Dec 19, 2024

hCCH-TOCSY_FL_3D.nan

DOI
https://dx.doi.org/10.17504/protocols.io.eq2lywpnpvx9/v1

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Protocol Citation: NAN KB, Alex Eletsky, John Glushka 2024. hCCH-TOCSY_FL_3D.nan. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2lywpnpvx9/v1

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

Created: April 22, 2024

Last Modified: December 19, 2024

Protocol Integer ID: 98595

Keywords: protein nmr, assignment, backbone, amides, 15N, hsqc, aliphatic 13c resonance, aliphatic 13c resonances via 1jcc coupling, 2d 13c hsqc, 13c spin, tocsy-fl-3d pulse sequence, tocsy correlations with the ca spin, hsqc pair, cb shifts from the backbone resonance assignment stage, backbone resonance assignment stage, tocsy correlation, hsqc peak, resonance assignment of aliphatic side, particular pair of spin, aliphatic 1h polarization, 3d phase, tocsy experiment, multiple 13c line, tocsy scheme, spins with extreme chemical shift spread, specialized nmr experiment, spin pairs from remote correlation, tocsy, 1jcc coupling, tocsy strip, spin pair, tocsy dimension, latter two spectra, ca spin, chain resonance assignment of larger system, chain resonance assignment, mhz, covalent network, spectra, spin, protonated labeling, methyl

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 (H)CCH-TOCSY_FL_3D pulse sequence with a FLOPSY-16 mixing sequence, gradient coherence selection and can be acquired with either a standard or a non-uniform (NUS) sampling scheme. This produces a 3D phase-sensitive dataset that correlates aliphatic ^{13}C resonances via $^{1}J_{CC}$ couplings. FLOPSY-16 mixing scheme offers improved broadband performance over the commonly used DIPSI-2.

Dimensions F3(^{1}H) and F2(^{13}C) represent an HSQC pair, while F1(^{13}C) is the TOCSY dimension that exhibits multiple ^{13}C lines for a given ^{13}C HSQC peak. 3D (H)CCH-TOCSY scheme is usually preferred over a related 3D H(C)CH-TOCSY experiment, since in the latter case the peak pattern is similar to the one observed in 3D ^{13}C-edited NOESY.

3D (H)CCH-TOCSY is used for resonance assignment of aliphatic side-chain ^{1}H and ^{13}C spins, combined with 2D ^{13}C HSQC, 3D (H)CCH-COSY, 3D ^{13}C-edited NOESY, H(CCCO)NH-TOCSY, and (H)C(CCO)NH-TOCSY. The CA and CB shifts from the backbone resonance assignment stage along with HA and HB shifts from 3D HBHA(CO)NH are used to bootstrap the side-chain assignment process.

(H)CCH-TOCSY has better sensitivity and higher information content than H(CCCO)NH-TOCSY and (H)C(CCO)NH-TOCSY. The latter two spectra only detect ^{13}C TOCSY correlations with the CA spin (relayed via CO, N, and HN), while (H)CCH-TOCSY delivers TOCSY strips for each covalently bonded aliphatic $^{13}C/^{1}H$ spin pair within a residue.

Peak intensities of (H)CCH-TOCSY have a complicated and not easily predictable behavior dependent on the covalent network of ^{13}C spins and the nature and the length of the mixing sequence. Though infrequently, certain expected correlations can appear undetectable due to low transfer efficiency between that particular pair of spins. Thus, (H)CCH-TOCSY is often complemented with (H)CCH-COSY, which has more predictable polarization transfer pathways and can be used to distinguish covalently bound ^{13}C spin pairs from remote correlations.

Required isotope labeling: U-^{15}N,^{13}C or U-^{13}C. Not suitable for samples with additional ^{2}H labeling due to starting with aliphatic ^{1}H polarization.

Optimal MW is ≤ 25 kDa. Side-chain resonance assignment of larger systems is usually performed using selectively protonated labeling on perdeuterated background (e.g. ILV-methyl) and specialized NMR experiments.

Field strength preference: High possible B_0 fields (800-1100 MHz) are preferred for better signal dispersion. There may be concerns about broadband performance of the TOCSY mixing element, but this mostly affects spins with extreme chemical shift spread, such as CA/CB and CG1 of Thr.

