

Affinity Screen 1.0

STARTING GUIDE



Document Number P/N 467 R1

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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.

O Advanced Plug-ins	?	\times
Filter:		
Available Updates Installed		
Name 🔿 Default Vers	sion	
Mnova Affinity Screen 1.0.0.12597		
	Insta	
Directory	/plugins	5
	Cl	ose



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

(🕑 Install Mnova Plug	-in	?	×
	Mnova	Affinity Screen		
	Description: Version: Installed Version: Release Date: Requires:	Affinity Screen brick for Mgears 1.0.0.12597 1.0.0.12597 Wednesday, 14 June 2023 MestReNova (>=14.3.1-30628 Mnova Gears BETA (>=2.5.0.1		
	Plug-in ready	to be installed.		•
		Install	Can	cel

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.

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Host	ID		
			h
			2
Licer	ises ——		
	State	Plug-in	
28	State	Plug-in Affinity Screen	Mestrel

2. The workflow

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

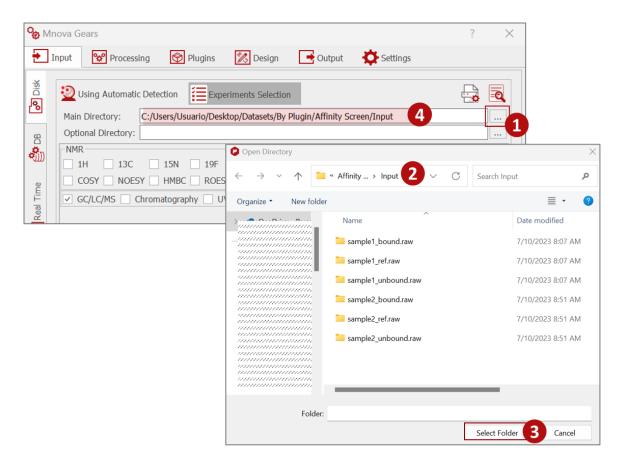




2.1. Input

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.

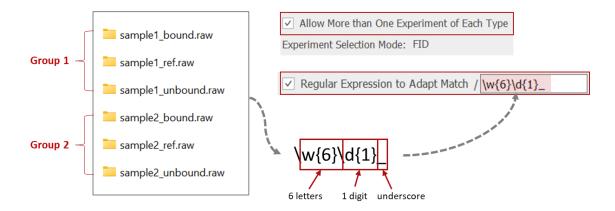


🧕 Using Automatio	Detection Experiments Selection 1
Main Directory:	C:/Users/Usuario/Desktop/Datasets/By Plugin/Affinity Screen/Input
Optional Directory:	
NMR	
□ 1H □ 13C	□ 15N □ 19F □ 31P □ HSQC □
	Y HMBC ROESY TOCSY H2BC
GC/LC/MS C	Chromatography 🗌 UV/IR/Raman/Fluorescence 🗌 Mnova Documer

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.



O N	Mnova Gears ?	×
÷	🕐 Input 🛛 😵 Processing 🚱 Plugins 🔀 Design 📑 Output 🌼 Settings	
oo Disk	Using Automatic Detection	
	Main Directory: C:/Users/Usuario/Desktop/Datasets/By Plugin/Affinity Screen/Input Optional Directory:	
2 Experiments Fou	Found: 2	7 ×
Experiment: MS(3): C:/Users Experiment:	ers/Usuario/Desktop/Datasets/By Plugin/Affinity Screen/L/Sample 1_bound.raw/_]1.DAT, C:/Users/Usuario/Desktop/Datasets/By Plugin/Affinity Screen/Inp(Sample 1_bound.raw/_]1.DAT, C:/Users/Usuario/Desktop/Datasets/By Plugin/Affinit	ple1_ref.raw/ o1_DAT_C:/Users/Usuario,Desktop/Datasets/By Plugn/Affinity Screen t/Sample1_unbound.raw/ sk2_ref.ram/_PUNC001_DAT_C:/Users/Usuario,Desktop/Datasets/By Plugn/Affinity Screen/Input/Sample2_unbound.raw/ FUNC
4) () () ()



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

🍫 Mnova G	ears				
🔶 Input	[™] Processing	🐞 Plugins	Design	📑 Output	Settings
Advised Proc	Templates				
Scripting: -	. .				
Processing Sc					
Script for Ren					

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.

