Mestrelab Research

Mnova Binding (Mnova 14.3)

STARTING GUIDE

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1. Introduction to Mnova Binding

In this Starting Guide, we will give you a brief overview of the main steps and functions available in Mnova Binding, which has been designed to carry out the quantitative chemical shift perturbation (CSP) analysis of 1D and 2D NMR titrations.

Mnova Binding deals with 1D and 2D NMR (typically HSQC-type) titration experiments where the target molecule (the receptor) spectrum is monitored in both the absence and the presence of increasing concentrations of a given species (the ligand) that binds to the receptor. The analysis of the CSP, as conducted according to the peaks of the receptor spectrum through the titration, provides quantitative information about the strength of the receptor-ligand interaction by means of the dissociation constant (K_D). In this way, for each peak selected, Mnova Binding tracks the peak movement, generates a binding curve (CSP vs. the ratio of ligand/protein concentration) and computes the associated K_D. With the individual K_D calculated for each peak, Mnova Binding performs statistical analyses to retrieve a single "representative" K_D based on a weighted average function. Mnova Binding allows the user to perform an initial curve fitting to a simple 1:1 model and, in a second step, a more sophisticated analysis is possible by exporting the curves to the AFFINImeter-NMR[1] module.

The main characteristics of the software include:

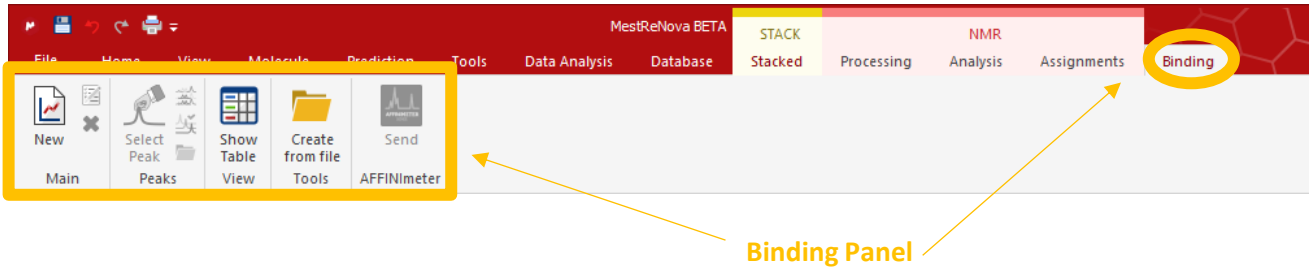
- Automated and manual peak tracking along the titration spectra.
- For each peak tracked, automatic generation of the corresponding binding curve and K_D calculation.
- Automatic calculation of average K_D.
- Semi-automated detection of "bad" K_D values and their exclusion from the calculation of the average K_D.
- Easy inspection of results, peak by peak.
- For a given target receptor, performs automated analysis of multiple titrations (for instance, involving different ligands), working in batch mode.
- Data exportation to the AFFINImeter-NMR module for an advanced and in-depth analysis.
- Saving and reporting of the analysis results.

Following in this Starting Guide, we will introduce you the main steps to the CSP analysis in Mnova Binding.

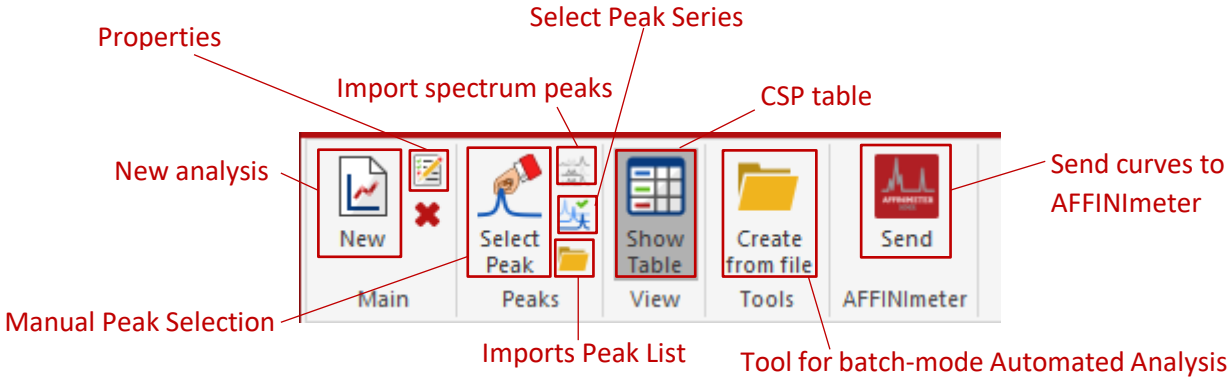
[1] AFFINImeter NMR is a functionality of Mnova Binding developed by the Company Software 4 Science Developments.

2. The Mnova Binding Panel

Before you start an analysis in Mnova Binding, the titration spectra must be loaded in an Mnova document, superimposed on the same page. Then, you can go to the Mnova Binding tab located in the main ribbon under "Binding".



This panel contains several options:



These options allow you to perform a manual or automated CSP analysis. In the following sections, we explain both approaches and how to export the binding curves to AFFINImeter-NMR in greater detail.

