NMR@CHEM.OX

Chemistry Research Laboratory

UNIVERSITY OF OXFORD

Introduction to MestreNova

28/10/2019

Outline

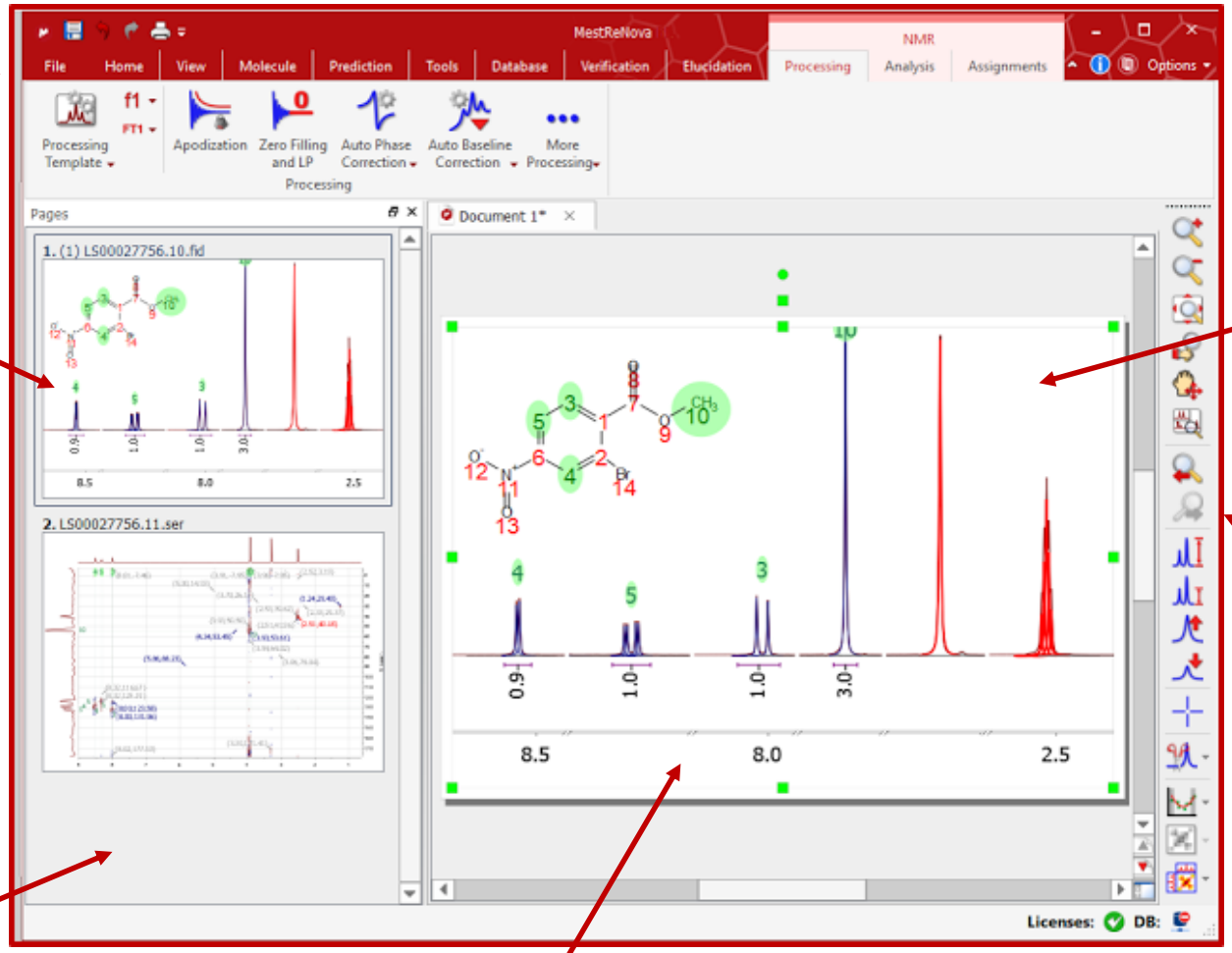
- Overview of Mnova
- Opening and processing 1D and 2D NMR data
- Peak picking and integration
- Multiplet Analysis for 1D H-1 NMR
- Report analysis results
- Assigning 1D and 2D peaks to a structure
- Stacking and superimposing spectra
- Predicting spectra from a structure
- What else can be done….

MestreNova v.12

All functions and options are available through the menus

"Pages" window allows you to browse files loaded into Mnova

Many other windows and tables can be docked here



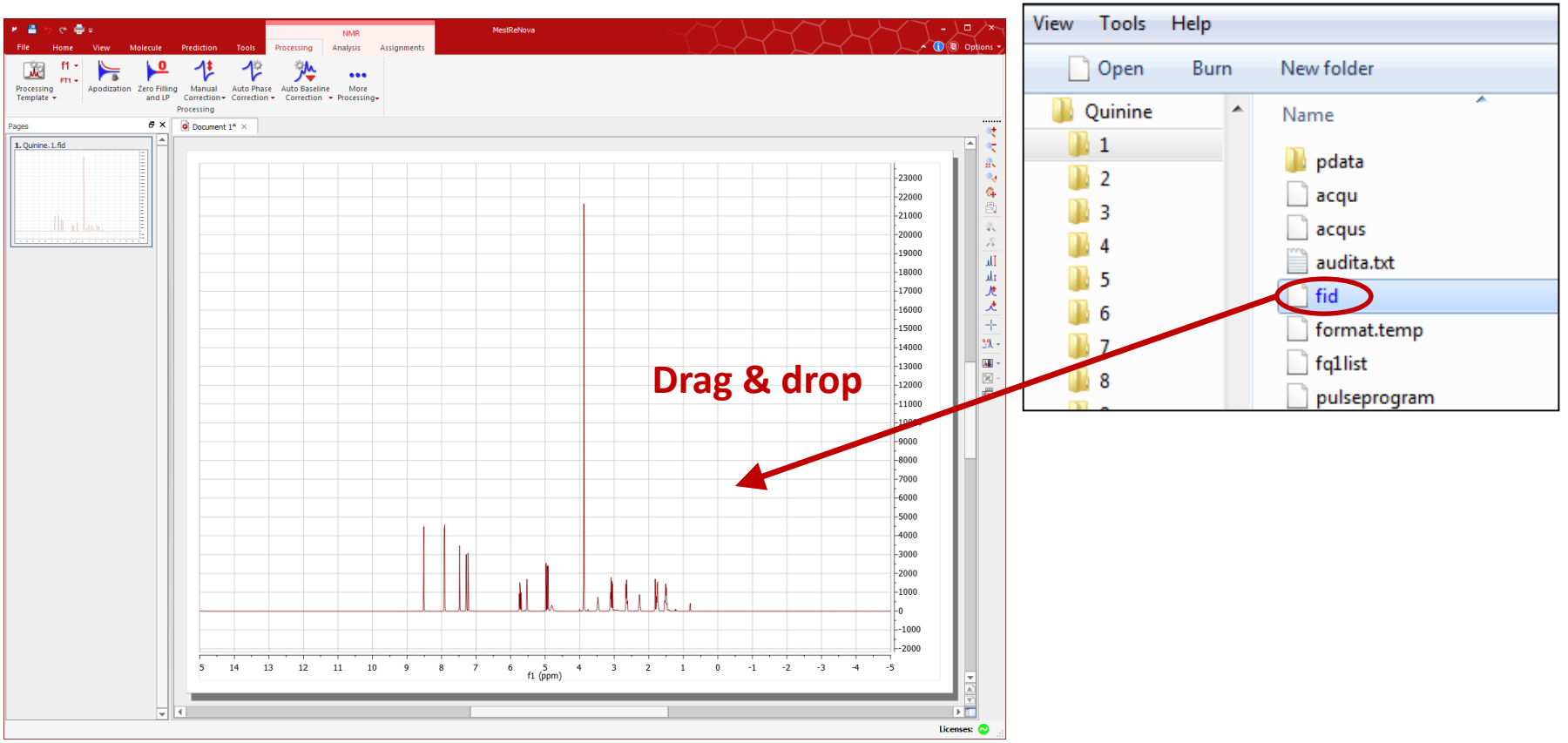
Main window

Quick links to common display options

Right-click in the spectra display window for more options

To open and transform your NMR data

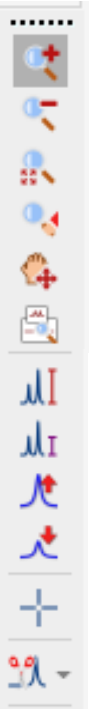
- Choose **File | Open** to open the raw data
- **Or** drag an **fid** file from a file browser to Mnova *
- Mnova automatically transforms the raw file into a spectrum (*including Windowing function, Fourier transform, phase correction etc*) **



*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 view or change the processing parameters by choosing ***Processing | Processing Parameters.

To visualise 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 intensity

Fit to highest compound

Increase Intensity (or rotate mouse wheel)

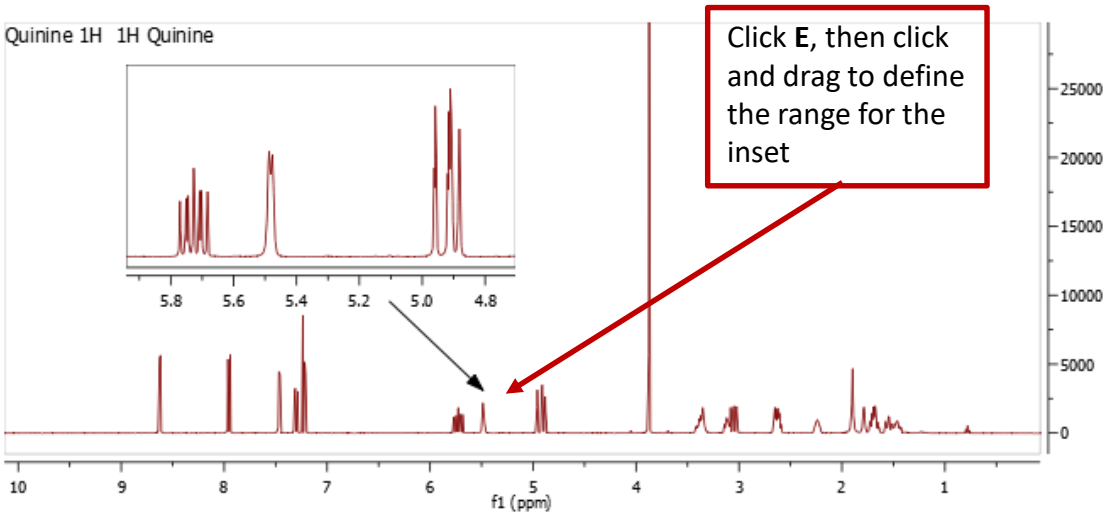
Decrease Intensity (or rotate mouse wheel)

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

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

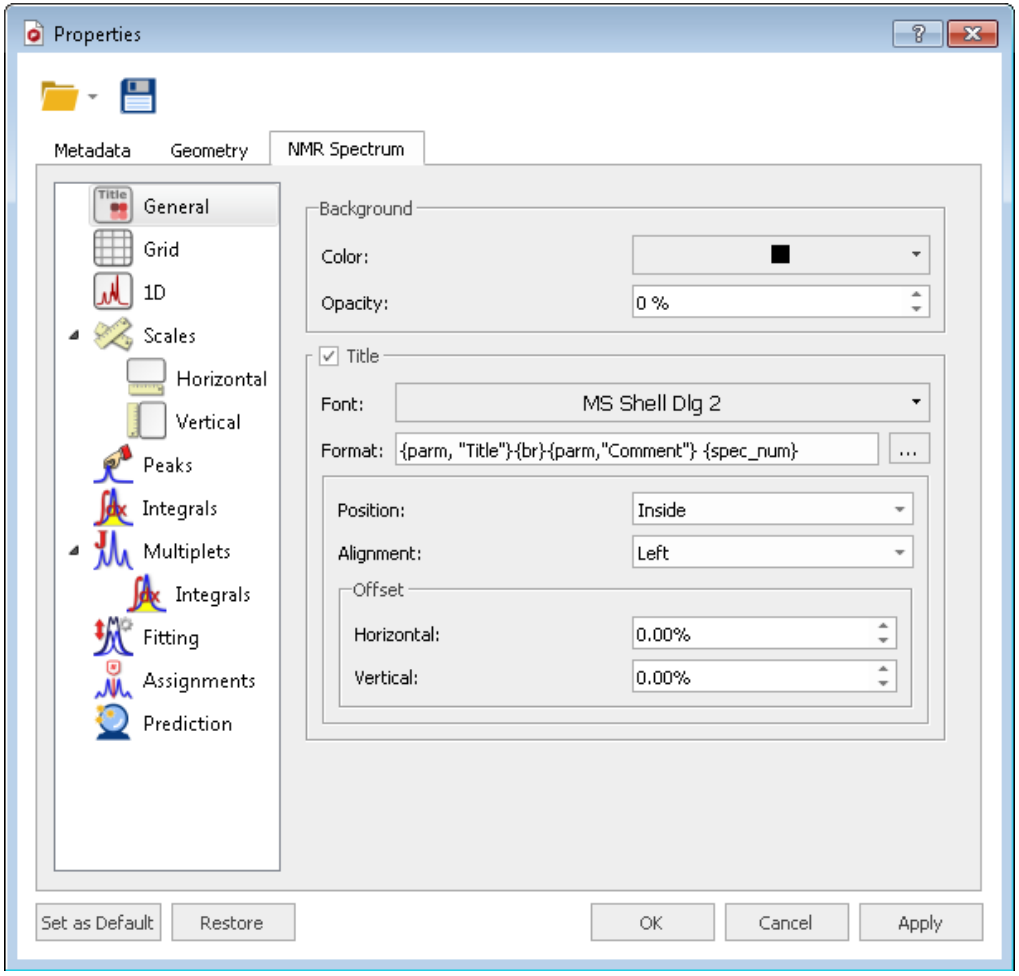
Press **Z several times to toggle between horizontal/vertical/box zoom*

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

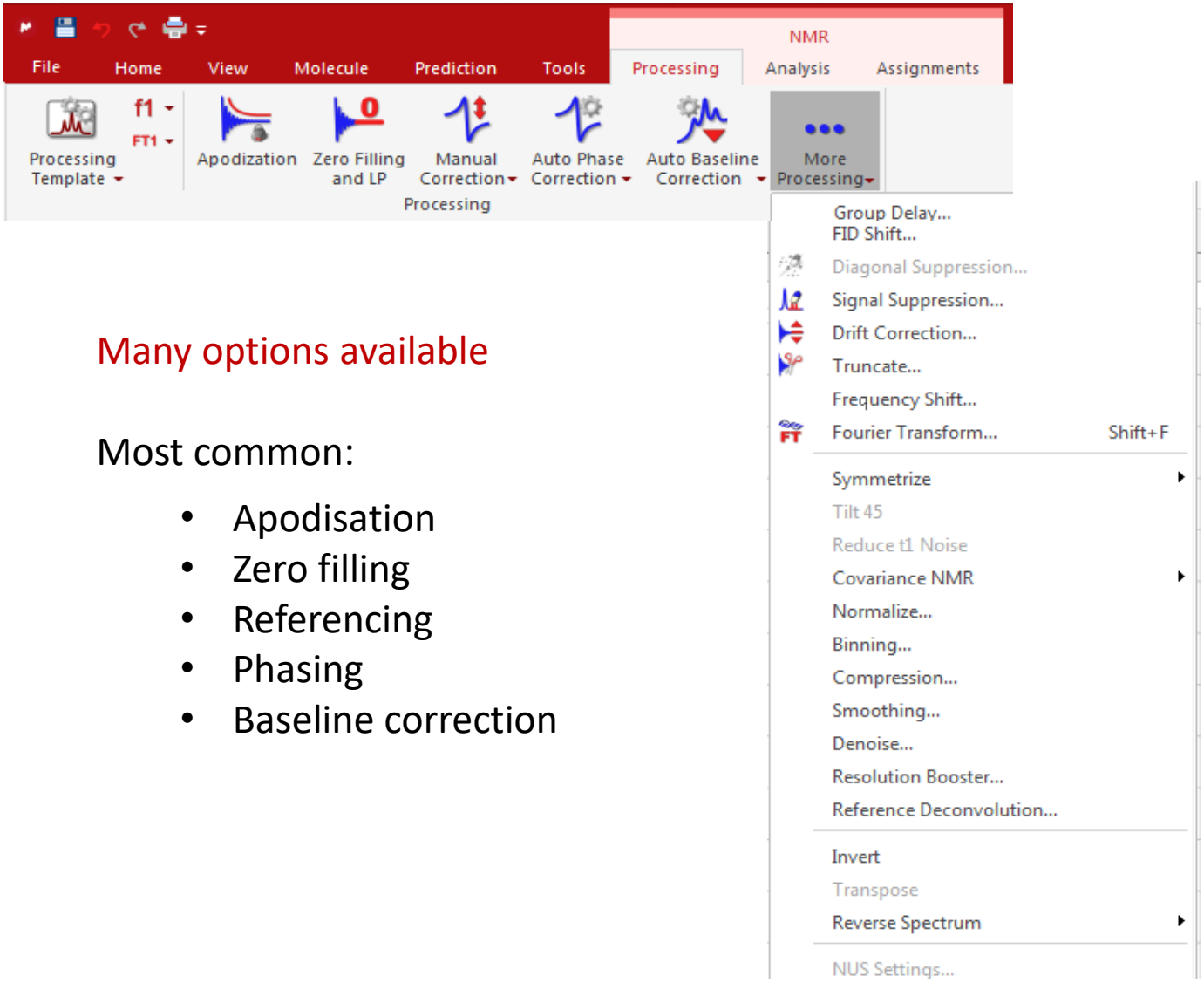


Editing your display preferences

Double click on spectrum or right-click and select “Properties”



Manual Processing

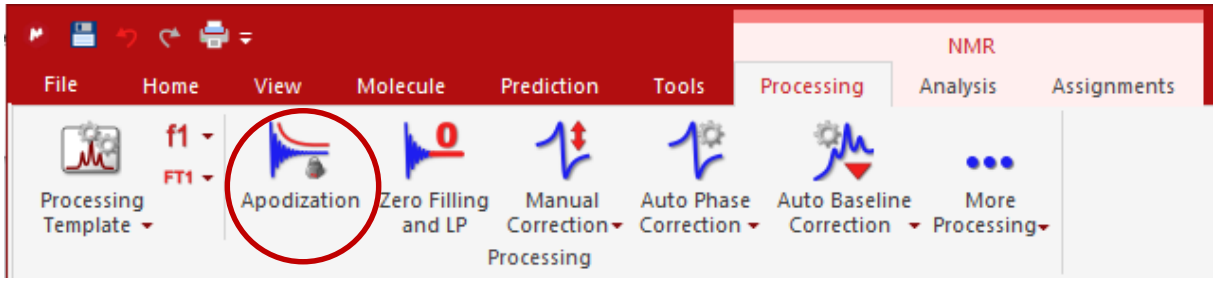


Many options available

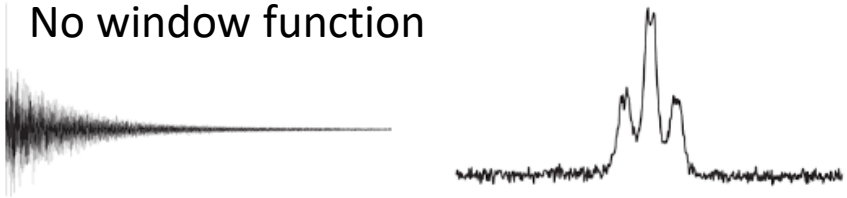
Most common:

