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trNOE.nan

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NAN KB[1], Alex Eletsky[2], John Glushka[2]

[1]Network for Advanced NMR (NAN); [2]University of Georgia

NAN-KB UGA
Network for Advanced NMR

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We use this protocol and it's working

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Disclaimer

This protocol is part of the knowledge base content of **NAN:** The Network for Advanced NMR (**https://usnan.nmrhub.org/**)
Specific filenames, paths, and parameter values apply to spectrometers in the NMR facility of the Complex Carbohydrate Research Center (CCRC) at the University of Georgia.

Abstract

This protocol describes running a 2D 1H-1H trNOE (NOESY) pulse sequence with ZQ coherence suppression and presaturation for water suppression. This produces a 2D phase-sensitive dataset with 1H-1H correlations due to NOEs.

This pulse sequence used is a standard 2D NOESY experiment but is applied to a mixture of a low MW ligand and a macromolecule to which it weakly binds. Since the sign of NOEs are dependent on the tumbling (correlation) time of the molecule, a low MW compound (fast tumbling) has generally weak 'positive' NOE signals whereas a high MW macromolecule has stronger 'negative' signals.

Generally two datasets are collected. The NOESY spectrum of the ligand alone is acquired with a suitably long NOE mixing time in order to see significant crosspeaks. Then a NOESY spectrum is acquired of the mixture of ligand and macromolecule in a molar ratio of 10-100: 1, generally with a shorter NOE mixing time. Under certain binding kinetics, the NOEs of the ligand while bound to the macromolecule will dominate and the crosspeaks will be of opposite sign from that of the ligand alone.
In some cases, additional control spectra of the macromolecule alone and of the ligand alone with the shorter mixing time are collected to resolve ambiguities.

Main applications
- measuring 1H-1H distances in small molecule ligands when bound to large molecules
- structure determination of bound conformations of small molecule ligands

Required isotope labeling: natural abundance.

Optimal MW: ligand < 2000 Da; macromolecule > 5 kDa.
Field strength and sample temperature are often chosen to achieve zero NOE transfer for free ligand ($\omega\tau c = 1.1$), though this may not always be possible.

Pulse sequence used is noesygpphzspr.

Attachments

PDF

biotop.pdf

2.5MB

Guidelines

Generally two datasets are collected. The NOESY spectrum of the ligand alone is acquired with a suitably long NOE mixing time in order to see significant crosspeaks. Then a NOESY spectrum is acquired of the mixture of ligand and macromolecule in a molar ratio of 10-100: 1, generally with a much shorter NOE mixing time. Under certain binding kinetics, the NOEs of the ligand while bound to the macromolecule will dominate and the crosspeaks will be of opposite sign from that of the ligand alone.
In some cases, additional control NOESY spectra of the macromolecule alone and of the ligand alone with the shorter mixing time are collected to resolve ambiguities.

Before start

A sample must be inserted in the magnet either locally by the user after training or by facility staff if running remotely.
This protocol requires a sample is locked, tuned/matched on the ^{1}H channel, and shimmed. At a minimum, ^{1}H 90° pulse width and offset O1 should be calibrated and a 1D proton spectrum with water suppression has been collected according to the **protocol PRESAT_bio.nan.**

Create trNOE experiment

1 Start with existing the Dataset containing 1D PRESAT data in EXPNO 1 acquired with protocol **PRESAT_bio.nan**

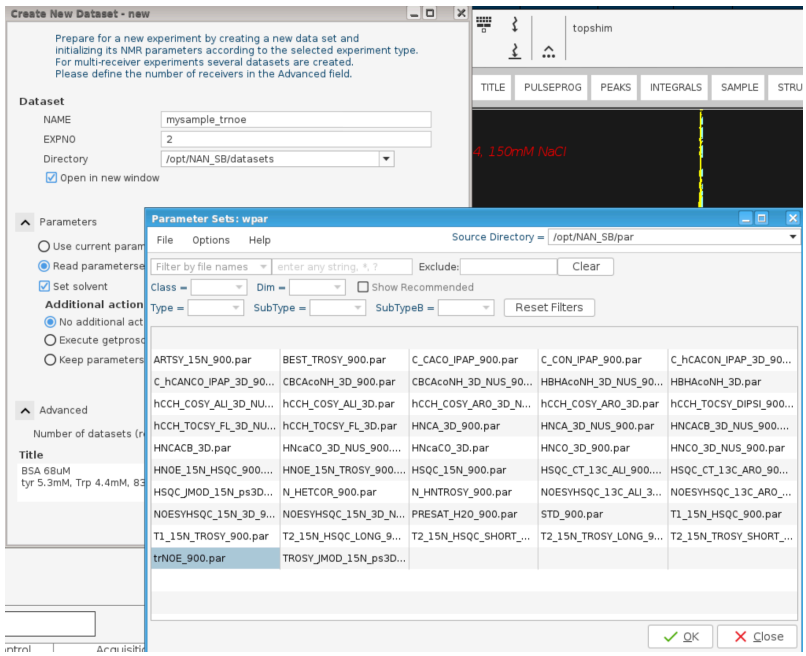
1.1 Click on Acquire → 'Create Dataset' button to open dataset entry box or type **edc** command.