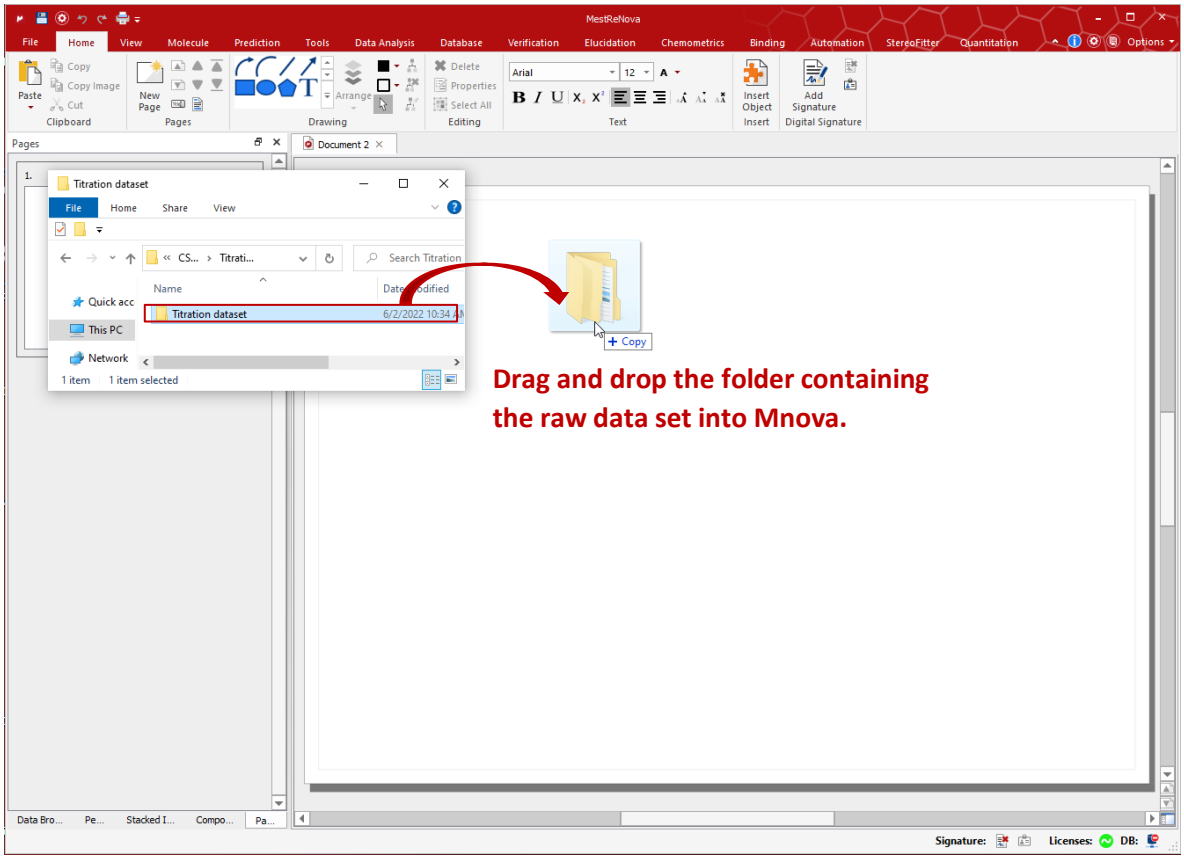
3. Manual Chemical Shift Perturbation Analysis

3.1. Superimpose the titration spectra in Mnova

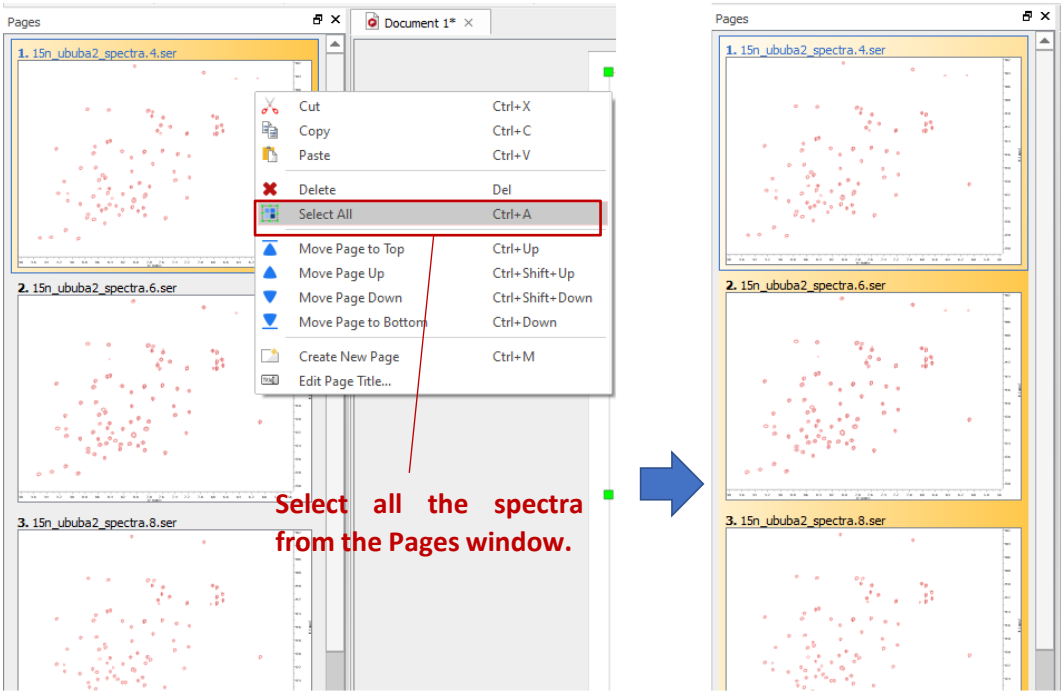
There are different approaches to superimposing the titration spectra in Mnova, as described in the Mnova Manual. This section explains two of these approaches: 1) manually, by loading the spectra into Mnova and using the options in the tab "Stacked", and 2) automatically, by using the "Import Directory Spectra Stack" script available in Mnova's Script tools section.

