



pyOPENMS for metabolomics

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Röst, H.L., Sachsenberg, T., Aiche, S., Bielow, C., Weisser, H., Aicheler, F., Andreotti, S., Ehrlich, H.-C., Gutenbrunner, P., Kenar, E., Liang, X., Nahnsen, S., Nilse, L., Pfeuffer, J., Rosenberger, G., Rurik, M., Schmitt, U., Veit, J., Walzer, M., Wojnar, D., Wolski, W.E., Schilling, O., Choudhary, J.S., Malmström, L., Aebersold, R., Reinert, K., Kohlbacher, O. *OpenMS: A flexible open-source software platform for mass spectrometry data analysis.* Nature Methods, vol. 13, 2016. [doi:10.1038/nmeth.3959](https://doi.org/10.1038/nmeth.3959)

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Computational Metabolomics/Proteomics

Hundreds of ***experimental methods*** and protocols

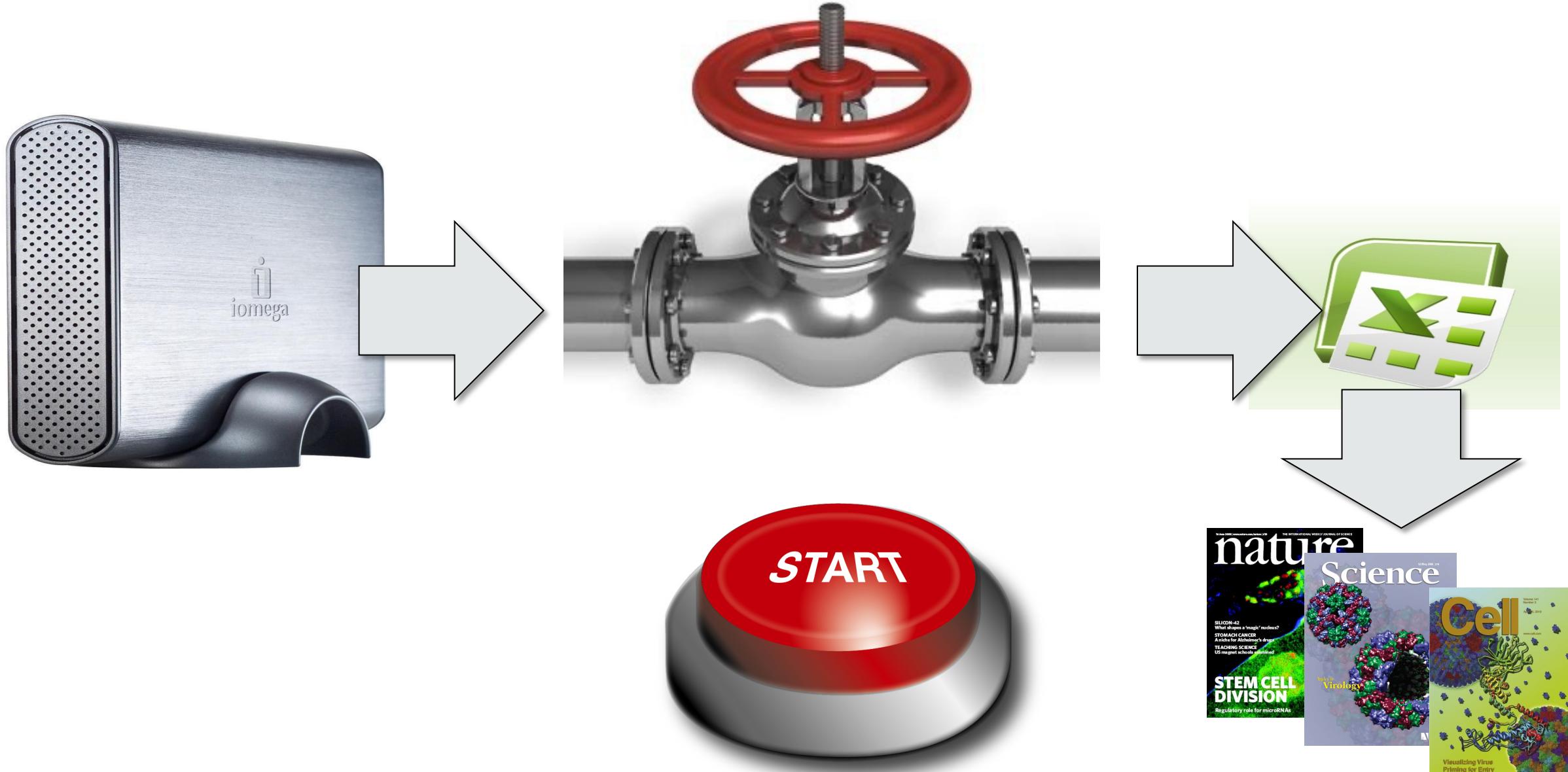
Many ***computational methods*** for

- Identification
- Quantification
- Statistical analysis

often tailored to experimental method

Goal: Extraction of maximum biological knowledge

Bioinformatics – The Holy Grail



Mass Spectrometry Workflows

Are diverse:

- Proteomics/metabolomics/interactomics, ...
- Separation methods/Acquisition
- with/without label
- Experimental designs

→ **A single workflow or tool is not enough**

Solution: A set of tools that can be combined to highly flexible workflows



What is OpenMS?

