Mestrelab Research

Affinity Screen 1.0

STARTING GUIDE

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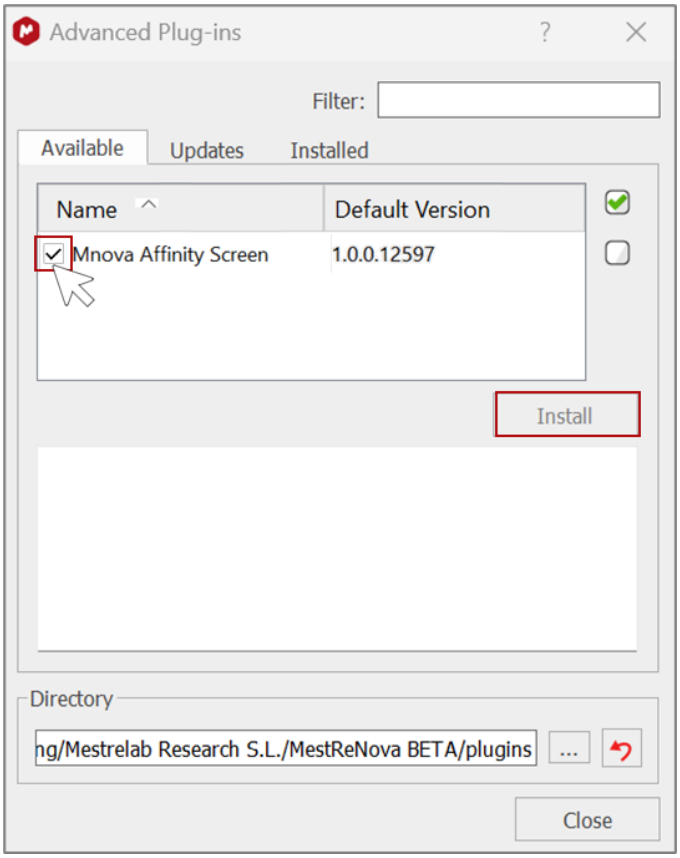
With the rapid advancements in proteomics research, Affinity Selection Mass Spectroscopy (AS-MS) has emerged as a powerful technique for studying protein-ligand interactions. However, the analysis of AS-MS data can be complex and time consuming, requiring specialized tools and expertise. That's where our software comes in. Affinity Screen is an automated solution that sorts input data files, processes the data, identifies and quantifies compound peaks, groups “bound”, “unbound” and “reference” samples together, and calculates a score to determine whether a molecule binds to a target or not. It generates human-readable reports where hits can be quickly identified, allows interactive visualization and review-by-exception of the results, and automatic recalculation of individual results and update of reports.

This comprehensive guide will walk you through the essential steps and functionalities of the software, empowering you to efficiently analyze and interpret your AS-MS experimental results.

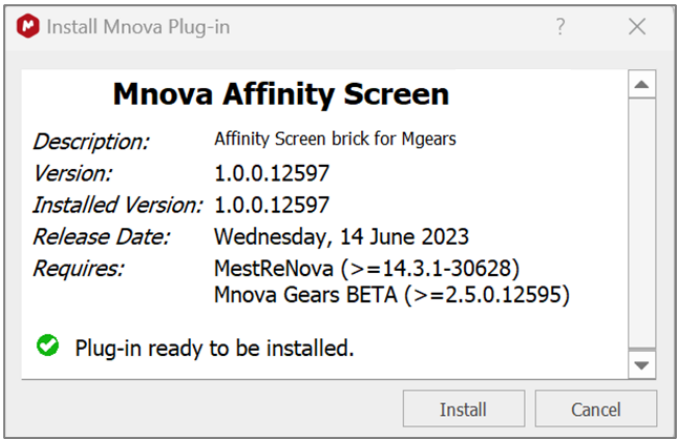
1. Installation

Before installing Affinity Screen, make sure you already have Mnova MSChrom (minimum version: 15.0) and Mgears (minimum version: 2.5) installed and running with valid licenses.

Go to **Files>Advanced Plug-ins>Available**. Tick the **Mnova Affinity Screen** plugin, then press **Install**.

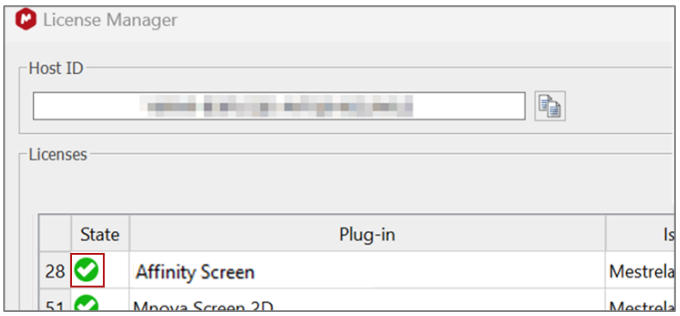


Another option is to drag and drop the Affinity Screen installer into the Mnova interface. The following dialog will open. Click on **Install**.



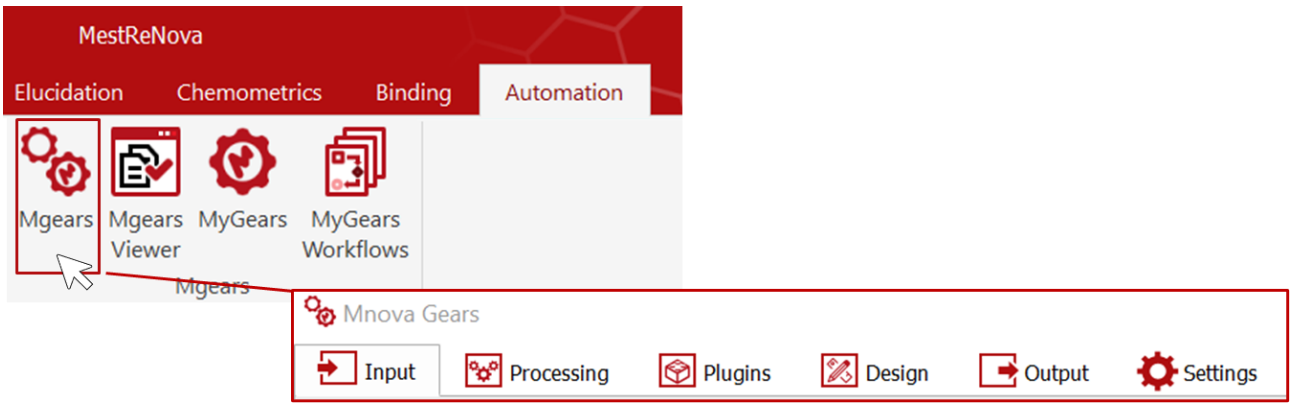
Restart Mnova.

Affinity Screen must now be installed. You can check your license status by going to **Files>Help>License Manager.** A green check must appear in the plugin's status column.



2. The workflow

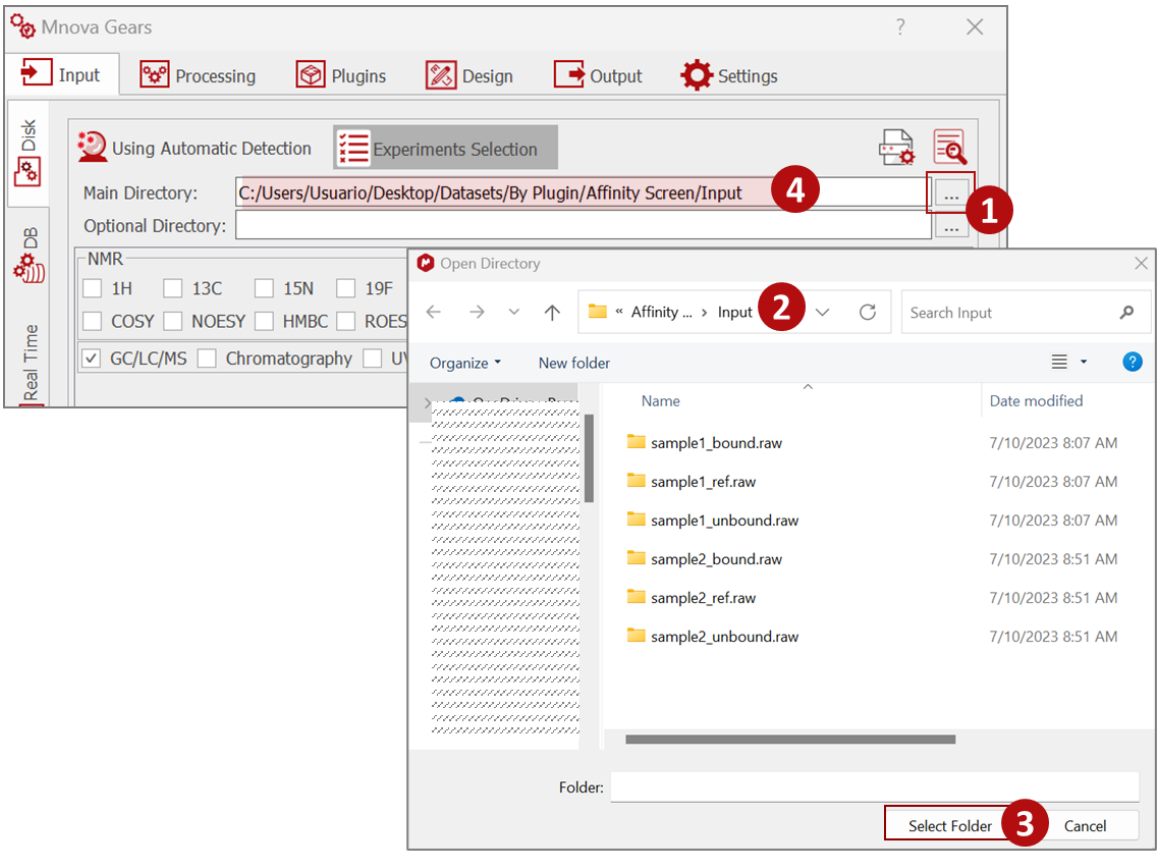
Launch Mgears from the Mnova **Automation** ribbon. The dialog with the usual six tabs will open.



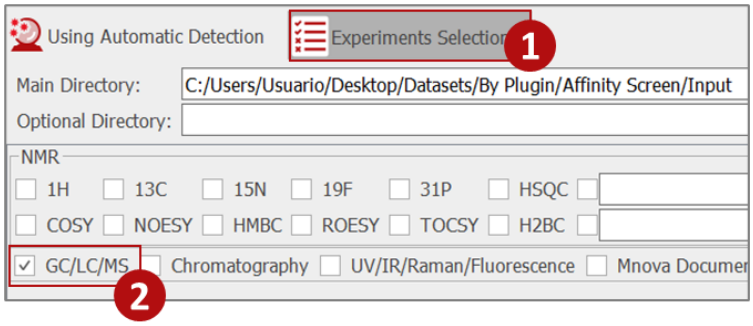
2.1. Input

Affinity Screen runs from within Mnova Gears and therefore follows the general setup workflow, starting with the input data. LCMS data can come from your local **Disk**, **Database**, or from **Real-Time** acquisition. In this guide, we will work with data from disk directories *(please refer to the Mnova Gears manual for more details about other input types).*

Click the ... button located next to the **Main Directory** box to choose the folder where your data files are stored. Once you've navigated to the desired folder, click **Select Folder** and the path will be displayed on the screen.



