



Mestrelab Research

Screen 2D

STARTING GUIDE



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1. Introduction to Mnova Screen 2D

In this starter guide we will give you a brief overview of the main steps needed to run Mnova Screen 2D. Screen 2D is a powerful tool for data processing and analysis, allowing one to obtain qualitative information from two-dimensional NMR (2D NMR) experiments used in screening campaigns.

Screen 2D deals with 2D NMR experiments (typically of the HSQC type) where the target macromolecule (i.e., a protein) spectrum is monitored both in the absence and the presence of certain concentrations of a compound that is, potentially, a ligand to the protein. The analysis of the chemical shift perturbation (CSP) experienced by the peaks of the protein spectrum upon ligand binding will provide qualitative information about the strength of the protein-ligand interaction.

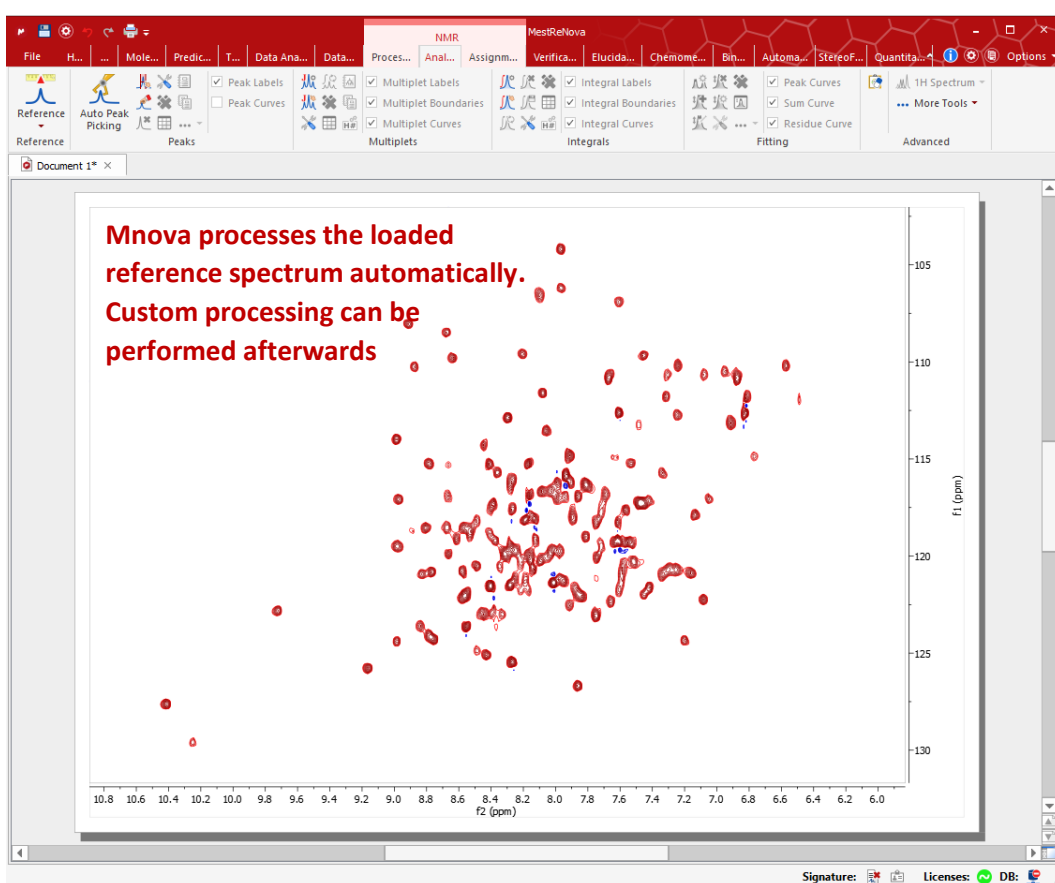
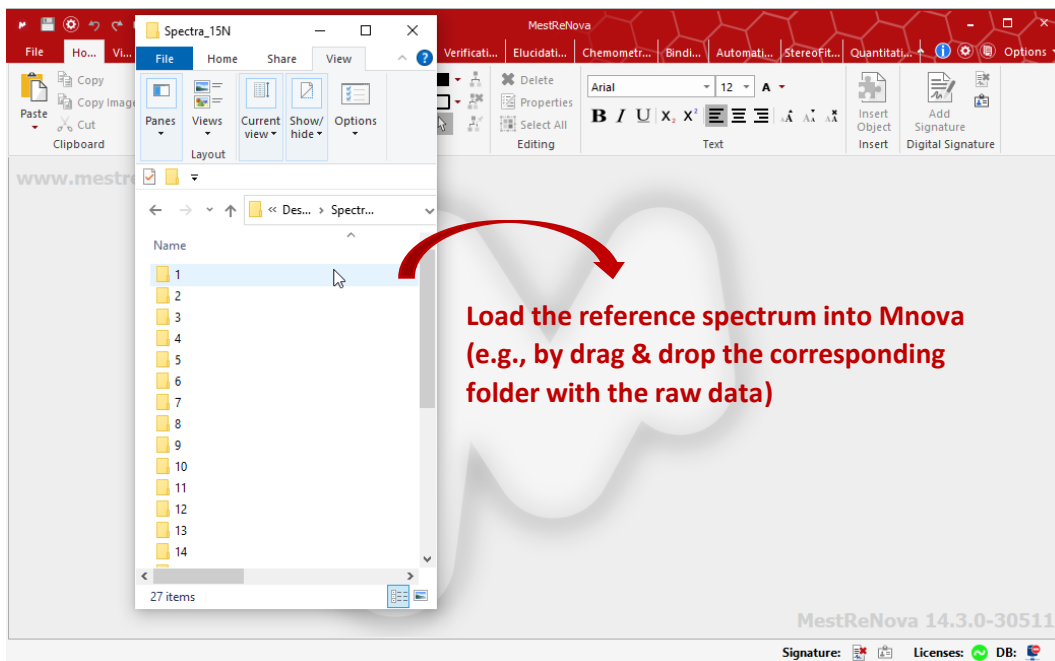
Screen 2D performs the automated processing and analysis of this type of experiment. Among the main characteristics of the software are:

