Measuring relaxation times

Inversion recovery for T_1

1. Overview

This handout describes procedures for the measurement of proton T_1 values using the inversion-recovery experiment. The T_1 relaxation time constant, also known as the longitudinal (or spin-lattice) relaxation time (constant), is an important NMR parameter which reflects how rapidly magnetisation returns to equilibrium after pulse excitation. A qualitative knowledge of T_1 times is required for all NMR experiments in order to ensure the repetition rate between scans is long enough (usually equal to the sum of the relaxation delay (D1) and the acquisition time (aq)).

For most NMR experiments in the Department of Chemistry, appropriate T_1 values have been taken into consideration when creating the parameter sets that are in common use and it is not normally necessary for users to measure these themselves. However, an accurate knowledge of T_1 values is important when setting up quantitative experiments and when measuring reaction kinetics. In particular, the longest T_1 value of all the nuclei of interest should be known. This will allow you to properly set the experimental parameters and to avoid errors in data analysis. Using recovery delays that are too short relative to T_1s leads to saturation of resonances and to inaccurate peak areas.

2. The inversion recovery experiment

In the inversion recovery experiment, the nuclei are first allowed to relax fully to their equilibrium states along the z-axis. A 180-degree pulse is then applied, which inverts the signals. The signals are then allowed to relax for a length of time τ that is varied between experiments. After each variable time, a 90-degree pulse is applied, and an NMR spectrum is recorded in which the peak intensities are a function of the variable delay τ and the individual T_1 relaxation rates (Figure 1). Fitting these data to a function will then yield the T_1 values. It is useful to remember that longitudinal relaxation is an exponential recovery process and these values are actually *relaxation time constants* for this process. Hence the recovery *rate* $R_1 = 1/T_1$.

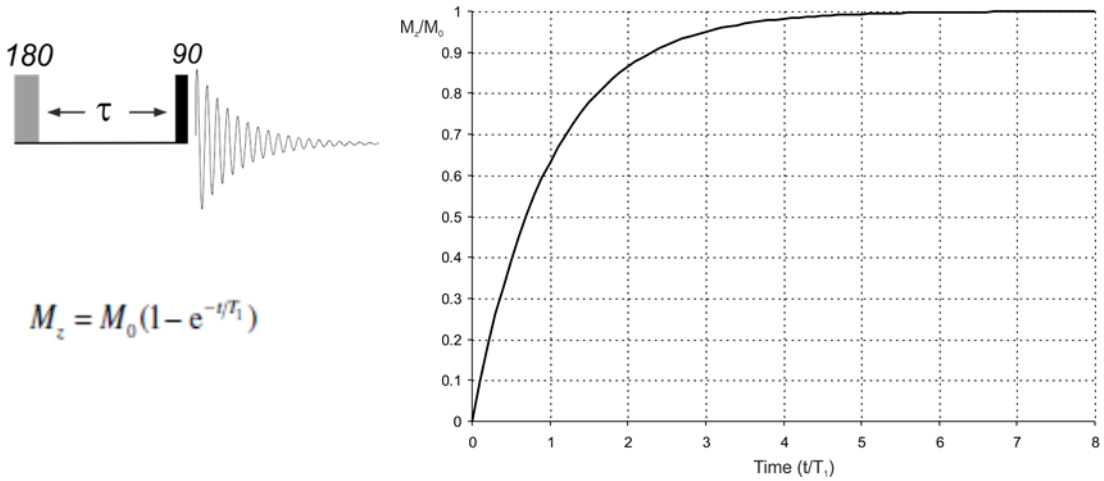

Figure 1: The inversion recovery sequence and the expression describing the recovery of magnetisation (Mz) after inversion back toward its equilibrium value (M_0) as a function of recovery time (t) and (right) the recovery profile.

The graph in Figure 1 shows that after a time equal to 3x T_1 the magnetisation has recovered by ~95% and after 5xT_1 by ~99% and is essentially complete.

3. Quantitative T_1 determination using the inversion recovery experiment

Although each individual inversion recovery experiment is 1D, the complete series is collected as a pseudo-2D experiment. Therefore in Topspin the 2D window will be displayed. Each individual 1D experiment is a "slice" of the pseudo-2D experiment, and can be inspected using the "rser" command.

