Dec 19, 2024

HNCO_3D.nan

DOI
https://dx.doi.org/10.17504/protocols.io.ewov19822lr2/v1

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

DOI: https://dx.doi.org/10.17504/protocols.io.ewov19822lr2/v1

Protocol Citation: NAN KB, Alex Eletsky, John Glushka 2024. HNCO_3D.nan. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov19822lr2/v1

Protocol status: Working
We use this protocol and it's working

Created: April 12, 2024

Last Modified: December 19, 2024

Protocol Integer ID: 98145

Keywords: protein nmr, assignment, backbone, amides, 15N, hsqc, 3d hnco pulse sequence with sensitivity enhancement, 2d 15n hsqc, resonance assignment of 13co spins chemical shift, 3d hnco, 13co spins chemical shift, spin system identification, backbone resonance assignment, hncogp3d, isotope labeling, required isotope labeling, spins system, hnco version, hnco, standard bruker topspin pulseprogram, sequential linking of spins system, 3d phase, other resonance, pulse sequence with sensitivity enhancement, resonances of residue, 3d trosy, spectrometer, resonance, pulse sequence, 13c, sensitive spectrum, resolving signal degeneracy

Funders Acknowledgements:
NSF
Grant ID: 1946970

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 HNCO 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 spectrum with H,N and C axes that correlates backbone ^{1}HN and ^{15}N resonances of residue (i) with ^{13}CO of residue (i-1).

Required isotope labeling: U-^{15}N,^{13}C (with or without ^{2}H).

Optimal MW is ≤ 25 kDa. Larger systems may benefit from 3D TROSY-HNCO 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}CO peak matching (in conjunction with 3D HN(CA)CO or (HCA)CONH spectra)
- resonance assignment of ^{13}CO spins
- chemical shift-based secondary structure prediction (e.g. TALOS), together with other resonances

Field strength preference: No significant preference - higher fields may yield better resolution, though this is rarely limiting, since peaks are dispersed in three dimensions. In many cases 600 MHz field strength may be sufficient.

It uses the standard Bruker Topspin pulseprogram 'hncogp3d'

Note: specific parameter values illustrated below may differ depending on the facility and spectrometer.

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(^{15}N) 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 delays. Normally it would be optimized by arraying it using **popt** with a ^{15}N HSQC experiment in 1D mode.

Since 3D HNCO is a very sparse spectrum at only 1 peak per residue, NUS sampling amount can be as low as 3-5%, provided your 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-HNCO or regular HNCO 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 ^{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.**

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. Biotop-Calibration and Acquisition setup

Create HNCO experiment

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

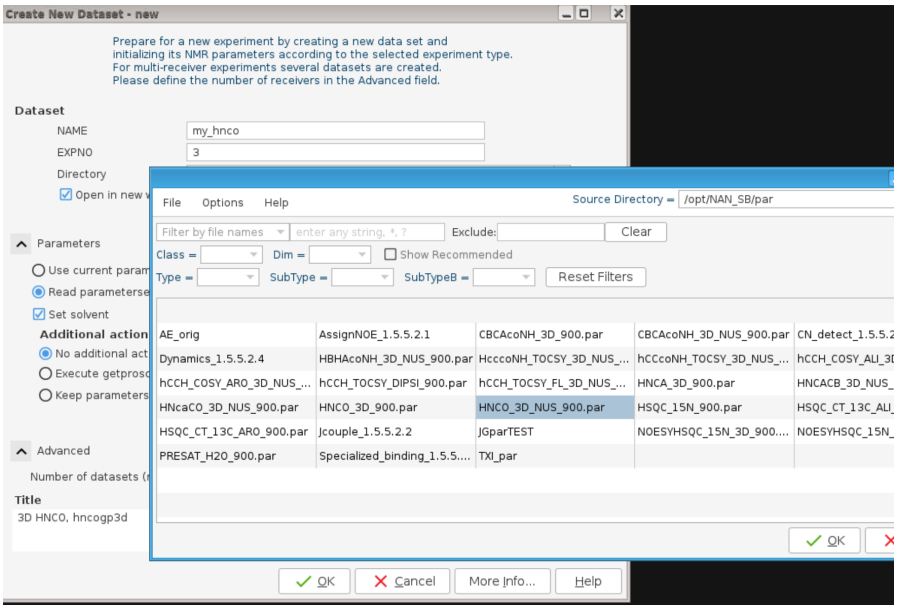
1.1 Click on Acquire → 'Create Dataset' button to open dataset entry box, or type the **edc** command.

