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 N_HETCOR.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 nitrogen detected HETCOR (inept) pulse sequence, which provides correlations between amide nitrogens and protons. It is complementary to ^{15}N HSQC and is best for high MW proteins and complexes.

^{15}N labeling is required, ^{13}C , ^2H labeling is optional.

It uses the pulseprogram 'n_hninept_f2ig.nan', modified from the original topspin library version.

Attachments



[biotop.pdf](#)

2.5MB



[NUS_poisson_gap_file...](#)

12KB



Guidelines

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%.

Due to poor sensitivity of ^{15}N , a suitable probe such as Bruker TXO is recommended.

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 to be 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**. If possible, 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 N_HETCOR experiment file

- 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 N_HETCOR_xxx.par, where xxx=900 or 600.
Click OK at bottom of window to create the new EXPNO directory.
- 1.3 If not done, tune Nitrogen and Carbon channels:
-on the Acquire menu, click Tune (or type atma on command line).

Calibrate pulses and adjust parameters

2

Note

Loading the N_HETCOR_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 Using 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 15N and 13C pulse widths and power levels from the 'edprosol' table, and are assumed to be sufficiently accurate.

Skip to step 2.3.**2.2 Use 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 parameters' and attached Bruker manual 'biotop.pdf' for details.

Inspect 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 two dimensions, F2 (15N) and F1 (1H) in columns.

Parameters to check:

2 TD: Number of ¹⁵N time domain real points (~512-2048, preferably 2^N, keep **2 AQ** <200 ms)

1 TD: Number of ¹H time domain real points (**1 AQ** ~35 ms, limited by ¹J_{HN,HA} coupling)

2 SW: ¹⁵N spectral width (~50 ppm, ~80 ppm if Arg HE/NE peaks are desired)

1 SW: ¹HN spectral width (~5 ppm, defined in **BioTop**)

O1P: ¹⁵N offset (~117 ppm, ~107 ppm if Arg HE/NE peaks are desired)

O2P: ¹³CO/CA offset (~110 ppm)

O3P: ¹HN offset (~8 ppm, defined in **BioTop**)

- 2.4** Then examine the parameters in Acqpars 'ased' mode (click 'pulse' icon), or **type 'ased'**;

Most of the default parameters are suitable, however it's useful to compare values in the fields against those proposed by the original parameter file and the pulseprogram.

CNST4 - ¹J_{NH} value (≥100 Hz), to calculate INEPT transfer delays

CNST11 - multiplicity factor (6 - NH,NH₂; 5 - NH only; 4 - NH,NH₂ with opposing signs)



D1: recycle delay (~1 s for protonated samples, ~2-3 s for perdeuterated samples)
DS: 32-128 'dummy' scans that are not recorded ; allows system to reach steady state equilibration.
NS: multiple of 4; increase for increased signal to noise (S/N increases as $\sqrt{NS}$)

ZGOPTNS flags:

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

channel f1:

P1 - ^{15}N 90° high power pulse (calibrated with **BioTop**)

channel f2:

- **CPDPRG2** - bi_p5m4sp_4sp.2 (adiabatic bilevel decoupling)

channel f3:

CPDPRG3 - ^1H decoupling (garp)

Acquire and Process Data

- 3 Type '**rga**' or click on 'Gain' in Topspin Acquire menu to execute automatic gain adjustment.
Type '**expt**' to calculate the expected run time
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 planes with NUS or uniformly sampled can be processed with Topspin: **xfb**

Protocol references

'nusPGSv8' written by Scott Anthony Robson 2013