3.1 Setup and acquisition

3.1.1 Calibrate 90 degree pulse and collect a 1D spectrum

Collect a normal 1D spectrum first, in order to ensure sample integrity. Calibrate the 90 degree pulse using the command "pulsecal". This will automatically set the "p1" parameter to the calibrated value. In most cases the prosol default p1 value should be suitable for use.

3.1.2 Load and edit parameters

The inversion recovery experiment can be run either manually or using Icon-NMR.

1. For manual setup, create a new experiment and load in the "Proton_T1" dataset (using the command "rpar"). If running under automation in Icon-NMR select the "Proton_T1" experiment from the drop-down list, and then click on **Parameters → Edit all Acquisition Parameters** from the top menu (Figure 2). Icon-NMR will then switch you to the "AcquPars" (Acquisition Parameters) panel in Topspin.

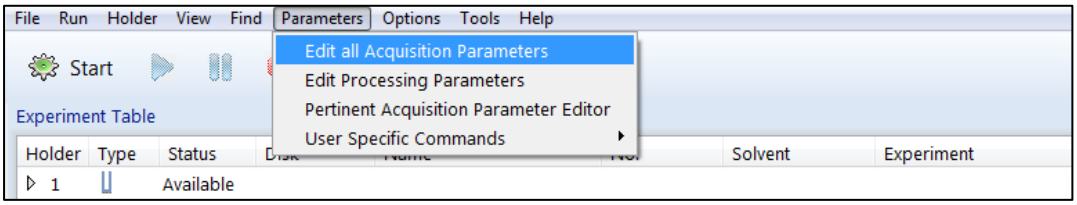

Figure 2. Editing experiment parameters within Icon-NMR

2. In the "AcquPars" panel, set the variable delay list by clicking on the  icon next to the VDLIST parameter (see figure 4), or by typing "edlist" and selecting "variable delay". You can create your own list or choose / edit one of the existing T_1 lists. An example vd list for inversion recovery is shown in figure 3. You should make sure that there are at least 8 delay values in order to accurately describe the recovery curve. The final value should be 4-5 times the expected T_1. Typical organic molecules under ambient conditions tend to have T_1 values in the range 0.5-4 seconds, and for these compounds the delay values listed in table 1 are a good choice. See the NMR staff if you require assistance with setting up variable delay values for non-typical samples, e.g. if you are working with very small compounds, if there is any paramagnetism in your sample, or if you have samples under inert atmosphere or extremes of temperature.

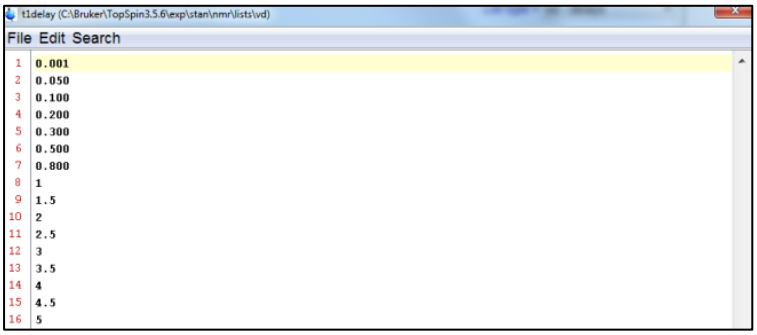

Figure 3. Example of a variable delay ("vd") list for proton T_1 measurement. Times are listed in seconds.

