

T₁ Relaxation Measurement: The Inversion-Recovery Experiment (Using ICON-NMR)

Revised 4-24-2018

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.


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

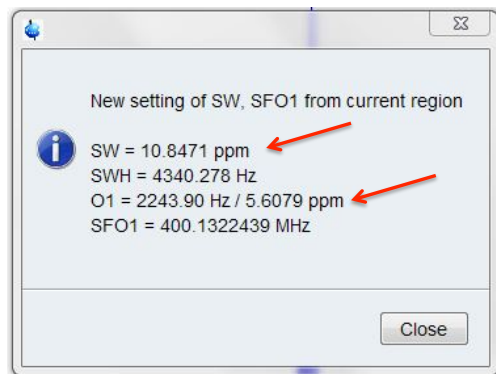
The pulse sequence used in the inversion-recovery experiment is shown below:

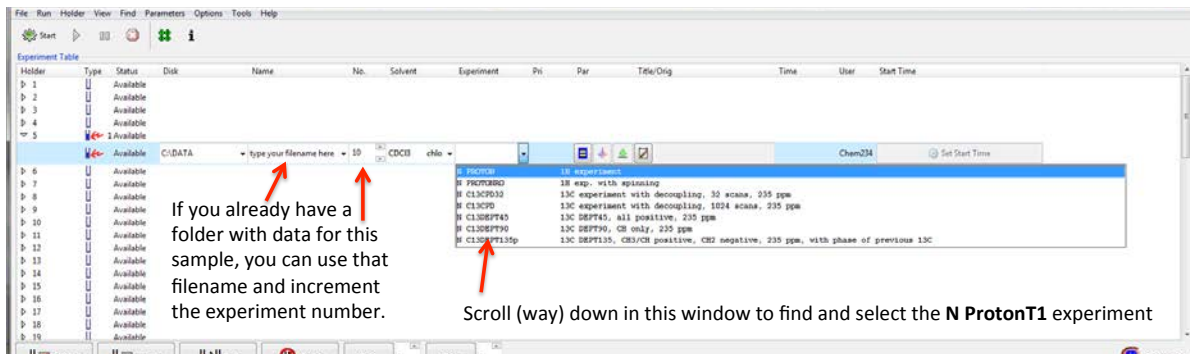
D1 - 180° - variable delay - 90° - FID

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 (**variable delay**) that is varied for each experiment. After the variable delay, a 90° pulse (**P2**) is applied and the FID is recorded. The FID records the spectrum intensity as a function of the variable delay, and the longer this delay is, the more the signal will have relaxed. The peak intensity will reflect the extent to which each signal has relaxed during the **variable delay** period.

