

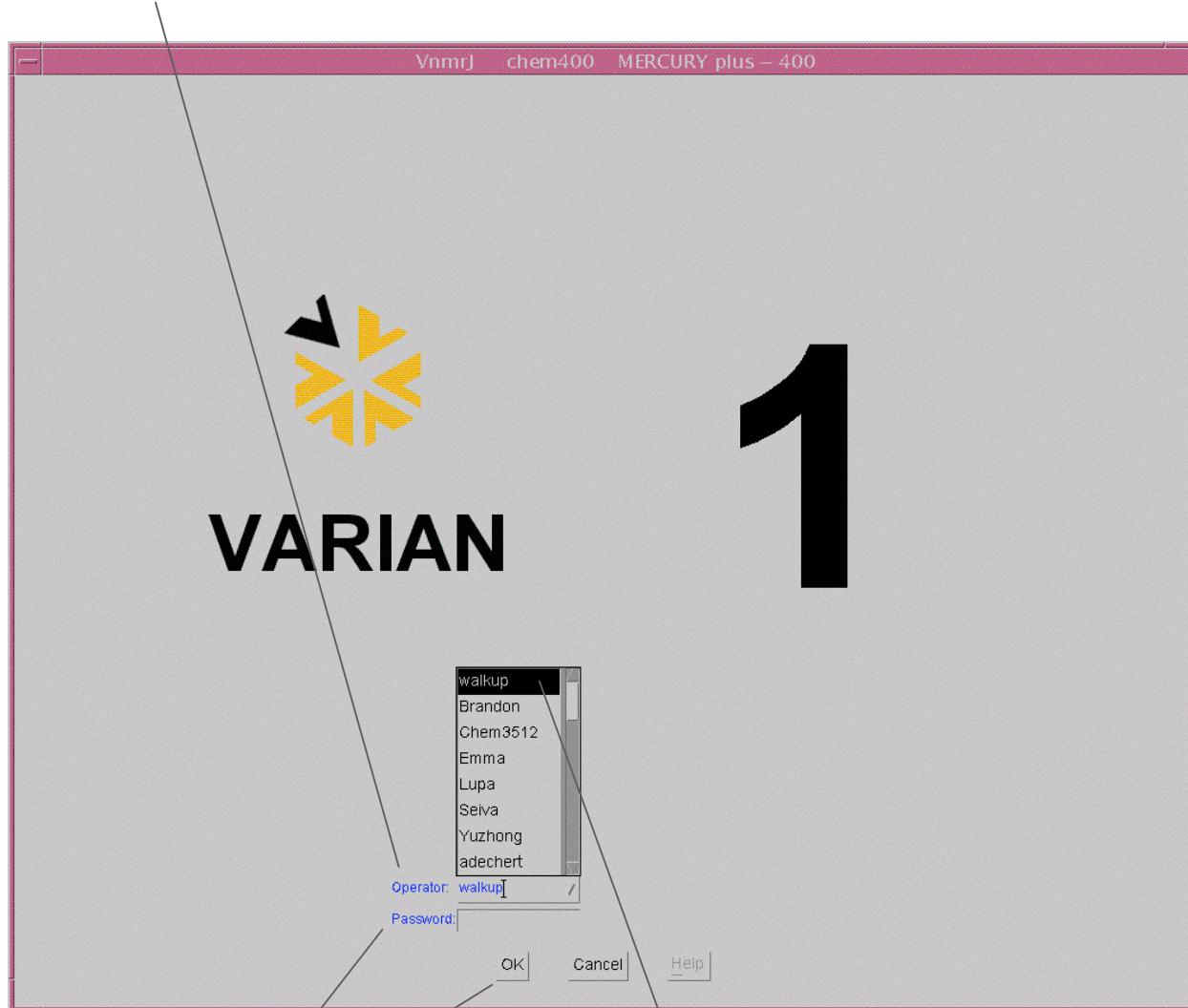
Lab #5

1D selective NOE and 2D ^1H , ^1H -TOCSY and NOESY

- 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 selective NOE spectra analysis
- 2D TOCSY and NOESY spectra collection and plotting

Login

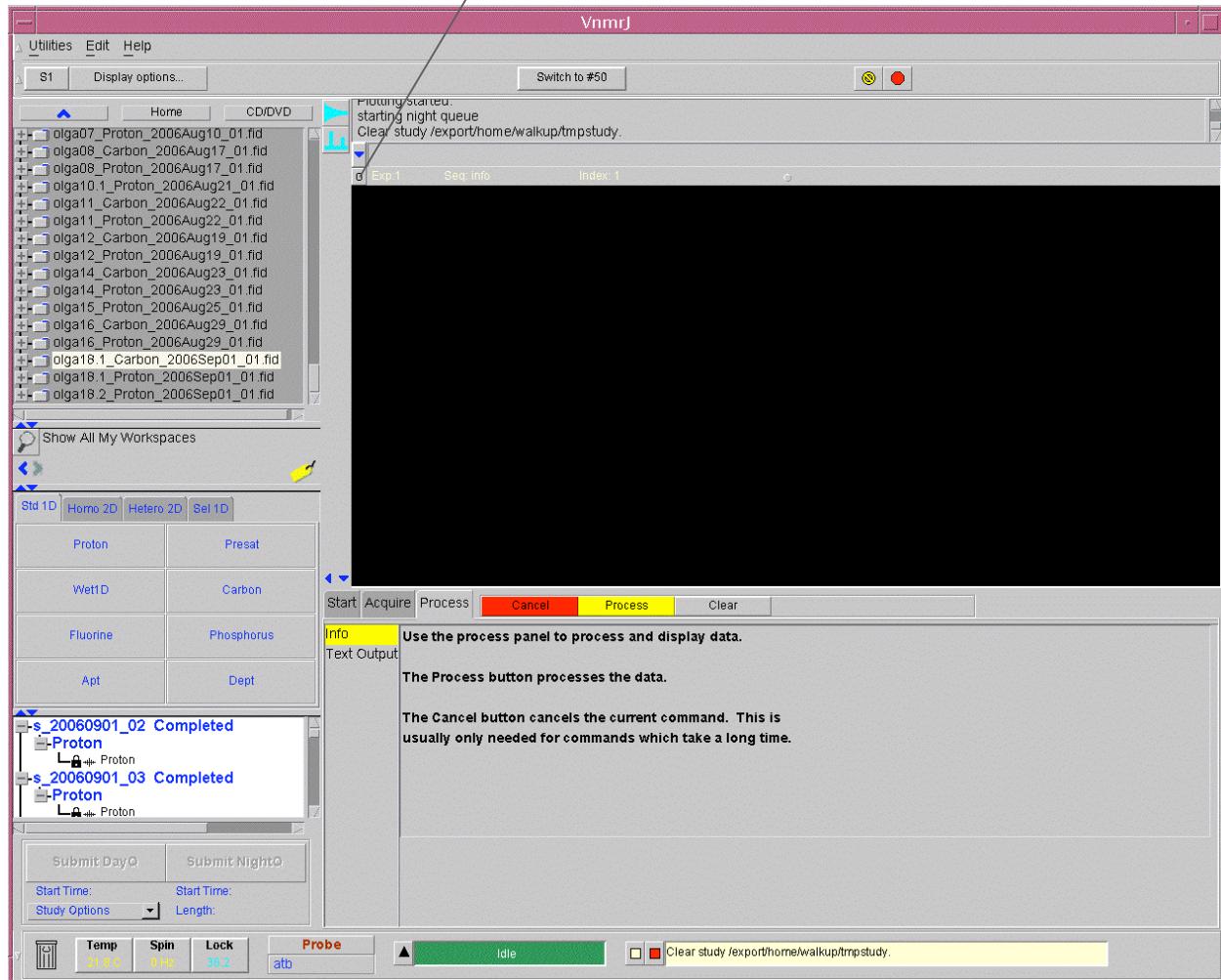
-the operator screen should be in view when you first sit down at the spectrometer console:



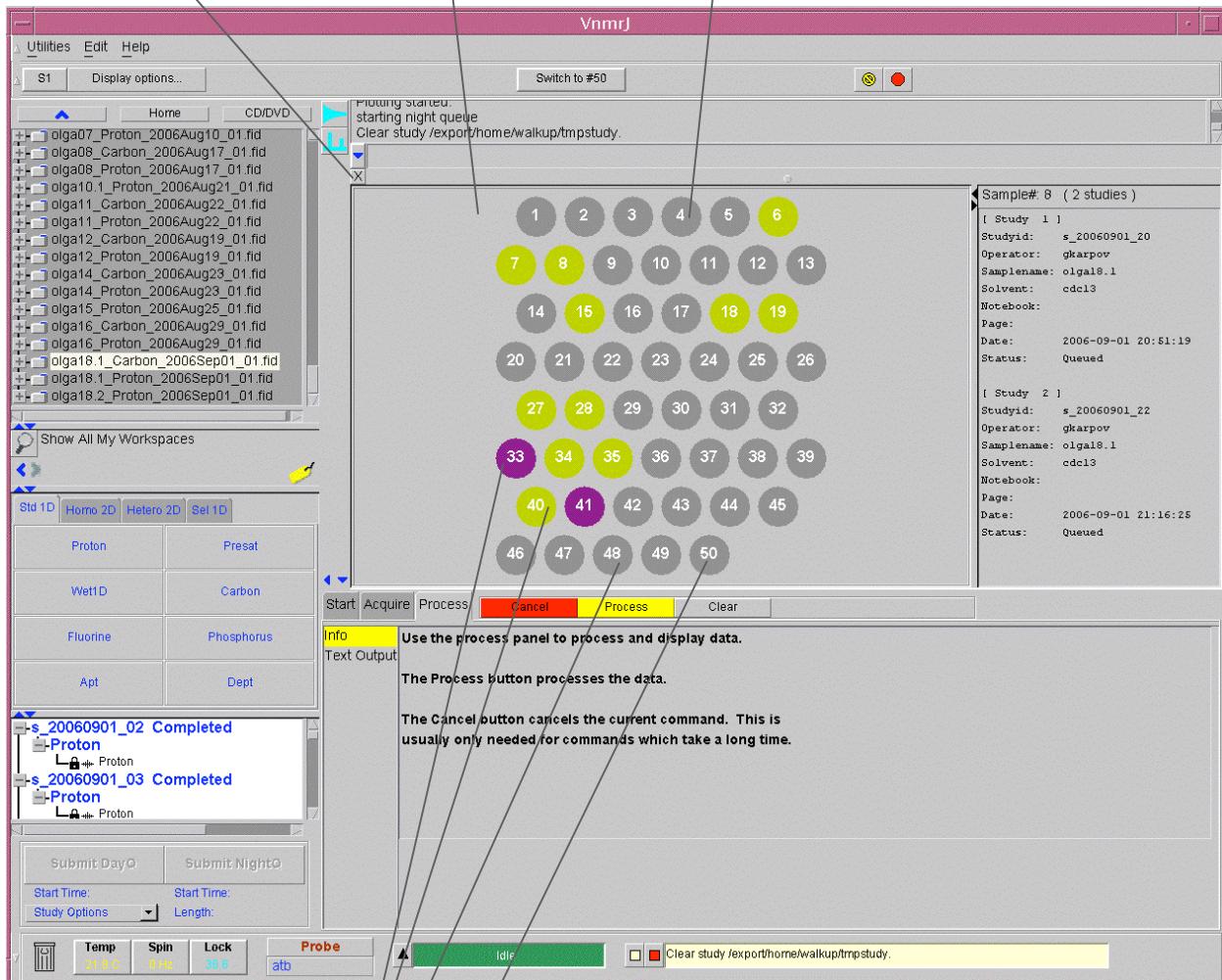
-from the list of operators (pull-down menu), **select Chem6190**
-type the **password** for the walkup account, which is **374robot**
-click **OK**

