Vanderbilt Small Molecule NMR Center

Instructions for Using TOPSPIN version 2.1

DRX-400: Setting up ^{1}H, ^{13}C (^{1}H decoupled), and ^{31}P NMR Experiments

By Markus Voehler, March 2011

1 Getting started

1.1 Sample Preparation

Make sure your sample has a **minimum volume of 0.5ml.** Samples with less volume will be more difficult to shim and may require large changes in Z^3 and Z^4 in order to see a "good" lineshape. **Please remember to use a NMR tube holder for transporting tubes to the NMR Vault**. They are available in the Chemistry Stockroom.

1.2 Logging in to Spectrometer

Log into the PC using the username and password that was assigned to you by the NMRStaff

Once you're logged in, double click on the TOPSPIN icon to launch the program:

1.2.1 Finding your data

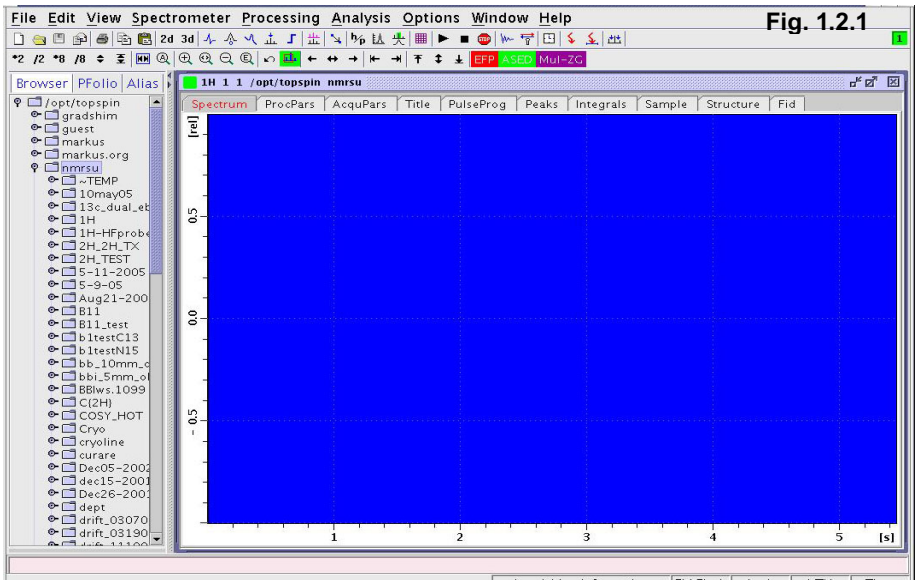


Fig. 1.2.1

All data is located in **/opt/topspin/data. (Fig. 1.2.1)**

Simply locate your username on the left-hand side and click on the icon to view the sub-directories.

To select a file, click on the file and drag into the active window on the right.

If you have multiple experiments in that directory the following window should appear (Fig. 1.2.2):

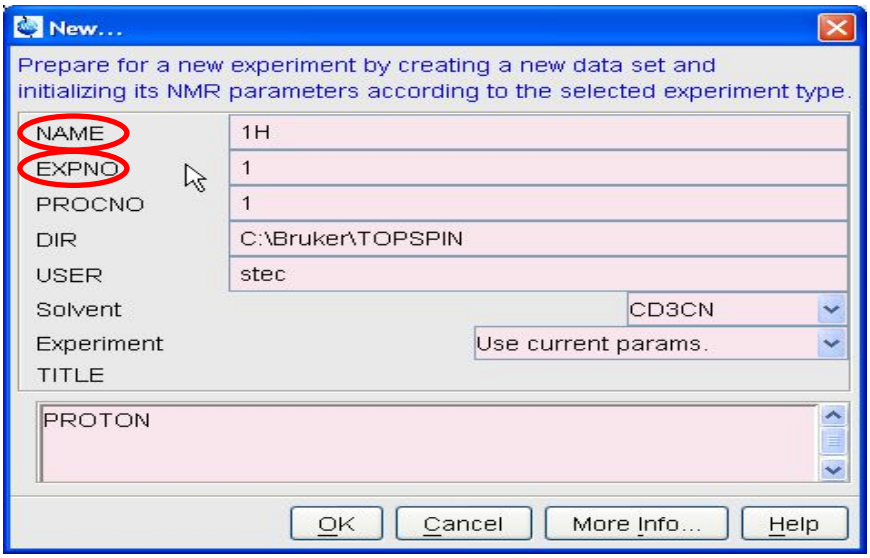
Fig 1.2.2

2 Acquisition

2.1 Creating a New File

There are 2 Ways To Open the Window That Creates New Files

1) type "**edc**"
2) Go under "**File**" than "**New**"



A **NEW** file can be created by either changing the Name OR by keeping the same Name and incrementing the EXPNO # (**Fig. 2.1.1**)

If the name is changed use up to a maximum of 13 characters (alphabetical or numeric). Do not use any special characters such as (!@#$%&?).

Also, **DO NOT** include any spaces since this will create problems when the data is transferred.

Since data files are overwritten once a new experiment is started it is very important to create a new file before a new acquisition is started!!

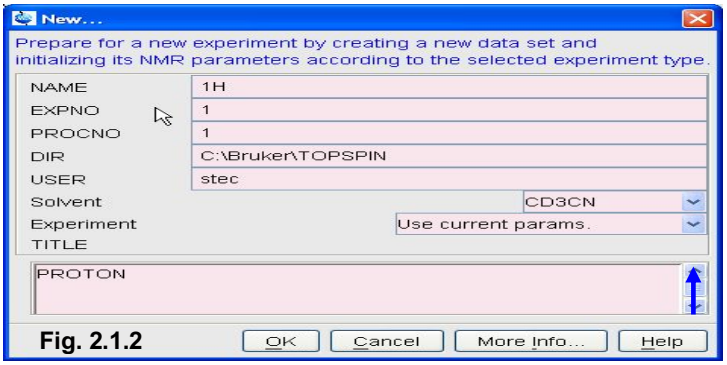
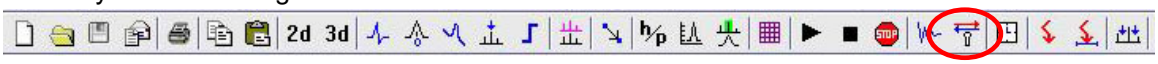


Fig. 2.1.2

The type of experiment can be selected by clicking on the icon across from "**Experiment**" (**Fig. 2.1.2**)
For ^{1}H experiments select AA_PROTON.MV
For ^{13}C experiments select A_C13CPD.MV
For Coupled ^{31}P experiments select A_P31.MV
For Decoupled ^{31}P experiments select A_P31CPD.MV

2.2 Inserting and Locking Sample

Next, open up the Lock Window by either typing in "**lockdisp**" or selecting the "**Lock**" icon found on the top bar or by double clicking on the lock icon on the bottom of the window



Once TOPSPIN is opened, the sample can be placed inside the magnet.

