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Bio - hNH 2D R1ρ V.2

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We use this protocol and it's working

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Abstract

Purpose
Pseudo-3D hNH dipolar correlation experiment with 15N T1ρ measurement at fast spinning rate for mapping the site-specific dynamics.

Scope
Site-specific dynamic measurement of protein using 1H detection.

Materials

Definitions:

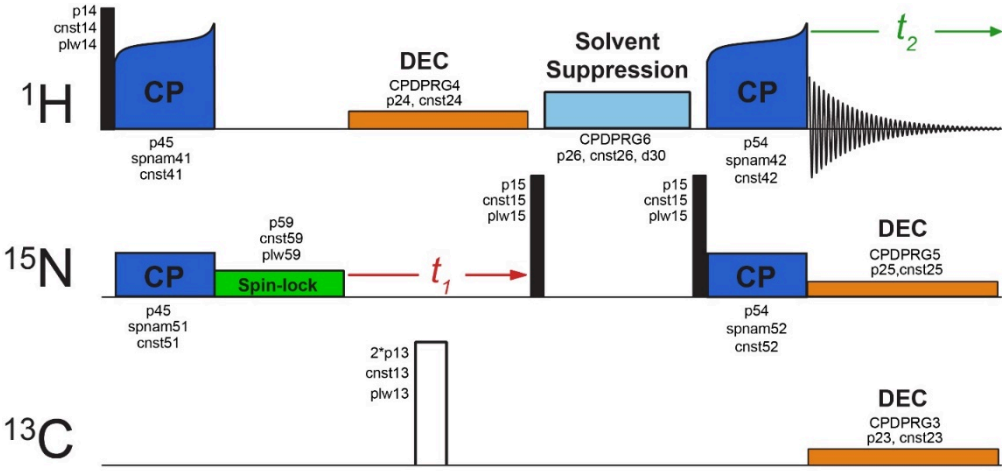
	Term	Definition
	hNH	CP based HN 2D correlation experiment
	CP	Cross Polarization
	MAS	Magic Angle Spinning

Instrument: The hNH2d-T1ρ Pseudo-3D dataset in this SOP was acquired using a 900MHz 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: 40 kHz

Sample: 25% ^{1}H back exchanged, ^{15}N, ^{13}C, ^{2}H -GB1

Pulse Parameters:

^{1}H 90°: (p14, plw14, cnst14) = (2.5 µs, 100W, 100 kHz)

^{13}C 90°: (p13, plw13, cnst13) = (5 µs, 108 W, 50 kHz)

^{15}N 90°: (p15, plw15, cnst15) = (9.6 µs, 100W, 26.0 kHz)

HN CP: [p45, spnam41, cnst41, spnam51, cnst51]
= [2.50 ms, tan70to100, 36.5 kHz, cw, 10 kHz]

NH-CP: [p54, spnam42, cnst42, spnam52, cnst52]
= [1.25 ms, tan70to100, 31.5 kHz, cw, 10 kHz]

15N spin-lock: [p59, cnst59]
= [vplist, 2.5 kHz]

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

^{13}C-decoupling - (CPDPRG3, p23, cnst23)
= (WALTZ16_p23, 100 µs, 2.5 kHz)

^{15}N-decoupling - (CPDPRG5, p25, cnst25)
= (WALTZ16_p25, 100 µs, 2.5 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 = 40

Rotor period (t_r): d20 = (1/cnst20) = 25 µs

Number of scans: ns = 8
Recycle delay: d1 = 1.5 sec

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

Carrier frequency: O1p = 9 ppm
Acquisition time: aq(F3) = 41 ms
Spectral width: sw(F3) = 111.14 ppm

^{15}N indirect dimension: t_2 or F2:

FnMode: States-TPPI

Carrier frequency: O3p = 118 ppm
Maximum evolution time: aq(F2) = 50 ms
Spectral width: sw(F2) = 43.869 ppm
Dwell time: IN_F (F2) = 250 µs
Total t_2 points: TD (F2) = 400

Spin-lock dimension: F1:
FnMode: QF
Total t_1 points: TD (F1)=9
vplist: 0.1, 36000, 72000, 108000, 144000, 216000, 288000, 360000, 540000 µs

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. τ

Below optimizations are required before setting up the Pseudo-3D hNH dipolar correlation experiment with 15N T1ρ measurement (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_T1rho” pulse program and parameter set “T1p-hNH-NAN_par”.

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

1.2 Input “hNH2d_T1rho” as PULPROG

1.3 Type “rpar”, then load file “T1p-hNH-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 Follow SOP to optimize and set all parameters for hNH2d experiment. Note that the F3 dimension in this pseudo-3D corresponds to F2 dimension of hNH 2D, and the F2 dimension in this pseudo-3D corresponds to F1 dimension of hNH 2D.

3 Set the spin-lock power.

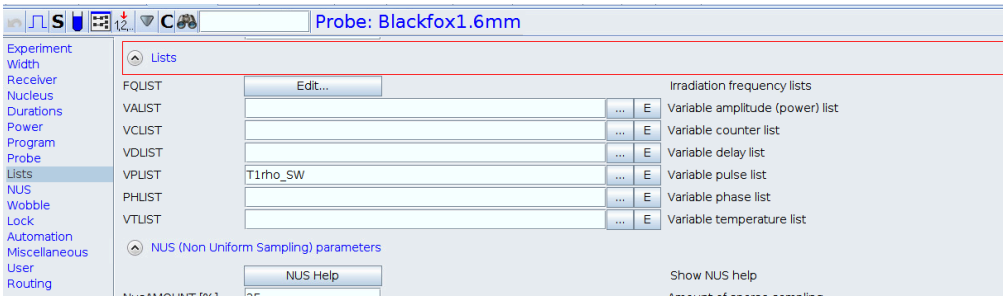
3.1 The parameter for spin-lock rf amplitude is cnst59. The unit is kHz. The default power is 10 kHz.