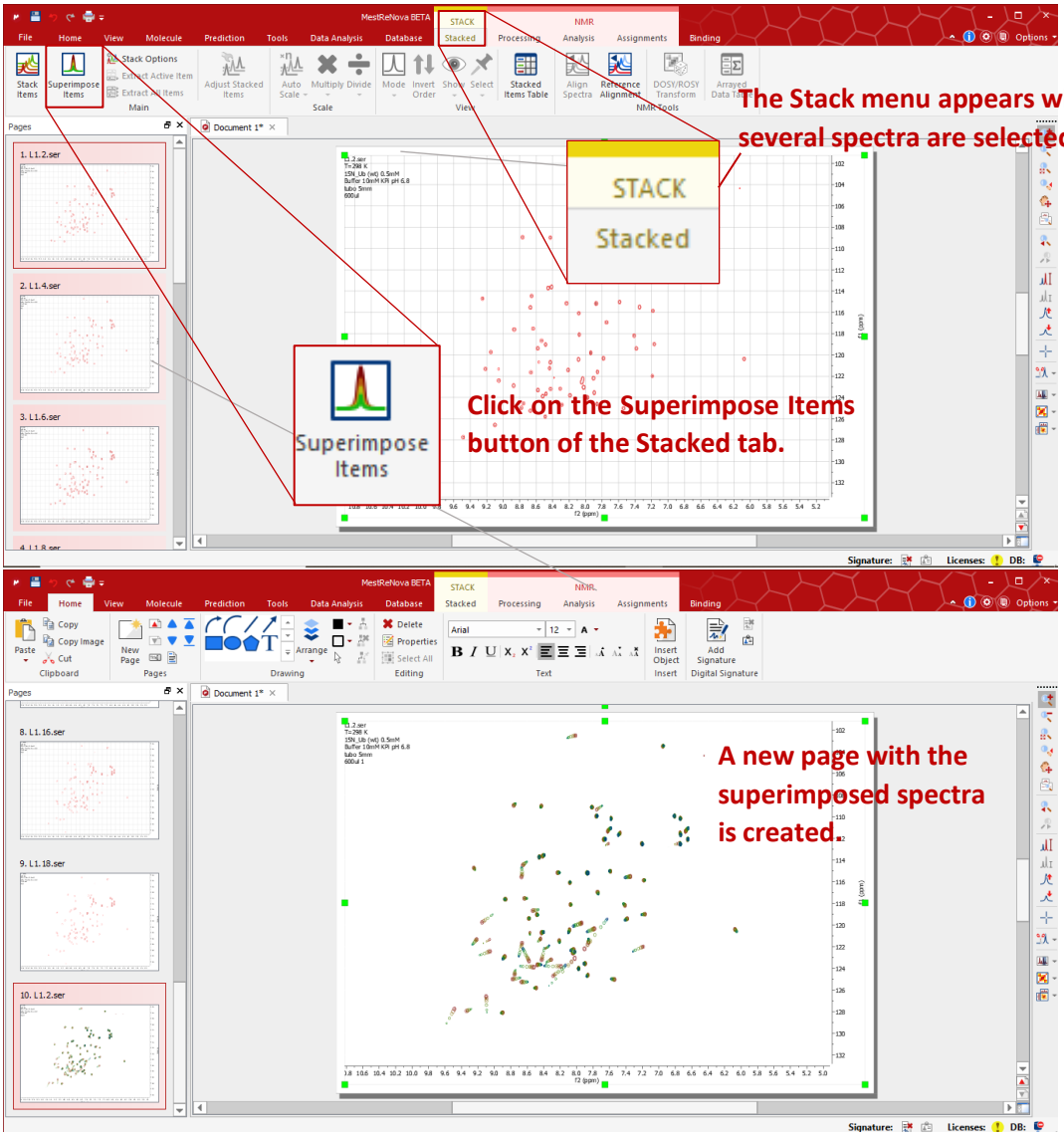
3.1.1. Manually

To superimpose the spectra manually, you need to import them into the same Mnova document (e.g., by dropping your raw data into Mnova). Once the relevant data has been imported, the spectra are automatically processed and individually displayed on different pages of the Mnova document.



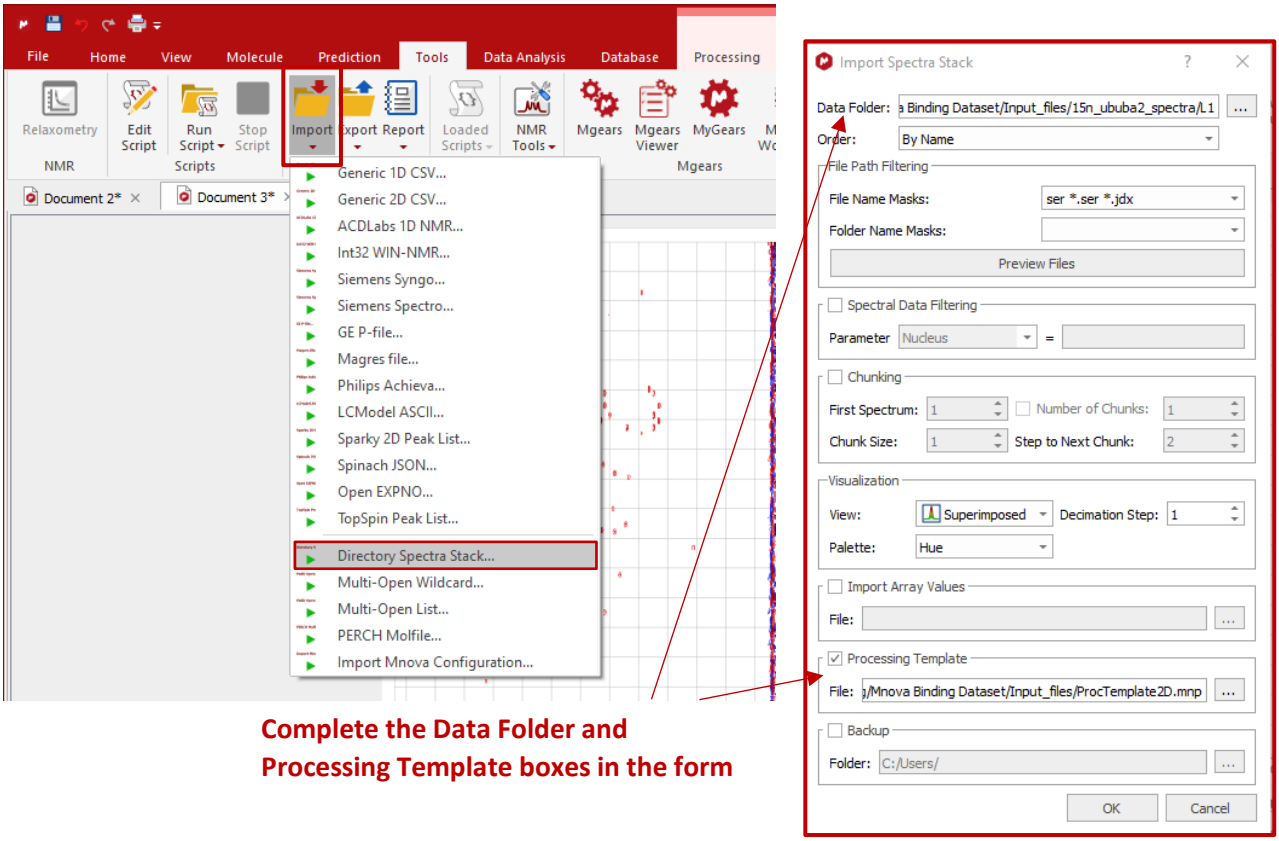
In the “Pages” window, select a page and right click with the mouse to use the option “Select All” (or press ctrl + A), which selects all the pages of the document. Use the “Superimpose Items” option in the Stacked tab to superimpose the spectra on the pages selected. The superimposed spectra will be displayed in a new document page.





