Jan 13, 2025

T2_15N_HSQC.nan

DOI
https://dx.doi.org/10.17504/protocols.io.n2bvjn35pgk5/v1

NAN KB[1], Alex Eletsky[2], John Glushka[2]

[1]Network for Advanced NMR (NAN); [2]University of Georgia

NAN-KB UGA
Network for Advanced NMR

DOI: https://dx.doi.org/10.17504/protocols.io.n2bvjn35pgk5/v1

Protocol Citation: NAN KB, Alex Eletsky, John Glushka 2025. T2_15N_HSQC.nan. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvjn35pgk5/v1

Protocol status: Working
We use this protocol and it's working

Created: May 29, 2024

Last Modified: January 13, 2025

Protocol Integer ID: 100787

Keywords: protein nmr, assignment, backbone, amides, 15N, hsqc, t2 hsqc experiment with cpmg spin, sampling t2 relaxation delay, larger systems 15n t2 trosy, t2 hsqc, t2 relaxation delay, systems 15n t2 trosy, t1 hsqc, protein backbone dynamics on the picosecond, hsqc experiment, t2 delay, required isotope labeling, isotope labeling, topspin library pulseprograms hsqct2etf3gpsitc3d, t2, studying protein backbone dynamic, t2 trosy, t1, 15n, protein backbone dynamic, nanosecond scale, picosecond, nanosecond scale in combination, temperature compensation, same total rf power for each t2 delay

Funders Acknowledgements:
NSF
Grant ID: 1946970

Disclaimer

This protocol is part of the knowledge base content of NAN: The Network for Advanced NMR (**https://usnan.nmrhub.org/**)
Specific filenames, paths, and parameter values apply to spectrometers in the NMR facility of the Complex Carbohydrate Research Center (CCRC) at the University of Georgia.

Abstract

This protocol describes running ^{15}N T_2 HSQC experiment with CPMG spin-lock, temperature compensation, sensitivity enhancement, gradient coherence selection, water flip-back, and optional ^{13}C decoupling. This is a pseudo-3D experiment, with the third dimension F1(T2) sampling T_2 relaxation delays, which are defined by a loop counter list provided in a separate text file. Temperature compensation involves application of pulses prior to recycle delay delivering the same total RF power for each T_2 delay.

This experiment is primarily used for studying protein backbone dynamics on the picosecond to nanosecond scale in combination with ^{15}N T_1 HSQC, $\{^1H\}$-^{15}N HNOE, etc.

Required isotope labeling: U-^{15}N, or U-^{15}N,^{13}C (with or without ^2H). Also suitable for samples with selective ("sparse") ^{15}N-labeling of certain amino acid types.

Optimal MW is ≤ 30 kDa. For larger systems ^{15}N T_2 TROSY may be more appropriate.

This protocol uses the Topspin library pulseprograms *hsqct2etf3gpsitc3d* (long CPMG cycle) or *hsqct2etf3gpsitc3d.2* (short CPMG cycle).

Attachments

PDF

biotop.pdf

2.5MB

Guidelines

The number of directly acquired points (**2 TD**) should be set so the acquisition time $t_{3,max}$ (**3 AQ**) is between ~50 ms (for larger proteins ~25 kDa) and ~120 ms (for smaller proteins). Longer times may cause excessive probe and sample heating during ^{15}N decoupling, and resolve undesirable $^{3}J_{HN,HA}$ splittings.

"Effective" $^{1}J_{NH}$ coupling value **CNST4** determines the length of the INEPT transfer delays. For larger proteins **CNST4** can be increased (i.e. > 92 Hz) to reduce losses due to relaxation. **CNST4** can be optimized by arraying using **popt** with ^{15}N HSQC experiment in 1D mode.

For samples with ^{13}C labeling use **-DLABEL_CN** ZGOPTNS flag to enable ^{13}C decoupling during ^{15}N evolution. ^{13}C channel offset **O2P** should be set ~110 ppm (middle of ^{13}C aliphatic and ^{13}CO shift range).

Since ^{15}N T_2 HSQC is a quantitative experiment, and the CPMG spin-lock generates significant probe heating, it is recommended to use longer **D1** recycle delays (> 2s) and a sufficient number of dummy scans **DS**. ^{1}H spectral width **3 SW** should be set a few ppm wider than required by the peak dispersion to allow for more "empty" noise regions for better baseline correction during FT processing.

Exact values of T_2 relaxation delays are equal to loop counter value (from **VCLIST**) times CPMG loop length. The latter value is stored as **D31** delay, computed internally by the pulseprogram and cannot be set directly by the user. The exact value of CPMG loop length **D31** depends on the **P30** CPMG 180° ^{15}N pulse length, and should be approximately 16 ms or 8 ms for long CMPG loop (*hsqct2etf3gpsitc3d*) and short CMPG loop (*hsqct2etf3gpsitc3d.2*) pulse sequences, respectively.

