Measuring ¹H T₁ Values in IconNMR

updated: 11 April 2025 (hh)

<u>Summary</u>

Knowledge of spin-lattice relaxation times, T₁, is important many experiments in NMR:

- a) Quantitative assays and analyses of reaction kinetics require having accurate knowledge of relaxation times. In particular the <u>longest T₁ of all protons of interest</u> must be known to properly optimize acquisition parameters and avoid systematic errors.
- b) All **NOESY** and **ROESY** experiments require at least semi-quantitative knowledge of proton T₁ values for proper setup and interpretation.
- c) All NMR experiments require qualitative knowledge of the *longest* T_1 of the nuclei of interest to properly setup repetition rates, typically equal to d1+aq.

 T_1 values accurate enough for b) and c) above can be obtained by an inversion-recovery null-crossing measurement. These were performed with regularity on our Varian spectrometers, with the array function in vnmr making such measurements straightforward. Similar procedures can be done in TopSpin (although to be honest, not in as easy a manner), as detailed in a companion document:

<u>http://www.chem.wisc.edu/~cic/nmr/Guides/Ba3vug/AV3_1H-T1null.pdf</u> In this document, a simple method for obtaining *quantitative* T₁ values using IconNMR is presented.

A. IconNMR's Inversion-Recovery Experiment – Setup and Acquisition:

- 1. In IconNMR, ADD the H1_T1invrec.UW experiment.
- 2. In the main menu, select PARAMETERS \rightarrow EDIT ALL ACQUISITION PARAMETERS

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▶ 6	L	Available	

- 3. IconNMR will switch you into the ACQUPARS panel in TopSpin; you will see ALL parameters. Press ☐ or type **ased**↓ to reduce the list to a reduced set (see Figure 1 next page).
- 4. Check the VDLIST by clicking ... and if not already set, select **t1uwchem** from the SOURCE = /home/topspin3.1/uwchem/lists/pp (in the upper right dropdown). Click Set selected item in editor then Close at the lower right. The list can be viewed and edited using the E button.
- 5. The list of delays is a good one for typical organic compounds under normal atmosphere. See NMR staff for more assistance if you are working with very small or paramagnetic compounds, or samples sealed under inert atmospheres. Otherwise the default list should work ok.

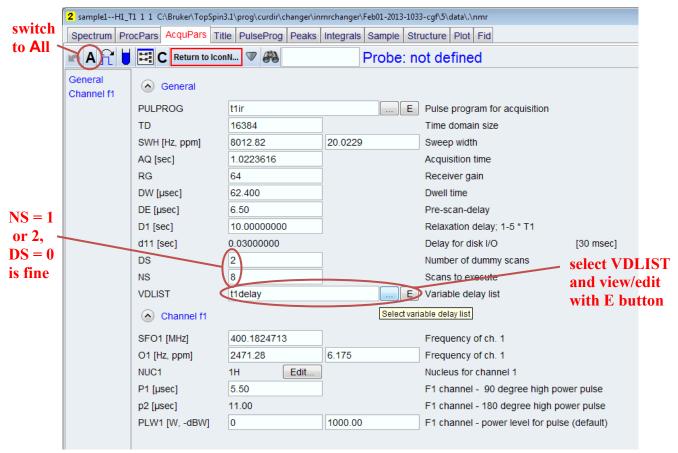
If you change the list, please save it under a different name and do NOT overwrite the tluwchem!

6. The # of values in VDLIST must equal TD[F1] (see Fig 1). On the command line, use TD, .

Figure 1:	All ACQUPARS	parameters.
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Figure 2: \square or ased reduced ACQU parameters.



7. D1 = 15 sec is the default. This value is too small for some compounds, and will need to be made longer when quantitative T₁ values are needed. If

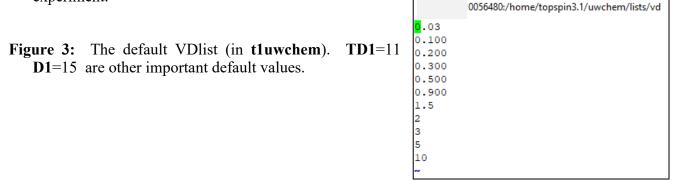
 $D1+AQ \le 5 \times T_1$ [for nuclei of interest; i.e., ignore TMS or other solvent peaks]

is found to be true in the analysis (see below), then the results are at best qualitative. The experiment would need to be rerun to obtain accurate results. When $D1+AQ \le 3 \times T_1$ the experiment should be rerun. For the most accurate results, using $D1+AQ = 10 \times T_1$ is recommended, as is randomizing the VDLIST values, and including 3 or more duplicates of the longest VD value.

- 8. Although the **t1ir** pulse sequences recommend **NS**=8×i and **DS**=4, **NS**=1 or 2 and **DS**=0 will work fine on our spectrometers (assuming your sample is at sufficient concentration).
- 9. Optional: IconNMR will perform an automated analysis of the data. You can check the processing set by clicking on the PROCPARS tab, then on the AUTOMATION link in the left-hand navigation bar. Make sure that a **<space>1** is at the end of the **AUNMP** name: **proc_2dt1**.

