Lab #2 1D ^{1}H Double Resonance (Selective Decoupling)

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

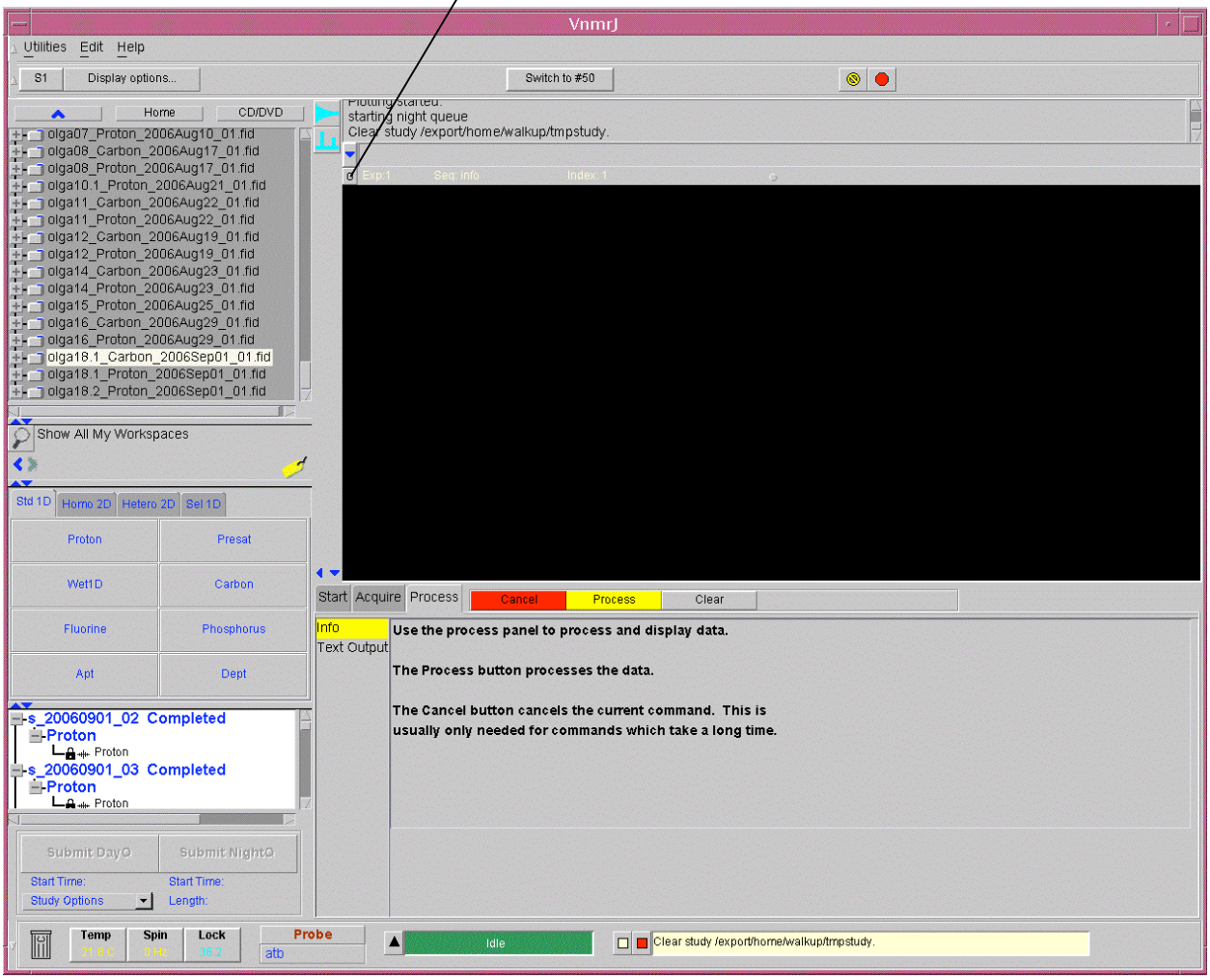
Login

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

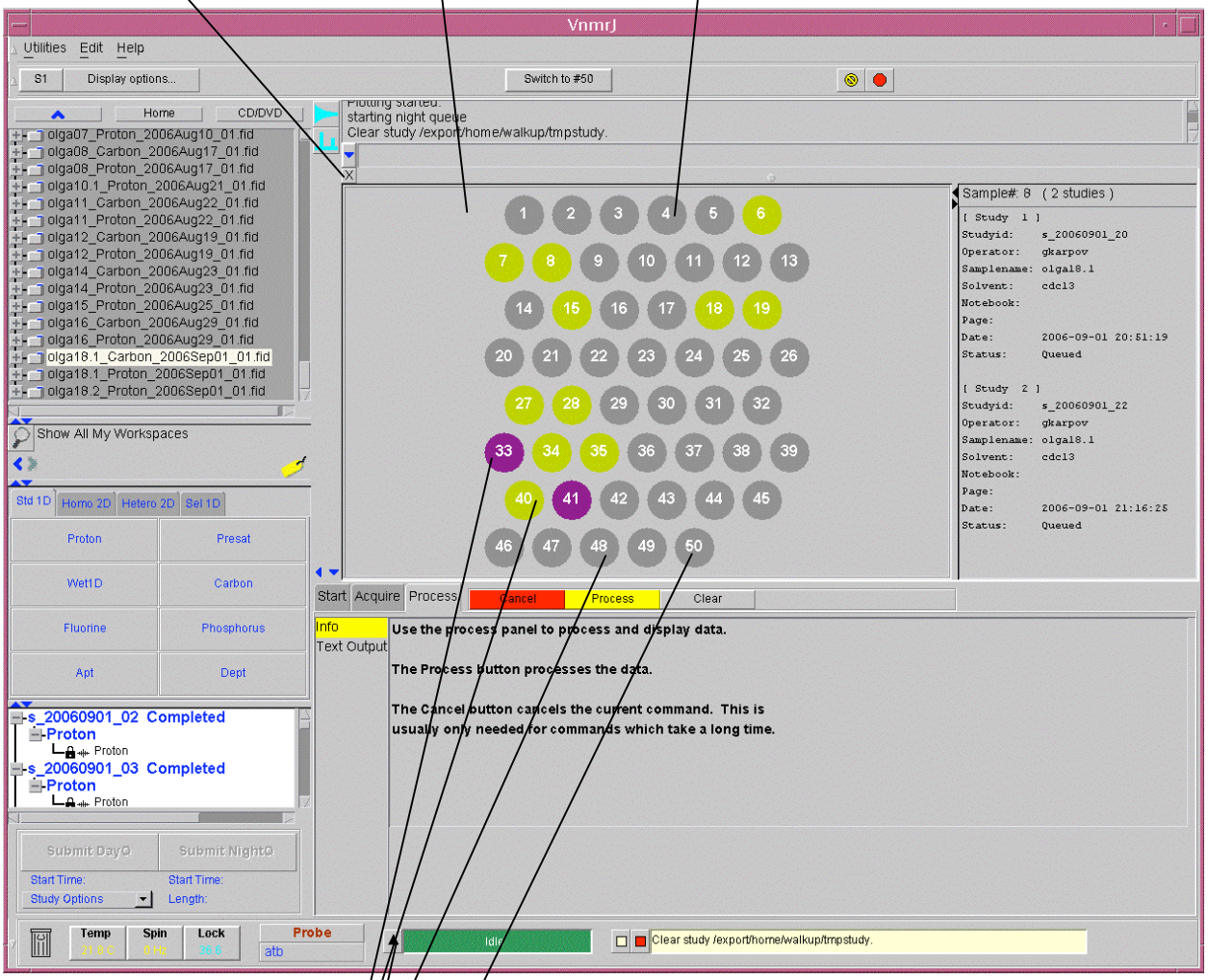
-from the list of operators (pull-down menu), **select chem6190**
-there is no password for this account
-click **OK**

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)
-you also can click on this arrow which will pop up a small window that tells you what sample currently is in the magnet

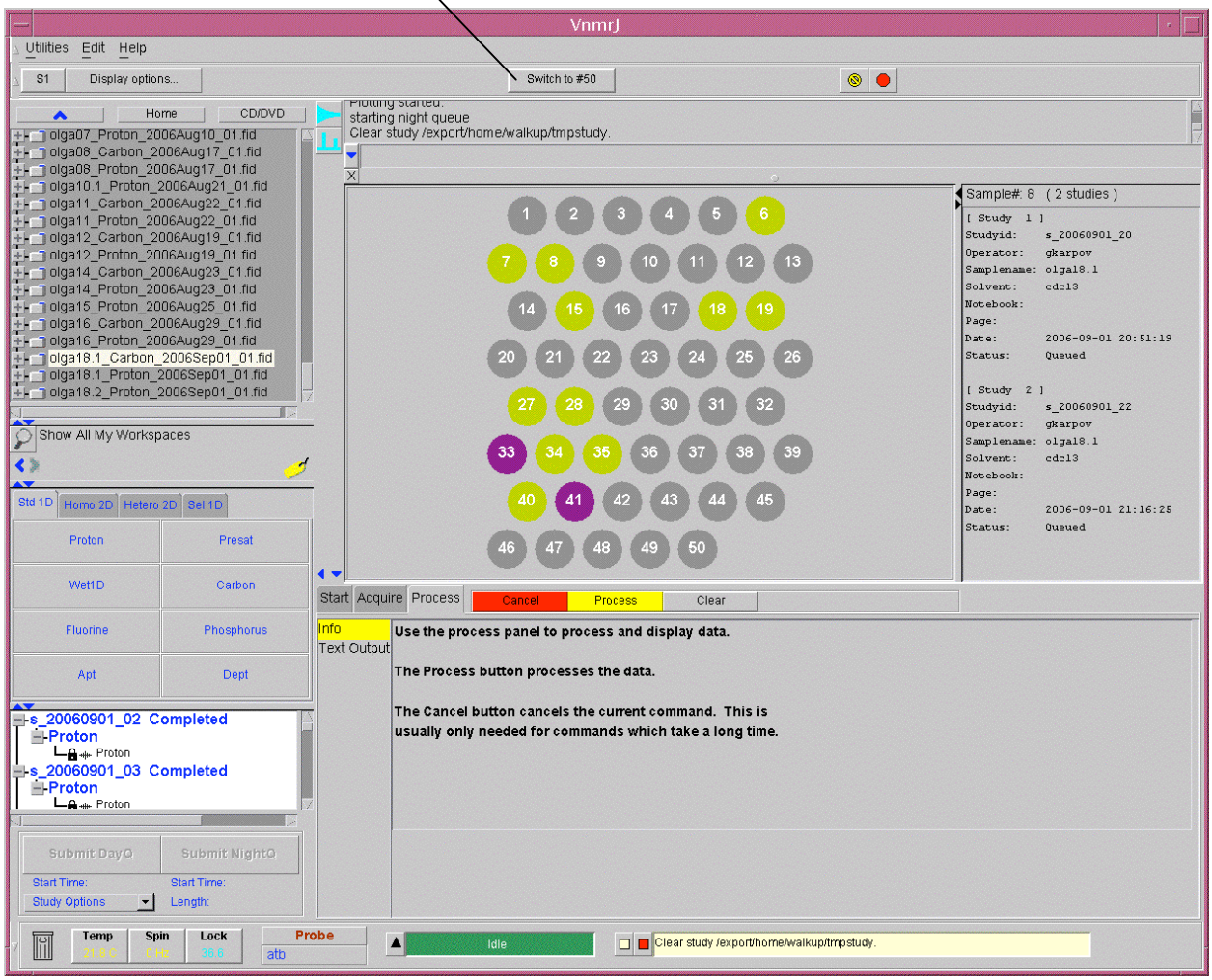
-click the **switch to #50 button** at the top of the screen

