# NMR Data Processing/Analysis Basics in MNova

Updated June 2025

## Getting started: installing MNova

1) Download MNova from the MestreLab website. Download the current UC MNova license from the chemistry software page.
2) Download the recommended display properties (MNova_default_display.mngp) from the NMR Lab website.
3) Install MNova. When you open it up for the first time, double click on the word **licenses** in the bottom right-hand corner: 

4) Click **install**, then navigate to the folder where you downloaded the UC license file. Select the .zip file and click **open**.
5) Close out of MNova, then re-open it. The red x symbol in the lower right-hand corner may change to a yellow exclamation point ! icon instead of a green checkmark but this is okay. You should now be able to load NMR data.

## Getting started: accessing and preparing the data

**NOTE: follow the below instructions carefully! You must use specific spectra from the ProcExer folder, not your own personal data, for the following exercises!**

1) Connect to the processing exercise file share **ProcExer**. Use the "Accessing your data…" instructions on the NMR lab website.

   **Folder**: \\nmrdata.ad.uc.edu\ProcExer on Windows
   smb://nmrdata.ad.uc.edu/ProcExer on Mac
   **Username**: Your full UC email address
   **Password**: Your UC password

2) Highlight the folder titled **Datasets** and ctrl+c or right click→copy to copy the folder.
3) Enter the folder titled **Completed** and ctrl+v or right click→paste to paste the folder into this directory.
4) Rename the folder you just pasted as your UC 6+2.

## Part 1: $0^{th}$ order phasing

Topspin performs automatic phasing (which MNova reads in) after most data collection, but this is often insufficient for careful analysis, particularly integration. MNova also offers automatic phasing, but it will often still be necessary to manually adjust the phasing, especially if there are large solvent peaks. Spectra with large, suppressed solvent peaks will almost always require manual phasing.

1) Enter your new directory. Drag and drop the folder titled **phasing_1** into the main MNova window. Right click the spectrum and select "Properties." Click on the "load" folder icon in the top left corner. Select the display properties you downloaded in the second step of "Installing MNova" above. I recommend clicking on "Set as Default" in the lower left corner to set these display properties as your default display properties [**NOTE: this is a matter of personal preference].**
2) Look at the spectrum. Describe the problem with it. Would you be able to obtain accurate integrals of these peaks? Why or why not?

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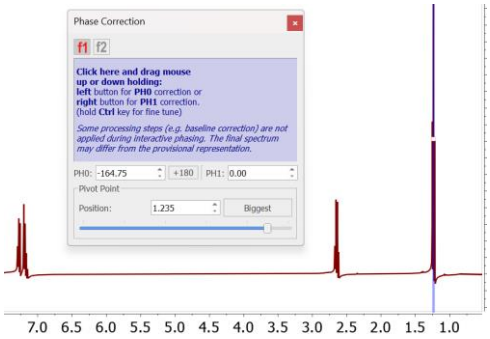
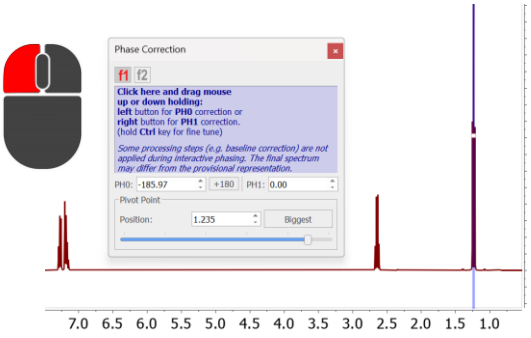
3) Either type shift+p or click on the "Processing" tab along the top of the screen and click on the **Manual Correction** icon. A purple window should appear and a vertical purple line should appear in the spectrum (the pivot point). Click and hold **in the purple window** with your **left mouse button**. Drag the mouse up and down until the peaks are all symmetric and all-positive, or **in-phase**. This is called **0th-order phasing**.




4) Using 0th order phasing, distort the spectrum so that it looks bad again. Along the top, click on "Processing" and "Auto Phase Correction." The peaks should all once again become in-phase. This is sometimes, but not always, sufficient.