For optimal fitting precision, use at least ~8 T_2 points with the longest delay should be at least as long at the expected T_2 relaxation time. This may vary from protein to protein, as T_2 decreases with MW. For safe operating limits regrading **P30** and **PLW23**/**PLdB23** power levels check the Bruker "Typical Pulses" manual for the particular probe installed with your instrument, or consult your NMR facility manager.

Before start

A sample must be inserted in the magnet either locally by the user after training, or by facility staff if running remotely.

This protocol requires a sample is locked, tuned/matched on 1H, 13C and 15N channels, and shimmed. At a minimum, 1H 90° pulse width and offset O1 should be calibrated and a 1D proton spectrum with water suppression has been collected according to the protocol **PRESAT_bio.nan**. Prior acquisition of a **2D 15N HSQC** is also recommended, according to protocol **HSQC_15N.nan.**

General aspects of the use of the **bioTop** module are described in more detail in the protocols listed below.

It is recommended to calibrate 1H carrier offset, 1H H2O selective flip-back pulse, as well as 1H, 13C, and 15N 90° pulse widths using the "Optimization" tab of BioTop. Alternatively, 1H 90° pulse width and offset can be calibrated using other methods, such as pulsecal or calibo1p1. Additional parameters, like 15N and 13C offsets and spectral width can be either optimized or manually entered in the "Optimization" tab of BioTop. Note that since BioTop optimizations are saved in the dataset folder, all experiments should be created under the same dataset name when using BioTop for acquisition setup.

Refer to protocols

1. Acquisition Setup Workflow, Solution NMR Structural Biology
2. PRESAT_bio.nan
3. HSQC_15N.nan
4. HNCO_3D.nan
5. Biotop-Calibration and Acquisition setup

Create T2_15N_HSQC experiment file

1 Start with existing dataset containing 1D 1H or 15N HSQC data for this sample.

1.1 Open create dataset window with **edc** command or through menu.
Edit text in Title box.

1.2 To select starting parameter set, check 'Read parameterset' box, and click Select.
For standard NAN parameter sets, change the Source directory at upper right corner of the window:
Source = /opt/NAN_SB/par
Click 'Select' to bring up list of parameter sets.
Select **T2_15N_HSQC_SHORT_xxx.par** or **T2_15N_HSQC_LONG_xxx.par**, where xxx=900,800 or 600.
Click OK at bottom of window to create the new EXPNO directory.

1.3 If not previously done, tune Nitrogen and Carbon channels. Return to the 'Acquire' menu and click 'Tune' (or type **atma** or **atmm** on command line). Re-shim after tuning.

Load pulse calibrations: use getprosol (step 2.1) or bioTop (steps 2.2)

2

Note

Loading the T2_15N_HSQC_short/long_xxx.par parameter set enters the default parameters into the experiment directory. While they represent a good starting point, they may not be fully optimal or accurate for your particular sample or spectrometer hardware. The probe- and solvent-specific parameters, specifically the 1H 90° pulse length, and possibly the 13C and 15N 90° pulse lengths, along with other dependent pulse widths and powers may need to be updated.

There are two ways of automatically updating experimental parameters:
1) Use **getprosol** command, which typically only updates proton pulse widths and power levels. It is most useful for running routine experiments using the default parameters.
2) Or use **bioTop** module that organizes calibrated and defined parameters for a dataset.

2.1 Loading pulse widths and power levels with **getprosol**:

Use the calibrated proton P1 value obtained from the proton experiment (protocol PRESAT_bio.nan) and note the standard power level attenuation in dB for P1 (PLW1);

otherwise type calibo1p1 and wait till finished.

Then execute the getprosol command:
getprosol 1H [calibrated P1 value] [power level for P1]
e.g. getprosol 1H 9.9 -13.14.
Where for example, the calibrated P1=9.9 at power level -13.14 dB attenuation

This also loads ^{15}N and ^{13}C pulse widths and power levels from the PROSOL table, and are assumed to be sufficiently accurate.

go to step #2.3 If not using BioTop

2.2 Loading experimental parameters from **BioTop**:

If you previously performed parameter calibrations using the "Optimizations" tab of the BioTop GUI, or entered parameters manually in the "Optimizations" tab, you can type **btprep** at the command line.

See protocol 'BioTop: calibration and acquisition setup' and attached Bruker manual 'biotop.pdf' for details.

Inspect and adjust parameters

2.3 Examine parameters by typing **'eda'**, or select the 'Acqpars' tab and get the 'eda' window by clicking on the '**A**' icon. This view shows the three dimensions, F3(1H), and F2(15N) and F1(T2) in columns.