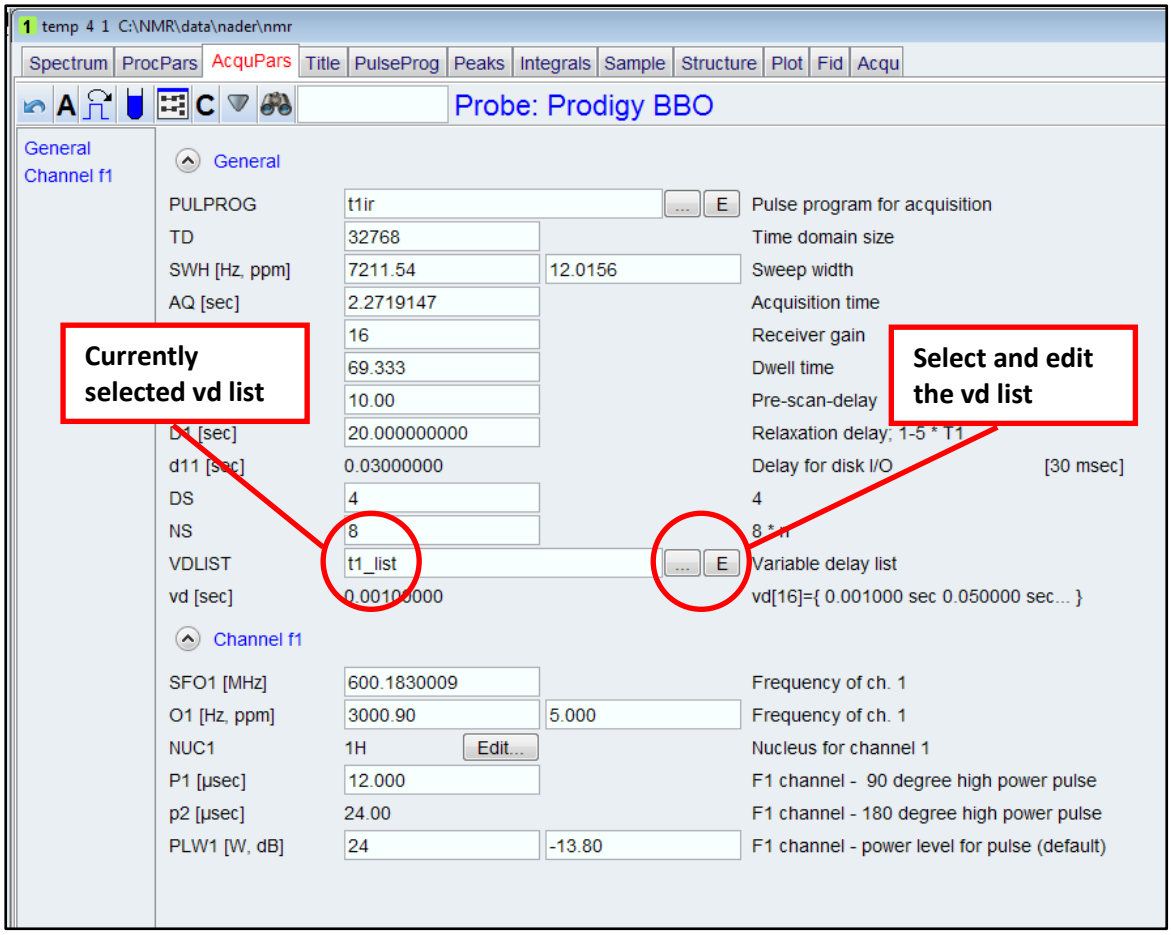

Figure 4. Selecting the vd list.

	Proton T_1 (seconds)		
vd #	**0.5**	**2**	**4**
1	0.01	0.05	0.05
2	0.02	0.06	0.10
3	0.025	0.09	0.20
4	0.03	0.12	0.30
5	0.05	0.20	0.40
6	0.07	0.30	0.70
7	0.10	0.40	1.00
8	0.15	0.60	1.50
9	0.25	1.00	2.00
10	0.35	1.50	2.50
11	0.50	2.00	4.00
12	0.8	3.00	6.00
13	1.0	4.5	9.0
14	1.5	6.5	13.5
15	2.5	10.0	20.0

Table 1. Example inversion recovery τ delays (in seconds) for a range of typical proton T_1 values. If you are uncertain of likely T_1s, set parameters for the longer T_1 value.

4. Other relevant parameters for data acquisition

The following points must be taken into consideration:

1. TD in F1 must be equal to the number of entries in the vd list. For example, if you have set 12 variable delays in the vd list, you must set TD to 12. You can do this either by clicking on the A button in the Acquisition parameters panel and editing the "TD" parameter, or by typing "td" in the command line (figure 5).