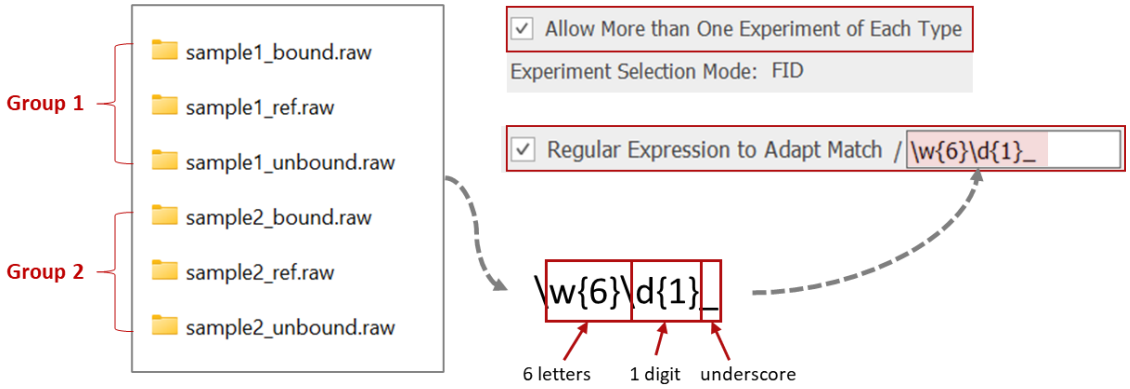
The detection of experiment files can be achieved manually by selecting the experiment type(s) and providing **Path Masks** to the relevant data, or automatically via Mgears. When using the **Automatic Detection** mode, **Experiment selection** is recommended if your data folder contains different types of data files to restrict detection to GC/LC/MS and avoid analysis of other undesired files. In this case, you must select the **GC/LC/MS** experiment type as shown below.



To group the datasets of the bound, unbound, and reference ligands for each sample, you must:

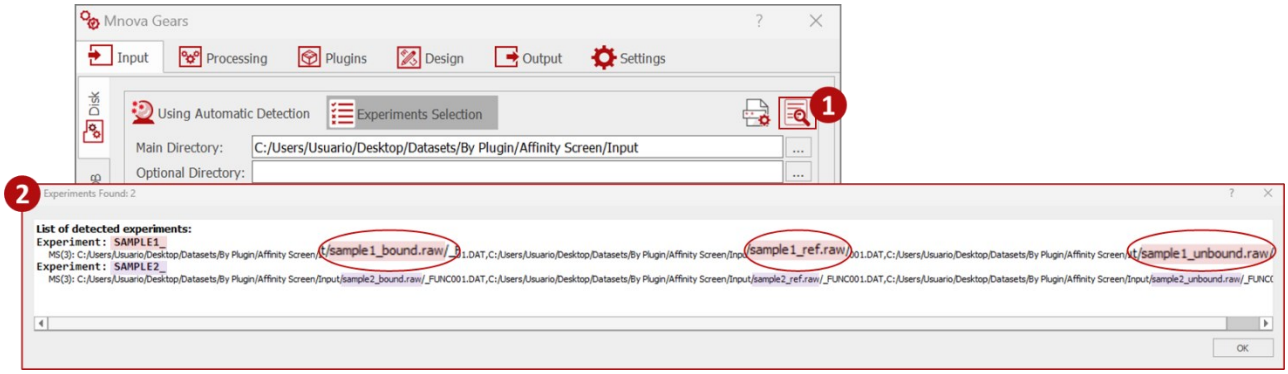
- **Allow More than One Experiment of each Type** to be detected by Mgears.
- Enable the **Regular Expression to Adapt match** option and type a regular expression that would allow the capture of the sample files to be grouped in a single experiment.

These two options are available under the **Advanced options** in the **Input** tab. In the example we show here, the datasets can be grouped using a regular expression that captures the first part of the files name, i.e., "sample1_".



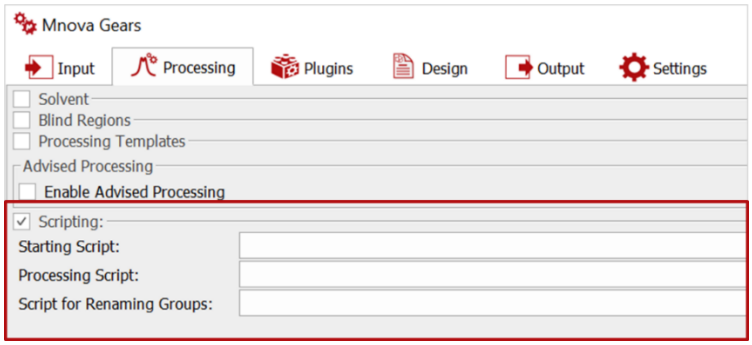
You can also opt for a custom script to organize the input files as needed instead of using a regular expression.

Use the automatic inspection button to check that Mgears has grouped the detected data as required.



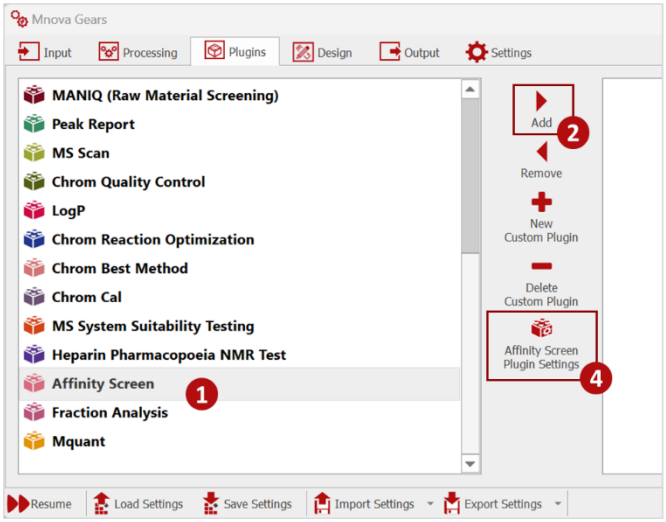
2.2. Processing

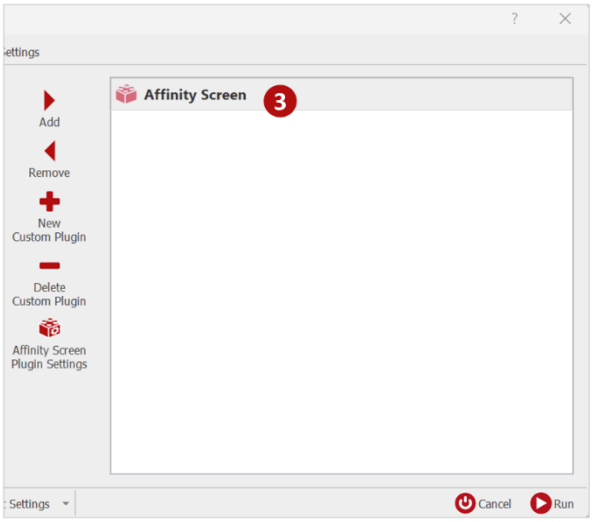
In the **Processing** tab, you can upload a script to apply customized processing options. This step is completely optional.



2.3. Plugins

In the **Plugins** section, select and add the Affinity Screen plugin. Then, click on **Affinity Screen Plugin Settings** to configure your analysis and reporting method.





A dialog with four main tabs should appear: the Input, Analysis, Quality Controls, and Output tabs.

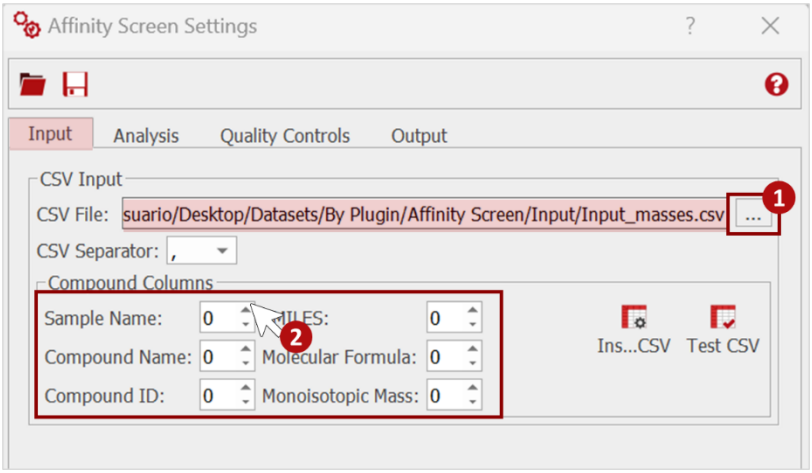
2.3.1. The Input tab

To function correctly, Affinity Screen relies on molecular information about the mixture compounds (potential ligands) to assign MS peaks and generate analytical EICs. This information must be provided in a CSV file and must include details such as Molecular Formula, Smiles, or Monoisotopic Weight.

To configure the CSV file, click on ... and select your input file, then proceed to assign the appropriate columns for each parameter listed below:

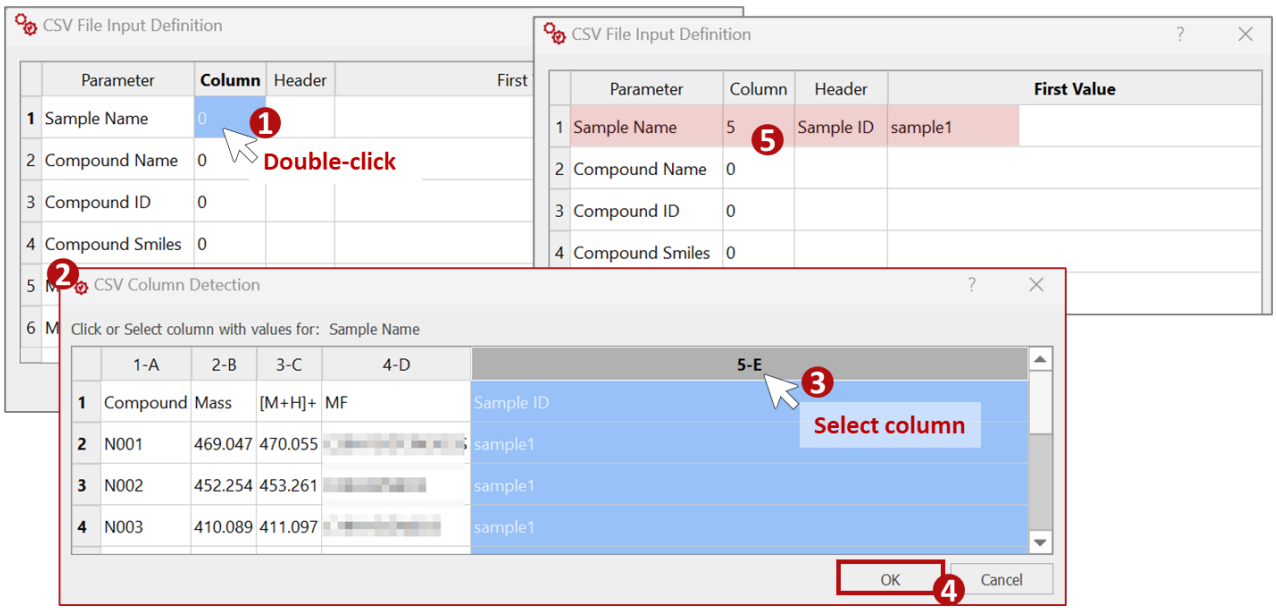
- **Sample name**: names of the sample (mixture of compounds/ligands) as indicated in the LCMS datafiles.
- **Compound name**: names of the compounds in the mixture.

