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 T1_15N_HSQC.nan

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We use this protocol and it's working

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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_1 HSQC experiments with 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 $F1(T_1)$ sampling a list of T_1 relaxation delays provided in a separate text file. ^1H BIP shaped 180° pulses are used to suppress cross-relaxation and cross-correlated relaxation between ^1H and ^{15}N spins during T_1 relaxation delay.

Temperature compensation involves application of pulses prior to recycle delay delivering the same total RF power for each T_1 delay. Total acquisition time thus strongly depends on the maximum T_1 delay.

This experiment is primarily used for studying protein backbone dynamics on the picosecond to nanosecond scale in combination with ^{15}N T_2 HSQC, $\{^1\text{H}\}$ - ^{15}N HNOE, etc.

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

Optimal MW is $\leq 30\text{--}35$ kDa. For larger systems ^{15}N T_1 TROSY may be more appropriate.

Field strength preference: detailed analysis of protein backbone dynamics often requires measurement of ^{15}N relaxation rates at multiple fields.

It uses the Topspin library pulseprogram 'hsqct1etf3gpsitc3d'.

Attachments



[biotop.pdf](#)

2.5MB



Guidelines

The number of directly acquired points (**3 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 $^3J_{\text{HN,HA}}$ splittings.

"Effective" $^1J_{\text{NH}}$ 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. **CNST21** and **CNST22** are used to define the ^{13}C channel offset for decoupling.

Since ^{15}N T_1 HSQC is a quantitative experiment, it is recommended to use longer **D1** recycle delays and a sufficient number of dummy scans **DS**. ^1H spectral width **3 SW** should be set a few ppm wider than required by the peak dispersion to allow for "empty" noise regions for proper baseline correction during FT processing.

T_1 relaxation delays should be multiples of 10 ms, and should include ~8 points or more. The shortest delay should be ≥ 20 ms, to avoid loop counter being less than 1. For optimal fitting precision, the longest delay should be at least as long as the expected T_1 relaxation time (usually in the range of 1-3 s). This may vary from protein to protein, as T_1 increases with MW. Due to temperature compensation, the longest delay also determines the overall experiment time, thus it should not be set excessively long.



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 , ^{13}C and ^{15}N 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 ^{15}N 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 H_2O selective flip-back pulse, as well as ^1H , ^{13}C , and ^{15}N 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 ^{15}N and ^{13}C 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 T1_15N_HSQC experiment

- 1 Start with existing dataset containing 1D proton 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 T1_15N_HSQC_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)

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Note

Loading the T1_15N_HSQC_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 ^{13}C and ^{15}N 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), F2(15N), and F1(T1) 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 $\sqrt{\text{NS}}$)
- **3 SW** - ^1H spectral width (~12-15 ppm, defined in BioTop)
- **2 SW** - ^{15}N amide spectral width (~25-40 ppm, defined in BioTop)
- **O1** - ^1H H₂O 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 ^1H 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 1 AQ at ~30-60 ms)
- **1 TD** - Number of T_1 delay points (should match number of lines in **VDLIST**)
- **DIGMOD** - 'baseopt' (zero 1st order phase correction in ^1H)

- 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.

General:

- **CNST4** - one-bond $^1J_{\text{NH}}$ coupling effective value (≥ 93 Hz); used to calculate INEPT transfer delays.
- **CNST11** - multiplicity factor; 4 - optimized for NH (default), or 8 - optimized for NH/NH₂
- **CNST12** - multiplicity factor; 4 - optimized for NH (default), or 8 - optimized for NH/NH₂
- **D1** - recycle delay (~2 s for protonated samples, ~3 s for perdeuterated samples). Usually set high for quantitative/relaxation experiments
- **VDLIST** - T₁ delay list file. Click on dots button '...' to select, or on 'E' to edit. Each delay must be a multiple of 10 ms; use at least 8 points. The longest delay should be at least as long as the expected T₁ relaxation time (~1-3 s, may need to be adjusted depending on protein MW). Also determines the duration of temperature compensation.

ZGOPTNS flags

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

Channel f1:

- **P1** - ^1H 90° high power pulse (calibrated with calibo1p1 or BioTop)
- **SPdB1** - power level [dB] for ^1H H₂O flip-back shaped pulse (calibrated with BioTop)

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

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

Channel f3:

- **P21** - ^{15}N 90° high power pulse (calibrated with BioTop)

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(T1)-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