

MassChroQ is one of the PAPPSO facility software projects

# MASSCHROQ USER MANUAL

FREE AND OPEN SOURCE MASS CHROMATOGRAM QUANTIFICATION SOFTWARE

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MASSCHROQ 2.4.23

# MASSCHROQ USER MANUAL: FREE AND OPEN SOURCE MASS CHROMATOGRAM QUANTIFICATION SOFTWARE

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January 30, 2024 , 2.4.23

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*MassChroQ*

[HTTP://PAPPSO.INRAE.FR/EN/BIOINFO/MASSCHROQ/](http://pappso.inrae.fr/en/bioinfo/masschroq/) 

This book is part of the *MassChroQ* project.

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## DEDICATION

To all the admirable people acting in the “*Free Software Movement*” for a better and more ethical computing world

To all the readers who helped with this manual.

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# PREFACE

## I SOFTWARE FEATURE OFFERINGS AND INTENDED AUDIENCE

This manual is about the *MassChroQ* proteomics quantification software project.

*MassChroQ* has the following features:

- Load an XML file mainly describing the precursor peptidic ions that were fragmented to produce tandem mass spectra that allowed to identify proteins.
- Compute extracted ion current (XIC) chromatograms for the precursor ions by looking into the mass spectrometry data files;
- Perform under-the-curve calculations to provide intensity values for the precursor ions.


## 2 FEEDBACK FROM THE USERS


We are always grateful to any constructive feedback from the users.

The PAPPSO software team might be contacted *via* the following contact page:

[HTTP://PAPPSO.INRAE.FR/EN/TRAVAILLER\\_AVEC\\_NOUS/CONTACT/](http://pappso.inrae.fr/en/travailler_avec_nous/contact/)  (search for team members having the “Bioinformatics” specialty mentioned, like Olivier Langella or Filippo Rusconi).


## 3 PROGRAM AND DOCUMENTATION AVAILABILITY AND LICENSE

The programs and all the documentation that are shipped along with the *MassChroQ* software suite are available at [HTTP://PAPPSO.INRAE.FR/EN/BIOINFO/MASSCHROQ/](http://pappso.inrae.fr/en/bioinfo/masschroq/) . Most of the time, a new version is published as source, and as binary install packages for *MS-Windows* (64-bit systems only).

For *GNU/Linux*, binary packages are created locally (see [HTTP://PAPPSO.INRAE.FR/EN/BIOINFO/MASSCHROQ/DOWNLOAD/](http://pappso.inrae.fr/en/bioinfo/masschroq/download/) ) but are also built in the *Debian*<sup>1</sup> autobuilders and are uploaded to the distribution servers. These packages are available using the system's software management infrastructure (like using the *Debian*'s `apt` command, for example, or the graphical application).

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<sup>1</sup> [HTTP://WWW.DEBIAN.ORG/](http://www.debian.org/) 

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# I GENERALITIES

In this chapter, some general concepts around the *MassChroQ* program and the reference to be used to cite the software in publications will be introduced.

## I.1 GENERAL CONCEPTS AND TERMINOLOGIES

This section describes the general concepts at the basis of the analysis of quantitative proteomics data that one needs to grok in order to properly assimilate the workings of the *MassChroQ* software.

### I.1.1 SPECTRAL COUNTS OR AREA UNDER THE CURVE?

*MassChroQ* is a program to perform quantitative mass spectrometry experiments in the context of proteomics projects. Quantitative proteomics projects can be handled in various ways, depending on the data that are used to actually quantify the identified peptides and proteins.

There are two main methods to perform quantitative assessments starting from proteomics data: spectral counting computations and area under the curve computations, as described below.

