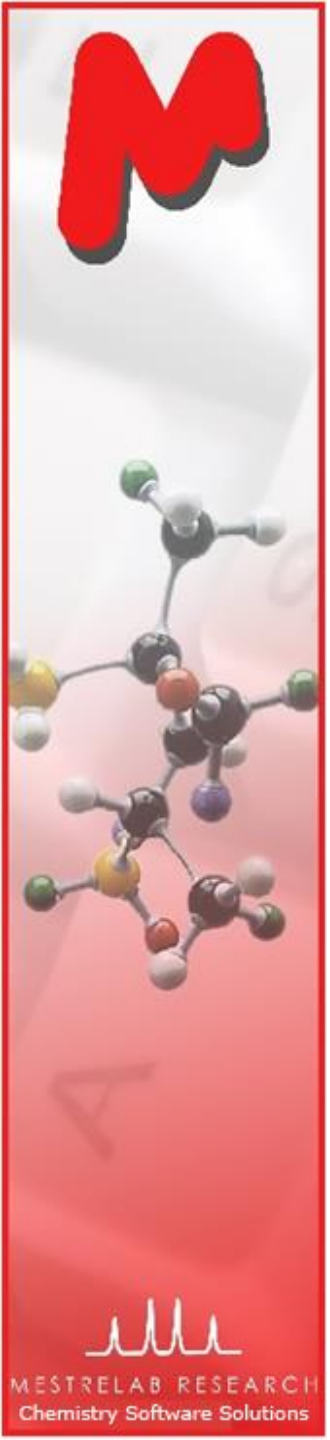

A Training for Chemists: Using Mnova to Process, Analyze and Report NMR on Your Desktop

Version 8.1
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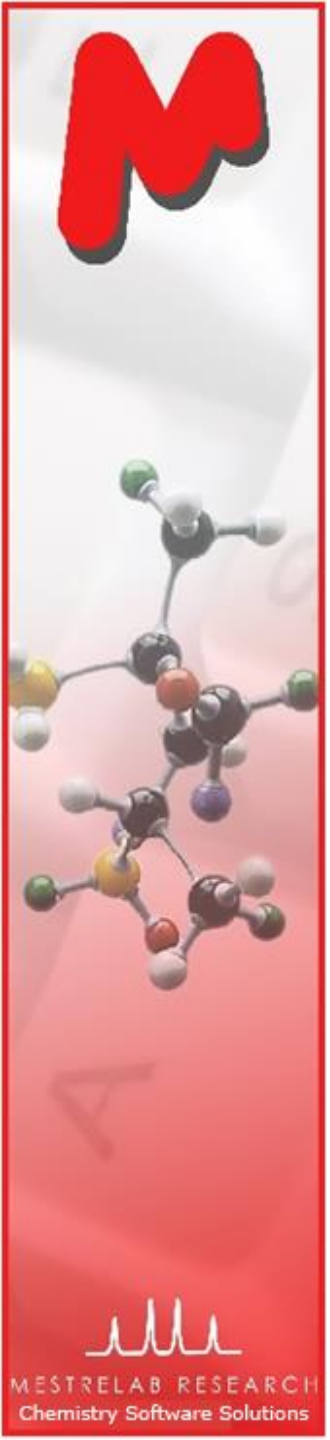
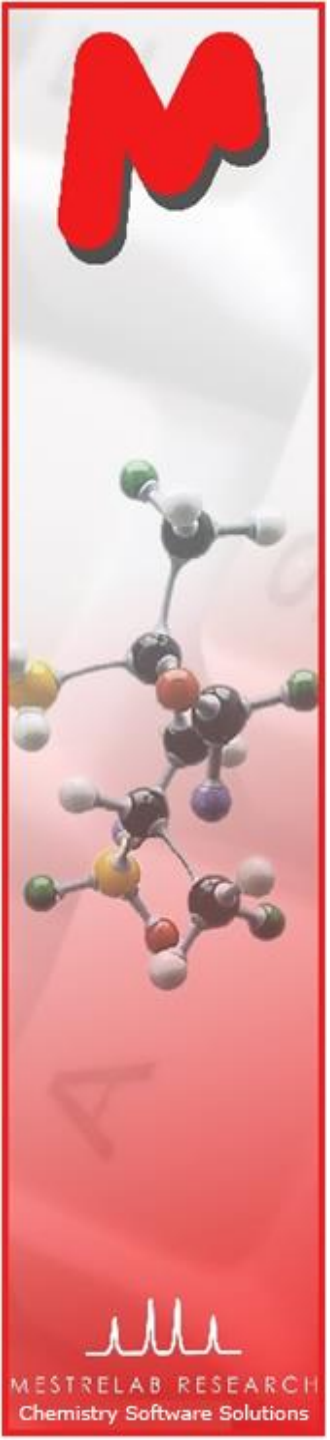


Outline

- Overview of Mestrelab and Mnova
- Open and process 1D and 2D NMR data
- Multiplet Analysis for 1D H-1 NMR
- Report analysis results
- Basic handling of multiple 1D and 2D spectra

Note: Only the basic features of Mnova NMR (and NMRPredict Desktop) are covered in this tutorial. For information about the advanced features of Mnova NMR, see http://mestrelab.com/software/mnova-nmr/. For information about other plugins in Mnova, such as MS, DB, Verify, qNMR, and Screen, please see http://mestrelab.com/software/ or write to chen.peng@mestrelab.com.

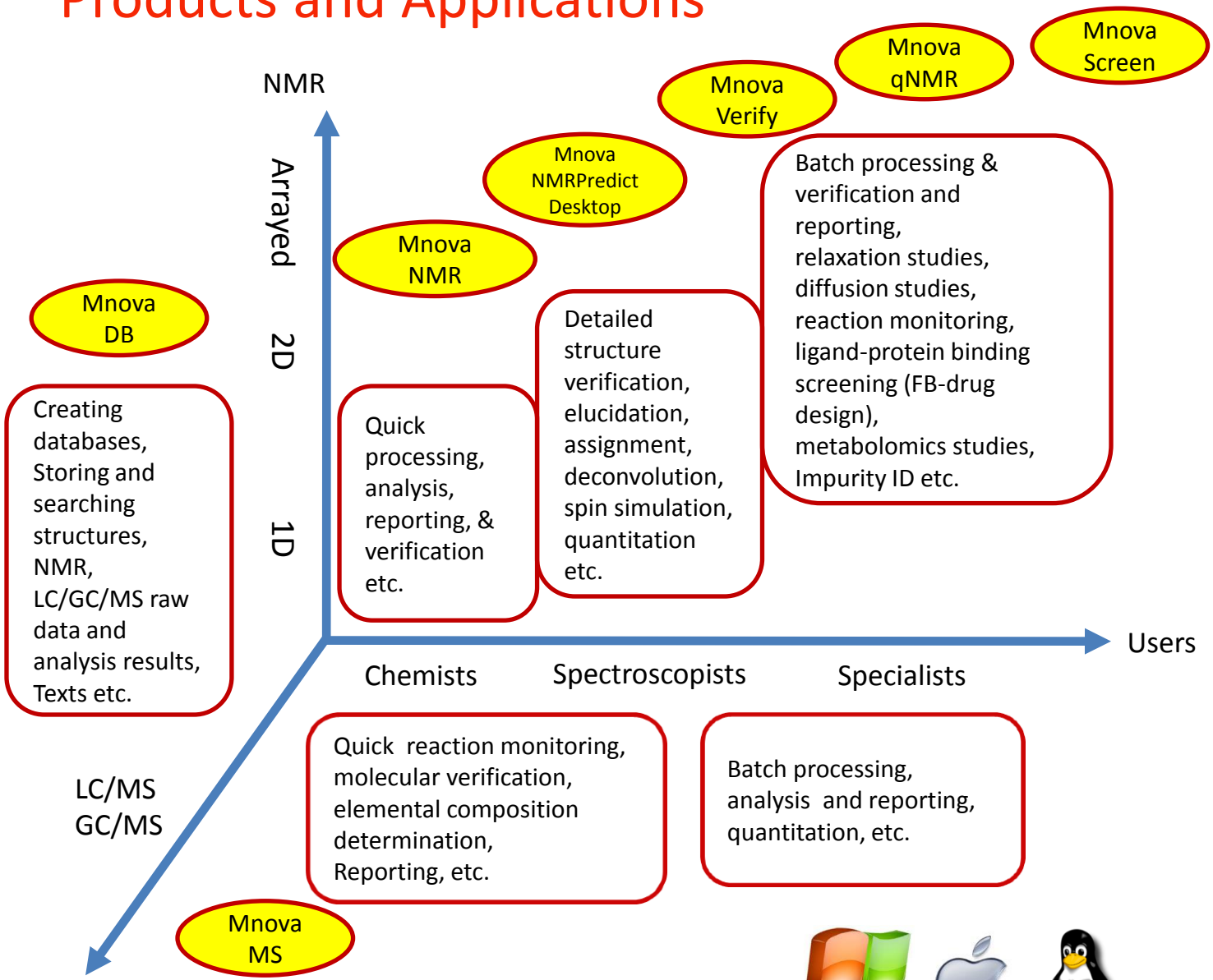


Mestrelab Research

- 1996: A research project in University of Santiago de Compostela, Spain, developed free **MestReC** software for NMR processing
- 2004: **Mestrelab Research** incorporated in Santiago de Compostela
- 2004: New **MestreNova (Mnova)** platform and **NMR** plugin released
- 2006: **NMRPredict Desktop** plugin released with Modgraph
- 2009: **LC/GC/MS** plugin released with Sierra Analytics
- 2009: Global Spectral Deconvolution (**GSD**) algorithm released with ExtraByte
- 2011: **DB** plugin for Database Management
- 2012: **Verify** plugin for auto structure verification and peak assignment
- 2012: **qNMR** plugin for quantitative NMR analysis
- 2013: **Screen** plugin for high-throughput ligand-protein binding analysis - to be released
- An **R&D company** with >20 people and >80,000 registered users

www.mestrelab.com

Products and Applications



Mnova is compatible with Mac, Windows and Linux

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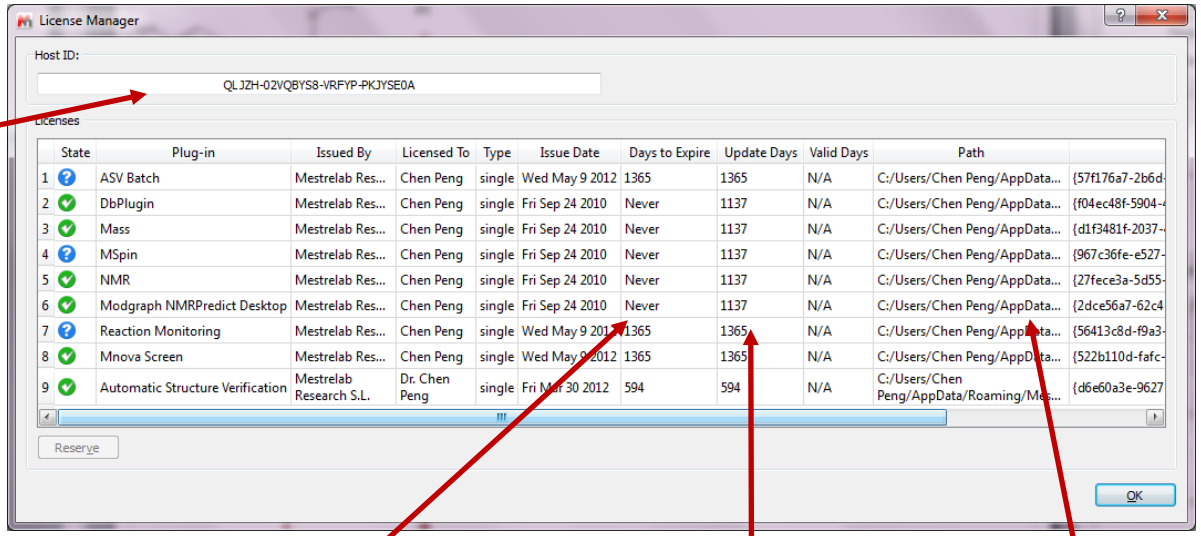
Before you start

- Make sure Mnova is properly installed and licensed on your computer.
- If you don't have license, you can download, install and free trial for 45 days*: http://mestrelab.com/software/mnova-suite/download/
- To verify your licensing status, choose **Help > License Manager**



Host ID (unique for this computer)

Make sure you have a positive number or "Never" (for perpetual license) here

Days before your Update and Support package expires**

Location of the license files

There is no difference between the free trial version and the released version of Mnova.

