^{13}C T_1 Acquisition & Analysis from HSQC Data Using TopSpin's Dynamics Center

updated: 20160324 (hh+cgf)

Because ^{13}C NMR is quite insensitive (even on callisto), measuring ^{13}C relaxation directly — e.g., using inversion recovery methods similar to the standard experiment used for ^{1}H, PROTONT1— can often not be done. ^{1}H–^{13}C HSQC provides greatly improved sensitivity, and can be used to measure ^{13}C relaxation of protonated carbons. The technique is routinely performed on proteins and similar macromolecules, where the relaxation times then provide sequence-specific probes of the atomic dynamic/motional behavior.[1] Three types of relaxation can be measured:

– spin-lattice relaxation, T_1, sensitive to fast motions (~10^9 Hz)

– spin-lattice relaxation in the rotating frame, $T_{1\rho}$, sensitive to kHz motions

– transverse relaxation, T_2, sensitive to slower motions

Here we discuss obtain $T_1(^{13}C)$, using the Bruker pulse sequence hsqct1etgpsi3d.2. Because we are acquiring ^{1}H-^{13}C hsqc spectra, quaternary carbons will not be observed and no T_1 values will be obtained for them.

A. Data Acquisition:

You will acquire a set of ^{1}H-^{13}C hsqc 2D spectra. The only difference between the different spectra will be a relaxation delay provided by a vd list. **d1** is *not* critically important in these experiments, although as always having **d1** not too short is important. The software does everything else, so acquisition is straightforward (at least, compared to the data processing).

1. Setup

• Acquire a normal ^{1}H 1D spectrum. Knowledge of the ^{1}H T_1 values (or at least the maximum T_1 of interest) is important.

• Acquisition is a ^{13}C 1D spectrum will be useful, but this is definitely not required.

2. Pseudo-3D acqusition

• Read in the parameter set (experiment): **HC_hsqc-C13T1.UW** . The pulse sequence hsqct1etgpsi3dp2.UW is identical to Bruker's (named above), except now **aq < 0.3s** is required.

• Use or edit (and save as another name) the vd list: **hsqcC13t1** . Note the number of entries: 7 is the standard set. Change the parameter **NBL** and **td1** to equal this number.

• # scans needs to be a multiple of 8: **ns = 8×*i*** .

• do a normal **rga↵** **zg↵**

• expt will not give a good estimate of the experiment time. Obtain the average value of the times in the vd list (or what you have changed it to), **Avd**, and use the equation below to get exp time:

exp time: (Avd+d1+aq) × ns × td2 × td1

example → **d1**=2 **aq**=0.2 **ns**=8 **td2**=64 **td1=NBL=7**

hsqcC13t1: **Avd** = (0.1+0.25+0.4+0.8+1.4+2.2+3.5)/7 = 1.1

exp time: (1.1+2+0.2)×8×64×7 = 11827 s = 3.3 hr [expt=2.2]

B. Data Processing:

When normal, direct-observe T_1 datasets are acquired, a series of 1D spectra are taken as a "pseudo-2D" experiment. All this means is that the 1D data are saved in the 2D format. A normal 2D is Fourier Transformed using **xfb**, which 1st FTs along t_2, and then along t_1. Pseudo-2D datasets are transformed using **xf2**, which only does the 1st FT along t_2. This leaves a set of 1D spectra, packed into a 2rr file. The peak(s) of interest is integrated in each 1D spectrum and the plotted and fit versus a relaxation delay to an exponential decay provides T_1.

The acquisition of a series of 2D hsqc spectra provides a direct pseudo-3D analog of the above. ftnd would FT all three dimensions of a 3D, but here we want to only get a series of 2D spectra. **tf3** will be 1st, transforming the t_3 (^1H) dimension. It is followed by **tf2**, which then transforms t_2, the ^{13}C dimension. Volume integrals of peaks through the stack of 2D spectra plotted and fit versus the relaxation delay again gives T_1.

1. Phase

• To phase the 2D spectra type **xfb**, select plane orientation 23 and plane 7 (last experiment). Save this in a different proc number (e.g. 999). You will see the raw 2D spectrum in this proc number.

• Phase the spectrum, make sure peaks are positive.

• Use the middle mouse button to adjust intensity so you can see all the peaks and minimize the noise. Right click and choose "edit contour levels" → apply, → ok.

