

# T<sub>1</sub> Relaxation Measurement: The Inversion-Recovery Experiment (Using IconNMR)

Revised 4-21-2015

Relaxation times are divided into two types: *longitudinal*, which concerns change in magnetization along the z-axis ( $T_1$ , an enthalpy effect), and *transverse*, which concerns change in magnetization in the x-y plane ( $T_2$ , an entropy effect). The inversion-recovery experiment is used to determine the time constant for the  $T_1$  process. One of the assumptions made in the  $T_1$  calculations is that the *equilibrium is approached exponentially*, and therefore, the magnetization along the z-axis is represented by equation 1. Here,  $M_0$  is the magnetization at thermal equilibrium,  $t$  is the time elapsed, and  $T_1$  is the time constant that is obtained by comparing different values of  $M_z$  at different times.


$$M_z = M_0(1 - e^{-t/T_1}) \quad (1)$$

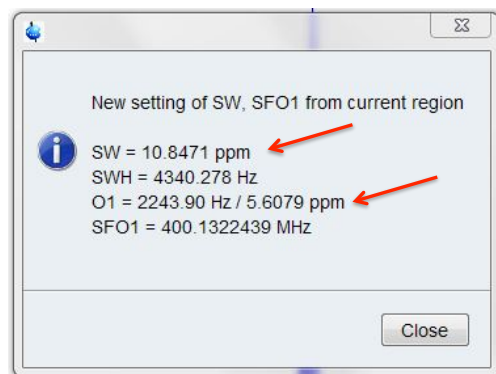
The pulse sequence used in the inversion-recovery experiment is shown below:

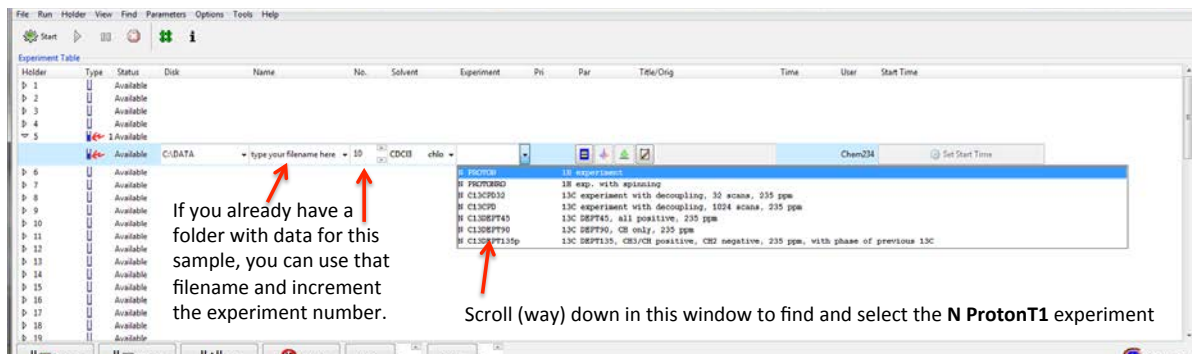
d1 - p1(180°) - d2 - p2(90°) - FID

In this experiment, the nuclei are first allowed to relax to equilibrium (**d1**, obviously, in the first run through the pulse sequence **d1** is superfluous). A 180° pulse (**p1**) is then applied to invert the signals. The signals are then allowed to relax for a length of time (**d2**) that is varied for each experiment. After the variable **d2**, a 90° pulse (**p2**) is applied and the FID is recorded. The FID records the spectrum intensity as a function of the variable delay **d2**, and the longer **d2** is, the more the signal will have relaxed. The peak intensity will reflect the extent to which each signal has relaxed during the **d2** period.

