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# HBHAcoNH_3D.nan

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NAN KB[1], Alex Eletsky[2], John Glushka[2]

[1]Network for Advanced NMR ( NAN); [2]University of Georgia

NAN-KB UGA
Network for Advanced NMR





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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 HBHA(CO)NH pulse sequence with sensitivity enhancement and gradient coherence selection, 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 $^{1}$HA and $^{1}$HB of residue (i-1). Note that HA and HB peaks will have opposite signs.

This experiment is somewhat less sensitive than CBCA(CO)NH, but more sensitive than H(CCCO)NH-TOCSY.

Required isotope labeling: U-$^{15}$N,$^{13}$C. Not suitable for samples with additional $^{2}$H labeling due to starting with $^{1}$HA and $^{1}$HB polarization. For larger systems CBCA(CO)NH-TROSY may be preferred instead.

This pulse sequence can be used for:

- spin system identification
- resolving signal degeneracy in 2D $^{15}$N HSQC.
- resonance assignment of $^{1}$HA and $^{1}$HB spins
- backbone resonance assignment: sequential linking of spins systems based on HA/HB peak matching (in conjunction with 3D $^{15}$N-edited NOESY.
- Bootstrapping side-chain resonance assignment via 3D HCCH-COSY, HCCH-TOCSY and $^{13}$C-edited NOESY-HSQC, by providing HA/CA and HB/CB strip anchoring

Field strength preference: Lower $B_0$ fields (600-700 MHz) are preferred, due to $R_2$ relaxation affecting $^{13}$CO coherences during long constant-time magnetization transfer periods. The $R_2$ relaxation rate of $^{13}$CO resonances is dominated by chemical shift anisotropy and is proportional to $B_0$ squared.

It uses the standard Bruker Topspin pulseprogram 'hbhaconhgp3d'

## Attachments


biotop.pdf
2.5MB




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.

"Effective" $^{1}J_{NH}$ parameter CNST4 defines the length of the INEPT transfer delays. For larger proteins CNST4 can be increased to compensate for relaxation losses during these delay. Normally it would be optimized by arraying it using **popt** with a $^{15}N$ HSQC experiment in 1D mode.

Since 3D HBHA(CO)NH only has 2 peaks per residue, NUS sampling amount can be as low as 5-10% provided there is sufficient S/N.

2D F1(Hab)-F3(HN) 2D plane can be acquired by setting **2 TD** to 1. Since HA and HB peaks have opposite signs, the 2D F2(N)-F3(HN) plane would have few signals apart from glycines, and is usually never recorded by itself.

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

This pulse sequence uses TPPI method to shift the apparent center of F1(Hab) dimension without frequency switching. The center of F1(Hab) in ppm is set approximately by **CNST20**. The exact frequency shift relative to **O1** in Hz is calculated and stored as **CNST62.** The exact center frequency of F1(Hab) in MHz is saved as **SFO1** status parameter for F1 dimension (in *acqus3* file).

For proper referencing in Topspin: increase the **1 SR** parameter by the value of **CNST62**.

For proper referencing in nmrPipe: in the conversion script (e.g. *fid.com*) set **zOBS** equal to **xOBS**; set **zCAR** to (**xCAR** - (**CNST62** / **xOBS**)). Make sure the resulting **zCAR** parameter is close to **CNST20**.

## 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_13C.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_13C.nan
5. HNCO_3D.nan
6. Biotop-Calibration and Acquisition setup

## Create HBHAcoNH experiment

1 Join an existing dataset and experiment (e.g. 1D proton, 2D 15N HSQC, 2D 13C HSQC,etc) for this sample.

1.1 Open create dataset window with **edc** command or through menu.
Edit text in Title box.

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 HBHAcoNH3D_NUS_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 HBHAcoNH_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, which typically only updates proton pulse widths and power levels. It is most useful for running routine experiments using the default parameters.
2) Load optimized parameters from **BioTop**. This module allows to optimize and define additional common parameters for a given dataset that can be applied to multiple experiments.

2.1 Loading pulse widths and power levels with **getprosol:**

Use the calibrated proton **P1** value obtained from 1D 1H experiment ( protocol PRESAT_bio.nan) and note the standard power level attenuation in dB for P1 (**PLdB1**). 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.
In this example, the calibrated **P1=9.9** us at power level -13.14 dB attenuation.

This also loads $^{15}$N and $^{13}$C pulse widths and power levels from the PROSOL table, which 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 or entered parameters values manually using the "Optimizations" tab of the **BioTop** GUI, you can simply 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; 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.
- **SW[ppm]**: 1H(F3) ~12-15 ppm; 15N (F2) ( ~25-40 ppm); 13Hab(~7-8 ppm); defined in bioTop
- **O1** - $^{1}$H $H_2O$ offset in Hz (calibrated with BioTop or calibo1p1)
- **O2P** - $^{13}$Cab offset (~42 ppm)
- **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*int(d30/in30); **2 AQ** ~24ms, CT dimension)
- **1 TD** - Number of $^{1}$Hab time domain real points ( **1 AQ** at ~12-15 ms)
- **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 are suitable, however it's useful to compare values in the fields against those proposed by the original parameter file and the pulseprogram.

- **D1** - 1-2 sec
- **P1** - 1H 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** - 13C 90º high power pulse (calibrated with BioTop)
- **CNST20**: $^{1}$Hab shift ( ~3 ppm, defined in bioTop)
- **CNST21**: $^{13}$CO shift ( ~173 ppm, defined in bioTop)
- **CNST22**: $^{13}$CA shift ( ~54 ppm, defined in bioTop)
- **CNST23**: $^{13}$Cab shift ( ~42 ppm, defined in bioTop)
- **P21** - 15N 90º high power pulse (calibrated with BioTop)

Note

The center of F1(Hab) dimension is defined by **CNST20** (in ppm). The **O1** parameter only defines the center of the directly detected dimension F3(HN)

## Configure NUS (non-uniform sampling) - optional

2.5 **Non-uniform sampling (NUS) parameter setup:**

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 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 HBHA(CO)NH yields approximately 3 peaks per residue, thus a 100-

residue protein would require more than 300 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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