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HSQC_15N.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 a 2D ^{15}N HSQC pulse sequence with sensitivity enhancement, gradient coherence selection and water flip-back. This produces a 2D phase-sensitive ^{15}N-^{1}H correlation dataset that displays signals for each backbone amide proton-nitrogen pair, as well as -CONH$_2$ side chains of Asn and Gln.

Required isotope labeling: U-^{15}N (with or without ^{13}C, ^{2}H)
Optimal MW is less than 25 kDa; larger systems generally benefit from 2D TROSY at high NMR fields.

This pulse sequence can be used for:

- resonance assignment of backbone and side-chain ^{1}HN and ^{15}N resonances
- spin system identification
- routine sample screening and stability monitoring
- chemical shift perturbation studies due to ligand binding, paramagnetic relaxation or pseudo-contact shifts
- optimization of ^{15}N offset and spectral width
- anchoring of 3D spectra (e.g. HNCO, ^{15}N-edited NOESY/TOCSY, etc.) during interactive visual analysis

It uses the pulseprogram 'hsqcetfpf3gpsi2.nan' (hsqc= heteronuclear single quantum correlation; et=echo-antiecho; fp=water signal flipback; f3=third channel for ^{15}N; gp=gradient pulse; si=sensitivity enhanced) modified from the original Bruker library sequence.

Attachments

PDF

biotop.pdf

2.5MB

Guidelines

The number of directly acquired points (**2 TD**) should be set so the acquisition time $t_{2,max}$ (**2 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.

NUS sampling is usually not required for 2D experiments, since time savings are small, unless running multiple 2D experiments. If using NUS keep sampling amount ~30-50%.

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).

The sampling in ^{15}N dimension is half-dwell by default. Such sampling has the advantage of easier identification of aliased(folded) peaks, since they change sign when aliased an odd number of times. Note that FT processing for half dwell sampling requires 90/-180 phasing (-90/180 in NMRPipe) and first point multiplier of 1.

Zero-dwell sampling in ^{15}N dimension can be enabled with **-DZERODWN** flag in ZGOPTNS. Phasing should then be 0/0 and first point multiplier of 0.5 .

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 ^{1}H, ^{13}C and ^{15}N channels, and shimmed. At a minimum, ^{1}H 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.**

It is recommended to calibrate ^{1}H carrier offset, ^{1}H H_2O selective flip-back pulse, as well as ^{1}H, ^{13}C, and ^{15}N 90° pulse widths using the "Optimization" tab of **BioTop**. Alternatively, ^{1}H 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 widths 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.

Familiarize yourself with the general workflow for NMR study of a protein sample is outlined in protocol "Acquisition Setup Workflow, Solution NMR Structural Biology".