-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***

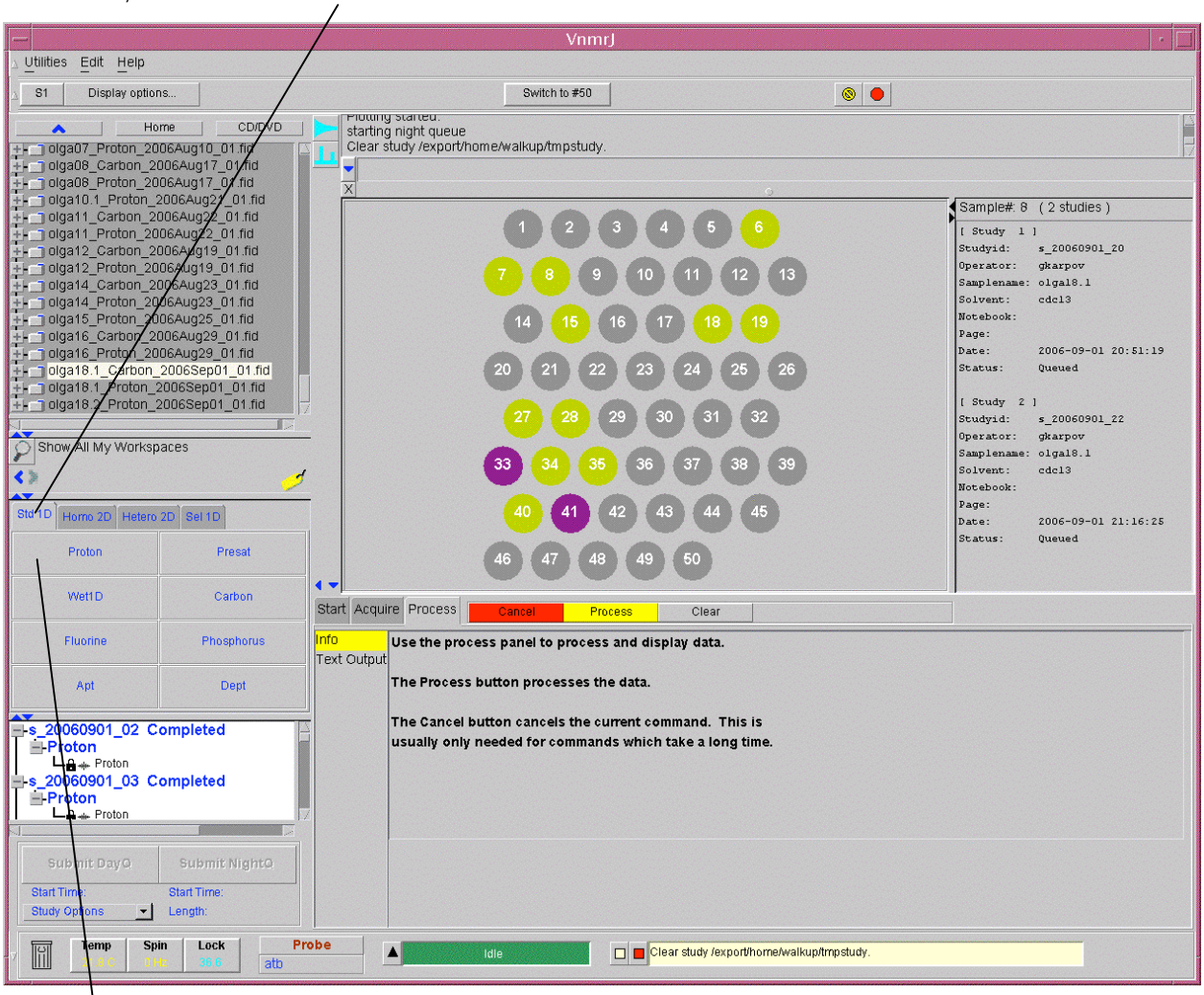


Sample placement

-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**

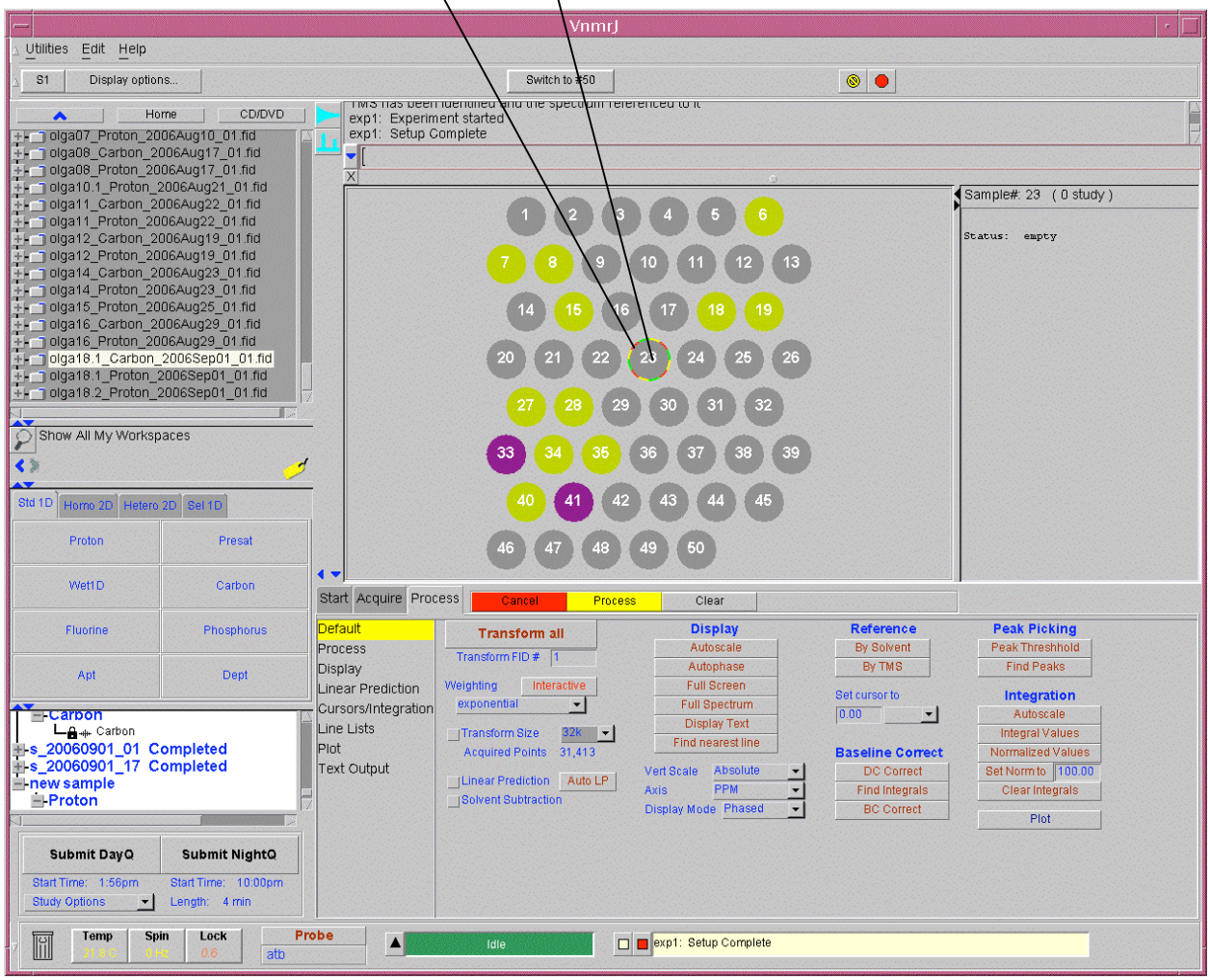


-click **Proton**
    -this will load the appropriate parameters for collecting a simple 1D ^{1}H experiment

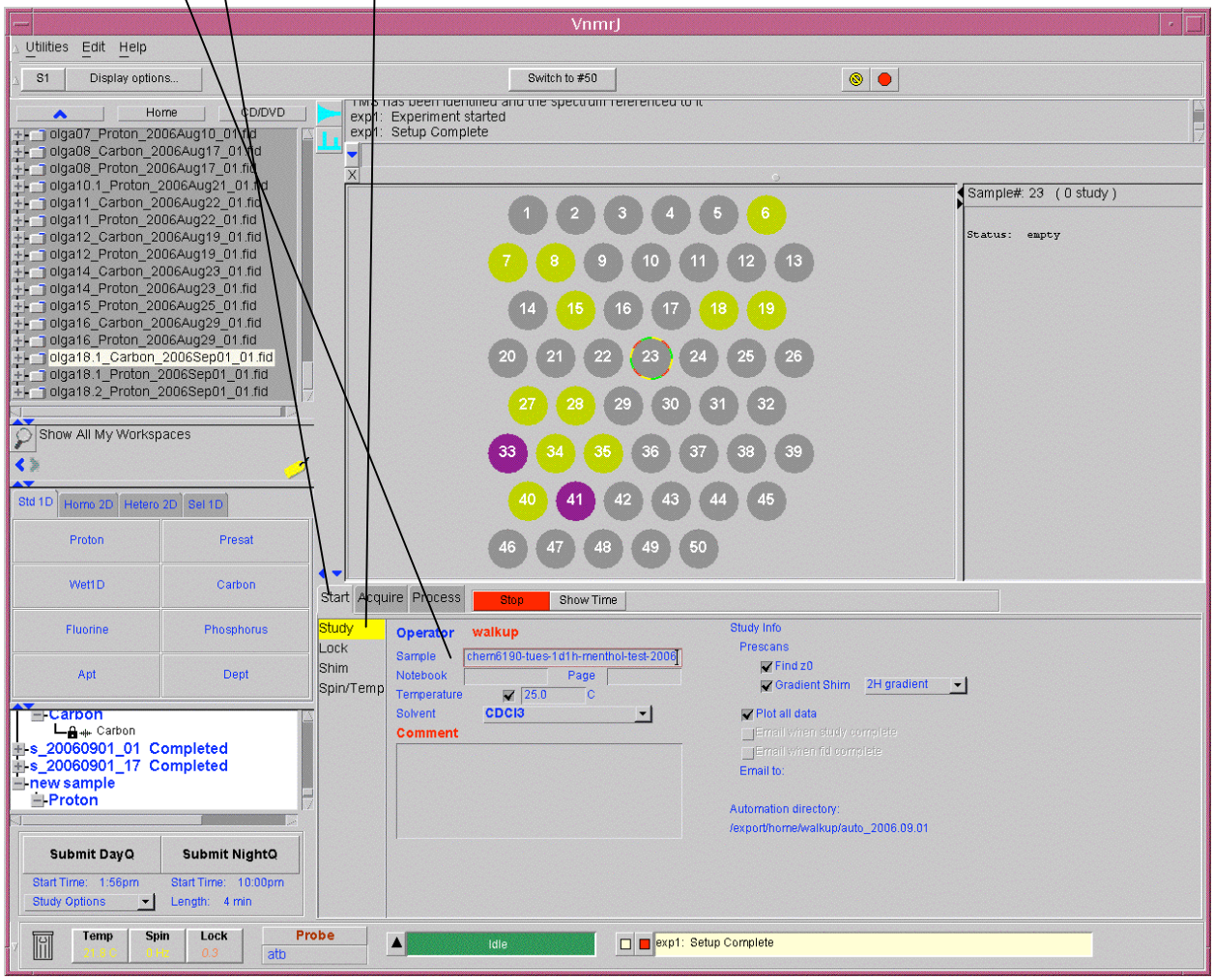
-in window below, **new sample** and **Proton** will appear (these will be colored yellow, indicating that the sample has not yet been submitted, and the experiment has not begun)

-on the **tray panel** click on the **appropriate slot** (an empty one that you have chosen)

-it will get a **multicolored highlight** once selected

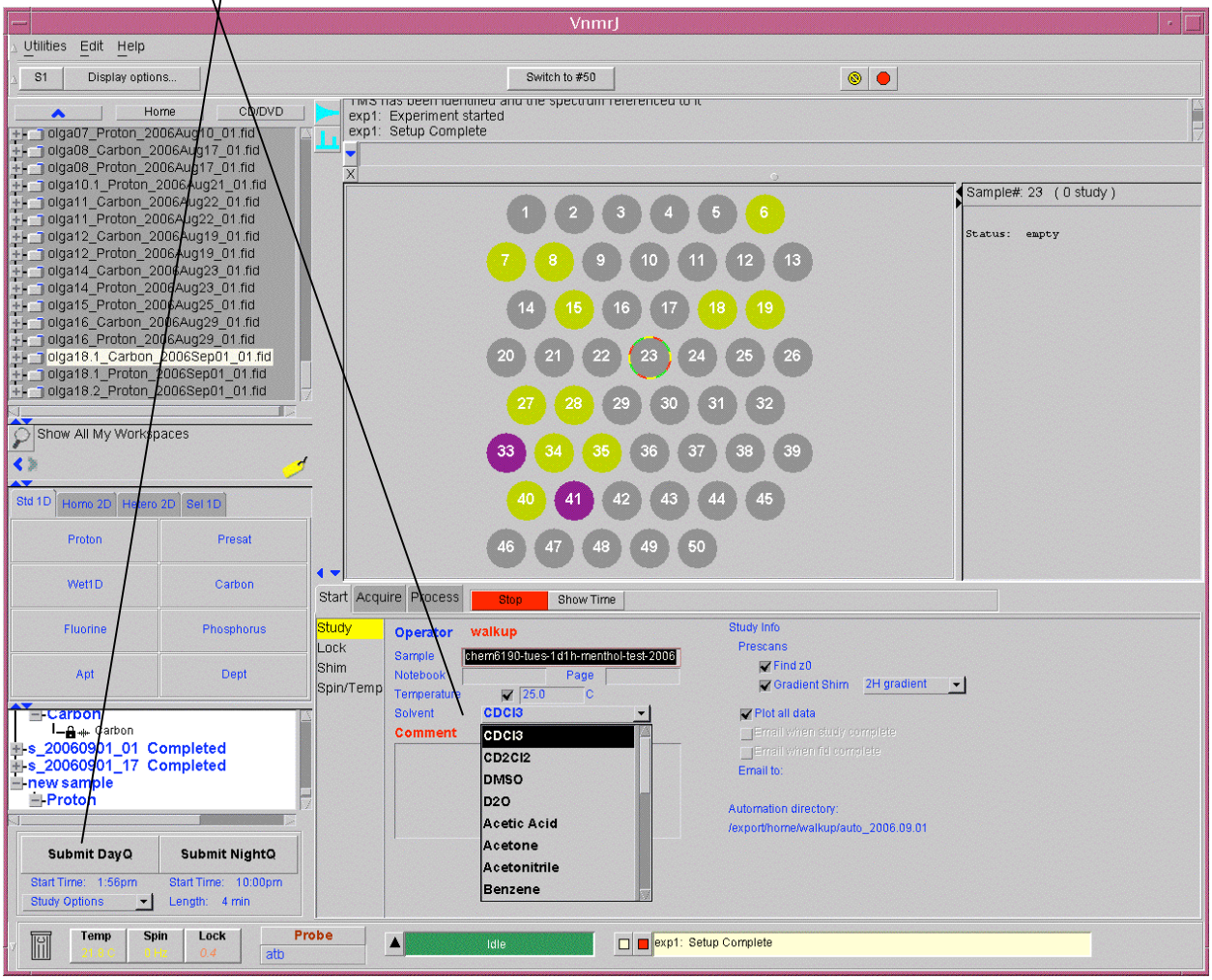


-click the **start tab**, and the **study** option
-in the **Sample** field, enter a **descriptive** sample name
-*no capital letters or spaces, no special characters other than dash (-) and underscore (_)*
-for instance, “chem6190-1d1h-090306’
-the **notebook**, **page**, and **comments** fields can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



