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 **STD.nan**

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Protocol status: Working

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 1D Saturation Transfer Difference (STD) pulse sequence with solvent suppression.

It is used to determine the degree and nature of binding of small molecules with macromolecules. This version can be run in D_2O or 90% H_2O . It uses the Bruker Topspin pulse sequence 'stdiffesgp.3'

For ligands that have important resonances in the water region ($\sim 4.7\text{ppm}$) it is best to exchange the sample with D_2O , use high quality D_2O as the solvent, and use the pulseprogram 'stdiff.3' without water suppression.

It is implemented as a pseudo-2D dataset where two spectra are collected. One spectrum has a saturation pulse ON, irradiating close to the range of alkyl methyls in macromolecules, typically 1 to -3 ppm , while avoiding irradiating the resonances of the ligand. A second reference spectrum has the saturation pulse OFF, which means far off-resonance at around -40 ppm . The two spectra are then subtracted.

The residual signals of the ligand indicate that (1) the ligand has spent time bound to the macromolecule and (2) the intensity of the residual signals correlate with those protons most closely bound to the macromolecule.



Attachments



[biotop.pdf](#)

2.5MB

Guidelines

This version includes water suppression to minimize residual water signal but will remove signals close to the water. An alternate version that does not include water suppression (stdiff.3) is also available.

Saturation frequency should be set close to the resonance range of macromolecules, but such that saturation does not directly perturb the ligand signals. You can test this by recording an STD experiment with varying saturation frequencies on a control sample with ligand or ligands only, without target proteins and macromolecules. Most common saturation ranges are methyl (**preferred** 0.5 to -2.5 ppm) and aromatic (6 to 8 ppm).

The sequence uses a second proton channel 'f2' for the saturation pulse which consists of multiple gaussian pulses. Its excitation frequencies are independent of the observe channel f1 frequency and the power level is low (e.g. ~27 db attenuation) for selective saturation.

Also make sure the power levels for the trim and spinlock pulses if f1 are also low (~ -7-8 db)

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 ^1H channel and shimmed.

At a minimum, ^1H 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 STD experiment

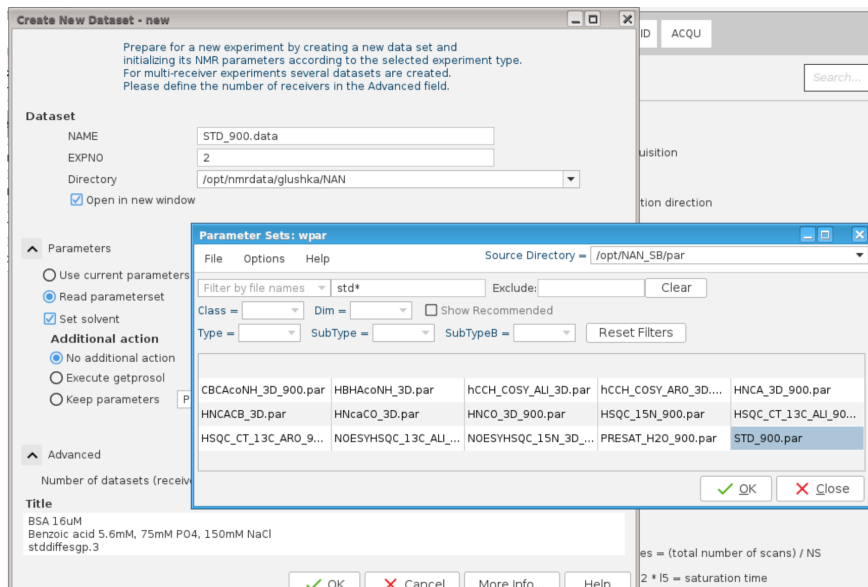
- 1 Start with existing Dataset containing 1D PRESAT data in EXPNO 1 collected with protocol **PRESAT_bio.nan**
- 1.1 Click on Acquire → 'Create Dataset' button to open dataset entry box or type **edc** command.