- **Compound ID**: identifiers for the compounds in the mixture (optional).
- **SMILES**: used to calculate m/z. If available, the molecular structures of the compounds will be available in Mgears viewer & reports (optional if Molecular Formulas or Monoisotopic Masses are provided).
- **Molecular Formula**: used to calculate m/z (optional if SMILES or Monoisotopic Masses are provided).
- **Monoisotopic Mass**: used to calculate m/z (optional if Molecular Formulas or SMILES are provided).



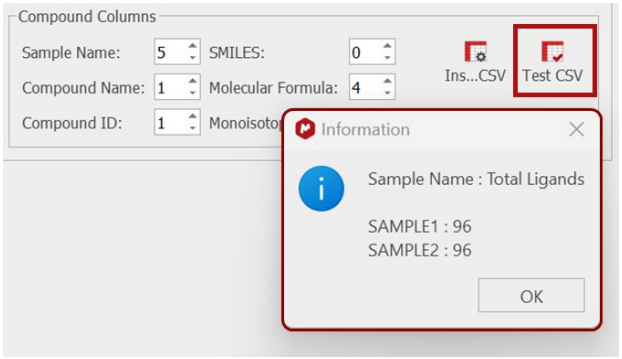
Important! Remember that in order to be correctly read by Mgears, the CSV separator (comma, semicolon, tab, or vertical line) should be correctly configured in the Mgears **Settings** tab.

To help you assign the columns correctly, you can click on **Inspect CSV**. A dialog box will appear, displaying a table with the following information: **Parameters**, assigned **column**, column **headers** in the CSV, and the **first value** of each column. Double-click on the **Column** cell (1). This action will open a preview of your CSV input file (2). From there, select the desired column (3) and click **OK** (4). The selected column number will appear in the parameters table (5).



Once all the **Compound Columns** are configured, you can test the validity of the CSV and its configuration by pressing the **Test CSV** button. Mgears will match the sample names with the experiment files and return the

number of ligands found for each sample. If this number is correct, you can move to the next tab; otherwise, you will need to review and correct your CSV configuration details.



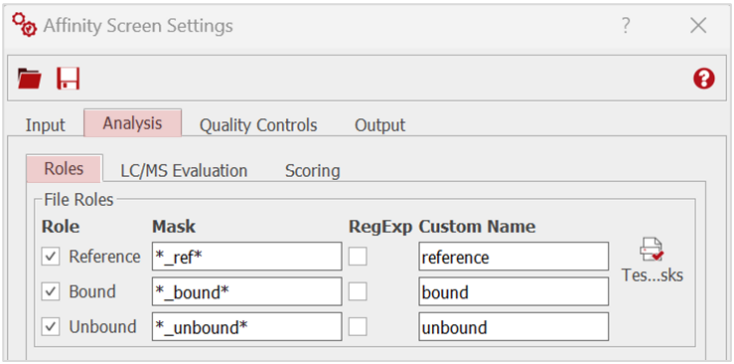
2.3.2. The Analysis tab

2.3.2.1. The Roles tab

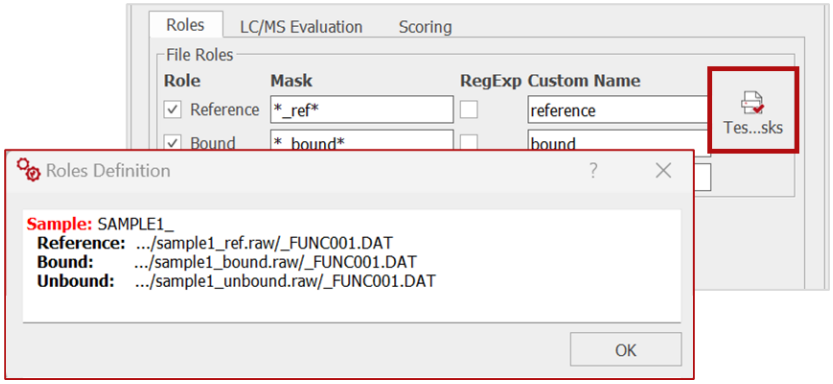
In the **Roles** tab, you need to provide the necessary information to assign a specific role to each dataset, such as *Reference*, *Bound*, or *Unbound* ligands samples.

To enable a role, simply check the corresponding checkbox and then enter a wildcard string or regular expression that matches the LCMS datasets. If you choose to use a regular expression, make sure to select the **RegExp** option.

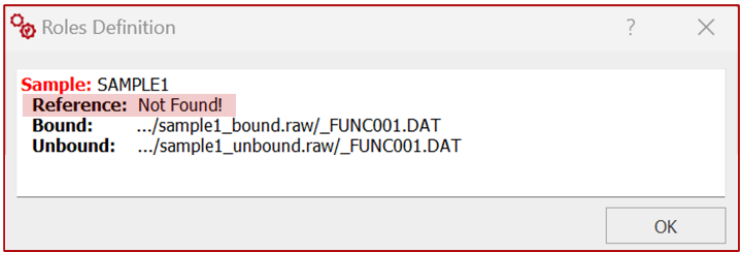
Additionally, you have the option to customize the name for each role. By providing a **Custom Name**, this will be used in the reports and Mgears Viewer instead of the default role names.



After configuring the roles, you can test the validity of the masks by clicking the **Test Masks** button. Mgears will attempt to match the first experiment's data files with the defined roles and display the results.



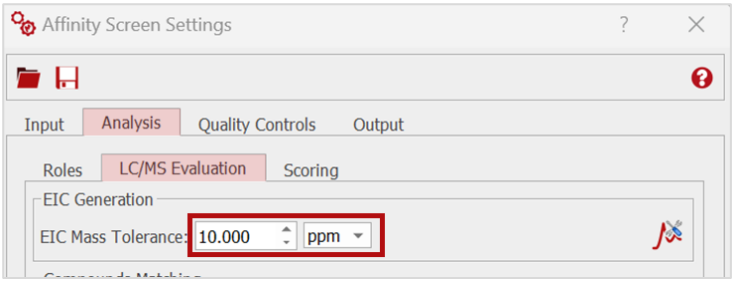
In case a data file for a specific role is not found, the label "Not found!" will be shown next to that role. This can occur if a mask does not match the file name, for example.



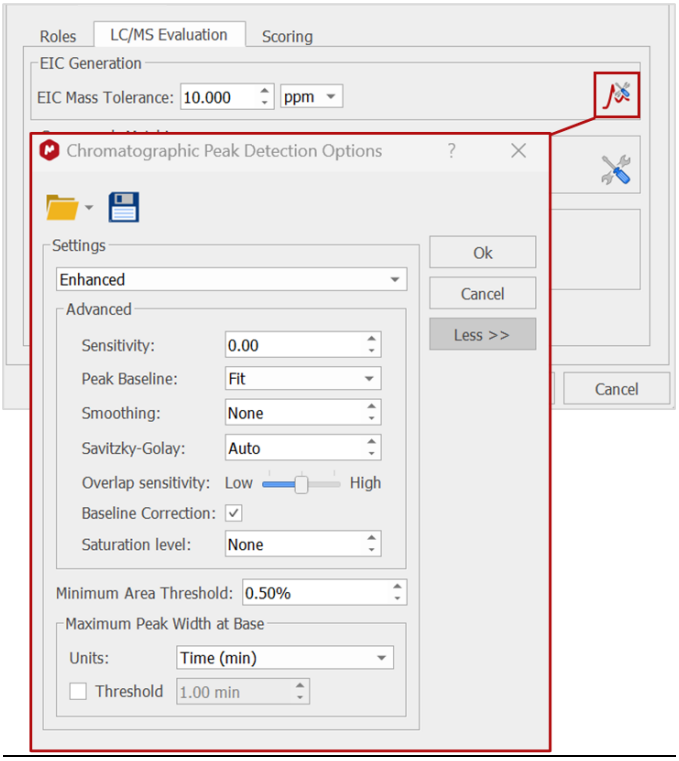
2.3.2.2. The LC/MS Evaluation tab

The EIC Generation

Configure the EIC extraction settings. Set the **EIC Mass Tolerance** that will be used to generate the EIC traces used for quantification.



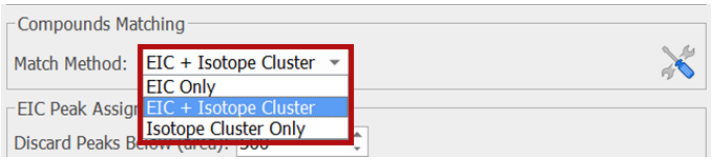
Click on the  button to access chromatogram peak detection options, including Minimum peak area threshold, Sensitivity & smoothing, and Peak width at base. *(Please, refer to the Mnova manual for a detailed explanation about the peak detection options.)* Click **OK** to save options.



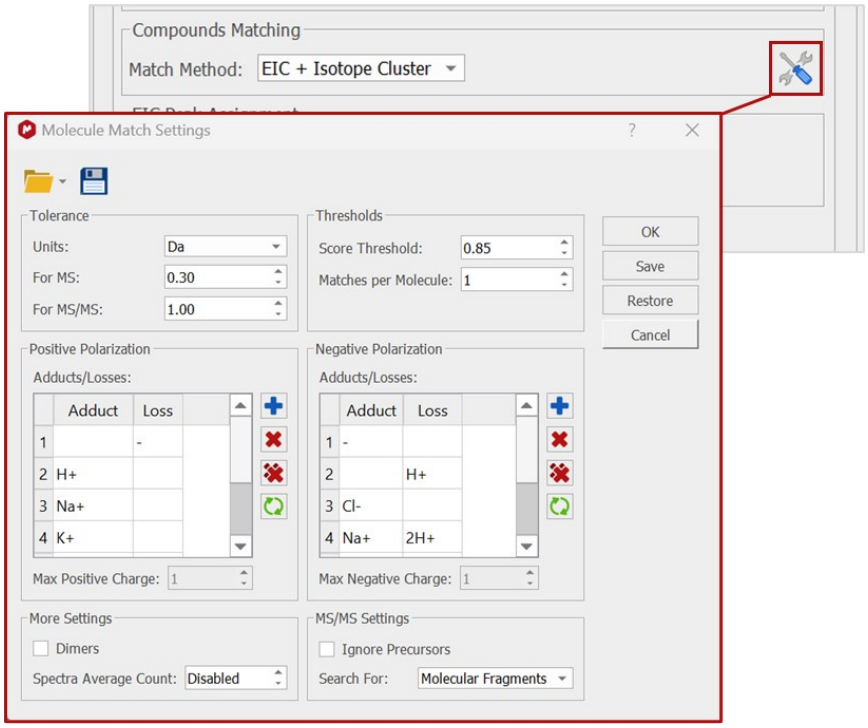
Compound Matching

Select a method for compound mass matching using the EIC. Three methods are available:

- **EIC only.** The largest peak in the EIC of the target mass is matched.
- **EIC + Isotope cluster.** If there are multiple peaks in the EIC (which may result in ambiguity in assignment), the peak identification will utilize the best-scored peak using the Molecule Match feature.
- **Isotopes only.** The Molecule Match feature is employed to identify peaks in the EIC of the different roles.