% Mnova Gears		?	×
🛃 Input 😵 Processing 🔯 Plugins 🔯 Design 📑 Output 🔅 Settings	ettings		
 MANIQ (Raw Material Screening) Peak Report MS Scan Chrom Quality Control LogP 	Add Affinity Screen 3		
Image: Chrom Reaction Optimization Custom Plugin Image: Chrom Best Method Delete Image: Chrom Cal Delete Image: Chrom Cal Image: Custom Plugin	Custom Plugin Delete Custom Plugin Custom Plugin Affinity Screen Plugin Settings		
Artinity Screen Fraction Analysis Mquant Nesume Load Settings Save Settings The proof Settings The	: Settings 👻	el (Run

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).

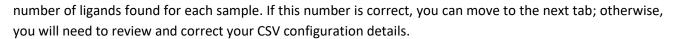
Affinity Screen Se	ttings	:
Input Analysis	Quality Controls Output	
CSV Input		
CSV File: suario/De	sktop/Datasets/By Plugin/Affinity Screen/Input/Input_n	nasses.csv
CSV Separator: ,	•	
Compound Column	S	
Sample Name:	0 🗘 🕂 📕 🚺	
Compound Name:	0 1 Molecular Formula: 0	CSV Test CSV
	0 C Monoisotopic Mass: 0 C	

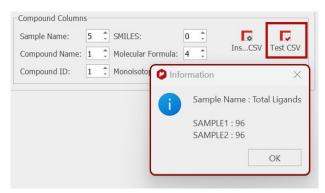
Important! Remember that in order to be correctly read by Mgears, the CSV separator (comma, semicolon, tab, or vertical line) should be correctly configured in the Mgears **Settings** tab.

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).

	Pa	rameter	Column	Heade		First		Parameter	Column	Header		Fir	rst Value	
s	Sample	Name	0	1			1	Sample Name	⁵ 6	Sample ID	sample1			
С	Compo	ound Name	0 1/3	Doub	le-click		2	Compound Name	0					
С	Compo	ound ID	0				3	Compound ID	0					
							_							
N	2.	CSV Column	Detection		Consta Norra		4	Compound Smiles	0		7	? X		
N	2.	CSV Column	Detection	alues for:	Sample Name		4	Compound Smiles			7		1	
N	2.	CSV Column k or Select colu 1-A	Detection Imn with v 2-B	alues for: 3-C	4-D	Sample I		Compound Smiles	0 5-E	8	7	2 ×		
N	2.	CSV Column	Detection Imn with v 2-B	alues for:	4-D	Sample II		Compound Smiles		B Select	column]	
N	Click	CSV Column k or Select colu 1-A Compound	Detection Imn with v 2-B Mass	alues for: 3-C [M+H]+	4-D		D	Compound Smiles		3 Select	column]	
N	2 (Click	CSV Column k or Select colu 1-A Compound N001	Detection Imn with v 2-B Mass	alues for: 3-C [M+H]+ 470.055	4-D MF		D	Compound Smiles		3 Select	column			

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





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.

⁰ Affinity Screen Settings	?	\times
		0
Input Analysis Quality Controls Output		
Roles LC/MS Evaluation Scoring		
Role Mask RegExp Custom Name	_	
✓ Reference *_ref*		
Bound *_bound* bound	Tess	sks
Unbound *_unbound*		



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.

	Roles LC/	MS Evaluation Scoring)			
	Role	Mask	RegExp	Custom Name		
	✓ Reference	*_ref*		reference		_ 🕀 _
	Bound	* bound*		bound	_	Tessks
🍫 Roles Defin	ition			? ×	<]
Bound:	/sample1_ref.ra /sample1_bound	w/_FUNC001.DAT J.raw/_FUNC001.DAT J.nd.raw/_FUNC001.DAT				
				ОК		

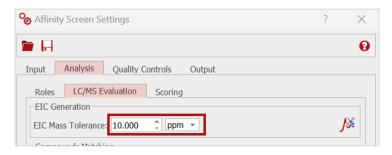
In case a data file for a specific role is not found, the label "Not found!" will be shown next to that role. This can occur if a mask does not match the file name, for example.