2. The number of scans (ns) should be a multiple of 2.

3. The relaxation delay (D1) should be set to 4-5 times the expected T_1. The default value for D1 is 10 seconds. This value is the minimum that should be used for typical organic compounds, particularly if quantitative T_1 values are required. If, having measured the T1 value for your compound, it turns out that $5xT_1$ >= D1+AQ (relaxation delay + acquisition time), the experiment should be repeated with a longer D1, as the measured T_1 value may not be accurate.

4. Set the 90 degree pulse length (P1) to the calibrated value (section 3.1.1).

5. Remember to set the receiver gain using the command "rga".

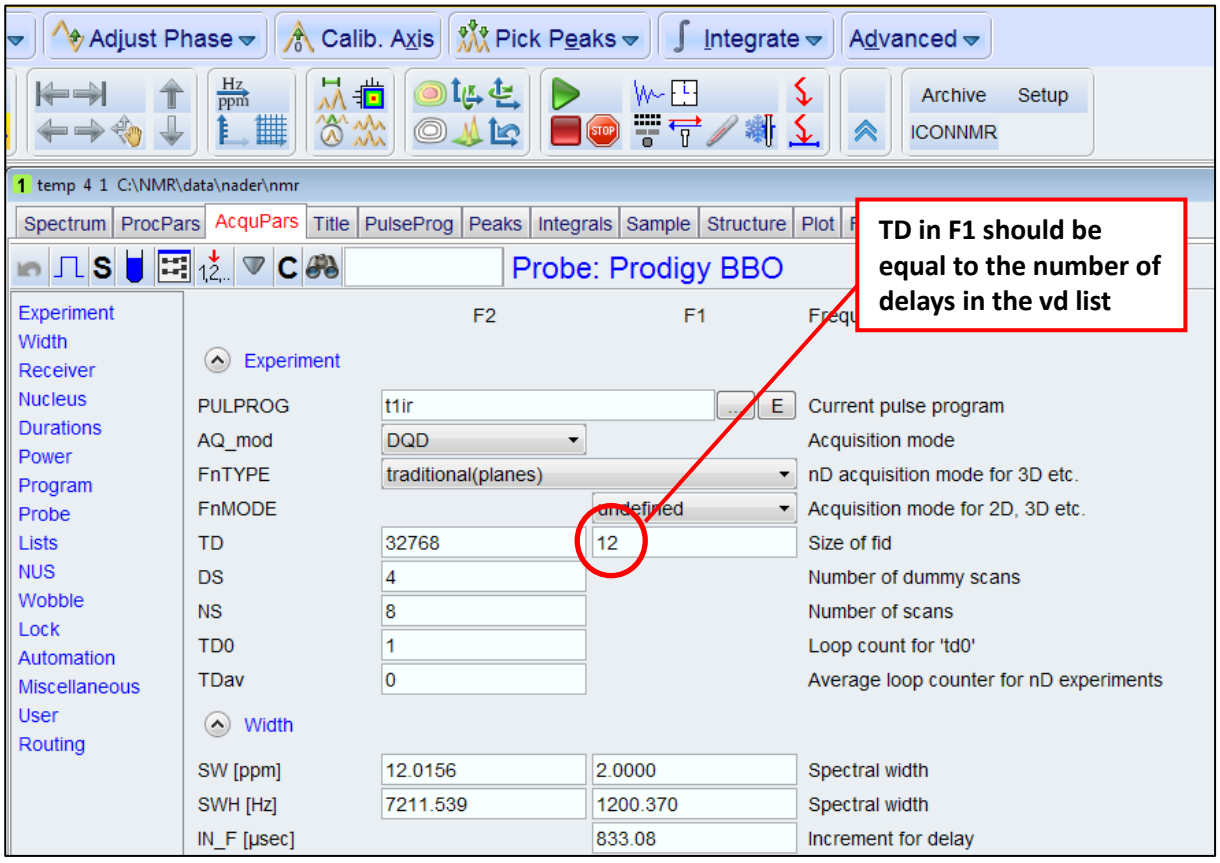

Figure 5. Setting TD[F1] for the inversion-recovery experiment.

5. Processing

1. Read the FID with the longest vd value. For example, if you had used 15 variable delays (and the last value was the largest), type **rser 15**.

