

1. Introduction to 1-D NMR Data Processing with MestReNova

The MestReNova program can do all of the routine NMR data processing needed for Chem 201 and 202 and will be available through the Reed downloads site or on the computers in Chem 203. This manual covers basic (“1-D”) data processing for ^1H NMR and guides you through a typical operating session.

2. What does an NMR spectrum look like?

The following spectrum is typical (Fig. 1).

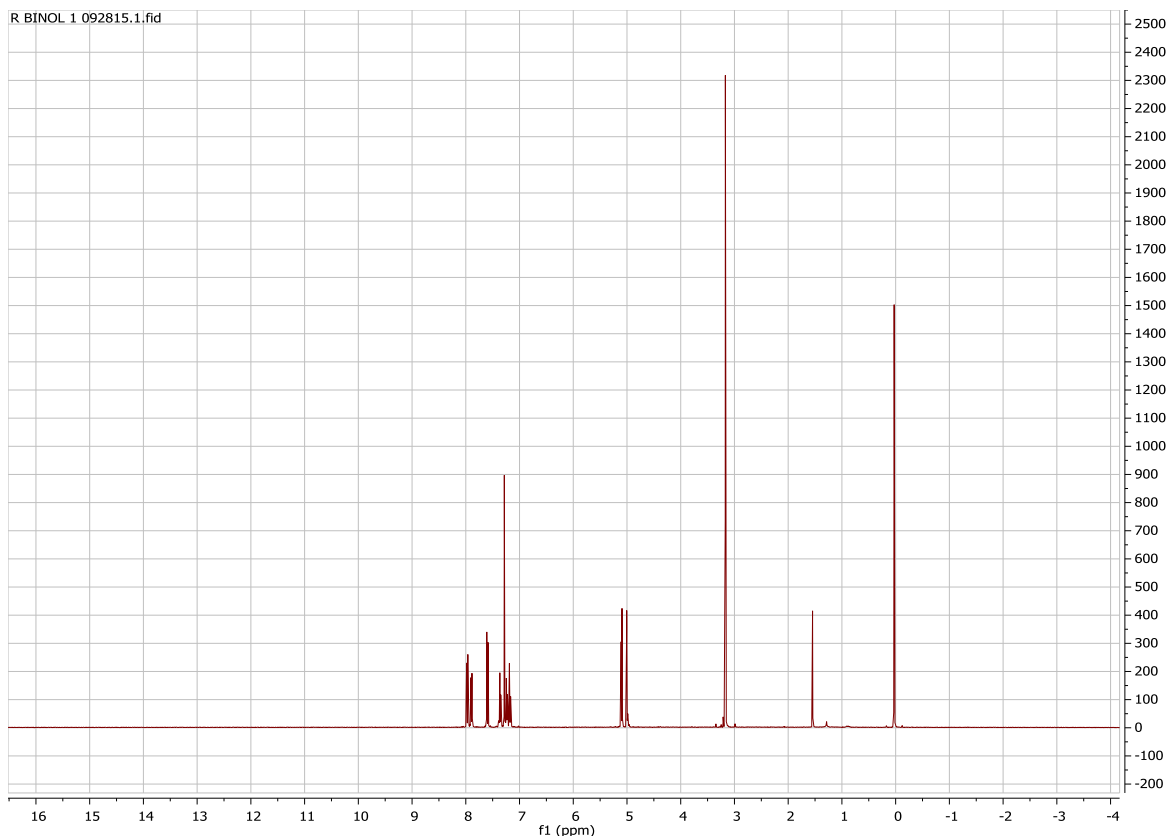


Figure 1

It contains several groups of signals separated by stretches of horizontal baseline. A chemist can extract information about molecular structure by noticing where signals appear, and also by noticing where they don't. Because 'absence of signal' is just as important as 'signal', it is essential to record and print a “full” spectrum, one that runs from -0.5 to about 10 ppm.

Another typical thing: the spectrum is extremely hard to read and not useful in its present format. We could correct this by (Fig. 2):

- printing the spectrum on a larger piece of paper
- zooming in on, or “expanding” selected regions of the spectrum.