Dataset Name: recommended to keep the same name when using BioTop for optimization and acquisition setup.

The EXPNO is automatically incremented by +1 by default.
The Title text box will copied from the previous experiment. Edit to designate the HNCO pulse program and add other details as appropriate.

1.2 Load the 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 HNCO_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 HNCO_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.

For example, clicking on the 'Prosol' button in the Acquire menu, or executing the **getprosol** command without arguments will load default values from the pre-configured spectrometer calibration table, including the default ^{1}H 90° pulse length and power level. However, for biological samples in aqueous solvents the optimal ^{1}H 90° pulse length can vary significantly depending on buffer conditions, sample geometry and temperature, and thus needs to be calibrated individually for each sample. ^{13}C and ^{15}N 90° pulse lengths do not typically exhibit large variations, but these can also be calibrated for best results.

There are two ways of automatically updating an entire range of experimental parameters. The first is using **getprosol** command, which only updates pulse widths and power levels without altering other parameters, such as spectral widths and offsets. This method is suitable for reproducing existing experiments or parameters sets with minimal variations.

The second method utilizes the **BioTop** module of TopSpin, and can load additional experimental parameters, such as spectral widths, offsets, and number of time-domain points. These additional parameters are set according to calibrations or definitions within the 'Optimization' tab of the BioTop GUI and the corresponding XML description files (*bt_hncogp3d.xml* in this case). This method has a lower dependency on the particular settings of the starting parameter set, and is suitable for setting up experiments from scratch. With this method nearly all important acquisition parameters can be optimized for a particular sample, and then applied consistently to multiple NMR experiments with a single command.

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

First, type **getprosol** without arguments on the command line.

This will load default pulse widths and power levels for all channels. The 90° high power pulses and power levels are P1/PL1 for ^{1}H, P3/PL2 for ^{13}C, and P21/PL3 for ^{15}N. The power levels can be entered in Watts (parameters **PLW1**, **PLW2**, etc.) or dB attenuation (parameters **PLdB1**, **PLdB2**, etc.), where lower dB values correspond to higher power. Note down the power levels (parameters **PLdB1**, **PLdB2**, **PLdB3** - in dB units) - these are the default power levels for high power pulses for a particular probe and should not be exceeded.

To load calibrated ^{1}H pulse widths enter the following command:

getprosol 1H [calibrated P1 value] [power level for P1 in dB (PLdB1)]

For example, *getprosol 1H 9.9 -13.14* - here the calibrated **P1** is 9.9 us at power level at -13.14 dB attenuation. Note that power level in dB argument is required even when using the default peak power level for a particular probe. This command will also update other dependent ^{1}H parameters (e.g power levels for ^{1}H shaped pulses and decoupling) and load default ^{13}C, ^{15}N pulse powers and widths from the PROSOL table.

If you also have separately calibrated 90° ^{13}C and ^{15}N pulses, these can be loaded with the following command

getprosol 1H [calibrated P1] [PLdB1] 13C [calibrated P3] [PLdB2] 15N [calibrated P21] [PLdB3]

In this case all dependent ^{1}H, ^{13}C, and ^{15}N power levels (shaped and decoupling pulses) are re-calculated based on the calibrated values provided.