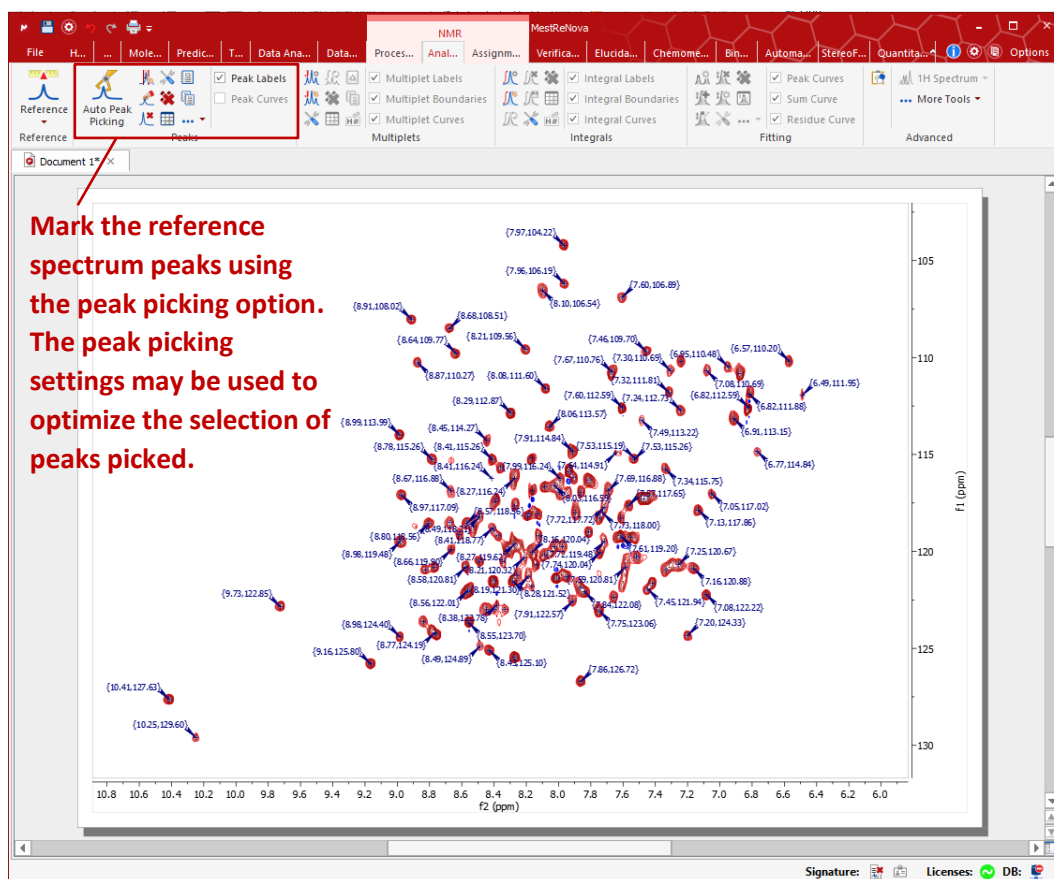
- For a given target protein, automated analysis of multiple ligands, working in batch mode, can be performed.
- Automatically identifies and matches the peaks selected in the protein spectrum with and without the presence of the ligand. It then calculates the CSP for every peak.
- Applies certain statistics to the CSP values such as Mean CSP, Max CSP, and CSP-Score.
- Classifies ligands in different levels according to their CSP-Scores. These levels are correlated with the affinity between the ligands and the protein.
- Permits the definition of a ligand as a hit or as an aggregator according to its CSP-Score.
- Allows for the easy inspection of results, peak by peak, for each ligand and the manual edition of the automated peak matching.