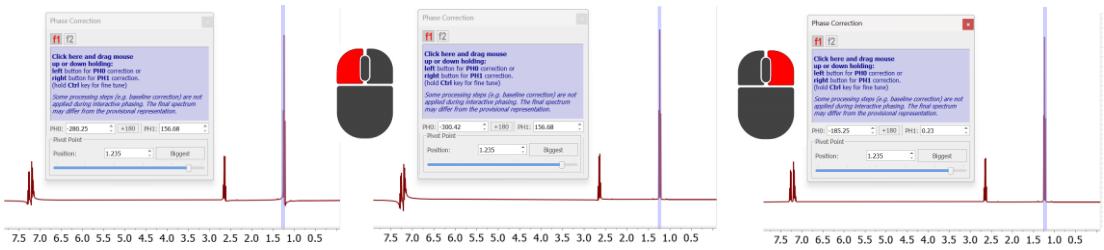



## Part 2: 1th order phasing

Sometimes the phase will vary linearly with chemical shift. In this case, you will need to adjust the 1st order phasing as well as the 0th order.

1) Drag and drop the folder titled **phasing_2** into the main MNova window. If it is not already open, open the phase correction window as you did for the previous spectrum.
2) Click and hold in the purple window with your left mouse button. Drag the mouse up and down until the peak **closest** to the vertical purple line (the pivot point) is symmetric and all-positive

(**0th-order phasing**). Next, click and hold in the purple window with you right mouse button. Drag the mouse up and down until the peak(s) **farthest** from the vertical purple line are **also** in-phase (**1st-order phasing**). All peaks in your spectrum should now be in-phase. If they are not, you may need to go back and forth between 0th and 1st-order phasing some more.



3) Drag and drop the folder titled **phasing_3** into the main MNova window. What happens to this spectrum when you click on "Auto Phase Correction?"

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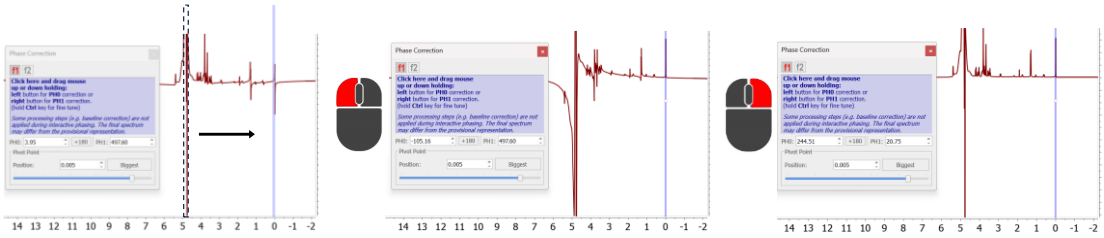
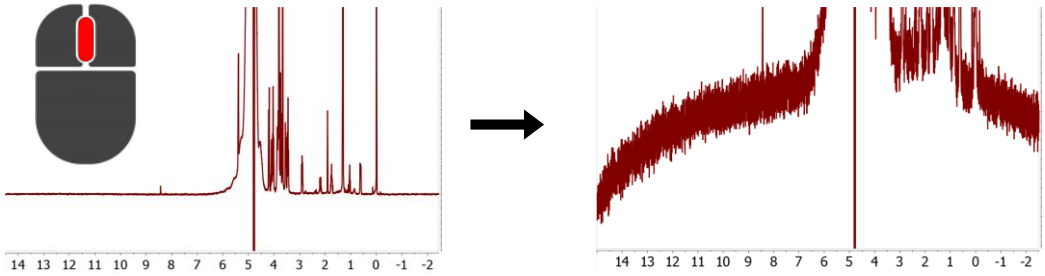
4) Use the **position slider** at the bottom of the Phase Correction window to move the purple line (pivot point) to the peak around 0 ppm. Next, manually adjust the 0th and 1st order phasing for this spectrum, **ignoring the appearance of the suppressed water peak at 4.78 ppm.** Keep going back and forth between 0th and 1st order phasing until all the (non-solvent) peaks are in-phase and the baseline is straight.