- Apodisation
- Zero filling
- Referencing
- Phasing
- Baseline correction

Apodisation

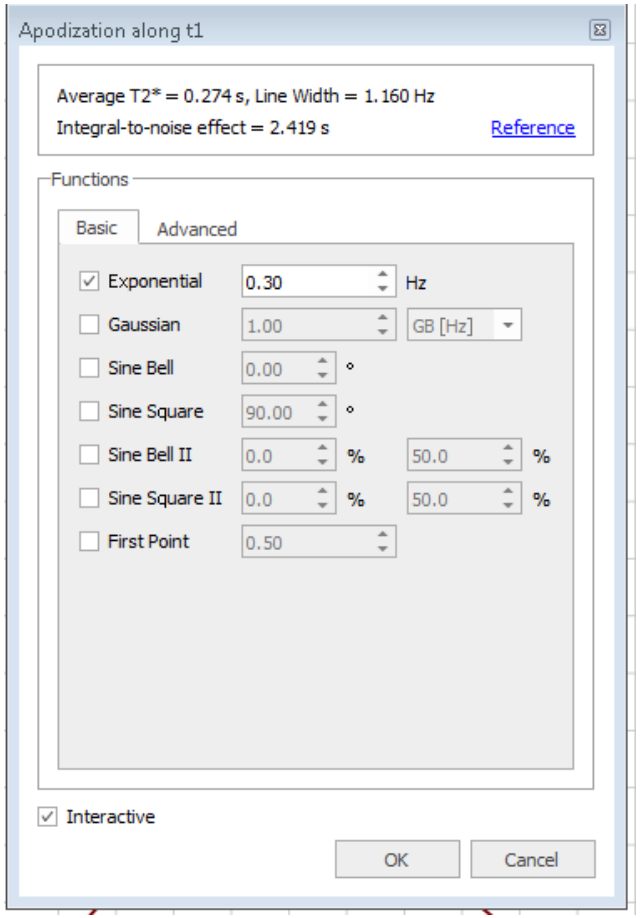


No window function

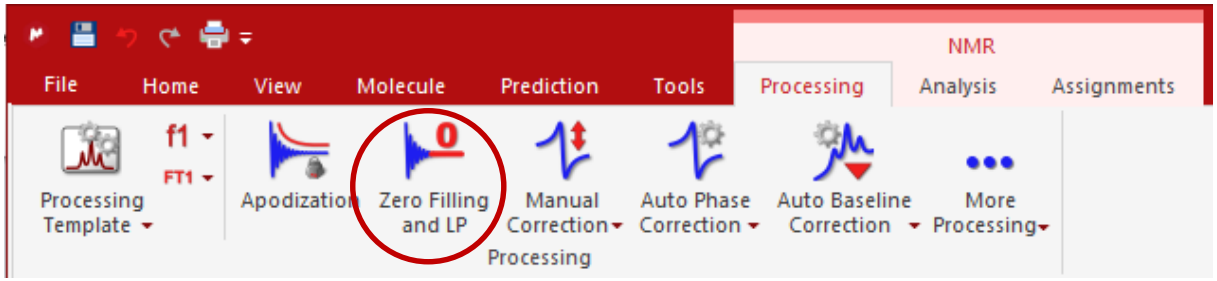


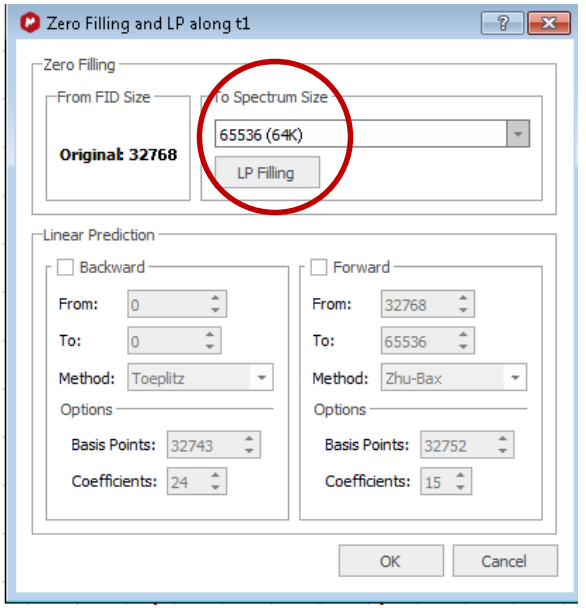
Exponential multiplication
lb = 0.3 Hz

Apodisation increases signal to noise at the expense of resolution

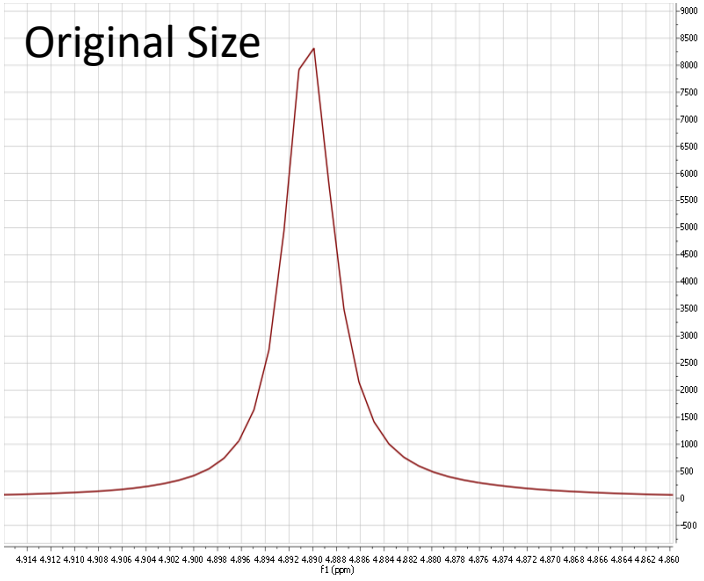


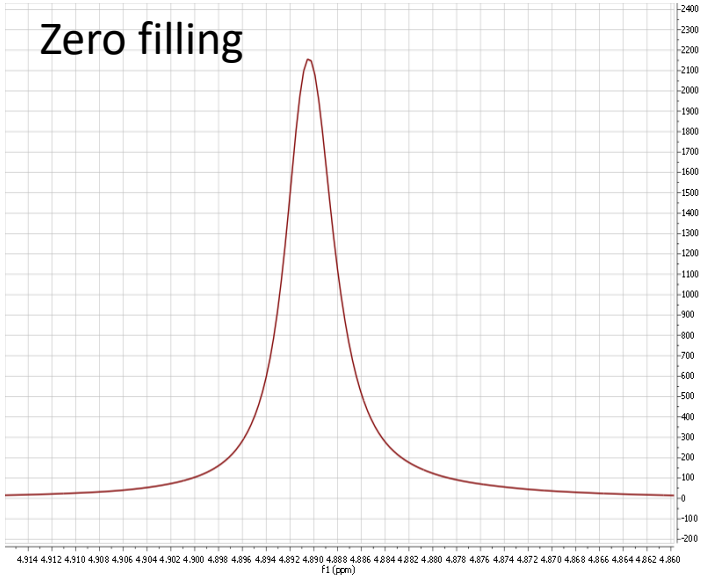
Zero Filling





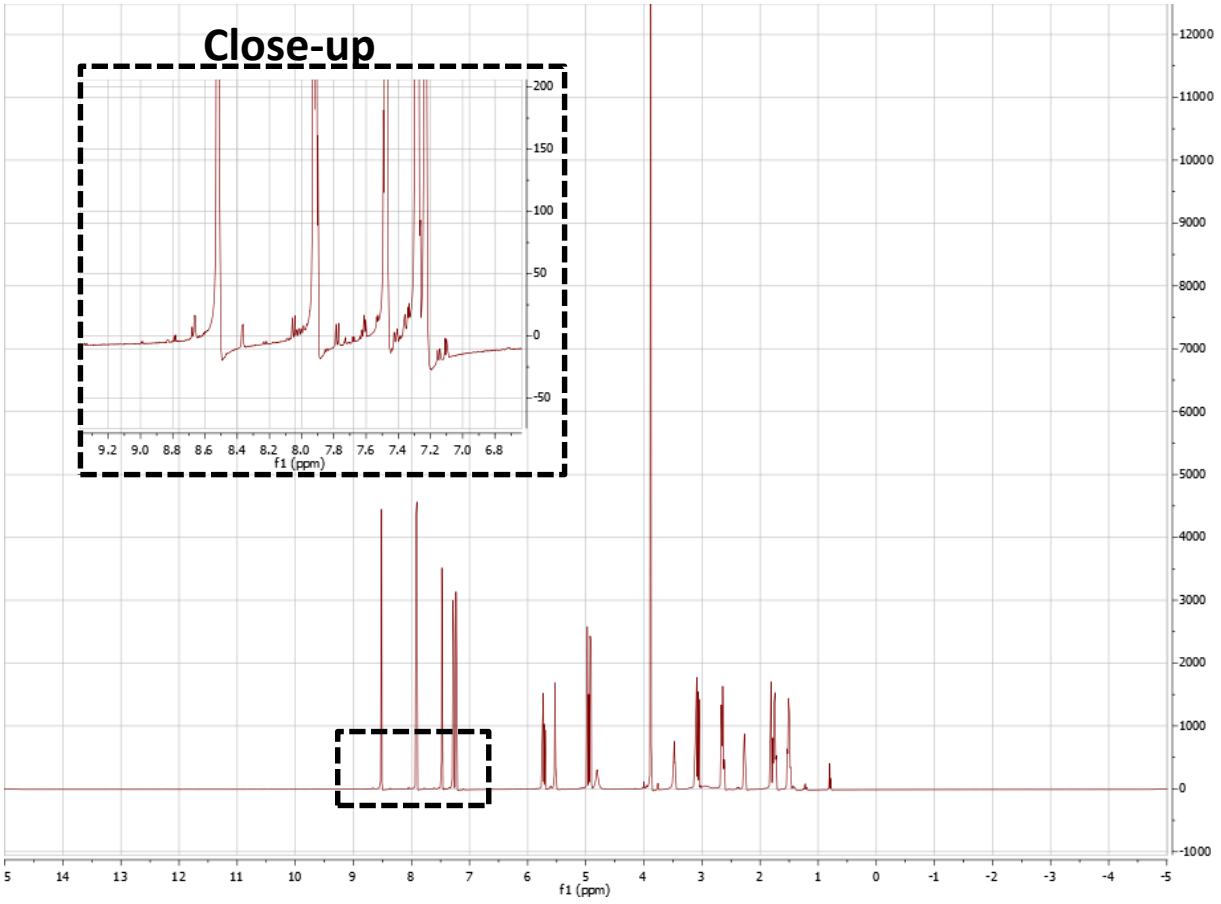
Zero filling increases the apparent acquired length of the FID, resulting in higher digital resolution and a “smoother” spectrum



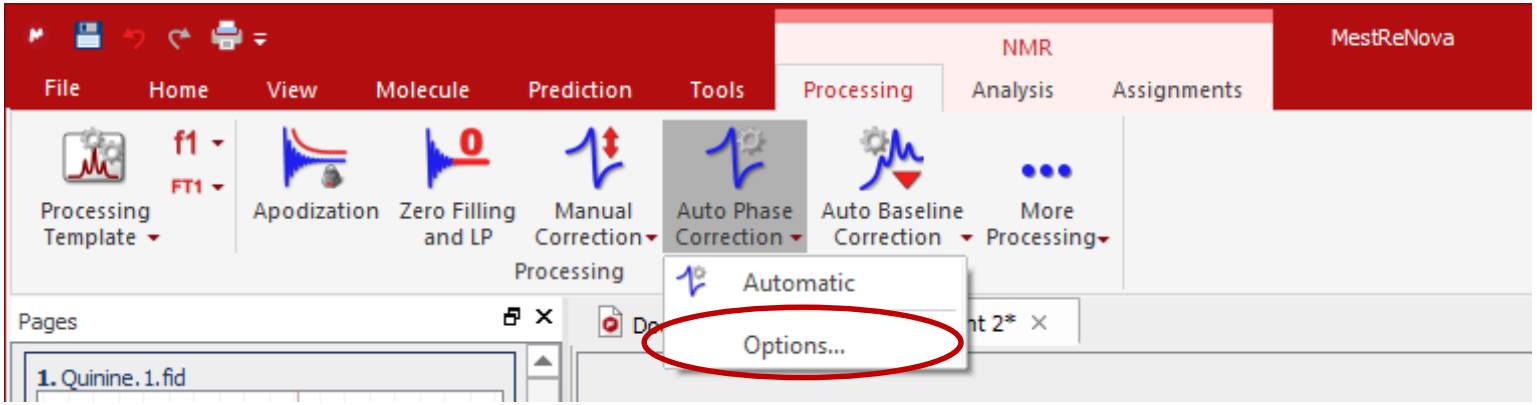


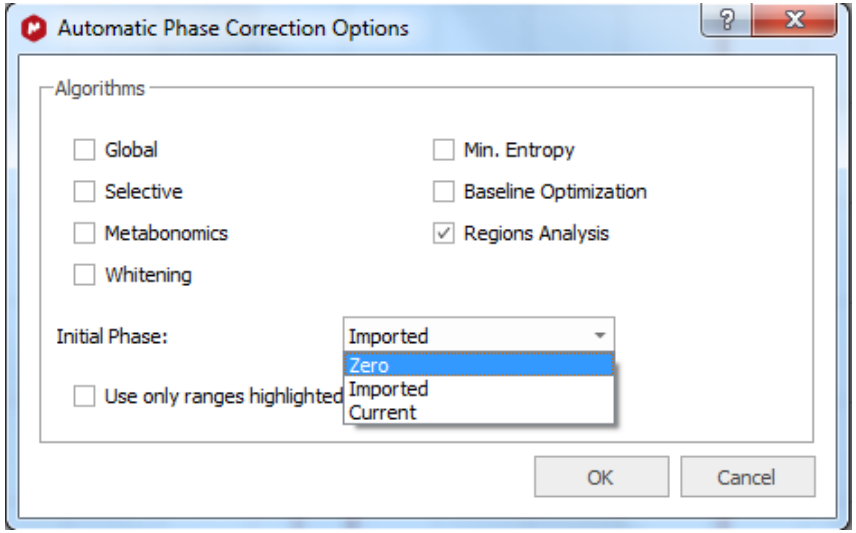
Phasing

- mNova does an initial phase correction when data is loaded
- Further phase correction is often needed



Automatic phasing



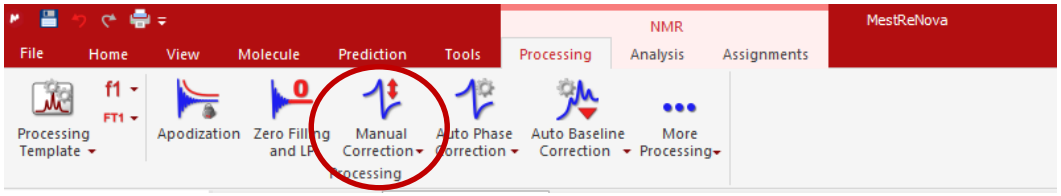


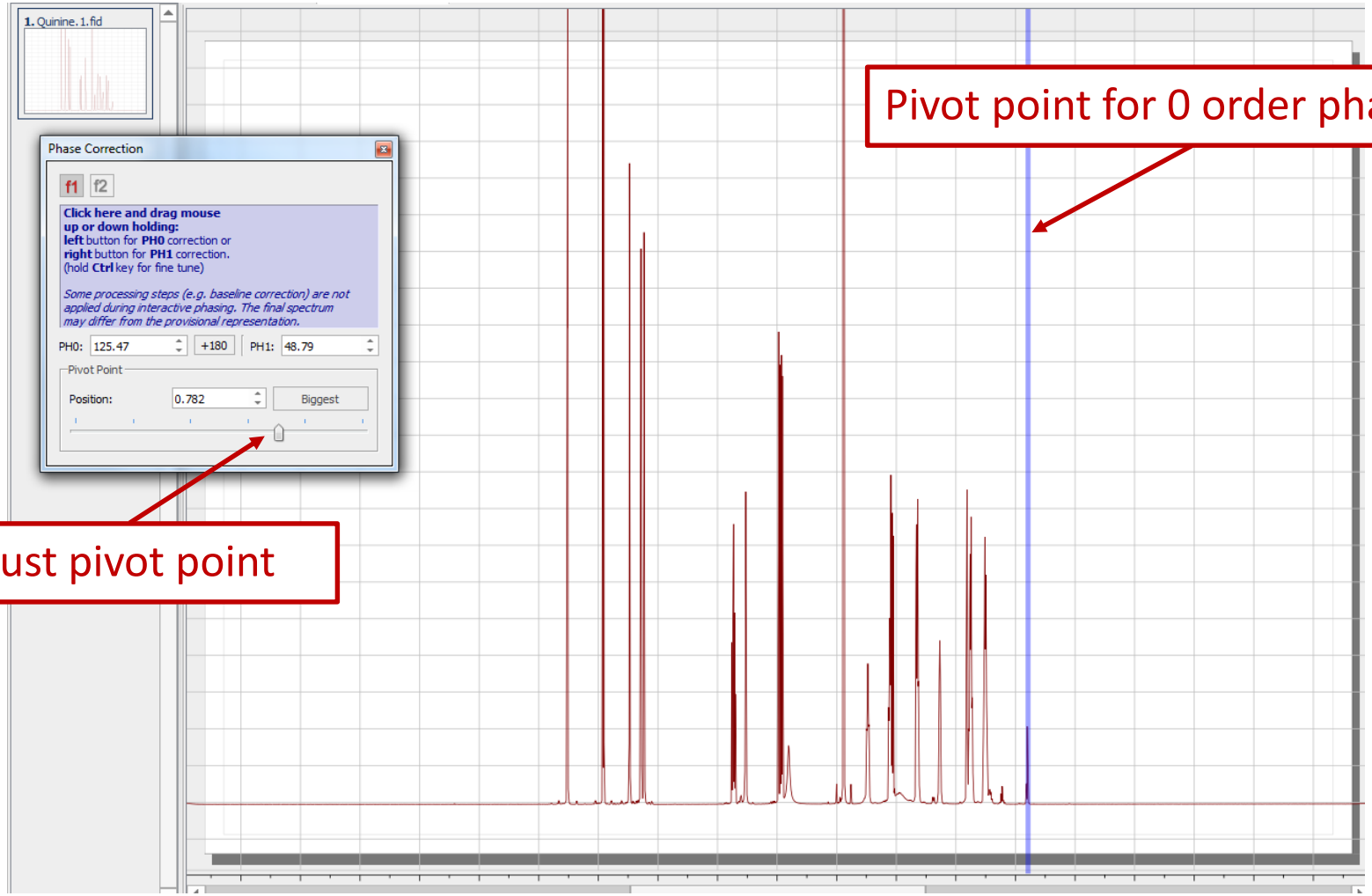
Automatic phase correction:

- Initial phase
- Algorithms

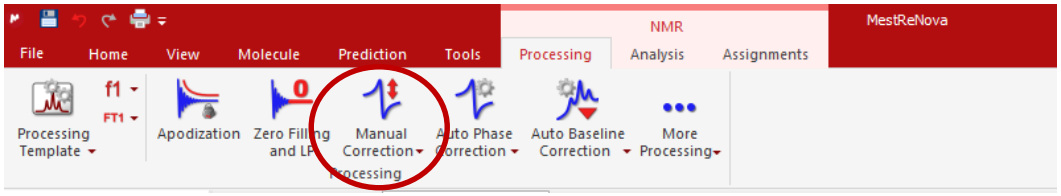
Experiment with different options for best results

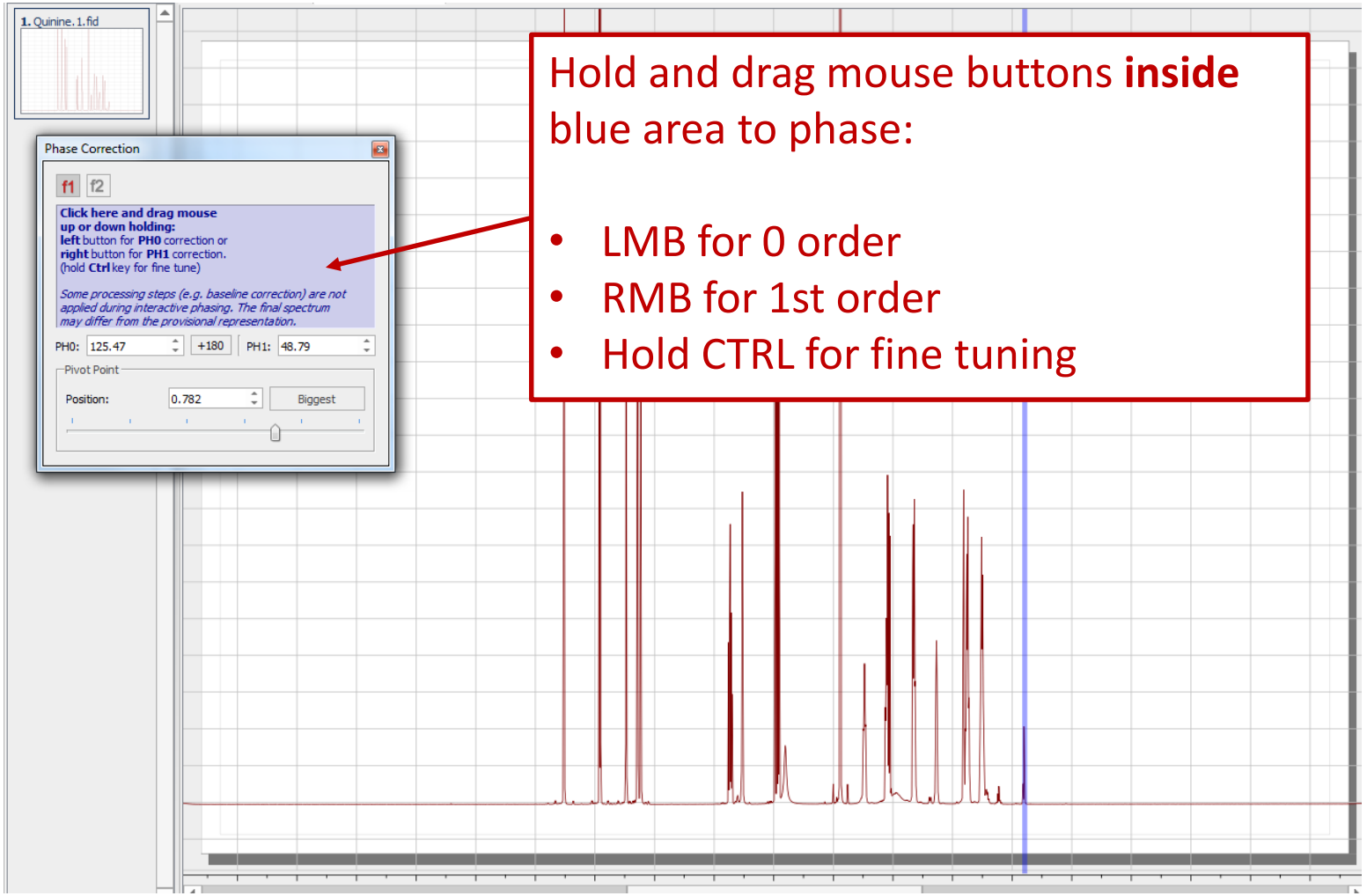
Manual phasing





Manual phasing

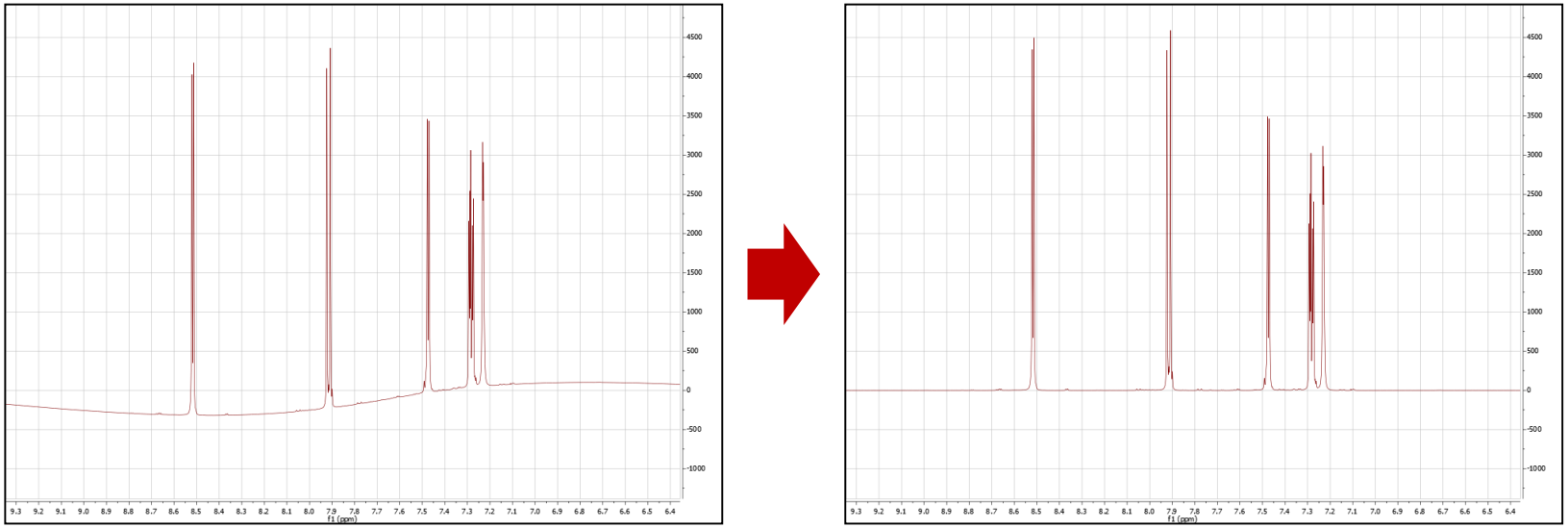




Hold and drag mouse buttons **inside** blue area to phase:

- LMB for 0 order
- RMB for 1st order
- Hold CTRL for fine tuning

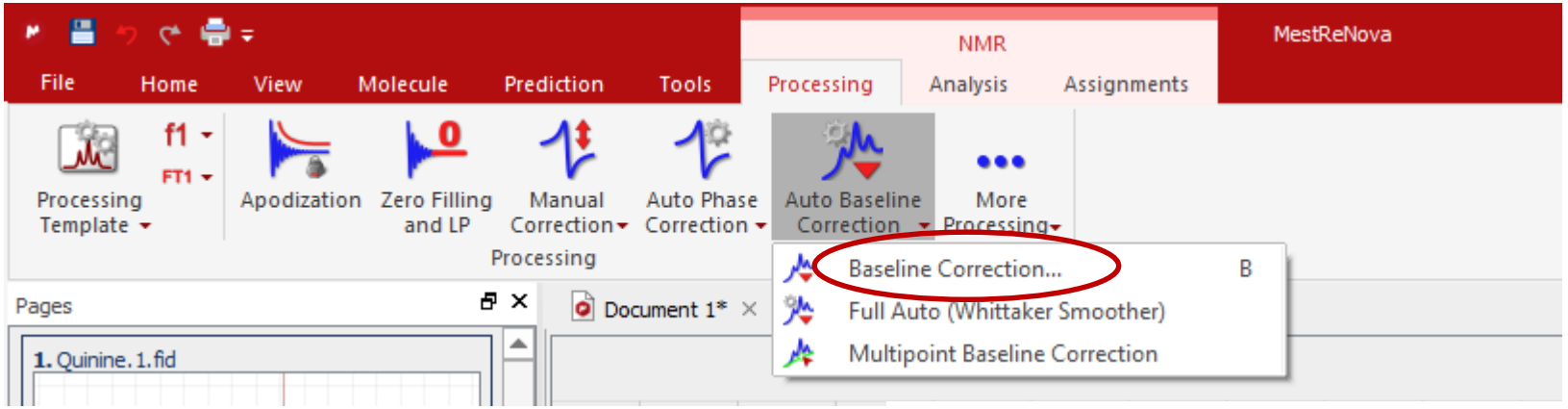
Baseline correction



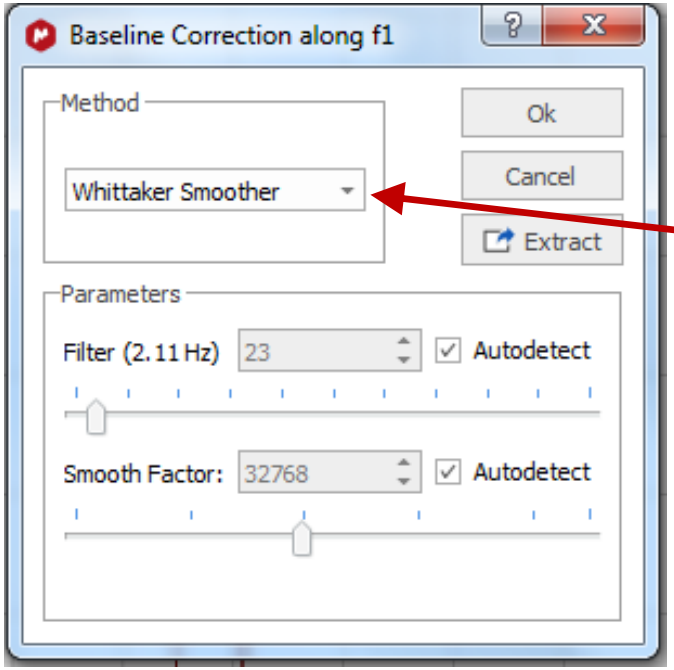
Poor baseline:

- Inaccurate integrals
- Difficult to analyse and compare spectra

Baseline correction algorithms



- Click on “Baseline Correction”
- **or** Right-click in spectrum and select

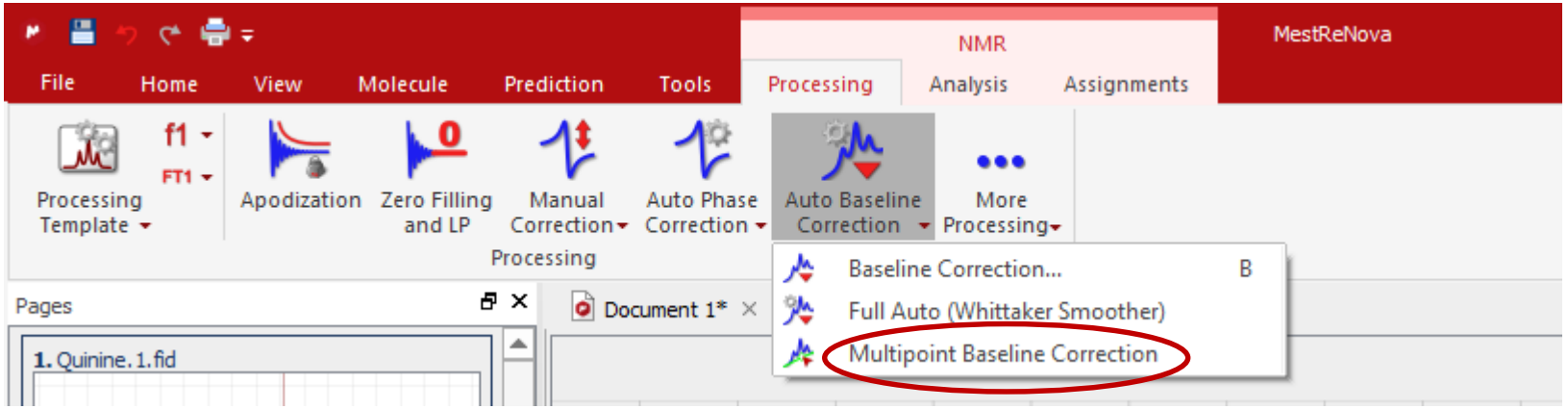


Many options available :

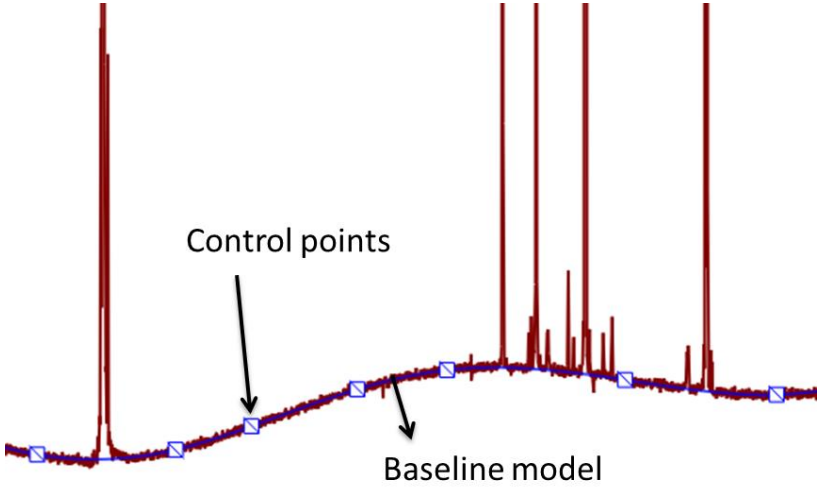
- Whittaker Smoother*
- Polynomial Fit
- Bernstein Polynomial Fit
- Ablative Splines

** Whittaker Smoother usually a good first choice*

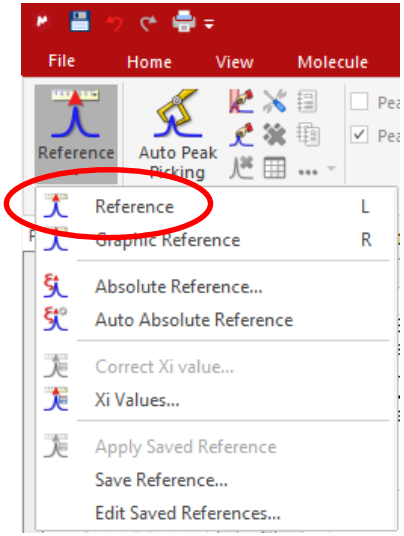
Baseline correction algorithms



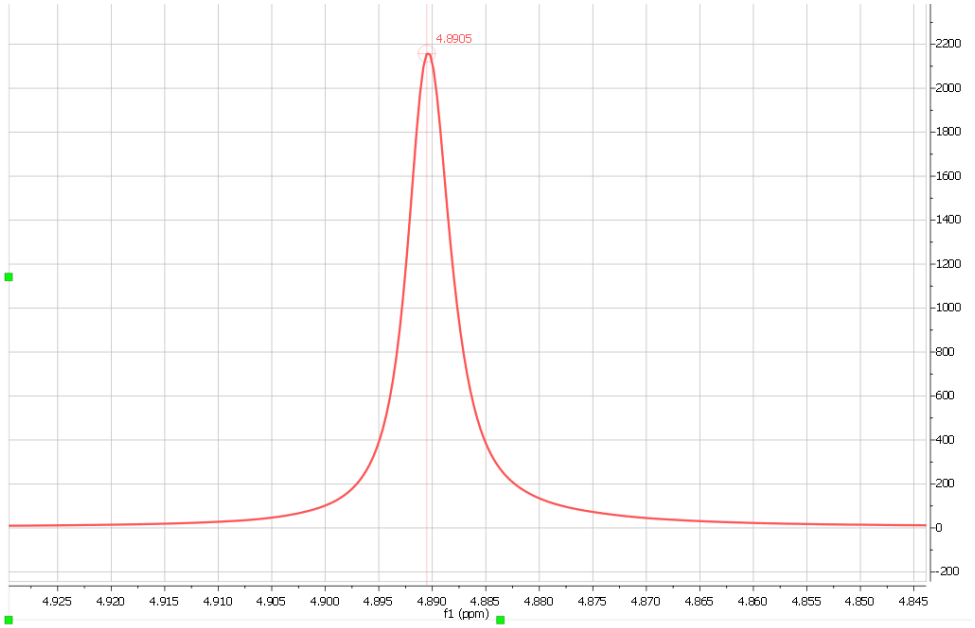
Model the baseline by selecting points that fall on the baseline and then interpolating between these points.