2. Fourier transform and apply line broadening using the "ef" command

3. Phase the spectrum automatically by typing **apk**. Enter manual phasing mode by typing **.ph**, and if necessary correct the phasing further. Once the spectrum has been phased (either automatically or manually), stay in manual phasing mode and store the phasing to 2D by clicking on the  button, and then save and exit by clicking the  button.

4. Return to the 2D experiment by clicking on the  button and Fourier Transform all the 1D traces using the **xf2** command.

6. Data Analysis

1. Enter relaxation analysis mode by clicking **Analyse → Dynamics** and selecting "T1/T2" from the drop-down menu (figure6).

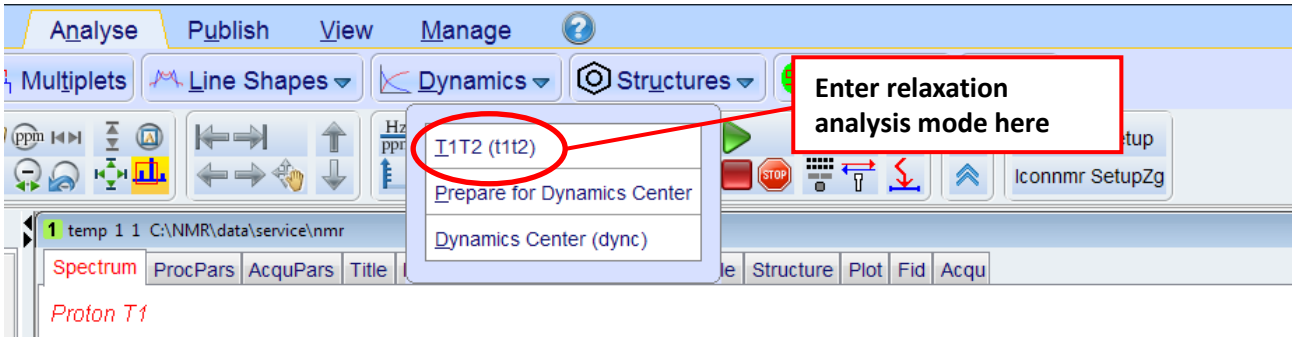

Figure 6. Entering relaxation mode analysis

2. Click on the "FID" button, and then select "Spectrum". In the dialogue box enter the number of the last FID (for example if you have used 12 variable delays, select spectrum number 12).

3. Click on the "Peaks/Ranges" button, and then select "Manual Integration". Integrate all peaks / multiplets for which you wish to calculate T_1. Export your integral regions to the Relaxation Module by clicking on the  button and selecting "Export Regions to Relaxation Module and .ret". This will allow you to use peak areas for determination of T_1.

4. Click on the "Peaks/Ranges" button, and then select **Manual Peak Picking**. Pick all peaks for which you wish to calculate T_1. Export your peak list to the Relaxation Module by clicking on the  button, and selecting **Export Regions and biggest peak within region to Relaxation Module and .ret**. This will allow you to use peak intensities for determination of T_1.

5. Return to the 2D experiment by clicking on the  button

6. Enter the Relaxation Module by clicking on **Analyse → Dynamics**, and selecting "T1/T2" from the drop-down menu (figure 6). Then select the "Relaxation" button (figure 7). This will open the fitting window (figure 8).

