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 (pages 2-5) and how to process the data using TopSpin (Dynamics Centre [pages 5-8] or T1/T2 relaxation mode [page 5,8-10]) and MestreNova [pages 11-13], henceforth known as MNova.

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 $R_1 = 1/T_1$.

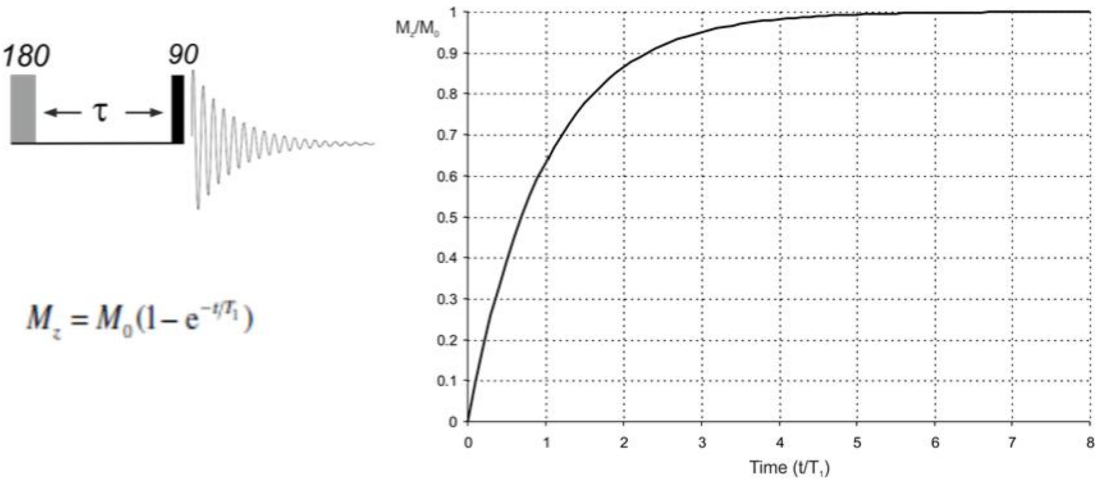


Figure 1: The inversion recovery sequence and the expression describing the recovery of magnetisation (Mz) after inversion back toward its equilibrium value (M_0) as a function of recovery time (*t*) and (right) the recovery profile.

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

4. Setup and Acquisition

4.1. Calibrate 90-degree pulse and collect a 1D ^{1}H NMR spectrum

For samples with high salt content or paramagnetic material, a p1 calibration is highly recommended. For standard samples, the standard 90-degree pulse duration (p1) should be adequate. 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 default p1 value obtained using the command ‘getprosol’ should be suitable for use.

4.2. Load and edit parameters

The inversion recovery experiment can be run either manually or using IconNMR. For manual setup, create a new experiment and load in the “h1invrec_t1.crl” dataset (using the command “rpar”). If running under automation in IconNMR, select the “h1invrec_t1.crl” 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.

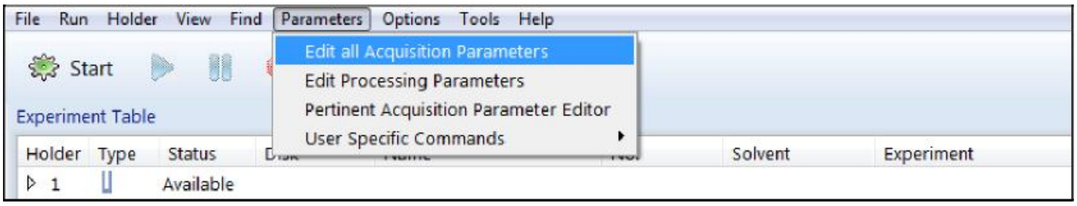
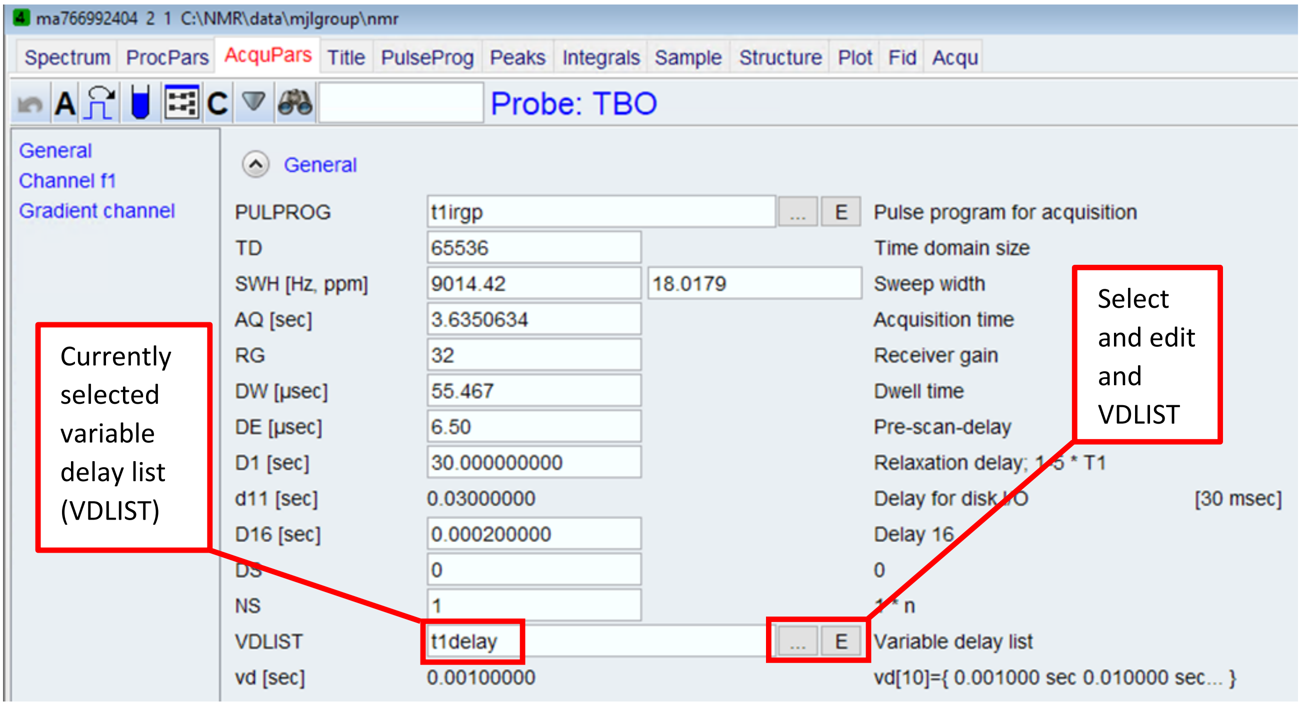


Figure 2. Editing experiment parameters within Icon-NMR

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 “t1delay”. You can create your own list or choose / edit one of the existing T_1 lists. **Do not change the values within the ‘t1delay’ vd list.** 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.