Create 15N HSQC experiment

1 Start with existing Dataset containing 1D PRESAT data in EXPNO 1 collected with protocol **PRESAT_bio.nan**

1.1 Click on Acquire -> 'Create Dataset' button to open dataset entry box or type **edc** command.

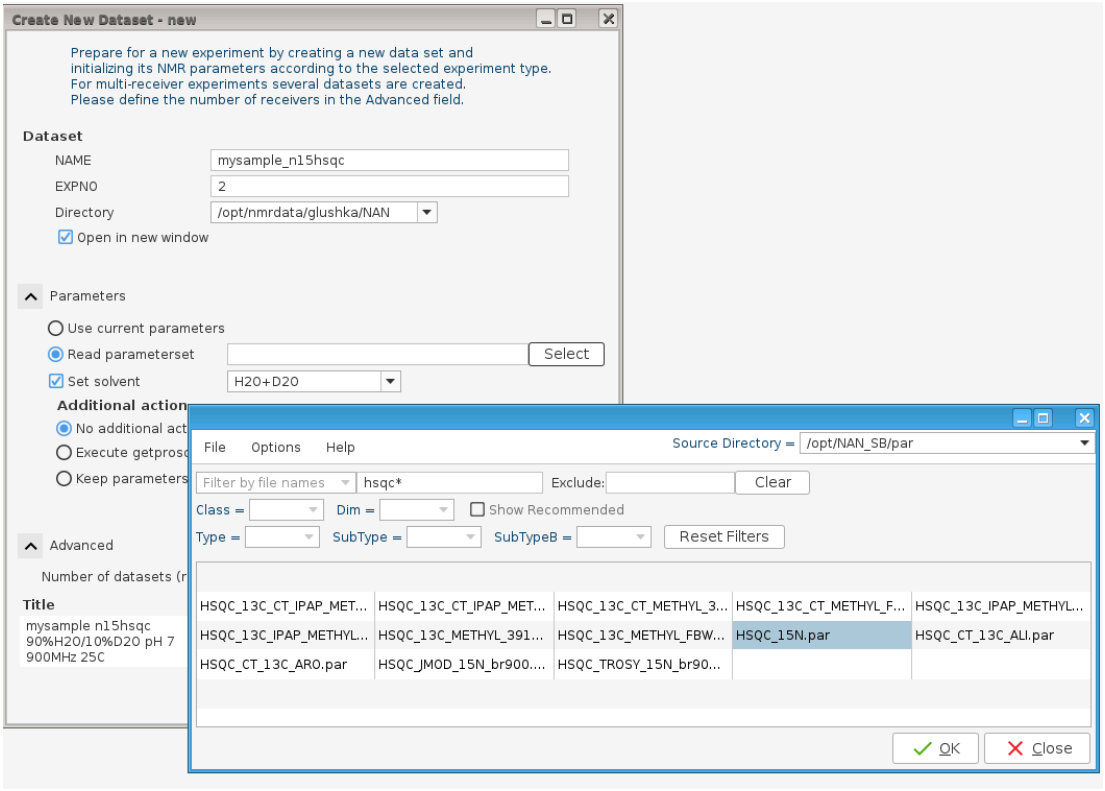
Dataset Name: recommended to keep the same name when using BioTop for optimization and acquisition setup.

The EXPNO is automatically incremented by +1 by default.
Directory should be the same as preliminary 1D.

The Title text box will copied from the previous experiment. Edit to designate the N15-HSQC pulse program and add other details as appropriate.

1.2 Load the 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 HSQC_15N_xxx.par, where xxx=900,800 or 600*.

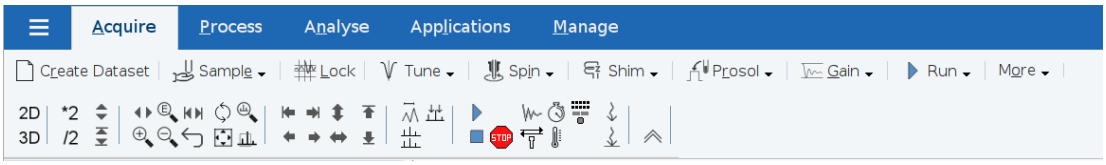


*Parameter names may differ depending on spectrometer

1.3 Click OK at bottom of window to create the new EXPNO directory.
It will be the active experiment in the acquisition window and should now be listed on your data browser.

1.4 **If not done, tune Nitrogen (and Carbon) channels.**

Return to the 'Acquire' menu and click 'Tune' (or type **atma** on command line).



This will start tuning of the nitrogen channel, then the carbon channel, followed by a re-tuning of the proton channel (which should not change).

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

2

Note

Loading the HSQC_N15_xxx.par parameter set enters the default parameters into the experiment directory. While 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 ^{1}H 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.

For example, clicking on the 'Prosol' button in the Acquire menu, or executing the **getprosol** command without arguments will load default values from the pre-configured spectrometer calibration table, including the default ^{1}H 90° pulse length and power level. However, for biological samples in aqueous solvents the optimal ^{1}H 90° pulse length can vary significantly depending on buffer conditions, sample geometry and temperature, and thus needs to be calibrated individually for each sample. ^{13}C and ^{15}N 90° pulse lengths do not typically exhibit large variations, but these can also be calibrated for best results.

There are two ways of automatically updating an entire range of experimental parameters. The first is using **getprosol** command (step 2.1), which only updates pulse widths and power levels without altering other parameters, such as spectral widths and offsets. This method is suitable for reproducing existing experiments or parameters sets with minimal variations.

The second method utilizes the **BioTop** module of TopSpin (step 2.2), and can load additional experimental parameters, such as spectral widths, offsets, and number of time-domain points. These additional parameters are set according to calibrations or definitions within the 'Optimization' tab of the BioTop GUI and the corresponding XML description files (*bt_hsqcetfpf3gpsi2.nan.xml* in this case). This method has a lower dependency on the particular settings of the starting parameter set, and is suitable for setting up experiments from scratch. With this method nearly all important acquisition parameters can be optimized for a particular sample, and then applied consistently to multiple NMR experiments with a single command.

