



Mestrelab Research

chemistry software solutions

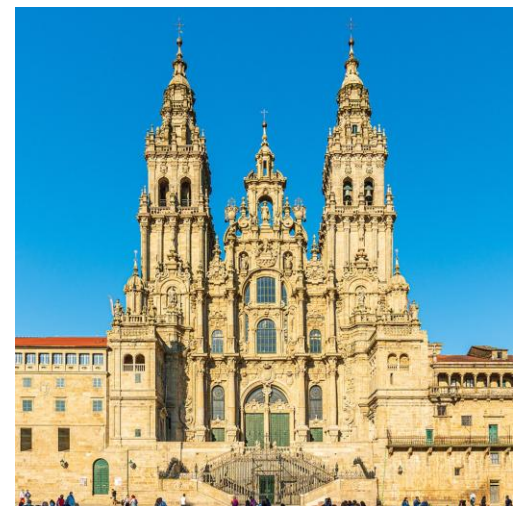
Mnova Training– Advanced

For Mnova v16.0
Oct. 2025

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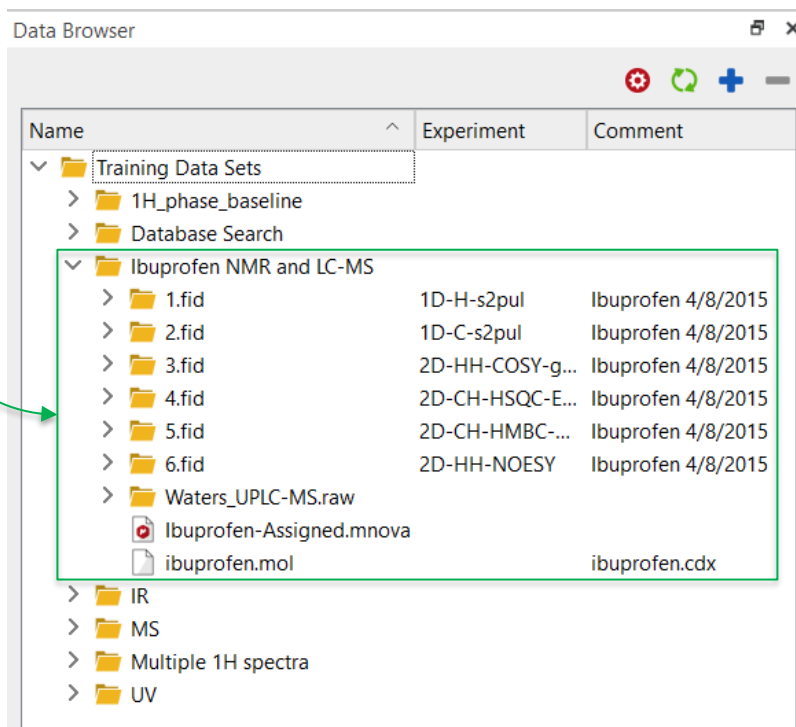
Main Topics

- Opening and processing 2D NMR
- Assigning peaks to atoms
- Reporting assignment results
- Analyzing arrayed spectra for reaction monitoring



Processing 1D & 2D NMR Spectra Together

Sample data

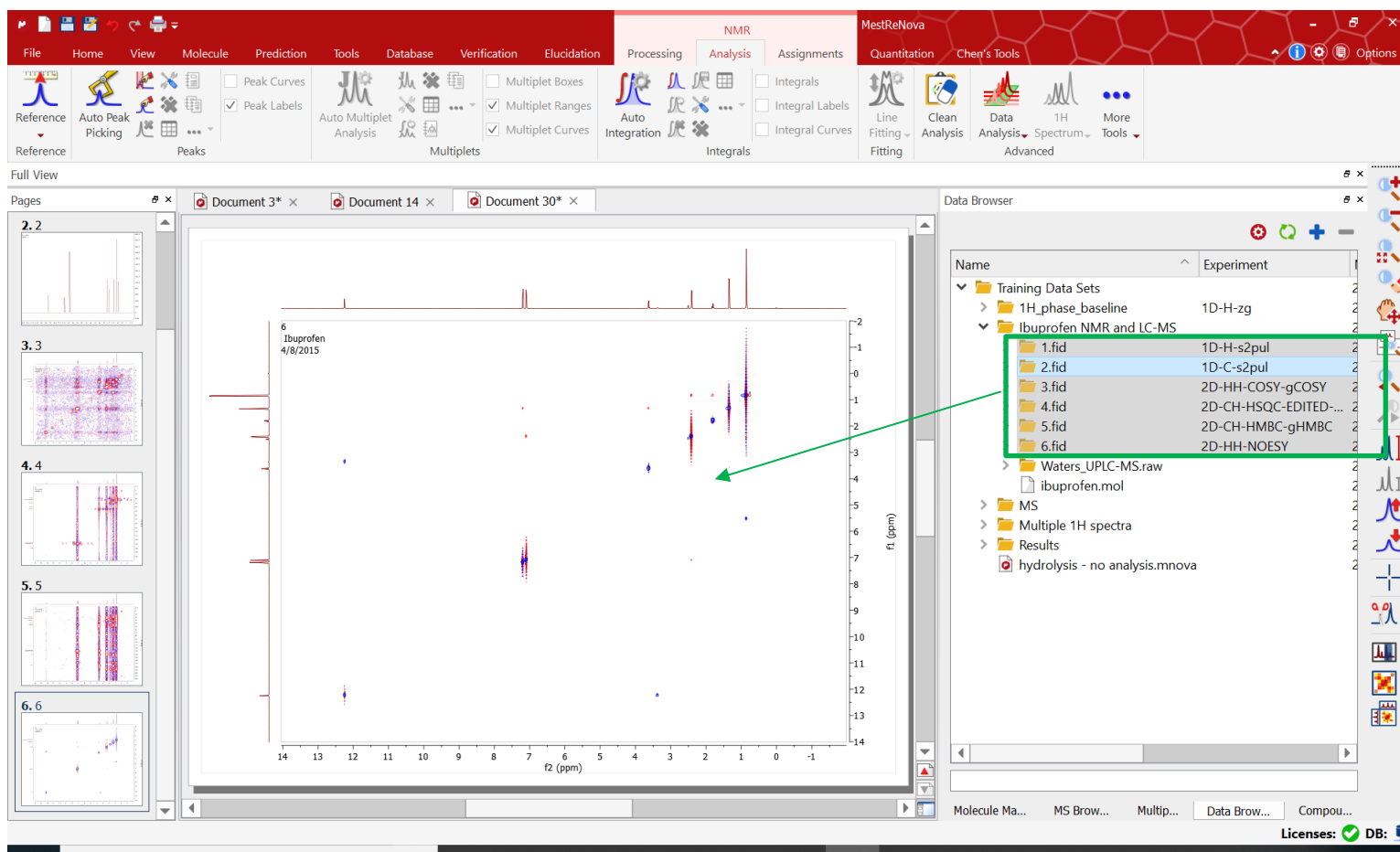


Name	Experiment	Comment
Training Data Sets		
1H_phase_baseline		
Database Search		
Ibuprofen NMR and LC-MS		
1.fid	1D-H-s2pul	Ibuprofen 4/8/2015
2.fid	1D-C-s2pul	Ibuprofen 4/8/2015
3.fid	2D-HH-COSY-g...	Ibuprofen 4/8/2015
4.fid	2D-CH-HSQC-E...	Ibuprofen 4/8/2015
5.fid	2D-CH-HMBC-...	Ibuprofen 4/8/2015
6.fid	2D-HH-NOESY	Ibuprofen 4/8/2015
Waters_UPLC-MS.raw		
Ibuprofen-Assigned.mnova		
ibuprofen.mol		ibuprofen.cdx
IR		
MS		
Multiple 1H spectra		
UV		

PROCESSING

Open a 1D and 2D spectra of Ibuprofen

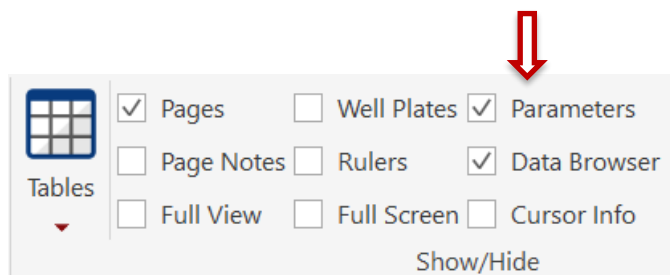
- Choose File > New to open a new blank document.
- In Data Browser, choose the 1D H/C, 2D HSQC, HMBC, COSY, and NOESY spectra and drag all of them to the main window.
- Re-process and analyze the H-1 and C-13 spectra according to the Basic Tutorial.



PARAMETERS

Which is which?

- Check View > Parameters Table to display the Parameters Table
- The Experiment and Pulse Sequence parameters usually indicate the type of NMR data



Parameters		
<div>Report Copy Setup Customize</div>		
	Parameter	Value
7	Instrument	vnmrs
8	Author	
9	Solvent	dmsd
10	Temperature	25.0
11	Pulse Sequence	gHSQCAD
12	Experiment	HSQC-EDITED
13	Probe	3mm_cold_HCN_P001
14	Number of Scans	8
15	Receiver Gain	44
16	Relaxation Delay	1.0000

Tip: You can display the Experiment as part of the spectrum title. Double click on the spectrum and setup the Title in the Properties Dialog.

PROCESSING

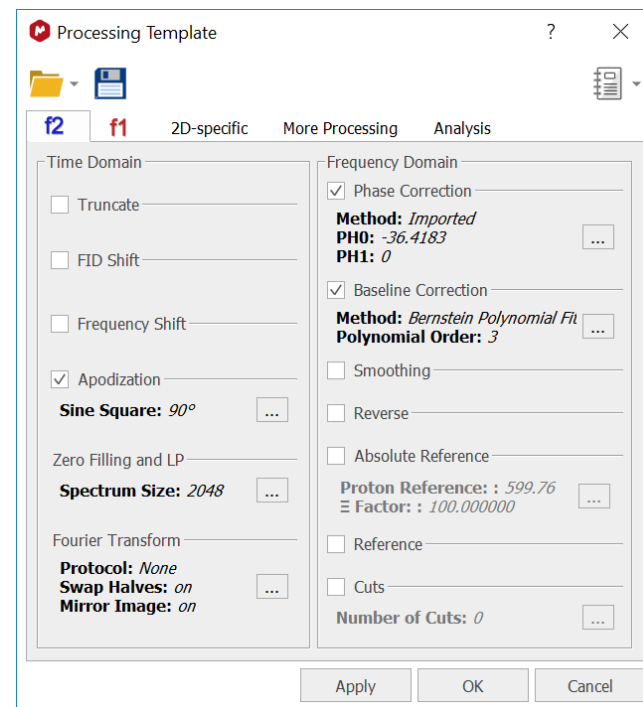
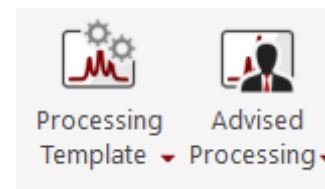
Rules of thumb for 2D processing

When a 2D is opened in Mnova, it is automatically processed starting from the raw data (*ser* or *fid*), using the parameters from the instrument.

- The used parameters can be displayed using the processing Template tool

If the automated processed spectrum is not satisfactory to you, you will need to adjust some of the processing parameters to improve it.

- The first tool you can try is the Advised Processing, which applies parameters suggested by Mnova and reprocesses the spectrum.
- If the results is still not satisfactory, follow the next pages to adjust the parameters manually.

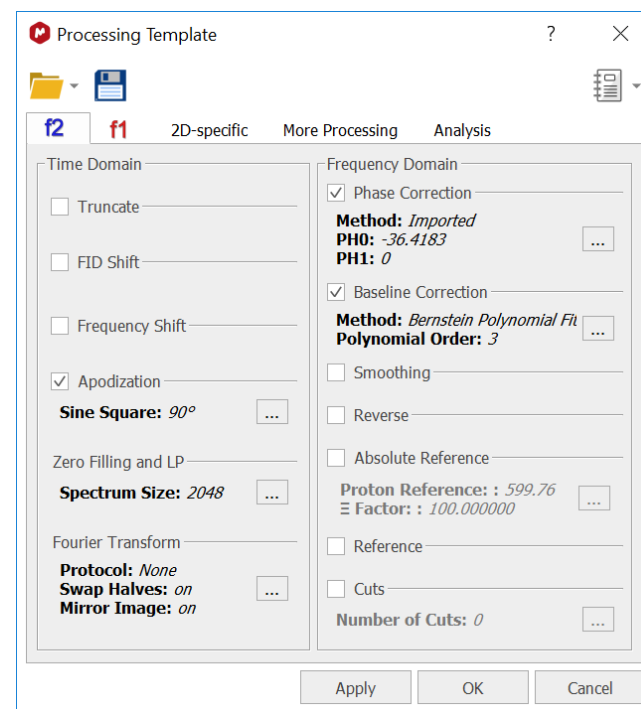
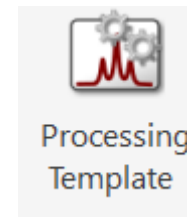


PROCESSING

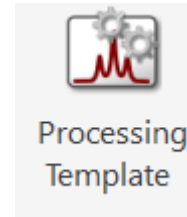
Rules of thumb for 2D processing

The following processing parameters are usually closed related the quality of the processed spectrum:

- Apodization: To improves the line shape, resolution and S/N ratio
 - COSY: Use Sine Square 0 for F2 & F1
 - Others: Use Since Square 90 F2 & F1
- Zero Fill and LP (Linear Prediction): To improve resolution
 - F2: at least double of the original datapoints, 2048 or 4096
 - F1: At least double of the original datapoints, 512 or 1024
 - F1: Do LP if original datapoints ≤ 128 (optional)
- Phase Correction: To improve line shape
 - Use Imported or Automated ("Regions") first
 - Do manual correction if needed.
- Baseline Correction: To reduce noise
 - Use Bernstein Polynomial Fit on either dimension
- Other 2D-Specific parameters (optional):
 - COSY, NOESY: Use Symmetrize with caution
 - HSQC, HMBC: Use Reduce T1 Noise Reduction when needed.



Rules of thumb for 2D processing



The 2D processing parameters that you may want to adjust and their recommended values*

	Apodi- zation	Zero-fill & Linear Prediction	Phase Correction	Baseline Correction	Others
COSY	Sine square 0 on both F2 and F1	At least double to 2K or 4K on F2; or 512 or 1K on F1. Do linear prediction on F1 if # of increments < 128	Use imported parameters first. Use auto or manual phasing if needed on both F1 and F2	Use Bernstein Polynomial Fitting (3 rd order) for F2 and F1	Do COSY-style symmetrization with caution
HSQC	Sine square 90 on both F2 and F1	Same as above	Same as above	Same as above	Use Reduce T1 Noise to reduce T1 noises with caution
HMBC	Same as above	Same as above	Same as above	Same as above	Same as above
NOESY/ ROESY	Same as above	Same as above	Same as above	Same as above	Do COSY-style symmetrization with caution

**These are recommended starting points for conventional 2D NMR processing. You can try many of other combinations as appropriate. Once you are satisfied with the results, you can save all the parameters as a processing template, and apply it to similar spectra later.*

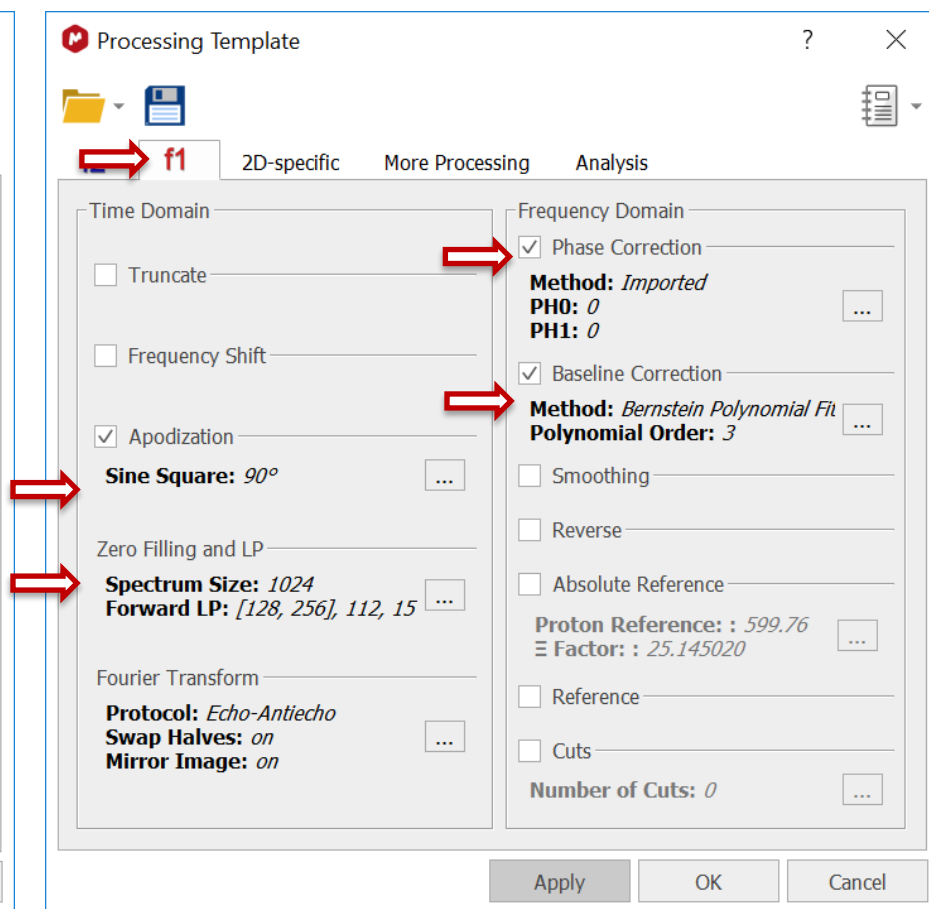
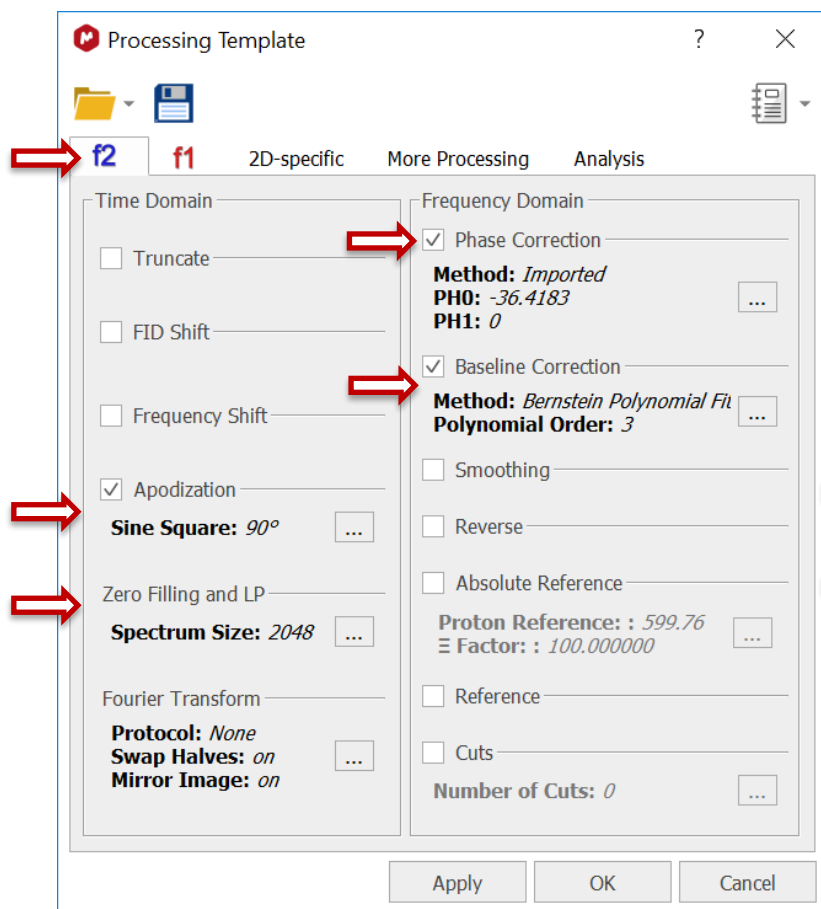
PROCESSING