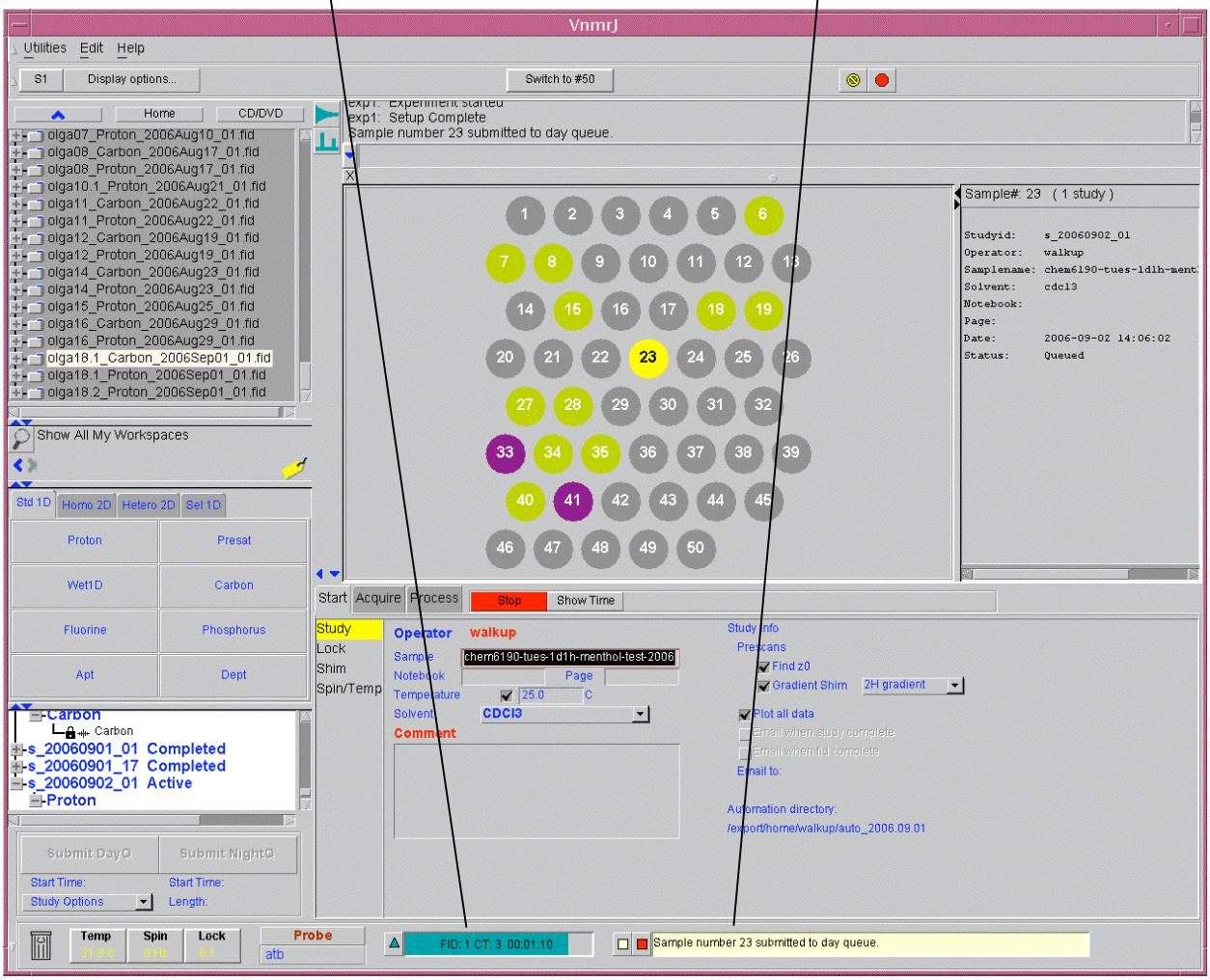
Setting parameters and submitting the sample

-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



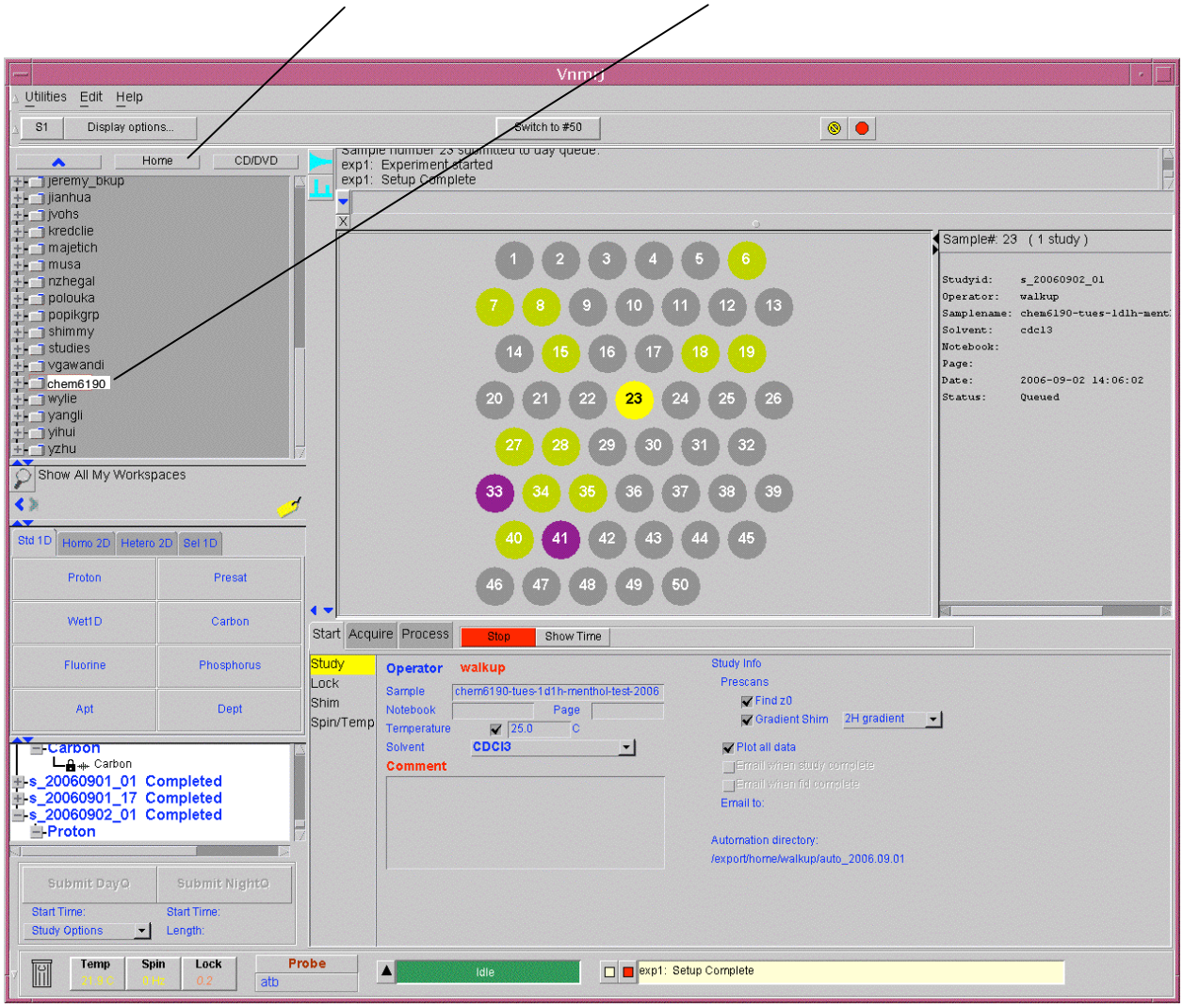
Locking and shimming

-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

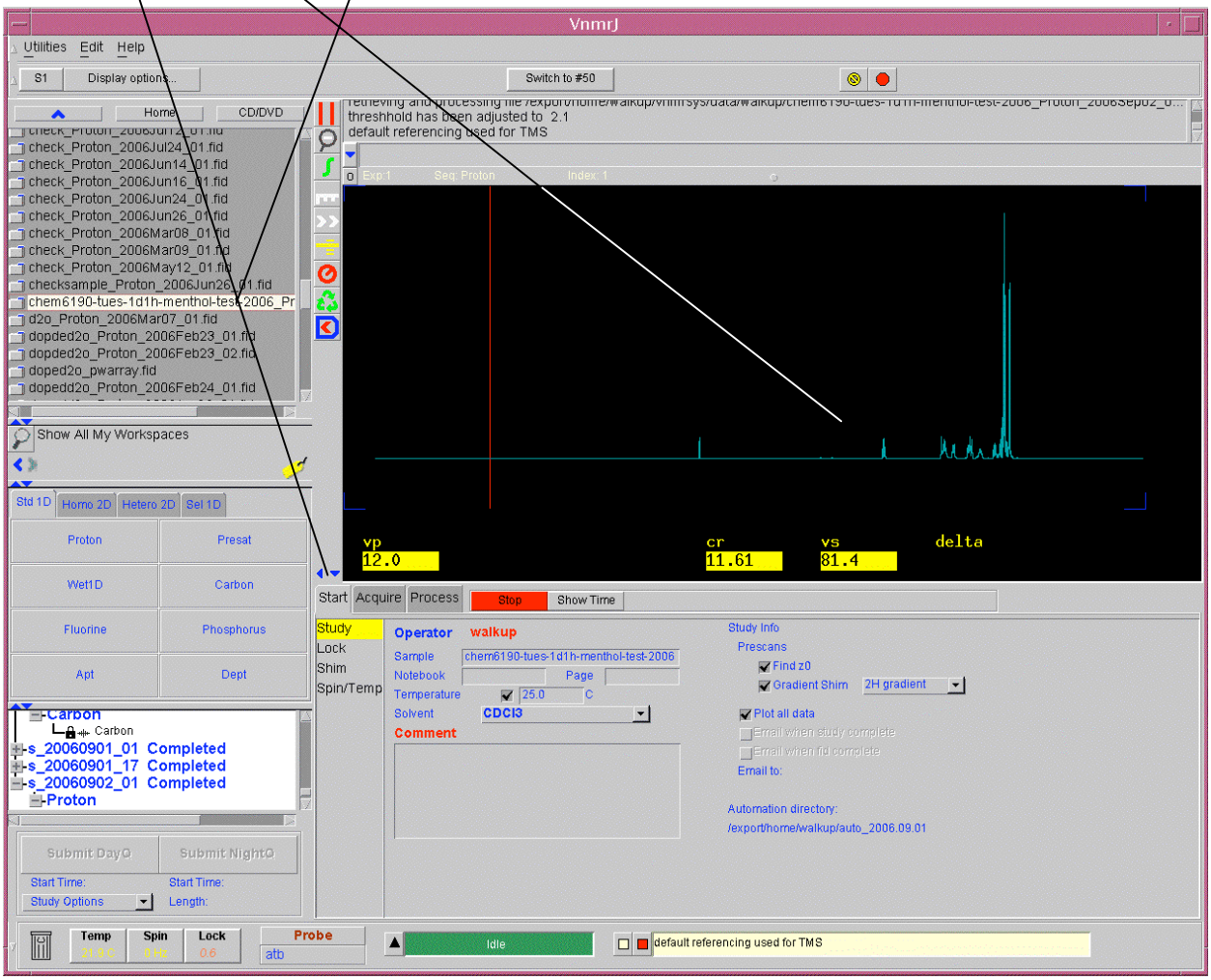


Retrieving the data and displaying the spectrum

-at the upper left, click the **Home** button, then double-click **chem6190**……..