The EXPNO is automatically incremented by +1 by default.
Directory should be the same as preliminary 1D.

The Title text box will copied from the previous experiment. Edit appropriately.

1.2 Load the starting parameter set: Check 'Read parameterset' box, and click Select.

For standard NAN parameter sets, change the Source directory at upper right corner of the window:
Source = /opt/NAN_SB/par
Click 'Select' to bring up list of parameter sets.
Select trNOE_xxx.par, where xxx=900,800 or 600*.



1.3 Click OK at bottom of window to create the new EXPNO directory.
It will be the active experiment in the acquisition window and should now be listed on your data browser.

Load pulse calibrations

2 Use **getprosol** to load correct pulse widths and power levels.

2.1 Loading pulse widths and power levels with **getprosol**:

Examine the **PRESAT** dataset acquisition parameters in Exp 1 (type 'ased'), previously collected according to the protocol **PRESAT_bio.nan** (see step 1). The P1 pulse width value should be calibrated - note its value (inspect table or type 'p1').
If necessary, it can be recalibrated by typing pulsecal.

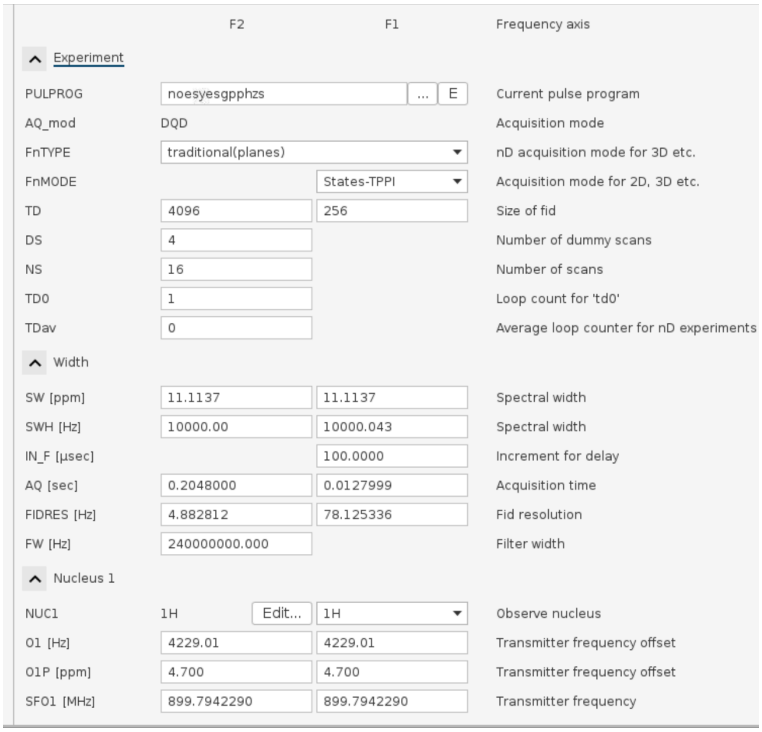
Then execute the getprosol command:
getprosol 1H [calibrated P1 value] [power level attenuation for P1 (PLdB1)]
e.g. getprosol 1H **9.9** -13.14.
Where for example, the calibrated **P1=9.9** at power level -13.14 dB attenuation

Inspect and adjust parameters

2.2 Other than the 1H pulsewidth, the default parameters from trNOE_xxx.par will provide suitable starting values.
Typically the only parameters to change will be NS = number of scans in order to increase the signal to noise.

2.3 Select the 'Acqpars' tab to display acquisition parameters. Two display modes can be selected, the full display mode (click on the '**A**' icon or type **eda**), or pulse program-specific mode (click on the 'pulse' icon, or type **ased**). The former gives you access to all parameters and provides an overview of all spectral dimensions at once, while the latter is useful because it only displays acquisition parameters used in the pulse sequence and can be parsed sequentially as a checklist.