1) You might want to check the temperature (type edte)
2) **Make sure the Lock, Autoshim and Spin are OFF before ejecting the sample from the magnet!!** Anything on the BSMS keypad that is illuminated green is on. To turn off the lock or spin, push the appropriate keypad button
3) **Remove the bore cap**
4) **Eject sample** (press lift on/off button, top left hand corner of keypad)
5) **Clean Sample and Place in depth gauge (make sure gauge is set for 18 mm)**
6) **Lower Sample** (by pressing the lift on/off button)

Click on "SHIM FILE" to call in default shim file (rsh chloshim.600)

(type) "lock" or click on the "LOCK" button

Fig. 2.2.1

When the solvent list displays, select the proper solvent by clicking on it. (**Fig. 2.2.1**) Computer will automatically adjust Field & auto-Lock

2.3 Probe Tuning

To tune the probe either type in the command "**atma**" or click on the "**TUNE**" button

This will automatically start the tuning program. The computer will notify you once the program has finished and the proper tune/match values have been achieved

2.4 Spinning Sample

Since the main probe on the high field instruments are inverse probes it is recommended **NOT** to spin the sample due to "Q" modulations that can be detected and often distort the baseline.

2.5 Shimming

Once the sample is spinning and you have locked on to the appropriate solvent, the next step is to shim the sample. The recommended method for sample shimming uses pulse field gradient shim routine which is fully automated.

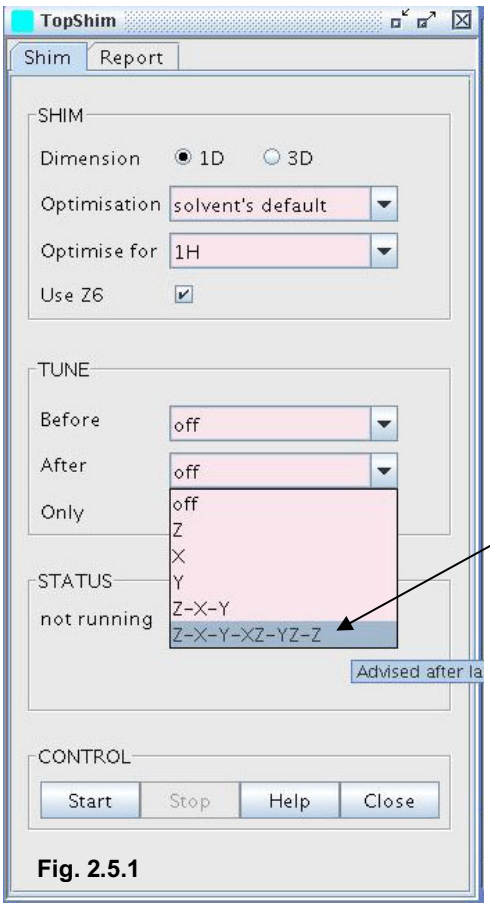


Fig. 2.5.1

Gradient Shimming with TOPSHIM

Before you start the gradient shimming program it is VERY important to return the shims to their default value. This is done by clicking on the "SHIM FILE" icon (see above):

Once the default shims values are recalled, the gradient shimming program is started by typing "**topshim gui**" or click on the "**SHIM**" button

Before starting the gradient shim make sure that you select Z-X-Y-XZ-YZ-Z under the "After" section in "TUNE" box. (**Fig. 2.5.1**) This will shim the off-axis shims once the on-axis shims are complete.

To begin shimming simply select the "Start" button located on the bottom. When the automatic gradient shimming is completed the Start button will again be available for selection.

To determine if the shim was successful, simply note the lock level before you started shimming and see if it increases upon completion of the shimming routine. If the lock level becomes too

high on the display and goes out of view, decrease “lock gain” until the signal is observable on the lock display.

An additional shim may be required if the lock increased dramatically. It is recommended to repeat the shimming procedure two times to ensure optimal lineshape.

2.6 Acquiring a Spectrum

After maximizing the lock level, take one scan and check the lineshape. Before you take a scan you want to set the receiver gain. The command to set the receiver gain is:

(Type) **“rga”** or simply click on the 

icon

Wait for the instrument to message that is has completed setting the receiver gain. Once it is done setting the gain you are ready to take a scan. The command to start an experiment is:

(Type) **“zg”** or click on the  icon located on the top-right of the TOPSPIN window

Type “**ns**” to set the number of scans or set the number of scans under the “AcquPars” window. The recommended number of scans is 16. (**Fig. 2.6.1**)

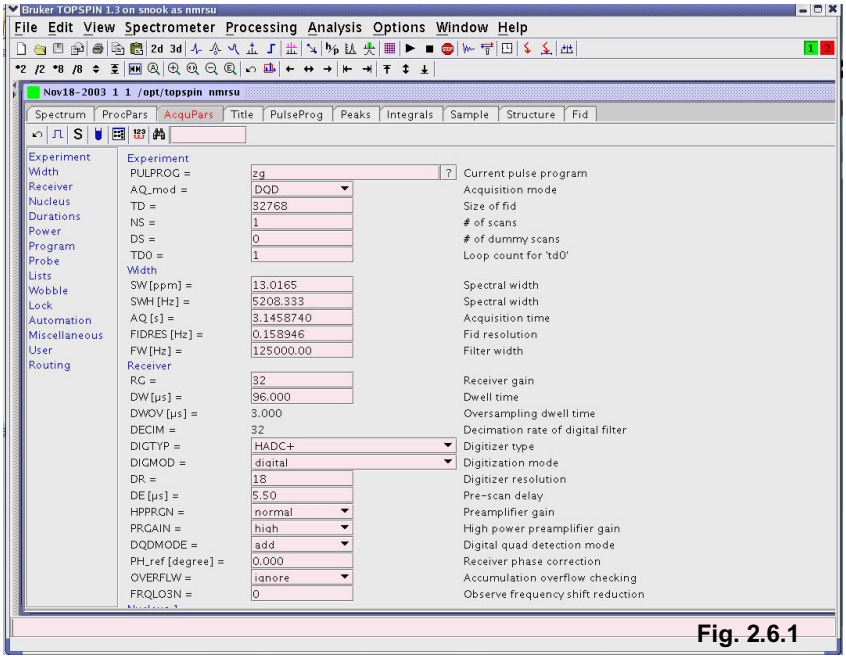


Fig. 2.6.1

After setting the number of scans start the acquisition as described above.

You might want ot check the shimming and parameter settings by acquiring a single scan (NS=1) experiment followed by a fourier transformation and phase correction as described below

3 Processing Spectrum

3.1 Fourier Transformation

(Type) **"efp"** or click on the **"EFP"** button
Keep in mind, that this uses the LB parameter (LB for 1H ~ 0.3 Hz, 13C ~ 2Hz).