## Part 3: Baseline correction

**In order to obtain accurate integrals, (good) baseline subtraction is almost always crucial**. MNova does not perform automatic baseline subtractions, so you must make sure to always do this manually before integrating. Furthermore, you should verify that the baseline being subtracted is smooth and free from artificial bumps or dips. With the now-phased spectrum from **phasing_3** in the MNova window:

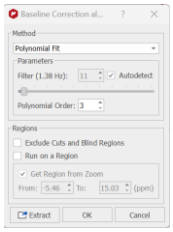
1) Use the middle wheel mouse button to increase the vertical scaling of the spectrum until the noise floor of the spectrum takes up an inch or so on your screen. You need to see the baseline to subtract it properly! You may need to use the hand icon:  under View to pull the spectrum back down into view.

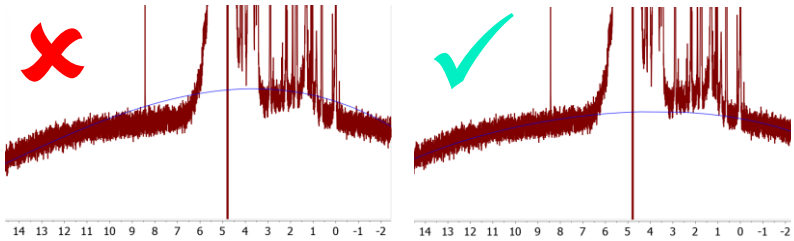



2) Press “b” or click on the red arrow on the bottom of the “Auto Baseline Correction” icon: 

and select “Baseline Correction…” Select different methods under “Method” and look at the **blue baseline** that has appeared along the bottom of the spectrum. Try adjusting the method-specific parameters for each.

**+ b**




3) A good baseline will be smooth and not contain any bumps. When subtracted, it should center the signal-free regions of your spectrum about 0 on the y axis without causing any dips in your spectrum below zero. (**NOTE:** you may find you need to touch up the phasing to get an acceptable baseline.) Select an appropriate baseline correction method and apply it by pressing “OK.” Which did you select?

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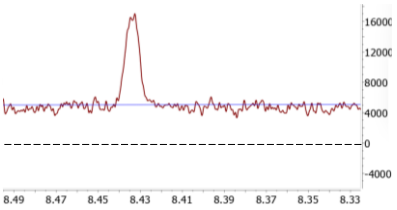
4) Integration (covered in part 7 below) in NMR describes measuring the area under peaks and relating it to the abundance of the nuclei giving rise to the peak. What would happen if you tried to integrate the small peak at 8.44 ppm before performing the baseline subtraction on the spectrum to the right? Would it be an over- or under-estimate of the true area?

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## Part 4: Referencing

Referencing is necessary for chemical shift precision, and must be performed manually. Referencing on an internal standard such as **TMS** (tetramethylsilane) or **DSS** (Sodium trimethylsilylpropanesulfonate, which unlike TMS is water soluble) is strongly recommended over referencing to solvent peaks such as CHCl3 or HOD. With the spectrum from **phasing_3** in the MNova window:

1) Use "View" + "Zoom in" to zoom in to the region between 3.5 and 3.9. What is the exact chemical shift of the singlet near the center of this range (about 3.7 ppm)?

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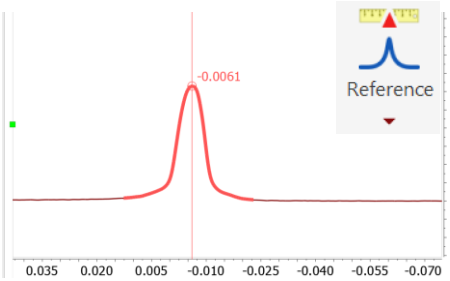
2) Use "View" + "Zoom in" to zoom in to the peak closest to 0 ppm. This is DSS.

3) Click on the "Analysis" tab along the top of the screen. Click the **Reference** icon:  Center the crosshairs on the peak (zoom in if necessary) and set it to exactly 0 ppm.

