Jul 08, 2025 Version 2

Bio - hCONH 3D V.2

DOI
https://dx.doi.org/10.17504/protocols.io.4r3l2q28jl1y/v2

Songlin Wang[1]

[1]NMRFAM, University of Wisconsin-Madison

NMRFAM Facility
UW-Madison

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Protocol Citation: Songlin Wang 2025. Bio - hCONH 3D. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l2q28jl1y/v2Version created by **NMRFAM Facility**

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

Created: July 08, 2025

Last Modified: July 08, 2025

Protocol Integer ID: 221999

Keywords: 3D hCONH, hconh 3d heteronuclear, hconh3d experiment, backbone chemical shift correlations of co, setting hconh3d experiment, hconh 3d purpose, 3d hconh, backbone chemical shift correlation, 3d hcanh, dipolar correlation 1h detection experiment, hconh, 1h chemical shift, 1h chemical shifts in conjugation, chemical shifts in conjugation, chemical shift, spinning rate, 1h detection, fast spinning rate, cnst41, same spectrometer, cnst32

Funders Acknowledgements:
National Science Foundation
Grant ID: 1946970

Abstract

Purpose
hCONH 3D heteronuclear dipolar correlation 1H detection experiment at fast spinning rate.

Scope
Intra-residue (COi-1, Ni, Hi) backbone chemical shift correlations of CO, N and H atoms. 3D hCONH is used for sequential assignment of 15N, 13C, and 1H chemical shifts in conjugation with 3D hCANH and 3D hCA(CO)NH experiments.

Checklist for setting hCONH3d 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, p54, spnam53, cnst53, spnam43, cnst43, p38, spnam38, cnst38, cpdprg4, p24, and cnst24, cpdprg3, p23, cnst23, cpdprg5, p25, cnst25, cpdprg6, p26, cnst26, and d30).
3. Set carrier frequencies for each channel (O1p, O2p, O3p).
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 under "Materials" to find corresponding parameter names if using other pulse sequences.

Materials

Definitions:

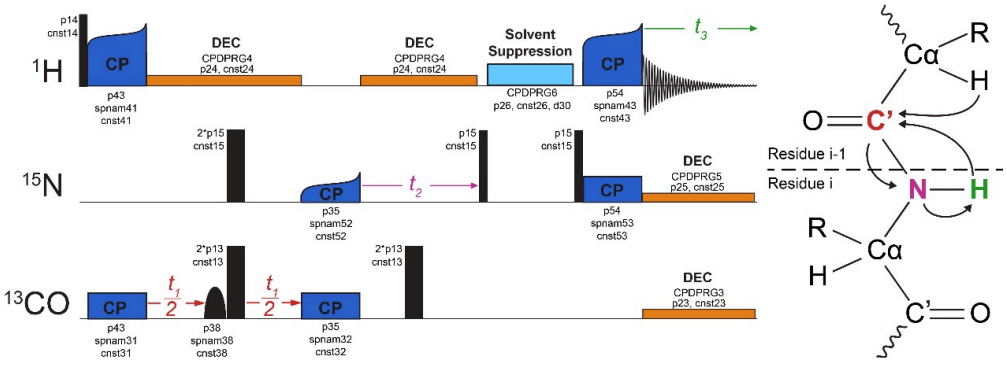
	Term	Definition
	hCANH	CP based HNCA 3D correlation experiment
	CP	Cross Polarization
	MAS	Magic Angle Spinning
	NUS	Non-Uniform Sampling

Instrument: The hCONH3d example dataset in this SOP was acquired using a 600 MHz NMR spectrometer with Bruker Avance III HD console. The probe used was a Phoenix 1.6mm HCN probe. For the best result, this experiment should be executed after properly adjusting the shimming and magic angle.

Sample:

1. Type: Protein
2. Labeling: Perdeuterated and ^{1}H back exchanged U-^{13}C,^{15}N, ^{2}H labeled samples (default) or fully protonated U-^{13}C,^{15}N labeling samples at spinning speed higher than 60 kHz.
3. Minimum amount: ~50 nmol for 1.6 mm rotor

hCONH3d schematic pulse sequence:



Example parameter set for a Phoenix 1.6mm probe using 38.462 kHz MAS on 600 MHz Bruker spectrometer (pulse sequence hCNH3d-NAN). Sample is 25% ^{1}H back exchanged, ^{15}N, ^{13}C, ^{2}H -GB1 protein:

Pulse Parameters:

^{1}H 90^{o}: (p14, cnst14) = (2.5 µs, 100 kHz)

^{13}C 90°: (p13, cnst13) = (2.5 µs, 100 kHz)

^{15}N 90°: (p15, cnst15) = (5 µs, 50 kHz)

HCA CP: [p43, cnst41(^{1}H), cnst31(^{13}C)]
= [5.0 ms, 77 kHz (tan70to100up.1000), 27 kHz (cw)]

CAN-CP: [p35, cnst32 (^{13}C), cnst52 (^{15}N)]
= [10.0 ms, 21 kHz (cw), 19 kHz (tan70to100up.1000)]

NH-CP: [p54, cnst43 (^{1}H), cnst53 (^{15}N)]
= [1.0 ms, 54.5 kHz (tan70to100up.1000), 11 kHz (cw)]

CA selective pulse: [p38, cnst38 (RSNOB)]
= [273 µs, 9 kHz]