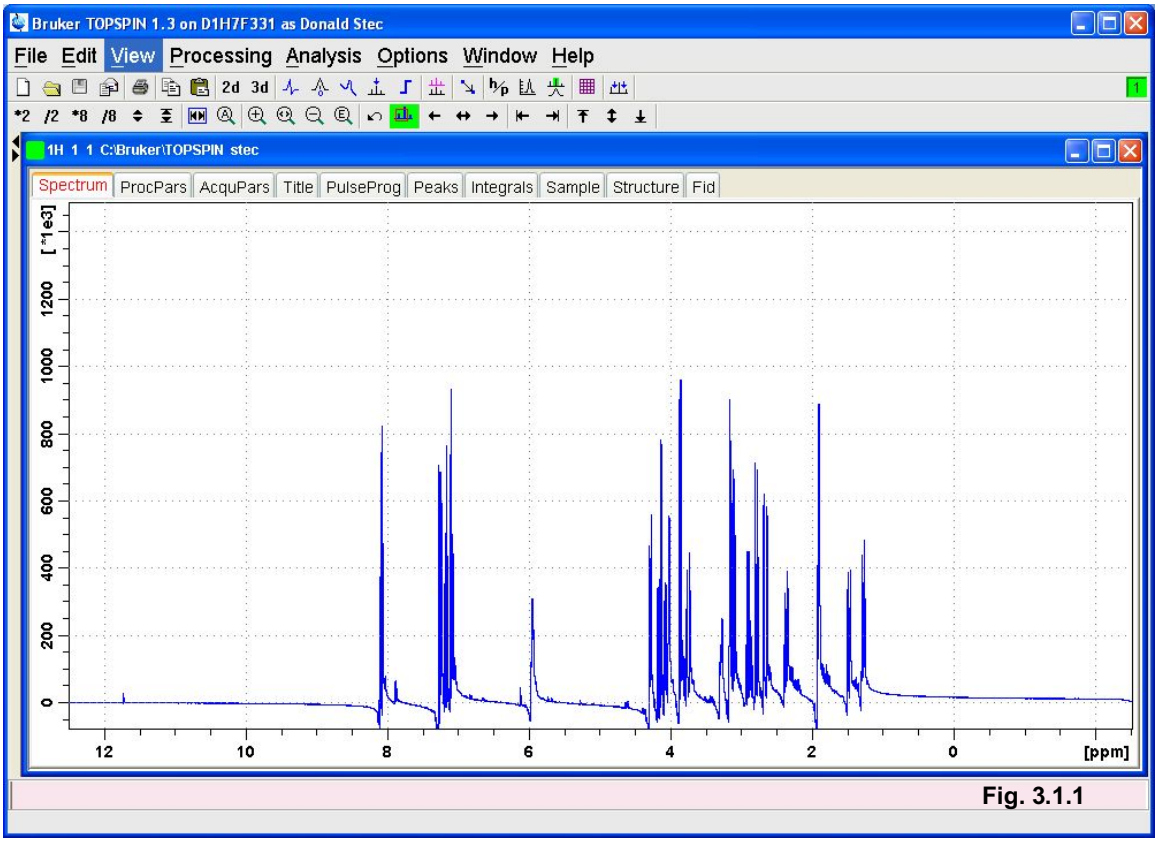
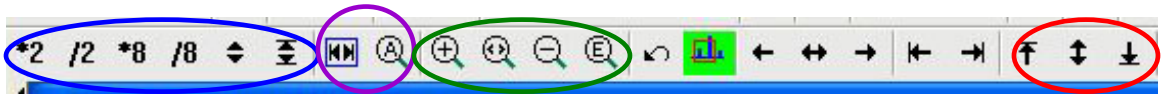


Fig. 3.1.1



- use these buttons to increase the scale so the baseline becomes easier to view
- use the "up" and "down" buttons to bring the spectrum to the center of the screen and to the bottom
- use these buttons to expand or contract your spectrum
- use this button to go back to the full display for your spectrum

3.2 Phase Correction

Click on the “Phase” icon (**Fig. 3.2.1**)

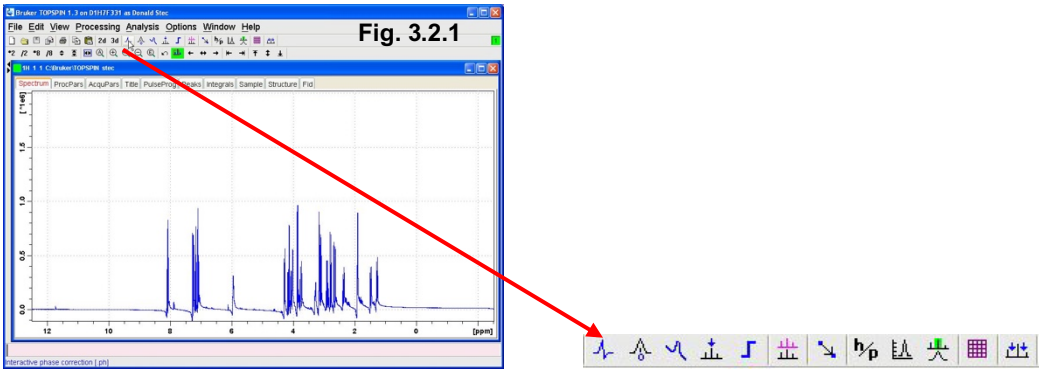


Fig. 3.2.1

This should open the following window (**Fig. 3.2.2**):

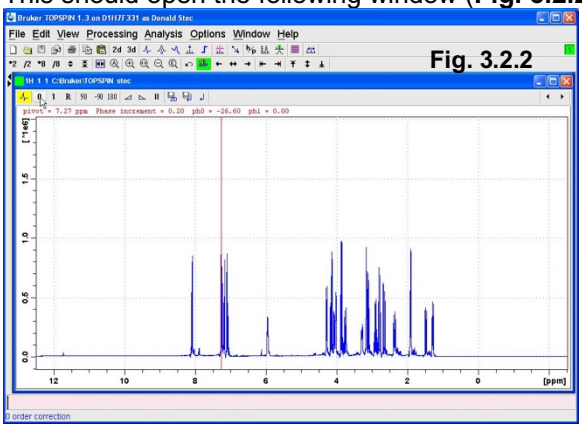


Fig. 3.2.2

To phase the spectrum, click and hold in on the “0” button for zero order phasing and the “1” button for 1st order phasing.

The “R” button resets the phase back to its original value while the 90, -90 and 180 buttons apply a 90, -90, and 180 degree phase automatically. Once the appropriate phase is applied click on the icon in the red box to save.

Check the solvent peak and other narrow peaks to make sure the lineshape is narrow and symmetric. This requires expanding the spectrum display.

3.3 Expanding Spectrum

Click mouse 1 on either side of the spectrum where you would like to expand and hold down while dragging the mouse. A second cursor should appear. The expansion will finish once you release mouse 1.

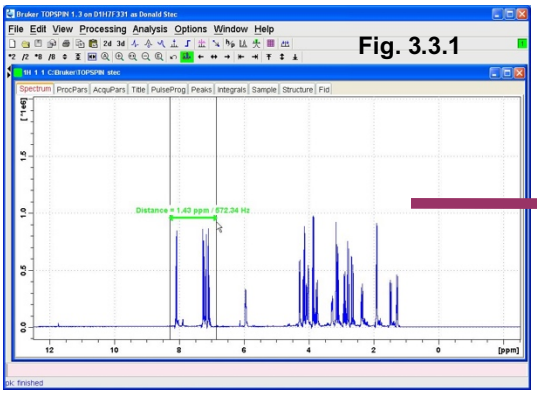


Fig. 3.3.1

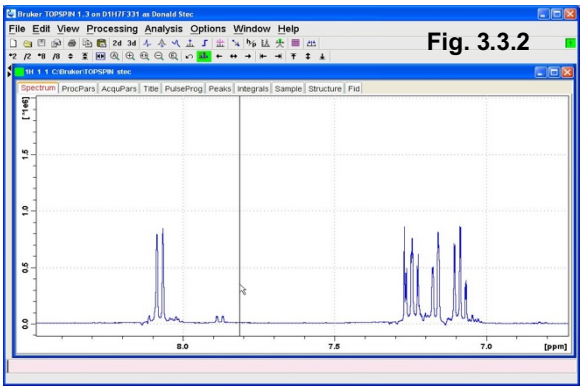
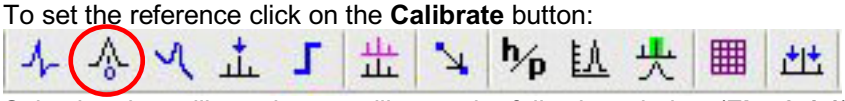


Fig. 3.3.2

3.4 Referencing

Now you can set the reference. The reference is usually set to a peak who's position you are certain of such as a solvent peak or TMS. The command sref does a rough calibration of the spectrum.