This functionality is also implemented as part of the **btprep** command (a component of BioTop) - see step 2.2

go to step #3 If not using BioTop

2.2 Loading experimental parameters from **BioTop**:

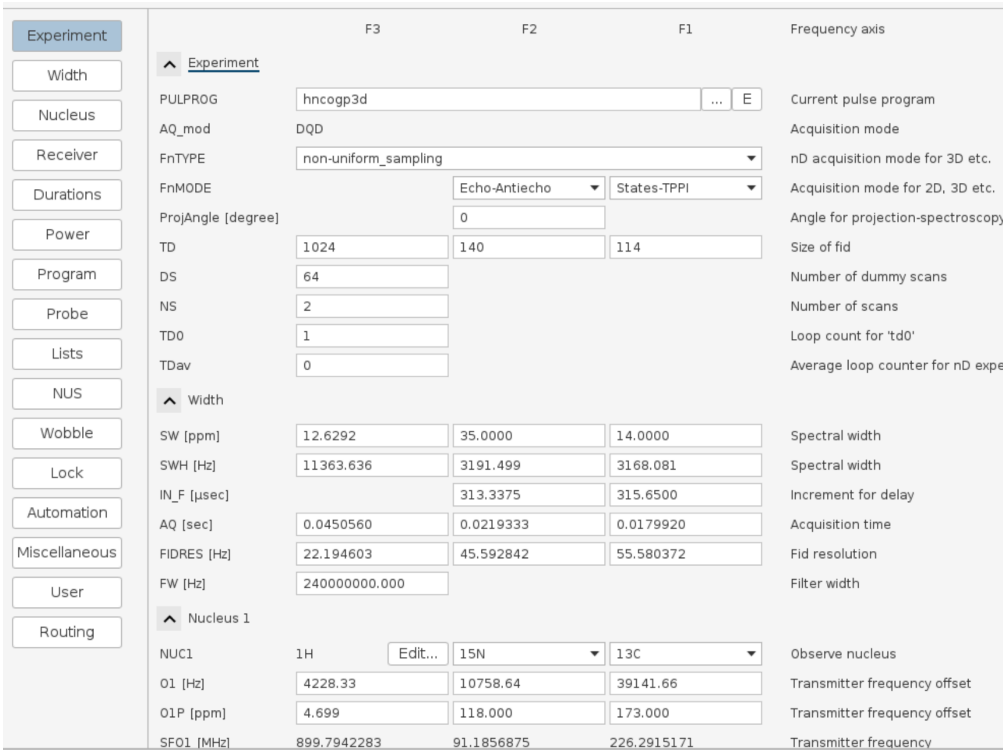
If you previously performed parameter calibrations using the "Optimizations" tab of the BioTop GUI, or entered parameters manually in the "Optimizations" tab, you can simply type **btprep** at the command line.

This is equivalent to calling **getprosol** with all ^{1}H, ^{13}C and ^{15}N optimized parameters followed by additional parameters loaded from the "Optimizations" tab in BioTop GUI. In this particular case these parameters are based on the *bt_hncogp3d.xml* description file: ^{1}H offset in Hz (**O1**), ^{1}H spectral width (**3 SW**), power level for ^{1}H sinc water-flipback pulse (**SPdB 1**), ^{15}N offset in ppm (**O3P**), ^{15}N spectral width (**2 SW**), ^{15}N max acquisition time (**2 AQ**), ^{13}CO offset in ppm (**O2P**), ^{13}CO spectral width (**1 SW**), ^{13}CO max acquisition time (**1 AQ**), ^{13}CA offset in ppm (**CNST22**).

Inspect and adjust parameters

3 Select the 'Acqpars' tab to display acquisition parameters. Two display modes can be selected, the full display mode (click on the '**A**' icon or type **eda**), or pulse program-specific mode (click on the 'pulse' icon, or type **ased**). The former gives you access to all parameters and provides an overview of all spectral dimensions at once, while the latter is useful because it only displays acquisition parameters used in the pulse sequence and can be parsed sequentially as a checklist.

3.1 First, inspect your parameters in the full '**eda**' mode. This view shows the three dimensions, F3 (1H), F2 (15N), and F1 (13C) in columns.