Dataset Name: recommended to keep the same name when using BioTop for optimization and acquisition setup.

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 to designate the N15-HSQC pulse program and add other details as appropriate.
- 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 STD_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 After collecting a PRESAT dataset according to the protocol **PRESAT_bio.nan**, the 90 degree P1 pulse width value will be calibrated - note its value. (If necessary, it can be recalibrated by typing **pulsecal**.)

Then execute the **getprosol** command to update the STD parameters with the correct P1 value:

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

- 3 The default parameters from STD_xxx.par will provide suitable starting values.
Often the only parameters to change will be **NS = number of scans** in order to increase the signal to noise.

- 3.1 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):

Parameters to check:

- **2 TD**: Number of 1H time domain real points (typical 1D proton size , 32768, >1 sec aq)
- **1 TD**: set to 2 for pseudo 2D collection of the 2 FIDs.
- **NS**: minimum 8; increase for for higher signal to noise (S/N increases as square root of NS)
- **DS**: 16 'dummy' scans that are not recorded; allows system to reach steady state equilibration.
- **SW[ppm]**: 1H(F2) ~12-15 ppm
- **O1**: ¹H H₂O offset in Hz (O1P is around 4.7 ppm depending on temperature)
- **DIGMOD** - 'baseopt' (zero 1st order phase correction)

- 3.2 Then examine the parameters in the pulse program-specific '**ased**' mode (click on the 'pulse' icon or type **ased**). The **ased** mode allows more convenient access to individual parameters within arrays, such as delays, pulse widths, constants, etc. It also displays parameter values computed internally within a pulse sequence, and provides context description from the relevant pulse program comment lines.

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 (~2 s)
- **D20**: saturation time (~ 2s)
- **FQ2LIST**: 'exam_std' is a list that contains 2 values, -40 and -1. The first value is used for FID #1 and is considered off-resonance or 'non-saturated'; the second value is used for FID #2 and is considered on-resonance or saturated on broad aliphatic protein signals that can extend beyond 0 ppm. This list can be edited (E) so that the second value is between 1 ppm and -3 ppm, depending on the protein and ligand.
- **NBL**: should be 2 ; ensures the 2 FIDS are stored separately
- **NS**: typically > 32; it is important to have excellent signal-to-noise since the resulting signals after subtraction may be small.



Probe: CP TXO 900SA N/C-H-D-05 Z

General

Channel f1

Channel f2

Gradient channel

F1 indirect dimension

General

PULPROG: stddiffesgp.3 ... E Pulse program for acquisition

TD: 32768 Time domain size

SWH [Hz, ppm]: 14705.88 16.3436 Sweep width in acquisition direction

AQ [sec]: 1.1141120 Acquisition time

RG: 101 Receiver gain

DW [μsec]: 34.000 Dwell time

DE [μsec]: 18.00 Pre-scan-delay

D1 [sec]: 2.000000000 Relaxation delay: 1-5 * T1

d11 [sec]: 0.0299999993 Delay for disk I/O [30 msec]

d12 [sec]: 0.0000200000 Delay for power switching [20 us]

D16 [sec]: 0.000200000 Delay for homospoil/gradient recovery

D20 [sec]: 2.000000000 Saturation time

d31 [sec]: 2.000000000 Saturation time as executed

DELTA1 [sec]: 0 DELTA1=d1-d31

DS: 8 4

FQ2LIST: exam_std ... E Frequency list 2

L4: 1 I4 = number of averages = (total number of scans) / (number of blocks of TD size)

I5: 40 loop for saturation: p42 * I5 = saturation time

NBL: 2 Number of blocks of TD size

NS: 32 8 * n

TAU [sec]: 0.0000757094 TAU=de+p1*2/3.1416+50u

ZGPTNS: -DCALC_POWER Options for zg

Channel f1

SF01 [MHz]: 899.7942290 Frequency of ch. 1

- **P1** - 1H 90° high power pulse (calibrated from presat 1D)
- **D29**: spinlock time (10-25ms) ; this is used to reduce the broad signals from the protein or other large molecule.
- **p17**: (2.5ms) trim pulses prior to acquisition pulses in order to minimize residual magnetization left over after each pulse
- **PLW10, PLW29** : lower power levels for trim and spinlock pulses (~-8 dB)