***If your Update and Support package has expired, you can only run the versions of Mnova that were released before the expiration date. Find previous versions of Mnova at http://mestrelab.com/software/mnova-suite/download/versions/.*

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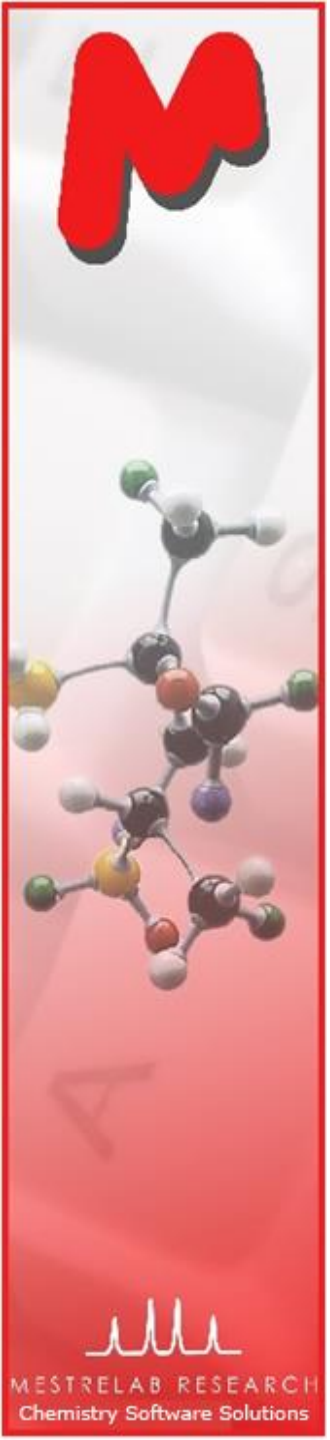


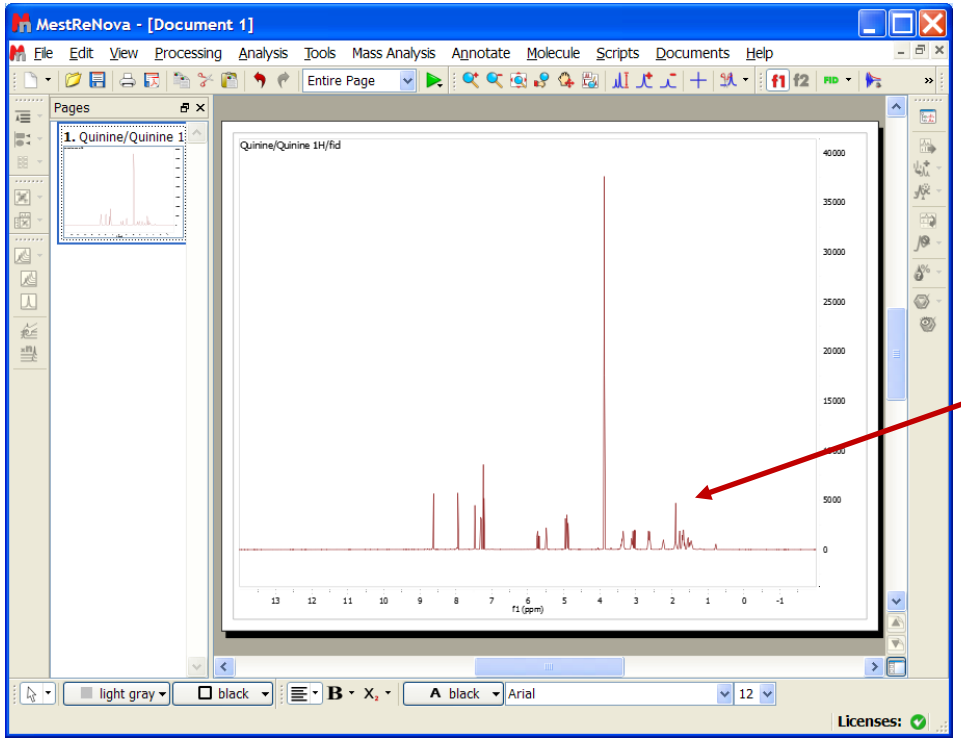
Sample data sets

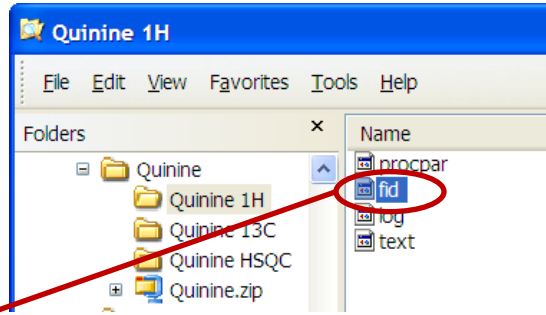
- A set of 1D ^{1}H, ^{13}C, 2D HSQC, and LC/MS* raw data of quinine are included when Mnova is installed. So is the .mol file. You can find them in a folder similar to the following:
  C:\Program Files (x86)\Mestrelab Research S.L\MestReNova\examples\datasets
- These data are used in many of the examples shown in this tutorial.
- When you use your own data, make sure you have all the original files in the folder of an NMR or MS experiment.

**You need the Mnova MS license to open and analyze LC/MS data. See http://mestrelab.com/software/mnova-ms/ for details.*

To open and transform your NMR data

- Choose **File | Open** to open the **fid** (or **ser**) file from the raw data
- Or drag an **fid** file from a file browser to Mnova *
- Mnova automatically transforms the raw file into frequency domain (*including Windowing function, Fourier transform, phase correction etc)* **





Drag & drop

You can drag **multiple folders that contain **fid** (or **ser**) files to Mnova to open multiple spectra simultaneously.*
Parameters from the raw data are used for processing. You can control the importing of some parameters (zero filling, phasing, baseline correction etc) by choosing **Edit > Preferences > NMR > Import**. You can view or change the processing parameters by choosing **Processing | Processing Parameters.

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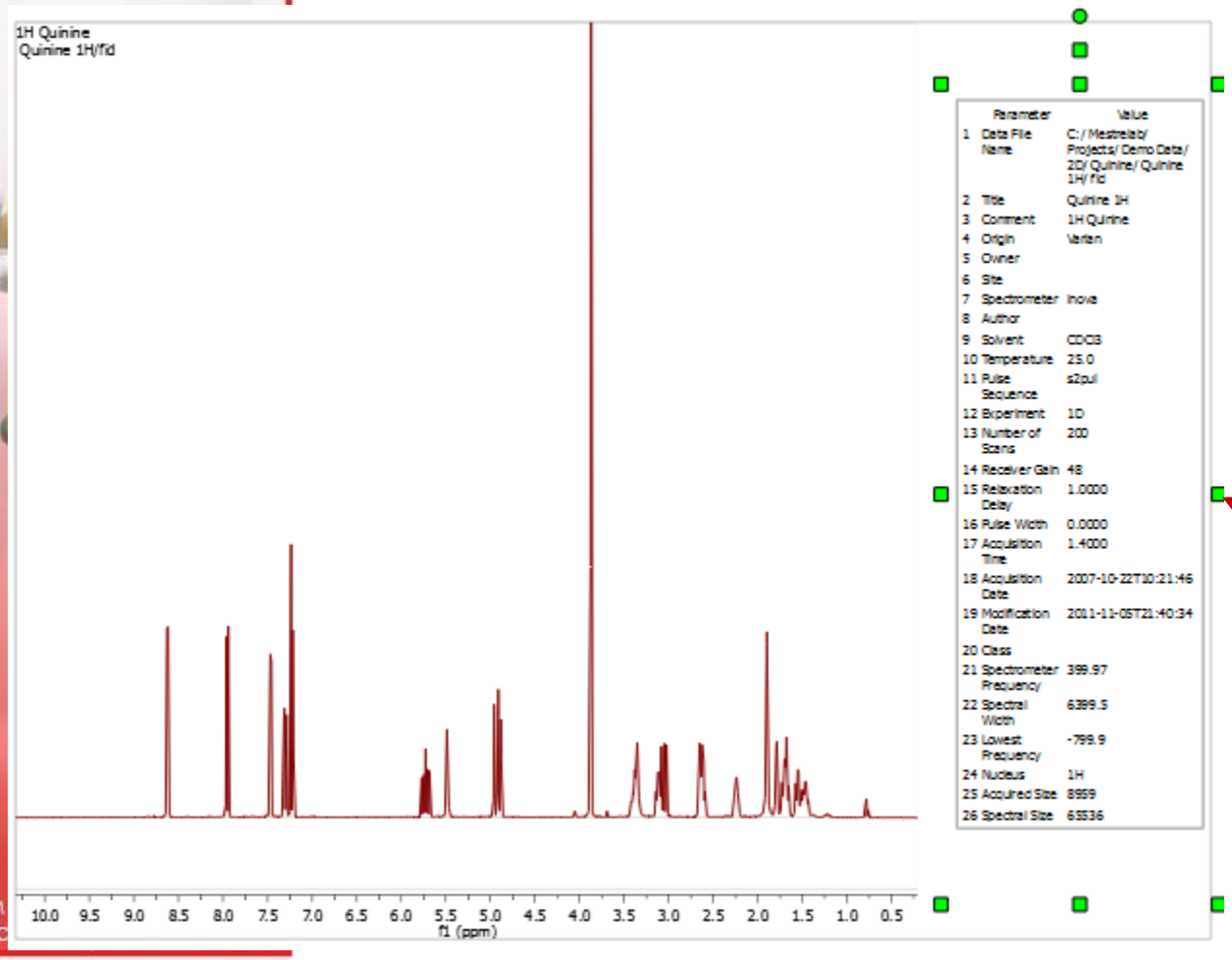
To see the parameters

- Choose **View | Tables | Parameters** to view the acquisition and processing parameters
- Click **Report** to report the parameters as a text box on the spectrum. Resize the text box and spectrum to make a better layout



Parameters

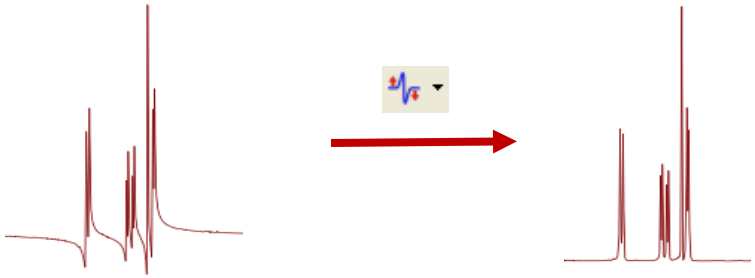
Report | Copy | Setup | Customize

	Parameter	Value
1	Data File Name	C:/Mestrelab/Projects/Demo Data/2D/Quinine/Quinine 1H/fid
2	Title	Quinine 1H
3	Comment	1H Quinine
4	Origin	Varian
5	Owner	
6	Site	
7	Spectrometer	inova
8	Author	
9	Solvent	CDCl3
10	Temperature	25.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Number of Scans	200
14	Receiver Gain	48
15	Relaxation Delay	1.0000