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 attenuation for P1 (PLdB1)]
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 default ^{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 simply type **btprep** at the command line.

This is equivalent to calling **getprosol** with all ^{1}H, ^{13}C and ^{15}N optimized parameters followed by additional parameters loaded from "Optimizations" tab in BioTop GUI. In this particular case these parameters are based on the *bt_hsqcetfpf3gpsi2.nan.xml* description file: ^{1}H offset in Hz (**O1**), ^{1}H spectral width (**2 SW**), power level for ^{1}H sinc water-flipback pulse (**SPdB 1**), ^{15}N offset in ppm (**O3P**), ^{15}N spectral width (**1 SW**), ^{15}N max acquisition time (**1 AQ**), ^{13}C offset in ppm (**O2P**).

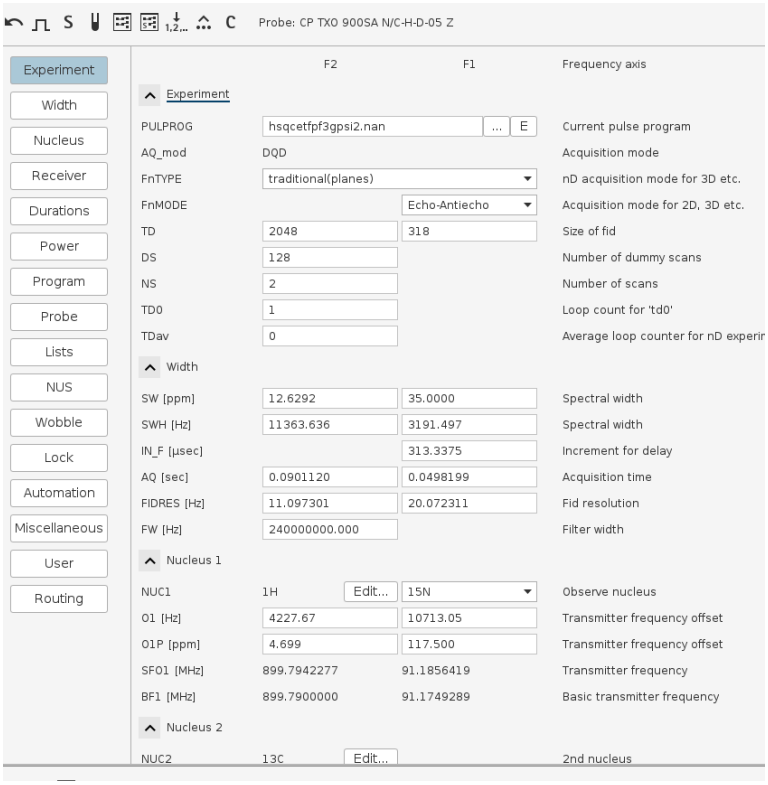
Inspect and adjust parameters

2.3 The default parameters from HSQC_15N_xxx.par will provide an 15N HSQC spectrum of a typical protein sample collected with the traditional sampling scheme (i.e. not using non-uniform sampling 'NUS')

Often the only parameters to change will be **NS = number of scans** in order to increase the signal to noise,
and **1 TD** (TD in F1) that changes the number of increments (points) and hence the resolution in the 15N dimension. Check the experiment time (type **expt**) after any change.
These and additional parameters can be accessed and changed on the parameter windows seen below.

2.4 Select the 'Acqpars' tab to display acquisition parameters. Two display modes can be selected, the full display mode (click on the '**A**' icon or type **eda**), or pulse program-specific mode (click on the 'pulse' icon, or type **ased**). The former gives you access to all parameters and provides an overview of all spectral dimensions at once, while the latter is useful because it only displays acquisition parameters used in the pulse sequence and can be parsed sequentially as a checklist.

