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Bio - CN-REDOR V.2

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We use this protocol and it's working

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Abstract

Purpose
13C/15N frequency selective REDOR for distance measurement.

Scope
Intermolecular or intramolecular 13C-15N distance measurement up to ~6 Å range. The selectivity of recoupling relies on 13C and 15N chemical shift resolution.

Checklist for setting CN-fsREDOR 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, const41, spnam31, cnst31, cpdprg4, p24, cnst24, spnam38, p38, cnst38, spnam58, p58, and cnst58).
3. Set flags for soft pulses (L3 and L5)
4. Set ^{13}C and ^{15}N frequencies for acquisition and selective pulses (o1p, o2p, o3p, cnst16, cnst17)
5. Set acquisition time (aq) and spectral width (sw) for direct dimension.
6. Set recycle delay (d1)
7. Set array to acquire psudo-2D dataset (L0 and L8)

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 section 6.8 to find corresponding parameter names if using other pulse sequences.

Materials

Definitions:

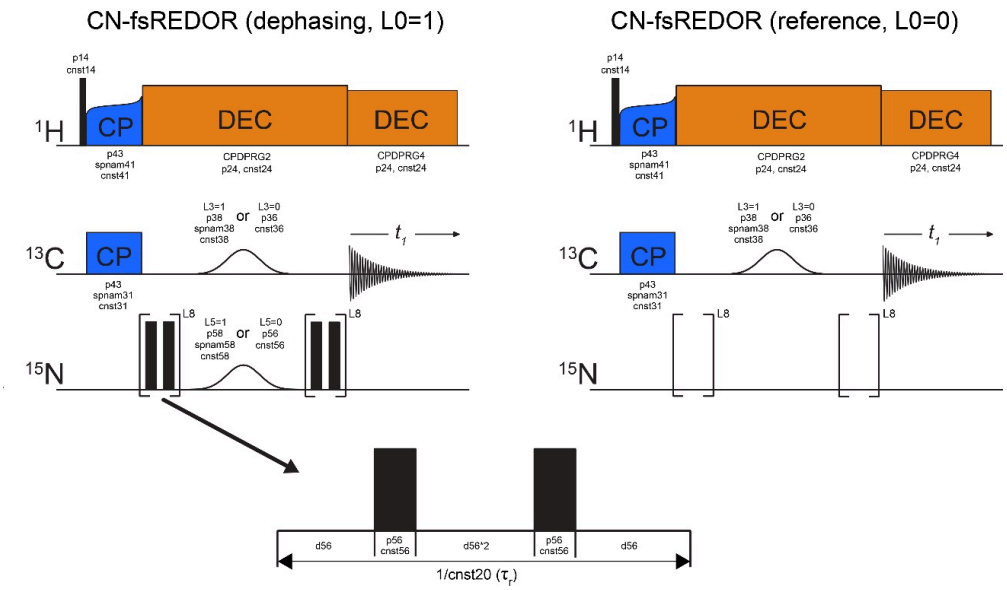
	Term	Definition
	REDOR	Rotational-echo double-resonance
	fs	Frequency selective
	CP	Cross Polarization
	MAS	Magic Angle Spinning

Instrument: The CN-fsREDOR example dataset in this SOP was acquired using a 600 MHz NMR spectrometer with 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: Peptide, protein, or small molecules with resolved ^{13}C and ^{15}N resonances.
2. Labeling: U-^{13}C,^{15}N labeling (small peptide or molecules), or sparsely labeled proteins.
3. Minimum amount: ~ 50 nmol for 1.6 mm rotor

CN-fsREDOR 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: CN-fsREDOR):

Pulse parameters:

^{1}H 90^{o}: (p14, plw14, cnst14) = (2.5 µs, 33W. 100 kHz)

^{13}C 90^{o}:(p13, plw13, cnst13) = (2.5 µs, 140W, 100 kHz)

^{15}N 90^{o}: (p15, plw15, cnst15) = (5 µs, 125W, 50 kHz)