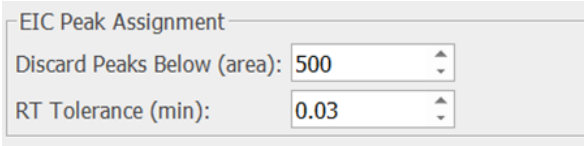
Click on the button to open and review the **Molecule Match Settings**.



The EIC Peak Assignment

Now configure the following options for peak assignment in the EICs from the different roles:

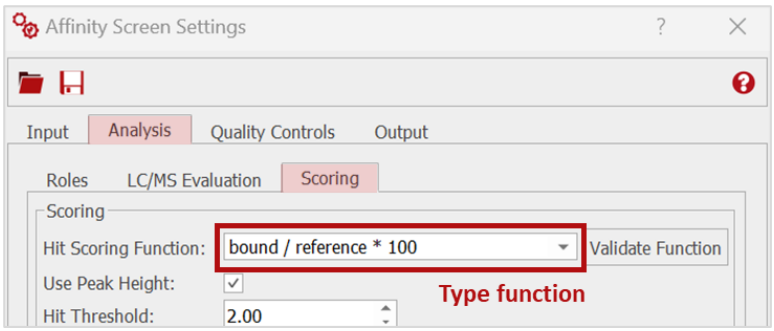
- **Discard Peaks Below (area):** EIC peaks below this threshold are discarded. This setting is used to distinguish real peaks from noise. Setting this parameter too high will lead to more false negatives, and too low to more false positives.
- **RT Match Tolerance (min):** Only peaks within this tolerance around the matched RT will be assigned. Setting this parameter too small can lead to false negatives.



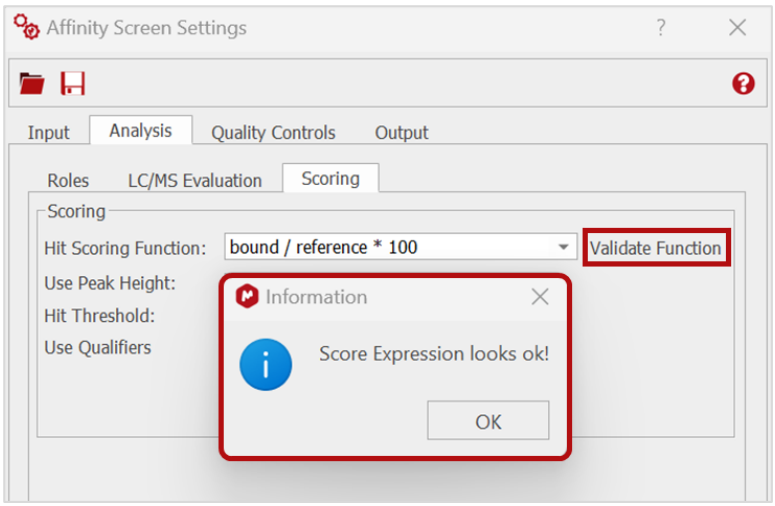
Note. We recommend tuning the parameters **Discard Peaks Below** and **RT Match tolerance** with a smaller sample set before evaluating the entire batch as these settings significantly affect the accurate assignment of EIC peaks. Utilize the **Peak Assignment Failures** control for this purpose.

2.3.2.3. The Scoring parameters

The **Hit Scoring Function** determines how the hits are scored and compared in the analysis. The default scoring function is the ratio of "*Bound/Unbound*". However, you have the flexibility to define any mathematical function using the different roles defined in the analysis, such as *Bound*, *Unbound*, and *Reference*. For example, you can define a custom scoring function like "*Bound/Reference x 100*" to compare the percentage of bound compounds relative to the reference sample.



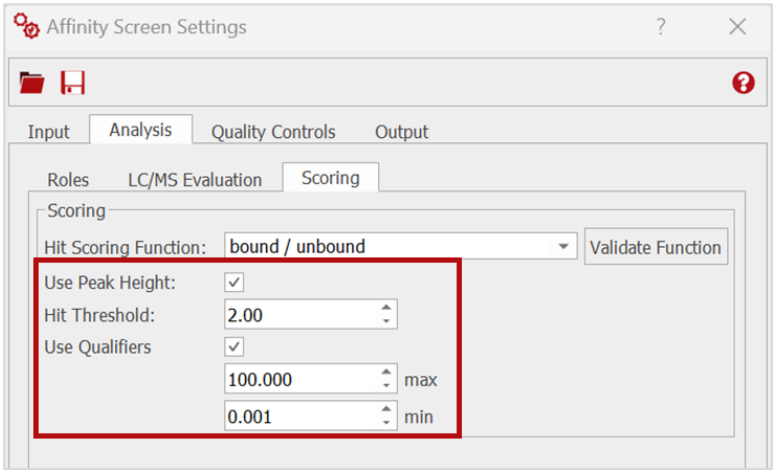
To test and validate the scoring function, simply click the **Validate Function** button. This will ensure that the scoring function is properly defined and can be used in the analysis.



Configure the following options, which will affect the scoring calculation and determine which peaks should be considered:

- **Use Peak Height**: if enabled, the peak height will be used in the score calculation instead of the peak area.
- **Hit Threshold**: this value sets the score limit above which a component is flagged as a HIT.
- **Use Qualifiers**: if enabled, a maximum and a minimum threshold for the calculated score must be set. These qualifiers help classify ligands based on their scoring values:
  - Above the defined "max" value: If a ligand's score is above the maximum threshold, the HIT score reported will be indicated as "> max".

- Below the "min" value: If a ligand's score is below the minimum threshold, the HIT score reported will be indicated as "< min".



2.3.3. The Quality Controls tab

In the **Quality Controls** tab, you can establish a set of controls to be executed on every ligand within the samples. Any controls that do not pass will trigger a flag for the specific ligand, which will be prominently displayed in the Affinity Screen Viewer. Moreover, when the percentage of ligands failing the same test within a single sample exceeds a predefined threshold, a flag is also raised at the sample level, providing visibility within the Mgears Viewer.

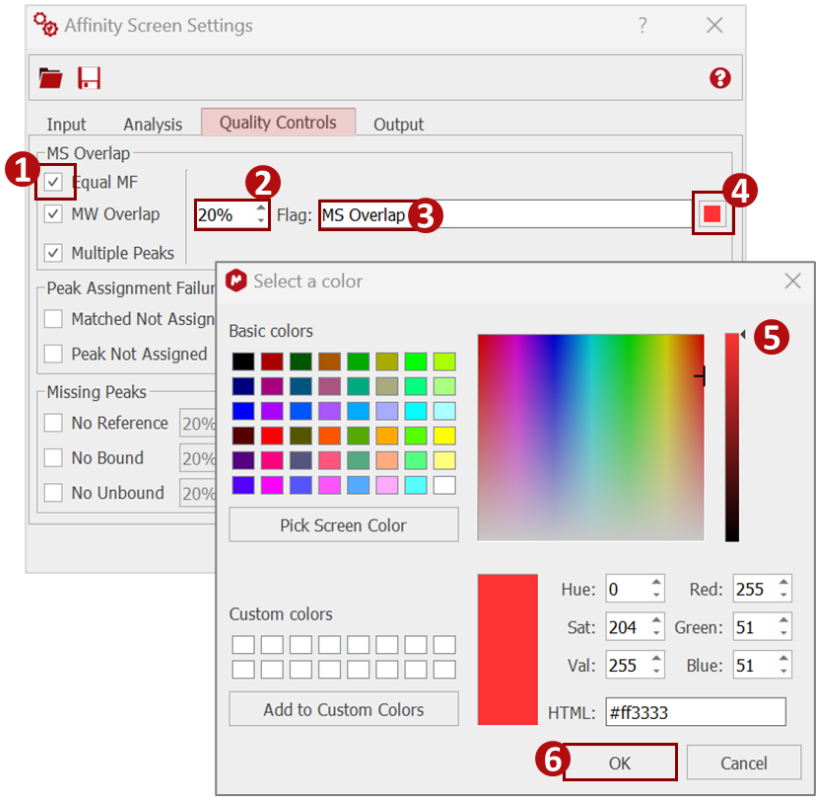
2.3.3.1. MS Overlap

The **MS Overlap** control aims to identify and report situations that result from the overlap in the MS spectra of different compounds, and which can lead to incorrect peak assignments. Such overlaps can occur when ligands have identical molecular formulae and/or monoisotopic masses.

There are three test options available, which can be configured by setting a threshold and a flag text:

- **Equal MF**: if enabled, a flag will be raised when a ligand has the same molecular formulae as another in a given sample. This test will only work when SMILES or Molecular Formulae are provided in the CSV Input.
- **MW overlap**: if enabled, a flag will be raised when a ligand has the same monoisotopic mass as another within the defined EIC RT range (as determined by the EIC tolerance).
- **Multiple Peaks**: if enabled, a flag will be raised when a ligand has more than one peak in the EIC. Note that the additional peaks are counted if their areas are above 30% of the assigned peak's area. This test is primarily conducted in the Reference sample EIC. However, if the Reference sample EIC is not available, the test is performed in the Bound or Unbound sample EIC instead.

To configure and enable the control, select the test you want to run (1), set the threshold (2), and enter the flag text you want to be displayed when the control raises a flag (3). Then, select a color to represent the flag that will be raised when the condition is met (4, 5, 6).

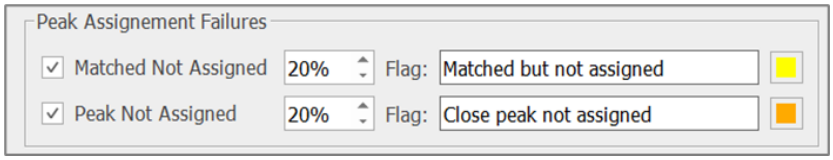


2.3.3.2. Peak Assignment Failures

The **Peak Assignment Failures** control allows you to identify and flag peak assignment issues.

There are two options available which can be configured by setting a threshold and a flag text:

- **Matched Not Assigned**: if enabled, a flag will be raised when a matched ligand has peaks that are not assigned.
- **Peak Not Assigned**: if enabled, a flag will be raised when a matched ligand has peaks that are discarded. This control can be used to tune the correct values for the "Discard Peaks Below" and "RT Match Tolerance" settings and avoid peaks being discarded that could be assigned to the ligand.

