Mestrelab Research
chemistry software solutions

Mnova Suite Tutorial

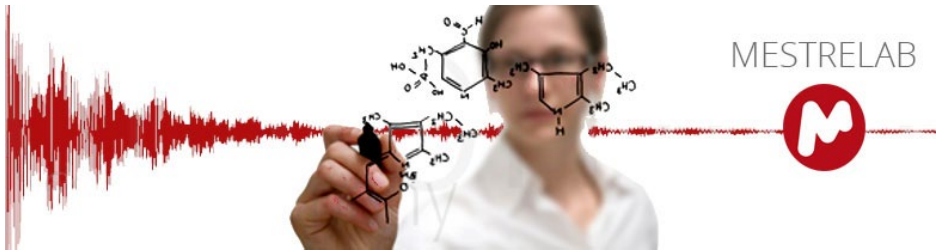
NMR
NMRPredict
MSChrom

Updated on 12/24/2022

Mnova Suite | Starting guide

- Open and process 1D and 2D NMR data
- Multiplet analysis for 1D ^{1}H NMR
- Assign 1D peaks to a structure
- Assign 1D and 2D spectra
- Report analysis results
- Basic handling of multiple spectra
- Predict, assign and verify
- LC/GC-MS data processing

Note: This tutorial covers only the NMR, NMRPredict, and MSChrom Mnova plugins

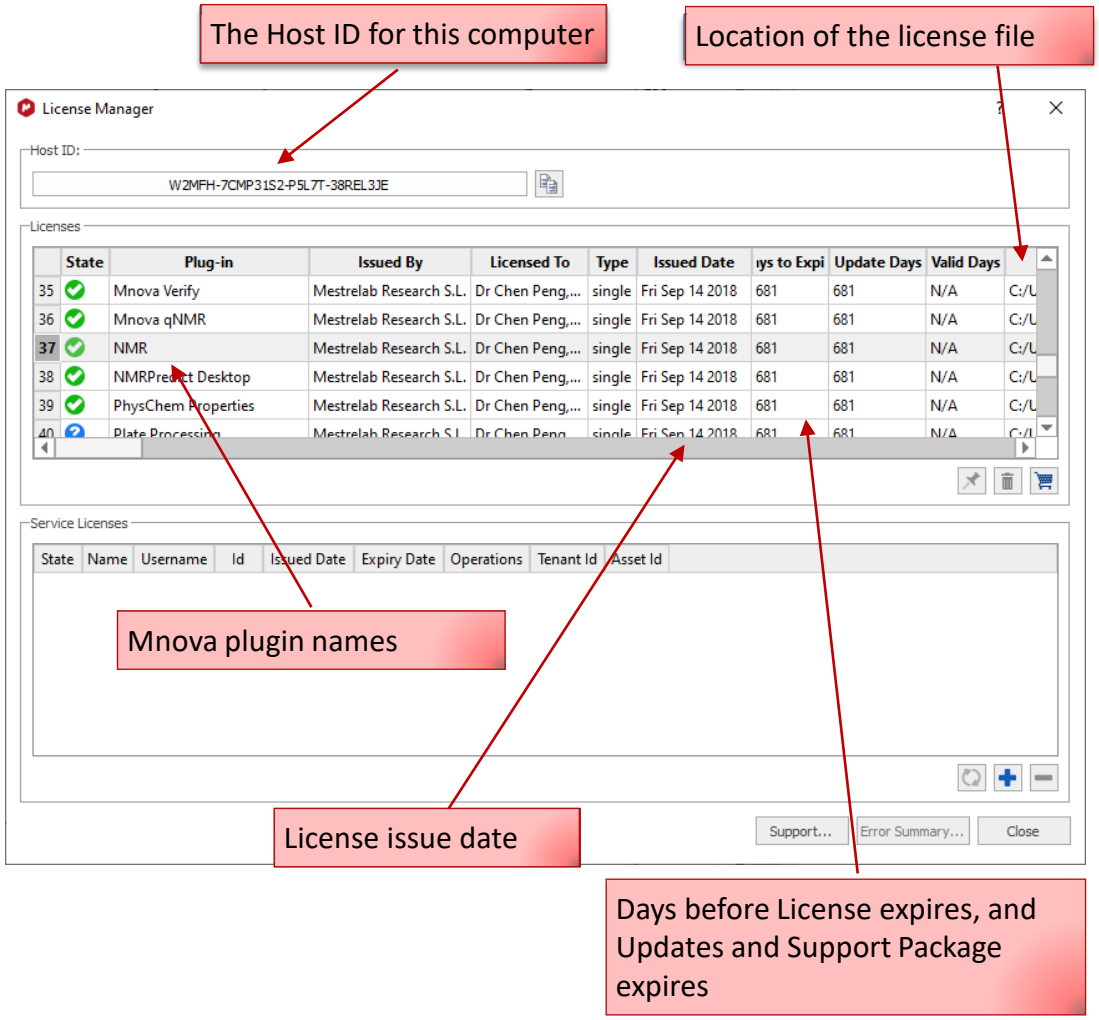


Installation and Activation of Mnova, and General Setup

You will need to have Mnova Suite license

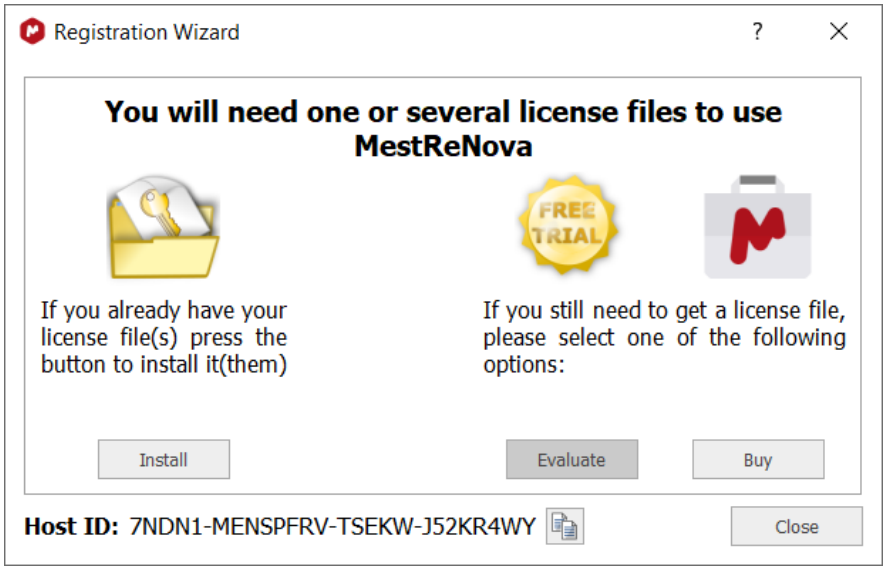
Download, install, and activate Mnova

- Download and install Mnova from www.mestrelab.com
- Choose File > Help > License Manager to open the License Manager dialog
- The status of the license activation of the plugins you've installed are listed. You can hover the cursor on the State icon, and it will display the status of that plugin
- To activate the plugins, click the button to open the Registration Wizard (see next page)



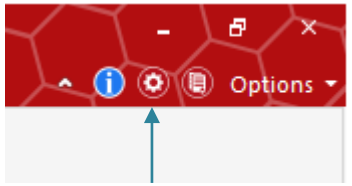
Activate Mnova

- If you have a license file (.lic or .zip), click *Install* to open it
- If you don't have license files, click *Evaluate* to apply for 45-day free trial licenses online, or otherwise click Buy to purchase a license
- To manage campus/site/concurrent licenses, click here



Turn on Auto Baseline Correction for 1D NMR

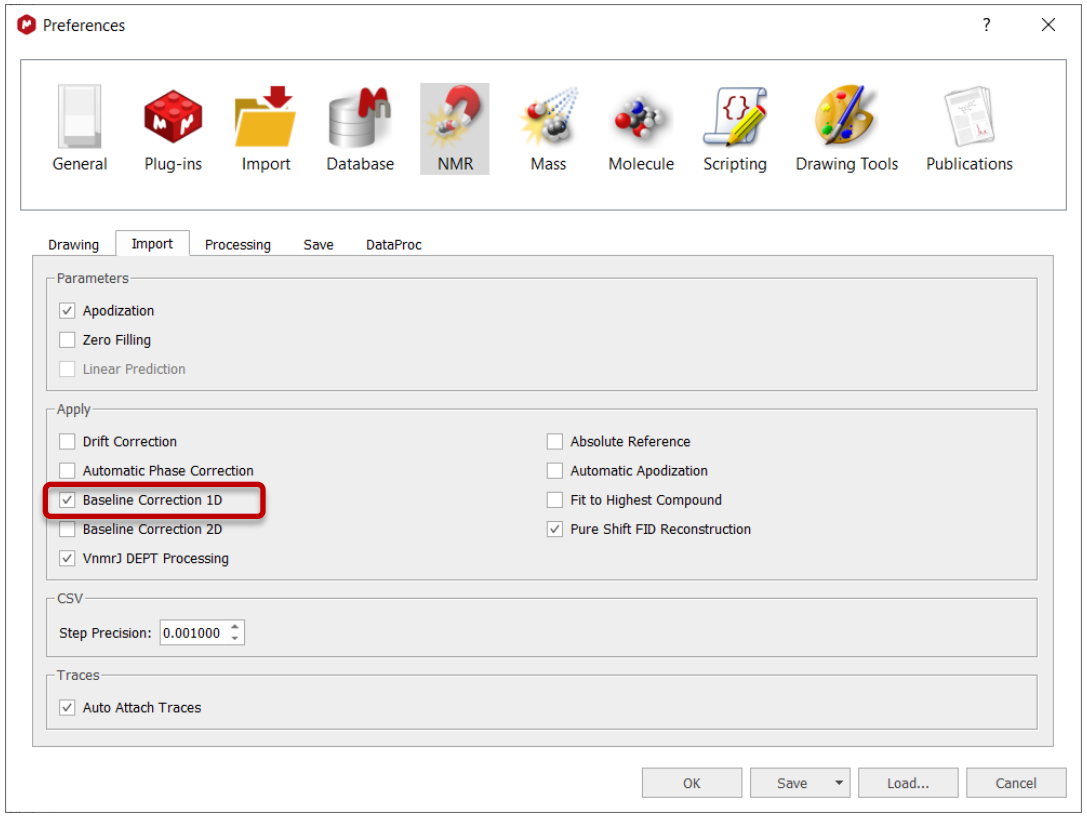
Choose File/Preferences. In the NMR > Import Tab, check Baseline Correction 1D so that baseline correction is automatically performed when you open an NMR spectrum.



Shortcut for Preferences

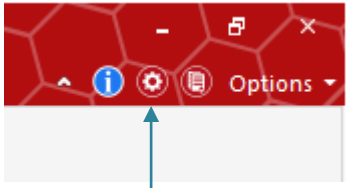
Note: Automatic Baseline Correction uses the default algorithm of "Bernstein Polynomial with order of 3", or the one that you used previously. Be aware of the default baseline algorithm it uses.
We don't recommend checking the Baseline Correction for 2D NMR because this may make manual phasing of 2D NMR sluggish. You can apply baseline correction manually after the phase has been corrected.

Tip: There are many other options and settings that you can change in the Preferences Dialog.



Setup the resolution for publishing spectra*

Choose File/Preferences. In the Drawing Tools tab, change the resolutions for Image Exporting and Image Copying to numbers similar to something shown below.

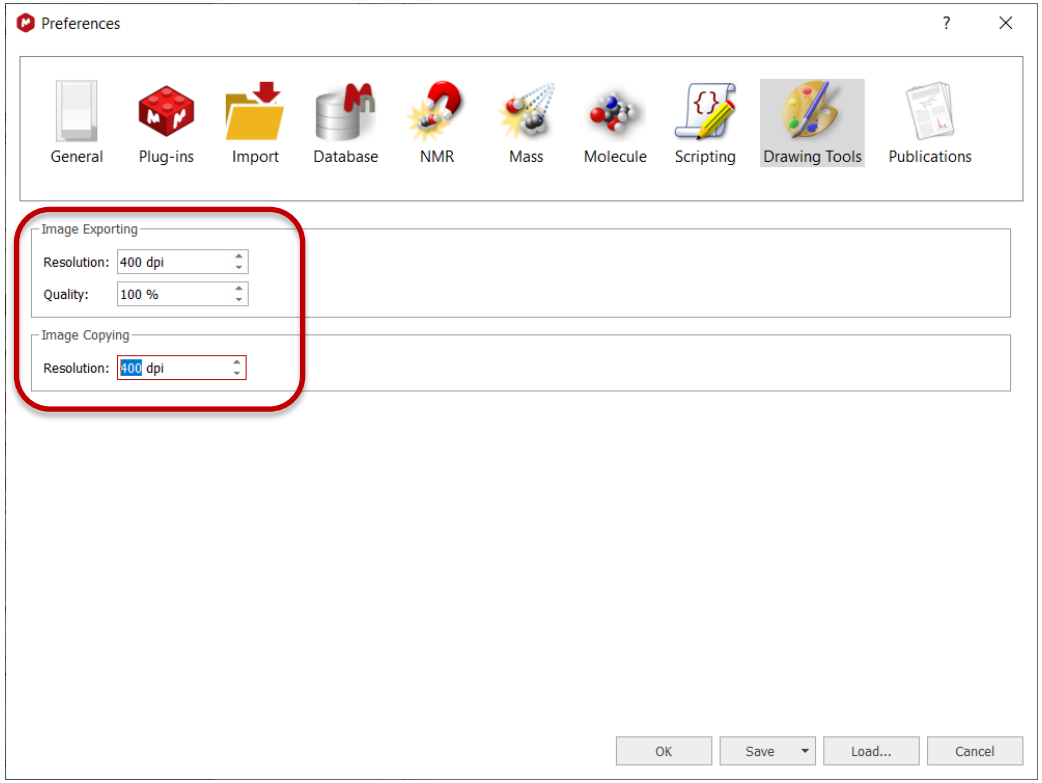


Shortcut for Preferences

The resolution for Image Exporting is used when you choose File > Save As and save the selected objects in Mnova as graphical image files.

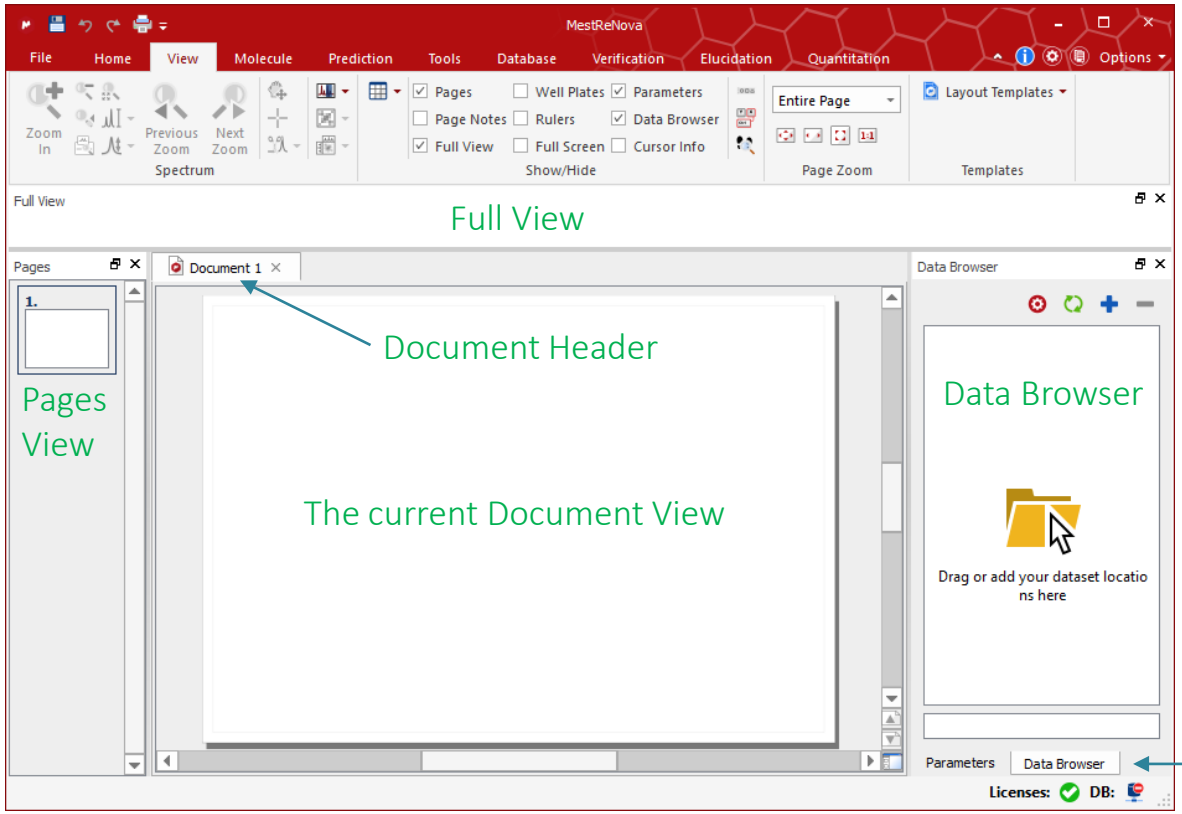
The resolution for Image Copying is used when you copy selected objects in Mnova and paste them into other applications.

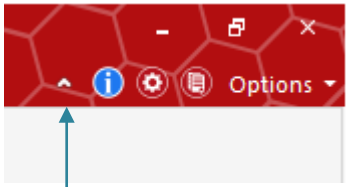
**Note: Increasing the graphics resolution can increase the size of the PDFs generated by Mnova. If you are mostly saving the reports in PDFs and want to conserve the disk space, skip this step.*



Setup the Workspace

- In the View Ribbon, check the Pages, Full View, Parameters, and Data Browser Views
- Dock and arrange them as shown below



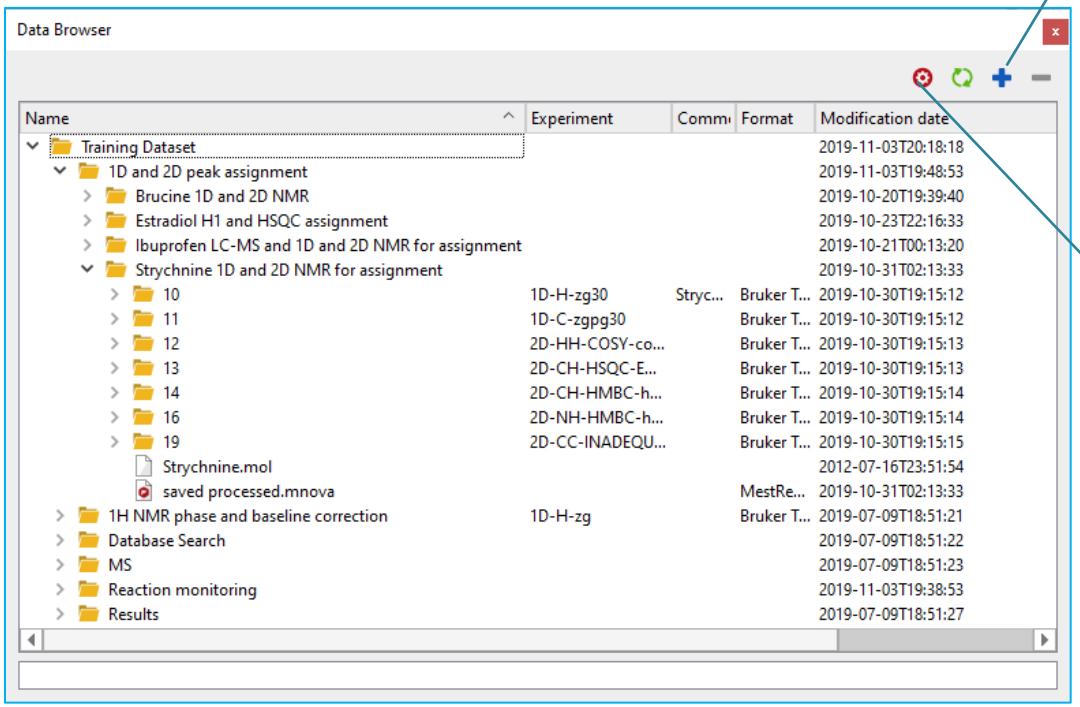


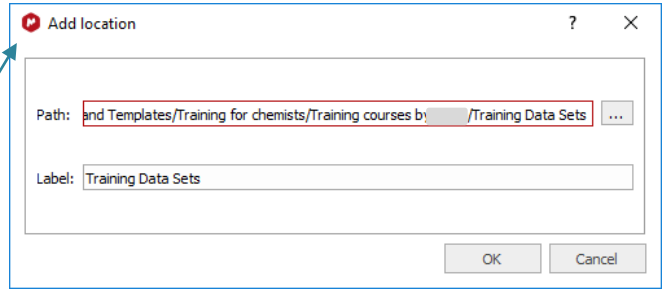
Click here to minimize the ribbon

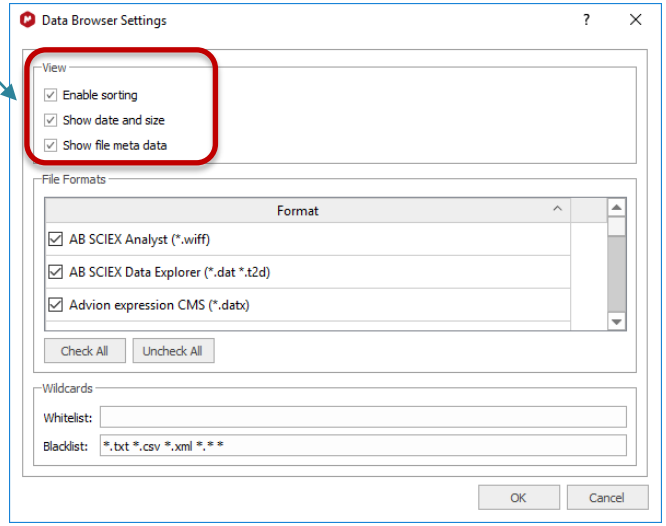
Click here to switch the panels or tables

Setup the Workspace

- Click “+” in the Data Browser, navigate to the directory where your NMR or LCMS data are located, and click OK to add it.
- Click the ‘Settings’ button to turn on the display of metadata, date and time, and enable sorting

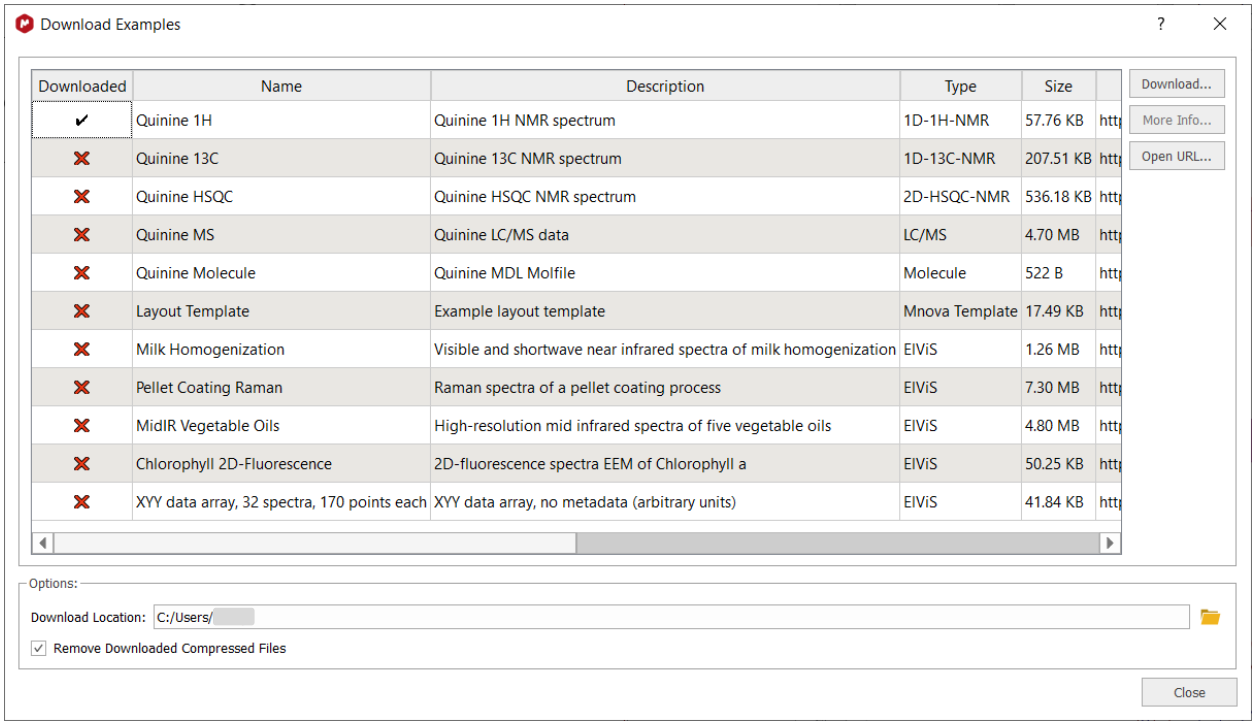






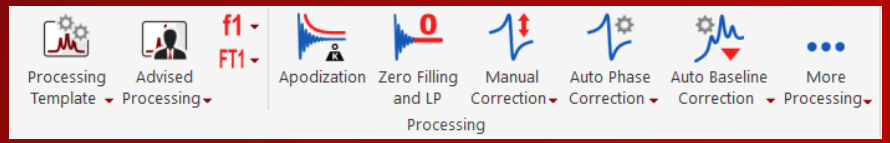
To download example datasets

If you need to download example datasets for practice, such as 1D and 2D NMR, LC/MS data, and the structure of quinine, choose File > Help > Download Examples



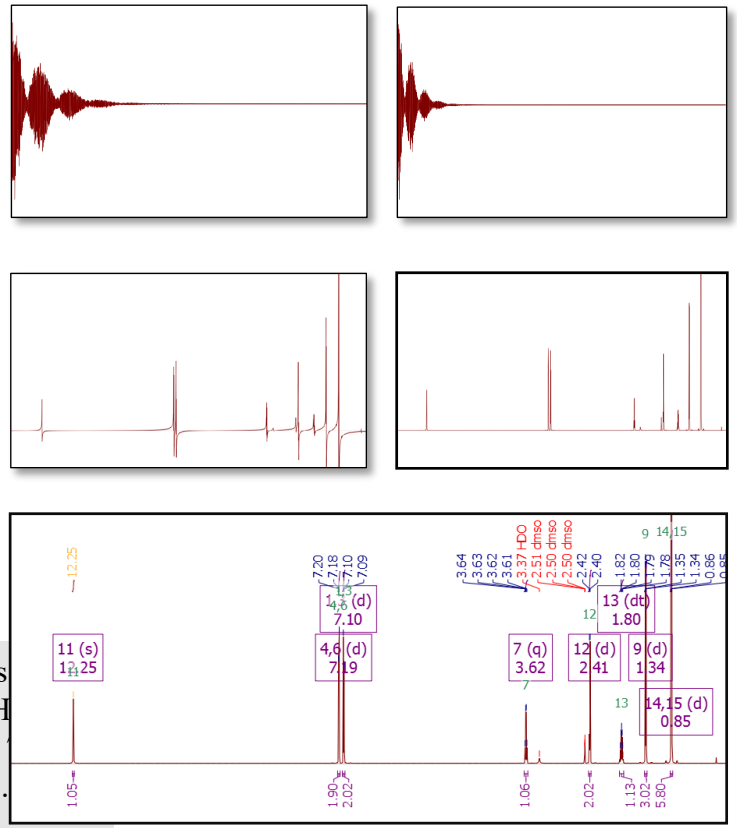
Basic ^{1}H NMR Processing

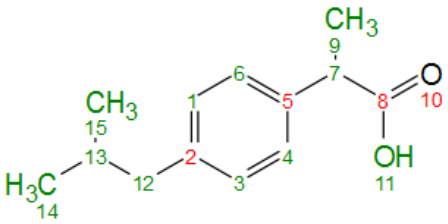
You will need to have an Mnova NMR license for this section



^{1}H processing and analysis: General procedure

- Open the raw data
- Pre-process the FID: apodize, zero fill, linear predict, etc.
- Fourier transform
- Phase correct and baseline correct
- Chemical shift reference
- Peak pick, integrate, multiplet analysis
- Peak assignment
- Report and publish



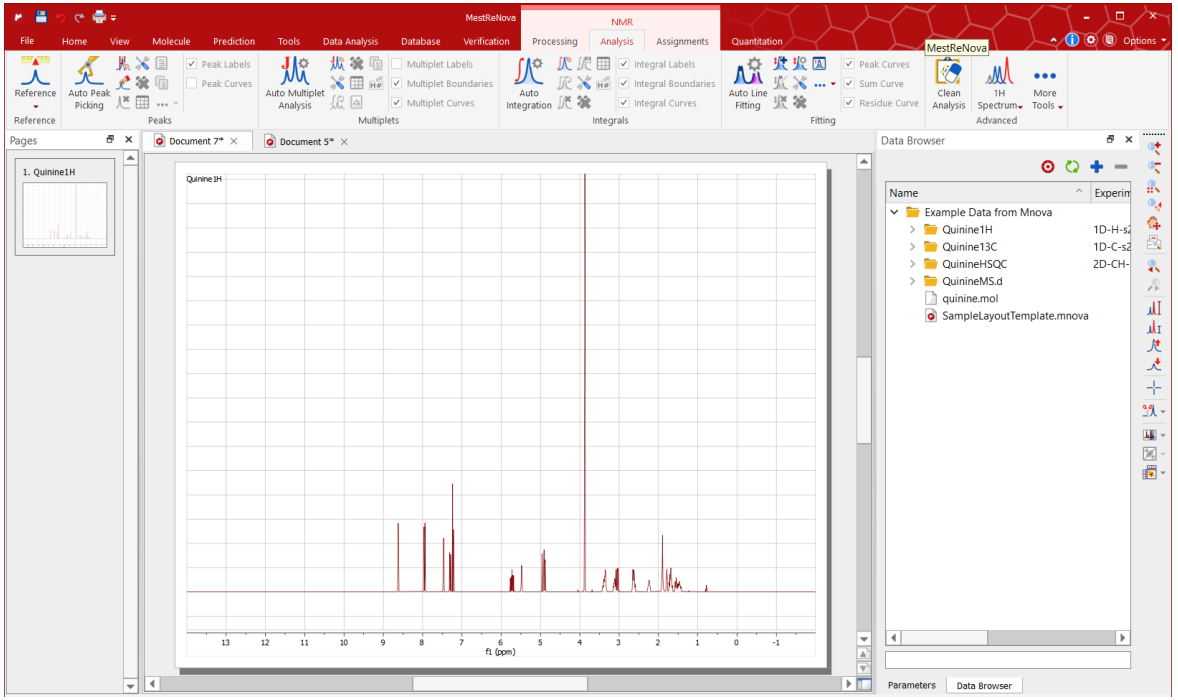


^{1}H NMR (600 MHz, DMSO-d_6) δ 12.25 (s
7.19 (d, J = 7.8 Hz, 2H), 7.10 (d, J = 7.9 H
2H), 3.62 (q, J = 7.1 Hz, 1H), 2.41 (d, J =
Hz, 2H), 1.80 (dt, J = 13.5, 6.8 Hz, 1H), 1.
J = 7.1 Hz, 3H), 0.85 (d, J = 6.7 Hz, 6H).

Note: Most of these steps are done automatically by Mnova. However, you retain full control at all times.