To set the reference click on the **Calibrate** button:



Selecting the calibrate button will open the following window (**Fig. 3.4.1**)

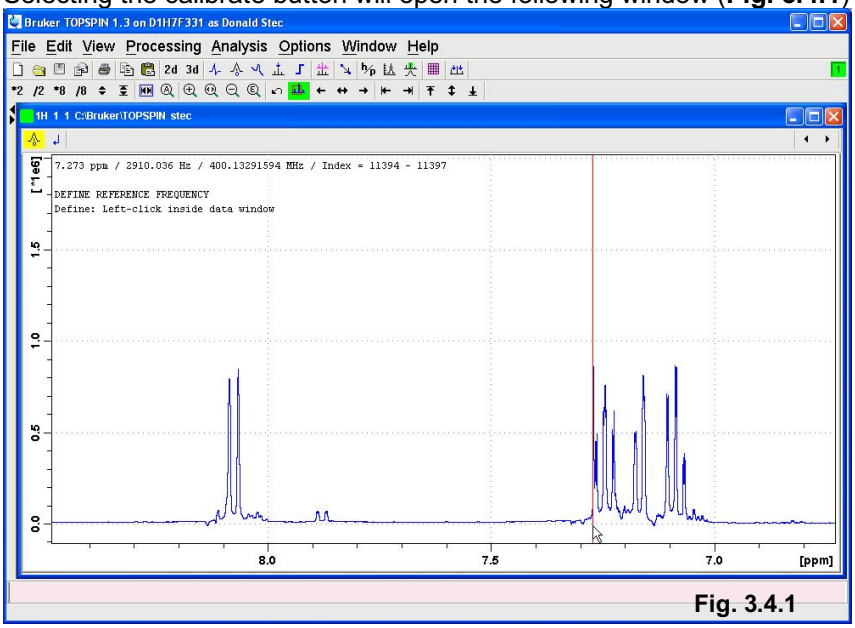
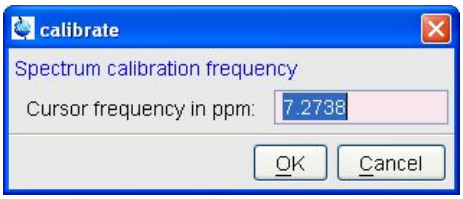


Fig. 3.4.1

With the red cursor in the center of the peak you would like to use as a reference click with mouse 1 and the following window should appear



Simply type the correct value into the box and select “OK”

3.5 Baseline Correction

Select the baseline correction icon and the following window will open (**Fig. 3.5.1**):

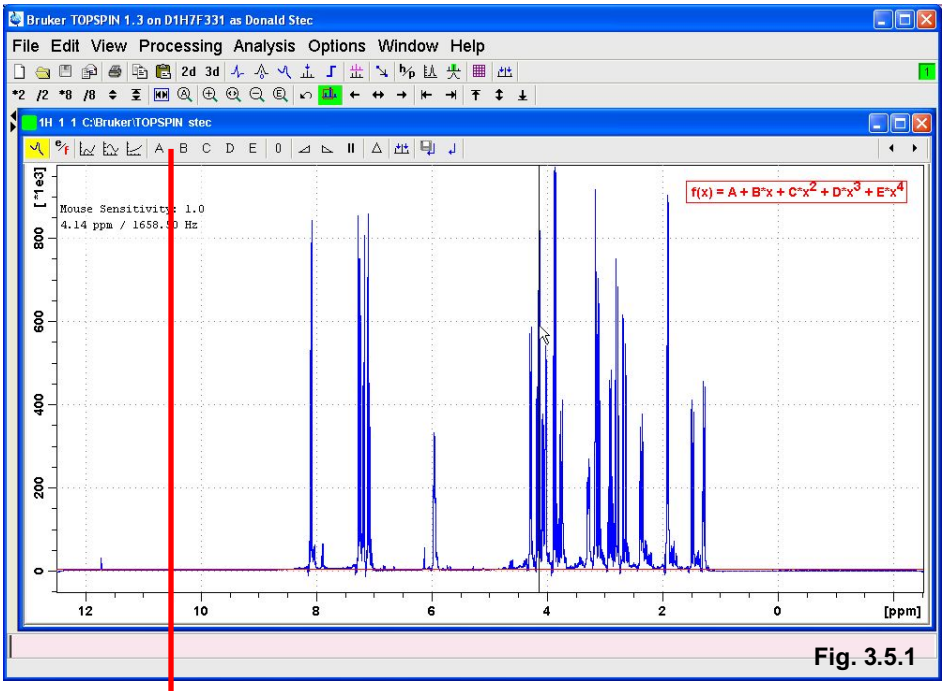


Fig. 3.5.1

- Selects the proper function (polynomial, sine, exponential). The default is polynomial
- Use these buttons to compensate of the offset (A) and to make sure the fit is running through the center of the spectral baseline (B,C,D,E)
- Once corrections are made select this button to SAVE and RETURN

3.6 Integration

Click on the “**Integrate**” icon

The following window should appear (**Fig. 3.6.1**):

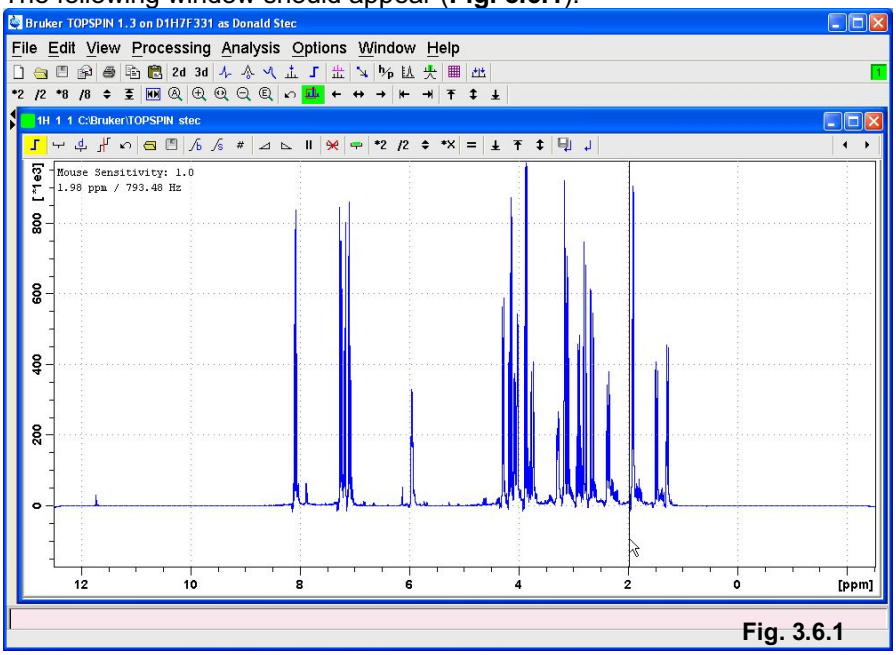


Fig. 3.6.1

Before you integrate, expand around the area of interest following the directions for expansion listed above.