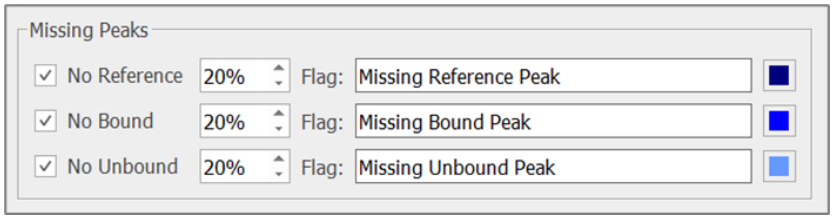


2.3.3.3. Missing Peaks

The **Missing Peaks** control aims to report situations where there is no peak assigned to the compound, while it was expected to have a peak.

There are three test options available, each of which can be configured by setting a threshold and a flag text:

- **No Reference**: if enabled, a flag will be raised when a component is not identified in the *Reference* sample TIC.
- **No Bound**: if enabled, a flag will be raised when a component is not identified in the *Bound* sample TIC.
- **No Unbound**: if enabled, a flag will be raised when a component is not identified in the *Unbound* sample TIC.

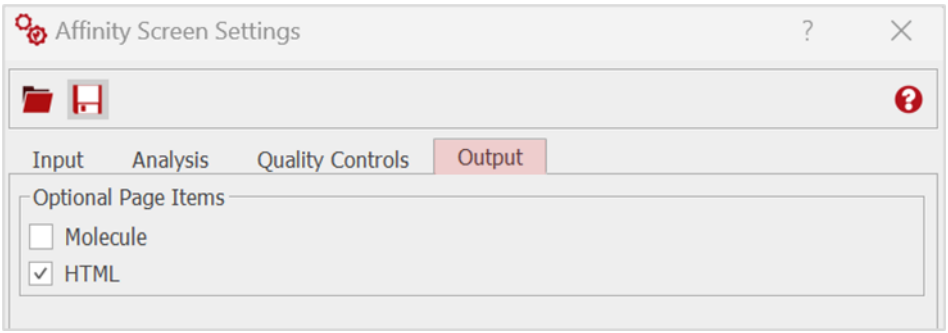


2.3.4. The Output tab

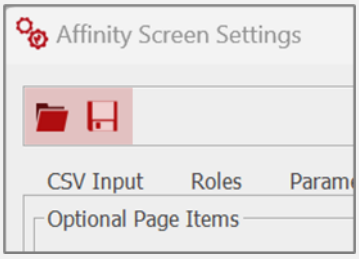
In the **Report** tab, you have the option to include the **Molecule** and/or a small **HTML** report to enhance your Mnova report pages.

- **Molecule option:** This option is only available when SMILES strings are provided in the CSV Input file. By selecting this option, the compound's molecular structure will be included in the report.
- **HTML option:** By selecting this option, a small HTML report will be added to each Mnova report page.

Regardless of the options selected, the molecular structure and the HTML report details will always be displayed in the result viewers.



Top tip! The analysis settings you just configured can be saved and reused in future analyses. Press the **Save** button on the top left side of the settings dialog box, then choose a location and press **Save**.

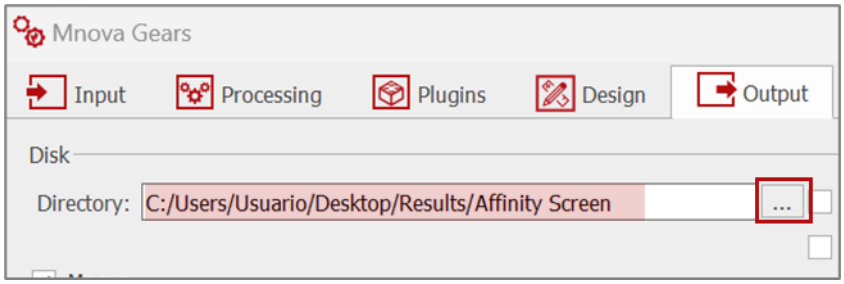


The next time you need to run Affinity Screen, you only need to press the folder button on the top left side of the settings dialog box, choose your settings file (*.data file) then press **Open**. Your saved settings will be loaded into the settings dialog. All you need to do now is to click **OK** and move to the next steps.

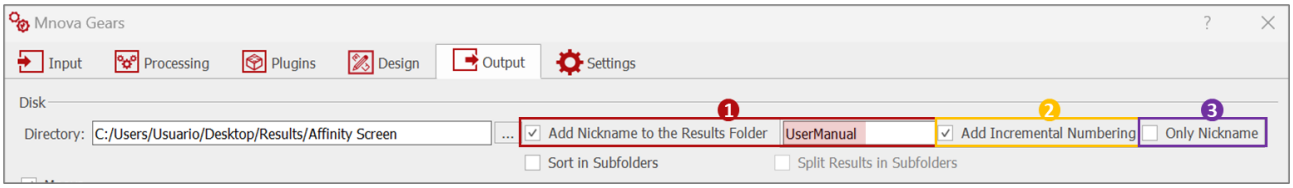
Now, if you are happy with the results you can click on **OK**, finalize your plugin settings setup, and move to the next step.

2.4. Output

Here, you must choose a directory in which to save your analysis results. Click on the ... button and select a results folder on your disk.



Optionally, enable the **Add Nickname to the Results Folder** and type the nickname of your choice (1), **Add Incremental Numbering** to your results folder (2), and/or decide to use **Only the Nickname** in the folder's name (3).



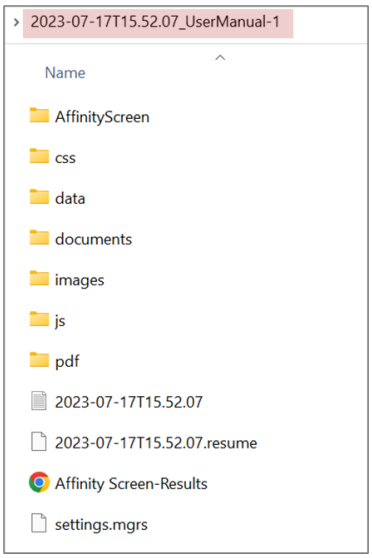
You can also choose to create an Mnova document, a PDF, or to save your results to a database.

Note. We strongly advise enabling the option to generate **Mnova** document output, particularly if you intend to examine or review the spectra alongside the results in the Mgears Result Viewer. Without this option enabled, when you load the results in the Mgears Result Viewer, only the data will be visible, without any spectra.

Once the configuration has been completed to your satisfaction, click on **Run** Run to launch the analysis.

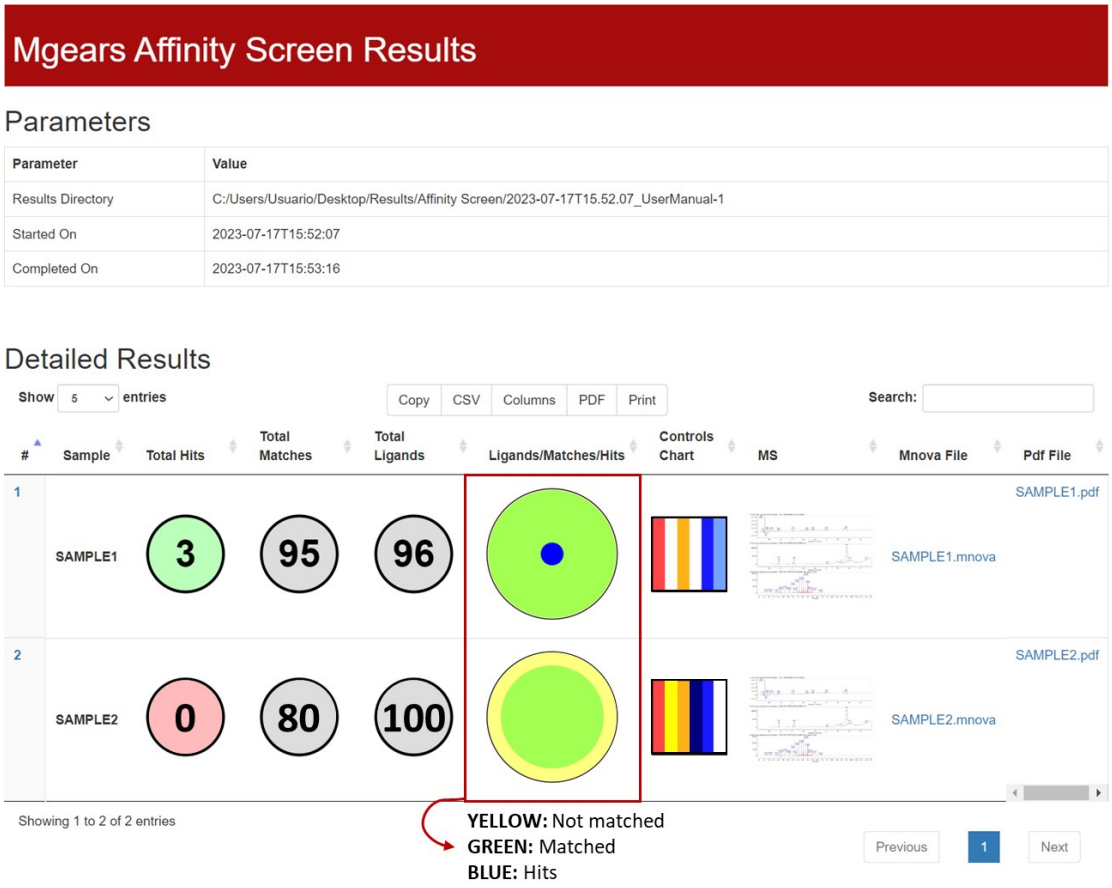
3. The output folder

The results folder is saved under the previously specified directory, as described above.



3.1. The HTML file

The HTML report offers a comprehensive overview of the results, including the total number of hits, matched ligands, and all ligands tested. It features visual graphics presenting the fractions of ligands, matches, and hits, as well as control charts. Moreover, the report provides convenient links to access the **Mnova** and **PDF** result files for further examination.



3.2. The PDF and Mnova

The PDF and Mnova documents, saved in the “Pdf” and “documents” subfolders, respectively, have similar structures and follow the applied layout template (default or custom one).

The default layout shows:

- The EICs from the *Reference*, *Bound*, and *Unbound* roles for each ligand on a separate page.
- The small HTML report if that option has been enabled in the Report tab.
- The molecular Structure if that option has been enabled in the Report tab (only possible when SMILES strings are provided in the CSV input file).