Re-process HSQC spectrum



Processing
Template

- Reprocess the HSQC spectrum as shown below.
- Note the apodization functions for F2 and F1
- Note the forward linear prediction for F1 applied here

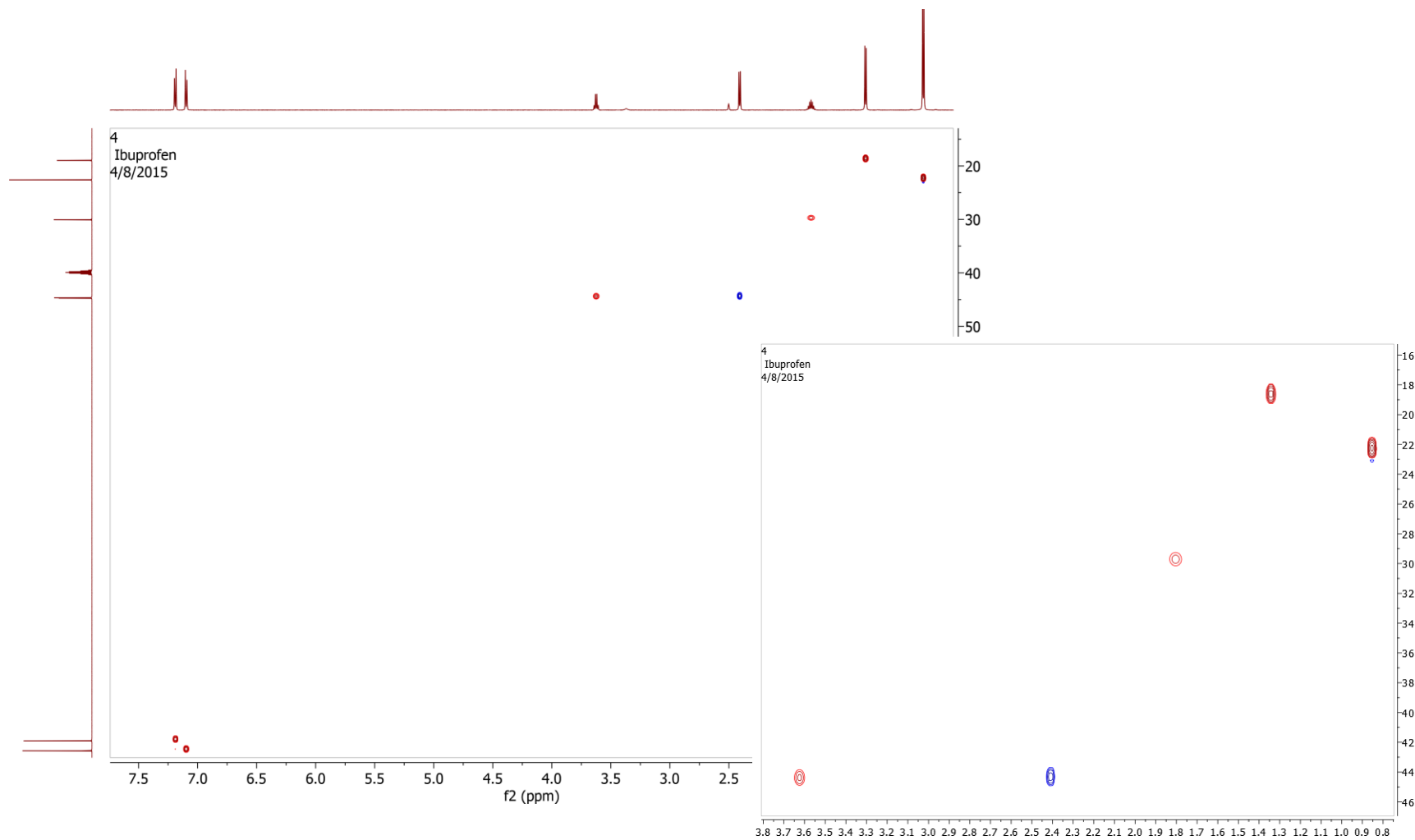


Note: The Fourier Transform method is automatically set and normally you don't need to change it.

PROCESSING

Re-process HSQC spectrum

- The re-processed HSQC spectrum shows better line shape, and higher resolution on the F1 dimension



PROCESSING

Re-process HMBC spectrum



Processing
Template

- Reprocess the HMBC spectrum as shown below.
- Note the apodization functions for F2 and F1
- Note the forward linear prediction for F1 applied here

Processing Template

f2 | f1 | 2D-specific | More Processing | Analysis

Time Domain

☐ Truncate

☐ FID Shift

☐ Frequency Shift

☒ Apodization
Sine Square: 90°

Zero Filling and LP
Spectrum Size: 2048

Fourier Transform
Protocol: None
Swap Halves: on
Mirror Image: on

Frequency Domain

☒ Phase Correction
Method: Magnitude

☒ Baseline Correction
Method: Bernstein Polynomial Fit
Polynomial Order: 3

☐ Smoothing

☐ Reverse

☐ Absolute Reference
Proton Reference: : 599.76
Factor: : 100.000000

☐ Reference

☐ Cuts
Number of Cuts: 0

Apply OK Cancel

Processing Template

f1 | 2D-specific | More Processing | Analysis

Time Domain

☐ Truncate

☐ Frequency Shift

☒ Apodization
Sine Square: 90°

Zero Filling and LP
Spectrum Size: 1024
Forward LP: [128, 256], 112, 15

Fourier Transform
Protocol: Echo-Antiecho
Swap Halves: on
Mirror Image: on

Frequency Domain

☒ Phase Correction
Method: Imported
PH0: 0
PH1: 0

☒ Baseline Correction
Method: Bernstein Polynomial Fit
Polynomial Order: 3

☐ Smoothing

☐ Reverse

☐ Absolute Reference
Proton Reference: : 599.76
Factor: : 25.145020

☐ Reference

☐ Cuts
Number of Cuts: 0

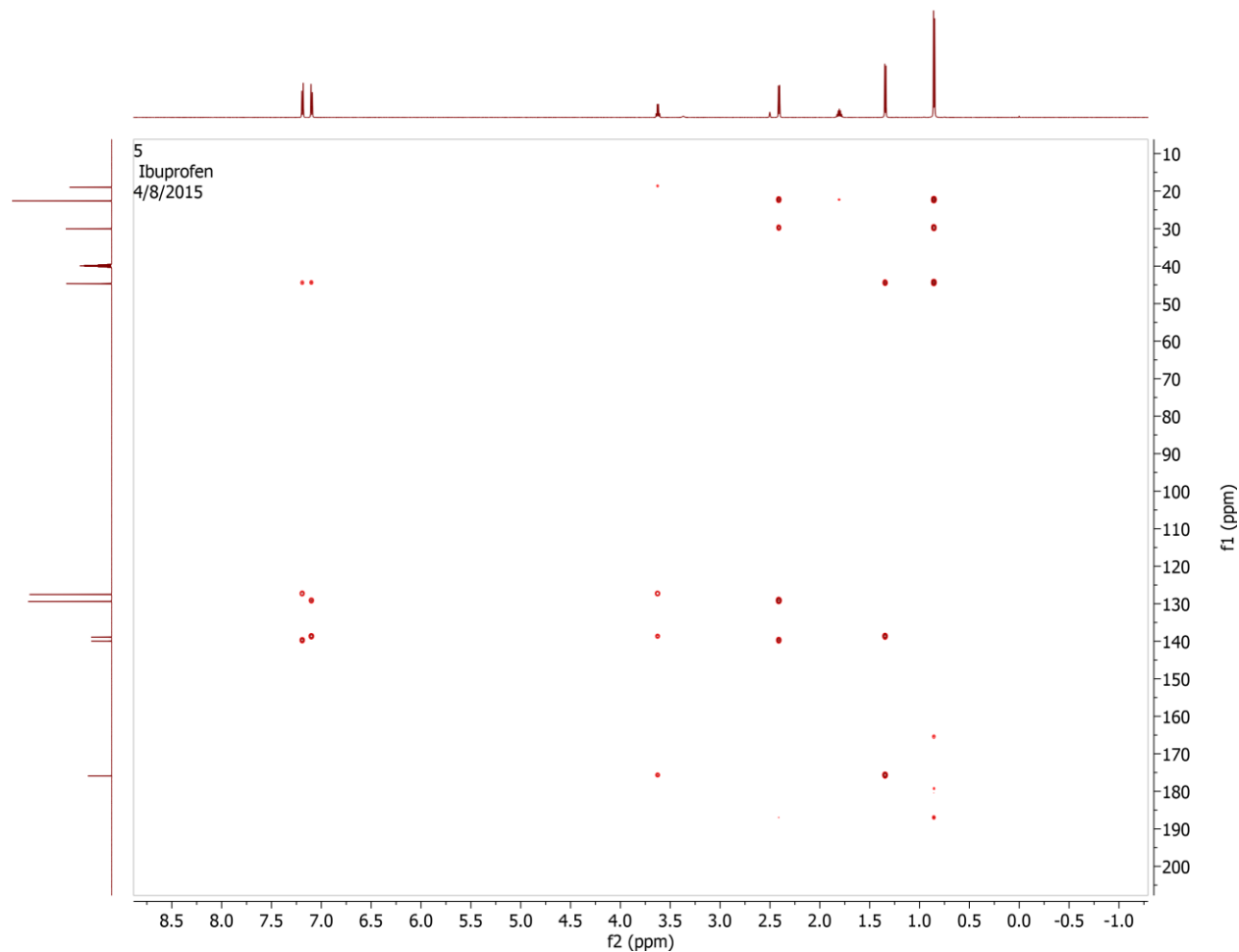
Apply OK Cancel

Note: The Fourier Transform method is automatically set and normally you don't need to change it.

PROCESSING

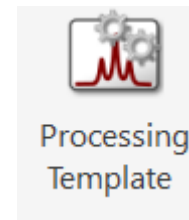
Re-process HMBC spectrum

- The re-processed HSQC spectrum shows better line shape, and higher resolution on the F1 dimension

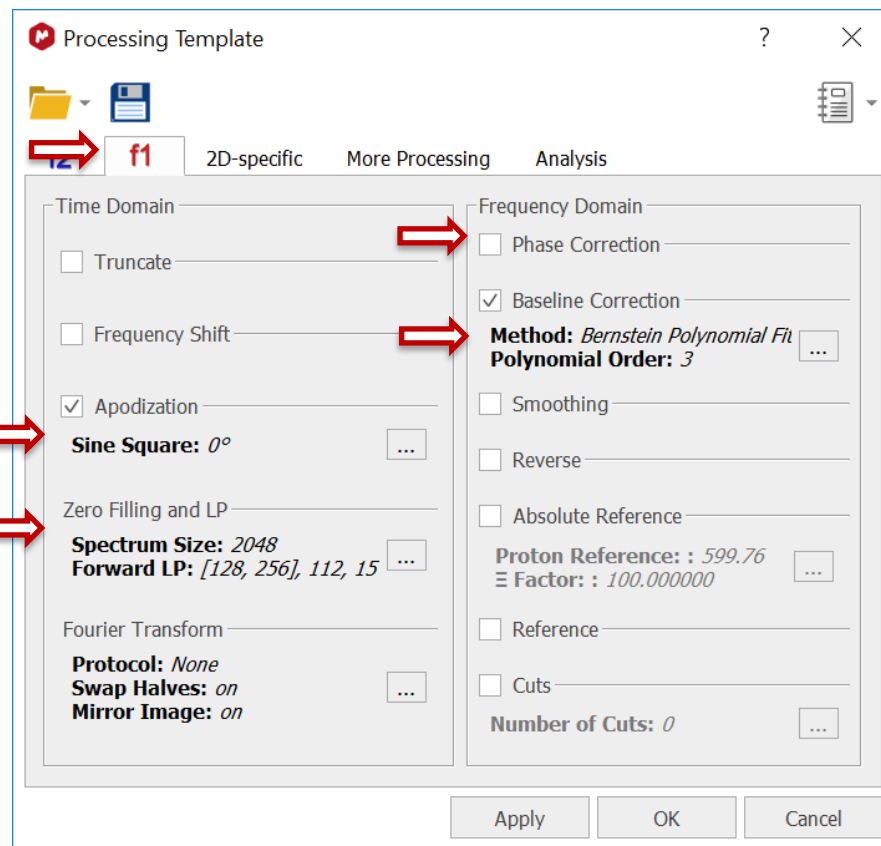
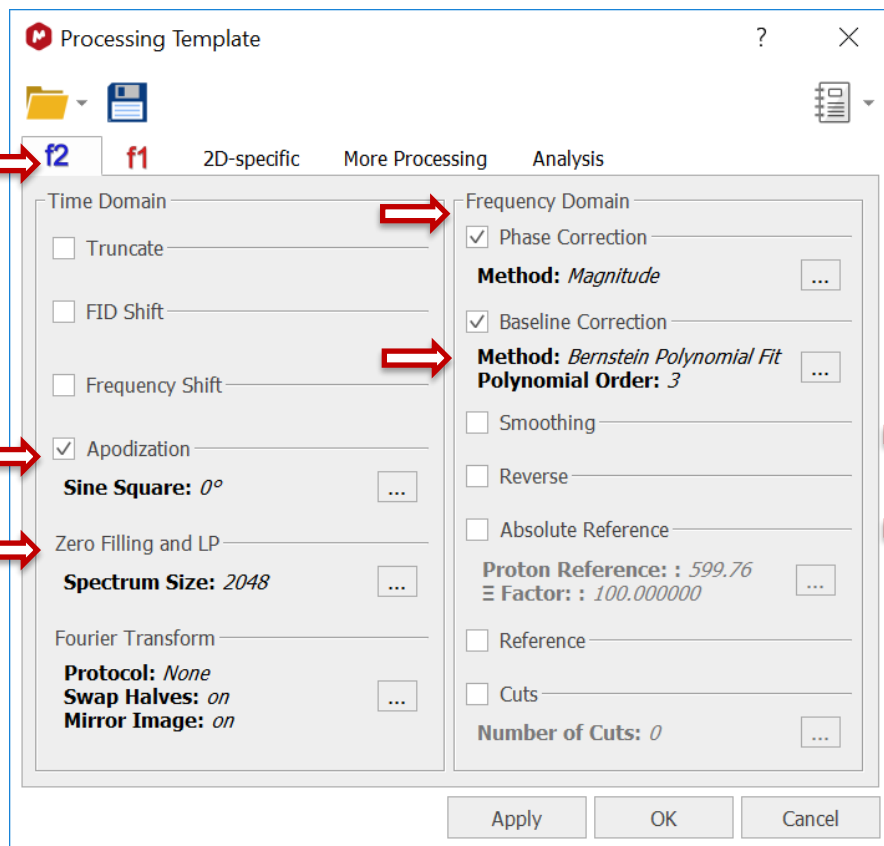


PROCESSING

Re-process COSY spectrum



- Reprocess the COSY spectrum as shown below.
- Note the apodization functions for F2 and F1
- Note the forward linear prediction for F1 applied here
- Turn off Symmetrize in the 2D-specific Tab

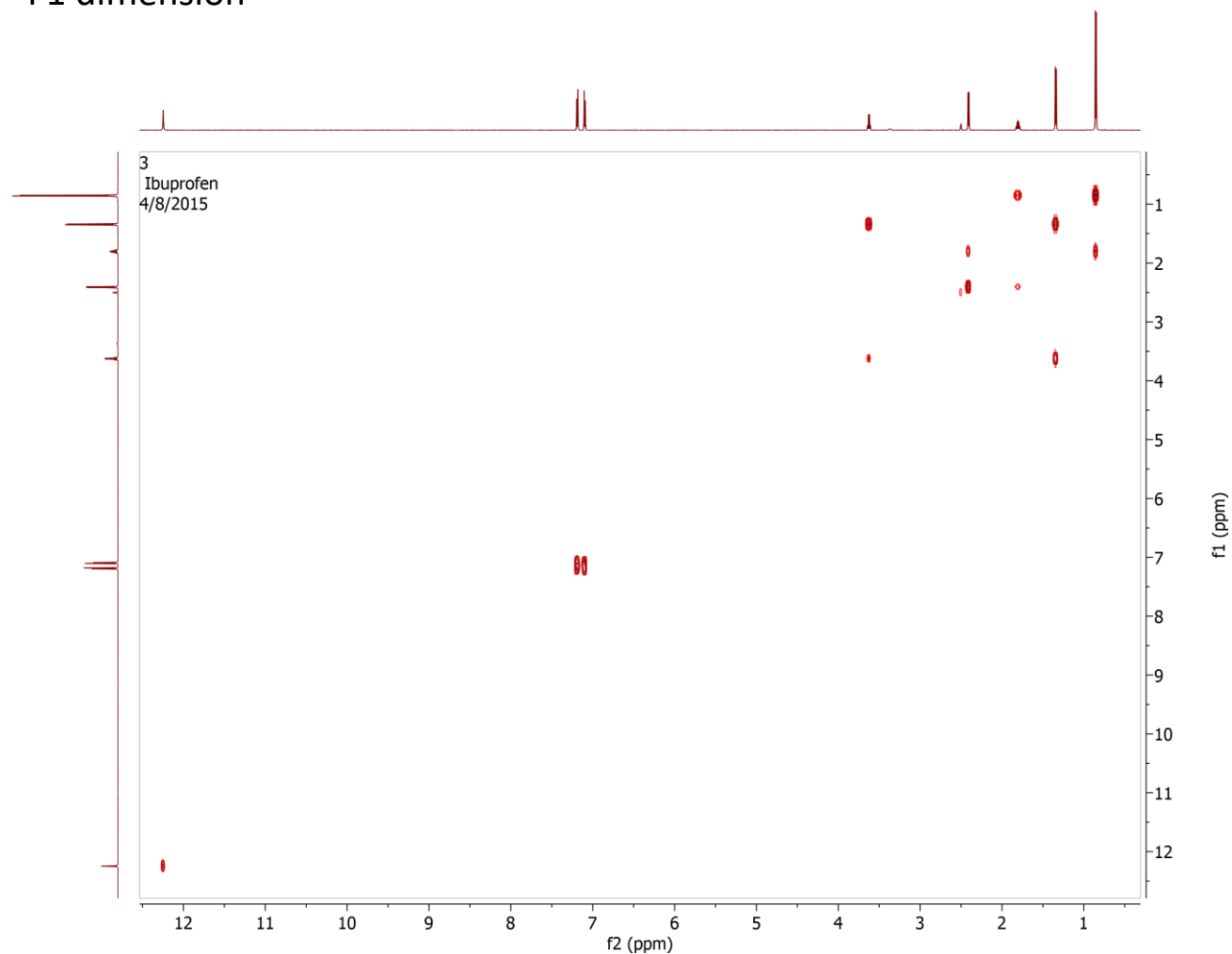


Note: The Fourier Transform method is automatically set and normally you don't need to change it.