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

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

^{13}C-soft pulse - [p38, spnam38, cnst38]
= [7.5 ms, Rsnob.1000, 0.28 kHz]

^{13}C frequency: [cnst16, cnst17]
= [100 ppm, 175.20 ppm]

Acquisition parameters:

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

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

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

^{15}N carrier frequency: O3p=118 ppm

Number of scans: ns = 8

Recycle delay: d1 = 1 sec

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

Carrier frequency: O1p = 100 ppm

Acquisition time: aq(F2) = 30.9 ms

Spectral width: sw(F2) = 663.0 ppm

Experimental time = 15 min

Safety warnings

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

Before start

MAS rate: 13.333 kHz at 600 MHz, 16.667 kHz at 750 MHz, or 20 kHz at 900 MHz (~88.9 ppm in ^{13}C).

Temperature: As determined for optimal sample sensitivity and resolution.

Below optimizations are required before setting up the CN-fsREDOR experiment (parameters needs to be updated are shown in parenthesis). Example SOP for each optimization is in 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). Also note that the soft pulse duration (p38 or p58 must be rotor synchronized, i.e., $2m\tau_r$).

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, spnam41, const41, spnam31, and cnst31)
3. Optimization of high-power ^{1}H decoupling (cpdprg4, p24, and cnst24)
4. Optimization of ^{13}C selective pulse (spnam38, p38, cnst38)
5. Optimization of ^{15}N selective pulse (spnam58, p58, cnst58)

Setup time: ~ 15 min, presuming all required optimizations/calibrations are done.

Procedure

1 Load pulse program and parameter set.

1.1 If a previous CN-fsREDOR 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 “CN-fsREDOR” as PULPROG. Type “rpar”, then load file “CN-fsREDOR-NAN_par”.

2 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.

3 Set the flags properly to switch pulse sequences.

3.1 The pulse sequence has option to turn on/off the soft pulses for $^{13}C/^{15}N$ channels (i.e., L3 for ^{13}C and L5 for ^{15}N. 0 is off and 1 is on). If the soft pulses are turned off, then regular REDOR (with non-selective hard pulses) will be executed.

4 Set frequency parameters

4.1 For ^{1}H, set the carrier frequency at ~5 ppm. On Topspin, type “O2p”, then input the value.

4.2 For ^{13}C, there are 3 frequency related parameters. First is carrier frequency “O1p” which should be either the center of the ^{13}C spectrum (i.e., 100 ppm for peptide), or the optimized one for ^{1}H-^{13}C CP transfer. Second is cnst16 which should be always the same as “O1p”. Third is cnst17 which should match the chemical shift of the ^{13}C resonance being selected.

4.3 For ^{15}N, set the carrier frequency “O3p” at the chemical shift of the ^{15}N resonance being selected.

5 Set the acquisition parameters for direct dimension t_1 (below parameters are typical for peptide samples).
1. Acquisition time: depending on ^{13}C T_2, default is ~15 ms
2. Spectral width: ~ 300 ppm.

6 Set the recycle delay

6.1 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.

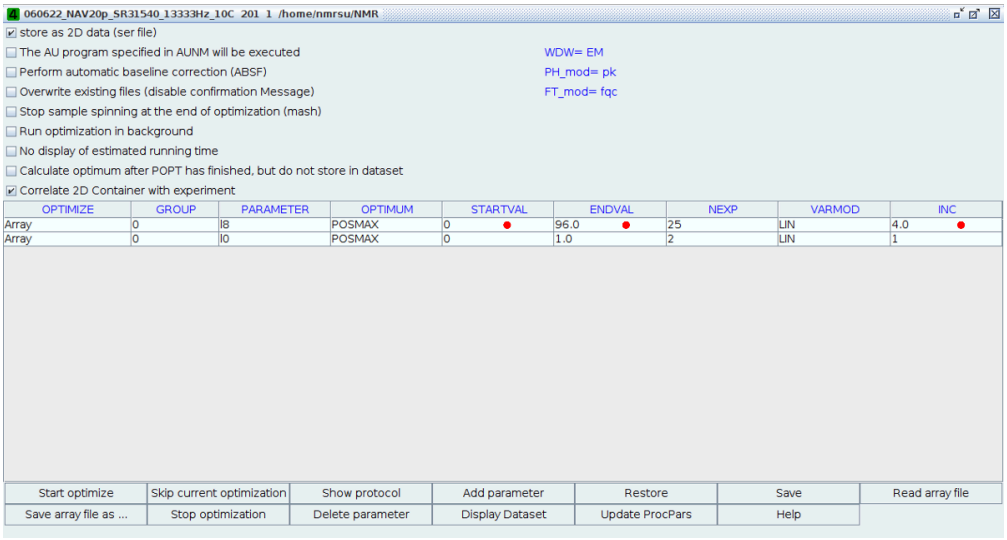
6.2 Due to high-power ^1H decoupling is applied, don't use recycle delay less than 1s.