• Under the processing flow bar, choose adjust phase (or type **.ph**). Right click on 2 to 3 peaks at different sides of the spectrum and "add" them.

• Use R button to phase the rows as you do in 1D spectra. Save the phase. Then process the columns C in a similar way. Save for 3D spectrum 3D and return. This will apply the same phase to all the 2D spectra in your pseudo 3D dataset.

• Return to the 3D dataset (or **.3d**).

2. Fourier Transform (+zerofill+apodization+linear_predict)

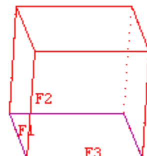
• Type **tf3**↵ and then **tf2**↵ to process these dimensions along the 3D dataset. **tf#** is the equivalent of efp, where zerofill, apodization, phasing, and linear prediction are completed as it's told in the PROCPARS panel. It is critical that you do not zerofill in F3, 1 zerofill in F2, and no zerofills in F1: i.e.,

SI3=TD3 SI2=2*TD2 SI1=TD1 (see staff if this is confusing you!)

Below is a common setup.

TD	2048	64	7	Size of fid
SI	2048	128	8	Size of real spectrum

• The 3D dataset is processed if you can see the cube in the 2D spectrum.



• Click on 12 23 31 to display the correct plane (23, or F2-F3). Your 2D spectrum should show again, but now with a "normal" hsqc appearance.

3. Dynamics Center

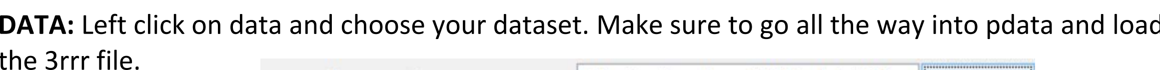
• In the ANALYSE flow bar, click the DYNAMICS CENTER on the dynamics tab (or type **dync**). This will open a separate window .

Method Center
Dynamics
T1
Sample
Data
Analysis
View
Report
Export
T2
T1 rho
Diffusion

• Expand the method tree by left clicking on the + sign next to T1. To complete the T1 analysis complete the components from top to bottom.

SAMPLE: Left click on sample and enter a description. Once you ok this, you can see a green square next to the sample.

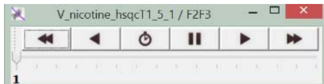
DATA: Left click on data and choose your dataset. Make sure to go all the way into pdata and load the 3rrr file.



• Select spectra type "pseudo 3D (N planes)" and ok.

• One of your 2D spectra (F2F3 plane of your 3D set) will open in the dynamics center window.

• You can use the second window that opens to move between the different 2D spectra.

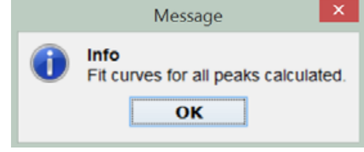


• Use the middle mouse wheel to adjust the intensity and check the automatic peak pick. You can zoom in by drawing a rectangle and double click to zoom out again. Rick clicking on a peak will allow to annotate, move or delete peaks.

• Choose between using Peaks or Integrals using the tabs in the Data panel. Under Integrals, you can choose "use peak are (user defined) integrals to obtain the most flexibility. The 2D spectrum will be applied, and integral boxed can be resize, or added or deleted by clicking the right mouse button on them.

ANALYSIS: Open the analysis method and choose $f(t) = I_0 * e^{-t/T1}$ (first option) as T1 fit function.

• Select error estimation method as "error estimation by weighted fit" and ok.



• The following message should appear on the screen. Click on Ok, then right click on the view method in the tree. If there is an error in the peak calculation, check your fitting method!

• Under the 2D plot tab, remove the tick for "display 2D plot based on fit" and ok.

• Hovering over different peaks will now pull up the fit for this peak in all spectra in a separate window.

• Click on report and make sure to put the correct path and name for the file you would like to save. This will save the report in pdf format.

1. KayLE etal - Pulse seq for heteronuc T1+T2 - JMR 97 (1992) 359-375; YamazakiT+MuhandiramR+KayLE - NMR for 13C relaxa in proteins - JACS 116 (1994) 8266-8278; FarrowNA etal (KayLE) - Backbone dyn of by 15N relaxa - Biochem 33 (1994) 5984-6003.