First examine the specific dimension parameters in Acqpars 'eda' mode (click 'A' icon):

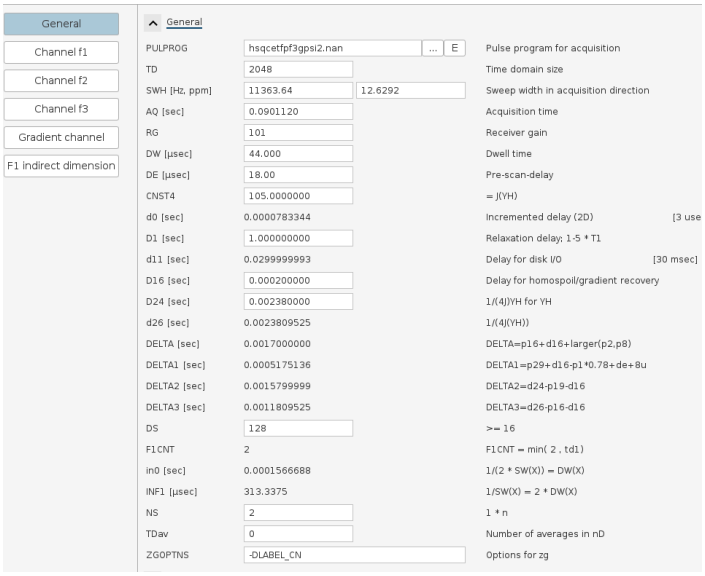


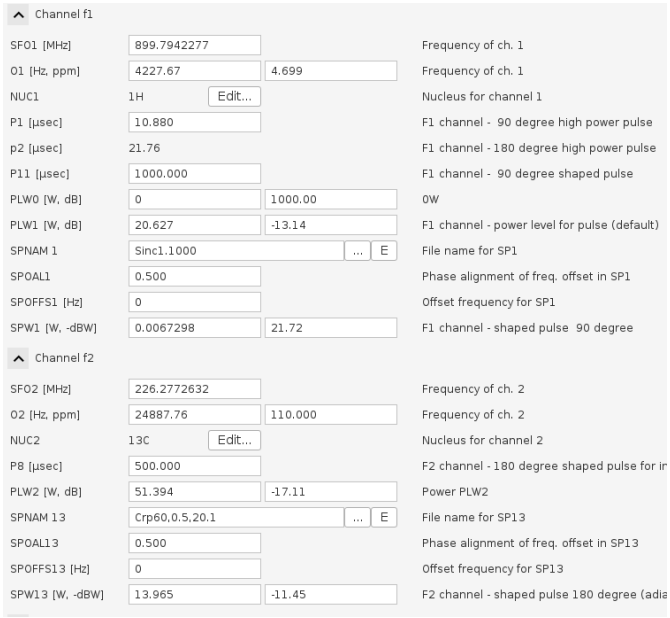
Parameters to check:

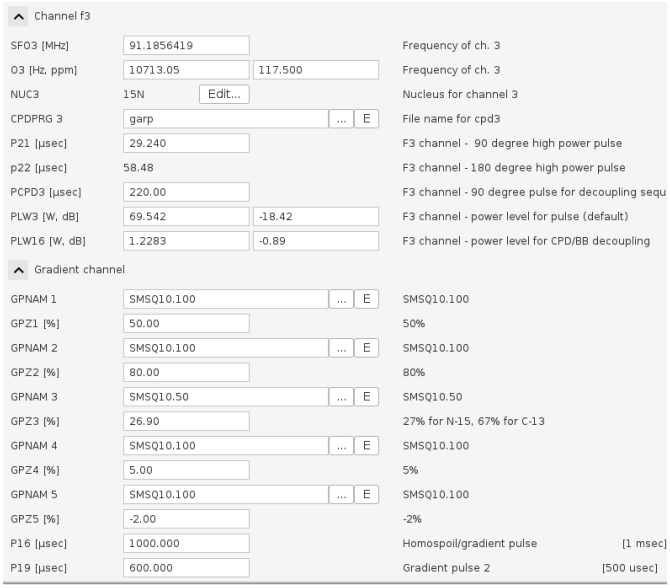
- **SW [ppm]** - F2(1H) ~12-15 ppm; F1(15N) ~25-40 ppm, defined in bioTop
- **O1** - ^{1}H H_2O offset in Hz (calibrated with BioTop or calibo1p1, **O1P** should be around 4.7 ppm depending on temperature)
- **O3P** - ^{15}N amide offset (~115-120 ppm, defined in bioTop)
- **O2P** - ^{13}C offset (~110 ppm, middle of ^{13}C aliphatic and ^{13}CO range, for decoupling with ZGOPTNS -DLABEL_CN)
- **2 TD** - Number of ^{1}H time domain real points (~1024-2048, preferably 2^N, keep **2 AQ** at ~50-120 ms)
- **1 TD** - Number of ^{15}N time domain real points (keep **1 AQ** at ~30-60 ms)
- **NS** - minimum 2; increase for for higher signal to noise (S/N increases as square root of NS)
- **DS** - 32-128 'dummy' scans that are not recorded; allows system to reach steady state equilibration. This is especially important for HSQC since ^{15}N decoupling during acquisition and can heat the probe and sample.
- **DIGMOD** - 'baseopt' (zero 1st order phase correction in ^{1}H)