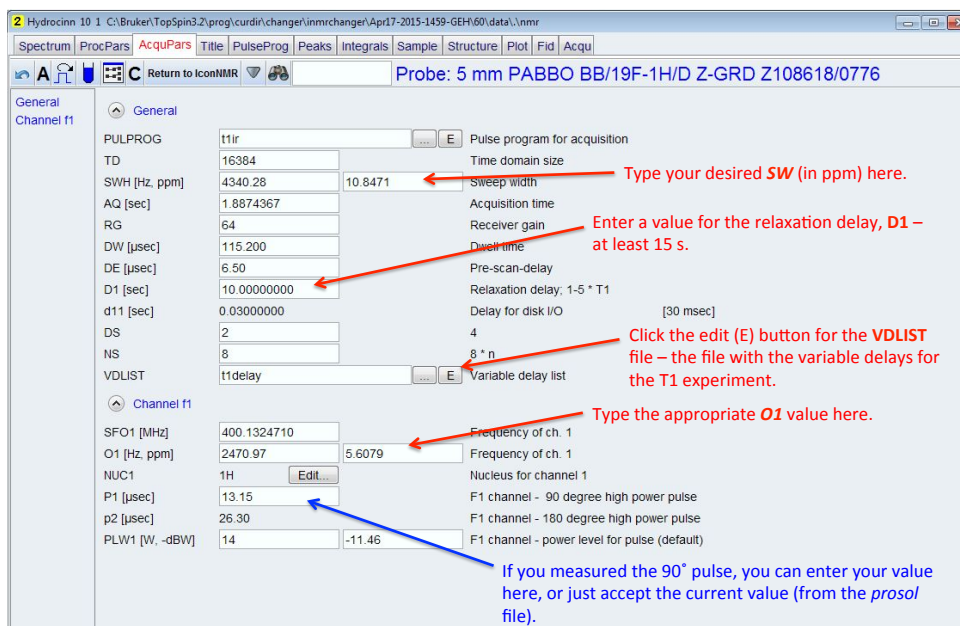
## Procedure using IconNMR

1. Working first in TopSpin:  
It is best to use a fairly minimal acquisition window (**SW**) when running the inversion-recovery experiment. If you already have a regular proton spectrum of your sample, you can use that to determine the proper acquisition window, otherwise, acquire a normal spectrum.
2. Open the spectrum file and process as usual. Expand around the peaks of interest, so that in the display, you leave about 0.5 ppm of baseline on either side of the spectrum. You may exclude the solvent peak, if desired, unless you want to know its  $T_1$  (it is likely to be quite long).  
After expanding the spectrum, click on the button “Set SW, SFO1 from current region”,  shown to the right.
3. A dialog box will appear and will give you the values of **SW** and **O1** that correspond to the displayed spectral region. Write down the values for SW and O1 (both in ppm). You'll need these to set up the T1 experiment. Close the dialog box.
4. Move to IconNMR and click on the appropriate entry line that corresponds to your sample in the sample changer. Fill in the filename, experiment number, solvent and select the experiment: **N ProtonT1**.





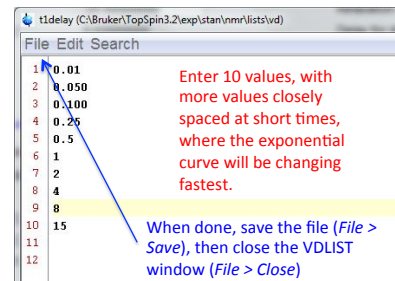
5. Various parameters need to be set for this experiment. From the menu items along the top of the Icon window, select *Parameters > Pertinent Acquisition Parameter Editor*, for a set of the relevant acquisition parameters for the **ProtonT1** experiment.



As indicated in the figure above, enter the values you noted earlier for **SW** and **O1**. You also need to enter the relaxation delay, **D1**. The default is 10 s, but ideally you want this to be 5 times your longest T1. The longer you make this, the longer the experiment will take. At minimum set to 15 s. If you determine that a proton has a very long T1 value, you may want to re-run the experiment with a longer **D1**. If you measured the 90° pulse, you can enter that value, too (or just accept the default value taken from the **Prosol** file).

Next create the list of variable delays to be used in the experiment. Click on the **E** (edit) button for the **VDLIST** (Variable Delay list), which will open the following dialog box shown to the right.