⁰ <mark>⊘</mark> Roles Defir	ition		?	\times
			Ok	(

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 Minimum peak cetection 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.

-	5 .	ak Detection Optic	ons	? X	\gg
Settings	1			Ok	
Enhanced			*	Cancel	
Sensitiv		0.00	•	Less >>	
Peak Ba	aseline:	Fit	•		Cancel
Smooth	ning:	None	÷		
Savitzk	y-Golay:	Auto	÷		
Overla	o sensitivity:		igh		
Baselin	e Correction:	\checkmark			
Saturat	ion level:	None	÷		
Minimum	Area Threshol	d: 0.50%	÷		
minimum A					

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.

Compounds Ma	tching	
	EIC + Isotope Cluster 💌	\sim
	EIC Only	
EIC Peak Assign	EIC + Isotope Cluster Isotope Cluster Only	•
Discard Peaks B	ciow (area). 500	*

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

		-Compound Match Meth			uster 👻				X
M	lolecule M	atch Settings	'			r.		? X	
	- 💾								
-	erance			Thresholds				ОК	
	ts:	Da	*	Score Thres		0.85	÷	Save	
or	MS:	0.30	\$	Matches per	Molecule:	1	:	Restore	
or	MS/MS:	1.00	÷						
	itive Polariz			Negative Pol Adducts/Los				Cancel	
	Adduct	Loss		Adduc	t Loss		- +		
1		-	×	1 -			×		
2	H+		*	2	H+		*		
3	Na+		0	3 CI-			0		
4	K+		-	4 Na+	2H+		-		
4a)	e Positive C	harge: 1	\$	Max Negativ	e Charge:	1	0		
lor	e Settings –			MS/MS Settin	ngs				
	Dimers			Ignore F	Precursors				
Spe	ectra Averag	e Count: Disab	led 🗘	Search For:	Molecu	lar Fragm	ents 👻		

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.

EIC Peak Assignment			
Discard Peaks Below (area):	500	*	
RT Tolerance (min):	0.03	* *	

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 <u>Peak Assignment Failures</u> 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.

⁰₀ Affinity Screen Settings	?	\times
🖆 🔒		0
Input Analysis Quality Controls Output		
Roles LC/MS Evaluation Scoring		
Hit Scoring Function: bound / reference * 100 Valida	te Functior	
Use Peak Height: Hit Threshold: 2.00		

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.

Affinity Screen Settings	?	\times
		0
Input Analysis Quality Controls Output		
Roles LC/MS Evaluation Scoring		
Hit Scoring Function: bound / reference * 100	/alidate Functi	ion
Use Peak Height: Hit Threshold:		
Use Qualifiers Score Expression looks ok!		
ОК		

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".

Affinity Screen Setti	ngs			? >
				(
nput Analysis (Quality Controls	Output		
Roles LC/MS Eval	uation Scoring			
Scoring				
Hit Scoring Function:	bound / unbound		-	Validate Function
Use Peak Height:	 Image: A start of the start of			
Hit Threshold:	2.00	÷		
Use Qualifiers	\checkmark			
	100.000	max		
		A .		

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 <u>Affinity Screen Viewer</u>. 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 <u>Mgears Viewer</u>.

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 <u>CSV Input</u>.
- **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 <u>EIC tolerance</u>).
- **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).

🍫 Affinity Screen Setti	ngs		?	\times
📁 🔚				•
-MS Overlap Cqual MF MW Overlap Multiple Peaks	Quality Controls Output			×
	Custom colors	Val:	204 ‡ (255 ‡	Red: 255 \$ Green: 51 \$ Blue: 51 \$ Cancel

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.

Peak Assignement Failures					
✓ Matched Not Assigned	20%	-	Flag:	Matched but not assigned	
✓ Peak Not Assigned	20%	*	Flag:	Close peak not assigned	

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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.