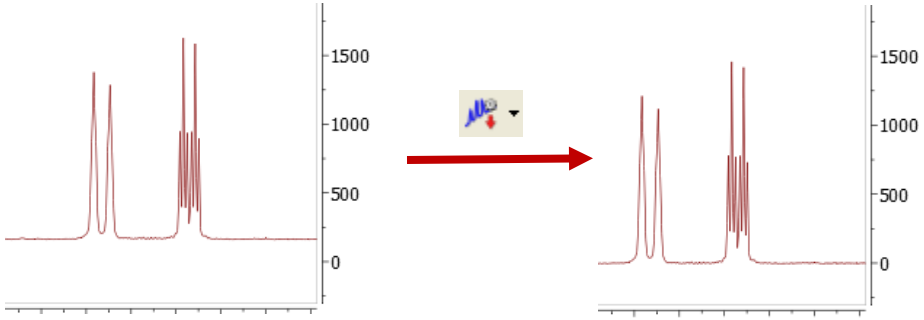
Use the green handles to move, rotate and resize the text box

To correct phase, baseline & reference

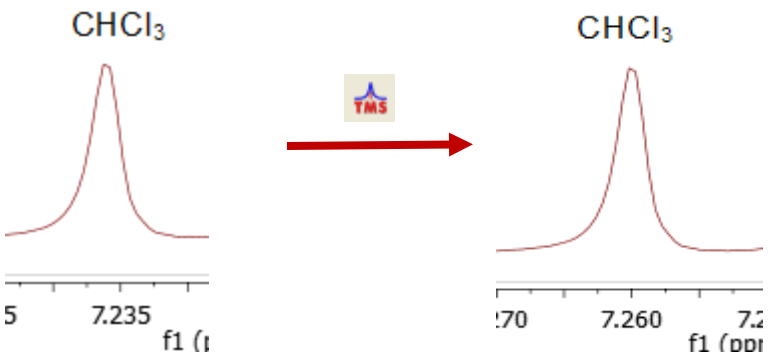
- Click  for **phase correction** if peaks are not symmetric*



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



- Click  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*

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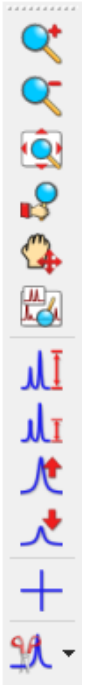
To visualize your spectrum



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&drag to draw an inset (or press E)

Fit to Highest Intensty (or press H)

Fit to highest compound peak

Increase Intensity (or rotate mouse wheel)

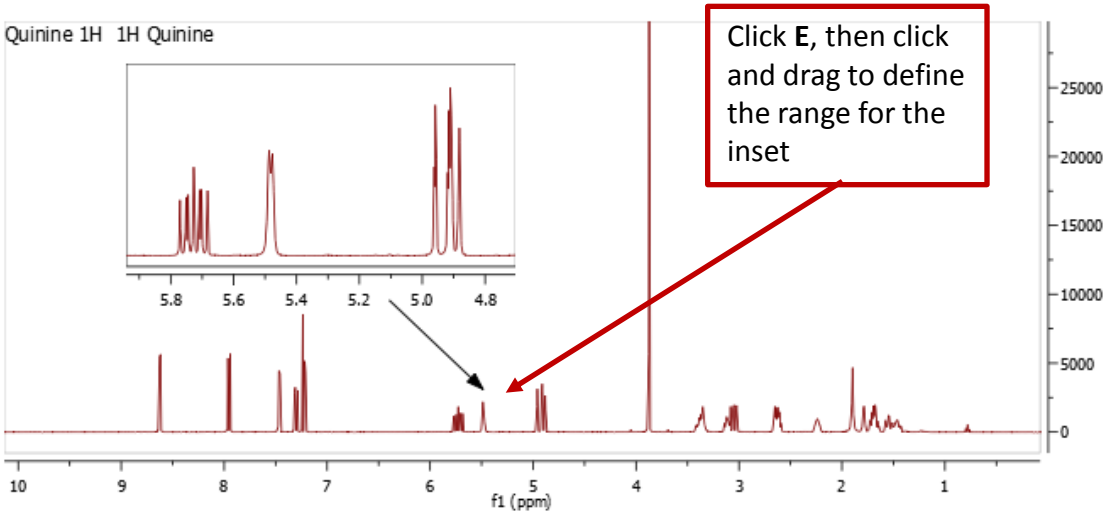
Decrease Intensity (or rotate mouse wheel)

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

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

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

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



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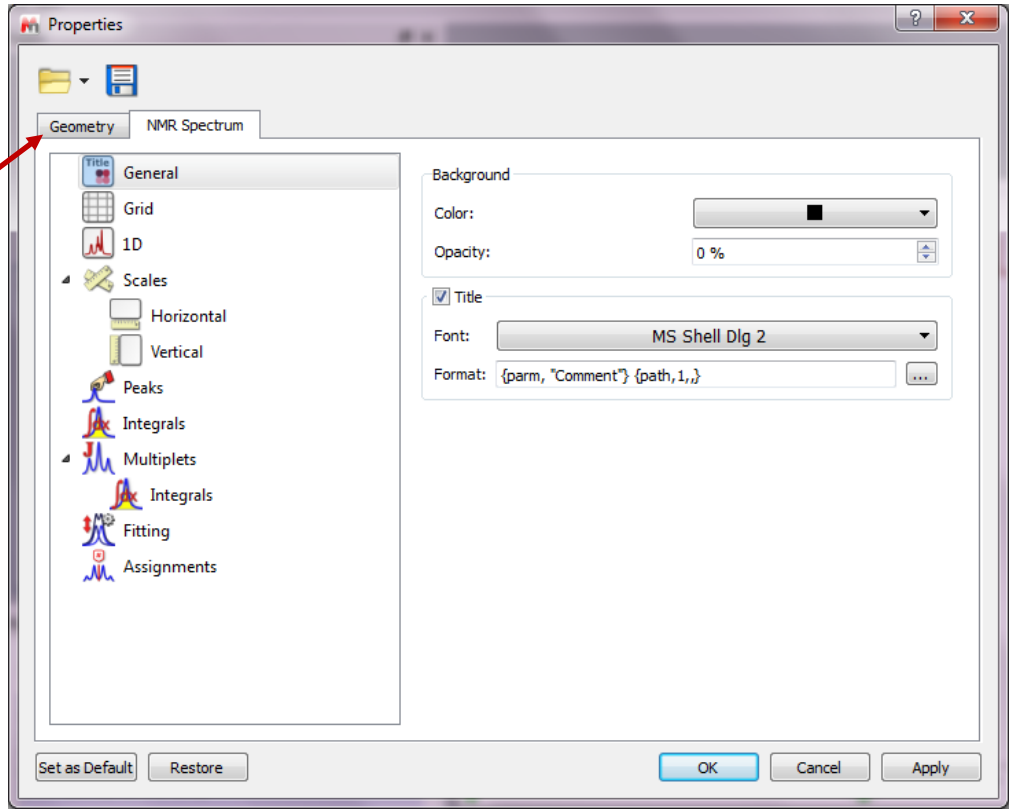
To change the display properties

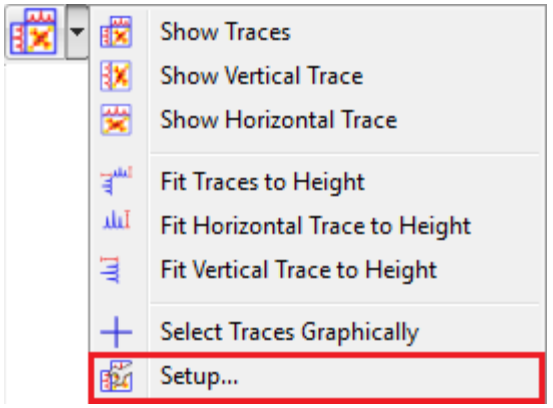
- Right click on a spectrum and choose **Properties** from the context menu
- Many display properties can be customized
- Click **Set as Default** to save the settings for spectra opened in the future
- Save the settings (e.g., for other users) using the **Save Properties** and **Load Properties** tools

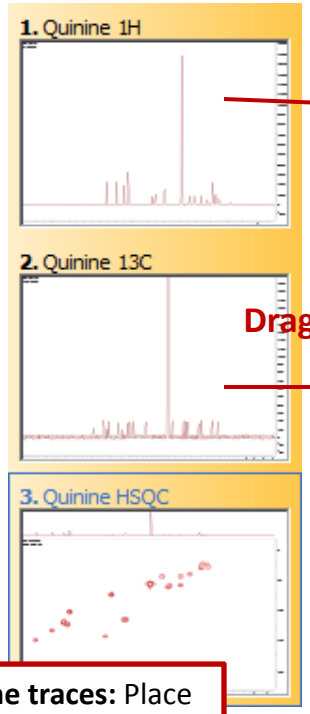
Tip: Use options in the **Geometry** Tab to change the size of a spectrum precisely



To attach 1D to 2D spectra

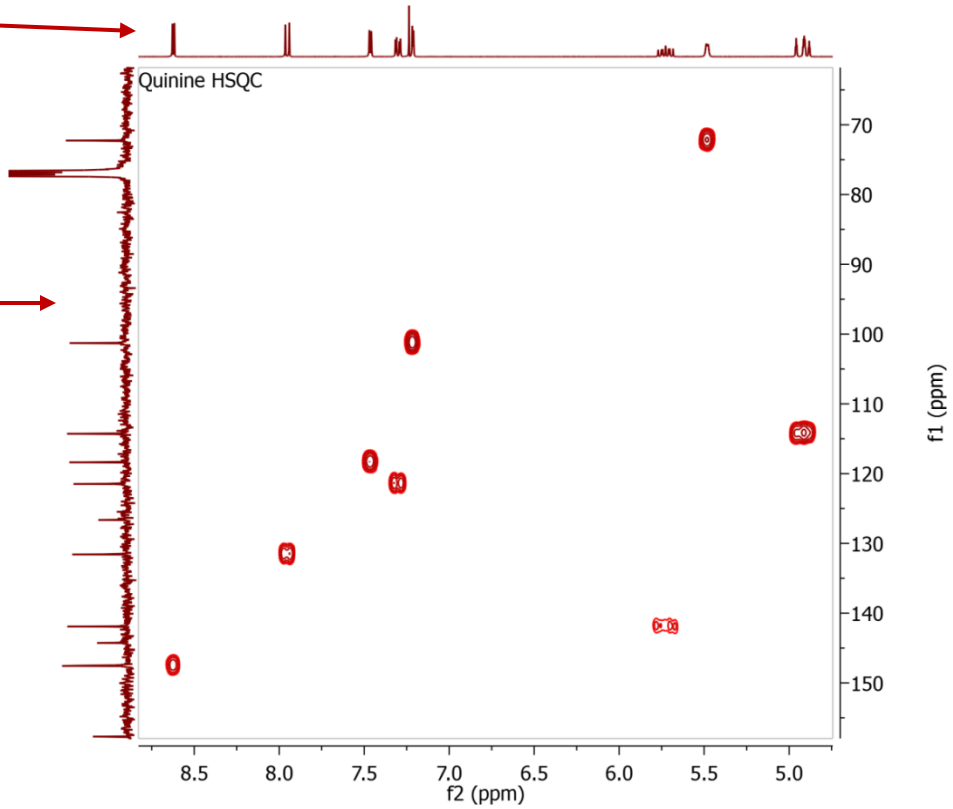
- Open 1D and 2D spectra in the same document (They are shown as separate pages)
- Display the 2D spectrum, click the **Traces** tool options and choose **Setup…**
- Choose a 1D in the Available 1D Spectrum, click to attach it to that axis