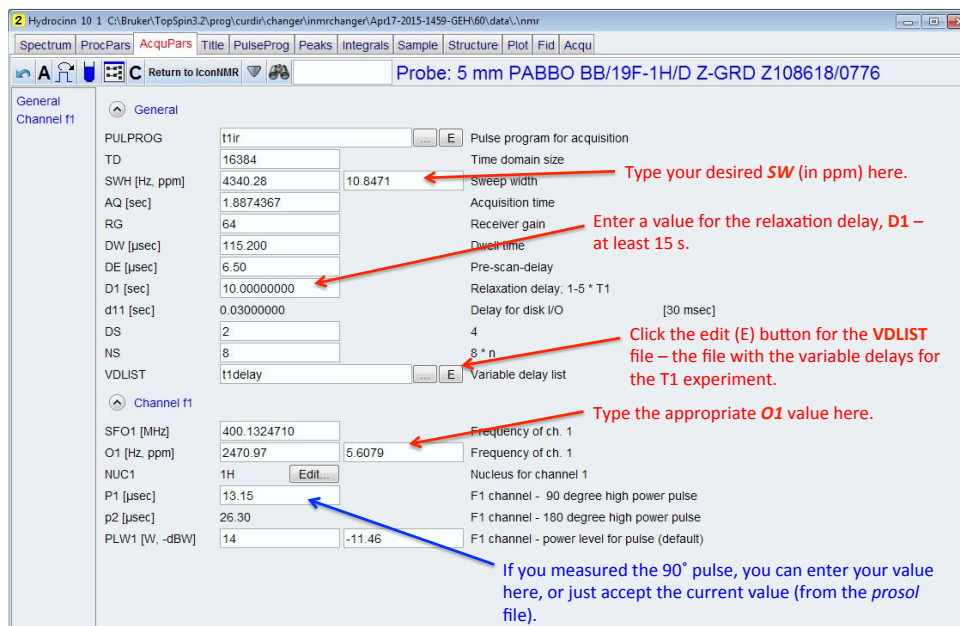
Procedure using IconNMR

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 . 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 ICON 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**.





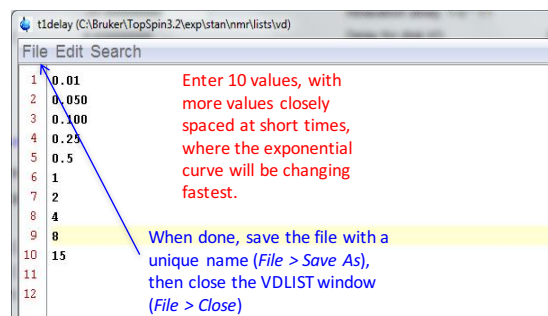
5. Various parameters need to be set for this experiment. From the menu items along the top of the Icon window, select *Parameters > Pertinent Acquisition Parameter Editor*. The program opens the *AcquPars* window in Topspin.



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. Start with 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). Also, check to see that the acquisition time (**AQ**) is set to a reasonable value. Around 2 s is adequate.

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.

The **VDLIST** file (default name **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 with a unique name of the form “*t1delay-your_name*” (*File > Save As...*) and close the VDLIST window (*File > Close*).

[Note: Because the default VDLIST that opens with the T1 experiment is *t1delay*, saving the VDLIST with a unique name makes it unlikely that someone else will change values in this VDLIST file before your experiment runs.]

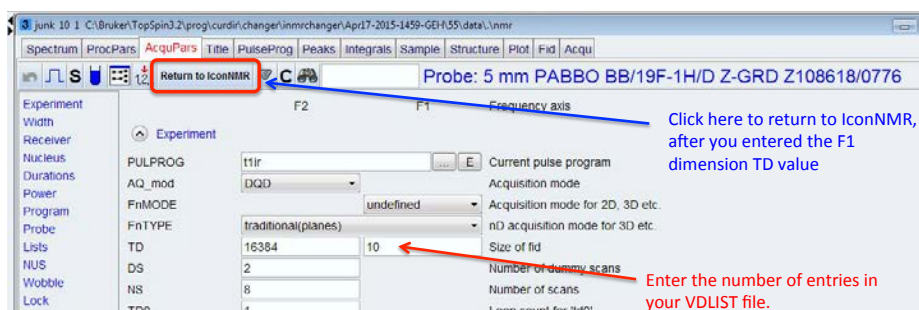
Double-check to make sure that the VDLIST file displayed is the one you created. If not, click on the button shown to the right to select your file.



- 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.