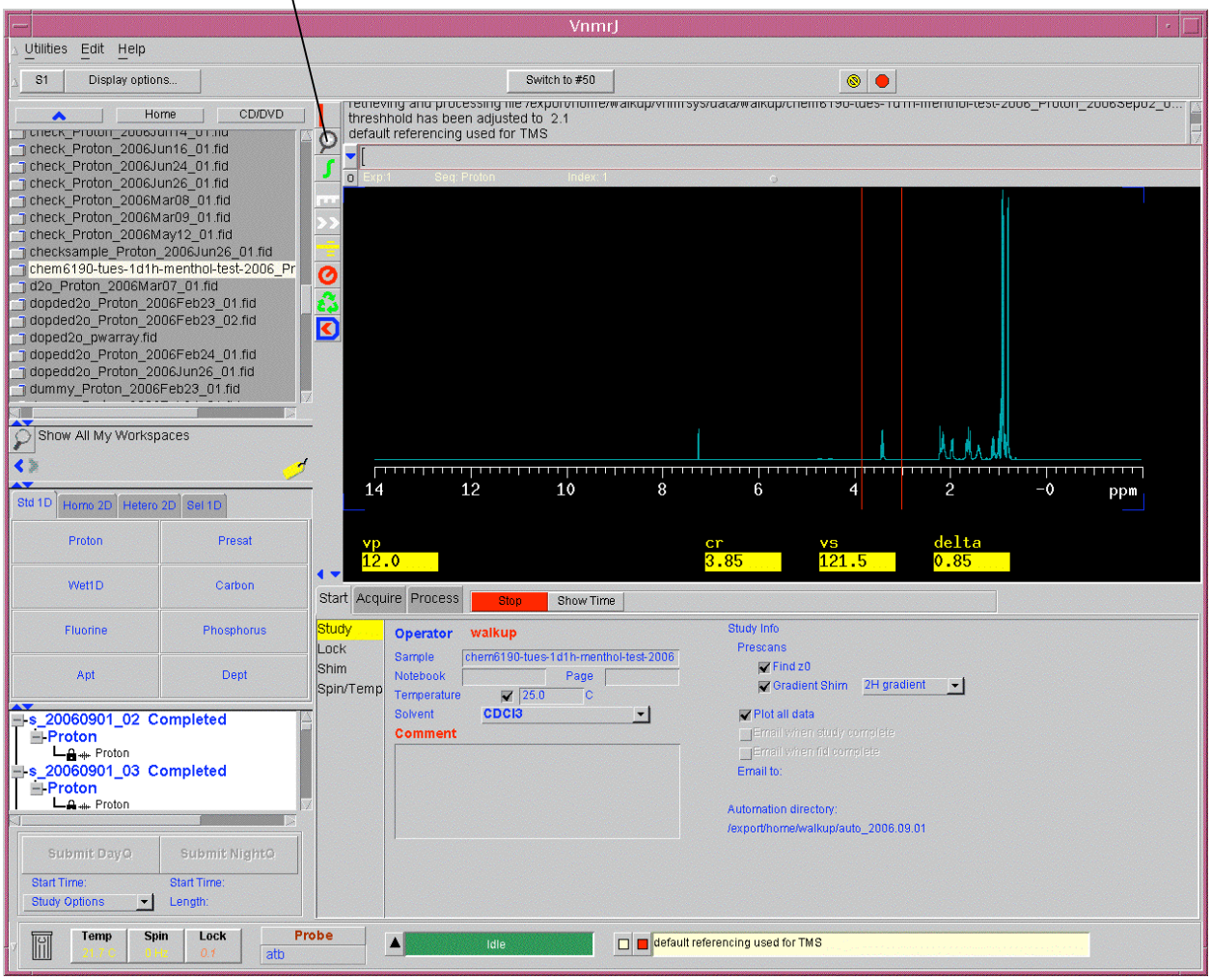
……..then double-click on the **filename** that you gave to **your data**
-the **spectrum** should appear (in the window previously occupied by the tray panel)
-the **two small blue arrow buttons** (◂ ▾) at the lower left of the spectral window permit this window to be expanded (to fill most of the screen)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



Zooming in and out

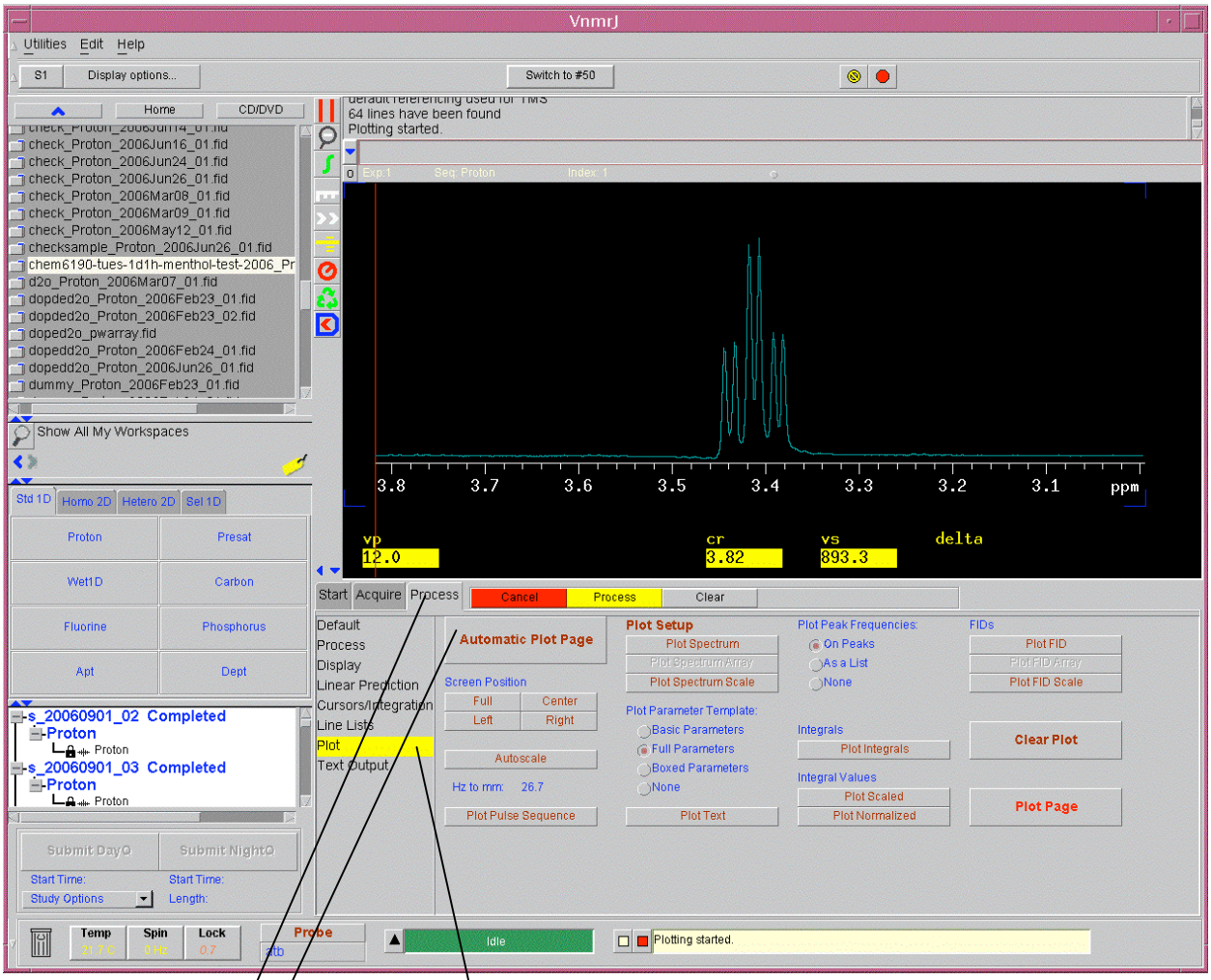
-to zoom in on a particular spectral region or peak of interest, place the **arrow cursor** at the **left edge** of this region and click the **left mouse button** (or drag the red cursor to the left side of the region of interest with the left mouse button), then move the **arrow cursor** to the **right 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*)

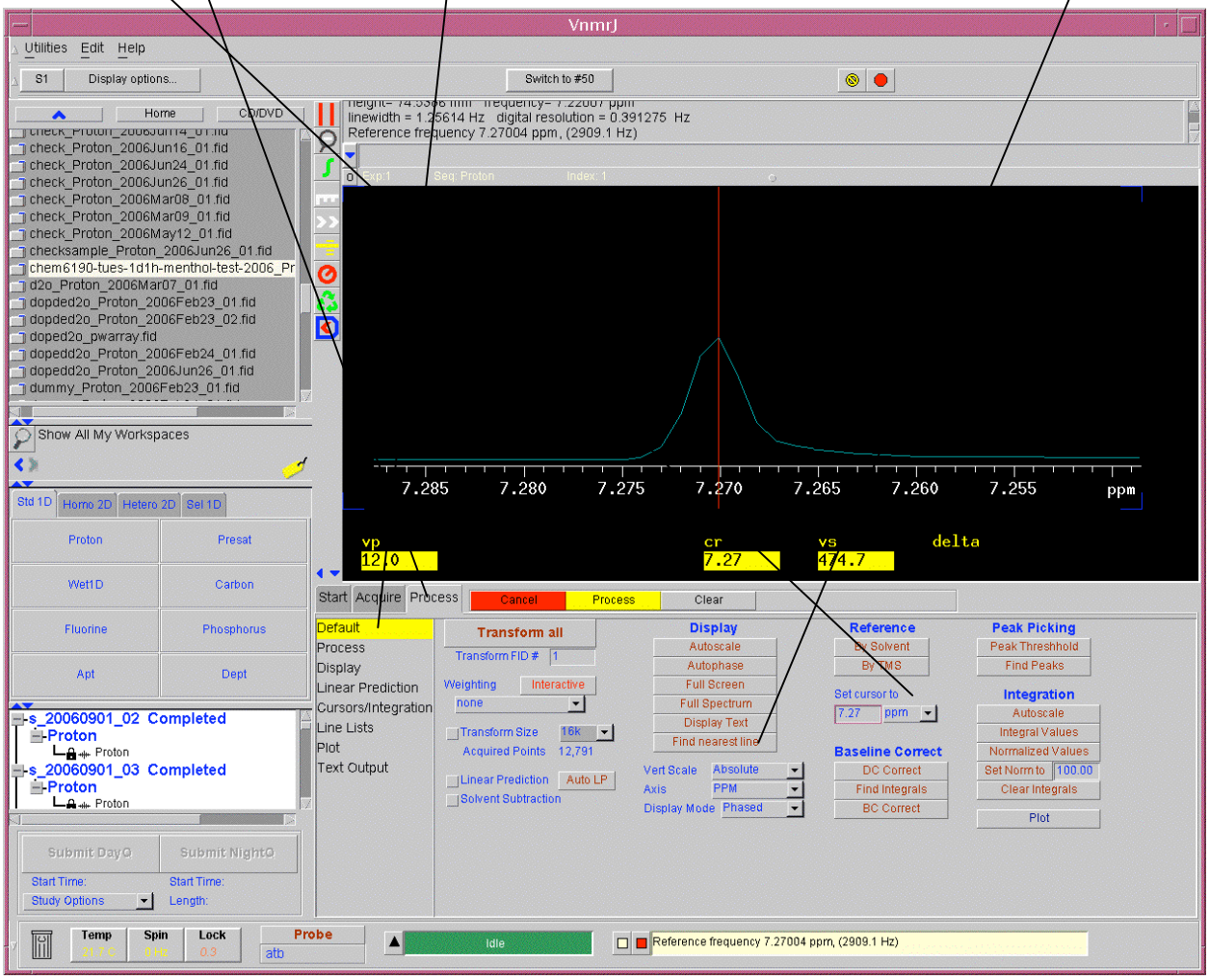


Quick plotting

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

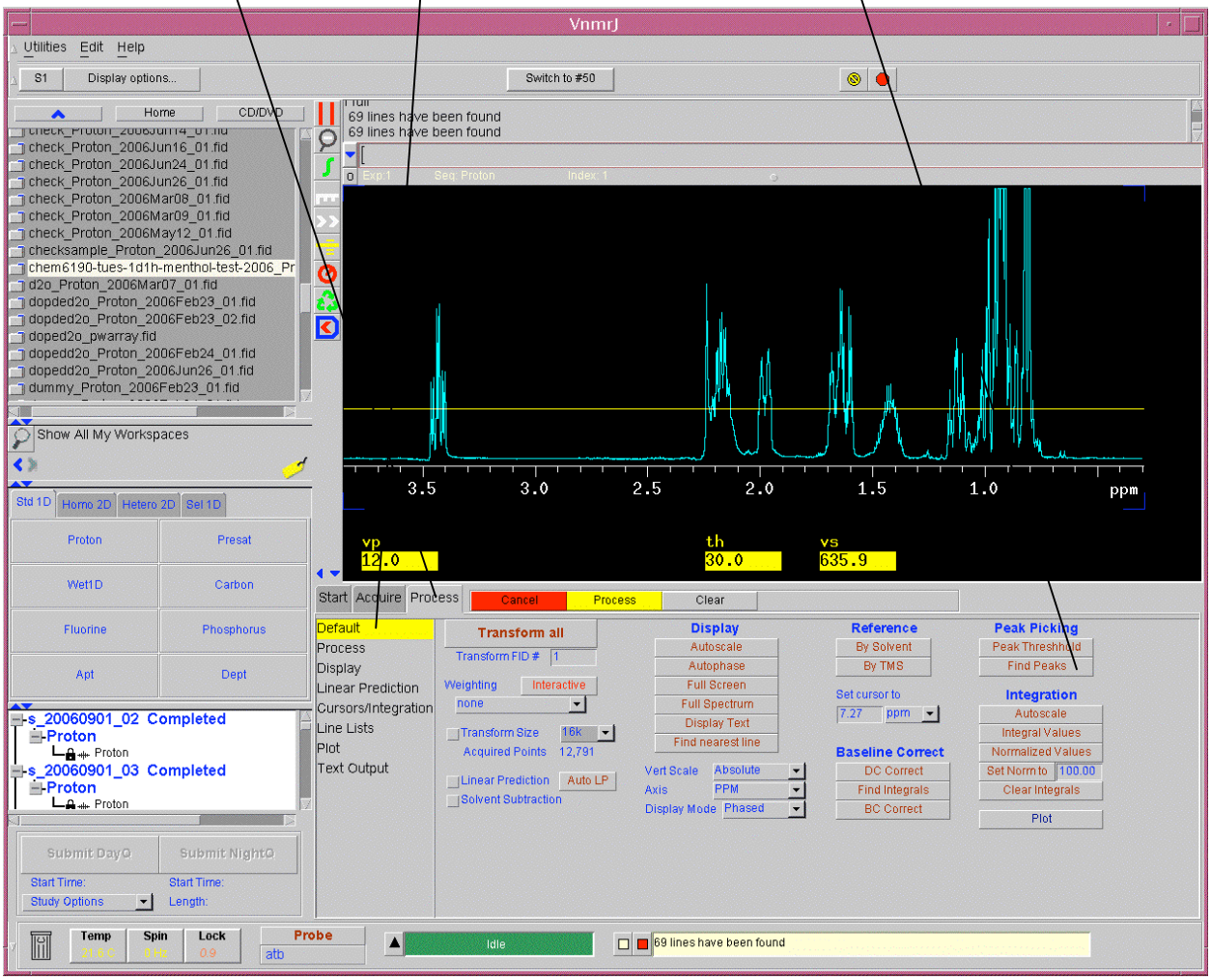
Chemical shift referencing

-the ^{1}H 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 ^{1}H 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**



