95.5	99.8	81.6	100	92.4
15	PC(16:0/18:1[1xDB,1xOH])	C42H82NO9P	C43H83NO11P	[M+HCOO]-	820.5704	0.45	4.6	94.9	95	99.7	81.6	100	98.4

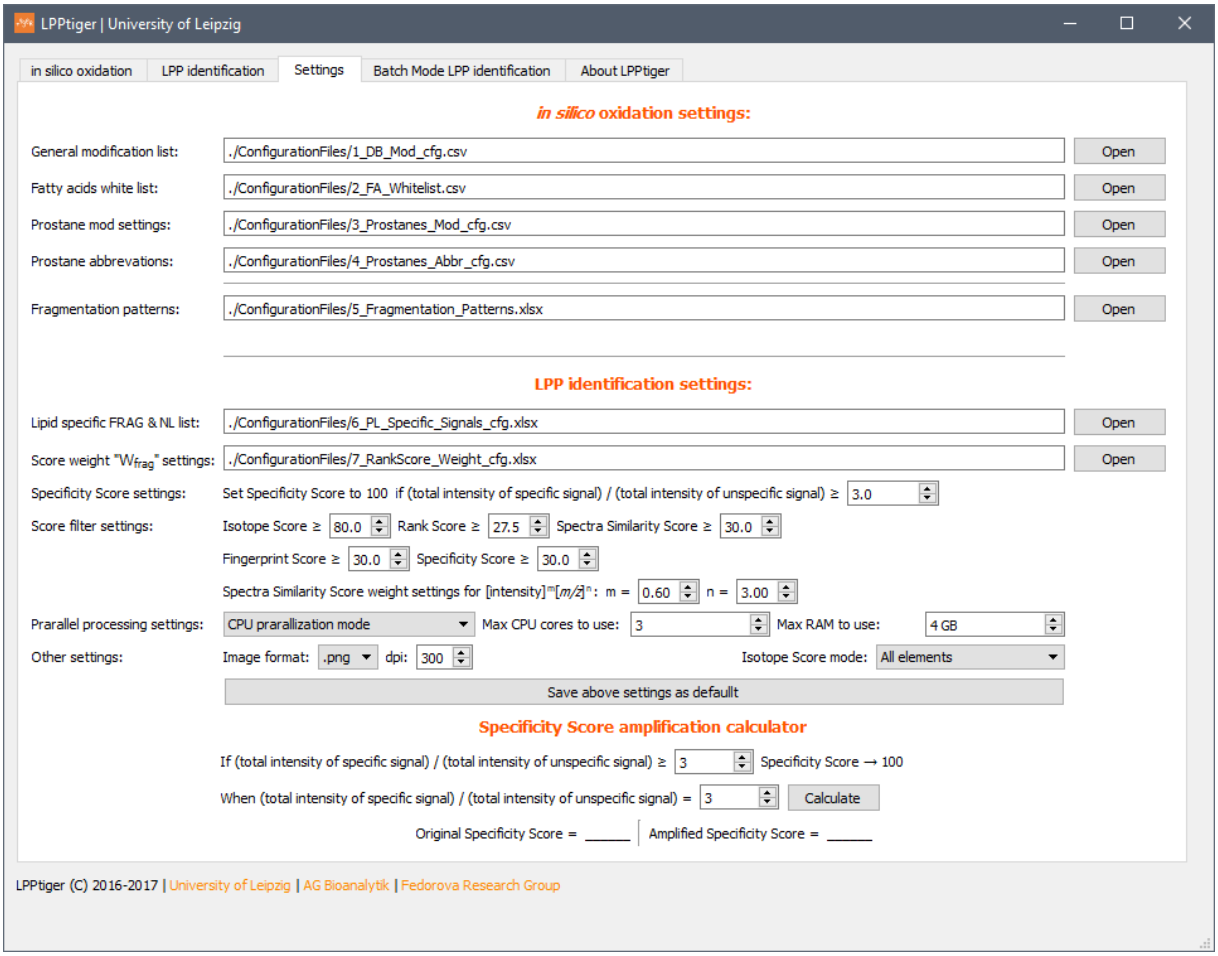
7 Configuration files

You can easily modify/create configuration files to define your own FA preferences using template files provided with LPPtiger.

You can also adapt PL specific fragments list and weight factors depending on the MS instruments, collision energies and ion adducts used in the study by modifying simple .xlsx tables provided with LPPtiger.