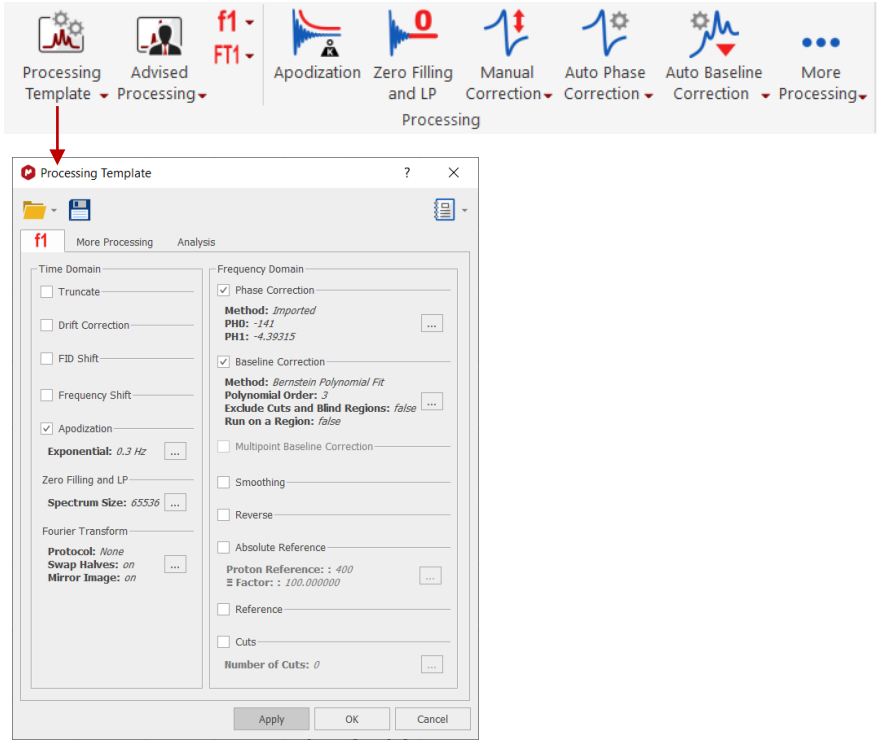
Open and transform NMR data

- Use '**File > Open**' to open one spectrum or drag and drop one or multiple data from the **Data Browser** onto the Mnova canvas
- When a raw data is brought in, it is automatically processed based on the original processing parameters and Mnova preferences



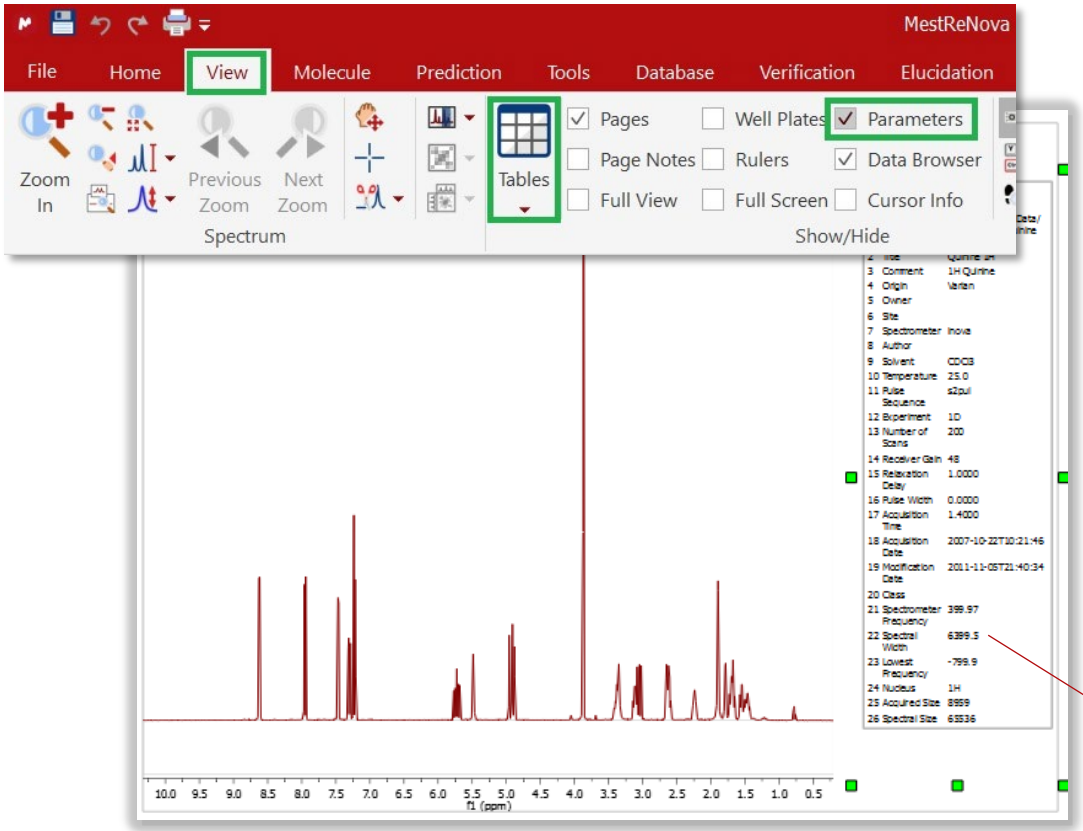
Reprocess the spectrum if needed

- If needed, use tools in **NMR/Processing Ribbon** to adjust any processing parameters to re-process the spectrum
- Or use the **Advised Processing** to reprocess the spectrum with suggested parameter settings
- The processing parameters can also be modified or imported using the **Processing Template**.



Display the acquisition parameters

- Go to **View/Tables… Parameters** to view the acquisition parameters
- Press **Report** to report the parameters as a text box on the page



Parameters

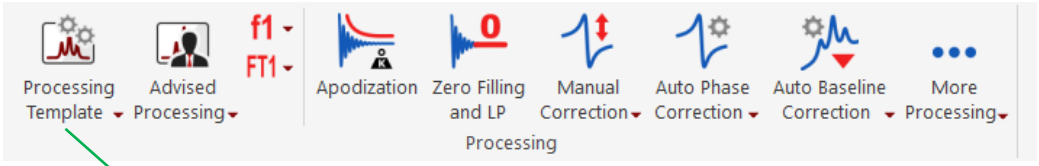
Report Copy Setup Customize

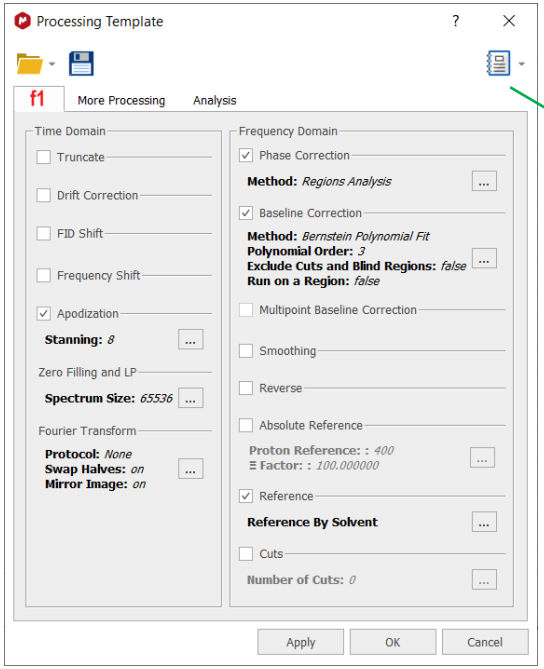
	Parameter	Value
1	Data File Name	C:/Program Files (x8...
2	Title	Quinine1H
3	Comment	1H Quinine
4	Origin	Varian
5	Owner	
6	Site	
7	Instrument	inova
8	Author	
9	Solvent	CDCl3
10	Temperature	25.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Probe	HF.CP

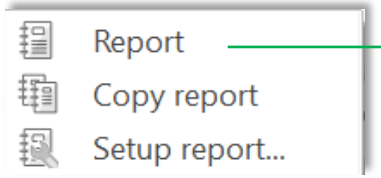
Use the green handles to move, rotate, and resize the text box. Every object in Mnova can be relocated and resized

Report a Processing Template

Use Processing Template to visualize the applied processing parameters, and report the processing parameters as needed. The parameters can also be saved as a template for processing similar spectra.



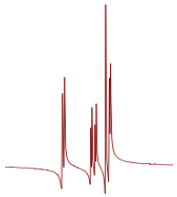
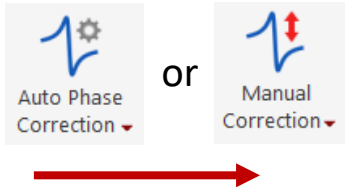


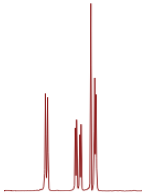


Apodization
Stanning: *8*
Zero Filling and LP
Spectrum Size: *65536*
Fourier Transform
Protocol: *None*
Swap Halves: *true*
Mirror Image: *true*
Real FT: *false*
Phase Correction
Method: *Regions Analysis*
PH0: *-141.50 °*
PH1: *-4.39 °*
Baseline Correction
Method: *Bernstein Polynomial Fit*
Polynomial Order: *3*
Exclude Cuts and Blind Regions: *false*
Run on a Region: *false*
Reference
Old shift: *7.236 ppm*
New shift: *7.260 ppm*
Autotune: *false*

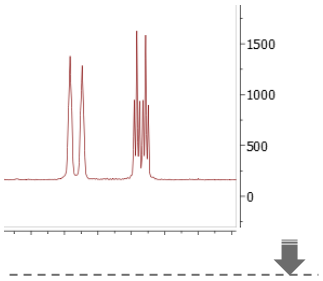
Phase, baseline correction, and reference

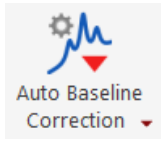
- Press  for **phase correction** if peaks are not symmetric*

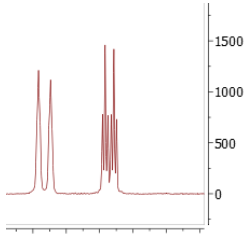





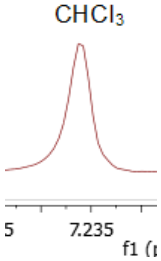
- Press  for **baseline correction** if baseline is not zero*

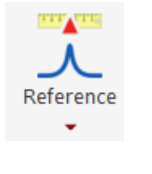


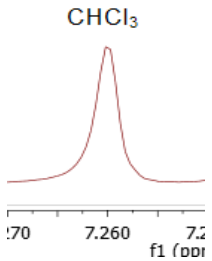




- Press  to calibrate the **chemical shift reference** if the solvent or TMS peak is not at the correct ppm

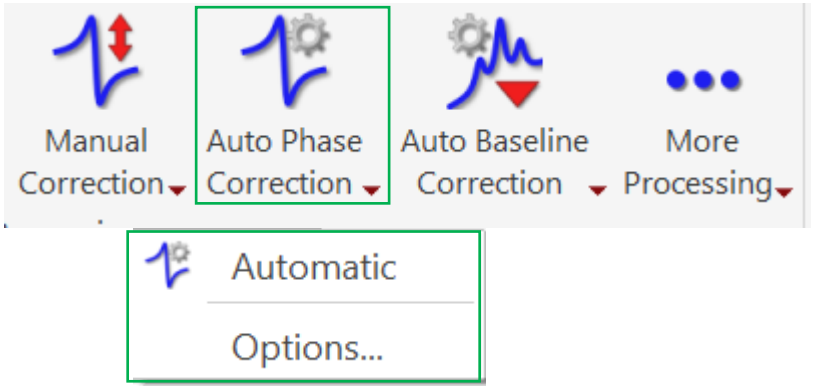


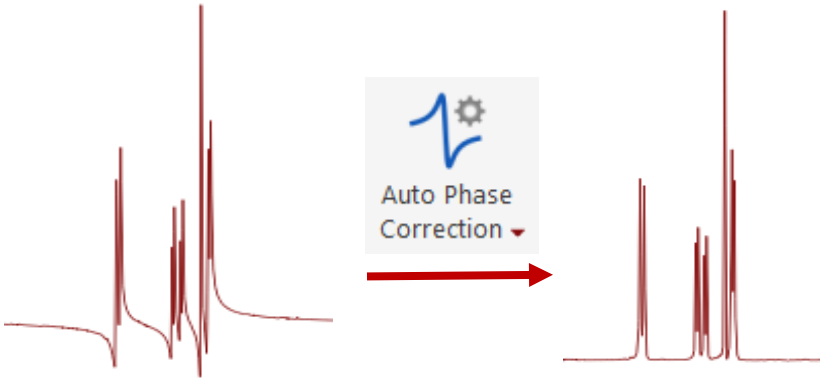


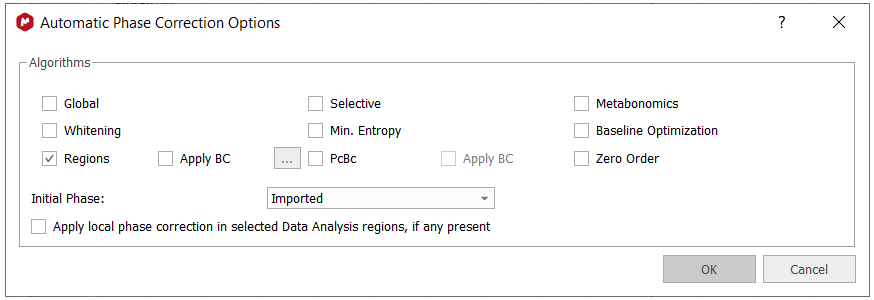


Note: Using these tools is equivalent to applying the corresponding options in the Processing Template Panel.

Automatic phase correction

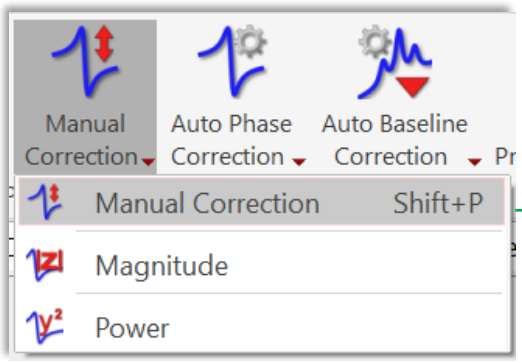


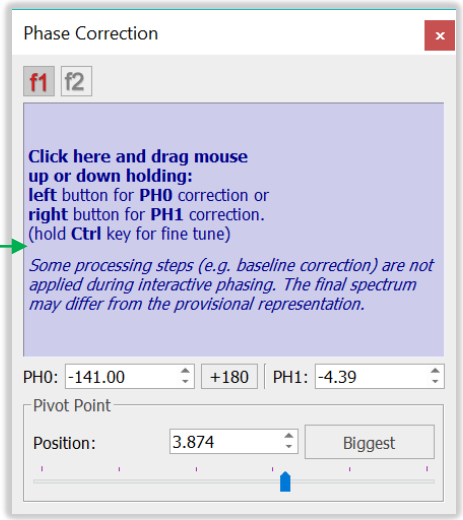


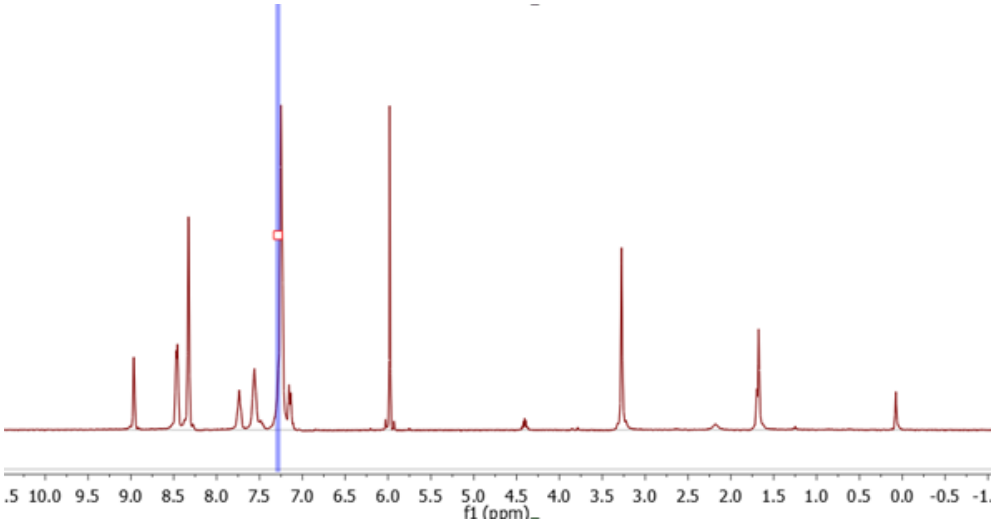


- **Regions Analysis:** good for most cases
- **Global:** good for spectra without negative and/or big solvent peaks
- **Selective:** DEPT type of spectra with negative peaks
- **Metabonomics:** spectra with big solvent peaks
- **Whitening:** usually for 2D

Manual phase correction

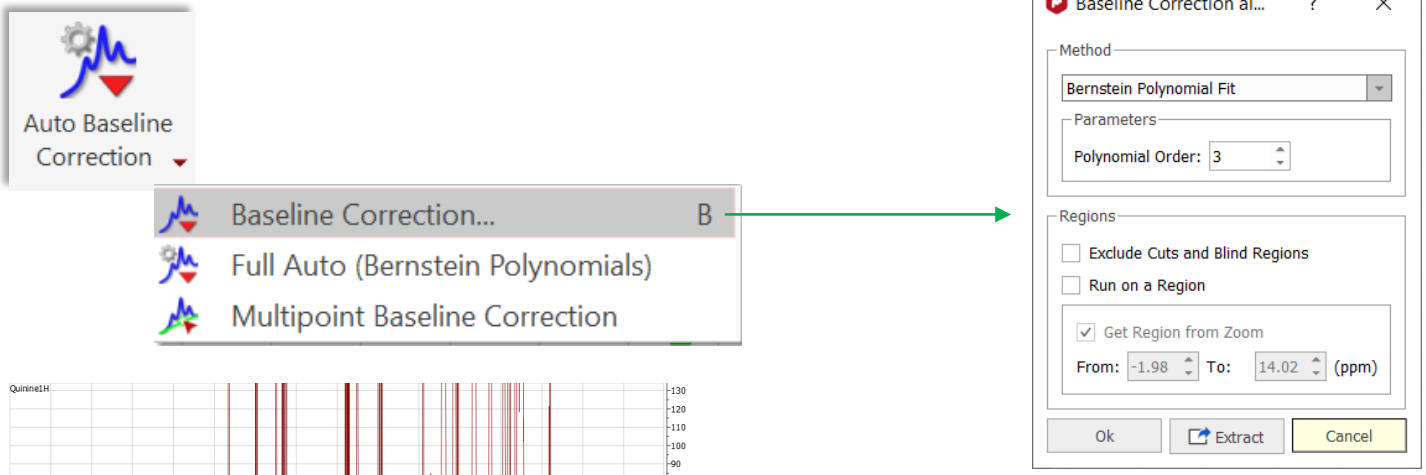


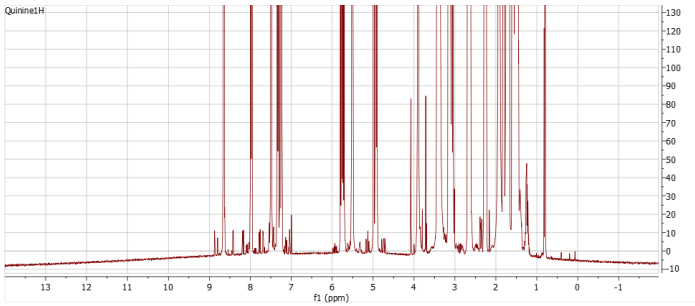


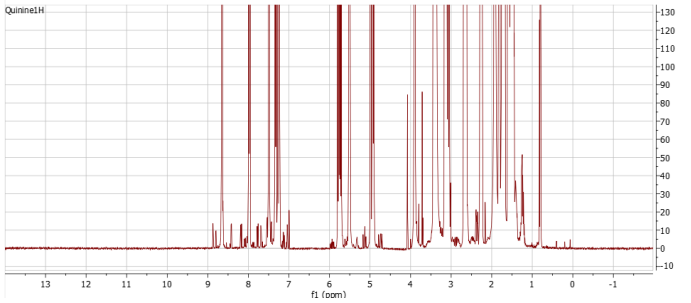


- Set the **Pivot Point**
- **PH0**: zero-order correction (left mouse button + scroll up/down)
- **PH1**: first-order correction (right mouse button + scroll up/down)
- **Ctrl + scrolling**: fine tuning

Baseline correction

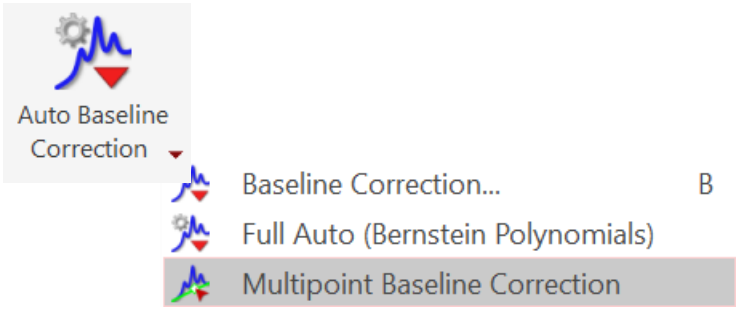


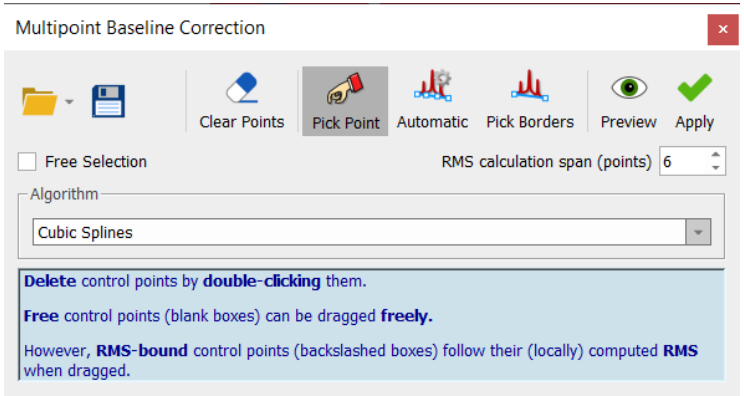




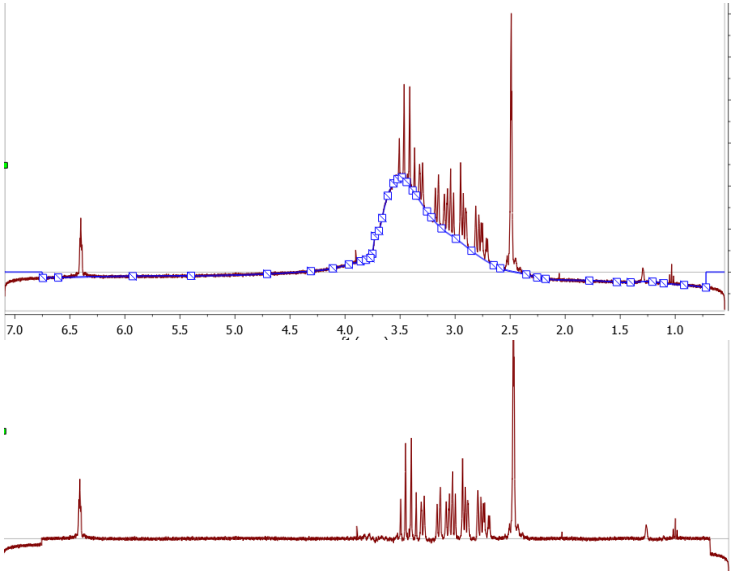
- Choose a function to model the baseline:
- **(Bernstein) Polynomial Fit**: small base errors
- **Splines or Ablative**: for medium base errors
- **Whittaker**: For more serious base errors. Use with caution and make sure the bases of peaks are not compromised. Use appropriate parameter values to tune the fit
- **Multipoint B.C.:** Manually define base points and choose a fitting algorithm

Multipoint baseline correction

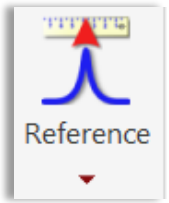


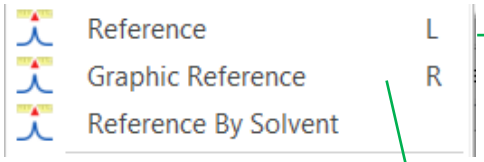


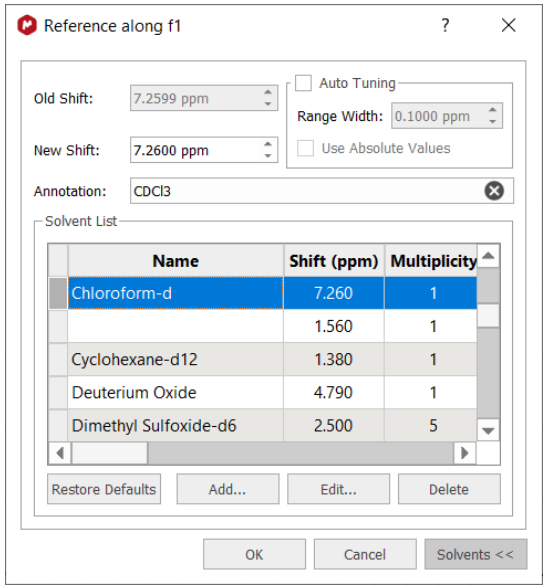
- Manually or automatically pick base points
- Double click on a basepoint again to remove it
- Choose the best algorithm to fit the basepoint to a baseline
- Click Apply to deduct the baseline from the spectrum



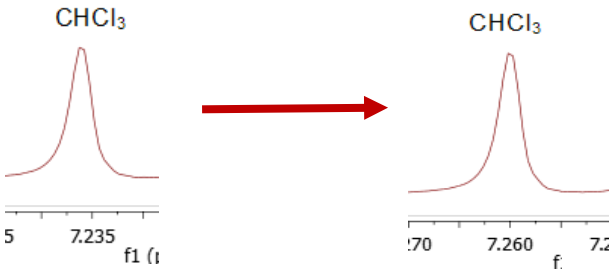
Referencing chemical shifts



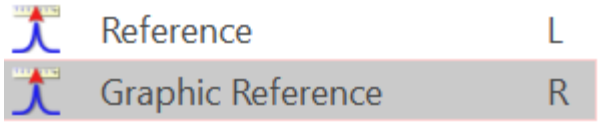


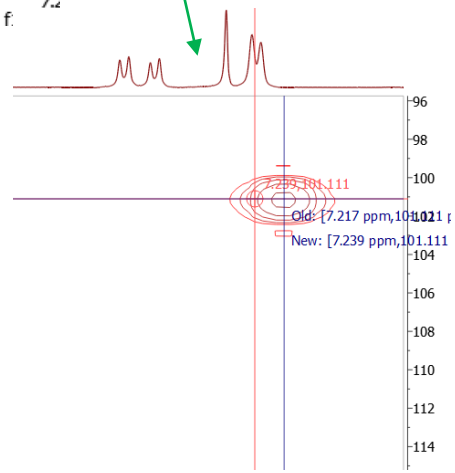


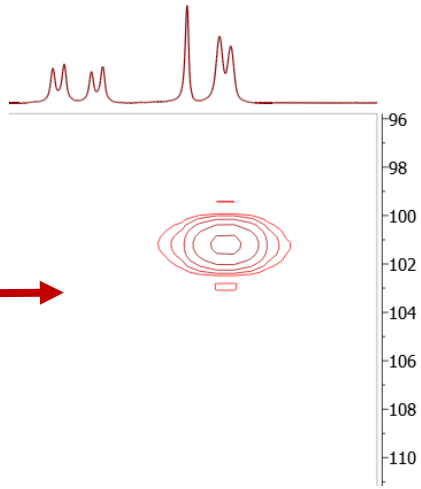
- **Reference** by entering the value



- **Graphic Reference** for 2D NMR (two clicks)



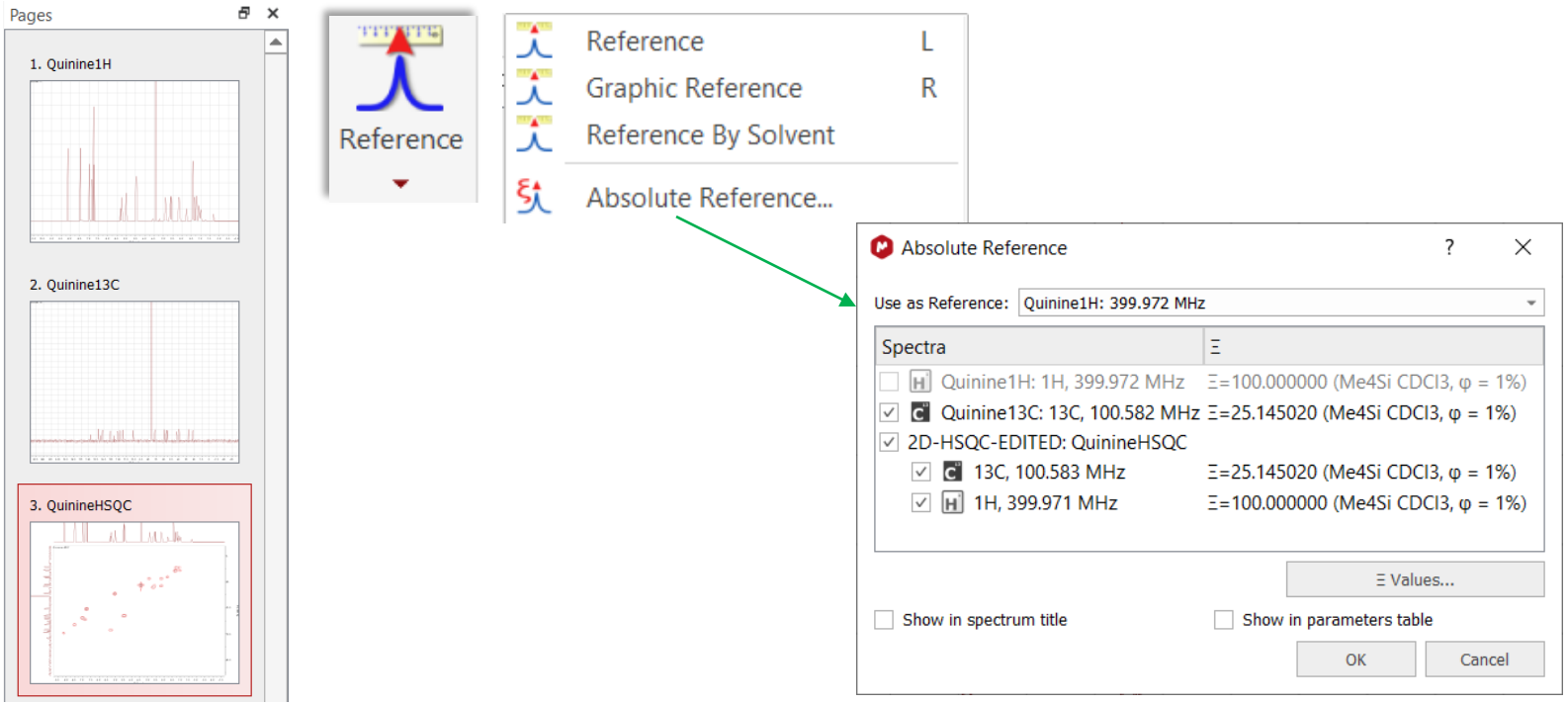




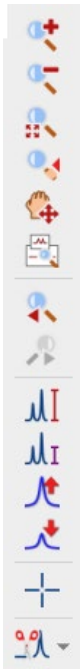
ANALYSIS

Absolute Reference

- Applies to multiple spectra of the same sample acquired under the same conditions
- Open all spectra in the same document
- Reference the H-1 spectrum manually first
- Next choose **Reference/Absolute Reference** to reference all other spectra (1D or 2D)