PROCESSING

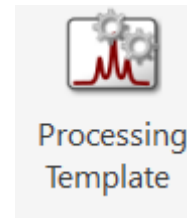
Re-process COSY spectrum

- The re-processed COSY spectrum shows better line shape, and higher resolution on the F1 dimension



PROCESSING

Re-process NOESY spectrum



- Reprocess the NOESY spectrum as shown below.
- Note the apodization functions for F2 and F1
- Note the forward linear prediction for F1 applied here

The image shows two side-by-side screenshots of the 'Processing Template' dialog box in Mestrelab Research software. The left window is for F2 processing, and the right window is for F1 processing. Red arrows highlight specific settings in both windows.

Left Window (F2):

- Time Domain:**
 - ☐ Truncate
 - ☐ FID Shift
 - ☐ Frequency Shift
 - ☒ Apodization: **Sine Square: 90°**
 - Zero Filling and LP: **Spectrum Size: 2048**
 - Fourier Transform: **Protocol: None**, **Swap Halves: on**, **Mirror Image: on**
- Frequency Domain:**
 - ☒ Phase Correction: **Method: Magnitude**
 - ☒ Baseline Correction: **Method: Bernstein Polynomial Fit**, **Polynomial Order: 3**
 - ☐ Smoothing
 - ☐ Reverse
 - ☐ Absolute Reference: **Proton Reference: : 599.76**, **Factor: : 100.000000**
 - ☐ Reference
 - ☐ Cuts: **Number of Cuts: 0**

Right Window (F1):

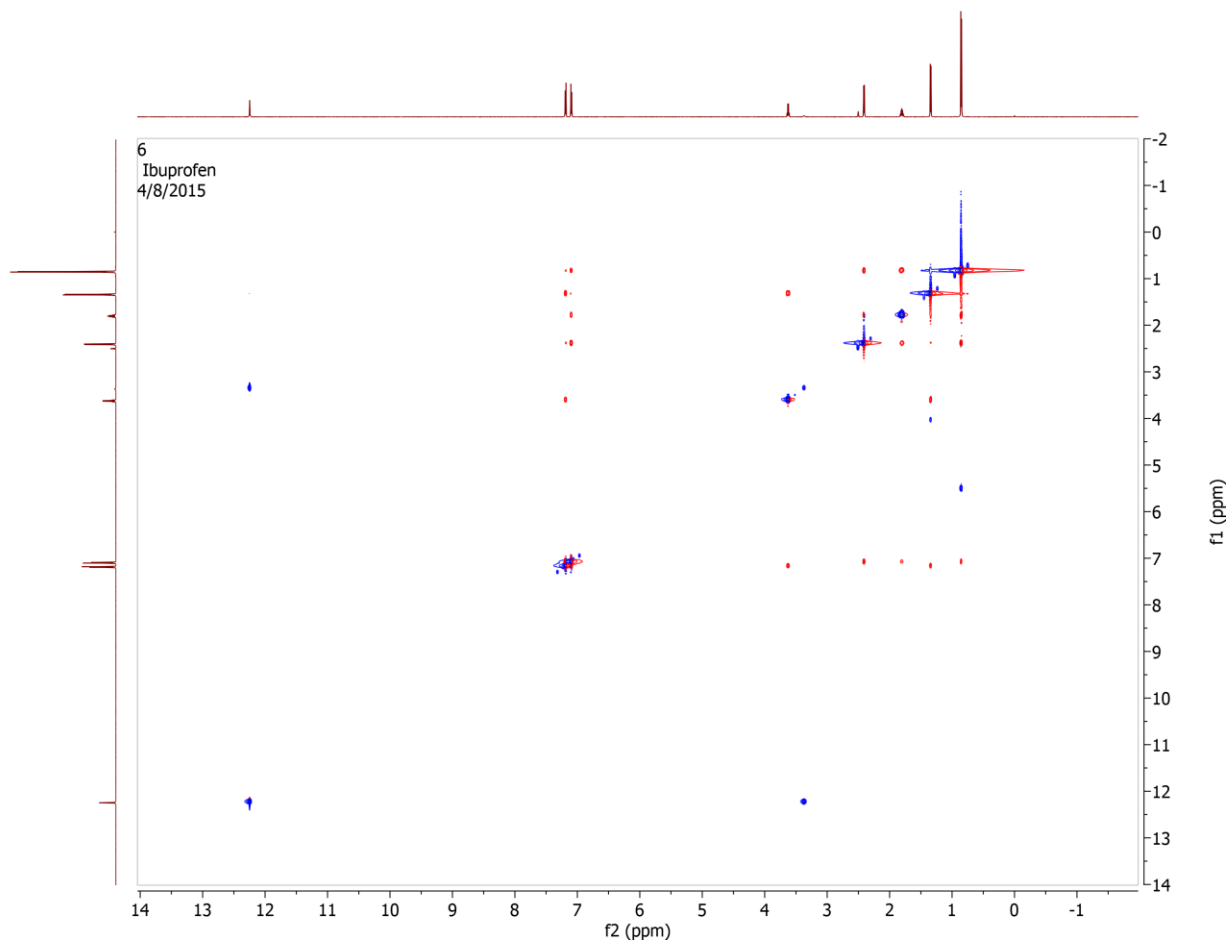
- Time Domain:**
 - ☐ Truncate
 - ☐ Frequency Shift
 - ☒ Apodization: **Sine Square: 90°**
 - Zero Filling and LP: **Spectrum Size: 1024**, **Forward LP: [128, 256], 112, 15**
 - Fourier Transform: **Protocol: Echo-Antiecho**, **Swap Halves: on**, **Mirror Image: on**
- Frequency Domain:**
 - ☒ Phase Correction: **Method: Imported**, **PH0: 0**, **PH1: 0**
 - ☒ Baseline Correction: **Method: Bernstein Polynomial Fit**, **Polynomial Order: 3**
 - ☐ Smoothing
 - ☐ Reverse
 - ☐ Absolute Reference: **Proton Reference: : 599.76**, **Factor: : 25.145020**
 - ☐ Reference
 - ☐ Cuts: **Number of Cuts: 0**

Note: The Fourier Transform method is automatically set and normally you don't need to change it.

PROCESSING

Re-process NOESY spectrum

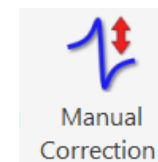
- The re-processed NOESY spectrum shows better line shape, and higher resolution on the F1 dimension, though there still some phase errors



PROCESSING

Phase correction for NOESY spectrum

- Do Manual phase correction for both dimensions.
- Also apply +180 for PH0 to make the cross peaks negative and diagonal peaks positive



Phase Correction

f1 f2

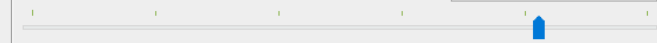
Click here and drag mouse
up or down holding:
left button for PH0 correction or
right button for PH1 correction.
(hold Ctrl key for fine tune)

Some processing steps (e.g. baseline correction) are not applied during interactive phasing. The final spectrum may differ from the provisional representation.

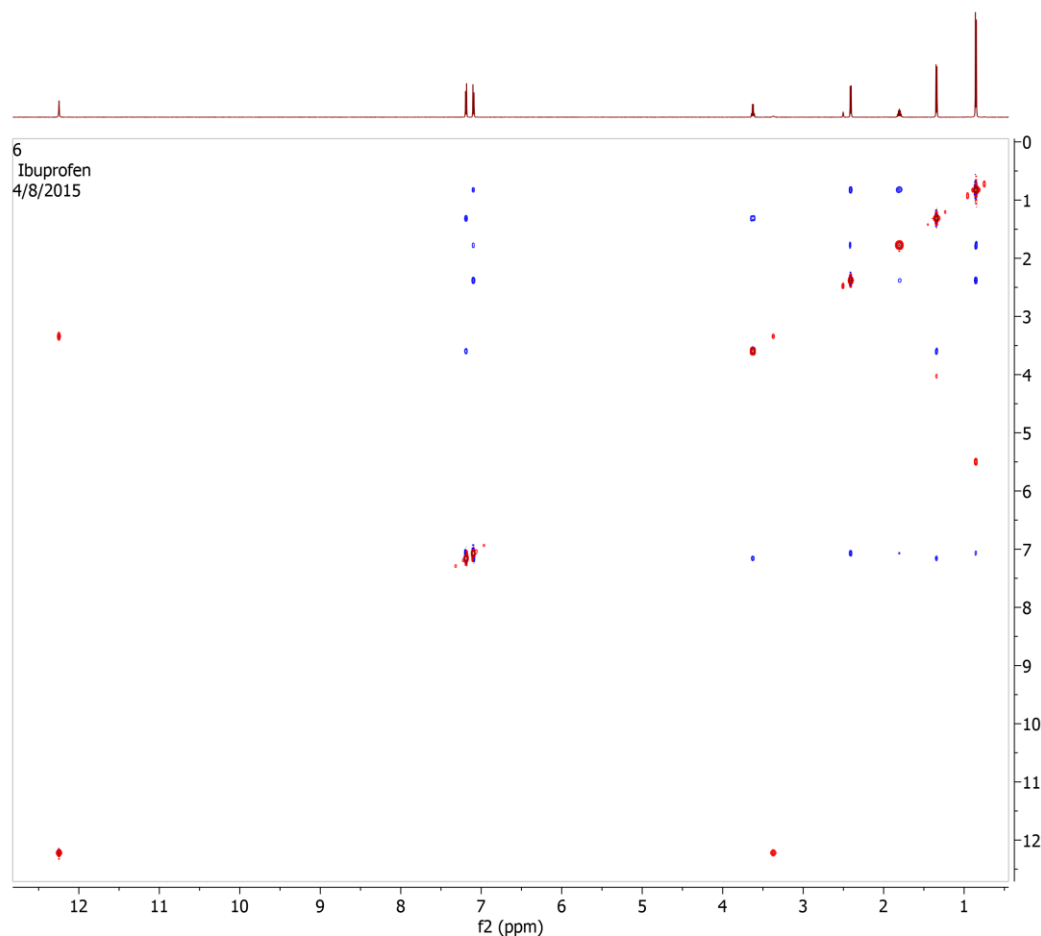
PH0: 54.60 +180 PH1: 0.00

Pivot Point

Position: 0.854 Biggest



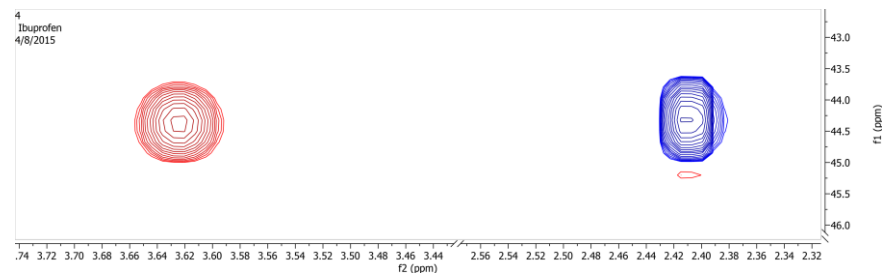
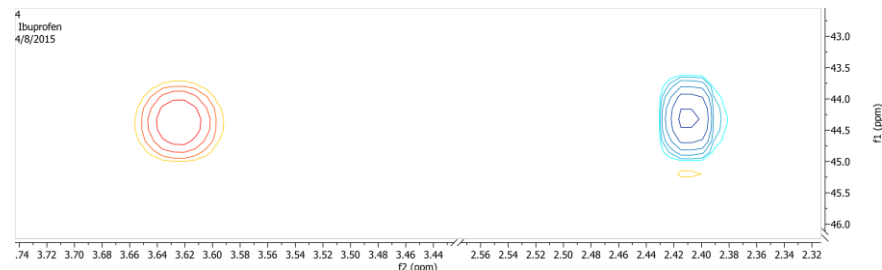
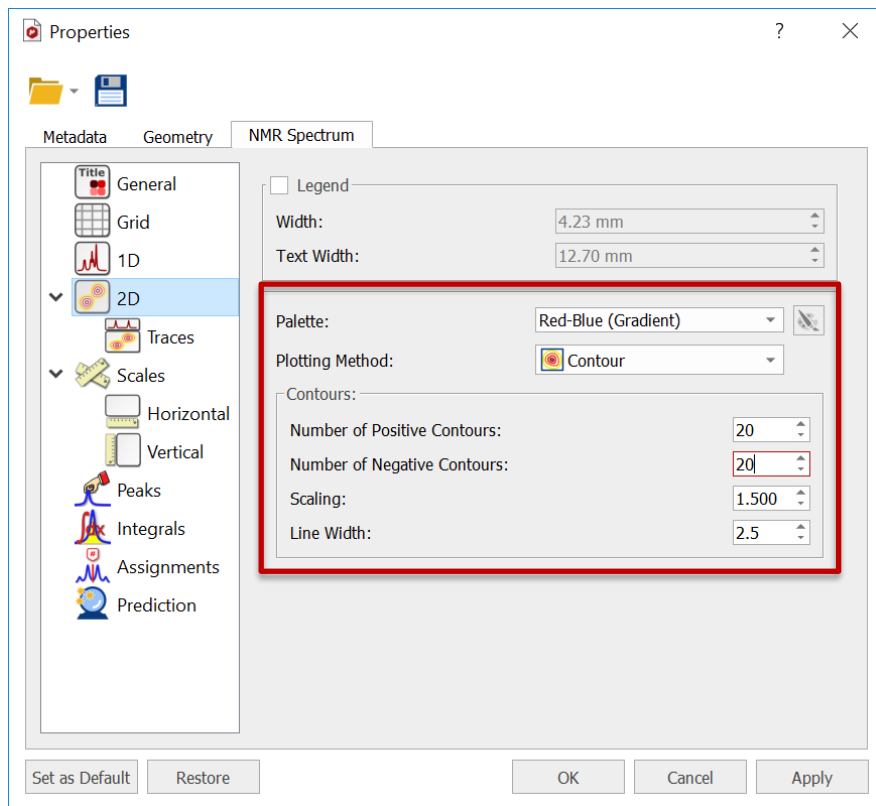
6
Ibuprofen
4/8/2015



PROCESSING

Change the Display Properties

- Right click on the spectrum to open the Properties Dialog, view the properties that can be changed
- In the 2D Category, adjust the highlighted parameters and click Apply to see the effects
- Click Set as Default to retain the settings for 2D spectra display in the future



Peak Assignment Using 1D & 2D NMR Spectra Together

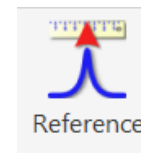
Sample data

This .mnova document has all 1D and 2D spectral peaks assigned to the atoms in Ibuprofen.

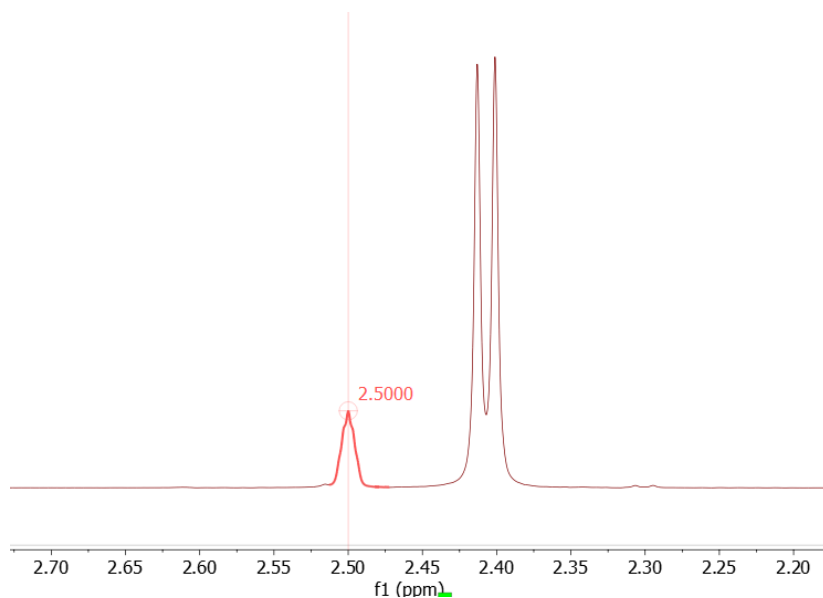
Name	Experiment	Comment
Training Data Sets		
1H_phase_baseline		
Database Search		
Ibuprofen NMR and LC-MS		
1.fid	1D-H-s2pul	Ibuprofen 4/8/2015
2.fid	1D-C-s2pul	Ibuprofen 4/8/2015
3.fid	2D-HH-COSY-g...	Ibuprofen 4/8/2015
4.fid	2D-CH-HSQC-E...	Ibuprofen 4/8/2015
5.fid	2D-CH-HMBC-...	Ibuprofen 4/8/2015
6.fid	2D-HH-NOESY	Ibuprofen 4/8/2015
Waters_UPLC-MS.raw		
Ibuprofen-Assigned.mnova		
ibuprofen.mol		ibuprofen.cdx
IR		
MS		
Multiple 1H spectra		
UV		

ANALYSIS

Chemical shift referencing for H-1



- This spectrum uses DMSO-d6 as the solvent. We can reference the chemical shifts by setting its middle peak to 2.5 ppm.
- Zoom to the DMSO peak at around 2.5 ppm. Choose Analysis > Reference, and click on the top of the middle peak.
- Set it to 2.5 ppm either manually or from the Solvent List.