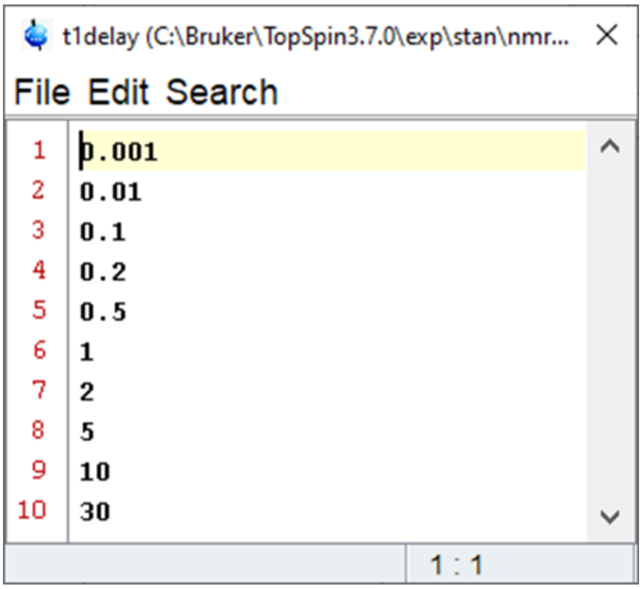


PULPROG	t1irgp		Pulse program for acquisition
TD	65536		Time domain size
SWH [Hz, ppm]	9014.42	18.0179	Sweep width
AQ [sec]	3.6350634		Acquisition time
RG	32		Receiver gain
DW [µsec]	55.467		Dwell time
DE [µsec]	6.50		Pre-scan-delay
D1 [sec]	30.000000000		Relaxation delay; 1-5 * T1
d11 [sec]	0.03000000		Delay for disk I/O [30 msec]
D16 [sec]	0.000200000		Delay 16
DS	0		0
NS	1		1 * n
VDLIST	t1delay		Variable delay list
vd [sec]	0.00100000		vd[10]={ 0.001000 sec 0.010000 sec... }

Currently selected variable delay list (VDLIST)

Select and edit and VDLIST

Figure 3: Selecting the vd list.



```
0.001
0.01
0.1
0.2
0.5
1
2
5
10
30
```

Figure 4: Example of a variable delay ("vd") list for proton T_1 measurement. Times are listed in seconds.

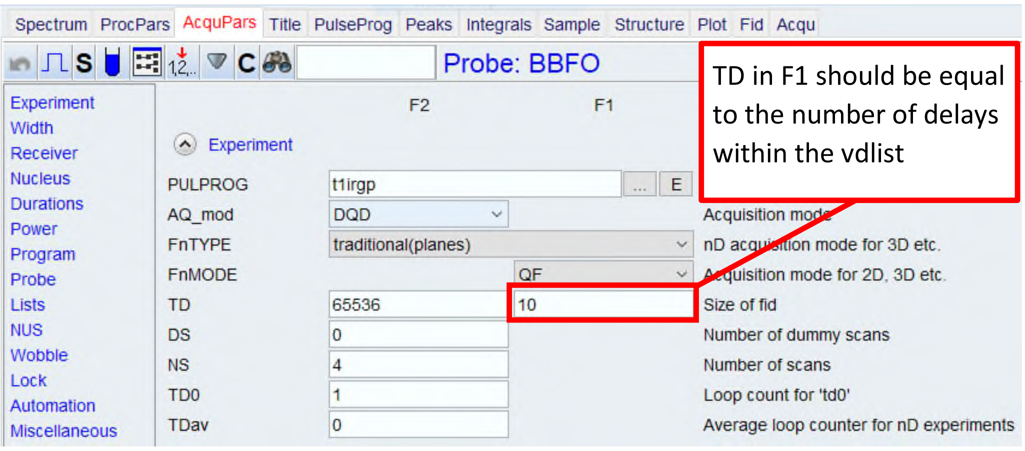
Table 1. Example inversion recovery τ delays (in seconds) for a range of typical proton T_1 values. If you are uncertain of likely T_1s, set parameters for the longer T_1 value.

	Proton T_1 (seconds)		
vd #	**0.5**	**2**	**4**
1	0.01	0.05	0.05
2	0.02	0.06	0.1
3	0.025	0.09	0.2
4	0.03	0.12	0.3
5	0.05	0.2	0.4
6	0.07	0.3	0.7
7	0.1	0.4	1.0
8	0.15	0.6	1.5
9	0.25	1.0	2.0
10	0.35	1.5	2.5
11	0.5	2.0	4.0
12	0.8	3.0	6.0
13	1.0	4.5	9.0
14	1.5	6.5	13.5
15	2.5	10.0	20.0

4.3. Other relevant parameters for data acquisition

The following points must be taken into consideration:

- 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 'AcquPars' button in the acquisition parameters panel and editing the "TD" parameter, or by typing "td" in the command line (Figure 5).
- The recommended number of scans (ns) is a multiple of 2.
- The relaxation delay (d1) should be set to 4-5 times the expected T_1. The default value for d1 is 30 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 T_1 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.
- Set the 90-degree pulse length (p1) to the calibrated value (section 3.1.1).
- Remember to set the receiver gain using the command "rga".