Visualize your spectrum



Zoom in/Zoom out (or press Z) *

Zoom out

Full spectrum (or press F)

Manual Zoom in to defined ppm range

Pan spectrum (or press P)**

Expansion – click and drag to draw an inset (or press E)

Previous Zoom

Next Zoom

Fit to Highest Intensity (or press H)

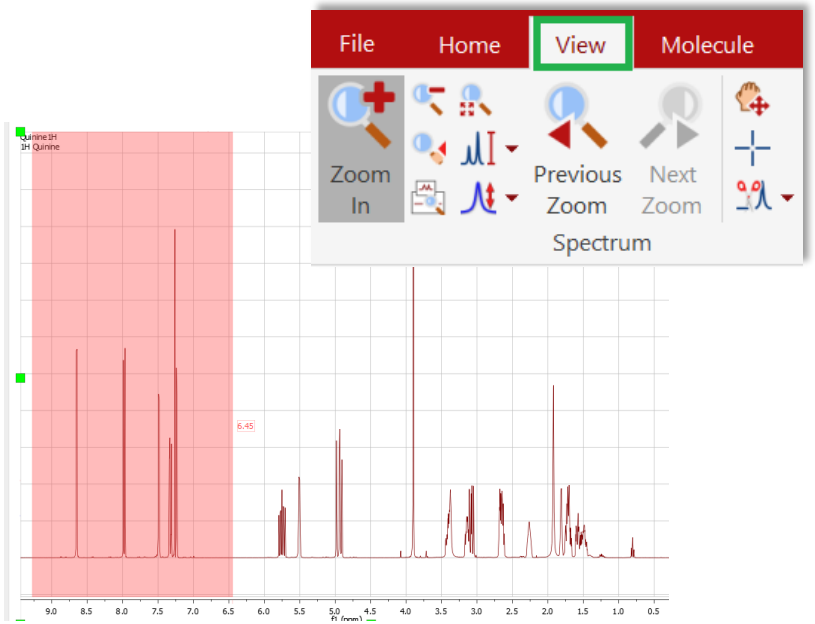
Fit to highest compound peak

Increase Intensity (or rotate mouse wheel)

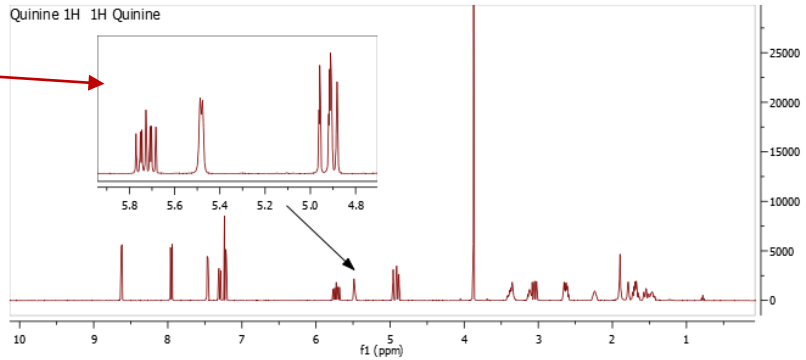
Decrease Intensity (or rotate mouse wheel)

Crosshair Cursor (or press C) for measuring *J*-couplings

Cut (or press X) to hide parts of the spectrum



Press **E**, then click and drag to define the range for the inset



Press **Z multiple times to toggle between horizontal/ vertical/ box zoom*

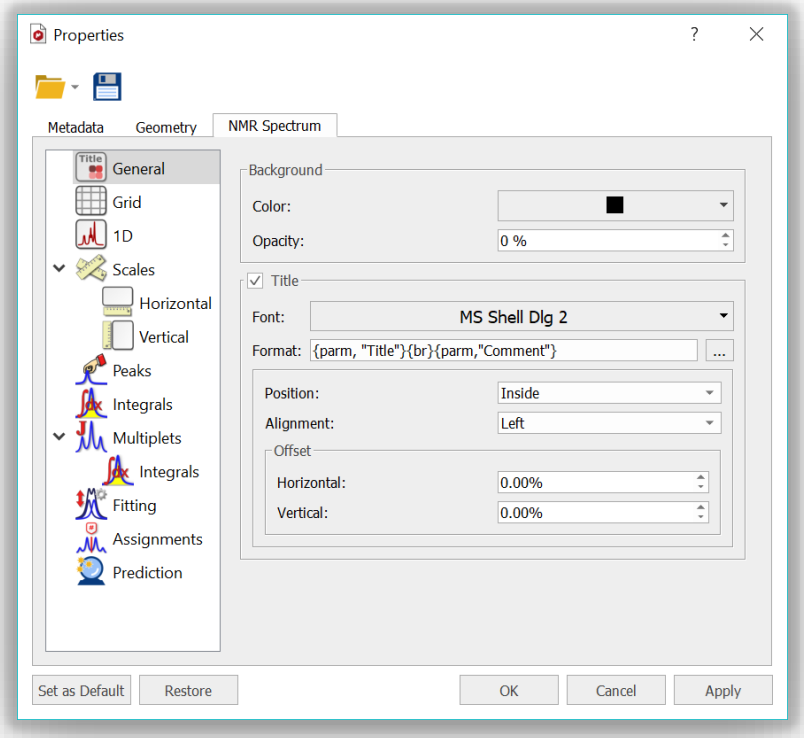
*** Press **P** multiple times to toggle between free/ horizontal/ vertical panning*

DISPLAY OPTIONS

Change spectrum display properties

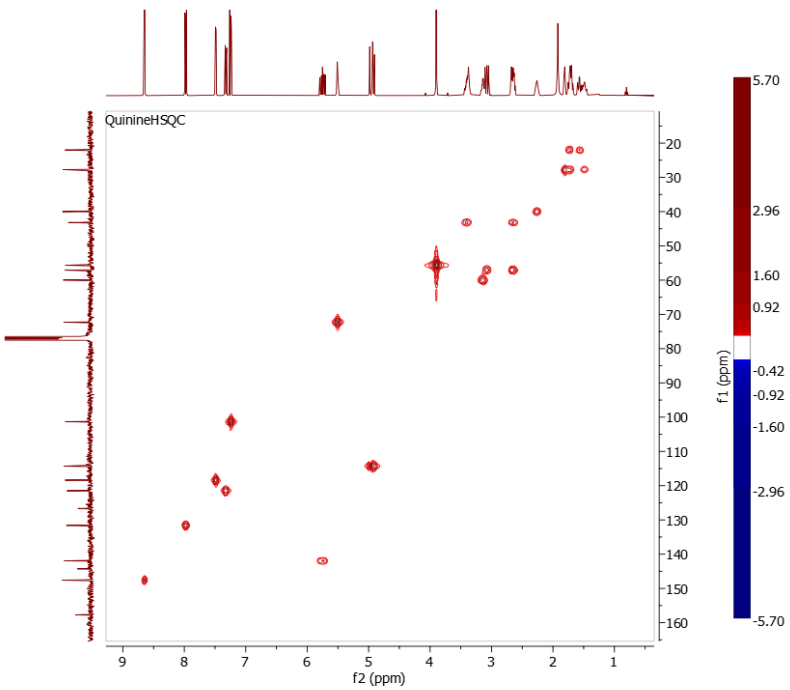
- Right click on the spectrum and choose Properties to open the **Properties** dialog
- Change the options as needed and click **Apply** to verify the effects.
- Click on **Set as Default** to save settings for spectra opened in the future

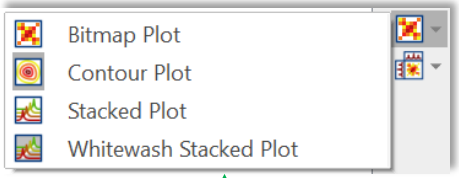
*Tip: Use the **Save** tool to save the properties to a file, and distribute it to other users for consistent display and reporting.*

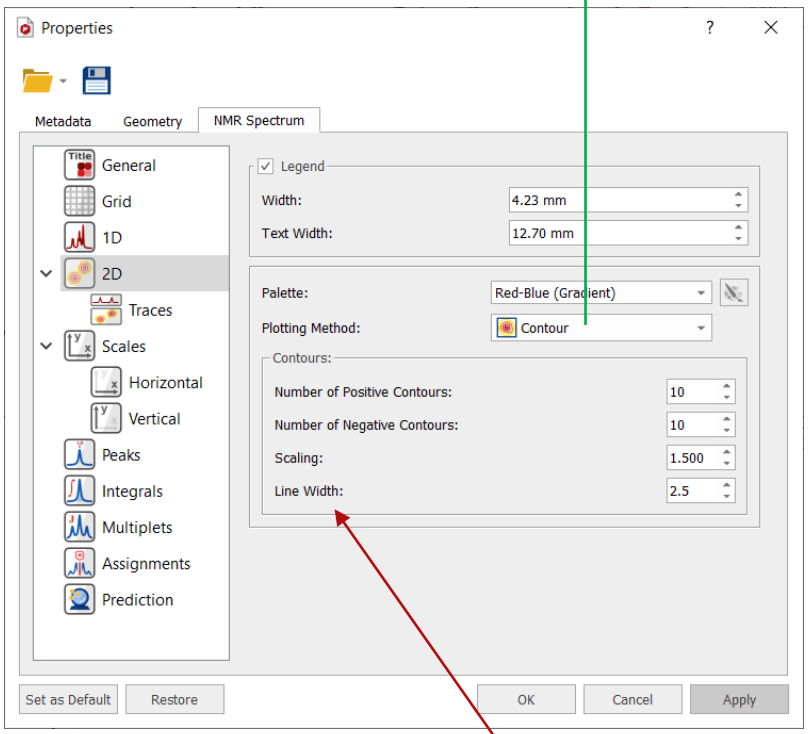


Display of 2D spectra

- For 2D NMR, there are extra properties to choose, such as:
  - Legend
  - Color Palette
  - Plotting Method
  - Contours



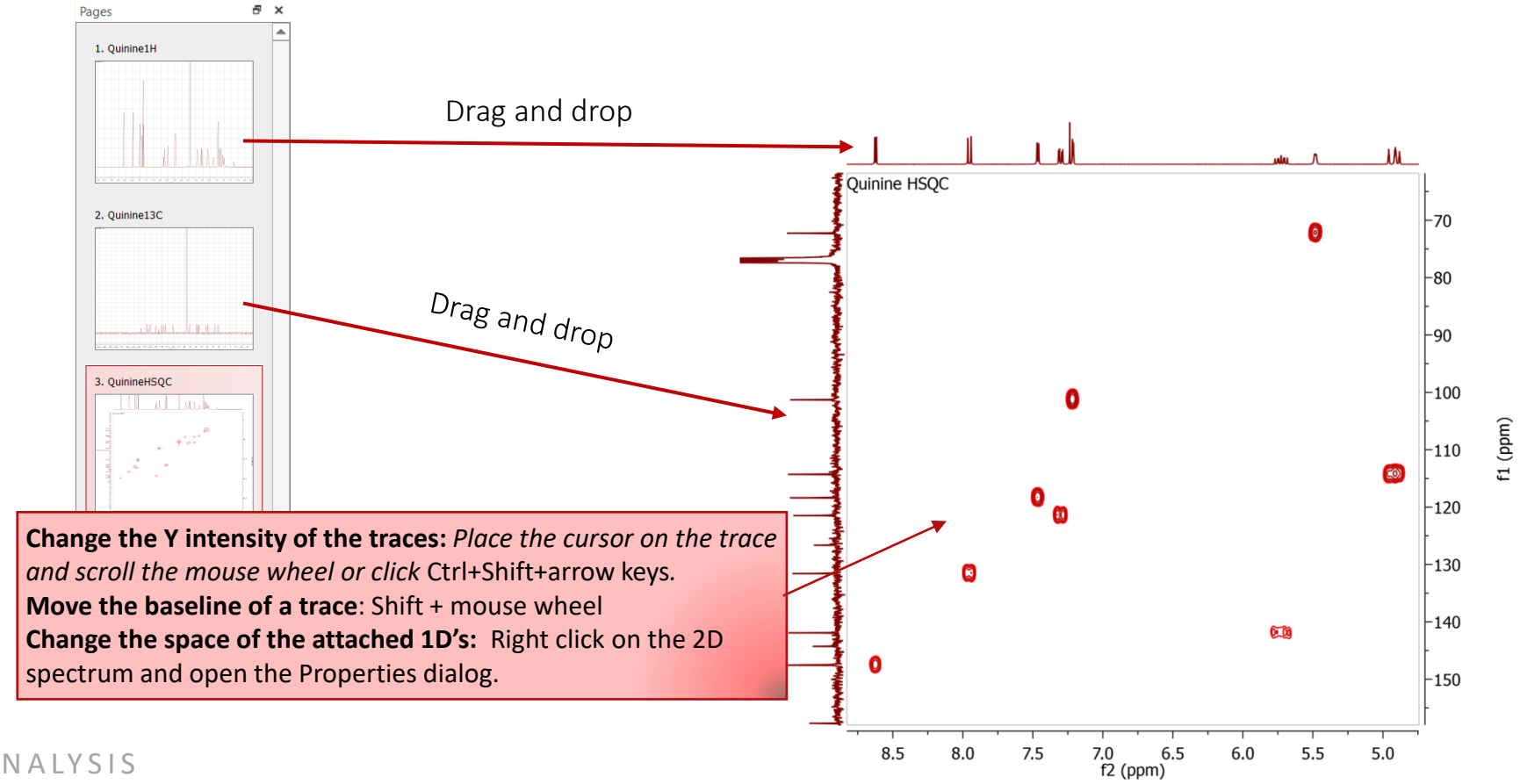




Tip: You can set a line width for 2D contours independent of that for 1D curves.

Attach 1D to 2D spectra

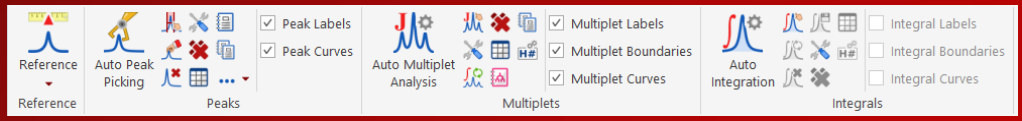
- If available, 1D spectra in the same document are automatically attached to 2D NMR when opened
- To achieve this manually, highlight a 2D spectrum, then drag a 1D from the Pages panel to attach it to the 2D as an external trace



Change the Y intensity of the traces: *Place the cursor on the trace and scroll the mouse wheel or click* Ctrl+Shift+arrow keys.
Move the baseline of a trace: Shift + mouse wheel
Change the space of the attached 1D's: Right click on the 2D spectrum and open the Properties dialog.

Basic Analysis of ^{1}H NMR

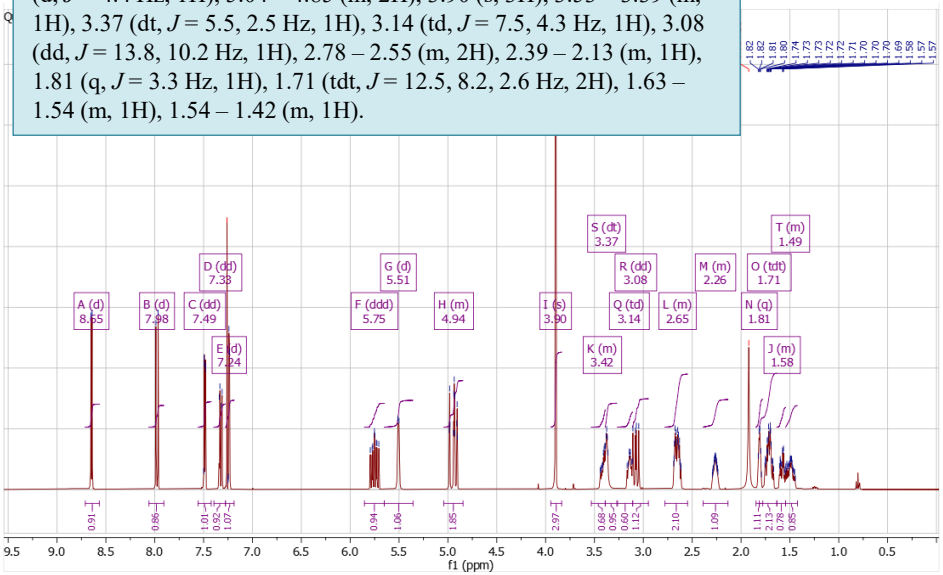
You will need to have an Mnova NMR license for this section

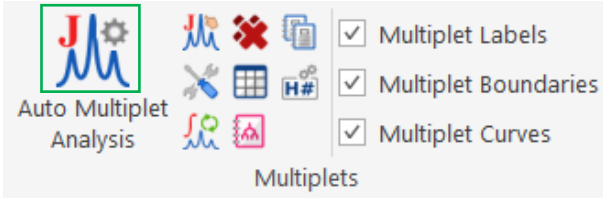


Analyze and report multiplets of 1H NMR

- Mnova provides two approaches to **multiplet analysis:**
  - **Fully automatic:** peak picking, integration, and multiplet analysis *all achieved in a single click*, with peaks deconvolved using GSD*
  - **Manual**: click and drag to pick each multiplet interactively
- In either case, you can refine the results interactively, and report them in the selected journal or patent formats

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, J = 4.5 Hz, 1H), 7.98 (d, J = 9.2 Hz, 1H), 7.49 (dd, J = 4.5, 0.7 Hz, 1H), 7.33 (dd, J = 9.2, 2.7 Hz, 1H), 7.24 (d, J = 2.7 Hz, 1H), 5.75 (ddd, J = 17.1, 10.3, 7.6 Hz, 1H), 5.51 (d, J = 4.4 Hz, 1H), 5.04 – 4.85 (m, 2H), 3.90 (s, 3H), 3.53 – 3.39 (m, 1H), 3.37 (dt, J = 5.5, 2.5 Hz, 1H), 3.14 (td, J = 7.5, 4.3 Hz, 1H), 3.08 (dd, J = 13.8, 10.2 Hz, 1H), 2.78 – 2.55 (m, 2H), 2.39 – 2.13 (m, 1H), 1.81 (q, J = 3.3 Hz, 1H), 1.71 (tdt, J = 12.5, 8.2, 2.6 Hz, 2H), 1.63 – 1.54 (m, 1H), 1.54 – 1.42 (m, 1H).

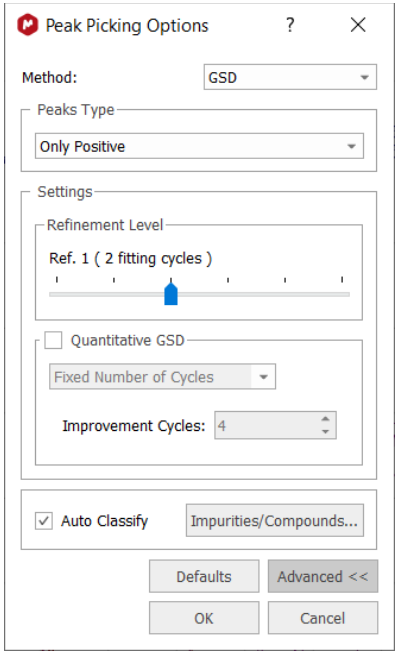


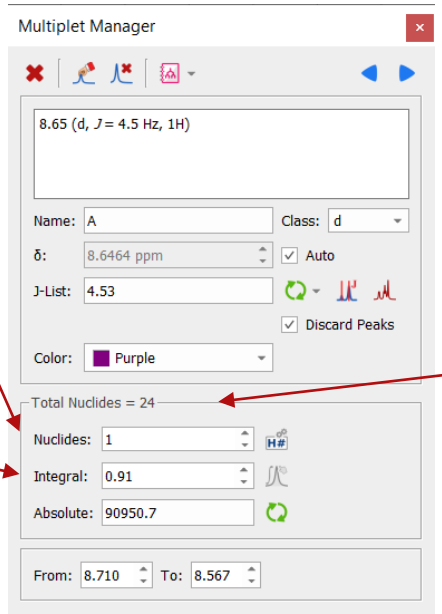


*See more details about GSD

Fully automatic multiplets analysis

- Click  to perform automatic multiplet analysis
- By default, Mnova does the following automatically:
  - Picks peaks using GSD* (if no peaks were picked) and classifies their types (compound, solvent, impurity peaks, etc.), all of which is controlled via **Peak Picking Options**
  - Groups picked peaks into multiplets and fits them to J-coupling patterns, then calculates their integrals, all controlled by **Multiplet Analysis Options**
  - Estimates the total number of nuclides (NN), and normalizes the integrals for each multiplet*

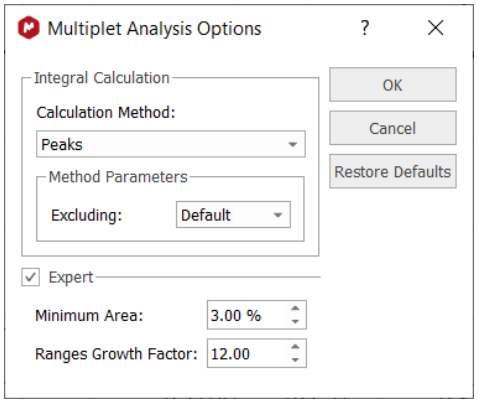




The number of nuclides (NN) of the multiplet

Normalized integral of the multiplet

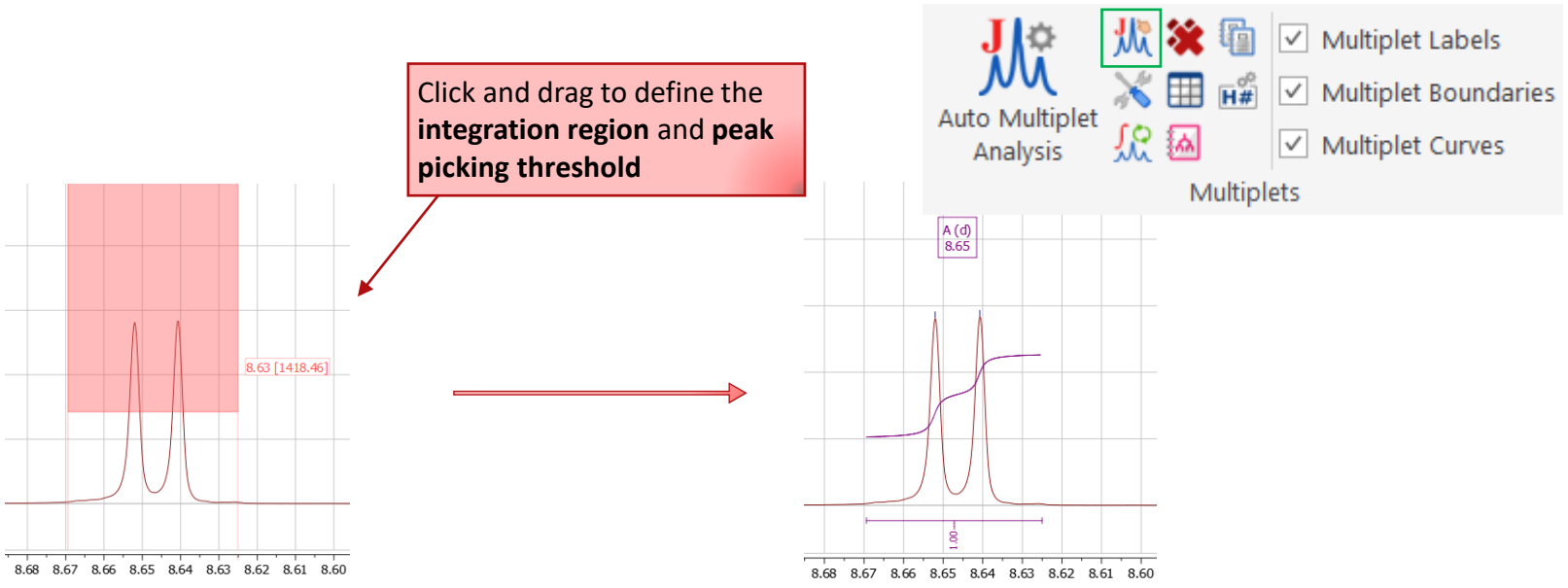
Total number of nuclides from all the multiplets and the number of protons in the molecule (if present)



Depending on the Calculation Method in the Multiplet Analysis Options, the integral of a multiple is calculated as the sum of the deconvoluted peaks or the sum of the data points within the range.

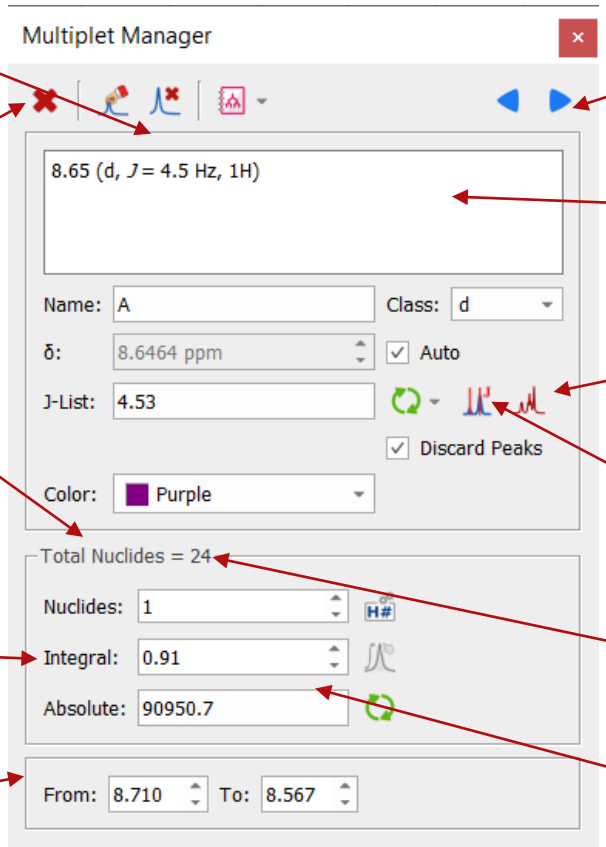
Pick multiplets manually

- Manual Multiplet Analysis offers more control to the user (**J** is the shortcut key)
- Zoom into each multiplet, click and drag to define the following:
  - Peak picking threshold
  - Integration region
- Mnova picks the peaks in the region, fits them to a *J*-coupling pattern, and defines the multiplet in the same way as in automatic multiplet analysis



Multiplet Manager

- Double click on a multiplet label to open the Multiplet Manager
- Use it to inspect and change the properties of the multiplets, including the normalization of the integrals, *J*-coupling patterns and constants, etc.



Add/Delete multiplet peaks

Delete the current multiplet

The number of protons this multiplet corresponds to. Changing this number affects only the current multiplet

Normalized integral of the multiplet. Changing it affects all multiplets

Integration region of the multiplet

Navigate to the Previous/Next multiplet

Properties of the current multiplet

Use this tool to simulate the multiplet

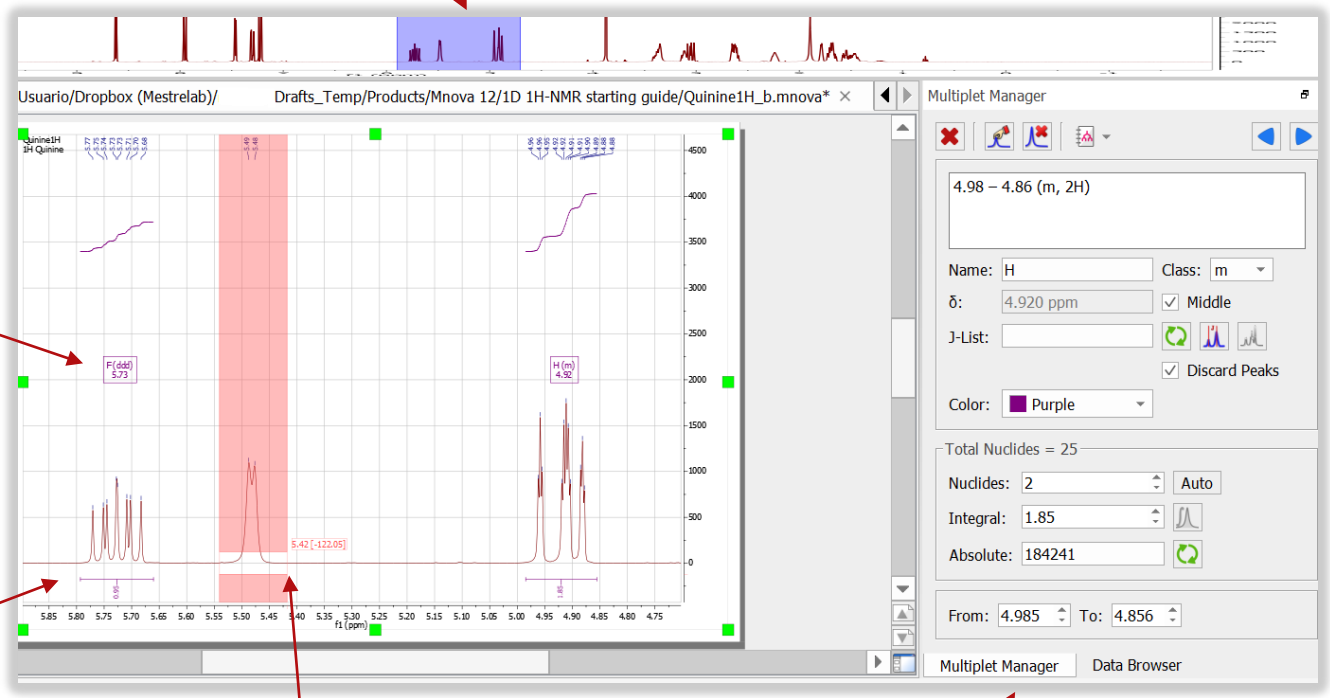
Use this tool to measure J constant manually

Number of protons in the molecule (if present)

Absolute integral of the multiplet

Handy tools for multiplet analysis

Full View: Display the whole spectrum and zoom-in area. Drag the purple box to move to other multiplets. Choose View/Full View to open the Full View panel, which can be docked as shown