Missing Peaks			
✓ No Reference	20%	Flag:	Missing Reference Peak
✓ No Bound	20%	; Flag:	Missing Bound Peak
✓ No Unbound	20%	Flag:	Missing Unbound Peak

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.

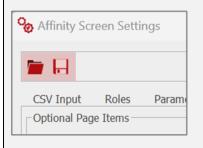
- **Molecule option:** This option is only available when SMILES strings are provided in the <u>CSV Input</u> file. By selecting this option, the compound's molecular structure will be included in the report.
- HTML option: By selecting this option, a small HTML report will be added to each Mnova report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

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

⁰⊗ Affinity Screen Settings				?	\times	
= 🔒						0
Input Optional F Molecu	-	Quality Controls	Output			



Top tip! The analysis settings you just configured can be saved and reused in future analyses. Press the **Save** button \square 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 and 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.

് ത്ര Mnova G	iears			
🛃 Input	💖 Processing	Plugins	🐹 Design	📑 Output
Disk				
Directory:	C:/Users/Usuario/Des	sktop/Results/Affi	nity Screen	

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).

°⊚ Mnova Ge	ears							?	\times
🛃 Input	vert Processing	Plugins	🐹 Design	Output	🛱 Settings				
Disk					n		2	ß	
Directory:	C:/Users/Usuario/Desk	top/Results/Affi	nity Screen	🗸	Add Nickname to the Results Folder	UserManual	🗸 Add Incremental Numbering 🗌	Only Nickna	me
					Sort in Subfolders	Split Results in Subfol	ders		

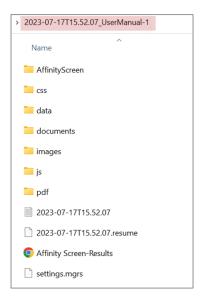
You can also choose to create an Mnova document, a PDF, or to save your results to a database.

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 <u>Mgears Result Viewer</u>. 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** \mathbf{v}^{Run} to launch the analysis.

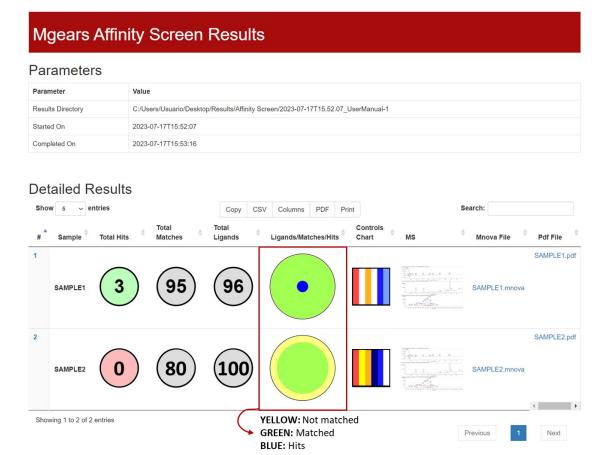


The results folder is saved under the previously specified directory, as described above.

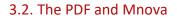


3.1. The HTML file

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.



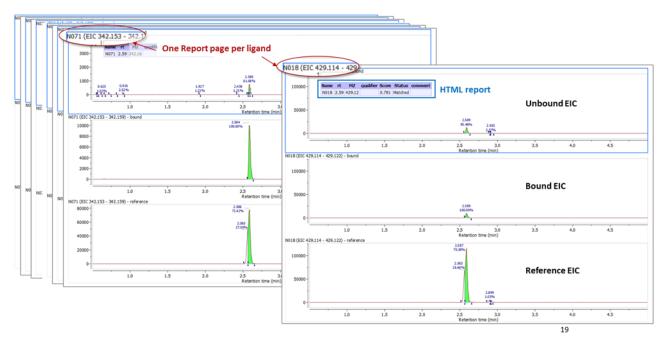




The PDF and Mnova documents, saved in the "Pdf" and "documents" subfolders, respectively, have similar structures and follow the applied layout template (default or custom one).

