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Bio - hCC 2D DARR V.2

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We use this protocol and it's working

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Abstract

Purpose
hCC 2D homonuclear dipolar correlation experiment with DARR mixing at moderate spinning rate.

Scope
Residue type identification with short mixing times (10-50 ms); inter-residue correlations with moderate mixing times (50-200 ms); distance measurements with long mixing times (>200 ms).

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 "Materials" to find corresponding parameter names if using other pulse sequences.

Materials

Definitions:

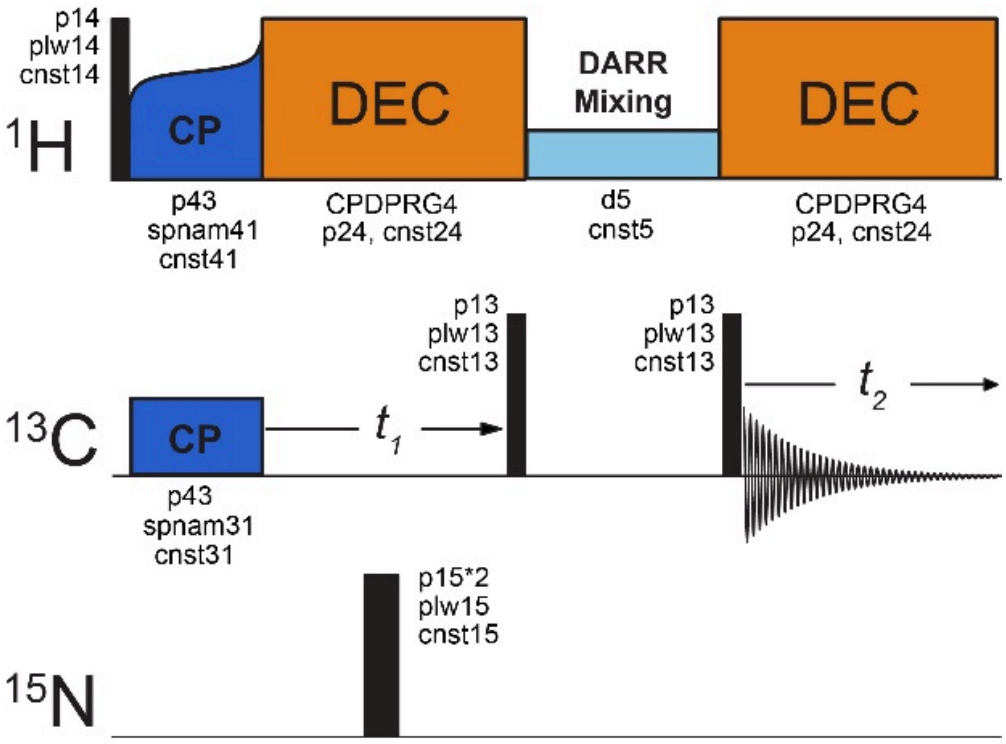
	Term	Definition
	hCC	Homonuclear 2D correlation experiment with CP
	DARR	Dipolar assisted rotational resonance
	CP	Cross Polarization
	MAS	Magic Angle Spinning
	SSB	Spinning side-band

Instrument: The CC 2D DARR 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 1.6 mm HCN probe. This experiment critically requires proper adjustment of the shimming and magic angle.

Sample:

1. Type: Protein
2. Labeling: U-^{13}C,^{15}N labeling (default), or ^{13}C-glycerol skip-labeled.
3. Minimum amount: ~50 nmol for 1.6 mm rotor.

hCC DARR 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: hCC-NAN):

Pulse Parameters:

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

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

^{15}N 90°: (p15, plw15, cnst15) = (5 µs, 115W, 50 kHz)

HN CP: [p43, cnst41 (^{1}H), cnst31(^{13}C)]
= [1 ms, 88 kHz (tan70to90.1000), 60 kHz (cw)]

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

^{1}H-decoupling - (CPDPRG4, p24, cnst24)
= (SPINAL64_p24, 5.2 ms, 90 kHz).

Acquisition Parameters:

MAS rate (w_r) in Hz: cnst20 =13.333 kHz

Rotor period (t_r): d20 = (1/cnst20) =75.0 ms

^{1}H carrier frequency: O2p = 6 ppm

Number of scans: ns = 4

Recycle delay: d1 = 1 sec

^{13}C Direct dimension: t_2 or F_2:

Carrier frequency: O1p = 100 ppm
Acquisition time: aq(F2) = 15.4 ms
Spectral width: sw(F2) = 331.5 ppm

^{13}C indirect dimension: t_1 or F_1:

Carrier frequency: O2p = 100 ppm
Maximum evolution time: aq(F1) = 12.8 ms
Spectral width: sw(F1) = 265.2 ppm
Total t1 points: TD (F1) = 1024
Complex t1 points: 512 (States-TPPI)

Experimental time = 2.5 hours

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 (~88.9 ppm in ^{13}C to avoid SSB overlap):

13.333 kHz at 600 MHz
16.667 kHz at 750 MHz
20 kHz at 900 MHz
25kHz at 1.1 GHz
Note that the transfer efficiency of DARR decreases significantly when MAS > 30 kHz, and alternatives such as CORD or RFDR mixing are preferred at higher MAS rates

Temperature: As determined for optimal sample sensitivity and resolution.

