Estimating T1s for ¹Hs in your sample on the Bruker NMR

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Estimates of T1 relaxation values of different ¹Hs in your sample are important for setting up more advanced NMR experiments, and essential when quantitative work is desired. The repetition time in any NMR experiment is determined by the relaxation delay (D1) and the acquisition time (AQ), ie. D1 + AQ. This guide will lead you in estimating T1 values for the ¹Hs in your sample. The table at the end of this guide will show you repetition delays for different NMR experiments based on your knowledge of these T1 values.

To determine T1 values, it is important to use an exact pw90 value. For Bruker spectrometers equipped with an automatic tune-and-match (ATM) probe, the calibration may be skipped, since the default calibration usually works quite well (P1 & PLW1).

1. <u>Acquire a ¹H 1D</u> experiment (uvm.1H1D) (see Bruker Speedy setup guide)
   DO NOT spin the sample! (OR turn spin off after you acquire the ¹H 1D)
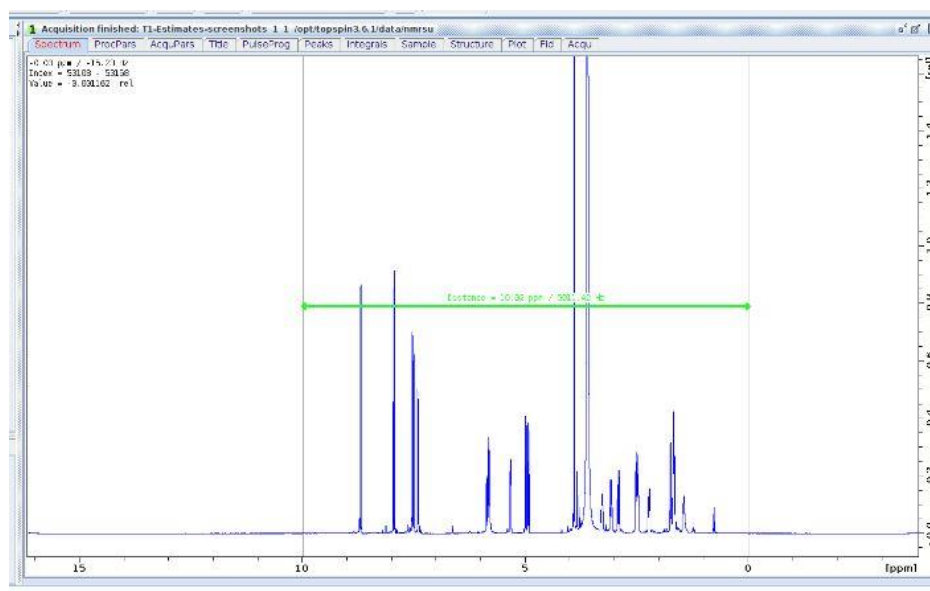

2. <u>Re-acquire a 1 scan ¹H 1D</u>: It is nice, although not essential, to optimize the sweep width (SW) and center of spectrum (O1) for the T1 estimation. Copy your ¹H 1D into a new experiment number ('**wra 2**') and join that experiment ('**re 2**'). With the cursor, zoom in on the area you want to acquire, leaving about 1ppm left of the left-most peak and same on the right side. Click [icon] which resets SW and O1. Before acquisition, change a few parameters. Set **NS = 1**, **DS = 0**, and change pulprog (at the top) from '**zg30**' to '**zg**'. Make sure to do a '**RGA**' (or click on 'Gain') before acquiring. Use '**efp**' to process, followed by '**apk**' and make sure all peaks are phased positive. (Note: You will not be using 'apk' again after this point.) It is important that the phasing is correct here, since the T1 estimates will **depend on these phases**!

3. Copy these parameters into a new experiment number by either using '**iexpno**' (increase experiment number), which copies all current parameters **or** by using '**wra #**' which copies the full dataset. With the latter you will need to use '**re #**' to get into the newly copied dataset.

4. Changing pulse sequence: Under acquisition parameters (AcquPars tab) change PULPROG (the pulse sequence) to do T1 inversion recovery: **t1ir1d** (type it in or select it). Then change P1 to the newly calibrated pw90 or just use the default value read in by **getprosol**. Leave NS = 1, DS = 0, though if this produces low S/N, then you will need NS > 1 (4 or 8 ideally), but then also ensure that D1 ≥ 5*T1 (relaxation delay needs to be long enough)!