- *Spectral counts* quantitation: this variant is based on the counting of the mass spectra that have allowed the identification of a given protein. It has the advantage of being a label-free quantitative technique, but it is also too rough to be considered a “robust and precise” technique.
- *Area under the curve* quantitation: this variant is based on the integration of the ion current that can be extracted from the mass data (“extracted ion current”, or XIC) for the precursors ions that, upon fragmentation, led to the protein identification. By integrating the XICs for all the peptide precursors that identified a given protein, the software can estimate the abundance of that specific protein. The software thus needs to be able to extract the ion current for all the precursor ions in the MS spectra, when these ions were fragmented and allowed identifying peptides that in turn identified proteins. This is the process that *MassChroQ* implements, which is why it is called *Mass Chromatogram Quantification*, with *Chromatogram* referring to the extracted ion current (XIC) chromatogram used to assign an intensity value to the peptide ion that was fragmented.



### NOTE

It should be noted that *MassChroQ* does not know anything about proteins. Instead, it does know about a list of peptidic precursor ions' m/z values, which is all it needs to perform these XIC extractions.

## 1.1.2 OUTLINE OF A *MassChroQ* WORKING SESSION

The very first step is to produce the `masschroqm1` file that lists all the peptides that need a XIC extraction. There are two possibilities for this:

- *X!TandemPipeline* can produce that file and has a *MassChroQ* configuration interface that is well documented in the user manual. The `masschroqm1` file contains all the data required for the *MassChroQ* operation.
- For scientists not using *X!TandemPipeline* for their proteomics projects, it is possible to craft the `masschroqm1` file starting from a rudimentary file and by pointing *MassChroQ* to a `tsv` file (a tab-separated values file) containing the data required by *MassChroQ*. A typical file is shown in [FIGURE 1.1, “THE PEPTIDE LIST FILE IN `tsv` FORMAT”](#).



### NOTE

It is important that the *mb* column be filled-in with the calculated  $[M+H]^+$  ion mass. The charge column (*z* column) represents the actual charge of the precursor peptide ion.

```

1 scan sequence mh z proteins mods
2 778 CCTKPESER 1166.4934 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
3 839 NYQEAQ 752.3585 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
4 1136 TCVADESHAGCEK 1463.5852 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
5 1585 SHCIAIEVEK 1072.5111 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
6 1935 NECFLSHK 1034.4729 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
7 1960 ECCDKPLLEK 1291.6019 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
8 1980 LCVLHEK 898.48236 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
9 2089 CCTESLVNR 1138.4973 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
10 2237 YICDNQDTISSK 1443.6373 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
11 2241 QNCDQFEK 1051.4155 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
12 2278 ETYGMADCCCK 1478.5223 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
13 2702 QEPERNECFLSHK 1656.7439 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
14 2713 YLEATLEECCAK 1502.61 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
15 2753 ECCHGDLLECADDR 1749.6615 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
16 2867 CCAADDKEACFAVEGPK 1927.797 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
17 2910 EACFAVEGPK 1107.5137 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
18 3267 DDPHACYSTVFDK 1554.6533 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
19 3494 YLYEIAR 927.49396 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
20 3629 RHPEYAVSVLLR 1439.8123 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
21 3818 LKPDPTLDCDEFK 1576.7699 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
22 3821 KVPQVSTPTLVEVSR 1639.9381 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
23 3870 KQATALVELLK 1142.715 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
24 4082 RHPEYAVSVLLR 1439.8177 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
25 4236 RPFCSALTPDETYVPK 1880.9225 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
26 4253 SLHTLFGDELCK 1419.6945 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
27 4460 LVNELTEFAK 1163.6317 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
28 4499 SLHTLFGDELCK 1419.6956 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
29 4972 RHPYFYAPELLEYANK 2045.0253 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
30 5084 LFTFHADICTLPDTEK 1907.9092 3 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
31 5308 LGEYGFQNALIVR 1479.807 2 "P02769|ALBU_BOVIN SERUM ALBUMIN PRECURSOR."
[peptide_example_tsv_file.txt] [text] [+] [Git(master)] --buf:1--tab:1/1--[ 5@ 1]/ 35

```

This file documents the peptides that led to the identification of proteins. It needs to be provided to *MassChroQ* in case the identification process has been carried over outside of *XITandemPipeline*.

FIGURE 1.1: THE PEPTIDE LIST FILE IN `tsv` FORMAT

FIXME: brief description of the steps of the quantification analysis.