First examine the specific dimension parameters in Acqpars 'eda' mode (click 'A' icon):

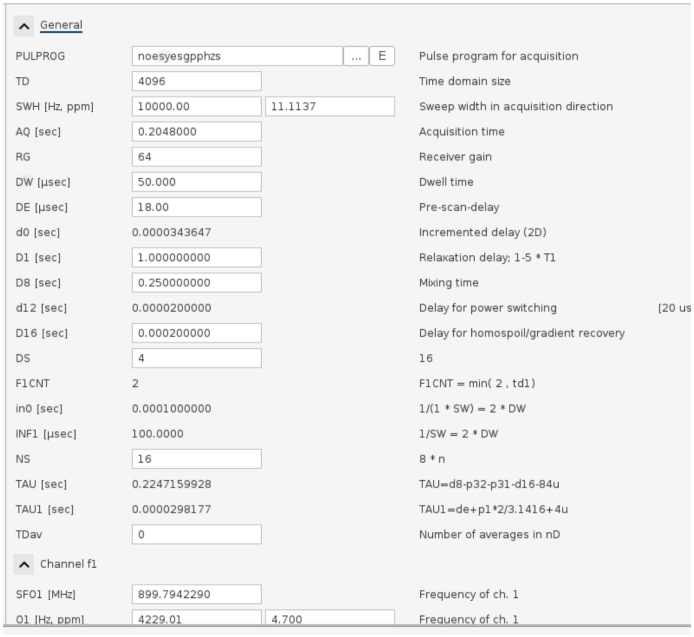


	F2	F1	Frequency axis
Experiment			
PULPROG	noesyesgpphzs ... E		Current pulse program
AQ_mod	DQD		Acquisition mode
FnTYPE	traditional(planes)		nD acquisition mode for 3D etc.
FnMODE		States-TPPI	Acquisition mode for 2D, 3D etc.
TD	4096	256	Size of fid
DS	4		Number of dummy scans
NS	16		Number of scans
TD0	1		Loop count for 'td0'
TDav	0		Average loop counter for nD experiments
Width			
SW [ppm]	11.1137	11.1137	Spectral width
SWH [Hz]	10000.00	10000.043	Spectral width
IN_F [µsec]		100.0000	Increment for delay
AQ [sec]	0.2048000	0.0127999	Acquisition time
FIDRES [Hz]	4.882812	78.125336	Fid resolution
FW [Hz]	240000000.000		Filter width
Nucleus 1			
NUC1	1H Edit...	1H	Observe nucleus
O1 [Hz]	4229.01	4229.01	Transmitter frequency offset
O1P [ppm]	4.700	4.700	Transmitter frequency offset
SFO1 [MHz]	899.7942290	899.7942290	Transmitter frequency

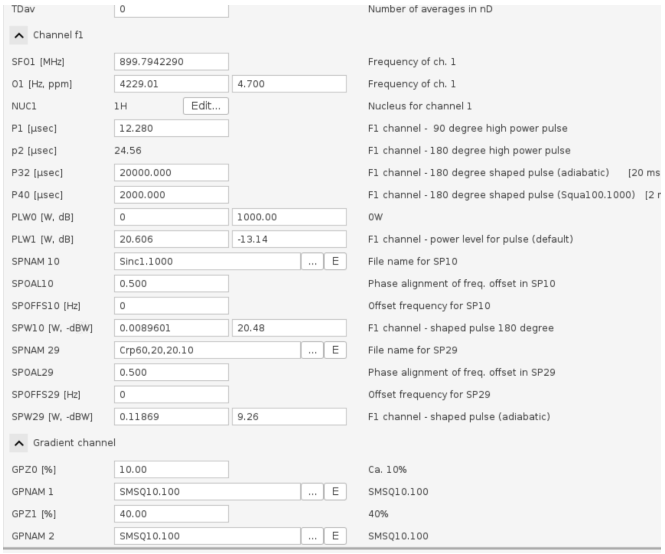
Parameters to check:

- **SW[ppm]**: 1H(F2 and F1) ~9-15 ppm
- **O1P:** ^{1}H H_2O offset, typically ~4.7.
- **2 TD:** Number of 1H time domain real points (2048-8192, depending on resolution desired)
- **1 TD:** 2 X number of 1H time domain real points (256-1024, depending on resolution desired and time available)
- **NS:** multiple of 4-16; increase for for higher signal to noise (S/N increases as square root of NS)
- **DS:** 8-32 'dummy' scans that are not recorded; allows system to reach steady state equilibration.
- **DIGMOD** - 'baseopt' (zero 1st order phase correction)