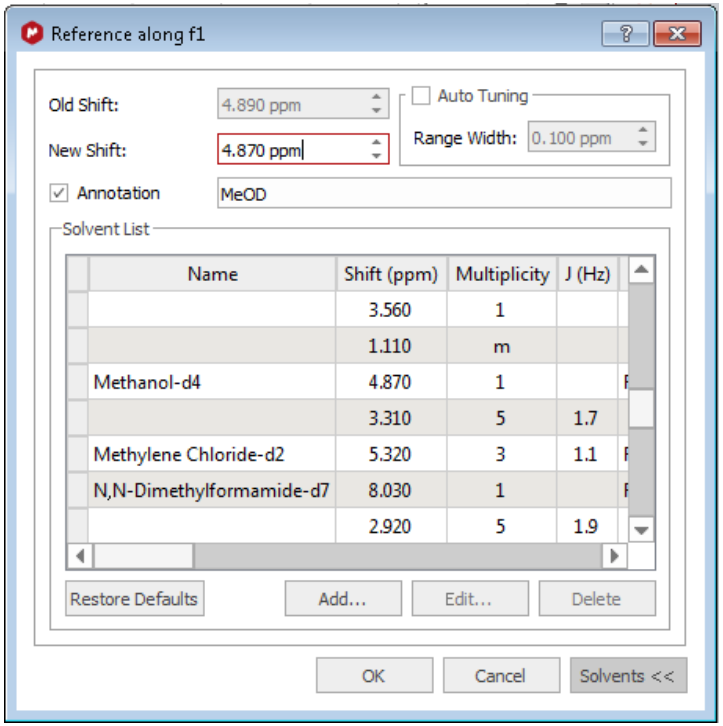


Referencing



- Select peak to use as reference
- Use the dialogue box to define the reference

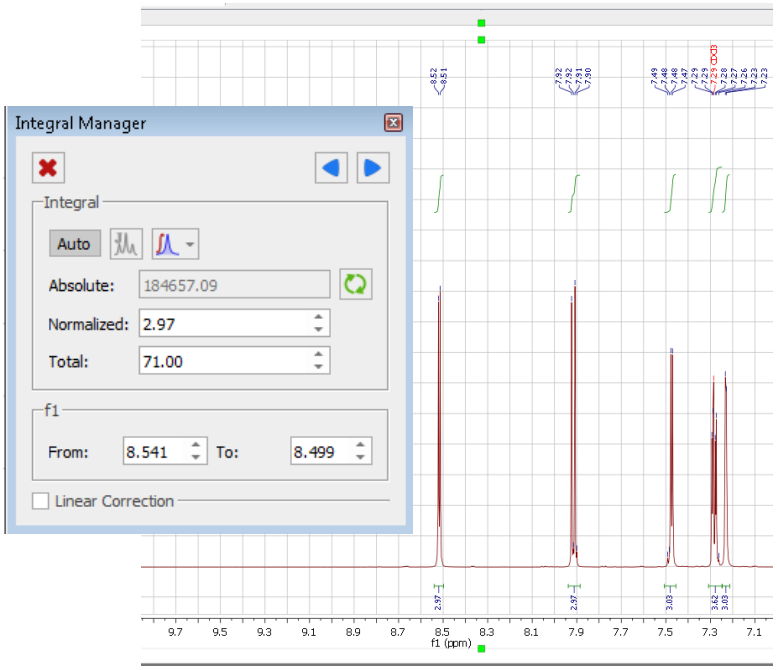


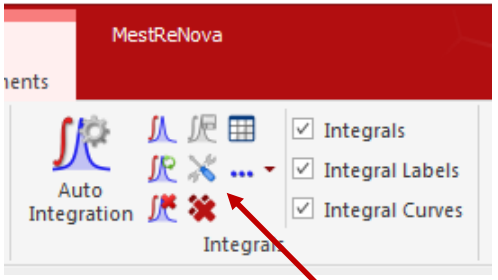


1D Integration

- Click 

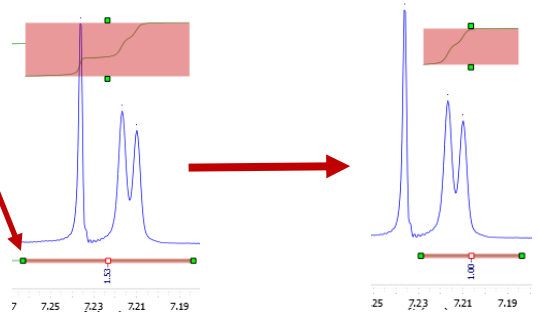
to do auto integration or click **I** to do it manually
- Double click on an integral curve to popup Integral Manager:





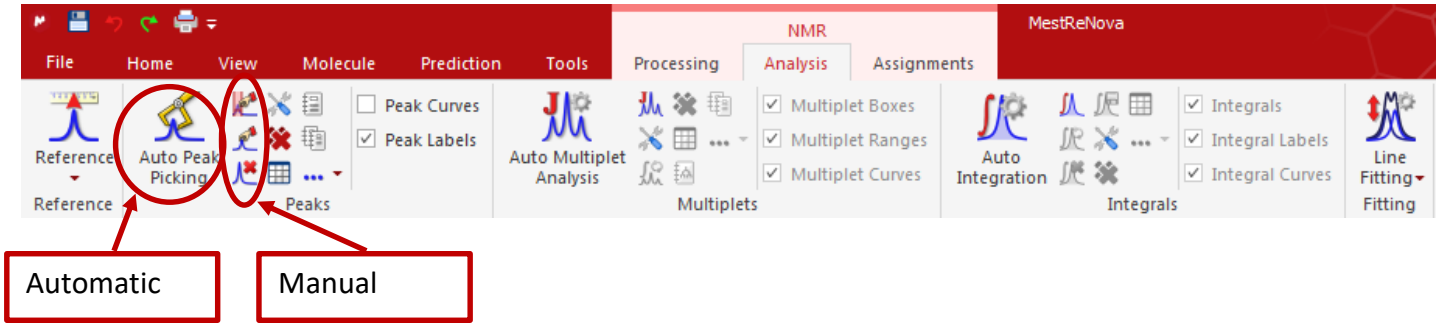
Use integration options to change the method and to specify other options

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



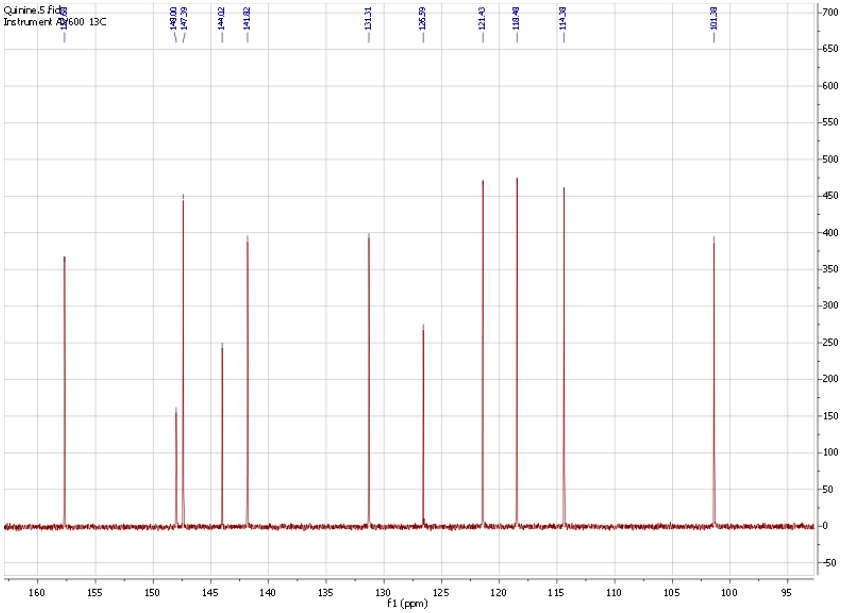
- Define a value to normalise the integrals
- Browse, delete, change, split integrals interactively if needed

1D Peak picking

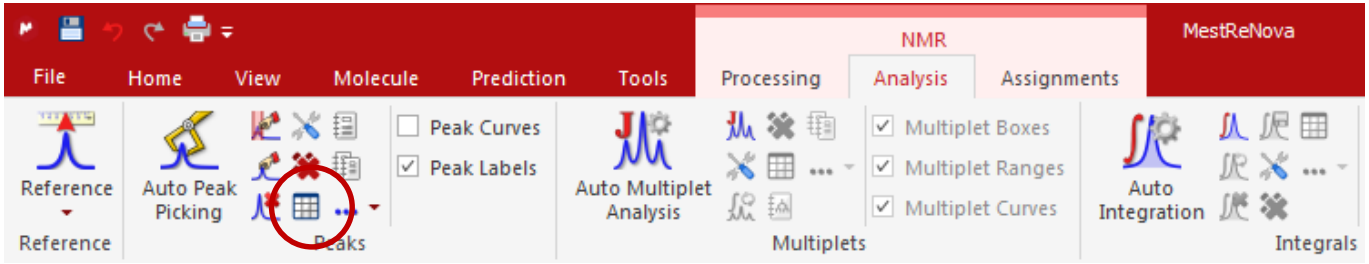


- Peak picking: Click on “Auto Peak Picking” for automatic peak picking. Click on  for options.

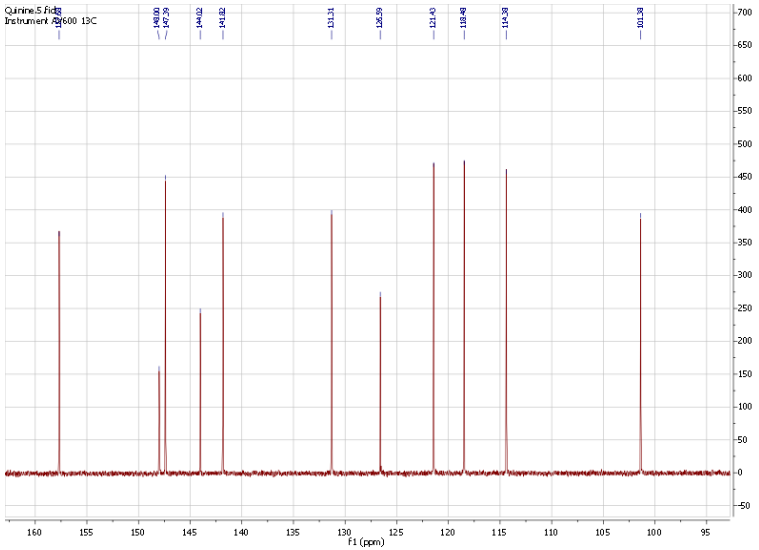
- Manual peak picking is also available. The manual threshold option (shortcut K) allows you to select groups of peaks with different thresholds. The peak by peak option (ctrl-K) is useful if you have shoulder peaks or ‘hidden peaks’ that were not selected in automatic peak picking.

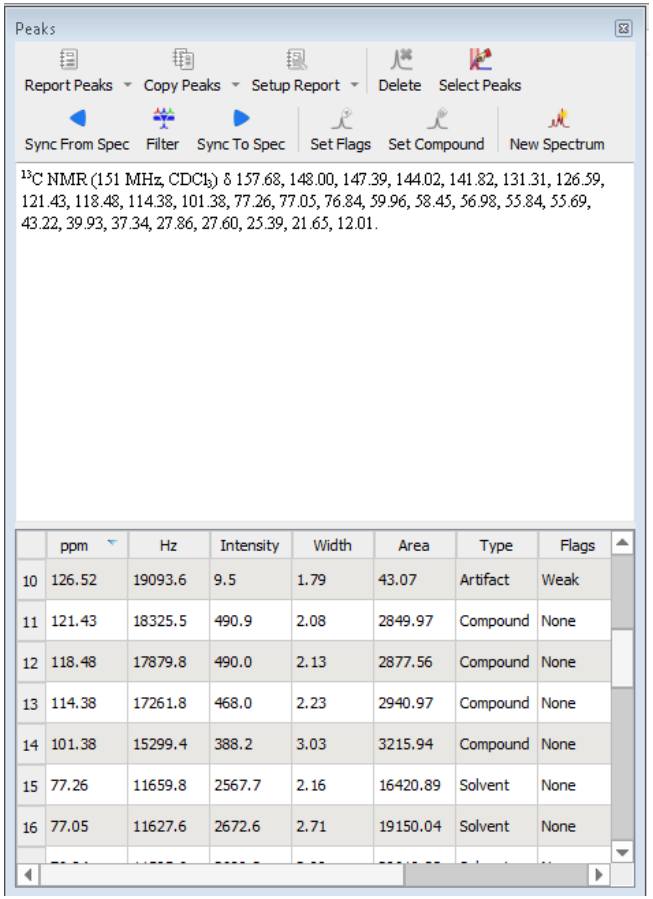


Peak information



- Click on  in the **NMR | Analysis** menu to access the Peaks table
- **Or** select “Peaks” from **View | Tables**
- This table gives information on peak area, intensity, linewidth, frequency (ppm and Hertz), etc.

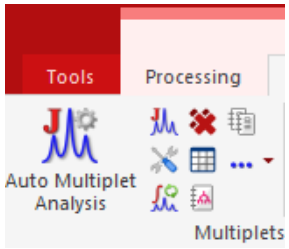




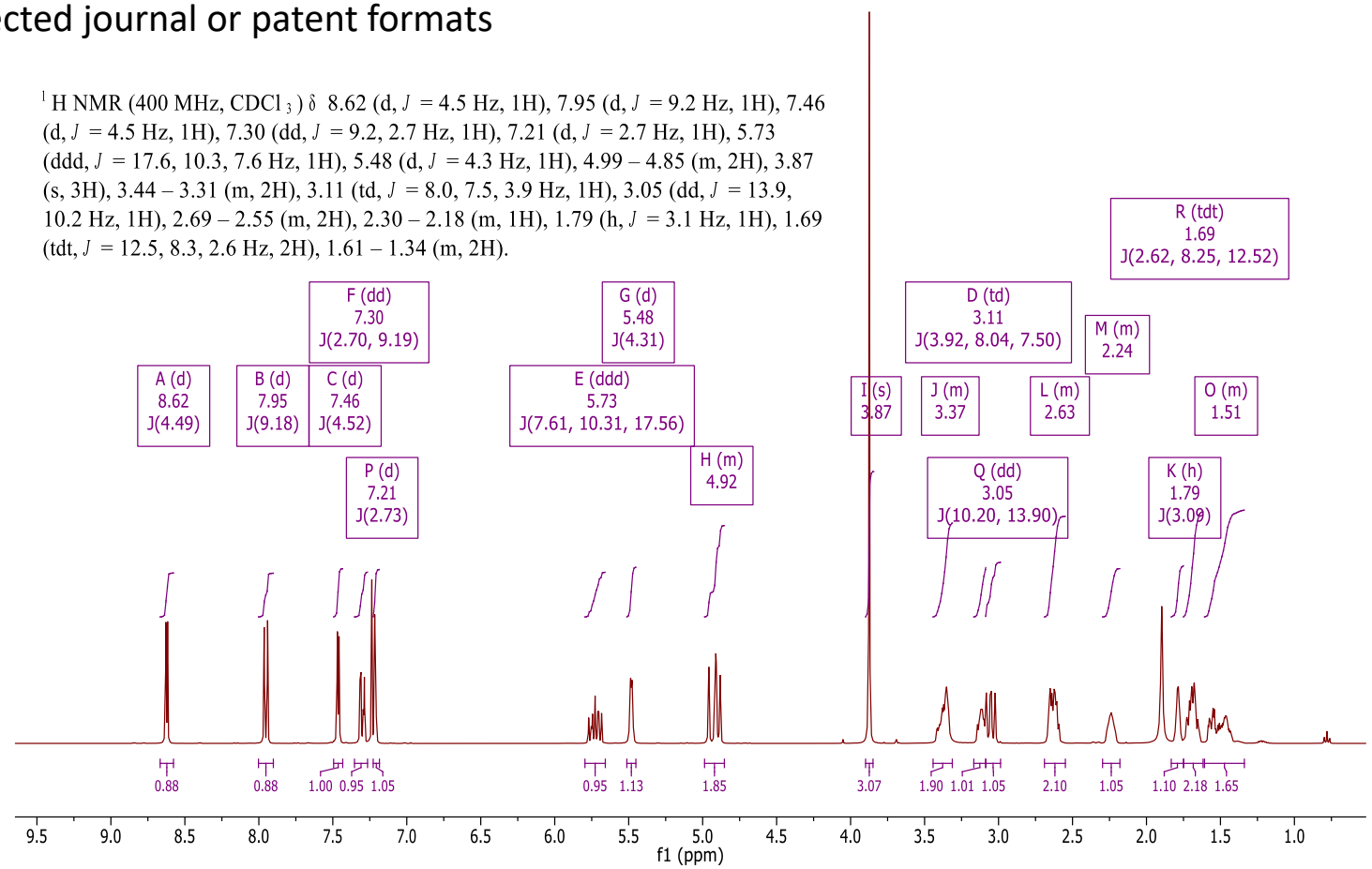
	ppm	Hz	Intensity	Width	Area	Type	Flags
10	126.52	19093.6	9.5	1.79	43.07	Artifact	Weak
11	121.43	18325.5	490.9	2.08	2849.97	Compound	None
12	118.48	17879.8	490.0	2.13	2877.56	Compound	None
13	114.38	17261.8	468.0	2.23	2940.97	Compound	None
14	101.38	15299.4	388.2	3.03	3215.94	Compound	None
15	77.26	11659.8	2567.7	2.16	16420.89	Solvent	None
16	77.05	11627.6	2672.6	2.71	19150.04	Solvent	None

Multiplet analysis

- Mnova provides two approaches to **multiplet analysis:**
  - **Fully automatic:** peak picking, integration and multiplet analysis *all done by one click*, with peaks deconvoluted using GSD and 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



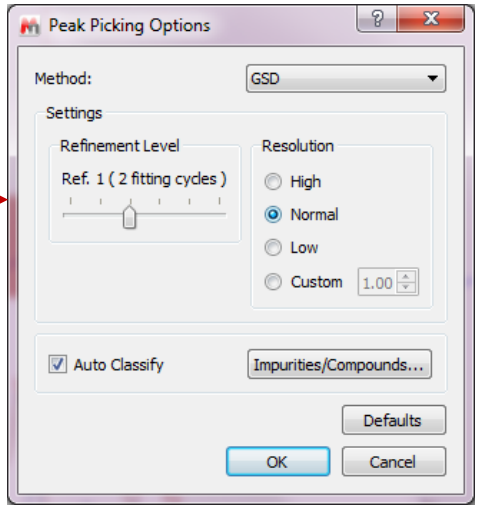
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, J = 4.5 Hz, 1H), 7.95 (d, J = 9.2 Hz, 1H), 7.46 (d, J = 4.5 Hz, 1H), 7.30 (dd, J = 9.2, 2.7 Hz, 1H), 7.21 (d, J = 2.7 Hz, 1H), 5.73 (ddd, J = 17.6, 10.3, 7.6 Hz, 1H), 5.48 (d, J = 4.3 Hz, 1H), 4.99 – 4.85 (m, 2H), 3.87 (s, 3H), 3.44 – 3.31 (m, 2H), 3.11 (td, J = 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).

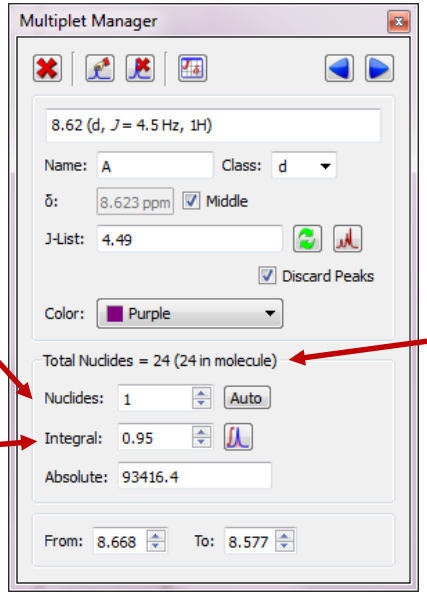


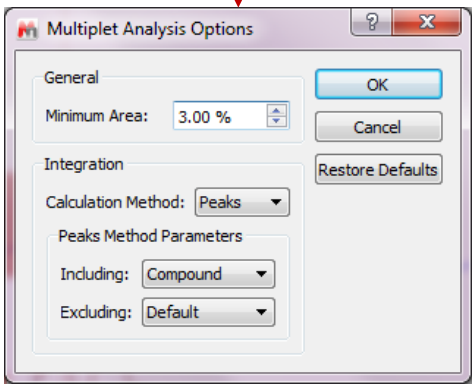
**GSD (Global Spectral Deconvolution): See Help > Contents > Analysis tools > Peak Picking > GSD for details*

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) in the multiplet

Normalised integral of the multiplet.

