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hCCH-COSY_aro_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 (H)CCH-COSY pulse sequence optimized for the aromatic spectral region. It uses semi-constant time (SCT) evolution in both indirect ^{13}C dimensions, and can be collected with a standard or a non-uniform (NUS) sampling scheme. This produces a 3D phase-sensitive dataset that correlates ^{1}H and ^{13}C resonances within aromatic rings of Trp, Phe and Tyr via $^{1}J_{HC}$ and $^{1}J_{CC}$ couplings.

Dimensions F3(^{1}H) and F2(^{13}C) represent an HSQC pair, while F1(^{13}C) is the COSY dimension that exhibits multiple ^{13}C lines for a given ^{13}C HSQC peak. 3D (H)CCH-COSY scheme is usually preferred over a related 3D H(C)CH-COSY experiment, due to better signal dispersion of aromatic ^{13}C as compared to ^{1}H.

Aromatic 3D (H)CCH-COSY is used for resonance assignment of aromatic side-chain ^{1}H and ^{13}C spins, combined with 2D ^{13}Caro CT-HSQC, 3D ^{13}Cali-edited NOESY, and 3D ^{13}Caro-edited NOESY. Assignment of aromatic resonances is usually performed after backbone and aliphatic side-chains assignment is complete.

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.

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 aromatic ^{1}H polarization.

Optimal MW is ≤ 25 kDa.

Field strength preference: Highest available B_0 field is preferred. Low dispersion of ^{13}C resonances in Phe and sometimes Trp residues may lead to strong coupling artifacts at low fields.

It uses a pulseprogram 'hcchcoctgp3d2_aro.nan' modified from the original 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 during ^{13}C decoupling, and resolve undesirable $^{3}J_{H,H}$ splittings.

If acquisition time in the indirect ^{13}C dimensions is kept under 4.5 ms, it defaults to purely constant-time (CT) evolution. Higher evolution time settings automatically enable semi-constant time evolution. Maximum useful evolution time is limited to ~6 ms due to $^{1}J_{CC}$ couplings. It is recommended to utilize the full 6 ms resolution due to high sensitivity of aromatic (H)CCH-COSY.

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.

Aromatic resonance assignment is usually initiated by identifying HD (Phe/Tyr) and HD1 (Trp) resonances among NOE cross-peaks within HB2/HB3 strips in ^{13}Cali-edited NOESY and confirmed via "mirror" cross-peaks in ^{13}Caro-edited NOESY. Alternatively, Yamazaki experiments, such as (HB)CB(CGCD)HD and (HB)CB(CGCDCE)HDHE, can be used to identify aromatic ^{1}H independently of the NOE data, though these experiments have very low sensitivity and are rarely required. Next, the corresponding CD (Phe/Tyr) and CD1 (Trp) aromatic ^{13}C resonances can be identified in 2D ^{13}Caro CT-HSQC and used to bootstrap resonance assignment via aromatic (H)CCH-COSY.

In rare cases when your protein does not contain Phe, Tyr, or Trp residues aromatic (H)CCH-COSY is not need. It also not suitable for assignment of HE1/CE1 resonances of His rings.

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 an aromatic 2D ^{13}C CT-HSQC is also recommended, according to protocol **HSQC_CT_13C_aro.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-COSY_aro 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 hCCH_COSY_aro_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_COSY_aro_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 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 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 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
- **DS** - 32-128 'dummy' scans that are not recorded (multiple of 8); allows system to reach steady state
- **3 SW** - ^{1}H spectral width (~12-15 ppm, defined in **BioTop**)
- **2 SW** - $^{13}Caro$ (INEPT) spectral width (~40 ppm)
- **1 SW** - $^{13}Caro$ (COSY) spectral width (~40 ppm)
- **O1** - ^{1}H H_2O offset in Hz (calibrated with BioTop or calibo1p1)

- **O2P** - ^{13}Caro offset (~125 ppm, defined in bioTop)
- **O3P** - ^{15}N offset, for Trp and His rings (~130 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 ^{13}Caro (INEPT) time domain real points (**2 AQ** ~4.5 ms for CT evolution only, or up to ~6ms for semi-CT evolution, limited by $^{1}J_{CC}$ couplings)
- **1 TD:** Number of ^{13}Caro (INEPT) time domain real points (**2 AQ** ~4.5 ms for CT evolution only, or up to ~6 ms for semi-CT evolution, limited by $^{1}J_{CC}$ couplings)
- **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 sec
- **P1** - ^{1}H 90º high power pulse (calibrated with calibo1p1 or BioTop)
- **P3** - ^{13}C 90º high power pulse (calibrated with BioTop)
- **P21** - ^{15}N 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).

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 in any of the 2D ^{13}C-^{13}C planes. Total number of aromatic (H)CCH-COSY peaks varies by residue type: 11 (Trp), 7 (Phe), 4 (Tyr), 2 (His, diagonal only). NUS sampling amount should thus be ~10-30% provided there is sufficient S/N.

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