1. .csv configuration file of modification types and levels for single double bond unit
2. .csv configuration file of fatty acids for oxidation
3. .csv configuration file of prostanes and other special LPP types for three or more double bond unit
4. .csv configuration file of abbreviation settings for prostanes and other special LPPs
5. .xlsx configuration file of phospholipid fragmentation patterns
6. .xlsx configuration file of phospholipid head group specific fragments and neutral losses
7. .xlsx configuration file for the fragmentation rank score settings

!!! Note: *All default configuration files are located under "LPPtigerFolder\ConfigureationFiles", Please click the "open" button, select the name accordingly. You can also save modified configuration files in different folders and change them accordingly for different data.*

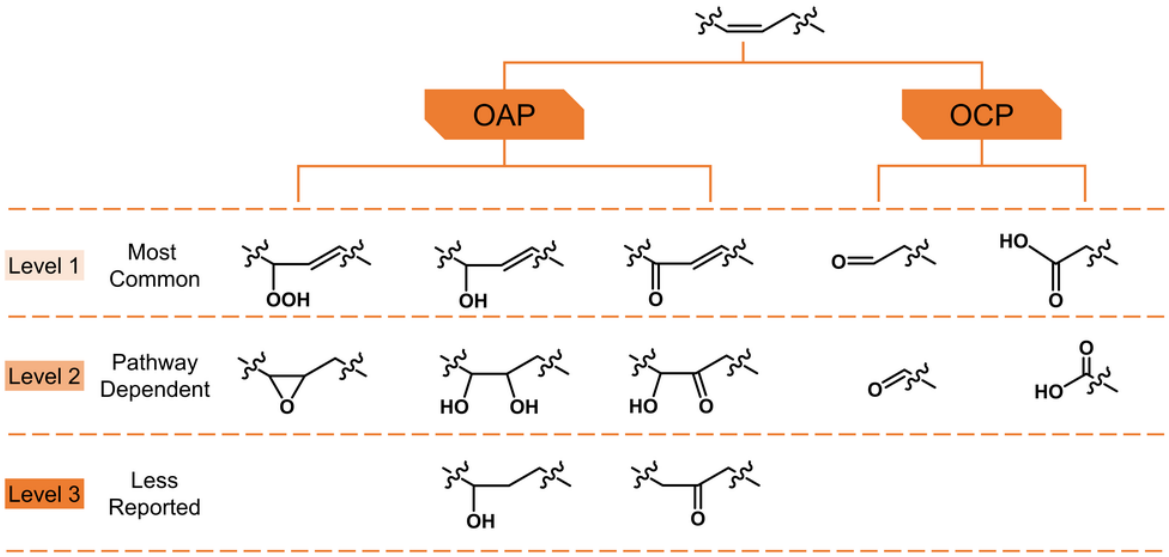


!!! Note: *If you edit any .csv configuration file in Excel, take care that FA abbreviation e.g. 14:0 are saved correctly and not converted to the time format (e.g. 14:00:00). Check modified .csv file by opening it with Notepad.*

1. Screenshot of .csv configuration file of modification types and levels for a single double bond unit

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		no_oxidation	hydroperoxyl	hydroxyl	keto	aldehyde	carboxylic_acid	aldehyde_short	carboxylic_acid_short	epoxy_no_db	keto_hydroxyl	dihydroxyl	hydroxyl_no_db	keto_no_db
2	SMILES	C/C=C/	C(OO)/C=C/	C(O)/C=C/	C(=O)/C=C/	CC=O	CC(O)=O	C=O	C(O)=O	C1C(O1)C	C(O)C(=O)C	C(O)C(O)C	C(O)CC	C(=O)CC
3	FRAG	C	C(OO)	C(O)	C(=O)	CC=O	CC(O)=O	C=O	C(O)=O	C	C(O)	C(O)	C(O)	C(=O)
4	OXIDATIO	1	1	1	1	1	1	2	2	2	2	2	3	3
5	OAP	0	1	1	1	0	0	0	0	1	2	2	1	1
6	OCP	0	0	0	0	1	1	1	1	0	0	0	0	0
7	DB	1	1	1	1	0	0	0	0	0	0	0	0	0
8	OH	0	0	1	0	0	0	0	0	0	1	2	1	0
9	KETO	0	0	0	1	0	0	0	0	0	1	0	0	1
10	CHO	0	0	0	0	1	0	1	0	0	0	0	0	0
11	COOH	0	0	0	0	0	1	0	1	0	0	0	0	0
12	EPOXY	0	0	0	0	0	0	0	0	1	0	0	0	0
13	OOH	0	1	0	0	0	0	0	0	0	0	0	0	0

