Zero(Null)-Crossing Estimates of ^{1}H T_1 Values

Estimates of proton spin-lattice relaxation times are important to the proper setup of all advanced NMR experiments, and critical whenever quantitative results are needed. Repetition times for most NMR experiments are dominated by ***d1*** *+* ***aq*** *. See the Table at the end of this document for guidance on setting repetition rates based on knowledge of T_1.*

Measurement of pw90 as specified in the guide Pulsewidth calibrations in TopSpin can often be skipped on Bruker spectrometers equipped with automatic tune-and-match (ATM) probes.[1,2]

The following procedure provides a simple method for estimating proton T_1 values in TopSpin. See the corollary document for obtaining quantitative T_1 values using IconNMR: AV3_1H-T1_Icon.

Estimating ^{1}H spin-lattice relaxation times, T_1, by zero-crossing/null-time experiments

In this section, a simple procedure is used to estimate proton T_1 values sufficient to setup most experiments. Other methods exist to measure T_1 values with higher precision, although careful setup and analysis of data produce by this procedure can provide accurate T_1 values for any sample.

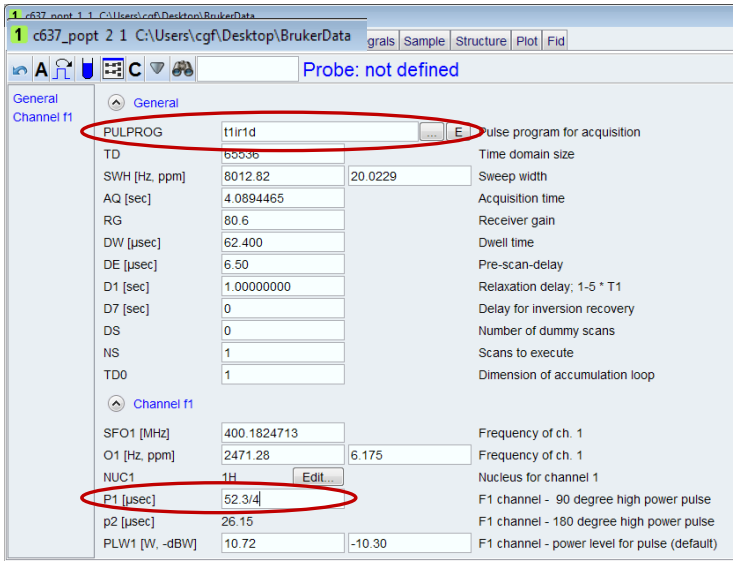
(a) You can copy an experiment, or experimental parameters, in a number of ways in TopSpin:

new ↵ (same as START → CREATE DATASET) can copy the parameters of the current experiment (via the appropriate check box) to the new experiment defined in the setup screen.

iexpno ↵ copies the current parameters to the next exp # (which cannot already exist) in the current folder. From **chem637_popt 1 1** , typing **iexpno** ↵ will create **chem637_popt 2 1** with a copy of the parameters from **1 1** . Note that **iexpno** also moves you to the new experiment **2 1**.

wra # is more general, in *copying the full dataset* (i.e., including the acquired fid) to the new exp #, e.g., **wra 40** ↵ makes a complete copy to experiment 40. It does not automatically move you there, so you have to drag in exp 40 form the TopSpin Browser, or type:

re 40 ↵ ;moves into experiment 40.



(b) **re 1 1** ↵

iexpno ↵

is the simplest way to copy the current parameters to experiment 2.

(c) In ACQUPARS , change PULPROG (the pulse sequence) to do T_1 inversion recovery:
t1ir1d ; type it in, or select via ... .