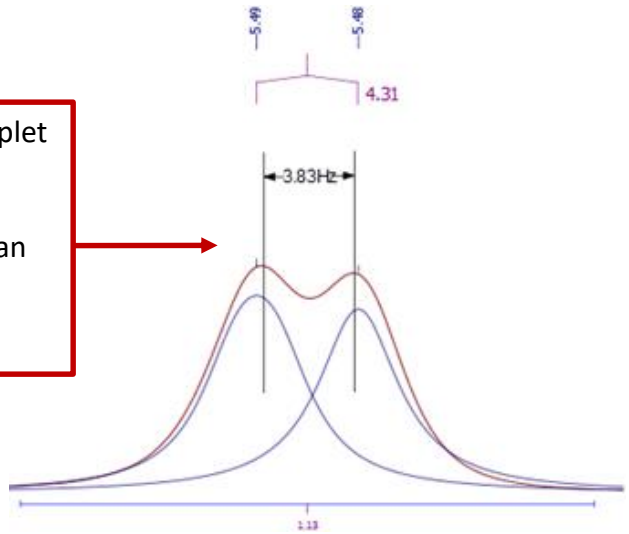
Total # of nuclides from all the multiplets and the # of protons in the molecule (if present)

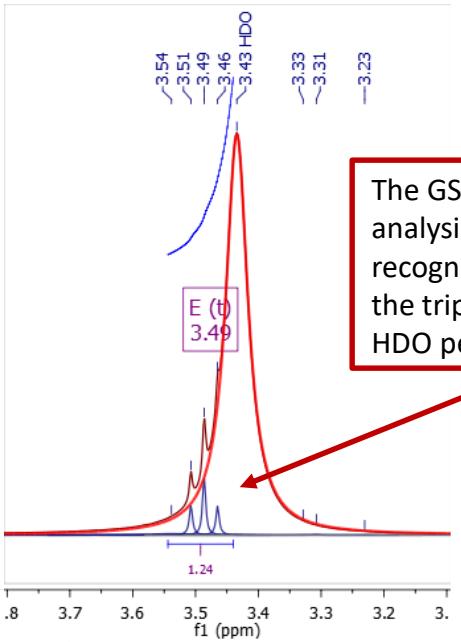
Advantages of GSD-based multiplet analysis

GSD extracts the spectral information from a ^{1}H spectrum automatically, without the need for peak picking and integration.

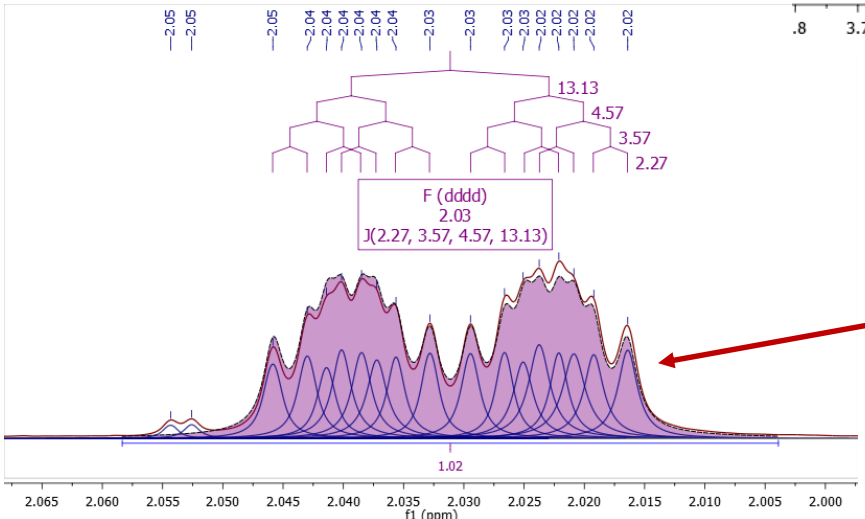
It usually gives good results when the spectrum is of decent quality and resolution, as shown here:

The GSD-based multiplet analysis gives a more accurate *J*-coupling constant (4.31 Hz) than the apparent peak separation (3.83Hz)





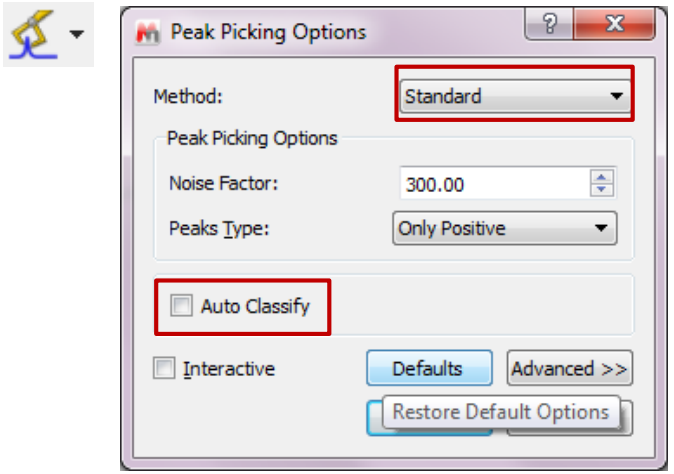
The GSD-based multiplet analysis successfully recognizes and separates the triplet from the large HDO peak



The GSD-based multiplet analysis successfully recognises a very complex (dddd) multiplet. Red: experimental spectrum; Blue: GSD peaks; Purple: simulated multiplet

Change the settings to traditional multiplet analysis

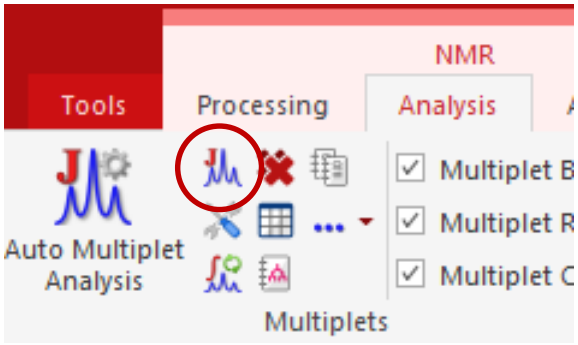
- If you do not wish to use GSD-based peak picking and multiplets analysis, you can change the options back to the traditional method.
- Open a 1D NMR, then do the following to turn off GSD-based peak picking and multiplet integration:
  - For **Peak Picking Options**, change the **Method** to **Standard** to use the traditional peak maxima-based method, also turn off the **Auto Classify** if you don't want to classify peak types automatically
  - For **Multiplet Analysis Options**, change the **Calculation Method** to **Sum***



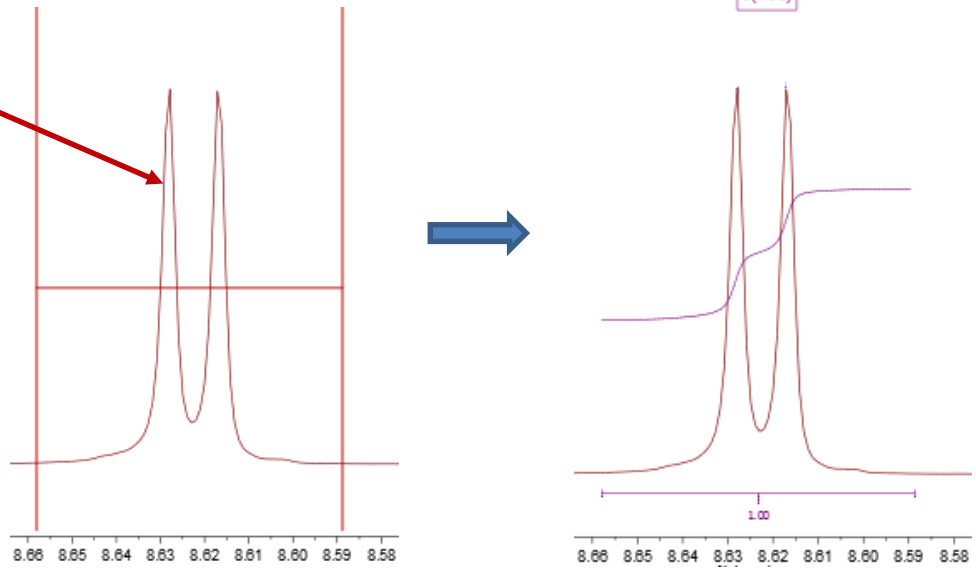
* *"Sum" is the traditional method of integration by summing up all points within the integration region.*

To pick multiplets manually

- Manual Multiplet Analysis allows you to have greater control of multiplet analysis (**J** is the shortcut key)
- Zoom into each multiplet, and 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



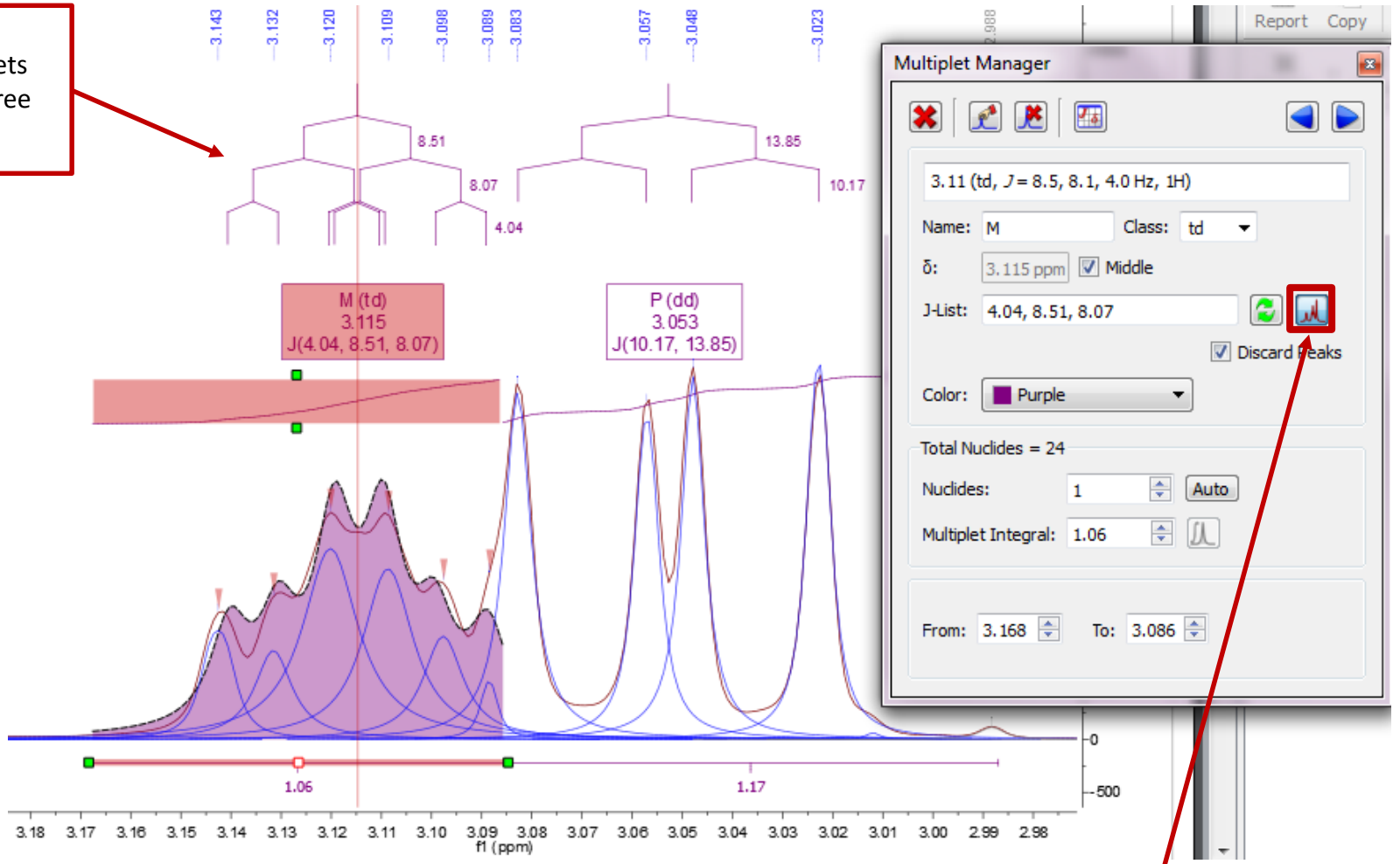
Click and drag to define the **integration region** and **peak picking threshold** and a doublet will be picked



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

Tools for verifying multiplet analysis results

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



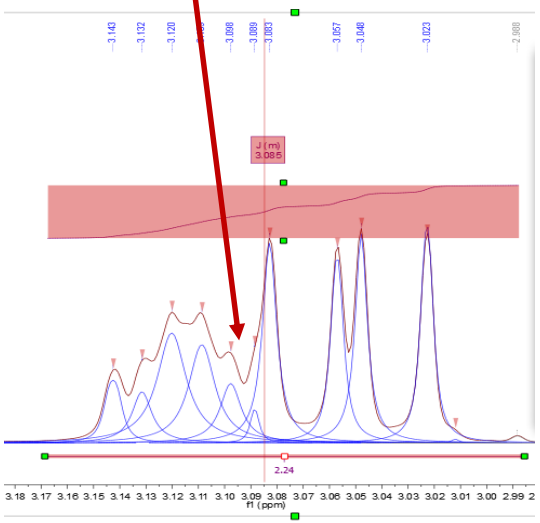
Use the simulation tool in the Multiplet Manager to simulate the multiplet and compare

Multiplet Analysis

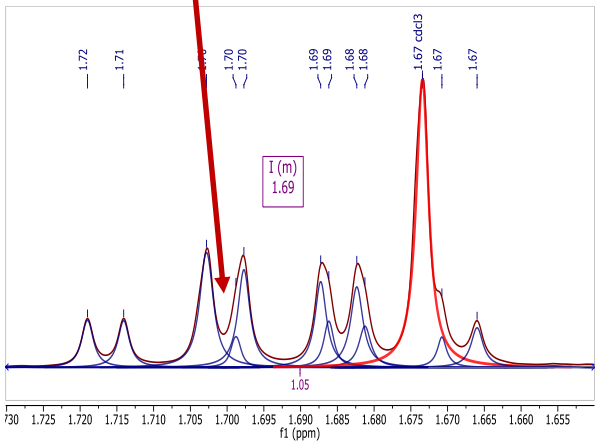
Many other multiplet analysis functions e.g.

- Split overlapping multiplets
- Add missing peaks to multiplets
- Re-assign peaks to multiplets (if asssigned incorrectly)

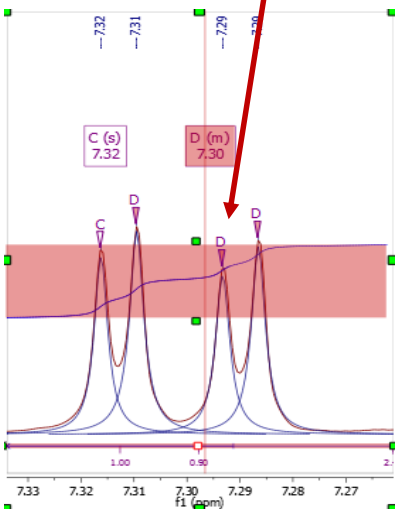
Overlapping multiplets



A small peak is missed by automated analysis

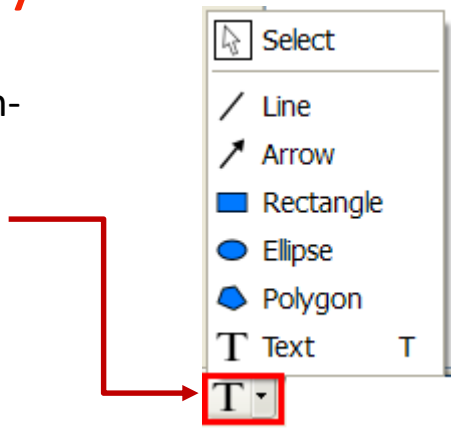


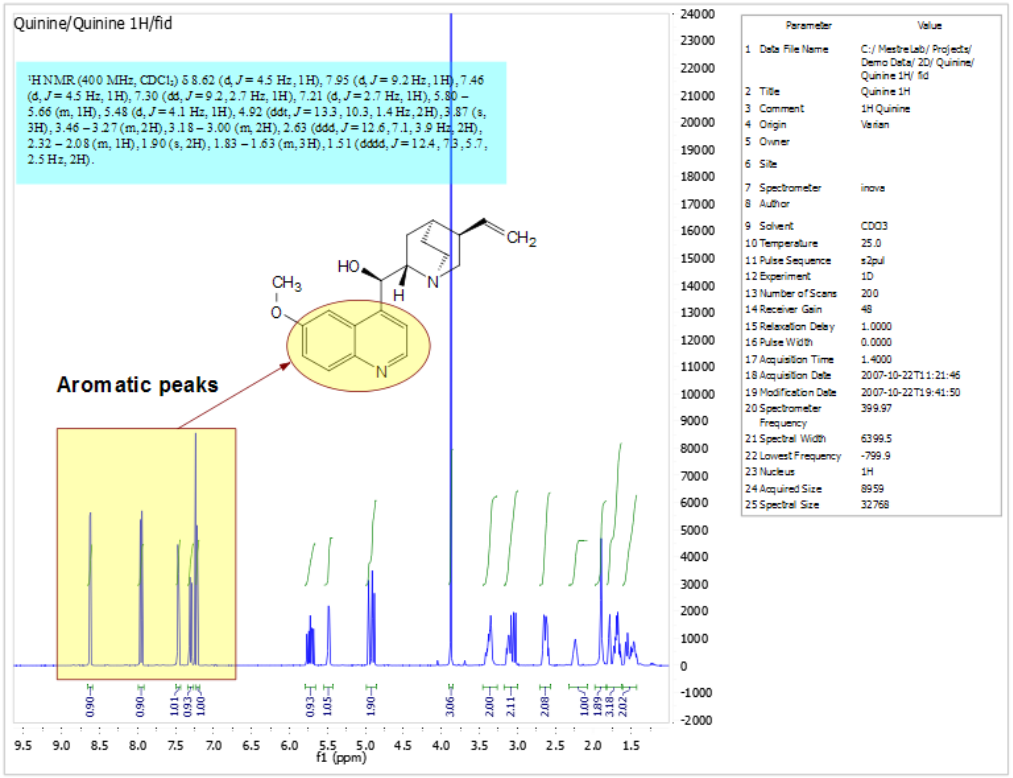
Incorrect assignment



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*

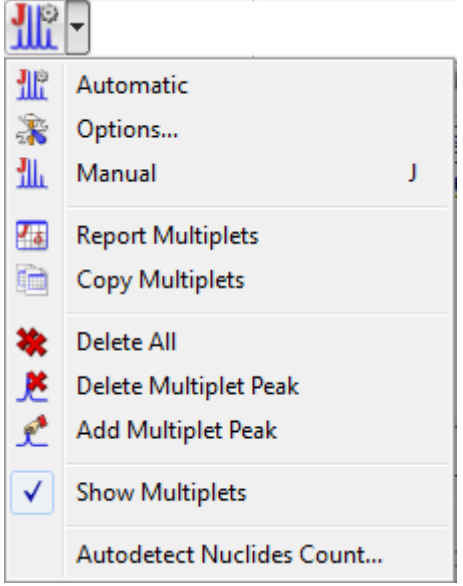
Use **View | Layout Templates menu to generate and apply layout templates, or request an auto **formatting script** from Mestrelab.*

