

Processing data with Bruker TopSpin

This exercise has three parts: a 1D ^1H spectrum to baseline correct, integrate, peak-pick, and plot; a 2D spectrum to plot with a ^1H spectrum as a projection; and three ^1H spectra to compare and plot against the same axis.

Some background information

1. In the Magnetic Resonance Facility, we have both Varian and Bruker spectrometers. Varian was purchased by Agilent in 2010 and the NMR business was shut down in 2014. Thus, you will hear reference to both Agilent and Varian NMR – for our purposes, they mean the same thing
2. Varian and Bruker hardware and software are each different from each other
3. TopSpin is published by Bruker and is used both to run the spectrometers (as a back end to IconNMR, the front end that we use) and to process data
4. Varian data must be converted to Bruker format before TopSpin can view it
5. TopSpin is very powerful for manipulating data exactly as desired, but it is not a WYSIWYG program and so processing and plotting are separate operations
6. It can be very helpful to type commands in TopSpin. This is done by entering the commands in the command line: for example, efp means process a 1D spectrum by applying exponential line broadening, a Fourier transform, and phasing.

efp	Nicotinamide_Liquid_DMSO 1 1	D:\admin\Downloads\NMRData	abs: finished
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7. Changes made to data processing are stored automatically, but changes made to plot layouts are not.

Preparation: download, unzip and/or convert, and save data

1. Think a little bit about where you would like to save your data.

The Bruker data you receive from the spectrometer is fairly easy to move around after you've downloaded it, because you can take the entire zip file, drag and drop it into TopSpin, and choose where you would like to unzip it to. Still, to be consistent with your Varian data, I recommend that you choose one of the two options below.

Varian data is a little more complicated. It must be converted to Bruker data in order to use it in TopSpin. During this process, you may choose where you would like to save the converted data. However, if you would like to keep the converted data with the original Varian data, I recommend that you use one of the two options below for your zipped data.

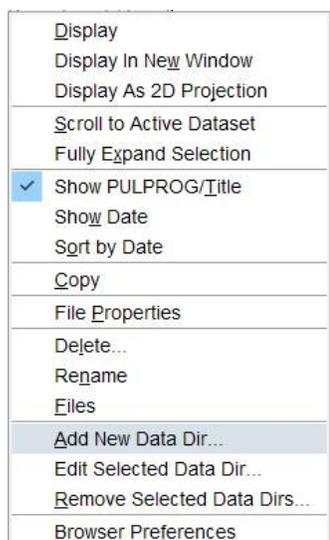
Option 1. Good if you don't want to classify your data into very many levels. Make a directory called (for example) NMRData within your Downloads folder. Save all your zipped data there. In TopSpin, in the data browser (left panel), right-click and choose Add New Data Dir... and enter the name of this directory (eg

c:\Users\Robin\Downloads\NMRData or /Users/Downloads/NMRData). You can do this with as many directories as you want.

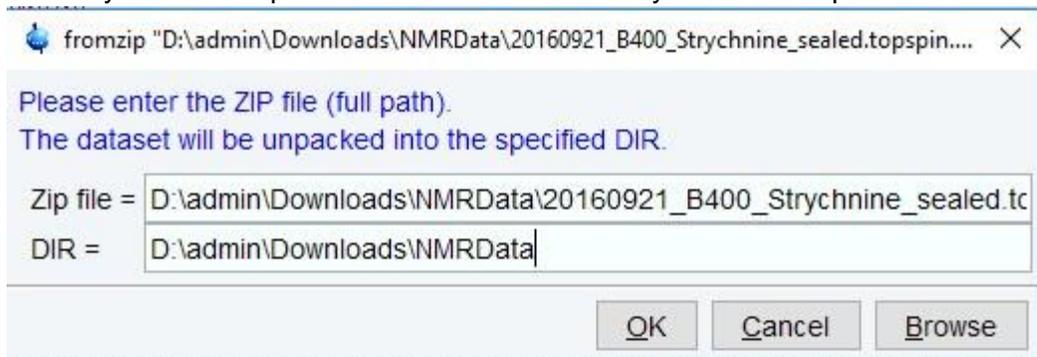
Option 2. Good if you want to classify your data into many levels.