Reference along f1

Old Shift: 2.5021 ppm

New Shift: 2.5000 ppm

Range Width: 0.1000 ppm

☐ Auto Tuning

☐ Annotation: DMSO-d6

Solvent List

Name	Shift (ppm)	Multiplicity	J (Hz)
Deuterium Oxide	4.790	1	
Dimethyl Sulfoxide-d6	2.500	5	1
	3.330	1	
Ethanol-d6	5.290	1	

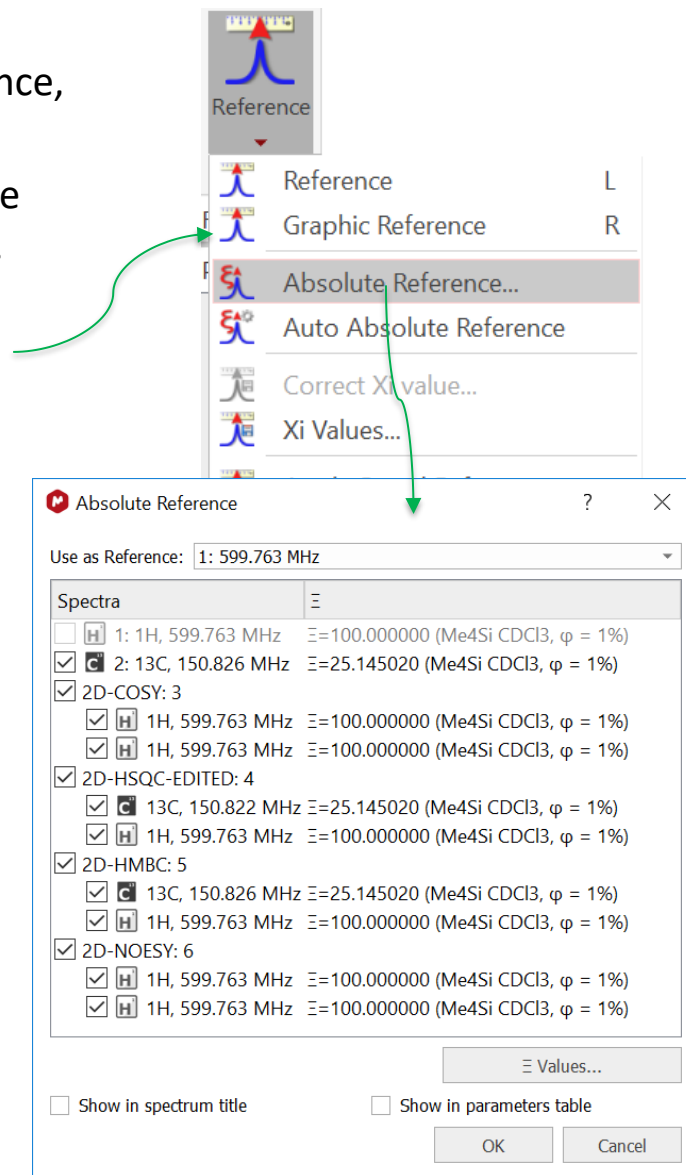
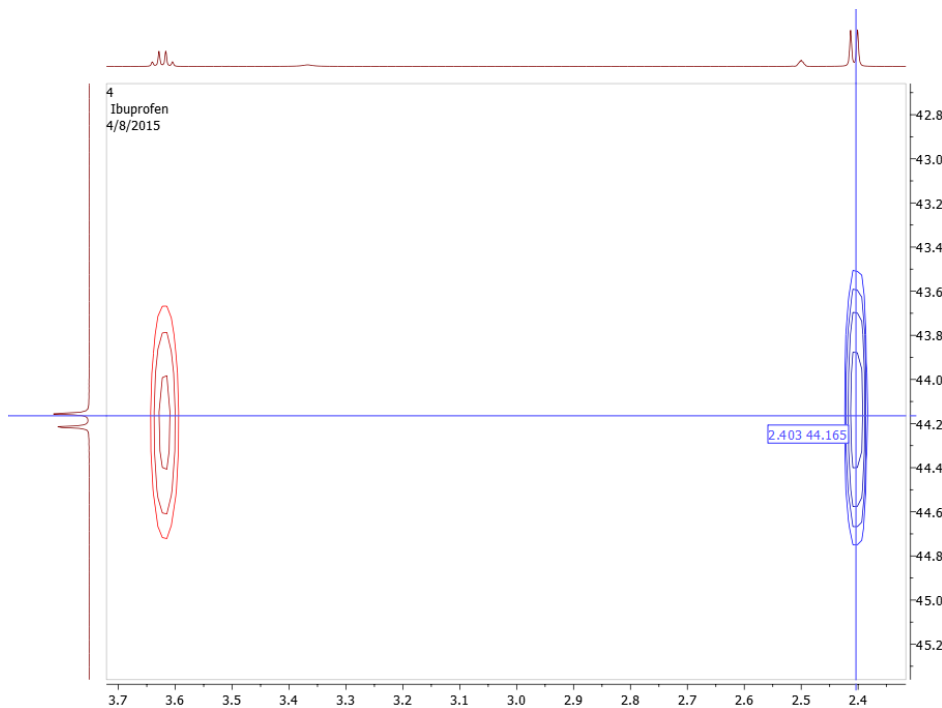
Restore Defaults Add... Edit... Delete

OK Cancel Solvents <<

ANALYSIS

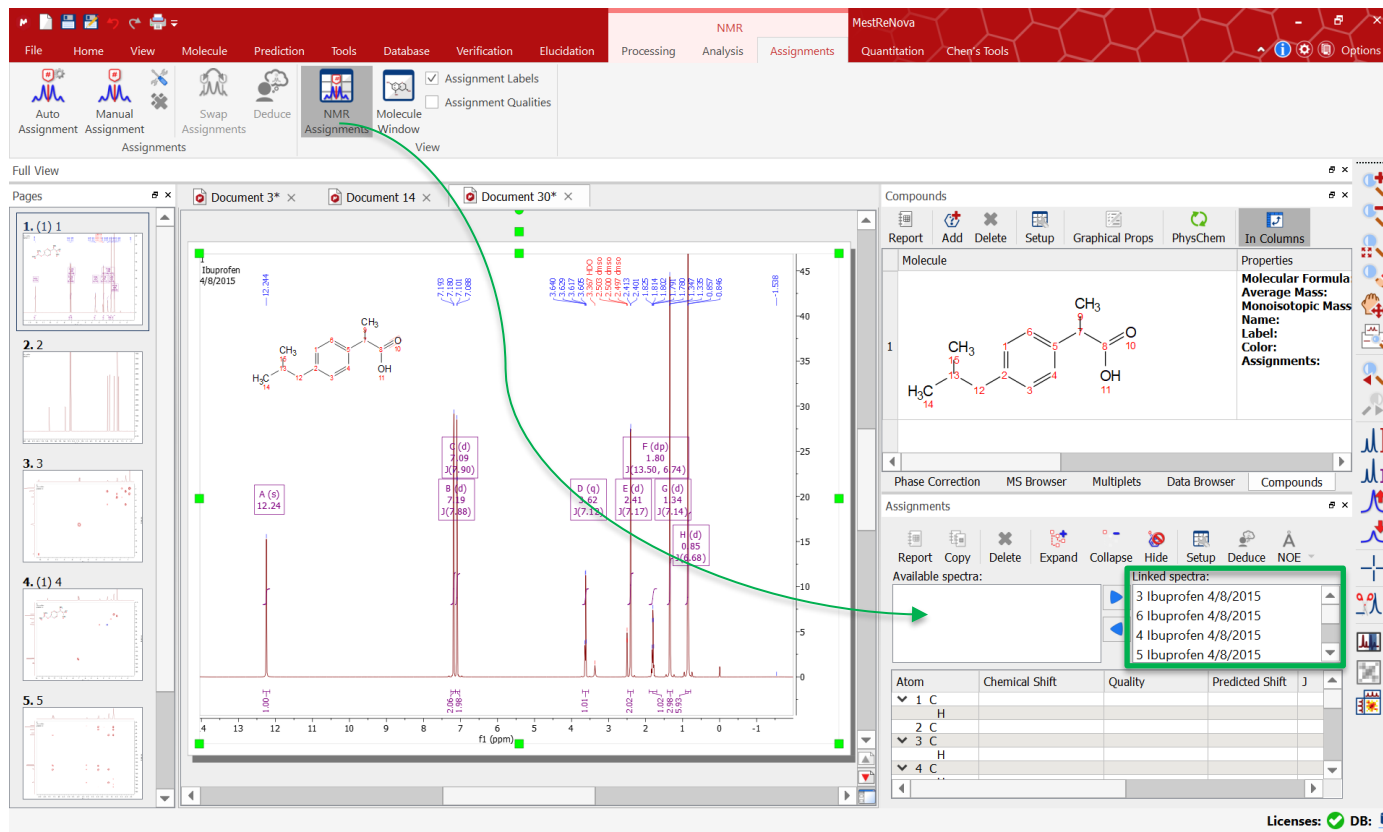
Chemical shift referencing for other spectra

- Choose Analysis > References > Absolute Reference, and click OK to the dialog.
- This applies referencing to all other spectra in the document using the H-1 spectrum as a standard.
- If needed, further align the 1D and 2D spectra manually using the Graphic Reference tool.



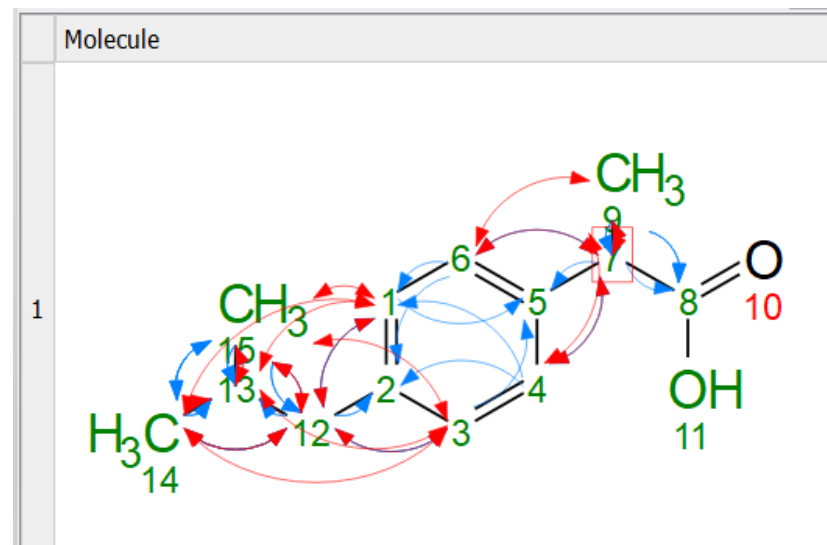
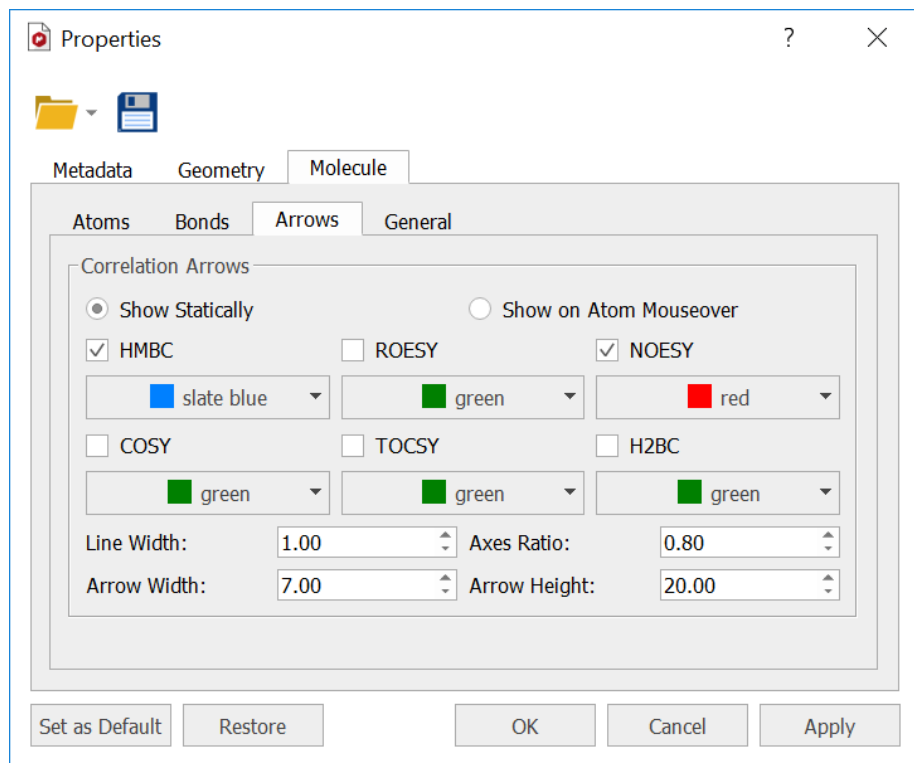
Open the structure for peak assignment

- Open the Ibuprofen.mol file from the Data Browser.
- Choose Molecule > Compound Table to show the structure on the side.
- Note: Open the same molecule only once. If needed, use Report on the Compound Table to report the structure to other pages. Do not open the same structure multiple times.
- Display the Assignment Table. Make sure all spectra are “linked” in it.



Show 2D correlation as arrows

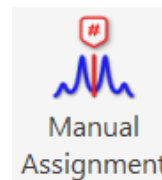
- Right-click on the structure (or click on Graphical Props in the Compounds Table)
- In the Arrows Tab, turn on the display of HMBC and NOESY correlation as different colors
- The correlations will be displayed when the assignments are added later



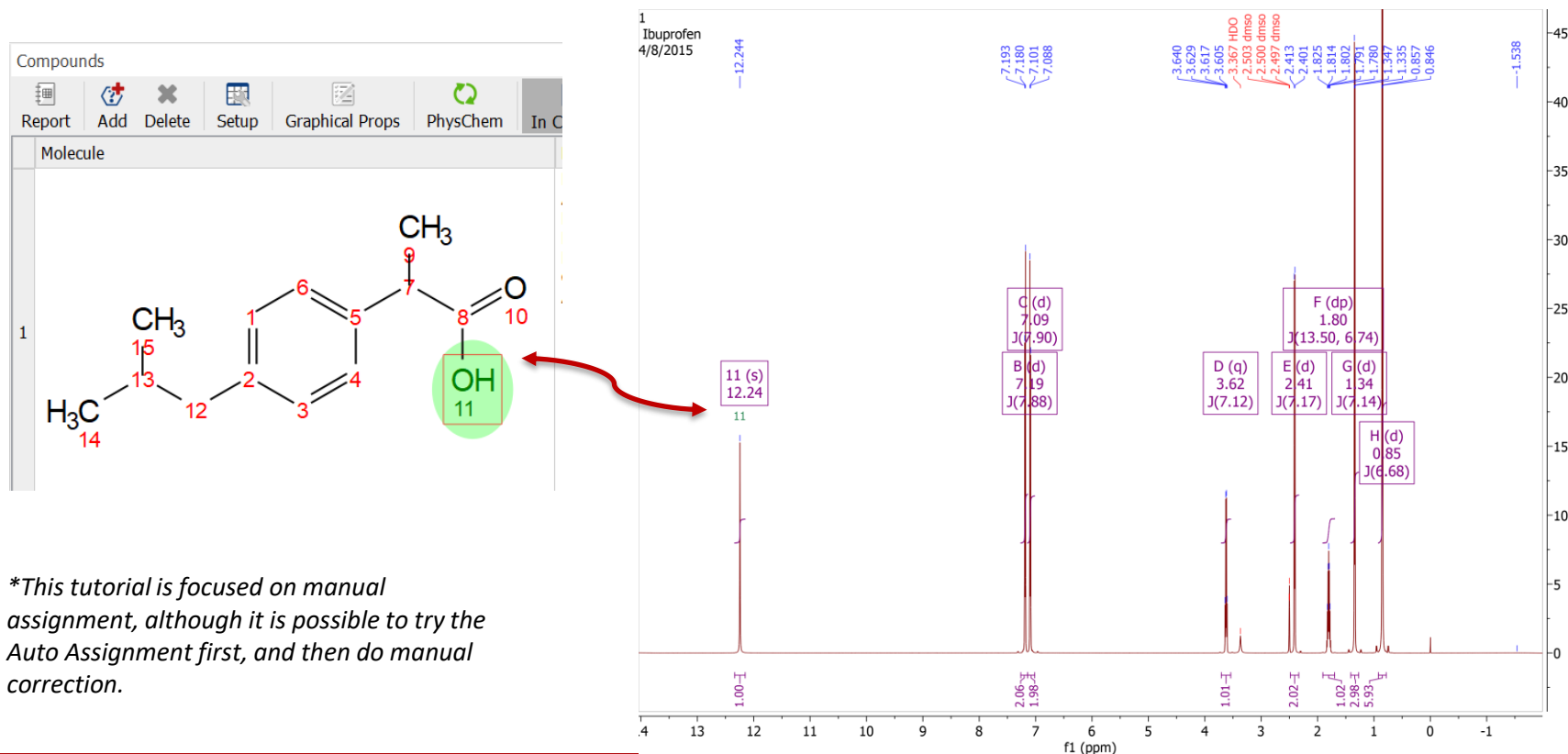
Tip: Click Save as Default button to save the settings

ANALYSIS

- Click A key to switch to Manual Assignment mode
- Click on a multiplet label and assign it to an atom (This is the most common way to assign H-1 peaks)
- Click on a peak and assign it to an atom
- Click and drag on the spectrum, and assign the range to an atom
- View chemical shift assignment grids on the other “linked” spectra

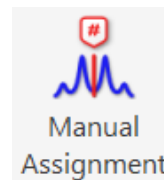


Shortcut = A



ANALYSIS

- Optionally, you can choose Predict > Predict and Compare to have a predicted H-1 spectrum stacked above the experimental one to guide your assignment of some peaks
- For example, the predicted quartet for H-7 is very close to the quartet observed at around 3.62ppm, so we can assign that observed quartet to H-7, by clicking the atom and then the multiplet label in the Manual Assignment mode.
- You can assign more multiplets in this way.