Drag & drop

Drag & drop

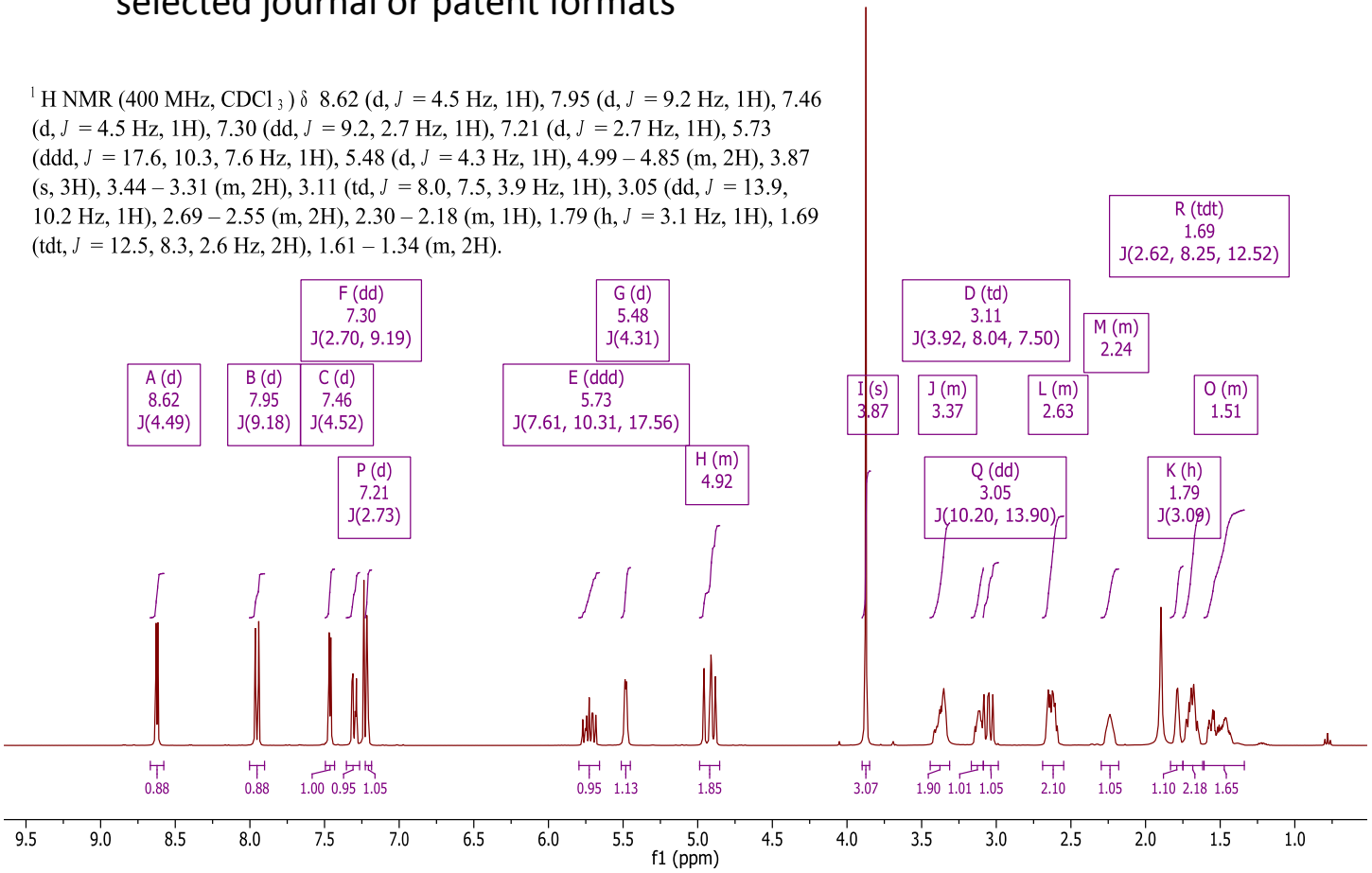


To change the Y intensity of the traces: Place the cursor on the trace and scroll the mouse wheel, or click **Ctrl+Shift+arrow keys.**
To move the baseline of a trace: Shift + mouse wheel.

To analyze and report multiplets of H-1 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 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 = 2.7 Hz, 1H), 5.73 (ddd, J = 17.6, 10.3, 7.6 Hz, 1H), 5.48 (d, J = 4.3 Hz, 1H), 4.99 – 4.85 (m, 2H), 3.87 (s, 3H), 3.44 – 3.31 (m, 2H), 3.11 (td, J = 8.0, 7.5, 3.9 Hz, 1H), 3.05 (dd, J = 13.9, 10.2 Hz, 1H), 2.69 – 2.55 (m, 2H), 2.30 – 2.18 (m, 1H), 1.79 (h, J = 3.1 Hz, 1H), 1.69 (tdt, J = 12.5, 8.3, 2.6 Hz, 2H), 1.61 – 1.34 (m, 2H).



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

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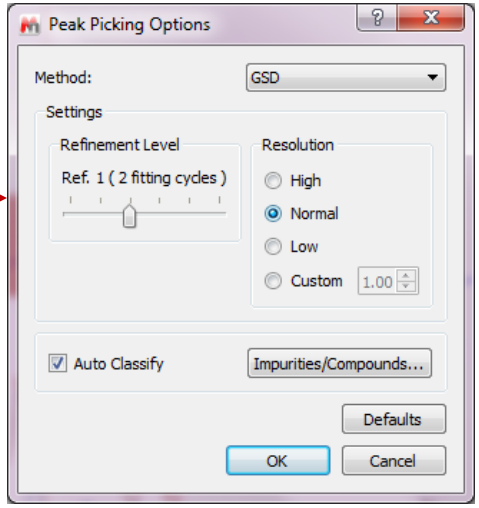
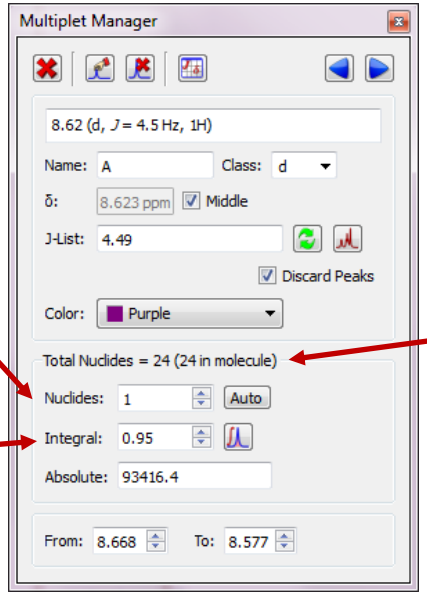
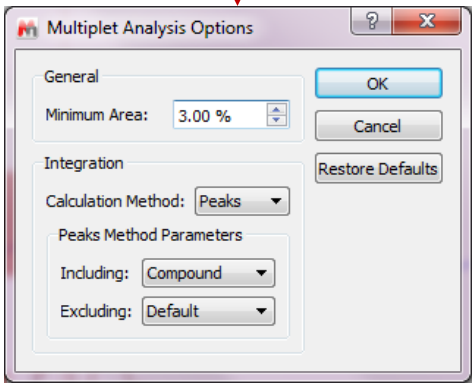
Fully automatic multiplet analysis

- Click  to do automatic multiplet analysis. By default, it does the following:
  - Picks peaks using GSD (if no peaks were picked) and classify their types (compound, solvent, impurity peaks etc.). Note these are controlled by the Peak Picking options
  - Groups the picked peaks into multiplets and fits them to *J*-coupling patterns, and calculates their integrals (depending on the Multiplet Analysis options). Note these are controlled by the 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*

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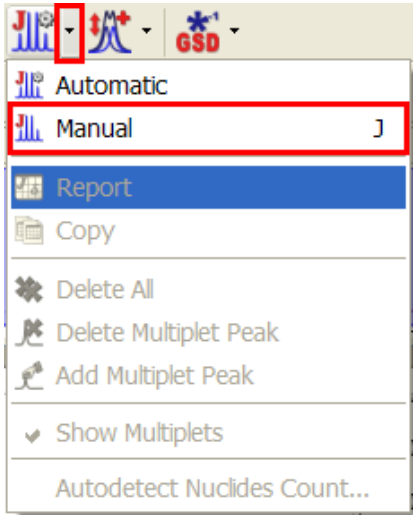
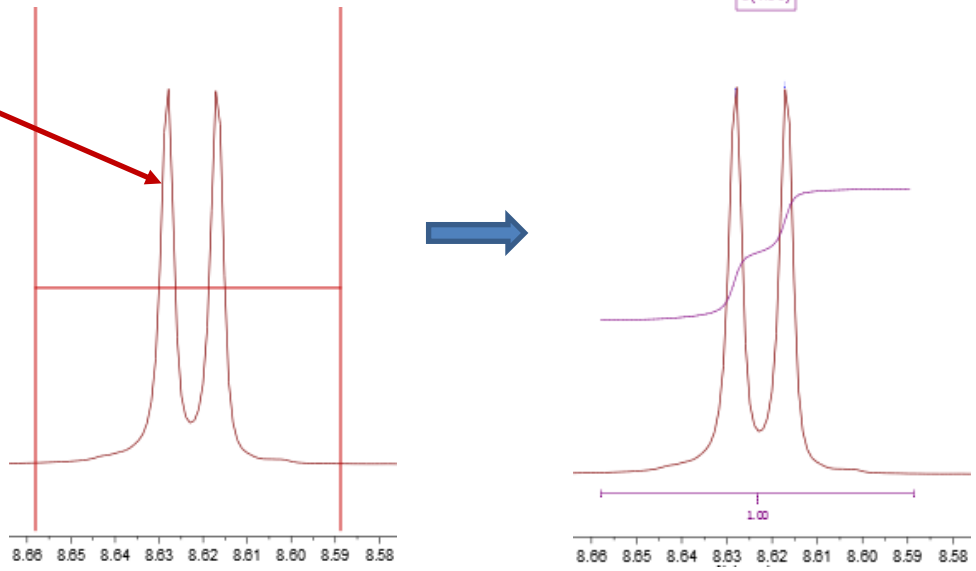
To pick multiplets manually

- Manual Multiplet Analysis allows you to have more control of the multiplet analysis (**J** is the shortcut key)
- You 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



Click and drag to define the **integration region** and **peak picking threshold** and a doublet will be picked



Tip: To turn on the integral curves, right click and select Properties, go to Multiplets > Integrals.

