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HNCA_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 a 3D HNCA 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 correlates backbone ^{1}HN and ^{15}N resonances of residue (i) with ^{13}CA of residues (i) and (i-1). The sequential (i-1) ^{13}CA peaks are typically weaker than intra-residue (i) 13CA peaks.

3D HNCA it often optional for backbone resonance assignment. It may be useful for challenging cases, by complementing HNCACB if it has low S/N. HNCA has better sensitivity than HNCACB and HN(CA)CO, though lower than HNCO. However, ^{13}CA shifts alone are rather limited in the ability to differentiate residue types - usually only Gly residues can be distinguished from non-Gly.

Required isotope labeling: U-^{15}N,^{13}C. Samples with additional ^{2}H labeling should use HNCA or TROSY-HNCA versions with ^{2}H decoupling.

Optimal MW is ≤ 25 kDa. Larger systems generally benefit from 3D TROSY-HNCA versions. Compare 2D H-N planes of HNCO and TROSY-HNCO to check which one yields better S/N.

This pulse sequence can be used for:

- spin system identification
- resolving signal degeneracy in 2D ^{15}N HSQC.
- backbone resonance assignment: sequential linking of spins systems based on ^{13}CA peaks matching (in conjunction with 3D CBCA(CO)NH, HN(CO)CA or HN(CO)CACB). Complements sequential linking of spins systems via HN(CA)CO and HNCACB.
- resonance assignment of ^{13}CA spins
- chemical shift-based secondary structure prediction (e.g. TALOS), together with other resonances

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

It uses the standard Bruker Topspin pulseprogram 'hncagp3d'

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.

Since the F2(15N) is a constant-time dimension, number of acquired points **2 TD** should be should be set to the largest possible value for optimal S/N. The maximum **2 TD** value is 2*int(d30/in30), where d30 is the decremented delay, and in30 is the decrement value. Both d30 and in30 are computed internally according to pulse program code and their values are shown in ased view. Note that in30 = in10 = 1(4∗SW(N)). In TopSpin 4.x the ased command would produce a warning if **2 TD** is set too large and reset the internal *td2* parameter to the maximum possible value.

As 3D HNCA only has 2 peaks per residue, NUS sampling amount can be as low as 5-10%, provided the sample delivers sufficient S/N.

Individual F1(CO)-F3(HN) or F2(N)-F3(HN) 2D planes can be acquired by setting, respectively, **2 TD** or **1 TD** to 1.

To check whether TROSY-HNCA or regular HNCA yields better S/N for your sample at a particular field strength, record and inspect the respective 2D F2(N)-F3(HN) planes of TROSY-HNCO or regular HNCO using identical spectral parameters.

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 and 2D ^{13}C HSQC are also recommended, according to protocols **HSQC_15N.nan** and **HSQC_CT_13C_ALI.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. HSQC_CT_13C_ALI.nan
5. HNCO_3D.nan
6. Biotop-Calibration and Acquisition setup

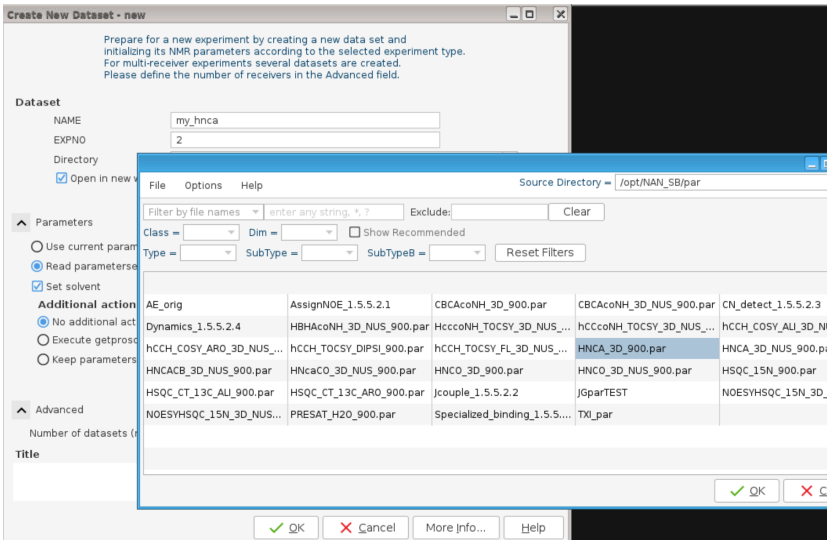
Create HNCA experiment file

1 Join an existing dataset and experiment (e.g. 1D proton, 2D 15N HSQC, 2D 13C 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 HNCA_3D_xxx.par, where xxx=900,800 or 600*.