Following in this starter guide, we introduce a few steps describing how to use Screen 2D efficiently.

2. Prepare the reference spectrum

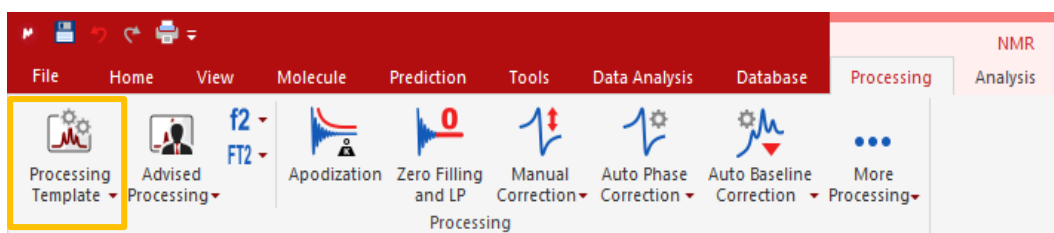
In Screen 2D, the reference spectrum (spectrum of the pure protein) is presented as a Mnova document where the peaks that will be monitored have been picked. You first need to open the reference spectrum in Mnova and use peak picking to determine the selected peaks.

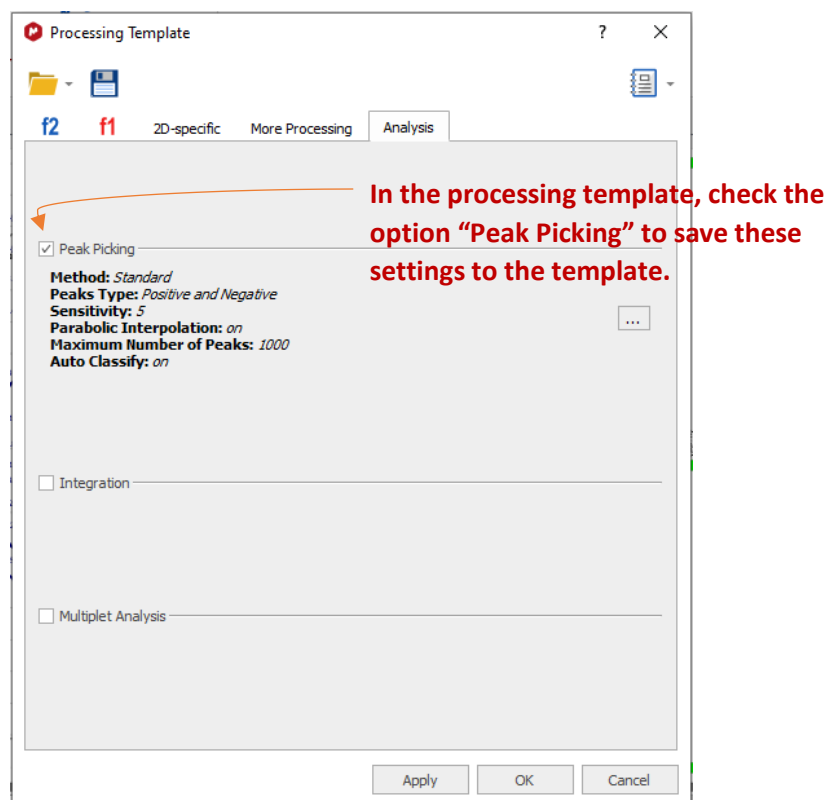




If you want to learn more about the NMR processing and peak picking you can look at this [Mnova Starting Guide](#) and the [MestreNova Manual](#).