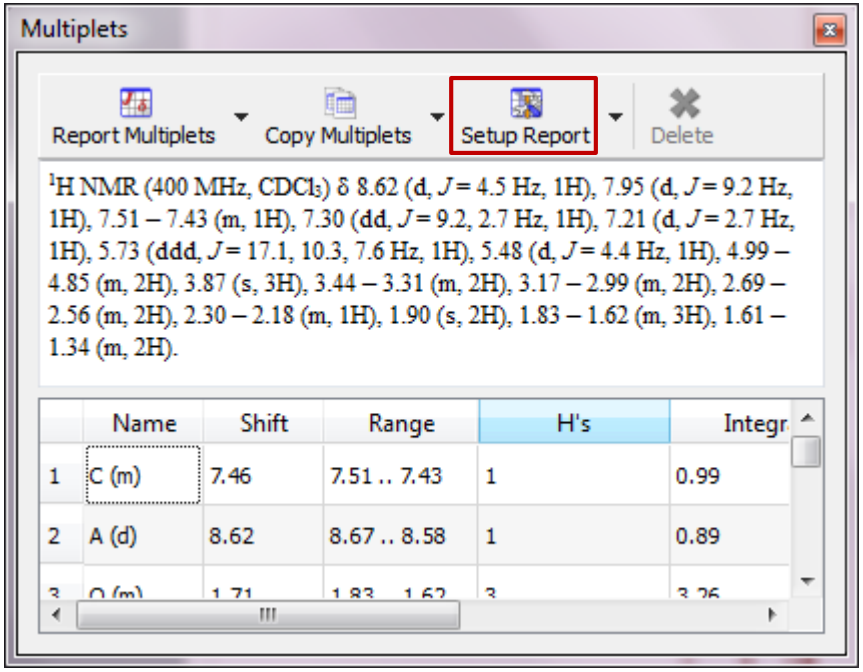
****Copy/paste** any object(s) to your document with high resolution*

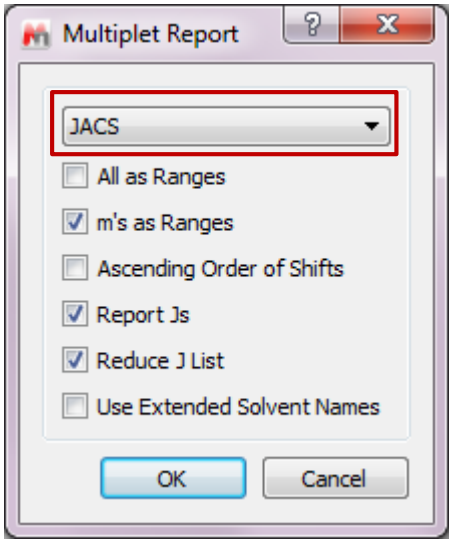
Click to export **PDF*

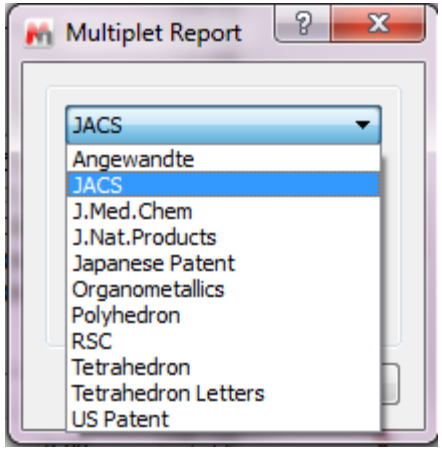
To report multiplets in journal format

- 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**



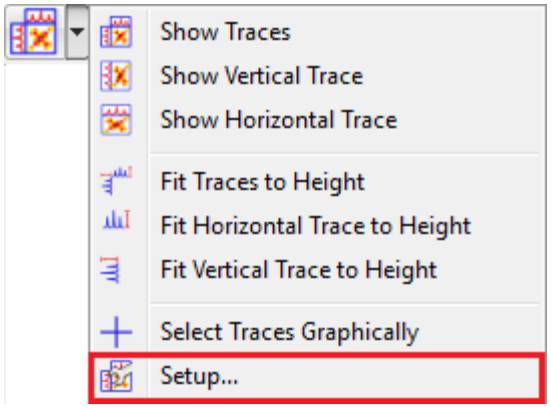




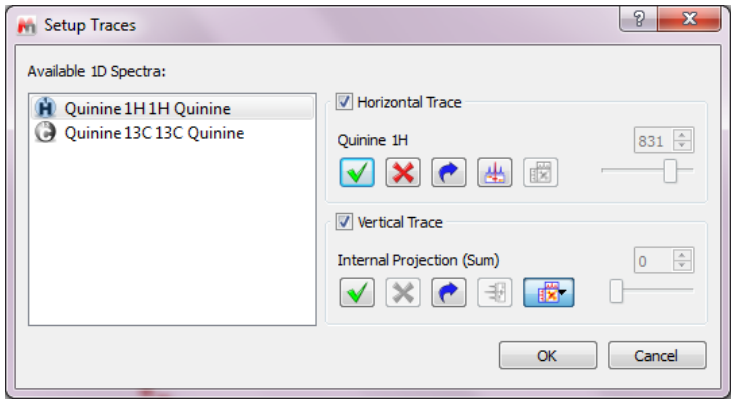


Tip: *From the Multiplet Table, click* ***Copy Multiplets*** *and then paste the texts to your document. Click* ***Copy Table*** *and then paste the spreadsheet to your document. The table can be customized using* ***Setup Table.***

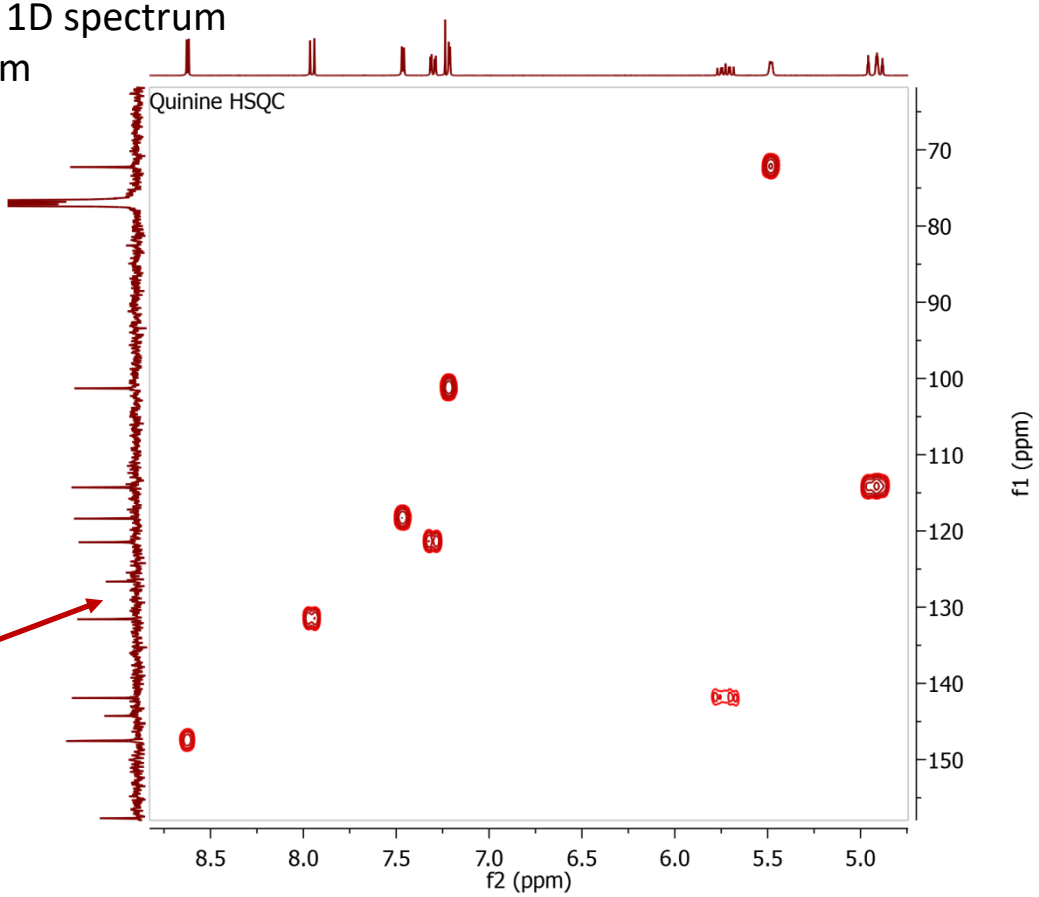
To attach 1D to 2D spectra



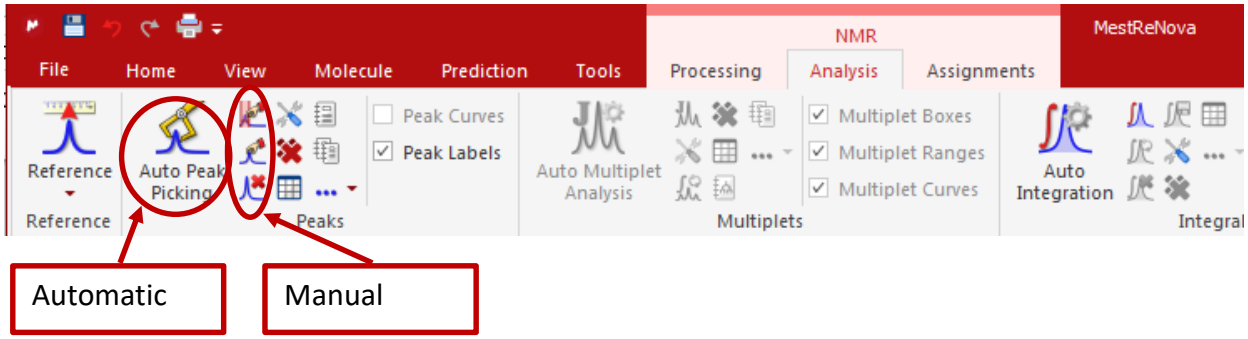
- Open 1D and 2D spectra in the same document (They are shown as separate pages)
- Display the 2D spectrum, click the **Traces** tool options and choose **Setup…**
- Choose a 1D in the Available 1D Spectrum, click to attach it to that axis
- Alternatively, simply drag and drop the 1D spectrum onto the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



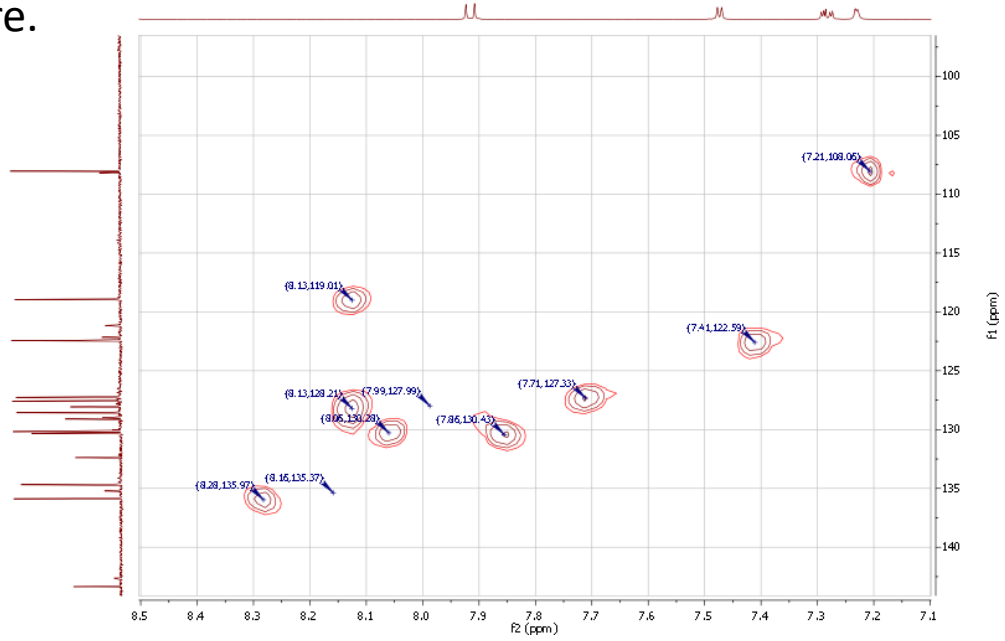
To change the Y intensity of 1D spectra: Place the cursor on a 1D and scroll the mouse wheel, or click **Ctrl+Shift+arrow keys**



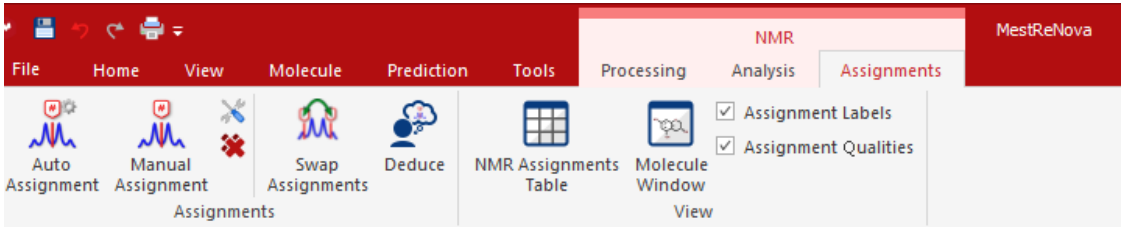
2D Peak picking



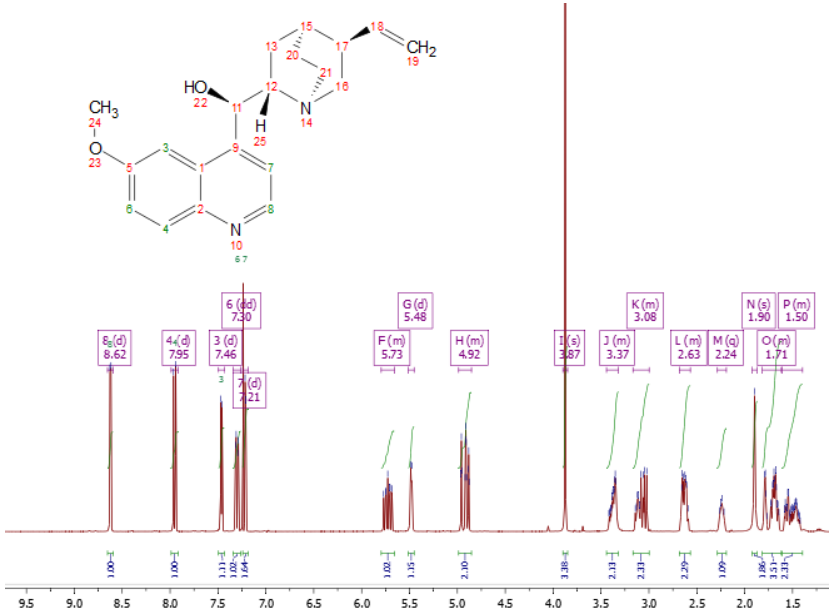
- Peak picking: Click on “Auto Peak Picking” for automatic peak picking. Click on  for options.
- To pick peaks manually, select either the manual threshold option, or the peak by peak option, which allows you to specify each pick by clicking on the peak centre.



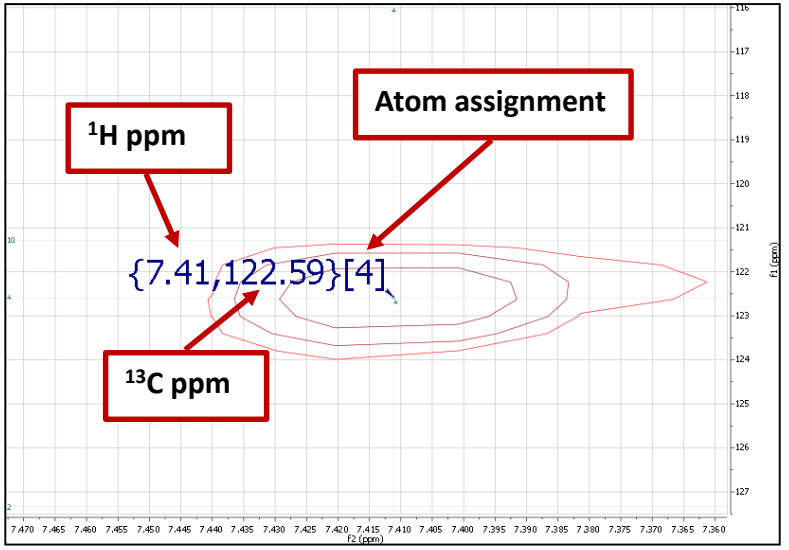
To assign spectra to a structure



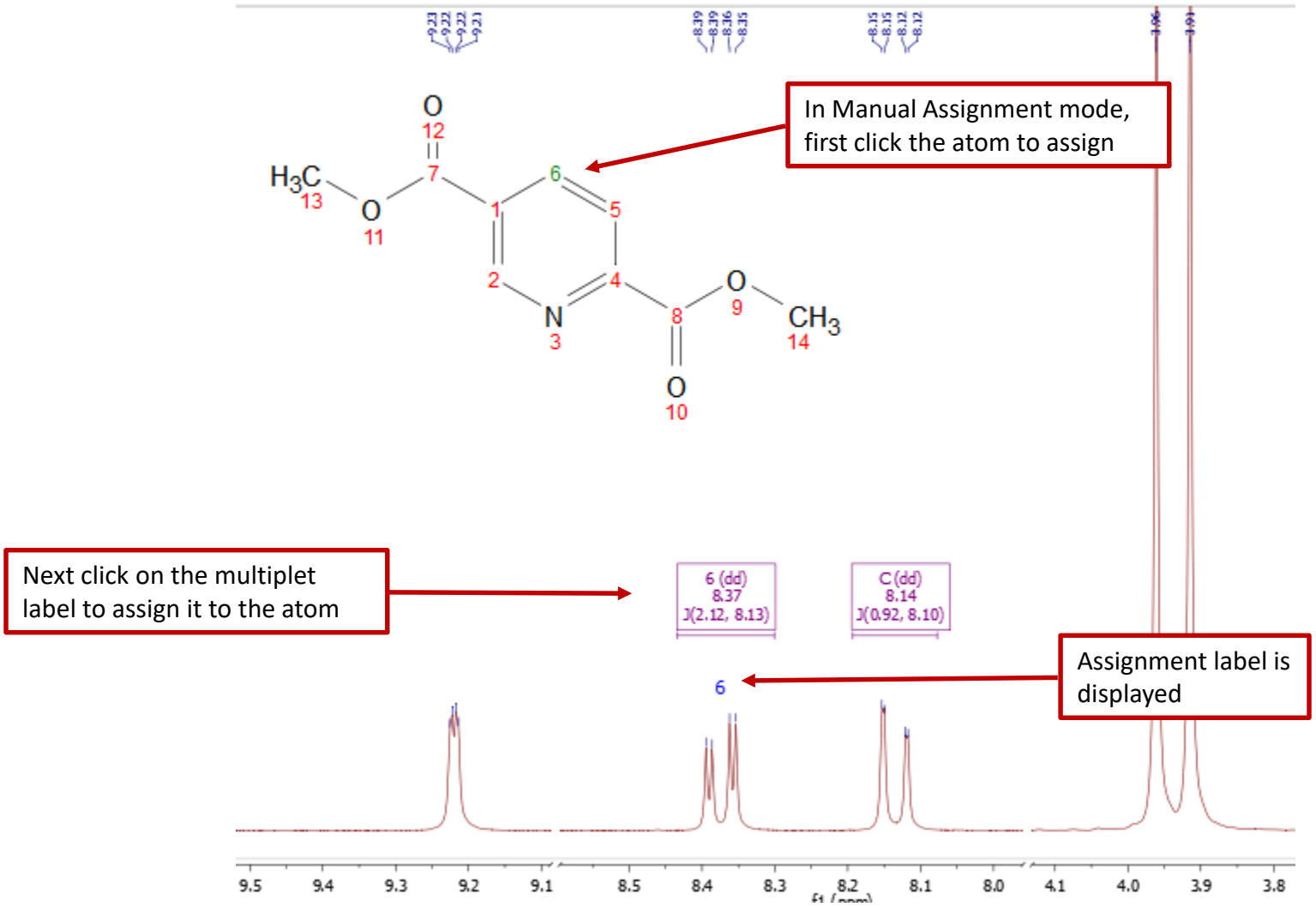
- Choose either automatic or manual assignment mode ("A" key is a shortcut for manual assignment mode)
- For manual assignment, click on an **atom** in the structure (cursor will change to) . Then choose the **peak** you want to assign. There are 3 ways to do it:
  - A picked **multiplet**, by clicking on the multiplet label, or
  - A **peak top** for 1D spectra / **peak centre** for 2D spectra, or
  - A **range** in the spectrum, by click-and-dragging to cover it