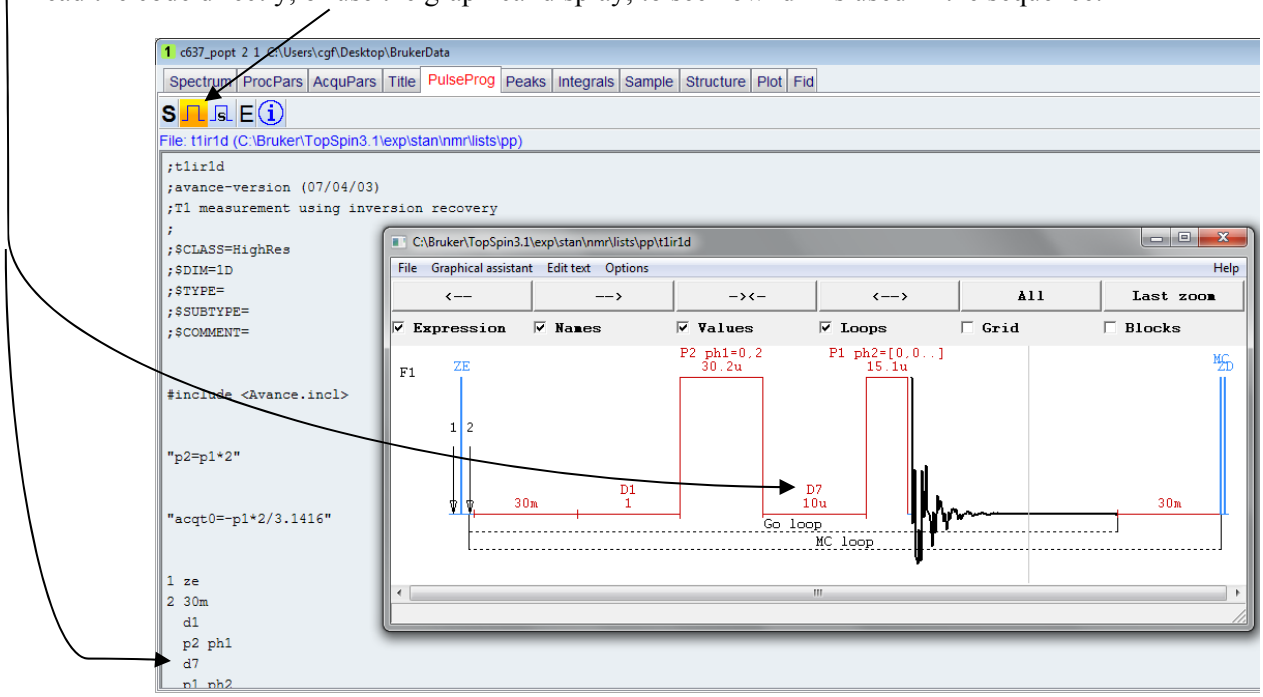
[1] The calibration procedure is recommended when using manually tuned probes — e.g., the Inova600 and Avance360 — and when using unusual samples, e.g., those having very high concentrations of salt.

[2] A calibrated 90° flip angle should be close to that given by **getprosol** . [This is typical on Bruker spectrometers with ATM; in such cases, the pw90 calibration can usually be skipped.] If pw90 ≡ p1(90°) is ≥ 1 µs from that given by **getprosol**, the sample is unusual (e.g., very high salt content) or you forgot to tune the probe with **atma**. Smaller variations are caused by sample differences; those variations likely will not impact any except the most complex experiments. See facility staff for more details.

(d) Change **p1** to match any new 90° calibration (typically just use the **getprosol** value).

(e) Check that **ns 1** and **ds 0** .[3] If low s/n requires **ns** > 1, make certain that **d1 ≥ 5×T_1** (i.e., insure that **d1** is "long enough").[4]

(f) **d7** is the key parameter for **t1ir1d**, being the interpulse delay. Click on the PULSEPROG tab and either read the code directly, or use the graphical display, to see how **d7** is used in the sequence:



(g) Set **d7** to a small value; delays are by default in seconds. So **d7 0.001** ↵ would set d7 to 1ms. The following are all equivalent: **d7 0.001s** ↵ **d7 1m** ↵ **d7 1000u** ↵ . Specifying the units can help avoid trouble. Pulse widths/lengths are set to µs by default, whereas delays are set to seconds by default. Note that hard pulse lengths[5] should never be made long (≥ 100 µs), unless you know exactly what you are doing (i.e., don't use long pulse lengths without staff consultation!).

