

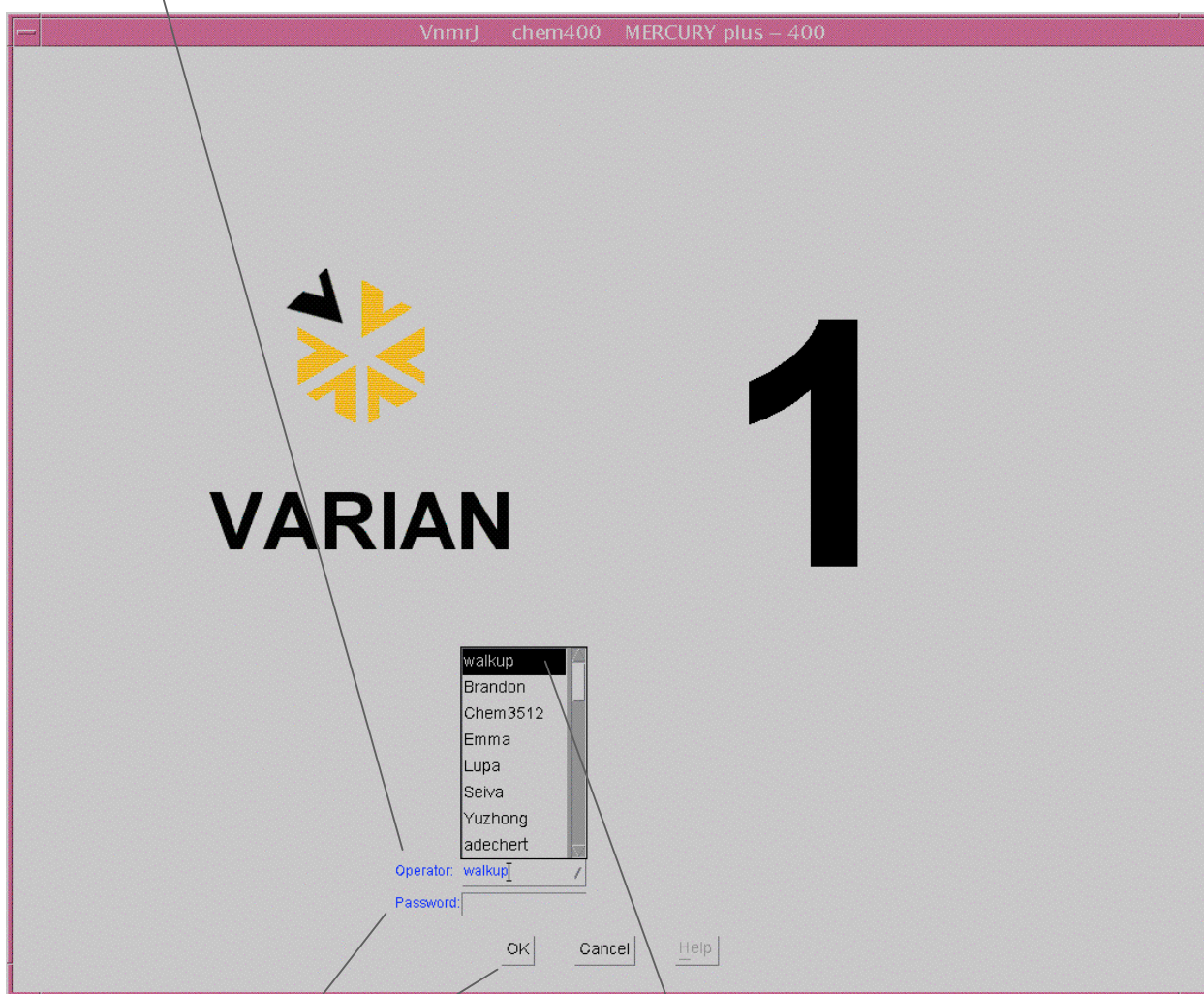
Lab #4

2D ^1H , ^1H -COSY and ^1H , ^{13}C -HSQC

- operation of the 400 MHz instrument using automated sample insertion (robot) and automated locking and shimming
- collection of 1D ^1H spectra
- retrieving data, peak picking, peak integration, plotting
- 1D ^{13}C spectra collection and plotting
- DEPT spectra collection and plotting

Login

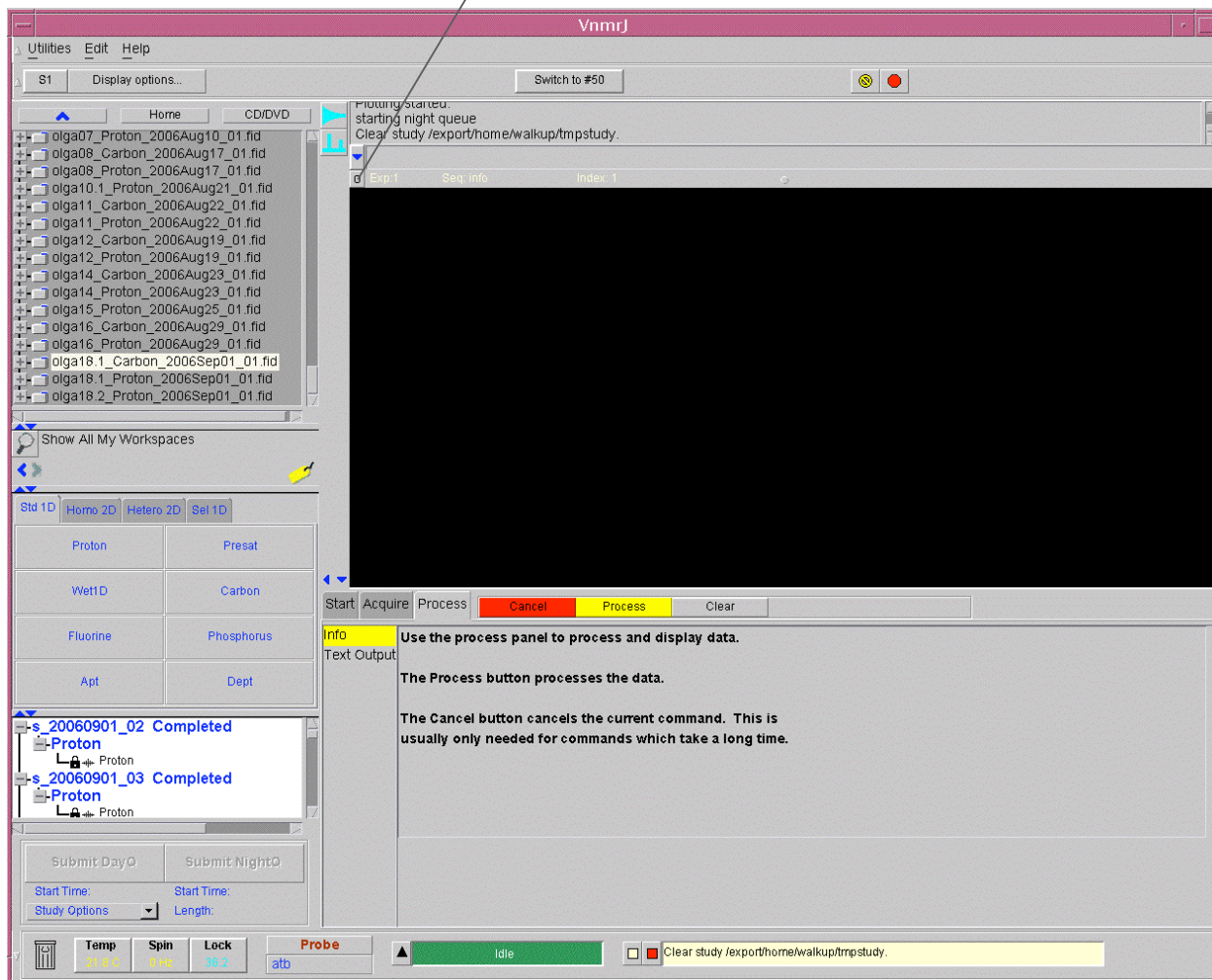
-the **operator** screen should be in view when you first sit down at the spectrometer console:



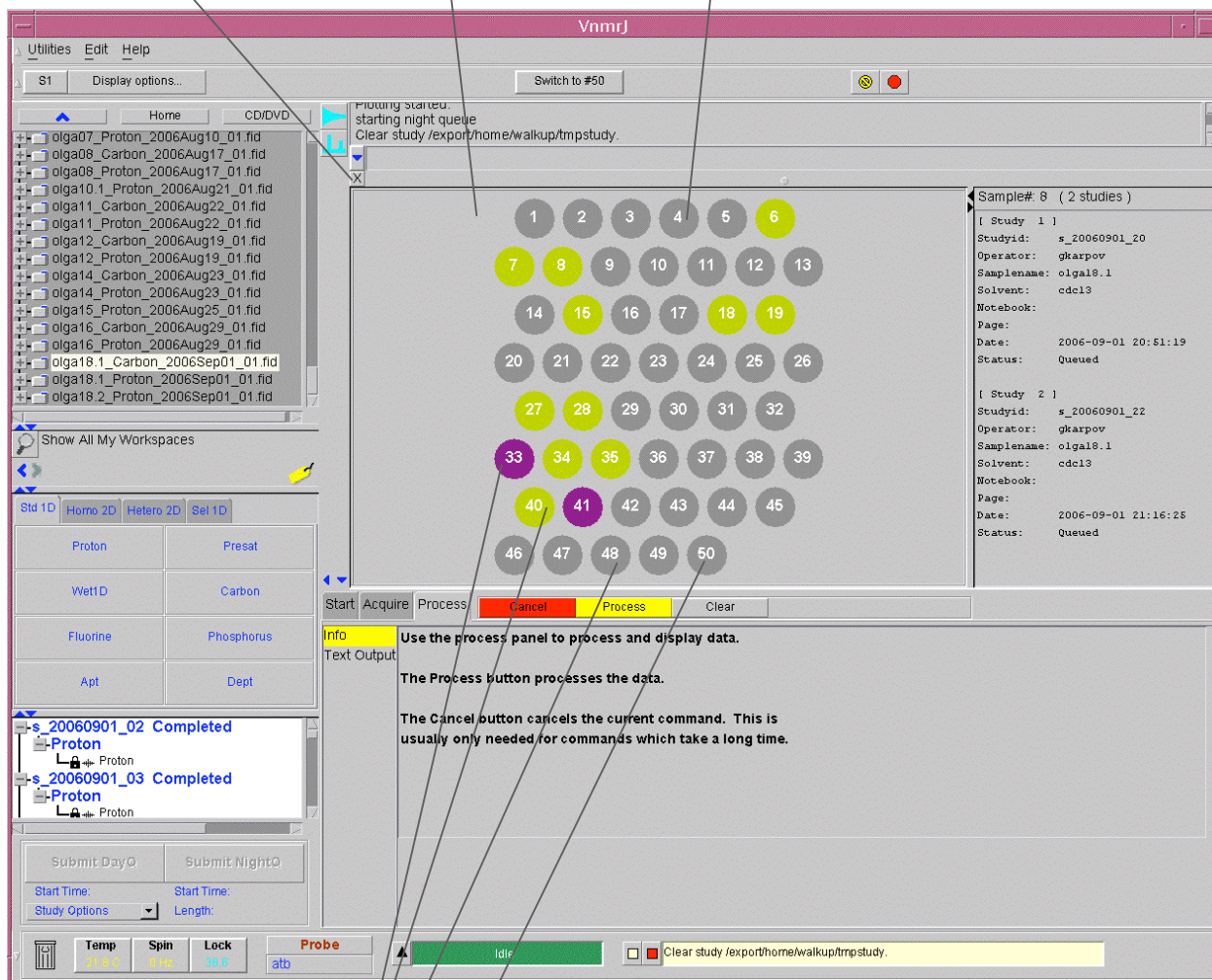
- from the list of operators (pull-down menu), **select Chem6190**
- type the **password** for the walkup account, which is **374robot**
- click **OK**

Sample slot selection

-if the **tray panel** is not present, click the “O” located above and to the left of the black spectral display window to display the tray panel

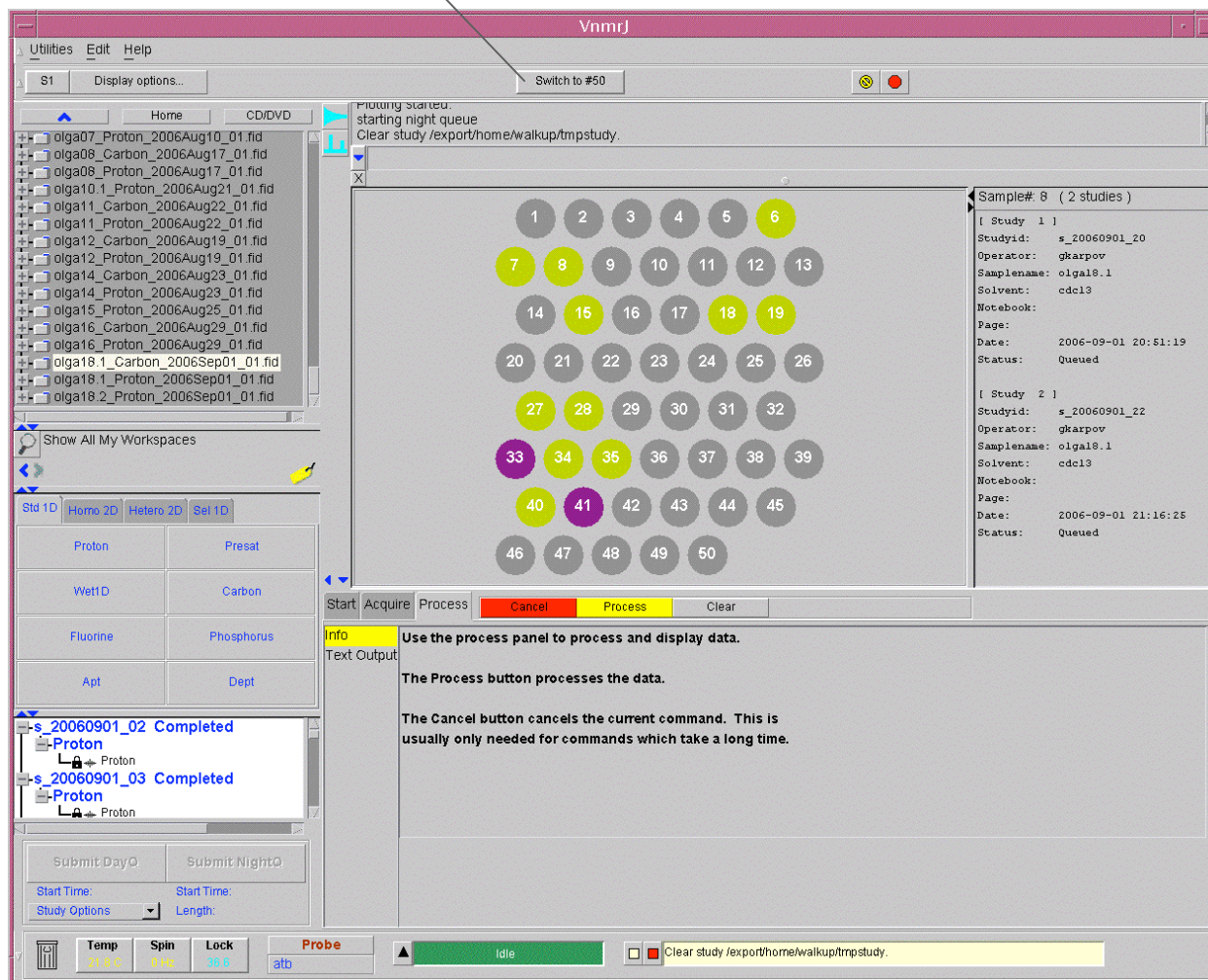


- the tray panel represents the **sample tray** located on top of the white platform next to the magnet
- the **X** in the upper left of the sample tray will toggle the display back to the black spectral display window
- the numbered circles on the tray panel correspond to **sample slots** in the sample tray