Parameters to check:

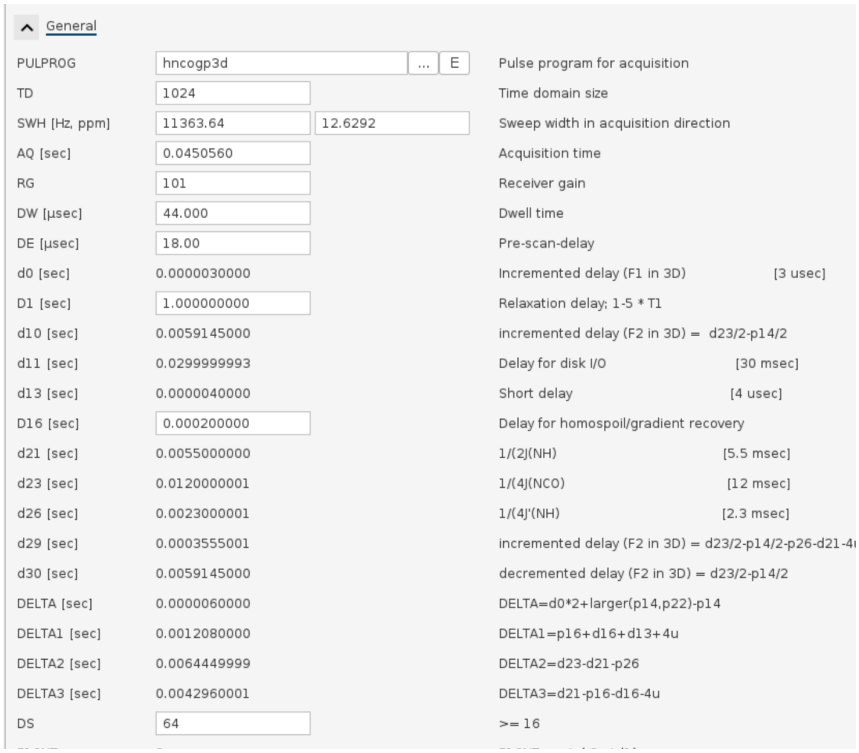
- **FnTYPE** - 'traditional planes' or 'non-uniform sampling' (see step 3.3 below)
- **NS** - minimum 2; increase for for higher signal to noise (S/N increases as square root of NS)
- **DS** - 32-128 'dummy' scans that are not recorded (multiple of 16); allows system to reach steady state equilibration. Important for HSQC-based experiments due to heating from ^{15}N decoupling during acquisition
- **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** ~23ms, CT dimension)
- **1 TD** - Number of ^{13}C time domain real points (**1 AQ** at ~20 ms)
- **SW [ppm]** - ^{1}H(F3) ~12-15 ppm, ^{15}N(F2) ~25-40 ppm, ^{13}CO(F1) ~12-20 ppm, all defined in BioTop
- **O1** - ^{1}H H_2O offset in Hz (calibrated with BioTop or calibo1p1)
- **O2P** - ^{13}C offset (~173 ppm, middle of ^{13}CO range, defined in bioTop)
- **O3P** - ^{15}N amide offset (~115-120 ppm, defined in bioTop)
- **DIGMOD** - 'digital' (pulse sequence does not use *acqt0* correction)

Note

To collect 2D planes:
For F3-F1 plane, set 2TD to 1; for F3-F2 plane, set 1TD to 1.
This may be useful prior to setting up a 3D to confirm SW and signal to noise.