4) Recheck the range from 3.5 and 3.9. What is the chemical shift of the central singlet near 3.7 ppm now?

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Referencing on a solvent peak is an alternative to using an internal standard (table 1 from this reference can be used for this), but it is generally less accurate because solvent peak shifts are more variable with sample conditions (temperature, acidity, etc.).

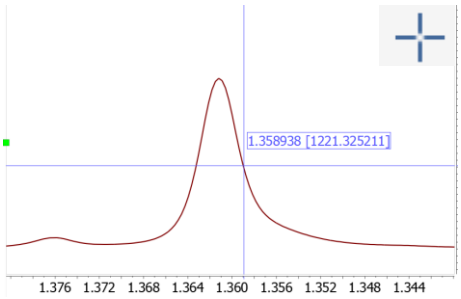
## Part 5: Apodization

The default window function used for the $^{1}H$ and $^{13}C$ spectra collected on our instruments is an exponential decay with line-broadening factors of 0.3 and 1.0 Hz, respectively. These are usually good settings, but they are not always appropriate when spectra have lineshapes that are not as narrow as can typically be achieved.

Load spectrum **apodization** into MNova and zoom into the region between 8.2 ppm and 11 ppm. Do you see any clear peaks in this range? _____

An optimal signal-to-noise ratio (SNR) is achieved when the line-broadening is **matched** to the natural linewidth of a spectrum. Measure the full width at half height (FWHH) of the peak at ~1.36 ppm and reprocess the data by doing the following:

1) Perform a baseline correction using what you learned in part 3.
2) Use "View" + "Zoom in" to zoom in to the region between 1.34 and 1.38 ppm. Scroll up with the mouse wheel until the peak comes close to the top of the screen.
3) Click the crosshair icon: or press "c" to select the crosshairs tool. Click the peak on its left side exactly where it is half as high as it is in the middle (its "half-height"). Drag the crosshairs over to the corresponding right side half-height. Note the value of $\Delta$f1 (in Hz) that appears on the screen when you do this. This known as the full width at half height FWHH. What is the FWHH?

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4) Next, inspect the current processing parameters by clicking on "Processing" + "Apodization." You should see the "Exponential" function selected with a line-broadening factor of 0 Hz.
5) Input the natural linewidth as the exponential line-broadening factor in place of 0 Hz. Press OK and zoom into the region between 8.2 ppm and 11 ppm again. Is a peak visible now? If so, what is its chemical shift?

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## Part 6: Dual display

When comparing two samples, it can be very useful to view their NMR spectra simultaneously. This is done in MNova by the "Superimpose" and "Stack" functions. Try these out by doing the following:

1) Load in spectra **dualdisplay_1** and **dualdisplay_2** into MNova. With **Ctrl+click**, simultaneously select **dualdisplay_1** and **dualdisplay_2** in the "Pages" window (note: if you don't see the Pages window, you can click on "View" along the top of the screen and check the "Pages" box in the resulting toolbar.) When both spectra are simultaneously selected, a new "Stacked" toolbar option appears along the top of the screen. Click on this. Click on "Stack Items" in the resulting toolbar.

2) Adjust the relative heights of the spectra so they can be compared. Click on "Stacked" (again) and the "Select" drop down: 

and pick "Select Graphically." Your cursor should become a push-pin like the Select icon. Click on the smaller of your two spectra and click on "Multiply" until the two spectra are close to the same height. **NOTE:** you will probably need to increase the scale of multiplication/division with the drop-down menu from the small arrow at the bottom of the icons.

3) Explore the different ways of viewing multiple spectra by clicking on Mode: 

Make sure to try "Superimposed" which is especially useful for spotting small differences (it is harder to select individual spectra in this mode, but it can be done with the "Stacked Items Table.")

4) These are $^{13}C$ spectra of the same compound with $^{1}H$ decoupling either on or off. What are the principal differences between these two spectra?