- sample slots that appear in **color** on the tray panel are **NOT** available for use
- also, sample **slot 50** is **never available** for use (this slot is reserved for a standard sample)
- sample slots that appear **gray** on the tray panel **may** be available for use, but this must be confirmed (see below)

- click the **switch to #50** button at the top of the screen
 - if nothing happens, this means that any of the gray slots can be used
 - if there is a sample in the magnet already, the robot will take it out and replace it with sample 50.
- At this point, all **gray slots** are **now available** for use (*the robot is slow, so be patient*)
- choose one of the available (gray) slots. **You must confirm that this slot is empty by checking the sample tray**

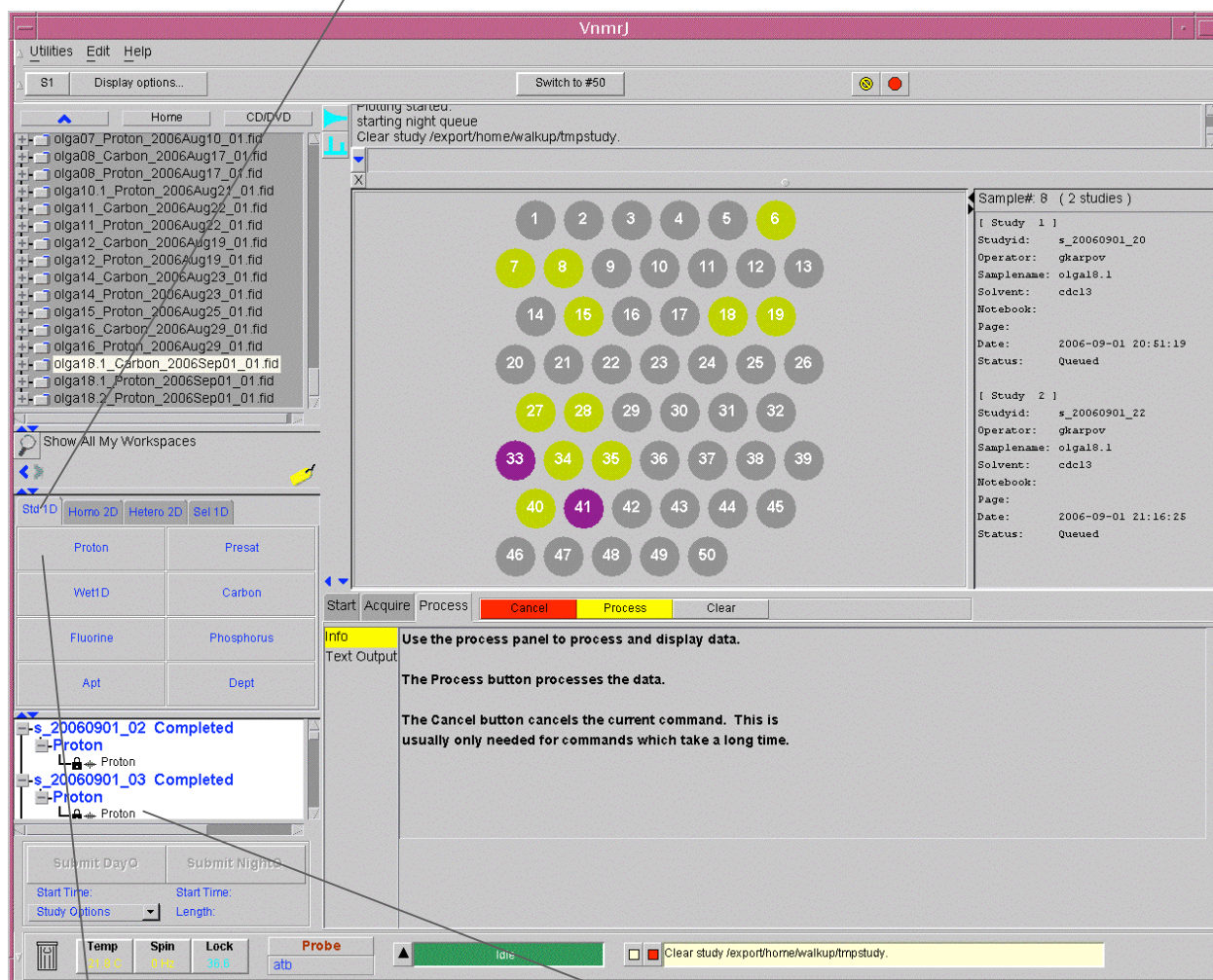


Sample placement

- hold the **sample tube** near the **middle of the tube** and in a **vertical position**
- push the tube through the hole on the large end of a **sample spinner turbine**
 - NEVER** push on the top of the tube
- the bottom of the tube should be pushed slightly through the bottom (small) end of the spinner turbine
- set the tube/spinner assembly in the black **sample depth gauge** in the sample tray
- push the tube (by its sides, NOT from the top) down until it stops
 - the tube is now properly positioned in the spinner turbine
- now you can place your tube/spinner assembly in one of the open slots (on the sample tray, the **slot number** is to the **right** of the slot/hole)

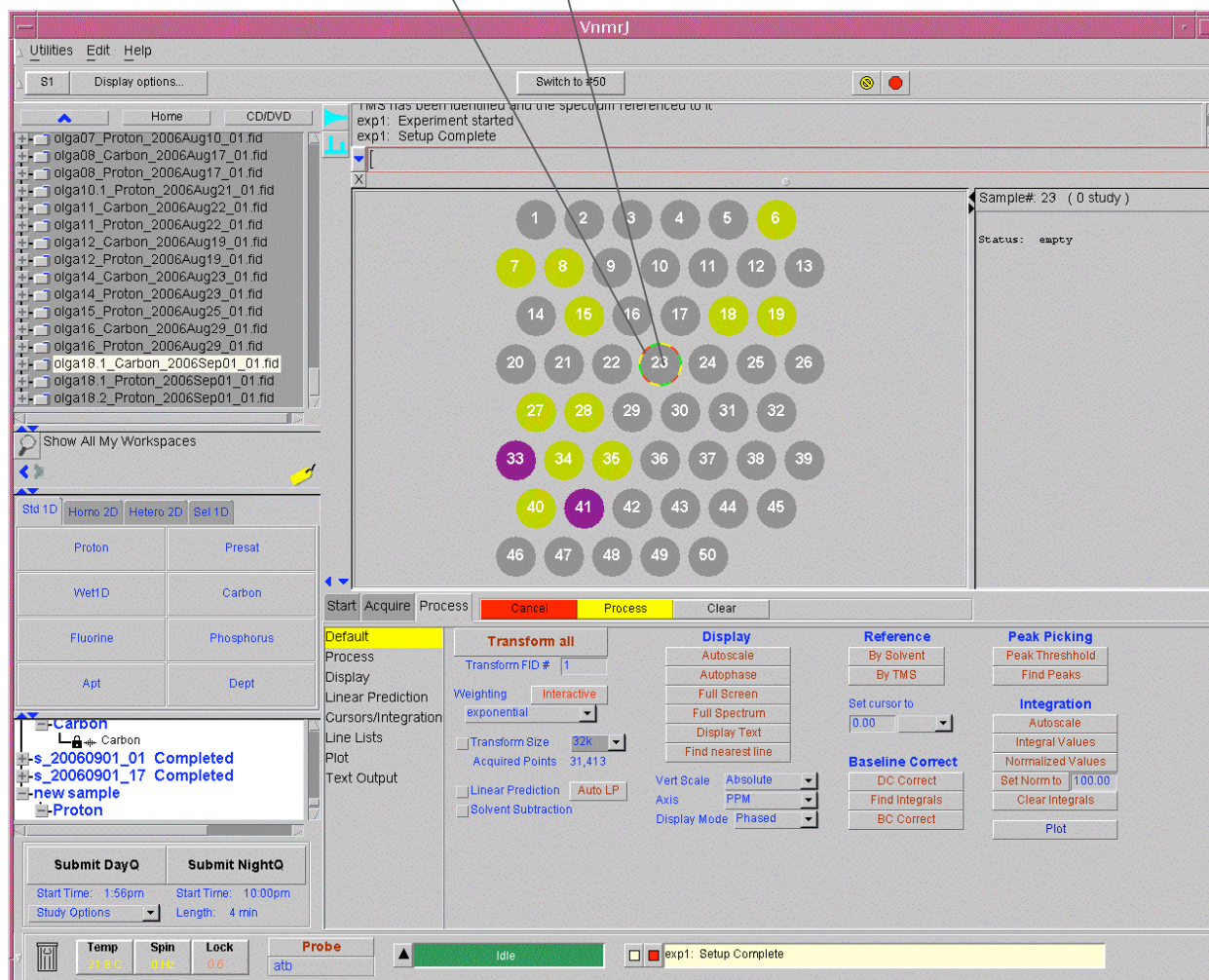
Experiment selection

- you can select from among the experiment tabs the one corresponding to the experiment that you will be acquiring
- in our case, we will select **std 1D**

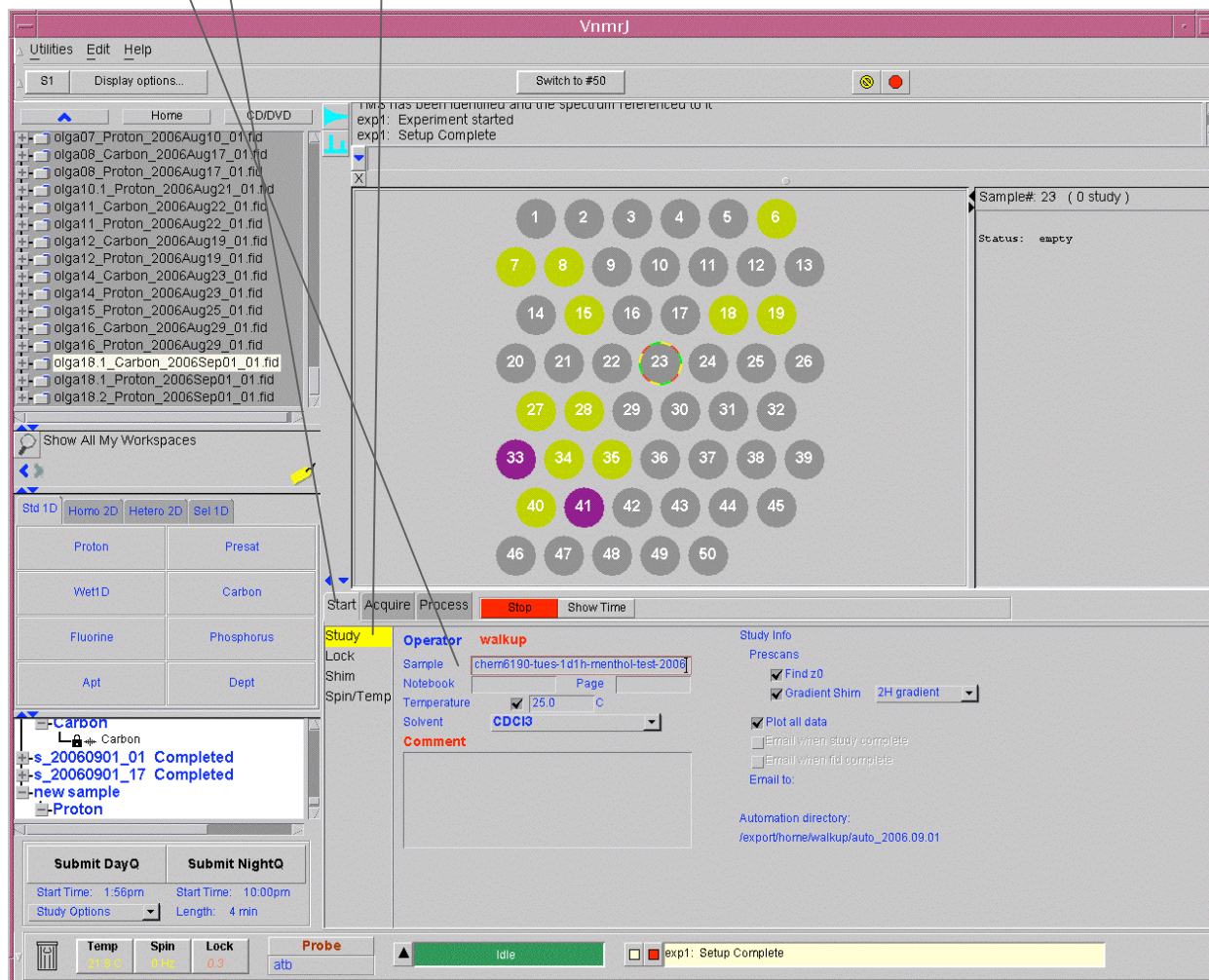


- click **Proton**, then **double click** on the **yellow highlighted Proton** in the window below
- this will load the appropriate parameters for collecting a simple 1D ^1H experiment
- in window below, **new sample** and **Proton** will appear (these will be colored yellow, indicating that the sample has not yet been submitted, and the experiment has not begun)

- on the **tray panel** click on the **appropriate slot** (an empty one that you have chosen)
- it will get a **multicolored highlight** once selected

