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 **HSQC_JMOD_15N_3D.nan**

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Protocol status: Working

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 pseudo-3D J-modulated ^{15}N HSQC for measuring backbone $^1\text{J}_{\text{H,N}}$ scalar and $^1\text{D}_{\text{H,N}}$ dipolar couplings.

This pulse sequence can be used for measurement of ^1H - ^{15}N residual dipolar couplings (RDCs) in large proteins ^{15}N isotope labeling is required; can also be applied to proteins with additional ^{13}C and ^2H labeling.

It uses the custom pulseprogram 'hsqcetfpf3jcgpsi2.nan' derived from the standard Topspin library version.

Attachments



[biotop.pdf](#)

2.5MB

Guidelines

Number of directly acquired points (2 TD) should be set to keep acquisition time $t_{2,max}$ (2 AQ) ~50-120 ms. For large proteins (~25 kDa) set closer to the lower range, higher values for small proteins.

For samples with ^{13}C labeling use -DLABEL_CN 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).

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 HSQC_JMOD_15N_3D 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 HSQC_JMOD_15N_3D_xxx.par, where xxx=900,800 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 HSQC_JMOD_15N_3D_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 (step 2.1), 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 (1H) and F2 (15N) in columns.

Parameters to check:

3 SW: 1H spectral width (~12-15 ppm, defined in BioTop)

2 SW: 15N amide spectral width (~25-40 ppm, defined in BioTop)

O1P: 1H offset defaults to ~4.7ppm (calibrated with bioTop)

O2P: 13C offset (~110 ppm, for decoupling when **ZGOPTNS -DLABEL_CN**)

O3P: 15N 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 **2 AQ** at ~30-60 ms)

1 TD: Number of delays in **VDLIST**

- 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: $^1\text{J}_{\text{NH}}$ value (≥ 93 Hz, to calculate INEPT transfer delays, can be optimized using **popt** in 1D mode to maximize S/N)

D1: recycle delay (~1 s for protonated samples, ~2-3 s for perdeuterated samples)

D24: $1/(4 \cdot \text{J}_{\text{NH}})$, normally set to same value as D28



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 $^{13}\text{C},^{15}\text{N}$ -labeled samples (default)

VDLIST: J-coupling evolution delay list. Delays should be no smaller than $(p21 \times 1.273 + 20\mu)$

P1 - ^1H 90° high power pulse (calibrated with calibo1p1 or BioTop)

SPdB1 - power level [dB] for ^1H H_2O flip-back shaped pulse (calibrated with BioTop)

P3 - ^{13}C 90° high power pulse (calibrated with BioTop)

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

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

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