** If Peaks is used as the Integration Method, the area of a GSD peak will be included in the integral as long as the peak top falls within the region.*

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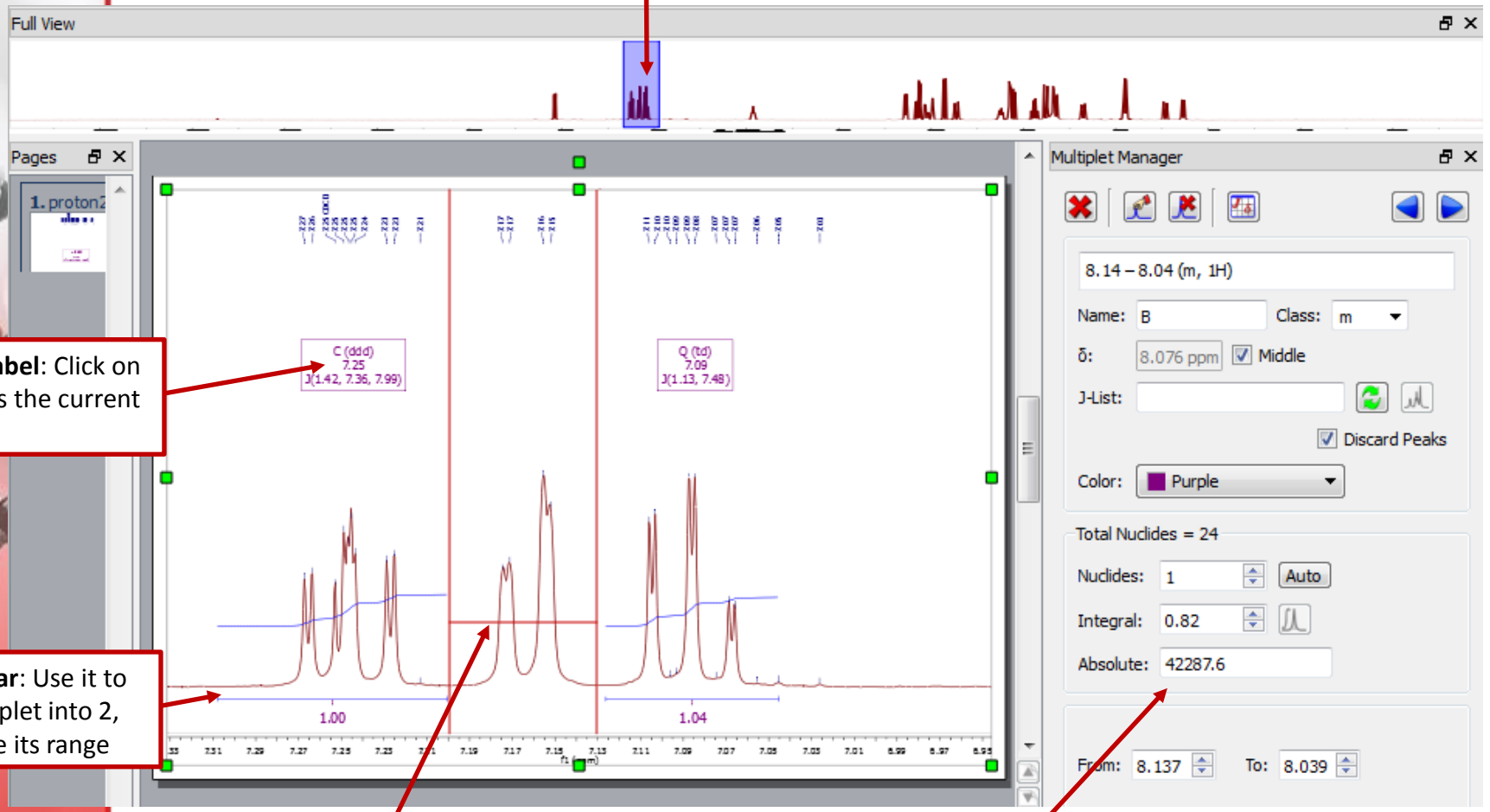
Handy tools for multiplet analysis

Full View: The whole spectrum and zoom-in area. Drag the blue box to move to other multiplets. (Choose **View | Full View** to open Full View)



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: Click **J**, then click and drag to define the range and peak picking threshold for a multiplet.

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

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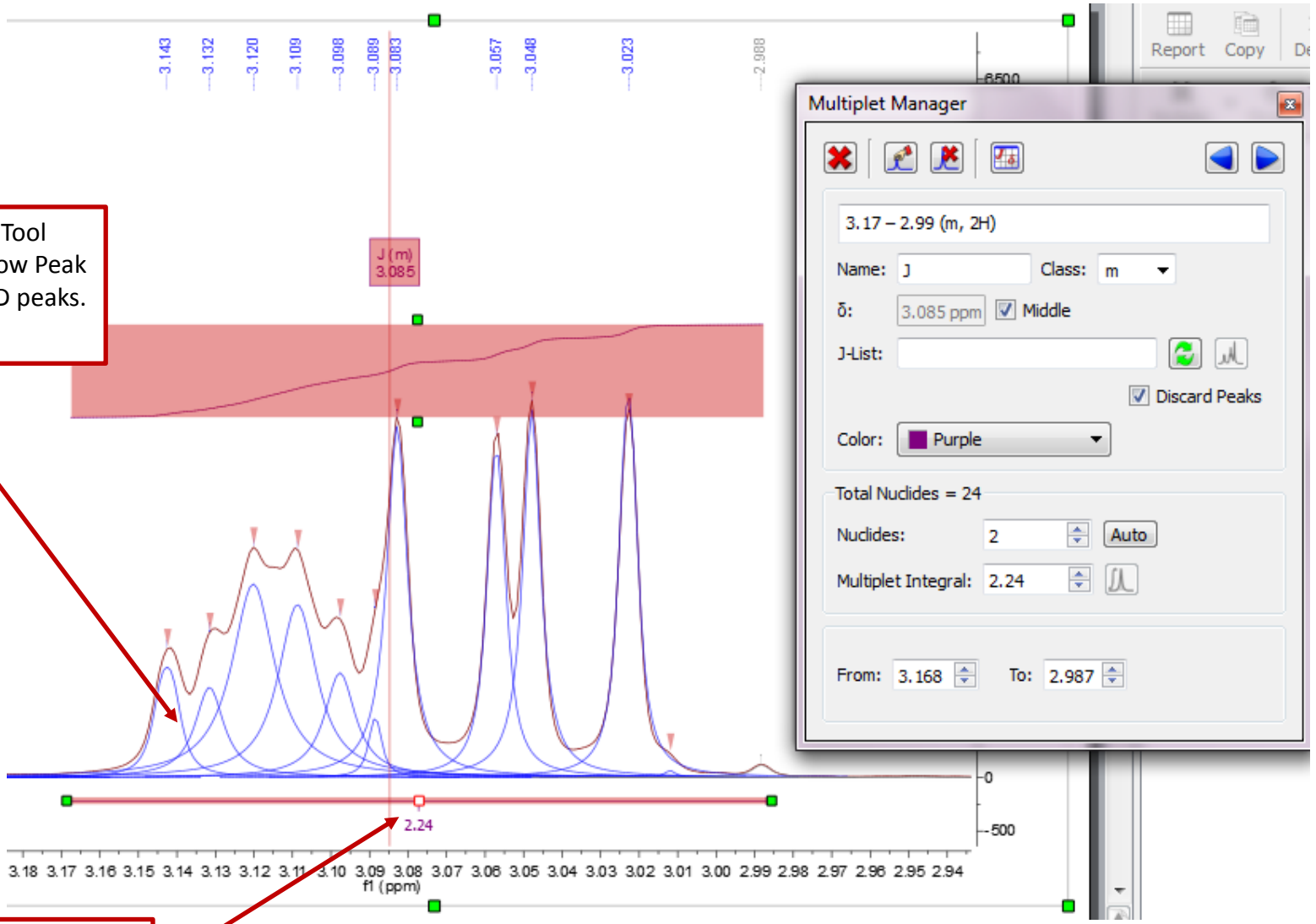
To split partially overlapping multiplets

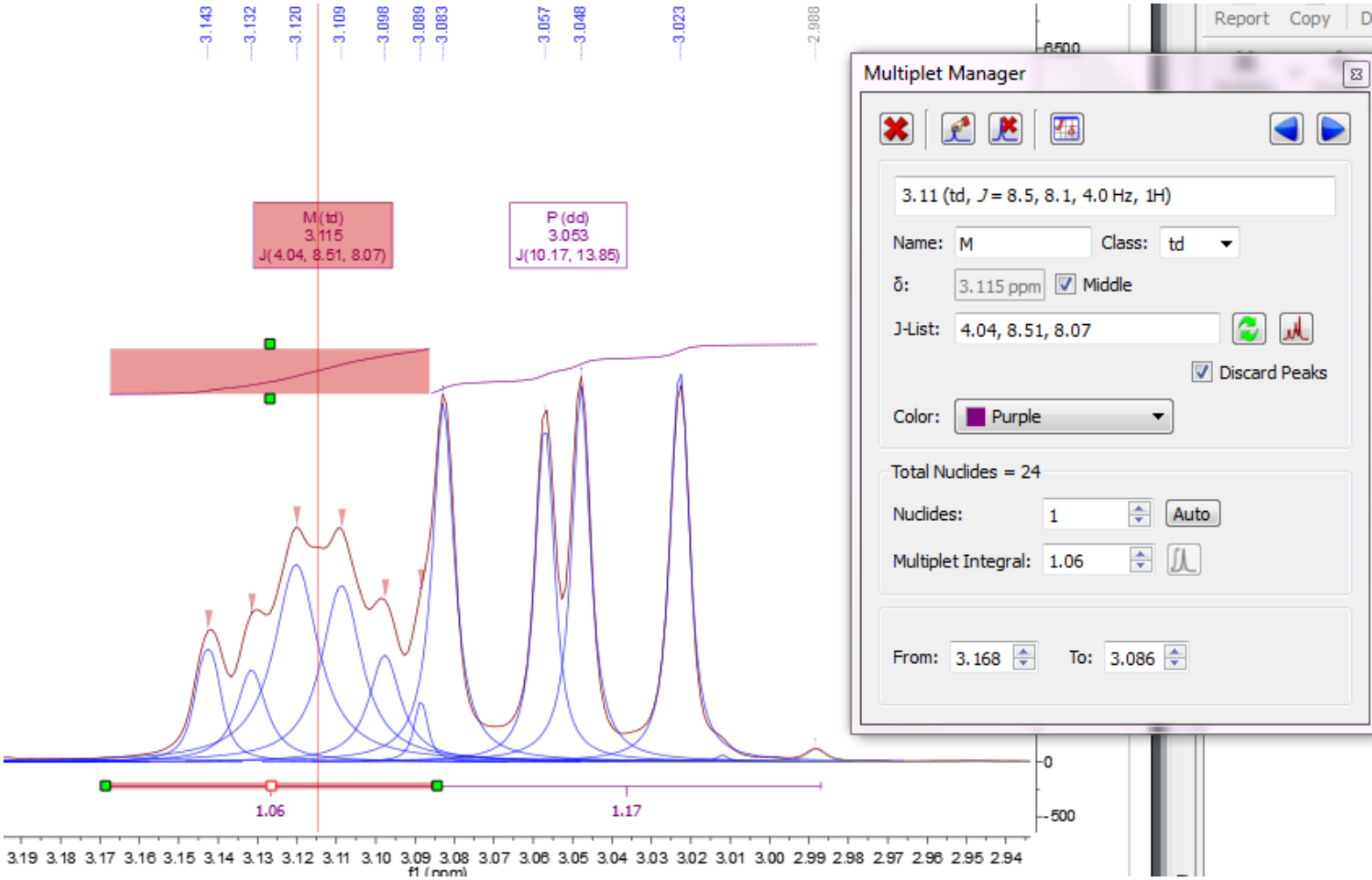
Expand the Peak Picking Tool menu , check Show Peak Curves to display the GSD peaks.