3.1.2. Automatically

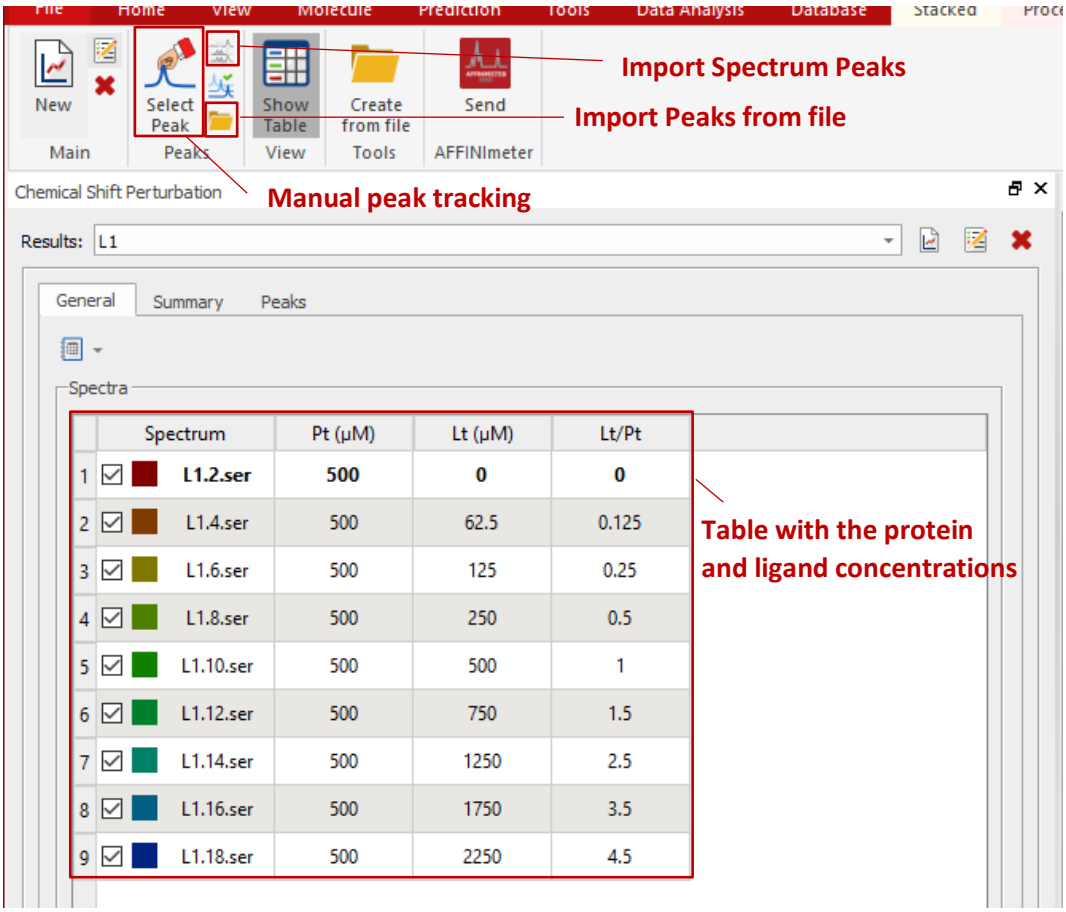
Use the “Import Directory Spectra Stack” script available in *Tools > Script Tools > Import > Directory Spectra Stack*. The Import Spectra Stack window that appears contains fields to gain the titration spectra from the folders where they are located, and options to set the final display of the spectra in the Mnova document. For subsequent use in Mnova Binding, use the “Superimposed” option. Additionally, you can include the path to a processing template file to apply defined processing to all the spectra involved in the titration. When the OK button is clicked, Mnova loads the spectra and automatically creates a document with a single page with the spectra superimposed.



Complete the Data Folder and Processing Template boxes in the form

3.2. Peak tracking

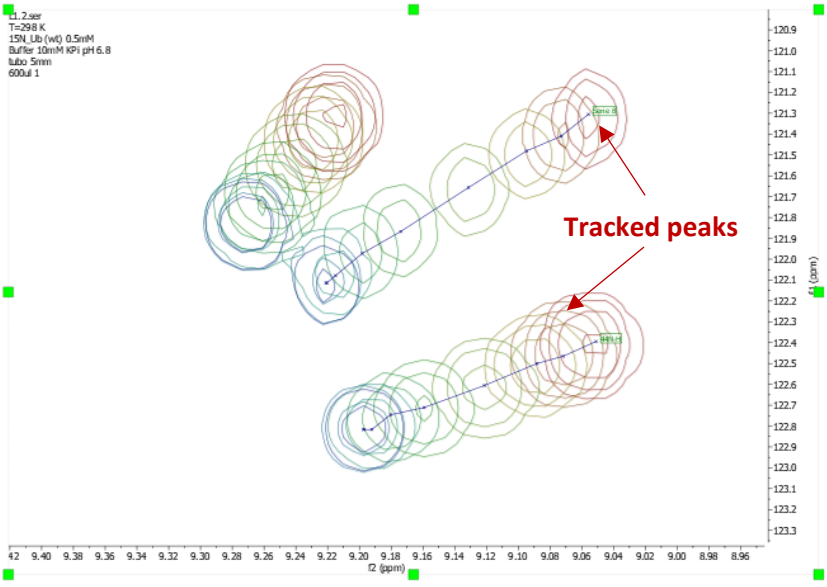
To begin the analysis, click on the “Show Table” button of the Mnova Binding menu to display the Chemical Shift Perturbation dialog. Then, click on “New” to start a new analysis project. In the General tab, a table with the list of superimposed spectra is shown, where the concentrations of the Receptor (Pt) and the Ligand (Lt) must be entered. An additional column giving the Lt/Pt concentration ratio is also included in this table. When the concentrations are added, appropriate options to track peaks are activated.