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 <u>Report tab</u>.
- The molecular Structure if that option has been enabled in the <u>Report tab</u> (only possible when SMILES strings are provided in the <u>CSV input</u> file).



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	В	С	D	E	F	G	Н	I.	J	K	L	М	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

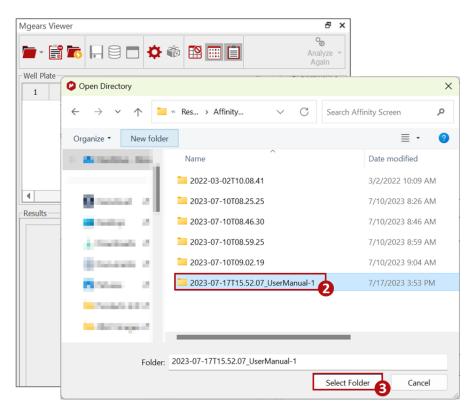
Affinity Screen has two different – but interconnected – result viewers, the <u>Mgears Viewer</u>, which displays overall results for the sample/well, and the <u>Affinity Screen Viewer</u>, which displays individual ligand results for each sample.

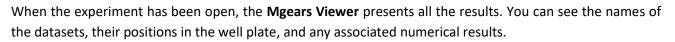
4.1. The Mgears Viewer

Open the Mgears Viewer from the Mnova Automation tab.



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

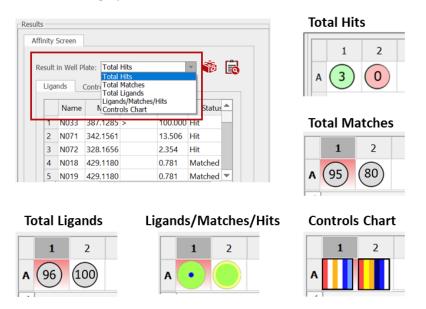




Results file name	Mg	gears Vi	iewer - 2	023-07-17	T15.52.07_U	UserManı	ual-1						
	_	•	î 📷		⊐ ≎	ê) III (İ					و Analy Agai	/ze 🔻
		#	Title	Docu	ment						Ŀ	ocation	-
Datasets list	1	S	AMPLE1	SAMPLE	l.mnova C	:/Users/U	suario/Desk	top/Resu	ults/Aff	inity Sc	reen/2023-	07-17T1	5.5
	2 1		AMPLE2	SAMPLE	2.mnova C	:/Users/U	suario/Desk	top/Resu	ults/Aff	inity Sc	reen/2023-	07-17T1	5.5 ▼
	We	ell Plate											
Well plate display		1	2	3 4	5	6	7 8	9	10	11	12		
tron place alopia,	A	3	0										
	Re	sults	-										
		Affinity	Screen										
		Result	t in Well P	late: Total	Hits						- 🏟		
		Lig	ands	Controls									
			Name	MZ	Qualifier	Score	Status	Ctrls			Comment	A	
Develo		1	N033	387.1285	>	100.000	Hit		no ur	bound	l peak		
Results		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	_				T	
		4									l l l l l l l l l l l l l l l l l l l		

4.1.1. The well plate view

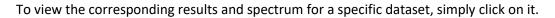
The **Well Plate** view offers flexibility in terms of displaying the results in various forms based on user preferences. It provides the option to choose from the following formats: **Total hits, Total Matches, Total Ligands, Ligands/Matches/Hits** graphics, or **Control Charts**.



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.



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	4
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 <u>Controls configuration</u>. 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%	1	
Peak Not Assigned	37.5%	×	Close peak not assigned
No Reference	1.0%	1	
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.

	Affinity Screen Settings ?	
7		
Ι	nput Analysis Quality Controls Output	
Г	CSV Input	
	CSV File: suario/Desktop/Datasets/By Plugin/Affinity Screen/Input/Input_masses.	CSV
	CSV Separator:	
	Compound Columns	_
	Sample Name: 5 \$ SMILES: 0 \$ InsCSV TO	
	Compound Name: 1 C Molecular Formula: 4 C InsCSV To	
	Compound ID: 1 C Monoisotopic Mass: 0 C	

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.