The following optimizations are required before setting up the CC 2D DARR experiment (parameters requiring updates are shown in parentheses).

Example SOPs for each optimization are attached at "References". Note that RF amplitudes in this pulse sequence are defined in units of kHz rather than Watts (i.e., input kHz number for RF powers and the pulse sequence code will calculate the corresponding values in Watts for Topspin to use). Only the hard pulses for calibration need to be input in units of Watts (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 HC CP (p43, spnam43, const41, and cnst31)
3. Optimization of high-power ^{1}H decoupling (cpdprg4, p24, and cnst24)

Setup time: ~ 10 min, presuming all required optimizations/calibrations are done.

Procedure

1 Load pulse program and parameter set.

1.1 If a previous CC 2D DARR experiment acquired using the same setup is available (i.e., same spectrometer, probe, and MAS rate), open the previous dataset and type “edc”. Input 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 “hCC-NAN” as PULPROG. Type “rpar”, then load file “hCC-NAN_par”.

2 Set the optimized pulse parameter values. The optimizations that need to be done and parameters need to be updated are listed at the "Before start" section under "Guidelines & Warnings".

3 Set the ^{1}H carrier frequency at ~5 ppm, and ^{13}C carrier frequency at ~100 ppm.

3.1 Ex: On Topspin, ^{1}H carrier frequency “o2p”, and ^{13}C carrier frequency “o1p”.

4 Set the spinning speed and DARR mixing.

4.1 Set the DARR ^{1}H condition. The ^{1}H rf power for DARR mixing should match the MAS rate. For the hCC NAN pulse sequence (hCC-NAN), below three parameters need to be changed. The spinning speed (cnst20, unit is Hz), ^{1}H rf power for DARR mixing (cnst5, unit is kHz, should match the spinning speed), and the DARR mixing time (d5, see the next section for details).

4.2 Choose DARR mixing time

1. For short range intra-residue correlations set the DARR mixing to 10-50 ms.
2. For medium range inter-residue correlations set the DARR mixing to 50-200 ms.
3. For long range (>5 Å) correlations set the DARR mixing to >200 ms.
4. Optimal mixing times increase with magnetic field. Refer to standard data sets for guidance.

5 Set the acquisition parameters.

5.1 Direct dimension (t_2)

1. Acquisition time (AQ [sec]): depending on ^{13}C T_2, default is ~15 ms (alternative for ^{13}C-glycerol labeling or crystalline samples: ~25 ms).
2. Spectral width (SW [ppm]): ~ 300 ppm.
3. Carrier frequency (O1P [ppm]): 100 ppm.

5.2 Indirect dimension (t_1)

1. Spectral width (SW [ppm]): coupled with dwell time, > 200 ppm is required to cover the full spectrum.
2. Hypercomplex scheme (FnMODE): States-TPPI
3. Increment of delay (IN_F [µsec]): 0.2 τ_R to 0.33 * τ_R, depending on magnetic field. (e.g., 25 µs at 600 MHz or 12.5 µs at 900 MHz). The spectral width must be an integer multiple of the MAS rate.
4. Maximum evolution time (AQ [sec]): ~10 ms. (This presumes a CA T_2 specification of 10 ms, recommend measuring CA T_2 prior to this experiment).
5. Number of rows (TD): coupled with increment of delay and maximum evolution time.

6 Set the recycle delay

6.1 Use recycle delay (d1) 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 use recycle delay less than 1s. Beware of heating limits for the probe and sample!

6.3 If duty cycle exceeds probe specification, an error should appear when attempting to start acquisition; if this is observed, increase the recycle delay until the error is resolved.

7 Determine experiment time

7.1 Minimal number of scans (phase cycle limited): 4.

7.2 Minimal measurement time: ~1 hr (depending on sample sensitivity).

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

8 Validation

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

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

Protocol references

Reference: Takegoshi, et al. ^{13}C-^{1}H dipolar assisted rotational resonance in magic-angle spinning NMR. *Chem. Phys. Lett.* 2001, 344, 631. https://doi.org/10.1016/S0009-2614(01)00791-6

SOP: **2.2.1.1 1H 90o pulse width calibration**

SOP: **2.2.1.2.1 1H-13C Cross Polarization**

SOP: **2.2.1.3.1.1 1H decoupling**

Example Data at 1.1 GHz: usnan.org/ark:/83454/e19dccb9f3-b169-43c5-b66e-2c354ff5895e
Example Data at 900 MHz: usnan.org/ark:/83454/e1eccdf207-a181-4d01-ace6-9acba8db6aec
Example Data at 600 MHz: usnan.org/ark:/83454/e11129cb25-3352-43e9-b59a-687a897cef5c