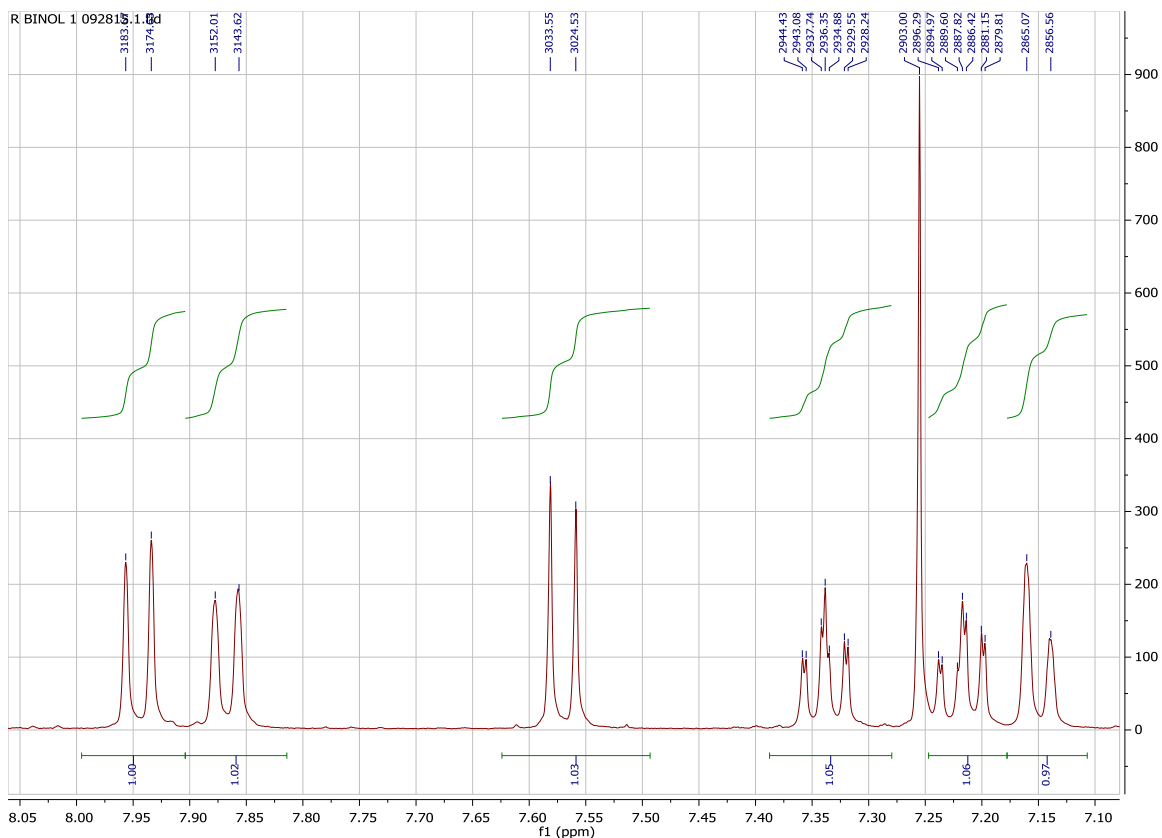


Figure 2



A complete “expansion” might show the shape of the signals, their chemical shifts (in ppm, scale shown on x-axis), integrals (rising green curves, blue numerical values below each set of peaks), and the exact frequency of each peak (in Hz, blue values above peaks). This manual shows you how to print a “full” spectrum and then how to create expansions like this one. To do this, you will need to:

- download and process your data to make a spectrum
- control which part of the spectrum is displayed (full vs. expansion)
- analyze the spectrum by integrating and “peak picking” (getting frequencies of individual peaks)
- print the spectrum

The description of each set of operations starts on a separate page so you can quickly find a desired set of instructions by flipping pages. The first time, however, you should go through the full menu without skipping any pages.

3. Downloading and processing your data

Your data is stored on a computer attached to the NMR spectrophotometer. It is stored in a read-only folder and you can download this folder as many times as you like. In the unlikely event that data processing makes a mess of your data, simply download a fresh copy of this folder.