2.4 Then examine the parameters in the pulse program-specific '**ased**' mode (click on the 'pulse' icon).



General			
PULPROG	noesyesgpphzs	... E	Pulse program for acquisition
TD	4096		Time domain size
SWH [Hz, ppm]	10000.00	11.1137	Sweep width in acquisition direction
AQ [sec]	0.2048000		Acquisition time
RG	64		Receiver gain
DW [µsec]	50.000		Dwell time
DE [µsec]	18.00		Pre-scan-delay
d0 [sec]	0.0000343647		Incremented delay (2D)
D1 [sec]	1.000000000		Relaxation delay; 1-5 * T1
D8 [sec]	0.250000000		Mixing time
d12 [sec]	0.0000200000		Delay for power switching [20 us
D16 [sec]	0.000200000		Delay for homospoil/gradient recovery
DS	4		16
F1CNT	2		F1CNT = min(2 , td1)
in0 [sec]	0.0001000000		1/(1 * SW) = 2 * DW
INF1 [µsec]	100.0000		1/SW = 2 * DW
NS	16		8 * n
TAU [sec]	0.2247159928		TAU=d8-p32-p31-d16-84u
TAU1 [sec]	0.0000298177		TAU1=de+p1*2/3.1416+4u
TDav	0		Number of averages in nD
Channel f1			
SFO1 [MHz]	899.7942290		Frequency of ch. 1
O1 [Hz, ppm]	4229.01	4.700	Frequency of ch. 1



TDav	0		Number of averages in nD
Channel f1			
SFO1 [MHz]	899.7942290		Frequency of ch. 1
O1 [Hz, ppm]	4229.01	4.700	Frequency of ch. 1
NUC1	1H Edit...		Nucleus for channel 1
P1 [µsec]	12.280		F1 channel - 90 degree high power pulse
p2 [µsec]	24.56		F1 channel - 180 degree high power pulse
P32 [µsec]	20000.000		F1 channel - 180 degree shaped pulse (adiabatic) [20 ms
P40 [µsec]	2000.000		F1 channel - 180 degree shaped pulse (Squa100.1000) [2 r
PLW0 [W, dB]	0	1000.00	0W
PLW1 [W, dB]	20.606	-13.14	F1 channel - power level for pulse (default)
SPNAM 10	Sinc1.1000 ... E		File name for SP10
SPOAL10	0.500		Phase alignment of freq. offset in SP10
SPOFFS10 [Hz]	0		Offset frequency for SP10
SPW10 [W, -dBW]	0.0089601	20.48	F1 channel - shaped pulse 180 degree
SPNAM 29	Crp60,20,20.10 ... E		File name for SP29
SPOAL29	0.500		Phase alignment of freq. offset in SP29
SPOFFS29 [Hz]	0		Offset frequency for SP29
SPW29 [W, -dBW]	0.11869	9.26	F1 channel - shaped pulse (adiabatic)
Gradient channel			
GPZ0 [%]	10.00		Ca. 10%
GPNAM 1	SMSQ10.100 ... E		SMSQ10.100
GPZ1 [%]	40.00		40%
GPNAM 2	SMSQ10.100 ... E		SMSQ10.100

Most of the default parameters should be appropriate, however it's useful to compare values in the fields against suggestions in the pulseprogram comments. In general, only a few may need to be changed.

- **D1:** recycle delay (~1-2 s)
- **D8**: NOE mixing time

- **NS:** multiple of 4-16; increase for for higher signal to noise (S/N increases as square root of NS)
- **P1** - calibrated 1H 90º high power pulse (see step 1)

Acquire and Process Data

3 Type '**rga**' or click on 'Gain' in Topspin Acquire menu to execute automatic gain adjustment.
Type '**zg**' or click on 'Run' in Topspin Acquire menu to begin acquisition.

3.1 You can always check the first FID by typing '**efp**' to execute an exponential multiplied Fourier transform. It will ask for a FID #, choose the default #1. You can evaluate the 1D spectrum for amide proton signal to noise and water suppression.

To take a look at the 2D, wait for >= 16 FIDs and then click on 'Proc.Spectrum' on the Topspin Process menu. This will execute an automated processing macro. Although the resolution will be poor, you can evaluate the signal to noise (S/N) and whether the ^{15}N offset and spectral width are appropriate.
This can be repeated at any time as additional FIDs are acquired.

3.2 The example spectrum region below shows both 'positive' crosspeaks for the tyrosine (blue, ~7.1 ppm), which does NOT bind to BSA, so behaves as a small molecule; and 'negative' crosspeaks for the tryptophan (yellow ~7.23 and 7.64 ppm) which does bind and behaves the same as the large BSA protein.

Protocol references

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