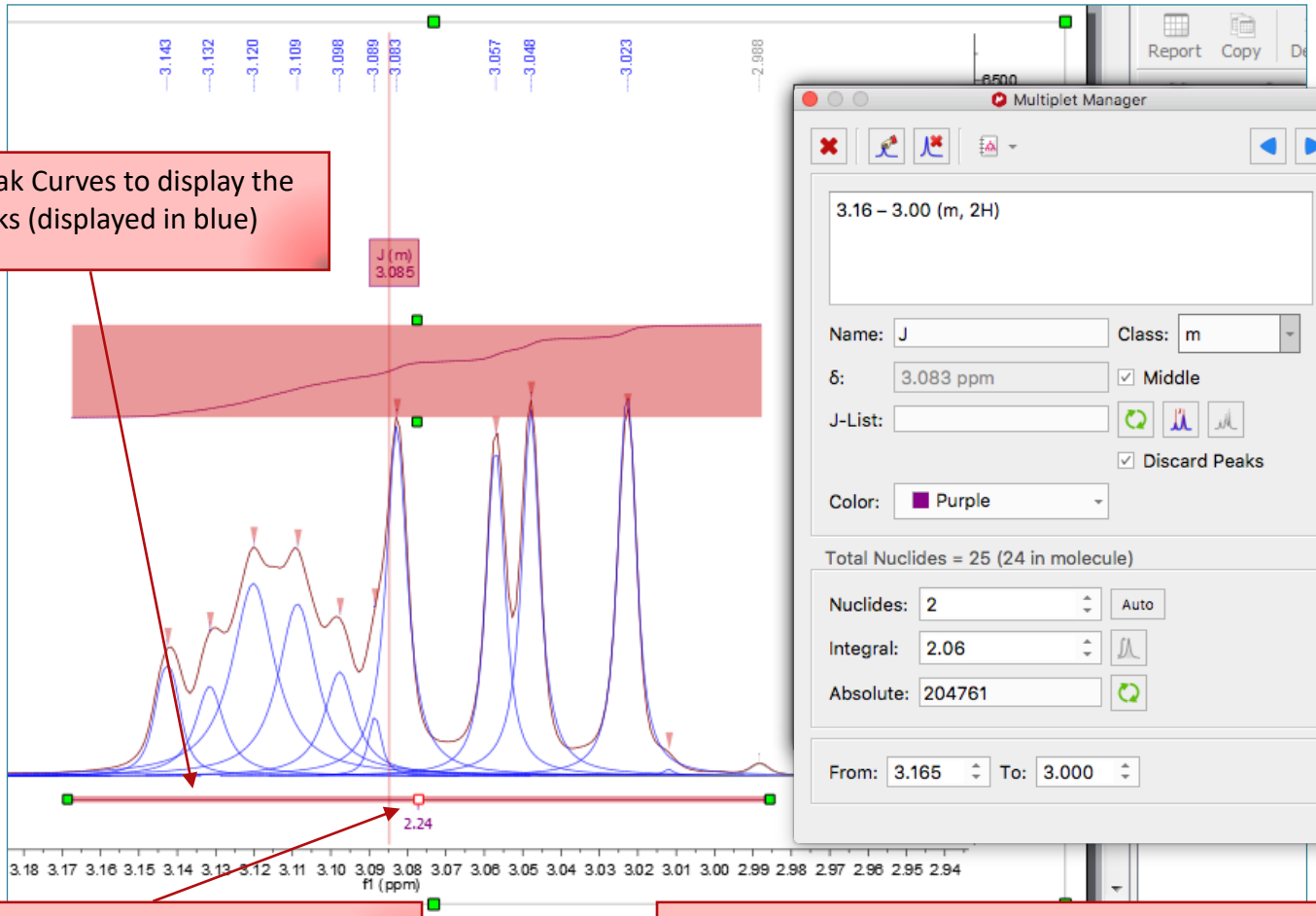
Multiplet label: Click on it to set it as the current active one

Multiplet bar: Use it to split a multiplet into two, or to change its range

Manual multiplet analysis: Press J, then click and drag across peaks to define the range and peak picking threshold

Multiplet Manager shows the properties of the currently picked multiplet. (Double click on a multiplet label to open it)

Split partially overlapping multiplets (1)

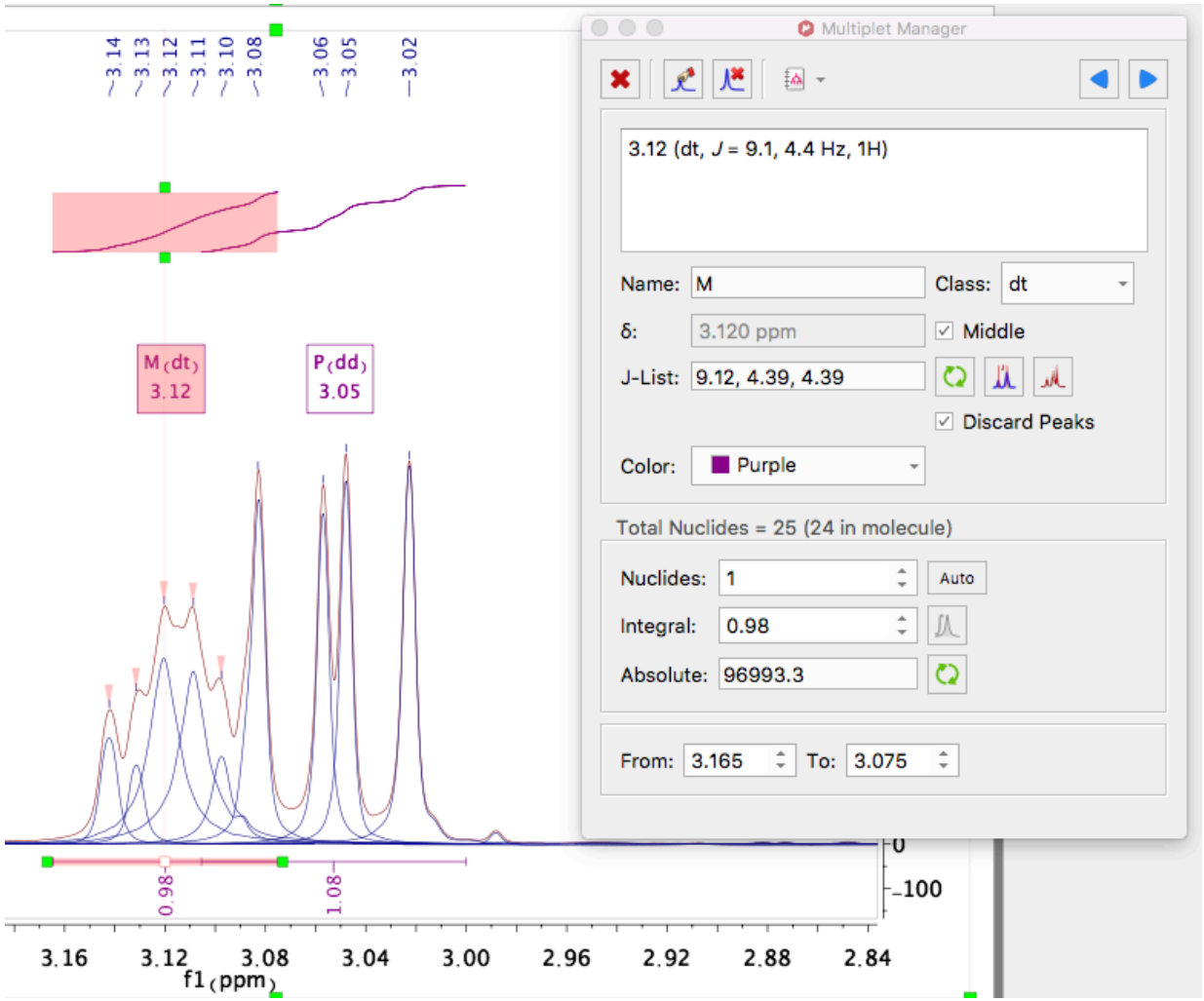


Show Peak Curves to display the GSD peaks (displayed in blue)

Drag this white box to split the multiplet into two distinct multiplets

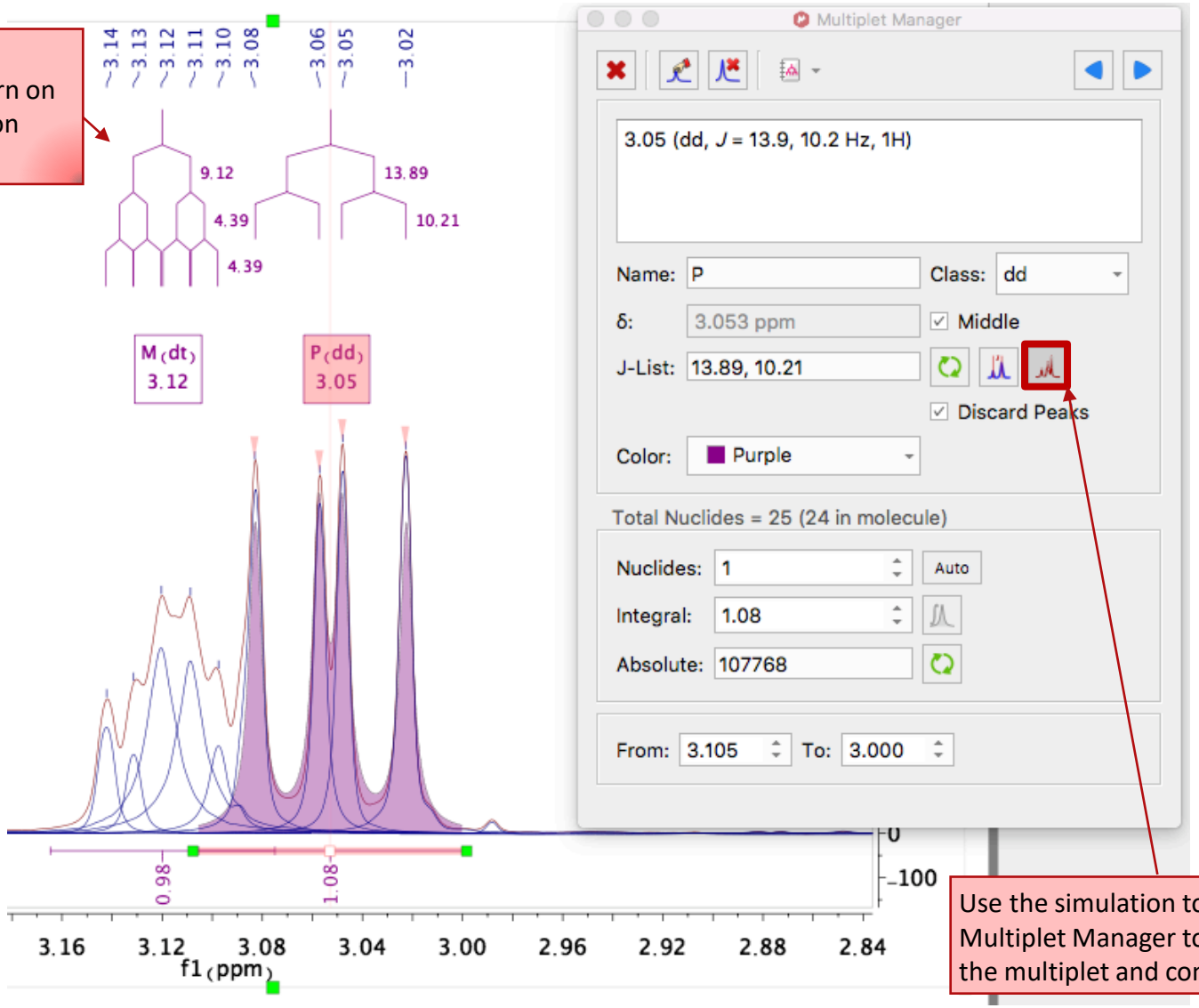
Tip: You can also change the display of the deconvolution peak curves in Properties Dialog > Peaks > Curve tab

Split partially overlapping multiplets (2)



Tools to verify multiplet analysis results

Go to Properties/ Multiplets and turn on the 'J's Tree' option

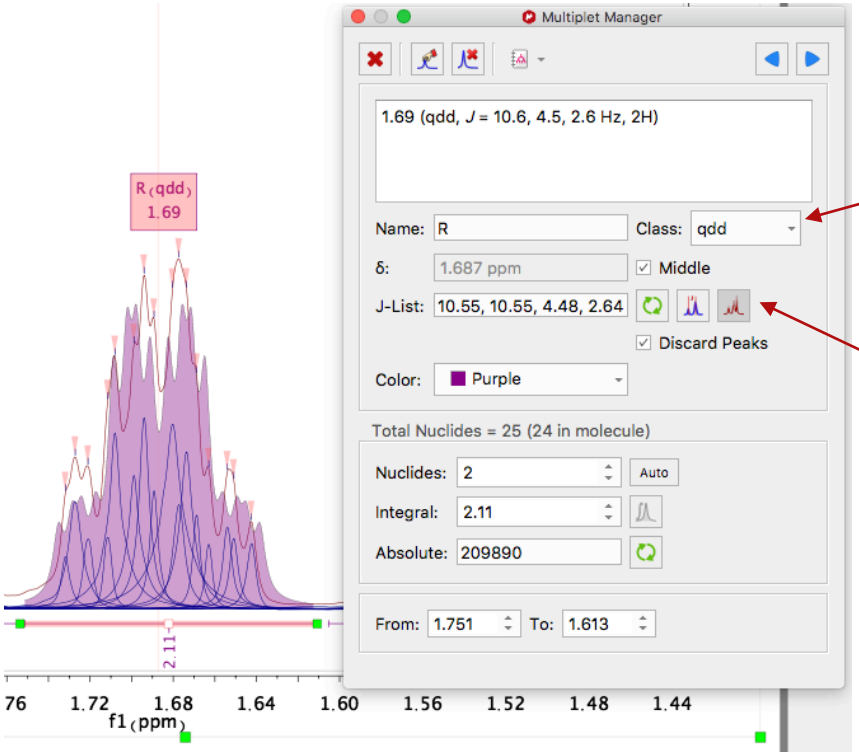


Use the simulation tool in the Multiplet Manager to simulate the multiplet and compare

ANALYSIS

Override the multiplet results with the Multiplet Manager

- Override the analysis results for a multiplet in **Multiplet Manager**
- In this example, the multiplet was estimated to be a “qdd”. The simulated multiplet does not agree with the observed spectrum, and hence it is wrong
- Select “m” from the ‘Class’ pull-down menu to override it

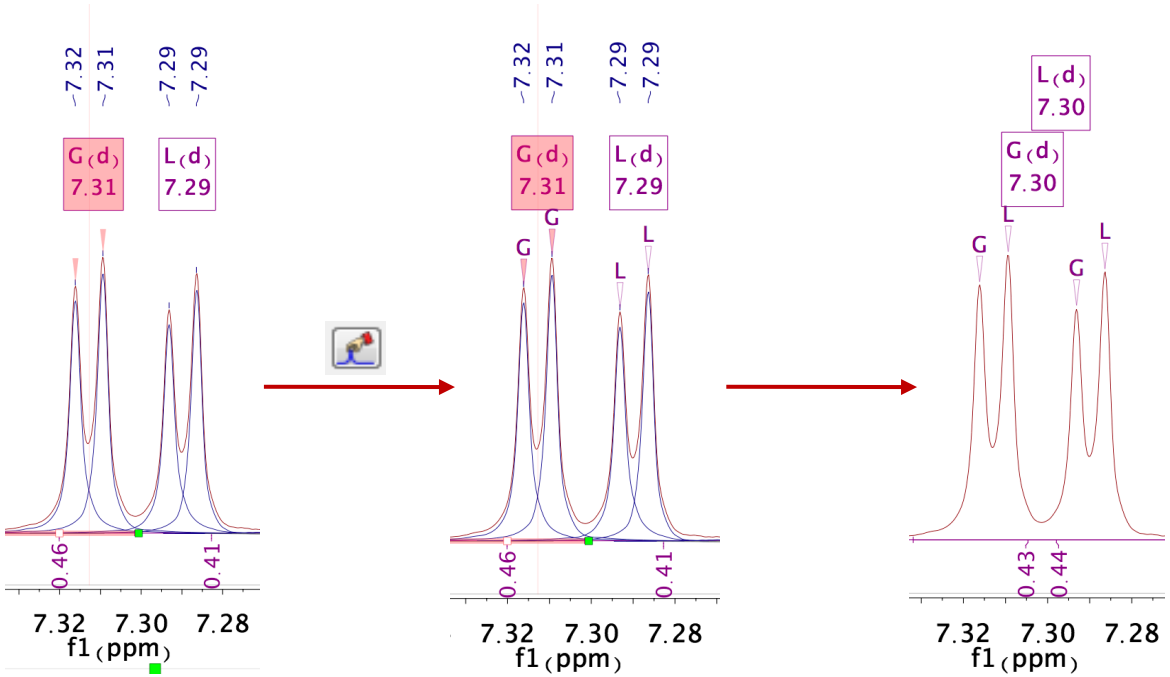


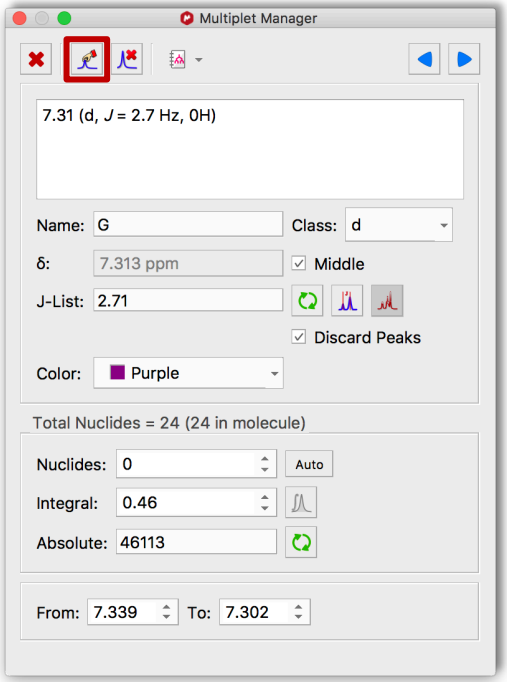
Choose “m” from the drop-down menu to override the results

Use the simulation tool to simulate the multiplet and compare

Reassign peaks to multiplets

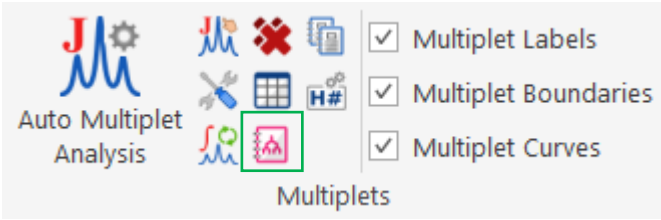
- If a peak is assigned incorrectly to a group, use the **Add Multiplet Peak** tool in the **Multiplet Manager** to reassign it to a different group
- Click on the pink wedge on a peak and drag it to the multiplet label
- In the following example, two peaks were reassigned, forming a different pair of doublets:

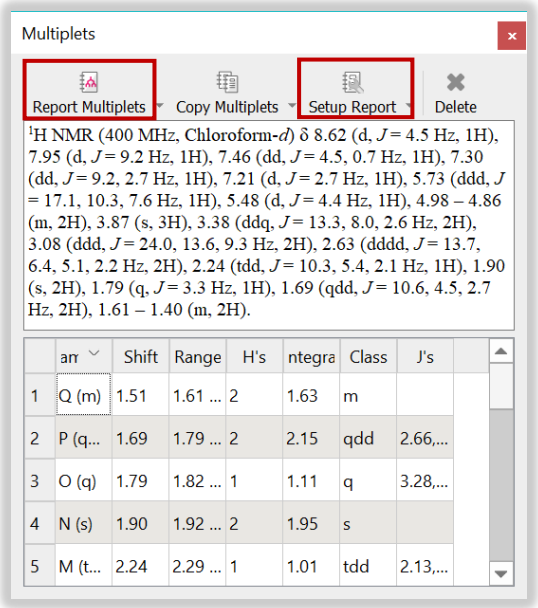


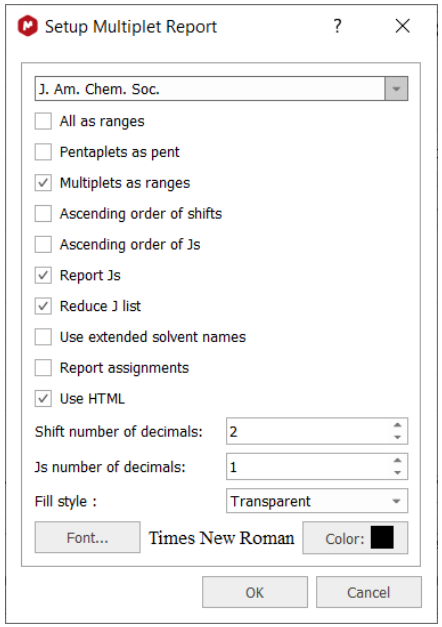


Report multiplets

- Click on **Report Multiplets** to report the results in a particular journal format
- To change the journal format: Go to **View/ Tables/ Multiplets** to display the Multiplets Table
- Then click on **Setup Report**



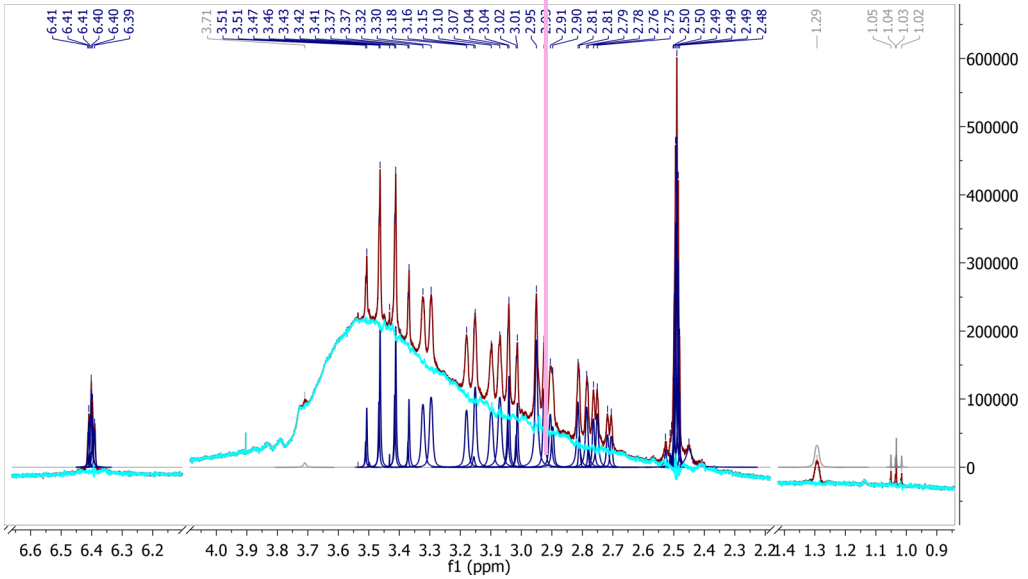


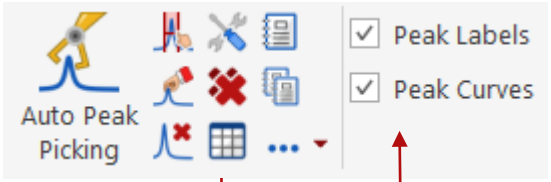


Tip: From the Multiplet Table, click Copy Multiplets and then paste the text to your document. Click on Copy Table and then paste the spreadsheet to your document. The table can be customized using Setup Table.

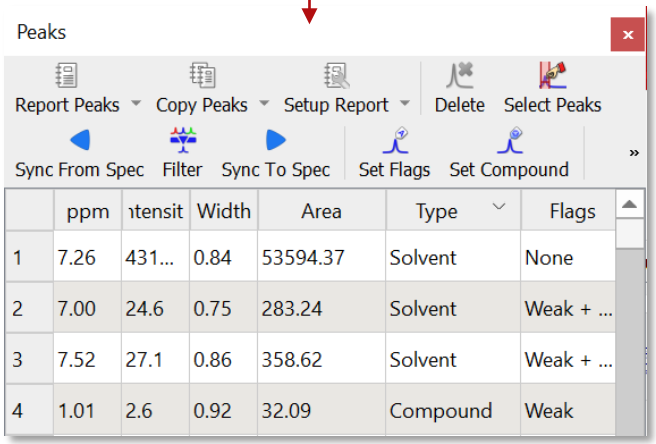
GSD peak picking

- When peak-picking or multiplet analysis is performed, Mnova does a global spectral deconvolution (GSD) by default, then uses the deconvolved peaks in the peak-picking results*
- Choose to display the deconvoluted peaks (blue) and the residuals (cyan), Peak Table using the relevant tools





To turn on/off the GSD peaks

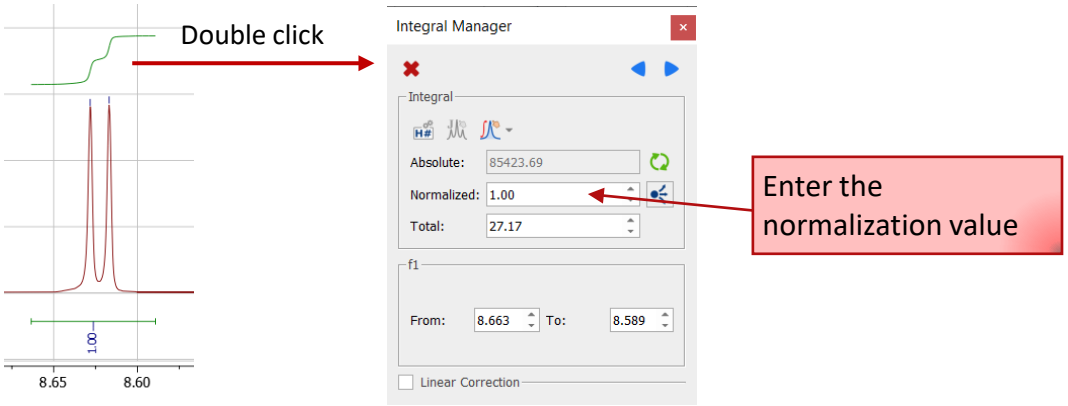


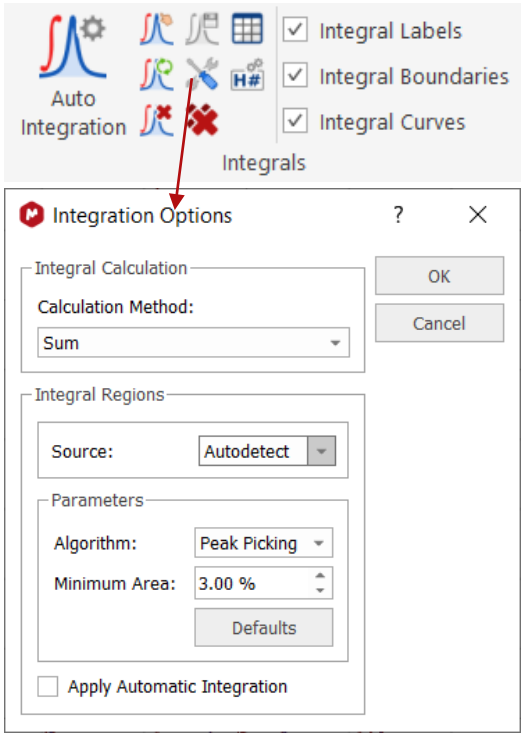
	ppm	ıtensit	Width	Area	Type	Flags
1	7.26	431...	0.84	53594.37	Solvent	None
2	7.00	24.6	0.75	283.24	Solvent	Weak + ...
3	7.52	27.1	0.86	358.62	Solvent	Weak + ...
4	1.01	2.6	0.92	32.09	Compound	Weak

*See more details about GSD

Integrate peaks independently of multiplet analysis

- The peak picking and multiplet analysis described previously both give integrals of deconvolved peaks or multiplets. However, in some situations, e.g., polymer or complex mixtures, peak- or multiplet-based integration is impossible, and we will use the direct integration tools
- By default, the integrals from these tools are “Sum-based”, i.e., by summing up each data point within a range. The results have nothing to do with the peak-picking results*
- Both automatic and manual integration tool are provided
- To normalize an integral, double click on the integral curve to open the Integral Manager, and set its Normalized value to 1

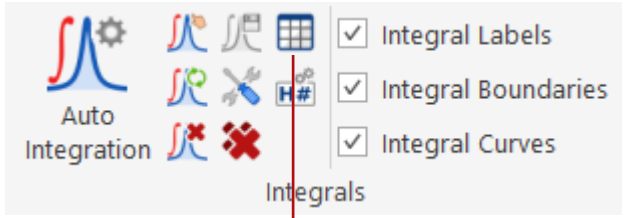


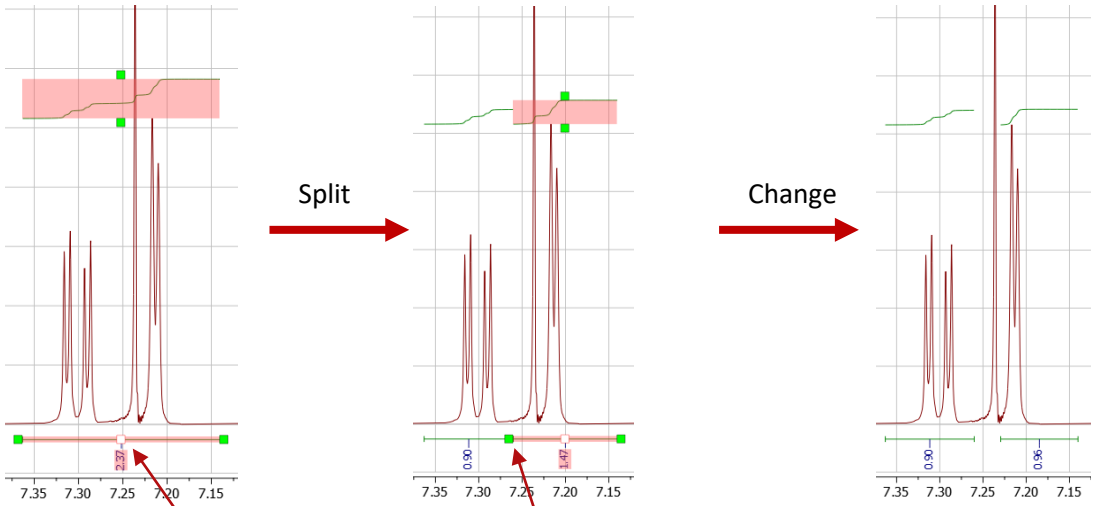


*Depending on the nature of the spectrum, different integration options can lead to significantly different results. Be aware of the options you are using.