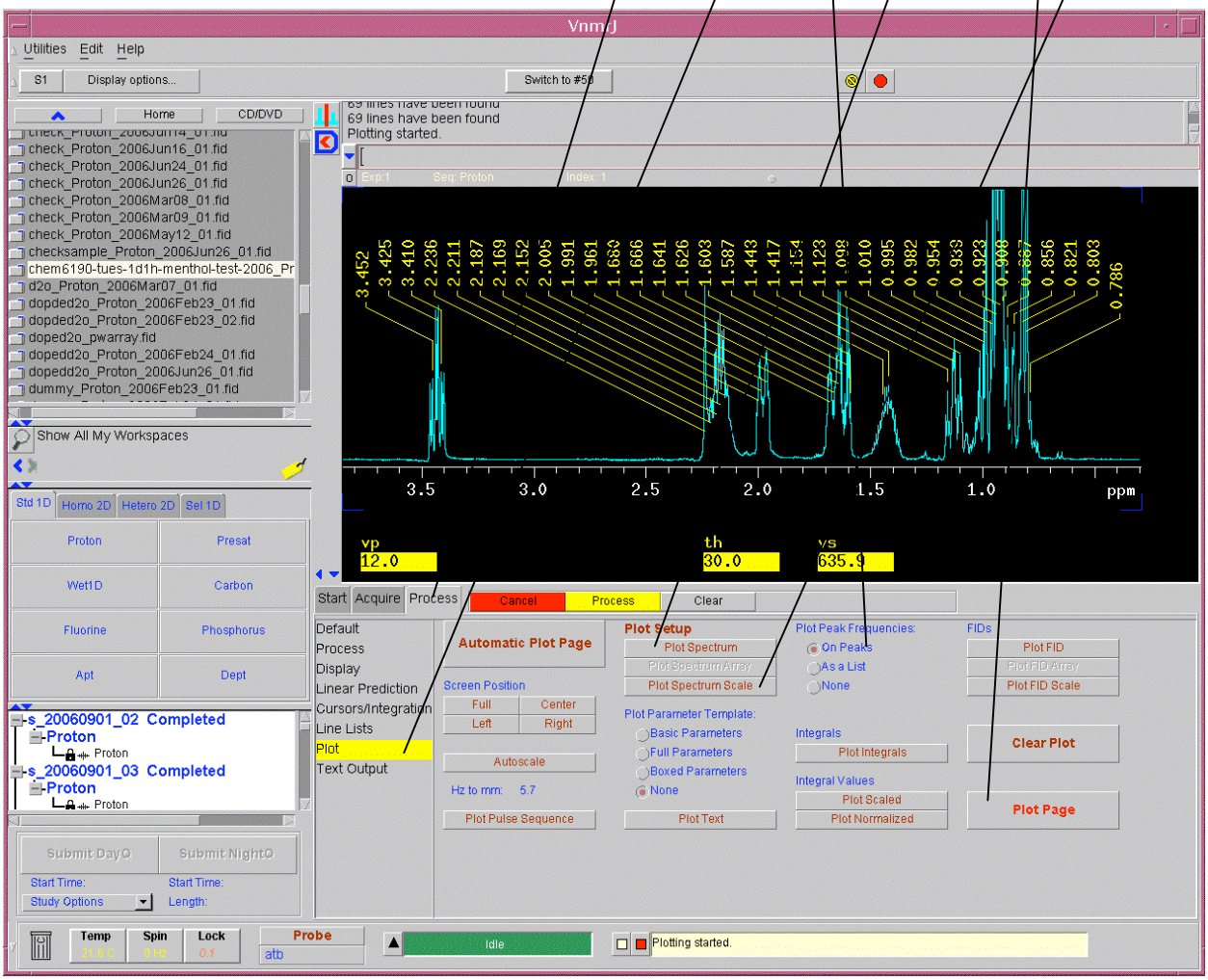
Peak picking

-**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**



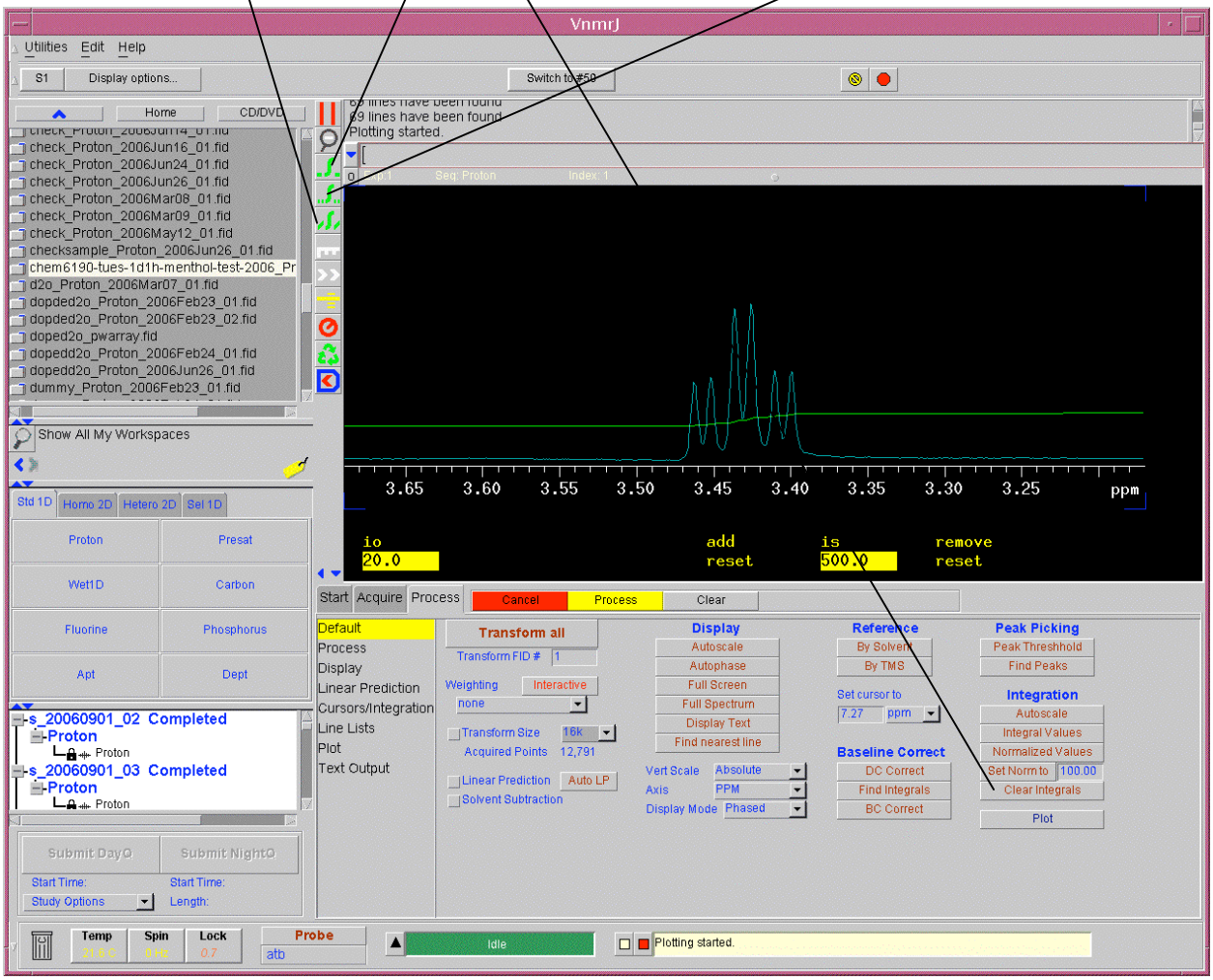
-in order to **print the peak positions** (hardcopy), under **Process**/**Plot**, click **Plot Spectrum**, then **Plot Spectrum Scale**, then under **Plot Peak Frequencies**, select **On Peaks**. Then click **Plot Page**