Probe: BBFO

Experiment

	F2	F1	
PULPROG	t1irgp		
AQ_mod	DQD		Acquisition mode
FnTYPE	traditional(planes)		nD acquisition mode for 3D etc.
FnMODE		QF	Acquisition mode for 2D, 3D etc.
TD	65536	10	Size of fid
DS	0		Number of dummy scans
NS	4		Number of scans
TD0	1		Loop count for 'td0'
TDav	0		Average loop counter for nD experiments

TD in F1 should be equal to the number of delays within the vdlist

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

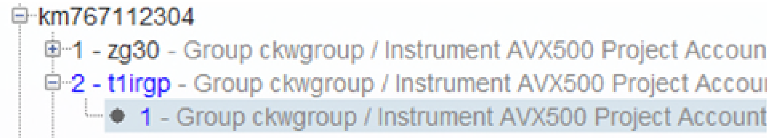
5. Processing T_1 data

T_1 inversion recovery data can be processed a number of ways. Below is how to process using TopSpin via *Dynamics Centre*, the *T1/T2 relaxation analysis* mode, or via MNova. If processing in TopSpin, spectra must be correctly phased prior to either mode.

5.1. Processing T_1 data using TopSpin

5.1.1. Phase and baseline correction of T_1 inversion recovery data in TopSpin

1. Open the processing number 1 of the 2D T_1 inversion recovery data (Figure 6).
2. Read the FID with the longest vd value. For example, if you had used 15 variable delays, type ‘rser 15’ in the command window and press enter.
3. Fourier transform and apply line broadening using the “ef” command.
4. Phase the spectrum automatically by typing apk. If necessary, correct the phasing further using the manual phasing mode (.ph). Do not exit manual phasing mode.
5. If not already, enter manual phasing mode and store the phasing to 2D by clicking on the ‘save-nD’ () button, and then save and exit by clicking the button.
6. Return to the 2D experiment by opening processing number 1 (Figure 6) and Fourier Transform all the 1D traces using the ‘xf2’ command.
7. Perform 2D baseline correction using the ‘abs2’ command.



km767112304
1 - zg30 - Group ckwgroup / Instrument AVX500 Project Accoun
2 - t1irgp - Group ckwgroup / Instrument AVX500 Project Accou
1 - Group ckwgroup / Instrument AVX500 Project Account

Figure 6: Example of T_1 data shown in TopSpin. The blue ‘2’ specifies the T_1 data set and the indented blue 1 is the processing number.

5.1.2. Processing T_1 data using Dynamics Centre

1. Enter the pre-Dynamics Centre window by clicking (TopSpin 4) *Applications – Dynamics – T1T2(t1t2)* or (TopSpin 3) *Analyse – Dynamics – T1T2 (t1t2)* from the drop-down menu (Figure 7).

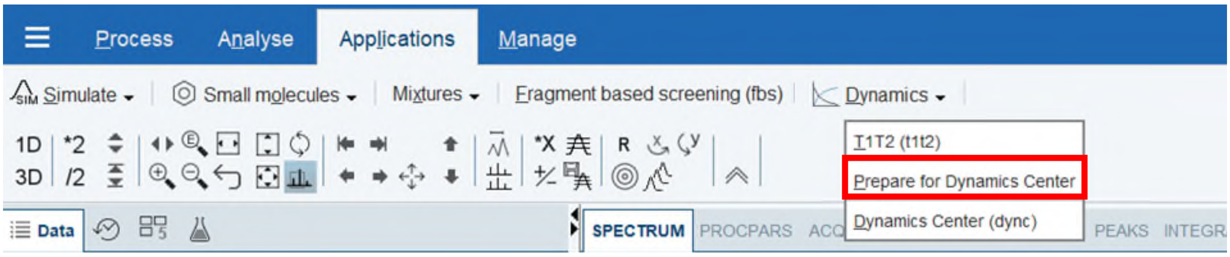
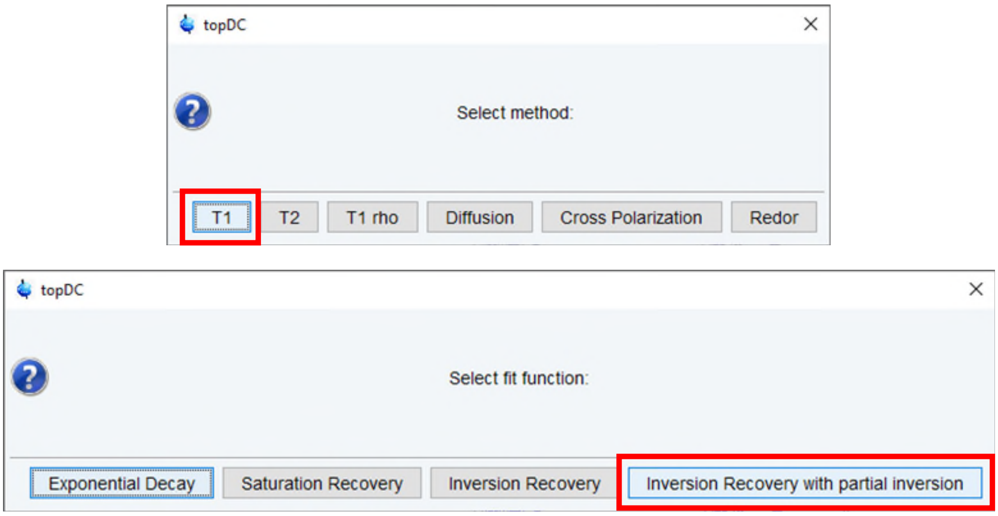


Figure 7: Entering relaxation mode analysis in TopSpin 4.

Data can be processed by integrating or peak picking signals of interest. If significant peak overlap is present in the spectrum, it is recommended to use the ‘peak picking’ option.

2. In the top banner, click ‘Manual Peak Picking’ and a pop-up window will appear. Click ‘T1’ then ‘Inversion Recovery with partial inversion’. If the data has previously been processed, these boxes won’t appear and instead the option of either ‘Manual Peak Picking’ or ‘Manual Integration’ will appear.



Select method:

Select fit function:

Figure 8: Specifying experiment used to generate T_1 data.