7 Acquire reference spectra and dephasing spectra, and store in one dataset.

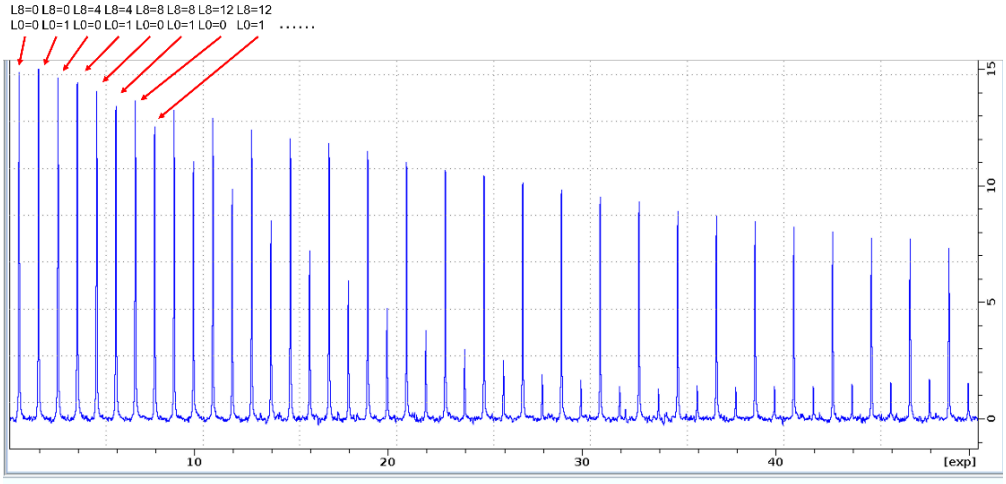
7.1 The parameter to switch between reference and dephasing spectra is L0 (L0=0 is referencing, L0=1 is dephasing). L8 is the number of REDOR blocks. The REDOR mixing is calculated using $L8*\tau_r*2$ (i.e., if spinning at 20kHz with L8 =16. Then the REDOR mixing time is $16*(1/20000)*2s$ = 1.6ms). See below schematic pulse sequence at section 6.10 for more details.

