Jun 25, 2025 Version 3

Bio - hNH 2D V.3

DOI
https://dx.doi.org/10.17504/protocols.io.rm7vzjx88lx1/v3

Songlin Wang[1]

[1]NMRFAM, University of Wisconsin-Madison

NMRFAM Facility
UW-Madison

DOI: https://dx.doi.org/10.17504/protocols.io.rm7vzjx88lx1/v3

Protocol Citation: Songlin Wang 2025. Bio - hNH 2D. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzjx88lx1/v3Version created by **NMRFAM Facility**

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

Created: June 25, 2025

Last Modified: June 25, 2025

Protocol Integer ID: 221067

Keywords: 2D hNH, dipolar correlation 1h detection experiment, protein nmr fingerprint, hnh 2d purpose, dimensional hnh, fast spinning rate

Funders Acknowledgements:
National Science Foundation
Grant ID: 1946970

Abstract

Purpose
Two-dimensional hNH dipolar correlation 1H detection experiment at fast spinning rate.

Scope
Sample quality assessment and protein NMR fingerprint.

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:

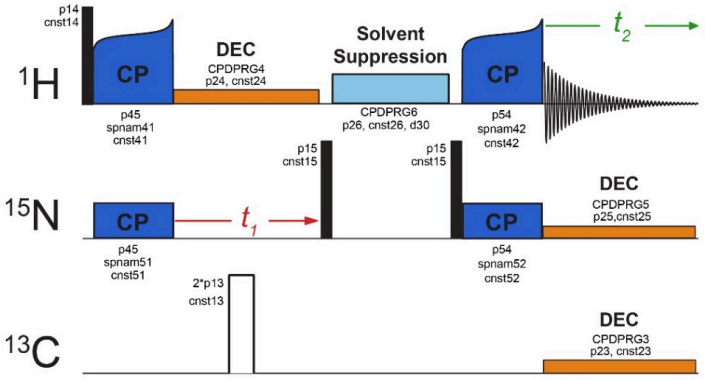
	Term	Definition
	hNH	CP based HN 2D correlation experiment
	CP	Cross Polarization
	MAS	Magic Angle Spinning

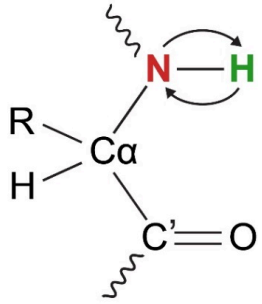
Instrument: The hNH2d experiment described in this SOP was acquired using a 600MHz NMR spectrometer with a Bruker Avance III console. The probe is a Phoenix 1.6 mm HCN probe. For the best result, this experiment should be executed after properly adjusting the shimming and magic angle.

Sample:

1. Type: Protein
2. Labeling: perdeuterated U-^{15}N labeling with ^{1}H back exchanged samples.
3. Minimum amount: ~ 50 nmol for 1.6 mm rotor

hNH2d schematic pulse sequence:





Example parameter set tested on protein sample:
Spectrometer: Fleckvieh (900 MHz) at NMRFAM
Probe: Phoenix 1.6 mm HCN probe
Spinning: 38.462 kHz
Sample: 25% ^{1}H back exchanged, ^{15}N, ^{13}C, ^{2}H -GB1

Pulse Parameters:

^{1}H 90°: (p14, cnst14) = (2.5 µs, 100 kHz)
^{13}C 90°: (p13, cnst13) = (5 µs, 50 kHz)
^{15}N 90°: (p15, cnst15) = (9.6 µs, 26.0 kHz)

HN CP: [p45, cnst41(^{1}H), cnst51(^{15}N)]
= [2.50 ms, 35.5 kHz (tan70to100), 10 kHz (cw)]

NH-CP: [p54, sp42 (^{1}H), sp52 (^{15}N)]
= [1.25 ms, 31 kHz (tan70to100), 10 kHz (cw)]

^{1}H-decoupling - (CPDPRG4, p24, cnst24)
= (SPINAL64_p24, 46 µs, 10 kHz)

^{13}C-decoupling - (CPDPRG3, p23, cnst23)
= (WALTZ16_p23, 25 µs, 10 kHz)

^{15}N-decoupling - (CPDPRG5, p25, cnst25)
= (WALTZ16_p25, 25 µs, 10 kHz)

^{1}H-solvent suppression - (CPDPRG6, p26, cnst26, d30)
= (MISSISSIPPI_p26, 50 ms, 30 kHz, 0.2 s)

Acquisition Parameters:
MAS rate (w_r) in Hz: cnst20 = 38462
Rotor period (t_r):
d20 = (1/cnst20) = 26 µs
Number of scans: ns = 4
Recycle delay: d1 = 1 sec