The **VDLIST** file (named **t1delay**) will have delay values from the last person who saved this file. The T1 experiment assumes you will enter 10 values. You can enter more (or less), but if you do you will have to set one more parameter to tell the instrument how many values are in your list (see below). The values shown in the figure are fine, but I like to go out a little longer if I'm not in a hurry, especially if you expect some long T1s. You want the values to be closely spaced at the short delays, where the exponential curve changes most rapidly. Example suggested values: 0.01, 0.06, 0.12, 0.3, 0.7, 1.3, 3, 7, 15, 25.



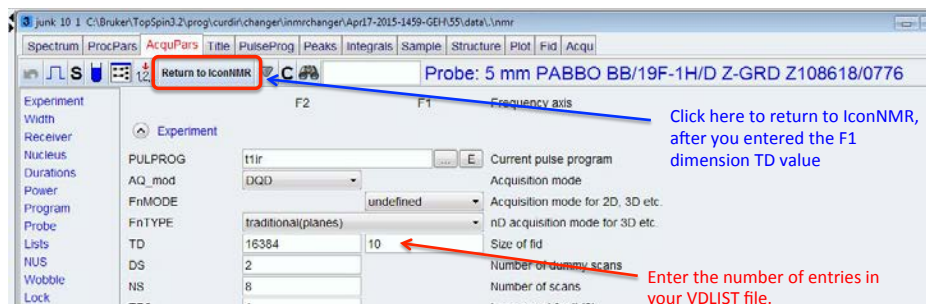
Save the VDLIST file (*File > Save*) and close the VDLIST window (*File > Close*).

[Note: Typically, all users are using the same VDLIST file (named *t1delay*, as indicated in the figure above). If this Icon experiment will not be running for some time and someone comes along and changes the VDLIST, they may overwrite the values you entered. You can give your VDLIST file a unique file name, if desired (e.g. *t1delay-dave*), and then there is little risk that someone would overwrite the list before your experiment runs.]

- Close the Parameter window and return to IconNMR by clicking on the **Return to IconNMR** button.



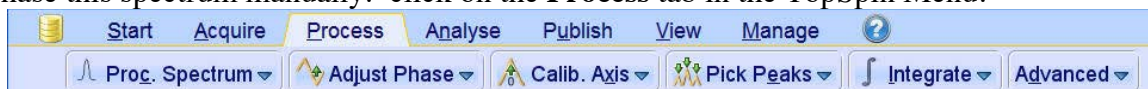
- If you entered more or fewer than 10 delays in your VDLIST, you need to change **TD** (number of time domain points) in the F1 dimension to match the number of entries in your list. To do so, along the top menu select *Parameters > Edit all Acquisition Parameters*. As before, you'll be bumped into TopSpin and a more extensive list of parameter will appear. Here you can enter the correct TD value in the F1 dimension. Then, return to IconNMR.



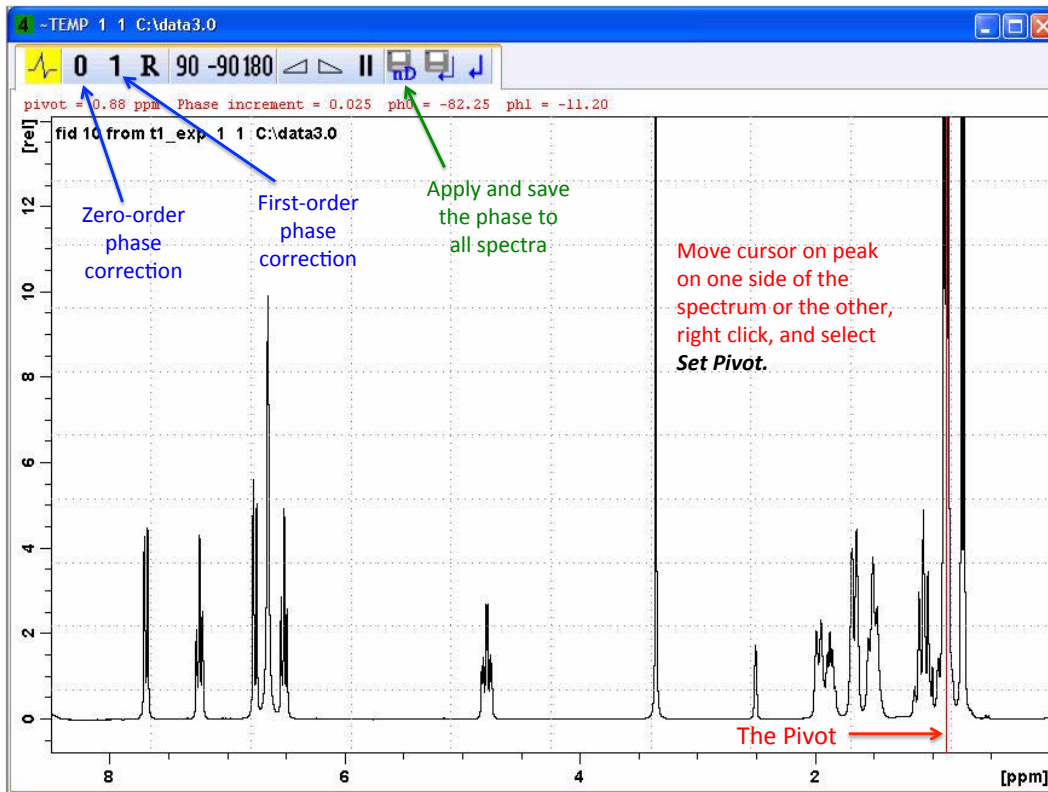
- Submit** your sample to be run, and start icon, if its not already running.