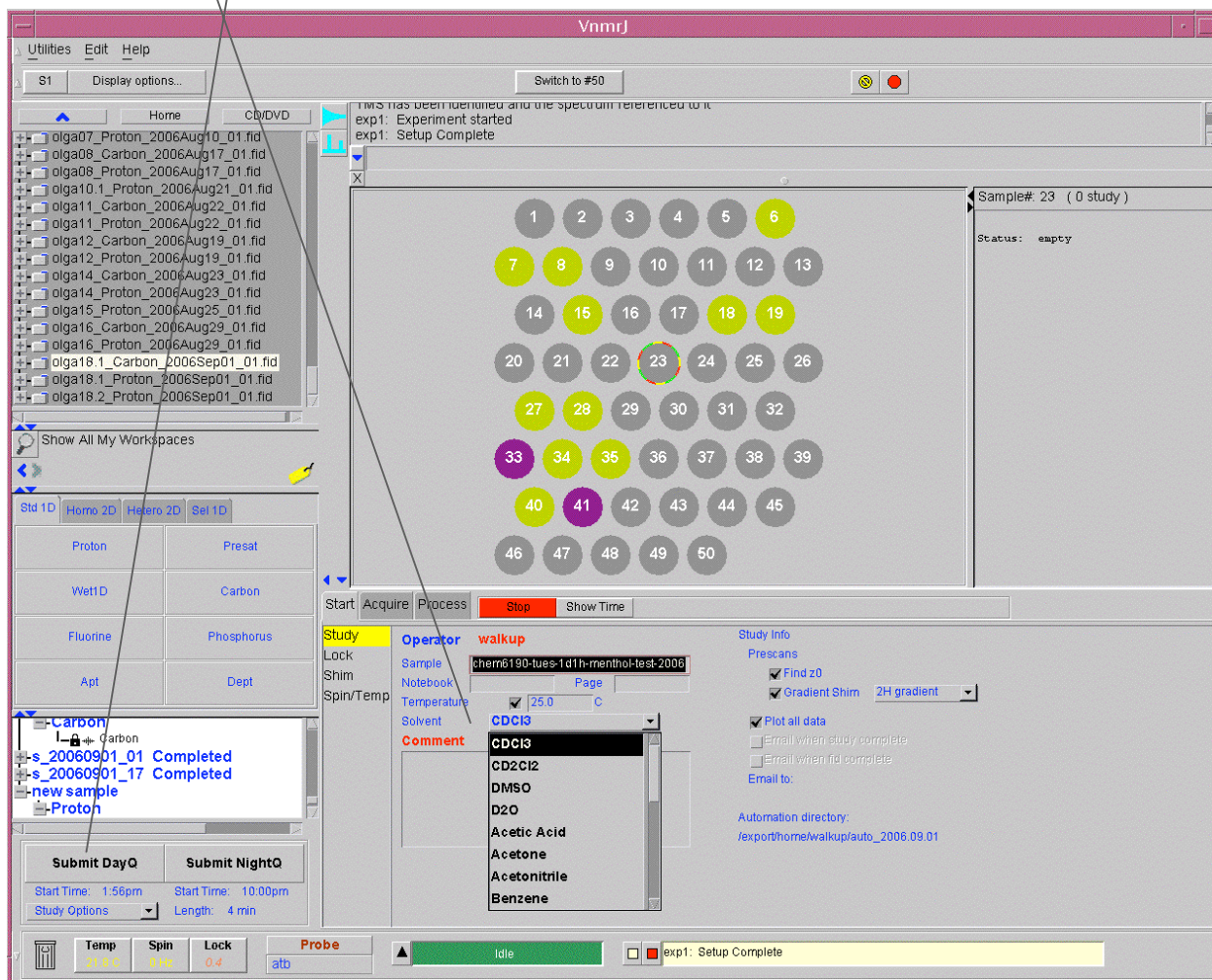


- click the **start tab**, and the **study** option
- in the **Sample** field, enter a **descriptive** sample name
 - no capital letters or spaces, no special characters other than dash (-) and underscore (_)
 - for instance, "chem6190-1d1h-090306"
- the **notebook**, **page**, and **comments** fields can be left blank



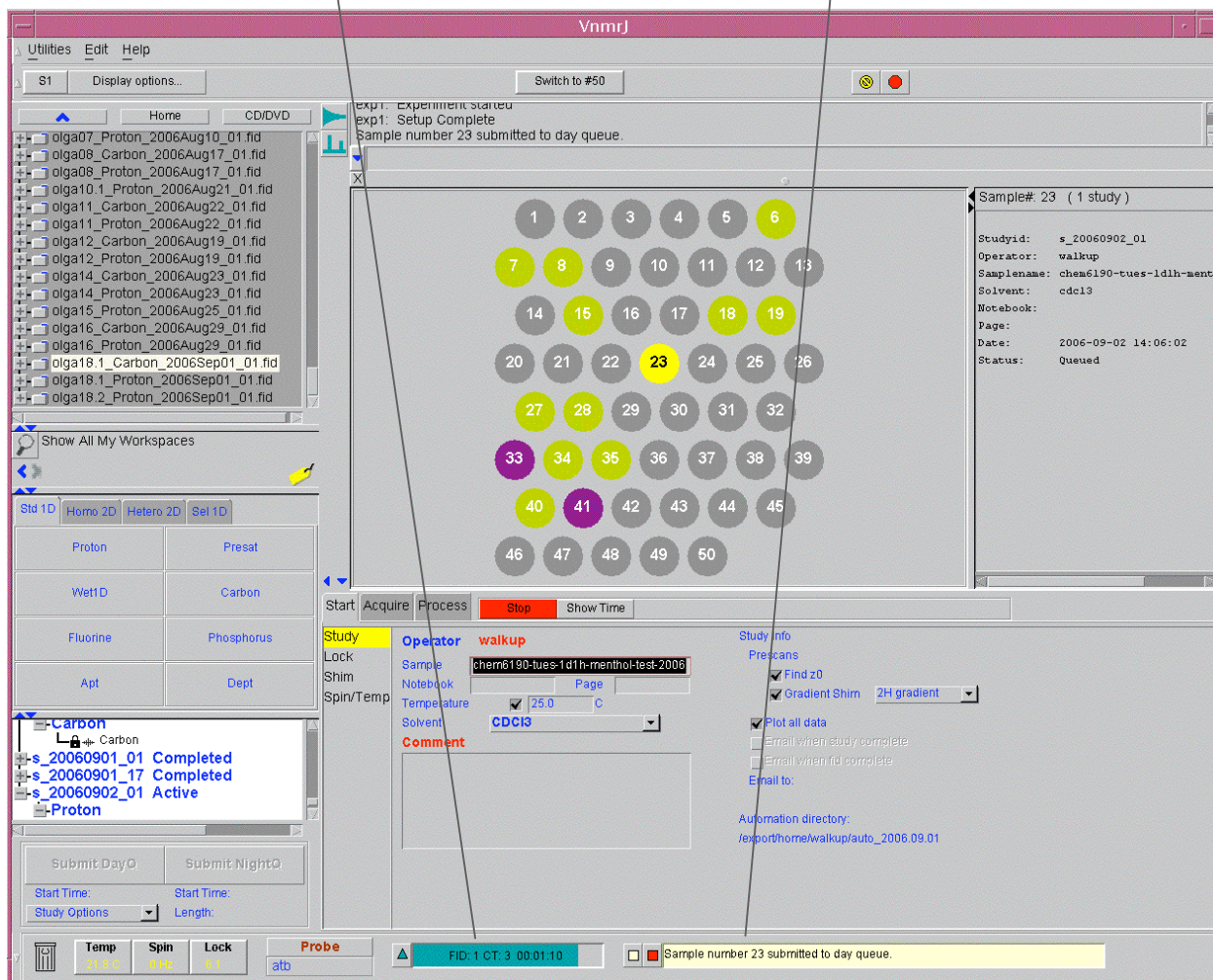
Setting parameters and submitting the sample

- do not change the Temp (temperature setting), or the Spin setting, or the Lock setting
- for **solvent**, select **CDCI3** (deuterated chloroform)
- click **Submit to Day Queue**
 - wait.....the robot is slow
 - the slot in the tray panel corresponding to your sample will turn yellow
 - the robot will eventually get your sample and place it in the magnet



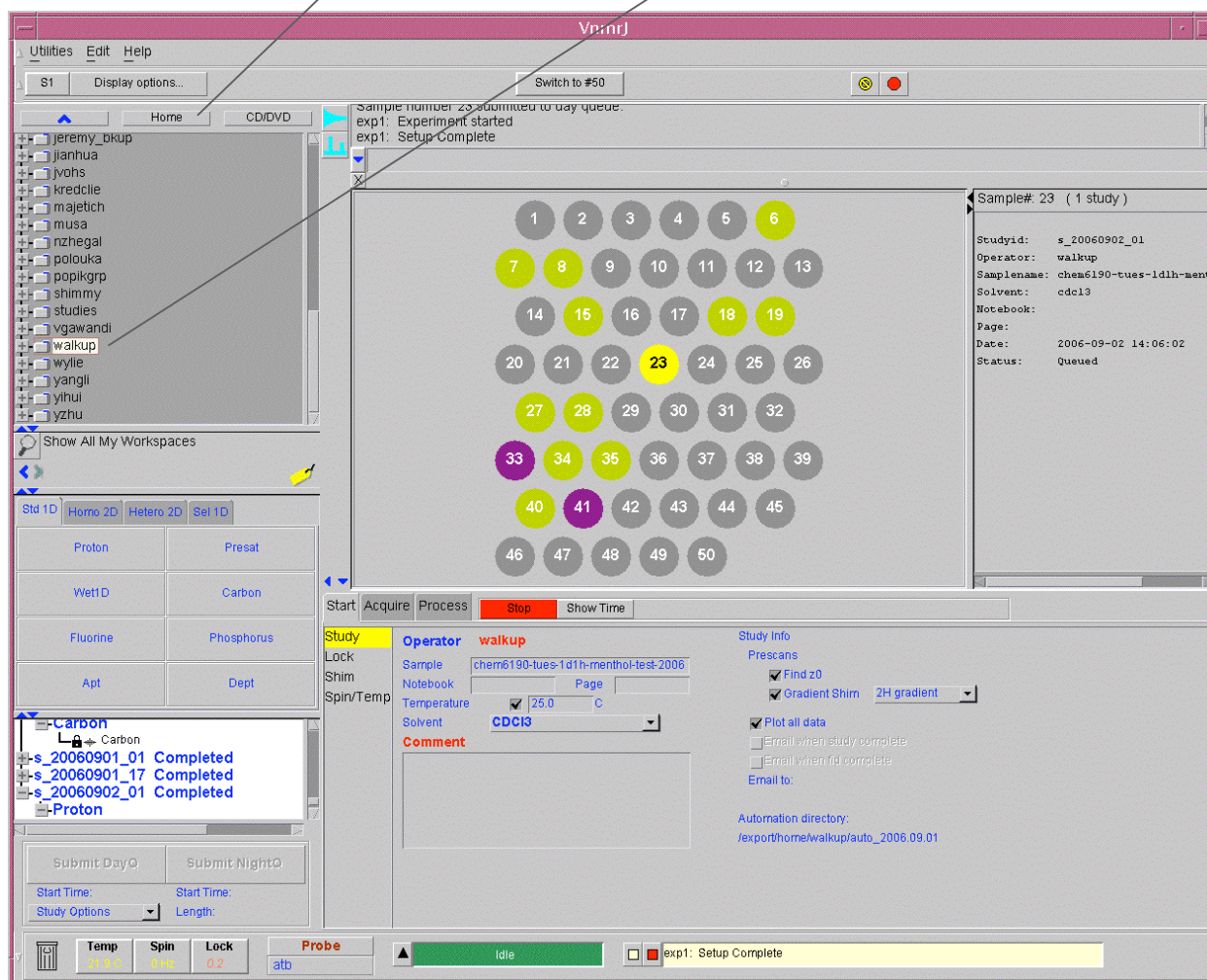
Locking and shimming

- in the automated mode, locking on the solvent and shimming (gradient shimming) are automated
- the **window** at the **bottom of the screen** keeps you informed on the locking and shimming progress
- another window at the bottom tells you that your **sample** has been **submitted** to the Day Queue

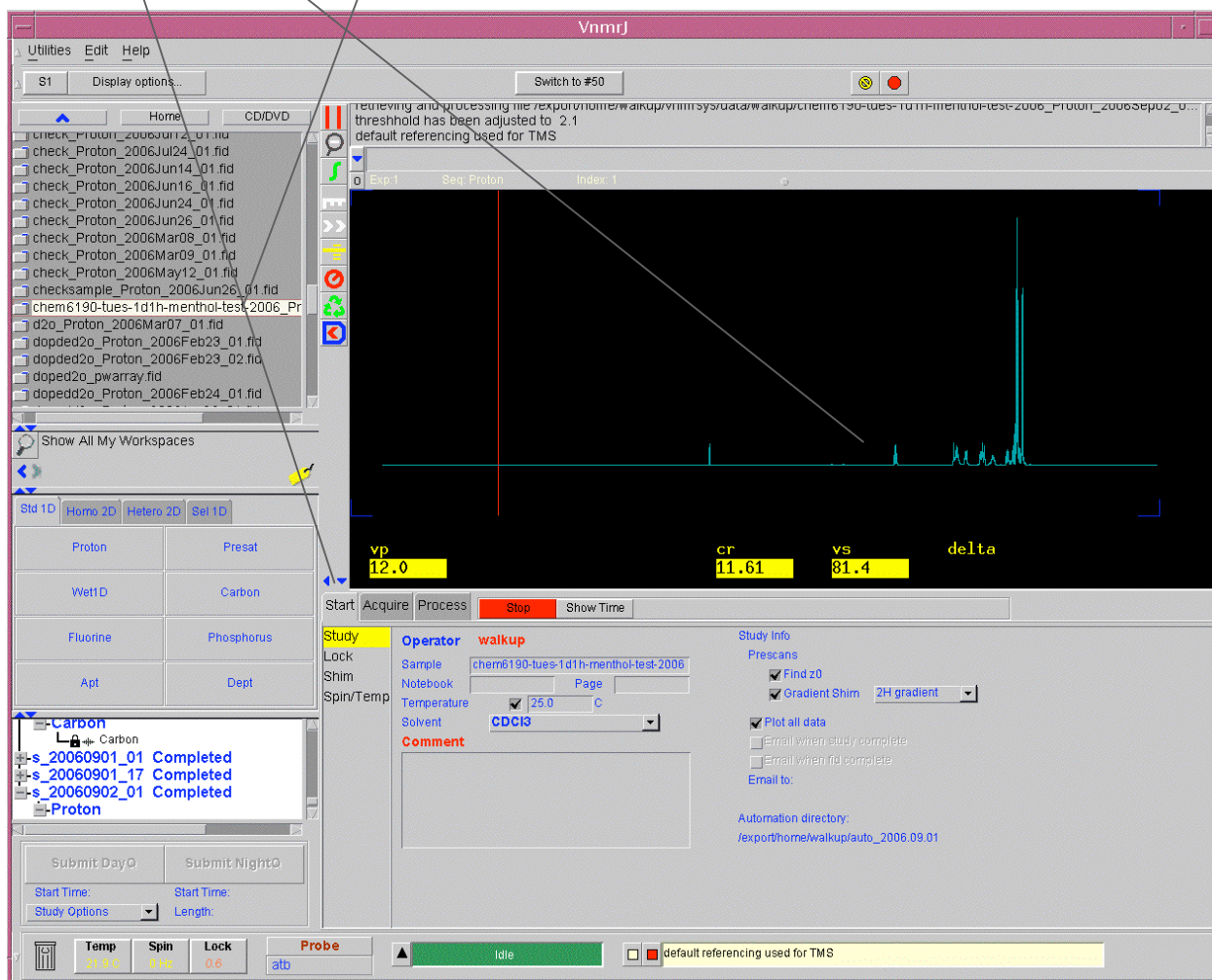


Retrieving the data and displaying the spectrum

-at the upper left, click the **Home** button, then double-click **walkup**.....