- Make a directory called data within your Downloads folder.
- Go into the data directory.
- Make a directory called (for example) Project1. Now go into the directory called Project1.
- Make a subdirectory called nmr.
- Save your zipped data to Downloads/data/Project1/nmr.
- In TopSpin, in the data browser (left panel), right-click and choose Add New Data Dir... and enter the first part name of this directory (eg c:\Users\Robin\Downloads\ or /Users/Downloads/).
- Click on the + sign to the left of this directory to see the Project 1 part appear. You can create as many directories of the form data/<projectname>/nmr as you want, and you don't have to add them individually to TopSpin.

Notes. You don't have to use the Downloads directory; you can use any directory you like. Also, you can mix and match the two forms; for example, you can use directories c:\users\robin\downloads\FirstYearWork\data\Project1\nmr, c:\users\robin\downloads\FirstYearWork\data\Project2\nmr, c:\users\robin\downloads\SecondYearWork\data\Project1\nmr, and c:\users\robin\downloads\SecondYearWork\data\Project2\nmr. In TopSpin, add both c:\users\robin\downloads\FirstYearWork and c:\users\robin\downloads\SecondYearWork to the databrowser.

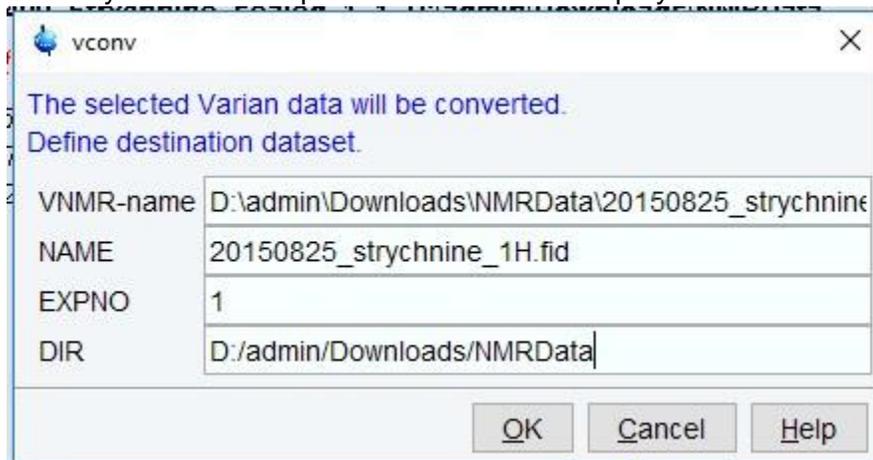


- Download the seven files linked to from <https://mcgill.ca/mc2/using-mc2/workshops/workshop-information>. If you use **Option 1**, save the files to Downloads\NMRData. If you use **Option 2**, save the files to Downloads\data\Project1\nmr.
- Unzip the Varian data (the Varian data ends with .fid.zip, rather than .topspin.zip – you may only see .fid or .topspin in the list of files in your File Browser). Don't add any extra directory levels while unzipping. I recommending using the program [7-zip](#) and the option Extract here.
- To view the Bruker data (data whose name ends in .topspin.zip (or .topspin)): drag and drop it from a File Browser into the middle TopSpin window. You will be prompted for the directory DIR to unzip it to. Use the same directory where the zip file was located.



If you used **Option 2**, your DIR should include /data/Project1/nmr

- To view the Varian data (data whose name ends in .fid.zip), type `vconv`. Browse to the zip file and click VNMR data conversion. When prompted, enter an EXPNO of 1 and the same DIR as you used in step 1. This should show up by default.