The three oxidation levels were defined by default as follows:



2. .csv configuration file of fatty acids for oxidation

FA_list.csv - Notepad

File Edit Format View Help

```
FA,Link,C,DB,elem,mass,[M-H]-,[M+FA-H]-,[M-W-H]-,[M+FA-W-H]-,NL-H2O,EXACT_ABBR,SMILES
14:0,A,14,0,C14H28O2,228.20893,227.2011,273.20658,245.21166,291.21714,210.19836,14:0,OC(CCCCCCCCCCCCC)=O
16:0,A,16,0,C16H32O2,256.24023,255.2324,301.23788,273.24296,319.24844,238.22966,16:0,OC(CCCCCCCCCCCCCCC)=O
16:1,A,16,1,C16H30O2,254.22458,253.21675,299.22223,271.22731,317.23279,236.21401,16:1(9Z),OC(CCCCCCC/C=C\CCCCCC)=O
18:0,A,18,0,C18H36O2,284.27153,283.2637,329.26918,301.27427,347.27975,266.26096,18:0,OC(CCCCCCCCCCCCCCCCC)=O
18:1,A,18,1,C18H34O2,282.25588,281.24805,327.25353,299.25862,345.2641,264.24531,18:1(9Z),OC(CCCCCCC/C=C\CCCCCCCC)=O
18:2,A,18,2,C18H32O2,280.24023,279.2324,325.23788,297.24296,343.24844,262.22966,"18:2(9Z,12Z)",OC(CCCCCCC/C=C\C/C=C\CCCCC)=O
18:3,A,18,3,C18H30O2,278.22458,277.21675,323.22223,295.22731,341.23279,260.21401,"18:3(6Z,9Z,12Z)",OC(CCCC/C=C\C/C=C\C/C=C\CCCCC)=O
20:3,A,20,3,C20H34O2,306.25588,305.24805,351.25353,323.25862,369.2641,288.24531,"20:3(5Z,8Z,11Z)",OC(CCC/C=C\C/C=C\C/C=C\CCCCCCCC)=O
20:4,A,20,4,C20H32O2,304.24023,303.2324,349.23788,321.24296,367.24844,286.22966,"20:4(5Z,8Z,11Z,14Z)",OC(CCC/C=C\C/C=C\C/C=C\C/C=C\CCCCC)=O
20:5,A,20,5,C20H30O2,302.22458,301.21675,347.22223,319.22731,365.23279,284.21401,"20:5(5Z,8Z,11Z,14Z,17Z)",OC(CCC/C=C\C/C=C\C/C=C\C/C=C\C/C=C\CC)=O
22:4,A,22,4,C22H36O2,332.27153,331.2637,377.26918,349.27427,395.27975,314.26096,"22:4(7Z,10Z,13Z,16Z)",OC(CCCCC/C=C\C/C=C\C/C=C\C/C=C\CCCCC)=O
22:5,A,22,5,C22H34O2,330.25588,329.24805,375.25353,347.25862,393.2641,312.24531,"22:5(4Z,7Z,10Z,13Z,16Z)",OC(CC/C=C\C/C=C\C/C=C\C/C=C\C/C=C\CCCCC)=O
22:6,A,22,6,C22H32O2,328.24023,327.2324,373.23788,345.24296,391.24844,310.22966,"22:6(4Z,7Z,10Z,13Z,16Z,19Z)",OC(CC/C=C\C/C=C\C/C=C\C/C=C\C/C=C\C/C=C\CC)=O
O-16:0,O,16,0,C16H34O,242.26097,241.25314,287.25862,223.24258,269.24805,224.2504,O-16:0,OCCCCCCCCCCCCCCCC
O-18:0,O,18,0,C18H38O,270.29227,269.28444,315.28992,251.27388,297.27935,252.2817,O-18:0,OCCCCCCCCCCCCCCCCCC
O-20:0,O,20,0,C20H42O,298.32357,297.31574,343.32122,279.30518,325.31065,280.313,O-20:0,OCCCCCCCCCCCCCCCCCCCC
P-16:0,P,16,0,C16H32O,240.24532,239.23749,285.24297,221.22693,267.2324,222.23475,P-16:0,O/C=C\CCCCCCCCCCCCCC
P-18:0,P,18,0,C18H36O,268.27662,267.26879,313.27427,249.25823,295.2637,250.26605,P-18:0,O/C=C\CCCCCCCCCCCCCCCC
P-20:0,P,20,0,C20H40O,296.30792,295.30009,341.30557,277.28953,323.295,278.29735,P-20:0,O/C=C\CCCCCCCCCCCCCCCCCC
```