1. Click the folder icon  (lower left corner of screen) to open the Libraries display
2. Click in the top bar where it says Libraries and replace this text by typing `\\nmr\data`
 - A (sometimes slower) alternative is to:
 - a. click Network on the left side of the display
 - b. Double-click NMR in the right side of the display
 - c. Double-click data in the right side of the display
3. Navigate to the folder containing your NMR data (usually Chem201labday).
4. Plow down into this folder until you find a folder labeled EXP.DAY.INI and drag this entire folder to the Desktop or to My Documents.
5. Close the NMR/data explorer window.
6. Double-click the desktop icon for MestReNova  or open from the programs in the start menu.
7. Drag your entire NMR data folder into the MestReNova window to load your data (your spectrum will be set up similar to Figure 1). An alternative way to open your data:
 - a. Under File, choose Open (Or Ctrl+O)
 - b. Select the fid file from your saved NMR data folder (NMR folder → 1 → fid)

Note: FID refers to “free induction decay” which is a signal vs. time spectrum and this is how your raw data looks when it is transferred from the NMR (Fig. 3). Mnova automatically processes your data and applies Fourier transformation (FT), which changes your data to a signal vs. frequency (chemical shift) spectrum.

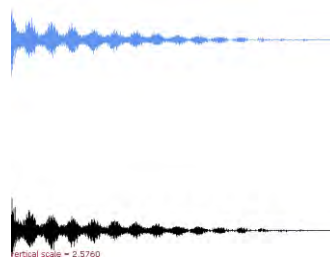


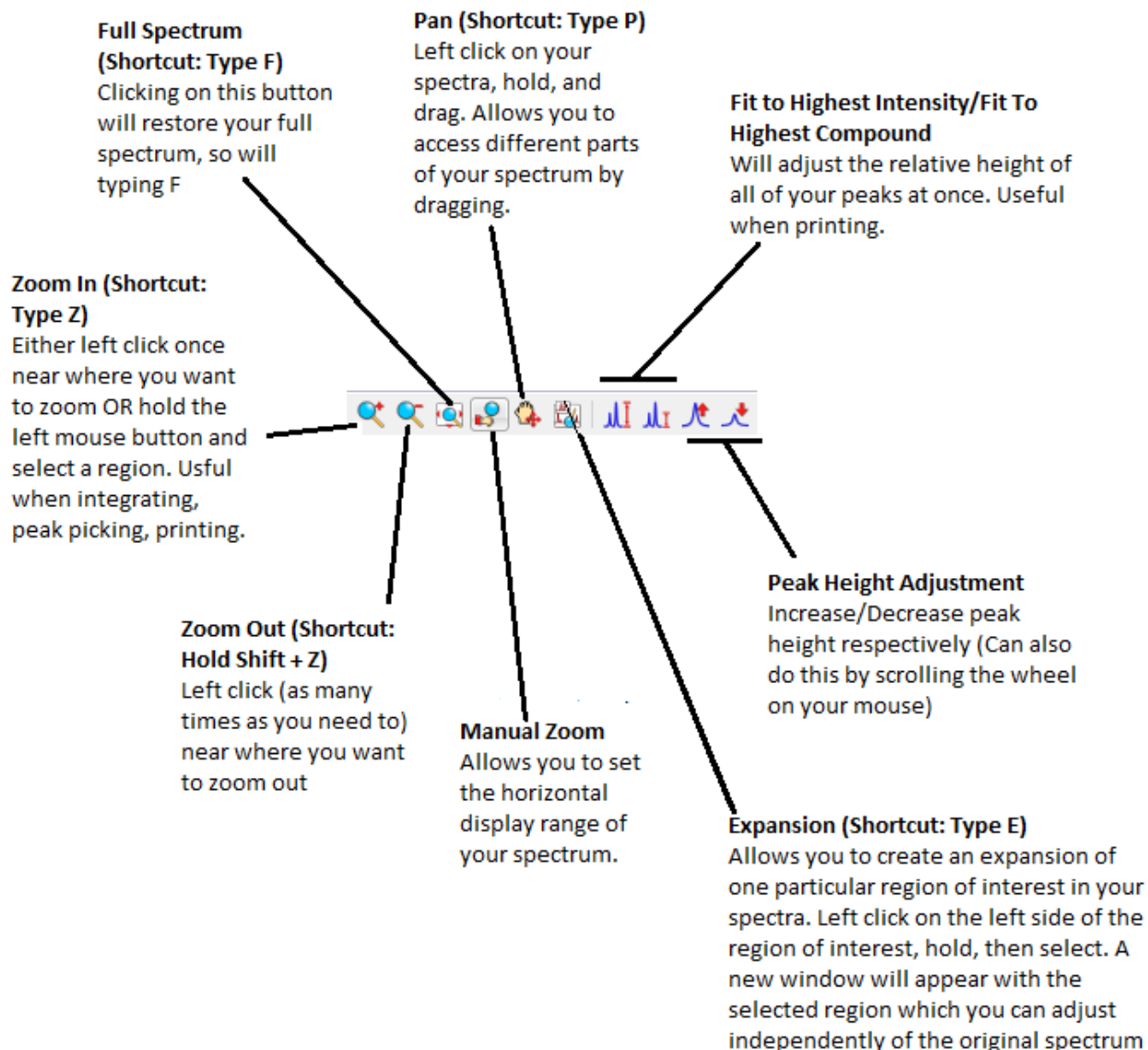
Figure 3

To obtain a copy of MestReNova for your own computer. In any web browser, go to **downloads.reed.edu**.

- a. From the menu on the left, select “Chemistry courseware”
- b. Click on Mnova
- c. Download the Mnova license
- d. Open MestReNova via the start menu under “programs”
- e. When prompted for the license file, select “install”; the license file will be called “nmr.lic” and will be found in the downloads folder
- f. Restart Mnova


4. Controlling the display

What you see in your display can be controlled by a variety of vertical and horizontal adjustments. Try each of the adjustment tools listed below. Once you have tried them, restore the full spectrum.



5. Phasing & Baseline Correction

A baseline correction is necessary when the baseline of the full spectrum is not flat and/or is offset from zero (**Shortcut: Type B**) which can affect the precision of the signal integration.


To apply an automatic baseline correction, click on the Baseline Correction icon 