^{1}H-decoupling - (CPDPRG4, p24, cnst24)
= (SPINAL64_p24, 184 µs, 2.5 kHz)

^{13}C-decoupling - (CPDPRG3, p23, cnst23)
= (WALTZ16_p23, 50 µs, 10 kHz)

^{15}N-decoupling - (CPDPRG5, p25, pl25)
= (WALTZ16_p25, 50 µs, 10 kHz)

^{1}H-solvent suppression - (CPDPRG6, p26, cnst26, d30)
= (MISSISSIPI_p26, 50 ms, 10 kHz, 0.2s)

Acquisition Parameters:

MAS rate (w_r) in Hz: cnst20 = 38.462 kHz

Rotor period (t_r): d20 = (1/cnst20) = 26.0 µs

^{1}H carrier frequency: O1p = 6 ppm
Number of scans: ns = 4
Recycle delay: d1 = 1 sec

^{1}H Direct dimension: t_3 or F3:

Carrier frequency: O1p = 6 ppm
Acquisition time: aq(F3) = 28 ms
Spectral width: sw(F3) = 30.5 ppm

CA indirect dimension: t_1 or F1:

Carrier frequency: O2p = 175 ppm

Maximum evolution time: aq (F1) = 18.3 ms
Spectral width: sw (F1) = 19.6 ppm
Total t_1 points: TD (F1) = 108
Complex t_1 points: 54

^{15}N indirect dimension: t_2 or F2:
Carrier frequency: O3p = 118 ppm
Maximum evolution time: aq (F2) = 13.1 ms
Spectral width: sw (F2) = 35.2 ppm
Total t_2 points: TD (F2) = 56
Complex t_2 points: 28

Safety warnings

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

Before start

MAS rate: ~40 kHz for 1.6 mm rotor or ~60 kHz for 1.2 mm rotor.

Temperature: As determined for optimal sample sensitivity and resolution.

Below optimizations are required before setting up the hCONH3d 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 HCO CP (p43, spnam41, spnam31, cnst41, and cnst31)
3. Optimization of CON CP (p35, spnam32, spnam52, cnst32, and cnst52)
4. Optimization of NH CP (p54, spnam53, spnam43, cnst53, and cnst43)
5. Optimization of CO selective pulse (p38, spnam38, cnst38)
6. Optimization of low-power ^{1}H decoupling (cpdprg4, p24, and cnst24)
7. Optimization of low-power ^{13}C decoupling (cpdprg3, p23, and cnst23)
8. Optimization of low-power ^{15}N decoupling (cpdprg5, p25, and cnst25)
9. Optimization of ^{1}H solvent suppression pulse (cpdprg6, p26, cnst26, and d30)

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

Procedure

1 Load “hCNH3d-NAN” pulse program and parameter set “hCONH3d-NAN_par”.

1.1 Use Topspin command “edc” to open a new experiment.

1.2 Input “hCNH3d-NAN” as PULPROG

1.3 Type “rpar”, then load file “hCONH3d-NAN_par”.

1.4 Note that the parameters will be off if using different spectrometers/probes. Please consult to your facility manager/staff to get proper starting parameter set.

2 Set the ^{1}H carrier frequency at ~5ppm (for the best water suppression performance). Set the ^{13}C carrier frequency at ~175 ppm, and ^{15}N carrier frequency at ~ 118 ppm.

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 acquisition parameters.

4.1 Direct ^{1}H dimension (t_3 or F3)

1. Default acquisition time: ~50 ms.
   Note that the acquisition time depends on the ^{1}H T_2. However, the low power decoupling allows us use longer acquisition time. So, we recommend acquiring ~50 ms if the ^{1}H T_2 is unknown. The FID can be truncated using data processing software (Topspin or NMRPipe) readily
2. Default spectral width: ~30 ppm.

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

1. Increment of delay (IN_F, s): n * t_r.
   For example, 182 µs can be used for 38.462 kHz MAS on 600 MHz. For a given MAS

rate, adjust the dwell time to integer multiples of t_r that covers at least 20 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 (O2p, ppm): 175 ppm.
4. Hypercomplex scheme (FnMODE): States-TPPI
5. Maximum evolution time (AQ, s): depending on ^{13}CO 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.

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

1. Increment of delay (IN_F, s): n * t_r.

   For example, 468 µs can be used for 38.462 kHz MAS on 600 MHz. For a given MAS rate, adjust the dwell time to integer multiples of t_r that covers at least 40 ppm ^{15}N spectral width.
2. Spectral width (SW, ppm): coupled with dwell time, >30 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 Set the recycle delay

5.1 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 for best practice.

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

7 Adjust measurement time as required by increasing number of scans in multiples of 16.

8 Validation

8.1 Start the experiment and monitor the first ~20-30 rows.

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

Protocol references

Reference: **Barbet-Massin et al. *J.Am.Chem.Soc.*, 2014, 136:12489**

Reference: **Zhou et al. J.AM.Chem.Soc., 2007, 129:11791**

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:**HN_NH_CP_optimization_1Hdetection_fast MAS**

SOP: **1H_15N_Dec_OPT_1Hdetection_fastMAS**

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/e10800837e-d1c7-4d2a-baa2-9e01112e75d5
Example Data at 900 MHz: usnan.org/ark:/83454/e132376708-2d6b-487b-85c7-130634fed13e
Example Data at 600 MHz: usnan.org/ark:/83454/e13e23021f-30c9-488a-bee6-816ac30e0282