Click on this icon to activate the integral mode and the following window will appear (once the icon is “active” it will change color to green) (**Fig. 3.6.2**)

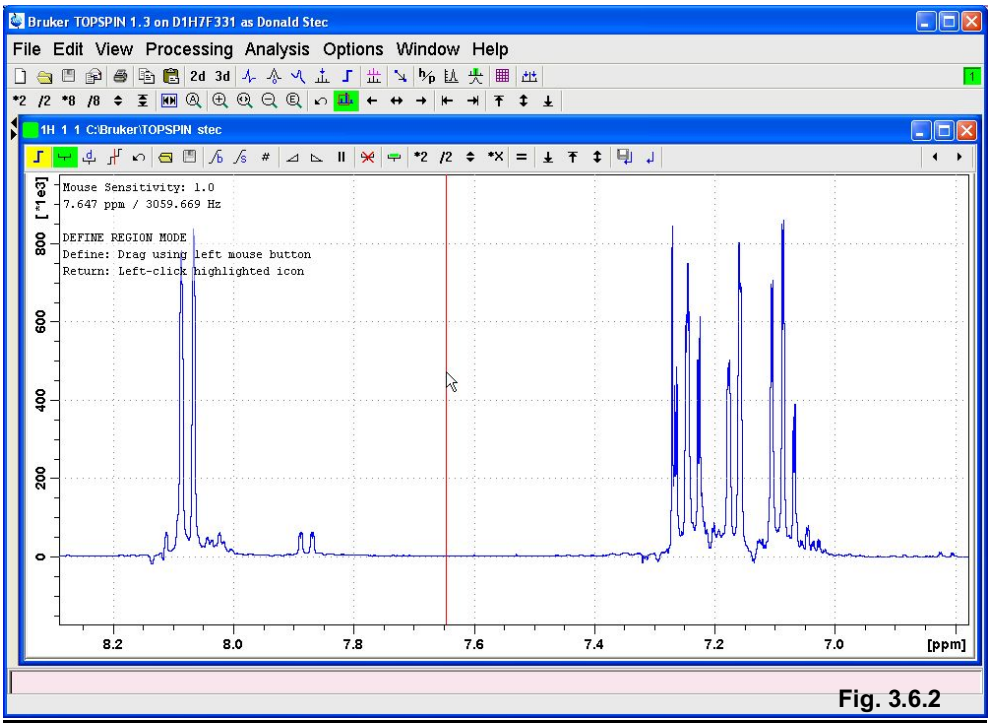


Fig. 3.6.2

To integrate, click on one side of the peak of interest using the left mouse and hold down. A second cursor will appear. Release the mouse when the cursor area covers the peak you would like to integrate. (**Fig. 3.6.3**)

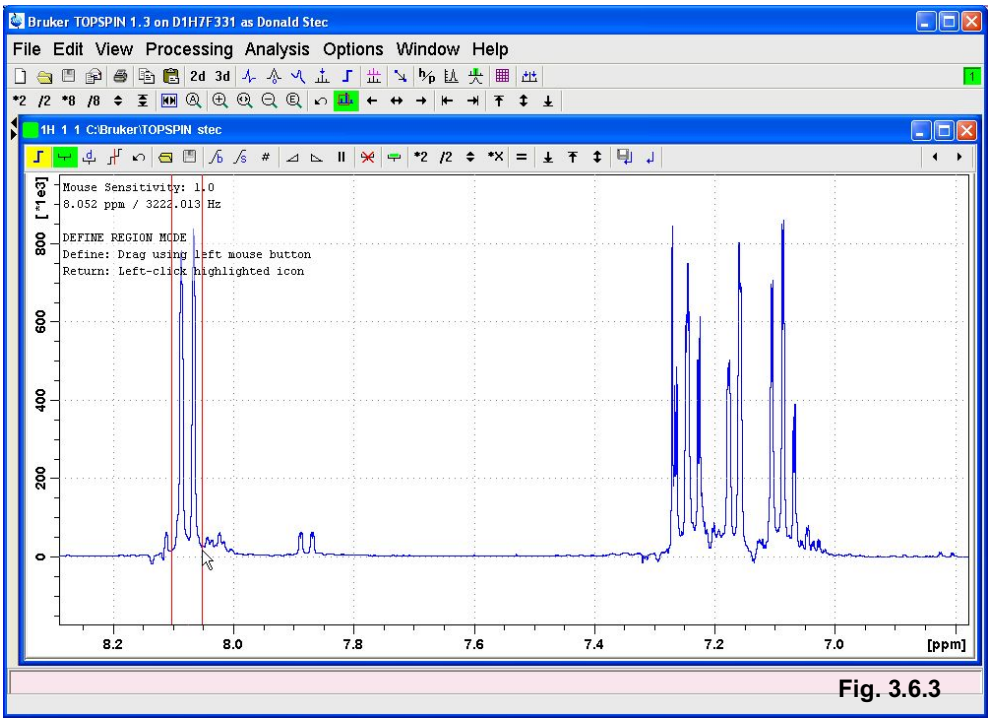


Fig. 3.6.3

Once an integral is set, a value will appear. To calibrate the integral to a reference number, click on the right mouse between the integral limits and select “Calibrate” in the window that appears. (**Fig. 3.6.4**) A new box will appear where you can modify the value. You can also delete the current integral using the same window

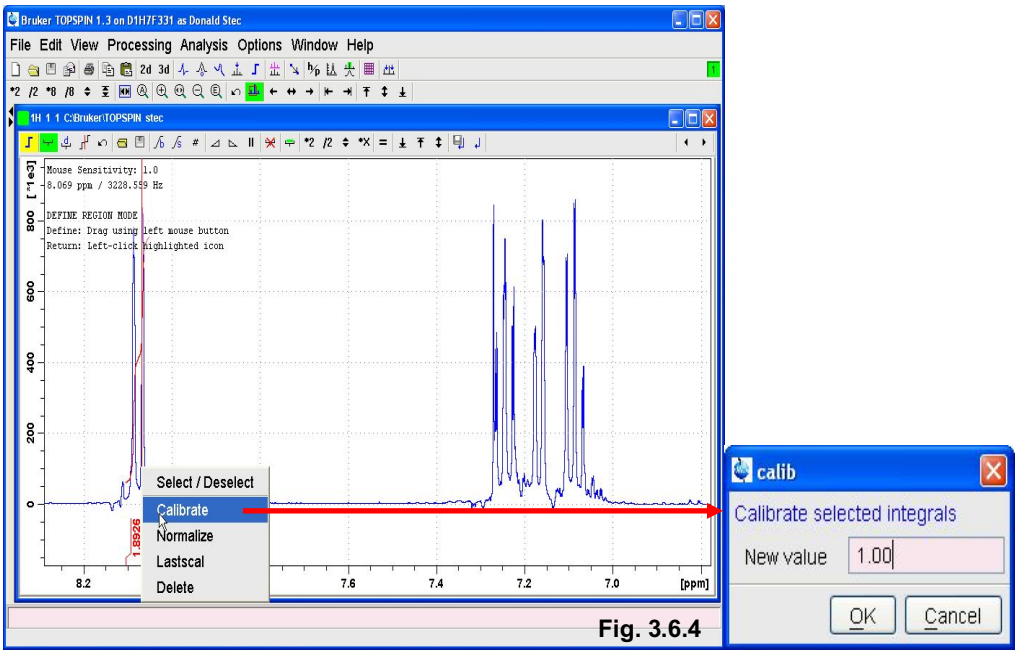


Fig. 3.6.4

- Use these keys to adjust the basis and slope of the integral
- Use these keys to define and delete integral regions
- Use these keys to manipulate vertical scale of integrals
- **USE THIS KEY TO SAVE INTEGRALS**