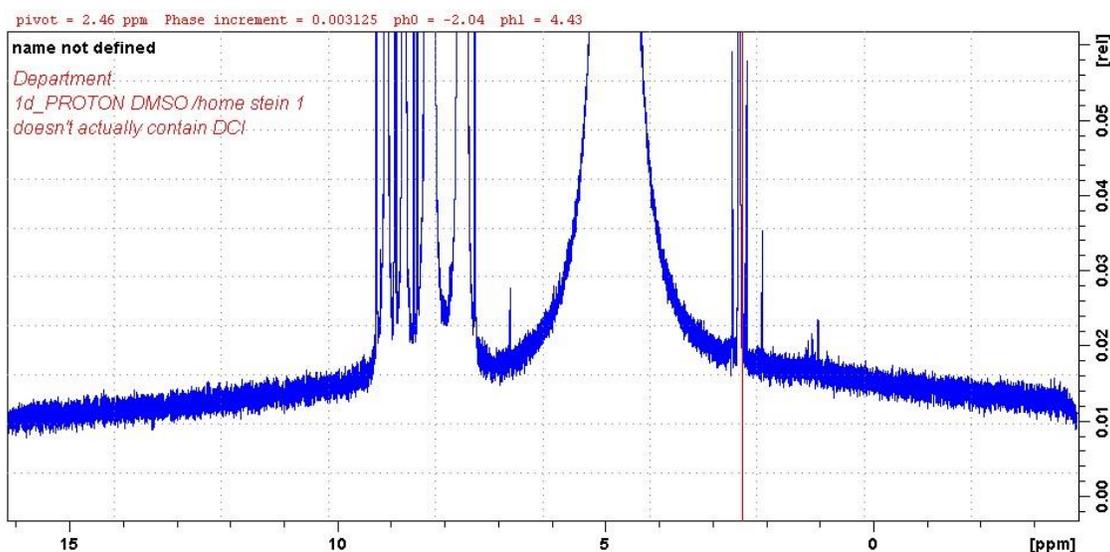


- A little customization goes a long way in TopSpin. Go to the settings menu (icon in upper right of screen) and enable the first option, "Auto-open last used dataset when restarting TopSpin."

Exercise 1. Processing and plotting a 1D dataset.

- View the dataset Nicotinamide_Liquid_DMSO/1 by dragging and dropping it from the left onto the spectrum window. If it isn't displayed there, find it in a Windows Explorer or Mac file browser window and drag and drop it into TopSpin, as above. The data should be processed automatically.
- Play with the spectrum viewing a little bit: click and drag to zoom in, double-click to view the whole spectrum, use the mouse wheel or touchpad gestures to scroll up and down.

3. Play with the icons on top to change the view and the axes. You have to click and hold on some of the icons, such as the scrolling icons, to make them take effect. One very useful icon is the previous view icon ().
4. Phase the spectrum. Phasing is the process of adjusting the shape of the peaks so that they all have an absorptive, positive lineshape.
 - a. Type `efp` to process and be sure that no baseline correction has been automatically applied. It is best to baseline correct after phasing.
 - b. Scale up vertically using the mouse wheel or touchpad gestures or the `*2` icon
 - c. Note that the peak at 2.5 ppm (the DMSO peak) is distorted – it dips down more on the right than on the left. If this were a peak you wanted to integrate, its integral would be incorrect.
 - d. Try typing `apk0` (0th order automatic phasing), `apk` (0th and 1st order automatic phasing), `apkm` (alternate algorithm for automatic phasing), or `apks` (another algorithm for automatic phasing, often good for ¹⁹F spectra)
 - e. If none of these work, you can phase manually – click on Process (at the top) and Adjust Phase.
 - i. Left-click on the DMSO peak (because it is on the edge of the spectrum), right-click, and choose Set Pivot Point. The red line should move onto the DMSO peak
 - ii. Click and hold on the 0 icon at the top of the window. Move the mouse forward and backward until the base of the peak is described by a smooth line from one side of the baseline to the other. Only worry about the DMSO peak, even though 0th order phasing affects all signals equally.
 - iii. Click and hold on the 1 icon at the top of the window to adjust the other peaks. 1st order phasing affects the signals furthest from the pivot point most.
 - iv. When you are done, you should see a baseline that rises in the middle of the spectrum but which doesn't change suddenly anywhere
 - v. Click on the floppy disk icon with the arrow () to save and return.

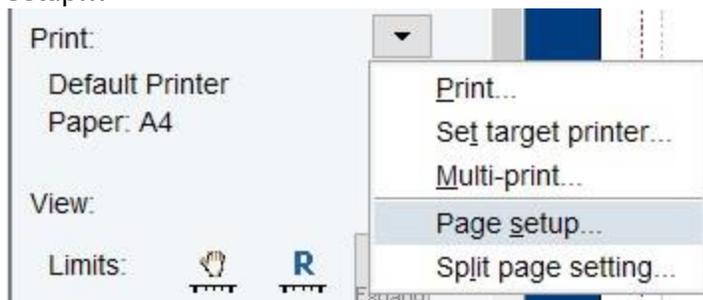


- f. Baseline correction. Baseline correction is the process of bringing the baseline to zero.