			T O	۴ 🏛		Ê						o ⊘ Analy	/ze 👻		Ligand		N03	33		- I		1
												Agai		N	/atched m/	387.12	85		X		λ.	2
	Title	Docu	nent								Loca	tion	-	Ν	Matched RT	2.187			Ň.			
SA	MPLE1	SAMPLE	.mnova C	/Users/U	suario	o/Deskt	op/Res	ults/Aff	finity Sc	reen/	2023-07	-17T1	5.5	C	Qualifier	>			1 &		ŝ	
SA	MPLE2	SAMPLE	.mnova C	:/Users/U	suario	o/Deskt	op/Res	ults/Aff	inity Sc	reen/	2023-07	-17T1	5.5 🕶	S	core	100.00	0		18		S.	
													•	S	Status	Hit		-	- ŝ	00.05	ŝ.	
te										_				n	o unbound p	eak			- 8		Ϋ́,	
	2	3 4	5	6	7	8	9	10	11	13	2							re-	• &			
	(0)																					
															Peaks M	S Overla	0					
	\smile														reaks ly	5 Overla	<i>,</i>					
															Assigned F		р 					
ity S	Screen														Assigned F	eaks	, 		Area	2		
						Click	ont	his h	uttor		>	-A-1			Assigned F	eaks RT			Area	a		
		late: Total	Hits	1		Click			uttor) [^] -		1			Assigned F Role Reference	eaks RT e 2.187	4.99e		Area	3		
sult i	in Well P	late: Total	Hits	1			C	or		Ť	*				Assigned F Role Reference Bound	eaks RT e 2.187 2.189			Area	a		
sult i	in Well P			1 Score	Do	ouble	o e-clicl	or k on	any c	ell	TRENT 4				Assigned F Role Reference	eaks RT e 2.187 2.189	4.99e		Area	3		
ult i .igai	in Well P nds	Controls	Qualifier	1 Score 100.000	Do Si		C	or con	any c	cell Com	- Cinc				Assigned F Role Reference Bound	eaks RT 2.187 2.189 1 -	4.99e 5.28e -		Area	3		
sult i Ligar	in Well P nds Name N033	Controls	Qualifier		Do Si Hit	ouble	o e-clicl	or con	any c	cell Com	- Cinc				Assigned F Role Reference Bound Unbound	eaks RT e 2.187 2.189 d -	4.99e 5.28e -					
igan 1	in Well P nds Name N033 N071	MZ 387.1285	Qualifier	100.000 13.506	Do Si Hit	ouble	o e-clicl	or con	any c	cell Com	- Cinc				Assigned F Role Reference Bound Unbound	eaks RT 2.187 2.189 1 -	4.99e 5.28e -			eason		
igan 1 2 3	Name N033 N071 N072	MZ 387.1285 342.1561	Qualifier >	100.000 13.506 2.354	Do Si Hit Hit	ouble	o e-clicl	or con	any c	cell Com	- Cinc				Assigned F Role Reference Bound Unbound	eaks RT 2.187 2.189 4 - ned Peak RT	4.99e 5.28e - s Area					



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 ⁹ to update the **Status** in the HTML report in the Mnova ligand page and on the Mgears viewer ligand table.

Ligand	N033			°0			Plate: Total	Hits				- 🐞	6
Matched m/z	387.1285	Ś	\$ 1 1	5 2	L	igands	Controls						
Matched RT	2.187	- X	- <u>3</u> -3	ξ.		Name	MZ	Qualifier	Score	Status		4	
Qualifier	>	in Soore		de la		1 N033	387.1285	>	100.000	Matched	3	no unbour	
Score	100.000	tees a		201		2 N071	342.1561		13.506	Hit			
Status	Hit	- 2	SAMPLE	1* ×								-	
no unbound pe	Not matched Matched Hit		N033 (EIC 38 10000-		t MZ qu				ment				
			6000- 4000-	N033 2.	19 387.13	> 100	0.000 Matche	3	und peak				
			2000-	0.625 0.822 6.91% 5.11%	1.149 3,15%				2.197 2 4.20% 2	2,414			

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 ⁹. This will update the comment both in the HTML report on the Mnova ligand page and on the Mgears viewer ligand table.

