Mestrelab Research

chemistry software solutions

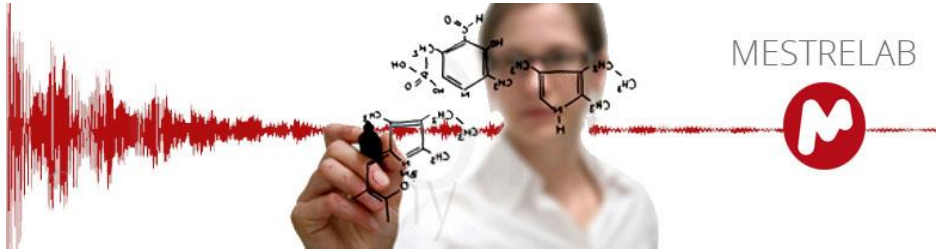
Mnova 12 – Starting guide
Includes Mnova NMR, NMRPredict, and MS

Released on 27SEP2017

Mestrelab Research
chemistry software solutions

Outline to this starting guide

- Overview of Mestrelab and Mnova
- 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

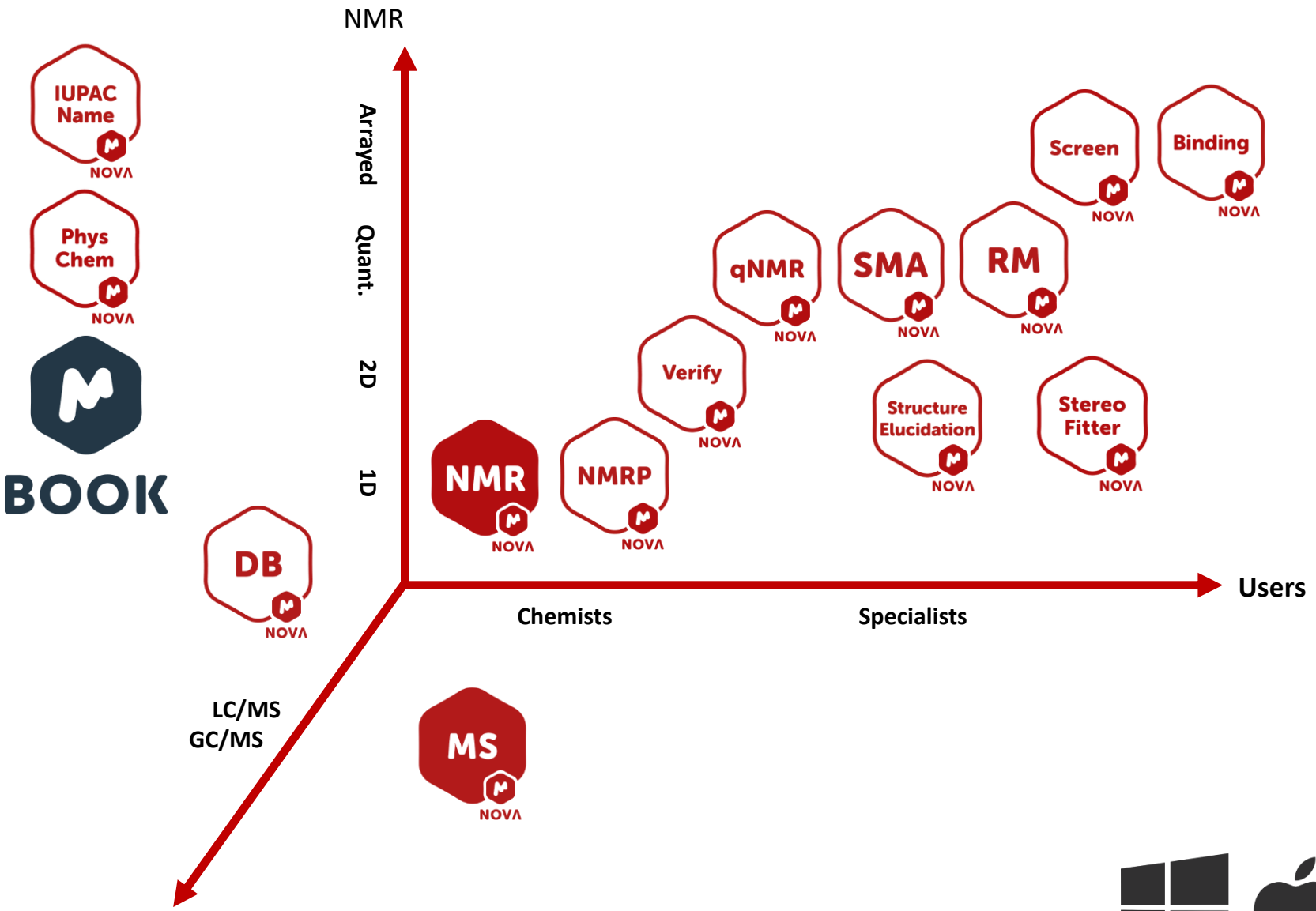


Note: This tutorial covers only the NMR, NMRPredict and Msplugins of Mnova

A Fast Growing Company

- 1996: A research project in University of Santiago de Compostela, Spain, developed a free Software, **MestReC**, for NMR processing.
- 2004: **Mestrelab Research** incorporated in Santiago de Compostela.
- 2004: New **MestReNova (Mnova)** platform and **NMR** plugin released.
- 2006: **NMRPredict Desktop** for NMR prediction.
- 2009: **MS** plugin for LC/GC/MS data analysis.
- 2009: **Global Spectral Deconvolution (GSD)** algorithm released for NMR.
- 2011: **DB** plugin for Database Management of NMR and MS.
- 2012: **Verify** plugin for auto structure verification.
- 2012: **qNMR** plugin for quantitative NMR analysis.
- 2013: **Reaction Monitoring** plugin for NMR-based reaction kinetics studies.
- 2014: **Screen** plugin for high-throughput ligand-protein binding analysis.
- 2015: **SMA** (plugin for simple mixture analysis) **Mbook (ELN)**, **Mnova app for tablets.**
- 2016: The office in Santiago doubles in size with a glorious table football!
- 2017: **Mnova 12**, **Structure Elucidation, Binding, IUPAC Name.**
- An R&D company with over 35 people and >80,000 registered users.

Mnova products and applications



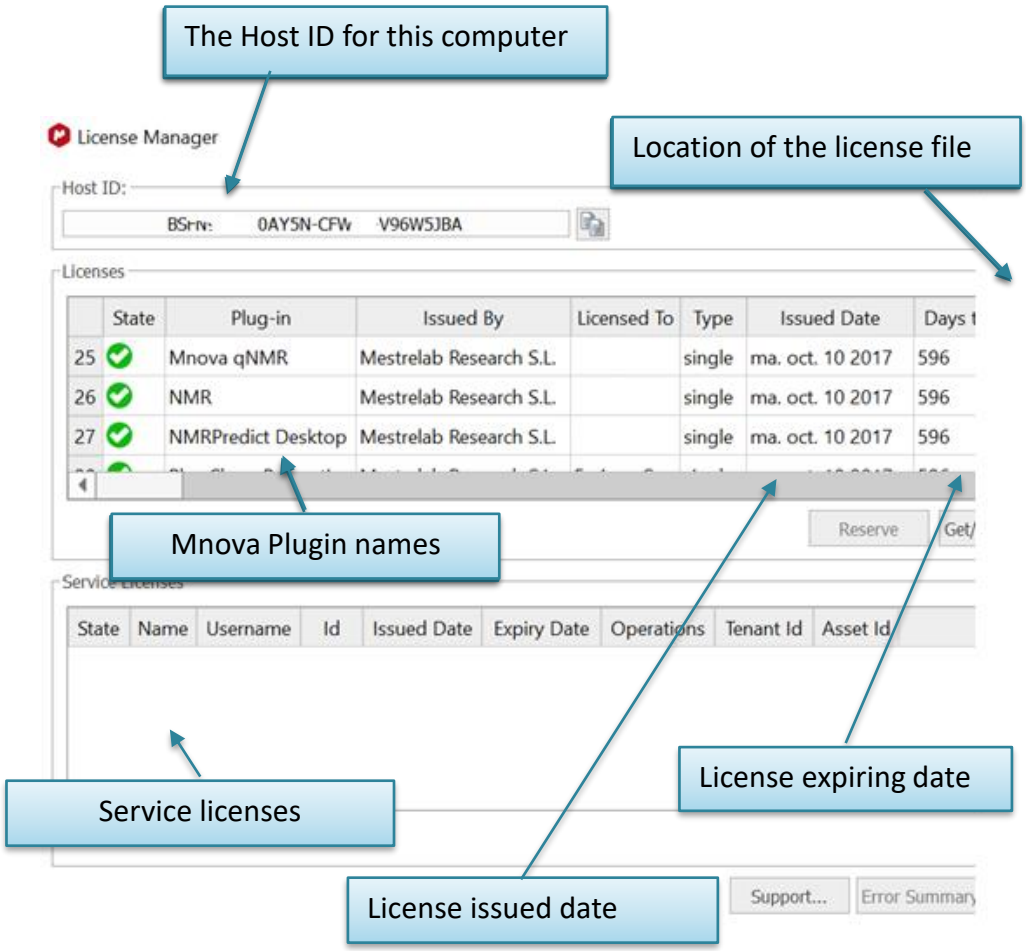
Note: This tutorial covers only the NMR, NMRPredict and MS plugins of Mnova

Mestrelab Research
chemistry software solutions

INSTALLATION

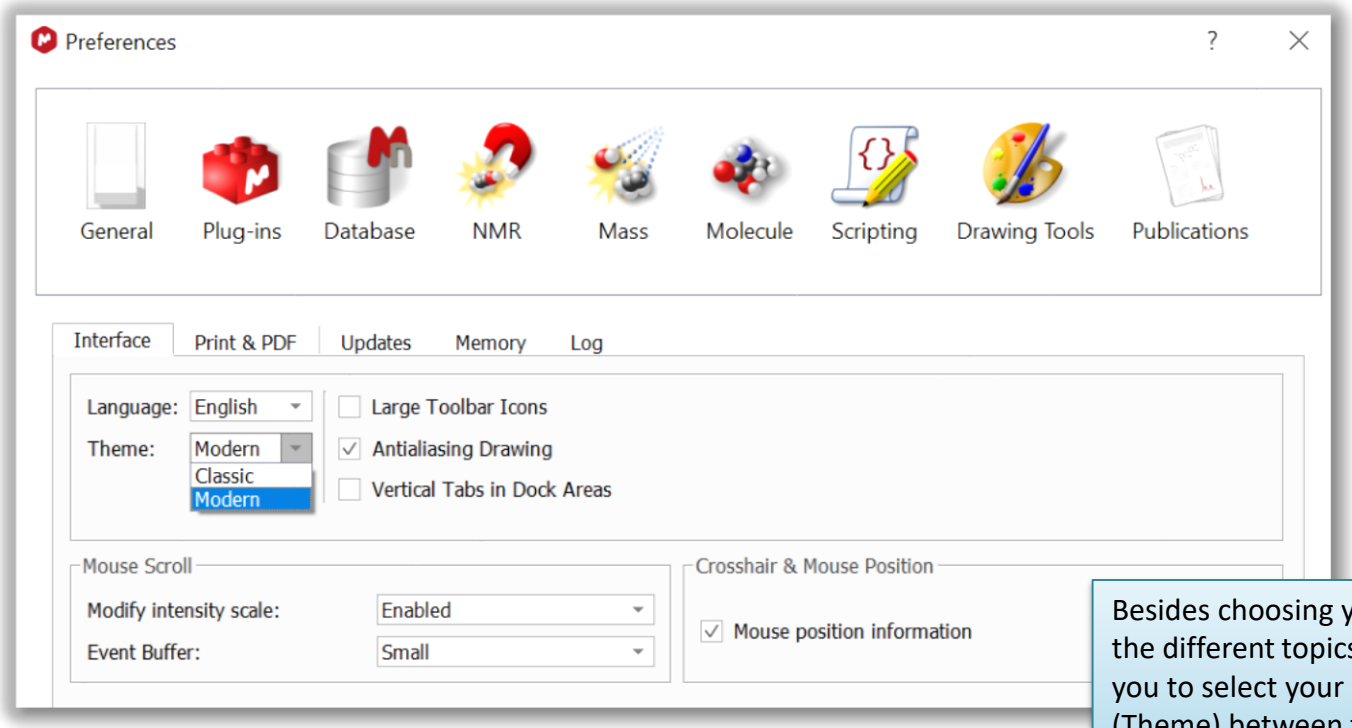
Download and activate your Mnova license

- Download and install Mnova www.mestrelab.com. Choose **Help > License Manager** to open the License Manager dialog.
- Activate Mnova using your purchased license files, or apply for 45 day free trial licenses (Click ***Get/Install Licenses***)
- Make sure that there are green checkmarks for NMR and other plugins that are supposed to be activated
- For managing campus/site/ concurrent licenses, see here



Mnova preferences

Mnova allows changes to Mnova's interface options for Plugins, Database, NMR, MS, Molecule, Scripting and Drawing Tools. They can be edited in **'File/Preferences'**



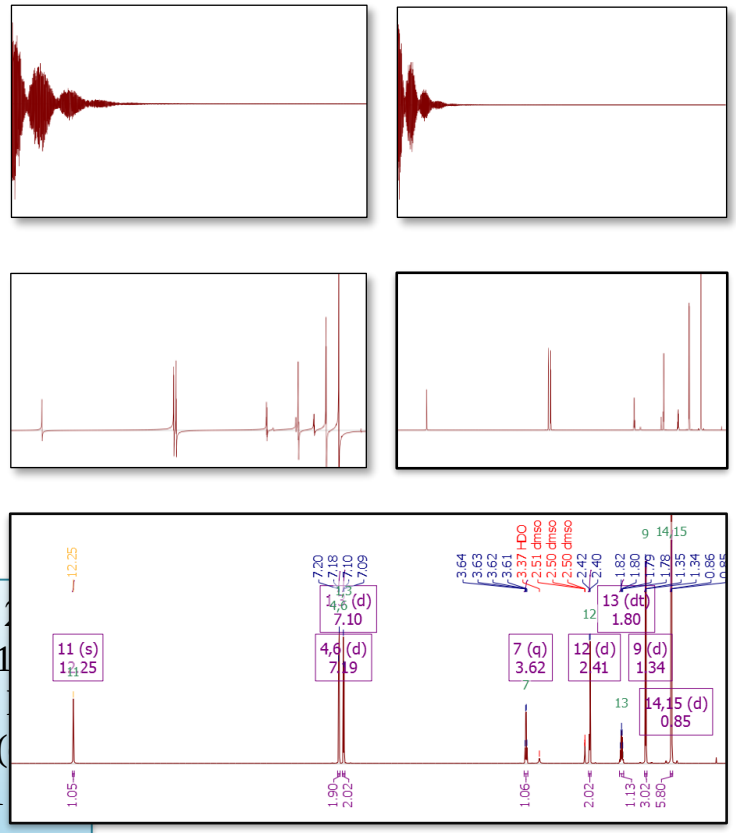
Besides choosing your preferences on the different topics, Mnova 12 allows you to select your preferred interface (Theme) between the classic or the new ribbon control.

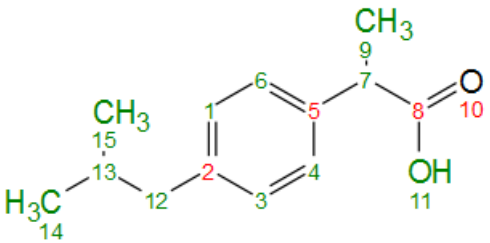
Mestrelab Research
chemistry software solutions

PROCEDURE

^{1}H processing and analysis: General procedure

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





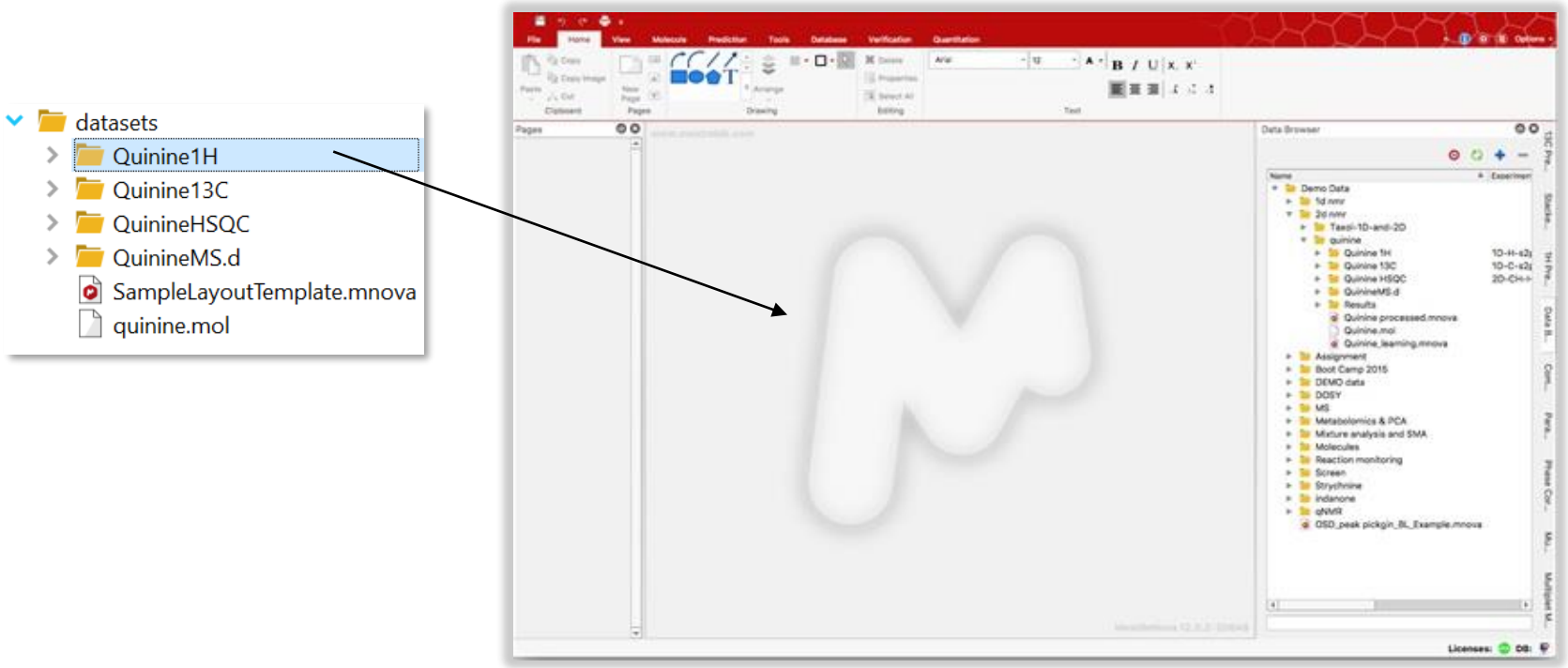
^{1}H NMR (600 MHz, DMSO-d_6) δ 1
(s, 1H), 7.19 (d, J = 7.8 Hz, 2H), 7.1
(d, J = 7.9 Hz, 2H), 3.62 (q, J = 7.1
1H), 2.41 (d, J = 7.2 Hz, 2H), 1.80 (
= 13.5, 6.8 Hz, 1H), 1.34 (d, J = 7.1
3H), 0.85 (d, J = 6.7 Hz, 6H).

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

Mnova comes with example datasets

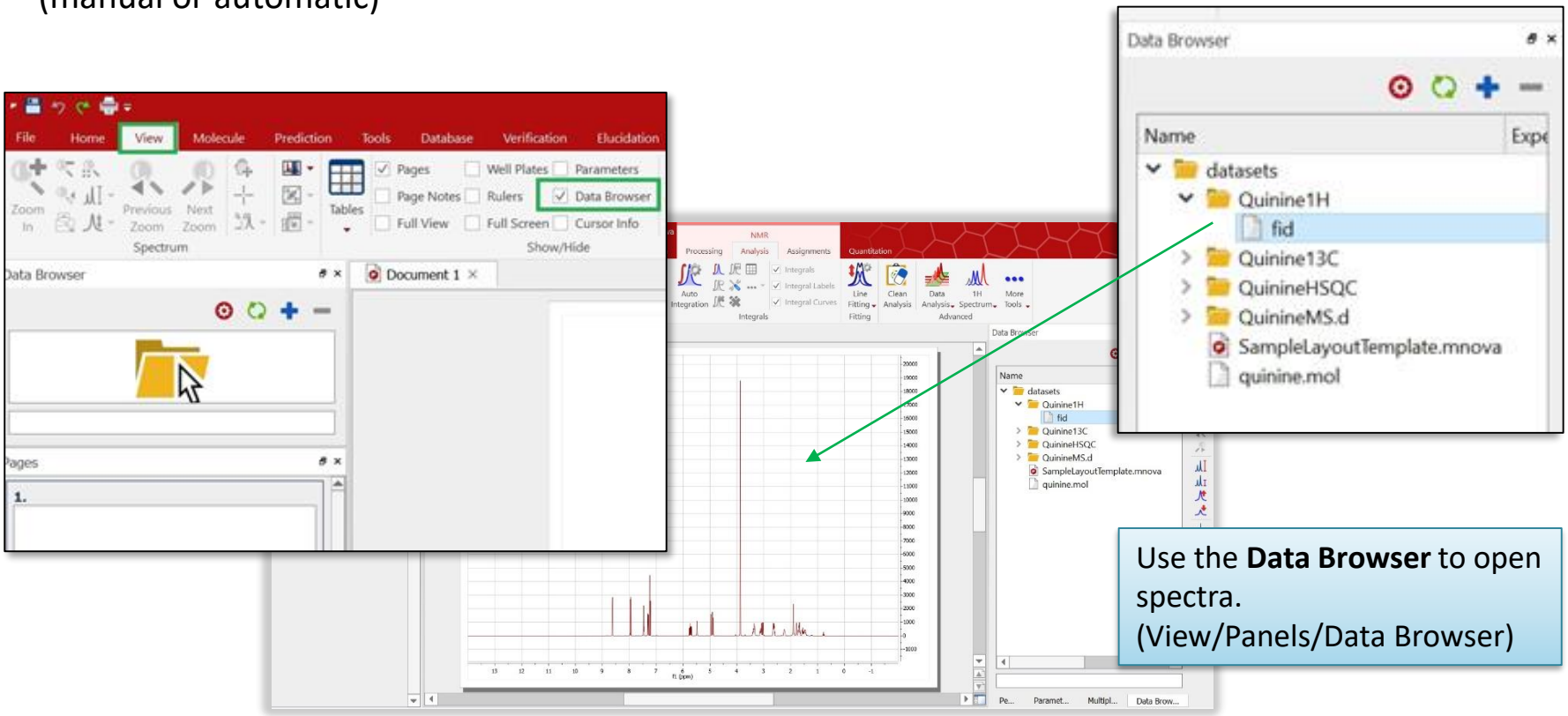
- An installation of Mnova comes with a set of 1D and 2D NMR, LC/MS data, and the structure of quinine for practice. On Windows, they are typically located in **C:\Program Files (x86)\Mestrelab Research S.L\MestReNova\examples\datasets**

- Drag the folders or individual files into Mnova to open these practical example



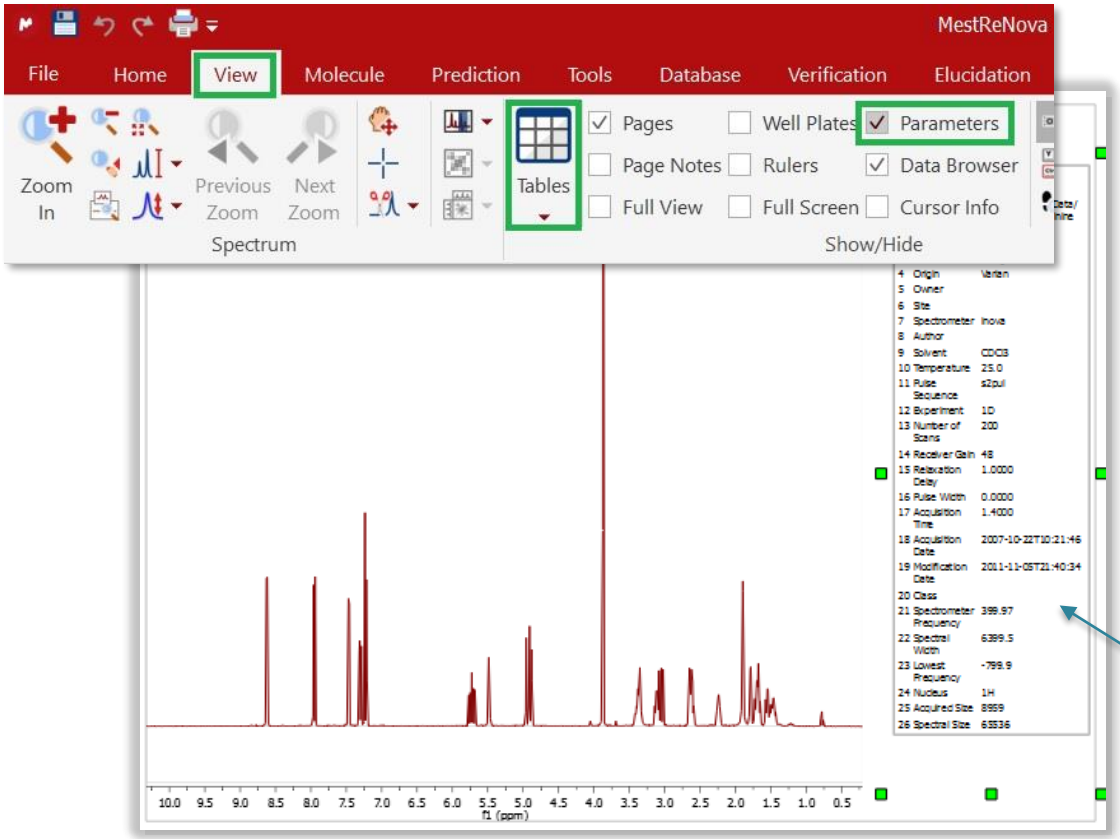
Open and transform NMR data

- Go to **File/Open** to open the **fid** (or **ser**) file of the raw data
- Or, drag & drop a folder from the **Data Browser** onto the Mnova canvas
- Mnova will automatically process the spectrum
- All data is brought in, and depending on your preferences, is processed to the desired extent. (manual or automatic)



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

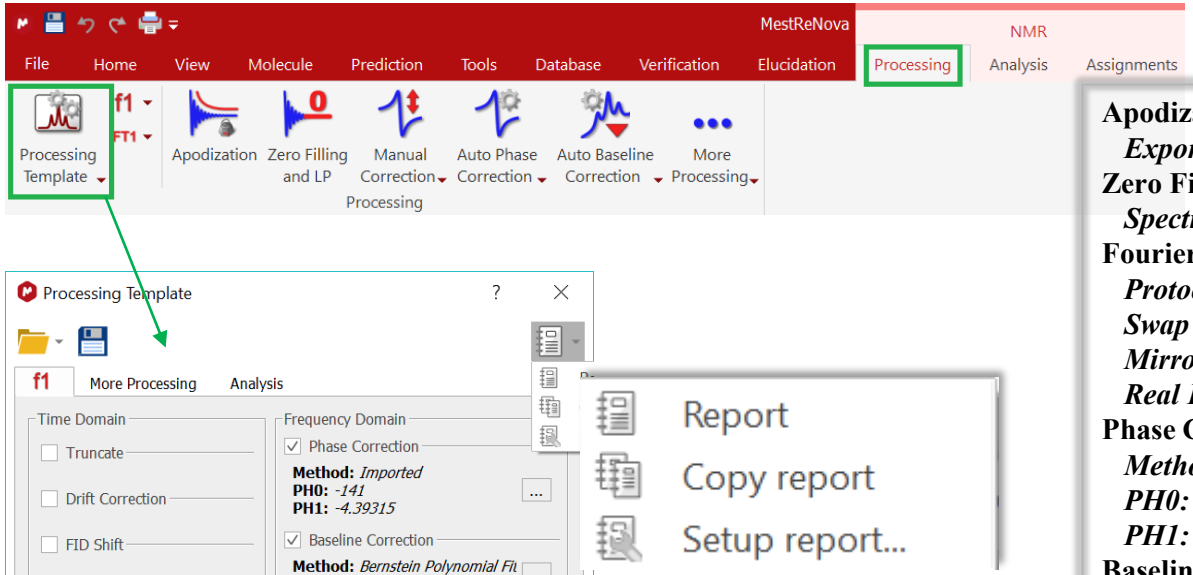
Report | Copy | Setup | Customize

	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

- A report of the Processing Parameters can be generated using your preferred report template for 1D and 2D spectra. A customized template can be easily added

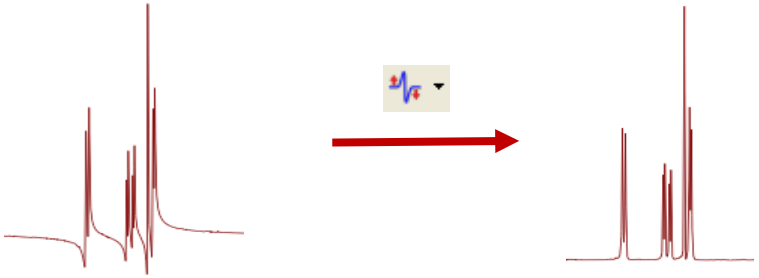


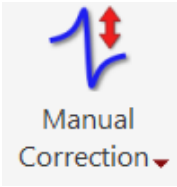
Apodization
Exponential: *0.50 Hz*
Zero Filling and LP
Spectrum Size: *65536*
Fourier Transform
Protocol: *None*
Swap Halves: *true*
Mirror Image: *true*
Real FT: *false*
Phase Correction
Method: *Imported*
PH0: *-141.00 °*
PH1: *-4.39 °*
Baseline Correction
Method: *Bernstein Polynomial Fit*
Polynomial Order: *3*

Peak Picking
Method: *GSD*
Refinement: *Ref1*
Auto Classify: *true*
Multiplet Analysis
Method: *Peaks*
Minimum Area: *0 %*

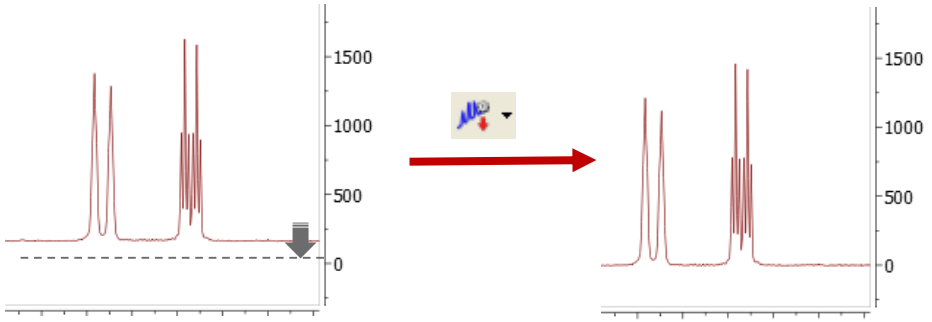
Phase, baseline correction & reference

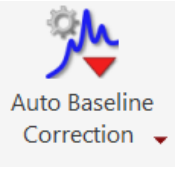
- Press  for **phase correction** if peaks are not symmetric*



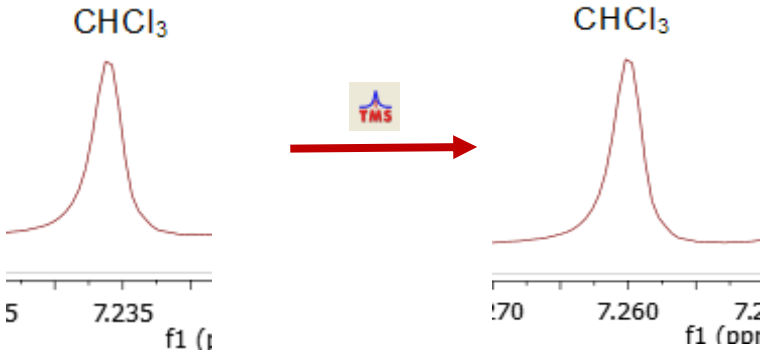


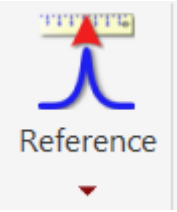
- Press  for **baseline correction** if baseline is not zero*