Integrate peaks independently of multiplet analysis

- The integration results can be displayed in an Integrals Table, and the results can be reported from there
- Browse, delete, change, split integrals interactively if needed
- To interactively change or split an integral:





Hover the cursor on the integral bar, drag the central white box to a basepoint and release to split the integral

Drag the left green box to the right to skip the $CHCl_3$ peak

Integrals

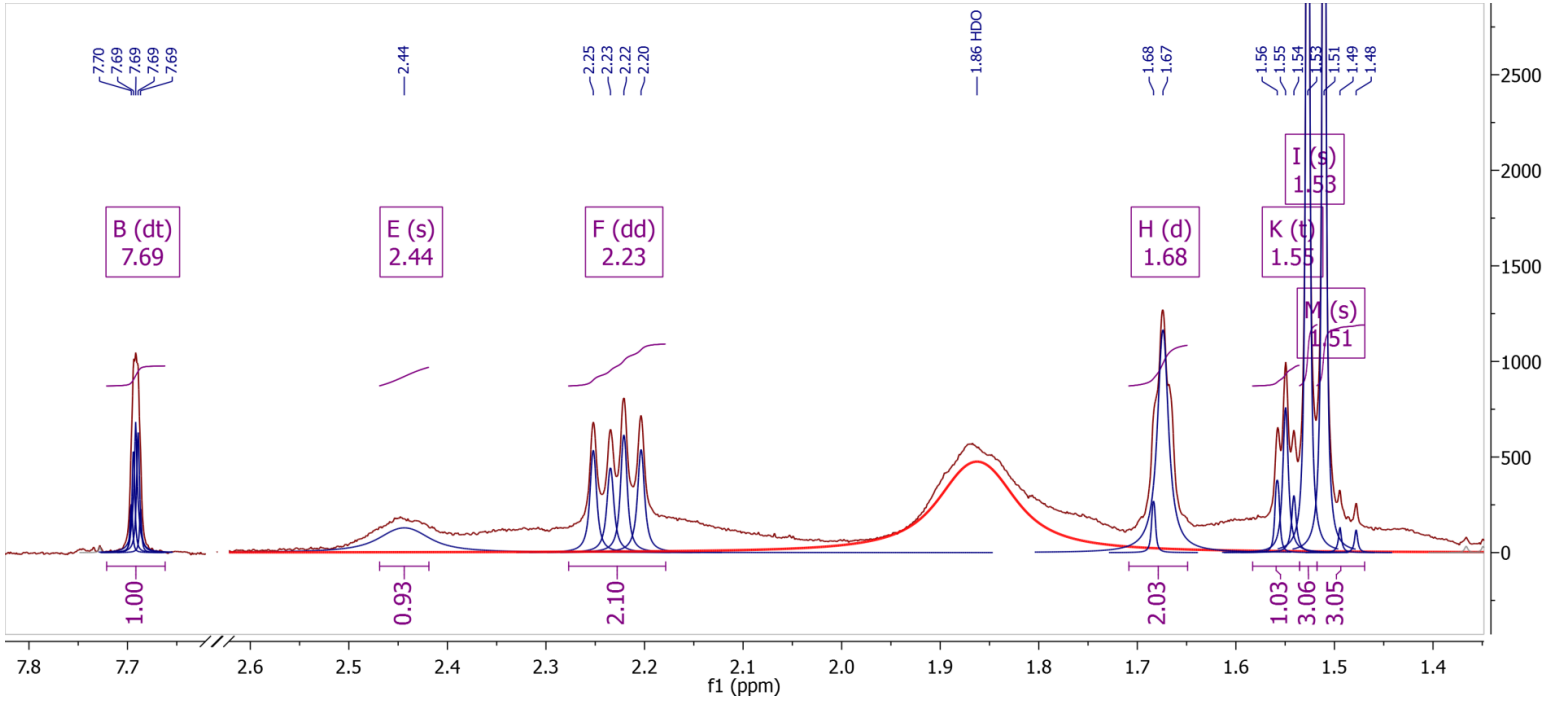
Report Copy Delete Setup

	Range	Normalized	Absolute
1	8.66 .. 8.59	1.00	85423.69
2	8.00 .. 7.92	1.00	85044.42
3	7.50 .. 7.43	1.12	94849.97
4	7.34 .. 7.26	1.02	86590.29
5	7.25 .. 7.19	1.66	141361.59
6	5.79 .. 5.66	1.02	86785.24
7	5.51 .. 5.45	1.14	97030.63
8	4.98 .. 4.86	2.09	177700.58

Why are integrals from multiplet analysis different from direct integration? (1)

(GSD) Peaks-based integration when running multiplet analysis

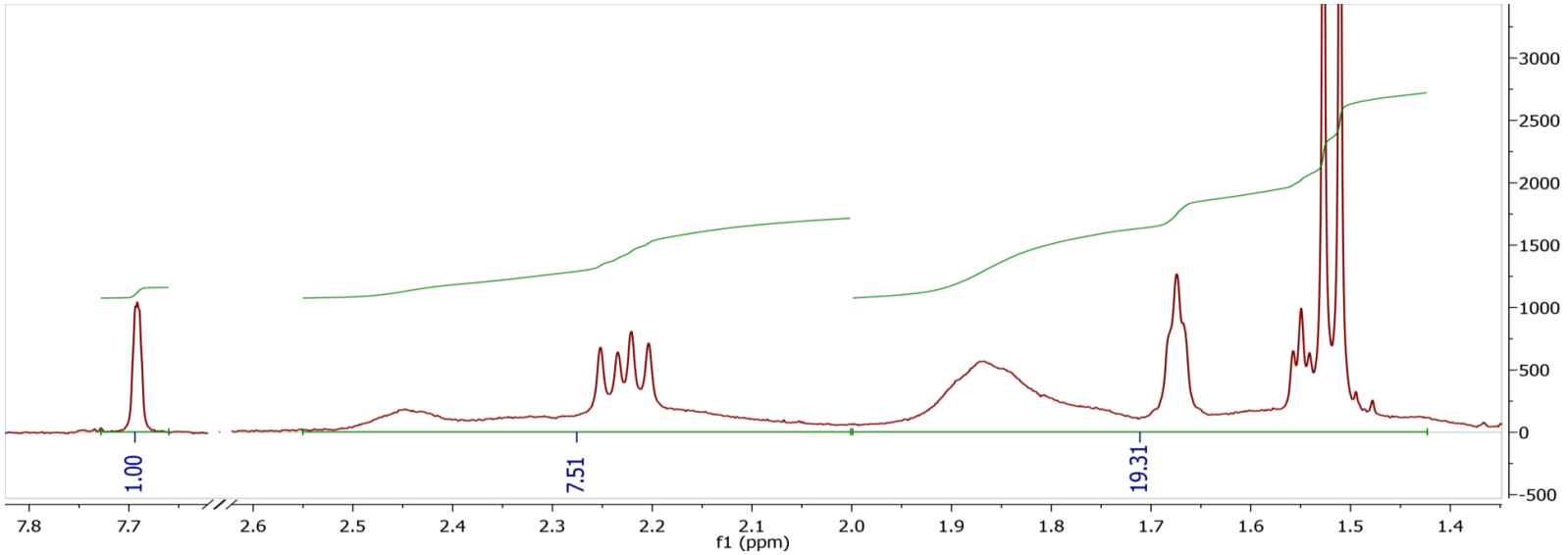
- When peaks have irregular shapes, Peaks-based multiplet analysis may give significantly different integration results than regular Sum-based integration
- In the example below, Peaks-based multiplet analysis extracts the regular peaks but ignores the irregular ones (that are usually) due to exchangeable protons



Why are integrals from multiplet analysis different from direct integration? (2)

Sum-based integration

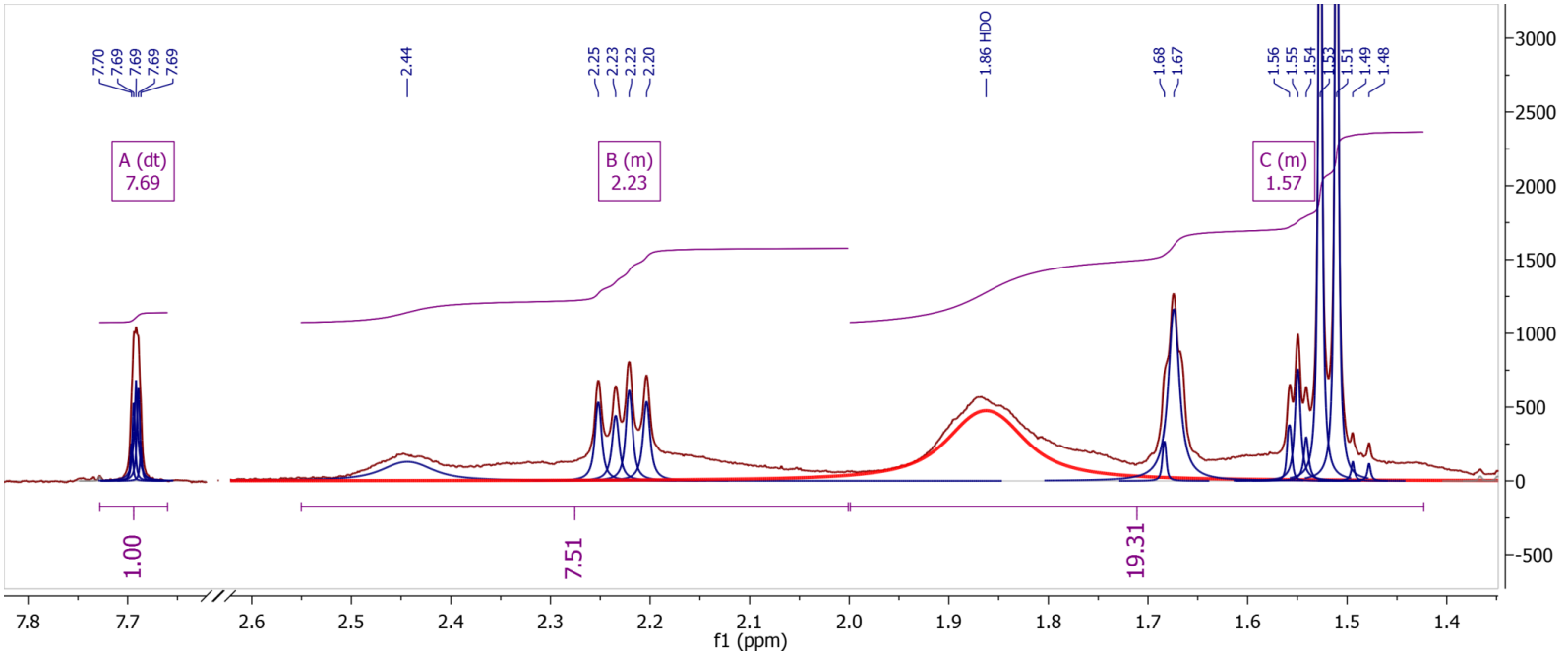
- When Sum-based integration is performed, all peaks are included by adding point intensity by point intensity within the integration region
- Depending on the goal of the analysis, one must choose the appropriate integration method



Force Mnova to use direct integration results in multiplet analysis

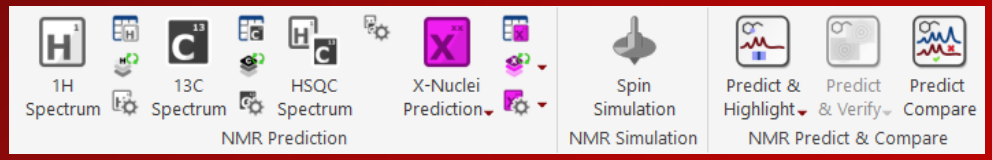
Combine Sum-based integration and multiplet analysis

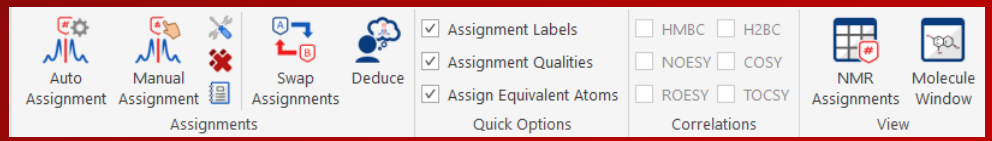
- If direct integration is performed prior to automatic multiplet analysis, the integration results (regions and integrals) will be preserved by the automatic multiplet analysis routine
- Compare the results below with those in the previous two slides:



More Advanced Analysis of 1D and 2D NMR

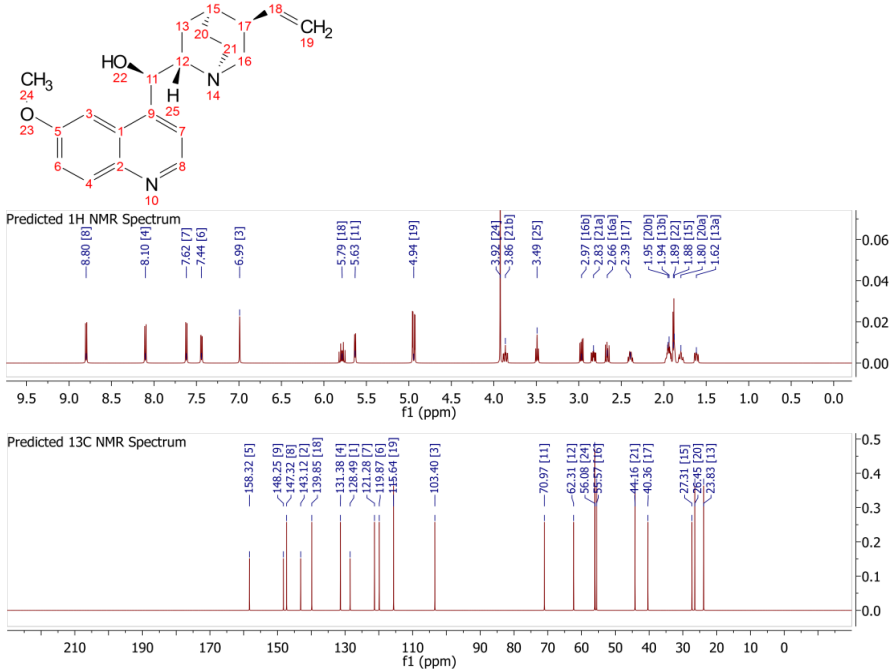
**You will need to have Mnova NMR and NMRPredict licenses for this section*

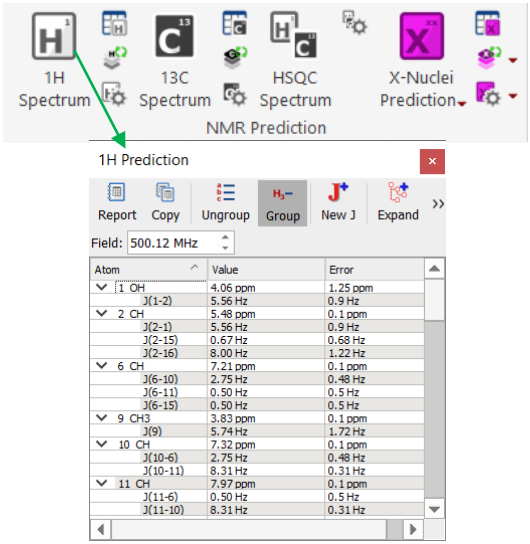




Predict NMR spectra from molecular structures

- Open a new document (**File/New**) or a new page (**Edit/Create New Page**)
- Copy a structure from ChemDraw or ChemSketch, then paste to Mnova, or open a .mol, .cdx, or .sdf file; or you can also sketch a structure in Mnova using the Molecule Ribbon
- Select a spectrum, and click the appropriate icon from the **Predict** ribbon



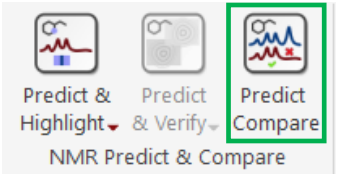


Tips:
1. Choose Prediction Options to change settings
2. You can turn on/off atom numbers by right-clicking on the structure and choosing Properties
3. You can open the Prediction Table to list the predicted shifts and J-couplings, and manually change them

A separate license for Mnova NMRPredict Desktop is needed.

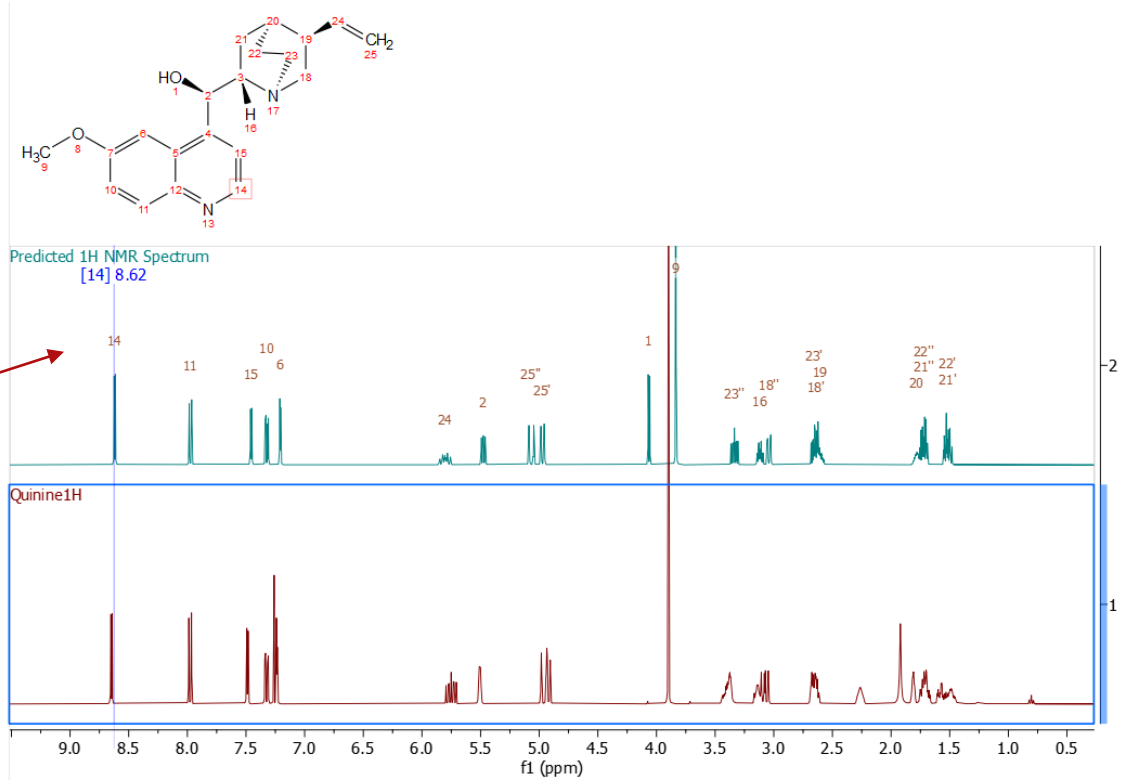
Predict and compare with the experimental spectrum

- Open a ^{1}H (or ^{13}C) **spectrum** on a new page
- Copy the **structure** from ChemDraw or ChemSketch
- Go to **Analysis/Predict and Compare.** The predicted spectrum is stacked with the experimental one for visual comparison



You can drag the label of a predicted peak to change its chemical shift. You can also change the predicted J-couplings in the 1H Prediction Table

To delete the predicted spectrum, open the Stacked Item Table from the Stacked Ribbon, and use the 'Delete' tool therein to delete it



Peak assignment for multiple spectra

Mnova provides a very convenient layout and a set of tools for assigning peaks from multiple spectra to the structure

The structure(s) is shared for all “linked” spectra and assigned peaks are color coded

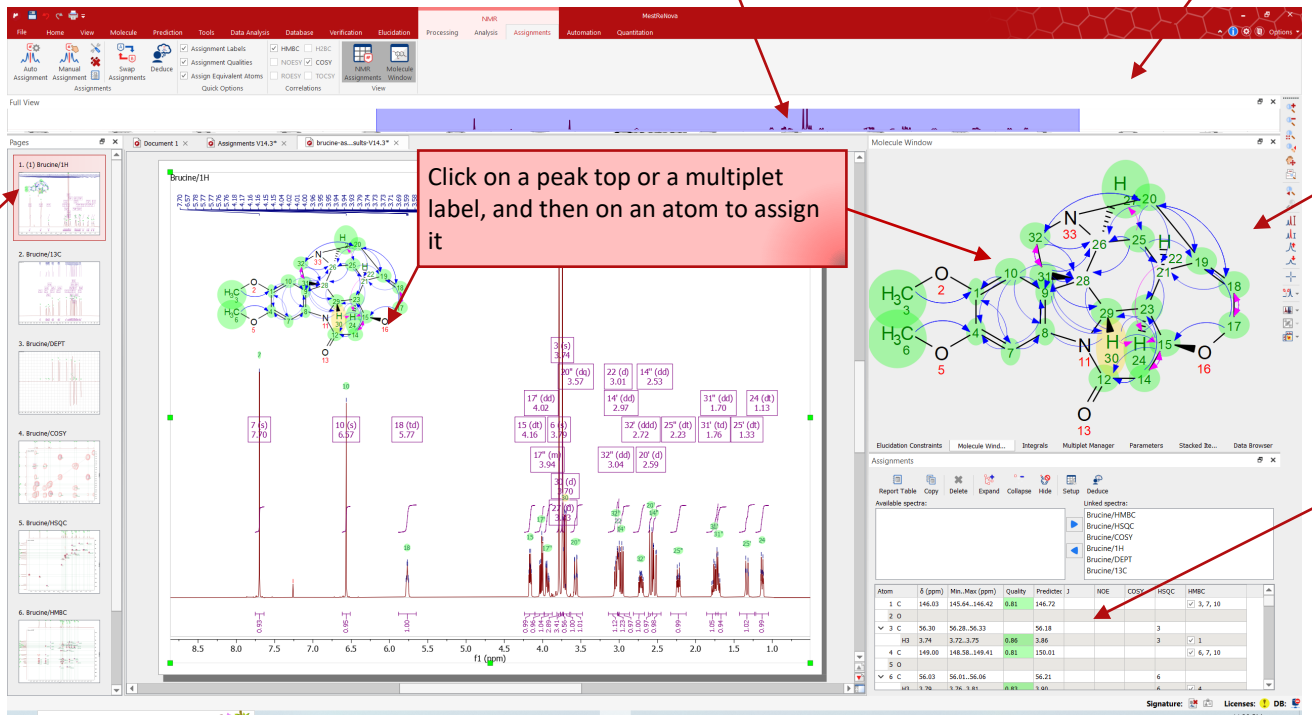
Full View allows you to easily navigate among peaks

Pages View allows you to easily navigate among spectra

Click on a peak top or a multiplet label, and then on an atom to assign it

The structure can be shown on the spectrum plot or in the Molecule Window

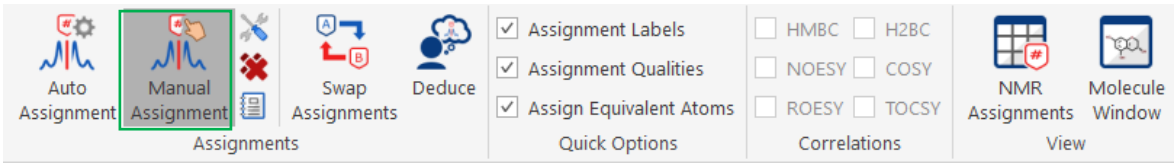
The assignment results are listed here. You can delete or change assignments here, and choose which spectra should be “linked”



Tip: Don’t mix spectra from different samples in the same document. Don’t open the same structure multiple times. Instead, use the Compounds Table to report the structure to the spectrum when needed. You can copy/paste and display multiple spectra side-by-side on the same page.

Assign a multiplet to an atom

- Press the **A** key (or choose **Assignments/Manual Assignment**) to enter Manual Assignment mode



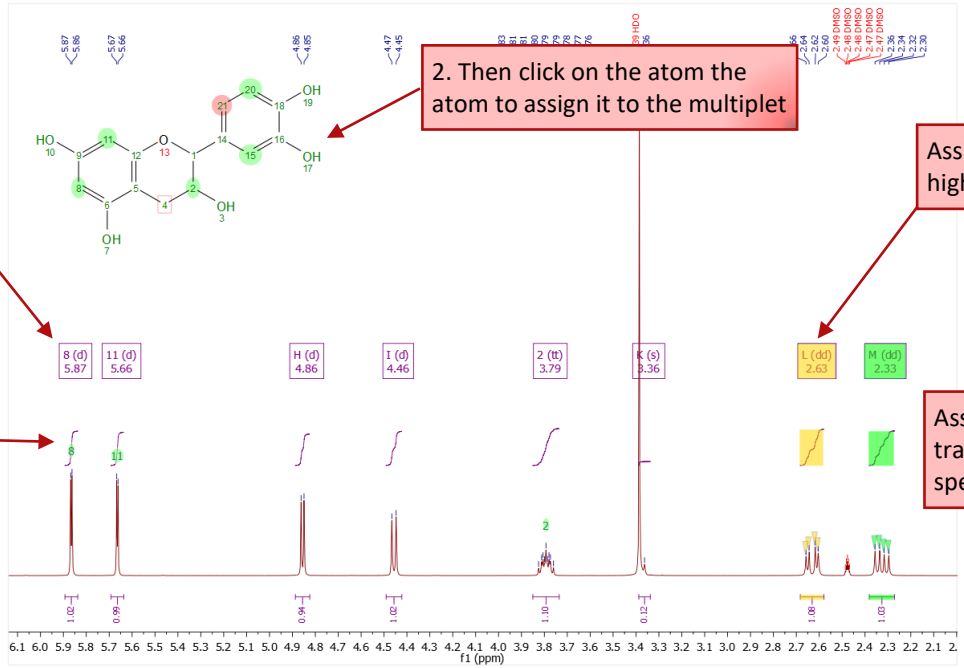
1. In Manual assignment mode, first click on the multiplet label. The cursor will change to 

2. Then click on the atom the atom to assign it to the multiplet

Assignments suggestions are highlighted by a suitable color code

3. Assignment label is displayed

Assignments are automatically transferred to the other 1D and 2D spectra in your document



Tip: After the assignment, the atom label is changed to green. The multiplet label shows the atom label. The multiplet label can be turned off by unchecking the Analysis/Multiplet Analysis/Multiplets Boxes.

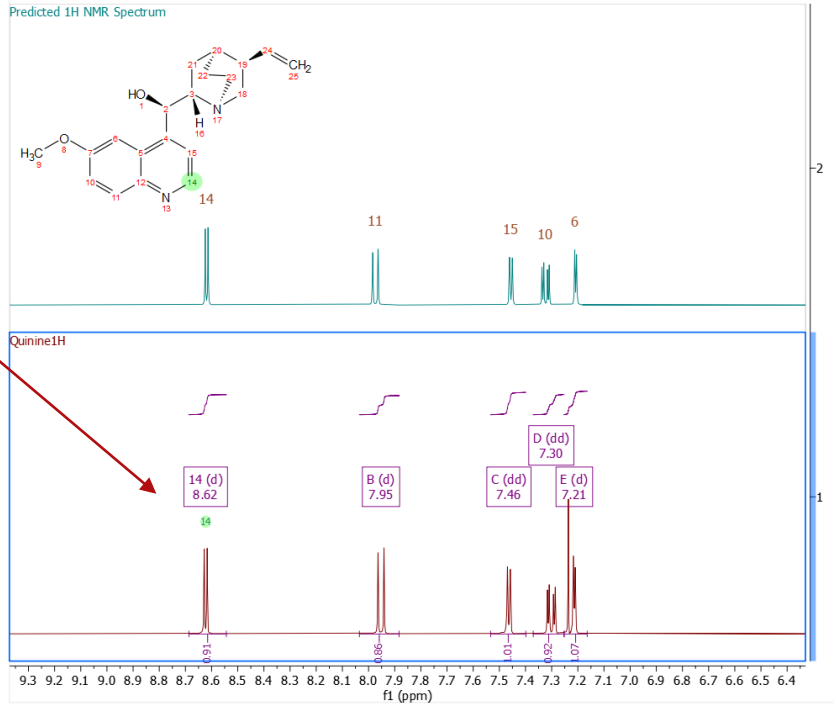
Predict NMR and help you assign peaks

- Open a ^{1}H (or ^{13}C) **spectrum** in a new page, do multiplet analysis or peak picking as usual
- Copy a **structure** from ChemDraw or ChemSketch
- Go to **Analysis/Predict & Compare.** The predicted spectrum will be stacked with the experimental one for visual comparison
- Switch to **Superimposed Mode** so you can assign the multiplets/peaks using the predicted peaks as a guide

Make sure the experimental spectrum is the active one

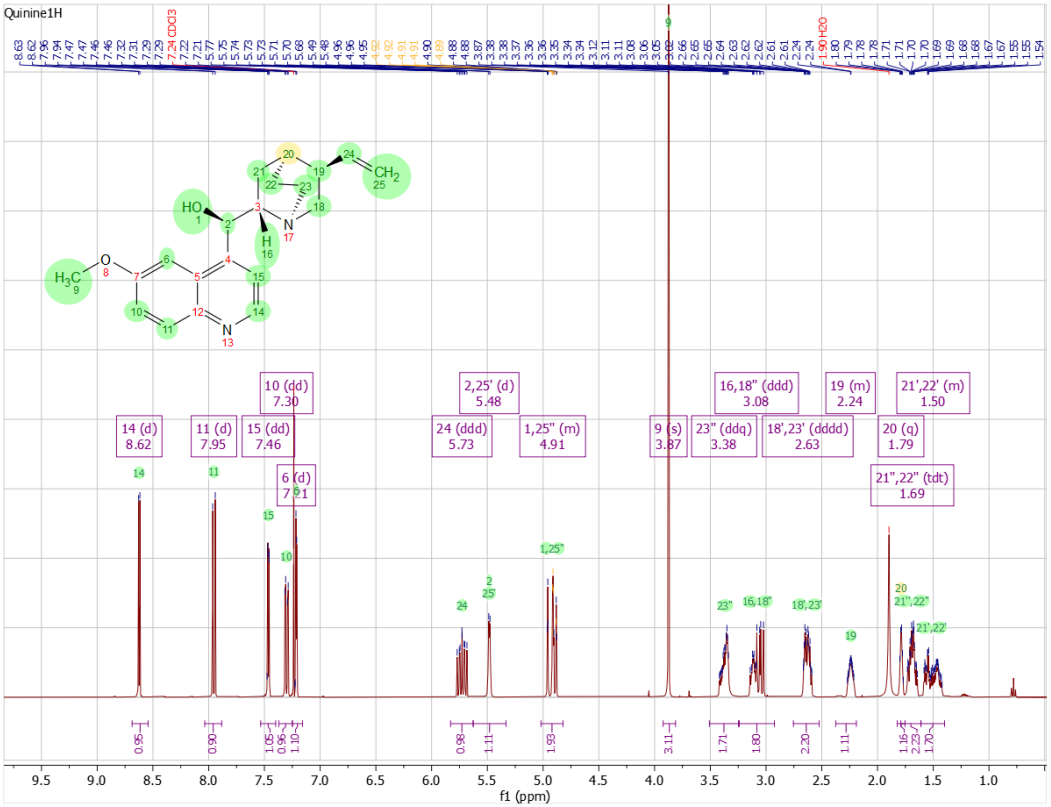
In Manual Assignment mode, click on a multiplet label and then on an atom to make the assignment

Blue: predicted peaks
Red: observed peaks



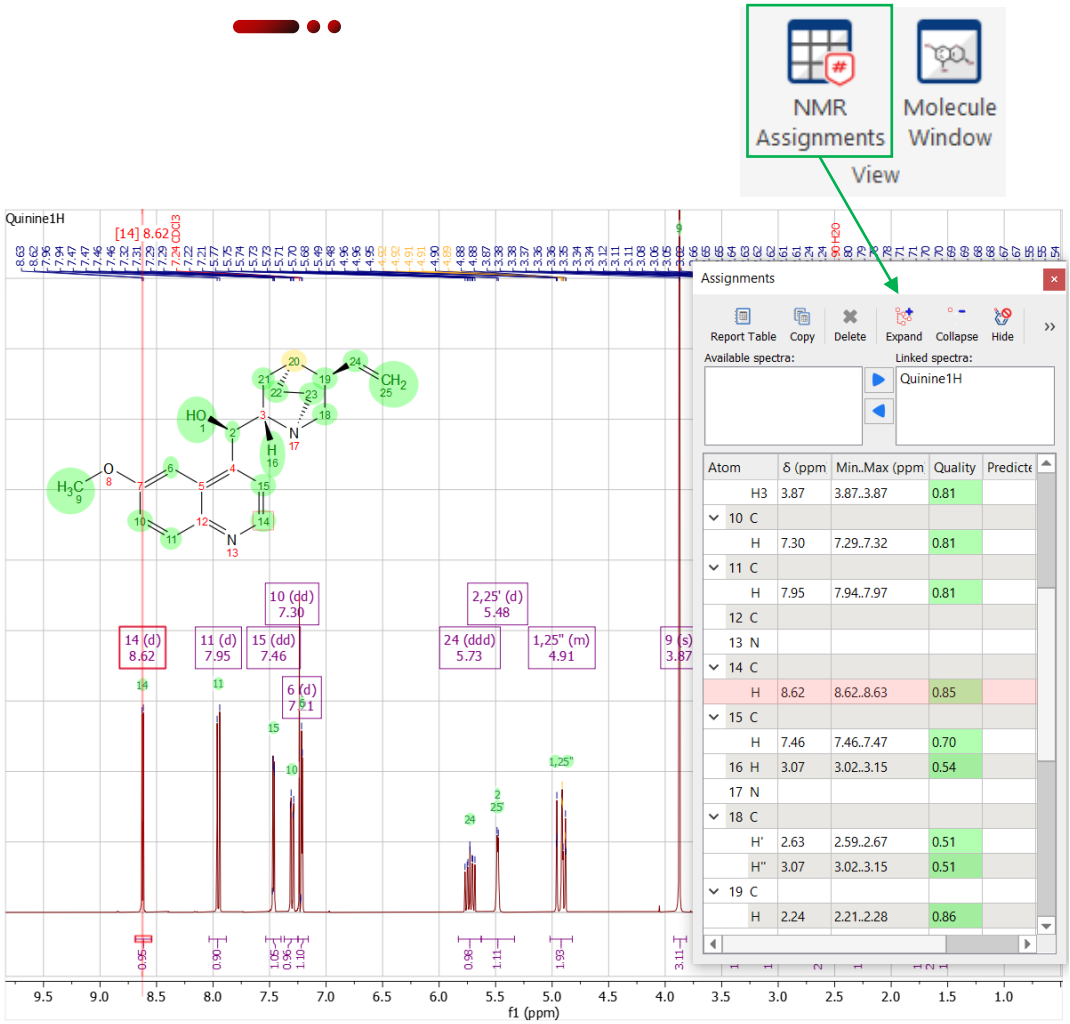
Automatic assignment of ^{1}H spectra

- Open a ^{1}H **spectrum** in a new page, and copy your **structure** from ChemDraw or ChemSketch
- Select **Analysis/Assignments/Automatic Assignment**. Mnova will do multiplet analysis (if not done yet), predict the ^{1}H spectrum, and automatically assign the ^{1}H peaks
- Automatic assignment is also available for 2D HSQC and ^{13}C spectra



Display and browse assignment results

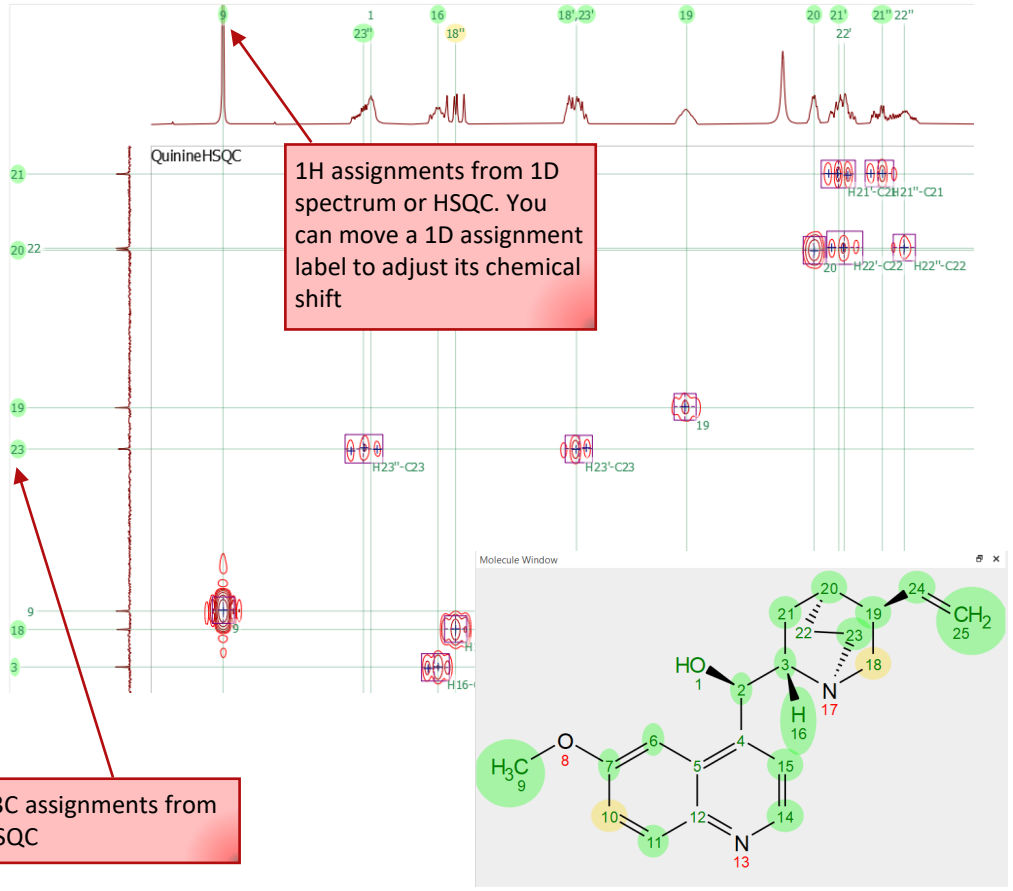
- In the Assignment Ribbon, press the NMR Assignments tool to open the Assignments Table
- The Table and the structure are correlated: you can click on a row to highlight the atom (and its assigned peak), or *vice versa*



Tip: right-click on an atom and go to Edit Atom Data from the pop-up menu to change its label. Changed labels will be used in the Assignments table and other relevant reports.