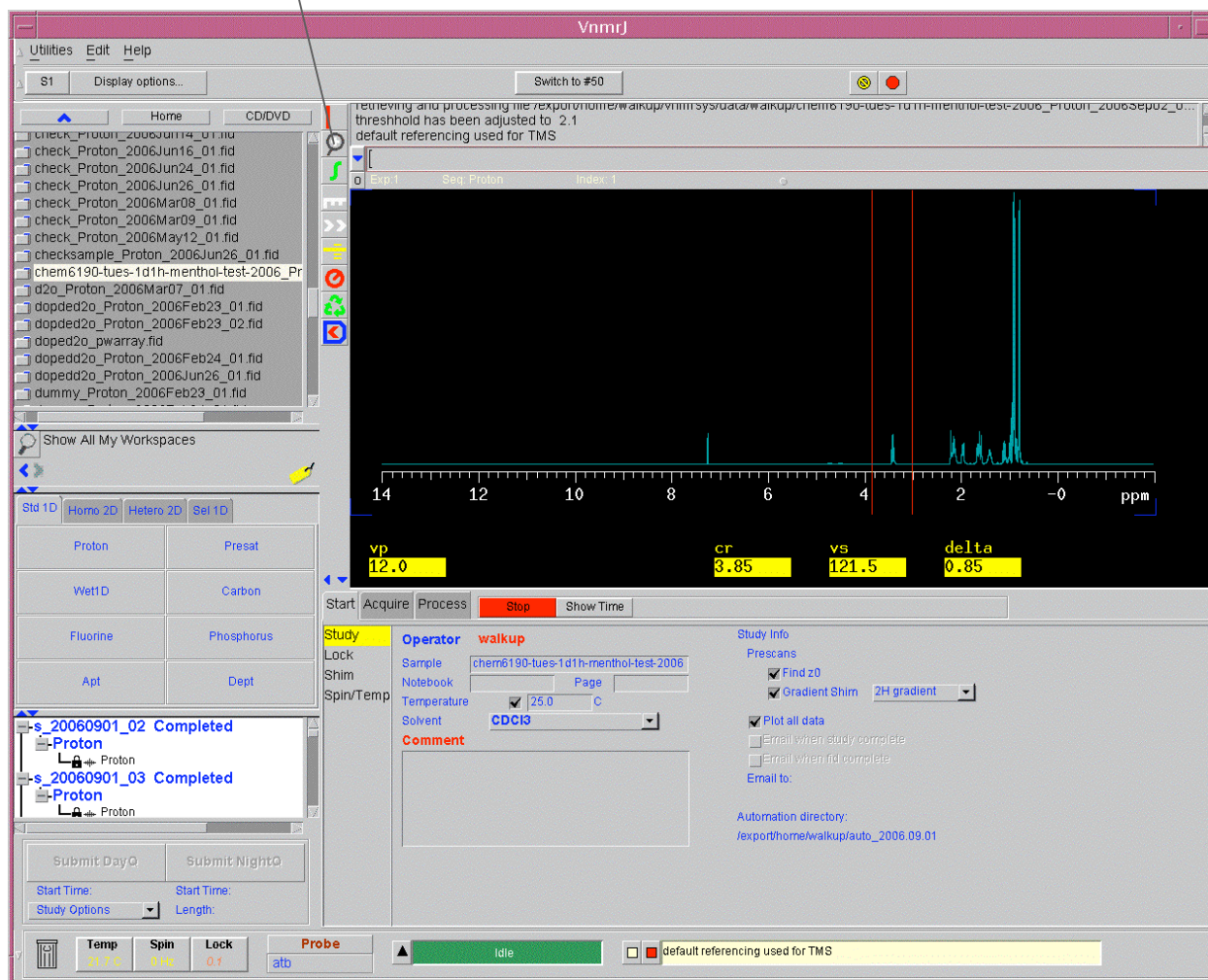


-then double-click on the **filename** that you gave to **your data**
- the **spectrum** should appear (in the window previously occupied by the tray panel)
 - the **two small blue arrow buttons** (◀ ▶) at the lower left of the spectral window permit this window to be expanded (to fill most of the screen) and contracted



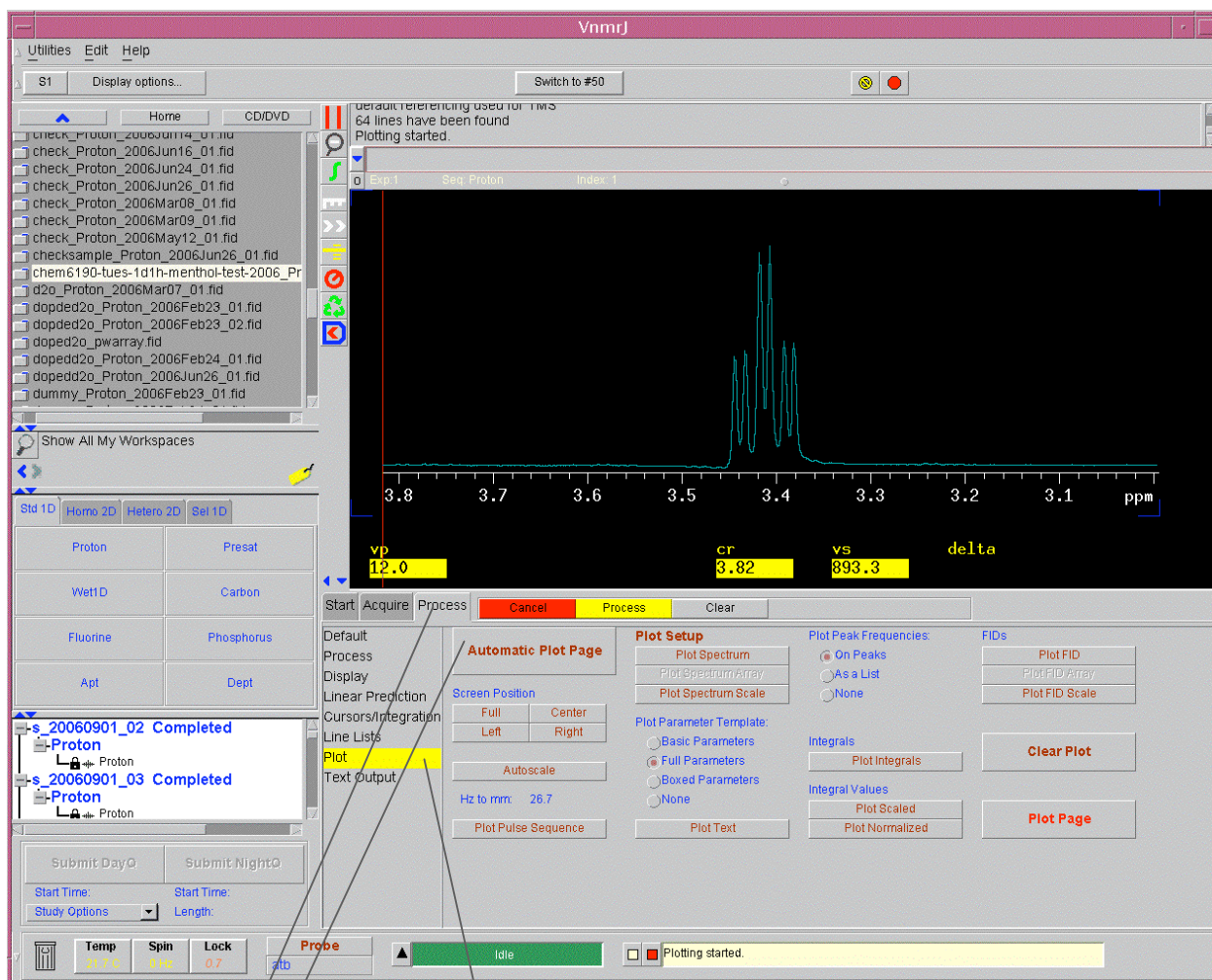
Zooming in and out

- to zoom in on a particular spectral region or peak of interest, place the **arrow cursor** at the **left edge** of this region and click the **left mouse button** (or drag the red cursor to the left side of the region of interest with the left mouse button), then move the **arrow cursor** to the **right edge** and click the **right mouse button**
- then click the **magnifying glass icon** (on the **vertical menu**) to toggle between the full display and the selected region



Adjusting scale

-to adjust the scale/intensity, **place the arrow cursor on a peak**, hold down the middle mouse button, and **move the mouse straight forward or backward** (*NOT sideways*)

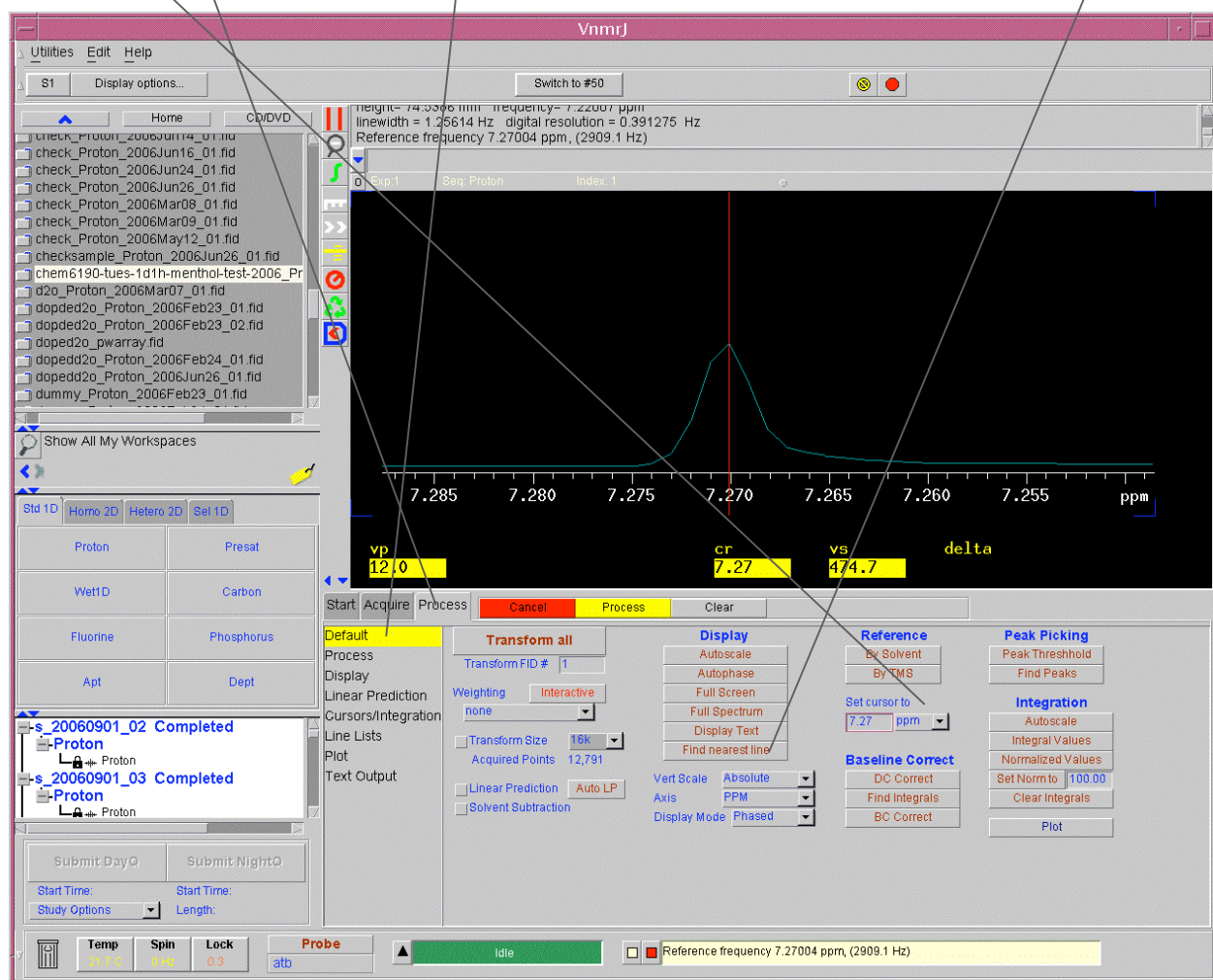


Quick plotting

-click on the **Process** tab and then the **plot option**
-click **Automatic plot page**