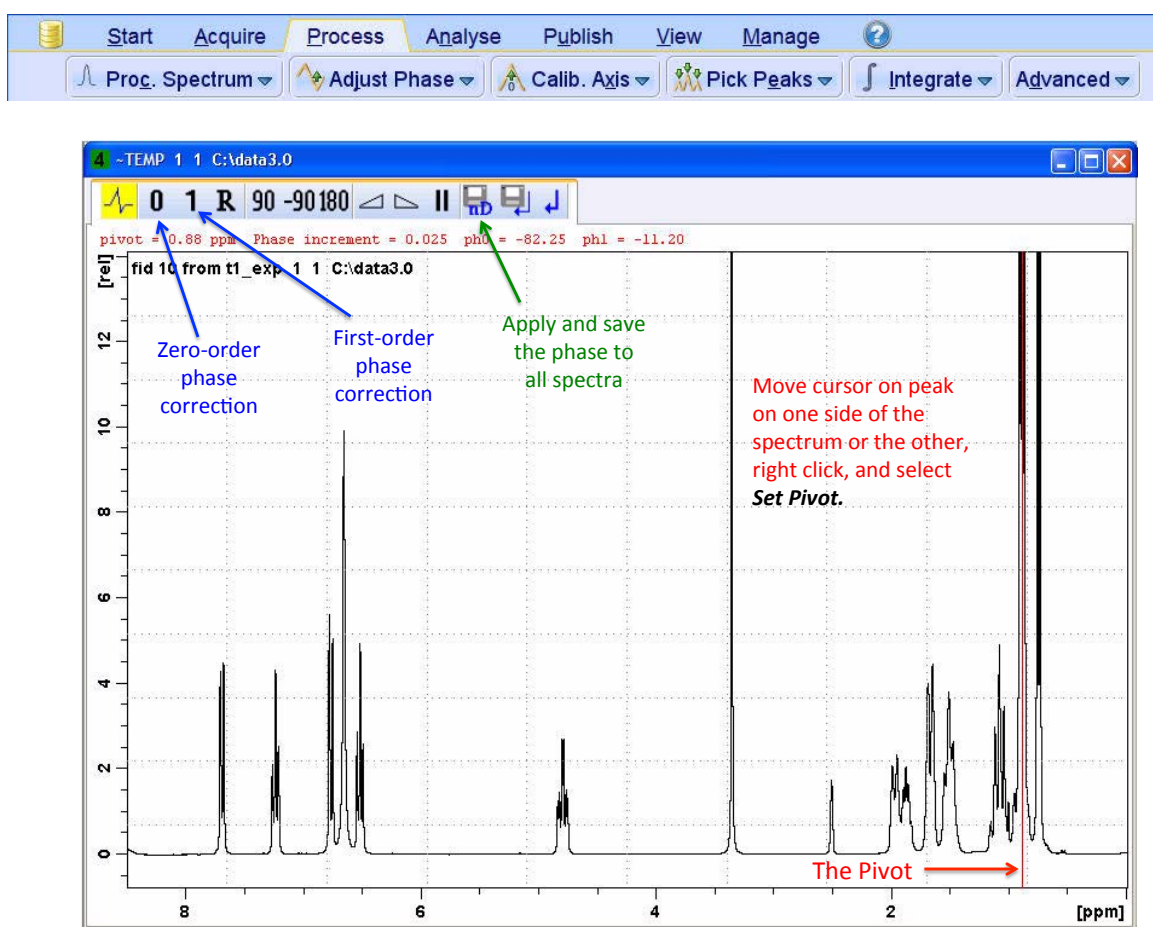
- Submit** your sample to be run, and start icon, if it's not already running.

Processing the T_1 data with TopSpin

1. In TopSpin, open your T1 experiment file.
2. 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 all spectra. Spectrum **10** (or *your* last spectrum) will be displayed, and this spectrum, with the longest variable delay, needs to be phased positively.

You can type the command *apk* to have the spectrum phased automatically. However, you still need to get into the manual phase mode in order to have the same phasing correction applied to all of the spectra acquired.

3. To enter the manual phase routine, click on the **Process** tab in the TopSpin menu then click the **Adjust Phase** button.



If phasing of the spectrum looks good and does not need to be tweaked, skip to step 4.

If you need to phase do the following.

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).

0

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.