Shortcut = A

Tip: Use the following short cut keys to accelerate your assignment:

A: Assignment mode

Z: Zoom in mode

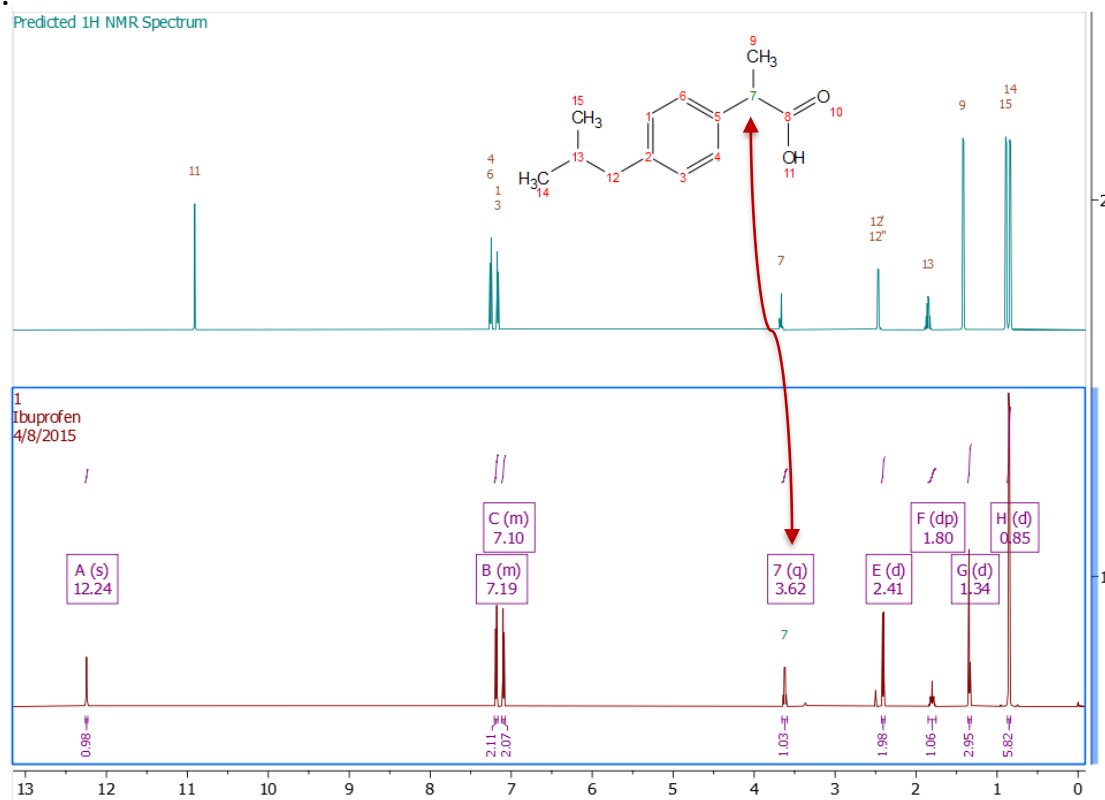
S: Swap the assignments of two atoms

<Ctrl+Space>: Switch to Zoom in mode temporarily

<ESC>: Exit from any of the modes above

If you need a copy of the structure in a page, click the structure in the Compounds Table and click Report.

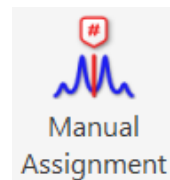
To delete the predicted C-13 spectrum, choose Stack > Stacked Items Table, and delete it from the Table.



ANALYSIS

- Make a copy of the C-13 spectrum.
- Choose Predict > Predict Compare to predict the 13C spectrum
- Use the prediction to guide the manual assignment

Assign C-13 peaks

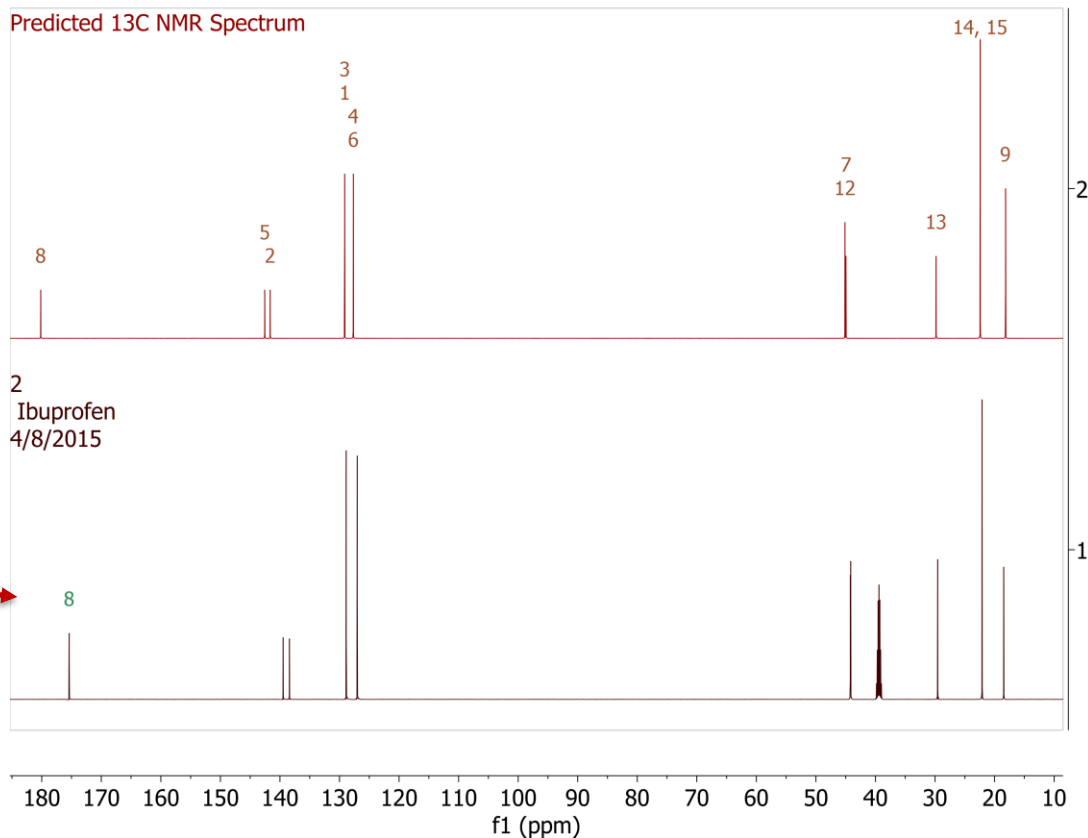
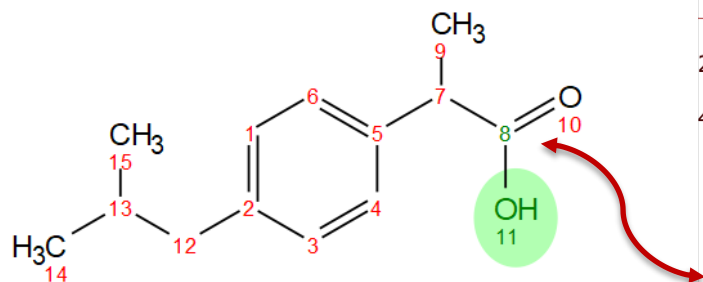


Shortcut = A

Compounds

Report Add Delete Setup Graphical Props PhysChem In

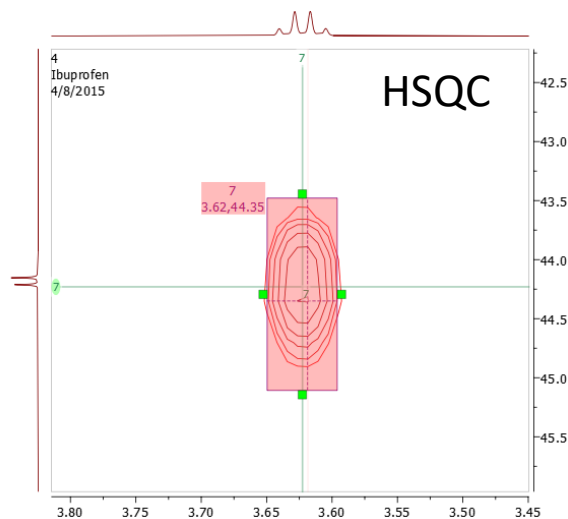
Molecule



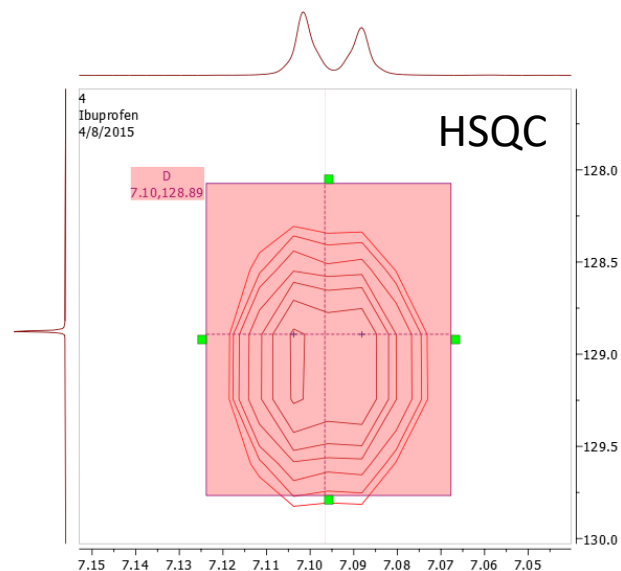
ANALYSIS

General guidance for assigning 2D spectra

- Since Mnova version 14.1, the assignment of a 2D peak is based on a “2D multiplet”, which can be either a single peak or a group of peaks.
- 2D multiplets can be automatically picked using Analysis > Auto Multiplet Analysis, and then manually corrected.
- If you don't pick any multiplets before assigning a 2D peak, it will automatically pick a multiplet around where you clicked in the 2D spectrum during the assignment.
- A 2D multiplet can be manually resized using the green blocks shown around it when you hover the cursor on it.



A HSQC multiplet with a single peak. It is assigned to CH(7)



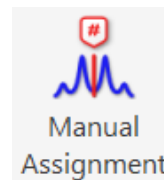
A HSQC multiplet with two peaks. It is not assigned to any atoms yet

Tip: For better visualization of the assignment results on a 2D spectrum, it is better to suppress the display of the 2D peaks and 2D multiplet labels. Right click on the spectrum and choose Properties, and turn off those options.

ANALYSIS

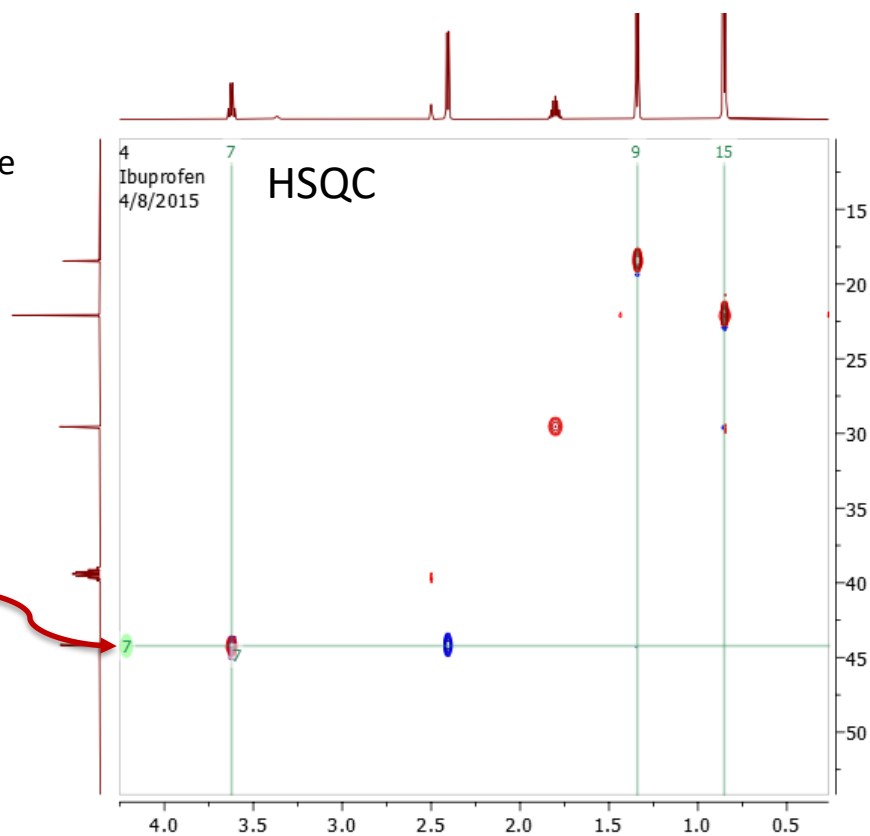
- If you assign a peak from a 1D ^1H -1 or ^{13}C spectrum, the assignment is displayed for the same nucleus on the 2D spectra automatically
- If you assign a cross peak from a 2D spectrum, the assignments for the corresponding nuclei/dimensions are also displayed on the corresponding 1D and 2D spectra, automatically
- The 1D and 2D assignment results are recorded in the Assignment Table (Assignment > NMR Assignments)
- You can click and drag an assignment line to change the chemical shift.

Assignment lines across the spectra



Shortcut = A

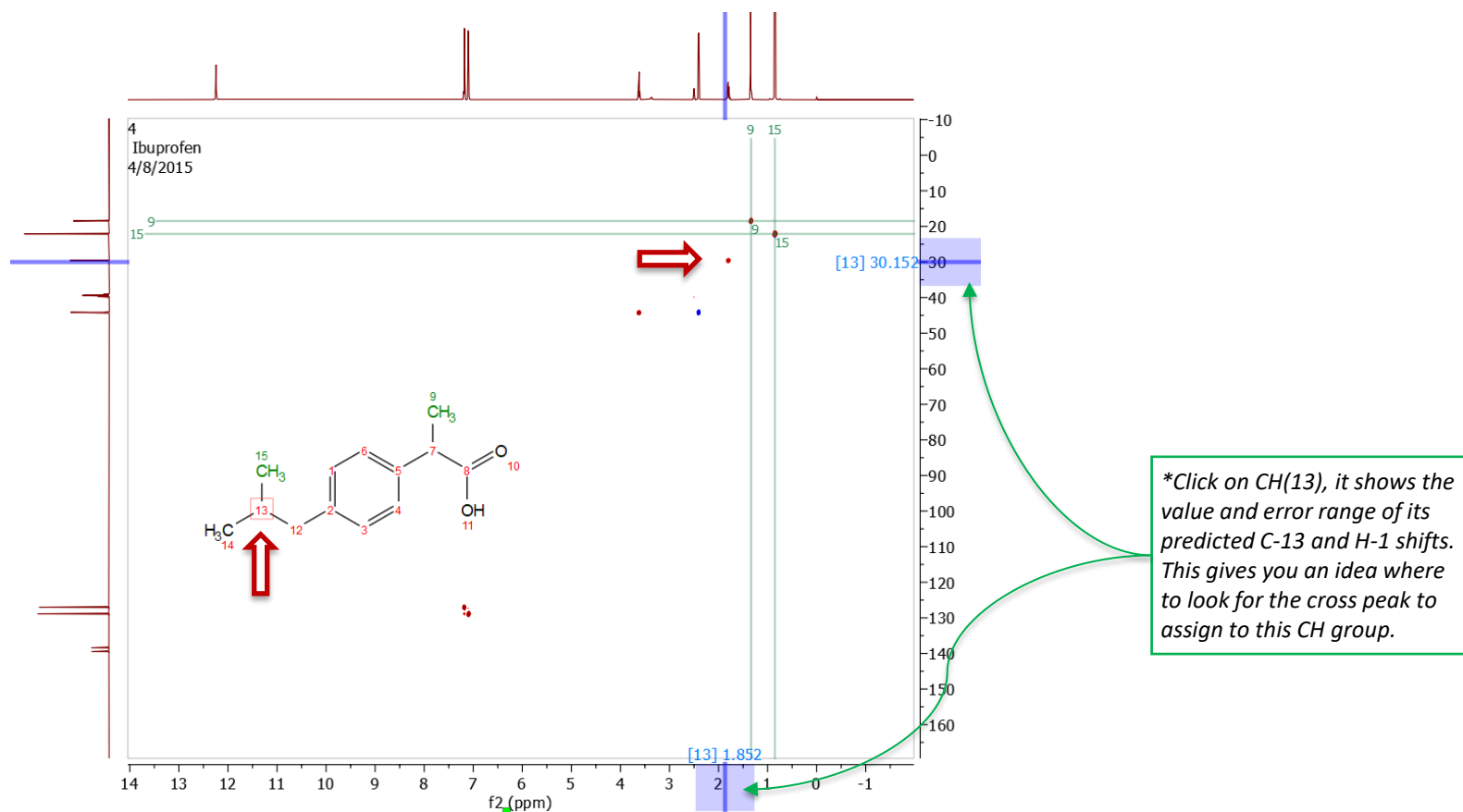
Tip: Click and drag the number ("7") to adjust the chemical shift value of this assigned peak in the ^{13}C dimension. The change will be reflected in all other spectra, as well as in the Assignment Table. You can do this on 1D spectra too.