2D peaks are labelled by frequency and assignment

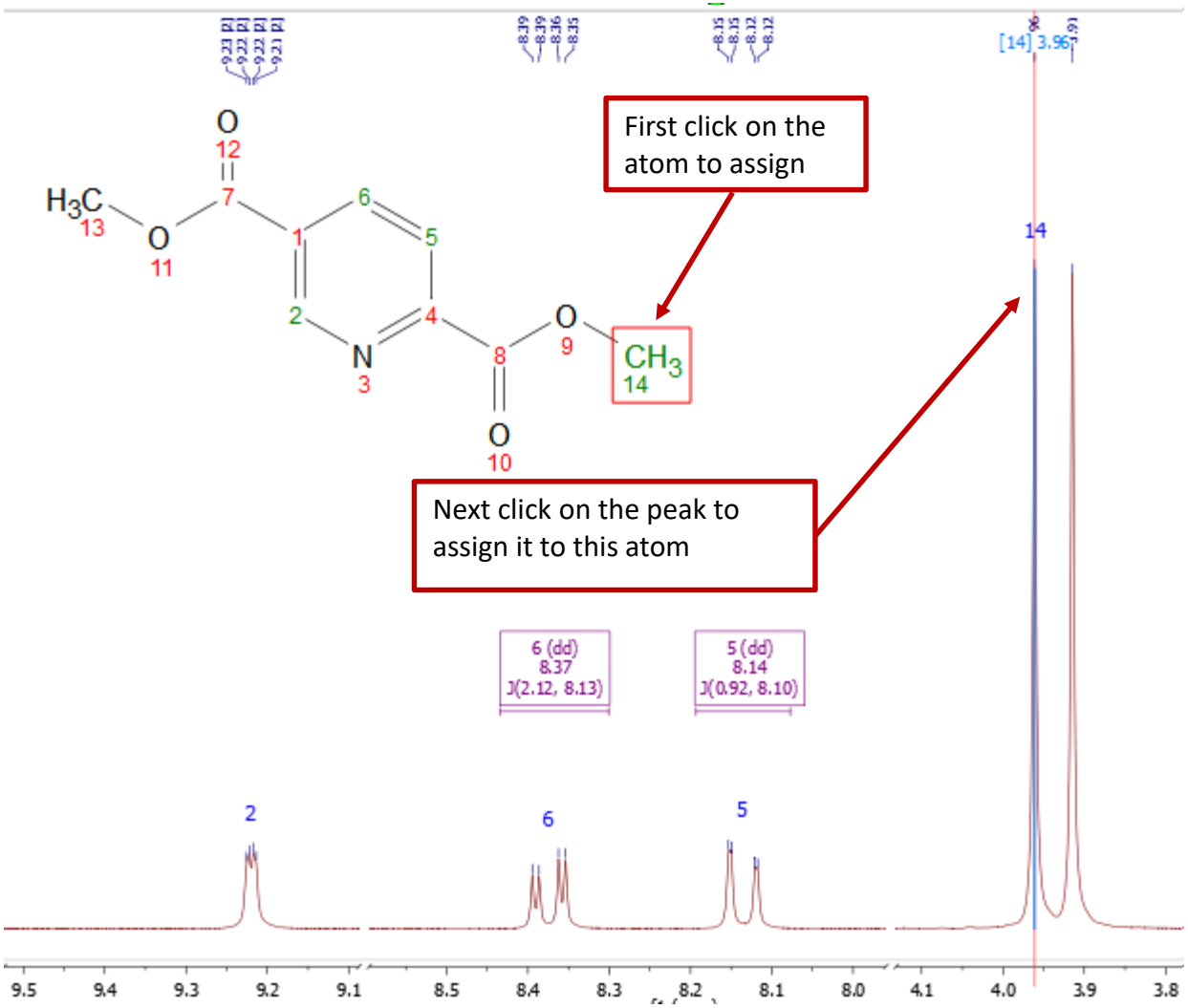


Example: assigning a multiplet to an atom



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 | Show Multiplets

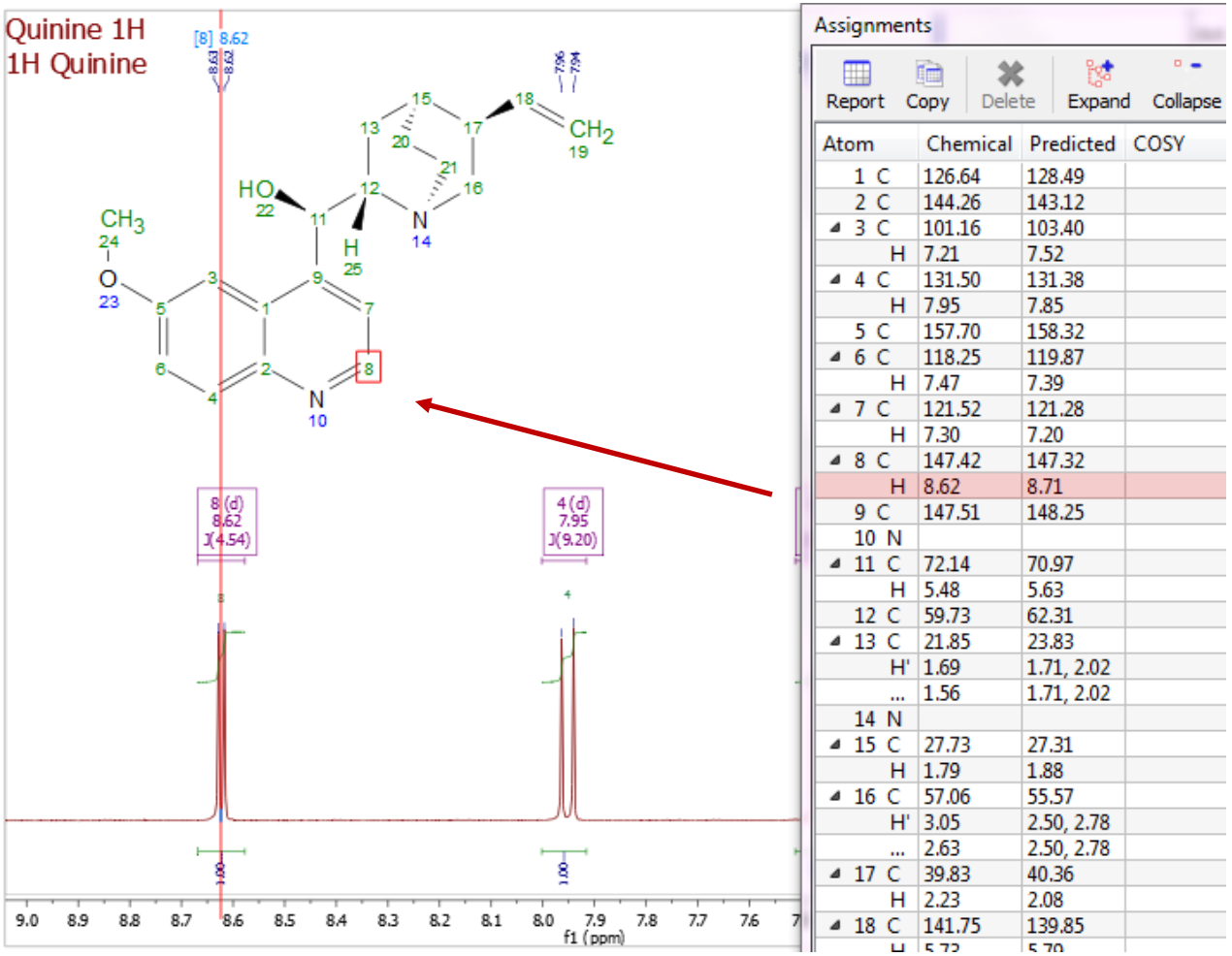
Example: assigning a peak to an atom



*Tip: By Default, Mnova automatically snaps to a peak top (with interpolation). Click **Shift** key one time to toggle it off if you want to choose a shoulder peak.*

To display and browse assignment results

- Choose **View | Tables | Assignments** to open the Assignments Table
- The Table and the structure are correlated: You can click a row to highlight the atom (and its assigned peak), and vice versa



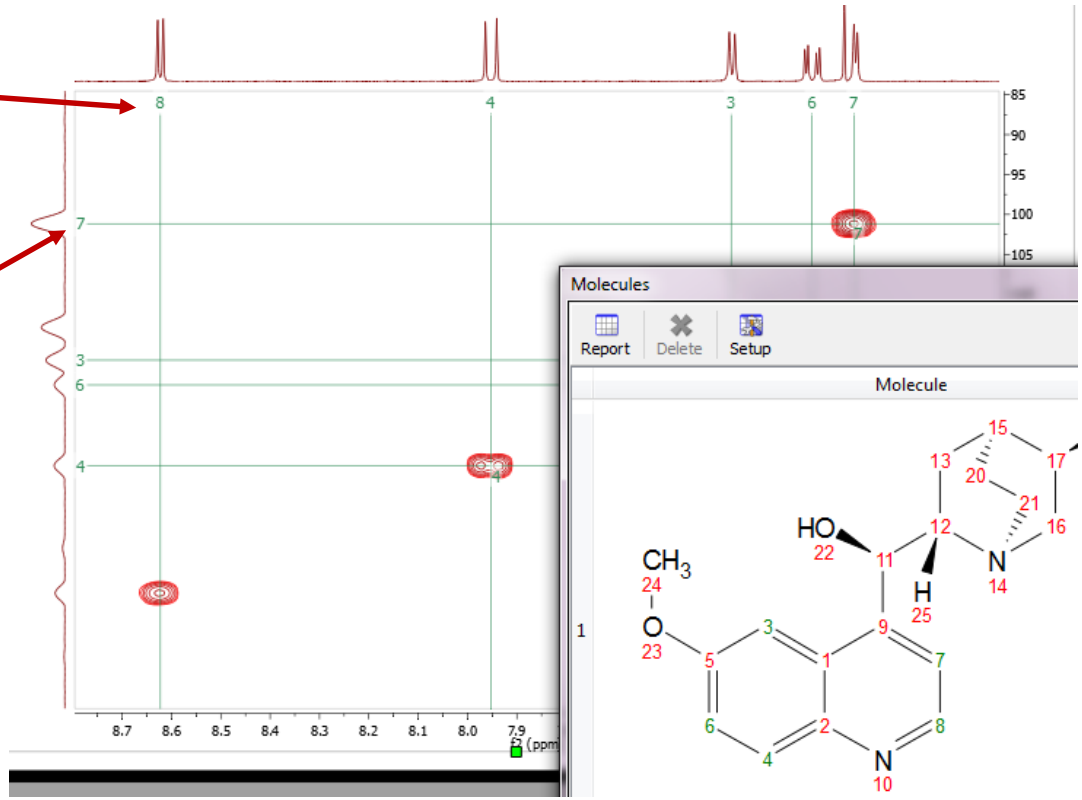
** You can right click on an atom and choose **Edit Atom Data** to change its label. Changed labels will be used in Assignments Table and other relevant reports.*

Example: assigning 2D peaks to an atom

- You can **either** first assign 1D H-1 peaks, and then assign HSQC cross peaks, **or** the opposite
- 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 in HSQC, click **A** key to enter Assignment mode. Click on an **atom** in the structure. Next click on the cross peak to assign to it*

H-1 assignments from 1D spectrum or HSQC

C-13 assignments from HSQC



By Default, Mnova automatically snaps to a peak top. Click **Shift key one time to toggle it off if you want to manually locate the peak center.*

The Assignment Table for multiple spectra

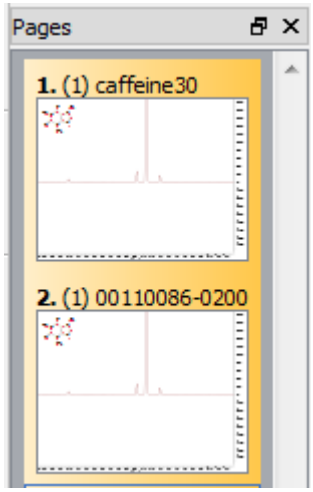
- Choose **View | Tables | Assignments** to open the Assignments Table if not yet
- The Table lists all assignment results, which can be copied to other documents
- Try **Script | Report | Assignments** to report the results in journal format

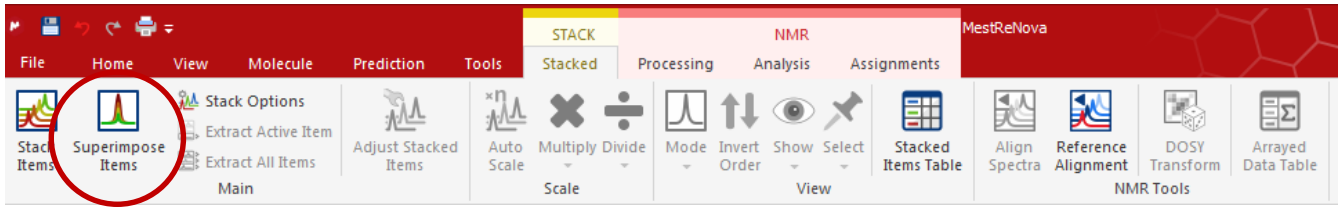
Assignments

Report | Copy | Delete | Expand | Collapse | Setup

Atom	Chemical Sl	Predicted S	COSY	TOCSY	HSQC	HMBC	H2BC	NOESY
1 C	36.08				1', 1"	7		
H'	2.35				1			
H"	2.29				1			
2 C	79.42					7, 26		
3 C	79.40					13', 13", ...		
4 C	133.54					57', 57", ...		
5 C	142.27					57', 57", ...		
6 C	72.46				6			
H	6.24				6			
7 C	75.13				7	26		
H	5.68		26		7	1, 2, 9, 28		
8 C	45.84				26			
9 C	58.92					7, 26, 21...		
10 C	203.84					11, 21', ...		
11 C	75.68				11			
H	3.82				11	10		26
12 O								
H								
13 C	27.02				13', 13", ...			
H3	1.25				13	3		
14 C	21.95				14', 14", ...			
H3	1.15				14			
15 O								
16 O								
17 C	81.46					24', 24", ...		

