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Bio - hNCOCX 3D V.2

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We use this protocol and it's working

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Abstract

Purpose

Three-dimensional hNCOCX dipolar correlation experiment with CC DARR mixing at moderate spinning rate.

Scope

Inter-residue (Ni, COi-1, CXi-1) backbone and sidechain chemical shift correlations of N, CO and CX atoms using DARR mixing times of 25 to 50 ms. Inter-residue correlations with moderate mixing times of 50-200 ms. 3D hNCOCX is used for sequential assignment of ¹⁵N and ¹³C chemical shifts in conjugation with 3D hNCACX and/or hCANCO.

Checklist for setting hNCOCX3d 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, p45, spnam41, cnst41, spnam51, cnst51, p53, spnam32, cnst32, spnam52, cnst52, p38, spnam38, cnst38, cpdprg4, p24, and cnst24).
3. Set carrier frequencies for each channel (o1p, o2p, o3p).
4. Set desired DARR rf-power and mixing time (cnst5, d5)
5. Set acquisition time (aq), dwell time (dw), spectral width (sw), and complex points (TD) for each dimension.
6. Set recycle delay (d1)
7. Set NUS schedule if applicable
8. 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:

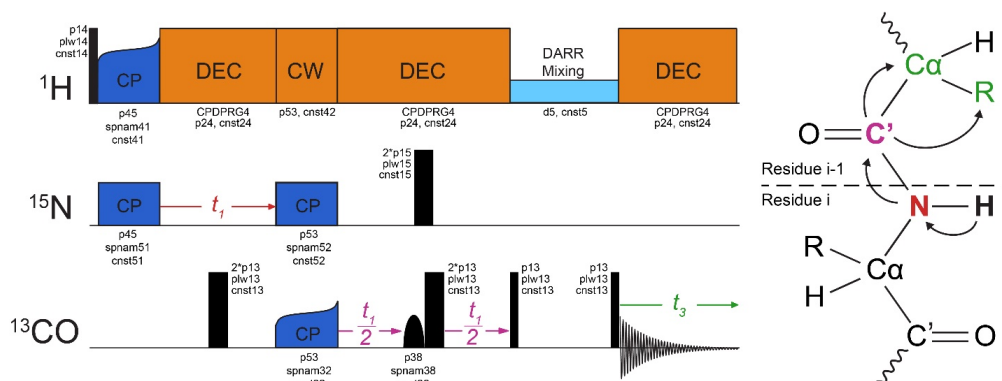
	Term	Definition
	hNCOCX	^1H - ^{15}N CP based NCOCX 3D correlation experiment
	DARR	Dipolar assisted rotational resonance
	MAS	Magic angle spinning
	CP	Cross polarization
	NUS	Non-uniform sampling

Instrument: The hNCOCX3d 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: ~ 50 nmol for 1.6 mm rotor

hNCOCX3d 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: NCC3d-NAN):

Pulse Parameters:

^1H 90°: (p14, plw14, cnst14) = (2.5 ms, 43W, 100 kHz)

^{13}C 90°: (p13, plw13, cnst13) = (2.5 ms, 150W, 100 kHz)

^{15}N 90°: (p15, plw15, cnst15) = (5 ms, 125W, 50 kHz)

HN CP: (p45, spnam41, cnst41, spnam51, cnst51)
= (1.8 ms, tan70to100, 69 kHz, cw, 33.33 kHz)

NCO CP: (p53, cnst42, spnam32, cnst32, spnam52, cnst52)
= (11.4 ms, 100 kHz, tan70to100, 21 kHz, cw, 33.33 kHz)

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

DARR mixing: (d5, cnst5) = (50 ms, 13.333 kHz)

^1H -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

^1H carrier frequency: O2p = 10 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) = 497.278 ppm

^{15}N indirect dimension: t_1 or F1:

Carrier frequency: O3p = 118 ppm

FnMODE: States-TPPI

Maximum evolution time: aq(F1) = 10.5 ms

Spectral width: sw(F1) = 43.9 ppm

Complex t1 points: TD (F1) = 56

^{13}C indirect dimension: t_2 or F2:

Carrier frequency: 175 ppm

FnMODE: States-TPPI

Maximum evolution time: aq(F1) = 7.2 ms

Spectral width: sw(F2) = 22.1 ppm

Complex t2 points: TD (F2) = 48

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). Note that the transfer efficiency of DARR decreases significantly when MAS > 30 kHz

Temperature: As determined for optimal sample sensitivity and resolution.

Below optimizations are required before setting up the hNCOCX3d 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 ^1H , ^{13}C , and ^{15}N solid pulses (p14, plw14, p13, plw13, p15, and plw15)
2. Optimization of HN CP (p45, spnam41, cnst41, spnam51, and cnst51)
3. Optimization of NCO CP (p53, spnam32, cnst32, , spnam52, and cnst52)
4. Optimization of CO selective pulse (p38, spnam38, cnst38)
5. Optimization of high-power ^1H decoupling (cpdprg4, p24, and cnst24)

Setup time: ~15 min, presuming above optimizations have already been completed.

Procedure

- 1 Load pulse program and parameter set. "NCC3d-NAN" pulse program and parameter set "hNCOCX3d-NAN_par".
 - 1.1 If a previous hNCOCX3d 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". In put 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 "NCC3d-NAN" as PULPROG. Type "rpar", then load "hNCOCX3d-NAN_par".
- 2 Set the ^1H 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 DARR mixing. Set the DARR mixing.
 - 4.1 Set the DARR ^1H condition. The ^1H rf power for DARR mixing should match the MAS rate. For the pulse sequence shown at "Materials", the ^1H rf power for DARR mixing is cnst5. The unit of cnst5 is kHz.
 - 4.2 Choose DARR mixing time
 1. For short range intra-residue correlations, set the DARR mixing to 10-50 ms which is optimal for sequential correlation of 2D planes along with other 3D data sets (i.e., hNCACX3d and hCANCO3d).
 2. For medium range inter-residue correlations set the DARR mixing to 50-200 ms.
 3. For long range ($>5 \text{ \AA}$) correlations set the DARR mixing to $>200 \text{ ms}$.
 4. The parameter name of DARR mixing time is d5 and the default unit is s. For example, if 50 ms is used, type "d5" and input 0.05. Optimal mixing times increase with magnetic field.
- 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 ~20 ms (alternative for ^{13}C -glycerol: ~30 ms).
2. Default spectral width: ~300 ppm.
3. Default carrier frequency: 175 ppm.

5.2 Indirect ^{15}N dimension (t_1 or F1)

1. Increment of delay (IN_F, s): $n * \tau_r$. For example, 375 ms can be used for 13.33 kHz MAS on 600 MHz. For a given MAS rate, adjust the dwell time to integer multiples of τ_r that covers at least 40 ppm ^{15}N spectral width.
2. Spectral width (SW, ppm): coupled with dwell time, >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.

5.3 Indirect ^{13}C dimension (t_2 or F2)

1. Increment of delay (IN_F, s): $n * \tau_r$. For a given MAS rate, adjust the dwell time to integer multiples of τ_r that covers at least 30 ppm ^{13}C spectral width.
2. Spectral width (SW, ppm): coupled with dwell time, >20 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 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 increasing 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 aspartate 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](https://doi.org/10.1002/1439-7633(20010401)2:4<272::AID-CBIC272>3.0.CO;2-2)

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/e179fa9177-8814-4614-b143-cff220e6bb99

Example Data at 900 MHz: usnan.org/ark:/83454/e1ec5e6563-e710-40f3-a1b6-6d088b904ac5

Example Data at 600 MHz: usnan.org/ark:/83454/e19da795c9-de23-492c-8738-a2f822bfc0f4