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3.3. The CSV file

The CSV file is saved under the “Affinity Screen” folder and includes information about the ligands, their peak area, and height in each condition (*Reference*, *Bound*, and *Unbound*). Additionally, it contains data about their status (Hit, Matched, Not Matched) and the score they achieved.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Sample Name	Ligand ID	MZ	Qualifier	Score	Status	rt	reference Area	bound Area	unbound Area	reference Height	bound Height	bound Hei	Comment
2	SAMPLE1	N033	387.1285	>	100	Hit	2.186698	49924.02148	5276.289307	0	10348.53679	1214.978052	0	no unbound peak
3	SAMPLE1	N071	342.1561		13.506	Hit	2.5861	566820.5325	64897.69727	2744.11499	79318.5572	10373.76972	768.0938	
4	SAMPLE1	N072	328.1656		2.354	Hit	3.244148	254676.6431	14339.27026	2535.427734	53110.33771	3688.916001	1566.849	
5	SAMPLE1	N018	429.118		0.781	Matched	2.587357	853041.5034	61885.93835	74413.4491	115483.4798	9653.97855	12357.67	
6	SAMPLE1	N019	429.118		0.781	Matched	2.587357	853041.5034	61885.93835	74413.4491	115483.4798	9653.97855	12357.67	
7	SAMPLE1	N063	453.1632		0.487	Matched	3.158503	150286.4386	628.8724365	3998.208984	28071.77282	539.8246342	1108.592	
8	SAMPLE1	N081	397.0884		0.396	Matched	2.911691	863767.2256	26802.87122	74201.59302	112182.176	4330.561684	10925.95	
9	SAMPLE1	N001	470.0547	<	0.001	Matched	2.916657	131299.3298	0	2009.771484	17165.05993	0	702.9165	no bound peak
10	SAMPLE1	N004	411.1275	<	0.001	Matched	2.50791	315243.9277	0	1564.62793	51297.30781	0	701.8115	no bound peak

3.4. Other output

- A “documents” directory, containing the output Mnova files (unless Mgears is configured to save Mnova files in another location).
- A “pdf” directory, containing the output PDF files (unless Mgears is configured to save PDF files in another location).
- A log file of the execution.

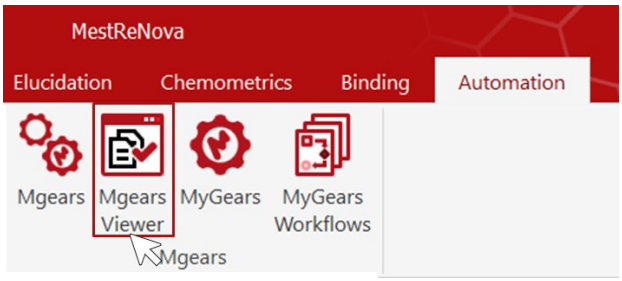
- A copy of the settings used in the current evaluation.
- A resume file of the steps followed in the execution.
- A CSS folder, a data folder, a JS folder, and an images folder.

4. Mnova Gears Results Viewer

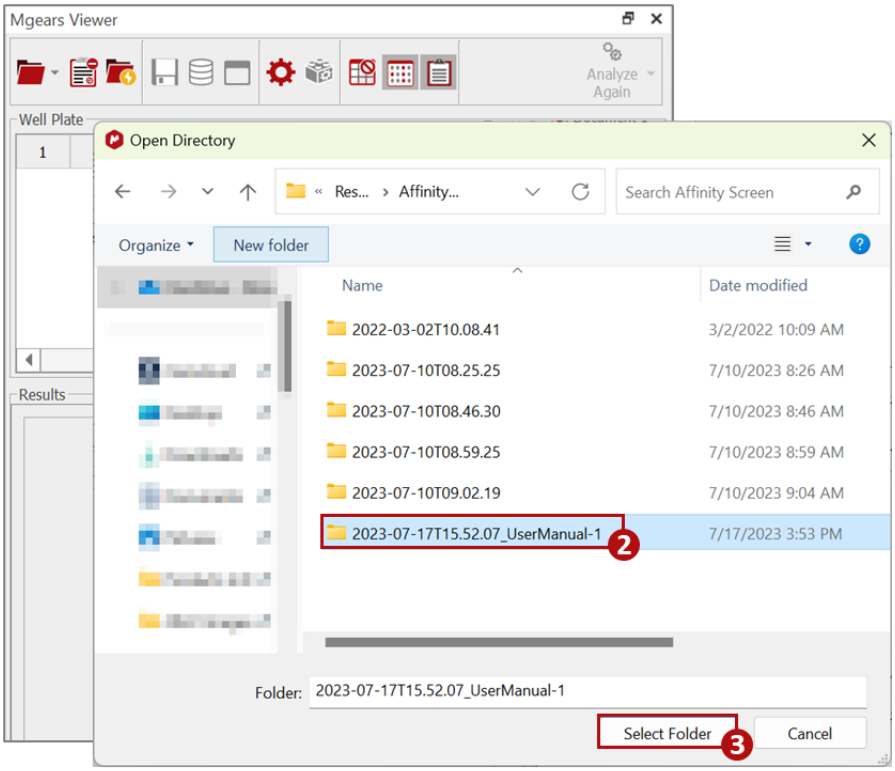
Affinity Screen has two different – but interconnected – result viewers, the Mgears Viewer, which displays overall results for the sample/well, and the Affinity Screen Viewer, which displays individual ligand results for each sample.

4.1. The Mgears Viewer

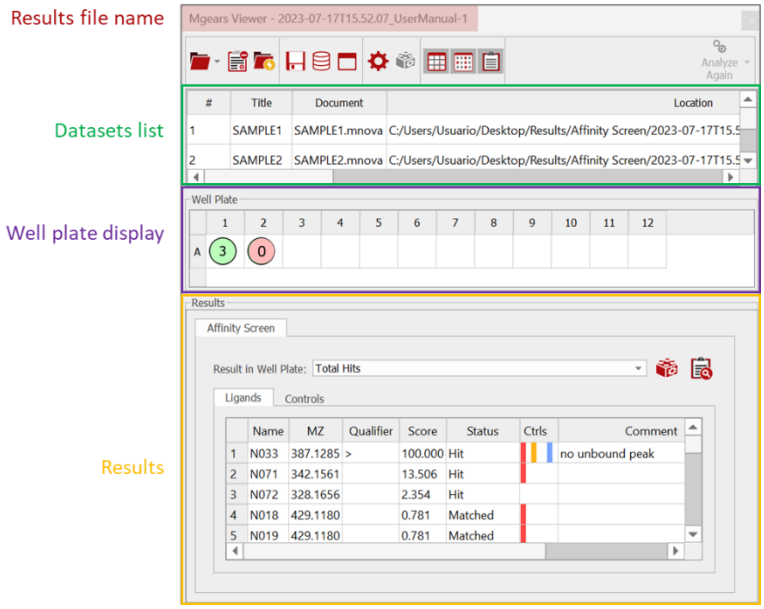
Open the **Mgears Viewer** from the Mnova **Automation** tab.



Click on  and select your analysis result folder to open it.

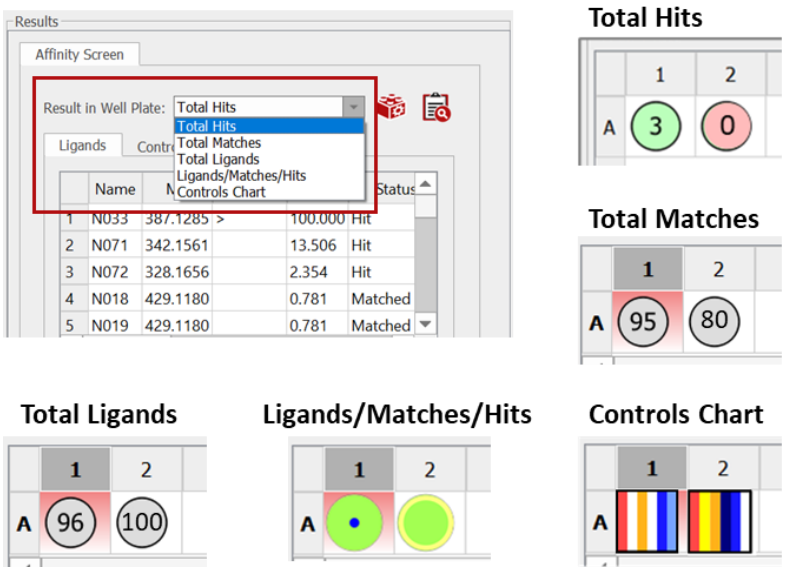


When the experiment has been open, the **Mgears Viewer** presents all the results. You can see the names of the datasets, their positions in the well plate, and any associated numerical results.



4.1.1. The well plate view

The **Well Plate** view offers flexibility in terms of displaying the results in various forms based on user preferences. It provides the option to choose from the following formats: **Total hits, Total Matches, Total Ligands, Ligands/Matches/Hits** graphics, or **Control Charts**.



The **Total Hits** graphics consist of colored circles that represent the number of identified hits. Circles are displayed in **green** when hits are present in the sample and in **red** when no hits have been identified.

The **Ligands/Matches/Hits** graphic is a diagram with concentric bubbles representing three different statuses in various colors: **yellow** (no matches), **green** (matches that are not hits), and **blue** (hits). The size of each bubble corresponds to the respective amount of each status.

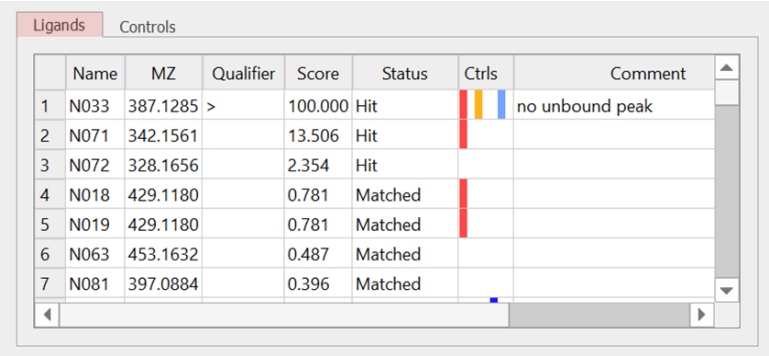
In the **Control Chart** graphic, a series of flags is displayed for each sample. When no controls have failed, the bars remain uncolored.

To view the corresponding results and spectrum for a specific dataset, simply click on it.

4.1.2. The Results section

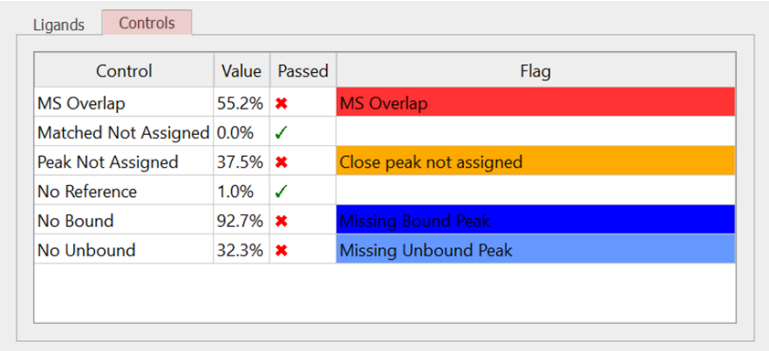
The **Results** section includes one or two tabs with numerical results:

- The **Ligands** tab displays a table listing the ligands present in the sample, along with their corresponding MZ, the achieved **Score,** the **Qualifier** (if this option has been enabled in the Scoring Parameters tab), and the **Status**. Additionally, a **Control Chart** is provided for each ligand to assist users in identifying and reviewing ligands with specific issues. The **Comment** column initially contains the flag text, providing more information about any failed tests, but can be edited as explained below.