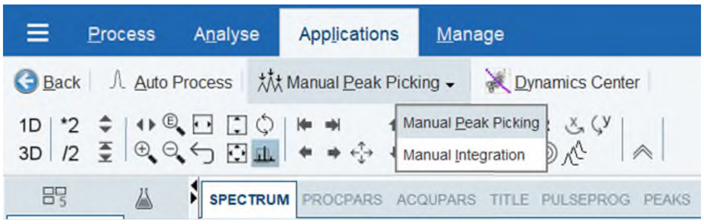


Figure 9: Top banner showing ‘Manual Peak Picking’ and ‘Manual Integration’ options.

3. [**Integration option**] Integrate all peaks / multiplets for which you wish to calculate T_1. Export your integral regions to the Relaxation Module by clicking on (TopSpin 4) the ‘save and return’ button or (TopSpin 3) the ‘save/export integration regions’ button and selecting “Export Regions to Relaxation Module and .ret”. This will allow you to use peak areas for determination of T_1.
4. [**Peak picking option**] 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 (TopSpin 4) the ‘save and return’ button or (TopSpin 3) the ‘save/export integration regions’ 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. In the top banner, only the ‘Dynamics Centre’ option should remain. Click to open Dynamics Centre.
6. Dynamics Centre should display three separate windows: spectrum overview, T_1 fitting window and the T_1 plot. To generate the T_1 fitting curve and plot, move the mouse cursor over a picked peak in the spectrum overview.

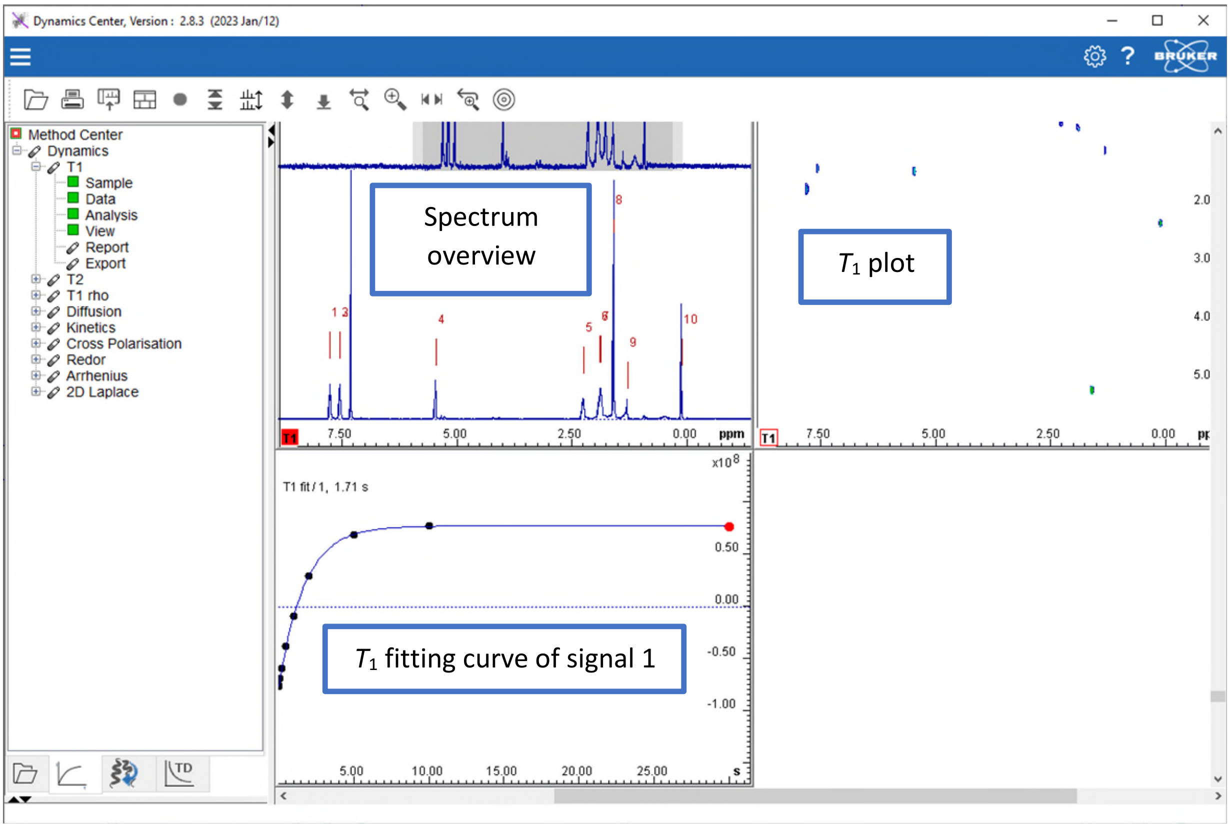
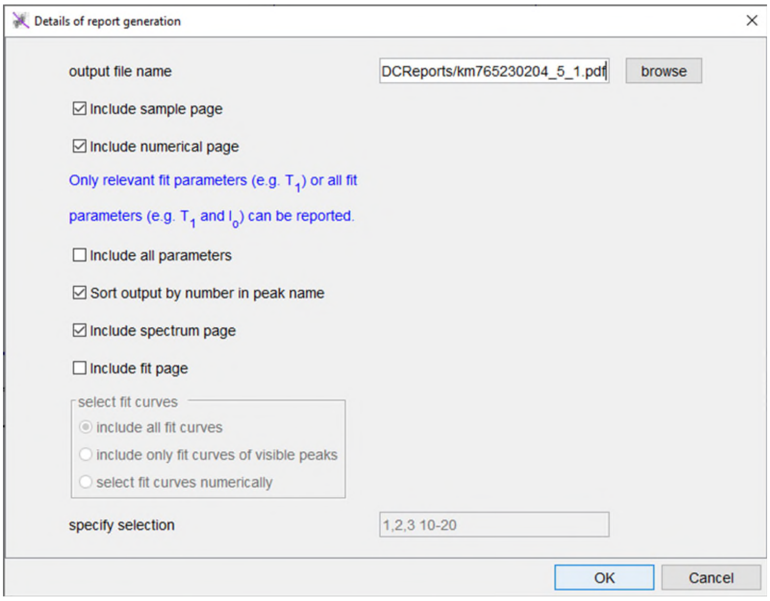


Figure 10: Dynamics Centre window.