5. Interpulse delay **D7**: **D7** is the key parameter for the **t1ir1d** experiment. You may click on the PulseProg tab and use the graphical display to see how D7 is used in the pulse sequence.
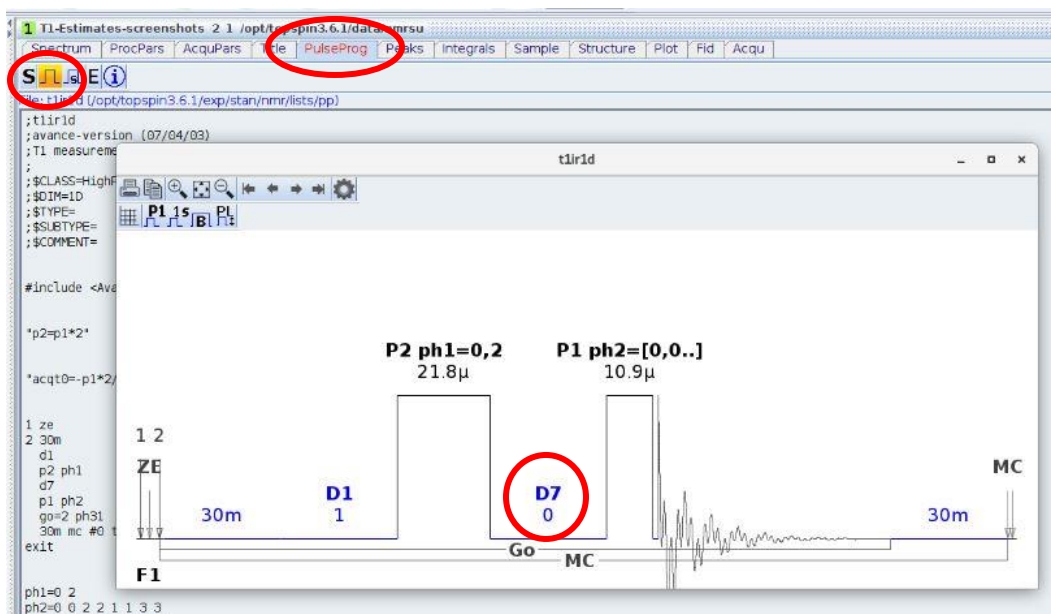

6. Adjusting D7: Start with a short D7 delay, say 1ms, so type **d7 0.001**. By default, delays are entered in seconds, but keep in mind that specifying the units can help avoid trouble. So, you can use **d7 0.001s** or **d7 1m** or **d7 1000u** to get the same value. With this short value for D7, acquire data with **zg** and transform with **efp**. All the peaks should be negative here! DO NOT apply an apk (phases come from the ^{1}H 1D you acquired in 2.) See my example of "quinine in DMSO-d_6" below, which unfortunately has a large water peak, that is a little bit out of phase. Otherwise all peaks are negative.
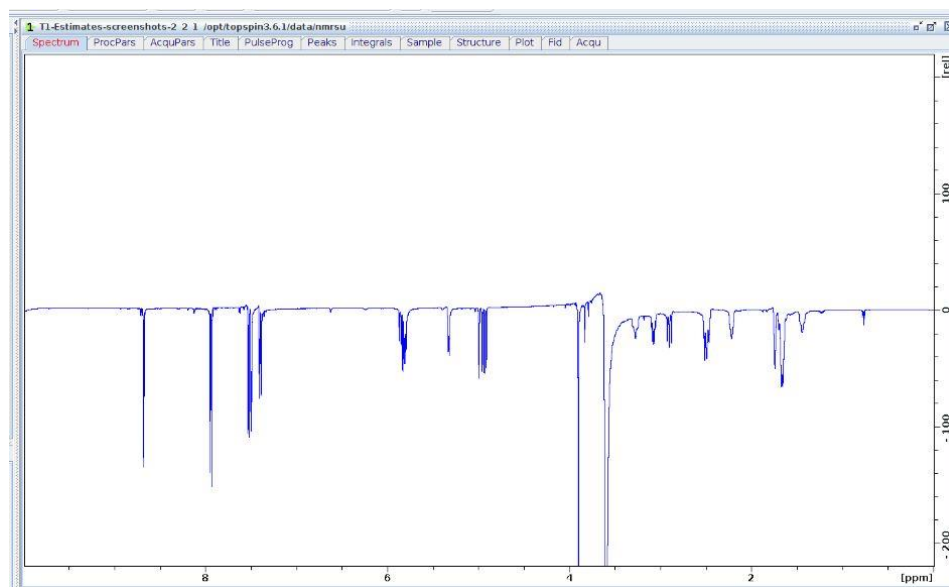
7. <u>Optional</u>: Get to a new experiment number (**iexpno**) and use a long **D7** delay, say **5s**, to see all the peaks recovered (or positive), though TMS and/or the solvent peaks could still be negative.

