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 CBCAcoNH_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 3D CBCA(CO)NH pulse sequence with sensitivity enhancement, gradient coherence selection, using either uniform or non-uniform sampling (NUS). This produces a 3D phase-sensitive dataset that correlates backbone ^1HN and ^{15}N resonances of residue (i) with ^{13}CA and ^{13}CB of residue (i-1).

Has higher sensitivity than HNCACB, and thus may provide $^{13}\text{CA}(i-1)$ and $^{13}\text{CB}(i-1)$ shifts in cases when the corresponding peaks are not detected in HNCACB.

Required isotope labeling: U- ^{15}N , ^{13}C . Not suitable for samples with additional ^2H labeling due to starting with ^1HA and ^1HB polarization; HN(CO)CACB or TROSY-HN(CO)CACB with ^2H decoupling should be used in this case.

Optimal MW is ≤ 25 kDa. Larger systems may benefit from 3D CBCA(CO)NH-TROSY version; compare 2D H-N planes 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 and ^{13}CB peak matching in conjunction with 3D HNCACB or HNCA spectra
- residue type information mainly based on CB shifts
- resonance assignment of ^{13}CA and ^{13}CB spins
- chemical shift-based secondary structure prediction (e.g. TALOS), together with other resonances

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 'cbcaconhgp3d'

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

Since both F1(^{13}C) and F2(^{15}N) dimensions are constant-time, numbers of acquired points **1 TD** and **2 TD** should be set to the largest possible values for optimal S/N. The maximum **1 TD** and **2 TD** values are $2 \cdot \text{int}(d20/in20)$ and $2 \cdot \text{int}(d30/in30)$, respectively, where d20 and d30 are the decremented delays, and in20 and in30 are the decrement values. The parameters D20, D30, IN20 and IN30 are computed internally according to pulse program code and their values are shown in 'ased' view. Note that $\text{in20} = \text{in0} = 1(2 \cdot \text{SW}(\text{C}))$ and $\text{in30} = \text{in10} = 1(4 \cdot \text{SW}(\text{N}))$. In TopSpin 4.x the **ased** command would produce a warning if **1 TD** or **2 TD** is set too large and reset the internal *td1* and *td2* parameters to the maximum possible values.

"Effective" $^1J_{\text{NH}}$ 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 CBCA(CO)NH only has 2 peaks per residue, NUS sampling amount can be as low as 5-10%

Individual F1(Cab)-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 CBCA(CO)NH-TROSY or regular CBCA(CO)NH yields better S/N for your sample at a particular field strength, record and inspect the respective 2D F2(N)-F3(HN) planes 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 ^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 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 ^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. **HSQC_13C.nan**
5. **HNCO_3D.nan**
6. Biotop-Calibration and Acquisition setup

Create CBCAcoNH 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 Open create dataset window with **edc** command or through menu Acquire → 'Create Dataset'.
Edit text in Title box.
- 1.2 To select starting parameter set, check 'Read parameterset' box, and click Select.
For standard NMR parameter sets, change the Source directory at upper right corner of the window:
Source = /opt/NMR_SB/par
Click 'Select' to bring up list of parameter sets.
Select CBCAcoNH_3D_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 CBCAcoNH_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 ^{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 parameter 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 ¹⁵N and ¹³C pulse widths and power levels from the 'edprosol' 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 parameters' 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 (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.
- **SW [ppm]** - 1H (F3) ~12-15 ppm; 15N (F2) ~25-40 ppm; 13Cab (F3) ~72-80 ppm; defined in bioTop
- **O1** - ¹H H₂O offset in Hz (calibrated with BioTop or calibo1p1)
- **O2P** - ¹³Cab offset (~42 ppm)
- **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 ($2 \cdot \text{int}(d30/in30)$; **2 AQ** ~ 24 ms, CT dimension)
- **1 TD** - Number of ^{13}C time domain real points ($2 \cdot \text{int}(d20/in0)$; **1 AQ** ~ 6.6 ms, CT dimension)
- **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 appropriate, however it's useful to compare values in the fields against those proposed by the original parameter file and the pulseprogram.

- **D1** - 1-2 s
- **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)
- **CNST21** - ^{13}CO shift for decoupling (~ 173 ppm, defined in bioTop)
- **CNST22** - ^{13}CA shift for decoupling (~ 54 ppm, defined in bioTop)
- **CNST23** - ^{13}Cab shift for decoupling (~ 42 ppm, same as **O2P**)
- **P21** - ^{15}N 90° 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).

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 CBCA(CO)NH 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