Drag this red box to where you want to split the multiplet into two

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

To split partially overlapping multiplets (2)



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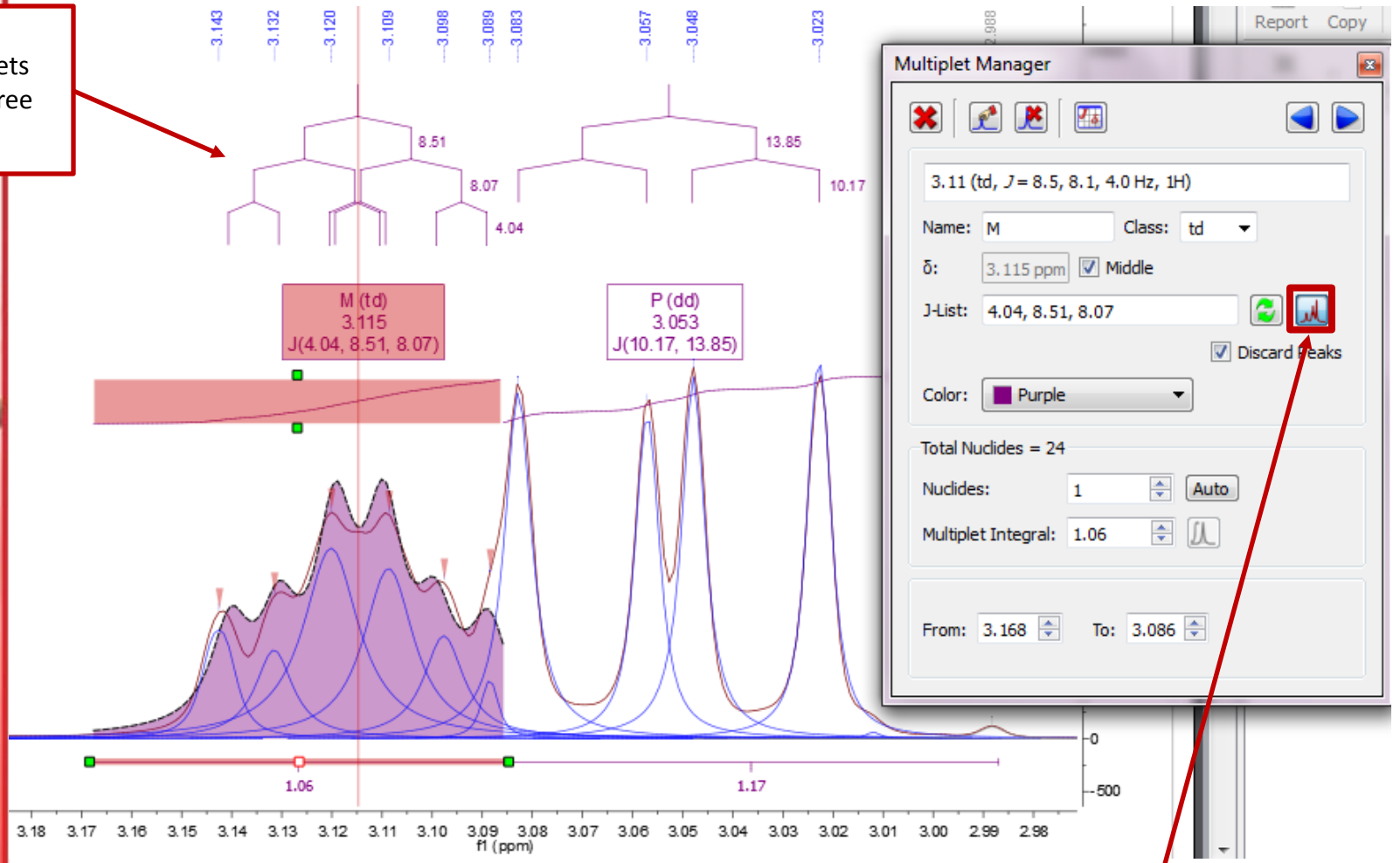
Tools for verifying multiplet analysis results

Choose View > Properties > Multiplets and turn on the J's Tree option



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

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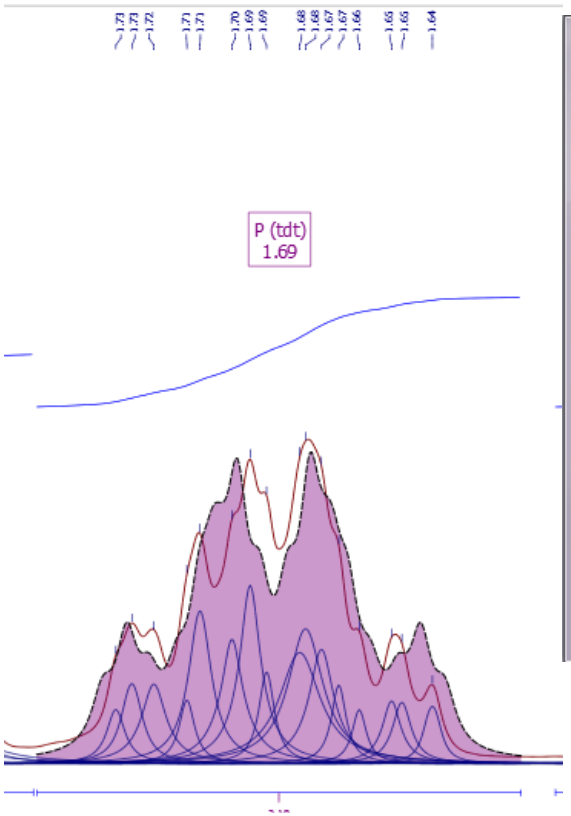
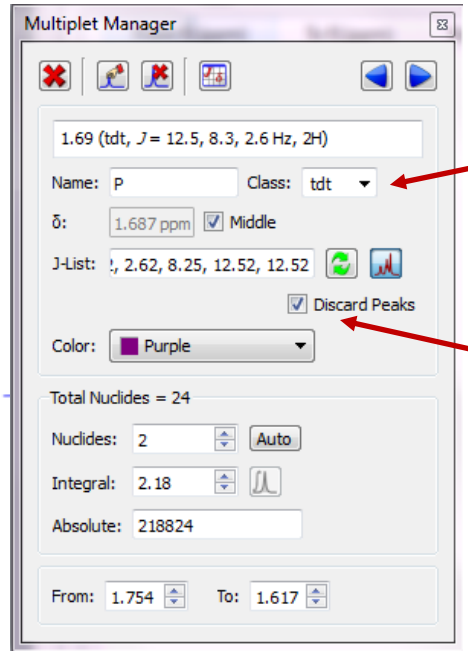
To override the multiplet results in Multiplet Manager

- You can override the analysis results of a multiplet in Multiplet Manager.
- In this example, the multiplet was over-fit as a “tdt”. The simulated multiplet does not agree with the observed spectrum and hence it is wrong.
- Click  to turn off the simulated multiplet first. Select “m” from the drag-down menu of Class to override it.
- Or you can turn off the Discard Peaks option to include all peaks to the multiplet (and you get an “m” in this case).





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

Or, you can turn off the Discard Peaks option to include all peaks and get a “m”

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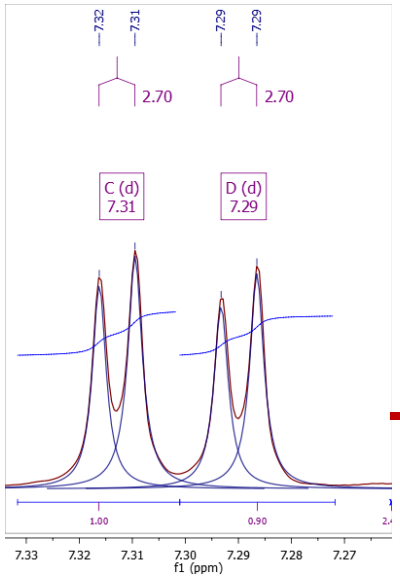
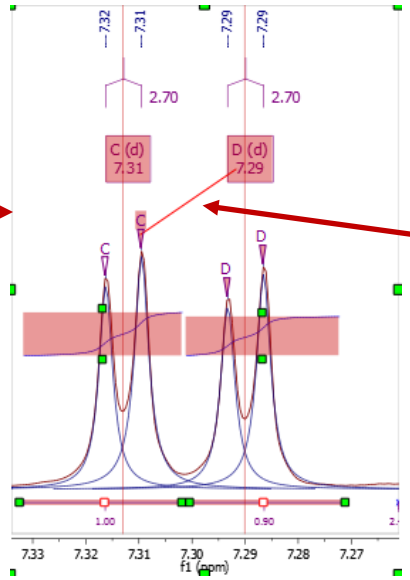
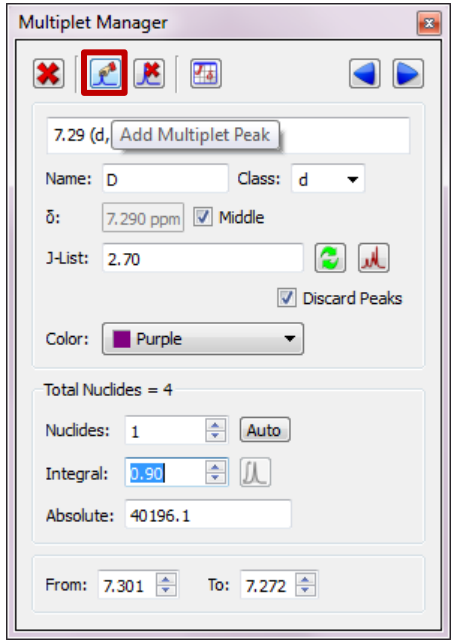
To re-assign peaks to multiplets

- 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:







Click on the triangle mark on top of the peak, drag it to the multiplet label “D” to assign it to a different group

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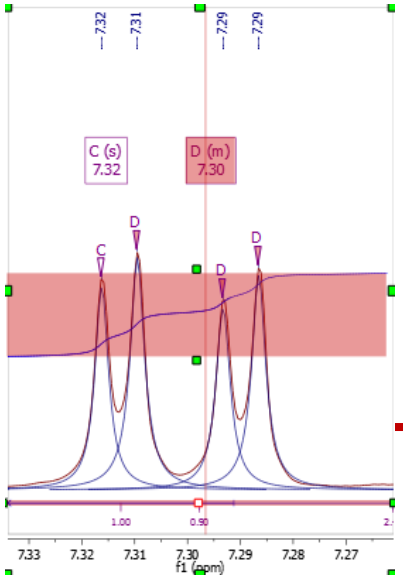
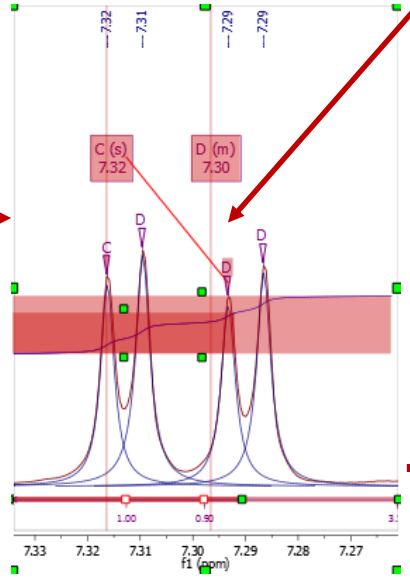
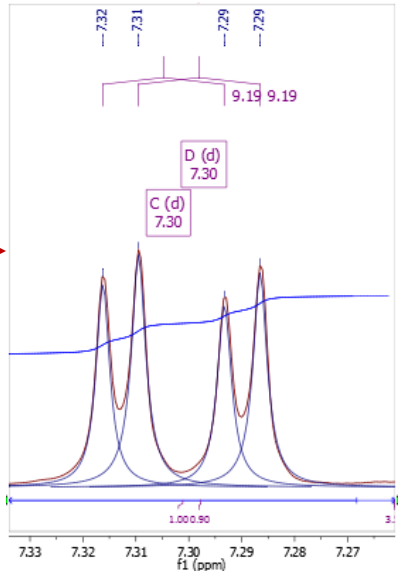
To re-assign peaks to multiplets (2)





Click on the triangle mark on top of the peak, drag it to the multiplet label “C” to assign it to Mutliplet “C”