7.2 Acquire a spectrum with L8=0 and L0=0, then phase it properly.

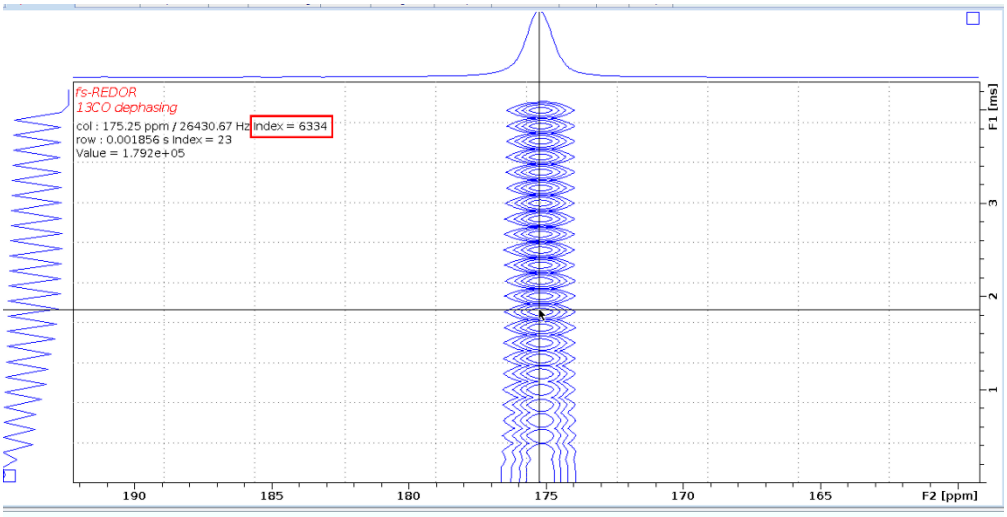
7.3 Type "popt" to open an optimization window. In this window, select "store as 2D data (ser file)" and "Correlate 2D Container with experiment" options. L0 and L8 will be arrayed together so add one more row by press "add parameter" button. Then set the two arrays as shown below. The only values can be changed are the start and end value of L8, and the increment (highlighted by red dots). Press "Start optimize" to initiate the acquisition.



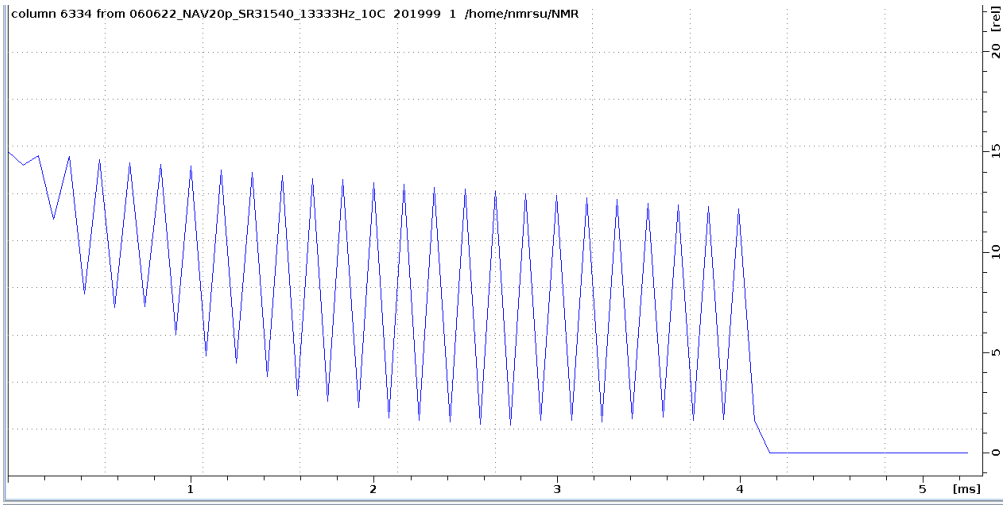
7.4 The above setting will acquire the reference and dephasing spectra for a L8 value, then increasing L8. The output result will look like below.