Assign HSQC peaks

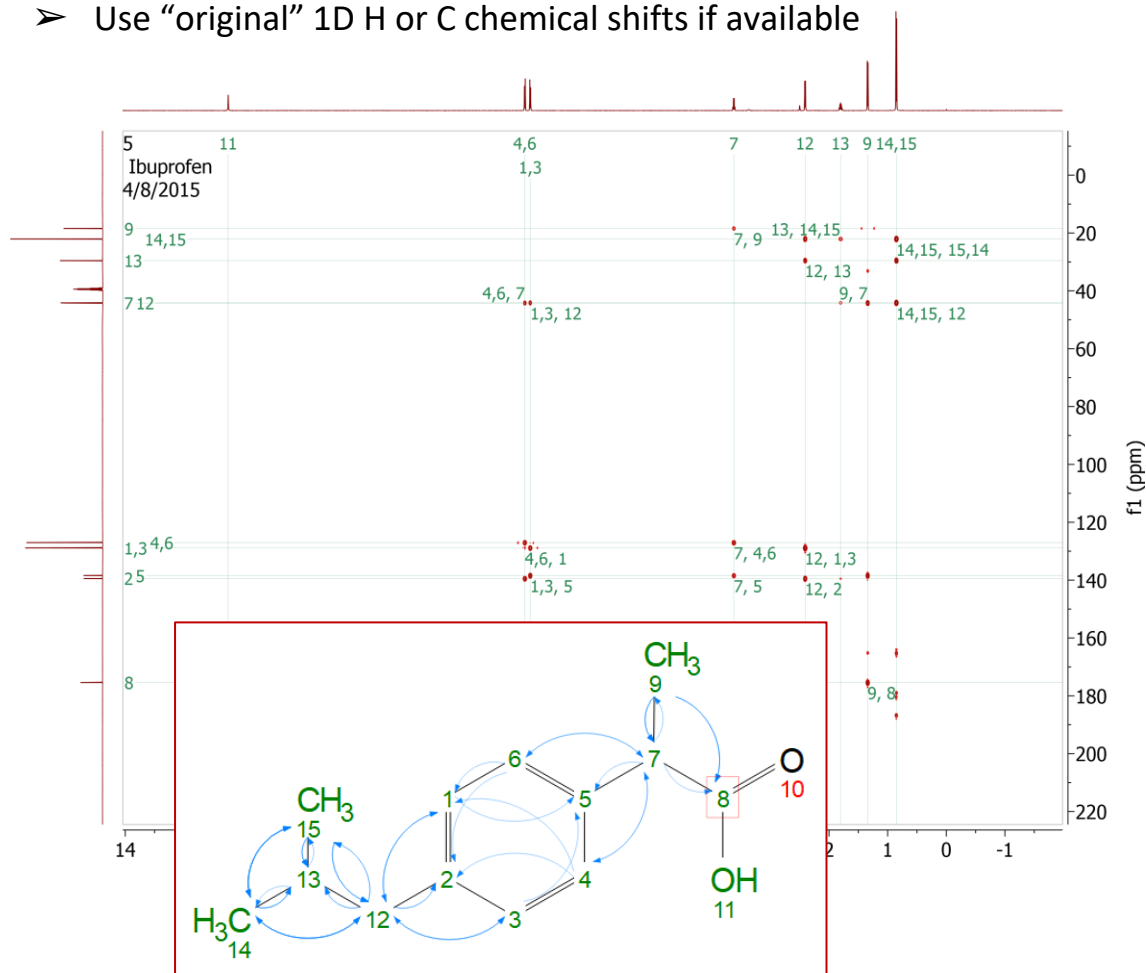
Use Predict & Highlight to help assigning HSQC peaks

- Optionally, you can choose Predict > Predict and Highlight to display the predicted range of HSQC peaks for CH groups. The ranges can be helpful for assigning HSQC peaks to their corresponding CH groups

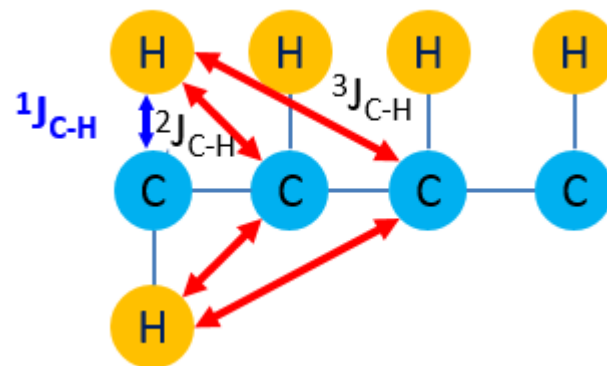


ANALYSIS

- Assign a cross peak to connected H-C with 2 or 3 bonds in between: Click the peak first, then the assigned H. Mnova will popup an Assign Dialog for you to choose the assigned C.
- Use "original" 1D H or C chemical shifts if available



Assign HMBC peaks

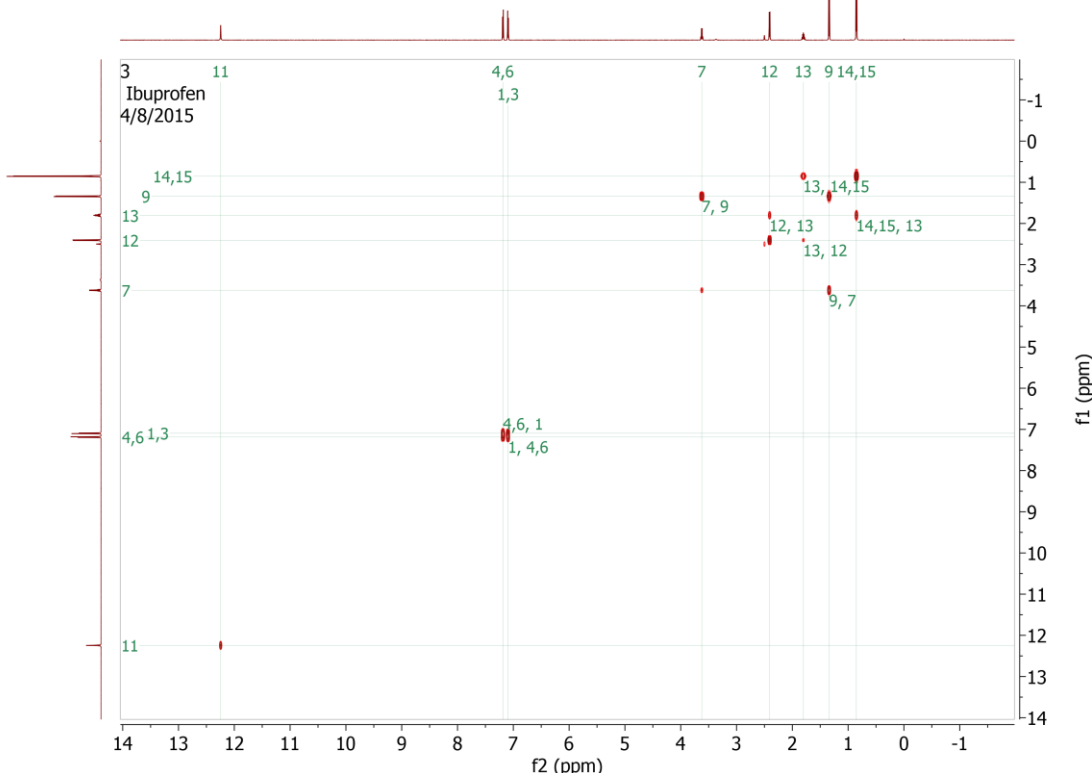
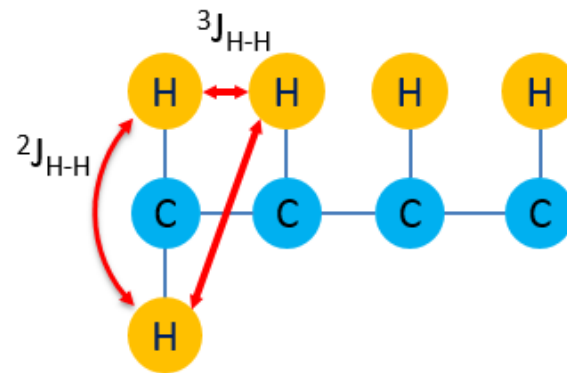


The screenshot shows the 'Assign' dialog box in the Mestrelab software. It is used to assign a specific cross-peak to a particular carbon atom. The dialog has two main sections. The top section is for the proton assignment, showing 'Atom 3:' and a dropdown for $\delta(^1\text{H})$ set to 'f2=7.095 ppm'. Below this, it shows 'Already assigned (3): 7.08-7.11' and three radio buttons: 'Replace', 'Add', and 'Keep Original' (which is selected). The bottom section is for the carbon assignment, with a checked box 'Assign f1'. It shows 'Atom: 12' in a dropdown and a dropdown for $\delta(^{13}\text{C})$ set to 'f1=44.2 ppm'. Below this, it shows 'Already assigned (12): 44.20-44.22' and the same three radio buttons, with 'Keep Original' selected. At the bottom are 'OK' and 'Cancel' buttons.

ANALYSIS

- Assign a cross peak to a H-H pair with 2 or 3 bonds in between:
Click the peak first, then the assigned H on F2. Mnova will popup an Assign Dialog for you to choose the other H.
- Note weak couplings between 4-5 bonds is also possible
- Use “original” 1D H-1 chemical shifts if available

Assign COSY peaks



Assign

Atoms 4,6:

$\delta(1H)$: f2=7.186 ppm

Already assigned (4):7.19 (6):7.19

☐ Replace
☐ Add
☒ Keep Original

☒ Assign f1

Atom: 1

$\delta(1H)$: f1=7.095 ppm

Already assigned (1):7.09

☐ Replace
☐ Add
☒ Keep Original

OK

Cancel

Tip: Displaying the diagonal line can be helpful to distinguish cross peaks and diagonal ones. To do that, right click and open the Properties Dialog, and check Grids > Show Diagonal.

REPORT

Assignments table

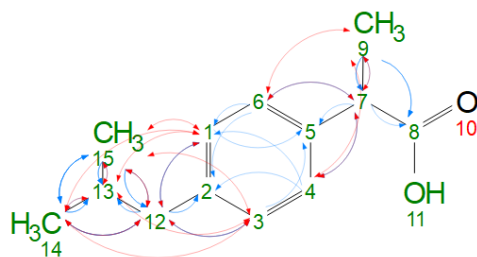
- Click Assignment > Assignment Table to display the Assignment Table
- The check boxes can be used to turn on/off the display of individual correlations on the structure

Assignments ×

Report Copy Delete Expand Collapse Hide Setup Deduce NOE ▾

Atom	Chemical Shift	Quality	Predicted Shift	J	NOE	COSY	HSQC	HMBC	NOESY
▼ 1 C	128.88		129.12				1	✓ 4, 6, 12	
H	7.09	0.71				✓ 4, 6	1	✓ 5, 12	✓ 12, 13, 14, 15
2 C	139.46		141.62					✓ 4, 6, 12	
▼ 3 C	128.88		129.12				3	✓ 12	
H	7.09	0.71					3	✓ 5, 12	✓ 12, 13, 14, 15
▼ 4 C	127.02		127.66				4	✓ 7	
H	7.19	0.71				✓ 1	4	✓ 1, 2, 7	
5 C	138.41		142.54					✓ 1, 3, 7	
▼ 6 C	127.02		127.66				6	✓ 7	
H	7.19	0.71				✓ 1	6	✓ 1, 2, 7	
▼ 7 C	44.22		44.96				7	✓ 4, 6, 9	
H	3.62	0.71				✓ 9	7	✓ 4, 5, 6, 8, 9	
8 C	175.40		180.15					✓ 7, 9	
▼ 9 C	18.45		18.15				9	✓ 7	
H3	1.34	0.71				✓ 7	9	✓ 7, 8	
10 O									
▼ 11 O									
H	12.24	0.56							
▼ 12 C	44.16		45.12				12	✓ 1, 3, 14, 15	
H2	2.41	0.71				✓ 13	12	✓ 1, 2, 3, 13, 14,...	✓ 1, 3, 14, 15
▼ 13 C	29.55		29.81				13	✓ 12, 14, 15	
H	1.80	0.25				✓ 12, 14, 15	13	✓ 14, 15	✓ 1, 3, 14, 15
▼ 14 C	22.09		22.41				14	✓ 12, 13, 15	
H3	0.85	0.71				✓ 13	14	✓ 12, 13, 15	✓ 1, 3, 12, 13
▼ 15 C	22.09		22.41				15	✓ 12, 13, 14	
H3	0.85	0.71				✓ 13	15	✓ 12, 13, 14	✓ 1, 3, 12, 13

- Choose Assignment > Report Assignments.
- Report the assignments on the spectrum or paste the table to another document



No	δ_H (Multiplicity, J)	δ_C	HSQC-EDITED	HMBC	COSY	NOESY
1	7.09 (d, 7.9 Hz)	128.9	128.9(1)	44.2(12), 128.9(1), 128.9(3), 138.4(5)	7.19(6)	0.85(14), 0.85(15), 1.80(13), 2.41(12)
2	-	139.5	-	2.41(12), 7.19(6)	-	-
3	7.09 (d, 7.9 Hz)	128.9	128.9(3)	44.2(12), 128.9(1), 128.9(3), 138.4(5)	-	0.85(14), 0.85(15), 1.80(13), 2.41(12)
4	-	127.0	-	-	-	-
5	-	138.4	-	1.34(9), 3.62(7), 7.09(1), 7.09(3)	-	-
6	7.19 (d, 7.9 Hz)	127.0	127.0(6)	44.2(7), 127.0(4), 127.0(6), 128.9(1), 128.9(3), 139.5(2)	7.09(1)	1.34(9), 3.62(7)
7	3.62 (q, 7.1 Hz)	44.2	44.2(7)	127.0(4), 127.0(6), 138.4(5), 175.4(8)	1.34(9)	7.19(6)
8	-	175.4	-	1.34(9), 3.62(7)	-	-
9	1.34 (d, 7.1 Hz)	18.5	18.5(9)	44.2(7), 138.4(5), 175.4(8)	3.62(7)	7.19(6)
11	12.24 (s)	-	-	-	-	-
12	2.41 (d, 7.2 Hz)	44.2	44.2(12)	22.1(14), 22.1(15), 29.6(13), 128.9(1), 128.9(3), 139.5(2)	1.80(13)	7.09(1), 7.09(3)
13	1.80 (dp, 13.5, 6.7 Hz)	29.6	29.6(13)	22.1(14), 44.2(12)	0.85(14), 0.85(15), 2.41(12)	7.09(1), 7.09(3)
14	0.85 (d, 6.7 Hz)	22.1	22.1(14)	29.6(13)	1.80(13)	7.09(1), 7.09(3)
15	0.85 (d, 6.7 Hz)	22.1	22.1(15)	29.6(13)	1.80(13)	7.09(1), 7.09(3)

Setup Assignments Report

?

✕

Options

☒ Include 13C and X-Nuclei Assignments

☒ Include 13C Multiplicity

☒ Include 1H Multiplicity

☒ Include Number of protons

☐ Order by Chemical Shift

☐ Report Mean Chemical Shift values

☐ Include Atom Type

☐ Only Copy to Clipboard

☐ Export To File:

☒ Text (TSV)

☐ HTML

Decimal Places For 1H:

2

Decimal Places For13C and X-Nuclei:

1

☒ 2D Correlations

Format:

☐ n

☒ $\delta(n)$

☐ Atom(δ)

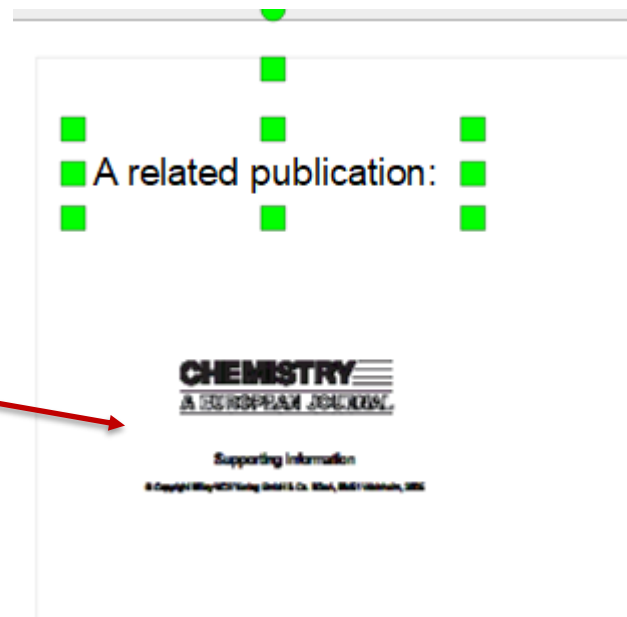
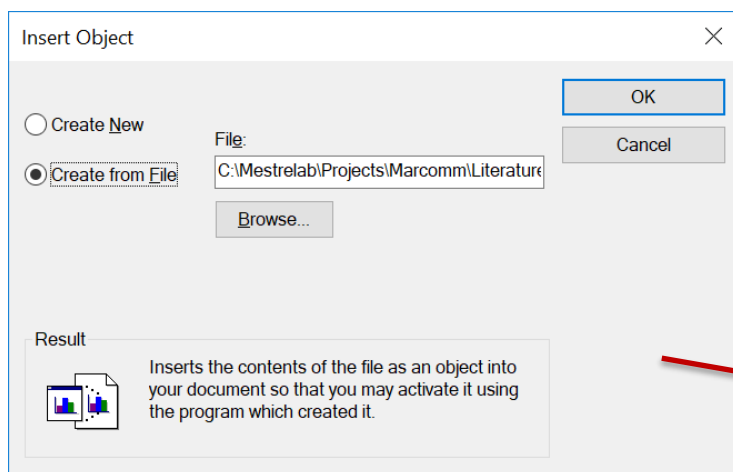
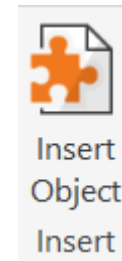
☐ Drop Lines Without Correlation

OK

Cancel

Insert a PDF to the document

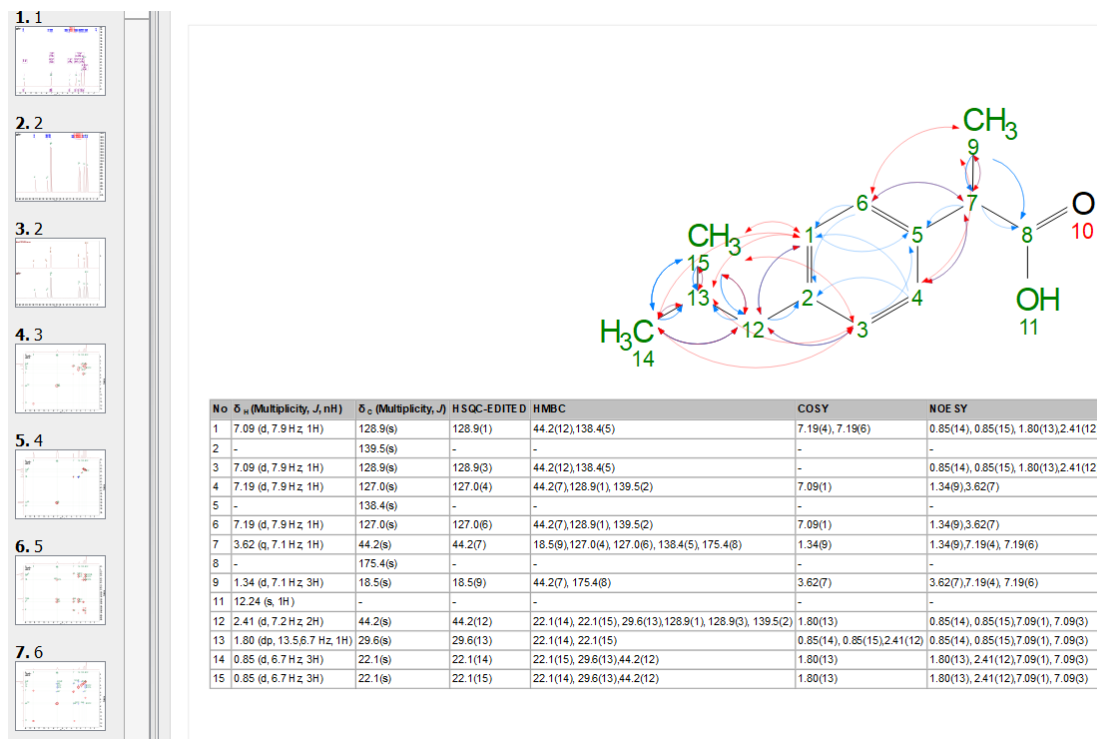
- Choose Home > Insert Object, choose Create from File, and insert a PDF to the document
- A preview logo of the document is displayed.
- Add a text box annotation to it
- You can double click on the preview to open it



REPORT

Save the results

- Choose File > Export to PDF to save a PDF report of the page.
- Chose File > Save as to save all the results to a .mnova file.
- Save all the results to a database (see steps later)
- Now you can close the document or continue to add other spectra to it.