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

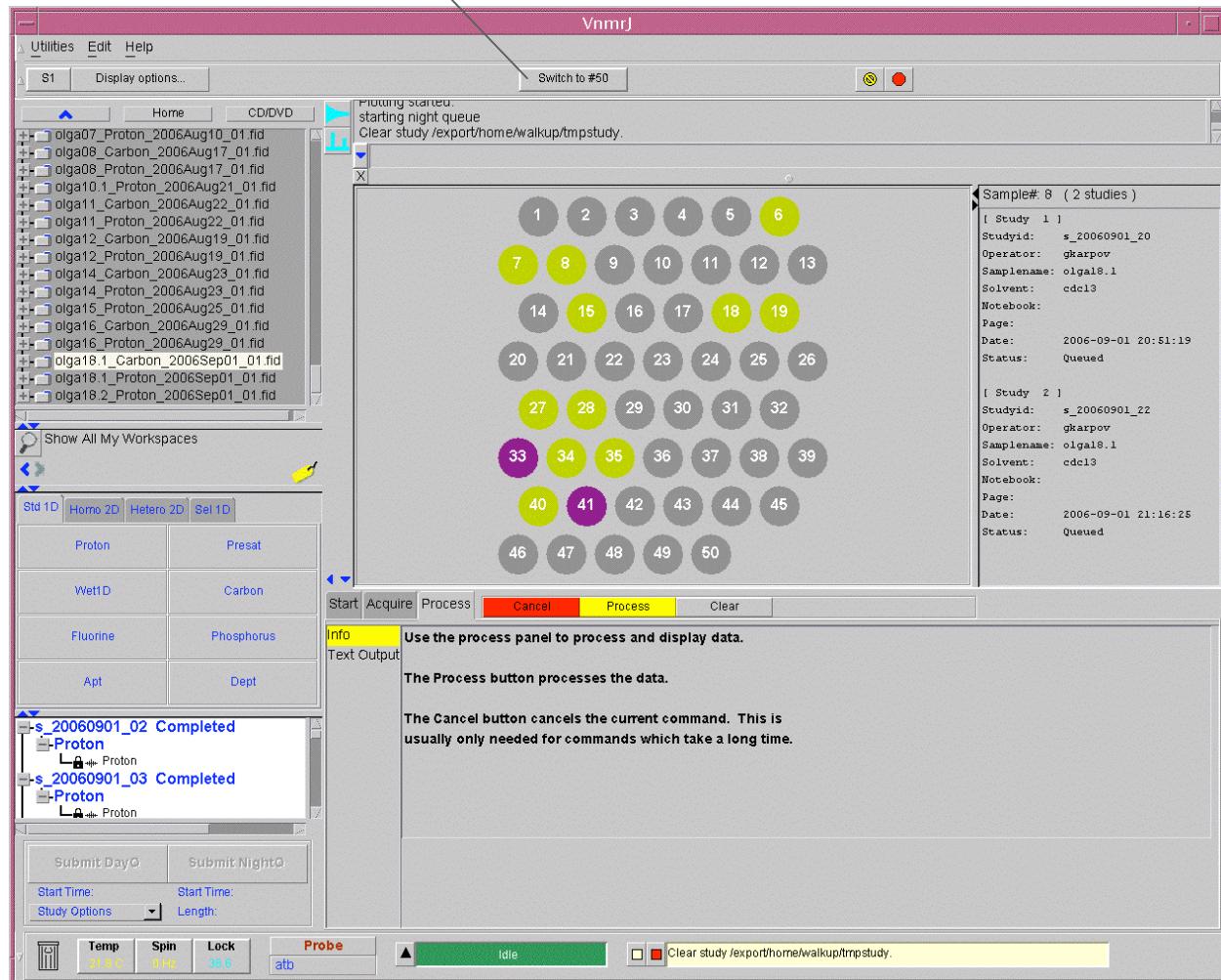


- the tray panel represents the **sample tray** located on top of the white platform next to the magnet
- the **X** in the upper left of the sample tray will toggle the display back to the black spectral display window
- the numbered circles on the tray panel correspond to **sample slots** in the sample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

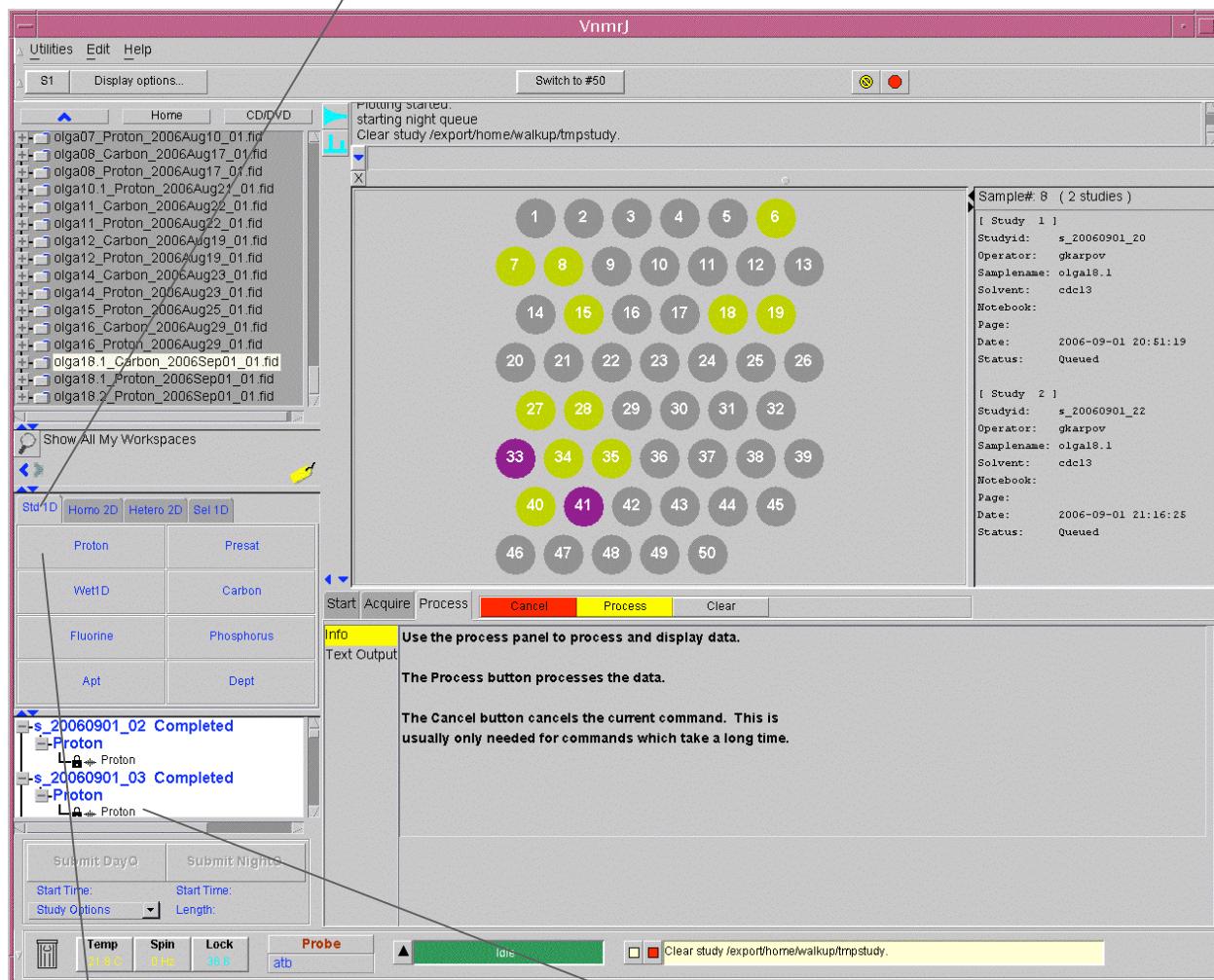


Sample placement

- 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)

