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NOESYHSQC_15N_3D.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 an ^{15}N-edited 3D NOESY-HSQC pulse sequence with sensitivity enhancement, gradient coherence selection and water flip-back, using either uniform or non-uniform sampling (NUS). This produces a 3D phase-sensitive dataset that provides NOE correlations between N-linked ^{1}H spins and remote ^{1}H spins resolved according to ^{15}N chemical shifts along the third dimension (F2). The N-linked ^{1}H spins typically include backbone amides, side-chain -$CONH_2$ groups of Asn/Gln, and HE1 of Trp rings. In some cases, signals from slow exchanging HE spins of Arg and HD2 or HE1 of His can be observed as well.

This pulse sequence can be used for:

- Aiding in backbone resonance assignment - sequential spin system linking can be validated by matching lineshapes of HA-HN, and HN-HN NOE cross-peaks.
- Side-chain resonance assignment of Asn/Gln -CONH2 groups, Trp HE1/NE1, Arg HE/NE.
- Generating distance restraints for structure calculation based on NOE cross-peak integrals or intensities

Required isotope labeling: U-^{15}N, or U-^{15}N,^{13}C. Also suitable for samples with selective ("sparse") ^{15}N-labeling of certain amino acid types.

Optimal MW is ≤ 30-35 kDa. For larger systems ^{15}N NOESY-TROSY may be more appropriate.

Field strength preference: highest available field strength is preferred for better sensitivity and peak dispersion.

It uses a pulseprogram 'noesyhsqcfpf3gpsi3d.nan' modified from the original Topspin version.

Attachments

PDF biotop.pdf 2.5MB

ZIP NUS_poisson_gap_file... 12KB

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 $^{3}J_{HN,HA}$ splittings.

NOE peak intensities depend on the mixing time. Initially the buildup rate is linear, reaching saturation as the mixing time increases. The buildup rate and saturation onset depend on protein MW. Short mixing times would yield more accurate distance restraints, at the cost of lower S/N and the loss of valuable long-range NOE peaks that are important for structure calculation. Very long NOE mixing times would result in peak intensities being no longer sensitive to internuclear distances. In practice, structure calculation algorithms work well with distance restraints generated even from somewhat saturated NOE data. The NOE mixing time is thus usually set to compromise values, for example, ~100 ms for small ubiquitin-sized proteins (~8 kDa), or ~60-70 ms for 20-25 kDa systems.

Due to large number of expected peaks and high dynamic range (strong diagonal peaks and weak cross-peaks) NUS sampling amount should be ~20-30% provided there is sufficient S/N.

2D F1(Hnoe)-F3(HN) plane can be acquired by setting **2 TD** to 1, and **ZGOPTNS** to "-DZERODWN".

2D F2(15N)-F3(HN) plane can be acquired by setting **1 TD** to 1, and **ZGOPTNS** to "-DZERODWH -DZERO".

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.** Prior acquisition of a 2D ^{15}N HSQC is also recommended, according to protocol **HSQC_15N.nan.**

General aspects of 3D setup and non-uniform sampling (NUS) options and use of the bioTop module are described in more detail in the protocol **HNCO_3D.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 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 15N NOESY-HSQC experiment

1 Join an existing dataset and experiment (e.g. 1D proton, 2D 15N HSQC, etc) for this sample.

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

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

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

2

Note

Loading the NOESYHSQC_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 ^{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.

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

Skip to step 2.3.

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' view by clicking on the '**A**' icon. This view shows the three dimensions, F3(1H), F2(15N) and F1(1H) in columns.

Parameters to check:

- **FnTYPE** - 'traditional planes' or 'non-uniform sampling' (see step 2.5 below)
- **NS** - minimum 2; optimally 4 to suppress axial peaks in both indirect dimensions. Increase for for higher signal to noise (S/N increases as square root of NS)
- **DS** - 32-128 'dummy' scans that are not recorded (multiple of 32); allows system to reach steady state equilibration. Important for HSQC-based experiments due to heating from ^{15}N decoupling during acquisition
- **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)

- **1 SW** - ^{1}H spectral width, indirect (NOE) dimension (usually same as **3 SW**, ~12-15 ppm, defined in BioTop)
- **O1** - ^{1}H H_2O offset in Hz (calibrated with BioTop or calibo1p1)
- **O2P** - ^{13}C aliphatic+aromatic offset (~69 ppm, same as **CNST60**)
- **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 (**2 AQ** ~23 ms)
- **1 TD** - Number of ^{1}H indirect (NOE) dimension time domain real points (**1 AQ** ≤16 ms, limited by J_{HH} couplings)
- **DIGMOD** - 'digital' (pulse sequence does not use *acqt0* correction)

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. For high MW proteins, increase the value of **CNST4** for shorter transfer delays and higher S/N.
- **D1** - 1-2 sec
- **D8** - NOE mixing time (~70-120 ms)
- **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 $CONH_2$
- **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)
- **P3** - ^{13}C 90º high power pulse (calibrated with BioTop)
- **CNST60** - ^{13}C aliphatic+aromatic offset (~69 ppm, same as **O2P**, for ^{13}C decoupling in F1(1H) Hnoe dimension, with ZGOPTNS -DLABEL_CN)
- **CNST61** - ^{13}C CA+CO offset (~110 ppm, for ^{13}C decoupling during ^{15}N evolution, with ZGOPTNS -DLABEL_CN)
- **P21** - ^{15}N 90º high power pulse (calibrated with BioTop)

ZGOPTNS flags:

- **-DLABEL_CN** - enable ^{13}C decoupling during indirect ^{1}H and ^{15}N evolution for ^{13}C,^{15}N-labeled samples (default)
- **-DZERO** - Apply 0º phase shift for initial ^{1}H pulse (instead of 45º default shift). Used for acquiring 2D F3(HN)-F2(15N) HSQC planes (optional)

- **-DZERODWH** - zero-dwell sampling in indirect F1(1H) NOE dimension. Used for acquiring 2D F3(HN)-F2(15N) HSQC planes (optional)
- **-DZERODWN** - zero-dwell sampling in F2(15N) dimension. Used for acquiring 2D F3(HN)-F1(Hnoe) planes (optional)

Configure NUS (non-uniform sampling) - optional

2.5

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).

Then select NUS in the 'eda' parameter winow and set the desired **NusAMOUNT** [%] sampling density.

For adequate reconstruction, number of NUS points should be larger than the number of expected peaks in the N-Hnoe planes. Due to large number of expected peaks and high dynamic range (strong diagonal peaks and weak cross-peaks) NUS sampling amount should be at least ~20-30%.

After this 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

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 3D NUS data usually cannot be processed in Topspin without a separate license (see note in step 2.5). 2D planes with NUS or uniformly sampled can be processed in Topspin, though.

Also, a 2D NUS plane can be extracted from a completed 3D NUS data set with the command '**rser2d**', and then processed within Topspin.

Full 3D NUS processing is usually performed using third-party processing software, such as NMRPipe together with specialized NUS reconstruction programs (SMILE, hmsIST, NESTA, etc.).

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

G.M. Clore (1989) *Biochemistry* **28** 6150-6156. (**Link to Article**)
D. Marion, L.E. Kay, S.W. Sparks, D.A. Torchia and A. Bax (1989) *J. Am. Chem. Soc.* **111** 1515-1517. (**Link to Article**)
E.R.P. Zuiderweg and S.W. Fesik (1989) *Biochemistry* **28** 2387-2391. (**Link to Article**)

'nusPGSv8' written by Scott Anthony Robson 2013