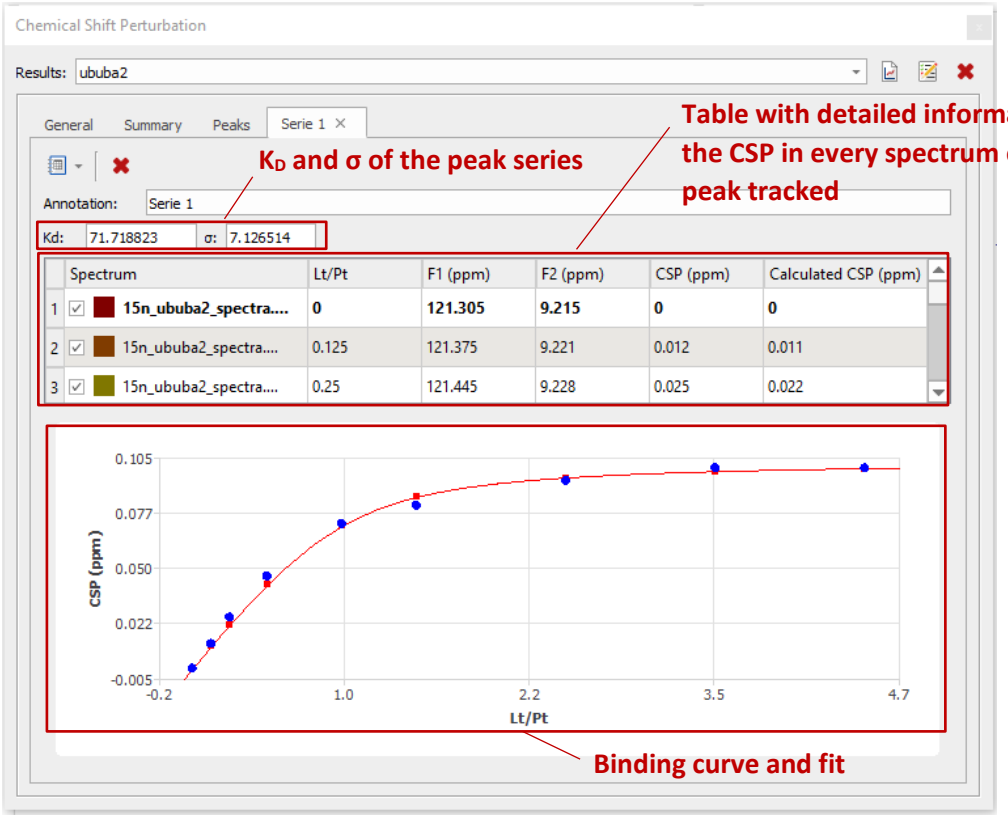
There are different ways to track the peaks:

3.2.1. Manual peak tracking:

- Click on the “Select peak” button and then click with the left mouse button on one peak of the first spectrum (ligand concentration = 0). Mnova Binding will then identify and track the analogous peak in the other spectra.
- Alternatively, after clicking on the desired peak with the left mouse button, you can hold down and drag the cursor in the direction in which the peak is moving.

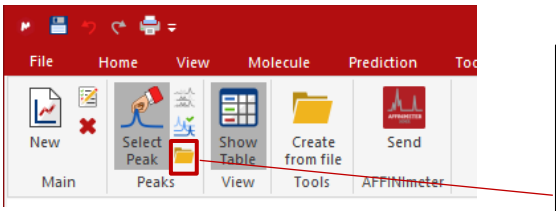


A new tab “Series n” is automatically generated with information on the peak being tracked. The corresponding binding curve is also displayed in this tab with the curve fitted to a 1:1 binding model and the resulting K_D value displayed. Users can also access this “Series n” tab by double clicking on the corresponding tracked peak in the Peaks table.

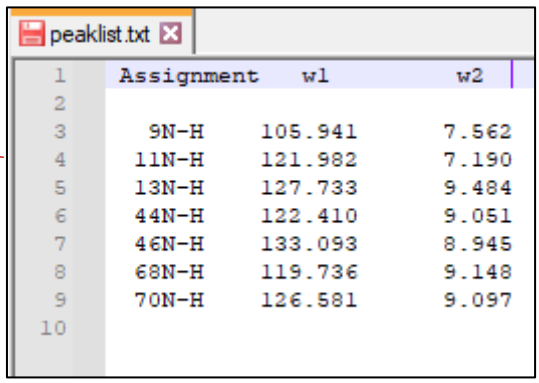


3.2.2. Importing a list of peaks:

You can import a text file with a list of peaks using the “Import Peaks From File” option. In this file, a label and chemical shift in each dimension of the peaks is included. The peaks in this file will be automatically identified and tracked.



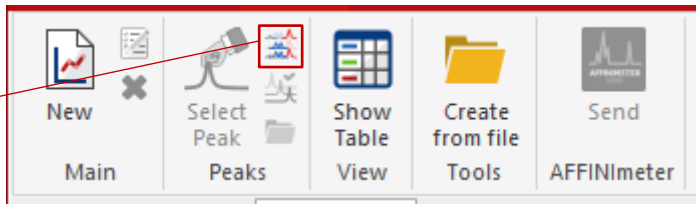
Tracks peaks listed in a txt file.



3.2.3. Importing the peaks picked in the spectra:

If there are peaks picked in any of the superimposed spectra, the “Import Spectrum Peaks” button is enabled and, when clicked, the peaks picked are automatically tracked. You can find more information about the peak picking options in the Mnova Manual.

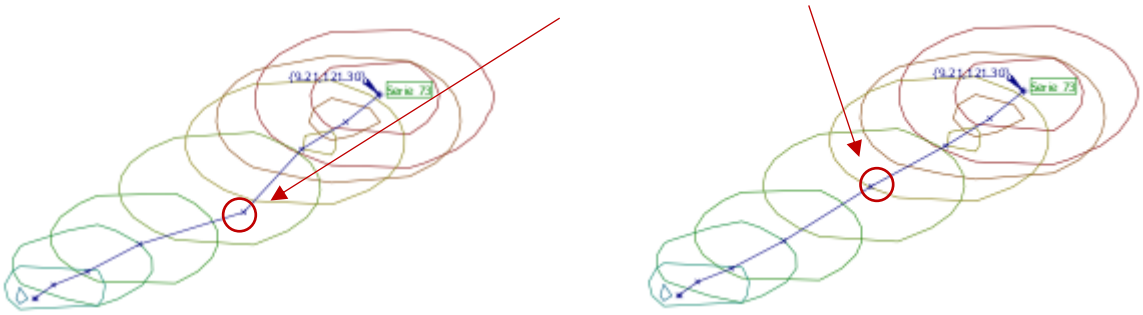
With this option, the peaks picked will be tracked automatically