The phasing adjustment affects the rise and fall of the baseline on each side of your signal. MestReNova attempts to make this adjustment for you *automatically* when you initially process your data, but you may need to make additional adjustments. The following diagram (Fig. 4) shows the same set of signals with “bad” and “good” phasing. Notice the difference in the rise and fall of the baselines.




Figure 4

Inspect the baseline around each of the signals in your spectrum. If the baseline around *all* of the peaks looks acceptable, proceed to **6. Calibrating** on the next page. Otherwise, additional phasing is required.

1. Click the Phasing icon  to adjust your spectrum
2. Select “Automatic Phasing” from the drop down menu
3. If your spectrum is still out of phase following this, select the “Manual Phasing” option from the menu
4. Follow the window instructions to adjust **ph0** and **ph1** (“zeroth-order” and “first-order” phase controls, respectively) and then close the window. These sliders have very different effects.
 - a. **ph0** – These sliders affect the entire spectrum in the same way. Adjust them so that the baseline around the *tallest* signal looks acceptable (a thick colored vertical bar underneath the spectrum marks the location of the *tallest* signal).
 - b. **ph1** – These sliders have little or no effect near the *tallest* signal, but their effect grows with increasing distance from this signal. Adjust them so that the baseline looks acceptable around signals that are *far* from the tallest signal.

6. Calibrating

Chemists use the signal provided by TMS (tetramethylsilane) to calibrate the chemical shift (horizontal) scale of an NMR spectrum. The TMS signal usually appears further right (upfield) than any other signal in the spectrum. The chemical shift of the TMS signal is arbitrarily set to 0.0 ppm (parts per million).

1. Zoom in on the TMS signal. You may need to do this a couple of times so that the signal looks quite broad (Fig. 5)
2. Click on the Calibration Tool icon  in the MestReNova menu (**Shortcut: Type L**).
3. Move your mouse cursor over the peak, and you should see a red line appear at the center of the peak, left click.
4. A box will appear titled "Reference along f1" (Fig. 6)
5. Either manually enter 0 for TMS or alternatively click on the "Solvents>>" button in the lower right of the box and select TMS from the list.
6. Restore the full spectrum display (**Type F**).