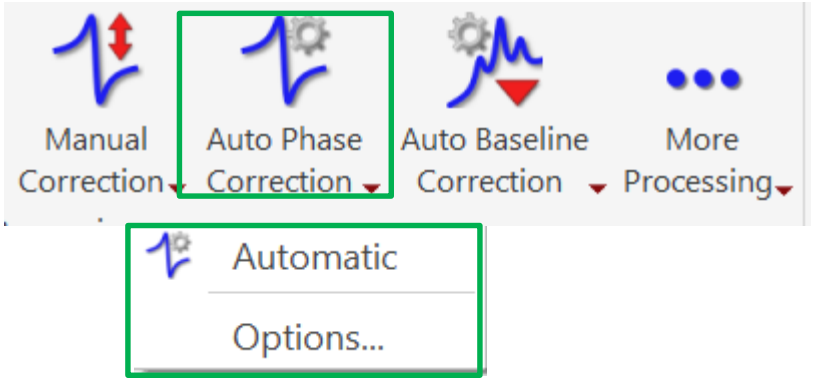
- Press  to calibrate the **chemical shift reference** if the solvent or TMS peak is not at the right ppm

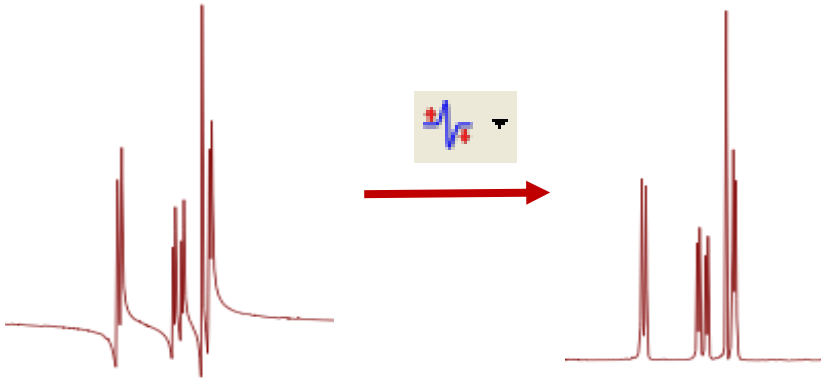


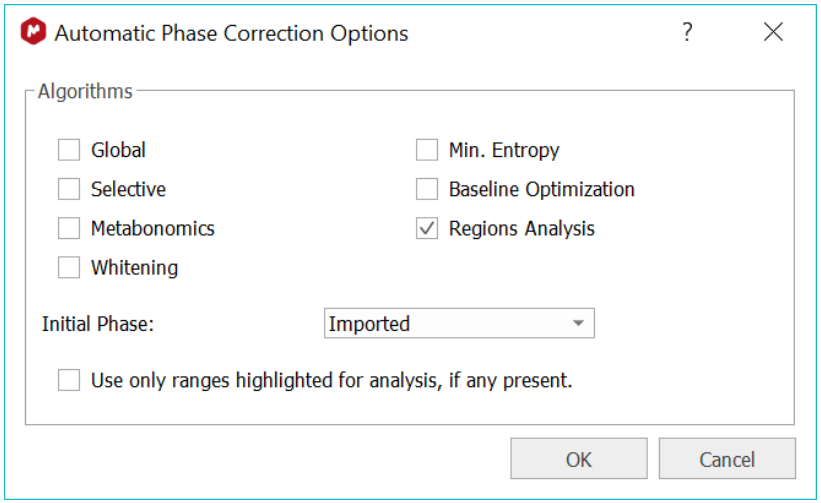


Click the arrow next to the tool icon for options, such as manual phasing and manual baseline correction.
*See **Help > Contents > Processing Basics** for more details.*

Automatic phase correction

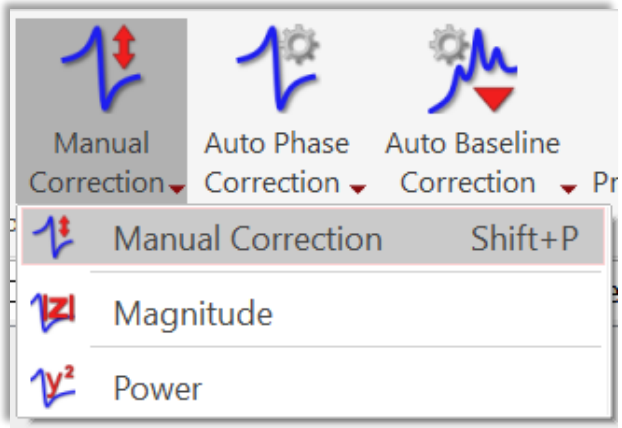


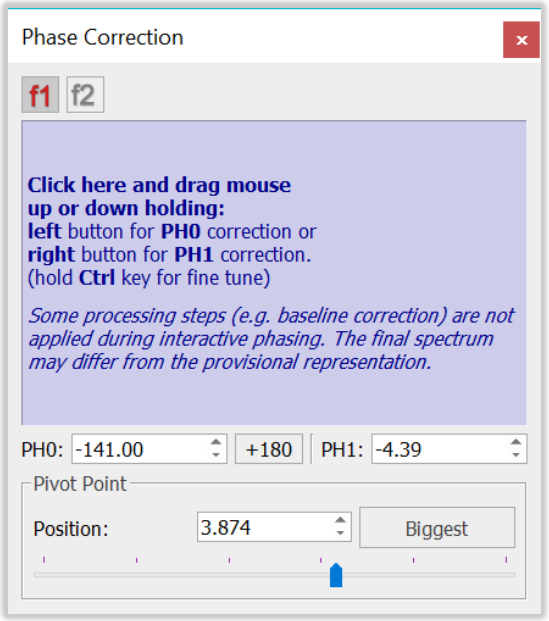


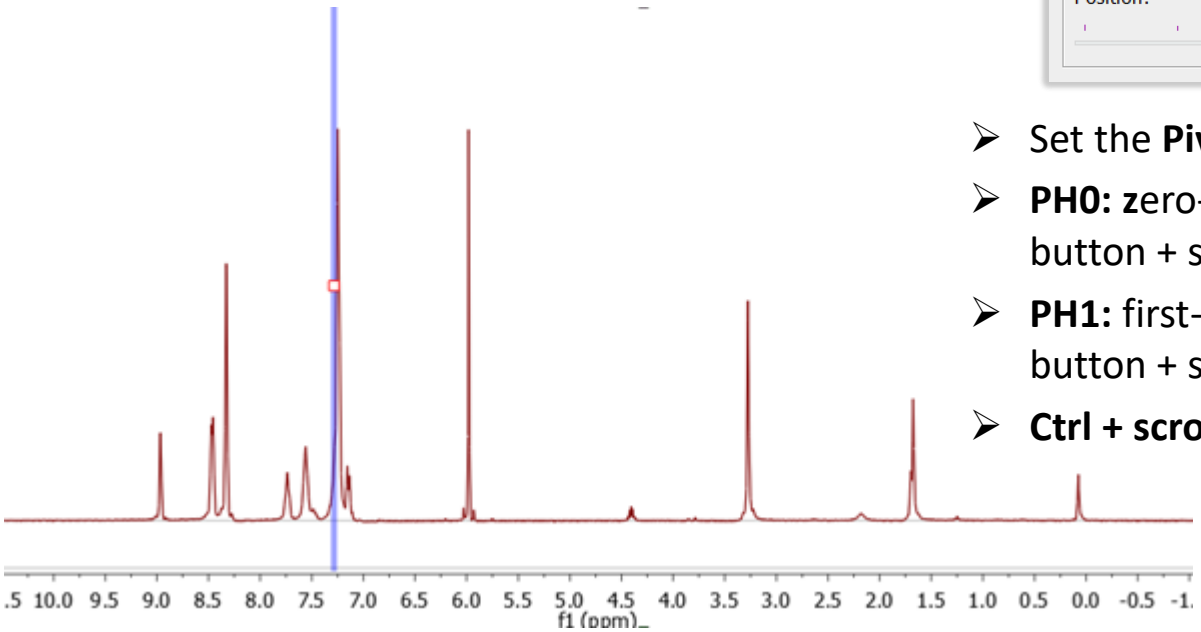


- **Regions Analysis:** *good for most cases*
- **Global:** good for spectra without negative and big solvent peaks
- **Selective:** DEPT type of spectra with negative peaks
- **Metabonomics:** spectra with big solvent peaks
- **Whitening**: usually for 2D

Manual phase correction

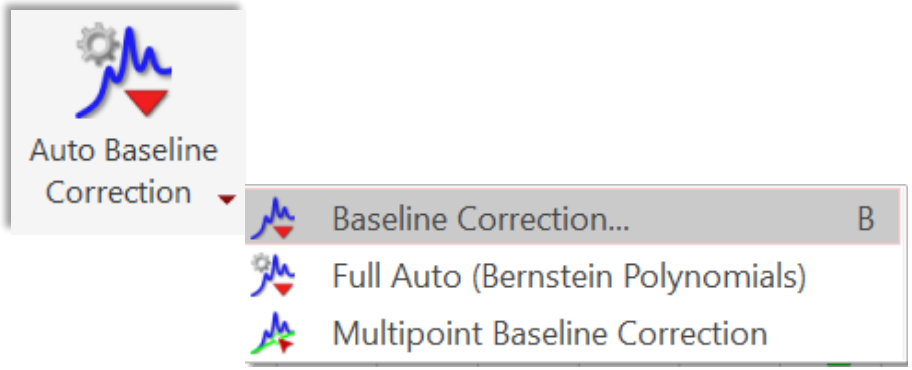


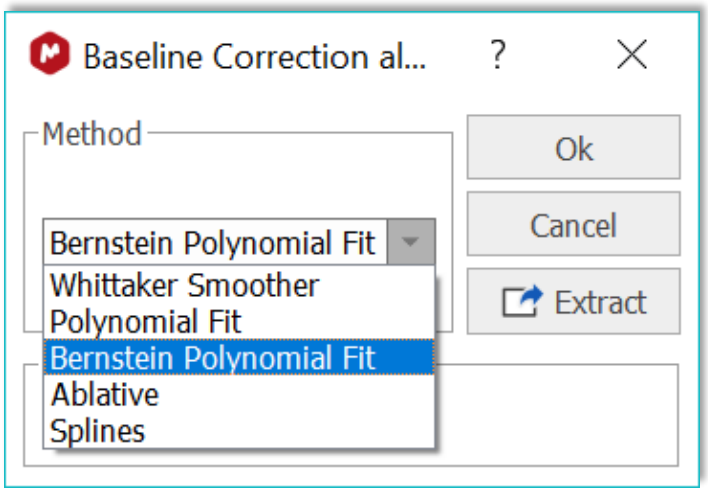


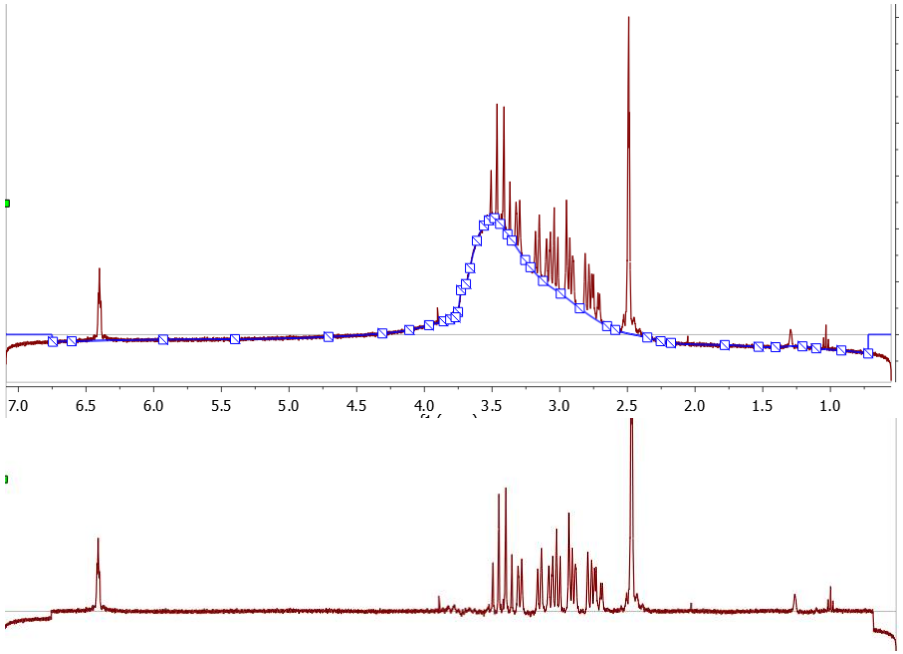


- Set the **Pivot Point**
- **PH0: z**ero-order correction (left mouse button + scroll up/down)
- **PH1:** first-order correction (right mouse button + scroll up/down)
- **Ctrl + scrolling:** fine tuning

Baseline correction



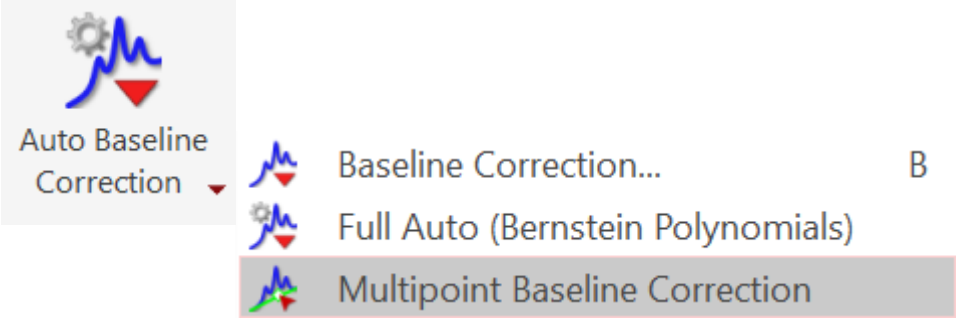




Choose a function to model the baseline:

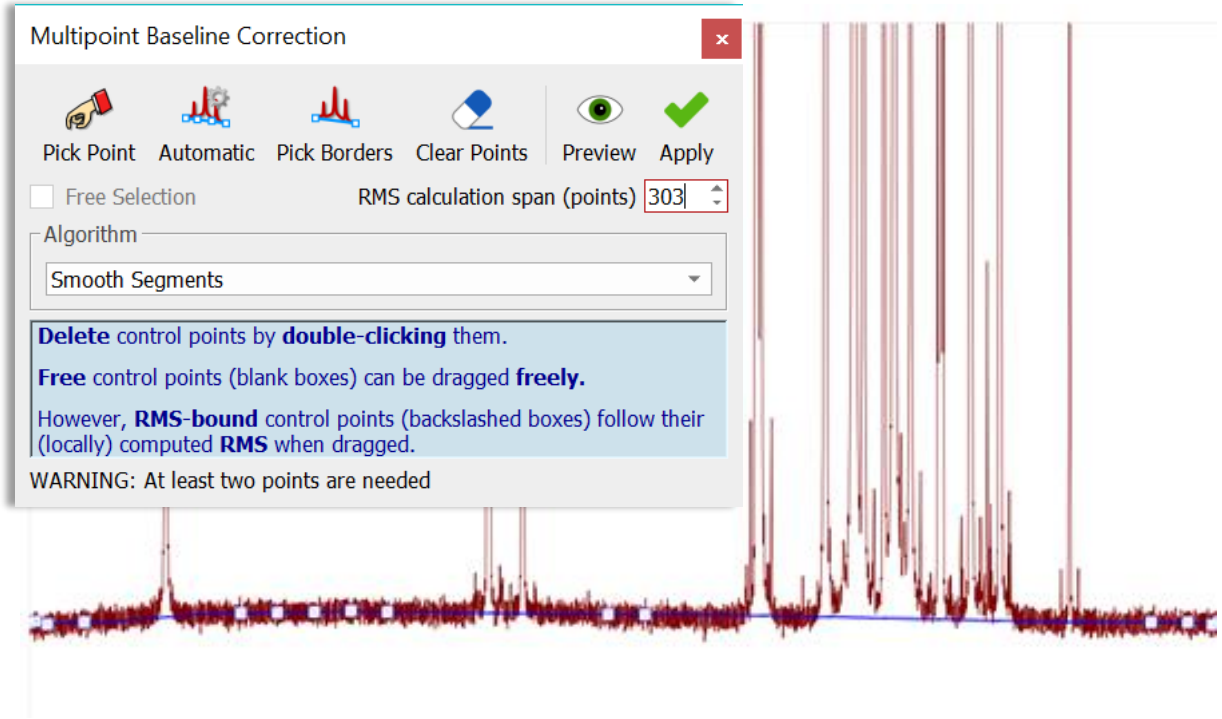
- **(Bernstein) Polynomial Fit: s**mall base errors
- **Splines or Ablative:** for medium base errors
- **Whittaker:** For more serious base errors. Use with caution and make sure the bases of peaks are not compromised. Use appropriate parameter values to tune the fit
- **Multipoint B.C.**: Manually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

Multipoint baseline correction



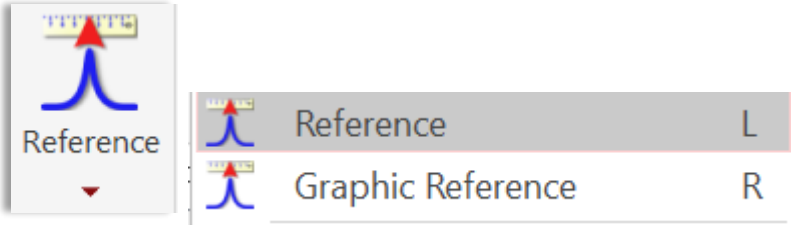
Improves the automatic detection of the control points

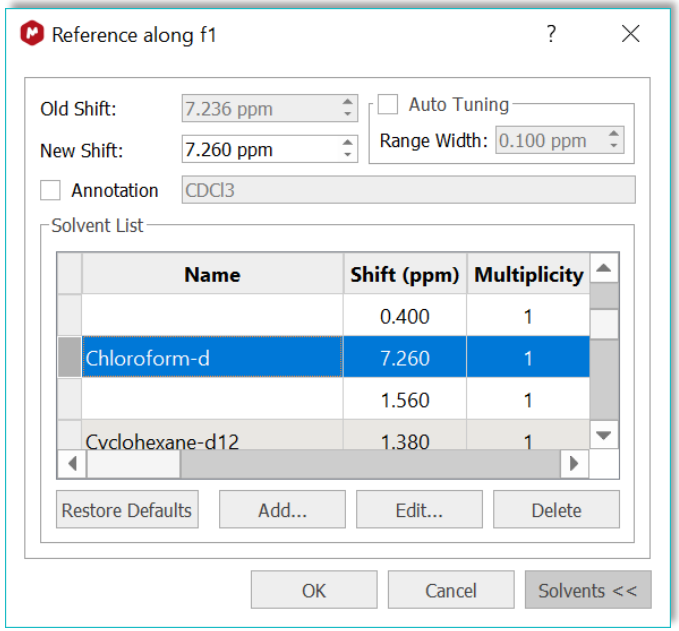
It estimates the noise regions and finds a lower number of control points for them



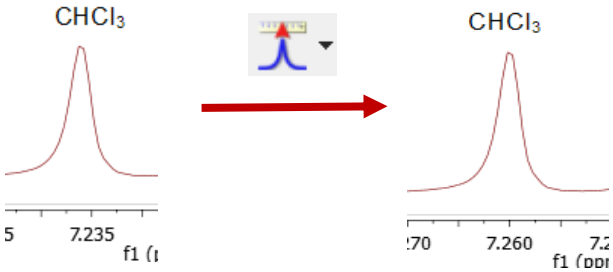
Referencing chemical shifts

ANALYSIS

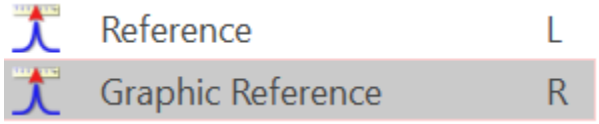


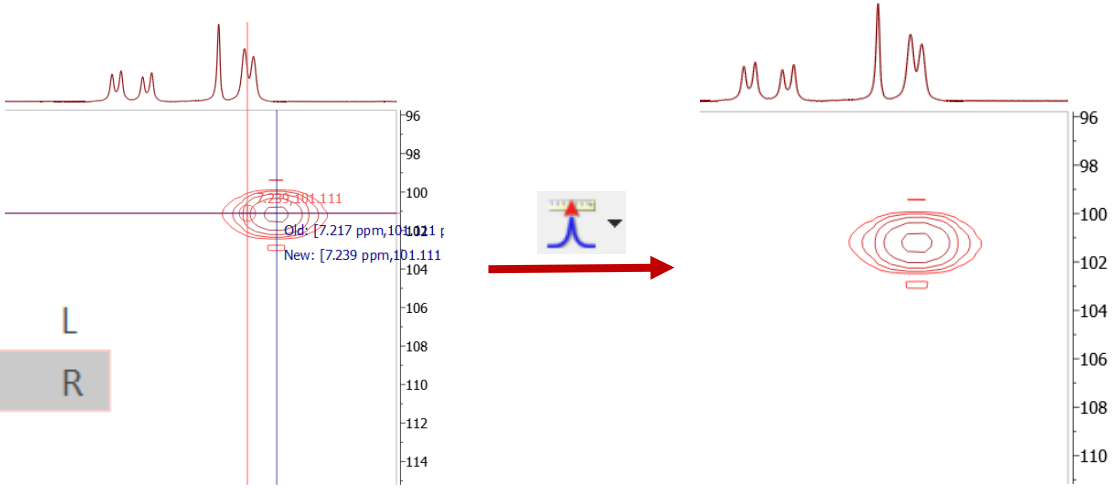


Reference by entering the value



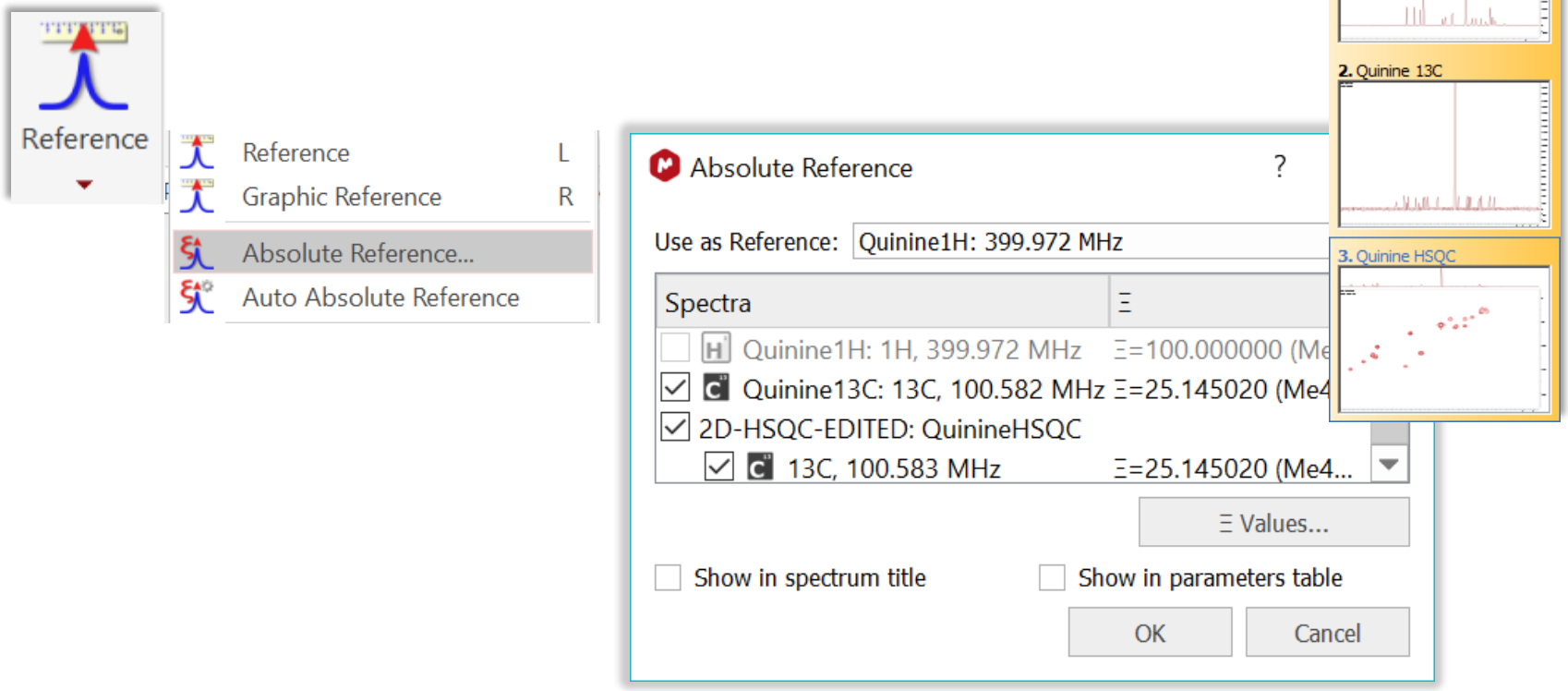
Graphic Reference
(two clicks)





Absolute Reference for automatically referencing multi-spectra/nuclei

- Use a referenced ^{1}H from the same instrument/probe/solvent/temperature
- Auto references other nuclei
- Auto-references other spectra (1D and 2D)
- Saves settings in **Preferences** to do it automatically

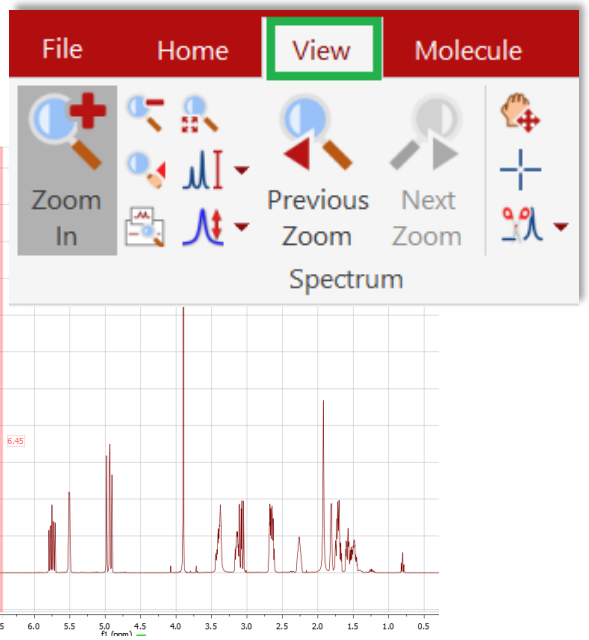


Mestrelab Research
chemistry software solutions

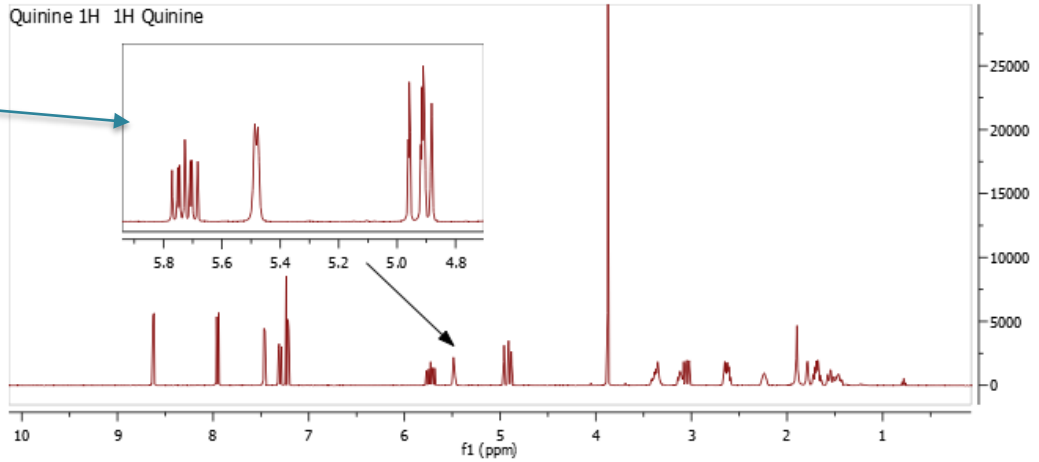
Visualize your spectrum

DISPLAY OPTIONS

- 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&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

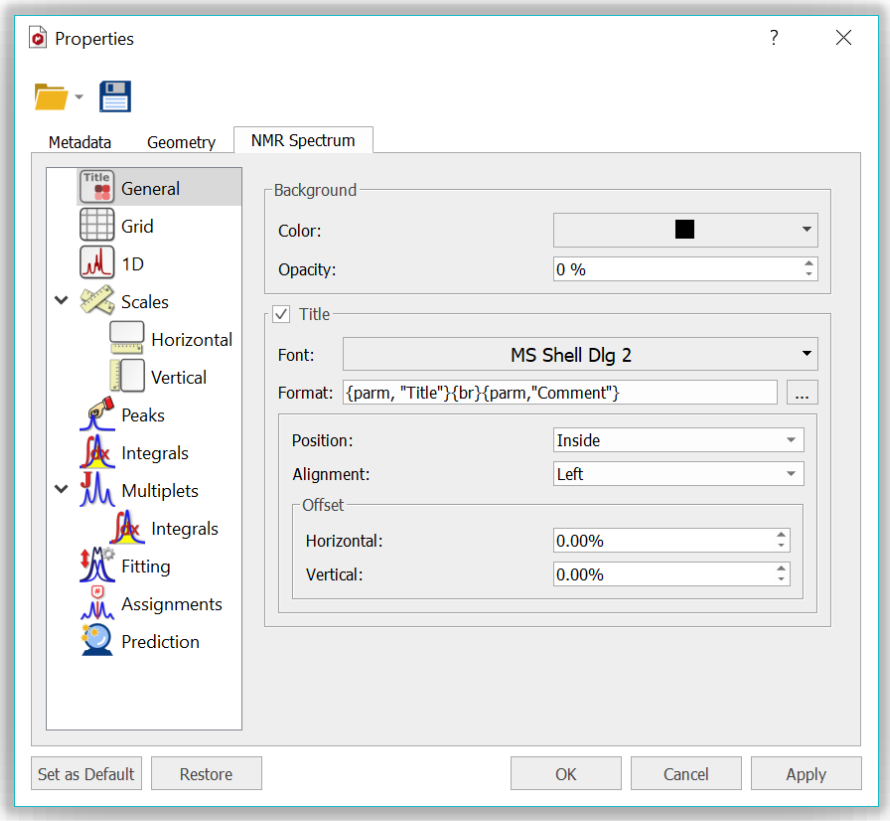


Press **Z several times to toggle between horizontal/vertical/box zoom*
*** Press **P** several times to toggle between free/horizontal/vertical panning*

Change spectrum display properties

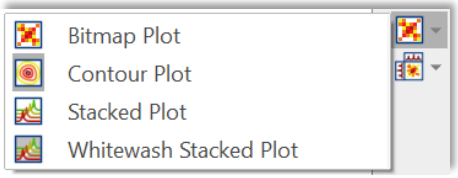
- Double click on the background of a spectrum to open the **Properties** dialog
- Display properties are divided into logical sections
- Click on Set as Default to save settings for spectra opened in the future

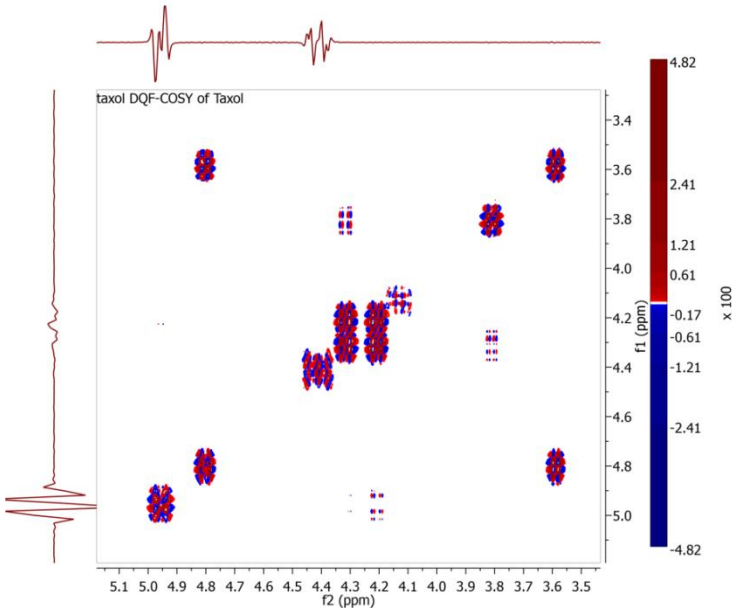
*Tip: Use the **Save** tool to save the properties to a file, and distribute it to other users for consistent display & reporting*