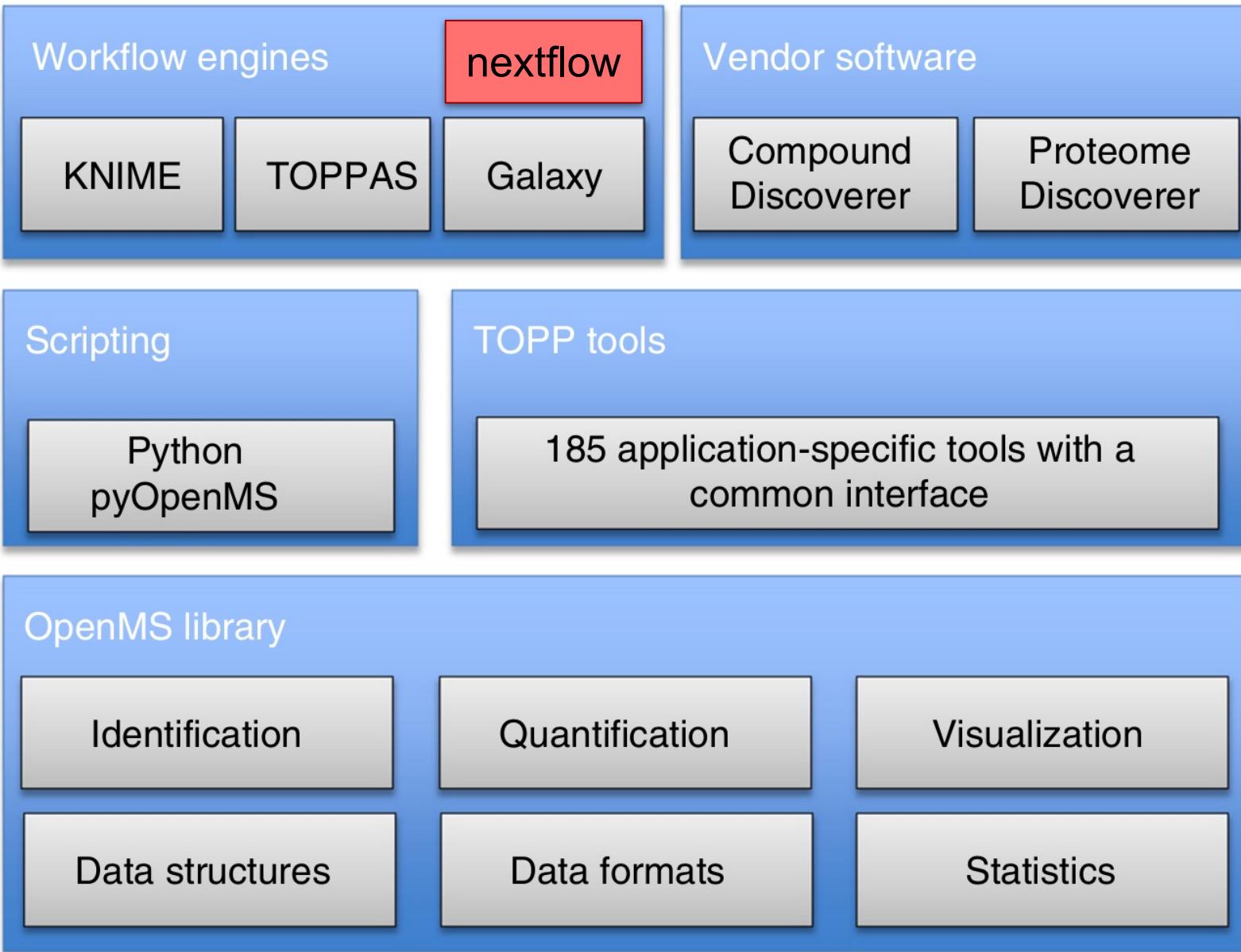
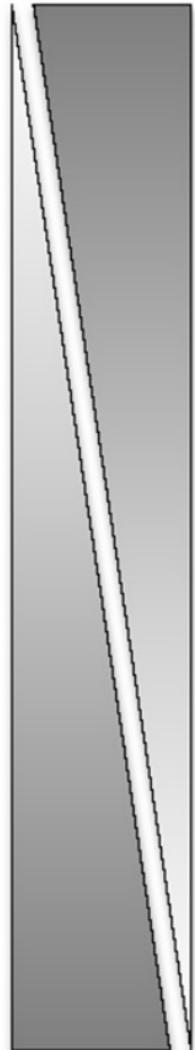
What is OpenMS?

OpenMS is:

- Computational **library**, **tools**, and **workflows** for the analysis of mass spectrometry data
- **Open-source** and free of charge
- **Vendor-independent**: Supports vendor-formats through conversion with proteowizard library
- **Standards**: Supports all major file formats (PSI)

The OpenMS framework

Usability and abstraction

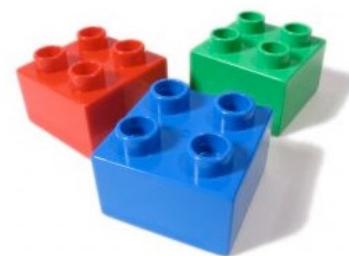


Extensibility

OpenMS Tools

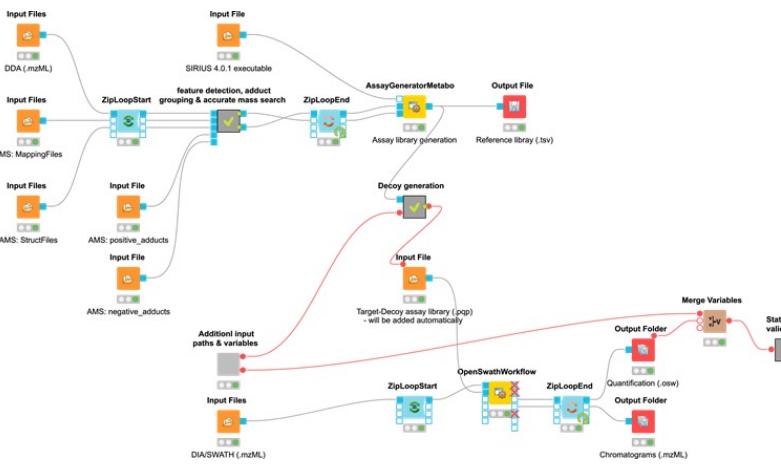
TOPP tools – The OpenMS PiPeline tools

- > 180 Building blocks: One application for each analysis step
 - All applications share **identical user interfaces**
 - Exchange data in **PSI standard formats**
- Easily integrated in various **workflow systems**
- **Support for other tools (e.g., SIRIUS, GNPS...)**



OpenMS workflows

Chaining of tools to build workflows
Facilitate *reproducible* and *reusable* research



Different workflow systems:

nextflow

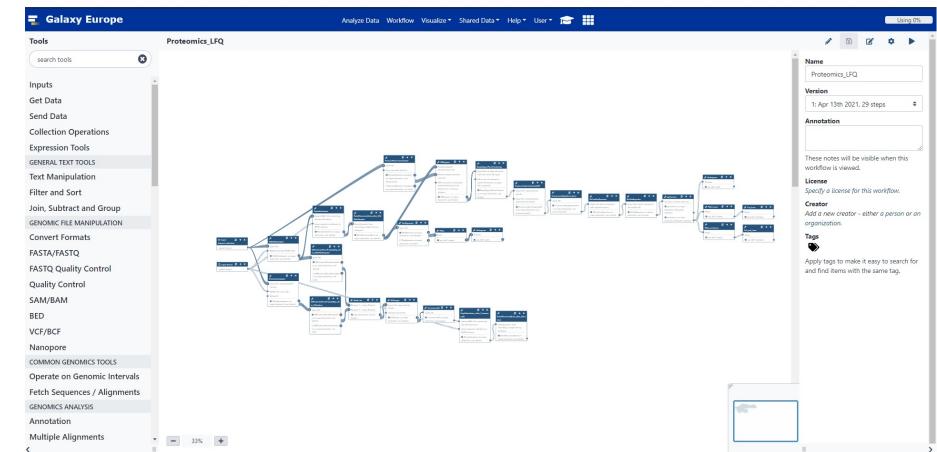
```
/*
 * STEP 1 - generate reversed decoy database
 */
process generate_decoy_database {
    input:
        file fastafile from input_fasta.mix(appended.fasta)

    output:
        file "${fastafile.baseName}_decoy.fasta" into (fastafile_decoy_1, fastafile_decoy_2)

    when:
        !params.skip_decoy_generation

    script:
        ===
        DecoyDatabase -in ${fastafile} \\
            -out ${fastafile.baseName}_decoy.fasta \\
            -decoy_string DECOY_ \\
            -decoy_string_position prefix
        ===
}
```