Parameters to check:

- **DS** - 32-128 'dummy' scans that are not recorded; allows system to reach steady state equilibration.
- **NS** - multiple of 8; increase for increased signal to noise (S/N increases as √NS)
- **3 SW** - ^{1}H spectral width (~12-15 ppm, defined in BioTop)
- **2 SW** - ^{15}N amide spectral width (~25-40 ppm, defined in BioTop)
- **O1** - ^{1}H H_2O offset in Hz (calibrated with BioTop or calibo1p1)
- **O2P** - Not used. ^{13}C offset set internally to the average of **CNST21** and **CNST22** (for ^{13}C decoupling with **ZGOPTNS -DLABEL_CN**)
- **O3P** - ^{15}N amide offset (~115-120 ppm, defined in bioTop)
- **3 TD** - Number of ^{1}H time domain real points (~1024-2048, preferably 2^N, keep **3 AQ** at ~50-120 ms)
- **2 TD** - Number of ^{15}N time domain real points (keep 2 AQ at ~30-60 ms)
- **1 TD** - Number of T_2 delay points (should match number of lines in **VCLIST**)

- **DIGMOD** - 'baseopt' (zero 1st order phase correction in ^{1}H)

2.4 Then examine the parameters in Acqpars 'ased' mode (click 'pulse' icon), or type **ased**. Most of the default parameters should be appropriate, however it's useful to compare values in the fields against those proposed by the original parameter file and the pulseprogram comments.

- **CNST4** - one-bond $^{1}J_{NH}$ coupling effective value (≥93 Hz); used to calculate INEPT transfer delays.
- **CNST11** - multiplicity factor; 4 - optimized for NH (default), or 8 - optimized for NH/NH_2
- **CNST12** - multiplicity factor; 4 - optimized for NH (default), or 8 - optimized for NH/NH_2
- **D1** - recyle delay (≥2s for protonated samples, ≥ 3s for perdeuterated samples)
- **D31** - length of single CPMG loop (~16 ms for *hsqct2etf3gpsitc3d*, or ~8 ms *hsqct2etf3gpsitc3d.2*); computed internally by the pulse program and cannot be changed; used to calculate exact values of T_2 delays
- **VCLIST:** Loop counter file for T_2 delays. Click on dots button '**...**' to select, or on '**E**' to edit. Length of each CMPG loop is given by **D31**; use at least ~8 points. The longest T_2 dealy should not exceed 250 ms.

ZGOPTNS flags

- **-DLABEL_CN** - enable ^{13}C decoupling during ^{15}N evolution for $^{13}C,^{15}N$-labeled samples (default)

channel f1:

- **P1** - ^{1}H 90º high power pulse (calibrated with calibo1p1 or BioTop)
- **SPdB1** - power level [dB] for ^{1}H H_2O flip-back shaped pulse (calibrated with BioTop)

Channel f2 (only for **ZGOPTNS -DLABEL_CN**):

- **CNST21** - 13CO offset (~173 ppm, calibrated with BioTop) for ^{13}C decoupling
- **CNST22** - 13CA offset (~53 ppm, calibrated with BioTop) for ^{13}C decoupling

channel f3:

- **P21** - ^{15}N 90º high power pulse (calibrated with BioTop) at **PLW3**/**PLdB3**
- **P30** - ^{15}N 180º pulse for CPMG spin-lock at **PLW23**/**PLdB23,** reduced power (defined in PROSOL). Should not be shorter than 80 us on most probes (90 us on 1.7 mm probes)!
- **PLW23**/**PLdB23** - power level for **P30** ^{15}N 180º pulse for CPMG spin-lock (calculated automatically with **getprosol** or **btprep**). Should not exceed **PLW3**/**PLdB3** power!

Note

^{15}N CPMG spin lock is the main source of probe/sample heating. Excessive heating may lead to poor quality spectra and in extreme cases cause probe damage. For safe operating limits regrading **P30** and **PLW23**/**PLdB23** check the Bruker "Typical Pulses" manual for the particular probe installed with your instrument, or consult your NMR facility manager.

To minimize sample/probe heating set the longest T_2 delay (largest **VCLIST** counter) as short as possible while allowing sufficient precision of T_2 decay fit. Use a large number of dummy scans (**DS** ≥128) for temperature equilibration. Enable autoshim to compensate for shim drift due to probe heating.

If probe heating is still an issue, you can also set longer **P30** pulse length (~100 us) than configured in PROSOL table. It should not be excessively long, or the refocusing performance would be reduced, especially at high magnetic fields. Recalculate **PLW23** power in watts according to $PLW23 = PLW3\left(\frac{P30}{2 \cdot P21}\right)^2$.

Acquire and Process Data

3 Type **'expt'** to calculate the expected run time

go to step #2.3 If necessary to re-adjust parameters

Type '**rga'** or click on 'Gain' in Topspin Acquire menu to execute automatic gain adjustment.
Type '**zg**' or click on 'Run' in Topspin Acquire menu to begin acquisition.

3.1 You can always check the first FID by typing **efp** to execute an exponential multiplied Fourier transform. It will ask for a FID #, choose the default #1. You can evaluate the 1D spectrum for amide proton signal to noise and water suppression.

3.2 2D F2(15N)-F3(1H) and F1(T2)-F3(1H) planes can be processed with Topspin commands **xfb** and **xf2,** respectively.

Protocol references

J.Cavanaugh, W.Fairbrother, A.Palmer, N.Skelton: Protein NMR Spectroscopy: Principles and Practice. Academic Press 2006 ; Hardback ISBN: 97801216449189, eBook ISBN: 9780080471037