Jul 08, 2025 Version 2

Bio - 2H/13C/13C Respiration CP 3D V.2

DOI
https://dx.doi.org/10.17504/protocols.io.j8nlk8o45l5r/v2

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Protocol Citation: Songlin Wang 2025. Bio - 2H/13C/13C Respiration CP 3D . **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlk8o45l5r/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: 222005

Keywords: 2H/13C/13C Respiration CP 3D , Protein dynamic, respiration cp 3d, specific dynamic measurement of protein, using respiration cp, respiration cp, specific dynamics for protein, 3d correlation experiment, protein

Funders Acknowledgements:
National Science Foundation
Grant ID: 1946970

Abstract

Purpose
2H/13C/13C 3D correlation experiment using Respiration CP for 2H/13C transfer and RFDR for 13C/13C transfer. This experiment is used to mapping the site-specific dynamics for proteins.

Scope
Site-specific dynamic measurement of protein.

Materials

Definitions:

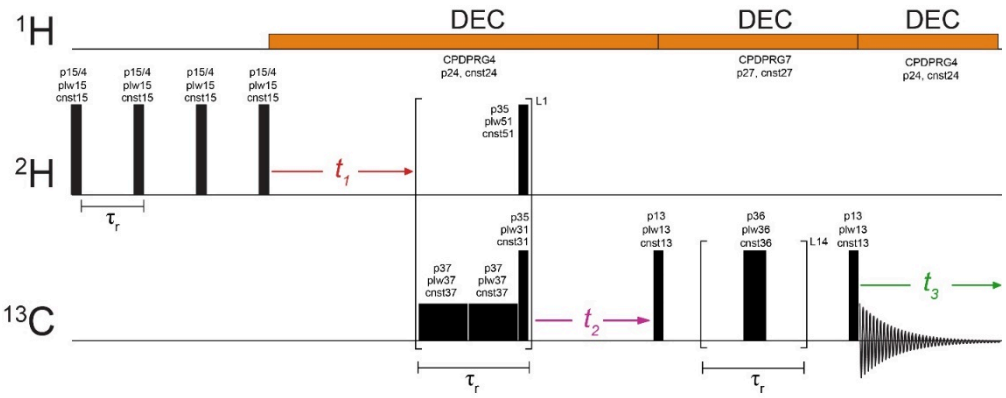
	Term	Definition
	CP	Cross Polarization
	MAS	Magic Angle Spinning
	RFDR	Radio-Frequency Driven Recoupling

Instrument: The $^{2}H/^{13}C/^{13}C$ Respiration CP 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-^{13}C labeling samples.
3. Minimum amount: ~50 nmol for 1.6 mm rotor.

Schematic pulse sequence of $^{2}H/^{13}C/^{13}C$ Respiration-CP 3D:



Example Parameter set for a Phoenix 1.6mm probe using 20 kHz MAS on 900 MHz Bruker spectrometer (pulse sequence: RespirationCP3d):

Pulse Parameters:

^{1}H 90°: (p14, plw14, cnst14) = (2.5 µs, 101 W, 100 kHz)

^{1}C 90°:(p13, plw13, cnst13) = (5 µs, 90 W, 50 kHz)

^{2}N 90^{o}: (p15, plw15, cnst15) = (8.8 µs, 100 W, 28.4 kHz)

Respiration CP: [p35, cnst37, cnst 51, cnst 31, L1]
=[2 µs, 40 kHz, 40 kHz, 55 kHz, 16]

RFDR: [p36, L14]
=[15 µs, 48]

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

^{1}H-decoupling for RFDR- (CPDPRG7, cnst27)
= (cw_p27, 0 kHz)

Acquisition Parameters:

MAS rate (w_r) in Hz: cnst20 = 20000

Rotor period (w_r): d20 = (1/cnst20) = 50 µs

Number of scans: ns = 192
Recycle delay: d1 = 0.1 sec

^{13}C Direct dimension: t_3 or F3:

Carrier frequency: O1p = 40 ppm
Acquisition time: aq(F3) = 20.5 ms
Spectral width: sw(F3) = 441.986 ppm

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

FnMode: States-TPPI
Carrier frequency: O1p = 40 ppm
Maximum evolution time: aq (F2) = 2.4 ms
Spectral width: sw (F2) = 88.397 ppm
Dwell time: IN_F (F2) = 50 µs
Total t_2 points: TD (F2) = 96

^{2}H indirect dimension: t_1 or F1:

FnMode: States-TPPI
Carrier frequency: O3p = 0 ppm
Maximum evolution time: aq(F1) = 0.23 ms
Spectral width: sw(F1) = 200 kHz
Dwell time: IN_F (F1) = 5 µs
Total t_1 points: TD (F1) = 92

Safety warnings

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

Before start

MAS rate: 10-20 kHz for 1.6 mm rotor

Temperature: Note that the temperature increases by 5 -10 °C due to frictional heating from spinning at 10-20 kHz for 1.6 mm rotors. Adjust the variable temperature (VT) control set point temperature accordingly 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 -10 to 0 °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. Example SOP for each optimization is attached at "References" section.