- Then do this: FIXME

## 1.2 CITING THE *MassChroQ* SOFTWARE.

Please, cite the software using the following reference:

Benoît Valot, Olivier Langella, Edlira Nano, Michel Zivy. 2011. MassChroQ: A versatile tool for mass spectrometry quantification. *Proteomics*, 11: 3572-3577. <https://doi.org/10.1002/pmic.201100120>.

## 1.3 INSTALLATION OF THE SOFTWARE

The installation material is available at [HTTP://PAPPSO.INRAE.FR/EN/BIOINFO/MASSCHROQ/DOWNLOAD/](http://pappso.inrae.fr/en/bioinfo/masschroq/download/).

### 1.3.1 INSTALLATION ON MS WINDOWS

The installation of the software is extremely easy on the MS-Windows platform: the installation program is standard and requires no explanation.

### 1.3.2 INSTALLATION ON DEBIAN- AND UBUNTU-BASED SYSTEMS

The installation on Debian- and Ubuntu-based GNU/Linux platforms is also extremely easy (even more than in the above situations). *MassChroQ* is indeed packaged and released in the official distribution repositories of these distributions and the only command to run to install it is:

```
$ 1 sudo apt install <package_name> 
```

In the command above, the typical *package\_name* is in the form `masschroq` for the program package and `masschroq-doc` for the user manual package.

Once the package has been installed, the program shows up in the *Science* menu. It can also be launched from the shell using the following command:

```
$ masschroq 
```

---

<sup>1</sup> The prompt character might be `%` in some shells, like *zsh*.

## 2 USAGE

In this chapter, the usage of *MassChroQ* will be introduced.

### 2.1 RUNNING MASSCHROQ

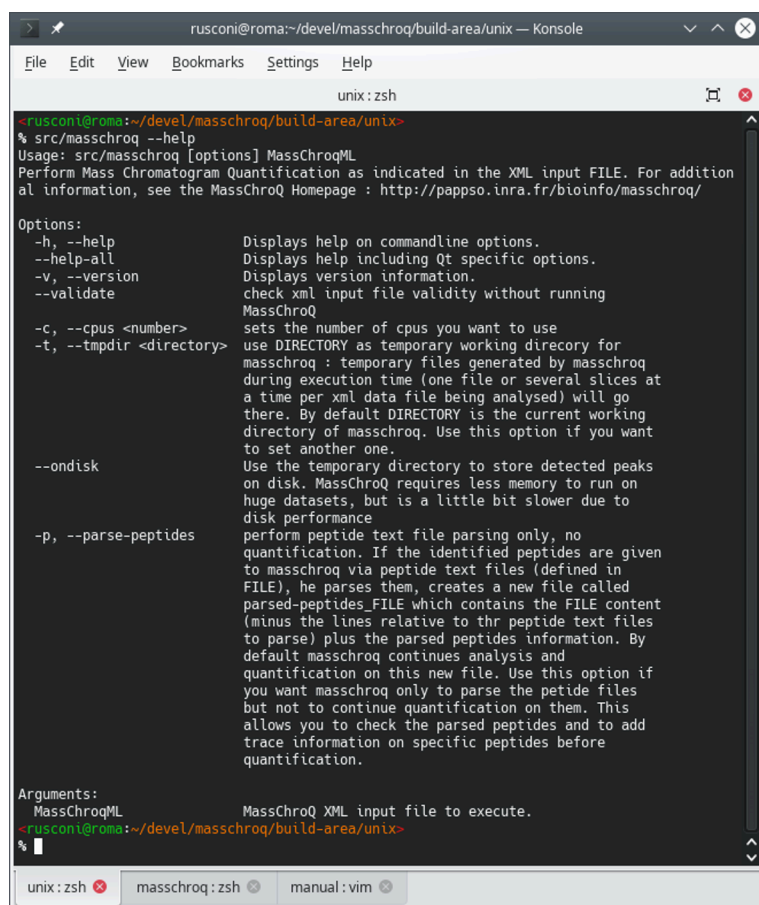
There are more than one ways to run the *MassChroQ* software, that will be described in the following sections.