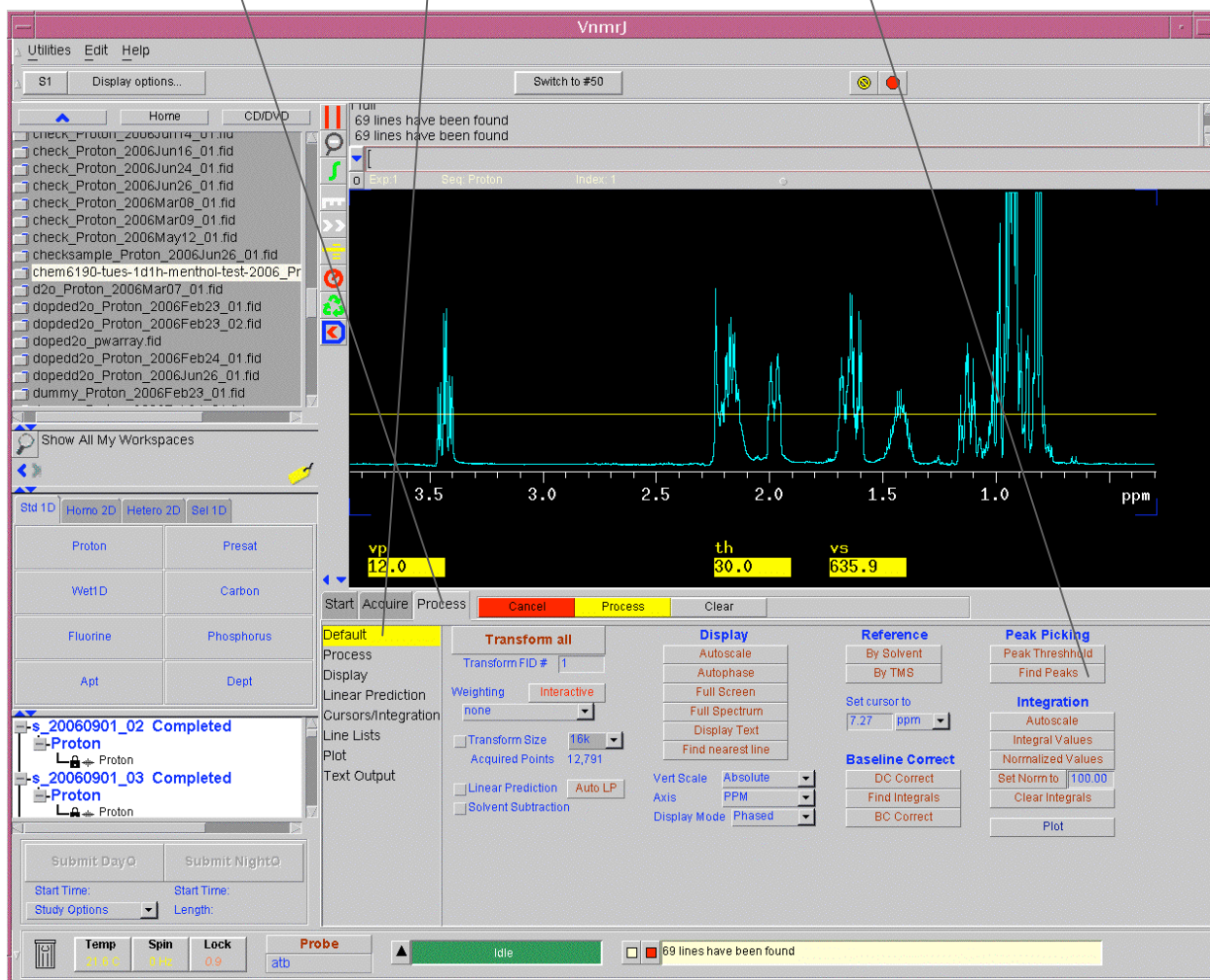
Chemical shift referencing

- the ^1H chemical shifts should be referenced relative to TMS at 0.0 ppm
 - when CDCl_3 is used as the solvent, there is a small amount of residual CHCl_3 in the sample, and the ^1H signal of CHCl_3 resonates at exactly 7.27 ppm relative to TMS
 - thus, we will reference the spectrum relative to this CHCl_3 peak (we will force the computer to recognize the chemical shift of this peak as 7.27 ppm).
- first, zoom in on the small signal at approximately 7.2 ppm
- click the **Process** tab, then select **default**
- set a red cursor (with the left mouse button) on the **center of the CHCl_3 signal** and click then **find nearest line** (under the **Display** options)
- in the **Set cursor to** field, select **ppm** from the drop down menu, enter **7.27** in the box and hit **return**

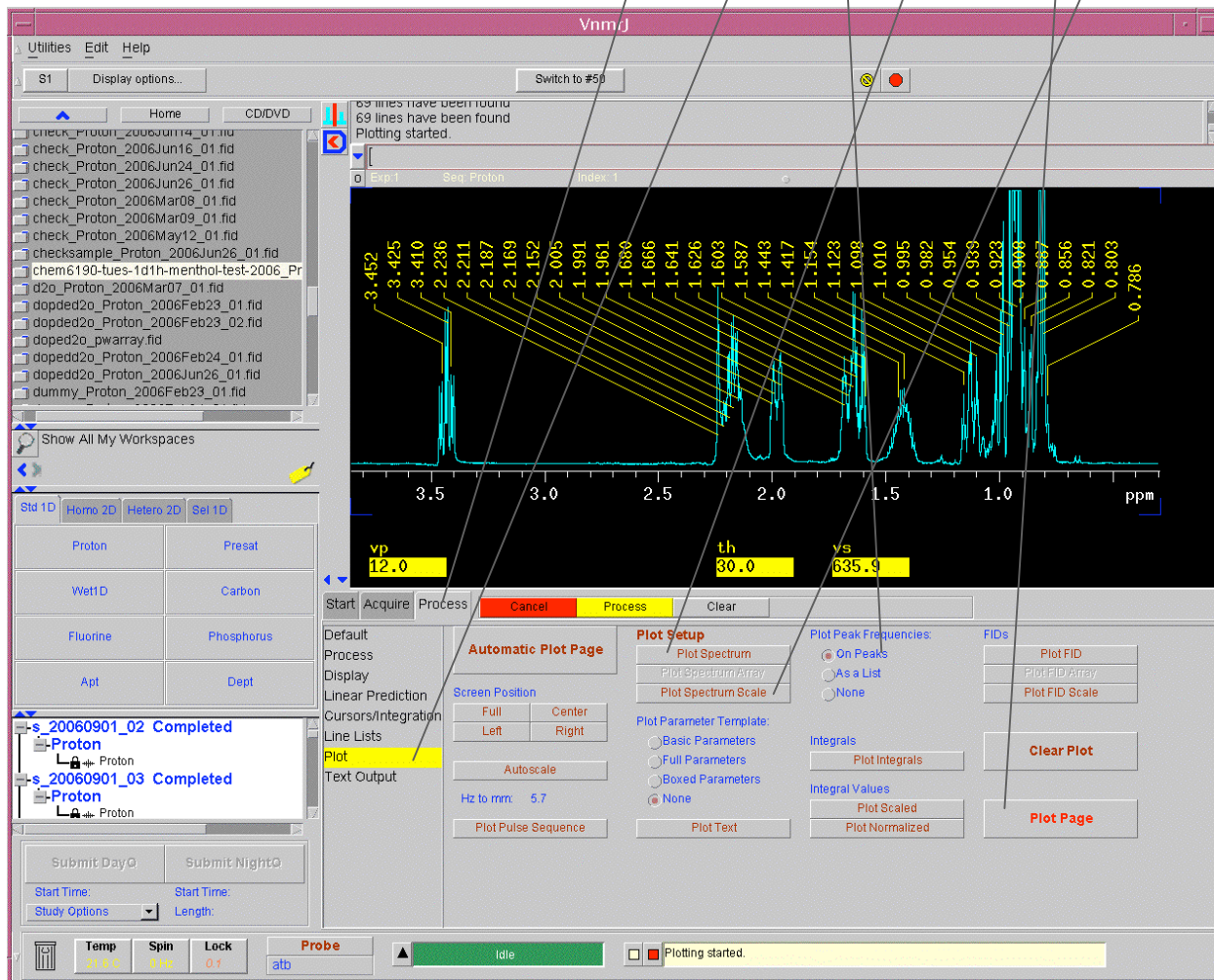


Peak picking

- peak picking** means determining what the chemical shifts are for the peaks in the spectrum
- first, zoom in on the peaks in your spectrum (the region from about 0 to about 4 ppm)
- first we need to set the **intensity threshold** (chemical shifts will be returned for all of the peaks with intensities above this threshold)
 - from the **vertical menu** at the left of the spectral display window, select the yellow **threshold icon** and move the **yellow line** up/down on the spectrum to the desired height
- click the **Process** tab, then the **Default** option, then click the **Find Peaks** button (under **Peak Picking**)
- the peak positions will be displayed on the screen
 - you can remove the displayed yellow lines and peak positions by selecting **Process/Display** and then clicking on **Full** under **Screen Position**

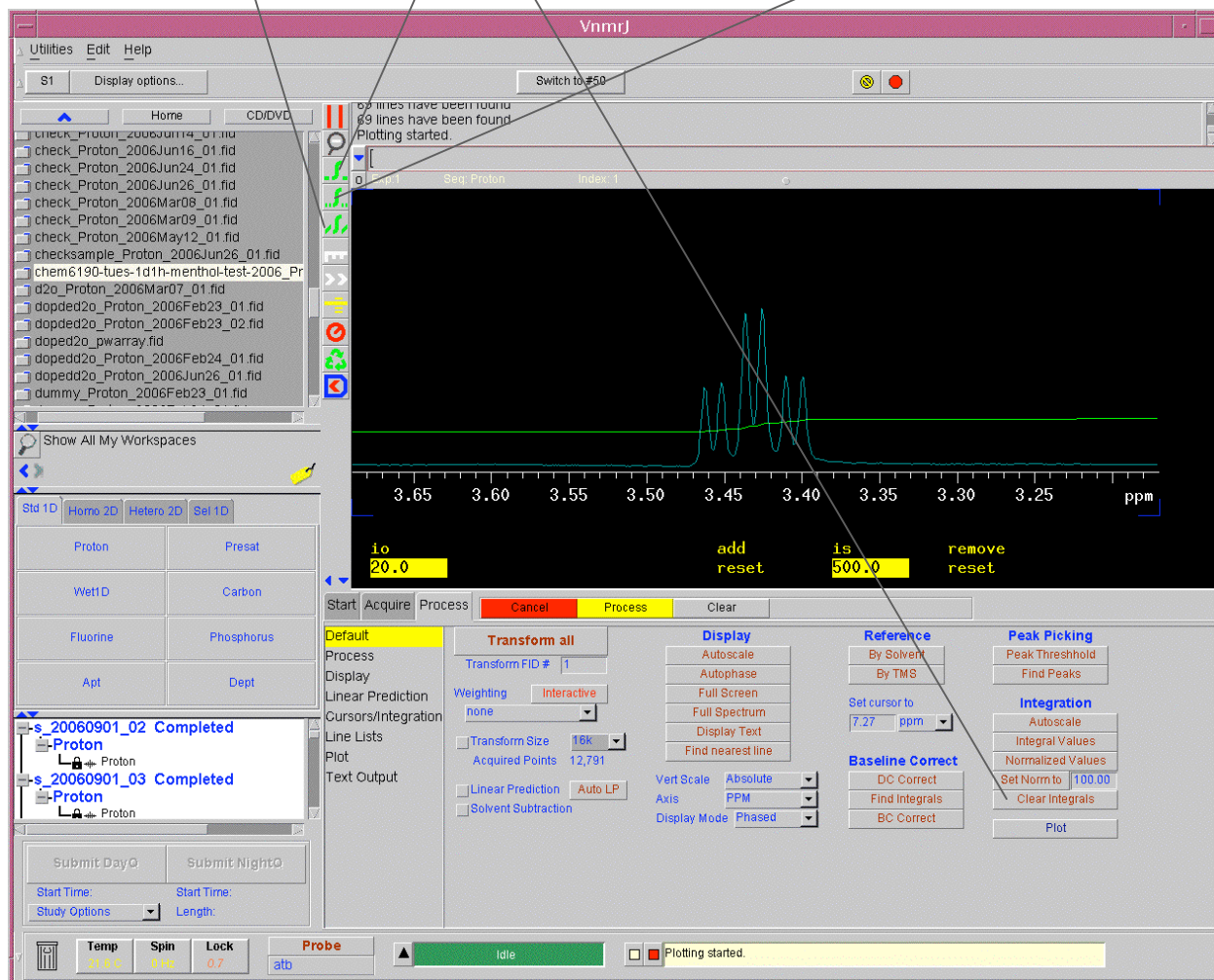


-in order to **print the peak positions** (hardcopy), under **Process/Plot**, click **Plot Spectrum**, then **Plot Spectrum Scale**, then under **Plot Peak Frequencies**, select **On Peaks**. Then click **Plot Page**
 -if you want to return to a display without the peak positions, select **Process/Display**, and then click **Full** under **Screen Position**. If the ppm scale disappears, click the **PPM** option under **Axis**