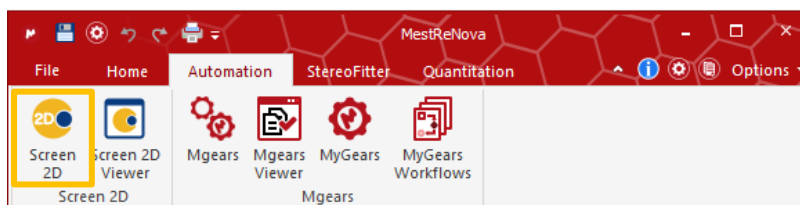
Once you have applied the desired processing and peak picking, save the processing template file. Using this processing template file, the goal is to apply the same processing and peak picking settings to the test spectra (the spectrum of the protein in the presence of a ligand) as those applied to the reference spectrum. You can save the processing template via Processing > Processing Template.





3. Select your data set and settings

Next, go to the Mnova panel and select Mnova Screen 2D (located under the tab Automation).



3.1. Entering Project details

The Screen 2D Project tab is displayed, where users can optionally complete the project information form. Here, it is necessary to give the path to the folder where the results of the analysis will be saved.

Screen 2D

Project Settings

Information

Project Name: Project Protein 1

Experiment Code: C-P1

Experiment Date: 5/19/2022

Author: Rick Astley

Ligand Concentration in $\mu\text{mol/l}$: 100

Source Organism:

Protein Used: Protein 1

Protein Concentration in $\mu\text{mol/l}$: 50

Results

Location to Save Results: C:/Screen2D_Result

Save Each Experiment as PDF

Save Parameters Load Parameters Reset OK Cancel

3.2. Entering Data Set and Analysis Settings

The next tab, Settings, is to indicate the paths to the reference spectrum, the test spectra, and the processing template previously saved. Additionally, you can include the path to a Spectra Label File to name the test spectra (for instance, with the names of the ligands).

Screen 2D

Project Settings

Input

Reference Spectrum

Reference Spectrum: \ValidationSet_Screen2D\Mnova_document\Reference_spectrum.mnova

Test Spectra

Project Data Folder: C:/ValidationSet_Screen2D/Test_spectra

Project Data Path Mask: */ser

Expert

Expert Options

Spectra Label File: C:/ValidationSet_Screen2D/Configuration_files/Labels.txt

Processing

Test Spectra Processing

Processing Template: \ValidationSet_Screen2D\Mnova_document\Reference_spectrum.mnova

Analysis

Save Parameters Load Parameters Reset OK Cancel

If you click on the “Settings” button (⚙️) of the Screen 2D Settings tab, a dialog is displayed in which you will be able to define your analysis parameters such as Regions of Interest, Peak Matching Algorithm, Chemical Shift Perturbation Parameters, and Scoring Parameters.

Screen2D Analysis Parameters

Region of Interest

F1 (from)	F1 (to)	F2 (1H) (from)	F2 (1H) (to)	Region type
16	26	-1	2.5	Included

Peak Matching

Peak Matching Method: Automatic Peak Tracing

Allow multiple Assignment in Test Peaks

Chemical Shift Perturbation Parameters

	Scaling Factors	Maximal Differences
1H	1.000	0.15
15N	0.156	2.00
13C	0.185	0.30

Scoring

Threshold for significant CSP: 0.010

Weighting Factors for CSP-Score:

CSP (Matched Reference Peaks): 1.0

Unmatched Reference Peaks: 0.5

Unmatched Test Peaks: 0.0

Use Shape Factors for CSP Scores

Thresholds for Q-Score:

Level 1: 0.030

Level 2: 0.031

Level 3: 0.032

Level 4: 0.033

Level 5: 0.050

Thresholds for Final Status:

Threshold for Hits: 0.050

Threshold for Aggregators: 0.060

OK

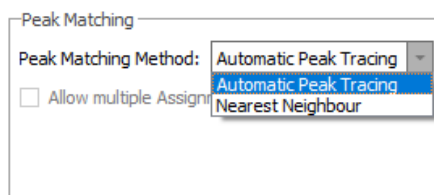
You can define one or several regions of interest in the spectra and categorize them as “included” or “excluded”. If you define one or more regions of interest of the type “included”, Screen 2D will consider only the peaks in these regions for analysis. Conversely, the peaks in a region of interest of the type “excluded” will not be considered in the analysis.