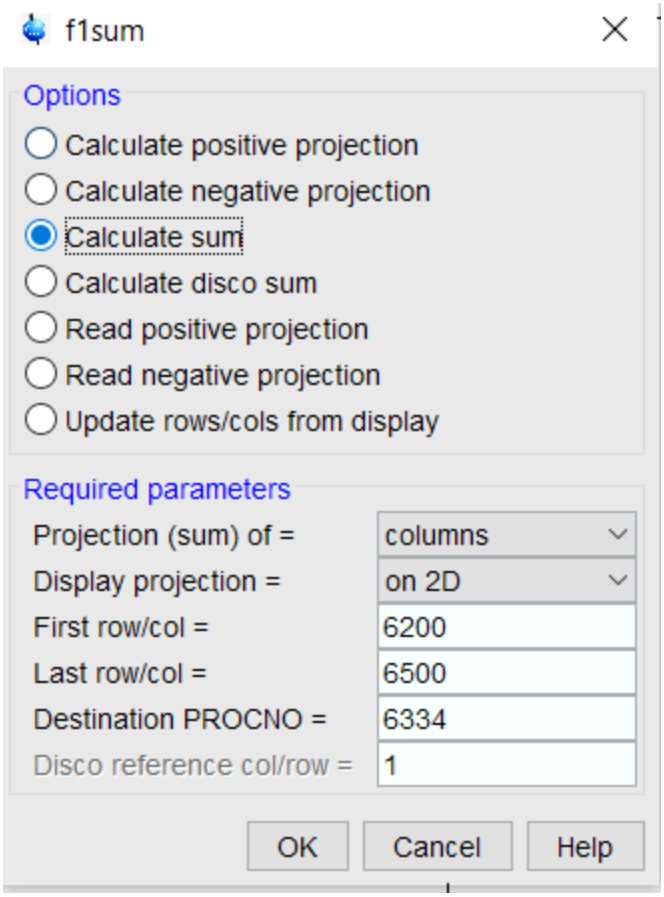
7.5 Topspin will output a pseudo 2D dataset named as “#999” (i.e., if the experiment number is 201, then the 2D dataset will be named as 201999). Open the dataset, then type “xf2” to process the direct dimension. Then locate the mouse curser to the position of the peak to find the slice number (in below example, the slice index is 6334). Or find the range of an integral (in below example, the range is ~6200 to ~6500).



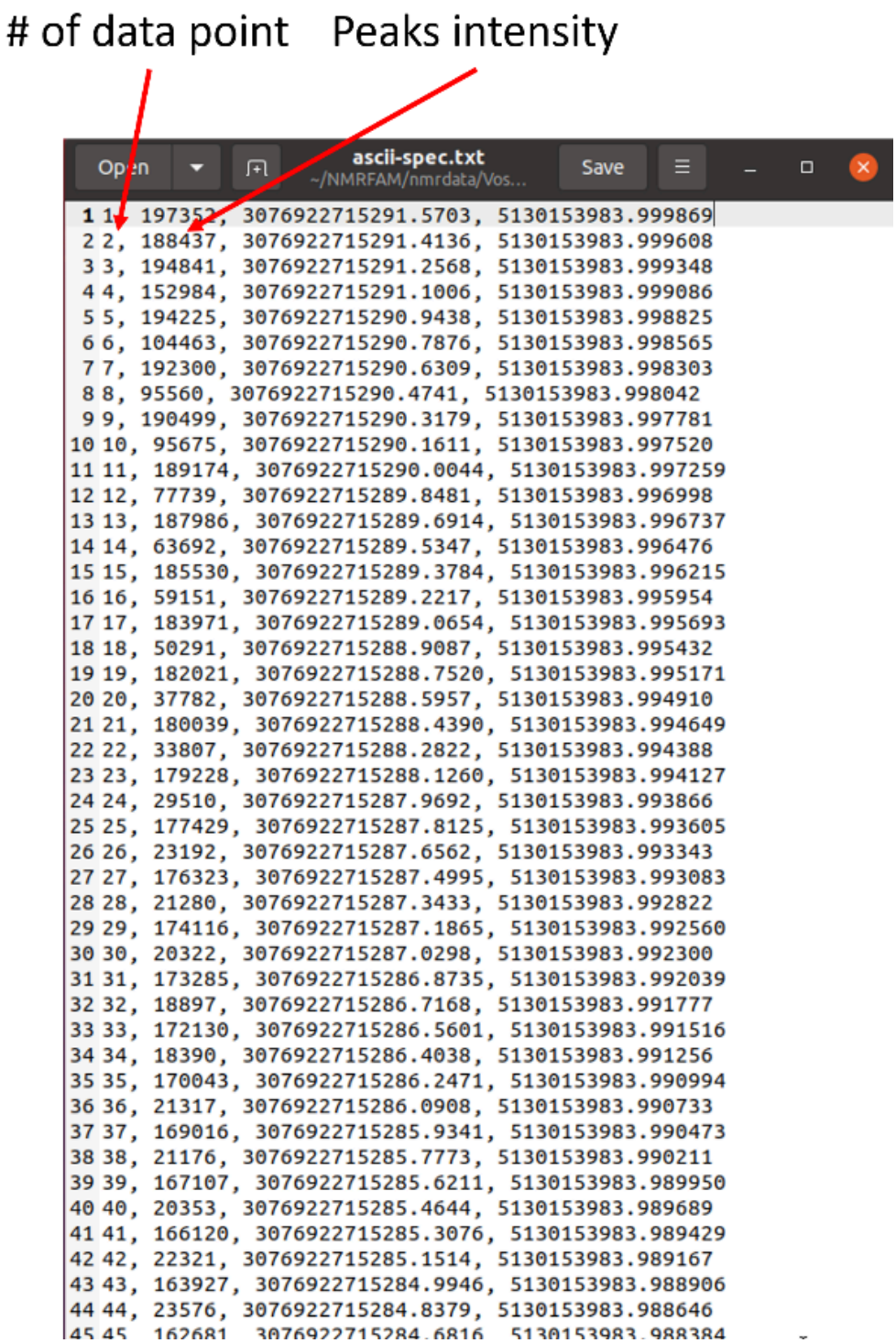
7.6 If peak heights are needed, then type “rsc # #” (in above example, type “rcs 6334 6334”). This will generate a 1D dataset for the slice with this index number. It looks like below. The top points correspond to the reference spectra, and the bottom points correspond to the dephasing spectra.