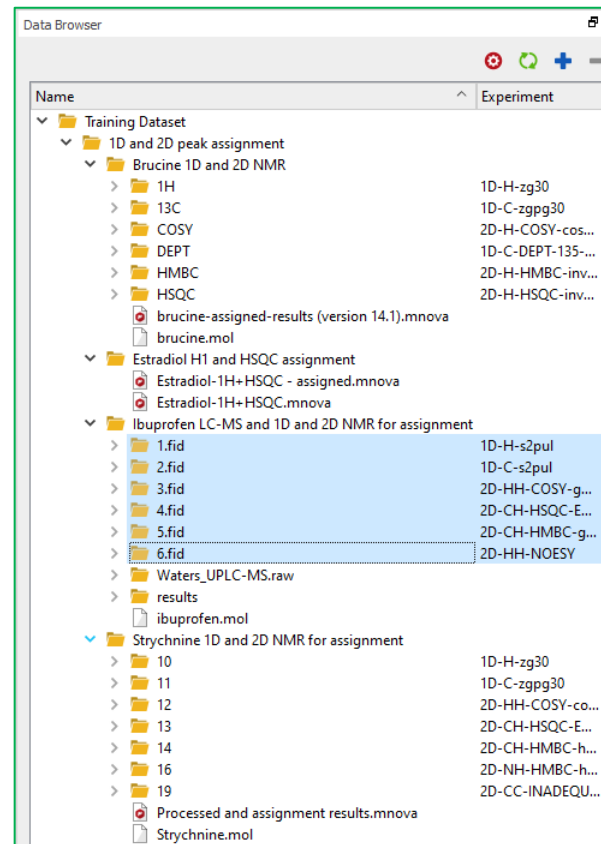
.pdf doc

.mnova doc

Database

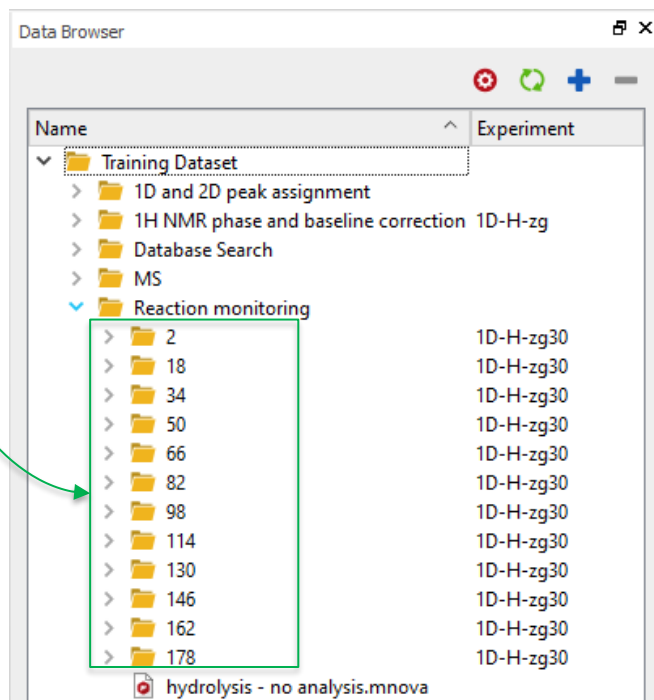
More about peak assignment

- Manual assignment of 1D and 2D peaks is not only an effective way to verify a proposed structure, but also a proven way to learn to understand and analyze 1D and 2D NMR.
- Usually you start with the ¹H, ¹³C and HSQC spectra, if available, with the assistance of the predicted peaks or ranges as a guidance. Then you extend the assignment to the other 2D spectra such as COSY and HMBC. While assigning COSY or HMBC peaks, conflicts with the previous assignments may be discovered and hence corrections can be done. If the conflicts cannot be resolved, it may imply that the structure is wrong.
- There are a total of 4 datasets with 1D and 2D NMR spectra for practicing spectral processing, peak assignment that come with this tutorial: Ibuprofen, Brucine, Strychnine, and Estradiol, along with results of full assignments. You can use them for practice.



Processing Arrayed Spectra for Reaction Monitoring etc.

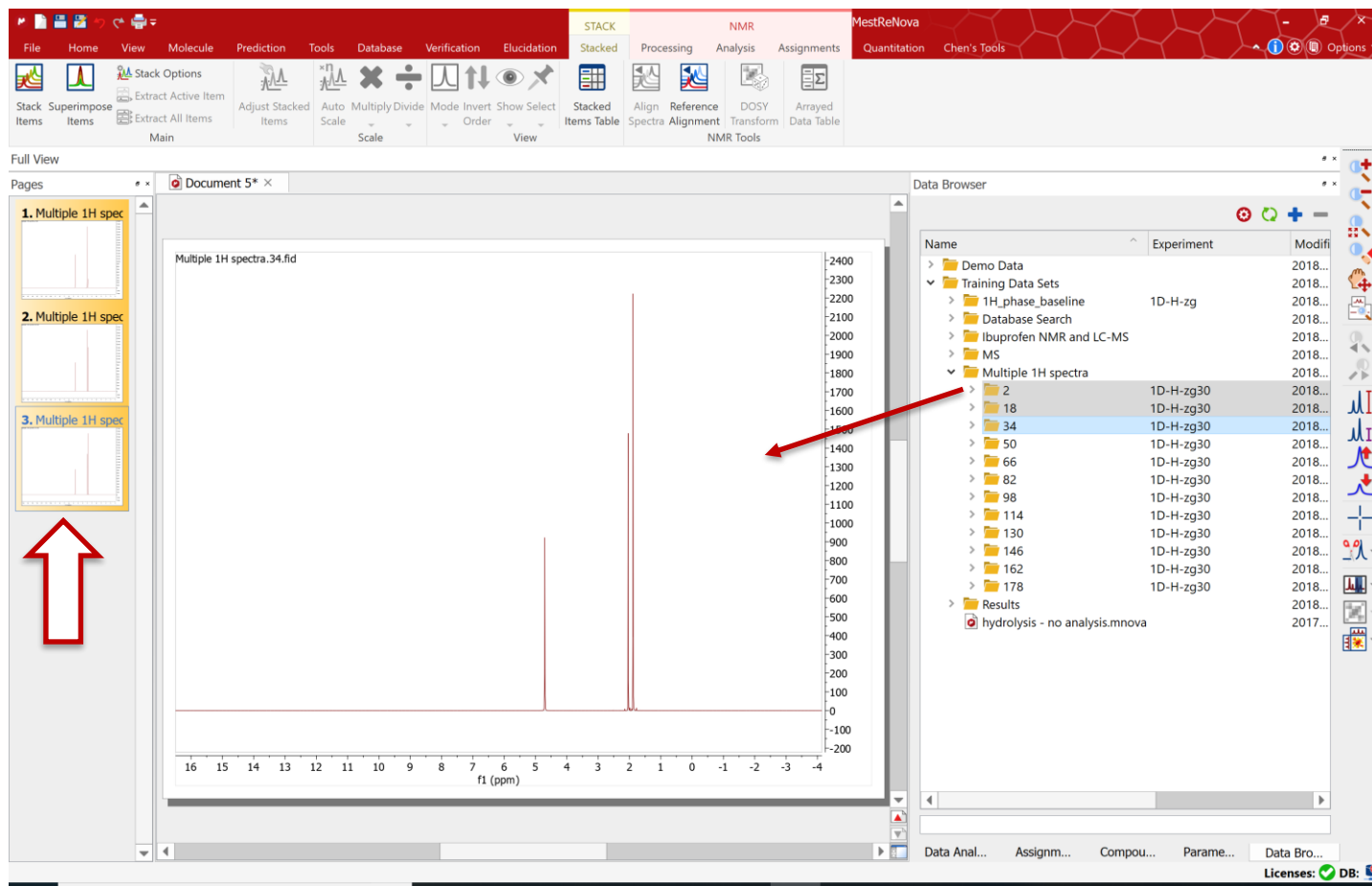
Sample data



ARRAYED SPECTRA

Stack a few spectra

- Open the first 3 spectra from the Multiple 1H spectra folder in Data Browser
- The Stacked Ribbon is visible if you highlight multiple spectra in the Pages View



ARRAYED SPECTRA

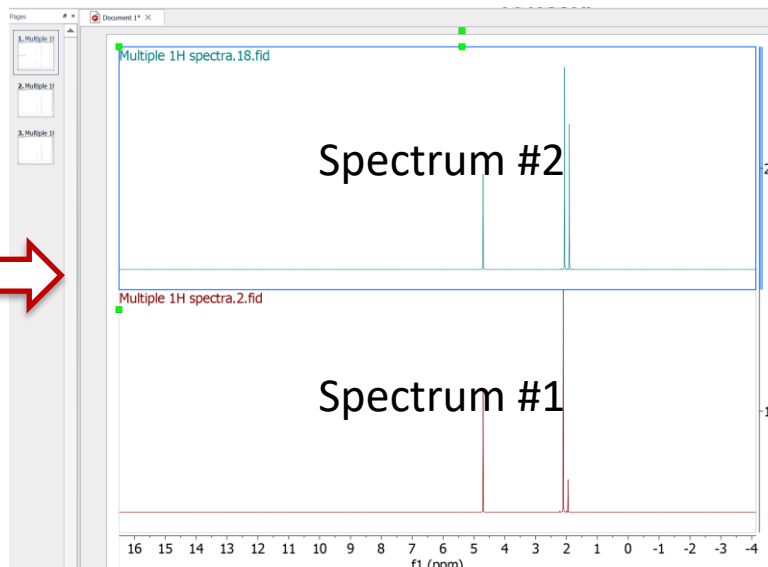
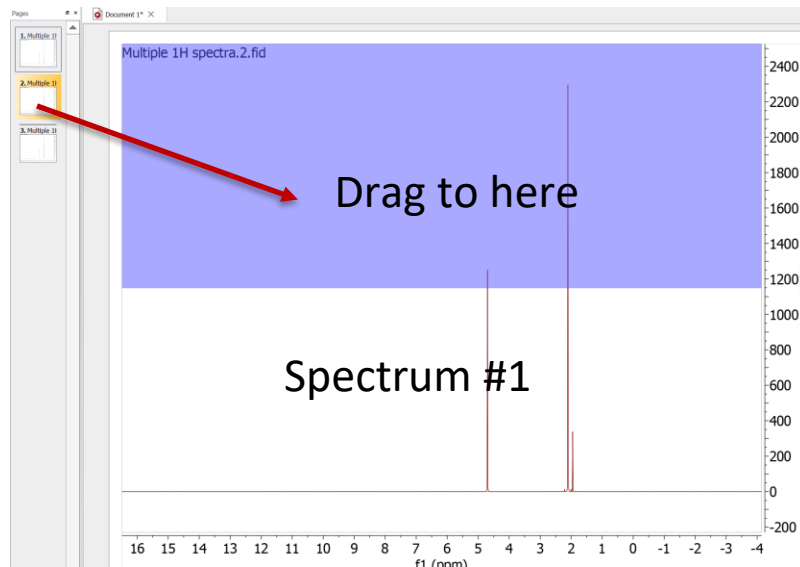
- You can use the Stack Items or Superimpose Items tools to stack or superimpose the highlighted spectra in the Pages View, or:
- Drag the thumbnail of another spectra from the Pages View to the current spectrum to stack them in desired way.
- Continue to drag the 3rd spectrum to the stack. Note you can put the spectrum to the top, middle or bottom, or to replace an existing spectrum in the stack.
- Try the different Stacking Mode, and other tools in the Stacked Ribbon



Stack
Items



Superimpose
Items



ARRAYED SPECTRA

Stack many spectra

- Choose Tools > Loaded Scripts > Directory Spectra Stack, navigate to the directory “Multiple H-1 Spectra” in the training dataset. Click OK to import and stack all of them.

Import Spectra Stack

Data Folder: ...

Order:

File Path Filtering

File Name Masks:

Folder Name Masks:

☐ Spectral Data Filtering

Parameter =

☐ Chunking

First Spectrum: ☐ Number of Chunks:

Chunk Size: Step to Next Chunk:

Visualization

View: Decimation Step:

Palette:

☐ Import Array Values

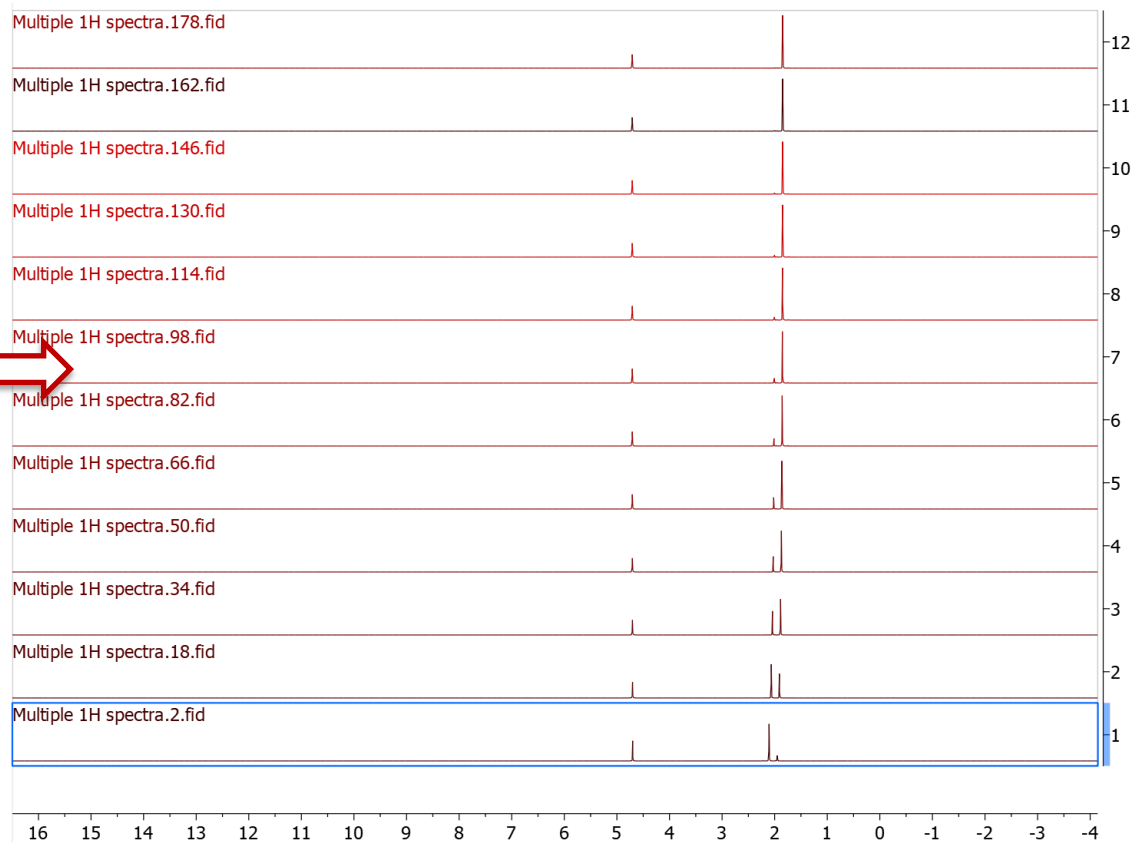
File:

☐ Processing Template

File:

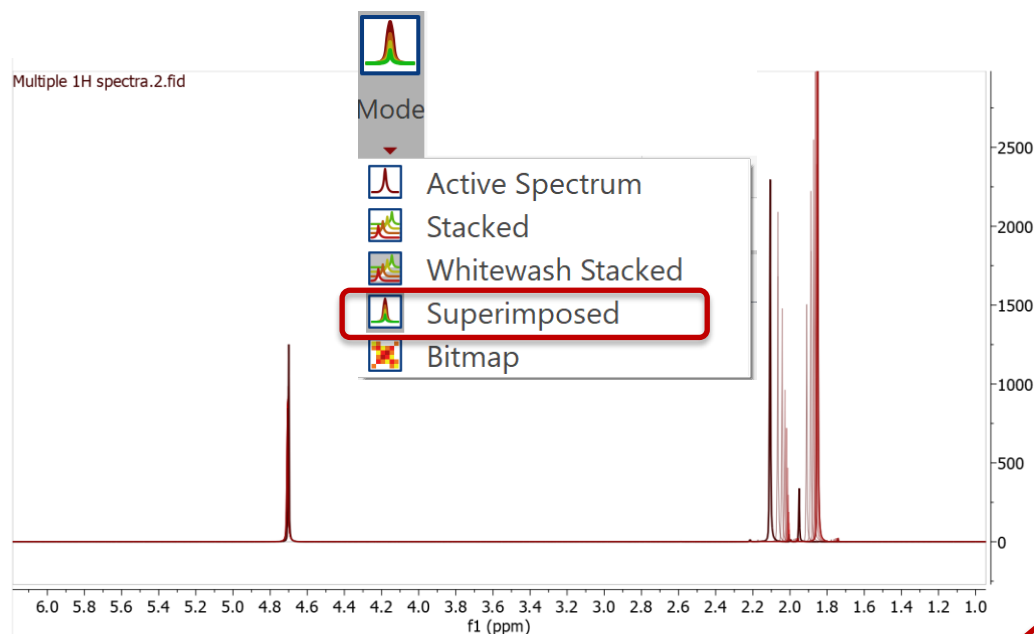
☐ Backup

Folder:



ARRAYED SPECTRA

- Choose Stacked > Mode to try different display modes. Choose Superimposed mode to make sure the baseline and phasing is OK for all spectra.
- Choose Stacked > Stacked Items Table to display the Table. You can manipulate the spectra in many ways using the tools on this Table.
- If needed, you can reprocess all or selected spectra



Stacking mode and Stacked Items Table

Stacked Items Table

Stacked Items

Report Copy Delete Invert Order Setup

Multiply Divide Show Select Adjust Stacked Items

	Eye	Title	Pin	T/G	Ratio	Norm. Factor	Δ (I)
12	<input checked="" type="checkbox"/>	Multiple 1H spectra.178.fid	<input type="checkbox"/>	0.00e+00	1.00e+00	1.00e+00	0.00
11	<input checked="" type="checkbox"/>	Multiple 1H spectra.162.fid	<input type="checkbox"/>	0.00e+00	1.00e+00	1.00e+00	0.00
10	<input checked="" type="checkbox"/>	Multiple 1H spectra.146.fid	<input type="checkbox"/>	0.00e+00	1.00e+00	1.00e+00	0.00
9	<input checked="" type="checkbox"/>	Multiple 1H spectra.130.fid	<input type="checkbox"/>	0.00e+00	1.00e+00	1.00e+00	0.00
8	<input checked="" type="checkbox"/>	Multiple 1H spectra.114.fid	<input type="checkbox"/>	0.00e+00	1.00e+00	1.00e+00	0.00
7	<input checked="" type="checkbox"/>	Multiple 1H spectra.98.fid	<input type="checkbox"/>	0.00e+00	1.00e+00	1.00e+00	0.00
6	<input checked="" type="checkbox"/>	Multiple 1H spectra.82.fid	<input type="checkbox"/>	0.00e+00	1.00e+00	1.00e+00	0.00
5	<input checked="" type="checkbox"/>	Multiple 1H spectra.66.fid	<input type="checkbox"/>	0.00e+00	1.00e+00	1.00e+00	0.00
4	<input checked="" type="checkbox"/>	Multiple 1H spectra.50.fid	<input type="checkbox"/>	0.00e+00	1.00e+00	1.00e+00	0.00
3	<input checked="" type="checkbox"/>	Multiple 1H spectra.34.fid	<input type="checkbox"/>	0.00e+00	1.00e+00	1.00e+00	0.00
2	<input checked="" type="checkbox"/>	Multiple 1H spectra.18.fid	<input type="checkbox"/>	0.00e+00	1.00e+00	1.00e+00	0.00
1	<input checked="" type="checkbox"/>	Multiple 1H spectra.2.fid	<input type="checkbox"/>	0.00e+00	1.00e+00	1.00e+00	0.00