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

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

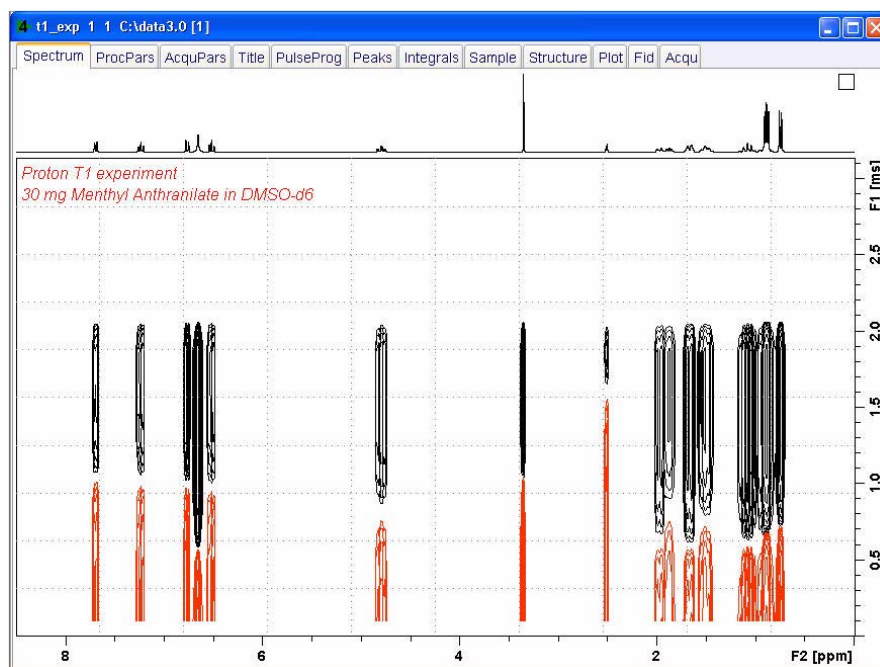


6. Click on the button shown to the right, to go to the 2D spectrum display.



7. Click on the *arrow* in the **Proc. Spectrum** 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)
Process Only F2 Axis (xf2)
Process Only F1 Axis (xf1)
Symmetrize Spectrum (sym)
Start Automation AU Program (xaup)


The T_1 Calculation

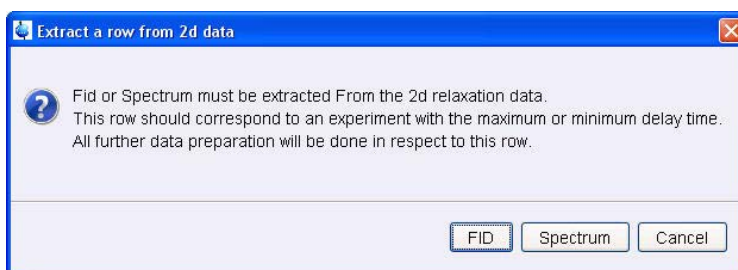
1. Click on the **Analyse** button on the TopSpin menu bar, click under the **Dynamics** button (click on the little triangle to see the options), select the **T1/T2 Module** button.

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

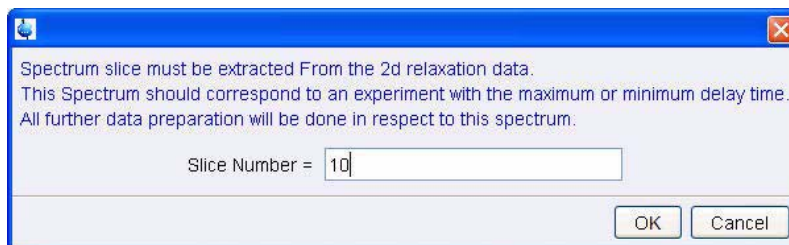


Note: A series of dialog boxes will appear as the following steps are executed. Read the messages and follow the instructions.