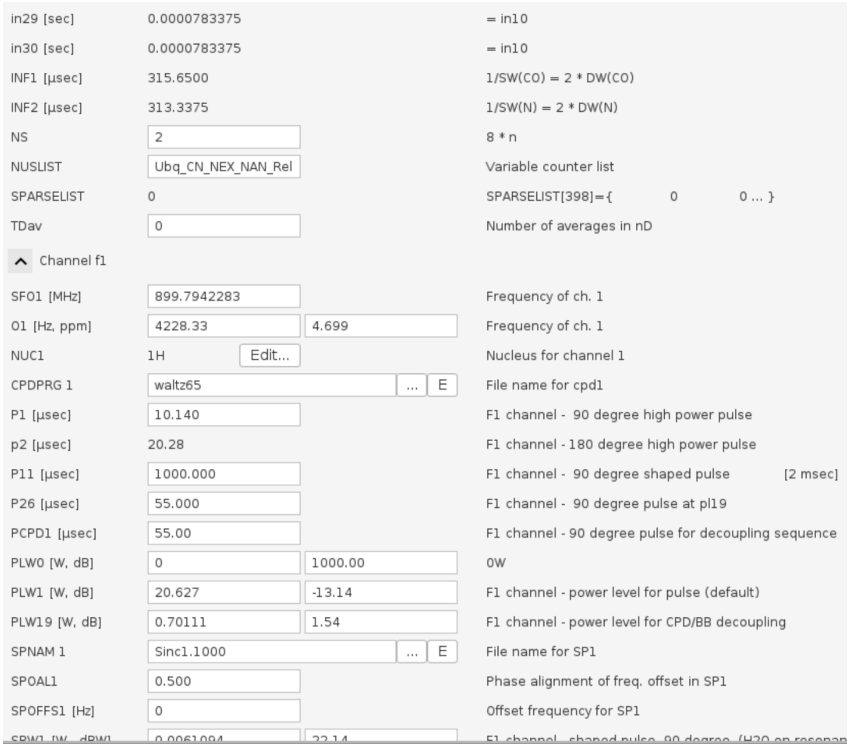
The planes can be acquired as NUS or traditional planes according to time available and signal intensity.

3.2 Then examine the parameters in the pulse program-specific '**ased**' mode (click on the 'pulse' icon). Most parameters are also accessible in the '**eda**' mode (step 3.1 above). However, the '**ased**' mode allows more convenient access to individual parameters within arrays, such as delays, pulse widths, constants, etc. It also displays parameter values computed internally within a pulse sequence, and provides context description from the relevant pulse program comment lines.



General

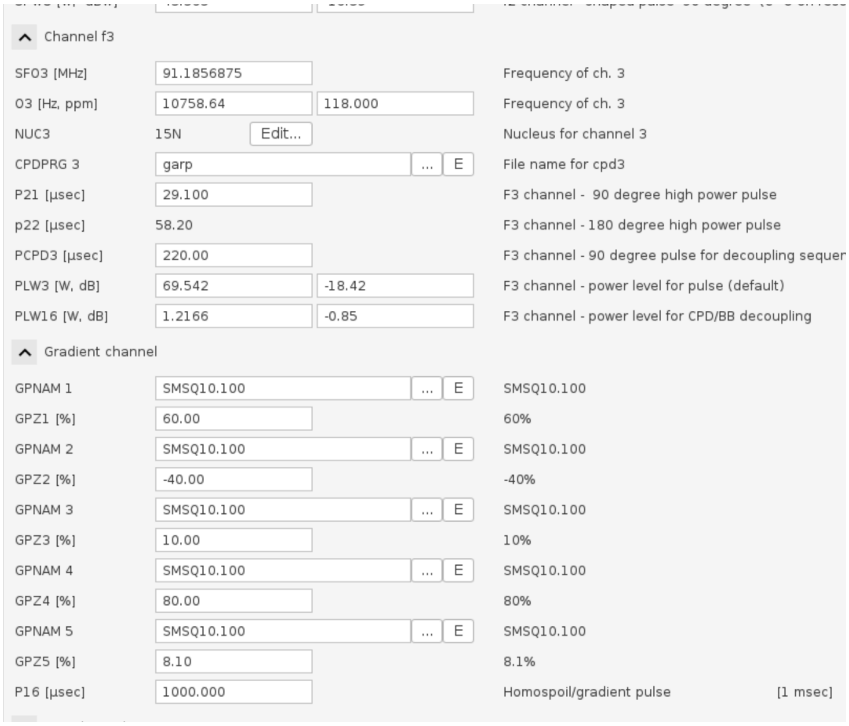
PULPROG	hncogp3d	... E	Pulse program for acquisition
TD	1024		Time domain size
SWH [Hz, ppm]	11363.64	12.6292	Sweep width in acquisition direction
AQ [sec]	0.0450560		Acquisition time
RG	101		Receiver gain
DW [µsec]	44.000		Dwell time
DE [µsec]	18.00		Pre-scan-delay
d0 [sec]	0.0000030000		Incremented delay (F1 in 3D) [3 usec]
D1 [sec]	1.000000000		Relaxation delay; 1-5 * T1
d10 [sec]	0.0059145000		incremented delay (F2 in 3D) = d23/2-p14/2
d11 [sec]	0.0299999993		Delay for disk I/O [30 msec]
d13 [sec]	0.0000040000		Short delay [4 usec]
D16 [sec]	0.000200000		Delay for homospoil/gradient recovery
d21 [sec]	0.0055000000		1/(2J(NH) [5.5 msec]
d23 [sec]	0.0120000001		1/(4J(NCO) [12 msec]
d26 [sec]	0.0023000001		1/(4J'(NH) [2.3 msec]
d29 [sec]	0.0003555001		incremented delay (F2 in 3D) = d23/2-p14/2-p26-d21-4
d30 [sec]	0.0059145000		decremented delay (F2 in 3D) = d23/2-p14/2
DELTA [sec]	0.0000060000		DELTA=d0*2+larger(p14,p22)-p14
DELTA1 [sec]	0.0012080000		DELTA1=p16+d16+d13+4u
DELTA2 [sec]	0.0064449999		DELTA2=d23-d21-p26
DELTA3 [sec]	0.0042960001		DELTA3=d21-p16-d16-4u
DS	64		>= 16