^{1}H Direct dimension: t_2 or F2:
Carrier frequency: O1p = 11 ppm
Acquisition time: aq(F2) = 39.9 ms
Spectral width: sw(F2) = 42.7 ppm

^{15}N indirect dimension: $t_1$or F1:
Carrier frequency: O3p = 120 ppm
Maximum evolution time: aq(F1) = 41.6 ms
Spectral width: sw(F1) = 42.181 ppm
Dwell time: IN_F (F1) = 260 µs

Total t_1 points: TD (F1) = 160

Complex t_1 points: 320

Safety warnings

Operator: User should be knowledgeable to operate MAS probes and use Topspin

Before start

MAS rate: 35-40 kHz for 1.6 mm rotor

Temperature: Note that the temperature increases by 15 -20 °C due to frictional heating from spinning at 35-40 kHz for 1.6 mm rotors. Adjust the variable temperature (VT) control set point temperature according to achieve desired sample temperature. For microcrystals and fibrils, typical sample temperature range is 0 to 10 °C, requiring a VT set point temperature of -20 to -5 °C. For membrane proteins and/or liposomes, the desired sample temperature depends on the phase transition of interest and can vary over a wide range depending on the sample.

Below optimizations are required before setting up the hNH2d 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 HN CP (p45, spnam41, spnam51, cnst41, and cnst51)
3. Optimization of NH CP (p54, spnam52, spnam42, cnst52, and cnst42)
4. Optimization of low-power ^{1}H decoupling (cpdprg4, p24, and cnst24)
5. Optimization of low-power ^{13}C decoupling (cpdprg3, p23, and cnst23)
6. Optimization of low-power ^{15}N decoupling (cpdprg5, p25, and cnst25)
7. Optimization of ^{1}H solvent suppression pulse (cpdprg6, p26, cnst26, and d30)

Setup time: ~0.5 hours, presuming initial calibrations have already been completed.

Procedure

1 Load “hNH2d-NAN” pulse program and parameter set “hNH2d-NAN_par”.

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

1.2 Input “hNH2d-NAN” as PULPROG

1.3 Type “rpar”, then load file “hNH2d-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 ~5 ppm (for the best water suppression performance). Set the ^{13}C carrier frequency at ~100 ppm, and ^{15}N carrier frequency ~ 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_2 or F2)

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 ^{15}N dimension (t_1 or F1)

1. Increment of delay (IN_F, s): n * t_r.
   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. For example, 468 µs can be used for 38.462 kHz MAS on 600 MHz.
2. Spectral width (SW, ppm): ~ 200 ppm. Note that the ^{15}N spectral width only need to cover the chemical shift range of amide ^{15}N which is typically from 100 to 140 ppm. However, if the spectral pattern is unknown, we recommend using ~200 ppm to prevent any side-chain resonances from folding back into amide region. Adjust "Increment of delay" to change SW.
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 ~20 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(^{1}H) for maximum sensitivity per unit time. If ^{1}H T_1 is unknown, use 2 s. ^{1}H T_1 measurement is recommended if it is unknown.

6 Determine experiment time

6.1 Minimal number of scans (phase cycle limited): 4

6.2 Minimal measurement time: ~10 min

6.3 Adjust measurement time as required for sample by incrementing number of scans in multiples of 4.

7 Validation

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

7.2 Process first dimension FT (xf2) to check for adequate signal correctly arraying indirect dimension.

Protocol references

Reference: **D. Zhou, G. Shah, M. Cormos, C. Mullen, D. Sandoz, and CM. Rienstra, Proton-Detected Solid-State NMR Spectroscopy of Fully protonated Proteins at 40 kHz Magic-Angle Spinning. *J.Am.Chem.Soc.*, 2007, 129:11791**

SOP: **2.2.1.1 1H and 13C Pulse Width Optimization**

SOP:**HN_NH_CP_optimization_1Hdetection_fast MAS**

SOP: **1H_15N_Dec_OPT_1Hdetection_fastMAS**

SOP: **15N T2 measurement SOP**

SOP: **1H T1 measurement SOP**

Example Data at 1.1 GHz: usnan.org/ark:/83454/e18631b0d7-1765-42ec-a9c7-1d2b3b0b0d67
Example Data at 900 MHz: usnan.org/ark:/83454/e1a1139e47-9d2f-45f8-872c-7078460baef7
Example Data at 600 MHz: usnan.org/ark:/83454/e1679c908d-c485-4a1c-ae7d-83c75cb5c8de