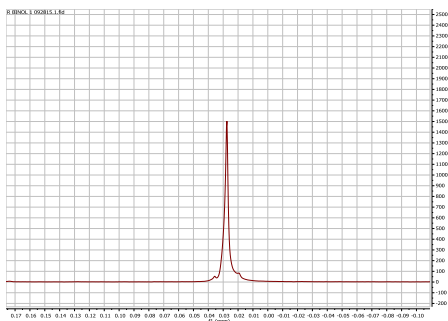


Figure 5

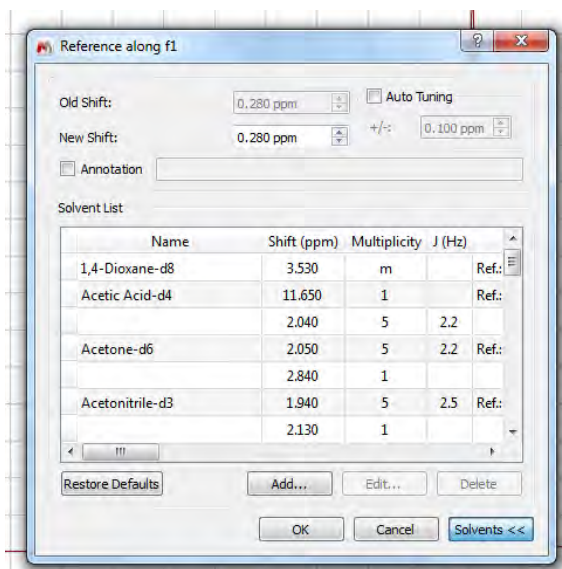





Figure 6

7. Printing a full spectrum


A proton NMR spectrum will contain signals from every hydrogen-containing compound in your sample. A full spectrum, one that displays chemical shifts (horizontal scale) from -0.5 and 10 ppm, will display all of these signals, both the signals that you think your compound has produced and also the signals produced by contaminants.

Organic chemists normally expect to “interpret” every signal in an NMR spectrum. Conversely, they expect to find a signal for every hydrogen nucleus in their sample. The assignment of signals to nuclei, and of nuclei to signals, means finding a satisfactory interpretation for every signal’s chemical shift, integration, and coupling pattern. A full spectrum reveals all of the signals and allows an unbiased observer to consider alternative interpretations of the NMR data.

Printing note: Although there is a printer in the Chemistry computer lab, it is strongly recommended that you print your spectra as PDF files, save these files in a secure location, and then print these files on another printer or copy the image directly into your lab report.

1. Click the Manual Zoom icon  to adjust horizontal display. Set the range from: -0.5 to 10 ppm.
2. Adjust the vertical scale so that tallest signal fits on screen using the mouse wheel or by clicking on the Fit To Highest Intensity icon .
3. Select “Edit” then “Edit Page Title...” and enter a descriptive title. Useful title components include: sample, NMR solvent, your name, date, and **EXP.INI.DAY** folder label. Click “OK” to close window.
4. Click on the Export to PDF icon . You will be asked to provide a name for your PDF file and a location where you want it saved.
5. Find your PDF file and open it. Inspect the *horizontal* scale (-0.5 to 10 ppm?). Inspect the *vertical* scale (Peaks large enough? Not too large?). Inspect the *paper alignment* (Landscape mode?). If everything seems satisfactory, go to the next page. Otherwise, make corrections and print your spectrum again.

Printing in the Chemistry computer lab. It is, in fact, possible to print on the printer in the computer lab and this could save you time in an emergency. That said, your instructors are not responsible for maintaining this printer and it seems to work wonderfully for long stretches and then behave very poorly for equally long stretches. Caveat printer. The time and money you waste on this printer is *your own*. If you want to try it, here’s what you must do:

1. Log in to the printer (printer name: **ChemLab**).
2. Open your PDF file and print it using the **ChemLab** printer (keep your fingers crossed) or select the Print icon  in MestReNova.
3. Don’t forget to *log out* when you are finished.

8. Integrating


Integrating the spectrum means finding the *area* underneath the peaks that interest you. This peak area, or *integral*, is proportional to the number of nuclei that create these signals. Therefore, an integral is useful only in comparison to another integral. Furthermore, only the *relative* integral size is meaningful; two integrals with values of 1 and 4 mean exactly the same thing as two integrals with values of 0.2 and 0.8. Remember, only integrate the signals that interest you. *Do not integrate contaminants* like TMS or the signals produced by solvent(s).

Reed College NMR Spectrometer disclaimer: The NMR spectra for Chem 201 & 202 are gathered in a way that can easily distort the values of integrals, making them artificially too large or too small (peak size distortion is an artifact of our "fast scan" procedure). These distortions need not affect all of the integrals in the same way and distortions of 10-20% are routine.

Integration involves three steps:

1. Proper phasing. We will assume this has been done already.
2. Selecting integration regions. Generally, a region should contain all of the signals produced by *one* type of hydrogen.
3. Calibrating the value of one integral (optional).

