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Bio - hCANCO 3D V.2

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Songlin Wang[1]

[1]NMRFAM, University of Wisconsin-Madison

NMRFAM Facility
UW-Madison

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We use this protocol and it's working

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Abstract

Purpose
Three-dimensional hCANCO dipolar correlation experiment at moderate spinning rate.

Scope
Inter-residue (CAi, Ni, COi-1) backbone chemical shift correlations of CA, N and CO atoms. 3D hCANCO is used for sequential assignment of 15N and 13C chemical shifts in conjugation with 3D hNCACX and hNCOCX.

Checklist for setting hCANCO3d experiment:
1. Load parameters file or previous standard experiment acquired using the same spectrometer, probe, and spinning rate (if exist).
2. Updated the optimized parameters (p14, plw14, p13, plw13, p15, plw15, p43, spnam41, cnst41, spnam31, cnst31, p35, spnam32, cnst32, spnam52, cnst52, p53, spnam33, cnst33, spnam53, cnst53, p38, spnam38, cnst38, cpdprg4, p24, and cnst24).
3. Set carrier frequencies for each channel (O1p, O2p, O3p, cnst16, cnst17).
4. Set acquisition time (aq), dwell time (dw), spectral width (sw), and complex points (TD) for each dimension.
5. Set recycle delay (d1)
6. Set NUS schedule if applicable
7. Set scan numbers (NS)

Guidelines

This SOP is written based on the pulse sequence developed at NMRFAM. If using other pulse sequences, the strategy is still applicable, but the parameter names will be different. Please refer to the schematic pulse sequence at section 6.10 to find corresponding parameter names if using other pulse sequences

Materials

Definitions:

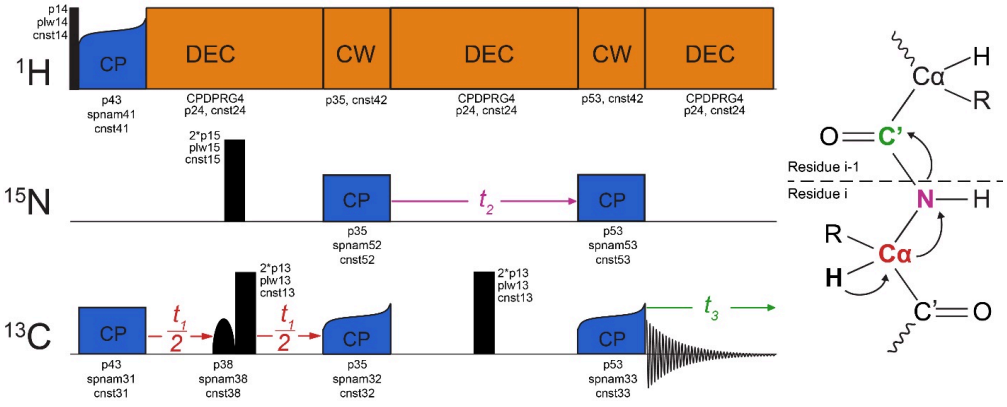
	Term	Definition
	hCANCO	1H-13C CP based CANCO 3D correlation experiment
	MAS	Magic Angle Spinning
	CP	Cross Polarization
	NUS	Non-Uniform Sampling

Instrument: The hCANCO3d example dataset in this SOP was acquired using a 600 MHz NMR spectrometer with Bruker Avance III console. The probe is a Phoenix 1.6 mm HCN probe. For the best results, this experiment should be executed after properly adjusting the shimming and magic angle.

Sample:

1. Type: Protein
2. Labeling: Fully protonated U-^{13}C,^{15}N labeling (default), or ^{13}C-glycerol skip-labeled (alternative conditions where noted). Not applicable to perdeuterated samples.
3. Minimum amount: 100 nmol (3.2 mm rotor) or 50 nmol (1.6 mm rotor)

hCANCO3d schematic pulse sequence:



Example Parameter set for a Phoenix 1.6 mm probe using 13.333 kHz MAS on 600 MHz Bruker spectrometer (pulse sequence: CNC3d-NAN):

Pulse Parameters:

^{1}H 90^{o}: (p14, plw14, cnst14) = (2.5 ms, 43W, 100 kHz)
^{13}C 90^{o}: (p13, plw13, cnst13) = (2.5 ms, 150W, 100 kHz)
^{15}N 90^{o}: (p15, plw15, cnst15) = (5 ms, 125W, 50 kHz)