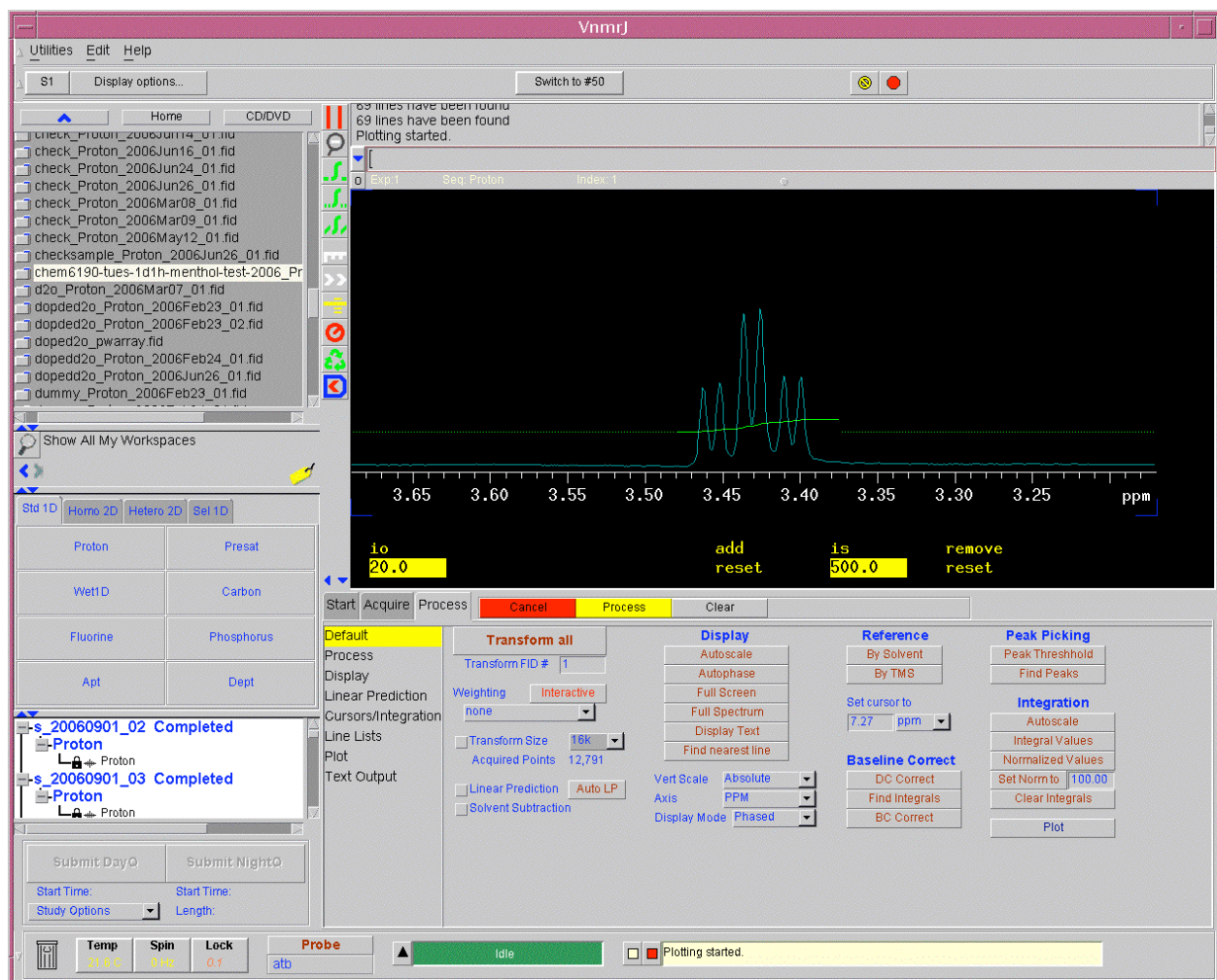


Integration

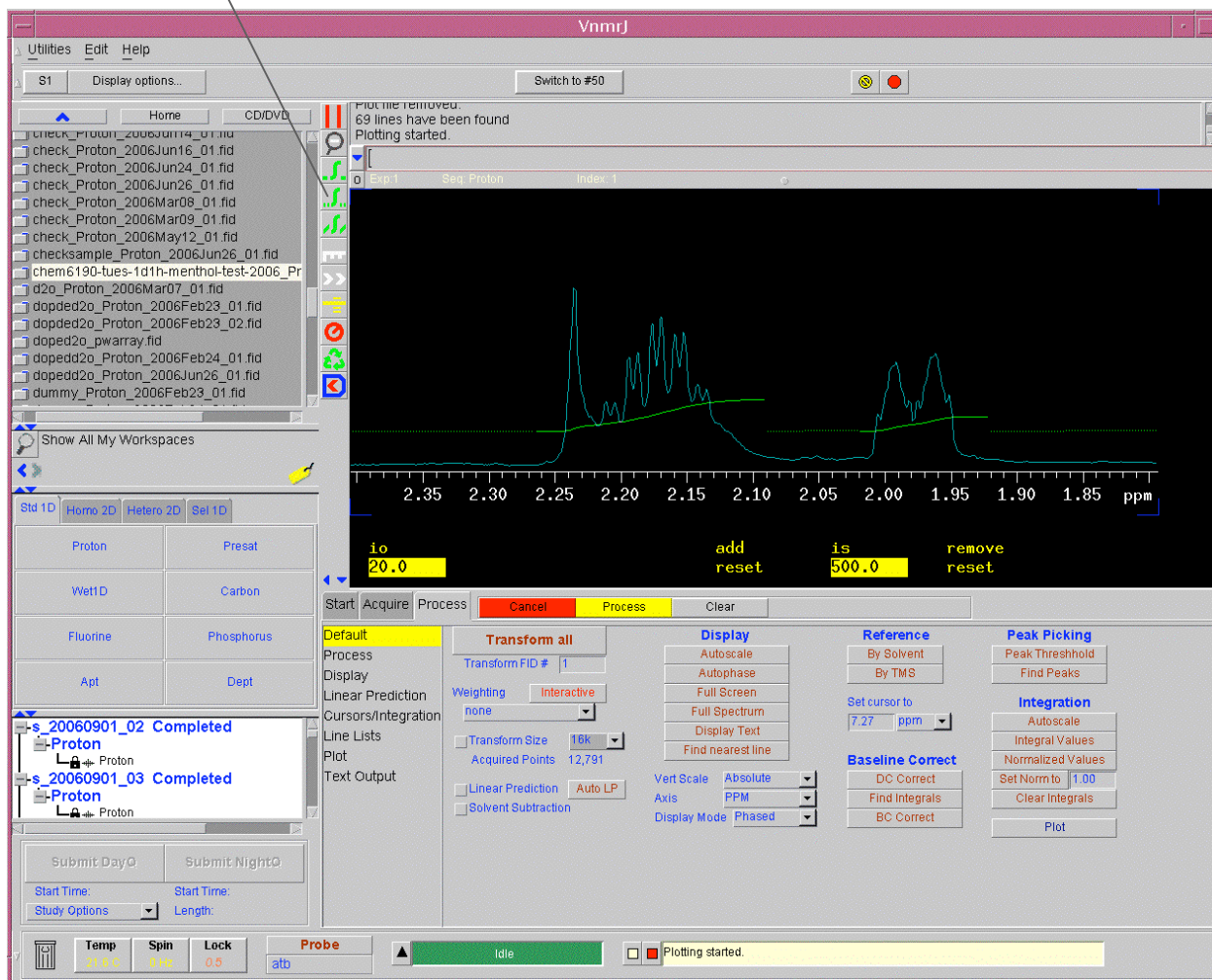
- first, **expand** the display around the multiplet signal centered at about 3.43 ppm
- on the vertical menu at the left is the **integral icon/button**
- as you click on this button you toggle through the **3 modes**: first is **part integral**, second is full integral, and third is **no integral**
- select the **part integral** mode
 - when this is selected, two more integral buttons will appear below the first: the **integral resets** and the **integral Lvl/Tlt**
- under **Process/default**, click **clear integrals** (under **Integration** options)
- then click the **integral resets** button



-then, place the cursor just to the **left of the peak** (at the baseline) and **click** (left mouse button) Then place the cursor just to the **right of the peak** and **click** (again, left mouse button).
 -you'll see that the green integral display changes to dashes except on the peak that you are integrating (which remains a solid green color)

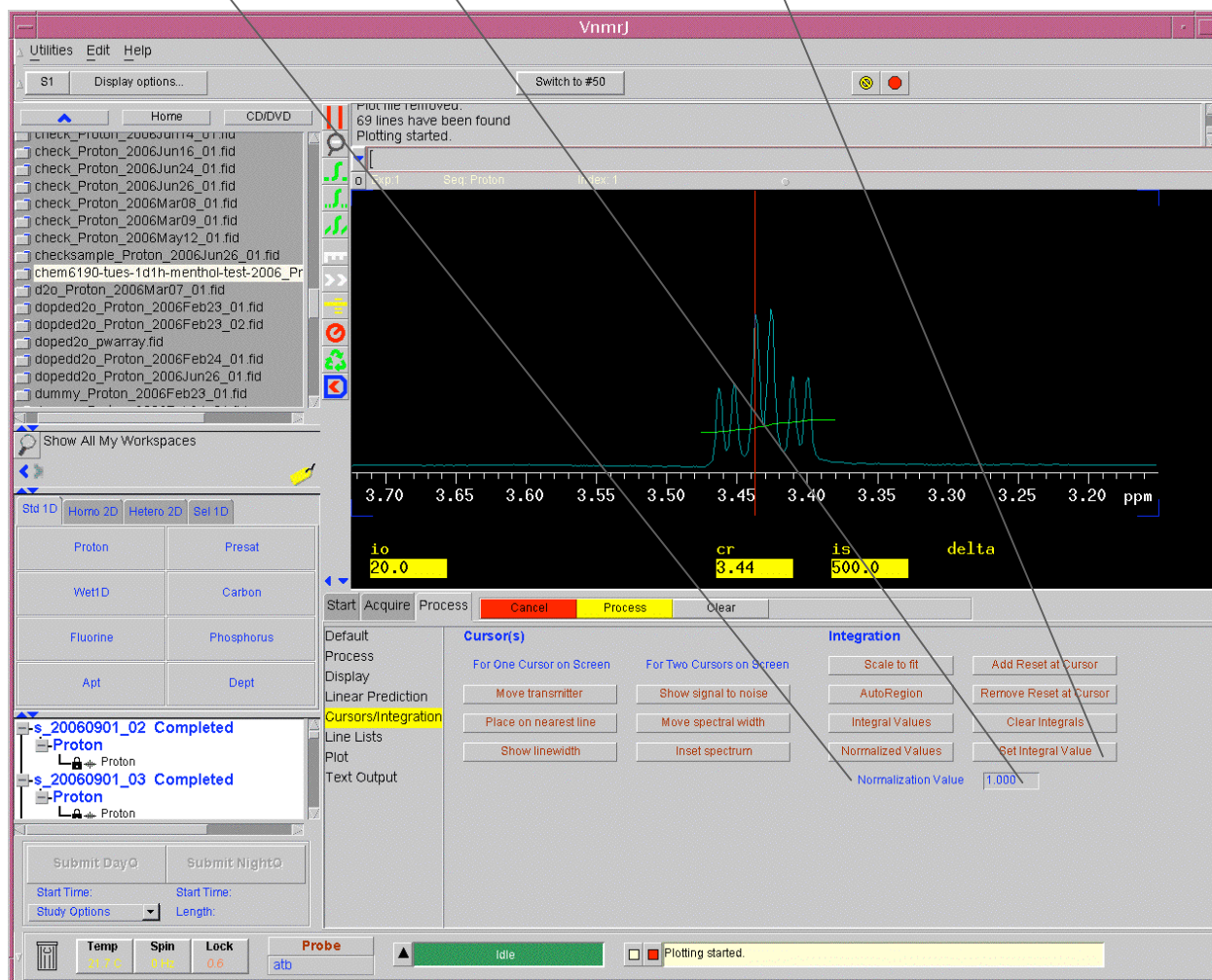


- expand around the next two signals in the spectrum (the two at approximately 2.2 and 2.0 ppm)
- click the **integral resets** button
- click on the right and left sides of each of these signals, as you have done for the previous signal
- repeat this for the rest of the signals in the spectrum



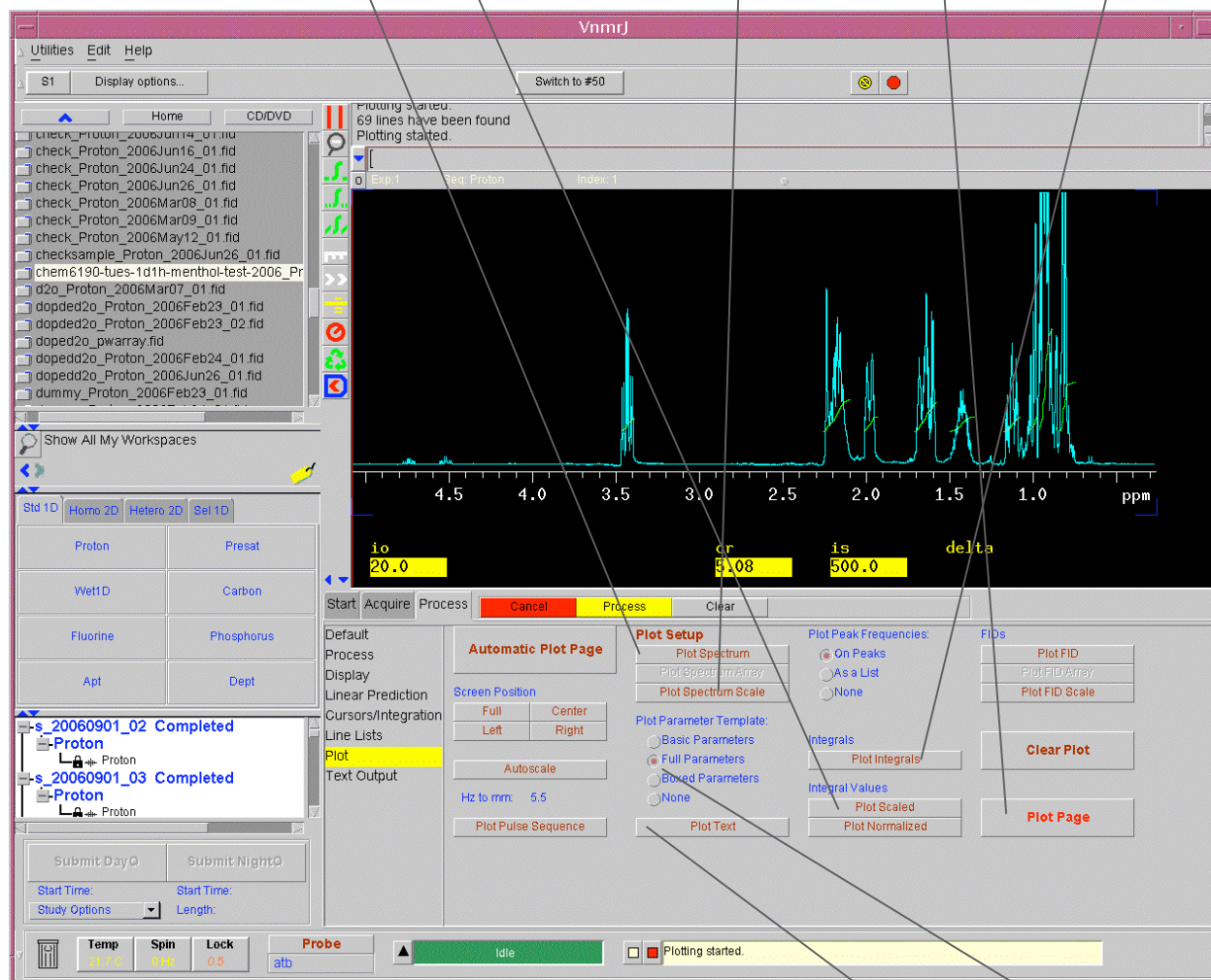
Integral calibration

- the **integrals** of signals are **proportional to the number of protons** giving rise to the individual signals
- it is convenient to **normalize the integration values** to simplify the analysis (enabling the analysis to be done by inspection)
- select a well-resolved signal, and, if possible, one that you suspect results from a single ^1H nucleus
- zoom on this peak**, put the **cursor on the peak**, select **Process** then **Cursors/Integration**, and in the **Normalization value field** put **1.0**. Then click **Set Integral Value**.