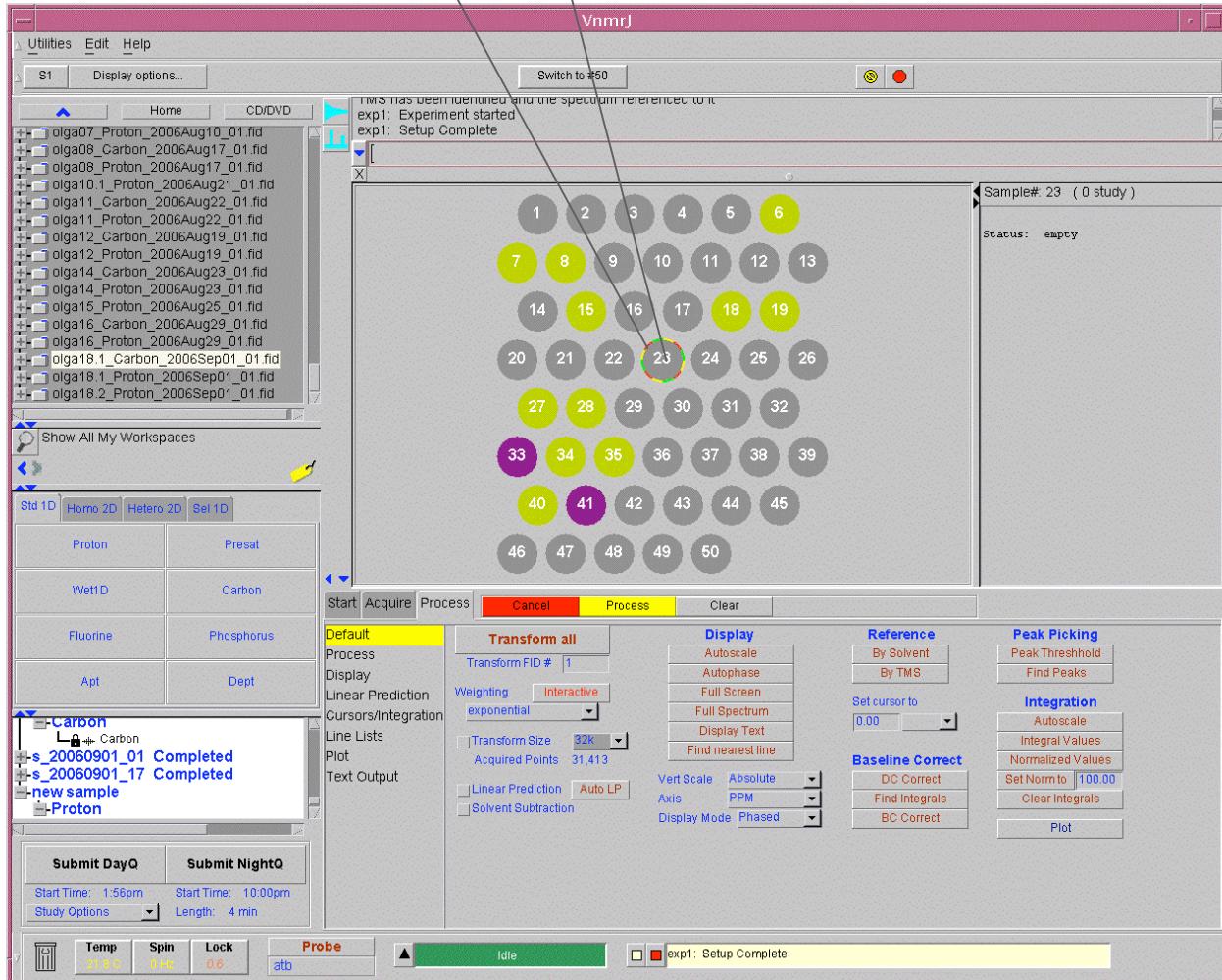
Experiment selection

- 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**

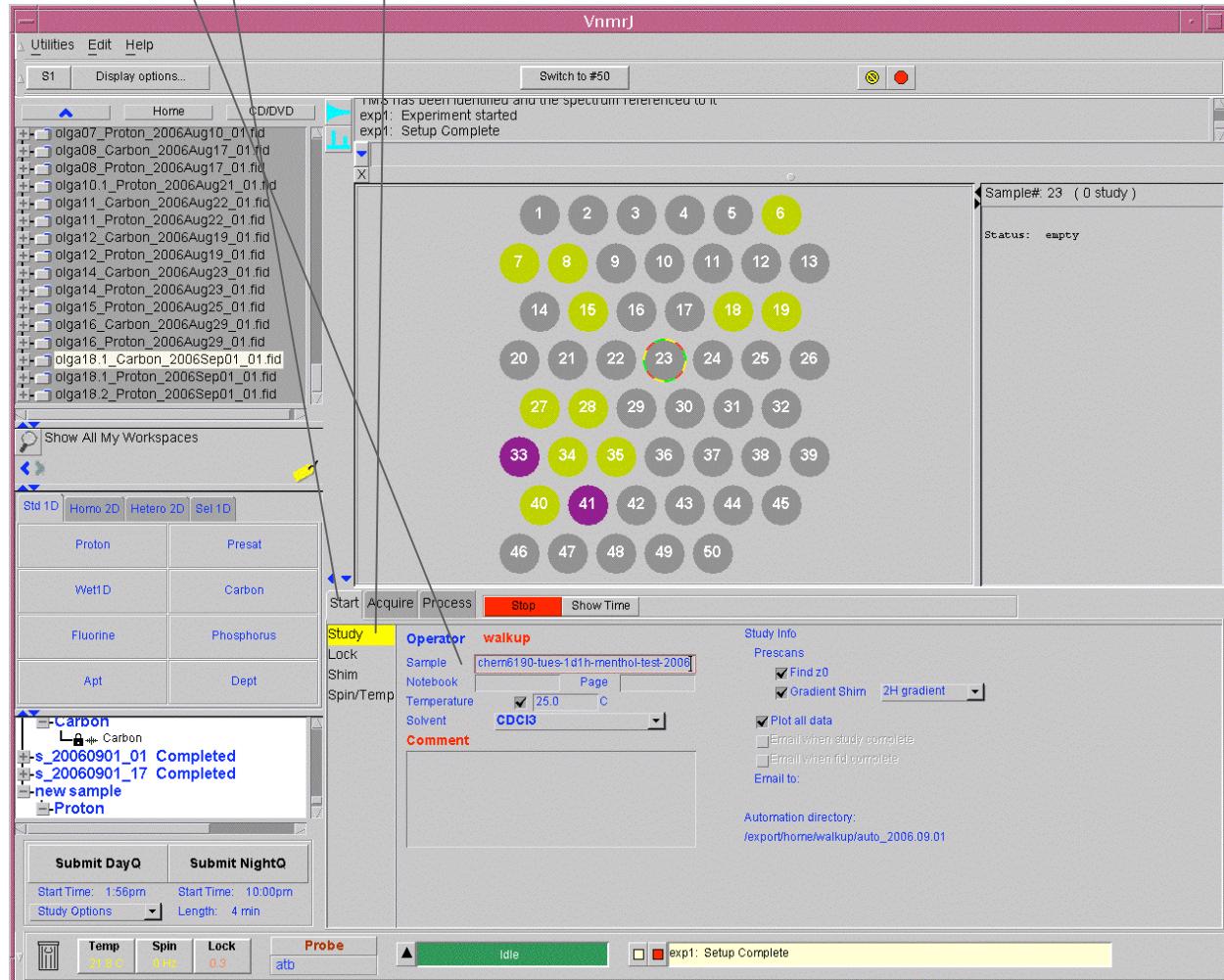


- click **Proton**, then double click on the **yellow highlighted Proton** in the window below
 - this will load the appropriate parameters for collecting a simple 1D ^1H experiment
 - in window below, **new sample** and **Proton** will appear (these will be colored yellow, indicating that the sample 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

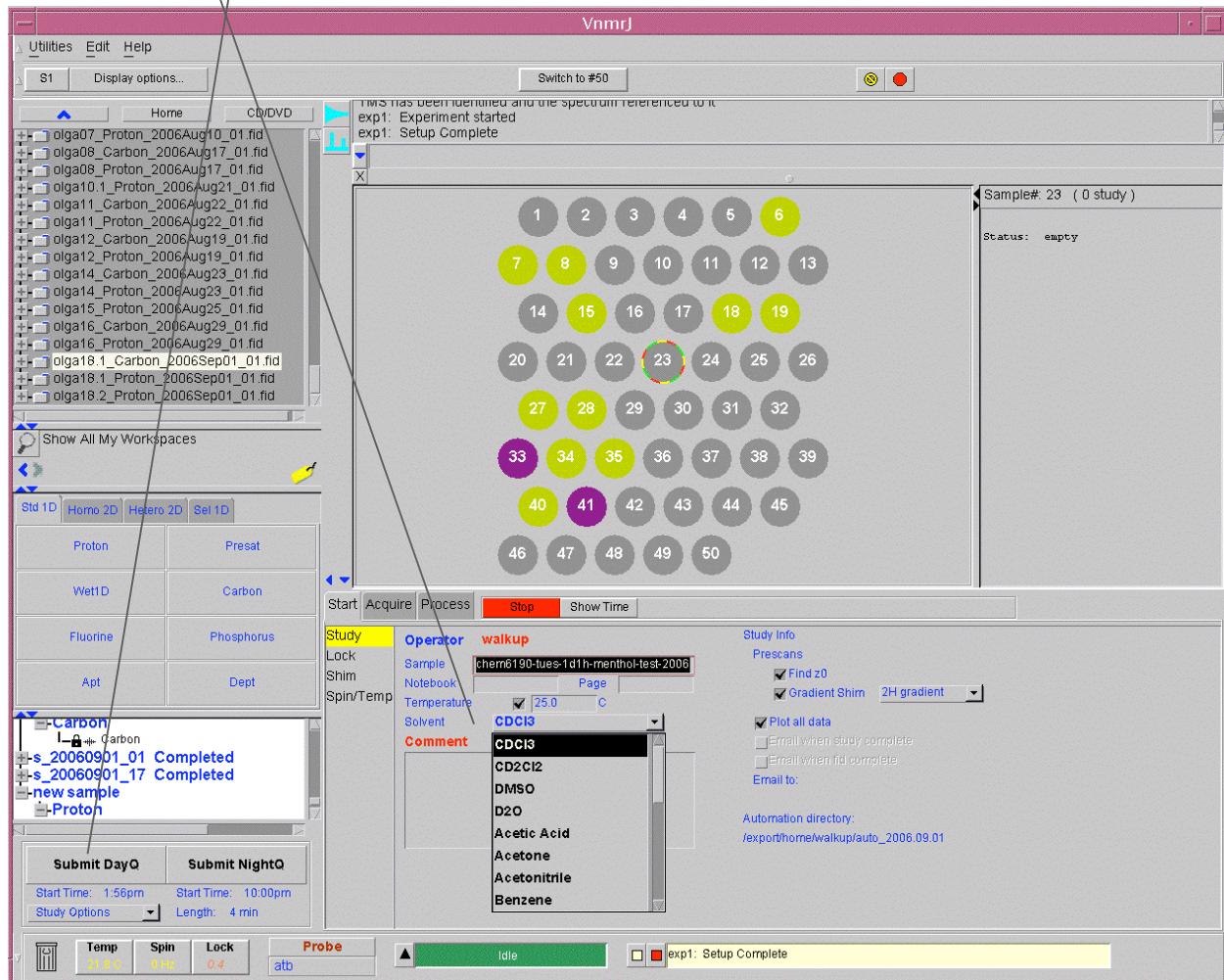
-do not change the Temp (temperature setting), or the Spin setting, or the Lock setting
-for solvent, select **CDCl₃** (deuterated chloroform)

-click **Submit to Day Queue**

-wait.....the robot is slow

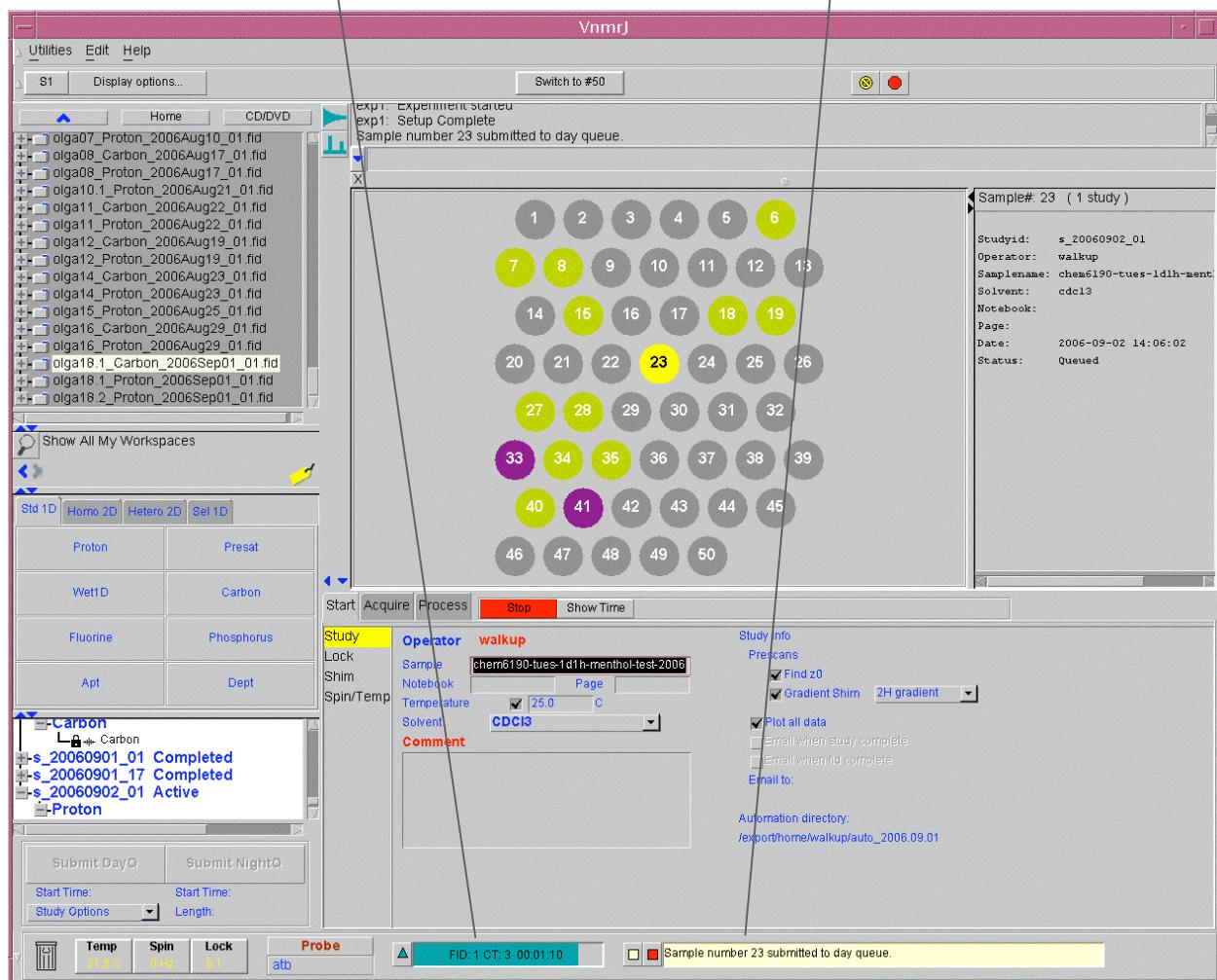
-the slot in the tray panel corresponding to your sample will turn yellow