in29 [sec]	0.0000783375		= in10
in30 [sec]	0.0000783375		= in10
INF1 [µsec]	315.6500		1/SW(C0) = 2 * DW(C0)
INF2 [µsec]	313.3375		1/SW(N) = 2 * DW(N)
NS	2		8 * n
NUSLIST	Ubq_CN_NEX_NAN_Rel		Variable counter list
SPARSELIST	0		SPARSELIST[398]={ 0 0 ... }
TDav	0		Number of averages in nD

Channel f1

SFO1 [MHz]	899.7942283		Frequency of ch. 1
O1 [Hz, ppm]	4228.33	4.699	Frequency of ch. 1
NUC1	1H Edit...		Nucleus for channel 1
CPDPRG 1	waltz65	... E	File name for cpd1
P1 [µsec]	10.140		F1 channel - 90 degree high power pulse
p2 [µsec]	20.28		F1 channel - 180 degree high power pulse
P11 [µsec]	1000.000		F1 channel - 90 degree shaped pulse [2 msec]
P26 [µsec]	55.000		F1 channel - 90 degree pulse at pl19
PCPD1 [µsec]	55.00		F1 channel - 90 degree pulse for decoupling sequence
PLW0 [W, dB]	0	1000.00	0W
PLW1 [W, dB]	20.627	-13.14	F1 channel - power level for pulse (default)
PLW19 [W, dB]	0.70111	1.54	F1 channel - power level for CPD/BB decoupling
SPNAM 1	Sinc1.1000	... E	File name for SP1
SPOAL1	0.500		Phase alignment of freq. offset in SP1
SPOFFS1 [Hz]	0		Offset frequency for SP1



Most of the default values should be appropriate, however it's useful to compare them against suggestions in the pulseprogram comments. In general, only a few may need to be changed.

- **D1** - recycle delay (~1 s for protonated samples, ~2-3 s for perdeuterated samples)
- **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)
- **CNST4** - effective $^{1}J_{NH}$ value (≥93 Hz)
- **CNST22** - ^{13}CA shift for decoupling (~54 ppm, defined in bioTop)
- **P21** - ^{15}N 90º high power pulse (calibrated with BioTop)

Configure NUS (non-uniform sampling) - optional

3.3 **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).

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 HNCO yields 1 peak per residue, thus a 100-residue protein would require more than 100 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

4 Type '**expt**' to calculate the expected run time.

go to step #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.

4.1 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.

4.2 3D NUS data usually cannot be processed in Topspin without a separate license. 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

L.E. Kay, G.Y. Xu & T. Yamazaki, J. Magn. Reson. A109, 129-133 (1994)

J. Schleucher, M. Sattler & C. Griesinger, Angew. Chem. Int. Ed. 32, 1489-1491 (1993)

S. Grzesiek & A. Bax, JMR 96, 432-440 (1992)

'nusPGSv8' written by Scott Anthony Robson 2013