HCA CP: (p43, spnam41, cnst41, spnam31, cnst31)
= (0.75 ms, tan70to100, 95 kHz, cw, 60 kHz)

CAN CP: (p35, cnst42, spnam32, cnst32, spnam52, cnst52)
= (7.5 ms, 100 kHz, tan70to100, 23.2 kHz, cw, 33.33 kHz)

NCO CP: (p53, cnst43, spnam33, cnst33, spnam53, cnst53)
= (11.4 ms, 100 kHz, tan70to100, 21 kHz, cw, 33.33 kHz)

CA selective pulse: (p38, spnam38, cnst38)
= (262.5 ms, RSNOB, 8.2 kHz)

^{1}H-decoupling - (CPDPRG4, p24, cnst24)
= (SPINAL64_p24, 5.2 ms, 90 kHz).

Acquisition Parameters:
MAS rate (w_r) in Hz: cnst20 =13333
Rotor period (t_r): d20 = (1/cnst20)=75 ms
^{1}H carrier frequency: O2p = 5 ppm
Number of scans: ns =16
Recycle delay: d1 =1.0 sec

^{13}C Direct dimension: t_3 or F3:
Carrier frequency: O1p = 175 ppm
Acquisition time: aq(F3) = 13.6 ms
Spectral width: sw(F3) = 497.287 ppm

^{13}CA indirect dimension: t_1 or F1:
Carrier frequency: 55 ppm
FnMODE: States-TPPI
Maximum evolution time: aq(F1) = 7.2 ms
Spectral width: sw(F1) = 29.5 ppm
Complex t_2 points: TD (F1) = 64

^{15}N indirect dimension: t_2 or F2:
Carrier frequency: O3p = 118 ppm

FnMODE: States-TPPI
Maximum evolution time: aq(F2) = 10.5 ms
Spectral width: sw(F2) = 43.9 ppm
Complex t_1 points: TD (F2) = 56

Safety warnings

Operator: User should be knowledgeable to operate MAS probes and use Topspin. User should know the power limit of the MAS probe being used.

Before start

MAS rate: 13.333 kHz at 600 MHz, 16.667 kHz at 750 MHz, or 20 kHz at 900 MHz (~88.9 ppm in ^{13}C).

Temperature: As determined for optimal sample sensitivity and resolution.

Below optimizations are required before setting up the hCANCO3d experiment (parameters needs to be updated are shown in parenthesis). Example SOP for each optimization is attached at References. Note that rf powers in this pulse sequence defined using kHz rather than Watt (i.e., input kHz number for rf powers and the code will calculate the corresponding Watt numbers for Topspin to use). Only the hard pulses for calibration need to be input as Watt (plw13, plw14, and plw15).

1. Calibration of ^{1}H, ^{13}C, and ^{15}N solid pulses (p14, plw14, p13, plw13, p15, and plw15)
2. Optimization of HCA CP (p43, spnam41, cnst41, spnam31, and cnst31)
3. Optimization of CAN CP (p35, spnam32, cnst32, spnam52, and cnst52)
4. Optimization of NCO CP (p53, spnam33, cnst33, spnam53, and cnst53)
5. Optimization of CA selective pulse (p38, spnam38, cnst38)
6. Optimization of high-power ^{1}H decoupling (cpdprg4, p24, and cnst24)

Setup time: ~15 min, presuming initial calibrations in the cited SOPs have already been completed.

Procedure

1 Load pulse program and parameter set. “CNC3d-NAN” pulse program and parameter set “hCANCO3d-NAN_par”.

1.1 If a previous hCANCO3d experiment acquired using the same setup is available (i.e., same spectrometer,
probe, pulse program and MAS rate), open the previous dataset and type “edc”. Input the directory for the new experiment and press “OK”.

1.2 If no previous data available, then use Topspin commend “edc” to open a new experiment. Input “CNC3d-NAN” as PULPROG. Type “rpar”, then load “hCANCO3d-NAN_par”.

2 Set the ^{1}H carrier frequency (O2p) at ~5 ppm, ^{13}C carrier frequency (O1p) at 175 ppm, and ^{15}N carrier frequency (O3p) at 118 ppm. For example, type “O1p” and enter, then input 175 to the opened window.

3 Optimize the pulse sequence parameters. The optimization needs to be done are listed in the "Before start" section under "Guidelines & Warnings". Example SOPs for the required optimization are attached at "References". Note that the parameters can be optimized use different methods/pulse sequences. Please consult to your facility manager to perform the optimization.