Add/delete regions of interest

Shift values that define the region of interest

Region type:
Included (peaks into this region are used in the analysis)
Excluded (peaks into this region are not used in the analysis)

In the peak matching section, you can choose between two different algorithms to perform the analysis: “Nearest Neighbor”, and “Automatic Peak Tracing”. The Automatic peak tracing algorithm¹ is currently the recommended one as it has been proved to match peaks more efficiently.



You can change the CSP parameters such as the scaling factors and the maximal CSP considered for each nucleus. These values will be used in Screen 2D to match each peak in the reference spectrum to the corresponding peaks in the test spectra, and to evaluate the CSP of the reference-test peak pair.

Chemical Shift Perturbation Parameters		
	Scaling Factors	Maximal Differences
1H	1.000	0.05
15N	0.156	0.32
13C	0.185	0.27

In the Scoring section, a group of settings are defined to calculate the following parameters:

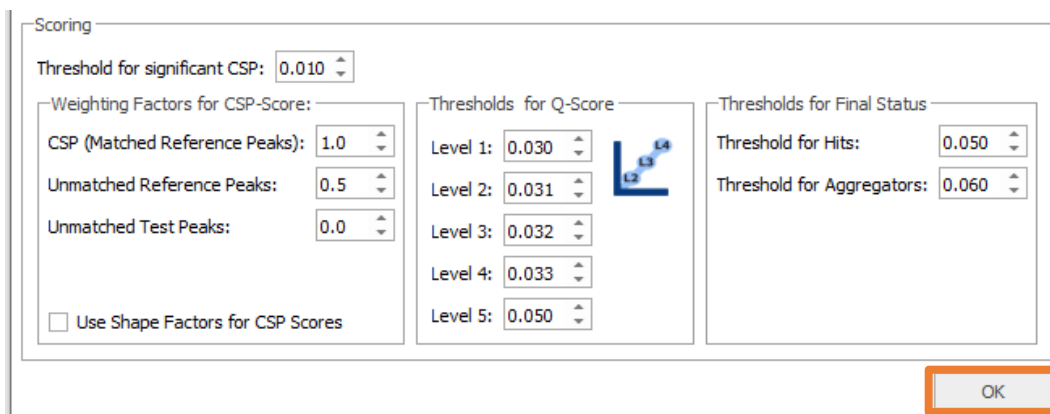
- Threshold for significant CSP: to determine the number of peaks that have a CSP greater than this threshold.
- Weighting factors for CSP-Score: The CSP-Score is a “global” CSP parameter to rank the ligands of the dataset. In the calculation of the CSP-Score, the matched/unmatched reference peaks, and the unmatched test peaks are considered. A weighting factor can be introduced for each one to confer more/less relevance in the CSP-Score calculation.
- Thresholds for Q-Score: the Q-Score is a metric of the ligand binding strength to the protein that organizes the ligands in levels 1 – 5 (from lower to higher affinity) based on rms (root mean square) CSP-Score values. In this way, the threshold for the Q-score for a given level (for instance, level 5) will assign that level to all ligands with an rms-CSP-Score larger greater than the set threshold.
- Threshold for Final Status: to categorize ligands as hits or aggregators based on the CSP-Score values. A ligand with an rms-CSP-Score greater than the specified threshold will be set as a hit/aggregator. In the results table, hits will be highlighted in blue and aggregators in red.

All these parameters are applied after the peak matching and CSP calculation performed by the algorithm². For this, they can be modified after the Screen 2D analysis to recalculate CSP-Score, Q-Score, number of significant CSPs, and hits/aggregators identification.