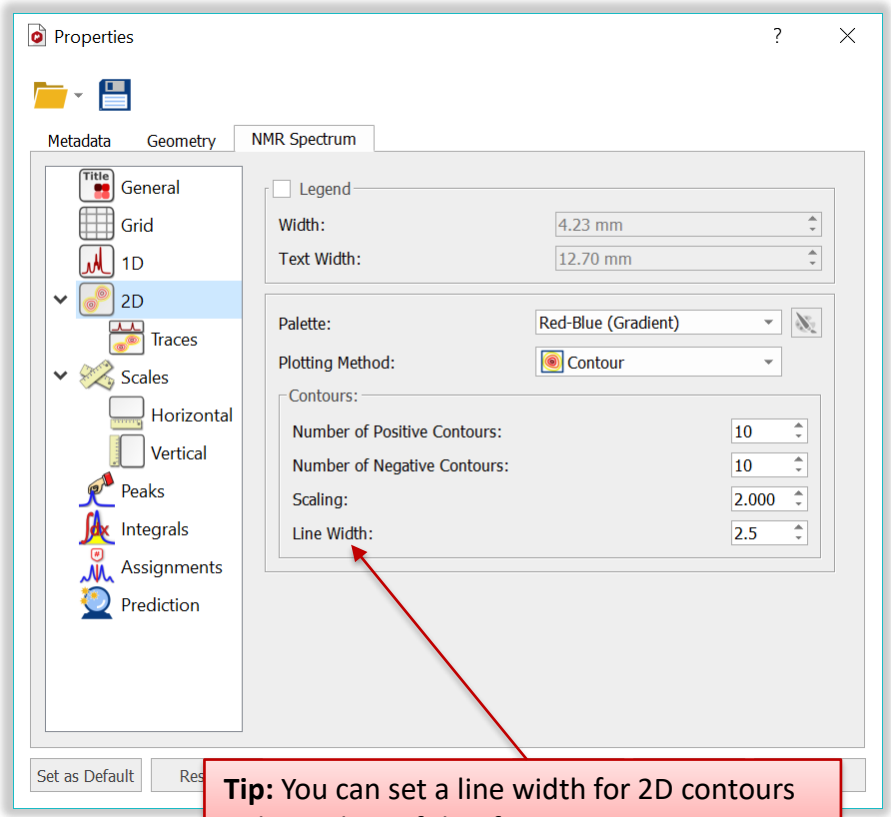


Display of 2D spectra

- Use the **Plot Mode** tools to change to bitmap or contour display, etc.
- Change other display properties by double-clicking on the spectrum to open the **Properties** dialog:
  - Legend
  - Color Palette
  - Contours
  - Traces





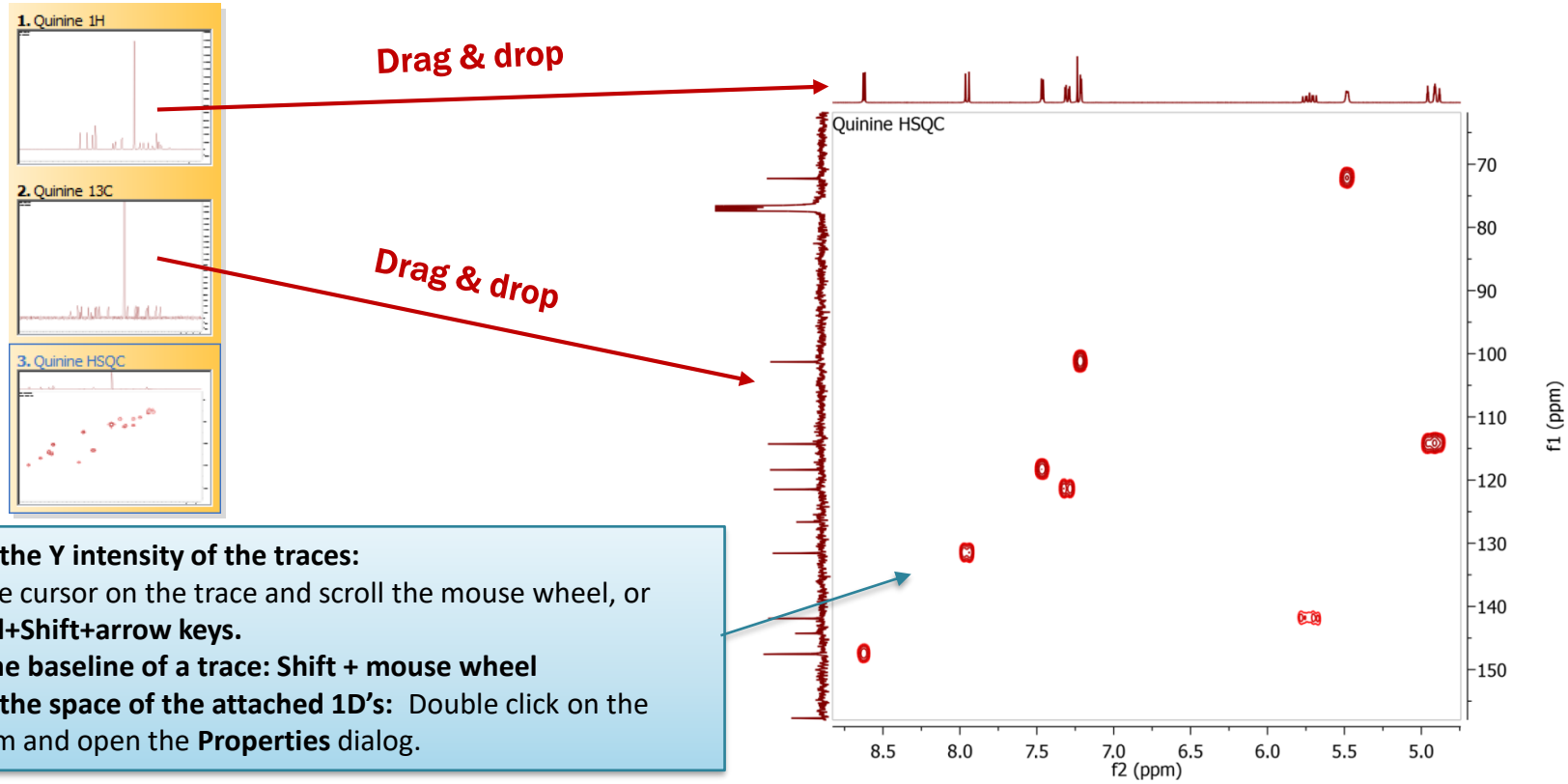


Tip: You can set a line width for 2D contours independent of that for 1D curves.

Attach 1D to 2D spectra

- Open 1D and 2D spectra in the *same* document. They are displayed in separate pages.

  If you don't see the **Pages** panel, choose **View/Pages**
- Select a 2D spectrum, then drag a 1D from the Pages panel to attach it to the 2D as an external trace
- It can be done automatically through **File/Preferences/NMR**

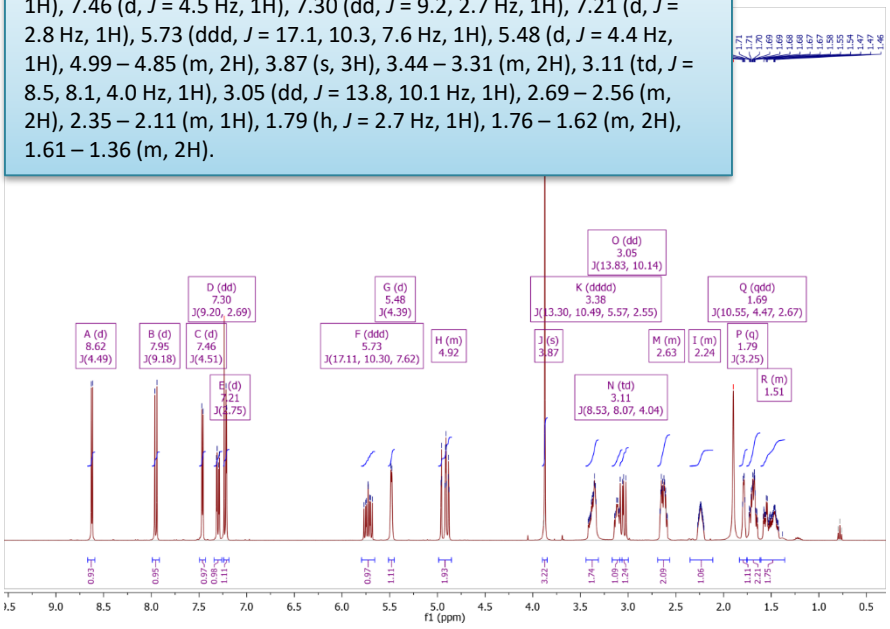


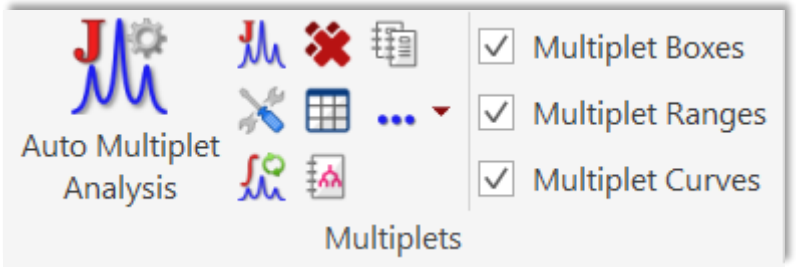
Change the Y intensity of the traces:
Place the cursor on the trace and scroll the mouse wheel, or click **Ctrl+Shift+arrow keys.**
Move the baseline of a trace: Shift + mouse wheel
Change the space of the attached 1D's: Double click on the spectrum and open the **Properties** dialog.

Analyze and report multiplets of ^{1}H NMR

- Mnova provides two approaches to **multiplet analysis:**
  - **Fully automatic:** peak picking, integration and multiplet analysis *all done by one click*, with peaks deconvolved using GSD* and types classified
  - **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 8.62 (d, *J* = 4.5 Hz, 1H), 7.95 (d, *J* = 9.2 Hz, 1H), 7.46 (d, *J* = 4.5 Hz, 1H), 7.30 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.21 (d, *J* = 2.8 Hz, 1H), 5.73 (ddd, *J* = 17.1, 10.3, 7.6 Hz, 1H), 5.48 (d, *J* = 4.4 Hz, 1H), 4.99 – 4.85 (m, 2H), 3.87 (s, 3H), 3.44 – 3.31 (m, 2H), 3.11 (td, *J* = 8.5, 8.1, 4.0 Hz, 1H), 3.05 (dd, *J* = 13.8, 10.1 Hz, 1H), 2.69 – 2.56 (m, 2H), 2.35 – 2.11 (m, 1H), 1.79 (h, *J* = 2.7 Hz, 1H), 1.76 – 1.62 (m, 2H), 1.61 – 1.36 (m, 2H).

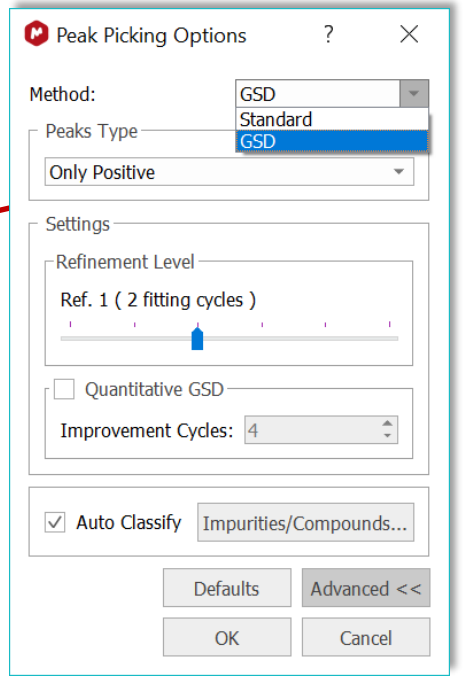


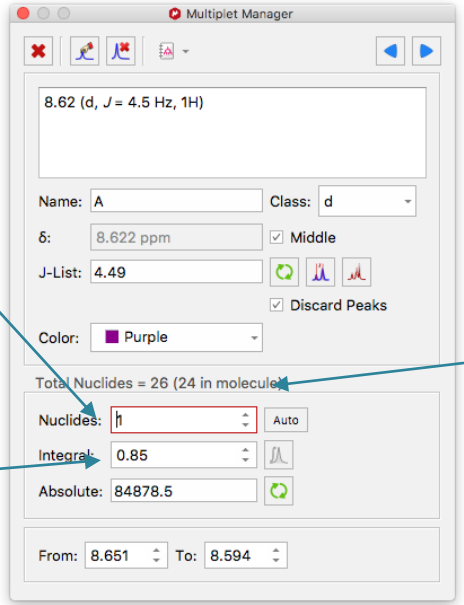


**GSD (Global Spectral Deconvolution): See Help > Contents > Analysis tools > Peak Picking > GSD for details.*

Fully automatic multiplets analysis

- Click  to do 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 controlled by **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

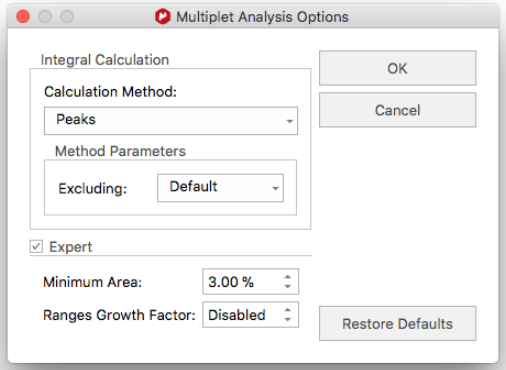




The number of nuclides (NN) of the multiplet

Normalized integral of the multiplet.

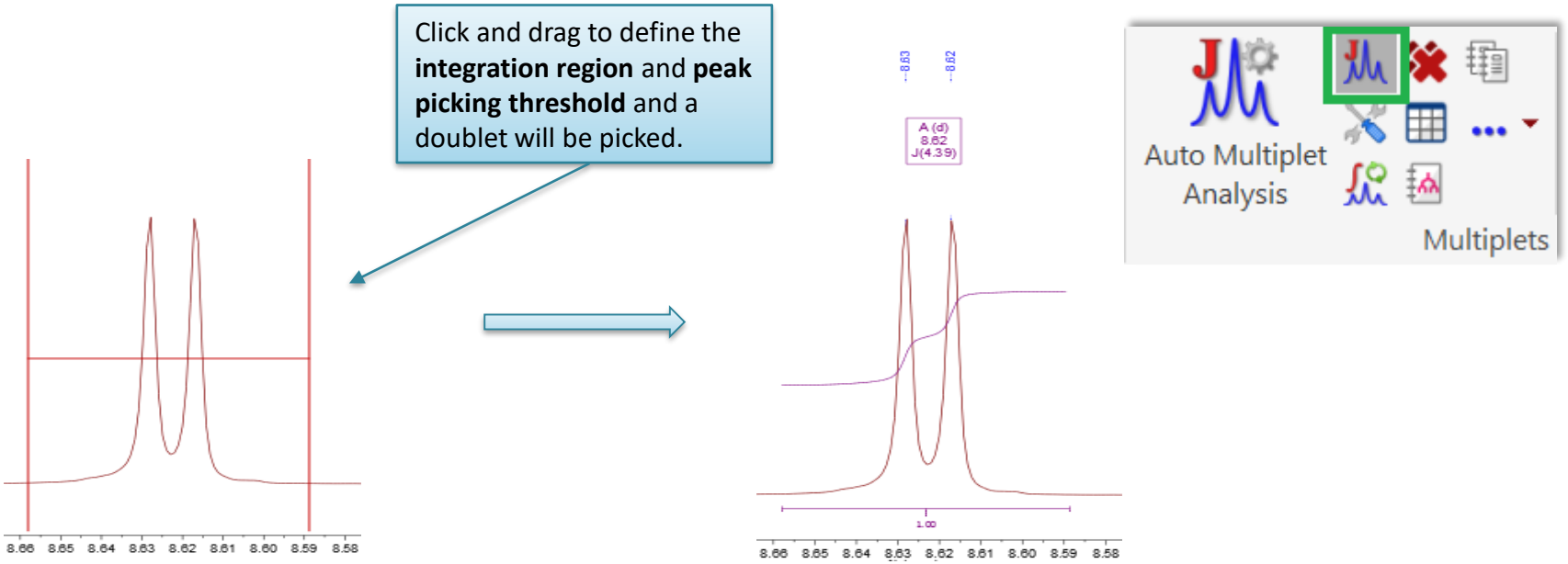
Total # of nuclides from all the multiplets and the # of protons in the molecule (if present)



***GSD (Global Spectral Deconvolution):** See Help > Contents > Analysis tools > Peak Picking > GSD for details*

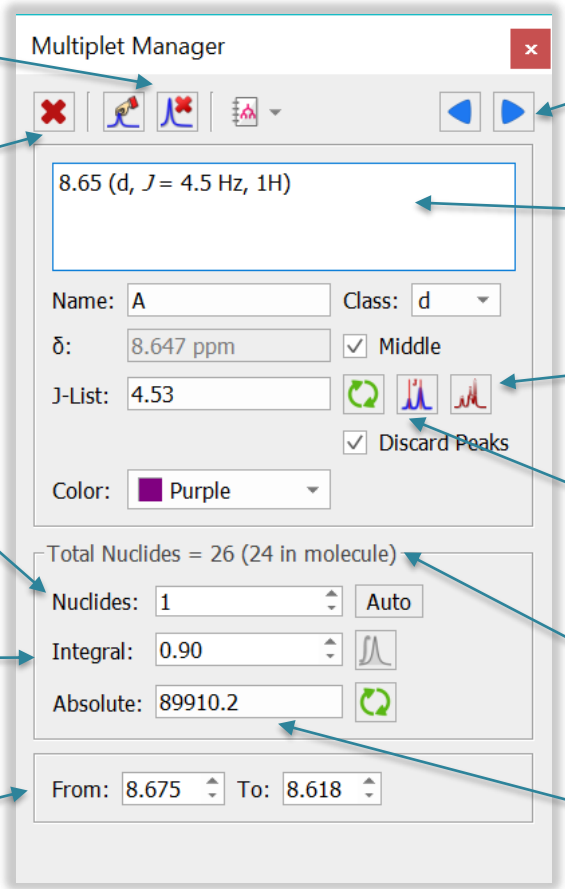
Pick multiplets manually

- Manual Multiplet Analysis offers more control (**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 # 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

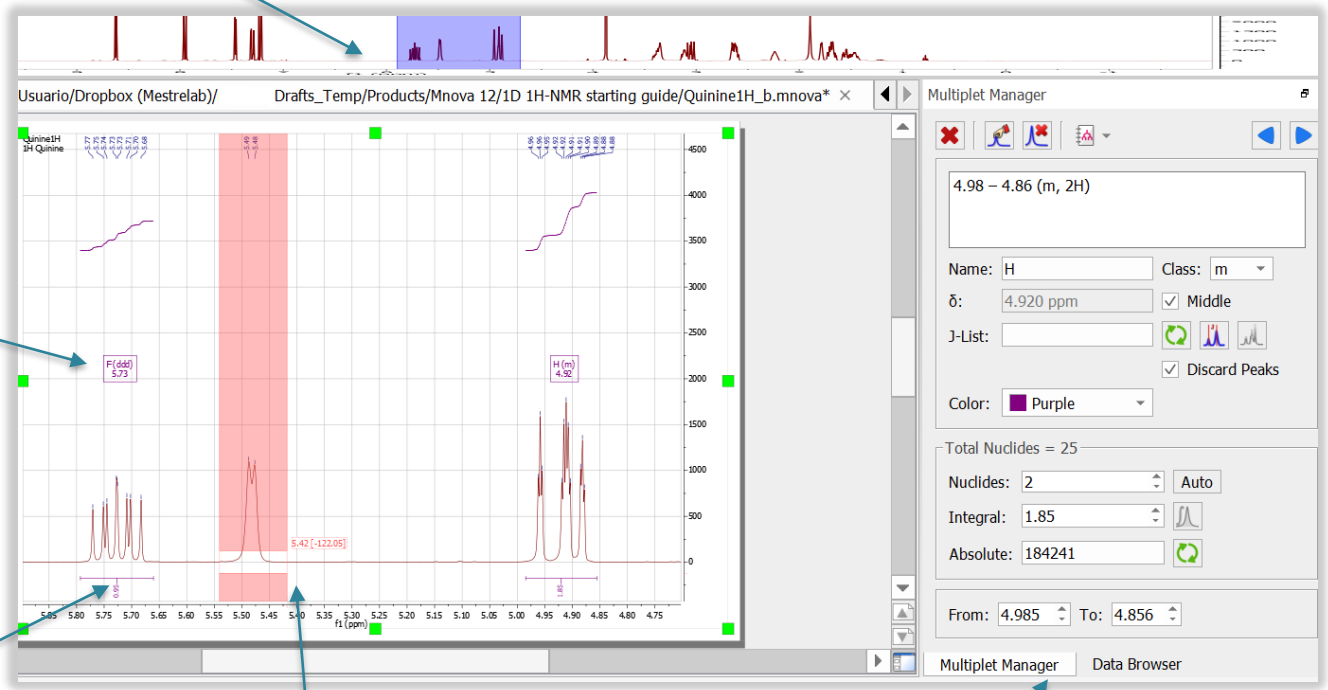
# of protons in the molecule (if present)

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

The **Auto Cut tool** will hide all noise-only regions of the spectrum. Choose **View/Cuts/Auto Cut**



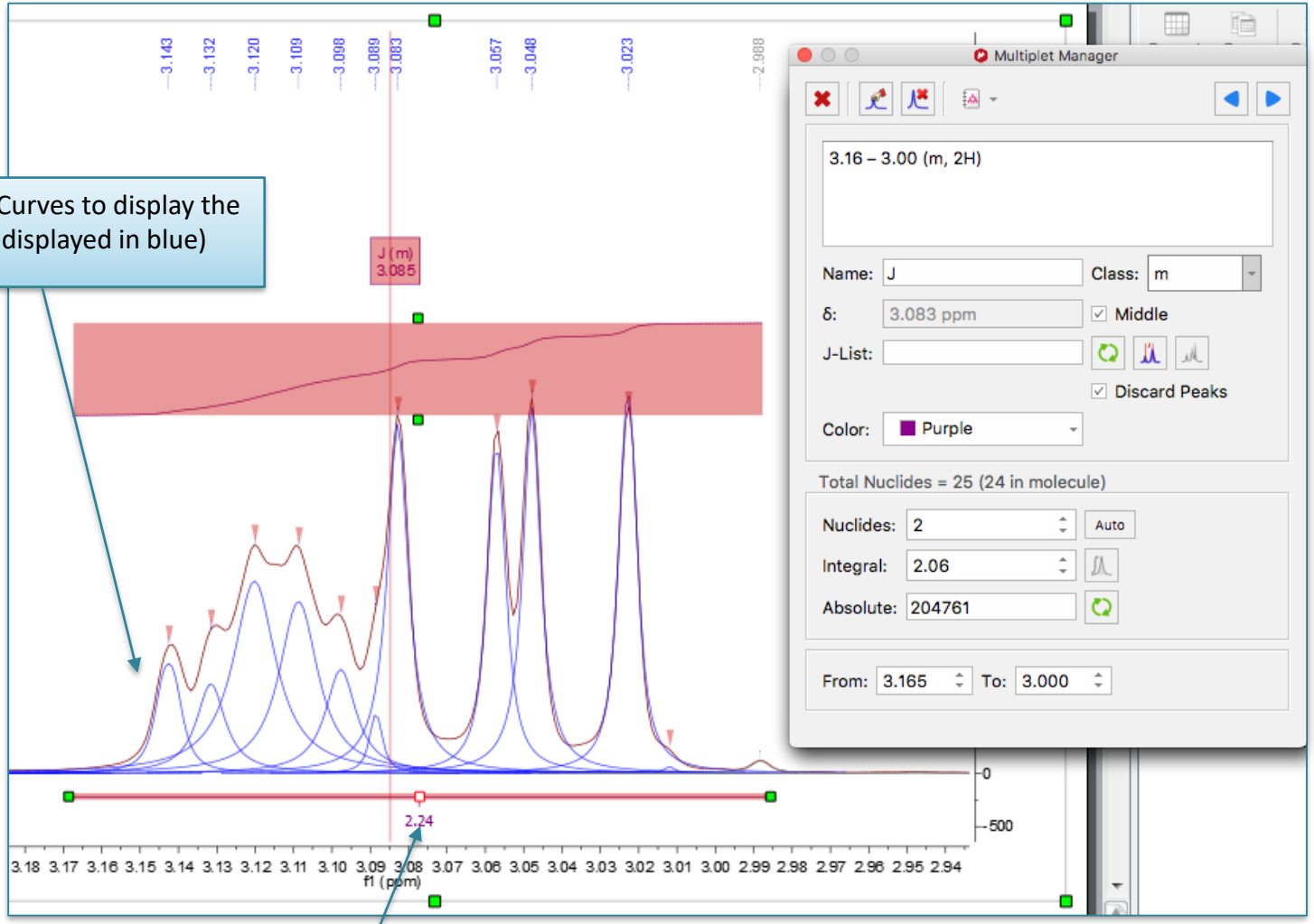
Multiplet label: Click on it to set it as the current active one

Multiplet bar: Use it to split a multiplet into 2, 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)

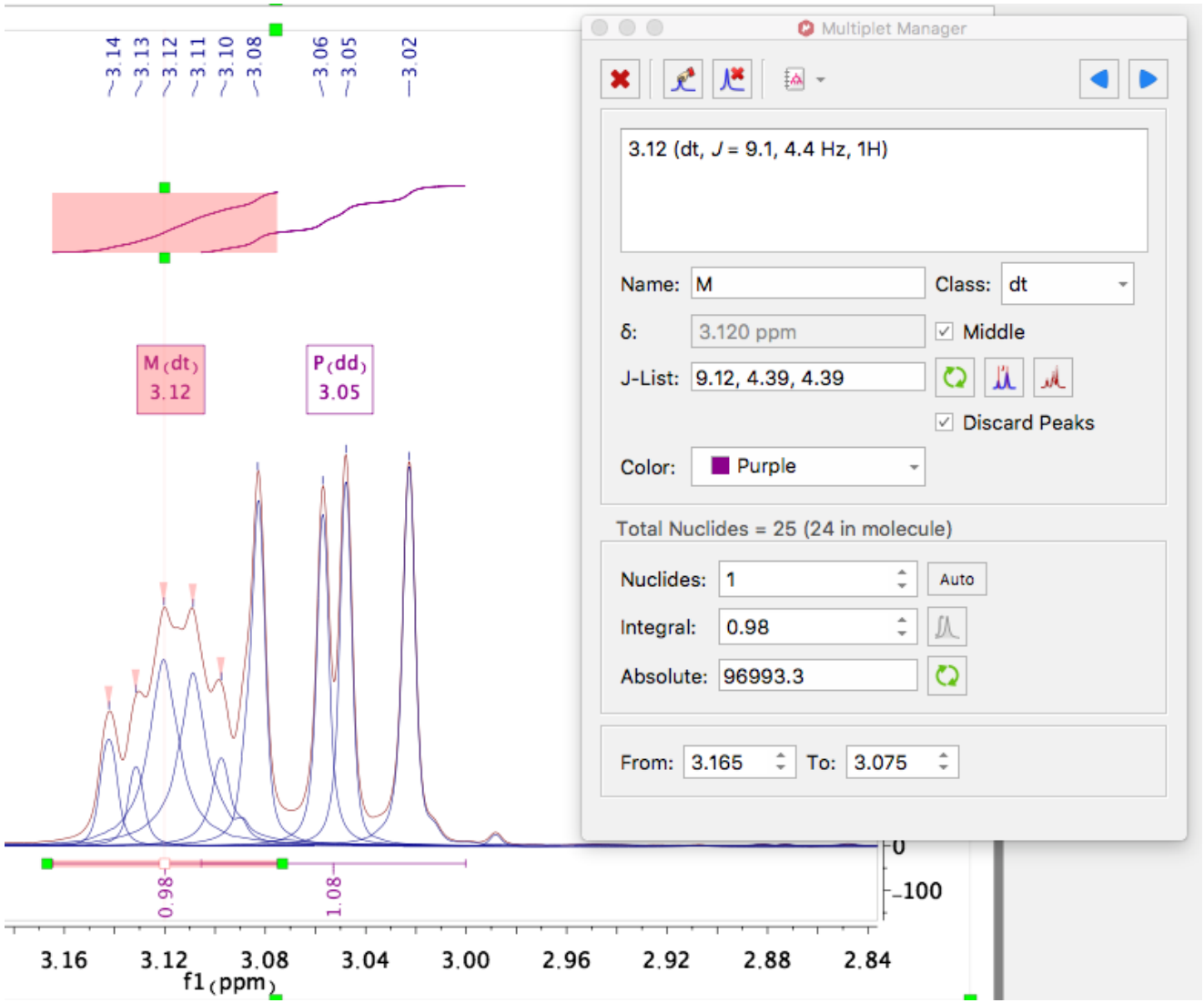


Show Peak Curves to display the GSD peaks (displayed in blue)

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

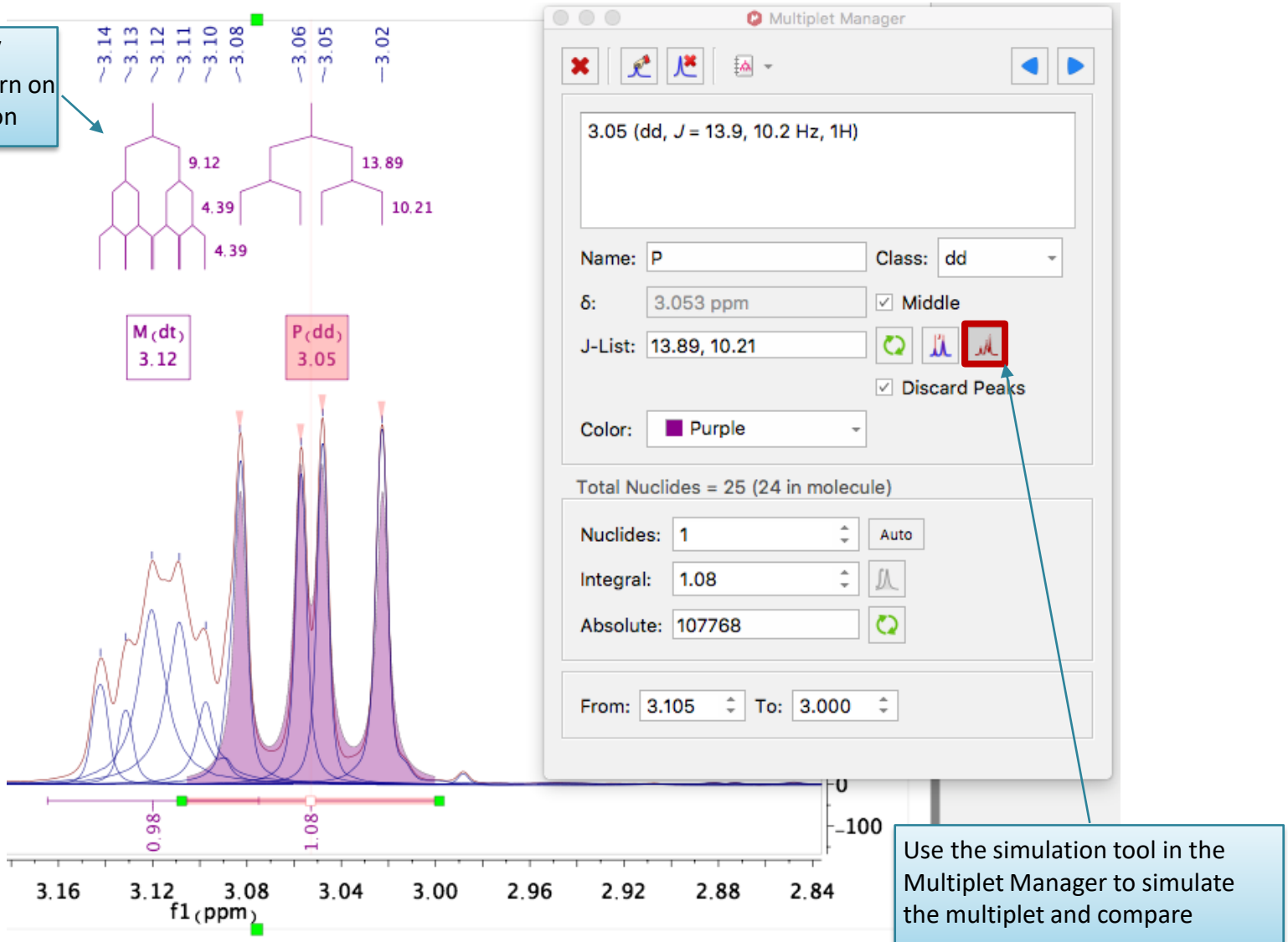
*Tip: You can also change the display of the deconvolution peak curves in **Properties Dialog > Peaks > Curve** tab*