-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**



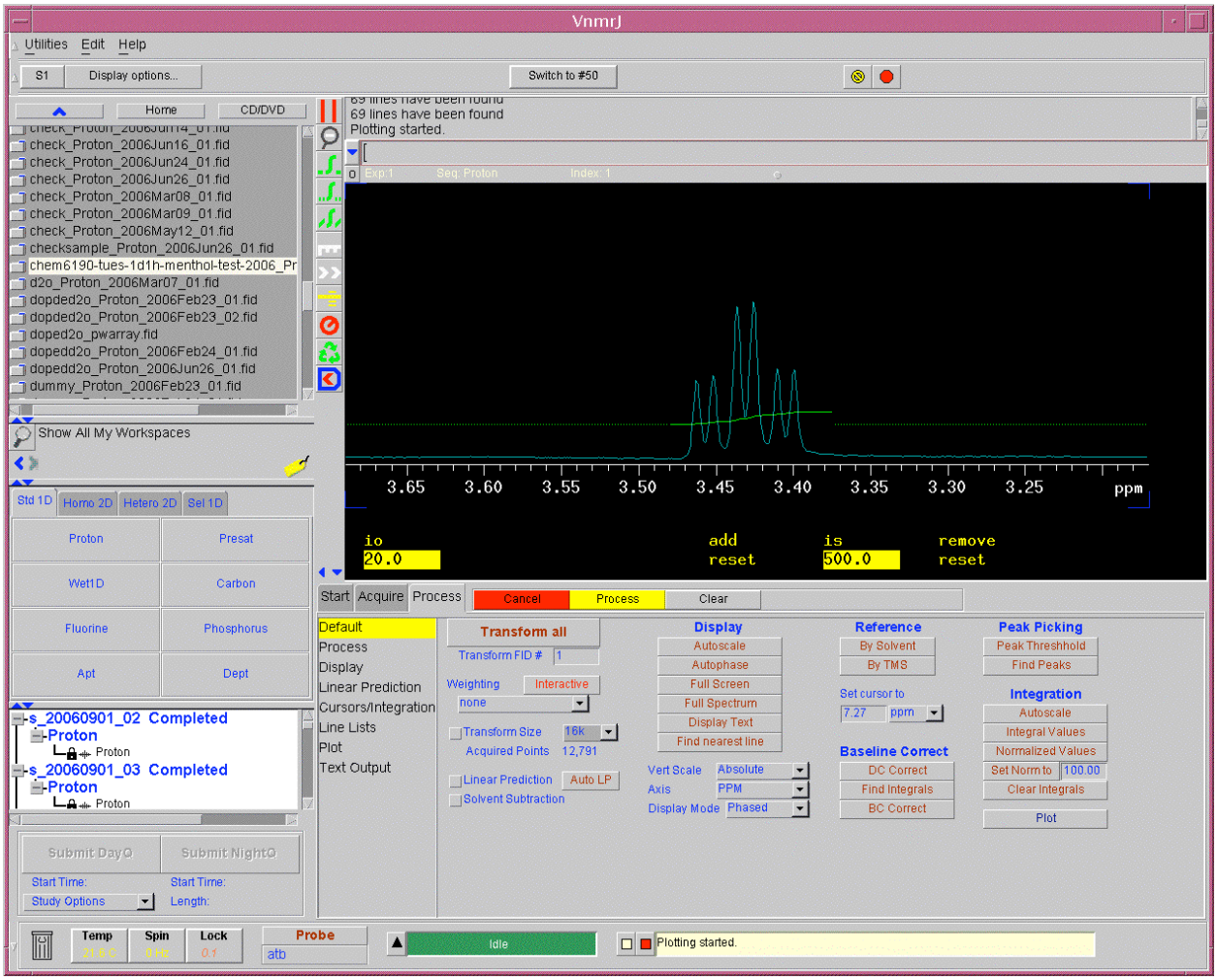
Integration

-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*

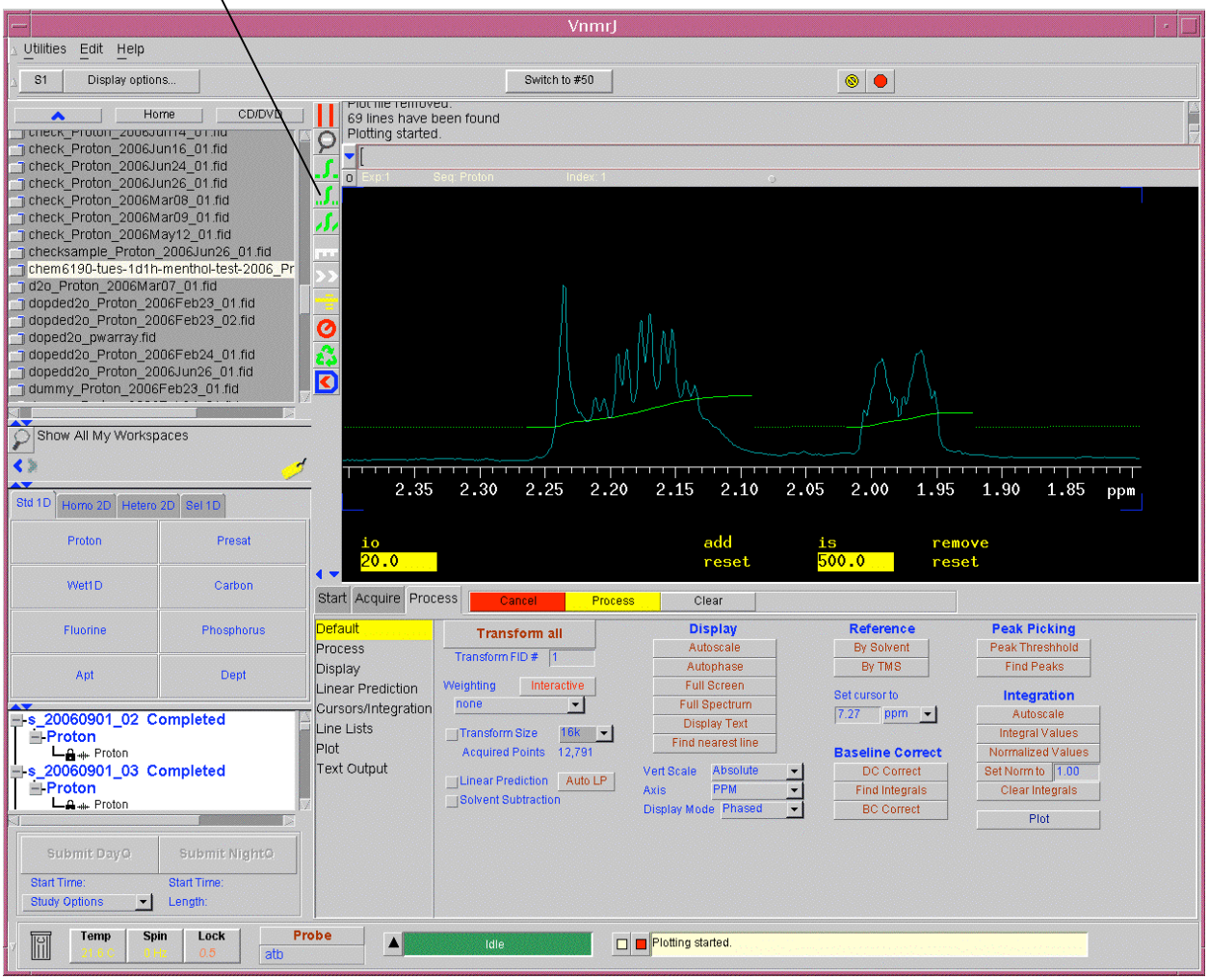


-then, place the cursor just to the **left of the peak** (at the baseline) and **click** (left mouse button) Then place the cursor just to the **right of the peak** and **click** (again, left mouse button).

-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)

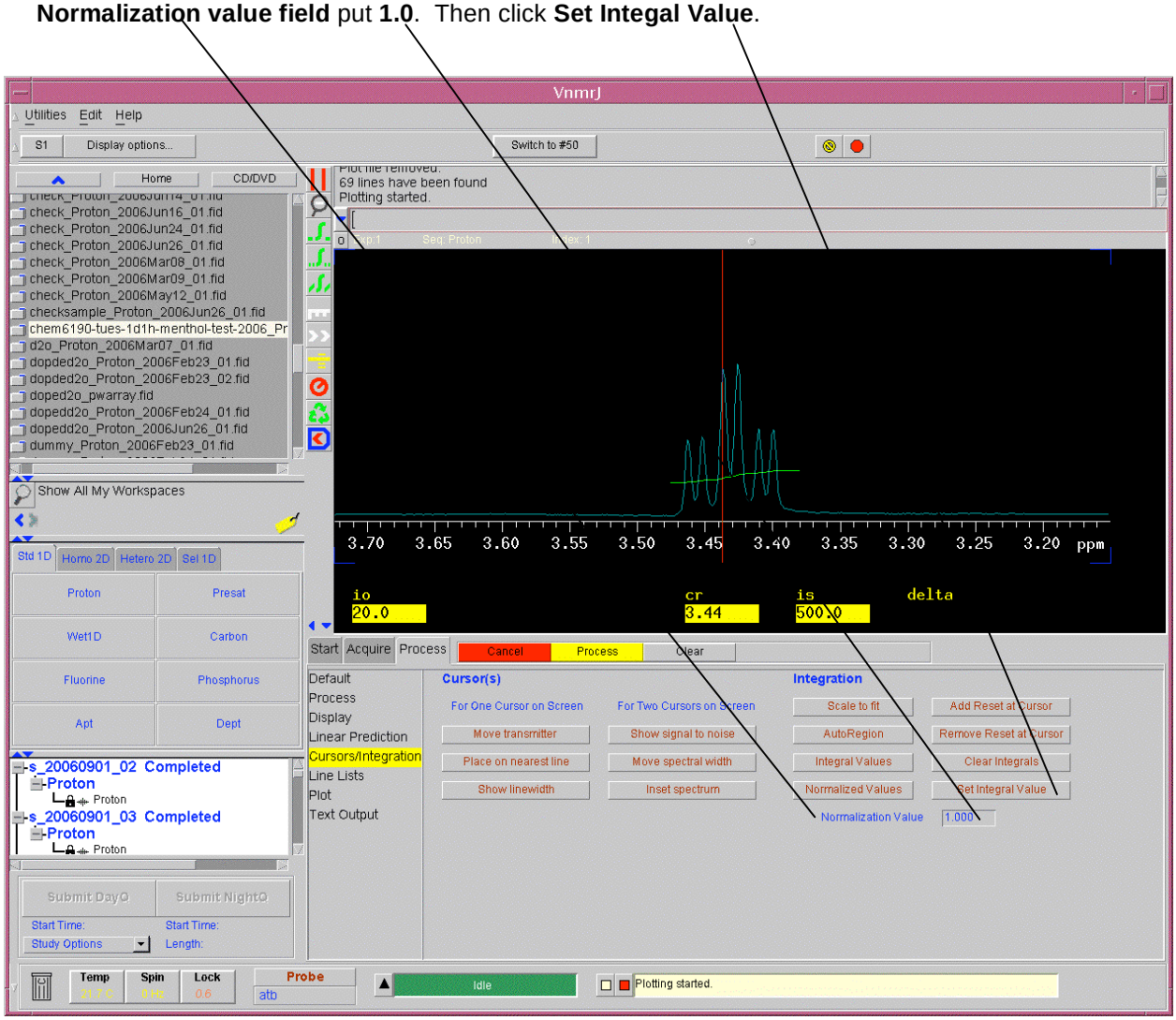


-expand around the next two signals in the spectrum (the two at approximately 2.2 and 2.0 ppm)
-*click the **integral resets button***
-click on the right and left sides of each of these signals, as you have done for the previous signal
-repeat this for the rest of the signals in the spectrum



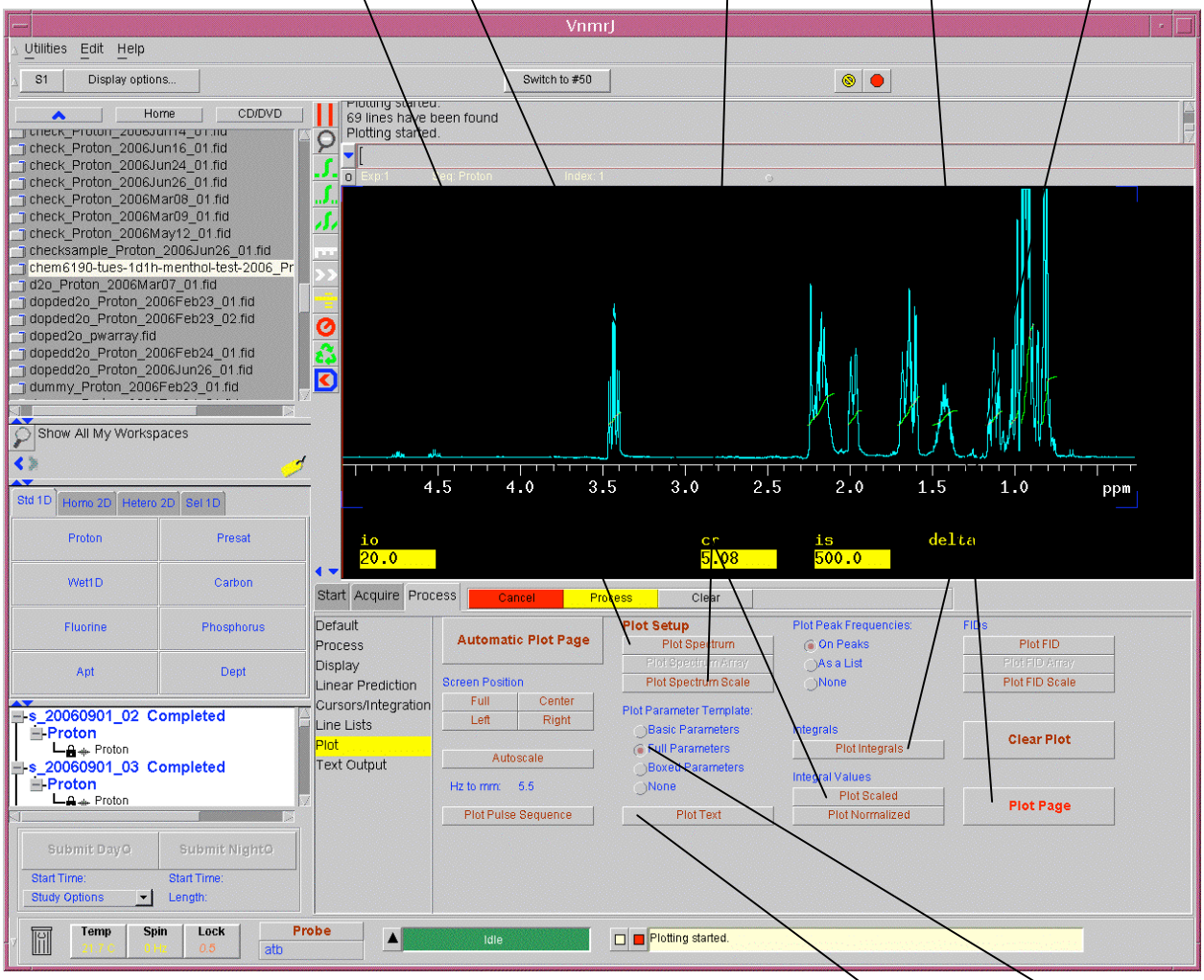
Integral calibration

-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 ^{1}H 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 Integal 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



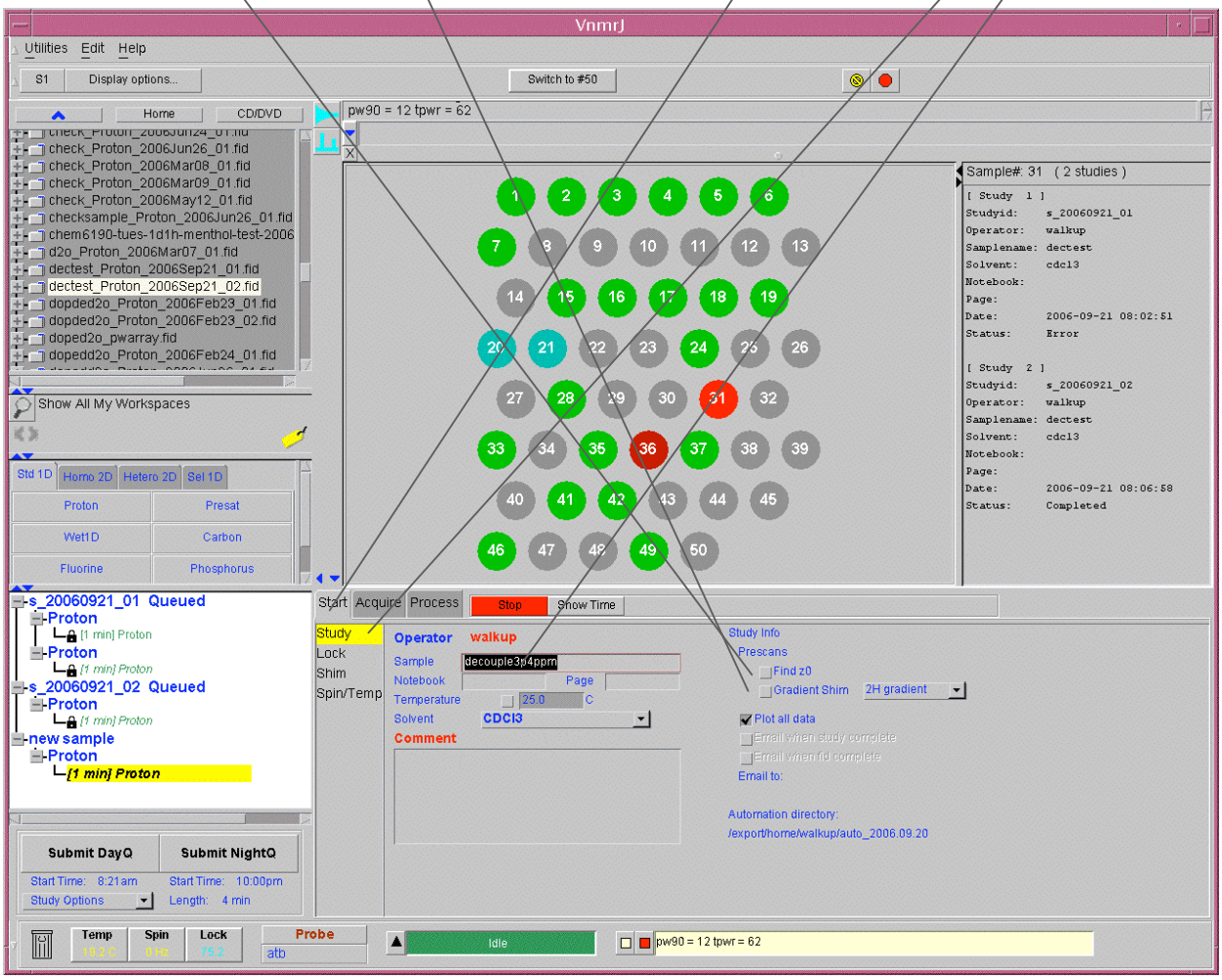
-try the above set of commands again, but in this case **include** clicking on **Plot Text** with the **Full Parameters** button selected
    -this will **print the acquisition parameters** on the hardcopy plot. *You will note that these parameters are printed on the left side of the spectrum, so it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