Once integrals are saved, they may disappear from your active window. To make your integrals appear in your active window, right click on the mouse and select "**DISPLAY PROPERTIES**". The following window will appear (**Fig. 3.6.5**):

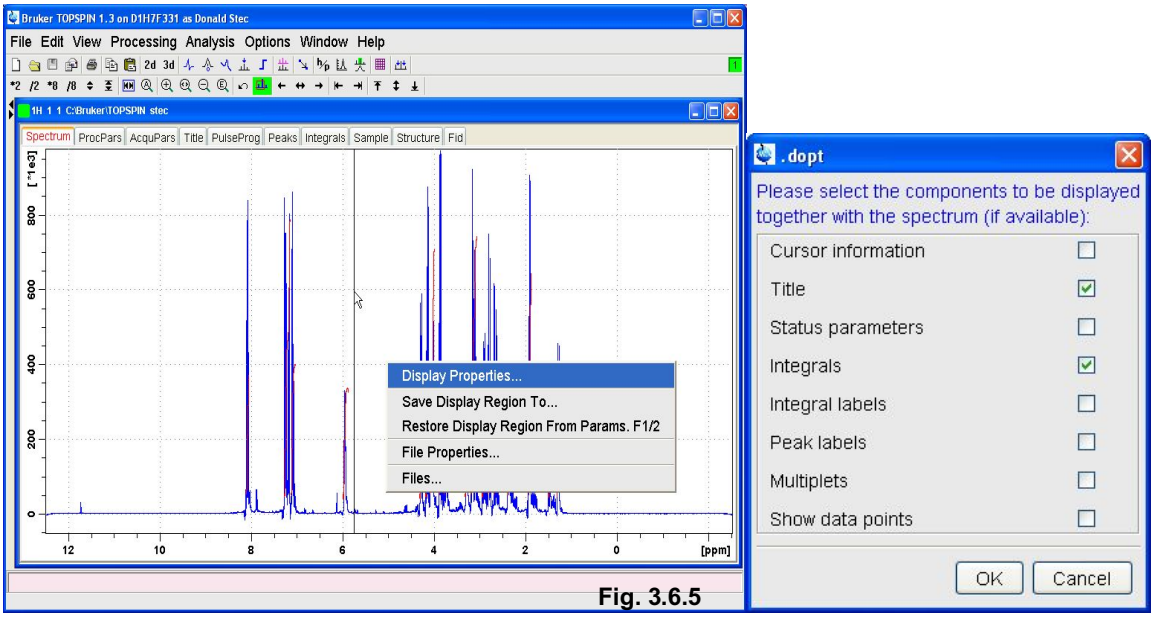


Fig. 3.6.5

Simply click on the box for the parameter you would like to appear on your active window.

3.7 Peak Picking

Click on the "**Peak Pick**" icon

The following window should appear (**Fig. 3.7.1**):

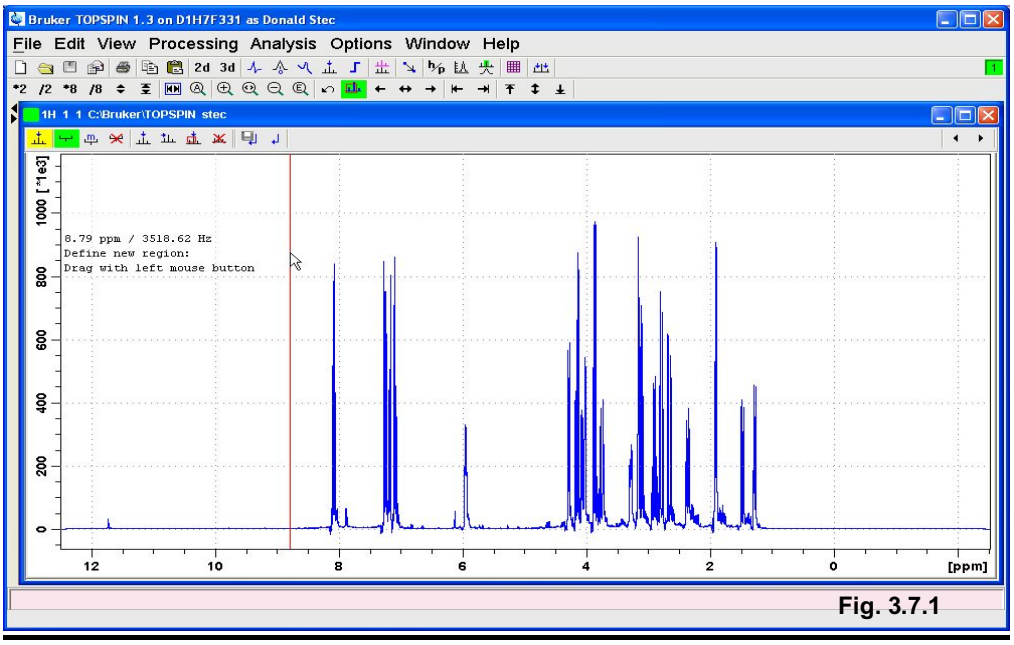


Fig. 3.7.1

To pick peaks, click on the left mouse and drag. A green box should appear as in the following (**Fig. 3.7.2**):

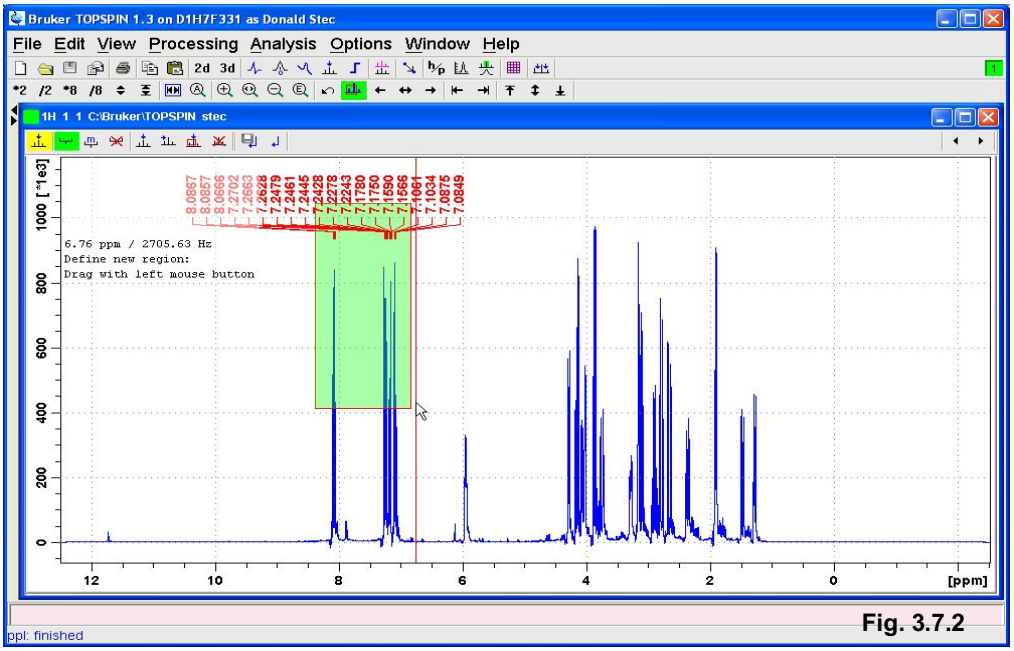


Fig. 3.7.2

Once you release the mouse the peaks should appear. Please remember, the lower the box is towards the baseline, the MORE peaks will be selected. To delete peaks, right click and select "**Delete All Regions**" (**Fig. 3.7.3**)