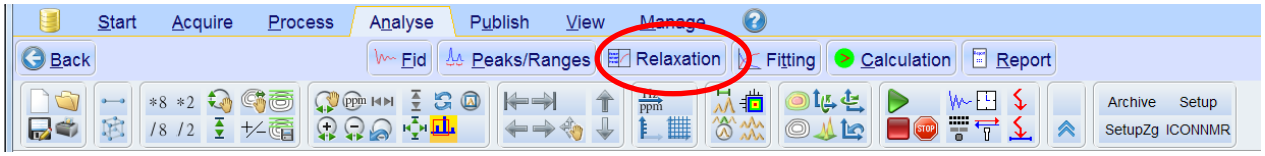

Figure 7. Entering the Relaxation Module

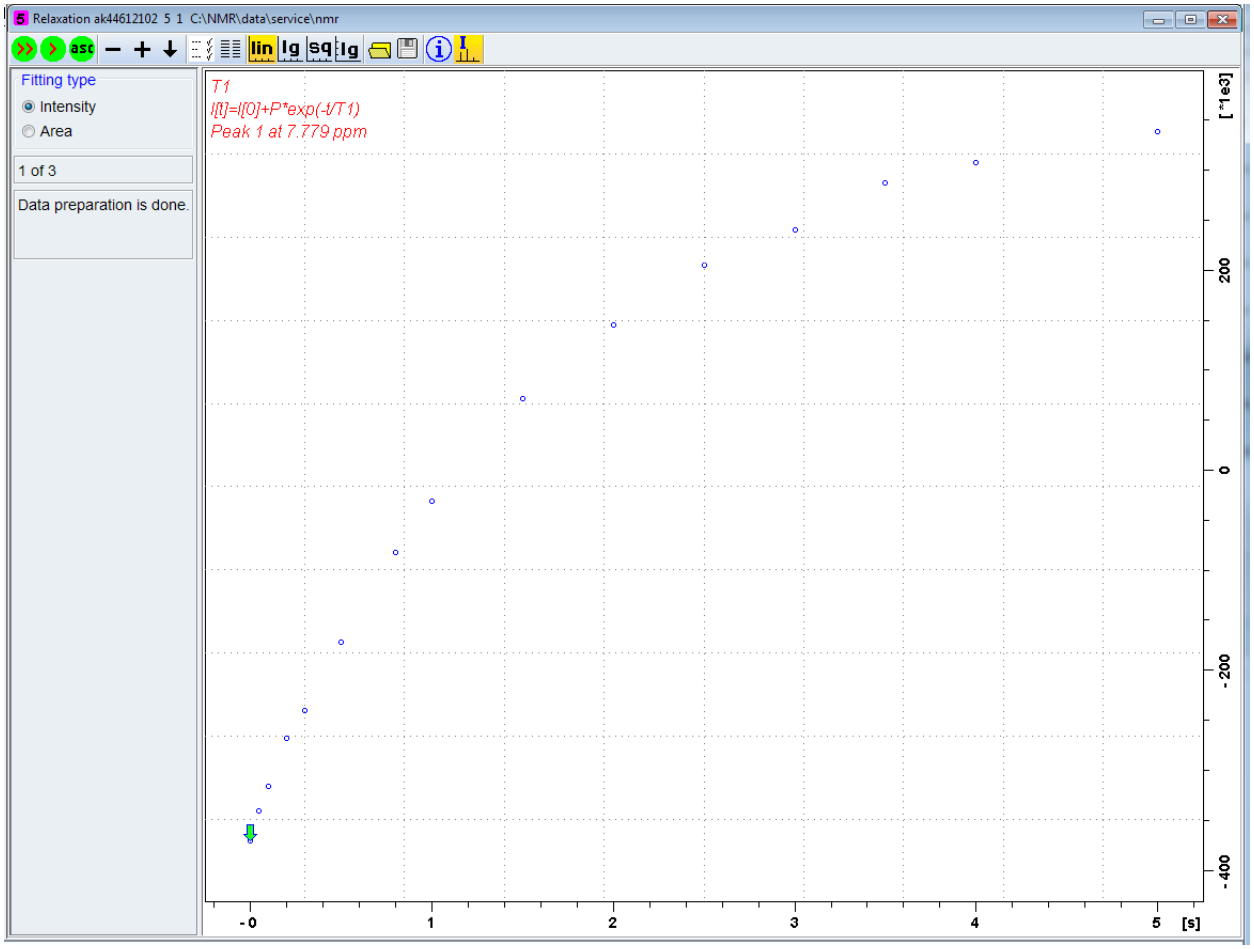

Figure 8. Peak fitting window.

7. Click on the “Show full spectrum button”  , or type “.all” in the command line, to ensure that you are observing the full curve.

8. Click on the “settings” button  located in the peak fitting window. This will open up a new window which allows you to set various parameters for the relaxation analysis and peak fitting (figure 9). Ensure that **Function Type** is set to “uxnmrt1”, and **List file name** is set to “vdlist”.