Galaxy PROJECT



Scripting with OpenMS: pyOpenMS



- Compiled language (fast!)
- Nontrivial to learn
- Verbose syntax
- Memory management
- Dependencies
(challenging for developers, easy for users)
- Wraps OpenMS
- Interactive scripting
- Easy to learn
- Dependencies easy to install



launch binder

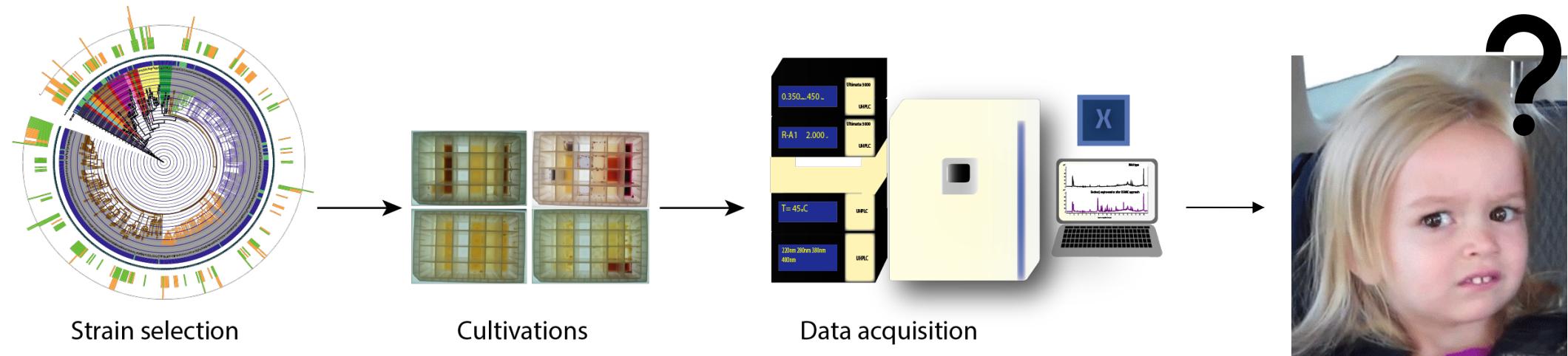
Brief intermission..

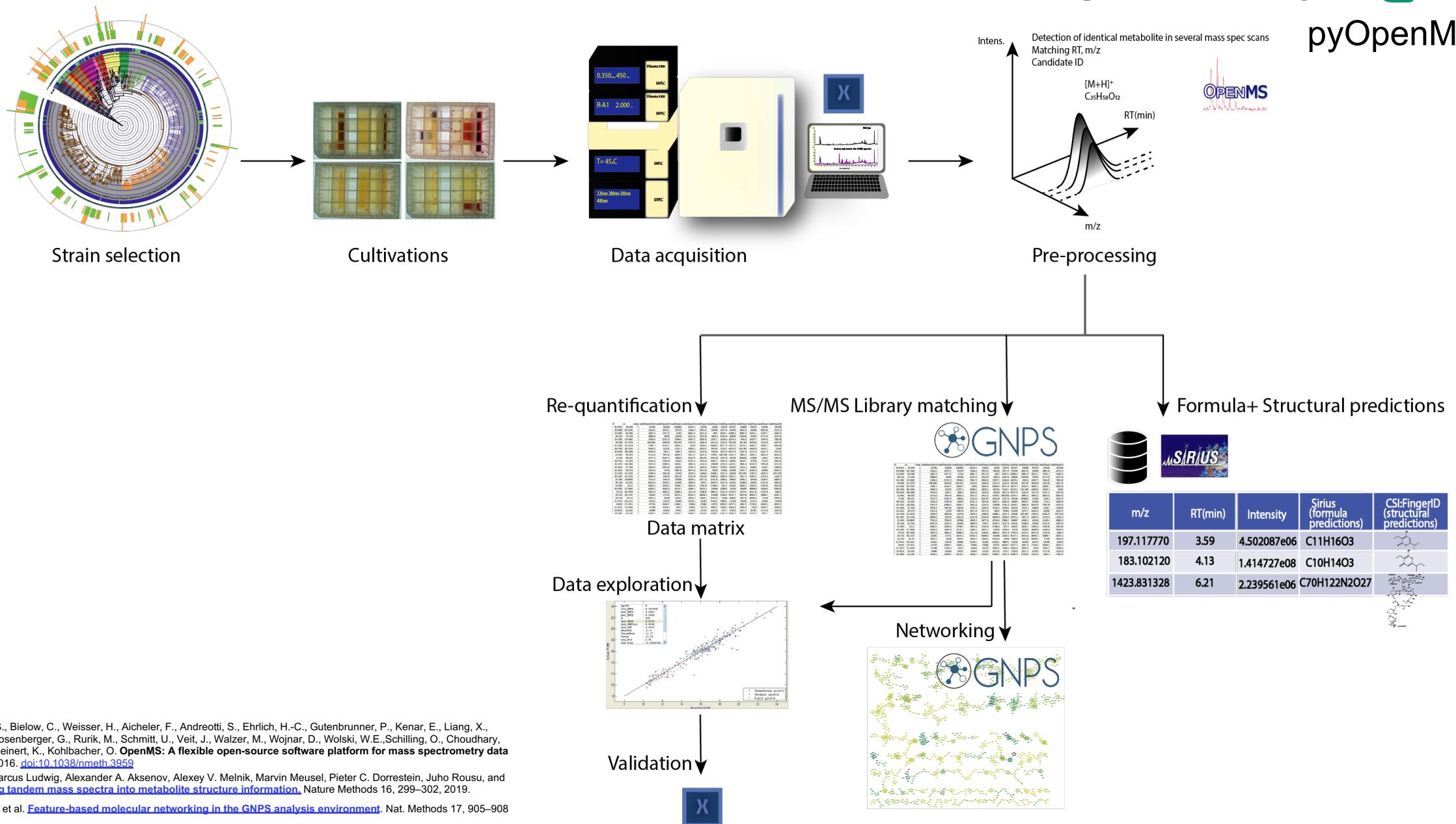
- <https://github.com/axelwalter/pyopenms-workshop>



- Click on the

Metabolomics for Specialized Metabolites, Ph.D. (Efi)



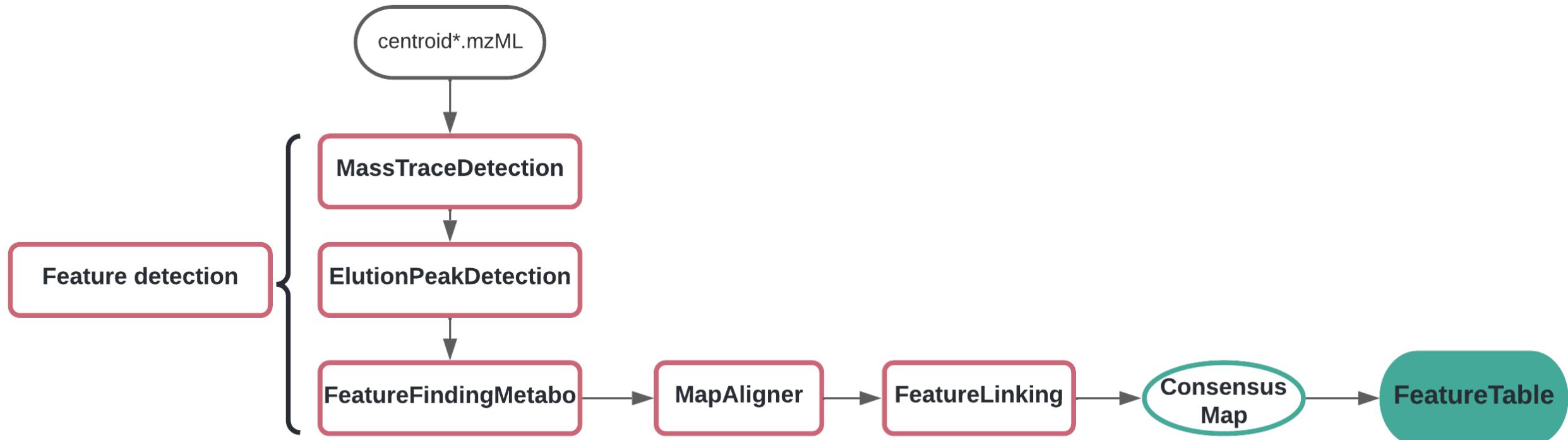


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Kai Dürkopp, Markus Fleischauer, Marcus Ludwig, Alexander A. Aksenenko, Alexey V. Melnik, Marvin Meusel, Pieter C. Dorrestein, Juhu Rousu, and Sebastian Böcker, [SIRIUS 4: Turning tandem mass spectra into metabolite structure information](#), Nature Methods 16, 299–302, 2019.