Split partially overlapping multiplets (2)



Tools to verify multiplet analysis results

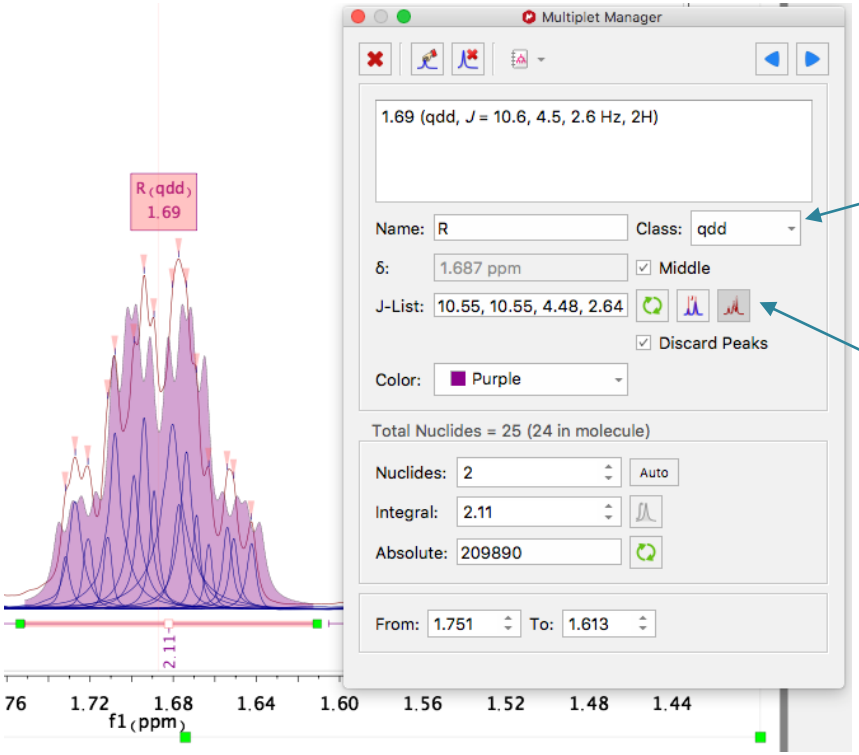
Go to **Properties/ Multiplets** and turn on the 'J's Tree' option



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

Override the multiplet results with the Multiplet Manager

- Override the analysis results of 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

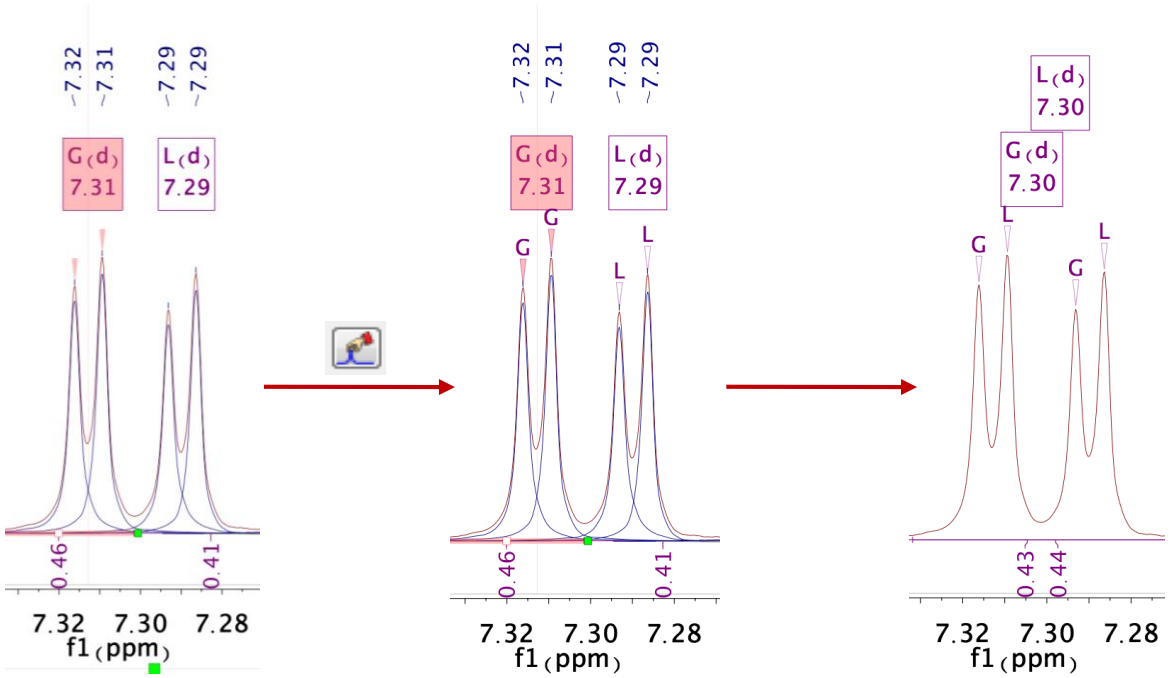


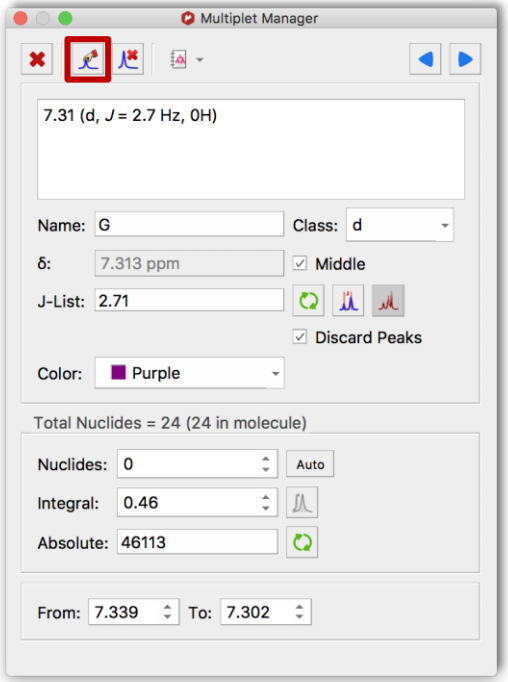
Choose “m” from the drop-down menu to override the results

Use the simulation tool to simulate the multiplet and compare

Re-assign peaks to multiplets

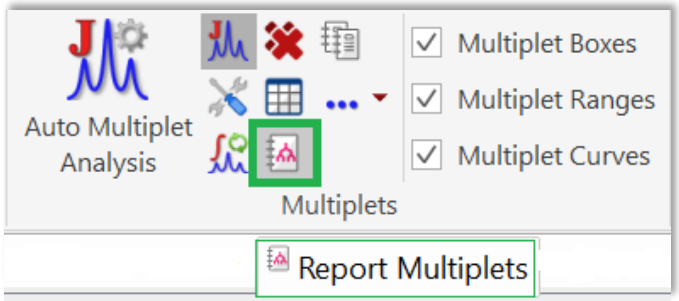
- If a peak is assigned to a wrong group, use the **Add Multiplet Peak** tool in the **Multiplet Manager** to re-assign it to a different group
- In the following example two peaks were re-assigned, forming a different pair of doublets:

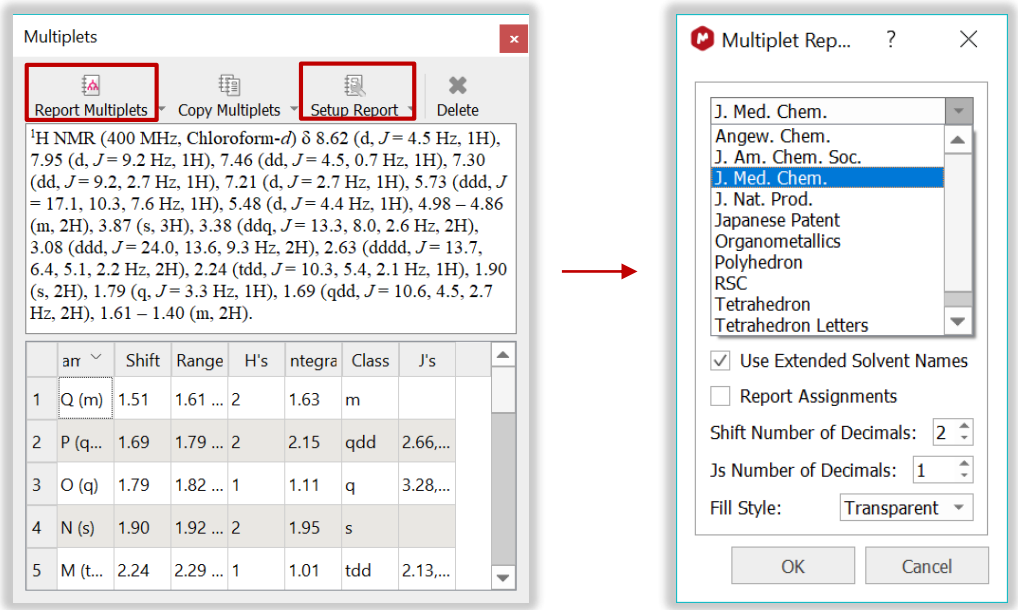




Report multiplets

- 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**

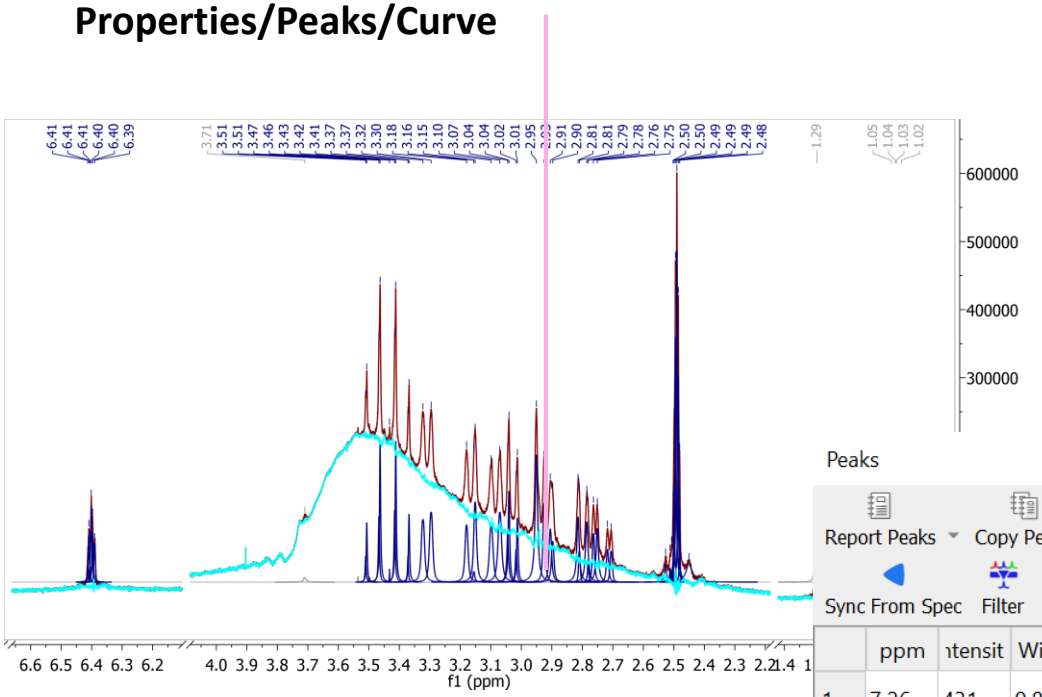


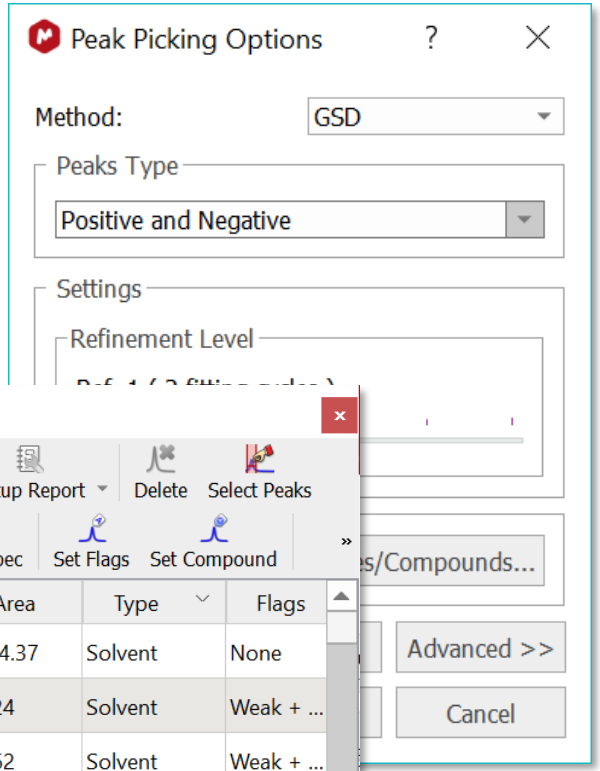


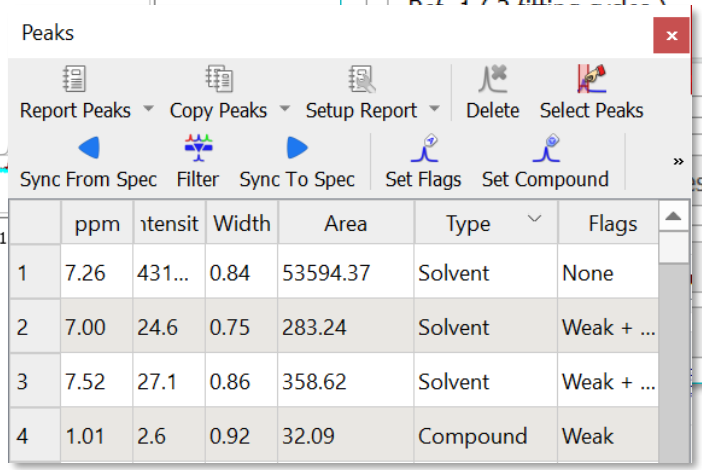
*Tip: From the Multiplet Table, click **Copy Multiplets** and then paste the texts to your document. Click on **Copy Table** and then paste the spreadsheet to your document. The table can be customized using **Setup Table.***

GSD Peak picking

- When peak-picking or multiplet analysis is done, by default Mnova does a global spectral deconvolution (GSD), then uses the deconvolved peaks as peak-picking results
- Go to **View/Tables… Peaks** to display the table
- Choose to display the deconvoluted peaks (blue) and the residuals (cyan) as shown below in **Properties/Peaks/Curve**





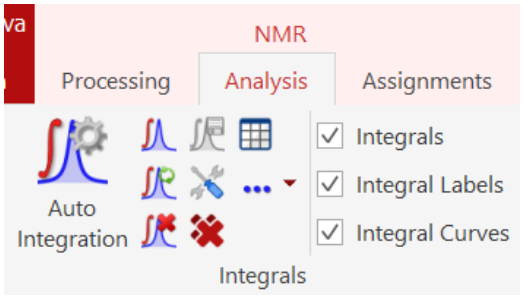


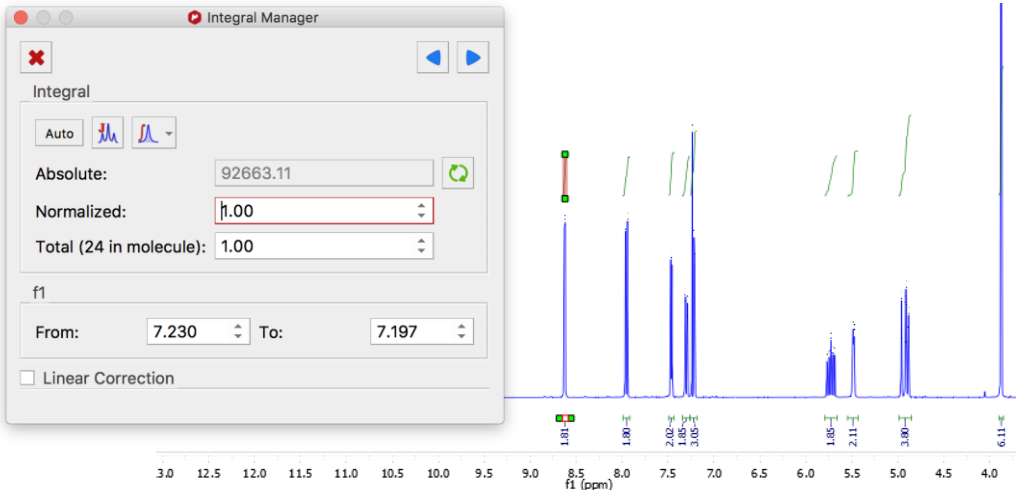
	ppm	ıtensit	Width	Area	Type	Flags
1	7.26	431...	0.84	53594.37	Solvent	None
2	7.00	24.6	0.75	283.24	Solvent	Weak + ...
3	7.52	27.1	0.86	358.62	Solvent	Weak + ...
4	1.01	2.6	0.92	32.09	Compound	Weak

See more details about GSD:
http://mestrelab.com/resources/gsd

To integrate peaks independently of multiplet analysis

- Press  to do auto integration or press "**I**" to do it manually
- Double click on an integral curve to popup the **Integral Manager:**

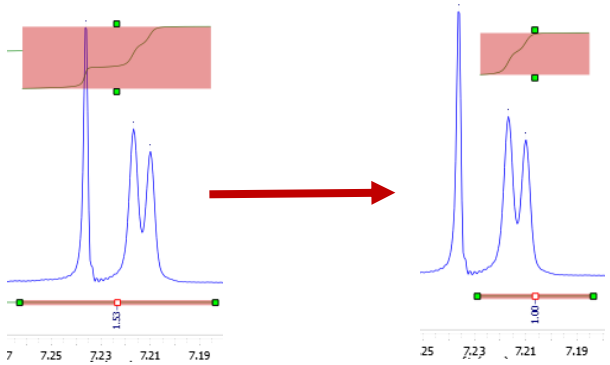




** Note: The results from Integration are independent of those from the Multiplet Analysis*
Use Integration Options to change the method and other parameters

- Type a value to **normalize** the integrals
- Browse, delete, change, split integrals interactively if needed

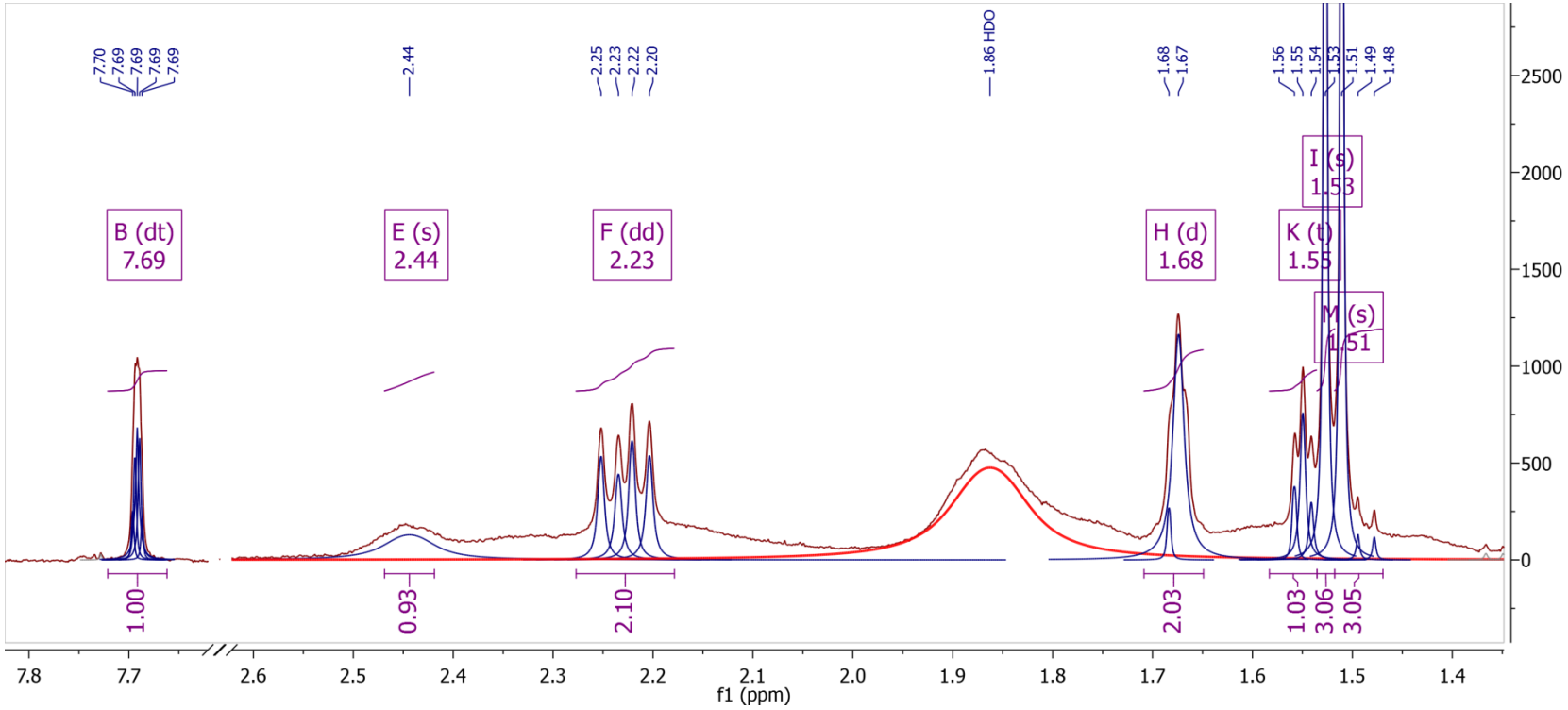
Click and drag the left green box to change the range of the integral.



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

(GSD) Peaks-based integration when running multiplet analysis

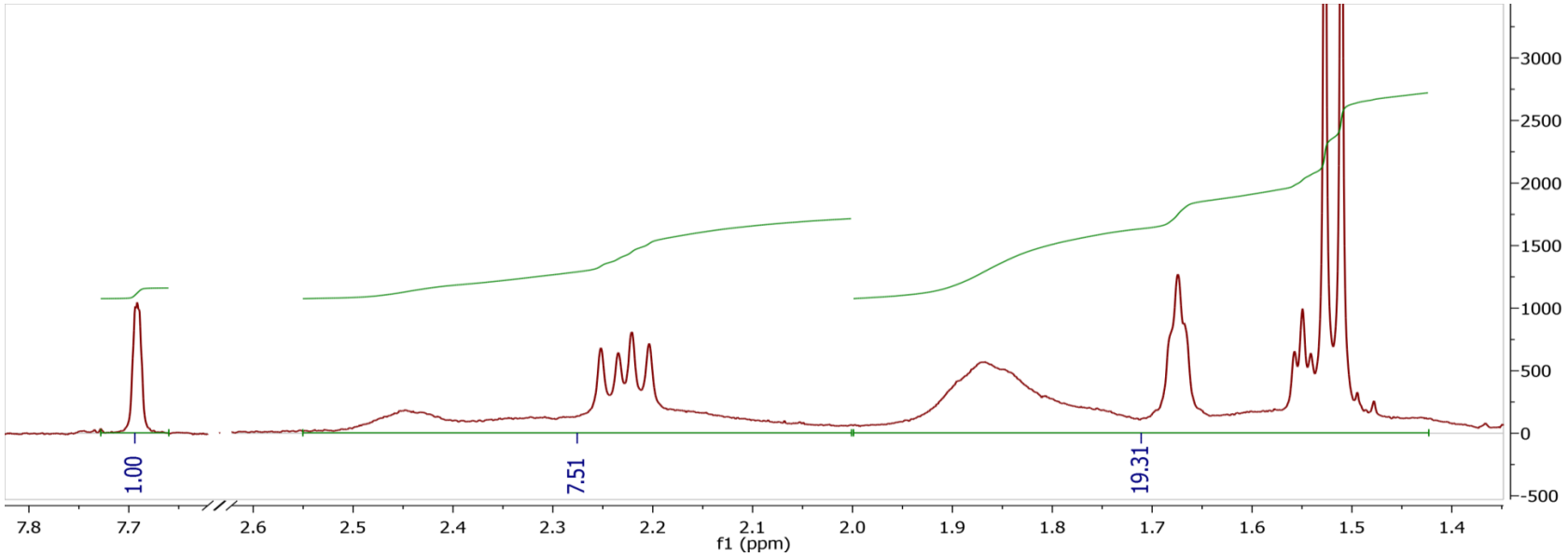
- When the peaks have irregular shapes, Peaks-based multiplet analysis may give significantly different integration results than regular Sum-based integration
- In the example below, Peaks-based multiplet analysis extracts the regular peaks but ignores the irregular ones (usually) due to exchangeable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



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

Sum-based integration

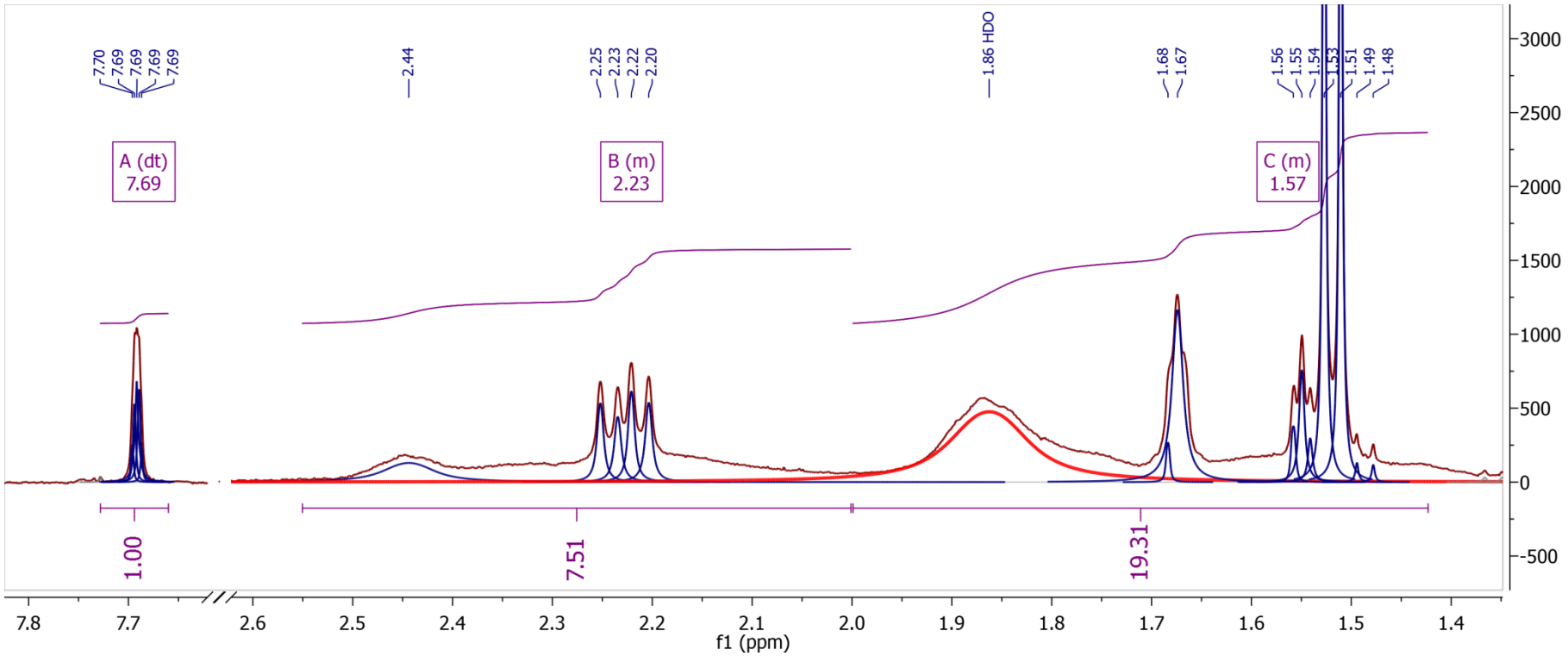
- When Sum-based integration is done, all peaks are included by adding point intensity by point intensity within the integration region
- Depending on the goal of the analysis, one must choose the appropriate integration method



Force Mnova to use Integration results in multiplet analysis

Combine Sum-based integration and multiplet analysis

- If Integration is done prior to automatic multiplet analysis, the Integration results (integration regions and integral values) will be used by the automatic **Multiplet Analysis** routine

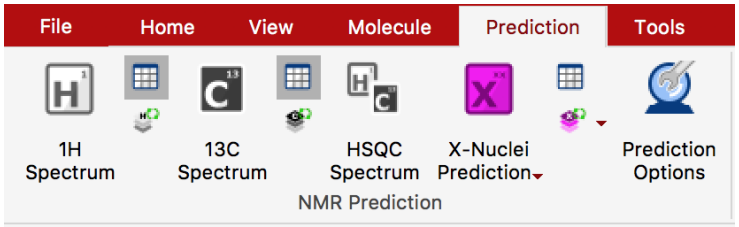


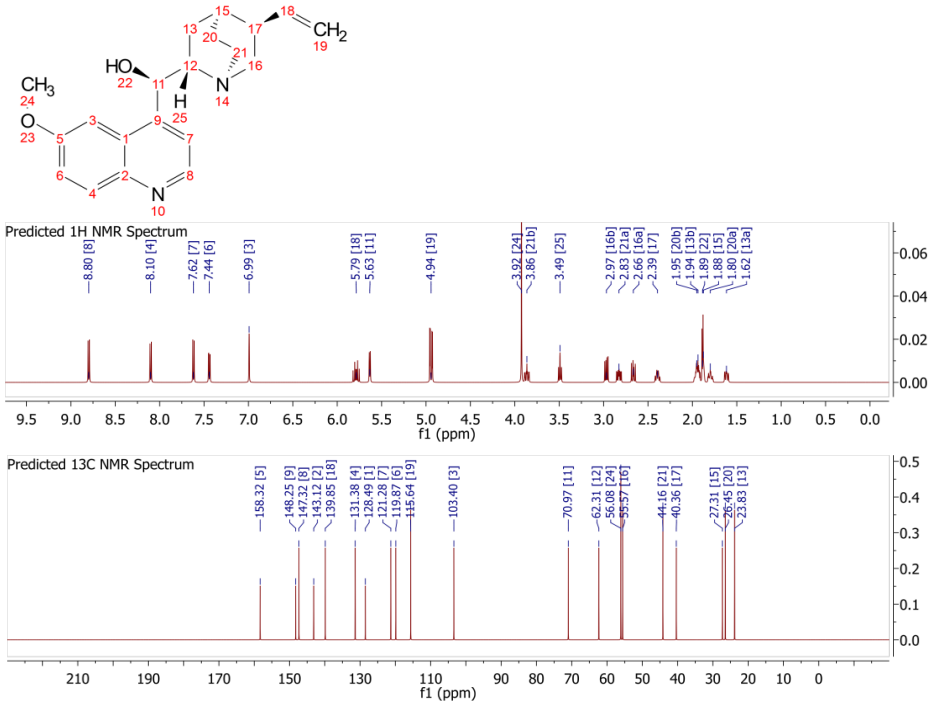
Mestrelab Research
chemistry software solutions

PREDICTION

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
- Select a spectrum, and click the appropriate icon from the **Predict** ribbon



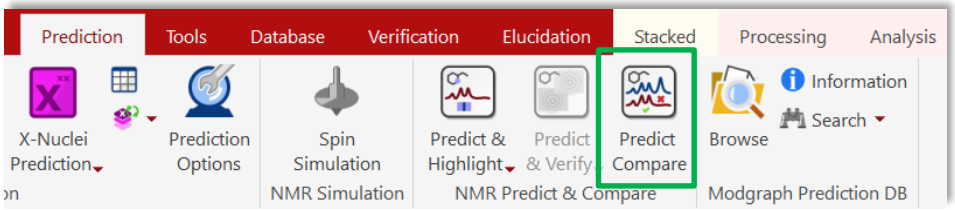


Tips:
*1. Choose **Prediction Options** to change settings*
2. You can turn on/off the atom numbers by right-clicking on the structure and choose Properties
*3. You can open the **Prediction Table** to list the predicted shifts and J-couplings, and manually change them*

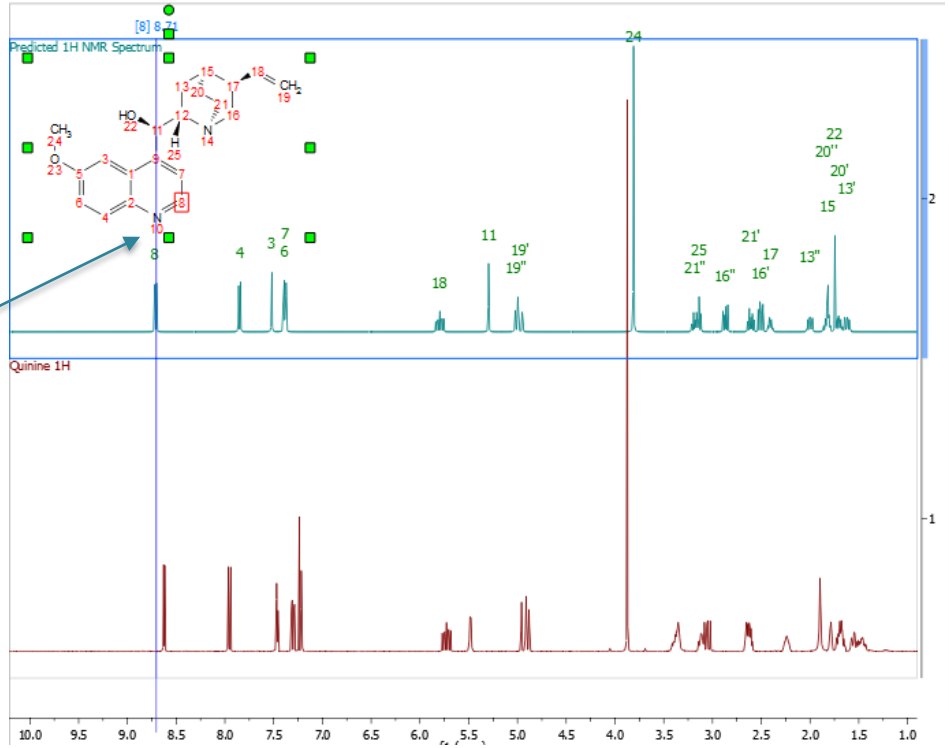
*A separate license of **Mnova NMRPredict Desktop** is needed*

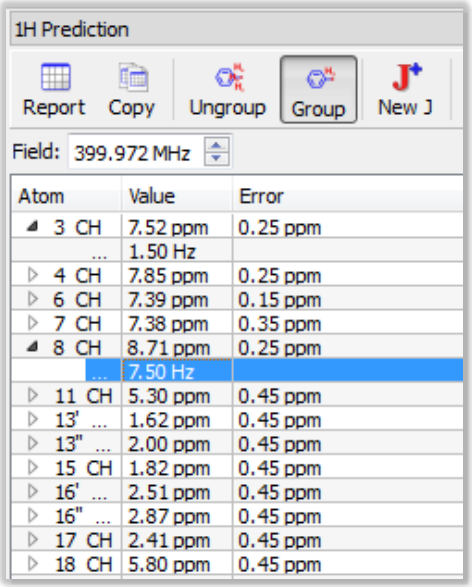
Predict NMR data & compare with the molecular structure

- Open a ^{1}H (or ^{13}C) **spectrum** in a new page
- Copy the **structure** from ChemDraw or ChemSketch
- Go to **Analysis/Predict & 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 ^{1}H Prediction Table.