Advanced Plotting

- expand around the peaks in your spectrum (0 – 4 ppm)
- under **Process/Plot**, click **Plot Spectrum** and **Plot Spectrum Scale** (both under **Plot Setup**), **Plot Integrals** (under **Integrals**), **Plot Scaled** (under **Integral Values**), and then **Plot Page**
- the relative integral values will appear below the signals on the (hardcopy) plot



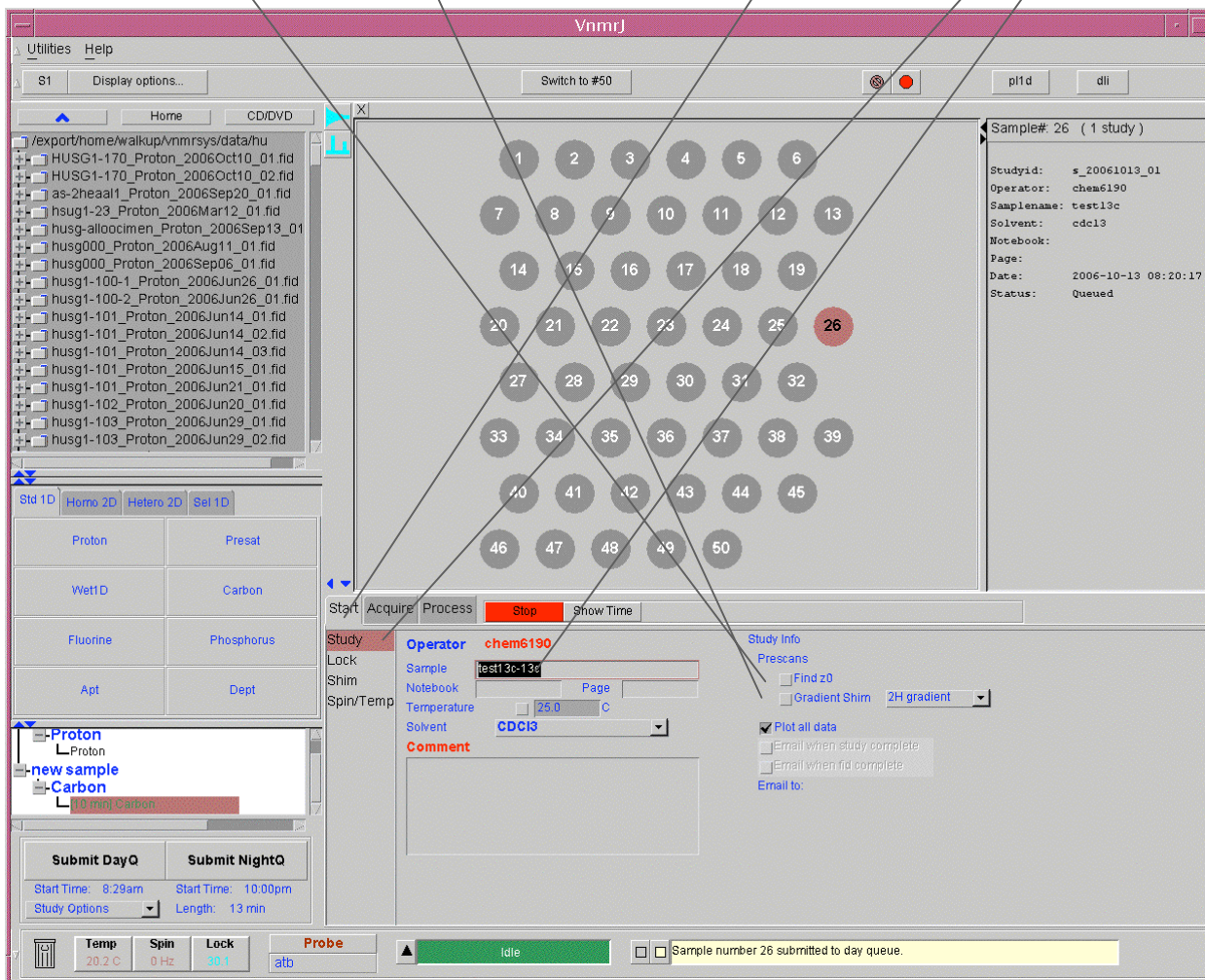
- try the above set of commands again, but in this case **include** clicking on **Plot Text** with the **Full Parameters** button selected
- this will **print the acquisition parameters** on the hardcopy plot. *You will note that these parameters are printed on the left side of the spectrum, so it is often a good idea to leave some blank space to the left of the peaks in your spectrum before you plot the parameters (i.e., in our case, zoom in on the region from about 5 ppm to 0 ppm, as above, in which case there is "blank space" between the left edge of the spectrum and the peak at 3.5 ppm)*

Logging out

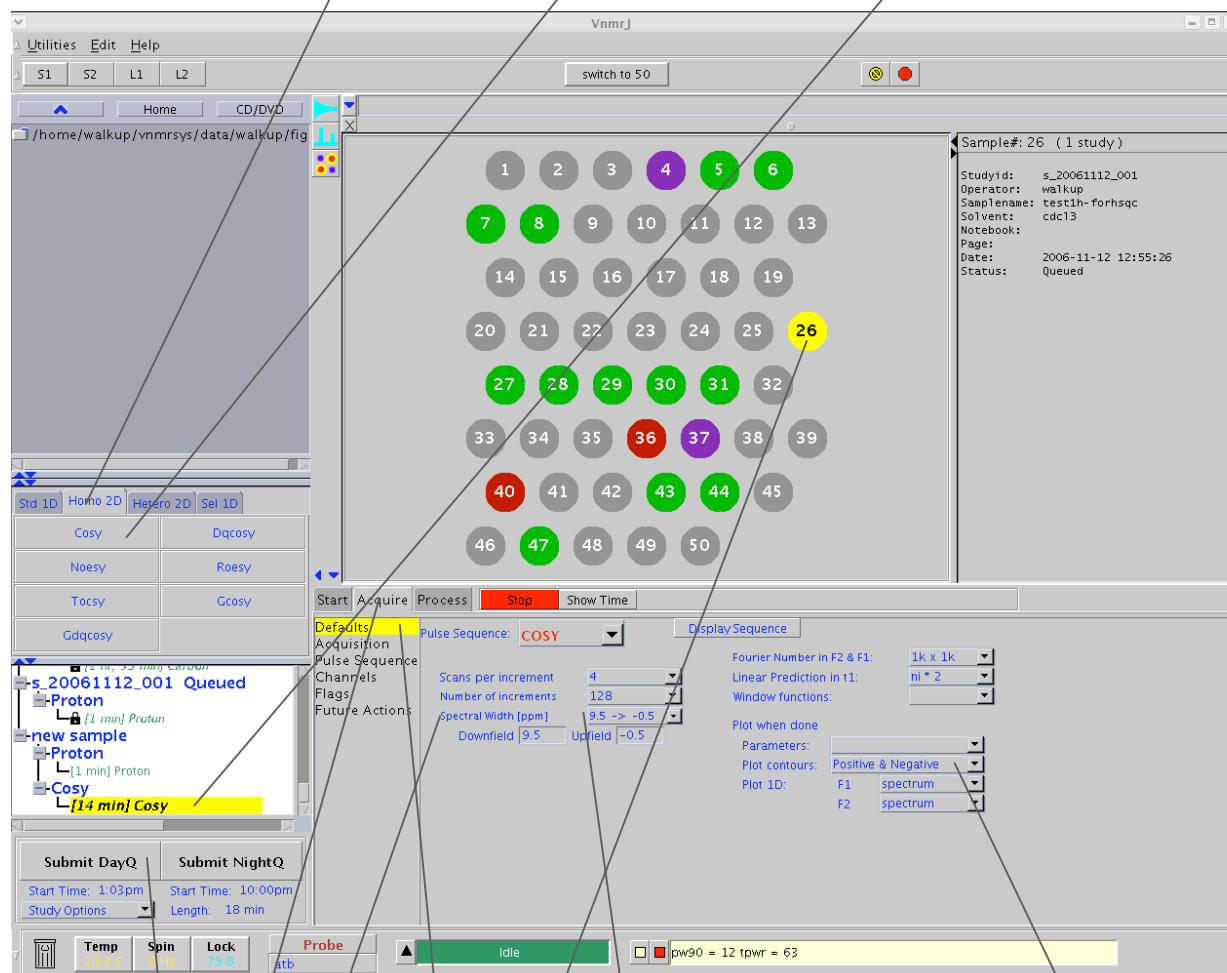
- it is best to remove your sample. Simply click the **Switch to #50** button
- in the extreme upper left of the screen click the **Utilities** button, and select **Change Operator** from the menu

2D ^1H , ^1H -COSY Spectrum Acquisition

- first, record a 1D ^1H spectrum of your sample (see pages 1-11)
- next, turn off the automated shimming routines by selecting the **Start** tab, then the **Study** option, and then **deselect Find Z⁰ and Gradient Shim**. Also, give your experiment a new name (**Sample**)



-then, as before, click the **Homo 2D** tab, then the **COSY** option, and then **double click** on the yellow-highlighted **COSY** selection

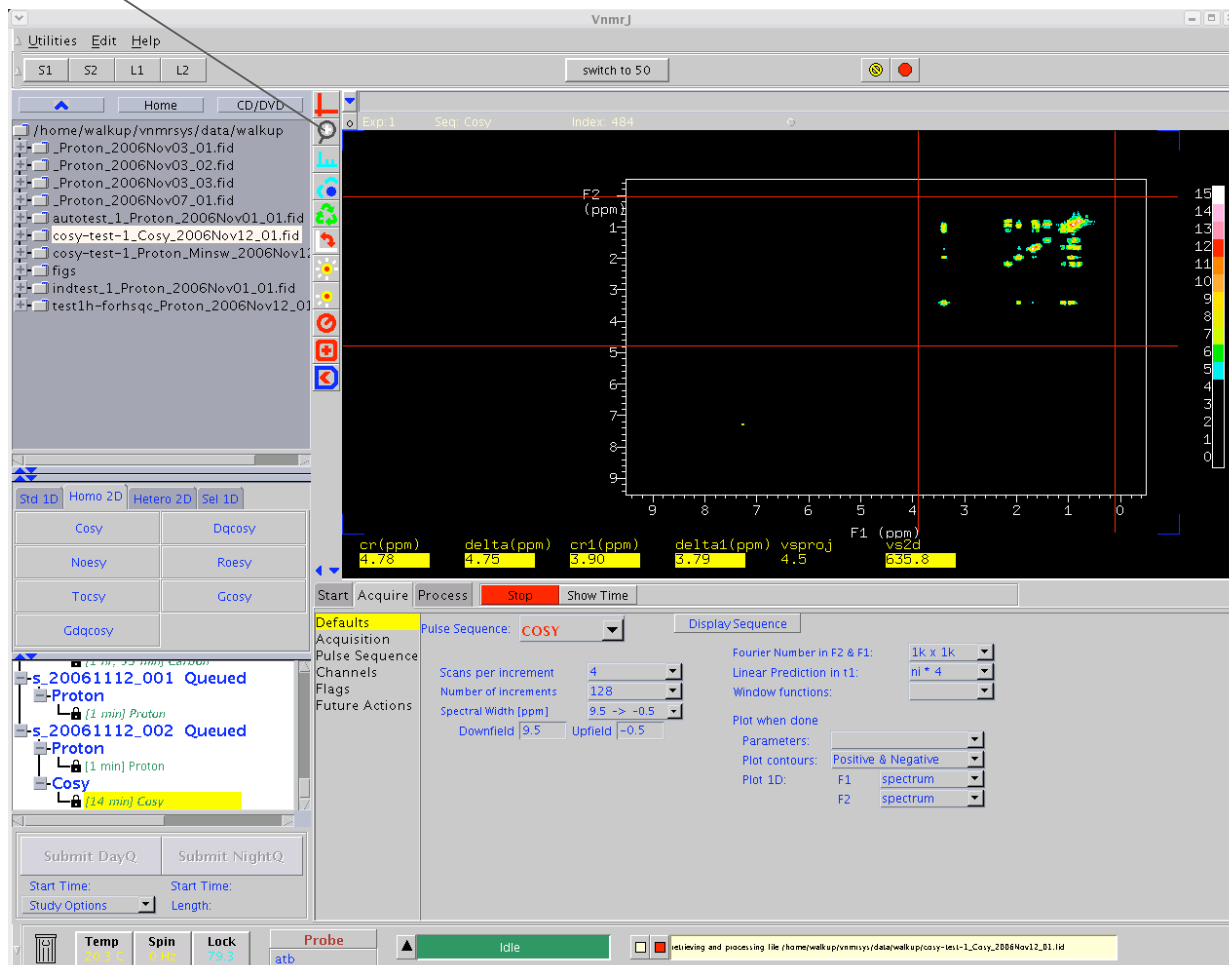


-then, click the **Acquire** tab and **Defaults**. You will see the default COSY parameters. From the drop-down menu for **Spectral Width [ppm]**, select **0.95 → -0.5**. Under **Plot when done**, select **Positive & Negative**.

-We will leave the other parameters at their default values as shown above.

-then, as before, we will **select our sample number** in the sample tray (26 if our sample was 26) and **Submit to Day Queue**

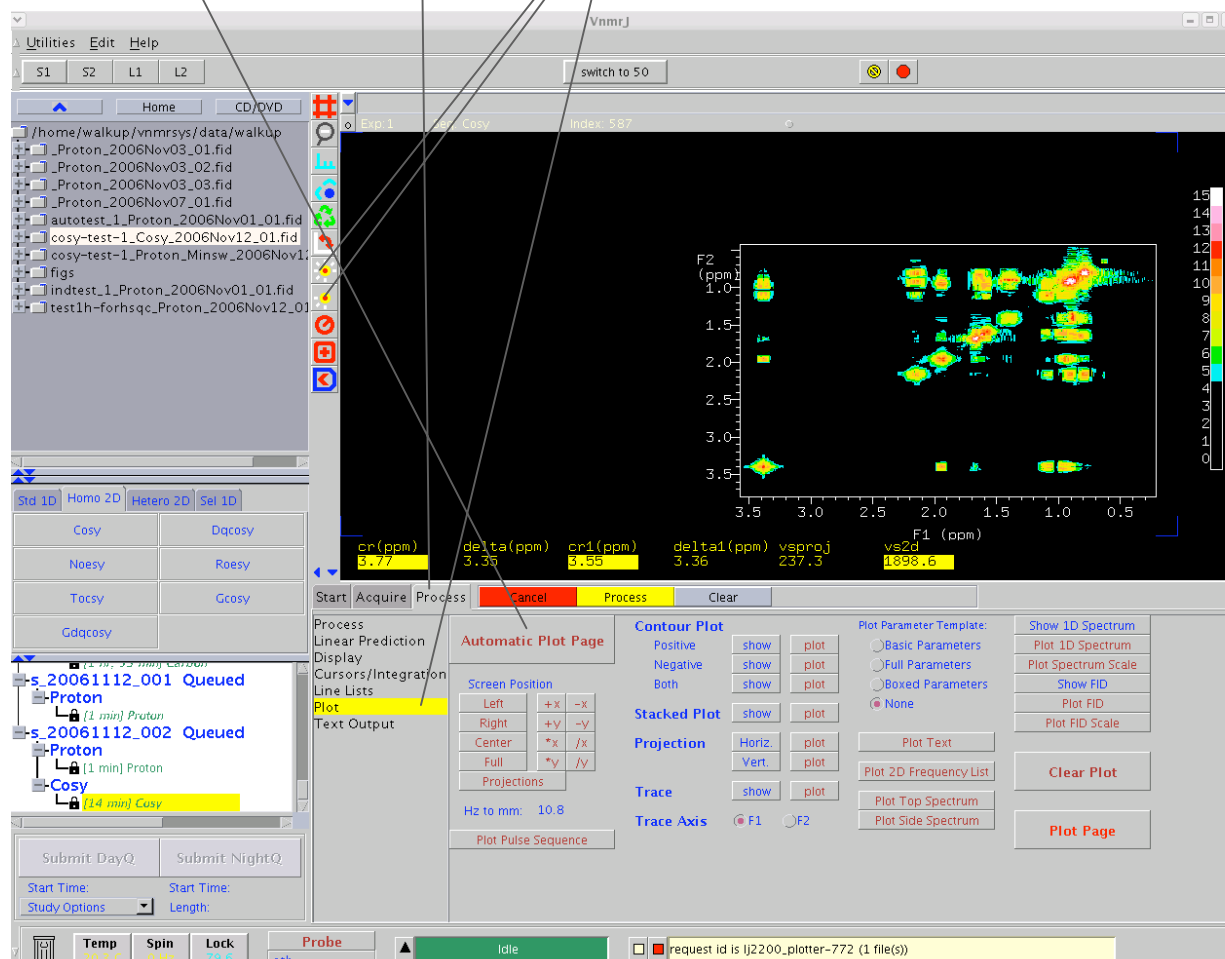
-retrieve the data as usual
 -you'll see the full spectrum plotted on the screen
 -expand around the region from about 4 ppm to about 0 ppm. You should use the **left mouse button** to **position the corners of a box** defined by the cursors to the **lower left** of the box defined by the data. Then use the **right mouse button** to position a new set of cursors to the **upper right**. Then **click the expand button**.