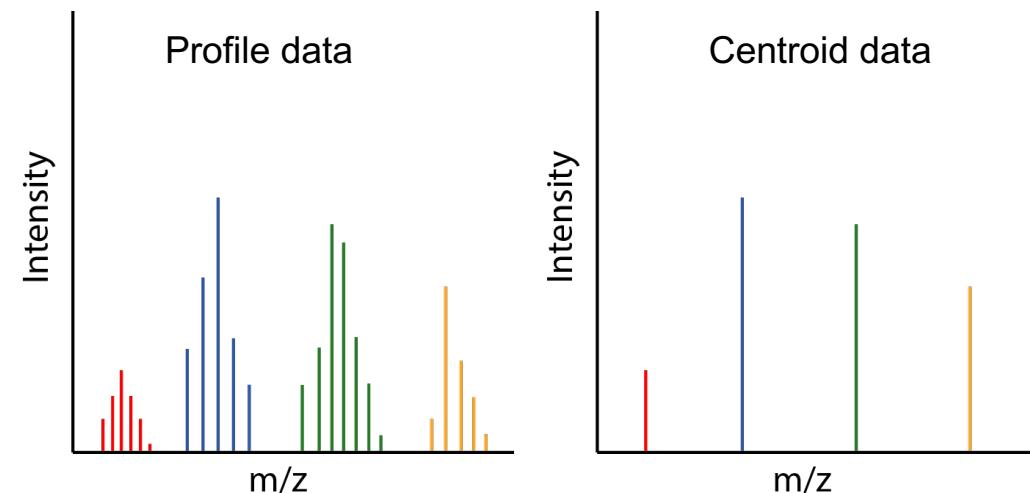
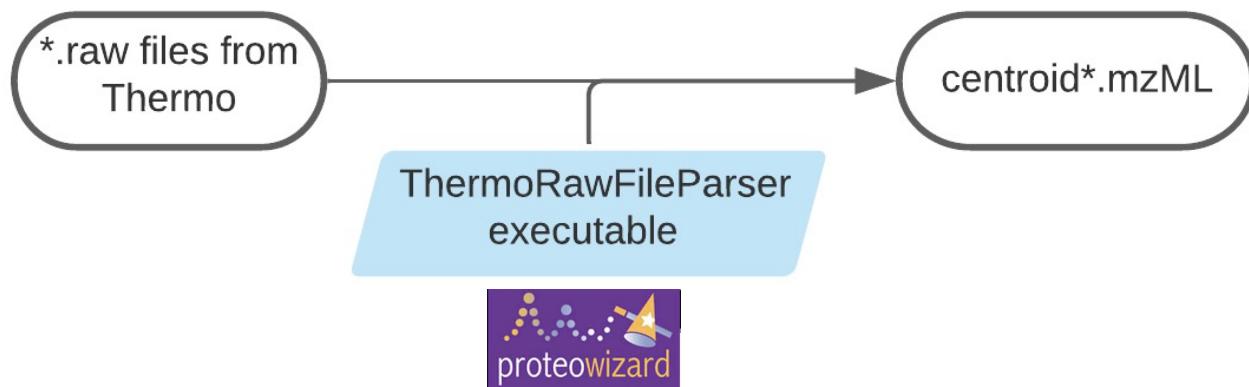
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Pre-processing: From raw data to a table of metabolic features