- i. Very frequently, the command `abs n` will correct the baseline well, especially with Bruker data. This applies a polynomial baseline correction. The order of the polynomial can be set to a number between 1 and 5 by changing the parameter `absg`.
 - ii. Other algorithms, including cubic spline fits, are available under the Process / Baseline (menu) / Adjust spectra baseline manually command, but they will not be described here. They may be useful for Varian data, in particular.
5. Calibrate the spectrum. By default, the spectrum is calibrated to the solvent, but sometimes it gets adjusted to TMS – or to vacuum grease! To reset it to the solvent, type `sr` and set the spectral reference parameter `sr` to 0. To reference to another signal, zoom in on it, then click on Process / Calib. Axis, click on the peak, and set its chemical shift.
6. Peak pick the spectrum.
 - a. Type `ppf` to peak pick the full spectrum automatically. This usually does a reasonable job. Sometimes it picks too low into the baseline. In this case, type `mi` and change the minimum intensity to a number greater than 0 – use the scale on the right to gauge. Hit enter and then do `ppf` again.
 - b. Another way to peak pick is graphically. Before doing graphical peak picking, double-click on the spectrum to see the whole thing, then zoom in around the spectrum by clicking and dragging over it. Go to the Analyze / Pick Peaks button. Now you have several options, using the icons that appear in the window. The main one is the second from the left (the one next to the yellow icon). When this icon () is highlighted, clicking and dragging to draw a box over the tops of the peaks will pick them. This makes it easy to pick some intense and some less intense signals. The other icons allow you to select individual peaks and specify ranges, or to delete ranges. Play around a bit. When you are satisfied, use the floppy disk icon () to save and return.
7. Integrate the spectrum,
 - a. The command `abs` does an automatic integration, and it also corrects the baseline. (The command `abs n` does the baseline correction without the automatic integration.)
 - b. To integrate graphically and/or to change the integrals generated automatically, go to Analyze / Integrate. Now you have several options, using the icons that appear in the window. Some of the especially useful ones are , which selects a region for integration, and , which cuts an integral in two. There is also an undo icon. The  icon is good for sharp signals on top of broad ones – it excludes the area between the ends of the integral and below the integral line. Try it.
 - c. Calibrate integrals by right-clicking over one of them and setting the number of protons it corresponds to.
 - d. When you are satisfied, use the floppy disk icon () to save and return.
8. Structure and multiplet analysis
 - a. Go to the tab in TopSpin that says Structure. Use the file folder open icon to browse to the nicotinamide.mol file you downloaded. Now use the Save As icon () to

save it to the dataset (accept the defaults; just say OK). Reread the entire dataset by dragging and dropping it from the left again. Now you should see the structure in the bottom left corner.

- b. The icon , in the main icon menu, makes it easy to measure the distance between two peaks in Hz
 - c. If you have peak-picked your spectrum, the multiplet analysis (mana) routine can be useful. Go to Analyze / Multiplets to enter it. The first icon is a good place to start. The success of the routine depends heavily on how well the signals are resolved and how well peak-picking is done. It is rare for it to be perfect, but you can modify it by selecting peaks for each multiplet manually. By carefully highlighting multiplets and sites on the structure, you can assign signals and output a report in various formats:
 - use the  icon. Click Exp. and OK to copy the experimental report output. Also try playing with finding connections.
9. Plotting. Plotting in TopSpin is done via the Plot Editor, which uses layout files to locate spectra, text, and other items on the page. Layout files can be heavily customized for color, color, font, etc., and they can be reused, so it is well worth taking the time to make some good layouts and saving them for reuse.
- a. One note: it is easy to export or copy the view of the spectrum in the spectrum window. To make a PDF or other image file, use the  icon. To copy the view, perhaps to paste it into Powerpoint, use the  icon.
 - b. To go to the plot editor, type `plotor` or click on PLOT. If you get an error message, ignore it.
 - c. To start customizing, start with the page size: change it to letter under Print / Page setup...