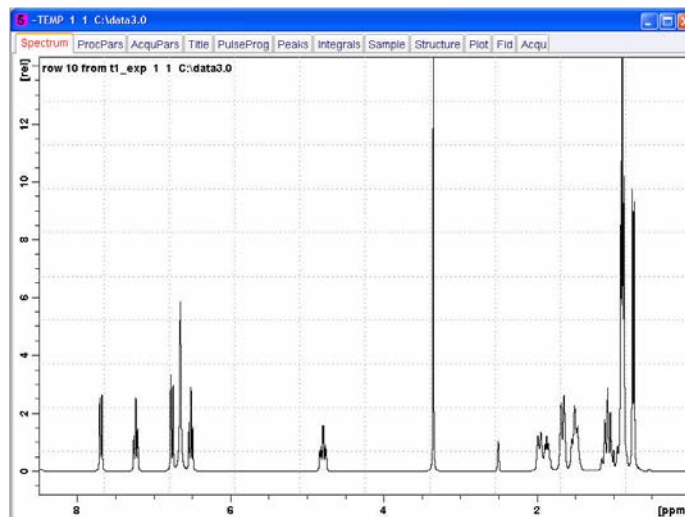
2. Click on  **Fid** (*Extract Slice*) and a dialog box appears:



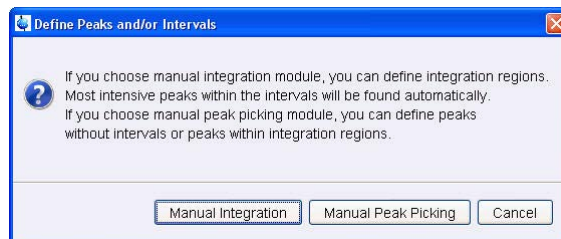
3. Click on **Spectrum**, then another dialog box appears:



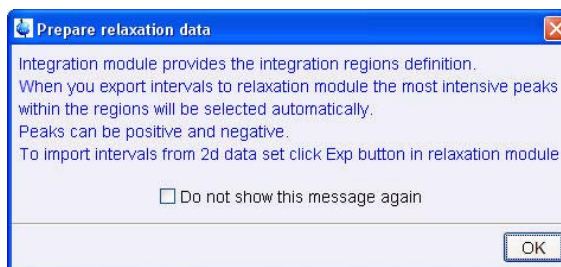
4. Input the slice number of your last spectrum (**10**, unless you had more or fewer entries in your VDLIST. Click **OK**. Spectrum **10** appears.



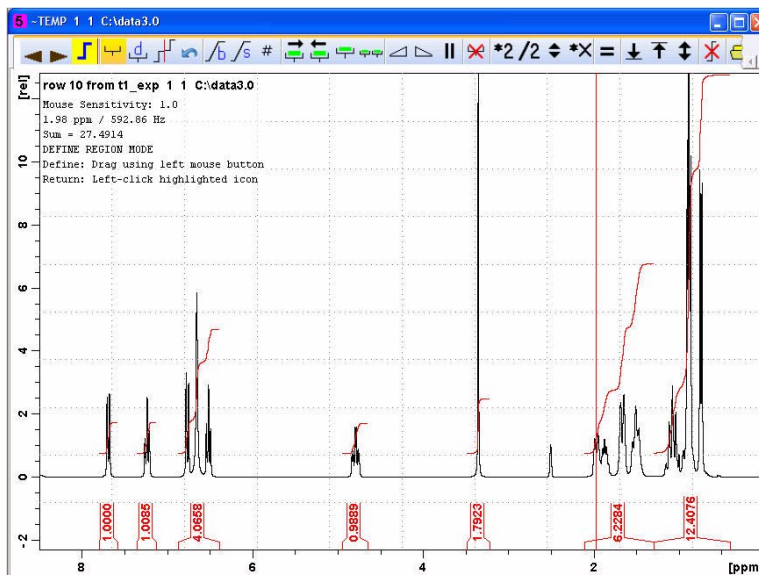
5. Click on  **Peaks/Ranges** (*Define Peaks/Ranges*). A dialog box appears:




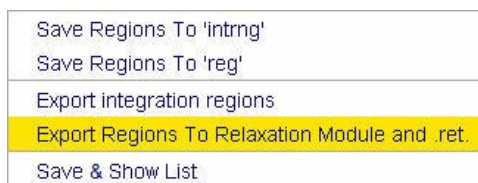
6. Click **Manual Integration**. Another dialog box appears:



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



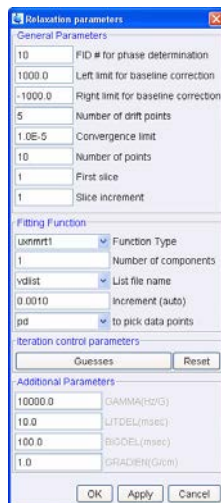
8. Use the cursor  to define the integral regions in the same way you would in MNova. Then click on the button:



Another dialog box appears:

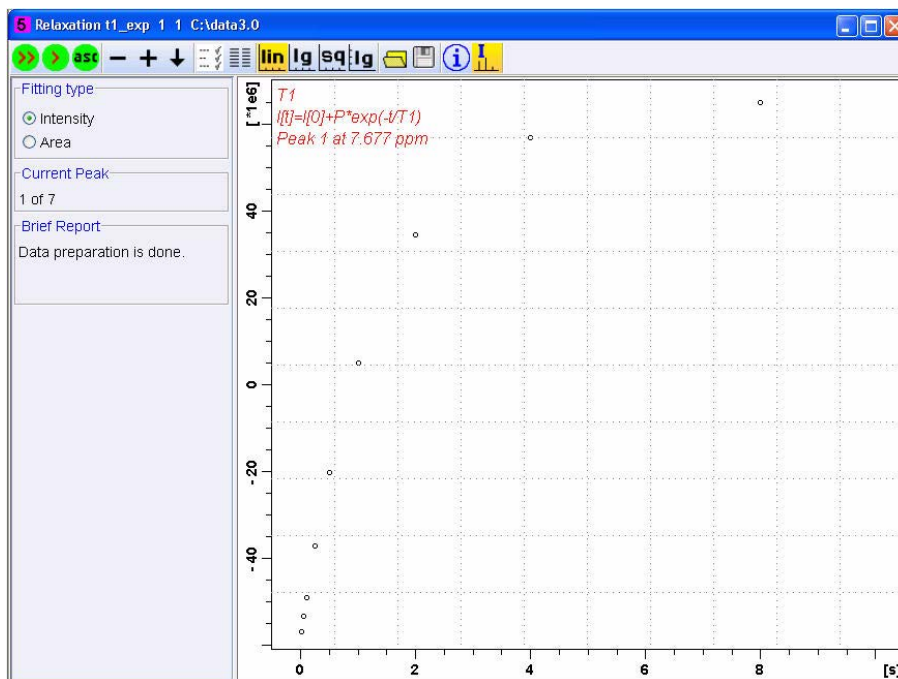
Select **“Export Regions to Relaxation Module and .ret.”**


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

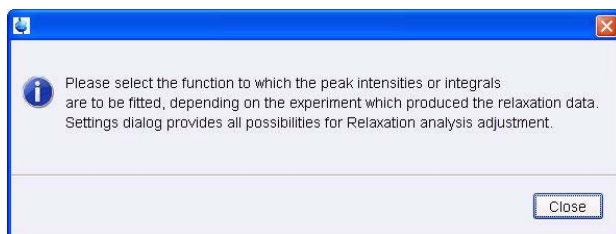


Click **OK**.

A plot will appear.