2.5 Then examine the parameters in the pulse program-specific '**ased**' mode (click on the 'pulse' icon). Most parameters are also accessible in the '**eda**' mode (step 2.4 above). However, the '**ased**' mode allows more convenient access to individual parameters within arrays, such as

delays, pulse widths, constants, etc. It also displays parameter values computed internally within a pulse sequence, and provides context description from the relevant pulse program comment lines.







Most of the default parameters should be appropriate, however it's useful to compare values in the fields against suggestions in the pulseprogram comments. In general, only a few may need to be changed.

- **CNST4** - effective one-bond $^{1}J_{NH}$ coupling value (≥93 Hz); used to calculate INEPT transfer delays. For high MW proteins, **CNST4** can be increased to yield shorter transfer delays and higher S/N.
- **D1** - recycle delay (~1 s for protonated samples, ~2-3 s for perdeuterated samples)
- **P1** - ^{1}H 90º high power pulse (calibrated with calibo1p1 or BioTop)
- **P3** - ^{13}C 90º high power pulse (calibrated with BioTop)
- **P21** - ^{15}N 90º high power pulse (calibrated with BioTop)
- **SPdB1** - power level [dB] for ^{1}H H_2O flip-back shaped pulse (calibrated with BioTop)
- **D24** - refocused INEPT transfer delay (<0.0027 s). Typically set to match **D26** or 1/(4***CNST4**), optimizing for backbone N-H at the expense of $CONH_2$
- **ZGOPTNS flags: -DLABEL_CN** by default. This assumes a double labeled ^{13}C,^{15}N protein, and enables ^{13}C decoupling during ^{15}N evolution.

Configure NUS (non-uniform sampling) - optional

3 After all other acquisition parameters (especially spectral widths and time-domain points) are set, change the **FnTYPE** parameter to 'non-uniform sampling' (type '**eda**' and select 'Experimental' to get correct parameter window).

Navigate down to the 'NUS' section in the 'eda' parameter window and set the desired **NusAMOUNT** [%] sampling density. For 2D ^{15}N HSQC sampling density can be around 30-50%.

You have the option of using either the built-in sampling schedule generator in Topspin or a third-party one. To use the built-in sampling schedule generator in TopSpin set the **NUSLIST** parameter to 'automatic'. The sampling schedule will then be generated at acquisition start, and will be purely random apart from point density weighting according to **NusJSP** and **NusT2** parameters.

A better way to generate the sampling schedule is with **nusPGSv8** AU program. This AU program uses **NusAMOUNT** and **TD** values of the current experiment to generate a random schedule with 'Poisson gap' point spacing, and offers additional options for point density weighting and sampling order. (see protocol **'**Poisson Gap NUS Acquisition Setup', and attached files **'nusPGSv8'** and **'poissonv3'**). To use this method, type **'nusPGSv8'** on the command line. You can typically accept the default values in pop-up dialog windows, since they are suitable for most applications. A schedule will be generated and will be stored to the parameter **NUSLIST**.

If **nusPGSv8** is not installed, copy the attached file 'nusPGSv8' to your user AU directory, */opt/topspin.X.X.X/exp/stan/nmr/au/src/user*, and copy the binary file 'poissonv3' to */opt/topspinX.X.X/prog/bin.*

Acquire and Process Data

4 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.

4.1 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.

To take a look at the 2D, wait for >= 16 FIDs and then click on 'Proc.Spectrum' on the Topspin Process menu. This will execute an automated processing macro. Although the resolution will be poor, you can evaluate the signal to noise (S/N) and whether the ^{15}N offset and spectral width are appropriate.

This can be repeated at any time as additional FIDs are acquired.

Protocol references

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