Channel f1

SF01 [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

D29 [sec]

0.025000000

Spinlock time

[10 - 50 msec]

P1 [µsec]

12.110

F1 channel - 90 degree high power pulse

p2 [µsec]

24.22

F1 channel - 180 degree high power pulse

P17 [µsec]

2500.000

F1 channel - trim pulse

[2.5 msec]

p29 [µsec]

25000.00

F1 channel - trim pulse

P40 [µsec]

2000.000

F1 channel - 180 degree shaped pulse (Squa100.1000) [2 msec]

PLW0 [W, dB]

0

1000.00

0W

PLW1 [W, dB]

20.606

-13.14

F1 channel - power level for pulse (default)

PLW10 [W, dB]

7.5549

-8.78

F1 channel - power level for TOCSY-spinlock

PLW29 [W, dB]

7.5549

-8.78

F1 channel - power level for trim pulse

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]

0.0087138

20.60

F1 channel - shaped pulse 180 degree

CNST62: effective bandwidth in Hz of selective gaussian saturation pulse (200 Hz)

P42: duration of selective gaussian pulse (50 msec)



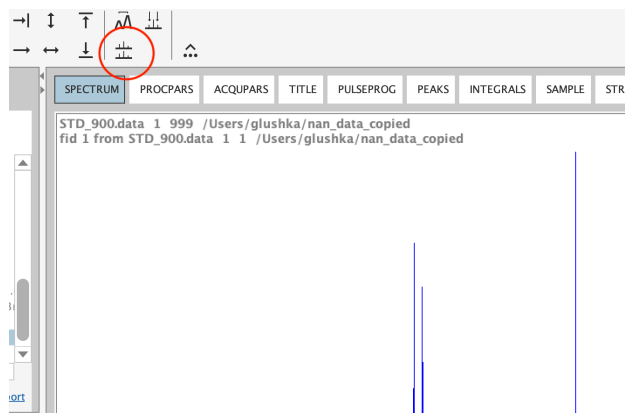
Channel f2		
SF02 [MHz]	899.7942290	Frequency of ch. 2
O2 [Hz, ppm]	4229.01 4.700	Frequency of ch. 2
NUC2	1H Edit...	Nucleus for channel 2
CNST62	200.0000000	GammaB1/2PI value for p42:sp9 (in Hz)
cnst63	10654.467773	Scaling factor for power (sp9)
P42 [μsec]	50000.000	F2 channel - shaped pulse for saturation [50 msec]
PLW2 [W, dB]	20.627 -13.14	F2 channel - power level for pulse (default) [120 dB]
P_in [μsec]	1250.00	$P_{in} = 1000000.0 / (cnst62 * 4)$
SPNAM 9	Gaus1.1000 ... E	Gaus1.1000
SPOAL9	0.500	Phase alignment of freq. offset in SP9
SPOFFS9 [Hz]	0	Offset frequency for SP9
spw9 [W, -dBW]	0.001934 27.14	$spw9 = plw1 / cnst63$
stdlist [MHz]	-0.0000400	$stdlist[2] = \{ -0.000040 \text{ MHz } -0.000001 \text{ MHz } \}$
Gradient channel		
GPNAM 1	SMSQ10.100 ... E	SMSQ10.100
GPZ1 [%]	40.00	40%
GPNAM 2	SMSQ10.100 ... E	SMSQ10.100
GPZ2 [%]	31.00	31%
GPNAM 3	SMSQ10.100 ... E	SMSQ10.100
GPZ3 [%]	11.00	11%
P16 [μsec]	1000.000	Homospoil/gradient pulse
P30 [μsec]	3000.000	Gradient pulse [3 msec]