*parameter file name may be different

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).

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

2

Note

Loading the HNCA_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.

go to step #2.3 If not using BioTop

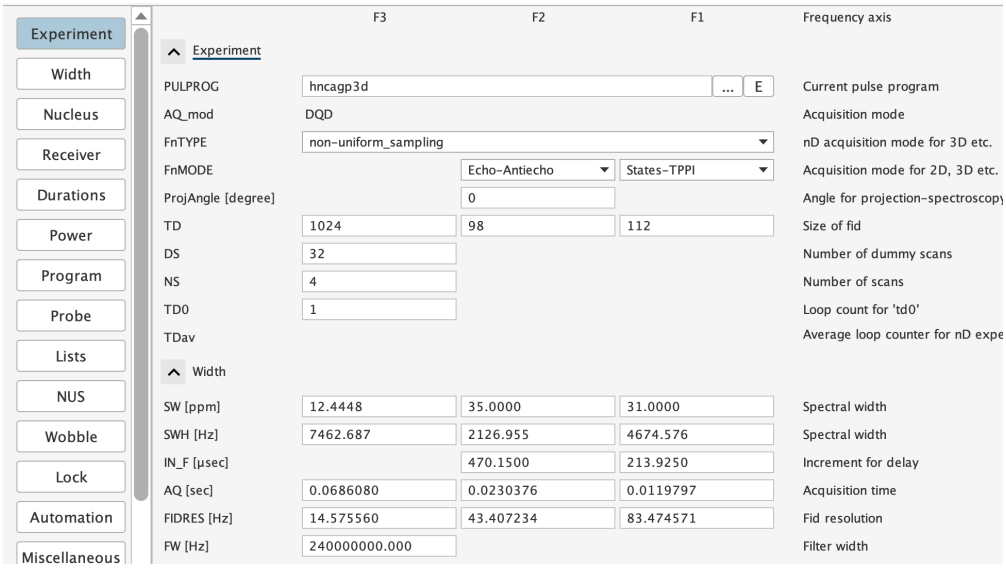
2.2 Loading experimental parameters from **BioTop**:

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 Selecting the 'Acqpars' tab will show lists of common and pulse program specific acquisition parameters.
First view the 'eda' list by clicking on the '**A**' icon or typing **eda**. This view shows the three dimensions, F3 (1H) and F2 (15N) and F1 (13C) in columns.



Parameters to check:

- **FnTYPE** - 'traditional planes' or 'non-uniform sampling' (see step 2.5 below)
- **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 (multiple of 16); allows system to reach steady state equilibration. Important for HSQC-based experiments due to heating from ^{15}N decoupling during acquisition
- **SW [ppm]** - 1H(F3) ~12-15 ppm; 15N (F2) ~25-40 ppm; 13CA (F1) ~32 ppm; defined in bioTop
- **O1** - ^{1}H H_2O offset in Hz (calibrated with BioTop or calibo1p1)
- **O2P** - 13CA offset (~54 ppm, middle of 13CA range, defined in bioTop)
- **O3P** - 15N amide offset (~115-120 ppm, defined in bioTop)
- **3 TD** - Number of ^{1}H time domain real points (~1024-4096, preferably 2^{N}, keep **3 AQ** at ~50-120 ms)
- **2 TD** - Number of ^{15}N time domain real points (2*int(d30/in30); **2 AQ** ~24ms, CT dimension)

- **1 TD** - Number of ^{13}CA time domain real points (**1 AQ** at ~12 ms, limited by $_{1}J_{CA,CB}$ coupling)
- **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 values should be appropriate, however it's useful to compare them against suggestions in the pulseprogram comments.

- **D1** - 1-2 sec
- **P1** - ^{1}H 90º high power pulse (calibrated with calibo1p1 or BioTop)
- **SPdB1** - power level [dB] for ^{1}H H2O flip-back shaped pulse (calibrated with BioTop)
- **P3** - ^{13}C 90º high power pulse (calibrated with BioTop)
- **CNST21** - ^{13}CO shift for decoupling (~173 ppm, defined in bioTop)
- **P21** - 15N 90º high power pulse (calibrated with BioTop)

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).

Navigate down to the 'NUS' section in the 'eda' parameter window and set the desired **NusAMOUNT** [%] sampling density.

For adequate reconstruction, number of NUS points should be larger than the number of expected peaks. 3D HNCA yields 2 peaks per residue, thus a 100-residue protein would require more than 200 NUS points.

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

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'nusPGSv8' written by Scott Anthony Robson 2013