#### 2.1.1 RUNNING MASSCHROQ FROM THE TERMINAL

*MassChroQ* can be run from the terminal. To get a review of the different options, run the following command at the prompt:

```
$ masschroq --help
```

The output of the command above is described in **FIGURE 2.1**, “PRINTING THE HELP MESSAGE IN THE TERMINAL”.



```
<rusconi@roma:~/devel/masschroq/build-area/unix>
% src/masschroq --help
Usage: src/masschroq [options] MassChroqML
Perform Mass Chromatogram Quantification as indicated in the XML input FILE. For addition
al information, see the MassChroQ Homepage : http://pappso.inra.fr/bioinfo/masschroq/

Options:
  -h, --help                Displays help on commandline options.
  --help-all               Displays help including Qt specific options.
  -v, --version             Displays version information.
  --validate                check xml input file validity without running
                           MassChroQ
  -c, --cpus <number>     sets the number of cpus you want to use
  -t, --tmpdir <directory> use DIRECTORY as temporary working directory for
                           masschroq : temporary files generated by masschroq
                           during execution time (one file or several slices at
                           a time per xml data file being analysed) will go
                           there. By default DIRECTORY is the current working
                           directory of masschroq. Use this option if you want
                           to set another one.
  --ondisk                  Use the temporary directory to store detected peaks
                           on disk. MassChroQ requires less memory to run on
                           huge datasets, but is a little bit slower due to
                           disk performance
  -p, --parse-peptides     perform peptide text file parsing only, no
                           quantification. If the identified peptides are given
                           to masschroq via peptide text files (defined in
                           FILE), he parses them, creates a new file called
                           parsed-peptides_FILE which contains the FILE content
                           (minus the lines relative to thr peptide text files
                           to parse) plus the parsed peptides information. By
                           default masschroq continues analysis and
                           quantification on this new file. Use this option if
                           you want masschroq only to parse the petide files
                           but not to continue quantification on them. This
                           allows you to check the parsed peptides and to add
                           trace information on specific peptides before
                           quantification.

Arguments:
  MassChroqML              MassChroQ XML input file to execute.
<rusconi@roma:~/devel/masschroq/build-area/unix>
% 
```

This is the help message that prints out in the terminal when the program is run with the `--help` option.

**FIGURE 2.1: PRINTING THE HELP MESSAGE IN THE TERMINAL**

There are two possibilities, either using the `--parse-peptides` option or not.

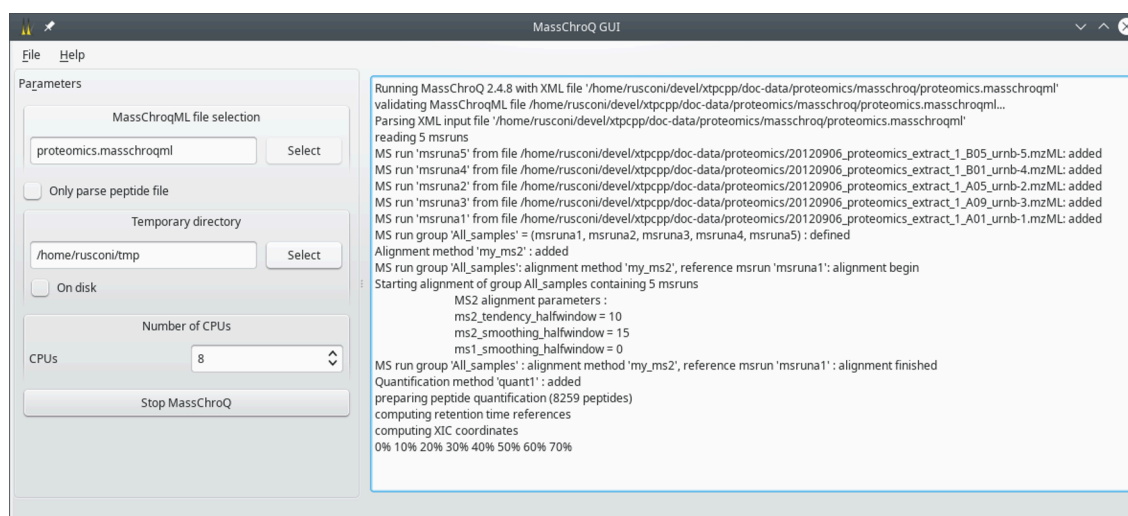
- Without the `--parse-peptides` option. In this case, the `masschroqml` file provided as parameter to the command is fully auto-sufficient: it contains both the *MassChroQ* configuration bits, the list of identified proteins and the list of identifying peptide precursor ions that need their ion current extracted (XIC).
- With the `--parse-peptides` option. In this case, the `masschroqml` file provided as parameter to the command is a partially empty shell: the list of peptide precursor ions is not provided as part of the file. The user must have crafted the peptide list file as described in [FIGURE 1.1, “THE PEPTIDE LIST FILE IN TSV FORMAT”](#). The name of that peptide list file must be provided in the `masschroqml` file provided as parameter to the command.