Ligand Matched m/z 387.128 Matched RT 2.187 Qualifier >			1	00							
Matched RT 2.187	35	2 A.F									
		2 A.									
Oualifier >			R -	2							
		Reverte		Sec.							
Score 100.000)	5	Affinity	Screen							
Status Matche	d 🔹 💌	Sec. R.	Annity	Screen							0-
no unbound peak	eak, reviewed 1				Plate: Total I Controls	1105					ð 🗟
		°c		Name			Score	Status	Ctrls	Comment	
	Type comment		1		387.1285 342.1561		00.000 3.506	Matched	111	no unbound peak, reviewe	
			3		328.1656			Hit	1		

8000

Name rt MZ qualifier Score Status

N033 2.19 387.13 > 100.000 Matched no unbound peak, review

comment



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**.

no unbound peak, reviewed	
🗞 Edit Comments ? X	Comments ? X
2 Comments	Comments
	1 Comment 1
	♣ 2 Comment 2
-	= 3 Comment 3
	4 Type comments
OK Cancel	OK Cancel

Now, in the **Affinity Screen viewer** press the little arrow in this button ⁶. 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.

no unbound peak, reviewed	□ Comment 1		y Screen It in Well P	late: Total	Hits					v	🏟 🗟
Peaks MS Overlap	Comment 2			Controls							
Assigned Peaks	Comment 3		Name	MZ	Qualifier	Score	Status	Ctrls	Cor	nment	
		1	N033	387.1285	>	100.000	Matched		Comment 2	3	
omment 2 3		2	N071	342.1561		13.506	Hit				
	€ -	3	N072	328.1656		2.354	Hit				
		NO	33 (EIC 10000	1	- 387.13 Name			ïer S	Score Statu	s commen	ıt
			8000						00.000 Matche		
			6000	4							

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 <u>Controls configuration</u>.

Deference	RT	Area 4.99e+4					
Bound Unbound		5.28e+3					
onbound		-					
lot Assigne	-						
Role	RT	Area	Reason				

• The **MS Overlap** tab will only be visible if the **MS overlap** test has been enabled in the <u>Controls</u> <u>configuration</u>. 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.

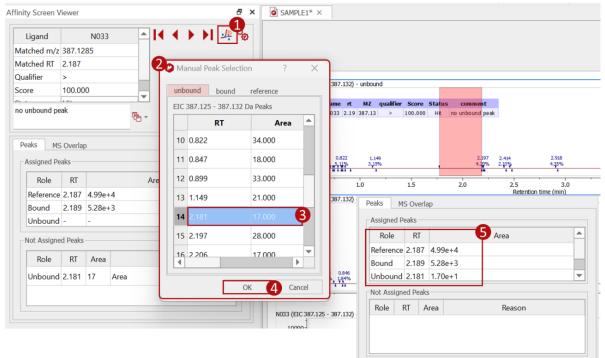
6	Peaks -	Double	click						
Affir	finity Screen Viewer			5	SAMPLE1* ×	* ×			
	Ligand	N019)) 🧏 🗞		N019 (EIC 429	9.114 - 429.122) - unbound		
N	atched m/z	429.1180				100000-		er Score Status comment	
N	latched RT	2.587					N019 2.59 429.12	0.781 Matched	
	alifier					50000-			
		0.781							2.589 95.48% 2.915 2.57%
S	tatus	Matched	—			어는	1.0	1.5 2.0	25 30
			~			N019 (EIC 429	9.114 - 429.122) - bound		Retention time (min)
						100000-			
	Peaks MS	Overlap							
	Test	Partner	Value			50000-			
	Equal MF	N018	C18H16F4N4O4						2.589
	Multiple EIC		2.563 (38%)			01	1.0	1.5 2.0	2.5 3.0
					N019 (EIC 429	9.114 - 429.122) - reference	10 10	Retention time (min)	
						100000-			2.587 70.38%
									2.563 26.66%
						50000-			



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 ¹/₂ 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 ^{Affinity} 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

H 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.