3.3. Manual refinement of peak tracking

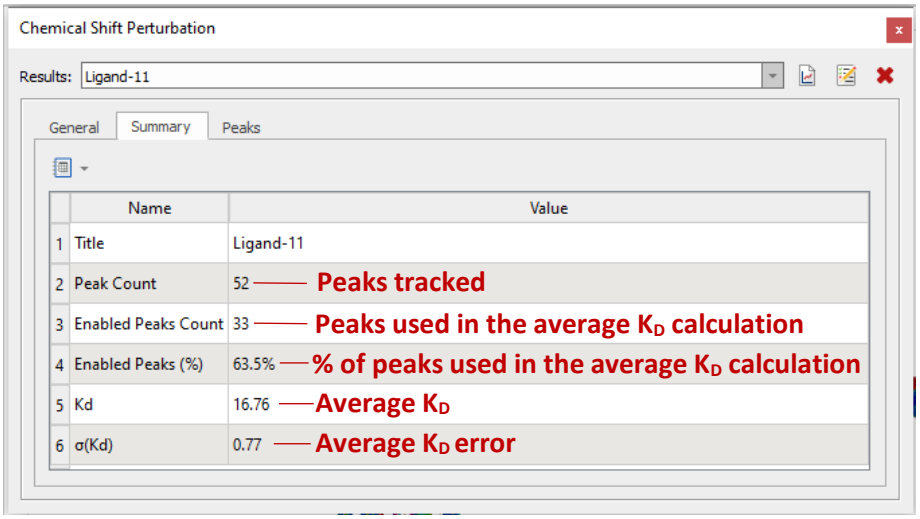
Manual refinement of peak tracking is also possible by placing the cursor on the position tracked for a given spectrum, clicking with the left mouse button, pressing the Shift key, holding down and dragging the cursor to the new position (note: in order to use this functionality, the “select peak” option must be off).

Manual refinement of the peak tracked

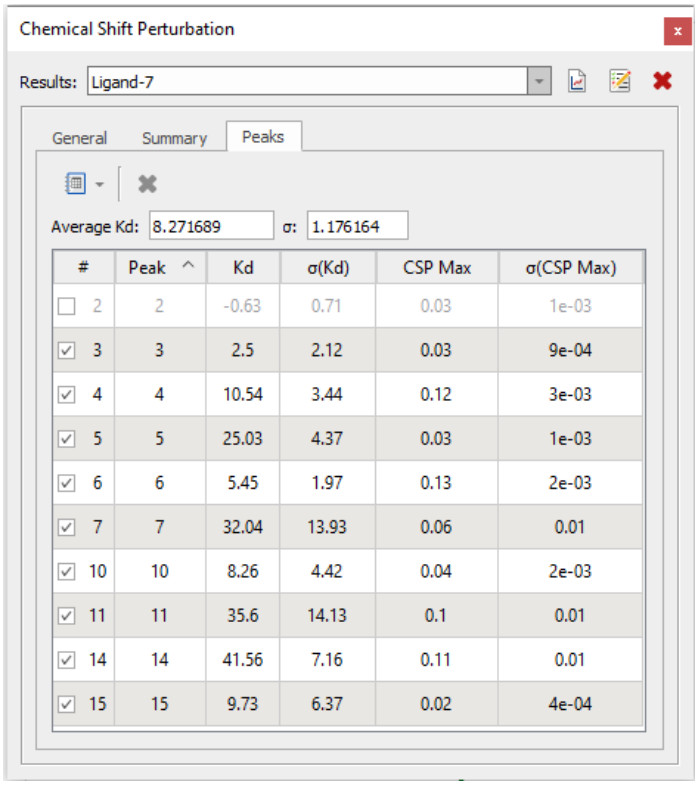


3.4. Binding curve generation and fitting

The results of your analysis will be displayed in the Summary and Peaks tabs. The Summary tab reports information about the statistical analysis performed to calculate a single “representative” averaged K_D value.



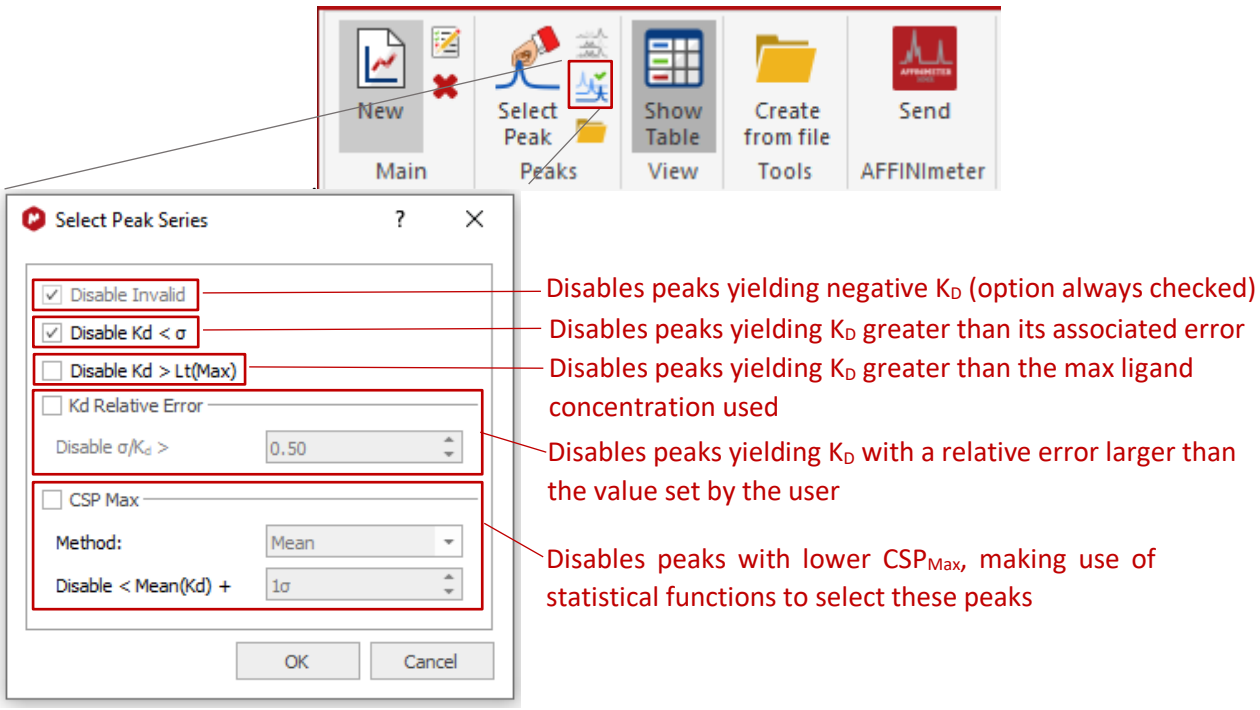
The Peaks tab shows a table listing all the peaks tracked, together with their individual K_D values and CSP_{Max} (maximum CSP at the saturation point) and respective errors, as calculated from the fitting of each curve. The average K_D is also shown at the top of this table. In this list, it is also possible to enable or disable the peaks manually to include/exclude certain peaks from the calculation of the average K_D. For this, use the checkbox next to the peak in the table. By default, Mnova Binding disables "unacceptable" results such as peaks that yielded negative K_D values (which corresponds to a situation where the fit did not converge to a solution). Disabled peaks are greyed out in the table.