7.7 If integrals are needed, type “f1sum”. It will open a window as below. Select “calculate sum” option, then specify the first row, last row, and destination number. In below example, it will calculate sum from slice 6200 to 6500, then store data into a dataset named as 6334. This will generate a similar 1D dataset as shown above.



7.8 Once get the 1D dataset, type “convbin2asc”. It will generate a txt file termed “ascii-spec.txt” in the raw data folder (i.e., in above example, the txt file will be in the /201999/pdata/6334 folder). Only the first two columns of this file are useful. The first column of the data file is the index of data point, and the second column of the data file is the peak intensity.


# of data point
Peaks intensity
Open
ascii-spec.txt
~/NMRFAM/nmrdata/Vos...
Save
1 1, 197352, 3076922715291.5703, 5130153983.999869
2 2, 188437, 3076922715291.4136, 5130153983.999608
3 3, 194841, 3076922715291.2568, 5130153983.999348
4 4, 152984, 3076922715291.1006, 5130153983.999086
5 5, 194225, 3076922715290.9438, 5130153983.998825
6 6, 104463, 3076922715290.7876, 5130153983.998565
7 7, 192300, 3076922715290.6309, 5130153983.998303
8 8, 95560, 3076922715290.4741, 5130153983.998042
9 9, 190499, 3076922715290.3179, 5130153983.997781
10 10, 95675, 3076922715290.1611, 5130153983.997520
11 11, 189174, 3076922715290.0044, 5130153983.997259
12 12, 77739, 3076922715289.8481, 5130153983.996998
13 13, 187986, 3076922715289.6914, 5130153983.996737
14 14, 63692, 3076922715289.5347, 5130153983.996476
15 15, 185530, 3076922715289.3784, 5130153983.996215
16 16, 59151, 3076922715289.2217, 5130153983.995954
17 17, 183971, 3076922715289.0654, 5130153983.995693
18 18, 50291, 3076922715288.9087, 5130153983.995432
19 19, 182021, 3076922715288.7520, 5130153983.995171
20 20, 37782, 3076922715288.5957, 5130153983.994910
21 21, 180039, 3076922715288.4390, 5130153983.994649
22 22, 33807, 3076922715288.2822, 5130153983.994388
23 23, 179228, 3076922715288.1260, 5130153983.994127
24 24, 29510, 3076922715287.9692, 5130153983.993866
25 25, 177429, 3076922715287.8125, 5130153983.993605
26 26, 23192, 3076922715287.6562, 5130153983.993343
27 27, 176323, 3076922715287.4995, 5130153983.993083
28 28, 21280, 3076922715287.3433, 5130153983.992822
29 29, 174116, 3076922715287.1865, 5130153983.992560
30 30, 20322, 3076922715287.0298, 5130153983.992300
31 31, 173285, 3076922715286.8735, 5130153983.992039
32 32, 18897, 3076922715286.7168, 5130153983.991777
33 33, 172130, 3076922715286.5601, 5130153983.991516
34 34, 18390, 3076922715286.4038, 5130153983.991256
35 35, 170043, 3076922715286.2471, 5130153983.990994
36 36, 21317, 3076922715286.0908, 5130153983.990733
37 37, 169016, 3076922715285.9341, 5130153983.990473
38 38, 21176, 3076922715285.7773, 5130153983.990211
39 39, 167107, 3076922715285.6211, 5130153983.989950
40 40, 20353, 3076922715285.4644, 5130153983.989689
41 41, 166120, 3076922715285.3076, 5130153983.989429
42 42, 22321, 3076922715285.1514, 5130153983.989167
43 43, 163927, 3076922715284.9946, 5130153983.988906
44 44, 23576, 3076922715284.8379, 5130153983.988646
45 45, 162681, 3076922715284.6816, 5130153983.988384