-you can adjust the contour level by using the two **contour adjustment level** buttons

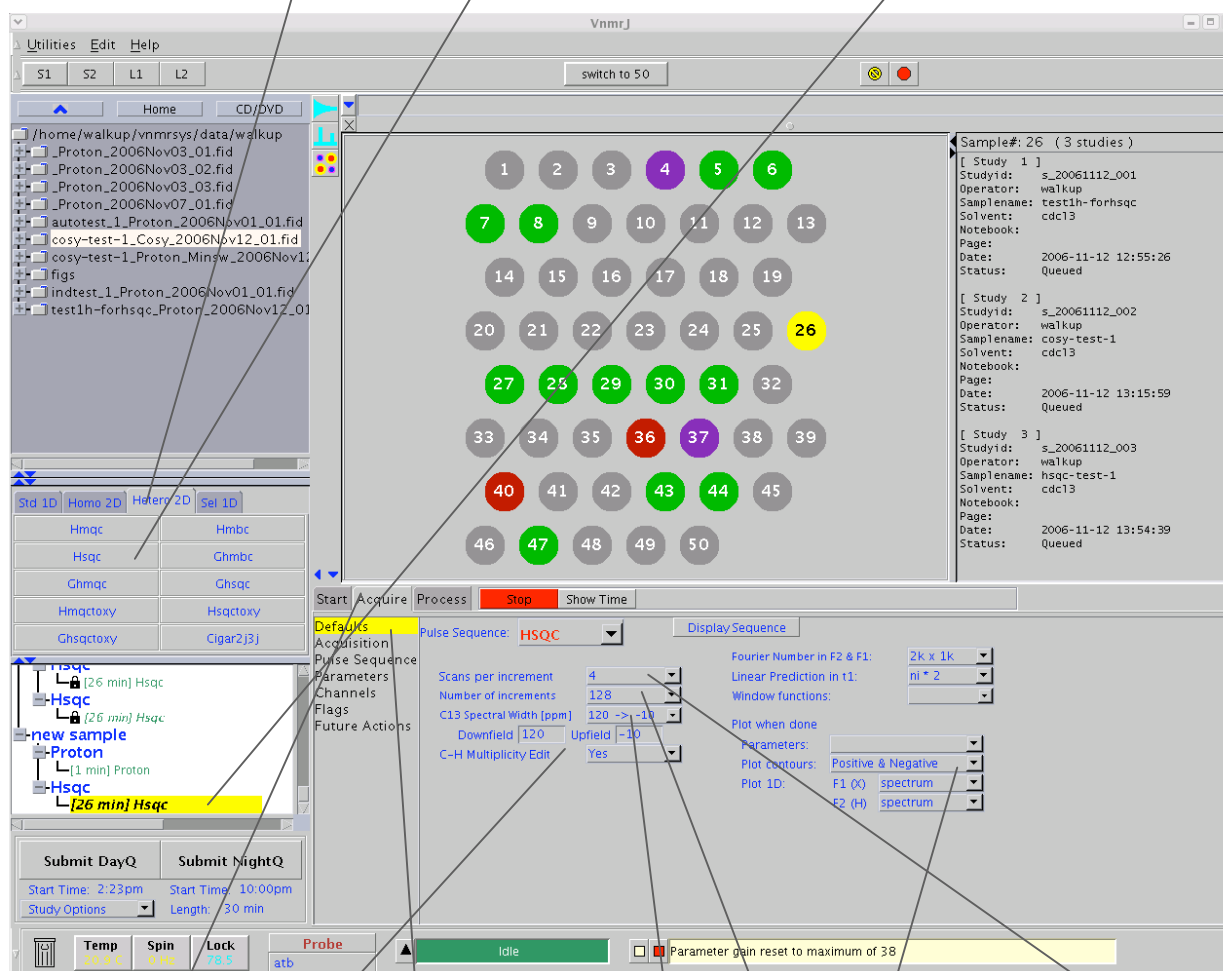
-to plot the spectrum click the **Process** tab and the **Plot** option to show the 2D plotting options. Click **Automatic Plot Page**.

-you should plot the spectrum at several different contour levels. First at a very high level so that only intense peaks can be seen, and then at successively lower levels to identify weak peaks



^1H , ^{13}C -HSQC Acquisition

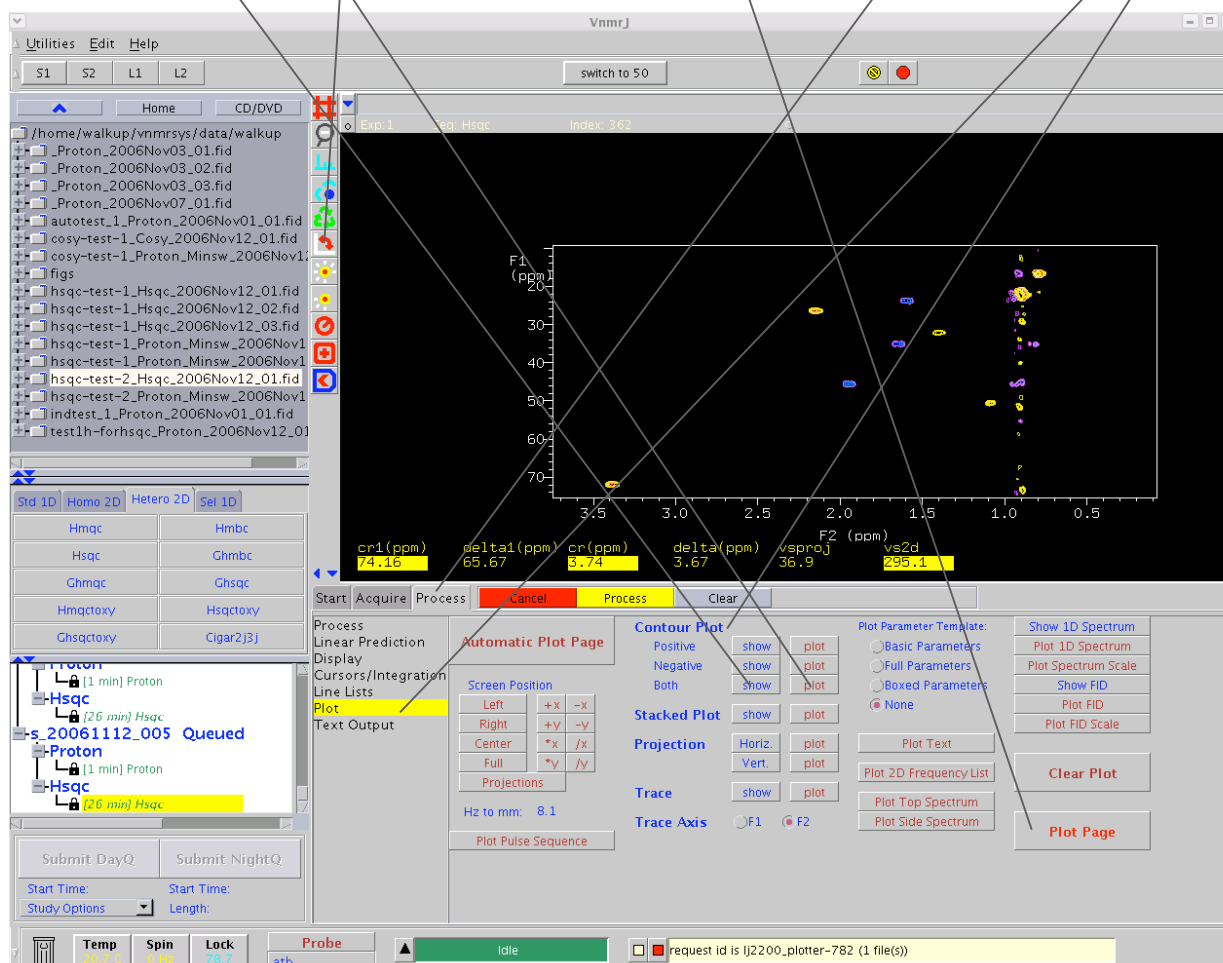
- once you have acquired a homonuclear COSY spectrum, acquire a 2D ^1H , ^{13}C -HSQC spectrum
- as before, you should select the **Start** tab and the **Study** option and name your experiment/data
- now, click the **Hetero 2D** tab, then **Hsqc**, then **double click** on the **yellow Hsqc** in lower window



- click the **Acquire** tab and the **Defaults** option to see the HSQC options
- under **C-H Multiplicity Edit** choose **Yes**. For **C13 Spectral Width [ppm]** choose **120 → -10**. For **Scans per increment** choose **8**, and for **Number of increments** choose **64**. For the rest of the options we will use the default parameters as shown above (make sure that **Plot contours** is set to **Positive & Negative**)
- select our sample in the sample tray menu and then click **Submit DayQ** as always.

-retrieve your data as usual

- as with the COSY spectrum, zoom in on the region of the spectrum where your peaks are
- you should click the **reverse axes** button to make the x axis the ^1H (F2) dimension
- then, plot the data at several different contour levels if necessary. Under the **Process** tab and the **Plot** option, you can simply choose **Automatic Plot Page** if you like. It is better, however, if, under **Contour Plot** you click **show** and **plot** under **Both**, and then click **Plot Page**.



Logging out

- first, go back to **Start/Study** and select **Find Z⁰** and **Gradient Shimming**
- it is best to remove your sample. Simply click the **Switch to #50** button
- in the extreme upper left of the screen click the **Utilities** button, and select **Change Operator** from the menu

Goals for Lab #4:

- 1). Acquire a 2D ^1H - ^1H COSY spectrum .
-plot the spectrum (contour plots at different elevations/levels)
- 2). Acquire a 2D ^1H , ^{13}C -HSQC spectrum of your sample
-plot all the spectra, etc.

Exercises and Questions for Lab #4:

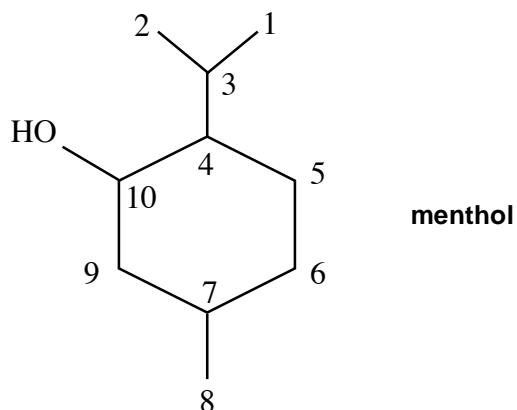
Please collect all the figures showing your 2D COSY data and 2D HSQC data and include them in your report. Please provide a typed report.

On the last page of this handout is shown the 1D ^1H spectrum of menthol with the peaks labeled. The labels correspond to the protons on the menthol molecule shown on that page. These are the correct assignments that most of you arrived at using the decoupling data from lab #2.

- 1). Using the spectrum and assignments on the last page of this handout, **make a table of the chemical shifts of the protons of menthol (these may be approximate shifts – do your best)**. Using these chemical shifts, **draw a complete ^1H , ^1H -COSY spectrum of menthol**. Make the simplifying assumption that each proton gives a diagonal peak centered at its Larmor frequency (no splitting) and each off-diagonal peak (cross-peak) appears as a single peak (not 4 peaks) centered at the Larmor frequencies of the nuclei giving rise to it. (NOTE: You should use a spreadsheet or graphing program to “draw” the spectrum. It is easiest to make a table of the chemical shifts in the spreadsheet and then simply “plot” the spectrum. Excel will work, as will any number of programs, like Origin, Kaleidagraph, etc.. If you like, you can draw it by hand). You should also assume that only 2- and 3-bond couplings give rise to crosspeaks.
- 2). On one of the 2D plots of the actual ^1H , ^1H -COSY of menthol that you acquired, **label every cross-peak using the assignments shown on the last page of this handout**. For instance, for the cross-peak between proton 10 (at 3.4 ppm) and one of the protons at C8, label it (10,8e). Label the cross-peak between proton 10 and the other proton at C8 (10,8a). Label the symmetric cross-peaks (8e,10) and (8a,10). **Compare the actual spectrum (i.e. all plots and all contour levels) with the one that you drew in question 1 above. Which crosspeaks do you not observe in the actual spectrum (provide a list of all of them)? In each case, why do you think that you do not observe a particular crosspeak? Are there any crosspeaks that appear in the actual spectrum that you did not predict? If so, what are these and why do you think that they are observed.**
- 3). For laboratory #3 you collected ^{13}C and DEPT spectra of menthol, and attempted to assign the ^{13}C chemical shifts of menthol based upon these data, etc. Now, **add these chemical shifts to the table you made in “1” above** (i.e. add a column or row to the table above, “ ^{13}C assignments from lab #3” or something). Then, using the ^1H and ^{13}C chemical shifts in your table, **draw the predicted ^1H , ^{13}C HSQC spectrum of menthol**.
- 4). Using the data from page 5 (the correct ^1H assignments for menthol) and the ^1H , ^{13}C -HSQC spectrum that you collected, **make the correct ^{13}C chemical shift assignments for menthol. Add these chemical shifts to the table that you made above** (make a new column or row, “correct ^{13}C assignments” or something). **Also, label each peak on the 2D ^1H , ^{13}C -HSQC plot with the correct assignment (using the structure and assignments on the last page of this handout).**
- 5). Using 250 words or more (please, not much more), discuss how you would attempt to make complete chemical shift assignments for a molecule like menthol, using what you now know about chemical shift assignment experiments. Discuss the strategy you would use, its strengths and weaknesses, including the experiments you would use, and perhaps those you would not, and the order that you would perform them and why.

SAMPLE:

Our sample is ~0.5M menthol in CDCl_3



position	^1H Chemical Shift or Shifts(ppm)	^{13}C shifts from lab # 3	^{13}C Chemical shifts from HSQC, lab # 4	Comments?
1	0.81			
2	Etc.			
3				
Etc.				