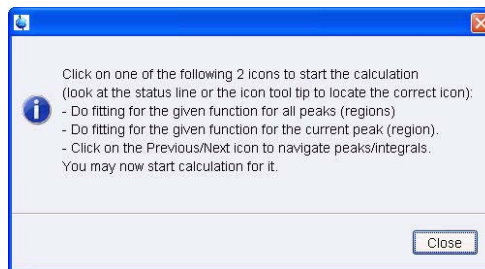


10. Click on  **Fitting** (**Fitting Functions**) to curve fit all the datasets. Another dialog box appears.



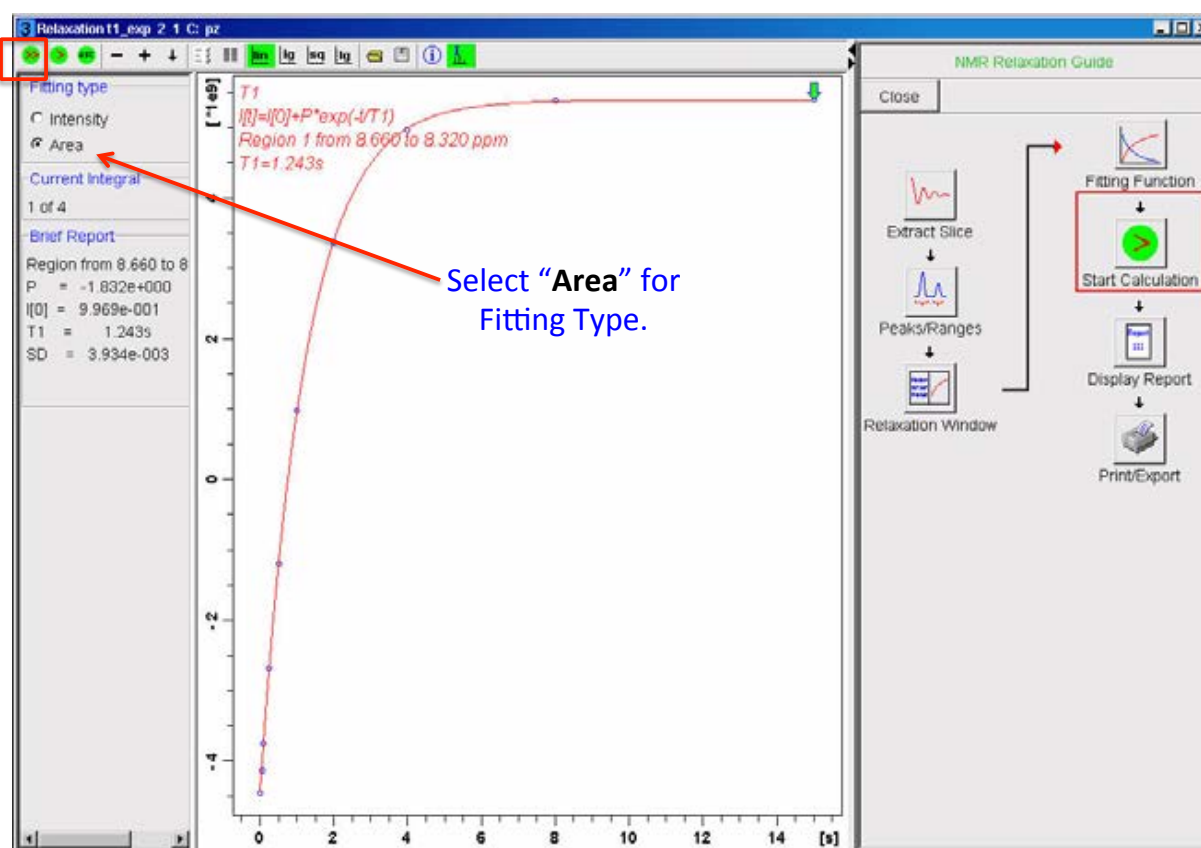
Click **Close**.