2D spectral assignment

- Assign the 1D 1H peaks, and then assign HSQC cross peaks, or *vice versa*
- Assignments in one spectrum are carried over to all other spectra in the same document. All spectra in the same document are "correlated" by default
- To assign atoms in a HSQC, press the "A" key to enter Assignment mode. Click on an atom in the molecular structure. Next click on the cross peak to assign it*

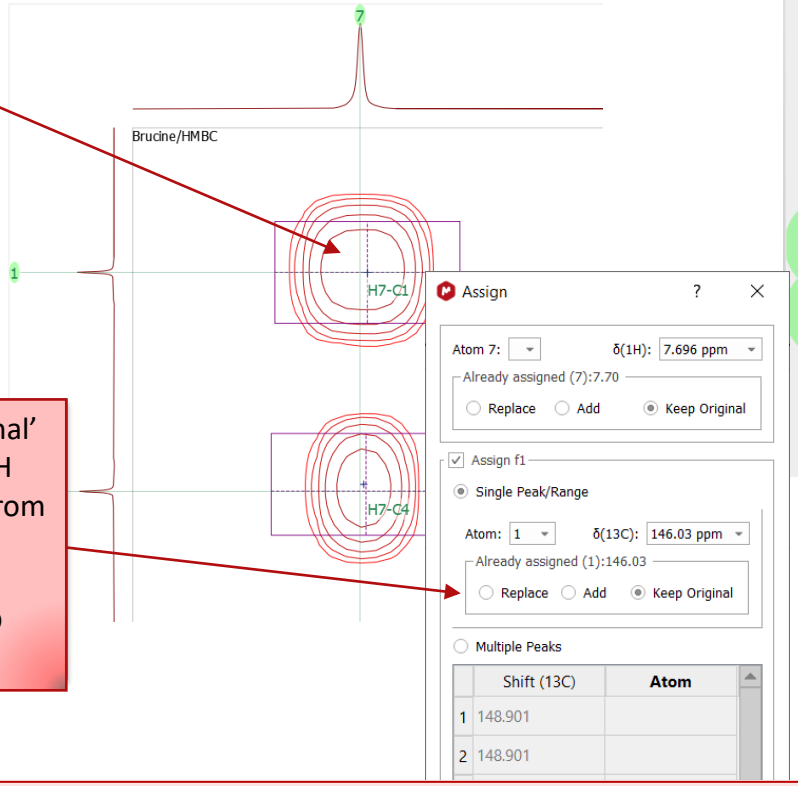


*By Default, Mnova automatically groups one or multiple 2D peaks into a "multiplet", similar to 1D NMR. When needed, it is very straightforward to manually change the size or center of a 2D multiplet, or to add a new one.

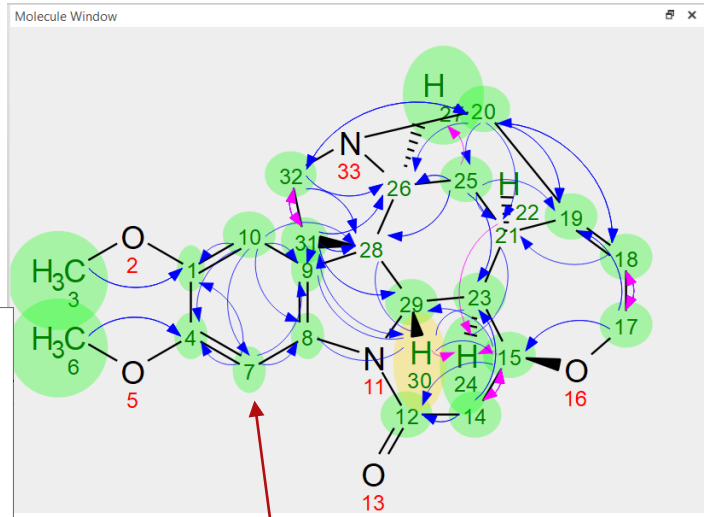
Assigning a HMBC peak

- In Assignment mode, click the center of an HMBC peak shown below. Then click on H7 while *holding the **Alt** key**
- In the Assign pop-up window, choose the options as shown below. Click OK to assign the peak to both H7 and C1
- COSY- and NOESY-type spectra can be assigned in a similar manner

1. Click the center of the peak in assignment mode



3. Choose 'Keep Original' for F2 to use the 1D 1H shift (instead of that from 2D). Choose C1 for F1, and choose 'Keep Original' to use the 1D 13C shift

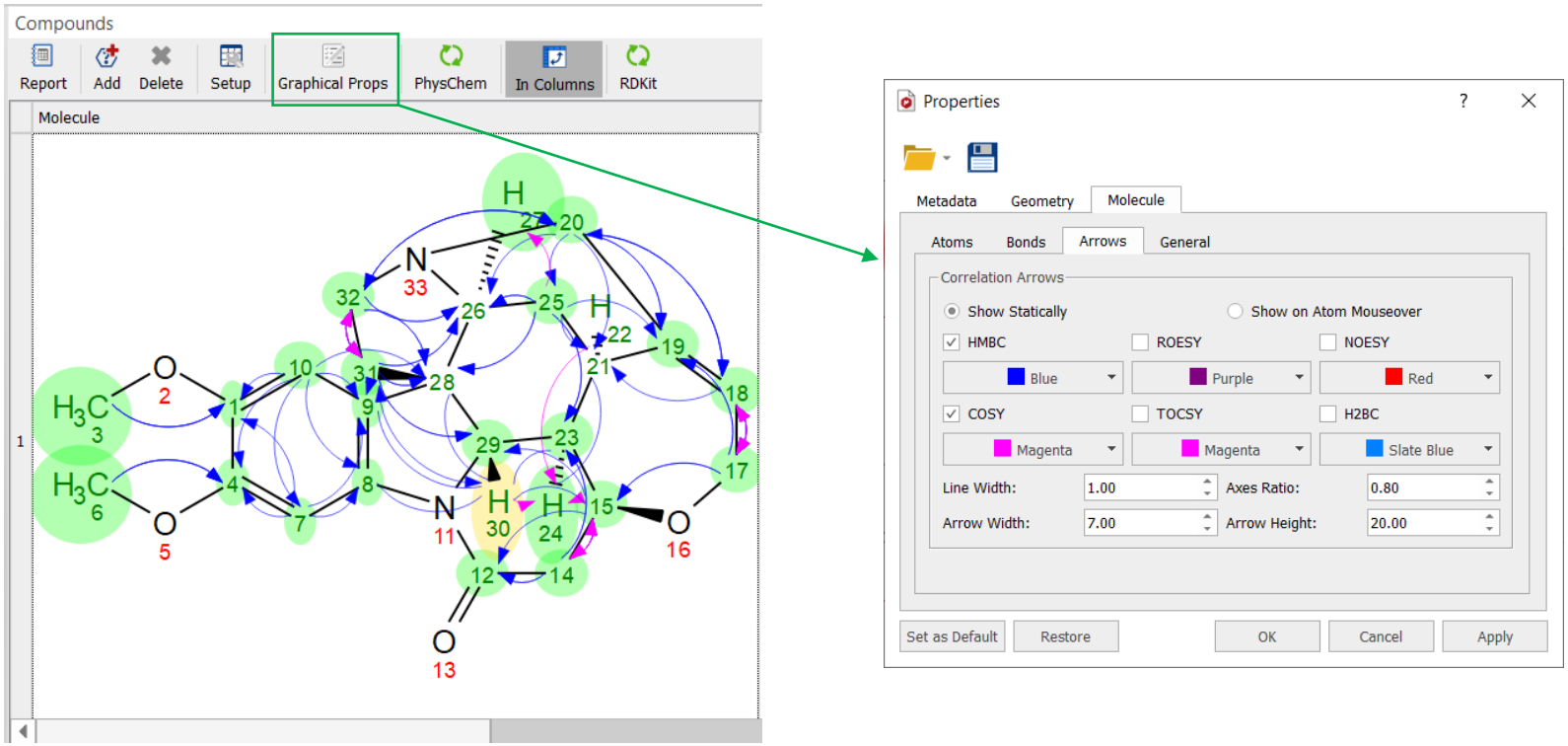


2. While holding Alt key, click H7

Since chemical shifts from 1D NMR are usually of higher resolution than 2D, we recommend you to use 1D shifts whenever possible. To access such choices, press and hold the **Alt key while assigning a peak*

Display 2D assignments on a molecular structure

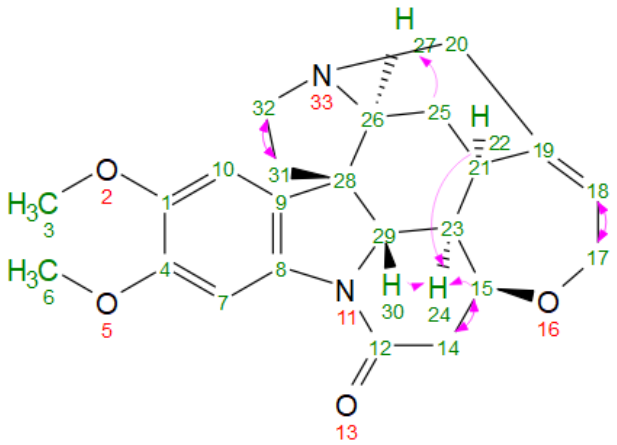
For a structure on the canvas, in the Molecule Window, or in the Compounds Table, you can choose Graphical Props Tool to choose display properties related to the assigned 2D NMR connectivity as shown below:



**Don't open the same structure multiple times. Instead, use the Compounds able to report the structure to the pages where needed*

Selective display of 2D spectral connectivities

Use the check boxes in the **Assignment** table to toggle the display of arrows



Assignments

Report Table | Copy | Delete | Expand | Collapse | Hide | Setup | Deduce

Available spectra:

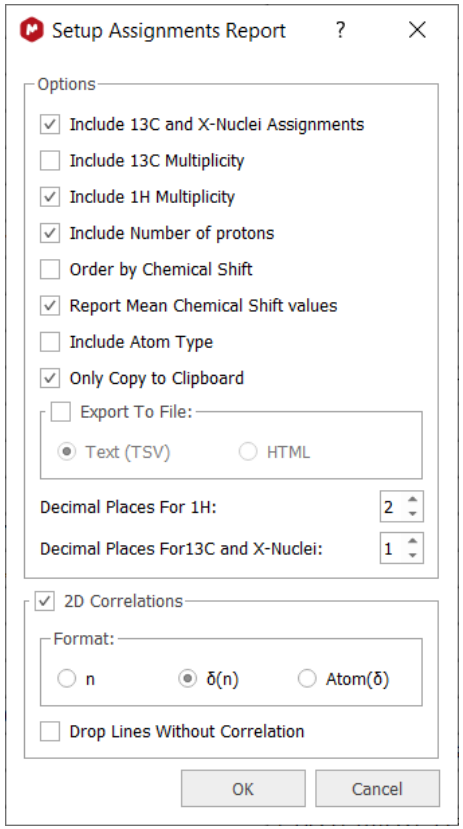
Linked spectra:
Brucine/HMBC
Brucine/HSQC
Brucine/COSY
Brucine/1H
Brucine/DEPT
Brucine/13C

Atom	δ (ppm)	Min..Max (ppm)	Quality	Predictec	J	NOE	COSY	HSQC	HMBC
H''	2.23	2.13..2.33	0.79	1.48, 1...			☑ 22, 25', 27	25	☑ 21, 23, 26, 28
26 C	59.78	59.75..59.80		59.92				27	☑ 20', 25', 25'', 31', 3...
27 H	3.73	3.71..3.76	0.81	3.93			☑ 25''	26	
28 C	51.75	51.72..51.77		51.98					☑ 10, 25', 25'', 30, 3...
29 C	60.21	59.99..60.66	0.80	62.34				30	☑ 15, 31', 31''
30 H	3.70	3.67..3.73	-0.16	4.44			☑ 24	29	☑ 8, 9, 15, 23, 28, 31
⌄ 31 C	42.32	42.26..42.84	0.81	41.62				31', 31''	☑ 30, 32'
H'	1.76	1.71..1.82	0.80	1.90, 2...			☑ 32', 32''	31	☑ 9, 26, 28, 29, 32
H''	1.70	1.64..1.74	0.30	1.90, 2...			☑ 32', 32''	31	☑ 9, 26, 28, 29, 32
⌄ 32 C	50.02	49.52..50.52	0.80	50.89				32', 32''	☑ 20', 20'', 31', 31''
H'	2.72	2.67..2.81	0.43	2.89, 3...			☑ 31', 31''	32	☑ 20, 26, 28, 31
H''	3.04	3.00..3.14	0.54	2.89, 3...			☑ 31', 31''	32	☑ 26, 28
33 N									

Uncheck here if you want to hide all the COSY connectivities related to H-32'' on the structure

Report spectral assignments in journal format

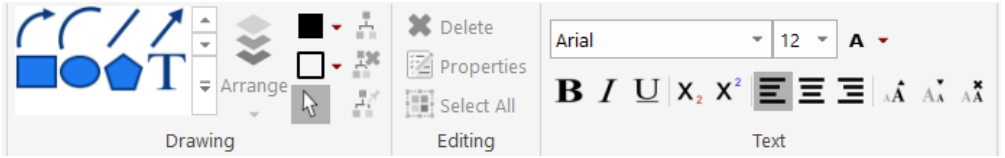
- Select **Tools/Report/Assignments** to report the assignment results in journal format
- The report can be copied and pasted into an external document

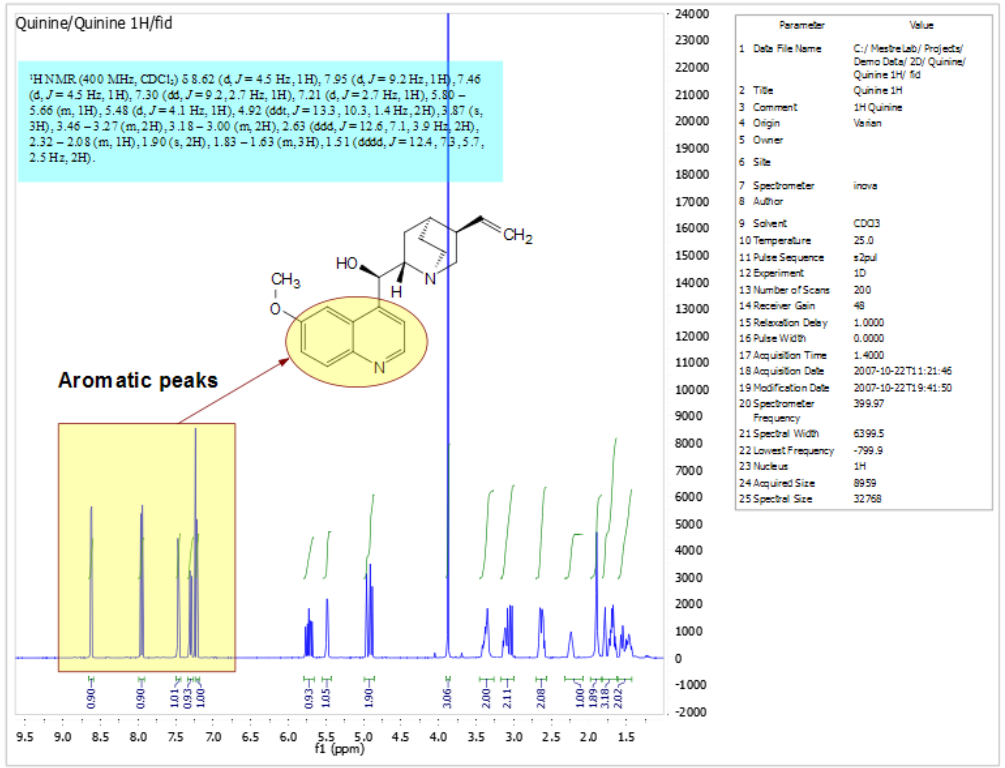


No	δ H (Multiplicity, J, nH)	δ C	HSQC	HMBC	COSY
1	-	146.0	-	3.74(3), 6.57(10), 7.70(7)	-
3	3.74 (s, 3H)	56.3	56.3(3)	146.0(1)	-
4	-	149.0	-	3.79(6), 6.57(10), 7.70(7)	-
6	3.79 (s, 3H)	56.0	56.0(6)	149.0(4)	-
7	7.70 (s, 1H)	100.8	100.8(7)	123.4(9), 135.8(8),146.0(1), 149.0(4)	-
8	-	135.8	-	3.70(30), 6.57(10),7.70(7)	-
9	-	123.4	-	1.70(31''), 1.76(31'), 3.70(30), 6.57(10),7.70(7)	-
10	6.57 (s, 1H)	105.5	105.5(10)	51.7(28), 100.8(7), 123.4(9), 135.8(8),146.0(1), 149.0(4)	-
12	-	168.8	-	2.54(14''), 2.97(14'), 4.16(15)	-
14'	2.97 (dd, 17.4,8.5 Hz, 1H)	42.2	42.2(14)	48.1(23), 77.6(15),168.8(12)	2.54(14''), 4.16(15)
14''	2.54	42.2	42.2(14)	48.1(23), 77.6(15),168.8(12)	2.97(14'), 4.16(15)
15	4.16 (dt, 8.4,3.3 Hz, 1H)	77.6	77.6(15)	60.2(29), 64.4(17),168.8(12)	1.13(24),2.54(14''), 2.97(14')
17'	4.02 (dd, 13.8,7.0 Hz, 1H)	64.4	64.4(17)	77.6(15), 127.0(18), 140.4(19)	5.77(18)
17''	3.94 (m, 1H)	64.4	64.4(17)	127.0(18), 140.4(19)	5.77(18)
18	5.77 (td, 6.1,3.3 Hz, 1H)	127.0	127.0(18)	31.4(21), 52.5(20),64.4(17)	3.94(17''), 4.02(17')
19	-	140.4	-	1.33(25'), 2.59(20'), 3.57(20''),3.94(17''), 4.02(17')	-
20'	2.59 (d, 14.8 Hz, 1H)	52.5	52.5(20)	26.7(25), 31.4(21), 50.0(32), 59.8(26),127.0(18), 140.4(19)	3.57(20'')
20''	3.57 (dq, 14.7,1.6 Hz, 1H)	52.5	52.5(20)	50.0(32),127.0(18), 140.4(19)	2.59(20')
21	-	31.4	3.01(22)	1.13(24), 2.23(25''), 2.59(20'),5.77(18)	-
22	3.01 (d, 4.1 Hz, 1H)	-	31.4(21)	-	1.13(24), 2.23(25'')
23	-	48.1	1.13(24)	1.33(25'), 2.23(25''),2.54(14''), 2.97(14'), 3.70(30)	-
24	1.13 (dt, 10.4,3.2 Hz, 1H)	-	48.1(23)	31.4(21)	3.01(22), 3.70(30),4.16(15)
25'	1.33 (dt, 14.4,2.1 Hz, 1H)	26.7	26.7(25)	48.1(23), 51.7(28), 59.8(26),140.4(19)	2.23(25'')
25''	2.23 (dt, 14.3,4.4 Hz, 1H)	26.7	26.7(25)	31.4(21), 48.1(23), 51.7(28), 59.8(26)	1.33(25'),3.01(22), 3.73(27)
26	-	59.8	3.73(27)	1.33(25'), 1.70(31''), 1.76(31'), 2.23(25''),2.59(20'), 2.72(32'), 3.04(32'')	-
27	3.73 (d, 2.3 Hz, 1H)	-	59.8(26)	-	2.23(25'')

Annotate and report manually

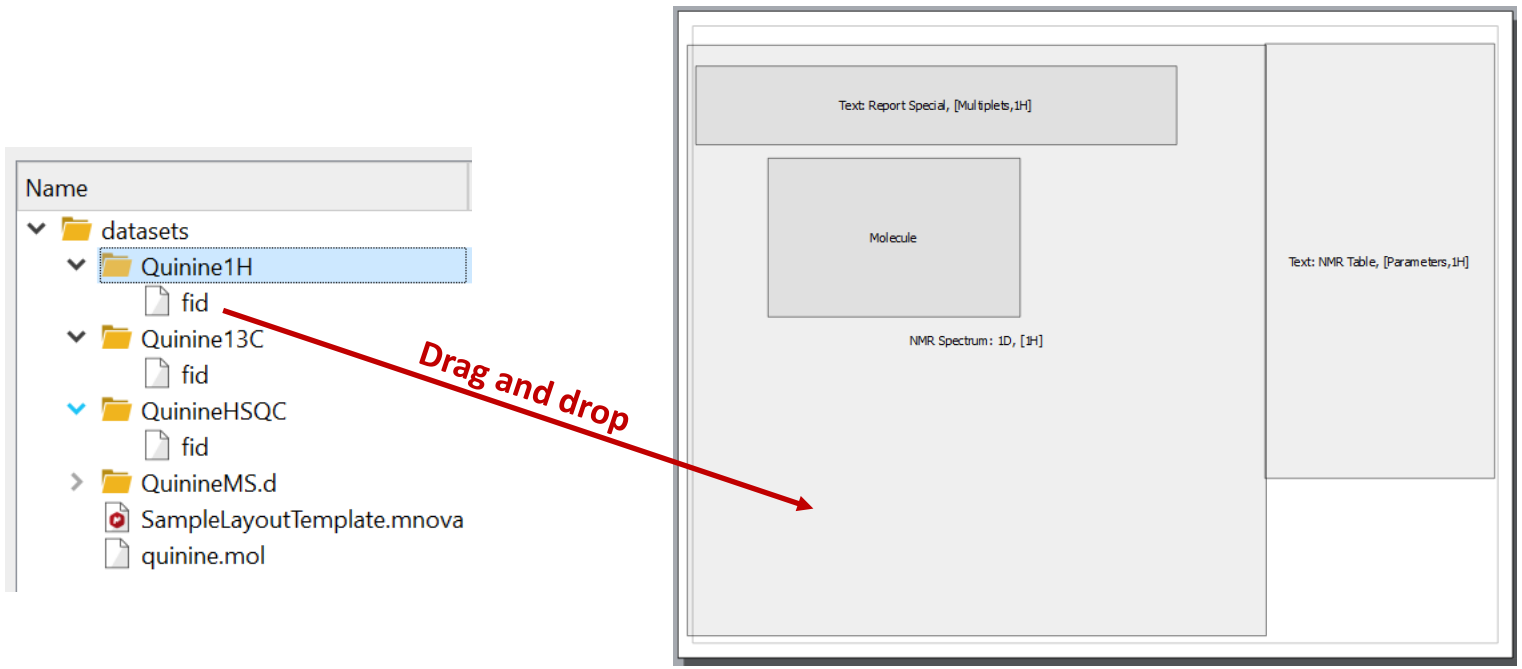
- **Annotations**, such as arrows, boxes, and text, etc. can be added using the tools in the Main Ribbon
- The display of objects can be customized by right-clicking, and then selecting **Properties**
- Tables of **Peaks, Integrals, Parameters**, etc. can be opened by selecting **View/Tables…** Contents can be reported or copied to other documents



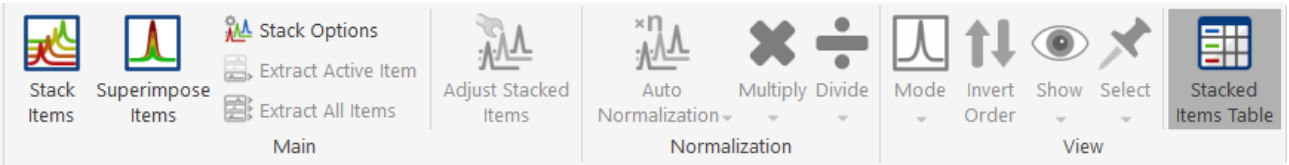


Create a layout template

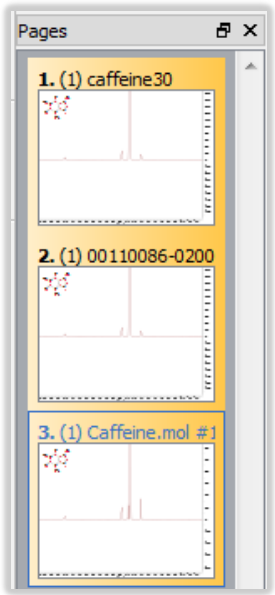
- Once all page objects are laid our correctly, choose **View/Layout Templates/Create Layout Template Document**…, and save the layout file to disk
- The content of all page items is removed to leave a template with placeholders
- To use a layout template, open a new FID and/or molecular structure onto the template, and it will be auto-formatted to the desired size and location
- If you have a spectrum already opened, choose **View/Layout Templates/Lay Out In Template Document**… to apply a template

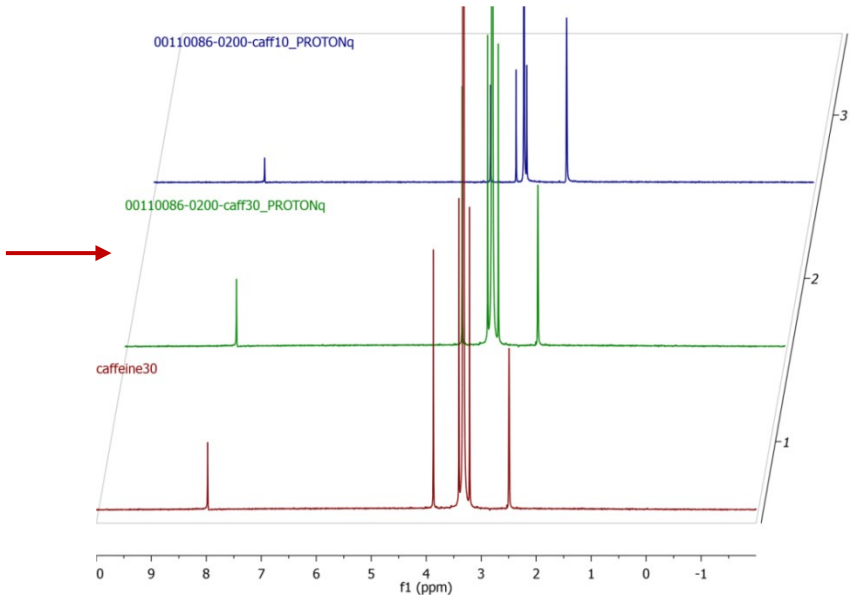


Open and stack multiple 1D spectra



- Open several 1D spectra in the same document
- Select some or all of them in the **Pages** view
- Press  to stack them in a new page
- Change the display to another Stack Mode, such as **Superimposed** mode

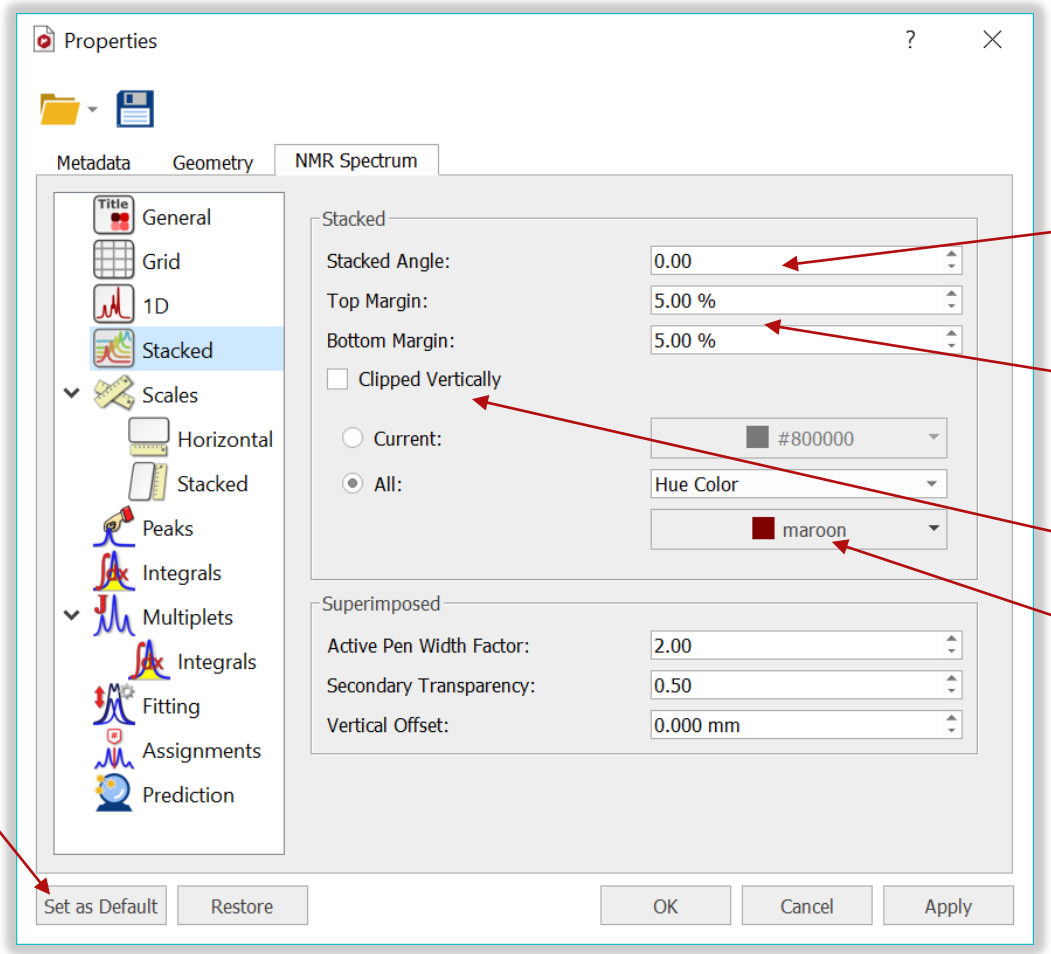




Tip: - You can also drag a 1D from a different page using the Pages view, which adds it to the current page as a new element in the stack.
- When multiple pages are selected, you can choose the Superimposed tool  to superimpose them directly.
- If you want to stack all the 1D spectra from a certain folder on the computer, select Tools/Import/Directory Spectra Stack.