1. Calibrate ^{1}H, ^{2}H, and ^{13}C solid pulses (p14, plw14, p13, plw13, p15, and plw15)
2. Optimize ^{2}H-^{13}C Respiration CP (p37, cnst37, p35, cnst31, cnst51, L1)
3. Optimize low-power ^{1}H decoupling (cpdprg4, p24, and cnst24)
4. Optimize ^{13}C/^{13}C RFDR mixing (p36, L14, cpdprg7, p27, and cnst27)

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

Procedure

1 Load “RespirationCP3d-NAN” pulse program and parameter set “RespirationCP3d-NAN_par”.

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

1.2 Input “RespirationCP3d-NAN” as PULPROG

1.3 Type “rpar”, then load file “RespirationCP3d-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 optimized pulse parameter values. The optimizations that need to be done and parameters need to be updated are listed in the "Before start" section under "Guidelines & Warnings".

3 Set the ^{2}H carrier frequency (O3p) at ~0ppm, ^{1}H carrier frequency (O2p) at ~4ppm, and ^{13}C carrier frequency (O1p) at 40 ppm. For example, type “O1p” and enter, then input 40 to the opened window.

4 Set acquisition parameters.

4.1 Direct dimension (t_3 or F3)

1. Acquisition time (AQ, s): depending on ^{13}C T_2, default is ~20ms.
2. Spectral width (SW, ppm): ~400 ppm.
3. Carrier frequency (O3p, ppm): 40 ppm.

4.2 Indirect ^{2}H dimension (t_1 or F1)

1. Spectral width (SW, Hz): the ^{2}H dimension has spin sidebands at $n*\omega_R$ (Hz, $n \geq 1$). So the spectral width should be able to cover most of the sidebands. The default n is 5, so the default spectral width is ~$5\omega_R$ in Hz.
2. Increment of delay (IN_F, s): coupled with spectral with. Increment of delay=1/SW.
3. Carrier frequency (O1p, ppm): 0 ppm.
4. Hypercomplex scheme (FnMODE): States-TPPI

5. Maximum evolution time (AQ, s): 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. The default value is ~0.25ms.

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

1. Spectral width (SW. ppm): For perdeuterated proteins, the region of interest is the aliphatic region, so the spectral width should cover from ~0 ppm to ~80ppm. The default spectral width is ~90 ppm.
2. Increment of delay (IN_F, s): coupled with spectral with and must be rotor synchronized. For example, 50 µs for 20 kHz MAS spinning rate.
3. Carrier frequency (O2p, ppm): 40 ppm for proteins.
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.

5 Set the recycle delay

5.1 Use recycle delay of 1.3∗T_1 (^2H) for maximum sensitivity per unit time. Note that the ^2H T_1 is very short. The default value is 0.1s.

5.2 Due to the short recycle delay, only low-power ^1H decoupling is allowed, which should be sufficient for perdeuterated proteins.

6 Adjust measurement time as required by increasing 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 to check for adequate signal correctly arraying indirect dimension.

Protocol references

Reference: **Low-power polarization transfer between deuterons and spin-1/2 nuclei using adiabatic RESPIRATION CP in solid-state NMR. *Phys. Chem. Chem. Phys.*, 2014, 16, 2827**

Reference: **Site-Specific Internal Motions in GB1 Protein Microcrystals Revealed by 3D 2H–13C–13C Solid-State NMR Spectroscopy. *J. Am. Chem. Soc.* 2016, 138, 12, 4105–4119**

SOP: **2.2.1.11H and 13C Pulse Width Optimization**

SOP: 2H-13C Respiration CP Optimization

SOP: **2.2.1.3.1.1 1H decoupling**

SOP: $^{13}C/^{13}C$ RFDR optimization

Example Data at 900 MHz: usnan.org/ark:/83454/e1921452a1-8645-4088-b98e-9d272c5f5c45
Example Data at 600 MHz: usnan.org/ark:/83454/e151a891b7-48f2-4ec6-b44e-9df5ed901fd5