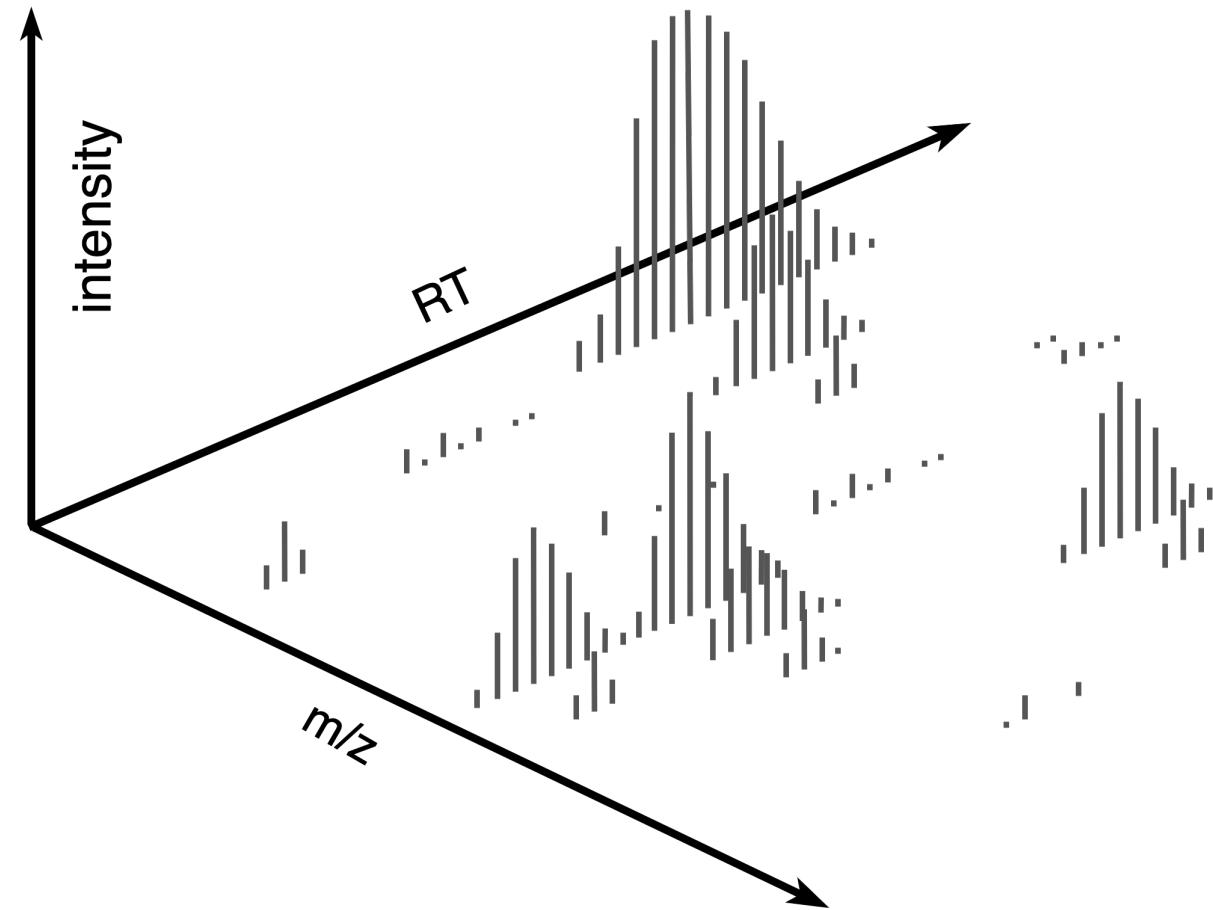


Data conversion

Vendor-format files to an **open community-driven format**

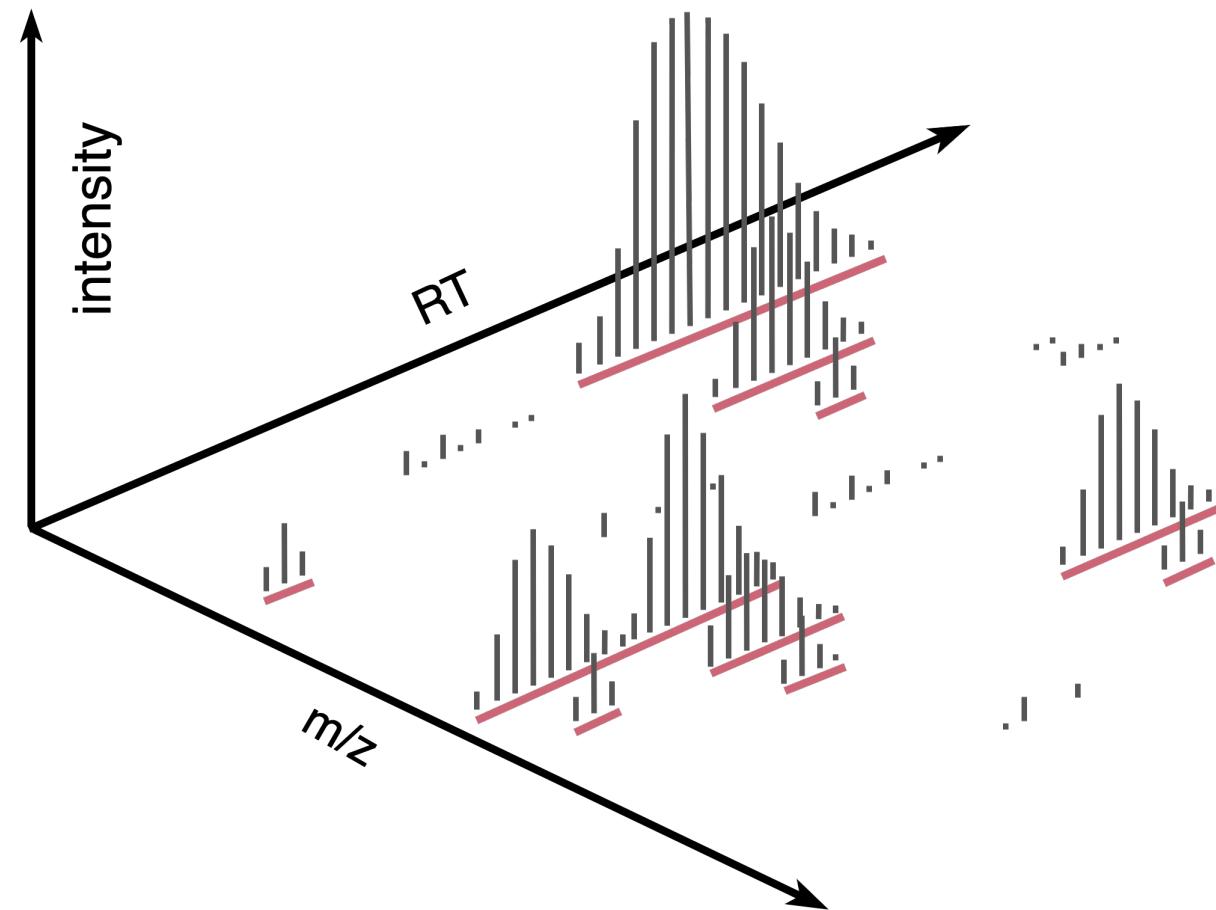


Feature detection (raw mzML)



1. Mass trace detection

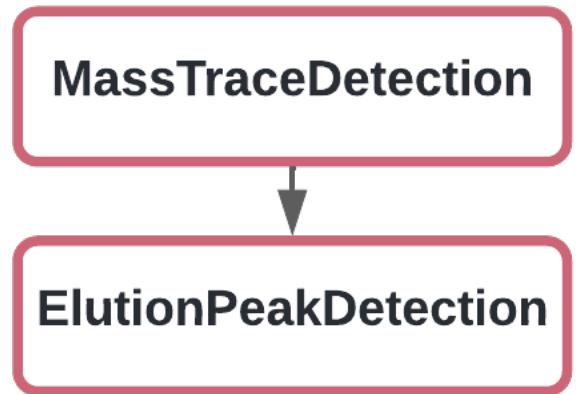
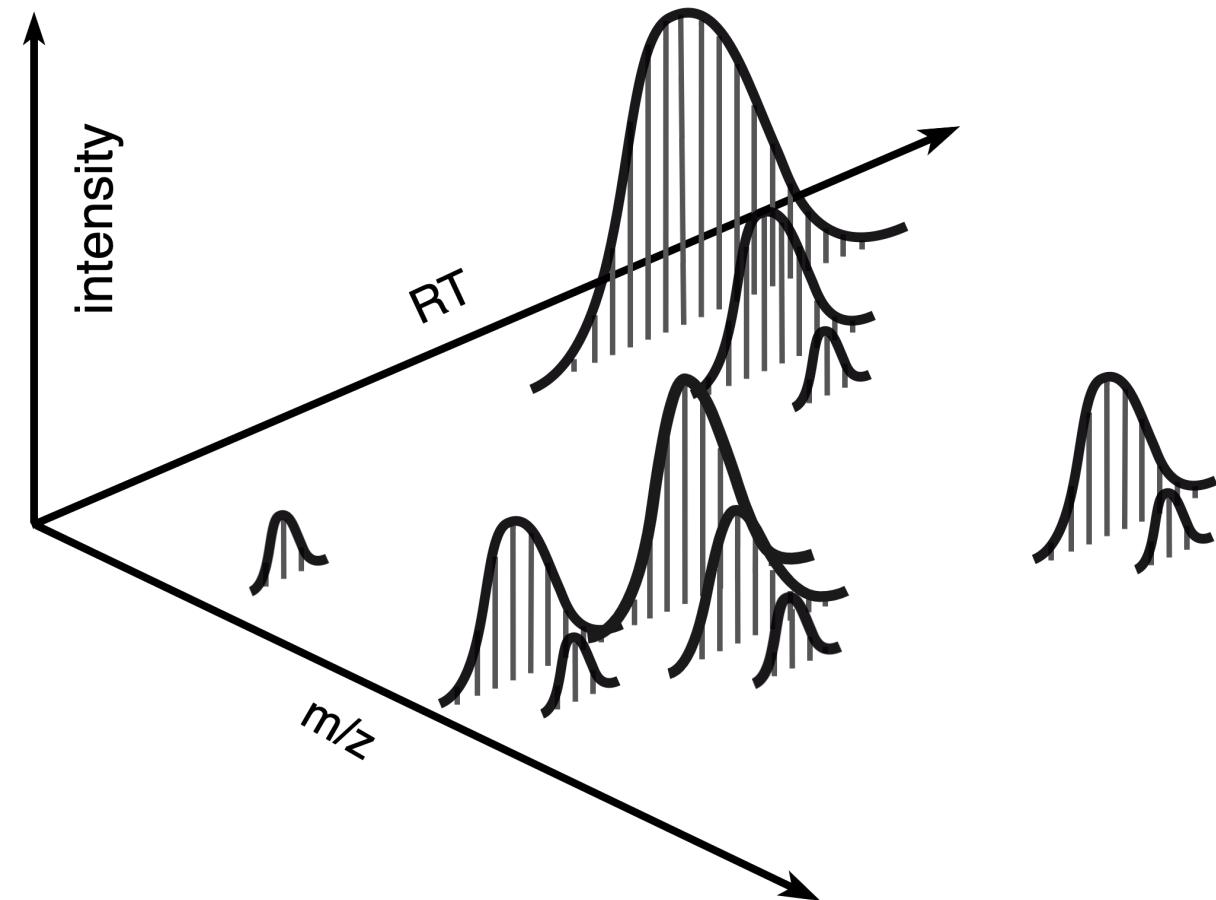
A mass trace extraction method that gathers peaks similar in m/z and moving along retention time.