### 2.1.2 RUNNING MASSCHROQ FROM THE GRAPHICAL USER INTERFACE

*MassChroQ* can be run from a graphical user interface. To this end, start the following command at the prompt:

```
$ masschroq_gui
```

The window that opens up is shown in [FIGURE 2.2, “THE MAIN WINDOW”](#).



The main window allows the user to easily configure the *MassChroQ* run. The output of the program is displayed in the text widget on the right hand side of the window.

**FIGURE 2.2: THE MAIN WINDOW**

The following configuration elements have to be set by the user:

- *MassChroQML file selection*: use the *Select* button to open a file selection dialog window. Choose the `masschroqml` file that specifies all the data required to run *MassChroQ*. This is the *input* file.
- *Only parse peptide file*: check this check box widget if the file set above does not contain the full list of peptide ion precursors that identified the proteins. Checking this widget is analogous to using the `--parse-peptides` option on the command line (see SECTION 2.1.1, “RUNNING MASSCHROQ FROM THE TERMINAL”).
- *Temporary directory*: use the *Select* button to open a directory selection dialog window. Choose the directory where *MassChroQ* will write the temporary data it needs to carry over its work.
- *On disk*: check this check box widget to write on disk the detected peaks to alleviate the consumption of random access memory. The program is slower because read/write operations on disk are considerably slower than their equivalent in memory.
- *Number of CPUs*: Enter the number of execution threads that *MassChroQ* is allowed to use when parallelizing its tasks.
- *Start MassChroQ*: click onto this button to start the program. When the program has started the label of this button changes to *Stop MassChroq*.  
When the program is running, the output is printed in the text widget on the right hand side of the window.

## 2.2 MASSCHROQ OUTPUT FILE

The output of *MassChroQ* is stored in a file named according to the `results_<input_file_name>.ods` scheme, where the `masschroqml` extension is removed. The file is written inside of the directory that contains the input `masschroqml` file.

The format of the output file is the *Libreoffice* spreadsheet format (*Open Document Spreadsheet*, ODS). That file contains three tabs, described below:

- *MassChroq informations - <id>*: the `<id>` suffix identifies the quantification set. This tab contains the configuration bits of the *MassChroQ* run.



## NOTE

The <id> suffix is not currently of use, actually. *MassChroQ* was designed to be able to perform multiple quantification runs with different settings on the same set of associated samples. However, this is not currently available in the *X!TandemPipeline* interface.

- *<Sample association group>\_proteins*: the <Sample association group> prefix identifies the group in which samples were associated. There can be one or more such groups. If there is only one group, it has the default *All\_samples* name. If there are more than one group, then they are named according to the configuration set by the user. This tab contains the *peptide*, *protein* and *protein\_description* columns, all self-explanatory.
- *peptides\_<id>\_<Sample association group>*: the <id> and <Sample association group> suffixes were described above. This tab contains the actual XIC data for each peptide precursor ion that was quantified. There are a large number of columns, described below.

The peptides tab in the results spreadsheet file contains a large number of columns that are described in the paragraphs below. Each row of the spreadsheet is related to a given peptide precursor ion that was fragmented and that allowed identifying a peptide, which in turn contributed to the identification of a protein.