-the robot will eventually get your sample and place it in the magnet



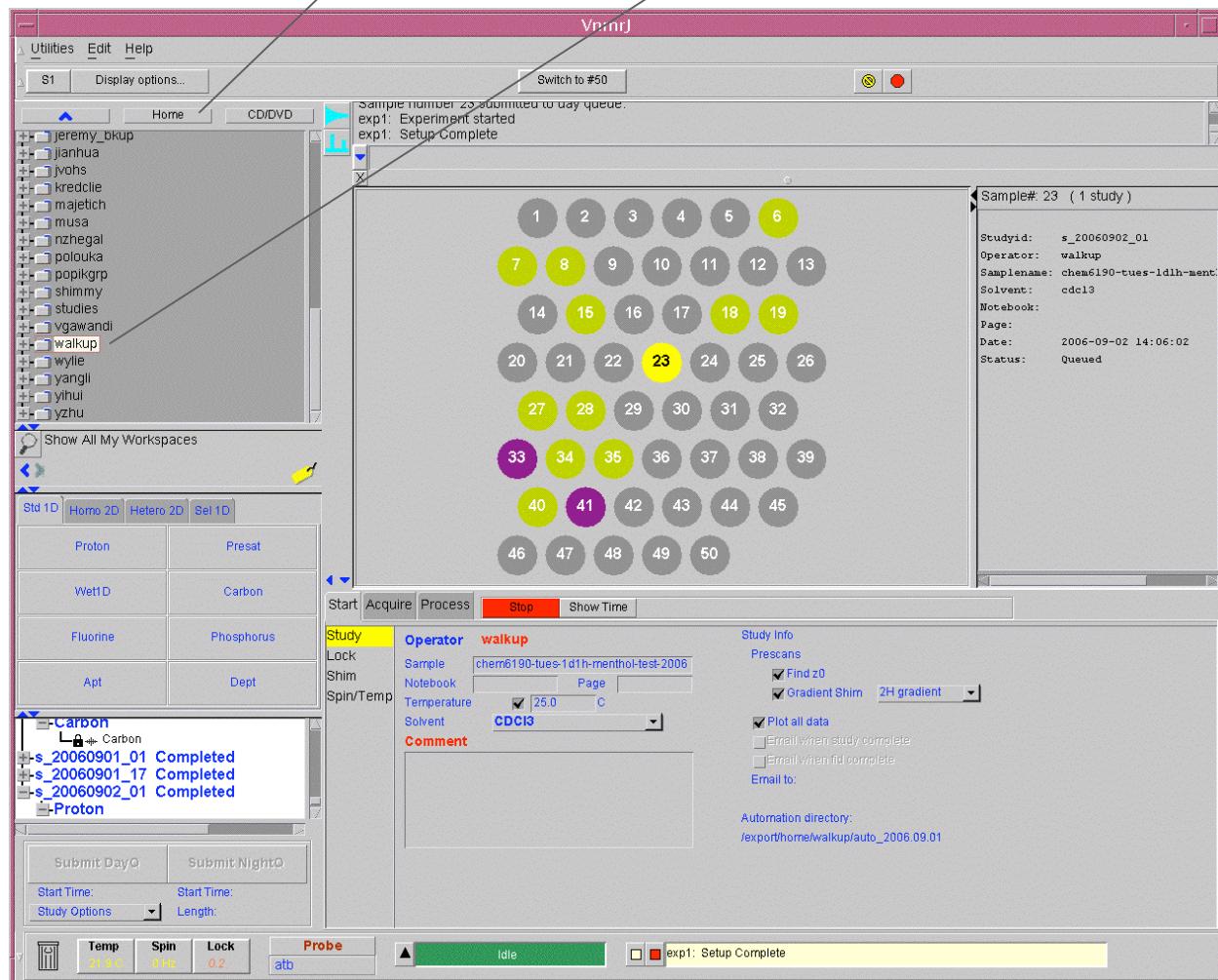
Locking and shimming

- 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

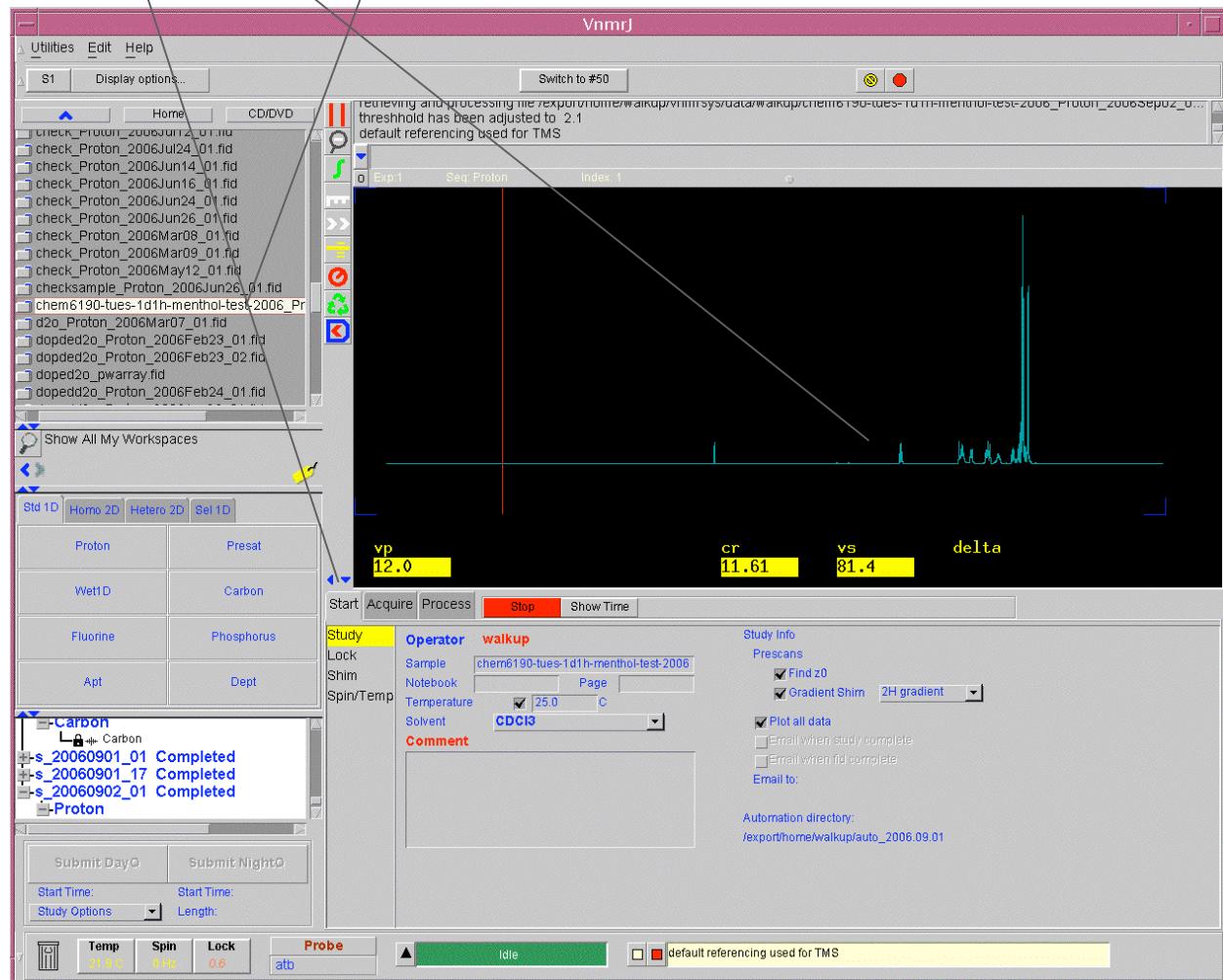
-at the upper left, click the **Home** button, then double-click **walkup**.....



.....then double-click on the **filename** that you gave to **your data**

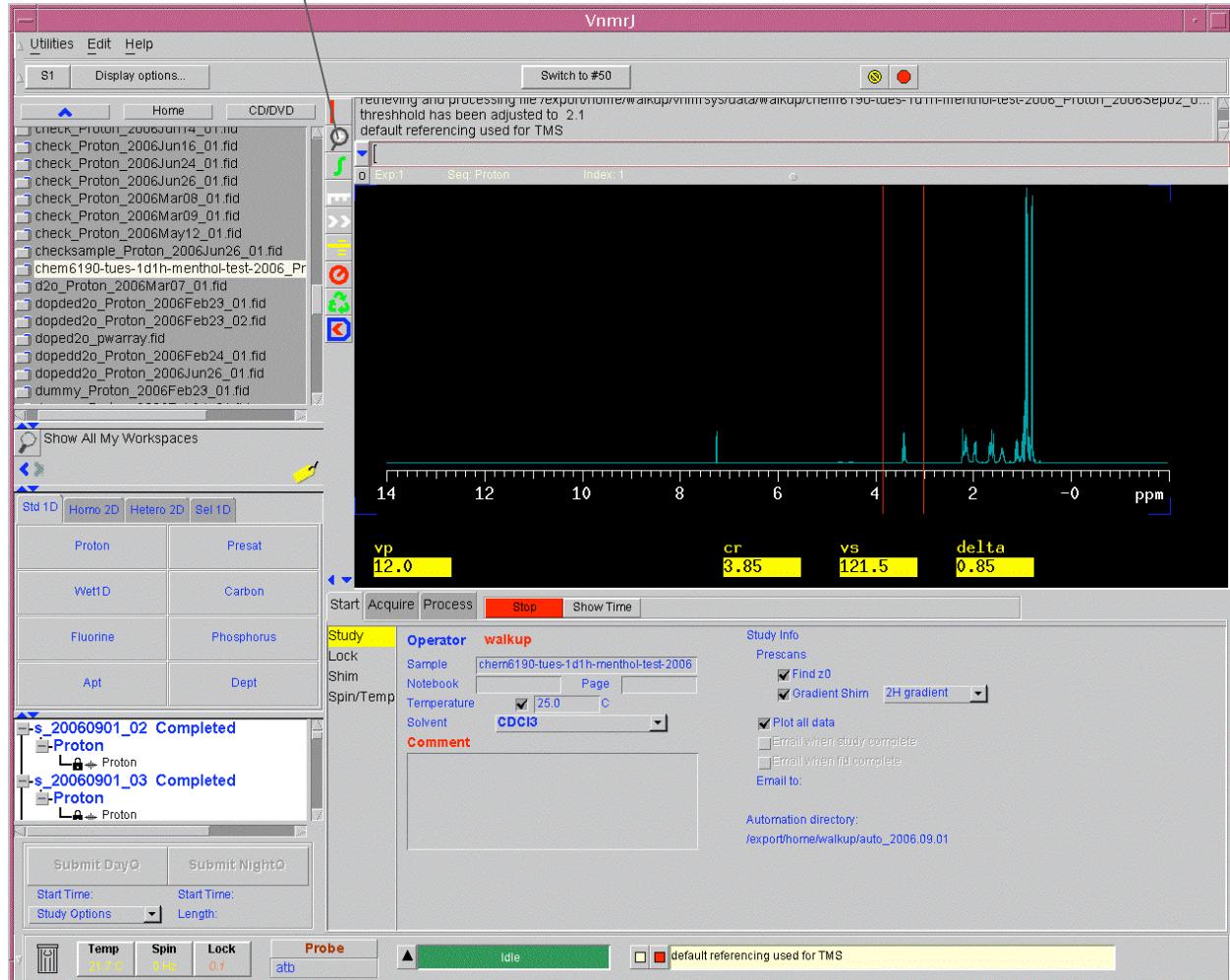
-the **spectrum** should appear (in the window previously occupied by the tray panel)