#	Peak	Kd	σ(Kd)	CSP Max	σ(CSP Max)
2	2	-0.63	0.71	0.03	1e-03
3	3	2.5	2.12	0.03	9e-04
4	4	10.54	3.44	0.12	3e-03
5	5	25.03	4.37	0.03	1e-03
6	6	5.45	1.97	0.13	2e-03
7	7	32.04	13.93	0.06	0.01
10	10	8.26	4.42	0.04	2e-03
11	11	35.6	14.13	0.1	0.01
14	14	41.56	7.16	0.11	0.01
15	15	9.73	6.37	0.02	4e-04

3.5. Screening options to detect and disable "bad" results for individual fittings

The button "Select Peaks Series" displays a dialog with various options to screen the peaks being tracked based on a series of filters set by the user. Here, the goal is to efficiently disable the peaks tracked when fitting does not fulfil the requirements set by the filters applied. The following options are available:

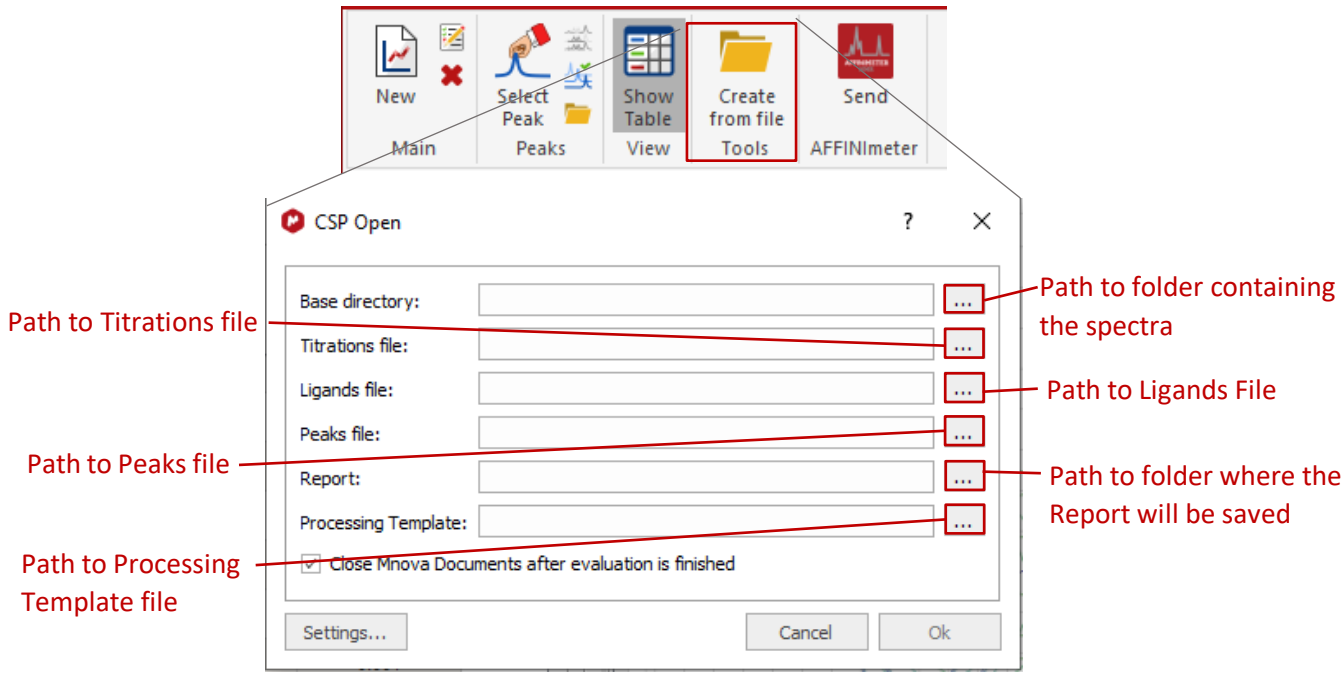


4. Automated Chemical Shift Perturbation Analysis

By clicking on the “Create from file” option, a “CSP Open” dialog containing a form is displayed. To begin the analysis, complete the form with the path to the folder containing the spectral dataset and, additionally, the paths to the following files:

- Titrations file: text file with the receptor and ligand(s) concentration.
- Ligands file: text file with the ligand(s) label.
- Peaks file: text file with the location of the peaks that will be tracked (ppm for each spectral dimension).

Optionally, the path to a folder in which to save the analysis report and the path to a processing template that will be applied to the spectral set can also be added.



See some illustrative examples of Titration, Ligands, and Peaks txt files below for the automated analysis of two titration datasets involving a receptor and two ligands, L1 and L2, where the labels “Ligand 1” and “Ligand 2” are used, respectively. The Titration file for all the spectra (located in the same folder) are organized in titration with L1 (spectra 4, 6, 8, 10, 12, 14, 16, and 18), titration with L2 (24, 26, 28, 30, 32, 34, 36, and 38), and the reference spectrum (spectrum 2, receptor without ligand), is the latter being common to both titrations. In the first line of the document, the concentration of the receptor is specified; in the following lines, next to each spectrum number, the corresponding ligand/receptor concentration ratio is given.