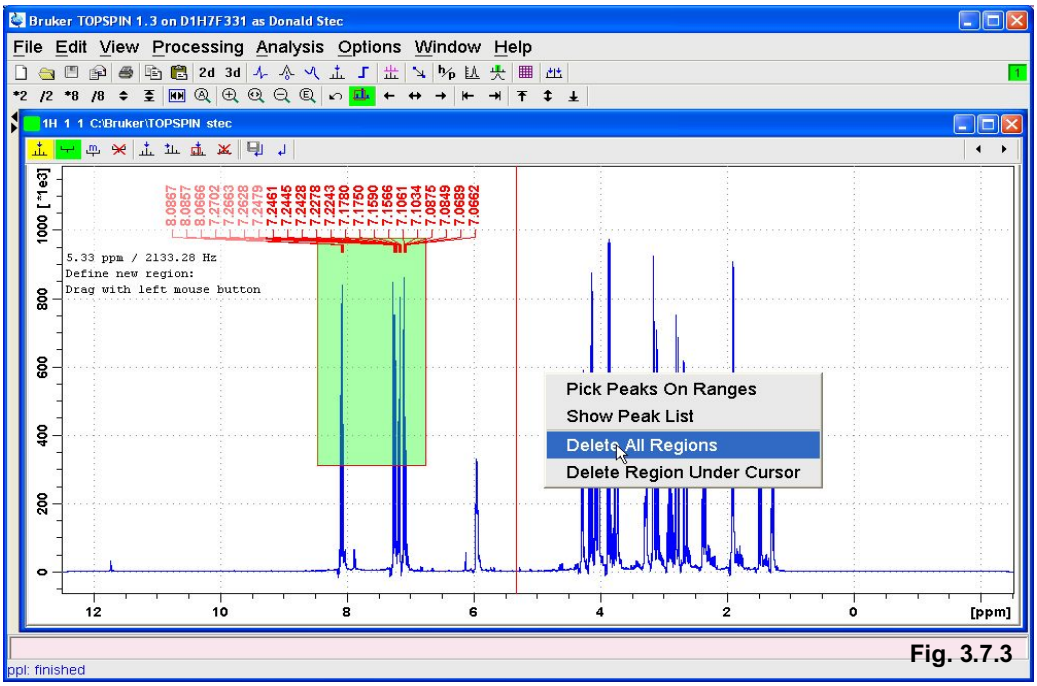


Fig. 3.7.3

● Use this icon to delete all peaks

● Use this icon to manually pick peaks

● USE THIS ICON TO SAVE PEAKS

To make your peaks appear in your active window, right click on the mouse and select "**DISPLAY PROPERTIES**" and make sure you select the "**Peak Labels**" box.

3.8 Setting the Title

Before printing you can set the title for the spectrum by selecting the "Title" icon. The following window should appear (**Fig. 3.8.1**):

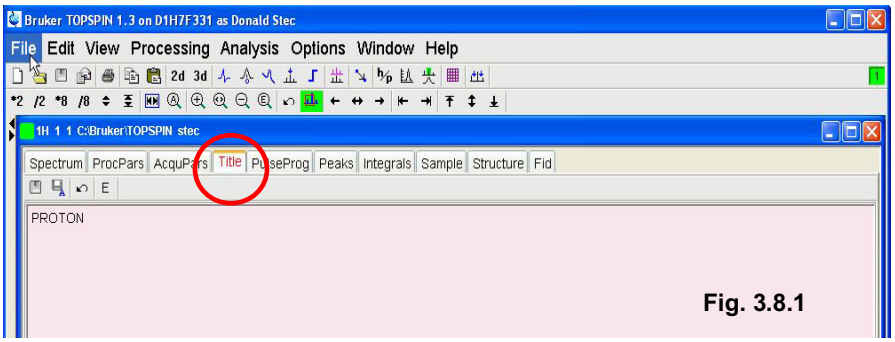


Fig. 3.8.1

To set the title, just click in the pink box and type the desired title. There are no character or space limitations.

3.9 Plotting

In version 2.1 of TOPSPIN, plotting is done using the Plot-editor.

To open up the plot editor click on the "**PLOT**" button

or

select "**File**" on the top left side of the window and than choose "**Print**" (**Fig. 3.9.1**)

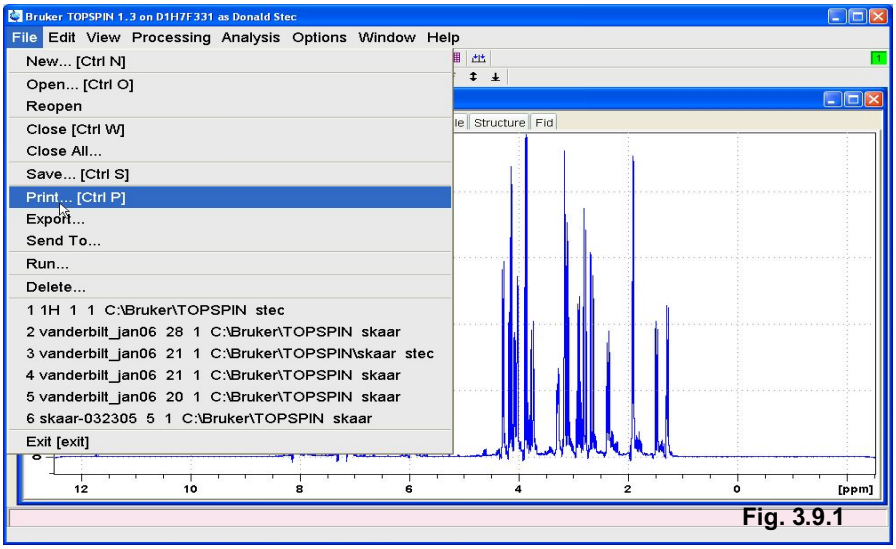


Fig. 3.9.1

Once you select "**Print**", the following box will appear (**Fig. 3.9.2**):

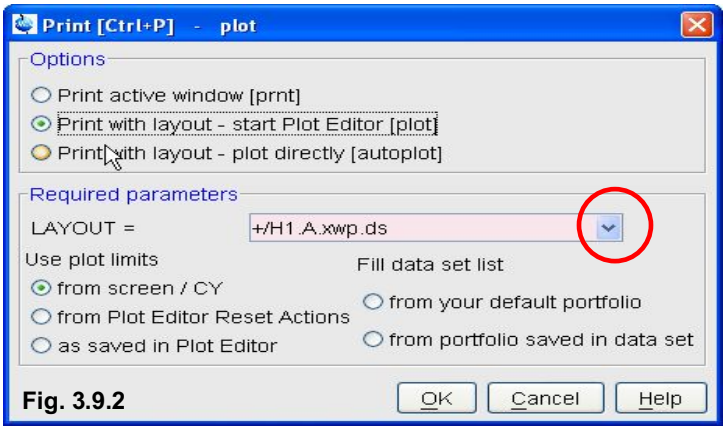


Fig. 3.9.2

Make sure "**Print with layout-start Plot Editor [plot]**" is selected and a Layout is selected as well. To select a layout, click on the arrow button. Once the proper layout is chosen the Plot-editor should open.

4 Finishing Up

1) **Lock = Off Spin = Off Eject Sample** (make sure bore cap is removed before ejecting)
Autoshim = off (if used)

2) **Put standard sample back inside the magnet**

3) Put the bore cap back in place!!!!