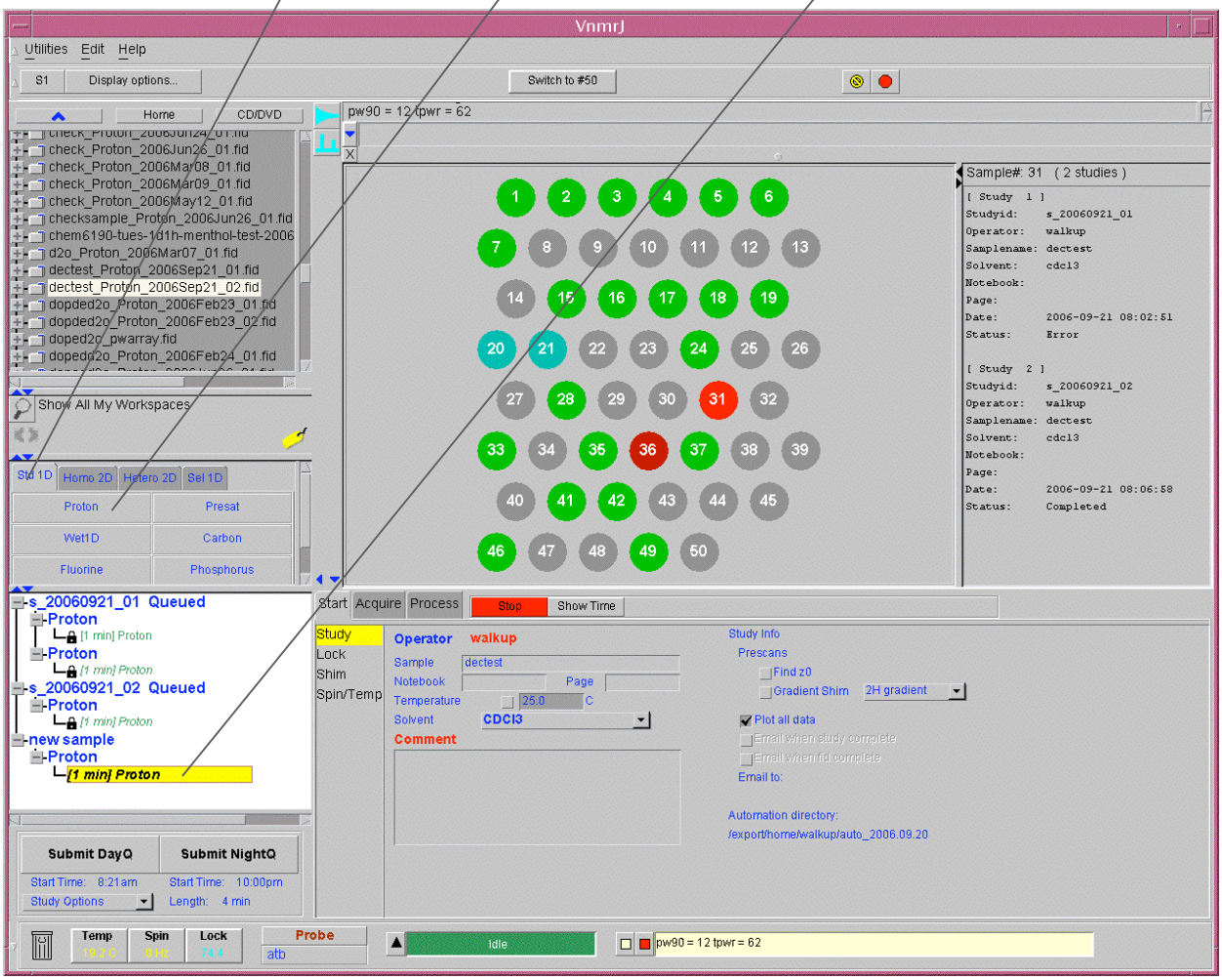
-it is best to remove your sample. Simply click the **Switch to #50** button
-in the extreme upper left of the screen click the **Utilities** button, and select **Change Operator** from the menu

Selective Homonuclear Decoupling

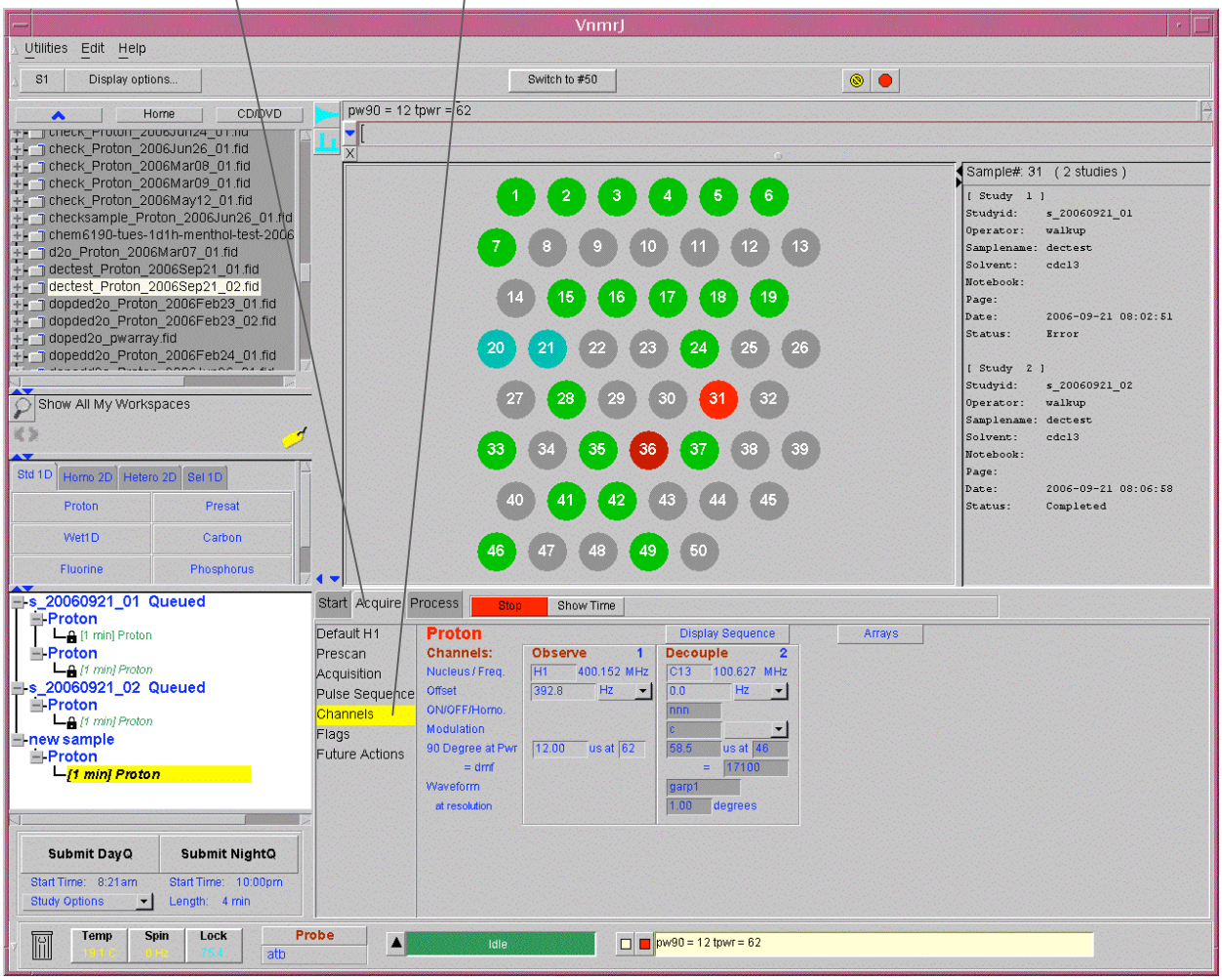
-first, record a 1D ^{1}H spectrum of your sample (see pages 1-11)
-next, turn off the automated shimming routines by selecting the **Start** tab, then the **Study** option, and then *de*select **Find Z^{0}** and **Gradient Shim**. Also, give your experiment a new name (**Sample**)



-then, as before, click the **Std 1D** tab, then the **Proton** option, and then **double click** on the yellow-highlighted ***(1 min) Proton*** selection



-then, click the **Acquire** tab, and the **Channels** option

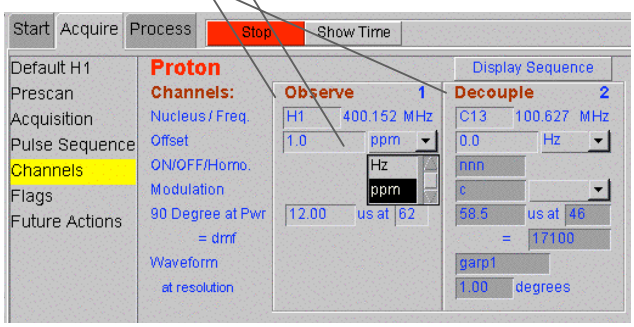


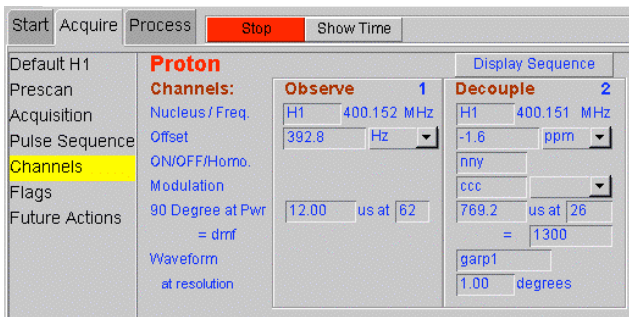
-here you will see two Channels: **Observe** (channel 1), the nucleus to be observed (^{1}H in our case), and **Decouple** (channel2), the nucleus to be decoupled (also ^{1}H in our case). The parameters for the observe channel will not change, but we need to adjust those for the decouple channel

-under **Observe**, you'll see the frequency and nucleus set to ^{1}H, etc

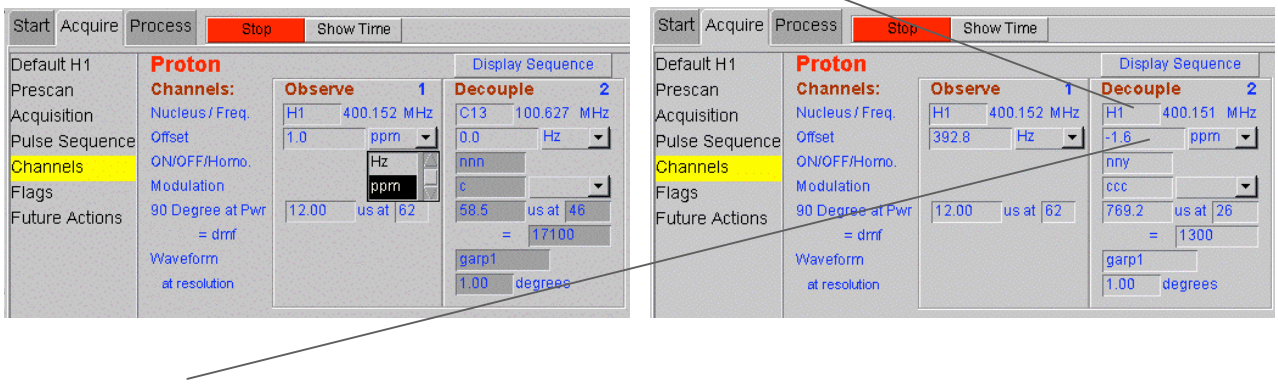
-for the **Offset** option, you'll see 398.2 Hz, or 1.0 ppm

-under **Decouple**, you'll see default parameters for decoupling ^{13}C – we need to change these for ^{1}H decoupling (next page)





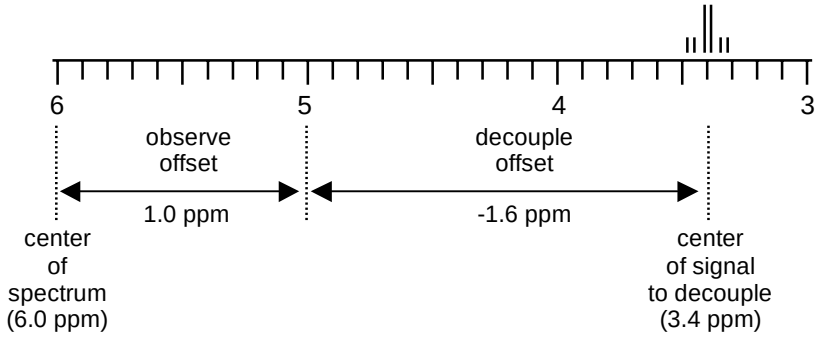
-for the **Decouple** channel, we'll set **Nucleus/Freq**. to **H1**