4 Set the carrier frequencies of ^{13}C channel.

4.1 Set cnst16 to 175 ppm. This constant is used as the frequency for detection and 3rd NCO CP. Type “cnst16”, then input 175.

4.2 Set cnst17 to 55 ppm. This constant is used as the frequency for the 1st hCA CP, 2nd CAN CP, and the soft selective pulse for CA. Type “cnst17”, then input 55.

5 Set the acquisition parameters.

5.1 Direct dimension (t_3 or F3)

1. Default acquisition time: depending on ^{13}C T_2, default is ~20ms (alternative for ^{13}C-glycerol: ~30 ms).
2. Default spectral width: ~300 ppm.
3. Default carrier frequency: ~175 ppm.

5.2 Indirect ^{13}C dimension (t_1 or F1)

1. Increment of delay (IN_F, s): n * τ_r. For a given MAS rate, adjust the increment of delay to integer multiples of τ_r that covers at least 30 ppm ^{13}C spectral width.
2. Spectral width (SW, ppm): coupled with increment of delay, >30 ppm is required to cover the full spectrum
3. Carrier frequency (O1p. ppm): 175 ppm.
4. Hypercomplex scheme (FnMODE): States-TPPI
5. Maximum evolution time (AQ, s): depending on ^{13}C T_2, the default is ~10 ms. The evolution time equals to (Increment of delay) * (TD). Once Increment of time is fixed as described above, change number of TD to adjust the total evolution time.

5.3 Indirect ^{15}N dimension (t_2 or F2)

1. Increment of delay (IN_F, s): n * τ_r. For example, 375 ms can be used for 13.33 kHz MAS on 600 MHz. For a given MAS rate, adjust the Increment of delay to integer multiples of τ_r that covers at least 40 ppm ^{15}N spectral width.
2. Spectral width (SW, ppm): coupled with increment of delay, >40 ppm is required to cover the full spectrum.
3. Carrier frequency (O3p, ppm): 118 ppm.
4. Hypercomplex scheme (FnMODE): States-TPPI
5. Maximum evolution time (AQ, s): depending on ^{15}N T_2, the default is ~10 ms. The evolution time equals to (Increment of delay) * (TD). Once increment of delay is fixed as described above, change number of TD to adjust the total evolution time.

6 Set the recycle delay

6.1 Use recycle delay of 1.3*T_1 for maximum sensitivity per unit time. If ^1H T_1 is not measured, use the default value which is 2 s. However, measuring ^1H T_1 before setting this experiment is highly recommended.

6.2 Due to high-power ^1H decoupling is applied, don't set recycle delay less than 1s.

7 Set NUS schedule. This step is optional, but 25% NUS is recommended for 3D experiments.

8 Adjust measurement time as required by incrementing number of scans in multiples of 16.

9 Validation

9.1 Start the experiment and monitor the first ~20-30 rows

9.2 Process first dimension FT to check for adequate signal correctly arraying indirect dimension.

Protocol references

Reference: J. Pauli, M. Baldus, B. van Rossum, H. de Groot, H. Oschkinat, Backbone and side-chain ^{13}C and ^{15}N signal assignments of the aspectrin SH3 domain by magic angle spinning solid-state NMR at 17.6 Tesla, ChemBioChem 2 (2001) 272–281. **https://doi.org/10.1002/1439-7633(20010401)2:4<272::AID-CBIC272>3.0.CO;2-2**

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SOP: **2.2.1.1 1H/13C/15N 90o pulse width calibration**

SOP: **2.2.1.2.1 1H-13C/1H-15N Cross Polarization**

SOP: **2.2.1.3.1.1 1H high-power decoupling**

SOP: **2.2.2.1.1.7.1 15N-13C specific CP**

SOP **2.2.1.1.2.1.1 13C selective pulse optimization**

SOP **2.2.2.1.1.1.1 1H T1 Measurement**

SOP **2.2.2.1.1.2.2.1 13C T2 Measurement**

Example Data at 1.1 GHz: usnan.org/ark:/83454/e1e8225078-71b2-4324-ad10-1a2677c0f2c4
Example Data at 900 MHz: usnan.org/ark:/83454/e1030c4007-9f70-4a75-9ada-ebcb7586ee57
Example Data at 600 MHz: usnan.org/ark:/83454/e1b46545e5-63fe-4f6a-b63a-a63401a4b004