- *quantification*: this is the <id> mentioned above. At the time of writing this identification bit is not significant.
- *group*: this is the name of the group of associated samples.
- *msrun*: automatically-assigned name that is unambiguously attached to the mass spectrometry file.
- *msrunfile*: mass spectrometry data file name for the current sample.
- *mz*: calculated (FIXME) m/z value.
- *rt*: retention time at which the peptide precursor ion was eluted (in seconds). FIXME (the rt for the most intense ion current, that is, the top of the curve or what ? see rtbegin below)
- *maxintensity*: maximum ion current intensity. FIXME
- *area*: area under the XIC chromatogram curve. FIXME
- *peak quality*: assessment on the quality of the peak. The more 'a' characters, the higher the quality. FIXME
- *rt begin*: the retention time at the beginning of the the precursor ion XIC chromatogram peak. FIXME c'est vrai ?
- *rt end*: the retention time at the end of the the precursor ion XIC chromatogram peak. FIXME c'est vrai ?



- *peptide*: the peptide id as set by the database search engine.
- *label*: label that might be attached to the peptide in labelling-based quantification. FIXME
- *sequence*: the sequence of the peptide precursor ion.
- *z*: the charge of the peptide precursor ion.
- *mods*: the chemical modifications encountered on the peptide. The format is like  $[C_{37} H_{55} O_{10} N_{10} S_o]$ , which is the net elemental composition of the modifications FIXME !!!!!
- *ninumber*: the isotopologue (0 for monoisotopic, 1 for 1 heavy isotope, 2 for 2 heavy isotopes).
- *nirank*: rank of the peak in the previous column in the isotopic cluster. FIXME
- *niratio*: ratio
- :
- :
- :
- :
- :
- :
- :
- :
- :
- :
- :

# A GNU GENERAL PUBLIC LICENSE VERSION 3

Version 3, 29 June 2007

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The precise terms and conditions for copying, distribution and modification follow.

## TERMS AND CONDITIONS

### 0. DEFINITIONS.

“This License” refers to version 3 of the GNU General Public License.

“Copyright” also means copyright-like laws that apply to other kinds of works, such as semiconductor masks.

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## I. SOURCE CODE.

The “source code” for a work means the preferred form of the work for making modifications to it. “Object code” means any non-source form of a work.

A “Standard Interface” means an interface that either is an official standard defined by a recognized standards body, or, in the case of interfaces specified for a particular programming language, one that is widely used among developers working in that language.

The “System Libraries” of an executable work include anything, other than the work as a whole, that (a) is included in the normal form of packaging a Major Component, but which is not part of that Major Component, and (b) serves only to enable use of the work with that Major Component, or to implement a Standard Interface for which an implementation is available to the public in source code form. A “Major Component”, in this context, means a major essential component (kernel, window system, and so on) of the specific operating system (if any) on which the executable work runs, or a compiler used to produce the work, or an object code interpreter used to run it.

The “Corresponding Source” for a work in object code form means all the source code needed to generate, install, and (for an executable work) run the object code and to modify the work, including scripts to control those activities. However, it does not include the work’s System Libraries, or general-purpose tools or generally available free programs which are used unmodified in performing those activities but which are not part of the work. For example, Corresponding Source includes interface definition files associated with source files for the work, and the source code for shared libraries and dynamically linked subprograms that the work is specifically designed to require, such as by intimate data communication or control flow between those subprograms and other parts of the work.

The Corresponding Source need not include anything that users can regenerate automatically from other parts of the Corresponding Source.

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## COLOPHON

**About the authors.** Filippo Rusconi is a senior research scientist at the French national research council (*Centre national de la Recherche scientifique*, CNRS). Filippo has a background in biochemistry and organic chemistry and was trained during his Ph.D. as a bioanalytical chemist. He has extensive knowledge of analytical techniques involved in the study of biopolymers.


Filippo Rusconi is the author of a handbook about mass spectrometry for biochemists (French). The book was published by the French sci/tech publisher **LAVOISIER** ([HTTPS://WWW.LAVOISIER.FR](https://www.lavoisier.fr)) .




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