MassTraceDetection

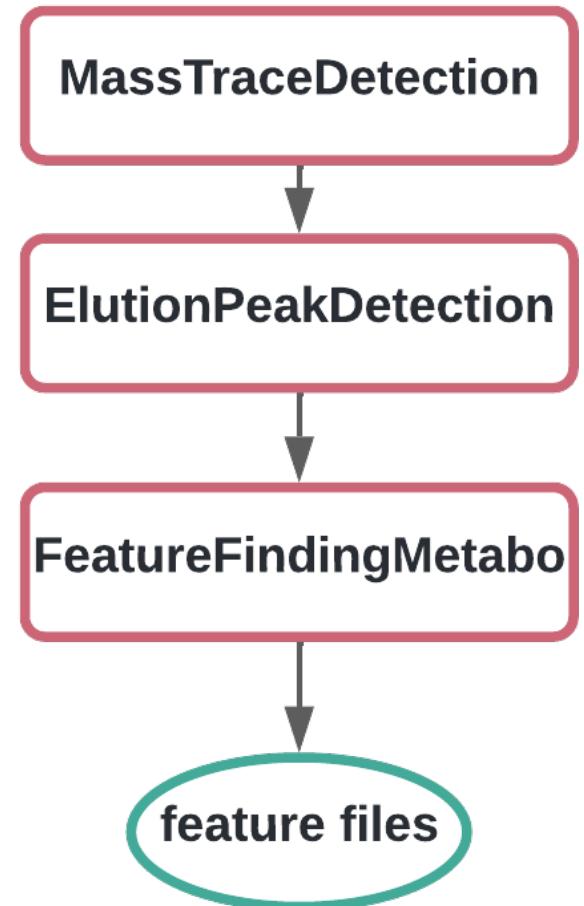
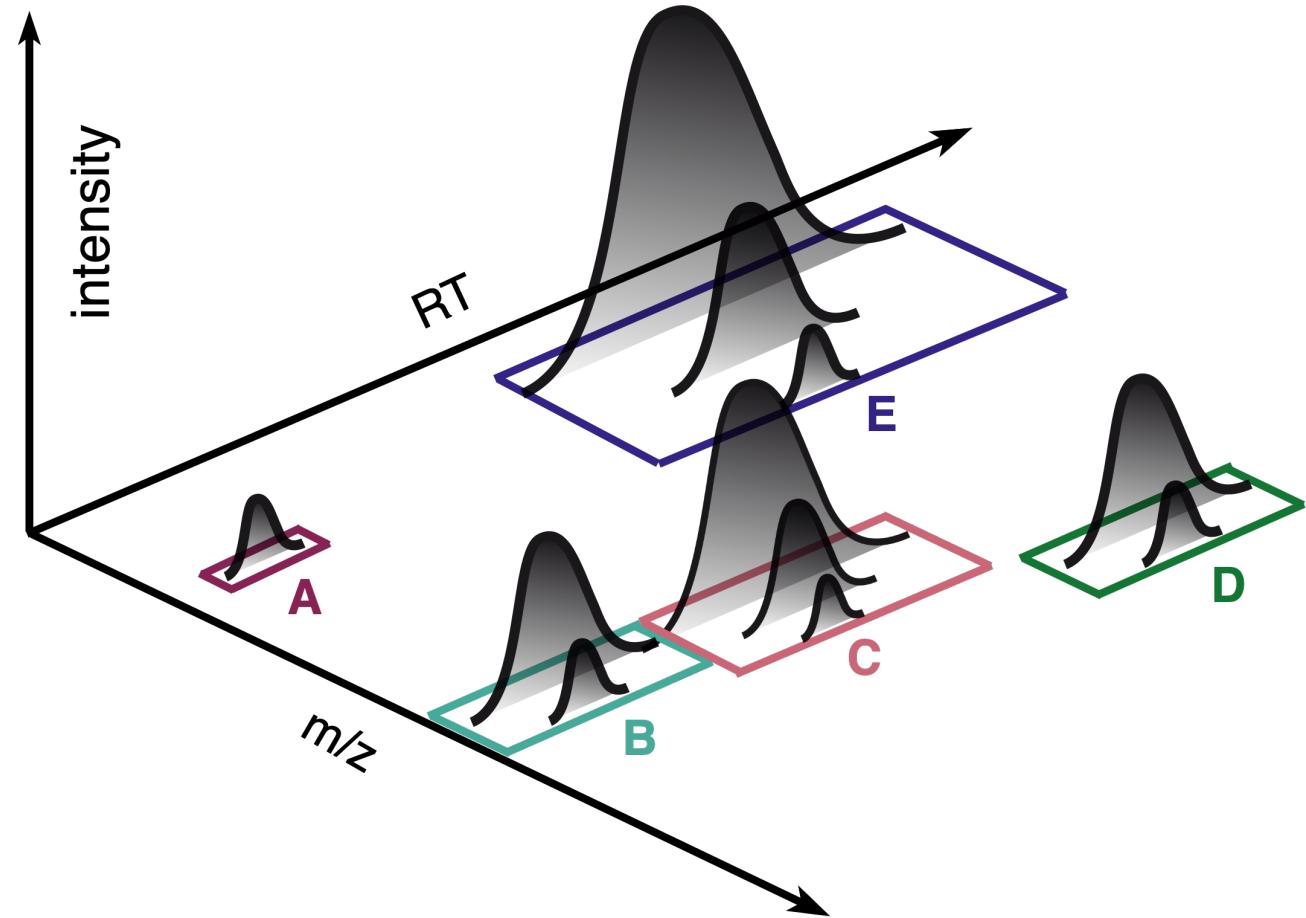
2. Elution peak detection

Extracts chromatographic peaks from a mass trace.