8. <u>Finding D7$_{null}$</u>: Do an '**iexpno**' and use a fairly short value for D7, say 0.1sec. (**d7 0.1**), acquire the spectrum (**zg**) then **efp** and see if any peaks flipped from negative to positive. If not, lengthen D7 to say 0.2 sec, and see how many of your solute peaks are flipped. The crossover point, where the peak goes from negative to positive is your **D7$_{null}$** value and is used to determine the T1 of that peak. In my "quinine in DMSO-d$_6$" example below, D7 = 0.3 sec, and we can see that a few of the peaks are flipped.
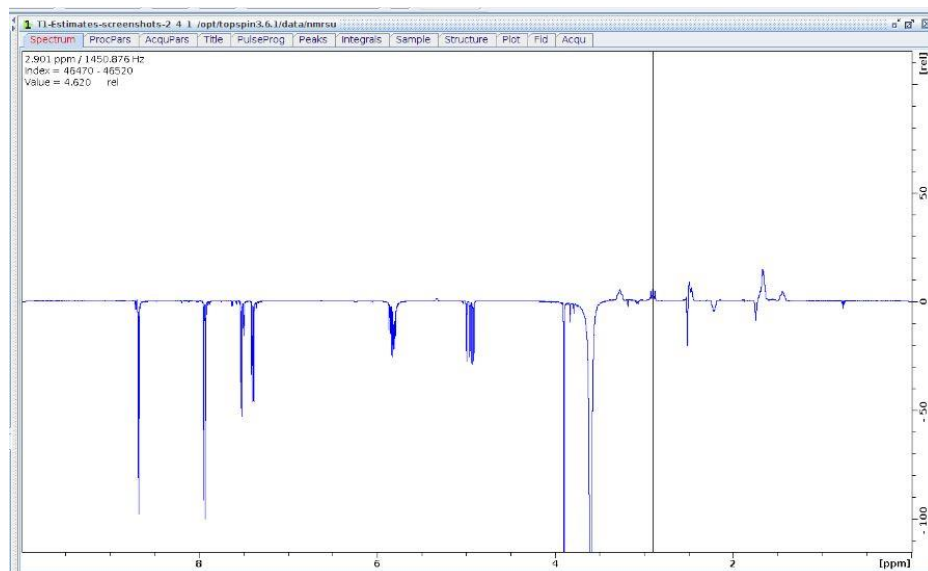
9. <u>Dual display</u>: Choose your peak of interest and narrow in on the D7 value that will make the peak appear null (either it disappears from the spectrum OR it will be equal parts negative as positive). Being able to view your ^{1}H 1D in the spectrum panel can be helpful, especially when looking for disappearing

peaks. Click on [icon] to enter this mode, then go to the directory in the left panel, find the ^{1}H 1D that you acquired in 1. or 2. and drag it into the spectrum screen. You may need to adjust the intensity, so choosing one of the spectra by clicking on the corresponding color square in the lower left and using the intensity icons in this submenu, will help you adjust intensity and position of the spectrum.
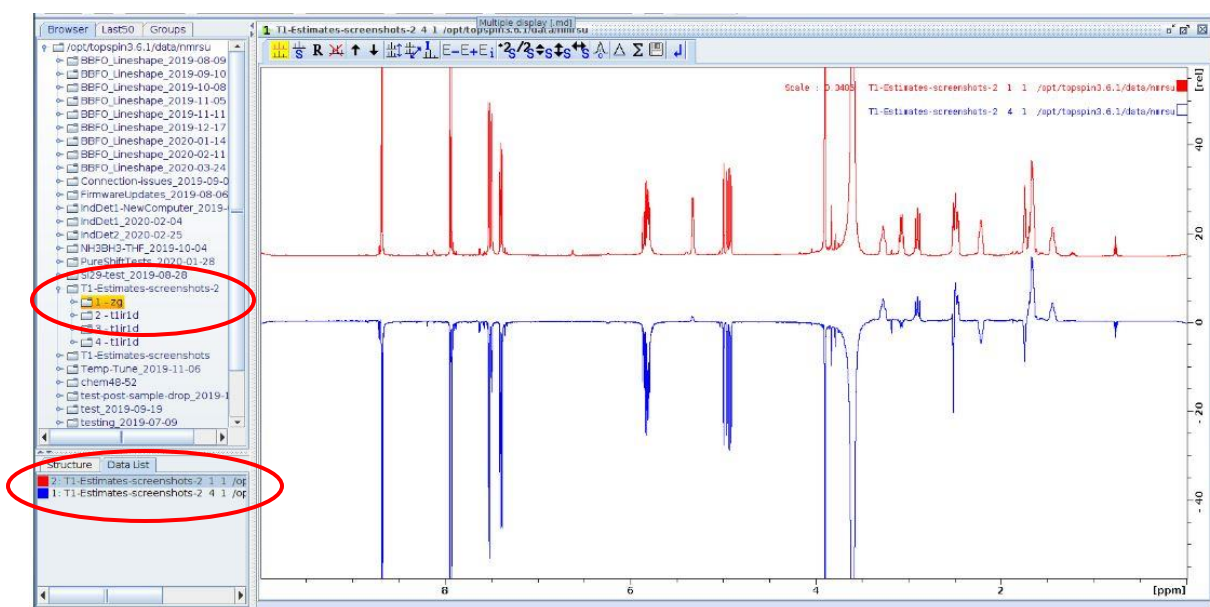