Protocol references

Reference: Jaroniec, et al. Frequency selective heteronuclear dipolar recoupling in rotating solids: accurate ^{13}C–^{15}N distance measurements in uniformly ^{13}C, ^{15}N-labeled peptides. *J. Am. Chem. Soc.* 2001, 123, 15, 3507–3519 https://doi.org/10.1021/ja003266e

SOP: **2.2.1.11H/13C/15N hard pulse width calibration**

SOP: **2.2.1.2.11H-13C Cross Polarization**

SOP: **2.2.1.3.1.11H high-power decoupling**

SOP: **2.2.1.1.2.1.113C-selective 180° pulse optimization**

Example Data at 1.1 GHz:
usnan.org/ark:/83454/e14246106d-3666-4e70-b975-ef1910bf5121
usnan.org/ark:/83454/e13513e8eb-88cb-4bd3-b4fa-9444c65f22e1
usnan.org/ark:/83454/e1136114de-d483-4d4c-8670-43100daa44a6
usnan.org/ark:/83454/e104b4edad-2e2e-4ea8-8878-8d0e3e73c024
usnan.org/ark:/83454/e1464c67c3-4efa-4207-ad44-3e3aade9f445
usnan.org/ark:/83454/e1f03306cb-8f81-4461-a5e0-807a4b66c45d

Example Data at 900 MHz:
usnan.org/ark:/83454/e13375c407-181d-42e9-b715-85aec43192bc
usnan.org/ark:/83454/e1a1a069a7-9f66-4d87-9bd0-69ce5e4dcacd
usnan.org/ark:/83454/e138a09848-b81f-4b10-b59e-6ac1f8b1aefa
usnan.org/ark:/83454/e12cf4a829-b8b6-4b18-8604-f21a19f330c1
usnan.org/ark:/83454/e15aff43a2-1eb8-487b-8e70-f7548a2d55ef

Example Data at 600 MHz:
usnan.org/ark:/83454/e123f09458-e1dd-416b-a0c5-baa82f83a672
usnan.org/ark:/83454/e1b0aac7ab-2c84-40da-9921-3fd2fe2b79a0
usnan.org/ark:/83454/e1330f0343-69c0-4d7c-ac23-bb9a8d045444
usnan.org/ark:/83454/e12692530b-b6bf-4ae1-aae2-e3ed4af5d4c3
usnan.org/ark:/83454/e113f94d68-eae1-4b7b-b6b4-f6986b185c25
usnan.org/ark:/83454/e1bf9652b7-99d1-45f8-995c-9223ee0c40cd