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## Part 7: Making Assignments

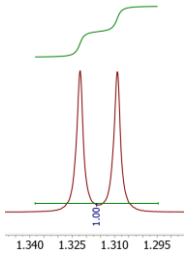
Standard $^{1}H$ spectra can often be considered "semi-quantitative" as the areas under $^{1}H$ peaks are usually more or less proportional to the number of nuclei giving rise to them. The default $^{1}H$ spectrum on our systems (PROTON) uses a 30-degree pulse and an inter-pulse delay of about 5 s to limit differential relaxation effects to achieve this. It is therefore very helpful to integrate the peaks of any new $^{1}H$ NMR spectrum. This can be critical to assignment of signals and help confirm or refute your expected chemical structure.

1) Load spectrum **Threonine** into MNova. This is the $^{1}H$ spectrum of threonine in $H_2O/D_2O$.

2) Use “View” + “Zoom in” to zoom in to the aliphatics region between 1 - 5 ppm. Notice that the peaks are not single peaks but rather have two or more components. These are called **multiplets** and are common in 1H NMR spectra. A multiplet can be described as a: singlet (s), doublet (d), triplet (t), quartet (q)…, or just multiplet (m), referring to groups of 1, 2, 3, etc. peaks.

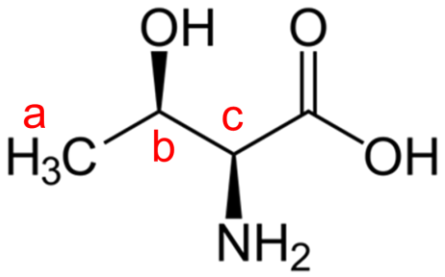
3) Phase and baseline correct this dataset for optimal integration accuracy.

4) Click on the “Analysis” tab along the top of the screen. Click the **Manual Integrate** icon:  Integrate the multiplets (at ~1.3, ~3.6, and ~4.2 ppm) in the display region by dragging over them, starting and ending right where the base of the peaks meet the baseline like so:




2) The peak at 1.31 ppm is a methyl group with three protons. Right click the integral value under the peak and select “Edit Integral.” Enter 3.0 next to “Normalized” and close the Integral Manager window.

Zoom out again and inspect your integral values, relating them to the structure of threonine:




The “n+1” rule states that a proton with n equivalent neighbors will be split into a multiplet with n+1 components, with intensities that follow Pascal’s Triangle:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| s | | | 1 | | | | |
| d | | | 1 | 1 | | | |
| t | | | 1 | 2 | 1 | | |
| q | | | 1 | 3 | 3 | 1 | |
| quintet | | 1 | 4 | 6 | 4 | 1 | |
| sextet | | 1 | 5 | 10 | 10 | 5 | 1 |
| heptet | 1 | 6 | 15 | 20 | 15 | 6 | 1 |
| octet | 1 | 7 | 21 | 35 | 35 | 21 | 7 | 1 |

Note: For this example, only protons attached to carbons should be considered. This is because the sample is in $H_2O/D_2O$ and the hydroxyl and amine protons are rapidly exchanging with solvent.

3) Fill in the table below, identifying the integration, # protons, multiplicity, and assignment for each multiplet. For multiplicity, you may simplify the more complicated mutiplet at 4.2 ppm as simply a s, d, t, q, etc. if you prefer.

| Peak Position (ppm) | Integration | # protons | Multiplicity (s, d, t, etc.) | assignment (a, b, or c) |
|---|---|---|---|---|
| 1.3 | | | | |
| 3.6 | | | | |
| 4.2 | | | | |

5) For high-sensitivity, well-resolved spectra, MNova is able to do a lot of analysis (peak picking, multiplet analysis, integration) automatically for you. Delete your integrals with the "Delete All" icon under Integrals:  Click on "Auto Multiplet Analysis" under Multiplets: 

Are there any differences between your analysis and Mnova's? If so, what are they?

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## Final Steps

After completing this, save your .mnova file in your /ProcExer/Completed/(UC6+2) directory. Email your answers to the questions to the NMR facility manager or bring them to your first training session. After both this exercise and your first training session are complete, you will be granted key access to the NMR facility.

**NOTE:** There are additional resources on the NMR facility website. Please take advantage of the resources available on the MNova website as well!