- d. As you make changes, use Layout / Save as... to give it a new name and save it.
- e. Change the region of the plot displayed using the icons in the plot panel and at the top of the TopSpin window, and use the icons to free up more of the screen

for the plot editor if you do not have a high-resolution monitor. You can always return to this panel display by clicking on the blue background on the sides of the plot editor.



- f. To modify objects already on the plot page, select them, then use the panels and the icons on the left to modify them. The colored squares are used to change fonts and colors. If you need to go back to the initial panel so you can make more of the window visible, click on the blue at the sides of the plot editor.
- Things to consider changing: displaying peaks; displaying integrals; changing axis font and color; changing title font and color; changing the size of various objects; deleting unwanted text or objects...
- g. Don't forget to save your work (see d. above) so you can reuse it
- h. To add other objects, click on the blue surrounding the plot editor to go back to the initial panel. Use the pull-down menus to add other spectra, the FID, text, shapes, etc. Once you chose an item, you usually have to click and drag to place the item, but for text you have to click once, then type.
10. When you are happy with the view you have created, then print or make a PDF, or a copy of the entire view with the icons at the top. The icons may be hidden under the three



11. Or, select an object, such as your spectrum, on the plot editor, and right-click to copy it. This creates a copy with a clear background that is very good for use in presentations.

Processing a Varian 2D spectrum and plotting a 2D spectrum with a 1D spectrum as a projection

- Go back to the SPECTRUM tab, then drag and drop the dataset 20150825_strychnine_1H.fid into the view on the right. It should process automatically. Scale up vertically and check that the phasing and baseline are correct – if not, see the instructions in Exercise 1
- Drag and drop the dataset 20180825_strychnine_gc2hsqcse_01.fid into the view on the right. It likely won't process properly. Instead, type `xfb`, which is the basic 2D processing command. The spectrum that appears is full of artifacts. An HSQC spectrum shows one-bond correlations between ^1H and ^{13}C , but the spectrum has horizontal mirror planes that indicate that something has gone wrong in the processing.
- To process Varian 2D datasets properly, try running the routines `varian2da`, `varian2db`, `varian2dc`, or `varian2dhmbc`. When you get the right one, the HSQC

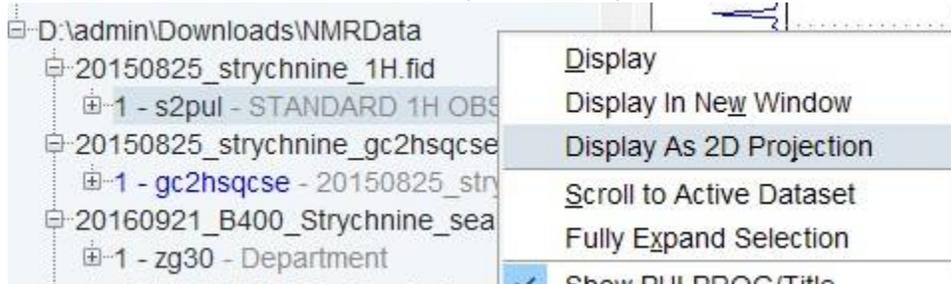
should show signals distributed roughly along the diagonal from upper right to lower left – there should be no symmetry in the spectrum.

4. Phasing. All signals in the HSQC used in the facility should be either positive (indicating CH or CH₃) or negative (CH₂). There should be no mixed signals. To correct the phasing, type `apk2d`. It is also possible to phase manually (not described here).
5. Contour levels. It can help to increase the number of contour levels by setting the parameter `nlev`, the number of contour levels, to a large number (say, 32) and running the routine `clev` to recalculate them. You can also use the mouse wheel to set the contour

levels, and the save contour levels icon  to use them for plotting.

6. Projections. Rather than use a projection of the 2D spectrum across the top, we will plot an actual 1D spectrum. In the data browser, locate the 1D spectrum you just opened (note: right-clicking on an empty space in the data browser and choosing “Scroll to active dataset”

is often useful, as is the history of the data browser under ). Right-click on the spectrum and choose Display as 2D Projection...