-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



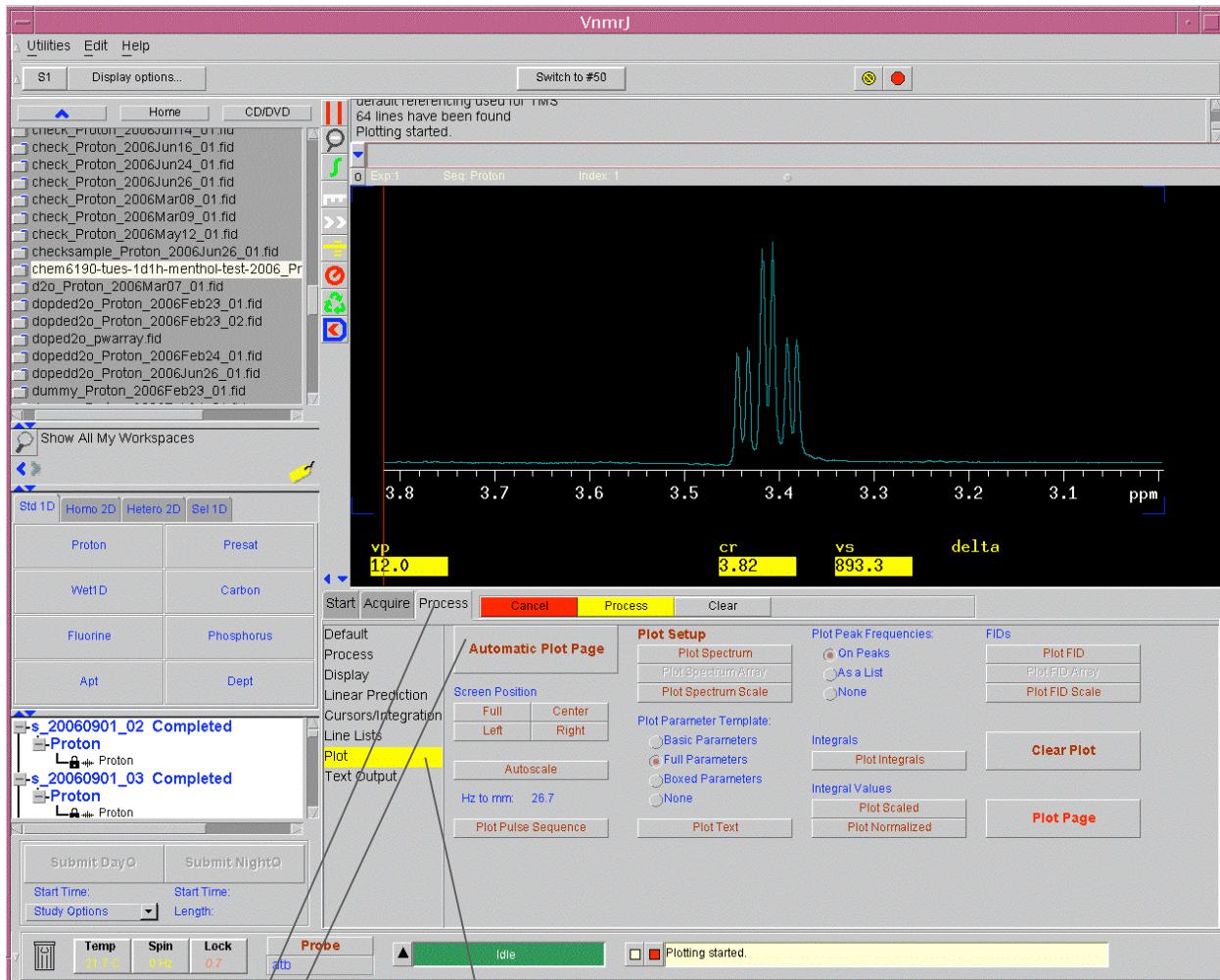
Zooming in and out

- 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)**

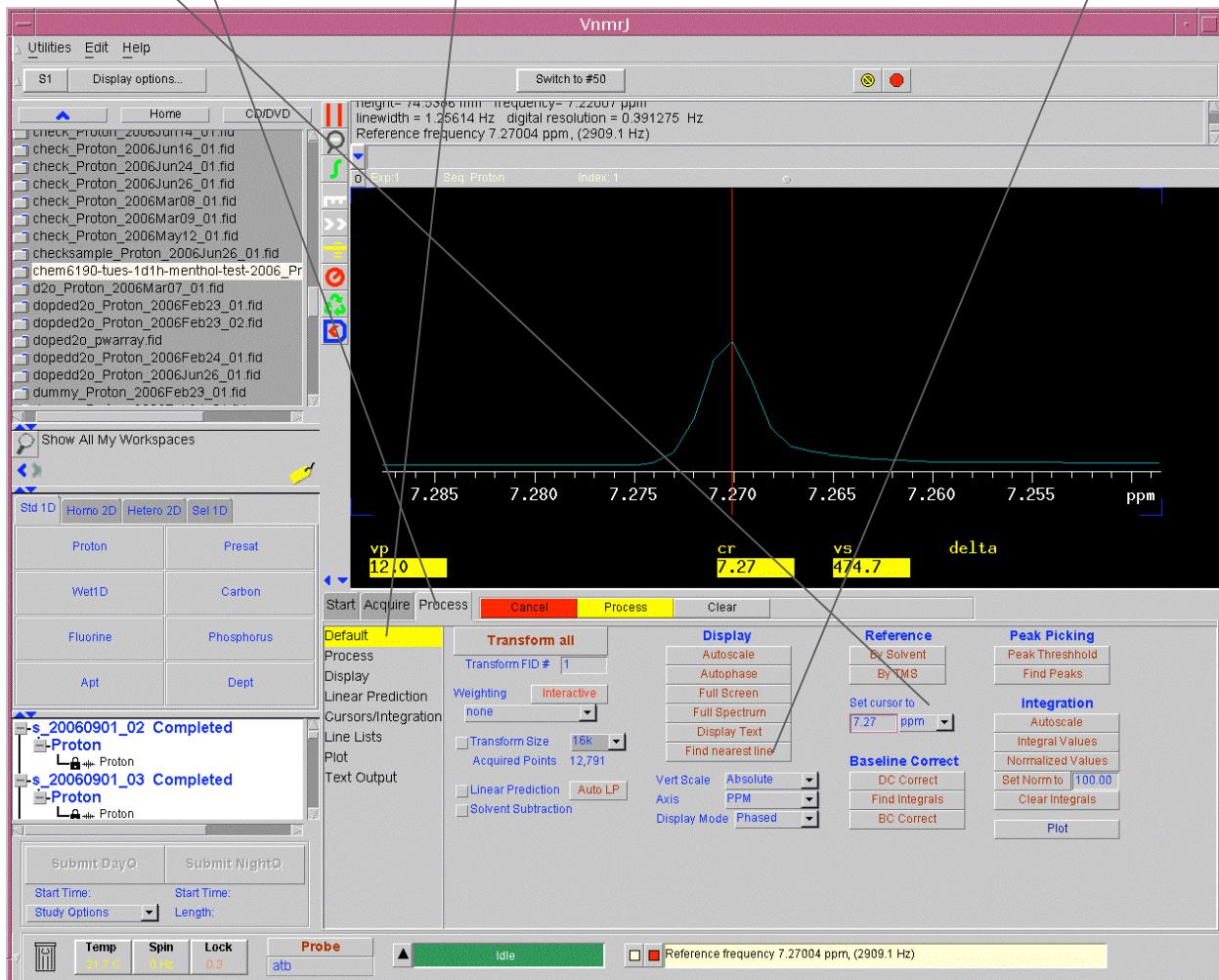


Quick plotting

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

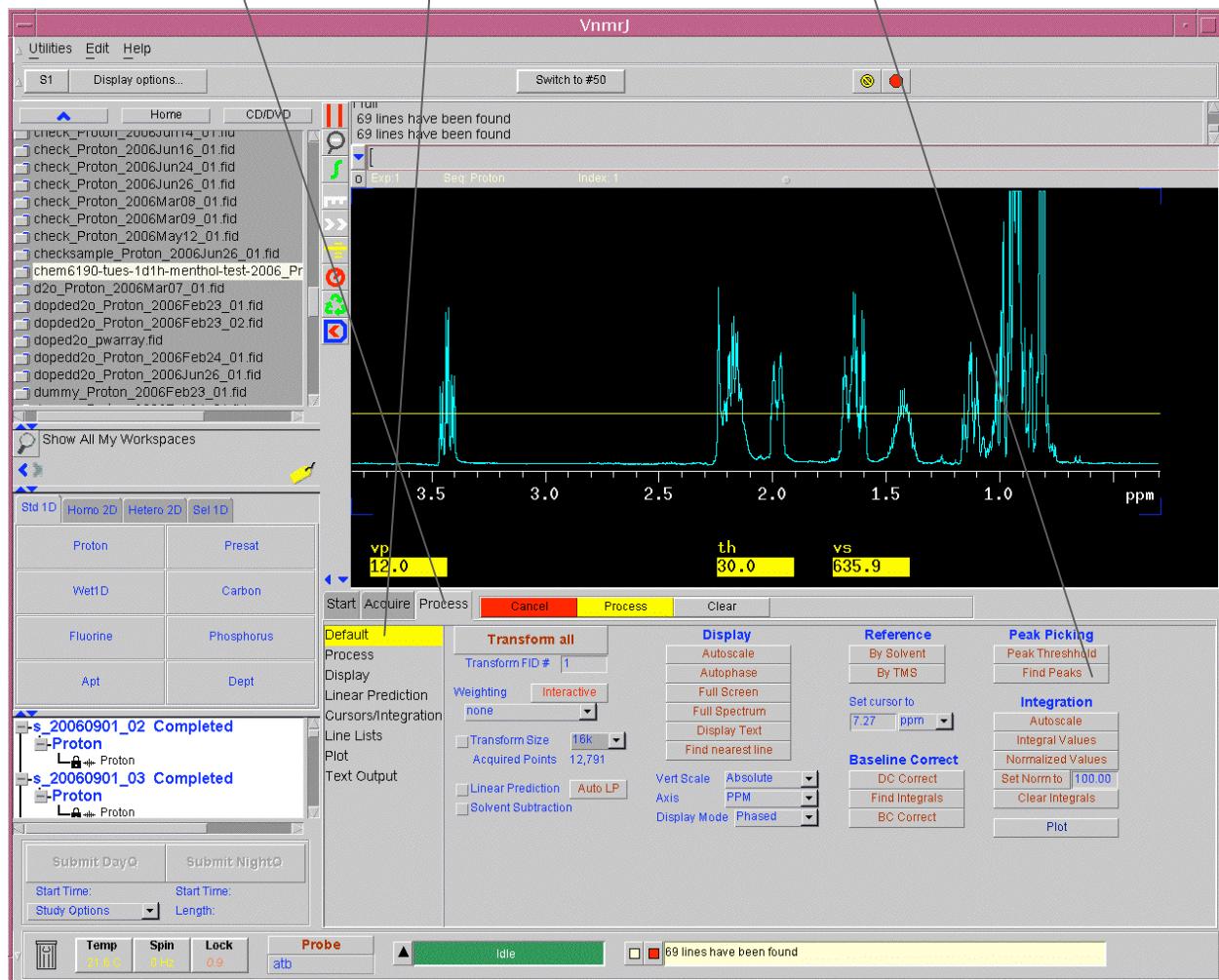
Chemical shift referencing

- 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**

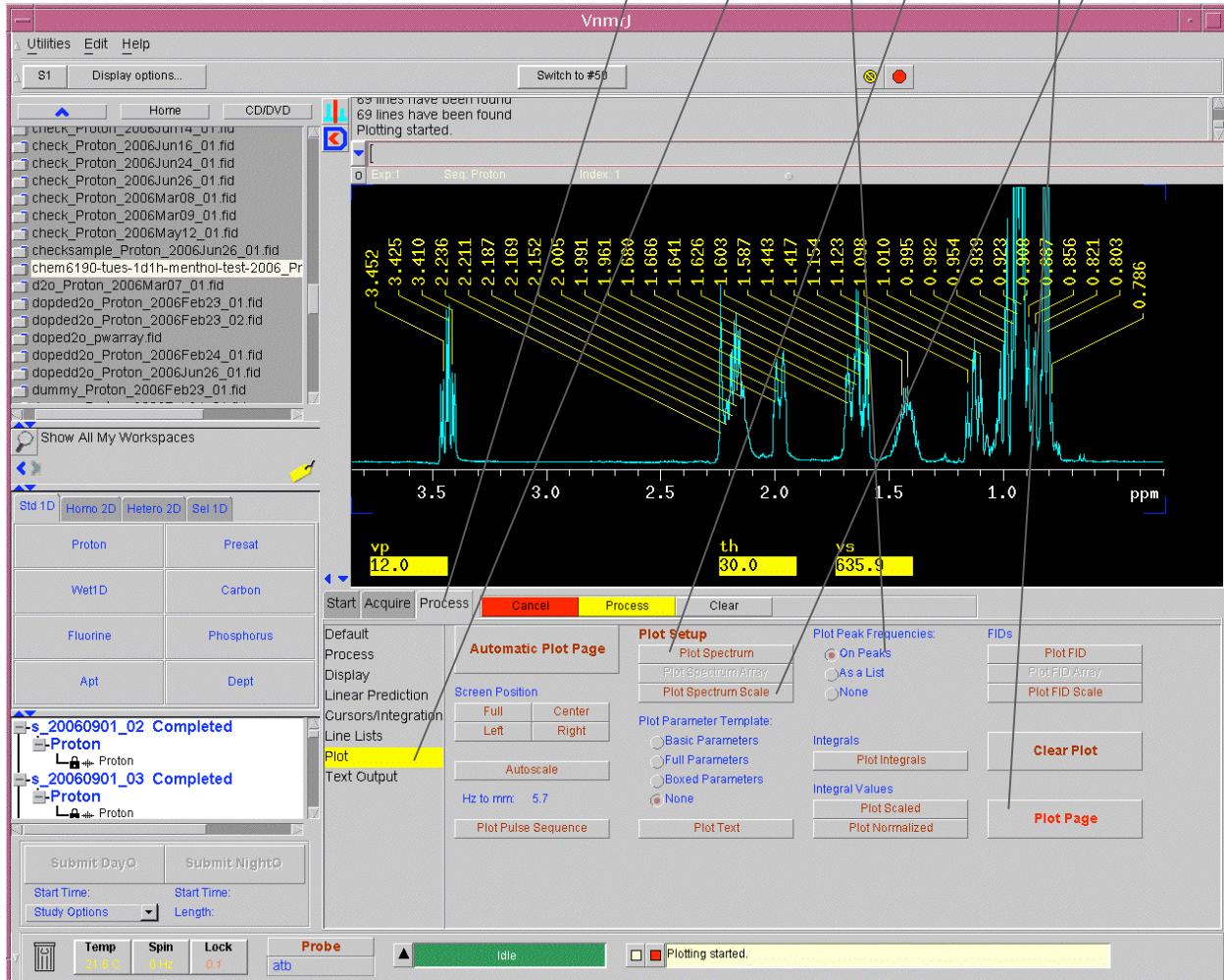


Peak picking

- 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**

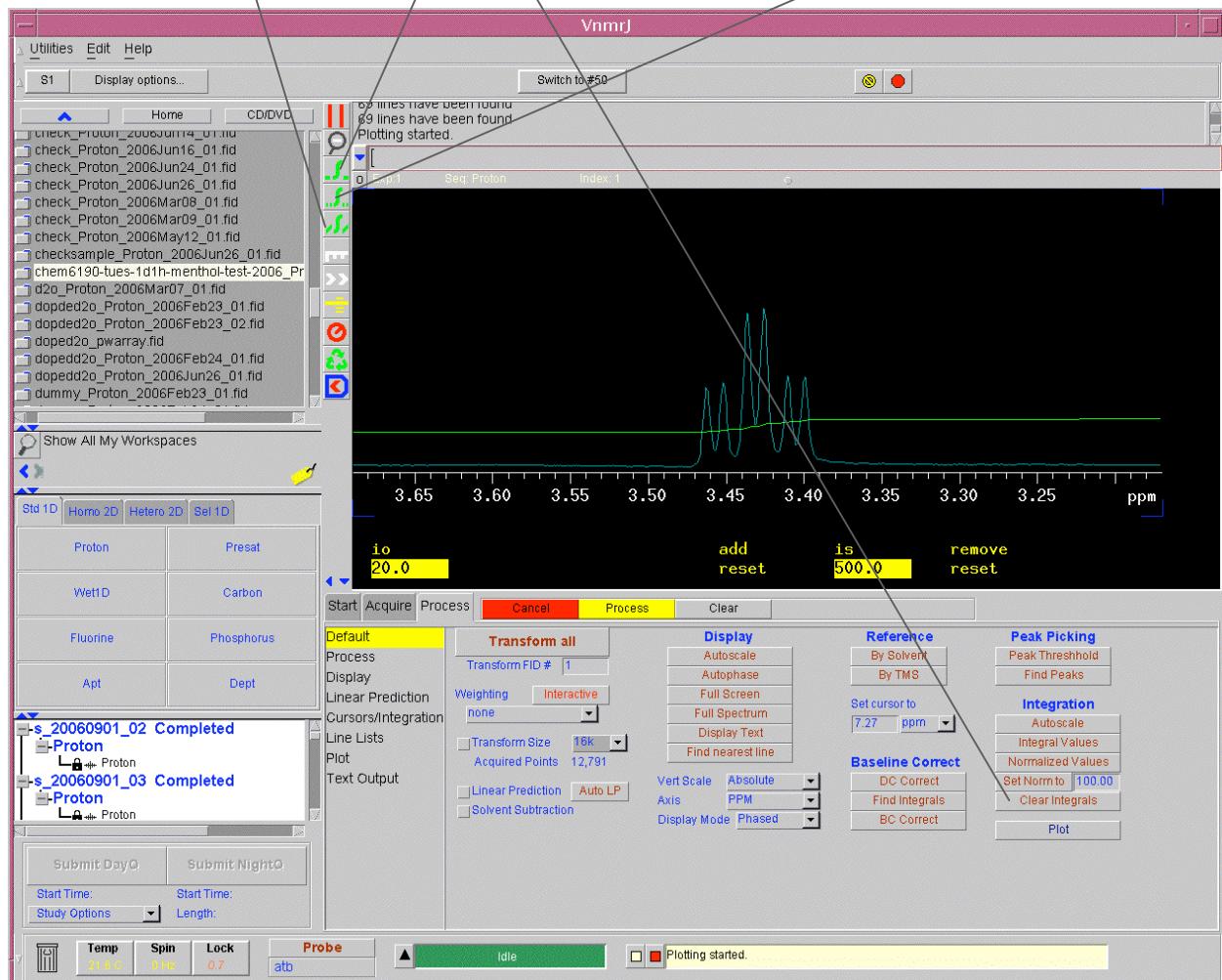


- in order to print the peak positions (hardcopy), under **Process/Plot**, click **Plot Spectrum**, then **Plot Spectrum Scale**, then under **Plot Peak Frequencies**, select **On Peaks**. Then click **Plot Page**