3. .csv configuration file of prostanes and other special LPP types for three or more double bound unit

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1		MAIN_SM	MAIN_SEF	REVERSE_	REVERSE_	ORIGIN_S	DB_NUM_	RING	RING_TYP	CLASS_TYI	OAP	OCP	DB	OH	KETO	OOH	CHO	COOH
2	PGA	CC1C(=O)/	8	CC(O)/C=(	1	C/C=C\C/(	3	1	A	P	1	0	1	1	1	0	0	0
3	PGB	C/C1=C(C(	8	CC(O)/C=(	1	C/C=C\C/(	3	1	B	P	1	0	1	1	1	0	0	0
4	PGC	CC1C(=O)(	8	CC(O)/C=(	1	C/C=C\C/(	3	1	C	P	1	0	1	1	1	0	0	0
5	PGD	CC1C(O)C(	8	CC(O)/C=(	1	C/C=C\C/(	3	1	D	P	1	0	1	2	1	0	0	0
6	PGE	CC1C(=O)(	8	CC(O)/C=(	1	C/C=C\C/(	3	1	E	P	1	0	1	2	1	0	0	0
7	PGF	CC1C(O)C(	8	CC(O)/C=(	1	C/C=C\C/(	3	1	F	P	1	0	1	3	0	0	0	0
8	PGG	CC1C2CC((	8	CC(OO)/C	1	C/C=C\C/(	3	2	G	P	1	0	1	0	0	1	0	0
9	PGH	CC1C2CC((	8	CC(O)/C=(	1	C/C=C\C/(	3	2	H	P	1	0	1	1	0	0	0	0
10	PGI	C/C=C1/C(	11		0	CC/C=C\C/	4	2	I	P	1	0	2	1	1	0	0	0
11	PGJ	CC1/C=C/(	8	CC(O)/C=(	1	C/C=C\C/(	3	1	J	P	1	0	1	1	1	0	0	0
12	TxA	CC1C2CC((	8	CC(O)/C=(	1	C/C=C\C/(	3	2		TxA	1	0	1	1	0	0	0	0
13	TxB	CC1C(O)C(	8	CC(O)/C=(	1	C/C=C\C/(	3	1		TxB	1	0	1	3	0	0	0	0
14	LGD	CC(C(=O))	8	CC(O)/C=(	1	C/C=C\C/(	3	0	D	K	1	0	1	1	2	0	0	0
15	LGE	CC(C(=O)C	8	CC(O)/C=(	1	C/C=C\C/(	3	0	E	K	1	0	1	1	2	0	0	0

4. .csv configuration file of abbreviation settings for prostanes and other special LPPs

	A	B	C
1	NUM_C	NUM_DB	CLASS_ABBR
2	18	3	Phyto
3	20	3	Iso
4	20	4	Iso
5	20	5	Iso
6	22	3	dihomo-Iso
7	22	4	dihomo-Iso
8	22	5	dihomo-Iso
9	22	6	Neuro