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10. Click on the RETURN TO ICONNMR tab, and SUBMIT the experiment.



NOTE: In ICON you will receive an error message after your experiment has run.

Date	User	Disk	Holder	No. Name	Experiment Load ATM Rotation Lock Shim Acq Proc T. Remarks
		/home/ahalder2/eos		10 tlinvrec_final_v2	H1_T1invrec.UW 🗸 🗸 🗸 🗸 🗸 🗸 ppt1: could not open file /opt/topspin3.6.2/exp/stan/nmr/lis
2024-04-22 20:05:01	analderz	/nome/analoer z/eos			was stopped by user

If you take a closer look, you will see that the data was acquired but not processed. You do not need to re-run the experiment!

B. Processing and Analysis of T₁ data:

- 1. <u>MestreNova</u>: Working up the data for visually inspection is (mostly) straightforward.
 - a) Some trouble occurs when importing raw data. Suppose the data folder name in TopSpin is:

e.g., D1404180938_sucrose_1HT1 10 1

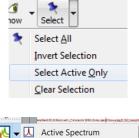
If you drag the **D1404180938_sucrose_1HT1/10** folder into MNova, it identifies that the dataset is pseudo-2D (i.e., comprised of a series of 1D spectra) and auto-processes it, including performing an autophase.

Recommended: drag the processed data folder in:

i.e., D1404180938_sucrose_1HT1/10/pdata/1

The phasing from TopSpin will then be used in MNova, and will (likely) be correct.

- b) or: To phase individual spectra in the stack, use the Stacked Spectra table view *i* in the spectrum you want to work on by checking the box under the pin icon *i* or choose Select → Select Active Only.
- c) phase the first spectrum negative. If the inversion (+180deg) causes the spectrum to disappear, increase the intensity and manually phase the spectrum.
- d) click on **Stacked** again to display all and the phase will automatically be applied to all spectra in the stack.
- e) You should be able to visually observe where the nulls occur. The d7 delays for each spectrum will be listed in the **Stacked Spectra** table under the T/G column. $T_1 = 1.4 \times d7_{null}$.



- 🎜	⊾	Active Spectrum	DOP4
	2	Stacked	<u>απ+4</u>
<u>z</u> C	×	Whitewash Stacked	
		Superimposed	
		Horizontally Stacked	
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If you see a contour instead of individual spectra, go to Stack \rightarrow 2D to 1D stack and preceed from there.

2. <u>Automatic processing in topspin</u>: If AUNMP is set correctly (as described in step 9 above), TopSpin will automatically process the data. Setup and results can be found in the following text files:

<datasetname>/10/vdlist</datasetname>	; contains the vdlist delay values
<datasetname>/10/pdata/1/dt1t2.txt</datasetname>	; contains a brief summary of the T ₁ values
<datasetname>/10/pdata/1/ct1t2.txt</datasetname>	; contains a detailed summary of the T ₁ values

The automated processing is somewhat problematic, especially ascertaining which multiplet regions are solute peaks, rather than impurities and solvent. The required information can be obtained, especially on very pure and relatively simple compounds. Otherwise, manual workup (MNova!) and processing will likely be preferred.

3. <u>Manual workup using TopSpin</u>: Bruker provides a good set of instructions in Chapter 8.2.4 of their "1D and 2D Step-by-Step – Advanced" manual, which can be accessed in TopSpin3.x by clicking the help button.:

ProtonT1: Quantitative T_1 measurements in *IconNMR*

🖕 Bruker TopSpin 3.1 on cgfthink as cgf
Start Process Analyse Publish View Manage
Create Dataset Find Dataset Open Click here to access TopSpin documentation: Manuals, release letters, functions keys, etc. Key shortcut to access this menu: ALT H
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A brief summary of the steps (use the TopSpin guide initially, but then this will likely suffice):
a) rser 10 (last fid) \rightarrow ef \rightarrow manually phase .ph \rightarrow store to 2D by clicking $\blacksquare \rightarrow \downarrow$
b) return to the 2d experiment by clicking \square and transfer with xf2
c) enter relaxation analysis with \bigwedge Analyse $\bigvee \rightarrow \bigvee$ which give the following menu:
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d) click $\xrightarrow{\text{Im} \text{Eid}} \rightarrow \text{SPECTRUM} \rightarrow \text{enter last fid } \# (10) \rightarrow \text{OK}$
e) click $\xrightarrow{\text{Peaks/Ranges}} \rightarrow \text{MANUAL INTEGRATION} \rightarrow \text{OK}$
f) integrate all interesting multiplets/peaks $\rightarrow \blacksquare$ then \bigcirc Save Regions To 'Introg' Save Regions To 'reg'
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j) note the BRIEF REPORT, or look at the full report by clicking Eport