7. To generate a report, click ‘Report’ in the left-hand panel. Specify where the report should be saved in the ‘output file name’. It is recommended not to use the default folder TopSpin and to change the filepath. Select ‘OK’.


Details of report generation

output file name: DCReports/km765230204_5_1.pdf

☑ Include sample page

☑ Include numerical page

Only relevant fit parameters (e.g. T_1) or all fit parameters (e.g. T_1 and I_0) can be reported.

☐ Include all parameters

☑ Sort output by number in peak name

☑ Include spectrum page

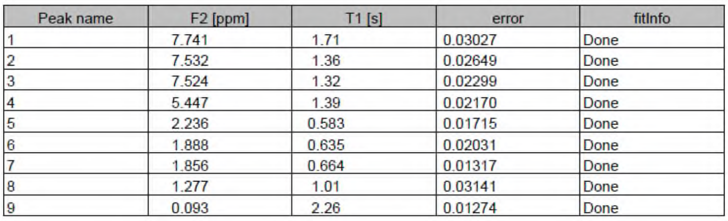
☐ Include fit page

select fit curves
- ● include all fit curves
- ○ include only fit curves of visible peaks
- ○ select fit curves numerically

specify selection: 1,2,3 10-20

Figure 11: Report generation window.

8. The report will include the ^{1}H NMR spectrum, a T_1 plot and a table summarising the results, including associated errors.



Peak name	F2 [ppm]	T1 [s]	error	fitInfo
1	7.741	1.71	0.03027	Done
2	7.532	1.36	0.02649	Done
3	7.524	1.32	0.02299	Done
4	5.447	1.39	0.02170	Done
5	2.236	0.583	0.01715	Done
6	1.888	0.635	0.02031	Done
7	1.856	0.664	0.01317	Done
8	1.277	1.01	0.03141	Done
9	0.093	2.26	0.01274	Done

Figure 12: Example of the table summarising T_1 results in the report summarising by Dynamics Centre.

5.1.3. Processing T_1 data using the T1/T2 relaxation analysis mode

9. Enter the relaxation analysis mode by clicking (TopSpin 4) *Applications – Dynamics – T1T2(t1t2)* or (TopSpin 3) *Analyse – Dynamics – T1T2 (t1t2)* from the drop-down menu (Figure 13).

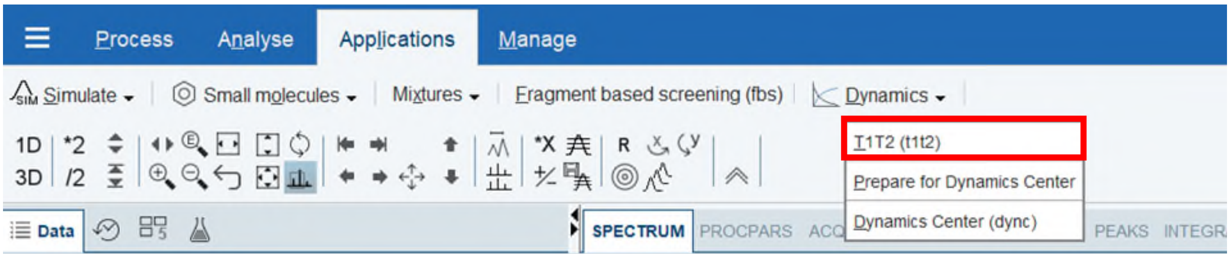
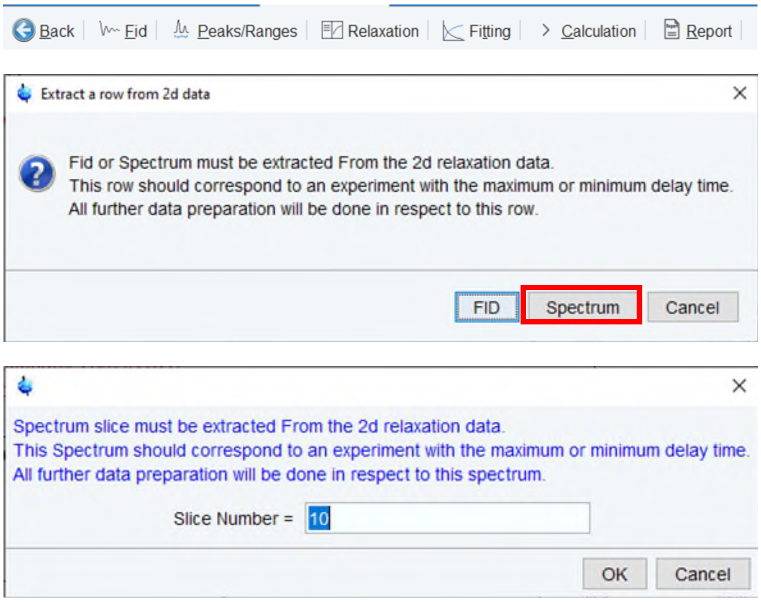


Figure 13: Entering relaxation mode analysis in TopSpin 4.

10. Click on the “FID” button in the top banner, and then select “Spectrum” in the drop-up window. In the dialogue box enter the number of the last FID (for example if you have used 10 variable delays, select spectrum number 10).



Extract a row from 2d data

Fid or Spectrum must be extracted From the 2d relaxation data.
This row should correspond to an experiment with the maximum or minimum delay time.
All further data preparation will be done in respect to this row.

Spectrum slice must be extracted From the 2d relaxation data.
This Spectrum should correspond to an experiment with the maximum or minimum delay time.
All further data preparation will be done in respect to this spectrum.