Efficient working environment for peak assignment for multiple spectra

Full View allows you to navigate among the peaks easily

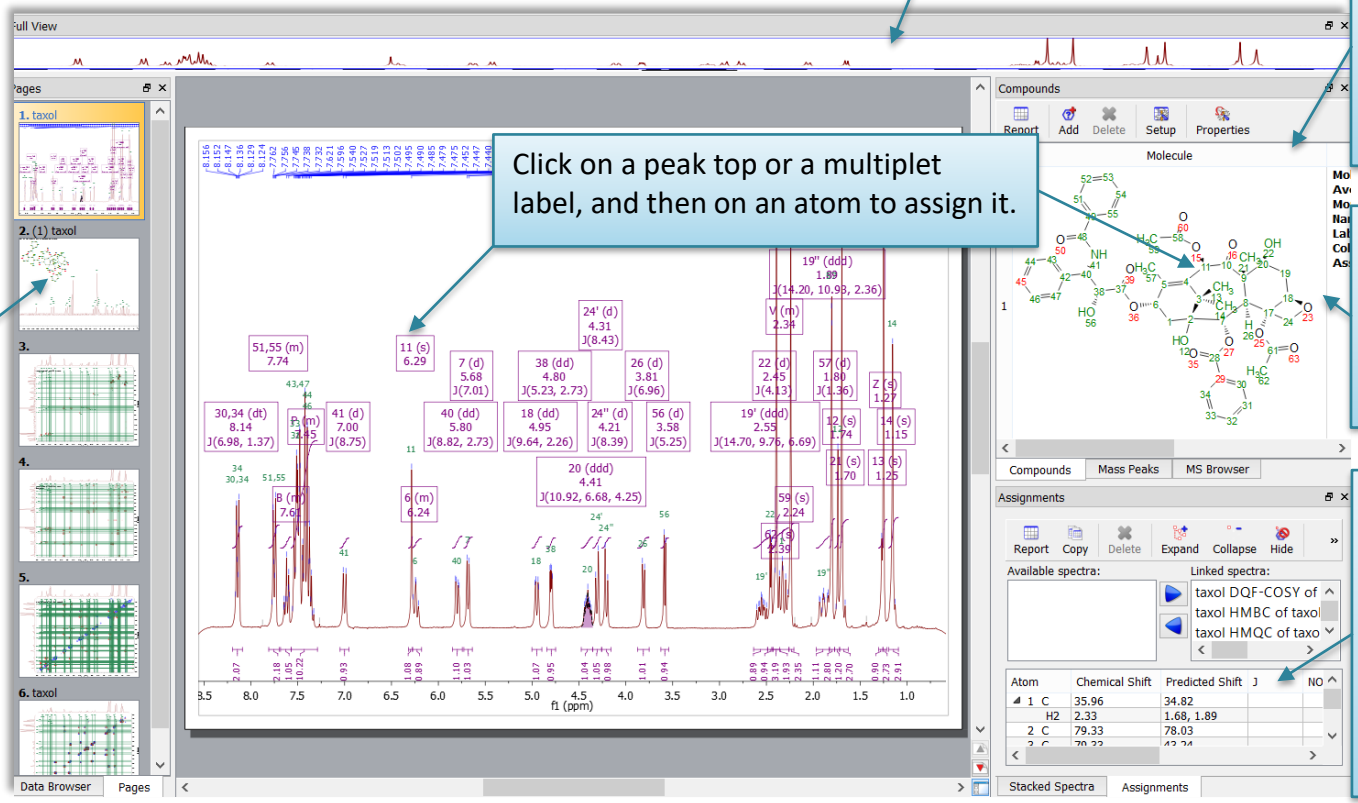
The structure(s) is shared for all "linked" spectra and assigned peaks are color coded

Pages View allows you to navigate among the spectra easily

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 compound window

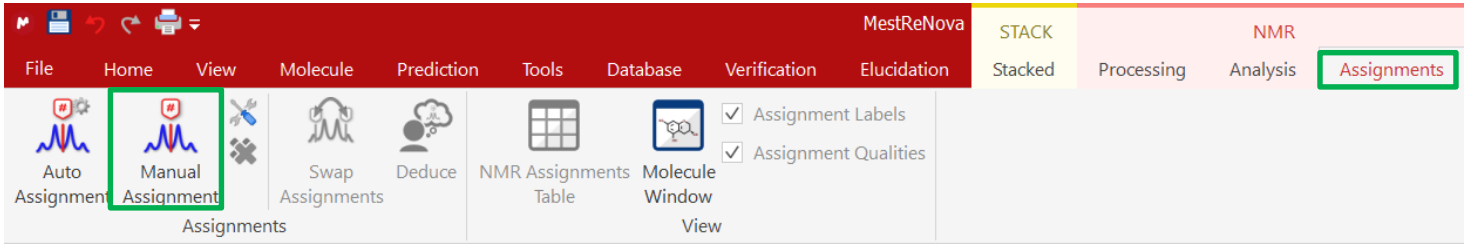
The assignment results are listed here. You can delete or change assignments here, and choose which spectra to be "linked"



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 rest of 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 **Analysis/Multiplet Analysis/Multiplets Boxes***

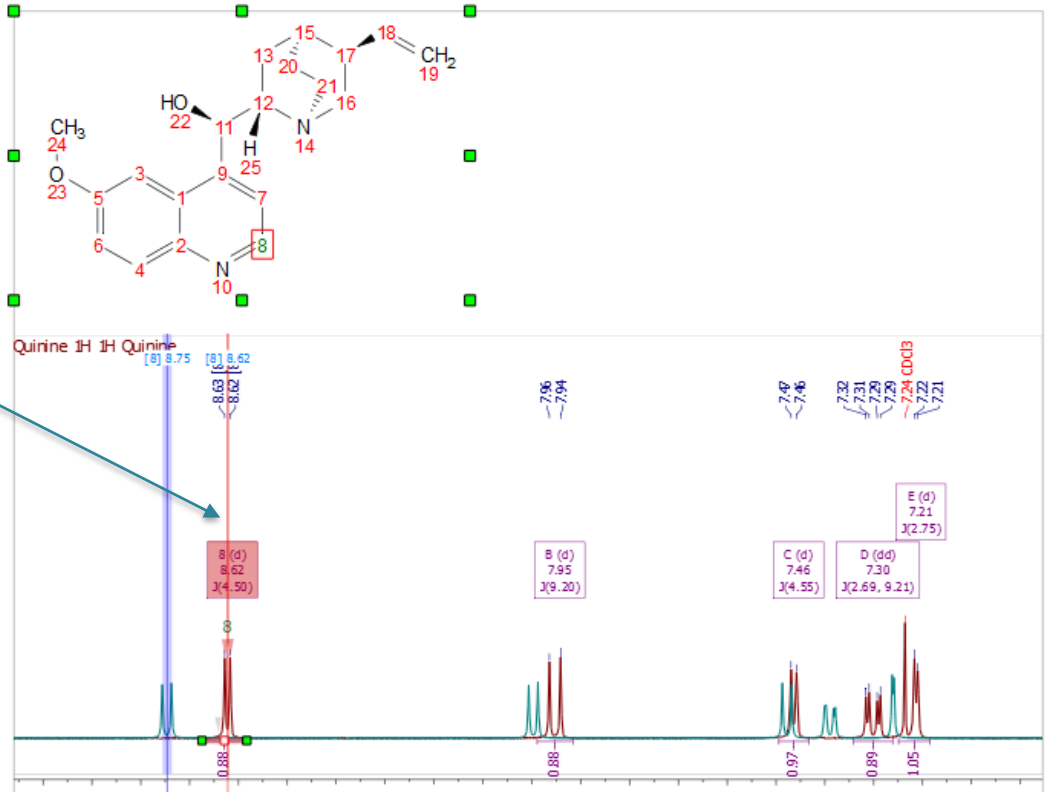
ASSIGNMENTS

Predict NMR & 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 guided by the predicted peaks

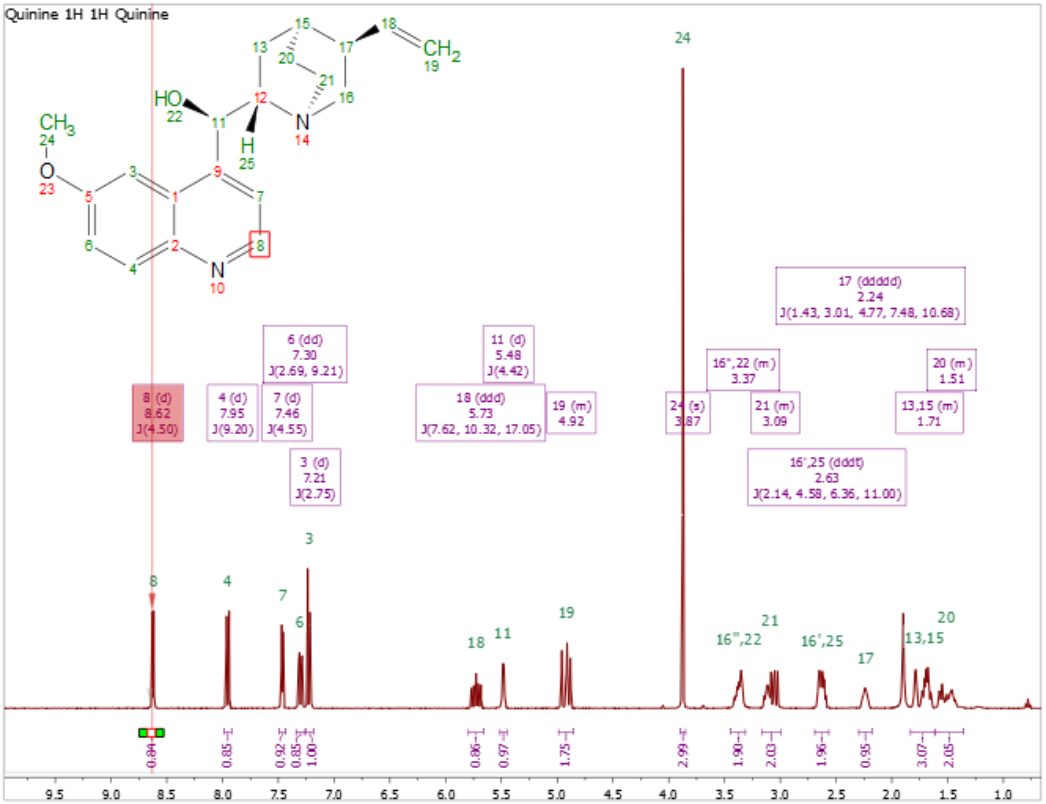
Use Shift + Up Arrow key to change the active spectrum and see the multiplet labels as well as predicted peak labels. In Assignment mode, click on a multiplet label and then on an atom to make the assignment.

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

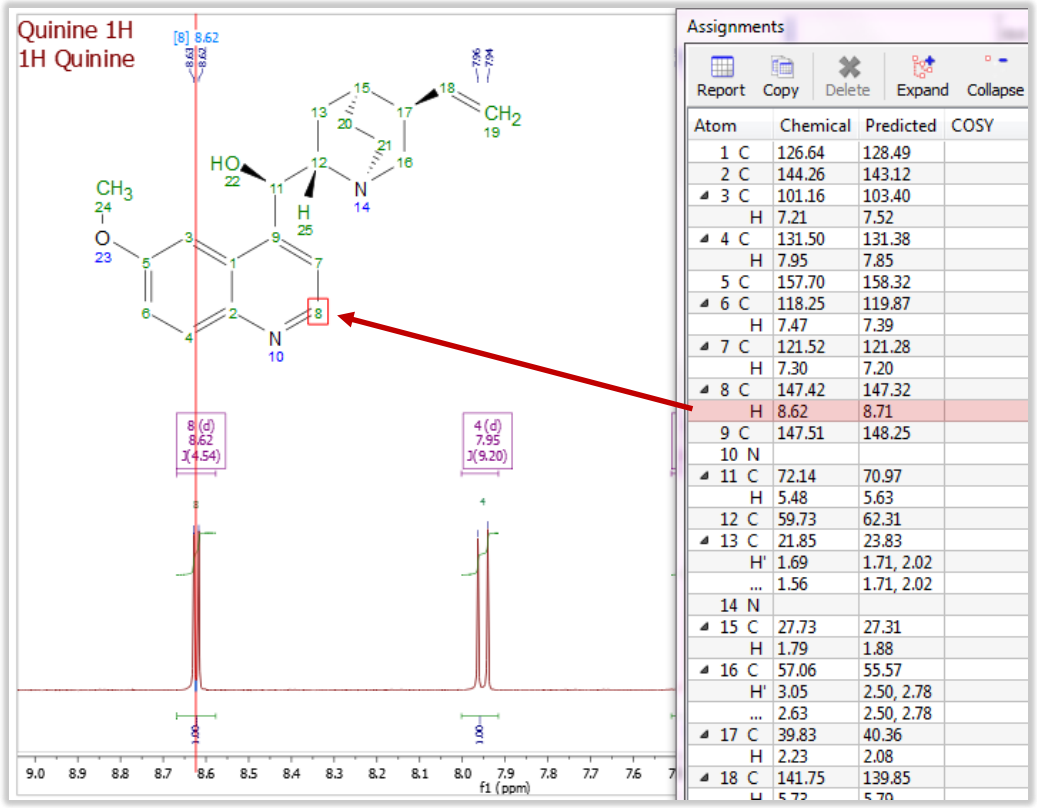


*A separate **Mnova NMRPredict Desktop** license is needed in addition to Mnova NMR*

*Tip: Multiplets can be cleaned up prior to automatic assignment. Also, try **Mnova Verify** to automatically verify proposed structures (http://resources.mestrelab.com/starting-guide-to-mnova-verify)*

Display and browse assignment results

- Go to **View/Tables…Assignments** to open the 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*.



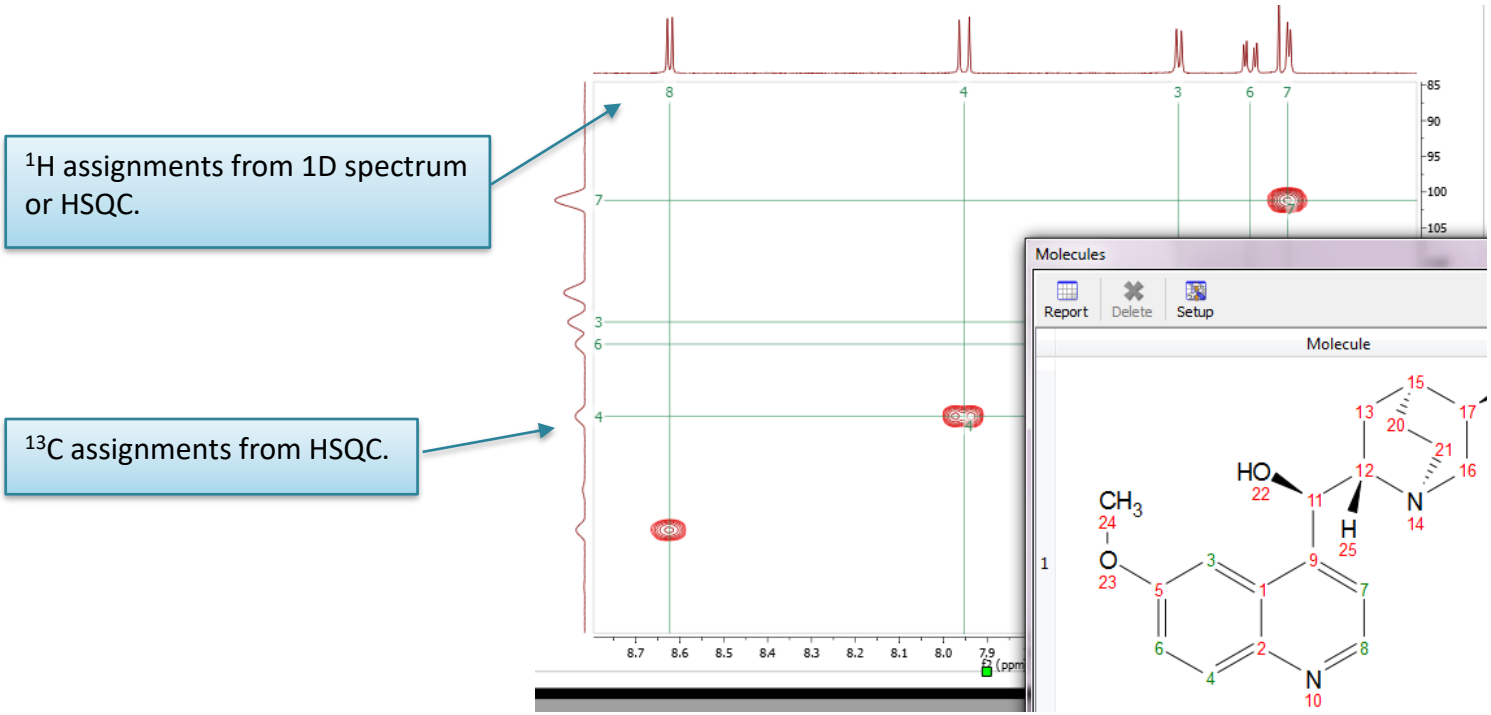
Atom	Chemical	Predicted	COSY
1 C	126.64	128.49	
2 C	144.26	143.12	
3 C	101.16	103.40	
H	7.21	7.52	
4 C	131.50	131.38	
H	7.95	7.85	
5 C	157.70	158.32	
6 C	118.25	119.87	
H	7.47	7.39	
7 C	121.52	121.28	
H	7.30	7.20	
8 C	147.42	147.32	
H	8.62	8.71	
9 C	147.51	148.25	
10 N			
11 C	72.14	70.97	
H	5.48	5.63	
12 C	59.73	62.31	
13 C	21.85	23.83	
H'	1.69	1.71, 2.02	
...	1.56	1.71, 2.02	
14 N			
15 C	27.73	27.31	
H	1.79	1.88	
16 C	57.06	55.57	
H'	3.05	2.50, 2.78	
...	2.63	2.50, 2.78	
17 C	39.83	40.36	
H	2.23	2.08	
18 C	141.75	139.85	

*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.*

ASSIGNMENTS

2D spectral assignment

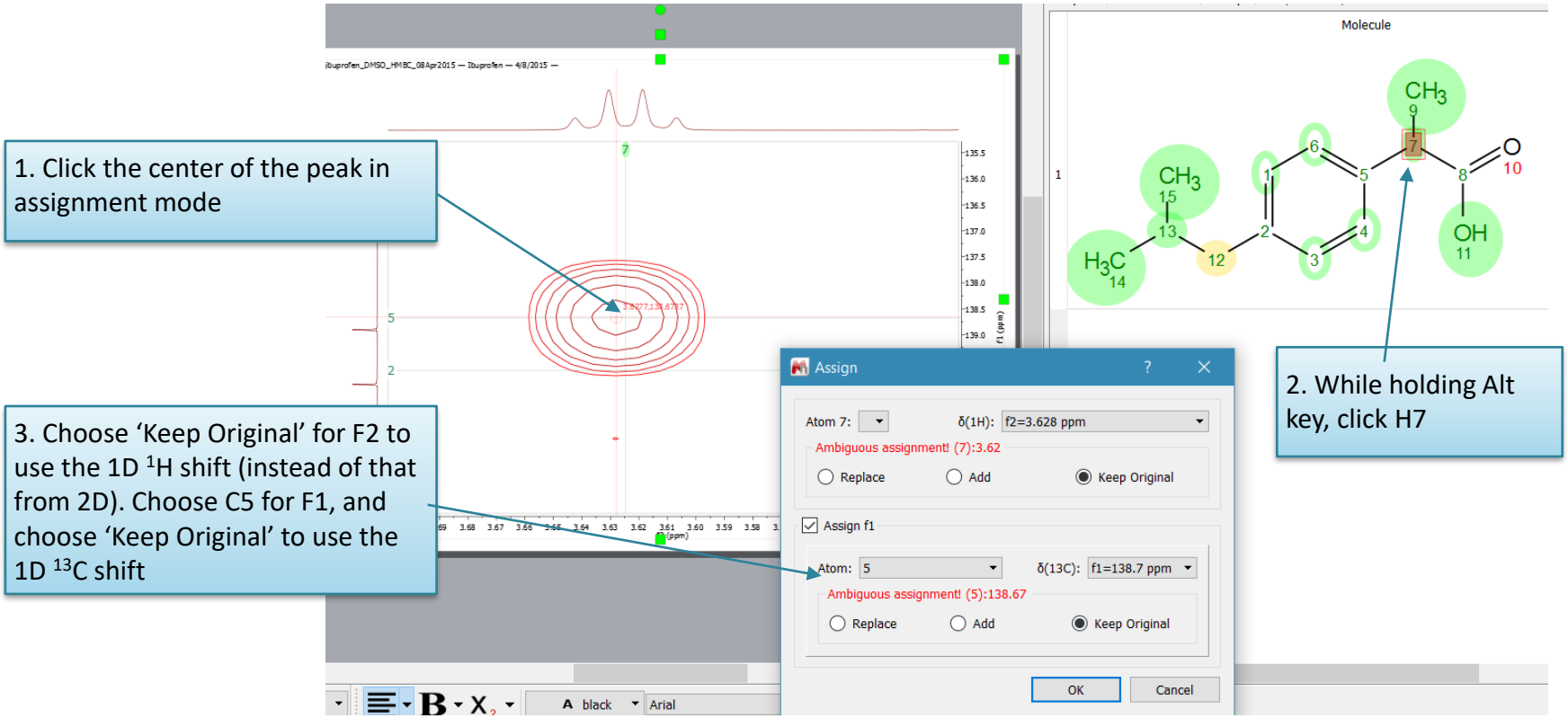
- Assign the 1D 1H peaks, and then assign HSQC cross peaks, or vice versa
- Assignments in one spectrum are carried over to all other spectra in the same document. All spectra in the same document are “correlated” by default
- To assign atoms in a HSQC, press the “A” key to enter Assignment mode. Click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



*By Default, Mnova automatically snaps to a peak top (with interpolation). Press the ***Shift*** key one time to toggle it off in order to manually locate the peak center. To see more choices, press and hold ***Alt*** key while assigning a peak*

Assigning a HMBC peak

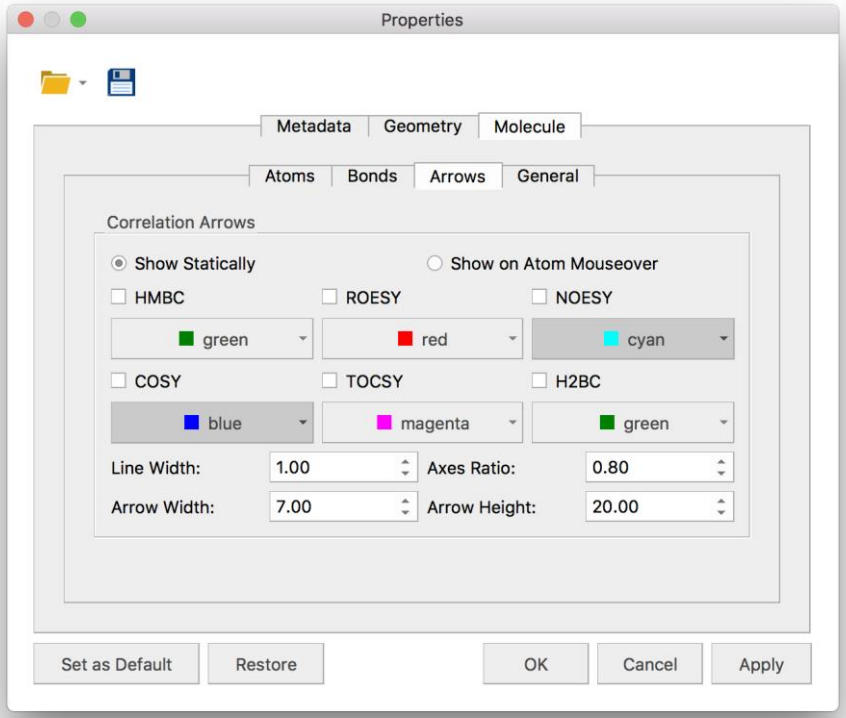
- In Assignment mode, click the center of the HMBC peak shown below, and then click on H7 while *holding the **Alt** key* *
- In the Assign pop-up window, choose the options as shown below. Click OK to assign the peak to both H7 and C5

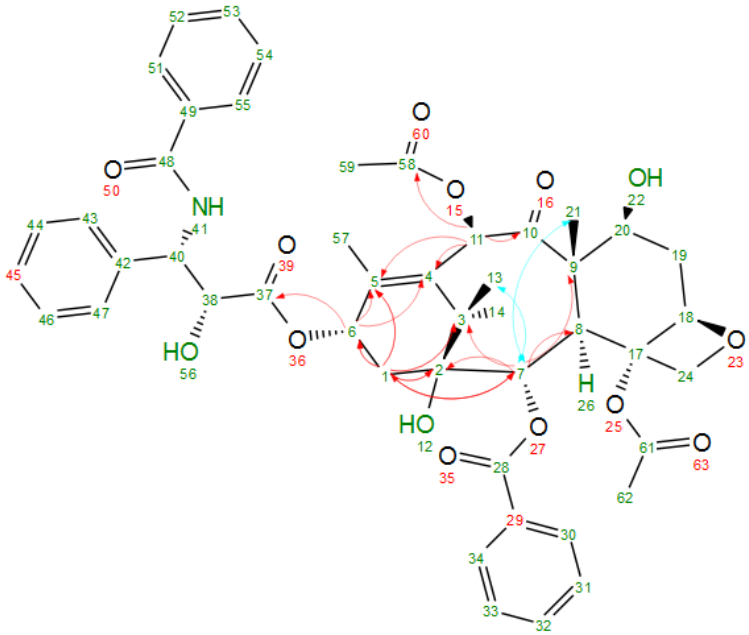


** Since chemical shifts from 1D NMR is usually of higher resolution than 2D, we recommend you to use 1D shifts whenever possible. To access such choices, press and hold the **Alt** key while assigning a peak*

Display 2D assignments on a molecular structure

- Report the structure from the **Compounds Table** *
- Select **Edit/Properties** to change the display properties of the structure
- Choose to display various connectivities for assigned atom pairs

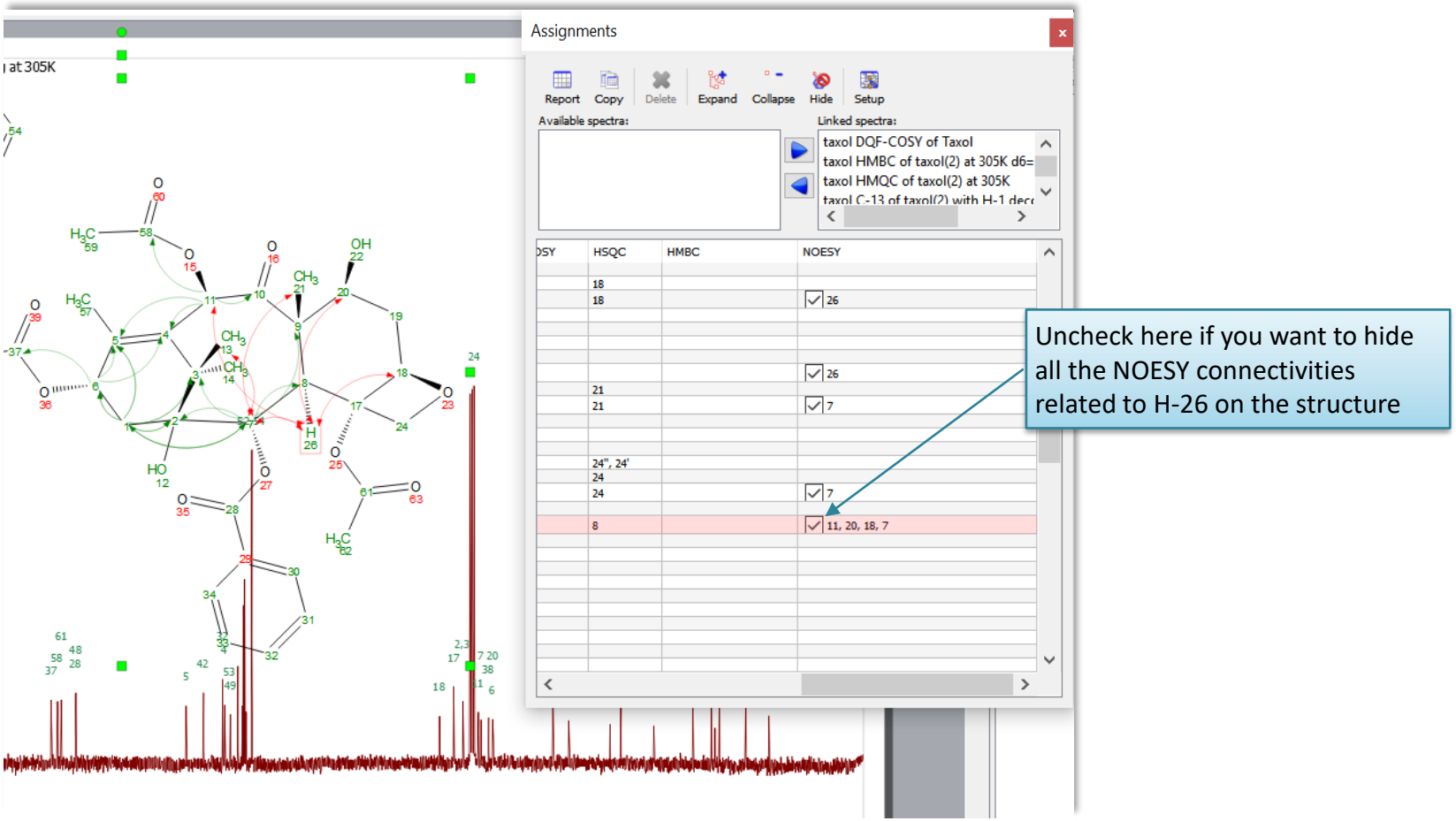




**Don't open the same structure multiple times. Instead, use the Compounds able to report the structure to the pages where needed*