Change display properties of stacked spectra

Right click on the spectra and select **Properties/Stacked:**



Enter 0 here if you don't like the tilt angle

Enlarge the top/bottom margins for better 3D effects

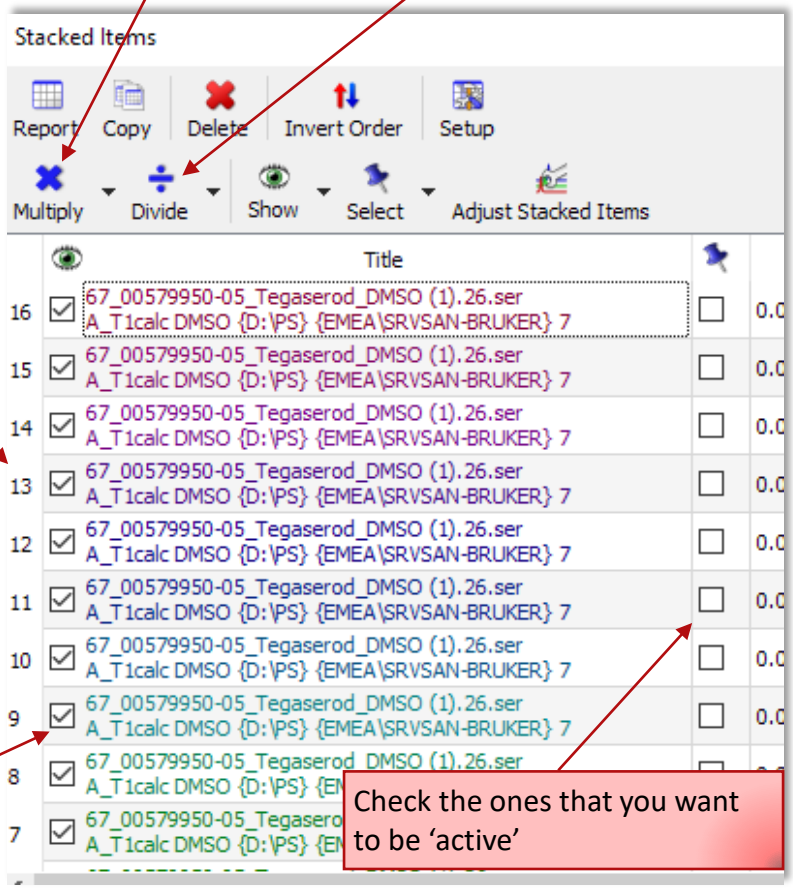
Check here if you want to clip the peaks

Change colors of spectra

Click here to set the changes as default

Handle stacked spectra (1)

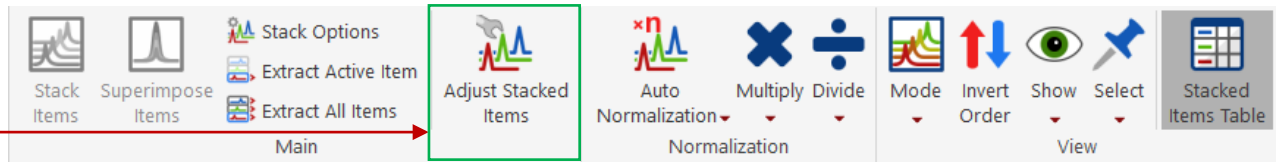
- Click  to toggle on the **Stacked Items** table.
- Use this table to do the following:
  - Delete spectra from the stack
  - Change order of the spectra in the stack
  - Change the Y-intensity of selected spectra
  - Choose which ones to display
  - Choose which ones to adjust



Tip: Read Help > Contents on more advanced data analysis, such as reaction monitoring, metabolomics, relaxation studies, DOSY processing, etc.

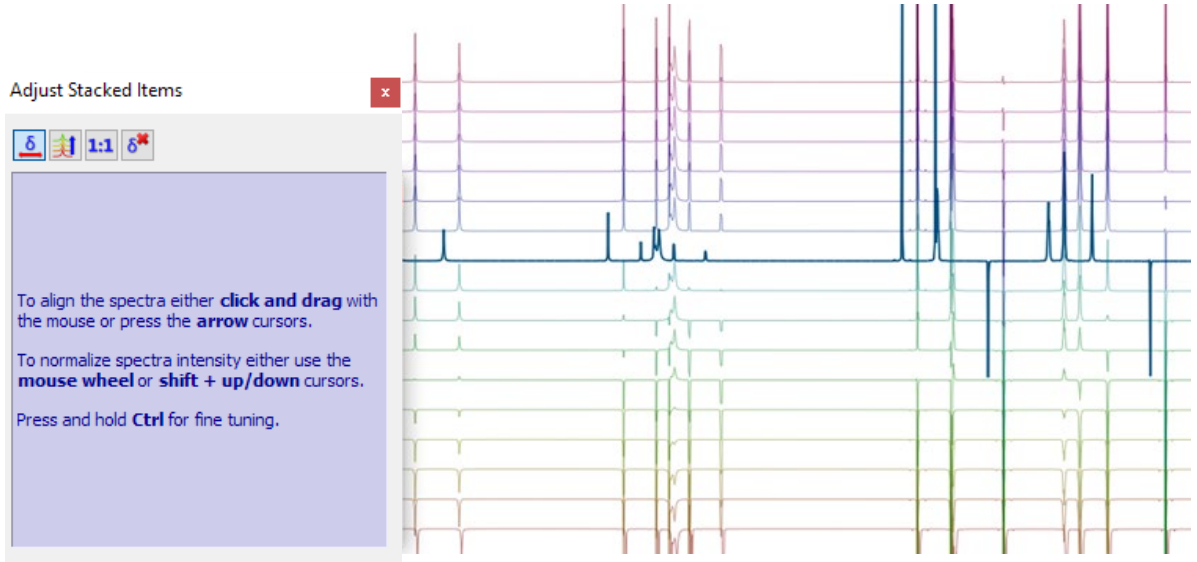
Handle the stacked spectra (2)

Click **‘Adjust Stacked Items ‘**



The cursor has to be inside the blue dialogue box

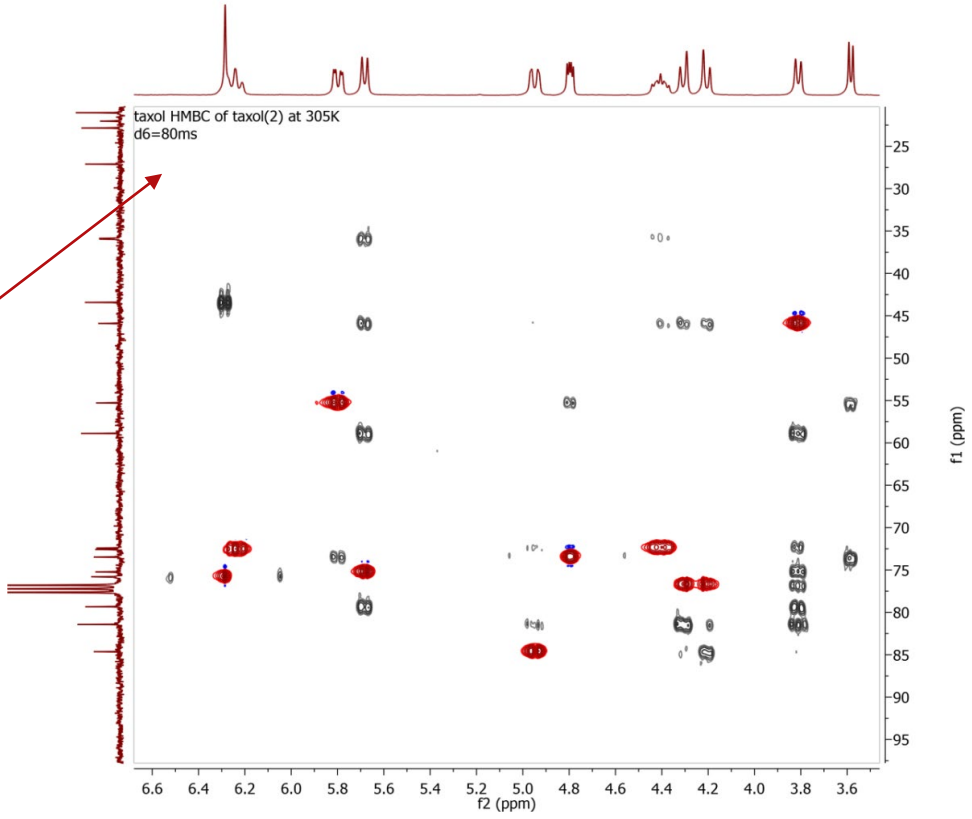
- Click and drag to shift an ‘active’ spectrum horizontally
- Click and drag to adjust the vertical offset between stacked spectra
- Reset intensities
- Reset shifts



Superimpose multiple 2D

- Multiple 2D can be stacked or superimposed in the same way as 1D spectra
- Press the Shift + Up Arrow key to change the active spectrum
- Right-click on the spectrum, and select Properties to change the color of the contours for the active spectrum

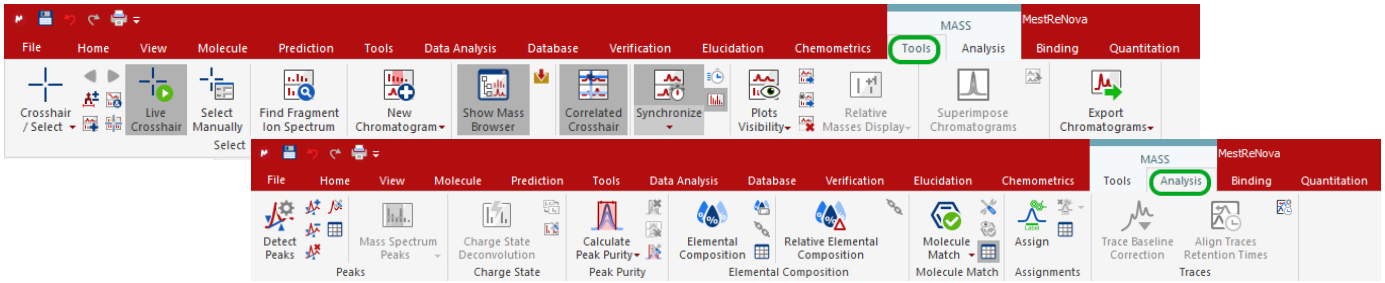
The title shows the currently ‘active’ spectrum



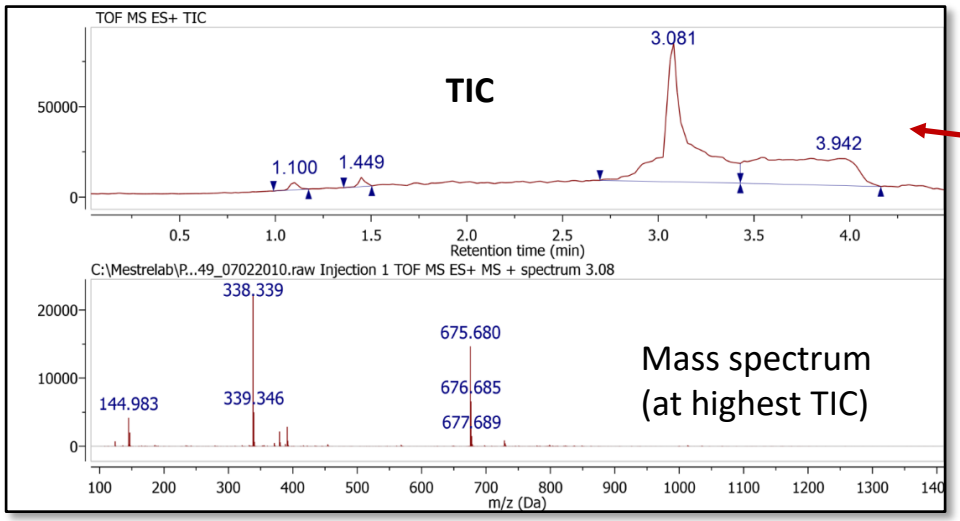
Basic Analysis of LC/GC-MS

*You will need to have Mnova MSChrom license for this section

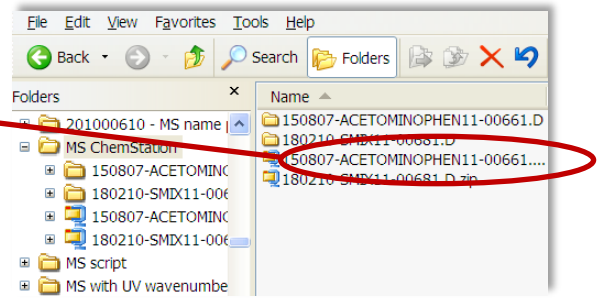
Open LC or GC-MS data



- Go to **File/Page Setup/Orientation** and change the page orientation to portrait if you wish
- Go to **Data Browser** to open any file in the folder containing raw data, or **drag and drop** the folder from Windows Explorer (or Finder) into Mnova
- Mnova will automatically convert your data and pick peaks



Drag & drop

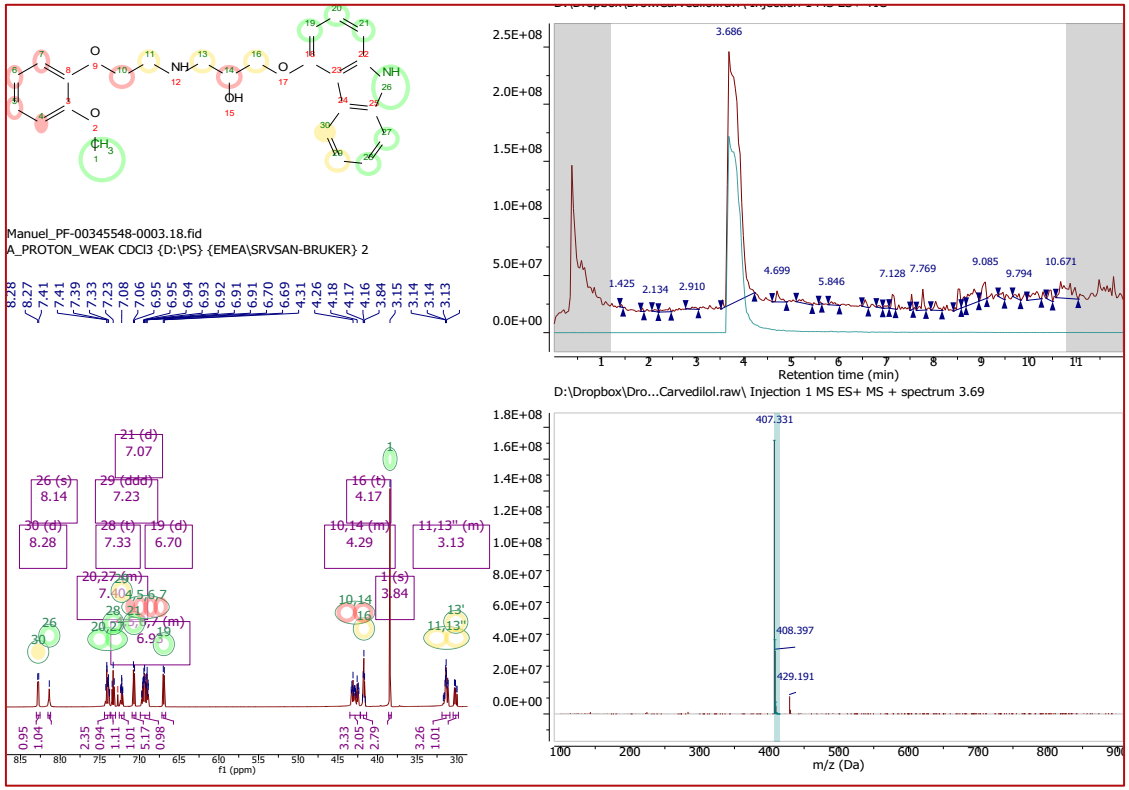


Details about the compatible data format:
http://mestrelab.com/resources/ms-supported-formats/

To download the full manual of Mnova:
https://mestrelab.com/downloads/mnova/manuals/MestReNova-14.2.3_Manual.pdf

Common interface for all analytical techniques

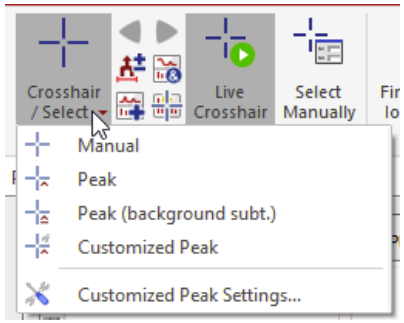
- Easily combine your MS and NMR data on the page in an Mnova document

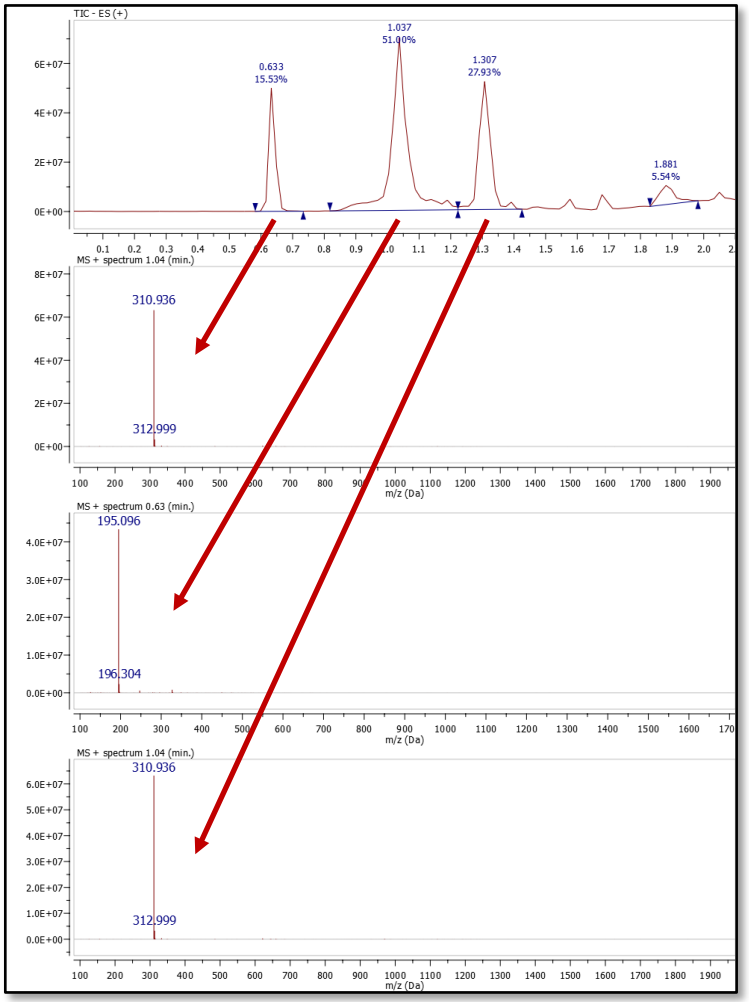


Note: When multiple spectral objects are opened they are loaded onto separate pages. You can copy (or cut) and paste them to the same page later.
Tip: Use the 'Bring to Back/Front', 'Align', and 'Tile' tools to arrange objects nicely.

Browse the mass spectra

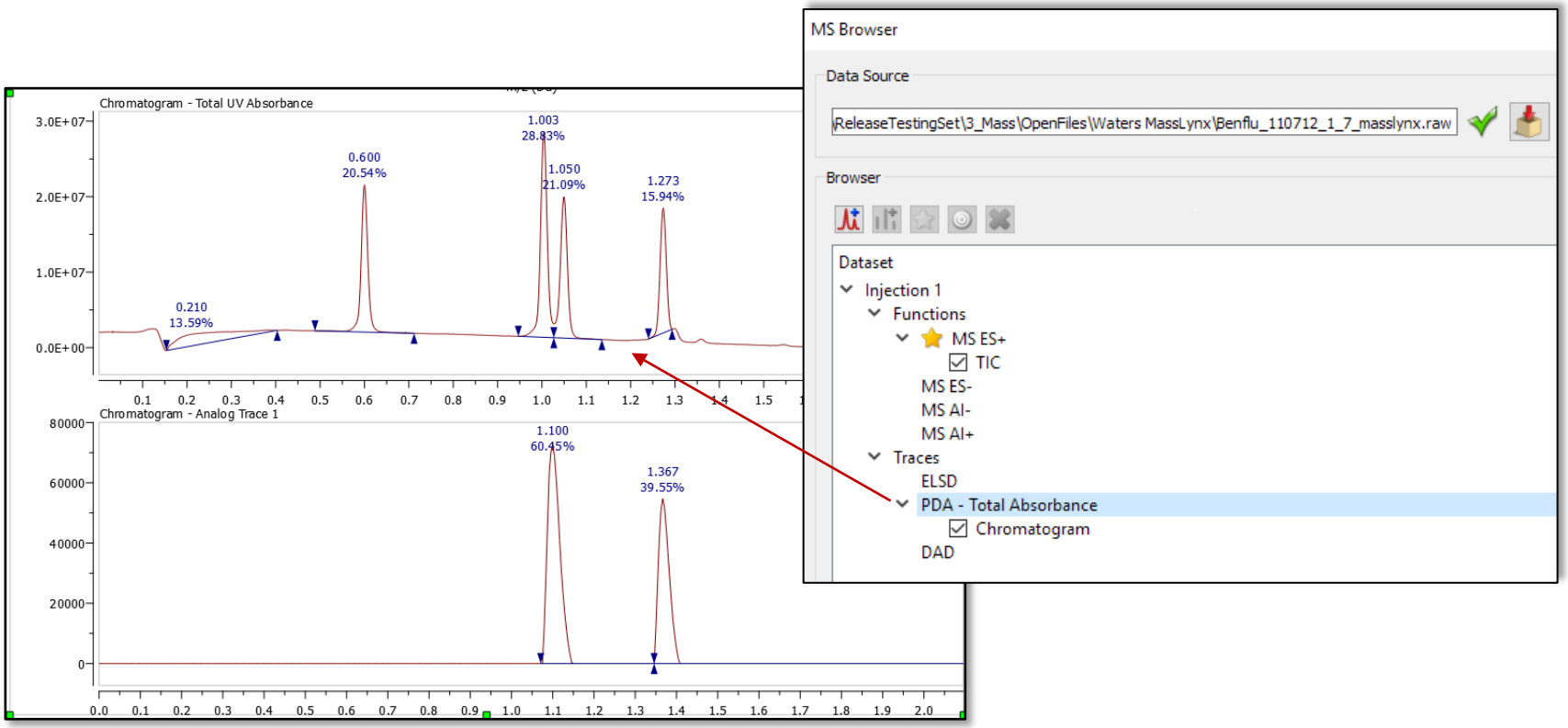
- Click  to switch to a crosshair cursor, and click on the TIC to display the mass spectrum at that retention time, or click and drag to display co-added spectra
- Press  to change to 'Append' mode to add a mass spectrum to the display
- Choose the **Crosshair/Select** drop-down menu to display mass in different ways:
  - **Manual mode**: Click to display a single MS, or click-and-drag to co-add multiple MS (default)
  - **Peak mode:** Click on a peak to display the co-added MS within the peak range
  - **Peak (Background subtraction) mode:** Click on a peak to display the co-added MS within the peak range with the background subtracted
  - **Customized Peak mode:** Customized co-added MS





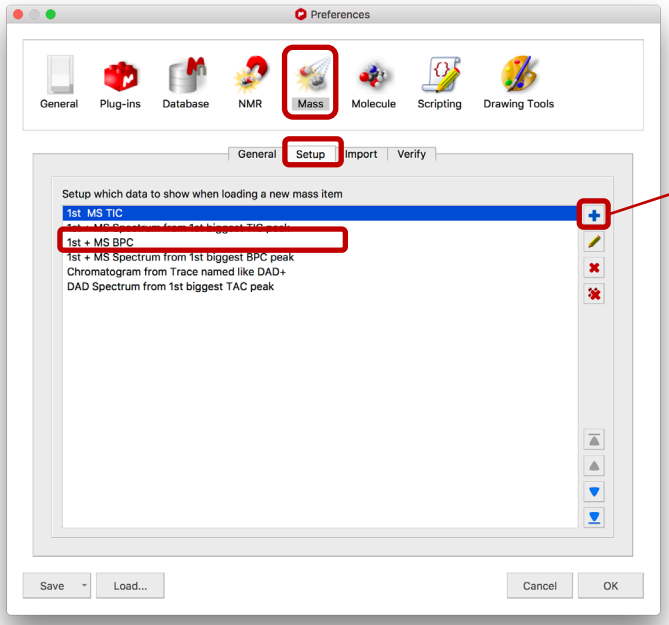
Browse UV traces

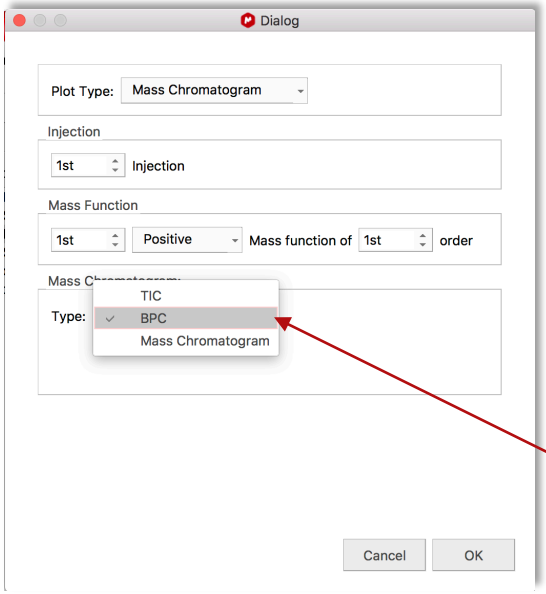
- Click  to show the MS Browser panel
- Double-click a ‘Total Absorbance’ item under ‘Traces’ to display a UV trace
- Click  to add more UV traces to the display



Setup MS import display preferences

- It is possible to control what to display when a dataset is first opened
- Choose **File/Preferences**, click the Mass icon, then the Setup tab
- Click “+” to add plots that you want to show when a dataset is opened
- Plots can be deleted or reordered



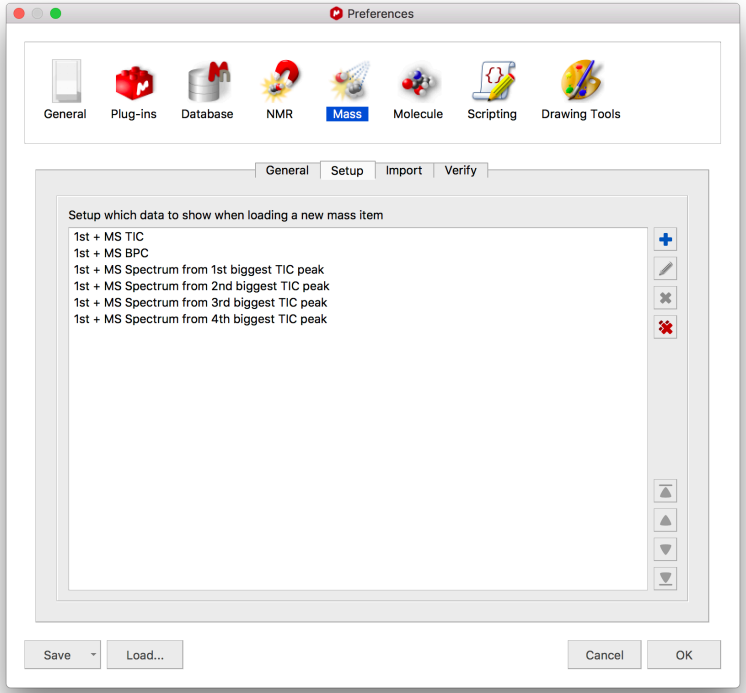


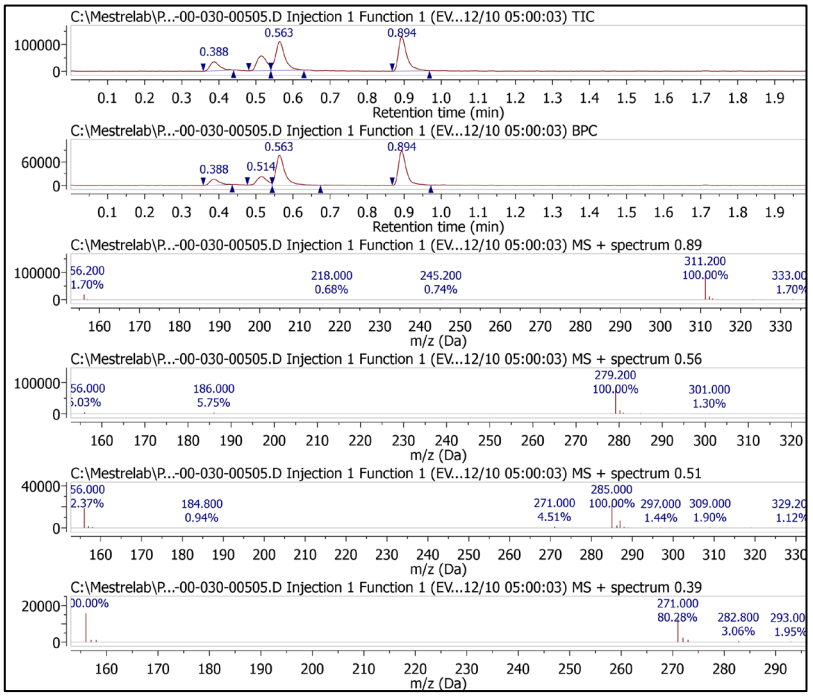
This dialog sets the display of the first positive base peak chromatogram (BPC) in the first injection (highlighted in the Preferences list)

Tips: Use MS Browser to see components available for display. Different preferences for different types of MS data may need to be defined. Save preferences to an .ini file for later use.

