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C_hCANCO_IPAP_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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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 carbon detected 3D IPAP-(H)CANCO pulse sequence, which provides correlations between CO(i)-N(i) with CA(i) and CA(i-1). [F2(HA) → F1(CA,t1) → F1(CO) → F3(N,t2) → F1(C=O,t3)]
Also detects CB-ND-CG and CG-NE-CD correlations for Asn and Gln side chains, respectively.

It yields two 2D sub-spectra, so use 'splitcomb ipap 2' to process in Topspin.

^{15}N and ^{13}C labeling are required.

It uses the Topspin library pulseprogram 'c_hcanco_ia3d'.

Attachments

biotop.pdf
2.5MB

NUS_poisson_gap_file...
12KB

Guidelines

IPAP loop is applied to F1(CA) dimension by default. Use ZGOPTNS -DLABEL_F2 flag to apply IPAP loop to F2(N) dimension. In TopSpin 3.x you need to double the number of TD points (and double the effective AQ time) in the dimension with the IPAP loop.

To test 2D F1-F3 (CA-CO) plane acquisition set TD2 to 1 and remove ZGOPTNS flags.

For 2D F2-F3 (N-CO) plane set TD1 to 1 and use ZGOPTNS -DLABEL_F2 flag to allow virtual decoupling of CO-CA splitting with 'splitcomb'.

Use 'splitcomb ipap 2' to process full spectrum in TopSpin.

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, 13C and 15N 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**. If possible, prior acquisition of a **2D 15N HSQC, 13C HSQC** and **3D HNCO** are also recommended, according to protocols **HSQC_15N.nan, 13C_HSQC** and **HNCO_3D.nan.**

General aspects of the use of the **bioTop** module are described in more detail in the protocols listed below.

It is recommended to calibrate 1H carrier offset, 1H H2O selective flip-back pulse, as well as 1H, 13C, and 15N 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 15N and 13C 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 C_hCANCO_IPAP_3D experiment file

1 Start with existing dataset containing 1D proton or 15N-HSQC data for this sample.

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

1.2 To 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 C_hCANCO_IPAP_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:
-on the Acquire menu, click Tune (or type atma on command line).

Calibrate pulses and adjust parameters

2

Note

Loading the C_hCANCO_IPAP_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 13C and 15N 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) Or use **bioTop** module that organizes calibrated and defined parameters for a dataset.

2.1 **Using 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]
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 15N and 13C pulse widths and power levels from the 'edprosol' table, and are assumed to be sufficiently accurate.

Skip to step 2.3.

2.2 **Use 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 Parameters

2.3 Examine parameters by typing **'eda'**, or select the 'Acqpars' tab and get the 'eda' window by clicking on the '**A**' icon. This view shows the three dimensions, F3 (13C), F2 (15N) and F1 (13C) in columns.
Parameters to check:

3 TD: Number of ^{13}CO time domain real points (~512-1024, preferably 2^N, keep **3 AQ** at ~50-100 ms to minimize heating from ^{1}H and ^{15}N decoupling)
2 TD: Number of ^{15}N time domain real points (CT dimension). **2 AQ** ~32 ms (TopSpin 4.x), or ~6
1 TD - Number of ^{13}CA time domain real points (CT dimension). **1 AQ** ~26 ms (TopSpin 4.x) or ~52 ms (TopSpin 3.x and ZGOPTNS flag not set, inner IPAP loop counts toward the TD points).
3 SW: ^{13}CO spectral width (~20 ppm, defined in **BioTop**)
2 SW: 15N amide spectral width (~46 ppm, defined in BioTop)
1 SW - ^{13}CA spectral width (~42 ppm, defined in **BioTop**)
O1P: ^{13}CO offset (~173 ppm, defined in **BioTop**)
O2P: ^{1}H offset (~4.7 ppm, defined in **BioTop**)
O3P: 15N amide offset (~122 ppm, defined in bioTop)

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: recyle delay (~1 s for protonated samples, ~2-3 s for perdeuterated samples)
DS: 32-128 'dummy' scans that are not recorded ; allows system to reach steady state equilibration.
NS: multiple of 4; increase for increased signal to noise (S/N increases as √NS)

channel f1:
CNST21 - ^{13}CO offset (~173 ppm, same as **O1P**, defined in **BioTop**)
CNST22 - ^{13}CA offset (~48 ppm, defined in **BioTop**)
channel f2:
CPDPRG2 - ^{1}H decoupling (garp)
channel f3:
P21 - 15N 90º high power pulse (calibrated with BioTop)
CPDPRG3 - ^{15}N decoupling (garp, garp4)

Acquire and Process Data

3 Type **'rga'** or click on 'Gain' in Topspin Acquire menu to execute automatic gain adjustment.
Type **'expt'** to calculate the expected run time
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 2D planes with NUS or uniformly sampled can be processed with Topspin: **xfb**

Protocol references

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'nusPGSv8' written by Scott Anthony Robson 2013