-**Offset** (**Decouple offset**) defines the frequency of the peak that we wish to (selectively) decouple
-this is calculated, in **ppm** as follows:

$$\text{decouple offset} = \text{center of peak to be decoupled} - \text{center of spectrum} + \text{observe offset}$$

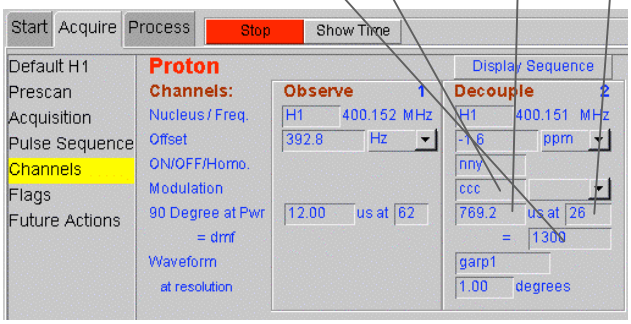
-for our case, to decouple the peak at 3.4 ppm, this is diagrammed as follows:



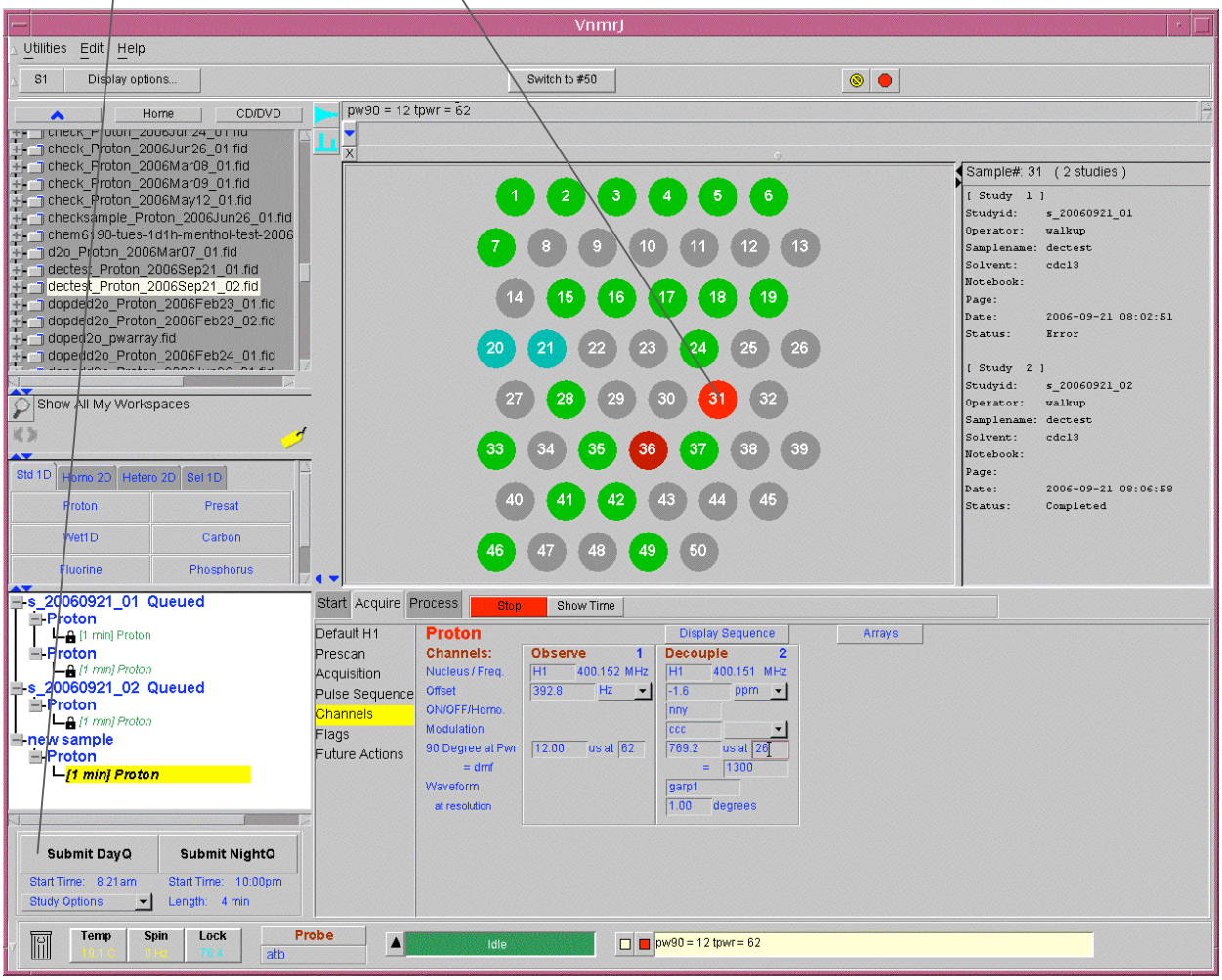
- the calculation shows that the decouple offset should be set to **-1.6 ppm** to decouple the peak at 3.4 ppm, assuming the center of the spectrum to be 6.0 ppm

-1.6 ppm = 3.4 ppm - 6.0 ppm + 1.0 ppm

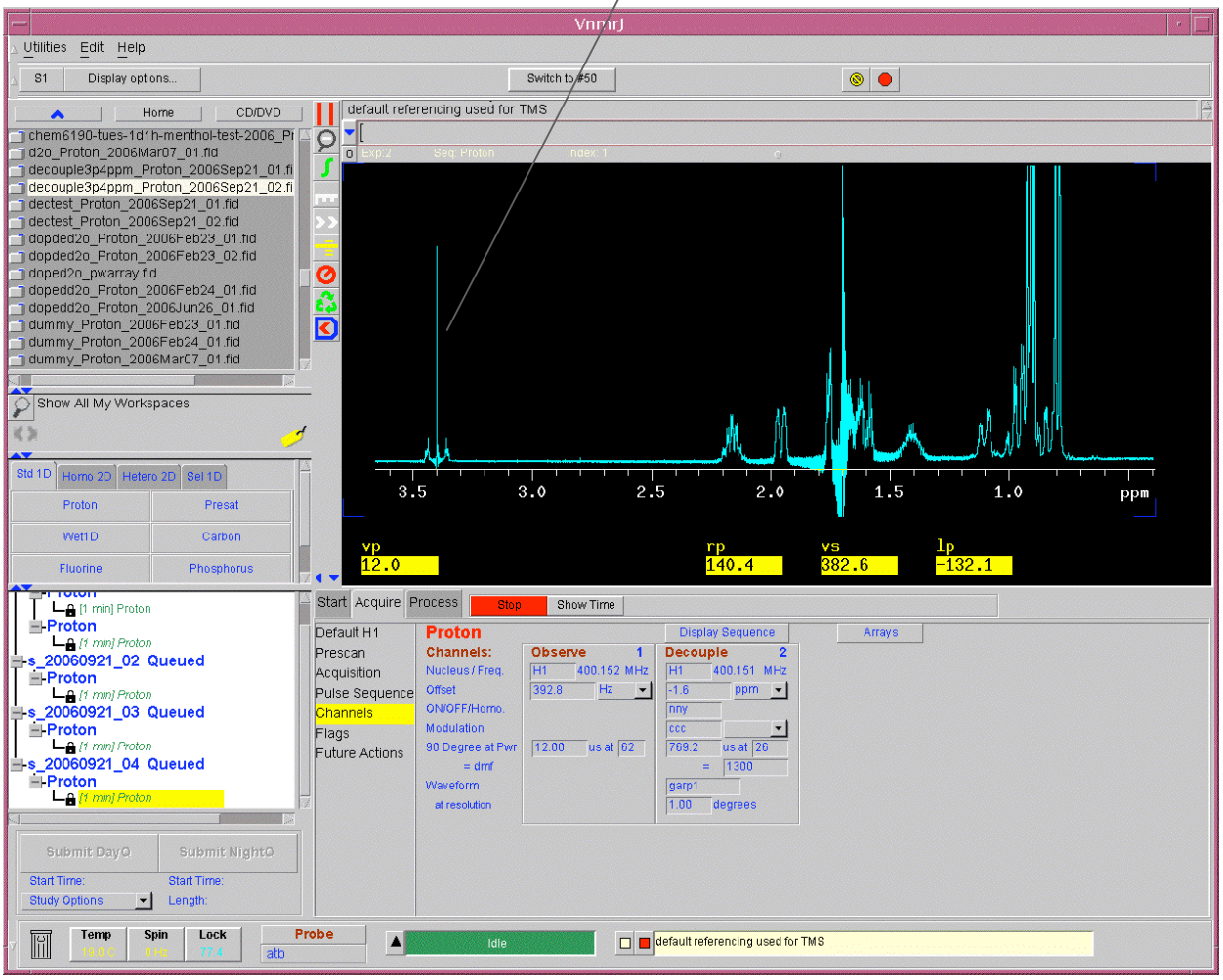
-**ON/OFF/Homo** is set to **nny** and **Modulation** is set to **c** or **ccc** for homonuclear decoupling
-we will set the 90 degree pulse width (**90 Degree**) and power (**Pwr**) to **300** and **34** respectively. The **=dmf** parameter will then be set automatically to ~**3300**. *NEVER SET PWR > 38*
-the remaining parameters, **Waveform** and **resolution** do not need to be adjusted



-then, as before, we will **select our sample number** in the sample tray (31 if our sample was 31) and **Submit to Day Queue**



-retrieve the data as usual. You will notice that the peak at 3.4 ppm is altered dramatically by the rf field applied at its chemical shift (including removal of splitting due to couplings to other protons)
-careful analysis of other peaks in the spectrum will reveal loss of couplings (removal of some splittings), indicating coupling to the hydrogen giving rise to the signal at 3.4 ppm



Logging out

-first, go back to **Start/Study** and *select* **Find Z^0** 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 #2:

1). Acquire a 1D ^{1}H spectrum of your sample. Plot (hardcopy) the full spectrum (the region that includes peaks from your molecule) and expansions of individual regions of the spectrum so that the effects of subsequent selective decoupling of individual peaks can be assessed.
2). Selectively decouple the peak at 3.4 ppm. Plot (hardcopy) the full spectrum (the region that includes peaks from your molecule). Plot expansions of the indidual regions so that the effect of this decoupling on all peaks in the spectrum can be assessed.
3). Do the same for the peaks at ~2.25 ppm, ~1.95 ppm, ~1.4 ppm, ~1.1 ppm, and ~0.8 ppm.

Exercises and Questions for Lab #2:

1. -Discuss, *in general*, which signals are good candidates for selective decoupling and which are not. Explain your reasoning.
-In menthol, considering the results of your decoupling experiments, which signals, when decoupled, gave valuable information and which did not. Elaborate on each specific case.

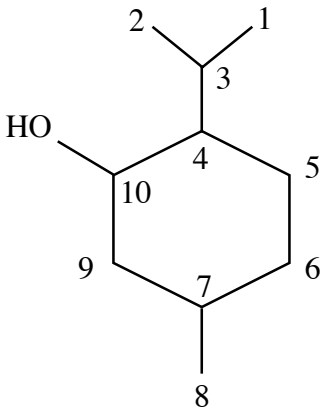
2. For lab #1, you created a table with information about the signals in the menthol spectrum including chemical shift, coupling constants, multiplet structure, and assignment. In the table (or in other parts of your report), you justified your assignments based on the information available to you.
-Now, revise/update your table. If you made mistakes the first time around, fix them. In addition, revise your assignments based on the new information that you have from the decoupling experiments. Try to assign as many signals as you can. You must thoroughtly justify your assignments.
-You will be scored based on all of the information in your table, and in particular your detailed justifications of assignments or revision of assignments based on your new decoupling information.

3. -In your own words, discuss the advantages/disadvantages and usefulness/utility of selective decoupling experiments.

4. For acquiring a simple 1D ^{1}H spectrum of our compound, we used $CDCl_3$ as the solvent. Suppose you don't have any $CDCl_3$, and you decide to use $CHCl_3$ instead.
-What is the concentration of $CHCl_3$ in 100% $CHCl_3$ (show your work)?
-What challenge will you face in acquiring a 1D ^{1}H spectrum of menthol (let's assume that the concentration of menthol in our sample is 1 mM) in pure $CHCl_3$ as the solvent?
-How might you overcome these problems (assuming that you can't switch solvents)?

SAMPLE:

Our sample is ~0.5M menthol in $CDCl_3$



menthol

SIGNAL (LABEL)	Chemical Shift (ppm)	Integral (normalized values)	Multiplicity	Coupling constants (Hz)	Connectivity / Assignments / Comments
a	3.43	1.0	Doublet of triplets	$^3J_{10,4}$=x Hz $^3J_{10,9}$=y Hz	Signal a corresponds to hydrogen 10 in menthol. This signal is a doublet of triplets (split by the two hydrogens at 9 into a triplet and the hydrogen at 4 into a doublet). Because of the inductive effect of the hydroxyl, it is the most downshielded hydrogen in the spectrum. Selective decoupling experiments show…
b	2.2				Incidentally, and are incorrect.
c					
Etc.					