The detailed instructions, beginning with "selecting integration regions," go like this:

1. Try to parse your spectrum by identifying signals that look like they come from a single type of hydrogen. Pay attention only to those signals that might be produced by your compound. Ignore contaminants like TMS, CHCl_3 , and H_2O .
2. Zoom into the peaks of interest
3. Click on the integration tool icon  in the MestReNova menu (**Shortcut: Type I**).
4. Click the baseline on the left and right sides of the signal pattern to select it (Fig. 7). This will produce an integration curve and the value of the integral underneath this curve will appear below the signal.
5. Move the spectrum horizontally to the left until you find the next pattern and repeat step #4.
6. When all of the integrals have been marked, calibrate one integral by selecting it, then left clicking, and selecting "Edit Integral" from the menu that appears.
7. A box will appear titled "Integral Manager" (Fig. 8)

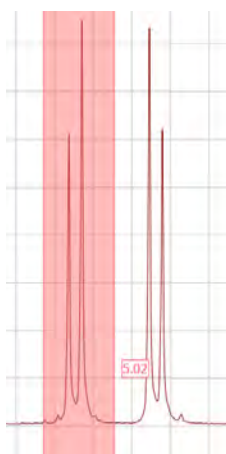


Figure 7

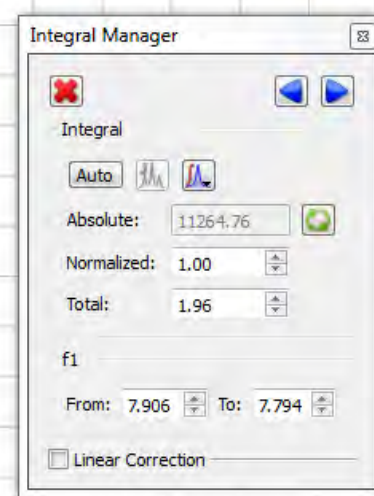


Figure 8

- Type the value you would like to assign to the integral in the “Normalized” slot, close the Integral Manager window. You should notice that the values of all the other integrals are reset, not just the integral you selected. Remember, all that counts with integrals are their relative values. Values of 0.634 and 1.902 have exactly the same meaning as values of 1.000 and 3.000. That said, the latter are easier to use.
- To adjust the green integration curve (optional), hover your mouse cursor over the curve, a red box should appear selecting it, then use the green boxes to adjust vertical height of the curve. To adjust positioning above the peak, left click, hold and drag the box (Fig. 9).
- Restore the full spectrum display (**Type F**).

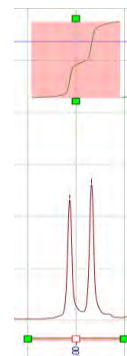
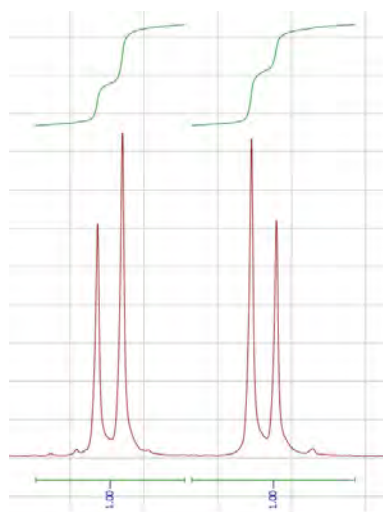


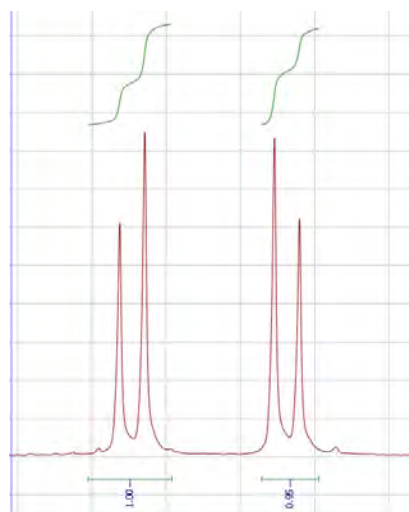
Figure 9

Note: The most common student mistake is to set the edges of the integral on the sloping curve of the signal instead of the flat baseline (Fig. 10). Notice that the bad integral starts and stops on the signal, not on the baseline. The good integral runs from baseline to baseline. Not only that, it encompasses enough baseline on each side of the signal so that the “flat” character of the baseline can be verified. If your integral is too narrow, delete it by moving your cursor over either the green integration curve above the peak or over the integration value (it will become highlighted), then left click and select ‘Delete Integral’ from the menu. If you want to delete all the integrals select ‘Delete All’.