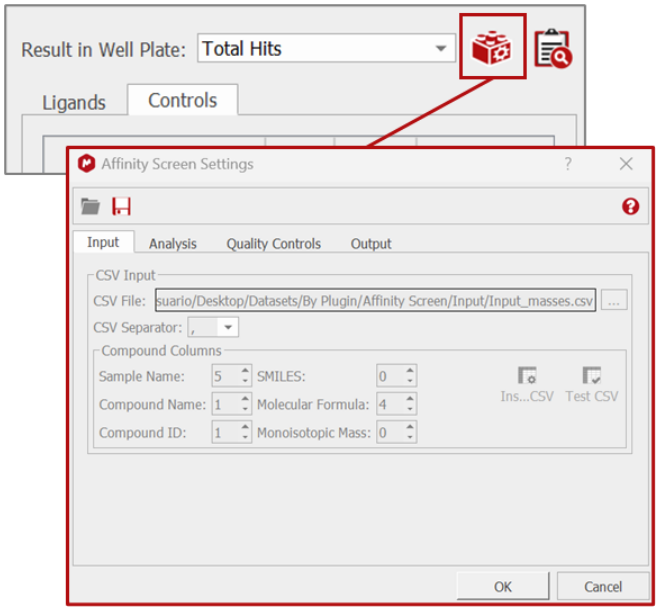
	Name	MZ	Qualifier	Score	Status	Ctrls	Comment
1	N033	387.1285	>	100.000	Hit		no unbound peak
2	N071	342.1561		13.506	Hit		
3	N072	328.1656		2.354	Hit		
4	N018	429.1180		0.781	Matched		
5	N019	429.1180		0.781	Matched		
6	N063	453.1632		0.487	Matched		
7	N081	397.0884		0.396	Matched		

- The **Controls** tab will only appear if controls have been enabled in the Controls configuration. This tab displays the results observed in the control chart, which includes the list of **Controls**, their respective calculated **Values**, and their status (either *passed* "✓" or *failed* "✖"). When a test is failed, the corresponding **Flag** is raised to indicate the occurrence.



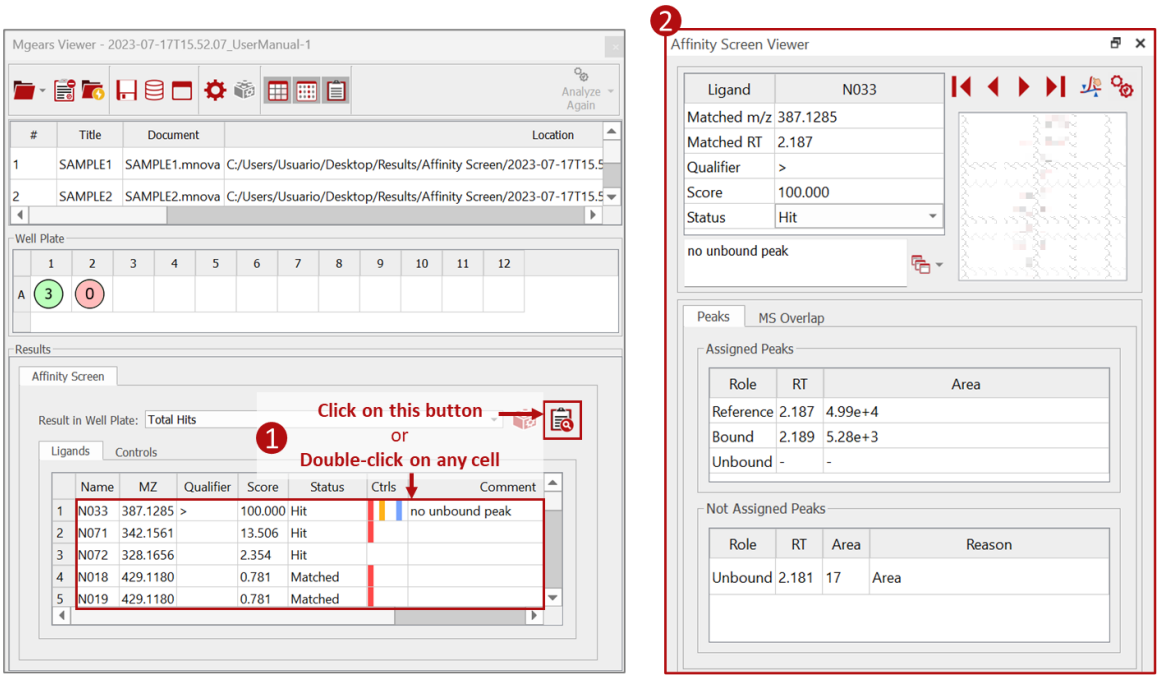
Control	Value	Passed	Flag
MS Overlap	55.2%	✖	MS Overlap
Matched Not Assigned	0.0%	✓	
Peak Not Assigned	37.5%	✖	Close peak not assigned
No Reference	1.0%	✓	
No Bound	92.7%	✖	Missing Bound Peak
No Unbound	32.3%	✖	Missing Unbound Peak

From the **Results** section you can also open the **Affinity Screen Setting** dialog box to edit certain settings and quickly relaunch the analysis on the same samples.



4.2. The Affinity Screen Viewer

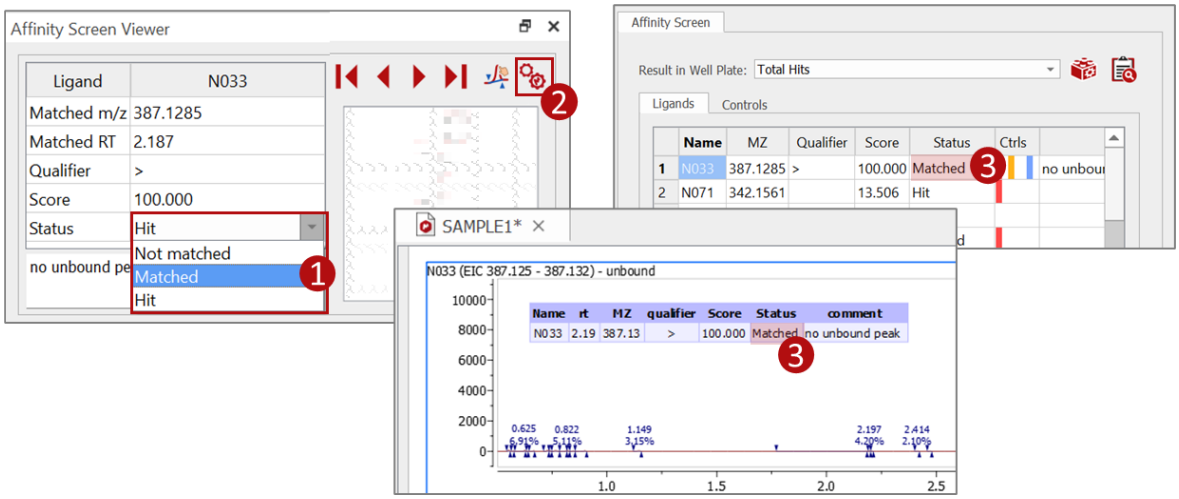
To open the **Affinity Screen Viewer,** you can either press the button in the results section or double click on any cell in the **Ligands** table to directly open the viewer with the result on the corresponding ligand.



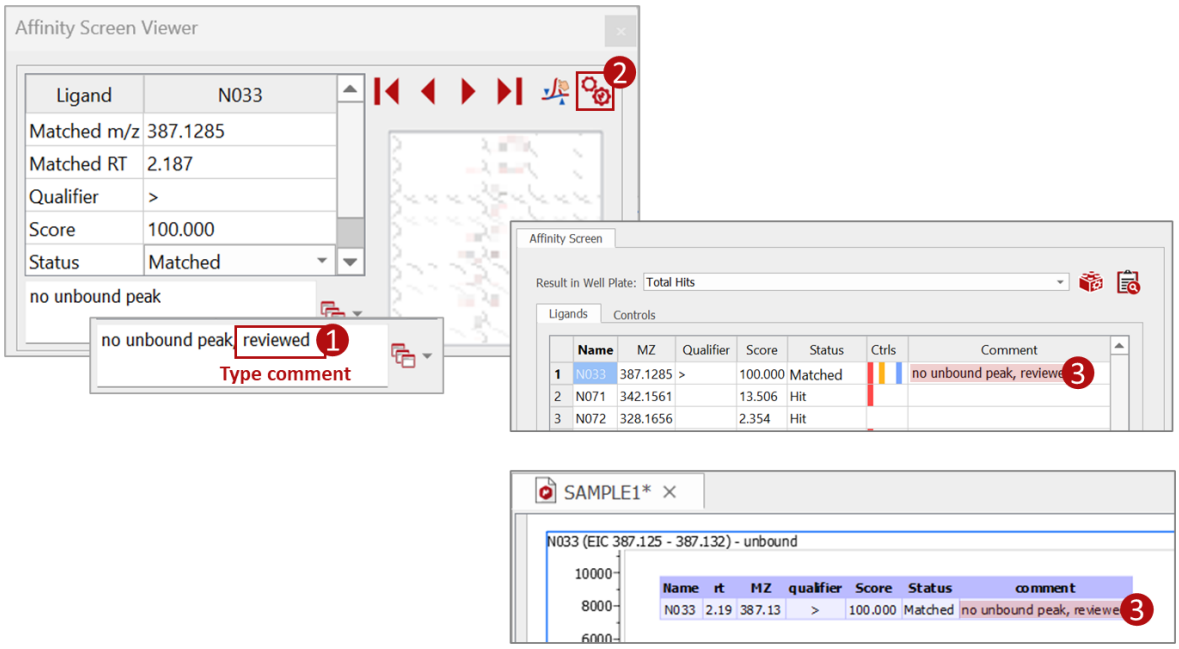
4.2.1. The Ligands details

The ligand details include:

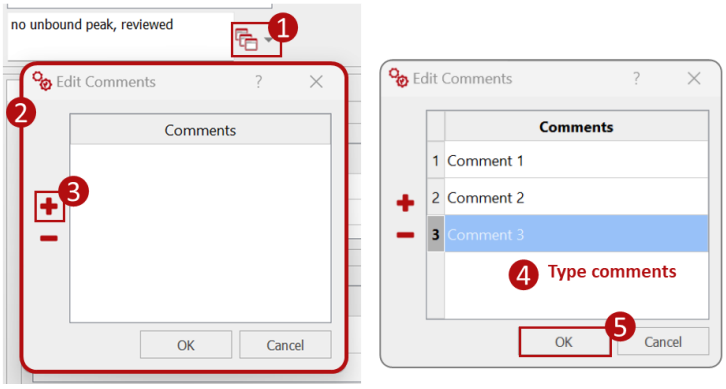
- The ligand details section with the **Ligand** name, **matched m/z** and **RT**, **Qualifier**, **Score**, **Status**, and **Molecular Structure** (when available in the input files).
  The **Status** field is editable, which allows you to override the results. You can simply click on the small arrow to open the list of options and select another status. Press the **Recalculate** button to update the **Status** in the HTML report in the Mnova ligand page and on the Mgears viewer ligand table.