- if you want to return to a display without the peak positions, select **Process/Display**,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



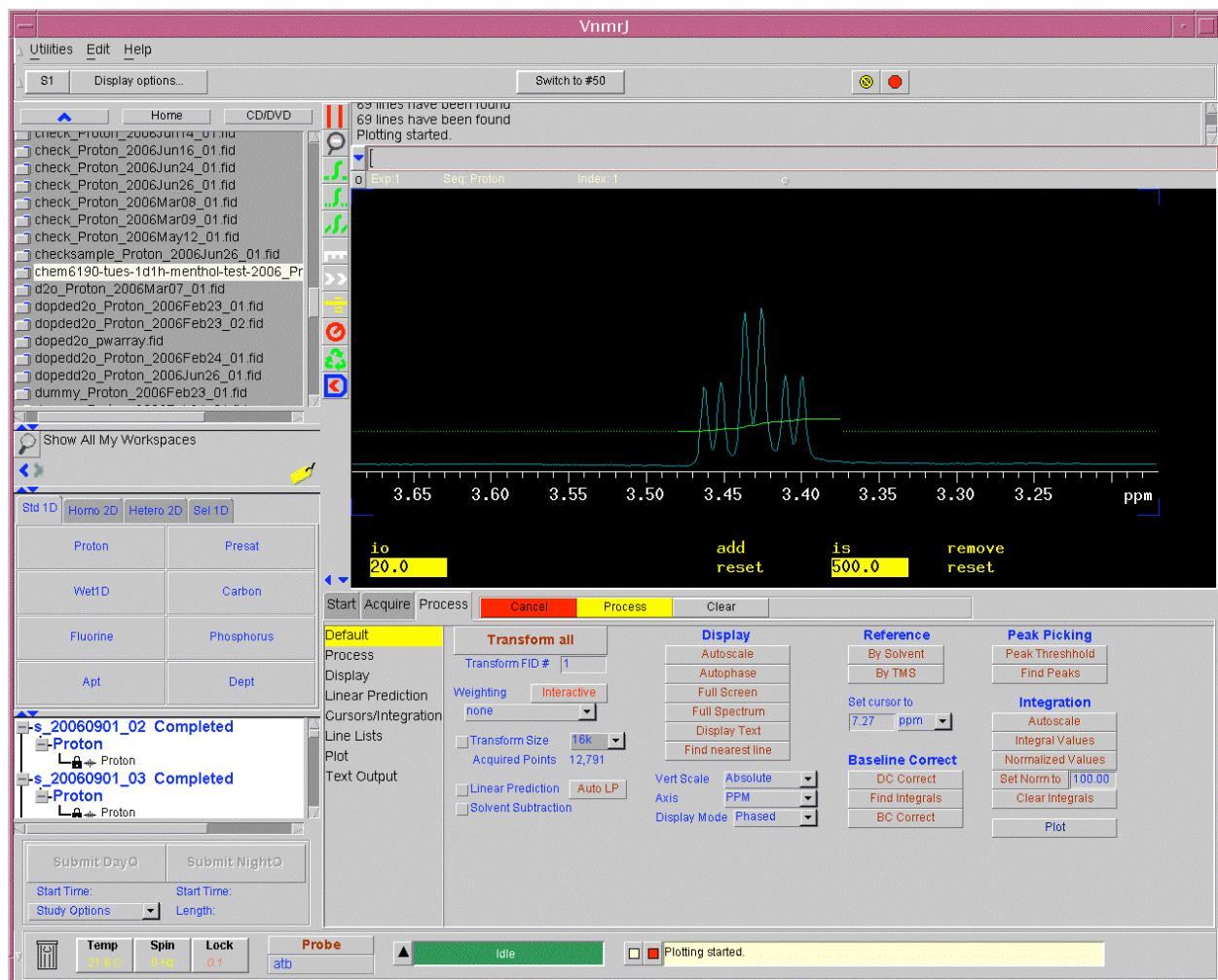
Integration

- 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)

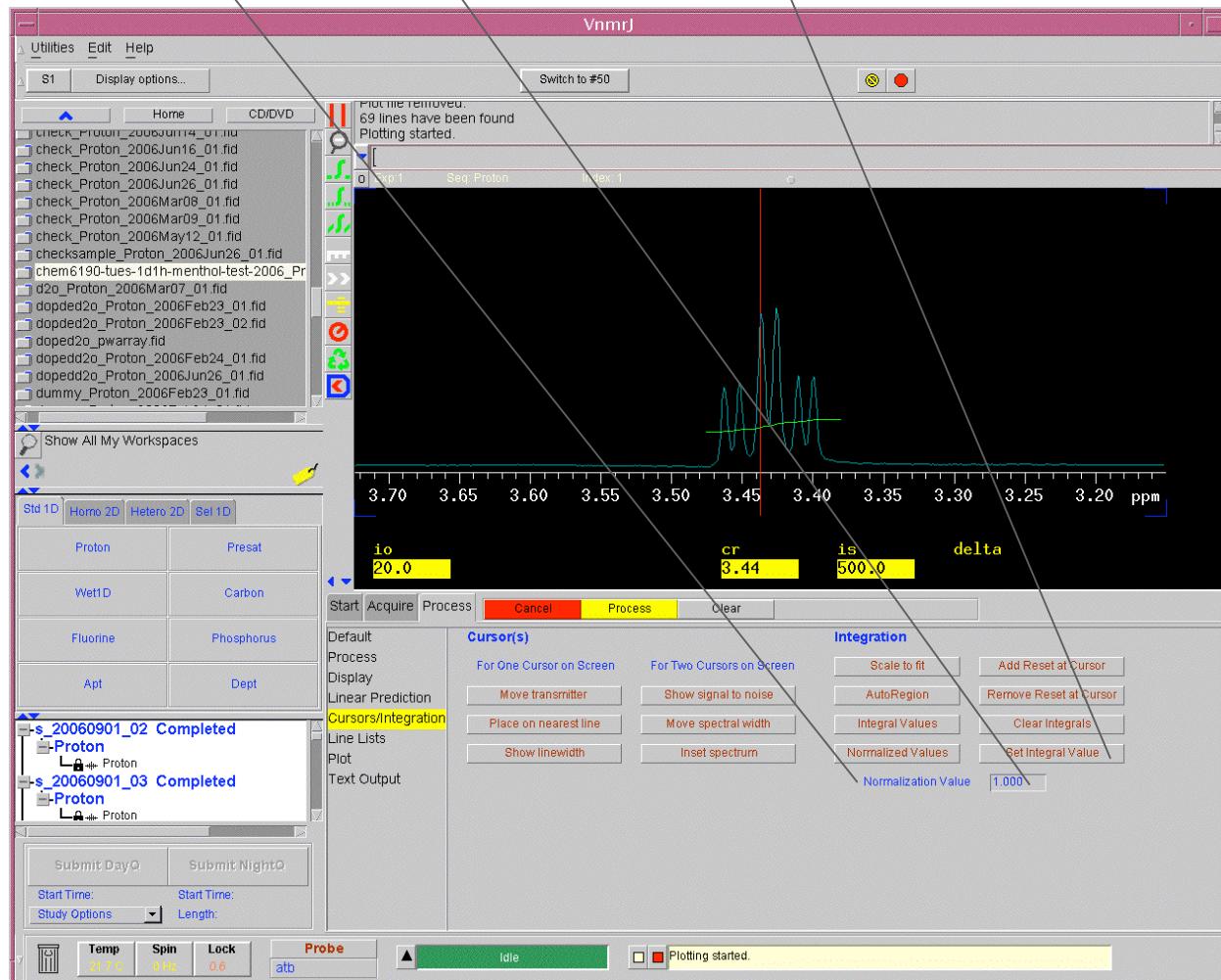


- expand around the next two signals in the spectrum (the two at approximately 2.2 and 2.0 ppm)
- click the **integral resets button**
- click on the right and left sides of each of these signals, as you have done for the previous signal
- repeat this for the rest of the signals in the spectrum