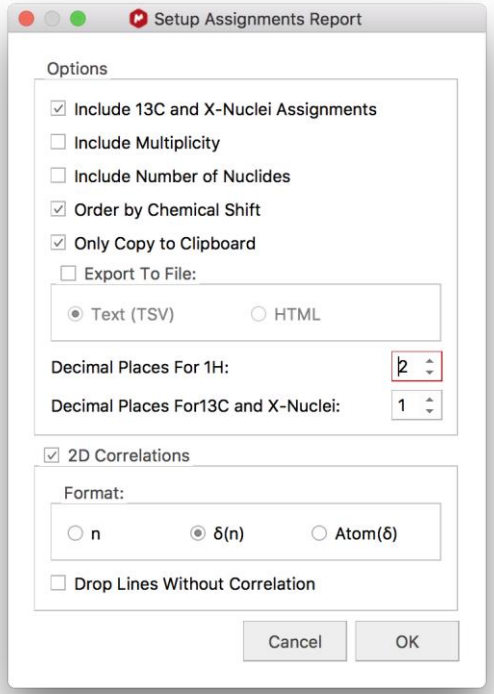
Selective display of 2D spectral connectivities

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



Report spectral assignments in journal format

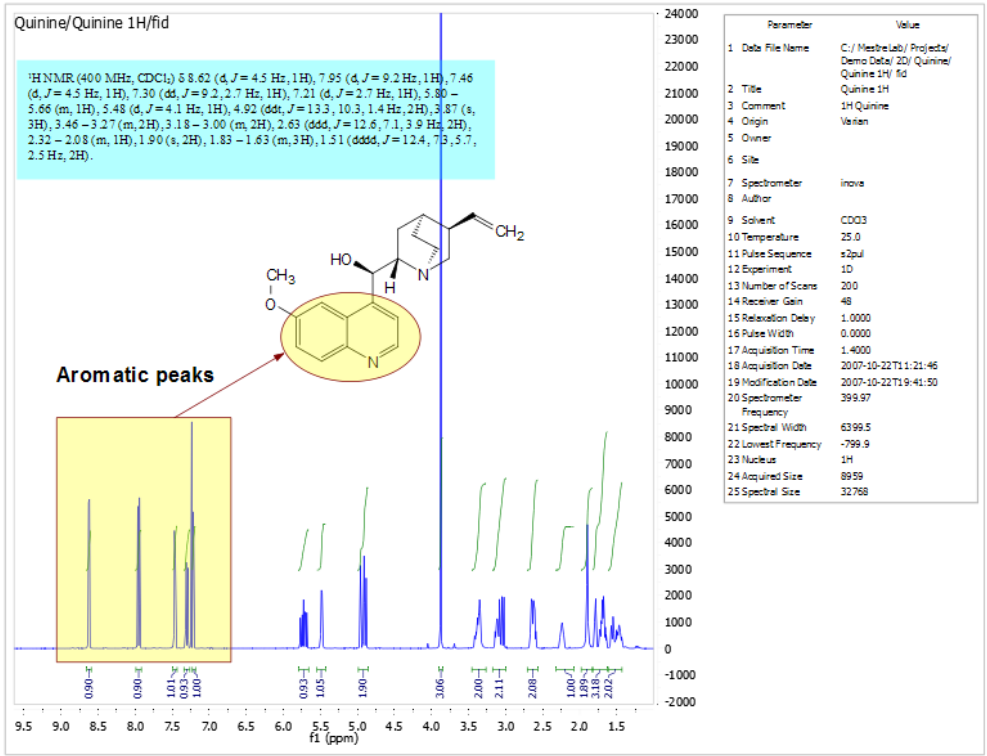
- Select **Scripts/Report/Peak Assignments**… to report the assignment results in journal format
- The report can be pasted into an external document

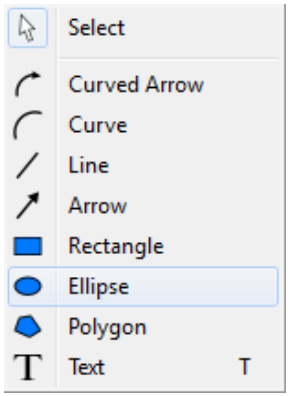


No	δ_H	Multiplicity, *J* (Hz)	δ_C	HSQC	HMBC	NOESY
1	2.33		35.96	35.96(1)	72.58(6), 75.23(7),79.33(2), 79.33(3), 142.19(5)	
2			79.33			
3			79.33			
4			133.45			
5			142.19			
6	6.24	m	72.58		133.45(4), 142.19(5), 172.90(37)	
7	5.68	d,J=7.01 Hz	75.23	75.23(7)	35.96(1), 45.90(8), 58.88(9), 79.33(2), 79.33(3)	3.81(26)
8			45.90			
9			58.88			
10			203.81			
11	6.29	s	75.80	75.80(11)	133.45(4), 142.19(5), 171.41(58), 203.81(10)	3.81(26)
12	1.74	s				
13	1.25	s	27.11	27.11(13)		
14	1.15	s	22.03	22.03(14)		
17			81.42			
18	4.95	dd,J=9.64,2.26 Hz	84.64	84.64(18)		3.81(26)

Annotate and report manually

- **Annotations**, such as arrows, boxes, and text, etc. can be added using the toolbar along the bottom of the Mnova window
- 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

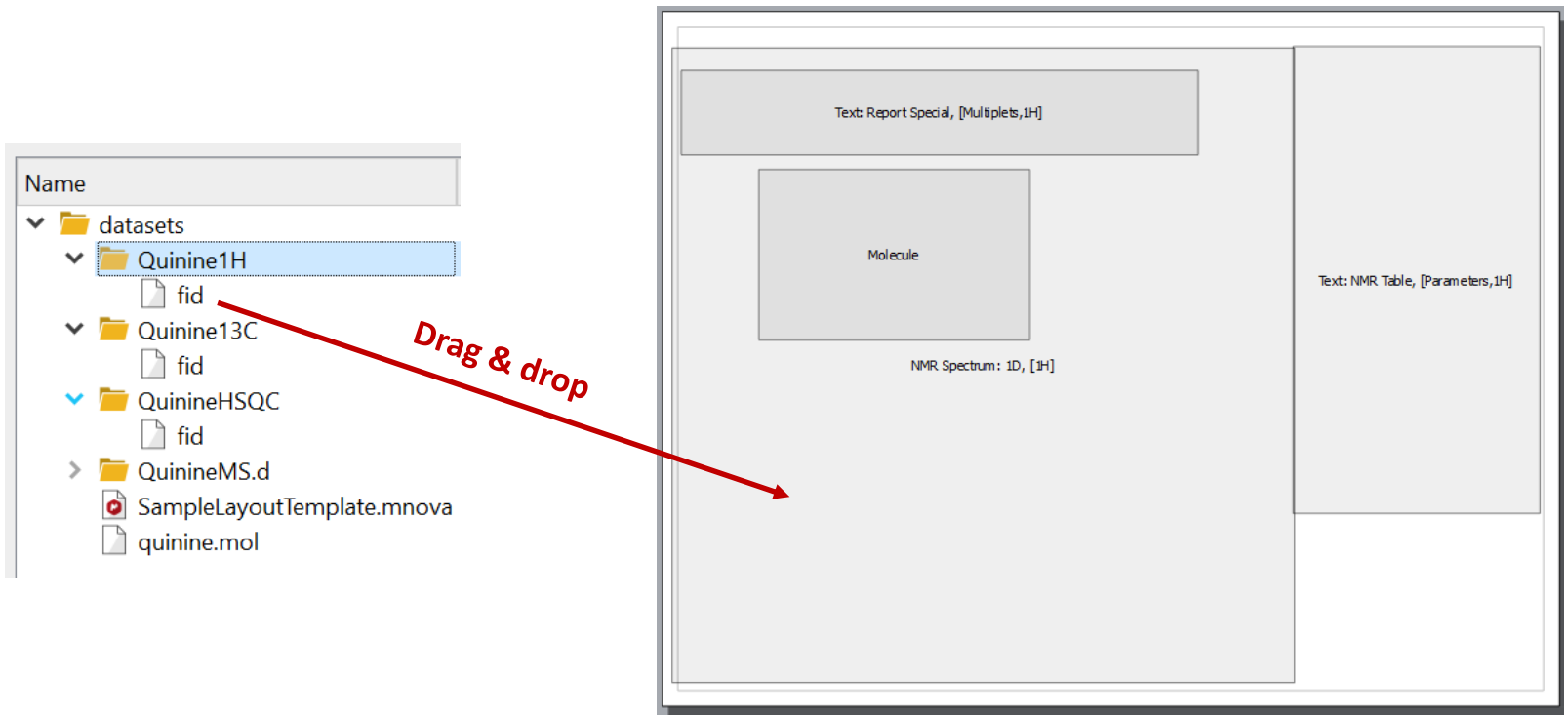




Tips:
Copy a **molecule from ChemDraw or ChemSketch, or open .mol or .sdf files*
Use **View/Layout Templates menu to generate and apply layout templates, or request an auto-**formatting script** from Mestrelab*
****Copy/paste** any object(s) to your document with high resolution*
Click to export to **PDF in the Quick Access toolbar*

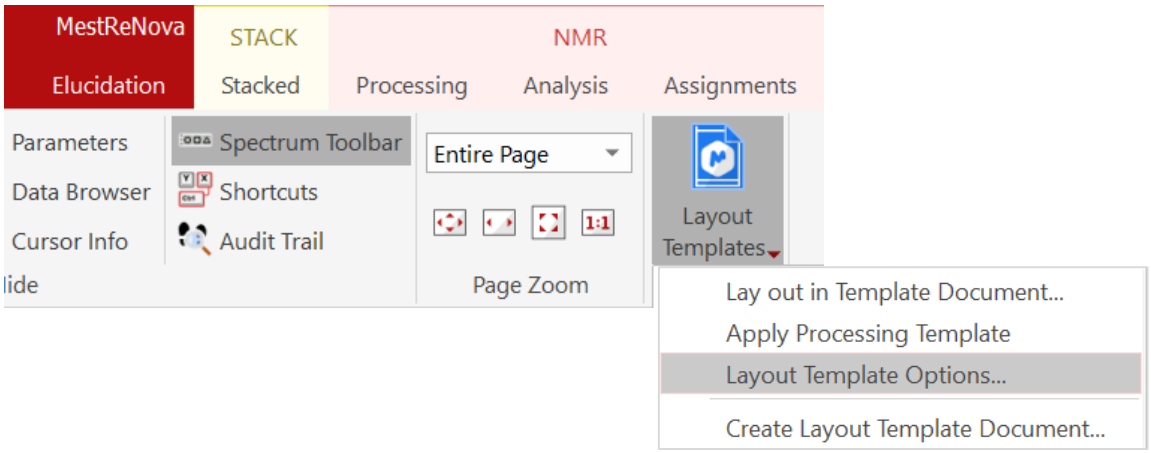
Create a layout template

- 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

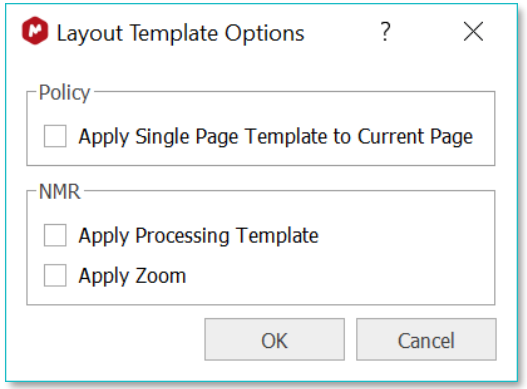


Apply layout templates to specific pages of a document

- A layout template can now be applied to a specific page of a document with several pages. The zoom ranges can also be set in the template

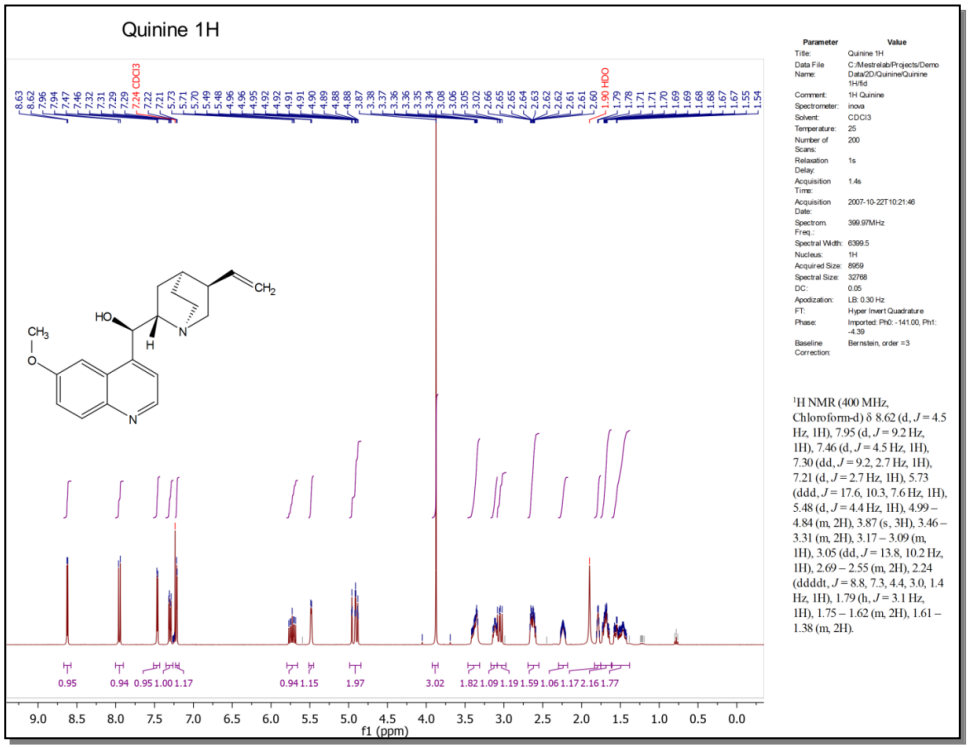


- A layout template can be applied to a specific page of a document that contains several pages
- Zoom ranges can also be set in the template



Auto format using Mnova script

- Mnova has a powerful scripting engine that allows you to automate many operations, including processing, analysis and reporting *
- Below is an sample result from running an Mnova script



^{1}H NMR (400 MHz, Chloroform-d) δ 8.62 (d, J = 4.5 Hz, 1H), 7.95 (d, J = 9.2 Hz, 1H), 7.46 (d, J = 4.5 Hz, 1H), 7.30 (dd, J = 9.2, 2.7 Hz, 1H), 7.21 (d, J = 2.7 Hz, 1H), 5.73 (ddd, J = 17.6, 10.3, 7.6 Hz, 1H), 5.48 (d, J = 4.4 Hz, 1H), 4.99 – 4.84 (m, 2H), 3.87 (s, 3H), 3.46 – 3.31 (m, 2H), 3.17 – 3.09 (m, 1H), 3.05 (dd, J = 13.8, 10.2 Hz, 1H), 2.69 – 2.55 (m, 2H), 2.24 (dddt, J = 8.8, 7.3, 4.4, 3.0, 1.4 Hz, 1H), 1.79 (h, J = 3.1 Hz, 1H), 1.75 – 1.62 (m, 2H), 1.61 – 1.38 (m, 2H).

** We provide development service for more complex batch processing and reporting requirements*

Auto Process, Analyze and Report

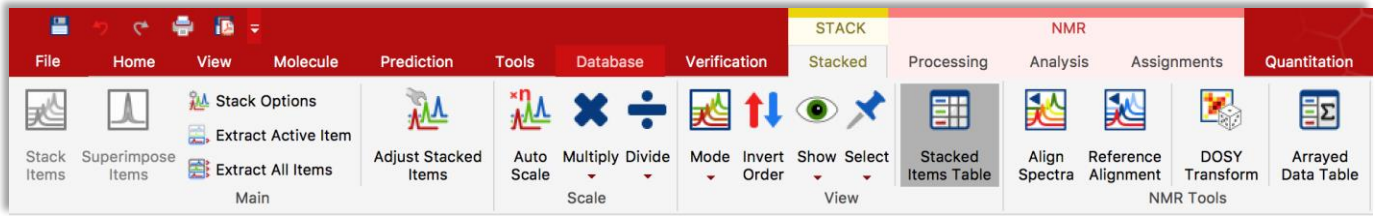
Example: Auto Process, Analyze, and Report a 1D spectrum using the Mnova script, PAR.qs

- Open a 1D 1H spectrum, run the script. It does the following: **
  - Re-processes the spectrum with a line-broadening value of 0.3 Hz; enhanced correction for Bruker Group Delay, if applicable; zero-filling to double the data size, or to at least 64K points; and, baseline correction using 3rd order Bernstein polynomial
  - Automated peak-picking and multiplet analysis using the current settings
- Manually verify and correct the multiplet analysis results
- Run the script again, and it will generate a report similar to the one on the previous slide
- Processing, analysis and reporting options can be easily customized by editing the script
- This script also works for 13C and other nuclei, though in a slightly different way (e.g., it picks and reports peaks instead of multiplets)
- This script only does formatting if it is a 2D

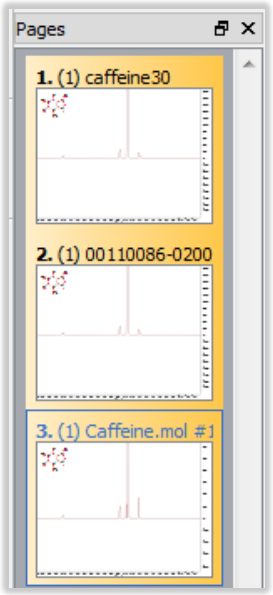
- *** The script can be edited, and the settings customized*

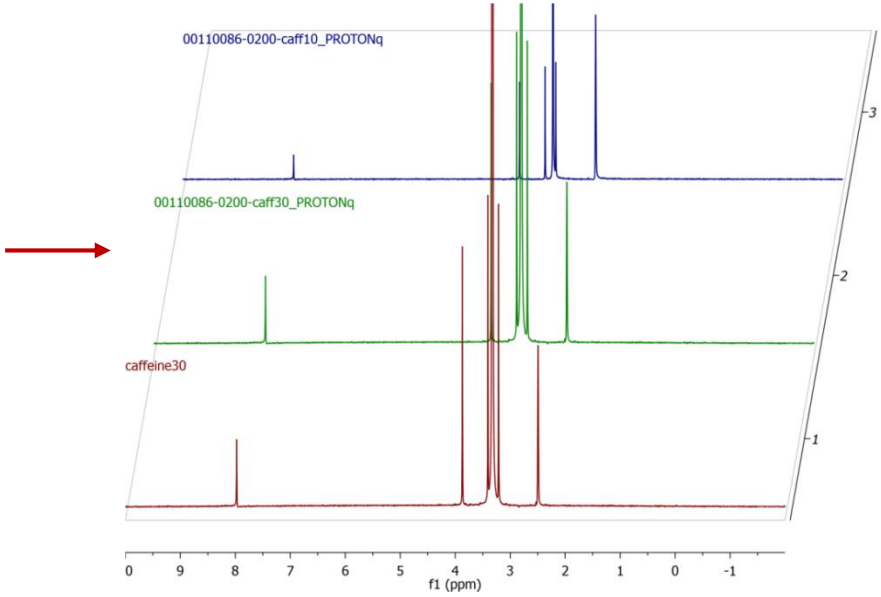
- *Write to support@mestrelab.com and ask for PAR.qs. It's free for academia*
- *To run the script, first save it to a directory on the computer*
- *Next, choose Scripts > Run Script, navigate to the directory, and open it*

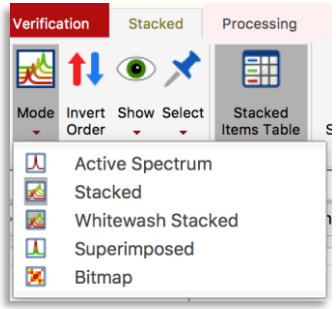
Open and stack multiple 1D spectra



- 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



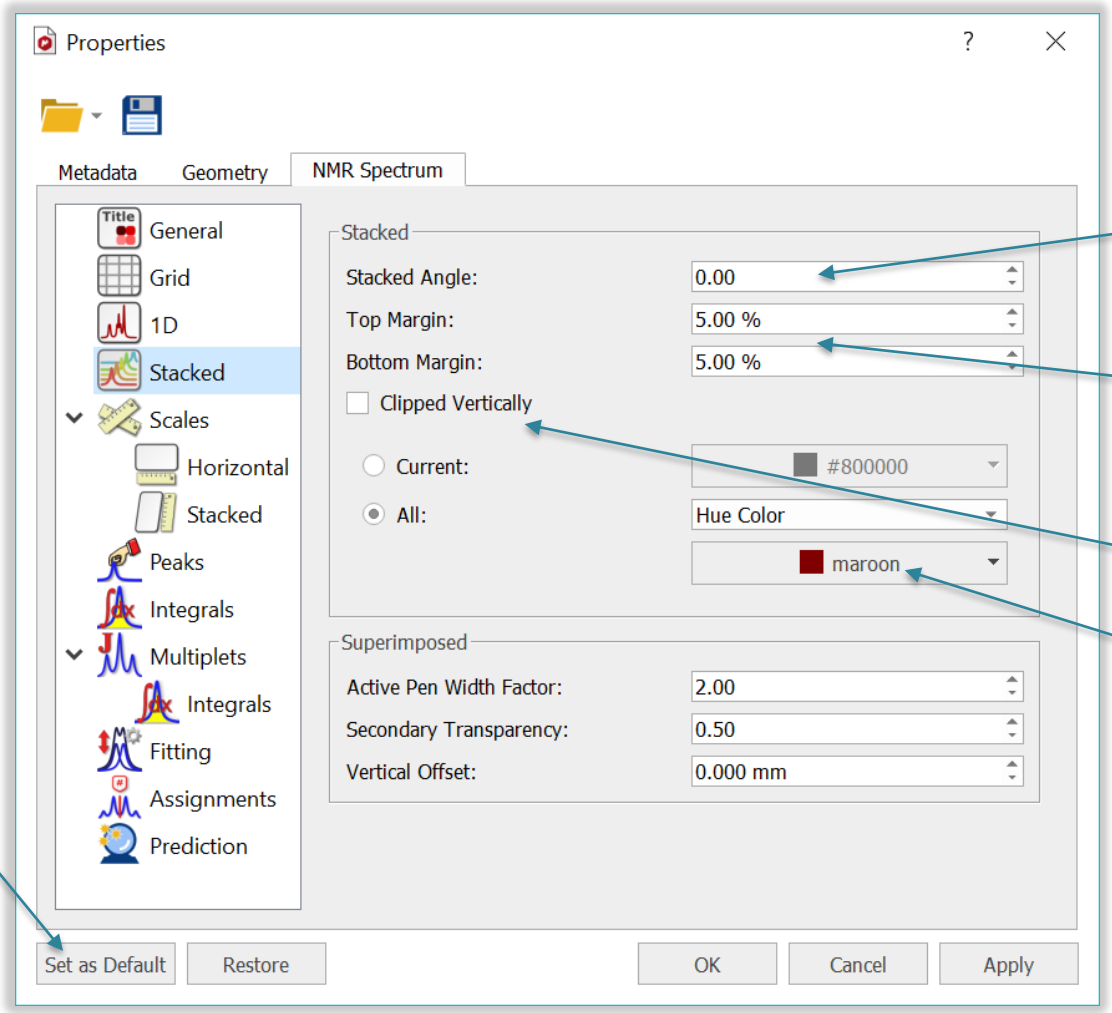




*Tip: - You can also drag a 1D from a different page using the **Pages** view, which add it to the current page as a new element in the stack*
*- When multiple pages are selected, you can choose the **Superimposed** tool*  *to superimpose them directly*
*- If you want to stack all the 1D spectra from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

Change display properties of stacked spectra

➢ Right click on it and select **Properties:** (or double click on the spectral window)



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

Enlarge the top/bottom margins for better 3D effects.

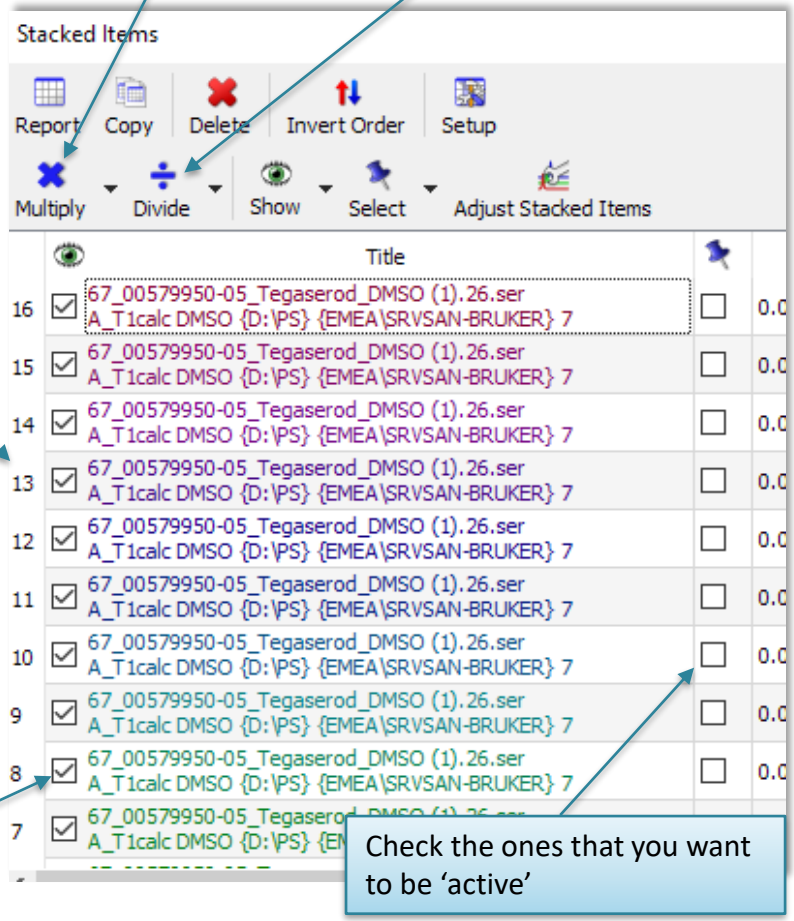
Check here if you want to clip the peaks.

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



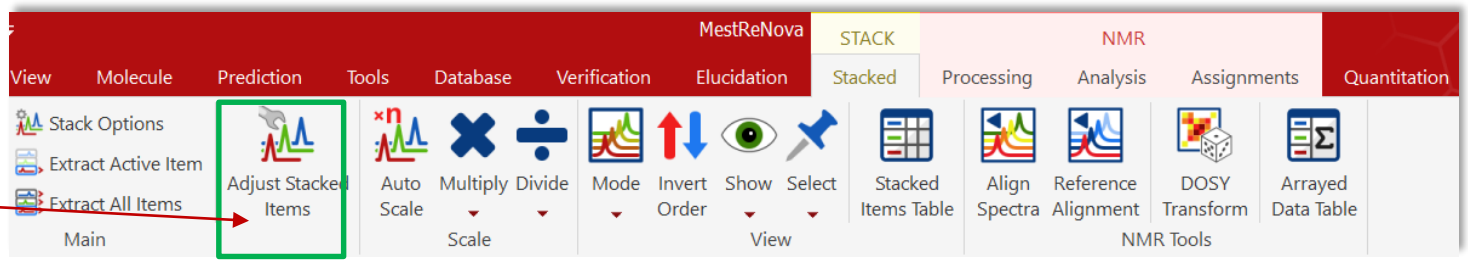
Tip: Read Help > Contents on more advanced data analysis, such as reaction monitoring, metabolomics, relaxation studies, DOSY processing etc.