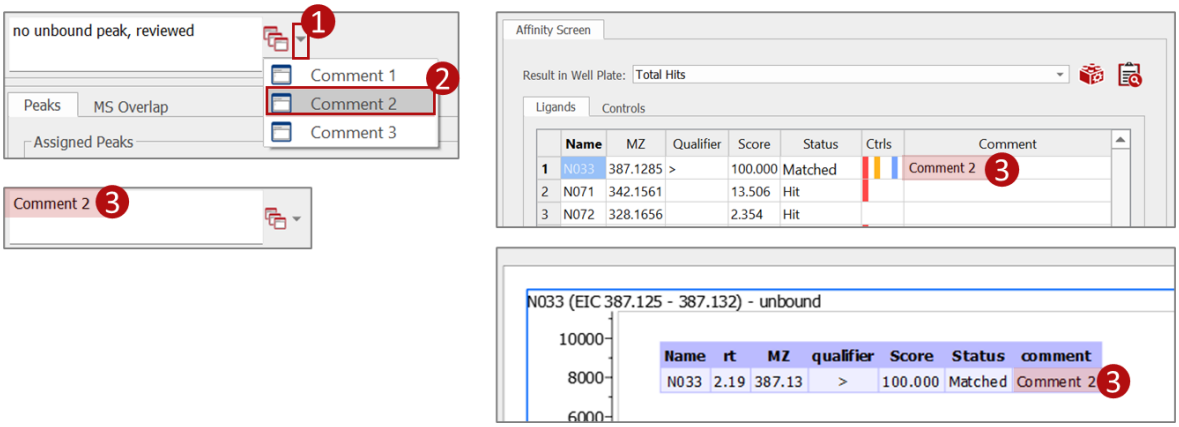
- A **Comment** section, providing details about any failed controls. This section is fully editable. To enter your comment, simply type it in the designated box and then press the **Recalculate** button . This will update the comment both in the HTML report on the Mnova ligand page and on the Mgears viewer ligand table.



To streamline the process, you can create a set of predefined comments that you can easily select for each ligand. Click on the button to open the **Edit Comments** dialog box. Initially, the dialog is empty. Press the **Add** button, type your comment, and then press **Enter** on your keyboard to add it. You can repeat this process to include further comments. Select a comment then press the button to remove it if needed. Once done, click on **OK**.



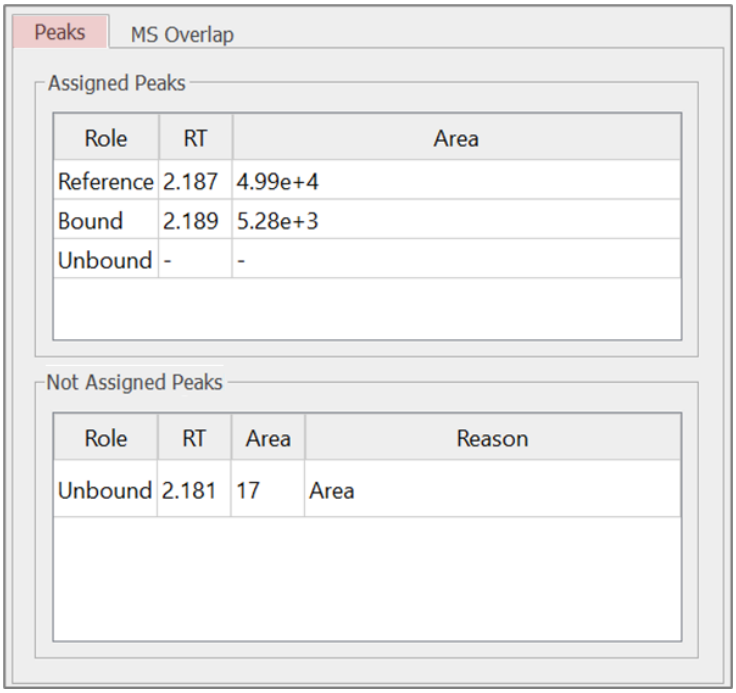
Now, in the **Affinity Screen viewer** press the little arrow in this button . All the added comments are available in the drop-down list and are saved in the registry. This means they will remain available whenever you open the dialog with other ligand results.
To display a specific comment, select it from the drop-down list. The comment will automatically populate all the fields where it is applicable, including the Affinity Screen viewer, HTML report, and the Mgears viewer. This ensures consistency and efficiency when managing comments for different ligands.



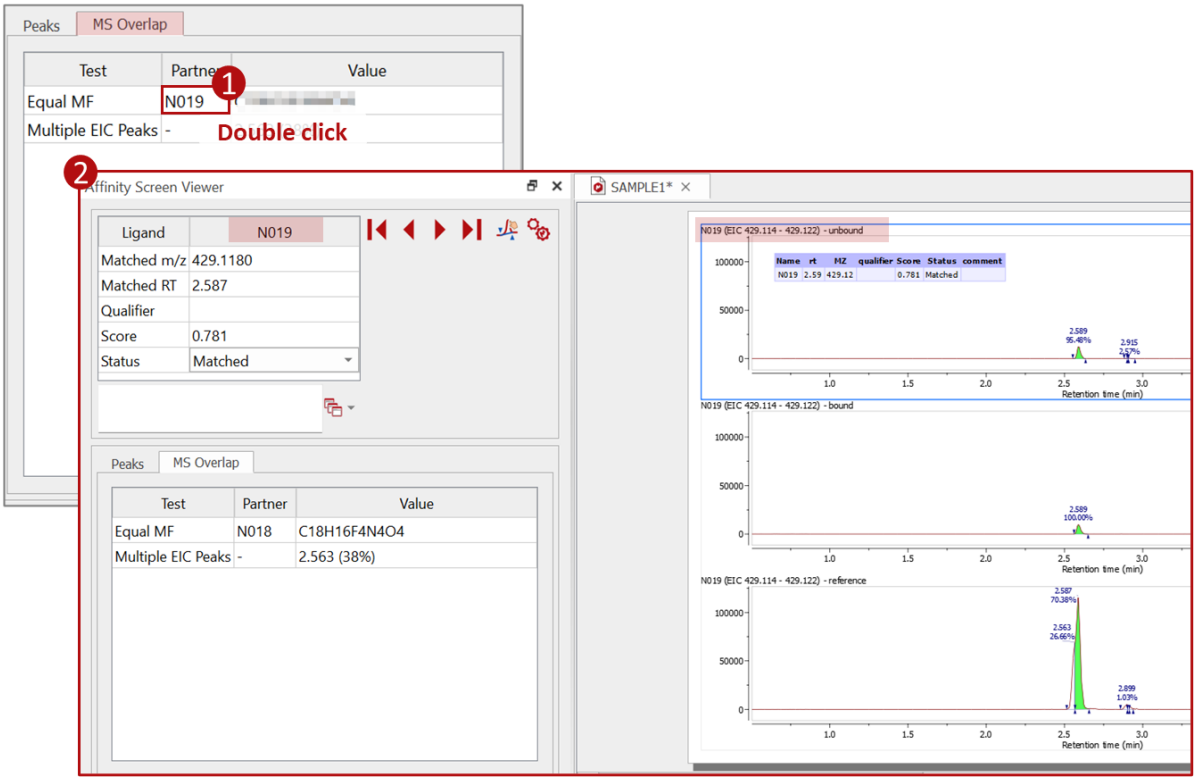
4.2.2. The peaks details

The Peaks details section includes one or two tabs:

- The **Peaks** tab with a table with the **RTs** and **Areas** of the Assigned peaks for the *Reference*, *Bound*, and *Unbound* samples. There is also another table with the **RTs** and **Areas** of the Unassigned peaks and the **Reason** for not assigning them. The second table is displayed only when a Peak Assignment control is enabled in the Controls configuration.



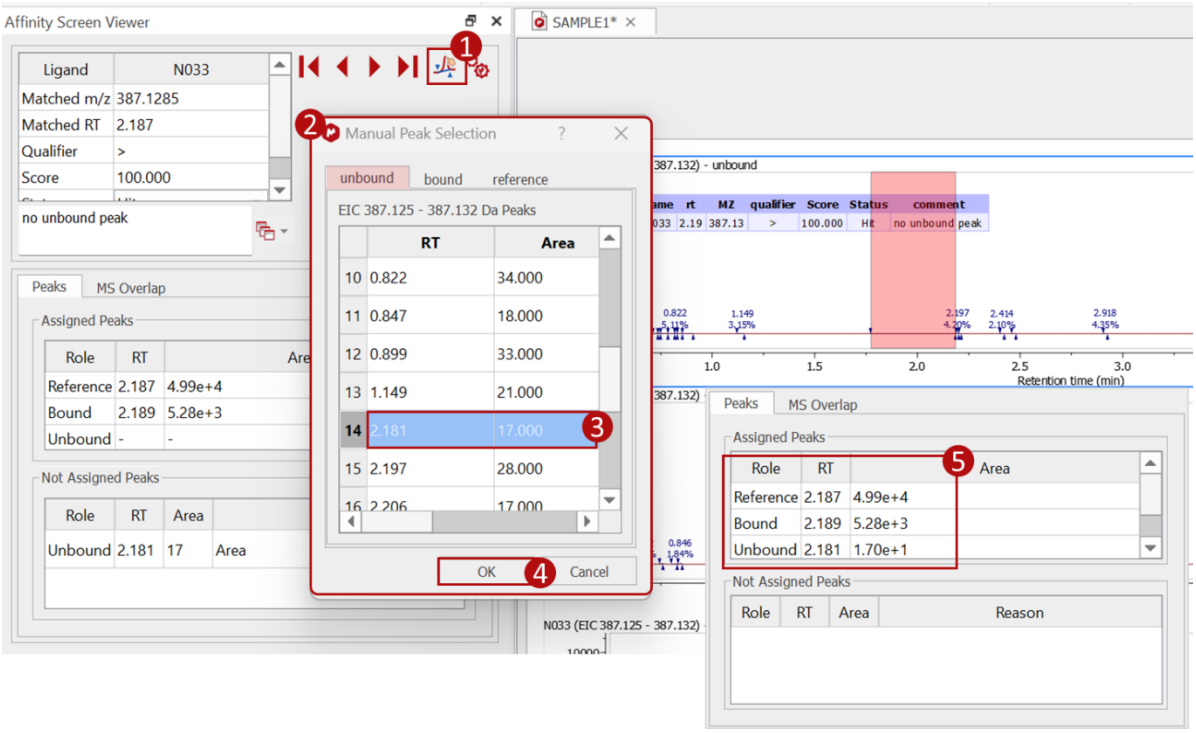
- The **MS Overlap** tab will only be visible if the **MS overlap** test has been enabled in the Controls configuration. Whenever an overlap is detected, and a partner ligand is identified, you can simply double-click on any cell in the partner column. This action will instantly display the corresponding results related to the partner ligand.



4.2.3. Recalculate results

Affinity Screen allows you to easily review and edit peak assignments manually, if needed. There are two methods you can use to achieve this:

- **Using Mnova tools:** You can pick peaks and assign them to the *reference*, *bound*, or *unbound* ligands. After making the necessary peak assignments, you must click the **Recalculate** button to update the results. This will trigger a reanalysis of the revised ligand.
- **Utilizing the Assign button**: Within the **Affinity Screen Viewer**, click on the button (1). This will open a dialog that allows you to select the peak you wish to assign (2). Once you've made the selection (3), click **OK** (4), and the results will be automatically updated (5).



The new results will be automatically saved to your output folder if the option **Save Automatically on Clicking** has been selected. **Analyze Again** is checked in the **Mgears Viewer settings**. Otherwise, press the **Save** button if you wish to update your output reports with the new results.

For more details on Mnova Gears' options, please refer to the Mnova Gears manual.