4) Exit Topspin program and log-out of instrument (right click on desktop)

If Problems Occur Please Contact Don or Markus

4.1 Copying Data onto a Flash Drive

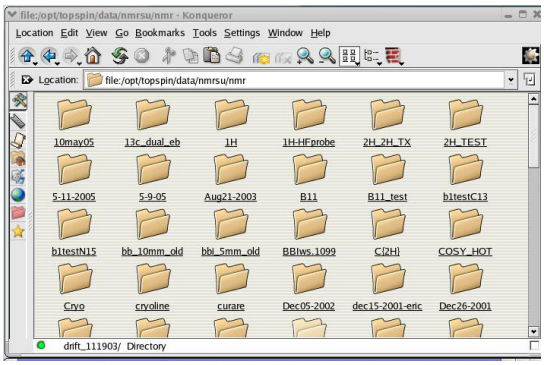
1) Open up data window by clicking on your home icon 

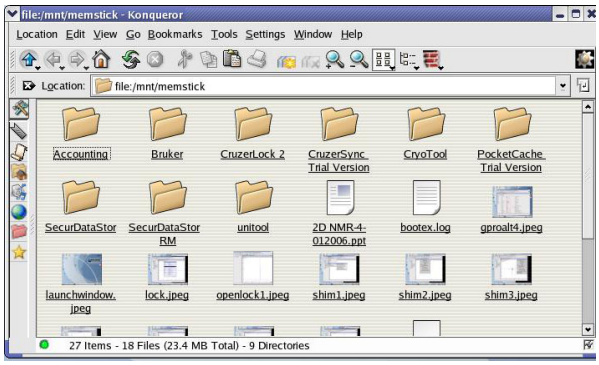
located on the Desktop.

2) All data is located in the following path**:/opt/topspin/data/*your_username*/nmr**

3) Open up data stick window by clicking on icon 

4) To transfer data:
Simply drag a file from your data directory to the USB Stick directory

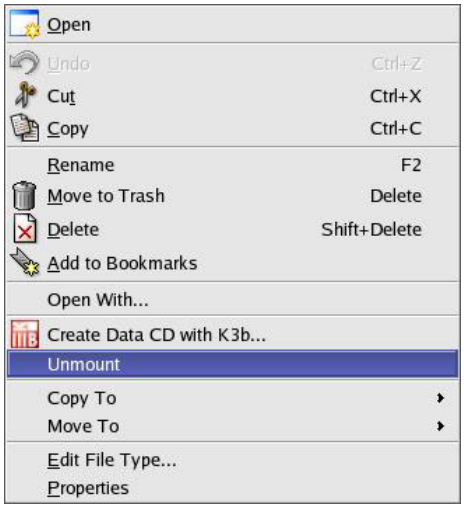




5) Once you drag the file over the following message will appear:

Select “Copy Here” and file should be saved on USB Stick

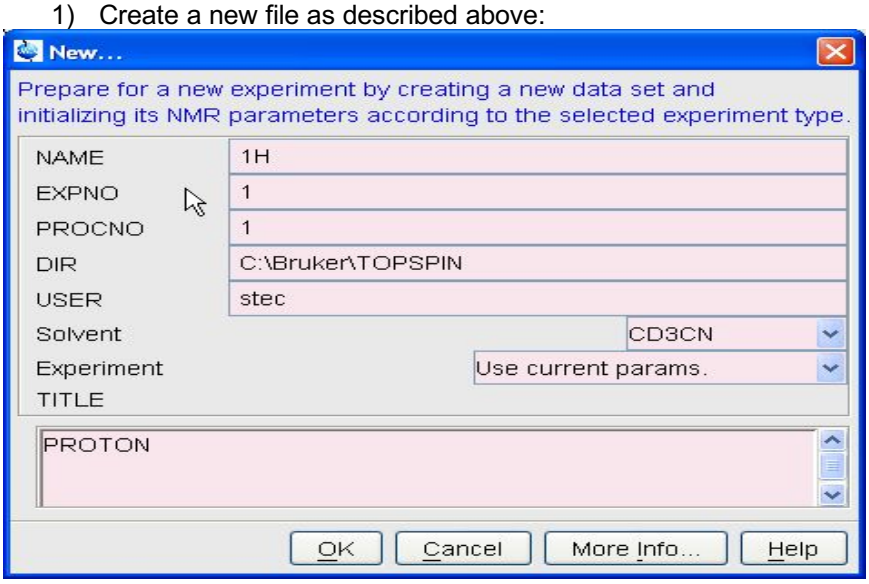
6) Remove USB Stick properly:
   a. Right click on USB drive icon
   b. Select the “unmount” option



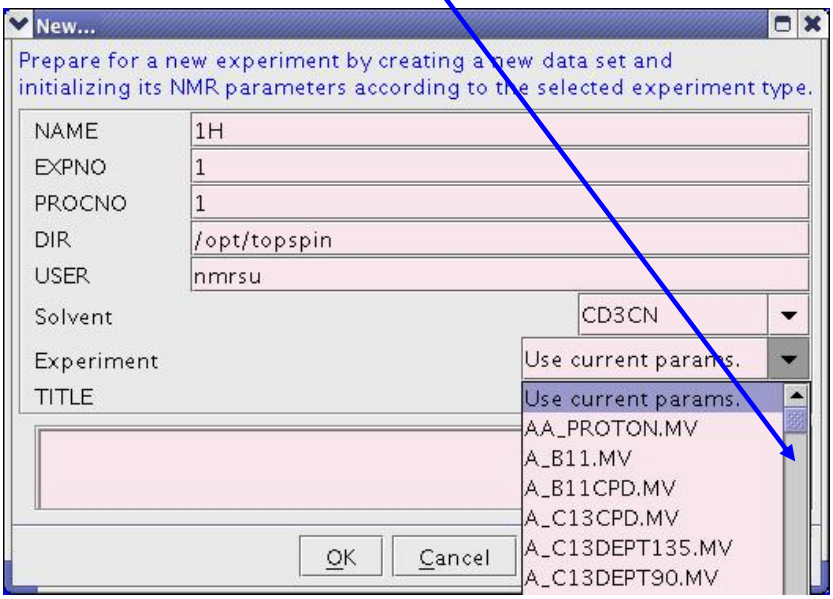
c. Remove USB stick

5 Acquiring $^{13}C/ ^{31}P/ ^{11}B$ NMR Spectrum

1) Create a new file as described above:



Under "Experiment" click on the  icon and select the following:



FOR ^{13}C: A_C13CDP.MV
A_C13DEPT135.MV
A_C13DEPT90.MV

FOR ^{31}P: A_P31.MV (no ^{1}H decoupling)
A_P31CPD.MV (with ^{1}H decoupling)

FOR ^{11}B: A_B11.MV (no ^{1}H decoupling)
A_B11CPD.MV (with ^{1}H decoupling)

Remember, the Probe MUST BE tuned before you start any "X" nucleus NMR experiment.

Type "atma" or click on the "TUNE" button

Once tuning is complete, you can begin the acquisition:

Set **ns for 13C**

128 scans = 6 mins 512= 24 mins 2000= 1.5 hrs 20000 = 15 hrs.

ZG or  starts the experiment

tr (lets you look at the data before it's finished)
wait for the message "checklockshift: finished" then type

efp (phase as needed)

halt to stop acquisition (do not use "stop", this will not save the FID)

efp to Fourier Transform and display the final spectrum