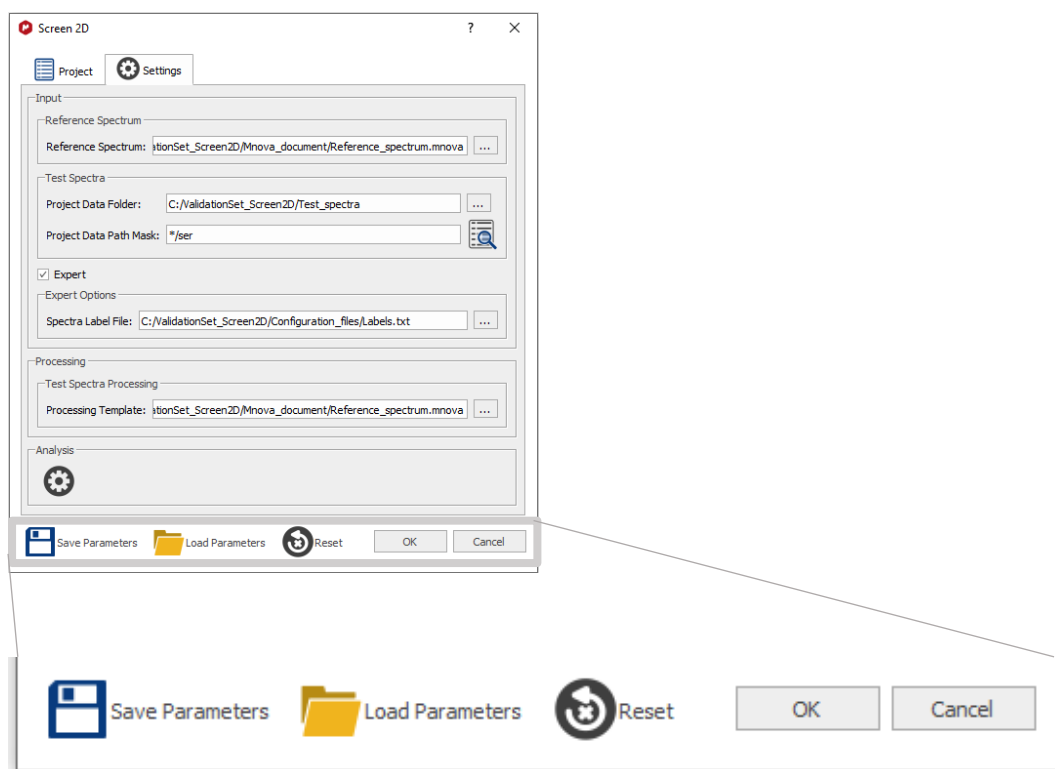
¹ This method is described in C. Peng, S. Unger, F. Filipp, M. Sattler, S. Szalma, Journal of Biomolecular NMR, 2004, 29, 491-504.

² For a detailed description of these parameters and how they are calculated, please go to the Screen 2D manual.

Once you have finished defining your settings you can select the OK button in the Screen 2D Analysis Parameters window.



You can save the input parameters for later reuse by clicking on “Save Parameters”; you can also load input parameters from a previously saved file using “Load Parameters”, or you can reset all parameters to their default values using “Reset”. You can then start the analysis by clicking on the OK button.



4. Results table and spectra

4.1. Screen 2D Results Editor

Once the analysis is complete, a Screen 2D Results Editor window can be displayed so you will be able to visualize the rms CSP-Score values, mean CSP-Score values, Q-Score, number of peaks with significant CSP, mean CSP, number of unmatched reference peaks, number of unmatched test peaks, along with a column for comments, for every ligand. Some of the options you will find in this window are:

- Load results from a previous analysis.

- Save current results.
- Report results.
- Configure report options.
- Clear current results.
- Modify parameters.

Test ID	Q-Score	rms CSP-Score	mean CSP-Score	# Significant CSP	Mean CSP	Max CSP	# Unm. Ref.	# Unm. Test	Comments
1 <input type="checkbox"/> L_Reference	0	0.0000	0.0000	0	0.000	0.000	0	0	
2 <input type="checkbox"/> L_01	3	0.0323	0.0219	22	0.016	0.094	4	5	
3 <input type="checkbox"/> L_05	4	0.0420	0.0323	29	0.022	0.082	8	6	
4 <input type="checkbox"/> L_07	4	0.0491	0.0391	28	0.026	0.110	11	6	

The option “Modify Parameters” displays the Screen 2D analysis parameters dialog, where the options for the “Scoring” section can be edited, with the consequent recalculation of the number of peaks with significant CSP, CSP-Score values, Q-Score rating and ligands categorized as hits/aggregators.