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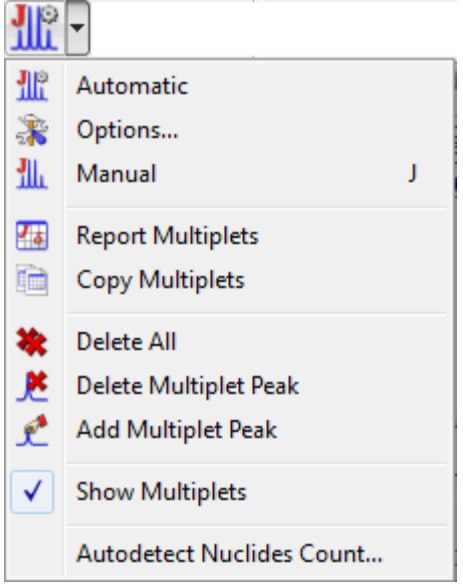
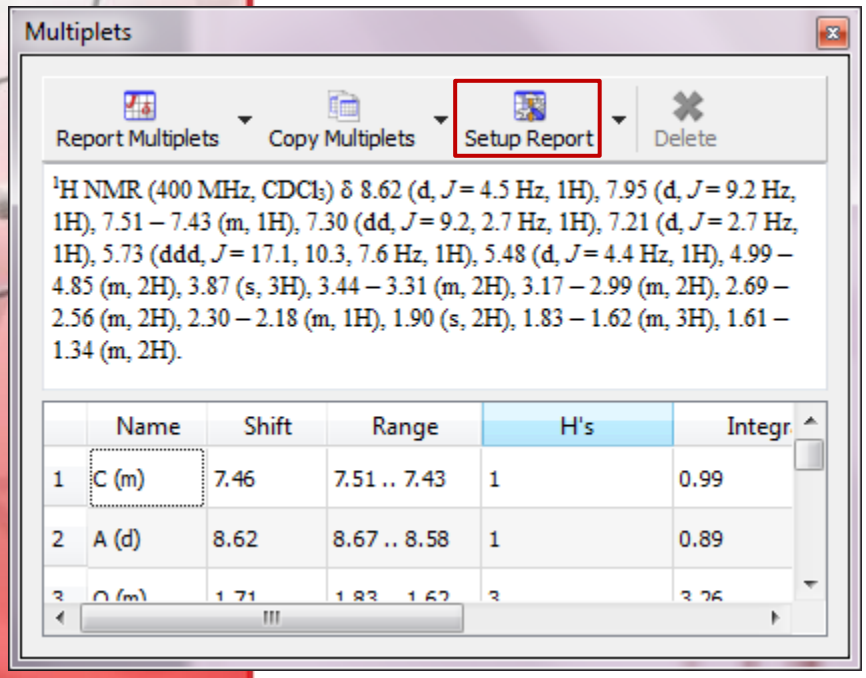
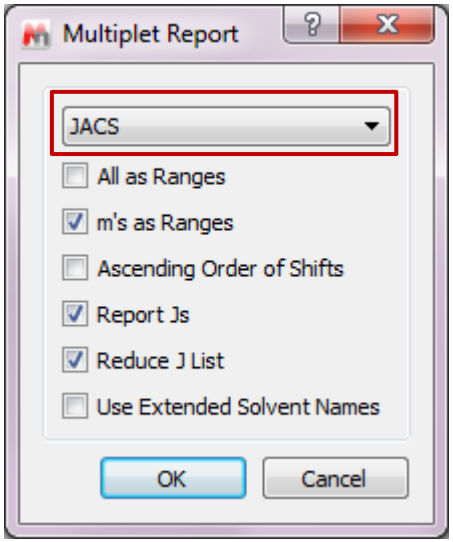
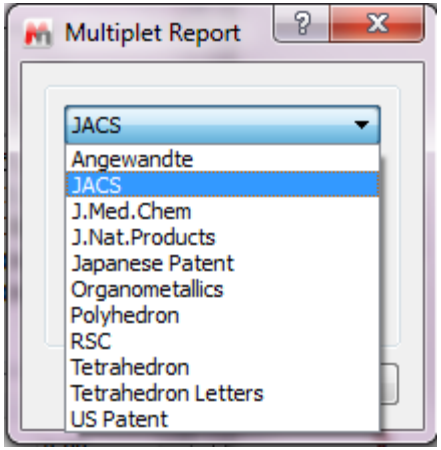
To report multiplets

- Click **Report Multiplets** to report the results in a journal format:
- To change journal format: choose **View | Tables | Multiplets** to display the Multiplets Table. Click **Setup Report**





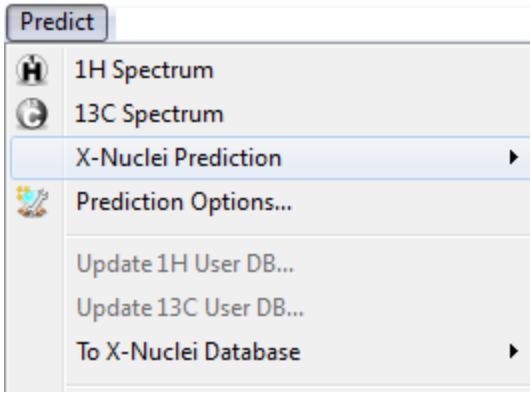


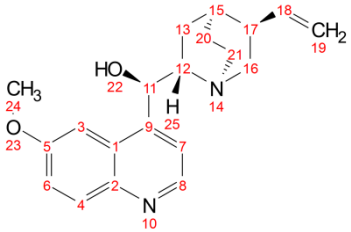


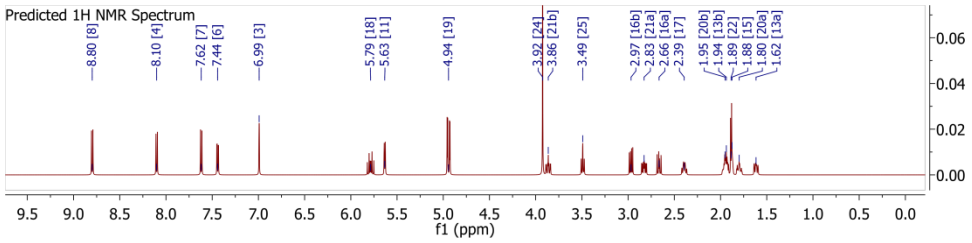
*Tip: From the Multiplet Table, click **Copy Multiplets** and then paste the texts to your document. Click **Copy Table** and then paste the spreadsheet to your document. The table can be customized using **Setup Table.***

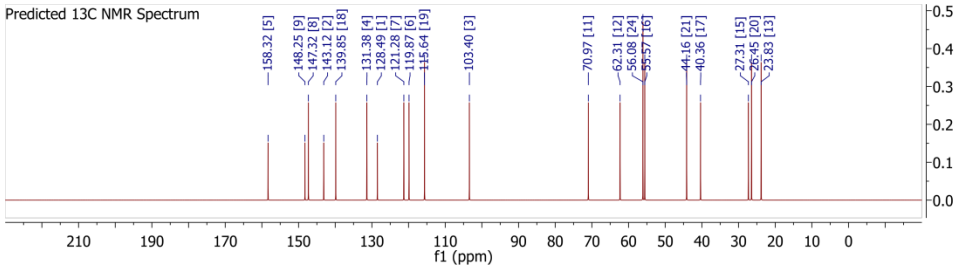
To predict NMR from a structure*

- Open a new document (**File | New**) or a new page (**Edit | Create New Page**)
- Copy a structure from ChemDraw, Isis/Draw or ChemSketch, and paste to Mnova, or open a .mol, .cdx or a .sdf file
- Choose an command from the **Predict** menu









Tips:
*1. Choose **Molecules | 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.*

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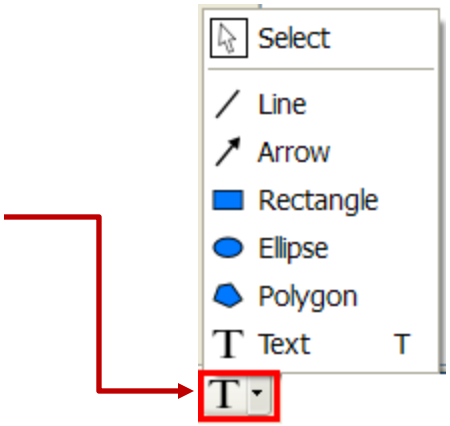
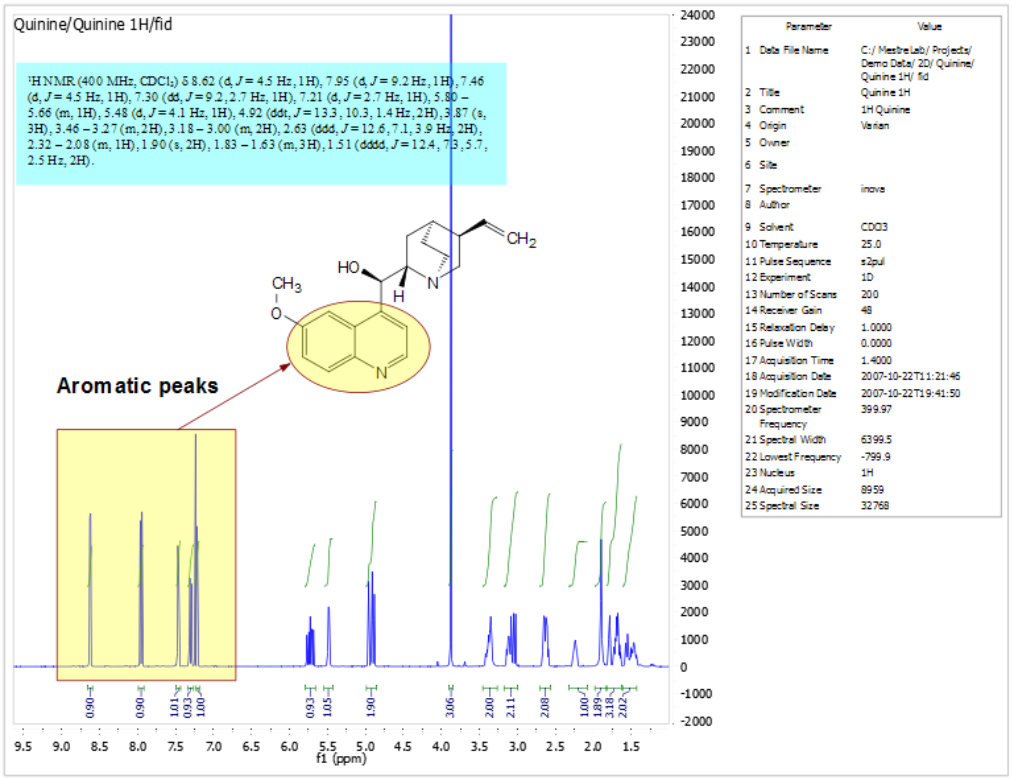
To annotate and report manually

- Click the **Annotation Options** button at the bottom-left corner of Mnova window
- Or press **T** to insert a text box
- All objects can be customized by right clicking on it and then selecting the **Properties** command
- **Tables of Peaks, Integrals, Parameters** etc can be opened by **View | Tables.** Report from there





Tips:
Copy a **molecule from ChemDraw or Isis/Draw, 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 **PDF*

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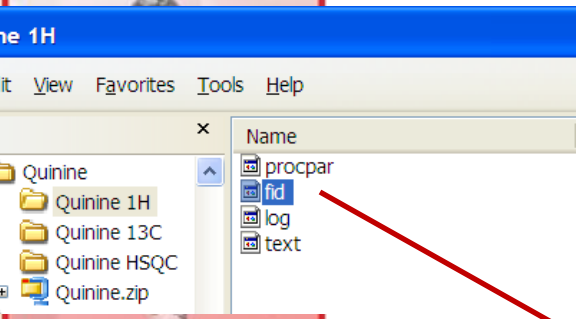
To create layout template

- Once you are satisfied with the layout, choose **View | Layout Template | Create Layout Template Document**, and save the layout
- You can continue to edit the template
- Once ready, open a new FID or structure to the template, and they will be auto formatted to the desired size and location.
- If you have a spectrum already opened, choose **View | Layout Template | Apply Layout Template Doc** to format it