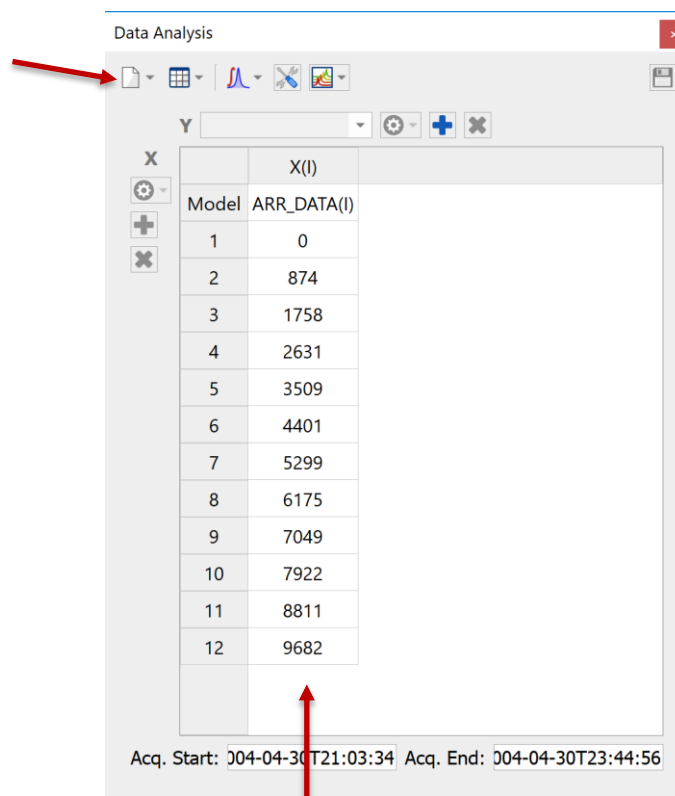
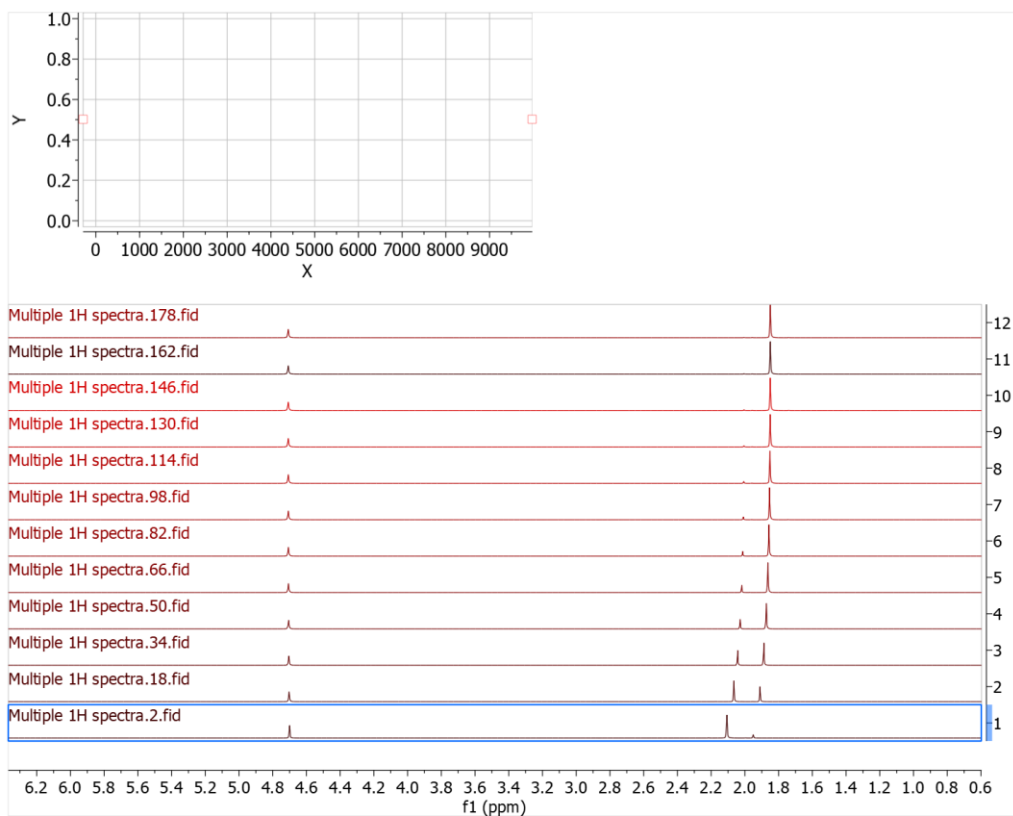
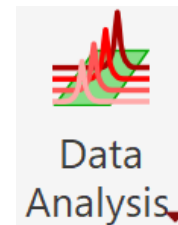
Click and drag here
to change the order

Check/uncheck
these to show/hide
spectra

Check these to
choose spectra to
change

ARRAYED SPECTRA

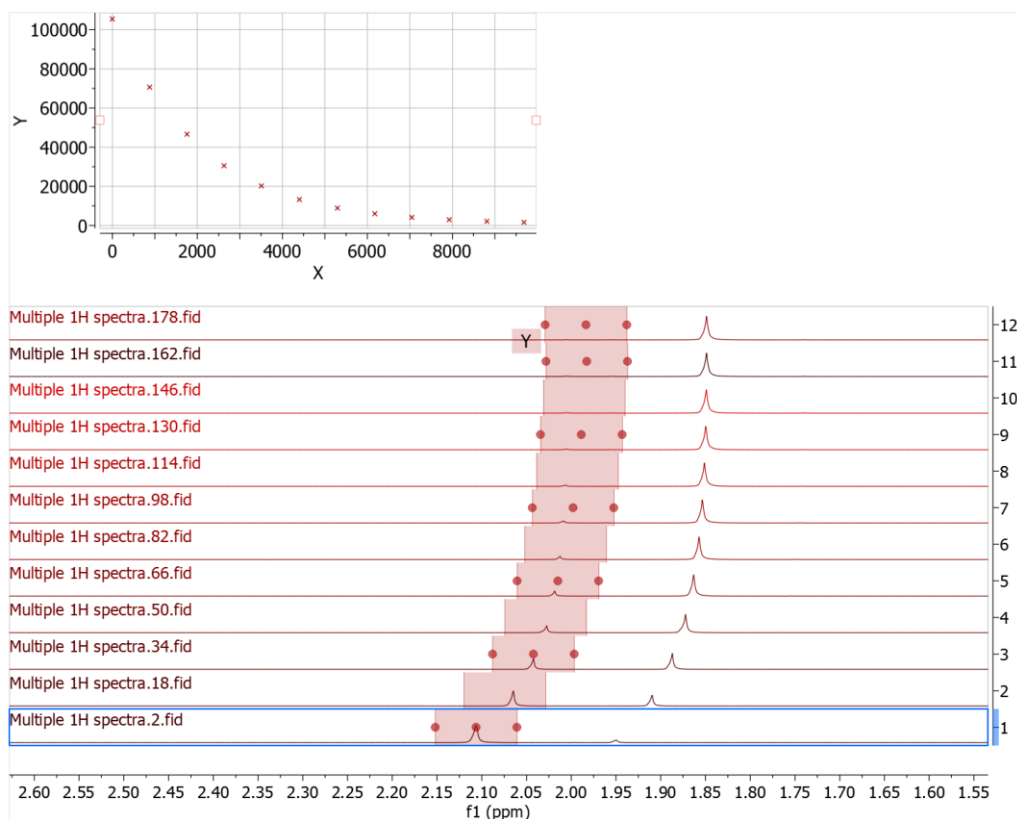
- Choose Analysis > Data Analysis > Show Table to display the Data Analysis Table.
- Click on the Empty Graph to import the X values (reaction time in this case) and display an empty XY graph.



Reaction time

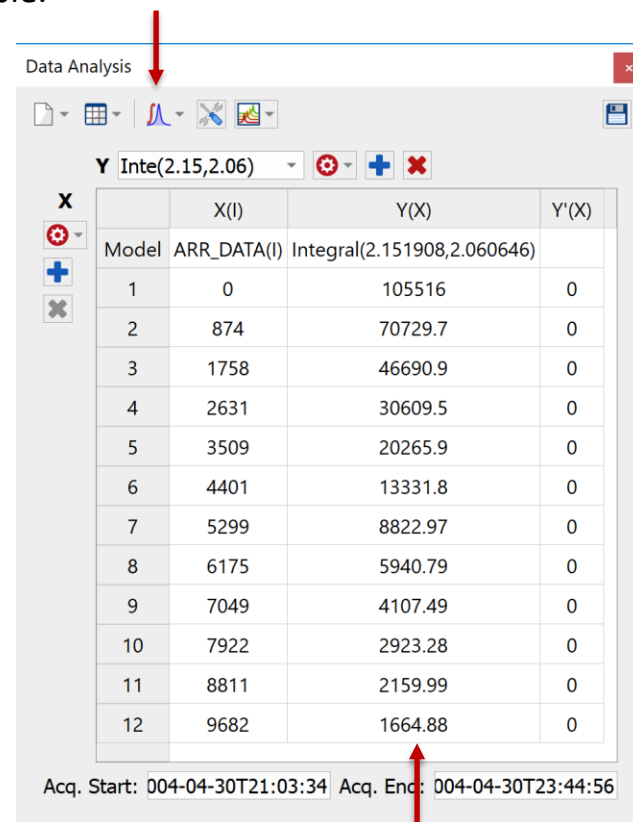
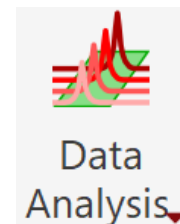
ARRAYED SPECTRA

- Click the Pick Integral tool.* Click and drag on first (bottom) spectrum to define the integration range
- If needed, adjust the handlers to change the integration range **
- The integrals are displayed on the XY graph and in the Data Analysis Table.



*Use Sum Method (default) for integration unless you are integrating overlapped peaks. Click Options for Integration in the Analysis Ribbon to verify. ** You can increase the # of handlers by using the Edit Model Option Tool.

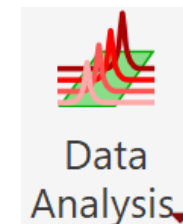
Integrate arrayed spectra



Integrals

ARRAYED SPECTRA

- Click the Model cell under $Y'(X)$ in Data Analysis.
- Choose the 3rd function, and click Calculate to fit the XY values to a first order reaction (with offset)



Double click here

Data Analysis

Y: Inte(2.15,2.06)

	X(I)	Y(X)	Y'(X)
Model	ARR_DATA(I)	Integral(2.151908,2.060646)	
1	0	105516	0
2	874	70729.7	0
3	1758	46690.9	0
4	2631	30609.5	0
5	3509	20265.9	0
6	4401	13331.8	0
7	5299	8822.97	0
8	6175	5940.79	0
9	7049	4107.49	0
10	7922	2923.28	0
11	8811	2159.99	0
12	9682	1664.88	0

Acq. Start: 004-04-30T21:03:34 Acq. End: 004-04-30T23:44:56

Y'-Column Model Function

Functions

	Name	Function	Initialization	Report	Description
1	Linear Fit	$A+B*x$	A= 0, B= 0		Zero Order Reaction Rate
2	Mono-exponential Fit	$B*\exp(-x*F)$			Exponential Decay, First Order Reaction Rate
3	Three Parameter Exponential Fit	$B+F*\exp(-x*G)$			Exponential Decay, First Order Reaction Rate With Offset
4	Inverse Linear Fit	$1/(A+B*x)$	A= 1, B= 0		Second Order Reaction Rate
5					

Restore Defaults

Fitted Parameters

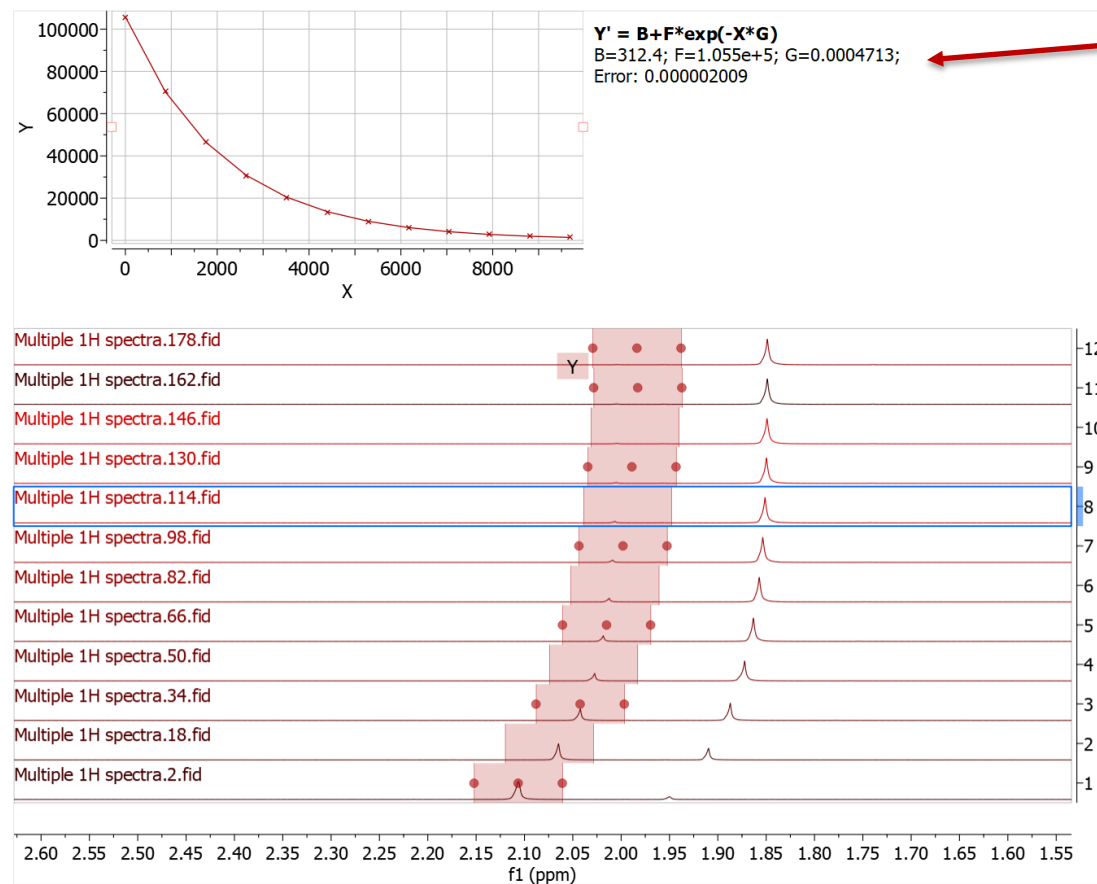
Calculate

B= 312.388, F= 105518, G= 0.000471256
rError = 2.00941e-06, probnotmono = 0.958368

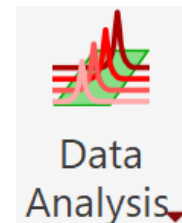
OK Cancel

ARRAYED SPECTRA

- Click the Report tools from the Data Analysis Panel to report the results next to the XY Graph
- Choose Report to Clipboard and paste the results to another document
- Repeat these steps for the other peaks around 1.91 ppm.



Report kinetic parameters



Data Analysis

Y: Inte(2.15,2.06)

X	X(l)	Y(X)	Y'(X)
Model	ARR_DATA(l)	Integral(2.151908,2.060646)	B + F * exp(-x * G) B = 312.388 F = 105518 G = 0.000471256
1	0	105516	105831
2	874	70729.7	70208.4
3	1758	46690.9	46394.2
4	2631	30609.5	30851.6
5	3509	20265.9	20503.6
6	4401	13331.8	13574.2
7	5299	8822.97	8998.29
8	6175	5940.79	6060.56
9	7049	4107.49	4120.01
10	7922	2923.28	2835.76
11	8811	2159.99	1972.11
12	9682	1664.88	1413.35

Acq. Start: 2004-04-30T21:03:34 Acq. End: 2004-04-30T23:14:56

Fitting results

HELP INFORMATION

- Use the Help Facility of Mnova: Help > Contents
- Visit www.mestrelab.com for manuals, tutorials, videos and publications
- Email support@mestrelab.com for technical questions

The screenshot displays the MestReNova software interface. On the left is a red sidebar menu with options: New, Close, Recent, Save, Save As..., Export to PDF..., Save To, Open..., Open Directory..., Open From, Print..., Page Setup..., Help (highlighted with a red arrow), Preferences..., Advanced Plug-ins..., and Exit. The main window is titled 'MestReNova' and has tabs for 'Help' and 'About'. The 'Help' tab is active, showing a 'MestReNova Manual' window. This window has a toolbar with 'Hide', 'Back', 'Forward', 'Home', 'Print', and 'Options'. Below the toolbar is a 'Contents' pane with a tree view of the manual's structure. The 'Help' menu is open, showing options: Help (Get help using Mnova), License Manager (Get licenses information like u...), Request Licenses (Buy or request evaluation lice...), and Check for Updates (Check if you are using the late...). The 'Contents' pane lists various topics, with 'Using GSD for multiplets analysis' highlighted. The main content area of the manual shows the title 'Using GSD for multiplets analysis' in a red header, followed by the text 'Exploiting the power of GSD for an improved Multi...' and 'By default Mnova uses Global Spectral Deconvolution (GS... picking and multiplet analysis.' Below this, it says 'Multiplet Analysis benefit directly from the exploitation of G... automatic analysis, with the enhanced peak picking capabilitie... automatic multiplicity identification and labeling.' and 'Here you can see an example of a triplet which is hidden unde...'. At the bottom, there is a plot of 'Fraction extracted' vs. 'Wavenumber (cm⁻¹)' showing a red curve with a peak at 3400 cm⁻¹.