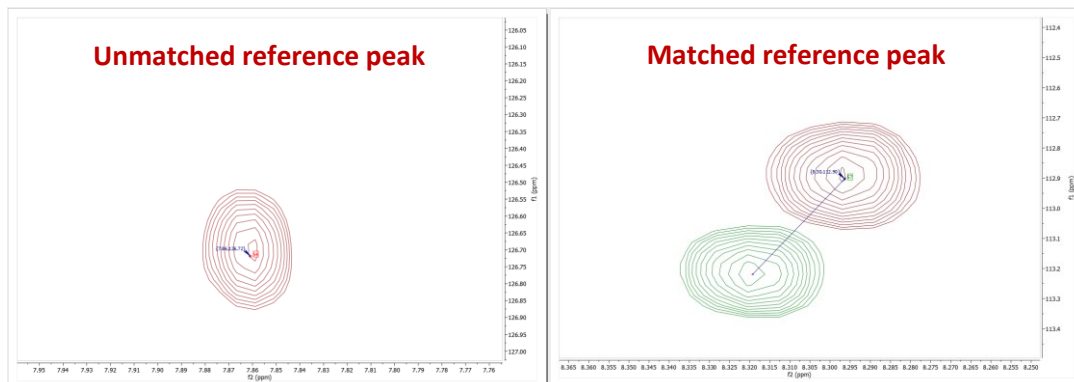
4.2. Screen 2D CSP Editor

If you double click on a ligand in the Results editor table (or select a ligand and then click on the CSP Table button) the CSP editor dialog is displayed, where a table with all the reference peaks and the analysis for this particular ligand is shown. Further, a Mnova document is displayed with the corresponding test spectrum stacked with the reference spectrum.

Ref. ID	Ref. δ_2	Ref. δ_1	Ref. Intensity	Test δ_2	Test δ_1	Test Intensity	$\Delta\delta_2$	$\Delta\delta_1$	CSP
9	0.935	24.123	2.466	-	-	-	-	-	0.160
10	1.013	19.062	1.628	0.920	19.296	2.272	-0.094	0.234	0.103
11	1.029	20.374	2.604	1.107	20.233	2.253	0.078	-0.141	0.082

4.3. Screen 2D Match Editor

If you double click on a peak in the CSP editor table (or select a peak from the table) and then click on the Match Table option, the Screen 2D Match Editor dialog is displayed and the region of the selected peak is zoomed. Matched peaks are shown with a line connecting the peak from the reference spectrum to the corresponding matched peak from the test spectrum.



The Match Editor table presents the information for the selected reference peak and its corresponding current match; you can also visualize a list of test peak candidates proposed by Screen 2D (peaks that fall into the region delimited by the “Maximal Differences” that were set for each nucleus). The Match Editor dialog allows the current peak result to be edited, offering different possibilities: unmatch the current matched peak, match the peak with a test peak from the list of candidates, and manual editing of a matched peak (allows a manual change, directly from the stacked spectra, of the current match test peak to a new one).

You can use the blue arrows to move to the next reference peak view and editing.

Screen 2D Match Editor

Current Test: L_11

Unmatch peak

	ID	δ_2	δ_1	Intensity	$\Delta\delta_2$	$\Delta\delta_1$	CSP
1	24	0.920	23.279	1.798			Manual mode of peak matching
2	Current	0.920	23.092	1.614	0.000	-0.187	0.035

Test Peak Candidates:

	Index	δ_2	δ_1	Intensity	$\Delta\delta_2$	$\Delta\delta_1$	CSP
1	1	0.920	23.092	1.614	0.000	-0.187	0.035
2	2	0.998	23.326	2.498	0.078	0.047	0.079
3	3	0.951	23.467	3.017	0.031	0.187	0.047

Exchange the current matched test peak for the candidate selected in the table. Double clicking on the candidate row has the same effect

Screen 2D Match Editor

Current Test: L_11

	ID	δ_2	δ_1	Intensity	$\Delta\delta_2$	$\Delta\delta_1$	CSP
1	9	0.935	24.123	2.466			
2	Current	-	-	-	-	-	0.160

Match with a test peak from the candidates in the table

Test Peak Candidates:

	Index	δ_2	δ_1	Intensity	$\Delta\delta_2$	$\Delta\delta_1$	CSP
1	1	0.951	24.170	2.343	0.016	0.047	0.018