Acquire and Process Data

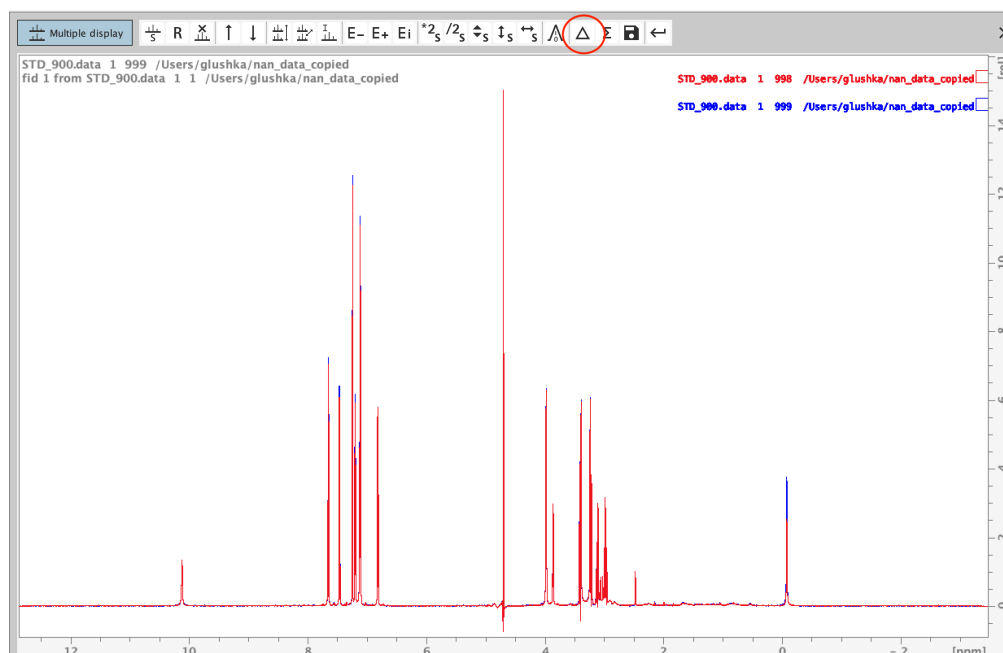
- 4 Type '**rga**' or click on 'Gain' in Topspin Acquire menu to execute automatic gain adjustment.
Type '**zg**' or click on 'Run' in Topspin Acquire menu to begin acquisition.
- 4.1 To see the difference spectrum in Topspin, first process each fid:
Open the std dataset in topspin and type 'efp' (em ft pk). This will prompt for a fid # and should default to 1, with a proc no. of 999. Click ok to process and show spectrum.
Type 'apk' to autophase the spectrum.

Re-open the std dataset and type efp again. This time choose fid #2 and change procno to 998.
Type efp, then phase with apk.