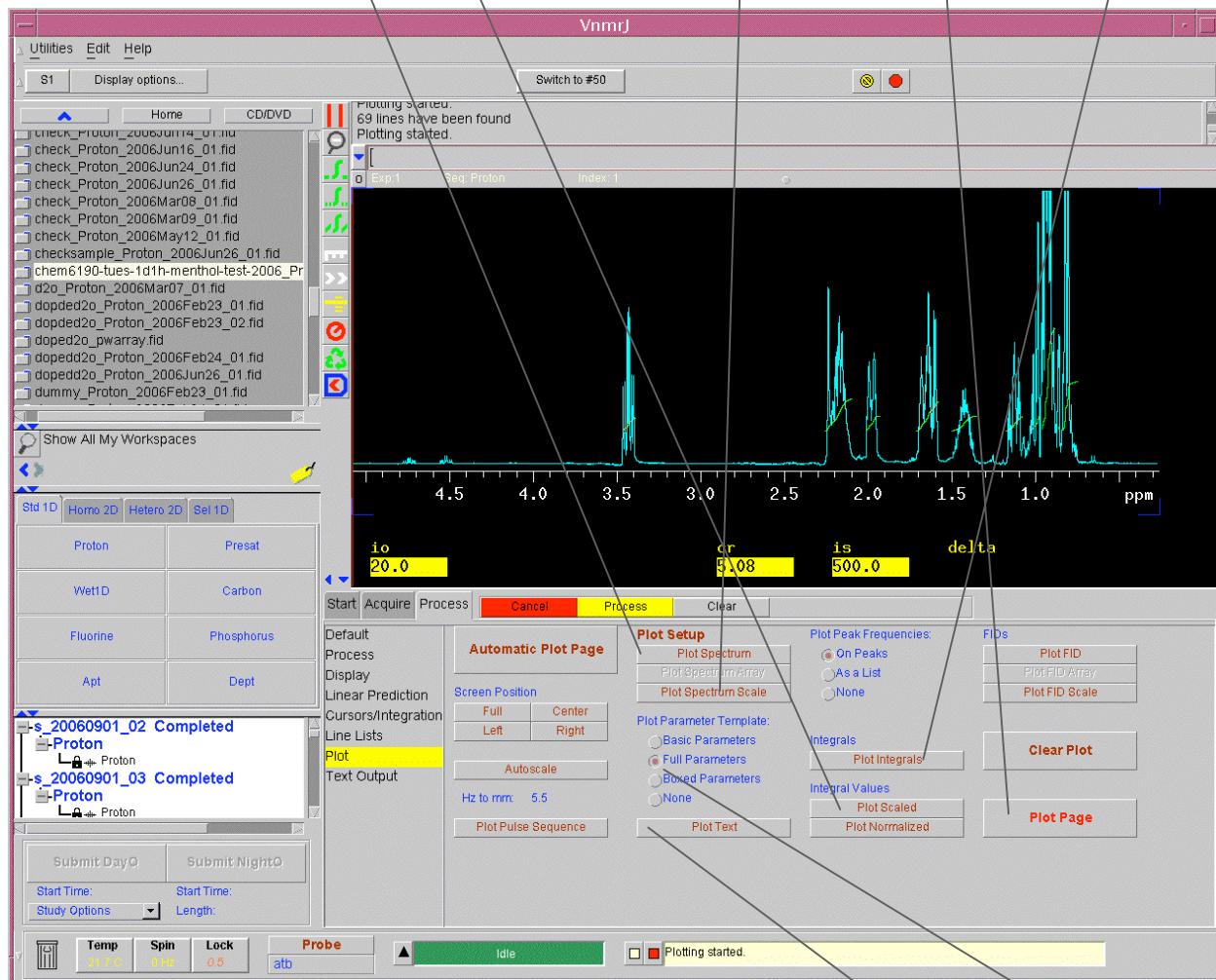
Integral calibration

- 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



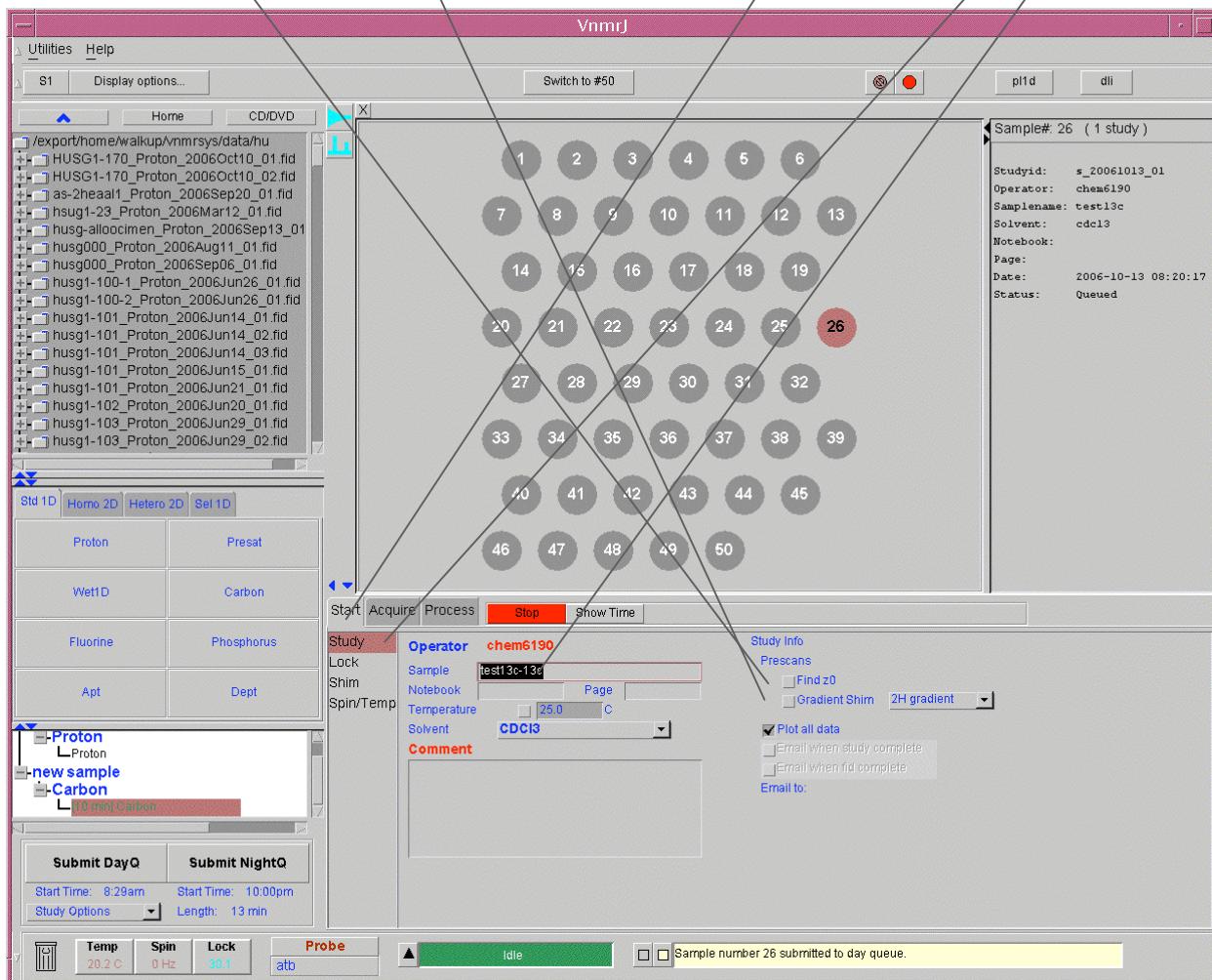
- try the above set of commands again, but in this case **include** clicking on **Plot Text** with the **Full Parameters** button selected
 - this will **print the acquisition parameters** on the hardcopy plot. You will note that these parameters are printed on the left side of the spectrum, so it is 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 -TOCSY 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 **TOCSY** option, and then **double click** on the yellow-highlighted **TOCSY** selection

-then, click the **Acquire** tab and **Defaults**. You will see the default TOCSY parameters. From the drop-down menu for **Spectral Width [ppm]**, select **0.95 → -0.5**. Under **Plot when done**, select **Positive & Negative**. For the **Mixing Time** select 30 ms

-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 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

-repeat with a 150 ms mixing time

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 #5:

- 1). Acquire two, 2D ^1H - ^1H TOCSY spectra at different mixing times (30 ms and 150 ms). .
-plot the spectrum (contour plots at different elevations/levels)
- 2). Analyze the TOCSY data, as well as 1D selective NOE and 2D NOESY data.

Exercises and Questions for Lab #5:

Please collect all the figures showing your 2D TOCSY data and include them in your report.

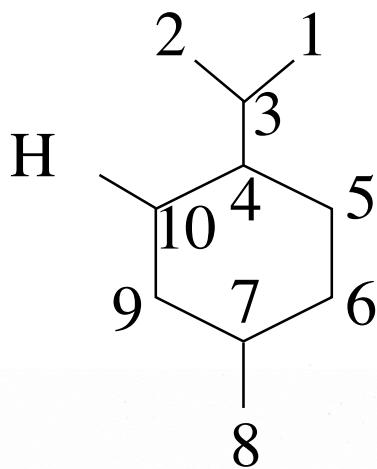
Please provide a typed report.

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

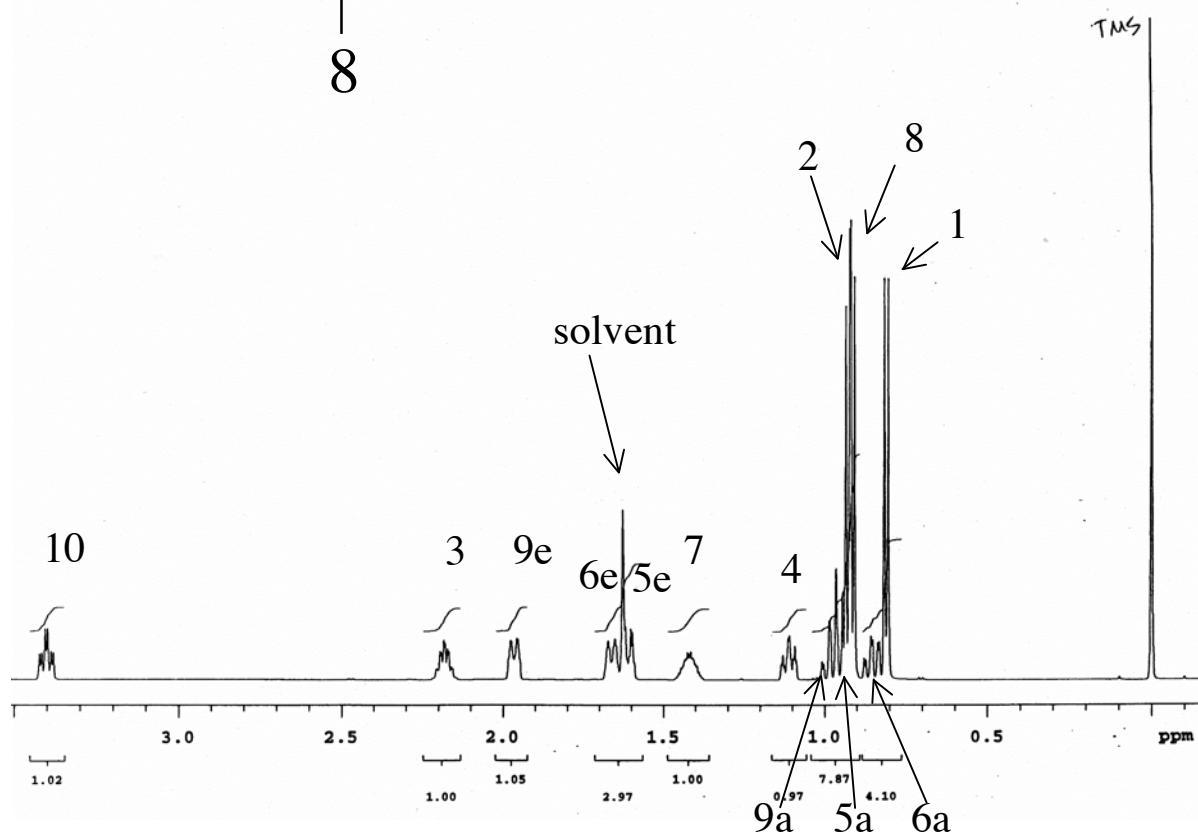
1). (**10 pts**) Examine the TOCSY spectrum with the 150 ms mixing time. For several reasonably well resolved signals (3.41, proton **10**; 2.18, proton **3**; 1.97, proton **9** 'e'; 1.42, proton **7**; 1.12, proton **4**, and 0.81, proton(s) **1**), **make a table indicating the protons to which each of these shares a cross-peak**. In cases of chemical shift degeneracy and crowding, if ambiguity exists, **make footnotes to your table explaining the ambiguities, etc.** Do all of the crosspeaks make sense based on what you know about how the TOCSY experiment works? Remember, you should plot the 2D TOCSY contour plots at several different contour levels to permit you to see very small peaks (very low contour level, near baseline) and also to permit you to remove most of the noise and look only at strong signals (very high contour level).