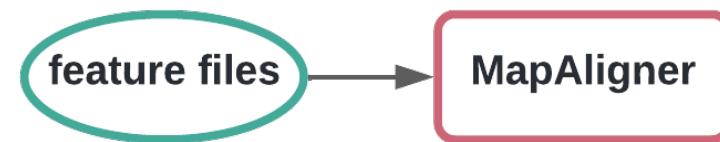


3. FeatureFinder

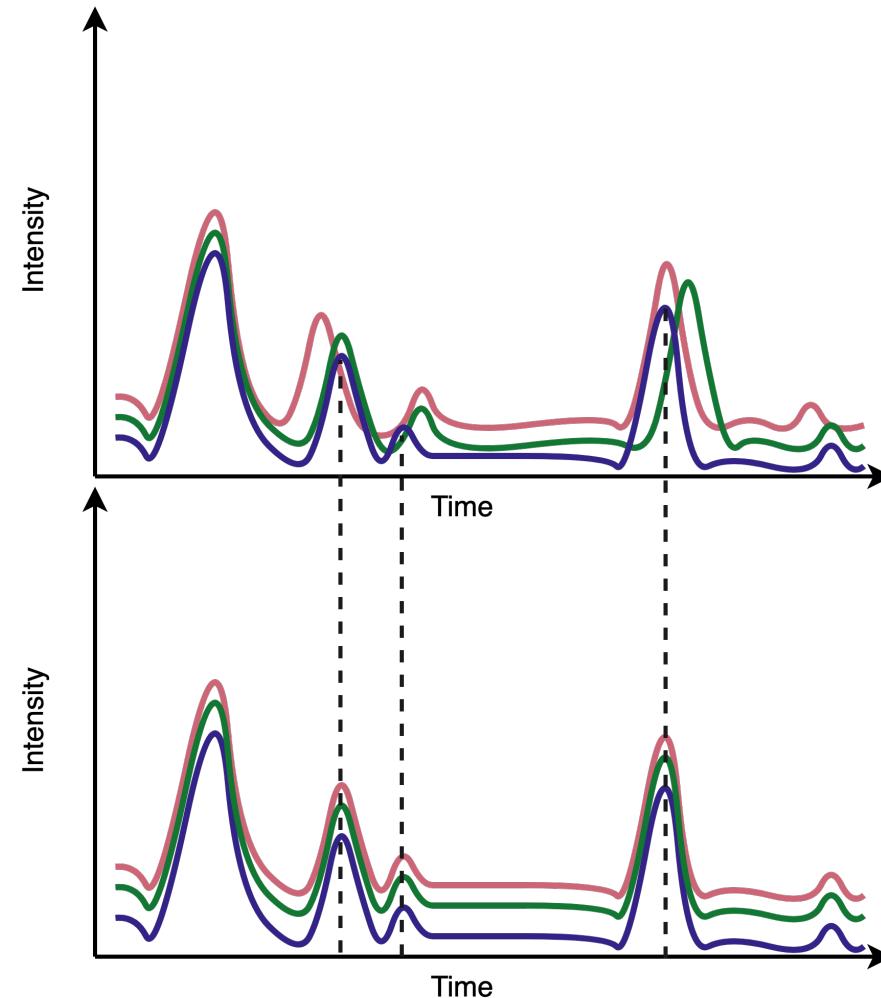
Assembles metabolite features from singleton mass traces.



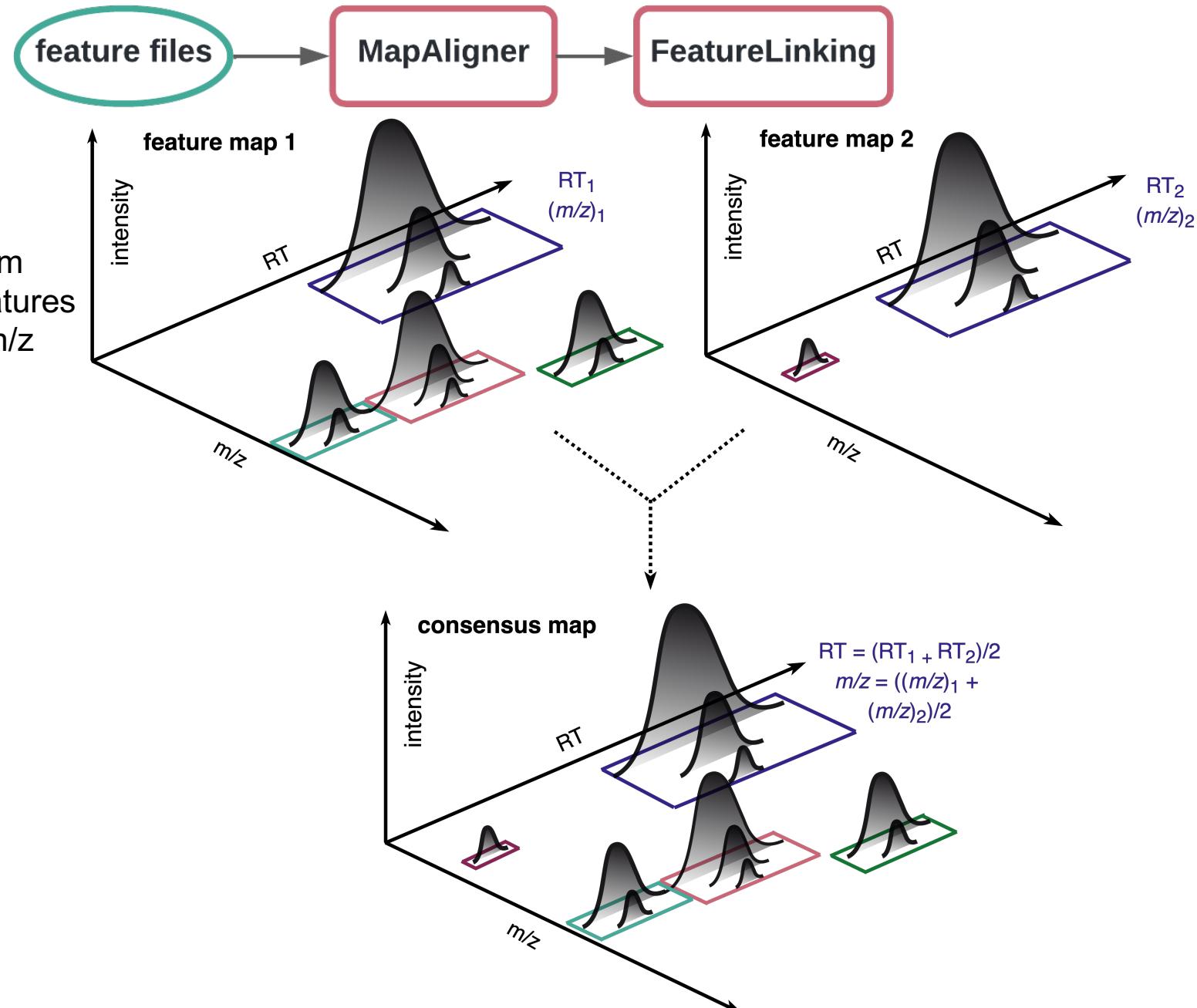
4. Map alignment



Performs a linear retention time alignment, to correct for linear shifts in retention time.