Slice Number = 10

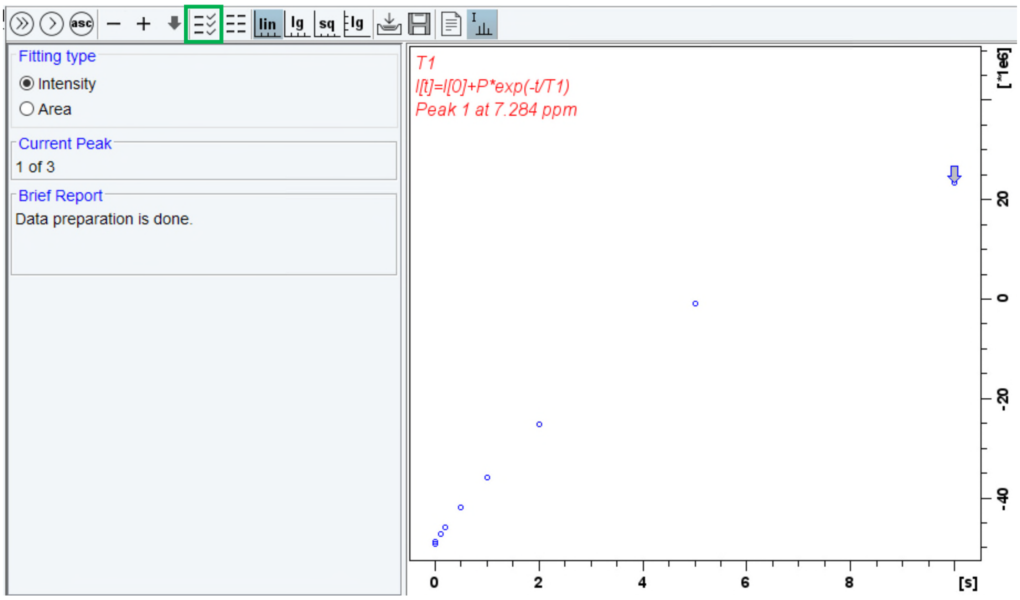
Figure 14: (top) Top banner of the T1/T2 relaxation analysis mode. (middle and bottom) pop-up windows requiring user input.

Data can be processed by integrating or peak picking signals of interest. If significant peak overlap is present in the spectrum, it is recommended to use the ‘peak picking’ option.

11. [**Integration option**] From the top banner, 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 ‘save/export integration regions’ button and selecting “Export Regions to Relaxation Module and .ret”. This will allow you to use peak areas for determination of T_1.

12. [**Peak picking option**] 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 ‘save/export integration regions’ 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.
13. Return to the 2D experiment by clicking on the ‘return’ button if required.
14. Select the “Relaxation” button from the top banner. This will open the fitting window (Figure 8).
15. Type ‘.all’ in the command window to ensure the full curve is observed.
16. Click on the ‘settings’ button (shown in Figure 15, green box) 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 16). Ensure that ‘Function Type’ is set to “uxnmrt1”, and ‘List file name’ is set to “vdlist”.



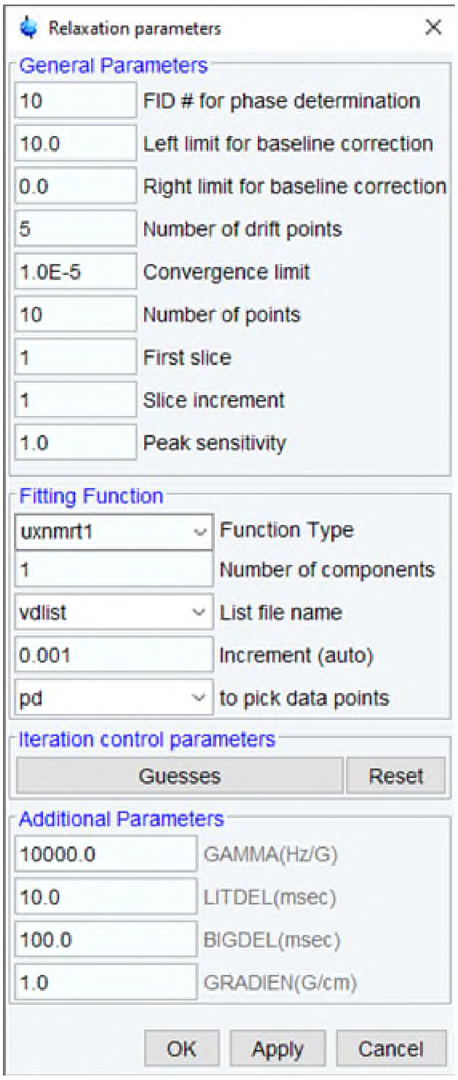
Fitting type
● Intensity
○ Area

Current Peak
1 of 3

Brief Report
Data preparation is done.

T1
I[t]=I[0]+P*exp(-t/T1)
Peak 1 at 7.284 ppm

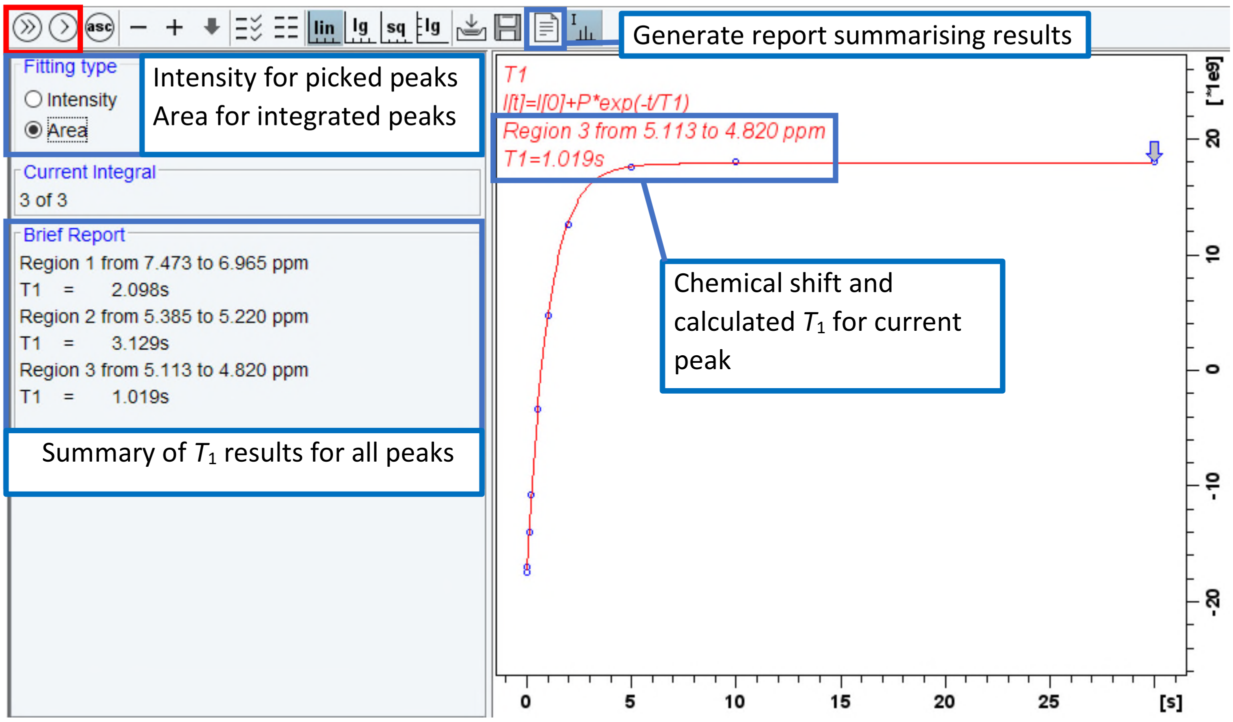
Figure 15: Peak fitting window. The settings window is shown within a green box.