Drag & drop

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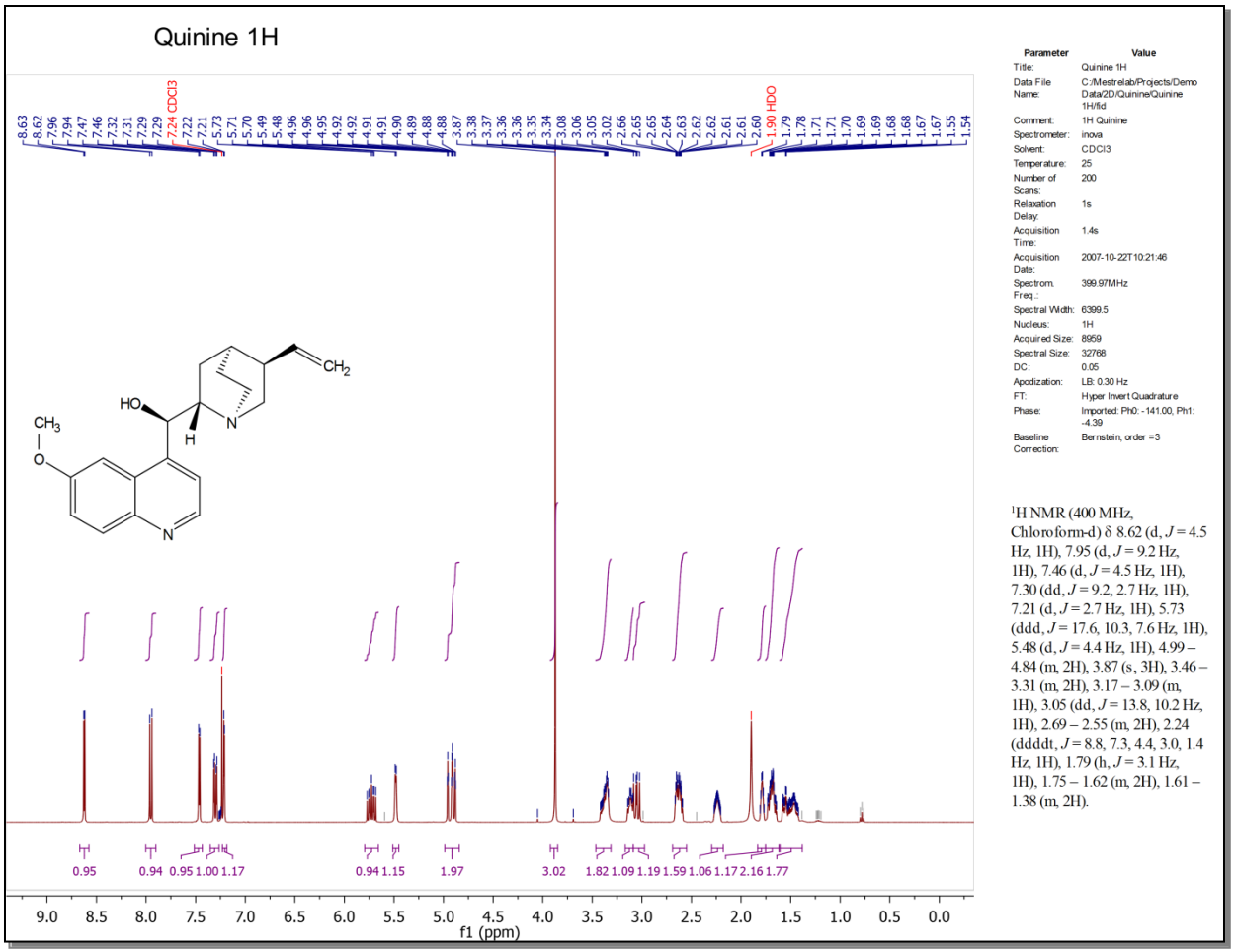
To auto format using Mnova script*

- Mnova has a powerful scripting engine that allows you to automate many operations, including processing, analysis and reporting
- The following is a sample output by running a Mnova script



** Click http://mestrelab.com/scripts/ to download free formatting scripts. We also provide service for more complex batch processing and reporting requirements*

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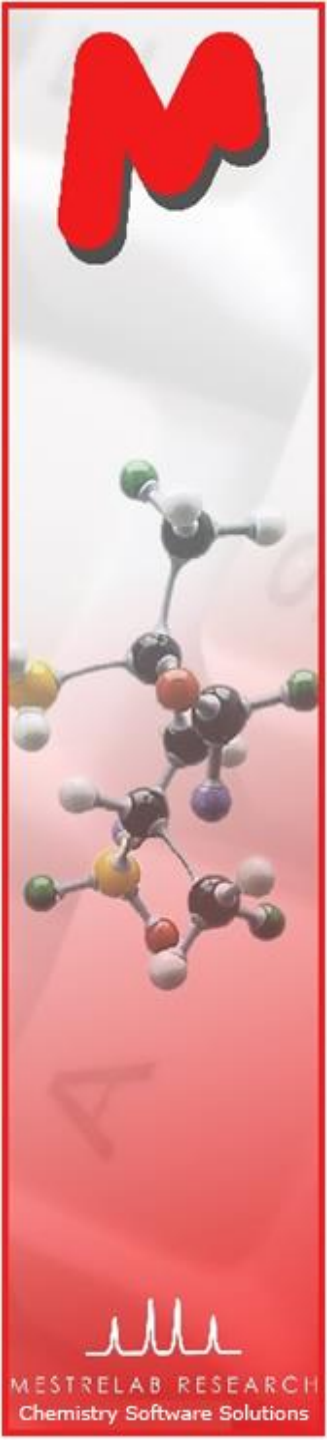


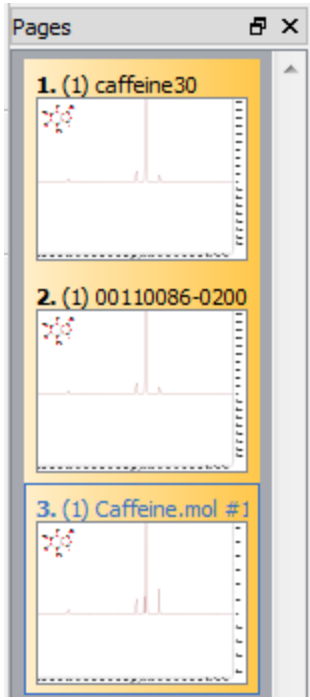
To auto **P**rocess, **A**nalyze and **R**eport a 1D spectrum using an Mnova script (PAR.qs)*

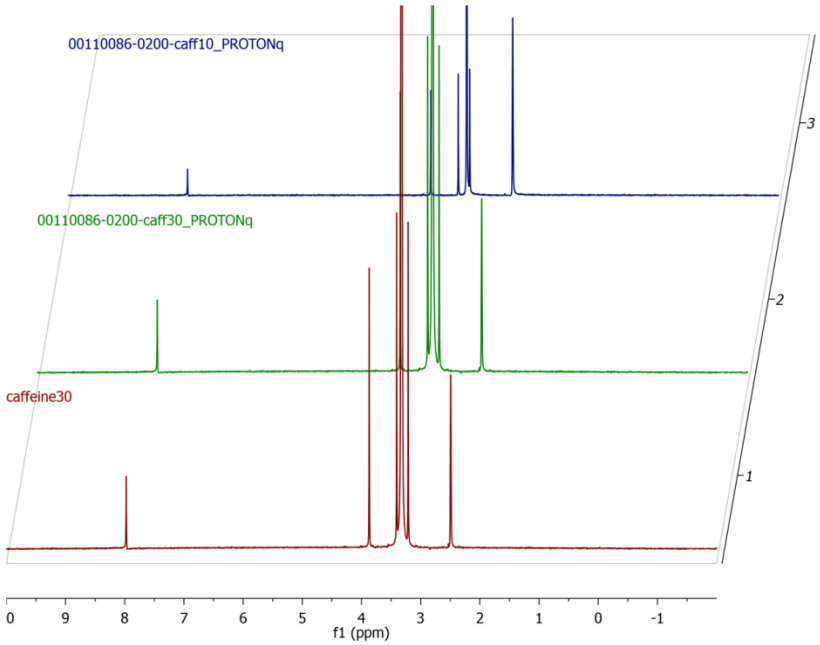
- You open a 1D H-1 spectrum, run this free script* for the first time. It does the following:
  - Re-processing the spectrum with line broadening of 0.3 Hz, enhanced correction for Bruker Group Delay if applicable, zero-filling to double the data size or at least 64K points, and baseline correction using 3rd order Bernstein Polynomial
  - Automated peak picking and multiplet analysis using the current options
- You manually verify and correct the multiplet analysis results
- You run the script *again*, and it generates a report similar to the one in the previous slide
- You can easily customize the processing, analysis and reporting options by editing the script.
- This script also works for C-13 and other nucleus, in slightly different way (e.g., it picks and reports peaks instead of multiplets).
- This script does formatting only if it is a 2D NMR

** Write to chen.peng@mestrelab.com and ask for PAR.qs. It's free.*
To run the script, first save it on your computer. Next choose Scripts > Run Script, and open it.

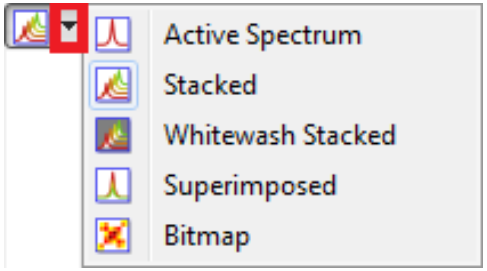
To open and stack multiple 1D spectra

- Open several 1D spectra in the same document
- Select some or all of them in the Pages View
- Click  to stack them in a new page:





- Click  to change the display to another Stack Mode, such as the Superimposed mode



*Tip: You can also choose the **Superimposed** tool*  *to superimpose selected spectra directly.*
*If you want to stack all the 1D spectra under a certain folder, use **Scripts > Import > Directory Spectra Stack***

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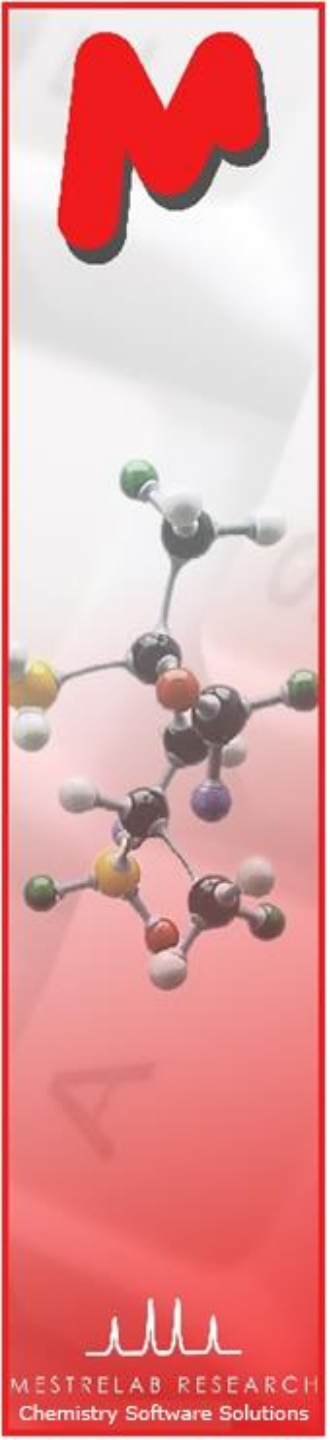

Thank you! For more information…

- Visit www.mestrelab.com for free trial, manual, tutorials, prices etc
- Check **Help > Contents** in Mnova for help on specific topics
- Email to chen.peng@mestrelab.com or support@mestrelab.com for questions.