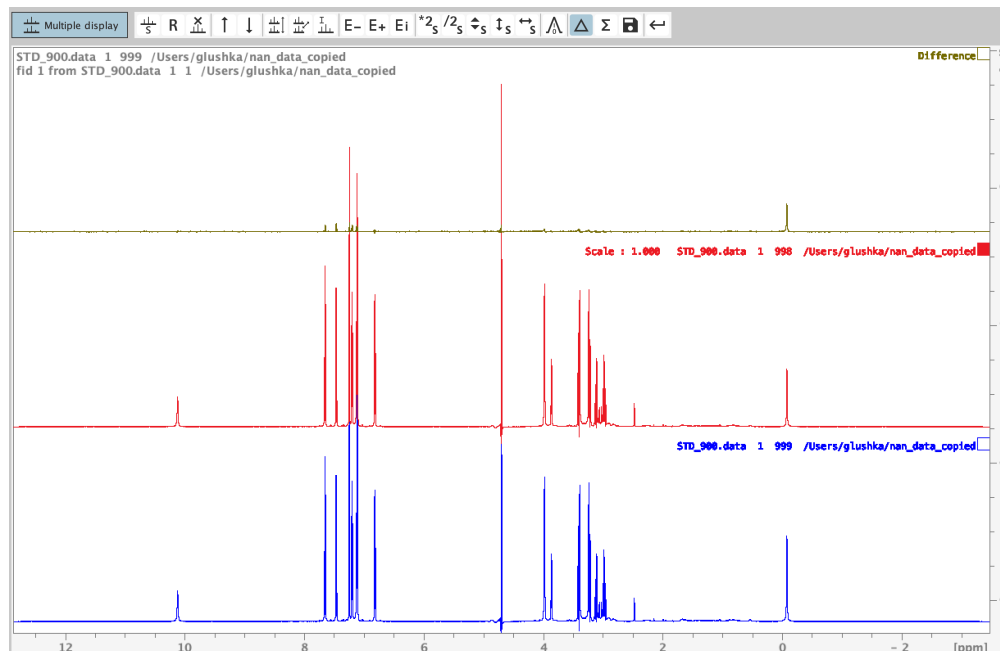
You should now have 2 additional subdirectories 998 and 999 in your topspin std dataset.
Click on 999 to open and choose the multiple display option (icon in red circle)



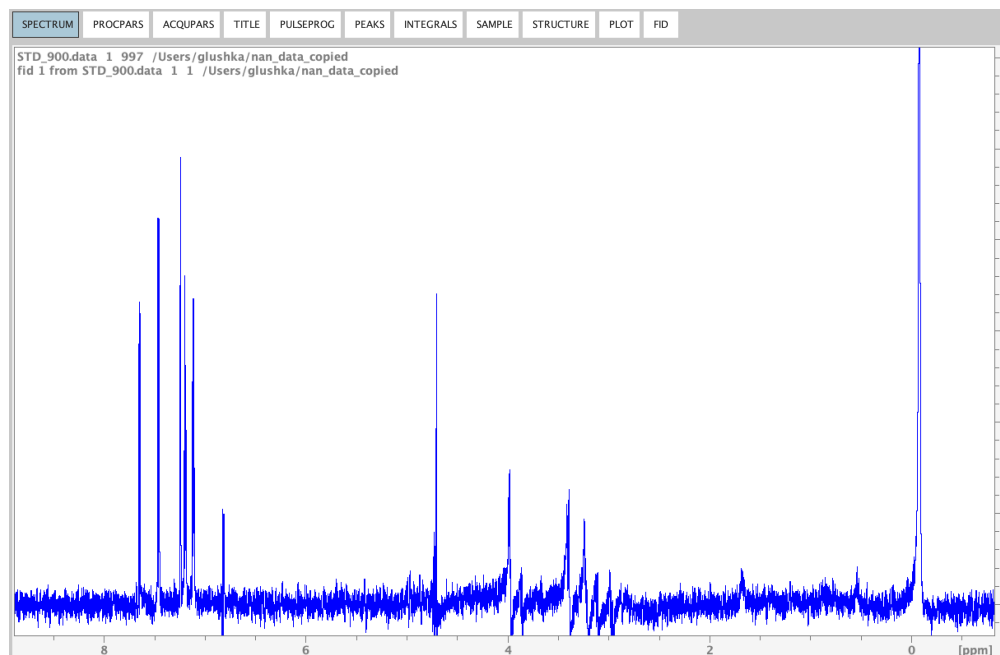
Then drag (or double click) the 998 directory entry in the data browser - its spectrum will be loaded on top of the 999 spectrum



Click on the subtraction symbol (red circle) to get the difference spectrum.



The difference spectrum can be extracted and saved separately by clicking on the disk symbol in the menu, then choosing another procno, e.g. 997





In this example (tryptophan and tyrosine mixed with Bovine Serum Albumin), only a subset of the original signals are clearly positive absorptive peaks, which indicate binding to the protein.

Protocol references

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T.-L. Hwang and A.J.Shaka, (1995) *J.Magn.Reson., Series A* 112, 275-279