Ligands file

```
L1 Ligand 1

L2 Ligand 2
```

Titrations file

```
[P]uM                500

EXPNO              [L]/[P]

REF
2                  0.000

L1
4                  0.125
6                  0.250
8                  0.500
10                 1.000
12                 1.500
14                 2.500
16                 3.500
18                 4.500

L2
24                 0.125
26                 0.250
28                 0.500
30                 1.000
32                 1.500
34                 2.500
36                 3.500
38                 4.500
```

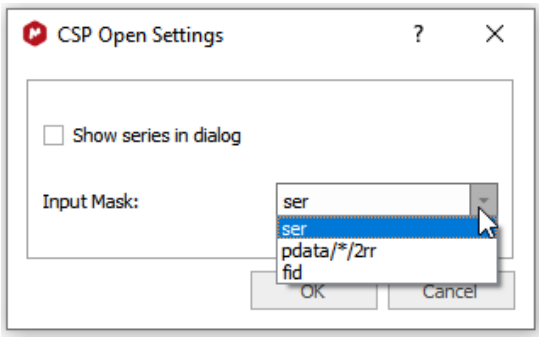
Peaks file

```
Assignment          w1          w2

      9N-H     105.941       7.562
     11N-H     121.982       7.190
     13N-H     127.733       9.484
     44N-H     122.410       9.051
     46N-H     133.093       8.945
     68N-H     119.736       9.148
     70N-H     126.581       9.097
```

From the CSP Open dialog, a window will be displayed. Using the “Settings” button requires the appropriate input mask to be chosen depending on whether your titration involves 1D or 2D NMR experiments (e.g., input

masks “Fid” and “ser” will recognize 1D and 2D raw NMR data, respectively). Custom input masks can also be used at this point.



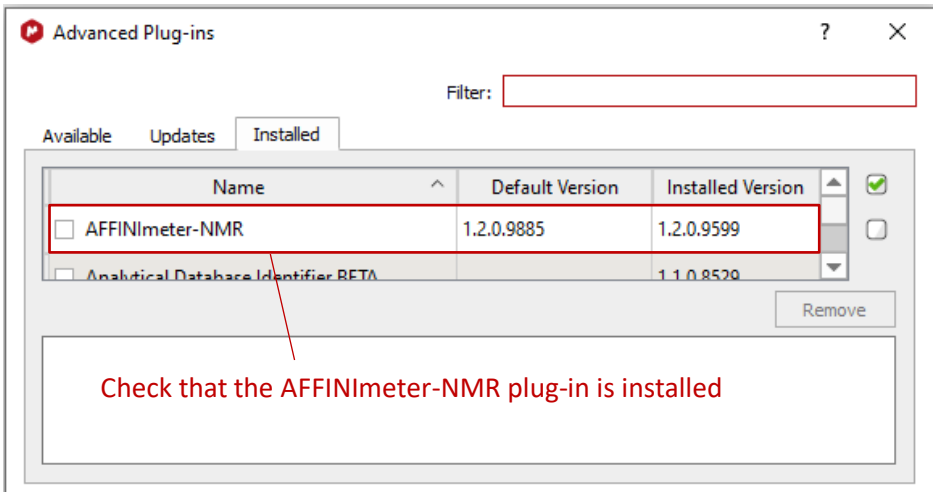
Once you have completed the form, click on “OK”. Mnova will load, process, and superimpose the spectra and the CSP analysis will be automatically performed in Mnova Binding based on the information provided in the text files.

5. Send to AFFINImeter

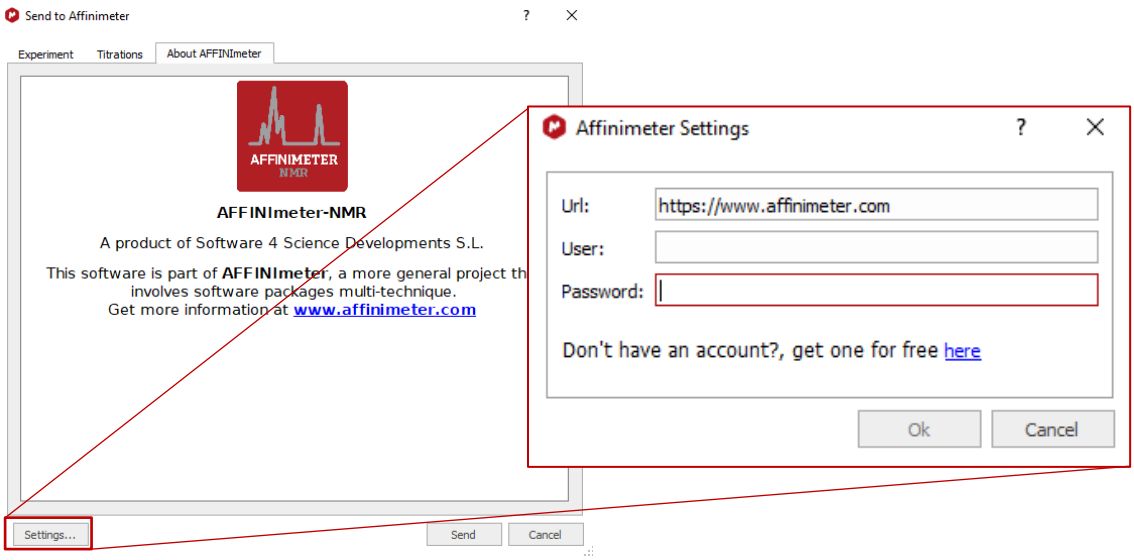
The “AFFINImeter” button exports the curves generated to the AFFINImeter-NMR module, which offers the possibility of performing an advanced analyses with the following options:

- Global fitting of the entire set of curves to retrieve a single global K_D value.
- Fitting to advanced binding models that involve, for example, stoichiometries other than 1:1 and the participation of a third species (e.g., a competitor or a cofactor).

If you do not find the AFFINImeter button, check in the Advanced Plug-ins dialog (*File > Advanced Plug-ins)* to ensure that the AFFINImeter-NMR plugin has been installed. If not, go to the *Available* tab, where it should be ready for installation.



When clicking on the AFFINImeter button, a “Send to AFFINImeter” window is displayed. Clicking on the Settings button displays a dialog where you are required to include your AFFINImeter user credentials.


Send to Affinimeter
Experiment
Titrations
About AFFINImeter
AFFINIMETER
NMR
AFFINImeter-NMR
A product of Software 4 Science Developments S.L.
This software is part of AFFINImeter, a more general project th
involves software packages multi-technique.
Get more information at www.affinimeter.com
Settings...
Send
Cancel
Affinimeter Settings
Url:
https://www.affinimeter.com
User:
Password:
Don't have an account?, get one for free here
Ok
Cancel