10	FID # for phase determination
10.0	Left limit for baseline correction
0.0	Right limit for baseline correction
5	Number of drift points
1.0E-5	Convergence limit
10	Number of points
1	First slice
1	Slice increment
1.0	Peak sensitivity
uxnmrt1	Function Type
1	Number of components
vdlist	List file name
0.001	Increment (auto)
pd	to pick data points
10000.0	GAMMA(Hz/G)
10.0	LITDEL(msec)
100.0	BIGDEL(msec)
1.0	GRADIEN(G/cm)

Figure 16: Relaxation parameters window.

17. Select either ‘Area’ or ‘Intensity’ from the left-hand side of the fitting window to use your peak integrals or picked peaks, respectively.
18. Select the single arrow button or the double-arrow button (Figure 17, red box) to fit the current peak or all peaks, respectively.
19. To cycle through the peak fittings, use the + and – buttons (Figure 17, next to red box).
20. To generate a report summarising all T_1 results, click ‘show report’ (Figure 17, top blue box).



Generate report summarising results

Fitting type
○ Intensity
● Area

Intensity for picked peaks
Area for integrated peaks

Current Integral
3 of 3

Brief Report
Region 1 from 7.473 to 6.965 ppm
T1 = 2.098s
Region 2 from 5.385 to 5.220 ppm
T1 = 3.129s
Region 3 from 5.113 to 4.820 ppm
T1 = 1.019s

Summary of T_1 results for all peaks

T1
I[t]=I[0]+P*exp(-t/T1)
Region 3 from 5.113 to 4.820 ppm
T1=1.019s

Chemical shift and calculated T_1 for current peak

Figure 17: Peak fitting for T_1 analysis. The red box highlights how to fit the data.

5.2. Processing data using MNova

1. Load 2D dataset into MNova. MNova will apply an appropriate weighting function, zero-filing, Fourier Transform and phase correction to the data, and stack all spectra as shown in Figure 18. The number of stacked spectra should equal the number of entries in the vd list.
2. Zoom in on the chemical shift range where signals are observed
3. Ensure the phase of all spectra is correct, i.e., the last spectra (spectrum number 10 in this case) is in phase and positive. If needed, correct using the ‘manual phase correction’ tool under the ‘*Processing*’ tab.
4. Perform automatic baseline correction.

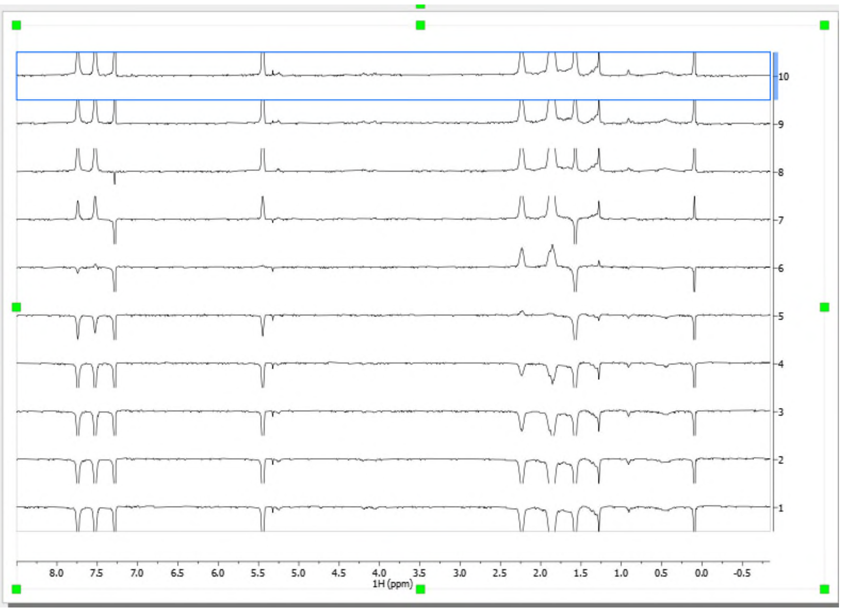


Figure 18: T_1 data showing 10 stacked spectra with the correct phasing for T_1 calculation.

5. Under ‘*Data Analysis*’, click on ‘*New*’, then ‘*Integrals Graph*’ (Figure 19)
6. Integrate the signals you wish to calculate the T_1 value of.