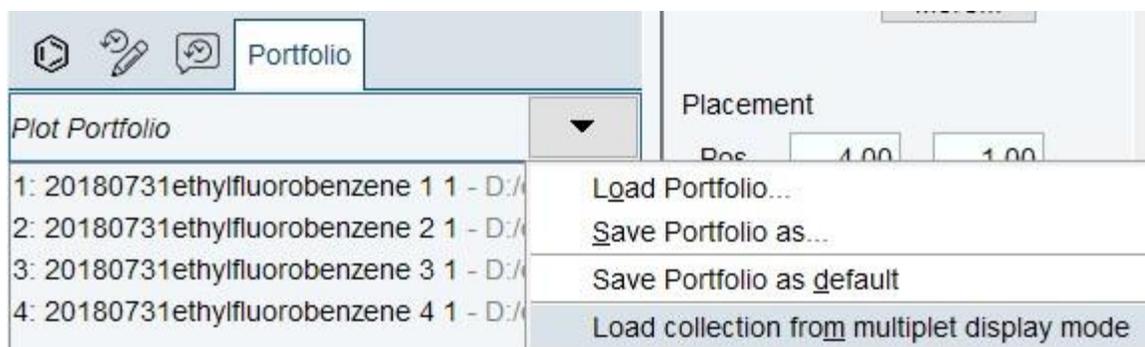
Choose F2 (horizontal dimension) when prompted.

7. Linear prediction. In 2D spectra, the vertical dimension (¹³C here) is usually acquired with very few points (say 256) relative to a normal 1D spectrum (often 32000 points). This saves time, but it means that resolution is poor, since only a few points are used to describe the spectrum. To linearly predict in the F1 (indirect or vertical dimension), go to the PROCPARS tab. Choose Fourier on the left. On the right (F1) column, set ME_mod to LPfc and NCOEF to 32. Type `xfb` to reprocess.
8. Spend a bit of time looking at the spectrum. Can you find any cases of inequivalent protons on CH₂ groups?
9. To plot, go to the PLOT tab. Many errors will pop up, because this is Varian data and TopSpin is confused. Ignore the errors. If the ¹H spectrum is not plotted along the top, go

back to the SPECTRUM tab. Go into multiple display mode by clicking on the  icon. Add the ¹H spectrum to the view by dragging and dropping it into the spectrum window. You should see the ¹H spectrum superimposed on the HSQC, and you should see two spectra

listed on the bottom left. Quit Multiple Display mode with the return  icon.

10. Go back to the PLOT tab. Ignore the errors. In the plot portfolio on the bottom left, click on the arrow and choose Load collection from multiple display mode...



Now 2 spectra should be listed.

- Open a more appropriate layout by clicking on the blue around the plot editor and then loading a new layout. 2D_inv.xwp is a good choice – it's used for HSQC, HMBC, and similar.
- Now the 2D and the 1D should appear in the correct positions, though you may need to select and deselect the top projection and/or right-click on the spectrum and choose Reset before it displays properly. Change the paper size. Add grid lines. And save your layout!

Exercise 3: Plotting multiple 1D spectra on the same page

- Go back to the SPECTRUM tab. Read in and process each of the three spectra we will use for the exercise: 20160921_B400_Strychnine_sealed, 20160921_B500_strychnine_sealed, and 20160921_M300_Strychnine_sealed_PROTON_01.fid. These used the default parameters on each spectrometer to compare sensitivity on the same sealed sample of strychnine in CDCl_3 . Roughly 3.5 mg of strychnine was in the sample. Be sure the spectra are phased and the baselines are correct. Check that the residual CHCl_3 signal has the same chemical shift in all spectra. If not, use Process / Calib. Axis until they are all the same.
- Go into multiple display mode by clicking on the multiple display icon (). Drag the other two spectra in until you can see all three spectra together. If you select one spectrum, you can use the icons inside the multiple display window to change its display; the icons outside of that window change all three spectra. Play around with the different views available to you. When you are finished, use the return icon to quit.
- Go to the PLOT tab. One spectrum should be displayed. Adjust its view to occupy one-third of the screen. Now copy and paste it twice. Arrange the three spectra on the page so that they appear stacked. You can use the arrows that appear to align them. Selecting each of the spectra and adjusting it vertically may be necessary.
- As with the 2D plotting exercise above, in the plot portfolio on the bottom left, click on the arrow and choose Load collection from multiple display mode... Now all three spectra should be listed. Drag and drop from the list onto the spectra at the right.