5. .xlsx configuration file of phospholipid fragmentation patterns

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	PL_CLASS	sn2_FA	sn2_DB	OH	KETO	TYPE	PR	[M-CH3]-	[sn1-H]-	[sn2-H]-	[sn2+CH3-H]-	[sn2-CO2-H2O-H]-	[sn2-H2O-H]-	[M-CH3-sn2]-	[M-H]-	[M-C3H9N-H]-	[M+HCOO]-
2	PC	18	2	0	1	OAP	[M+HCOO]-	550	650	999	0	0	0	175	0	0	0
3	PC	18	2	-1	0	OAP	[M+HCOO]-	800	700	999	0	0	300	100	0	0	0
4	PC	18	2	-1	1	OAP	[M+HCOO]-	999	950	300	0	0	400	120	0	0	0
5	PC	18	1	0	1	OAP	[M+HCOO]-	500	500	999	0	0	0	50	0	0	0
6	PC	18	1	-1	0	OAP	[M+HCOO]-	475	600	999	0	0	0	75	0	0	0
7	PC	18	1	-1	1	OAP	[M+HCOO]-	999	800	500	0	0	0	100	0	0	0
8	PC	-1	-1	0	1	OCP_CHO	[M+HCOO]-	600	500	999	0	0	0	130	0	0	0
9	PC	-1	-1	0	1	OCP_COOH	[M+H]-	0	999	0	375	350	0	0	250	100	0
10	PC	-1	-1	0	1	OAP	[M+HCOO]-	500	500	999	0	0	0	100	0	0	0
11	PC	-1	-1	-1	1	OCP_CHO	[M+HCOO]-	600	500	999	0	0	0	130	0	0	0
12	PC	-1	-1	-1	1	OCP_COOH	[M+H]-	0	999	0	375	350	0	0	250	100	0
13	PC	-1	-1	-1	1	OAP	[M+HCOO]-	999	900	400	0	0	400	100	0	0	0
14	PC	-1	-1	-1	-1	UNMOD	[M+HCOO]-	550	650	999	0	0	0	100	0	0	0

6. .xlsx configuration file of phospholipid head group specific fragments and neutral losses

	A	B	C	D	E	F	G	H	I	J
1	CLASS	TYPE	EXACTMASS	FORMULA	CHARGE_MODE	PR_CHARGE	LABEL	REMARKS		
2	PA	NL	97.9769	H3O4P	NEG	[M-H]-	PA:-98	-PA Head Group		
3										
4	PC	FRAG	168.0458	C4H11O4NP-	NEG	[M+HCOO]-	PC:168	demethylated PC [M-H]-		
5	PC	FRAG	224.0688	C7H15O5NP-	NEG	[M+HCOO]-	PC:224	demethylated PC dehydrated glycerol ester [M-H]-		
6	PC	FRAG	242.0794	C7H17O6NP-	NEG	[M+HCOO]-	PC:242	demethylated PC glycerol ester [M-H]-		
7	PC	NL	60.0211	C2H4O2	NEG	[M+HCOO]-	PC:-60	-methyl formate (-CH3COOH)		
8	PC	NL	183.0660	C5H14NO4P	NEG	[M+HCOO]-	PC:-183	-PC Head Group		
9										
10	PE	FRAG	140.0113	C2H7O4NP-	NEG	[M-H]-	PE:140	PE Head Group [M-H]-		
11	PE	FRAG	196.0375	C5H11O5NP-	NEG	[M-H]-	PE:196	Deprotonated doubly dehydrated glycerol phosphocholine		
12	PE	NL	141.0191	C2H8NO4P	NEG	[M-H]-	PE:-141	-PE Head Group		
13	PE	NL	43.0422	C2H5N	NEG	[M-H]-	PE:-43	-PE Head Group part		
14										
15	PG	FRAG	171.0059	C3H8O6P-	NEG	[M-H]-	PG:171	PG Head Group [M-H]-		
16	PG	FRAG	152.9953	C3H6O5P-	NEG	[M-H]-	PG:153	PG Head Group [M-H2O-H]-		
17	PG	NL	172.0137	C3H9O6P	NEG	[M-H]-	PG:-172	-PG Head Group		
18										
19	PI	FRAG	241.0113	C6H10O8P -	NEG	[M-H]-	PI:241	PI Head Group [M-H]-		
20	PI	NL	162.0528	C6H10O5	NEG	[M-H]-	PI:-162	-inositol		
21										
22	PS	FRAG	184.0011	C3H7NO6P-	NEG	[M-H]-	PS:184	PS Head Group [M-H]-		
23	PS	NL	87.0320	C3H5NO2	NEG	[M-H]-	PS:-87	-serine		
24										

7. .xlsx configuration file for the fragmentation rank score settings

	A	B	C	D	E	F
1	Type	Weight	[M-H]-	[M+HCOO]-	FA	H2O
2	sn1	25	0	0	1	0
3	sn2	25	0	0	1	0
4	[M-H]-sn1	15	1	0	-1	1
5	[M-H]-sn2	15	1	0	-1	1
6	[M-H]-sn1-H2O	10	1	0	-1	0
7	[M-H]-sn2-H2O	10	1	0	-1	0