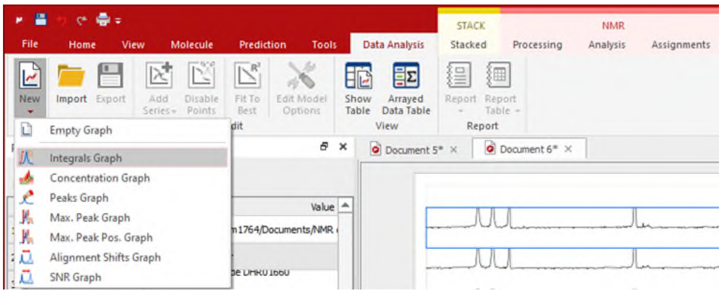
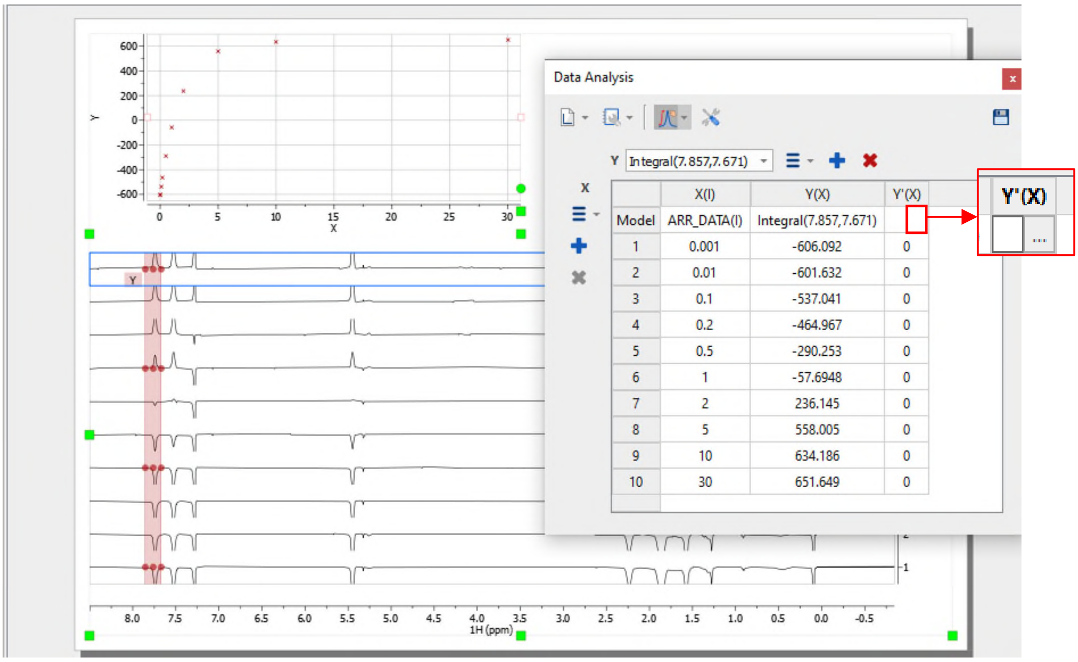


Figure 19: How to open the integration function to process T_1 data.

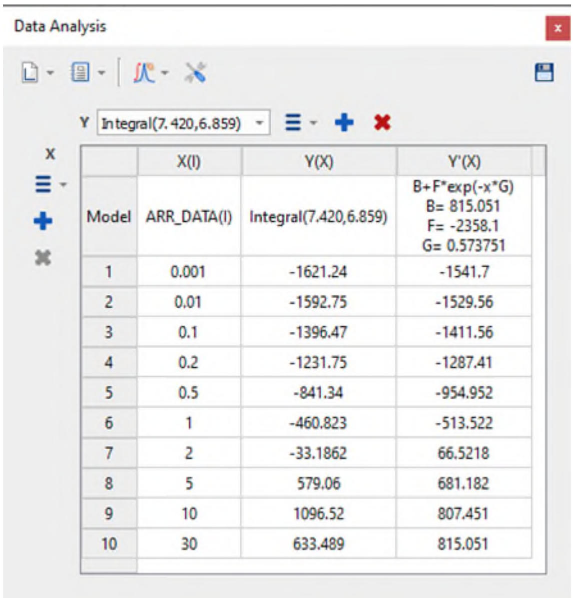
7. A data analysis window will appear when a signal is integrated with time point [X(I)] and integration value [Y(X)]. Confirm the time point values for each timepoint (under *Model*) correspond to those within the vdlist. An XY plot will also be generated plotting these time points against integration values.
8. In the ‘Data Analysis window’ click in the empty box on the right-side hand under Model/Y’(X) (red box in Figure 20) and click on the box with three dots that appears.



X	X(I)	Y(X)	Y'(X)
Model	ARR_DATA(I)	Integral(7.857,7.671)	
1	0.001	-606.092	0
2	0.01	-601.632	0
3	0.1	-537.041	0
4	0.2	-464.967	0
5	0.5	-290.253	0
6	1	-57.6948	0
7	2	236.145	0
8	5	558.005	0
9	10	634.186	0
10	30	651.649	0

Figure 20: T_1 data showing the (top) XY plot of time (x-axis) vs integration (y-axis), (right) the data analysis window showing the x- and y- values, and the (bottom left) T_1 data array. The red boxes highlights how to open the Y’ column model function window.

9. Select the function **B+F*exp(-x*G)**. Depending on the version of MNova, this may be called ‘Three parameter exponential fit’ or ‘mono exponential fit with offset’.
10. Click ‘Calculate’ then OK.



Y Integral(7.420,6.859)

	X(I)	Y(X)	Y'(X)
Model	ARR_DATA(I)	Integral(7.420,6.859)	B+F*exp(-x*G) B= 815.051 F= -2358.1 G= 0.573751
1	0.001	-1621.24	-1541.7
2	0.01	-1592.75	-1529.56
3	0.1	-1396.47	-1411.56
4	0.2	-1231.75	-1287.41
5	0.5	-841.34	-954.952
6	1	-460.823	-513.522
7	2	-33.1862	66.5218
8	5	579.06	681.182
9	10	1096.52	807.451
10	30	633.489	815.051

Figure 21: Data Analysis window showing the G value ($1/T_1$) value for an integrated ^{1}H signal.

11. The signal intensity at time (t) is defined by the equation $M(t)=M_0(1-2e^{-t/T_1})$. The fitting function used here is $S=B+Fe^{-xG}$. Therefore, to calculate the relaxation rate, $T_1 = 1/G$. In this case, the T_1 value for the ^{1}H signal at 7.42 – 6.86 ppm is 1.74 seconds.
12. If multiple signals were generated, repeat steps 8-11.