### **Processing the T<sub>1</sub> data with TopSpin**


- In TopSpin, open your T1 experiment file.
- Type *rser 10* (or the number that corresponds to your last VDLIST entry, if you entered more or fewer than 10 delays). Type *ef* to process your spectra. Spectrum **10** (or *your* last spectrum) will be displayed, and this spectrum, with the longest variable delay, needs to be phased positively.
- Phase this spectrum manually: click on the **Process** tab in the TopSpin Menu.





Click the **Adjust Phase** button. Your spectrum will probably be 180° out of phase, unlike the one below.




Set the red **pivot** to a peak at one side of the spectrum or the other by moving the cursor to that peak, right click, and select **Set Pivot**. The red pivot should now be on that peak.


Focusing on the peak with the pivot, put the cursor on the zero-order phase correction button and, while holding down the left mouse button, slide up or down to phase this peak positively (similar to what you do in MNova, to manually phase). 


Now, without moving the pivot, focus on the peaks at the other end of the spectrum and apply the first-order phase correction by placing the cursor over the First-order phase correction button and, while holding down the left mouse button, slide the cursor up or down to phase these peaks. 

Once phase properly, click on the button shown to the right, to save the 2-D phase values for all of the spectra in your file. 

The spectrum will go back to being unphased, but don't worry about it, the phases are saved for the 2D spectrum processing to come.

4. Click on the button shown to the right to exit the phase routine. 

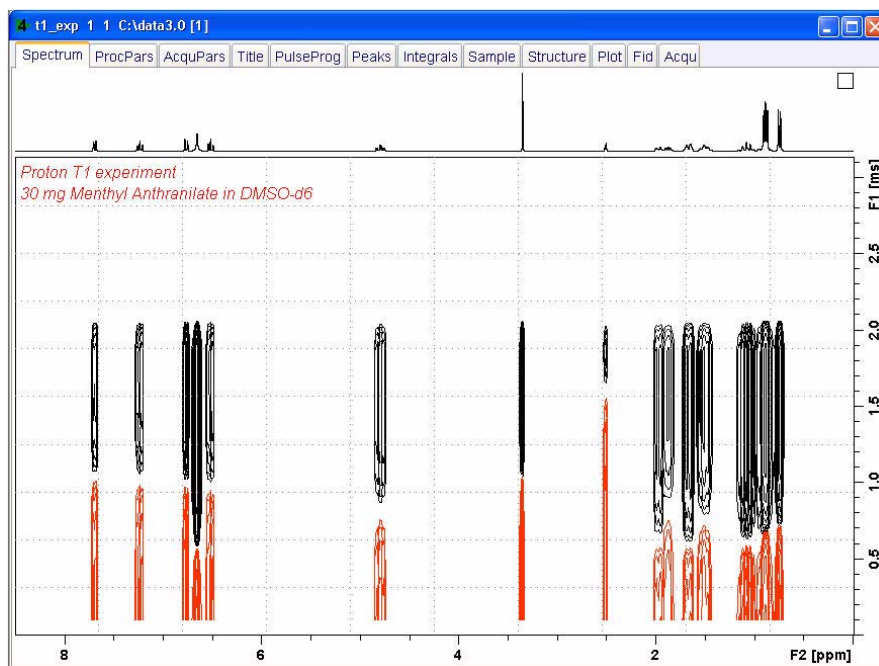
5. Click on the button  to go to the 2D spectrum display.