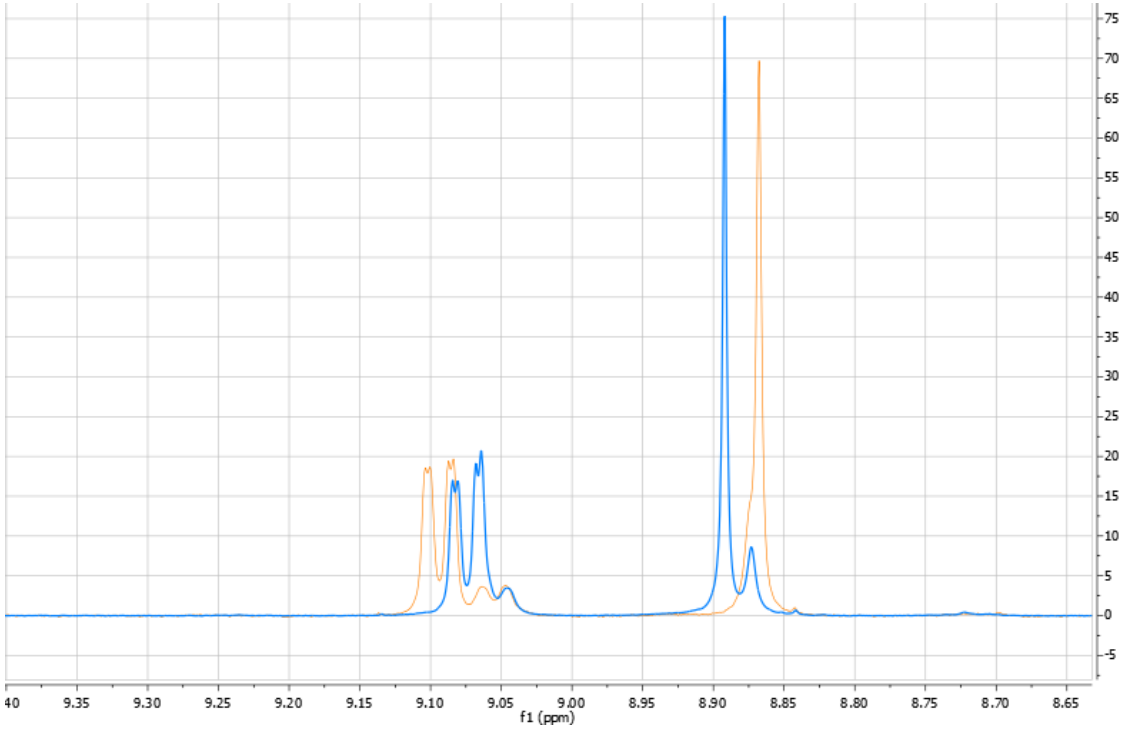
To superimpose multiple 1D spectra



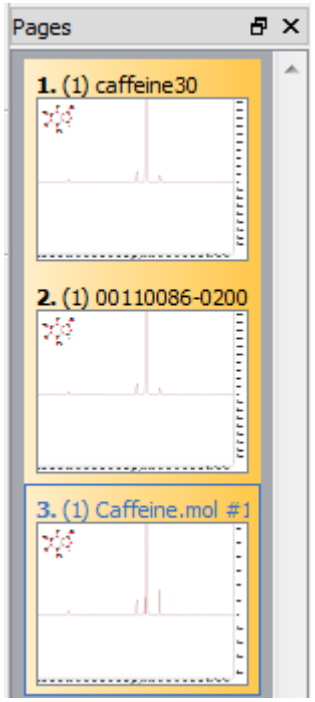


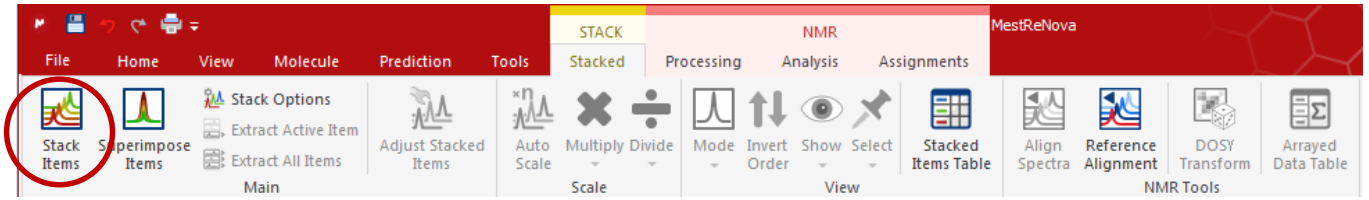
- Open several 1D spectra in the same document
- Select some or all of them in the Pages View
- Click “Superimpose Items” to stack them in a new page:

*Right-click on the spectra and choose **Properties** to change display properties, e.g. colours, transparency, etc.*

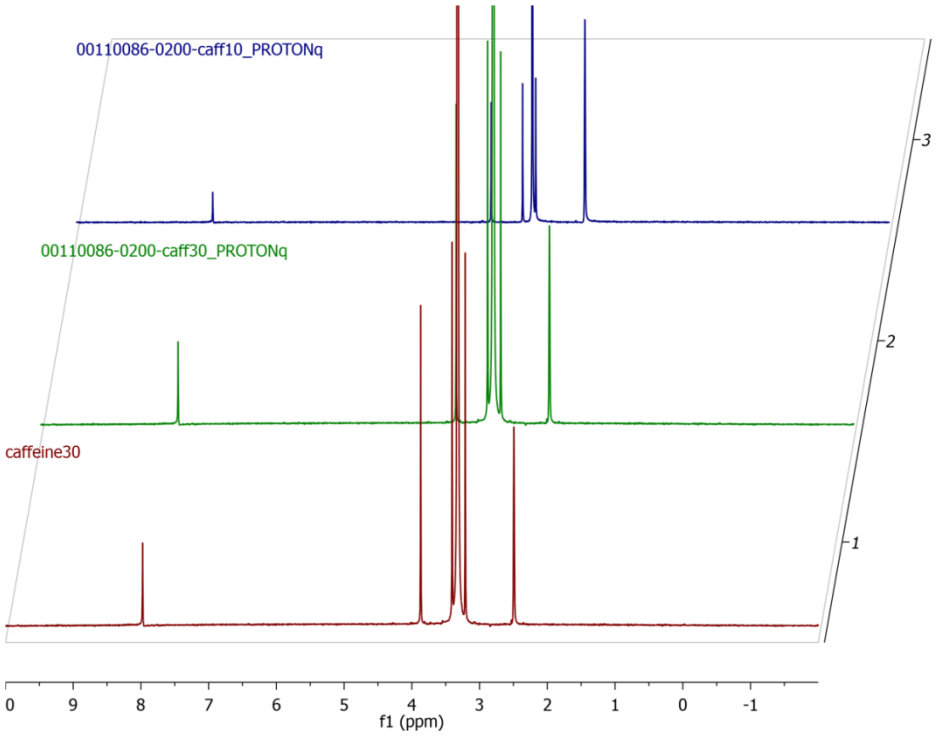


To open and stack multiple 1D spectra





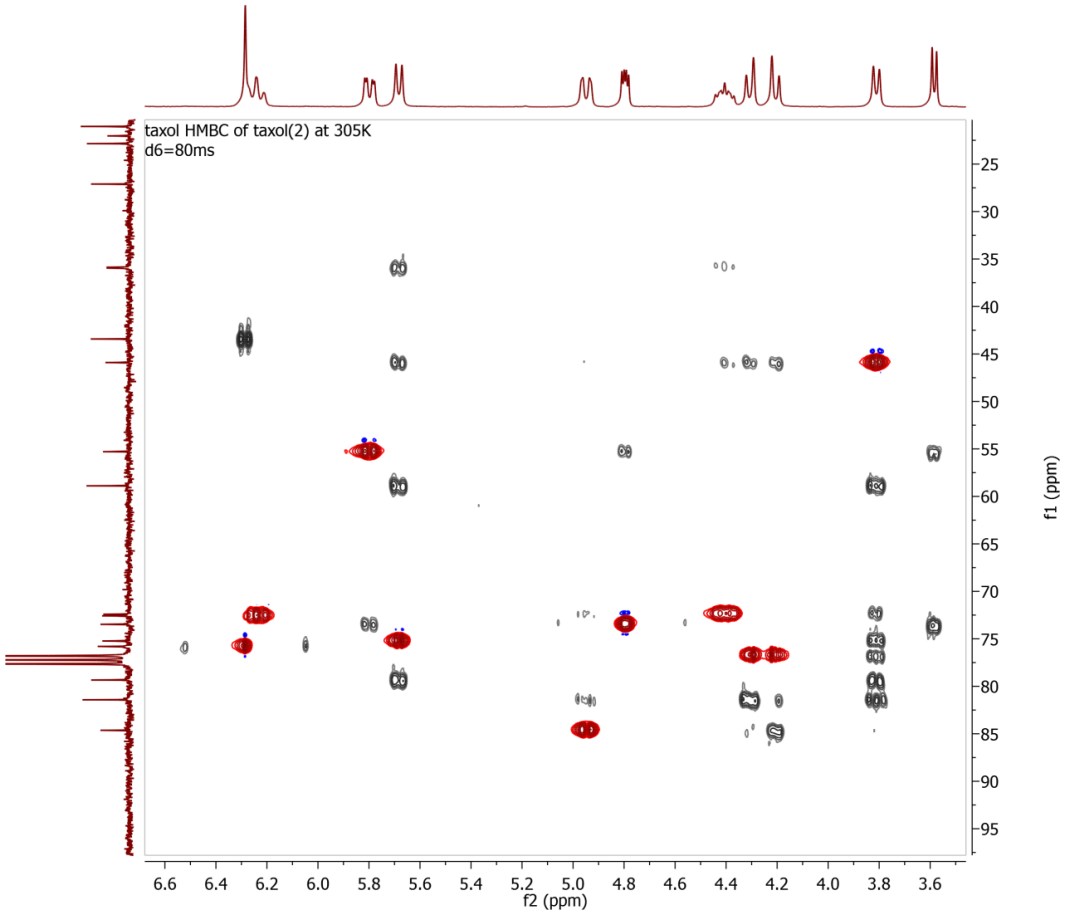
- Open several 1D spectra in the same document
- Select some or all of them in the Pages View
- Click “Stack Items” to stack them in a new page:



*Right-click on the spectra and choose **Properties** to change display properties, e.g. tilting angle, colours, titles, clipping vertically etc.*

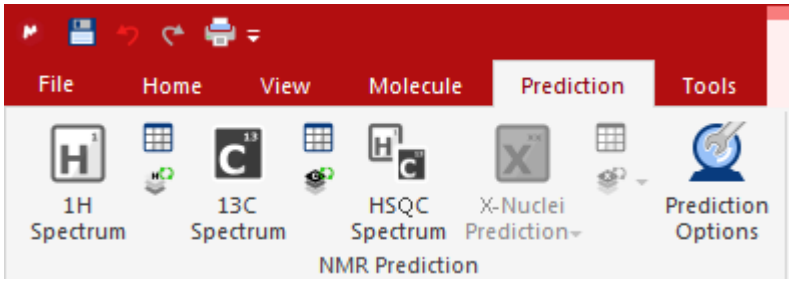
To superimpose multiple 2D spectra

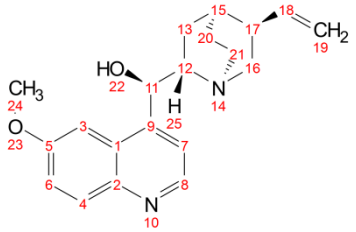
- Multiple 2D spectra can be stacked or superimposed in the same way as 1D spectra
- Click **Shift + Up Arrow** key to change the active spectrum
- Right click on it and select **Properties** to change the color of the contours for the active spectrum

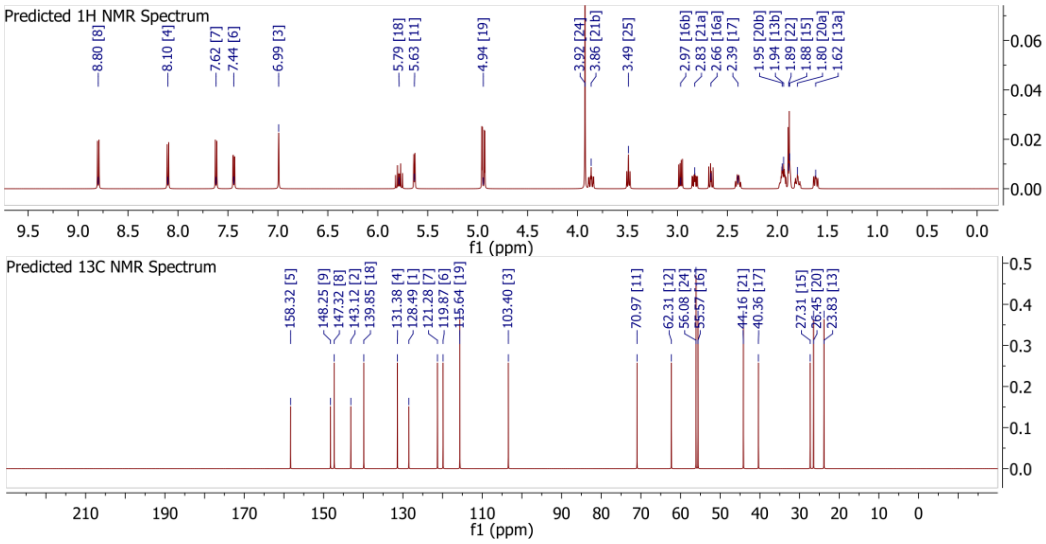


Predicting spectra from a structure

- 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 a command from the **Predict** menu







Tips:

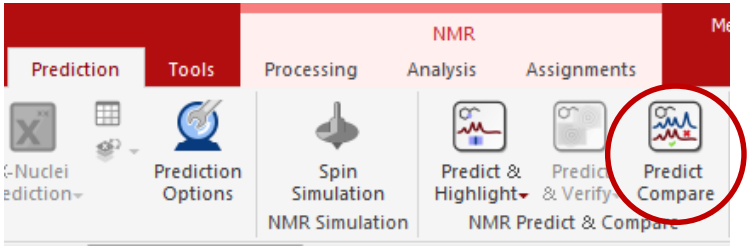
1. *Choose **Molecules | Prediction Options** to change settings*

2. *You can turn atom numbers on/off by right-clicking on the structure and choosing Properties.*

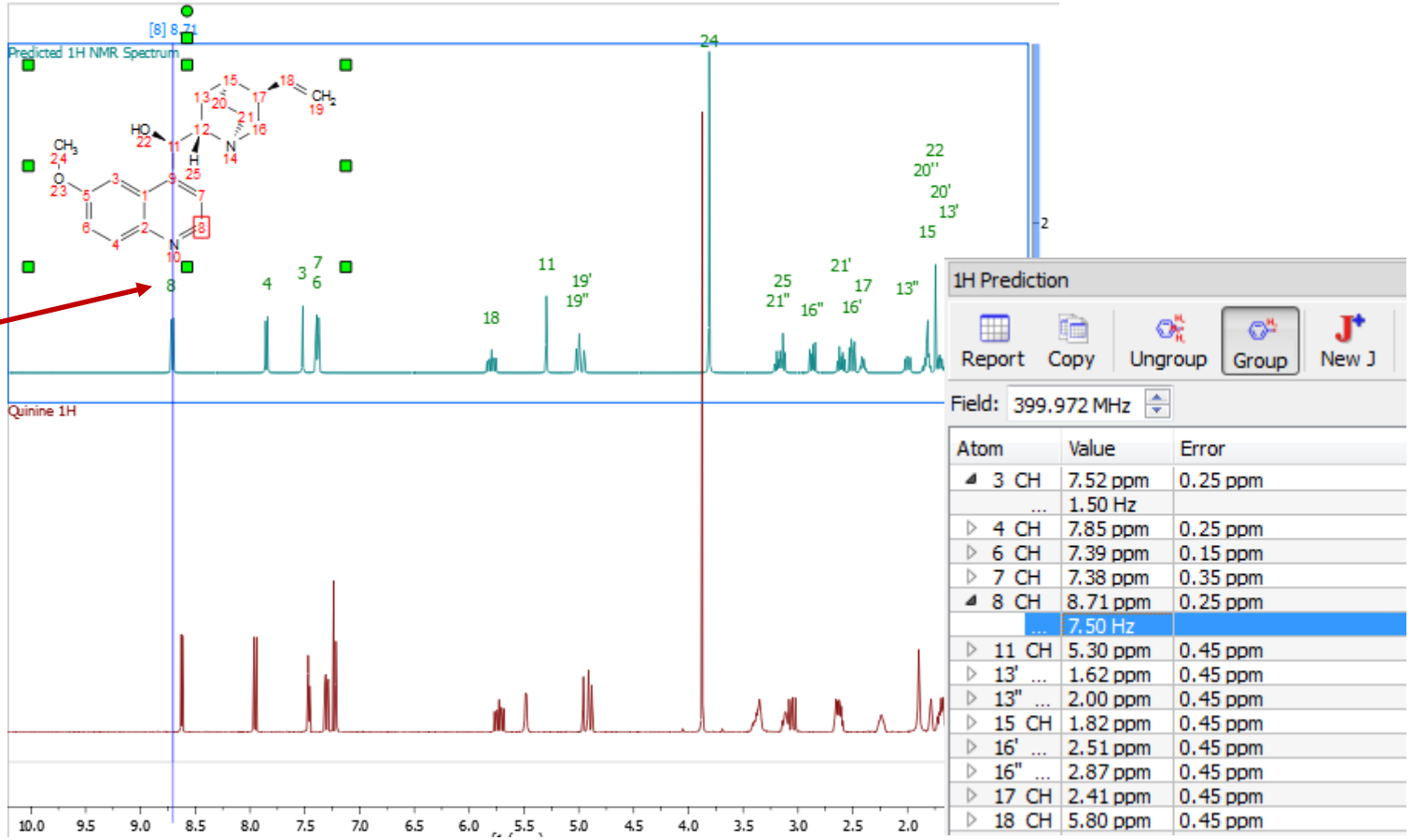
3. *You can open the **Prediction Table** to list the predicted shifts and J-couplings, and manually change them.*

Predicting a spectrum & verifying your structure

- Open your ^{1}H (or ^{13}C) **spectrum** in a new page
- Copy your **structure** from ChemDraw or Isis/Draw
- Choose **Analysis | Predict & Compare.** The predicted spectrum is stacked with the experimental one for visual comparison



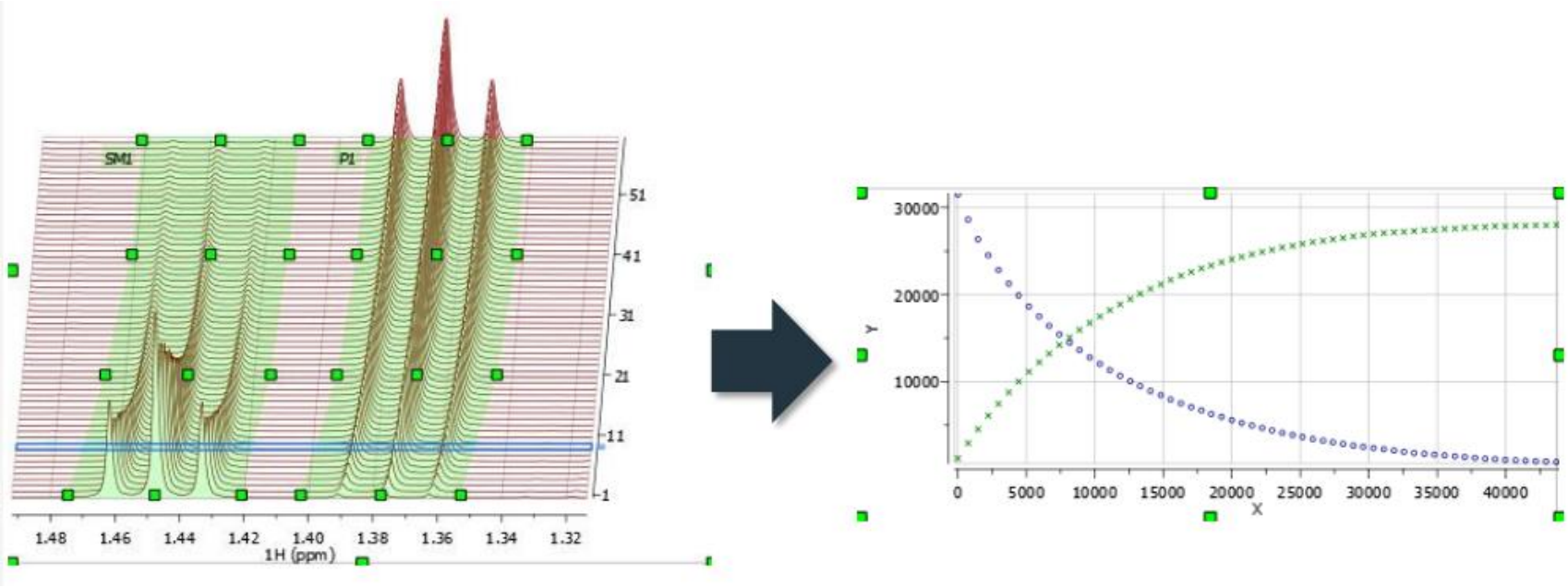
You can drag the label of a predicted peak to change its chemical shift. You can also change the predicted J-couplings in the 1H Prediction Table.



Other things you can do in Mnova… *not covered today!*

- Draw chemical structures
- Analyse Mass Spec data
- Copy and paste spectra directly in Word, Powerpoint etc
- Analyse kinetic time course data
- Simulate spectra from chemical shift and coupling data

e.g. analysing kinetic time course data:



For more information

mNova:
http://resources.mestrelab.com/

Oxford CRL NMR:
http://nmrweb.chem.ox.ac.uk/

NMR@CHEM.OX

Chemistry Research Laboratory