It uses a pulseprogram 'hcchflgp3d2.nan' modified from the Topspin version.

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 ^{13}C decoupling, and resolve undesirable $^{2,3}J_{H,H}$ splittings.

The FLOPSY-16 mixing scheme has better broadband performance than the commonly used DIPSI-2, and should work well even at 1.1 GHz field strength. The mixing time is defined by the L1 loop counter, according to the formula (P9*188.448*L1). L1=3 is a good overall choice as it gives a total mixing time of 14 ms for P9=25u, sufficient to produce complete TOCSY correlations even for long-chain amino acids.

(H)CCH-TOCSY is a fairly dense spectrum, with significantly more peaks (~16 per residue on average) than the triple-resonance spectra. NUS sampling amount should ideally be ~10-30% provided there is sufficient S/N.

To reduce minimal measurement time in the uniformly sampled mode the HSQC/INEPT dimension F2(C13) can be "folded" approximately 3X (to ~27 ppm). Note that this may complicate further data analysis, as some visualization software (e.g. Sparky, etc.) may be less suited for working with folded/aliased spectra than others (e.g. CARA). Also, some signals may be negatively affected by spectral edge artifacts.

Dimension folding does not significantly benefit NUS acquisition apart from reducing the processed spectrum file size. Since the number of expected peaks stays the same, the total number of NUS points needed for accurate reconstruction remains the same.

Individual F2(13C)-F3(1H) and F1(13C)-F3(1H) 2D planes can be acquired by setting, respectively, **1 TD** or **2 TD** to 1.

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 ^{13}C HSQC is recommended, according to protocol **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 hCCH-TOCSY_FL experiment

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 hCCH-TOCSY_FL_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 hCCH-TOCSY_FL_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) 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 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 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 if not default. This view shows the three dimensions, F3(1H), F2(13C), and F1(13C) in columns.

Parameters to check:

- **FnTYPE** - 'traditional planes' or 'non-uniform sampling' (see step 2.5 below)
- **NS** - minimum 2 with full **2 SW**; 4 when folding is used in **2 SW**
- **DS** - 32-128 'dummy' scans that are not recorded (multiple of 16); allows system to reach steady state
- **3 SW** - ^{1}H spectral width (~12-15 ppm, defined in **BioTop**)
- **2 SW** - ^{13}Cali (INEPT) spectral width (~80 ppm, ~27 ppm if folded)
- **1 SW** - ^{13}Cali (TOCSY) spectral width (~80 ppm)
- **O1** - ^{1}H H_2O offset in Hz (calibrated with BioTop or calibo1p1)
- **O2P** - ^{13}C aliphatic offset (~38 ppm, defined in bioTop)

- **O3P** - ^{15}N 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 ^{13}Cali (INEPT) time domain real points (**2 AQ** ~12ms, limited by $^1J_{CC}$ couplings)
- **1 TD** - Number of ^{13}Cali (TOCSY) time domain real points (**1 AQ** ~12ms, limited by $^1J_{CC}$ couplings)
- **DIGMOD** - 'baseopt' (zero 1st order phase correction in F3(^{1}H))

2.4 Then examine the parameters in Acqpars 'ased' mode (click 'pulse' icon or type **ased**).

Most of the default parameters should be 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
- **L1** - Loop counter for FLOPSY-16 mixing cycle (3 or 4). Mixing time is given by (P9*188.448*L1), ~14 ms for P9=25u and L1=3
- **P1** - ^{1}H 90º high power pulse (calibrated with calibo1p1 or BioTop)
- **P3** - ^{13}C 90º high power pulse (calibrated with BioTop)
- **CNST21**: ^{13}CO shift (~173 ppm, defined in bioTop)
- **P21** - ^{15}N 90º high power pulse (calibrated with BioTop)

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. (H)CCH-TOCSY yields approximately 16 peaks per residue, thus a 100-residue protein would require more than 1600 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

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, A.U. Singer, D.R. Muhandiram & J. D. Forman-Kay, J. Magn. Reson. B 101, 333 - 337 (1993))

W.Peti, C. Griesinger & W. Bermel, J. Biomol. NMR 18, 199 - 205 (2000).

'nusPGSv8' written by Scott Anthony Robson 2013