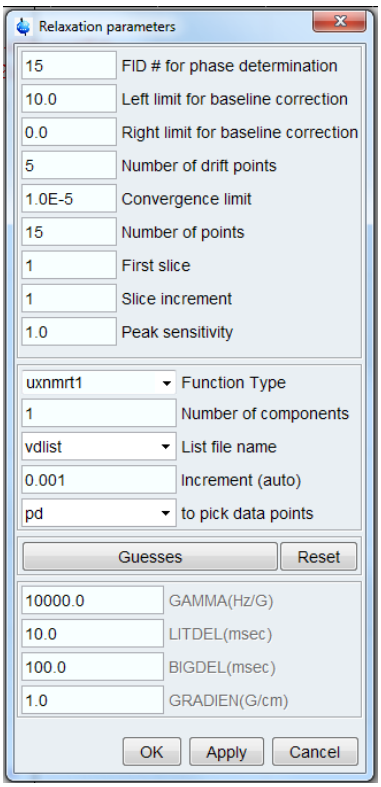

Figure 9. Relaxation parameters window.

9. Select either “Area” or “Intensity” from the left-hand side of the fitting window to use your peak integrals or picked peaks, respectively. Select the single arrow button to fit the current peak, or the double-arrow button to fit all peaks. You can cycle through the peaks using the + and – buttons (figure 10).

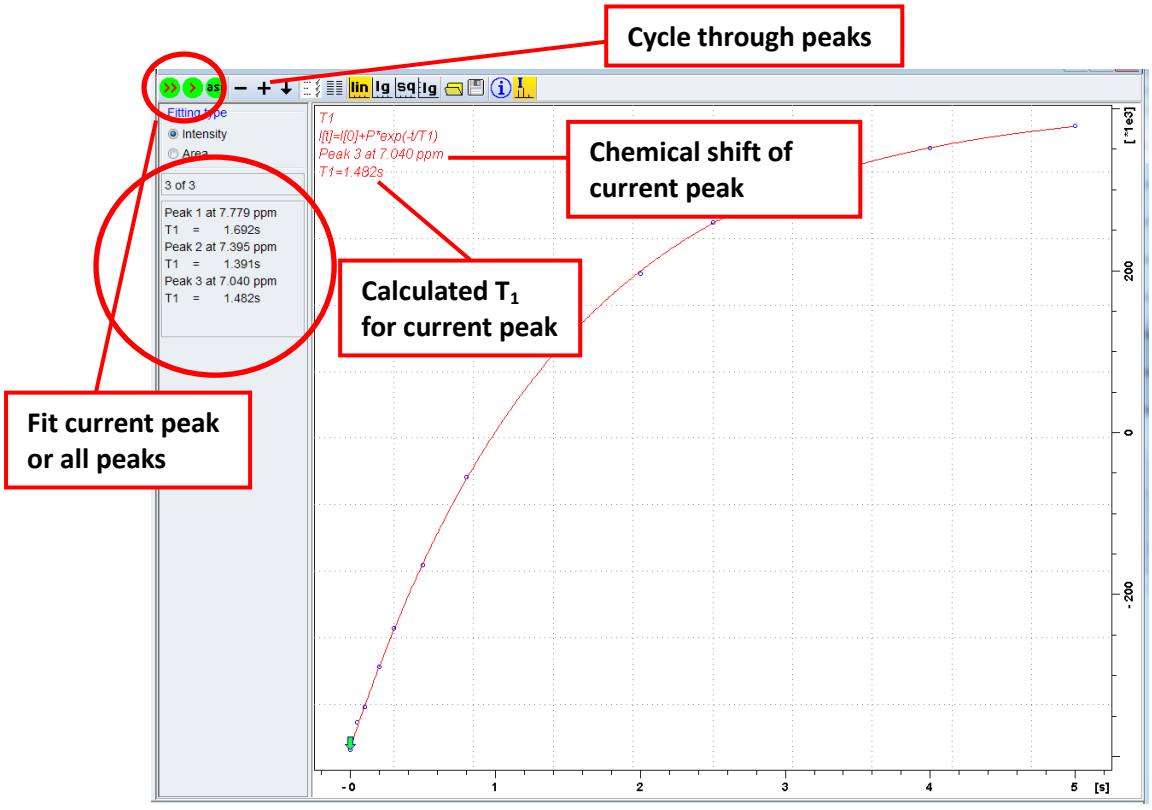

Figure 10. Peak fitting for T_1 analysis.