10. <u>Finding nulled peaks</u>: There will be a range of T1 values in your spectrum. The peak with the shortest T1 will be the first to flip from negative to positive, while the peak with the longest T1 will be the last to flip. For most NMR experiments, you will need to know the value of the longest T1 in your sample. *Be sure to exclude the TMS peak or any solvent peak in your spectrum.* Since these molecules are of different size than your compound, they will tumble at a different rate, and most often this results in longer T1 values. In the example of "quinine in DMSO-d_6" below, I narrowed in on D7 = 0.285 sec to see the peak at 5.30ppm nulled.
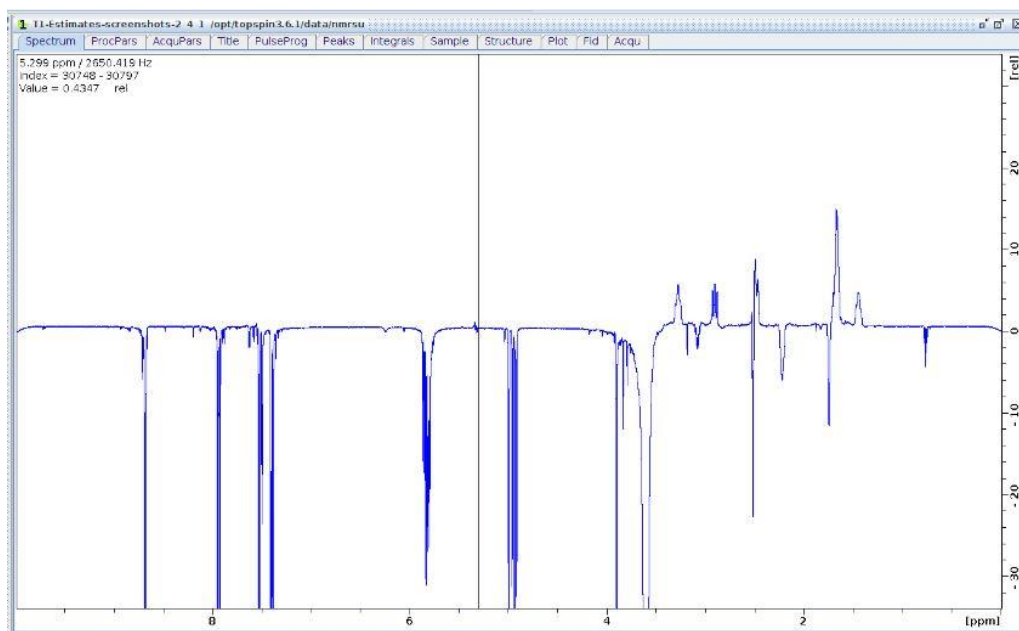
11. <u>Finding the longest T1</u>: Typically ^{1}Hs that are surrounded by other ^{1}Hs will have short T1s, while isolated ^{1}Hs will have longer T1s. Thus, aliphatic peaks have shorter T1 values than aromatic peaks. To find your longest T1 relaxing ^{1}H, use a longer D7 value, say 1.5 or 2 sec and acquire the spectrum (**zg**) followed by **efp**. If ALL the peaks are already positive, back up on the D7 value. Get to the point where the longest T1 relaxing ^{1}H peak is slightly negative. Play around with D7, so that you get this peak to null. Record this **D7$_{null}$** value, which will be the one of the longest relaxing ^{1}H in your sample.