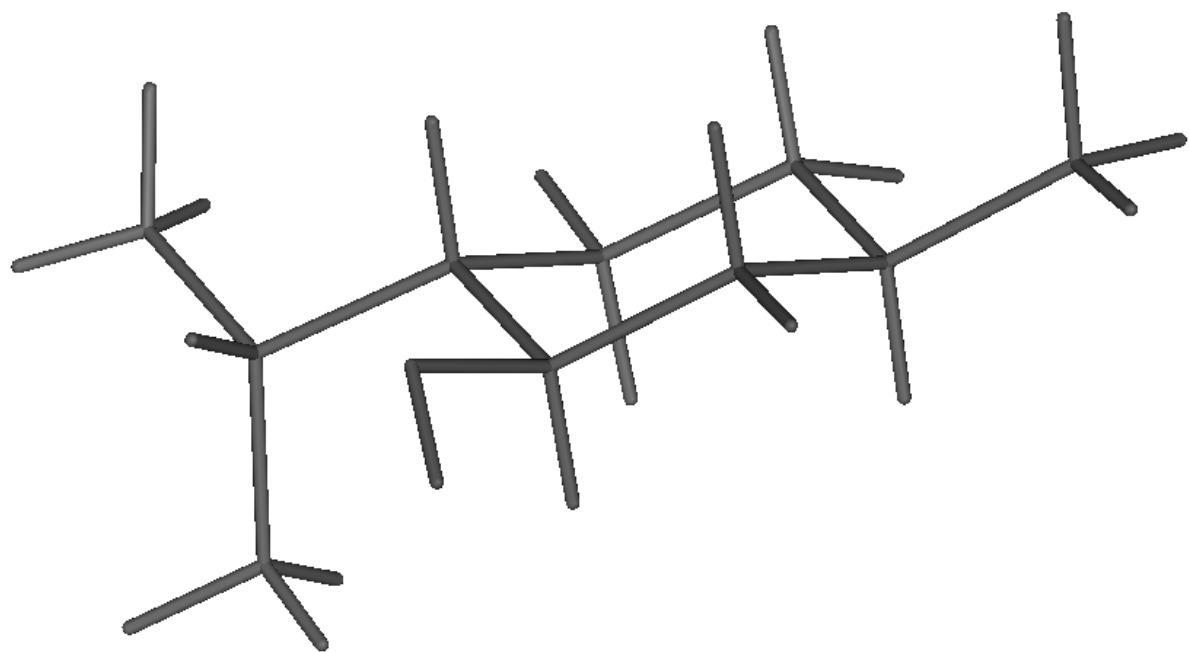
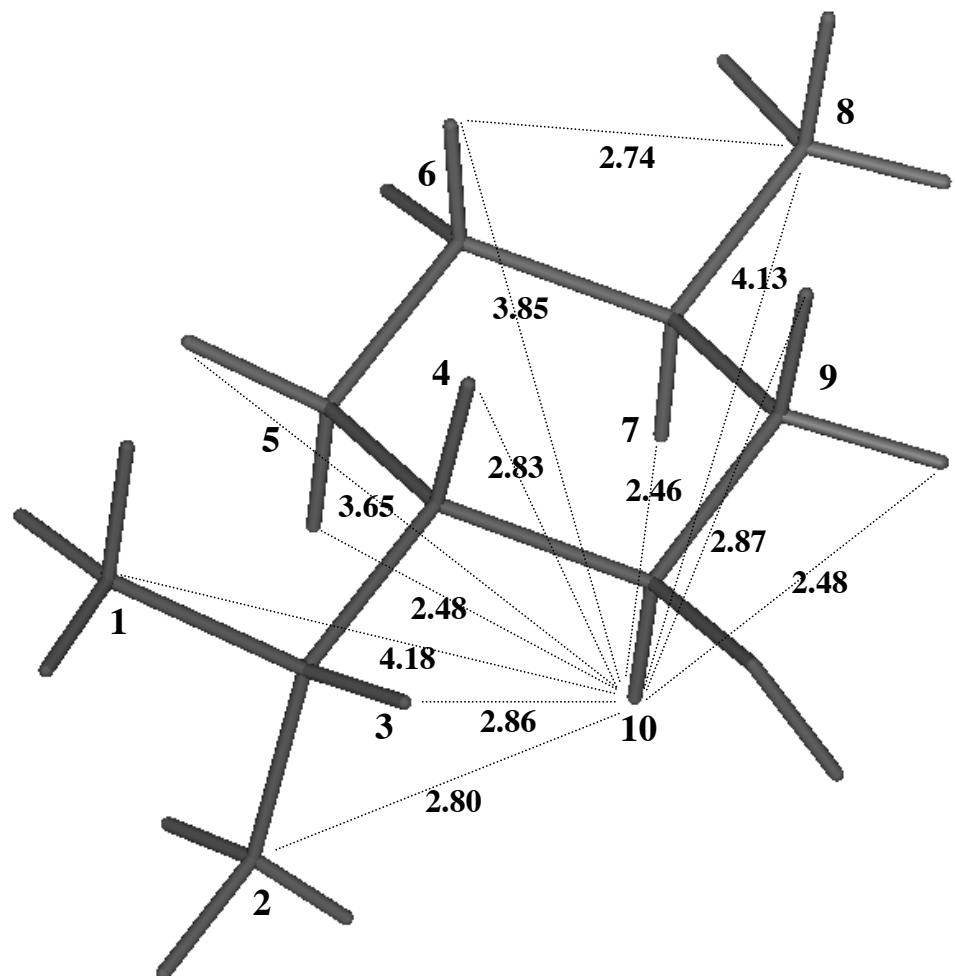
2). (**10 pts**) Examine the TOCSY spectrum with the 30 ms mixing time. You will notice immediately that there are many fewer peaks in this spectrum, and that this is precisely what you would expect based on the fact that the mixing time is much shorter. **In the table that you made for question #1, circle entries (corresponding to peaks) that are missing in the TOCSY spectrum with the 30 ms mixing time** (but are present in the 150 ms mixing time TOCSY spectrum). **Do you have any cases (in either the 150 or 30 ms spectrum) where a crosspeak from a neighboring proton is missing but one from a more distant proton still shows up?** If so describe them. Also, include in your report a copy of your 150 ms TOCSY spectrum plotted near the baseline, and a copy of your 30 ms TOCSY plotted near the baseline. **Circle in the 150 ms TOCSY all of the crosspeaks involving a given well-resolved signal/proton (for instance, the proton at 3.41 ppm) that appear in the 150 ms TOCSY but that do not appear in the 30 ms TOCSY.**

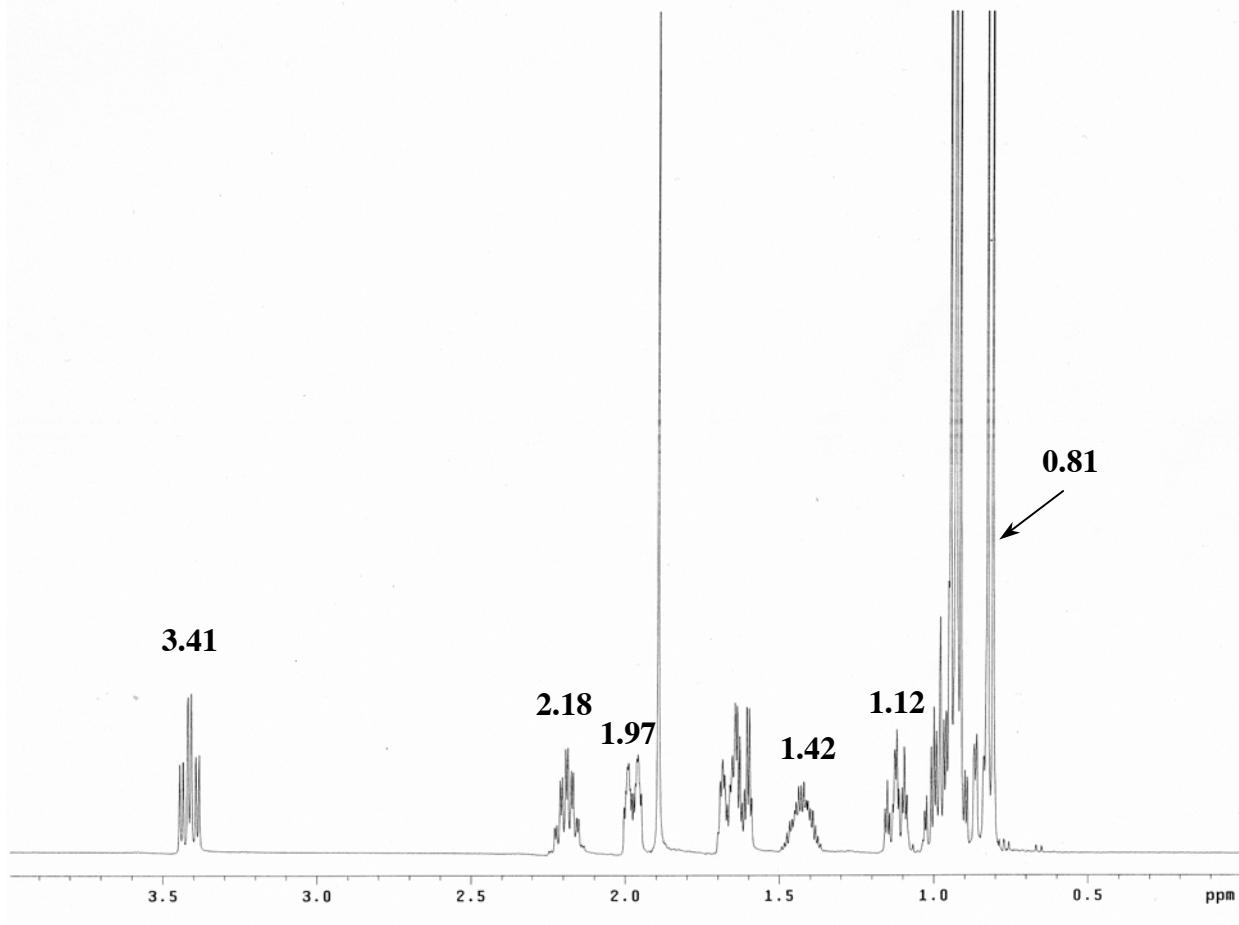
3). (**30 pts**) On the following pages is shown two views of the x-ray crystal structure (model) of menthol. Our standard identifiers are shown, as are representative distances. On the following pages is the 1D ^1H spectrum of menthol indicating the signals that you chose for selective inversion in order to elicit NOE perturbation of the signals from neighboring protons. The following pages show the 1D NOE data (difference spectra) when each of these signals is inverted. Here are your jobs. **You must assign the axial and equatorial positions at 9, 6, and 5**, i.e. we know that one of the protons at C9 gives rise to a signal at 1.97, and the other gives rise to a signal at about 0.95. Which (axial, equatorial) gives rise to the signal at 1.97, and which (axial, equatorial) gives rise to the signal at 0.95 (do the same for 6 and 5). You must carefully analyze the NOE spectra on pages 9-11 to do this. **You will not merely supply me with an answer, but you will describe your logic and rationale, and you will refer to specific peaks in the spectra on pages 9-11 to make you points.** You will also refer to the X-ray crystal structure model shown on the following pages and the measured distances shown there, and you will use these in your **detailed explanations**. Also, based on the NOE spectra, **is the assignment of methyl groups 1 and 2 on the following pages correct?** Why (explain in detail, using the NOE spectra, etc.)? Next, the 2D NOESY spectrum of menthol is shown on the following pages, plotted at two contour levels. The lower plot was plotted at a higher contour level, and only shows very large crosspeaks (disregard diagonal peaks). **In the lower spectrum circle 3 or 4 unique crosspeaks (do not pick a crosspeak and its symmetrically related crosspeak) and tell me why they should be large, based on the crystal structure of menthol shown below, the distances, etc.** Then, in the top figure (plotted at a lower contour level, showing strong and weaker crosspeaks), pick (circle) some weak crosspeaks (those that do not show up in the lower plot) and explain to me, based on the x-ray structure on the following pages, why the crosspeaks should be weaker. Finally, there appears to be an artifact in the 2D NOESY spectrum.....a vertical "streak" at about 1.9 ppm. What do you suppose this is due to?



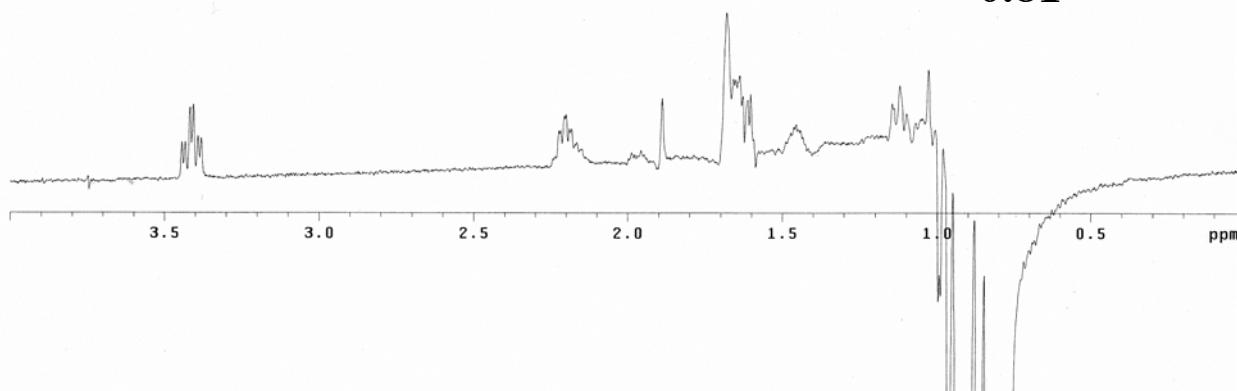
menthol







0.81



3.41