Mestrelab Research
chemistry software solutions

STACKED SPECTRA

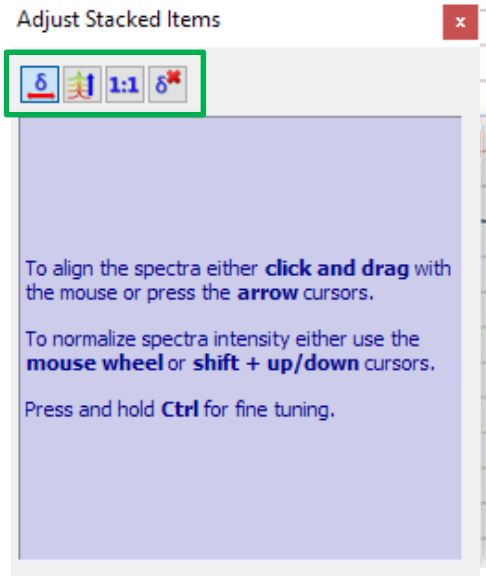
Handle the stacked spectra (2)

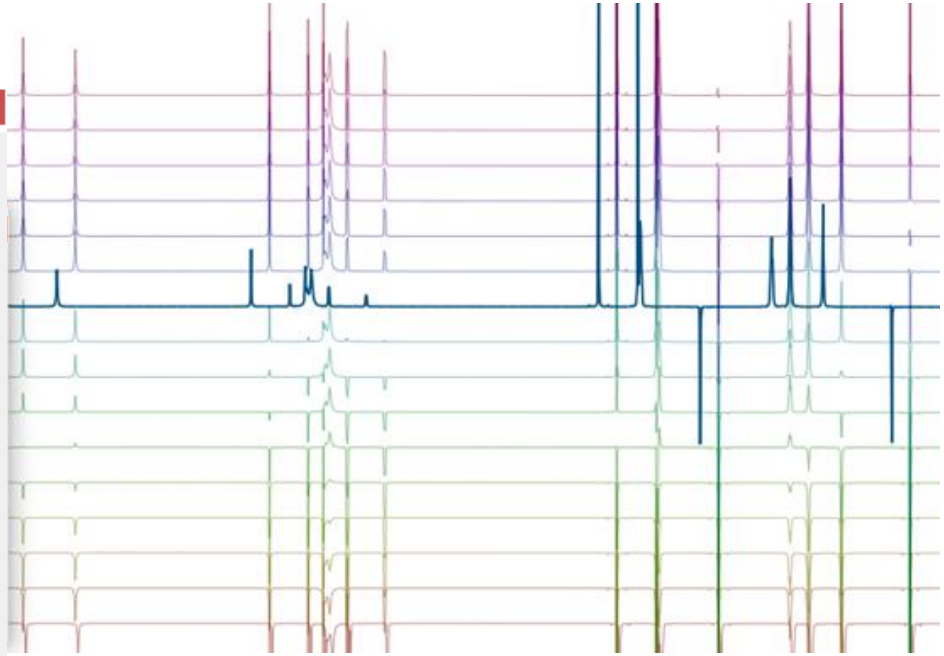
Click **Adjust Stacked Items**



The cursor has to be inside the blue dialogue box

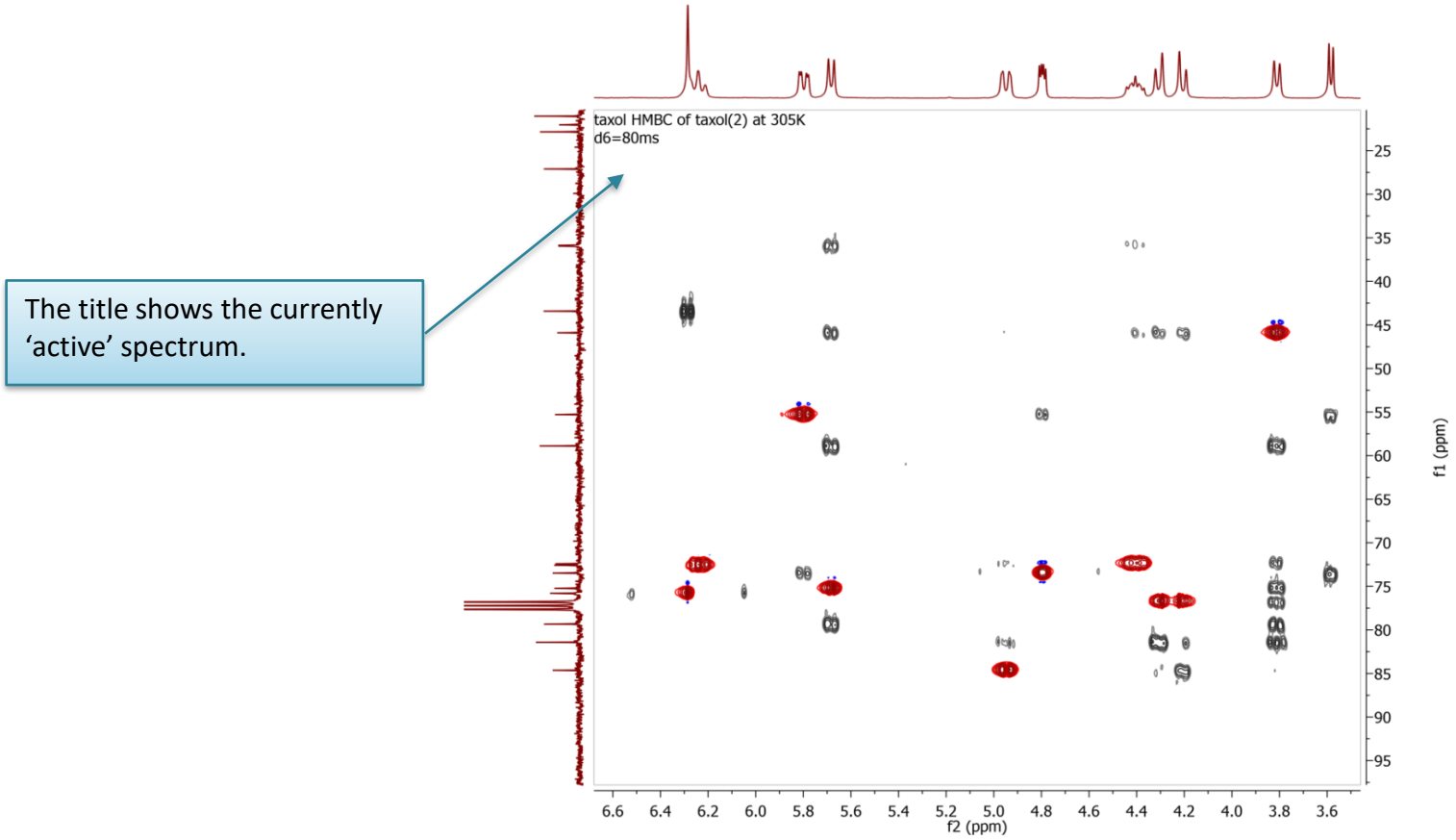
- Click&drag to shift an 'active' spectrum horizontally
- Click&drag to adjust the vertical offset between stacked spectra
- Reset intensities
- Reset shifts





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



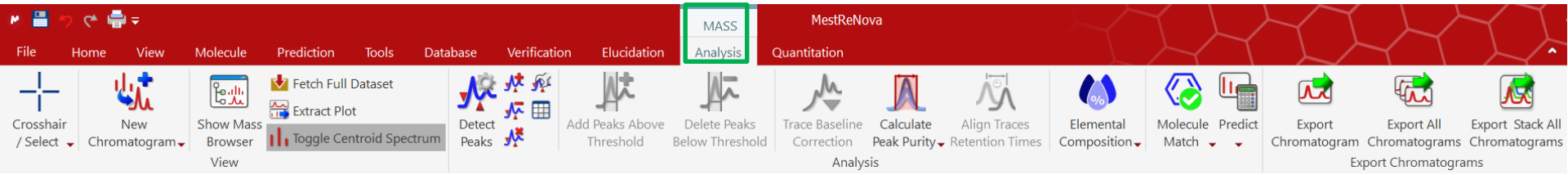
LC/GC-MS data formats compatibility

Vendor	Windows			Mac	Linux
Agilent	Chemstation	MassHunter	Ion Trap		
Waters	MassLynx	Compass	Openlynx	MassLynx	MassLynx
Thermo	Xcalibur	Exactive	Q-Exactive		
Bruker[1]	XMass	Compass		XMass	XMass
JEOL	MSQ 1000	FastFlight			
AC SCIEX	Analyst	Data Explorer			
Shimadzu[2]	LabSolutions v3	Labsolution v5			
mzData, mzXML	mzData, mzXML			mzData, mzXML	mzData, mzXML
NetCDF ANDI-MS	NetCDF ANDI-MS				
Advion Expression	Data Express				

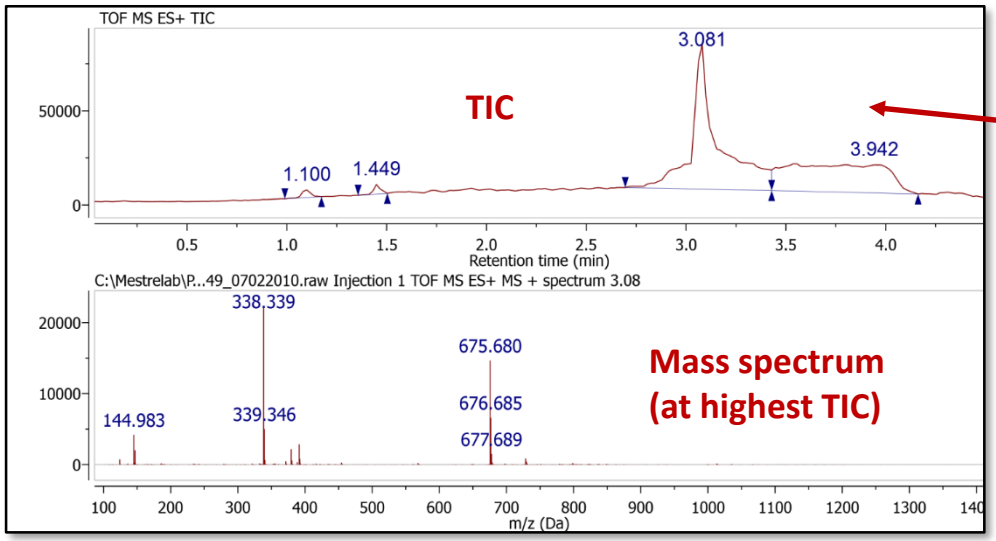
1. *Bruker software is required to be installed on the same computer (or users can download and install CompassXtract, as instructed* ***in*** *http://mestrelab.com/resources/bruker-compass-mnova-ms/).*
2. *LabSolutions software is required to be installed on the same computer*

Note: In all of the formats above, raw data can be opened in Mnova on the same computer with vendor software installed, convert it to an Mnova binary file, then send it to other users with Mnova. This can also be done in batch mode or in real-time using an Mnova script. This is an effective workaround for Mac and Linux users

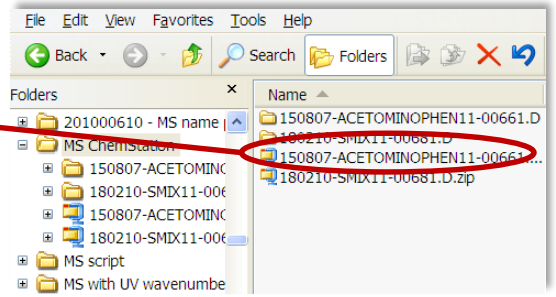
Open LC or GC-MS data



- 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&drop** the folder from Windows Explorer (or Finder) into Mnova
- Mnova will automatically convert your data and pick peaks

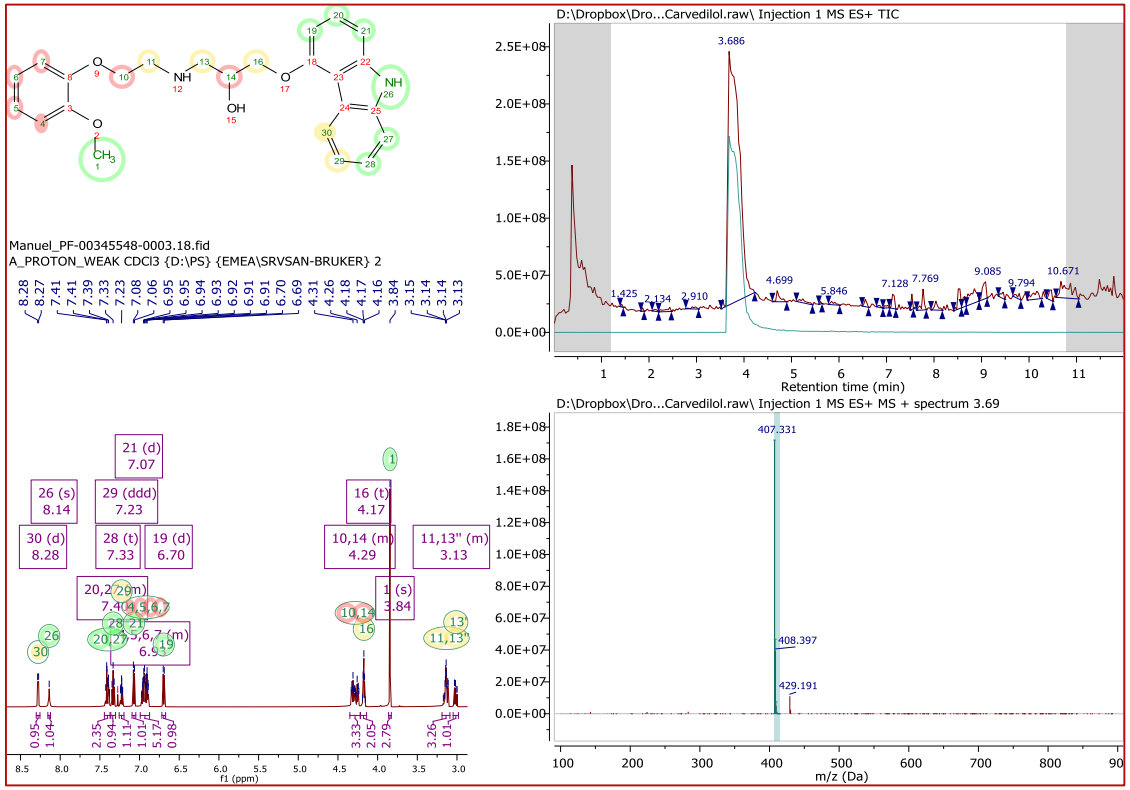


Drag & drop



Common interface for all analytical techniques

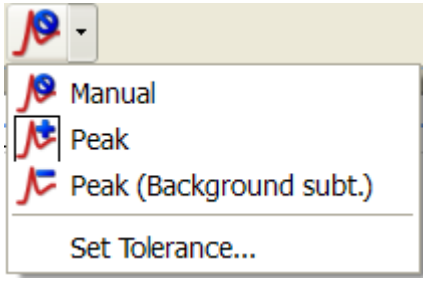
Easily combine your MS & NMR data on the page in an Mnova document

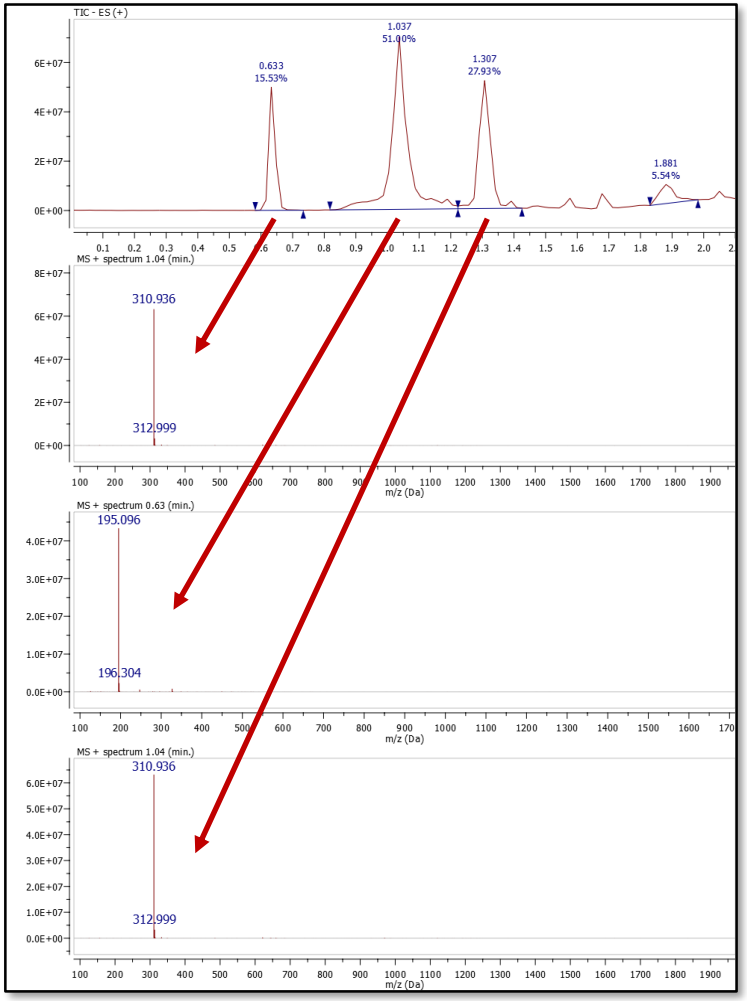


Note: When multiple spectral objects are opened they are loaded onto separate pages. You can copy (or cut) and paste them to the same page later
Tip: Use the 'Bring to Back/Front', 'Align', and 'Tile' tools to arrange objects nicely

Browse the mass spectra

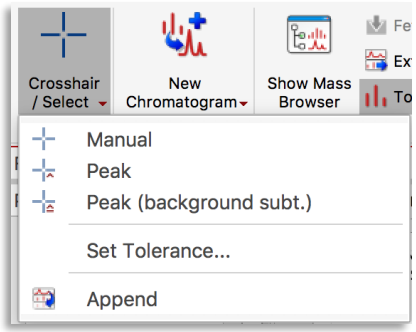
- Press  to switch to crosshair cursor, and click on the TIC to display the mass spectrum at that retention time, or click-and-drag to display co-added spectra.
- Press  to change to appending mode if you want to display multiple mass spectra.
- Choose the **Spectrum Selection Mode** to display mass spectra conveniently:
  - **Manual mode**: Click to display a single MS, or click-and-drag to co-add multiple MS.
  - **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.

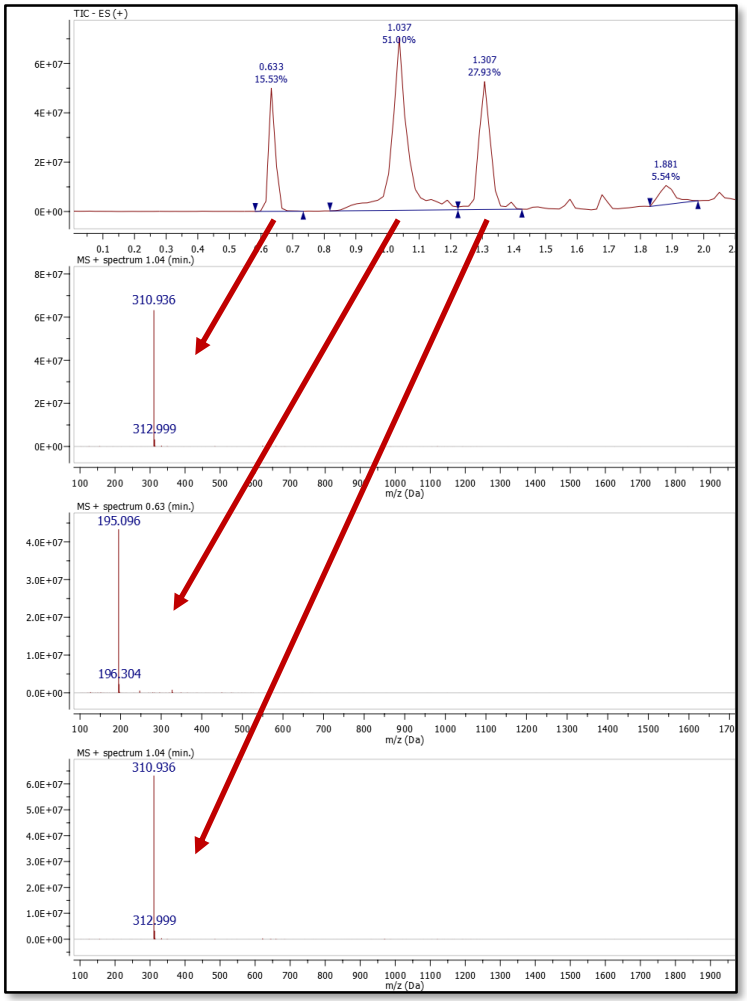




Browse the mass spectra

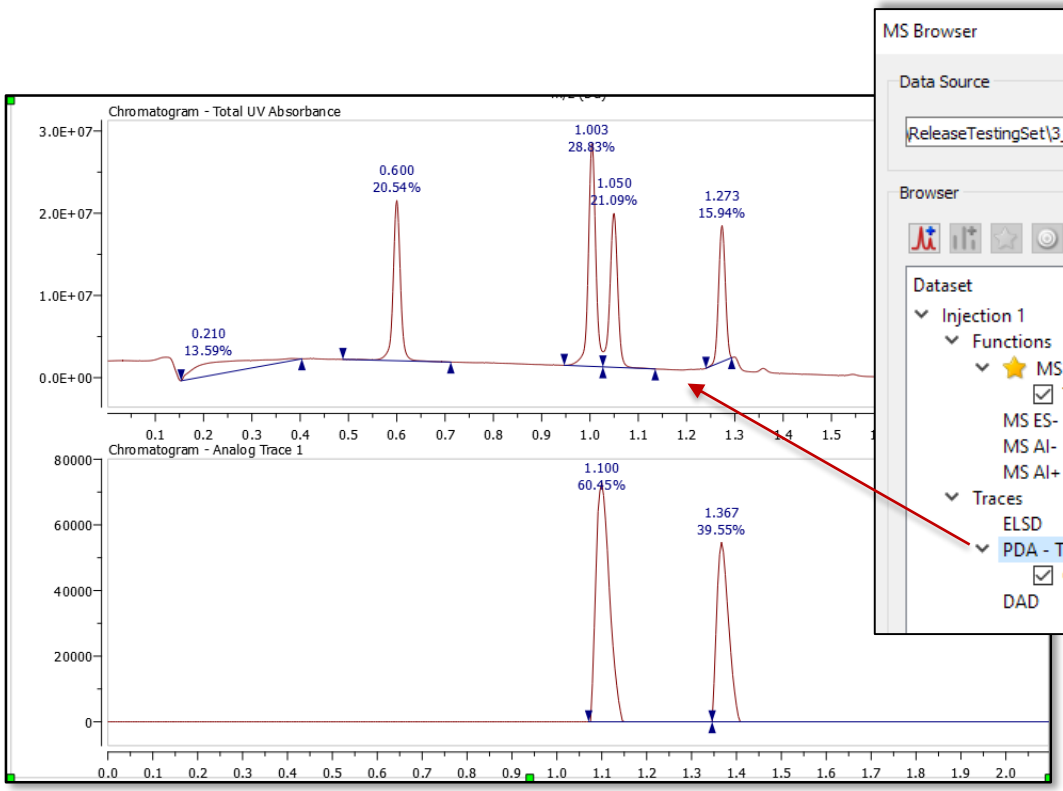
- Click  to switch to a crosshair cursor, and click on the TIC to display the mass spectrum at that retention time, or click-and-drag to display co-added spectra
- Press  to change to 'Append' mode to add a mass spectrum to the display
- Choose the **Crosshair/Select** drop-down menu to display mass 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

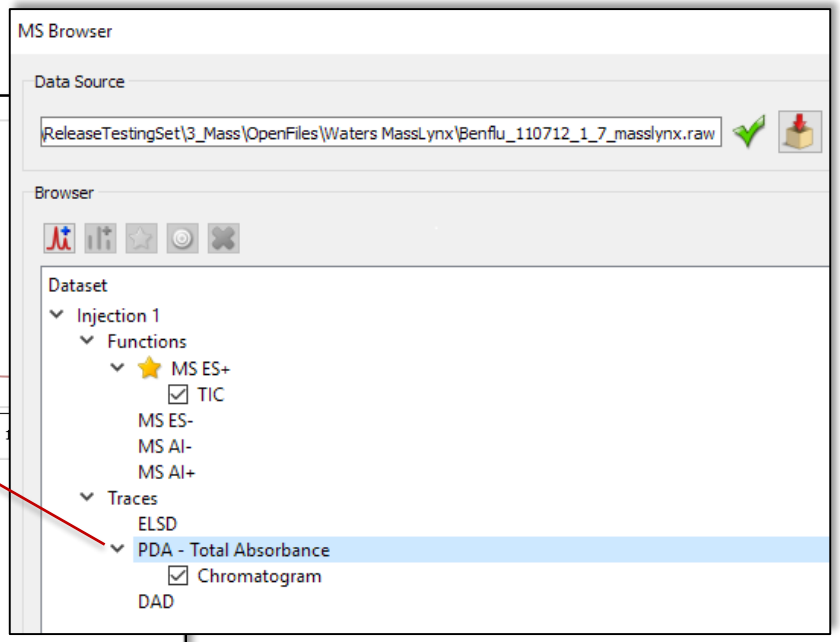




Browse UV traces

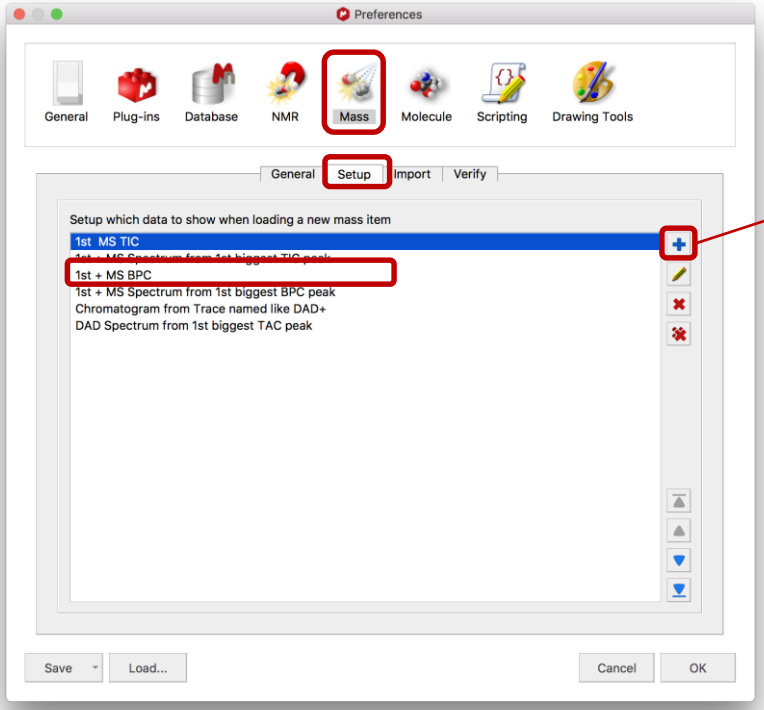
- Click  to show the MS Browser panel
- Double-click a 'Total Absorbance' item under 'Traces' to display a UV trace.
- Click  to add more UV traces to the display

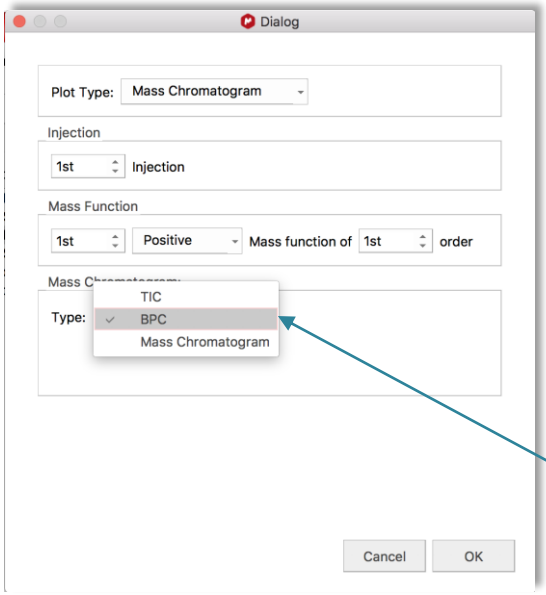




Setup MS import display preferences

- 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



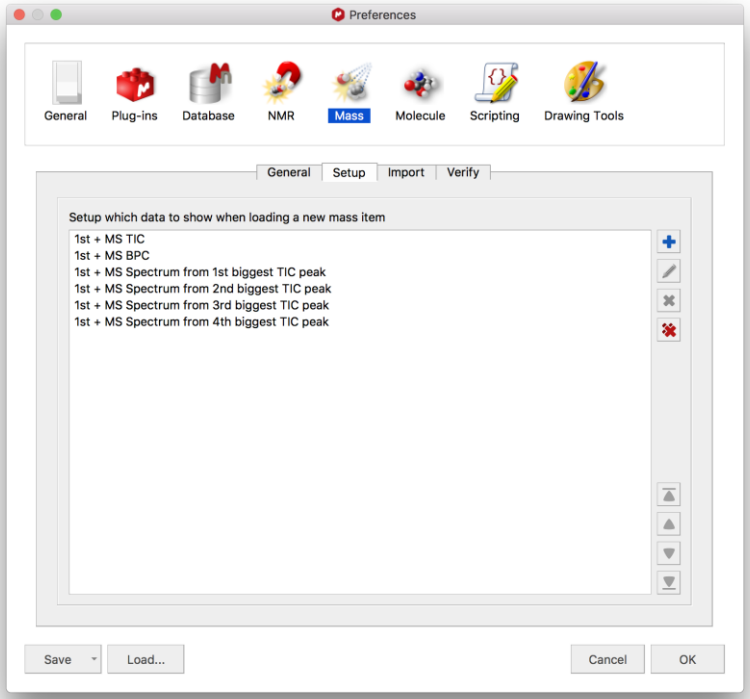


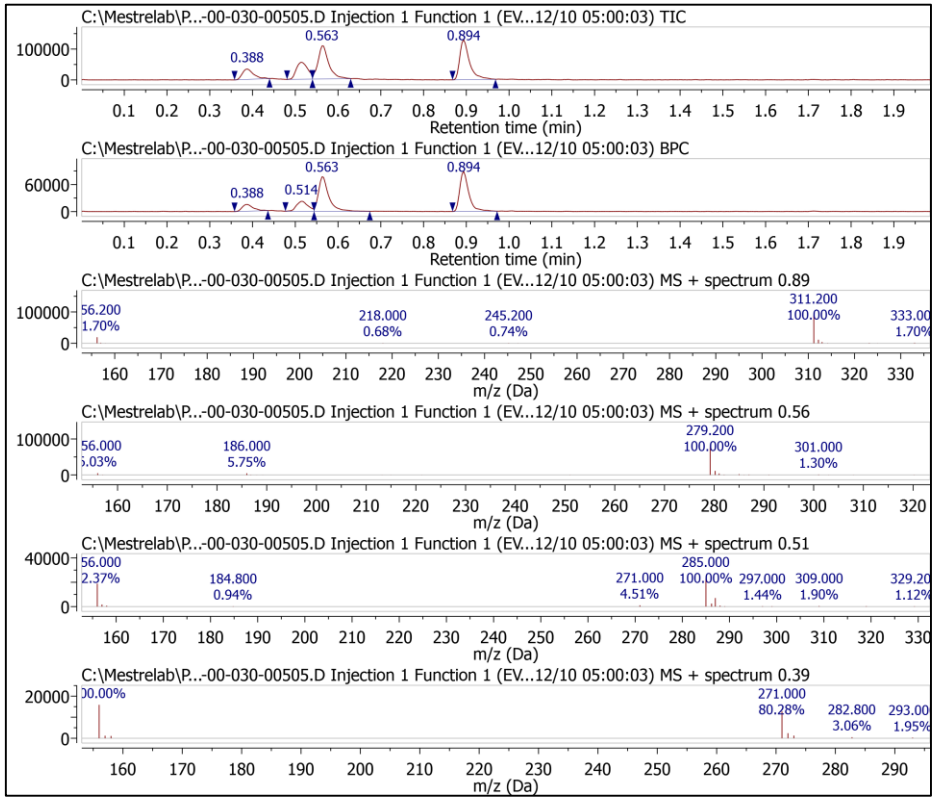
This dialog sets the display of the first positive base peak chromatogram (BPC) in the 1st injection (highlighted in the Preferences list)

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

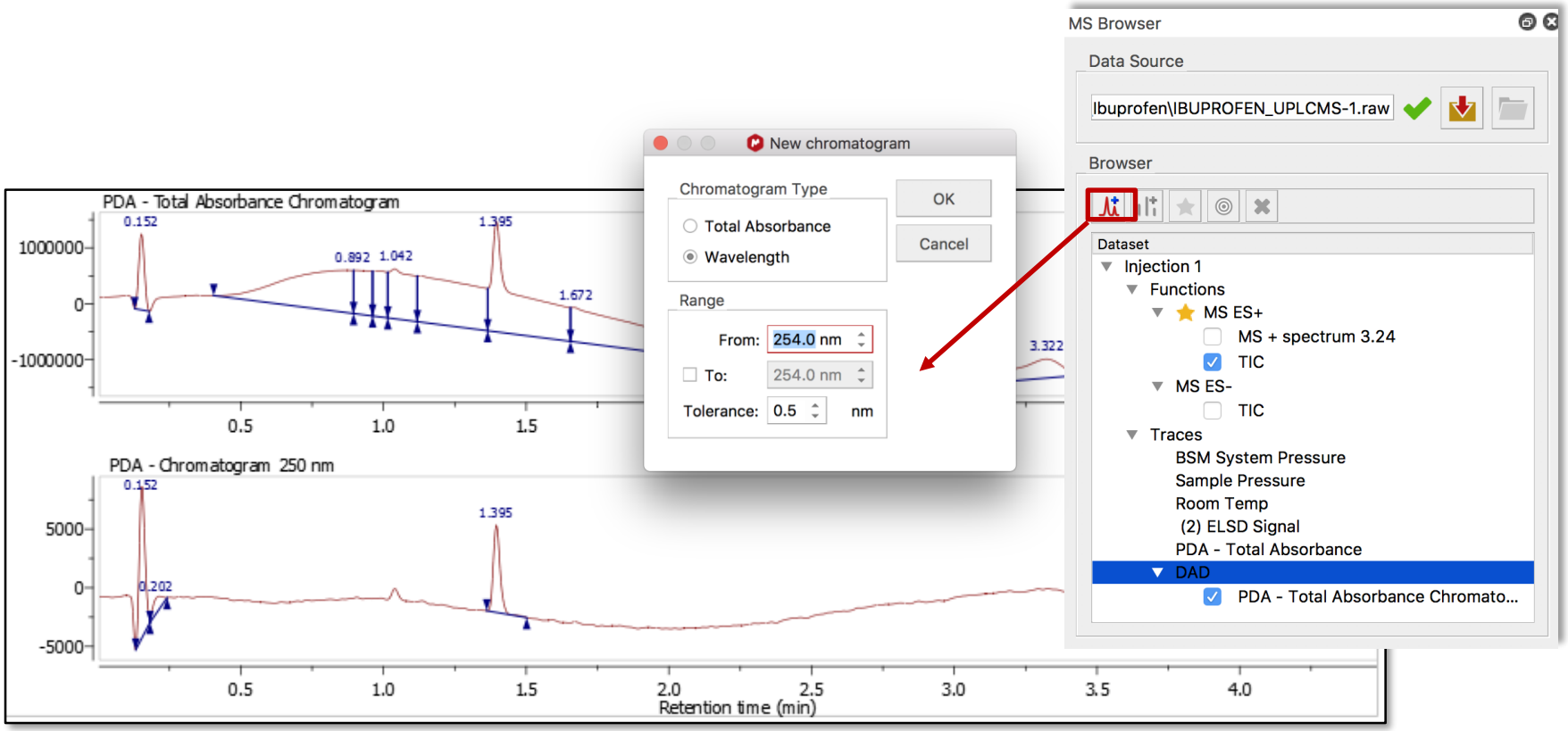
- Choose **File/Preferences**, click the Mass icon, then the Setup tab
- Define the display of the TIC, BPC, and the mass spec corresponding to the top 4 TIC peaks, as shown below
- Open a new MS dataset, and observe the display