3.2 The signal decay will be slower as the spin-lock power increase when the power is smaller than half of the spinning speed (1/2 ω_R, Bloch-McConnell relaxation), while the signal decay will be dramatically faster when the spin-lock power is close to the spinning speed (ω_R, near-rotary-resonance relaxation). Note that this is a general observation since the signal decay also depends on the dynamic of the sample.

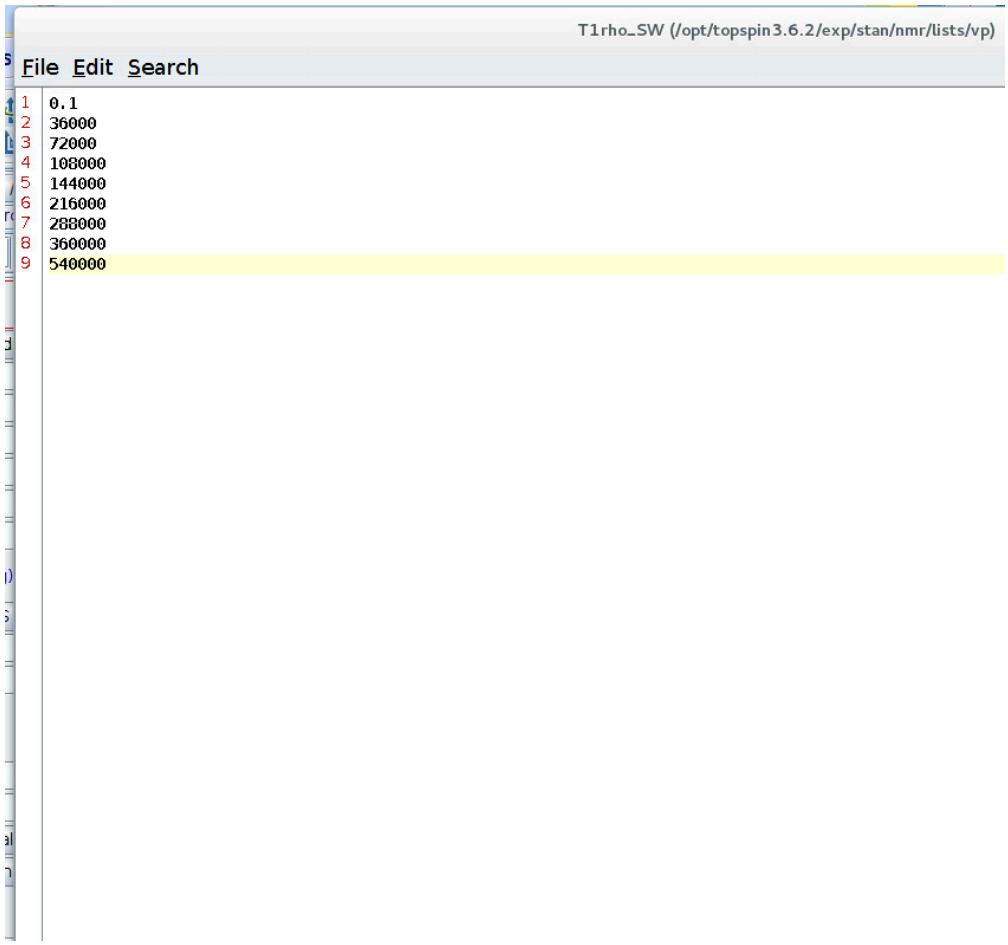
3.3 When set cnst59, check plw59 as well which corresponds to the rf power in unit of Watts. Note that the spin-lock duration could be long (e.g. a few hundreds milliseconds), so using high rf power for spin-lock may damage the sample and probe. So, the plw59 should not be a large number (<10W is recommended for sample safety if long spin-lock durations are used). Please consult with your facility staff to find out the best combination of the spin-lock power and duration.

4 Make a vplist for the durations of spin-lock

4.1 Input a list name in “VPLIST”. For example, T1rho_SW is the vplist for the example dataset.



4.2 After input a name, press the “E” button to edit the list. Input all the spin-lock durations for the $T_{1\rho}$ measurement. Note that the unit is micro-second, and each number is one line. See below example. Save and quite after that.



5 Set F1 dimension regarding the vplist.

5.1 Set FnMODE as “QF”

5.2 Set TD as the number of spin-lock durations in the vplist (e.g. 9 for the above example).

5.3 The experiment will generate a series of hNH 2D spectra with different spin-lock durations and store them in a Pseudo-3D dataset. The number of hNH 2D spectra corresponds to the number of spin-lock durations in the vplist.

6 Determine experiment time

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

6.2 Note that a decrease of the hNH 2D sensitivity is expected as the spin-lock duration increase. For better sensitivity, adjust measurement time as required 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 the first hNH 2D spectrum to check for adequate signal correctly arraying indirect dimension.

Protocol references

Reference: **P. Rovo, CA. Smith, D. Gauto, BL. de Groot, P. Schanda, and R. Linser, Mechanistic insight into microsecond time-scale motion of solid proteins using complementary ^{15}N and ^{1}H relaxation dispersion techniques. *J.Am.Chem.Soc.*, 2019, 141:858**

SOP: **hNH2d setup SOP**

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/e10413fea8-c2fc-448b-89c8-311a915919f0
Example Data at 900 MHz: usnan.org/ark:/83454/e1c9700717-6709-4488-8e79-1dda3681f24e
Example Data at 600 MHz: usnan.org/ark:/83454/e16f3e52c4-7235-427a-ba02-67533d30cd6a