Figure 10



Good Integrals –
Regions run baseline to baseline



Bad Integrals –
Integral regions are too narrow

9. Peak picking

The most reliable way to obtain coupling constants (J values) is to measure the distance between signals (“peaks”) in Hz. Peak picking prints the frequencies of selected peaks above those peaks. The tops of the peaks are marked with small vertical lines. Peak picking is applied only to the peaks that 1) are currently visible in the window, and 2) rise above a threshold value so peak picking requires the following steps:

1. To display the peak labels in Hertz, under “Edit” select “Properties...”
2. A box will appear (Fig. 11).
3. On the left hand side under the “NMR Spectrum” tab select “Peaks”.
4. Then on the right hand side, where it says “Units:” select Hz from the dropdown menu.
5. Click “Apply” then “Ok” to close the window.
6. On your spectrum bring the signals of interest (and *only* these signals) into the window.
7. Click on the manual threshold peak picking

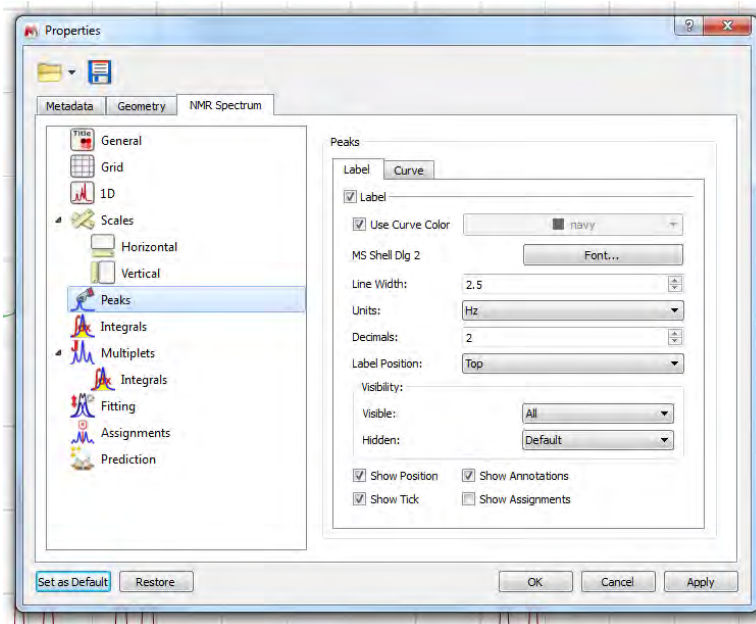





Figure 11

tool icon  in the MestReNova menu (**Shortcut: Type K**).

8. Left click to the side of the peaks you want to pick, hold the mouse button, and then drag the box over the tops of all the peaks (the box you create is the “threshold” and peaks below this box will not be selected).
9. Repeat steps 6-8 for different signals of interest. To delete a single peak, move your cursor over the peak label to select it (it will turn red), then left click, and select “Delete Peak” from the menu that appears. To delete all the peaks, click on the downward triangle on the peak picking button, and select “Delete All” from the dropdown menu.

10. Printing expansions

Once you load up a spectrum with integrals and peak picks, you will want to print your data. Unfortunately, a full spectrum will cram all of this information into a small space rendering it unreadable in most cases (see pages 2-3 above). A better alternative is to expand regions of interest so that the integrals and peak picks in these regions can be read clearly.

1. Zoom in on the chemical shift region of interest (**Type Z, then highlight the region**).
2. Adjust the vertical scale so that tallest signal fits on screen using the mouse wheel or by clicking on the Fit To Highest Intensity icon .
3. Select "Edit" then "Edit Page Title..." and enter a descriptive title. Useful title components include: sample, NMR solvent, your name, date, and **EXP.INI.DAY** folder label. Click "OK" to close window.
4. Click on the Export to PDF icon . You will be asked to provide a name for your PDF file and a location where you want it saved.
5. Find your PDF file and open it. Inspect the *horizontal* scale (The region you intended?). Inspect the *vertical* scale (Peaks large enough? Integral and peak pick data easy to read?). Inspect the *paper alignment* (Landscape mode?). If everything seems satisfactory, expand and print another region by repeating steps 1-5.