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



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


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

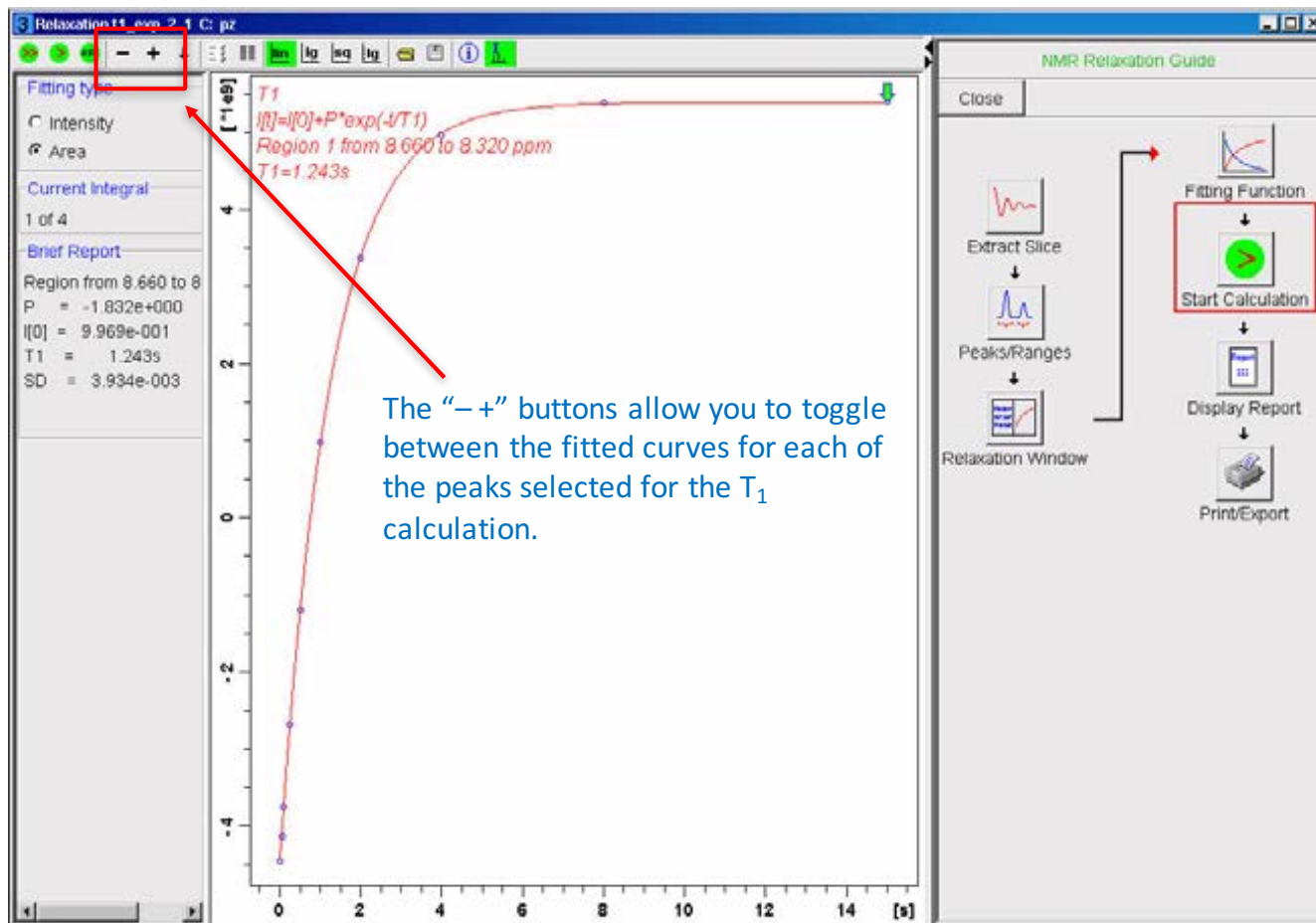


13. In the upper left corner of the data display, above, click on the  button, to calculate all the regions selected.

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.
14. You can toggle between the curves for each peak using the “- +” buttons.



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