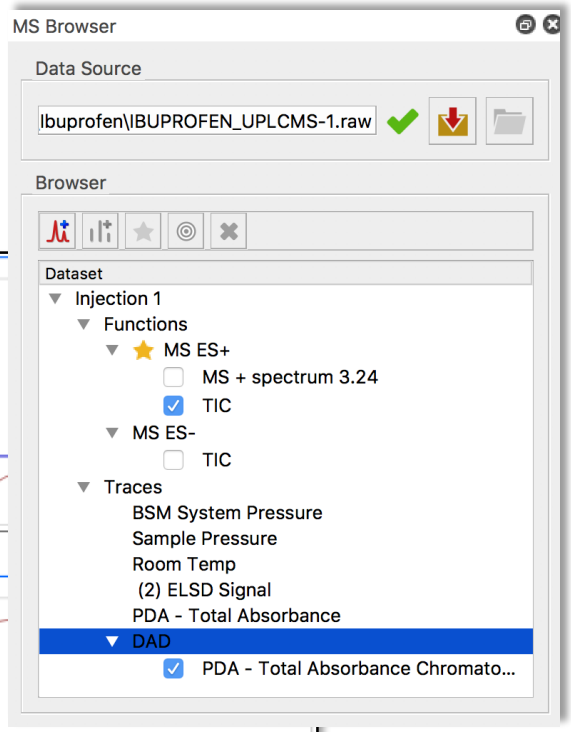
Extract a UV trace at selected wavenumber

- 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

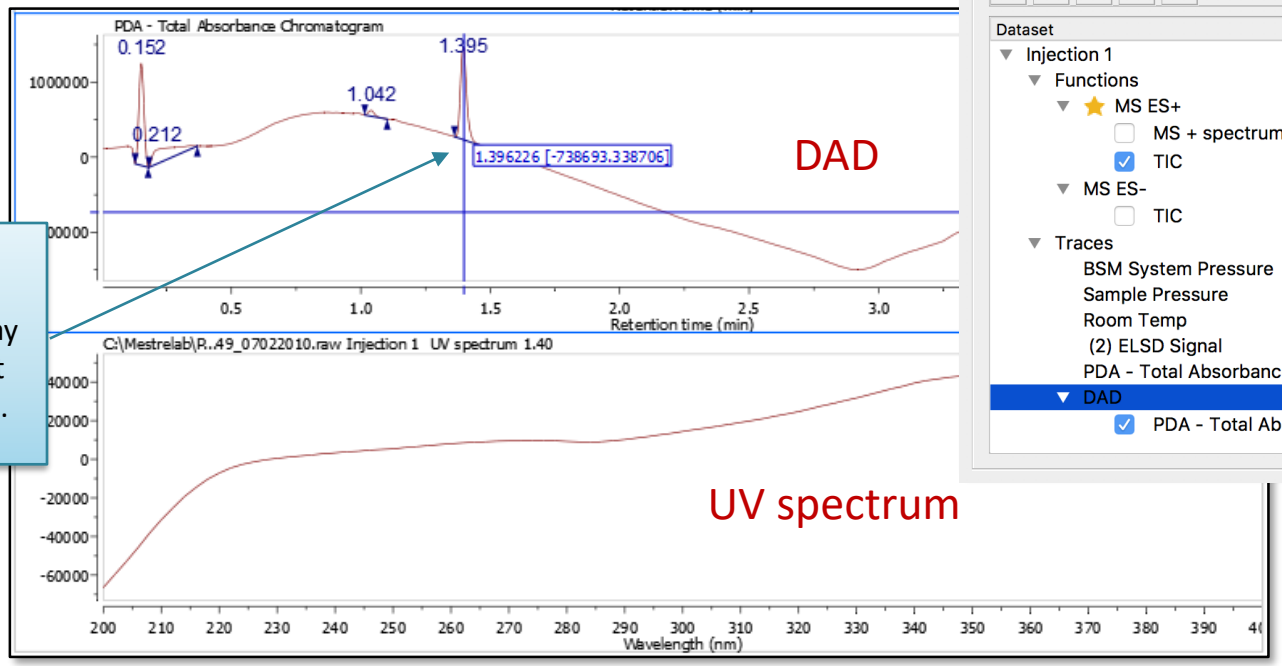


Display a UV spectrum at a selected retention time

- 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

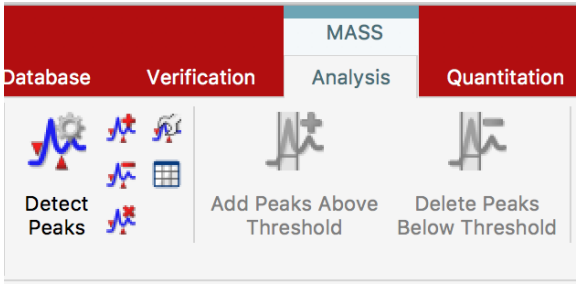


In crosshair cursor mode, click on the PDA curve to display the UV spectrum at that retention time.



Edit and report peak integration results

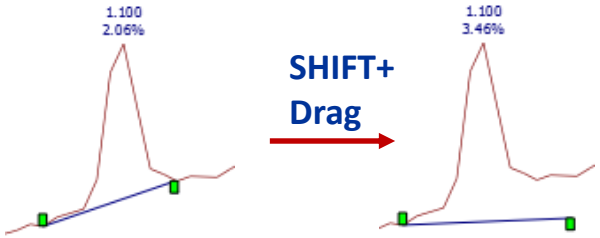
- Peaks are automatically integrated when you open a chromatogram
- Use the **Detect Peaks** ribbon icons to re-detect peaks, add, delete, or clear peaks
- Hover the cursor over a blue wedge, then click&drag the green boxes to change the range of a peak
- Or press **Shift +** click&drag green boxes to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



Mass Peaks

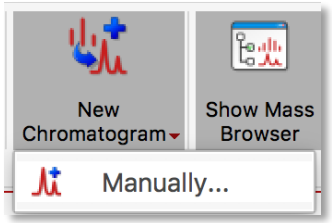
Report Copy Setup

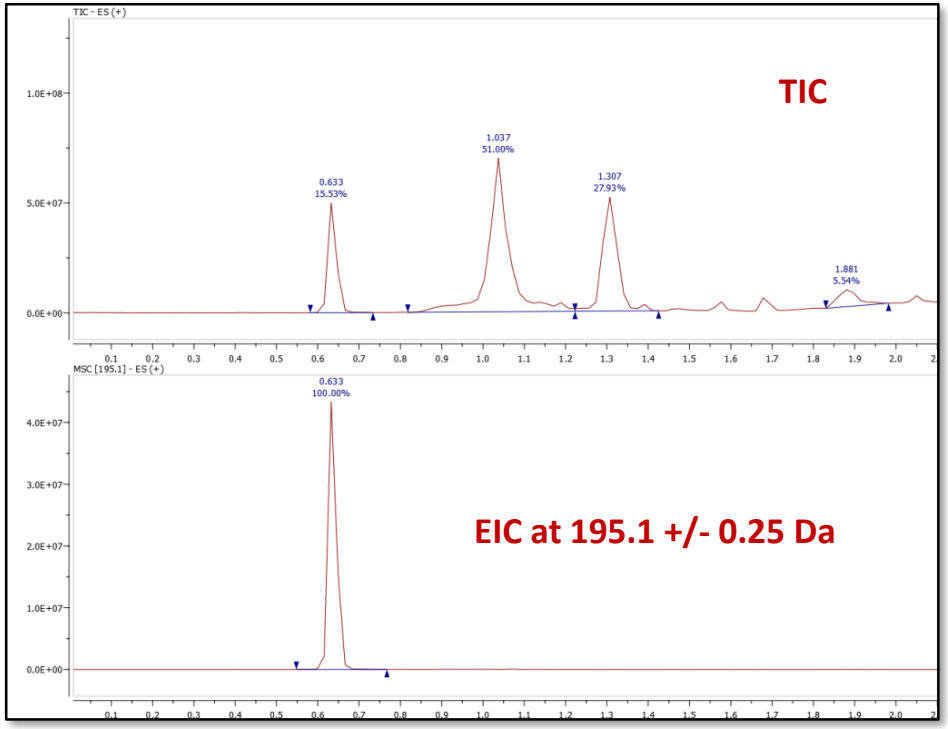
	RT	Scan	Type	Height	Area	Total Height %	Total Area %	Start time	End time
1	4.31	483	VB	65677.5	3184.9	1.52	0.67	4.312	4.392
2	4.14	464	BV	160655.3	31687.9	3.71	6.64	4.036	4.312
3	3.90	437	BB	227958.5	30589.4	5.26	6.41	3.787	4.018
4	3.75	421	BB	157293.0	3864.0	3.63	0.81	3.724	3.787
5	2.26	253	BB	3309241.0	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

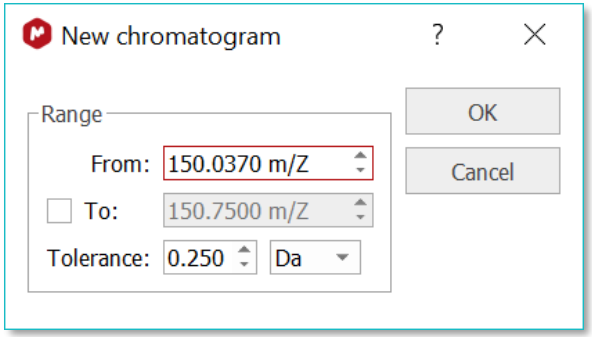


Display an extracted ion chromatogram from a specific *m/z* value

- 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 EIC

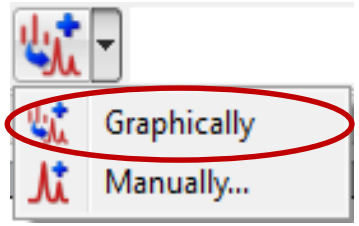




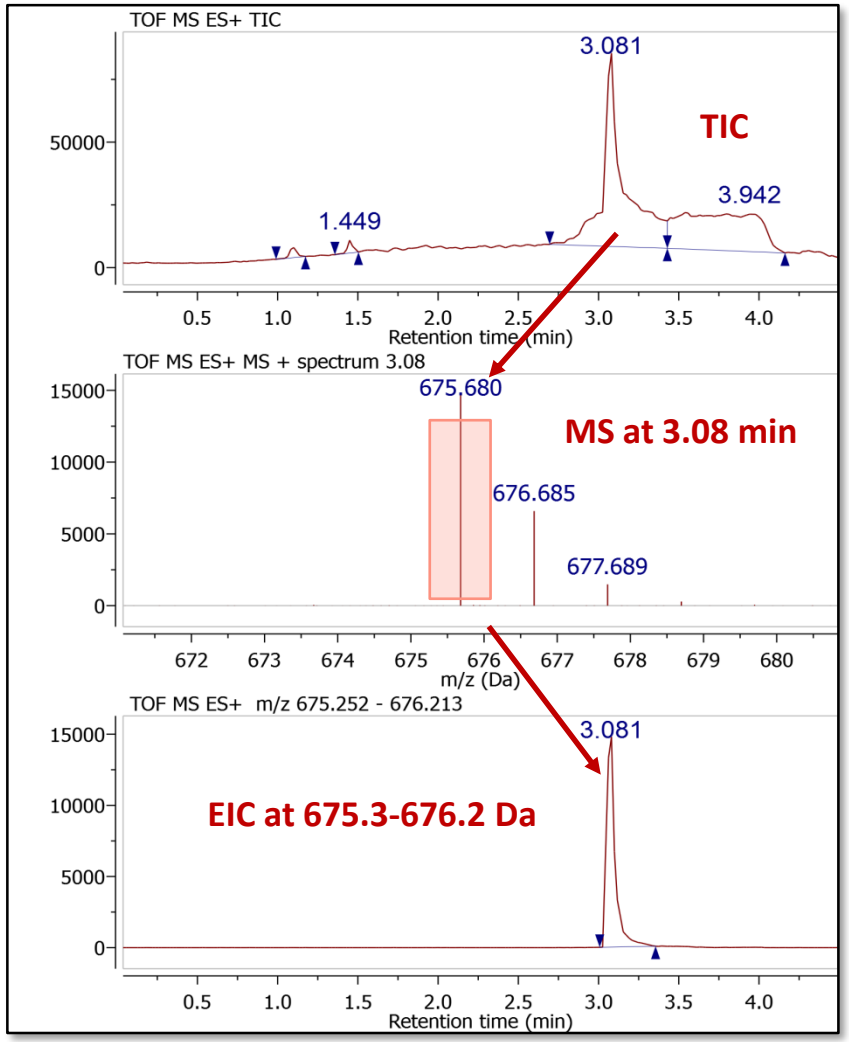


Tip: You can also go to Mass **Analysis/Spectrum Prediction** to run a mass prediction from a molecular formula.

Display extracted ion chromatogram for an MS peak

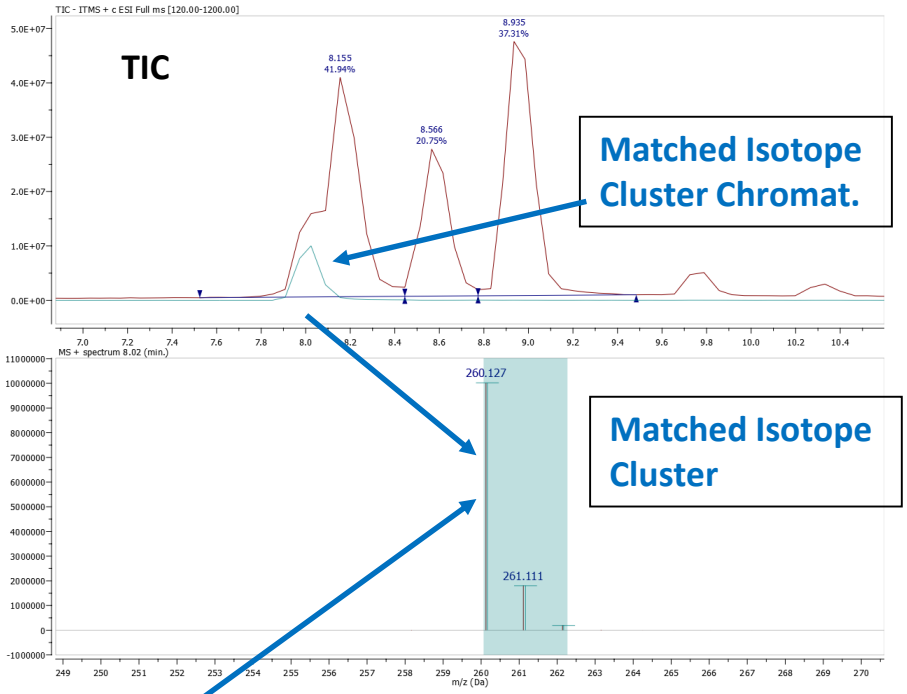


- First display the MS trace and zoom into the molecular ion peak that you are interested
- Next press (or go to **Mass Analysis/ New Mass Chromatogram/Graphically**), click-and-drag around the peak to define a mass range
- An EIC will be displayed within the mass range



Confirm proposed structures using Molecule Match (1)

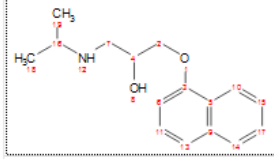
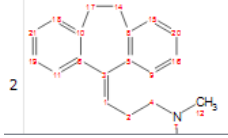
- Import one or several structures by copy/pasting from ChemDraw, Isis/Draw or ChemSketch, or by opening .mol or .sdf files
- Click  (or go to **Mass Analysis/ Molecule Match/Calculate from molecules**)
- In the Molecule Match Table, click on a molecule to see the matching results



Molecule Match

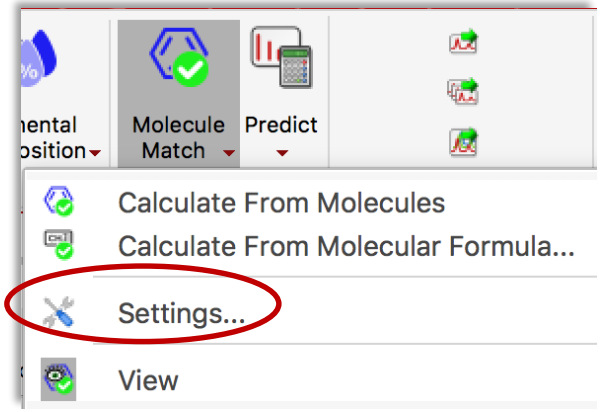
Report | Calculate | View | Settings | Setup

Mol Match Results

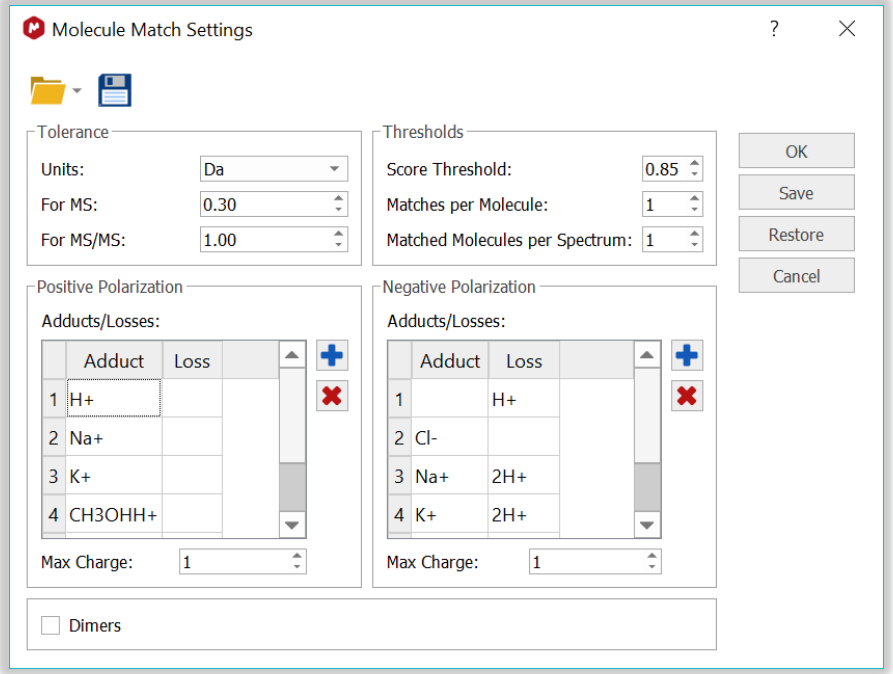
	Molecule	Formula	ecular Wei	Match	latch Score	Similarity	MS Purity	RT	Scan	Purity
1		$C_{16}H_{21}NO_2$	259.157	✓	1.000	1.000	0.756	8.02	171	10.00%
2		$C_{20}H_{23}N$	277.183	✓	1.000	1.000	0.450	8.57	180	11.39%

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

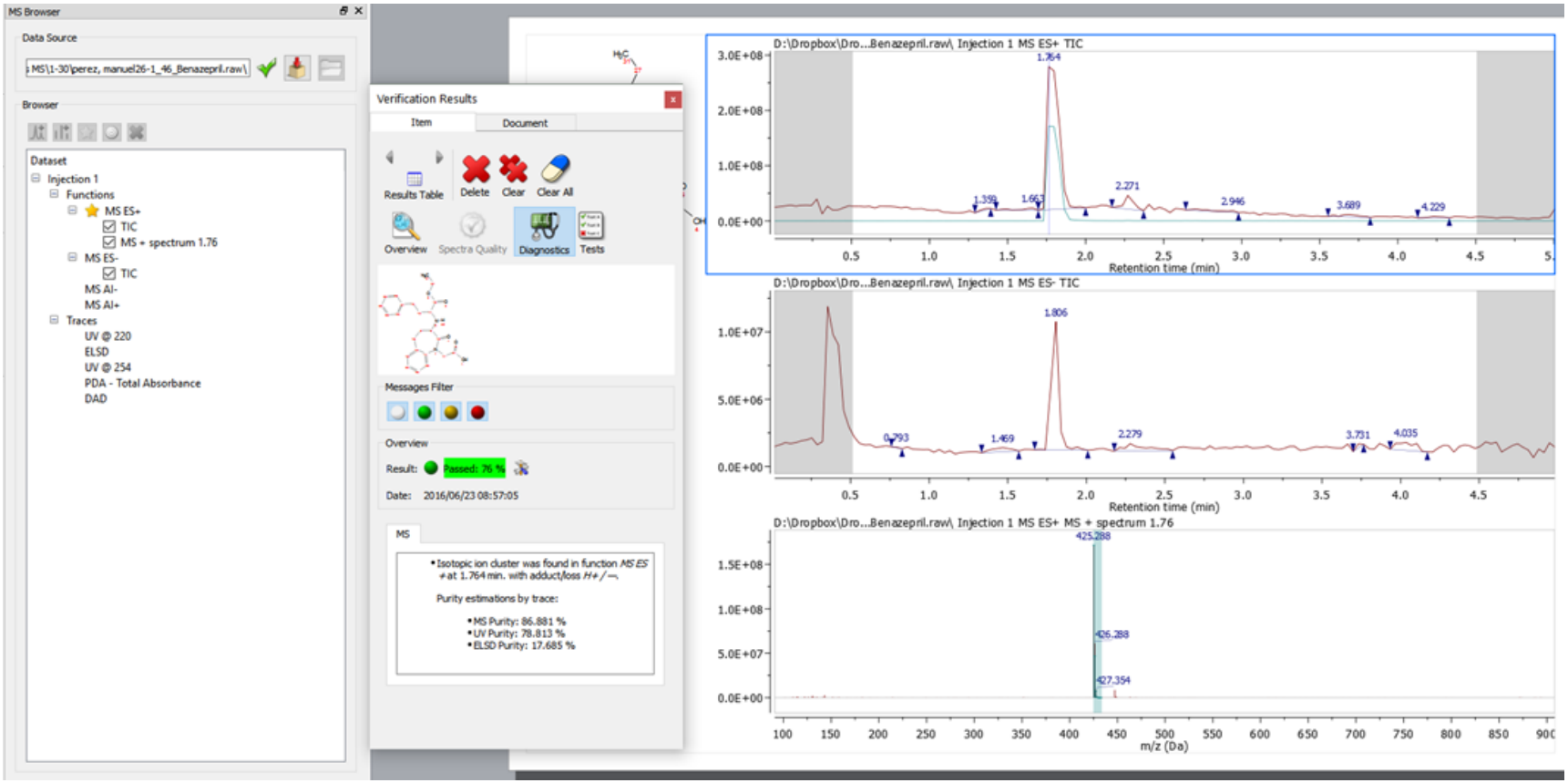


Tip: Click the "+" buttons to add a new adduct. Enter "+" for a radical cation. Highlight one and click the "x" button to remove it. Click Restore to reset to the default or previously saved settings.



MS driven structural verification

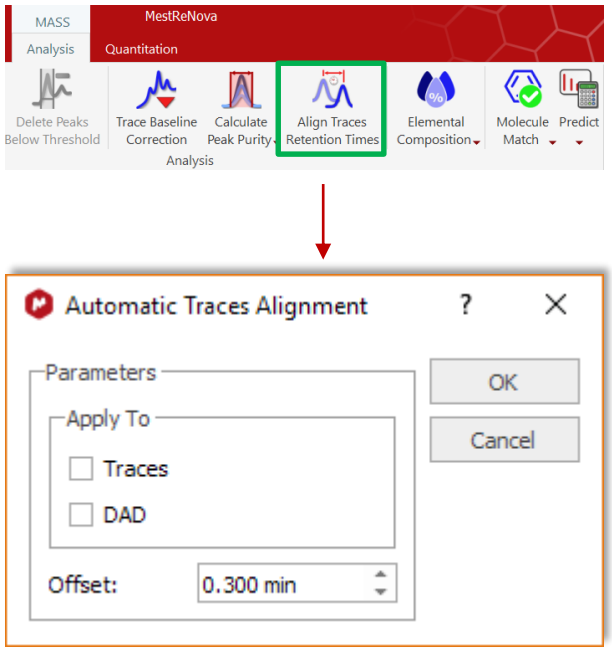
MS data can be combined with NMR data to improve structure verification results *

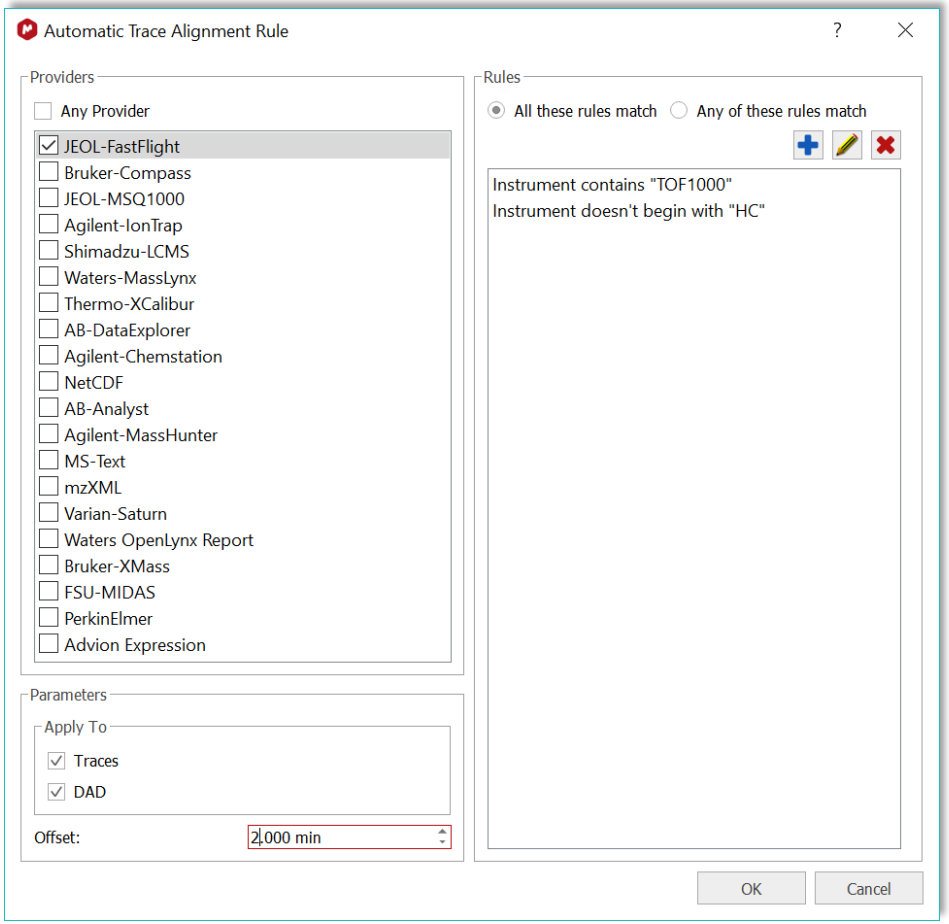


A separate Mnova Verify license is required

Automatic trace alignment: Instrument-specific

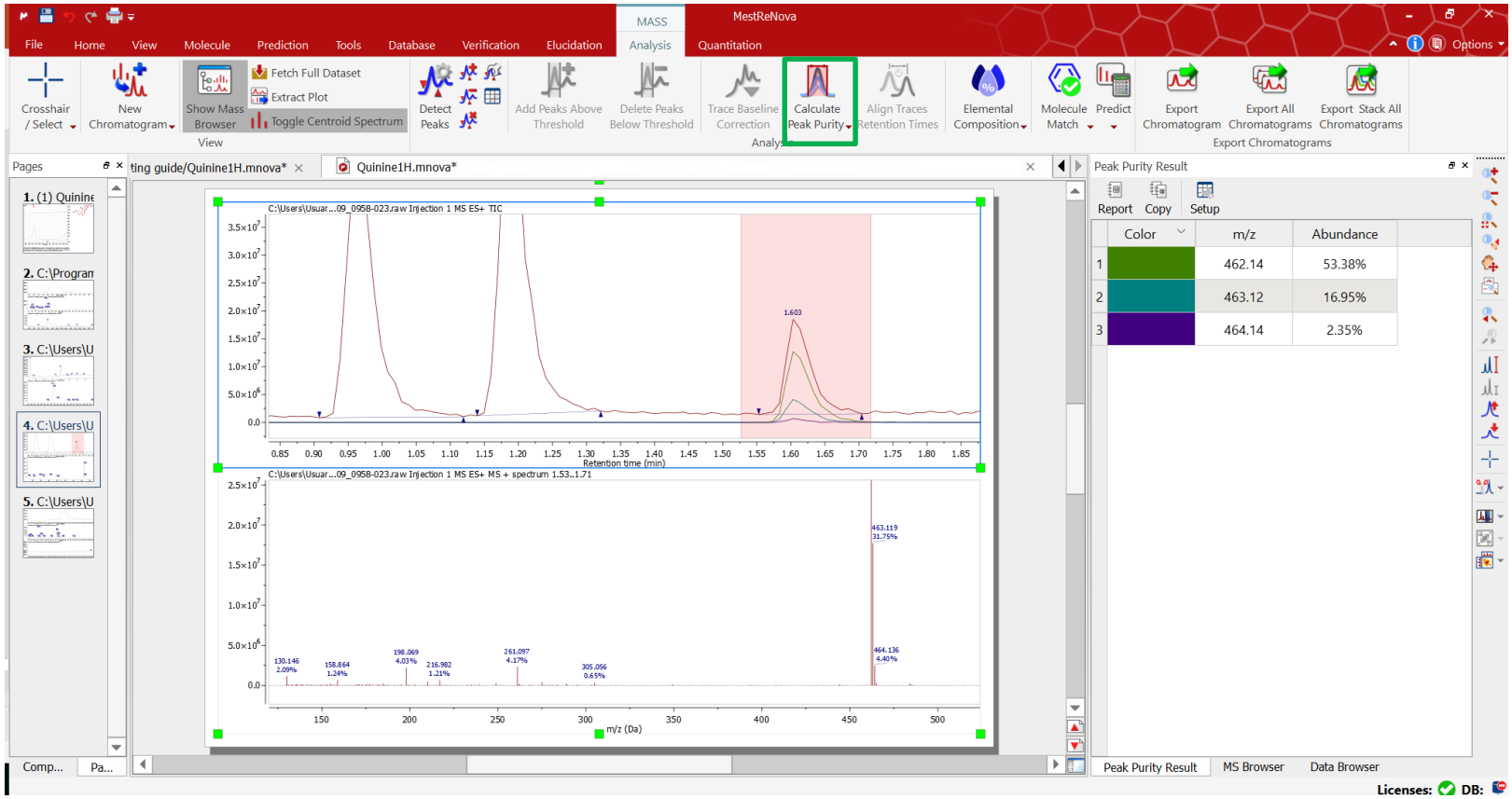
- Align a DAD, or another trace, to a TIC using the auto-alignment settings
- Set the rules to specifically identify the instrument and apply the correct alignment automatically





Peak purity calculation

- It shows the curves associated to the most abundant mass peaks under the selected chromatogram peak



Just the tip of the iceberg!

Thank you for your time!

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

Mestrelab Research

chemistry software solutions

Mnova 12 – Starting guide

Thank you for trusting our software solutions!