6. Click on the arrow in the  button and select the “**Process Only F2 Axis (xf2)**” option.

You'll see a 2D spectrum something like the one shown below...

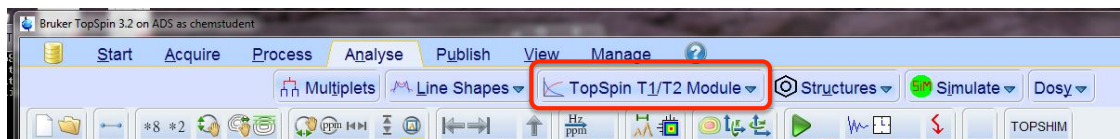
Configure Standard Processing (proc2d)
Process F2+F1 Axis (xfb)
<b>Process Only F2 Axis (xf2)</b>
Process Only F1 Axis (xf1)
Symmetrize Spectrum (sym)
Start Automation AU Program (xaup)





### The $T_1$ Calculation

1. Click on the **Analyse** button on the TopSpin menu bar, then click on the **TopSpin T1/T2 Module** button.

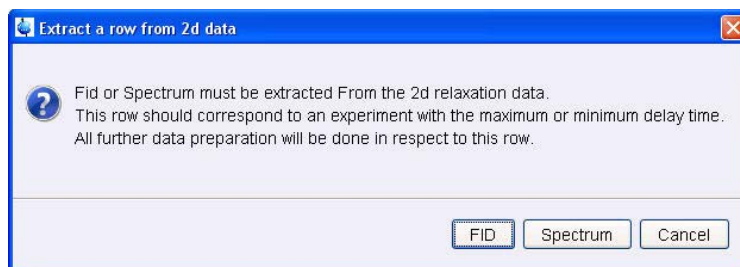


Note: the flow of buttons change, as shown below.

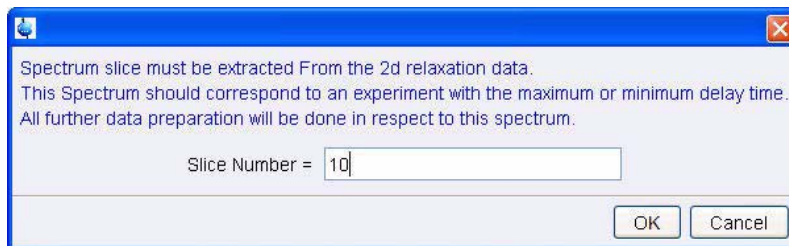


Note: A series of dialog boxes will appear as the following steps are executed. Read the messages and follow the instructions.

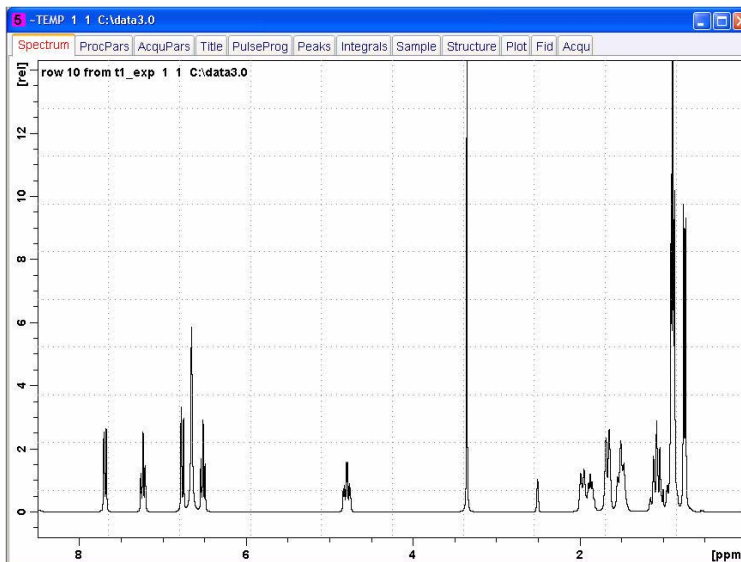
2. Click  **Fid** on **(Extract Slice)** and a dialog box appears:



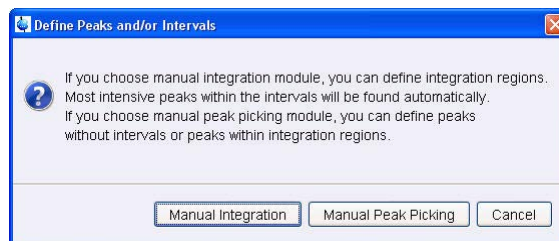
3. Click on **Spectrum**, then another dialog box appears:



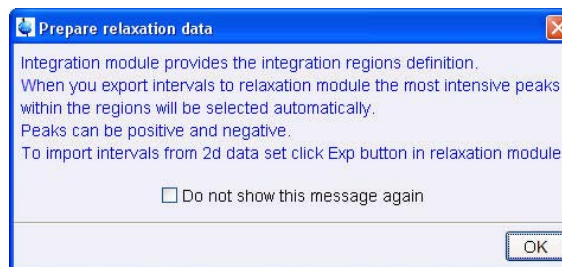
4. Input the slice number of your last spectrum (**10**, unless you had more or fewer entries in your VDLIST). Click **OK**. Spectrum **10** appears.



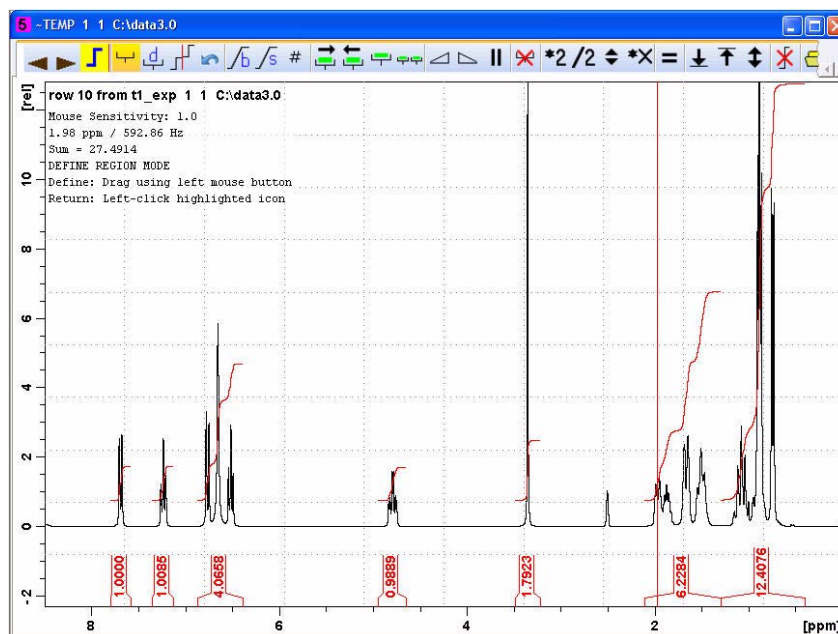
5. Click on  **Peaks/Ranges** (**Define Peaks/Ranges**). A dialog box appears:




6. Click **Manual Integration**. Another dialog box appears:

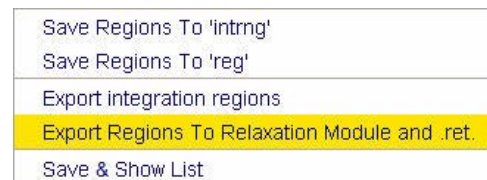


- Click **OK**, then a spectrum appears with the Integral routine activated.