Example of a customized display

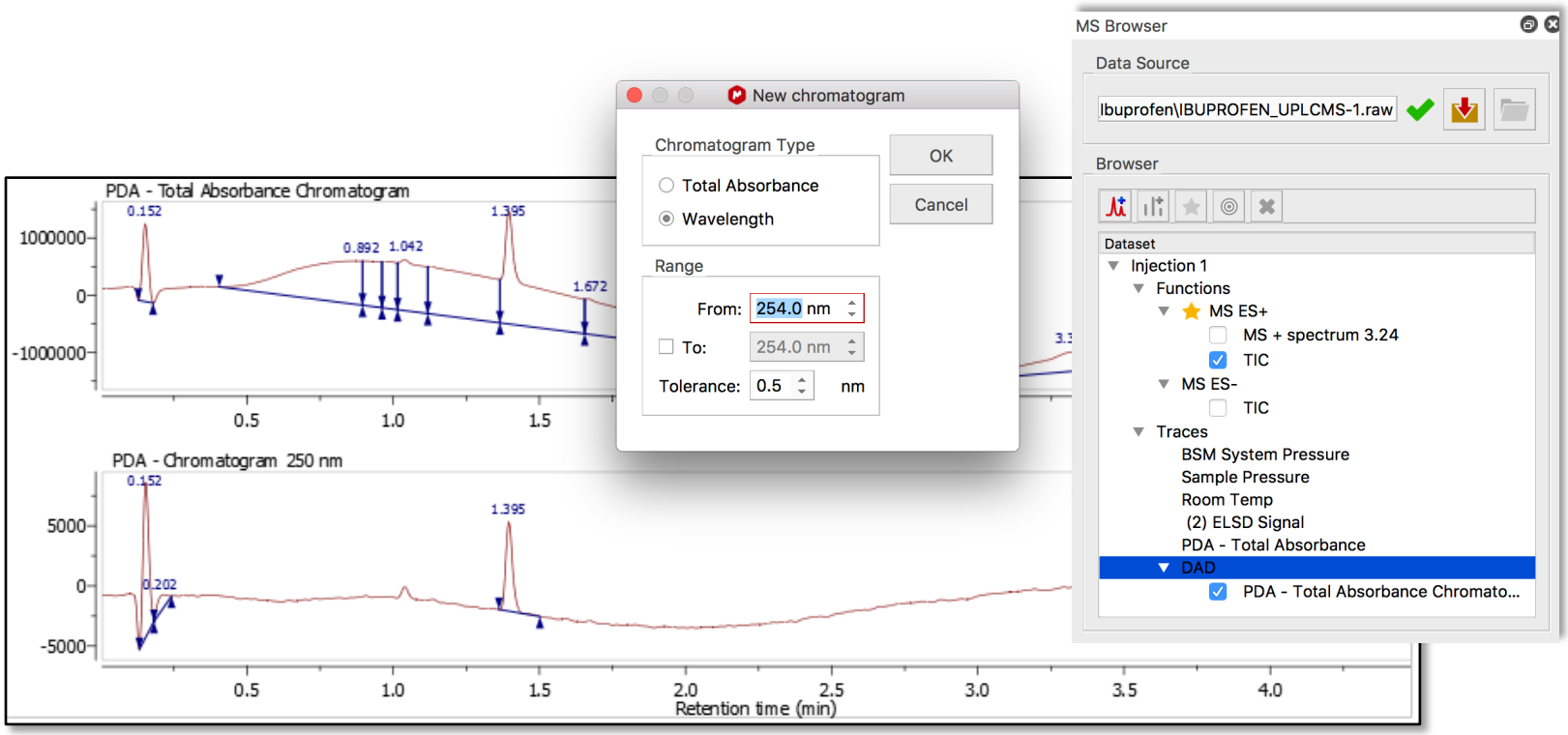
- Choose **File/Preferences**, click the Mass icon, then the Setup tab
- Define the display of the TIC, BPC, and the mass spectrum corresponding to the top four TIC peaks, as shown below
- Open a new MS dataset, and observe the display





Extract a UV trace at a selected wavenumber

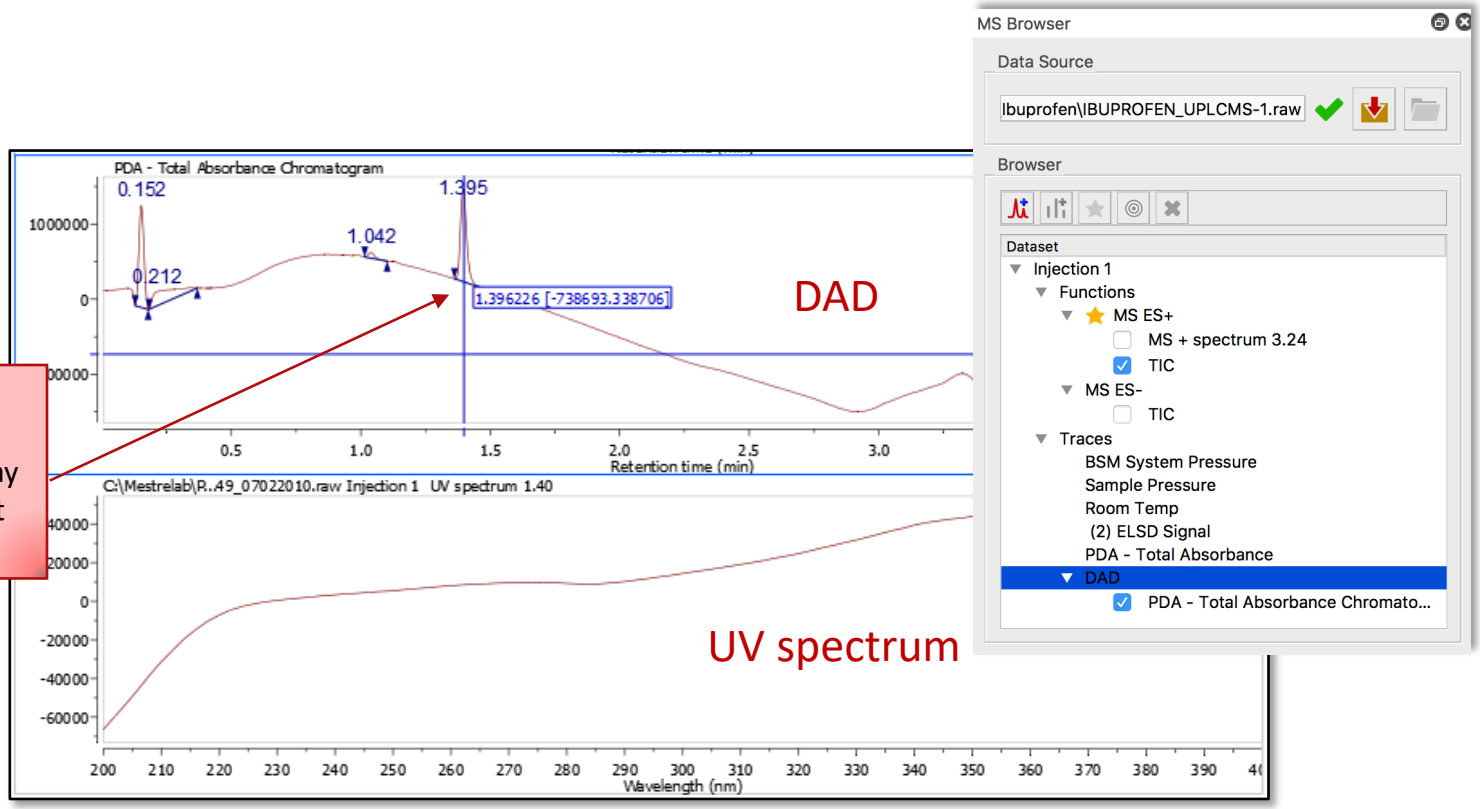
- Double click the DAD in the **MS Browser** to display it
- In the **New Chromatogram** dialog, choose **Wavelength**, and enter a wavelength and a tolerance to display the extracted UV trace



Display a UV spectrum at a selected retention time

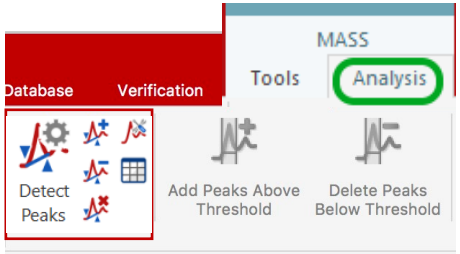
- Double click the DAD Trace in the **MS Browser** to display it
- Press for Crosshair Cursor, press and hold the **Alt** key, then click on the DAD trace to display the UV spectrum at that retention time

In crosshair cursor mode, click on the PDA curve to display the UV spectrum at that retention time



Edit and report peak integration results

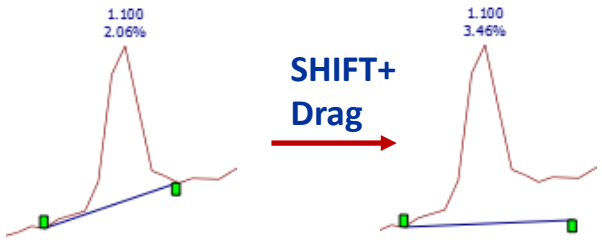
- Peaks are automatically integrated when you open a chromatogram
- Use the **Detect Peaks** ribbon icons to redetect, add, delete, or clear peaks
- Hover the cursor over a blue wedge, then click and drag the green boxes to change the range of a peak
- Or press **Shift** + click and drag green boxes to change the baseline of a peak
- Go to **View/Tables… Mass Peaks** to display or report the **Mass Peaks** table



Mass Peaks

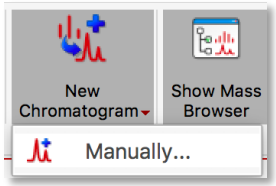
Report Copy Setup

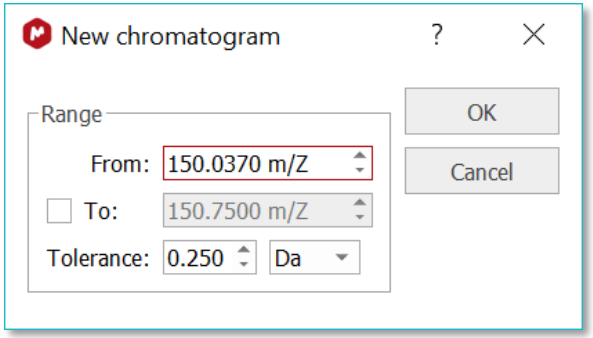
	RT	Scan	Type	Height	Area	Total Height %	Total Area %	Start time	End time
1	4.31	483	VB	65677.5	3184.9	1.52	0.67	4.312	4.392
2	4.14	464	BV	160655.3	31687.9	3.71	6.64	4.036	4.312
3	3.90	437	BB	227958.5	30589.4	5.26	6.41	3.787	4.018
4	3.75	421	BB	157293.0	3864.0	3.63	0.81	3.724	3.787
5	2.26	253	BB	3309241.0	373467.8	76.38	78.23	2.169	2.471
6	0.70	78	BB	376270.8	31871.3	8.68	6.68	0.631	0.862
7	0.12	12	BB	35336.2	2729.3	0.82	0.57	0.062	0.213

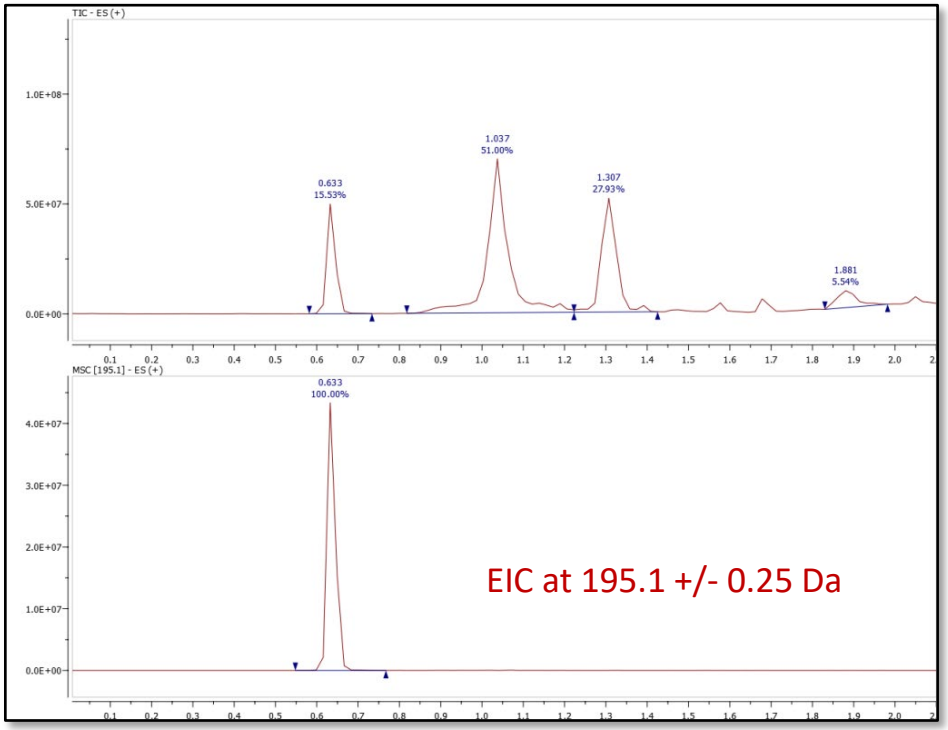


Display an EIC for a specific m/z value

- Click  or go to **Mass Analysis/New Mass Chromatogram/Manually…**
- In the **New Chromatogram** dialog, enter an m/z value and a suitable Tolerance
- Click OK to display the Extracted Ion Chromatogram (EIC)

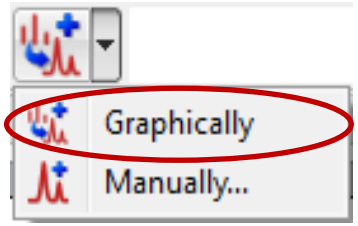




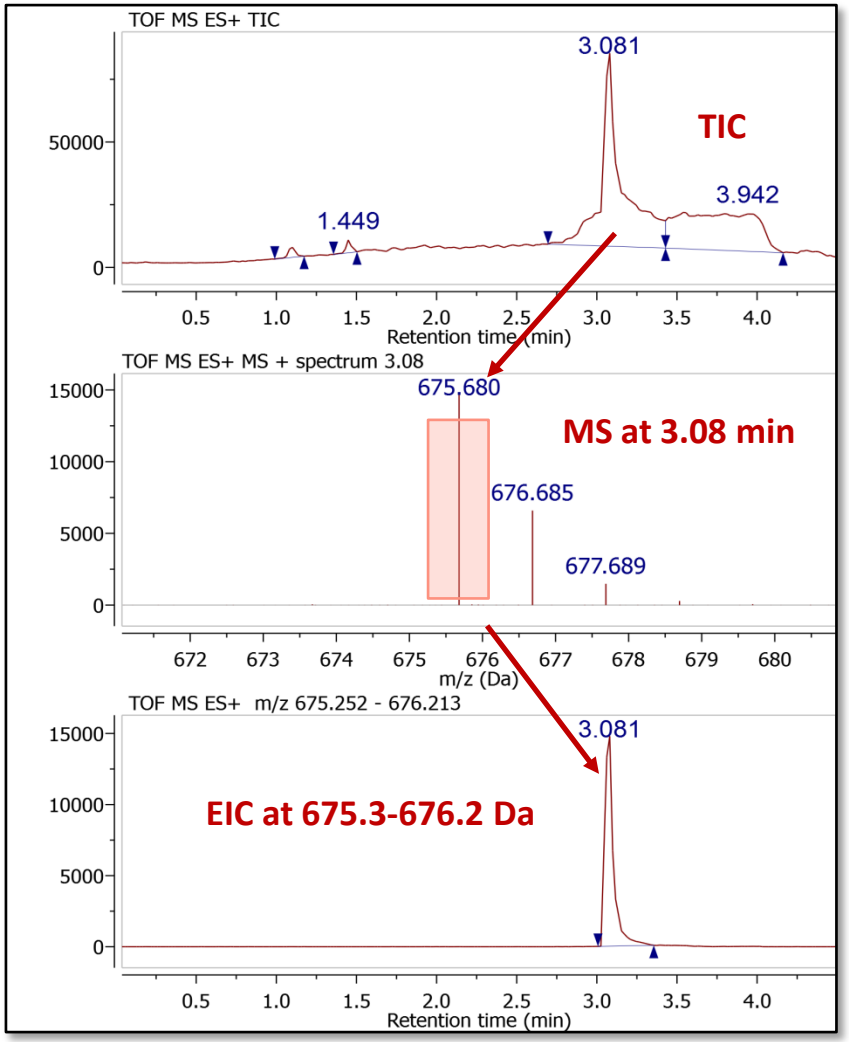


Tip: You can also go to **Mass Analysis/Spectrum Prediction** to run a mass prediction from a molecular formula.

Display extracted ion chromatogram for an MS peak

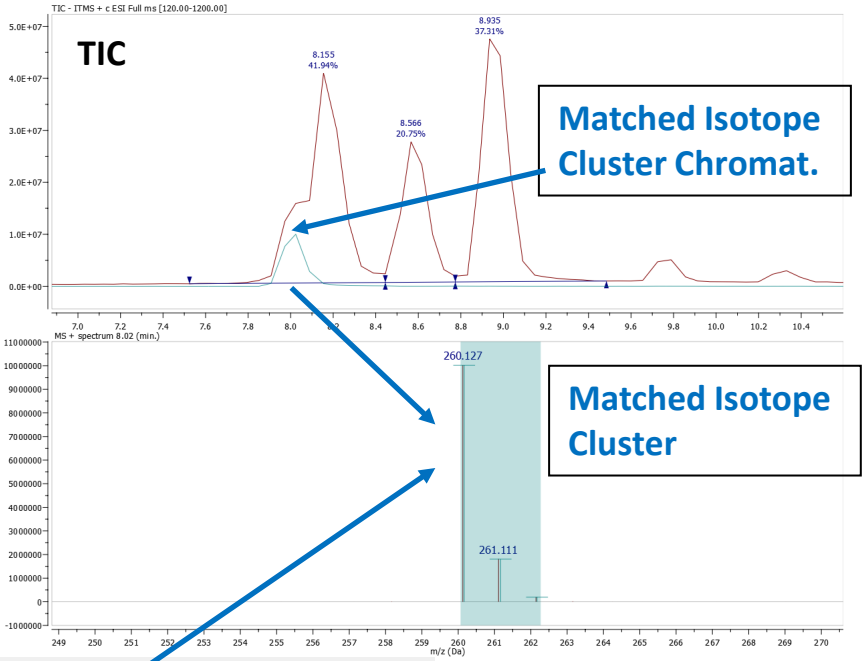


- First display the MS trace and zoom into the molecular ion peak that you are interested
- Next, press  or go to **Mass Analysis/ New Mass Chromatogram/Graphically**, click-and-drag around the peak to define a mass range
- An EIC will be displayed within the mass range



Confirm proposed structures using Molecule Match (1)

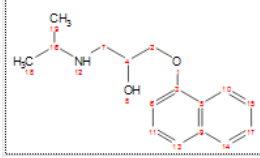
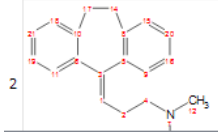
- Import one or several structures by copying/pasting from ChemDraw, Isis/Draw or ChemSketch, or by opening .mol or .sdf files
- Click  or go to **Mass Analysis/ Molecule Match/Calculate from molecules**
- In the Molecule Match Table, click on a molecule to view the matching results



Molecule Match

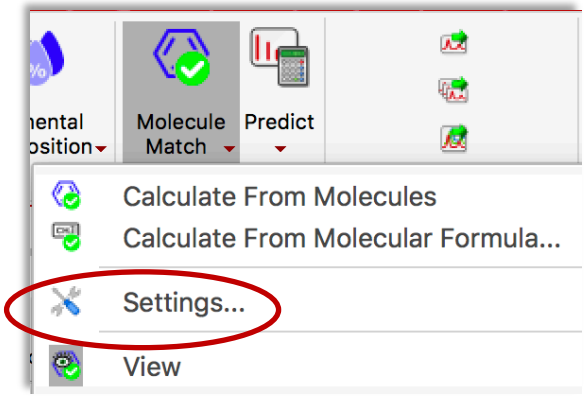
Report | Calculate | View | Settings | Setup

Mol Match Results

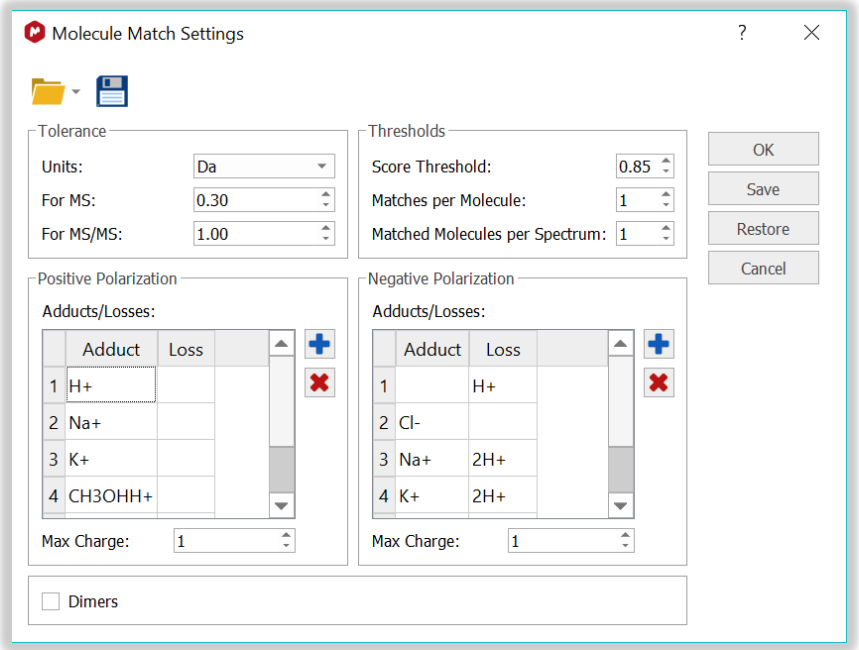
	Molecule	Formula	ecular Wei	Match	latch Score	Similarity	MS Purity	RT	Scan	Purity
1		$C_{18}H_{21}NO_2$	259.157	✓	1.000	1.000	0.756	8.02	171	10.00%
2		$C_{20}H_{23}N$	277.183	✓	1.000	1.000	0.450	8.57	180	11.39%

Confirm proposed structures using Molecule Match (2)

- You can go to **Mass Analysis/Molecule Match/Settings** to change the settings for Molecule Match
- The default settings are for low-resolution MS. Change Tolerance to 5-10 ppm if you are using high-resolution MS
- Edit the **Adducts/Losses** and other parameters, if required
- Click  to run the **Molecule Match** again

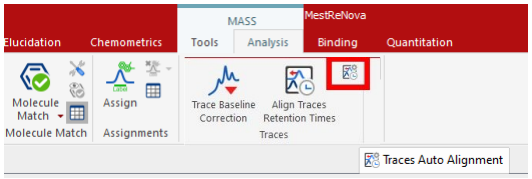


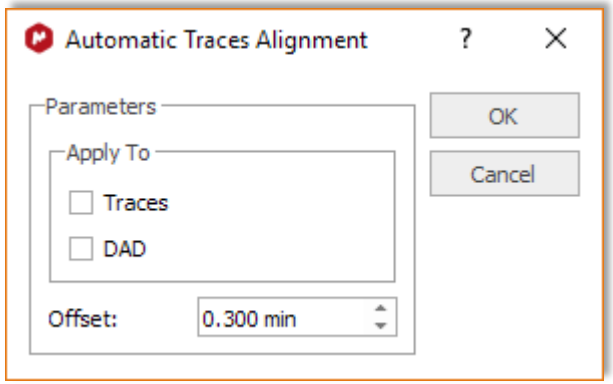
Tip: Click the "+" buttons to add a new adduct. Enter "+" for a radical cation. Highlight one and click the "x" button to remove it. Click Restore to reset to the default or previously saved settings.

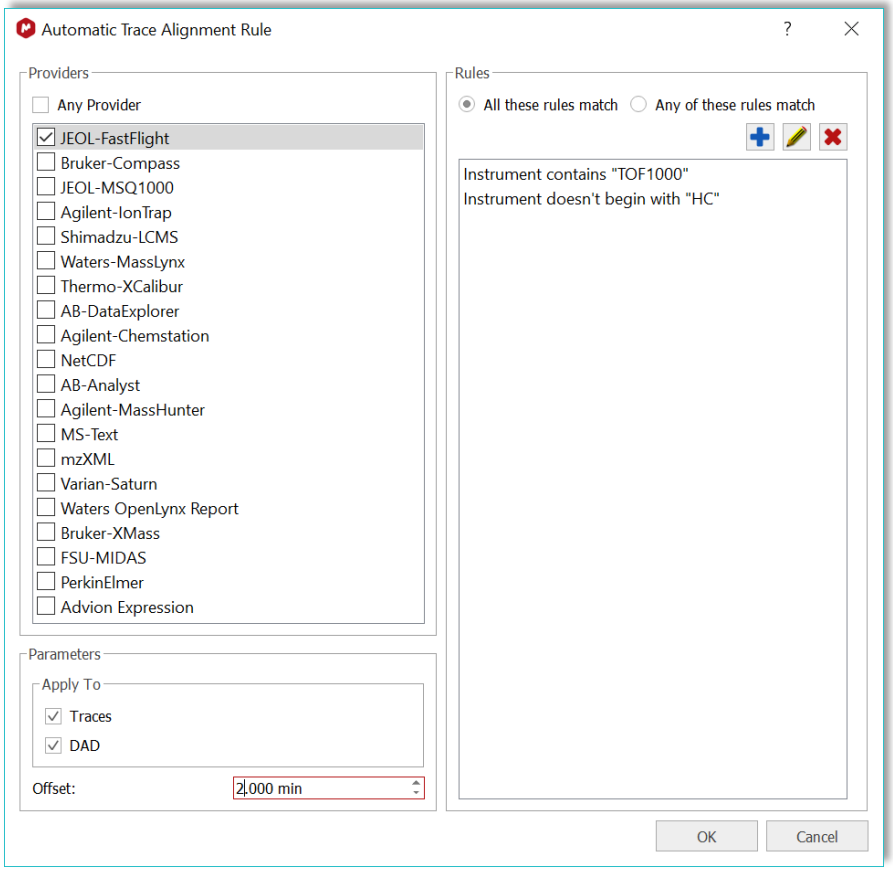


Automatic trace alignment: Instrument-specific

- Align a DAD, or another trace, to a TIC using the auto-alignment settings
- Set the rules to specifically identify the instrument and apply the correct alignment automatically

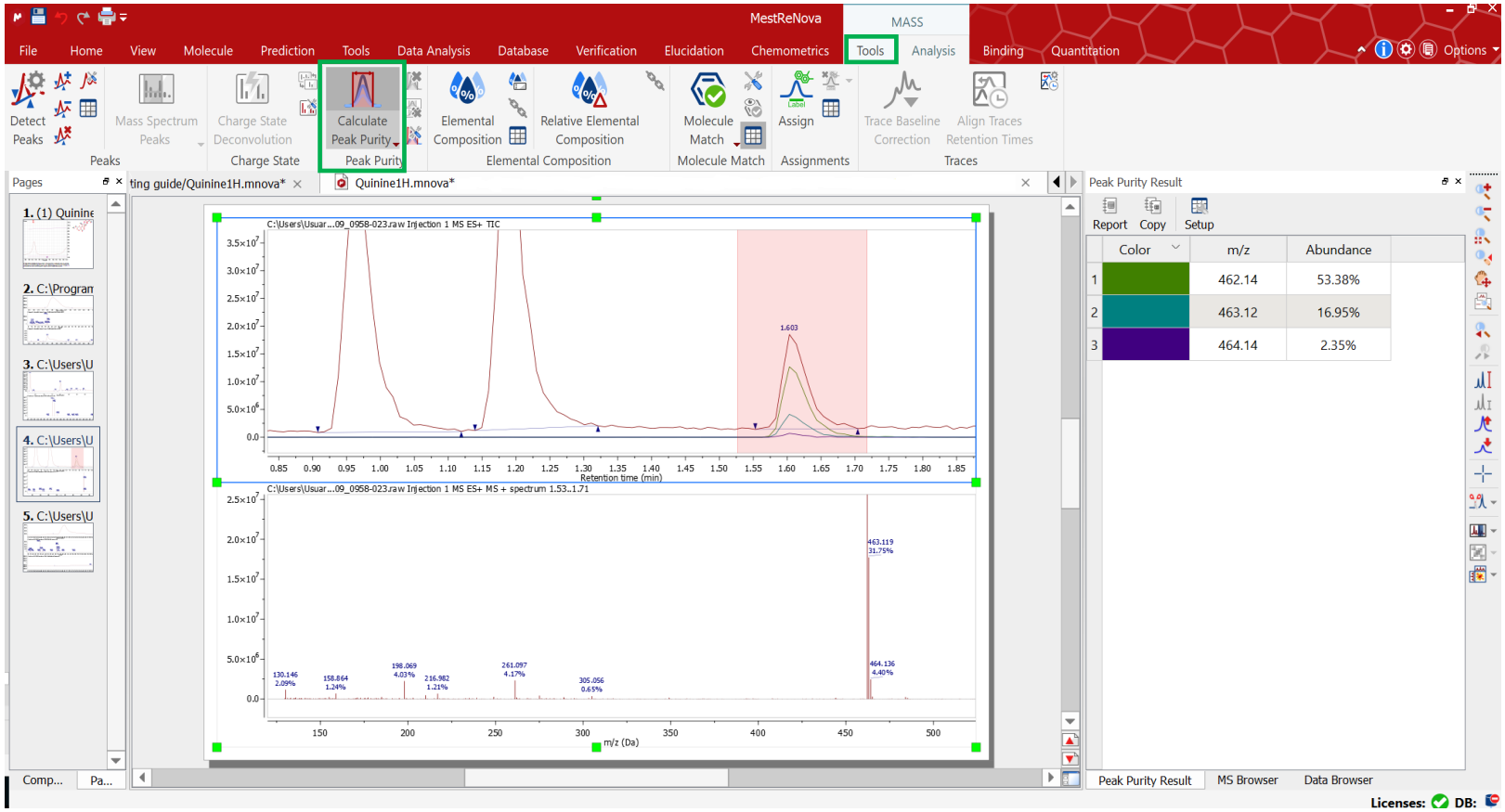






Peak purity calculation

- This shows the curves associated with the most abundant mass peaks under the selected chromatogram peak



MESTRELAB RESEARCH

This was just was just the tip of the iceberg!

For more information:

- Visit www.mestrelab.com for information about manuals, tutorials, and many more Mnova plugins
- Check **Help > Contents** in Mnova for help information
- Email support@mestrelab.com for technical questions
- Email sales@mestrelab.com for sales related queries

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thank you