(h) With a small setting (e.g., 1ms) for **d7** , take data (*finally*!), and transform with **efp** ↵ . All magnetization should be inverted, as this spectrum takes the phase from the previous experiment where a single 90° pulse was phased positive. *Do not apply an apk* .

(i) Use **iexpno** and make **d7** long (e.g., 5s). Acquire the new fid (you now be in exp 3). This spectrum should be positive (TMS and other solvent peaks might still be negative, having larger T_1 values).

(j) Use **iexpno** and experiment with **d7** (start small, 0.1s). Acquire the new fid (you now be in exp 4).

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[3] When **NS**=1 and **DS**=0, the time forced between each experiment — processing, inspecting the new spectrum, etc. — is usually long enough such that the repetition delay is not an issue. Of course, **this assumption insists that one not rush!**

[4] In this event one has to guess as to the T_1(longest-of-interest), and set **d1** accordingly. As the data is being acquired, re-estimate T_1(longest-of-interest) and lengthen **d1** if required, as well as retaking important intermediate spectra.

[5] Hard pulses are rectangular pulses run at high power. Other pulses, such as spin-locks, can have longer pulse lengths. There will always be advice as to how long and how strong such pulses can get; again, consult staff if you are unsure.

(k) Continue as in (j), and look for values where the magnetization crosses over from negative to positive. These are **d7$_{null}$** values for the multiplets as they crossover from negative to positive. Continue until all protons excluding solvent and impurity peaks have crossed over. A typical set of values is:

d7 = 0.001, 0.1, 0.2, 0.35, 0.6, 1, 2, 3.5

(l) There will always be a *range* of T_1 values in your samples. Different protons relax at different times, depending on the proximity of other protons, and the local mobility of that part of the molecule. It is typical to determine the following values:

T_1(fastest of interest) = smallest **d7$_{null}$** × 1.4

T_1(longest of interest) = largest **d7$_{null}$** × 1.4

In general,	**d1 = 1 to 3 [sometimes up to 7] × T_1 (longest of interest)**
and	**mix ≤ T_1 (fastest of interest)**

The phrase "… of interest" is important: it is not unusual for aromatic and vinyl protons to have significantly longer relaxation times than aliphatic protons. If the aromatic part of the compound is uninteresting to the question(s) you want answered, then by ignoring these longer-relaxing protons the experiment might run 2 to 4× faster.

T_1 "Abusability" vs Experiment Repetition (d1)

[modified from Varian course notes] The likelihood of artifacts increases as you go down the table.

gcosy, cosy, gCOSY (magnitude mode)	1-1.5 × T_1
gDQCOSY	≥ 1-2 × T_1
INADEQUATE	≥ 1.5 × T_1(^{13}C)
HSQC, HMQC	~ 1.5 × T_1
HOM2DJ, HET2DJ, HETCOR, COSYPS	~ 1.5 × T_1
TOCSY, HMBC	~ 1.5-3 × T_1
NOESY & ROESY (qualitative)	~2-3 × T_1
NOESY & ROESY (quantitative)	3-5 × T_1
QNMR: quantitative ^{1}H 1D[6]	> 3-7 × T_1 with accuracies: 3×→95%, 5×→99%, 7×→99.9%

[6] There are a variety of issues involved in obtained the most accurate quantitative data. PULPROG = zg30 is usually better than zg, as one example, since a 30° nutation greatly reduces dependence on repetition-times. ^{13}C decoupling can considerably improve the accuracy of ^{1}H quantitation by removed overlap of ^{13}C satellite peaks from other peaks. Processing can be equally or more important for QNMR: accurate baseline correction is critical if one is using standard (sum) integration. Peak fitting / deconvolution will be significantly better than standard integration in most cases when overlap of peaks is involved. For absolute quantitation, proper make-up of a standard sample is likely the defining portion of the experiment for accuracy. And so on….