- Use the cursor to define the integral regions in the same way you would in MNova. Then click on the button:  Another dialog box appears:

Select **“Export Regions to Relaxation Module and .ret.”**

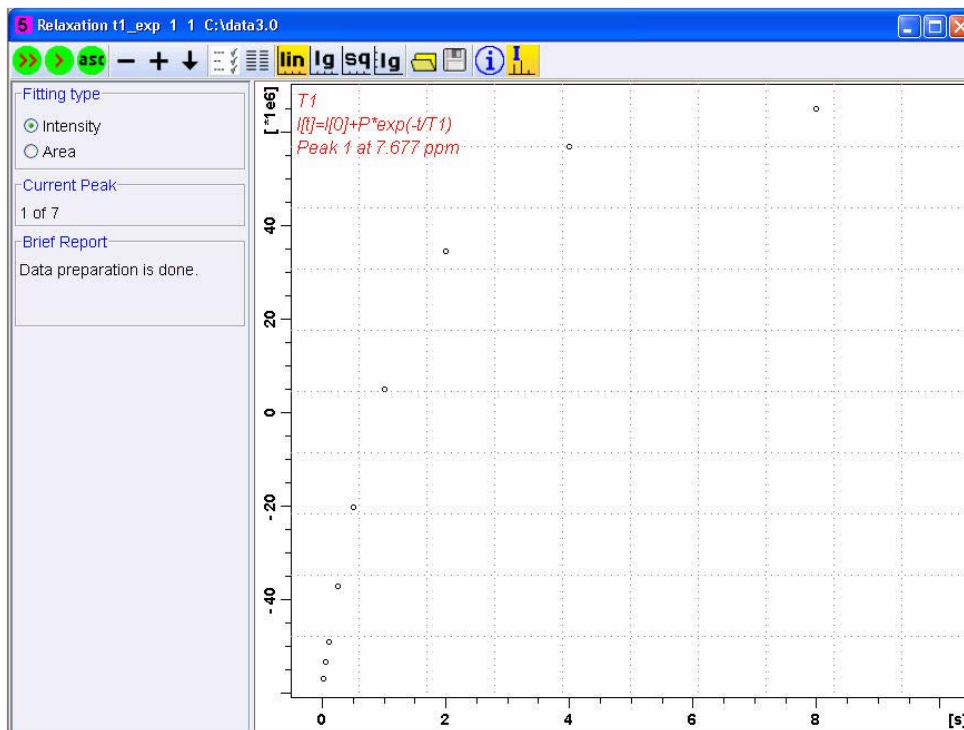



- Click on  (**Relaxation Window**), and a dialog box appears:

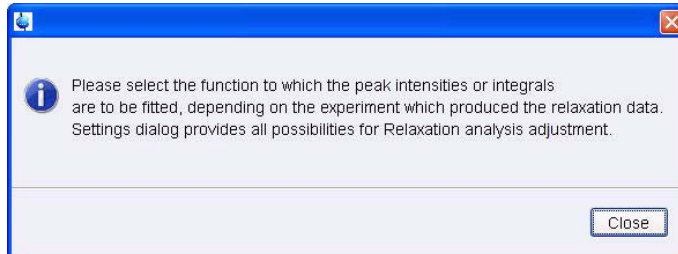
Click **OK**.



A plot will appear.

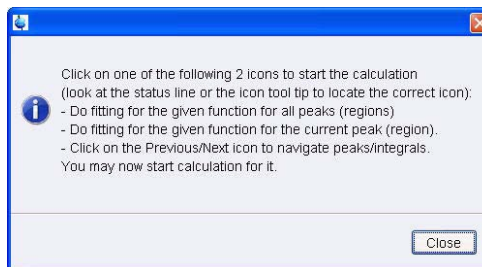


10. Click on  **(Fitting Functions)** to curve fit all the datasets. Another dialog box appears.



Click **Close**.