In my "quinine in DMSO-d$_6$" example below, the peak at 7.9ppm is the longest relaxing peak in the spectrum, with a null at D7 = 1.53 sec. I've shown it with the ^{1}H 1D in red for comparison.
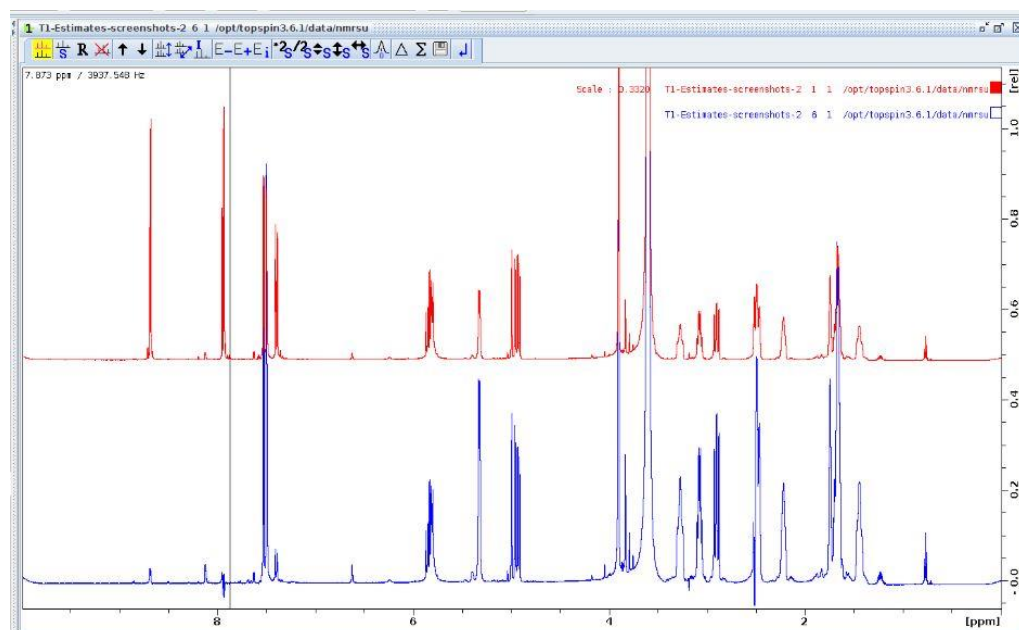
12. <u>Converting D7$_{null}$ to T1</u>: Next, calculate the longest T1 of your sample, since D7 ≠ T1. The T1 relaxation time is a function of magnetic field strength, temperature, solvent and sample preparation. When the intensity is nulled for a multiplet, the spin-lattice relaxation time for that proton is: *T1 ~ D7 × (1/ln2)*

Thus, you will need to multiply **D7$_{null}$** by about 1.4 to get the T1 value for your ^{1}H of interest. Folks typically do the T1 determination to find the longest T1 relaxing ^{1}H in their sample, since this value is then used to acquire a quantitiative ^{1}H 1D (in which D1 should be set to 5*longest T1 of your sample).

13. <u>Determining D1 for NMR experiment</u>: Use this 'rule of thumb' when deciding on relaxation delay (D1) values for various NMR experiments and mixing delays for NOE experiments:

$$D1 = 1 – 3 \text{ (and up to 7)} × T1 \text{ (longest of interest)}$$

$$mix ≤ T1 \text{ (fastest of interest)}$$

T1 "Abusability" vs. Relaxation delay (D1)
(modified from Varian course notes)

NMR experiment	relaxation delay D1 (in terms of T1)
gcosy, cosy, gCOSY	1 - 1.5 × T1
gDQCOSY	≥ 1 - 2 × T1
HSQC, HMQC	~ 1.5 × T1
HOM2DJ, HET2DJ, HETCOR, COSYPS	~ 1.5 × T1
TOCSY, HMBC	~ 1.5 - 3 × T1
NOESY & ROESY (qualitative)	~ 2 - 3 × T1
NOESY & ROESY (quantitative)	3 - 5 × T1
quantitative ^{1}H 1D	> 3 - 7 × T1 with accuracies: 3× → 95%, 5× → 99%, 7× → 99.9%