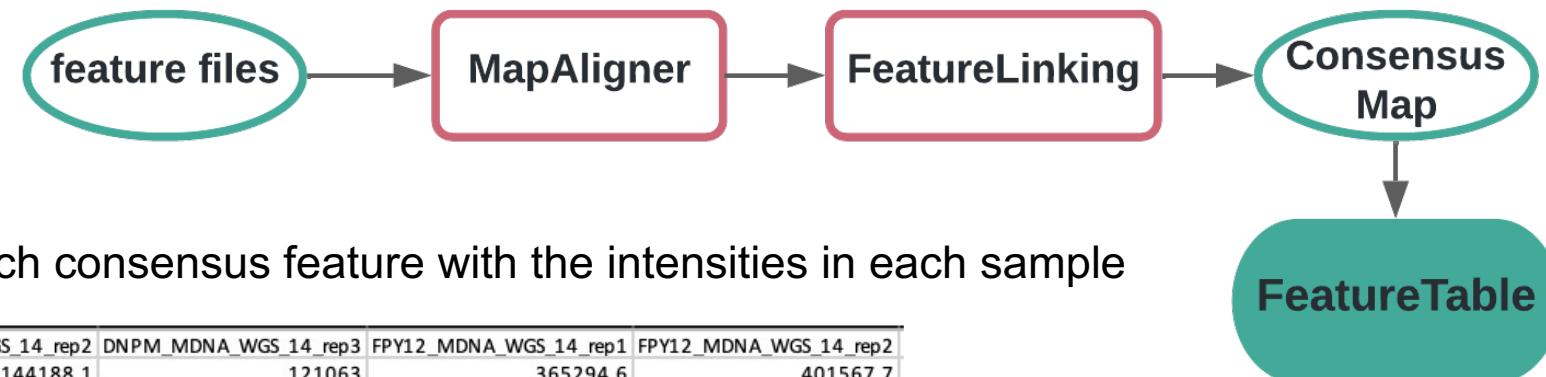


5. Feature linking



FeatureLinking clusters the feature information (from single files) into a ConsensusFeature, linking features from different files together, which have similar m/z and RT (MS1 level)

6. Table of features

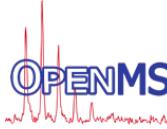


Dataframe with m/z and RT information from each consensus feature with the intensities in each sample

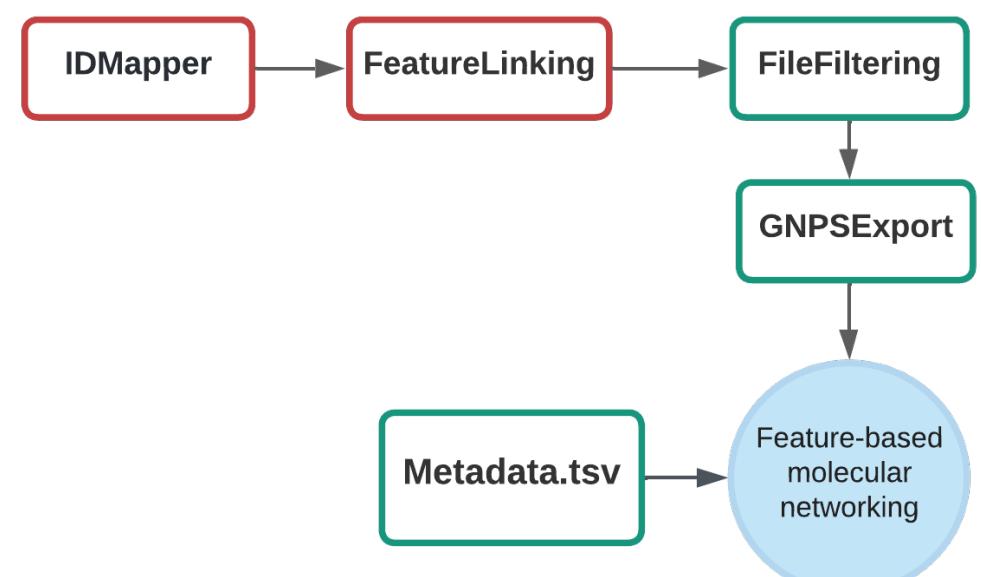
mz	RT	DNPM_MDNA_WGS_14_rep1	DNPM_MDNA_WGS_14_rep2	DNPM_MDNA_WGS_14_rep3	FPY12_MDNA_WGS_14_rep1	FPY12_MDNA_WGS_14_rep2
416.2637	347.19	136219.6	144188.1	121063	365294.6	401567.7
1441.839	389.335	540440.3	58655.34	408005.8	1033793	5176443
966.6142	394.971	284705.5	149096.8	140170	29289.28	247274.7
684.2365	67.7326	124401.3	32565.46	28127.74	932534.5	1040187
1423.831	405.735	2249281	1614707	1636310	2324051	37097830
705.9128	388.712	1686535	1109222	1095907	51761.03	838351.3
787.9419	394.949	115049.2	131840.9	76413.18	349033.8	7193028
1020.645	392.771	413206.9	266186.6	284185.1	195431.9	2686807
1047.42	398.641	272859.3	432108.1	533610.7	274664.6	7865175
1162.709	411.64	183676.2	131980.2	166135	148731.6	3300304
719.9283	410.999	695653.4	534247.3	484984.5	487374.9	4233446
1000.358	403.499	196254.7	153696.9	186016.1	29293.06	58768.09
206.1534	475.921	61302.61	22137.95	21526.22	15900.39	14530.26
819.9009	401.672	114797.2	106675.7	107820.8	399065.8	3710466
801.4605	391.291	61343.05	88985.27	61679.61	192527.3	5325955
1180.719	395.529	34702.9	61262.11	26275.12	104274.9	3261669
657.2782	61.5897	618277.2	380894.1	367958.9	8468848	28313430
988.6195	388.865	148853	137188.4	340571.3	6243.7017	1518407
807.2531	259.516	22518.6	15335.66	23392.15	2158250	1640954
1442.844	390.495	441195.7	370601.3	353536.6	877375.1	40558810

Suggestions after pre-processing

- **SiriusAdapter:** Generate formula and structural predictions with **SIRIUS** and **CSI:FingerID** (MS1& MS2) 

- **Adduct annotation:** Assign adducts & reconstruct neutral masses 

- **GNPSExport:** Generate all the files necessary for Feature-Based Molecular Networking (FBMN) and  Ion Identity Molecular Networking (IIMN)



<https://pyopenms.readthedocs.io/en/latest/index.html>

Kai Dührkop, Markus Fleischauer, Marcus Ludwig, Alexander A. Aksenov, Alexey V. Melnik, Marvin Meusel, Pieter C. Dorrestein, Juho Rousu, and Sebastian Böcker. [SIRIUS 4: Turning tandem mass spectra into metabolite structure information](#). Nature Methods 16, 299–302, 2019.

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Hands-on time!

- <https://github.com/axelwalter/pyopenms-workshop>



- Click on the

Visit our documentation:

<https://pyopenms.readthedocs.io/en/latest/>

The screenshot shows a dark-themed documentation page for pyopenms. On the left sidebar, under 'GETTING STARTED', are links for Installation, Import pyopenms, Getting help, and First look at data. Under 'MASS SPECTROMETRY CONCEPTS', are links for MS Data, Chemistry, Peptides and Proteins, Oligonucleotides: RNA, Fragment spectrum generation, and a link to GitHub.

The main content area has a light background. At the top, there are navigation links: a home icon followed by '» Feature Detection', a 'TRY ONLINE WITH' button, a 'BINDER' button, and an 'Edit on GitHub' button.

Metabolomics - untargeted

For the untargeted detection of small molecule features we can use the `FeatureFindingMetabo` with prior `MassTraceDetection` and `ElutionPeakDetection`.

```
from pyopenms import *
from urllib.request import urlretrieve

gh = "https://raw.githubusercontent.com/OpenMS/pyopenms-docs/master"
mzML_path = gh + "/src/data/FeatureFinderMetaboIdent_1_input.mzML"
urlretrieve(mzML_path, "ms_data.mzML")

exp = MSEExperiment()
MzMLFile().load("ms_data.mzML", exp)
```