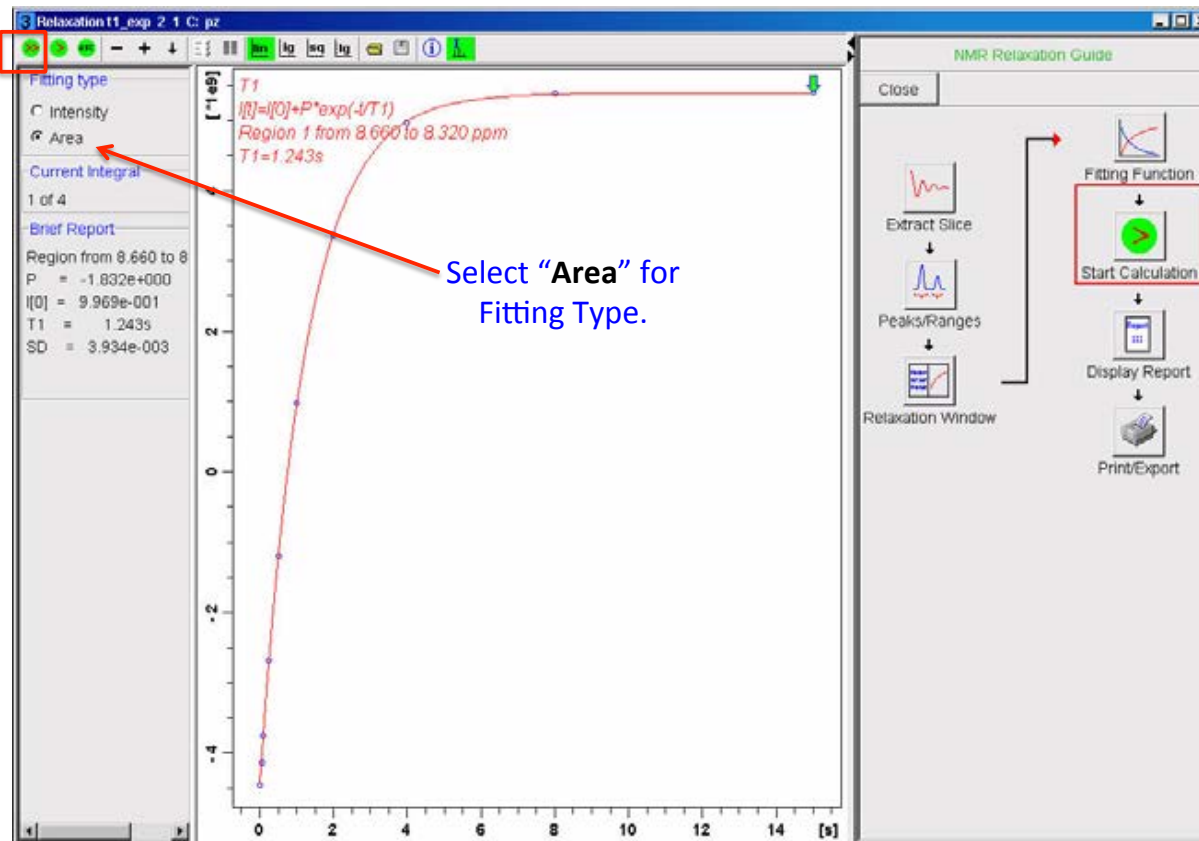
11. Click on  **(Start Calculating)**. Another dialog box appears.



Click **Close**, and a plot appears.

12. Select **Area** for *Fitting Type* (see next page).





13. In the upper left corner of the data display, above, click on the




button, to calculate all the regions selected.

The T1s will be displayed in the “Brief Report”.

```

Brief Report
Peak 1 at 7.677 ppm
T1 = 1.427s
Peak 2 at 7.234 ppm
T1 = 1.312s
Peak 3 at 6.653 ppm
T1 = 237.766m
Peak 4 at 4.784 ppm
T1 = 641.009m
Peak 5 at 3.352 ppm
T1 = 1.411s
    
```

13. Click on  (Display Report) for a detailed report. This report can be printed from the File menu.

**Note:** saved VDLISTS are stored in: C:\Bruker\TopSpin3.2\exp\stan\nmr\lists\vd